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UPLC Method Development and Validation for Simultaneous Estimation

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Abstract

UPLC is modern technique which gave a new direction for liquid chromatography. UPLC refers to ultra, performance liquid chromatography, which enhances mainly in three areas: "speed", "resolution" and "sensitivity". UPLC applicable for particle less than 2micro meter in diameter to acquire better resolution, speed and sensitivity compared to HPLC.[29] UPLC technology is now applied throughout the world produce quality data with reproducible and robust method as compared to convention HPLC. UPLC can hyphenate with other techniques such as Mass spectrometer (MS), Ion Chromatography (IC), Nuclear magnetic resonance spectrometer (NMR), and Infrared spectrometer (IR), etc. This technique provides unique end-to- end solution for all the industries and has found application in various field such as pharmaceutical, food, environmental, forensic, toxicology and pesticide. [26]

Keywords: - *UPLC method development, Method Validation*

INTRODUCTION

HPLC from past 30 year is a technique which used for analysis in Laboratory but thanks to significant advances and innovation in instrumentation, detector designs, processing and particle size technology, results in the event of Ultra Performance Liquid Chromatography (UPLC). Principle of UPLC basically remains same as HPLC only with the assistance of technology; it achieves dramatic increase in resolution, speed and sensitivity of the liquid chromatography. UPLC Technology is applied throughout

the planet for providing advantage of your time, cost, and quality.

Beside this it's also reproducible result and robust method as compared to standard HPLC 26].

The casual ideology of this advancement is governed by what is called the Van Deemtre equation. By the use this technology the benefit in Chromatographic principle to encourage separation utilizing columns chock full of tinier particle and superior flow rates for higher speed with exceptional resolution and excellent sensitivity [48].

- To develop a simple, precise, accurate, rapid, and economical and stability indicating analytical method for simultaneous qualification of Active Ingredient in Technical as well as in single as well as in combine formulation sample using UPLC method.
- To perform forced degradation study on formulation sample.
- To validate the developed method for simultaneous qualification of AI in formulation sample using UPLC method.

AIMS AND OBJECTIVE

Quantitative analysis is an important tool in an industry. The number of pesticides and pesticides formulation introduced into the market has been increased at an alarming rate.

UPLC are most sophisticated, faster sensitive technique than HPLC. UPLC is the new category of analytical separation science which works on same principle of HPLC. As compare to HPLC, UPLC have advantage in the term of speed of analysis, sensitivity and resolution with a significant decrease in the time and solvent consumption.

The objective for research work to develop and validated and analytical method for the determination of single or combined form and estimation of degradant generated during storage of finished product using technique like High performance Liquid Chromatography, Gas Chromatography and also by using the Instruments like UPLC. Which are superior to prior developed method with respective to cost of analysis, sensitivity and simplification.

FORMULATION ASPECT AND METHOD OF DEVELOPMENT

Working

The UPLC is based on the principle of the use of stationary phase consist of particle less than 2micrometer. The principle of this technique is governed by the Van Deemtre, Emperical formula that describes the relationship between linear velocity and plane height.

H= A+B/v + Cv

Instrumentation

The Ultra Performance Liquid Chromatography have the ability to work more efficiently with higher speed, sensitivity and resolution at a much wider range of linear velocities, flow rates and backpressures to obtain superior results.

The Acquity UPLC system consist of

- Binary solvent manager
- Sample manager including the column heater
- Optional Sample manager
- Pumps
- Detector

Binary Solvent Manager

Two individual serial flow pumps is used by Binary solvent manager to deliver a parallel binary gradient High pressure pump that move solvent through the system. Flow rates of 1 ml/min at 103421 Kpa [1034 bar, 1500 psi] in binary solvent manager and up to 2ml/min at reduced pressure to 62053 Kpa [621 bar, 9000 psF]. The solvent manager can pump two solvent immediately.

Sample Manager

The Acuity sample manager injects the sample it draws from Micro titer or vials in to the chromatography flow stream. A locating mechanism uses a probe to access sample location and draw sample from them. An injection performs by the sample manager in approximately in 15second. The sample manager also controls the column heater. Column temperature up to 65℃ can be attained.

Column Heater

The column heater is of a modular design and also its foot print is identical to that of the sample manager. Thus its connect to the top sample manager and serves as that instrument's top cover.

