VOLUME 11 SPECIAL ISSUE 1 2025

ISSN 2454 - 3055

Manuscripts under Special issue are published as Conference Proceedings organized by Department of Pharmaceutical Technology, Brainware University, India under the theme "Drug Discovery: Advances, Challenges and Perspectives"

GUEST EDITOR: Dr. Shaikh Ershadul Haque,
Professor, Department of Pharmaceutical Technology, Brainware University, Barasat, WB-700125, Kolkata, India

GUEST CO-EDITORS: Dr. Saptarshi Samajdar,
Associate Professor, Department of Pharmaceutical Technology, Brainware University, Barasat, WB-700125, Kolkata, India

Mr. Sreejan Manna,
Associate Professor, Department of Pharmaceutical Technology, Brainware University, Barasat, WB-700125, Kolkata, India

INTERNATIONAL JOURNAL OF ZOOLOGICAL INVESTIGATIONS

Forum for Biological and Environmental Sciences

Published by Saran Publications, India

International Journal of Zoological Investigations Vol. 11 (Special Issue 1), 150-158 (2025)

International Journal of Zoological Investigations
Contents available at Journals Home Page: www.ijzi.net
Editor-in-Chief: Prof. Ajai Kumar Srivastav
Published by: Saran Publications, Gorakhpur, India

ISSN: 2454-3055

Reverse Phase High Performance Liquid Chromatographic Method and Method Validation of Empagliflozin by using Single Mobile Phase

Choudhury Tirthankar[1] and Pathak Baisnab Das[2]*

[1]Department of School of Pharmacy, ITM University, Gwalior 474011, M.P, India
[2]Department of Pharmaceutical Technology, Brainware University, 398 Ramkrishnapur Road, Barasat, West Bengal 700125, India

**Corresponding Author*

Received: 14th September, 2024; **Accepted:** 5th October, 2024; **Published online:** 27th June, 2025

https://doi.org/10.33745/ijzi.2025.v11ispl1.019

Abstract: In the pharmaceutical industry, employing computerized methods for development and validation significantly improves drug analysis. This study describes the establishment, validation, and utilization of a simple, sensitive, and accurate high-performance liquid chromatography (HPLC) technique for measuring Empagliflozin with UV detection at 284 nm. Empagliflozin tablets from two different brands, namely Empaone 25 by MSN Laboratories Ltd., Hyderabad, India and Jardiance 25 by Boehringer Ingelheim, Mumbai, were obtained for examination. By utilizing a Hypersil ODS C18 reversed-phase column and a mobile phase comprising phosphate buffer (pH 6.8) and water (95:5 v/v) at a flow rate of 1.0 ml/min, effective separation of compounds was achieved. The linear range for Empagliflozin was found to be 50-200 µg/ml, with limits of quantitation (LOQ) at 8.51 µg/ml and limits of detection (LOD) at 4.155 µg/ml. This investigation conclusively illustrates the possibility of analysing Empagliflozin using a single mobile phase via reversed-phase liquid chromatography, providing both sensitivity and selectivity.

Keywords: Empagliflozin, ODS column, RP-HPLC, Empaone, Jardiance

Citation: Choudhury Tirthankar and Pathak Baisnab Das: Reverse phase high performance liquid chromatographic method and method validation of empagliflozin by using single mobile phase. Intern. J. Zool. Invest. 11(Special Issue 1): 150-158, 2025.

https://doi.org/10.33745/ijzi.2025.v11ispl1.019

Introduction

The process of ensuring that analytical methods are appropriate for their intended purposes is known as methods validation. The initial stage involves carefully collecting and organizing validation data to support the analytical procedures. Following this, a review chemist assesses both the validation data and analytical methods. A validated method is one that consistently produces a product meeting predetermined specifications under controlled

conditions. In the pharmaceutical industry, validation studies are essential components of Good Manufacturing Practice (GMP) and Good Laboratory Practice (GLP), and they must comply with established standards. A comprehensive written report summarizing the findings and recommendations should be created and maintained. Guidance on adhering to GMP and GLP requirements is provided by organizations such as the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH), the United States Food and Drug Administration (USFDA), and the European Union, specifically the European Medicines Agency.

