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REVIEW ON THE ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY A NEW CHROMATOGRAPHIC METHOD FOR ANALYSIS OF PHARMACEUTICALS AND DRUG MOLECULES

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ABSTRACT

Ultra Performance Liquid Chromatography (UPLC) methods, a systematic approach was taken in order to optimize the chromatographic conditions, including the selection of the UPLC column and the choice of mobile phase. Ammonium acetate buffer was chosen for its organic nature and wide pH range compatibility, with its pH being adjusted using formic acid to enhance resolution and make the mobile phase compatible with LC-MS. The flow rate, injection volume, and column oven temperature were optimized to achieve the required resolution and peak shape, ensuring effective separation and detection of impurities. Stress studies showed the stability-indicating characteristic of the developed method, specifically for the determination of gliclazide and its potential impurities in pharmaceutical dosage forms. The method was validated for system suitability, linearity, precision, specificity, accuracy, solution stability, filter interference, and robustness, meeting the criteria specified by the International Conference on Harmonization (ICH) guidelines. The developed UPLC method was demonstrated to be sensitive, precise, accurate, and stability-indicating, providing a comprehensive and specific approach for the analysis of pharmaceutical drugs and their impurities.

KEYWORDS: Analysis of Pharmaceuticals, System suitability Method development, Accuracy and Precision, ICH Gridlines, RP-UPLC.

INTRODUCTION

Most medications in multicomponent dosage forms may be analysed by the UPLC system thanks to its many benefits, including speed, specificity, consistency, accuracy, precision, and simplicity of automation. The UPLC method saves repeating extraction and isolation procedures. There are several modes of differentiation in UPLC. These are Size Exclusion Chromatography, Chromatography of Reversed Phase Ion Phase, Chromatography of Affinity, Normal Phase Mode, and Inverted Phase Mode.

The effectiveness and safety of a medicine are significantly influenced by the quality of the drug. For customers to have access to safe and effective medicinal formulations, quality assurance and control of pharmaceutical and chemical formulations are crucial. Hence When determining whether a chemical is suitable for use in patients, analysis of both the pure drug material and its pharmaceutical dose forms is crucial. The calibre of the processes used to generate the data are what determines the calibre of the analytical data. To ensure that pharmaceuticals and their formulations are legally certified by regulatory bodies, it is crucial to establish tough and reliable analytical procedures.

Depending on the type and characteristics of the substance, a broad range of obstacles are encountered when creating the procedures. This, along with the significance of achieving selectivity, speed, cost, simplicity, sensitivity, reproducibility, and accuracy of results, provides researchers with an opportunity to find solutions to the problems associated with getting new analytical techniques adopted by the chemical and pharmaceutical industries. Different physico-chemical methods (1) are used to study the physical phenomenon that occurs as a result of chemical reactions. Among the physico-chemical methods, the most important are optical (refractometry, polarimetry, emission and fluorescence methods of analysis), photometry (photocolorimetry and spectrophotometry covering UV-Visible, IR Spectroscopy and nepheloturbidimetry) and chromatographic (column, paper, thin layer, gas liquid and high-performance liquid chromatography) methods. Methods such as nuclear magnetic resonance (NMR) and para magnetic resonance (PMR) are becoming more and

more popular. The combination of mass spectroscopy (MS) with gas chromatography is one of the most powerful tools available. The chemical methods include the gravimetric and volumetric procedures which are based on complex formation; acid-base, precipitation and redox reactions. Titrations in non-aqueous media and complexometry have also been used in pharmaceutical analysis. The number of new drugs is constantly growing. This requires new methods for controlling their quality. Modern pharmaceutical analysis must need the following requirements.

1. The analysis should take a minimal time.
2. The accuracy of the analysis should meet the demands of Pharmacopoeia.
3. The analysis should be economical.
4. The selected method should be precise and selective.

CHROMATOGRAPHY

Chromatography (Chroma means 'color' and graphein means to 'write') is the collective term for a set of laboratory techniques for the separation of mixtures. It involves passing a mixture dissolved in a "mobile phase" through a stationary phase,(2-4) which separates the analyte to be measured from other molecules in the mixture based on differential partitioning between the mobile and stationary phases. Differences in compounds partition coefficient results in differential retention on the stationary phase and thus changing the separation.

Different types of chromatographic techniques were summarized in

Table No. 1: Different types of chromatographic techniques.

Sl. No	Basic principle involved	Type of Chromatography
1.	Techniques by chromatographic bed shape	Column chromatography
		Paper chromatography
		Thin layer chromatography
2	Techniques by physical state of mobile phase	Gas chromatography
		Liquid chromatography
3	Affinity chromatography	Supercritical fluid chromatography
4	Techniques by separation mechanism	Ion exchange chromatography
		Size exclusion chromatography
5	Special techniques	Reversed phase chromatography
		Simulated moving -bed chromatography
		Pyrolysis gas chromatography
		Fast protein liquid chromatography
		Counter current chromatography
		Chiral chromatography

Chromatography may be preparative or analytical. The purpose of preparative chromatography is to separate the components of a mixture for further use (and is thus a form of purification). Analytical chromatography is done normally with smaller amounts of material and is for measuring the relative proportion of analytes in a mixture**.**

INTRODUCTION TO UPLC

UPLC

UPLC is an emerging area of analytical separation science which retains the practicality and principles of UPLC while increasing the overall interlaced attributes of speed, sensitivity and resolution. Speed and peak capacity can be extended to new limits, termed Ultra Performance Liquid Chromatography, or UPLC by using fine particles. UPLC takes full advantage of chromatographic principles to run separations using columns packed with smaller particles and/or higher flow rates for increased speed, sensitivity and superior resolution.

In this article we explored the potential of UPLC to improve the analysis of the samples that are encountered during pharmaceutical development and manufacturing. Particular emphasis has been placed on determining whether UPLC can reduce analysis times without compromising the quantity and quality of the analytical data generated compared to UPLC.

Here particular emphasis is given on principle involved, instrumentation encountering different UPLC columns, different particle chemistries, detectors and various applications. UPLC generated higher separating efficiencies through the use of a smaller diameter particle packing and higher operating pressures. A commercial system capable of generating much higher pressures (1000 bar) than used in standard UPLC has been evaluated to determine its potential in routine analysis. UPLC has been shown to generate high peak capacities in short times and this is found to be quite beneficial in analyzing the complex mixtures that constitute metabolism samples. The application of UPLC resulted in the detection of additional drug metabolites, improved the spectrum quality and separation efficiency.[1,2]

PRINCIPLE

The UPLC is based on the principal of use of stationary phase consisting of particles less than 2µm, while UPLC columns are typically filled with particles of 3 to 5µm.

