DESIGN AND FABRICATION OF DIGITAL IMAGE COLORIMETER FOR SOME CHEMICALS DETECTION IN TURMERIC AND FOOD

JUTHATHIP WONGTHANYAKRAM

A Thesis Submitted to the Graduate School of Naresuan University
in Partial Fulfillment of the Requirements
for the Doctor of Philosophy Degree in Chemistry
September 2020

Thesis entitled "DESIGN AND FABRICATION OF DIGITAL IMAGE COLORIMETER FOR SOME CHEMICALS DETECTION IN TURMERIC AND FOOD"

by Ms. Juthathip Wongthanyakram

has been approved by the Graduate School as partial fulfillment of the requirements for the Doctor of Philosophy in Chemistry of Naresuan University

Oral Defense Committee

..... Chair

(Associate Professor Atitaya Siripinyanond, Ph.D)

..... Advisor

(Associate Professor Prinya Masawat, Ph.D.)

..... Co – Advisor

(Assistant Professor Yuthapong Udnan, Ph.D.)

..... Internal Examiner

(Assistant Professor Wipharat Chuachuad Chaiyasith, Ph.D.)

..... Internal Examiner

(Assistant Professor Filip Kielar, Ph.D.)

Approved

.....

(Professor Paisarn Muneesawang, Ph.D.)

Dean of the Graduate School

23 SEP 2020

ACKNOWLEDGEMENT

I would like to thank to the people who have helped and supported me so much throughout this education.

Firstly, I would like to express my sincere appreciation to Associate Professor Dr.Prinya Masawat, my great advisor for her kind inspiration, supervision, reinforcement, generous guidance and support made it possible to me to work on a topic that was of great interest to me. I am greatly delight working with her. Besides my advisor, my thankfulness is also expressed to my co-advisor, Assistant Professor Dr.Yuthapong Udnan for ideas and comments. My sincere appreciation is extended to the external examiner of the thesis defense, Associate Professor Dr.Atitaya Siripinyanond from Mahidol University, for her kindness to give the suggestions and comments. Moreover, I would like to thank internal examiner, Assistant Professor Dr.Wipharat Chuachuad Chaiyasith for her comments, questions and suggestions over the completion of my dissertation. I also would like to thank Assistant Professor Dr.Filip Kielar for helpful comments and English corrections of the manuscript for international publications and also being an internal examiner.

I gratefully acknowledge grant from the Science Achievement Scholarship of Thailand (SAST) to provide me Ph.D. education opportunity that I otherwise would not have been able to develop my scientific discoveries. I also would like to thank Department of chemistry, Faculty of science, Naresuan university for giving an access to the laboratory and research facilities. Without they precious support, it would not be possible to conduct this research.

I thank my friends for the discussions, and for all the fun we have had in the past time. Last but not least, I would like to thank my family and my parents for supporting me spiritually throughout making this research, writing this thesis, and my life in general.

Juthathip Wongthanyakram

Title	DESIGN AND FABRICATION OF DIGITAL IMAGE COLORIMETER FOR SOME CHEMICALS DETECTION IN TURMERIC AND FOOD
Author	Juthathip Wongthanyakram
Advisor	Associate Professor Prinya Masawat, Ph.D.
Co - Advisor	Assistant Professor Yuthapong Udnan, Ph.D.
Academic Paper	Thesis Ph.D in Chemistry, Naresuan University, 2020
Keywords	Curcumin, Acrylamide, Digital image colorimeter, Application, Turmeric, Food, Fluorescein, Fluorescence

ABSTRACT

This dissertation consists of two parts. The first part focuses on design and fabrication of digital image colorimeter for curcumin detection in turmeric samples. The second part is design and fabrication of digital image colorimeter for acrylamide detection in food samples. The aim of this dissertation was to develop novel, convenient and simple method. Digital image colorimeter (DIC) represents a low cost option for analyzing curcumin and acrylamide in samples. The advantages of DIC are small, inexpensive and portable instrument. However, it can detect several samples at the same time and saves reagents. The results of this research were validated by comparing the results with measurements performed using ultraviolet- visible spectrophotometer (microplate reader) and high performance liquid chromatography.

In the first part, a fast, plausible, and novel analysis using smart device-based digital image colorimeter (smart device- based DIC) method based on color measurements obtained from digital images of curcumin solutions to determine curcumin in turmeric is proposed. Digital images were obtained using a smart device and daylight white light emitting diodes (LED) light source placed in a photography lightbox as a measurement device. Images in red, green, and blue (RGB) color space were processed to extract relevant colors from the image and the color values were used to analyze curcumin concentrations. Curcumin solutions extracted from turmeric samples using a 60:40 (v/v) mixture of ethanol and hydrochloric acid were captured and the concentration was estimated by comparing color values with those collected in a

database. The proposed smart device-based DIC is able to provide a limit of detection (LOD) of 0.48 mg L^{-1} and a limit of quantitation (LOQ) of 1.61 mg L^{-1}. The color values of curcumin were tested for accuracy using turmeric sample solution spiked with 1.00, 5.00, and 7.00 mg L^{-1} of standard curcumin. The average percentage recoveries were acceptable in all samples and the percentage recoveries of the method were in the range of 98.6-110.0%. The method was validated by comparing the results with measurements performed using ultra violet-visible microplate reader (UV–Vis MR) and high performance liquid chromatography (HPLC). There was no significant difference observed between the three methods at 95% confident level. Consequently, smart device-based DIC provided a convenient, easy-to-use, low-cost, accurate, precise, and efficient approach for rapid detection of curcumin in turmeric.

In the second part, a novelty of this research work was presented/relied on a simple, fast, and plausible analysis of acrylamide in food samples accomplished using an iOS gadgets-based digital imaging colorimeter (iOS gadgets-based DIC). This method was based on fluorescence color measurements obtained from digital images of derivatized acrylamide solution extracted from snack, seasoning, and refreshment food samples (fried banana chip, fried potato chip, fried taro chip, fried durian chip, cayenne pepper, paprika seasoning, tea, and instant coffee). This device represented a convenient and low cost detection for immediate and simultaneous determination. In addition, there was no reported research using an iOS gadgets-based DIC for the analysis of acrylamide in food samples. In this research, acrylamide was degraded through Hofmann reaction, and a fluorescent product is produced by the vinyl amine reacts with fluorescein, resulting in strong fluorescence emission spectra at 590 nm. Fluorescein was chosen instead of fluorescamine because fluorescein is about 10 times cheaper than fluorescamine. The linear range for the relationship between color value and acrylamide concentration was from 1.00-10.0 mg L^{-1} with the correlation coefficient (R^2) of 0.9985. The limits of detection and quantitation were 0.53 and 1.78 mg L^{-1}, respectively. The color values of acrylamide solution were tested for accuracy using food samples spiked with 1.00, 3.00, and 5.00 mg L^{-1} of standard acrylamide solutions. The recoveries for food samples are from 82.74%-113.3%. There was no significant difference observed when comparing with fluorescence microplate reader and high performance liquid chromatography at 95% confident level.

LIST OF CONTENTS

Chapter **Page**

LIST OF CONTENTS (CONT.)

Chapter **Page**

LIST OF CONTENTS (CONT.)

Chapter **Page**

LIST OF TABLES

Table **Page**

LIST OF TABLES (CONT.)

Table **Page**

LIST OF TABLES (CONT.)

Table **Page**

LIST OF FIGURES

Figure **Page**

LIST OF FIGURES (CONT.)

Figure **Page**

LIST OF FIGURES (CONT.)

Figure **Page**

LIST OF FIGURES (CONT.)

Figure **Page**

LIST OF FIGURES (CONT.)

Figure **Page**

CHAPTER I

INTRODUCTION

Curcumin

1. Introduction to curcumin

Curcumin (diferuloylmethane) is an important component of the spices turmeric. These spices are very favourite, especially in Indian, Mexican cuisine (1-3) and Thailand. Curcumin, has been found to be useful in humans at dose up to 10 g day^{-1} (4) and it possesses a variety of remarkable pharmacological properties (5). By reason of its high medicinal potential with practically no side effects, curcumin has attracted many interest in various years (1-3, 6-9). Curcumin is unstable in basic condition, and its degrades within 30 minute to 4-dioxo-5-hexanal, ferulic acid, trans-6-(40-hydroxy-30-methoxyphenyl)-2, feruloylmethane and vanillin (10). Under acidic pH, the degradation of curcumin is much slower, with less than 20% of curcumin decomposed at 1 hour. Curcumin is more stable in cell culture medium existing 10% serum or in blood, with less than 20% decomposition within 1 hour compared with 90% within 30 minute in serum-free medium (11). The major active compounds of turmeric are curcuminoids consisting of curcumin (60-80%), demethoxycurcumin (15-30%), and bisdemethoxycurcumin (2-6%) (12-14). The chemical structures of these three curcuminoids are shown in Figure 1.1 (15).

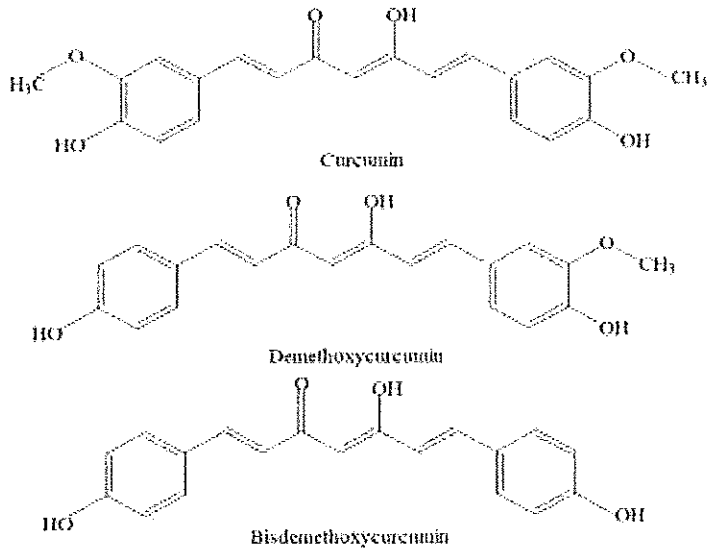


Figure 1.1 **Chemical structure of curcumin, demethoxycurcumin, and bisdemethoxycurcumin.**

Source: Re-drawn from Monton, et al., 2016 (15)

Curcumin has molecular formula $C_{21}H_{20}O_6$, molecular weight is 368.91 g mol^{-1} and melting point is 183°C (16). Curcumin is poorly soluble in hydrocarbon solvents. It is an oil soluble pigment, practically insoluble in acidic and neutral pH but, soluble in dimethylsulfoxide, methanol, ethanol, acetone, and oils. It has the absorption maximum at 420 nm (17-18). Curcumin is widely used in food coloring. It is recorded for use in fats, oils, and fat emulsions, dairy products, fruit and vegetable products, edible ices, confection, bakery, cereal, fish, meat, and eggs products, spices and herbs, mildly seasoned soup, and flavouring (19).

2. Curcumin in turmeric

Thailand is climatically endowed with a diversity of medicinal plants with athletic likely for therapeutic usability. Turmeric (*Curcuma longa L.*) is herbaceous plant that belongs to family *Zingiberaceae* and originating from India, Southeast Asia (12). It is normally vegetative as all year round crop. It requires a warm and a mildy humid atmosphere with a temperature between 20-30°C. It has 60-90 centimeters in height and the color of flower is yellow or dark orange. The leaf is ovate and thin. Turmeric have no fruits upper the ground. The fruits or rhizomes of turmeric have finger like protrusions. The central fruits or rhizomes are mainly large and thickened like a

tuber and it has massive roots (20). The fruits or rhizomes color is yellow and it is mostly 2.6-7 centimeters in length and 2-2.4 centimeters in width (21). The rhizomes and powder of turmeric are shown in Figure 1.2 (22).

Turmeric rhizomes Turmeric powder

Figure 1.2 The rhizomes and powder of turmeric.

Source: Himesh, et al., 2011 (22)

Turmeric is one of the most popular medicinal plants, and it is registered as one of the produce champions of Thailand (23). The powdered turmeric has been used for 1,000 years as flavoring, nurture, and coloring agent in food (24). Latterly, turmeric is universally used as a nutritional products and coloring agent in medicines and cosmetics. Turmeric has been found to be a refreshing source of curcuminoids (curcumin, demethoxycurcumin, and bisdemethoxycurcumin) (25).

Turmeric is regarded as native plant of India which was called harida and haldi (26). It is vegetative commercially in various countries of South Asia, India, China, and Thailand. Turmeric is a rapid source of bioactive chemicals like flavonoids, antioxidants, and polyphenols, which is the represent of antibiotics used in food products. Turmeric contains turmerone, ar-turmerone, zingiberene, and curlone which are volatile and curcuminoids which are nonvolatile (27). The yellow color of turmeric is because of curcuminoids which are the phenolic chemical compounds that are eminently present in turmeric. Amongst curcuminoids, curcumin is the main complement which is responsible for biological functions and features of turmeric (28).

In Thailand, turmeric is planted all over the country, mainly in the south. Thai herbal medicine organization has suggested that powdered turmeric should comprise or

not less than 5% w/w of curcuminoids. It has been informed that turmeric from diverse provincial part in Thailand has high differentiation of curcuminoids (29).

3. The biological properties of curcumin

Curcumin has shown a range of biological activities; its biological properties and interesting. It is a strong antioxidant and also occupies anti- inflammatory, antibacterial, anti- platelet, anti- fungal including cholesterol lowering properties. It encompasses a combination of strenuous antioxidant such as curcuminoids and it stops the cancer (30). It has substantiated as an effective anticarcinogenic chemical and decreased tumor development, angiogenesis, and carcinogenesis. It shown its repress mitogen-induced amplification of blood cell and diverse reactions of lymphocytes. It stops the mutagens of smooth muscle cells and protects the stimulation of neutrophils (31). Curcumin demonstrated a variety of therapeutic properties like antiangiogenic, antineoplastic, cytotoxic, anti- apoptotic, anti- lithogenic, anti- thrombotic, wound healing, immunomodulatory, anti-stressor and anti-diabetic actions (32). It has several pharmacologic and therapeutic properties. The following is the major significant pharmacologic and therapeutic properties of curcumin.

3.1 Antioxidant properties

Curcumin has been presented to be a strong keeper of oxygen free radicals. Its antioxidant property is the same as to vitamins C and E. It protects hemoglobin and lipid from oxidation reaction. In addition, it inhibits the propagation of reaction oxygen species for example H_2O_2, superoxide anions, and nitrite radical propagation by activating macrophages. Moreover, its derivatives such as bisdemethoxycurcumin or demethoxycurcumin also have antioxidant properties (33). Curcumin has been displayed to reduce ischemia-induced oxidative stress and changes in the heart system (24). An in vitro study showed the results of curcumin on an inducible stress protein resulting in adding cellular defiance to oxidative damage (34).

3.2 Inflammatory and edematous disorders

Curcumin has the ability to anti- inflammatory with particular lipoxygenase- and cyclooxygenase-2-inhibiting properties. In vitro studies have shown its effect at reducing both acute and chronic inflammation. Curcumin has inhibited edema at dosage between 50-200 mg kg^{-1}. A 50% of reduction in edema was succeeded with dosage of 48 mg kg^{-1} of body weight, with curcumin nearly as effective as

phenylbutazone or cortisone at the same dosage. In mice, a lower dosage of 20-80 mg kg^{-1} reduced inflammation or edema. Curcumin has inhibited formaldehyde induced rheumatism in mice at a dosage of 40 mg kg^{-1} and shown no acute toxicity at dosage up to 2 g kg^{-1} day^{-1} (35). In an animal study, rheumatoid arthritis induced by streptococcal cell wall, intraperitoneal injection of turmeric extract containing 4 mg total curcuminoids kg^{-1} day^{-1} for 4 days prior to induction of arthritis, inhibited joint inflammation in both acute (75%) and chronic (68%) phases. To test the efficacy of an oral preparation, a 30 fold higher dose of the curcuminoids preparation, given to rats 4 days prior to arthritis induction, reduced joint inflammation by 48% (36).

3.3 Anti-cancer effect

Many animal studies have diagnosed curcumin influence on the carcinogenesis. Many studies have shown that curcumin is able to restrain carcinogenesis at 3 phases; 1. angiogenesis, 2. tumor promotion, and 3. tumor growth. In the studies of prostate and colon cancer, curcumin was demonstrated to restrain cell amplification and tumor growth. Curcumin is able to repress the work of many common carcinogens or mutagens. The anticarcinogenic results of curcumin has been connected to direct antioxidant and free-radical scavenging results, as well as that their capability to increase glutathione levels, thus abetting and aiding in hepatic detoxification of carcinogens or mutagens, and stopping nitrosamine creation (35-39).

3.4 Antimicrobial activity

Curcumin has been demonstrated to be able to stop the development of bacteria, parasites and pathogenic fungi. A study of chickens polluted with Eimera maxima showed that foods supplemented with 1% curcumin resulted in a reduction in enteric lesion and weight gain (38). In another study, application of curcumin oil stopped pathogenic fungi in pigs at 7 days of post-curcumin application (40). Curcumin has been found to have medium activity against Leishmanial major organisms and Plasmodium falciparum (41).

3.5 Alzheimer

Epidemiological studies have proposed a decreased risk of Alzheimer' s disease in patients with long-time use of nonsteroidal drugs which may present the role of cerebrum inflammation in Alzheimer's disease. It also has been shown with increased cytokines and activated microglia. Moreover, it has been shown that curcumin is a

nonsteroidal drug, which can reduce oxidative damage. To estimate that curcumin could influence Alzheimer-like pathology, the effect of 160-5000 mg L^{-1} dosages of curcumin on inflammation, plaque pathology, and oxidative damage were studied. Total dosage decreased oxidized proteins and interleukin-1, a proinflammatory cytokine normally elevated in the brains of mice. Both doses significantly lowered oxidized proteins and interleukin-1, a proinflammatory cytokine usually elevated in the brains of these mice. For checking of its capability and palpable low toxicity, it is accepted for the protection of Alzheimer's disease (42-43).

3.6 Cardiovascular and anti-diabetic effects

Curcumin exerts cardio- preventive effects mostly by antioxidant property, decreasing lipid peroxidation, antidiabetic property and stopping platelets aggregation. In the study of rabbits given 1.6-3.2 mg kg^{-1} day^{-1} of curcumin extract has shown to reduce pusillanimity of low-density lipoprotein to lipid peroxidation and to lower triglyceride and plasma cholesterol levels. Curcumin effect on plasma cholesterol levels was due to the reduction of cholesterol uptake in intestine and in addition of transformation of cholesterol to bile acids in liver. Prevention of platelets aggregation by curcumin composition is thought to be via potentiation of prostacyclin and prevention of thromboxane synthesis including curcumin reduces glucose level in diabetic mice and complications in diabetic patient. Further clinical trials need to be operated in this area to find optimal dosages for cardiovascular prevention and glucose or lipid reducing properties (44).

3.7 Gastrointestinal effects

Curcumin exerts several protective effects on the gastrointestinal tract. Turmeric also inhibits ulcer formation caused by stress, alcohol, Indomethacin, reserpine, pyloric ligation, increasing gastric wall mucus in rats subjected to these gastrointestinal insults. Curcumin adds bicarbonate and stops enteric spasm, secretin, gastrin, and pancreatic enzyme secretion. Phase 2 trial operated on 25 patients with diagnosed gastric ulcer, received 600 mg of powdered curcumin 5 times daily, presented completely treated in 48% of patients. No prejudicial reactions and blood anomalies were reported (44). Curcumin decreased mucosal perniciousness in mice with colitis. A 10 days prior to induction of colitis, with 1,4,6- trinitrobenzene sulphonic acid compound, dispensation of 50 mg kg^{-1} of curcumin resulted in important reduction of

diarrhea, neutrophil permeation and lipid peroxidation in colonc. Likewise, all indexes inflammation was decreased and the attitudes improved (37). Furthermore, in mice models of pancreatitis, curcumin was able to reduce inflammation and stop inflammatory mediators, resulting in assisting disease solemnity as measured by pancreatic trypsin, serum amylase, histology, and neutrophil permeation (38).

3.8 Renoprotective and hepatoprotective effects

Curcumin has been shown to have renoprotective and hepatoprotective properties similar to silymarin. Animal studies have demonstrated renoprotective and hepatoprotective effects of turmeric from a variety of hepatotoxic insults. The hepatoprotective and renoprotective effects of turmeric are mainly due to its antioxidant properties, as well as its ability to decrease the formation of pro-inflammatory cytokines (24- 25, 33). Turmeric and curcumin have also reversed fatty changes, biliary hyperplasia and necrosis induced by aflatoxin production (25). Sodium curcuminate, a salt of curcumin, also exerts choleretic effects by increasing biliary excretion of bile salts, cholesterol, and bilirubin, as well as increasing bile solubility, therefore, possibly preventing and treating cholelithiasis (33).

3.9 Photo-protector property

Photo- protector property of curcumin is due to its antioxidant property. A major part of skin is unsaturated thus they are simply assaulted by free radicals. The ultraviolet rays of sun infiltrate skin and speed harm caused by free radicals. Protracted exposure to these radiations may degrade lipids hence begetting degeneration in texture of the skin. In lab studies, extract of turmeric was indicated to be effective in inhibiting inflammation and preventing the skin cells from damages caused by ultraviolet B radiation. Moreover, curcumin with a small dosage of turmeric was proved to prevent damage caused by gamma radiation (44).

4. Literature reviews

The literature reviews are focused on extraction and determination method for curcumin or curcuminoids, particularly, the overviews of extraction, purification, and determination from turmeric samples.

4.1 Extraction method

Plant samples are currently an essential significance due to their extra characteristics such as a big source of phytochemicals that may lead to amplification of

drugs. Major phytochemicals from turmeric samples named curcumin or curcuminoids have been presented to have positive effect on health (45). The study of plant sample starts with pre-extraction or the extraction process, which is a significant step in the processing of bioactive ingredient from plant samples. Extraction methods such as maceration and soxhlet extraction are normally used at small research level. In addition, there are other methods of plants extraction such as microwave-assisted, ultrasound-assisted extraction, and supercritical fluid extraction, in which these methods are aimed to increase percentage yield. The following are the description of the regular extraction used for turmeric samples (46).

4.1.1 Soxhlet extraction

In soxhlet extraction method, elegantly ground sample is placed in a porous bag made from a cellulose or filter paper then placed in a chamber of the soxhlet apparatus as shown in Figure 1.3 (47). Solvent heated the bottom flask, evaporates into sample thimble, then it condenses in the condenser and drips back. When the liquid solution reaches the siphon arm, the liquid solution emptied into bottom flask again and the procedure is continued repeating many times (46).

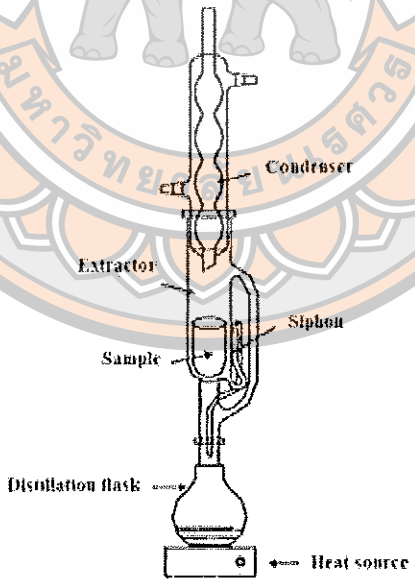


Figure 1.3 Conventional soxhlet extractor.

Source: Luque de Castro, et al., 2010 (47)

The drawbacks of soxhlet extraction as compared with the other methods for solid sample preparation are long time used and the large amount of solvent wasted, which is not only extravagant to eliminate off, but also the origin of environmental problems. Samples are extracted at the solvent boiling point over long time, which can produce in thermal decomposition of target species. In addition, the large amounts of extractant used call for an evaporation– concentration step after extraction. Finally, the Soxhlet technique is limited by extractant and difficult to automate (47).

Several soxhlet extraction for determination of curcumin or curcuminoids are briefly listed in Table 1.1

Table 1.1 Soxhlet extraction for determination of curcumin or curcuminoids.

Type of extractant	Yield (%w/w)	References
Curcuminoids	2.34-9.18	(13)
Curcuminoids	2.74-4.59	(48)
Curcuminoids	0.18-3.42	(28)
Curcuminoids	4.30-5.60	(19)
Curcumin	41.5-69.67	(49)
Curcumin	0.610-0.820	(50)
Curcuminoids	4.30-5.60	(51)
Curcumin	2.20-3.49	(52)

4.1.2 Microwave assisted extraction

Microwave assisted extraction uses microwave energy to accommodate partition of analytes from the sample into the solvent (53). Microwave energy interacts with dipoles of solvents or sample causes heating near the surface of the sample and heat is staggered by conduction. Dipole rotation of molecules induced with microwave electromagnetic destroys hydrogen bonding; supplementing the migration of dissolved ions and helps solvent broaching into the matrix. While in nonpolar solvents, low heating arises as the energy is transferred by dielectric absorption (54).

The advantages of microwave assisted extraction are decreasing time and solvent volume in comparison with the other methods. Moreover, it improves efficiency of extraction, recoveries of samples and reproducibility at the suitable conditions (54). However, microwave assisted extraction is limited to phenolic acid compounds such as gallic and ellagic acid, isoflavin, and quacertin because theirs molecules were stable under heating conditions up to 100°C for 20 minutes. Additional cycles of microwave assisted extraction such as from 2 × 10 - 3 × 10 second can be resulted in drastic reduce in the yield of flavanones and phenolic acid compounds, mostly gotten by the oxidation of compounds (53).

Several microwave assisted extraction for determination of curcumin or curcuminoids are briefly listed in Table 1.2

Table 1.2 **Microwave assisted extraction for determination of curcumin or curcuminoids.**

Type of extractant	Yield (%w/w)	References
Curcumin	4.49-12.89	(55)
Curcumin	2.10-62.6	(56)
Curcuminoids	0.79-0.95	(57)
Curcumin	23.0-35.0	(58)
Curcumin	82.4	(59)
Curcumin	3.51	(60)
Curcuminoids	1.30-1.70	(61)

4.1.3 Ultrasound-assisted extraction or sonication extraction

Ultrasound-assisted extraction or sonication extraction involves the use of ultrasound ranging from 20-2000 kilohertz (54). The mechanic results of acoustic cavitation from ultrasound increase the surface catch between samples and solvents. The chemical and physical properties of samples and solvents subjected to ultrasound are changed and destroy these properties which facilitating unleash of compounds and

supplementing mass transport of the solvents into samples (62). The process is easy and low cost. It can be used in both small and larger scale of sample extraction.

The advantage of ultrasound- assisted extraction or sonication extraction is mostly due to the reduction of time and solvent volume. Moreover, use of ultrasound energy more than 20 kilohertz may have an effect on the phytochemicals through formation of free radicals (54).

Several ultrasound-assisted extraction or sonication extraction for determination of curcumin or curcuminoids are briefly listed in Table 1.3

Table 1.3 Ultrasound-assisted extraction or sonication extraction for determination of curcumin or curcuminoids.

Type of extractant	Yield (%w/w)	References
Curcuminoids	1.14-7.51	(63)
Curcumin	1.72-2.99	(64)
Curcuminoids	81.90–99.86	(65)
Curcumin	0.004-0.012	(66)
Curcumin	12.44-20.95	(67)
Curcuminoids	0.012-0.028	(68)
Curcuminoids	0.78-0.97	(57)
Curcuminoids	4.72-6.40	(69)
Curcuminoids	0.0024-2.331	(70)

4.1.4 Accelerated solvent extraction

Accelerated solvent extraction is an efficient form of solvent extractions comparison with soxhlet extraction as the technique uses minimum amount of liquid solvent. The sample is packed with inert material to protect it from consolidating and blocking the system tubing (71). The pack of accelerated solvent extraction cell includes layers of inert material-sample mixture in between inert material layers and cellulose filter paper. Accelerated solvent extraction is able to control pressure and temperature for each sample and demand less time for extraction. Similar

to other liquid solvent extraction, accelerated solvent extraction is also critically base on the liquid solvent types.

Several accelerated solvent extractions for determination of curcumin or curcuminoids are briefly listed in Table 1.4

Table 1.4 Accelerated solvent extraction for determination of curcumin or curcuminoids.

Type of extractant	Yield (%w/w)	References
Curcuminoids	0.94-1.80	(72)
Curcumin	4.17-5.17	(73)
Curcumin	2.40-8.60	(74)
Curcumin	0.47-5.73	(75)

4.1.5 Supercritical fluid extraction

Supercritical fluid is a substance that combines the physical properties of both liquid and gas at its critical point. Pressure and temperature are the factors that push a substance into its critical point. Supercritical fluid conducts more like gas but have the solvating property of a liquid or solution. An example of supercritical fluid is carbon dioxide which become supercritical fluid at over 31.1°C and 7380 kilopascals. Attention in supercritical-carbon dioxide extraction is due to it' s an excellent solvent for nonpolar compounds and carbon dioxide is widely available. It also has low toxicity and low cost. Even though, supercritical-carbon dioxide has low solubility for polar compounds, adaptation such as increasing few amount of methanol or ethanol enable it to extracts polar compounds. Supercritical-carbon dioxide also produces sample at concentrate form while carbon dioxide evaporates at ambient temperature. Supercritical-solvents can be easily changed by adjusting the pressure and temperature. Moreover, by increasing modifiers it can lead to decreasing extraction time. A major drawback of supercritical fluid extraction is a very high cost of the initial apparatus (76).

Several supercritical fluid extractions for determination of curcumin or curcuminoids are briefly listed in Table 1.5

Table 1.5 Supercritical fluid extraction for determination of curcumin or curcuminoids.

Type of extractant	Yield (%w/w)	References
Curcuminoids	0.34-8.43	(77)
Curcuminoids	1.36-5.69	(78)
Curcuminoids	1.72-1.96	(79)
Curcumin	0.255-2.645	(80)
Curcuminoids	0.46-2.08	(81)
Curcuminoids	5.21-5.62	(69)
Curcumin	0.000445	(82)

All the methods that use solvents in the process are influenced by the liquid solvent types. Microwave assisted extraction and ultrasound-assisted extraction or sonication have no significant effect caused by the solvent volume used on the biologically active samples (53). Although, all these extraction methods resulted in crude extracts containing a mixture of metabolites, the efficacy of those crude extracts using nano-encapsulated processing in Centella asiatica showed to have similar efficacy as those purified (83). This particular fact suggests that further isolation and purification of extracts, which is rather complex and time using is not necessary if proper preparation and extraction are done.

Proper conditions for each extraction are important. Definite temperature and light need to be estimated to extracts thermo- labile chemical compounds. Among factors such as solvent strength (70% ethanol is used in turmeric extraction (56), triterpenoids extraction (84), and phenolic extraction (85)), solvent types, agitation speed, extraction time, sample-solvent ratio, and temperature were examined using the designed experiment.

Among those optimization processes, the most influential factors in all extraction are solvent strength and type of solvent. Moreover, solvent-sample ratio has no significant effect, recommending inessential large volume of liquid solvents can be avoided. Each optimized process is characteristic to the plants or samples. All the

factors such as solvents, temperature, and agitation speed might have the potentiality to enhance extraction, but without suitable decision may cause degradation of chemical compounds. Therefore, considering extractions that has least factors might be an intelligent decision step in choosing suitable extraction. Other than, if purity is concern, accelerated solvent extraction is the most advanced extraction technology (46).

All procedures for samples preparation or extractions are relatively important in the experiment of medicinal plants. The sample preparation such as drying and grinding affected the phytochemical substance and efficiency of the final samples extractions; that ultimately have an effect on the final sample extracts. It can be summarized that there is no universal extraction technique which is the ideal technique and each extraction step is the characteristic of the medicinal plants. Previously optimized technique can be applied for the decision of selection of suitable extraction. Moreover, evaluation and decision of samples preparation and samples extraction depend on the objectives, target compounds or samples (46).

4.2 Determination method

4.2.1 High Performance Liquid Chromatography (HPLC)

High Pressure or High Performance Liquid Chromatography; HPLC is a favorite analytical method used for the identification, separation, and quantification of analytes present in various the samples. HPLC is an advanced method of column liquid chromatography. The liquid solvent flows through column but in HPLC method the liquid solvent is controlled under high pressures up to 400 atmospheres so that matrix can be separated from the sample with the help of difference in relative affinities (86-89).

In HPLC method, pumps are used to pass pressurized solvent and the mixture which is approved to enter into a column filled with solid adsorbent. The reaction of each sample constituent is varie and sample constituent with difference flow rates finally leads to separation of each constituent from the column (90).

Chromatographic method can be depicted as a mass changed process including adsorption. HPLC method depends on pumps which used for passing samples and a pressurized fluid through a section loaded with solid adsorbent, stimulating the partition of the sample segments. The dynamic portion of the solid adsorbent and the section, are granular material made of silica or polymers, 2- 50

micrometers in size. The fragment of the sample mixture is isolated from each other forasmuch of their exclusive degrees of relation with the retentive particles. The pressurized fluid known as mobile phase is a blend of solvents such as acetonitrile, water, or methanol. Its temperature and organization plays a main section in the partition process by affecting the relations occurring between adsorbent and sample component (91-96).

HPLC method is recognized from traditional liquid chromatography because operational pressures are 50-350 bar, while normal liquid chromatography depends on the power of gravity to pass the portable stage through the segment. Because of the few sample amount isolated in scientific HPLC method, column portion measurements are 2.1-4.6 millimeter in diameter, and 30-250 millimeter in length. In addition, HPLC segments are made with smaller sorbent particles (2 μm to 50 μm in normal molecule size). This gives HPLC high determining or resolving power (the capacity to recognize components) while isolating mixtures, which makes it a prominent chromatographic method (97-104).

The sample blend to be isolated and dissected is presented, in a discrete little volume, into the stream of mobile phase permeating through the column. The segments of the sample travel through the column at various speeds, which are a component of particular physical connections with the adsorbent (likewise called stationary phase). The velocity of every component relies on its compound nature and composition of mobile phase. The time at which a particular analyte elutes is called its retention time. The retention time measured under specific conditions is a distinguishing normal for a given analyte (105-114).

Various sorts of columns are available, loaded with adsorbents varying in molecule size, and in the nature of their surface. The utilization of small molecule size packing materials requires the application of higher operational pressure (backpressure) and regularly enhances chromatographic resolution (i.e. the degree of division between sequential analytes rising up out of the column). Sorbent particles might be hydrophobic or polar in nature. Standard mobile phases are any miscible mixture of liquid with different natural solvents such as acetonitrile and methanol. The aqueous section of the mobile phase may contain trifluoroacetic, formic, or phosphoric acid and salts to help separating the components. Mode of separations are isocratic and

gradient elution. Isocratic elution is normally successful in the partition of sample components that are not altogether different in their proclivity for the stationary phase. In gradient elution mode, the composition of the mobile phase is changed normally from low to high eluting quality. The eluting quality of the mobile phase is reflected by analyte maintenance times with high eluting quality delivering quick elution (90).

The chosen structure of the mobile phase relies on the control of relations between different analytes and stationary phase. Dependent upon their nature of the mobile phase and stationary phase, analytes were separated between the two. During the release process arising in the sample, it is like what happens in a liquid-liquid extraction, however, it is continuous, not step- wise. In this case, using an acetonitrile/water, more hydrophobic parts will elute (fall off the column) late, once the mobile phase gets more packed in acetonitrile (i.e. in a versatile period of higher eluting quality) (90, 115-120).

1) Instrumentation

The instrumentation connects solvent reservoir, pump, sample injector, column, detector, estimator or integrator and display system. The instrument parts comprise of:

1.1) Solvent Reservoir: The volume of mobile phase is presented in glass container. Mobile phase is a mixture of non-polar and polar solvents. Depending on the composition of sample, the non-polar and polar solvents will be variegated.

1.2) Pump: The pump pulls mobile phase or solvent from solvent reservoir and forces mobile phase or solvent to column then sends to detector. 1000-6000 pound per square inch (psi) is the operating pressure of pump. This pressure depends on flow rate, column dimensions or particle size, and composition of mobile phase or solvent.

1.3) Sample Injector: The injector can be a solitary or computerized infusion framework. A sample injector for framework should give infusion of the liquid specimen inside the sample loop of 10-100 microliter (μL) with good reproducibility and under high pressure.

1.4) Columns: Columns are normally made of stainless steel. It is wide around 50 millimeters and 300 millimeters long and it have diameters around 2-5 millimeters. They are universally loaded with a stationary phase with a molecule size of

3-10 micrometers. Columns with inner diameters of <2 millimeters are regularly alluded as microbore section. Especially the temperature of the column and the mobile phase or solvent should be kept consistent during determination.

1.5) Detector: The detector situated toward the end of column distinguishes the samples as they elute from the chromatographic column. Ordinarily used detectors are fluorescence, mass spectrometric, UV- spectroscopy, and electrochemical identifiers.

1.6) Integrator: Signals from the detectors might be compiled on graph that fluctuate in their capacity to process and in many-sided quality, it stores and presents chromatographic information (90).

The schematic of a HPLC instrument normally incorporates a sample injector, pumps, and a detector. The sample was dissolved in the mobile phase then injected to the column. The pumps usher the mobile phase through the column. The detector generates a signal which relative to measure the sample component rising up out of the section, thereby, taking into consideration quantitative determination of the sample parts. A computerized microchip or software controls instrument and gives data. A few models of pumps can combine various solvents in a ratio changing in time, creating a synthesis slope in the portable stage. Most of HPLC instruments have a column broiler that considers transitioning the temperature at which the partition is operated (121-123).

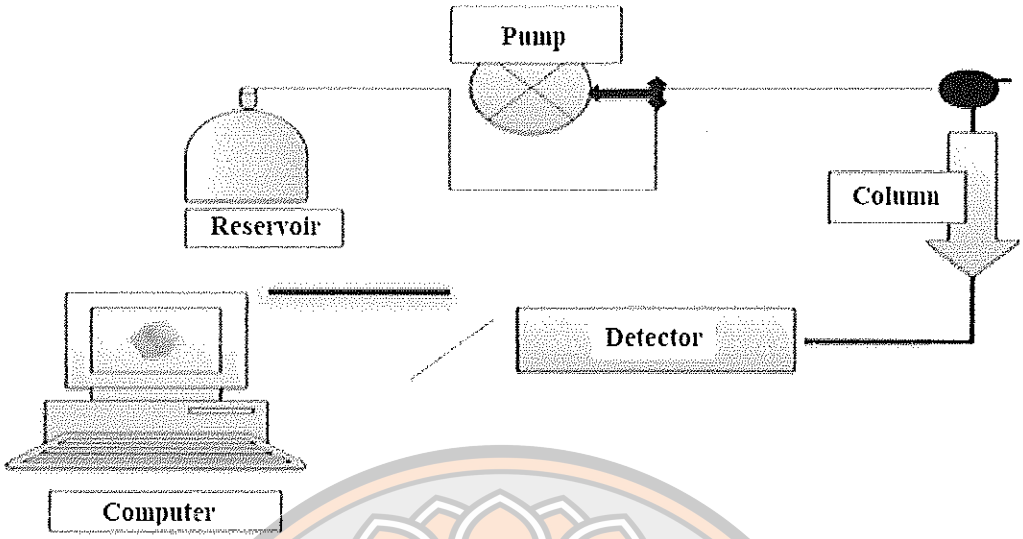


Figure 1.4 The schematic of HPLC instrumentation.

Source: Gawhari, F. 2013 (124)

2) Types of HPLC

Depending on the substrate used (stationary phase and mobile phase), the HPLC is differentiated into 4 types as the following (125-132).

2.1) Normal Phase: In this system the separation is based on polarity. The stationary phase is polar, mainly silica is utilized and the mobile phase is non-polar; hexane, diethyl ether, and chloroform. The polar samples are retained on column (128).

2.2) Reverse Phase: It is reverse to normal phase. The stationary phase is non-polar and the mobile phase is polar. The sample is the non-polar, it will be retained on column.

2.3) Size-exclusion: The stationary phase will be incorporating with accurately controlled substrate molecules. Based on the unlikeness in molecular sizes the separation of composition will arise.

2. 4) Ion- exchange: The stationary phase is having charged surface reverse to sample charge. The mobile phase used is buffer solution which is controlled the pH or ionic strength.

3) Applications of HPLC

The HPLC has many applications in the fields of chemistry, industry, environment, forensic, pharmacy, and clinical. These application helps in the separation or purification of chemical compound (133-134).

3.1) Pharmacy: The pharmacy applications include controlling of medicine stability, quality control and dissolution studies.

3.2) Environment: Determining of pollutants include detecting compositions of drinking and consumption water.

3.3) Forensic: Quantification of medicine and steroids in biological samples.

3.4) Flavour and food: Detecting polycyclic compounds in vegetables sample. Sugar analysis in fruit juices sample, analysis of preservatives in food sample.

3.5) Clinical: Analysis of biological samples such as blood, serum, urine, and feces. Detecting endogenous neuropeptides.

Several HPLC method for determination of curcumin or curcuminoids are briefly listed in Table 1.6

Table 1.6 HPLC method for determination of curcumin or curcuminoids.

Type of analyte	Sample	LOD/LOQ	Amount Found	QA/QC report	Ref.
Curcuminoids	Turmeric powder	LOQ 0.05 µg	2.34-9.18%w/w	-	(13)
Curcumin	Dog plasma	LOQ 2 ng mL^{-1}	4.29-63.75 ng mL^{-1}	%recovery 98.7-105.0 %RSD<7.3	(135)
Curcuminoids	Turmeric rhizome	LOD 21.81-31.91, LOQ 66.10-96.72 ng mL^{-1}	32-371 ng mL^{-1}	%recover 98.4-101.8 %RSD 1.21-2.52	(136)
Curcumin	Rat plasma	LOD 20 ng mL^{-1}	47.1-1015 ng mL^{-1}	%recovery 58.12-98.17 %RSD 2.06-12.7	(137)
Curcuminoids	Curcuma longa	-	0.72-2.19 %w/w	%recovery 92.9-97.3	(138)
Curcumin	Commercial capsules of curcumin	LOD 0.06, LOQ 0.21 µg mL^{-1}	2.06-6.01 µg mL^{-1}	%recovery 97-103 %RSD 0.07-0.58	(139)
Curcuminoids	Curcuma zedoaria rhizome	LOD 0.14-0.33, LOQ 0.47-1.11 µg mL^{-1}	6.09-16.83 %w/w	%recovery 100.70-101.85 %RSD	(140)
Curcumin	Rat biomatrices	LOD 12.5 ng g^{-1}	1.4-3671.8 ng g^{-1}	0.63-1.66 %recovery 94-106 %RSD	(141)

Table 1.6 (cont.)

Type of analyte	Sample	LOD/LOQ	Amount Found	QA/QC report	Ref.
Curcumin	Commercial capsules of curcumin	LOD and LOQ 1-50 ng mL^{-1}	3.68-8.125 ng mL^{-1}	0.7-5.5 %recovery 95-105 %RSD 1.274-1.316	(142)
Curcumin	Rat plasma	LOQ 1 ng mL^{-1}	1.81-407.37 ng mL^{-1}	%recovery 94.15-107.05 %RSD 1.74-9.24	(143)
Curcumin	Vicco turmeric cream and haridrakhand churna	LOD 52.9 ng mL^{-1}, LOQ 160 ng mL^{-1}	5.608-18.59 µg mL^{-1}	%recovery 96-104.17 %RSD 0.672-0.704	(144)
Curcumin	Rat plasma	LOQ 10 ng mL^{-1}	9.98-1615 ng mL^{-1}	%recovery 92.83-107.83 %RSD 2.79-8.20	(145)
Curcumin	Rat plasma	LOQ 5-10 ng mL^{-1}	12.3-1259 ng mL^{-1}	%recovery 93.9-108 %RSD 1.63-18.7	(146)
Curcumin	Turmeric powder	LOD 49, LOQ 148 ng $spot^{-1}$	1.82-34.50 %w/w	%recovery 99.60-99.73	(16)
Curcumin	Crude Powder	LOD 103.91,	3.12-3.69 %w/w	%recovery 100.28-101.61	(147)

Table 1.6 (cont.)

Type of analyte	Sample	LOD/LOQ	Amount Found	QA/QC report	Ref.
	Mixture and Ayurvedic Formulation	LOQ 314.88 ng		%RSD 1.98	
Curcuminoids	Commercial foods	-	18.68-617.98 $\mu g\ g^{-1}$	%recovery 3.31-98.9	(148)
Curcumin	Human plasma	-	1.88-86.10 $ng\ mL^{-1}$	%recovery 81.3-119.9 %RSD 2.91-13.10	(149)
Curcumin	Curcumin encapsulated in the Eudragit E 100 nanoparticles	LOD 0.3, LOQ 0.4 $mg\ L^{-1}$	4.5925-4.7778 mg	%recovery 98.97-100.90 %RSD<2	(150)
Curcumin	Pharmaceutical dosage forms	LOQ 10 $\mu g\ L^{-1}$	10.31-1002.85 $ng\ mL^{-1}$	%recovery 99.98-104.24 %RSD 0.16-13.26	(103)
Curcumin	Different polyherbal formulations	LOD 0.01, LOQ 0.0246 $\mu g\ mL^{-1}$	21.39 $\mu g\ mL^{-1}$	%recovery 99.34-99.89 %RSD 0.3397-0.7848	(151)
Curcuminoids	Turmeric	LOD 0.18-0.27, LOQ 0.55-0.84	10.13-124.00 $\mu g\ mL^{-1}$	%recovery 97.7-104.0 %RSD < 2.84	(152)

Table 1.6 (cont.)

Type of analyte	Sample	LOD/LOQ	Amount Found	QA/QC report	Ref.
		µg mL^{-1}			
Curcumin	Turmeric	LOD 26, LOQ 77 µg $band^{-1}$	1362.33-3720 µg $band^{-1}$	%recovery 98.88-99.61 %RSD 1.54-1.74	(153)
Curcumin	Curcuma longa extract and emulsion formulation	LOD 0.305, LOQ 2 µg mL^{-1}	99.45-100.20 %w/w	%recovery 98.03-98.24, %RSD 0.11-1.99	(154)
Curcuminoids	Turmeric	LOD 3, LOQ 10 ng mL^{-1}	-	%RSD < 1	(155)
Curcumin	Rat plasma	LOQ 6 ng mL^{-1}	5.07-201.97 ng mL^{-1}	%recovery 92.47-105.70 %RSD 5.37-11.26	(156)
Curcuminoids	Turmeric	LOD 16.9-18.0, LOQ 31.1-33.8 nM	136-162 µM	%RSD 1.64-2.25	(157)
Curcumin	Pharmaceutical dosage form	LOD 3.21, LOQ 9.75 µg mL^{-1}	24.62-76.62 µg mL^{-1}	%recovery 98.53102.91 %RSD 0.31-2.04	(158)
Curcumin	Curcumin nanoparticulate formulation	LOD 0.018, LOQ 0.056 µg mL^{-1}	0.78 mg	%recovery 97.8-102.36	(159)

Table 1.6 (cont.)

Type of analyte	Sample	LOD/LOQ	Amount Found	QA/QC report	Ref.
Curcumin	Various dosage forms	LOD 0.0104, LOQ 0.0118 mg mL^{-1}	-	%recovery 100.78 %RSD 0.603	(160)
Curcumin	In vitro performance tests	LOD 11.61, LOQ 500 ng mL^{-1}	1.01-71.00 µg mL^{-1}	%recovery 99.7-108 %RSD < 5	(161)
Curcumin	Mammalian samples	-	0.55-2.32 mg g^{-1}	%recovery 91.66-99.00, %RSD 1.82-2.98	(162)
Curcuminoids	Curcuma syrup formulation	LOD 0.031-0.056, LOQ 0.095-0.169 µg mL^{-1}	0.116-1.868 mg 5 mL $syrup^{-1}$	%recovery 97.04-100.51 %RSD 1.73-2.16	(163)
Curcuminoids	Tablet dosage form	LOD 0.126-0.074, LOQ 0.223-0.382 µg mL^{-1}	0.22-3.09 mg $taplet^{-1}$	%recovery 99.02-103.78 %RSD < 2	(164)
Curcumin	Pharmaceutical samples	LOD 0.25, LOQ 0.5 µg mL^{-1}	3.206-78.2 %w/w	%recovery 98.9-100.5 %RSD < 0.638	(165)
Curcumin	Polymeric micelles	LOQ 7.6 ng mL^{-1}	2.02-1566.67 ng mL^{-1}	%recovery 94.94-98.44 %RSD	(166)

Table 1.6 (cont.)

