IJPSR (2014), Vol. 5, Issue 2 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 14 September, 2013; received in revised form, 24 October, 2013; accepted, 10 January, 2014; published 01 February, 2014

METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS DETERMINATION OF FLUPIRTINE MALEATE AND PARACETAMOL BY RP – HPLC TECHNIQUE

P. Haritha [1], B. Sreenivasa Rao*[2] and Y. Sunandamma [3]

Department of Chemistry, JNTUK [1], Kakinada, Andhra Pradesh, India
GITAM University [2], Visakhapatnam, Andhra Pradesh, India
Department of Chemistry, Vikrama Simhapuri University [3], Nellore, Andhra Pradesh, India

Keywords:

RP-HPLC, Isocratic, Flupirtine maleate, Paracetamol

Correspondence to Author:

B. Sreenivasa Rao

GITAM University, Visakhapatnam, Andhra Pradesh, India

E-mail: srbattula@gmail.com

ABSTRACT: The present study describes a simple, accurate and precise RP-HPLC Technique for the simultaneous determination of Flupirtine maleate and Paracetamol in pharmaceutical dosage form. The method involves an isocratic elution of drug in a stationary phase of Phenomenex, C18 (150mm × 4.6mm, 5µm) column using a mobile phase composition of methanol and 0.1% (v/v) orthophosphoric acid in the composition ratio of 60:40 v/v with a flow rate of 0.8 mL/min at 270 nm of detection. The injection volume is 20 µL. the method has been validated for specificity, linearity, range, precision, accuracy, limit of detection, limit of quantification, ruggedness and robustness. The retention times for Flupirtine maleate and Paracetmol are about 3.07 and 4.63 minutes respectively. Quantitative linearity was observed over the concentration range of 10.08 to 302.51 µg/mL for Flupirtine maleate and 4.99 to 99.80 for Paracetamol respectively. The regression equations of concentration of Flupirtine maleate and Paracetamol are found to be y = 1774x+4755, y = 39182x + 64154 respectively where y is the peak area and x is the concentration of drug (µg/mL). The % recovery of Flupirtine maleate and Paracetamol are found to be in the range of 97% to103 %. All the validation parameters are within the acceptance range

INTRODUCTION: Flupirtine maleate is pyridine derivative with the chemical name of ethyl 2 amino 6 (4 - fluorobenzylamino) 3- pyridyl-carbamate maleate (**Fig. 1a**).

Flupirtine maleate is used as an analgesic for acute and chronic pain, in moderate to severe cases.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.5(2).463-72

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.5(2).463-72

Its muscle relaxant properties make it popular for back pain and other orthopedic uses, but it is also used for migraines, in oncology, postoperative care and gynecology. Paracetamol is chemically named asN – acetyl – p – aminophenol (**Fig. 1b).** It is used as pain reliever and antipyretic. The combination of flupirtine maleate and Paracetamol was found to be more effective in relieving pain.

Flupirtine maleate and Paracetamol tablets are available in the combination of 100 + 500 mg, 1000+500 mg. Literature survey revealed spectro-fluorimetric method [1], spectrophotometric method [2] and RP- HPLC method [3], for the validation of Paracetamol drug alone.

Paracetamol in combination with other drugs is reported to be estimated by spectrophotometric method [4], HPLC method [5], and RP-HPLC method [6, 7]. Flupirtine maleate estimatation in pharmaceutical dosage forms is reported by UV – VIS spectrophotometry [8].

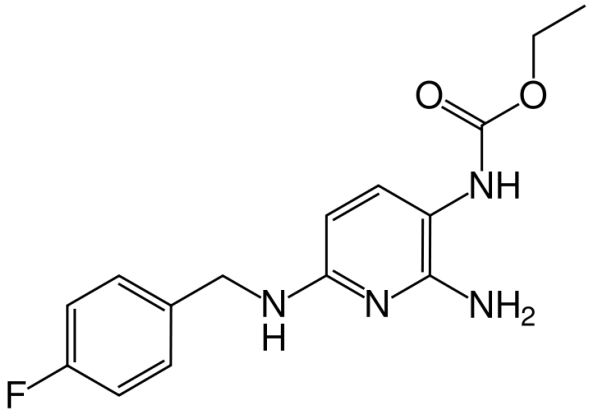


FIG-1A: STRUCTURE OF FLUPIRTINE MALEATE

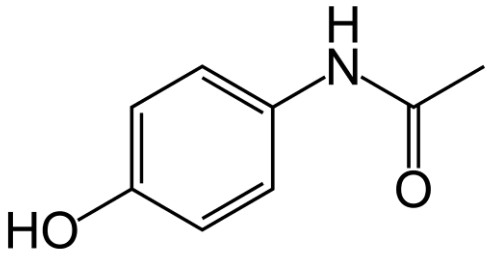


FIG-1B: STRUCTURE OF PARACETAMOL

The present work describes a validated reverse phase HPLC method for simultaneous determination of Flupirtine maleate and Paracetamol in tablets. The proposed method is validated as per ICH guidelines [9].

MATERIALS AND METHODS:

Reagents and Chemicals: Orthophosphoric acid (AR grade, SD Fine chem limited), methanol (HPLC grade, Merck limited), Milli–Q water, Flupirtine maleate (99.8% w/w procured from Lupin Pharma) and Paracetamol (99.8% w/w procured from Unichem Laboratories Ltd). All other chemicals are of the highest grade commercially available unless otherwise specified.

