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A REVIEW ON THE CURRENT TRENDS IN THE RECENT DEVELOPMENT OF HPLC/UHPLC/UPLC METHODS FOR THE EVALUATION OF VARIENT IMPURITIES IN VARIOUS PHARMACEUTICAL DRUGS

Abdul Saleem Mohammad[1]*, Dr. B. Jayanthi[2], Dr. Fazil Ahmad[3]

1Assistant Professor, Department of Pharmaceutical Analysis and Quality Assurance, Nizam Institute of Pharmacy, Deshmukhi (V), Pochampally (M), Behind Mount Opera, Yadadri Bhuvanagiri (Dist)-508284, Telangana, India.

2 Assistant Professor, Department of Pharmaceutics (Drug Delivery System), Annamalai University, Annamalainagar – 608002, Tamil Nadu, India.

3Associate Professor, Department of Pharmacology, Nizam Institute of Pharmacy, Deshmukhi (V), Pochampally (M), Behind Mount Opera, Yadadri Bhuvanagiri (Dist)-508284, Telangana, India.

Abstract:

New chromatography technology for the analytical laboratory is being driven by the ever expanding need and challenge to get more and better information faster, all in an economic climate where cost control is a primary concern. At the same time, samples have become more and more complex, detection limits are being driven increasingly lower, and regulatory concerns, particularly for biotherapeutics, are being increasingly scrutinized. To answer today's challenges in the laboratory, chromatographers are taking advantage of available new technology, often in rather unique ways. To tackle a particular issue they sometimes simply apply a new approach to a problem, like a new type of column or detector, or other times use a combination of new technologies, where the end result is greater than the sum of the individual parts. From new columns and detectors to multidimensional and orthogonal applications of technology, chromatographers have an ever expanding repertoire of tools available.

Keywords: *Pharmaceutical drugs, chromatography, analytical laboratory, HPLC, UPLC, UHPLC.*

Corresponding Author:

Abdul Saleem Mohammad,

Department of Pharmaceutical Analysis and Quality Assurance, Nizam Institute of Pharmacy, Deshmukhi (V), Pochampally (M), Behind Mount Opera,Yadadri Bhuvanagiri (Dist)-508284, Telangana, India.

Phone number: - 9700880519

E-mail address: - mohdsaleempharma@gmail.com

QR code

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INTRODUCTION:

Developments of Liquid Chromatography

In this *Recent Developments in HPLC/UHPLC* supplement to *LCGC*, several articles dealing with a broad range of technology and approaches were discussed. The first three articles deal with bioanalytical assays from small molecules to biotherapeutics in a regulatory environment. The first is an article by Pekol and colleagues that discusses the high sensitivity liquid chromatography–tandem mass spectrometry (LC–MS-MS) analysis of a novel ibuprofen dosage form from plasma and synovial fluid samples in preclinical (miniature swine) studies. The next article by Aboul-Enein and colleagues utilizes fast LC, highlighting their work for the identification and quantitative analysis of some third-generation cephalosporin antibiotics in human plasma. Completing the bioanalytical trifecta is the article by Chambers and colleagues that presents a systematic approach to bioanalytical method development for therapeutic peptides, using ultrahigh-pressure liquid chromatography (UHPLC)–MS-MS. They use a high-sensitivity, high-throughput approach and describe the sample preparation, separation, and detection of a set of 12 diverse peptide therapeutics (1-2).

Fast LC is one way to get more productivity out of existing high performance liquid chromatography (HPLC) technology and prepare for the next generation of UHPLC systems with more efficient separation schemes. Continuing on the high-throughput, fast-LC theme, the article by Joe Helble incorporates the use of faster mobile phase flow rates and smaller particles to achieve separations in less time and with equivalent resolution to traditional HPLC. The article by Xue and colleagues proposes a solution to the challenge of tracking peaks during method development for the assessment of impurity profiles of active pharmaceutical ingredients. Using multiple orthogonal HPLC–MS-MS methods, automated peak tracking, and the application of chemometric component data analysis, their approach takes only minutes to interpret all the MS spectral data of interest and reduces the time required to obtain a comprehensive impurity profile from weeks to hours (2-4).

