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Development and Application of HPLC and UFLC Methods for the Analysis of Anti Histaminic Drugs

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Abstract:
Research focused on medications used to treat allergic reactions. The results of this inquiry were contrasted with those that had already been released. In comparison to other works in the literature, it was determined that the current study had the best complete baseline separation and lowest detection limit. The retention, separation, and resolution factors were discovered to have ranges of 0.07-9.14, 1.44-4.21, and 2.15-18.66, respectively. The SPE and UFLC techniques created and verified for this purpose were used to examine human plasma samples for the presence of antihistaminic drugs. Peak retention, separation, resolution, and symmetry all matched the reference samples' values. The results for symmetry, separation, and resolution were all in agreement with the reference samples. The methods used to separate and identify Phenylephrine, cetirizine, loratadine, montelukast, and Ebastine in human plasma were found to be selective, effective, hardy, affordable, environmentally friendly, and reproducible, according to the authors. The selectivity of the SPE technique was validated by the absence of any secondary signal in the plasma samples. Additionally, there was no evidence of these drugs degrading in the plasma sample. This is the initial report on the simultaneous isolation and identification of these eight medications. The well-known SPE and UFLC techniques were successfully used to monitor these drugs in human plasma. Therefore, these drugs can be detected in any plasma sample using SPE or UFLC methods.

Keywords: Solid Phase extraction, Ultra fast liquid chromatography, Anti histaminic drugs

1. Introduction

The primary objective of the pharmaceutical business is to produce safe, effective, and high-quality pharmaceuticals. One of the various pharmaceutical procedures involved in creating a new medicine is analytical testing. The analytical results help decide how to proceed with development or if a therapeutic product should be available to the public. Each development or production process must be monitored and tweaked as needed to ensure it consistently produces high-quality, believable outcomes. When the quality of the method used to make a medicine is improved, the quality of the drug that results is enhanced as well. Analytical processes are very important in the development of medications. They play an essential role in supporting a wide range of other processes throughout the whole drug research and production lifecycle. High-performance liquid chromatography (HPLC) and ultra-performance liquid chromatography (UPLC) are two common LC methods; both are often used in reversed-phase mode and

detected using ultraviolet (UV) absorbance. Identification of varying amounts, significances, and relevance of analytes serves a variety of analytical applications. Assaying an API or identifying its metabolites and breakdown products often use analytical methods (1-4).

This study aims to develop and validate optimized Solid Phase Extraction (SPE) and High-Performance Liquid Chromatography (HPLC) methods for analysing anti-histaminic drugs in human plasma. The study will focus on achieving efficient extraction, separation, and quantification of the target drugs while adhering to regulatory guidelines for method validation. By analyzing human plasma samples, this research aims to provide valuable insights into drug concentrations, pharmacokinetic profiles, and potential drug-drug interactions, contributing to the advancement of clinical pharmacology and therapeutic drug monitoring.

2 Materials and Methods:

2.1 Drugs

Indian pharmaceutical companies have standardised the following: Phenylephrine, Cetirizine, Loratidine, Montelukast, and Ebastine (Nutra Specialities Pvt. Ltd., Bharathi Nagar, India). The urea, methanol, and HPLC-grade aprotic amine were kindly provided by Merck (Bombay, India). Both the o-H3PO4 and the NH4COOCH3 were supplied by Merck (in Bombay, India), and both were of A.R. quality. Manufacturer's Licence No. 504 (fresh frozen human plasma) was graciously provided by the Rotary Blood Bank located in New Delhi, India. Millipore Milli-Q (Bedford, MA, USA) was the company that produced Millipore water.

2.2 Methodology

2.2.1 Preparation of the Standard Solutions

These compounds were dissolved in eluent to form standard solutions of entity (1.0 mgmL-1) and mixture (0.0001-0.025 mgmL-1). The storage solutions were wrapped in aluminum foil to prevent them from touching the beam sample vials and kept cool (at 4 degrees Celsius). To achieve the range of concentrations required, these molecules were sequentially diluted using pipettes and a 10.0 mL volumetric flagon (5).

