Determination Methods for Biologically Active Compounds by Ultra-Performance Liquid Chromatography Coupled with Mass Spectrometry: Application to the Analyses of Pharmaceuticals, Foods, Plants, Environments, Metabonomics, and Metabolomics

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Abstract

This review summarizes the determination methods for biologically active compounds, such as pharmaceuticals, agrochemicals, and biogenic amines, by ultra-performance liquid chromatography coupled with mass spectrometry. The typical applications using the method are also described in this study, together with the characteristics of the methods.

Introduction

High-performance liquid chromatography (HPLC) coupled with various detection systems has been widely used for the analyses of various compounds in many research fields such as biological, pharmaceutical, food, and environmental. The broad use of HPLC in the last three decades seems to be due to the progress of column resins, detection systems, and data processing. Many compounds are satisfactorily separated by HPLC utilizing columns packed with porous 3–5 µm particles. However, the simultaneous separation of multi-components such as metabonomics is very difficult in the conventional HPLC system. The efficiency and speed of analysis have become of great importance in many application areas of LC. The pharmaceutical industry is particularly interested in rapid and efficient procedures for qualitative and quantitative analysis, in order to cope with a large number of samples. The simplest approach available to shorten an analytical run is to shorten the column length and increase the flow velocity. The approach utilizing a conventional stationary phase made of 3–5 µm particles is not recommended because the chromatographic performance is much lower and the separation of multi-components will be insufficient. Therefore, the method can be applied only for the analysis of a limited number of substances in simple matrices (e.g., quality control of drug formulations). Another strategy is the use of monoliths instead of porous particles (1). Monolithic rods, made of silica or polymeric materials, can accept high flow-rates in conventional column lengths without generating high backpressures. Although rapid analysis is possible with monolith columns due to their bimodal structures with macropores and mesopores (2), in general, the simultaneous separation of multi-components is fairly difficult. Therefore, their use is particularly adapted to simple analysis such as the quality control of drugs, the same as conventional silica columns.

The second way to shorten the run time is to increase the temperature. High-temperature liquid chromatography (HTLC) is a valuable tool for reducing the analytical time (3,4). At a high temperature, the operation at higher flow rate is possible due to a decrease in the mobile phase viscosity. HTLC can be used to perform rapid analysis with conventional column lengths due to the low backpressure. However, HTLC is not routinely used in spite of these advantages. The major limitation is low temperature stability of the packing materials often used in HPLC (5,6). Of course, unstable compounds at high temperatures cannot be adapted.

The third means to shorten run time is to decrease the particle size. The method utilizing small particle resins allows rapid analysis with high efficiency, but the negative aspect is a high backpressure generation, which is not acceptable for conventional HPLC instruments and conventional columns. According to the van Deemter equation, which shows the correlation between linear velocity and Height Equivalent to Theoretical Plate (HETP), a decrease in particle size minimizes the HETP value (7,8). This equation means that the separation efficiency depends on the particle size of the resin; the sharp peak is obtained from the use of a smaller particle. However, the use of short columns packed with small particles (e.g., 3 µm) does have practical limits to the combination of the chromatographic resolution and speed because of the low performance of the pumping systems. The

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problem is solved by both the development of a column packed with particle sizes less than 2 µm and an instrument that can operate at a high pressure. Reducing the particle diameter was expected to result in an increase in the efficiency and speed as well as increases in the resolution and sensitivity. Some research groups reported dealing with lab-made ultra high-pressure liquid chromatography (UHPLC) connected with columns packed with 1.5 µm particles (9,10). Recently, an improvement in chromatographic performance has been achieved by the introduction of an ultra-performance liquid chromatography (UPLC) system utilizing 1.7 µm porous particles as the column. The UPLC technology provides a higher peak capacity, greater resolution, increased sensitivity, and higher speed, compared to 3 µm material (11). This approach allows results to be achieved similar to those obtained previously by conventional HPLC, but in one-tenth of the run time. The radical shortening of the analytical time opens up the possibility of a relatively high throughput screening for samples containing multiple components (12–16).

In the UPLC system, several detectors such as UV–vis, fluorescence, and mass spectrometry (MS), are essentially possible to use, the same as in conventional HPLC. The important characteristics of a suitable detector are a long path-length and a low volume detection cell to enable the highest sensitivity. Furthermore, the system volumes should be minimized in order to maintain the speed, resolution, and sensitivity of the analysis. The reduction in peak width increases the peak capacity, significantly reducing the spectral overlap and the analytical sensitivity (17). Other requirements are a high sampling rate and a high acquisition rate. For instance, the typical peak widths generated by the UPLC system are in the order of a few seconds for a 10-min separation. Therefore, a detector that possesses a rapid sampling rate is recommended for drawing the UPLC performance.

A UV detector that is widely used as a universal detector might not be the best choice, because at least a few µL of the cell volume is necessary to obtain high sensitivity. In these viewpoints, the MS system seems to be most reliable as the detector for multi-components separated from UPLC. Thus, the present review mainly describes the analytical method for biologically important compounds by UPLC with MS detection.

MS detection

Over the past 20 years, HPLC coupled with MS detection has become a powerful technique for various fields in the pharmaceutical, biotechnology, food, agriculture, and chemical industries. Many applications, such as rapid analysis for drug discovery, impurity analysis, metabolite identification, regulatory science analysis, and combinatorial screening, utilize HPLC–MS as one of the main techniques.

The invention of an atomospheric pressure ionization (API) interface in the late 1980s allowed the facile connection of HPLC to quadrupole (Q) MS. Various detection modes of MS [e.g., triple Q, ion trap (IT), time-of-flight (TOF), hybrid Q-TOF, linear IT] have been developed in the following years. The functionality and capability of MS has gradually increased until now. Although Q-MS provides *m/z* information, the triple-Q-MS allows not only *m/z* of the parent ion, but also information about the fragments generated from the MS–MS analysis. The measurements of the multiple reaction monitoring (MRM) and precursor ion scanning available from a triple-Q-MS are powerful means for highly sensitive detection and for simplifying the complex samples, respectively. Because multi-step MS–MS data (MS^n) is obtained from IT-MS, both the *m/z* information of the parent and fragment ions are also detected similar to the triple Q. Furthermore, the ionic intensity is generally strong. Recently developed TOF-MS instruments (e.g., TOF, hybrid Q-TOF) allow the generation of the exact mass information (generally 3–5 ppm error) with greater accuracy and precision. The exact mass values can be used to speculate the candidate empirical formulae, which significantly reduces the number of the possible structure. Several hybrid-MS were also developed and used for the detection of various compounds in following years. To increase the overall performance of the MS detection, the separation efficiency of LC has to be enhanced. The UPLC utilizing sub-2-µm particles is more predominant in resolution, sensitivity, and speed of analysis. In the following sections, the applications of UPLC–MS are classified by the detection method and the analyzed sample.

