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

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Abstract

A simple, selective, rapid, precise and economical reverse phase high performance liquid chromatographic (RP-HPLC) method has been developed for the simultaneous estimation of Paracetamol (PARA) and Flupiritine Maleate (FLU) from pharmaceutical formulation. The method is carried out on Agilent C18 (25 cm x 4.6 mm i.d., 5 µ) column with a mobile phase consisting of Methanol : Water (0.2% TEA, adjusted to pH 3.0 using orthophosphoric acid) in the ratio of 90:10 v/v. The retention time of Paracetamol and Flupiritine Maleate is 3.2 min and 5.1 min respectively with the flow rate of 1mL/ min with VWD detection at 239 nm. The linear regression analysis data for the linearity plot showed good linear relationship with correlation coefficient value for Paracetamol and Flupiritine Maleate were R2=0.9995 and R2=0.9996 in the concentration range of 9-63 µg. mL-1 , 3-21 µg. mL-1 respectively. The relative standard deviation for intra-day precision has been found to be lower than 2.0 %. The method is validated according to the ICH guidelines. The developed method is validated in terms of specificity, selectivity, accuracy, precision, linearity, limit of detection, limit of quantitation and solution stability. The proposed method can be used for simultaneous estimation of these drugs in marketed dosage forms.

Keywords: RP-HPLC, Paracetamol and Flupiritine Maleate

Introduction

Paracetamol (PARA) is chemically N-(4-Hydroxy Phenyl) Acetamide (amide derivative). It functions as a weak inhibitor of the synthesis of prostaglandins (PGs).[1] However, the in vivo effects of paracetamol are similar to those of the selective cyclooxygenase-2 (COX-2) inhibitors.[2] Paracetamol also decreases PG concentrations in vivo.Structure of Paracetamol (PARA) were shown in Figure-1. Flupiritine Maleate (FLU) is chemically ethyl {2-amino-6-[(4-fluorobenzyl) amino] pyridin-3-yl} carbamate acts as selective neuronal potassium channel opener that also has NMDA receptor antagonist properties.[3] Structure of Flupiritine (FLU) was shown in Figure-2. The review of literature revealed that various analytical methods involving spectrophotometry, HPLC, have been reported for Paracetamol.[5-10] Several analytical methods have been reported for Flupiritine including simultaneous estimation of Flupiritine and its metabolites in human plasma, human serum, and urine.[11-15] UV method is reported for Flupiritine.[16] But there is no HPLC method was reported for these drugs. Hence the necessity of developing simple and cost effective RP-HPLC method always a continuing interest.

In the proposed method, methanol and water (0.2%TEA) with change in pH with orthophosphoric acid was used elute analytes with greater efficiency and reduced run time, thus making the method economic, simple, reliable, more precise and reliable for stability. The proposed method is validated as per ICH guidelines.

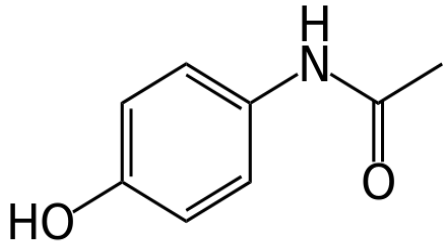

Figure 1: Paracetamol

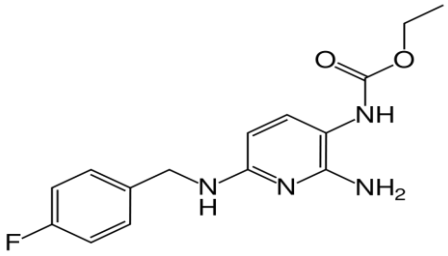

Figure 2: Pioglitazone

Materials and Methods

Instrumentation

HPLC system (Agilent HPLC Model-1120 with Ezchromelite Software) containing C18 (Agilent, 250 x 4.6 mm. 5 µ) column with UV- VWD detection. LABINDIA-3000+ UV-Visible double beam spectrophotometer with a fixed slit width 1nm and 1cm matched quartz cells was used for all the spectral measurements.

Chemicals and reagents

Analytically pure PARA and FLU were kindly provided by Lupin Pharmaceuticals Ltd, Mumbai as gift samples. Analytical grade methanol was purchased from Merck and Co. Glasswares used in each procedure were soaked overnight in a mixture of chromic acid and sulphuric acid rinsed thoroughly with double distilled water and dried in hot air oven. Water (HPLC grade) was purchased from Merck, India. Triple distilled water is used for all purpose.

