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Importance of Ultra Pressure Liquid Chromatography

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Commentary

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Importance of Ultra Pressure Liquid Chromatography

Ultra-Performance Liquid Chromatography has vital advances in instrument and column technology was made to give good resolution, speed and sensitivity in liquid chromatographic technique [1-3]. In this UPLC chromatographic technique very fine particles with less than 2.5µm are used for sample preparation, so therefore mechanically it will decrease the length of the column, and reduces solvent consumption and time saving method [4,5].

The UPLC principal is relies on the use of stationary phase consisting of particles less than 2 µm. Ultra pressure liquid chromatography will make the most of the separation performance (by reducing dead volumes) and according to the pressures about 8000 to 15,000 PSI, compared with 2500 to 5000 PSI in HPLC [6-9]. Due to the use of fine particle the column length can be reduced then automatically it will give more efficiency in result. The application of UPLC resulted in the detection of additional Pharmaceutical products metabolites, prior separation and enhanced spectral quality. The column temperature maintained in the ultra-pressure liquid chromatography is 65°C [8-11].

Ultra pressure liquid chromatography expands the scope of Multiresidual ways and it will rapidly quantify the related and unrelated compounds [12-14]. This Chromatographic technique requires less amount of solvent. Increases sample throughput and enables manufacturers to produce more material that consistently meet or exceeds the product specifications, potentially eliminating variability, failed batches of medication, or the necessity to re-work material [15].

In this chromatographic technique there would be some disadvantages, due to the increased pressure which require more maintenance and column life time [16]. Ultra pressure liquid chromatography is widely used for the analysis of natural products and traditional herbal medicine, identification of metabolites, study of metabolomics, screening of absorption, distribution, metabolism and excretion, bio analysis or bioequivalence studies, to analyse the samples of Dissolution, stability or forced degradation studies. Method development and validation for the sample solutions of assay, related substances, and impurity studies. UPLC also used for the analysis of dosage forms, amino acids, pesticides etc [17-20].

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UPLC enhances the productivity of both chemistry and instrumentation by giving more resolution, speed and high sensitivity of liquid chromatography [21-23]. Scientists those who are doing the research work on analytical techniques by chromatographic methods may experiences the separation barriers with conventional high performance liquid chromatographic technique, in those conditions UPLC extends and expands the utility of chromatography by main advantage is reduction of analysis time which also results less solvent utilization and It is clearly proved that the quality of the UPLC-MS spectra is better than that of the Capillary LC-MS spectra with much improved signal-to-noise ratio. UPLC instrument is designed in a special way to withstand high system pressures [24-26]. The quality analyses of various pharmaceutical formulations are transferred from HPLC to UPLC system due to its more advanced applications [27].

In every chromatographic technique detector plays major role to give the peak area result [28]. Detectors which are used in Ultra pressure liquid chromatography are:

- Optical detectors
- Tunable ultra violet detectors
- Fluorescence detector
- Refractive index detectors
- Light scattering detectors
- Mass spectrometric detectors

This UPLC technique takes full advantage of chromatographic principles to run the separation using columns which are packed with smaller particles and higher flow rate [29]. UPLC principal is based on the "**Van Deemter equation"** and it explains the relationship between flow rate and HEPT (height equivalent to theoretical plates) or efficiency of column [30].

The common chromatographic conditions for UPLC are:

1. Columns like C_{18} ACQUITY UPLC BEM, BEH C_8 or Phenyl column and dimensions of 2.1X50mm 1.7µm.
2. Mobile phase A1: 20mm NH_4COOH in water with pH of 3.0 and Mobile phase A2: 20mm NH_4COOH in water with pH of 10.0
3. Mobile phase B1 is Acetonitrile and Mobile phase B2 is Methanol.
4. Flow rate should be maintained as 0.5ml/min and the injection volume is 10.0 µl
5. To maintain the proper flow rate temperature should be maintained as 30°C and detection is measured at UV 254nm [31-33].

UPLC method improves the quality of data and resulting in more definitive map. The separation of analytical samples on UPLC is performed at very high pressure i.e. up to 15000psi [34]. Factors which

affect the performance of UPLC instrument and method are pressure, column, particle size of packing material, and temperature. Pressure responses are more pronounced for both retention and peak shape than the temperature effects for the polar or charged compounds.

Due to the very sharp and narrow peaks there are more number of peaks appear in short time which might facilitate in analysis of complex mixtures and which may give more information regarding the samples should be analysed [35-38]. Present scenario aspects many of the pharmaceutical industries searching for the new ideas to reduce the cost and time for the analysis of drugs, in such cases UPLC has been invented and now it serve as best alternative for previous analytical chromatographic techniques [39-40].

The effects of pressure and resistance heating deserve serious consideration in ultra-high-pressure liquid chromatography (UHPLC) separations, because the pressures used will be thrice bigger than those in conventional high-performance LC (HPLC) [41-43]. The effects of pressure alone will give useful selectivity effects, particularly when separating the molecules of various sizes. Frictional heating effects will cause serious losses in column potency and may also give changes in the selectivity of the separation [44-47].

A new methodology has been fabricated for the determination of organic substances like chlorine, synthetic pyrethroid, organophosphate and carbamate pesticide precipitates in fruit juices by ultra-performance liquid chromatography with atmospheric pressure photoionization–high resolution mass spectroscopy technique [48-52]. Food products contaminant analysis carried out by the ultra-pressure liquid chromatography which connected to quadruple mass spectroscopy (MS/MS), into analytical applications.

In comparison to traditional HPLC research studies showed that UPLC can decrease the run time and give high resolution applications. UPLC – MS has been widely used to the bioanalysis which involves stages of drug discovery and development [53-55]. Pharmaceutical development is an important part of the phase in between the identification of a chemical entity with therapeutic potential and the launch and routine use of a new medicine [56]. UPLC have been investigated as an alternative chromatographic technique to HPLC for the analysis of Pharmaceutical development products [57].

Abbreviations

UPLC – Ultra pressure liquid chromatography
HPLC – High performance liquid chromatography
MS – Mass spectroscopy

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