A SYSTEMATIC EVALUATION OF ULTRA-PERFORMANCE LIQUID CHROMATOGRAPHY FOR ASSESSING DRUG CONCENTRATIONS IN ALLERGY AND IMMUNE SYSTEM INTERVENTIONS

MRS. KAVITA KULKARNI
Research Scholar
Department Of Pharmacy
S JJT University
Rajasthan.

DR. ABHINANDAN KUMAR DANODIA
Guide
Department Of Pharmacy
S JJT University
Rajasthan.

DR. SRIDHAR SIDDIRAJU
Co-Guide
Department Of Pharmacy
S JJT University
Rajasthan

ABSTRACT

The accurate assessment of drug concentrations is paramount in evaluating the efficacy, safety, and therapeutic outcomes of allergy and immune system interventions. Ultra-Performance Liquid Chromatography (UPLC) has emerged as a promising analytical technique for the quantification of drugs due to its high sensitivity, speed, and resolution. This systematic review aims to critically evaluate the utility of UPLC in assessing drug concentrations in allergy and immune system interventions. A comprehensive literature search was conducted across major scientific databases to identify relevant studies published up to the present date. The search strategy included terms related to UPLC, drug concentration measurement, allergy, and immune system interventions. Studies encompassing a wide range of drug classes and intervention types were included in the analysis.

***Keywords**: UPLC, drug, allergy, immune system.*

Introduction

The field of allergy and immune system interventions has witnessed remarkable advancements over the years, leading to improved therapeutic outcomes and enhanced patient care. Central to the success of these interventions is the precise assessment of drug concentrations in biological matrices, a fundamental requirement for ensuring both efficacy and safety. In this context, analytical techniques play a pivotal role in quantifying drug levels, facilitating pharmacokinetic studies, and guiding dose optimization strategies. Among the diverse array of analytical techniques available, Ultra-Performance Liquid Chromatography (UPLC) has emerged as a powerful tool for quantifying drug concentrations with unprecedented sensitivity, speed, and resolution. UPLC represents a significant evolution beyond traditional high-performance liquid chromatography (HPLC), offering the ability to analyze complex samples in shorter timeframes and with improved separation efficiency. These attributes render UPLC particularly suitable for the analysis of drugs involved in allergy and immune system interventions, where accurate and rapid assessment of drug concentrations is essential for informed decision-making. This systematic evaluation aims to comprehensively explore the role of UPLC in the quantification of drug concentrations within the context of allergy and immune system interventions. By critically reviewing existing literature, we intend to shed light on the advantages, challenges, and applications of UPLC in this specialized field. Furthermore, the evaluation will encompass a range of drug classes and intervention types, highlighting

the versatility of UPLC in addressing the unique analytical demands of diverse therapeutic agents.

Literature review

Hoda M. Marzouk et al(2022) reported that favipiravir (FAV), a new and promising antiviral alternative in the treatment of COVID-19, may be determined using a comprehensive stability-indicating HPLC-DAD approach that has been developed and validated.

Prakash M et al ,(2022) reported simple, accurate, precise and highly selective RP-HPLC method was developed and velaidated for pregabalin and etoricoxib.

Hassan M. Albishri et al (2022) Benzodiazepines (BZDs) are well regarded among the medications used to treat cognitive disorders. Drug addicts still misuse BZDs despite their legitimate usage in medicine. Therefore, forensic and clinical toxicology need a straightforward and trustworthy method for detecting and measuring BZDs in the human plasma matrix.

Imran Ali et al (2023) Allergies appear to be among the most problematic diseases affecting humans. Stress and anxiety are often the result of living with an allergy condition. It is treated with a wide variety of drugs, both nonracemic and racemic. In order to properly assess drug reactions, plasma profiles, and efficacy, one needs precise analysis methods. Since HPLC is the most effective analytical approach, it is imperative that both the conventional and chiral HPLC procedures for antiallergic medicines be reviewed. This article provides an overview of the global situation, topics covered include: what triggers allergies, how they manifest in humans, what treatments are available, what types of pharmaceuticals are used, what makes some drugs "chiral," how to analyse them using high-performance liquid chromatography, and how to recognize them.

CHROMATOGRAPHY

The term chromatography, which refers to a series of laboratory procedures used to separate substances, comes from the Greek words chroma (colour) in addition to graphene (to write). A number of techniques exist where the analyte of interest is transferred from a mixture including a "mobile phase" (2-4) to a "stationary phase," where its molecular mixture is separated into a mobile phase in addition to a stationary phase using differential partitioning. Due to the variety of chemicals in addition to partition coefficients, several different approaches have been developed for keeping the stationary phase stable.

To separate components of a mixture, chromatography utilises melting them in a mobile segment and then passing the resultant solution through a stationary stage. Strongly interacting molecules travel more slowly through the resin than weakly interacting ones. There are analytical in addition to preparatory uses for separation. Analytical chromatography's purpose is to separate in addition to identify individual substances in complex mixtures, in addition to in some situations to quantify those substances. Separating in addition to purifying molecules of interest for further use is the goal of preparative chromatography.

