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METHOD DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF PARACETMOL AND FLUPIRTINE MALEATE IN COMBINED TABLET DOSAGE FORMS

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ABSTRACT

A novel validated Reverse phase HPLC method for the simultaneous determination of Paracetamol and Flupirtine maleate in pharmaceutical dosage form was developed and validated. Proposed research work was developed by selecting Inertsil, C_{18}, (250 x 4.6mm, 5µ) column as stationary phase and Methanol: Ortho Phosphoric acid (65:35 v/v) as mobile phase. Separation was achieved at flow rate of 1mL/ min at ambient temperature throughout the experiment. Quantification was achieved with ultraviolet (PDA) detection at 280 nm. The retention times of Paracetamol and Flupirtine maleate were found as 4.7 min and 3.37 min respectively. The linearity concentration of 50-150µg/mL for both Paracetamol and Flupirtine maleate respectively, and the regression coefficients found as 0.998 and 0.999 for Paracetamol and Flupirtine maleate respectively. The % recovery of pharmaceutical formulations for both within the accepted limits and there is no interference with any excepients in the formulation. This method was validated according to ICH guidelines.

KEYWORDS

Paracetamol and Flupirtine maleate, RP-HPLC, Analytical method validation and Lupirtine-P tablets.

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INTRODUCTION

Paracetamol

Paracetamol[1] is a common analgesic and antipyretic drug that is used for the relief of fever, headaches and other minor aches and therapeutic concentrations of Paracetamol inhibit PG synthesis in intact cells *in vitro*. Since an overdose of Paracetamol can cause fulminating hepatic necrosis and other toxic effects. It has the structural formula and shown in Figure No.1.

The chemical name of Paracetamol is N-(4-hydroxyphenyl) acetamide. The molecular formula of paracetamol is $C_8H_9NO_2$ and it has the molecular weight of 151.1 g/mol. It is freely soluble in methyl alcohol. It has the melting point of 169-171^oC, pH of 5.5-6.5 and pKa of 9.38.

Flupirtine Maleate

Flupirtine Maleate[2] is a non-opioid centrally-acting analgesic agent, structurally dissimilar from ther analgesics. It has the structural formula and shown in Figure No.2.

The chemical name Flupirtine Maleate is Ethyl N-(2- amino-6-{[(4-fluorophenyl) methyl] amino} pyridin-3-yl) carbamate. The molecular formula of Flupirtine Maleate is $C_{15}H_{17}FN_4O_2$ and it has the molecular mass of 304.31 g/mol. It is soluble in methanol. It has the melting point of 115.5^oC, pH of 2.61 and pKa of 12.9. From the literature survey, we found that Paracetamol and Flupirtine maleate were estimated by different analytical methods like RP-HPLC[3-8] LC-MS[9] and UV spectrophotometry[10]. Only one HPLC method for simultaneous estimation Paracetamol and Flupirtine maleate is available, but it show high RT Values (time consuming) method. So, there is a need to develop a validated RP-HPLC method for simultaneous estimation of Paracetamol and Flupirtine maleate which can complete the estimation in short time and with highly accurate recovery results. This study aimed to develop a simple, precise, accurate and validated Reversed-Phase HPLC method for the simultaneous estimation of Paracetamol and Flupirtine maleate in bulk and pharmaceutical dosage form as per ICH guidelines. The statistical analysis proved that method is reproducible and selective for the simultaneous analysis of Paracetamol and Flupirtine maleate in bulk and formulations

Method Development

Chromatographic Conditions

Chromatographic separation was achieved by using Inertsil, C_{18}, (250 x 4.6mm, 5µ) column as stationary phase and composition of Methanol: Ortho Phosporic acid (65:35 v/v) as mobile phase. The detection was carried out at 280 nm. Mobile phase is used as diluent the samples were filtered through Whatman filter paper (0.45µm) and degassed before injected into the system. Typical chromatogram of blank, standard drug and Samples were as shown in Figure No.3-6.

Preparation of stock solution

10mg of Flupirtine maleate and 32.5mg of Paracetamol RS drugs were weighed individually and taken into a 100mL clean dry volumetric flask and added about 70mL of diluent. It was sonicated to dissolve completely and made volume up to the mark with the same diluents (100, 325µg/mL). From this, 4ml of the solution was pipette into another 10ml volumetric flask and diluted up to the mark with diluent (40, 130µg/mL).