Empagliflozin is a sodium glucose co-transporter-2 (SGLT-2) inhibitor used alongside diet and exercise to improve glycaemic control in adults with type 2 diabetes. SGLT2 co-transporters facilitate glucose reabsorption from the kidney's glomerular filtrate. Chemically, empagliflozin is (1S)-1,5-Anhydro-1-C-[4-chloro-3-[[4-[[(3S)-tetrahydro-3-furanyl] oxy]phenyl] methyl]phenyl]-D-glucitol, with the molecular formula $C_{23}H_{27}ClO_7$. Classified as a flavonoid, empagliflozin acts as an antioxidant. While sparingly soluble in water, it readily dissolves in phosphate buffer (pH 6), appearing as a yellowish-white crystalline powder.

The literature review indicates that developed methods were primarily used for biological fluids and buffer solutions. Challenges are particularly pronounced when working with buffer solutions and biological fluids due to several factors: (i) Biological fluids such as blood, urine, or tissues often contain trace amounts of drugs or their metabolites; (ii) Presence of endogenous pigments can lead to analytical errors if not adequately controlled; (iii) Drug-protein binding can result in slow recovery; (iv) Preparation of buffer solutions from scratch is necessary; (v) Monitoring medication plasma levels in patients poses challenges, requiring substantial plasma samples for each patient; and (vi) Therapeutic Drug Monitoring (TDM) involves prospective clinical investigations, yielding conflicting findings and emphasizing the importance of considering multiple factors during TDM (Marzolini *et al*., 2002).

Although there is no necessity to prepare phosphate buffer-water from scratch due to its high recovery rate, our objective was to simplify and economize testing procedures by developing a single, straightforward, rapid, reliable, and cost-effective method for analyzing Empagliflozin using Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) in individual pharmaceuticals.

Materials and Methods

Chemicals and Reagents

S.D. Fine Pvt. Ltd. (Mumbai, India) provided HPLC-grade phosphate buffer, while Universal Laboratories Pvt. Ltd. (Mumbai, India) provided triple-distilled water. The standard marketed dugs were purchased from MSN Laboratories Ltd., Hyderabad (Empaone 25) and Boehringer Ingelheim, Mumbai (Jardiance 25), respectively (Ramachandran *et al*., 2006). The pure bulk drugs were supplied from MSN Laboratories Ltd., Hyderabad, India.

Instrumentation

Shimadzu Corporation, Switzerland, HPLC apparatus from the LC-10AT VP series, which includes a UV-Visible detector, manual injector with a 20-l loop, and Hypersil ODS C18 column (250 mm X 4.6 mm i.d., 5 m particle sizes), was used (Ramachandran *et al*., 2006).

Chromatographic condition

Base deactivated silyl bonded Hypersil C18 reversed phase column (250 mm x 4.6 mm i.d., 5 µm) was used for the chromatography. Phosphate buffer (PH-6.8) and water were the mobile phase in a 95:5 (v/v) ratio (Ramachandran *et al*., 2006). There was a 1 ml/min flow. During the analysis, the column was maintained at 25.0± 0.10 °C; the injection volume was 20 µl, and the detection

Table 1: Condition applied

Column Mode	:	C18 RP-HPLC
Detector	:	UV – Visible
Type of Analysis	:	Peak area and peak height
Detection Limit	:	284 nm
Flow Rate	:	1.0 ml/min
Run Time	:	10 min
Injection volume	:	20 µl

wavelength was 284 nm (Table 1).

Solution Preparation

Empagliflozin (100 mg) was weighed in a 100 ml volumetric flask, dissolved in phosphate buffer, and diluted to volume with the same solvent of these solutions. 1 ml was then further diluted to 100 ml with mobile phase to create the stock and working standard solutions for Empagliflozin (Persiani *et al*., 1996; Malaty and Kupper, 1999).

Stock and working standard solution

Empagliflozin (100 mg) was weighed in a 100 ml volumetric flask, dissolved in 95 ml of methanol, and then diluted to a volume of 100 ml using the same solvent. To create working standard solutions containing Empagliflozin (10 g/ml) from these solutions, 1 ml was further diluted to 100 ml with mobile phase.

Preparation of internal standard solution

Empagliflozin standard, accurately weighed at 100 mg, was taken into 100 ml volumetric flasks, mixed with 60 ml of methanol in each, and then sonicated for 30 min to obtain 1 mg/ml (1000 µg/ml) standard stock solution (Barry *et al*., 1999; Rezk *et al*., 2002).

