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Development and validation of HPTLC method for simultaneous estimation of cinitapride and omeprazole in combined dosage form

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ABSTRACT

A simple, precise, specific and accurate high performance thin layer chromatographic method has been developed for the simultaneous determination of Cinitapride (CNT) and Omeprazole (OMZ) in pharmaceutical dosage form. The separation was carried out on Merck HPTLC aluminum plates of silica gel G60 F254, (20 × 10 cm) with 250 µm thickness using chloroform : ethyl acetate: methanol (7.3: 2: 0.7, v/v/v) as mobile phase. HPTLC separation of the two drugs followed by densitometric measurement was carried out in the absorbance mode at 277 nm. The drugs were resolved satisfactorily with R_f values of 0.46 ± 0.01 and 0.68 ± 0.01 for CNT and OMZ, respectively. The linear regression analysis data for the calibration plots showed good linear relationship with R^2=0.999 and 0.999 for CNT and OMZ, respectively in the concentration range of 30-180 ng/spot for CNT and 200-1200 ng/spot for OMZ. The method was validated for accuracy, precision, specificity and robustness. The limit of detection and quantitation were 2.79 and 8.46 ng/spot, respectively for CNT and 18.22 and 55.1 ng/spot, respectively for OMZ. The proposed developed HPTLC method can be applied for identification and quantitative determination of OMZ and CNT in bulk drug and drug formulation.

Keywords: cinitapride; omeprazole; HPTLC; validation.

INTRODUCTION

Omeprazole is substituted benzimidazole (RS)-6- methoxy-2-((4-methoxy-3,5dimethylpyridin -2-yl) methyl sulfinyl)-1H-benzoimidazole that function as proton pump inhibitors. It is anti-secretory drug effective for rapid healing peptic ulcer and corrosive esophagitis [1-5].

Cinitapride Hydrogen Tartrate is benzamide class of drug (4–Amino–N- [1-(3 cyclohexen-1-ylmethyl)-4-piperidinyl]-2- ethoxy-5-nitrobenzamide Hydrogen L-(+)-tartrate) that function as pro-kinetic agent and antiemetic. Combination of Omeprazole and Cinitapride is approved in 12th May - 2010 by CDSCO India and manufactured & marketed by Zydus Cadila Healthcare as Burpex Capsule with dosage regimen of 20 mg Omeprazole and 3 mg Cinitapride Hydrogen Tartrate. Combination is used in treatment of gastric ulcer, gastro esophageal reflux disease (GERD) & Dyspepsia not responding to omeprazole alone.

Omeprazole is official in Indian Pharmacopoeia, British Pharmacopoeia, US Pharmacopoeia [1-3] while Cinitapride is not official in any pharmacopoeia. Deep literature survey reveals that numbers of analytical methods are reported for the estimation of Omeprazole and Cinitapride in single dosage forms. Reported methods for estimation of Omeprazole are Spectrophotometric method[10,18-19], HPLC[11-13,20-21], HPTLC[14], Non-aqueous titration[15-16], Polarographic method[17] and similarly for estimation of Cinitapride are Spectrophotometric method[22], Colorimetric method[23], RP-HPLC[24], UHPLC[25], LC-MS/MS[26] and First Derivative Spectrophotometric Method[27].

But, there is no any analytical method has been reported yet for combinaton of these drugs. There for the present research work aims to develop a simple, sensitive, accurate and reproducible method for simultaneous estimation of Cinitapride and Omeprazole in combined dosage form by HPTLC method.

