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An Updated Review on Analytical Method Development of Various Drugs use in Ultra Performance Liquid Chromatography

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ABSTRACT:

Ultra-performance liquid chromatography (UPLC) is a new advance technique giving a new possibility in liquid chromatography, especially decreases of time, and solvent consumption. The principle of UPLC is based on Van Deemter equation which describes the Relationship between linear velocity with plate height. UPLC, which improves the three areas: "speed, resolution and sensitivity". UPLC chromatographic system is designed in a specific way to withstand high system back- pressures in this system uses a fine particle (less than 2.5 µm) so, decrease the length of column and significantly gain the efficiency. Separation efficiency maintained or is improved by UPLC. Speed allows a greater number of analyses to be performed in a shorter amount of time thereby increasing sample output and lab productivity. Today's pharmaceutical industries are looking for new ways to reduce cost and save time for the development of various categories of drugs while at the same time improving the quality of their products. As compared to HPLC, UPLC has more advantages and pharmaceutical application. This review introduces the theory of UPLC, Principle, Instrumentation, comparison with HPLC and summarizes some of the most recent work on various drugs in a pharmaceutical field.

KEY WORDS: UPLC, HPLC, Van deemter, high pressure

INTRODUCTION

Chromatography is a non-destructive procedure for separating a mixture into their components with the help of a porous medium under the influence of solvents. Liquid chromatography is an analytical chromatographic technique that is useful for separating ions or molecules that are dissolved in a solvent. [1]

Liquid chromatography techniques have been widely used for the fast, accurate, sensitive, and selective determination of trace amounts of pharmaceutical active compounds in biological samples and their dosage forms.

HPLC and UPLC are the commonly used as a separation technique. Both can be applied for the various modes of LC, including the normal and reverse phases, and can be paired with mass spectrometry systems for better results, but the instrumentation needed, and performance delivered differs significantly in both the systems. [3]

High performance liquid chromatography (HPLC) is a well-known technique that has been used in laboratories worldwide from more than last 30 years. The factor responsible for the development of the technique was evolution of packing materials used to affect the separation, but due to some limitation a new technique has been introduced by the scientist in 2004 which is highly efficient and advanced and also overcome some of the limitation of HPLC and the technique popularly known as "Ultra Performance Liquid Chromatography (UPLC)"[1]

"Ultra-Performance Liquid Chromatography," was introduced by Waters Corporation when they introduced their Acquity LC system. UPLC is a new invention in liquid chromatography. A well-known technique has been used in laboratories from last 10 years.

Ultra-Pressure Liquid chromatography, it improves in three areas: Resolution, Speed, and Sensitivity. UPLC instrument

operates at high pressure than that of HPLC. System uses fine particles (< than 2.5 µm) and Mobile phase at high linear velocities decreases the length of column, this also reduces solvent consumption and saves time. By using smaller particles, speed and peak capacity of the (Number of peaks resolved per unit time) can be extended to new a limit, which is known as Ultra performance.

Principle 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 (column efficiency), according to van deemter equation, as particle size decreases to less than 2.5 µm, not only there is a significant gain in efficiency, but the efficiency does not diminish at increased flow rate or linear velocities. [4]

The van Deemeter 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 HPLC system can operate only up to 400 bars. That is why short columns filled withparticles of about 2µm are used with these systems, to accelerate the analysis without loss of efficiency, while maintaining an acceptable loss of load. [5]

UPLC has many advantages like robustness; ease of use, changeable sensitivity selectivity but the main limitation is lack of efficiency in comparison to gas chromatography or capillary electrophoresis. [6]

What is UPLC ?

"UltraPerformance Liquid Chromatography, "it was introduced by Waters Corporation when they introduced their Acquity LC system. The biggest change was the use of sub- 2 µm, particles, which were operated at higher flows and pressures than a conventional system. This concept resulted in significantly shorter analysis times. [6]

PRINCIPLE

Principle of UPLC is based on using stationary phase which has particles less than 2 µm (while HPLC columns are typically filled with particle size of 3 to 10 µm). It is governed by van deemter equation, which describes the relationship between linear velocity (flow rate) and plate height (HETP or column efficiency).

$$H=A+B/v+Cv$$

Where A, B and C are constants and V is linear velocity, the carrier gas flowrate,

A = Eddy mixing

B = axial diffusion

C = solute's mass transfer

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 natural diffusion tendency of molecules. This effect is diminished at higher 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.[4]

INSTRUMENTATION

The UPLC (Ultra performance liquid chromatography) System has been specially designed to resist higher system pressures during the analysis of chromatographic methods and to match the performance needs of innovative column chemistries with easy-to-use software, robust hardware and specific support services.

