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Stability indicating method development and validation for the simultaneous estimation of rabeprazole sodium and ketorolac tromethamine in bulk and synthetic mixture by RP-HPLC

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ABSTRACT

A new, simple, fast and economical stability indicating reverse phase high performance liquid chromatographic method for the simultaneous determination of Rabeprazole sodium (RPZ) and Ketorolac tromethamine (KTR) in bulk and synthetic dosage form. Acetonitrile and potassium dihydrogen phosphate buffer was used as a solvent to extract drugs from the synthetic mixture. Subsequently samples were evaluated directly by simultaneous estimation method by using Inertsil C_{18} (125×4.6 mm, 5µm) column by isocratic elution by using acetonitrile and potassium dihydrogen phosphate buffer (50:50) as a mobile phase. The simultaneous determination of RPZ and KTR was carried out with a flow rate of 1 mL/min and UV detection at 292 nm wavelength. The method was validated according to ICH guidelines with respect to linearity, precision, accuracy, robustness, ruggedness, specificity and limit of detection and limit of quantification. Further the stability of RPZ and KTR were accessed using various stressed conditions like acidic, alkaline, oxidative, thermal and photolytic degradations in bulk drugs (APIs) and formulation. Degradation products produced as a result of stress studies did not interfere with the detection of RPZ and KTR, so the assay can thus be stability-indicating and can be used for the routine analysis.

Keywords: RP-HPLC, Rabeprazole sodium or RPZ , Ketorolac tromethamine or KTR, Inertsil C_{18} column and, acetonitrile and potassium dihydrogen phosphate buffer.

INTRODUCTION

Ketorolac tromethamine is a pyrrolizine carboxylic acid derivative, structurally related to Indomethacin. It is coming under the category of NSAIDs and used mainly for its analgesic activity. KTR is a racemic mixture of (-)S and (+)R-enantiomeric forms, with the S-form having analgesic activity. Its anti-inflammatory effects are believed to be due to inhibition of enzymes both cyclooxygenase-I (COX-I) and cyclooxygenase-II (COX-II) which leads to the inhibition of prostaglandin synthesis leading to decreased formation of precursors of prostaglandins and thromboxanes from arachidonic acid. Analgesia is probably produced via a peripheral

action in which blockade of pain impulse generation results from decreased prostaglandin activity.

Rabeprazole sodium is an antiulcer drug in the category of proton pump inhibitors. It is a prodrug in nature but in the acidic environment of the parietal cells it turns into active sulphenamide form. Rabeprazole inhibits the H^+/ K^+ ATPase of the coating gastric cells and dose-dependent oppresses basal and stimulated gastric acid secretion. Rabeprazole belongs to a class of anti-secretory compounds that do not exhibit anticholinergic or histamine H_2-receptor antagonist properties, but suppress gastric acid secretion by inhibiting the gastric H^+/ K^+ ATPase at the secretory surface of the gastric parietal cell.

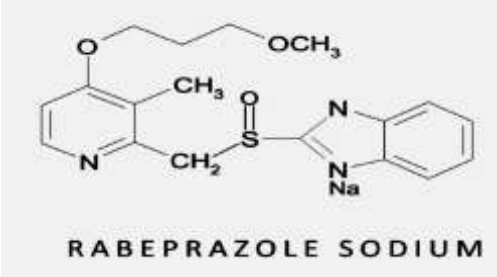


Fig. no-1: Rabeprazole Sodium

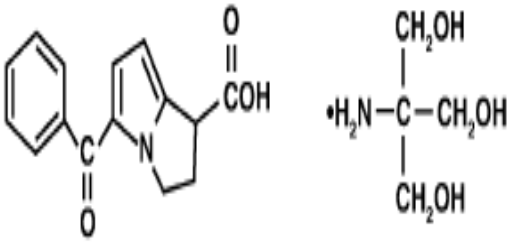


Fig. no-2: Ketorolac Tromethamine

Review of literature reveals that there are many methods reported for the estimation of KTR and RPZ individually but very few methods reported as in combination form. From the literature it was also found that few UV-spectrophotometric, HPLC, UPLC methods were developed and reported on these drugs in combination with other category drugs. But till now there is no stability indicating RP-HPLC method reported for simultaneous estimation of both the drugs in combination form.

