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DETERMINATION OF DRUGS OF ABUSE IN BLOOD BY HYPHENATED CHROMATOGRAPHIC TECHNIQUES

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ABSTRACT
The detection and quantitation of drugs of abuse in blood is of growing interest in forensic and clinical toxicology. Hyphenated techniques combine chromatographic and spectral methods to exploit the advantage of both. Chromatography produces pure or nearly pure fractions of chemical components in a mixture. Spectroscopy produces selective information for identification using standards or library spectra. The term forensic science covers those professions which are involved in the application of the social and physical sciences to the criminal justice system. Forensic experts are required to explain the smallest details of the methods use, to substantiate the choice of the applied techniques and to give their unbiased conclusions. All under the critical and often mistrustful gaze of the servants of the justice, as well as the general public and the media. Recent years have seen the development of powerful technologies that have provided forensic scientists with new analytical capabilities, unimaginable only a few years ago. The aim of this article is to present an overview of some of the most recent applications of hyphenated liquid chromatography to forensic analysis. Focuses on trace analysis (including chemical warfare agents, explosives and dyes Drugs of abuse in alternative matrices, trace chemicals, systemic toxicological analysis, doping agent and related compounds, therapeutic drugs of toxicological relevant, environmental poisons, Natural toxins). It is not the intention to provide an exhaustive review of the literature but rather to provide the reader with a „flavour" of the versatility and utility of the technique within the forensic sciences. Also a number of drugs/drug metabolites that are structurally close to these substances are included in the tables. Basic information about the biosample assayed, work-up, GC column or LC column and mobile phase, detection mode, reference data and validation data of each procedure is summarized in the tables. Examples of typical applications are presented. This review also includes methods for the determination of the most commonly occurring illicit drugs and their metabolites, which are important for the assessment of drug abuse: Methamphetamine, amphetamine, 3,4-methylenedioxymethamphetamine (MDMA), N-ethyl-3,4-methylenedioxyamphetamine (MDEA), 3,4-methylenedioxy-amphetamine (MDA), cannabinoids (delta-9-tetrahydrocannabinol, 11-hydroxy-delta-9-tetrahydrocannabinol, 11-nor-9-carboxy-delta-9-tetrahydrocannabinol), cocaine, benzoylecgonine, ecgonine methyl ester, cocaethylene and the opiates (heroin, 6-monoacetylmorphine, morphine, codeine and dihydrocodeine).

KEYWORD: Hyphenated chromatography, Forensic toxicology, Drug Abus, Metabolites, Analysis.

INTRODUCTION
Hyphenated techniques combine chromatographic and spectral methods to exploit the advantage of both. Chromatography produces pure or nearly pure fractions of chemical components in a mixture.[1] Spectroscopy produces selective information for identification using standards or library spectra. The term "forensic science" covers those professions which are involved in the application of the social and physical sciences to the criminal justice system. Forensic experts are required to explain the smallest details of the methods used, to substantiate the choice of the applied technique and to give their unbiased conclusions. All under the critical and often mistrustful gaze of the servants of the justice, as well as the general public and the media.[2] The final result of the work of the forensic scientist exerts a direct influence on the fate of a given individual. This burden is a most important stimulus, and one which determines the way of thinking and acting in forensic sciences. Consequently, the methods applied in forensic laboratories should assure a very high level of reliability and must be subjected to extensive quality assurance and rigid quality control programs. The legal system is based on the belief that the legal process results in justice. This

has come under some question in recent years. He or she can, however, contribute to restoring faith in the judicial processes by using science and technology in the search for facts in civil, criminal and regulatory matters. The purpose of this article is to review some of the most recent applications of hyphenated liquid chromatography to forensic analysis with special focus on the following; trace analysis, the use of alternative specimens for monitoring drugs of abuse, systematic toxicological analysis and high-throughput analysis. The aim of this article is to present an overview of some of the most recent applications of hyphenated liquid chromatography to forensic analysis.

Hyphenated techniques combine chromatographic and spectral methods to exploit the advantage of both. Chromatography produces pure or nearly pure fractions of chemical components in a mixture. Spectroscopy produces selective information for identification using standards or library spectra. The term forensic science covers those professions which are involved in the application of the social and physical sciences to the criminal justice system. Forensic experts are required to explain the smallest details of the methods use, to substantiate the choice of the applied techniques and to give their unbiased conclusions. All under the critical and often mistrustful gaze of the servants of the justice, as well as the general public and the media. Recent years have seen the development of powerful technologies that have provided forensic scientists with new analytical capabilities, unimaginable only a few years ago.