The advancements in Chromatographic separations involve

- Small, pressure-tolerant particles
- Minimized system volume.
- High-pressure fluidic modules (up to 100 MPa or 15000 psi)
- Reduced cycle time.
- Negligible carryover
- High-speed detectors
- Integrated system software (novel communication protocols and advanced diagnostics) [8]

Figure 1 Ultra Performance Liquid Chromatography [10]

The components in UPLC are:

- ✓ Sample injector
- ✓ UPLC columns
- ✓ Pump device
- ✓ Column heater
- ✓ Sample manager
- ✓ Solvent delivery system
- ✓ Detectors
- ✓ Software
- ✓ Sample Injector

In UPLC, sample introduction is critical. The use of the injector is to add earlier measured, a small volume of solution containing the sample in the mobile phase. The injection must be done reproducibly and accurately.

The UPLC system with its flow through-needle design sample manager addresses three design challenges for reliable performance: robust sealing of the needle at higher pressure, minimizing the extra column band spread for narrow peaks and for performing pulse free injection process to protect column from extreme pressure fluctuations.

To protect the column from extreme pressure fluctuations, the injection process must be relatively pulse-free, and the swept volume of the device 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 of the system.

The volume of the sample in UPLC is normally 2-5 µL. Nowadays, direct injection approaches are employed for the biological samples.

There are different types of injectors available with different amounts of injection ports. The zesport injector is most commonly used in single column analyses. [2,5,6,7,9]

UPLC Column

The UPLC columns are made up of small particles having size less than 2 µm. The particles are bonded in matrix, as the bonded stationary phase is required for providing both retention and selectivity.

Resolution is increases in a particle packed column (1.7µm) because efficiency is better. Separation of the components of a sample requires a bonded phase that provides both retention and selectivity.

Four bonded stationary phase columns manufactured by ACQUITY are available in the market, which can be used by UPLC technique.

A. ACQUITY UPLCTM BEH C8,
B. ACQUITY UPLCTM BEH C18,
C. ACQUITY UPLC BEH Shield RP18 and
D. ACQUITY UPLC BEH Phenyl (phenyl group tethered to the silyl functionality with a C6 alkyl), (BEH-Bridged Ethane Hybrid)

Based on technology columns are differentiated as follows

BEH [Ethylene Bridged Hybrid] technology: BEH C18, BEH C8, BEH shielded RP18, BEH Phenyl, and BEH amide columns.

Peptide separation technology: BEH 130, BEH 300.

Protein separation technology: BEH123, BEH200, BEH450 SEC columns.

Glycan separation technology: BEH Glycan column.

Oligo nucleotides separation technology: OST C18 columns

Charged surface hybrid technology: CSH C18, CSH Phenyl-Hexyl and FlouroPhenyl columns.

HSS technology: HSS T3, HSS C18, HSS C18 SB, HSS PFP, HSS Cyno columns.[2]

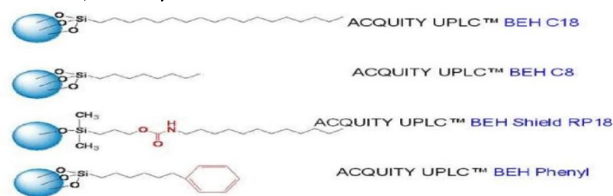

Figure 2 BEH Column [12]

BEH C18 COLUMNS:

These are straight alkyl chain, bonded and columns are considered the universal columns with leading mobile phase pH (1-12) and temperature (80℃).

These columns provide the wide pH range. They integrate trifunctional ligand bonding chemistry, which produce low pH stability.[6]

BEH C8 columns:

Columns are considered the universal columns of choice for most UPLC separations by providing the widest pH range. They incorporate trifunctional ligand bonding chemistries, which produce superior low pH stability. This low pH stability is combined with the high pH stability in 1.7 µm. BEH particle to deliver the broad usable pH operating range. Provides long column lifetimes and excellent peak shape.

Due to its shorter alkyl chain length, these columns exhibit low hydrophobicity than C18 column, resulting in lower retention and faster elution of analyte peaks.