2.2.2 Extraction of the drugs from plasma by Solid Phase Extraction

Individually and in combination, the medicines mentioned here showed plasma diversification (6). In combination, 1.0 mL (1.0 mgmL-1) of each medication was thorned with 5.0 mL human plasma. The various plasma samples were stored in an incubator at 37 degrees Celsius for 24 hours. Each sample vial was given 15.0 mL of CH3COCH3 and set aside for 30 minutes. The supernatant was removed by centrifuging the samples at 10,000 rpm (11,180 g) for 10.0 minutes. The remaining pellet was resuspended in 10.0 mL of phosphate buffer (25 mM, pH 7.0) while the supernatant was evaporated under pressure. Independently and proportionally, 2.0 mL of MeOH and 5.0 mL of Millipore water were used to pre-condition 1.0 mL Sep- Pac C18 cartridges. 0.1 mLmin-1 flow rates of buffers containing a mixture of medicines were used, and the cartridges were tracked while being washed with Millipore (2.0 mL) water. In addition, hot air was used to dry these cartridges. A final volume of 10.0 mL of methanol at a flow rate of 0.1 mLmin-1 was used to elute the declared medicines. These medicines were eluted into MEOH solutions and concentrated to 0.5 mL under vacuum. The UFLC analyses utilized these samples.

2.2.3 UFLC Conditions

The UFLC mentioned above system was used for the experiments (7). Individually and proportionally, 5.0 L aliquots of the reference solutions for each medication and the combination were put into the UFLC apparatus. The eluent was a mixture of ammonium acetate (pH 7.0 with H3PO4) and acetonitrile (60:40, v/v) with a concentration of 0.05% TEA. To create a pH 7.0 acetate buffer (for mobile phase), 3.08 g NH4COOCH3 (0.05 M, Mol. Wt. 77.08) was weighed into a conical flask, followed by 800 mL of water and 0.4 mL of CH3CH2NH2. For 5 minutes, it was sonicated. O-phosphoric acid was used to raise the pH level to 7.0. The eluent was prepared by mixing 300 mL of acetate buffer solution with 200 mL of acetonitrile (ACN). Before each day's usage, it was filtered and degassed. The temperature was 45 1oC, and the flow rate was 1.0 mLmin-1. "The detection was at 210 nm. The retention (k), separation (a), and resolution (Rs) characteristics of ultra-high-performance liquid chromatography were calculated for the medications in question". Using UFLC, we determined to what extent we had control over the eluted peaks for each medication. Retention periods and peak regions were used to their fullest potential for qualitative and quantitative evaluation. The UFLC procedure was perfected and verified. The formulated and verified UFLC technique analyzed these medications in human plasma samples.

2.2.4 Validation

Adjusting several UFLC settings (FDA, 1995, 1997, 2000, 1994, 1987; USP, 2000) confirmed the reliability of the UFLC approach. Precision, specificity, robustness, accuracy, and ruggedness were determined; in addition to linearity, limit of detection (LOD), limit of quantitation (LOQ), precision, and limit of detection (LOQ). Minimum concentrations of these medications were used to establish the LOD and LOQ. "Microsoft Excel was used to conduct statistical analysis on the experimental data, including the calculation of confidence intervals, correlation coefficients, and the relative standard deviation (SD)." The correlation coefficients and relative standard deviations were calculated based on the calibration plots' linearity and the experimental points' low scatter. The wide range of experimental variables confirmed the method's reliability in determining the peak regions (8).

2.2.5 Linearity

Calibration plot least-squares linear regression analysis verified the UFLC method's linearity (9). Furthermore, the linearities of calibration plots (peak area versus quantity) were optimized and compared across various concentration ranges for metformin HCl, vildagliptin, gliclazide, linagliptin, sitagliptin, pioglitazone, glimepiride, repaglinide standards. The UFLC was filled with 5.0 L of the usual as described above. Statement drug UFLC chromatograms were generated separately and in tandem. "The observed peak areas vs. apparent quantities of metformin HCl, vildagliptin, gliclazide, linagliptin, sitagliptin, pioglitazone, glimepiride, and repaglinide were used to construct calibration curves."

2.2.6 Detection and Quantitation Limits

The limits of detection and quantification for the medicines mentioned were determined to be three and five epochs, respectively, above and below the baseline noise. The process adhered to the guidelines established by the United States Pharmacopoeia (USP, 2000) (10).

2.2.7 Specificity

The process's sensitivity was determined by monitoring the HPLC findings for any alterations brought on by a few adulterations in the standard samples. Very little uncut drug data was published, enough to throw off the standard models (11).

