

SIMULTANEOUS ESTIMATION OF PARACETAMOL, ACECLOFENAC AND RABEPRAZOLE SODIUM IN COMBINED TABLET DOSAGE FORM BY USING RP-HPLC**NAGARAJU PAPPULA* AND PODILI RAVITEJA**

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ABSTRACT

For the simultaneous estimation of Paracetamol, Aceclofenac and Rabeprazole Sodium in combined tablet dosage form, a practical, sensitive RP-HPLC technique has been developed and validated by choosing chromatographic parameters. On Kromasil C18 (250 x 4.6 ID, 5 µm) with mobile phases containing a mixture of Buffer: ACN (50:50 %v/v), isocratic elution mode was used to separate the drugs Paracetamol, Aceclofenac and Rabeprazole Sodium from their process related substances. With a detection wavelength of 285 nm, the flow rate was set to 1 mL/minute, and the injection volume was set at 10 µL with a 8-minute run time. The developed method's appropriateness was examined and confirmed in accordance with the ICH (Q2 R2) guidelines for studies on specificity, Linearity, Accuracy, Precision, Detection limit, Quantification limit and Robustness. Paracetamol, Aceclofenac and Rabeprazole Sodium had retention times of 3.121 minutes, 3.670 minutes and 4.505 minutes, respectively. For Paracetamol, Aceclofenac and Rabeprazole Sodium, linearity was found in the concentration ranges of 6.5 - 390 µg/mL, 20-120 µg/mL and 2 - 12 µg/mL, respectively. For Paracetamol, Aceclofenac and Rabeprazole Sodium, the percentage recoveries ranged from 99.13-100.8%, 98.02-101.30% and 98.012-101.6%, respectively. The method was proven to be useful for determining and validating the presence of Paracetamol, Aceclofenac and Rabeprazole Sodium in combined tablet dosage form.

Keywords: RP-HPLC, Paracetamol, Aceclofenac and Rabeprazole Sodium.**1. INTRODUCTION**

Paracetamol is chemically N-(4-hydroxyphenyl)acetamide. It is a centrally and peripherally acting non-opioid analgesic and antipyretic. It functions as a weak inhibitor of the synthesis of prostaglandins (PGs). However, the in vivo effects of paracetamol are similar to those of the selective cyclooxygenase-2 (COX-2) inhibitors. Paracetamol also decreases PG concentrations in vivo. It is official in the Indian Pharmacopoeia [1].

Aceclofenac, is chemically, 2-[(2,6-dichlorophenyl)amino]phenyl acetoxy acetic acid is a phenyl acetic acid derivative with potent analgesic and anti-inflammatory properties. It is largely used in the symptomatic treatment of pain and of inflammatory or degenerative arthropathies like osteoarthritis, rheumatoid arthritis and ankylosing spondylitis. It has been widely used for the treatment of arthritis. It works by blocking the action of Cyclo oxygenases. Cyclo oxygenases (Cox) are involved

in the production of various prostaglandins. It is official in Indian Pharmacopoeia [2].

Rabeprazole sodium is chemically known as 2-[[4-(3-methoxypropoxy)-3methyl-2pyridinyl]-methyl] sulfinyl]-1H-benzimidazole sodium salt. Rabeprazole is a substituted benzimidazole that inhibits gastric acid secretion and primarily used in the treatment of ulcerative gastro-oesophageal reflux disease. Rabeprazole belongs to a class of anti-secretory compounds that suppress gastric acid secretion by inhibiting the gastric H⁺-K⁺-ATPase at the secretory surface of the gastric parietal cell. As this enzyme is regarded as the acid pump within the parietal cell, Rabeprazole has been characterised as a gastric proton pump inhibitor. It has a faster onset of action and lower potential drug interaction compared to Omeprazole. It is official in Indian Pharmacopoeia [3]. Detailed survey of literature revealed that analytical methods are reported by using UV- Spectrophotometry,

4-5 RP-HPLC, 10-18 and HPTLC 19 for the estimation of Paracetamol alone as well as in combination with other drugs using pharmaceutical formulations.

Literature for Aceclofenac revealed that few methods include Spectrophotometric method, 4-7 RP-HPLC, 14 and HPTLC, 19 and Liquid Chromatography in biological fluids [21] for the estimation of Aceclofenac alone as well as with other drugs in different pharmaceutical formulations.

