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## Chapter

# UPLC-MS: An Emerging Novel Technology and Its Application in Food Safety

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## Abstract

Over the past decade, food safety has become an important issue worldwide due to higher incidences of food contamination. Currently, one of the great challenges in food safety is the analysis of emerging food contaminants. Moreover, the scope, relevance, and level of food safety and testing have never been in such complexity than in today's global marketplace. In recent years, a novel technology ultra performance liquid chromatography (UPLC) coupled with mass spectroscopy (MS) has been developed to estimate the food contaminants, as well as food components with better accuracy, sensitivity, precision, and high throughput. UPLC-MS works on van Deemter principle, which states that, the flow rate of smaller particles are much faster in compare with large particles as well as unfolding the correlation of flow rate and plate height. Additionally, various food components as well as food contaminants such as vitamins, amino acid, metabolite identification, adulteration, forensic testing, toxicity studies, phytoconstituents, pesticide in agriculture, antibiotic residue, hormones, dyes and pigment analysis can be performed using UPLC-MS. Moreover, uniqueness of UPLC-MS and its wide range of application makes it an important tool for food safety laboratory around the world.

**Keywords:** food safety, food contaminants, liquid chromatography, van Deemter, UPLC-MS

## 1. Introduction

Food safety has become an important key issue worldwide, because of the emergence of several new chemical hazards present in food [1]. In addition to that, maintaining food safety has become very challenging at the operational level, as production of food and their consumptions are currently involved in a series of events that must be adequately accomplished to ensure the safety of food [2]. Therefore, food safety has become an increasingly important public health issue all over the world and due to which governments are escalating their efforts to improve and ensure food safety. These efforts can also be recognized in response to a growing number of food safety problems and

increasing consumer health safety concerns [3]. A very well-known proverb from nutritionists or dietitians is “we are what we eat”. Definitely, it does not mean that if we eat apple we become apple, but for good or for ill, the components we eat must be incorporated, transformed, and/or excreted by our bodies. Because, food is an indispensable ingredient of life, and access to food is often the limiting factor in the size of a given populace [4]. There are several incidents of food safety outbreak, which has received major attention from all parts of the world such as occurrence of benzene in carbonated drinks (UK), foods contaminated with pesticides (Japan), presence of dioxins in milk products and pork sample (Belgium), incidence of pesticides in soft drinks (India) and occurrence of melamine in dairy products (China). Such incidents have made people distressful of their food consumption worldwide [5]. In addition to that, such contemporaneous incidents are growing concerns, mainly because of mass production of agronomic products and industrialization at a very fast pace to meet the requirement of current population. Moreover, it has been considered that mainly increasing worldwide population is making farming people to force mass production of agronomic products without giving ample consideration to the safety and quality of food produce. In addition to that, changes in life style patterns of consumers have been called responsible for food safety hazards [6]. Due to fast-paced urbanization, food products such as ready-to-eat, processed food and junk foods has increased, but due to rise in application of chemicals usage, such processed food has also come under the scanner of food safety professionals [1, 7].

Moreover, the scope, relevance, and level of food safety and testing have never been in such complexity than in today’s global marketplace. In recent years, a novel technology UPLC-MS has been developed to estimate the food contaminants as well as food components with better accuracy, sensitivity, precision, and high throughput. In addition to that, this advanced novel technique provided the platform to estimate different analytes at very lower levels, with better accuracy, and more importantly in less time. Moreover, the uniqueness of UPLC-MS has marked several applications to food safety. Various food safety parameters such as residual analysis, vitamins, amino acid, metabolite identification, adulteration, forensic testing, toxicity studies, phytoconstituents analysis, pesticide in agriculture, antibiotic residue, hormones, dyes and pigment analysis can be performed by using UPLC-MS [8, 9]. In addition to that, wide range of analysis makes UPLC-MS as an integral part of food safety laboratory around the globe. Moreover, in this chapter a detailed study and exploration has been made for better understanding of principles and applicability of UPLC-MS in food safety.

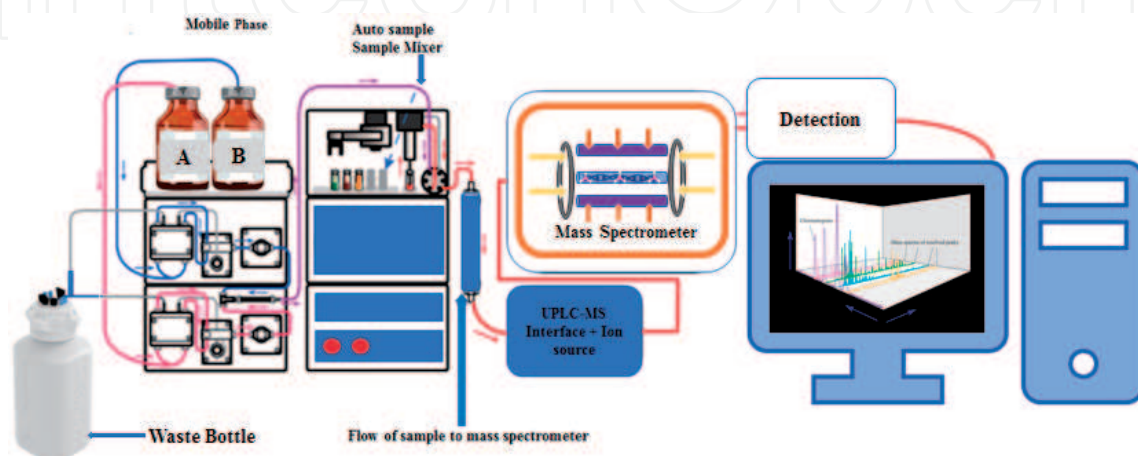
## **2. Chromatography and food safety**

Today, our food supply is more diverse and highly processed than ever before. However, to ensure the nutritive value and to improve the food safety several states have disseminated regulations that states the acceptable limit for each components likewise, food additives, food residues and contaminants in food or food products. Consequently, a better and safe food can only be ensured when we have good approach to analyze such food components, contaminants, or chemical contaminants. In past few decades, chromatography has been recognized as one of important tool to identify and quantify food contaminants to ensure food safety. This novel technique allows the separation, purification, and identification from

a mixture of the components for both qualitative and quantitative analysis. In current years, a unique technology UPLC-MS has been developed to estimate the food contaminants as well as food components for improving food safety. Therefore to obtain such targets, in 2004 Waters launched a brand of liquid chromatography (LC) called UPLC having a significant advancement in column particle size and column dimension having a small and porous particle (sub 2  $\mu\text{m}$ ) [10, 11].

