International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 4 July-Aug 2024, pp: 879-888 www.ijprajournal.com ISSN: 2456-4494

# New RP- HPLC Method Development and Validation for the Estimation of Feropenam

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Date of Submission: 25-07-2024 Date of Acceptance: 05-08-2024

**ABSTRACT**
The present developed and validated High Performance Liquid Chromatographic (HPLC) method is to determine Faropenem in tablet dosage form. Faropenem was identified and characterized as it was bitter, odorless and yellow colored powder melted at 134 °C. Faropenem was freely soluble in methanol, soluble in ethanol, slightly soluble in 0.1 N NaOH and 0.1 N HCl, sparingly soluble in water and phosphate buffer. RP-HPLC method was developed Retention Time was 7.47 min at flow rate of 1.0 ml/min. Mobile phase was selected methanol:Water (70:30) pH-3 total run time was 12 min. Developed method was validated on various parameters according to ICH guideline. System suitability parameters were found tailing factor 1.253, capacity factor 2.03, similarity factor1.0 and % RSD of STD Faropenem for Area 0.064% and % RSD of STD Faropenem for Retention time was 0.061%. The values of linearity were determined with $R^2$ value 0.999. Precision was found within 2.0% of RSD for both Repeatability of sample and Repeatability of Injection. Limit of detection (LOD) and limit of quantification (LOQ) were found. Accuracy was performed on three spiked labels. Robustness for change in analyst was also under limit. Estimation of Faropenem in marketed formulation was obtained. Over all this developed method was linear, accurate, précised and robust according to ICH guideline.

**KEYWORD:** Vlidation, HPLC, Faropenem, ICH guideline, Repeatability

## I. INTRODUCTION

The concept of drug treatment, which was earlier "right drug for right person" is now changing from "right does for the right person" to "right time of the does for the right person"[1]. It is necessary to find the concept of each drug either in bulk or single or combined dosage forms for purity testing[2]. It is also essential to know the concentration of the drug and it's metabolites in biological fluids after taking the dosage for treatment. Analytical methods can be separated into classical and instrumental[3]. Classical methods (also known as wet chemistry methods) use separations such as precipitation, extraction, and distillation and qualitative analysis by color, odor, or melting point[4]. Instrumental methods use an apparatus to measure physical quantities of the analyte such as light absorption, fluorescence, or conductivity[5]. Analytical chemistry is also focused on improvements in experimental design, chemometrics, and the creation of new measurement tools to provide better chemical information[6].

Method validation can be defined as (ICH) "Establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics"[7]. Method Validation, however, is generally a one-time process performed after the method has been developed to demonstrate that the method is scientifically sound and that it serves the intended analytical purpose[8]. The scope of developing and validating and analytical method is to ensure a suitable method for a particular analyte more specific, accurate and precise[9]. The main objective for that is to improve the condition and Parameters, Which should be followed in the development and validation[10].

Review of literature reveals that good analytical methods are not available for the drug like Feropenam. There are very few methods in the literature for the estimation of Feropenam in dosage forms. The development of analytical

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 4 July-Aug 2024, pp: 879-888 www.ijprajournal.com ISSN: 2456-4494

method for the determination of drugs in bulk, in dosage forms or in body fluids have received attention in recent years because of their importance in quality control, bioavailability and pharmacokinetic study etc.

## II. MATERIALS AND METHOD

All solvent and reagents used in this experiment were belongs to HPLC grade and A.R. grade.

**(A) Procedure of Method Development**

**(a) Selection of Solvent:** The ideal properties of the solvent to be used in UV-Visible Spectrophotometry include:

1. Faropenem should show solubility in the solvent used.
2. Drug should show stability in the selected solvent.
3. Drug should obey Beer-Lambert's Law over a appropriate range of analytical Concentrations.
4. The solvent should be to the extent, be economic.

After taking above consideration on various solvents, Analytical Grade Methanol was selected as a solvent of analysis.

**(b) Selection of Wavelength:** Dissolve 10mg of Faropenem in methanol to produce 100 ml. 1 ml of the stock solution was taken and was further diluted with methanol up to 10 ml to produce a conc. of 10µg/ml. It was scanned on UV-Visible spectrophotometer between wavelength ranges of 200 nm to 400 nm.

**(c) Preparation of Mobile Phase:** Mobile phase prepare by mixing Methanol (HPLC grade) and water (HPLC grade) in selected proportion. Prepared Mobile phase taken separately filtered through membrane nylon filters of size 4.5 µ, to the filtered solution 1.5 ml of Ammonium Hydroxide Solution added and the mixed solution then sonicated for 15 minutes and filtered through membrane nylon filters of size 4.5 µ.

**(d) Determination of Retention Time:** Weigh accurately 10 mg of Faropenem into 10 ml volumetric flask, add some amount of methanol, sonicate to dissolve and further diluted to 10 ml with methanol. The working standard solution of Faropenem was injected into the chromatograph separately and their retention time recorded at detection wavelength of 309 nm.