Type of analyte	Sample	LOD/LOQ	Amount Found	QA/QC report	Ref.
Curcumin	Rat plasma	LOD 1.5625, LOQ 3.125 $\mu g\ mL^{-1}$	5.183-47.610 $\mu g\ mL^{-1}$	5.37-8.66 %recovery 95.22-103.66 %RSD	(167)
Curcumin	Ayurvedic formulation	LOD 0.183, LOQ 0.550 $mg\ L^{-1}$	4.673-4.676 $mg\ L^{-1}$	0.28-0.86 %recovery 98.70-100.32 %RSD	(168)
Curcumin	Plasma and lung tissue	LOQ 0.05-0.1 $mg\ L^{-1}$	0.1-5 $mg\ L^{-1}$	0.08-0.21 %recovery 101.1-101.4, %RSD 4.5-12.0	(169)
Curcumin	Curcumin at Polymeric microparticles	LOD 91.61, LOQ 128.71 $\mu g\ L^{-1}$	96.33-97.86 %w/w	%recovery 97.0 %RSD 1.38	(170)
Curcumin	Curcumin solutions	LOD 0.04, LOQ 0.14 $mg\ L^{-1}$	0.3125-60 $mg\ L^{-1}$	%RSD < 2	(171)

4.2.2 UV-visible spectrophotometry (UV-vis)

The evolution of analytical methods has conducted hand in hand with the introduction of new instruments or techniques. In the end of the 19th century, the creation of the spectroscopic methods were increased to the list of available steps. At first, they were used only for semiquantitatively and qualitatively, but in the 1930's, fast development of photoelectric tubes, vacuum tube amplifiers, integrated circuits, transistors, and semiconductor devices were used which adding levels of complexity. Physical property is the characteristic of sample which can be the basis of analytical

method. In the spectroscopy, the absorption at particular wavelengths is the property used for mensuration.

Spectrophotometry refers to the measurements of relative number of light as the function and feature of wavelength. Normally, measurements are made by analogy with some scale, the instrument indirectly or directly acts as comparison. The sample is estimated relative to the standard because the reading received from the sample is rationed to the reading received from the standard. The standard or reference in spectrophotometry is usually a colorless solution (water or deionized water) (172).

Ultraviolet (UV) and Visible (vis) absorption spectrophotometry (UV-vis) is the method based on intensity of electromagnetic radiation mensuration by an absorbing sample or reference. The radiation has a spectrum range of 190–800 nanometers, which different in terms of energy ranges. This concentration results from the scattering, reflection, interferences or absorption. However, precise measurements of the intensity can be done recording only the absorbance value. The absorbance value is proportional to concentration of the sample or reference to measure and to distance of the light source when it passes through sample or reference. This relation is called "Beer's law" and it is written as Equation 1.1.

$$A = \varepsilon \times b \times c \quad \text{..... (Equation 1.1)}$$

where A is an absorbance value, ε is the molar absorptivity in mol^{-1} L cm^{-1}, b is the path length in centimeter, and c is the concentration in mol L^{-1}.

This linear relation can be influenced by different parameter such as the photodegradation of the molecules, the characteristics of the spectrophotometer, the fluorescent compounds in the sample, the presence of scattering or absorbing interferences in the sample, an interaction between the sample and the solvent, and the pH value (173).

1) Instrumentation

The scientist studied in molecular absorbance measurements in ultraviolet, visible, and near-infrared has hundred and more instrument manufactures and modes to select from usability. Some instrument is easy and low cost. The simple instruments provide data as rapid and as satisfactory as that received by the more complicated instruments. On the other hand, the more sophisticated instruments have developed to operate with tough, time-consuming, or unimaginable with the simpler

ones. Instruments for measuring the absorbance of ultraviolet, visible, and near-infrared radiation are made up of source, wavelength selectors, sample containers, radiation transducers, signal processors and readout devices (174). A brief detail of each parts are as the following;

1.1) Source: for the aims of molecular absorbance mensuration, continuum source is required which energy does not convert sharply over a massive range of wavelengths.

1.1.1) Deuterium and Hydrogen Lamps: A continuum spectral in ultraviolet region is generated by electrical excitation of hydrogen or deuterium lamps at low pressure. Most modern lamps of this deuterium are low-voltage type in which is formed between heated, metal electrode, and oxide-coated filament. The heated filament provides electrons to maintain current when around 40 volts is applied; an imposed power supply is used for stable intensities. An essential feature of deuterium or hydrogen release lamps is shape of the hole between the 2 electrodes, which shrinks the release to a strait path. So, an intensive ball of radiation about 1-1.5 millimeter in diameter is made. Both deuterium and hydrogen lamps create a salutary continuum spectral in the range of 160-375 nanometer.

1.1.2) Tungsten Filament Lamps: The most joint source of visible and near-infrared radiation is tungsten filament lamp. The power diffusion of source approximates that of blackbody radiation and is temperature dependent. The most absorbance instruments, the working filament temperature is 2,870 kelvins; this lamp is use in the range of 350-2500 nanometer. In the visible range, the power output of tungsten filament lamp varies approximately as the 4^{th} power of the working voltage. A stable voltage transformers or electronic voltage regulators are normally employed to receive the desired invariability. As a selective, tungsten filament lamp can be maintained at a 6 volt, which gives an exceptionally stable voltage source if it is stored in good condition.

1.1.3) Xenon Arc Lamps: The xenon arc lamp manufactures intense radiation by the way of current perpetually an atmosphere of xenon. The spectral is continuum in the range of 200-1000 nanometer. In some instrumental, the xenon arc lamp is worked periodically by normal liberates from capacitor; high intensities are received.

1.2) Wavelength selector: for most spectroscopy analyses, radiation is limited and ranged with continuum group of wavelengths. A narrow bandwidth increases the sensitivity of measurements, may give selectivity to both absorption and emission methods, and it is desire from the standpoint of receiving linear relationship between the concentration and optical signal. Moreover, the output from wavelength selector would be a radiation of a frequency or single wavelength. Herein, the percentage of radiation of a provided wavelength that is transmitted by selector is written as an operation of wavelength. The bandwidth is converse measure of the quality of device. 2 types of wavelength selectors are discovered; monochromators and filters.

1.2.1) Monochromator: for most spectroscopy methods, it is pleasurable or mandatory to be able to differ the wavelength of radiation consistently over a massive range. This step is called scanning spectral. Monochromators are created for spectral scanning. This wavelength selector for ultraviolet, visible, and infrared radiation are overall homologous in mechanical creation in the sense that they use prisms or gratings, slits, window, lenses, and mirrors.

1.2.2) Filters: 2 types of this wavelength selector are used for wavelength selection; absorption and interference filters. Absorption filters are limited to the visible range of the spectral; on the other hand, interference filters are used for ultraviolet, visible, and infrared region.

1.3) Sample containers: it is used for overall spectroscopy studies unless emission spectroscopy. In common with optical elements of wavelength selectors (monochromators), the cuvettes or cells that hold the sample must be created of material that is pellucid to radiation in spectrum region of attention. Silicate glasses can be used in the range of 350-2000 nanometer. Plastic containers have found application in visible range and crystalline sodium chloride is the most normal substance used for cell windows in infrared range.

1.4) Radiation transducers: the detectors for preliminary spectroscopy instruments were human eye or film or photographic plate. These detectors have been mainly supplanted by radiation transducers that transform light signal into an electrical signal (174). It must reciprocate in a wide wavelength region, reciprocate with low noise and with high sensitivity, have a rapid response, have a linear response region, and a

low consumption of sample. In order to reach an ideal detector, many detectors are explained as the following; (173).

1.4.1) Photomultipliers: Photomultiplier tubes are comprised by photoemissive cathode, series of electrodes and anode. A photoemissive cathode when stricken by a photon, emits many electrons that are quicken towards dynodes, since they are a discharge of many secondary electrons. Those ones are quicken to next the electrode where each one discharges more electrons. Finally, they are collected on the anode and thereby the last output is henceforward electronically amplified (175). The consume of photomultiplier tubes that are very sensitive, authorize the detection of very low levels of light source, and good wavelength resolution since they authorize the use of a narrow slit widths. Photomultiplier tubes have been universally used for detections in ultraviolet and visible regions.

1.4.2) Photodiode array detector: The function of a photodiode array detector is depended on the truth that each diode in array is reverse-biased. Before the exposition of the detector to the light source, the diodes are finished charging through transistor switch. When the light source is arriving a photodiode array detector, charge carriers will be created in the silicon. These carriers will neutralize the stored charges that have the adversative polarity. So, this lost amount of charge is frankly proportional to the light source acquired by the detector. It is checked during the recharge procedure of each diode, gauging the amount of current need for recharging (176). Photodiode array detector have the skill to determine at all wavelengths. So, they have been popularly consumed as multichannel detectors. Moreover, they can note a perfect spectrum in a very short length of time, since the motion of the diffractor does not influence the scan time. Therefore, they are able to measure provisional signals. In addition, a photodiode array detector shows some usefulness such as the skill to quantify large photon fluxes, high height-to-width aspect ratio, and high quantum proficiency. Notwithstanding these advantages, a photodiode array detector shows some disadvantages, such as a high read noise and a relevant dark current (177).

1.4.3) Charged-coupled device: The explanation presented by the Royal Society of Chemistry for this device is a detector that uses a silicon chip to transform light into electric signal. The silicon chip discharges a single electron and absorbs a photon. The silicon chip surface is covered by the electrodes that hold the

electrons in array of pixels. The charge creates up corresponds to a scheme of light (173). So, charged-coupled device shows some usefulness such as a low read noise, a low dark count estimation, and a high ultraviolet and visible quantum proficiency (177), that make charged-coupled device intelligent spectroscopy detectors (173).

1.4.4) Paired emitter detector diode-based photometry: The Light-Emitting Diode have been used as an interesting component in optical detectors (178-180), since they are cheap, the stoutness, permit the miniaturization, and a high proficiency. Despite their use started to be as light sources, nowadays they have been applied in a reverse mode, as detectors (181-182). When the light is incident on the p-n junction, a current is generated. This current is proportional to the light intensity (183-184). Thus, since they can be used as a light source and detector, several devices have been described based on Paired Emitter Detector Diodes. Paired Emitter Detector Diode optical sensors have presented several usefulness such as the low power consumption, the reduced size, the sensitivity, the response in a large wavelength range with a high signal-noise ratio.

1.5) Signal processor and readout: the signal processor is normally an electronic device that expands the signal from the detector. Moreover, it may solve the signal from direct current to alternating current (or from alternating current to direct current), permute the phase of the signal, and percolate it to eliminate unwanted components. Other than, the signal processor may be called upon to practice such mathematical operation on the signal transition, integration, and transformation to logarithm. Several kinds of readout device are discovered in fashionable instruments. Some of readout device include the digital and d' Arsonval meter movement (In 1881 Jacques d' Arsonval developed a moving coil based on principle known as the moving coil galvanometer as shown in Figure 1.5. The basic principle of the d'Arsonval meter movement is much like that of a permanent magnet motor. As current passes through the windings of the moving coil, north and south poles are set up on the coil, also known as the armature. These poles interact with the poles of the permanent magnet and cause the needle to deflect. The direction of rotation depends on the direction of current flow. So, the polarity of the meter movement is crucial, and the terminals on the movement are marked accordingly. The spring that is attached to the moving coil is used to retract

the needle to the ''zero'' position after current is removed) (185), recorders, scales of potentiometers, and cathode-ray tubes.

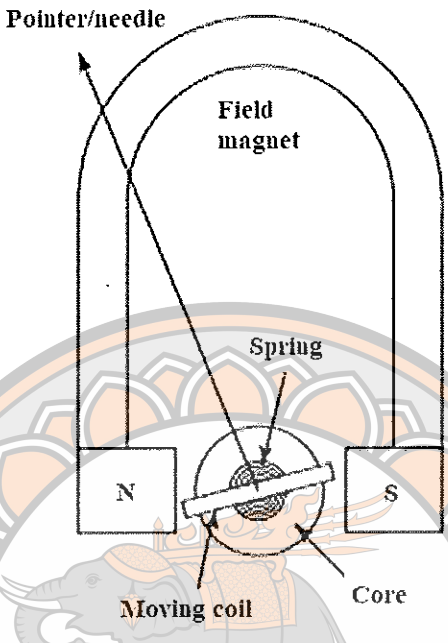


Figure 1.5 Basic diagram of the d'Arsonval meter movement.

Source: Re-drawn from Melhorn, 1999 (185)

2) Types of instruments

In types of instruments, it considers three general types of spectroscopy instruments: single-beam, double-beam, and multichannel (174).

2.1) Single-beam instruments (Figure 1.6 (A)): it consists of one of radiation sources, a wavelength selector, one of the detectors, an amplifier, and a readout device. Ordinarily, single-beam instrument demands a steadied voltage procure to avoid errors resulting from convertion of the beam intensity during the time used to produce the 100% transmittance improvement and determine % transmittance for the analyte.

2.2) Double-beam instruments (Figure 1.6 (B)): it consists of radiation sources, a beam splitter. One beam passes through reference solvent to detector, and the second concurrently detects the sample solution to a second, matched

detector. The two outputs are amplified, and their ratio is measured electronically and shown by a readout device. Double- beam instrument is a two- step procession associating first the zero improvement with a shutter in place between selector and beam splitter. In the last step, the shutter is opened and the absorbance or transmittance is read from signal processors and readouts.

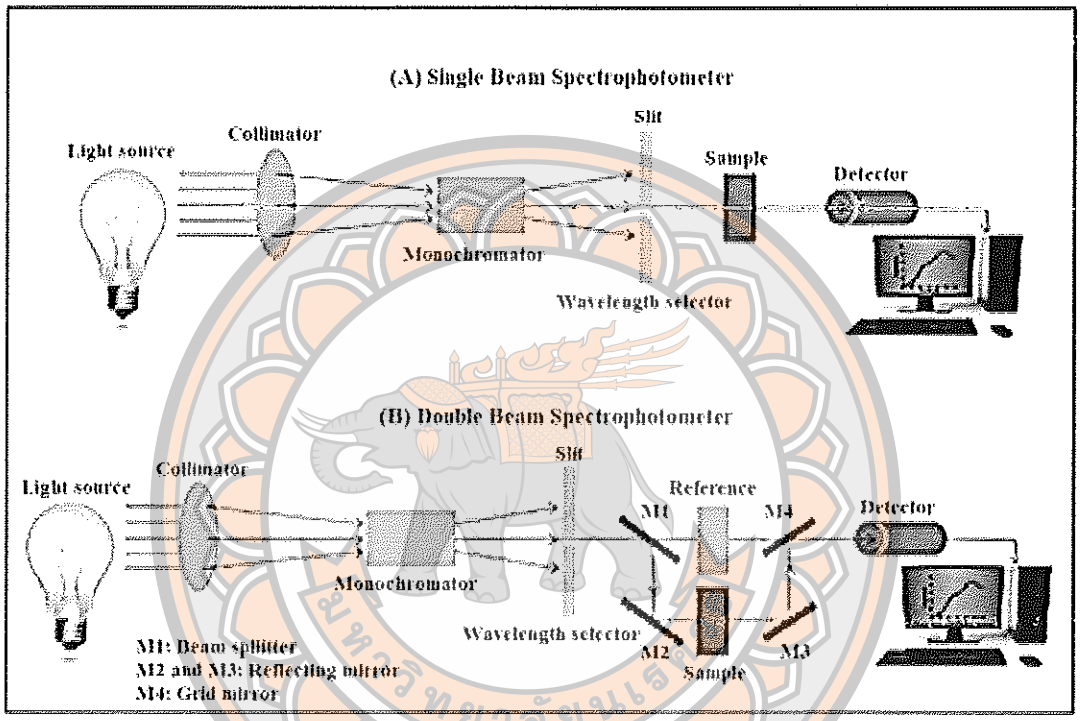


Figure 1.6 **Instrumental designs for UV-visible spectrophotometer; (A) single beam and (B) double beam.**

Source: Re-drawn from Skoog, et al., 1998 (174)

2.3) Multichannel instruments (Figure 1.7): the most recent type of spectrophotometer, it is a single beam instrument based on the diode array detector. Radiation from a light source is focused upon sample container and then passes into wavelength selector with a fixed grating. The cleaved off radiation falls on the diode array detector, which, as cited earlier, consist of linear array. It has been formed along the length of silicon chip. A silicon chip also contains a capacitor and an electronic

switch for every diode. Radiation impacting on any diode surface sources partial release of its capacitor. This lost charge is replaced during next switching cycle. The effecting charging currents, which are proportional to the radiant energy, amplified, digitized, and stored in computer.

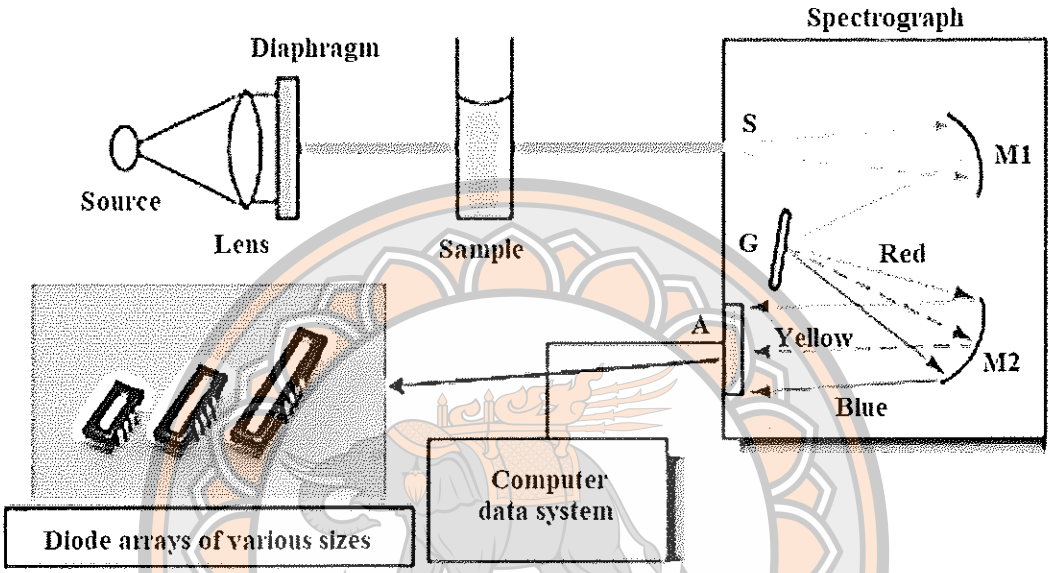


Figure 1.7 Instrumental designs for multichannel spectrophotometer.

Source: Re-drawn from Skoog, et al., 1998 (174)

3) Applications of UV-visible spectrophotometry

3.1) Qualitative analysis can be operated in the ultraviolet or visible range to identify exact classes of chemical compound both in the fresh state and in chemical and biological mixture.

3.2) Quantitative analysis may be operated by making use of the fact that certain chromophores, for example the aromatic amino acids in proteins and the heterocyclic bases in nucleic acids, absorb at specific wavelengths.

3.3) The amounts of chemical compound with overlapping spectral such as chlorophylls A or B in diethylether may be measured if their extinction coefficients are known at 2 wavelengths.

3.4) Used to follow enzyme kinetic by determining the extent of reaction between its substrate and an enzyme.

3.5) Structural studies of biological samples such as nucleic acids and proteins can be operated.

3.6) Aromatic amino acids are strong chromophores in the ultraviolet range. Procedures such as denaturation of polypeptide chain by temperature, pH value, and ionic strength can be detected as more of these residues become declarative to the incident radiation (174).

Several UV-visible spectrophotometric methods for determination of curcumin or curcuminoids are briefly listed in Table 1.7

Table 1.7 UV-visible spectrophotometric method for determination of curcumin or curcuminoids.

Type of analyte	Sample	LOD/LOQ	Amount Found	QA/QC report	Ref.
Curcumin	Mustard and curry	LOD 0.076-0.136, LOQ 0.25-0.45 mg L^{-1}	1.53-2.99 mg g^{-1}	%recovery 97.28-101.81, %RSD	(186)
Curcumin	Bulk drug and pharmaceutical dosage forms	LOD 0.05, LOQ 0.172 mg L^{-1}	2.48-5.49 mg L^{-1}	1.22-1.46 %recovery 99.1-101.4,	(187)
Curcumin	Poly (L-lactic acid) nanoparticles	LOD 0.04, LOQ 1 mg L^{-1}	170-1028 mg L^{-1}	%RSD 0.39 %recovery 77.21-97.28, %RSD	(188)
Curcumin	Nano-formulation	LOD 0.4, LOQ 1.21 mg L^{-1}	-	0.50-1.05 %recovery 99.2-101.1, %RSD	(189)

Table 1.7 (Cont.)

Type of analyte	Sample	LOD/LOQ	Amount Found	QA/QC report	Ref.
Curcumin	Liposomal formulation	LOD 0.45, LOQ 0.48 mg L^{-1}	3.59-8.38 mg L^{-1}	0.235-0.425 %recovery 99.2, %RSD 0.87	(190)
Curcumin	Water, wastewater, and food samples	LOD 0.23, LOQ 0.78 µg L^{-1}	91.76-99.97 µg L^{-1}	%recovery 91.76-100, %RSD < 3	(191)
Curcumin	Food	LOD 1.31, LOQ 4.38 mg L^{-1}	0.07-1.56 %w/w	%recovery 79.37-88.89, %RSD < 5	(192)
Curcumin	Drug	LOD 0.18, LOQ 0.65 mg L^{-1}	1.8-2 mg L^{-1}	%recovery 99.25-100.88, %RSD 0.01-0.09	(193)
Curcumin	Turmeric powder	LOD 7, µg L^{-1}	0.048-2.014 µg L^{-1}	%recovery 98.3-99.0, %RSD 2.72	(194)
Curcumin	Drug	LOD 0.38, LOQ 0.99 mg L^{-1}	2-3 mg L^{-1}	%recovery 101.05-105.94, %RSD	(195)
Curcumin	Cream and gel	LOD 0.11-0.19, LOQ 0.31-0.57 mg L^{-1}	39.228-40.698 mg L^{-1}	0.03-0.09 %recovery 99.01-100.1, %RSD < 0.5	(196)

4.2.3 Spectrofluorometric Method

Fluorescence occurs in simple as well as in complex gaseous, solid, and liquid chemical system. The simplest type of fluorescence is that showed by dilute atomic vapors. This type of fluorescence, in which the absorbed radiation is reemitted without a change in frequency, is seen as a resonance fluorescence. Many molecular species also show resonance fluorescence. However, molecular fluorescence bands center at wavelengths longer than the resonance line. (174).

1) Instrumentation

The diverse components of instruments for determining fluorescence are homologous to those found in ultraviolet and visible spectrophotometers. Figure 1.8 shows a typical configuration for these components of spectrofluorometers (174).

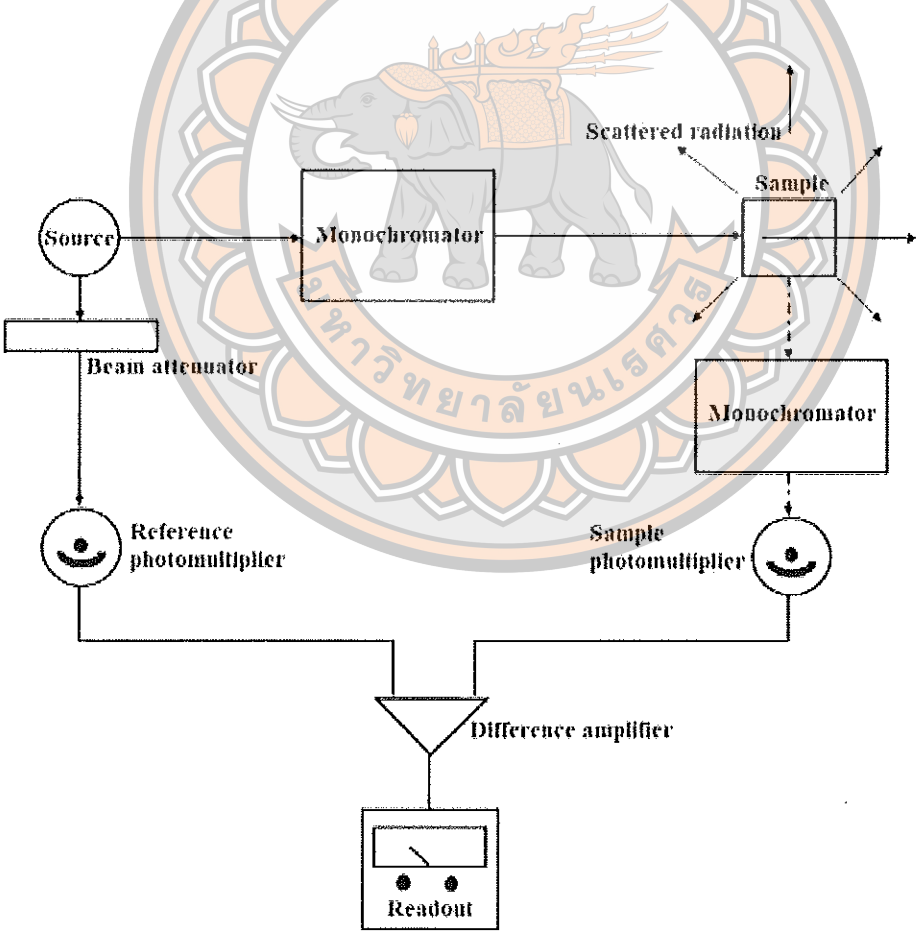


Figure 1.8 Components of spectrofluorometer.

Source: Re-drawn from Skoog, et al., 1998 (174)

1.1) Source: In major applications, more intense source is needed than the hydrogen or tungsten lamp used for the detection of absorbance. The scale of the output signal, and so the sensitivity, is proportional to the source power P_0. The most conjunct source for filter is low-pressure mercury vapor lamp installed with a merged silica window. This source creates valuable lines exciting fluorescence at 254, 302, 313, 546, 578, 691, and 773 nanometers. Private lines can be separated with interference filters or proper absorbance. Since fluorescence can be induced in most fluorescing chemical compounds by a diversity of wavelengths, at lowest one of the mercury lines normally proofs suitable. For spectrofluorometers, where a source of continuum radiation is used, a 75-450 watt of high pressure xenon arc lamp is regularly employed. Such lamps demand a large energy procure capable of creating direct currents of 5-20 ampere at 15-30 volt. The spectral from a xenon arc lamp is continuum from 300-1300 nanometer. In some instruments, capacitor is released through lamp at a stable frequency to give flashes that are normally spaced in time. Apart from that, the outputs of the transducers are then ac signal that can be readily amplified and processed.

1.2) Monochromator and filter: Both absorbance and interference filters have been utilized in spectrofluorometers for wavelength selection of both the excitation beam and the resulting radiation. The most spectrofluorometers are installed with at least one and often two grating monochromators.

1.3) Cell compartment: Both rectangular and cylindrical cells created of silica or glass are employed for fluorescence detection. Care must be taken in the style of cell compartment to decrease the amount of scattered radiation entering detector. Baffles are frequently introduced into compartment for this objective. Even more than in absorbance detections, it is essential to evade fingerprints on cells because oils frequently fluorescence.

1.4) Detector: The generally fluorescence signal is of low intensity, large amplifier gains are thus wanted for its detection. The photomultiplier tubes are the farthest conjunct detectors in sensitive spectrofluorometer. Frequently, these are performed in the photo counting mode to provide developed signal-to-noise ratios. Cooling of detectors is also employed sometimes to develop signal-to-noise ratios. Charge- transfer and diode- array detectors have also been demonstrated for

spectrofluorometers (197-198). This kind of detector permits the fast recording of both excitation and emission spectral.

2) Applications of spectrofluorometers

Fluorescence methods have inherently lower limits of detection than absorption based spectrophotometric measurements. They are among the most sensitive analytical techniques available to the scientist. The increased sensitivity happens from the concentration-related parameter for fluorometry and phosphorimetry which F being directly proportional to the source radiant power P_0. The fluorescence intensity can be measured independently of P_0. In agreement, an absorbance detection wants assessment of both P_0 and P, due to absorbance, which is proportional to concentration, depends on the ratio of these two quantities. The sensitivity of a fluorescence method can be developed by increasing P_0 or by further amplifying the fluorescence signal (174).

2.1) Fluorometric determination of inorganic species: Inorganic spectrofluorescence methods are of two kinds; cations forming fluorescing chelates and fluorometric reagents. Direct methods involve the formation of a fluorescence chelate and the determination of its emission wavelength. The second group is depended on the reduction of fluorescence resulting from the quenching rendition of the compound being measured. The latter technique has been most widely used for anion analysis (174).

2.1.1) Cations forming fluorescing chelates: Two parameters greatly limit the number of transition metal ions that form fluorescing chelates. First, many of these ions are paramagnetic, which increases the rate of intersystem crossing to the triplet state. Deactivation by fluorescence is thus unlikely, although phosphorescence may be observed. A second parameter is that transition-metal complexes are characterized by many closely spaced energy levels, which enhance the possibility of deactivation by internal conversion. The principal inorganic applications of fluorimetry are thus to nontransition-metal ions, which are less susceptible to these deactivation processes. Note that such cations are generally colorless and tend to form colorless chelates. Hence, fluorometry often complements spectrophotometry.

2.1.2) Fluorometric reagents: The most successful fluorometric reagents for cation analyses have aromatic structures with two or more donor functional groups that permit chelate formation with the metal ion.

2.2) Fluorometric determination of organic species: The number of works of fluorometric analysis for biochemical and organic species are impressive. The most essential works of fluorescence methods are in the analyses of clinical or food samples, natural products, and pharmaceuticals (199).

Several fluorescence methods for determination of curcumin or curcuminoids are briefly listed in Table 1.8

Table 1.8 Fluorescence method for determination of curcumin or curcuminoids.

Type of analyte	Sample	LOD/LOQ	Amount Found	QA/QC report	Ref.
Curcumin	Curry	LOD 0.08 $\mu g\ L^{-1}$	0.0179-0.0187 $\mu g\ mL^{-1}$	%recovery 99.5, %RSD 1.9	(200)
Curcumin	Lipid and polymeric nanocapsule suspensions	LOD 0.03, LOQ 0.10 $mg\ L^{-1}$	2.5-10.0 mg	%recovery 98.33-100.00, %RSD 0.21-3.13	(201)
Curcuminoids	Turmeric	LOD 0.005-0.081, LOQ 0.016-0.270 $mg\ L^{-1}$	0.43-55.7 %w/w	%recovery 90.0-104, %RSD 0.23-4.75	(202)
Curcumin	Commercial herbal medicines	LOD 0.0165, LOQ 0.0549 $mg\ mL^{-1}$	0.4-2.4 $mg\ mL^{-1}$	-	(203)
Curcumin	Urine	-	1.93-4.21 µM	%recovery 96.5-105.5, %RSD 2.58-4.56	(204)

4.2.4 Other methods

Moreover, other methods for determination of curcumin or curcuminoids are briefly listed in Table 1.9. These methods are not as popular as those previously described.

Table 1.9 Other methods for determination of curcumin or curcuminoids.

Type of analyte	Sample	Method	LOD/LOQ	Found	Ref.
Curcuminoids	Curcuma longa L. and Zingiberaceae	Capillary Electrophoresis	LOD 0.01 mg mL^{-1}	1.0-9.1 %w/w, %recovery 98, %RSD<6	(205)
Curcuminoids	Curcumin longa	TLC-densitometry	-	67.13-96.25 %w/w, %RSD <8.31	(48)
Curcuminoids	Curcuma rhizome	Near-infrared Spectroscopic Analysis	-	0.07-3.84 %w/w, %recovery 100.22-101.14, %RSD 0.89-1.72	(206)
Curcuminoids	Turmeric	Flow injection analysis	LOD 0.6, LOQ 1.8 µg mL^{-1}	0.9-4.3 %w/w, %recovery 94.3-108.0	(207)
Curcumin	Curcumin in model	Voltammetry	LOD 0.1 µM	0.68-3.08 %w/w,	(208)

Table 1.9 (cont.)

Type of analyte	Sample	Method	LOD/LOQ	Found	Ref.
	solutions			%RSD 1.2-2.2	
Curcumin	Biological important phenolic compounds	Voltammetry	-	%recovery 97-100, %RSD 3.5	(209)
Curcumin	Turmeric	Voltammetry	-	10.29-60.26 μM, %recovery 92-108	(210)

These methods have been used for the determination of curcumin or curcuminoids content in samples. It exhibits high performance in terms of quantitative and qualitative analysis in many chemical analyses methods. However, these techniques employ big and expensive equipment and they are inconvenient as they use off-site analysis. Therefore, this research proposes a high-throughput, rapid, low-cost, and on-site analysis method for the detection of curcumin in turmeric samples.

Acrylamide

1. Introduction to acrylamide

Thermal methods are frequently used in food producing to obtain secure products with a protracted shelf-life and have strong effect on the last quality of foods. Grilling, toasting, frying, roasting, baking, sterilization result in undesired and desired impacts due to many chemical reactions being Maillard reaction, lipid oxidation, strecker decomposition, and caramelisation which is the most important. One of the aim of thermal method is to develop the sensory properties of food products, their lusciousness and to extend the range of tastes, colors, textures, and aromas in foods produced from same raw materials. Heating also destroys micro-organisms and enzymes

and lowers the water (H_2O) activity of the food, therefore, protecting the food products. It is a good known that some chemical compound happening from the heating methods can show a helpful role on human health. Many formed chemical compounds presenting antioxidant, antiallergenic and antimicrobial impacts as well as modulating activity in vitro have been measured in heated food products (211-213). Beside to these helpful impacts, some deleterious consequences of thermal methods must be cautiously estimated. The loss of thermal labile substance such as vitamins as well as important amino acids and off-flavors are good created phenomena begetting about a loss in the sensorial quality and nutritional value of heated food products (214).

The main concern happening from heating methods comes from the formation of substance that are not spontaneously shown in foods, but that may improve during preservation or heating methods and that disclose deleterious impacts such as cytotoxic, mutagenic, and carcinogenic impacts. Well known examples of these substances are nitrosamines, polycyclic aromatic hydrocarbons, and heterocyclic amines (215-216). Lately, one formed contaminant has obtained many attentions because of wide occurrence in food products and high toxicological potential, it is acrylamide. Acrylamide can be considered as the most major heat-induced contaminants arising in bakery and bread products (214).

Acrylamide has been included on the list of food-borne toxicants since 2002. Swedish National Food Administration discovered out relevant amount of acrylamide in carbohydrate-rich foods and heat treated food such as crisps and potato chips, bread and coffee (217). Shortly after its detection in foods, it has been obviously established that the important pathway for acrylamide formation in food products is Maillard reaction with free asparagine as a major precursor (218-221). An asparagine can decompose by decarboxylation but when a carbonyl source is show, the yield of acrylamide from asparagine is much higher describing the high concentration of acrylamide measured in food products in decreasing sugars and free asparagine such as bakery products and fried potatoes (220, 222-223). Other reaction routes for acrylamide formation in food products have been confirmed from acrolein and acrylic acid (224), and from wheat gluten (225). Moreover, acrylamide can be created by deamination of 3-aminopropionamide which is a mediator in Maillard reaction (226). It can also form by decarboxylation of free asparagine and it can yield acrylamide at heating even in

absence of a carbonyl source (227-228). Finally, a feasible pathway for the formation of acrylamide in food product is the Strecker decomposition (Strecker aldehyde) of asparagine (229) as demonstrated in Figure 1.9. This decomposition takes place at high temperatures such as when frying and roasting potatoes. Although not all biochemical decomposition procedures leading from an asparagine to an acrylamide, they have been completely uncovered, this decomposition probably proceeds via a transamination-decarboxylation that yields the Strecker aldehyde. Listings of quantities of acrylamide in food products are, for example, procured by several consumer associations (229).

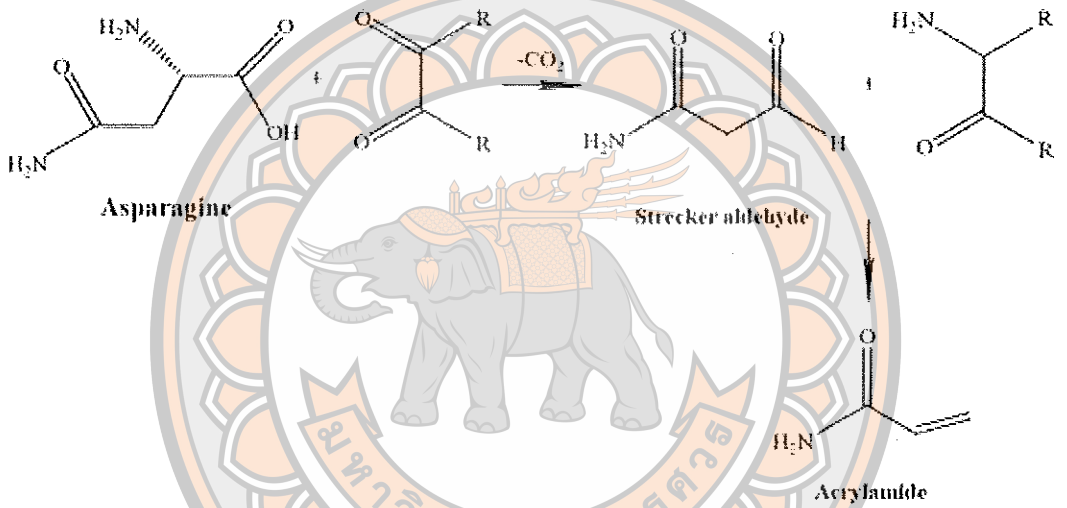


Figure 1.9 Strecker decomposition of asparagine.

Source: Re-drawn from Kleefisch, et al., 2004 (229)

2. Acrylamide in foods and dietary exposure

In 2009, the European Food Safety Agency (230) proposed the results of the measuring of acrylamide levels in food products in response to the request of the European commission (231). Data collected in this report concern with foods sampled in 2007 and submitted to the commission by 21 members from Norway. Two additional reports depended on food product in 2008 and 2009 are expect published by European Food Safety Agency in 2011. A brief of the effects latterly reported by European Food Safety Agency is given in Table 1.10. Acrylamide is formed during roasting, frying, or baking and it is not normally found in microwaved or boiled foods.

Table 1.10 Acrylamide levels (μg kg^{-1})[x] in different food commodities (230).

Food commodities	N[y]	Median	Mean	Maximum
Biscuits	227	169	317	4200
Bread	272	50	136	2430
Breakfast cereals	128	100	156	1600
Cereal-based baby food	76	42	74	353
Coffee	208	188	253	1158
French fries	529	253	350	2668
Jarred baby foods	84	31	44	162
Other products	854	169	313	4700
Potato crisps	216	490	628	4180
Home cook potato products	121	150	319	2175

[x] Values between LOD and LOQ were set to the LOD or the LOQ value.

[y] Number of data analyzed for each food commodities.

Acrylamide has been found in food products besides those listed in Table 1.10 such as almonds, hazelnuts (232), and dried fruits (233). Heat treated animal products such as fish and meat, mainly exhibit low levels of acrylamide concentration (217, 230, 234). Moreover, a large variance in acrylamide level between different food products of each food as well as between different food brands of the same food product has been reported. The difference in acrylamide concentration of free asparagine and reducing sugars in raw materials, difference in food composition and procedure conditions applied can readily describe the observed variance (235).

Estimates of dietary acrylamide input have been made for peoples in many countries. A great variance between peoples has been found according to people's eating habits and the route of foods processing. Dybing, et al. (236) presented the average daily input of acrylamide concentration for adults close to 0.5 mg kg^{-1} of body weight, with 95th percentile values of about 1 mg kg^{-1} of body weight. The World Health Organization (237) estimates a dietary input of acrylamide concentration in the range of 0.3-2.0 mg

kg^{-1} of body weight for the general people and up to 5.1 mg kg^{-1} of body weight for the 99^{th} percentile consumers. The daily inputs of dietary acrylamide concentration for the general people and high consumers are estimated to be on average 1 and 4 mg kg^{-1} of body weight, respectively. Children consume more acrylamide than adults possibly because of their higher consumption of certain acrylamide-rich foods, such as potato crisps and French Fries, as well as their higher caloric input relative to body weight (236). Heudorf, et al. (238) evaluated the dietary of acrylamide in 5-6 years aged by method of urinary excretion. They presented a median daily intake of acrylamide concentration in children of 0.54 mg kg^{-1} of body weight which confirming that children are susceptible subgroup of people. Arribas-Lorenzo and Morales (239) evaluated the dietary from potato chips in the Spanish people. They reported a daily dietary exposure from potato crisps of 0.053 mg kg^{-1} of body weight for the adult and of 0.142 mg kg^{-1} of body weight for children. In most of the peoples, the main contribution to acrylamide input are potato chips and potato crisps, coffee and bread which each accounting for nearly one third of an overall input (237). Other foods can account for up to 10 percentage of an overall input of acrylamide. However, some concern on the dependability of the dietary evaluation has been shown. It is possibly that consumption data are rather than the chemical compound analysis which mainly supported to the instabilities of the analysis methods for quantification of acrylamide concentration in bakery and potato products (240-241). Lately, two European reference materials for the determination of acrylamide concentration in food products have been improved (242). These European reference materials could be applied to validate or develop the current, available analysis methods for bakery products. Moreover, it would be valuable that additional European reference materials could be released in the future for coffee and potato products.

Furthermore, several factors may complicate the precision in estimation of the actual dietary exposure to acrylamide. The acrylamide concentrations of commercially available food products mostly vary between production batches and between brands. Moreover, storage conditions and duration as well as domestic catering or cooking conditions might stably affect the acrylamide concentration of food products as they are eaten. Food frequency questionnaires based processes for the estimation of acrylamide input are normally not designed for the precise evaluation of the acrylamide

exposure. Prior studies presented that food frequency questionnaires that are not designed to estimate acrylamide dietary input did not related to the concentration of urinary acrylamide metabolites and acrylamide-Hb adducts (243-245). Nevertheless, in 2005, the Joint FAO/ WHO Expert Committee on Food Additives concluded that "exposure estimates are consistent and that new calculations or product analyses are not likely to change exposure estimates" (237). This conclusion has been recently reaffirmed by the expert panel of European Food Safety Agency in the scientific colloquium on acrylamide on 2008 for which "the analytical methods for establishing occurrence data and estimating human exposure appear adequate and, even with better data, it seems unlikely that the currently estimated margins of exposure will change dramatically" (246).

3. Health risk of dietary acrylamide

Many studies show that acrylamide is neurotoxic in humans and animals and that it is toxicant, carcinogen and germ-cell mutagen in rats (247-249).

3.1 Metabolism

Acrylamide metabolism has been completely examined by means of toxico kinetic studies in rats, mice, and humans. After ingestion, acrylamide is quickly absorbed and spreaded in humans and animals all over the body. Acrylamide can be found in breast milk (250) and human afterbirth (251), therefore, it can be readily transferable to newborn or fetus infants. Moreover, it can be found in many organs (e.g. the liver, brain, thymus, heart, and kidneys) (252). Acrylamide can bind in hemoglobin, DNA, serum albumins, and enzymes (249, 253). Glutathione-conjugates of acrylamide is further changed to mercapturic acids conjugates, N-acetyl-S-(3-amino-3-oxopropyl)-cysteine (AAMA), N-(R,S)-acetyl-S-(3-amino-2-hydroxyethyl-3-oxopropyl)cysteine (GAMA), S-(3-amino-3-oxopropyl)cysteine, N-acetyl-S-(3-amino-3-oxopropyl)-cysteine-S-oxide, and N-acetyl-S-(1-carbamoyl-2-hydroxyethyl) cysteine which are defecated with urines (253-255). N-acetyl-S-(3-amino-3-oxopropyl)-cysteine, its sulfoxide, and N-(R,S)-acetyl-S-(3-amino-2-hydroxyethyl-3-oxopropyl)cysteine have been obviously confirmed in humans (255-257). Toxicokinetic studies on humans presented that nearly 60 percent of absorbed acrylamide is excrete in the urine mostly (86 percent) as glutathione-conjugates and that unprocessed acrylamide accounts only for 4.4 percent of the up taken dosage.

The hemoglobin adducted with acrylamide in blood and the mercapturic acids metabolites of acrylamide in the urine can be used as biomarker of acrylamide exposure (258-259). The urinary concentration ratio of N-acetyl-S-(3-amino-3-oxopropyl)-cysteine and N-(R,S)-acetyl-S-(3-amino-2-hydroxyethyl-3-oxopropyl) cysteine can be considered as a detecttion of the scope of transformation of acrylamide to glycidamide (257).

DNA adducted with acrylamide can be considered as a biomarker of biologically active internal dosage of acrylamide which have not been measured yet in humans. Three models have been presented so far to explain acrylamide metabolism and absorption in test species and humans (260-262). The physiologically based toxicokinetic or toxicodynamic multi-compartmental model that have been lately improved by Young et al. (262) for glycidamide, acrylamide, and their glutathione-conjugates in rats and mice is popularly considered as the most useful available model. In this model, liver glycidamide-DNA adducted and hemoglobin adducted with acrylamide and glycidamide were included as pharmacodynamic components of the model and their levels as well as their decay rates extrapolated to humans. The stable-state human liver glycidamide-DNA adducted concentration from exposure to background concentration of acrylamide in the food was estimated to be between 0.06-0.26 adducts per 10^8 nucleotides. Such a low number of DNA adducts as estimated by model may illustrate why DNA adduct has not yet been measured in humans. Enzymes involved in acrylamide biotransformation are the phase I enzyme cytochrome P450 2E1, the microsomal epoxide hydrolase, and Glutathione-S-Transferases enzymes. The role of the phase I enzyme cytochrome P450 2E1 in epoxidation of acrylamide to glycidamide and formation of glycidamide-DNA adduct has been shown by using the phase I enzyme cytochrome P450 2E1-null mice. When mice were exposed to acrylamide, higher concentrations of acrylamide adducted were observed and compared to difference type of mice (254, 263). The biotransformation of acrylamide to glycidamide can be regarded thereof as the crucial procedure for the toxicity of acrylamide. The hydrolysis of glycidamide to glyceramide as well as the type addition of acrylamide to glutathione is considered as detoxification paths. Since enzymes involved in either stimulation or detoxification of carcinogens and acrylamide are polymorphically shown with the alleles displaying different enzymatic activities.

Different activity of these enzymes or acrylamide may correct the value of transformation of a xenobiotic into its metabolite or their inactivation or detoxification (264). Duale, et al. (265) examined the relationship between the ratio of acrylamide-hemoglobin and polymorphic differences in genes codification for metabolizing enzymes. They reported an important association between particular combinations of genes and the ratio of acrylamide-hemoglobin. This finding proposed that genotype is fundamental in measuring the individual pusillanimity to cancer following acrylamide input. This study was depended on a few individuals thus further data are wanted to assert these findings. Moreover, further data is needed on the operation of the enzymes involved in acrylamide metabolism in various people by subgroups or individuals as well as the duty of non-genetic parameters that may potentially influence their work (e.g. medication, alcohol, and diseases) which has still to be obviously explained.

3.2 Neurotoxicity

Neurotoxicity of acrylamide results from high levels of exposure and concerns workers occupationally exposed to acrylamide through inhalation or dermal absorption. It is characterized by ataxia and skeletal muscle sickliness. In mice and rat studies, the NOEL (No Observable Effect Level) for neurotoxic effect has been estimated which ranging from 0.2-10 mg kg^{-1} of body weight day^{-1} and is far above dietary exposure (237). Lately, it has been postulated that neurotoxicity of acrylamide might be progressive and so that dietary exposure might not be negligible (266).

3.3 Carcinogenicity

In 1994, acrylamide has been classified by the International Agency for Research on Cancer as a possibly carcinogen to humans (247). Several studies in rats promote the premise that acrylamide is a multi-organ carcinogen, being capable to cause neoplasms to many organs (e.g. uterus, mammalian gland, lung, brain, and skin) (267). Two lifetime carcinogenicity studies in F344 rats and B6C3F1 mice and F344 rats have been carried out so far (268-269). Information presented that the tumor sites were straight in both earlier studies on rats and consisted fibroadenoma and carcinoma of the mammary gland, tunica vaginalis mesotheliomas of the male testicle, and thyroid. In contrast, a difference pattern of goal organs has been remarked in mice (e.g. lung, liver, and Harderian gland). It is popularly accepted that the carcinogenicity of acrylamide would stem from its transformation in mammalians to glycidamide. The acrylamide-

induced DNA conjunction has been assumed as the key procedure in acrylamide carcinogenicity although the chain of incidences starting from glycidamide- DNA adducts formation and guiding to mutation has not been directly examined. Supplemental evidences of the duty of glycidamide are the results from experimentations on CYP2E1-mice. Until now, suggestions on the mode of action of acrylamide-induced tumors have been created for mammary and testis tumors in rats (249) whereas no reliable explanation for thyroid tumor in rats and mice is available. Non-genotoxic mechanisms for acrylamide carcinogenicity have been presented as well. Lamy, et al. (270) offered that conjugation of glutathione would lead to a diminution of glutathione store, adding cell oxidative stress. Another report explained the first evidence of acrylamide inhibition of a mitotic or meiotic motor protein that this could be alternative mechanism to DNA adduction in the potential carcinogenicity and the creation of cell division disadvantages (271-272). A hormonal effect in rat thyroid and mammary glands has also been assumed for carcinogenicity of acrylamide (237). Albeit it is impossible to summarize that all of the acrylamide-induced tumors in the test species are ascribable to genotoxic mechanism, there is no convincing premise for non-genotoxic mechanisms at acrylamide relevant volume. The study also summarized that acrylamide is a "carcinogen at multifarious anatomic sites in both mice and rats, though the tumor sites and different between the two species". A supplemental main result of this study was that the tumors show early following neonatal exposure in mouse. Since metabolic stimulation of acrylamide to glycidamide through the phase I enzyme cytochrome P450 2E1 is crucial for acrylamide toxicity and the enzyme is preliminary shown in humans, there is a concern about an added cancer risk following acrylamide exposure for the babys (214).

