Comprehensive Development and Validation of a RP-UPLC Method for the Determination of Flupirtine Maleate in Pharmaceutical Dosage Forms

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Abstract

This research paper presents the development and validation of a rapid and efficient Reverse Phase-Ultra-Performance Liquid Chromatography (RP-UPLC) method for the analysis of Flupirtine Maleate in a pharmaceutical dosage form. The chromatographic separation employs a high-pressure isocratic elution mode with specific column chemistry and a mobile phase composed of 0.05% TFA (pH 2.4) and acetonitrile in a 70:30 (v/v) ratio. Rigorous validation demonstrates high precision, accuracy, and specificity, meeting the requirements of the United States Pharmacopeia (USP) and the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH). The method's robustness is confirmed against minor variations, making it an invaluable asset for routine analysis in quality control laboratories, contributing to increased productivity and solvent conservation. This UPLC method offers significant advantages over standard HPLC techniques, benefiting both businesses and end-users in pharmaceutical quality control.

Keywords: RP-UPLC, Flupirtine Maleate, Pharmaceutical Dosage Form, Validation, Assay, Analytical Method, Chromatography

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Introduction

Flupirtine maleate, an atypical non-opioid analgesic, has gained significant attention within the pharmaceutical industry due to its unique mechanism of action, which involves the selective modulation of potassium channels, thereby exerting analgesic effects without the risk of opioid-related side effects, such as dependence and respiratory depression. Flupirtine maleate has been widely used for the management of acute and chronic pain, making it a vital component of many pharmaceutical formulations[1]. As pharmaceutical manufacturers strive to ensure the quality, safety, and efficacy of medications containing flupirtine maleate, it is imperative to establish robust analytical methods for its quantification [2]. The reliable determination of the active pharmaceutical ingredient (API) is not only a regulatory requirement but also an essential component in maintaining consistent product quality, which directly impacts patient safety and therapeutic outcomes [3]. High-Performance Liquid Chromatography (HPLC), and its advanced variant, Reversed-Phase Ultra-Performance Liquid Chromatography (RP-UPLC), are instrumental techniques widely employed for the analysis of pharmaceutical compounds. These methods offer high sensitivity, precision, and accuracy in quantifying drug substances in various dosage forms[4,5]. In this context, the development and validation of an RP-UPLC method for the quantification of flupirtine maleate in pharmaceutical formulations become particularly relevant[6,7]. This research paper aims to elucidate the comprehensive development and validation of an RP-UPLC method for the determination of flupirtine maleate in pharmaceutical dosage forms. Through this study, we intend to provide an analytical

foundation that ensures the accurate and reproducible assessment of flupirtine maleate content in pharmaceutical products[8]. Such analytical rigor is fundamental not only for regulatory compliance but also for guaranteeing that patients receive medications that consistently meet the prescribed standards for efficacy and safety[9]. Additionally, the study will emphasize the importance of establishing reliable analytical methods in pharmaceutical development, which can ultimately translate to enhanced patient care and drug product quality assurance.

Material and method

Materials

All solvents and chemicals employed in this study were of LCMS-grade quality. LCMS-grade water (Milli-Q) and PTFE syringe filters with a 0.22 µm pore size were consistently utilized throughout the experimental procedures. The standard drug, Flupirtine, was sourced from Sigma-Aldrich.

Method

Primary Characterization of the Drug

This phase encompassed the evaluation of the drug, or Active Pharmaceutical Ingredient (API), in accordance with in-house specifications, which involved the following aspects:

Description: The physical appearance of the drug was examined.

Solubility: Solubility tests were conducted using various solvents, including water, methanol, ethanol, chloroform, acetone, and ethyl acetate. In each test tube, 3 mg of the drug was placed, and 3 mL of the respective solvents were slowly added, followed by sonication. The solubility was assessed visually.

UV Scanning: A stock solution was prepared in methanol, and drug FPT concentrations were adjusted for each dilution to reach 200 µg/mL. Subsequently, the solution was scanned in the range of 400 nm to 190 nm using a UV-Visible spectrophotometer, and the results were analyzed.

Mass Spectroscopy: The drug, with a concentration of 200 µg/mL FPT, was diluted and analyzed using an LCMS system to determine the molecular mass of the drug.

NMR Spectroscopy: A small quantity of pure drug (3 mg) was placed in a sample tube, and 6 mL of deuterated-DMSO was added as a solvent. The resulting solution was subjected to NMR spectroscopy for analysis.

RP-UPLC Method Development

Preparation of Standard Solutions

Flupirtine (FPT) Stock Solution: A precise amount of Flupirtine (50 mg) was accurately weighed and transferred into a 50 mL volumetric flask. Methanol was added, and the mixture was sonicated for 15 minutes to ensure complete dissolution of the contents. The volume was then adjusted to the mark using methanol, resulting in a final concentration of 1 mg/mL (1000 µg/mL)[10].

Method Optimization Strategy

Primary Screening

Primary screening involved testing different solvent compositions in various combinations, taking into account the pH of the mobile phase buffer solution. Retention may be influenced by the choice of the mobile phase. Analyte ionization can be affected by the surface properties of the adsorbent, which can be manipulated to prevent ionization and promote it as needed. Various buffers, with varying compositions of acetonitrile (ACN) and/or methanol (MeOH), were tested over a wide pH range to determine the most effective elution conditions for the analytes[11]. The objective was to achieve optimal retention time, resolution, and peak symmetry by maximizing ionization.