### 3. Ultra performance liquid chromatography

UPLC is a novel technique that offers a new pathway for LC. UPLC enhances the capability of LC in four main areas like increasing speed, sensitivity, resolution and accuracy. UPLC is also known as ultra high-performance liquid chromatography (UHPLC). In comparison to high-performance liquid chromatography (HPLC), UPLC has been upgraded with column packing materials of less than 2  $\mu\text{m}$  in diameter, which increases the speed, accuracy, resolution and sensitivity. Moreover, particle size used in HPLC, UPLC column ranges from 3 to 5  $\mu\text{m}$  and < 2 respectively as well as mobile phase flow rate in HPLC is usually 3.0 ml/min compared to UPLC flow rate 0.6 ml/min. The basic difference in the principle of UPLC and HPLC is the column packing material, which makes a huge difference over the sensitivity and accuracy of the novel techniques. Apart from the principle involved in the LC, there is not much change in basic principle except the pressure generated or created in the instruments make it a more efficient technology. The development of UPLC techniques has urged the scientists to improve the prevailing instrumentation capability for LC, which has the advantage of improved parting performance and constant pressure. Efficiency of this technique is equivalent to the dimension of the column and inversely proportional to the radius of the atoms. As the name suggest ultra performance or ultra-pressure, UPLC works under very high pressure up to 1000 bars, however for HPLC, pump pressure not go more than 300–400 bars. A schematic diagram of UPLC and its internal diagram are presented here in **Figure 1**. In recent years, UPLC has become an integral part of any food safety laboratories, as it reduces the time of run as well as cost of analysis for any analysis [9, 12, 13].



**Figure 1.**  
*Flow diagram of ultra performance liquid chromatography-mass spectrometry.*

### 3.1 Principle

UPLC works on the van Deemter principle, which describes the correlation between the flow rate and height of chromatogram. The van Deemter states that, “the flow rate of smaller particles are much faster in compare with large particles as well as unfolding the correlation of flow rate and plate height”. According to van Deemter equation, when the porous particle size reduced to less than 2.5  $\mu\text{m}$ , there will be increase in efficiency; however, the efficiency does not weaken at increased flow rates or linear velocities.

The following equation describes the relationship between linear velocity (flow rate) and plate height [13, 14].

$$H = A + B/v + C_v \quad (1)$$

where,

A, B and C = Constants.

$v$  = Linear velocity of carrier gas flow rate.

A = It is independent of velocity and represents “eddy” mixing. This is smallest when the packed column particles are small and uniform.

B = It stands for axial diffusion or the natural diffusion tendency of molecules. This effect is diminished at high flow rates and so this term is divided by  $v$ .

C = It represent kinetic resistance to equilibrium in the separation process.

According to van Deemter equation, resistance of kinetics is the time lag involved in traveling from the gas phase to the packing stationary phase and back again. Moreover, higher the gas flow, greater will be a molecule to lag behind in the mobile phase on packed stationary phase. Therefore, the term is proportional to  $v$ . Moreover, there will be a chance to surge throughput, and thus the rapidity of analysis without affecting the chromatographic performance [15]. However, UPLC performance is not much efficient until unless it is coupled with tandem mass spectrometry or other spectrometry techniques as it helps in molecular analysis by using mass-by-charge ratio [16, 17].

### 3.2 Mass spectrometry

Spectrometry method for the molecular analysis of any compound requires mass spectrometry (MS). The principle of MS was first proposed by Dr. Wien, which suggests that, refraction of charged particle in electric or magnetic field can analyzed by using MS. Mass spectrometer is an important tool to for the molecular mass analysis [18]. MS methods identifies the ionized molecules in gaseous phase in different ways

- Qualitative analysis of unknown compounds or mixture
- Quantitative estimation of any mixture or solution
- Structure characterization
- Molecular weight determination

MS works on the principle of fragmentation of molecule and separation or filtration of ions on the basis of their mass-to-charge ( $m/z$ ) ratio. The molecular mass resulting from mass spectrum and produced ions are a function of mass by

charge ratio [19]. Consequently, fragmentation of molecular mass in MS make it principally a very important technique over any other traditional chromatographic techniques. Notwithstanding that, on account of the capacity of MS to create m/z proportion, it considered as an exceptionally novel, straightforward, sensitive, accurate, and particular for the quantitative investigation of any mixture or blend [20, 21].

### 3.3 Tandem mass spectrometry (MS/MS)

There are mainly five techniques for analyzing mass of any compound by using MS like, quadrupole mass filter (single and triple), time of flight, quadrupole ion trap and Fourier transform ion-cyclotron resonance instruments. Furthermore, MS gave a thought of molecular mass, however on the other hand it does not give authentication of molecular structure. In this way, to conquer the restriction of past mass spectrometry, improvement of couple mass spectroscopy (MS/MS) rises. This MS/MS system work into two stages, first to choose parent ions generated from parent ion cells and to disintegrate into daughter ions after the collision of parent ion into at least one daughter ions. In mass spectrometry parent ions and daughter ions gets isolated, divided, and distinguished into single ion cell. In addition to that, fast collisions of compounds performed in argon cell, where translational energy gets transformed into ion internal energy to make ions in excited state and unimolecular decay progresses [22]. The breaking of compound in ion cell of MS/MS spectrum is selected based upon parent and daughter ions. Collision of compound can be performed in in single ionization cell or triple quadrupole system (TQS). TQS is the most frequently used now a day MS/MS techniques as compared to other mass analyzer [23].