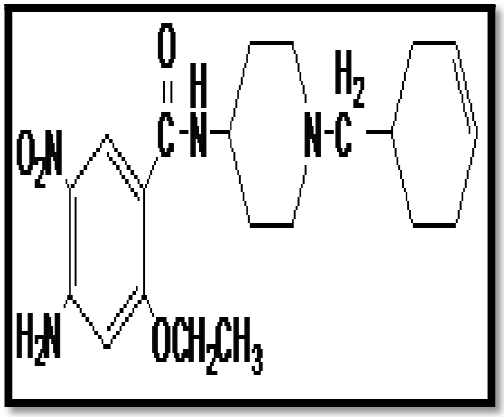

Fig. 1: Structural of cinitapride

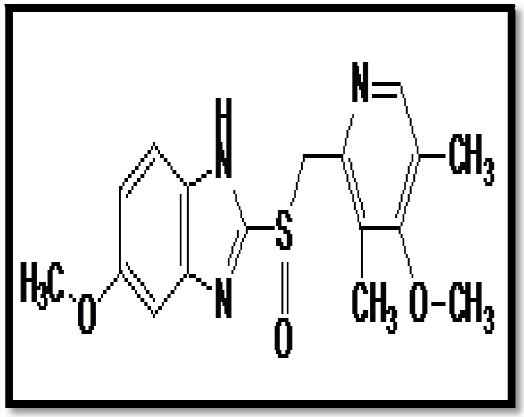

Fig. 2: Structural of omeprazole

MATERIALS AND METHODS

2.1. Materials:

Working standards of pharmaceutical grade OMZ (99.80 %, w/w) and CNT (99.9 %, w/w) were obtained as gift samples from Galpha laboratories ltd. Ankleshwer and Symed labs ltd., Hyderabad. All chemicals and reagents of analytical grade were purchased from Merck Chemicals, Mumbai, India.

2.2. Selection of analytical wavelength:

Stock solutions of drugs were prepared in methanol separately. UV spectrum of 100µg/mL of individual drug was taken. Further, in situ HPTLC spectral overlain of OMZ and CNT was taken as depicted in figure no.3

2.3. Instrumentation and chromatographic conditions:

The HPTLC plates were prewashed with methanol and activated at 110 ^{o}C for 5 min prior to chromatography. The samples were spotted in the form of bands 6 mm width with a Camag 100 microlitre sample syringe (Hamilton, Bonaduz, Switzerland) on silica gel precoated HPTLC aluminum plate 60 F254, [(20 × 10 cm) with 250 µm thickness; E. Merck, Darmstadt, Germany, supplied by Anchrom Technologies, Mumbai] using a Camag Linomat 5 applicator (Switzerland). A constant application rate of 0.1µL/sec was used and the space between two bands was 7 mm. Linear ascending development was carried out in 20 cm × 10 cm twin trough glass chamber (Camag, Muttenz, Switzerland) saturated with the mobile phase. The mobile phase was consisted of chloroform: ethyl acetate: methanol (7.3: 2: 0.7, v/v/v) and 20 mL was used per chromatography run. The optimized chamber saturation time with mobile phase was 30 min using saturation pads at room temperature (25 ^{o}C ± 2). The length of chromatogram run was 80 mm and run time was 20 min. Densitometric scanning was performed using a Camag TLC scanner III in the reflectance-absorbance mode and operated by winCATS software (V1.1.4, Camag). The slit dimension was kept at 5mm × 0.45 mm and the scanning speed was 10 mm/sec. The source of radiation used was a deuterium lamp emitting a continuous UV spectrum between 200 and 400 nm. All determinations were performed at ambient temperature with a detection wavelength of 277 nm. Concentrations of the compound chromatographed were determined from the intensity of the diffused light. Evaluation was by peak areas with linear regression.

