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Research Article

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Development and validation of RP-HPLC method for the simultaneous estimation of paracetamol, aceclofenac and rabeprazole sodium in bulk and its pharmaceutical dosage form

G. Saravanan*, Mohammad Yunoos and B. Pooja

Department of Pharmaceutical Chemistry, Bapatla College of Pharmacy, Bapatla, Andhra Pradesh, India

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ABSTRACT

A novel, simple, precise, accurate, sensitive, rapid, economic and isocratic RP-HPLC method has been developed for the simultaneous estimation of paracetamol, aceclofenac and rabeprazole sodium in bulk and its pharmaceutical dosage form. The chromatographic separation was achieved on waters 2675 HPLC separation module equipped with Agilent CN column (250 X 4.6 mm, 5 µ particle size) and UV detector using ammonium acetate buffer (pH 7.5) and acetonitrile in the ratio of 70:30 % v/v as mobile phase at a flow rate of 1.0 ml/min. The detection was carried out at 213 nm. The retention time of paracetamol, aceclofenac and rabeprazole sodium was found to be 3.678, 5.556 and 9.572 min respectively. Linearity was observed in the concentration range of 16-488 µg/ml for paracetamol, 5-150 µg/ml for aceclofenac and 0.5-16.8 µg/ml for rabeprazole sodium. % Recoveries for paracetamol, aceclofenac and rabeprazole sodium were found to be 100.45 %, 100.47 % and 100.47 % respectively. The % RSD below 2.0 shows the high precision of the proposed method. The method was validated for precision, linearity, accuracy, specificity and robustness in accordance with ICH guidelines and can be applied for routine analysis of paracetamol, aceclofenac, and rabeprazole in bulk and its pharmaceutical dosage form.

Keywords: Paracetamol, Aceclofenac, Rabeprazole, RP-HPLC, Simultaneous estimation.

INTRODUCTION

Paracetamol, a centrally and peripherally acting non-opiod analgesic and antipyretic which acts by inhibiting the synthesis of prostaglandins, chemically it is *N*-(4-hydroxyphenyl) acetamide (Figure 1).

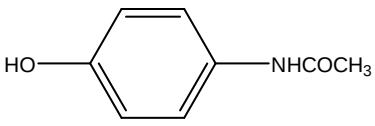

Fig. 1: Structure of paracetamol

Aceclofenac is a phenyl acetic acid derivative with potent analgesic and anti-inflammatory properties. Chemically it is 2-[2-[2-[(2, 6 dichlorophenyl) amino] phenyl] acetyl] oxyacetic acid (Figure 2). It is largely used in the symptomatic treatment of pain and of inflammatory or degenerative orthropathies like osteoarthritis, rheumatoid arthritis and ankylosing spondilities.

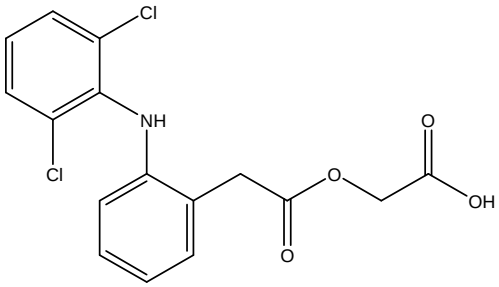

Fig. 2: Structure of aceclofenac

Rabeprazole sodium is chemically known as 2-([4-(3-methoxypropoxy)-3-methylpyridin-2-yl] methylsulfinyl)-1*H*-benzo[*d*] imidazole (Figure 3). It is proton pump inhibitor that suppresses gastric H^+, K^+ ATPase at the secretory surface of the gastric parietal cell and used in the treatment of duodenal ulcers.

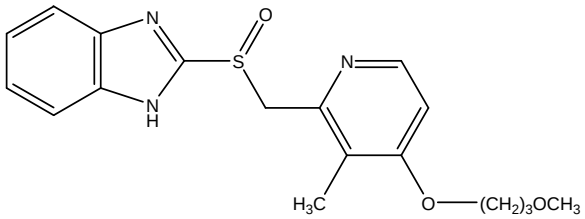

Fig. 3: Structure of rabeprazole sodium

Literature review reveals that only spectrophotometric [1] and HPTLC [2-3] methods have been reported for the simultaneous estimation of paracetamol, aceclofenac and rabeprazole sodium in pharmaceutical dosage forms and several HPLC [4-13] and spectrophotometric methods [14-15] are reported in combination with other drugs for their estimation in biological fluids and pharmaceutical dosage forms. However, there was no RP-HPLC method reported for the simultaneous estimation of paracetamol, aceclofenac and rabeprazole sodium in pharmaceutical dosage form. Hence, the present study was aimed to develop simple, precise, accurate, sensitive, rapid and economic RP-HPLC-UV method for the simultaneous analysis of paracetamol, aceclofenac and rabeprazole sodium in bulk drug and pharmaceutical dosage forms.