Instrumentation: The Chromatographic system consisted of a Shimadzu Class VP Binary pump LC-10ATvp, SIL-10ADvp Auto sampler, CTO-10Avp Column Temperature Oven, SPD-10Avp UV-Visible Detector. All the components of the system are controlled using SCL-10Avp System Controller. Data acquisition was done using LC Solutions software.

The mobile phase consisted of 60:40 % (v/v) of Methanol and 0.1% Orthophosphoric acid operated on isocratic mode. The flow rate is 0.8mL/min. Chromatographic determination of Flupirtine maleate and Paracetamol was performed on Phenomenex® Prodigy C18 column (150 X 4.6 mm, 5µm). The wavelength of detection is 270 nm. The injection volume is 20µL.

Preparation of standard solutions, Calibration Standards & Quality Control Samples: Stock solutions of Flupirtine maleate (10mg/mL), & Paracetamol (1mg/mL) were prepared separately in a volumetric flask using methanol and labeled accordingly. Suitable dilutions were then prepared using 50:50 %v/v methanol & Milli-Q water as diluent solution. For the linear calibration curve, seven non-zero standards were prepared using diluent solution in the concentration range of 10.08 to302.51 µg/mL for Flupirtine maleate and 4.99 to 99.80 µg/mL for Paracetamol.

The calibration standard sample is then transferred into the auto sampler for analysis. Samples for specificity (Sample with Flupirtine maleate alone, sample with Paracetamol alone, Blank Sample and sample containing both the drugs) were also prepared accordingly.

For the preparation of quality control samples, a separate stock containing approximately the same concentration of the Flupirtine maleate and Paracetamol were prepared and labeled as quality control stocks.

From these stocks, quality control samples containing Flupirtine maleate and Paracetamol were prepared at three concentration levels namely LQC, MQC, and HQC so as to obtain low, medium and high concentration quality control samples. The performance of the linear calibration curve is then evaluated using quality control samples.

Assay: The assay of tablets containing Flupirtine maleate and Paracetamol is done using the procedure given in Indian Pharmacopoeia under tablets. The active ingredients in each of 10 dosage units is taken by random sampling and analyzed by the developed method.

The tablets are said to be compliance if the each individual content is 90 – 110 % of the average content or labeled claim.

For the current assay ten tablets were randomly taken and transferred separately into 100ml volumetric flasks and dissolved in 20 ml methanol. The solution was then ultrasonicated for 10min and then made up to volume. Required amount of solution is then taken and filtered through 0.45µ nylon membrane and diluted with diluent solution so that the resultant concentrations are within the calibration range of the developed method. The samples are then analyzed by using the validated method. The sample is then injected in triplicate.

Method Validation:

System Suitability: A sample containing mixture of Flupirtine maleate (approximate concentration of 151µg/ml) and Paracetamol (approximate concentration of 50µg/ml) was used as system suitability sample. System suitability was assessed by six replicate analysis. A percent coefficient of variation (% CV) less than 1 % for retention times for the drugs is taken as the acceptance criterion.

Detection and Quantification Limits (Sensitivity): Limits of detection (LOD) and Limit of quantification (LOQ) (**Fig. 2**) were estimated from both linearity calibration curve method and signal to noise ratio method. The detection limit was defined as the lowest concentration level resulting in a peak area of three times the baseline noise. The quantification limit was defined as the lowest concentration level that provided a peak area with signal to noise ratio higher than 5, with precision (%CV) and accuracy with (±) 20%.

Linearity (Calibration Curve): The Linearity of detector response to different concentrations of both the drugs was studied with a series of working standard solutions prepared by diluting the stock solution. The standard plots were then constructed between concentration Vs. Peak area using seven non-zero standards ranging from 10.08 to 302.51 µg/mL for Flupirtine maleate and 4.99 to 99.80 µg/mL for Paracetamol. The linearity was evaluated by linear regression analysis, which was calculated by least square method. It is depicted in (**Fig. 3**).

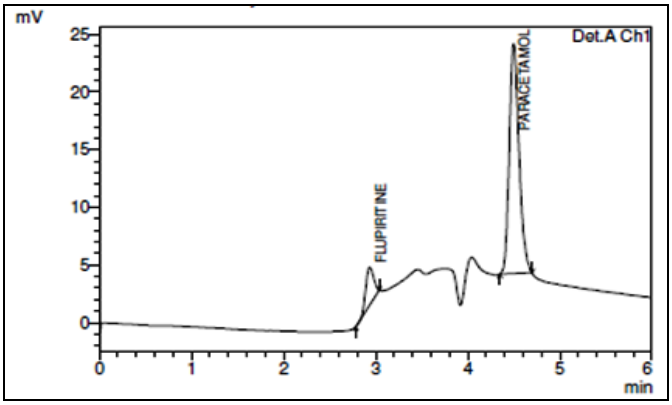
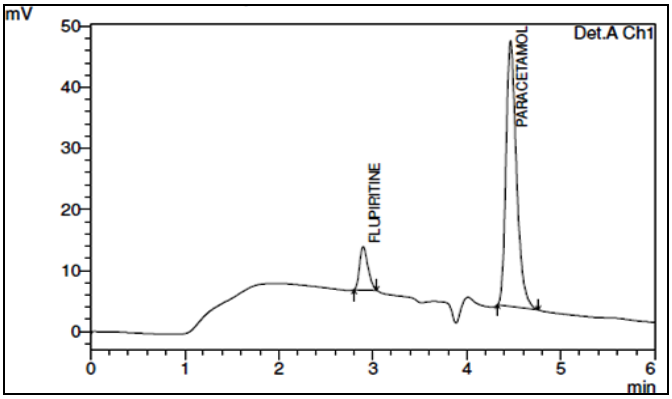


FIG. 2: CHROMATOGRAM FOR LOD SAMPLE



CHROMATOGRAM FOR LOQ SAMPLE

Accuracy and Precision: According to ICH guidelines, repeatability should be assessed by using a minimum of nine determinations covering the specified range for the procedures (i.e. three concentrations and three replicates of each concentration). Precision was studied to find out intra and inter day variations of the proposed method at three different levels.The %CV values less than 2% indicate that the method was precise.

