Original Article

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Development and Validation of Stability Indicating RP-HPLC Method for Simultaneous Estimation of Paracetamol and Flupirtine maleate in Pure and Pharmaceutical Dosage Forms

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ABSTRACT

Objective: The objective of the proposed method was to develop a simple, fast, sensitive, and validated high-performance liquid chromatography (HPLC) method for the simultaneous estimation of Paracetamol and Flupirtine Maleate in combined-dosage form. **Materials and Methods:** A Hypersil BDS C18, 150 x 4.6, 5 µ column with mobile phase containing Phosphate buffer (Ph 6.2): Acetonitrile (600:400) was used. The flow rate was 1.0 mL/min, column temperature was 30°C and effluents were monitored at 245 nm. The retention times of Paracetamol and Flupirtine Maleate were 3.1 min and 5.2 min respectively. **Results:** The correlation co-efficient for Paracetamol and Flupirtine Maleate were found to be 0.99 and 1 respectively. The proposed method was validated with respect to linearity, accuracy, precision, specificity, and robustness. Recovery of Paracetamol and Flupirtine Maleate in formulations was found to be 100% and 100% respectively confirms the non-interferences of the excipients in the formulation. Degradation studies reveals that purity threshold is greater than the purity angle hence the peak is said to be pure. **Conclusion:** Due to its simplicity, rapidness and high precision, this method was successfully applied to the estimation of Paracetamol and Flupirtine Maleate in combined dosage form.

Key words: Flupritine maleate, method development, paracetamol, RP-HPLC, stability indicating and validation.

Access this article online	
Journal Sponsor	Website: www.jyoungpharm.org
www.phcog.net	DOI: 10.5530/jyp.2015.2.5

INTRODUCTION

Paracetamol is chemically known as N-(4-hydroxyphenyl) acetamide. The empirical formula is $C_8H_9NO_2$. Paracetamol is thought to act primarily in the CNS, increasing the pain threshold by inhibiting both isoforms of cyclo oxygenase, COX-1, COX-2, and COX-3 enzymes involved in prostaglandin (PG) synthesis. It functions as a weak inhibitor of the synthesis of prostaglandins (PGs).[1] The in vivo

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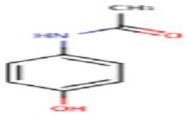

Figure 1: Chemical structure of Paracetamol

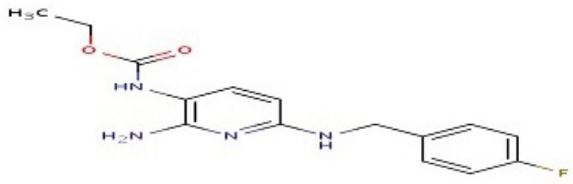

Figure 2: Chemical structure of Flupritine maleate

effects of paracetamol are similar to those of the selective cyclooxygenase-2 (COX-2) inhibitors.[2] Unlike NSAIDs, acetaminophen does not inhibit cyclooxygenase in peripheral tissues and, thus, has no peripheral anti-inflammatory affects. The antipyretic properties of acetaminophen are likely due to direct effects on the heat-regulating centers of the hypothalamus resulting in peripheral validation, sweating and hence heat dissipation. The chemical structure of Paracetamol was shown in Figure 1.

Flupirtineis an aminopyridine that functions as a centrally acting non-opioid analgesic. It is chemically ethyl N-(2-amino-6-{[(4-fluorophenyl) methyl] amino} pyridin-3-yl) carbamateacts as selective neuronal potassium channel opener that also has NMDA receptor antagonist properties.[3] The empirical formula is $C_{15}H_{17}FN_4O_2$. Flupirtine up regulates Bcl-2, increases glutathione levels, activates an inwardly rectifying potassium channel, and delays loss of intermito chondrial membrane calcium retention capacity. Flupirtine acts like a NMDA receptor antagonists, but does not bind to the receptor. One study concluded that the discriminative effects of flupirtine are neither of opioid nor of alpha-1 adrenergic type, but are primarily mediated through alpha-2 adrenergic mechanisms. The chemical structure of Flupirtine Maleate was shown in Figure 2.

Literature survey revealed that few analytical methods such as and LC-MS,[4] UV spectrophotometric,[5-8] and HPLC,[9-14] methods have been reported for the estimation of Flupirtine and hence we tried to develop and validate a new stability indicating RP-HPLC methodas per ICH guidelines,[15,16] for the estimation of Flupirtine maleate and paracetamol in bulk and pharmaceutical dosage forms.