### 3.4 Small-size particles and their chemistry

Small-size particles not only enhance proficiency, nonetheless it also increases the flexibility to enhance linear velocity without losing efficiency of the column. Moreover, efficiency is the essential separation factor in UPLC, as it depends on the selectivity and retention activity as in HPLC. Below equation shows that: ( $R_s$ ) resolution is directly proportional to the square root of  $N$ .

$$R_s = \frac{\sqrt{N}}{4} \left( \frac{\alpha - 1}{\alpha} \right) \left( \frac{k}{k + 1} \right) \quad (2)$$

However,  $N$  is inversely proportional to particle size ( $dp$ ): as the particle size is lowered by a factor of three, from, for example, 5  $\mu\text{m}$  (HPLC scale) to 1.7  $\mu\text{m}$  (UPLC-scale),  $N$  is increased by three and resolution by the square root of three or 1.7.  $N$  is also inversely proportional to the square of the peak width:

$$N \propto \frac{1}{w^2} \quad (3)$$

This demonstrates that the narrower the peaks are, the easier would be to separate from each other. Moreover, peak width height is inversely proportional to the peak height:

$$H \propto \frac{1}{w} \quad (4)$$

Therefore, decrease in particle size increases  $N$  and subsequently  $R_s$ , and by virtue of which sensitivity increased, taller peak as well as narrower peak mean

more peak capacity per unit time in gradient separations, as per the requirement in several food safety application notes. Moreover, another equation comes into play when migrating toward smaller particles:

$$F_{\text{xxx}} \alpha = \frac{1}{\text{dpc}} \quad (5)$$

Van Deemter equation revealed that, as particle size decreases, the optimum flow  $F$  opt to reach maximum  $N$  increases. However, flow rate is directly proportional to back pressure as smaller particle sizes needed much higher operating pressures. Efficiency is inversely proportional to the particle size however proportional to column length.

$$N \alpha \frac{L}{dp} \quad (6)$$

Moreover, the column can be shortened by the same factor as the particle size without loss of resolution. Although non-porous, high-efficiency 1.5- $\mu$  particles are easily available in market, but these non-porous particles suffer poor loading capacity as well as poor retention because of low surface area. However, silica-based column have good mechanical strength nonetheless, it can undergo to a number of disadvantages, such as limited pH range and tailing of basic analytes. In addition to that, polymeric columns can overcome pH limitations. Moreover, packed column bed and their uniformity are also important, mainly if shorter columns have to uphold resolution while achieving the objective of faster separations [9, 13, 15].

#### 4. Application of ultra performance liquid chromatography in food safety

In recent years, the demand of UPLC-MS/MS in food analysis has increased, because of the novel characteristics of UPLC with good resolution, better accuracy and sensitivity and reproducibility. Since its inception, it has reduces the time of food scientists as well as cost of the analysis because of its capability of producing more valuable, reliable, and reproducible data. The UPLC sensitivity has reached to ppb and ppt levels by virtue of which a food analyst would be more confident in ensuring safe food for consumption. Analysis of several food components as well as food contaminants has been performed using UPLC-MS/MS technique. By using this technique, below-mentioned food matrices can be tested for ensuring better food safety and we can also get more accurate qualitative and quantitative data of samples with high standards [11].

- Determination of antibiotic residue in food matrices
- Quantification of pesticides residues in food [24–26]
- Amino acid profiling [9, 27]
- Multi-drug residue quantitation in food matrix [28]
- Metabolomics study in food safety [9]
- Analysis of food contaminants in food matrices
- Determination of phytoconstituents

- Analysis of natural medicine and herbal medicine [9]
- Determination of acrylamide in food matrix [29]
- Analysis of mycotoxin in food [30, 31]
- Determination of bromate in drinking water [32]
- Pesticide in fruit and vegetables [33]
- Determination of food-borne carcinogens heterocyclic amines [34]
- Capsaicinoids analysis in capsicum species [35]
- Analysis of vitamin in food
- Determination of alkaloids in cocoa
- Lactose content determination in milk
- Phenolic content determination in fruits and vegetables
- Analysis of food based coloring agent [36]

#### **4.1 Determination of antibiotic residue in honey**

Several antibiotic residues such as streptomycin (**Figure 2**), chloramphenicol, tetracycline etc. has been identified and quantified in honey by using UPLC-MS coupled along with electron spray ionization [37, 38].