Specificity: For demonstration of specificity, 4 samples namely blank sample, sample containing Flupirtine maleate alone, sample containing Paracetamol alone and sample containing the mixture of Flupirtine maleate and Paracetamol were prepared separately. Specificity of the method was determined by comparing results of all the samples (**Fig. 4**). The developed method is said to be specific if the % interference calculated as peak area (if any) at the retention time of each of the analytes in the blank sample is less than 20% of peak area at the corresponding retention times of each of the drugs in the lowest calibration standard. Sample Specificity is also observed in the degradation study of the drug. None of the degraded products must interfere with the quantification of the drug.

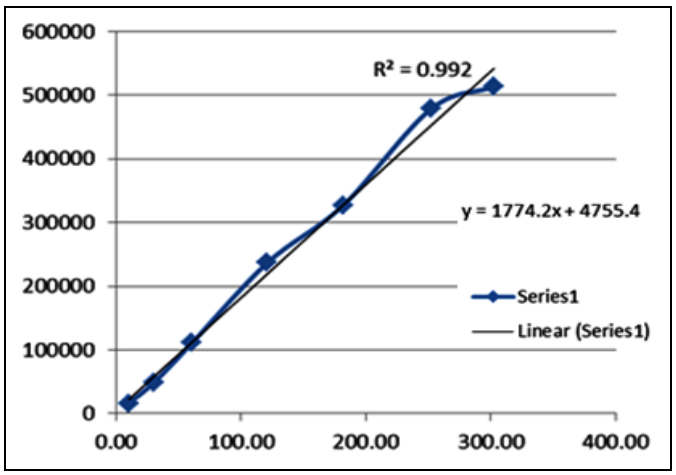


FIG. 3A: LINEAR CALIBRATION CURVE OF FLUPIRTINE MALEATE

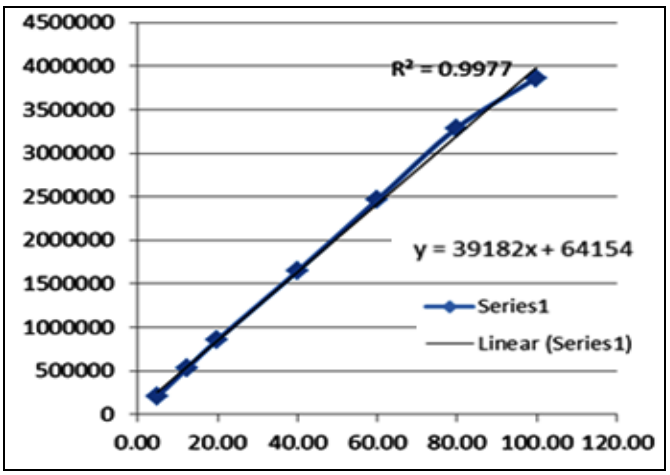


FIG. 3B: LINEAR CALIBRATION CURVE OF PARACETAMOL

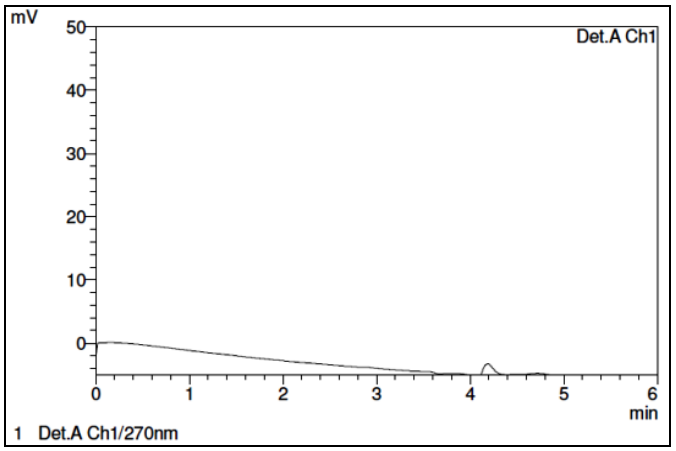


FIG. 4A: BLANK CHROMATOGRAM

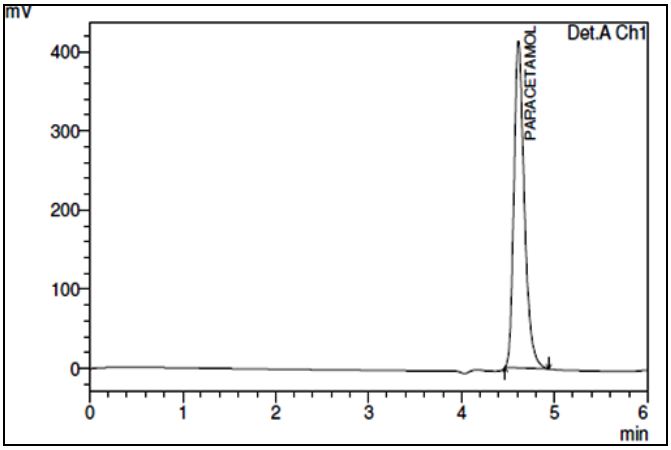


FIG. 4C: CHROMATOGRAM OF PARACETAMOL ALONE

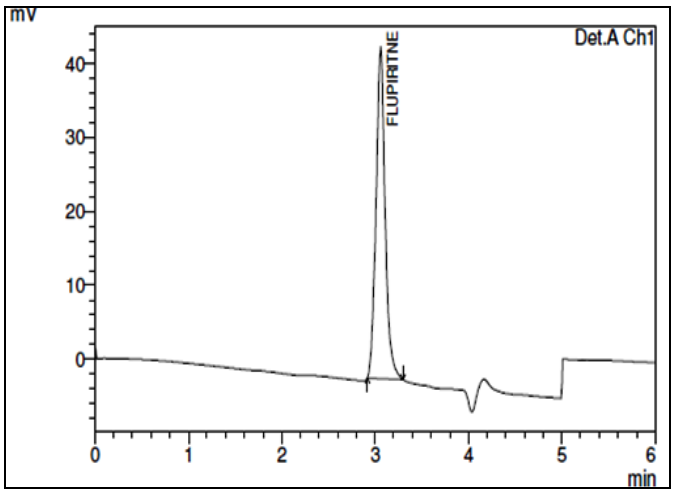


FIG. 4B: CHROMATOGRAM OF FLUPIRTINE MALEATE ALONE

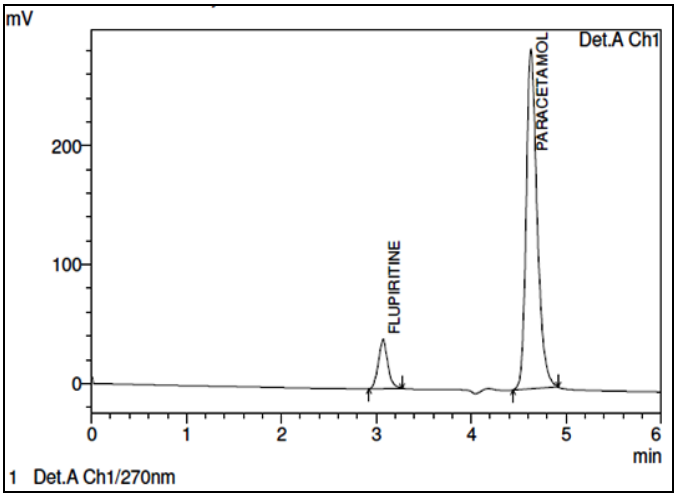


FIG. 4D: CHROMATOGRAM OF BOTH FLUPIRTINE MALEATE AND PARACETAMOL

FIG. 4: COMPARISON OF (A) BLANK CHROMATO-GRAM, (B) FLUPIRTINE MALEATE ALONE, (C) PARACETAMOL ALONE AND (D) SAMPLE CONTAINING BOTH FLUPIRTINE MALEATE AND PARACETAMOL

Stability: The stability of the drug is determined by placing the MQC samples for the short term stability at room temperature up to 12 hours and then comparing the obtained peak area with that of the similarly prepared fresh sample. Further, auto-sampler stability for up to 24 hrs was studied and established.