Process related impurities

An extensive survey of the literature published in various analytical and pharmaceutical chemistry related journals has been conducted and the high-performance liquid chromatography (HPLC) methods which were developed and used for determination of process-related impurities in drugs have been reviewed. This review covers the time period from 1995 to 2016 during which around 450 analytical methods including all types of chromatographic and hyphenated techniques were reported. HPLC with UV detection was found to be the technique of choice for many workers and more than 200 methods were developed using LC-UV alone. A critical analysis of the reported data has been carried out and the present state-of-art of HPLC for determination of impurities of analgesic, antibiotic, anti-viral, anti-hypertensive, anti-depressant, gastro-intestinal and anti-neoplastic agents has been discussed.

CURRENT USAGE RATE OF INSTRUMENTS

To understand the current usage rate of instruments, a question was asked pertaining to number and type of instruments personally used per respondent per week. Questions in previous surveys were less specific and, therefore, laboratory managers occasionally responded with estimates for their entire laboratory, which tended to skew the results (4-7).

According to the survey results, a user of conventional HPLC instruments is responsible for a weighted average of 2.6 units. Because each respondent may have been responsible for a single instrument or for multiple instruments, this weighted average was derived from looking at the total number of instruments of this type identified by all respondents and then dividing this number of the total number of respondents. For example, some respondents reported that they have only one conventional HPLC system while others reported that they have five instruments for which they are personally responsible (7-9).

EMERGING TRENDS IN HPLC ANALYSIS

Ever since its evolution High Performance Liquid Chromatography has stood on a rock hard foundation and has seen several innovations which have met the growing expectations of chromatographers.

Some of the desirable features and expectations of scientists are:

1) High separation efficiencies with lowest column back pressures
2) Reduced solvent consumption and high speed analysis
3) Low levels of detection and quantification
4) Separations over wide temperature ranges
5) Availability of almost complete pH range for analysis
6) Greater mechanical robustness of packed columns
7) Longer useful lifespan of columns
8) Large sample load capacities of columns

It has been possible to achieve the desirable features through several innovations which have made a remarkable contribution to the popularity of the HPLC technique in laboratories across the globe (10-13).

Advances in Stationary Phases

Stationary phase characteristics are the main deciding factors contributing to efficient separation of mixture component by High Performance Liquid Chromatography. Silica-based columns are to this day predominantly used in majority of separations. However, recently developed packaging materials have have opened up new fields of applications. The advantages offered by core shell packings, monolith packings and zirconia packings have already been discussed in earlier articles (13-15).

Ultra Pressure Liquid Chromatography (UPLC)

Column efficiency increases with reduction in particle size. Sub- 2µm particle sizes were introduced in 2003 and paved the way for fast separations. It became possible to reduce column lengths and diameters to give faster separations with increased sensitivities. Small size particles pose problems of increased column back pressures which could be overcome by advances in instrumentation to permit operation under high pressures. Nano- bore and Micro- bore High Performance Liquid Chromatography. Analysis of limited amounts of samples has always posed a challenge. Nano bore High Performance Liquid Chromatography offers solutions to allow femtomole levels with good quality resolution for reliable identification and quantification.Nano columns have id's as small as 75 µm and flow rates up to 300 nL/ minute are used in nano bore applications. Micro bore HPLC columns have id's around 1 mm and flow rates of 50- 75 µL per minute. Both nano bore and micro bore columns have been used to their full potential with mass spectrometry detection systems particularly in analysis of peptides in biological matrices (15-17).

Hyphenated LC techniques

Several hyphenated techniques such as LC-MS, LC-FTIR, LC-AAS have been developed but LC-MS has been vastly applied to a range of separations and quantification of complex molecules particularly in bioanalytical separations (16-19).

Fast Protein liquid chromatography

This mode has main application in purification of proteins. It has potential for milligrams scale to kg scale separations. Separation and isolation is based on charge separation between resin and a protein. Elution is based on variation of buffer composition (19-21).

Affinity chromatography

Affinity chromatography has potential in separation of biochemical molecules such as a nucleic acids and protein purification in biological fluids such as blood. It is based on interactions between antigen-antibody, enzyme and substrate or receptor and ligand (19-21).