2.2.8 Precision

The described medications' precision values were determined at three different amounts or concentration

0.04, 0.05, and 0.06 mgmL-1. All three values were analyzed by HPLC using five resting-state tests (12).

2.2.9 Accuracy

HPLC accuracy was evaluated using varying amounts of the probe molecules. The range of concentrations used was from 0.04-0.06 mgL-1. Five separate optimum HPLC runs were performed (n = 5). Interpreting peak regions from five replicates of these reported medications allowed us to determine their accuracy (13).

2.2.10 Robustness

Robustness was determined by making a minor adjustment to variables like flow rate, temperature, eluent components, and max in chromatographic experiments. Peak area, peak form, and retention duration were compared between standard and slightly off-center experimental conditions (14).

2.2.11 Ruggedness

The method's durability was tested by introducing random variables into the experiments, such as various handlers and time intervals (15, 16).

2.2.12 Quantitative Analysis

The medications' quantitative examination used standard evaluation methods (17, 18). Quantitative drug determination was confirmed by comparing drug peak regions in both conventional and plasma samples. Limits of detection and quantification were determined using a range of drug concentrations. “Microsoft Excel was used to compute the results of the statistical analysis of the experimental data, including the average correlation coefficient, standard deviation, and confidence intervals.” The medication dosages were determined using the following equation. Standardization of the procedure using tyrosine as an internal standard allowed for determining the % recoveries of the medicines. Tyrosine was also used to determine the method's flaw. By comparing the spiked plasma amount to the actual amount recovered in the UFLC trial, we can calculate the % recoveries of the medications.

3. Results and Discussion

The repercussions and defense are divided into two portions, each dedicated to UFLC and solid phase extraction.

3.1 Solid Phase Extraction

These medications were extracted from the plasma samples using a solid-phase extraction method. We calculated the percent recoveries of phenylephrine HCl, cetirizine HCL, loratadine HCl, montelukast sodium, and Ebastine by doing blank tests. Recoveries of 80%, 78%, 85%, 94%, and 71% were targeted for phenylephrine HCl, cetirizine HCl, loratadine HCl, montelukast sodium, and Ebastine in laboratory-created samples in H2O. Table 1 shows 10, 12, 15, 06, and 29% plasma concentrations. The need to combine these drugs with plasma proteins led to low concentrations being found in the plasma samples. The SPE process was optimized by adjusting the flow rates of the plasma samples, the phosphate buffer, and the eluting solvents. That meant that MeOH was the optimal solvent for elution. Phosphate buffer (20.0 mM, pH 7.0) at a 0.1 mL/min flow rate recovered the maximum percentage of each medication. Table 2 displays the range of possible values for the correlation coefficient (R), the relative standard deviation (RSD), and the confidence level for these pharmaceuticals.

3.2 Optimization

Solid phase extraction is the gold standard technique for obtaining desired compounds. Cardio antihistamine medications in human Plasma were extracted using the same approach. Antihistamine

medication recoveries from reference samples varied between 71% and 94%. In plasma samples, these numbers ranged from 6% to 29%. These findings suggest that solid-phase extraction is the best approach for extracting cardiovascular medicines. Since antihistamine medications in plasma samples interacted with the protein molecules and prevented their extraction using SPE, the recoveries were lower than in reference samples. In addition, the selectivity of the solid phase extraction process was shown by the absence of secondary peaks in HPLC chromatograms. The Plasma's pH, flow rate, and eluting solvent flow rate were all optimized throughout the SPE process. Methanol, dichloromethane, ethanol, acetone, and ethyl acetate were all utilized as eluting solvents throughout the solid phase extraction method's optimization process. The following sections detail the experimental approach used to optimize SPE conditions.

3.3 Effect of pH of Plasma

Analyte recoveries as a percentage will shift depending on the Plasma's pH. Plasma analyte purity is achieved by adsorption on the silica gel of the C18 cartridge during solid phase extraction. Changes in plasma pH primarily control adsorption. This extraction procedure employed a wide pH range of 1.0 to 10.0 to determine how much the Plasma's pH affected the proportion of recovered material. Antihistamine medication recoveries as a percentage are shown in Figure 5.2 for various pH values. The chart shows that the percentage recoveries are low for low pH values and rise to a pH of 7.0. However, the % recoveries from extraction did not improve with further increases in pH. Maximum drug recoveries at pH 7.0 varied between 71% and 94%. Differences in ionic strength between the medicines account for the wide range of readings at pH 7.0.