Preparation of the sample solutions

In a mortar and pestle, the twenty tablets (Empaone 25 and Jardiance 25) were precisely weighed and finely ground. A 100 mg equivalent was taken in a volumetric flask of 100 ml from the powder (Hollanders *et al*., 2000; Cenacchi *et al*., 2000, 2011). This was sonicated for 30 min with internal shaking after being dissolved in 60 ml of phosphate buffer. Finally, 100 ml of mobile phase were added to the volume to produce a clear solution containing 1 mg/ml. Aforementioned solution was centrifuged at 3000 rpm for 5 minutes (Little *et al*., 1989; Barry *et al*., 1999).

Results and Discussion

Method Validation

There were numerous mobile phase compositions tested in order to optimise the HPLC parameters. To improve reproducibility and repeatability, a mobile phase made up of phosphate buffer and water (95:5 v/v) led to a successful separation and peak symmetry for Empagliflozin. Based on the peak area, quantification was accomplished using UV detection at 284 nm. It was discovered that the peaks had better resolution and a distinct base line separation (Fig. 1). Empagliflozin has limits of detection (LOD) of 4.155 µg/ml and limits of quantitation (LOQ) of 8.51 µg/ml. The work demonstrated that Empagliflozin can be determined using a single mobile phase utilising reversed-phase liquid chromatography, which is sensitive and selective.

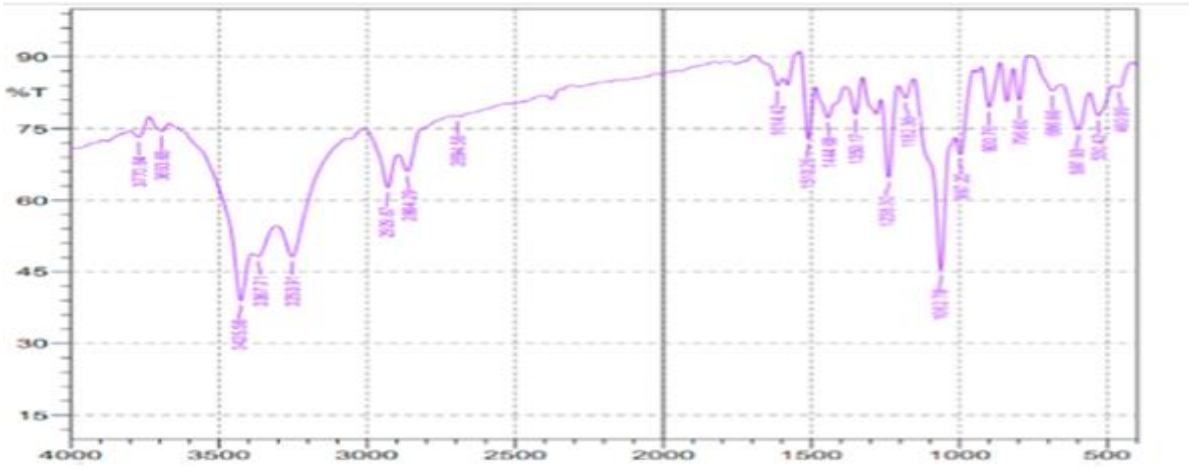


Fig.1: IR Spectrum for Empagliflozin.

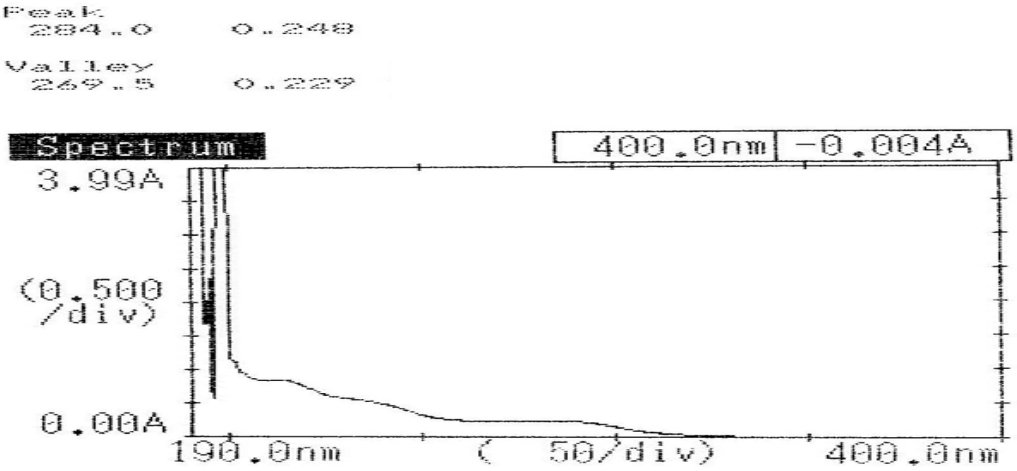


Fig. 2: UV Spectrum for Empagliflozin (λmax= 284 nm).