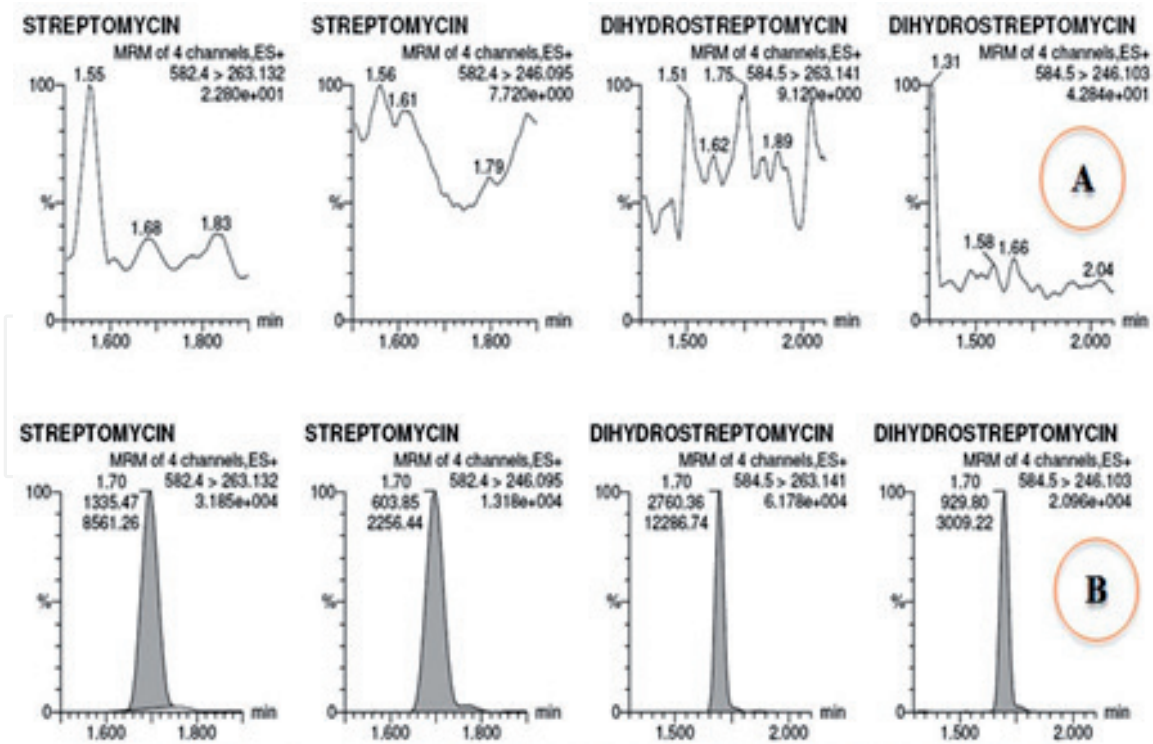
#### **4.2 Multi pesticide residue analysis in cereal grains**

Pesticides are chemicals widely used against plant pests in agriculture and farming to increase crop production, either against plant diseases or prophylactic usage. Currently, more than 350 pesticides are known, which are used to protect plants or plant products; however these pesticide are not allowed more than the permitted level. In addition to that, these chemicals could be dangerous to human health. The function of full scan UHPLC-Orbitrap-MS/UPLC-MS is adequate enough to enable detection and accurate analysis of mass measurement of a broad range pesticides residue at very lowest concentration in complex sample matrices [24–26].

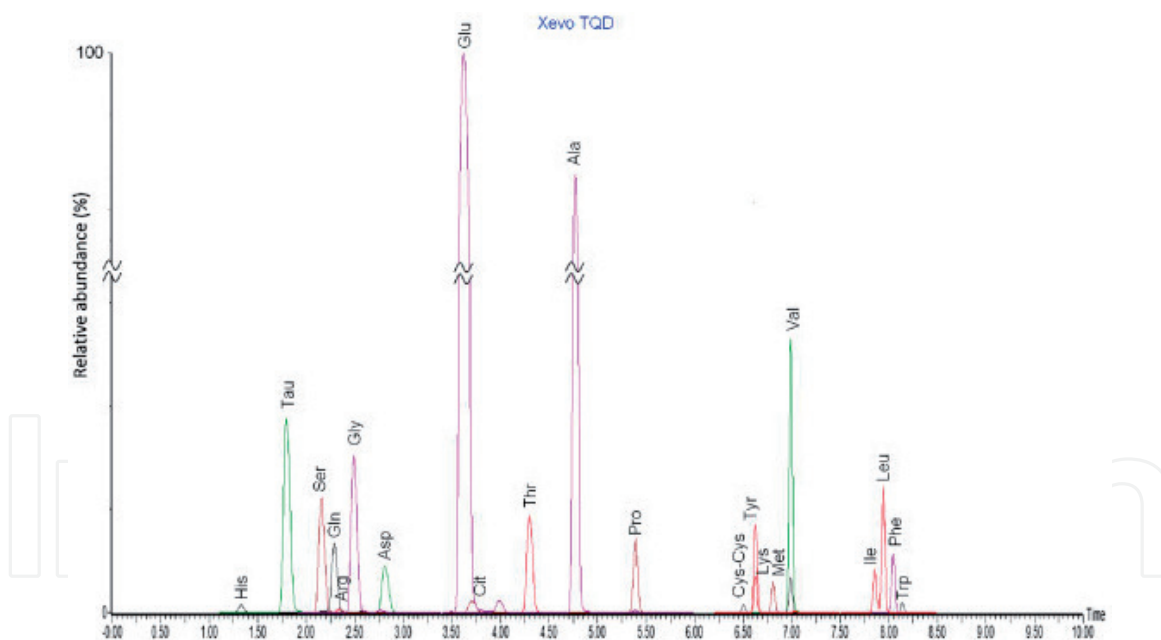
#### **4.3 Amino acid profiling**

Amino acid profiling is one of the important proximate analyses parameter in food safety, as it contributes major portion of protein and an essential component of human diet. However, among the several protein food resources mammalian milk is purest food available over the globe. However, free amino acids are calculated from total nitrogen present in milk. UPLC coupled to electrospray ionization tandem mass spectrometry (ESI-MS/MS) system has been estimated for free amino acid analysis in milks of human, rat, and cow as presented in **Figure 3**. Moreover, UPLC-ESI-MS/MS allowed the quantitation of 21 free amino acids in 10-minute run time using labeled amino acids as internal standard in mammalian milk [27].





