

Development and Validation of RP-HPLC Method for the Simultaneous Estimation of Paracetamol and Flupirtine Maleate in Pharmaceutical Dosage Form

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ABSTRACT

Objective: To develop and validate a simple, efficient and reproducible RP-HPLC method for the simultaneous determination of paracetamol and flupirtine maleate in bulk and in pharmaceutical formulations. **Materials and Method:** The chromatographic analysis was performed on a Thermo BDS hypersil C₁₈ (250 x 4.6 mm i.d, 5 μ) column in isocratic mode using water: methanol(pH was adjusted to 3.35 \pm 0.02 with ortho-phosphoric acid) in the ratio of 50:50 v/v as eluent. The flow rate was 1 ml/min and eluent was detected at 250 nm. The retention time of paracetamol and flupirtine maleate were 3.5 and 5.4 min, respectively. **Results:** The linear dynamic range was 650-1950 μ g/ml and 200-600 μ g/ml for paracetamol and flupirtine maleate, respectively. Percentage recoveries for paracetamol and flupirtine maleate were 100.50 and 99.76 %, respectively. **Conclusion:** All the analytical validation parameters were determined and found in the limit as per ICH guidelines, which indicates the validity of the method. The developed method is also found to be precise and robust for the simultaneous determination of paracetamol and flupirtine maleate in tablet dosage forms..

Key words: Accuracy, flupirtine, paracetamol, validation.

INTRODUCTION

Paracetamol or acetaminophen is a widely used over-the-counter analgesic (pain reliever) and antipyretic (fever reducer). It is classified as a mild analgesic. It is commonly used for the relief of headaches and other minor aches and pains and is a major ingredient in numerous cold and flu remedies. In combination with opioid analgesics, paracetamol can also be used in the management of more severe pain such as post-surgical pain and providing palliative care in advanced cancer patients. Chemically paracetamol is N-(4-hydroxyphenyl)acetamide [Figure 1]. It is official in Indian Pharmacopoeia,¹ British Pharmacopoeia² and United States Pharmacopoeia.³

Flupirtine maleate⁴ is a non-opioid centrally-acting analgesic agent, structurally dissimilar from other analgesics. It is used as an analgesic for acute and chronic pain, in moderate to severe cases. Its muscle relaxant properties make it popular for back pain and other

orthopaedic uses, but it is also used for migraines, in oncology, postoperative care, and gynaecology. It is a pyridine derivative with a chemical name of ethyl {2-amino-6-[(4-fluorobenzyl)amino]pyridin-3-yl} carbamate [Figure 2].

Literature survey reveals a few spectrophotometric and bioanalytical methods for the estimation of both drugs as a single component and in combination with other drugs.⁵⁻¹⁰ However no method has been reported for analysis of these drugs in combined dosage form. The objective of present work is to develop simple, rapid and precise RP-HPLC method for the estimation of paracetamol and flupirtine maleate in combined tablet dosage form.

MATERIALS AND METHODS

Reagents and chemicals

Paracetamol and flupirtine maleate reference substances are gifted by Lara Drugs

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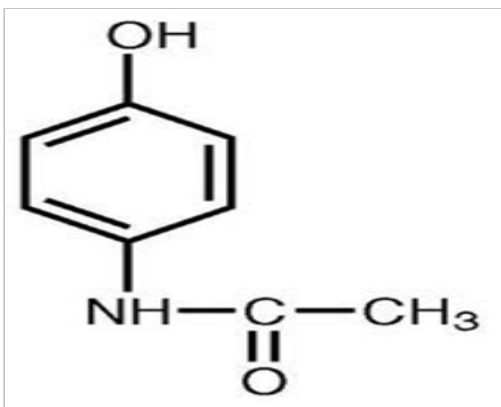


Figure 1: Structure of paracetamol

Pvt Limited, Hyderabad, India. Tablet dosage forms (KETOFLAM-P, LupinMaxter, Mumbai) were procured from the local market, each tablet containing 325 mg of paracetamol and 100 mg of flupirtine maleate. HPLC grade water and Methanol were purchased from E.Merck (India) Ltd., Mumbai. Ortho-phosphoric acid of AR Grade were obtained from S.D. Fine Chemicals Ltd., Mumbai.

