

Development and Application of HPLC and UFLC Methods for the Analysis of Cardiovascular Drugs

Kiran Joshi¹, Rajpurohit Bhavesh², Priyadarshini Ravindra Kamble³

^{1,2,3}Bhupal Nobles' College of Pharmacy, Faculty of Pharmacy, Bhupal Nobles' University, Udaipur, (Raj). 313001

Abstract:

Separation and identification of amiloride, metoprolol, hydrochlorothiazide, Carvedilol and amlodipine, in human plasma using the presented SPE and HPLC procedures was selective, efficient, robust, economical, environmentally friendly, and repeatable. Because plasma samples showed no evidence of a secondary peak, it was determined that the claimed drug-drug interaction between cardiovascular medications did not occur. Furthermore, no new peak was generated, indicating no metabolic product. This is the first report on isolating and identifying these nine cardiovascular medicines. Thus, SPE and HPLC techniques may be used to investigate these 5 cardiovascular medications. Successful monitoring of these medications in human plasma was achieved using the established SPE and HPLC technologies. 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 cardiovascular 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 amiloride, metoprolol, hydrochlorothiazide; Carvedilol and Amlodipine 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, cardiovascular drugs

1. Introduction

The development of methods for the estimate of those medications existing separately or in mixed dosage forms using various analytical techniques has a broad application. Quantitative and qualitative analysis are two fields of analytical chemistry that use a variety of suitable analytical techniques to

complete the goal of analysis. Qualitative analytical techniques reveal the identities of the atomic, molecular, or functional groups present in the material. Quantitative techniques reveal the relative concentration of one or more of these substances. Finding out what compounds are contained in a sample when it is completely unknown is typically the first necessity. Studies on the same issue can be utilised in a different method to determine whether contaminants are present in a given sample or to confirm that a sample is free of a particular impurity. Qualitative chemical analysis is the field that deals with these kinds of issues. Analysts are commonly asked to ascertain the amount of the specified constituent(s) present in the supplied sample, and these kinds of determinations fall under the purview of quantitative analytical chemistry. 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).

Our goal is to develop validated analytical methods in compliance with ICH guidelines using contemporary analytical instruments for active pharmaceutical ingredients (APIs), drug substances (DS), pharmaceutical formulations, and drug products (DP) for which there are no available methods. Many pharmaceutical analyses have established methods that are either not available in the literature or, in certain situations, require a lot of work and time to complete. Any development endeavour or project must use well-developed and tested analytical procedures for all analyses. It is impossible to guarantee the work's exceptional quality if such a procedure is not provided. Our goal is to develop validated analytical methods that use many validation variables, such as stability indicating research, to assess new pharmaceuticals from bulk and pharmaceutical formulations.

Therefore, analytical research is a difficult subject of study due to the difficulties involved in developing analytical methods and their enormous importance in drug discovery and development. 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

Standard Cardiovascular drugs (Amiloride, Metoprolol, Hydrochlorothiazide, Carvedilol and Amlodipine) were procured from the Nutra Specialities Pvt. Ltd., Bharathi Nagar, India. The urea, methanol, and HPLC-grade aprotic amine were kindly provided by Merck (Bombay, India). Both the $\text{o-H}_3\text{PO}_4$ and the $\text{NH}_4\text{COOCH}_3$ 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 ($0.0001\text{-}0.025 \text{ mgmL}^{-1}$). 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 flask (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 °C, 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 Amiloride, Metoprolol, Hydrochlorothiazide, Carvedilol and Amlodipine 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 Amiloride, Metoprolol, Hydrochlorothiazide, Carvedilol and Amlodipine 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 concentrations: 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 defence are divided into two portions, each dedicated to UFLC and solid phase extraction.