The aim of this article is to present an overview of some of the most recent applications of hyphenated liquid chromatography to forensic analysis. Focuses on trace analysis (including chemical warfare agents, explosives and dyes Drugs of abuse in alternative matrices, trace chemicals, systemic toxicological analysis, doping agent and related compounds, therapeutic drugs of toxicological relevant, environmental poisons, Natural toxins). It is not the intention to provide an exhaustive review of the literature but rather to provide the reader with a 'flavour' of the versatility and utility of the technique within the forensic sciences.

An ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS-MS) method for screening of drugs in whole blood has been developed and validated. Samples were prepared by supported liquid-liquid extraction on ChemElute(®) columns with ethyl acetate/heptane (4:1). LC separation was achieved with an Acquity HSS T3-column (2.1 100 mm, 1.8-µm particle).

Mass detection of blood content performed by positive ion mode electrospray MS-MS and included the following drugs/metabolites: morphine, codeine, ethyl morphine, oxycodone, buprenorphine, methadone, cocaine, methylphenidate, amphetamine, methamphetamine, 3,4-methylenedioxymethamphetamine (MDMA), Δ(9)-tetrahydrocannabinol (THC), fentanyl, alprazolam, bromazepam, clonazepam, diazepam, nordiazepam, 3-OH-diazepam, fenazepam, flunitrazepam, lorazepam, nitrazepam, oxazepam, zopiclone, zolpidem, carisoprodol, and meprobamate.

AVAILABLE HYPHENATED TECHNIQUES

Double hyphenated techniques LC-MS• LC-NMR• LC-IR• HPLC-DAD• GC-MS• GC-IR• GC-FTIR• CE-MS•

Triple hyphenated techniques LC-API-MS• APCI-MS-MS• LC-ESI-MS-MS• LC-ESI-MS• ESI-MS-MS• LC –MS-TSPLC-UV-NMR-MS• LC-UV-NMR-MS-ESI• LC-NMR-MS• LC-DAD-API-MS• LC –DAD-MS• LC-PDA-MS• LC-PDA-NMR-MS• SPE-LC-MS• LVI-GC-MS•

GC-MS

GC-MS, which is a hyphenated technique developed from the coupling of GC and MS. Mass spectra obtained by this hyphenated technique offer more structural information based on the interpretation of fragmentation. The fragment ions with different relative abundances can be compared with library spectra. Compounds that are adequately volatile, small, and stable in high temperature in GC conditions can be easily analyzed by GC-MS. In GCMS a sample is injected into the port of GC device vaporized, separated in the GC column, analyzed by MS detector and recorded.

LC-IR

The hyphenated technique developed from the coupling of an LC and the detection method infrared spectroscopy (IR) or (FTIR) is known as LC-IR or HPLC–IR. A useful spectroscopic technique for the identification of organic compound, because in the mid-IR region the structures of organic compounds have many absorption band that are characteristic of particular functionalities eg.-OH, -COOH.

LC-MS

LC-MS or HPLC-MS refers to the coupling of an LC with a mass spectral data. The separated sample emerging from the column can be identified on the basis of its mass spectral data. An LC-Ms combines the chemical separating power of LC with the ability of an MS to selectively detect and confirm molecular identity. CE-MS MS detector linked to a CE system for acquiring on-line MS data of the separated compound, the resulting combination is termed as CE-MS. CE analysis is driven by an electrical filed, performed in narrow tubes, and can result in the rapid separation of many hundreds of different compounds .Separation is achieved through channels etched on the surface of the capillary connected to an external high voltage power supply that delivers sample to ESIMS.

LC-NMR

Technological developments have allowed the direct parallel coupling of HPLC system to NMR.LC-NMR promises to be of great value in the analysis of complex mixtures of all types, particularly the analysis of natural products and drug related metabolites biofluids. The main prerequisites for on-line LC-NMR, in addition to the continuous-flow probe for recording either continuousflow or stopped flow NMR spectra. For the high sensitivity, new RF system for multiple solvent suppression and improved dynamic range gradient elusion capability and automatic peak piking/storing capability.

Fig 1. Image Of Ultra Performance Liquid Chromatogarphy

What is UPLC?

The term UPLC, meaning "Ultra Performance Liquid Chromatography," was introduced by Waters Corporation when they introduced their Acquity LC system. The biggest change was the use of sub-2 um particles, which were operated at higher flows and pressures than a conventional system. This concept resulted in significantly shorter analysis times.