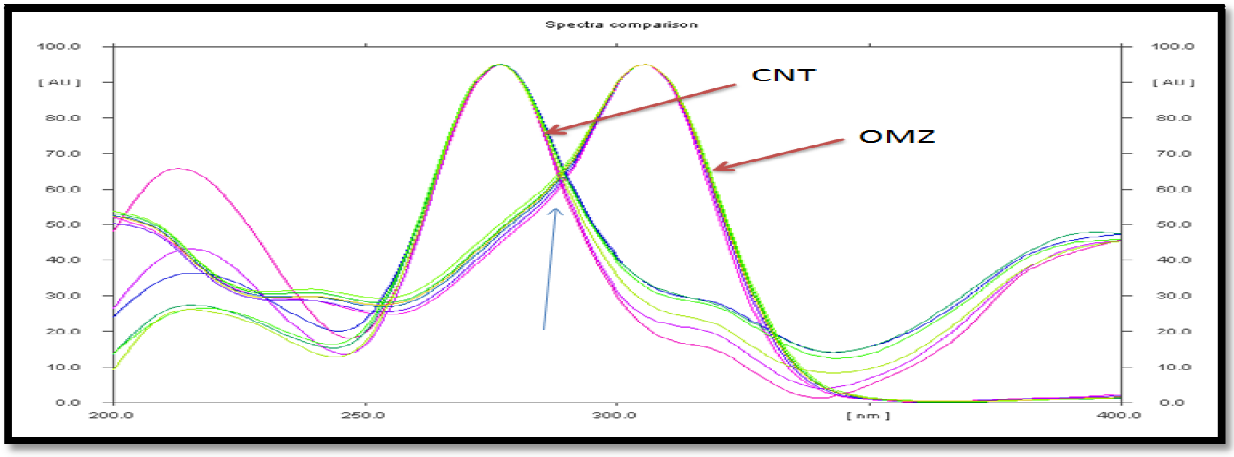

Figure 3: Overlain spectra of comparison of CNT and OMZ

2.4. Standard solutions and calibration graphs:

Mixed stock standard solution containing 30 µg/mL of CNT and 200 µg/mLof OMZ was prepared in methanol by dissolving 3 mg of CNT and 20 mg of OMZ in 100 mL methanol. Mixed stock standard solution was further diluted with methanol to obtain working standard solutions in a concentration range of 30-180 ng/spot for CNT and 200-1200 ng/spot for OMZ. Each concentration was applied six times on the HPTLC plate. The plate was then developed using the previously described mobile phase. The peak areas were plotted against the corresponding concentrations to obtain the calibration graphs. Linear calibration curves were generated using least-squares linear-regression analysis.

2.5. Sample preparation:

Twenty Tablets were weighed and finely powdered. The powder equivalent to 20 mg (OMZ) of tablet formulation were accurately weighed and transferred to volumetric flask of 50 mL capacity. 15mL of methanol transferred to volumetric flask and sonicated it for 5 mins. The flask was shaken and volume was made up to the mark with methanol.

The above solution was carefully centrifuged at 4000 rpm for 15 min. It was filtered through vacuum filter using Whatman filter paper (No.41). The aliquot (5 mL) was transferred into 10 mL volumetric flask. and volume was made up to the mark with methanol to give a solution containing 30 µg/mL of CNT and 200 µg/mL of OMZ. The plate was developed in the previously described chromatographic conditions. The peak area of the spots were measured at 277 nm for OMZ and CNT, respectively and the concentrations in the samples were determined using multilevel calibration developed on the same plate under the same conditions using linear regression equation and amount of drug found were calculated in terms of % Assay as depicted in Table no.2

2.6 VALIDATION PARAMETER OF THE DEVELOPED METHODS:-

2.6.1 Linearity

The linearity of analytical method is its ability to elicit test results that are directly proportional to the concentration of analyte in sample within a given range. The range of analytical method is the interval between the upper and lower levels of analyte that have been demonstrated to be determined within a suitable level of precision, accuracy and linearity.

Standard mixture solution of CNT and OMZ having concentration of 30 to180 ng/spot for CNT and 200 to 1200 ng/spot for OMZ were spotted and developed as described in proposed method. Developed plates were subjected to densitometric measurements in absorbance mode at wavelength 277.0 nm using Camag TLC Scanner 3 and calibration curve shown in figure no. 4 & 5.