Instrumentation

The HPLC system consisted of Waters Alliance 2695 separations module, integrated with Auto Sampler and equipped with Waters 2998 Photodiode Array Detector. The output of signal was monitored and integrated using waters Empower 2 software.

Chromatographic conditions

Thermo BDS hypersil C₁₈ column (250 x 4.6 mm i.d, 5 μ) was used for separation. The mobile phase containing water and methanol (adjusted to pH 3.35 with ortho-phosphoric acid) in the ratio of 50:50 v/v was delivered at a flow rate of 1.0 ml/min with detection at wavelength 250 nm. The Injection volume was 20 μl and the analysis was performed at ambient temperature.

Standard stock solution

Stock solutions of paracetamol and flupirtine maleate (1 mg/ml) were prepared separately using methanol as solvent. From the standard stock solutions, mixed standard solutions of different concentrations ranging from 650 to 1950 μg/ml of paracetamol and 200 to 600 μg/ml of flupirtine maleate were prepared by diluting with mobile phase. With the optimized chromatographic conditions, a steady base line was recorded. Twenty micro litres of each mixed standard solution was injected six times and chromatograms were recorded. The retention time of paracetamol and flupirtine maleate were found to be 3.5 and 5.4 min, respectively. Calibration curves were constructed by plotting the average peak areas against the respective concentrations and found to be linear in

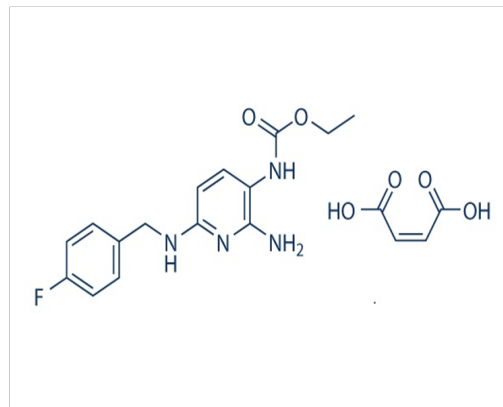


Figure 2: Structure of flupirtine maleate

the above range with the correlation coefficients (r^2) 0.999 and 0.999 for paracetamol and flupirtine maleate, respectively.

Analysis of paracetamol and flupirtine maleate in combined dosage form

Twenty tablets were weighed and average weight was determined and finely powdered. Tablet powder equivalent to 325 mg of paracetamol and 100 mg of flupirtine maleate was accurately weighed and transferred to 100 ml volumetric flask. The contents were sonicated after adding 50 ml of methanol and the volume was made up to the mark with same. The sample solution was filtered through whatmann filter paper. Suitable dilutions with the diluent were made to prepare tablet solutions containing 1300 μg/ml of paracetamol and 400 μg/ml of flupirtine maleate. Twenty micro liters of the solution was injected into the chromatographic system and the peak areas were measured and the quantitation was carried out by keeping these values to the regression equation of corresponding calibration curve.

VALIDATION¹¹⁻¹²

The method was validated for accuracy, precision, linearity, limit of detection, limit of quantitation and robustness as per ICH guidelines.

Linearity

The linearity of the method was determined at six concentration levels ranging from 650 to 1950 μg/ml of paracetamol and 200 to 600 μg/ml of flupirtine maleate. The regression equation of calibration curves were $Y = 1069x - 1243$ for paracetamol and $Y = 4655x - 12538$ for flupirtine maleate.