Method Optimization

The effectiveness of a separation process relies on the chemical properties of the analyte and the attributes of the mobile and stationary phases employed. The selectivity of a RP-UPLC separation can be adjusted or enhanced by manipulating several key parameters. Various technique parameters, including column chemistry, the pH/composition of the mobile phase, flow rate, and detection wavelength (treated as independent variables), were manipulated to optimize the methods and achieve the most desirable results during the development process[12].

Validation of Developed RP-UPLC Method

The validation of the UPLC method followed USP and ICH standards, involving the preparation of working standard solutions by accurately weighing work standard materials and meeting the specified target volume. Specificity was assessed to ensure well-separated Flupirtine (FPT) peaks in test concentrations without interference from matrix components, with matching chromatograms and no interfering peaks during the analyte's retention time as acceptance criteria. Placebo and blank interferences were investigated, confirming the absence of interfering peaks during analyte retention times for placebos and blanks. Peak purity was verified through diode array and mass spectrum analysis, confirming that the analyte's peak attributed to a single component and its molecular mass established[13]. This rigorous validation process underscored the UPLC method's reliability and accuracy for Flupirtine analysis.

Specificity

Specificity was assessed to determine the method's ability to generate well-separated Flupirtine (FPT) peaks in test concentrations (working standard and sample solutions) without interference from matrix components. Standard and sample solutions were prepared according to the prescribed test protocol and injected into the UPLC system, with resulting chromatograms recorded. The acceptance criteria involved close resemblance of chromatograms for sample and standard solutions in terms of retention times[14].

Placebo Interference

An investigation was conducted to evaluate placebo interference. This involved injecting a solution containing common placebos, such as Microcrystalline Cellulose, Lactose Monohydrate, and Calcium Phosphate, into the UPLC system, with the resulting chromatogram documented. Acceptance criteria included the absence of peaks in the placebo chromatogram during the analyte's peak retention period and the absence of interference due to placebos during the analyte's retention times[15].

Blank Interference

A study was conducted to examine blank interference, characterized by disruption or distortion of

information processing resulting from the absence of relevant stimuli or cues. A diluent was introduced into the UPLC system as part of the test procedure, and the blank sample's chromatogram was recorded. The criteria for acceptance were the absence of peaks in the blank chromatogram during the analyte's peak retention period and the assurance that blank did not interfere with analytes during their retention times[16].

Linearity and Range

In the evaluation of linearity and range, standard drug solutions spanning concentrations from 100 to 400 µg/mL were prepared by diluting their respective standard solutions and injected into the UPLC system three times with a 1.0 mL injection volume to assess the proposed method's linearity and range[17]. The procedure involved the preparation of a stock solution by accurately measuring the working standards and ensuring complete dissolution through sonication. Subsequently, solutions at various concentrations (100, 150, 200, 250, and 300 µg/mL) were prepared in volumetric flasks. Chromatograms of these solutions were recorded, and a correlation between peak response (area) and concentration was established to determine the correlation coefficient, y-intercept, slope of the regression line, and residual sum of squares. The minimum acceptable correlation coefficient was set at 0.99.

Detection Limit (LOD)

The determination of the Limit of Detection (LOD) followed ICH recommendations and was calculated using the equation:

$$LOD = 3.3\ \sigma / S$$

Where:

- σ represents the standard deviation of the y-intercepts of the regression line.
- S corresponds to the slope of the calibration curve.

Quantitation Limit (LOQ)

The calculation of the Limit of Quantitation (LOQ) also adhered to ICH guidelines and was determined utilizing the equation:

$$LOQ = (10 * \sigma) / S$$

Where:

- σ signifies the standard deviation of the y-intercepts of the regression lines obtained from the calibration curve.
- S stands for the slope of the calibration curve[18].

Application of the Method in Formulation Assay:

For the assay of commercial formulations, twenty capsules of "LUPIN'S KATADOL CAPS," each containing 100 mg of FPT, were utilized. The content of one capsule, equivalent to 10 mg of FPT, was extracted in a 100 mL volumetric flask using methanol. This extraction process involved agitation and

sonication for thorough dissolution, followed by calibration with methanol and subsequent filtration using a PTFE syringe filter. The resulting supernatant contained a concentration of 200 µg/mL of FPT, which was collected in 2 mL UPLC vials. The estimation of drug content was performed by injecting standard and sample solutions into the stationary phase and measuring the peak area of the sample analytes in the chromatograms[19,20]. This allowed for the determination of FPT content within the studied dosage form by comparing it to a standard of known concentration.

Result and discussion

Characterization of Drugs

An initial characterization of the drugs was conducted to evaluate their purity and identity. The observations were as follows:

Description: The drug presented as a solid, white-colored, and odorless powder.

Solubility: It exhibited solubility in MeOH and EtOH, being slightly soluble in chloroform, while insoluble in water.

UV Scanning: UV scanning of the drug was performed in the range of 400 nm to 190 nm using MeOH as the solvent. The sample solution of FPT displayed two absorption maxima at wavelengths of 247 nm and 346 nm. Since the UV detection absorbance of FPT at 346 nm was found to be higher than at 247 nm, absorbance at 346 nm in MeOH was selected for further investigations (refer to Figure 1).