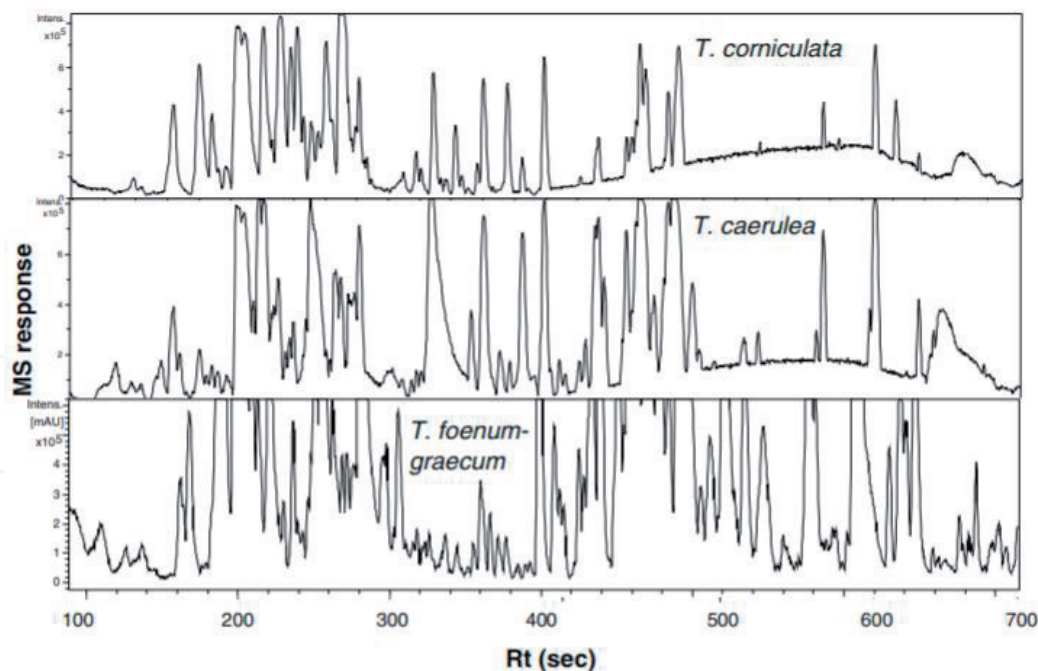
**Figure 2.**  
Chromatogram showing blank honey sample (A) vs. spiked honey sample (B).



**Figure 3.**  
Free amino acid ion chromatogram obtained in human milk (cumulative).

#### 4.4 Metabolomics study in food safety

In recent years, the performance of UPLC has set the stage for a myriad of metabolomics analysis in plants and plant products. UPLC along with qTOF (quadrupole time of flight) system has been applied for semi-polar metabolite analysis in tomato fruit model. Moreover, UPLC coupled with qTOF mass spectrometer produces high-resolution and mass accuracy, good dynamic range, and a fast spectral acquisition capacity, which makes UPLC one of the most appropriate techniques for extensive profiling of many plant metabolites. In addition to that UPLC-MS



**Figure 4.**  
Metabolomic analysis of *T. caerulea*, *T. corniculata*, and *T. foenum-graecum* using UPLC-qTOF-MS.

along with multivariate data analysis has been used for metabolomics profiling of Trigonella seed. Metabolomic study of all the three Trigonella species *T. caerulea*, *T. corniculata*, and *T. foenum-graecum* identified 93 metabolites including 26 saponins, 5 peptides, 22 C/O-flavonoid conjugates, and 9 fatty acids as determined in **Figure 4**. Out of which, various novel compounds such as dipeptides, flavonoids were reported for first time [39–41].

#### 4.5 Multi-drug residue quantitation in poultry muscle

In recent year, poultry industries have become million dollar industries due to higher consumption among the world population. However, multi-drug residue is very common in poultry muscles as poultry husbandry people illegally feed several drugs such as quinolones, amantadine, sulfonamides, tetracycline, amoxicillin, lincomycin, and so on. UHPLC-ESI-MS/MS has been used to analyses such veterinary drug residues in poultry muscle ranging from very polar to nonpolar compounds. UHPLC-ESI-MS/MS operating in positive multiple reactions monitoring (MRM) has been operated to quantify most of the multi-drug residue in sample [28].

#### 4.6 Method development and validation

Method development plays a great role in concluding for any analytical method. In quantitative evaluation, development of method can roughly divided into three parts

- Optimization of chromatography conditions
- Mass spectrometry parameters
- Preparation of sample

Depending upon physical or chemical characteristics of analyzing components method development could be easily performed considering the following factors

like selection of column, mobile phase, pH, and particle size and flow rate in any chromatographic setting.

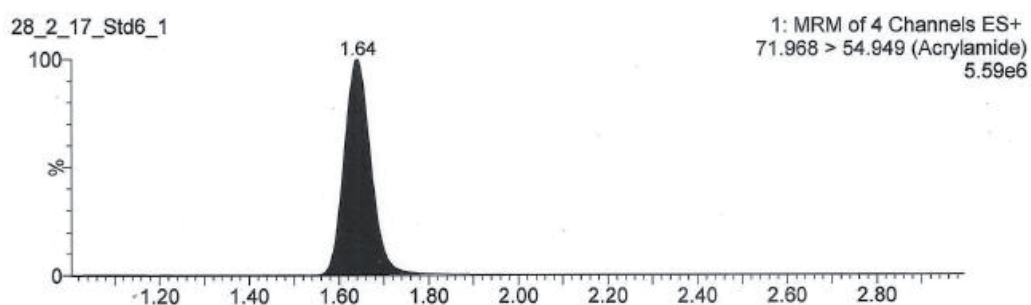
The benefits of using UPLC-MS method over others were better recovery, good repeatability, and amount of extraction solvent volume. The selection of ionization techniques is depending on analytical results with pretreated samples. UPLC-MS/MS tuning parameters and scan modes are decided by uninterrupted infusion of standard solution, depending on the sensitivity and specificity needed. Few key elements for method development are sample pre-treatment, chromatography, internal standard, choice between electrospray ionization (ESI) and APCI, and mass spectrometry [42]. On the other hand, method validation results support for new analytical procedures or new drug development such as Carnosol, Carnosic acid, and Rosmarinic acid in food matrices. Validation required defining performance of developed method and reliability of obtained results. The analytical developed method could be utilized for quantitation application then it would be better to be validated to ensure minimum requirement of validation experiments along with satisfactory results [43].