Accuracy

Method accuracy was performed by adding known amounts of paracetamol and flupirtine maleate to the preanalysed synthetic mixture solution and then comparing the added concentration with the found con-

Table 1: System Suitability and Validation Parameters

Parameters	Paracetamol	Flupirtine maleate
Theoretical plates	5660	6482
Resolution	-----	6.43
Tailing factor	1.11	1.02
Retention Time (min)	3.5	5.4
Linearity range ($\mu\text{g/ml}$)	650-1950	200-600
Regression Equation $Y=mx+c$	$Y = 1069x - 1243$	$Y = 4655x - 12538$
Slope (m)	1069	4655
Intercept (c)	1243	12538
Correlation coefficient	0.999	0.999
Percent RSD		
Intra day (n=3)	0.16	0.16
Inter day (n=3)	0.09	0.04
LOD ($\mu\text{g/ml}$)	6.6	2.39
LOQ ($\mu\text{g/ml}$)	21.78	7.96

*Average of six determinations, Y-Area under the curve and x is the concentration, RSD-Relative standard deviation, LOD-Limit of detection, LOQ-Limit of quantitation

centration. Three levels of solutions were made which correspond to 50%, 100%, 150% of the nominal analytical concentration (650 $\mu\text{g/ml}$ for paracetamol and 200 $\mu\text{g/ml}$ for flupirtine maleate). Each level was made in triplicate [Table 3]. The percentage recovery in each case was calculated. As per the ICH guideline, the %recovery must be between 98% and 102%.

Precision

Precision was studied to find out intra and inter day variation in the proposed method at three different levels on the same day and on three different days, respectively. The %RSD was calculated for intra-day and inter-day precision was found to be less than 1%.

Limit of detection (LOD) and limit of quantitation (LOQ)

Calibration curves were prepared using concentrations in the range of 650 to 1950 $\mu\text{g/ml}$ of paracetamol and 200 to 600 $\mu\text{g/ml}$ of flupirtine maleate (expected detection limit range). The standard deviation of Y intercepts of regression lines were determined and kept in the following equation for the determination of LOD and LOQ.

$$\text{Detection limit} = 3.3 \sigma/S$$

$$\text{Quantitation limit} = 10 \sigma/S$$

where,

σ is the Standard deviation of Y intercept of regression lines

S is the slope of calibration curve

The LOD was found to be 6.6 $\mu\text{g/ml}$ for paracetamol and 2.39 $\mu\text{g/ml}$ for flupirtinemaleate. LOQ was found

to be 21.78 $\mu\text{g/ml}$ for paracetamol and 7.96 $\mu\text{g/ml}$ for flupirtine maleate, respectively.

Robustness

Robustness of the method was determined by making slight changes in the composition of mobile phase $\pm 2\%$, flow rate by ± 0.2 ml, detection wavelength by ± 2 nm and temp by ± 2 $^{\circ}\text{C}$. It was observed that there were no marked changes in the retention time and area of the chromatograms and the % RSD was less than 1%, which demonstrated that the developed RP-HPLC method was robust.

Specificity

Commonly used excipients (starch, lactose, magnesium stearate etc.) were spiked into a pre weighed quantity of drug mixture. The chromatogram was taken by appropriate dilutions and the amount of each drug present the sample mixture was determined.

Stability

In order to demonstrate the stability of the both the standard and sample solutions during analysis, both the solutions were analyzed over a period of 5 hours at room temperature. The peak areas and retention time of both the drugs remained almost unchanged and no significant degradation within the indicated period.

RESULTS AND DISCUSSION

The goal of this study was to develop and validate a novel RP- HPLC method for simultaneous analysis of paracetamol and flupirtine maleate in its bulk and pharmaceutical formulations using a commonly used reverse phase C_{18} column. To develop an effective method for