Preparation of standard stock solutions

Accurately weighed 10 mg of PARA and FLU standard were transferred to separate 10 mL volumetric flask and dissolved in 10 mL Methanol. The flasks were shaken and volume was made up to the mark with Methanol to give solutions containing 1000 µg. mL-1 PARA and 1000 µg. mL-1 FLU. From this solution 1 mL was transferred to volumetric flask of 100 mL capacity. Volume was made up to the mark to give a solution containing 100 µg.mL-1 PARA and 100 µg. mL-1 FLU.

Calibration of standards

The standard calibration curve was constructed for Paracetamol and Flupiritine Maleate. Different volumes of stock solutions of each were accurately transferred in to 10 mL volumetric flasks and diluted to mark to yield a concentration range of 9-63 µg. mL-1 solutions of Paracetamol and 3-21 µg. mL-1 solutions of Flupiritine Maleate. The calibration line was obtained by plotting the peak area against concentration of drug.

Determination of Paracetamol and Flupiritine Maleate in their Combined Dosage

Sample preparation

Twenty tablets of marketed formulation Pruf-P (Safetab Life Sciences) containing FLU 100 mg and PARA 325 mg formulation were weighed, and finely powdered. Tablet powder equivalent to 100 mg FLU with relevant quantities of PARA was weighed and transferred to a 100 ml volumetric flask, extracted for 30mins with methanol and volume was made up to 100 ml with diluent. 0.12 ml of above solution was taken in 10 ml volumetric flask and volume was made up to 10 ml with mobile phase, and final solution was filtered through 0.45 µ syringe filter and it was analysed. The results of the assay were shown in Table 7.

Results

Method Development and Optimization

Some important parameters like pH of the mobile phase, concentration of the acid or buffer solution, percentage and type of the organic modifier, etc., were tested for a good chromatographic separation. Trials showed that an acidic mobile phase with reverse phase Agilent C18 column gives symmetric and sharp peaks. Methanol was chosen as the organic modifier because it dissolves drugs very well. Mobile phase composition of 90:10 (v/v) at a flow rate of 1.0 mL/min showed good resolution. When orthophosphoric acid was used as modifier, resolution between PARA and FLU was much better at pH 3.0, 0.2%TEA was used to decrease the peak tailing. Retention times of the drugs obtained under these conditions were

3.2 and 5.1 min for PARA and FLU, respectively. For the quantitative analytical purposes the wavelength was set at 239 nm. The typical chromatogram of the sample is shown in Figure 3 and the results are shown in the table 1.

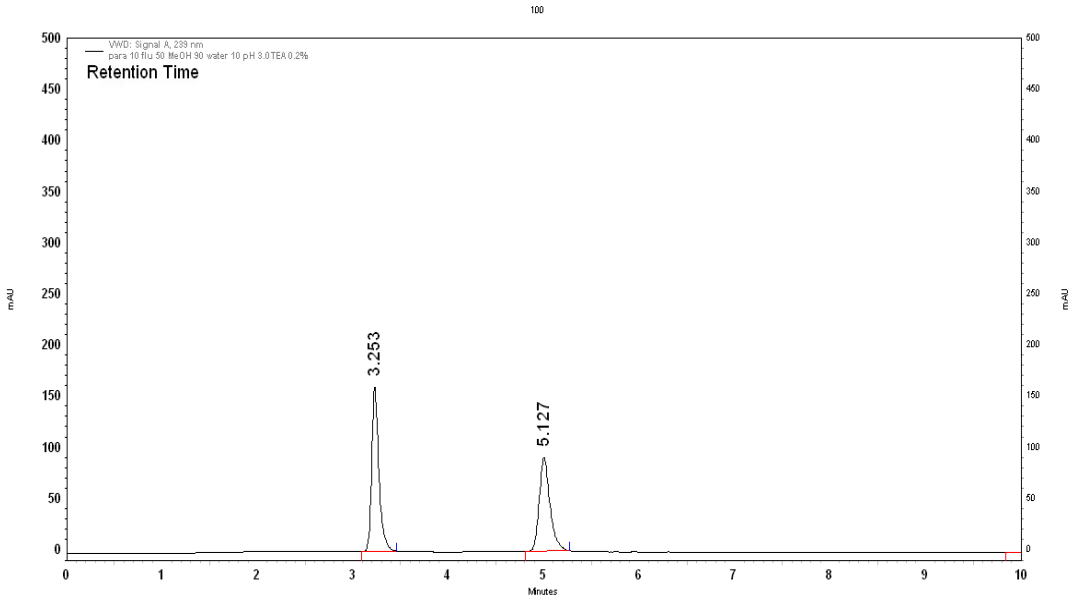

Figure 3: Optimized chromatogram of Paracetamol and Flupiritine Maleate

Table 1: Optimized Chromatographic conditions of Paracetamol and Flupiritine Maleate