Chiral chromatography

Developments in stereoactive column packings mainly polysaccharide derivatives like cellulose, amylase and cyclodextrin have been used for a wide range of optically active molecules. In earlier days separation of such molecules was not possible using conventional separation modes. The article is briefly introduction to the recent innovative trends in High Performance Liquid Chromatography technique. Please share your comments and leave suggestions on this article. High performance liquid chromatography (HPLC) is the premier analytical technique used in many pharmaceutical applications including potency/purity/performance assays, pharmacokinetics/ bioanalytical testing, purification, high-throughput screening (HTS), In Process Control (IPC) Monitoring and Quality Control (QC) testing (1-6). The pharmaceutical industry is the major consumer segment of HPLC (7) and has been the primary driving force for higher throughput and performance. This article provides a brief review of significant developments in HPLC impacting pharmaceutical analysis in the last decade. Instrumentation: Ultra-high pressure LC (UHPLC) going mainstream (21-23).

1) Columns: Sub-2 µm, sub-3µm core-shell and hybrid particles; novel bonding chemistries; hydrophilic interaction chromatography (HILIC); immobilized polysaccharide chiral phases; columns for biomolecules and biopharmaceuticals.
2) Others: Liquid chromatography – Mass Spectrometry (LC/ MS) - particularly High-Resolution MS (HRMS) or Hybrid MS; Charged Aerosol Detector (CAD); Automated Method Development Systems (AMDS) (24-25).

ULTRA-HIGH PRESSURE LIQUID CHROMATOGRAPHY (UHPLC)

The "revolution" in ultra-high pressure LC (UHPLC) began in 1997 with the proof-of-concept study by Professor James Jorgenson (8), followed by the first commercial system introduced in 2004 (1, 9-11).

Today, the transformation from HPLC to UHPLC is mostly complete with all major manufacturers having some type of UHPLC offerings. Detailed reviews of UHPLC systems, columns and applications are available elsewhere (4, 10-11). Fundamentals, benefits, potential issues and best practices of UHPLC in pharmaceutical analysis are well documented (4, 11-14).

Another important benefit of UHPLC is its superior separation of complex samples. This aspect is often overlooked and appears to be "under-reported" in the literature (17-18). Peak capacities (PC) in the range of 400 to 1000 in a reasonable time span (~1h) have been demonstrated using UHPLC. Peak capacity is the number of peaks that can be resolved in the chromatogram with a resolution of 1.0; typically ~200 for conventional HPLC (1). For the first time ever, UHPLC can offer more effective assays, in a single dimension, for complex pharmaceuticals, natural materials, and other difficult sample matrices (4, 11, 17-18).

Other benefits of UHPLC include substantial solvent savings (5-15 fold), increased mass sensitivity (3 – 10 fold) and precision performance for both retention times (2 - 3 fold) and peak areas (<0.1% RSD) (11, 14, 18). Note that reports on UV detection sensitivity increase with UHPLC are often misleading because mass sensitivity (amount of analytes injected) is primarily related to column void volumes. UHPLC typically does not increase concentration sensitivity (the most desirable kind of sensitivity) because one does not expect that the use of small flow cells can actually increase signal/noise ratio unless extended pathlength flow cells (e.g., 60 mm) are used. Potential issues such as viscous heating, baseline perturbation from pump blending, and method transfer have been described and can be instrument-specific (4, 11, 18). In general, these technical issues are well understood and can be readily mitigated by judicious choice of system configuration (e.g., mixer volume). Nevertheless, the implementation of UHPLC in QC labs remains to be time-consuming due to training, compatibility to "validated" data systems, and other method transfer issues (4, 18).

One often hears arguments that UHPLC is perhaps not needed because other approaches (high-temperature LC, core-shell columns or 2-D LC) can be more cost-effective for enhancing speed or resolution. The reasoning is not truly valid since UHPLC can be used in combination with one or more of these approaches with superior results than those from conventional HPLC, as they are options rather than alternatives. Also, the term "UHPLC" may eventually go away in a few years since all newer HPLC will be UHPLC (25-26).