Preparation of mobile phase

The mobile phase was prepared by mixing 850 ml of methanol, 150 ml of O-Phosphoric acid, filtered through Whatman filter paper (0.45µm) and degassed before use.

Preparation of working sample solution

Twenty tablets containing Paracetamol and Flupirtine maleate combination of marketed formulation was taken and powdered. The powder equivalent to 10mg of Flupirtine maleate and 32.5mg of Paracetamol RS drugs were weighed individually and taken into a 100mL clean dry volumetric flask and added about 70mL of diluent. It was sonicated to dissolve completely and made volume up to the mark with the same diluents (100, 325µg/mL).

From this, 4ml of the solution was pipette into another 10ml volumetric flask and diluted up to the mark with diluent (40, 130µg/mL). A typical chromatogram of Lupirtine- P tablet formulation (sample) drug was shown in Figure No.4. The assay results are shown in Table No.1.

METHOD VALIDATION

Method validation was carried out as per ICH guidelines[11].

System Suitability

A standard solution was prepared by using Paracetamol and Flupirtine maleate working standards as per test method and was injected five times into the HPLC system. The system suitability parameters were evaluated from standard

chromatograms by calculating the % RSD from five replicate injections for Paracetamol and Flupirtine maleate retention times and peak areas. Table No.2.

Specificity

Blank and sample were prepared and are injected into chromatographic system.

Linearity

A Series of solutions are prepared using Paracetamol and Flupirtine maleate working standards at concentration levels from 50-150µg/ml of target concentration.

Precision

Repeatability

Accuracy (Recovery)

Accuracy done by recovery studies and Drug Assay was performed in triplicate as per test method with equivalent amount of Paracetamol and Flupirtine maleate into each volumetric flask for each spike level to get the concentration of Paracetamol and Flupirtine maleate equivalent to 50%, 100%, and 150% of the labeled amount as per the test method. The average % recovery of Paracetamol and Flupirtine maleate were calculated.

Robustness

Effect of variation of flow rate

Prepared standard solution as per the test method was injected into the HPLC system using flow rates, 0.8ml/min and 1.2mL/min.

Limit of Detection and Quantitation (LOD and LOQ)

From the linearity data calculate the limit of detection and quantitation, using the following formula 4. The Tailing factor (T) for Paracetamol and Flupirtine maleate peaks is NMT 2.0.

LOD for Paracetamol = 3.769700 LOD for Flupirtine maleate = 3.182503.

LOQ for Paracetamol = 10.136540 LOQ for Flupirtine maleate = 10.259800.

Table No.1: System suitability data of Paracetamol and Flupirtine maleate

S.No	Parameter	Paracetamol	Flupirtine maleate	Acceptance criteria
1	Retention time	4.731	3.353	
2	Theoretical plates	13958	9156	>2500
3	Tailing factor	1.24	1.29	<2.00
4	% RSD	0.1	0.1	<2.00

Table No.2: Calibration data of Paracetamol

S.No	Conc (µg/mL)	RT	Peak Area
1	50	4.741	545934
2	75	4.745	545471
3	100	4.738	1089953
4	125	4.733	1363163
5	150	4.729	1646171

Table No.3: Calibration data of flupertine

S.No	Conc (µg/mL)	Retention time	Peak area
1	50	3.362	452965
2	75	3.359	681710
3	100	3.359	917312
4	125	3.357	1145499
5	150	3.356	1362576

Table No.4: Precision data for Paracetamol

S.No	RT	Peak Area	%Assay
injection1	4.739	1078871	99
injection2	4.734	1064983	98
injection3	4.746	1083323	99
injection4	4.727	1080612	99
injection5	4.725	1078487	99
injection6	4.740	1072778	98
Mean			99
Std. Dev.			0.61
% RSD			0.62

Table No.5: Precision data for flupertine

S.No	RT	Peak Area	%Assay
injection1	3.357	911468	99
injection 2	3.356	910692	99
injection 3	3.365	913771	99
injection 4	3.354	914557	99
injection 5	3.342	910623	99
injection 6	3.359	910986	99
Mean			99
Std. Dev.			0.19
%RSD			0.19