MATERIALS AND METHOD

Instrumentation

The separation was carried out on HPLC system with Waters 2695 alliance with binary HPLC pump, UV-Visible detector, Waters Empower 2 software and column Hypersil BDS C18, 150 x 4.6, 5 µ.

Chemicals and reagents

The working standards of Paracetamol, Flupirtine Maleate were provided as gift samples from Lara drugs Pvt. Ltd., Hyderabad. Marketed formulation of combination was purchased from local market. Methanol of HPLC grade was purchased from E. Merck (India) Ltd., Mumbai. Orthophosphoric Acid and Acetonitrile of AR grade were obtained from S.D. Fine Chemicals Ltd., Mumbai and milli Q water.

HPLC conditions

The mobile phase consisting of phosphate buffer (p^H 6.5 ± 0.1 adjusted with dilute orthophosphoric acid or dilute potassium hydroxide solution) Acetonitrile (HPLC grade) were filtered through 0.45 µm membrane filter before use, degassed and were pumped from the solvent reservoir in the ratio of 600:400 v/v was pumped into the column at a flow rate of 1.0 mL/min. The column temperature was 30°C. The detection was monitored at 245 nm and the run time was 10 min. The volume of injection loop was 20 µL prior to injection of the drug solution the column was equilibrated for at least 30 min. with the mobile phase flowing through the system.

Preparation of standard solution

Paracetamol

Accurately weighed quantity 32.5 mg of Paracetamol was transferred into 100 mL of volumetric flask, add 30 ml diluent and sonicated to dissolve and diluted to volume with diluent. (Stock solution). From the above solution 10 ml was taken into 100 ml volumetric flask and diluted to volume with diluent.

Table 1: System suitability

Actual method	Sample name	RT	Tailing	Plate count
Flow (1ml)	Metformin	3.048	1.147	6782
Temp(30°C)				
PH(4.5)	Linagliptin	4.457	1.096	8231
Mobile phase(600:400 %V/V)				

RT=Retention Time

Table 2: Recovery studies for Paracetamol

Spiked Level	Sample Weight mg/L	Sample Area	Amount Recovery	% recovery
50%	16.25	423769	50.15	50.15
50%		424436		
50%		424554		
100%	32.5	845094	100.02	100.02
100%		845902		
100%		846132		
150%	48.75	1271094	150.07	150.07
150%		1271435		
150%		1265789		

Table 3: Recovery studies for Flupritine maleate

Spiked Level	Sample Weight mg/L	Sample Area	Amount Recovery	% recovery
50%	5	140998	50.05	100.11
50%		141323		
50%		142096		
100%	10	281325	100.28	100.28
100%		283029		
100%		283435		
150%	15	422675	149.39	99.59
150%		422132		
150%		421854		