Application

Drugs and their metabolites

HPLC is one of the standard and reliable techniques for drug analysis such as qualitative and quantitative determinations. Of course, the technique utilizing HPLC is applicable to the compounds not only for drug discovery but also for drug development. In the last decades, 3–5 µm resin columns have been used for HPLC analysis. Recently, the UPLC method utilizing 1.7 µm porous silica has gradually increased for the drug analysis because of its high peak capacity, high resolution, short run time, etc. For instance, amphetamine-type drugs (amphetamine and methanphetamine), ketamine, and several designer drugs (i.e., 3,4-methylenedioxyamphetamine [MDA], 3,4-methylenedioxymethamphetamine [MDMA], *para*-methoxyamphetamine [PMA], 4-methylthioamphetamine [4-MTA], and *N*-methyl-1-[3,4-methylenedioxyphenyl]-2-butanamine [MBDB]) were identified by product ion mass spectra in less than 4 min using UPLC–MS–MS (18). The same group also reported 3 min analysis of eight drugs (amphetamine, methanphetamine, ephedrine, pseudoephedrine, phentermine, ketamine, MDA, MDMA, and MDEA) by UPLC–MS–MS (19). The multi-residue analysis of pharmaceuticals in wastewater is carried out by UPLC–Q–TOF-MS (20). Pharmaceuticals, including veterinary drugs, are continuously being released into the environment mainly as a result of the manufacturing process, the disposal of unused or expired products, and the excreta. They are interesting as environmental contaminants because they often have physico-chemical behavior similar to other harmful xenobiotics. The prerequisite for proper risk assessment and monitoring of waste and drinking water quality is a method for multi-residual analysis that permits measurement at low nanograms per liter. Furthermore, the exact mass information is required for the structural elucidation of the contaminant. Thus, the TOF-MS

and Q–TOF-MS instruments, which enable accurate mass measurement with accuracy of less than 5 ppm, are suitable for such purposes. Twenty-nine pharmaceutical compounds belonging to different therapeutical classes (analgesics and anti-inflammatories, lipid regulating agents, cholesterol lowering agents, psychiatric drugs, anti-ulcer agents, histamine H2 receptor antagonist, antibiotics, and β-blockers) in wastewater and river water were identified within 10 min by UPLC–Q–TOF-MS (20). The trace level determination of nine priority pesticides (simazine, atrazine, isoproturon, diuron, terbutylazine, alachlor, pentachlorophenol, chloropyrifor, and trifluralin) in water was also performed by UPLC coupled with triple Q tandem MS–MS (21). The separation time of nine target compounds was less than 4 min, and the limits of detection were in the range between 0.11 and 7.8 ng/L. Fast analysis of three bromine-containing preservatives (bronopol, bronidox, and methyldibromo glutaronitrile), which are often used in cosmetics and pharmaceutical preparations, was carried out by UPLC with inductively coupled plasma mass spectrometry (ICP–MS) (22). The quantitations were performed on the basis of the less interfered ^{79}Br isotope, and both isotopes (^{79}Br and ^{81}Br) were monitored simultaneously in order to detect matrix-based interferences. The coupling of UPLC to ICP–MS described in this study is a very rare case. The short run times seem to be advantageous for ICP–MS detection due to the reduced consumption of expensive argon.

Although the previously mentioned examples are of the analysis of drug and related compounds in a simple matrix, the bioanalysis of various drugs are also reported. Troglitazone in mice plasma (23), amlodipine (24), and doxazosine (25) in human plasma were determined by the UPLC–MS–MS method. Ethynylestradiol in human plasma, labeled with dansyl chloride, was also determined by a similar MS–MS (26). The bioanalysis of drugs [e.g., epirubicin in human plasma (27) and corticosteroids in urine (28)] were also performed by UPLC–TOF-MS. These drugs in biological specimens such as plasma and urine are successfully determined. However, the mass spectrometric responses for an analyte in the standard solution and the same analyte in the biological matrix (plasma and urine, etc.) are sometimes different, and the influence of the matrix is thus inevitable for bioanalysis. The matrix effects result from coeluting the matrix substances that affect the ionization of the target analytes, resulting either in ion suppression or ion enhancement, in some cases. The matrix effects are caused by numerous factors and are analyte specific. Furthermore, they are highly variable and can be difficult to predict and control. The interference by the matrix can be reduced from sample pre-treatments such as protein precipitation, liquid–liquid extraction, and solid-phase extraction. On the other hand, UPLC separation provides significant advantages for reducing the matrix effects resulting from matrix components and in improving the sensitivity. Chambers et al. (29) show the reduction of the matrix effect by the analysis of 8 drugs (propranolol, atenolol, chlorpheniramine maleate, amitriptyline, pseudoephedrine, terfenadine, imipramine, and clozapine) in rat plasma utilizing UPLC–MS–MS.

HPLC is adaptable for the assessment of the purity of new drug candidates in the pharmaceutical industry. For instance, the quality evaluation of active compounds during the development of a synthetic route, and the stability assessment of the drug in trial formulations are performed by HPLC. The identification of any impurities generated during the synthesis and/or of any degradation products on the stability testing are undertaken by spectroscopic techniques such as nuclear magnetic resonance (NMR) and MS. The profiling of impurities and degradation products is an essential part of the pharmaceutical research and development process. It is a regulatory requirement to characterize the impurities of a manufacturing process to enable the scale-up from pilot plant manufacturing and to ensure the quality of the final product (30,31). The regulatory requirement also gives information regarding the quality over the lifetime of the product at various temperatures, light, relative humidity, and storage conditions. Because the concentration of impurities and degradation products are generally at low levels, the separation and detection method requires a high sensitivity and high resolution. Furthermore, a high-throughput method is recommended because many samples should be analyzed in limited periods. HPLC coupled with MS is the first choice for the identification and determination. Of course, the HPLC–UV method is adaptable for the purpose. Because the structural information cannot be obtained from the UV detection, the detector is not selected for the identification of the samples including unknown impurities. UPLC seems to be suitable for the determination and identification of the impurities because of the performance of the high peak capacity, high resolution, and rapid separation.

The detection and identification of drug metabolites is also crucial to both the drug discovery and development processes. The emphasis is slightly different in both areas. Because the number and diversity of compounds is very high in drug discovery, the focus is on the rapid detection and identification of the major metabolites. The data give information on the route of the metabolism and the cytochrome (CYP) p450 isoforms (32). In drug development, the detection and characterization of all metabolites has to be done for the efficiency and safety of the drug candidate. If a new compound is detected during the human metabolism study, the safety assessment study is necessary, as the metabolite itself may be toxic. Biotransformation is a part of the elimination pathway for many drugs. An understanding of the metabolic pathways of a drug is important for addressing the pharmacokinetic issues. Thus, the structural information on metabolites and an understanding of the major routes of metabolism play important roles in the overall drug discovery process. As CYP p450 enzymes, which are present in subcellular liver fractions, are some of the most important enzymes, test compounds are usually incubated in liver microsome fractions to identify the metabolites and the metabolic route. The metabolite identification studies require a high resolution, because diverse metabolites exist in low concentrations. As a result, longer run times are seen in the chromatograms utilizing 3–5 µm resin columns. To solve the drawback, the UPLC technique utilizing a 1.7-µm column is adopted for fast metabolite identification. Walles et al. (33) compared the precision and separation efficiency of three drugs (verapamil, propranolol, and fluoxetine) by the method using UPLC–Q–TOF-MS. The separation efficiency and the MS–MS spectral quality were also evaluated from the separation and detection of verapamil and major metabolites (norverapamil