3.4 Effect of Flow Rate of Plasma

Regarding solid phase extraction, the flow rate is one of the most crucial optimizing elements. When the plasma flow rate is low, the proportion of analytes recovered is high, and vice versa when the flow rate is high. This is why work has optimized the solid phase extraction for the best possible drug recoveries. This was accomplished by experimenting with several extraction flow rates, including 0.025, 0.05, 0.075, 0.10, 0.125, and 0.15 mL/min. Figure 3 displays the experimental findings, which reveal that the proportion of anti-histaminic medications recovered varied from 71 to 94 percent over various flow rates. Although the highest % recoveries were achieved at a flow rate of 0.025 mL/min, this rate was much too low to be used in experiments. Considering everything, a flow rate of 0.10 ml/min was optimal, with maximum percentage recoveries ranging from 71% to 94%. Therefore, our investigation relied on this flow rate throughout.

3.5 Effect of Other Solvents

The choice of eluting solvent also controls the optimality of the solid phase extraction. Therefore, several solvents were tested to find the most effective eluting solvent for the elution of critical amino acids via the C18 cartridge. In order to get the highest recoveries of the anti-histaminic medications from the C18 cartridge, methanol, dichloromethane, ethanol, ethanol, acetone, and ethyl acetate were utilized as the eluting medium. Figure 4 shows the relative percentages of drug recoveries achieved by using the five different solvents. This chart shows that methanol has the highest % recovery, followed by dichloromethane, ethanol, acetone, and ethyl acetate. After carefully analyzing these tests, methanol and ether yielded the highest and lowest drug recoveries. Maximum recoveries of the pharmaceuticals via the C18 cartridge were achieved using methanol as the eluting solvent. These solvents' unique characteristics may be attributed to their varying polarity and dielectric constants. Because of its high dielectric constant value and polarity, methanol is the most effective of these solvents for desorbing

analytes from the C18 cartridge. Dichloromethane, like methanol, has a relatively high dielectric constant and polarity, and hence it, too, yields respectable drug recovery percentages. However, methylene dichloride was not used in the trials since its flammability makes it dangerous for the researcher conducting the tests.

Furthermore, the remaining solvents The observed drug molecules did not dissociate from the C18 material of the solid phase cartridge at values of dielectric constants and polarities considered to be significant. Because of this, the reported drug recoveries were understated. Since methanol was shown to be the most effective eluting solvent, it was consistently employed in all of the tests.

3.6 Ultra Fast Liquid Chromatography

The ultra-fast liquid chromatographic parameters for phenylephrine HCl, cetirizine HCL, loratadine HCl, montelukast sodium, and Ebastine were calculated. Table 3 provides the values for various UFLC parameters. Retention factors varied from 2.00 to 11.00, separation factors from 1.15 to 2.31, and resolution factors from 1.00 to 6.07. Figures 5.5 and 5.6 show the UFLC chromatograms of the reference standards and plasma samples, respectively, for these medications. The baseline separation of the medications discussed is evident in this diagram. The retention durations of phenylephrine HCl, cetirizine HCL, loratadine HCl, montelukast sodium, and Ebastine were run and compared to identify the separated medicines. The lack of any observable secondary peak in the plasma samples confirmed the selectivity of the SPE technique.

3.7 Validation

Below, we describe the Linearity, LOD, LOQ, specificity, Precision, accuracy, robustness, and ruggedness of UFLC's validation metrics.

3.7.1 Linearity

Phenylephrine HCl, Cetirizine HCl, Loratadine HCl, Montelukast sodium, and Ebastine all had their Linearity of calibration curves (peak area versus concentration) evaluated over the concentration ranges of 0.01322 to 0.01925 mg mL-1, 0.003848 to 0.011544 mg mL-1, 0.003926 to 0.011778 mg mL-1, 0.007954 to 0. The graphs were linear over these reported concentration levels (n = 7). Graphs showed the relationship between the peak regions and the concentrations of phenylephrine HCl, cetirizine HCl, loratadine HCl, montelukast sodium, and Ebastine. The established curves were used to conduct the linear regression analysis. Phenylephrine HCl, Cetirizine HCl, Loratadine HCl, Montelukast sodium, and Ebastine all had correlation coefficients (r) (n = 7) of 0.9990, 0.9995, 0.9999, and 0.9990, respectively. In the concentration ranges studied, the 95% confidence interval (CI) and the relative standard deviation (RSD) values varied from 0.481 to 1.433% and 100.104 to 101.732%.