3.4 Biomarkers of exposure

The use of biomarkers of exposure would be gainful mainly to a more accurate approximation of the dietary input and in turn to create epidemiological studies more plausible but also to explore metabolic differences at person level and help extrapolation between humans and animals. In epidemiological studies, DNA adduct would be more instructive since they reflex the biological maneuverable dose of acrylamide. Today, their determination still has high costs and technical troubles (214).

Urinary biomarkers reflex a short-term exposure and cannot be considered as enough biomarkers in epidemiological studies (244). Hemoglobin adducted in the blood could give helpful insight on long-term exposure to acrylamide. Although hemoglobin adducted reflects a recent exposure period and cancer enlargement desires long-term exposure, single detection of blood hemoglobin adducted are supposed to well reflect longer exposures because population's diet is justly stable. Nevertheless, a main shortcoming of using biomarkers of exposure is that they reflect exposure to overall the feasible sources of the goal compound as well as individual differences in metabolism and absorption. The main non-dietary sources of acrylamide are cosmetics, water, and smoking. In particular, given the strong correlation between hemoglobin adducts levels and smoking behavior (273), it should be suggested the epidemiological studies to be restrained to non-smokers or to allow a classification by smoking status.

Metabolic work of enzymes involved in acrylamide biotransformation is depending on enlargement stage, genetic polymorphism and on the presence of inducers or inhibitors. Lately, it has been hypothesized that acrylamide might form endogenously that are linked to oxidative stress (e.g. diabetes or Alzheimer's disease). This presumption is encouraged by the observation that acrylamide adducted concentrations were added in mice cured with chemical compounds that induce oxidative stress (274). Tareke, et al. (275) reported that acrylamide can form in vitro from asparagine by the reaction of H_2O_2 at physiological pH, temperature, and ionic strength while the H_2O_2 concentration applied in their study was far higher than the H_2O_2 concentration normally detected in human blood. It is known that H_2O_2 concentration increases in long-term chronic and pathological conditions (276-277). Besides, several reactive species other than H_2O_2 (278-279) might support to endogenous acrylamide formation, thus, the probability that acrylamide could form endogenously under pathological conditions and cannot be restrained out which wants further inquisition.

Lately, 2 antibodies specific to acrylamide-adducted hemoglobin proper for applying in a high-throughput biomarker immunoassay to measure acrylamide exposure have been improved (280). The possibility to use immunological techniques for the study of biomarkers of exposure gives new powerful equipment for future determination in this field.

3.5 Epidemiological data

Although substantial results contribute the evidence that acrylamide is carcinogenic and genotoxic in vitro and vivo, epidemiological information have not yet unambiguously responded the question that dietary acrylamide exposure can add cancer risk for humans. The amount of epidemiological studies addressed to different goal organs and different peoples have been published over the last 17 years (Table 1.11). Today, the results of these epidemiological studies approve in acting no positive connection between overall dietary acrylamide input and risk of colorectal cancer (281-284), esophageal cancer (282-283), bladder cancer (281, 283), prostate cancer (245, 282-284), brain cancer (285), oropharyngeal, laryngeal (282), gastric, and pancreatic (283). An anticipatory Swedish mammography study found no connection between acrylamide input and either ovarian cancer (284) whereas Hogervorst, et al. (286), in a case-cohort study under a Netherlands colony, reported a statistically important added risk of ovarian cancer among postmenopausal women and an importantly added risk of endometrial cancer among never smoking women in connection with dietary exposure to acrylamide. In the same group, there was suggestion of an added risk of renal cancer among women and men in connection with dietary acrylamide exposure (283). Moreover, 2 people-based case control studies in Sweden found no connection between renal cancer risk and dietary acrylamide (281). In addition, for breast cancer, studies depended on food frequency questionnaires have constantly reported no positive connection between risk of pre- or postmenopausal breast cancer and estimated dietary acrylamide input, or breast cancer defined by progesterone and estrogen receptor status (282, 284, 286).

Table 1.11 Summary of epidemiological studies on dietary acrylamide input and cancer risk.[x]

Cancer site	Design	people	Dietary exposure assessment	RR for highest vs. lowest quintile (95% confidence interval)	Reference
Bladder	Case-control	Swedish women and men	Semi quantitative FFQ	0.9 (0.5-1.5) for highest vs. lowest quartile	(281)
	Case-cohort	Dutch women and men	Baseline FFQ	0.91 (0.73-1.15)	(283)
Brain	Case-cohort	Dutch women and men	Baseline FFQ	HR 1.02 (0.89-1.16) per 10 mg day^{-1} increment ACR input	(285)
Breast	Case-control	Italian/Swiss women	FFQ	1.06 (0.88-1.28)	(282)
	Case-cohort	Dutch women	Baseline FFQ	0.93 (0.73-1.19); 1.10 (0.80-1.52) for never smokers	(286)
	Cohort	Swedish women-postmenopausal	Updated FFQ	0.91 (0.80-1.02) for highest vs. lowest quartile	(284)
	Cohort	US women-premenopausal	Updated FFQ	0.92 (0.76-1.11)	(287)

Table 1.11 (Cont.)

Cancer site	Design	people	Dietary exposure assessment	RR for highest vs. lowest quintile (95% confidence interval)	Reference
Colorecta	Case-control	Swedish women and men	Semi quantitative FFQ	0.7 (0.4-1.0) for highest vs. lowest quartile	(281)
	Case-control	Italian/Swiss women	FFQ	0.97 (0.80-1.18)	(282)
	Cohort	Swedish men	FFQ	0.95 (0.74-1.20) for colorectal for highest vs. lowest quartile; 0.97 (0.71-1.31) for colon; 0.91 (0.62-1.34) for rectal cancer	(283)
	Case-cohort	Dutch men	Baseline FFQ	HR=1.00 (0.96-1.06) per 10 mg day^{-1} increment ACR input	(283)
Endometrial	Case-cohort	Dutch women	Baseline FFQ	HR=1.29 (0.81-2.07); 1.99 (1.12-3.52) per 10 mg day^{-1} increment ACR input for never smokers	(286)

Table 1.11 (Cont.)

Cancer site	Design	people	Dietary exposure assessment	RR for highest vs. lowest quintile (95% confidence interval)	Reference
	Cohort	Swedish women	Updated FFQ	1.05 (0.68-1.63) for highest vs. lowest quartile	(284)
Esophageal	Case-control	Italian/Swiss women	FFQ	1.10 (0.65-1.86)	(282)
	Case-cohort	Dutch men	Baseline FFQ	HR=0.96 (0.85-1.09) per 10 mg day^{-1} increment ACR input	(283)
Gastric	Case-cohort	Dutch men	Baseline FFQ	HR=1.02 (0.94-1.10) per 10 mg day^{-1} increment ACR input	(283)
Laryngeal	Case-control	Italian/Swiss women	FFQ	1.23 (0.80-1.90)	(282)
Lung	Case-cohort	Dutch women and men	Baseline FFQ	HR=1.03 (0.96-1.11) for men and 0.82 (0.69-0.96) for women per 10 mg day^{-1} increment ACR input;	(285)

Table 1.11 (Cont.)

Cancer site	Design	people	Dietary exposure assessment	RR for highest vs. lowest quintile (95% confidence interval)	Reference
				1.03 (0.77-1.39) for men and 0.45 (0.27-0.76) for highest vs. lowest quintile	
Oropharyngeal	Case-control	Italian/Swiss women	FFQ	1.12 (0.76-1.66)	(282)
Ovarian	Case-control	Italian/Swiss women	FFQ	0.97 (0.73-1.31)	(282)
	Case-cohort	Dutch women	Baseline FFQ	HR=1.78 (1.10-2.88) per 10 mg day^{-1} increment ACR input; 2.22 (1.20-4.08) for never smokers	(286)
	Cohort	Swedish women	Updated FFQ	0.86 (0.63-1.16) for highest vs. lowest quartile	(284)
Panceatic	Case-cohort	Dutch men	Baseline FFQ	HR=1.06 (0.96-1.17) per 10 mg day^{-1} increment ACR input	(283)

Table 1.11 (Cont.)

Cancer site	Design	people	Dietary exposure assessment	RR for highest vs. lowest quintile (95% confidence interval)	Reference
Prostate	Case-control	Swedish men	FFQ; Hb adducts	0.97 (0.75-1.27) based on FFQ 0.93 (0.47-1.85) based on Hb adducts	(245)
	Case-cohort	Dutch men	Baseline FFQ	1.06 (0.87-1.30)	(283)
	Case-control	Italian/Swiss women	FFQ	0.92 (0.69-1.23)	(282)
	Cohort	Swedish men	Updated FFQ	0.88 (0.70-1.09) for total prostate cancer; 1.07 (0.87-1.32) for localized prostate cancer; 0.98 (0.78-1.22) for advanced prostate cancer	(284)
Renal	Case-control	Swedish women and men	Semi quantitative FFQ	0.7 (0.3-1.3) for highest vs. lowest quartile	(281)
	Case-cohort	Dutch women and men	Baseline FFQ	1.59 (1.09-2.30)	(283)

[x] FFQ = Food Frequency Questionnaires; ACR=Acrylamide; GA=Glycidamide; Hb=Hemoglobin; HR=The multivariable-adjusted hazard ratio, RR=Relative risk.

The image changes if biomarkers of input instead of dietary approximation are considered. Olesen, et al. (288) studied a positive connection between the concentrations of acrylamide-hemoglobin adduct and estrogen receptor positive breast cancer with an approximated incidence rate ratio of 2.7 per 10 fold added in acrylamide-hemoglobin concentration, after adjustment for smoking actions. Since acrylamide-hemoglobin adduct concentrations in smokers are by far higher than in non-smokers, the relevancy of these findings is questionable. There are many reasons that could explain the conflicting affects from epidemiological studies. The first reasoning could be that the relative risk for cancer is thus low even at high exposure concentrations that no epidemiological studies could readily measure the effect (281). The second explanation could stem from an inconstant approximation of acrylamide input. Since acrylamide concentrations in food products are extremely variable even in the same type of examine and the accuracy with which population reported their consumption information could be not well, the actual approximation of dietary input from self-reported food product questionnaire could be subject to misclassification.

On the whole, the conclusion that can be drawn from available epidemiological information is that, at least for some goal organs and some peoples, acrylamide input might cause a health risk and the level of cancer risk deriving from acrylamide input might be organ- or people-dependent. The actual connection between risk of some cancers and acrylamide exposure may be greater than the relative risks approximated in the epidemiological studies because of the non-differential viciousness tending to underestimate the risk. In addition, epidemiological studies carried out in different peoples might lead to different effects because of the difference in acrylamide exposure. For that reason, more expected studies in additional peoples and additional cancer sites should be carried out. The sickliness of individuals and subgroups of people because of the genetic polymorphisms that enzymes effect involved in acrylamide metabolism should be further examined as well as to refine the accuracy of epidemiological studies (214).

4. Mitigation options

The amount of mitigation options to decrease the acrylamide content in food products have been proposed and tested up to now. Acrylamide have been mostly focused on cereal and potato products that are two of the main contributors to dietary

exposure in most of the peoples. Conversely, only limited procedure options for coffee products are available for decreasing acrylamide concentrations without effecting the final quality (289). The mitigation methods include changing in formulations and recipes, changing in technologies and procedure conditions. The revocation of acrylamide after formation by means of vacuum has been demonstrated but its impact on manufacturing practices and food quality has not yet been obviously established (290).

One of the most promising equipment to control acrylamide concentration in heat treated food products is the addition of the asparaginase enzyme. The asparaginase enzyme is the enzyme which could be able to catalyze hydrolysis of asparagine in ammonia and aspartic acid thus lowering the concentration of leader asparagine. Asparaginase enzyme has been successfully used at lab scale both to cereal-based products (291-292) and potato products (221) with percentage of reduction up to 85-90 percentage and no effect on food products taste and appearance. Some introductory results obtained at lab scale highlight that asparaginase pretreatment of green beans may supersede a practicable way to decrease acrylamide levels in roasted coffee.

It should be noted that most of the mitigation detection presented so far were only tested at the pilot and laboratory scale. Therefore, for those mitigation detection, it is not obviously known whether the percentage of letdown in acrylamide cited at laboratory scale which could ever be achievable in food production at an industrial scale. When many mitigation options are used to the same food product, the overall percentage of letdown in acrylamide concentration is not merely the sum of the percentages achievable when each single detection is used. The interactions among different detection are not apparently known well and should be taken into account (214).

It has been underscored that some mitigation options are associated with a loss in benefits and increasing in other risks. For example, elongating yeast fermentation can effectively decrease acrylamide levels in bread but it is associated to increase in the concentrations of 3-monochloropropandiol (293). Likewise, replacing of ammonium bicarbonate with sodium bicarbonate as rising agent for fine bakery products results in enhancing of sodium input (294). There is a wide concurrence that the behavior aiming at lowering acrylamide concentration of food products should be accompanied by a risk-benefit or risk-risk analysis to explain overall the side effects and their effects on human

health. In Table 1.12, some of the available mitigation options for acrylamide reduction in bakery products are presented along with the concomitant effect on 5-hydroxymethylfurfural level. Moreover, some of the mitigation options that have been represented bring about changing in organoleptic properties of food products that can dramatically result in the final quality and consumer's acceptance (292-293). This is a fundamental point for the future considering that mitigation options are fully not helpful if sensorial reasoning consumers do not like the "mitigated" products giving their preference to the "conventional" ones. In that consider, the knowledge of the kinetics of acrylamide accumulation in food products is the maximum importance. Acrylamide concentration in food products should be considered as the result of concomitant actions of elimination and formation. Acrylamide begins to form at a temperature >100°C after an initial lag phase during which no acrylamide forms. Afterwards, the acrylamide level increases exponentially with time to a maximum level, after which it can reduce again because of the languidness of one of the reactants or by the elimination of acrylamide. However, the mechanisms of elimination have not been completely explained and needed further inquisition. Acrylamide dominates 2 functional groups, an electron-deficient vinylic double bond and an amide group that makes it available for a wide range of actions, including nucleophilic, radical actions, and Diels-Alder additions. Acrylamide may achieve Michael addition type actions to the vinylic double bond with nucleophiles, including thiol and amino groups of proteins and amino acids, respectively. On the other hand, the amide group can accomplish many actions including dehydration, alcoholysis, hydrolysis, and condensation with aldehydes (261). Many kinetic models have been presented to explain acrylamide elimination and formation and to forecast its final level in model systems and food products. Single-response models depended on empirical total action kinetics have been popularly employed. Acrylamide formation has been modeled as a first order action (295-296), as a pseudo-first order action (297) or as a second order action (298) according to action conditions and reactant contents. Acrylamide elimination has been normally modeled as a first order kinetics (297). Empirical models such as those presented by Corradini and Peleg (299) and Kolek, et al. (300) have been demonstrated and proved to satisfactorily explain acrylamide level in potato chips (301) and model systems (302). Such empirical models are not depended on an underlying chemical compound mechanism and

extrapolation is also not feasible outside the region of variant for which the function has been derived. On the other hand, multiresponse models using acrylamide information supplemented with information on action precursors, end products and intermediates including mechanistic information in the chemistry involved have been presented (301). With such model systems, not only acrylamide formation but also that of other relevant Maillard-related chemical compounds can be modeled and the approximation of kinetic factors is much more accurate.

Table 1.12 Some acrylamide mitigation options available in literature for bakery products and their effect on 5-hydroxymethylfurfural (HMF) formation.

Acrylamide mitigation strategy	Effect on HMF[x]	Reference	Comments
Asparaginase addition	No effect	(291-292)	-
Ca^{2+} addition	+	(303)	Cations prevent the formation of Schiff base, changing the reaction path toward the dehydration of glucose leading to HMF and furfural
Changing of time temperature profile toward lower thermal input	-	(303)	The overall thermal input is pivotal in determining the final concentration of both acrylamide and HMF
Glycine addition	+	(292)	Overall Maillard Reaction is accelerated when glycine is added
Replacement of reducing sugars with sucrose	-	(303)	Increasing HMF if baking temperatures >250°C
Selection of refined flours	No effect	(304)	Refined flours have lower free Asparagine and free amino acids content

[x] + = Increased HMF content; - = Decreased HMF content.

5. Literature reviews

The literature reviews are focused on extraction and determination method for acrylamide, particularly, the overviews of extraction, purification, and determination from food products and samples.

5.1 Extraction method

Precise quantification of acrylamide contained in food products by employing the modern methods such as electrophoresis or chromatography requires complete and difficult purification from substances and isolation of this analyte which can interfere with its examine. However, sample preparation process frequently involves both its derivatization and analyte extraction from food matrix which recovers precision of analysis. In the look of the complex manner of food products, isolation of individual chemical compounds is extremely difficult for the analyst (305-307). Separation of acrylamide from other chemical compound extracted from food product is difficult and thus numerous amounts of internal standards are used. Consequently, only a few laboratories are installed with suitable and high-performance analytical equipment which are applicable to acrylamide detection (307-308). Quantification of acrylamide in food products by utilizing biosensors requires only simple processes of sample preparation before the examination. Preparation of food products include defatting and extraction of food material by using hexane and water and in purification of resulting extracts by using Carrez reagents (309). This protocol of sample preparation demands only a little amounts of reagents and toxic organic solvents, effectively transfers matrix components and assures high recovery of quantified chemical compounds (309-310). Lately, Quan, et al. (311) developed a simple and fast method of sample preparation which does not require derivatization and extraction clean-up procedures. Its benefit is the low consumption of reagents and easement because the food samples are only centrifuged and shaken (312). Commonly, the sample preparation process involves conventional extraction in the liquid–solid method. Many variants of this method are applied prior to acrylamide analysis by high performance liquid chromatography, gas chromatography, and capillary electrophoresis or immunological methods, or by using biosensors. For example, extraction can be intensified by ultrasounds or accelerated by solvent (accelerated solvent extraction) or conducted by utilizing a supercritical fluid

extraction (313-314). Proteins are precipitated with sodium chloride, acetonitrile, methanol, ethanol, or Carrez reagents (315-317).

Because of the low selectivity of extract, it is additionally purified by means of solid phase extraction, most frequently on modified silica gel packed in solid phase extraction columns (318-320). Clean-up of acrylamide extracts by means of solid phase extraction prior to electrophoresis, chromatography, or biological examine eliminates multiple interfering chemical compounds and increases accuracy and precision of instrumental analyses. The new version of solid phase extraction is dispersive solid phase extraction (321). This method is depended on extraction by consuming organic solvents (e.g. acetone, acetonitrile, and ethyl acetate). Despite of the mentioned above, solid phase extraction advantage as well as liquid–liquid extraction is carried out to enhance acrylamide level in water extracts. The liquid–liquid extraction and solid phase extraction are relatively quickly method. One of the modern isolation techniques which is suitable to compounds with strongly bound with the matrix is the matrix solid phase dispersion method comprising in simultaneous extraction and purification of food product. Matrix solid phase dispersion has been utilised in isolation of pesticides, drugs, and food components. According to Dias Soares, et al. (322), matrix solid phase dispersion can be used to extract acrylamide from several food products. Matrix solid phase dispersion increased the sensitivity of analysis, reduced consumption of solvents and shortened the time of extraction. Different techniques of samples preparation for chromatography were published. One of the newest techniques of sample preparation before chromatography analysis is solid phase micro extraction. Solid phase micro extraction gives isolation of chemical compound from liquid and gaseous samples depended on its separation between a stationary phase and a matrix (323). This method has been increasingly used in isolation of acrylamide and its derivatization because of the short time of extraction and a very small amounts of solvents (324).

The multi- stage preparation of food products before acrylamide quantitative mensuration by chromatography or electrophoresis provided some easy, fast to operate, sensitive and inexpensive analytical processes were developed. Although classical extraction processes such as liquid-liquid extraction and solid phase extraction have been normally used, the newer processes like supercritical fluid extraction and

matrix solid phase dispersion become increasingly favorite because they propose savings of compounds and faster preparation before the analysis (325).

5.1.1 Accelerated solvent extraction

Details are explained in introduction of curcumin (section 4.1.4). Accelerated solvent extraction method includes in extraction of analytes by consuming a little amounts of a solvent. This procedure is conducted at elevated pressure and temperature (313, 326) Thus, solvents consumption in accelerated solvent extraction system is very low while acrylamide extraction from many food products is efficient (327-329). The disadvantage of accelerated solvent extraction is the formation of acrylamide in cocoa and powdered milk taking place during extraction at elevated temperature, and imperfect extraction of the analyte from these products which in aftereffect can lead to false examine results (330-331). The most normally used solvent for extraction is water, less often used are mixtures of polar organic solvents. Because the yield of extraction is reduced by high fat level, foods rich in fat are defatted by utilizing toluene, cyclohexane, or hexane (332).

Several accelerated solvent extraction for determination of acrylamide are briefly listed in Table 1.13.

Table 1.13 Accelerated solvent extraction for determination of acrylamide.

Samples	LOD/LOQ	Amount Found	QA/QC report	Ref.
French Fries, potato chips, tortilla chips, wheat snacks with bacon flavor, and crisp bread	-	N.D.-1.57 mg kg^{-1}	%recovery 95.1-96.4	(333)
Heat-processed foods	LOD 0.015 µg mL^{-1}	N.D.-956 µg kg^{-1}	%recovery 66.0-110.6	(334)

Table 1.13 (cont.)

Samples	LOD/LOQ	Amount Found	QA/QC report	Ref.
Brown, Fino, and Baladi bread	LOD 0.71 ng g^{-1}	0.11-10.12 µmol L^{-1}	%recovery 98.0-110.0, %RSD 1.14-3.42	(335)
Chips, crackers, and cereal-based baby foods	LOD 0.08, LOQ 0.28 µg kg^{-1}	10.2-145 µg kg^{-1}	%recovery > 95, %RSD < 6.3	(336)
Potato chips	LOD 0.6, LOQ 2 ng g^{-1}	116.85-139.84 ng g^{-1}	%recovery 97-99, %RSD 6.8	(337)

5.1.2 Supercritical fluid extraction

Details are explained in introduction of curcumin (section 4.1.5). Several supercritical fluid extraction for determination of acrylamide are briefly listed in Table 1.14.

Table 1.14 Supercritical fluid extraction for determination of acrylamide.

Samples	LOD/LOQ	Amount Found	QA/QC report	Ref.
Coffee and chocolate	LOD 0.20-0.30, LOQ 0.60-1.00 µg kg^{-1}	15.0-385 µg kg^{-1}	%recovery 81.0-105, %RSD 2.00-5.00	(338)
Roasted Malaysian tropical almond nuts	LOD 0.004-0.382, LOQ 0.014-1.270	1.050-157.8 mg kg^{-1}	%recovery 89.7-99.7	(339)

Table 1.14 (cont.)

Samples	LOD/LOQ	Amount Found	QA/QC report	Ref.
	mg L^{-1}			
Coffee	-	0.37-0.90 mg kg^{-1}	-	(340)
Beverages, grains, and confectioneries	LOD 0.12-5.00, LOQ 0.41-17.0 µg kg^{-1}	1.40-300 µg kg^{-1}	%recovery 100.7-102.7, %RSD 0.60-2.00	(341)

5.1.3 Solid-Phase Extraction

Solid-Phase Extraction is depended on the partition of the sample between a sorbent in a column and a liquid phase that is a solvent with analytes. As a result of the diffusion by adsorption of molecules on sorbent, an equivalence is set up and the sample retains on the sorbent in compliance with the diffusion coefficient. Set of sample from the solid-phase extraction can be carried out through procuring a more likeable environment to the sample than solid-phase. Desorption of chemical compounds with favoured liquid can be operated via passing of the sample into liquid phase and exiting from the solid phase extraction system. After this elution step, one or more rinsing or washing steps can be used in order to transfer undesirable chemical compounds which retained together with the sample on the sorbent (342).

Particle size of solid-phase extraction sorbents commonly ranges between 10-60 micrometers and their formats can be a syringe, cartridges, and pipette types (343-344). Various types of sorbent are available and the selection can be created according to the food component. Different mechanisms which are depended on different reactions (e. g. van der Waals, hydrogen-bonding, dipole-dipole, or electrostatic) play an essential role during the extraction of related sample. Normal-phase involves a polar stationary phase and retention happening from reaction between polar groups of the sample and polar groups on the sorbent. Reversed-phase involves a nonpolar stationary phase with a polar mobile phase and normally applied for liquid

samples which main reaction force is van der Waals. Ion-exchange can be used for chemical compounds which are charged. Lastly, in mixed-mode, two different groups on the same sorbent are utilized. (342, 344). Silica sorbent is the most normal one because of its respectability for stability and modification (343).

Solid- Phase Extraction is a helpful method for separation and isolation applications which preconcentration is one of the aims in its application field. The sample leaves the pretreated cartridge which filled with needed sorbent in a small volume of solvent solution. Relatively teeny surface of solid phase, big volume of sample, which may provide a perfect retention, and the spend of volatile solvents are the main parameters for utilizing solid-phase extraction for preconcentration. Moreover, solid-phase extraction can be a beneficial equipment for clean-up that is essential for removal interfering chemical compounds and taking obviously identifiable signals in chromatography (342).

Foods consist of wide diversity of chemical components (e. g. fats, proteins, carbohydrates, vitamins and minerals). Before the determination of a specific chemical compound in food component, homogenization, extraction, and expulsion interferences are the important practices. Solid-phase extraction is widely applied for the determination of different chemical compounds in various food components. Solid-phase extraction application for the acrylamide determination in food products is a good-documented topic in the literature review (319, 345-346). Preferably for the clean-up method, solid-phase extraction is frequently used by many researchers traversed for acrylamide determination. Roach et al. (347) and Andrzejewski et al. (348) have studied the determination of acrylamide which depended on a liquid extraction of acrylamide and a clean-up method firstly with oasis and secondly with an Accucat column. Results of the studies were the limit of quantitation of the methods which was 10 $\mu g\ kg^{-1}$ for the first column. The second column provides 10 $ng\ g^{-1}$ for ground and instant coffees and 1.0 $ng\ mL^{-1}$ for brewed coffee. Zhang et al. (349) have experienced another study for cereal foods. For enhancing the concentration estimated by percentage recovery, different cartridge kinds were assessed. Detection was operated by liquid chromatography coupled with electrospray ionization tandem mass spectrometry. As respects to the report results, oasis HLB showed a good performance in percentage recovery.

Several solid-phase extraction for determination of acrylamide are briefly listed in Table 1.15.

Table 1.15 Solid-phase extraction for determination of acrylamide.

Samples	LOD/LOQ	Amount Found	QA/QC report	Ref.
Heated foods	LOD 25.00 $\mu g\ kg^{-1}$	110.0-5870 $\mu g\ kg^{-1}$	%RSD 3.500	(345)
Cereal, bread crumbs, potato chips, and coffee	LOQ 100 $\mu g\ kg^{-1}$	62.0-684 $\mu g\ kg^{-1}$	%recovery 92.0, %RSD 0.90-6.90	(347)
Coffee	LOD 2.00 $\mu g\ L^{-1}$	2.16 $\mu g\ L^{-1}$	%recovery 96.0-104, %RSD 4.00-14.0	(346)
Coffee	LOQ 10.0 $ng\ g^{-1}$ for ground and instant coffees, 1.00 ng mL^{-1} for brewed coffee	45.0-374 $ng\ g^{-1}$ in ground coffee, 172-539 $ng\ g^{-1}$ in instant coffee, 6.00-16.0 ng mL^{-1} in brewed coffee	-	(348)
Infant cereal-based foods	-	3.30-37.1 $\mu g\ kg^{-1}$ for infant rice cereals, 10.900-1568.9 $\mu g\ kg^{-1}$	%recovery 87.0-96.0, %RSD < 6.50	(349)

Table 1.15 (cont.)

Samples	LOD/LOQ	Amount Found	QA/QC report	Ref.
		for cereal-based food		
Thermally processed foods	LOD 2.00, LOQ 6.00 ng g^{-1}	195.4 µg kg^{-1}	%recovery 92.80-101.5, %RSD < 4.100	(350)
Chocolate	LOD 0.30, LOQ 1.00 µg kg^{-1}	23.0-537 µg kg^{-1}	%recovery 86.0-93.0, %RSD < 3.50	(351)
Starch-based foods	30.000 µg kg^{-1}	213.20-2868.2 µg kg^{-1}	%recovery 92.500-104.00	(352)
Coffee	-	131.79-2191.1 µg kg^{-1}	%recovery 74.6-84.1	(353)
Roasted coffee	LOD 5.00, LOQ 16.0 µg kg^{-1}	150-327 µg kg^{-1}	%recovery 92.0-95.0, %RSD < 5.00	(354)
Various Belgian potato products	LOD 25.00, LOQ 50.00 µg kg^{-1}	N. D. – 2311 µg kg^{-1}	-	(355)
Baked and deep-fried Chinese foods	LOD 8.00, LOQ 25.0 µg kg^{-1}	86.3-151 µg kg^{-1}	%recovery 89.0-103	(356)
Starchy foodstuffs	-	13.00-28.00 ng g^{-1}	%recovery 73.13-98.00, %RSD 0.500-1.630	(357)

5.1.4 Liquid-liquid extraction

Liquid-liquid extraction, depended on move of analyte from the liquid sample to water-immiscible solution, is being popularly employed for sample preparation. However, some flaws such as the emulsion formation, the consumption of big sample volumes and toxic solutions and hence, the generation of big amounts of pollutants makes liquid-liquid extraction expensive, environmentally unfriendly, and time-consuming. Miniaturization of this extraction method can be accomplished by a severe reduction of the extraction phase volume. Depended on this basis, three new methods have happened; single-drop microextraction, hollow fibre liquid-phase microextraction, and dispersive liquid-liquid microextraction. Single-drop microextraction is a preconcentration method depended on the utilization of a micro drop of extractant exposed to the sample solution, to a thin organic phase leveled over the aqueous sample, or to the headspace above the sample when analytes are volatile. In hollow fibre liquid-phase microextraction using two phase sampling form, a hydrophobic hollow fibre is employed to prevent and expose a certain amount of extractant to the sample solution. The extraction procedure arises in the holes of the fibre, where the solution is immobilized. In the three-phase sampling form, the analytes are extracted into an organic solvent phase which fills the holes of the fibre and then back-extracted into an aqueous or liquid phase placed inside the hollow fibre. Though hollow fibre liquid-phase microextraction has been popularly used for the detection of organic chemical compounds, the application of this method in the inorganic is still meager. Dispersive liquid-liquid microextraction is depended on the clouded solution formed when a suitable mixture of extraction solvent and disperser solvent is speedily injected into the liquid sample. The extraction solvent should have higher density than water, while the disperser solvent should be miscible in the extraction solvent and the liquid sample. The fine drips of extraction solvent are diffused throughout the liquid sample, allowing its reaction with the analyte. The evolution of the different forms of single-drop microextraction along with hollow fibre liquid-phase microextraction and dispersive liquid-liquid microextraction proposes new feasibilities over the basic liquid-liquid extraction. Researchers have named microextraction methods discussed here in many different methods. For example, single-drop microextraction is known as solvent drop microextraction, liquid-phase microextraction, solvent microextraction, single

drop extraction, and single drop liquid phase microextraction. Furthermore, in some cases the same name has been given to different microextraction methods. This is the case of the term liquid-phase microextraction, which has been employed to cite the single-drop microextraction and the hollow fibre liquid-phase microextraction (358).

Several liquid-liquid extraction for determination of acrylamide are briefly listed in Table 1.16.

Table 1.16 Liquid-liquid extraction for determination of acrylamide.

Samples	LOD/LOQ	Amount Found	QA/QC report	Ref.
Starch-based foods	LOD 15.00, LOQ 50.00 $\mu g\ kg^{-1}$	145.0-2522 $\mu g\ kg^{-1}$	%recovery 89.60-102.0, %RSD 2.100-7.400	(359)
Water	LOD 1.0 $ng\ L^{-1}$	0.0-1.2 $\mu g\ L^{-1}$	%recovery 91, %RSD 3.6	(360)
Environmental and drinking waters	LOD 2.40, LOQ 17.0 $ng\ L^{-1}$	21.0-280 $ng\ L^{-1}$	%recovery 89.0-102, %RSD < 6.00	(361)
Bread and biscuit samples	LOD 3.0, LOQ 9.0 $\mu g\ L^{-1}$	N.D. – 70 $ng\ g^{-1}$	%recovery > 90, %RSD < 9.0	(362)
Potato chips	LOD 0.100, LOQ 0.330 $ng\ mL^{-1}$	7.340-19.45 $ng\ mL^{-1}$	%recovery 91.48, %RSD 1.460	(363)
Brewed coffee	LOD 0.90, LOQ 3.00 $\mu g\ L^{-1}$	10.5-28.5 $\mu g\ L^{-1}$	%recovery 97.0-106, %RSD 6.00-9.00	(364)
Cereal products	LOD 0.6000,	100.22-233.94 $ng\ g^{-1}$	%recovery 95.000,	(365)

Table 1.16 (cont.)

Samples	LOD/LOQ	Amount Found	QA/QC report	Ref.
	LOQ 2.00 ng g^{-1}		%RSD 2.9- 5.6	
Tap and well water samples	LOD 0.10, LOQ 0.30 ng mL^{-1}	<LOQ-9.80 ng mL^{-1}	90.8-94.1, %RSD 2.10-5.50	(366)

5.1.5 Matrix solid-phase dispersion

In 1989 (367), a new procedure for the extraction of solid sample was introduced. This procedure, merges aspects of several methods for sample disruption while also creating a material that dominates unique chromatographic feature for the extraction of chemical compounds from a given sample (368). Matrix solid-phase dispersion connects interweaving a viscid, solid sample with a solid contribute. For matrix solid-phase dispersion, it is more easily being applied in a different feature than formerly intended. In this feature, the solid contribute serves the same aim as the utilization of sand as an incisive: the chopping forces of blending with a pestle and mortar disrupt all architecture of the solid sample, ruining the sample into smaller pieces. The owning of the bound organic phase provides forward dimension to the procedure: sample compositions melt and dissipate into the bound organic phase on the surface of the molecule, leading to the perfect annihilation of the sample and its dispersion over the surface. Sample compositions spread over the surface relied on their relation polarities. Non-polar compositions dissipate into the non-polar organic phase depended on their spreading coefficients with the phase and the dynamic changes that occur when this procedure proceeds. Smaller, greatly polar molecules are envisioned to participate with silanols on the surface of silica and inner the holes of silica solid contribute as well as with matrix compositions capable of hydrogen bonding while

larger, less polar molecules are diffused across the surface, bonded-phase or spread-sample-lipid structure (368).

Several matrix solid- phase dispersion for determination of acrylamide are briefly listed in Table 1.17.

Table 1.17 Matrix solid-phase dispersion for determination of acrylamide.

Samples	LOD/LOQ	Amount Found	QA/QC report	Ref.
Potato chips	LOD 12.8, LOQ 38.8 $\mu g\ kg^{-1}$	186.42-1890.9 $\mu g\ kg^{-1}$	%recovery 98.0-111, %RSD 2.60-5.50	(369)
Coffee and coffee substitutes	LOD 5.00, LOQ 15.0 $\mu g\ kg^{-1}$	23.6-330 $\mu g\ kg^{-1}$	%recovery 84.0-97.0, %RSD 2.00-10.0	(370)
Breakfast cereals, bread, toasted bread, biscuits, cookies, baby food, salty snacks, and chocolates	LOD 5.20, LOQ 15.7 $\mu g\ kg^{-1}$	15.0-996 $\mu g\ kg^{-1}$	%RSD 1.00–7.00	(371)
Potato, eggplant, gorrasa, minnan, taamia, and Hilumur	LOD 9.10-12.8, LOQ 27.8-38.9 $\mu g\ kg^{-1}$	N.D.-752 $\mu g\ kg^{-1}$	%recovery 88.0–103, %RSD < 6.60	(372)
Twisted cruller, potato chip, and toast samples	LOD 1.500 $\mu g\ L^{-1}$	209.0-530.0 $\mu g\ kg^{-1}$	%recovery 85.30-94.60, %RSD 4.300	(373)

Table 1.17 (cont.)

Samples	LOD/LOQ	Amount Found	QA/QC report	Ref.
Biscuits and breads	LOD 14.5-16.1, LOQ 40.1-40.5 ng g^{-1}	-	%recovery 83.7-98.0, %RSD 1.23-6.72	(374)

5.2 Determination method

5.2.1 High Performance Liquid Chromatography (HPLC)

Details are explained in literature reviews of curcumin (section 4. 2. 1) . Several high performance liquid chromatography for determination of acrylamide are briefly listed in Table 1.18.

Table 1.18 HPLC method for determination of acrylamide.

Samples	LOD/LOQ	Amount Found	QA/QC report	Ref.
Heated foodstuffs	LOD 5.000, LOQ 10.00 µg kg^{-1}	146.0-2273 µg kg^{-1}	%recovery 97.00-112.0, %RSD 1.600-8.900	(375)
Heated foods	-	1036- 3936 µg kg^{-1}	-	(220)
Dry heating of gluten	LOD 90.0 µg g^{-1}	53.4-63.9 µg kg^{-1}	-	(225)
Gouda cheese before and after thermal treatment	-	N.D.-189 µg kg^{-1}	%RSD 9.80	(226)
Human urine	-	-	%recovery 51-52, %RSD 5.0	(256)

Table 1.18 (cont.)

Samples	LOD/LOQ	Amount Found	QA/QC report	Ref.
Cocoa, coffee, and cereal products	-	43.400- 1492.4 $\mu g\ kg^{-1}$	%RSD 0.5000-14.000	(228)
Cocoa and coffee products	LOD 10.0, LOQ 20.0 $\mu g\ kg^{-1}$	175-859 $\mu g\ kg^{-1}$	%recovery 93.0-99.0, %RSD 3.40	(376)
Tea	LOD 1.0, LOQ 5.0 $ng\ mL^{-1}$	N.D.-94 $ng\ g^{-1}$	%recovery 74.0-79.0, %RSD 1.6-8.3	(377)
Deep-fried flour-based indigenous Chinese foods	LOD 6.00, LOQ 23.0 $\mu g\ kg^{-1}$	27.0–198 $\mu g\ kg^{-1}$	%recovery 78.0-107, %RSD 2.10-10.9	(378)
Popular Iranian brands of potato and corn products	LOD <10.00, LOQ <30.00 $\mu g\ kg^{-1}$	244.0-1688 $\mu g\ kg^{-1}$	-	(379)
Baked and deep-fried Chinese foods	LOD 8.00, LOQ 25.0 $\mu g\ kg^{-1}$	86.3–151 $\mu g\ kg^{-1}$	%recovery 89.0–103	(356)
Coffee	LOQ 5.00 $mg\ L^{-1}$	7.70-40.0 $mg\ L^{-1}$	-	(380)
Baby food	LOQ 20.0 $\mu g\ kg^{-1}$	35.0 $\mu g\ kg^{-1}$	%recovery 94.0–110, %RSD < 10.0	(381)
Cereals, legumes, roots and tubers	-	161.12- 5562.6 $mg\ kg^{-1}$	-	(382)

Table 1.18 (cont.)

Samples	LOD/LOQ	Amount Found	QA/QC report	Ref.
Dried fruits and edible seeds	-	10.00-124.3 $\mu g\ kg^{-1}$	%recovery 61.0-82.0, %RSD < 20.0	(383)
French Fries	20.0 $\mu g\ kg^{-1}$	303 $\mu g\ kg^{-1}$	-	(384)

5.2.2 Gas chromatography

Gas chromatography is a kind of chromatography in which the mobile phase is gas, normally an inert gas (helium) or an un-reactive gas (nitrogen) and the stationary phase is a microscopic level of polymer or liquid on an inert solid, inside metal tubing or glass which called the column. The column is filled with a stationary phase; a fine solid coated with a non-volatile liquid or polymer. The sample is passed through the column by a stream of helium gas or nitrogen gas. Mass spectrometry is one of the detector for gas chromatography. As the sample outlets at the end of the gas chromatographic column, it is separated by ionization and the segments are sorted by mass to form a separation pattern. Like the retention time, the separation pattern for a given composition of sample is unique and thus is an identifying characteristic of that composition. It is so particular that it is frequently cited to a molecular fingerprint. Gas chromatography-mass spectrometry is an analytical technique that merges the properties of mass spectrometry and gas-liquid chromatography to identify different chemical compounds within a test sample. Gas chromatography can separate semi-volatile and volatile compounds with large resolution, but it cannot identify them. Mass spectrometry can provide detailed structural information on most compounds such that they can be exactly identified, but it cannot readily separate them (385).

1) Principle of gas chromatography.

Gas chromatography and mass spectrometry are the combination of 2 different analytical methods which used for detection of biochemical mixtures and organic complex (386). The gas chromatography-mass spectrometry instrument consists of 2 main compositions. The gas chromatography segment separates different chemical compounds in the sample into pulses of pure chemicals relied on their volatility by flowing an inert gas, which carries the sample, through a stationary phase filled in the column (386). Spectrum of chemical compounds are collected as they vent off the column by mass spectrometer, which quantifies and identifies the chemical compounds according their mass-to-charge ratio.

2) Instrumentation of gas chromatography-mass spectrometry.

The Fig. 1.10 is the schematic diagram of gas chromatography-mass spectrometry. Its different sections and their operations are discussed below.

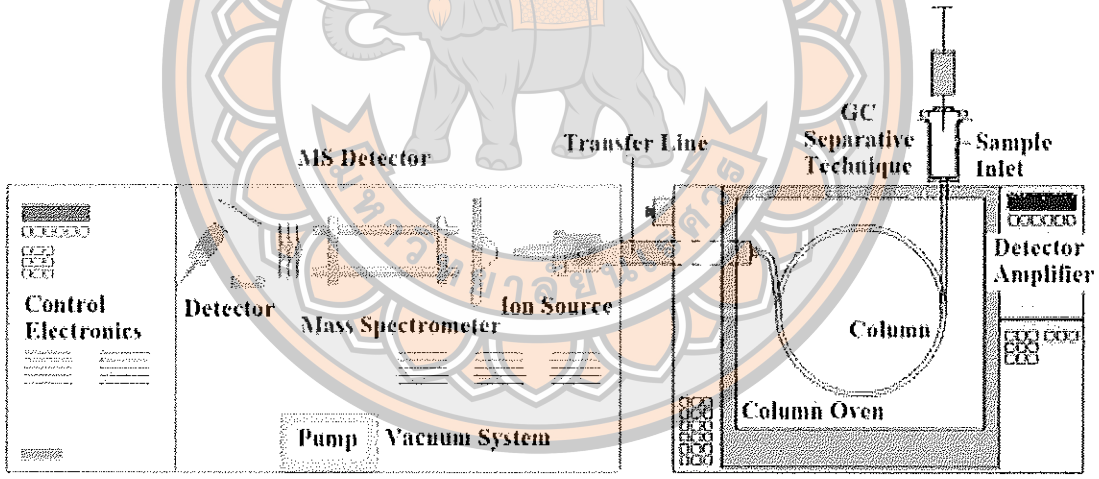


Figure 1.10 Schematic diagram of gas chromatography-mass spectrometry.

Source: Re-drawn from Hussain, et al., 2014 (385)

2.1) Gas supply: Gas is fed from the cylinders through the regulators and tubing to the instrument. It is routine to purify the gas to assure high gas purity and gas supply pressure.

2.2) Injector: Here the sample is volatilized gas entrained into the stream approaching the gas chromatographic column.

2.3) Column: gas chromatography uses a gas mobile phase to transport sample compositions through columns either packed with coated silica or hollow capillary columns comprising the stationary phase coated onto the internal wall. Capillary columns are normally several meters long with an inside diameter of 0.10-0.50 millimeters while packed columns tend to be 1-5 meters in length with either 2-4 millimeters inside diameter.

2.4) Oven: This method has oven that is temperature programmable part. The temperature of ovens normally ranges from 5-400°C.

2.5) Mass spectrometer: The partitioning of the ions phase is obtained within the mass spectrometer using magnetic or electrical fields to individualize ions.

2.6) Ion source: The products are ionized before detection in the mass spectrometer.

2.7) Mass analyzer: There are several kinds of mass analyzer participatory with routine gas chromatography-mass spectrometry analysis and the overall vary in the fundamental path in which they separate species on a mass-to-charge basis.

2.8) Vacuum system: Mass analyzers need high levels of vacuum in order to work in a predictable and efficient path.

2.9) Detector: The ion stream that emerges from the mass analyzer have to be analyzed and converted into a usable signal. Detector is a significant element of the mass spectrometer that generates a signal from incident ions by either manufacturing secondary electrons, which are forwards amplified, or by inducing a current.

2.10) Electronic control: The mass spectrometry factors can be selected and controlled from this electronic control. Modern apparatuses will also permit to control mass spectrometry factors from a computer by employing extraordinarily designed software.

The mobile-phase is called carrier gas which helium gas is the most normally used, moreover, nitrogen, hydrogen, and argon are also utilized.

These gases are held in pressurized tanks and its use pressure regulators, gauges, and flow meters to manage the flow rate of the mobile-phase. Flow rate normally ranges from 25-150 milliliter min^{-1} for packed columns and 1-25 milliliter min^{-1} for open tubular capillary columns, and are assumed to be strong if inlet pressure is strong. This is frequently accompanied by a molecular sieve to purify the gas before it is applied. Samples are introduced as a plug of vapor. Liquid samples are introduced using calibrated micro syringes to inject sample through a septum and into a heated sample port which should be about 50°C above the boiling point of the volatile composition of the sample, then, it is carried to the column by carrier gas. The temperature of the column is a significant variant, so the oven is installed with a thermostat that manages the temperature to a few tenths of a degree. Boiling point of the sample and the amount of separation required determines the temperature the sample should be run with. As the carrier gas carrying the sample is passed through the stationary phase, the different compositions of the sample are separated. Then, the sample is run through detector (386), which ionizes the sample and then segregates the ions depended on their mass-to-charge ratio. This information is then sent to a computer to be analyzed. The computer joined to the gas chromatography-mass spectrometry has a data source of samples to assist in determining this information (385). Information for the gas chromatography-mass spectrometry is showed in several paths. One is the total ion chromatogram, which summarizes the total ion abundances in each spectra and plots them as a function of time. Another is the mass spectra at a specific time in the chromatogram to identify the specific composition that was eluted at that time. A mass spectrum of selected ions with a particular mass-to-charge ratio, called a mass chromatogram, can also be obtained.

Several gas chromatography for determination of acrylamide are briefly listed in Table 1.19.

Table 1.19 Gas chromatography for determination of acrylamide.

Samples	LOD/LOQ	Amount Found	QA/QC report	Ref.
Fried food	LOD 10.0 μg kg^{-1}	200 μg kg^{-1}	-	(387)
Chinese foods	LOD 5.00 μg kg^{-1}	20.0-900 μg kg^{-1}	%recovery 102-110	(388)
Green tea	-	27.00-1880 ng g^{-1}	-	(389)
Breakfast cereals, bread, toasted bread, biscuits, cookies, baby food, salty snacks, and chocolates	LOD 5.20, LOQ 15.7 μg kg^{-1}	15.0-996 μg kg^{-1}	%RSD 1.00–7.00	(371)
Foods	-	7.00-980 μg kg^{-1}	-	(390)
Espresso coffee	-	131.79-2191.2 μg kg^{-1}	%recovery 74.600-84.100	(353)

5.2.3 Other methods

Moreover, other methods for determination of acrylamide are briefly listed in Table 1.20. These methods are not as popular as those previously described.

Table 1.20 Other methods for determination of acrylamide.

Samples	Method	LOD/LOQ	QA/QC report	Ref.
Potato chips	Capillary chromatography	LOD 0.100, LOQ 0.330 μg mL^{-1}	%recovery 90.86-99.60, %RSD 0.860-9.650	(332)

Table 1.20 (cont.)