BEH shield RP 18 columns

This contains an embedded polar group that combines the hydrophobicity of a straight- chain alkyl ligand C18 with hydrophilicity of an embedded polar group (carbamate).

This unique and patented bonding chemistry provides complementary selectivity to a C18 column and enhances the peak shape for basic compounds and yielding compatibility with 100% aqueous mobile phases.

BEH phenyl columns

This columns provide complementary selectivity to straight chain alkyl phases, particularly the molecule contains aromaticity due to pi-pi interactions.

This employs trifunctional-bonded phenyl hexyl ligand, which provides reproducibility, chemical stability and peak shape for all analyte types.

BEH Amide columns

The combination of trifunctional bonded amide phase with BEH small particles provides exceptional column lifetime. The use of a wide range of phase pH i.e., from pH 2 to 11.

This column is ideal for the analysis of carbohydrates due to its exceptional chemical stability at high pH and high temperature to collase reducing sugars anomers as well as improved quantitation accuracy due to the lack of Schiff-base formation. [2,5]

Factors affecting column efficiency :

According to chromatographic theory, column efficiency (N) is inversely proportional to particle size (dp). Thus, smaller particles provide higher resolution. The highly efficient 1.7µm BEH particles allow chromatographers to maximize the efficiency (N) of their separation when used in the Acquity UPLC system.

The factors affecting column efficiency are:

- Column length
- Particle size
- Linear velocity
- Retention factor
- Temperature of the column
- Solvents [2]

Pump Device

The UPLC pump is one of the most important components in a liquid chromatography system, which provides continuous constant flow of the eluent through the UPLC injector, column, and detector.

Standard UPLC pump requirements:

Sample injection volume is as less as 3 – 5 microliters

Pump operates at 15000-psi pressure.

Particle size in stationary phase packing material is less than 2 micrometre.

An ideal pump for UPLC has a capacity of delivering solvent at higher pressure around 15000 psi for the optimum flow rate with maximum efficiency across 15 cm long column packed with 1.7 µm particles. Hence, at these pressures, the pump was suitable for delivering solvent reproducibly and smoothly. UPLC uses two serial pumps with pressure limit of 1000 bar and have inbuilt solvent selector valves, which have the capability to choose the accurate solvent ratio up to four solvents.[2,4,8,12]

Column heater

It is attached to the top of the sample manager and serves as instrument's top cover. The column heater heats the column compartment to any temperature from 5℃ to 65℃.[2,4]

Sample Manager

The Acuity sample manager injects the sample, it draws from Micro titer plates or vials into the chromatographic flow stream.

The Sample manager can perform an injection within 15 seconds. The sample manager also has control over column heater. Column can attained temperature up to 65 °C.[4]

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.

In binary solvent manager, two individual serial flow pumps deliver a parallel binary gradient. A high-pressure pump moves solvent through the system.

It provides pulse free solvent flow and delivers solvent at flow rates of 1ml/min at 103421 Kpa [1034 bar, 1500 psi] and up to 2 ml/min at reduced pressures to 62053 Kpa [621 bar, 9000 psi]. The solvent manager can pump two solvents immediately. [4,7]

Detector

The UPLC technique provides the sensitivity of separation of two to three times more than the previous analytical method perfumed on HPLC, which is also due to the method employed for the detection.

Types Of Detectors:

Detectors can be classified as:

- Optical detector
- Photo diode array [PDA] detector
- Tunable ultraviolet [TUV] detector
- Evaporative light scattering [ELS] detector
- Fluorescence [FLR] detector

A. Optical Detectors :

Optical Detectors are used in UPLC analytical techniques, which has low dispersion characteristics, simple operation, and high data acquisition rates as well as cost effective maintenance and support. The system can be configured with a TUV, PDA or ELS optical detector or any combination of the three.[4]

B. Tunable Ultraviolet Detector :

The TUV optical detector is a two channel ultraviolet / visible absorbance detector, which is design for use in acquity UPLC system.

The detector has two flow cell options.

The analytical cell flow, with a volume of 500 nano liters and a path length of 10 nm and

The high sensitivity flow cell with a volume of 2.4 micro litres and 25 mm path length

Both utilizes the waters patented light guiding flow all technology. The TUV detector operates at wavelength ranging from 190 to 700 nm.