**(B) Preparation of Working Standard Mixture**

**(a) Standard stock solution:** Weigh accurately Faropenem into a 10 ml volumetric flask. Add sufficient amount of methanol, sonicate to dissolve, cool and dilute up to the mark with methanol.

**(b) Standard solution:** above stock solutions were further diluted 1 ml to 10 ml with methanol.

**Method Development:** The method development and establishment phase defines the chemical assay. The fundamental parameters for an analytical method validation are accuracy, precision, selectivity, sensitivity, reproducibility, and stability. Typical method development and establishment for a analytical method include determination of (1) selectivity, (2) accuracy, precision, recovery, (3) calibration curve, and (4) stability of analyte in spiked samples.

## III. RESULTS AND DISCUSSION

**Identification of drugs:** Faropenem was identified and characterized as it was bitter, odorless and yellow colored powder melted at 134 °C. Faropenem was freely soluble in methanol, soluble in ethanol, slightly soluble in 0.1 N NaOH and 0.1 N HCl, sparingly soluble in water and phosphate buffer. RP-HPLC method was developed using $C_{18}$ Column (25×0.46cm, i.d,5 µm), and Retention Time was 7.47 min at flow rate of 1.0 ml/min, $\lambda_{max}$ of Faropenem was found to be 309 nm by UV spetrophotometric investigation. Solution was stable about 20 hrs.

**Method Development for Assay by RP-HPLC Method:**

**(A) Selection of mobile phase:** Different combinations of solvents using isocratic mode is used for mobile phase selections.

1. Mobile Phase: Water and Acetonitrile (90:10)
2. Mobile Phase: Methanol and Water (50:50).
3. Mobile Phase: Methanol and water (60:40) and pH- 3 attained with O -Phoshphoric acid
4. Mobile Phase: Methanol and Water (70:30) and pH- 3 attained with O -Phoshphoric acid

Satisfactory separated single peak for Faropenem was obtained with solvent system of Methanol and Water (70:30) and pH- 3 attained with O - Phoshphoric acid.

**(B) Method Optimization:**

**Chromatographic conditions:** to develop method the for assay of Faropenem chromatographic conditions used were as Column $C_{18}$ Column (25×0.46cm, i.d,5 µm), maintained temperature $25^0$ C, Flow rate 1.0ml/min, Isocratic mode, Injection Volume was 20µl, total run time was 12 Min,

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 4 July-Aug 2024, pp: 879-888 www.ijprajournal.com ISSN: 2456-4494

Wavelength used 309 nm with Methanol and Water (70:30) and pH- 3.0 as mobile phase.

**(C) Effect of ratio of mobile phase:** After confirming the mobile phase, change in the ratio of mobile phase was done for the optimization of the peak. The ratio of Water: Acetonitrile 90:10, Water: Methanol 50: 50, 60: 40, 70:30 were tried. In that case 70:30 v/v shows good retention time and resolution.

**(D) Effect of flow rate:** After confirming the ratio of mobile phase, flow rate of the mobile phase was changed, at 0.8ml/min it shows increased retention time the flow rate of 1.2 ml/min resulted in fronting of the peak. The flow rate of 1ml/min has given a good result.

**(E) Selection of column:** The literature review showed the usage of C-18 column for the determination of Faropenem. Mostly C-18 column is used for analytical purpose and the column selected was YMC pack pro C18column. The columns with different dimensions were tried but that showing shifting of retention time. YMC pack pro C18 (100 × 4.6mm, 5µ) column shows good result.

**(F) Selection of detector wavelength:** The sensitivity of the HPLC method that uses UV detector depends upon the proper selection of wavelength. An ideal wavelength selected by UV-spectra of Faropenem that was the point gives maximum absorbance and good response for the drugs to be detected. A UV spectrum of Faropenem was recorded at $\lambda_{max}$ at 309 nm. Also, 309 nm was selected for the proposed study.

**(G) Prerparation of Solvent mixture:** 700.0ml of Methanol (HPLC grade), 300ml of water (HPLC grade) was taken separately filtered through membrane nylon filters of size 4.5 µ, to the filtered of Ortho phosphoric acid was added and the mixed solution was sonicated for 15 minutes and filtered through membrane nylon filters of size 4.5 µ.

**(H) Determination of Retention Time (RT) Faropenem:** Weigh accurately 10 mg of Faropenem into 10 ml volumetric flask add some amount of methanol, sonicate to dissolve and further diluted to 10 ml with methanol.

The working standard solution (100µg/ml) of Faropenem was injected into the chromatograph separately and their retention time recorded at detection wavelength of 309 nm. The result is presented below.