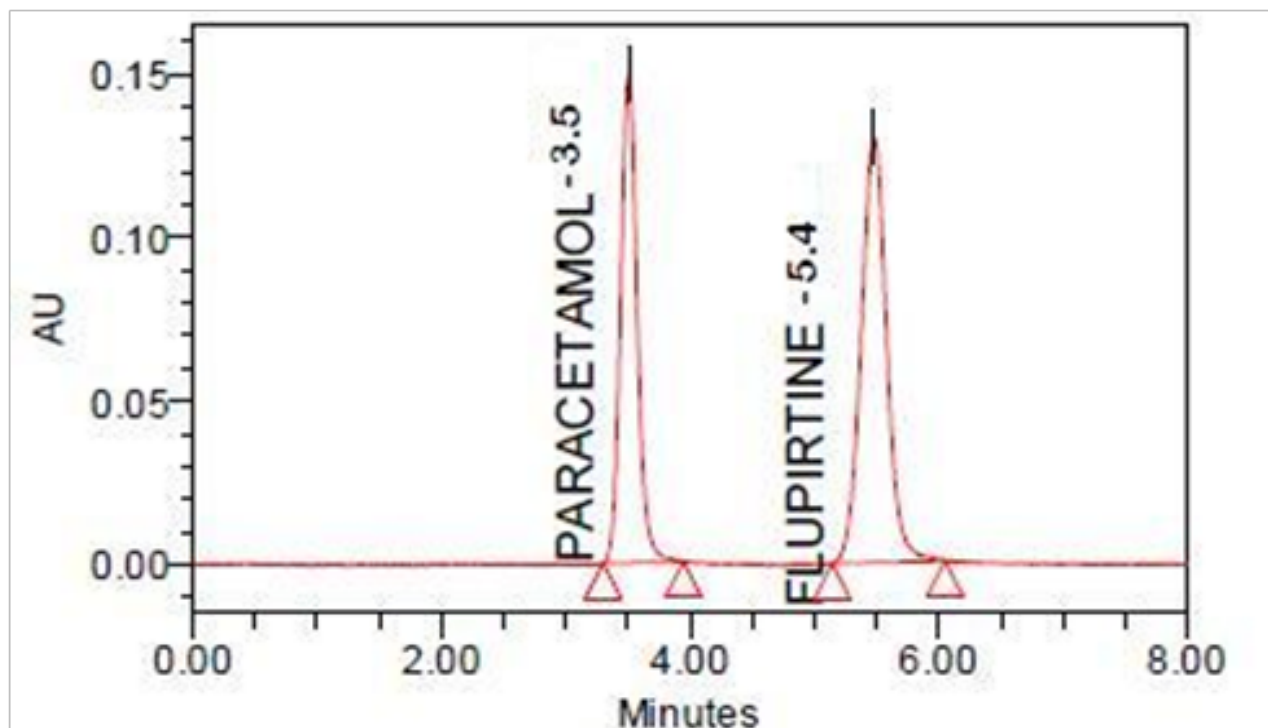


Figure 3: Typical chromatogram showing separation of Paracetamol (1300 µg/ml) and Flupirtine maleate (400 µg/ml) in combined dosage formulation