Samples	Method	LOD/LOQ	QA/QC report	Ref.
Bake rolls, potato chips, and crackers	Voltammetry	-	%recovery 98.7-99.7, %RSD 0.70-2.00	(391)
Food products	Microchip electrophoresis	LOD 1.000, LOQ 2.000 ng mL^{-1}	%recovery 94.70-110.7, %RSD 2.400-7.500	(392)
Chips, crackers, and cereal-based baby foods	Flame atomic absorption spectrometry	LOD 0.08, LOQ 0.28 mg kg^{-1}	%recovery 95.0, %RSD < 6.30	(393)
Heat-processed foods	Fluorescence	LOD 0.015 µg mL^{-1}	%recovery 66.00-110.6	(334)
Food products	Fluorescence	LOD 0.5×10^{-6} M	%recovery 82.00-103.6, %RSD 0.770-3.120	(394)
Potato chips	A computer vision based	-	%recovery 94	(395)
Potato chips	An imaging	-	%recovery 97	(396)
Fried potato chip	Image processing based	-	%recovery 98.33	(397)
Fried food	Raman spectroscopy	LOD 2.00, LOQ 5.00 µg kg^{-1}	%recovery 73.4–92.8, %RSD 4.20	(398)

These methods have been used for the determination of acrylamide content in food samples. Its exhibit high performance in terms of quantitative and qualitative analysis in many chemical analyses methods. However, these techniques employ big and expensive equipment and they are inconvenient as they use off-site analysis. Consequently, this research proposes a high-throughput, rapid, lowcost, and on-site analysis method for the detection of acrylamide in food samples.

Instrumentation

1. Microplate Reader

The microplate reader is a versatile measurement tool such as luminescence or fluorescence, and absorbance. It can be measured in the wavelength range 300-900 nm. The working principle and components of microplate reader are the same as UV-visible spectrophotometer except sample cell. As shown in Figure 1.11, sample cell of the microplate reader can be used at the microliter or milliliter level and it is named microwell plate or microtiter plate (399).

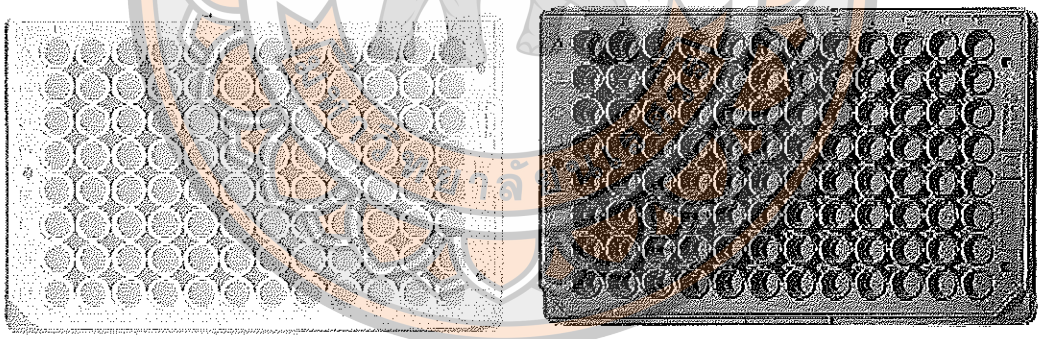


Figure 1.11 A: transparent 96-well microtiterplate and B: black 96-well microtiterplate (400).

Characteristics of microplate reader used in this research work is shown in Figure 1.12 (401-402).

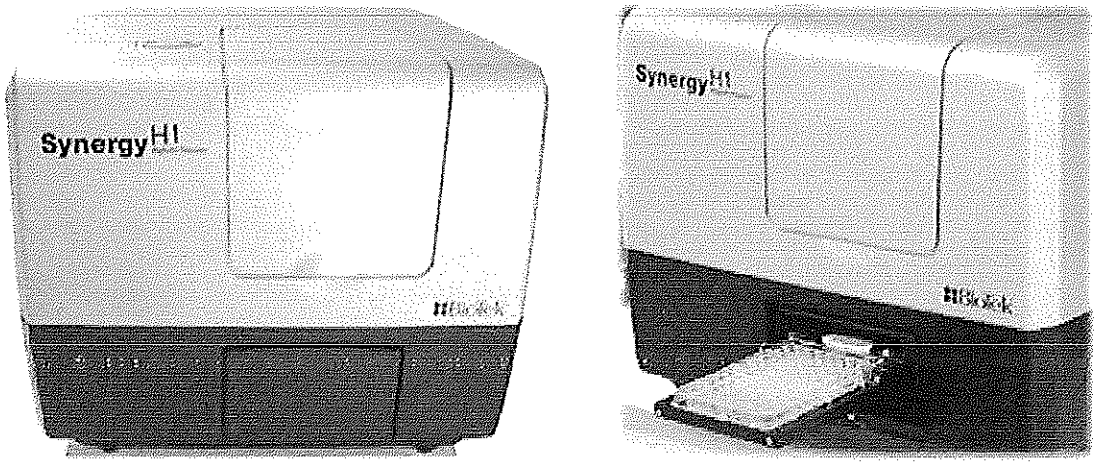


Figure 1.12 Characteristics of microplate reader (401-402).

Synergy™ H1 is a configurable multi-mode microplate reader. It chooses monochromator-based optics for flexibility or filter-based optics for sensitivity. BioTek patented Hybrid Technology™ offers high-performance and applications versatility in a modular platform to expand as the laboratory's needs change. UV-visible absorbance, fluorescence intensity, and luminescence detection are available to meet many life science research requirements. Technical details and detection modes are briefly listed in Table 1.21-1.26 (403).

Table 1.21 Technical details of Synergy H1 Hybrid Multi-Mode Reader (403).

General	
Detection modes	UV-Vis absorbance
	Fluorescence intensity
	Luminescence
	Fluorescence polarization
	Time-resolved fluorescence
Read methods	Endpoint, kinetic, spectral scanning, well area scanning

Table 1.21 (cont.)

General	
Microplate types	6-384 well plates
Other labware supported	Petri and cell culture dishes Take3 Micro-Volume Plates
Temperature control	4-Zone™ incubation to 45 °C with Condensation Control™ ±0.2 °C at 37 °C
Shaking	Linear, orbital, double orbital
Software	Gen5™ Data Analysis Software
Automation	BioStack and 3rd party automation compatible BioSpa 8 Automated Incubator compatible
CO_2 and O_2 control (option)	Range: 0 - 20% (CO_2); 1 - 19% (O_2) Control Resolution: ±0.1% (CO_2 and O_2) Stability: ±0.2% at 5% CO_2; ±0.2% at 1% O_2 Models for both CO_2 and O_2 or CO_2 only are available

Table 1.22 UV-Vis absorbance mode (403).

UV-Vis absorbance	
Light source	Xenon flash
Detector	Photodiode
Wavelength selection	Monochromator
Wavelength range	230 - 999 nm, 1 nm increments
Monochromator bandwidth	4 nm (230-285 nm), 8 nm (>285 nm)
Monochromator wavelength accuracy	±2 nm
Monochromator wavelength repeatability	±0.2 nm
Stray light	0.03% at 230 nm

Table 1.22 (cont.)

UV-Vis absorbance	
Reading speed (kinetic)	96 wells: 11 seconds
	384 wells: 22 seconds

Table 1.23 Fluorescence intensity mode (403).

Fluorescence intensity	
Light source	Xenon flash
Detector	PMT for monochromator system
	PMT for filter system
Wavelength selection	Quad monochromators (top/bottom)
	Filters (top)
Wavelength range	Monochromators: 250 - 700 nm (850 nm option), Filters: 200 - 700 nm (850 nm option)
Monochromator bandwidth	16 nm
Reading speed (kinetic)	96 wells: 11 seconds
	384 wells: 22 seconds

Table 1.24 Luminescence mode (403).

Luminescence	
Wavelength range	300-700 nm
Dynamic range	>6 decades

Table 1.25 Fluorescence polarization mode (403).

Fluorescence polarization	
Light source	Xenon flash
Detector	PMT
Wavelength selection	Filters
Wavelength range	280 - 700 nm

Table 1.26 Time-resolved fluorescence mode (403).

Time-resolved fluorescence	
Light source	Xenon flash
Detector	PMT
Wavelength selection	Quad monochromators (secondary mode), Filters (top) 280 - 700 nm
Wavelength range	Filters: 200 - 700 nm

2. Digital image colorimeter

In this research, digital image colorimeter for curcumin in turmeric and acrylamide for food determination were designed and fabricated as shown in Figure 1.13 and Figure 1.14, respectively. Figure 1.13, the photography lightbox was constructed from aluminum with the dimensions of 16 × 25 × 30 cm (width × length × height) and the internal walls were padded with black leather. The light source is flexible strip of super bright light emitting diodes (LEDs). The lightbox uses only 11.1 V lithium polymer (LiPo) battery (Wild Scorpion, Rohs, China). The switch on the side of the lightbox is used to increase or decrease the super bright light emitting diodes illumination. The image acquisition using the developed ColorConc2 application operating on iPod touch can be performed in order to obtain the images of sample solution placed in transparent 96-well microtiter plate. Figure 1.14, the photography lightbox was made from wood with the dimensions of 14.5 × 32.5 × 15 cm (width × length × height) and the internal walls were sprayed with black paint. The light source

is 2 bulbs of blacklight illumination (8 watt, Haichao, Thailand). The image acquisition using the developed ColorConc2 application operating on iPod touch can be performed in order to obtain the images of sample solution placed in black opaque 96- well microtiter plate.

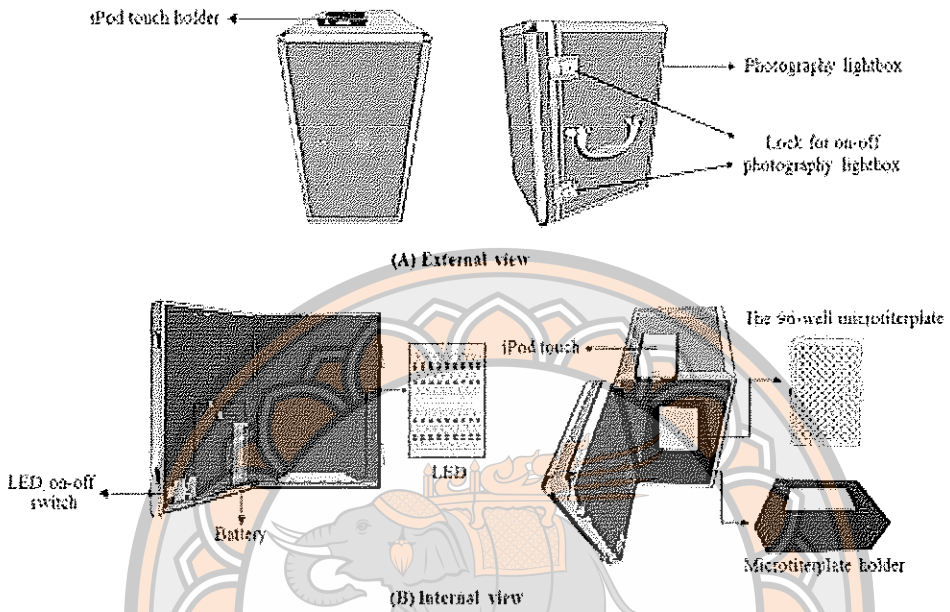


Figure 1.13 **Schematic diagram of the developed digital image colorimeter for curcumin detection; (A) external view and (B) internal view.**

Source: Wongthanyakram, et al., 2019 (404)

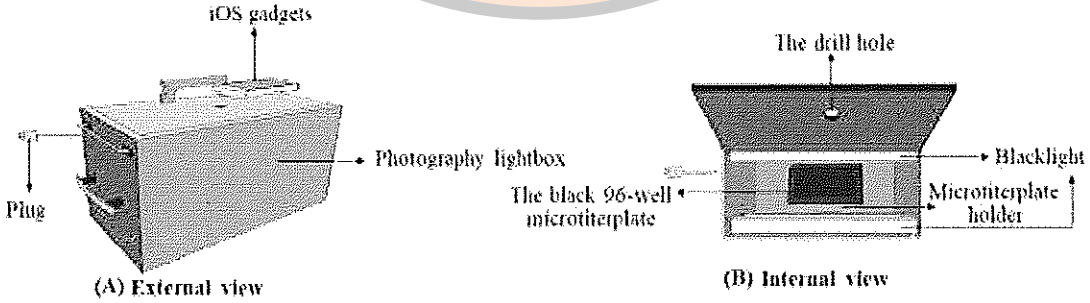


Figure 1.14 Schematic diagram of the developed digital image colorimeter for acrylamide detection; (A) external view and (B) internal view.

Determination of curcumin in turmeric and acrylamide in food samples

Nowadays, many scientists and organizations have concerned towards curcumin in turmeric and acrylamide in food samples, which has reinforced in the evolution of more precise, accurate, and sensitive methods for the determination of curcumin and acrylamide in wide range of samples as can be seen from literature reviews for curcumin and acrylamide detection. Research scope are as the following;

1. The digital image colorimeter for curcumin in turmeric and acrylamide in food sample was designed and fabricated. The size of photography lightbox, light position, type of light, and brightness of light for the determination of curcumin and acrylamide was optimized.

2. The analytical conditions for the determination of curcumin by digital image colorimetry such as solvents of curcumin, time and temperature of extraction, extraction method for determination of curcumin were optimized. The analytical conditions for the determination of acrylamide such as effect of NaOH and NaClO concentrations, effect of derivatization time and temperature, effect of fluorescamine concentration, effect of the buffer volume in derivatization procedure will be optimized.

3. The method validation of the proposed digital image colorimetry for the quantitative determination of curcumin in turmeric and acrylamide in food sample including linearity, limit of detection, limit of quantitation, % recovery, interday precision, and intraday precision will be evaluated. The results will be compared with ultraviolet-visible spectrophotometry using microplate reader and high performance liquid chromatography.

4. Turmeric samples for curcumin determination and snack, seasoning, and refreshment food samples for acrylamide determination will be detected with the propose digital image colorimeter and compared the results obtained with microplate reader and high performance liquid chromatograph.

Objective

1. To design and fabricate the digital image colorimeter for curcumin in turmeric and acrylamide in food samples determination.

2. To optimize the analytical parameters for the determination of curcumin and acrylamide by the developed digital image colorimeter.

3. To validate the proposed digital image colorimeter for the quantitative determination of curcumin in turmeric and acrylamide in food sample.

4. To determine the curcumin content in commercial turmeric samples and acrylamide content in commercial food samples by applying the proposed digital image colorimeter and comparing the results with ultraviolet-visible spectrophotometer using microplate reader and high performance liquid chromatography.

CHAPTER II

DESIGN AND FABRICATION OF THE DIGITAL IMAGE COLORIMETER FOR CURCUMIN IN TURMERIC SAMPLES DETERMINATION

Introduction

Thailand is a country endowed with a variety of medicinal plants and herbs with strong potentiality for treatment applications (49). Turmeric is an agent of the most popular medicinal herbs (23) as it has been used for family medicine for a numerous amount of time. Moreover, it has been used as an insect repellent and a food coloring agent (24). Turmeric belongs to *Zingiberaceae* family and it has various helpful properties such as antioxidant activities, treatment of several diseases (*e. g.* psoriasis, Alzheimer' s disease, and myelodysplastic syndrome) (405), and anti- human immunodeficiency virus cycle replication (406-407). Further medical applications of curcumin in food include muscle and skin anti-inflammatory, antimicrobial, antifungal, and anti-bacterial agents. Finally, the anti-carcinogenic (408-410) effects of curcumin resulted in its recent notable use among anticancer chemical compounds and as a preventive agent to minimize the risk of lung (411-412), duodenum, liver, and kidney cancers (413- 414). The essential active chemical compounds of turmeric are curcuminoids (curcumin, demethoxycurcumin, and bisdemethoxycurcumin). The chemical structures of curcuminoids are shown in Figure 1.1 (15).

Several techniques including nuclear magnetic resonance (NMR) (415), cyclic voltammetry (CV), differential pulse voltammetry (DPV) (416), ultra violet-visible spectrophotometry (UV–Vis) (416-417), and high performance liquid chromatography (HPLC) (15) have been used for the detection of curcumin content in turmeric samples. These techniques show high performance in terms of quantitative and qualitative analysis in various chemical analyses techniques. Anywise, these techniques utilize big and expensive tool and are inconvenient as they use off-site analysis. Consequently, this work proposes a rapid, high-throughput, low-cost, and on-site analysis method for the determination of curcumin in turmeric samples.

The previous developers who applying digital images for quantitative colorimetry used this technique for the detection of starch (418), urine (419), blue food dye, lemon-lime sports drink, and iron (III) chloride (420). Presently, digital image colorimetry is popularly used for the detection of many samples such as water (421-423), natural rubber latex and medical latex gloves (424), bovine milk (425-426), soil (427), and cassava samples (428). The method has excellent stoutness, high sensitivity, wide determination range, and easy operation. Thus, it is a good choice to be developed into a detector, which transforms the color value to concentration value. Nowaday, smartphones or smart devices are used for chemical analysis (423, 426-428). In some applications of chemical analysis, a smartphone or a smart device can replace the traditional tools and perform chemical determination. Using, the camera of a smartphone or a smart device for optical determination is one of the most common techniques in smartphone and smart device determination and it is applied in various fields such as fluorescent imaging (429), colorimetric determination (423, 426-428, 430), and microscopy imaging (431).

In the present work, a novel chemical analysis technique for the rapid determination of curcumin was developed by combining an iOS application named ColorConc2 and a portable colorimetric process. The application displays real-time image color value and concentration value. In addition, it operates data storage, data display, and instantaneous data sharing via e-Mail. Finally, the results from the smart device-based digital image colorimeter were compared with UV-Vis microplate reader, and HPLC. The smart device-based digital image colorimeter exhibits efficient, convenient, low-cost, and fast determination for simultaneous and immediate detection of curcumin in turmeric samples.

Experimental

1. Instrumentation

1.1 Smart device-based digital image colorimeter (smart device-based DIC)

A schematic of the smart device-based DIC (weight 1.5 kg) is shown in Figure 1.13. In order to prevent the system from outside light, a photography lightbox was made from aluminum (16 × 25 × 30 cm; width × length × height) with the internal

walls being padded with black leather. The developed ColorConc2 application working on a smart device (iPod touch; Apple Inc., USA) was utilized for capturing digital images from outside the lightbox via a drilled hole. The curcumin standard or sample solutions filled in the 96-well microtiterplate were placed on the microtiterplate holder made from black sponge placed on top of the microtiterplate holder made from white acrylic. A light source prepared from rows of flexible strips of daylight white light emitting diodes (LED) connected to the electric circuits was placed underneath the microtiter plate holder. When the lithium polymer battery (Wild Scorpion, Rohs, China) is plugged in (2200 mAh, which when fully charged provides 11.1 V), the photography lightbox can be closed and the daylight white LED switch on the side of the box can be turned on. To adjust the brightness of the light source, the Lux changeable switch located on the side of the box can be utilized in conjunction with the Light Meter application (whitegoods, available free on App Store) equipped on the smart device. When light intensity is 400 Lux, which is proper for image capture, the image acquisition using the ColorConc2 application can be performed in order to acquire the images of curcumin standard or sample solutions placed in the 96-well microtiterplate.

1.2 Ultraviolet-visible spectrophotometer (microplate reader)

All absorption spectrum recordings and absorbance measurements were performed on a double beam Synergy H1 Hybrid Multi-Mode Reader ultraviolet-visible spectrophotometer or microplate reader (BioTex, Vermont, USA) by monitoring the absorbance changes in the wavelength region 300-700 nm. The optimum wavelength for curcumin determination was established as 430 nm. A yellow soluble curcumin was presented in all samples. Sample solutions were measured at 430 nm for all curcumin concentrations.

1.3 High-Performance Liquid Chromatograph (HPLC)

Analysis was performed on a HPLC instrument, specifically an Agilent 1100 series that is installed with a photodiode array detector and autosampler. Data analysis was performed using Plumbagin software (Agilent Technologies, Waldbronn, Germany). The isocratic separation was performed on a VertiSep UPS C18 column (250mm×4.60mm internal diameter, 5μm) (Vertical Co., Ltd, Bangkok, Thailand). The column temperature was controlled at 33°C. The mobile phase consisted of acetonitrile and 2%v/v aqueous solution of acetic acid in the ratio of 40:60 v/v with a flow rate of 1

mL min^{-1}. The injection volume was 20 µL. The quantitation wavelength was selected as 430 nm.

2. ColorConc2 application

ColorConc2 was developed for smart devices using the iOS working system. Screenshots displaying how the application works are shown in Figure 2.1(A-D) and explained in detail below. ColorConc2 application consists of a "learning" and "testing" mode. It stores data in a local database so that multiple works can be maintained independently.

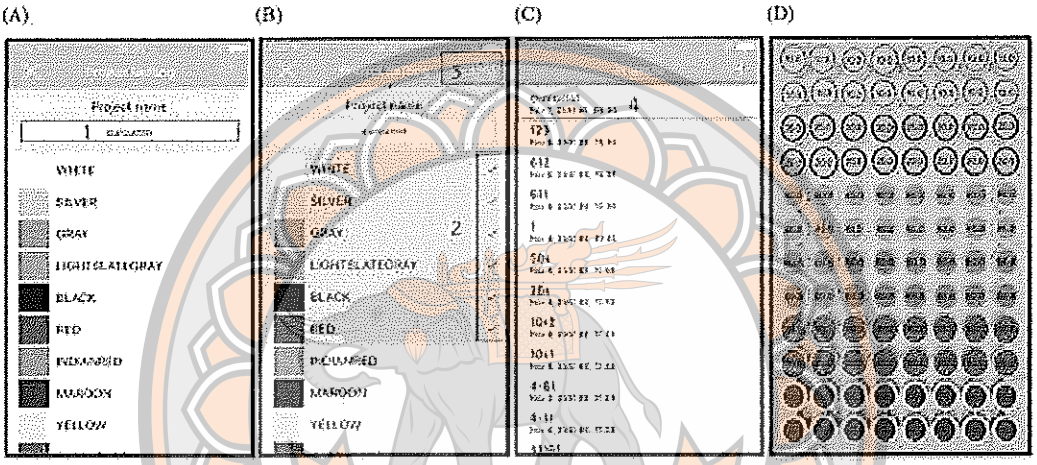


Figure 2.1 Screenshots of the ColorConc2 application used to quantify curcumin in turmeric samples.

Source: Wongthanyakram, et al., 2019 (404)

2.1 Learning mode

The learning mode calibrates the software with a set of images obtained from solutions with standard concentrations. Each image will be defined to a concentration level. Upon the establishing of a new work, the set of images will be empty. First, the user will establish a new work by establishing the name of the new work (Figure 2.1A). Then, the user will have to select a set of colors to represent the concentration levels (Figure 2.1B). The number of colors should be coincident to the number of standard concentrations in the learning set. After saving the work, it will become available from the project list (Figure 2.1C). Therefrom, the user will prepare the standard solutions and place them within the photography lightbox. Next, the user

will place the device into position such that the device camera is at the top of the photography lightbox and push down a button in the ColorConc2 application to take the photo. The image data is then passed through a Hough transform to determine the circular pits (432). For each determined pit in the 96-well microtiterplate, the image data is cropped to the inside area of the pit. The crop radius, calculated from Equation 2.1, is significantly less than the plate radius because the edges of the image contain shadows.

$$q = \frac{r}{2} \quad \text{...(Equation 2.1)}$$

where q is crop radius, r is plate pit radius.

After that, an average color value is calculated from the individual red, green, and blue color components of the pixels inner the crop radius. Red, green, and blue color values are calculated from Equation 2.2-2.10.

$$R_1 = \frac{R}{255}\text{; R from 0-255} \quad \text{...(Equation 2.2)}$$

$$\text{If } R_1 > 0.04045;\ R_2 = ((R_1+0.055)/1.055)\text{^}2.4 \quad \text{...(Equation 2.3)}$$

$$\text{or } R_1 \leq 0.04045;\ R_2 = R_1/12.92 \quad \text{...(Equation 2.4)}$$

$$G_1 = \frac{G}{255}\text{; G from 0-255} \quad \text{...(Equation 2.5)}$$

$$\text{If } G_1 > 0.04045;\ G_2 = ((G_1+0.055)/1.055)\text{^}2.4 \quad \text{...(Equation 2.6)}$$

$$\text{or } G_1 \leq 0.04045;\ G_2 = G_1/12.92 \quad \text{...(Equation 2.7)}$$

$$B_1 = \frac{B}{255}\text{; B from 0-255} \quad \text{...(Equation 2.8)}$$

$$\text{If } B_1 > 0.04045;\ B_2 = ((B_1+0.055)/1.055)\text{^}2.4 \quad \text{...(Equation 2.9)}$$

$$\text{or } B_1 \leq 0.04045;\ B_2 = B_1/12.92 \quad \text{...(Equation 2.10)}$$

Later, red, green, and blue color components are transformed to X, Y, and Z values (Equation 2.11-2.13). Next, an average L* a* b* value is calculated from Equation 2.14-2.22.

$$X = ((R_2*0.4124)+(G_2*0.3576)+(B_2*0.1805)) \quad \text{...(Equation 2.11)}$$

$$Y = ((R_2*0.2126)+(G_2*0.7152)+(B_2*0.0722)) \quad \text{...(Equation 2.12)}$$

$$Z = ((R_2*0.0193)+(G_2*0.1192)+(B_2*0.9505)) \quad \text{...(Equation 2.13)}$$

$$\text{If } (X/Xr) > 0.008856,$$

$$fx = (X/Xr)\text{^}(1/3);\ Xr = 0.95 \quad \text{...(Equation 2.14)}$$

$$\text{or } (X/Xr) \leq 0.008856,$$

fx = (903.3*(X/Xr)+16/116 ...(Equation 2.15)

If (Y/Yr) > 0.008856,

fy = (Y/Yr)^(1/3); Yr = 1.00 ...(Equation 2.16)

or (Y/Yr) ≤ 0.008856,

fy = (903.3*(Y/Yr)+16/116 ...(Equation 2.17)

If (Z/Zr) > 0.008856,

fz = (Z/Zr)^(1/3); Zr = 1.08 ...(Equation 2.18)

or (Z/Zr) ≤ 0.008856,

fz = (903.3*(Z/Zr)+16/116 ...(Equation 2.19)

CIE-L* = (116*fy)-16 ...(Equation 2.20)

CIE-a* = 500*(fx-fy) ...(Equation 2.21)

CIE-b* = 200*(fy-fz) ...(Equation 2.22)

The final step of the learning mode is for the user to explain the concentration value corresponding to the concentration of the curcumin standard solution in the photo (Figure 2.1D). The curcumin standard solutions are stored in a database.

2.2 Testing mode

The procedure of determining samples is similar to the procedure of the learning mode. The concentration of unknown samples will be estimated through the comparison with the database of images. Primary, the user takes a photo of the unknown samples under the same conditions as explained earlier. The sample pixels are automatically tested as per the learning mode, by cropping circle area of the pit in the 96-well microtiterplate and calculating the average L* a* b* values (Figure 2.2). To detect the concentration, the Euclidean distance algorithm is applied to find the closest match to the given image. The Euclidean distance algorithm is the distance between 2 points on a plane. The calculated L* a* b* values consists of 3 components, hence there are 3 dimensions in the Euclidean distance calculation (Equation 2.23).

$$\Delta E^*ab = \sqrt{(L_2^* - L_1^*)^2 + (a_2^* - a_1^*)^2 + (b_2^* - b_1^*)^2} \quad \text{...(Equation 2.23)}$$

where L*2 is L* of reference, L*1 is L* of sample, a*2 is a* of reference, a*1 is a* of sample, b*2 is b* of reference, and b*1 is b* of sample.

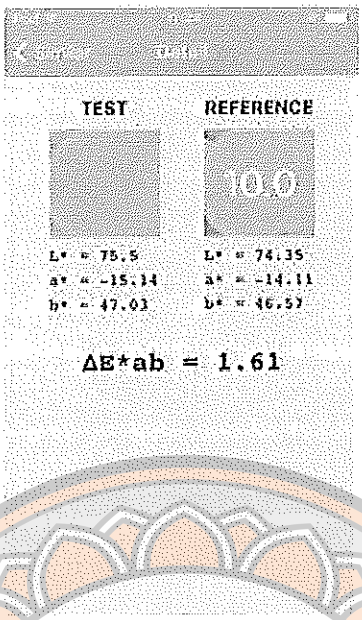


Figure 2.2 **The average L* a* b* values of unknown sample and reference.**

Source: Wongthanyakram, et al., 2019 (404)

In order to find the curcumin standard solution image with the closest match, a linear search algorithm is performed on the learning mode. For each curcumin standard solution image in the learning mode, the Euclidean distance is calculated. After the linear search algorithm has checked each curcumin standard solution image, the result of the algorithm will be the image with the lowest Euclidean distance to the unknown sample image. The concentration of the image with the lowest Euclidean distance is the estimated concentration of the unknown sample image.

3. Reagent and standard solutions

Stock standard solution (1000 mg L^{-1}) of curcumin was prepared by dissolving curcumin powder (from curcuma longa; turmeric, Sigma aldrich, Missouri, USA) in a 60: 40 (v/v) mixture of ethanol and hydrochloric acid (RCL Labscan, Bangkok, Thailand). The stock standard solution was stored in a wide mount amber reagent glass bottle. The working standard solutions were prepared daily by dilution with a 60:40 (v/v) mixture of ethanol and hydrochloric acid to create calibration standards with concentration of 10-100 mg L^{-1}.

To minimize contamination, all glassware was cleand and soaked at least 24 hours in 10% (v/v) of nitric acid, thoroughly rinsed with deionized water.

Method development

1. Design and fabrication the photography lightbox

The photography lightbox as shown in Figure 1.13 was designed to prevent the system from outside light and it was made from aluminum with the internal walls being padded with black leather. A drilled hole was utilized for capturing digital images from outside the photography lightbox. The photography lightbox can be closed and the daylight white LED or the Lux changeable switch on the side of the box can be turned on.

2. Optimization parameters for curcumin determination

The parameters of dissolution solvent, extraction time, ratio of ethanol and hydrochloric acid, number of extraction, and extraction method were studied in order to improve the determination efficiency.

3. Quantitative analysis of curcumin

Turmeric samples aged between 9-12 months were bought from 3 provinces; Phitsanulok, Nakhonsawan, and Phatthalung, which are the representative of north, middle, and southern parts of Thailand, respectively. Turmeric samples aged between 9-12 months are the age of use and the harvesting period. The extraction method is a method developed specifically for this research. Three samples from the 3 provinces were weighed (10 g) to make 5 replicates. The weighed samples were peeled, cut into small pieces, ground until fine, and weighed (1 g) into a beaker. Each sample was extracted using 10.00 mL of a 60:40 (v/v) mixture of ethanol and hydrochloric acid for 1 min. The resulting suspensions were filtered through No. 5 (110 mm Ø) Whatman filter paper, 1 mL of each sample solution was then transferred to a 10.00 mL volumetric flask and diluted to the mark with a 60:40 (v/v) mixture of ethanol and hydrochloric acid and used for subsequent smart device-based DIC, UV-Vis MR, and HPLC.

4. Analytical performance

Under optimized conditions, data for calibration curves for curcumin standard containing 10.00-100.00 mg L^{-1} were obtained from 1000 mg L^{-1} of curcumin stock standard solution. The linear regression equation relating green values with curcumin

concentrations was used to determine a limit of detection (LOD) and a limit of quantitation (LOQ). The precision (intraday and interday) are expressed as percentage relative standard deviation ± standard deviation (%RSD ± SD). The recoveries were evaluated by three different types of turmeric spiked with 1.0, 5.0, and 7.0 mg L^{-1} standard curcumin solution.

Results and discussion

1. Optimization of the photography lightbox

The parameters of optimization are listed in Table 2.1. The results are shown in Figure 2.3-2.10, respectively.

Table 2.1 Optimization parameters for photography lightbox.

Conditions Studied	Optimum Conditions
Size of the photography lightbox	16 × 25 × 30 cm (width × length × height)
Position of daylight white LED	Below of the photography lightbox
Number of daylight white LED	54 bulbs
Photography brightness	379 Lux
Suitable distance for shooting	8 cm
Magnification in photography	4x
Distance (in application)	7

1.1 Size of the photography lightbox

Size of the photography lightbox could have an effect on the image therefore two different sizes were fabricated: (A) 19 × 25 × 30 cm and (B) 16 × 25 × 30 cm (width × length × height) and the results are presented in Figure 2.3. Although the sensitivity (slope) and the correlation coefficients (R^2) of the green value corresponded with curcumin concentrations from both sizes are similar, the digital images of curcumin standard solutions (10.00-100.00 mg L^{-1}) when using the photography lightbox size (B) are brighter than that obtained when using size (A). Therefore, the box size (B) was chosen for the next study.

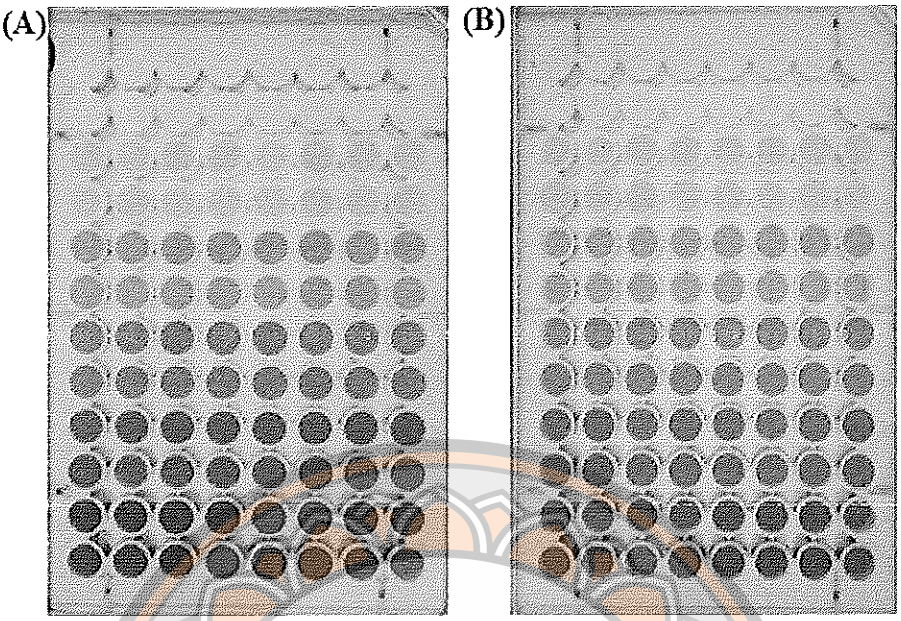


Figure 2.3 **The digital images of curcumin standard solutions when using the photography lightbox size 19 × 25 × 30 cm (A) and 16 × 25 × 30 cm (B) (width × length × height).**

1.2 Position of daylight white LED

The light illumination is critical to the color of an image of curcumin solution. The position of daylight white LED was studied in 2 points; (A) at the top and (B) at the below of the photography lightbox. The results from Figure 2.4 reveal that the image obtained from position (A) has a reflected light on the center of an image giving not homogeneous image. It was also found that beside of image obtained from position (A) is darker than middle of image. The best image was obtained by photographing in the position (B) which gave true color of curcumin solution. Therefore, the set-up of daylight white LED position at the below of the box was selected as the optimum position.

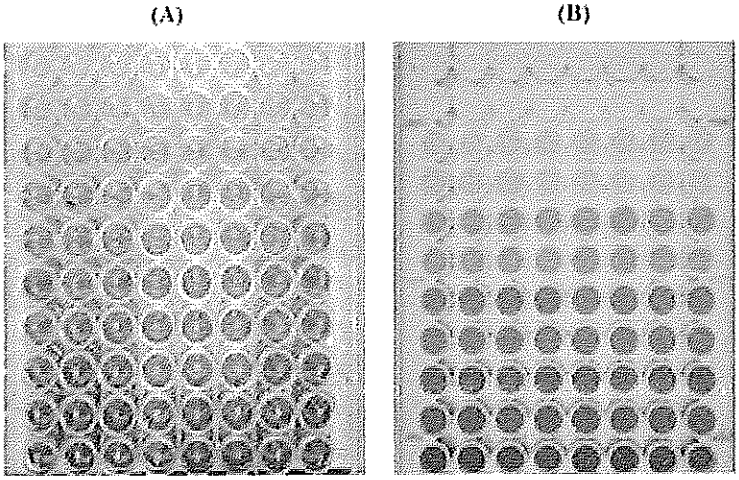


Figure 2.4 **The digital image of curcumin standard solutions obtained when setting daylight white LED (A) at the top and (B) at the below of the photography lightbox.**

1.3 Number of daylight white LED

Number of daylight white LED could have an effect on the image thus it was studied at: (A) 24, (B) 36 and (C) 54 bulbs. The image obtained when using 54 bulbs of LED provide a homogeneous image due to lighting throughout the 96-well microtiterplate as shown in Figure 2.5 (C).

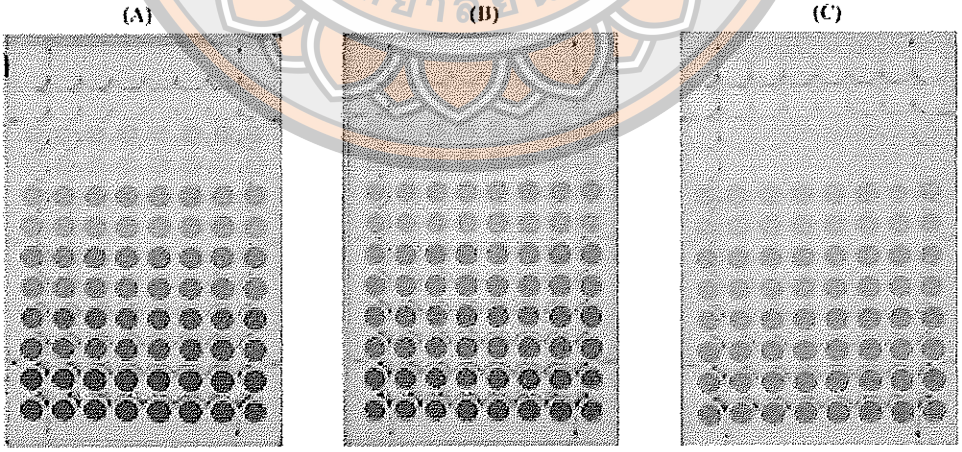


Figure 2.5 **The digital image of curcumin standard solutions obtained when using three different number of daylight white LED; (A) 24, (B) 36, and (C) 54 bulbs.**

1.4 Photography brightness

The results in Figure 2. 6 showed that the digital images of curcumin standard solutions when using 379 Lux is the same as brightness those obtained when using 592 Lux, 897 Lux and 1. 15 kLux. In addition, processing of application when using 1. 15 kLux are incorrect because the applications cannot specify the correct concentration. Processing through the application of curcumin standard solutions obtained from 379, 592, and 897 Lux are correct and the application can specify the correct concentration. Hence, the 379 Lux was chosen for the next study because it could save more battery.

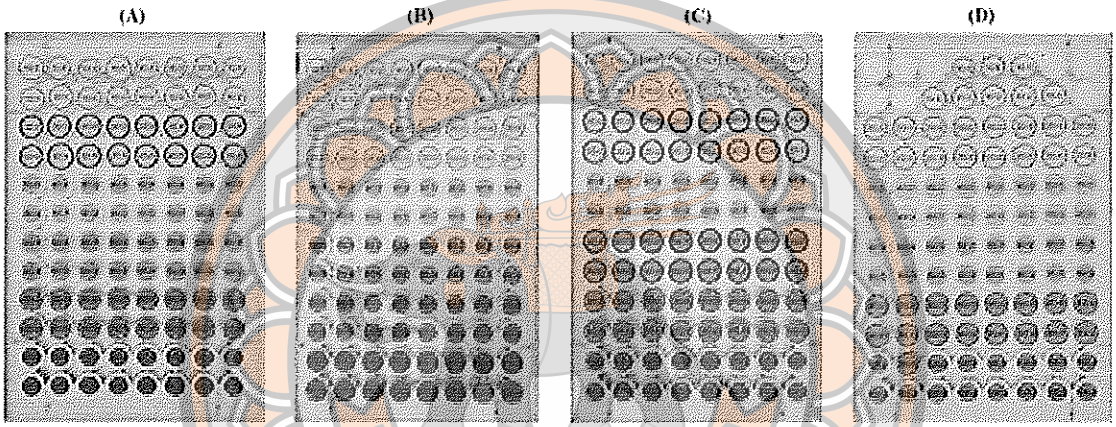


Figure 2.6 **The digital image of curcumin standard solutions obtained when using four different photography brightness; (A) 379, (B) 592, (C) 897 Lux, and (D) 1.15 kLux.**

1.5 Suitable distance for shooting

Suitable distance for shooting could have an effect on the image therefore ten different distance were studied: (A) 2, (B) 4, (C) 6, (D) 8, (E) 10, (F) 12, (G) 14, (H) 16, (I) 18, and (J) 20 cm. The results in Figure 2.7 indicated that the image obtained from distance = 8 cm has a reflected light on the center of an image less than other distance. Thus, 8 cm is the optimum distance for photography.

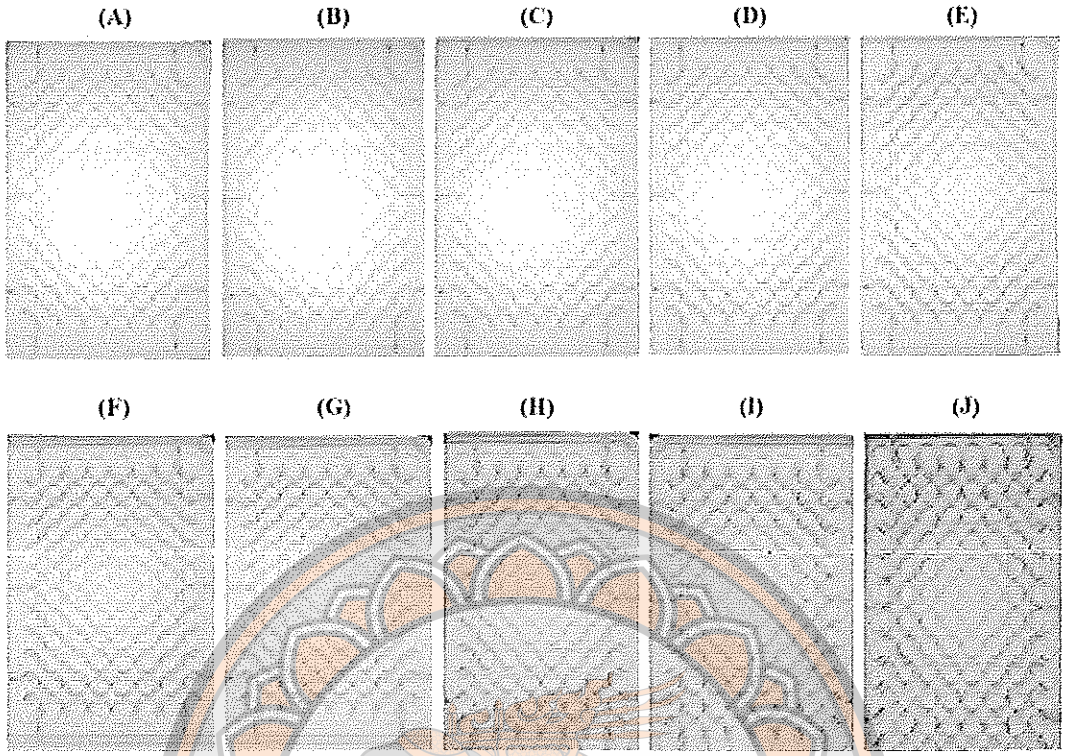


Figure 2.7 **The digital image of blank microtiterplate obtained when using ten different distances for photography; (A) 2, (B) 4, (C) 6, (D) 8, (E) 10, (F) 12, (G) 14, (H) 16, (I) 18, and (J) 20 cm.**

1.6 Magnification in photography

The magnification in photography could have an effect on the image accordingly five different magnification were studied: (A) 1X, (B) 2X, (C) 3X, (D) 4X and (E) 5X. The results as shown in Figure 2.8 indicated that the digital images of curcumin standard solutions when using 1X-3X are inappropriate because the distance of the camera to focus are inappropriate. Thus, processing of application is incorrect. The digital images of curcumin standard solutions when using 5X is also inappropriate because the camera cannot focus fully on the 96-well microtiterplate. Hence, the magnification of 4X was chosen for photography with the applications in this research, because the camera can focus fully on the 96-well microtiterplate.

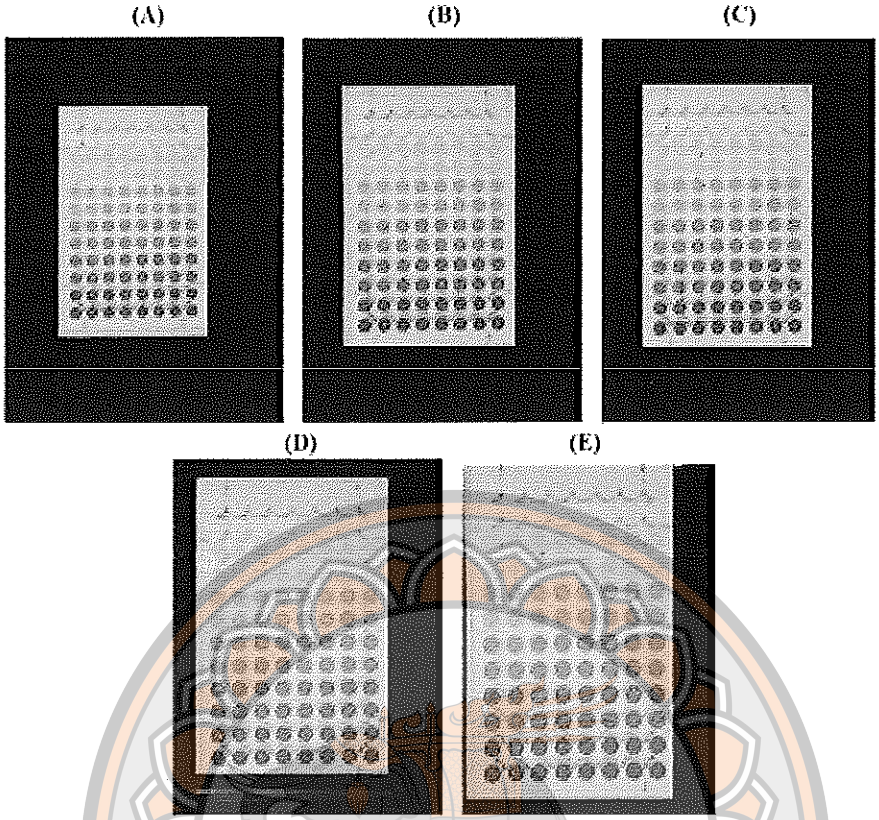


Figure 2.8 **The digital image of curcumin standard solutions obtained when using five different magnification in photography; (A) 1, (B) 2, (C) 3, (D) 4, and (E) 5X.**

1.7 Distance (in application)

Distance (in application) provides good retrieval accuracy and it is adopted for computation. In ColorConc2 application, distance is Euclidean distance calculation (Equation 2.23) as explained in section 2.2. Distance value which approached 1 shows that the application has a very color differences while distance value which reached 30 indicates that the application has a little color differences. The distance in application could have an effect on the image therefore eight different distance were studied: (A) 1, (B) 3, (C) 5, (D) 7, (E) 10, (F) 12, and (G) 14. Processing of the application when using 1, 3, 5, 10, 12, and 14 are incorrect because the applications cannot specify the correct concentration. Processing of the application when using distance = 7 is correct because the application can specify the correct concentration as shown in Figure 2.9.

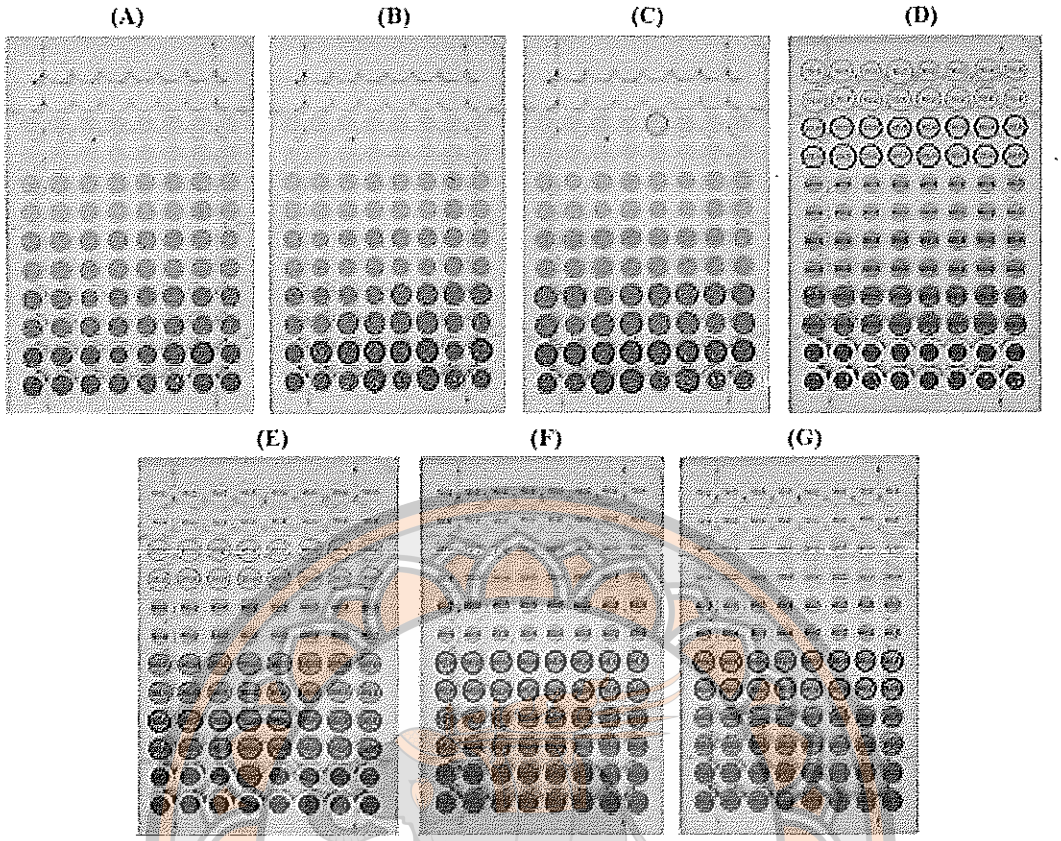


Figure 2.9 **The digital image of curcumin standard solutions obtained when using seven different distance; (A) 1, (B) 3, (C) 5, (D) 7, (E) 10, (F) 12, and (G) 14.**

2. Optimization for curcumin determination

The parameters for optimization are listed in Table 2.2. The results are shown in Figure 2.10-2.13 and Table 2.3, respectively.