Therefore in this present project work an attempt was made to develop a simple, sensitive, precise, accurate stability indicating RP-HPLC method for simultaneous estimation of KTR and RPZ in bulk and synthetic mixture.

EXPERIMENTAL

Chemicals and reagents: KTR and RPZ reference standards were obtained as gift samples from Aurobindo Pharma Ltd. Hyderabad. Due to unavailability of the Mkd. formulation in the local market during the time of experimentation KTR and RPZ synthetic mixture was prepared by placebo technique and formulated as tablet. HPLC grade methanol, acetonitrile and water were procured from Merck (Mumbai, India) and potassium dihydrogen phosphate, dipotasssium hydrogen phosphate and potassium hydroxide of analytical grade were used for the studies. The solvents and mobile phases after preparation were filtered using Millipore 0.45 µm filter medium.

Instrumentation

A Waters Alliance 2695 Separation Module-HPLC system comprising of quaternary, low pressure mixing pump and inline vacuum degasser with auto sampler and programmable temperature control, and a PDA detector using Waters (Alliance) Empower-2 software. For normal UV absorbance estimations during trials were done by a Lab India UV- VIS spectrophotometer UV 3000^+, and weighing of drugs and chemicals were done by a Sartorius digital balance of model no- BSA 2245-cw, Biotecnic India ultra sonicator of model no-9l250H, Make Polmon pH meter of model no- LP 1398 and Vacuum filter.

Chromatographic conditions

An isocratic separation was carried out using a mobile phase consisting acetonitrile and potassium dihydrogen phosphate (50:50, pH 6.5) was used at the flow rate of 1 mL/min with UV detection was done at 292 nm. The column was heated to 30^0C and an injection volume of 20 µl was used. The mobile was filtered through 0.4µ membrane filter and degassed in an ultrasonic bath prior to use.

Buffer preparation

To prepare 0.01N Potassium dihydrogen phosphate (pH 6.5), weigh accurately 0.27 gms of potassium dihydrogen phosphate diluted in 200 mL of distilled water, and pH adjusted by using triethylamine (TEA).

Mobile phase preparation

Mix the prepared phosphate buffer and acetonitrile at 50:50 ratio, and filter it by using vacuum filtration through 0.4 micron membrane filter and degas it by sonicating the resulting solution.

Standard stock solution preparation

Weigh and transfer 40 mg of KTR-RS and 20 mg of RPZ-RS into 100 mL volumetric flask, add 50 mL of diluent and sonicate to dissolve and dilute to volume with diluent.

Standard working solution preparation

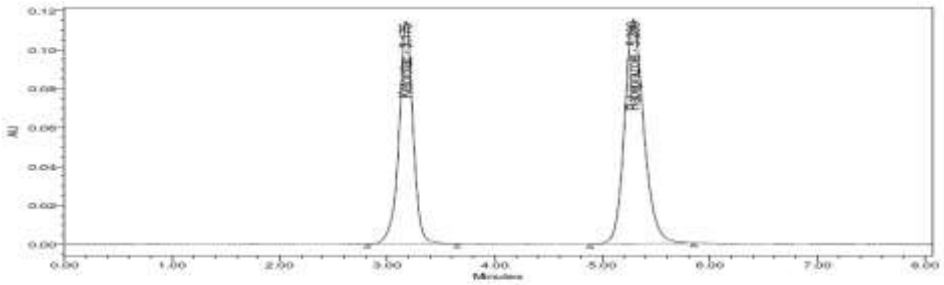
Transfer 10 mL of standard stock solution into 100 mL volumetric flask and dilute to volume with diluent.

Synthetic mixture sample preparation

Finely grind pre weighed 20 tablets. Transfer grinded sample quantitatively equivalent to 40 mg of KTR and 20 mg of RPZ in to 100 mL volumetric flask add 50 mL of diluent, sonicate to dissolve for 10 minutes and dilute to volume with diluent. Further filter the solution through filter paper. Dilute 10 mL of filtrate to 100 mL with mobile phase.

