



# Open Chemistry Journal

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DOI: 10.2174/1874842201603010001



## Principle, Instrumentation, and Applications of UPLC: A Novel Technique of Liquid Chromatography

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Received: April 03, 2015

Revised: March 03, 2016

Accepted: March 09, 2016

**Abstract:** The key focus of the pharmaceutical or chemical industries is to reduce the cost involved in the development of new drugs and to improve the selectivity, sensitivity, and resolution for their detection. The purpose can now be solved by the separation method called UPLC which is the modified HPLC method comprising high pressure and small sized particles (less than 2  $\mu\text{m}$ ) used in the column, so the length of the column decreases leading to time saving and reduction in the consumption of solvent. The underlying principle of UPLC is based on van Deemter statement which describes the connection between linear velocity with plate height. UPLC contributes to the improvement of the three areas: speed, resolution, and sensitivity. This is a new advanced category of the HPLC which has the same basic principle and methodology with improved chromatographic performance. This review is an effort to compile the principle, instrumentation, and applications of UPLC.

**Keywords:** Flow rate, HPLC, Plate height, UPLC, Van Deemter equation.

### INTRODUCTION

High-performance liquid chromatography (HPLC) is an important liquid chromatography (LC) technique used for the segregation of different components in mixtures. It is also used for the identification and quantification of compounds in the process of drug development and has been used over the world since decades. To further achieve the dramatic increase in resolution, speed and sensitivity in LC, a significant advancement in the instrumentation and column technology (column particle size and column dimension) were made [1]. To achieve the above targets, Waters in 2004, launched and trademarked Ultra Performance Liquid Chromatography (UPLC) which is based upon small, porous particles (sub 2micron particles). Van Deemter equation is the principle behind this evolution which correlates the connection between linear velocity and plate height. The small particles require a high pressure to work with UPLC *i.e.*, 6000 psi which is typically the upper limit of conventional HPLCs. It was observed that when the particle size is decreased below 2.5  $\mu\text{m}$ , there is a remarkable increase in the effectiveness and this effectiveness does not lessen on increasing the linear speed or rate of flow [2]. The use of speed, particles with a small radius and maximum number of resolvable peaks (peak capacity) comprehends the efficiency together with resolution. This method reduces the mobile phase volume consumption by at least 80% compared to HPLC with a shorter runtime of about 1.5 min. The smaller sized particles increase the pressure up to 1000 bars or more which can alone increase the retention factor of the separation. Lower injection volume is required for UPLC which results in higher efficiency and increase in resolution. The higher column temperature reduces the mobile phase viscosity resulting in the high diffusion coefficient and flow rate without significant loss in efficiency and increase in column back pressure. UPLC is a special version of HPLC which has the advantage of technological strides made in particle chemistry performance, system optimization, detector design, data processing and control [3]. These achievements lead to a very significant increase in resolution, sensitivity and efficiency with faster results and less consumption of solvents which lowers the cost and make the technology

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environment friendly also [4, 5]. Table 1 shows the comparison between UPLC and HPLC.

**Table 1. Comparison Between UPLC and HPLC**

Characteristics	HPLC	UPLC
Particle size	3 to 5 $\mu$ m	Less than 2 $\mu$ m
Maximum backpressure	300-400 bars	1000 bars
Analytical column	C18	UPLC BEH C18
Column dimensions	150 X 3.2 mm	50 X 2.1 mm
Injection volume	5 $\mu$ L	2 $\mu$ L
Column temperature	30 ° C	65 °C
Total run time	10 min.	1.5 min
USP resolution	3.2	3.4
Plate count	2000	7500
Flow rate	3.0 ml/min	0.6ml/min

## PRINCIPLE

The underlying principle of UPLC is based on the van Deemter relationship which explains the correlation between flow rate and plate height [5]. The van Deemter equation (i) shows that the flow range with the smaller particles is much greater in comparison with larger particles for good results [6 - 9].

$$H=A+\frac{B}{v}+Cv \quad \text{(i)}$$

Where H represents height equivalent to the theoretical plate (HETP), A, B & C are the constants and v is the flow rate (linear velocity) of the carrier gas. The aim is to minimize HETP to improve column efficiency. The term A does not depend on velocity and indicates eddy mixing. It is smaller if the columns are filled with small and uniform sized particles. The term B denotes the tendency of natural diffusion of the particles. At high flow rates, this effect is smaller, so this term is divided by v. The term C represents the kinetic resistance to equilibrium during the process of separation. The kinetic resistance is the time lag involved in moving from the mobile phase to the stationary phase and back again. The higher the flow rate of the mobile phase, the more a molecule on the packing material inclines to lag behind molecules in the mobile phase. Thus, this term is inversely proportional to linear velocity. Consequently, it is likely to enhance the throughput, and without affecting the chromatographic performance, the separation can be speeded up. The emergence of UPLC has necessitated the improvement of existing instrumentation facility for LC, which takes the benefit of the separation performance (by decreasing dead volumes) and consistent pressures (about 500 to 1000 bars, compared with 170 to 350 bars in HPLC). Efficiency is proportionate to the length of the column and inversely proportional to the radius of the particles [9]. Consequently, the column length can be reduced by the similar factor as the particle radius without affecting the resolution. The use of UPLC has helped in the detection of drug metabolites and enhancement of the quality of separation spectra [10, 11].

## CHEMISTRY OF SMALL SIZE PARTICLES [12 - 14]

The chemistry of the particles used in this course of the method contributes the increased efficiency and potential to work at amplified linear velocity, thereby, providing both the speed and the resolution. Efficiency is one of the important separation parameters which plays a significant role in UPLC since it depends on the same selectivity and retentivity as HPLC. This may be understood with the help of the following basic resolution ( $R_s$ ) equation (ii):

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

Where  $\alpha$  represents the selectivity factor, N is efficiency and K denotes proportionality constant. According to this, resolution increases with increase in efficiency. Since efficiency (N) is inversely proportional to particle size (dp) equation (iii):

$$N \propto \frac{1}{dp} \quad \text{(iii)}$$

As the size of the particle decreases by a factor of three, there is three times increase in the efficiency and in

the resolution, there is an increase of the square root of three (nine times). So, there is an increase in efficiency with consequent increase in resolution and sensitivity as the particle size decreases. Similarly, efficiency (N) is directly proportional to the length of the column (L). So, the equation (iii) may be written as equation (iv) as follows:

$$N \propto \frac{L}{dp} \quad \text{(iv)}$$

Therefore, the length of the column (L) may be reduced by the same ratio as the size of the particle without losing the resolution.