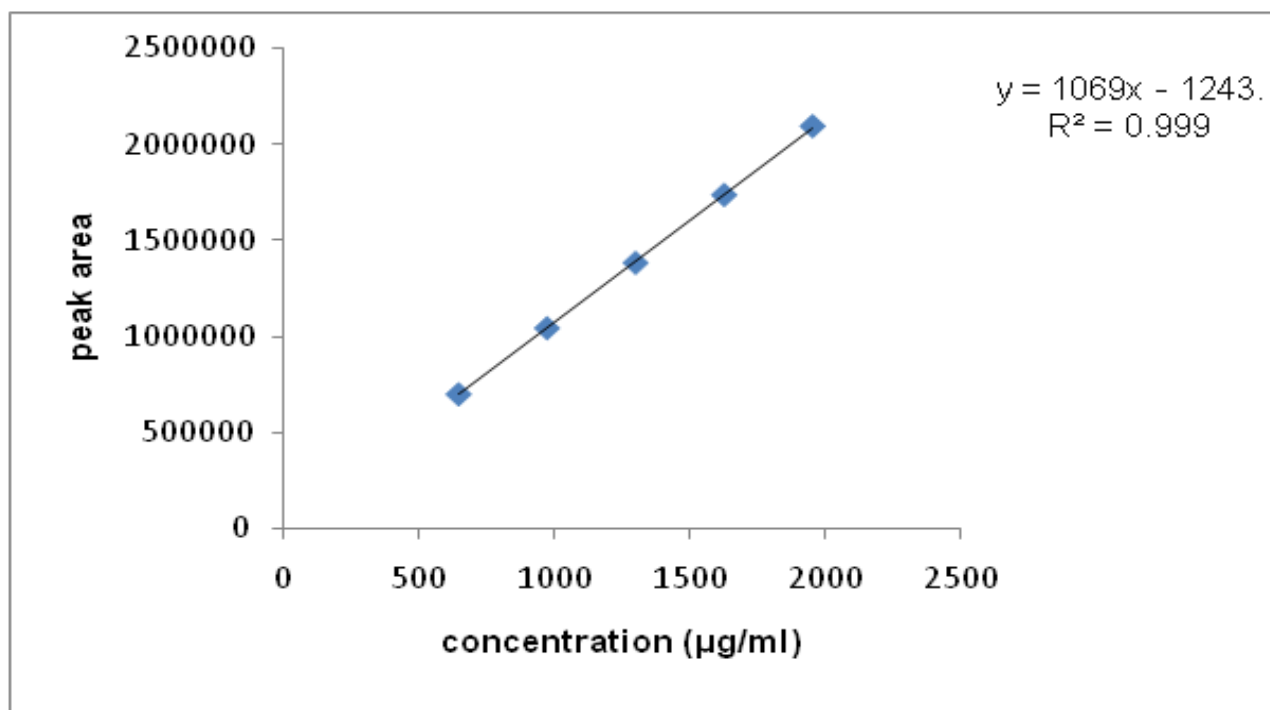


Figure 4: Calibration curve of paracetamol

the analysis of the drugs, preliminary tests were performed in order to select adequate and optimum conditions. Parameters such as detection wavelength, ideal mobile phase and its combination, optimum pH and concentration of the standard solution were studied. The mobile phase containing water and methanol (pH was adjusted to 3.35 ± 0.03 with ortho-phosphoric acid)

in the ratio of 50:50 v/v with a flow rate of 1.0 ml/min was selected for analysis after preliminary tests. The retention time of paracetamol and flupirtine maleate were 3.5 and 5.4 min, respectively. Resolution between paracetamol and flupirtine maleate was found to be 6.43, which indicate good separation of both the compounds.

