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A validated stability indicating RP-HPLC method for simultaneous estimation of paracetamol, aceclofenac and rabeprazole sodium in bulk and combined tablet dosage form

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ABSTRACT

To develop a simple, sensitive, specific, precise and accurate stability indicating RP-HPLC method and subsequent validation of the method for the simultaneous estimation of paracetamol, aceclofenac and rabeprazole sodium in bulk and combined tablet dosage form. The chromatographic separation was carried out using waters 2675 HPLC separation module equipped with Agilent CN column (250 X 4.6mm, 5µ particle size) and mobile phase consisting of ammonium acetate buffer (pH adjusted to 7.5 with ammonia solution) and acetonitrile in the ratio of 70:30 % v/v at a flow rate of 1.0 ml/min was used. UV 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. The developed method illustrated excellent linearity 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 respectively. Drugs were subjected to stress conditions including acidic, alkaline, oxidation, photolysis and thermal degradation. No chromatographic interference from the tablet excipients was found. The % recoveries were found to be 100.45 % for paracetamol, 100.47 % for aceclofenac and 100.47 % for rabeprazole sodium respectively which shows accuracy of the method. The developed method was validated in accordance with ICH guidelines and was found to be accurate, precise, reproducible and specific and can be successfully applied for the quantitative estimation of paracetamol, aceclofenac and rabeprazole in bulk and pharmaceutical dosage form and in routine quality control analysis.

Keywords: Aceclofenac, Paracetamol, Rabeprazole, RP-HPLC, Method validation.

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 (Fig.1).

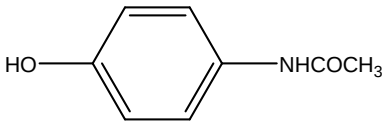

Figure 1: Structure of Paracetamol

Aceclofenac, 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 (Fig. 2). It is largely used in the symptomatic treatment of pain and of inflammatory or degenerative orthropathies like osteoarthritis, rheumatoid arthritis and ankylosing spondilities.

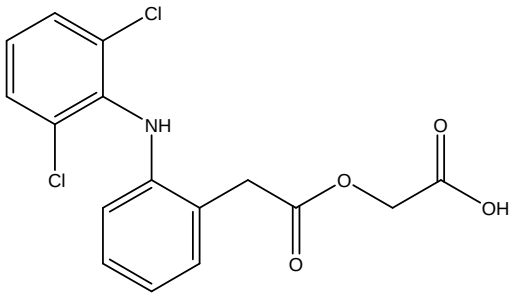

Figure 2: Structure of aceclofenac

Rabeprazole sodium is chemically known as 2-([4-(3-methoxypropoxy)-3-methylpyridin-2-yl] methylsulfinyl) - 1*H*-benzo[*d*] imidazole (Fig. 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.

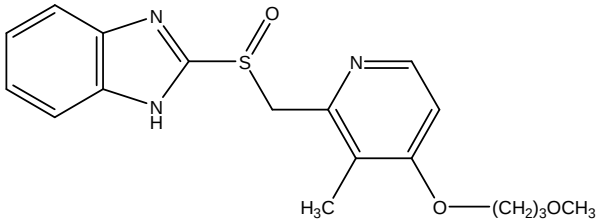

Figure 3: Structure of rabeprazole sodium

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 but several HPLC [4-8] and Spectrophotometric methods [9] are reported in combination with other drugs for their estimation in biological fluids and pharmaceutical dosage forms. However, there was no stability indicating RP-HPLC method has been reported for the simultaneous estimation of paracetamol, aceclofenac and rabeprazole sodium in tablet dosage form. Hence, the present study was aimed to develop a simple stability indicating RP-HPLC method for the simultaneous analysis of paracetamol, aceclofenac and rabeprazole sodium in bulk and combined tablet dosage forms. The developed method was validated as per ICH and USP guidelines [10].