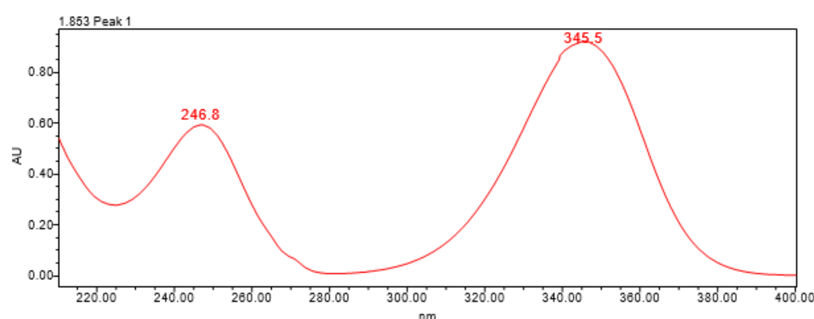

Figure 1 UV-spectrum of Flupirtine API

a) Mass Spectroscopy

The molar mass of the standard FPT-API was determined to be 305.29 g/mol, confirming both the purity and identity of the drug, as supported by reported literature (as shown in Figure 2).

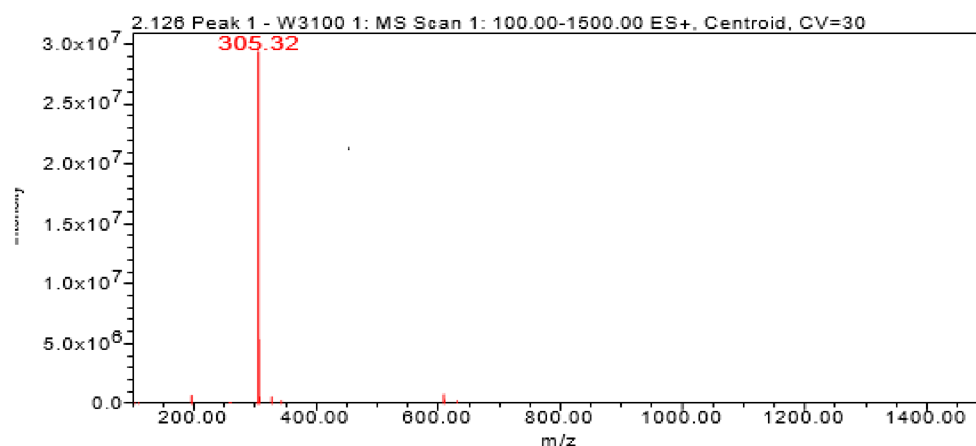

Figure 2 Mass-spectrum of Flupirtine API

e) NMR Spectroscopy

The NMR spectra of the standard FPT-API show 17 protons, which are indicative of the drug's purity and identity, a fact confirmed by existing literature. These spectra are presented in Figure 3.

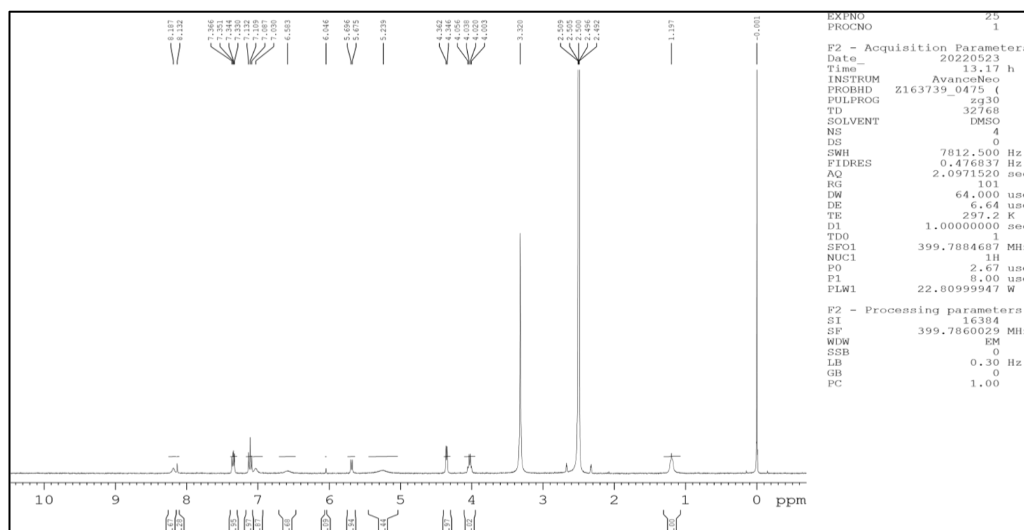

Figure 3 NMR spectrum of Flupirtine API

RP-UPLC APPROACH FOR METHOD DEVELOPMENT

Method Optimization Strategy

Primary screening was performed on the standard solution (laboratory mixture) by taking different mobile phase buffer solutions of various pH ranges in combination with organic solvents so as to check peak purity passing, good resolution between the sample peaks and repeatability with no co-elution of any impurity.

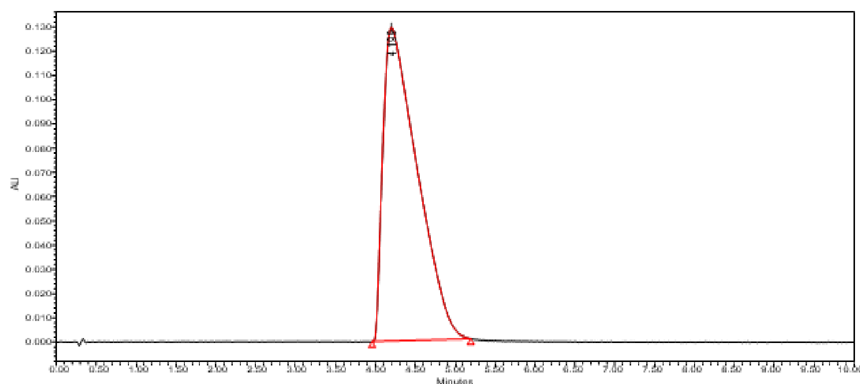

Figure 4 Elution observations of Acidic-pH (FA)