and *O*-desmethylverapamil) in the incubation of human liver microsome. Testosterone and its metabolites, 6β-hydroxytestosterone (6β-OH-T), 16β-hydroxytestosterone (16β-OH-T), 16α-hydroxytestosterone (16α-OH-T), and 2α-hydroxytestosterone (2α-OH-T) (34,35), isosorbide-5-mononitrate (an active metabolite of isosorbide dinitrate) (36), desloratidine, and 3-hydroxydesloratadine (a metabolite) (37), and 4 antipsychotics (quetiapine, perospirone, aripiprazole, and quetiapine sulfoxide) and some metabolites (38), were successfully determined by the UPLC–MS–MS. Two major API sources, electrospray ionization (ESI), and atomospheric pressure chemical ionization (APCI), are used for LC–MS (39). ESI and APCI have their own benefits and limitations. ESI is especially well suited for the analytes that can be ionized in the liquid phase such as polar, non-volatile, high-molecular mass, and thermally labile compounds (40). On the other hand, APCI is effective in the analysis of medium- and low-polarity compounds (41). Many compounds can be ionized by the ESI method, but APCI may be suitable for a couple of compounds. Based upon such ideas, Yu et al. (42) are recommending the combination use of ESI and APCI sources (ESCi multi-mode ionization source). The mass spectrometer was switched rapidly among four ionization modes (ESI^+, ESI^-, $APCI^+$, and $APCI^-$) during the chromatographic run for each drug (acetophenone, corticosterone, daspone, 17α-hydroxyprogesterone, ibuprofen, nortriptyline, sulfadimethoxine, or tolbutamide). The UPLC–ESCi–MS–MS technique offers time savings in analysis and can therefore further improve the analytical throughput. The studies of metabolite identification involve the detection and structural characterization of the biotransformation products of drug candidates. These experiments are necessary throughout the drug discovery and development process. Bateman et al. (43) show the use of mass defect filtering in combination with UPLC and MS^E (E represents colligion energy) is a powerful approach for both in vitro and in vivo metabolite identification studies. The collection of fragmentation data through the use of MS^E data acquisition allows for the rapid assessment of metabolite structures.

As in the determination by the TOF-MS method, the metabolism studies of acetoaminophen (44), dextromethrophan (45), etc. are reported. TOF-MS has been recognized as a low-resolution technique, providing a very limited dynamic range. However, some technical innovations have dramatically changed this perception. The orthogonal acceleration of the ions significantly narrowed the width of the ion packets pushed into the flight tube. The temperature stabilization of the flight tube and the electronics improved the reproducibility of the flight time measurements. Furthermore, the developments of novel detectors broaden the dynamic range and increased the sensitivity. The high resolutions more than 10,000 FWHM (Full Width at Half Maximum) permit significant selectivity and sensitivity. Furthermore, the combination of UPLC–TOF-MS provides increased advantages concerning selectivity, sensitivity, and speed. The TOF detection offers two main advantages over the MS–MS detection for bioanalysis. One advantage is that there is no requirement for the development of distinct and specific MS methods for each analyte. The specificity for the analyte is derived from the extraction of a narrow window extracted ion chromatograms from full-scan data. The second advantage is that the data related to the metabolic routes are collected to the target compound. Consequently, UPLC–TOF-MS seems to provide an important role for metabolism study.

According to the Food and Drug Administration's policy statement for the development of new isomeric drugs (stereoisomer and optical isomer, etc), the pharmacokinetics of a single isomer or mixture of isomers have to be evaluated (46). Therefore, the quantitative assays for individual isomers in vivo are required early in the drug development. As the separation of isomers is generally very difficult owing to the similarity of the physicochemical property, a method with high resolution is essentially required. Wang et al. (47) developed a fast, sensitive, and specific UPLC–tandem-MS–MS method for the determination of the diastereomers of the drug (SCH503034) in monkey plasma. The de-proteinized samples were separated with a mixture of methanol–acetonitrile–water containing 40mM perfluoropentanoic acid (PFPA) as an ion-pair reagent and was detected by selected reaction monitoring (SRM) in the positive ionization mode using APCI. The two diastereomers were well separated within 5 min under an isocratic elution. The proposed UPLC–MS–MS method was applied to a high-throughput pharmacokinetic study in monkeys.

The assessment of drug permeability is an important task in order to predict the transport properties in humans. The permeability test has to be estimated in early drug discovery in a fast and cost-efficient way. Because the number of compounds with poor aqueous solubility is increasing with the rapid development of combinatorial chemistry, the permeability analysis has become a "bottleneck" in this drug screening. Therefore, the analysis for this assay has to be high-throughput. Mensch et al. (48) developed and validated the UPLC–tandem–MS–MS method for a high-throughput quantitation of 6 drugs (i.e., caffeine, propranolol, ampicillin, atenolol, griseofulvin, and carbamazepine) generated during the permeability assessment. Using UPLC combined with automated MS–MS analysis, a four-fold increase in throughput (1.5 min versus 6.5 min of chromatographic run time), as well as a significant increase in sensitivity (20-fold), was obtained compared to the traditional in house generic LC–MS methodology. The example applications to drug analysis using UPLC–MS are listed in Table I.

Metabonomics and metabolomics

Metabonomics and metabolomics are very similar in terms arising from different areas of bioscience research, mainly animal biochemistry and microbial/plant biochemistry, respectively. However, these terms are sometimes used interchangeably. Metabonomics (metabolomics) are defined as the quantitative measurement of the dymanic multi-parametric metabolic response of living systems to pathophysiological stimuli or genetic modification. Metabonomics is an approach to understanding the metabolic regulation of an organism and is based on the determination of global metabolite profiles in biological specimens with subsequent data analysis via a range of multivariate statistical approaches. This technology has rapidly developed and demonstrated a tremendous potential in many fields such as function genomics, drug efficacy and toxicology, disease diagnosis, drug discovery, and genotype discrimination. One important aspect of the metabonomics approach is the reli-