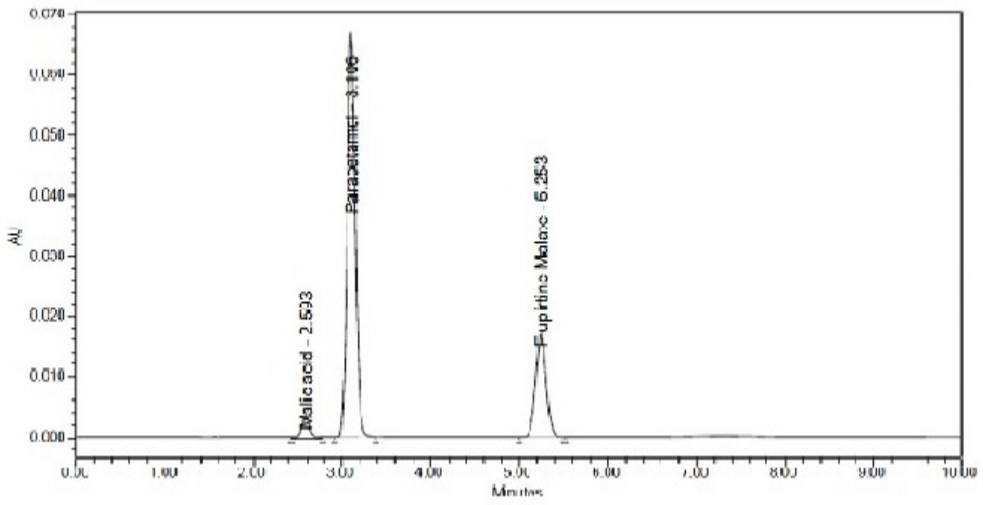

Figure 3: Accuracy at 50% level

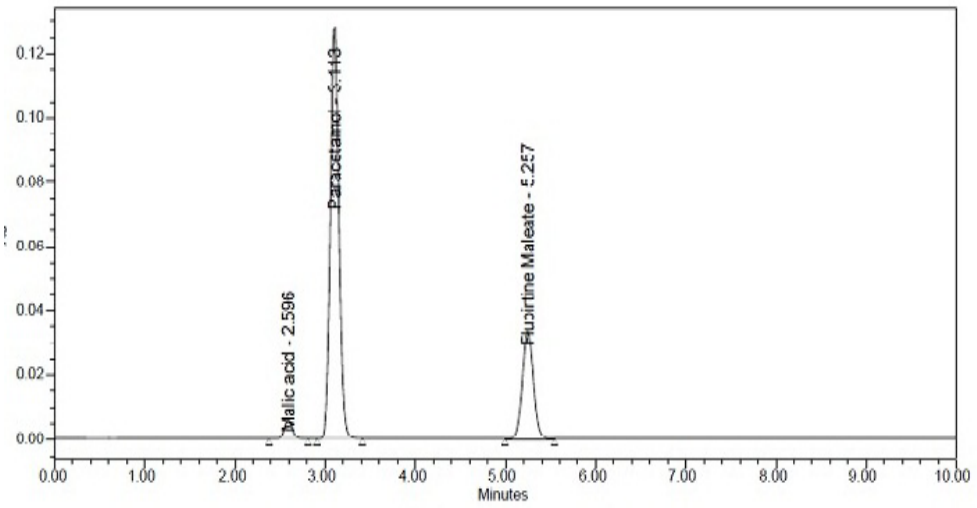

Figure 4: Accuracy at 100% level

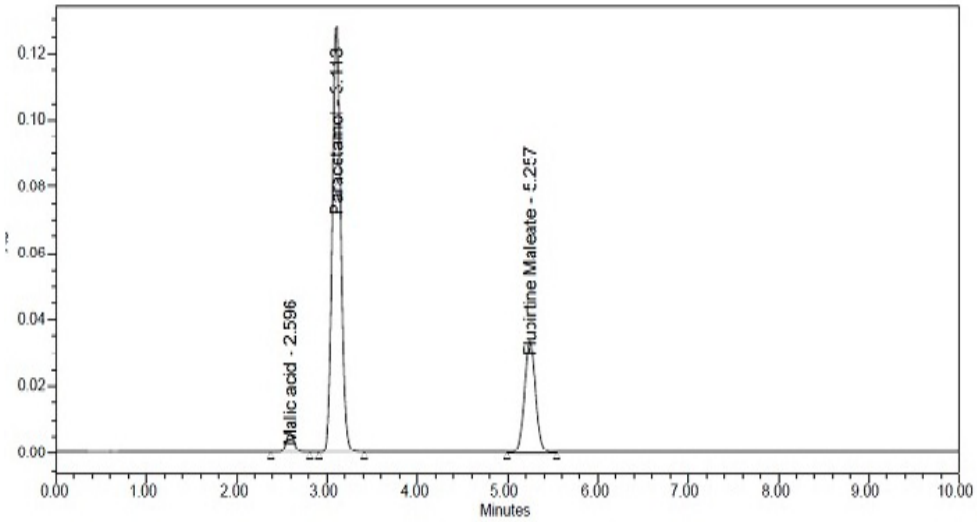

Figure 5: Accuracy at 150% level

Table 4: Intraday precision of paracetamol and flupritine maleate

Sample Weight(mg)	Paracetamol	Flupritine	% Assay (Paracetamol)	% Assay (Flupritine)
68	844123	281326	99.5	99.9
68	844905	282136	99.6	100.2
68.1	845213	282675	99.5	100.2
68.3	845540	283125	99.3	100.1
68.8	845658	283340	98.6	99.4
68.6	844890	283905	98.8	99.9
		Average Assay	99.2	100.0
		%RSD	0.42	0.30

Table 5: Interday precision of Paracetamol and Flupritine maleate

Sample Weight	Paracetamol	Flupritine	%Assay Paracetamol)	% Assay (Flupritine)
68	854365	290143	100.7	103
68	859904	288951	101.4	102.6
68.9	866436	291254	100.8	102.1
68.9	857650	289045	99.8	101.3
68.9	869054	292109	101.1	102.4
68.8	869434	293246	101.3	102.9
		Average Assay	101.0	102.4
		%RSD	0.3	0.61

Flupirtine Maleate

Accurately weighed quantity 10 mg of Flupirtine Maleate was transferred into 100mL of volumetric flask, add 30 ml diluent and sonicated to dissolve and dilute to volume with diluent. (Stock solution). From the above solution 10 ml was taken into 100 ml volumetric flask and diluted to volume with diluent.