Acidic pH - TFA

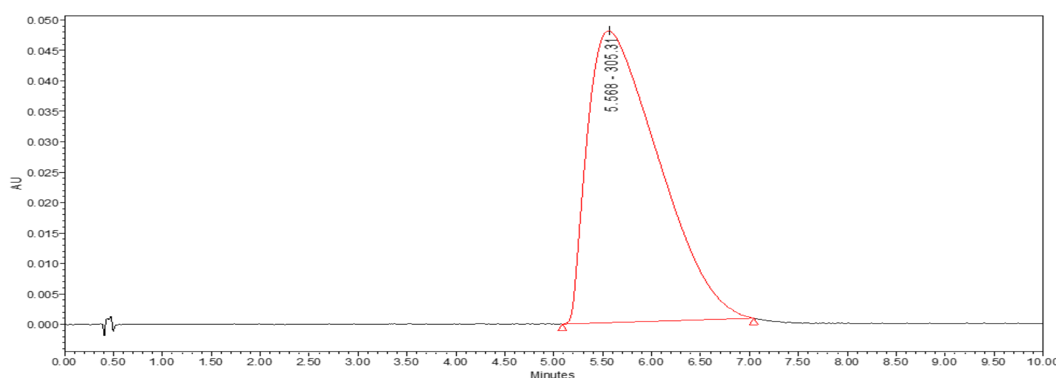

Figure 5 Elution observations of Acidic pH (TFA)

Table 1Retentention time and observations

S. No	Mobile Phase (Ratio)		Retention Time (min)	Observations
	Buffer (TFA)	ACN		
1	10	90	0.288	Peak eluted at void with poor retention
2	20	80	0.304	Peak eluted at void with poor retention
3	30	70	0.310	Peak eluted at void with poor retention
4	40	60	0.334	Peak eluted at void; splitting occurs withpoor retention
5	50	50	0.341	Splitting occurs with poor retention
6	60	40	0.463	Splitting occurs with poor retention
7	70	30	0.629	Peak shape is good with good resolution

8	80	20	2.06	Peak shape is good and late eluted
9	90	10	5.56	Peak shape is broad and late eluted

As a result of primary screening, ***0.05% Trifluoroacetic Acid (in water) and ACN*** with acidic pH ***range (2.4)*** was selected for the mobile phase buffer. Screening also indicated that further optimization of mobile phase was required by changing pH, typeand composition of organic solvent(s).

VALIDATION OF DEVELOPED UPLC METHOD

Through the use of RP-UPLC, we attempt to develop a new analytical technique for measuring FPT. As of yet, studies have not proven that RP-UPLC can be used to estimate FPT. A new analytical technique for quantifying pharmaceutical compounds has been invented: reversed-phase ultra-performance liquid chromatography (RP-UPLC). ICH's Q2 (R1) guidelines and USP's guidelines state that this approach is valid, as documented in the United States Pharmacopeia (USP).The validation attempts and parameters covered for estimation of FPT are discussed further in present manuscript.

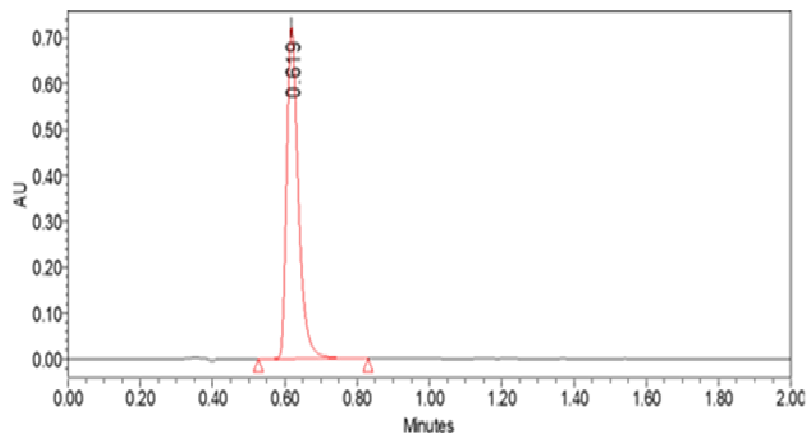

Figure 6 Chromatogram showing optimized result

Validation Parameters

The resulting data is presented in Figure 7, which displays the overlay chromatogram of FPT, along with the linearity graph.

A. LINEARITY AND RANGE

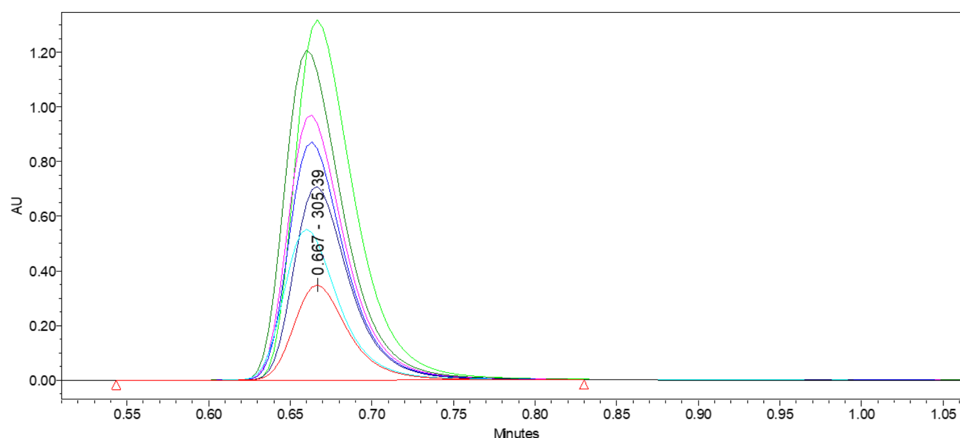

Figure 7 Overlay chromatogram of FPT solutions for linearity (100-400 µg/mL)

As shown in Figure 8, FPT gives a linear response from 100-400 µg/mL in RP- UPLC. The correlation coefficient value was found to be 0.9997. The high value of correlation coefficient approaching to 1.0 validates the Beer Lambert's law. Thevalues of area obtained with respect to conc. are given in Table 2 clearly signify thatthe conc. range selected for FPT is linear. It indicates good linearity over the given concentration range.