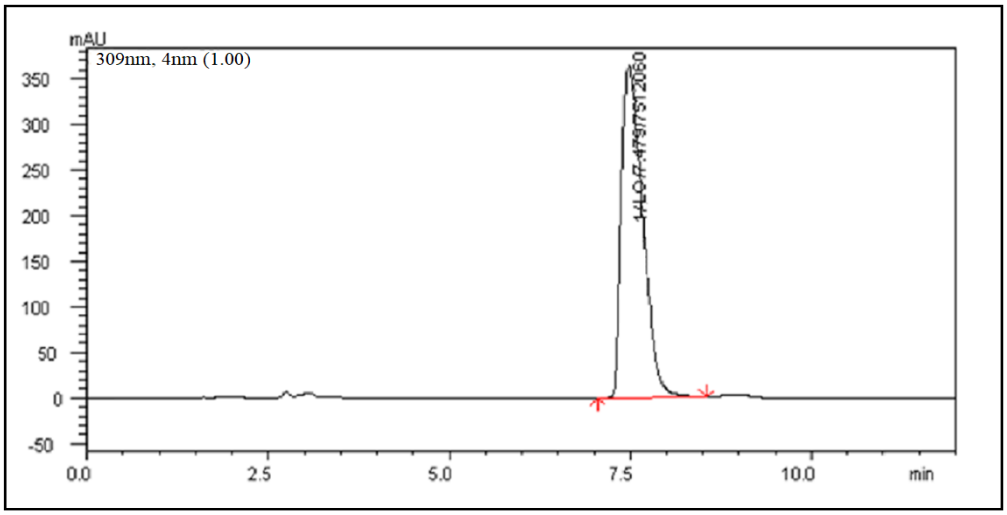



**Figure 1: HPLC chromatogram of Faropenem Working STD**

**Observation:** Retention time of Working Standards of Faropenem was 7.47 min.

**Method Validation of Faropenem estimation by RP-HPLC**

**(A) Specificity and system suitability:**

**Stationary phase:** $C_{18}$ 250 mm X 4.6 mm, 5µ, Inertsil ODS 3V.

**Mobile phase:** 700 ml of Methanol, 300ml of water (HPLC grade)

**Detector parameter:** UV at wavelength 309 nm.

**Flow rate:** 1 ml/min.

**Injection volume:** 20 µl

**Column oven temperature:** $25^0$ C.

**Mode:** Isocratic

**Retention time:** 7.47 min.

**Blank:** Methanol:water (70:30)

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 4 July-Aug 2024, pp: 879-888 www.ijprajournal.com ISSN: 2456-4494

**Run Time:** 12 minutes

**Table No. 1: Data for Specificity test of Faropenem**

| Sample Name | Area µAU*sec Faropenem | Retention Time Faropenem | Similarity factor for Faropenem |
|---|---|---|---|
| STD 1 | 1428838 | 7.580 | 1.00 |
| STD 2 | 1427258 | 7.572 | |
| STD 3 | 1428844 | 7.580 | |
| %RSD | 0.064 | 0.061 | |

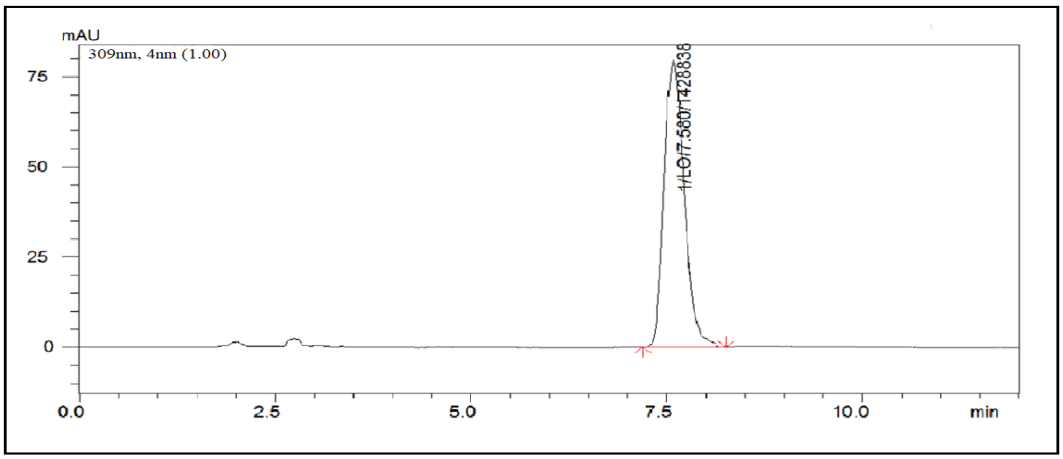



**Figure 2: HPLC chromatogram of Specificity of Faropenem**

**Table No. 9: Data for System Suitability**

| **Parameter** | **Acceptance Criteria** | **Faropenem** |
|---|---|---|
| Tailing Factor | **NMT 2** | 1.253 |
| Capacity Factor | **NLT 2** | 2.03 |
| Similarity Factor | **0.98 to 1.02** | 1.0 |
| %RSD of STD Faropenem for Area | **NMT 2** | 0.064% |
| %RSD of STD Faropenem for Retention time | **NMT 2** | 0.061% |

**Result:** The given method is specific.

**(B) Linearity:** Weigh accurately 10mg of Faropenem into 10 ml of volumetric flask. Add sufficient amount of methanol, sonicate and dilute up to mark with methanol (1000µg/ml).