3.1 Solid Phase Extraction

The medication combination and any novel compounds or interferences were removed from the plasma samples using the SPE method. The percent recoveries of each cardiology drug were determined to monitor the efficacy of the stated SPE processes. “Amiloride, metoprolol, hydrochlorothiazide, carvedilol and amlodipine had their percent recoveries determined by blank experiments.” Table 1 displays the computed % revivals of various cardiac pharmaceuticals from plasma. Percentage recoveries for Amiloride, metoprolol, hydrochlorothiazide, carvedilol and amlodipine are shown in the following table to be 60.5, 30.0, 30.0, 30.0, 10.10, and 100. The percentages of these medicines still in circulation (after being bound to plasma proteins) ranged from 40% to 90%. “Varying the concentration and pH of the phosphate buffer and the flow rates of the plasma samples, phosphate buffer, and eluting solvents led to the optimization of SPE.” The alternatives of MeOH, EtOH, CH₃COOCH₂CH₃, and CH₂Cl₂ as eluting solvents were also tried. Extensive testing revealed that MeOH was the optimal eluting solvent. Methanol's polarity is sufficient for eliminating these cardioactive medicines from the C18 cartridges. Phosphate buffer (20.0 mM, pH 7.0) at a flow rate of 0.1 mLmin⁻¹ was shown to be optimal for reviving cardiovascular medicines at their maximal percentages individually and together. Table 2 displays the ranges of RSD, R, and confidence levels for these medications. The values are between 1.2 and 1.6, 0.9994-0.9995, and 99.2-99.5, respectively.

3.2 Optimization

Solid phase extraction is the gold standard technique for obtaining desired compounds. Cardio medications in human Plasma were extracted using the same approach. Cardiovascular medication reference samples had recoveries between 90% and 95% of the time. The results ranged from 10% to 65% in the plasma samples tested. These findings suggest that solid-phase extraction is the best approach for extracting cardiovascular medicines. Since some of the cardiovascular medicines in the plasma samples interacted with the protein molecules and prevented their extraction using SPE, the recoveries in the plasma samples were lower than in the 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. In addition, the solid phase extraction technique was optimized using several eluting solvents, including MeOH, CH₂Cl₂, EtOH, and CH₃COCH₃. The following sections detail the experimental methodology used to determine the optimal SPE settings via considerable testing.

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. 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. Adjustment of plasma pH is a central regulator of this sorption process. The pH range employed in this elimination method was from 1.0 to 10.0 to determine how the pH of the plasma affected the percentage of material recovered. Cardiovascular medication recoveries as a percentage for various pH values. The chart shows that the

percentage recoveries are low and increased to a pH of 7.0. Further increases further reduced percentage recoveries in the standards in pH. At a pH of 7.0, the highest drug recoveries were in the 90-95% range. The pH 7.0 values vary because the medicines have varied ionic strengths (Figure 1).

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. The results of the trials, which show that the percentage of revived heart function from the cardiovascular medications varied from 99 to 64 throughout the tested flow rates. Although the highest rate of revival in percentage occurred at a flow rate of 0.025 mLmin⁻¹, this rate was too low to be used in experimental settings. Considering everything, a flow rate of 0.10 mLmin⁻¹ was optimal, with the highest percentage recoveries in the 90-95% range. This flow rate was thus employed consistently throughout the analysis (Figure 2).

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. The C18 cartridge was used to elute MeOH, CH₂Cl₂, EtOH, CH₃COCH₃, and CH₃COOCH₂CH₃ to their maximum recoveries of cardiovascular medications. Figure 4 displays the percentage of drug recoveries achieved using these five solvents. The relative percentages of recovered analytes are MeOH > CH₂Cl₂ > EtOH > CH₃COCH₃ > CH₃COOCH₂CH₃. Based on a careful analysis of these tests, MeOH and CH₃COOCH₂CH₃ produced the highest and lowest drug revival percentages, respectively. Methanol was the most effective eluting solvent for reviving the medicines to their maximum levels in the C18 cartridge. These solvents' unique characteristics may be attributed to their varying polarity and dielectric constants. MeOH, with its superior dielectric constant evaluation and polarity, outperformed the others in the desorption process of the molecules throughout the C18 cartridge. In addition to MeOH, CH₂Cl₂ possesses a high dielectric constant and polarity, allowing for an excellent percentage of drug revivals. However, methylene dichloride was not used in the trials since its flammability makes it dangerous for the researcher conducting the tests. Bond dissociation between the analytes of the reported medications and the C18 material of the solid phase cartridge was not significantly affected by the magnitude of dielectric constants or polarity for the other solvents. This is why the stated drug revival percentages were given such low amounts. As a result, MeOH was shown to be the most effective eluting solvent, and it was consistently used throughout the tests (Figure 3).