Stress Degradation Studies: For Stress Degradation Analysis, 1 mL aliquots (in duplicate) of samples containing MQC level concentration are treated separately with 100 µL of 0.1N HCl (Acid stress), 0.1N NaOH (Alkaline stress), 5% v/v Hydrogen Peroxide (Oxidative Stress), for 24 Hrs. Samples for Photolytic stress are placed in a transparent glass vial & placed in a UV chamber for 24 Hrs. Samples are then injected for analysis. The results of analysis are then compared with similarly prepared fresh samples. The analysis is performed in triplicate.

RESULTS AND DISCUSSION:

Method Development and Validation: The HPLC procedure was optimized with a view to develop a stability indicating assay method. Chromatographic behavior at different pH values ranging from pH 3.0 to pH 6.5 using various columns like Hypersil-BDS-C18, Symmetry C18, Ymc-pack C18, Ymc-pack pro, Spherisorb C18, Phenomenex C18 have been tried with different buffer salts such as ammonium Formate, orthophosphoric acid, di-potassium hydrogen orthophosphate, in combination with acetonitrile, methanol and tetrahydrofuran is done. However less tailing and high theoretical plates are obtained with Phenomenex Prodigy ODS2 C18,(150 X 4.6 mm), 5µm column.

The final mobile phase composition consisted of (60:40 v/v) of Methanol and 0.1% Orthophosphoric acid on isocratic mode. The flow rate of the method is 0.8 mL/min. Calibration standards were prepared in diluents solution containing 50:50 % v/v of Methanol and Milli-Q water.The wavelength of detection is 270nm. The column temperature is maintained at 25^{O}C. At the reported flow rate, peak shape was excellent; however increasing or decreasing the flow rate resulted in unacceptable tailing factor and poor peak shape. Hence, 0.8 mL/min was optimized flow rate decreasing the consumption of the mobile phase, which in turn proves to be cost effective for long term routine quality control analysis. To evaluate the feasibility of the experiment under regular lab conditions, the assessment of the stability of Flupirtine maleate and Paracetamol under room temperature and under normal light conditions is done.