On C $_{18}$ Column

S.No	Parameters	Paracetamol	Flupiritine
1	Mobile Phase Optimized	methanol: H_2O (90 : 10,0.2%TEA, pH 3.0)	methanol : H_2O (90 : 10,0.2%TEA, pH 3.0)
2	Flow Rate (mL/ min)	1	1
3	Run Time (min)	10	10
4	Column Temperature ^{O}C	23	23
5	Volume of Injection (µL)	20	20
6	Detection Wavelength (nm)	239	239
7	Retention time Rt	3.2	5.1

Method Validation parameters

Method was validated as per ICH (Q2) guidelines with respect to linearity, accuracy, precision, specificity, and robustness, limit of detection and limit of quantification.[17-20]

a) System Suitability Criteria

It is defined as tests to measure the method that can generate result of acceptable accuracy and precision. The system suitability was carried out after the method development and validation have been completed. The system suitability was assessed by five replicate analyses of the drugs at concentrations of 36 µg. mL−1 of PARA and 12 µg. mL−1 of FLU and for this, parameters like plate number (n), tailing factor, HETP, peak asymmetry of samples were measured.

b) Linearity

Appropriate volume of aliquot from PARA and FLU standard stock solution was transferred to volumetric flask of 10 mL capacity. The volume was adjusted to the mark with methanol to give solutions containing 9-63 µg. mL-1 PARA and 3-21 µg. mL-1 FLU. The slope, Y-intercept and correlation coefficient were calculated. The regression line relating standard concentrations of drug using regression analysis, the calibration curves were linear in the studied range and equations of the regression analysis were obtained: Y=62807x+22055; R2=0.9995 for PARA and Y=137410x-394335; R2=0.9996 for FLU respectively. The mean and correlation coefficient of standard curves (n=3) were calculated. The represented data was shown in below and Table 2 and 3.

Table 2: Linearity study of Paracetamol and Flupiritine Maleate

Concentration of Paracetamol (µg. mL^{-1})	Peak Area mean± SD (n=3) of Paracetamol	Concentration of Flupiritine (µg. mL^{-1})	Peak Area mean± SD (n=3) of Flupiritine	% RSD	
				PARA	FLU
9	575669±4935	3	34609 ± 431	0.8573	1.2440
18	1142181 ± 15420	6	417202 ± 4059	1.3501	0.9731
27	1760611 ± 34356	9	813040 ± 12456	1.9514	1.5321
36	2261726 ± 36305	12	1270751 ± 14728	1.6052	1.1590
45	2838375 ± 27921	15	1675616 ± 10573	0.9837	0.6311
66	4048382 ± 38014	22	2594542± 36716	1.1024	1.7563
84	5154699 ± 52514	28	3376339 ± 18797	1.3279	0.7591

Table 3: Linearity Study

Drug	Range*	Slope	Intercept	R	LOD*	LOQ*
Paracetamol	09-63	62807	22055	0.9995	0.036	0.109
Flupiritine	03-21	137410	394335	0.9996	0.057	0.173

*** µg. mL^{-1}**

c) LOD and LOQ

LOD and LOQ were calculated from the formula 3.3 x (σ/S) and 10 x (σ/S), respectively where, σ is standard deviation of intercept and S is the mean of slope. The LOD and LOQ can also be determined by S/N. The value for LOD should be 3-5 whilst for LOQ 10-15. The results are shown in table 4.

Table 4: LOD and LOQ

Drug	LOD μg. mL^{-1}	LOQ μg. mL^{-1}
Paracetamol	0.036	0.109
Flupiritine	0.057	0.173

d) Accuracy

Accuracy was assessed by determination of the recovery of the method by addition of standard drug to the pre-quantified placebo preparation at 3 different concentration levels 80%, 100% and 120%, taking into consideration percentage purity of added bulk drug samples. Each concentration was analyzed 3 times and average recoveries were measured. Results of accuracy and recovery were presented in the table 5.

Table 5: Accuracy Report of Paracetamol and Flupiritine Maleate

Drug	**Amount taken (μg. mL^{-1})**	**Recovery Level**	**Amount of Drug Added**	**Amount of Drug Found (μg. mL^{-1}) Mean± S.D**	**% RSD**	**% Recovery**
PARA	36	80%	28.8	65.85 ± 1.006	1.5278	101.62
		100%	36	71.92 ± 0.457	0.4571	99.89
		120%	43.2	78.16 ± 0.744	0.9519	98.68
FLU	12	80%	9.6	21.69 ± 0.419	1.9350	100.41
		100%	12	23.96 ± 0.208	0.8697	99.87
		120%	14.4	26.09 ± 0.347	1.3314	98.84

e) Precision

The repeatability was evaluated by assaying 6 times of sample solution prepared for assay determination. The intraday and interday precision study of PARA and FLU was carried out by estimating same concentration of PARA (36 μg. mL-1) and FLU (12 μg. mL-1), 6 times on the same day and on 3 different days (first, second, third) and the results are reported in terms of C.V. The results are shown in Table 6a and 6b.

