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STABILITY INDICATING RP-HPLC METHOD FOR DETERMINATION OF OMEPRAZOLE AND CINITAPRIDE IN COMBINED PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, precise and accurate isocratic high-performance liquid chromatography method is developed for the simultaneous estimation of omeprazole and cinitapride in bulk drug and pharmaceutical dosage form. The separation and quantification is carried out using Phenomenex C18 (150 mm × 4.6 mm; 5µm) analytical column. The mobile phase comprises of 0.1% orthophosphoric acid and methanol (55:45 *v/v*). The flow rate is 1.0 mL/min. The eluent is monitored at 256 nm. The retention time of omeprazole and cinitapride are 2.564 min and 3.904 min, respectively. The method is validated in terms of linearity, sensitivity, precision, accuracy, specificity, selectivity and robustness. The stress testing is carried out under acidic, alkaline, oxidation, photolytic and thermal degradation conditions. The degradation products are well resolved from the omeprazole and cinitapride peaks.

Keywords: Stability indicating, HPLC, omeprazole, cinitapride

INTRODUCTION

Omeprazole[1-3], chemically known as, 5-methoxy-2-[[(4-methoxy-3, 5-dimethyl-2-pyridinyl) methyl] sulphinyl]-1H-benzimidazole (Fig. 1), belongs to proton pump inhibitor group of drugs. Omeprazole is widely used for the prophylaxis and treatment of both gastro-duodenal ulcers and symptoms of gastrooesophageal reflux. Also, it is effective in healing erosive esophagitis. In combination with antibiotics, omeprazole may also be given to treat gastritis caused by infection with *Campylobacter pylori*.

Cinitapride[4-6], chemically known as 4-amino-N-[1-(cyclohex-3-en-1-ylmethyl) piperidin-4-yl]-2-ethoxy-5-nitrobenzamide (Fig.-1), is an antiulcer and gastroprokinetic agent. It belongs to the benzamide class of drugs. Cinitapride exerts its activity by acting as an agonist for the 5-HT1 and 5-HT4 receptors and as an antagonist for the 5-HT2 receptor. Cinitapride is effective in treating the patient with gastroesophageal reflux, functional dyspepsia and irritable bowel syndrome.

The combination of the above two drugs is used in treatment of gastric ulcer, gastro esophageal reflux disease and dyspepsia when they are not responding to omeprazole alone. The combination of omeprazole and cinitapride is not official in any pharmacopoeia. There are few references in the literature describing the simultaneous quantification of omeprazole and cinitapride in the combined dosage form using UV-spectrophotometry[7-9], RP-HPLC[10-14] and HPTLC[15].

To the best of our knowledge, one stability indicating HPLC method was reported for the simultaneous analysis of omeprazole and cinitapride [16]. The disadvantages of the reported stability indicating HPLC method is lack of accuracy and lesser precision. The retention time of the analytes and total runtime in the reported method is more than the proposed method which leads to a longer runtime for a single sample. The reported stability indicating HPLC method used acetonitrile in their mobile phase which probably will raise the cost of the method. The preparation of buffer and maintaince of pH of the buffer makes the method cumbersome. Further more the reported stability indicating HPLC method does not reported the spectral homogeneity of omeprazole and cinitapride peaks.

Due to the above facts, the aim of the present work is to develop and validate a simple, rapid, economical, precise and accurate stability indicating HPLC method for the simultaneous estimation of both the drugs in combined capsule dosage form.