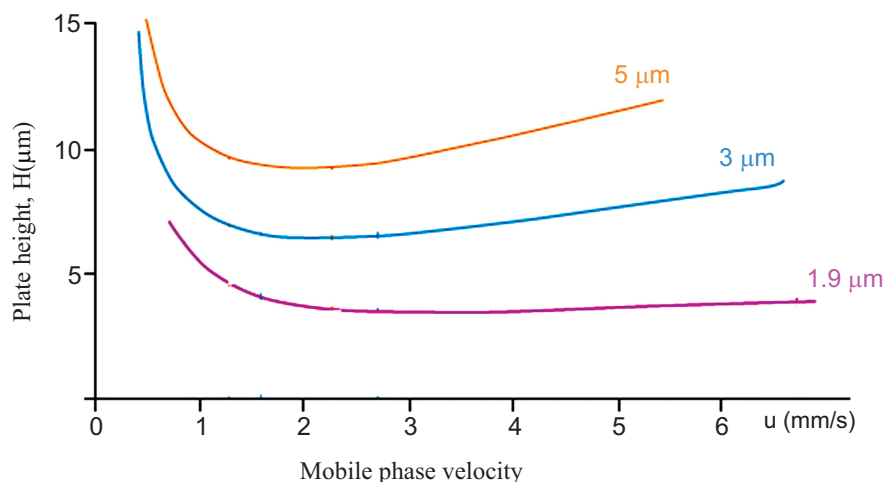
Efficiency also has indirect relationship with the peak width (w) according to the equation (v):

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

This means that narrower the peaks are, easier is their separation from each other. Peak height (H) also has opposite relationship with peak width (w) according to equation (vi):

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

Based on the above facts, in UPLC, by reducing the particle size ( $1/3^{\text{rd}}$ ), the column length is reduced ( $1/3^{\text{rd}}$ ), the flow rate is increased (3 times) and the separation is done faster ( $1/9^{\text{th}}$  time) with maintaining the resolution [12]. For this reason, short columns packed with small size particles (about  $2 \mu\text{m}$ ) are used with these systems, to quicken the separation with higher efficiency, while maintaining a tolerable loss of load. The effect of particle size on HETP and linear velocity has been illustrated in Fig. (1). by using van Deemter plot. Fig. (1) shows that the particles with a smaller diameter are contributing less to band broadening compared to larger particles and are less affected by higher column flow rate [15, 16].



**Fig. (1).** Van Deemter Plot illustrating the effect of particle size (in  $\mu\text{m}$ ) on plate height (H). Smaller particle size provides higher overall peak efficiencies and a much wide range of flow rates.

#### ADVANTAGES OF UPLC [17 - 19]

It is more selective and sensitive with high resolution performance and faster resolving power. It also reduces process cycle time and assures end-product quality with reduced cost of operation and decreased run time. It increases sensitivity and provides quick analysis through the use of a novel column material of very small particle size. It decreases the consumption of solvent and increases sample throughput and also provides real-time analysis in step with manufacturing processes.

## DISADVANTAGES [20 - 22]

A major disadvantage of UPLC is the higher back pressures compared to conventional HPLC which decreases the life of the columns. Increasing the column temperature reduces the back pressure problem in UPLC. Moreover, the particles of less than 2  $\mu\text{m}$  are mostly non-regenerable and, therefore, have a narrow use.

## INSTRUMENTATION

The instrumentation of UPLC includes- sample injection, UPLC columns and detectors.

### Sample Injection

The use of the injector is to add precisely measured, a small volume of solution containing the sample in the mobile phase. The injection must be done reproducibly and accurately. Conventional injection valves may be manual or programmed and to guard the column from extreme pressure instabilities, the injection process must be comparatively pulse-free. To reduce the potential band spreading, the swept volume of the device is desired to be minimal. A quick injection cycle time is required to fully avail the speed afforded by UPLC. To increase the sensitivity, low volume injections with minimal carryover are required [23]. The volume of the sample in UPLC is usually 2-5  $\mu\text{L}$ . Nowadays, direct injection approaches are utilized for the biological samples [24 - 26].

### UPLC Column

Columns used for UPLC have been developed and manufactured by the following different companies:

1. Waters: Acquity UPLC columns and Vanguard Pre-columns have been produced [27].
2. Agilent technology provides highest performing columns that provide fast and reproducible results. These include Poroshell 120 columns, ZORBAX Rapid Resolution High definition columns, ZORBAX Eclipse plus columns and ZORBAX Rapid Reduction High Throughput columns [28].
3. Altech Associate [29].
4. Phenomenex provides Kinetex<sup>®</sup> Coreshell HPLC/UHPLC columns of high efficiency and performance [30].

Different types of columns being used in UPLC are packed with particles which are produced through different technologies [27]. These are as follows:

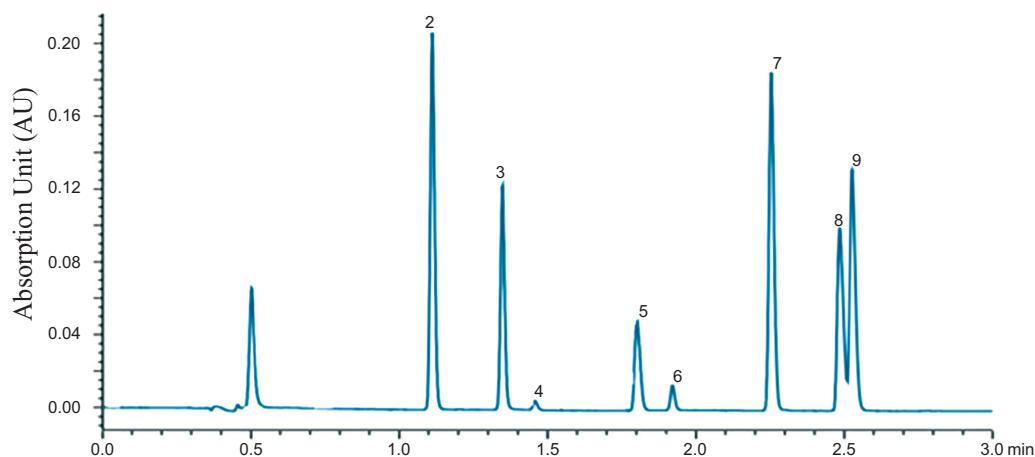
1. Charged Surface Hybrid [CSH] particle technology,
2. Ethylene Bridged Hybrid [BEH] particle technology,
3. High Strength Silica [HSS] particle technology and
4. Peptide Separation Technology (PST).