The analytical methods are reported for the estimation of Rabeprazole Sodium alone and in combination which include Spectrophotometric method, 4, 9 RP-HPLC, 17-20 HPTLC, 19 and Liquid Chromatography in biological fluids [21-22].

The present research work is aimed to develop a simple, rapid, accurate, sensitive and stability indicating method for simultaneous estimation of the above mentioned three drugs for routine analysis with shorter run time. The proposed method is optimized and validated as per the International Conference on Harmonization (ICH) guidelines [23].

2. EXPERIMENTAL METHODS

2.1 Chemicals and Reagents Used

The chemicals and reagents used in the study were **Potassium Dihydrogen Phosphate** (Merck Life Science Pvt. Ltd.), **HPLC-grade Methanol** (Rankem), **HPLC-grade Acetonitrile** (Rankem), **HPLC-grade Water** (Merck Life Science Pvt. Ltd.), and reference standards of **Paracetamol**, **Aceclofenac**, and **Rabeprazole Sodium**, which were obtained as gift samples from **Zydus Lifesciences Ltd., Ankleshwar, India**.

HPLC Instrumentation

The chromatographic analysis was performed using a **Shimadzu LC-2010** HPLC system equipped with a **10 μ L fixed-loop injector** and a **UV detector**. Data acquisition and processing were carried out using **LC Solution** software.

2.2 Preparation of Mobile Phase

The mobile phase consisted of **buffer and acetonitrile in the ratio of 50:50 (v/v)**. The prepared mobile phase was filtered through a **0.45 μ m membrane filter** under vacuum to remove particulate matter and subsequently degassed before use.

2.3 Preparation of Standard Solution

The standard stock solution of **Paracetamol** and **Aceclofenac** was prepared by accurately weighing **26 mg of Paracetamol** and **8 mg of Aceclofenac** and transferring them into a **10 mL volumetric flask** containing approximately **7 mL of diluent (Acetonitrile:Water, 50:50 v/v)**. The solution was sonicated for **30 minutes** to ensure complete dissolution of the drugs, and the volume was then made up to the mark with the same diluent. The resulting

standard stock solution contained **2600 μ g/mL of Paracetamol** and **800 μ g/mL of Aceclofenac**.

The standard stock solution of **Rabeprazole Sodium** was prepared by accurately weighing **4 mg** of the drug and transferring it into a **50 mL volumetric flask** containing approximately **35 mL of diluent (Acetonitrile: Water, 50:50 v/v)**. The solution was sonicated for **30 minutes** to ensure complete dissolution, after which the volume was made up to the mark with the same diluent to obtain a standard stock solution containing **80 μ g/mL of Rabeprazole Sodium**.

2.4 Preparation of Sample Solution

Twenty tablets of Acelist-PR (Paracetamol 325 mg, Aceclofenac 100 mg, and Rabeprazole Sodium 10 mg) were accurately weighed and finely powdered. Tablet powder equivalent to 500 mg of Aceclofenac (approximately 4 g of powder) was accurately weighed and transferred into a 250 mL volumetric flask. About 200 mL of diluent (Acetonitrile:Water, 50:50 v/v) was added, and the mixture was sonicated for 30 minutes to ensure complete dissolution of the drugs. The solution was then diluted to volume with the same diluent and mixed thoroughly. The resulting sample stock solution contained 6500 μ g/mL of Paracetamol, 2000 μ g/mL of Aceclofenac, and 200 μ g/mL of Rabeprazole Sodium. The solution was filtered through a 0.45 μ m membrane filter to remove insoluble excipients and other particulate matter before chromatographic analysis.

2.5 Validation of RP-HPLC Method

2.5.1 Linearity

The linearity of the developed RP-HPLC method was evaluated by preparing standard solutions at different concentration levels. Each solution was injected into the HPLC system, and the corresponding peak areas were recorded. Calibration curves were constructed by plotting peak area versus concentration and the correlation coefficient (R^2) was calculated to assess the linear relationship between concentration and detector response.