EXPERIMENTAL SECTION

Chemicals and solvents

Paracetamol, aceclofenac and rabeprazole sodium were obtained as a gift samples from Aurobindo pharma limited, Hyderabad. HPLC grade methanol and acetonitrile were purchased from E.Merck. chem. ltd. Mumbai and HPLC grade water was used throughout the study. All the chemicals (Merck. chem. ltd. Mumbai) used were of analytical grade. Fixed dose combination tablet formulation (SAFENAC-XP) containing 325 mg of paracetamol, 100 mg of aceclofenac and 10 mg of rabeprazole sodium was procured from local market.

Instrumentation

All the chromatographic measurements were made on HPLC (Waters 2675) separation module equipped with Agilent CN column (250 X 4.6 mm, 5 µm) and UV detector (Waters). Ultra Sonicator (Enertech SE60US), Weighing balance (Single pan balance, Ascoset ER200A) and pH meter (Unichem AD102U) were used throughout the study.

Chromatographic conditions

Mobile phase consisting of ammonium acetate buffer (pH 7.5) and acetonitrile (70:30 % v/v) was used in isocratic mode and the mobile phase was filtered through nylon disc filter of 0.45 µm (Millipore) and sonicated for 5 min before use. The flow rate was 1.0 ml/min. UV detection was carried out at 213 nm and separation was achieved at ambient temperature.

Preparation of buffer:
Ammonium acetate buffer pH 7.5 is prepared by adding 0.385 gm of ammonium acetate in 100 ml double distilled water and then adjusted to pH 7.5 with ammonia solution.

Preparation of standard solution:
Weighed accurately and transferred about 203.5 mg of paracetamol, 62.6 mg of aceclofenac and 6.8 mg of rabeprazole sodium of working standard into a 50 ml volumetric flask, dissolved and diluted with mobile phase and filtered through 0.45 µ nylon syringe filter. Further 4.0 ml of the above stock solution was transferred into a 50 ml volumetric flask and then made up to the volume with mobile phase.

Preparation of sample solution:
20 tablets, each containing 325 mg of paracetamol, 100 mg of aceclofenac and 10 mg of rabeprazole sodium were weighed and powdered. A quantity of tablet powder equivalent to 325 mg of paracetamol, 100 mg of aceclofenac and 10 mg of rabeprazole sodium was accurately weighed and transferred into a 50 ml volumetric flask, about 30 ml of mobile phase was added and sonicated for 15 min. Then volume was made up to the mark with mobile phase and filtered through 0.45 µ nylon syringe filter. Further 2.5 ml of the above solution was transferred into a 50 ml volumetric flask and then made up to the volume with mobile phase.

Procedure for assay:
A steady base line was recorded with the optimized chromatographic conditions after equilibrating the column for 30 min using mobile phase and then standard and sample solutions of 10 µl were separately injected into the HPLC system and the chromatograms were recorded as shown in Figure 4 and Figure 5. The amount present in the each tablet was quantified by comparing the peak area of standard drug with that of the sample.

Method validation
The optimized chromatographic method was completely validated according to the procedures described in ICH Q2 (R1) guidelines.

Linearity:
A linear relationship was evaluated across the range of the analytical procedure. A series of standard dilutions were prepared from the working standard solution in the concentration range of 16-488 µg/ml of paracetamol, 5-150 µg/ml of aceclofenac and 0.5-16.8 µg/ml of rabeprazole sodium. 10 µl of each solution was injected into HPLC system. Linearity is evaluated by plotting the peak area as a function of analyte concentrations.

Accuracy:
Recovery studies were performed by standard addition method by spiking at three different levels 50 %, 100 % and 150 % of the known quantities of standard within the range of linearity to sample solution of drug product and these solutions were analyzed by developed method in triplicate.

Precision:
System precision:
Six standard solutions were injected into the chromatographic system and % RSD was calculated.

Method precision:
Six assay samples of drug product at 100 % of the test concentration were prepared and injected into the chromatographic system and % RSD was calculated.

Ruggedness:
Six assay samples of drug product at 100 % of test concentration were prepared and injected into the chromatographic system on different days by using different column and equipment and % RSD were calculated.

Robustness:
Robustness was performed at different flow rates (± 0.2 ml/min), different wavelengths (± 5 nm), different mobile phase ratio (± 5 %), and different mobile phase pH (± 0.2) by using working standard solution of paracetamol, aceclofenac and rabeprazole sodium. The results obtained were unaffected by small variations in these parameters.