Table IA. Application of UPLC–MS to Drug Analysis

Analyte	UPLC condition			MS condition		Sample	Reference
	Column	Elution	Flow rate	Instrument	Detection		
β-Blockers (oxprenolol, metoprolol, acebutolol, propranolol, pindolol, alprenolol)	Acquity BEH C18 (1.7 µm, 100 × 2.1 mm) Temp. 35°C	Gradient: A, 0.1% TFA in H_2O B, 0.1% TFA in CH_3CN	0.5 mL/min	Micromass ZQ single Q	ESI^+	Drug standard	(88)
Amphetamine, methanphetamine, ephedrine, pseudoephedrine, MDA, MDMA, MDEA, ketamine	Acquity BEH C18 (1.7 µm, 50 × 2.1 mm) Temp. 40°C	Isocratic: Aqueous pyrrolidine–methanol	0.4 mL/min	Micromass Quattro Micro	ESI^+	Whole blood extract	(19)
Isosorbide 5-mononitrate	Acquity BEH C18 (1.7 µm, 100 × 2.1 mm), Temp. 35°C.	Isocratic: CH_3CN–H_2O (20:80)	0.3 mL/min	Micromass Quattro Micro	ESI^-	Human plasma	(36)
Amlodipine, Nimodipine (I.S.)	Acquity BEH C18 (1.7 µm, 50 × 2.1 mm) Temp. 45°C.	Gradient: A, 0.3% HCOOH in H_2O; B, 0.3% HCOOH in CH_3CN	0.35 mL/min	Micromass Quattro Micro	ESI^+ MRM	Human plasma (Pharmacokinetic study)	(24)
Doxazosine, tamusulosin (I.S.)	Acquity BEH C18 (1.7 µm, 50 × 2.1 mm), Temp. 40°C.	Gradient: A, 0.05% pentadeca-fluorooctanoic acid in H_2O; B, 0.05% pentadeca-fluorooctanoic acid in CH_3CN	No data	Micromass Quattro Micro	ESI^+ MRM	Human plasma	(25)
Epirubicin, epidaunorubicin (I.S.)	Acquity BEH C18 (1.7 µm, 50 × 1 mm), Temp. 30°C.	Gradient: A, 0.1% HCOOH in H_2O; B, 0.1% HCOOH in CH_3CN	0.2 mL/min	Quattro Premier Micromass	ESI^+ MRM	Human plasma	(27)
Troglitazone, rosiglitazone (I.S.)	Acquity BEH C18 (1.7 µm, 50 × 2.1 mm)	Isocratic: 50% MeOH in H_2O	0.5 mL/min	2000Q-TRAP	ESI^- MRM	Mice plasma (Pharmacokinetic study)	(23)
Acetophenone, corticosterone, ibuprophen, nortriptyline, sulfadimethoxine, tolbutamide, 4,4′-diaminodiphenyl sulfone, 17α-hydroxyprogesterone,	Acquity BEH C18 (1.7 µm, 50 × 2.1 mm) Temp. 40°C.	Gradient: A, 10mM CH_3COONH_4 in CH_3CN–H_2O (10:90); B, 10mM CH_3COONH_4 in CH_3CN–H_2O (95:5)	1.0 mL/min	Micromass Quattro Premier	ESI-APCI multimode	Microsomal incubation	(42)
Caffeine, propranolol, ampicillin, atenolol, griseofulvin, carbamazepine	Acquity BEH C18 (1.7 µm, 50 × 2.1 mm) Temp. 55°C	Isocratic and Gradient: A, 0.1% HCOOH in H_2O; B, 0.1% HCOOH in CH_3CN	0.2 mL/min (Isocratic) 0.6 mL/min (Gradient)	Micromass Quattro Premier	ESI^+ MRM	Permeability assessment	(48)
SCH 503034 diastereomers	Acquity BEH C18 (1.7 µm, 50 × 2.1 mm), Temp. 40°C.	Isocratic: 40mM PFPA in MeOH–H_2O	0.7 mL/min	API 4000 triple Q	$APCI^+$ SRM	Monkey plasma (Pharmacokinetic study)	(47)
Diphenhydramine, alprazolam, prednisolone, naproxen, ibuprofen	Acquity BEH C18 (1.7 µm, 50 × 2.1 mm) Temp. 40°C	Gradient: A, 10mM CH_3COONH_4 in CH_3CN–H_2O (20:80); B, 10mM CH_3COONH_4 in CH_3CN–H_2O (80:20)	0.6 mL/min	Micromass Quattro Premier tandem Q	ESI^+ and ESI^- MRM	Rat plasma	(89)

Table IB. (Continued) Application of UPLC–MS to Drug Analysis

Analyte	UPLC condition			MS condition		Sample	Reference
	Column	Elution	Flow rate	Instrument	Detection		
MDA, MDMA, PMA, 4-MTA, MBDB, ketamine	Acquity BEH C18 (1.7 µm, 50 × 2.1 mm) Temp. 40°C	Isocratic and gradient: Aqueous pyrrolidine–MeOH	0.4 mL/min	Micromass Quattro Micro	ESI^+ MRM	Drug standard	(18)
Dansyl derivatives of ethinyl estradiol, 19-norethindrone, levonorgestrel	Acquity BEH C18 (1.7 µm, 50 × 2.1 mm), Temp. 45°C	Gradient: A, 0.1% HCOOH in CH_3CN; B, 50% CH_3CN in H_2O	0.65 mL/min and 0.75 mL/min	API 4000 triple Q	ESI^+ MRM	Human plasam (Pharmacokinetic study)	(26)
Desloratadine, 3-hydroxydesloratadine	Acquity C18 (1.7 µm, 50 × 2.1 mm).	Gradient: A, 10mM $HCOONH_4$ with 0.2% HCOOH; B, 10mM $HCOONH_4$ in MeOH with 0.2% HCOOH	0.5 mL/min	API 4000 triple Q	ESI^+ MRM	Human plasma	(37)
Testosterone (T), 6β-OH-T, 16α-OH-T, 16β-OH-T, 2α-OH-T	Acquity BEH C18 (1.7 µm, 50 × 2.1 mm) Temp. 40°C	Gradient: A, 0.1% HCOOH in H_2O; B, 4mM CH_3COONH_4 and 0.02% CH_3COOH in CH_3CN–H2O (5:95)	0.5–1.0 mL/min	API 4000 triple Q Turbo ion spray	$APCI^+$, SRM	In vitro sample	(34)
Quetiapine, perospirone, aripiprazole, quetiapine sulfoxide	Acquity BEH C18 (1.7 µm, 100 × 2.1 mm), Temp. 40°C	Isocratic: 62% CH_3CN and 38% CH_3COONH_4	0.3 mL/min	Micromass Quattro Premier XE tandem Q	ESI^+ MRM	In vitro sample	(38)
Propranolol, atenolol, chlorpheniramine maleate, amitriptyline, pseudoephedrine, terfenadine, imipramine, clozapine	Acquity BEH C18 (1.7 µm, 50 × 2.1 mm), Temp. 50°C.	Gradient: A, 0.1% HCOOH, 10mM CH_3COONH_4 or 10mM ammonium bicarbonate; B, MeOH	0.4–0.6 mL/min	Micromass Quattro Premier tandem Q	ESI^+ MRM	Rat plasma (Matrix effect study)	(29)
Sulfanilamide, sulfadimethoxine	Acquity BEH C18 (1.7 µm, 100 × 2.1 mm), Temp. 40°C.	Gradient: A, 0.1% HCOOH; B, MeOH	0.3 mL/min	Micromass Quattro Ultima Pt	ESI^+ MRM	Electron irradiation study	(90)
Pesticides: simazine, atrazine, isoproturon, diuron, terbutylazine, alachlor, chlorpyrifos, trifluralin	Acquity BEH C18 (1.7 µm, 100 × 2.1mm).	Gradient: A, 0.1% HCOOH in H_2O; B, CH_3CN	0.5 mL/min	Micromass Quattro Premier	ESI^+ and ESI^- MRM	Groundwater	(21)
Azithromycin	Acquity BEH C18 (1.7 µm, 50 × 2.1 mm), Temp. 40°C	Gradient: A, 50mM CH_3COONH_4; B, CH_3CN	0.35 mL/min	Micromass Quatro Micro API MS	ESI^+ MRM	Human plasma (Pharmacokinetic study)	(95)
Oxymatrine and matrine	Acquity BEH C18 (1.7 µm, 50 × 2.1 mm), Temp. 40°C	Gradient: A, 0.01% CH_3COOH; B, MeOH	0.3 mL/min	Micromass Quatro Micro API MS	ESI^+ MRM	Beagle dog plasma (Pharmacokinetic study)	(96)
Testosterone hydroxyl metabolites	Acquity BEH C18 (1.7 µm, 50 × 2.1 mm), Temp. 40°C	Gradient: A, 0.1% HCOOH; B, 0.1% HCOOH in MeOH	0.5 mL/min	PE Sciex 4000Q Trap	ESI^+ MRM	Rat liver microsome	(35)
28 basic/neutral pharmaceuticals, illicit drugs	Acquity BEH C18 (1.7 µm, 100 × 1 mm), Temp. 22°C	Gradient: A, pH 2.8 in H_2O–MeOH–CH_3COOH (94.5:5:0.5); B, pH 3.2 MeOH–CH_3COOH (99.5:0.5)	70 µL/min	Micromass Quatro Micro triple-Q	ESI^+ MRM	Surface water	(97)