Optional Sample Organizer

The optional sample organizer stores vial plates or micro meter and transfer them to and from the sample manages, automating their processing and increasing through put. [2]

Pumps

The UPLC pumps is consider to be one of the most important in a liquid

chromatography system which has to provide a continuous constant flow of the eluent through the UPLC injector, and detector.

The two basic classic classifications are

- Constant pressure pump
- Constant flow pump

Detector

Type of Detector

Detector can be classified as:

- Optical detectors
- Tunable ultra violet detector
- Evaporative light scattering detectors
- Florescence detector

Method Development & Validation

According to FDA, validation is defined as a process of establishing documentary evidence demonstrating that a procedure, process, activity carried out in testing and then production maintains the desire level of compliance at all stage. UPLC provide efficiency in method development. Using UPLC, analysis times become as short as one minutes can be optimized in just one or two hours, significantly reducing the time required to develop and validate.

Method Development

The developed and validated method was aimed to established chromatography condition, capable of qualitative and quantitative impurities substances. The chromatographic condition was initially based on the method provided by pharmacopoeial.

The tailing factor got worst after replicate injection, caused the relatively high backpressure to the system and resulted in poor peak shape. To overcome this problem, we transferred the method from HPLC to UPLC and varied the condition until met the required criteria. Various mobile phase composition and flow rates were adjusted. An isocratic RP-UPLC method was develop for screening all the content with UV detection at 275 nm. The compared results of UPLC and HPLC were given in Fig.3 as an example of triamcinolon cream. The improved USP tailing factor and reduced analyzing time demonstrated that the UPLC- based method is more efficient than HPLC.

In general, the sustained released PLGA microspheres would be dissolved in organic solvent for determination of the encapsulation efficiency which are directly subjected for HPLC analysis. However, for the measurement of the impurities content, a large amount of the drug product should be injected into the column to satisfy the limit of quantization with trace substance.

Avoid injecting PLGA polymer into the UPLC column is one of the key concern in the sample preparation. It may easily lead to physical clogging of the column even though the pre-column has been installed.

To determine whether the PLGA precipitation depend on organic solvent composition, the transmission of the PLGA solution under different concentration were measured. At high volume of organic phase, a clear sample solution and high transmission were observed. However, at the high volume of aqueous phase large amount of PLGA polymer has been precipitated. By these features, PLGA polymer could be exhaust by organic- aqueous phase transfer. Meanwhile, the contained drug substance were dissolved and recovered under both solution systems for further analysis.

Specificity

The diluents sample, stress degraded and placebo (without API) were analyzed under the proposed chromatography condition to prove the selectivity. The UV spectrum of stress degraded and shows the UV spectrum obtained from direct analysis of the sample diluents and forced degradation placebo. No signal of solvent was presented in the spectrum.