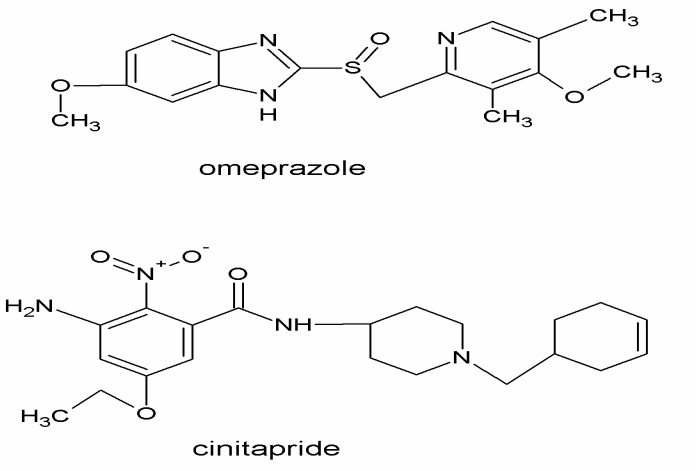

Fig.-1: Chemical structure of the drugs

EXPERIMENTAL

Apparatus

The analysis of omeprazole and cinitapride was carried out using a Waters 2695 alliance HPLC system with binary pump and Waters 2998 PDA detector. Waters Empower2 software was used for recorded the data. Phenomenex C18 (150mm × 4.6; 5µm) analytical column used to achieve chromatographic separation

Commercial capsule dosage forms

Burpex capsule manufactured by Cadila Pharmaceuticals Ltd., India was purchased from local market. Each capsule claimed to contain 20 mg of omeprazole and 3 mg of cinitapride.

Solvents

Methanol of HPLC grade was purchased from Merck (India) Ltd., Mumbai. Ortho phosphoric acid of analytical reagent grade was obtained from Sd Fine Chemicals Ltd., Mumbai. Mille Q water was used through out the process.

Mobile phase

The mobile phase consisting of 0.1% orthophosphoric acid: methanol was degassed and pumped from the solvent reservoir in the ratio of 55:45 (*v/v*).

Chromatographic conditions

The mobile phase was pumped from the reservoir into the column at a flow rate of 1.0 mL/min. The column temperature was set at 30°C. The detection was monitored at 256 nm and the run time was 6 min. The volume of injection loop was 10 µL. Prior to injection of the drug solution, the column was equilibrated for at least 15 min. with the mobile phase.

Standard solutions

Stock solutions of omeprazole and cinitapride (each, 1mg/mL), were prepared by dissolving the drugs in mobile phase then completing in 100 mL volumetric flasks. Series of working solutions of omeprazole and cinitapride were prepared by the appropriate dilution of the stock solutions with mobile phase to reach the concentration ranges of 20- 60 µg/mL for omeprazole and 3-9µg/mL for cinitapride.

Procedure for calibration graph
Ten µL injections were made for each working concentration and chromatographed under the condition described above. The peak area of each concentration was plotted against the corresponding concentration to get the calibration graph and regression equation was derived.

Procedure for pharmaceutical dosage sample
The contents of twenty capsules, labeled to contain 20 mg of omeprazole and 3 mg of cinitapride, were weighed, mixed and finely powdered in a mortar. An amount of the powder equivalent to 20 mg of omeprazole and 3 mg of cinitapride was accurately weighed, transferred into 100 mL volumetric flask and diluted with mobile phase. The sample solution was filtered using 0.45 µm filter. An aliquot of filtrate was pipetted and diluted to obtain concentrations 40 µg/mL of omeprazole and 6 µg/mL of cinitapride. The procedure was completed as mentioned above. The nominal concentration of omeprazole and cinitapride was obtained either from calibration graph or from corresponding regression equation.

Specificity (Forced degradation)
The specificity of the proposed method was assessed to prove the absence of interference from the degradants of omeprazole and cinitapride. Degradation study was performed by subjecting the capsule powder to degradations such as acid, alkaline, oxidation, thermal and photolytic conditions to evaluate the interference of degradants. All forced degradation studies were analyzed at 40 µg/mL omeprazole and 6 µg/mL cinitapride concentration levels. Thermal degradation was performed by keeping the sample in petri dish and then placed them in an oven at 105^{o}C for 30 minutes. The photolytic study was carried out by placing the sample in petri dish and exposed to sun light for 24 hours. Acid, base and oxidation degradations were performed by adding 10 mL of 0.1N HCl, 10 mL of 0.1N NaOH and 10 mL of 30% peroxide solution, respectively to the sample and sonicate for 30 minutes. The acid degraded sample and base degraded sample are neutralized with 0.1 N NaOH and 0.1 N HCl, respectively.

RESULTS AND DISCUSSION

The main objective of the HPLC method was to develop a validated stability indicating method for the estimation of omeprazole and cinitapride simultaneously in bulk and capsule dosage form and to obtain well resolved peaks of omeprazole, cinitapride and their degradants.