OPTIMIZED METHOD CONDITIONS

Column : Inertsil-ODS, C-18, 125 X 4.6, 5µ.
Run Time : 10 min
Flow Rate : 1.0 mL/min
Wave length : 292 nm
Column temperature : 30°C
Injection volume : 20 µL
Diluent solvent : Mobile Phase
Elution type : Isocratic
Needle wash solution : Water: Acetonitrile (90:10)
Mobile Phase : 0.01N potassium dihydrogen phosphate (pH 6.5): Acetonitrile (50:50)



	Peak Name	RT	Area	Height	% Area	USP Resolution	USP Tailing	USP Plate Count
1	Ketorolac	3.175	1041592	115099	41.915		0.94	3126.31
2	Rabeprazole	5.296	1443429	115366	58.085	7.45	1.18	4267.15

Fig. no-3: Optimized chromatogram for RABEPRAZOLE and KETOROLAC

Method Validation

As per ICH guidelines, the method validation parameters were performed for specificity, linearity, accuracy, precision, limit of detection and limit of quantification, robustness and ruggedness.

Linearity and Range

Aliquots of standard stock solutions of RPZ and KTR were taken in 10 mL volumetric flasks and diluted with diluent to get final concentrations in range of 05-30 µg/mL for RPZ and 10-60 µg/mL for KTR. Triplicate injections were made five times for each concentration for each drug separately. The peak areas of the chromatograms were plotted against the concentrations for RPZ and KTR both to obtain the respective calibration curves.

Sensitivity

Limit of detection (LOD) and Limit of quantification (LOQ) were estimated in order to obtain signal-to-noise ratio of 3:1 for LOD and 10:1 for LOQ, based on the standard deviation of response and slope. So 5 sets of serial dilutions of RPZ and KTR working standard solutions were prepared in the linearity-range and 5 set calibration curves were obtained. The LOD and LOQ may be calculated as –

LOD = 3.3XSD/Slope and **LOQ = 10XSD/Slope**

Where, SD= standard deviation of the Y-intercepts of the 5 calibration curves.

Slope= Average of slopes of the 5 calibration curves.

Precision

The precision of the analytical method was studied by analysis of multiple sampling of homogeneous sample. The precision results were expressed as standard deviation or relative standard deviation. Precision study was assessed by injection repeatability tests, by injecting standard solution of RPZ and KTR in replicate nos. In this method precision was confirmed by obtaining %RSD values of peak area for all components within acceptance limit.

Accuracy

The different between theoretical added amount and practically achieved amount is called accuracy of analytical method. To ensure the reliability (accuracy) of the method, recovery studies were carried out by mixing standard quantity of standard drug with the pre-analyzed sample formulation and the contents were reanalyzed by the proposed method. The accuracy was determined at three levels 50%, 100%, 150% of target concentrations by calculating recoveries of RPZ and KTR by standard addition method.

Robustness and Ruggedness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters, and provides an indication of it's during normal usage. It is done by small, deliberate changes in flow rate, mobile phase ratio and detection wavelength. The ruggedness of an analytical method is the degree of reproducibility of the test results obtained by the analysts of the same samples under a variety of normal test conditions such as different laboratories, different analysts using same operational and environmental conditions that may differ but are still within the specified parameters of the assay.

Stability studies

System stability testing is essential for the assurance of the quality performance of the chromatographic system. The earlier prepared solutions for chromatographic conditions were tested for system stability studies. The degradation studies were performed under various conditions such as acidic, alkaline, oxidative & thermal.

RESULTS AND DISCUSSIONS

Linearity

The linear detector response for KTR and RPZ were demonstrated by conc. Vs peak area, over the range of 10-60 µg/mL for KTR and 5-30 µg/mL for RPZ. The correlation coefficient (r^2) value was found to be 0.9990 for both KTR and RPZ**.** The results are presented in **Table-1 and Table-2.**

Accuracy

The accuracy of the test method is demonstrated by % of recovery. The sample preparations are spiked with known amount of standard drugs dilutions at three concentration levels and injected three times at 50% (10 µg/mL RPZ & 20 µg/mL KTR), 100% (20 µg/mL RPZ & 40 µg/mL KTR) and 150% (30 µg/mL RPZ & 60 µg/mL KTR). The obtained results were reported in **Table-6**.

Precision

System Precision: Dilute 10 mL of standard stock solution with 100 mL of diluent. Prepare the similar concentration six times and inject the above solutions repeatedly.