Fig. 1: Ultra performance liqiud chromatography

Fig. 2: Ultra perfromance liquid chromatography

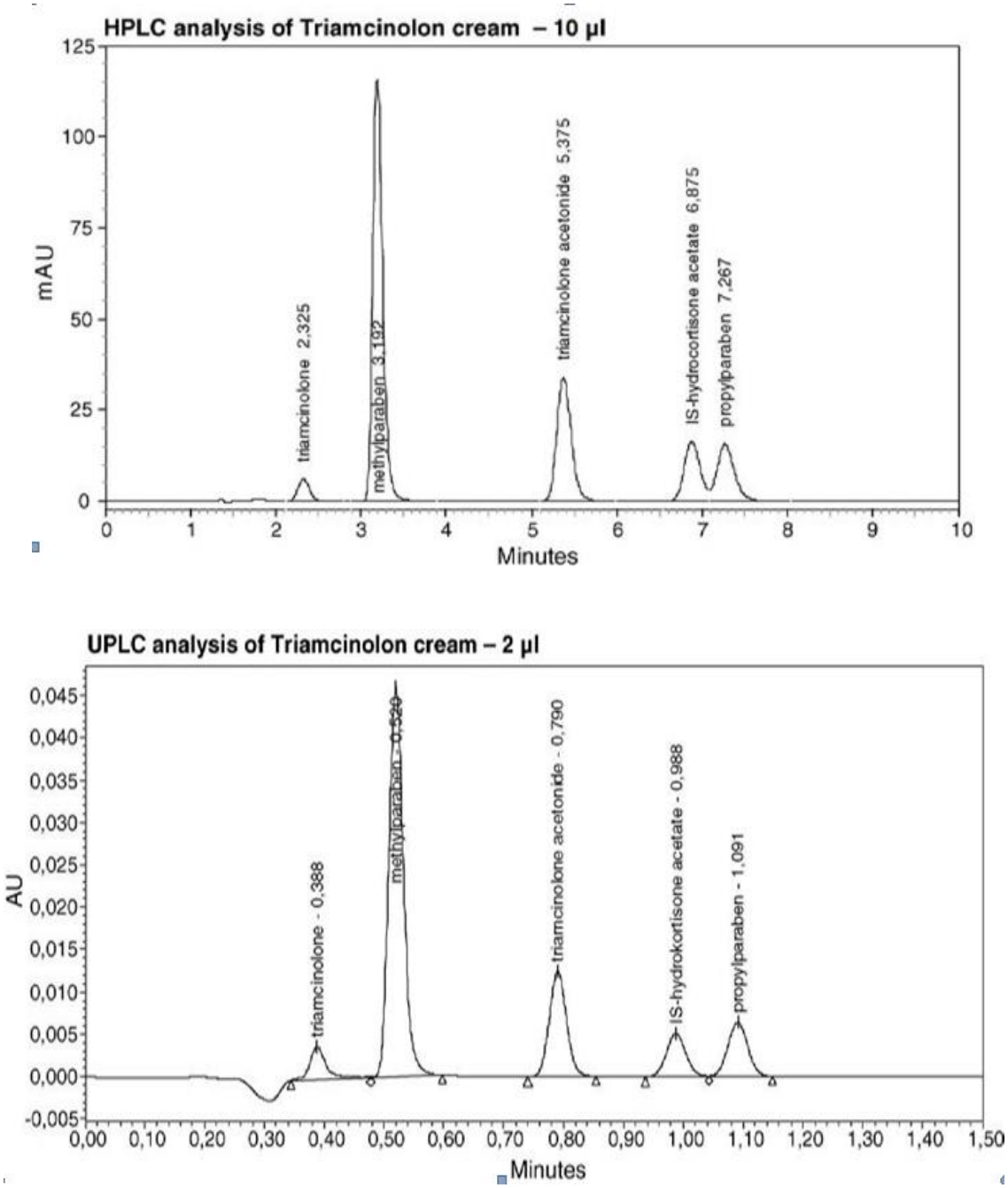

Fig. 3: Sample chromatogram of HPLC & UPLC

Standard Solution Preparation

Accurately weighted drug reference standard (10mg) was transferred to 50 ml volumetric flask, dissolved in and diluted to the mark with 0.01 N HCL.

Standard solution was prepared by diluting the stock solution to obtain suitable concentration for chromatographic measurements. All solutions were filtered by a 0.22 micrometer PTFE syringe filter before analysis.

The Solution for System Suitability

Accurately weighed related compound mixture was transferred to a 10ml volumetric flask, dissolved in and diluted to the mark with diluents. The chemical contained in it are depend on the drug used for validation were filtered by a 0.22 micrometer PTFE syringe before analysis.

Sample Preparation

50mg Compound/Drug are accurately weighed and transferred to 100 ml volumetric flask, 10 ml of basic compound was added into the flask, followed by 10 min sonication to dissolve PLGA polymer. 90 ml of 0.01 N acidic solutions was slowly added and resulted in the precipitation of PLGA polymer. After well- mixed the suspension, 2ml of sample solution was centrifuged at 8000x g for 10 min to remove most of the polymer. For the remaining polymer depletion, the supernatant was filtered through a μ-YM10 filter with a 10,000- Da molecular mass cutoff. All the solutions ere filtered by a 0.22 micrometer PTFE syringe solution containing drug substance or impurities is stables over one week.

VALIDATION PROCESS

Validation of the newly developed method was construction of linearity, range, precision, accuracy and robustness, limit of quantization and limit of detection as per guidelines. Since the lack of the qualified purity impurities, the diluted solution was used for all studies in term of quantization e.g., linearity, sensitivity, precision and accuracy.