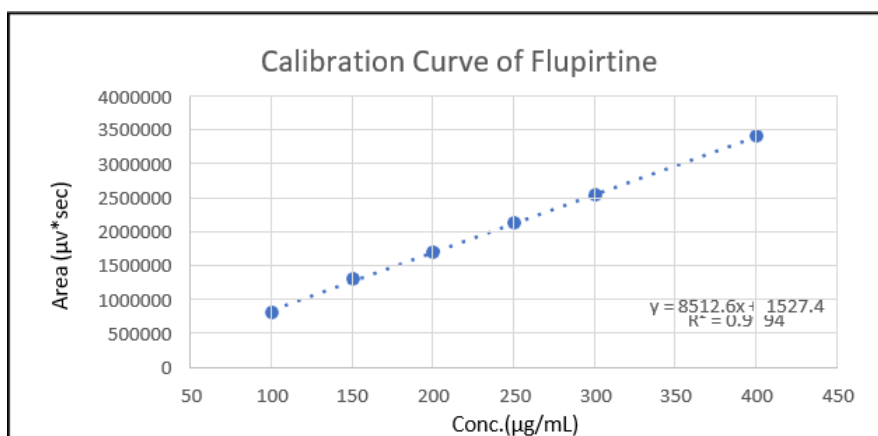

Figure 8 Calibration Curve of FPT

Table 2 Linearity data for FPT

Conc. (µg/mL)	Area (µV*sec)		
	Inj # 1	Inj # 2	Avg. Area
100	820085	820590	820338
150	1313077	1314399	1313738

200	1707041	1708121	1707581
250	2141876	2141041	2141459
300	2589616	2489656	2539636
400	3403971	3404168	3404070
Correlation Coefficient			**0.9997**
Slope of Regression Line			**8512.6**
y-Intercept			**1527.4**

B. SPECIFICITY

The method's specificity was assessed, and the resulting interference data is presented in Table 3.

Table 3 Specificity data of FPT

Conc. (µg/mL)	Area before addition of excipients	Area after additionof excipients	% Interference
100	820123	820083	0.005
200	1738063	1736719	0.077
400	3352376	3345335	0.210

The absence of interference from excipients and the specificity of the devised approach are evident from the values of percentage interference of excipients for FPT. Moreover, the chromatograms obtained from the standard and sample exhibit a high degree of similarity, with virtually equal retention times of around 0.6 minutes. No interference was found from the placebo, which consisted of a combination of Microcrystalline Cellulose, Lactose Monohydrate, and Calcium Phosphate, during the retention period of the analyte. This observation indicates that the technique employed was specific. The chromatograms illustrating the specificity investigations, including the standard, sample, placebo, and blank, are depicted in Figures 9,10,11,12, respectively.