**Sample 1:** 1ml of Stock Solution of drug in 10ml volumetric flasks and diluted it up to 10ml with methanol (100µg/ml).

**Sample 2:** 7.5ml of Sample 1 solution of drug was taken in 10 ml of volumetric flasks with pipette and diluted with methanol up to the mark (75µg/ml).

**Sample 3:** 5 ml of sample1 solution of drug was taken in 10 ml of volumetric flasks with pipette and diluted with methanol up to the mark (50µg/ml).

**Sample 4:** Take 2.5ml of sample1 solution of each drug in two, 10 ml of volumetric flasks with pipette and dilute them with methanol up to the mark (25µg/ml).

**Table No. 2: Linearity of Standard Faropenem**

| **Sample Name** | **Concentration Faropenem (µg/ml)** | **Area µAU*mi Faropenem** |
|---|---|---|
| Sample 4 | 25 | 379544 |
| Sample 3 | 50 | 744941 |
| Sample 2 | 75 | 1062460 |
| Sample 1 | 100 | 1428838 |

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 4 July-Aug 2024, pp: 879-888 www.ijprajournal.com ISSN: 2456-4494

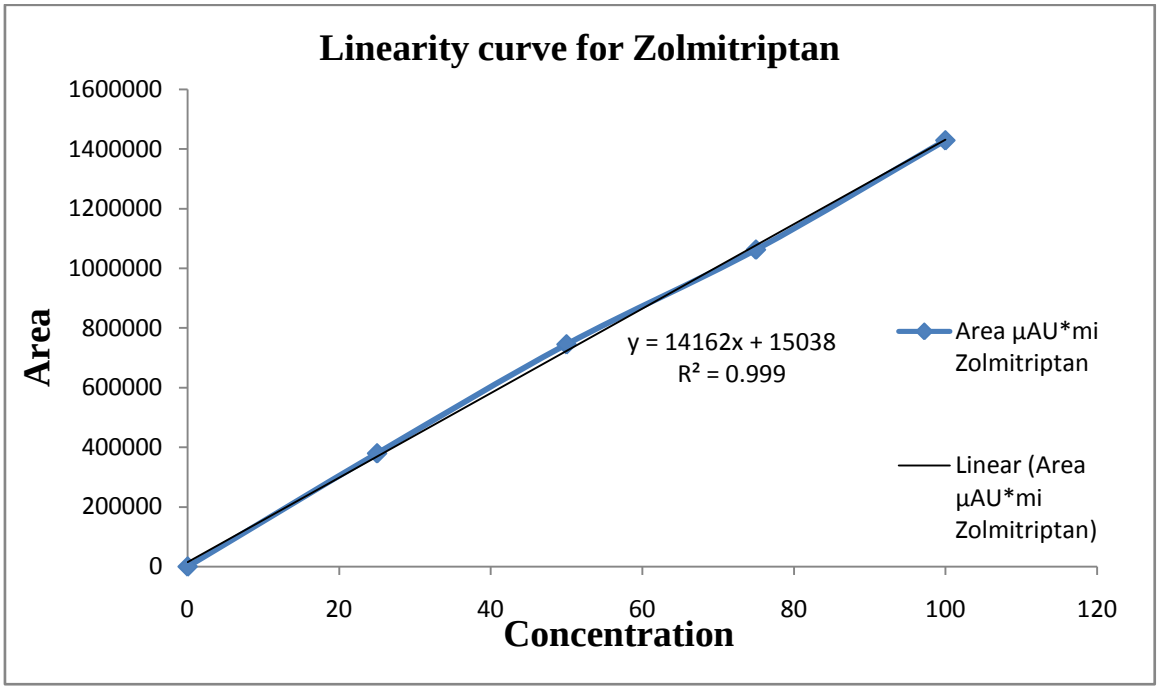
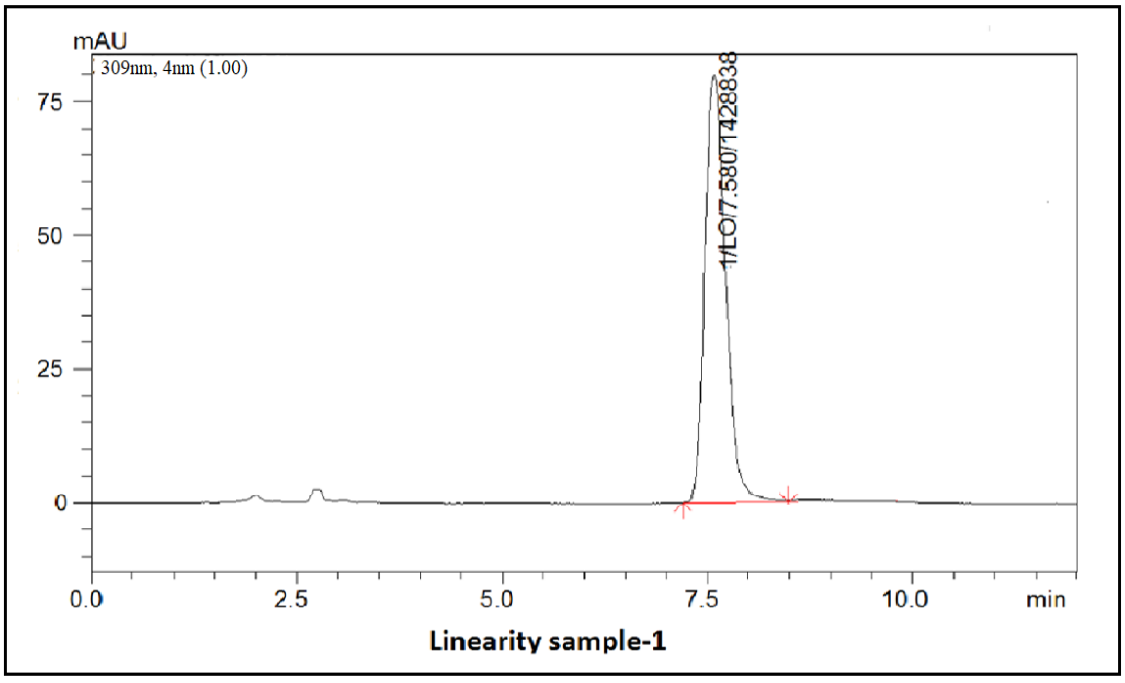