MATERIALS AND METHODS

Chemicals and Solvents:

Paracetamol, aceclofenac and rabeprazole sodium were obtained as 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 and Chromatographic Conditions:

All the chromatographic measurements were made on HPLC (waters 2675) separation module equipped with Agilent CN column (250 X 4.6mmX 5µm) 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.
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 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 minutes 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 fig. 4 and fig. 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. Then 10 µl of each solution was injected into HPLC system. Linearity is evaluated by plotting the peak area as a function of analyte concentrations.

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 (Intermediate precision):
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 was determined.

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.

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 the system suitability parameters.

Limit of detection and quantitation:
Series of diluted standard solutions were prepared and analyzed by both methods. The limit of detection (LOD) and limit of quantitation (LOQ) were separately determined based on standard deviation of the y-intercept and the slope of the calibration curve.

Specificity and Forced degradation studies:
The study was intended to ensure the effective separation of paracetamol, aceclofenac and rabeprazole sodium and their degradation peaks of formulation ingredients at the retention time of respective drugs.

Acid degradation was carried out in 0.1N HCl and similarly alkaline degradation was conducted using 0.1N NaOH and refluxed for 30 min at 60 ^{o}C. After cooling, the solutions were neutralized and diluted with mobile phase.

Solutions for oxidative stress studies were prepared using 3% H_2O_2 and subjected to reflux for 30 min at 60 ^oC, cooled and diluted accordingly with the mobile phase. For thermal stress testing, the drug solution was heated at 60 ^oC for 30 min, cooled and used. The drug solution for photo stability testing was exposed to UV light for 6h in a UV light chamber (365nm) and analyzed.

RESULTS AND DISCUSSION

Method Development and optimization

Preliminary tests were performed to select adequate optimum conditions. The parameters such as detection wavelength, ideal mobile phase and their proportions, flow rate and concentration of the standard solutions were studied. After several trails, it was found that mixture of ammonium acetate buffer (pH 7.5) and acetonitrile gave sharp, well resolved peaks with symmetry within the limits and significant reproducibility as compared to other mobile phase compositions.

The chromatographic separation was carried out using Agilent CN column (250 X 4.6mmX 5µ) and 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 1.0 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.