Method Validation:

System Suitability: The % CV of the peak area for both drugs is within the acceptable criteria (**Table 1**). The efficiency of the column was expressed as the number of theoretical plates for the six replicate injections was around 10456.3±366.41 for Flupirtine maleate and 20225.3±351.80 for Paracetamol. The USP tailing factor was 0.948 ± 0.0147 for Flupirtine maleate while that of Paracetamol is 1.252 ± 0.02.

Determination and Quantification Limits (Sensitivity): Fig. 2 represents the chromatogram of limit of detection and limit of quantification. The method is found to be sensitive which can be determined from the data obtained from the (**Table 2)**.

Linearity: The linearity was demonstrated in triplicate. The results of the best fit line (y = mx + c) for the triplicate analysis is given in **Table 3**. The accuracy of the calibration standards was evaluated from the back calculated concentrations **(Table 4)**. All the standards were found to be within the range of 97 – 103 %.

Accuracy and Precision: Accuracy and precision calculated for the QC samples during the intra- and inter –day run are given in the **Table 5**.The intra-day (day-1) and inter-day accuracy for Flupirtine maleate ranged from 97.24 – 100.41 %. While that of Paracetamol ranged from 97.37 – 103.82 %. The results obtained from intermediate precision (inter-day) also indicated a good method precision. All the data were within the acceptance criteria.

Specificity: Specificity was determined by comparison of the Blank chromatogram with that of the Standard chromatogram (**Fig. 4**).

Stress Degradation: Stress studies revealed that Flupirtine maleate is not susceptible to degradation under acid, oxidative stress, light (UV) stress conditions (**Fig. 5a**).

However, in alkaline conditions (0.1N NaOH), the drug was unstable and the degradation peak eluted earlier accompanied with a drastic peak distortion and increased tailing. Except for alkaline conditions, the drug content was within 97 –106 % for all stress conditions indicating the stability and specificity of the analytical method to differentiate the degradation peaks.

Room Temperature Stability: Stability studies were done for short term stability up to 12 hrs on the bench top for the MQC levels conditions. Stability is calculated as the ratio of the mean peak area of the stability sample to the mean peak area of the fresh sample and expressed as the percentage (n=6). The room temperature stability was found to be 93.91% for Flupirtine maleate and 98.69 % for Paracetamol. The results are tabulated in **Table 6**. Stress studies on Paracetamol indicated the stability of drug under acid, oxidative stress, light (UV) and alkaline (0.1N NaOH) conditions (**Fig. 5b**). This has been clearly demonstrated by the help of overlap spectra of all the stress samples as compared with that of freshly prepared sample of similar concentration **(Fig. 6b)**. For all the stress conditions the Paracetamol content was within 100 –103 % indicating the stability and specificity of the analytical method to differentiate the degradation peaks.

TABLE 1A: SYSTEM SUITABILITY FOR FLUPIRTINE MALEATE

Sample ID	Peak Retention Time	Peak Area	Theoretical Plates	Tailing Factor
1	3.08	441216	9992	0.93
2	3.08	422723	10443	0.94
3	3.05	383959	11024	0.96
4	3.08	422138	10607	0.95
5	3.07	430282	10538	0.94
6	3.01	417526	10134	0.97
MEAN	**3.062**	**419640.7**	**10456.3**	**0.948**
STDEV	**0.0279**	**19350.06**	**366.41**	**0.0147**
%CV	**0.91**	**4.61**	**3.50**	**1.55**

TABLE 1B:SYSTEM SUITABILITY FOR PARACETAMOL

Sample ID	Peak Retention Time	Peak Area	Theoretical Plates	Tailing Factor	Resolution
1	4.60	2319467	20221	1.26	22.17
2	4.60	2283901	20579	1.26	22.16
3	4.58	2100210	19965	1.27	22.93
4	4.61	2284941	20564	1.23	22.76
5	4.60	2351352	20343	1.26	22.66
6	4.54	2096696	19680	1.23	22.69
MEAN	**4.588**	**2239427.8**	**20225.3**	**1.252**	**22.56166667**
STDEV	**0.0256**	**112014.80**	**351.80**	**0.02**	**0.321211249**
%CV	**0.56**	**5.00**	**1.74**	**1.38**	**1.42370355**

TABLE 2: SENSITIVITY OF FLUPIRITINE MALEATE & PARACETAMOL

	LOD Flupirtine maleate			**LOD Paracetamol**	
S. No.			**S. No.**		
	Retention Time	**Peak Area**		**Retention Time**	**Peak Area**
1	2.93	19911	1	4.5	147881
2	2.89	19984	2	4.46	130193
3	2.89	18943	3	4.46	142499
MEAN	**2.9**	**19612.7**	**MEAN**	**4.5**	**140191.0**
ST DEV	**0.02**	**581.10**	**ST DEV**	**0.02**	**9067.05**
% CV	**0.80**	**2.96**	**% CV**	**0.52**	**6.47**
	LOQ Flupirtine maleate:			**LOQ Paracetamol**	
S. No.			**S. No.**		
	Retention Time	**Peak Area**		**Retention Time**	**Peak Area**
1	2.89	43712	1	4.46	337629
2	2.94	43991	2	4.5	325434
3	2.93	43990	3	4.47	337630
MEAN	**2.9**	**43897.7**	**MEAN**	**4.5**	**333564.3**
ST DEV	**0.03**	**160.79**	**ST DEV**	**0.02**	**7041.08**
% CV	**0.91**	**0.37**	**% CV**	**0.47**	**2.11**