3.6 Ultra Fast Liquid Chromatography

For amiloride, metoprolol, hydrochlorothiazide, carvedilol, amlodipine, the retention (k), separation (α), and resolution (Rs) factors were determined. Parameter values are listed in Table 3.3. Retention factors varied from 0.19 to 3.40, separation factors from 1.20 to 3.60, and resolution factors from 2.43 to 12.37. Retention periods varied from 2.64 to 11.60 hours, tailing factors from 1.20 to 1.34, and the number of theoretical plates from 25,883 to 14,482. Figure 3.5 and Figure 4 show HPLC chromatograms of various cardiovascular medications in reference materials and plasma, respectively. The baseline separation of the reported medications may be seen clearly in these numbers. "Running and comparing the retention durations of amiloride, metoprolol, hydrochlorothiazide, carvedilol, amlodipine, individually allowed us

to identify the separated medications”. No other peak was seen in plasma samples, indicating that cardiovascular medications did not interact. However, at higher concentrations of acetonitrile (40-50 mL), the peaks merged into a single new peak. Therefore, 35 mL of acetonitrile was determined to be optimal for the highest level of separation. Solvent system flow rates between 0.2 and 1.0 mLmin⁻¹ were tested. At low flow rates (0.2, 0.3, and 0.4 mLmin⁻¹), it was found that the peaks were poorly defined and had increased retention durations. On the other hand, when the flow rates were increased to 0.6-1.0 mLmin⁻¹, the peaks merged into a single, more prominent peak. In a nutshell, the peaks were separated at a flow rate of 0.5 milliliters per minute.

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

The Linearity of calibration curves (peak the area vs. concentration) for amiloride HCl, metoprolol tartrate, a drug called hydro amiloride, metoprolol, hydrochlorothiazide, carvedilol, amlodipine, standards were checked over the concentration ranges of 4.018-6.026 µgmL⁻¹, 4.504-6.756 µgmL⁻¹, 3.910-5.866 µgmL⁻¹, 3.936-5.894 µgmL⁻¹ and 4.099-6.149 µgmL⁻¹ respectively. Over these stated concentration ranges (n = 5), the displayed curves for the cardiovascular medications were linear. “As a function of concentration, the peak regions of amiloride, metoprolol, hydrochlorothiazide, carvedilol, amlodipine, were graphed.” The resulting curves were used to estimate the linear regression analysis. “Amiloride HCl, metoprolol tartrate, hydrochlorothiazide, carvedilol, amlodipine besylate, frusemide, telmisartan, losartan potassium, and olmesartan were all found to have correlation coefficients (r) (n = 5) of 0.9997, 0.9995, 0.9998, 0.9992, 0.9998, 0.9997, and 0.9998, respectively”. RSD values and confidence intervals ranged from 0.322 to 0.722 percent and 98.277 to 100.964 percent, respectively, over the tested concentration ranges.

3.7.2 Limits of Detection and Quantitation

Lowest Detectable Dose (LDD) and Limit of Quantitative Determination (LOQ) values for amiloride, metoprolol, hydrochlorothiazide, carvedilol, amlodipine, were 0.2273, 0.6886, 0.2285, 0.6924 and 0.3019.

3.7.3 Specificity

All compounds' retention durations were almost identical between the reference solutions and the plasma samples. The contaminants in the standards did not alter these compounds' retention periods and peak shapes. The results showed that the described approach was rather specific.