Table 6a: Intra-day Precision

Drug	Conc (µg/ml)	Peak Area Mean±SD (n=6)	%RSD
Paracetamol	36	2176535±25095	1.153
Flupiritine	12	1268670±9226	0.7272

Table 6b: Inter-day Precision

Drug	Conc (µg/ml)	Peak Area Mean±SD (n=3)	%RSD
Paracetamol	36	2262763±31635	1.3961
Flupiritine	12	1293857±13113	1.013

f) Robustness

The robustness of the method was evaluated by analyzing the system suitability standards and evaluating system suitability parameter data after varying the HPLC pump flow rate (±0.1 mL) and organic solvent content (±2 mL) and pH (±0.1) .The alterations caused a significant change in peak area R.S.D (%), USP tailing factor and retention times.

g) Assay

The proposed method was successfully applied to the analysis of marketed products (PRUF P) and the results obtained are given in Table 7.

Table 7: Assay report of formulation

S. No.	Brand name	Content	Peak Area mean ± S.D	Assay	%RSD
1	PRUF P	36mg-Paracetamol	2306345 ± 20053	101.02%	0.8695
		12mg-Flupiritine	1239469 ± 7351	99.08%	0.5625

h) Solution stability and Mobile phase stability

The stability of PARA and FLU in solution was determined by leaving test solutions of the sample and reference standard in tightly capped volumetric flasks at room temperature for 3 days during which they were assayed at 12 hr intervals. Stability of mobile phase was determined by analysis of freshly prepared sample solutions at 12 hr intervals for 48 hrs and comparing the results with those obtained from freshly prepared reference standard solutions. The mobile phase was prepared at the beginning of the study period and not changed during the experiment. The % assay of the results was calculated for both the mobile phase and solution-stability experiments.

Discussion

In order to fulfill the objective of the proposed RP-HPLC method for PARA and FLU, the mobile phase at different ratio of methanol and water with ion pair reagent (0.2%TEA), pH with OPA were tested in trial and error basis for a good chromatographic separation. Initial trials with methanol as organic phase showed that response the peak was poor, at pH 6, observed co-eluting of drugs respectively for PARA and FLU. An acidic mobile phase with methanol and water at 90:10 % (v/v) and pH 3.0 with reverse phase Agilent C18 column gives symmetric and sharp peaks and eluted at 3.2 and 5.1 min as Rt for PARA and FLU, respectively. The method was validated as per ICH Q2 guidelines. The linearity range 9-63 µg. mL-1 for PARA and 3-21 µg. mL-1 for FLU was investigated between and the respective regression coefficient was R2=0.9995 and R2=0.9996. LOD and LOQ were calculated from the formula 3.3 x (σ/S) and 10 x (σ/S), respectively. LOD was found to be 0.036 µg. mL-1 and 0.057 µg. mL-1 whilst LOQ was 0.109 µg. mL-1 and 0.173 µg. mL-1 for PARA and FLU. The interday and intra precision was found to be less than 1.4 %. The % recovery investigated at three levels and was in between 98-101%. Solution stability studies showed that the active pharmaceutical ingredients remained stable for 24 hrs at room temperature. High percentage recovery of drug shows the method is free from interference of excipients present in the formulation. Thus the method is simple, rapid, sensitive, specific, accurate, and precise and does not involve complicated sample preparation procedures. The method is robust for all parameter. Allowed variation in pH is about 0.1 unit and % organic phase < 2%. The method exhibit many significance like reduced run time, economic, simple, reliable, more precise and even reliable for stability as per ICH guidelines.

Conclusion

The developed method gave good resolution between PARA and FLU with short analysis time (10min), high efficiency and complies with modified SST specifications of USP. The use of C18 column in the present work has shown better elution of analytes with good resolution, improved plate count, capacity factor. So the C18 column can be used to achieve high specificity in shorter time of analysis of Paracetamol and Flupiritine Maleate as per ICH Q2 (R2) guidelines. the proposed method was found to be simple, precise, accurate, linear, robust and rapid for simultaneous determination and quantification of Paracetamol and Flupiritine maleate. The sample recoveries were in good agreement with their respective label claims suggested non-interference in the estimation. Hence, the method can be easily and conveniently adopted for routine analysis of Paracetamol and Flupiritine maleate in combined dosage forms.

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