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A REVIEW ON: ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY (UPLC)

Ankita A. Muli[*1], Pavan V. Salve[*2], Pratik P. Ughade[*3], Dhanshree K. Maske[*4], Bhakti V. Kalaskar[*5], Chandrakant R. Bedke[*6]

[*1,4,5,6]Department Of Quality Assurance, Shreeyash Institute Of Pharmaceutical Education And Research, CH. Sambhaji Nagar, Maharashtra, India.

[*2,3]Department Of Pharmaceutics, Shreeyash Institute Of Pharmaceutical Education And Research, CH. Sambhaji Nagar, Maharashtra, India.

ABSTRACT

UPLC is a modern technique which gives a new direction for liquid chromatography. UPLC refers to ultra-performance liquid chromatography which enhances mainly in three areas: "speed, resolution and sensitivity. Ultra performance liquid chromatography (UPLC) applicable for particle less than2μm in diameter to acquire better resolution, speed, and sensitivity compare with high-performance liquid chromatography (HPLC). In twenty first centenary pharmaceutical industries are focusing for new ways to in economy and shorten time for development of drugs. UPLC analysis at the means time gives the betterquality of their products and analytical laboratories are not exception in this trend. The separation and quantification in UPLC is done under very high pressure (upto 100M Pa). As compare to HPLC, under high pressure it is observed that not Any negative influence on analytical column and also other components liketime and solvent consumption is less in UPLC.

Keywords: Ultra Performance Liquid Chromatography, High Separation, High Pressure, Drug Analysis Quality, Automated.

I. INTRODUCTION

Over the years, the high-performance liquid chromatography (HPLC) technique has gained immense popularity in most analytical laboratories. The liquid chromatography is believed to be the third most popular laboratory equipment, right after balance andpH meters.

Of course, as with any technique, it is constantly being improved. At the beginning of the 1970s, columns (filled with non-porous, irregularly shaped silicate gel of about 40 μm in size) with very low efficiency (the number of theoretical plates was about1000 per 1 m bed) were commercially available. Later, columns with a grain of 10 μm in diameter were produced, followed by silica gel columns with spherical, porous grains with a diameter of 5 μm. These increased the yield to about 12,000 theoretical plates with a column length of 150 mm. In the 1990s, columns with grains of 3 μm in diameter were created. Subsequently, it was found that further grain reduction was not justified due to the costs and problems associated with their use.

The breakthrough year was 2004 when a completely new model of the Waters UPLC liquid chromatograph equipped with columns with a grain diameter of 1.7 μm appeared on the market. It can now be concluded that UPLC (e.g., ultra-performance liquid chromatography) has proven to be a milestone in liquid chromatography This technique, serving to separate the components present in mixtures, has found application particular to the analysis of thermally labile or low-volatile compounds.

In recent years, it has gained particular popularity due to the possibility of faster separation of small molecules Chromatographic columns with particles <2 μmare used here, applied in equipment capable of working under high pressure. The flow rates are lower than in classical HPLC, but due to the increase in yield, the total separation time is shortened. This allows the particles to be separated quickly with high efficiency. UPLC is therefore an effective chromatography technique that offers a wide flow range and significantly reduces analysis time.

The basic principle upon which UPLC is based is that as the size of the fill particles decreases, so does the efficiency and hence the resolution. After particle size reduction toless than 2 μm, the efficiency shows a significant increase and does not decrease at increased line velocities or flow rates, in accordance with the van Deemeter equation. It is known that the smaller the grain diameter of the column packing, the lower the height of the theoretical plate, i.e., the higher the column efficiency, will be. The minimum of the van Deemter curve corresponds to the ideal flow velocity at which the highest column efficiency is obtained.

In addition, to improve the efficiency, an increased temperature range should be used (this increases the flow rate of the mobile phase by reducing its viscosity, i.e., significantly lowering the back pressure) and monolithic columns (consisting of a solid piece with flow paths connected by skeletons, so-called passage pores).