### CSH Particle Technology [27]

CSH Technology is the newest methodology in the development of hybrid materials which utilizes low-level surface charged particles for the enhancement of the selectivity and sharpness of the peaks. Hybrid based packing material approach provides sharp peaks specially for basic compounds under low pH with higher efficiency and chemical stability. CSH C<sub>18</sub>, CSH Phenyl hexyl, and CSH Fluoro phenyl are the different types of CSH particles being widely used.

These columns have the advantage of exceptional peak shape, increased loading capacity (CSH C<sub>18</sub>); complementary selectivity to straight chain alkyl phases (CSH-phenyl-hexyl); selectivity for positional isomers, halogenated and polar compounds (CSH-fluoro phenyl). The other advantages include- higher stability at a wide range of pH, improved batch to batch reproducibility and fast column equilibration after any change in the pH of the mobile phase.

Applications of CSH technology based columns include the analysis of basic compounds even in their ionized form. While analyzing the basic compounds under low pH and reversed phase conditions, poor peak shape and retention often result. Whereas, CSH Phenyl hexyl columns provide exceptional peak shape for basic drugs under acidic mobile phase conditions. Fig. (2) demonstrates the analysis of basic drugs using CSH technology based columns.

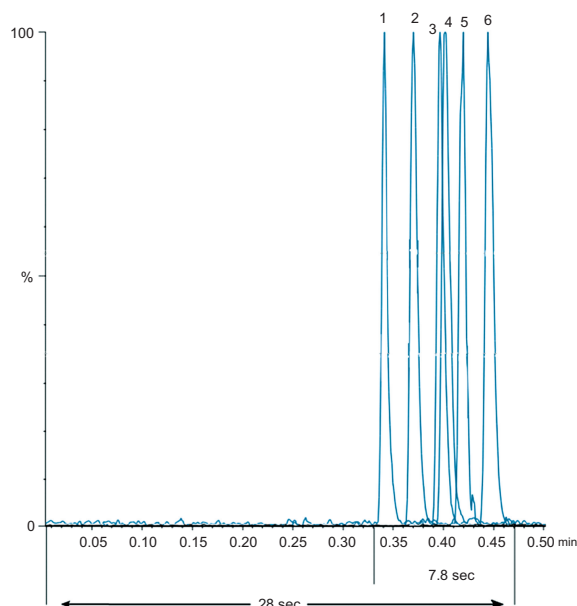


**Fig. (2).** Chromatogram of the analysis of basic drugs using the Acquity CSH-phenyl hexyl particles. Peaks obtained: 2-aminopyrazine (1), Pindolol (2), Quinine (3), Quinine impurity (4), Labetolol (5), Diltiazem impurity (6), Diltiazem (7), Verapamil (8), Amitriptyline (9)

### BEH Particle Technology [27]

For more than a decade, hybrid particle technology [HPT] has delivered incomparable versatility and performance, enabling chromatographers to push the limits of LC separations. The XTerra particle was the first commercially available option to improve the issues (poor peak shape for basic compounds and column longevity due to chemical instability) without the drawbacks of unpredictable selectivity produced by alternative materials such as zirconia, organic polymers, and graphitic carbon. With the commercialization of 2.5  $\mu\text{m}$  XTerra particles, the concept of fast HPLC with small particles was born, improving the productivity of chromatographic laboratories globally.

Straight chain alkyl columns (BEH  $\text{C}_{18}$  and  $\text{C}_8$ ), Embedded polar group column (BEH Shield RP18) and UPLC BEH Phenyl (phenyl group tethered to the silyl functionality with a  $\text{C}_6$  alkyl) are the different types of BEH particle technology based columns which are being widely used.



**Fig. (3).** Chromatogram of the rapid assay of cytochrome p450 isoenzymes in BEH C18. Peaks of the major metabolites include 4-acetaminophenol (1), 1-hydroxybufuralol (2), 4-hydroxymephenytoin (3), 7-hydroxycoumarin (4), 4-hydroxytolbutamide (5), 1-hydroxyimidazolam (6)

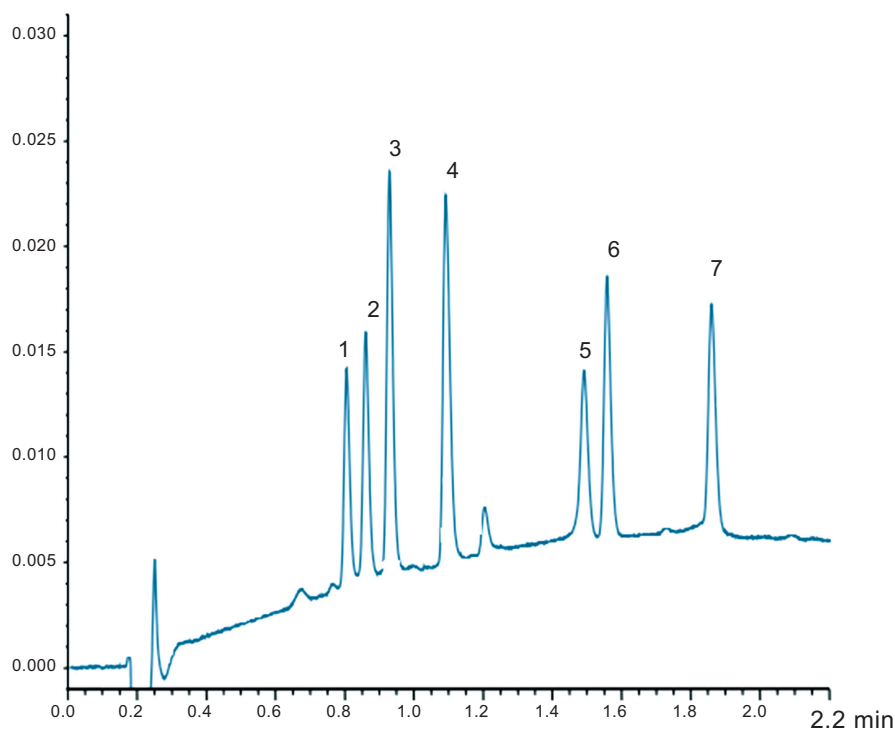
These columns provide higher retention, selectivity ( $C_{18}$ ); exceptional efficiency, peak symmetry, chemical stability ( $C_8$ ); enhanced peak shape and higher compatibility with 100% aqueous mobile phase (RP18); chemical stability, reproducibility and peak shape (phenyl). Applications include the rapid assay of cytochrome p450 isoenzymes which are responsible for more than 90% of drug metabolism on the market today. Cytochrome p450 isoenzymes are used to determine the level of drug inhibition, induction or drug-drug interaction that takes place. The ultra-low dispersion and system dwell volume of UPLC technology enables the rapid analysis of these enzymes in less than 30 seconds. (Fig. 3). shows the chromatogram of an assay of cytochrome P450.

