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RP-HPLC Method for the Determination of Cinitapride in the Presence of its Degradation Products in Bulk Drug

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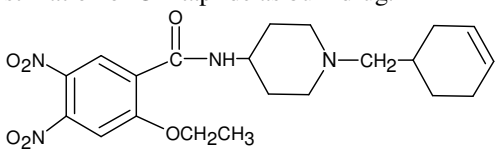
Abstract: A reverse phase HPLC method is described for the determination of cinitapride hydrogen tartrate in the presence of its degradation products in bulk drug. A drug was subjected to all stress conditions such as reduction, oxidation acidic and alkaline medium. Chromatography was recorded on an Intersil ODS-3 column using mixture of acetonitrile and phosphate buffer, pH adjusted to 6.7 in the ratio (70:30 v/v) as the mobile phase at the rate of 1.0 mL/min with detection at 260 nm. Glimepride was used as internal standard. The retention time of drug cinitapride was 3.8 min and glimepride an internal standard was 2.5 minute. The drug was found to degrade extensively in reduction conditions and mild degradation in the presence of in alkaline, acidic and oxidative but the drug was stable in thermal stress. The method was validated by determining its specificity, linearity, precision and accuracy. The developed method with good separation of all degradation products from drug could be successfully applied for the determination of cinitapride in the presence of its degradation products in the bulk drug**.** The proposed method is simple, fast, accurate and precise and hence applied for routine quality control of cinitapride in bulk drug. It can be used for analysis of samples during stability testing.

Keywords: RP-HPLC, Cinitapride, Determination of cinitapride, Degradation products.

Introduction

Cinitapride, chemically 4-amino-*N*-[3-(cyclohexan-1-yl-methyl)-4-piperidinyl]-2-ethoxy-5-nitrobenzamide has the molecular formula $C_{21}H_{30}N_4O_4$ and molecular weight 402.49 g.mol^{-1}. Cinitapride is a drug that has against action to the serotoninergic 5-HT2 and D2 dopaminergic receptors that has been indicated in the gastroesophageal reflux and in the

functional disorders of gastrointestinal motility treatment. The therapeutic effect of cinitapride lies on the capacity of increasing lower esophageal sphincter tone and has strong gastrokinetic activity, which generates significant increases in the gastric emptiness; besides, through the serotoninergic system it stimulates the intestinal activity. The use of cinitapride is efficient and safe in treatment of patients with disorders in the gastric emptiness related to gastroesophageal reflux and functional dyspepsia as well as in individuals that present irritable bowel syndrome with constipation and abdominal pain[1-3].The ICH guideline Q1A (R2) emphasizes that the testing of those features which are susceptible to change during storage and are likely to influence quality, safety and efficacy, must be done by validated stability indicating testing method. As per Q1 (R2) information on the stability of the drug substance is an integral part of the systematic approach to stability evaluation. Stress testing of the drug substance can help identify the likely degradation products, which can in turn help establish the degradation pathways and the intrinsic stability of the molecule and validate the stability indicating power of the analytical procedures used. The nature of the stress testing will depend on the individual drug substance and the type of drug product involved. Stress testing is likely to be carried out on a single substance. The main objective was to develop a suitable method of analysis which is stability indicating and to get an idea of how drug substance or product degrades, degenerates and behaves under changing conditions. By keeping all this in view, it was thorough worthwhile to develop stability indicating HPLC method**.** Literature survey reveals some chromatographic method has been used for determination by polarographic and in biological fluid by LC-MS/MS[4-6]. Since, article on method has not been reported for the estimation of Cinitapride as bulk drug. The present paper aims at reporting an isocratic RP-HPLC method for the estimation of Cinitapride as bulk drug.



Experimental

Working standard of cinitapride hydrogen tartrate and glimepride was obtained from Cadila healthcare Limited, Ankleshwar, India. Acetonitrile of HPLC grade were used of J.T. Baker Potassium dihydrogen phosphate and tri ethylamine of AR grade were obtained from Merck.

Instrumentation and chromatographic conditions

High performance liquid chromatography, Water Alliance separation module 2695, equipped with auto sampler injector, ultra-violet detector Water 2487 with Empower software. Chromatographic separation was achieved using Intersil ODS-3 column (150 mm x 4.6 mm, 3 µm) analytical column. The mobile phase consisting of acetonitrile and 0.1 M potassium dihydrogen phosphate buffer, pH adjusted to 6.7 with triethylamine in the ratio (70:30 v/v).The flow rate was maintained at 1.0 mL/min and the measurement were made at 260 nm. The column and auto sampler were kept at ambient temperature.