Table 2.2 Optimization parameters for curcumin determination.

Conditions studied	Optimum conditions
Dissolution solvent	Ethanol and hydrochloric acid
Ratio of ethanol and hydrochloric acid	60:40 (v/v)
Extraction time	1 min
Sample preparation	Ground until fine
Number of extraction	1 time
Extraction method	Soaked with solvent

2.1 Dissolution solvent

The selection of dissolution solvent used in the smart device-DIC is an important parameter that affects the extraction efficiency. Two types of dissolution solvents were studied; ethanol and mixture of ethanol and hydrochloric acid. The results from Figure 2.10 reveal that mixture of ethanol and hydrochloric acid helps to increase the yellow color of the solution when curcumin concentration was increased. Curcumin dissolves well in ethanol, however, ethanol is very volatile, therefore hydrochloric acid was used in a mixture with ethanol for the extraction. In addition, hydrochloric acid helps to reduce the volatility of ethanol.

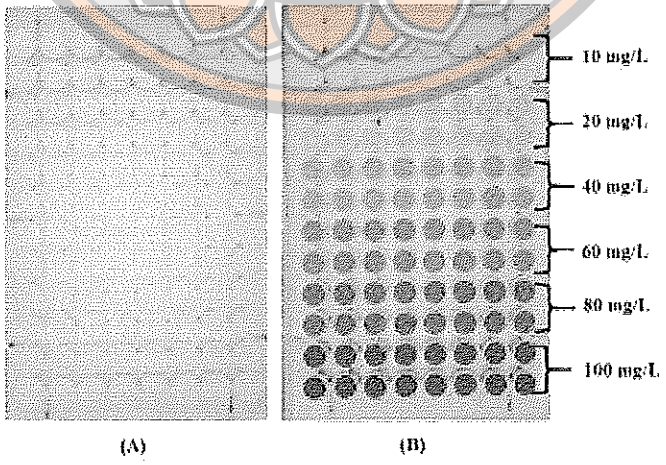


Figure 2.10 The digital image of curcumin standard solutions obtained when using two types of dissolution solvents; (A) ethanol and (B) mixture of ethanol and hydrochloric acid.

2.2 Ratio of ethanol and hydrochloric acid

The ratio of ethanol and hydrochloric acid was studied at 50:50, 60:40, 70:30, 80:20, 90:10, and 0:100 (v/v). It was found that 60:40 (v/v) resulted in darker-yellow curcumin solutions than the other ratio as shown in Figure 2.11. Furthermore, when curcumin concentration was increased, the yellow color will increase at this proportion of ethanol and hydrochloric acid. Other ratios of ethanol and hydrochloric acid result in the color of the curcumin extracts not being suitable for calculation by the developed application. So, the appropriate ratio of ethanol and hydrochloric acid was found to be 60:40 (v/v).

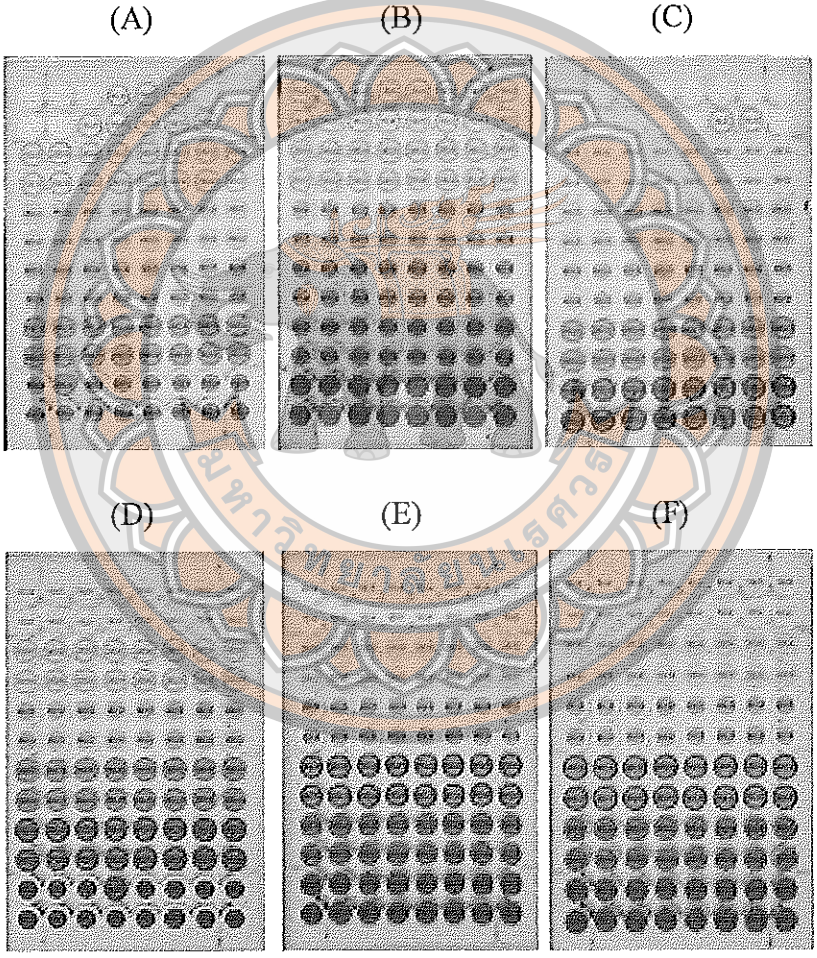


Figure 2.11 The digital image of curcumin standard solutions obtained when using six ratios of ethanol and hydrochloric acid; (A) 50:50, (B) 60:40, (C) 70:30, (D) 80:20, (E) 90:10, and (F) 0:100 v/v.

2.3 Extraction time

Extraction time is a considerable effect on extraction efficiency and an appropriate extraction time is therefore necessary to be investigated. The results as shown in Figure 2.12 demonstrated that absorbance values steadied when the extraction time was increased. Consequently, 1 min extraction time was employed for this study, due to maintaining a fast process.

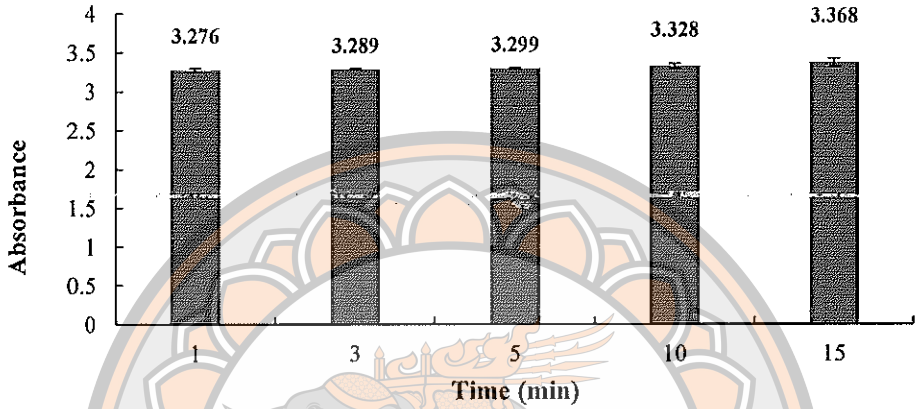


Figure 2.12 Effect of extraction time on the absorbance of curcumin solutions.

2.4 Sample preparation

The sample preparations for curcumin in turmeric samples was studied. The turmeric samples were peeled, cut into small pieces and ground until fine. The results as shown in Figure 2. 13 indicated that absorbance values increased when turmeric samples were ground. Consequently, turmeric samples were ground until fine for this study due to increasing the area of the extraction.

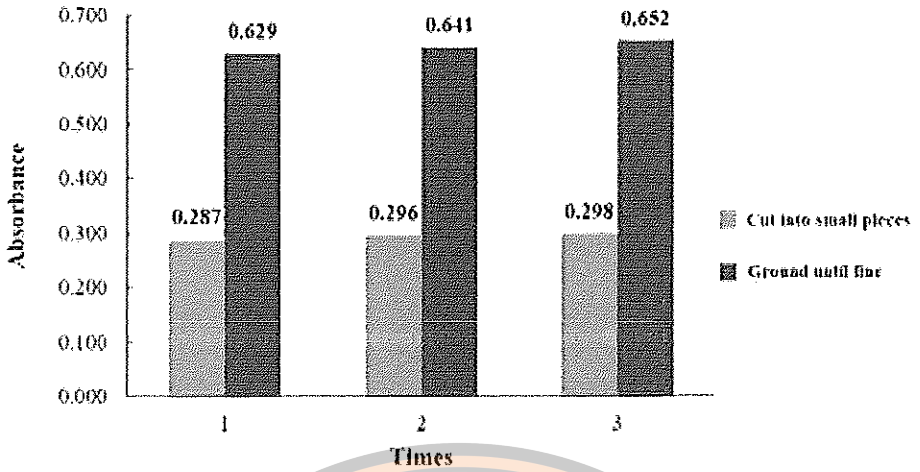


Figure 2.13 Effect of sample preparation on the absorbance of curcumin solutions.

2.5 Number of extraction

Repetition could apparently affect the extraction efficiency, consequently, the effect of extraction number was studied within the range of 1-10 times. The results were described in Figure 2. 14 that the absorbance values remained steady at 1-7 extraction times but the absorbance values decreased a little at 8-10 extraction times. Therefore, 1 time of extraction was selected as the optimum number for further studies due to a fast preparation time.

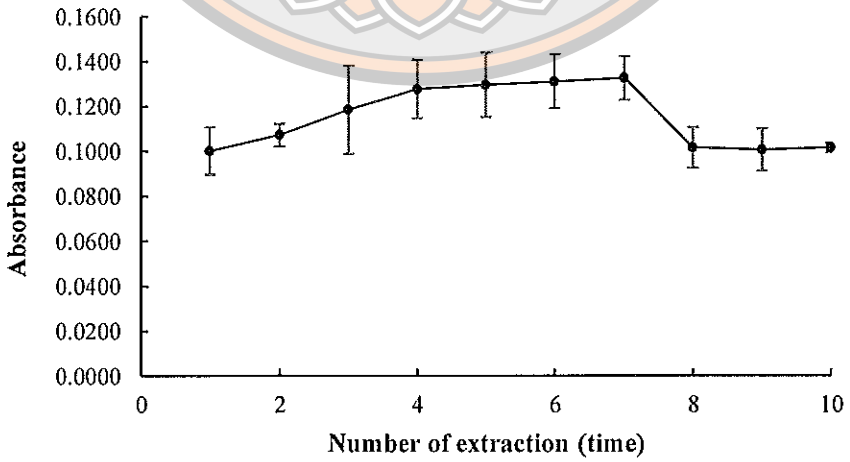


Figure 2.14 Effect of extraction number on the absorbance of curcumin solutions.

2.6 Extraction method

The turmeric samples were peeled, cut into small pieces, ground until fine, and weighed about 1 g into a beaker. Each sample was extracted using vortex mixer, ultrasonic bath, and leaving at a room temperature. The results were described in Table 2.3 which the absorbance values remained steady for overall experiment. Therefore, leaving at room temperature was selected as the optimum method for further studies to save energy.

Table 2.3 Effect of extraction method on the absorbance values of curcumin solution.

Time	Absorbance values		
	Vortex mixer	Ultrasonic bath	Leaving at a room temperature
1	0.557	0.629	0.596
2	0.569	0.641	0.613
3	0.566	0.652	0.614
Average	0.564	0.641	0.608

3. Quantitative analysis of curcumin

3.1 Quantitation by UV-Vis MR and HPLC

The comparison of spectrum of curcumin extracted from turmeric sample, 10 mg L^{-1} standard curcumin sample, and turmeric sample spiked with 10 mg L^{-1} standard curcumin is shown in Figure 2.15. The calibration curves for curcumin standard solutions were linear over the concentration range of 1.00-10.00 mg L^{-1}, with correlation coefficients (R^2) of 0.9997 and 0.9998 for UV-Vis MR and HPLC, respectively. The results for the determination of aqueous extractable curcumin from 3 different sources by UV-Vis MR and HPLC are presented in Table 2.4. Moreover, the average percentage recoveries of curcumin from turmeric sample spiked with concentrations of 1.0, 5.0, and 7.0 mg L^{-1} of standard curcumin were determined, and it was found that the average percentage recovery was acceptable as illustrated in Table 2.5.

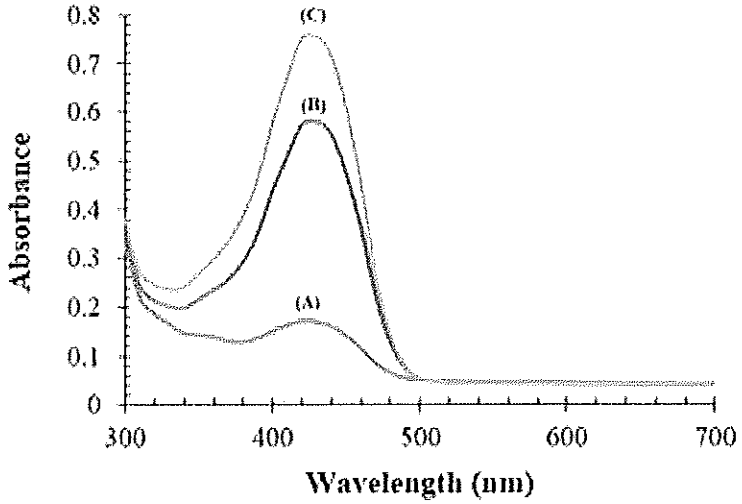


Figure 2.15 The spectra of (A) curcumin extracted from turmeric sample, (B) 10 mg L^{-1} standard curcumin, and (C) turmeric sample spiked with 10 mg L^{-1} standard curcumin obtained by UV-Vis MR.

Table 2.4 Results of curcumin assay in turmeric samples determined using the developed smart device- based digital image colorimeter (smart device-based DIC) and compared with Ultra Violet-Visible microplate reader (UV–Vis MR) and high performance liquid chromatograph (HPLC).

Source of turmeric sample	Curcumin Found (mg g^{-1}, n=5)		
	Smart device -based DIC	UV-Vis MR	HPLC
Phitsanulok	5.00±1.33	5.49±1.32	5.52±0.25
Nakhonsawan	4.97±0.65	5.30±1.96	4.73±1.00
Phatthalung	4.95±0.05	5.33±1.21	5.74±0.62

3.2 Quantitation by smart device-based DIC

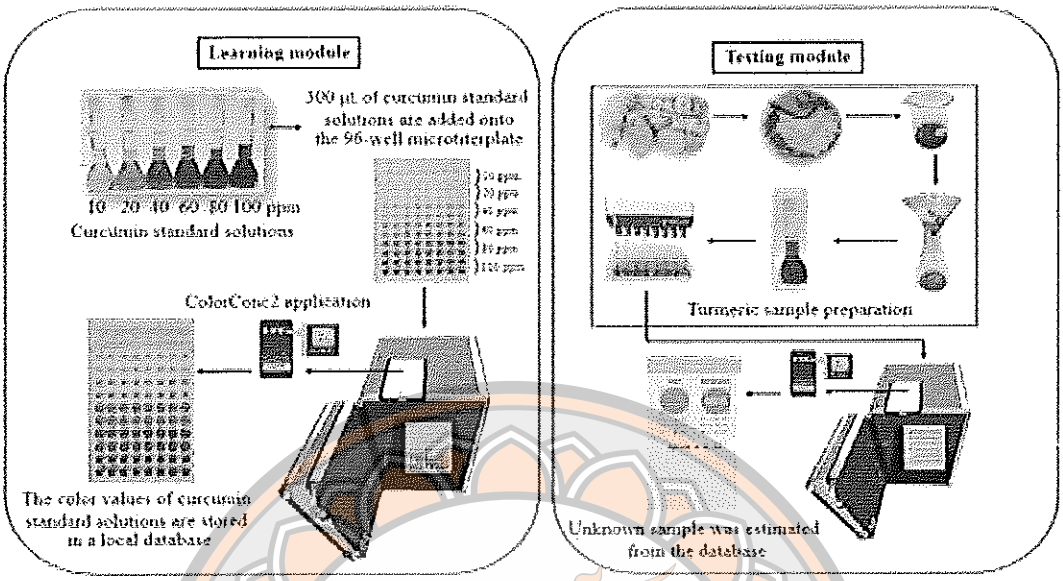


Figure 2.16 **The workflow schematic of curcumin measurement using the developed smart device-based digital image colorimeter (smart device-based DIC).**

Figure 2. 16 demonstrates the workflow schematic of curcumin measurement using the smart device-based DIC. Solutions with standard curcumin concentrations 10.0-100.0 mg L^{-1} were filled to the 96-well microtiterplate then the image of the microtiterplate was captured, and the color values were recorded in the database. All of the data in the database was used for estimating the curcumin concentration of the unknown solutions. Consequently, the unknown solutions were prepared for images capturing and analyzing the color values by the ColorConc2 application. Curcumin concentrations of the unknown extracted solutions were immediately reported on the screen of the smart device-based DIC. Another option for concentration calculation is to transfer the color values of each standard curcumin solution to Microsoft excel program. In addition, the graph plotted between standard curcumin concentrations and color values as shown in Figure 2. 17 was used for quantitative calculations reported in Table 2.4-2.5. The result in Figure 2.17 showed that the green value decreases with increasing standard curcumin concentration. The

green value was, therefore, used to calculate the limit of detection (LOD) and limit of quantitation (LOQ).

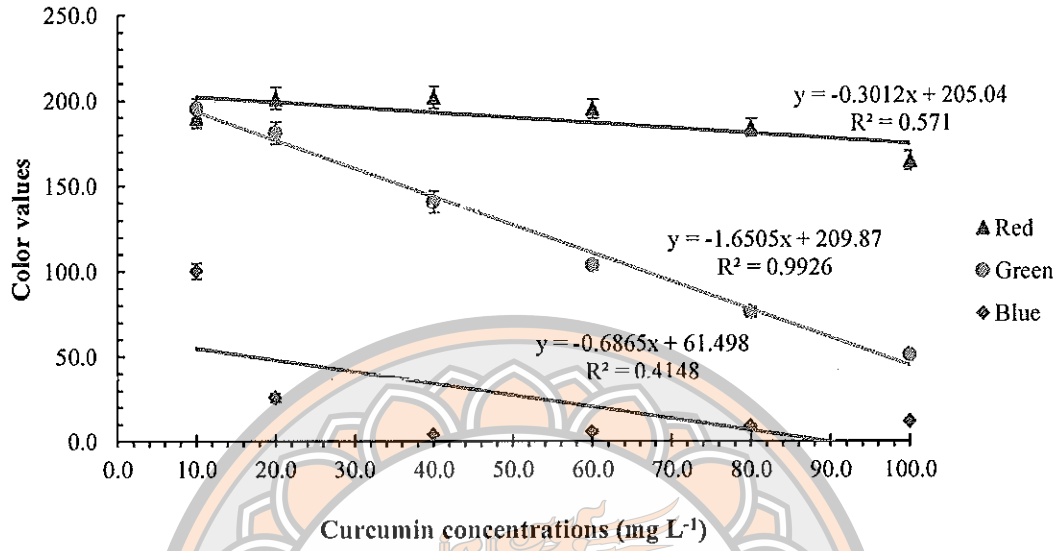


Figure 2.17 Correlation between curcumin concentration and color components (red, green and blue).

4. Analytical performance of smart device-based DIC

The calibration curve was linear over the concentration range of 10.0-100.0 mg L^{-1}, with a correlation coefficient (R^2) of 0.9926. Three different types of turmeric spiked with 1.0, 5.0, and 7.0 mg L^{-1} standard curcumin solution were determined by the smart device-based DIC. It was found that the recoveries are in the range of 98.57-110.0% as shown in Table 2.5. These results demonstrate that the developed method has a good potential for the determination of curcumin in real samples.

Table 2.5 **Recoveries study of curcumin in turmeric performed utilizing the developed smart device- based digital image colorimeter (smart device-based DIC), Ultra Violet-Visible microplate reader (UV–Vis MR), and high performance liquid chromatograph (HPLC).**

Method	Standard curcumin Added (mg L^{-1})	Curcumin Found (mg L^{-1}, n=5)	%RSD	%Recovery
Smart device	0.00	59.2±0.80	1.35	-
-based DIC	1.00	60.3±0.10	0.15	110.0
	5.00	64.4±0.54	0.84	104.0
	7.00	66.1±0.33	0.50	98.57
UV-Vis MR	0.00	1.21±0.01	0.51	-
	1.00	2.02±0.06	3.18	81.00
	5.00	7.13±0.03	0.41	118.4
	7.00	9.07±0.01	0.12	112.3
HPLC	0.00	1.25±0.01	0.73	-
	1.00	2.18±0.06	2.60	93.00
	5.00	6.90±0.05	0.71	113.0
	7.00	8.51±0.05	0.62	103.7

The repeatability (intraday precision) was investigated by performing five replicate analyses for each concentration on the same day but different times (n=5) and the intermediate precision (interday precision) was performed for each concentration on five different days (n=5). The precision is expressed as percentage relative standard deviation ± standard deviation (%RSD ± SD). It was found that %RSD ± SD values for intraday and interday precision were 0.41% ± 0.01 and 0.38% ± 0.01, respectively. The low values of %RSD reflect the high precision of the method. The low error rates imply that a single calibration can be used for more than one day (i.e. the system does not need to be calibrated every day). The linear regression equation relating green values with curcumin concentrations was used to determine the LOD and LOQ and were found to be 0.48 and 1.61 mg L^{-1}, respectively. These results suggest that the method provides

adequate sensitivity for curcumin assay in turmeric. A comparison of the percentage recoveries obtained with the three methods can be seen in Table 2.5. The concentration of curcumin found in the blank sample (reported in mg L^{-1}) determined by our proposed technique was different from those obtained by the UV-Vis MR and HPLC techniques. This is due to the difference in dilutions performed before determination by the three techniques (10 times dilution for smart device-based DIC but 500 times dilution for UV-Vis MR and HPLC) and the linearity range used (10.0-100.0 mg L^{-1} for smart device-based DIC but 1.00-10.00 mg L^{-1} for UV-Vis MR and HPLC). However, when the concentration is calculated and reported using the mg g^{-1} units, the curcumin concentration found by the proposed method is not different from those found by the comparison methods. The results from the determination of aqueous extractable curcumin from the three methods are presented in Table 2.4. The single factor ANOVA was applied for the comparison of the proposed method with the other two methods and it was found that the results obtained from the three techniques showed positive agreement with no statistically significant difference at the 95% confidence level. The amount of curcumin in turmeric collected from 3 provinces at 5 different times were found to be in the range of 4.73-5.74 mg g^{-1}. Furthermore, the application on a smart device offers a rapid, sensitive, precise, reliable, and simple procedure for the determination of analytes, which have intrinsic color like curcumin or analytes, which can form colored complex solutions. Moreover, the analysis of other types of turmeric samples, such as turmeric capsules or turmeric powder, can be studied as well. These samples can be analyzed with this technique but it should be taken into account that the matrix in these samples can affect the analysis.

Conclusions

In this research work, we report the development of a portable smart device-based DIC system for the quantification of curcumin in turmeric samples. The analysis is carried out after extraction with a 60:40 (v/v) mixture of ethanol and hydrochloric acid. The advantage of this method is its rapidity as the calibration curve needs to be measured only once using ColorConc2 application and can be used in all subsequent measurements. The limitation of this technique is that the uniformity of the photographic conditions such as option of the smart device, size of photography lightbox, distance,

and brightness for image recording need to be ensured at all times. However, the evidence suggests that the proposed closed-box system is sufficient for maintaining the conditions without recalibration from day to day. Albeit the size and price of the smart device- based DIC are many- fold smaller than for a commercial equipment, the developed equipment provides comparable analytical performance. This method is generalizable to any device that can capture a reasonably good digital image such as smart mobile devices (android or iOS system). Nevertheless, when a researcher uses a given device, this device needs to be used throughout the experiment.

CHAPTER III

FLUOROMETRIC DETERMINATION OF ACRYLAMIDE IN SNACK, SEASONING, AND REFRESHMENT FOOD SAMPLES WITH AN iOS GADGETS-BASED DIGITAL IMAGE COLORIMETER

Introduction

In 1994, the International Agency for Research on Cancer (IARC) designated acrylamide as probably carcinogenic to humans (247). Since then food security has received increasing amount of attention, for example, its toxicity, identification, degradation, dietary exposure, and metabolism have been widely studied (214, 375, 380, 396, 433). In 2002, researchers reported that carbohydrate-rich foods contain relatively high levels of acrylamide as a result of being baked and fried at high temperatures which above 120 °C. The maximum formation peaks for acrylamide happen at temperatures between 160-180°C through interaction of amino acids, especially asparagine, with reducing sugars like glucose (218, 375). Many foods contain high levels of acrylamide, such as processed cereal, toasts, bread, snacks, cookies, biscuits, breakfast cereals, chocolates, baby foods, French fries, steamed buns, coffee, fired chicken rolls, cakes, potato flour, semolina, banana chips, and potato and corn products. (370, 379, 433-435). Acrylamide can be absorbed by animals and humans via ingestion, through the skin, or inhalation, and it can be rapidly distributed to all tissues and found mainly in liver, brain, heart, placenta, kidneys, and in breast milk. Acrylamide and glycidamide can bind to DNA, hemoglobin, enzymes, and serum albumins and can produce neurotoxic effects in animals and humans. Typical symptoms of acrylamide exposure include skeletal and ataxia muscle weakness (214). Therefore, many researchers have turned their attention to the detection of acrylamide in food samples.

Based on literature review, mass spectrometry (MS) coupled with gas chromatography (GC) is the most commonly used technique for the determination of acrylamide (306, 370, 375, 388). Although derivatization of the target analyte in GC-MS can increase the sensitivity of acrylamide determination, the disadvantages are time-

consuming and inconvenient derivatization process. Likewise, mass spectrometry coupled with liquid chromatography (LC) is also utilized for acrylamide determination (345, 436-438). LC-MS techniques have received more attention for the quantitative analysis of acrylamide due to the fact that the analysis can be carried out without derivatization steps and it exhibits high sensitivity. However, bothersome operations as well as high cost of instrumentation and maintenance limit the application of this technique. Capillary electrophoresis (CE) is another chromatographic technique which has been used for acrylamide determination (332, 439-442). The advantages of CE include minimized volumes of samples, the short time of analysis, and the fact that complex cleanup of samples prior to the instrumental analysis is not required. Nevertheless, CE exhibits similar disadvantages as LC-MS. Furthermore, GC-MS, LC-MS, or CE are not suitable for convenient and rapid on-site detection of acrylamide. Fluorescence is very interesting for quantitative analysis of acrylamide due to its high sensitivity and rapidity but there are only three literature reports for acrylamide determination using fluorescence (334, 394, 443). In this research, we selected to follow Liu et al. (334) because Hu et al. (443) utilized a complex procedure including polymerization of acrylamide and preparation of functional quantum dots with N-acryloxysuccinimide, whereas, Asnaasharia et al. (394) have to synthesize water resuspended gold nanoparticles equipped with DNA. Both of these techniques are complicated and time-consuming. On the other hand, Liu et al. (334) only utilized Hofmann reaction in which acrylamide is degraded to vinyl amine, and pyrrolinone is produced when the vinyl amine reacts with fluorescamine which is expensive. Therefore, in this research, researcher chose to use fluorescein instead of fluorescamine because fluorescein is about 10 times cheaper than fluorescamine. It is the novelty of this research. Moreover, researcher found that it can be used interchangeably because Hofmann reaction is the key step for the fluorescence determination of acrylamide (444).

Nowaday, smartphones, smart gadget, or smart devices are being used for the analysis of chemical (423, 426-428). Using the camera of smartphone, smart gadget, and smart device for optical measurement is one of the most common techniques in smartphone, smart gadget, or smart device detection and it is practical in many fields such as colorimetric detection (427, 430, 445-447), microscopy imaging (431), and

fluorescent imaging (429, 431, 448). In this research work, which uses acrylamide-derivatization depended on Hofmann reaction (444) producing vinyl amine, which in turn reacts with fluorescein, we propose easy, quick, convenient, and low-cost an iOS gadgets- based DIC (a new laboratory- made portable colorimetric system) , in combination with ColorConc2 iOS application (404) , method for acrylamide determination in food samples. Concluding, the results obtained from an iOS gadgets-based digital image colorimeter are compared with measurements obtained using fluorescence microplate reader (fluorescence MR), and HPLC. An iOS gadgets-based digital image colorimeter exhibits low- cost, convenient, and rapid detection for instantaneous and synchronous determination of acrylamide in food samples.

Experimental

1. Instrumentation

1.1 An iOS gadgets-based digital image colorimeter (iOS gadgets-based DIC)

A schematic of a laboratory made iOS gadgets- based digital imaging colorimeter (under petty patent, Thailand, 2019) (300$, weight 1.0 kg) is depicted in Figure 1.14. The photography lightbox was made from wood and has the following dimensions: 14.5 × 32.5 × 15 cm (width × length × height). The internal walls were smeared with black paint. An iOS gadget (iPod touch; Apple, USA) which has ColorConc2 iOS application (404) was utilized for images capturing from outside the lightbox via the drilled aperture. The acrylamide standard and sample solutions filled in the black 96- well microtiterplate (Flat bottom, China) , which was located in microtiterplate holder made from wood, was photographed under 2 bulbs of blacklight illumination (8 watt, Haichao, Thailand). The screenshots, which demonstrate how the application operates, are shown in Figure 3.1. The application consists of a calibration or 'learning' module and a 'testing' module. These modules were explained in section of experimental of chapter 2.

Briefly, a series of acrylamide standard solutions with concentration of 1.00-10.0 mg L^{-1} were photographed and color values were stored in the database on ColorConc2 iOS application (learning module). Color values in the database were used to estimate acrylamide concentration of extracted food sample solutions. The

ColorConc2 iOS application uses all 3 components (red, green, and blue) to find the closest match by Euclidean distance theory. The color values of the image with the lowest Euclidean distance is the estimated acrylamide concentration of food samples image (testing module).

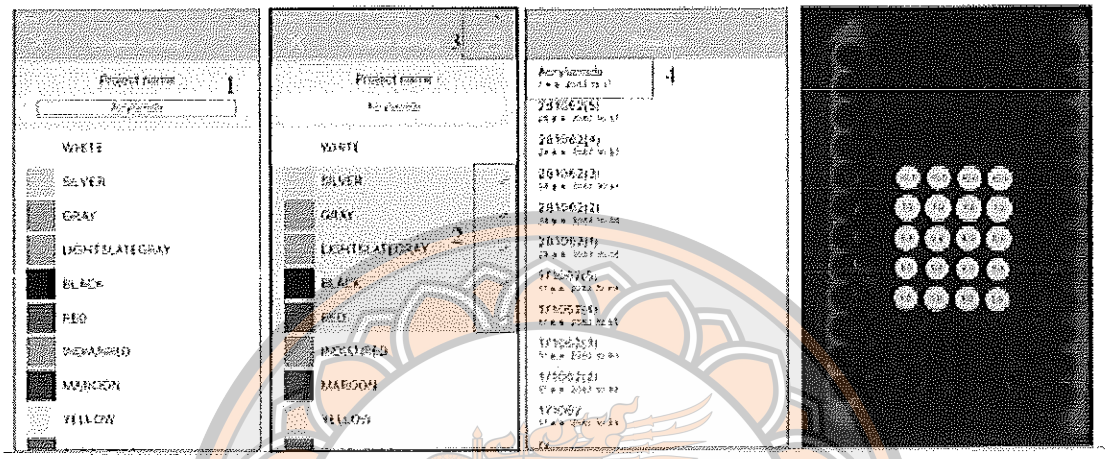


Figure 3.1 **Screenshots of the ColorConc2 application used to quantify acrylamide in food samples.**

1.2 Fluorescence microplater reader

All fluorescence emission spectra were recorded and measured using the double beam Synergy H1 Hybrid Multi-Mode Reader Fluorescence MR (BioTex, USA) by monitoring the spectral changes in the wavelength range 300- 700 nm. The fluorescence intensity of fluorescein, after reaction with decomposed acrylamide, was measured at 590 nm with the excitation wavelength at 470 nm.

1.3 High-Performance Liquid Chromatograph (HPLC)

Comparative measurement which used without the derivatization steps was carried out using HPLC instrument. The HPLC system (Agilent Technologies, Germany) equipped with an autosampler and a photodiode array detector. The enhancers separation was performed using a VertiSep UPS C18 column (250 mm x 4.60 mm, 5 μm) (Vertical Co., Ltd, Thailand) and the column was controlled at 30°C. The mobile phase was a mixture of acetonitrile and 0.1% formic acid in water (10:90) at

flow rate of 0.5 mL min^{-1}. 20 µL was used as the injection volume. The wavelength for acrylamide quantification was selected at 210 nm.

2. Reagent and standard solutions

The stock solution of acrylamide standard (1,000 mg L^{-1}) was prepared by dissolving acrylamide (>98.5%) (Loba chemie, India) in deionized (DI) water (Milli Q Integral 3, Merck, Germany). The acrylamide standard solutions were prepared daily from the stock solution by dilution with DI water to create calibration standards.

The stock solution of fluorescein (1000 mg L^{-1}) was prepared by dissolving fluorescein (>99.0%) (Honeywell Fluka, USA) in DI water, stored at 4°C and protected from sunlight. 0.05 mol L^{-1} borate buffer solution was prepared using 0.2 mol L^{-1} H_3BO_3, 0.2 mol L^{-1} KCl and suitable amount of 0.1 mol L^{-1} NaOH.

To minimize contamination, all glassware were cleaned and soaked at least 24 hours in 10% (v/v) of nitric acid, thoroughly rinsed with deionized water before used.

3. Sample preparation procedures

Snack samples are Phitsanulok provincial souvenir, which were purchased from Phra sri rattana mahathat vora maha viharn temple. Seasoning samples and instant coffee were purchased from supermarkets in Phitsanulok province with different brands. Tea samples were purchased from Chiang Rai province.

3.1 Cayenne pepper, paprika seasoning, tea, and instant coffee preparation

Cayenne pepper, paprika seasoning, tea, and instant coffee were weighed (0.05 g), in three replicates, dissolved with 25.0 mL of DI water, and ultrasonicated at 50°C for 15 min. The sample solutions were then passed through No. 5 (110 mm diameter) Whatman filter paper, and stored for derivatization step. (449).

3.2 Fried banana, potato, taro, and durian chips preparation

Fried banana, potato, taro, and durian chips were ground in a blender and dried at 60°C. Each sample were weighed (0.05 g), in three replicates, dissolved with 10 mL of hexane, and centrifuged at 7,000 rpm for 10 min followed by a 10 min of ultrasonication. The top solution of centrifugate layer was discarded, and another 10 mL of hexane was added to the remainder to iterate the defatting step. Then, 10 mL of water was added into the remainder for the extraction of acrylamide and the mixture was subjected to ultrasonic mixing for 10 min and centrifuging at 7,000 rpm for 10 min.

The sample solutions were passed through No. 3 (125 mm diameter) Whatman filter paper. The extraction method was carried out double. Finally, filtrate was gathered and stored for derivatization step. (450).

4. Derivatization step

The derivatization step utilized in this research work was established according to Hofmann reaction (444). The reactions were carried out in water bath at various temperatures and with different reaction times. Subsequently, the fluorescence intensity and color values were measured. 1.00 mL of the acrylamide standard and extracted sample solution was placed in a centrifuge tube and allowed to reach thermal equilibrium. NaOH solution (the rate of N-chlorination is considerably depended on the NaOH concentration) was added before the addition of NaClO solution (acrylamide undergoes chlorination on the N atom by NaClO). The concentration ranges of NaOH and NaClO were selected between 0.10-1.00 mol L^{-1} and 0.002-0.100 mmol L^{-1}, respectively. After that total reagents were mixed, then the derivatization was performed in a controlled water bath for a fixed amount of time.

Method development

1. Design and fabricate the photography lightbox

The photography lightbox as shown in Figure 1.14 was designed to prevent the system from outside light and it was made from wood with the internal walls being smeared with black paint. A drilled hole was utilized for capturing digital images from outside the photography lightbox.

2. Optimization parameters for Hofmann reaction condition

The parameters of phosphor, NaOH, NaClO, derivatization temperature, derivatization time, fluorescein concentration, and buffer solution were studied in order to improve the determination efficiency.

3. Quantitative analysis of acrylamide

2.00 mL of 0.05 mol L^{-1} H_3BO_3-NaOH-KCl buffer were added into the derivatization product solution, then, 2.00 mL of 100 mg L^{-1} fluorescein solution was added. The fluorescence intensity and color value of the solutions were measured at 590 nm with the excitation wavelength at 470 nm by fluorescence MR and an iOS gadgets-based DIC, respectively.

4. Analytical performance

Under optimized conditions, data for calibration curves for acrylamide standard containing 1.00-10.00 mg L^{-1} were obtained from acrylamide standard 1000 mg L^{-1}. The linear regression equation relating green values with acrylamide concentrations was used to determine limit of detection (LOD) and limit of quantitation (LOQ). The precision (intraday and interday) are expressed as percentage relative standard deviation ± standard deviation (%RSD ± SD). The recoveries were evaluated by different types of food samples spiked with 1.0, 3.0, and 5.0 mg L^{-1} standard acrylamide solution.

Results and discussion

1. Optimization of the photography lightbox

The parameters of optimization are listed in Table 3.1. The results are shown in Figure 3.2-3.6, respectively.

Table 3.1 Optimization parameters for photography lightbox.

Conditions	Optimum Conditions
Size of the photography lightbox	14.5 × 32.5 × 15 cm (width × length × height)
Lamp type	2 bulbs of blacklight illumination (8 watt)
Method of photographing	Zoom, not focus
Distance (in application)	2

1.1 Size of the photography lightbox

Size of the photography lightbox could have an effect on the image therefore three different sizes were fabricated: (A) 20 × 35 × 15 cm, (B) 14.5 × 32.5 × 15 cm, and (C) 15 × 38 × 15 cm (width × length × height). The result presented in Figure 3.2 demonstrated that the digital images of acrylamide standard solution (1,000.00 mg L^{-1}) when using the photography lightbox size (B) are brighter than that obtained when using size (A) and (C). Moreover, application can work properly with this size. So, the photography lightbox size (B) was selected for the next study.

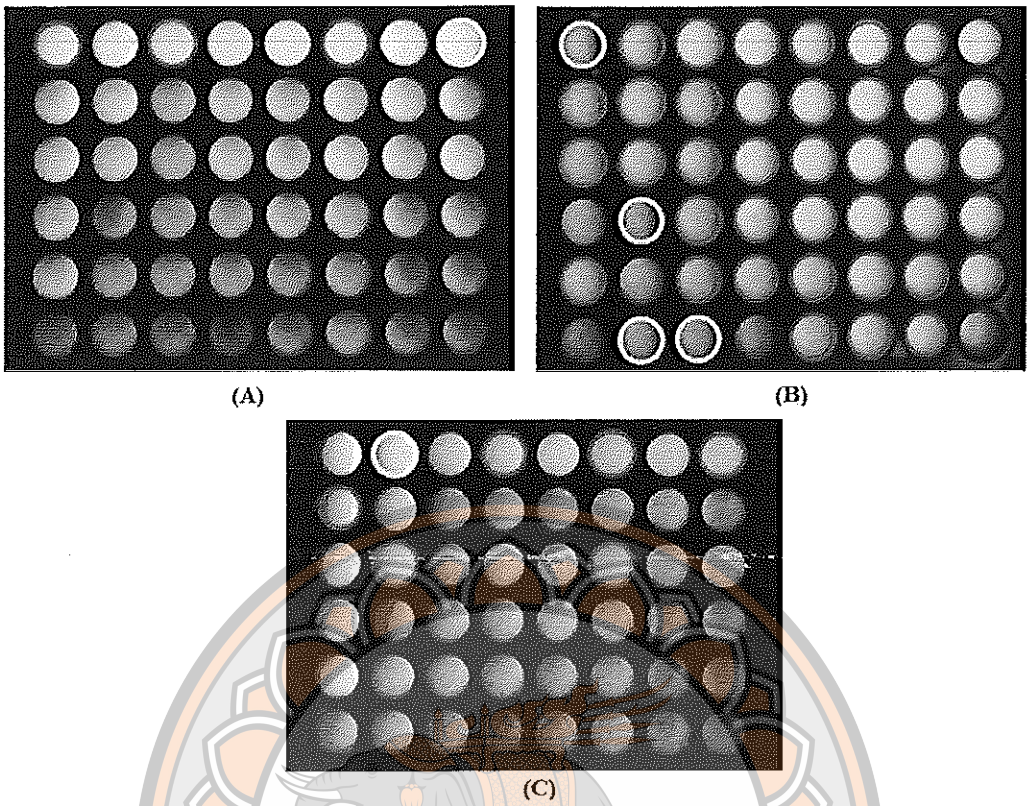


Figure 3.2 **The digital images of acrylamide standard solution (1,000 mg L^{-1}) when using the photography lightbox size 20 x 35 x 15 cm (A), 14.5 x 32.5 x 15 cm (B), and 15 x 38 x 15 cm (C) (width x length x height).**

1.2 Lamp type

The light source is critical to the color of an image of acrylamide solution thus three different types which each types use 2 bulbs were studied; (A) compact-fluorescence (CFL) bulbs (40 watt), (B) blacklight bulbs (30 cm, 8 watt), and (C) spiral violet lamps (20 watt). The results from Figure 3.3 reveal that the image obtained from light source type (A) and (C) have a reflected light on the side of an image giving not homogeneous image. In addition, it was found that the image obtained from light source type (A) and (C) are darker than that from light source type (B). The best image was obtained when photographing with the light source type (B) which gave bright color of acrylamide solution. Hence, 2 bulbs of blacklight (30 cm, 8 watt) was selected as the optimum type of light source of fluorescence illumination.

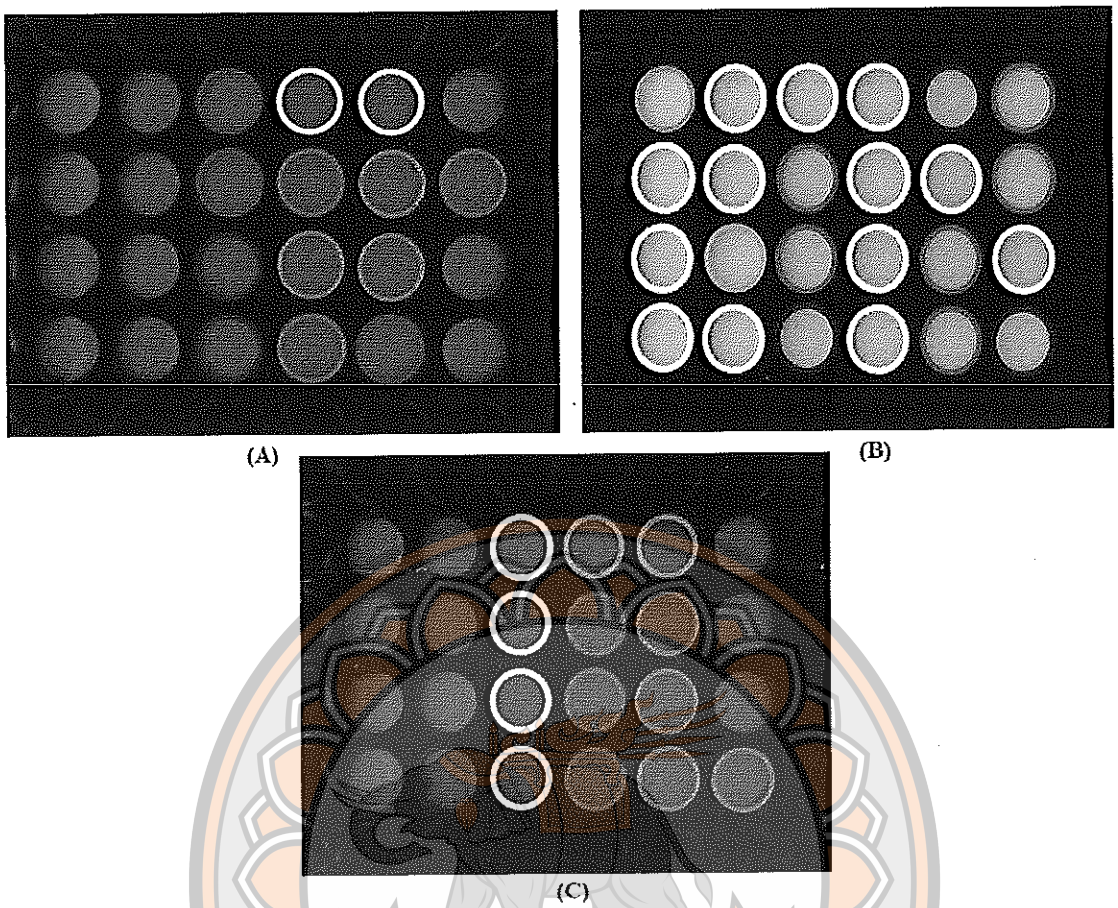


Figure 3.3 **The digital image of acrylamide standard solutions (100.0 mg L^{-1}) obtained from 3 different type of light source (A) compact-fluorescence (CFL) bulbs (40 watt), (B) blacklight bulbs (30 cm, 8 watt), and (C) spiral violet lamps (20 watt).**

1.3 Method of photographing

1.3.1 Zoom and unzoom

Method of photographing could have an effect on the image therefore two different methods were studied: (A) 4X zoom and (B) unzoom. The application can specify the correct concentration of acrylamide standard solutions when using 4X zoom. On the other hand, the applications cannot specify the correct concentration when using unzoom. Accordingly, 4X zoom was chosen for the next study.

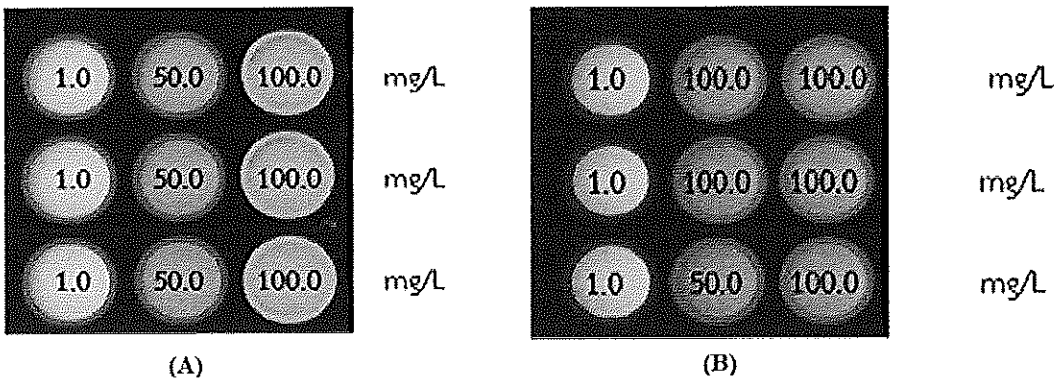


Figure 3.4 **The digital image of acrylamide standard solutions obtained from two different methods of photographing; (A) 4X zoom and (B) unzoom.**

1.3.2 Focus and unfocus

In addition, two more different methods of photographing were studied: (A) focus and (B) unfocus. Processing through the application of acrylamide standard solutions obtained from both methods are correct which application can specify the correct concentration. Consequently, unfocus was chosen for photographing because it will reduce the method of experimentation. Moreover, focusing at different point may cause the inaccurate result.

