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COMPARATIVE STUDY OF NEW TRENDS IN HPLC

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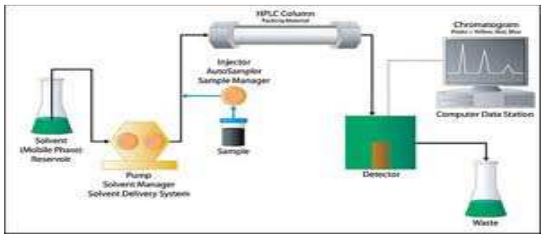
Abstract

This article represents a brief review of new trends in HPLC along with its principle and instrumentation. It describes about comparative study of new trends in HPLC such as RRLC, UPLC, UFLC & Nano LC. Mainly we discuss in terms of instrumental operating conditions such as column temperature, flow rate, injection volume and also cover the applications and advantages over HPLC of each technique. Superiority of modern instruments consists of internal diameter reduction, increases sensitivity and/or less sample requirement, significantly cheaper, quicker than 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 sample analysis.

INTRODUCTION:

Chromatographic process can be defined as separation technique involving mass-transfer between stationary and mobile phase. HPLC is one mode of chromatography, one of the most used analytical techniques. The stationary phase can be a liquid or a solid phase. HPLC utilizes a liquid mobile phase to separate the components of a mixture [1].



Block diagram of HPLC instrumentation

PRINCIPLE:

The underlying principles of this evolution are governed by the van Demeter equation, which is an empirical formula that describes the relationship between linear velocity (flow rate) and plate height (HETP or 1/column efficiency) [2].

H=A+B/u + Cu

A-Eddy's diffusion

B- Longitudinal diffusion

C-Concentration

u- Linear Velocity

NEW AMENDMENTS IN HPLC TECHNIQUE:

HPLC is compared with the classical techniques are characterized by [3]:

- Rapid Resolution Liquid chromatography (RRLC)
- Ultra Performance Liquid chromatography (UPLC)
- Ultra Fast Liquid chromatography(UFLC)
- Nano Liquid chromatography(NANO LC)

Rapid resolution liquid chromatography:

RRLC system was designed to provide highest analysis speed, resolution & pressure at a minimum. Fastest and most efficient and flexible LC system in the world. It has become an increasingly useful approach to achieve higher throughput, improve sensitivity and reduce costs. The RRLC system enables faster analysis (theoretically up to 20x) than with conventional HPLC while maintaining equivalent resolution. This is achieved by using sub-2 micron column particle chemistry and high flow rates. Often higher

temperatures are employed to minimize system back-pressure. With the widespread adoption of RRLC comes the question of HPLC detector compatibility. Presented here is the use of Charged Aerosol Detection with conventional HPLC and RRLC. Rapid Resolution Liquid chromatography system back-pressure. With the wide spread adoption of RRLC comes the question of HPLC (RRLC) [3].

Advantages over HPLC: The major advantages of high–throughput HPLC are the increase in throughput and the reduction in the analysis cost. The shortening in analysis time is due to the use of a shorter column length [4].

Applications of RRLC: RRLC-tandem mass spectrometry method for the determination of endocrine disrupting chemicals (EDCs), pharmaceuticals and personal care products (PPCPs) in waste water irrigated soils. RRLC analysis for quality control of Rhodiolarosea roots and commercial standardized products.

Ultra performance liquid chromatography:

UPLC is comes from HPLC. HPLC has been the evolution of the packing materials used to effect the separation. An underlying principle of HPLC dictates that as column packing particle size decreases, efficiency and thus resolution also increases. As particle size decreases to less than 2.5µm, there is a significant gain in efficiency and it's doesn't diminish at increased linear velocities or flow rates according to the common Van Demeter equation. By using smaller particles, speed and peak capacity (number of peaks resolved per unit time) can be extended to new limits which is known as Ultra Performance [4, 5].

Advantages over HPLC: It decreases run time, increases sensitivity and expands scope of Multi residue Methods UPLC's fast resolving power quickly quantifies related and unrelated compounds.

Applications of UPLC: UPLC used in Identification of Metabolite, impurity Profiling Bio analysis / Bioequivalence studies Analysis of amino acids, natural products and traditional herbal medicine [5].

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 even at normal pressure levels.

By maximizing the column and performance of the entire system UFLC minimizes the deviation from the vandemeter theory.

Advantages over HPLC: It maximizing Data Reliability and making Ultra-Fast analysis more accessible.

Applications: It is used for analysis of Isoflavones, enables the use of high-viscosity mobile phase and analysis under lower temperature. Simple, selective, and sensitive measurement of urea in body fluids of mammals by reversed-phase ultra-fast liquid chromatography. Analysis of artificial colorants by UFLC- mass spectroscopy. Separation of major components in PanaxGinseng [5].

Nano liquid chromatography:

Defined Nano LC as chromatographic modality having mobile phase flow rate at Nano ml per minute. A modality of chromatography involving samples in Nano liters, mobile phase flow rates in nano milli liter per minute, with detection at nano grams per milli liter. This sort of modality is generally carried out in microchips and hence, has also been termed as Lab on chip chromatography.

Advantages over HPLC: Internal diameter reduction increases sensitivity and/or less sample requirement. Increased detection sensitivity in mass spectroscopy, because of lower flow rates in smaller columns. Recent developments have significantly increased the resolution power for complex sample analysis.

Applications: It is used in the analysis of peptides/proteins in proteomics, Biological and environmental samples Determine dyessotoxin for the determination of Yessotoxin in marine phytoplankton by Nano LC with hybrid quadruple time-of-flight mass spectrometry.

Characteristics	HPLC	RRLC	UPLC	UFLC	NANOLC
Particle size(column)	3 to 10µ	1.8µ	<2.5µ	1.7-2.2µ	1.7-3µ
Analytical column	XTerraC18, Allitma C18	ZORBAX EclipseXDB –C18 RRHT	AcquityUPLCb eh c18,c8,rp	Shim-pack XR-ODS column	Capillary HPLC Micro HPLC
Column Dimensions (length x I.D)	150× 3.2 mm	2.1-4.6mm	150× 2.1mm	3.0mmI.D.x 75 mm	125 mm x. 0.05mm - 4.6mm
Column temperature	30^0C	Upto 100^0C	65^0C	40^0C	25^0C-35^0C
Injection volume	5µL	1.5µL	2 µL	0.1-100 µL	10Nl- 125 µL
Flow Rate	0.01-5mL/min	0.2-20 µL/min	0.6mL/min	3.7 nL/min	20-200nL/min

CONCLUSION:

RRLC offers improved run times and increased sensitivity over conventional HPLC based methods. In RRLC High Sensitivity - Low limit of detection, Excellent Reproducibility, Broad Applicability, Ease of

Use - Easy setup.¾ At a time when many scientists have reached separation barriers with Conventional HPLC, UPLC presents the possibility to extend and expand the utility of chromatography.¾ Columns with small internal diameters and / or short column lengths are more susceptible to extra-column band-broadening for high-speed separation in UPLC.¾ Ultra fast analysis means a significant enhancement in sample throughput (5-10times) & productivity compared to a conventional HPLC.¾ Nano LC is the latest innovation in separation science in which detections can achieved at nano gram or lower levels.

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