Preparation of standard stock solution

The stock solution of cinitapride (100 µg/mL) was prepared by dissolving 13.80 mg cinitapride hydrogen tartrate sample (99.7%) which is equivalent to 10.00 mg cinitapride with mobile phase in a standard 100 mL volumetric flask. The stock solution of glimepride (100.0 µg/mL) was prepared by dissolving 10.01 mg of glimepride as internal standard (99.5%) with mobile phase in a standard 100 mL volumetric flask.

Preparation of sample solution

Accurately weighed cinitapride hydrogen tartrate sample, which is equivalent to 10.00 mg of cinitapride with mobile phase was dissolved in a standard 100 mL volumetric flask. Further 3.0 mL of cinitapride drug and 5.0 mL of internal standard (100 µg/ mL) with mobile phase was diluted in a standard 10.0 mL volumetric flask.

Forced degradation studies (stress testing)

The drug concentration for all stress studies was taken 1 mg/mL as per standard literature. The bulk drug was subjected to alkaline studies by adding 1.0 mL of the 1 N NaOH for 12 hours and neutralized with 1.0 mL of 1 N HCl acid. Similarly, the acidic studies was performed by adding 1.0 mL of 1 N HCl for 12 hours and neutralized with 1.0 mL of 1 N NaOH. Oxidation studies was performed on bulk drug by adding 1.0 mL of 5 % H_2O_2 for 12 hours and reduction studied was performed on bulk drug by adding 1.0 mL 1 % sodium borohydrate for 12 hours. Bulk drug was subjected to heat at 80 ºC for 12 hours. All samples were taken in different 10 mL volumetric flasks and dissolved in mobile phase. Final assay drug concentration and external internal standard was made up with mobile phase and injected in the chromatographic system. All stressed sample were analyzed by developed HPLC method[7-13].

Method validation

Validation of developed analytical method was performed as per ICH guideline Q2 B, over the linearity, accuracy, precision, specificity, limit of detection, limit of quantitation[7-13].

Results and Discussion

Analysis of stressed samples

The ICH stability guideline Q1A (R2) defines stress testing for new drug substances and drug products, to elucidate the intrinsic stability of the drug substances and drug products. The stress testing may also provide information about degradation pathways and selectivity of the applied analytical method. The drug was found to degrade extensively on reduction (Figure 2) and in acidic medium 1 N HCl.(Figure 3). Mild degradation was observed, when exposed to oxidation (Figure 1), basic (Figure 4) and thermal condition (Figure 5). Degradation % assay value given in Table 1.

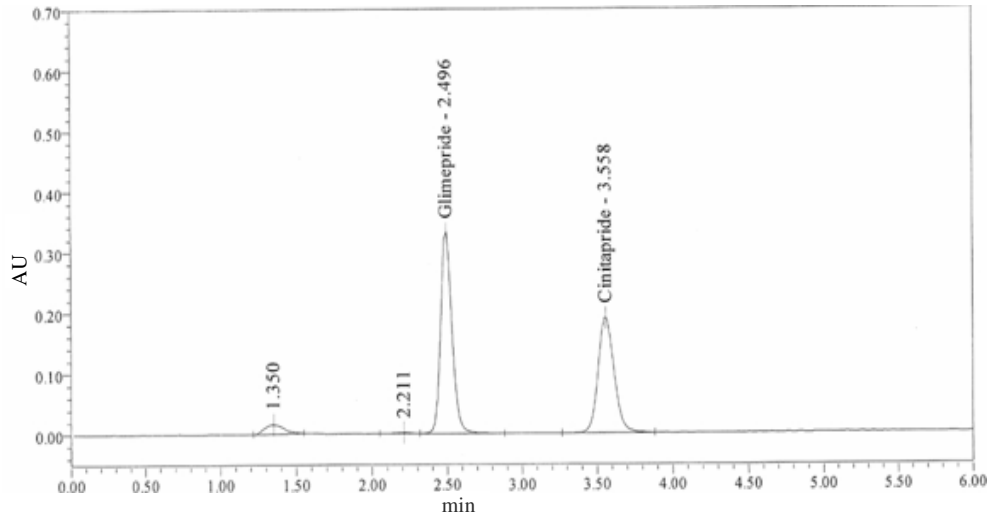


Figure 1. HPLC Chromatogram representing degradation behavior of cinitapride in oxidation.