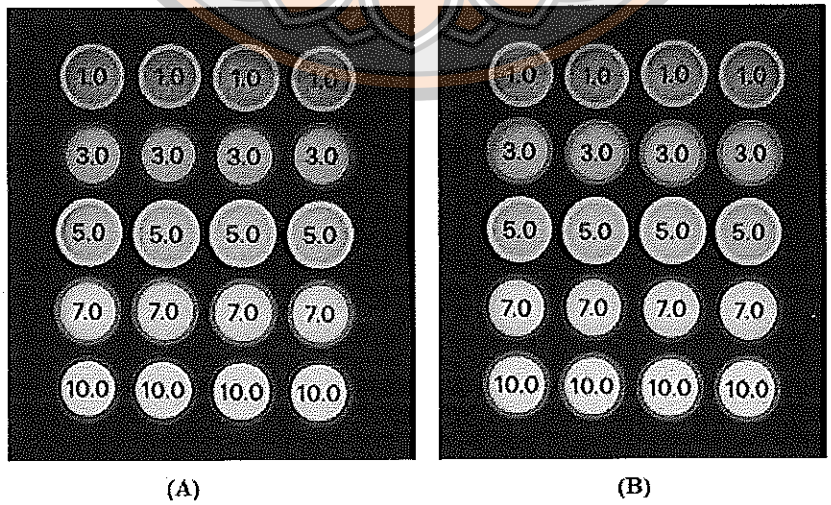


Figure 3.5 **The digital image of acrylamide standard solutions obtained from two different methods of photographing; (A) focus and (B) unfocus.**

1.4 Distance (in application)

The distance in application could have an effect on the image thence nine different distance were studied: (A) 1, (B) 2, (C) 3, (D) 4, (E) 5, (F) 6, (G) 7, (H) 8, and (I) 9. Processing of application when using 1, 3, 4, 5, 6, 7, 8 and 9 are incorrect because application cannot specify the correct concentration. Processing of application when using distance = 2 is correct because applications can specify the correct concentration as shown in Figure 3.6.

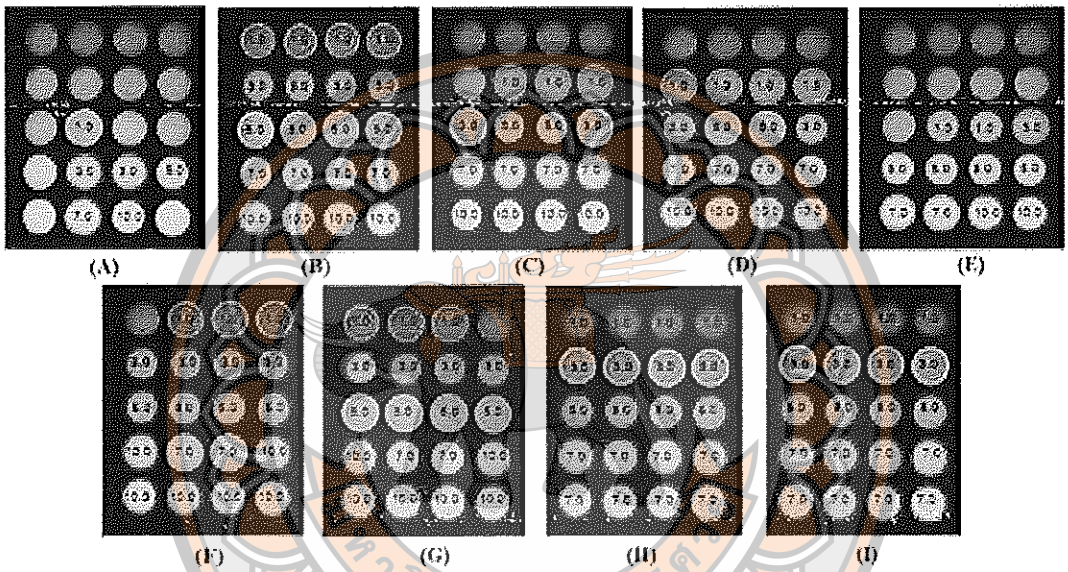


Figure 3.6 **The digital image of acrylamide standard solutions obtained from nine different distance (in application); (A) 1, (B) 2, (C) 3, (D) 4, (E) 5, (F) 6, (G) 7, (H) 8, and (I) 9.**

2. Optimization parameters for Hofmann reaction

The optimization parameters are listed in Table 3.2. The results are shown in Figure 3.7-3.13, respectively.

Table 3.2 Optimization parameters for Hofmann reaction.

Conditions Studied	Optimum Conditions
Phosphor	Fluorescein
NaOH concentration	0.5 mol L^{-1}
NaClO concentration	0.004 mmol L^{-1}
Derivatization temperature	60°C
Derivatization time	5 min
Fluorescein concentration	100 mg L^{-1}
Buffer solution	Buffer pH 7.8

2.1 Effect of phosphor

Liu et al. (333) utilized Hofmann reaction in which acrylamide is degraded to vinyl amine, then the vinyl amine reacts with fluorescamine and pyrrolinone is produced. Since fluorescamine is expensive, two phosphors were studied; (A) fluorescamine and (B) fluorescein. The best image was obtained when using fluorescein as shown in Figure 3.7(B) which gave bright color of acrylamide standard solutions that can easily process through the application. Moreover, fluorescein is much cheaper than fluorescamine thus it is the optimum phosphor in this research work.

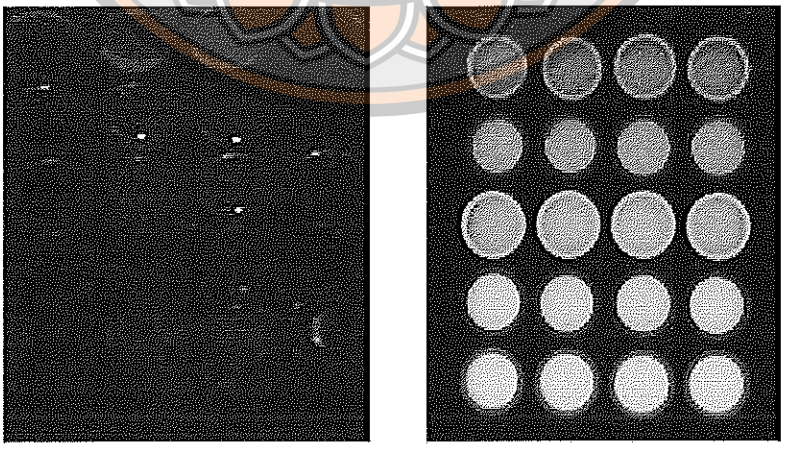
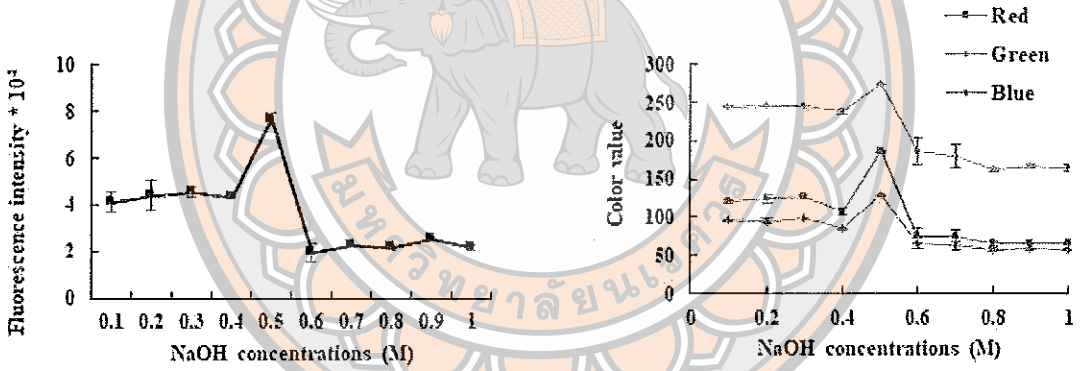


Figure 3.7 The digital image of acrylamide standard solutions obtained from two different phosphors; (A) fluorescamine and (B) fluorescein.

2.2 Effect of NaOH concentration

NaOH is significant determinant that effects Hofmann reaction. The fluorescence intensity and color value steadied with increasing NaOH concentration in the ranges of 0.1-0.4 mol L^{-1}. Normally, the rate of N-chlorination is considerably depended on the NaOH concentration (451). Other than, the isocyanato groups, formed as an intermediate of the degradation reaction, can react with water to yield amines in the presence of base. Meanwhile, the amine product can itself react with the isocyanoto intermediate resulting in the formation of urea (445). Therefore, excess NaOH is helpful to underwrite rapid formation of the amine product and limiting the side reaction with the isocyanato intermediate. However, further increases of NaOH content (for example, over 0.5 mol L^{-1}) result in decreased activation of fluorescein, leading to decreased fluorescence intensities and color values as demonstrated in Figure 3.8. Therefore, 0.5 mol L^{-1} NaOH was selected for further work.



(A) (B)

Figure 3.8 **Effect of NaOH concentrations when using 10.0 mg L^{-1} acrylamide standard corresponding with fluorescence intensity (A) and color value (B) Optimization studied by fluorescence microplate reader with the excitation wavelength of 470 nm and iOS gadgets-based digital imaging colorimeter, respectively.**

2.3 Effect of NaClO concentration

NaClO or (ClO-) is the significant reagent and anion in Hofmann reaction. The effect of NaClO concentration was studied in the range of 0.002-0.100 mmol L^{-1}. As shown in Figure 3.9, the fluorescence intensity and color value increased with increasing NaClO concentration until 0.004 mmol L^{-1}, since the halogen for Hofmann reaction was provided with suitable concentration of NaClO. The fluorescence intensity and color value steadied when NaClO concentration was over 0.004-0.06 mmol L^{-1} and decreased when NaClO concentration was over 0.06-0.08 mmol L^{-1} because the excess of the strong oxidant, NaClO, affectations the consistency of fluorescent product. Therefore, 0.004 mmol L^{-1} NaClO was chosen for further study.

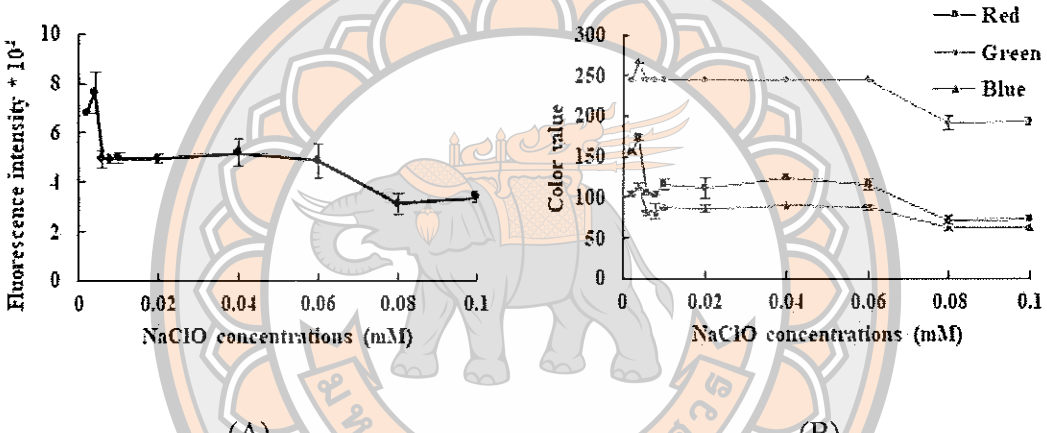
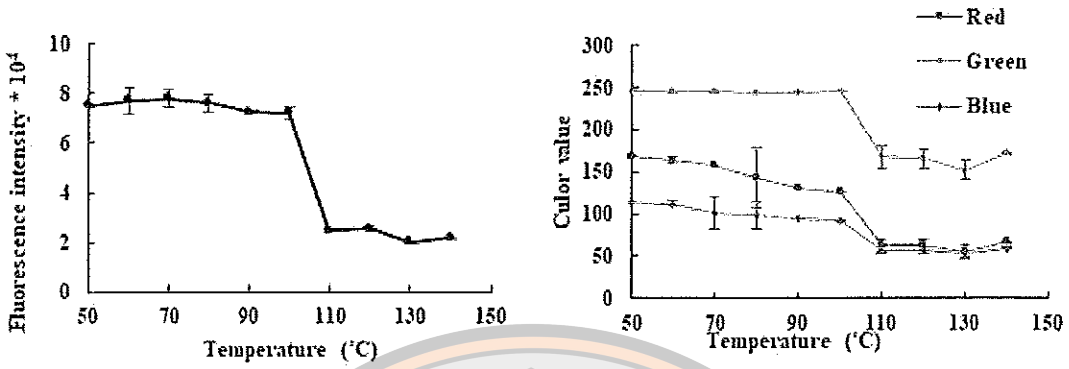


Figure 3.9 **Effect of NaClO concentrations when using 10.0 mg L^{-1} acrylamide standard corresponding with fluorescence intensity (A) and color value (B) Optimization studied by fluorescence microplate reader with the excitation wavelength of 470 nm and iOS gadgets-based digital imaging colorimeter, respectively.**

2.4 Effect of derivatization temperature

The reaction temperature effectively influences derivatization process (452), hence, the effect of temperature was investigated between 50-140°C. As shown in Figure 3.10, the fluorescence intensity and color value were steady as temperature increased up to 100°C (the reaction was almost to the limit rate) and then decreased when the temperature was over 100°C. For convenience and save energy, 60°C was

selected as the reaction temperature for the Hofmann reaction step. In addition, carrying out the Hoffman reaction at 60°C could cause the high frequency of collisions between molecules, which accelerates the reaction.

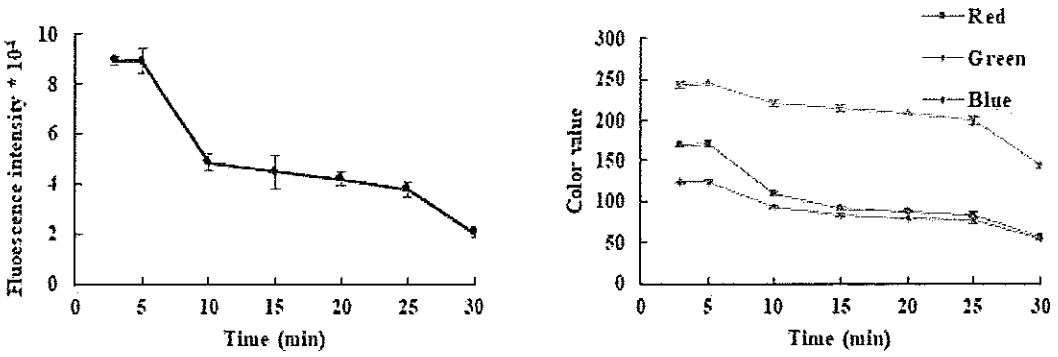


(A) (B)

Figure 3.10 Effect of derivatization temperature when using 10.0 mg L^{-1} acrylamide standard corresponding with fluorescence intensity (A) and color value (B) Optimization studied by fluorescence microplate reader with the excitation wavelength of 470 nm and iOS gadgets-based digital imaging colorimeter, respectively.

2.5 Effect of derivatization time

The optimal reaction time for Hofmann reaction was established by plotting against the fluorescence intensity and color values versus the derivatization time with a range from 3-30 min. As shown in Figure 3.11, the fluorescence intensity and color value were steady between 3 and 5 min and then decreased when the derivatization time was longer than 5 min. The change presented that a period of time was wanted to assure the reaction sufficiently. Therefore, derivatization times in the range of 10-30 min were not beneficial for the formation of vinyl amine forasmuch some of the amine could undergo oxidation of the nitrogen atom. Based on these results, 5 min was selected for further study to guarantee adequate outcome of the derivatization. Furthermore, the derivatization time selected in this work is shorter than the time used in the research of Lui et al. (335).



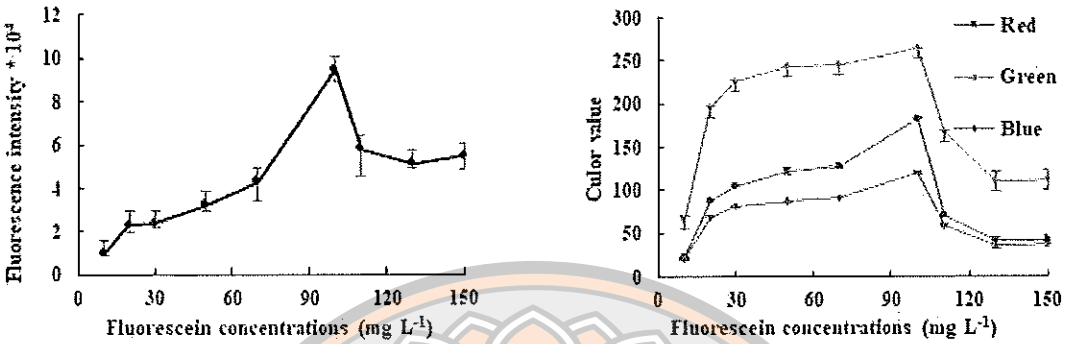
(A) (B)

Figure 3.11 Effect of derivatization time when using 10.0 mg L^{-1} acrylamide standard corresponding with fluorescence intensity (A) and color value (B) Optimization studied by fluorescence microplate reader with the excitation wavelength of 470 nm and iOS gadgets-based digital imaging colorimeter, respectively.

2.6 Effect of fluorescein concentration

Fluorescein solution is the luminophore starting point in the fluorescence mensuration and fluorescein concentration is important for fluorescence intensity. The effect of the fluorescein concentration from 10-150 mg L^{-1} was investigated. As shown in Figure 3.12, the fluorescencae intensity and color value increased with the increasing fluorescein concentration, but decreased when fluorescein concentration was higher than 100 mg L^{-1}. Besides, high optical turbidity or densities could also result in decreasing fluorescence intensities. The fluorescence emission spectrum shown in Figure 3.14(C) looks a bit odd. This is likely because the fluorescein concentration of 100 mg L^{-1} is quite high for fluorescence MR measurements. Nevertheless, this concentration is suitable for the developed method because when the fluorescein concentration was less than 100 mg L^{-1} it caused problems with photography, such as faint fluorescence of the solution and images that are not suitable for concentration calculation using the ColorConc2 iOS application. As shown in Table 3.3, the concentration values of acrylamide found in the blank food samples determined by the proposed technique corresponded to those obtained by the fluorescence MR. Moreover, when the concentration values are computed and reported using the units of mg kg^{-1}, the

acrylamide concentration values found by the proposed technique are not different from found by fluorescence MR. Based on this experiment, the fluorescein concentration of 100 mg L^{-1} was chosen for further study.

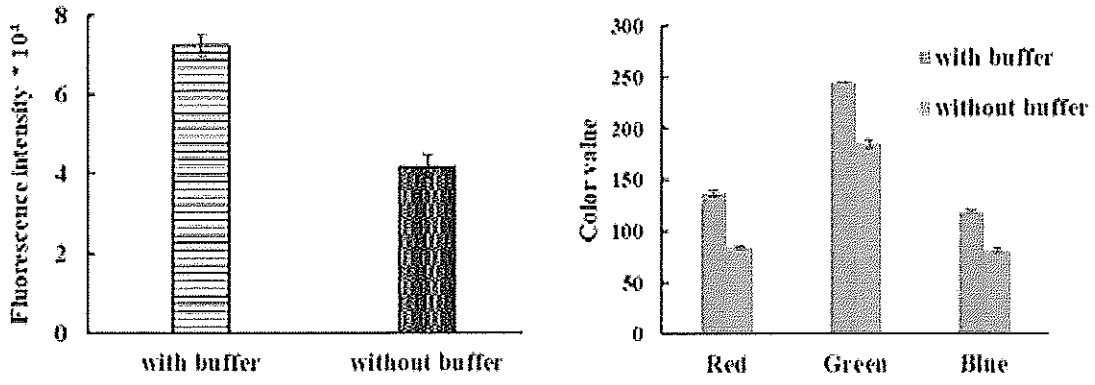


(A) (B)

Figure 3.12 Effect of fluorescein concentrations when using 10.0 mg L^{-1} acrylamide standard corresponding with fluorescence intensity (A) and color value (B) Optimization studied by fluorescence microplate reader with the excitation wavelength of 470 nm and iOS gadgets-based digital imaging colorimeter, respectively.

2.7 Effect of buffer solution

pH value is a precious determinant to augment the confines of the fluorescence reaction. Lui et al. (335) have reported that the fluorescence intensity varies considerably with different buffer solutions and maximum fluorescence intensity could be received at pH 7.8. To confirm these results reported by Lui et al., researcher further verify that the pH result on the fluorescence reaction by carrying out the reaction in solutions with and without a buffer solution as depicted in Figure 3.13. Fluorescence intensity and color values were lower for reactions where no buffer was added. This could be due to the protonation of the amine, which could hinder its reaction with fluorescein. Hence, buffer was added in this experiments.



(A) (B)

Figure 3.13 **Effect of buffer solution when using 10.0 mg L^{-1} acrylamide standard corresponding with fluorescence intensity (A) and color value (B) Optimization studied by fluorescence microplate reader with the excitation wavelength of 470 nm and iOS gadgets-based digital imaging colorimeter, respectively.**

3. Quantitative analysis of acrylamide

3.1 Possible reaction mechanism including fluorescence characteristics

In the presence of excess NaOH solution, acrylamide undergoes chlorination on the N atom by NaClO, and then rearranged to form isocyanato group, which decomposed to a vinyl amine (444). It was expected that this primary amine could act as base shifting the equilibria of fluorescein species from its mono anion form to its dianion form by deprotonation as demonstrated in Figure 3.14(A). Such change in fluorescein speciation leads to increased amount of fluorescence as the dianion form has a higher quantum yield (93%) than the mono anion form (36%) (453). Indeed, emission of fluorescence at 590 nm was observed after excitation at 470 nm as illustrated in Figure 3.14(B) and its intensity increased in response to the addition of acrylamide. Illustrative fluorescence emission spectra for samples of acrylamide extracted from cayenne pepper A, 10 mg L^{-1} standard acrylamide, and cayenne pepper A spiked with 10 mg L^{-1} standard acrylamide prepared at optimal conditions are shown in Figure 3.14(C).

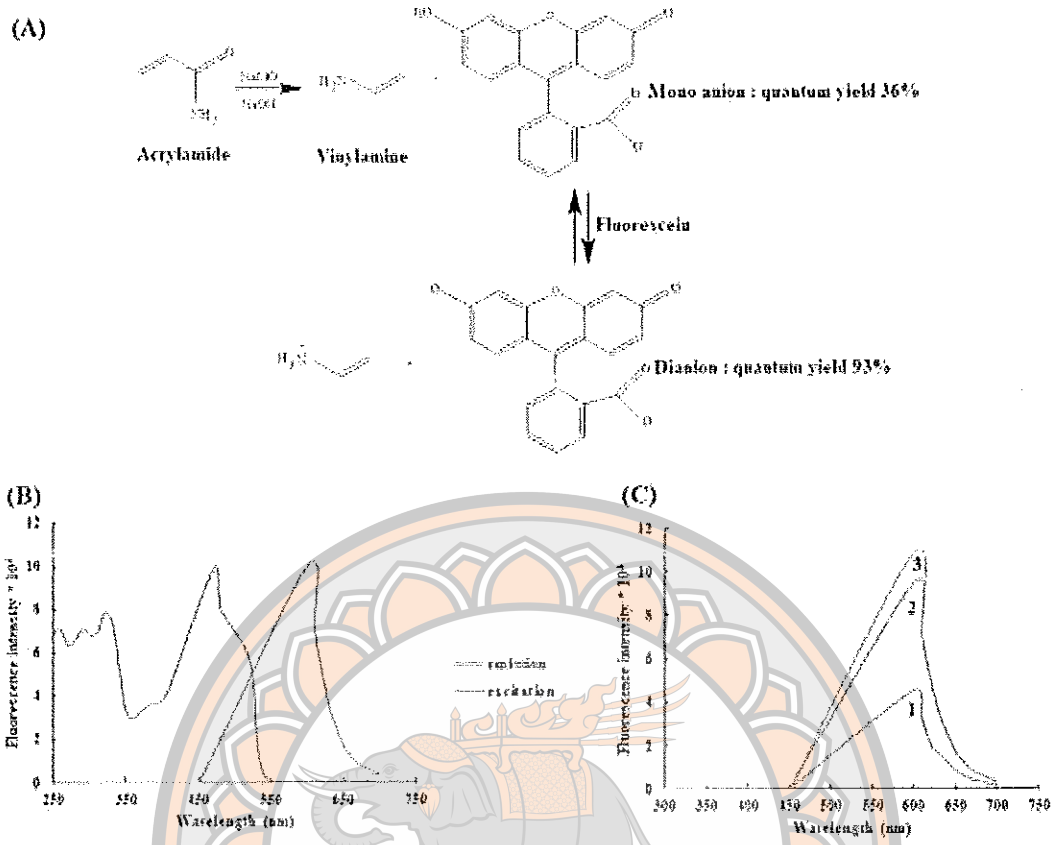


Figure 3.14 (A) The generation process of the fluorescent from this research, (B) fluorescence excitation and emission spectra of fluorescein, and (C) fluorescence emission spectra (excitation at 470 nm) of (1) acrylamide extracted from cayenne pepper A, (2) 10 mg L^{-1} acrylamide standard, and (3) cayenne pepper A spiked with 10 mg L^{-1} acrylamide standard obtained by fluorescence microplate reader.

3.2 Application to acrylamide assay in food samples

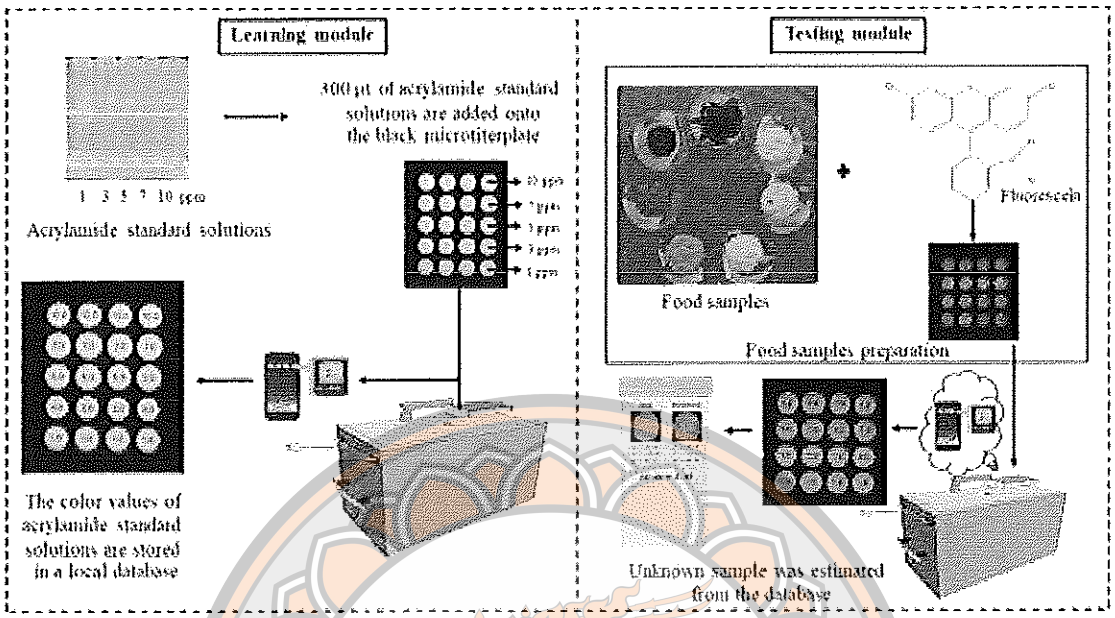


Figure 3.15 The workflow schematic of acrylamide measurement using an iOS gadgets-based digital imaging colorimeter (iOS gadgets-based DIC).

Figure 3.15 demonstrates the workflow process of acrylamide mensuration using an iOS gadgets-based DIC. 300 µL of acrylamide standard in the concentrations range of 1.00-10.0 mg L^{-1} were filled in the black microtiterplate then put in the lightbox. The image of the black microtiterplate was photographed, and the color values were saved and used for estimation the acrylamide concentration of the food sample solutions. The food sample solutions were made as described previously for catching the images and analyzing the color data by ColorConc2 iOS application (404). Acrylamide concentrations of the extracted food sample solutions were instantaneously displayed on the screen of iOS gadgets. Other options for acrylamide concentration calculation is to send the color data of each acrylamide standard concentration to Microsoft excel. The calibration graphs plotted between acrylamide standard concentrations versus color values (red, green, and blue) derived from this data, shown in Figure 3.16, was applied for quantitative calculations presented in Table 3.4. In addition, color values (red, green, and blue) can be changed to color parameters (L^*, a^*, and b^*). Therefore, changed color parameters can be compared with the commercial colorimeter. Furthermore, the

application of an iOS gadgets offers a rapid, precise, and simple method for the determination of different analytes.

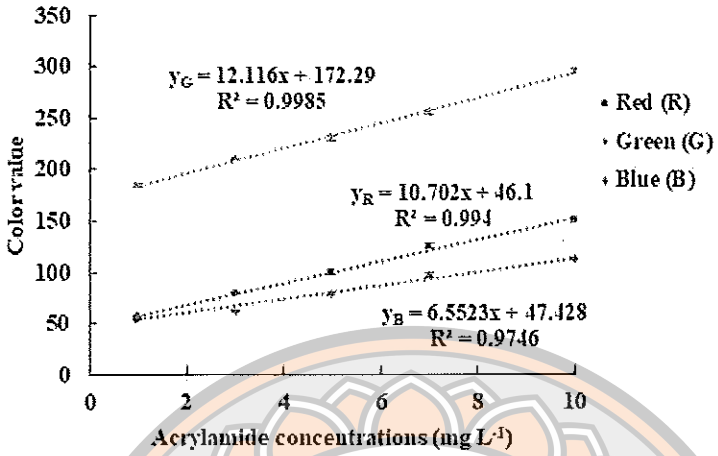


Figure 3.16 **Correlation between acrylamide concentration and color value (red, green and blue).**

Table 3.3 **Recoveries study of acrylamide in snack, seasoning, and refreshment food samples performed using an iOS gadgets-based digital imaging colorimeter (iOS gadgets-based DIC), fluorescence microplate reader (Fluorescence MR), and high performance liquid chromatograph (HPLC)[a]**

Samples	Acrylamide standard Added (mg L^{-1})	iOS gadgets-based DIC			Fluorescence MR			HPLC		
		Acrylamide Found (mg L^{-1})	%RSD	%Recovery	Acrylamide Found (mg L^{-1})	%RSD	%Recovery	Acrylamide Found (mg L^{-1})	%RSD	%Recovery
Fried banana chip	0	1.06±0.01	1.00	-	1.05±0.01	0.15	-	1.04±0.01	0.60	-
	1	1.99±0.04	2.11	92.44	1.98±0.01	0.42	92.94	2.03±0.01	0.27	98.91
	3	4.16±0.04	1.01	103.1	4.10±0.01	0.24	101.7	3.94±0.07	1.84	96.60
	5	6.22±0.02	0.29	103.2	6.14±0.01	0.08	101.6	6.05±0.13	2.11	100.2
Fried potato chip	0	1.05±0.01	0.75	-	1.05±0.01	0.18	-	1.04±0.02	1.56	-
	1	2.02±0.08	3.91	96.36	1.98±0.01	0.32	92.68	2.10±0.03	1.62	106.1
	3	4.19±0.06	1.45	104.6	4.11±0.01	0.19	102.1	4.09±0.02	0.60	101.9
	5	6.25±0.06	0.94	104.0	6.15±0.01	0.16	101.9	6.19±0.02	0.32	103.1
Fried taro chip	0	1.06±0.01	1.10	-	1.05±0.01	0.51	-	1.05±0.01	0.59	-
	1	2.02±0.04	2.04	96.15	1.98±0.01	0.39	93.49	2.11±0.07	3.44	105.8
	3	4.16±0.06	1.41	103.5	4.12±0.01	0.23	102.4	4.07±0.03	0.68	100.8
	5	6.21±0.02	0.38	103.1	6.17±0.01	0.20	102.0	6.11±0.06	0.99	101.2
Fried durian chip	0	1.06±0.01	1.33	-	1.06±0.02	0.76	-	1.06±0.01	0.88	-
	1	2.01±0.06	2.76	95.12	1.98±0.01	0.44	92.47	2.03±0.02	0.78	96.71
	3	4.10±0.06	1.38	101.3	4.11±0.01	0.19	101.2	3.96±0.11	2.67	96.61
	5	6.14±0.04	0.72	101.4	6.15±0.05	0.19	101.9	6.22±0.14	2.25	103.2
Cayenne pepper A	0	3.02±0.01	0.22	-	3.04±0.02	0.59	-	2.99±0.01	0.37	-
	1	4.01±0.07	1.78	98.42	4.10±0.05	1.12	105.3	4.01±0.01	0.16	101.5
	3	6.06±0.08	1.30	101.4	6.09±0.01	0.21	101.5	6.05±0.04	0.69	101.8
	5	8.06±0.11	1.41	100.7	8.17±0.01	0.07	102.4	8.28±0.11	1.31	105.7

Table 3.3 (Cont.)

Samples	Acrylamide standard Added (mg L^{-1})	iOS gadgets-based DIC			Fluorescence MR			HPLC		
		Acrylamide Found (mg L^{-1})	%RSD	%Recovery	Acrylamide Found (mg L^{-1})	%RSD	%Recovery	Acrylamide Found (mg L^{-1})	%RSD	%Recovery
Cayenne pepper B	0	3.12±0.01	0.37	-	3.17±0.01	0.13	-	3.11±0.02	0.53	-
	1	3.95±0.07	1.71	82.74	4.06±0.03	0.62	88.46	4.03±0.19	4.63	91.97
	3	6.08±0.06	0.96	98.77	6.10±0.01	0.18	97.74	6.04±0.27	4.49	97.67
	5	8.11±0.14	1.71	99.70	8.16±0.01	0.08	102.3	8.09±0.24	3.02	99.50
Cayenne pepper C	0	3.04±0.01	0.31	-	3.09±0.01	0.26	-	3.03±0.01	0.36	-
	1	4.06±0.07	1.65	101.9	4.10±0.01	0.13	100.4	4.13±0.01	0.30	110.3
	3	6.11±0.07	1.21	102.3	6.12±0.01	0.31	100.9	6.35±0.03	0.40	110.5
	5	8.37±0.03	0.33	106.7	8.16±0.01	0.08	101.9	8.55±0.13	1.46	110.3
Paprika seasoning	0	2.96±0.01	0.36	-	3.01±0.01	0.06	-	2.95±0.02	0.84	-
	1	4.10±0.14	3.33	113.3	4.11±0.01	0.13	110.1	4.01±0.14	3.49	105.4
	3	6.09±0.08	1.35	104.4	6.12±0.01	0.08	103.5	6.12±0.02	0.28	105.5
	5	8.19±0.11	1.38	104.5	8.16±0.01	0.13	102.5	8.27±0.44	5.28	106.3
Green tea	0	1.06±0.01	0.87	-	1.09±0.01	0.81	-	1.06±0.02	2.21	-
	1	2.01±0.09	4.60	94.92	1.98±0.01	0.39	88.77	2.08±0.08	3.97	101.7
	3	4.13±0.06	1.53	102.4	4.10±0.01	0.34	100.4	4.11±0.01	0.08	101.8
	5	5.97±0.04	0.62	98.18	6.10±0.01	0.09	100.7	6.15±0.05	0.81	101.8
Jasmine tea	0	3.13±0.01	0.37	-	3.18±0.02	0.68	-	3.12±0.02	0.62	-
	1	4.14±0.16	3.81	101.5	4.08±0.01	0.20	89.90	3.94±0.19	4.80	82.63
	3	6.14±0.06	0.92	100.3	6.11±0.01	0.57	97.67	5.80±0.01	0.25	89.35
	5	8.29±0.23	2.74	103.3	8.19±0.01	0.07	102.9	8.09±0.31	3.78	99.43
Oolong tea	0	1.07±0.01	1.00	-	1.08±0.01	0.84	-	1.07±0.02	1.77	-
	1	2.06±0.05	2.65	98.84	1.94±0.02	0.95	85.76	2.11±0.06	2.95	104.0
	3	4.14±0.08	2.04	102.2	4.11±0.01	0.41	101.0	4.20±0.03	0.65	104.3
	5	6.11±0.06	1.00	100.8	6.10±0.01	0.11	101.4	6.28±0.02	0.37	104.2

Table 3.3 (Cont.)

Samples	Acrylamide standard Added (mg L^{-1})	iOS gadgets-based DIC			Fluorescence MR			HPLC		
		Acrylamide Found (mg L^{-1})	%RSD	%Recovery	Acrylamide Found (mg L^{-1})	%RSD	%Recovery	Acrylamide Found (mg L^{-1})	%RSD	%Recovery
Instant coffee	0	1.06±0.01	1.17	-	1.07±0.02	1.44	-	1.05±0.01	0.78	-
	1	2.01±0.13	4.02	99.87	2.00±0.01	0.34	92.66	2.06±0.08	4.00	100.6
	3	4.11±0.05	1.28	101.7	4.12±0.01	0.65	101.7	4.27±0.11	2.63	107.1
	5	6.10±0.03	0.54	100.8	6.11±0.01	0.11	101.9	6.40±0.04	0.61	107.0

[a] Data are not significantly different according to ANOVA at $p < 0.05$

Table 3.4 **Results of acrylamide assay in snack, seasoning, and refreshment food samples determined using an iOS gadgets-based digital imaging colorimeter (iOS gadgets-based DIC) and compared with fluorescence microplate reader (Fluorescence MR) and high performance liquid chromatograph (HPLC)[a]**

Samples	Acrylamide Found (mg kg^{-1}) ±SD, n=3		
	iOS gadgets-based DIC	Fluorescence MR	HPLC
Fried banana chip	209.8±2.11	210.5±0.32	207.7±1.24
Fried potato chip	208.5±1.56	209.9±0.37	207.4±3.23
Fried taro chip	208.9±2.31	209.3±1.07	209.9±1.24
Fried durian chip	210.2±2.79	211.3±1.60	211.7±1.87
Cayenne pepper A	601.9±1.34	598.1±1.41	599.8±1.63
Cayenne pepper B	621.6±2.33	620.1±0.72	622.7±3.29
Cayenne pepper C	605.2±1.90	604.8±1.89	606.2±2.16
Paprika seasoning	589.6±2.12	589.5±0.29	590.3±4.98
Green tea	211.4±2.12	212.6±1.47	211.7±4.36
Jasmine tea	623.3±2.33	622.4±4.92	623.4±3.89
Oolong tea	213.1±2.12	212.7±1.75	214.2±3.79
Instant coffee	211.0±2.47	210.3±3.58	210.2±1.65

[a] Data are not significantly different according to ANOVA at $p< 0.05$

4. Analytical performance

4.1 Analytical performance of the fluorescence MR and HPLC reference instruments

Fluorescence MR and HPLC were used as reference instruments in this experiments. The calibration curve was linear for acrylamide over the concentration range of 1.00-10.0 mg L^{-1}, with correlation coefficients (R^2) of 0.9977 and 0.9960 for fluorescence MR and HPLC, respectively. As shown in Table 3.3, the recoveries of acrylamide from food samples spiked with 1.00, 3.00, and 5.00 mg L^{-1} acrylamide standard solution were determined and found to be in the range of 85.76-105.3% and 82.63-110.5% for fluorescence MR and HPLC, respectively. The comparison of fluorescence emission spectra of acrylamide extracted from food sample (for example, cayenne pepper A), 10.0 mg L^{-1} acrylamide standard solution, and cayenne pepper A spiked with 10.0 mg L^{-1} standard acrylamide is illustrated in Figure 3.14(C). It was found that all three samples exhibit emission at the same wavelength, which means that the acrylamide extraction method used in this research is reliable.

4.2 Analytical performance of an iOS gadgets-based DIC

The calibration graphs were linear over the acrylamide standard concentration range of 1.00-10.0 mg L^{-1}, with a correlation coefficient (R^2) of 0.9985. The series of acrylamide standard solution images were recorded as the calibration series for examining the extracted food samples. The different types of food samples (snack, seasoning, and refreshment) spiked with 1.00, 3.00, and 5.00 mg L^{-1} acrylamide standard solution were analyzed by an iOS gadgets-based DIC. It was encountered that the recoveries are in the range of 82.74-113.3% as shown in Table 3.3. These results indicate that an iOS gadgets- based DIC has a good possibly for the analysis of acrylamide in food samples.

The intraday and interday precision were investigated by preparing series of acrylamide standard solutions five times in the same day and once a day for 5 days, respectively. The percentage relative standard deviations (%RSD) for the correlation coefficient and slope of green color values with acrylamide standard concentrations (1.00-10.0 mg L^{-1}) were used to assess the error in the arrangement of the calibration graphs. The intraday and interday precision values were 3.02%±0.34 and 1.85%±0.19, respectively. %RSD at low values indicate suitable and reflect the good precision of the

proposed apparatus. Therefore, this apparatus was not imperative to recalibrate every day. The experiment has displayed that the green color value increases with increasing acrylamide standard concentration. Accordingly, the green color value was used to compute the limit of detection (LOD (3SDblank/slope, n=25)) and limit of quantitation (LOQ (10SDblank/slope, n= 25)) for acrylamide and were found to be 0.53 and 1.78 mg L^{-1}, respectively. These results represent that this apparatus gives sufficient sensitivity for acrylamide assay in food samples. In addition, when acrylamide concentration is calculated and reported using the mg kg^{-1} units, the acrylamide concentration found by an iOS gadgets-based DIC is not different from those found by fluorescence MR and HPLC. The results for the measurement of the extractable acrylamide solution from the three instruments are indicated in Table 3.4.

The statistical analysis, ANOVA at $p<0.05$, was used for the analogy with the comparison instruments and it was found that those results obtained from an iOS gadgets-based DIC, fluorescence MR, and HPLC presented good agreement with no statistical difference at the 95% confidence level.

Conclusions

This research provides a quick, uncomplicated, and accurate fluorescence method for acrylamide detection depending on Hofmann reaction followed by iOS gadgets-based DIC, fluorescence MR, and HPLC detection. An iOS gadgets-based DIC proposed excellent work such as convenient and environmentally friendly procedure, short detection time, good selectivity, and low cost equipment. The developed methodology is appropriate for routine analysis or including point-of-care measurements. Moreover, the calibration needs to be gauged only once and its can be used in all future measurements. These properties are in accordance with the high desire for monitoring of acrylamide in food samples.

CHAPTER IV

CONCLUSIONS AND FUTURE WORKS

Conclusions

In the first work, a novel method was developed and successfully applied for determination of curcumin in turmeric samples by a smart device-based DIC. In this method, turmeric was initially extracted in a 60:40 (v/v) mixture of ethanol and hydrochloric acid. Then curcumin solutions extracted from turmeric samples were captured and the concentration was estimated by comparing color values with those collected in a database. The results demonstrated that the method provide several advantages, such as convenient, easy-to-use, low-cost, accurate, precise, and good reproducibility. Therefore, smart device-based DIC is a suitable equipment for rapid determination of curcumin in various turmeric samples by the optimized extraction parameters.

In the second work, an efficient method was based on fluorescence color measurements obtained from digital images of derivatized acrylamide solution extracted from food samples. In this research, acrylamide was degraded through Hofmann reaction, and a fluorescent product was produced by the vinyl amine reacts with fluorescein and it was detected by an iOS gadgets-based DIC. The developed technique offers several advantages, such as simple, fast, plausible, and use of inexpensive reagents. Consequently, an iOS gadgets-based DIC is a proper equipment for detection of acrylamide in various food samples by the optimized extraction parameters and the Hofmann reaction conditions.

Comparison of the analytical performances of two developed equipment are described in Table 4.1

Table 4.1 Comparison of analytical performances of two developed equipment

Analytical performance	smart device-based DIC	iOS gadgets-based DIC
	Curcumin	Acrylamide
Linearity (mg L^{-1})	10.0-100.0	1.00-10.0
Calibration curve	y = -1.6505X + 209.87	y = 12.116X + 172.29
R^2	0.9926	0.9985
RSD (%)	0.38-0.41	1.85-3.02
LOD (mg L^{-1})	0.48	0.53
LOQ (mg L^{-1})	1.61	1.78
Recovery (%)	98.57-110.0	82.74-113.3

Future works

The first part focused on extraction using a 60:40 (v/v) mixture of ethanol and hydrochloric acid combined with a smart device-based DIC for determination of curcumin in turmeric samples. Based on this techniques and compared with earlier reports and recent works by practical optimization, this technique is very well suited for extraction of curcumin analytes from a broad range of samples, including turmeric capsules or turmeric powder. In addition, several reports demonstrated acceptable validation data for the technique. With all this information, a smart device-based DIC is in principle nature for implementation in routine analytical laboratories.

However, the combination of extraction and smart device-based DIC is currently limited by the unavailability of extraction method. Work is in progress in this area, and soon, it will show commercial equipment of extraction and smart device-based DIC. Moreover, development of generic method for extraction method is important, to reduce the time required for laboratories and to develop on-site smart device-based DIC. Finally, commercial equipment of extraction and a smart device-based DIC can be used on-site for the detection of curcumin in turmeric farm or market.

The second part focused on the Hofmann reaction conditions for determination of acrylamide in food samples. The acrylamide contents were detected by using an iOS

gadgets-based DIC and the Hofmann reaction conditions for the developed method were investigated.

In derivatization step, the selection of the appropriate phosphor reagent is necessary to achieve complete fluorescence of the interested analytes, at the same time with reducing the price of reagent. For this purpose, a new of alternative phosphors have been used in several fluorescence determinations. Indeed, replacing expensive reagents with fluorescein is the main advantages of fluorescence techniques such as low cost and enhancing color. Furthermore, the selectivity and sensitivity accomplished by the new phosphor and iOS gadgets-based DIC designed for acrylamide analysis were better than those obtained by fluorescamine reagent.

Therefore, the utilization of fluorescein reagent in acrylamide analysis will increase in the near future. Forthcoming studies and developments employing fluorescein may focus on the following areas; (I) fluorescein at low concentration and (II) analyze acrylamide at low concentrations ($0.10\text{-}1.00$ mg L^{-1}). Nevertheless, the development of new fluorescence reaction utilizing other phosphor will help to develop a new analytical method and tool that will simplify the analytical process and to detect analytes selectively.

REFERENCES

REFERENCES

1. Kempaiah RK, Srinivasan K. Influence of dietary curcumin, capsaicin and garlic on the antioxidant status of red blood cells and the liver in high-fat-fed rats. Ann Nutr Metab. 2004;48:314-20.

2. Srinivasan K. Role of spices beyond food flavoring: Nutraceuticals with multiple health effects. Food Rev Int. 2005;21:167-88.

3. Ahuja KDK, Kunde DA, Ball MJ, Geraghty DP. Effects of capsaicin, dihydrocapsaicin, and curcumin on copper-induced oxidation of human serum lipids. J Agric Food Chem. 2006;54:6436-9.

4. Jana NR, Dikshit P, Goswami A, Nukina N. Inhibition of proteasomal function by curcumin induces apoptosis through mitochondrial pathway. J Biol Chem. 2004;279(12):11680-5.

5. Fulisawa S, Atsumi T, Ishihara M, Kadoma Y. Cytotoxicity, ROS-generation activity and radical-scavenging activity of curcumin and related compounds. Anticancer Res. 2004;24:563-70.

6. Anand P, Thomas SG, Kunnumakkara AB, Sundarama C, Harikumar KB, Sung B, et al. Biological activities of curcumin and its analogues (Congeners) made by man and mother nature. Biochem Pharmacol. 2008;76(11):1590-611.

7. Manaia MAN, Diculescu VC, de Souza Gil E, Oliveira-Brett AM. Guaicolic spices curcumin and capsaicin electrochemical oxidation behaviour at a glassy carbon electrode. J Electroanal Chem. 2012;682:83-9.

8. Rajesh J, Rajasekaran M, Rajagopal G, Athappan P. Analytical methods to determine the comparative DNA binding studies of curcumin--Cu(II) complexes. Spectrochim Acta A. 2012;97:223-30.

9. Li J, Dai CX, Sun H, Jin L, Guo CY, Cao W. Protective effects and mechanisms of curcumin on podophyllotoxin toxicity in vitro and in vivo. Toxicol Appl Pharmacol. 2012;265(2):190-9.

10. Lin JK, Pan MH, Shiau SYL. Recent studies on the biofunctions and biotransformations of curcumin. Biofactors. 2000;13(1-4):153-8.

11. Wang YJ, Pan HM, Cheng AL, Lin LI, Ho YS, Hsieh CY, et al. Stability of curcumin in buffer solutions and characterization of its degradation products. J Pharm Biomed Anal. 1997;15(12):1867-76.