### HSS Particle Technology [27]

HSS particle technology is the advanced technology, born from an innovative synthetic process in which the mechanical stability is improved while the pore volumes remain similar to that of HPLC silica-based materials. This results in an advanced technique which provides higher retention in comparison to the hybrid particles. HSS T3, HSS  $C_{18}$ , HSS  $C_{18}$  SB, HSS PFP and HSS CN are the different types of HSS particles being widely used.

These columns provide balanced retention of polar and hydrophobic molecule (T3); exceptional peak shapes, increased retention ( $C_{18}$ ); greater retention of basic compounds ( $C_{18}$  SB); ideally suited for planar aromatic, positional, halogenated compounds (PFP); ultra stable retention and compatible with both reversed-phase and normal-phase techniques (CN).

Applications of this technology include the analysis of tetracycline antibiotics which are commonly prescribed for the treatment of bacterial infections. The ACQUITY UPLC HSS C8 column enables a single UV-based method for the simultaneous separation of oxytetracycline including its degraded and related products (which are produced due to drug fermentation) as well as additional veterinary antibiotics (Fig. 4).



**Fig. (4).** Chromatogram of the separation of tetracycline and related antibiotics on HSS  $C_{18}$ . Peaks obtained: 4-epioxytetracycline (1), oxytetracycline (2), tetracycline (3), alpha-apo-oxytetracycline (4), chlortetracycline (5), beta-apo-oxytetracycline (6), doxycycline hydrochloride (7)

### PST Columns [27]

PST utilizes the  $C_{18}$  BEH Technology<sup>TM</sup> particles whose particles sizes range from 1.7  $\mu\text{m}$  to 10  $\mu\text{m}$  and the column dimension ranges from 75  $\mu\text{m}$  to 30 mm internal diameter (i.d) and column length from 50 mm to 250 mm. They are

used in all kind of research and development that involves analysis and isolation of peptides. The PST columns provide sharp symmetrical peaks.

### Solvent Delivery System

The solvent delivery system must perform reproducible high pressure pumping with a smooth and constant flow of solvents. UPLC systems routinely operate at 8000-15000 psi. The delivery system must also remunerate for a variety of solvents used in isocratic, linear & nonlinear gradient elution and solvent compressibility for a wide range of pressures. The Acquity UPLC binary solvent manager has two solvent delivery modules operating in parallel for high pressure merging of two solvents in <140  $\mu\text{L}$  internal system volume. The dissolved gases are removed by vacuum up to four eluents plus two wash solvents [24, 26].

### The Detector

The detector employed for the UPLC should be able to give a high sampling rate with narrow obtainable peaks (<1 s half-height peak width) and the dispersion of the peaks should be minimum so that the wastage of the separated solute is less on the column. The UPLC technique provides the sensitivity of separation two to three times more than the previous analytical method HPLC, which is also due to the method employed for the detection. The detectors employed in the UPLC are Acquity photodiode array (PDA) and Tunable Vis-UV (TUV) in which Teflon AF is used which provides an internally reflective surface and enhances the light transmission efficiency by eliminating the internal absorptions. These have path lengths 10 nun, acquisition rates 20 (PDA) and 40 (TUV) points, and total internal volume 500 nL. Mass spectrometric detection has also been used with UPLC [24, 26].

### Applications of UPLC

#### *Determination of Pesticides in Groundwater [31]*

UPLC coupled with triple quadrupole tandem mass spectrometry (UPLC<sup>TM</sup>-MS/MS) can be utilized to determine the trace level pesticides in groundwater in less time and speedy manner. The technique has enhanced the analysis speed, sensitivity, and resolution.

#### *Improved Resolving Power in Peptide Maps [27, 32]*

Peptide mapping is an essential technique for the characterization of proteins. Due to exceptionally reduced instrument and column dispersion, the analyzes of tryptic digest of phosphorylase by UPLC technology provides significantly improved resolution, peak capacity, and sensitivity compared to HPLC, allowing the detailed characterization of the protein (Fig. 5).

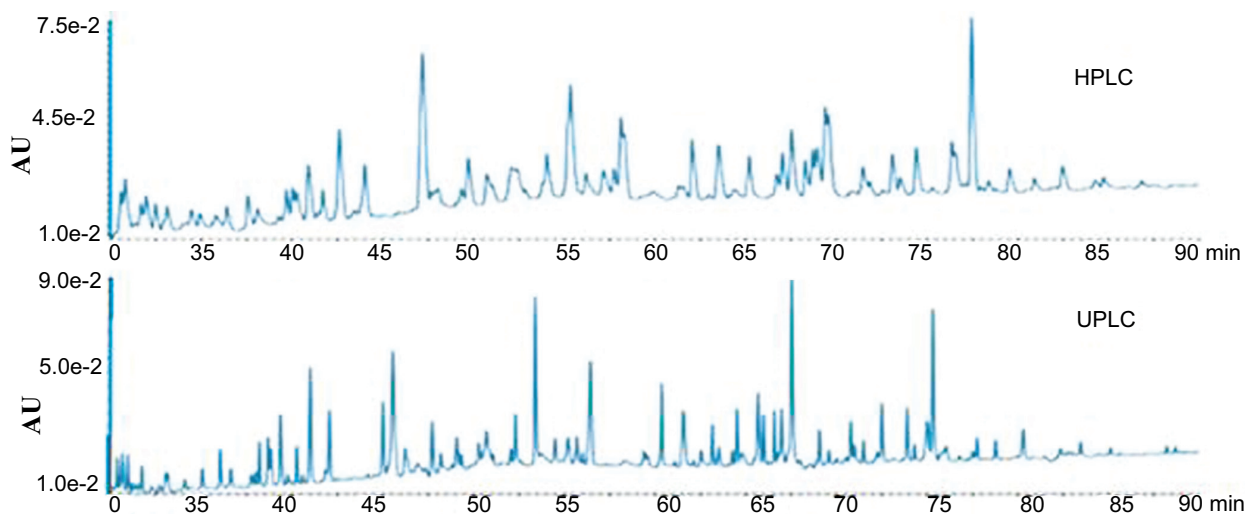


Fig. (5). Improved resolving power in peptide maps by using UPLC.