Method development and optimization
Chromatographic parameters such as mobile phase composition, wavelength of detection, column and column temperature were optimized to achieve better efficiency of the chromatographic system. Two HPLC analytical columns, Phenomenex C18 (150 mm x 4.6 mm x 5 µm) and Zorbax C18 (250 mm x 4.6 mm x 5 µm) were tested during method development. The system suitability parameters like tailing factor, resolution, and plate count were taken into consideration. Based on the above said parameters Phenomenex C18 (150 mm x 4.6 mm x 5 µm) column was finalized for simultaneous analysis. Different composition of mobile phases containing a mixture (*v/v*) of 0.1 M NaH_2PO_4, methanol and 0.1% orthophosphoric acid in water were evaluated in order to obtain suitable composition of mobile phase. Finally the mixture of 1% orthophosphoric acid in water and methanol in the ratio of 55:45 (*v/v*) was selected as optimal as it produced well defined and well resolved peaks of omeprazole and cinitapride at a flow rate of 1 mL/min and with column temperature of 30^{o}C.
For the detection and quantification of omeprazole and cinitapride, 256 nm was selected as the optimum wavelength. At this wavelength best detector response for both omeprazole and cinitapride was obtained. The retention time for omeprazole and cinitapride was found to be 2.564 min and 3.904 min, respectively. A typical chromatogram is given in Fig.-2.

Method validation
The developed method was validated as per the guidelines given by International Conference on Harmonization[17].

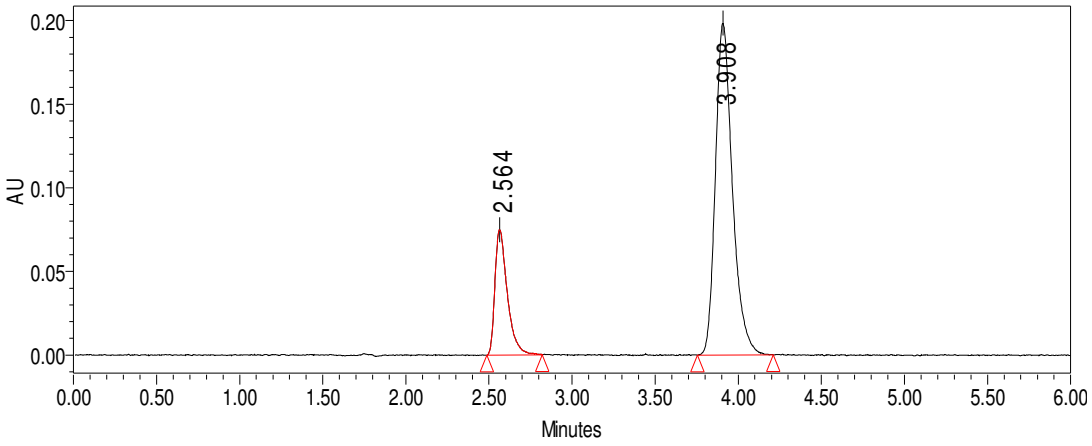

Fig.-2: Typical chromatogram of omeprazole (tR – 2.564) and cinitapride (tR – 3.908)

System suitability

For system suitability testing, five replicates of omeprazole (40 µg/mL) and cinitapride (6 µg/mL) standard solutions were injected. The retention time, peak area, USP plate count and USP tailing factor of each replicate were established. The results of system suitability in comparison with the required limits are shown in Table-1 and are found to be within the accepted limits.

Table-1: System suitability

Parameters	Omeprazole	Cinitapride	Recommended limits
Retention time	2.566	3.904	-
Peak area	390103 (%RSD – 0.7)	1423526 (%RSD – 0.6)	RSD ≤1
USP resolution	-	8.22	> 1.5
USP plate count	5937	7214	> 2000
USP tailing factor	1.33	1.32	≤ 2

Linearity

The proposed method was tested for linearity by plotting peak area against concentration of drug. The plot of peak area *vs* the respective concentrations of omeprazole and cinitapride were found to be linear in the concentration range of 20-60 µg/mL and 3-9µg/mL respectively. The results of linearity and regression equations for omeprazole and cinitapride were given in Fig.-3. The results shows that an excellent correlation exists between area and drug concentration within the concentration range indicated above.