12. Ahsan H, Parveen N, Khan NU, Hadi SM. Pro-oxidant, anti-oxidant and cleavage activities on DNA of curcumin and its derivatives demethoxycurcumin and bisdemethoxycurcumin. Chem-Biol Interact. 1999;121(2):161-75.

13. Jayaprakasha GKL, Rao LJM, Sakariah KK. Improved HPLC method for the determination of curcumin, demethoxycurcumin, and bisdemethoxycurcumin. J Agric Food Chem. 2002;50(13):3668-72.

14. Jayaprakasha GK, Jagan Mohan Rao L, Sakariah KK. Chemistry and biological activities of C. longa. Trends Food Sci Technol. 2005;16(12):533-48.

15. Monton C, Charoenchai L, Suksaeree J, Sueree L. Quantitation of curcuminoid contents, dissolution profile, and volatile oil content of turmeric capsules produced at some secondary government hospitals. J Food Drug Anal. 2016;24:493-9.

16. Gantait A, Barman T, Mukherjee PK. Validated method for estimation of curcumin in turmeric powder. Indian J Tradit Know. 2011;10(2):247-50.

17. Gupta AP, Gupta MM, Kumar S. Simultaneous determination of curcuminoids in curcuma samples using high performance thin layer chromatography. J Liq Chromatogr Relat Technol. 1999;22:1561-9.

18. Abas F, Lajis NH, Shaari K, Israf DA, Stanslas J, Yusuf UK, et al. A labdane diterpene glucoside from the rhizomes of curcuma manga. J Nat Prod. 2005;68(7):1090-3.

19. Kulkarni SJ, Maske KN, Budre MP, Mahajan RP. Extraction and purification of curcuminoids from Turmeric (curcuma longa L.). Int J Pharmacol Pharmaceut Tech. 2012;1(2):81-4.

20. Ahmad W, Hassan A, Ansari A, Tarannum T. Cucurma longa L.- A review. Hippocratic J Unani Med. 2010;5:179-90.

21. Lal J. Turmeric, Curcumin and Our Life: A Review. Bull Environ Pharmacal Life Sci. 2012;1(7):11-7.

22. Himesh S, Sharan PS, Mishra K, Govin N. Qualitative and quantitative profile of curcumin from ethanolic extract of Curcuma longa. Int Res J Pharm. 2011;2(4):180-4.

23. Gritsanapan W. Popular natural cosmetics in Thailand and their natural components. J Health Res. 2004;18(1):23-9.

24. Ammon HPT, Wahl MA. Pharmacology of Curcuma longa. Planta Med. 1991;57(1):1-7.

25. Govindarajan VS, Stahl WH. Turmeric — chemistry, technology, and quality. CRC Crit Rev Food Sci Nutr. 1980;12(3):199-301.

26. Hanif R, Qiao L, Shiff SJ, Rigas B. Curcumin, a natural plant phenolic food additive, inhibits cell proliferation and induces cell cycle changes in colon adenocarcinoma cell lines by a prostaglandin-independent pathway. J Lab Clin Med. 1997;130(6):576-84.

27. Chainani-Wu N. Safety and Anti-Inflammatory Activity of Curcumin: A Component of Tumeric (Curcuma longa). J Altern Complement Med. 2003;9(1):161-8.

28. Naz S, Jabeen S, Ilyas S, Manzoor F, Aslam F, Ali A. Antibacterial activity of Curcuma longa varieties against different strains of bacteria. Pak J Bot. 2010;42(1):455-62.

29. Nisar T, Iqbal M, Raza A, Safdar M, Iftikhar F, Waheed M. Turmeric: a promising spice for phytochemical and antimicrobial activities. Am-Euras J Agric & Environ Sci. 2015;15(7):1278-88.

30. Luthra PM, Singh R, Chandra R. Therapeutic uses of Curcuma longa (turmeric). Ind J Clin Biochem. 2001;16:153-60.

31. Huang HC, Jan TR, Yeh SF. Inhibitory effect of curcumin, an anti-inflammatory agent, on vascular smooth muscle cell proliferation. Eur J Pharmacol. 1992;221(2-3):381-4.

32. Jayaprakasha GK, Negi PS, Anandharamakrishnan C, Sakariah KK. Chemical composition of turmeric oil-a byproduct from turmeric oleoresin industry and its inhibitory activity against different fungi. Z Naturforsch C. 2001;56(1-2):40-4.

33. Ammon HPT, Anazodo MI, Safayhi H, Dhawan BN, Srimal BC. Curcumin: a potent inhibitor of leukotriene B_4 formation in rat peritoneal polymorphonuclear neutrophils (PMNL). Planta Med. 1992;58:226.

34. Golding BT, Pombo E, Samuel CJ. Turmerones: isolation from turmeric and their structure determination. J Chem Soc Chem Commun. 1982;(6):363-4.

35. Rao CV, Rivenson A, Simi B, Reddy BS. Chemoprevention of colon carcinogenesis by dietary curcumin, a naturally occurring plant phenolic compound. Cancer Res. 1995;55:259-66.

36. Shpitz B, Giladi N, Sagiv E, Lev-Ari S, Liberman E, Kazanov D, et al. Celecoxib and curcumin additively inhibit the growth of colorectal cancer in a rat model. Digestion. 2006;74(3-4):140-4.

37. Perkins S, Verschoyle RD, Hill K, Parveen I, Threadgill MD, Sharma RA, et al. Chemopreventive efficacy and pharmacokinetics of curcumin in the min/+ mouse, a model of familial adenomatous polyposis. Cancer Epidemiol Biomarkers Prev. 2002;11(6):535-40.

38. Kwon Y, Magnuson BA. Age-related differential responses to curcumin-induced apoptosis during the initiation of colon cancer in rats. Food Chem Toxicol. 2009;47(2):377-85.

39. Zeng Y, Qiu F, Takahashi K, Liang J, Qu G, Yao X. New sesquiterpenes and calebin derivatives from Curcuma longa. Chem Pharm Bull. 2007;55(6):940-3.

40. Dujic J, Kippenberger S, Ramirez-Bosca A, Diaz-Alperi J, Bereiter-Hahn J, Kaufmann R, et al. Curcumin in combination with visible light inhibits tumor growth in a xenograft tumor model. Int J Cancer. 2009;124(6):1422-8.

41. Huang MT, Lou YR, Xie JG, Ma W, Lu YP, Yen P, et al. Effect of dietary curcumin and dibenzoylmethane on formation of 7,12-dimethylbenz a anthracene-induced mammary tumors and lymphomas/leukemias in sencar mice. Carcinogenesis. 1998;19(9):1697-700.

42. Rabiei Z, Rafieian-kopaei M, Heidarian E, Saghaei E, Mokhtari S. Effects of zizyphus jujube extract on memory and learning impairment induced by bilateral electric lesions of the nucleus basalis of meynert in rat. Neurochem Res. 2014;39(2):353-60.

43. Rabiei Z, Rafieian-Kopaei M, Mokhtari S, Alibabaei Z, Shahrani M. The effect of pretreatment with different doses of lavandula officinalis ethanolic extract on memory, learning and nociception. Biomed Aging Pathol. 2014;4(1):71-6.

44. Khajehdehi P. Turmeric: reemerging of a neglected Asian traditional remedy. J Nephropathol. 2012;1(1):17-22.

45. Venugopal R, Liu RH. Phytochemicals in diets for breast cancer prevention: the importance of resveratrol and ursolic acid. Food Sci Hum Wellness. 2012;1:1-13.

46. Azwanida NN. A review on the extraction methods use in medicinal plants, principle, strength and limitation. Med Aromat Plants. 2015;4(3):196-201.

47. Luque de Castro MD, Priego-Capote F. Soxhlet extraction: past and present panacea. J Chromatogr A. 2010;1217:2383-9.

48. Pothitirat W, Gritsanapan W. Quantitative analysis of curcumin, demethoxycurcumin and bisdethoxycurcumin in the crude curcuminoid extract from curcumin longa in Thailand by TLC-densitometry. Pharm Sci Asia. 2005;32(1-2):23-30.

49. Popuri AK, Pagala B. Extraction of curcumin from turmeric roots. Int J Innovative Res Stud. 2013;2(5):289-99.

50. Avram M, Stroescu M, Stoica-Guzun A, Floarea O, Dobre T. Optimization of curcumin extraction from turmeric powder using a Box-Behnken Design (BBD). Rev Chim (Bucharest). 2015;66(1):78-82.

51. Waghmare P, Patingrao D, Kadu P. Extraction, isolation, purification and identification of curcumin: a review article. Europ J Biomedical & Pharm Sci. 2015;2(3):108-23.

52. Haldar S, Mishra HN, Majumdar GC. Optimization of Oleoresin extraction from Curcuma Longa L. using RSMand determination of equilibrium constant. J Food Process Preserv. 2016;40:1188-98.

53. Trusheva B, Trunkova D, Bankova V. Different extraction methods of biologically active components from propolis: a preliminary study. Chem Cent J. 2007;1(13):1-4.

54. Kaufmann B, Christen P. Recent extraction techniques for natural products: microwave-assisted extraction and pressurised solvent extraction. Phytochem Anal. 2002;13:105-13.

55. Sogi DS, Sharma S, Oberoi DPS, Wani IA. Effect of extraction parameters on curcumin yield from turmeric. J Food Sci Technol. 2010;47(3):300-4.

56. Paulucci PV, Couto RO, Teixeira CCC, Freitas LAP. Optimization of the extraction of curcumin from curcuma longa rhizomes. Rev Bras Farmacogn. 2013;23(1):94-100.

57. Li M, Ngadi MO, Ma Y. Optimisation of pulsed ultrasonic and microwave-assisted extraction for curcuminoids by response surface methodology and kinetic study. Food Chem. 2014;165:29-34.

58. Bener M, Özyürek M, Güçlü K, Apak R. Optimization of microwave-assisted extraction of curcumin from curcuma longa L. (turmeric) and evaluation of antioxidant activity in multi-test systems. Rec Nat Prod. 2016;10(5):542-54.

59. Rezaei S, Najafpour GD, Mohammadi M, Moghadamnia AA, Kazemi S. Formic acid and microwave assisted extraction of curcumin from turmeric (Curcuma longa L.). Int J Eng Transactions B: App. 2016;29(2):145-51.

60. Ebadi T, Moghadamnia AA, Kazemi S, Darzi GN. Enhanced microwave extraction of curcumin from turmeric (Curcuma longa L.). American-Eurasian J Agric & Environ Sci. 2016;16(4):757-64.

61. Liang H, Wang W, Xu J, Zhang Q, Shen Z, Zeng Z, et al. Optimization of ionic liquid-based microwave-assisted extraction technique for curcuminoids from curcuma longa L. Food Bioprod Process. 2017;104:57-65.

62. Dhanani T, Shah S, Gajbhiye NA, Kumar S. Effect of extraction methods on yield, phytochemical constituents and antioxidant activity of Withania somnifera. Arab J Chem. 2013;10(1):S1193-9.

63. Sotanaphun U, Phattanawasin P, Sriphong L. Application of Scion image software to the simultaneous determination of curcuminoids in turmeric (curcuma longa). Phytochem Anal. 2009;20:19-23.

64. Mandal V, Dewanjee S, Sahu R, Mandal SC. Design and optimization of ultrasound assisted extraction of curcumin as an effective alternative for conventional solid liquid extraction of natural product. Nat Prod Commun. 2009;4(1):95-100.

65. Wichitnithad W, Jongaroonngamsang N, Pummangura S, Rojsitthisak P. A simple isocratic HPLC method for the simultaneous determination of curcuminoids in commercial turmeric extracts. Phytochem Anal. 2009;20:314-9.

66. Kim HJ, Jang PY. Direct analysis of curcumin in turmeric by DART-MS. Phytochem Anal. 2009;20:372-7.

67. Zhan PY, Zeng XH, Zhang HM, Li HH. High-efficient column chromatographic extraction of curcumin from curcuma longa. Food Chem. 2011;129:700-3.

68. Long Y, Zhang W, Wang F, Chen Z. Simultaneous determination of three curcuminoids in curcuma longa L. by high performance liquid chromatography coupled with electrochemical detection. J Pharm Anal. 2014;4(5):325-30.

69. Haiyee ZA, Shah SHM, Ismail K, Hashim N, Ismail WIW. Quality parameters of curcuma longa L. extracts by supercritical fluid extraction (SFE) and ultrasonic assisted extraction (UAE). Malaysian J Anal Sci. 2016;20(3):626-32.

70. Felipe Osorio-Tobón J, Carvalho PIN, Barbero GF, Nogueira GC, Rostagno MA, Meireles MAA. Fast analysis of curcuminoids from turmeric (Curcuma longa L.) by high-performance liquid chromatography using a fused-core column. Food Chem. 2016;200:167-74.

71. Rahmalia W, Fabre JF, Mouloungui Z. Effects of cyclohexane/acetone ratio on bixin extraction yield by accelerated solvent extraction method. Procedia Chem. 2015;14:455-64.

72. Braga MEM, Meireles MAA. Accelerated solvent extraction and fractioned extraction to obtain the curcuma longa volatile oil and oleoresin. J Food Process Eng. 2007;30:501-21.

73. Barnwal P, Singh KK, Sharma A, Choudhary AK, Zachariah TJ, Saxena SN. Biochemical, antioxidant and thermal properties of cryogenic and ambient ground turmeric powder. Int Agri Eng J. 2014;23(1):38-46.

74. Mainum SH, Shashikala P. Extraction, nanoformulation and evaluation of curcumin. Int J Pharm. 2014;1(8):520-7.

75. Sahnea F, Mohammadia M, Najafpoura GD, Moghadamnia AA. Enzyme-assisted ionic liquid extraction of bioactive compound from turmeric (Curcuma longa L.): isolation, purification and analysis of curcumin. Ind Crop Prod. 2017;95:686-94.

76. Naudé Y, De Beer WHJ, Jooste S, Van Der Merwe L, Van Rensburg SJ. Comparison of supercritical fluid extraction and soxhlet extraction for the determination of DDT, DDD and DDE in sediment. Water SA. 1998;24:205-14.

77. Braga MEM, Leal PF, Carvalho JE, Meireles MAA. Comparison of yield, composition, and antioxidant activity of turmeric (Curcuma longa L.) extracts obtained using various techniques. J Agric Food Chem. 2003;51:6604-11.

78. Li S, Yuan W, Deng G, Wang P, Yang P, Aggarwal BB. Chemical composition and product quality control of turmeric (Curcuma longa L.). Pharm Crops. 2011;2:28-54.

79. Martins RM, Pereira SV, Siqueira S, Salomão WF, Freitas LAP. Curcuminoid content and antioxidant activity in spray dried microparticles containing turmeric extract. Food Res Int. 2013;50:657-63.

80. Pyo D, Kim E. Rapid and efficient extraction of curcumins from curry powder using supercritical CO_2. Bull Korean Chem Soc. 2014;35(10):3107-10.

81. Song W, Qiao X, Liang WF, Ji S, Yang L, Wang Y, et al. Efficient separation of curcumin, demethoxycurcumin, and bisdemethoxycurcumin from turmeric using supercritical fluid chromatography: from analytical to preparative scale. J Sep Sci. 2015;38:3450-3.

82. Arango-Ruiz Á, Martin Á, Cosero MJ, Jiménez C, Londoño J. Encapsulation of curcumin using supercritical antisolvent (SAS) technology to improve its stability and solubility in water. Food Chem. 2018;258:156-63.

83. Kwon MC, Choi WY, Seo YC, Kim JS, Yoon CS, Lim HW, et al. Enhancement of the skin-protective activities of Centella asiatica L. Urban by a nanoencapsulation Process. J Biotechnol. 2012;157:100-6.

84. Wei L, Zhang W, Yin L, Yan F, Xu Y. Extraction optimization of total triterpenoids from Jatropha curcas leaves using response surface methodology and evaluations of their antimicrobial and antioxidant capacities. Electron J Biotechnol. 2015;18(2):88-95.

85. Vongsak B, Sithisarn P, Mangmool S, Thongpraditchote S, Wongkrajang Y, Gritsanapan W. Maximizing total phenolics, total flavonoids contents and antioxidant activity of Moringa oleifera leaf extract by the appropriate extraction method. Ind Crops Prod. 2013;44:566-71.

86. Mulubwa M, Rheeders M, Du Plessis L, Grobler A, Viljoen M. Development and validation of high performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS) method for determination of tenofovir in small volumes of human plasma. J Chromatogr Sep Tech. 2015;6(7):1-6.

87. Lin G, Jian J, Yuliang R. Determination of Sodium Tanshinone IIA Sulfonate in rat plasma by high performance liquid chromatography and its application to pharmacokinetics studies. Pharm Anal Acta. 2015;6(6):1-4.

88. Myron P, Siddiquee S, Azad SA, Yong YS. Tributylamine facilitated separations of fucosylated chondroitin sulfate (Fucs) by high performance liquid chromatography (HPLC) into its component using 1-Phenyl-3-Methyl-5-Pyrazolone (PMP) derivatization. J Chromatogr Sep Tech. 2014;6(1):1-4.

89. Tang ML, Nguyen JF, Sadoun M, Ruse ND. HPLC analysis of monomer release from conventionally and high temperature high-pressure polymerised urethane dimethacrylate intended for biomedical applications. J Chromatogr Sep Tech. 2014;5(3):1-3.

90. Thammana M. A review on high performance liquid chromatography (HPLC). Res Rev J Pharm Anal. 2016;5(2):22-8.

91. Hafez HM, Elshanawane A, Abdelaziz LM, Mohram MS. Development and validation of HPLC method for simultaneous estimation of Brimonidine Tartrate and Timolol Maleate in bulk and pharmaceutical dosage form. J App Pharm. 2014;6(4):398-407.

92. Mustafa S, Hashim W, Khaliq S, Azizuddin, Khan RA. An improved high performance liquid chromatographic method for tryptophan analysis in rat brain administrated by seaweed. J Anal Bioanal Tech. 2014;5(2):1-7.

93. Caglar S, Alp AR. A validated high performance liquid chromatography method for the determination of saxagliptin and metformin in bulk, a stability indicating study. J Anal Bioanal Tech. 2014;12:1-5.

94. Abdallah NA. Validated stability-indicating HPLC and thin layer densitometric methods for the determination of pazufloxacin: application to pharmaceutical formulation and degradation kinetics. J Chromatogr Sep Tech. 2014;5(2):1-8.

95. de Figueiredo NB, Oiye ÉN, de Menezes MMT, de Andrade JF, Silva MCB, de Oliveira MF. Determination of 3,4-methylenedioxymethamphetamine (MDMA) in confiscated tablets by high-performance liquid chromatography (HPLC) with diode array detector. J Forensic Res. 2010;1(2):1-3.

96. Gurupadayya BM, Disha NS. Stability indicating HPLC method for the simultaneous determination of ceftriaxone and vancomycin in pharmaceutical formulation. J Chromatogr Sep Tech. 2013;4(10):1-5.

97. Akan JC, Sodipo OA, Mohammed Z, Abdulrahman FI. Determination of organochlorine, organophosphorus and pyrethroid pesticide residues in water and sediment samples by high performance liquid chromatography (HPLC) with UV/visible detector. J Anal Bioanal Tech. 2014;5(6):1-5.

98. Parbhunath OL, Rautenbach F, Davison G, Marnewick JA. Optimization and validation of a reverse-phase high performance liquid chromatography assay with ultra-violet detection for measuring total L-ascorbic acid in food and beverage products. J Anal Bioanal Tech. 2014;5(4):1-11.

99. Szterk A, Roszko M, Najman K, Kruk M, Mroczek E, Zarodkiewicz M, et al. Comparison of various detection systems coupled to high performance liquid chromatography for determination of tocopherols in meat. The influence and comparison of the most popular sample preparation method. J Anal Bioanal Tech. 2013;2:1-10.

100. Lories IB, Mostafa AA, Girges MA. High performance liquid chromatography, TLC densitometry, first-derivative and first-derivative ratio spectrophotometry for determination of rivaroxaban and its alkaline degradates in bulk powder and its tablets. J Chromatogr Sep Tech. 2013;4(9):1-6.

101. Sun H, Hao Y, Wu X. A rapid and effective method for simultaneous determination of residual sulfonamides and sarafloxacin in pork and chicken muscle by high performance liquid chromatography with accelerated solvent extraction-solid phase extraction cleanup. J Chromatogr Sep Tech. 2012;3(8):1-6.

102. Virkar PS, Pingale SG, Mangaonkar KV. Development and validation of a high performance liquid chromatography method for determination of telmisartan in rabbit plasma and its application to a pharmacokinetic study. J Anal Bioanal Techniques. 2012; 3(3):1-5.

103. Gugulothu DB, Fernandes CB, Patravale VB. A versatile high performance liquid chromatography method for simultaneous determination of three curcuminoids in pharmaceutical dosage forms. Pharm Anal Acta. 2012;3(4):1-7.

104. Devika GS, Sudhakar M, Rao JV. Simultaneous determination of eprosartan mesylate and hydrochlorthiazide in pharmaceutical dosage form by reverse phase high performance liquid chromatography. Pharm Anal Acta. 2011;2(3)1-5.

105. Harmita, Mansur U, Sartika J. Optimation and validation of analytical method of cotrimoxazole in tablet and plasma in vitro by high performance liquid chromatography. J Bioanal Biomed. 2012;4(2):26-9.

106. Nardulli P, Capparelli E, Perrone MG, Ferraiuolo S, Laforgia MR, Crapolicchio C, et al. A combined HPLC and LC-MS approach for evaluating drug stability in elastomeric devices: a challenge for the sustainability in pharmacoeconomics. J Pharmacovigilance. 2014;3(1):1-6.

107. Shintani H. Immobilized enzyme column combined with HPLC and column switching method for the analysis of complicated matrix such as body fluids. Pharmaceut Reg Affairs. 2014;3(5):1-2.

108. Murthy TGK, Geethanjali J. Development of a validated RP-HPLC method for simultaneous estimation of Metformin hydrochloride and Rosuvastatin calcium in bulk and in-house formulation. J Chromatogr Sep Tech. 2014;5(6):1-7.109.

109. Suresh Babu VV, Sudhakar V, TEGK M. Validated HPLC method for determining related substances in compatibility studies and novel extended release formulation for Ranolazine. J Chromatogr Sep Tech. 2014;5(1):1-7.

110. Arayne MS, Shahnaz H, Ali A, Sultana N. Monitoring of pregabalin in pharmaceutical formulations and human serum using UV and RP-HPLC techniques: application to dissolution test method. Pharm Anal Acta. 2014;5(2):1-7.

111. Praveen C, Ranganath MK, Divakar P. Method development and validation for simultaneous estimation of ethinyl estradiol and drospirenone and forced degradation behavior by HPLC in combined dosage form. Pharm Anal Acta. 2013;4(5):1-5.

112. Abdulla SA, El-Shal MA, Attia AK. Validated HPLC method for the determination of Nisoldipine. Pharm Anal Acta. 2013;S1:1-4.

113. Sawsan Mohammed AH, Azhar Abdul JB, Hammed A. Effects of blood collection tubes on determination vitamin-a by HPLC. J Chromatogr Sep Tech 2013; 4(5):1-3.

114. Subbaiah PR, Kumudhavalli MV, Saravanan C, Kumar M, Chandira RM. Method development and validation for estimation of Moxifloxacin HCl in tablet dosage form by RP-HPLC method. Pharm Anal Acta. 2010;1(2):1-2.

115. Ahir KB, Patelia EM, Shah A. Simultaneous estimation of Metformin Hydrochloride and Repaglinide in pharmaceutical formulation by HPTLC-densitometry method. J Chromatogr Sep Tech 2013;4(1):1-5.

116. Vali1 SJ, Kumar SS, Sait SS, Garg LK. Separation and quantification of Octahydro-1H-indole-2-carboxylic acid and its three isomers by HPLC using refractive index detector. J Chromatogr Sep Tech. 2012;3(5):1-6.

117. Fayyad MK, Misha AK, Al-Musaimi OIY. Effect of temperature, wavelength, pH, ion pair reagents and organic modifiers' concentration on the elution of Cystatin C. Stability of mobile phase. J Anal Bioanal Techniques. 2010;1(1):1-4.

118. Ndorbor T, Wang Y, Huaijing D, Zhizhang D, Kolawole JA, Hua H. Chromatographic and molecular simulation study on the chiral recognition of atracurium besylate positional isomers on Cellulose tri-3, 5-dimethylphenycarbamate (CDMPC) column and its recognition mechanism. J Chromatogr Sep Tech. 2013;4(3):1-8.

119. Hua Z, Yuesheng D, Ge X, Menglu Y, Liya D, LiJia A. Extraction and purification of anthocyanins from the fruit residues of *Vaccinium uliginosum Linn*. J Chromatogr Sep Tech. 2013;4(2):1-5.

120. Flores HE, Galston AW. Analysis of polyamines in higher plants by high performance liquid chromatography. Plant Physiol. 1982;69:701-6.

121. Mesbah M, Premachandran U, Whitman WB. Precise measurement of the G + C content of deoxyribonucleic acid by high-performance liquid chromatography. Int J SystEvol Micr. 1989;39(2):159-67.

122. Tamaoka J, Komagata K. Determination of DNA base composition by reversed-phase high-performance liquid chromatography. FEMS Microbiol Lett. 1984;25:125-8.

123. Shintani H. Validation study in membrane chromatography adsorber and phenyl hydrophobic membrane chromatography adsorber for virus clearance and removal of many other components. Pharm Anal Acta. 2013;2:1-8.

124. Gawhari, F. Development of a non-ionic surfactant vesicles formulation of gemcitabine for pulmonary delivery. [cited 2020 July 17] Available from: file:///C:/Users/it%20corner/Downloads/Developmentofnonionicsurfactantvesiclesformulationofgemcitabineforpulmonarydelivery%20(1).pdf.

125. Badgujar DC, Sawant U, Mahadik H, Gadewal N, Varma AK. Pathogenicity of mutations discovered in BRCA1 BRCT domains is characterized by destabilizing the hydrophobic interactions. J Cancer Sci Ther. 2012;4(11):386-93.

126. Ukuku DO, Geveke DJ, Cooke PH. Effect of thermal and radio frequency electric fields treatments on *Escherichia coli* bacteria in apple juice. J Microb Biochem Technol. 2012;4(3):76-81.

127. Pandarinath P, Rao AA, Devi GL. A python based hydrophilicity plot to assess the exposed and buried regions of a protein. J Proteomics Bioinform. 2011;4(7):145-6.

128. Lu M, Whitelegge JP, Whelan SA, He J, Saxton RE, Faull KF. Hydrophobic fractionation enhances novel protein detection by mass spectrometry in triple negative breast cancer. J Proteomics Bioinform. 2010;3(2):1-10.

129. Morgante PG, Vicente FF, Coffani-Nunes JV, Moreno PRH. Establishment of simple and efficient methods for plant material harvesting and storage to allow DNA extraction from a Myrtaceae species with medicinal potential. Int J Genomic Med. 2013; 1(1):1-5.

130. Shah DA, Patelia EM, Mori A. Simultaneous estimation of Pregabalin and Methylcobalamine in pharmaceutical formulation by HPTLC-densitometry method. J Chromatogr Sep Tech. 2013;4(2):1-4.

131. Mehta FA, Patelia EM, Bhoya PN. Simultaneous estimation of Ambroxol Hydrochloride and Doxofylline in pharmaceutical formulation by HPTLC-desitometric method. J Chromatogr Sep Tech. 2013;4(2):1-4.

132. Boadu RF, Agyare C, Adarkwa-Yiadom M, Adu F, Boamah VE, Boakye YD. In vitro activity and evaluation of quality of some selected penicillins on the Ghanaian market using developed HPLC methods. Med chem. 2015;5(1):1-14.

133. Sultana N, Arayne MS, Shah SN. Development and validation for the simultaneous quantification of prazosin, amlodipine, diltiazem and verapamil in API, dosage formulation and human serum by RP-HPLC: Application to in vitro interaction studies. Med chem. 2014;4(12):770-7.

134. Olbrich J, Corbett J. Development and utilization of reversed phase high performance liquid chromatography methods for a series of therapeutic agents. Mod Chem Appl. 2013;1(2):1-6.

135. Hao K, Zhao XP, Liu XQ, Wang GJ. LC determination of curcumin in dog plasma for a pharmacokinetic study. Chromatographia. 2006;64:531-5.

136. Jadhav BK, Mahadik KR, Paradkar AR. Development and validation of improved reversed phase-HPLC method for simultaneous determination of curcumin, demethoxycurcumin and bis-demethoxycurcumin. Chromatographia. 2007;65:483-8.

137. Ma Z, Shayeganpour A, Brocks DR, Lavasanifar A, Samuel J. High-performance liquid chromatography analysis of curcumin in rat plasma: application to pharmacokinetics of polymeric micellar formulation of curcumin. Biomed Chromatogr. 2007;21:546-52.

138. Paramasivam M, Aktar MW, Poi R, Banerjee H, Bandyopadhyay A. Occurrence of curcuminoids in *Curcuma longa* : A quality standardization by HPTLC. Bangladesh J Pharmacol. 2008;3:55-8.

139. Dandekar PP, Patravale VB. Development and validation of a stability-indicating LC method for curcumin. Chromatographia. 2009;69:871-7.

140. Paramapojn S, Gritsanapan W. Free radical scavenging activity determination and quantitative analysis of curcuminoids in *Curcuma zedoaria* rhizome extracts by HPLC method. Current Science. 2009;97(7):1069-73.

141. Marczylo TH, Steward WP, Gescher AJ. Rapid analysis of curcumin and curcumin metabolites in rat biomatrices using a novel ultraperformance liquid chromatography (UPLC) method. J Agric Food Chem. 2009;57:797-803.

142. Ramshankar YV, Suresh S, Pandit V, Puranik SB. Reverse phase high performance liquid chromatographic method for the estimation of curcumin. Orient J Chem. 2009;25(1):105-10.

143. Li J, Jiang Y, Wen J, Fan G, Wu Y, Zhang C. A rapid and simple HPLC method for the determination of curcumin in rat plasma: assay development, validation and application to a pharmacokinetic study of curcumin liposome. Biomed Chromatogr. 2009;23:1201-7.

144. Kishore CN, Shrivastava A, Anurekha J. Stability-indicating RP-HPLC determination of curcumin in Vicco Turmeric cream and Haridrakhand churna. Phcog J. 2010;2(6):90-101.

145. Wahajuddin SSP, Jain GK. Determination of curcumin in rat plasma by liquid–liquid extraction using LC–MS/MS with electrospray ionization: assay development, validation and application to a pharmacokinetic study. J Bioanal Biomed. 2010;2(4): 79-84.

146. Han YR, Zhu JJ, Wang YR, Wang XS, Liao YH. A simple RP-HPLC method for the simultaneous determination of curcumin and its prodrug, curcumin didecanoate, in rat plasma and the application to pharmacokinetic study. Biomed Chromatogr. 2011; 25:1144-9.

147. Vyas N, Gamit K, Khan MY, Panchal S, Pundarikakshudu K. Simultaneous estimation of curcumin and piperine in their crude powder mixture and ayurvedic formulation using high performance thin layer chromatography. Int J Pharm BiomedSci. 2011;2(1):230-6.

148. Lee JH, Choung MG. Determination of curcuminoid colouring principles in commercial foods by HPLC. Food Chem. 2011;124:1217-22.

149. Wang XM, Zhang QZ, Yang J, Zhu RH, Zhang J, Cai LJ, et al. Validated HPLC–MS/MS method for simultaneous determination of curcumin and piperine in human plasma. Trop J Pharm Res. 2012;11(4):621-9.

150. Moorthi C, Kumar CS, Mohan S, Krishnan K, Kathiresan K. Application of validated RP-HPLC-PDA method for the simultaneous estimation of curcumin and piperine in Eudragit E 100 nanoparticles. J Pharm Res. 2013;7:224-9.

151. Purohit PJ, Kapupara PP, Shah KV. Development and validation of analytical method for simultaneous estimation of curcumin and gallic acid in different polyherbal formulations by HPLC. Res J Pharm and Tech. 2014;7(7):748-53.

152. Li HX, Zhang HL, Zhang N, Wang N, Yang Y, Zhang ZZ. Isolation of three curcuminoids for stability and simultaneous determination of only using one single standard substance in turmeric colour principles by HPLC with ternary gradient system. LWT-Food Sci Tech. 2014;57(1):446-51.

153. Siddiqui NA. Evaluation of thermo sensitivity of curcumin and quantification of ferulic acid and vanillin as degradation products by a validated HPTLC method. Pak J Pharm Sci. 2015;28(1):299-305.

154. Syed HK, Liew KB, Loh GOK, Peh KK. Stability indicating HPLC–UV method for detection of curcumin in Curcuma longa extract and emulsion formulation. Food Chem. 2015;170:321-6.

155. Puttaiah K, Harshan HR, Nitin MS, Kumar S, Lingam Y. A simple binary reverse phase high performance liquid chromatographic method for the determination of curcumin, demethoxycurcumin, and bisdemethoxycurcumin. Nat Prod: An Indian J. 2016;12(1):26-30.

156. Setyaningsih D, Murti YB, Martono S, Hinrichs WLJ, Hertiani T, Fudholi A. A novel reversed phase high performance liquid chromatography method to accurately determine low concentrations of curcumin in rat plasma. Int J Pharm Clinic Res. 2016;8(5):377-86.

157. Shervington L, Ingham O, Shervington A. Purity determination of three curcuminoids found in ten commercially available turmeric dietary supplements using a reverse phase HPLC method. Nat Prod Chem Res. 2016;4(6):1-6.

158. Kalra R, Diwan A, Kumar J, Sharma S. Simultaneous estimation of Artemether & Curcumin by RP-HPLC method. Pharmacophore. 2016;7(3):141-51.

159. Panigrahi S, Hirlekar R. A new stability-indicating RP-HPLC method for determination of curcumin: an application to nanoparticulate formulation. Int J Pharmacy Pharm Sci. 2016;8(12):149-55.

160. Korany MA, Haggag RS, Ragab MAA, Elmallah OA. A validated stability-indicating HPLC method for simultaneous determination of Silymarin and Curcumin in various dosage forms. Arab J Chem. 2017;10:S1711-25.

161. Fonseca-Santos B, Gremiao MPD, Chorilli M. A simple reversed phase high-performance liquid chromatography (HPLC) method for determination of *in situ* gelling curcumin-loaded liquid crystals in *in vitro* performance tests. Arab J Chem. 2017;10: 1029-37.

162. Mishra J, Tripathi A, Misra K, Sarada SKS. HPTLC: a tool for determination of curcumin in mammalian samples. Int J Pharmacognosy and Phytochem Res. 2017;9(4): 498-501.

163. Prabaningdyah NK, Riyanto S, Rohman A, Siregar C. Application of HPLC and response surface methodology for simultaneous determination of curcumin and desmethoxycurcumin in Curcuma syrup formulation. J Appl Pharm Sci. 2017;7(12):58-64.

164. Siregar C, Prabaningdyah NK, Choiri S, Riyanto S, Rohman A. Optimization of HPLC using central composite design for determination of curcumin and demethoxycurcumin in tablet dosage form. Dhaka Univ J Pharm Sci. 2017;16(2):137-45.

165. Amanolahi F, Mohammadi A, Oskuee RK, Nassirli H, Malaekeh-Nikouei B. A simple, sensitive and rapid isocratic reversed-phase high-performance liquid chromatography method for determination and stability study of curcumin in pharmaceutical samples. Avicenna J Phytomed. 2017;7(5):444-53.

166. Wang J, Li Y, Ma W, Wang X, Tu P. Validated LC–MS/MS method for simultaneous determination of doxorubicin and curcumin in polymeric micelles in subcellular compartments of MCF-7/Adr cells by protein precipitation–ultrasonic breaking method. Biomed Chromatogr. 2017;31(6):1-9.

167. Kim DW, Yousaf AM, Li DX, Kim JO, Yong CS, Cho KH, et al. Development of RP-HPLC method for simultaneous determination of docetaxel and curcumin in rat plasma: validation and stability. Asian J Pharm Sci. 2017;12(1):105-13.

168. Shaikh S, Jain V. Development and validation of a RP-HPLC method for the simultaneous determination of curcumin, piperine and camphor in an ayurvedic formulation. Int J Pharmacy Pharm Sci. 2018;10(4):115-21.

169. Mahale J, Howells YM, Singh R, Britton RG, Cai H, Brown K. An HPLC-UV method for the simultaneous quantification of curcumin and its metabolites in plasma and lung tissue: potential for preclinical applications. Biomed Chromatogr. 2018;32:1-8.

170. Reolon JB, Brustolin M, Haas SE, Bender EA, Malesuik MD, Colomé LM. Development and validation of high-performance liquid chromatography method for simultaneous determination of acyclovir and curcumin in polymeric microparticles. J Appl Pharm Sci. 2018;8(1):136-41.

171. Treesinchai S, Pitaksuteepong T, Sungthongjeen S. Determination of curcumin stability in various gastro-intestinal pH by Arrhenius equation using HPLC method. Pharm Sci Asia. 2020;47(1):86-96.

172. Galen WE. Instrumental Methods of Chemical Analysis. J Chem Educ. 1960;37(11):A750-2. 173.

173. Passos MLC, Saraiva MLMFS. Detection in UV-visible spectrophotometry: detectors, detection systems, and detection strategies. Measurement. 2019;135:896-904.

174. Skoog DA, Holler FJ, Nieman TA. Principles of instrumental analysis. 5th ed. Mississippi State, United States of America: RR Donnelley; 1998. p. 312-25.

175. Christian GD, Dasgupta PK, Schug KA. Analytical Chemistry.7th ed. Washington, United States of America: Wiley; 2014. p. 504-13.

176. Grossman WEL. A comparison of optical-detectors for the visible and ultraviolet. J Chem Edu. 1989;66(8):697-700.

177. Sweedler JV, Jalkian RD, Denton MB, A linear charge-coupled device detector system for spectroscopy. Appl Spectrosc. 1989;43(6):953-62.

178. Dasgupta PK, Eom IY, Morris KJ, Li JZ. Light emitting diode-based detectors-Absorbance, fluorescence and spectroelectrochemical measurements in a planar flow-through cell. Anal Chim Acta. 2003;500(1-2):337-64.

179. Kovac J, Peternai L, Lengyel O. Advanced light emitting diodes structures for optoelectronic applications. Thin Solid Films. 2003;433(1-2):22-6.

180. Dasgupta PK, Bellamy HS, Liu HH, Lopez JL, Loree EL, Morris K, et al. Lightemitting diode based flow-through optical absorption detectors. Talanta. 1993; 40(1):53-74.

181. Mims FM. Sun photometer with light-emitting diodes as spectrally selective detectors. Appl Opt. 1992;31(33):6965-7.

182. Miyazaki E, Itami S, Araki T. Using a light-emitting diode as a high-speed, wavelength selective photodetector. Rev Sci Instrum. 1998;69:3751-4.

183. Tymecki L, Koncki R. Simplified paired-emitter-detector-diodes-based photometry with improved sensitivity. Anal Chim Acta. 2009;639(1-2):73-7.

184. Tumecki L, Brodacka L, Rozum B, Koncki R. UV-PEDD photometry dedicated for bioanalytical uses. Analyst. 2009;134:1333-7.

185. Melhorn CJ. Multimeters. Wiley Encyclopedia of Electrical and Electronics Engineering; 1999. p. 634.

186. Tang B, Ma L, Wang HY, Zhang GY. Study on the supramolecular interaction of curcumin and â-cyclodextrin by spectrophotometry and its analytical application. J Agric Food Chem. 2002;50(6):1355-61.

187. Sharma K, Agrawal SS, Gupta M. Development and validation of UV spectrophotometric method for the estimation of curcumin in bulk drug and pharmaceutical dosage forms. Int J Drug Dev Res. 2012;4(2):375-80.

188. da Silva-Buzanello RA, Ferro AC, Bona E, Cardozo-Filho L, de Araújo PHH, Leimann FV, et al. Validation of an ultraviolet–visible (UV–Vis) technique for the quantitative determination of curcumin in poly (L-lactic acid) nanoparticles. Food Chem. 2015;172:99-104.

189. Hazra K, Kumar R, Sarkar BK, Chowdary YA, Devgan M, Ramaiah M. UV-visible spectrophotometric estimation of curcumin in nano-formulation. Int J Pharm. 2015;2(3): 127-30.

190. Sharma S, Sapaogi GK, Kumar V. Development of spectrophotometric methods for simultaneous determination of artesunate and curcumin in liposomal formulation. Int J App Pharm. 2015;7(1):18-21.

191. Asfaram A, Ghaedi M, Alipanahpour E, Agarwal S, Gupta VK. Application of response surface methodology and dispersive liquid-liquid microextraction by microvolume spectrophotometry method for rapid determination of curcumin in water, wastewater, and food samples. Food Anal Methods. 2016;9:1274-83.

192. Liu X, Zhu L, Gao X, Wang Y, Lu H, Tang Y, et al. Magnetic molecularly imprinted polymers for spectrophotometric quantification of curcumin in food. Food Chem. 2016;202:309-15.

193. Savale SK. Simultaneous determination of curcumin and Gefitinib in pure form by using UV spectrophotometric method. Hygeia J D Med. 2017;9(1):1-8.

194. Afkhami A, Pirdadeh-beiranvand M, Madrakian T. A method based on ultrasound-assisted solidification of floating drop microextraction technique for the spectrophotometric determination of curcumin in turmeric powder. Anal Bioanal Chem Res. 2017;4(1):1-10.

195. Savale SK. UV spectrophotometric method development and validation for quantitative estimation of curcumin. Asian J Biomater Res. 2017;3(4):14-8.

196. Rapalli VK, Kaul V, Gorantla S, Waghule T, Dubey SK, Pandey MM, et al. UV spectrophotometric method for characterization of curcumin loaded nanostructured lipid nanocarriers in simulated conditions: Method development, in-vitro and ex-vivo applications in topical delivery. Spectrochim Acta A. 2020; 224:117392.

197. Talmi Y. Spectrophotometry and spectrofluorometry with the self-scanned photodiode array. Appl Spectrosc. 1982;36(1):1-18.

198. Epperson PM, Jalkian RD, Denton MB. Molecular fluorescence measurements with a charge-coupled device detector. Anal Chem. 1989;61(3):282-5.

199. Bright FV. Bioanalytical applications of fluorescence spectroscopy. Anal Chem. 1988;60(18):1031A-9A.

200. Wang F, Wu X, Wang F, Liu S, Jia Z, Yang J. The Sensitive fluorimetric method for the determination of curcumin using the enhancement of mixed micelle. J Fluoresc. 2006;16(1):53-9.

201. Mazzarino L, Bellettini IC, Minatti E, Lemos-Senna E. Development and validation of a fluorimetric method to determine curcumin in lipid and polymeric nanocapsule suspensions. Braz J Pharm Sci. 2010;46(2):219-26.

202. Kalaycioglu Z, Hashemi P, Günaydın K, Erim FD. The sensitive capillary electrophoretic-LIF method for simultaneous determination of curcuminoids in turmeric by enhancing fluorescence intensities of molecules upon inclusion into (2-hydroxypropyl)-β-cyclodextrin. Electrophoresis. 2015;36:2516-21.

203. Nugraha SN, Batubara I, Rahmat M, Alatas H. Fluorometric based procedure for measuring curcumin concentration in commercial herbal medicines. Optik. 2017; 149:125-31.

204. Bian W, Wang X, Wang Y, Yang H, Huang J, Cai Z, et al. Boron and nitrogen co-doped carbon dots as a sensitive fluorescent probe for the detection of curcumin. Luminescence. 2018;33(1):174-80.

205. Lechtenberg M, Quandt B, Nahrstedt A. Quantitative determination of curcuminoids in Curcuma rhizomes and rapid differentiation of Curcuma domestica

Val. and Curcuma xanthorrhiza Roxb. by capillary electrophoresis. Phytochem Anal. 2004;15(3):152-8.

206. Tanaka K, Kuba Y, Sasaki T, Hiwatashi F, Komatsu K. Quantitation of curcuminoids in curcuma rhizome by near-infrared spectroscopic analysis. J Agric Food Chem. 2008;56(19):8787-92.

207. Thongchai W, Liawruangrath B, Liawruangrath S. Flow injection analysis of total curcuminoids in turmeric and total antioxidant capacity using 2,20-diphenyl-1-picrylhydrazyl assay. Food Chem. 2009;112:494-9.

208. Ziyatdinova GK, Nizamova AM, Budnikov HC. Voltammetric determination of curcumin in spices. J Anal Chem. 2012;67(6):591-4.

209. Zhang D, Ouyang X, Ma J, Li L, Zhang Y. Electrochemical behavior and voltammetric determination of curcumin at electrochemically reduced graphene oxide modified glassy carbon electrode. Electroanalysis. 2016;28:749-56.

210. Chaisiwamongkhol K, Ngamchuea K, Batchelor-McAuley C, Compton RG. Multiwalled carbon nanotube modified electrodes for the adsorptive stripping voltammetric determination and quantification of curcumin in turmeric. Electroanalysis. 2017;29:1049-55.

211. Lindermeier M, Hofmann T. Influence of baking conditions and precursor supplementation on the amounts of the antioxidant pronyl-L-lysine in bakery products. J Agric Food Chem. 2004;52(2):350-4.

212. Borrelli RC, Fogliano V. Bread crust melanoidins as potential prebiotic ingredients. Mol Nutr Food Res. 2005;49:673-8.

213. Van Boekel M, Fogliano V, Pellegrini N, Stanton C, Scholz G, Lalljie S, et al. A review on the beneficial aspects of food processing. Mol Nutr Food Res. 2010;54:1215-47.

214. Capuano E, Fogliano V. Acrylamide and 5-hydroxymethylfurfural (HMF): A review on metabolism, toxicity, occurrence in food and mitigation strategies. LWT-Food Sci Tech. 2011;44:793-810.

215. Tricker AR, Preussmann R. Carcinogenic N-nitrosamines in the diet: occurrence, formation, mechanism and carcinogenic potential. Mutat Res. 1991;259(3-4):277-89.

216. Skog KI, Johansson MAE, Jägerstad MI. Carcinogenic heterocyclic amines in model systems and cooked foods: a review on formation, occurrence and intake. Food Chem Toxicol. 1998;36(9-10):879-96.

217. Swedish National Food Administration. Information about acrylamide in food. [updated 2002; cited 2020 Mar 16] Available from: http: //www.slv.seS.

218. Mottram DS, Wedzicha BL, Dodson AT. Acrylamide is formed in the Maillard reaction. Nature. 2002;419(6906):448-9.

219. Stadler RH, Blank I, Varga N, Robert F, Hau J, Guy PA, et al. Acrylamide from Maillard reaction products. Nature. 2002;419(6906):449-50.

220. Zyzak DV, Sanders RA, Stojanovic M, Tallmadge DH, Eberhardt BL, Ewald DK, et al. Acrylamide formation mechanism in heated foods. J Agric Food Chem. 2003; 51(16):4782-7.

221. Stadler RH, Robert F, Riediker S, Varga N, Davidek T, Devaud S, et al. In-depth mechanistic study on the formation of acrylamide and other vinylogous compounds by the Maillard reaction. J Agric Food Chem. 2004;52(17):5550-8.

222. Weisshaar R, Gutsche B. Formation of acrylamide in heated potato products-model experiments pointing to asparagine as precursor. Deut Lebensm-Randsch. 2002;98(11):397-400.

223. Yaylayan VA, Wnorowski A, Perez-Locas C. Why asparagine needs carbohydrates to generate acrylamide. J Agric Food Chem. 2003;51(6):1753-7.

224. Yasuhara A, Tanaka Y, Hengel M, Shibamoto T. Cromatographic investigation on acrylamide formation in browning model systems. J Agric Food Chem. 2003;51(6): 1753-7.

225. Claus A, Weisz GM, Schieber A, Carle R. Pyrolytic acrylamide formation from purified wheat gluten and gluten-supplemented wheat bread rolls. Mol Nutr Food Res. 2006;50:87-93.

226. Granvogl M, Schieberle P. Thermally generated 3-amminopropionammide as a transient intermediate in the formation of acrylamide. J Agric Food Chem. 2006;54(16): 5933-8.

227. Granvogl M, Jezussek M, Koehler P, Schieberle P. Quantification of 3-amminopropionammide in potatoes-a minor but potent precursor in acrylamide formation. J Agric Food Chem. 2004;52(15):4751-7.

228. Granvogl M, Schieberle P. Quantification of 3-aminopropionamide in cocoa, coffee and cereal products. Correlation with acrylamide concentrations determined by an improved clean-up method for complex matrices. Eur Food Res Technol. 2007; 225:857-63.

229. Kleefisch G, Kreutz C, Bargon J, Silva G, Schalley CA. Quartz microbalance sensor for the detection of acrylamide. Sensors. 2004;4:136-46.

230. European Food Safety Agency (EFSA) (2009). Scientific report of EFSA prepared by data collection and exposure unit (DATEX) on "Results on the monitoring of acrylamide levels in food". The EFSA Scientific Report, 285, p. 1-26.