Different types of chromatographic techniques

In order to achieve an acceptable separation in a reasonable amount of time, chromatography, which is also utilized for quantitative analysis, is applied. There are several chromatographic techniques

available for this purpose.

Chromatography is a technique that may be used for preparation as well as analysis. Preparative chromatography produces a pure product by separating the mixture's constituent parts. To ascertain the quantity of analytes in a sample, analytical chromatography is employed as opposed to preparative chromatography.

When chemicals are dissolved in a liquid mobile stage, the liquid is passed over a solid stationary phase, such as media or resin, with which the compounds interact in different ways. Since this is the case, certain components of the mobile phase are somewhat slowed down as they go through the chromatographic medium. LC can be further classified into planar chromatography and column chromatography.

METHODOLOGY

Materials:

Tamsulosin, deflazacort, methotrexate, folic acid, and the like are all ingredients used in various pharmaceuticals. Rankem produces all of the aforementioned chemicals and solvents.

4.1.4 Instruments:

- The BVK Enterprises pH meter from India. Electronic Balance by Denver
- Ultrasonicator-BVK Corporations
- The Binary pumps, TUV detector, and auto sampler that make up the WATERS ACQUITY UPLC System are all completely compatible with the Empower 2 software.
- Absorbances were taken with a PG Instruments T60 UV-VIS spectrophotometer that had been fitted with quartz cells that were a perfect match, as well as UV win 6 software. There was a special 2mm and 10mm bandwidth on the spectrophotometer.

Methodology of sample and validation parameters:

For Fluticasone and Azelastine:

- **Preparation of Standard SS:** In a 10ml volumetric flask, we poured 0.5mg of Fluticasone and 1.37mg of Azelastine that had been precisely weighed. After adding 3/4 of diluents, the flask was sonicated for 10 minutes. Standard stock solution was prepared in a flask and diluted with solvents. (50mcg of Fluticasone and 137mcg of Azelastine per milliliter).
- **SWS 100% (standard):** Each stock solution was diluted to 10 milliliters by pipetting 1 milliliter into a volumetric flask. Fluticasone (5 g/ml) and Azelastine (13.7 g/ml) inhalation solution.
- **Creating Test Stock Solutions:** After properly combining the contents Dymista spray for the nose was prepared by adding the contents of five bottles to a separate container, then transferring enough of the combination to fill one container to the volumetric flask of 100 mL holding 50 mL of solvent and sonicating the mixture for 20 minutes. Makeup was then carefully applied to the scars and carefully blended. The supernatant was used in future research and development after being separated from the suspension using centrifugation at 4000 rpm for 15 minutes. (Fluticasone 500 g/ml

with Azelastine 1370 g/ml)

- **SWS (100% solution)** They were created by putting 1 ml of each stock solution into a volumetric flask with a volume of and filling it up to 10 ml with water. Fluticasone (5 g/ml) with Azelastine (13.7 g/ml)

Preparation of buffer:

0.01N NA_2HPO_4 buffer: In a 1000 ml VF, accurately weigh 1.42 g of disodium phosphate or sodium hydrogen phosphate, Degas to sonicate, add around 900 ml of milli-Q water, and top off with water to the desired volume.

Validation:

System suitability parameters:

Standard solutions of Fluticasone and Azelastine (13.7 ppm, 5.0 ppm) were prepared and injected six times to evaluate critical system components such peak residue, resolution, and the number of USP plates.

Results from six standard injections should show no more than a 2% RSD in the area.

Specificity: Interference testing for the best approach. In theory, the blank and placebo peaks during the medications' retention durations should not interfere with each other using this strategy. Therefore, this strategy was seen to be targeted.

RESULTS AND DISCUSSIONS

Precision chromatogram system

Six injections were performed using the same volumetric flask of working standard solution, and their results will be analyzed below. Averages, spreads, and percent variations between two drugs were calculated.

Since the value of 2 was less than the RSD values for both Fluticasone and Azelastine, the system's precision was over the threshold.

Repeatability:

Table : Repeatability table of Fluticasone and Azelastine

S. No	Area of Fluticasone	Area of Azelastine
1.	547657	1043364
2.	549919	1044247
3.	543345	1047385
4.	542301	1035416
5.	548827	1041319
6.	542733	1036444
Mean	545797	1041363
S.D	3383.9	4650.8
%RSD	0.6	0.4

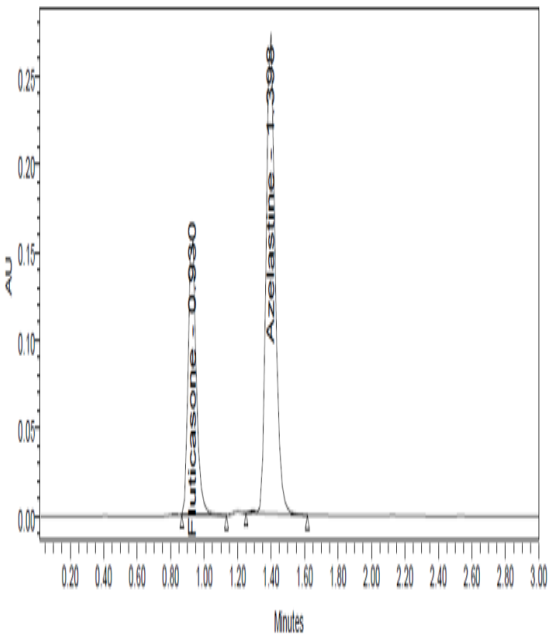

Table : Fluticasone with azelastine

intermediate accuracy (Day Precision)