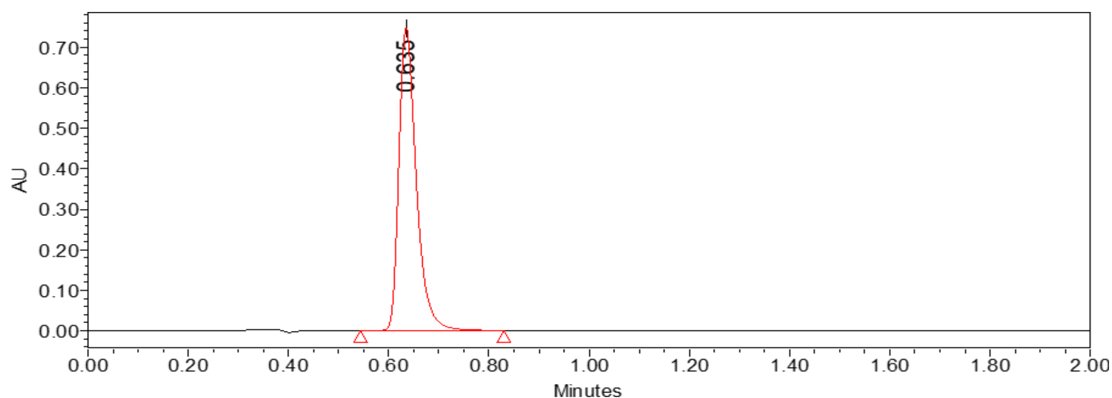

Figure 9 Standard chromatogram for FPT

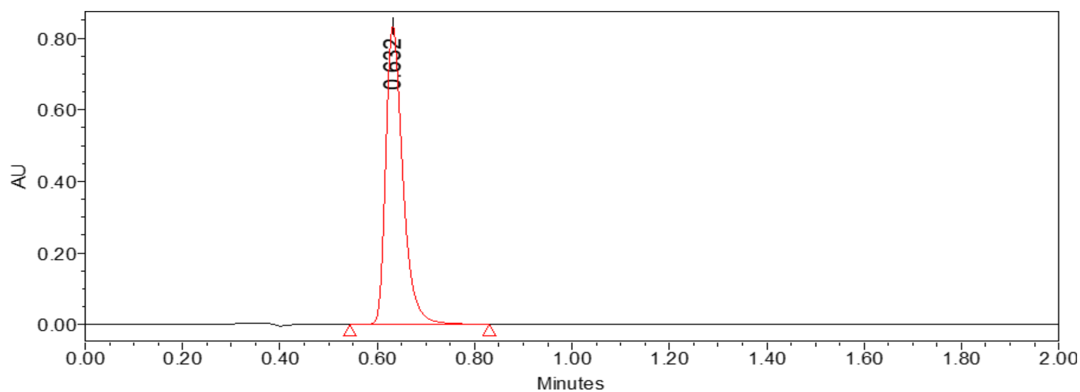

Figure 10 Sample chromatogram for FPT

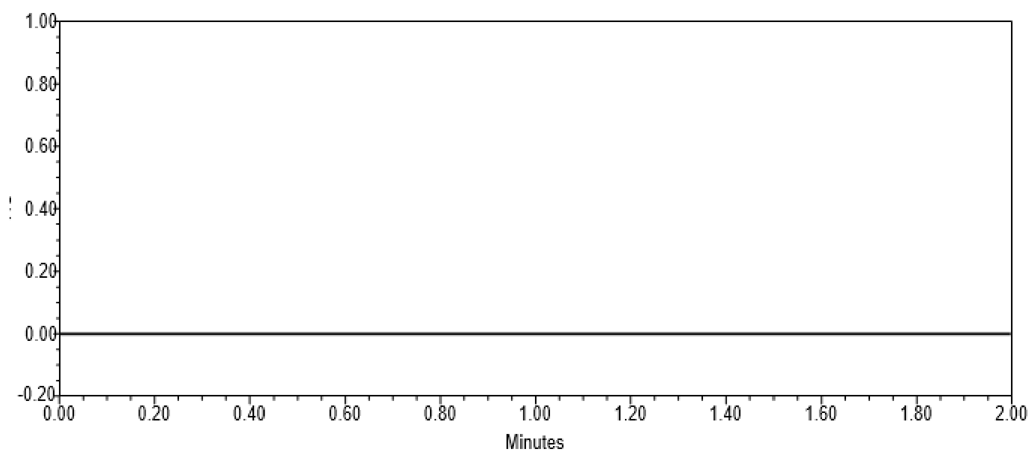

Figure 11Chromatogram for Placebo interference

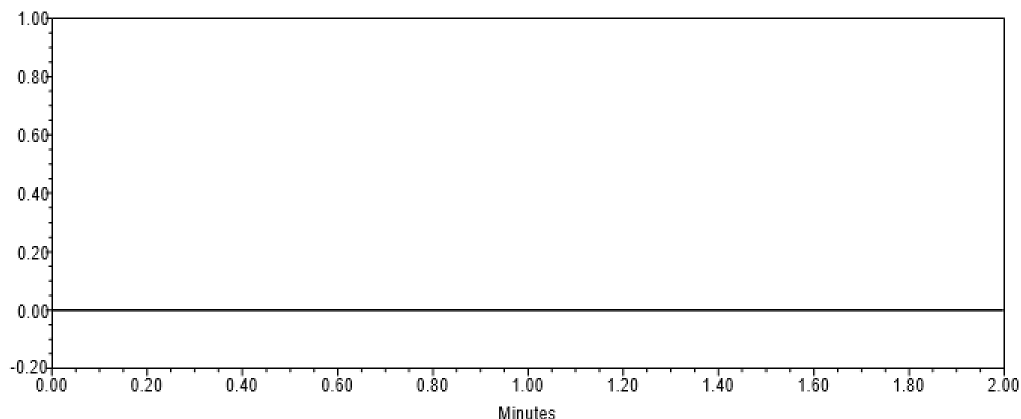

Figure 12 Chromatogram for blank interference

C. Accuracy

Table 4 provides a summary of the percentage recovery findings obtained for FPT. The recovery study conducted for FPT yielded results within the range of 98-102%. These findings align with the standard limit given in the ICH recommendations, suggesting that the devised approach is accurate.

Table 4 Recovery data of FPT

Level of Recovery %	Amountof Sample (µg/mL)	Amount of std. FPT spiked (µg/mL)	Total amount of FPT (µg/mL)	Amount of FPT Found (µg/mL)	Amount of FPT Recovered (µg/mL)	% Recovery	Mean Recovery ± SD (n=3)
80	201.02	160.00	360.00	360.02	159.00	99.38	99.79±0.39
	199.67	160.00	360.00	359.89	160.22	100.14	
	200.85	160.00	360.00	360.63	159.78	99.86	
100	198.21	200.00	400.00	398.74	200.53	100.27	99.61±0.91
	199.95	200.00	400.00	399.92	199.97	99.99	
	201.78	200.00	400.00	398.93	197.15	98.58	
120	201.52	240.00	440.00	438.83	237.31	98.88	99.41±0.46
	200.48	240.00	440.00	439.73	239.25	99.69	
	198.83	240.00	440.00	438.02	239.19	99.66	

KATADOL CAPS (for FPT estimation):