Specificity:
The specificity of the method was evaluated by comparing the chromatograms obtained from the standard with that obtained from tablet formulation. The retention times of drug substance and drug product were observed and absence of interference of excipients in the tablet formulation indicates the specificity of the proposed method.

RESULTS AND DISCUSSION

Method development
A satisfactory separation and good peak symmetry was obtained with stationary phase Agilent CN column (250 X 4.6mm, 5 µ), mobile phase comprising of ammonium acetate buffer (pH 7.5) and acetonitrile in the ratio of (70:30 % v/v) at a flow rate of 1 ml/ min. The detection was carried out at 213 nm. The retention time of paracetamol, aceclofenac and rabeprazole sodium were found to be 3.678, 5.556 and 9.572 min respectively as shown in Figure 4.

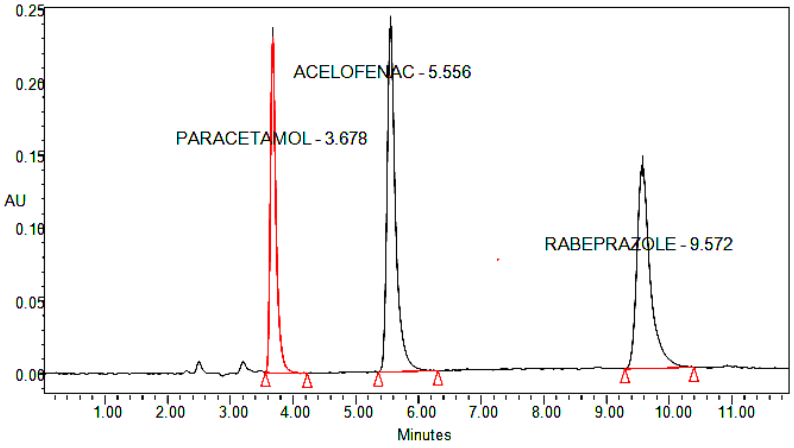

Fig. 4: A representative chromatogram of standard

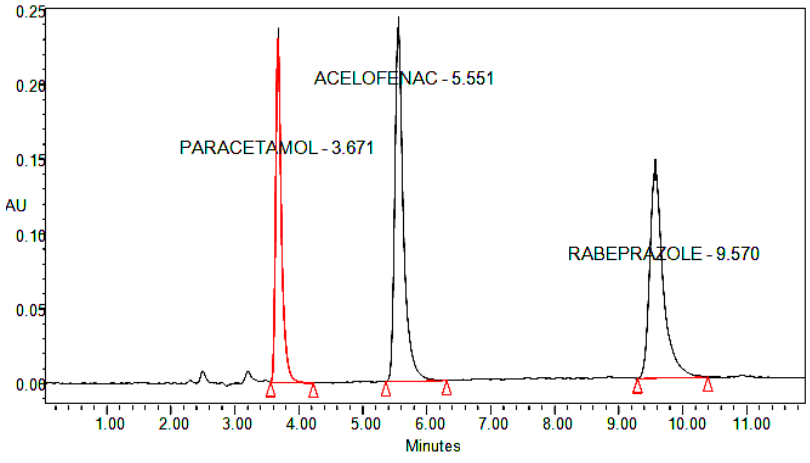

Fig. 5: A representative chromatogram of sample

Method validation
The proposed method was validated according to ICH guidelines.

Linearity:
The calibration curves obtained showed good linear relationship over the concentration range of 16-488 µg/ml of paracetamol, 5-150 µg/ml of aceclofenac and 0.5-16.8 µg/ml of rabeprazole sodium as shown in Figure 6, Figure 7 and Figure 8. Peak areas and concentrations were subjected to least square regression analysis to calculate regression

equation. Correlation coefficient was found to be 0.9996 for paracetamol, 0.9995 for aceclofenac and 0.9992 for rabeprazole sodium indicating a linear response over the range used. The data from the calibration curve was given in Table 1.