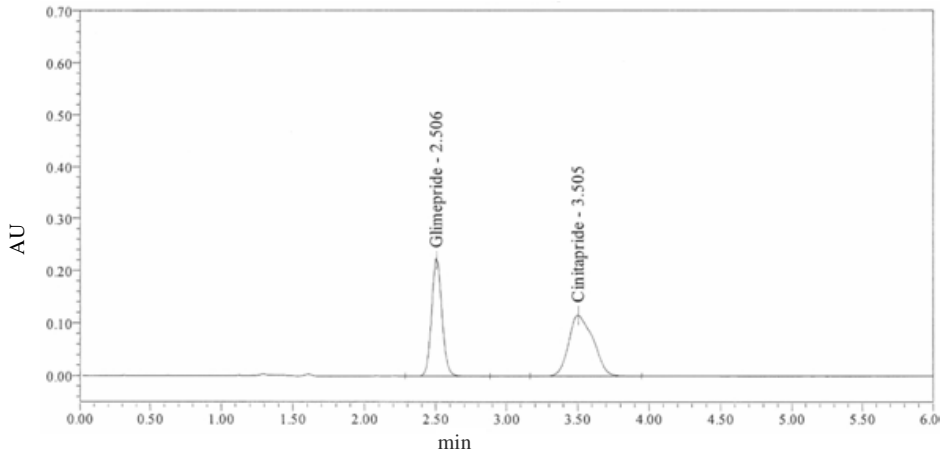


Figure 2. HPLC Chromatogram representing degradation behavior of cinitapride in reduction.

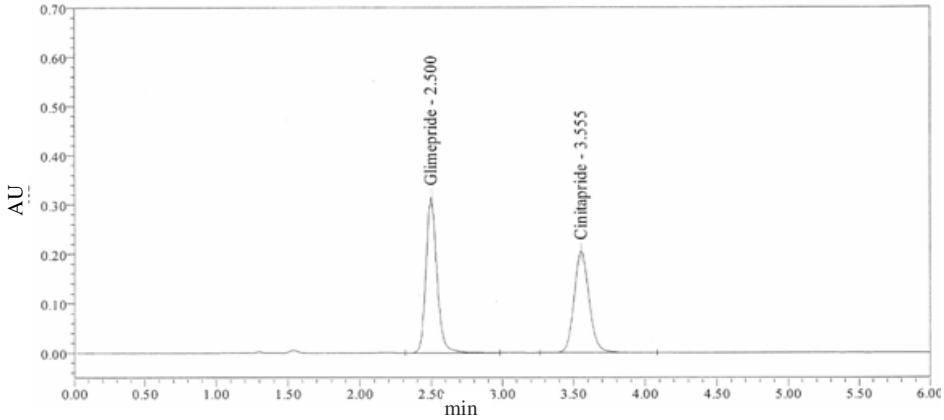


Figure 3. HPLC Chromatogram representing degradation behavior of cinitapride in acid.

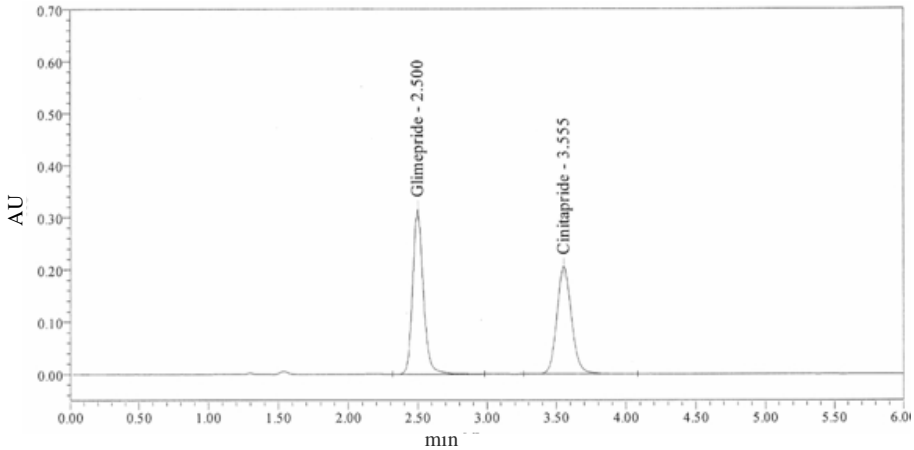


Figure 4. HPLC Chromatogram representing degradation behavior of cinitapride in base.