Features:

Maximum signal-to-noise response enabled by light-guiding flow cell technology, which eliminates internal absorption, for minimal bandspreading and for maintaining concentration.

High sensitivity for low-level detection for simultaneous quantitation of major and minor.[4,6]

C. (Photo Diode Array) detector:

It is an optical detector absorbs UV-Visible light that operate between 190-500nm. The detector offers two flow cell options.

The analytical cell, with a volume of 500 nano liters and path length of 10 mm, and high sensitivity flow cell, with a volume of 2.4 micro liters and a 25mm path length, both utilizes the flow cell technology. [5,13]

D. ELS (Evaporative Light Scattering) detector:

ELS detector is an evaporative light scattering detector designed for use in UPLC system.

The detector, Controlled by Empower or Mass Lynx software, which incorporates a flow-type nebulizer that is optimized for UPLC system performance and detector offers a convenient stackable design, easy maintenance, and long lamp lifetimes.

The detector incorporates a flow type nebulizer that is optimized for acquity UPLC system performance.

The UPLC ELS Detector helps to analyse more molecules (including sugars, triglycerides, phospholipids, antibiotics, and natural products) in a single run.

Features

Provides reproducible, reliable results with precise control over nebulization and desolvation processes for the measurement of temperature sensitive molecules.

Ensures the benefits of UPLC performance across the entire flow rate range with high data capture rates.

One nebulizer for the entire flow rate range provides simplified set-up and the benefit of maximized performance. [4,5,6]

E. **FLR (Fluorescence) detector:**

It is a multi-channel, multi wavelength detector, which has an excitation wavelength that ranges from 200 to 890 nm, an emission wavelength that ranges from 210-900 nm, offers 3D scanning capability for easier method development.

Delivers sensitivity and selectivity to fluorescence-based applications. Transfer your HPLC fluorescence methods to Ultra Performance LC for greater throughput without compromising sensitivity.

Features

Advanced optical design to maximize light throughput, and reduce light scatter, allowing for better signal-to-noise performance.

Intuitive system console provides simple navigation to manage instrument parameters for easy system control.

Intuitive software interface and diagnostic tools instill confidence that the detector is performing optimally[4,5,13].

Softwares

ACQUITY UPLC Systems can be easily controlled, diagnosed and monitored via a graphical system console interface with **Empower™** and **MassLynx™** software.

Both Empower and MassLynx provide the dynamic data processing and information management tools to convert the results generated by the ACQUITY UPLC System into valuable knowledge. [14]

3. COMPARISON OF HPLC AND UPLC TECHNIQUES : [2,4,5]

The comparison of HPLC and UPLC techniques are shown in table no 1.

ACQUITY UPLC BEH C18 and **C8** columns are **universal columns** of choice for most of UPLC separations by providing the widest pH range.[4,10,11]

Table 1 Comparison between HPLC and UPLC

CHARACTERISTICS	HPLC	UPLC
Particle Size	3–5 µm	Sub-2 µm
Maximum back pressure	35 to 40 Mpa	103.5 MPa
Analytical column	Alltima C18	Acquity UPLC BEH C18
Column dimensions	150 ×3.2 mm	150 X 2.1 mm
Column temperature	30°C	65°C
Sample throughput	Less	More
Sample preparation	Simple	Tedious
Column coagulation	Does not takes place	Takes place
Analysis time	More	Less
Sensitivity	Less	Higher
Plate count	More than 2000	More than 7500
Injection volume	5-20Ml	2-5µL
Pressure limit	Up to 4000 psi	15000 psi
Total run time	10 min	1.5 min

