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COMPARATIVE STUDY OF RECENT ADVANCEMENT IN HPLC: A REVIEW

Tahir Nizami*[1], Namrata Rathore[1], Neelesh Malviya[1]

[1*]Smriti College of Pharmaceutical Education, Indore, Madhya Pradesh, India.

ABSTRACT

In present era, chromatography is the most important science of separation which is being used in all research centers, laboratories, agriculture, chemical, dyes and pharmaceutical industries of the world. Among all available chromatography techniques, High Performance Liquid Chromatography or HPLC is one of the widest and commonly used chromatographic techniques for analytical purpose. Recent improvement separation techniques and advancement in the instrumentation for liquid chromatography producing fast very efficient analytical separations. This article on chromatography will produces a short review of recent advancement in HPLC along with its principle and instrumentation. This study focus about recent emerging techniques in field of analytical chemistry, specifically chromatography.

KEYWORDS

Liquid chromatography, HPLC, RRLC, UPLC, UFLC and Nano LC.

Author for Correspondence:

Tahir Nizami,

Smriti College of Pharmaceutical Education,

Indore, Madhya Pradesh, India.

Email: tahir.nizami@scopeindore.info

INTRODUCTON

Chromatography is an advanced separation method in which various parts or elements of any mixture are differentiated on the basis of affinity of substance in between two different compartment system in which there is a fixed immovable phase known as stationary phase while the other is a phase which tends to rise in column known as mobile phase. Liquid chromatography is an advanced technique of chromatography in which mobile phase nature is liquid.

Principle

The basic theory of this separation mechanism is governed by the van Demeter equation, which is a mathematical equation which explains the

correlation between the flow rate and height of plate that is HETP or simply 1/column efficiency.

$$H=A+B/u + Cu$$

A represents Eddy's diffusion
B represents longitudinal diffusion
C represents concentration,
u- represents linear Velocity[1].

New Amendments in HPLC Technique

In this review HPLC technique is correlated with some advanced techniques which are characterized by:

- Rapid Resolution Liquid chromatography or RRLC
- Ultra-Performance Liquid chromatography or UPLC
- Ultra-Fast Liquid chromatography or UFLC
- Nano Liquid chromatography or Nano LC

RAPID RESOLUTION LIQUID CHROMATOGRAPHY

Rapid Resolution Liquid Chromatography or RRLC system is advanced form of HPLC which is designed to provide highest speed chromatography, resolution and at a minimum pressure[3]. This method of analysis is now become a common and routine method in the chemical and pharmaceutical industry. This advance method produce excellent peak shapes, increased reproducibility, high sensitivity, high-speed detection with reduced analysis cost, and is valuable for the quality control of convectional, traditional and herbal medicines. HPLC provide added advantage of improved sensitivity and comparative lesser time of analysis of components. For further improvement, column efficiency must be increased.

Applications of Rapid Resolution Liquid Chromatography (RRLC)

Estimation of paeoniflorin extract from Paeonia Sinjiang K. Y. Pan.8

Paeonia Sinjiang K. Yaphank root is a vital drug substance in Chinese herbal medicine which is commonly used for heat-clearing, blood-cooling, activating blood, absorbing clots and treating carbuncle. The separation was done using advanced RRLC technology with the use of analytical Agilent C-18 column using mixture of methanol and potassium phosphate monobasic as mobile phase.

RRLC method for estimation and QC study of plant *Rhodiola rosea* roots and standardization of its marketed products

Novel, accurate, simple and sensitive analytical method foe estimation of some therapeutically useful compounds of plant *Rhodiola rosea* was performed by using RRLC. The method was validated for estimation of various plant product and root extract like salidroside, osarin, rosavin, rosin and rosiridin etc in plant *Rhodiola rosea*. The separation was done using Phenomenex C18 column at temperature of 40 degrees with a flow rate of 1.0mL/min. The RRLC method is accurate and sensitive.

ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY

This novel chromatographic technique provides more advanced: chromatographic resolution, improved speed and provide high sensitivity. It includes application of very fine particles and reduces solvent consumption. UPLC is advanced form of HPLC. As principle of convectional HPLC determines the relation between particle packing and resolution it confirms that as stationary phase packing particle size is reduced, the efficiency and hence resolution also improved. As the size of particle reduced to less than 2.5µm, this produce a makeable gain in efficiency.

Advantage of UPLC over convectional HPLC

- Ensures decrease run time with high and improved sensitivity
- Improved resolution performance
- Reduced cost of operation
- Decreased process cycle times, so that more product can be produced with existing resources

Applications of UPLC

Drug Discovery and Development

UPLC accelerates drug discovery process by virtue of its high throughput screening, combinational chemistry, high throughput *in vitro* screening to

determine physiochemical and drug's pharmacokinetics.

High throughput quantitative estimation

This novel technique is associated with mass spectroscopy for give the metabolic and chemical stability assay.