The underlying principles of this evolution are governed by the van Deemter equation, which is an empirical formula that describes the relationship between linear velocity (flow rate) and plate height (HETP or column efficiency).[3] The Van Deemter curve, governed by an equation with three components shows that the usable flow range for a good efficiency with a small diameter particles is much greater than for larger diameters.[4,5]

$$H = A + \frac{B}{v} + Cv$$

Where A, B and C are constants and v is the linear velocity, the carrier gas flow rate. The A term is independent of velocity and represents "eddy" mixing. It is smallest when the packed column particles are small and uniform. The B term represents axial diffusion or the natural diffusion tendency of molecules. This effect is diminished at high flow rates and so this term is divided by v. The C term is due to kinetic resistance to equilibrium in the separation process. The kinetic resistance is the time lag involved in moving from the gas phase to the packing stationary phase and back again. The greater the flow of gas, the more a molecule on the packing tends to lag behind molecules in the mobile phase. Thus, this term is proportional to v.

Chemistry of Small Particles

Smaller particles provide not only increased efficiency, but also the ability to work at increased linear velocity without loss of efficiency, providing both resolution and speed. Efficiency is the primary separation parameter behind UPLC since it relies on the same selectivity and retentivity as UPLC. In the fundamental resolution (Rs) equation:

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

Resolution is proportional to the square root of N. But N is inversely proportional to particle size (dp):

$$N \propto \frac{1}{dp}$$

N is also inversely proportional to the square of the peak width:

$$N \propto \frac{1}{w^2}$$

This illustrates that the narrower the peaks are, the easier they are to separate from each other. Also, peak height is inversely proportional to the peak width:

$$H \propto \frac{1}{w}$$

So as the particle size decreases to increase N and subsequently Rs, an increase in sensitivity is obtained, since narrower peaks are taller peaks. Narrower peaks also mean more peak capacity per unit time in gradient separations, desirable for many applications. Eg: peptide maps.

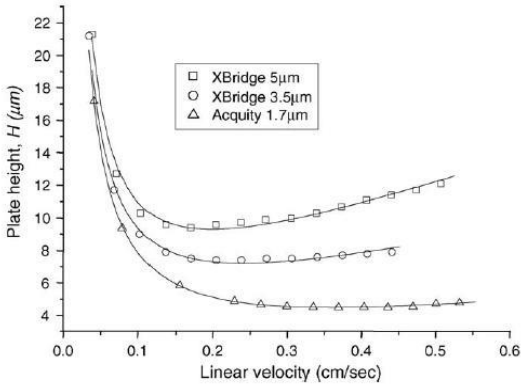
Efficiency is proportional to column length and inversely proportional to the particle size[3]:

$$N \propto \frac{L}{dp}$$

Therefore, the column can be shortened by the same factor as the particle size without loss of resolution. Using a flow rate three times higher with smaller particles and shortening the column by one third (again due to the smaller particles), the separation is completed in 1/9 the time while maintaining resolution.

An underlying principle of UPLC dictates that as column packing particle size decreases, efficiency and thus resolution also increases. As particle size decreases to less than 2.5µm, there is a significant gain in efficiency and it doesn't diminish at increased linear velocities or flow rates according to the common Van Deemter equation.[6]

The Van Deemter equation shows that efficiency increases with the use of smaller size particles but this leads to a rapid increase in back pressure, while most of the UPLC system can operate only upto 400 bar. That is why short columns filled with particles of about 2µm are used with these systems, to accelerate the analysis without loss of efficiency, while maintaining an acceptable loss of load. The effect of particle size on HETP and linear velocity was illustrated using Van Deemter plot in Fig.1.



H–u plots obtained for acetophenone on Acquity and XBridge columns. Columns: Acquity BEH C18, 1.7 µm, 10 cm × 2.1 mm ID; XBridge C18, 3.5 µm, 15 cm × 4.6 mm ID; XBridge C18, 5 µm, 25 cm × 4.6 mm ID.

Fig. 1: Van Deemter Plot illustrating the effect of particle size on plate height.

INSTRUMENTATION

The specifications used for UPLC and UPLC for different characteristics have been listed in Table 1.

Table 1: Comparison between HPLC and UPLC.

Characteristics	HPLC	UPLC
Particle size	3 to 5µm	Less than 2µm
Maximum backpressure	35-40 MPa	103.5 MPa
Analytical column	Alltima C_{18}	UPLC BEH C_{18}
Column dimensions	150 X 3.2 mm	150 X 2.1 mm
Column temperature	30 °C	65 °C
Injection volume	5µL (Std. In 100% MeOH)	2µL (Std.In100% MeOH)

Sample Injection

In UPLC, sample introduction is critical. Conventional injection valves, either automated or manual, are not designed and hardened to work at extreme pressure. To protect the column from extreme pressure fluctuations, the injection process must be relatively pulse-free and the swept volume of the device also needs to be minimal to reduce potential band spreading. A fast injection cycle time is needed to fully capitalize on the speed afforded by UPLC, which in turn requires a high sample capacity. Low volume injections with minimal carryover are also required to increase sensitivity.[3] There are also direct injection approaches for biological samples.[7,8]

UPLC Columns

Separation of the components of a sample requires a bonded phase that provides both retention and selectivity. Recently, columns used in UPLC are packed with particles produced through different technologies like Ethylene Bridged Hybrid [BEH] particle technology, Charged Surface Hybrid [CSH] particle technology, High Strength Silica [HSS] particle technology. An internal dimension (ID) of 2.1mm column is used. For maximum resolution, choose a 100mm length and for faster analysis, and higher sample throughput, choose 50mm column.

Ethylene Bridged Hybrid [BEH] Particle Technology

Four bonded phases are available for UPLC separations: UPLC BEH C_{18} and C_8 (straight chain alkyl columns), UPLC BEH Shield RP_{18} (embedded polar group column) and UPLC BEH Phenyl (phenyl group tethered to the silyl functionality with a C_6 alkyl).[3] Each column chemistry provides a different combination of hydrophobicity, silanol activity, hydrolytic stability and chemical interaction with analytes.

For more than a decade, hybrid particle technology [HPT] has delivered unsurpassed versatility and performance, enabling chromatographers to push the limits of LC separations. This first generation organic/inorganic methyl hybrid was illustrated in Fig.2 which provides significant improvement to the most problematic characteristics plaguing silica-based column: poor peak shape for basic compounds and column longevity due to chemical instability. The XTerra particle was the first commercially available option to improve these issues without the drawbacks of unpredictable selectivity produced by alternative materials such as organic polymers, zirconia and graphitic carbon. With the commercialization of 2.5µm XTerra particles, the concept of fast UPLC with small particles was born, improving the productivity of chromatographic laboratories globally. Different columns produced through this BEH particle technology is represented in Table 2.