2.5.2 Precision

a) **Repeatability:** Repeatability was evaluated by injecting the standard solution six times under identical chromatographic conditions. The peak areas obtained from the six replicate injections were recorded, and the percentage relative standard deviation (%RSD) was calculated.

b) **Intraday Precision:** Intraday precision was determined by analyzing three different standard concentrations in triplicate ($n = 3$) on the same day. The %RSD of the assay results was calculated to evaluate the precision of the method.

c) **Interday Precision:** Interday precision was assessed by analyzing three different standard concentrations in triplicate ($n = 3$) on three different days. The %RSD

values obtained were used to evaluate the reproducibility of the method.

2.5.3 Accuracy

The accuracy of the method was evaluated by the recovery study at three concentration levels, namely 50%, 100%, and 150% of the target concentration. Standard solutions of Paracetamol, Aceclofenac, and Rabepirazole Sodium were prepared at these levels and analyzed using the developed RP-HPLC method. The percentage recovery for each analyte was calculated, and the mean recovery values were determined to assess the accuracy of the method.

2.5.4 Robustness

The robustness of the developed method was evaluated by introducing deliberate small variations in chromatographic conditions, including wavelength, flow rate, and column temperature. Standard and sample solutions containing Paracetamol, Aceclofenac, and Rabepirazole Sodium were analyzed under these modified conditions. The effects of these changes on system suitability parameters such as resolution, tailing factor, asymmetry, and theoretical plate count were evaluated. No significant variation was observed, indicating that the method is robust.

2.5.5 System Suitability

System suitability was evaluated by injecting the standard solution six times before sample analysis. The tailing factor for the peaks of Paracetamol, Aceclofenac, and Rabepirazole Sodium was required to be not more than 1.5, while the theoretical plate count for each peak was required to be not less than 2000. The obtained system suitability parameters complied with the acceptance criteria, confirming the suitability of the chromatographic system.

2.5.6 Detection Limit (DL) and Quantitation Limit (QL)

The Detection Limit (DL) and Quantitation Limit (QL) were estimated from the calibration curves obtained during the linearity study.

The Detection Limit (DL) was calculated using the following equation:

$$DL = 3.3 \times (SD / \text{Slope})$$

Where:

- SD = Standard deviation of the y-intercepts of six calibration curves
- Slope = Mean slope of the six calibration curves

The Quantitation Limit (QL) was calculated using the following equation:

$$QL = 10 \times (SD / \text{Slope})$$

Where:

- SD = Standard deviation of the y-intercepts of six calibration curves
- Slope = Mean slope of the six calibration curves

3. RESULTS AND DISCUSSION

3.1 Validation

The developed RP-HPLC method was validated in accordance with the ICH Q2 (R2) guidelines. The validation parameters included linearity, precision, accuracy, robustness, system suitability, detection limit, and quantitation limit. The results obtained are presented below.

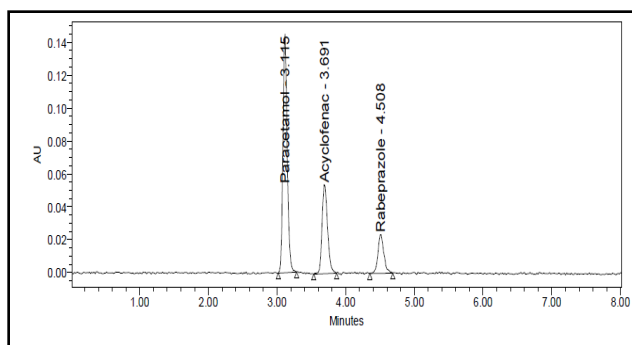
3.1.1 Linearity

The linearity of the developed RP-HPLC method was evaluated by analyzing six concentration levels for each drug. The concentration ranges were 65–390 µg/mL for Paracetamol, 20–120 µg/mL for Aceclofenac, and 2–12 µg/mL for Rabepirazole Sodium, as presented in Table 1. Each concentration level was injected into the HPLC system, and calibration curves were constructed by plotting peak area against concentration (Figures 1–9). The correlation coefficient (R^2) obtained for all three drugs was 0.999, demonstrating excellent linearity and satisfying the acceptance criterion of $R^2 \geq 0.999$.