The main advantages of the UPLC technique include the reduction of analysis time and increased sensitivity and resolution. These changes became possible thanks to the new design of chromatograph elements, including columns, pumps, dozers, and detectors with a reduced volume of measuring cells. The use of short columns and their low packing (1.7 μm) significantly shortened the analysis time. Small column packing forced the use of high pressures (about 1200 bar), and heating the column lowers the viscosity of the solutions, thus increasing the sample flow rate through the system. These changes make it possible to obtain very fast measurement cycle times a reduction of the dead volume and a shorter stabilization time of the system. The reduction in time reduced analysis costs through more efficient use of the equipment and reduced solvent consumption. At the same time, the increased efficiency of the system allows more information to be obtained than in HPLC.[1]

Comparison between UPLC and HPLC:

Sr.No	Parameter	HPLC	UPLC
1	Particle size	3-5 micro m	Less than 2 micro m
2	Mobile phase flow rate	More	Less
3	Injection volume	5 micro lit	2 micro lit
4	Column	ALLTIMA C18 ZORBAX C8	ACQUITY UPLCBEH C18 and C8
5	Column dimension	150 x 3.2	150 x 2.1
6	Column temperature	30* C	65*C
7	Maximum back pressure	35 -40 MPa	103.5

The principles of UPLC are same principle as HPLC, the basic difference is in designer of the column material particle size which less than 2-μm. Which make a big deference in performance and to maximize the advantages of these columns, creating a powerful, robust and reliable solution? The familiar design of UPLC H-class's Quaternary Solvent Manager (QSM) and Sample Manager (SMFTN), with flow-through needle design, gives all the flexibility and usability of your current HPLC while still achieving the highly efficient separations that only UPLC can provide.[2]

To improve the UPLC efficiency following measures need to be performed:

1. By employing high temperature which reduce the viscosity of mobile phase and ultimately flow rate if high. Significantly back pressure is reduced.

2. The unique feature of UPLC analysis is interconnected skeletons and interconnected flow paths (through-pores) which are found in monolithic columns make UPLC technique different from HPLC. In UPLC chromatogram it is found that better resolution and separation are found as compared to HPLC along with perform more sensitive analysis, reduce consumption of solvent and has high speed of analysis.[3]

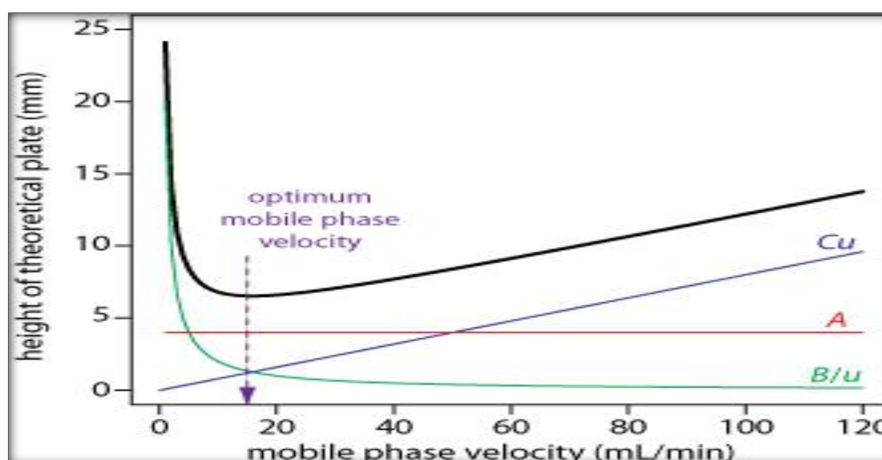
Contribution of small particles in UPLC:

The small particle size (i.e., less than 2 μm) of stationary phase is the basis of UPLC technique. The basal principle of this technique is governed by an empirical formula known as Van Deemter equation that describes the relationship between linear velocity (flow rate) and plate height (HETP or column efficiency). This equation shows that enhancement of efficiency of UPLC technique cannot be accomplished without using smaller particle size than those used in conventional HPLC technique.[4]

Van Deemter plot demonstrates that as the particle size decreases less than 2 μm there is a significant gain in the efficiency, and this condition is well maintained even if there is an increase in flow rate or linear velocities. This plot indicates that the usable flow range which shows good efficiency is much greater for smaller particles as compared to the larger particles.[2]

