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DEVELOPMENT AND VALIDATION OF FIRST ORDER DERIVATIVE SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF OMEPRAZOLE AND CINITAPRIDE IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

The use of first order derivative spectrophotometry allowed simultaneous estimation of Omeprazole and Cinitapride, at their respective zero crossing point (ZCP) in fixed dose combination products. The absorbance values at 263.2 nm (ZCP of cinitapride) and 253.8 nm (ZCP of omeprazole) of first derivative spectrum were used for the estimation of Omeprazole and Cinitapride respectively without mutual interference in methanol. The method was found to be linear (r2>0.9995) in the range of 7-42 µg/ml for Omeprazole. The linear correlation was obtained (r2>0.9992) in the concentration range of 1-6 µg/ml for Cinitapride. The method was validated for as per ICH Q2 (R1) guidelines. The limit of determination was 0.06µg/ml and 0.33µg/ml for Omeprazole and Cinitapride, respectively. The limit of quantification was 0.18µg/ml and 1.00µg/ml for Omeprazole and Cinitapride, respectively. The proposed method is simple, rapid, precise and accurate and hence can be successfully applied for the simultaneous determination of Omeprazole and Cinitapride in formulations.

KEYWORDS : ***Omeprazole, Cinitapride, Derivative Spectrophotometry, Zero crossing point, Pharmaceutical Dosage form.***

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INTRODUCTION

Gastroesophageal reflux disease (GERD) or No- ulcer dyspepsia (NUD) is a recurrent and chronic disease for which long-term medical therapy is usually effective. It is important to recognize that chronic reflux does not resolve itself. There is no cure for GERD yet. Long-term and appropriate treatment is necessary with essential drugs that include proton pump inhibitors and gastroprokinetic agents[1]. Omeprazole (OMZ),5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl) methyl] sulphinyl]-1*H*-benzimidazole(Fig.1a),a substituted benzimidazole compound and prototype anti-secretary agent, is the first of the "proton pump inhibitors" widely used for the prophylaxis and treatment of gastro-duodenal ulcers and for the treatment of symptomatic gastro-esophageal reflux disease[2]. Cinitapride (CNT),4-amino-N-[1-(cyclohex-3-en-1-ylmethyl)piperidin-4-yl]-2-ethoxy-5-Nitrobenzamide (Fig 1b), is a substituted benzamide gastroenteric prokinetic agent acting via complex, but synergistic effects on serotonergic 5-HT_2 (inhibition) and 5-HT_4 (stimulation) receptor and dopaminergic D_2 (inhibition) receptors in the neuronal synapses of the myenteric plexus, a stimulating gastrointestinal moiety agent and commercially successful anti-ulcerative drug substance[3,4]. Combination of these two drugs into fix dose combination (FDC_S) has been essential constituent of Gastroesophageal reflux disease (GERD) or No- ulcer dyspepsia (NUD) therapy.