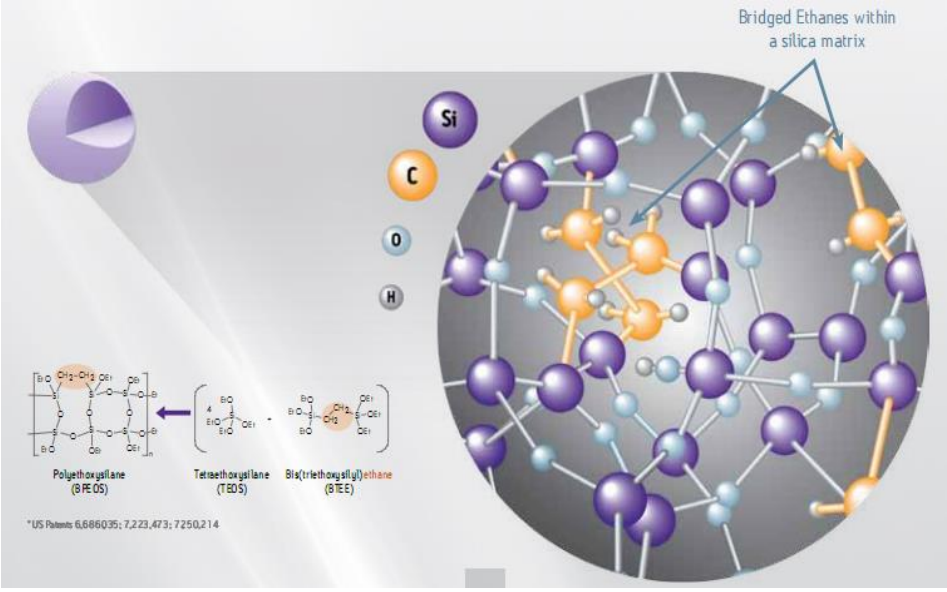
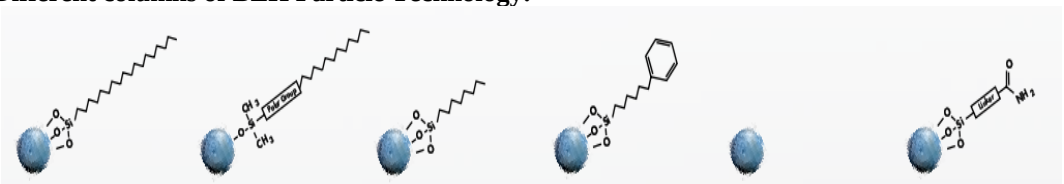


Fig. 2: UPLC column of BEH Particle Technology.

Table 2: Different columns of BEH Particle Technology.



	BEH C_{18} 1.7µm	BEH ShieldRP18 1.7µm	BEH C_8 1.7µm	BEH Phenyl 1.7µm	BEH HILIC 1.7µm	BEH Amide 1.7µm
Ligand Type	Trifunctonal C_{18}	Monofunctio-nal Embedded Polar	Trifunctional C_8	Trifunctional Phenyl-Hexyl	Unbonded BEH Particle	Trifunctional Carbamoyl
Ligand Density	3.1µmol/m^2	3.3µmol/m^2	3.2µmol/m^2	3.0µmol/m^2	n/a	7.5µmol/m^2
Carbon Load	18%	17%	13%	15%	Unbonded	12%
Endcap Style	Proprietary	TMS	Proprietary	Proprietary	n/a	none
USP Classification	L1	L1	L7	L11	L3	-
pH Range	1-12	2-11	1-12	1-12	1-9	2-11
Low pH Temp.Limit	80°C	50°C	60°C	80°C	60°C	90°C
High pH Temp.Limit	60°C	45°C	60°C	60°C	45°C	90°C
Pore Diameter	130A°	130A°	130A°	130A°	130A°	130A°
Surface Area	185m^2/g	185m^2/g	185m^2/g	185m^2/g	185m^2/g	185m^2/g
UPLC Column Equivalent	XBridge BEH C_{18}	XBridge BEH Shield RP18	XBridge BEH C_8	XBridge BEH Phenyl	XBridge BEH HILIC	XBridge BEH Amide
UPLC Particle Sizes	2.5,3.5,5,10µm	2.5,3.5,5,10µm	2.5,3.5,5,10µm	2.5,3.5,5µm	2.5,3.5,5µm	2.5,3.5µm

Forced degradation studies are performed to identify likely degradation products and establish degradation pathways as well as the intrinsic stability of a drug substance. Forced degradation analysis of Glimepiride on BEH C_{18} was shown in Fig.3. In the later stage of drug development, forced degradation studies are used to distinguish between degradation products related to the drug substance in formulation from excipients. The increased resolution capability of UPLC technology enables an improved characterization of complex samples.

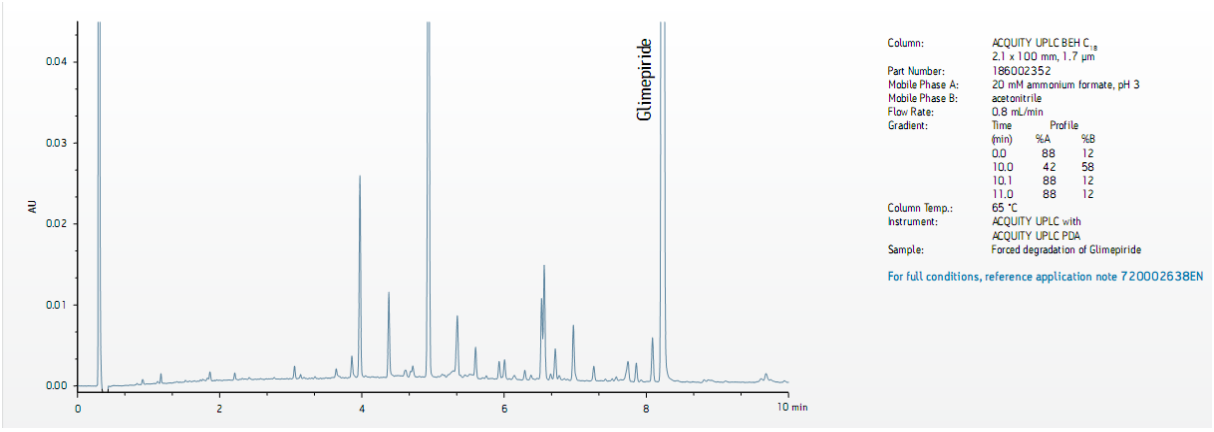


Fig.3: High Resolution Analysis of Glimepiride Forced Degradation on BEH C_{18}.