Table No.6: Accuracy data for Paracetamol

S.No	Accuracy level	Injection	Sample area	RT
1	80%	1	541598	4.733
		2	546689	4.726
		3	541728	4.709
2	100%	1	1089965	4.728
		2	1080210	4.732
		3	1085854	4.715
3	120%	1	1667553	4.755
		2	1666002	4.743
		3	1666117	4.732

Table No.7: Accuracy Data for Flupertine

S.No	Accuracy level	Sample Name	Sample Peak area	RT
1	80%	1	459327	3.348
		2	456469	3.358
		3	450190	3.335
2	100%	1	910952	3.345
		2	916393	3.366
		3	916445	3.337
3	120%	1	1367985	3.370
		2	1364944	3.367
		3	459327	3.348

Table No.8: Robustness Data for Paracetamol

S.No	Parameter	RT	Theoretical plates	Asymmetry
1	Decreased flow rate(0.6ml/min)	5.270	14731	1.27
2	Increased flow rate(1.0ml/min)	4.321	13704	1.21
3	Decreased temperature(20^0c)	5.237	13997	1.25
4	Increased temperature(30^0c)	4.310	13146	1.22

Table No.9: Robustness Data for Flupertine

S.No	Parameter	RT	Theoretical plates	Asymmetry
1	Decreased flow rate(0.6ml/min)	3.726	9384	1.31
2	Increased flow rate(1.0ml/min)	3.050	8891	1.26
3	Decreased temperature(20^oc)	3.710	9499	1.31
4	Increased temperature(30^oc)	3.049	9005	1.26

Table No.10: Summary of validation data for Paracetamol

S.No	Parameter	Result	Acceptance Criteria
1	System suitability Theoretical plates Asymmetry Retention time %RSD	13958 1.24 4.731 0.1	Not less than 2500 Not more than2
2	Specificity a) Blank interference b) Placebo interference	Specific	Specific
3	Method precision(%RSD)	0.62	Not more than 2.0%
4	Linearity parameter Slope Correlation coefficient(r^2)	50-150 mcg/ml 43363 0.999	Not less than 0.999
5	Accuracy Mean % recovery	101.00	97 - 103%
6	Robustness a) Flow rate variation b) Temperature variation	All the system suitability parameters are within the limits.	

Table No.11: Summary of validation data for Flupertine

S.No	Parameter	Result	Acceptance criteria
1	System suitability Theoretical plates Asymmetry Retention time %RSD	9156 1.29 3.353 0.1	Not less than 2500 Not more than 2
2	Specificity a) Blank interference b) Placebo interference	Specific	Specific
3	Method precision(%RSD)	0.19	Not more than 2.0%
4	Linearity parameter Slope Correlation coefficient(r^2)	50-150 mcg/ml 10569 0.999	Not less than 0.999
5	Accuracy Mean % recovery	100.00	97 - 103%
6	Robustness a) Flow rate variation b) Temperature variation	All the system suitability parameters are within the limits.	