Principle

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

$H=A+B/v+Cv$

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*.

Therefore it is possible to increase throughput, and thus the speed of analysis without affecting the chromatographic performance. The advent of UPLC has demanded the development of a new instrumental system for liquid chromatography, which can take advantage of the separation performance (by reducing dead volumes) and consistent with the pressures (about 8000 to 15,000 PSI, compared with 2500 to 5000 PSI in HPLC). Efficiency is proportional to column length and inversely proportional to the particle size. Therefore, the column can be shortened by the same factor as the particle size without loss of resolution. The application of UPLC resulted in the detection of additional drug metabolites, superior separation and improved spectral quality.

Advantages of UPLC

The advantages of UPLC are:

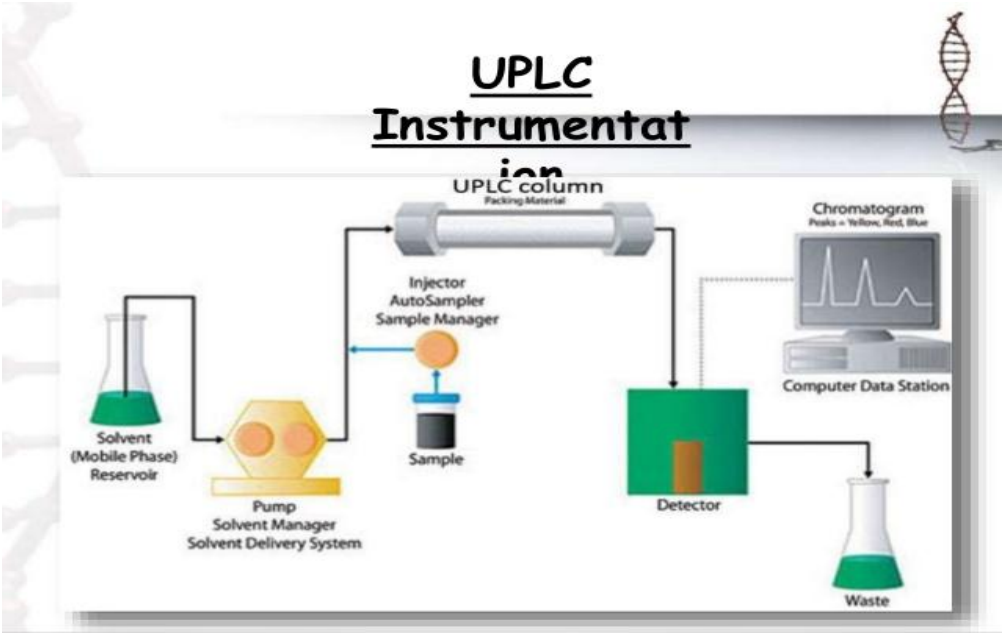
- Decreases run time and increases sensitivity
- provides the selectivity, sensitivity, and dynamic range of LC analysis
- maintaining resolution performance.
- Expands scope of Multiresidue Methods
- UPLC's fast resolving power quickly quantifies related and unrelated compounds
- Faster analysis through the use of a novel separation material of very fine particle size
- Operation cost is reduced
- Less solvent consumption

 Reduces process cycle times, so that more product can be produced with existing resources
 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 (27,28)
 Delivers real-time analysis in step with manufacturing processes
 Assures end-product quality, including final release testing

Disadvantages
The dis advantages of UPLC are
 Due to increased pressure requires more maintenance and reduces the life of the columns of this type.
 So far performance similar or even higher has been demonstrated by using stationary phases of size around 2 µm without the adverse effects of high pressure.
 In addition, the phases of less than 2 µm are generally non-regenerable.

Draw Backs
 Cost mixing
 Solvent pumping
 Lack of variety in commericial columns at 1.7 µm

Instrumentation



The Acquity UPLC system consists of
 Binary solvent manager
 Sample manager including the column heater
 Optional Sample manager
 Pumps
 Detector

Binary Solvent Manager
The binary solvent manager uses two individual serial flow pumps to deliver a parallel binary gradient. The binary solvent manager is a high pressure pump that moves solvent through the system. It provides steady (pulse free) solvent flow at analytical flow rates. The binary solvent manager delivers solvent at flow rates of 1 ml/min at 103421 Kpa [1034 bar, 1500 psi] and up to 2 ml/min at reduced pressures to 62053 Kpa [621 bar, 9000 psF] . The solvent manager can pump two solvents immediately.