The Van Deemter plot is governed by the equation: $H = A + B/v + Cv$, where v is linear velocity and A, B and C are constants. A is independent of velocity and represents the eddy mixing and when column particles are uniformly small, the value of A is the lowest. B is axial diffusion or the natural diffusion tendency of molecules

and this effect is diminished at high flow rates, so this term is divided by v. C is due to kinetic resistance to equilibrium in the separationprocess. The design and development of required 1.7 µm particles was a challenging task, on the contrary, researchers have shown keen interest in the development of these particles in order to capitalize their advantages.[5] Despite, the availability of high efficiency non porous 1.5 µm particles in the market; they were not employed in UPLC columns due to their poor loading capacity and retention due to low surface area. UPLC required porous particles, which can withstand the high pressure in order to maintain their retention and capacity similar to that of HPLC Silica particles possess good mechanical strength but their application was limited by narrow pH application range and generally exhibit tailing during analysis of basic analytes. However, polymeric columns did not have any pH limitations but found to have low efficiency. In 2000, the first generation hybrid chemistry utilizes the classical silica-gel synthesis method to create durable columns that incorporated carbon in the form of methyl groups. These columns exhibit several advantages such as mechanical strength, high efficiency and are operative over an extended pH range. However, they do not possess enough mechanical stability necessitated by UPLC. Consequently, the second generation bridged ethane hybrid (BEH) technology was developed). This technology increases the mechanical stability of 1.7 µm particles by bridging the methyl groups in the silica matrix that lead to the production of the columns which can withstand high pressure and ph. These BEH columns are highly efficient as efficiency of a column is directly proportional to its length and is inversely proportional to the particle size.[6] The application of BEH column resulted in the detection of additional drug metabolites, superior separation and improved spectral quality.



Principle:

The underlying principle of UPLC is based on the van Deemter relationship which explains the correlation between flow rate and plate height. The van Deemter equation-

(i) Shows that the flow range with the smaller particles is much greater in comparison with larger particles for good results.

$$H.E.T.P = A+B/v +Cv$$

(I)Where H represents height equivalent to the theoretical plate (HETP), A, B & C are the constants and v is the flow rate (linear velocity) of the carrier gas. The aim is to minimize HETP to improve column efficiency. The term A does not depend on velocity and indicates eddy mixing. It is smaller if the columns are filled with small and uniform sized particles. The term B denotes the tendency of natural diffusion of the particles.[7] At high flow rates, this effect is smaller, so this term is divided by v. The term C represents the kinetic resistance to equilibrium during the process of separation. The kinetic resistance is the time lag involved in moving from the mobile phase to the stationary phase and back again. The higher the flow rate of the mobile phase, the more a molecule on the packing material inclines to lag behind molecules in the mobile phase. Thus, this term is inversely proportional to linear velocity. Consequently, it is likely to enhance the throughput, and without affecting the chromatographic performance, the separation can be speeded up.[21] The emergence of UPLC has necessitated the improvement of existing instrumentation facility for LC, which takes the benefit of the separation performance (by decreasing dead volumes) and consistent pressures (about 500 to 1000 bars, compared with 170 to 350 bars in HPLC). Efficiency is proportionate to the length of the column and inversely proportional to the radius of the particles. Consequently, the column length can be reduced by the similar factor

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as the particle radius without affecting the resolution. The use of UPLC has helped in the detection of drug metabolites and enhancement of the quality of separation spectra.[8]

Instrumentation:

A) Sample injection
B) UPLC columns
C) Solvent delivery system
D) Detector

A) 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. There are also direct injection approaches for biological samples. The injection must be done reproducibly and accurately.[20]

B) UPLC Column:

Resolution is increased in a 1.7µm particle packed column because efficiency is better. Separation of the components of a sample requires a bonded phase that provides both retention and selectivity. Four bonded phases are available for UPLC separations:

(i) ACQUITY UPLCTM BEH C8 (straight chain alkyl columns)

(ii) ACQUITY UPLCTM BEH C18 (straight chain alkyl columns)

(iii) ACQUITY UPLC BEH Shield RP18 (embedded polar group column)

(iv) ACQUITY UPLC BEH Phenyl (phenyl group tethered to the silyl functionality with a C6 alkyl),ACQUITY UPLC BEH Phenyl (phenyl group tethered to the silyl functionality with a C6 alkyl).[9]

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

1) Waters: Acquity UPLC columns and Vanguard Pre-columns have been produced.

2) Agilgent technology provides highest performing columns that provide fast and reproducible results. These include Poroshell 120 columns, ZORBAX Rapid Resolution High definition columns, ZORBAX Eclipse plus columns and ZORBAX Rapid Reduction High Throughput columns.

3) Altech Associate.