S. No	Area of Fluticasone	Area of Fluticasone
1.	543730	1039223
2.	545535	1042608
3.	541173	1042468
4.	546568	1046506
5.	546851	1036377
6.	549480	1045173
Mean	545556	1042059
S.D	2851.1	3748.9
%RSD	0.5	0.4

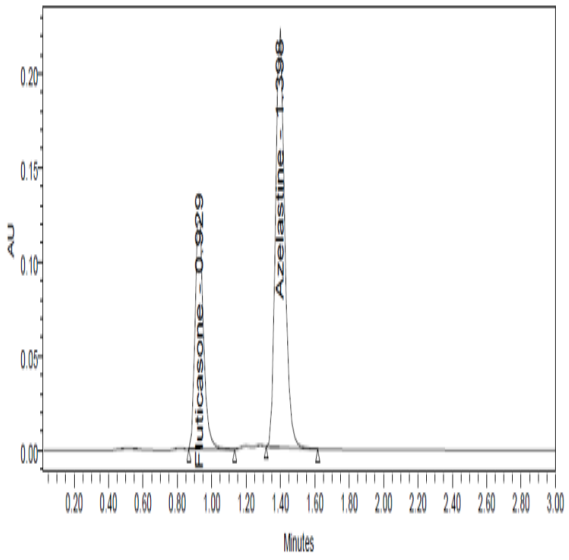

Table: Accuracy table of Fluticasone

% Level	Spiked amount (µg/mL)	Quantity retrieved (in g/mL)	% Recovery	Mean %Recovery
50%	2.5	2.5	99.9	
	2.5	2.5	100.8	
	2.5	2.5	100.4	
100 %	5	5.0	100.8	
	5	5.0	99.3	
	5	5.0	100.3	
150 %	7.5	7.4	99.1	99.94%
	7.5	7.5	99.8	
	7.5	7.4	99.0	

Table : Accuracy table of Azelastine

% Level	Quantity of Spikes (ng/mL)	Quantity retrieved (in g/mL)	% Recovery	Mean % Recovery
50%	6.85	6.87	100.34	99.73 %
	6.85	6.80	99.26	
	6.85	6.88	100.40	
100%	13.7	13.58	99.13	
	13.7	13.77	100.50	
	13.7	13.71	100.09	
150%	20.55	20.36	99.08	
	20.55	20.47	99.59	
	20.55	20.38	99.16	

Discussion: The samples for each of the

three Accuracy levels were prepared using a standard addition technique. Mean %Recovery values of 99.94% and 99.73% were achieved when each dose of Fluticasone and Azelastine was injected three times for precision.

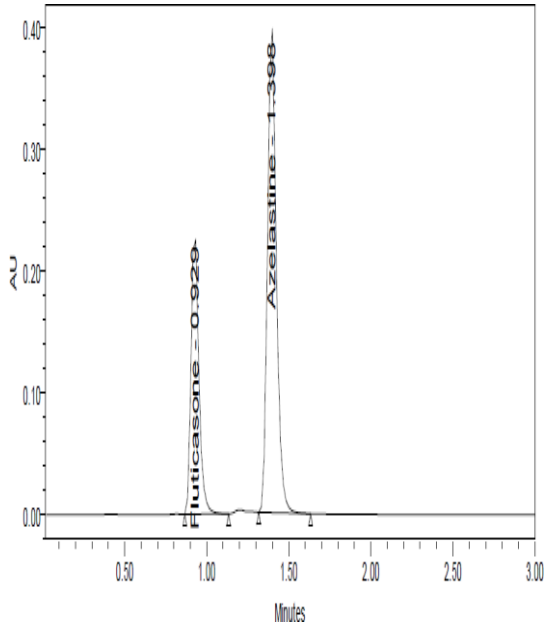

Figure : Fluticasone and Azelastine Chromatogram, 50% Accuracies

Conclusion

The systematic evaluation presented herein underscores the remarkable utility of Ultra-Performance Liquid Chromatography (UPLC) as a pivotal analytical technique for assessing drug concentrations in the realm of allergy and immune system interventions. Through an in-depth exploration of existing literature and case studies, this evaluation has elucidated the strengths, challenges, and wide-ranging applications of UPLC in this specialized field. UPLC's rapid analysis times, heightened sensitivity, and enhanced resolution have been revealed as crucial attributes that address the unique demands of drug concentration assessment in allergy and immune system interventions. The ability of UPLC to provide precise and accurate measurements even within complex biological matrices empowers researchers and clinicians to make informed decisions regarding therapeutic dosing and patient care. The high throughput nature of UPLC enables efficient processing of large sample sets, facilitating both preclinical research and clinical studies.

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