Table 01: Linearity Results of Paracetamol, Aceclofenac, and Rabepirazole Sodium

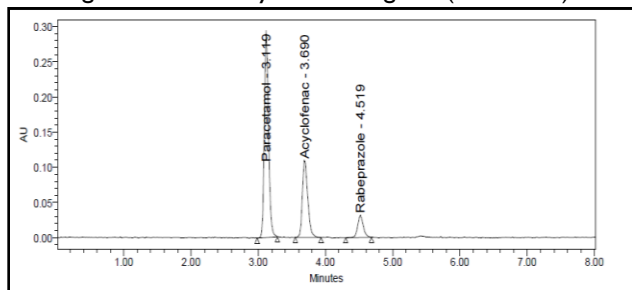
Paracetamol		Aceclofenac		Rabepirazole Sodium	
Concentration in µg/mL	Peak area*	Concentration in µg/mL	Peak area*	Concentration in µg/mL	Peak area*
65	626220.7	20	306680	2	95131
130	1261981	40	603442	4	188024
195	1912202	60	911850	6	282887
260	2515919	80	1217746	8	369743
325	3146839	100	1531262	10	464588
390	3789259	120	1813661	12	564513

*Mean of three determinations



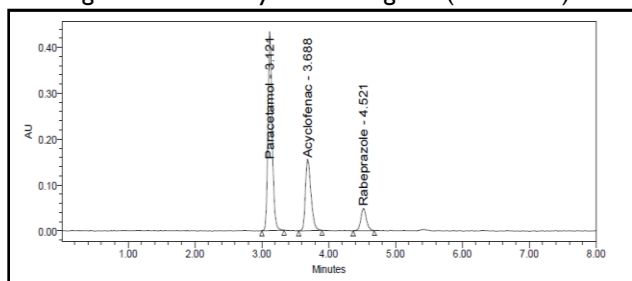
25% Working Standard Solution containing 65 µg/mL of Paracetamol, 20 µg/mL of Acyclofenac and 2 µg/mL of Rabeprazole Sodium.

Figure 01: Linearity chromatogram (25 % level)



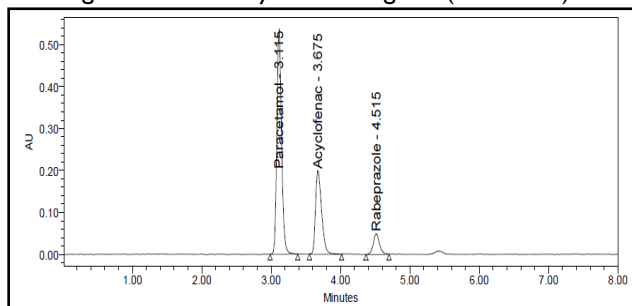
50% Working Standard Solution containing 130 µg/mL of Paracetamol, 40 µg/mL of Acyclofenac and 4 µg/mL of Rabeprazole Sodium

Figure 02: Linearity chromatogram (50 % level)



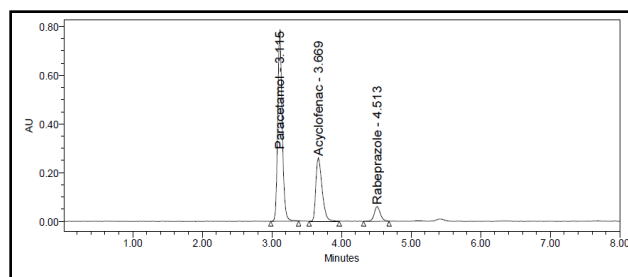
75% Working Standard Solution containing 195 µg/mL of Paracetamol, 60 µg/mL of Acyclofenac and 6 µg/mL of Rabeprazole Sodium.

Figure 03: Linearity chromatogram (75 % level)



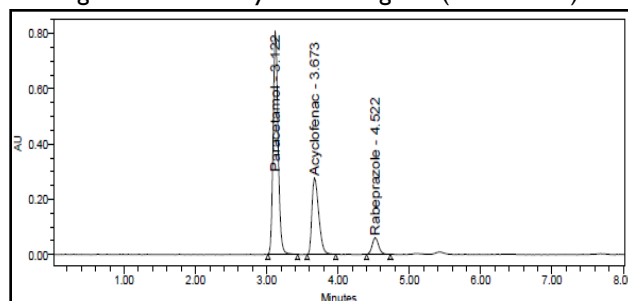
100% Working Standard Solution containing 260 µg/mL of Paracetamol, 80 µg/mL of Acyclofenac and 8 µg/mL of Rabeprazole Sodium.