Method Precision: Dilute 10 mL of standard stock solution, with 100 mL of diluent. Prepare six solutions and inject each solution reported in **Table 5**. The % RSD values were within 2 and the method was found to be precise.

Sensitivity

Limit of detection (LOD) and Limit of quantification (LOQ) were estimated from the based on the standard deviation of response and slope. The detection limit was defined as the lowest concentration level resulting in a peak height of three times the baseline noise. The quantification limit was defined as the lowest concentration. LOD and LOQ values reported in Table. 3& 4. The values were found to be within the range.

Robust and Ruggedness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but

deliberate variations in method parameters and provides an indication of its during normal usage. When Ruggedness was performed on two different days and by different analysts there was minute change in R_t. reported in Table 7 & 8.

System stability testing is essential for the assurance of the quality performance of the chromatographic system the earlier prepared solutions for chromatographic conditions were tested for system stability studies reported in table 9.

System stability

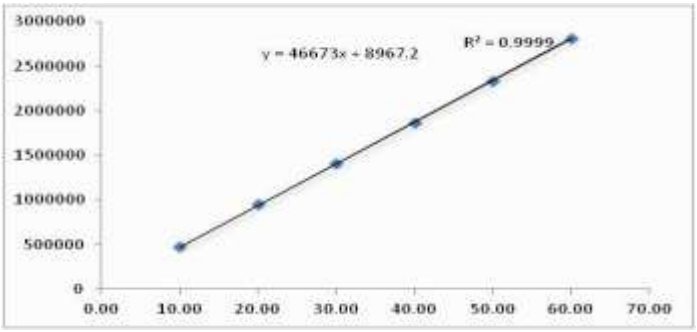


Figure no- 4: Calibration graph of KETOROLAC

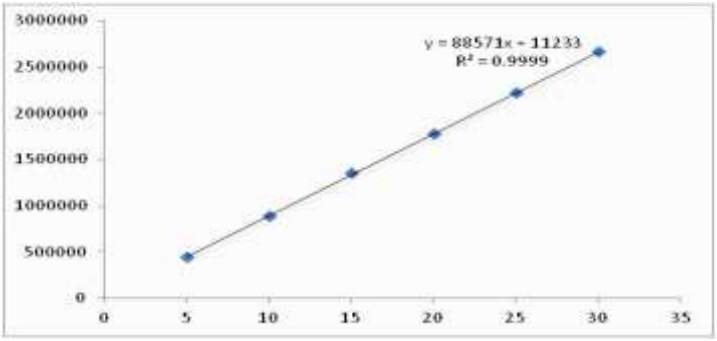


Figure no- 5: Calibration graph of RABEPRAZOLE

Table no- 1: Linearity results for KETOROLAC

Sl. No.	Linearity Level	KTR	
		Conc. (µg/mL)	Area
1	I	10.00	472080
2	II	20.00	949708
3	III	30.00	1413345
4	IV	40.00	1866118
5	V	50.00	2338563
6	VI	60.00	2815324
Correlation coefficient		0.999	

Table no- 2: Linearity results for RABEPRAZOLE

Sl. No.	Linearity Level	RPZ	
		Conc. (µg/mL)	Area
1	I	5	445429
2	II	10	895915
3	III	15	1357556
4	IV	20	1781302
5	V	25	2222275
6	VI	30	2664835
Correlation coefficient		0.999	

LOD = 3.3*STDEV/SLOPE

Table no- 3: LOD results for KETOROLAC and RABEPRAZOLE

S.NO	DRUG	LOD Value
1	KTR	0.4964 MCG/ML
2	RPZ	0.3325 MCG/ML

LOQ = 10*STDEV/SLOPE

Table no-4: LOQ results for KETOROLAC and RABEPRAZOLE

S.NO	DRUG	LOD Value
1	KTR	0.3325 MCG/ML
2	RTZ	1.0078 MCG/ML

Table no-5: System precision & Method Precision Results for RABEPRAZOLE and KETOROLAC