Charged Surface Hybrid [CSH] Particle Technology

The progression and evolution of materials science has led to significant advances in chromatographic materials, the most recent of which being hybrid particle technology. Hybrid-based packing materials offer exceptional peak shape and efficiency as well a industry-leading chemical stability. Charged Surface Hybrid [CSH] Technology as shown in Fig.4 is the latest advancement in hybrid materials that utilizes a controlled, low-level surface charge to provide enhanced selectivity and exceptional peak shape, particularly in low-ionic-strength mobile phase.

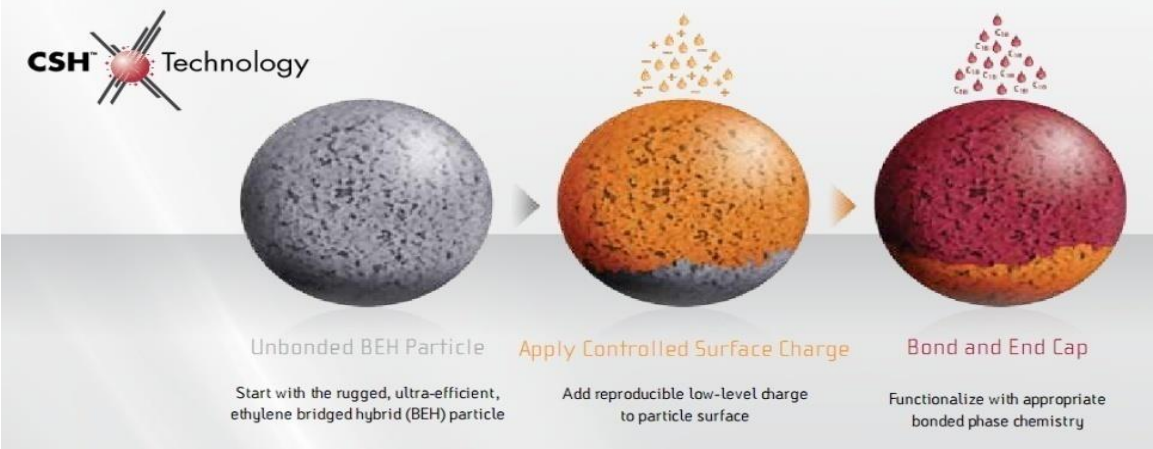


Fig. 4: Acquity UPLC column of CSH Particle Technology.

Advantages of CSH Technology include

- Unique column selectivity with industry-leading reproducibility.
- Exceptional peak shape and loading capacity for basic compounds at low and high pH without the need for ion-pair reagents.
- Exceptional stability and advanced column equilibration at low and high pH.

Ziprasidone is an anti-psychotic drug primarily used to treat the symptoms of schizophrenia, mania and bipolar disorder by altering the activity of specific natural chemicals present in the brain. The UPLC CSH C_{18} was used to successfully characterize the peroxide degradation products of ziprasidone in a simple formic acid mobile phase while demonstrating exceptional peak shape and peak-to-peak resolution in a rapid analysis time as shown in Fig.5.

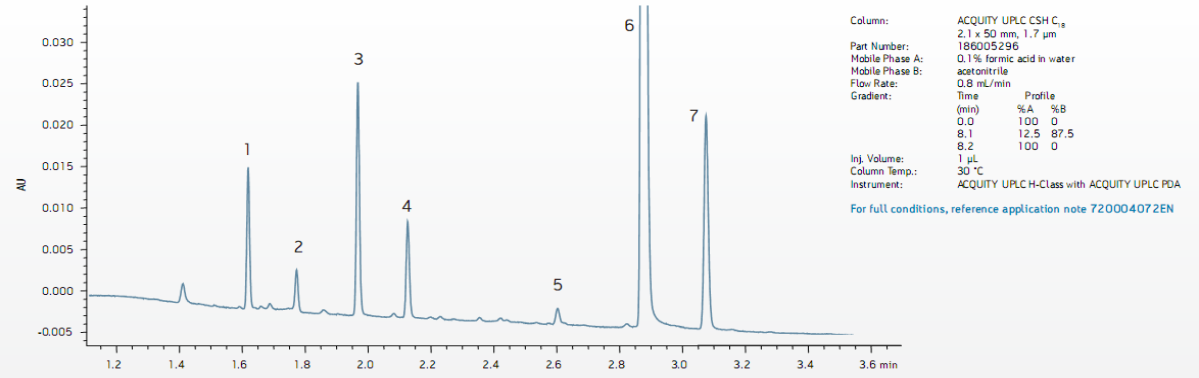


Fig. 5: Analysis of Ziprasidone Peroxide Degradation on CSH C_{18}.

High Strength Silica [HSS] Particle Technology

To complement revolutionary Hybrid Particle Technology [HPT], a mechanically tolerant, silica-based material as shown in Fig.6 was designed to withstand UPLC pressures. High Strength Silica [HSS] particle technology was born from an innovative synthetic process that significantly increases the mechanical stability of silica while maintaining pore volumes similar to that of UPLC silica-based materials. The result is a novel particle technology that provides increased retentivity compared to hybrid particles while serving as the ideal substrate to create stationary phases that provide alternate selectivity.

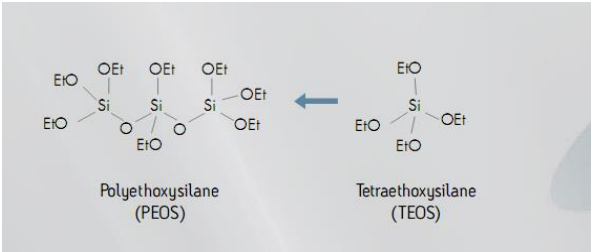


Fig.6: UPLC column of HSS Particle Technology.

Xanthine alkaloids are commonly used as stimulants of the central nervous system to temporarily reduce fatigue and drowsiness. Additionally, this class of compounds has been used as bronchodilators for the treatment of asthma. The UPLC HSS C_{18} Column allows for the rapid analysis of several xanthine alkaloids as shown in Fig.7 that are part of the metabolic pathway of caffeine.