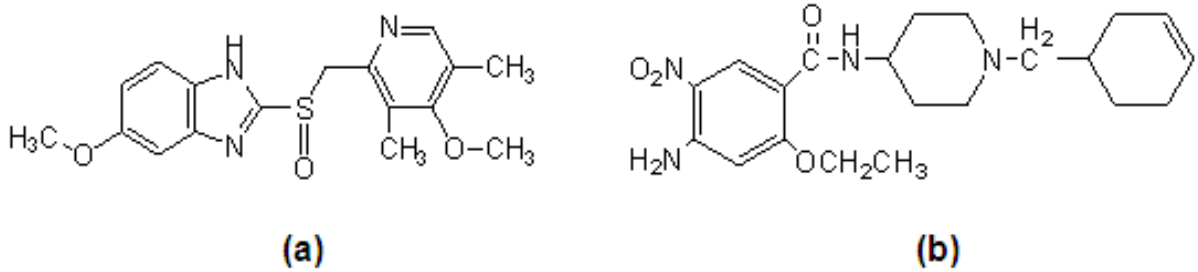

Figure 1
Chemical structures of (a) omeprazole (b) cinitapride

A literature survey reveals that there are a number of various analytical methods available for the quantitative individual determination of omeprazole, or combination with other drugs, mainly using chromatographic methods such as HPLC[5,7], RP-HPLC[6,7], TLC[8,9],HPTLC[9] and other methods include Spectrophotometric method,[2,10,11], Polarographic method[12]. On the other hand, various analytical methods available for the quantitative individual determination of cinitapride, or combination with other drugs are RP-HPLC[13], LC-MS/MS[14], Spectrophotometric method.[15,16,17] No spectrophotometric method has been reported in the literature and no official or reported procedure is present for the simultaneous determination of omeprazole and cinitapride in their commercial formulations. It would therefore be beneficial to provide accurate, precise and reliable methods for determining for simultaneous determination of omeprazole and cinitapride in commercial formulations. The present work describes analytical procedures for the quantitation of omeprazole and cinitapride commercial formulation on basis of zero-crossing measurement with using derivative spectroscopy.

MATERIALS AND METHODS

(i) Chemicals and reagents

The bulk drug of Omeprazole and cinitapride hydrogen tartrate were obtained as gift sample from Galpha laboratories Ltd., (Ankleshwar, India) and Symed labs Ltd., (Hyderabad, India),respectively. The

commercial fixed dose combination product BURPEX (omeprazole 20 mg, cinitapride hydrogen tartrate 3 mg) was procured from the local market. Methanol AR Grade was used as solvent.

(ii) Instrumentation

A double beam UV/Visible spectrophotometer (Shimadzu model 2450, Japan) with spectral width of 2 nm, 1 cm quartz cells was used to measure absorbance of all the solutions. Spectra were automatically obtained by UV-Probe system software. An analytical balance (Sartorius CD2250, Gottingen, Germany) was used for weighing the samples. Class 'A' volumetric glassware were used.

(iii) Preparation of stock solution

An accurately weighed quantity of omeprazole (10 mg) and cinitapride hydrogen tartrate (10 mg) were transferred to a separate 100 ml volumetric flask, respectively and dissolved and diluted to the mark with methanol to obtain standard solution having concentration of OMZ (100 µg/ml) and CNT (100 µg/ml).

(iv) Validation of proposed Method

The method was validated according to International Conference on Harmonization Q2 (R1) guidelines.[18,19]

Linearity

Standard stock solutions of CNT (100 µg/ml), and OMZ (700 µg/ml) were prepared separately by dissolving 5 mg and 35 mg of drug in 50mL methanol respectively. Several aliquots of these standard stock solutions were taken in different 10ml volumetric flask and diluted up to mark with methanol, such that the final linearity concentration of CNT and OMZ were 1-6 µg/ml and 7-42 µg/ml, respectively (table 1). First-derivative absorbance (D_1) was measured at 253.8 nm for CNT and 263.2 nm for OMZ .The calibration curves for derivative were constructed by plotting absorbance versus drug concentration and the regression equation was computed(fig. 5).

Precision

The precision of the developed method was assessed in terms of repeatability (intra-day) and intermediate precision (inter-day) by analyzing three replicate of standard stock solutions at three levels that cover the calibration ranges for OMZ and CNT[20,21].The precision of the developed method was assessed by analyzing samples of the same batch in nine determinations with three concentrations (2, 4, 6 µg/ml for CNT and 14, 28, 42 for OMZ) and three replicate (n=3) each on same day for intra-day and in triplicate (n=3) per day for consecutive 3 days for inter-day precision. The % RSD value of the results corresponding to the absorbance was expressed for intra-day precision (table 2) and on 3 days for intermediate (inter-day) precision (table 3).

Robustness and Ruggedness

Robustness and Ruggedness of the method was determined by subjecting the method to slight change in the method condition, individually, the volumetric flask (10 ml, 50 ml and 100 ml), Change in instrument (UV-Vis Spectrophotometer model 1800 and 2450), and analyst. Three replicates were made for the same concentration (6 µg/ml of CNT and 42 µg/ml of OMZ) in 10 ml, 50 ml and 100 ml volumetric flasks and the recording of absorbances were done on both the UV-Vis spectrophotometer. The result is expressed in % RSD (table 4).