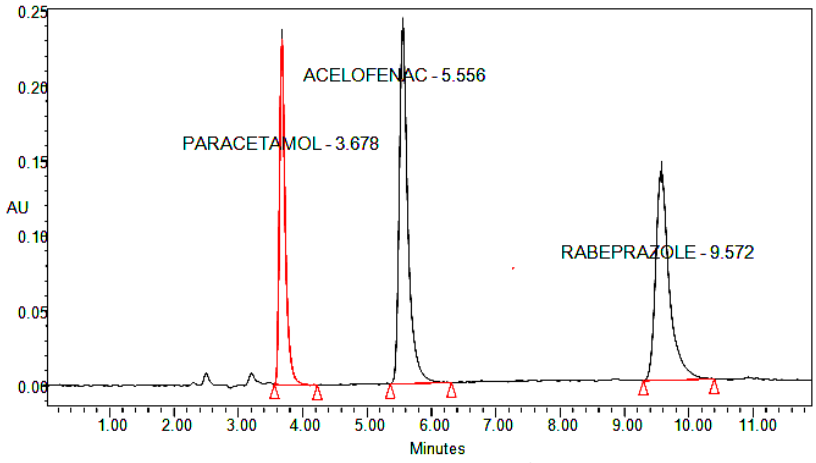

Figure 4: A representative chromatogram of standard solution

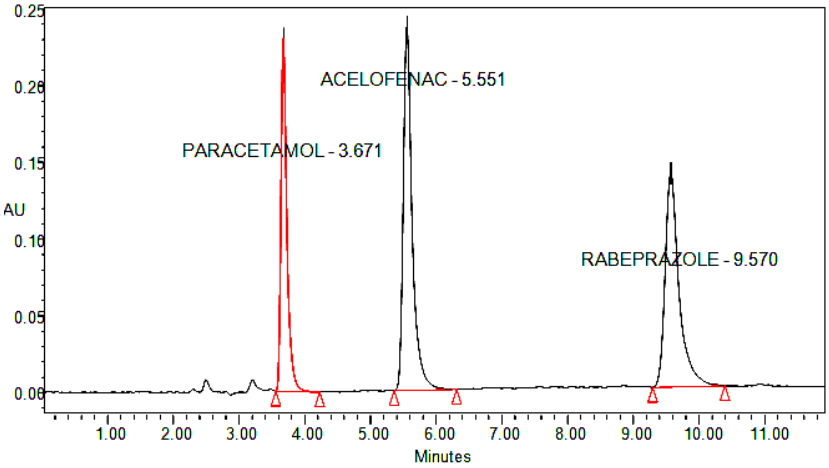

Figure 5: A representative chromatogram of sample solution

Linearity:

The calibration curves obtained shows good linear relationship over the concentration range of 16-488 µg/ml, 5-150 µg/ml and 0.5-16.8 µg/ml of paracetamol, aceclofenac and rabeprazole sodium respectively (fig. 6, fig. 7 and fig. 8). Peak areas and concentrations were subjected to least square regression analysis to calculate regression equation. Correlation coefficient was found to be 0.9996, 0.9995 and 0.9992 for paracetamol, aceclofenac and rabeprazole sodium indicating a linear response over the range used. The data from the calibration curve is given in Table 1.

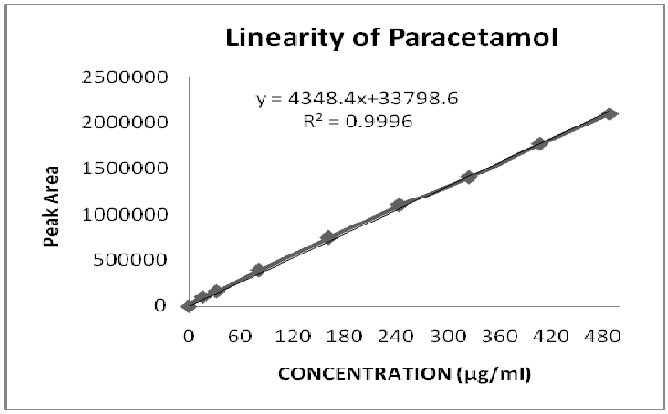

Figure 6: Calibration curve of paracetamol

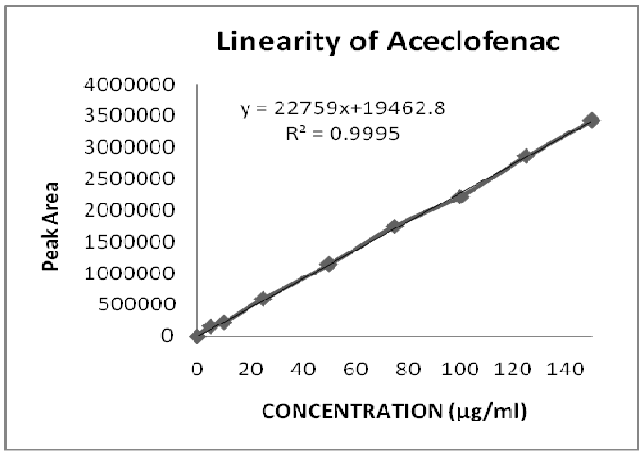

Figure 7: Calibration curve of aceclofenac

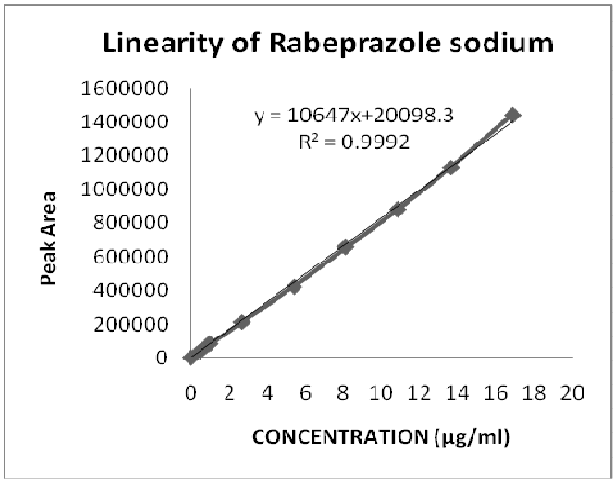

Figure 8: Calibration curve of rabeprazole sodium

Accuracy:

The accuracy of the proposed method was evaluated by performing recovery studies. The %RSD and % recovery were within the acceptable limits in all 3 levels. The % 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 are 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 in tablet dosage form.

Table 1: Data of Linearity studies

Level	Paracetamol		Aceclofenac		Rabeprazole sodium	
	Conc. (µg/ml)	Peak area	Conc. (µg/ml)	Peak area	Conc. (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

Table2: Accuracy data for Paracetamol

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

Table 3: Accuracy data for Aceclofenac

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

Table 4: Accuracy data for Rabeprazole Sodium

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

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.

Ruggedness:

The % 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 organic ratio and mobile phase pH. The results indicated that the method is robust as the % RSD shows below 2.0.

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

Specificity:

To evaluate stability indicating properties and specificity of the method, the drug product is subjected to forced degradation studies under different conditions like acidic (fig. 9), alkaline (fig. 10), oxidation (fig .11), photolysis (fig. 12) and thermal degradation (fig. 13) and checking the chromatograms for appearance of any extra peaks at the retention time of Paracetamol, aceclofenac and rabeprazole sodium and the results shows that there were no co-eluting or degradation peaks at the peak of analytes, as shown in Table 7 which indicates free from interference from the excipients present in the drug product and % degradation was calculated as shown in Table 8.