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 cardiovascular drugs 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. Separation and identification of amiloride, metoprolol, hydrochlorothiazide, carvedilol, amlodipine, in human plasma using the presented SPE and HPLC procedures was selective, efficient, robust, economical, environmentally friendly, and repeatable. Because plasma samples showed no evidence of a secondary peak, it was determined that the claimed drug-drug interaction between cardiovascular medications did not occur. Furthermore, no new peak was generated, indicating no metabolic product. This is the first report on isolating and identifying these nine cardiovascular medicines. Thus, SPE and HPLC techniques may be used to investigate these nine cardiovascular medications. Successful monitoring of these medications in human plasma was achieved using the established SPE and HPLC technologies. Most of the world's population is ill or troubled in some way. The prevalence of cardiovascular disease is of particular concern. The rising number of people using antidepressants and antihistamines is a problem for everyone. That is why creating and developing rapid, low-cost, selective, efficient, and effective analytical techniques for analyzing these medicines in various matrices and core-shell porous columns is crucial. Chromatography stands above other analytical methods owing to its speed, accuracy, and reliability. A literature search revealed several HPLC approaches that may be used to test cardiovascular, antidiabetic, and antihistamine medications. It was discovered that these techniques had several flaws. The main drawbacks are expensive experiments, large chemical consumption, slow processing times, adverse environmental effects, and low quantification precision. In addition, pharmaceuticals need sample preparation prior to HPLC analysis. A review of the available literature suggests that liquid-liquid extraction is used to prepare the samples. This approach has several drawbacks, such as the high cost of the chemicals used, the length of time required, the lack of concern for the environment, and the low efficiency of the extraction process. In light of the above, scientists throughout the globe have been working on perfecting eco-friendly SPE, HPLC, and UFLC technologies for analyzing cardiovascular, antidiabetic, and antihistamine medications.

5 Conflict of Interest

None

6 References

- Jehangir M, Ahmed D, Ahmed M, Naeem M. Validated stability indicating HPLC and UFLC assay for the determination of azithromycin: Application to solid dosage and biological samples. *Lat. Am. J. Pharm.* 2020 Jan 1;39(8):1491-9.
- Ramaswamy S, Gowthamarajan K, Priyanka Dwarampudi L, Bhaskaran M, Kadiyala M. Analytical method development, validation and forced degradation studies for rutin, quercetin, curcumin, and piperine by RP-UFLC method. *Drug Development and Industrial Pharmacy.* 2021 Apr 3;47(4):562-8.
- Purvini K, Chethan IA, Vaijanathappa J. Novel Analytical Method Development, Validation and Stability Study of Anticancer Drug Erlotinib in Tablet Dosage Form by RP-UFLC. *Indian Journal of Pharmaceutical Education & Research.* 2021 Apr 2;55.
- Qureshi HK, Veeresham C, Srinivas C. Analytical Method Development and Validation of Filgrastim by UV and RP-UFLC Methods. *American Journal of Analytical Chemistry.* 2021 Oct 22;12(10):333-46.
- Srinivasa MS, Chandan RS, Koganti VS. Validated RP-UFLC Method Development of Paclitaxel in Pure and Pharmaceutical Dosage Form. *Pharmaceutical Methods.* 2017 Jan 1;8(1).
- Shivabasappa PM, Diksha H, Chouhan MK, Satyappa S, Jalalpure SS. Method development and validation of ciprofloxacin HCl and ornidazole by UFLC in combined dosage form. *Indian J Pharm Educ Res.* 2019 Jul 1;53(3):S373-9.
- Panda SS, Ravi Kumar Bera VV, Beg S, Mandal O. Analytical quality by design (AQbD)-oriented RP-UFLC method for quantification of lansoprazole with superior method robustness. *Journal of Liquid Chromatography & Related Technologies.* 2017 May 28;40(9):479-85.
- Aboras SI, Korany MA, Abdine HH, Ragab MA. HPLC/Fluorescence-Diode Array Detection for Rapid and Reliable Determination of Illegal Synthetic Drugs in Male Sexual Herbal and Honey Remedies: Comparative Study with UFLC-MS. *Journal of AOAC International.* 2022 Sep 1;105(5):1288-98.
- NAGAVI JB, Gurupadaya BM. RP-UFLC Method Development and Validation for Simultaneous Estimation of Clopidogrel, Pantoprazole and Rosuvastatin in Human Plasma: Drug Interaction Studies. *Int. J. Pharm. Pharm. Sci.* 2014;6:490-6.
- Lingamaneni K, Annapurna MM. Development and validation a new stability indicating RP-UFLC method for the quantification of Voriconazole. *Research Journal of Pharmacy and Technology.* 2021;14(1):420-6.
- Girme A, Pawar S, Ghule C, Shengule S, Saste G, Balasubramaniam AK, Deshmukh A, Hingorani L. Bioanalytical method development and validation study of neuroprotective extract of Kashmiri saffron using ultra-fast liquid chromatography-tandem mass spectrometry (UFLC-MS/MS): In Vivo pharmacokinetics of apocarotenoids and carotenoids. *Molecules.* 2021 Mar 23;26(6):1815.
- Panda SS, RAVI KUMAR BV, Mohanta G, Dash R, Patel PK. New stability-indicating RP-UFLC method for determination of tiroprisium chloride in tablet dosage form. *Scientia pharmaceutica.* 2012 Dec;80(4):955-64.