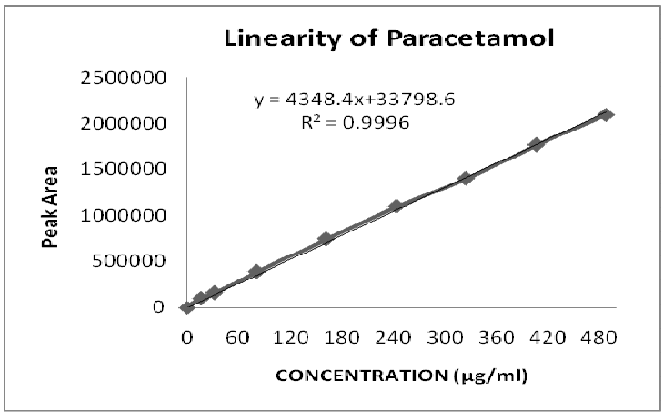

Fig. 6: Calibration curve of paracetamol

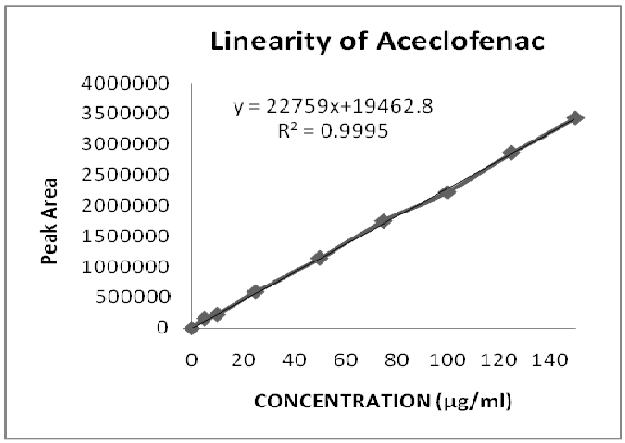

Fig. 7: Calibration curve of aceclofenac

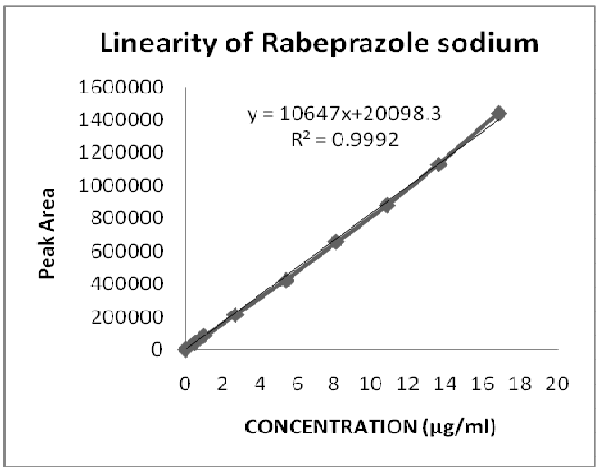

Fig. 8: Calibration curve of rabeprazole sodium

Table-1: Data of linearity studies

Level	Paracetamol concentration (µg/ml)	Peak area	Aceclofenac concentration (µg/ml)	Peak area	Rabeprazole sodium concentration (µg/ml)	Peak area
1	16	100709	5	152141	0.5	41590
2	32	154830	10	218044	1.0	81693
3	81	388419	25	587393	2.7	212796
4	162	743035	50	1139678	5.4	402344
5	244	1097666	75	1750213	8.1	604426
6	325	1402362	100	2217023	10.8	849736
7	407	1768792	125	2858212	13.6	1179716
8	488	2096312	150	3429851	16.8	1441240
Slope		4348.4		22759		106478
Intercept		33798.69		19462.82		20098.33
R		0.9996		0.9995		0.9992

Accuracy:

The accuracy of the proposed method was evaluated by performing recovery studies and the % RSD and % recovery were within the acceptable limits in all 3 levels and % recovery was found to be 100.45 % for paracetamol, 100.47 % for aceclofenac and 100.47 % for rabeprazole sodium. It is evident from the results of accuracy study given in the Table 2, Table 3 and Table 4 that the proposed method enables very accurate for quantitative estimation of paracetamol, aceclofenac and rabeprazole sodium.

Table-2: Data of accuracy for paracetamol

Accuracy	Peak area	% recovery	Mean % recovery	Overall mean % recovery
50 %	728747	100.9	Mean=100.7	
50 %	733737	100.7	S.D = 0.163	
50 %	725504	100.5	% RSD = 0.161	
100 %	1401192	100.8	Mean=100.56	Mean= 100.45
100 %	1403724	100.6	S.D = 0.204	S.D = 0.149
100 %	1398253	100.3	% RSD = 0.202	% RSD = 0.144
150 %	2077466	100.1	Mean=100.1	
150 %	2050485	100.0	S.D = 0.08	
150 %	2059855	100.2	% RSD = 0.07	