Table IC. (Continued) Application of UPLC–MS to Drug Analysis

	UPLC condition			MS condition			
Analyte	Column	Elution	Flow rate	Instrument	Detection	Sample	Reference
17 corticosteroids	Acquity BEH C18 (1.7 µm, 50 × 2.1 mm), Temp. 35°C	Gradient: A, 0.1% HCOOH in H_2O; B, 0.1% HCOOH in CH_3CN	0.35 mL/min	Waters LCT Premier TOF-MS	ESI^+, CID W-optics mode	Urine (sport dopind and veterinary control)	(28)
Veterinary drugs (more than 100)	Acquity BEH C18 (1.7 µm, 50 × 2.1 mm)	Gradient: A, H_2O–HCOOH–CH_3CN (947:3:50); B, H_2O–HCOOH–CH_3CN (50:3:947)	0.3 mL/min	Waters LCT Premier TOF-MS	ESI^+, CID	Urine (multi-residue screening)	(91)
Dextromethrophan and metabolites	Acquity C18 (1.7 µm, 100 × 2.1 mm).	Gradient: A, 0.1% HCOOH in H_2O; B, 95% CH_3CN in H_2O	0.4 mL/min	Micromass Q-Tof-Micro	ESI^+ MRM	In vitro study (Microsomal incubation)	(45)
Acetaminophen and metabolites	Acquity BEH C18 (1.7 µm, 50 × 2.1 mm), Temp. 40°C	Gradient: A, 0.1% HCOOH in H_2O; B, 0.1% HCOOH in CH_3CN	0.5 mL/min	Micromass Q-Tof-Micro	ESI^+ EIC	Human urine	(44)
Verapamil, proplanolol, fluoxetine, and their metabolites	Acquity BEH C18 (1.7µm, 50 × 2.1 mm), Temp. 40°C. Zorbax Eclipse C18 RRHT , (1.8 µm, 50 × 2.1 mm) Temp. 40°C.	Gradient: A, 0.1% HCOOH in H_2O; B, 0.1% HCOOH in CH_3CN	0.5 mL/min	Waters Q-TOF Premier	ESI^+ MS^E mode (collision energy switching), EIC	In vitro study (Human liver microsomal incubation)	(33)
Midazolam and metabolites	Acquity C18 . (1.7 µm, 100 × 2.1 mm or 100 × 1.0 mm)	Gradient: A, 0.1% HCOOH in H_2O; B, 95% CH_3CN in H_2O	0.4–0.5 mL/min for 2.1 mm column; 0.1 mL/min for 1 mm colum	Micromass Q-Tof-Micro	ESI^+, EIC MS and MS–MS modes	Rat bile	(92)
Indinavir, L-006235 (cathepsin K inhibitor) and their metabolites,	Acquity C18 (1.7 µm, 100 × 1.0 mm), Temp. 40°C	Gradient: A, 0.1% HCOOH in H_2O; B, 0.1% HCOOH in CH_3CN	0.25 mL/min	Waters Q-TOF Premier	ESI^+ MS^E mode (collision energy switching)	In vitro and in vivo study (Rat plasma and hepatocyte incubation)	(43)
Compound 1 (novel cognition-enhancing agent)	Acquity BEH C18 (1.7 µm, 50 × 2.1 mm)	Gradient: A, 0.25mM formate buffer (pH 3); B, CH_3CN	0.5 mL/min	Micromass Q-Tof2	ESI^+	Rat plasma	(93)
29 drugs	Acquity BEH C18 (1.7 µm, 50 × 2.1 mm)	Gradient: A, 5mM ammonium acetate buffer (pH 4.8); B, CH_3CN–MeOH (2:1)	0.4 mL/min	Micromass Q-Tof-Micro	ESI^+ and ESI^-	River water and wastewater	(20)
Ranitidine and its impurities	Acquity BEH C18, C8, phenyl, C18 Shield (1.7 µm, 100 × 2.1 mm), Temp. 50°C	Gradient: A, 20mM ammonium bicarbonate; B, MeOH	0.45 mL/min	Micromass Q-Tof-Micro	ESI^+ MS–MS	Heat degradation study	(94)
Bronopol, bronidox, methyl-dibromoglutaronitrile	Acquity BEH C18 (1.7 µm, 100 × 2.1 mm), Temp. 25°C	Isocratic: 0.1% HCOOH in 40% MeOH	50–90 µL/min	ICP-MS (PE-SCIEX ELAN 6000)	^{79}Br and ^{81}Br	Cosmetic products	(22)

ability and significance of the identified biomarkers. Many technologies have been used in metabonomics research. NMR is widely accepted and the most popular method, characterized by its high speed and no requirement for complicated sample pretreatment. FT–MS was recently used for the metabonomics research as a high-throughput determination. The combinations of MS and separation techniques (e.g., gas chromatography, LC, and capillary electrophoresis) have been widely employed as the tool in metabolite fingerprinting and metabolite identification. Recently, LC–MS based methods have increased in metabonomics research. As described in the previous section, UPLC has been applied to the determination of multi-components in complex matrices, such as biological specimens. The characterization (e.g., rapid separation and the high resolution of peaks) has been considered to offer a more compatible combination with MS for use in metabonomics research. Nordstrom et al. (49) demonstrated the efficiency of the UPLC technology for nontargeted metabonomics applications. Indeed, 20% more components compared to HPLC were separated by UPLC. Moreover, UPLC displayed better retention time reproducibility and signal-to-noise ratios over the HPLC. Wilson et al. (50) also show that UPLC offers significant advantages over conventional HPLC amounting to more than doubling of peak capacity, an almost 10-fold increase in speed and a 3–5-fold increase in sensitivity. A main theme of this research is related to the multivariate metabolic profiling of urines from males and females of two groups of phenotypically normal mouse strains and a nude mouse strain. From the results of the principal component analysis (PCA) derived from the UPLC–TOF-MS data for male and female urine samples, black and white mouse samples were clearly classified as distinct from those of the nude mice. A significant separation of the black and white mouse urines is also achieved as well as of males and females (50,51). The metabolite profiles for urine obtained from three strains of Zucker rats (Zucker lean, Zucker [fa/fa] obese and Zucker [lean/fa] cross [52]). The age- and strain-related differences were clearly noted with the leptin-difficient (fa/fa) obese animal showing significant differences from both the other Zucker rat strains. Recently, Inagaki et al. (53) reported a significant difference in the metabolite profiling in hairs of normal and hypertensive rats (SHR/Izm and SHRSP/Izm). Many other studies concerning metabonomics and metabolomics utilizing the UPLC–MS system have been published (54–56). The biomarker discovery by statistical analysis based upon the difference of metabolite features obtained from UPLC–MS has been reported for cancer (57), kidney cancer (58), and metabolic diseases (59). For the determination of organic acid markers relevant to inherited metabolic diseases, a derivatization reagent, 4-[2-(*N*,*N*-dimethylamino)ethylaminosulfonyl]-7-(2-aminoethylamino)-2,1,3-benzoxadiazole (DAABD-AE), for carboxylic acid group was used for the selected analysis closely related and clinically important dicarboxylic acids (glutaric and 3-hydroxyglutaric acids) by UPLC–MS–MS (59). Ross et al. (60) also described a UPLC–MS–MS method for the rapid quantitation (less than 3 min) of organic acids to monitor the metabolism during microbial fermentation because organic acids are important metabolites in fermentation.