#### *Rapid Dose Formulation Analysis [33, 34]*

Nowadays, the use of UPLC together with UV and MS detection has been widely utilized in pharmaceutical

applications. Several commercial drug formulations were used as models to study the efficiency of separations with the change of flow rate. The efficiency was judged on the parameters of resolution, theoretical plates, column ruggedness, retention time, and peak area. For example, mefenamic acid and chloramphenicol separation was studied in dimethylacetamide/ polyethylene glycol-200 vehicle.

#### ***Analysis of Traditional Chinese Medicines (TCM) [35]***

The identification and quantification of components of TCM by chromatographic analysis is one of the major challenges. TCM is a complex matrix in which all the constituents play a specific role for the overall efficacy. Therefore, the analysis of all the constituents is synchronously necessary for the quality control. The new technique UPLC is used for the quality control of the TCM.

#### ***Multi-Residue Analysis of Pharmaceuticals in Waste Water [36]***

The water used in the pharmaceutical companies is found to have the traces of various cholesterol-lowering statin agents, anti-ulcer agents, antibiotics, beta-blockers, analgesics, anti-inflammatory agents, lipid regulating agents, psychiatric drugs, and histamine H<sub>2</sub> receptor antagonists. UPLC coupled with Q-TOF-MS is used to confirm and screen these drugs in the samples of waste water treatment plant.

#### ***Identification of Metabolites [37 - 39]***

The identification and detection of all the possible metabolites of the candidate drugs for the discovery of new chemical entities is a very important step. For the identification of the metabolites, a high sample throughput is required to be maintained by the analysts to provide quick results to the medicinal chemists. UPLC-MS/MS is helpful in biomarker discovery as it meets tough analytical requirements and provides sensitivity, mass accuracy, dynamic range, and resolution.

#### ***In Manufacturing / Quality Assurance (QA) / Quality Control (QC) [40]***

Identification, quantification, purification, efficacy and safety are key parameters to be evaluated during manufacturing of a drug product and pharmaceutical dosage form. Material stability is also observed as a component of QA and QC. UPLC is used as an important tool in QA/QC laboratories for the quantitative and extremely regulated analysis.

#### ***Impurity Profiling [41 - 43]***

Impurity profiling should be efficient for consistent detection and separation of all the impurities present in the active compound. The drug development and formulation process demand accurate measurement/testing of low-level impurities present with the active pharmaceutical ingredients or the excipients or the raw materials used in the preparation of the final product. Thus, the presence of excipients in the sample makes the profiling difficult and with HPLC method, it takes longer time for analysis to achieve sufficient resolution. Thus, the combination of UPLC with mass spectrometry has been useful for the documentation of drug and endogenous metabolites in the final product.

#### ***Method Development / Validation [41, 44, 45]***

Method development and validation is a complex process and consumes a lot of time. For the development of a robust and reliable method, the labs are required to study many combinations of different parameters *e.g.* mobile phase, temperature, pH, column and gradient chemistry *etc.* UPLC is an important method used in the laboratory which reduces the cost and increases the efficiency of analysis required for developing and validating the method. With UPLC, the speed of the separation increases and efficiency improves, which results in the fast development of methodologies. High stability of the UPLC columns provides the possibility of selection of column temperature and pH from a wide range.

#### ***Forced Degradation Studies (FDS) [27, 46]***

This study is done to access the chemical stability of the candidate compound in the pharmaceuticals. Usually, it is performed at the preliminary stage in the process of drug development. Forced degradation/ stress testing is performed under accelerated environment. The experimental conditions cause the candidate compound to degrade under extreme conditions like acid and base hydrolysis, peroxide oxidation, photo-oxidation and thermal stability to identify the resultant degradation products. This helps to establish degradation pathways and thus intrinsic stability of a drug substance. The stability of product describes shelf life and storage conditions and helps in the selection of appropriate formulations and their suitable packaging. This is compulsory for regulatory documentation. The commonly used



analytical approach for FDS is HPLC with UV and/ or MS but these techniques consume a lot of time and not provide high resolution to confirm the precise detection of degradation products. Use of UPLC with photodiode array and MS analysis supports the identification of degradation products and also reduces the time needed to evolve stability indicating methods. Fig. (6) is a chromatogram of FDS of glimepiride done on UPLC BEH C<sub>18</sub> column. It shows the sharp peaks of different degradation products along with glimepiride.