14. Ali I, K Dutta K, K Jain A, A Alothman Z, Alwarthan A. Synchronized fast SPE and UFLC methods for the analyses of eight antihistaminic drugs in human plasma. *Combinatorial Chemistry & High Throughput Screening*. 2017 Mar 1;20(3):208-14.
15. Yasaswini RS, Annapurna MM, Sheela AS. New stability indicating RP-UFLC method for the determination of Trifluridine–A potent antiviral drug. *Research Journal of Pharmacy and Technology*. 2020;13(6):2881-5.
16. Aluri SG, Annapurna MM. New stability indicating RP-UFLC method for the quantification of Efavirenz in pharmaceutical dosage forms. *Research Journal of Pharmacy and Technology*. 2022;15(5):1981-8.
17. Tuzimski T, Szubartowski S, Gadzała-Kopciuch R, Miturski A, Wójtowicz-Marzec M, Kwaśniewski W, Buszewski B. Comparison of DAD and FLD detection for identification of selected bisphenols in human breast milk samples and their quantitative analysis by LC-MS/MS. *Journal of AOAC International*. 2020 Jul 1;103(4):1029-42.
18. Vogeser M. Quantification of circulating 25-hydroxyvitamin D by liquid chromatography–tandem mass spectrometry. *The Journal of steroid biochemistry and molecular biology*. 2010 Aug 1;121(3-5):565-73.
19. Zhou B, Xiao JF, Tuli L, Resson HW. LC-MS-based metabolomics. *Molecular BioSystems*. 2012;8(2):470-81.

Table 1. The percentage recoveries of cardiovascular drugs from human plasma.

Name of drugs	% Recovery by SPE from human plasma	% of drug interaction with human plasma
Amiloride HCl	60.0	40.0
Metoprolol tartrate	65.0	35.0
Hydrochlorothiazide	30.0	70.0
Carvedilol	10.0	90.0
Amlodipine besilate	30.0	70.0

Table 2. Validation data of SPE method.

Drugs	%RSD	Correlation Coefficient (r)	Confidence Level (%)
Amiloride HCl	1.5	0.9995	99.5
Metoprolol tartrate	1.6	0.9995	99.5
Hydrochlorothiazide	1.2	0.9994	99.3
Carvedilol	1.3	0.9994	99.3
Amlodipine besilate	1.6	0.9995	99.5

Table 3: Tailing factor (T), number of theoretical plates (NTP), separation factor (Rs), resolution factor (Rs), and retention time (Rt).

S.No.	Drugs	Rt	k	α	Rs	Tfactor	NTP/meter
1.	AmilorideHCl	2.64	1.33	025883
2.	Metoprololtartrate	3.13	0.19	..	2.86	1.34	034283
3.	Hydrochlorothiazide	3.58	0.36	1.91	2.52	1.34	042686
4.	Carvedilol	6.01	1.28	3.59	12.37	1.33	084358
5.	Amlodipinebesilate	6.64	1.52	1.19	2.93	1.29	096847