According to a report from the World Health Organization, 65–80% of the world's population intakes traditional medicine as a primary form of healthcare. Herbal medicine is a commonly practiced form of traditional medicine. Because the quality and contents of herbs are highly variable depending upon the species, geographical origins, cultivation and harvesting procedures, and post-harvesting formulation processes, the differential analysis of metabolite profiling is important with regards to quality control, safety assessment, and formulation of herbal medicinal products. UPLC–TOF-MS analysis was applied to identify the relationship between Sugihiratake mushrooms and acute encephalopathy cases based upon the multivariate statistical analysis of mass features of toxic and safe mushrooms (61). Chen et al. (62) report on the discrimination of herbal extracts and the ginsenoside biomarker discovery of raw and steamed *Panax notoginseng* by multivariate PCA based on the UPLC–TOF-MS data. The assessment of Chinese herbal medicine based upon the detection of aristolochic acids by UPLC–MS was also reported by Jacob et al. (63). Although the metabonomics method has been applied to the pharmacodynamics study, toxicity evaluation, and discrimination of the pharmaceutical preparations of other plant medicines, the combined study on the therapeutic basis and metabolic effects of traditional Chinese medicine is very rare. Li et al. (64) employed a metabonomic approach for the investigation of the therapeutic basis and metabonomic effects of *Epimedium brevicornum* Maxim. on an animal model induced by a high dose of hydrocortisone. A significant difference in the endogenous metabolite profiles was observed in the intervention rats and the abnormality of the metabolism recovered towards the normal level after the administration of the *Epimedium brevicornum* Maxim. extract. The metabolomic approach is a potentially powerful means for exploring the therapeutic basis and clarifing the possible action mechanism of traditional medicine. The UPLC–MS–MS method was also developed for the quantitative and qualitative determination of the constituents of the flower of *Trollius ledibouri* Reichb (65), but not for the metabolomics study. From more than 50 peaks detected in 95% ethanol extract, 15 constituents (e.g., orientin, 2"-O-β-L-galactopyranosylorientin, 2"-O-β-arabinopyranosylorientin, and vitexin) were deduced from the MS–MS spectra.

In many studies for metabonomics and metabolomics utilizing UPLC, ESI-MS, and TOF-MS have been used as the instrument of mass detection. Although the choice depends on the research purpose, in the case of the biomarker discovery based upon the difference of the multivariate MS features, TOF-MS and Q–TOF-MS seem to be more suitable in terms of an accurate mass measurement. The details of metabonomics and metabolomics studies are shown in Table II.

Biological compounds

Lipids are an important class of biomolecule that exist in great variety in higher organisms. The great diversity exhibited by these molecules is most likely due to their many biochemical functions. Therefore, the analysis and profiling of lipids has become important in the fields of food analysis. The resolution of lipid profiling in biological fluids is also important for the analysis of the effects of many candidate pharmaceuticals of metabolic pathways, such as cholesterol synthesis. A majority of chromatographic separations are based upon reversed-phase LC.

Table IIA. Application of UPLC–MS to Metabonomics and Metabolomics Studies

Purpose	UPLC condition			MS condition		Data Analysis	Sample	Ref
	Column	Elution	Flow rate	Instrument	Detection			
Hydrazine induced liver toxicity (Biomarker discovery)	Acquity BEH C18 (1.7 µm, 100 × 2.1 mm), Temp. 40°C	Gradient: A, 0.1% HCOOH in H_2O; B, 0.1% HCOOH in CH_3CN.	0.5 mL/min	Micromass Q-Tof Premier	ESI^+ MS–MS	O-PLS-DA	Rat urine	(55)
Hydrazine induced liver toxicity (Metabonomic toxicology)	Acquity BEH C18 (1.7 µm, 100 × 2.1 mm), Temp. 40°C	Gradient: A, 0.1% HCOOH in H_2O; B, 0.1% HCOOH in CH_3CN	0.5 mL/min	Micromass Q-Tof Micro	ESI^+ MS–MS	SHY	Rat urine	(56)
Kidney cancer (Biomarker discovery)	Acquity BEH Shield C18 (1.7 µm, 150 × 2.1 mm), Temp. 40°C.	Gradient: A, CH_3CN; B, 13mM CH_3COONH_4 (pH 5.5)	0.5 mL/min	Finnigan LTQ linear ion trap	ESI^+ and ESI^-	MZmine, XCMS ANOVA, PLS, PCA	Human urine	(58)
Metabonomics application	Acquity BEH C18 (1.7µm, 100 × 2.1mm), Temp. 40°C.	Gradient: A, 0.1% HCOOH in H_2O; B, 0.1% HCOOH in CH_3CN	0.5 mL/min	Micromass Q-Tof Micro	ESI^+	XCMS	Human serum	(49)
Cancer patient (cis-diol metabolites)	Acquity BEH C18 (1.7 µm, 100 × 2.1 mm), Temp. 40°C	Gradient: A, 0.1% HCOOH in H_2O; B, CH_3CN	0.25–0.35 mL/min	Micromass Q-Tof	ESI^+	PCA	Human urine	(57)
Metabolic disease (organic acid marker)	Acquity BEH C18 (1.7 µm, 50 × 2.1 mm), Temp. 40°C	Gradient: A, 0.05% PDOFA in H_2O; B, 0.05% PDOFA in CH_3CN	0.4 mL/min	Micromass Quattro Micro triple Q	ESI^+ MRM		Human urine labeled with DAABD-AE (glutaric acidimia type I)	(59)
Metabonomics (Zucker rats, mice)	Acquity BEH C18 (1.7 µm, 50 × 2.1 mm), Temp. 40°C	Gradient: A, 0.1% HCOOH in H_2O; B, 0.1% HCOOH in CH_3CN	1 mL/min	Micromass LCT Premier TOF-MS	ESI^+	PCA PLS-DA	Urine (normal/obese Zucker rats, and black, white, nude mice)	(51)
Metabonomics (Zucker rats: lean, fa/fa obese, lean/fa cross)	Acquity BEH C18 (1.7 µm, 100 × 2.1 mm)	Gradient: A, 0.1% HCOOH in H_2O; B, 0.1% HCOOH in CH_3CN	0.6 mL/min	Micromass LCT Premier TOF-MS W-optics	ESI^+	PCA, PLS-DA	Urine (strain and age differences)	(52)
Metabolic pathway profiling (male, female, and strain different mice)	Acquity BEH C18 (1.7 µm, 50 or 100 × 2.1 mm), Temp. 40°C	Gradient: A, 0.1% HCOOH in H_2O; B, 0.1% HCOOH in CH3CN	0.5–0.8 mL/min	Micromass LCT Premier TOF-MS W-optics	ESI^+	PCA	Urine	(50)
Metabolite profile (Chinese medicinal herb) (detection of aristolochic acids)	Acquity BEH C18 (1.7 µm, 100 × 2.1 mm), Temp. 65°C	Gradient: A, 0.1% HCOOH in H_2O; B, 0.1% HCOOH in CH3CN/isopropyl alcohol (1:1)	0.4 mL/min	Micromass Quattro Premier tandem Q	ESI^+ MRM	PCA PLS-DA	Herb preparations	(63)
Metabolomics (raw and steamed *Panax notoginseng*)	Acquity BEH C18 (1.7 µm, 100 × 2.1 mm), Temp. 45°C	Gradient: A, 0.1% HCOOH in H_2O; B, 0.1% HCOOH in CH_3CN	0.5 mL/min	Micromass LCT Premier TOF-MS	ESI^+ and ESI^-	PCA	Herbal extracts	(62)