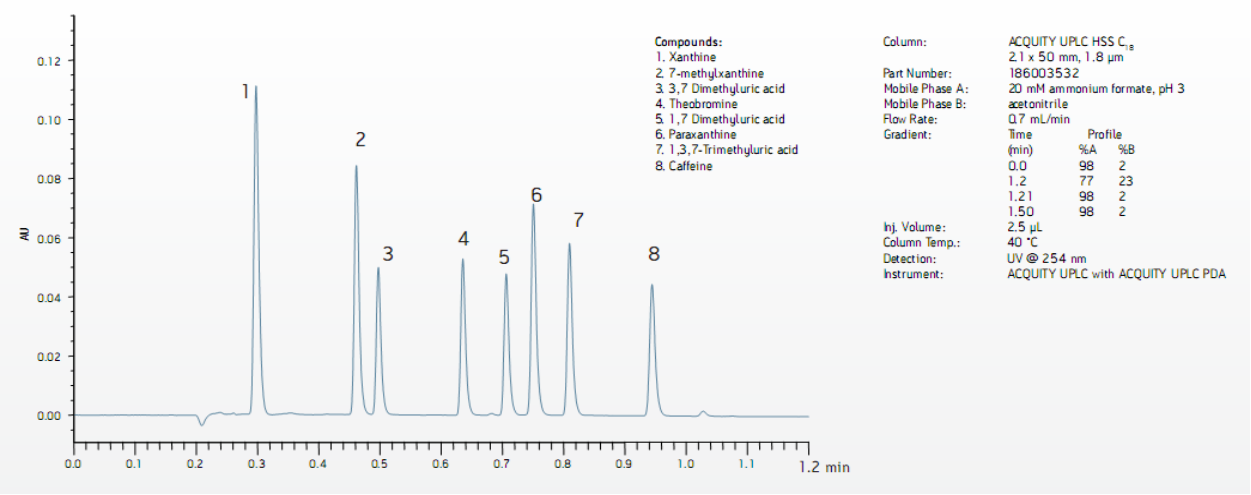


Fig.7: Separation of Xanthine Alkaloids on HSS C_{18}.

Column Choices for Alternate Selectivity

When selecting a column for a specific separation, it is important to match the properties of your analytes with the separation capabilities of a specific column. When screening multiple columns, it is important to select columns that provide differences in selectivity and hydrophobicity to maximize the potential separation capability. Fig.8 represents the reversed-phase column selectivity chart that compares these differences under low-pH conditions. The further apart the columns are on the y-axis, the more different in selectivity they are. Additionally, the columns are plotted in increasing hydrophobicity (retention) from left to right on the x-axis.

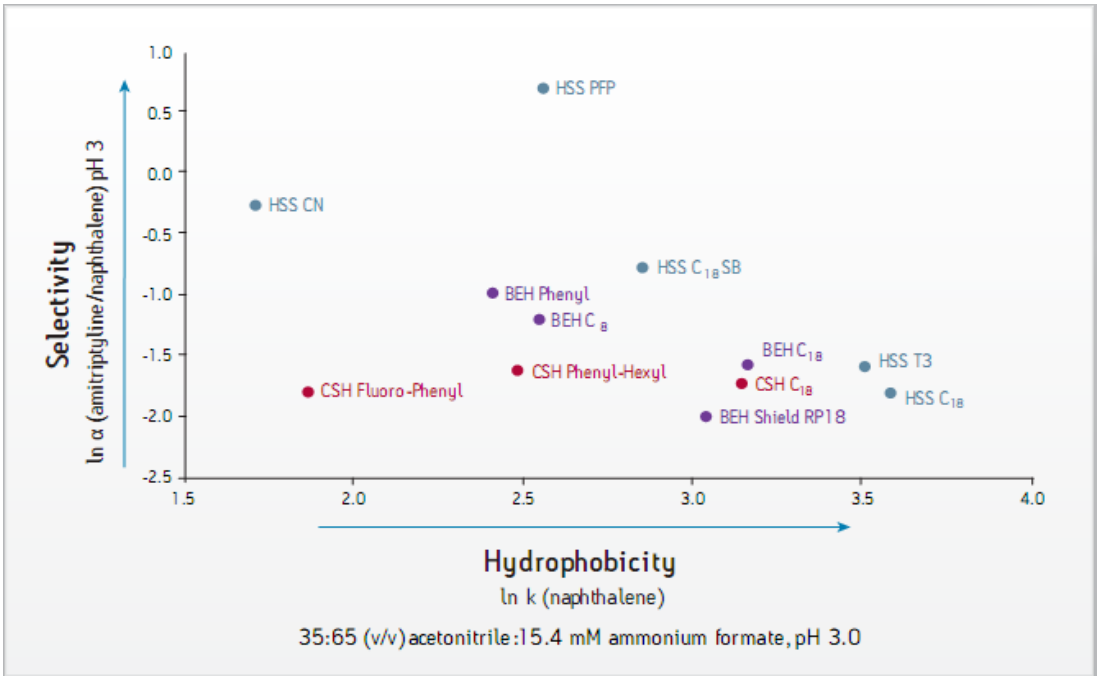


Fig. 8: Chart representing the UPLC column selectivity.

UPLC Column Protection – VanGuard Pre-Columns

Contamination resulting from the analysis of samples present within complex matrices, or that are particulate-laden, may result in reduced column lifetime if not properly addressed. VanGuard Pre-Columns are ideally suited for the physical and chemical protection of UPLC Columns. The key features and benefits of this VanGuard Pre-columns are listed in Table 3.

Directly compatible with UPLC pressures upto 18,000psi [1241bar], this ultra-low dispersion direct connect guard column is specifically engineered to preserve the lifetime of an UPLC Column without negatively impacting its separation performance.[9]

Table 3: Key Features and Benefits of VanGuard Pre-Column.

Feature	Benefit
First pre-column for UPLC applications	Guaranteed compatibility with pressures upto 18,000psi
Patent pending, ultra-low volume design	Minimal chromatography effects
Manufactured using UPLC column hardware, particles and chemistries	Superior UPLC column protection and performance
Connects directly to UPLC column	Leaks and connection voids are eliminated

The UPLC System consists of a binary solvent manager, sample manager including the column heater, detector, and optional sample organiser. The binary solvent manager uses two individual serial flow pumps to deliver a parallel binary gradient. There are built-in solvent select valves to choose upto four solvents. There is a 15,000psi pressure limit (about 1000bar) to take full advantage of the sub-2µm particles. The sample manager also incorporates several technology advancements. Using pressure assisted sample introduction, low dispersion is maintained through the injection process, and a series of pressure transducers facilitate self-monitoring and diagnostics. It uses needle-in-needle sampling for improved ruggedness, and needle calibration sensor increases accuracy. Injection cycle time is 25 seconds without a wash and 60sec with a dual wash used to further decrease carry over. A variety of microtiter plate formats likedeep well, mid height, or vial can also be accommodated in a thermostatically controlled environment. Using the optional sample organiser, the sample manager can inject upto 22 microtiter plates. The sample manager also controls the column heater. Column temperatures upto 65°C can be attained. To minimize sample dispersion, a "pivot out" design allows the column outlet to be placed in closer proximity to the source inlet of an MS detector.