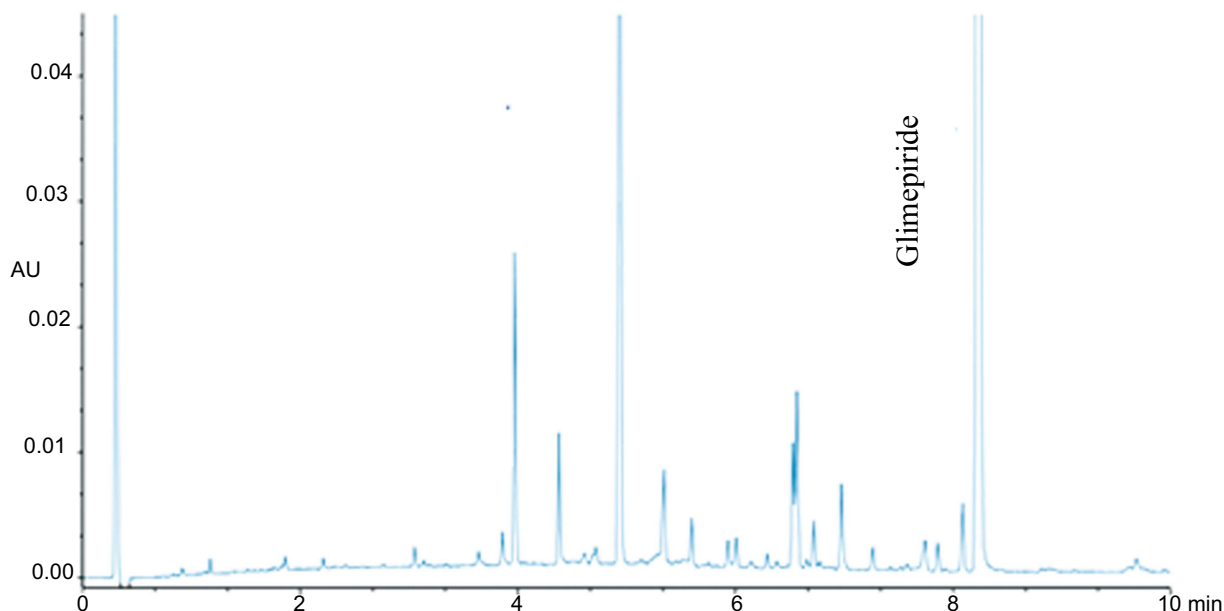


Fig. (6). Chromatogram of high resolution analysis of Glimepiride forced degradation on BEH C<sub>18</sub>.

#### **Dissolution Testing [47]**

Dissolution testing is one of the most important step carried out during formulation and manufacturing process to test the drug release. The dissolution data provides understanding to validate consistency and uniformity of the active ingredient in every batch. Testing of potent drugs in sustained release dosage form is very important as their dissolution studies data can affect the delivery of the medicine. Moreover, new and potent formulations require higher separation sensitivity. UPLC method provides accurate and consistent automated online sample acquirement.

#### **Bioequivalence / Bioanalysis Studies [48, 49]**

Bioequivalence studies are pharmacokinetic studies needed for the quantitation of drugs in biological samples. This is an important step to compare the rate and exposure level of newly developed formulations of prevailing drugs with that of the original formulation. The selectivity and sensitivity of UPLC-MS/MS produce reliable and precise data. UPLC- MS/MS solutions have increased efficacy, output, and profitability for the bioequivalence laboratories. UPLC sample manager enhances the effectiveness by considering a huge number of samples in a temperature controlled atmosphere, confirming maximum throughput which increases the sensitivity and quality of data acquisition rates of tandem quadrupole MS systems.

#### **Toxicity Studies [50]**

During the drug development process, toxicity issue causes a fall out of drug candidates and this causes monetary loss to the organization. It is a complicated task to estimate candidate drugs for possible inhibition or initiation of metabolizing enzymes, toxicity or drug-drug interactions in the body. UPLC allows precise detection due to its high resolution. Further, its sensitivity also allows the detection of the peaks at low concentrations. These factors lessen the time for analysis and decrease failure of sample analysis.

#### **Iodinated Disinfection Byproducts (IDBPs) [51]**

Till date, a few numbers of IDBPs have been characterized in drinking water by using GC/MS. But with the help of coupling UPLC to the electrospray ionization-triple quadrupole mass spectrometer (ESI-tqMS), pictures of IDBPs in samples of water, treated with chlorine and chlorine- ammonia have been collected and 17 IDBPs structures were provisionally projected.

### ***Therapeutic Drug Monitoring [52, 53]***

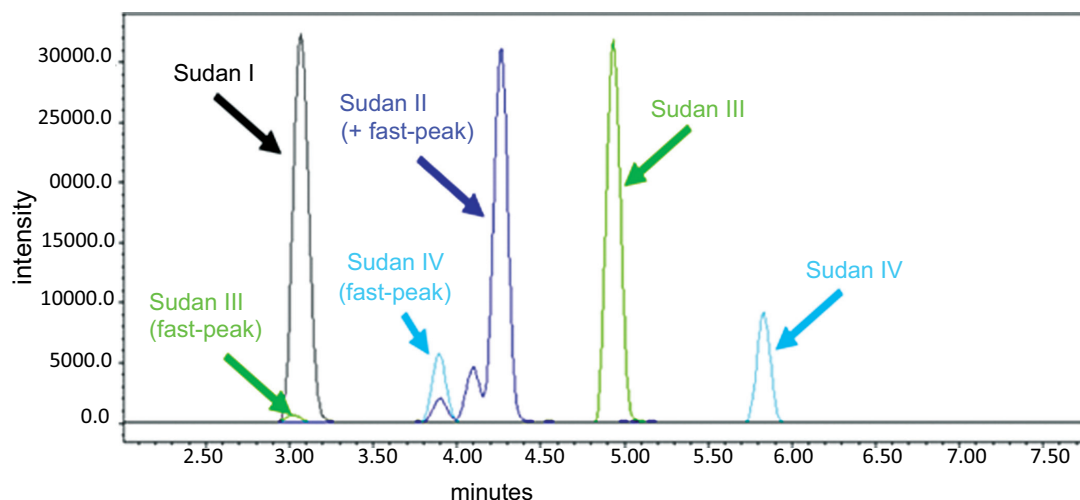
Carlier *et al* in 2012 reported the monitoring of  $\beta$ -lactam antibiotic concentration in plasma of patients with different pharmacokinetics. In their studies, they tried to validate a UPLC-MS/MS method for the simultaneous estimation of two  $\beta$ -lactamase inhibitors and seven  $\beta$ -lactam antibiotics in human plasma. The main benefit of the technique is the faster speed of analysis (5.5 min /sample) compared to other approaches used for this type of multiple analytes.

### ***Analysis of Explosives [54]***

UPLC proved to be an enhanced procedure to analyze various explosives. In addition, analysis of explosive remains from hand swipes can also be detected by UPLC with minimal sample preparation requirement (less than 9 min), thus enhancing the lab output as well as freeing up valuable MS time for further analysis

### ***Analysis of Contaminants in Foodstuffs [55]***

Sudan I-IV is a red colored dye which is commonly used in petrochemical industries. The intensive color of Sudan dyes lured frauds for improving the color of several spices and food stuffs which can form DNA adducts causing mutations. UPLC coupled to tandem mass spectrometry allows the identification of Sudan at low ppb levels in spices and chilli containing food stuffs. Fig. (7) shows the chromatogram of Sudan I-IV and it also contains the fast peaks of sudan II, III and IV. These fast peaks are additional peaks as these eluted a few minutes before the main peak of the compounds.