Preparation of sample solution

Previously grinded powder equivalent to 68.094 mg weight was transferred into 100 mL volumetric flask, added 30 mL of diluent, sonicated to dissolve for 10 minutes and diluted to volume with diluent. The solution was filtered through 0.45 µ filter. 10 ml of filtrate was diluted to 100 ml with diluent.

Method validation

System suitability studies

The concept behind the system suitability test is to ensure that the complete testing system is suitable for the intended application. The results of system suitability are presented in Table 1.

Specificity

Specificity is the ability of the developed method to detect the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc.

Accuracy

The accuracy of an analytical procedure expresses the closeness of the agreement between the actual values to the mean analytical value. The accuracy of the method was determined by recovery experiments. The recovery studies were carried out at three concentration levels. Each level was repeated for three times. The percentage recovery and standard deviation was calculated. The mean recoveries of Paracetamol, Flupirtine Maleate were found between 98% to 102%. The results indicated good accuracy of the method for the determination of analyzed drugs as revealed by mean recovery data. The results are presented in Table 2 & 3. The accuracy at three levels was shown in Figures 3, 4, & 5.

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The precision of the method was demonstrated by intra-day and inter-day variation studies. In the intra-day studies the assay on six test preparations were performed by injected into the chromatographic system as per the test method. The % assay of drug and percentage RSD were calculated. The inter-day variations of the method also determined by using six replicate injections of the same concentration and analyzed on two consecutive days and response factor of drugs peaks and percentage RSD was calculated. From the data obtained, the developed RP-HPLC method was found to be precise. The results are presented in Table 4 & 5.

Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. Chromatographic method was tested for linearity by plotting peak area against concentration of solutions. The plot of peak area versus the respective concentrations of Paracetamol and Flupirtine Maleate were found to be linear in the concentration range of 8-50 µg/mL and 2.5-15 µg/mL respectively. The regression equation for Paracetamol is y=26287x – 4353 with a coefficient of correlation (R^2) of 0.99. The regression equation for

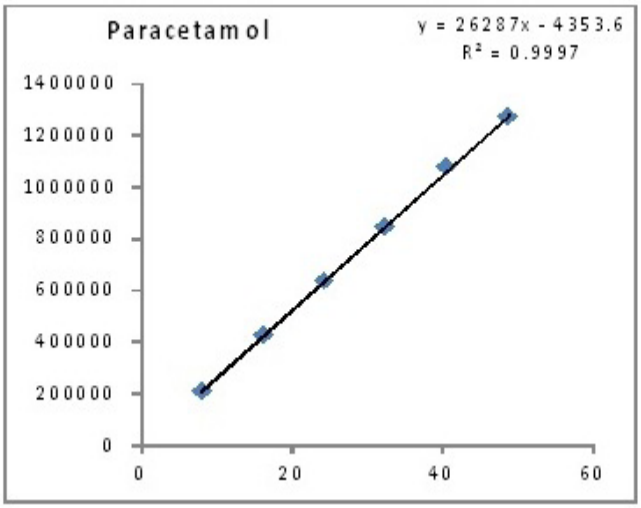

Figure 6: Linearity of Paracetamol

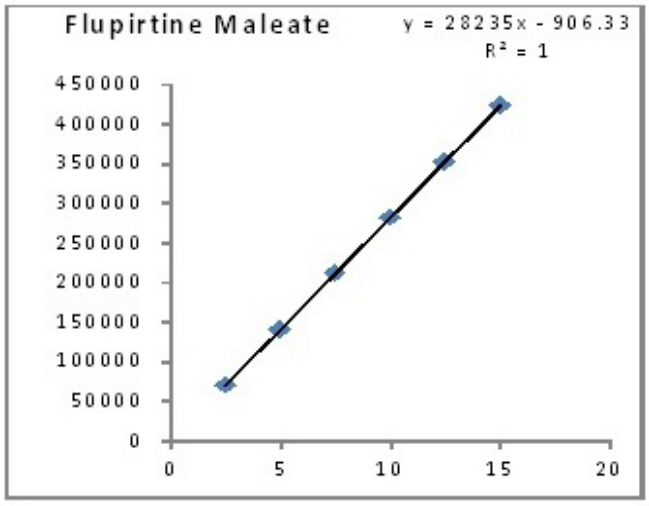

Figure 7: Linearity of Flupritine maleate

Flupirtine Maleate is y = 28235x-906.3 with a coefficient of correlation (R^2) of 1. The results shows that an excellent correlation exists between areas and concentration of drugs within the concentration range indicated above. And the results for calibration curves are given in Figure 6 & 7. The results are presented in Table 6.