#### 4.7 Determination of acrylamide in food matrices

Acrylamide as a risk factor come to scientists attention recently, as its discovery in food was accidental. Formation of acrylamide in different types of cooked food or processed food at high temperatures reported recently. Several researchers have validated an analytical method for the analysis of acrylamide in food by UPLC-MS/MS as determined in **Figure 5**. Various reports suggests that processed food such as potato, coffee, bakery and other human dietary products contain acrylamide. One of the study carried out in Cyprus found that potato crisp had highest amount of acrylamide (642 ppb), followed by French fries and biscuits. Concurrently, regular consumption of such food products may lead to carcinogenicity [29].

#### 4.8 Determination of phytoconstituents

Determination of phytoconstituents analysis involves usage of several analytical techniques for the isolation and characterization of phytoconstituents. Primitive techniques basically involved usage of UPLC-MS for the isolation and determination of phytoconstituents. Analysis and identification of chemical constituents of fenugreek by UPLC-MS and UPLC-Q-TOF-MS revealed that, 57 saponins and 19 flavonoid components. In addition to that, characterizations and quantitation of phytoconstituents has been reported in *Piper betle*. Moreover, quantitative data revealed significant variances in the contents of the major bioactive components in *Piper betle* species [44, 45].



**Figure 5.** UPLC-MS chromatogram for acrylamide standard solution at  $500 \text{ ng ml}^{-1}$ .

## 4.9 Determination of food contaminants in food matrices

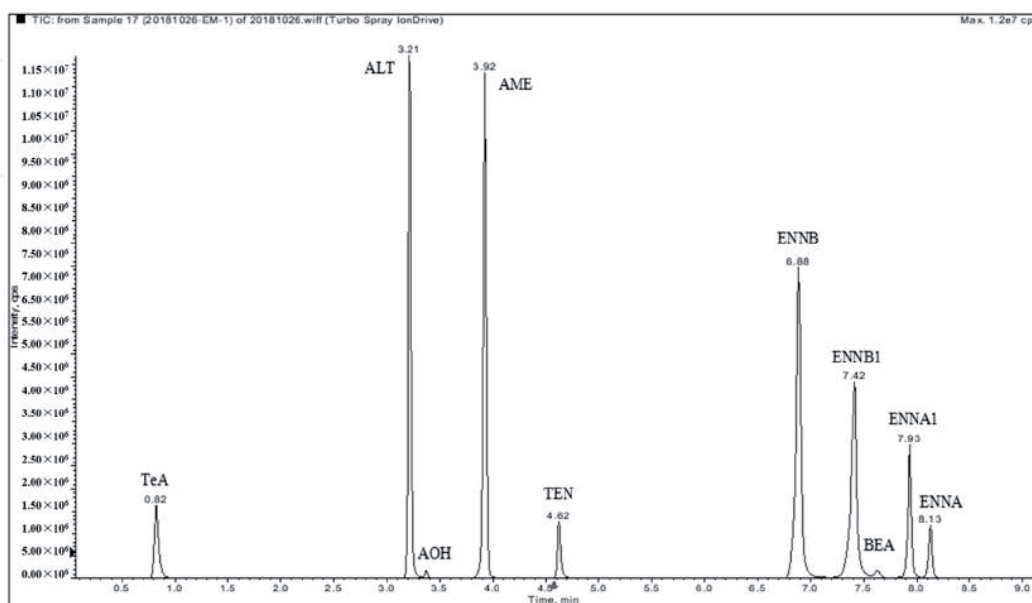
In current years, various food such as legumes, cereals, potatoes, eggs, aquatic foods, dairy products, vegetables, fruits, and beverages reported to have several mycotoxins such as beauvericin, enniatin A, enniatin B, alternariol, tentoxin, and tenuazonic acid (**Figure 6**). These mycotoxins have been considered as a major food contaminants. In recent years, UPLC-MS has emerged as one of the most suitable method for the determination of these food contaminants. UPLC-MS has advantages over other instruments because of having better detection level, fast and accurate. UPLC-MS has emerged as a powerful tool for monitoring and measuring dietary exposure assessment of such mycotoxins [30].

## 4.10 Analysis of antioxidant and phenolic compound using UPLC-MS

It's been well-known that antioxidant has ability to fight against free radicals since free radicals are considered as a causative agent for several diseases. However, use of antioxidant has increased in food industry due to its antimicrobial property. Nowadays, natural as well as synthetic antioxidant such as butylated hydroxyanisole (BHA), butylated hydroxytoluene has been extensively used in food industry. However, the safety and toxicity of synthetic antioxidant is still a matter of concern for human health. On the other hand, several phenolic compounds have been well known for human nutrition. Moreover, these components are used for retarding microbial growth, increasing shelf life, reducing undesirable fragrances, enhancing nutritional value as well as delaying the formation of toxic oxidation. Phenolic profiling as well as antioxidant activities can be analyzed UPLC-ESI-MS/MS in *Salvia* species in some of the medicinal plants from South West Anatolia, Turkey. Moreover, it is assumed that, it was first reported for the analysis of individual phenolic profiles of *S. potentillifolia*, *S. albimaculata*, and *S. nydeggeri* [46].

## 4.11 Bromate in drinking water

Most of the drinking water contains bromide, as the primary source of bromide is soils containing bromide or sea water containing excess amount of



**Figure 6.** Chromatogram (100 ng/mL) showing complete separation of a mixture of 10 mycotoxin standards at (100 ng/mL) using UPLC-MS method.