4) Phenomenex provides Kinetex® Core shell HPLC/UHPLC columns of high efficiency and performance.[10]

Different types of columns being used in UPLC are packed with particles which are produced through different technologies:

These are as follows:

A) Charged Surface Hybrid [CSH] particle technology

B) Ethylene Bridged Hybrid [BEH] particle technology

C) High Strength Silica [HSS] particle technology and

D) Peptide Separation Technology (PST)

C) Solvent Delivery System:

The solvent delivery system must perform reproducible high pressure pumping with a smooth and constant flow of solvents. UPLC systems routinely operate at 8000-15000 psi. The delivery system must also remunerate for a variety of solvents used in isocratic, linear & nonlinear gradient elution and solvent compressibility for a wide range of pressures. The Acquity UPLC binary solvent manager has two solvent delivery modules operating in parallel for high pressure merging of two solvents in <140 µL internal system volume. The dissolved gases are removed by vacuum up to four eluents plus two wash solvents.[12]

D) Detector:

The detectors are use in UPLC analysis is UV/Visible detector. Detection of analytes is conventionally based on absorbance that is concentration sensitivity detectors. In UPLC the flow cell volume would have to be reduced to maintain concentration and signal.[19]

Based on Beer's Law, smaller volume conventional flow cells would also reduce the path length upon which the signal strength depends. A reduction in cross-section means the light path is reduced, and transmission drops with increasing noise. Therefore, if a conventional HPLC flow cell were used, UPLC sensitivity would be compromised.[18]

The ACQUITY 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 problem.[13]

Mainly Used Detector are as follows :-

- UV detector
- Fluorescent
- Refractive index detector
- Light Scattering detector
- Mass spectrometric detector
- Electrochemical detector

Fig No.1 – instrumentation of UPLC

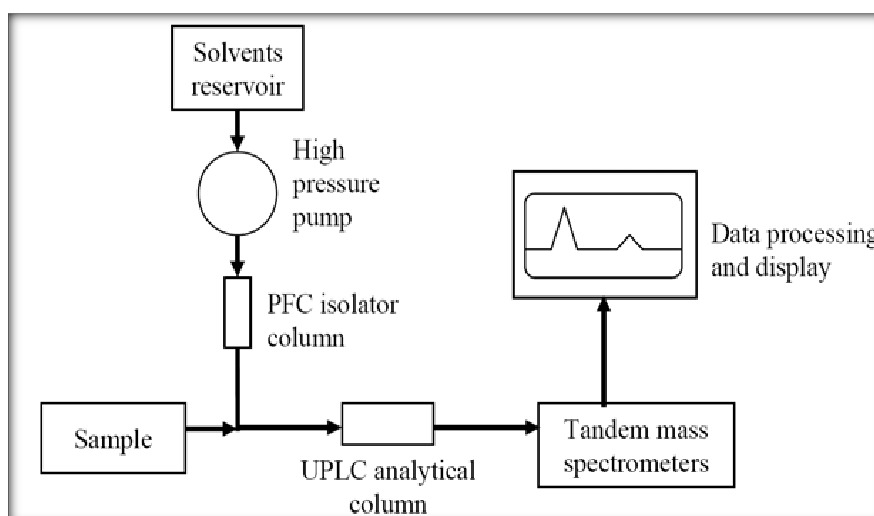

Fig no.2 -Ray digram of UPLC Instrumentation

Advantages of UPLC:

- Various advantages of UPLC are as follows:
- Require less run time and enhance sensitivity.
- Provides the selectivity, sensitivity, and dynamic range of LC analysis.

- In chromatogram resolved peaks are obtained.
- Multi residue methods are applied.[22]
- Speedy analysis, quantify accurately analytes and related products.
- Uses of fine particle (2µm) for packing of stationary phase make analysis fast.
- Time and cost both are reduced.
- Consumption of solvents is less.[14]

Disadvantage of UPLC:

1) In UPLC analysis the main disadvantage occurs are life of columns, during analysis high pressure developed because the particle size. Increase pressure reduces the life of the columns. Due to increased pressure requires more maintenance and reduces the life of the columns of these types. Using stationary phase of particle size 2µm performbetter analysis without the adverse effects of high pressure.[16]

2) UPLC has better column technology, which can work under extremely high pressure conditions, have faster rate of detection and moreover, is based on already established HPLC technique so there is a hope that UPLC will increase the quality of pharmaceutical analyses and productivity of research scientists. While it may take some years for UPLC to become full time analytical technique, we remain optimistic that this technique will live up to expectations.[17]

3) UPLC operates at ultra-high pressure up to 1000 bars so column requires more maintenance and life of column is reduced.[23]