Specificity (*Selectivity*)

By contrasting the chromatograms produced from samples and the equivalent placebo, the selectivity of the RP-HPLC technique was evaluated (Barry *et al*., 1999; Avolio *et al*., 2007). While the active ingredients are easily soluble in phosphate buffer or the mobile phase, tablet additives are essentially insoluble in these media (Saha *et al*., 2013). The tablet ingredients had no effect on the results.

Linearity

Analysis of standard plots connected to the nine-point standard calibration curve was used to establish the linearity of the procedure. Regression analysis of the calibration standard and the measurement of the drug concentration were both used to compute the analytes' concentrations using a straightforward linear equation (Barry *et al*., 1999; Avolio *et al*., 2007; Parameswari *et al*., 2023). The calibration standards' peak area ratio values correlated with the medicines.

Each sample's peak area was plotted against its relative Empagliflozin concentration, and it was discovered that this relationship was linear between 25 and 200 g/ml. Regression analysis was used to generate the linear equation Y= 15.67x + 3.2132 with an R^2 = 0.9999. By analysing

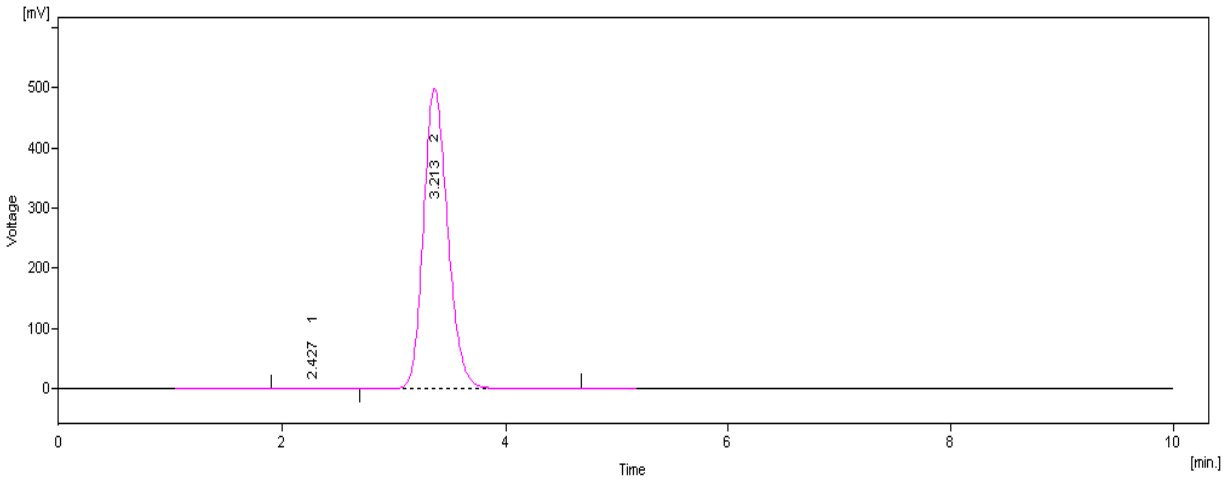


Fig. 3: Peak area and peak height Empagliflozin (Pure drug).

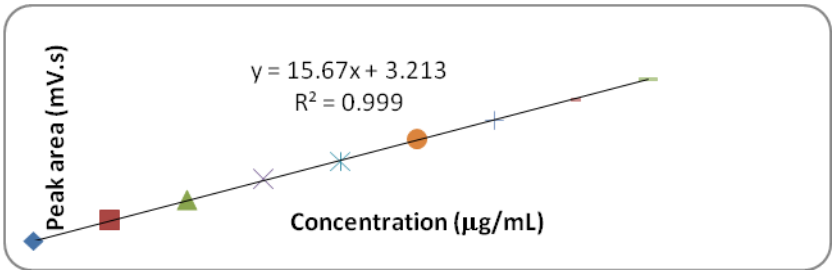


Fig. 4: Standard calibration curve of Empagliflozin (Pure drug).

standard plots connected to an eight-point standard calibration curve, the linearity of the procedure was confirmed (Fan and Stewart 2002; Mistri *et al*., 2007; Mukthinuthalapati and Bukkapatnam, 2015).