**Figure 3: Calibration Curve for Faropenem**



International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 4 July-Aug 2024, pp: 879-888 www.ijprajournal.com ISSN: 2456-4494

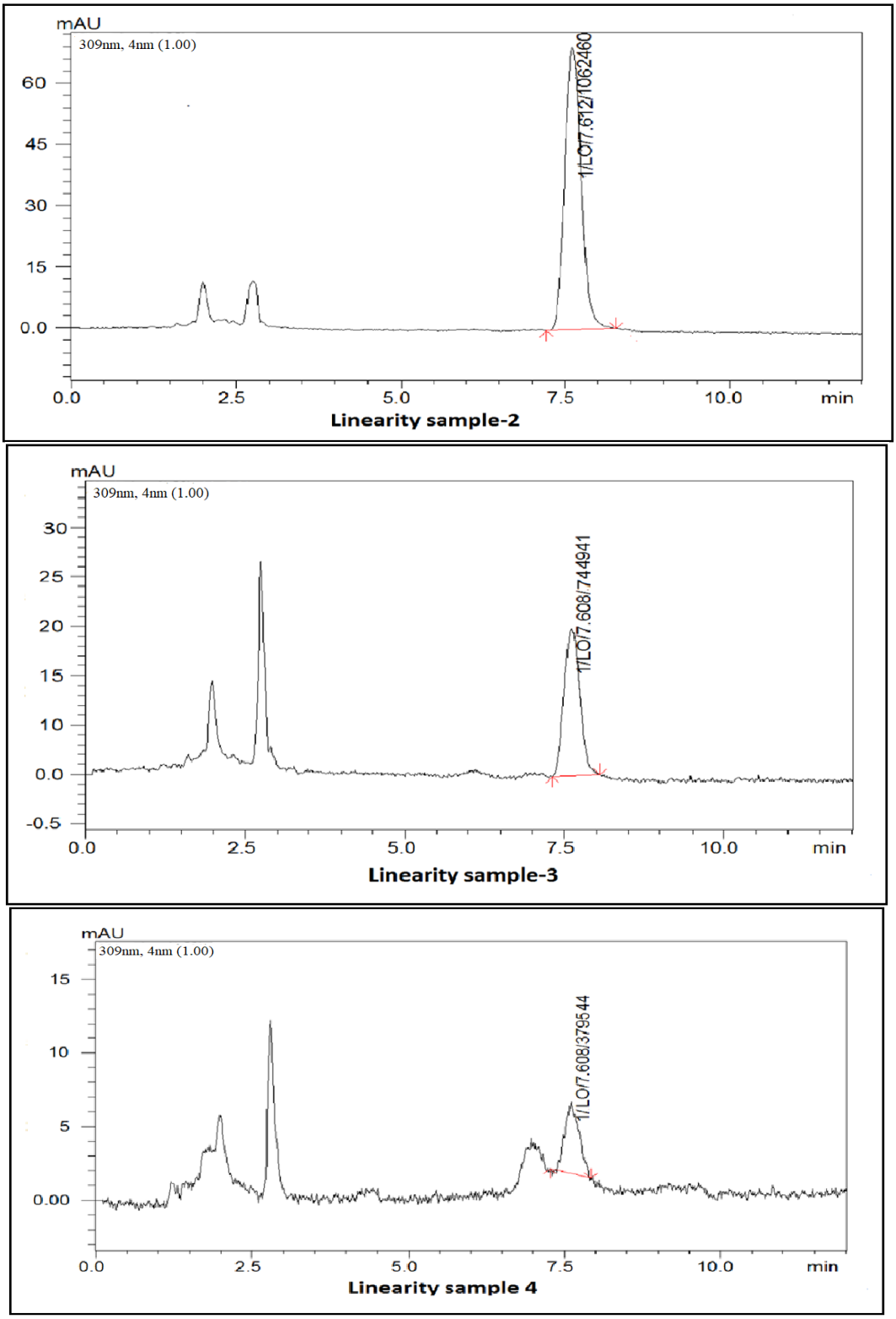



**Figure 4: HPLC chromatograms (sample 01-04) of linearity for calibration curve**

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 4 July-Aug 2024, pp: 879-888 www.ijprajournal.com ISSN: 2456-4494

**Result:** The given method is linear with Correlation Coefficient = 0.999 and Linearity equation (Y = 14162x + 15038).

**(C) Precision:**

**(a) Repeatability of sample:**

**Table No. 3: Repeatability of for Faropenem sample**

| Concentration (in ppm) | Retention time | RSD in % | Area | RSD in % | Avg RSD in % |
|---|---|---|---|---|---|
| 100 | 7.580<br>7.572<br>7.580 | 0.050 | 1428838<br>1427258<br>1428844 | 0.052 | |
| 75 | 7.612<br>7.609<br>7.608 | 0.022 | 1062460<br>1062577<br>1062032 | 0.022 | **0.048** |