Sl. No	Name	KTR		RPZ	
		RT	Area	RT	Area
1	S-Precision-1	3.177	1881304	5.208	1773893
2	S-Precision-2	3.177	1871215	5.209	1776486
3	S-Precision-3	3.176	1865454	5.208	1779018
4	S-Precision-4	3.176	1867103	5.209	1780792
5	S-Precision-5	3.177	1860701	5.209	1776876
6	S-Precision-6	3.178	1866636	5.211	1786688
7	M-Precision-1	3.173	1854767	5.203	1774365
8	M-Precision-2	3.175	1854210	5.206	1774899
9	M-Precision-3	3.175	1851098	5.207	1774204
10	M-Precision-4	3.176	1857435	5.209	1776908
11	M-Precision-5	3.174	1858989	5.208	1777233
12	M-Precision-6	3.174	1858665	5.209	1778044
Average		**3.176**	**1862298**	**5.208**	**1777451**
Standard Deviation		**0.001**	**8477.385**	**0.002**	**3569.241**
RSD		**0.047**	**0.455**	**0.038**	**0.201**

Table no- 6: Accuracy results for RABEPRAZOLE and KETORLAC

	Accuracy-- 50%		Accuracy-- 100%		Accuracy-- 150%	
Sl. No.	KTR Area	RPZ Area	KTR Area	RPZ Area	KTR Area	RPZ Area
Injection-1	1419803	1338099	1886210	1785066	2363460	2228790
Injection-2	1420546	1338603	1891238	1786215	2358698	2228098
Injection-3	1422110	1339099	1885097	1788965	2370232	2276708
Avg.	1420820	1338600	1887515	1786749	2364130	2244532
Amt. Recovered	49.67	49.47	98.56	99.74	148.99	150.59
%Recovery	99.35	98.95	98.56	99.74	99.33	100.40

Table 7- Study of Robustness (Flow Rate and oven Temperature)

Sl. No.	KTR		RPZ	
	RT	AREA	RT	AREA
1.	STANDARD			
	3.175	1897882	5.205	1781175
2.	robust-1 flow-1			
	2.833	1677314	4.638	1588884
3.	robust-2 flow-2			
	3.618	2157154	5.943	2061305
4.	robust-3 column oven temperature-1			
	3.163	1914838	4.971	1800726
5.	robust-4 column oven temperature-1			
	3.188	1910875	5.486	1810386

Table no-8: Ruggedness Day-1 & Day-2

S No	Name	KTR		RPZ	
		RT	Area	RT	Area
1	Ruggedness-(Day-1)-1	3.173	1854767	5.203	1774365
2	Ruggedness-(Day-1)-2	3.175	1854210	5.206	1774899
3	Ruggedness-(Day-1)-3	3.175	1851098	5.207	1774204
4	Ruggedness-(Day-1)-4	3.176	1857435	5.209	1776908
5	Ruggedness-(Day-1)-5	3.174	1858989	5.208	1777233
6	Ruggedness-(Day-1)-6	3.174	1858665	5.209	1778044
7	Ruggedness-(Day-2)-1	3.173	1864656	5.205	1785455
8	Ruggedness-(Day-2)-2	3.177	1864098	5.209	1785878
9	Ruggedness-(Day-2)-3	3.179	1863255	5.211	1785909
10	Ruggedness-(Day-2)-4	3.179	1866569	5.212	1786456
11	Ruggedness-(Day-2)-5	3.180	1867945	5.213	1787023
12	Ruggedness-(Day-2)-6	3.180	1868033	5.212	1781650
Average		**3.176**	**1860810.000**	**5.209**	**1780668.667**
Standard Deviation		**0.0027**	**5732.9606**	**0.0031**	**5222.2731**
% RSD		**0.084**	**0.308**	**0.059**	**0.293**

Table no- 9: Stability Study Results of RABEPRAZOLE and KETOROLAC

		% of degradation				
S no.	**Drug sample**	**Acid**	**Base**	**Peroxide**	**Thermal**	**Uv**
1	RABE	27 %	9 %	33 %	13 %	4 %
2	KETO	24 %	9 %	35 %	19 %	3 %

CONCLUSION

The proposal study describes a new RP-HPLC method using mobile phase for the estimation of RPZ and KTR in combined synthetic mixture. The method was validated and found to be simple, sensitive, accurate and precise.

It was also proved to be convenient and effective for the determination of RPZ and KTR in the bulk and synthetic mixture. The % Recovery shows that the method is free from interference of the excipients used in formulation. Moreover, the lower solvent consumption along with the short analytical run time leads to cost effective chromatographic method.

It inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision

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