Accuracy

The conventional addition approach was used to conduct the recovery studies. For Empagliflozin (Brand 1 and Brand 2), the percent ages of the recoveries were 99.703% and 100.186%, respectively, and the outcomes are displayed in (Table 2). The technique recovered well.

Precision

System precision

When the method is used repeatedly to do multiple sampling of a homogeneous sample, the accuracy of an analytical method is measured by the degree of agreement among the individual test findings. The relative standard deviation (% RSD) or percentage coefficient of variation (% CV) were used to calculate the accuracy.

Repeatability is the application of the analytical technique over a brief period of time (within a single day) in a laboratory, using the same analytes with the same tools. By injecting three different concentrations of the same standard medication three times on the same day, repeatability was tested. The repeatability test for the technique was successful since the RSD was less than 2% (Table 3, Fig. 5).

Intermediate precision

Participate in the assessment of analytical variance when a method is used within a laboratory, such as on various days (inter-day). By injecting three distinct standard concentrations in triplicate on three separate days, intermediate accuracy was examined. Since

Table 2: Recovery studies (for accuracy)

Labeled claim 75mg	Amount added (mg)	% Recovery*
Brand 1 (Empaone 25)	10 20 30	99.38 100.12 99.1
Brand 2 (Jardiance 25)	10 20 30	100.25 99.85 100.06

*Mean of five determinations

Table 3: Intraday precision of Empagliflozin by the proposed RP- HPLC method

Conc. Empagliflozin (µg/ml)	Mean area (n= 3)	Standard deviation	%RSD (n= 3)
50	740.666	10.689	1.43
100	1557.629	25.568	1.641
200	3179.414	39.857	1.253

Table 4: Inter- Day precision of Empagliflozin by the proposed RP- HPLC Method

Conc. of Empagliflozin (µg/ml)	Mean area (n= 9)	Standard deviation	%RSD (n= 9)
50	782.0973	4.093	0.523
100	1557.834	22.003	1.412
200	3144.218	44.302	1.409

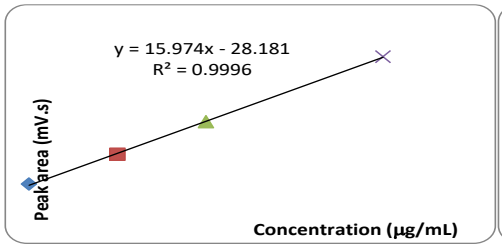


Fig. 5: Intraday- graph of Empagliflozin.

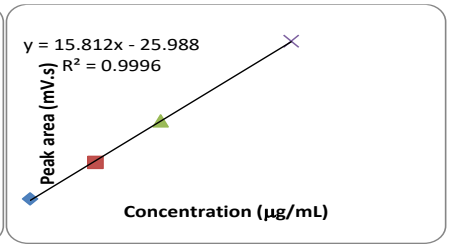


Fig 6: Inter day- graph of Empagliflozin

the RSD was less than 1.5% (Table 4, Fig. 6), the approach passed the test for intermediate accuracy.

Ruggedness

The technique graph was tested on a different analyte with a different batch of chemicals. The results did not vary significantly. These investigations demonstrated the method's rugged.

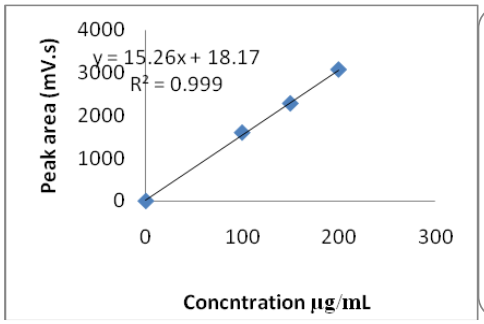
Robustness

By intentionally making tiny but purposeful changes to the flow rate, mobile phase, and temperature, the method's robustness was examined. At three distinct concentrations and temperatures (ranging from (±1˚C to ±5˚C), the effects of flow rate modification (±1 ml/min) were investigated. At three different concentrations, the impact of varying the mobile phase ratio was also investigated (Table 7).

ratio was also investigated (Table 7).