Sample Manager
The Acuity sample manager injects the sample it draws from Micro titer plates or vials in to the chromatographic flow stream. A locating mechanism uses a probe to access sample locations and draw sample from them. The Sample manager can perform an injection in approximately 15 seconds. The sample manager also controls the column heater. Column temperatures up to 65°C can be attained.

Column Heater
The column heater is of a modular design and its foot print is identical to that of the sample manager. Thus it attaches to the top of the sample manager and serves as that instrument's top cover.

Optional Sample Organizer
The optional sample organizer stores micro miter or vial plates and transfers them to and from the sample manages, automating their processing and increasing through put.

Pumps
The UPLC pump is considered to be one of the most important components in a liquid chromatography system which has to provide a continuous constant flow

of the eluent through the UPLC injector, column, and detector.
The two basic classifications are
 Constant pressure pump
 Constant flow pump

Constant pressure pump - The constant pressure is used only for column packing.
Constant flow pump - This type is mostly used in all common UPLC applications

Standard UPLC pump requirements
 Sample injection volume is as less as 3 – 5 micro liters
 Pump operates at 10000 psi pressure
 Particle size in stationary phase packing material is less than 2 micro meter

Types of Pumps
Reciprocating Piston Pumps
The basic principle of the reciprocating single piston pumps is that it expels liquid through a one-way valve (check valve). The pumping rate is usually adjusted by controlling the distance the piston retracts, thus limiting the amount of liquid pushed out by each stroke, or by the cam rotating speed. Schematic of the reciprocating single piston pump. CAM is pushing a sapphire piston back and force. When the piston is moving backwards it sucks the eluent through the inlet check valve (on the bottom). The sapphire ball is lifted and opens the path for the eluent. When the piston moves forward, the liquid pushes the inlet ball down and closes the path, but the outlet ball is lifted and opens the outlet valve (upper).

Disadvantage
The main disadvantage of this type of pump is sinusoidal pressure pulsations which lead to the necessity of using pulse dampers.

Dual Piston Pumps
A more efficient way to provide a constant and almost pulse free flow is the use of dual-headed reciprocating pumps. Both pump chambers are driven by the same motor through a common eccentric cam; this common drive allows one piston to pump while the other is refilling. As a result, the two flow-profiles overlap each other significantly reducing the pulsation downstream of the pump; this is visualized below. Since the acceleration/deceleration profile is somewhat non-linear, the more efficient types of these pumps use eccentricity-shaped cams to obtain the best overlapping of the pressure curves and to obtain smooth flow.

Comparison between UPLC and HPLC

Characteristics	HPLC	UPLC
Particle size	3 to 5µm	Less than 2µm
Maximum backpressure	35-40 MPa	103.5 MPa
Analytical column	Alltima C_{18}	Acquity 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	2µL

Dual-head reciprocating pumps
The advantages of this pump are the unlimited solvent reservoir allowing long-term unattended use and quick changeover and clean out capability. However, unless special care has been exercised in manufacture, these pumps may have several disadvantages. There is a tendency for the incompletely compensated pulsations to be observable at high refractive index detector sensitivities, especially at low flow rates where piston cycles are widely spread. Furthermore, since each head has two check valves, pump reliability depends on the cleanliness of the mobile phase and continued sealing capability of four check valves on each cycle, with cycles normally occurring several times per minute.

Check valves on the reciprocating pump are the weakest part. It may be easily contaminated or clogged which leads to the pump misfunction. Most of the recent HPLC instruments use improved dual piston pumps which have three or even two check valves.

The schematic of this pump is shown above. The first piston, called low pressure, is sucks the liquid from the reservoir while the second (high pressure piston) is supplying the eluent to the system. Then the first piston refills the second piston very fast, during 1/100 of the whole pump cycle. This scheme allows the use of only 3 check valve, one of which is working under low pressure. There are no cavitation effects. Because the piston volumes are small (~100 l), pressure pulastions are small and sharp and easy to damp Another type of dual piston pump uses only two check valves, but piston volumes are different. While the smaller piston dispenses an eluent in the HPLC system, the bigger piston is sucking an eluent. When pistons change their direction, the bigger piston simultaneously refills the smaller chamber and dispenses eluent into the system. This set-up allows only two check valves for the dual piston pump.