Limit of quantification and detection

Limit of quantification (LOQ) and detection (LOD) were predicted by plotting linearity curve for different nominal concentrations of omeprazole and cinitapride. The LOQ and LOD values were predicted using following formulae (a) and (b)-

(a) $LOQ = 10\ \sigma / S$
(b) $LOD = 3.3\ \sigma / S$

Where σ = residual standard deviation of response; S = slope of the calibration curve.

The LOD and LOQ for omeprazole were found to be 1.165 µg/mL and 3.883 µg/mL, respectively. The LOD and LOQ for cinitapride were found to be 0.0657 µg/mL, 0.2190 µg/mL, respectively.

Precision

The precision of the proposed method was determined by the analysis of a fixed concentration of the selected drugs (omeprazole: 40 µg/mL and cinitapride: 6 µg/mL), within the linearity range, by six replicate analyses. The precision was expressed as percent standard deviation. The results were illustrated in Table-2.

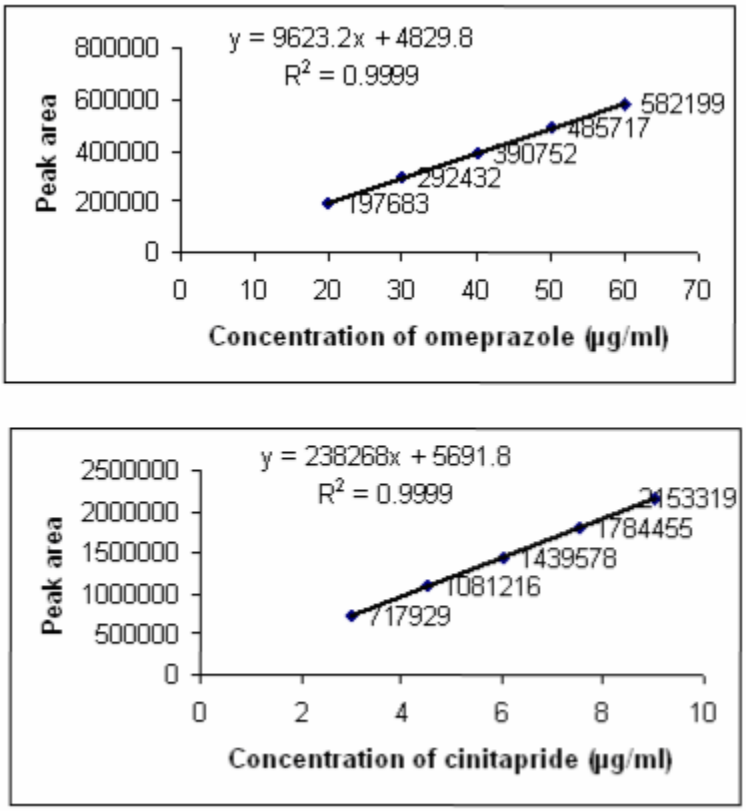

Fig.-3: Linearity curves and regression equations

Table-2: Precision

Omeprazole		Cinitapride	
Peak area	%RSD	Peak area	%RSD
393965	0.31	1430442	0.29
392641		1425668	
395268		1435388	
393872		1433915	
392746		1426429	
395543		1434320	

Accuracy

To study the accuracy of the proposed method, recovery studies were conducted at three concentrations of 50%, 100%, and 150% levels by standard addition method. The accuracy expressed as percentage recoveries was shown in Table-3. The results indicated that the method is accurate.

Specificity

The degradation study was carried out using the capsule powder containing omeprazole and cinitapride. Specificity of the method was performed by injecting the stressed degradation samples into the HPLC

system. The chromatograms of the samples after forced degradation treatment are shown in Fig.-4. The samples submitted to all degradation conditions showed significant alteration in the peak areas. In all the degradation conditions two peaks, in addition to the omeprazole and cinitapride peaks, were observed expect in photolytic degradation where three additional peaks were observed. The degradation peaks were well resolved from that of omeprazole and cinitapride peaks. The degradation results of various stress conditions were shown in Table-4.