**Fig. (7).** Chromatogram of Sudan I – IV at 50  $\mu\text{g/L}$ .

### ***Dendrimers Characterization [56, 57]***

Dendrimers are highly branched symmetric polymers having a compact round structure (diameter 1.1nm to 9 nm) and unique behavior. They are normally synthesized from a central polyfunctional core by repetitive addition of polymers. Dendrimers surfaces provide a brilliant stage for the attachment and appearance of cell specific targeting groups, solubility modernizers and stalth moieties that decrease immunological interactions. Polyamidoamine (PAMAM) dendrimers are one of the widely used dendrimers. HPLC has been utilized to isolate and to check the purity of many PAMAM dendrimer generation or conjugates. This technique also helps to study the solubility of multi functionalized dendrimers and the interactions between them and biomolecules. UPLC reduces the retention time of analytes with an improvement of the resolution proficiency during dendrimers studies

### ***Determination of Phytoconstituents [58 - 62]***

UPLC can be used to identify and quantify procyanadines, phenolic compounds, monomers, oligomers, isoflavones, flavonoids, coumarins and alkaloids such as caffeine and theobromine. Fig. (8) shows the comparison of chromatograms for analysis of coumarins using HPLC and UPLC methods. It is clear from this figure that UPLC method completes the process in less time and sharp peaks are obtained.

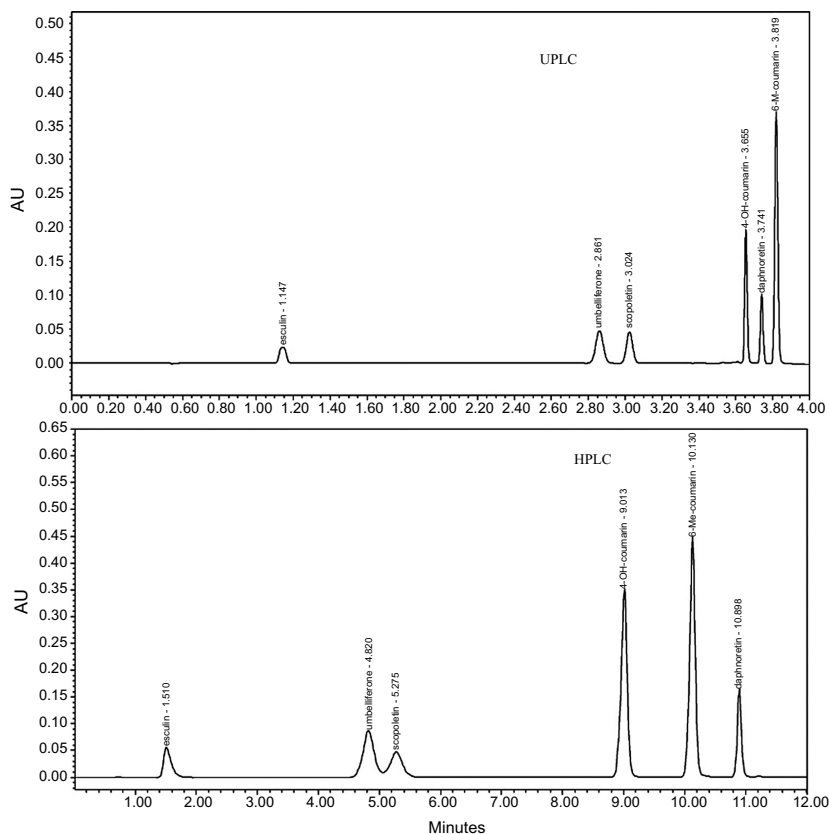


Fig. (8). Chromatogram of the Analysis of Coumarins by HPLC and UPLC.

#### Identifying Static and Kinetic Lipid Phenotype [63]

High resolution UPLC-MS is employed to study the concentration of lipids and their endogenous production. Therefore, this technique was found to be useful in determining the contribution of different pathways and synthesis that could affect lipid biology.

#### Analysis of Free Amino Acids (FAA) in Wines [64]

The production of FAA in the less aged white wines can be determined by the UPLC. The UPLC<sup>TM</sup> method is an established method for the analysis of amino acids using 6-aminoquinolyl. This new UPLC<sup>TM</sup> method has made the separations quick and reliable for 24 amino acids within 23 minutes. This method proved to be superior compared to original HPLC method due to much improvement in resolution with reduced run time. Fig. (9) shows the contribution of UPLC in monitoring the complex autolysis processes during the on-lees aging of wines.