Limit of Detection and Limit of Quantification (LOD&LOQ)

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products.

(a) LOD= 3/s/n*100% conc

(b) LOQ=10/s/n*100% conc

LOD= 3/2415*2000 =2.484 (Paracetamol)

LOQ= 10/2415*2000=8.281(Paracetamol)

LOD= 3/128*10=0.2344 (Flupirtine Maleate)

LOQ= 10/128*10=0.7813 (Flupirtine Maleate)

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. The Robustness of the method was determined by making slight changes in the chromatographic conditions i.e. flow variation ± 10% (0.9 ml and 1.10 ml/min), temp variation ± 5% variation (25°c and 35°c). It was observed that there were no marked changes in the system suitability results, which demonstrated that the RP-HPLC method developed was robust.

Forced Degradation Studies

The stability studies were determined by applying the physical stress (acid, base, thermal and light) to the product. It was observed that there were marked degradation in the chromatograms, and the data given in Tables 7& 8.

RESULTS AND DISCUSSION

System suitability parameters are retention time, resolution, tailing and plate count were shown uniformity and %RSD was less than 1 (limit of % RSD is less than 2.0%) so we can say that system is suitable for the analysis. Method specificity was concluded by standard chromatogram and formulation chromatogram. There was no interference from placebo and excipients peaks with standard and analytic peaks. These results demonstrate the absence of interference from other materials. Hence it confirms the specificity of the proposed method. The method accuracy was evaluated by recovery studies. Paracetamol and Flupirtine Maleate recovery was found between 98%-102% and also percentage RSD was very low so method

Table 6: Linearity of Paracetamol and Flupritine maleate

Paracetamol		Flupritine	
Conc(μg)	**Area**	**Conc(μg)**	**Area**
8.125	209068	2.5	69326
16.25	423491	5	140749
24.375	635779	7.5	211283
32.5	844012	10	280920
40.625	1075477	12.5	351635
48.75	1271319	15	422976

Table 7: Forced degradation for Flupirtine maleate

Mode of degradation	Conditions	Area	% Assay	% Deg.	Purityangle	Purity Threshold
Control	No treatment	-	-	-	-	-
Acid degradation(5 N HCl)	40°C/5 min	257127	81	-19	0.433	1.023
Alkali degradation (1N NaOH)	80°C/1 hr	240724	80	-20	0.387	0.895
Light (200 watts hrs/min)	105°C/72 hrs	214259	90	-10	0.285	0.566
Heat (105°C/72 hr	25°C/72 hrs	274441	93	-7	0.354	0.565

Table 8: Forced degradation for Paracetamol

Mode of degradation	Conditions	Area	% Assay	% Deg.	Purity angle	Purity Threshold
Acid degradation(5N HCl)	40°C/5 min.	823568	80	-20	0.324	0.675
Alkali degradation (1N NaOH)	80°C/1 hr.	817961	84	-16	0.332	0.702
Light (200 watts hrs/min)	821226	92	-8	0.276	0.465	
Heat (105°C/72 hr)	25°C/72 hr.	820010	92	-8	0.314	0.657

is accurate. Calibration curve was plotted concentration versus areas. Linear correlation was found to be y = 26287x – 4353 for Paracetamol, y=28235x-906.3 for Flupirtine Maleate. The intra-day and inter-day variations were calculated in terms of % RSD. The low % RSD results revealed that method was precise. Method robustness was performed by making small deliberal changes in the chromatographic conditions. The robustness results proved that the method was robust. Degradation studied were performed under different conditions and in each condition it was observed that the purity threshold value was found to be greater than the purity angle value which indicates that the peak is pure i.e. no interference of degradants with the analyte peak.

CONCLUSION

The proposed HPLC method was found to be simple, precise, accurate and sensitive for the simultaneous estimation of Paracetamol, Flupirtine Maleate in pharmaceutical dosage forms. Degradation studies reveals that the developed method was stability indicating Hence, this method can easily and conveniently adopt for routine quality control analysis of Paracetamol, Flupirtine Maleate in pure and its pharmaceutical dosage forms.

CONFLICT OF INTEREST

This study was unfunded. There were no conflicts of interest.

ACKNOWLEDGEMENT

We acknowledge the pharmacy departments of Jawaharlal Nehru technological university, Kakinada and Andhra university for their assistance.

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