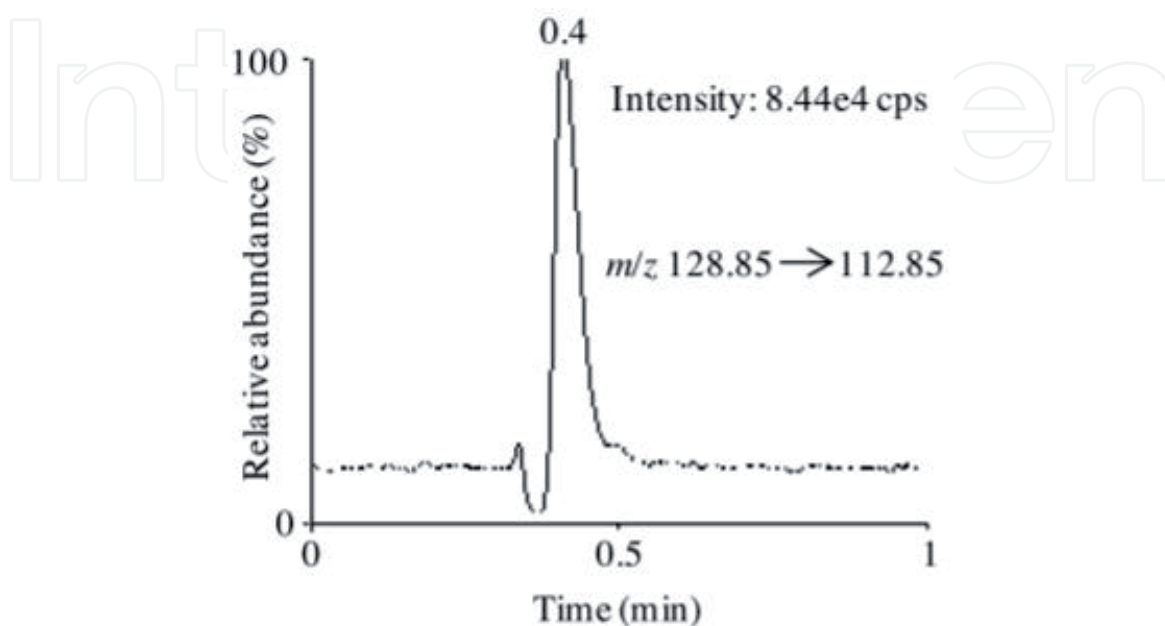
bromide. During the ozonation process bromide gets converted into carcinogenic bromate [47]. International Cancer research agency has found that, bromate has carcinogenic property in human beings. UPLC-MS techniques have been reported to quantify bromate at very low detection levels, that is, 0.01 ng/mL as found in **Figure 7**. UPLC-MS method is found to be rapid, selective, and sensitive for routine analysis of bromate at very low level in drinking water as well as sea water [32].

#### 4.12 Analysis of capsaicinoids in capsicum species

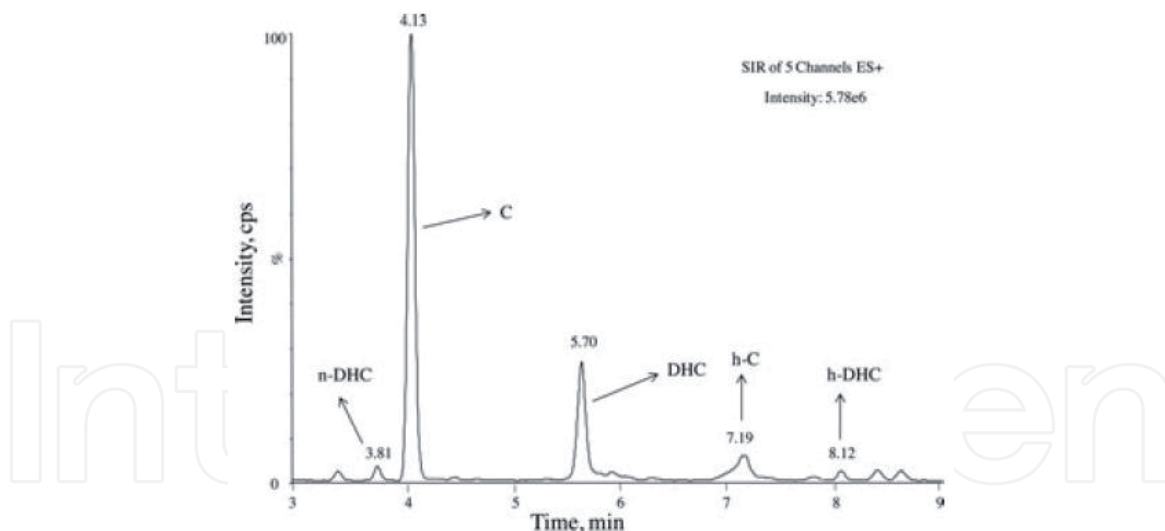
Capsaicinoids are the pungent metabolites of the fruit capsicum. Capsaicinoids are a group of more than 13 alkaloids having structure of vanillylamide with branched fatty acid in the 9–11 carbons. Moreover, the most predominant capsaicinoids are capsaicin and dihydrocapsaicin. These two major capsaicinoids are responsible for the spiciness of capsicum (**Figure 8**). UPLC-MS is used to analyze capsaicinoids in various capsicum species. Analysis is carried out to measure the amount of all the capsaicinoids such as capsaicin, dihydrocapsaicin, nordihydrocapsaicin, homocapsaicin, and homodihydrocapsaicin in different species of capsicum. Based upon the UPLC-MS analysis limit of detection is calculated 0.05, 0.06, 0.15, 0.2, and 0.1 g/g for capsaicin, dihydrocapsaicin, nordihydrocapsaicin, homocapsaicin, and homodihydrocapsaicin, respectively [35].

#### 4.13 Foodborne carcinogens

Foodborne carcinogens are a metabolic product of food after food processing (e.g., heating, curing, smoking) and during food preparation (e.g., baking, frying, grilling). Sometimes, fungi and plant-derived products also tend to produce foodborne carcinogens. Dietary carcinogens produced by chemical and physical food processing are N-nitroso compounds, heterocyclic aromatic amines, polycyclic aromatic hydrocarbons, and acrylamide. However, infected grains and peanuts have been reported to contain mold *Aspergillus flavus* and *Aspergillus parasiticus*, which is considered for producing secondary metabolite such as aflatoxins (carcinogenic potential) [48].