3.7.2 LimitsofDetectionandQuantitation

Phenylephrine HCl, Cetirizine HCl, Loratadine HCl, Montelukast sodium, and Ebastine each had a LOQ and LOD of 0.8292, 2.5126, 0.1553, and 0.4705, 0.3403, and 1.0312, and 0.6542, respectively, at 1.9823g mL-1.

3.7.3 Specificity

The provided procedure is rather detailed. All the compounds' retention periods in the reference samples were similar to those in the plasma samples. The contaminants added to the standards did not affect these medications' retention times or peak forms. The described UFLC technique was shown to have high specificity in these tests.

3.7.4 Precision

All described compounds' accuracy was measured at three concentrations: 0.001, 0.05, and 0.10 mg mL-1. For each of the three concentrations, five separate UFLC runs were performed. RSD values and confidence intervals varied between 0.16 and 1.06 percent and 99.0 and 99.0 percent, respectively.

3.7.5 Accuracy

The range of reported molecule concentrations was used to evaluate the performance of the chromatographic technique. Five UFLC tests were conducted with 0.001, 0.05, and 0.10 mg mL-1 (n = 5). Interpolating the peak regions of these medications in five replicates allowed us to determine their accuracies. The absolute error values fluctuated between 1.60 and 1.88%.

3.7.6 Robustness

We determined the method's stability by subjecting the UFLC tests to a variety of tweaks. Variations in solvent system composition, flow rate, temperature, and wavelength were used in the various experiments. “The retention time, peak area, and peak shape were examined in the established, slightly modified experimental circumstances.”

3.7.7 Ruggedness

Experimental settings, such as the number of days and number of operators, were varied to determine the robustness of the UFLC approach.

4 Conclusions

The results presented here indicate that the SPE and UFLC methods reported effectively separated and identified phenylephrine HCl, cetirizine HCL, loratadine HCl, montelukast sodium, and Ebastine in human Plasma. These methods were also robust, cost-effective, environmentally friendly, and reproducible. The lack of any secondary signal in the plasma samples confirmed the selectivity of the SPE technique. In addition, no deterioration of these medications was seen in the plasma sample. These five medicines have never before been reported to have been isolated and identified at the same time. Successful monitoring of these medications in human Plasma was achieved using the established SPE and UFLC techniques. This means that SPE and UFLC procedures may be used to test for the presence of these medicines in plasma samples.

5 Conflict of Interest:

None

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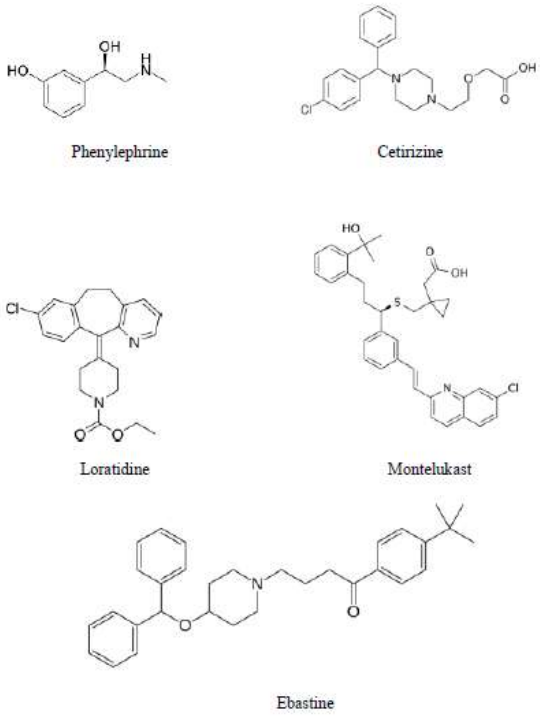