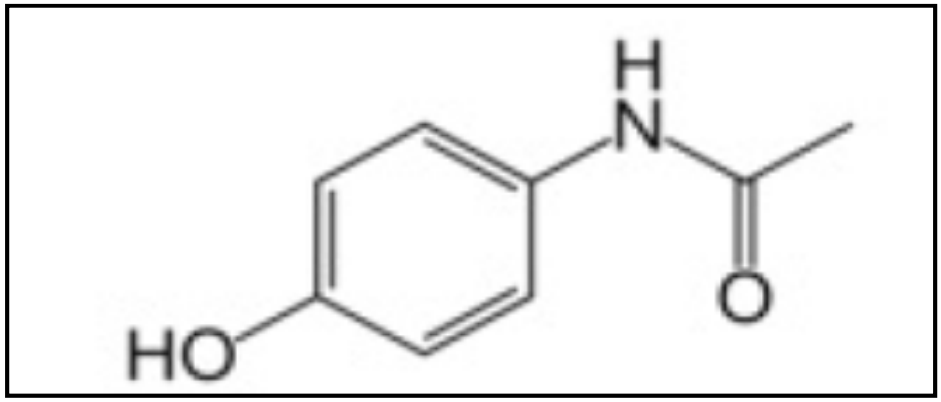

Figure No.1: Chemical Structure of Paracetamol

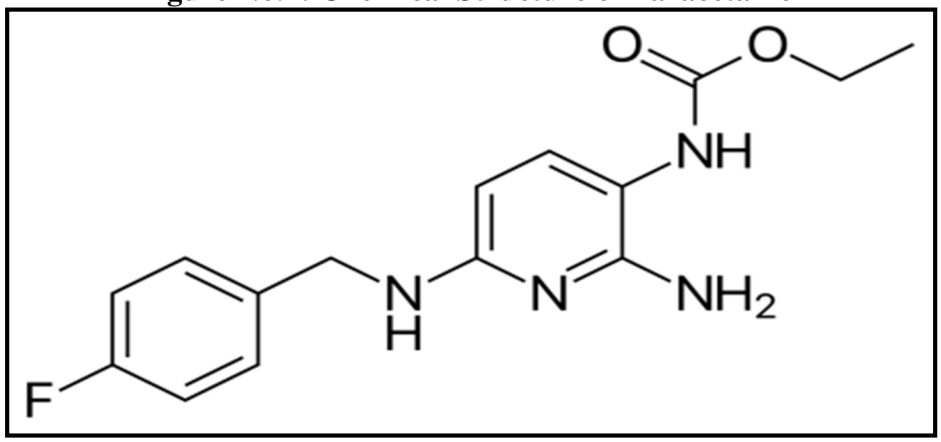

Figure No.2: Chemical structure of Flupertine

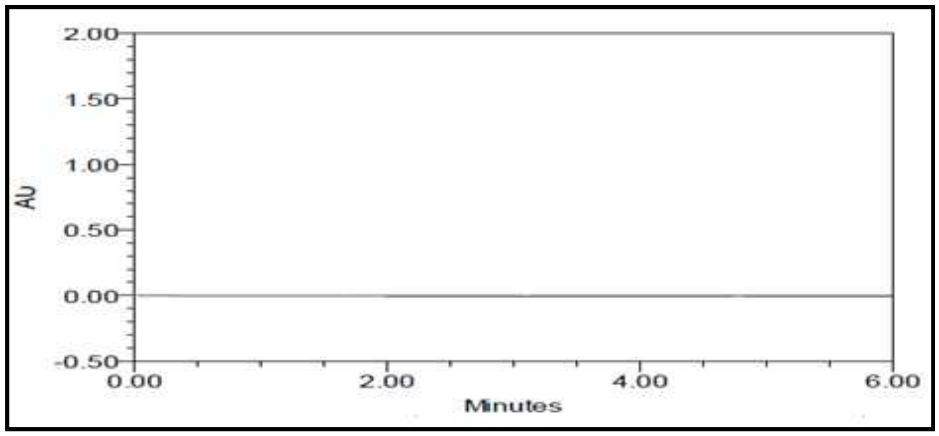

Figure No.3: Chromatogram of Blank (solvent system)