231. European Commission. Commission recommendation 2007/331/EC: on the monitoring of acrylamide levels in food; L123, 33-40. [updated 2007; cited 2020 Mar 16] Available from:https://eur-lex.europa.eu/legal content/EN/TXT/?uri=CE L E X %.

232. Amrein TM, Lukac H, Andres L, Perren R, Escher F, Amadò R. Acrylamide in roasted almonds and hazelnuts. J Agric Food Chem. 2005;53(20):7819-25.

233. Amrein TM, Andres L, Escher F, Amadò R. Occurrence of acrylamide in selected foods and mitigation options. Food Addit Contam. 2007;24(1):13-25.

234. Lineback D, Wenzi T, Ostermann OP, Calle B, Anklam E, Taeymans D. Overview of Acrylamide Monitoring Databases. J AOAC Int. 2005;88(1):246-52.

235. Boon PE, de Mul A, van der Voet H, van Donkersgoed G, Brette M, van Klaveren JD. Calculation of dietary exposure to acrylamide. Mutat Res. 2005;580(1-2): 143-55.

236. Dybing E, Farmer PB, Andersen M, Fennell TR, Lalljie SP, Muller DJ, et al. Human exposure and internal dose assessments of acrylamide in food. Food Chem Toxicol. 2005;43(3):365-410.

237. World Health Organization. Summary report of the sixty-fourth meeting of the Joint FAO/WHO expert committee on food additive (JECFA). Rome, Italy: World Health Organization press; 2005.

238. Heudorf U, Hartmann E, Angerer J. Acrylamide in children-exposure assessment via urinary acrylamide metabolites as biomarkers. Int J Hyg Environ Health. 2009;212(2):135-41.

239. Arribas-Lorenzo G, Morales FJ. Dietary exposure to acrylamide from potato crisps to the Spanish population. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 2009;26(3):289-97.

240. Eriksson S, Karlsson P. Some analytical factors affecting measured levels of acrylamide in food products. Adv Exp Med Biol. 2005;561:285-91.

241. Wenzl T, Lachenmeier DW, Gökmen V. Analysis of heat-induced contaminants (acrylamide, chloropropanols and furan) in carbohydrate-rich food. Anal Bioanal Chem. 2007;389:119-37.

242. Koch M, Bremser W, Koeppen R, Siegel D, Toepfer A, Nehls I. Development of two certified reference materials for acrylamide determination in foods. J Agric Food Chem. 2009;57(18):8202-7.

243. Hagmar L, Wirfalt E, Paulsson B, Tornqvist M. Differences in hemoglobin adduct levels of acrylamide in the general population with respect to dietary intakes, smoking habits, and gender. Mutat Res. 2005;580(1-2):157-65.

244. Bjellaas T, Stølen LH, Haugen M, Paulsen JE, Alexander J, Lundanes E, et al. Urinary acrylamide metabolites as biomarkers for short-term dietary exposure to acrylamide. Food Chem Toxicol. 2007;45(6):1020-6.

245. Wilson KM, Balter K, Adami HO, Grönberg H, Vikström AC, Paulsson B, et al. Acrylamide exposure measured by food frequency questionnaire and hemoglobin adduct levels and prostate cancer risk in the Cancer of the Prostate in Sweden Study. Int J Cancer. 2009;124(10):2384-90.

246. European Food Safety Agency. EFSA's 11th Scientific Colloquium-Acrylamide Carcinogenicity-New evidence in relation to dietary exposure-22 and 23 May 2008. Tabiano, Italy: European Food Safety Agency press; 2008.

247. International Agency for Research on Cancer. Acrylamide. In IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Lyon, France: International Agency for Research on Cancer; 1994. p. 389-433.

248. European Commission. Join research centre: Acrylamide-Summary risk assessment report. Oxfordshire, United Kingdom: European Commission; 2002.

249. Shipp A, Lawrence G, Gentry R, McDonald T, Bartow H, Bounds J, et al. Acrylamide: review of toxicity data and dose-response analyses for cancer and noncancer effects. Crit Rev Toxicol. 2006;36(6-7):481-608.

250. Sorgel F, Weissenbacher R, Kinzig-Schippers M, Hofmann A, Illauer M, Skott A, et al. Acrylamide: increased concentrations in homemade food and first evidence of its variable absorption from food, variable metabolism and placental and breast milk transfer in humans. Chemotherapy. 2002;48(6):267-74.

251. Schettgen T, Kütting B, Hornig M, Beckmann MW, Weiss T, Drexler H, et al. Trans-placental exposure of neonates to acrylamide-a pilot study. Int Arch Occup Environ Health. 2004;77(3)-213-6.

252. Abramson-Zetterberg L, Wong J, Ilbäck NG. Acrylamide tissue distribution and genotoxic effects in a common viral infection in mice. Toxicology. 2005;211(1-2):70-6.

253. Doerge DR, da Costa GG, McDaniel LP, Churchwell MI, Twaddle NC, Beland FA. DNA adducts derived from administration of acrylamide and glycidamide to mice and rats. Mutat Res. 2005;580(1-2):131-41.

254. Sumner SCJ, Fennell TR, Moore TA, Chanas B, Gonzalez F, Ghanayem BI. Role of cytochrome P450 2E1 in the metabolism of acrylamide and acrylonitrile in mice. Chem Res Toxicol. 1999;12(11):1110-6.

255. Fennell TR, Sumner SC, Snyder RW, Burgess J, Spicer R, Bridson WE, et al. Metabolism and hemoglobin adduct formation of acrylamide in humans. Toxicol Sci. 2005;85(1):447-59.

256. Boettcher MI, Bolt HM, Drexler H, Angerer J. Excretion of mercapturic acids of acrylamide and glycidamide in human urine after single oral administration of deuterium-labelled acrylamide. Arch Toxicol. 2006;80(2):55-61.

257. Fuhr U, Boettcher MI, Kinzig-Schippers M, Weyer A, Jetter A, Lazar A, et al. Toxicokinetics of acrylamide in humans after ingestion of a defined dose in a test meal to improve risk assessment for acrylamide carcinogenicity. Cancer Epidemiol Biomark Prev. 2006;15(2):266-71.

258. Hagmar L, Tornqvist M, Nordander C, Rosen I, Bruze M, Kautiainen A, et al. Health effects of occupational exposure to acrylamide using hemoglobin adducts as biomarkers of internal dose. Scand J Work Environ Health. 2001;27(4):219-26.

259. Friedman M. Chemistry, biochemistry and safety of acrylamide: A review. J Agric Food Chem. 2003;51(16):4504-26.

260. Kirman CR, Gargas ML, Deskin R, Tonner-Navarro L, Andersen ME. A physiologically based pharmacokinetic model for acrylamide and its metabolite, glycidamide, in the rat. J Toxicol Environ Health. 2003;66(3):253-74.

261. Walker K, Hattis D, Russ A, Sonawane B, Ginsberg G. Approaches to acrylamide physiologically based toxicokinetic modeling for exploring child-adult dosimetry differences. J Toxicol Environ Health. 2007;70(24):2033-55.

262. Young JF, Luecke RH, Doerge DR. Physiologically based pharmacokinetic/pharmacodynamic model for acrylamide and its metabolites in mice, rats, and humans. Chem Res Toxicol. 2007;20(3):388-99.

263. Ghanayem BI, McDaniel LP, Churchwell MI, Twaddle NC, Snyder R, Fennell TR, et al. Role of CYP2E1 in the epoxidation of acrylamide to glycidamide and formation of DNA and hemoglobin adducts. Toxicol Sci. 2005;88(2):311-8.

264. Autrup H. Genetic polymorphisms in human xenobiotica metabolizing enzymes as susceptibility factors in toxic response. Mutat Res. 2000;464(1):65-76.

265. Duale N, Bjellaas T, Alexander J, Becher G, Haugen M, Paulsen JE, et al. Biomarkers of human exposure to acrylamide and relation to polymorphisms in metabolizing genes. Toxicol Sci. 2009;108(1):90-9.

266. Lopachin RM. Changing view of acrylamide neurotoxicity. Neurotoxicology, 2004;25(4):617-30.

267. Rice JM. Carcinogenicity of acrylamide. Mutat Res. 2005;580(1-2):3-20.

268. Johnson KA, Gorzinski SJ, Bodner KM, Campbell RA, Wolf CH, Friedman MA, et al. Chronic toxicity and oncogenicity study on acrylamide incorporated in the drinking water of Fisher 344 rats. Toxicol Appl Pharmacol. 1986;85(2):154-68.

269. Friedman MA, Dulak LH, Stedham MA. A lifetime oncogenicity study in rats with acrylamide. Toxicol Sci. 1995;27(1):95-105.

270. Lamy E, Volkel Y, Roos PH, Kassie F, Mersch-Sundermann V. Ethanol enhanced the genotoxicity of acrylamide in human, metabolically competent HepG2 cells by CYP2E1 induction and glutathione depletion. Int J Hyg Environ Health. 2008;211(1-2):74-81.

271. Sickles DW, Sperry AO, Testino A, Friedman M. Acrylamide effects on kinesin- related proteins of the mitotic/meiotic spindle. Toxicol Appl Pharmacol. 2007;222(1):111-21.

272. Chatzizacharias NA, Kouraklis GP, Theocharis SE. Disruption of FAK signaling: a side mechanism in cytotoxicity. Toxicology. 2008;245(1-2):1-10.

273. Schettgen T, Broding HC, Angerer J, Drexler H. Hemoglobin adducts of ethylene oxide, propylene oxide, acrylonitrile, and acrylamide-biomarkers in occupational and environmental medicine. Toxicol Lett. 2002;134(1-3):65-70.

274. Tareke E, Lyn-Cook B, Robinson B, Ali SF. Acrylamide: a dietary carcinogen formed in vivo?. J Agric Food Chem. 2008;56(15):6020-3.

275. Tareke E, Heinze TM, Da Costa GA, Syed A. Acrylamide formed at physiological temperature as a result of asparagine oxidation. J Agric Food Chem. 2009;57(20):9730-3.

276. Armstrong D, Abdella N, Salman A, Miller N, Abdel RE, Bojancyzk M. Relationship of lipid peroxides to diabetic complications with conventional laboratory tests. J Diabetes Complicat. 1992;6(2):116-22.

277. Góth L. The hydrogen peroxide paradox. Orv Hetil. 2006;147(19):887-893.

278. Murray AK. The expanding role of oxygen free radicals in clinical medicine. West J Med. 1986;144(4):441-6.

279. Forstermann U, Munzel T. Endothelial nitric oxide synthase in vascular disease: from marvel to menace. Circulation. 2006;113(13):1708-14.

280. Preston A, Fodey T, Douglas A, Elliott CT. Monoclonal antibody development for acrylamide-adducted human haemoglobin; A biomarker of dietary acrylamide exposure. J Immunol Methods. 2009;341(1-2):19-29.

281. Mucci LA, Dickman PW, Steineck G, Adami HO, Augustsson K. Dietary acrylamide and cancer of the large bowel, kidney, and bladder: absence of an association in a population-based study in Sweden. Br J Cancer. 2003;88(1):84-9.

282. Pelucchi C, Galeone C, Levi F, Negri E, Franceschi S, Talamini R, et al. Dietary acrylamide and human cancer. Int J Cancer. 2006;118(2):467-71.

283. Hogervorst JG, Schouten LJ, Konings EJ, Goldbohm RA, van de Brandt PA. Dietary acrylamide intake is not associated with gastrointestinal cancer risk. J Nutr. 2008;138(11):2229-36.

284. Larsson SC, Kesson A, Bergkvist L, Wolk A. Dietary acrylamide intake and risk of colorectal cancer in a prospective cohort of men. Eur J Cancer. 2009;45(4):513-6.

285. Hogervorst JG, Schouten LJ, Konings EJ, Goldbohm RA, van de Brandt PA. Dietary acrylamide intake and brain cancer risk. Cancer Epidemiol Biomarkers Prev. 2009;18(5):1663-6.

286. Hogervorst JG, Schouten LJ, Konings EJ, Goldbohm RA, van den Brandt PA. A prospective study of dietary acrylamide intake and the risk of endometrial, ovarian, and breast cancer. Cancer Epidemiol Biomarkers Prev. 2007;16(11): 2304-13.

287. Wilson KM, Mucci LA, Cho E, Hunter DJ, Chen WY, Willett WC. Dietary acrylamide intake and risk of premenopausal breast cancer. Am J Epidemiol. 2009;169(8):954-61.

288. Olesen PT, Olsen A, Frandsen H, Frederiksen K, Overvad K, Tjonneland A. Acrylamide exposure and incidence of breast cancer among postmenopausal women in the Danish Diet, Cancer and Health Study. Int J Cancer. 2008;122(9): 2094-100.

289. Guenther H, Anklam E, Wenzl T, Stadler RH. Acrylamide in coffee: review of progress in analysis, formation and level reduction. Food Addit Contam. 2007;24(1):60-71.

290. Anese M, Suman M, Nicoli MC. Acrylamide removal from heated foods. Food Chem. 2010;119(2):791-4.

291. Capuano E, Ferrigno A, Acampa I, Ait-Ameur L, Fogliano V. Characterization of Maillard reaction in bread crisps. Eur Food Res Tech. 2008;228:311-9.

292. Capuano E, Ferrigno A, Acampa I, Serpen A, Açar ÖÇ, Gökmen V, et al. Effect of flour type on Maillard reaction and acrylamide formation during toasting of bread crisp model systems and mitigation strategies. Food Res Int. 2009;42(9):1295-302.

293. Hamlet CG, Sadd PA. Effects of yeast stress and pH on 3-monochloropropanediol (3-MCPD)-producing reactions in model dough systems. Food Addit Contam. 2005;22(7):616-23.

294. Sacks FM, Svetkey LP, Vollmer WM, Appel LJ, Bray GA, Harsha D, et al. Effects on blood pressure of reduced dietary sodium and the dietary approaches to stop hypertension (DASH) diet. DASH-Sodium Collaborative Research Group 1. N Engl J Med. 2001;344(1):3-10.

295. Gökmen V, Senyuva HZ. A simplified approach for the kinetic characterization of acrylamide formation in fructose-asparagine model system. Food Addit Contam. 2006;23(4):348-54.

296. Hedegaard RV, Frandsen H, Granby K, Apostolopoulou A, Skibsted LH. Model studies on acrylamide generation from glucose/asparagine in aqueous glycerol. J Agric Food Chem. 2007;55(2):486-92.

297. Amrein TM, Limacher CP, Amadò R, Escher F. Influence of thermal processing conditions on acrylamide generation and browning in a potato model system. J Agric Food Chem. 2006;54(16):5910-6.

298. De Vleeschouwer K, Van der Plancken I, Van Loey A, Hendrickx M. Impact of pH on the kinetics of acrylamide formation/elimination reactions in model systems. J Agric Food Chem. 2006;54(20):7847-55.

299. Corradini MG, Peleg M. Linear and non-linear kinetics in the synthesis and degradation of acrylamide in foods and model systems. Crit Rev Food Sci Nutr. 2006;46(6):489-517.

300. Kolek E, Simon P, Simko P. Nonisothermal kinetics of acrylamide elimination and its acceleration by table salt--a model study. J Food Sci. 2007;72(6):E341-4.

301. Knol J, Viklund GÅI, Linssen JPH, Sjöholm IM, Skog KI, et al. A study on the use of empirical models to predict the formation of acrylamide in potato crisps. Mol Nutr Food Res. 2008;52(3):313-21.

302. Zhang Y, Zhang Y. Effect of natural antioxidants on kinetic behaviour of acrylamide formation and elimination in low-moisture asparagine-glucose model system. J Food Eng. 2008;85(1):105-15.

303. Gökmen V, Senyuva HZ. Effects of some cations on the formation of acrylamide and furfurals in glucose-asparagine model system. Eur Food Res Tech. 2007;225(5):815-20.

304. Capuano E, Garofalo G, Napolitano A, Zielinski H, Fogliano V. Rye flour extraction rate affects Maillard reaction development, antioxidant activity, and acrylamide formation in bread crisps. Cereal Chem. 2010;87(2):131-6.

305. Gökmen V, Morales FJ, Atac B, Serpen A, Arribas-Lorenzo G. Multiple-stage extraction strategy for the determination of acrylamide in foods. J Food Comp Anal. 2009;22(2):142-7.

306. Zhang Y, Zhang G, Zhang Y. Occurrence and analytical methods of acrylamide in heat-treated foods: Review and recent developments. J Chromatogr A. 2005;1075(1-2):1-21.

307. Delatour T, Risset AP, Goldmann T, Riediker S, Stadler RH. Improved sample preparation to determine acrylamide in difficult matrixes such as chocolate powder, cocoa, and coffee by liquid chromatography tandem mass spectroscopy. J Agric Food Chem. 2004;52(15):4625-31.

308. Zhang Y, Ren Y, Zhang Y. New research developments on acrylamide: analytical chemistry, formation mechanism, and mitigation recipes. Chem Rev. 2009;109(9):4375-97.

309. Stobiecka A, Radecka H, Radecki J. Novel voltammetric biosensor for determining acrylamide in food samples. Biosens Bioelectron. 2007;22(9-10):2165-70.

310. Krajewska A, Radecki J, Radecka H. A voltammetric biosensor based on glassy carbon electrodes modified with single-walled carbon nanotubes/hemoglobin for detection of acrylamide in water extracts from potato crisps. Sensors (Basel). 2008;8(9):5832-44.

311. Quan Y, Chen M, Zhan Y, Zhang G. Development of an enhanced chemiluminescence ELISA for the rapid detection of acrylamide in food products. J Agric Food Chem. 2011;59(13):6895-9.

312. Watanabe E, Miyake S, Baba K, Eun H, Endo S. Immunoassay for acetamiprid detection: application to residue analysis and comparison with liquid chromatography. Anal Bioanal Chem. 2006;386(5):1441-8.

313. Watanabe E, Tsuda Y, Watanabe S, Ito S, Hayashi M, Watanabe T, et al. Development of an enzyme immunoassay for the detection of plant growth regulator inabenfide in rice. Anal Chim Acta. 2000;424(2):149-60. 314. Morozova VS, Levashova AI, Eremin SA. Determination of pesticides by enzyme immunoassay. J Anal Chem. 2005;60(3):202-17.

315. Lagalante AF, Felter MA. Silylation of acrylamide for analysis by solid-phase microextraction/gas chromatography/ion-trap mass spectrometry. J Agric Food Chem. 2004;52(12):3744-8.

316. Gökmen V, Senyuva HZ, Acar J, Sarıoglu K. Determination of acrylamide in potato chips and crisps by high-performance liquid chromatography. J Chromatogr A. 2005;1088(1-2):193-9.

317. Bermudo E, Moyano E, Puignou L, Galceran MT. Determination of acrylamide in foodstuffs by liquid chromatography ion-trap tandem mass-spectrometry using an improved clean-up procedure. Anal Chim Acta. 2006;559(2):207-14.

318. Roach JAG, Andrzejewski D, Gay ML, Nortrup D, Musser SM. Rugged LC-MS/MS survey analysis for acrylamide in foods. J Agric Food Chem. 2003;51(26):7547-54.

319. Mastovska K, Lehotay SJ. Rapid sample preparation method for LC–MS/MS or GC–MS analysis of acrylamide in various food matrices. J Agric Food Chem. 2006;54(19):7001-8.

320. Zhu Y, Li G, Duan Y, Chen S, Zhang C, Li Y. Application of the standard addition method for the determination of acrylamide in heat-processed starchy foods by gas chromatography with electron capture detector. Food Chem. 2008;109(4):899-908.

321. Zhang X, Mobley N, Zhang J, Zheng X, Lu L, Ragin O, et al. Analysis of agricultural residues on tea using d-SPE sample preparation with GC-NCI-MS and UHPLC-MS/MS. J Agric Food Chem. 2010;58(22):11553-60.

322. Soares CMD, Alves RC, Casal S, Oliveira M, Fernandes JO. Development and validation of a matrix solid-phase dispersion method to determine acrylamide in coffee and coffee substitutes. J Food Sci. 2010;75(3):T57-63.

323. Augusto F, Valente ALP. Applications of solid-phase microextraction to chemical analysis of live biological samples. Trends Anal Chem. 2002;21(6–7):428-38.

324. Lee MR, Chang LY, Dou J. Determination of acrylamide in food by solid-phase microextraction coupled to gas chromatography–positive chemical ionization tandem mass spectrometry. Anal Chim Acta. 2007;582(1):19-23.

325. Oracz J, Nebesny E, Zyżelewicz D. New trends in quantification of acrylamide in food products. Talanta. 2011;86:23-34.

326. Keramat J, LeBail A, Prost C, Soltanizadeh N. Acrylamide in foods: chemistry and analysis. A review. Food Bioprocess Tech. 2011;4:340-63.

327. Ridgway K, Lalljie SPD, Smith RM. Sample preparation techniques for the determination of trace residues and contaminants in foods. J Chromatogr A. 2007;1153(1-2):36-53.

328. Liu LB, Hashi Y, Qin YP, Zhou HX, Lin JM. Development of automated online gel permeation chromatography–gas chromatograph mass spectrometry for measuring multiresidual pesticides in agricultural products. J Chromatogr B. 2007;845(1):61-8.

329. He Y, Liu YH. Assessment of primary and secondary amine adsorbents and elution solvents with or without graphitized carbon for the SPE clean-up of food extracts in pesticide residue analysis. Chromatographia. 2007;65(9-10):581-90.

330. Pedersen JR, Olsson JO. Soxhlet extraction of acrylamide from potato chips. Analyst. 2003;128(4):332-4.

331. Hoenicke K, Gatermann R, Harder W, Hartig L. Analysis of acrylamide in different foodstuffs using liquid chromatography–tandem mass spectrometry and gas chromatography–tandem mass spectrometry. Anal Chim Acta. 2004;520(1-2):207-15.

332. Zhou X, Fan LY, Zhang W, Cao CX. Separation and determination of acrylamide in potato chips by micellar electrokinetic capillary chromatography. Talanta. 2007;71(4):1541-5.

333. Cavalli S, Maurer R, Höfler F. Fast determination of acrylamide in food samples using accelerated solvent extraction followed by ion chromatography with UV or MS detection. [updated 2003; cited 2020 Feb 24] Available from:https://assets.thermofisher .com/TFS-Assets/CMD/Application-Notes/AN-409-ASE-Acrylamide-Food-Samples-AN70241-EN.pdf

334. Liu C, Luo F, Chen D, Qiu B, Tang X, Ke H, et al. Fluorescence determination of acrylamide in heat-processed foods. Talanta. 2014;123:95-100.

335. EI-Hady DA, Albishri HM. Simultaneous determination of acrylamide, asparagine and glucose in food using shot chain methyl imidazolium ionic liquid based ultrasonic assisted extraction coupled with analyte focusing by ionic liquid micelle collapse capillary electrophoresis. Food Chem. 2015;188:551-8.

336. Altunay N, Gurkan R, Orhan U. A preconcentration method for indirect determination of acrylamide from chips, crackers and cereal-based baby foods using flame atomic absorption spectrometry. Talanta. 2016;161:143-50.

337. Zokaei M, Abedi AS, Kamankesh M, Shojaee-Aliababadi S, Mohammadi A. Ultrasonic-assisted extraction and dispersive liquid-liquid microextraction combined with gas chromatography-mass spectrometry as efficient and sensitive method for determining of acrylamide in potato chips samples. Food Chem. 2017;234:55-61.

338. Pardo O, Yusa V, Coscolla C, Leon N, Pastor A. Determination of acrylamide in coffee and chocolate by pressurised fluid extraction and liquid chromatography–tandem mass spectrometry. Food Addit Contam. 2007;24(7):663-72.

339. Lasekan O, Abbas K. Analysis of volatile flavor compounds and acrylamide in roasted Malaysian tropical almond (Terminalia catappa) nuts using supercritical fluid extraction. Food Chem Toxicol. 2010;48(8-9):2212-6.

340. Banchero M, Pellegrino G, Manna L. Supercritical fluid extraction as a potential mitigation strategy for the reduction of acrylamide level in coffee. J Food Eng. 2013;115(3):292-7.

341. Yoshioka T, Izumi Y, Nagatomia Y, Miyamoto Y, Suzuki K, Bamba T. A highly sensitive determination method for acrylamide in beverages, grains, and confectioneries by supercritical fluid chromatography tandem mass spectrometry. Food Chem. 2019;294:486-92.

342. Thurman EM, Mills MS. Solid-phase extraction principles and practice. New York: Wiley; 1998. p.25-46.

343. Buldinia PL, Riccib L, Sharmac JL. Recent applications of sample preparation techniques in food analysis. J Chromatogr A. 2002;975(1):47-70.

344. Żwir-Ferenc A, Biziuk M. Solid phase extraction technique-trends, opportunities and applications. Polish J Environ Studies. 2006;15(5):677-90.

345. Ahn JS, Castle L, Clarke DB, Lloyd AS, Philo MR, Speck DR. Verification of the findings of acrylamide in heated foods. Food Addit Contam. 2002;19(12):1116-24.

346. Granby K, Fagt S. Analysis of acrylamide in coffee and dietary exposure to acrylamide from coffee. Anal Chim Acta. 2004;520(1-2):177-82.

347. Roach JAG, Andrzejewski D, Gay ML, Nortrup D, Musser SM. Rugged LC-MS/MS survey analysis for acrylamide in foods. J Agric Food Chem. 2003;51(26):7547-54.

348. Andrzejewski D, Roach JAC, Gay ML, Musser SM. Analysis of coffee for the presence of acrylamide by LC-MS/MS. J Agric Food Chem. 2004;52(7):1996-2002.

349. Zhang Y, Jiao J, Ren Y, Wu X, Zhang Y. Determination of acrylamide in infant cereal-based foods by isotope dilution liquid chromatography coupled with electrospray ionization tandem mass spectrometry. Anal Chim Acta. 2005;551(1-2):150-8.

350. Gökmen V, Senyuva HZ. A generic method for the determination of acrylamide in thermally processed foods. J Chromatogr A. 2006;1120(1-2):194-8.

351. Ren Y, Zhang Y, Jiao J, Cai Z, Zhang Y. Sensitive isotope dilution liquid chromatography/electrospray ionization tandem mass spectrometry method for the determination of acrylamide in chocolate. Food Addit Contam. 2006;23(3):228-36.

352. Geng Z, Jiang R, Chen M. Determination of acrylamide in starch-based foods by ion-exclusion liquid chromatography. J Food Compos Anal. 2008;21(2):178-82.

353. Alves RC, Soares C, Casal S, Fernandes JO, Oliveira MBPP. Acrylamide in espresso coffee: influence of species, roast degree and brew length. Food Chem. 2010;119(3):929-34.

354. Bortolomeazzi R, Munari M, Anese M, Verardo G. Rapid mixed mode solid phase extraction method for the determination of acrylamide in roasted coffee by HPLC-MS/MS. Food Chem. 2012;135(4):2687-93.

355. Douny C, Widart J, Maghuin-Rogister G, Pauw ED, Scippo ML. Quantification of acrylamide in various Belgian potato products using solid phase extraction and liquid chromatography tandem mass spectrometry detection. Food Public Health. 2012;2(5): 137-41.

356. Wang H, Feng F, Guo Y, Shuang S, Choi MMF. HPLC-UV quantitative analysis of acrylamide in baked and deep-fried Chinese foods. J Food Compos Anal. 2013;31(1):7-11.

357. Zhang W, Deng Z, Zhao W, Guo L, Tang W, Du H, et al. Determination of trace acrylamide in starchy foodstuffs by HPLC using a novel mixed-mode

functionalized calixarene sorbent for solid-phase extraction cleanup. J Agric Food Chem. 2014;62(26):6100-7.

358. Templeton DM, Ariese F, Cornelis R, Danielsson LG, Muntau H, Van Leeuwen HP, et al. Guidelines for terms related to chemical speciation and fractionation of elements: definitions, structural aspects, and methodological approaches (IUPAC Recommendations 2000). Pure Appl Chem. 2000;72(8):1453-70.

359. Geng Z, Wang P, Liu A. Determination of acrylamide in starch-based foods by HPLC with pre-column ultraviolet derivatization. J Chromatogr Sci. 2011;49(10):818-24.

360. Yamini Y, Ghambarian M, Esrafili A, Yazdanfar N, Moradi M. Rapid determination of ultra-trace amounts of acrylamide contaminant in water samples using dispersive liquid–liquid microextraction coupled to gas chromatography-electron capture detector. Inter J Environ Anal Chem. 2012;92(13):1493-505.

361. Backe WJ, Yingling V, Johnson T. The determination of acrylamide in environmental and drinking waters by large-volume injection – hydrophilic-interaction liquid chromatography and tandem mass spectrometry. J Chromatogr A. 2014;1334:72-8.

362. Faraji M, Hamdamali M, Aryanasab F, Shabanian M. 2-Naphthalenthiol derivatization followed by dispersive liquid–liquid microextraction as an efficient and sensitive method for determination of acrylamide in bread and biscuit samples using high-performance liquid chromatography. J Chromatogr A. 2018;1558:14-20.

363. Mohebi S, Mazloomifar A. Preconcentration of acrylamide with dispersive liquid-liquid microextraction based on solidification of floating organic drop prior to determination by HPLC. Iran Chem Commun. 2018;6(1):8-18.

364. Galuch MB, Magon TFS, Silveira R, Nicácio AE, Pizzo JS, Bonafe EG. Determination of acrylamide in brewed coffee by dispersive liquid–liquid microextraction (DLLME) and ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS). Food Chem. 2019;282:120-6.

365. Nematollahi A, Kamankesh M, Hosseini H, Ghasemi J, Hosseini-Esfahani F, Mohammadi A. Investigation and determination of acrylamide in the main group of

cereal products using advanced microextraction method coupled with gas chromatography-mass spectrometry. J Cereal Sci. 2019;87:157-64.

366. Sayah M, Kiarostami V. Rapid analysis of acrylamide in tap and well water samples by solvent terminated dispersive liquid–liquid microextraction followed by GC–FID. Bull Environ Contam Toxicol. 2019;102(4):560-6.

367. Barker SA, Long AR, Short CR. Isolation of drug residues from tissues by solid phase dispersion. J Chromatogr A. 1989;475(2):353-61.

368. Barker SA. Matrix solid-phase dispersion. J Chromatogr A. 2000;885(1-2):115-27.

369. Fernandes JO, Soares C. Application of matrix solid-phase dispersion in the determination of acrylamide in potato chips. J Chromatogr A. 2007;1175(1):1-6.

370. Soares CMD, Alves RC, Casal S, Oliveira MBPP, Fernandes JO. Development and validation of a matrix solid-phase dispersion method to determine acrylamide in coffee and coffee substitutes. J Food Sci. 2010;75(3):57-63.

371. Soares CMD, Fernandes JO. MSPD method to determine acrylamide in food. Food Anal Methods. 2009;2(3):197-203.

372. Omar MMA, Ibrahim WAW, Elbashir AA. Sol–gel hybrid methyltrimethoxysilane–tetraethoxysilane as a new dispersive solid-phase extraction material for acrylamide determination in food with direct gas chromatography–mass spectrometry analysis. Food Chem. 2014;158:302-9.

373. Zhao H, Li N, Li J, Qiao X, Xu Z. Preparation and application of chitosan-grafted multiwalled carbon nanotubes in matrix solid-phase dispersion extraction for determination of trace acrylamide in foods through high-performance liquid chromatography. Food Anal Methods. 2014;8(5):1363-71.

374. Arabi M, Ghaedi M, Ostovan A. Development of dummy molecularly imprinted based on functionalized silica nanoparticles for determination of acrylamide in processed food by matrix solid phase dispersion. Food Chem. 2016;210:78-84.

375. Tareke E, Rydberg P, Karlsson P, Eriksson S, Tornqvist M. Analysis of Acrylamide, a carcinogen formed in heated foodstuffs. J Agric Food Chem. 2002;50(17):4998-5006.

376. Arisseto AP, Toledo MCF, Govaert Y, van Loco J, Fraselle S, Degroodt JM. A modified sample preparation for acrylamide determination in cocoa and coffee products. Food Anal Methods. 2008;1(1):49-55.

377. Liu J, Zhao G, Yuan Y, Chen F, Hu X. Quantitative analysis of acrylamide in tea by liquid chromatography coupled with electrospray ionization tandem mass spectrometry. Food Chem. 2008;108(2):760-7.

378. Wang H, Lee AWM, Shuang S, Choi MMF. SPE/HPLC/UV studies on acrylamide in deep-fried flour-based indigenous Chinese foods. Microchem J. 2008;89(2): 90-7.

379. Boroushaki MT, Nikkhah E, Kazemi A, Oskooei M, Raters M. Determination of acrylamide level in popular Iranian brands of potato and corn products. Food Chem Toxicol. 2010;48(10):2581-4.

380. Mesías M, Morales FJ. Acrylamide in coffee: Estimation of exposure from vending machines. J Food Compos Anal. 2016;48(8-12)1-9.

381. Petrarca MH, Rosa MA, Queiroz SCN, Godoy HT. Simultaneous determination of acrylamide and 4-hydroxy-2,5-dimethyl-3(2H)-furanone in baby food by liquidchromatography–tandem mass spectrometry. J Chromatogr A. 2017;1522:62-9.

382. Galani JHY, Patel NP, Talati JG. Acrylamide-forming potential of cereals, legumes and roots and tubers analyzed by UPLC-UV. Food Chem Toxicol. 2017;108 (Pt A):244-8.

383. Paola ELD, Montevecchi G, Masino F, Garbini D, Barbanera M, Antonelli A. Determination of acrylamide in dried fruits and edible seeds using QuEChERS extraction and LC separation with MS detection. Food Chem. 2017;217:191-5.

384. Mesias M, Delgado-Andrade C, Holgado F, Morales FJ. Acrylamide content in French fries prepared in food service establishments. LWT-Food Sci Tech. 2019;100(83-91)1-19.

385. Hussain SZ, Maqbool K. GC-MS: Principle, technique and its application in food science. Int J Curr Sci. 2014;13:116-26.

386. Skoog DA, Holler FJ, Crouch SR. Principles of Instrumental Analysis.6th ed. Canada: Thomson Brooks/Cole; 2007. p. 788-789.

387. Tareke E, Rydberg P, Karlsson P, Eriksson S, Törnqvist M. Acrylamide: a cooking carcinogen?. Chem Res Toxicol. 2000;13(6):517-22.

388. Cheng WC, Hsiao SW, Chou SS, Sun-Hwang L, Lu TJ, Yeh AI. Determination of acrylamide in Chinese foods by GC-ion trap MS using 2-bromopropenamide and 2-bromopropenamide-$^{13}C_3$. J Food Drug Anal. 2006;14(2):207-14.

389. Mizukami Y, Kohata K, Yamaguchi Y, Hayashi N, Sawai Y, Chuda Y, et al. Analysis of acrylamide in green tea by gas chromatography-mass spectrometry. J Agric Food Chem. 2006;54(19):7370-7.

390. Koch M, Bremser W, Koeppen R, Siegel D, Toepfer A, Nehls I. Development of two certified reference materials for acrylamide determination in foods. J Agric Food Chem. 2009;57(18):8202-7.

391. Vesela H, Šucman E. Determination of acrylamide in food using adsorption stripping voltammetry. Czech J Food Sci. 2013;31(4):401-6.

392. Wu M, Chen W, Wang G, He P, Wang Q. Analysis of acrylamide in food products by microchip electrophoresis with on-line multiple-preconcentration techniques. Food Chem. 2016;209:154-61.

393. Altunay N, Gürkan R, Orhan U. A preconcentration method for indirect determination of acrylamide from chips, crackers and cereal-based baby foods using flame atomic absorption spectrometry. Talanta. 2016;161:143-50.

394. Asnaasharia M, Kenari RE, Farahmandfar R, Taghdisi SM, Abnous K. Fluorescence quenching biosensor for acrylamide detection in food products based on double-stranded DNA and gold nanoparticles. Sens Actuator B. 2018;265:339-45.

395. Dutta MK, Singh A, Ghosal S. A computer vision based technique for identification of acrylamide in potato chips. Comput Electron Agric. 2015;119:40-50.

396. Dutta MK, Singh A, Ghosal S. An imaging technique for acrylamide identification in potato chips in wavelet domain. LWT - Food Sci Tech. 2016;65: 987-98.

397. Yadav A, Sengar N, Issac A, Dutta MK. Image processing based acrylamide detection from fried potato chip images using continuous wavelet transform. Comput Electron Agric. 2018;145:349-62.

398. Chenga J, Zhang S, Wang S, Wang P, Su XO, Xie J. Rapid and sensitive detection of acrylamide in fried food using dispersive solid-phase extraction combined with surface-enhanced Raman spectroscopy. Food Chem. 2019;276:157-63.

399. Microplate reader. [cited 2018 Mar 3] Available from:https://www.biotek.com/products/detection/?Product_Interest_Source=PPC%20-%20Adwords&source=PPC%20%20Adwords&Lead_Campaign_Source=7011O000002TCTU&Campaign_Source=7011O000002TCTU&utm_source=biotek&utm_medium=cpc&utm_campaign=7011O000002TCTU&gclid=EAIaIQobChMIwMSFuIi46QIVBsEWBR067gtMEAAYASAAEgL5kPD_BwE

400. Eppendorf Microplates. [cited 2018 Mar 6] Available from: https: //onlineshop.eppendorf.com/OC-en/Laboratory-Consumables-44512/Plates-44516/Eppendorf-Microplates-PF-8861.html.

401. Synergy H1 Hybrid Multi-Mode Microplate Reader. [cited 2020 May 16] Available from:https://www.biocompare.com/20131-Microplate-Reader-ELISA-Plate-Reader/1541259-Synergy-H1-Hybrid-MultiMode-Microplate-Reader/.

402. Synergy H1 Hybrid Multi-Mode Reader. [cited 2020 May 20] Available from: https://niom.no/materials-testing/testing-facilities/multi-mode-reader/

403. Synergy H1 Hybrid Multi-Mode Reader. [cited 2020 May 16] Available from: https://www.biotek.com/products/detection-hybrid-technology-multi-mode-microplate-readers/synergy-h1-hybrid-multi-mode-reader/technical-details/.

404. Wongthanyakram J, Harfield A, Masawat P. A smart device-based digital image colorimetry for immediate and simultaneous determination of curcumin in turmeric. Comput Electron Agric. 2019;166:104981.

405. Hatcher H, Planalp R, Cho J, Torti FM, Torti SV. Curcumin: from ancient medicine to current clinical trials. Cell Mol Life Sci. 2008;65(11):1631-52.

406. Vlietinck AJ, De-Bruyne T, Apers S, Pieters LA. Plant-derived leading compounds for chemotherapy of human immunodeficiency virus (HIV) infection. Planta Med. 1998;64(2):97-109.

407. Ferreira VH, Nazli A, Dizzell SE, Mueller K, Kaushic C. The anti-inflammatory activity of curcumin protects the genital mucosal epithelial barrier from disruption and blocks replication of HIV-1 and HSV-2. PLoS One. 2015;10(4):1-18.

408. Péret-Almeida L, Cherubino APF, Alves RJ, Dufossé L. Separation and determination of the physico-chemical characteristics of curcumin, demethoxycurcumin and bisdemethoxycurcumin. Food Res Int. 2005;38(8-9):1039-44.

409. Martins CV, da Silva DL, Neres AT, Magalhães TF, Watanabe GA, Modolo LV, et al. Curcumin as a promising antifungal of clinical interest. J Antimicrob Chemother. 2009;63(2):337-9.

410. Patil MB, Taralkar SV, Sakpal VS, Shewale SP, Sakpal RS. Extraction, isolation and evaluation of anti-inflammatory activity of curcuminoids from Curcuma longa. Int J Chem Sci Appl. 2011;2:172-4.

411. Cheng Y, Li HL, Wang HF, Sun HF, Liu YF, Peng SX, et al. Inhibition of nicotine-DNA adduct formation in mice by six dietary constituents. Food Chem Toxicol. 2003;41(7):1045-50.

412. Abbasi MA, Ilyas M, Aziz-ur-Rehman A, Sonia D, Shahwar MA, Raza KM, et al. Curcumin and its derivatives: Moderate inhibitors of acetylcholinesterase, butyrylcholinesterase and trypsin. Sci Iran. 2012;19(6):1580-3.

413. Chen YS, Ho CC, Cheng KC, Tyan YS, Hung CF, Tan TW., et al. Curcumin inhibited the arylamines N-acetyltransferase activity, gene expression and DNA adduct formation in human lung cancer cells (A549). Toxicol in Vitro. 2003;17(3):323-33.

414. Vietri M, Pietrabissa A, Mosca F, Spisni R, Pacifici GM. Curcumin is a potent inhibitor of phenol sulfotransferase (SULT1A1) in human liver and extrahepatic tissues. Xenobiotica. 2008;33(4):357-63.

415. Gören AC, Çıkrıkçı S, Çergel M, Bilsel G. Rapid quantitation of curcumin in turmeric via NMR and LC–tandem mass spectrometry. Food Chem. 2009;113(4):1239-42.

416. Masek A, Chrzescijanska E , Zaborski M. Characteristics of curcumin using cyclic voltammetry, UV-vis, fluorescence and thermogravimetric analysis. Electrochim Acta. 2013;107:441-7.

417. Silva-Buzanello RA, Ferro AC, Bona E, Cardozo-Filho L, Araújo PH, Leimann FV, et al. Validation of an Ultraviolet-visible (UV-Vis) technique for the

quantitative determination of curcumin in poly(L-lactic acid) nanoparticles. Food Chem. 2015;172:99-104.

418. Mathews KR, Landmark JD, Stickle DF. Quantitative assay for starch by colorimetry using a desktop scanner. J Chem Educ. 2004;81(5):702-4.

419. Wang SQ, Zhao XH, Khimji I, Akbas R, Qiu WL, Edwards D, et al. Integration of cell phone imaging with microchip ELISA to detect ovarian cancer HE4 biomarker in urine at the point-of-care. Lab Chip. 2011;11(20):3411-8.

420. Kehoe E, Penn RL. Introducing colorimetric analysis with camera phones and digital cameras: an activity for high school or general chemistry. J Chem Educ. 2013;90(9):1191-5.

421. Andrade SE, Limal MB, Barreto IS, Lyra WS, Almeida LF, Araújo MCU, et al. A digital image-based flow-batch analyzer for determining Al(III) and Cr (VI) in water. Microchem J. 2013;109:106-11.

422. Firdaus ML, Alwi W, Trinoveldi F, Rahayu I, Rahmidar L, Warsito K. Determination of chromium and iron using digital image-based colorimetry. Procedia Environ Sci. 2014;20:298-304.

423. Masawat P, Harfield A, Srihirun N, Namwong A. Green determination of total iron in water by digital image colorimetry. Anal Lett. 2017;50(1):173-85.

424. Bang-iam N, Udnan Y, Masawat P. Design and fabrication of artificial neural network-digital image-based colorimeter for protein assay in natural rubber latex and medical latex gloves. Microchem J. 2013;106:270-5.

425. Urapen R, Masawat P. Novel method for the determination of tetracycline antibiotics in bovine milk based on digital-image-based colorimetry. Int Dairy J. 2015;44:1-5.

426. Masawat P, Harfield A, Namwong A. An iPhone-based digital image colorimeter for detecting tetracycline in milk. Food Chem. 2015;184:23-9.

427. Moonrungsee N, Pencharee S, Jakmunee J. Colorimetric analyzer based on mobile phone camera for determination of available phosphorus in soil. Talanta. 2015;136:204-9.

428. Wongthanyakram J, Masawat P. Rapid low-cost determination of lead(II) in cassava by an iPod-based digital imaging colorimeter. Anal Lett. 2019;52(9):550-61.

429. Zhu H, Isikman SO, Mudanyali O, Greenbaum A, Ozcan A. Optical imaging techniques for point-of-care diagnostics. Lab Chip. 2013;13(1):51-67.

430. Coskun AF, Nagi R, Sadeghi K, Phillips S, Ozcan A. Albumin testing in urine using a smart-phone. Lab Chip. 2013;13(21):4231-8.

431. Tseng D, Mudanyali O, Oztoprak C, Isikman SO, Sencan I, Yaglidere O, et al. Lensfree microscopy on a cellphone. Lab Chip. 2010;10(14):1787-92.

432. Gao ZC, Lin YL, Xu B, Pan Y, Xia SJ, Gao NY, et al. Degradation of acrylamide by the UV/chlorine advanced oxidation process. Chemosphere. 2017;187:268-76.

433. Mulla ML, Bharadwaj VR, Annapure US, Singhal RS. Effect of formulation and processing parameters on acrylamide formation: A case study on extrusion of blends of potato flour and semolina. LWT-Food Sci Tech. 2011;44(7):1643-48.

434. Tekkeli SEK, Önal C, Önal A. A Review of Current Methods for the determination of acrylamide in food products. Food Anal Method. 2012;5:29-39.

435. Mulla MZ, Annapure US, Bharadwaj VR, Singha RS. A study on the kinetics of acrylamide formation in banana chips. J Food Process Preserv. 2017;41(1):1-10.

436. Croft M, Tong P, Fuentes D, Hambridge T. Australian survey of acrylamide in carbohydrate-based foods. Food Addit Contam. 2004;21(8):721-36.

437. Fohgelberg P, Rosén J, Hellenäs KE, Abramsson-Zetterberg L. The acrylamide intake via some common baby food for children in Sweden during their first year of life--An improved method for analysis of acrylamide. Food Chem Toxicol. 2005;43(6):951-9.

438. Yusa V, Quintas O, Pardo O, Marti P, Pastor A. Determination of acrylamide in foods by pressurized fluid extraction and liquid chromatography-tandem mass spectrometry used for a survey of Spanish cereal-based foods. Food Addit Contam. 2006;23(3):237-44.

439. Bermudo E, Núñez O, Puignou L, Galceran MT. Analysis of acrylamide in food products by in-line preconcentration capillary zone electrophoresis. J Chromatogr A. 2006;1129(1):129-34.

440. Zheng Y, Sun Y, Ren J. Identification and quantitation of iodotyrosines and iodothyronines in hydrolysate of iodinated casein by capillary electrophoresis. Talanta. 2006;69(1):107-12.

441. Font G, Ruiz MJ, Fernández M, Picó Y. Application of capillary electrophoresis-mass spectrometry for determining organic food contaminants and residues. Electrophoresis. 2008;29(10):2059-78.

442. Tezcan F, Erim FB. On-line stacking techniques for the nonaqueous capillary electrophoretic determination of acrylamide in processed food. Anal Chim Acta. 2008; 617(1-2):196-9.

443. Hu Q, Xu X, Li Z, Zhang Y, Wang J, Fu Y, et al. Detection of acrylamide in potato chips using a fluorescent sensing method based on acrylamide polymerization-induced distance increase between quantum dots. Biosens Bioelectron. 2014;54:64-71.

444. Hiroo T, Ryoichi S. Preparation of polyvinylamine by the Hofmann degradation of polyacrylamide. B Chem Soc Jpn. 1976;49(10):2821-3.

445. Hong G, Luo MR, Rhodes PA. A study of digital camera colorimetric characterization based on polynomial modeling. Color Res Appl. 2001;26(1):76-84.

446. Minamisawa RA, Sentos LER, Parada MA, Daghastanli KRP, Ciancaglini P, De Almeida A. Digital image analysis to standardize a photometric method in colorimetric quantification. Instrum Sci Tech. 2007;36(1):97-104.

447. Shin J, Choi S, Yang JS, Song J, Choi JS, Jung HI. Smart forensic phone: colorimetric analysis of a bloodstain for age estimation using a smartphone. Sens Actuator B-Chem. 2017;243:221-5.

448. Liu Z, Goodwin M, Ellwood RP, Pretty IA, McGrady M. Automatic detection and classification of dental fluorosis in vivo using white light and fluorescence imaging. J Dent. 2018;74(S1):S34-41.

449. Barbero GF, Ruiz AG, Liazid A, Palma M, Vera JC, Barroso CG. Evolution of total and individual capsaicinoids in peppers during ripening of the cayenne pepper plant (Capsicum annuum L.). Food Chem. 2014;153:200-6.

450. Becalski A, Lau BPY, Lewis D, Seaman SW. Acrylamide in foods: occurrence, sources, and modeling. J Agric Food Chem. 2003;51(3):802-8.

451. Tanaka H. Hofmann reaction of polyacrylamide: relationship between reaction condition and degree of polymerization of polyvinylamine. J Polymer Sci: Polymer Chem Ed. 1979;17(4):1239-45.

452. Arryanto Y, Bark LS. Improved and rapid method for the spectrofluorimetric determination of trace amounts of polyacrylamides in waters. Analyst. 1991;116(11):1149-53.

453. ThermoFisher Scientific. Molecular Probes™ Handbook, A Guide to Fluorescent Probes and Labeling Technologies 11th ed. 2010. Chapter 20. p. 886. [cited 2020 May 22] Available from: https://www.thermofisher.com/th/en/home/references/molecular-probes-the-handbook/ph-indicators.html