Estimation and quantification of drug dosage form

UPLC enables improved speed, with high levels of accuracy and capable of producing reproducible results both isocratic and gradient estimation samples.

Separation and estimation of amino acids

UPLC has been now days used to as accurate, reliable and reproducible for the separation and estimation of amino acids in the areas of cell culture monitoring and the nutritional analysis of foods.

Analysis of agricultural products

UPLC is associated with triple Quadra-pole tandem mass spectroscopy will help in identification of trace level of pesticides from water.

Estimation of Herbal and Traditional Medicine

UPLC is now days commonly applied for estimation analysis of herbal products and herbal medicines. The main objective of this is to detect drug samples come from the complexity of the mixture and variability from sample to sample.

ULTRA FAST LIQUID CHROMATOGRAPHY

It is ten times higher speed and three times better separation than other LC techniques and offers outstanding speed and separation at ordinary level of column pressure. By potentiating the stationary phase and overall performance of the whole system UFLC reduces the changes from the theoretical concept of van Demeter. The Prominence UFLC series provides ultrafast analysis, while maintaining high analytical precision and reliability

Advantages over HPLC

- Reduce analysis time by 75% over regular LC system
- Increased separation performance

Applications of UFLC

Determination of iodiconazole in micro-dialysis samples

Iodiconazole estimation is now routinely done by UFLC technique. In order to estimation this anti-fungal drug in dermal microdialysate, the drug is assayed using UV detection at 230nm. This novel antifungal is being nowdays estimated by using instrument Shimadzu Prominence UFLC and C18 column using mixture of acetonitrile and tri-ethylamine solution, at pH 3.6 which was adjusted with phosphoric acid (65:35, v/v), and a flow rate of 0.5ml/min.

Determination of podophyllotoxin in dermal and blood micro-dialysis samples of rats

The micro-dialysis samples were prepared by liquid-liquid extraction using ethyl acetate along with etoposide which used as the internal standard (IS). Analytical column Agilent ZORBAX XDB-C18 column was used. In this method acetonitrile and ammonium acetate in (40:60, V/V) was used as mobile phase. The UFLC-MS/MS system was operated in the mode of multiple reactions monitoring using the electro spray ionization technique in positive mode.

Estimation of Catechins in Green Tea

Estimation of Catechins in Green Tea was performed with the help of UFLC. In this Shim-pack XR-ODS (50mm L × 2.0mm i.d) column was used. 0.1 % formic acid aqueous solution and acetonitrile was used as mobile phase at the flow rate of 0.5mL /min, and sample injection volume is 2µL at 50^{o}C temperatures.

NANO LIQUID CHROMATOGRAPHY

Few definitions have been found in the literature based on column diameter and mobile phase flow rates. Some researchers defined NLC as advanced chromatographic technique having mobile phase flow rate at nano ML per minute. However, the detection aspect of this advanced chromatography which is very vital in analytical technology was not taken into consideration until then.

Advantages of Nano Liquid Chromatography over HPLC

- Markedly decrease the consumption of mobile phase and subsequent production of waste.
- Reduction in internal diameter improves the sensitivity and less sample requirement
- Comparatively more cheaper, faster than its traditional counterpart
- Improved sensitivity of detection in MS since lower flow rates comparatively smaller columns
- Greater separation efficiency and ability to analyses very small amount of solute
- Current advancement shows marked improvement in resolution power for sample analysis

Applications of Nano LC

Separation of sulfonamides

Nano-liquid separation technique associated with mass spectrometry and was routinely used for the simultaneous estimation compounds like sulfonamides with the aid of capillary column with a size of 100µm internal diameter. For this separation Kinetex C18 analytical column was used as the stationary phase and mobile phase was mixture of water and acetonitrile, containing 0.1% (v/v) formic acid, was used at flow rate of 190nL /min.

Separation of organic peptides

Advanced nano liquid chromatography techniques is also now been used for separation and estimation of organic protein and peptide separation through online catalization with the help of immobilized enzymatic reactor, and is routinely used for analysis of such compounds.

Glycomics as biomarker using nano-LC

Novel Nano flow liquid chromatography, markedly produce very sensitive and accurate method of separation and profiling of these substance.

Nano-LC for glycobioanalysis

Structural heterogeneity of glycoconjugates and glycans in biological matrices. C18 column containing carbon was used as stationary phase which is applied to nanoflow level and on electronic chip format, producing to increased sensitivity of data on highly complex materials such as glycans and glycol conjugate mixtures.

Other applications

Analysis of biological products: when minute quantity of samples are provided like as blood sample, CSF fluid, hormones, biochemical enzymes and xenobiotics at nano levels.

High throughput screening (HTS) and drug discovery where the limits of detection are very low.

Proteomic and genomic research.

Detection of accumulated drug samples in the body.