Figure 1: Chemical Structures of Anti Histaminic Drugs

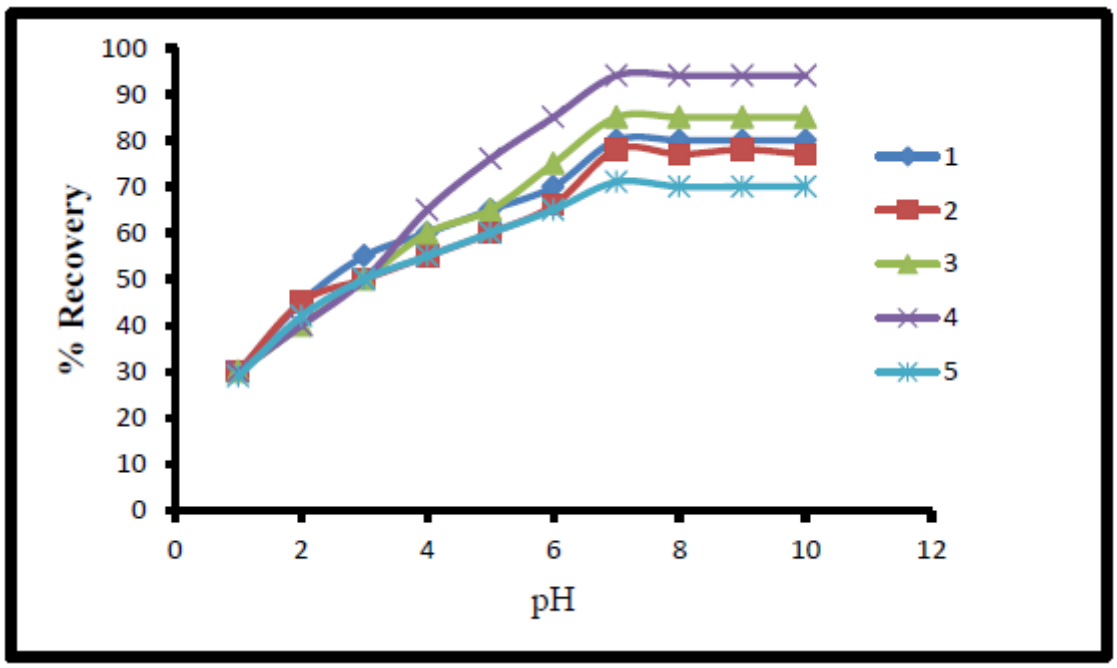

Figure 2:Variations in plasma pH and their impact on antihistamine medication recoveries. (1-Ebastine, 2-Montelukast sodium, 3-Loratidine HCl, 4-Phenylephrine HCl, and 5-Cetirizine HCl.)