| 50 | 7.608<br>7.596<br>7.600 | 0.066 | 744941<br>745106<br>743915 | 0.071 | |

**(b) Repeatability of Injection:**

**Preparation of solution:** From the Stock Solutions 1ml was pipette out in 10ml of volumetric flask and the volume was made up to 10 ml with methanol (100µg/ml) and injected into HPLC.

**Table No. 4: Data of Repeatability Test for Faropenem**

| Sample Name | Retention time | Area µAU*sec Atenolol | Area % |
|---|---|---|---|
| Sample 1 | 7.580 | 1428838 | 66.836 |
| Sample 2 | 7.572 | 1427258 | 66.844 |
| Sample 3 | 7.580 | 1428844 | 67.031 |
| Mean | 7.577 | 1428313 | 66.904 |
| % RSD | 0.050 | 0.052 | 0.135 |

**(c) Sample for Repeatability:**

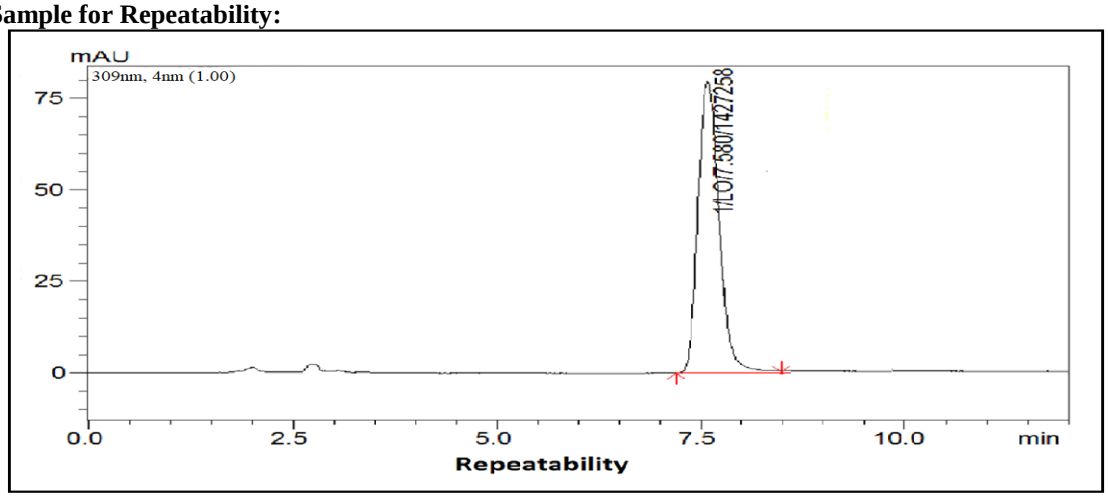



**Figure 5: HPLC chromatogram of Repeatability sample**

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 4 July-Aug 2024, pp: 879-888 www.ijprajournal.com ISSN: 2456-4494