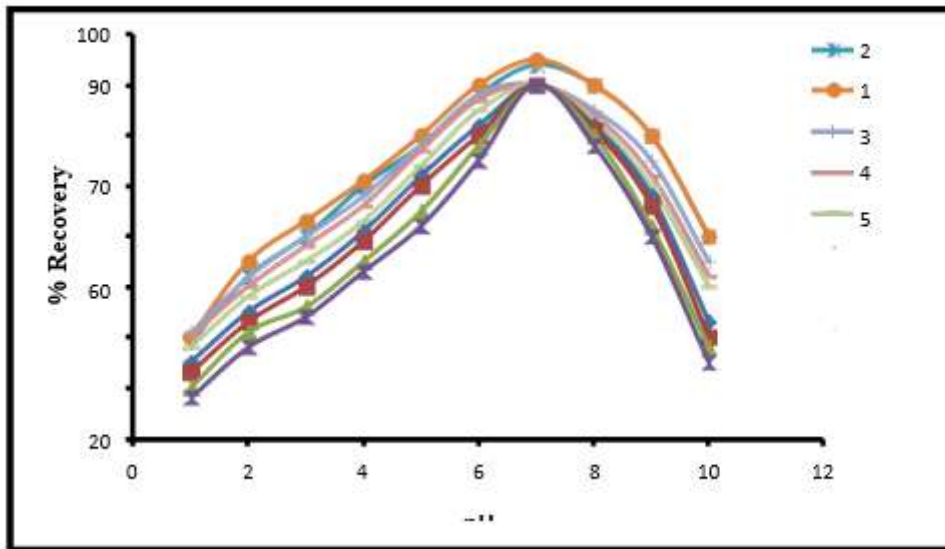


Figure 1: The Effect of pH of Plasma on the percentage recoveries of cardiovascular drugs. 1. Amiloride, 2 Hydrochlorothiazide, 3 Metoprolol, 4 Carvedilol and 5 Amlodipine

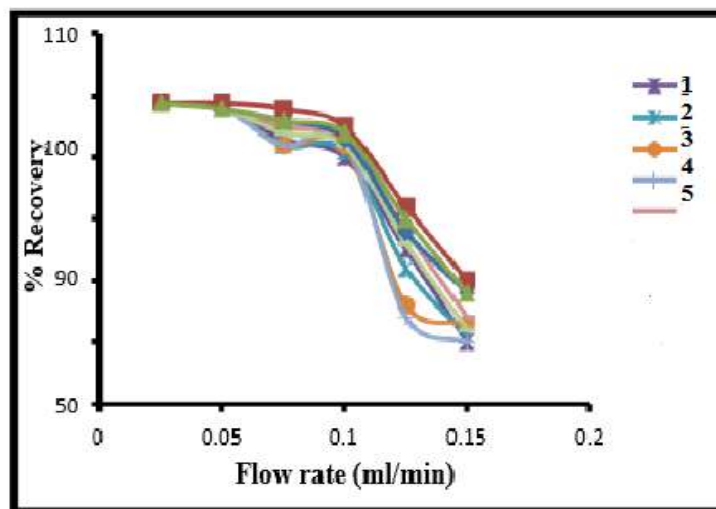


Figure 2: The Effect of different plasma flow rates on the percentage recoveries of cardiovascular drugs. 1. Amiloride, 2 Hydrochlorothiazide, 3 Metoprolol, 4 Carvedilol and 5 Amlodipine

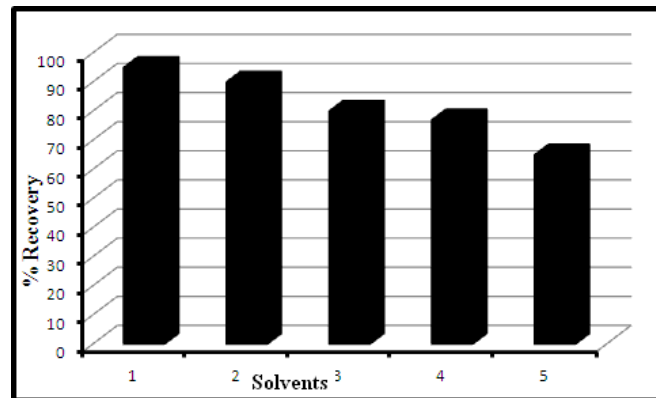


Figure 3: The Effect of different solvents on the percentage recoveries of cardiovascular drugs. Ethyl acetate, acetone, methanol, dichloromethane, ethanol, and methanol.