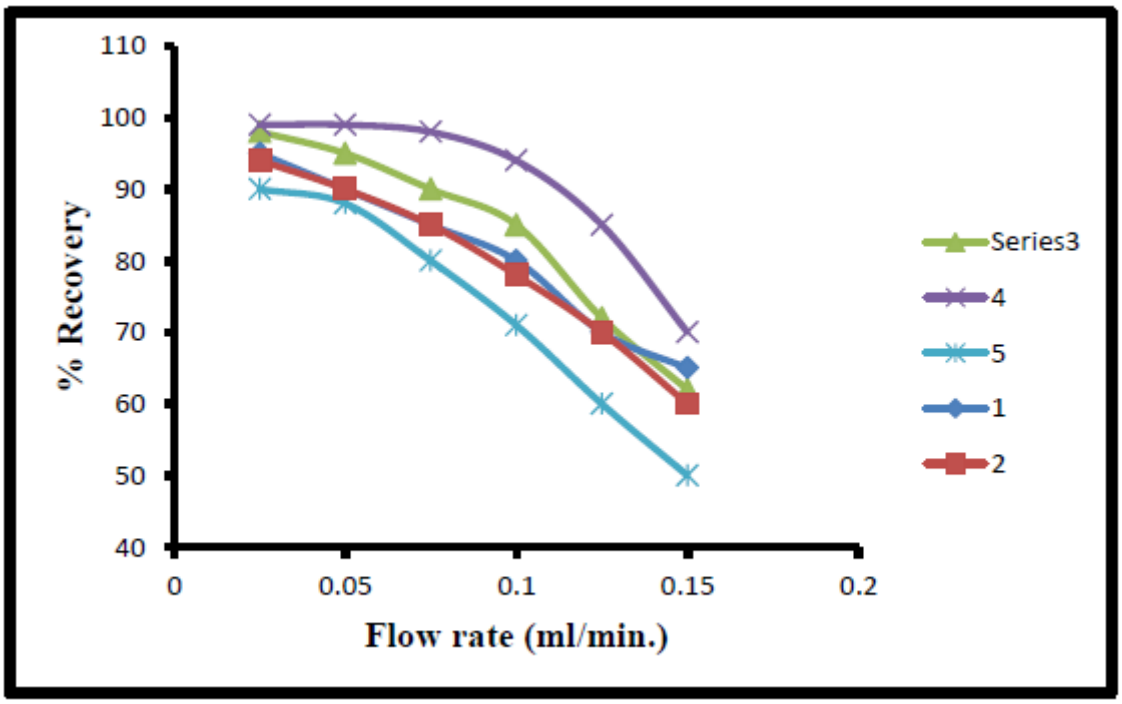

Figure 3: The variation in antihistamine medication recoveries across plasma flow rates. (1-Ebastine, 2-Montelukast sodium, 3-Loratidine HCl, 4-Phenylephrine HCl, and 5-Cetirizine HCl.)

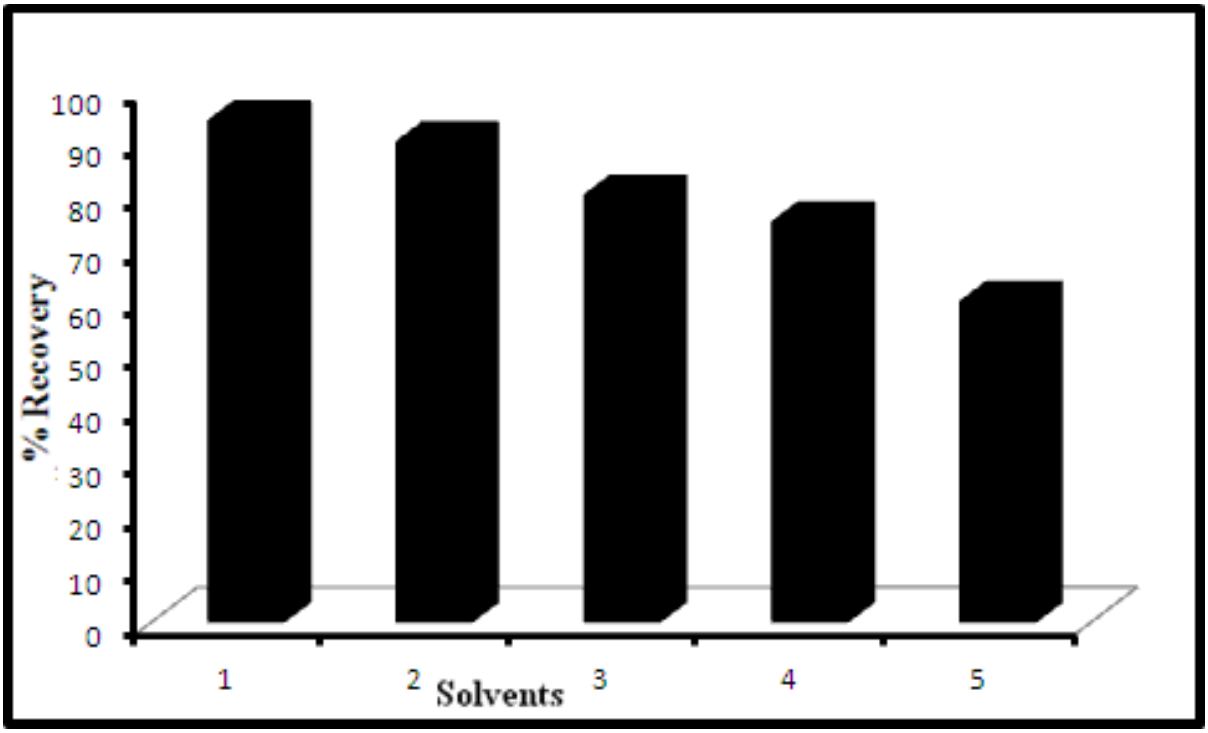

Figure 4:Percentage recoveries of antihistamines and their impact on the choice of solvent. (1-Ebastine, 2-Montelukast sodium, 3-Loratidine HCl, 4-Phenylephrine HCl, and 5-Cetirizine HCl.)