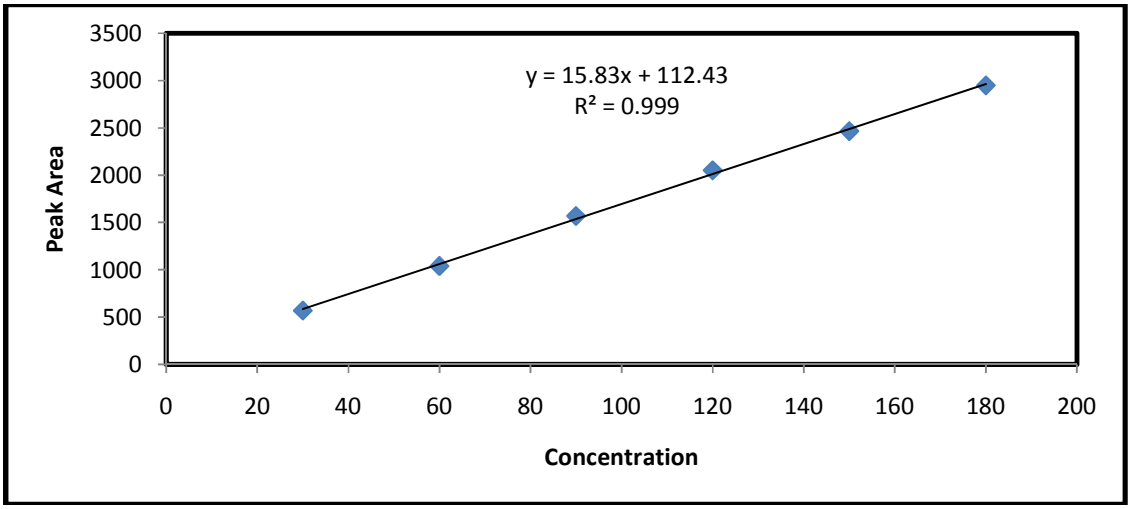

Figure 4: Calibration curve for Cinitapride

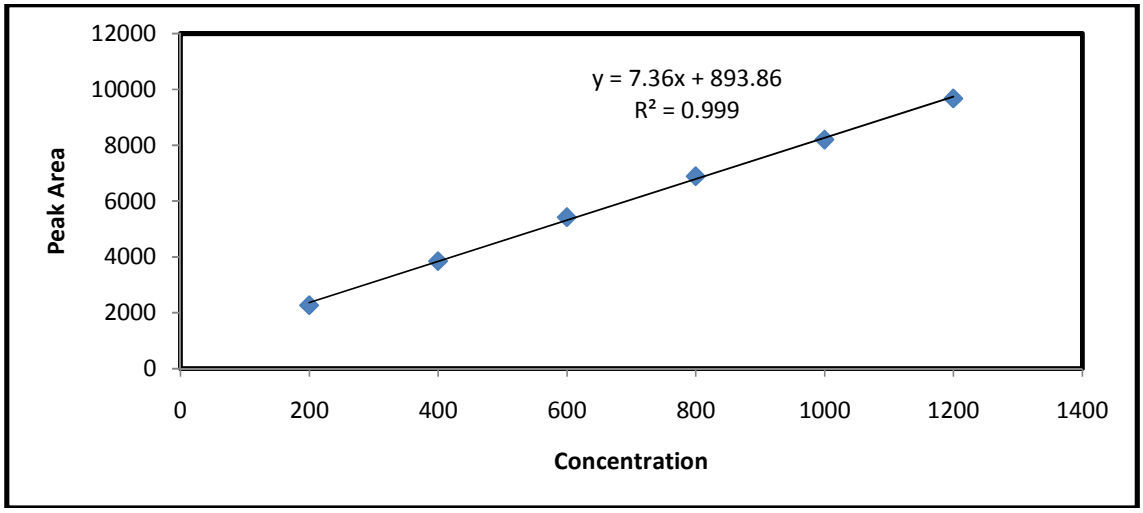

Figure.5: Calibration curve for Omeprazole

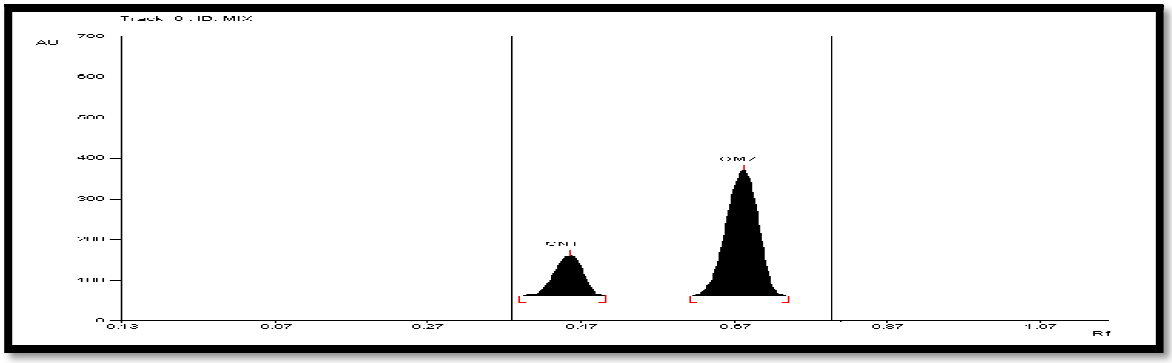

Figure 6: Chromatogram of Standard mixture Omeprazole and Cinitapride in Chloroform : Ethyl acetate : Methanol (7.3: 2 : 0.7 v/v/v)