#### Identification of Metabolic Biomarkers to Diagnose Epithelial Ovarian Cancer (EOC) [65]

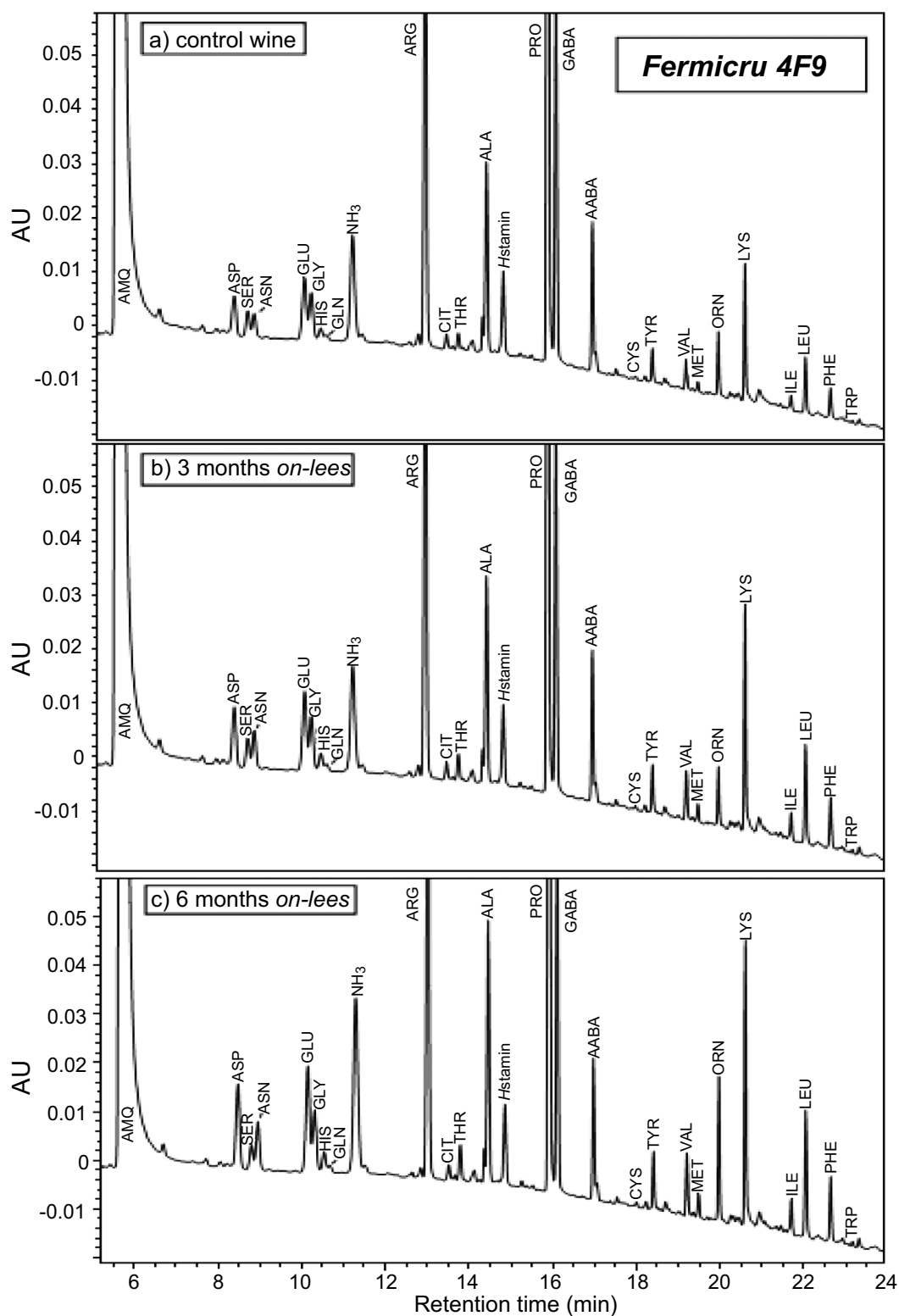
Currently available tests are insufficient to distinguish patients with EOC from normal individuals. Plasma specimens of EOC patients and normal individuals were analyzed using UPLC/QTOF/MS. Eight biomarkers were identified which may serve as novel biomarkers for diagnosis. Fig. (10) shows the application of UPLC in the diagnosis of EOC.

#### ADME [66, 67]

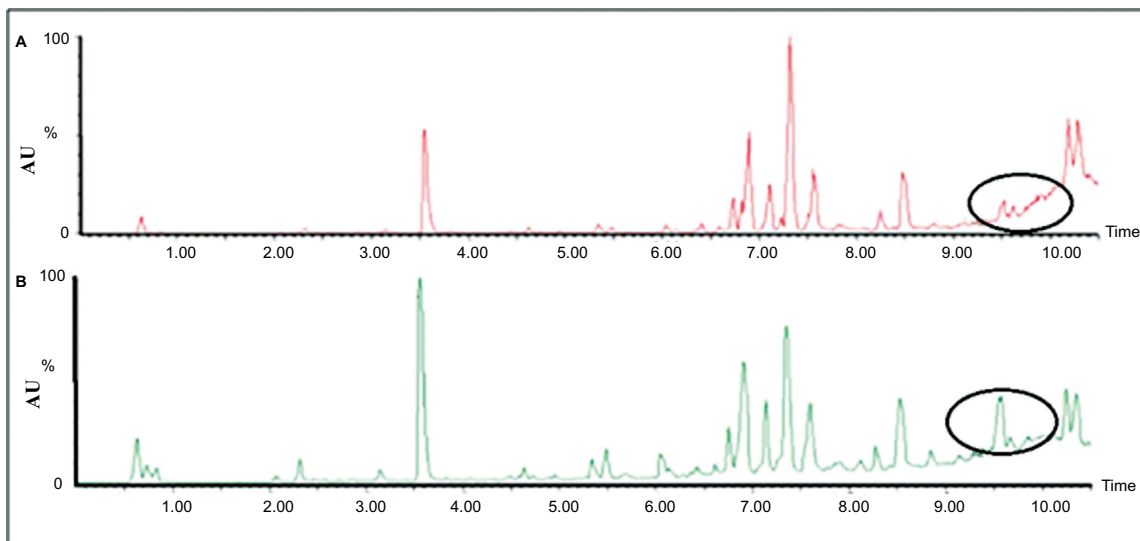
ADME studies include absorption, digestion, metabolism, and elimination. Yan developed and validated the sensitive UPLC-MS/MS method for the pharmacokinetic studies of HZ08 liposome injection in plasma and tissues of rats to study plasma kinetic and tissue distribution respectively.

#### Drug Abuse [68]

UPLC-MS/MS method can be used to develop and evaluate a fast, robust and specific screening platform for the determination and quantification of a variety of commonly used drugs of abuse (opioids, benzodiazepines *etc*) in urine.



**Fig. (9).** UPLC<sup>TM</sup> chromatograms of FAA profiles for the wine set fermented with the yeast *Fermicru 4F9*, (a) control wine, (b) 3 months, and (c) 6 months on-lees maturation.



**Fig. (10).** Plots from UPLC/QTOF/MS. A and B shows the typical total ion current chromatograms of plasma obtained from an EOC patient and a normal control, respectively.

## CONCLUSION

UPLC is one of the most important tools in analytical chemistry which increases the speed, resolution, and sensitivity of the chromatographic analysis and decreases the time, solvent consumption and cost involved. The peaks obtained through UPLC have decreased noise and better signal to noise ratio. It gives sharp and narrow peaks of more or less all categories of pharmaceutical drugs. It also facilitates the analysis of complex mixtures in less time and the peaks obtained through this method depicts more information which is more clearer in comparison to the peak obtained through HPLC. This method is widely used for the analysis of different pharmaceuticals such as amino acids, peptide mapping, glycans analysis, phenotyping, drug discovery, metabolomics *etc.* This technology thus creates a new opportunity for business profitability in highly efficient manner and allows the product to be introduced to the market in less time.

## CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

## ACKNOWLEDGEMENTS

Declared None.

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