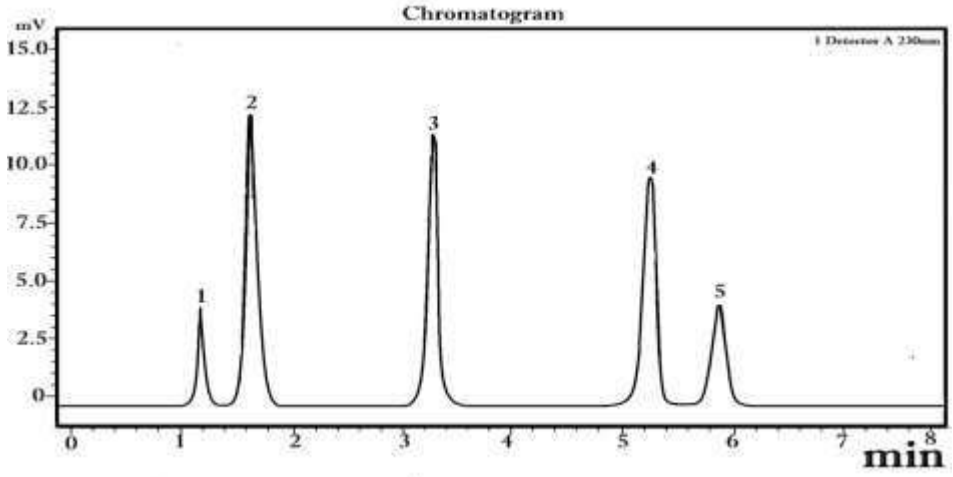

Figure 5:Chromatogram of Standard Antihistamine Solutions by Ultra-Fast Liquid Chromatography (1-Ebastine, 2-Montelukast sodium, 3-Loratidine HCl, 4-Phenylephrine HCl, and 5-Cetirizine HCl.)

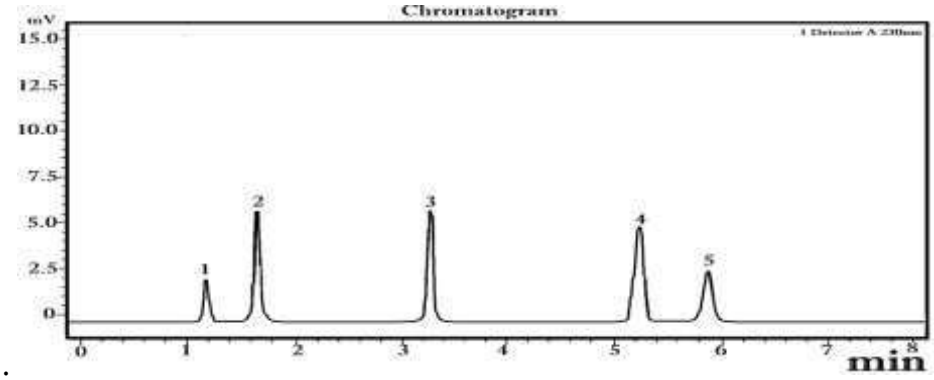

Figure 6:Antihistamines in Plasma, as seen by a UFLC chromatogram. (1-Ebastine, 2-Montelukast sodium, 3-Loratidine HCl, 4-Phenylephrine HCl, and 5-Cetirizine HCl.)

Table 1.Solid phase extraction % recoveries of synthetic and natural antihistamines from water and human plasma samples.

S.No.	Nameof drugs	% Recovery in water	%Recovery in humanPlasma
1.	Phenylephrine HCl	80	10
2.	Cetirizine HCl	78	12
3.	Loratidine HCl	85	15
4.	Montelukast sodium	94	06

5.	Ebastine	71	29

Table 2.Anti-histaminic medication SPE technique validation data.

S.No.	Drugs	%RSD	CorrelationCoefficient(r)	ConfidenceLevel(%)
1.	Phenylephrine HCl	1.5	0.9996	99.0
2.	CetirizineHCl	1.6	0.9997	99.0
3.	LoratidineHCl	1.3	0.9995	99.1
4.	Montelukast sodium	1.2	0.9994	99.1
5.	Ebastine	1.4	0.9996	99.0

Table 3: Capacity factors (k), separation factors (), and resolution factors (Rs) of antihistamine medications that may be determined using chromatography.

S. No.	Anti-histamines	k	α	R_s
1.	Phenylephrine-HCl	2.00	-	-
2.	Cetirizine-HCl	2.60	1.30	1.00
3.	Loratidine	6.00	2.31	6.07
4.	Montelukast sodium	9.60	1.60	4.95
5.	Ebastine	11.00	1.15	1.67