Detectors
Types of Detectors
Detectors can be classified as:
- Optical detectors
- Tunable ultra violet detectors
- Evaporative light scattering detectors
- Fluorescence detector

Optical Detectors: Optical Detectors are used in ultra performance liquid chromatography analytical techniques, featuring low dispersion characteristics, simple operation, and high data acquisition rates as well as cost effective maintenance, support and parts.

The system can be configured with a TUV, PDA or ELS optical detector or any combination of the three.

Tunable Ultra Violet Detector
For UPLC detection, the TUV [tunable ultra violet] detector is used which includes new electronics and firm ware to support. The TUV optical detector is a two channel ultra violet / visible absorbance detector designed for the use of in the acquity UPLC system.

The detector offers 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 utilize the waters patented light guiding flow all technology.
The TUV detector operates at wave length 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 maintained concentration
- High sensitivity for low-level detection for simultaneous quantitation of major and minor components

Injectors of UPLC
The injector is comprised of six miniature air actuated needle valves, plumbed to simulate the flow path of a conventional rotor/stator injector. An integral controller sends the on/off positioning signals to each valve, coordinating them to perform load, inject, and flush functions.
There are three methods for sending positioning commands to the injector:

- Manual control with the pushbuttons on the controller
- Laboratory computer via serial port communication
- Contact closure inputs

Selecting the Right column
A 1.7µm particle packed column provides significant improvements in resolution because efficiency is better. Separation of the components of a sample, however, still requires a bonded phase that provides both retention and selectivity. Four bonded phases are available for UPLC separations.

Differences between UPLC and HPLC
HPLC
- Broader peak width provides less resolution
- Less sample throughput comparatively.
- Sample injection volume is 20 micro liters.
- Pump operates at 2000-6000 psi pressure
- Particle size in stationary phase packing material is between 5-12 micrometers.

UPLC
- Smaller peak width provides better resolution and more number of peaks getting identified.
- Higher sample throughput with more information per sample.
- Sample injection volume is as less as 3-5 micro liters.
- Pump operates at 10,000 psi pressure
- Particle size in stationary phase packing material is less than 2 micromete

APPLICATIONS OF HYPANATED TECHNIQUES
Drug Discovery
- UPLC improves the drug discovery process by means of high throughput screening, combinational chemistry, high throughput in vitro screening to determine physiochemical and drug's pharmacokinetics.

High throughput quantitative analysis
- UPLC coupled with time of flight mass spectroscopy give the metabolic stability assay.

Analysis of Dosage form
- It provides high speed, accuracy and reproducible results for isocratic and gradient analysis of drugs and their related substance. Thus method development time decrease.

Analysis of amino acids
- UPLC used from accurate, reliable and reproducible analysis of amino acids in the areas of protein characterizations, cell culture monitoring and the nutritional analysis of foods

Determination of Pesticides
- UPLC couples with triple Quadra-pole tandem mass spectroscopy will help in identification of trace level of pesticides from water.
- Thus Ultra Pressure Liquid Chromatography set a new standard in the science of chromatography. Working range with 15000 to 16000 psi pressure and column packed with less than 2 micrometer in size helped in various fields.

Analysis of Natural Products and Traditional Herbal Medicine
 UPLC is widely used for analysis of natural products and herbal medicines.. The main purpose of this is to analyze drug samples arise from the complexity of the matrix and variability from sample to sample.. UPLC provides high-quality separations and detection capabilities to identify active compounds in highly complex samples that results from natural products and traditional herbal medicines.

Identification of Metabolite
 UPLC/MS/MS addresses the complex analytical requirements of biomarker discovery by offering

unmatched sensitivity, resolution, dynamic range, and mass accuracy.

ADME (Absorption, Distribution, Metabolism, Excretion) Screening

 The high resolution of UPLC enables accurate detection and integration of peaks in complex matrices and extra sensitivity allows peak detection for samples generated by lower concentration incubations and sample pooling.. UPLC/MS/MS provides following advantages:- UPLC can more than double throughput with no loss in method robustness. UPLC is also simpler and more robust than the staggered separations sometimes applied with HPLC methods.

 UPLC operating with rapid, generic gradients has been shown to increase analytical throughput and sensitivity in high throughput pharmacokinetics or bioanalysis studies, including the rapid measurement of potential p450 inhibition, induction, and drug-drug interactions.

Bioanalysis / Bioequivalence Studies

UPLC delivers excellent chromatographic resolution and sensitivity. The sensitivity and selectivity of UPLC at low detection levels generates accurate and reliable data that can be used for a variety of different purposes, including statistical pharmacokinetics analysis. UPLC solutions are proven to increase efficiency, productivity and profitability for bio equivalence laboratories.