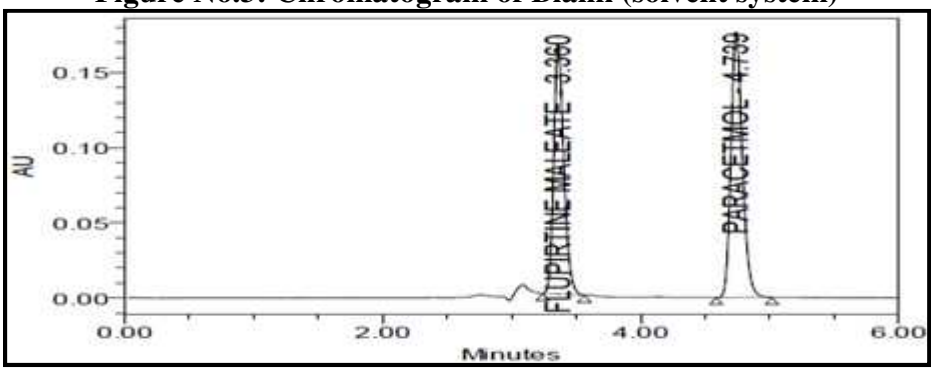

Figure No.4: Typical Chromatogram of Flupertine and Paracetamol

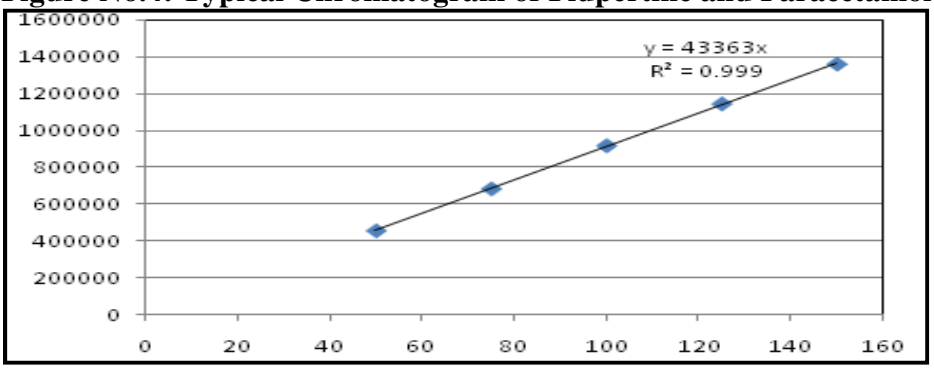

Figure No.5: Calibration curve of Paracetamol

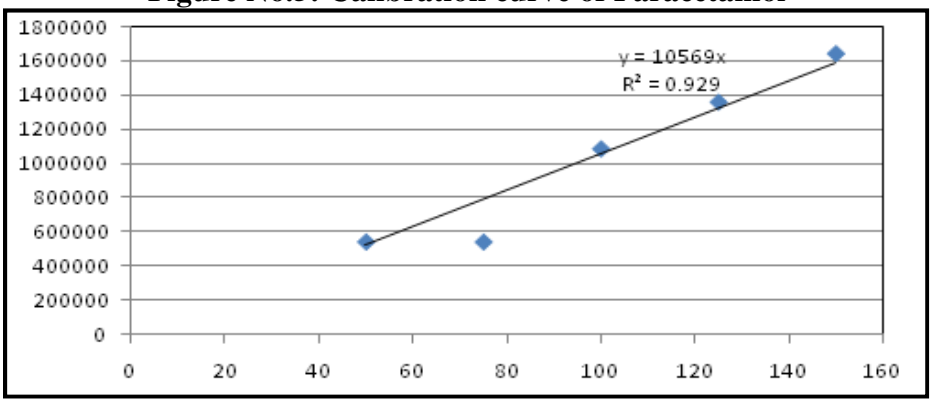

Figure No.6: Calibration curve of Flupertine

SUMMARY AND CONCLUSION

A new method was developed for simultaneous estimation of Paracetamol and Flupirtine maleate by RPHPLC method. The sample preparation was simple and analysis time was short. The analytical procedure was validated as per ICH guidelines and shown to be accurate, precise and specific. Maximum absorbance was found to be at 239nm for Paracetamol and Flupirtine maleate and the peak purity was excellent. Injection volume was selected to be 20µl which gave a good peak area. The column used for study was Inertsil C18 chosen good peak shape. The flow rate was adjusted to 1.0mL/ min because of good peak area and satisfactory retention time. Different ratios of mobile phase were studied, and mobile phase with ratio of 65:35(methanol: o-phosporic acid) was fixed due to good symmetrical peak. Methanol was selected because of maximum extraction, sonication time was fixed to be 5min at which all the drug particles were completely soluble and showed good recovery. Run time was selected to be 5min because analyse gave peak around 2.78 and 3.47 also to reduce the total run time.

The present recovery was found to be 98.86-100.73 % w/v was linear and precise over the same range. Detection limit was found to be 8.23 for PCM and 4.15µg/mL for Flupirtine maleate. Linearity study correlation coefficient 0.999 and curve fitting was found. The analytical method was found linearity over the range of 50-150 µg/mL of the target concentration. The analytical passed both robustness and ruggedness tests. This method represents a fast analytical procedure for the simultaneous quantitation of Paracetamol and Flupirtine maleate. This method can be applicable to the routine analysis of large numbers of sample with good precision and accuracy.

Hence this method was found to be simple, accurate, economical and rapid and they can be applied for routine analysis in laboratories and is suitable for the quality control department.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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