**Table No. 5: Summary of Repeatability**

| Parameter | Acceptance Criteria | Faropenem |
|---|---|---|
| % RSD of Area | **NMT 2%** | 0.052 |
| Similarity Factor | **0.98 to 1.02** | 0.98 |

**Result:** The given method is repeatable.

**(D) Limit of Detection:** The limit of detection (LOD) and The Limit of Quantification (LOQ) were calculated:

**Table No. 6: LOD for Faropenem**

| Sample Name | LOD | LOQ |
|---|---|---|
| Faropenem | 33.31 µg/ml | 333.104 µg/ml |

**(E) Accuracy:** The accuracy of an analytical procedure expresses the closeness of results obtained by that method to the true value. The results of accuracy showed percentage recovery at all three levels. The results of percentage recovery and %RSD were within the accepted limits from 98.0% to 102.0% and not more than 2.0%, respectively, which indicates the applicability of the method for routine drug analysis.

**Table No. 7: Recovery data of Faropenem**

| S. No. | % Spiked Label | Peak Area | % Recovery | Mean ± % RSD |
|---|---|---|---|---|
| 1. | 80 | 1143103<br>1143088<br>1143048 | 99.568<br>99.566<br>99.563 | 99.565 ± 0.002 |
| 2. | 100 | 1428943<br>1429045<br>1428853 | 99.838<br>99.845<br>99.832 | 99.838 ± 0.005 |
| 3. | 120 | 1714822<br>1714911<br>1714907 | 100.020<br>100.025<br>100.025 | 100.0233<br>±<br>0.0024 |

**(F) Robustness (Change in Analyst):**

**Table No. 8: Data for Change in Analyst**

| S. No. | Analyst | Sample | Area | % of Label Claim | Tailing factor |
|---|---|---|---|---|---|
| 1 | Analyst1 | Sample1 | 1429425 | 98.5% | 1.741 |
| 2 | Analyst2 | Sample2 | 1428557 | 99.0% | 1.744 |
| 3 | Analyst3 | Sample3 | 1428889 | 98.8% | 1.742 |
| | %RSD | | 0.025 | | 0.057 |

**Estimation of Faropenem in marketed formulation**

In this experiment Faronac™ Marketed formulation was used having 200 mg/tablet of Faropenem as label claim. Equivalent to 1 mg of Faropenem was dissolved in 2ml of methanol and sonicated then filtered and volume was made up to 10ml final concentration was 100µg/ml then injected into HPLC. Quantity of Faropenem present in tablet was determined using linearity equation.

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 4 July-Aug 2024, pp: 879-888 www.ijprajournal.com ISSN: 2456-4494

**Table No. 9: Assay of Faropenem in tablet Formulation**

| **Brand Name** | **Faronac™** | |
|---|---|---|
| | **Faropenem** | **% Assay** |
| **Label Claim (mg)** | 200 mg | 99.9 |
| | 200 mg | 99.8 |
| | 200 mg | 99.7 |
| | 200 mg | 99.9 |
| | 200 mg | 99.8 |
| **Mean** | 0 | 99.82 |
| **SD** | 0 | 0.074833 |
| **%RSD** | 0 | 0.07497 |