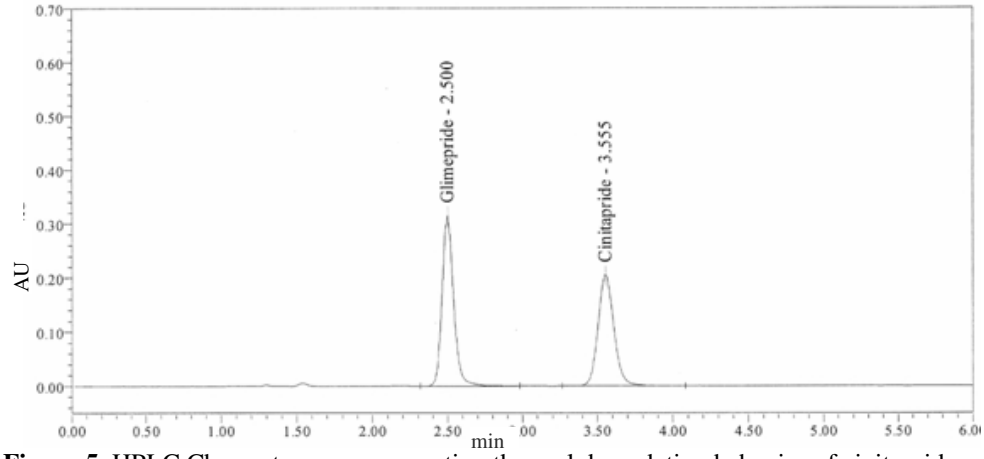


Figure 5. HPLC Chromatogram representing thermal degradation behavior of cinitapride.

Table 1. Degradation % assay value.

Stress conditions	% assay of drug after exposed to stress condition	% of degradation impurity observed	% assay (impurity observed+% assay after exposed
Acidic-12 h	95.2	5.5	100.7
Basic-12 h	96.2	4.4	100.7
Oxidation-12 h	99.6	1.0	100.7
Reduction-12 h	92.1	8.6	100.7
Thermal -12 h	99.3	1.3	100.7

Linearity

Several aliquots of standard stock solution (1.25, 1.5, 2.4, 3.0, 3.6, and 4.5 mL; 1 mL= 100 µg mL^{-1}) of cinitapride drug and 5.0 mL glimepride internal standard were taken in different six standard 10.0 mL volumetric flasks and diluted up to mark with mobile phase. Evaluation was performed with UV detector at 260 nm. The peak area was recorded for all the peaks and calibration graph was obtained by plotting peak area ratio *versus* concentration of cinitapride. The plot of peak area ratio against concentration of cinitapride was found to be linear in the range of 12.5 to 45 µg/ mL with correlation coefficient of 0.9978.The respective slope, intercept and correlation coefficient given in Table 2.

Limit of detection (LOD) and limit of quantitation (LOQ)

LOD and LOQ were determined from standard deviation and slope method as per ICH guideline, for cinitapride LOD was found to be 2.841 µg/ mL and LOQ was found to be 8.610 µg/ mL. The respective limit of detection, limit of quantitation and LOQ level precision is given in Table 3.

Accuracy

To study accuracy of the method, recovery experiment was carried out by spiked concentrations. A known quantity of drug substance corresponding to 80, 100 and 120% was spiked, to determine accuracy (recovery) against 100% working standard. The accuracy was expressed as the percentage of analytes recovered by the assay. The results indicate the method is highly accurate for determination of the cinitapride. Table 4 lists the recoveries of the drugs from a series of spiked concentrations.

Table 2. The calibration graph peak area ratio, slope, intercept and correlation coefficient.