**Figure 7.** Chromatograms showing a UPLC-MS/MS peak of bromate in drinking water.



**Figure 8.**  
UPLC-MS chromatogram showing different capsaicinoids extracted from red chili.

Researchers have developed an analytical method for biomonitoring of cooked meat carcinogens and their metabolites in human urine [34].

#### 4.14 Vitamin analysis using UPLC-MS

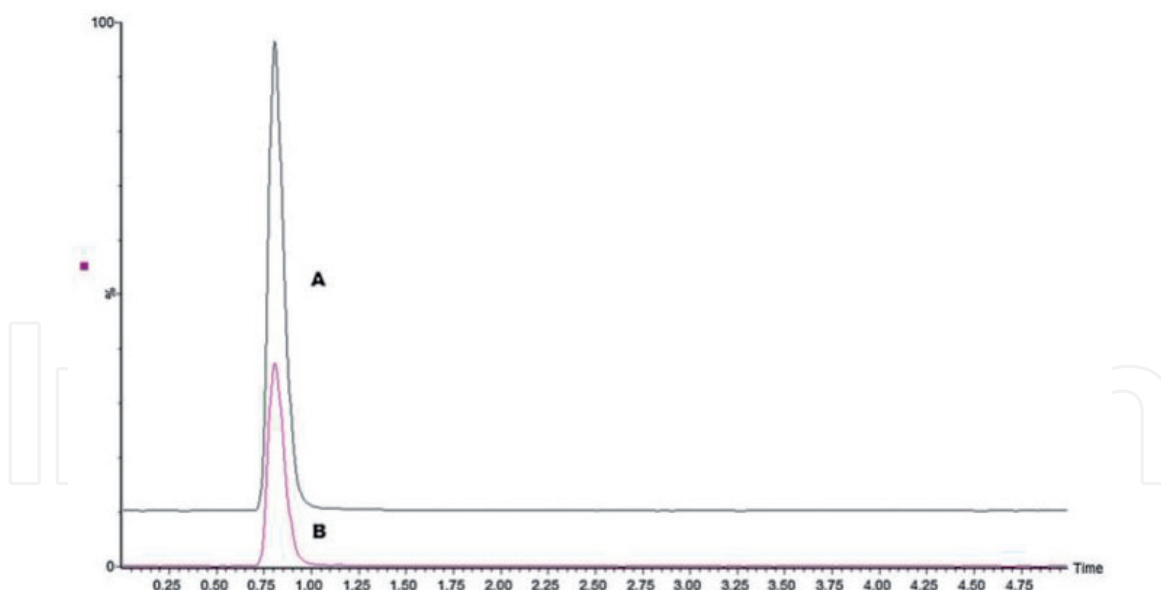
Vitamins can be defined as biologically active organic compounds that have a relatively low molecular weight. Vitamins are present in minute quantity; however it is very important for human health and overall growth. Vitamin can be fulfilled only from regular diet or nutrition supplement, because these nutrients help in the metabolism of carbohydrates, fat, and proteins. In addition to that, it is also reported that, it reduces damage from free radicals. On the other hand deficiency in vitamin may lead to various diseases. UPLC-MS is very well known for the analysis of vitamins. Several UPLC-MS methods have been reported for the analysis of vitamin B complex (thiamin, riboflavin, biotin, nicotinic acid, pyridoxine, pyridoxamine, pyridoxal, pantothenic acid, FAD, and nicotinamide) analysis in human milk. UPLC-MS coupled with ESI techniques is used to analyze vitamin B from milk sample [49].

#### 4.15 Determination of alkaloids, theobromine, and caffeine in cocoa

Ortega et al. [50] reported identification and quantification of alkaloids, theobromine, and caffeine in cocoa sample using UPLC-MS/MS. UPLC instrumentations are the most common techniques for routine analysis of such components in field of trace analysis. On the other hand, UPLC-MS has also been reported for alkaloid profiling of medicinal plants having cytotoxic properties. It is used for analysis of various alkaloids such as sanguinarine, berberine, protopine, and chelidone [50].

#### 4.16 Analysis of lactose in human and cow's milk

Sugars are found in a variety of food matrices as either naturally or artificially added. Fructose, glucose, and sucrose are important constituents of various fruit juices. Maltose is found in products derived from corn and grain products. Lactose, also known as milk sugar, exists in dairy products. This set of sugars is known as the five food sugars. Analysis of these sugars is important for quality control purposes, or to determine authenticity or adulteration of food products (**Figure 9**).



**Figure 9.**  
Chromatogram showing standard lactose (A) vs. milk sample (B).

In addition to that, lactose is most important source of sugar for infant, kids as well as adult. UPLC-MS/MS can be easily used for determination of lactose in cow's or human milk as well as other food products [51].

#### 4.17 Pesticides in fruit and vegetables

Fruit and vegetables are important crops of horticulture, as they are an integral part of the human diet. Fruit and vegetables provide carbohydrate, protein, vitamins, minerals, fiber and help in the maintenance of a healthy life style. However, in current years demand of fruit and vegetables has increased tremendously, because of high consumption and population demand. Therefore to boost the production, farmers are using so many chemicals in terms insecticides, fungicides, herbicides, acaricides, and rodenticides for prophylactic use or in diseased condition. However, it has been reported that, these chemical has very harmful effect on human health [52]. Savini et al. [33] reported a quick and sensitive UPLC method coupled with Orbitrap for determining highly polar pesticides and contaminants in processed fruits and vegetables.

#### 4.18 Analysis of food-based dyes using UPLC-MS

Synthetic oil-soluble mono-azo coloring agents such as Sudan dyes and Para Red are very common in food industries. Due to minimal expense and high intensity color it is very commonly used as food additives particularly in chili. However, International Agency for Research on Cancer (IARC 1975) categorized these dyes a potential cancer-causing agent. Moreover, illegal use of these dye such as Sudan Red 7B, Sudan I-IV and Para Red have been still found in food impacting consumer health. UPLC-MS has been reported as one of best choice of instrument analysis of such dyes due to their highest sensitivity.

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