Detectors

Half-height peak widths of less than one second are obtained with 1.7µm particles, which gives significant challenges for the detector. In order to integrate an analyte peak accurately and reproducibly, the detector sampling rate must be high enough to capture enough data points across the peak. The detector cell must have minimal dispersion (volume) to preserve separation efficiency. Conceptually, the sensitivity increase for UPLC detection should be 2-3 times higher than UPLC separations, depending on the detection technique. MS detection is significantly enhanced by UPLC; increased peak concentrations with reduced chromatographic dispersion at lower flow rates promotes increased source ionization efficiencies.

UPLC detectors enhance ability to analyze a variety of compounds. It includes-The Photodiode Array (ACQUITY TUV), Tunable UV (TUV) and Evaporative Light Scattering (ELS).

Tunable UV (TUV)

The Tunable UV/Visible detector cell consists of a light guided flow cell equivalent to an optical fibre. Light is efficiently transferred down the flow cell in an internal reflectance mode that still maintains a 10mm flow cell path length with a volume of only 500mL. Tubing and connections in the system are efficiently routed to maintain low dispersion and to take advantage of leak detectors that interact with the software to alert the user to potential problems.[10]

UPLC is an ideal inlet for the sensitivity and specificity offered by mass spectrometry. The low dispersion, high-speed detection performance of MS Technologies, in combination with the performance characteristics of UPLC, can dramatically extend detection capabilities.

Electronic Tools to Facilitate Method Transfer

Based on the concept of maintaining column length [L] to particle size [dp] ratio [L/dp], the UPLC Columns Calculator enables methods to be transferred from UPLC to UPLC or from UPLC to UPLC while preserving the integrity of the separation as shown in Fig.9. In addition, this intuitive software program compensates for differences in gradient dwell volume, thus replicating the gradient profile independent of the LC system type being used.

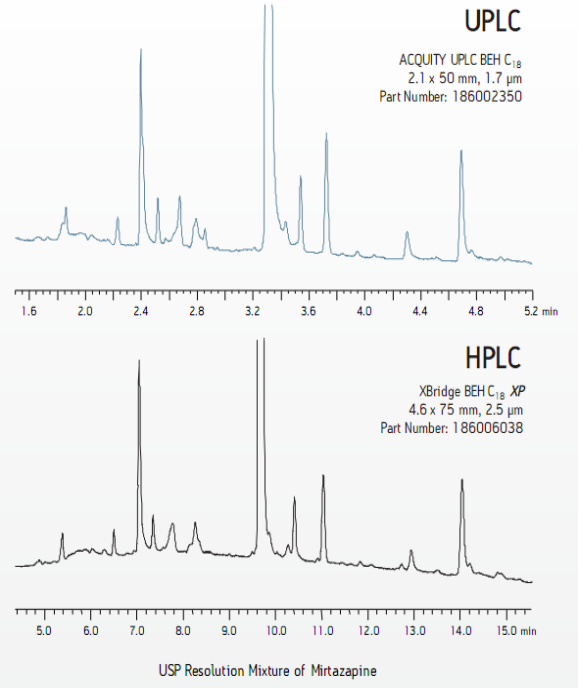


Fig.9: Chromatogram of HPLC and UPLC indicating method transfer.

ADVANTAGES

- Drastically decreases the run time compared to UPLC.
- Provides the selectivity, sensitivity, and dynamic range of LC analysis.
- The time spent on optimizing new methods can also be greatly reduced.
- Expands the scope of Multi residue methods.
- UPLC's fast resolving power quickly quantifies related and unrelated compounds.
- Use of very fine particle size of novel separation materials reduces the analysis time.
- Operation cost is reduced and less solvent consumption.
- Increases sample throughput and enables manufacturers to produce more material that consistently meet or exceeds the product specifications, potentially eliminating variability, failed batches, or the need to re-work material[2,4].
- The time needed for column equilibration while using gradient elution and during method validation is much shorter.

DISADVANTAGES

One of the major disadvantages in UPLC is the higher back pressures compared to conventional UPLC which in turn may reduce the life of the columns. This backpressure can be reduced by increasing the column temperature.

In addition, the phases of less than 2µm are generally nonregenerable and thus have limited use.[11,12]

APPLICATIONS[13-28]

1. Enhanced efficiency compared to UPLC

The analysis time and solvent consumption is greatly reduced in UPLC compared to UPLC as depicted in Fig.10.

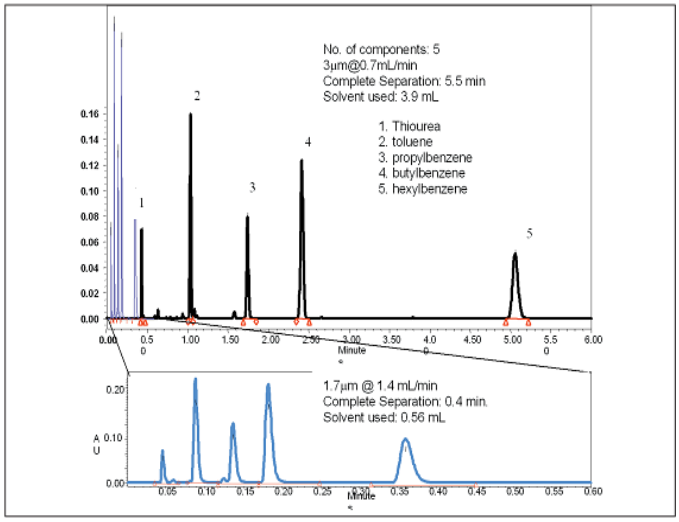


Fig.10: The top chromatogram is an overlay of both conventional (3 µm) UPLC and 1.7µm UPLC for a five component sample mixture[13]. The bottom is an expansion of the first 0.6 minutes of the overlay to show the increased speed of UPLC, while resolution is still maintained. Solvent use is also greatly reduced with UPLC.

Another example of an reduced run time in UPLC by using 2.1 by 100mm 1.7 mm ACQUITY BEH C_{18} at 288C and gradient solvent at a flow rate of 0.3mL/min as compared to UPLC using 2.1 by 100mm 5.0mm prototype BEH C_{18} at 288C and gradient solvent at a flow rate of 1.0mL/min is shown in Fig.11.

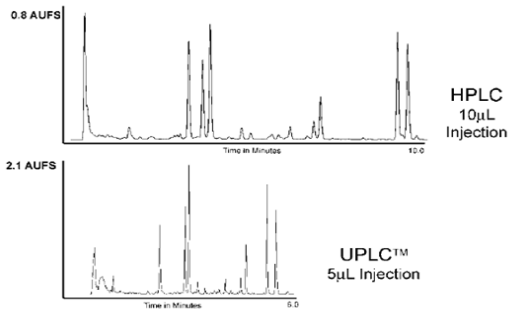


Figure 11: Comparison of HPLC and UPLC for the separation of a ginger root extract.[14]

2. Analysis of Traditional Chineese Medicines

Ultra Performance Liquid Chromatography (UPLC), through its chromatographic principles, was used in the quality control of Panax ginseng.[15]

Table 4: Gradient Mobile Phases of HPLC and UPLC.