Figure 04: Linearity chromatogram (100 % level)



125% Working Standard Solution containing 325 µg/mL of Paracetamol, 100 µg/mL of Acyclofenac and 10 µg/mL of Rabeprazole Sodium

Figure 05: Linearity chromatogram (125 % level)



150% Working Standard Solution containing 390 µg/mL of Paracetamol, 120 µg/mL of Acyclofenac and 12 µg/mL of Rabeprazole Sodium.

Figure 06: Linearity chromatogram (150 % level)

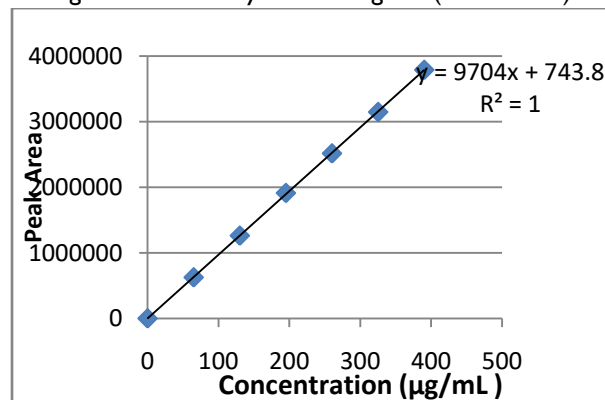


Figure 07: Standard Calibration Curve of Paracetamol

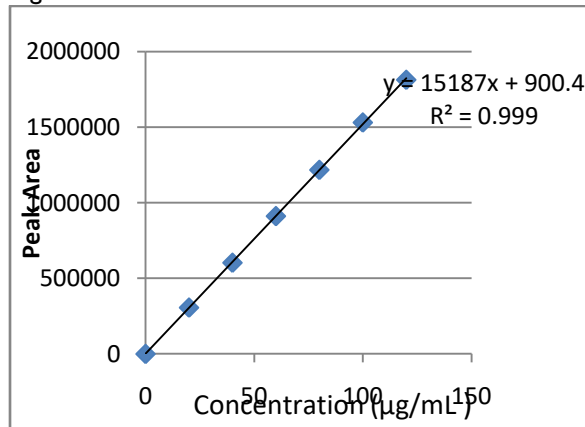


Figure 08: Standard Calibration Curve of Acyclofenac

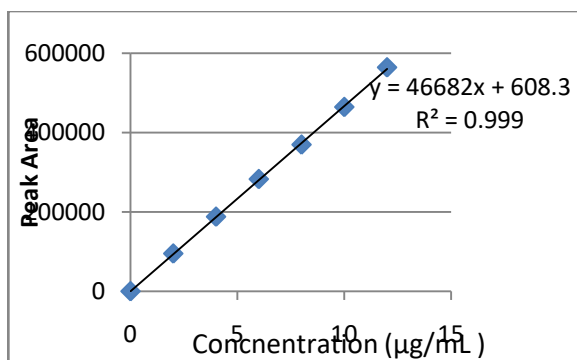


Figure 09: Standard Calibration Curve of Rabeprazole Sodium

3.1.2 Precision

Repeatability: The data of repeatability for Paracetamol, Aceclofenac and Rabeprazole Sodium are shown in (Table 02). Intraday precision: The data for intraday precision for Paracetamol, Aceclofenac and Rabeprazole Sodium are depicted in the (Table 03).

Interday precision: The data for interday precision for Paracetamol, Aceclofenac and Rabeprazole Sodium are depicted in the (Table 04).