Dissolution Testing

For quality control and release in drug manufacturing, dissolution testing is essential in the formulation, development and production process. UPLC provides precise and reliable automated online sample acquisition. It automates dissolution testing, from pill drop to test start, through data acquisition and analysis of sample aliquots, to the management of test result publication and distribution.

Method Development / Validation

According to FDA, validation is defined as establishing documented evidence that provides a high degree of assurance that a specific process will consistently produce a product meeting its predetermined specifications and quality attributes UPLC help in critical laboratory function by increasing efficiency, reducing costs, and improving opportunities for business success. UPLC provide efficiencies in method development. Using UPLC, analysis times become as short as one minute, methods can be optimized in just one or two hours, significantly reducing the time required to develop and validate.

CHROMATOGRAPHIC SOFTWARE'S

Name	Publisher	Control	Analysis	Types
Malcon	Schlumberger	No	Yes	GC/GC-MS
Chemstation	Agilent	Yes	Yes	LC/GC,LC/MS,GC-MS
Ezchromelite	Agilent	Yes	Yes	LC/GC
Open lab cds	Agilent	Yes	Yes	LC/GC/LC-MS/GC-MS
Chromeleon	Dionex	Yes	Yes	GC/LC
Empower	Waters	Yes	Yes	GC/LC
Openchrom	Philip wening		Yes	GC/LC
Atlas cds	Thermoscientific	Yes	Yes	GC/LC
Clarity	Data apex	Yes	Yes	GC/LC/MS
Mass frontier	Highchem		Yes	GC/LC/MS

UPLC SYSTEMS AND ITS IMPLEMENTATION CURRENT SCENARIO

The introduction of new LC systems or UPLC systems having reduced system volumes (i.e. low dwell and dispersion volumes), along with reduction of particle size of columns from 10 µm/5 µm to 3.5 µm and then to =1.8µm, led to improvement of the chromatographic performance w.r.t. sensitivity, speed and resolution.

To effectively leverage the power of smaller particles in narrow column formats while accelerating flow rate requires systems that operate at higher pressures and that are designed for both lower volumes and lower dispersion. The first design considerations involved the quality of tubing that could withstand UPLC operating conditions, so as not to dilute the magnitude gain in resolution brought about by UPLC column, hence extra-column volumes were minimized.

The UPLC pump delivers higher pressures, but retention time precision remains extremely important. The Waters UPLC system uses an automatic and continuous compressibility compensation algorithm that does not require user intervention to cope with these demands, resulting in retention time reproducibility. Also, electronically controlled pump-check valves ensure that the pump check valves close quickly, thus improving performance and priming reliability.

Since the introduction of binary pump UPLC about a decade ago and with the industrial success of UPLC-technology, we have now in market Quaternary pump UPLC's & the UPLCs with further low dispersion

volumes. The latest UPLCs can be used to run ballistic gradients that are gradient programmes even below 1 minute and chromatography columns of dimensions 1 mm can be used thus leading to faster developments and analysis. This throughput advancement has lead to increased implementation of UPLC in both Diagnostics and Research Labs also.

The implementation of UPLC has increased significantly in both Active Pharmaceutical Ingredient (API) and Formulation industries, in both their Research and Development centers (R&D's) and Quality Control (Q.C.) Labs. The performance and savings (return on investment) associated while performing the typical tests such as reaction monitoring, assay, dissolution and related substances during the release and In-house testing by UPLC is significantly high. This saving in time in R&D centers further leads to faster product development that means products reach "early to market" and in Quality Control saving time during analysis leads to decrease in quarantine time of intermediates, faster release results for In-process testing and hence faster release of finished batches.

The QbD approach as emphasized by US-FDA during analytical method development is an important reason for UPLC success in Pharmaceutical R&D's, because of its capability to screen-n-scout the analytical methods much faster than the conventional HPLC. There is also a greater emphasis on future-proofing based procurement approach, where-in Quality control Labs and R&D centers have started budgeting and procuring latest UPLC's which has capability to run conventional HPLC and UPLC analytical methods.

The adoption of UPLC methods for various tests by different regulators like US-FDA, EMEA as release methods or alternative methods and adoption of UPLC by EP, USP in their monographs and medicinal compendia clearly reflects the reason for the increase in trend w.r.t implementation of UPLC technology in pharmaceutical industry.

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