HPLC COLUMN AND STATIONARY PHASE DEVELOPMENTS

HPLC column is the heart of the chromatographic system. The pharmaceutical industry has been the primary driver for HPLC columns towards higher speed, resolution, and better peak shapes for basic analytes. In addition, QC laboratories have demanded improved column batch-to-batch reproducibility. From the 1970s to 1990s, there were steady quality improvements of the packing materials accompanied by a gradual reduction of "standard" particle sizes from 10 to 3 µm (1-3). The introduction of high-purity type B silica materials (with low metallic content) in the late 1980s was a huge step and resulted in reduced silanol activity and substantial improvements in lot-to-lot consistency (21). The use of high-purity silica is now the norm for all modern silica-based columns.

Sub-2 µm Particles

The use of very small particles for fast and efficient separations was predicted in 1956 by Van Deemter (23). Typical particle size of packings has been decreasing in the past five decades cumulating to the use of sub-2 µm microparticulate silica in the early 2000s. As predicted, these particles (e.g., 1.7 µm) yield excellent efficiency performance (~280,000 plates/m or plate height of ~4 µm). However, columns packed with sub-2 µm particles generate high back pressures and are typically packed in a 2.1-mm ID format to reduce efficiency loss from viscous heating effects (4, 9, 14). The system requirements for high pressures and low dispersion (to reduce extracolumn band broadening) were responsible for the current characteristics of modern UHPLC systems. Further reduction of particle size to less than 1.5 µm may be advantageous for even higher speed and performance. However, it must be accompanied by a substantial increase of system pressures and a reduction of column ID to capillary formats.

Core-shell Particles

The concept of fused-core or core-shell particles for reducing resistance to mass transfer was first described by Kirkland (24). The first core-shell particles had these characteristics: 2.7 µm superficially porous silica materials with nonporous cores (1.7 µm) and porous shells (0.5 µm thick) (24-25). These sub-3 µm particles appear to have similar efficiencies as the sub-2 µm fully porous materials but generate much lower pressure drops. The

exceptional performance may be due to their shorter diffusion paths of the shells and/or the narrower distribution of the packings. Core-shell columns are rapidly gaining wide acceptance for fast separations (HTS, IPC and cleaning verification) and for biomolecules (25-26). Myriad bonded phases and particle sizes (1.3, 1.7, 2.6, 2.7 and 5 µm) are currently available from an increasing number of manufacturers (6+). We fully expect these columns to be highly competitive in all applications to those from porous microparticulates (25).

Hybrids

The concepts of hybrid particles with organic groups incorporated into the inorganic silica matrices were first described by Unger (21) in late 1970s, though the first commercial columns with methyl groups only became available in 1999 (22). Bonded phases from these hybrids were demonstrated to have superior pH stability (from 1 to 12 with novel bonding chemistries vs. 2 to 8 for conventional silica with traditional monofunctional bonding), and lower silanophilic activity. A second-generation bridged ethylene hybrids (BEH) was introduced in 2005 and enjoyed immediate "mainstream" success, particularly for applications with high-pH mobile phase applications and UHPLC (9-10, 23).

Novel Bonding Chemistries

While the traditional monofunctional C18 silica-based bonded phase remains to be the main "staple" with perhaps the best batch-to-batch reproducibility (1, 3), newer bonding chemistries have brought benefits such as a wider pH stability range (from polyfunctional silane chemistry or silanes with isopropyl protective groups) and enhanced selectivity for difficult separations (polarembedded, phenyl, hexylphenyl, cyano, pentylfluorophenyl bonding) (1,3). One recent innovation, termed charged surface hybrids (CSH) technology introduced in 2010, appears to have gained immediate acceptance for pharmaceutical analysis due to improved peak shapes of highly basic analytes under acidic, low ionic-strength mobile phases conditions (e.g., 0.1% formic acid) (11). The proprietary technology involves the introduction of a low level of positive charges to the surface of the stationary phases. This is somewhat analogous to the addition of an amine additive such as triethylamine to the mobile phases in earlier days which is no longer acceptable in LC/MS due to ion suppression (11-13)

Hydrophilic Interaction Chromatography (HILIC)

The retention of many highly polar compounds is simply unachievable or problematic due to phase collapse (bonded phase "dewetting") under reversed-phase LC (RPLC) conditions in mobile phases with low organic contents (1-3). The HILIC mode, first developed by Alpert in 1990s (12), uses a hydrophilic stationary phase (silica, diol, cyano, amide, ztwitterionic) with an RPLC-like aqueous buffer and acetonitrile mobile phases, has enjoyed increasing popularity for the analysis of polar drugs, secondary drug metabolites, amino acids, peptides, neurotransmitters, oligosaccharides, carbohydrates, nucleotides, or nucleosides (13). The actual retention mechanism in HILIC can be the "partitioning" of analyte molecules to the water layer adhering to the hydrophilic bonded groups. Other prominent benefits of HILIC include "orthogonal" selectivity to RPLC where sample preparations are compatible to both modes, higher electrospray ionization sensitivity for MS (5-15 fold), and lower operating pressures vs. RPLC (13).