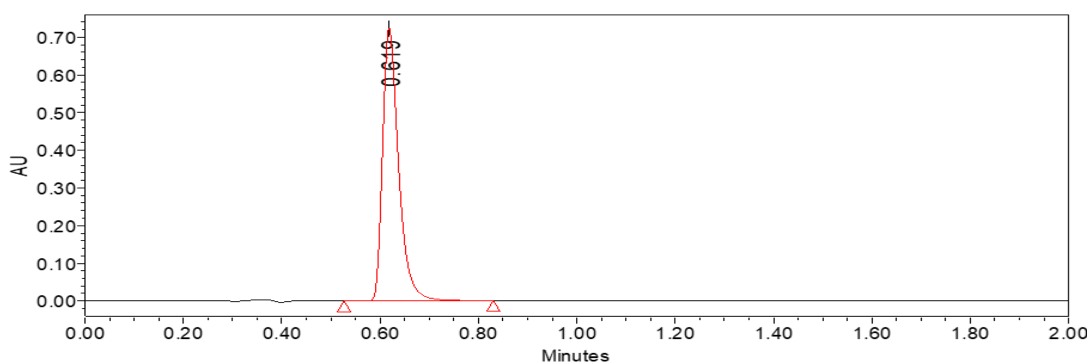

Figure 13 Recovery performed on Katadol Caps (FPT) with 80 % spiking

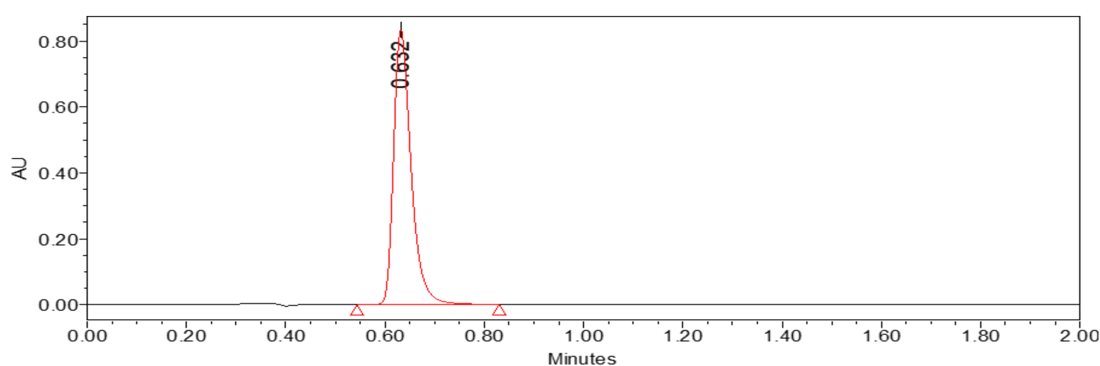

Figure 14 Recovery performed on Katadol Caps (FPT) with 100 % spiking

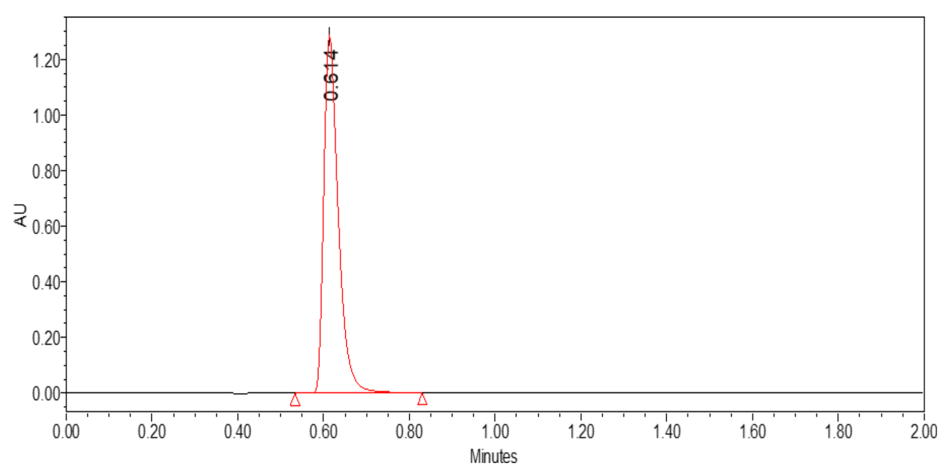

Figure 15 Recovery performed on Katadol Caps (FPT) with 120 % spiking

D. PRECISION

Precision was assessed by analyzing six repeated runs on the same day of testconcretion of 200 µg/mL of standard solution. Chromatograms demonstrating the precision parameter of the developed method for FPT at a test concentration of 200 µg/mL (standard solution) are displayed in Figure 16.