Table 02: Precision of recommended procedure using working standard solution

Injection	Paracetamol		Aceclofenac		Rabeprazole Sodium	
	Retention Time in Min	Peak Area	Retention Time in Min	Peak Area	Retention Time in Min	Peak Area
1	3.117	2635719	3.656	1237840	4.487	200959
2	3.118	2563313	3.669	1231615	4.502	208875
3	3.12	2595543	3.67	1211680	4.504	206355
4	3.123	2631311	3.673	1250831	4.507	204619
5	3.124	2589661	3.675	1239252	4.508	205402
6	3.129	2608790	3.679	1232213	4.513	207759
Mean	3.12	2604056	3.67	1233905	4.50	205661.5
Std. dev	0.00	27230.39	0.00	12904.43	0.00	2773.97
%RSD	0.14	1.04	0.21	1.04	0.19	1.34

Table 03: Intraday Precision of working sample solution

Sample Preparation ns	%Assay		
	Paracetamol	Aceclofenac	Rabeprazole
Sample-1	101.29	100.04	99.20
Sample-2	99.22	99.87	100.75
Sample-3	100.92	100.82	99.88
Sample-4	98.53	100.03	99.15
Sample-5	99.87	100.06	100.43
Sample-6	100.91	98.45	100.47
Mean	100.12	99.87	99.98
Std. dev	1.09	0.775	0.68
%RSD	1.09	0.77	0.68

Table 04: Inter day Precision of working sample solution

Sample Preparations	% Assay		
	Paracetamol	Aceclofenac	Rabeprazole
Sample-1	98.82	99.19	100.04
Sample-2	99.68	99.70	99.92
Sample-3	99.57	99.70	100.66
Sample-4	99.59	100.13	99.26
Sample-5	100.58	99.51	99.53
Sample-6	100.95	99.44	100.39
Mean	99.87	99.61	99.97
Std. dev	0.772	0.32	0.52
%RSD	0.77	0.32	0.52

3.1.3 Accuracy

The % Recovery study was conducted using the Standard Addition Method. Known quantities of Paracetamol, Aceclofenac and Rabeprazole Sodium standard solutions were added at 50%, 100%, and 150% levels to pre-measured sample solutions containing Paracetamol, Aceclofenac and Rabeprazole Sodium. The amounts of Paracetamol, Aceclofenac and Rabeprazole Sodium were determined using the regression equation from the calibration curve. The low standard deviation values indicate the accuracy of the proposed method. The recovery study results are presented in (Table 05).

Table 05: Recovery studies of Paracetamol, Aceclofenac and Rabeprazole Sodium in tablet dosage form

% Conc	Paracetamol			Aceclofenac			Rabeprazole Sodium		
	Amount Added ($\mu\text{g/mL}$)	Amount Found ($\mu\text{g/mL}$)	% Recovery	Amount Added ($\mu\text{g/mL}$)	Amount Found ($\mu\text{g/mL}$)	% Recovery	Amount Added ($\mu\text{g/mL}$)	Amount Found ($\mu\text{g/mL}$)	% Recovery
50	130	128.9	99.13	40	40.53	101.30	4	4.06	101.6
50	130	131	100.8	40	39.2	98.02	4	4.02	100.6
50	130	130.2	100.1	40	40.33	100.8	4	3.98	99.62
100	260	260.8	100.3	80	79.74	99.68	8	8.13	101.6
100	260	262.7	101	80	80.44	100.6	8	7.84	98.12
100	260	259.8	99.9	80	79.55	99.45	8	7.95	99.49
150	390	389.5	99.88	120	118.3	98.58	12	12.08	100.7
150	390	393.9	101	120	121.2	101	12	12.11	100.9
150	390	392.3	100.6	120	121.1	101	12	11.97	99.79
Average	100.31			100.04			100.28		
SD	0.62			1.17			1.13		
RSD	0.62			1.17			1.13		

* Mean of three determination

3.1.4 Robustness

Some deliberate modifications were made to the test parameters to carry out the robustness investigation. typical variations are Wavelength: ± 1 nm, Flow rate: ± 0.1 mL/min, Column temperature: ± 2 °C (Table 6 to 8).