Table 2 Summary of UPLC methods on various Pharmaceutical Dosage form

Sr No	DRUG NAME	DOSAGE FORM	METHOD	DESCRIPTION	Ref No.
1	Zolpidem	Tablet	UPLC	**Mobile Phase-** Potassium di-hydrogen phosphate: Acetonitrile [40:60 %v/v] **Column**- Waters Acquity HSS T-3 C18 stationary phase (100 × 2.1 mm, 1.8µm) **Wavelength**-243 nm **Detector** – UV	15
2	Semaglutide	Tablet	UPLC	**Mobile Phase-** 0.01N potassium dihydrogen phosphate:acetonitrile [50:50 %v/v] **Column**- Acquity BEH C18 (50mm x 1.6 mm) 1.8µm column **Wavelength**-292 nm **Detector** –UV	16
3	Topiramate	Tablet	UPLC	**Mobile Phase-** Methanol: water [80:20% v/v] **Column-** Inertsil ODS, (250×4.6) 5µm **Wavelength**-276 nm **Detector** – UV	17
4	Erythromycin	Tablet	UPLC	**Mobile Phase**- **A]**a mixture of Buffer solution (Disodium hydrogen phosphate and Ortho phosphoric acid solution) and Methanol 35:65 [% v/v] **B**] Methanol **Column** - BEH C_{18}, (50 mm x 2.1 mm) ,1.7 µm particle size **Wavelength**-210 nm **Detector** –PDA	18
5	Velpatasvir and Sofosbuvir	Tablet	UPLC	**Mobile Phase**- NaH2PO4 [sodium dihydrogen phosphate], pH 2.5 (with phosphoric acid) and acetonitrile in a ratio of [60:40% v/v] **Column**- **A]**Acclaim RSLC 120C18, 5.0 µm, 4.6 × 150 mm **B]** Acclaim RSLC 120 C18, 2.2 µm, 2.1 × 100mm **Wavelength**-260 nm **Detector** - UV	19
6	Duloxetine HCl	API	UPLC	**Mobile phase-** Mixture of buffer [Sodium di hydrogen orthophosphate buffer (0.01M) and 0.1% triethyl amine with pH 7.0]and acetonitrile in the ratio [78:22 %v/v] **Column**- XR-ODS II (3.0× 100)mm, 2.2 µm **Wavelength-**230nm **Detector-**PDA	20
7	Crizotinib	Capsule	STABILITY INDICATING UPLC	**Mobile phase-** 0.1%Ortho-phosphoric acid and acetonitrile (45:55% v/v) **Column**- Hibra C18 (100 mm × 2.1 mm, 2 µm) **Wavelength-**327nm **Detector –**UV	21
8	Alogliptin and pioglitazone	Tablet	STABILITY INDICATING RP-UPLC	**Mobile phase**-phosphate buffer (pH 3) and methanol[45:55% v/v] **Column**- BEH C18 (2.1× 50 mm, 1.7 µ) **Wavelength**-280nm **Detector**-PDA	22
9	Lenvatinib Mesylate	Tablet		**Mobile phase-** 0.1% ortho phosphoric acid and acetonitrile [50:50% v/v]	23

			STABILITY INDICATING UPLC	**Column**- HSS C18 (100 mm × 2.1 mm, 1.8 µ) column **Wavelength**-240nm **Detector**- UV	
10	Emtricitabine ,Tenofovir, and Efavirenz	Tablet	STABILITY INDICATING UPLC	**Mobile phase** – 0.01N potassium dihydrogen phosphate buffer (pH 4.5) and acetonitrile (40:60, V/V). **Column**-HSS C18 (100×3 mm, 1.7 µ) **Wavelength**-265nm **Detector** – UV	24
11	Dolutegravir and rilpivirine	Tablet	STABILITY-INDICATING RP-UPLC	**Mobile phase** – 0.1% orthophosphoric acid and acetonitrile as solvent in the ratio of [55:45%v/v] **Column**- Sb c8 column (100 x 3 mm, 1.8 mm) **Wavelength**-260nm **Detector**- PDA	25
12	Ledipasvir and Sofosbuvir	Tablet	STABILITY-INDICATING UPLC	**Mobile phase-** Buffer (0.01 N w/v potassium di-hydrogen orthophosphate buffer of pH 3.5 modified with dilute orthophosphoric acid) and (acetonitrile) in the ratio of [50:50% v/v]. **Column**- BEH C18 column (50 mm × 2.1 mm ,1.6 µm particle size) **Wavelength**-220nm **Detector** –UV	26
13	Donepezil	Human plasma	UPLC-MS/MS	**Mobile phase** -20 mM ammonium acetate buffer (pH 3.3) and 100% acetonitrile 60:40 (v/v) **Column**- Thermo Hypersil Gold C18 column **M/Z** - 380.6 → 91.1 **Detector**-triple quadrupole tandem Xevo Q-MS mass spectrometer	27
14	Telmisartan	Human plasma	UPLC – MS/MS	**Mobile phase**- acetonitrile: 8 mM ammonium acetate containing 0.15 % formic acid [70:30%v/v] **Column** -Acquity UPLC BEHTM C18 column (50 x 2.1 mm) 1.7 µm **m/z** - 515.27→276.13 **Detector**- TQD triple quadrupole mass spectrometer	28
15	Tamoxifen And Sulphoraphan	Rat plasma	UPLC – MS/MS	**Mobile phase-** (0.1%)formic acid in acetonitrile)and(0.1%)formicacid in water)(80: 20%v/v) **Column**- UPLCBEHC18 column(50mm×2.1mm,1.7µm) **M/Z –** • For Tamoxifen m/z=372.0[M+H]+ →71.92for • For sulphoraphane m/z=177.9[M+H]+→113. 9 **Detector** – WatersZsprayTMXevoTQD detector	29