Immobilized Polysaccharide Chiral Stationary Phases

Improved versions of the highly successful coated polysaccharides chiral stationary phases (CSPs) were available in the late 2000s. They brought similar versatility as the earlier coated CSPs but are more robust to aggressive solvents and can be used in normal phase, polarorganic and reversed-phase modes (5, 24).

Columns for Biomolecules

Wide-pore silica and polymeric packings first available in 1980s were effective for separations of large biomolecules (1, 15). With the advent of recombinant proteins as biopharmaceuticals such as monoclonal antibodies (mAb), the need for detailed characterization by HPLC or capillary electrophoresis for QC purposes has become more pressing (16). Recent developments of sub-2 µm microparticulates and coreshell wide-pore particles as well as several innovative ion-exchange and size exclusion phases have proved useful for separations of these large biologics (17).

High Resolution Mass Spectrometry (HRMS)

The combination of HPLC with mass spectrometry (LC/MS) has been touted as the perfect analytical tool, combining the separation power of HPLC and the unsurpassed sensitivity/selectivity of MS. LC/MS is the preferred technique for identification of impurities and degradants,HTS in drug discovery,

bioanalytical assays (LC/MS/MS for drugs and metabolites in biofl uids), and in-progress monitoring during process scale-up for the synthesis of drug substances (1-2, 5, 26). LC/MS is becoming the standard platform technology for cleaning verification of highly-potent drugs (26) and determination of potential genotoxic impurities (2). The last decade has seen the rapid evolution of HRMS (such as time-of-flight (TOF), OrbiTrap MS) and hybrid MS (such as Quadrupole-TOF or ion trap-OrbiTrap). The combination of HRMS with UHPLC and 2-D LC has enabled active research in metabonomics, proteomics, de nova protein sequencing, and characterization of biopharmaceuticals (5, 12). Perhaps the most exciting opportunity for LC/MS lies ahead as a generic platform technology for the determination of disease biomarkers and clinical diagnostics in the emergent fi eld of personalized medicine.

Charged Aerosol Detection (CAD)

The lack of an ideal universal detector is often cited as a limitation of HPLC, though the UV/Vis detector comes fairly close for chromophoric compounds. The refractive index detector is not gradient compatible and does not have sufficient sensitivity (1-3). Evaporative light scattering detection (ELSD) using nebulizer technology with laser light scattering detection is an option and is gradient compatible but has been recently surpassed by CAD (uses nebulizer with corona discharge detection), which offers better sensitivity (low ng) and improved linearity. CAD is becoming a mainstream detector for HTS in medicinal chemistry, reaction monitoring, and raw material/excipient testing (19).

Automated HPLC Method System (AMDS)

HPLC method development for complex mixtures is time-consuming due to the need to optimize many operating parameters (column dimension; type of bonded phase and mobile phase A and B (organic solvent/buffer type, pH, and ionic strength), gradient time and range, column temperature, and flow rate) (1, 5, 6). A common example is the stability-indicating or purity assay of active pharmaceutical ingredients (API) in which all impurities and degradants must be separated for accurate quantitation by UV detection. Software or automated systems based on simulation, prediction, simplex optimization, and column/mobile phase screening have been available to facilitate HPLC method development for many years. Continued improvements have enhanced their capability and ease-of-use though they never appeared to be very popular (2, 6). The latest market entry was an add-on software package compatible to two of the commonly used chromatography data systems. The software addresses the most time-consuming portion of the HPLC method development process (optimization) by automating method sequence from a user-defined design space using principles of Design of Experiments (DoE) and Quality by Design (QbD) (20-21).