Table-3: Data of accuracy for aceclofenac

Accuracy	Peak area	% recovery	Mean % recovery	Overall mean % recovery
50 %	1149329	100.5	Mean=100.66	
50 %	1151965	100.8	S.D = 0.122	
50 %	1152844	100.7	% RSD = 0.121	
100 %	2259891	100.7	Mean=100.36	Mean= 100.47
100 %	2245916	100.2	S.D = 0.234	S.D = 0.216
100 %	2263236	100.2	% RSD = 0.233	% RSD = 0.215
150 %	3437674	100.3	Mean=100.4	
150 %	3375615	100.1	S.D = 0.294	
150 %	3401080	100.8	% RSD = 0.292	

Table-4: Data of accuracy for rabeprazole sodium

Accuracy	Peak area	% recovery	Mean % recovery	Overall mean %recovery
50 %	424025	100.5	Mean=100.56	
50 %	410072	100.3	S.D = 0.248	
50 %	422888	100.9	% RSD = 0.246	
100 %	843148	100.9	Mean=100.53	Mean= 100.47
100 %	841216	100.4	S.D = 0.251	S.D = 0.25
100 %	830822	100.3	% RSD = 0.249	% RSD = 0.248
150 %	1200030	100.7	Mean=100.33	
150 %	1255253	100.2	S.D = 0.251	
150 %	1258669	100.1	% RSD = 0.250	

Precision:
The % RSD of method precision and system precision were found to be 1.612 and 0.558 for paracetamol, 0.838 and 0.589 for aceclofenac and 1.104 and 0.961 for rabeprazole sodium respectively. The % RSD below 2.0 shows high precision of proposed method as shown in Table 5 and Table 6.

Table-5: Data of system precision

Injection	Retention time			Peak area		
	Paracetamol	Aceclofenac	Rabeprazole sodium	Paracetamol	Aceclofenac	Rabeprazole sodium
1	3.667	5.025	9.500	1426789	2216795	843726
2	3.680	5.533	9.562	1432357	2237082	843723
3	3.677	5.525	9.555	1443975	2168077	842768
4	3.676	5.516	9.547	1431282	2205090	832876
5	3.675	5.510	9.541	1416281	2182720	843689
6	3.672	5.502	9.536	1427870	2207320	847939
Mean				1429759	2202847	842453
SD				79728.05	12974.77	8095.97
% RSD				0.558	0.589	0.961

Table-6: Data of method precision

Injection	Retention time			Peak area		
	Paracetamol	Aceclofenac	Rabeprazole sodium	Paracetamol	Aceclofenac	Rabeprazole sodium
1	3.675	5.441	9.548	1392869	2253656	799772
2	3.677	5.440	9.550	1396841	2226909	824362
3	3.671	5.433	9.547	1428735	2267793	813508
4	3.672	5.431	9.546	1439493	2265756	823568
5	3.677	5.438	9.555	1392570	2275776	798929
6	3.673	5.430	9.553	1437156	2278154	804336
Mean				1414611	2261341	810745
SD				22803.5	18950	8950.6
% RSD				1.612	0.838	1.104

Ruggedness:
% RSD obtained on different days by using different column and equipment were found to be 0.712 and 0.504 for paracetamol, 1.695 and 0.801 for aceclofenac and 1.774 and 1.199 for rabeprazole sodium respectively. The % RSD below 2.0 shows rugged method.

Robustness:
The robustness of the method is used to determine the capacity of the intended method to remain unaffected by changing flow rates, wavelengths, mobile phase ratio and mobile phase pH. The results indicated that the method is robust as the % RSD shows below 2.0.

Specificity:
The specificity of the method was established by comparing the chromatograms of standard and drug product, as there were no co-eluting peaks at the retention time of paracetamol, aceclofenac and rabeprazole sodium, which shows that peak of analytes, were free from interference from the excipients present in the drug product.

System suitability:
System suitability was carried out by injecting six standard concentrations at optimized chromatographic conditions. The system suitability parameters were noted as shown in Table 7.

Table-7: Data of system suitability parameters

S. No	Name	Retention time (min)	Area (A.U)	% Area	Height (A.U)	USP resolution	USP tailing	USP plate count
1	Paracetamol	3.678	1443269	25.12	228179	-	1.507	8596
2	Aceclofenac	5.556	2248725	39.15	237942	9.36	1.538	9400
3	Rabeprazole sodium	9.572	849736	19.83	139707	13.15	1.546	11506

CONCLUSION

From this study, a simple, precise, accurate and efficient RP-HPLC method was developed and validated for the analysis of paracetamol, aceclofenac and rabeprazole sodium in pharmaceutical dosage form. The developed method was validated as per ICH guidelines and found to be applicable for routine quality control analysis for the simultaneous estimation of paracetamol, aceclofenac and rabeprazole sodium in bulk and pharmaceutical dosage form.

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