System suitability test

Using this approach, the relative standard deviation (% RSD) for Empagliflozin was



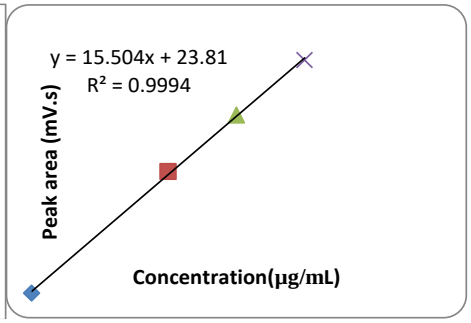


Fig. 7: Analyst-1 Empagliflozin. Fig. 8: Analyst -2 Empagliflozin.

Table 5: Analyst-1 for Empagliflozin

Concentration	**Peak area (mV.s)***	**SD**	**%RSD***
100	1595.588	4.005	0.251
150	2277.222	25.512	1.120
200	3067.112	29.163	0.950

*Average of three values

Table 6: Analyst-2 for Empagliflozin

Concentration	**Peak area (mV.s)***	**SD**	**%RSD***
100	1615.169	4.216	0.261
150	2362.835	25.541	1.080
200	3094.138	10.674	0.344

*Average of three values

Table 7: Effect of variations in the flow rate of (-1.0 ml/min) for Empagliflozin

Concentration	Retention Time*	Peak area (mV.s)*	SD	%RSD
50	3.547	779.509	1.971	0.252
100	3.543	1486.747	11.066	0.747
200	3.557	2718.083	22.777	0.837

*Average of three values

Table 8: System suitability test for Empagliflozin

Parameters	Values
Retention Time	3.22
HETP (mm)	0.009
Tailing factor	1.113
Theoretical plates/m	110099.84
LOD (µg/ml)	3.155
LOQ (µg/ml)	9.61

Table 9: Empagliflozin regression statistics

Concentration range(µg/ml)	Regression equation	Regression coefficient (R^2)
50-200	Y=15.97X – 28.18	R^2=0.999
50-200	Y=15.81X-25.98	R^2=0.999

determined to be 1.03. The entire set of outcomes fell inside the permitted range. Chromatographic procedures often include system suitability studies to ensure that the system's resolution and repeatability are sufficient for the analysis to be carried out. All of the criteria for system appropriateness had values that fell within acceptable bounds.

Regression analysis

Empagliflozin regression statistics for intraday and inter-day are shown in Table 9. With a regression value of 0.999, the method's linearity was shown in the concentration range of 50-200 µg/ml, indicating its acceptability for analysis.

Assay of the tablet dosage form

Empagliflozin in pharmaceutical items was effectively identified using the suggested validated approach. The findings for Empagliflozin were equivalent to the levels that were labelled (Table 2).

In the foregoing study, the liquid chromatographic technique was explained, and the outcomes were provided. Limits of detection (LOD) were determined to be 3.155 µg/ml, limits of quantitation (LOQ) were 9.61 µg/ml, and recovery rates for two commercially available L Ornithine L Aspartate Silymarin, Zinc, Amino Acid and Vitamin based formulations, respectively, were 99.703% and 100.186%, with a regression coefficient of 0.9999 (Intraday and Inter-day). So, the technique was demonstrating its validity. The regression coefficient (r2) value of 0.999 was obtained from the standard calibration curve using the concentration of 50-200 µg/ml (Y=15.97X - 28.18, Y=15.81X-25.98).

Conclusion

For the study of Empagliflozin in pharmaceutical formulations, a high-performance liquid chromatographic approach has been developed that is straightforward, dependable, repeatable, and affordable. The disclosed approach may be utilised to analyse Empagliflozin in tablet or other pharmaceutical formulations both qualitatively and quantitatively. The obtained limits of detection (LOD) were 4.155 µg/ml and 8.51 µsg/ml for the limits of quantitation (LOQ).

Ethical Statement

No animal or microbes have been used or sacrificed for this study, hence Ethical Approval not required.

Author Contributions

Choudhury Tirthankar: Conceptualization; research work; *Pathak Baisnab Das*: Writing; Planning. All authors have read and agreed to the published version of the manuscript.

Acknowledgements

The authors acknowledge ITM University's School of Pharmacy (SOP), Gwalior and The Central Instrumentation Laboratory of Brainware University, 398 Ramkrishnapur Road, Barasat, Kolkata:700125, West Bengal, India, for providing the resources necessary to conduct the research.