TABLE 3: RESULTS OF BEST-FIT LINE FOR TRIPLICATE ANALYSIS FOR FLUPIRTINE MALEATE (ABOVE) AND PARACETAMOL (BELOW)

Flupirtine maleate			
curve	**Slope**	**Intercept**	**r^2**
1	1774	4755	0.992
2	1783	4632	0.993
3	1764	4842	0.991
Mean	1773.666	4743	0.992
Paracetamol			
Curve	**Slope**	**Intercept**	**r^2**
1	39182	64154	0.997
2	39331	64140	0.998
3	39280	63280	0.996
Mean	39264.33	63858	0.997

TABLE 4: LINEARITY AND RANGE FOR FLUPIRITINE MALEATE (ABOVE) AND PARACETAMOL (BELOW)

Flupirtine maleate					
Sample ID	**Concentration (Microgram/mL)**	**Retention Time**	**Peak Area**	**Back Calc Concentration**	**% Accuracy**
Blank	0.00	**NA**	**0**	NA	**NA**
CC - 01	10.08	2.94	16415	6.57	65.21
CC - 02	30.25	2.95	49388	25.16	83.17
CC - 03	60.50	2.97	110903	59.84	98.90
CC - 04	121.00	2.99	237123	130.99	108.25
CC - 05	181.51	2.99	326931	181.61	100.06
CC - 06	252.09	3.02	478695	267.16	105.98
CC - 07	302.51	3.02	513458	286.75	94.79
CC - 08	Blank	**NA**	0	**NA**	NA
Paracetamol					
Sample ID	**Concentration (Microgram/mL)**	**Retention Time**	**Peak Area**	**Back Calc Concentration**	**% Accuracy**
Blank	0.00	**NA**	**0**	NA	NA
CC - 01	4.99	4.51	212605	3.79	75.93
CC - 02	12.48	4.51	537437	12.08	96.79
CC - 03	19.96	4.52	858443	20.27	101.56
CC - 04	39.92	4.52	1643484	40.31	100.97
CC - 05	59.88	4.50	2461586	61.19	102.18
CC - 06	79.84	4.51	3288073	82.28	103.06
CC - 07	99.80	4.51	3863172	96.96	97.15
CC - 08	Blank	**NA**	0	NA	NA

TABLE 5A: RESULTS OF INTER AND INTRA-DAY ACCURACY&PRECISION FOR FLUPIRTINE MALEATE

Flupirtine maleate	**Nominal concentration(µg/mL)**		
	75.63 (LQC)	**151.26 (MQC)**	**226.88 (HQC)**
DAY 1 (Intraday)			
MEAN (n=6)	100.41	97.57	97.44
STDEV	2.24	5.46	6.65
% CV	2.23	5.59	6.82
DAY 2			
MEAN (n=6)	99.85	97.48	97.34
STDEV	0.58	5.23	1.12
% CV	0.58	5.26	1.12

DAY 3			
MEAN (n=6)	99.7	98.62	97.24
STDEV	0.43	0.97	0.21
% CV	0.41	0.96	0.21

TABLE 5B: RESULTS OF INTER AND INTRA-DAY ACCURACY&PRECISION FOR PARACETAMOL

Paracetamol	**Nominal Concentration (µG/ML)**		
	24.95 (LQC)	**49.90 (MQC)**	**74.85 (HQC)**
DAY 1 (INTRA DAY)			
MEAN (N=6)	103.82	97.37	101.08
STDEV	3.23	5.59	3.72
% CV	3.11	5.74	3.68
DAY 2			
MEAN (N=6)	101.28	99.68	100.52
STDEV	0.25	0.63	0.52
% CV	0.24	0.63	0.52
DAY 3			
MEAN (N=6)	102.62	100.93	100.23
STDEV	0.43	0.32	0.92
% CV	0.5	0.32	0.92

TABLE 6A: ROOM TEMPERATURE STABILITY OF FLUPIRTINE MALEATE (n=6)

Fresh Sample (Flupirtine maleate)				**Stability Sample (Flupirtine maleate)**			
S. No.	**Sample ID**	**Drug**		**S. No.**	**Sample ID**	**Drug**	
		Retention Time	**Peak Area**			**Retention Time**	**Peak Area**
1	Fresh sample	3.00	271522	1	Stability sample	2.96	250641
2	Fresh sample	3 .00	269945	2	Stability sample	2.96	261237
3	Fresh sample	2.99	281613	3	Stability sample	2.97	279401
4	Fresh sample	2.98	293168	4	Stability sample	2.99	224583
5	Fresh sample	2.98	285909	5	Stability sample	2.95	268613
6	Fresh sample	2.98	269852	6	Stability sample	2.98	285814
MEAN			**278668.17**	**MEAN**			**261714.83**
SD			**9759.44**	**SD**			**22103.33**
% CV			**3.50**	**% CV**			**8.45**
					% Stability	93.916301	

TABLE 6B: ROOM TEMPERATURE STABILITY OF PARACETAMOL (n=6)

Fresh Sample (Paracetamol)				**Stability Sample (Paracetamol)**			
S. No.	**Sample ID**	**Drug**		**S. No.**	**Sample ID**	**Drug**	
		Retention time	**Peak area**			**Retention Time**	**Peak Area**
1	Fresh sample	4.51	2237944	1	Stability sample	4.46	2223425
2	Fresh sample	4.51	2284659	2	Stability sample	4.47	2321257
3	Fresh sample	4.5	2305508	3	Stability sample	4.5	2229891
4	Fresh sample	4.49	2382254	4	Stability sample	4.48	2282418
5	Fresh sample	4.49	2356244	5	Stability sample	4.49	2398554
6	Fresh sample	4.49	2356384	6	Stability sample	4.49	2285642
MEAN			**2320498.83**	**MEAN**			**2290197.83**
STDEV			**54228.56**	**STDEV**			**64625.90**
% CV			**2.34**	**% CV**			**2.82**
					% Stability	98.694203	