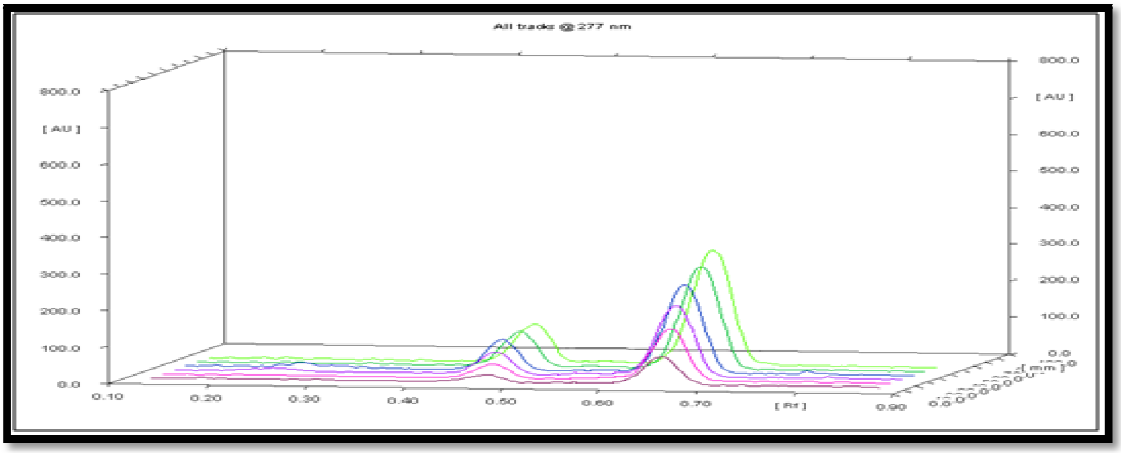

Figure 7: Three Dimension Chromatogram of calibration curve.

2.6.2 Precision

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of homogenous samples. It provides an indication of random error results and was expressed as coefficient of variation(CV).

Intra and inter day precision

Intraday and interday precision was determined in terms of % RSD. Intraday precision was determined by analyzing in combined solution their respective calibration range for five times in the same day. Interday precision was determined by analyzing CNT and OMZ in for five days.

⇒**Procedure for Intraday Precision:** From combined standard solution of CNT and OMZ 60+400ng/spot, 120+800 ng/spot, 180+1200 ng/spot were spotted 5 times on same day and developed as described in proposed method. Developed plates were subjected to densitometric measurements in absorbance mode at wavelength 277.0 nm using Camag TLC Scanner 3 and % RSD was calculated.

⇒ **Procedure for Interday Precision:** From combined standard solution of CNT and OMZ 60+400ng/spot, 120+800 ng/spot, 180+1200 ng/spot were spotted 5 times on different days and developed as described in proposed method. Developed plates were subjected to densitometric measurements in absorbance mode at wavelength 277.0 nm using Camag TLC Scanner 3 and % RSD was calculated.

2.6.3 Accuracy

Accuracy may often be expressed as percentage recovery. It was determined by calculating the recovery of CNT and OMZ by application of the analytical method to mixtures of the drug product contents to which known amount of analyte have been added within the range ofthe method.

Tablet formulation solution:-

Weigh accurately 200 mg equivalent weight of tablet powder and dissolved in 50 mL of methanol to get concentrations 600 µg/mL of CNT and 4000 µg/mL of OMZ. from this solution 1 mL was transferred into a series of 10 ml volumetric flask i.e. labeled as 50%, 100%, 150%.

Standard stock solution:-

Accurately weigh 100 mg of OMZ and 15 mg of CNT standard were transferred to 50 mL of volumetric flask. The volume was adjusted to the mark with methanol. Thus final solution of mixture of CNT and OMZ obtained contain 300+2000 µg/mL. from this solution 1.0 mL, 2.0 mL, 3.0 mL were transferred into a series of above 10 ml volumetric flask. Samples were spotted and developed as described in proposed method. Developed plates were subjected to densitometric measurements in absorbance mode at wavelength 277.0 nm using Camag TLC Scanner 3.The mean % recovery from of peak areas calculated.

2.6.4 Specificity
Specificity of the method was determined by means of complete separation of pure drugs in the presence of other excipients normally present in the formulation. The specificity of the method was ascertained by peak purity profiling studies. Peak purity of CNT and OMZ was assessed by comparing their respective spectrum at peak start (S), peak apex (M) and peak end (E) position of the spots as shown in Table no. 3. The peak purity was determined on winCATS software using statistical equation.