Mobile phase	Acetonitrile (%) / Water (%)	18 / 82	18 / 82	29 / 71	29 / 71	40 / 60	19 / 81	19 / 81
Elution Time (min)	UPLC (min)*	0	35	55	70	100	101	110
	UPLC (min)^	0	8.8	13.8	17.5	25	25.1	27
	UPLC (min)#	0	3.7	5.8	7.4	10.5	10.6	11.5

*Flowrate=1.0mL/min ^Flowrate=0.21mL/min #Flowrate =0.50mL/min

In comparison to UPLC, the UPLC has obvious advantages in the TCM analysis. The main advantage is that the significant reduction of analysis time as shown in Table 4 and Fig.12, which means reduction in solvent consumption but keeping the equivalent separation power.

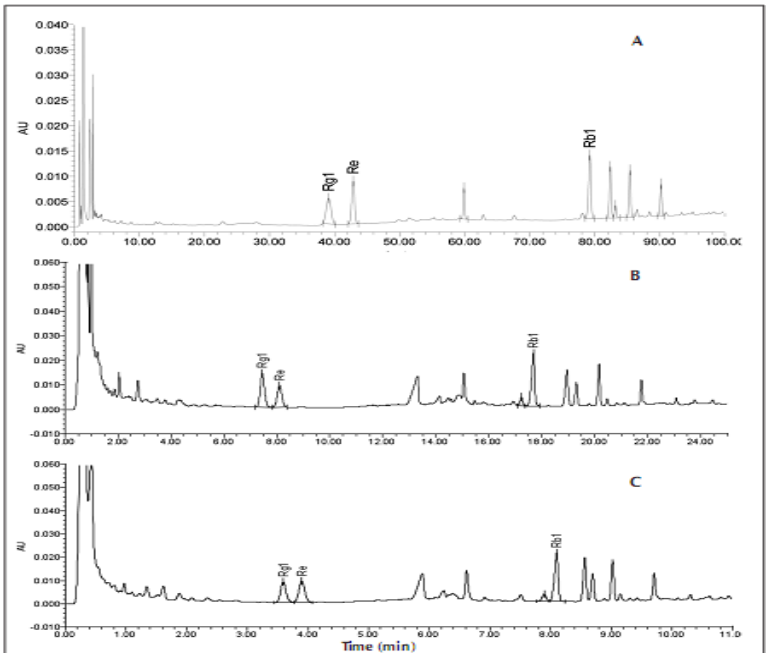


Figure 12: The (A) HPLC and (B and C) UPLC analysis of Panax ginseng.

Conditions A: Hypersil ODS-2 column(200 mm × 4.6 mm, 5 µm); the mobile phases shown in Table 4; flow rate 1.0mL/min; detection 203nm; temperature 25°C; injection 10µL; acquisition rate 1Hz. Conditions B: Acquity BEH C_{18} column (50mm×2.1 mm,1.7µm); the mobile phases shown in Table 4; flow rate 0.21mL/min; detection 203nm; temperature 25°C; injection 2µL; acquisition rate 20Hz. Conditions C: flow rate 0.5mL/min (other conditions same as conditions B).

3. Application of UPLC Amino Acid Analysis Solution to Foods and Feeds.

Amino acid analysis has been used in the food and feed industries to determine and characterize materials and processes.[16]

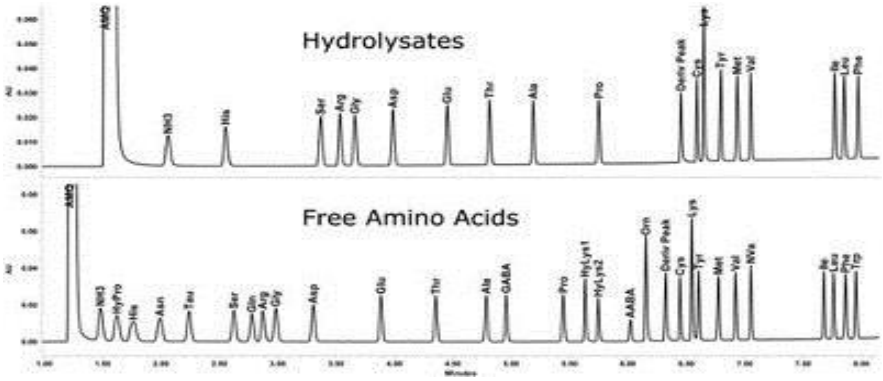


Fig.13: Representative chromatograms for hydrolysate amino acids and free amino acids using the UPLC Amino Acid Analysis Solution methods, both at 10pmol on column.

Amino acid content has been determined by a variety of techniques. With the continuing need for improved accuracy, precision, and ruggedness, preferably with higher throughput Ultra Performance LC has been combined with the well-established pre-column derivatization of AccQ·Tag Ultra. A robust turnkey solution, the UPLC Amino Acid Analysis Solution, has been developed to meet the needs of the food and feed industries. The improved resolution and sensitivity provide unequivocal identification and consistent quantitation of the amino acids. Standard methods are involved in the analysis of hydrolysate amino acids as well as for the separation of a larger set of free amino acids. These standard separations are shown in Figure 13.

Three applications indicate the utility of this method. The amino acid composition of a hydrolyzed poultry feed was measured which determine its nutritional content. Starting fermentation barley malts were identified and characterized through the analysis of free amino acids to characterize the raw materials used in a manufacturing process. Lastly, the progress of fermentation was monitored with amino acid analysis at different stages to illustrate the role of metabolic processes.