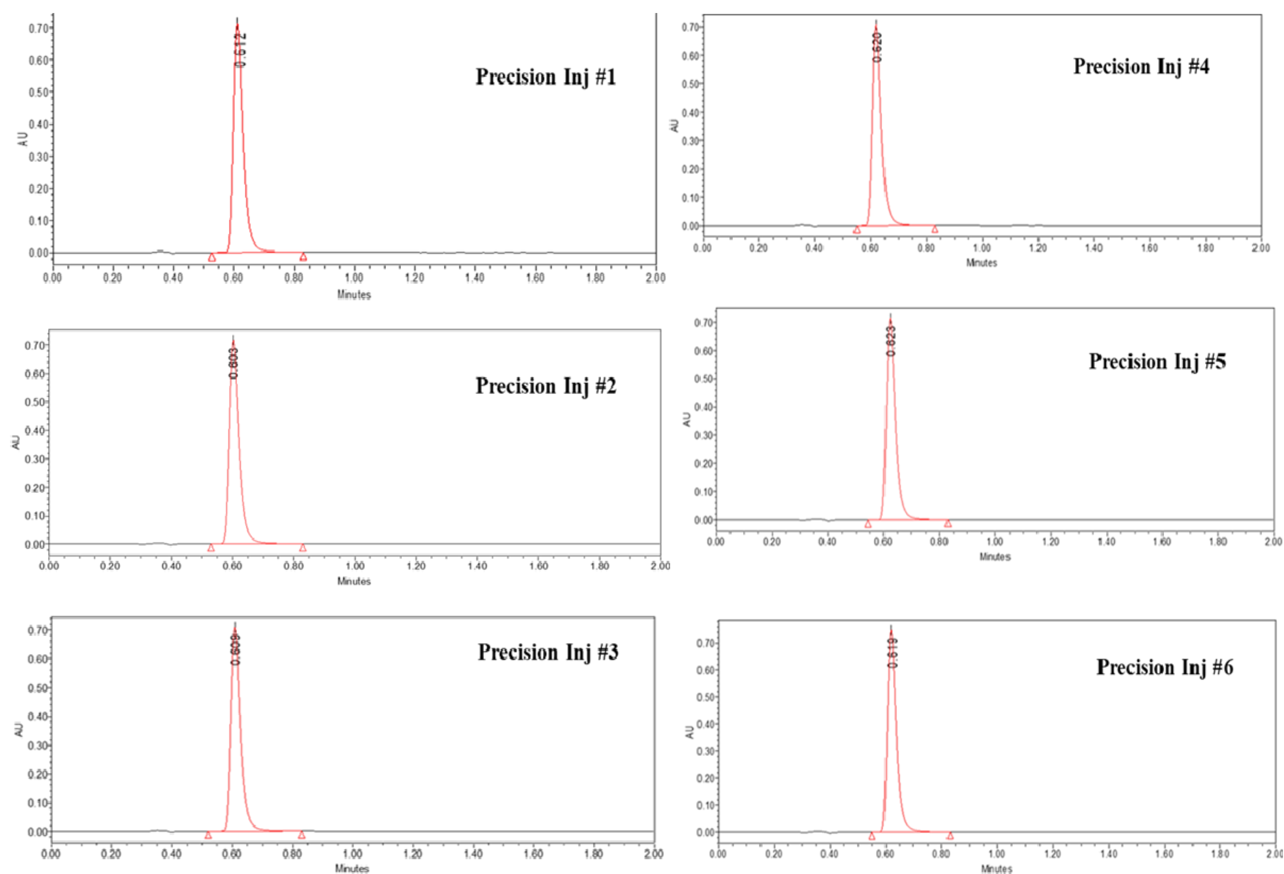

Figure 16 Chromatograms showing the precision parameter of the developed method for FPT at test conc. of 200 µg/mL (standard solution)

Table 5 Precision data of FPT

Tabel showing Precision study of FPT Standard Solution		
Conc.(µg/mL)	**Area (µV*sec)**	**Retention Time (min)**
200	1707041	0.665
200	1704780	0.665
200	1738063	0.665
200	1713697	0.665
200	1699722	0.666
200	1708121	0.664
Average	1711904	0.665
Std. Dev.	13598.68	0.0006
% RSD	0.79	0.095

During the precision study of six replicate injections of standard solution, the % R.S.D. of the area

and retention time of the FPT peak were estimated to be 0.79 % and 0.095 %, respectively. Less than 2.0% of the value was determined. According to the Precision study, the observed values fell within the parameters of acceptable values. This method was then found to be an accurate method of determining FPT by RP-UPLC.

DETECTION LIMIT

The limit of detection (LOD) for FPT, calculated using the equation $LOD = 3.3\sigma/S$, where σ represents the standard deviation of y-intercepts of the regression line, and S is the slope of the calibration curve, was determined to be 10.08 µg/mL.

QUANTITATION LIMIT

The Limit of Quantitation (LOQ) was determined using the formula $LOQ = 10\sigma/S$, with σ representing the standard deviation of the y-intercepts of the regression line and S being the slope of the calibration curve, resulting in an observed LOQ value of 30.54 µg/mL for FPT.

Assay of marketed pharmaceutical dosage form (capsule)

The validated method shows % assay of FPT in marketed formulation “KATADOL as 99.58 % (w/w) ±0.69 (n=3). The presented RP-UPLC analytical method for quantifying FPT in capsules is straightforward, accurate, and rapid, making it suitable for routine analysis. It demonstrates high precision and repeatability, as evident from the low standard deviation and satisfactory percentage recovery in the assay of marketed formulations. The robustness of the optimized method was assessed by varying column oven temperature, flow rate, and mobile phase composition. Changes in mobile phase composition influenced the retention time, emphasizing the need for careful adjustment when altering the mobile phase composition. Similarly, changes in the flow rate affected retention time, with an optimal flow rate of 0.35 mL/min recommended. However, alterations in column oven temperature had minimal impact on retention time, indicating its stability.

Conclusion

Conclusion, the RP-UPLC approach has demonstrated exceptional efficiency, enabling the accurate quantification of Flupirtine Maleate within a remarkably short analysis time of just 2 minutes, extendable to 1 minute if needed—an "Ultra-short method" of analysis. This efficiency translates into heightened sample throughput and increased productivity. The method employs a high-pressure isocratic elution mode with an "Acquity UPLC BEH Shield RP18" column and a 70:30 (v/v) mobile phase of 0.05% TFA (pH 2.4): ACN, with detection at 346 nm using a Photodiode Array detector. Rigorous validation attests to its precision, accuracy, and specificity, complying with USP and ICH guidelines. The method's robustness is demonstrated against minor variations in relevant factors. Notably, this UPLC method offers time and solvent savings, making it a valuable asset for routine analysis in quality control laboratories, benefiting both businesses and end-users alike.

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