Table-3: Accuracy

Drug	Spiked Level	µg/mL added	µg/mL found	% Recovery	% Mean
Omeprazole	50%	20.103	20.00	100	100
	50%	20.103	20.07	100	
	50%	20.103	20.04	100	
	100%	40.205	40.29	100	100
	100%	40.205	40.12	100	
	100%	40.205	40.40	100	
	150%	60.308	59.82	99	99
	150%	60.308	59.45	99	
	150%	60.308	59.87	99	
Cinitapride	50%	3.015	3.00	100	100
	50%	3.015	3.01	100	
	50%	3.015	3.01	100	
	100%	6.031	6.02	100	100
	100%	6.031	6.00	100	
	100%	6.031	6.05	100	
	150%	9.046	9.01	100	100
	150%	9.046	9.06	100	
	150%	9.046	9.08	100	

Table-4: Forced degradation studies

Type of Degradation	Omeprazole			Cinitapride		
	Peak area	% Assay	% Degradation	Peak area	% Assay	% Degradation
Undegraded	390103	100	-	1423526	100	-
Acid	372574	95	5	1355024	95	5
Base	374919	95	5	1376348	96	4
Peroxide	375649	95	5	1356875	95	5
Heat	377285	96	4	1360163	95	5
Sunlight	376678	96	4	1370705	96	4

Spectral homogeneity of omeprazole and cinitapride in the presence of their stress degradation products was checked. Peak purity passed for both the omeprazole and cinitapride. The results are presented in Table-5. Purity angle value was less than the purity threshold for omeprazole and cinitapride peaks indicating both the peaks are spectrally homogeneous.

Table-5: Spectral homogeneity of omeprazole and cinitapride

Type of Degradation	Omeprazole		Cinitaride	
	Purity Angle	Purity Threshold	Purity Angle	Purity Threshold
Acid	1.176	1.473	0.262	0.475

Base	1.304	1.553	0.251	0.462
Peroxide	1.282	1.591	0.249	0.464
Heat	1.319	1.654	0.266	0.477
Sunlight	1.281	1.656	0.275	0.485