4) Phases 1.7 µm sized are non-generable and thus have limited use.[15]

Application of UPLC:

- **Determination of Pesticides in Groundwater**:- UPLC coupled with triple quadruple tandem mass spectrometry (UPLCTM-MS/MS) can be utilized to determine the trace level pesticides in groundwater in less time and speedy manner. The technique has enhanced the analysis speed, sensitivity, and resolution.[24]
- **Improved Resolving Power in Peptide Maps:-**Peptide mapping is an essential technique for the characterization of proteins. Due to exceptionally reduced instrument and column dispersion, the analyses of tryptic digest of phosphorylase by UPLC technology provides significantly improved resolution, peak capacity, and sensitivity compared to HPLC, allowing the detailed characterization of the protein.[25]
- **Rapid Dose Formulation Analysis:-**Nowadays, the use of UPLC together with UV and MS detection has been widely utilized in pharmaceutical applications. Several commercial drug formulations were used as models to study the efficiency of separations with the change of flow rate. The efficiency was judged on the parameters of resolution, theoretical plates, column ruggedness, retention time, and peak area. For example, mefenamic acid and chloramphenicol separation was studied in dimethylacetamide/ polyethylene glycol-200 vehicle.
- **Analysis of Traditional Chinese Medicines (TCM):-**The identification and quantification of components of TCM by chromatographic analysis is one of the major challenges. TCM is a complex matrix in which all the constituents play a specific role for the overall efficacy. Therefore, the analysis of all the constituents is synchronously necessary for the quality control. The new technique UPLC is used for the quality control of the TCM.
- **Multi-Residue Analysis of Pharmaceuticals in Waste Water:-**The water used in the pharmaceutical companies is found to have the traces of various cholesterol-lowering statin agents, anti-ulcer agents, antibiotics, beta-blockers, analgesics, anti-inflammatory agents, lipid regulating agents, psychiatric drugs, and histamine H2 receptor antagonists. UPLC coupled with Q-TOF-MS is used to confirm and screen these drugs in the samples of waste water treatment plant.
- **Identification of Metabolites:-**The identification and detection of all the possible metabolites of the candidate drugs for the discovery of new chemical entities is a very important step. For the identification of the metabolites, a high sample throughput is required to be maintained by the analysts to provide quick results to the medicinal chemists. UPLC-MS/MS is helpful in biomarker discovery as it meets tough analytical requirements and provides sensitivity, mass accuracy, dynamic range, and resolution.

- **In Manufacturing / Quality Assurance (QA) / Quality Control (QC):-**Identification, quantification, purification, efficacy and safety are key parameters to be evaluated during manufacturing of a drug product and pharmaceutical dosage form. Material stability is also observed as a component of QA and QC. UPLC is used as an important tool in QA/QC laboratories for the quantitative and extremely regulated analysis.
- **Impurity Profiling:** Impurity profiling should be efficient for consistent detection and separation of all the impurities present in the active compound. The drug development and formulation process demand accurate measurement/testing of low-level impurities present with the active pharmaceutical ingredients or the excipients or the raw materials used in the preparation of the final product. Thus, the presence of excipients in the sample makes the profiling difficult and with HPLC method, it takes longer time for analysis to achieve sufficient resolution. Thus, the combination of UPLC with mass spectrometry has been useful for the documentation of drug and endogenous metabolites in the final product.
- **Method Development / Validation:-**Method development and validation is a complex process and consumes a lot of time. For the development of a robust and reliable method, the labs are required to study many combinations of different parameters e.g. mobile phase, temperature, pH, column and gradient chemistry etc. UPLC is an important method used in the laboratory which reduces the cost and increases the efficiency of analysis required for developing and validating the method. With UPLC, the speed of the separation increases and efficiency improves, which results in the fast development of methodologies. High stability of the UPLC columns provides the possibility of selection of column temperature and pH from a wide range.[26]

II. CONCLUSION

UPLC is based on much proven technology of HPLC technique and it not only enhances but flourishes the utility of conventional HPLC technique.

When many scientists started experiencing separation obstacles with it. The information provided per Unit work by UPLC is much greater than HPLC as ithas higher speed, resolution and sensitivity. Also theanalysis time and solvent consumed in UPLC is lessthan in all previously used chromatographic techniques. On the contrary, in UPLC the factors like Resolution, tailing factors and peak area/peak retention time repeatability are similar to those of HPLC.