Conflict of Interest

The authors declare no conflicts of interest for this study.

References

Avolio DA, Siccardi M, Sciandra M, Lorena B, Bonora S, Trentini L and Perri GD. (2007) HPLC–MS method for the simultaneous quantification of the new HIV protease inhibitor darunavir, and 11 other antiretroviral agents in plasma of HIV-infected patients.J. Chromatographia 859: 234.

Barry M, Mulcahy F, Merry C, Gibbons S and Back D.

(1999) Pharmacokinetics and potential interactions amongst antiretroviral agents used to treat patients with HIV infection. Clin Pharmacokinet. 36: 289.

Cenacchi V, Baratte S, Cicioni P, Frigerio E, Long J and James C. (2000) LC–MS–MS determination of exemestane in human plasma with heated nebulizer interface following solid-phase extraction in the 96 well plate format. J Pharm Biomed Anal. 22(3): 451-460.

Cenacchi V, Barattè S, Cicioni P, Frigerio E, Long J and James C. (2011) LC-MS-MS determination of exemestane in human plasma with heated nebulizer interface following solid-.chromatography. J Chromatogr Sci. 49(8): 634-639.

Fan B and Stewart JT. (2002) Determination of zidovudine/lamivudine/nevirapine in human plasma using ion-pair HPLC. Pharm Biomed Anal. 28: 903.

Hollanders RMF, Kolmer EB, Burger DM, Wuis EW, Koopmans PP and Hekster YA. (2000) Determination of nevirapine, an HIV-1 non-nucleoside reverse transcriptase inhibitor, in human plasma by reversed-phase high-performance liquid chromatography. J Chromatogr B Biomed Sci Appl. 744: 65-71.

Little BB, Bawdon RE, Christmas JT, Sobhi S, Gilstrap LC. (1989) Pharmacokinetics of azidothymidine during late pregnancy in Long-Evans rats. Am J Obstet Gynecol. 161(3):732-734.

Malaty LI and Kupper JJ. (1999) Drug interactions of HIV protease inhibitors. Drug Saf. 20(2): 147-169.

Marzolini C, Beguin A, Telenti A, Schreyer A, Buclin T, Biollaz J and Decostered LA. (2002) Determination of lopinavir and nevirapine by high-performance liquid chromatography after solid-phase extraction: application for the assessment of their transplacental passage at delivery. J Chromatogr B 774: 127.

Mistri HN, Jangid AG, Pudage A, Gomes N, Sanyal M and Shrivastav P. (2007) High throughput LC–MS/MS method for simultaneous quantification of lamivudine, stavudine and nevirapine in human plasma. J Chromatogr B 853: 320.

Mukthinuthalapati MA and Bukkapatnam V. (2015) A novel validated stability-indicating RP-HPLC method for the determination of exemestane (steroidal aromatase inhibitor). J Bioequiv Availab 7: 288-292.

Parameswari SA, Alagusundaram M, Keshri P, Venkateswarlu G and Ratnam MV. (2023) Development of an analytical method and validation of exemestane tablet by uv spectrophotometry. Curr Topics Chem Biochem. 8: 1-11.

Persiani S, Broutin F, Cicioni P, Stefanini P and Benedetti MS. (1996) Determination of the new aromatase inhibitor exemestane in biological fluid by automated high-performance liquid chromatography followed by radioimmunoassay. European J Pharm Sci. 4(6): 331-340.

Ramachandran G, Hemanthkumar AK, Kumaraswami V and Swaminathan S. (2006) A simple and rapid liquid chromatography method for simultaneous determination of zidovudine and nevirapine in plasma. J Chromatography B Analytical Technol Biomed Life Sci. 843: 339-344.

Rezk NL, Richard R, Tidwell and Kashuba, AD. (2002) Simple and rapid quantification of the non-nucleoside reverse transcriptase inhibitors nevirapine, delavirdine, and efavirenz in human blood plasma using high-performance liquid chromatography with ultraviolet absorbance detection. J Chromatogr B 774: 79.

Saha S, Dey BK, Chakrabotry S and Choudhury T. (2013) Development of reverse phase high performance liquid chromatographic method and method validation of paracetamol by using economical single mobile phase. J Appl Pharm Res. 1(1): 36-41.