Table 06: Results of robustness by variations in flow rate, column temperature and Mobile phase composition of Paracetamol

S. No.	Parameter	Conditions	Peak Area*	Retention Time**	Plate count	Tailing Factor
Optimized Conditions		Buffer : Acetonitrile (50:50 %v/v); 1.0 mL/min; 30°C	2531388	3.098	12166	1.17
1	Flow Rate (± 0.1 mL/min)	0.9 mL/min	2782141	3.505	9510	1.20
		1.1 mL/min	2417582	2.805	9031	1.19
2	Column Temperature (± 5 °C)	25 °C	2539043	3.120	8257	1.23
		35 °C	2635719	3.117	7797	1.20
3	Mobile phase composition	45:55 %v/v	2574128	3.115	6884	1.26
		55:45 %v/v	2647839	3.152	6622	1.29

* Mean of three determinations, **Retention time in min

Table 07: Results of robustness by variations in flow rate, column temperature and Mobile phase composition of Aceclofenac

S.No	Parameter	Used	Peak Area*	Retention Time**	Plate count	Tailing Factor
Optimized Conditions		Buffer : Acetonitrile (50:50 %v/v) 1.0 mL/min; 30°C;	1192526	3.654	8604	1.37

1	Flow Rate (± 0.1 mL/min)	0.9 mL/min	1331933	4.133	8137	1.36
		1.1 mL/min	1122487	3.318	7751	1.30
2	Column Temperature ($\pm 5^\circ\text{C}$)	25°C	1210259	3.674	7689	1.29
		35 °C	1237840	3.656	8015	1.34
3	Mobile phase composition	45:55 %v/v	1230027	3.607	7390	1.30
		55:45 %v/v	1217363	3.793	7461	1.30

* Mean of three determinations, **Retention time in min

Table 08: Results of robustness by variations in flow rate, column temperature and Mobile phase composition of Rabeprazole Sodium

S.No	Parameter	Used	Peak Area*	Retention Time**	Plate count	Tailing Factor
Optimized Conditions		Buffer : Acetonitrile (50:50 %v/v) 1.0 mL/min; 30°C;	384645	4.487	13423	1.13
1	Flow Rate (± 0.1 mL/min)	0.9 mL/min	337398	5.081	12409	1.15
		1.1 mL/min	263260	4.070	11067	1.17
2	Column Temperature ($\pm 5^\circ\text{C}$)	25°C	341374	4.514	10470	1.14
		35 °C	200959	4.487	9674	1.17
3	Mobile phase composition	45:55 %v/v	229739	4.427	8573	1.55
		55:45 %v/v	259286	4.662	8066	1.27

* Mean of three determinations, **Retention time in min

3.1.5 DL and QL

The DL and QL for Paracetamol was found to be 0.169 $\mu\text{g/mL}$ and 0.511 $\mu\text{g/mL}$ respectively. The DL and QL for Aceclofenac was found to be 0.036 $\mu\text{g/mL}$ and 0.108 $\mu\text{g/mL}$ respectively. The DL and QL for Rabeprazole Sodium was found to be 0.010 $\mu\text{g/mL}$ and 0.030 $\mu\text{g/mL}$ respectively.

4. CONCLUSION

The availability of literature on the determination of Paracetamol, Aceclofenac and Rabeprazole Sodium in bulk and combined tablet dosage form is scarce prior to commencement of this work. Hence, the author has developed a sensitive, accurate and precise RP-HPLC for the estimation of these three drugs in bulk and combined tablet dosage form. A mixture of Potassium dihydrogen phosphate buffer: Acetonitrile (50:50 %v/v) as mobile phase was found to be most suitable to obtain a good chromatographic separation with well-defined peaks, free from tailing. From the typical chromatogram, it was found that the retention times were 3.092, 3.655 and 4.488 minutes for Paracetamol, Aceclofenac and Rabeprazole Sodium respectively. A good linear relationship of $r^2=0.999$ for all the three drugs was observed between the concentration range of 65-390 $\mu\text{g/mL}$, 20-120 $\mu\text{g/mL}$ and 2-12 $\mu\text{g/mL}$ for Paracetamol, Aceclofenac and Rabeprazole Sodium respectively. Low values of standard deviation for retention time and peak areas are indicative of the high precision of the method. The assay of Paracetamol, Aceclofenac and Rabeprazole Sodium was found to be 100.13, 99.88, and 99.98

respectively. From the recovery studies, it was found that the % RSD of the three drugs was within the limits, which indicate high accuracy of the method. The absence of additional peaks in the chromatogram indicates non-interference of the common excipients used in the formulation. This demonstrates that the developed HPLC method is simple, linear, precise, reproducible and sensitive. Thus, the developed method can be easily used for the routine quality control of bulk and combined tablet dosage form in a shorter run time with virtually no interference of the usual additives present in the pharmaceutical formulation.

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