Table No.1: Comparison between novel chromatographic techniques

S.No	Characteristics	HPLC	RRLC	UPLC	UFLC	Nano LC
1	Particle size	3 to 10µ	1.8µ	Less than 2µ	1.7 – 2.2µ	1.7 – 3µ
2	Analytical column	XTerraC_{18}, Alltima C_{18}	ZORBAX Eclipse XDB−C18 RRHT	Acquity UPLC beh C_{18}, C_8, rp	Shim-pack XR-ODS column	Capillary HPLC, Micro HPLC
3	Column dimensions (length x I.D)	150 X 3.2mm	2.1-4.6mm	150 X 2.1mm	75mm X 3.0mm	125mm X 0.05mm - 4.6mm
4	Column temperature	30^oC	Up to 100^oC	65^oC	40^oC	25-35^oC
5	Injection volume	5µL	1.5µL	2µL	0.1-100µL	10 nL-125µL
6	Flow rate	0.01-5mL/min	0.2-20µL/min	0.6mL/min	3.7nL/min	20-200nL/min

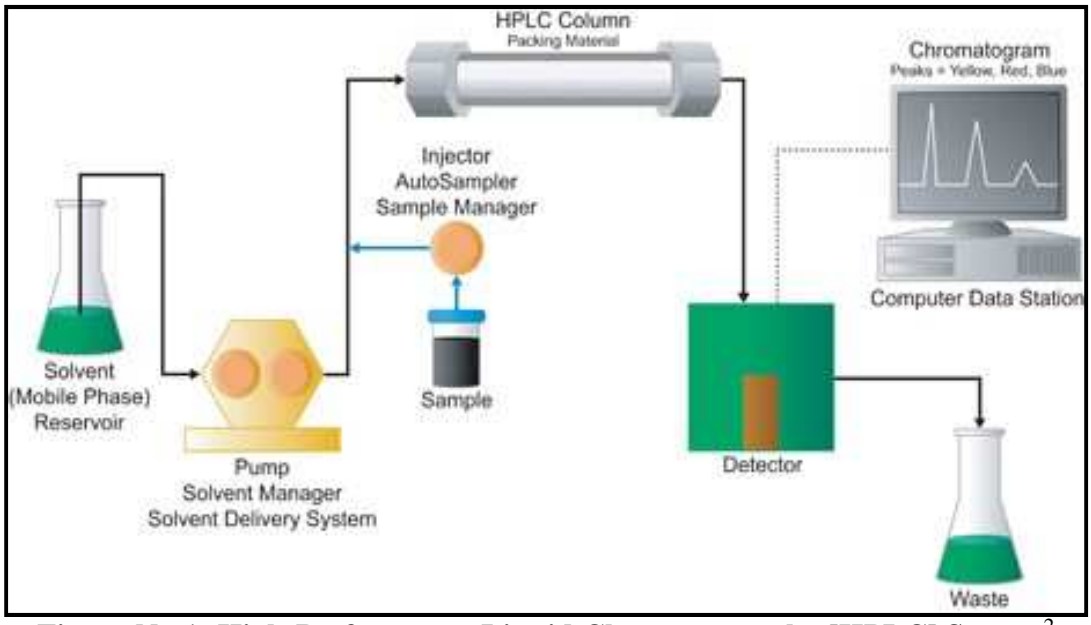

Figure No.1: High-Performance Liquid Chromatography [HPLC] System[2]

CONCLUSION

From this article we conclude that RRLC produce improved run times and increased sensitivity over old HPLC based methods. RRLC offers High Sensitivity with Low limit of detection, Excellent Reproducibility and Broad Applicability. At a condition when a group of analyst have reached separation barriers with traditional and conventional separation methods like HPLC, UPLC offers the possibility to improve, extend and increase the utilization of chromatography. Ultra-fast analysis means a significant enhancement in sample throughput (510times) and productivity compared to a conventional HPLC. Nano LC is the most advanced innovation in field of separation science in which separation and estimation can achieved at very minute nano gram or even at lower levels.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

BIBILIOGRAPHY

1. Yoshida T, Ronald E M and Kumagai H. High-Speed Analyses using Rapid Resolution Liquid Chromatography on ZORBAX column packed 1.8µm Particles, *Chromatography,* 28(2), 2007, 81-87.
2. www.waters.com/webassets/cms/category/media/other_i mages/primer_e_lcsystem.jpg
3. Ian A, Darwin A, Eddie G, Christensen J, and Ryan M. Rapid Resolution Liquid Chromatography with Charged Aerosol Detection, *Magellan Biosciences,* Available from: URL:http://www.esainc.com/docs/spool/RRLC_CAD_Pittco n_2008.pdf.
4. Ettre L S M S. Tswett, The invention of chromatography, *LC-GC,* 21(5), 2003, 458-467.
5. Andrea Weston and Phyllis R. Brown, HPLC and CE Principles and Practice, *Academic Press,* 1997, 01.

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