[**PDA-** photo diode array**, TQD-** triple quadrupole detector, **API** – Active pharmaceutical ingredient]

Advantages

✓ Faster analysis through the use of a novel separation material of very fine particle size.

✓ Provides the selectivity, sensitivity, and dynamic range of LC analysis.

✓ Maintaining resolution performance.

✓ It gives increased peak capacity (number of peaks resolved per unit time).

✓ Separation on UPLC is performed under the high pressures up to 100 MPa.

✓ UPLC's fast resolving power and is fast quantifies related and unrelated.

✓ Compounds Delivers real-time analysis in step with manufacturing processes.

✓ Assures end-product quality, including final release testing.

- ✓ UPLC dramatically improves the quality of the data.
- ✓ Expands scope of Multi residue Methods.
- ✓ Operation cost is minimize.
- ✓ Less solvent consumption.
- ✓ Require less run time and enhance sensitivity.
- ✓ In chromate ogram, resolved peaks are obtained.
- ✓ 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. [4,10,11]

Disadvantages

- ✓ High backpressure compared to HPLC, which reduces the life of the column.
- ✓ In addition, the phases of less than 2 µm are generally non-regenerable and thus have limited use.
- ✓ So far, performance similar o even higher has been demonstrated by using stationary phases of size around 2 µm without the adverse effects of high pressure. Due to increased pressure requires more maintenance.
- ✓ Higher price of instruments, spare parts and columns.
- ✓ Detector and data collection system (CDS) may not cope withsharper peaks (data acquisition rate).[4,10]

Use of UPLC system

1. Elevated-temperature chromatography is also allowed for high flow rates by lowering the viscosity of the mobile phase, which importantly reduces the column backpressure.
2. Monolithic columns contain a polymerized porous support structure that provides lower flow resistances than conventional particle-packed columns.[5]

Drawbacks of UPLC are

- ✓ Cost mixing,
- ✓ Solvent pumping, and
- ✓ Lack of variety in commercial columns at 1.7 µm

Application:

- ✓ Drug discovery
- ✓ Analysis of Dosage form
- ✓ In Forced Degradation Studies
- ✓ Study of metabonomics /metabolomics
- ✓ Dissolution Testing
- ✓ Manufacturing / QA / Qc
- ✓ Impurity Profiling
- ✓ Analysis of amino acids
- ✓ Determination of Pesticides
- ✓ Drug Abuse [30]
- ✓ UPLC fingerprint analysis [31]
- ✓ Method Development / Validation
- ✓ Bioequivalence / Bioanalysis Studies
- ✓ Analysis of Traditional Chinese Medicines (TCM)[32]
- ✓ **A**DME (Absorption, Distribution, Metabolism, Excretion) Screening
- ✓ Identification of Metabolite
- ✓ Toxicity Studies

CONCLUSION

UPLC increases productivity in both chemistry and instrumentation by providing more information per unit of work as it increases resolution, speed and sensitivity for liquid chromatography.

The sensitivity of UPLC was much higher than conventional HPLC. Tailing factors and resolution were similar for both HPLC and UPLC techniques. Peak area repeatability as a (RSD) and peak retention time repeatability (RSD) were similar for both techniques.

A negative point of UPLC could be the higher backpressure than in conventional HPLC. This Backpressure reduced by increasing the column temperature. Overall, the UPLC can offer significant improvements in speed, sensitivity and resolution compared with conventional HPLC.

The main advantage is a reduction of analysis time which also reduces solvent consumption, Analysis time and analysis cost which are very important in many analytical laboratories.

This technology creates a new opportunity for business advantageous in highly efficient manner and allows the product to be introduced to the market in lesstime.

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