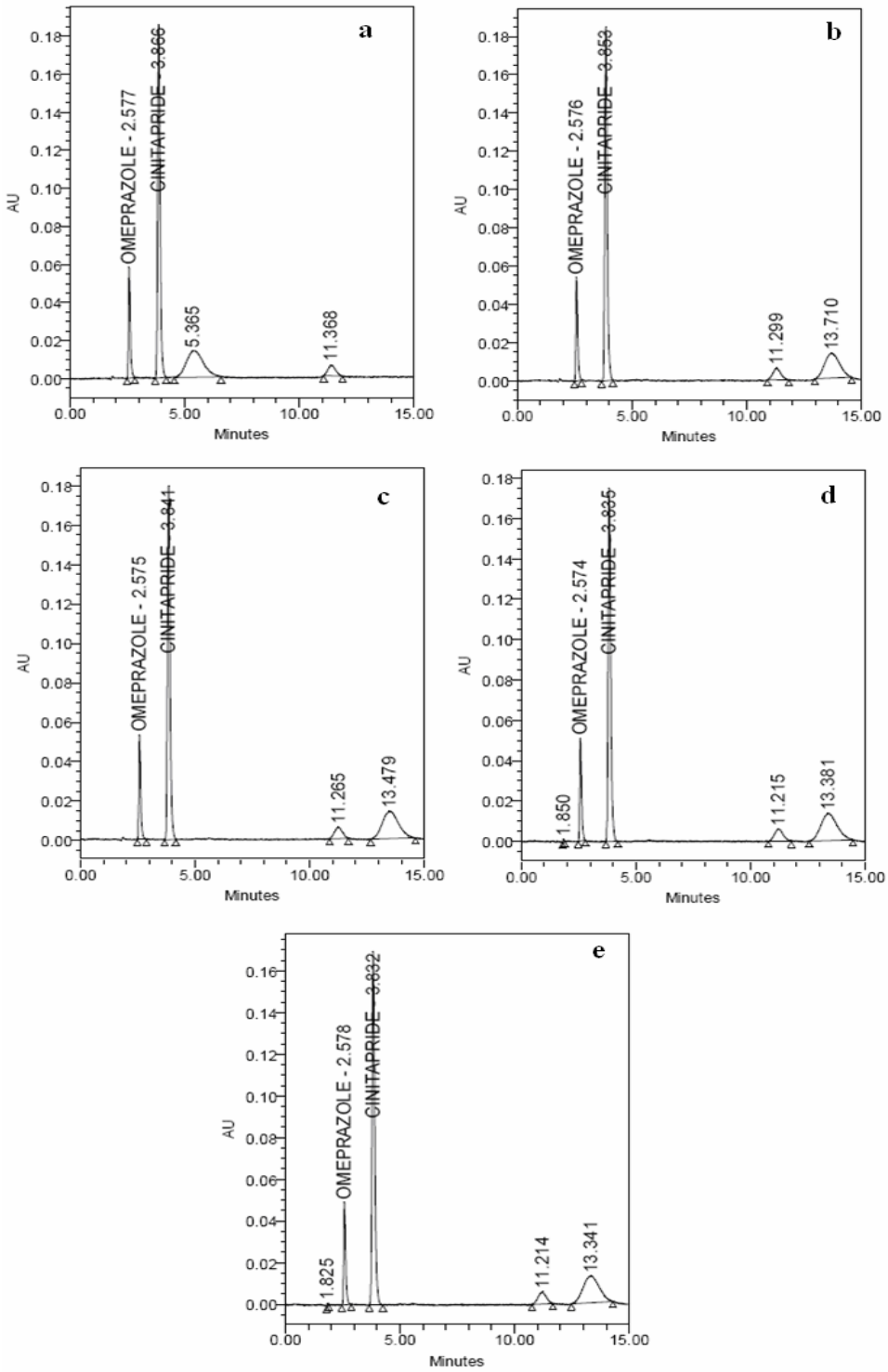

Fig.-4: Chromatograms omeprazole and cinitaride after (a.) Acid hydrolysis (b.) Alkali hydrolysis (c.) Peroxide degradation (d.) Thermal degradation (e.) Photolytic degradation

Selectivity

To confirm the noninterference of placebo, placebo solution was prepared in the same way of the capsule sample solution in the presence of all excipients of the capsule dosage form but without omeprazole and cinitapride. The chromatograms of blank, placebo, test sample and standard were compared to give reason for the selectivity of method. The method was selective since excipients in the formulation and components of the mobile phase did not interfere in the simultaneous analysis of omeprazole and cinitapride (Fig.-5).