Concentration in µg /mL	Area		Area ratio
	Cinitapride	Glimepride	
12.55	738395	993241	0.743
15.06	884819	995672	0.889
24.1	1192660	999956	1.193
30.12	1489744	994231	1.498
36.14	1770422	997862	1.774
45.18	2208769	992456	2.226
Correlation coefficient			0.9978
Slope			0.0446
Intercept			0.1741

Table 3. Results of LOD and LOQ.

Concentration in µg/mL	Area
12.55	738395
15.06	884819
24.10	1192660
30.12	1489744
36.14	1770422
45.18	2208769
Correlation	0.9981
Slope	44302.352
SD	38146.214
LOD	2.841
LOQ	8.610
% RSD for LOQ level	4.52

Table 4. Results of the study accuracy.

Level %	Spiked conc., ppm	Recovered conc., ppm	% Recovery	Mean	S.D.	% RSD
80	24.10	24.39	101.21	101.21	0.29	0.29
		24.32	100.92			
		24.46	101.51			
100	30.12	30.68	101.86	101.08	1.78	1.76
		29.83	99.04			
		30.82	102.33			
120	36.14	36.62	101.31	100.66	0.81	0.81
		36.05	99.75			
		36.48	100.92			

Precision

Repeatability was studied by carrying out system precision and method precision. System precision was determined from results for six replicate injections of the system suitability standard solutions. The relative standard deviations were less than 2%. Method precision was determined by estimated the percentage assay of Cinitapride. The relative standard deviation was 0.83.Refer Table 5.

Table 5. Results of the study of relative standard deviation.

S.No.	% Assay
1	100.29
2	101.72
3	101.63
4	99.84
5	100.55
6	99.88
Mean	100.65
SD	0.83
%RSD	0.83

Specificity

Specificity is the ability to assess unequivocally the analytic in the presence of components that may be expected to be present. Typically, these might include impurities, degradedness, *etc*. injected individually and combined mixture of cinitapride and glimepride as internal standard (Figure 6-8). The method was said to be specific, there should not any interference at retention time of cinitapride and glimepride as internal standard in blank mobile phase solution.

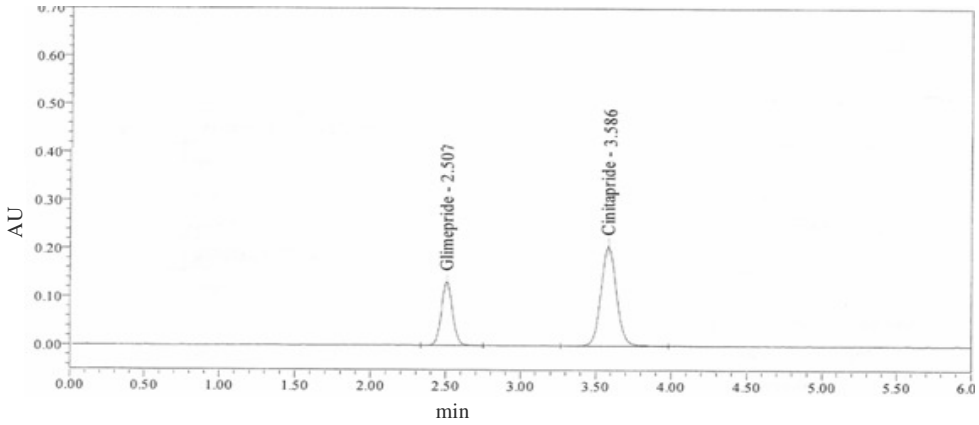


Figure 6. Specificity chromatogram of cinitapride and glimepride (Internal standard).

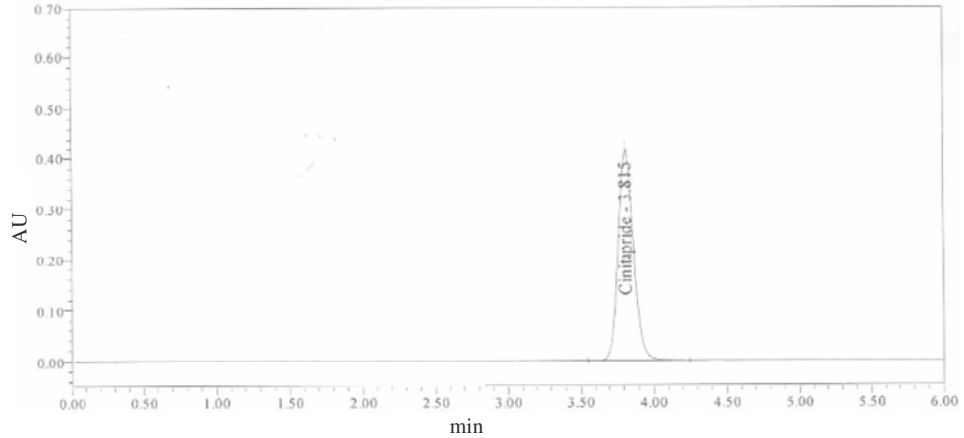


Figure 7. Specificity chromatogram of cinitapride.