Table IIB. (Continued) Application of UPLC–MS to Metabonomics and Metabolomics Studies

Purpose	UPLC condition			MS condition		Data Analysis	Sample	Ref
	Column	Elution	Flow rate	Instrument	Detection			
Pharmaco-metabonomic study	Acquity BEH C18 (1.7 µm, 50 × 2.1 mm), Temp. 40°C	Gradient: A, 0.1% HCOOH in H_2O; B, 0.1% HCOOH in CH_3CN	0.25mL/min	Micromass Quattro Micro	ESI^+ and ESI^-	PCA	Rat urine and serum (hydrocortisone and herbal medicine administration)	(64)
Metabonomics (± arecoline 1-oxide administration to mice)	Acquity BEH C18 (1.7 µm, 50 × 2.1 mm).	Gradient: A, 0.1% HCOOH in H_2O; B, 0.1% HCOOH in CH_3CN	0.6 mL/min	Waters Q-TOF Premier	ESI^+ MS–MS	PCA, PLS-DA	Mice urine	(54)
Metabolomics (Toxicity of Angel's Wing Mushroom)	Acquity BEH C18 (1.7 µm, 100 × 2.1 mm)	Gradient: A, 5mM CH_3COONH_4; B, MeOH	0.3 mL/min	Waters LCT Premier TOF-MS W-optics	ESI^+	PCA, SIMCA	MeOH extracts	(61)
Metabolomics (organic acid profiling during fermentation)	Hypersil Gold C18 (1.9 µm, 50 × 2.1 mm), Temp. 30°C	Isocratic: H_2O–CH_3CN (97:3)	0.3 mL/min	Waters Quattro Premier XE triple Q	ESI^- MRM	Matlab v6.5	Filtered fermentation samples	(60)
Metabolomic effects of hepatotoxic doses of pravastin (identify potential markers)	Acquity BEH C18 (1.7 µm, 100 × 2.1 mm or 1 mm), Temp. 40°C	Gradient: A, 0.1% HCOOH; B, 0.1% HCOOH in CH_3CN	0.5 mL/min 136 µL/min	Micromass Q-Tof Micro	ESI^+	PCA	Rat urine	(98)
Stress-induced metabolome modifications	Acquity BEH C18 (1.7 µm, 150 × 2.1 mm)	Gradient: A, 0.1% HCOOH; B, 0.1% HCOOH in CH_3CN	0.3 mL/min	Micromass LCT Premier TOF MS	ESI^+	PCA and HCA	Leaf extract of *Arabidopsis thaliana*	(99)

However, lipid analysis is often carried out utilizing a normal-phase chromatography because of the high hydrophobic property. Rainville et al. (66) applied UPLC technology to lipid analysis in rat plasma. UPLC coupled with Q–TOF-MS operating in the MS^E mode gave both fragment and parent information the phospholipids in a single chromatographic run. The UPLC–Q–TOF-MS system may provide an attractive means to the lipidmics study as well as targeted lipid analysis (67).

The importance of oligosaccharides in biological systems has been recognized and, thus, much research concerning the structural elucidation has been carried out until now. Although HPLC is one of the important techniques for the determination of oligosaccharides, the simultaneous analysis of oligosaccharides by conventional LC utilizing 3–5 µm columns is very difficult due to the similarity and diversity. Kurihara et al. (68,69) used UPLC–TOF-MS for multi-component analysis of the oligosaccharides. Fifteen species of oligosaccharides in ovalbumin were satisfactorily separated and detected by the method. The same group also applied the UPLC–TOF-MS system to the separation and detection of histamine and the metabolites in mice hairs (70,71). As in the other applications to biological components, apolipoproteins in human serum (72), *N*-acylhomoserine lacton metabolites (73), and 5-hydroxytryptophol glucuronide, and 5-hydroxyindoleacetic acid (74) were determined by UPLC–tandem MS.

Food analysis and others

The main research fields for the use of UPLC–MS technology are pharmaceutical analysis and bioanalysis, as described in the previous sections. Food analyses such as food components, food additives, and hazardous compounds in food, are also inevitable. According to the EC residue monitoring program 2005–2007, 55 pesticides in various foods including potatoes, oranges, and cereal-based baby foods have to be monitored. The analysis required efficiency, speed, and sensitivity for multi-residues in real samples. The UPLC method, which gives superior chromatographic resolution and reduced run times, seems to be suitable for multi-residue analysis. The low degree of band broadening in UPLC also benefits mass spectrometric detection, concentrating the analyte at the peak center and thereby increasing the response. Leandro et al. (75) developed the determination method for 52 pesticides in foods, including baby foods, by UPLC–MS–MS with fast polarity switching. Forty-four and eight compounds were determined by the polarity switching method in positive and negative ionization modes in a single run, respectively. The multi-residue analyses of priority pesticides were also reported by other papers (76,77). The UPLC method coupled with MS offers improvements in performance for quantitative analysis over the existing HPLC–MS–MS methodology.