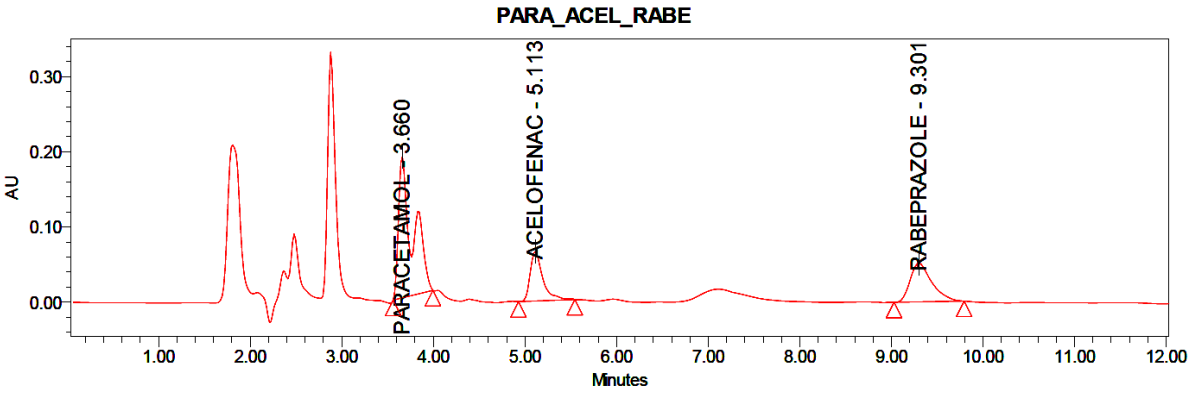

Figure 9: A typical chromatogram of acid hydrolysis degraded sample

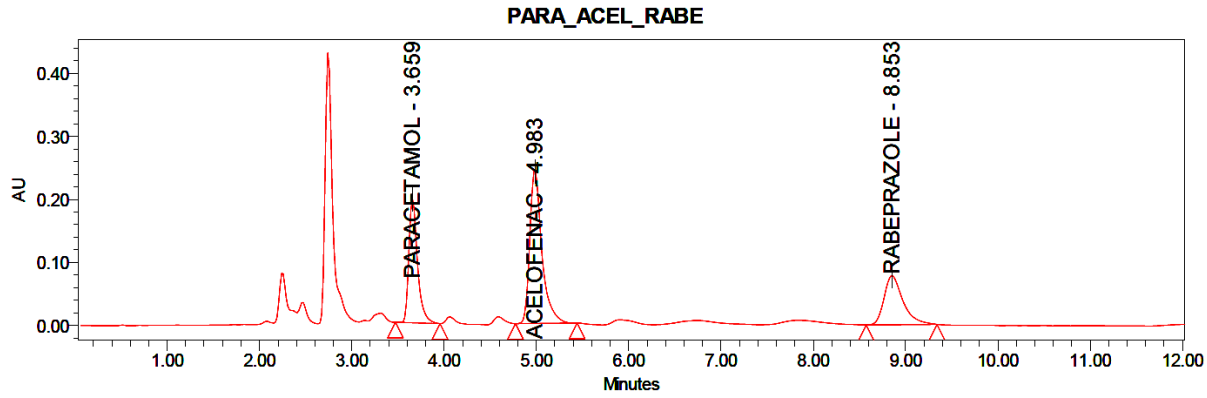

Figure 10: A typical chromatogram of base hydrolysis degraded sample

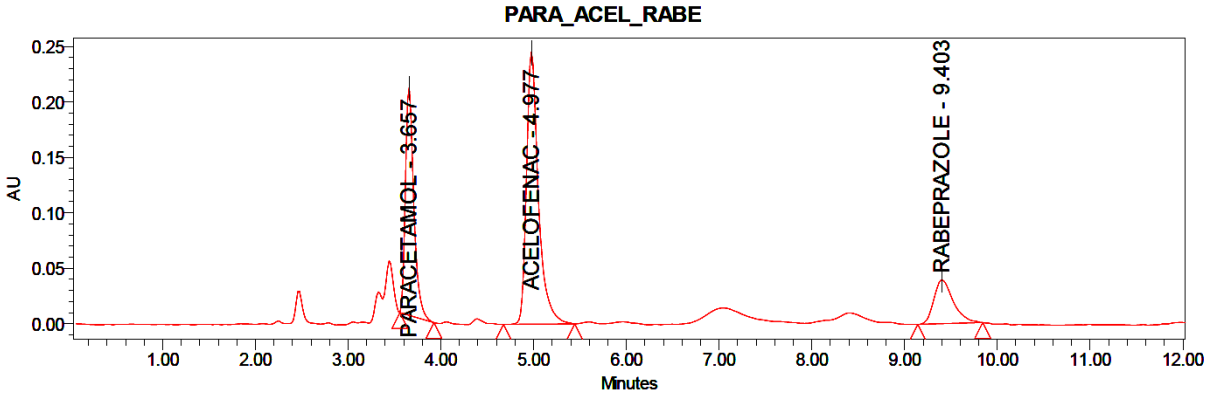

Figure 11: A typical chromatogram of oxidative degraded sample

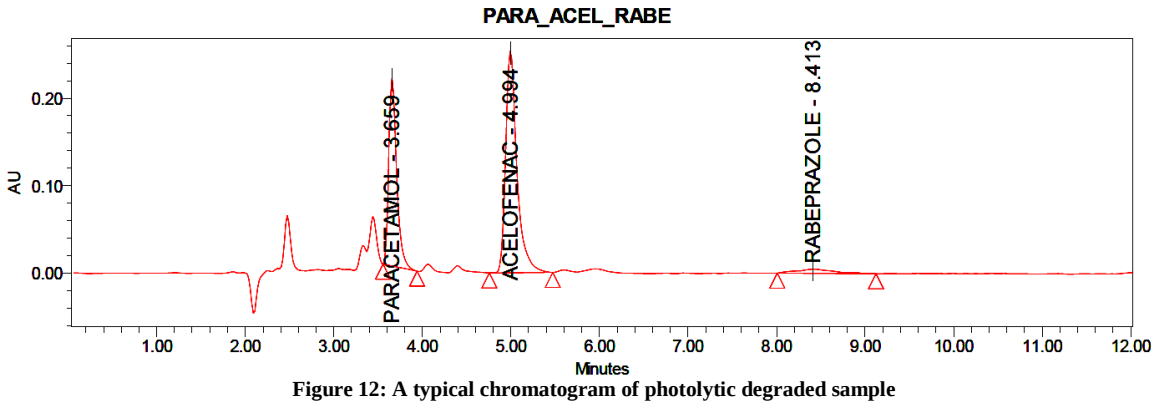

Figure 12: A typical chromatogram of photolytic degraded sample

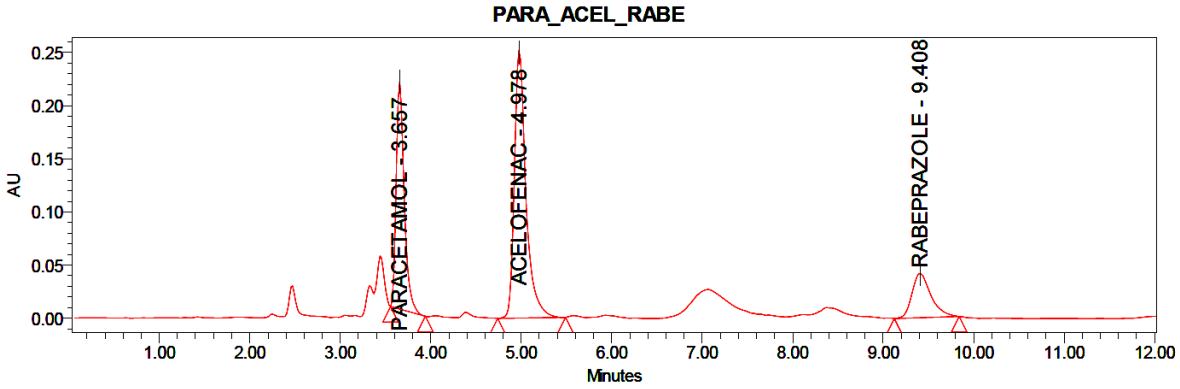

Figure 13: A typical chromatogram of thermal (heat) degraded sample

Table 7: Data of forced degradation studies

Stress condition	Paracetamol			Aceclofenac			Rabeprazole sodium		
	Retention time (Rt)	USP Plate count	USP Tailing	Rt	USP Plate count	USP Tailing	Rt	USP Plate count	USP Tailing
Acidic	3.660	6879	1.462	5.113	7596	1.401	9.301	7291	1.429
Alkali	3.659	7857	1.471	4.983	7525	1.493	8.853	9517	1.435
Thermal	3.657	8378	1.480	4.978	8212	1.482	9.408	10191	1.343
Photolytic	3.659	8080	1.481	4.994	7854	1.470	8.413	9933	1.389
Oxidative	3.657	7857	1.471	4.977	7525	1.493	9.403	9517	1.435

Table 8: Data of % degradation of Paracetamol, Aceclofenac and Rabeprazole sodium

Stress condition	% degradation			% drug recovered		
	Paracetamol	Aceclofenac	Rabeprazole sodium	Paracetamol	Aceclofenac	Rabeprazole sodium
Acidic	9.4	1.2	1.8	90.6	98.8	98.2
Alkali	6.2	1.6	1.4	93.8	98.4	98.6
Thermal	4.1	0.7	0.8	95.9	99.3	99.2
Photolytic	3.7	0.4	2.2	96.3	99.6	97.8
Oxidative	5.2	1.4	1.2	94.8	98.6	98.8

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 9.

Table 9: Data of System Suitability Parameters

S. No.	Name of the drug	Rt (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 and accurate stability indicating 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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