Robustness study: Robustness is the measure of method capacity to remain unaffected by deliberate small changes in the chromatographic conditions. The experimental conditions were deliberately altered to evaluate the robustness of the method. The impact of flow-rate (0.8 ± 0.1 mL/min), and effect of mobile-phase composition (± 5%) on chromatographic parameters such as retention time,

theoretical plates, and tailing factor, were studied**.** At normal flow rate, the retention time of Flupirtine maleate was 3.602 ± 0.02 minutes (n=6) while that of Paracetamol was 4.588 ± 0.02 minutes. At normal flow rate, the tailing factor for Flupirtine maleate is 0.948 ± 0.01 while that of Paracetamol was 1.25 ± 0.02. At higher flow rate, tailing factor for both Flupirtine maleate and Paracetamol remained unchanged as compared to normal flow. At a lower flow rate of 0.7 mL/min, Flupirtine maleate and Paracetamol eluted at 3.4 ± 0.01 and 5.1± 0.01 minutes respectively. The tailing factor of Flupirtine maleate and Paracetamol were 1.033 ± 0.0 and 1.250 ± 0.0 respectively (n=6). At mobile phase composition of 65: 35 % v/v of Methanol and 0.1% v/v orthophosphoric acid the retention times of Flupirtine maleate and Paracetamol were 2.9 ± 0.00 and 4.3 ± 0.01 minutes (n=6).

At mobile phase composition of 55: 45 % v/v of Methanol and 0.1% v/v orthophosphoric acid the retention times of Flupirtine maleate and Paracetamol were 3.0 ± 0.00 and 4.7± 0.00 minutes (n=6)

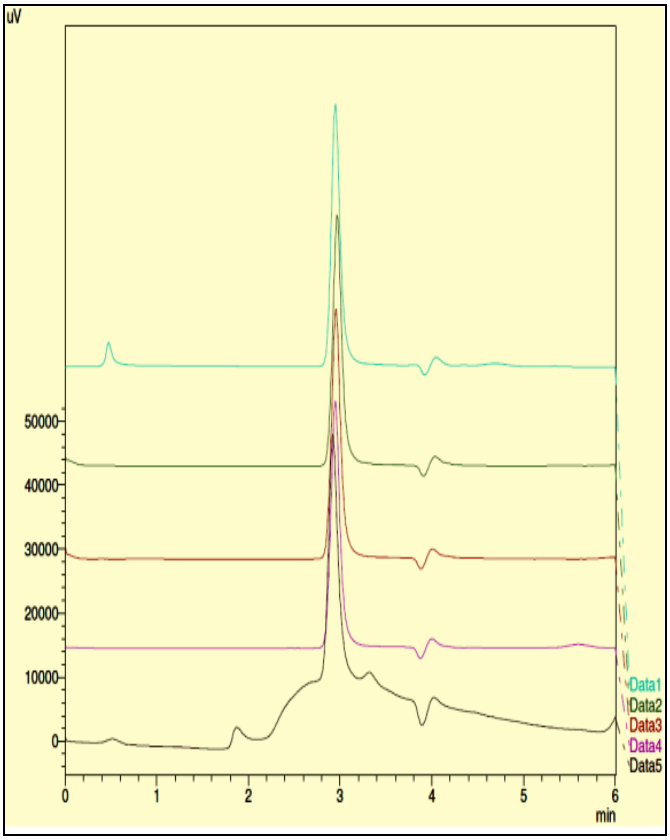


FIG. 5a: OVERLAP CHROMATOGRAM SHOWING THE INFLUENCE OF VARIOUS STRESS CONDITIONS ON FLUPIRTINE MALEATE. Data 1-Fresh sample; Data 2-Acid stress; Data 3- Oxidative stress; Data 4-Photolytic stress; Data 5-Alkaline stress

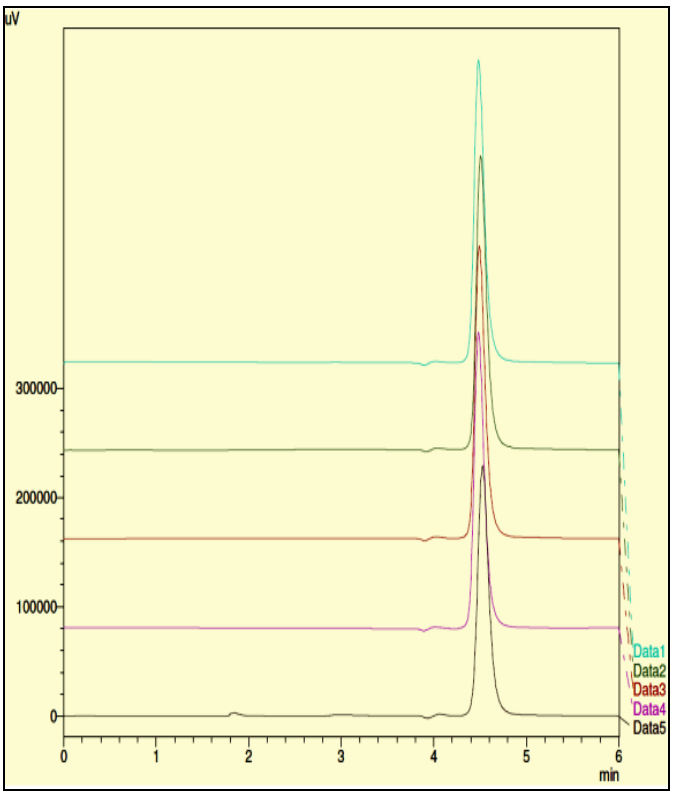


FIG. 5B: OVERLAP CHROMATOGRAM SHOWING THE INFLUENCE OF VARIOUS STRESS CONDITIONS ON PARACETAMOL. Data 1-Fresh sample; Data 2-Acid stress; Data 3- Oxidative stress; Data 4-Photolytic stress; Data 5-Alkaline stress