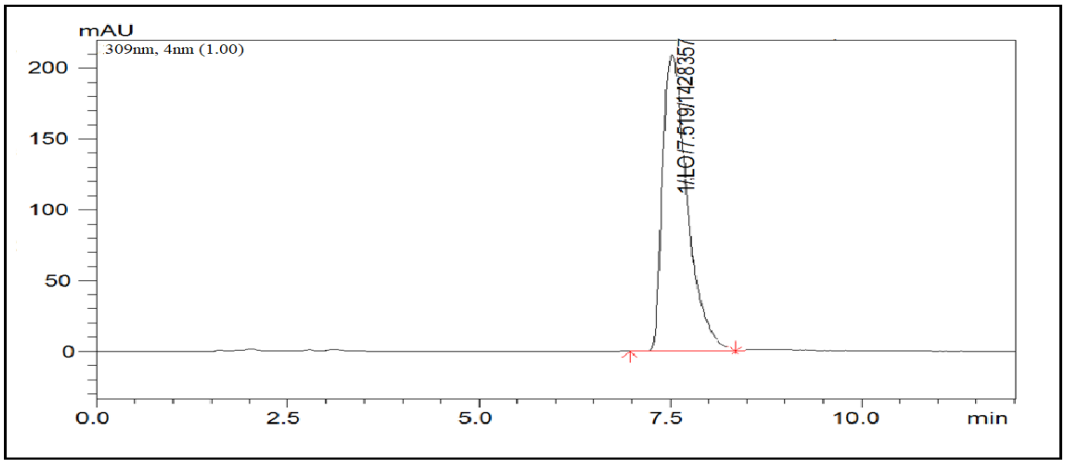



**Figure 6: Chromatogram of tablet formulation of Faropenem**

## IV. DISCUSSION

The present developed and validated High Performance Liquid Chromatographic (HPLC) method is to determine Faropenem in tablet dosage form. Faropenem was identified and characterized as it was bitter, odorless and yellow colored powder melted at 134 °C. Faropenem was freely soluble in methanol, soluble in ethanol, slightly soluble in 0.1 N NaOH and 0.1 N HCl, sparingly soluble in water and phosphate buffer. RP-HPLC method was developed using $C_{18}$ Column (25×0.46cm, i.d,5 µm), and Retention Time was 7.47 min at flow rate of 1.0 ml/min, $\lambda_{max}$ of Faropenem was found to be 309 nm by UV spetrophotometric investigation. Solution was stable about 20 hrs. Mobile phase was selected by hit and try method and final obtained solvent system was methanol:Water (70:30) pH-3 total run time was 12 min and injection was 20 µl. Developed method was validated on various parameters according to ICH guideline. System suitability parameters were found tailing factor 1.253, capacity factor 2.03, similarity factor1.0 and %RSD of STD Faropenem for Area 0.064% and %RSD of STD Faropenem for Retention time was 0.061%. The values of linearity, was determined by preparing four different concentrations 25, 50, 75 and 100 µg/ml and area under the curve (AUC) linearity equation was found Y=14162x + 15038 with $R^2$ value 0.999. Precision was found within 2.0% of RSD for both Repeatability of sample and Repeatability of Injection. Limit of detection (LOD) and limit of quantification (LOQ) were found at 33.31µg/ml and 333.104µg/ml respectively. Accuracy was performed on three spiked labels 80%, 100% and 120% and Recovery found under limit e.g. 0.002%, 0.005% and 0.0024% of RSD respectively. Robustness for change in analyst was also under limit. Estimation of Faropenem in marketed formulation (Faronac™ 200mg) was obtained by preparation of 100µg/ml concentration then injected into HPLC and quantified using linearity equation and found mean % assay was 99.8% and % RSD also under limit 0.07497. Over all this developed method was linear, accurate, précised and robust according to ICH guideline.

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 4 July-Aug 2024, pp: 879-888 www.ijprajournal.com ISSN: 2456-4494

## V. CONCLUSION

The present High Performance Liquid Chromatographic method is to determine Faropenem. Various experiments were carried out to establish the method. The mobile phase was methanol:water (70:30) pH-3 attained by Ortho phosphoric acid is found to be ideal for the estimation of Faropenem. Retention time was 7.47 min and total run time was 12 min at flow rate of 1.0 ml/min. method was evaluated at various parameters like linearity, Precision, accuracy, LOD, LOQ and accuracy and percentage of standard deviation show that the proposed method was reproducible, accurate, precise and robust.

**CONFLICTS OF INTERESTS**

There are no conflicts of interests

## REFERENCES

[1]. R.M. Verma, "Analytical Chemistry (Theory and Practice)", 3rd Edition, Pp- 1-7.

[2]. Ravi Shankar, "Instrumental Method of Analysis", 2nd Edition, Pp- 1-2.

[3]. A.H. Beckett, J.B. Stenlake, "Practical Pharmaceutical Chemistry," Part – II, 4th Edition, pp- 269- 288.

[4]. Gurdeep R. Chatwal, Sham K. Anand, "Instrumental Method of Chemical Analysis," 5st Edition, pp- 2.566- 624.

[5]. Jyothi Mirjapuram, & Gade Sammaiah. Development and validation of a new analytical rp-hplc method for the quantitative estimation of finerenone in api form and marketed pharmaceutical dosage form. International Journal of Allied Medical Sciences and Clinical Research, 2023;11(2):210–218.

[6]. Sankar PR, Kishore KP, Babji B and Sulthana MS: Analytical method development and validation for the determination of mirabegron in pharmaceutical dosage form by RP-HPLC. Int J Pharm Sci & Res 2020;11(5):2223-28.

[7]. Dhaval N Darji, D G Desai, Aarti Zanwar, Ashim Kumar Sen and A K Seth. Development and validation of uv spectroscopy method forthe estimation of faropenem sodium in bulk and dosageform. Pharma science monitor, 2013;4(3): 10-17.

[8]. J. Cielecka-Piontek, A. Krause, M. Paczkowska. An application of high performance liquid chromatographic assay for the kinetic analysis of degradation of faropenem. Pharmazie, 2012;67:912-916.

[9]. Menon, Shobhana & Panchal, Jayesh & Patel, Ravindra. Development and Validation of a Stability Indicating LC Method for the Determination of Faropenem in Pharmaceutical Formulations. Chromatographia, 2009; 69:1012-1018.

[10]. Saibaba S V, and S. P. P. "Method Development and Validation of RP-HPLC Method for the Determination of Olanzapine in Bulk and Tablet Dosage Form". Asian Journal of Pharmaceutical and Clinical Research, 2017;10(5):281-4.