SUMMARY AND CONCLUSIONS:

In summary, HPLC remains a highly dynamic field with numerous innovations in instruments, column technologies, and approaches in recent years. Pharmaceutical scientists are early adopters and beneficiaries of these newer technologies for research, development and quality control. UHPLC is becoming the standard HPLC platform with rapid adoption by research & development, albeit slower implementation in QC labs. Newer column technologies allow faster and more efficient analysis of complex samples, chiral molecules and biomolecules. Finally, the rapid advancements of UHPLC and 2-D LC in combination with high-resolution MS have revolutionized life science research and promise to have even greater impact for clinical diagnostics. These developments are welcomed progress for the analytical chemist working in this rapid evolving world of drug development.

As a perspective for LC in the next years, some innovative works can be mentioned. As described by Professor Jim Jorgenson: “Moving to still higher pressures (50,000 psi) will enable the use of smaller particles and/or longer columns, and yield faster and better separations. This will almost certainly require the use of sub-mm bore (capillary) columns due to issues with heat generation and dissipation. This won’t be easy, but the separation potential in terms of high speed with high resolution is enticing.” Alternatively, the use of extremely uniform packing of sub-micron silica particles in capillaries could be employed to generate plate heights well below 1 µm and impressive separations of protein variants. These two research findings are unlikely to be ready for mainstream analytical work or QC very soon but the first results are encouraging and thus worth mentioning here.

An extensive survey of the literature published in various analytical and pharmaceutical chemistry related journals has been conducted and the high-performance liquid chromatography (HPLC) methods which were developed and used for determination of

process-related impurities in drugs have been reviewed. This review covers the time period from 1995 to 2016 during which around 450 analytical methods including all types of chromatographic and hyphenated techniques were reported. HPLC with UV detection was found to be the technique of choice for many workers and more than 500 methods were developed using LC-UV alone. A critical analysis of the reported data has been carried out and the present state-of-art of HPLC for determination of impurities of analgesic, antibiotic, anti-viral, anti-hypertensive, anti-depressant, gastro-intestinal and anti-neoplastic agents has been discussed.

Superiority of modern instruments consists of internal diameter reduction, increases sensitivity and/or less sample requirement, its conventional counterpart and increased detection sensitivity in MS because of lower flow rates in smaller columns. Recent developments have significantly increased the resolution power for complex

Innovations and Future Trends in HPLC Column Technology

An average high performance liquid chromatography (HPLC) instrument uses 6-8 columns per year; as the number of instruments grows, so does the market for columns. The overall market for columns (analytical, preparative/process, capillary/nano, bulk packing, and accessories) is now estimated to be $1.3 billion with an overall growth of 3.5 % (1). Growth in the high performance liquid chromatography (UHPLC) segment is higher. Developments in column technology to deliver greater efficiency, speed and inertness benefit the drug development process from discovery to manufacturing and quality control. For new methods, superficially porous particle (SPP) columns have become the favoured column type in pharmaceutical laboratories thanks to the lower pressure, enhanced efficiency and equal load ability offered in comparison to smaller totally porous particles. The development of even smaller SPP particles can also be anticipated. If SPP columns continue to dominate, the need to further increase instrument pressure limits may not be necessary, but in chromatography, pressure is always a useful commodity.

If researchers are able to improve efficiency without great increases in backpressure and can make longer length columns for difficult separations, monolithic columns will still have great promise. In particular, polymeric monoliths could be quite attractive because their wider operating range gives them some advantages. Monoliths may become the favoured approach for laboratory-on-a-chip systems since they can be synthesized in situ inside the narrow channels where efficient packing of particulates may prove exceedingly difficult. With its orthogonal separation power, supercritical-fluid chromatography (SFC) has made a comeback in the rapid analysis of small pharmaceutical compounds. Initially, SFC made its contributions in the preparative arena for chiral drugs but now has been applied to more general small molecule applications. For many separations, SFC can be superior to HPLC/UHPLC, especially in the speed of analysis. The phases used for SFC are different than those used for liquid chromatography so additional polar phases are required to exploit this technology.

The trend in the use of monoclonal antibodies and peptide-based compounds in drug development requires columns capable of providing high recovery separations of biologically derived compounds, oligonucleotides and biosimilars, both neat and in biological fluids. Column manufacturers are already responding with biocompatible columns that provide more selective separations with higher recovery.

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