4. Application of UPLC-SRM/MS method to quantify urinary eicosanoids Eicosanoids are considered to be the key mediators and regulators of inflammation and oxidative stress often used as biomarkers for diseases and pathological conditions such as cardiovascular and pulmonary diseases and cancer. Analytical quantification of different eicosanoid species in a multi-method approach is problematic as most of these compounds are relatively unstable and may differ in their chemical properties. Here a novel ultra-performance liquid chromatography-selected reaction monitoring mass spectroscopy (UPLC-SRM/MS) method for simultaneous quantification of key urinary eicosanoids, including the prostaglandins (PG) tetranor PGE-M, 8-iso-, and 2,3-dinor-8-iso-$PGF_{2\alpha}$; the thromboxanes (TXs) 11-dehydro- and 2,3-dinor-TXB_2; leukotriene E_4; and 12-hydroxyeicosatetraenoic acid[17].Chromatographic separation was performed on a Waters (Eschborn, Germany) Acquity ultra performance liquid chromatography (UPLC) BEH C_{18} column (2.1 × 50 mm) with a 1.7µm particle size. The column was maintained at 30°C, and the injection volume was set to 5µl. Eluent A consisted of 0.1% formic acid in water; eluent B was 0.1% formic acid in acetonitrile. An efficient chromatographic separation is crucial for eicosanoid analysis because these compounds often occur in isobaric forms. In addition, there may occur ion suppression and misquantification due to interfering matrix components present within urine. Previous studies have shown that RP UPLC is an adequate technique for separation of most eicosanoid species. Based on these data, chromatographic separation using a C_{18} RP analytical column was established. In contrast to previously published methods, short UPLC column containing very small particles (50×2.1mm; 1.7µm) allowing shorter runtimes (14min including re-equilibration compared with >20min [UPLC column; 100 × 3.0 mm; 3.5 µm]) are proved to be beneficial**.**

5. Iodinated disinfection by product Iodinated disinfection by products (DBPs) are generally more toxic than their chlorinated and brominated analogues. Until now, only a few iodinated DBPs in drinking water have been identified by gas chromatography/mass spectrometry. Faster detection of polar iodinated DBPs was developed using an electrospray ionization-triple quadrupole mass spectrometer (ESI-tqMS) by conducting precursor ion scan of iodide at m/z 126.9. Through this picture of polar iodinated DBPs in chlorinated, chloraminated, and chlorine-ammonia treated water samples were achieved. By coupling ultra performance liquid chromatography (UPLC) to the ESI-tqMS, tentatively structures of 17 iodinated DBPs were proposed. The results demonstrate that, with respect to the DBP number/levels among the three disinfection processes, chloramination generated the highest iodinated DBPs, chlorination produced the lowest iodinated DBPs, and chlorine-ammonia sequential treatment formed iodinated DBPs lying in between. The ratio of total organic iodine levels in chlorine-ammonia sequential treatment and chloramination could be expressed as a function of the lag time of ammonia addition[18].

6. Identification of Metabolite Biotransformation of new chemical entities (NCE) is necessary for drug discovery. Compound after reaching the development stage, metabolite identification becomes a regulated process. It is of utmost importance to detect and identify all circulating metabolites of a candidate drug. Discovery studies are generally carried out in vitro to identify major metabolites so that metabolic weak spots on the drug candidate molecule can be recognized and protected by structure changes of the compound. In metabolite identification major aspect is maintaining high sample throughput and providing results as soon as they are available to medicinal chemists. UPLC/MS/MS addresses the complex analytical requirements of biomarker discovery by offering unmatched sensitivity, resolution, dynamic range, and mass accuracy.

7. Study of Metabonomics / Metabolomics

Development of new medicines are carried out in labs to accelerate the Metabonomics studies. The ability to compare and contrast large sample groups provides insight into the biochemical changes that occur when a biological system is exposed to a new chemical entity (NCE). A rapid and robust method for detecting these changes was provided by metabonomics, improves understanding of potential toxicity, and allows monitoring the efficacy. The perfect implementation of metabonomic and metabolomic information helps similar discovery, development, and manufacturing processes in the biotechnology and chemical industry companies.

Taking these into consideration, scientists are better able to visualize and identify fundamental differences in sample sets. The UPLC/MS System combines the benefits of UPLC analyses, high resolution exact mass MS, and specialized application managers to rapidly generate and interpret information-rich data, allowing rapid decisions to be made.

8. Bioanalysis / Bioequivalence Studies For pharmacokinetic, toxicity, and bioequivalence studies, quantitation of a drug in biological samples is an important part of development programs. The drugs are usually of low molecular weight and are tested during both preclinical and clinical studies. Several biological matrices are used for quantitative bioanalysis, among which the most common being blood, plasma, and urine19. The primary technique for quantitative bioanalysis is LC/MS/MS. The sensitivity and selectivity of UPLC/MS/MS at low detection levels generates accurate and reliable data that can be used for a wide variety of purposes, including statistical pharmacokinetics (PK) analysis.

9. Forced Degradation Studies The analytical technique most commonly used for monitoring forced degradation experiments is UPLC with UV and/or MS detection for peak purity, mass balance, and identification of degradation products but the main drawback is that these UPLC-based methodologies are time-consuming and provide only medium resolution to ensure that all of the degradation products are accurately detected. ACQUITY TUV /MS (photodiode array and MS), allows for faster and higher peak capacity separations, for complex degradation product profiles also. Combining the speed, resolution, and sensitivity of UPLC chromatographic separations with the high-speed scan rates of UPLC-specific photodiode array and MS detection helps in the identification of degradation products and thus shortening the time required to develop stability-indicating methods.

Table 1.1: System Suitability Parameters and their recommended limits.

Parameter	Recommendation
Capacity Factor (K')	The peak should be well-resolved from other peaks and the void volume, generally K' > 2
Repeatability	RSD ≤ 1% N ≥ 5 is desirable
Relative Retention	Not required as the resolution is stated
Resolution (Rs)	Rs of > 2 between the peak of interest and the closest eluting potential interferent
Tailing Factor(T)	T ≤ 2
Theoretical Plates(N)	In general, should be > 2000

Table 1.2: Characteristics to be validated in UPLC.

Characteristics	Acceptance Criteria
Accuracy/trueness	Recovery 98-102% (individual)
Precision	RSD < 2%
Repeatability	RSD < 2%
Intermediate Precision	RSD < 2%
Specificity / Selectivity	No interference
Detection Limit	S/N > 2 or 3
Quantitation Limit	S/N > 10
Linearity	Correlation coefficient R2 > 0.999
Range	80 –120 %

CONCLUSION

UPLC extends and expands the utility of chromatography compared with conventional UPLC as it increases productivity in both chemistry and separation barriers. The main advantage is that it provides more information per unit of work as it gives increased resolution, speed, and sensitivity for liquid chromatography. During separation of Phthalates, UPLC and UPLC were compared and this study showed analysis time was reduced by a factor of 2.5 and solvent consumption by a factor of 6.4 with UPLC. Analysis time, solvent consumption, and analysis cost are considered very important in many analytical laboratories. Sensitivity can be compared by studying the peak width at half height. It was found that UPLC sensitivity was much higher than that of conventional UPLC. Tailing factors and resolution were found to be similar for both techniques. Peak area repeatability (as RSD) and peak retention time repeatability (RSD) were also similar for both techniques. Overall, it can be stated that UPLC can offer significant improvements in speed, sensitivity and resolution compared with conventional UPLC.

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