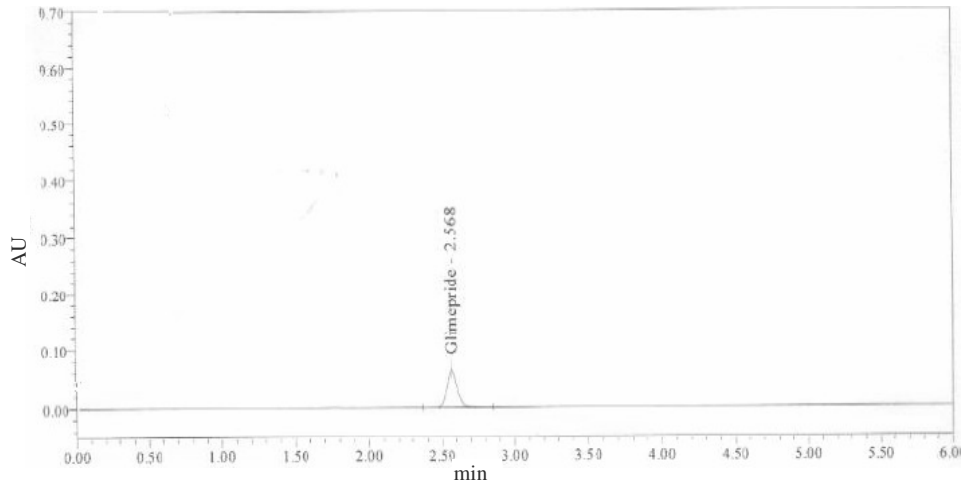


Figure 8. Specificity chromatogram of glimepride (Internal standard).

System suitability

System suitability testing is an integral part of analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analysed constitute an integral system that can be evaluated as such. System suitability testing is performed to ensure system performance before and during the analysis, which demonstrates that the system is operating properly and is ready to deliver results with acceptable accuracy and precision. System suitability tests were performed as per the USP 31 to confirm the suitability and reproducibility of the system. The test was carried out by injecting 10 µL six replicate injections of standard solutions of cinitapride and glimepride as internal standard of 30.0 and 50.00 µg mL^{-1} respectively. The relative standard deviation values were found to be satisfactory and meeting the requirements of USP 31. Theoretical plates, resolution, tailing factor were determined and are presented in Table 6.

Table 6. Theoretical plates, resolution, tailing factor.

Replicate n=6	Area ratio	Tailing factor		Resolution
		Cinitapride	Glimepride	
Mean	2.122	1.08	1.08	6.44
% RSD	0.83			

Conclusion

A stability indicating reversed phase liquid chromatograph was developed for determination of Cinitapride during stability testing as per ICH recommended stress studies. Composition of mobile phase as well pH value of mobile phase was played important role in method development**.** Several mobile phases such as phosphate buffer-methanol/acetonitrile in different ratios were tried at different pH value. It was observed that cinitapride was less retained in acidic pH valve, while glimepride was more retained in acidic pH value. Finally, good peak shape and good resolution between Cinitapride and Glimepride were observed using the mobile phase mentioned in chromatographic conditions. Forced degradation studies revealed that possible degradation products do not interfere with the determination of cinitapride. The developed method after being completely validated showed satisfactory data for all the method validation parameters. The method was found to be specific. The low

values of relative standard deviation for method precision suggested that the method is precise. Linearity evaluated for the analyte peak showed a good linear response over a wide range of concentration. The proposed reversed phase liquid chromatographic method was validated over the linearity, precision, accuracy and specificity, proved to be convenient and effective for the determination of cinitapride during stability testing of the active substances. Moreover, the lower solvent consumption along with the short analytical run time of 6.0 min leads to cost effective chromatographic method.

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