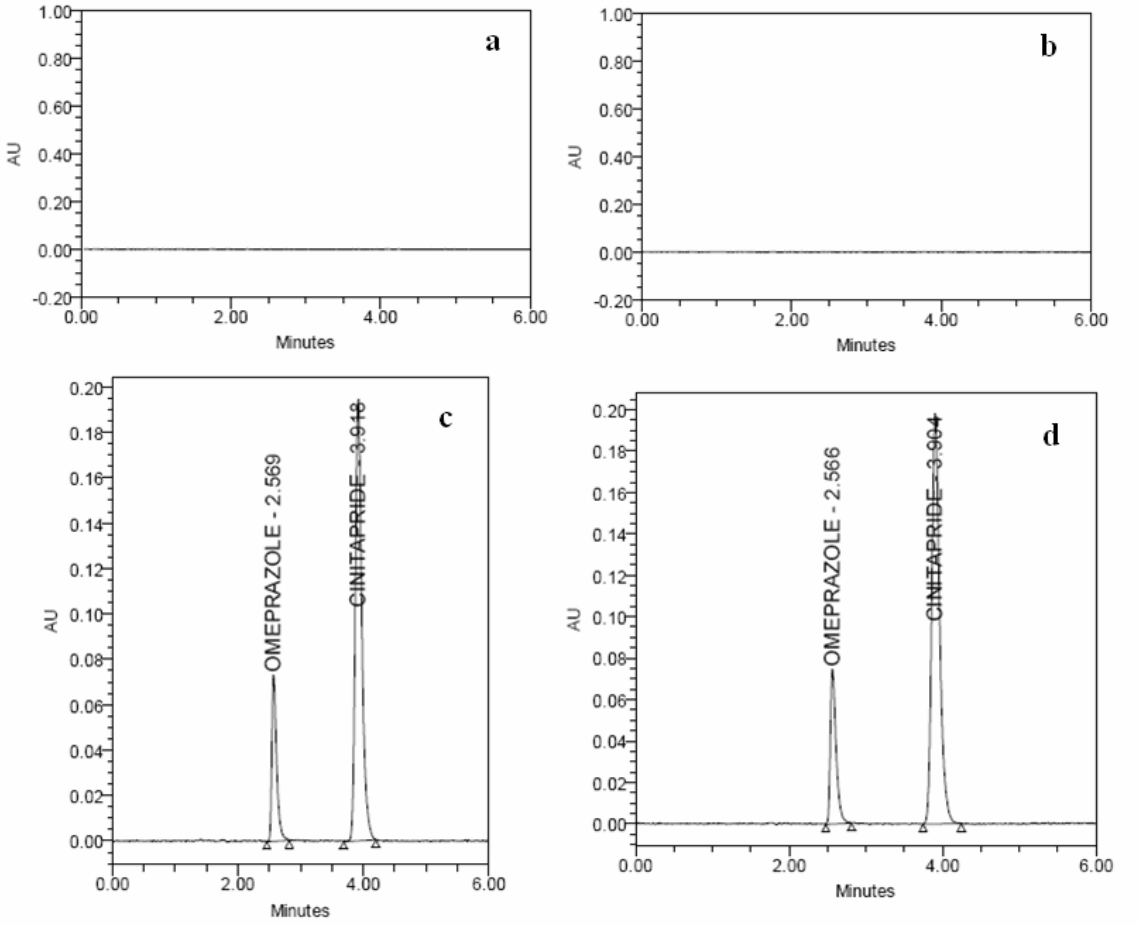

Fig.-5: Chromatograms of (a.) Blank mobile phase (b.) Placebo blank (c.) Test sample (d.) Standard

Robustness

To establish the robustness of the method, ratio of the mobile phase, flow rate of the mobile phase and column temperature was slightly varied instead of optimized values. The robustness was studied at two different concentration levels. The results are summarized in Tables-6 & 7. The low values of percent relative standard deviation (<1%) indicate that the method is robust.

Table-6: Robustness of the method for the assay of omeprazole

Experimental variable	Investigated range	20 µg/mL omeprazole			60 µg/mL omeprazole		
		Peak area	Mean Peak area (n=3)	%RSD	Peak area	Mean Peak area (n=3)	%RSD
Mobile phase ratio* (*v/v*)	53:47	193248	193911	0.592	585624	584255	0.203
	55:45	193248			583624		

	57:43	195238			583518		
Temperature of the column (^oC)	28	194359	193630	0.351	582648	584466	0.272
	30	193015			585127		
	32	193518			585624		
Flow rate of mobile phase (mL/min)	0.9	194532	195177	0.570	588546	585717	0.534
	1.0	198513			586248		
	1.1	192486			582357		

Table-7: Robustness of the method for the assay of cinitapride

Experimental variable	Investigated range	3 μg/mL cinitapride			9 μg/mL cinitapride		
		Peak area	Mean Peak area (n=3)	%RSD	Peak area	Mean Peak area (n=3)	%RSD
Mobile phase ratio* (*v/v*)	53:47	714851	715479	0.308	2184621	2169042	0.628
	55:45	717929			2163185		
	57:43	713658			2159321		
Temperature of the column (^oC)	28	717584	715574	0.258	2176294	2173353	0.955
	30	715184			2151285		
	32	713956			2192482		
Flow rate of mobile phase (mL/min)	0.9	715691	715943	0.357	2168426	2151165	0.854
	1.0	713518			2153218		
	1.1	718621			2131852		

CONCLUSION

A simple, rapid, economical stability indicating HPLC method was developed for the separation and simultaneous quantification of omeprazole and cinitapride in the presence of its stress degradation products in bulk and in its pharmaceutical dosage forms. Degradation behavior of omeprazole and cinitapride was studied under various degradation conditions like acid, base, peroxide, thermal and sunlight. Degradation peaks were observed in all stress conditions. All the stress degradation products were well separated from omeprazole and cinitapride revealing the stability-indicating capability of the method. The developed method can be used for the simultaneous quantification of omeprazole and cinitapride in routine analysis.

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