So, the transfer of an existing HPLC method to new UHPLC method is preferable as well as profitable in the pharmaceutical industry and this has been successfully demonstrated in the literature. Extraction of above data has revealed that UPLCwill prove to be an essential and crucial tool for enhancing the quality of pharmaceutical analysis and also the productivity of the research scientists.

III. REFERENCES

[1] Van Deemter JJ, Zuiderweg EJ, Klinkenberg A. Longitudinal diffusion & resistance to mass transfer or cause of non ideality in chromatography. Chem. Eng. Sci. 1965; 5: 271-289.

[2] Srivastava B, Sharma BK, Baghel US, Yashwant, Sethi N. Ultra Performance Liquid Chromatography (UPLC): A Chromatography Technique. International Journal of Pharmaceutical Quality Assurance.2010; 2: 19-25.

[3] Zhang YH, Gong XY, Zhang HM, Larock RC, Yeung ES. Combinatorial screening of homogeneous catalysis and reaction optimization based onmultiplexed capillary electrophoresis. Journal of Combinatorial Chemistry.

[4] Zhou C, Jin Y, Kenseth JR, Stella M, Wehmeyer KR, Heineman WR. Rapid pKa estimation using vacuum-assisted multiplexed capillary electrophoresis (VAMCE) with ultraviolet detection.J Pharm Sci. 2005; 94: 576-589.

[5] Zhu J, Goodall DM, Wren SAC. Ultra-High Performance Liquid Chromatography and Its Applications.LCGC.2005; 23: 54-72.

[6] Greibrokk T, Andersen T. High-temperature liquid chromatography. J Chromatogr A. 2003; 1000: 743-755.

[7] Gerber F, Krummen M, Potgeter H, Roth A, Siffrin C, Spoendlin C. Practical aspects of fast reversed-phase high-performance liquid chromatography using 3 microm particle packed columns and monolithic columns in pharmaceutical development and production working under current good manufacturing practice. J Chromatogr A. 2004; 1036: 127-133.

[8] Tanaka N, Kobayashi H, Nakanishi K, Minakuchi H and Ishizuka N. Monolithic columns-a new type of chromatographic support for liquid chromatography. Anal.Chem. 2001; 73: 420-429.

[9] Wu N, Dempsey J, Yehl PM, Dovletoglu A, Ellison A. Wyvratt. Practical aspects of fast HPLC separations for pharmaceutical process development using monolithic columns. Journal of Analytical Chemistry. 2004; 523: 149-156.

[10] Jerkovich AD, Mellors JS, Jorgenson JW. LCGC 2003; 21: 606-611. Narasimham L.Y.S., Barhate V.D.: J. Chem. Metrol. 4, 1 (2010).

[11] Yang Y., Hodges C.C.: LCGC Europe 18, 28 (2005).

[12] Plumb R., Castro P.J., Granger J., Beattie I., boncour K., Wright A.: Rapid Commun. MassSpectrom.18, 2331 (2004).

[13] Castro P.J., Plumb R., Granger J.H., Beattie I., Joncour K., Wright A.: Rapid Commun. Mass Spectrom.19, 843 (2005).

[14] Wilson I.D., Nicholson J.K., Castro P.J.,GrangerJ.H., Johnson K.A., Smith B.W., Plumb R.S.: J. Proteome Res. 4, 591 (2005).

[15] Goodwin L., White S.A., Spooner N.: J. Chromatogr. Sci. 45, 298 (2007).

[16] Tolley L., Jorgenson J.W., Moseley M.A.: Anal. Chem. 73, 2985 (2001).

[17] Lippert J.A., Xin B., Wu N., Lee M.L.: J. Microcolumn Sep. 11, 631 (1999).

[18] Cintron J.M., Colon L.A.: Analyst 127 ,701(2002)

[19] Martin M., Guiochon G.: J. Chromatogr. A 1090, 16 (2005).

[20] Sun X., Wrisley L.A.: Am. Drug Discov. 2, 22 (2007).

[21] Wren S.A.C., Tchelitcheff P.: J. Chromatogr. A 1119, 140 (2006)

[22] Acquity UPLC column. Available https://www.waters.com/webassets/cms/library/docs/720001140en.pdf

[23] Agilent Technologies. Available at: https://www.agilent.com/cs/library/catalogs/public/5991-1059EN%20LC_Columns.pdf

[24] Global Manufacturer pages. Available at: http://http://www.mfgpages.com/

[25] Phenomenex. Available at: http://http://www.phenomenex.com/