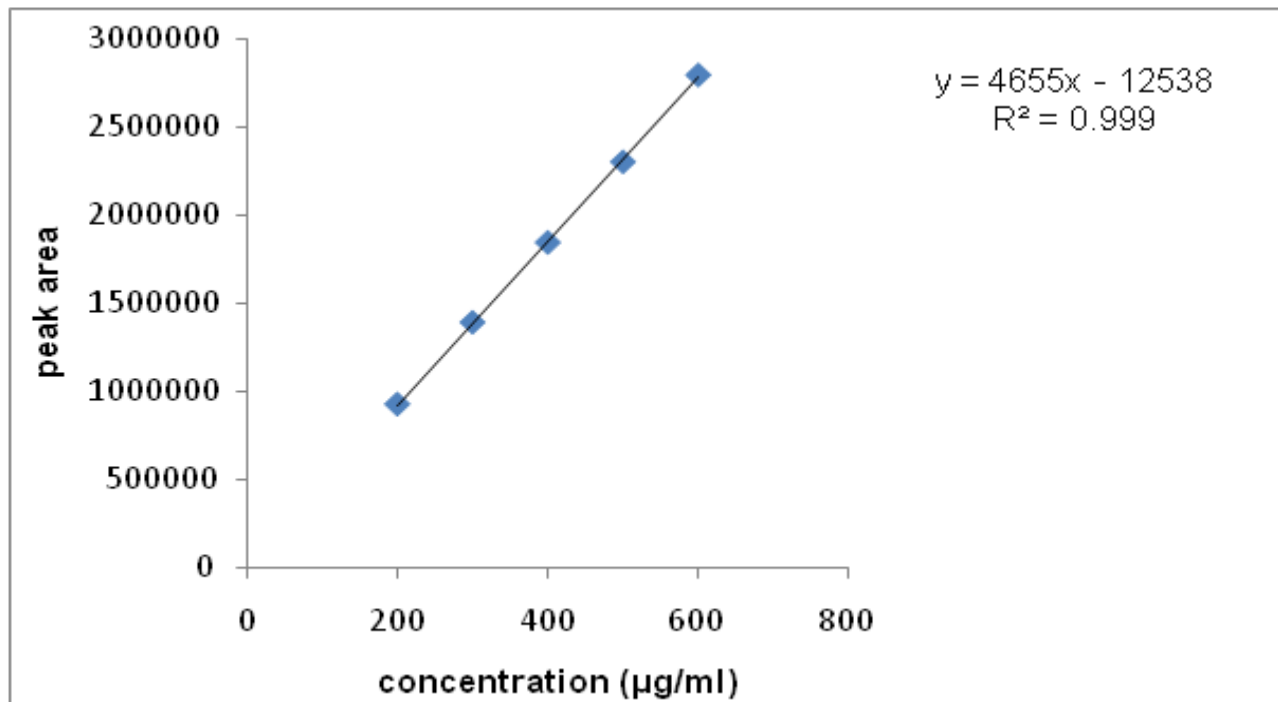


Figure 5: Calibration curve of flupirtine maleate

Table 2: Robustness data

Parameter	Proposed	Variation	Paracetamol	Flupirtine maleate
			% RSD*	
Wavelength (nm)	250	252	0.087	0.171
		248	0.068	0.165
Mobile phase	50:50 v/v	48:52	0.129	0.175
		52:48	0.183	0.260
Flow rate (ml/min)	1 ml/min	1.2	0.164	0.101
		0.8	0.154	0.239
Temperature	30° C	28	0.100	0.078
		32	0.120	0.053

*%RSD –Percent Relative Standard Deviation (Six determinations)

Table 3: Results of the recovery studies

Drug	Recovery Level	Amount added (µg/ml)	Amount recovered* (µg/ml)	%Recovery±SD*	%RSD*
Paracetamol	50%	325	324.77	99.83±0.11	0.11
	100%	650	650.75	100.08±0.04	0.03
	150%	975	974.50	99.94±0.02	0.02
Flupirtine	50%	100	100.09	100.09±0.03	0.02
	100%	200	199.72	99.86±0.19	0.19
	150%	300	300.86	100.28±0.36	0.35

* Mean of three replicates, SD-Standard deviation, %RSD- Relative standard deviation

Table 4: Results of analysis of commercial formulation

Drug	Amount mg/tablet		%RSD	%Drug estimated
	Label claim	Amount found±SD		
Paracetamol	325	324.21±0.38	0.11	99.76
Flupirtine maleate	100	100.15±0.17	0.16	100.15

*Average of six determinations, SD-Standard deviation

System suitability tests were carried out on freshly prepared standard solutions and the parameters are summarized in Table 1. The values obtained demonstrated that the suitability of the system for analysis of these drugs in combined dosage form. Typical chromatogram showing separation of paracetamol and flupirtine maleate is given in Figure 3. The calibration curves of paracetamol and flupirtine maleate were constructed by plotting the peak area and the drug (Y-axis) to the concentration (x-axis). It was found to be linear with a correlation coefficient of 0.999 and 0.999 which shows that good correlation exists between area of the peak and the concentration [Figure 4 and 5]. This method is validated for its intra-day and inter-day precision. The results obtained were in the acceptable limit [Table 1]. Robustness of the method was studied by changing the chromatographic conditions slightly and the results were presented in the Table 2. Detection limit for paracetamol and flupirtine maleate was 6.6 µg/ml and 2.39 µg/ml and quantitation limit was 21.78 µg/ml and 7.96 µg/ml, which suggest that a microgram quantity of both the compounds can be estimated accurately. The developed RP-HPLC method in present study was used to quantify paracetamol and flupirtine maleate in combined dosage form and the results of assay were comparable with the corresponding labeled amounts [Table 4]. High recovery values and no additional peaks, in the chromatogram indicate that the proposed procedure is free from interference of the commonly used excipients in the formulation. So the proposed method is accurate and specific and can be used for routine analysis of paracetamol and flupirtine maleate in their combined dosage form.

CONCLUSION

Proposed study describes a new RP-HPLC method for the estimation of paracetamol and flupirtine maleate in combined dosage form. The method gives good resolution between the compounds with a short analysis time.

The method was validated and found to be simple, sensitive, accurate and precise.

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