2.6.5 Selectivity
Selectivity is the procedure to detect qualitatively the analyte in presence of components that may expected to be present in the sample matrix. Commonly used excipients present in selected tablet formulation were spiked into a preweighed quantity of drugs. The absorbance was measured, and calculations determined the quantity of the drugs as shown in table no.3

2.6.6 Limit of Detection (LOD)
The L.O.D. was estimated from the set of 5 calibration curves.
LOD = 3.3 × (S.D./Slope)
Where,
S.D. = Standard deviation of the Y- intercepts of the 5 calibration curves.
Slope = Mean slope of the 5 calibration curves.
LOD of CNT and OMZ is described in table 1

2.6.7 Limit of Quantification (LOQ)
The L.O.Q. was estimated from the set of 5 calibration curves.
LOQ = 10 × (S.D./Slope)
Where,
S.D. = Standard deviation of the Y- intercepts of the 5 calibration curves.
Slope = the mean slope of the 5 calibration curves.
LOQ of CNT and OMZ is described in table 1

2.6.7 Robustness
The robustness of the method was established by introducing small changes in mobile phase composition and chromatograms were run. The amount of mobile phase, chamber saturation time, time from spotting to chromatography and from chromatography to scanning (±10 min) and development distance from spot application was varied in the range of ± 5%. The The robustness of the method was determined at three different concentration levels of 30, 60, 90 ng/spot for CNT and 200, 400, 600 ng/spot for OMZ and %RSD calculated as shown in Table no.1

RESULTS AND DISCUSSION

The TLC procedure was optimized with a view to develop an assay method for the simultaneous estimation of CNT and OMZ. The standard solutions of both the drugs were spotted on the TLC plates and run in different solvent systems. The mobile phase consisting of chloroform: ethyl acetate: methanol (7.3: 2: 0.7, v/v/v) gave sharp and symmetrical peaks with the Rf values of 0.46 ± 0.01 and 0.68 ± 0.01 for CNT and OMZ, respectively. Well defined spots were obtained when the chamber was saturated with mobile phase for 20 min at room temperature (27 ± 3^0C). A combined densitogram of mixed standards and 3-D chromatogram showing peaks of CNT and OMZ in different concentrations at 277 nm are depicted in Figure 6 and Figure 7, respectively.

TABLE 1: Regression analysis data and summary of validation parameter for the proposed method

Sr. No.	Parameters	Results	
		Cinitapride	Omeprazole
1	Linearity Range	30-180 ng/spot	200-1200 ng/spot
2	Regression equation	y = 15.83x + 112.4	y = 7.36x + 893.8
3	Correlation coefficient (R^2)	0.999	0.999
4	% Recovery	98.80 – 100.74	99.14 – 100.88
5	Precision (%RSD) Interday	0.50 – 0.65	0.28 – 0.49
	Intraday	0.59 – 0.82	0.57 – 0.87
6	LOD (ng/spot)	2.79	18.22
7	LOQ (ng/spot)	8.46	55.21
8	Robustness (%RSD)	0.52 – 0.92	0.19 – 0.65

Table 2: Analysis of marketed formulations

Capsule	Label claim (mg)	Amount of drug found ± RSD (%) n=6	
	CNT : OMZ	* CNT	* OMZ
(BURPEX)	3:20	99.93±1.28	100.06±0.77

Table 3 : Specificity and Selectivity study

Parameter	CNT	OMZ
Specificity	99.26 %	99.16%
Peak purity	r (S, M) = 0.9998 r (M, E) = 0.9999	r (S, M) = 0.9999 r (M, E) = 0.9997
Selectivity	Selective	Selective

CONCLUSION

The developed HPTLC technique is precise, specific, robust and accurate method for analysis of OMZ and CNT in pharmaceutical preparations. Statistical analysis proves that the method is suitable for the analysis of Omeprazole and Cinitapride as bulk drug and in pharmaceutical formulation without any interference from the excipients. It may be extended to study the degradation kinetics of Omeprazole and Cinitapride and also for its estimation in plasma and other biological fluids. The proposed TLC method is less expensive, simpler, rapid, and more flexible than HPLC.

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