The compound mixture solution was subjected to prove system suitability. The USP tailing factor of a peak should not be more than 2.0. For linearity, the peak response was carried out at five concentration levels from LOQ to 150% of the specification (0.10%).

The calibration curves were generated and regression parameter was calculated by Excel. The precision of the method was determined by checking the intra-day and inter-day precision for five independent preparations.

To determine the robustness of the developed method, different experimental conditions including mobile phase composition (±2%), column temperature

(±5°C) and flow rate (±0.02 ml/min) were altered. All specificity of the UPLC assay method was evaluated by stress test.

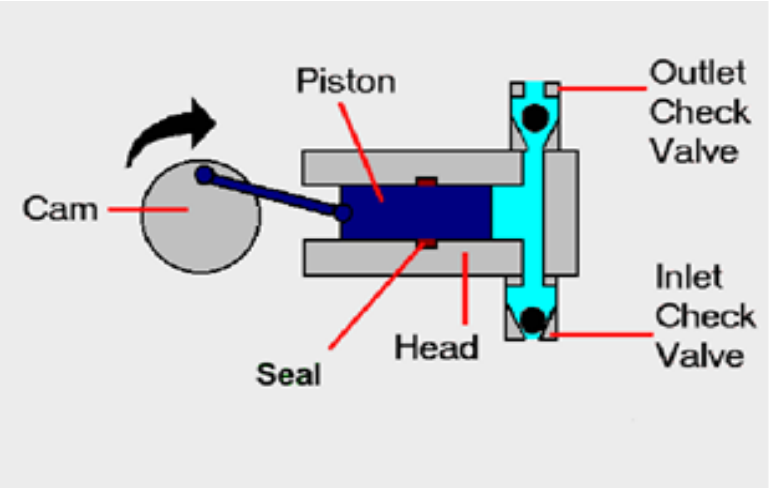

Fig. 4: Reciprocating Piston

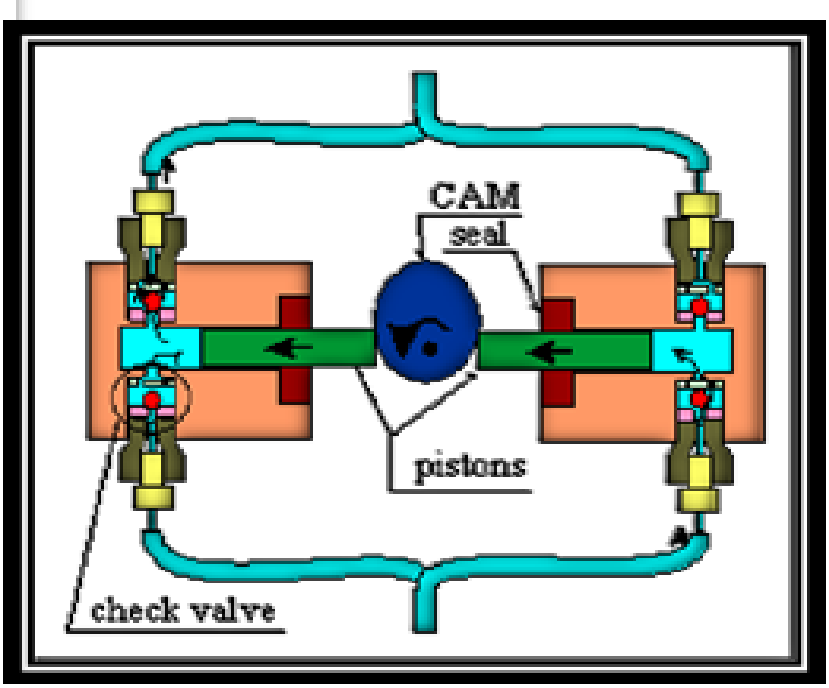

Fig. 5: Dual piston pump pump

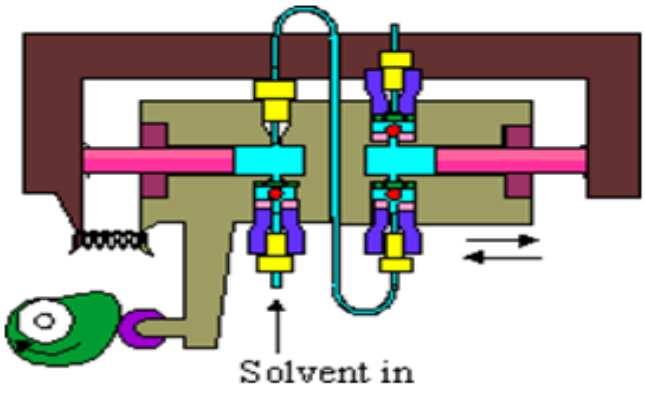

Fig. 6: Dual-head reciprocating pump

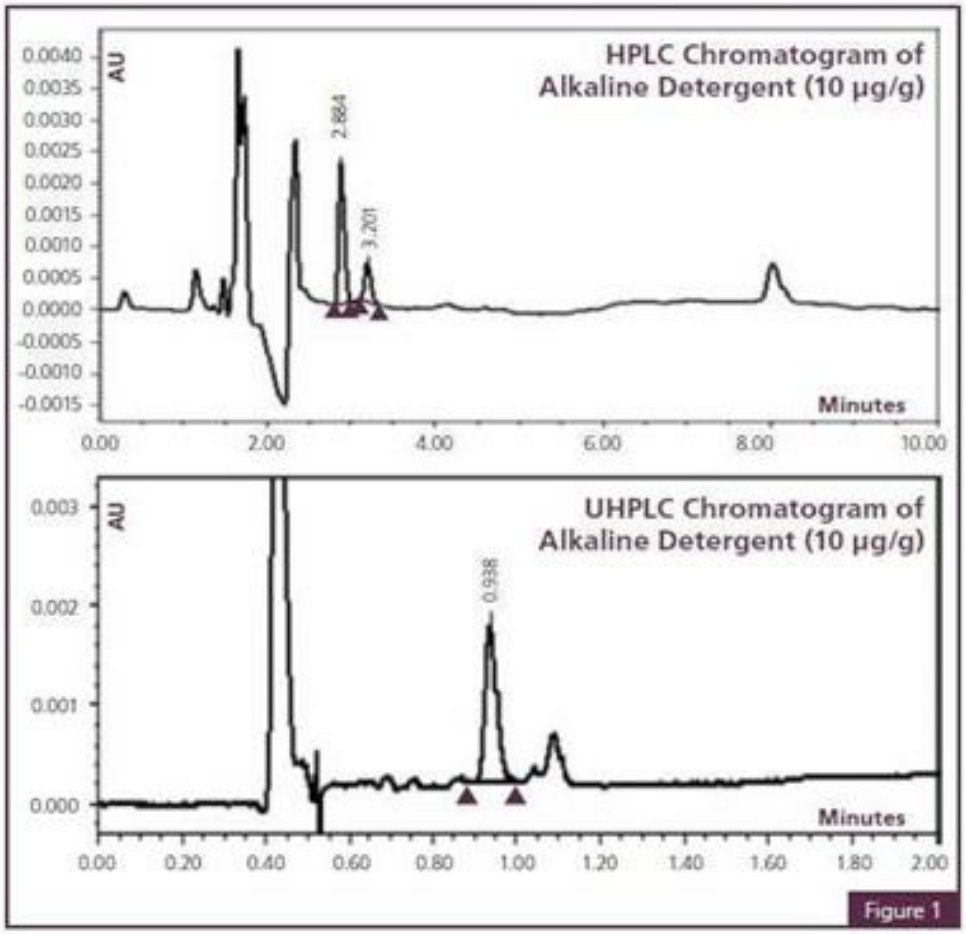

Fig. 7: Difference between HPLC &UPLC

CONCLUSION & SUMMARY

UPLC increasing productivity in both chemistry and instrumentation by providing more information per unit of work as it gives increased resolution, speed, and sensitivity for liquid chromatography.

The main advantage is a reduced solvent consumption, which is due to reduction of analysis time. UPLC can offer significant

improvement in sensitivity, resolution and speed compared with conventional HPLC. The method development process include gathering sample information, defining method goals, scouting column and mobile phase, analyzing counting results, selecting separation conditions, optimizing the method and validation the method. Each step discussed in detail, and column temperature ad gradient and the example of simultaneous optimization of gradient and mobile phase buffer pH are given.

The process of converting an HPLC method to UPLC includes selecting a column with the same chemistry; calculating flow rate, holding time and gradient segment time; performing experiment for verification and validation methods.

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