Glycocorticoids have metabolic and anti-inflammatory prop-

Table III. Application of UPLC–MS to Food Analysis

Purpose	UPLC condition			MS condition		Sample	Reference
	Column	Elution	Flow rate	Instrument	Detection		
16 Mutagenic heterocyclic amines	Acquity BEH C18 (1.7 µm, 50 × 2.1 mm)	Gradient: A, CH_3CN; B, 30mM HCOOH–$HCOONH_4$ (pH4.75)	1 mL/min	Micromass Quattro Premier triple Q	ESI^+ MS–MS SRM	Lyophilized meat extracts	(82)
17 Glucocorticoids	Acquity BEH C18 (1.7 µm, 100 × 2.1 mm), Temp. 40°C	Gradient: A, MeOH; B, 0.1% HCOOH in H_2O	0.3 mL/min	Micromass Quattro Ultima	ESI^- MS/MS	Milk and egg	(78)
17 Mycotoxins	Acquity BEH C18 (1.7 µm, 100 × 2.1 mm), Temp. 35°C	Gradient: A, 10mM CH_3COONH_4 (for ESI^+ mode), 0.1% NH_4OH (for ESI^- mode); B, MeOH	0.3 mL/min	Micromass Quattro Ultima triple Q	ESI^+ and ESI^- MS–MS MRM	Peanut butters and corn feeds	(79)
16 Priority pesticides and transformation products	Acquity BEH C18 (1.7 µm, 100 × 2.1 mm), Temp. 40°C	Gradient: A, 20mM CH_3COONH_4 in H_2O–MeOH (90:10); B, 20mM CH_3COONH_4 in H_2O–MeOH (10:90)	0.3 mL/min	Micromass Quattro Premier triple Q	ESI^+ MS–MS MRM	Baby foods (fruit and rice, potato and pork, oats, and cream)	(76)
17 (semi)polar pesticides	Acquity BEH C18 (1.7 µm, 100 × 2.1 mm), Temp. 25°C	Gradient: A, H_2O; B, CH_3CN	0.3 mL/min	Micromass Quattro Premier triple Q	ESI^+ MS–MS MRM	Apples	(77)
52 Pesticides	Acquity BEH C18 (1.7 µm, 50 × 2.1 mm), Temp. 40°C	Gradient: A, 17.5mM CH_3COOH in H_2O; B, 17.5mM CH_3COOH in CH_3CN	0.6 mL/min	Waters Quattro Premier XE tandem Q	ESI^+ and ESI^- (polarity switching) MS–MS MRM	Cereal-based baby foods, oranges, and potatoes	(75)
Acrylamide	Acquity BEH C18 (1.7 µm, 50 × 2.1 mm), Temp. 25°C	Isocratic: 0.1% HCOOH in H_2O–MeOH (90:10)	0.2 mL/min	Micromass Quattro Ultima triple Q	ESI^+ MS–MS MRM	Potato crisps	(83)
Phytosterols (plant sterols)	Acquity BEH C18 (1.7 µm, 100 × 1.0 mm), Temp. 35°C.	Gradient: A, MeOH; B, 1% CH_3CN in H_2O	0.1 mL/min	Micromass Quattro Ultima triple Q	$APCI^+$ SIM	Corn, sesame, oat, and peanut	(85)
Macrolide antibiotics	Acquity BEH C18 (1.7 µm, 100 × 2.1 mm), Temp. 45°C.	Gradient: A, CH_3CN; B, 10mM CH_3COONH_4	0.4–0.5 mL/min	Micromass Q-Tof Premier	$APCI^+$ MS and MS–MS	Egg, raw milk, and honey	(100)

erties. Artificial glycocorticoids have been used in many veterinary therapeutic drugs for inflammatory diseases. For the animals that will be used for human consumption, the residual level is a topic for human healthcare. Cui et al. (78) developed a UPLC–MS–MS method for the simultaneous determination of 17 glycocorticoid residues in eggs and milk. In this case, ESI tandem MS operating in a negative ion mode was adopted.

Mycotoxins, which are a series of secondary metabolites generated from molds (e.g., *Aspergillus*, *Fusarium*, and *Penicillium*), widely contaminate plant origin products such as crops and feeds. Because many kinds of mycotoxins have been found, the simultaneous separation is very difficult in conventional HPLC. Ren et al. (79) developed a UPLC–MS–MS method for the simultaneous quantitation of mycotoxins in various foods and feeds. Ten positive and 7 negative ions of mycotoxins were separated by gradient elution with 6.5 and 4 min, respectively. The limit of quantitation (LOQ) (0.01–0.7 µg/kg) was lower than the criteria of EU, USA, and other countries on the determination of the minimum limiting levels of various mycotoxins in foods. Even with the use of the UPLC technology, total analysis of more than 200 mycotoxin species still seems to be difficult. As in the analysis of toxins, the lipophilic marine toxins (80) and microcystins (81) were also determined by UPLC–MS–MS.

Heterocyclic amines such as Trp-P-1 and -P-2, formed from cooking of protein-rich foodstuffs, have been proven to be mutagenic and sometimes shown to be carcinogenic in animal exper-

iments. The analytical method has to be detected at a low ppb level with a short run time, which requires a high throughput. Sixteen mutagenic heterocyclic amines in meat extract were separated in less than 2 min and detected by UPLC–MS–MS (82). The LODs were 10-fold lower than those obtained with the HPLC–MS–MS method. The discovery of an undesirable acrylamide in heat-treated foods attracted wide attention. The acrylamide contents under the asparagine-sugar low-moisture system were quantitated by the UPLC–MS–MS system (83,84).

As in the plant component analysis utilizing UPLC technology, the identification and quantitation of 10 diversiform phytosterols in food materials (e.g., corn, sesame, oat, and peanut) were carried out by UPLC–APCI–MS (85). The determination of flavonoids (86) and ginsenosides (87) were also performed by UPLC–MS. The phytosterols which are a "triterpene" family and bioactive components occurring in all vegetable foods are important compounds in terms of decreased serum cholesterol levels in humans. The examples for food analysis are shown in Table III.

Conclusion and Prospect

The miniaturization of resin particle size and column dimension is one of the major current trends in separation sciences. This leads to a shortening of the analytical run time and great savings in solvent consumption. UPLC is a fast commercially available ultra-high pressure system in LC, which allows using a small particle-packed column (< 2 µm). As described in this study, the UPLC technology provides a higher peak capacity, greater resolution, increased sensitivity, and higher speed, compared to 3–5 µm material. The radical shortening of the analytical time opens up the possibility of a relatively high throughput determination for the samples containing multiple components. In the HPLC system, various detectors such as UV–vis, FL, and MS are possible to use. However, in the UPLC system, the important characteristics for detecting have to possess a long pathlength and low volume detection cell to obtain the highest sensitivity. The reduction in peak width increases the peak capacity, significantly reduced the spectral overlap and analytical sensitivity. Both the high sampling rate and the high acquisition rate are important in UPLC performance. Therefore, the detector possessing a rapid sampling rate is recommended to assess the UPLC performance. From such observations, the MS system is currently most reliable as the detector of multi-components separated from UPLC. Indeed, many applications by UPLC coupled to MS have demonstrated the efficiency of the UPLC separation and MS detection systems, such as tandem MS–MS and Q–TOF-MS. However, the ion suppression based upon endogenous substances in a biological specimen is sometimes observed in MS detection. To avoid the unfavorable ion suppression, sample pretreatment to eliminate the endogenous substances and/or to concentrate the analytes, such as LLE and SPE, is most important, and high sensitivity, accuracy, precision, and robustness are obtained as the results.

Because chromatography systems using sub-2-µm particles are becoming increasingly popular due to the potential for increasing the speed, resolution, sensitivity, and peak capacity, high-pressure LC systems similar to UPLC, operating at pressures up to 15,000 psi have been developed with several companies. Thermo Accera (pressure limit, 15,000 psi: Thermo Electron) and X-LC (pressure limit, 15000 psi: JASCO) are very high-pressure LC systems and can to operate at pressures up to 15,000 psi to accommodate the elevated backpressures associated with using sub-2-µm particles. On the other hand, the pressure limits of the Agilent 1200SL (Agilent) and UFLC (Shimadzu) are 9,000 psi and 5,000 psi, respectively, so the use of columns with lower than 2 µm particles seems to be difficult now. Overall, each instrument is capable of providing a reliable and reproducible chromatography. According to the development of the LC systems, various columns packed with 1.5–2.5 µm particles are now on the market. Consequently, ultra-high pressure LC such as UPLC offers an excellent chromatographic performance and will become a standard LC technique.

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