Application of the method to dosage forms: The HPLC method developed is sensitive and specific for the quantitative determination of Flupirtine maleate and Paracetamol. Also the method is validated for different parameters; hence it has been applied for the simultaneous estimation in pharmaceutical dosage forms. The amount of Flupirtine maleate and Paracetamol in the commercial tablet dosage form is within the pharmacopoeial specifications. None of the tablets ingredients interfered with the analyte peak. The spectrum of Flupirtine maleate and Paracetamol in the extracted tablet was matching with that of standard compounds indicating the purity of the compounds in the tablets.

CONCLUSIONS: The method gave accurate and precise results in the concentration range of 10.08 to 302.51µg/mL for Flupirtine maleate and 4.99 to 99.80 µg/mL for Paracetamol. The mobile phase composition consists of 60:40 % v/v of Methanol and 0.1 % Orthophosphoric acid at the flow rate of 0.8 mL/min. The retention time of Flupirtine maleate is 3.062 ± 0.02 minutes and that of Paracetamol is 4.588 ± 0.02 minutes.

The column is Phenomenex Prodigy ODS 2, (150 X 4.6mm), C18 column with the particle size of 5µm.A rapid sensitive and specific method for the simultaneous estimation of Flupirtine maleate and Paracetamol in the pharmaceutical tablet formulations has been developed and validated.

REFERENCES:

1. Maria delos A.Oliva , Roberto A. Olsina , Adriana N. Masi, selective spectrofluorimetric method for Paracetamol determination through coumarinic compound formation , *Elsevier* , Volume 66, Issue1 , 31 March2005, pages 229-235.
2. Andre Flavorante,cherrine k.pires, Boaventura F Reis, Multicommuted flow system employing pinch solenoid valves and micro pumps :spectrophotometric determination of Paracetamol in pharamaceutical formulations , *Journal of pharmaceutical and biomedical analysis* , volume 42 , Issue 4 ,11 october 2006, pages 423-429.
3. T.A. Phazna devi, Aravind Setti, Method Development and validation of Paracetamol drug by RP-HPLC, *Journal of Medical and Allied sciences*, volume 3, Issue 1, Year 2013, Pages 08-22.
4. Chandani Joshi, Jasmin Mansuri, Hetal Hariyani, Pooja Gandhi, Rakesh Radadiya, Dr. Shital Faldu, Q-Absorbance ratio spectrophotometric method for the simultaneous estimation of Paracetamol and Tapentadol hydrochloride in bulk drug and in pharmaceutical dosage form, *International Bulletin of Drug Research*, volume 3, Issue 4, year 2013, pages 37-45.
5. J.T. Franeta, D. Agbaba, S. Eric, S. Pavkav, M. Alersic, S. Vladimrov, HPLC assay of acetylsalicylic acid , Paracetamol , Caffeine and Phenobarbital in tablets, *Elsevier*, Volume 57, Issue 9, September 2002, Pages 709-713.
6. R.Gopinath, S.Rajan, S.N. Meyyanathan, N. Krishnaveni, B.Suresh, A RP-HPLC method for simultaneous estimation of Paracetamol and aceclofenac in tablets, *Indian Journal of Pharmaceutical sciences*, volume 69, Issue 1, year 2007, pages 137-140.
7. D. Samson Israel, K. Krishna Chaitanya, D. Gowri Sankar, A. Vijayalakshmi, Method Development and Validation for simultaneous Determination A multiple Drug dosage form of Paracetamol, Orphenedrine, Ibuprofen by RP-HPLC, *Journal of Global Trends in Pharmaceutical Sciences*, Volume 4, Issue 3, July-September 2013, Pages 1153-1162.
8. Amal D, Aneesh TP,Method Development and Validation for the estimation of Flupirtine maleate in bulk and Pharmaceutical dosage forms using UV-VIS spectrophotometry, *International Research Journal of Pharmacy*, volume 2, Issue 12, year 2011, pages 179-182.
9. International Conference on Harmonization (ICH Q2(R1)). Validation of Analytical procedures: Text and Methodology, IFPMA, Geneva, Switzerland, 2005.

How to cite this article:
Haritha P, Rao BS and Sunandamma Y: Method development and validation for simultaneous determination of Flupirtine maleate and Paracetamol by RP – HPLC Technique. *Int J Pharm Sci Res* 2014; 5(2): 463-72.doi: 10.13040/IJPSR. 0975-8232.5(2).463-72

All © 2013 are reserved by International Journal of Pharmaceutical Sciences and Research. This Journal licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 3.0 Unported License.

This article can be downloaded to **ANDROID OS** based mobile. Scan QR Code using Code/Bar Scanner from your mobile. (Scanners are available on Google Playstore)