Accuracy

The accuracy of the method, which is defined as the nearness of the true value and found value.[20,21] To study the reliability and suitability of the developed method, recovery experiments were carried out. Standard stock solutions were spiked with different amount to marketed formulation of OMZ and CNT at 80, 100 and 120%.Data from nine determinations over three concentration levels covering the specified range was determined. Recovery for pharmaceutical formulations should be within the range 100±5%. The RSD percent and percent recovery of individual measurements was also determined (table 5).

Limit of detection and quantitation

The limit of detection (LOD) is defined as the lowest concentration of an analyte that an

analytical process can reliably differentiate from background levels. The limit of quantification (LOQ) is defined as the lowest concentration of the standard curve that can be measured with acceptable accuracy, precision and variability[22]. The LOD and LOQ were calculated as LOD = 3.3σ/*S,* and LOQ = 10σ/*S,* where σ is the standard deviation of the lowest standard concentration and *S* is the slope of the standard curve (table 6).

Analysis of OMZ and CNT in combined capsule dosage form

Twenty commercial capsules were weighed, emptied in a glass mortar and powdered. An amount of powdered equivalent to 3 mg of CNT and 20 mg of OMZ was accurately weighed in a 50 ml volumetric flask. About 20 ml of methanol was added to this flask and sonicated for 10 min. and volume was made up to the mark with same solvent. The solution was filtered through Whatman filter paper No. 41 and appropriate aliquot of this standard stock solution of commercial formulation was taken into a 10 ml volumetric flask and volume was made up to mark with methanol to obtain final solution containing 3 µg/ml of CNT and 20 µg/ml of OMZ. A first order derivative spectrum of the sample solution was recorded and the absorbance at 253.8 nm and 263.2 nm were noted for estimation of CNT and OMZ, respectively. The concentration of CNT and OMZ in capsules were determined using the corresponding calibration graph.

RESULT AND DISCUSSION

Method Development

The standard solution of CNT and OMZ were scanned separately between 200-400 nm, and zero-order spectra (fig.2) were not showed overlapping peaks, thus obtained spectra was then processed to obtain first-derivative spectra. The overlain first order spectra (fig.3) of OMZ and CNT reveal that OMZ showed zero crossing at 253.8 nm, while CNT showed zero crossing at 263.2nm. At zero crossing point (ZCP) of OME (253.8 nm), CNT showed a first-derivative absorbance, whereas at ZCP of CNT (263.2 nm), OMZ showed a first-derivative absorbance. Hence 253.8 nm and 263.2 nm were selected as analytical wavelengths for determination of cinitapride and omeprazole, respectively. The First-derivative spectra (fig.4) showed linear absorbance at 253.8 nm (ZCP of OMZ) for CNT and 263.2 nm (ZCP of CNT) for OMZ. First-derivative spectra give satisfactory ZCPs and good quantitative determination of both the drugs at their respective without any interference from the excipients in their combined dosage form.

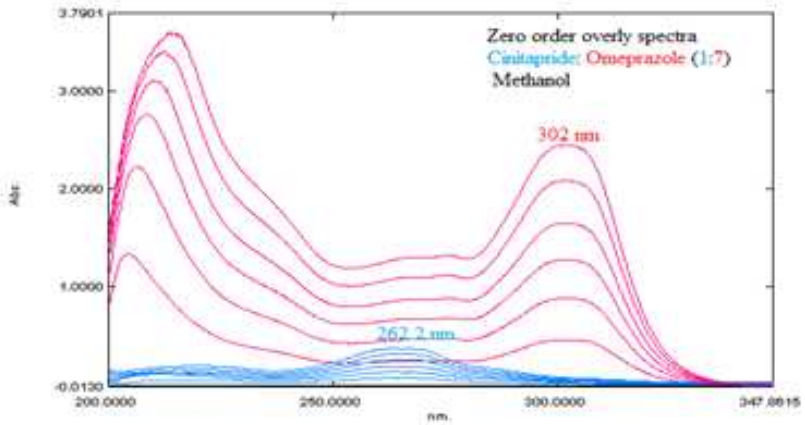

Figure 2

Overlain zero order spectra of CNT: OMZ (1:7) ratios, respectively.

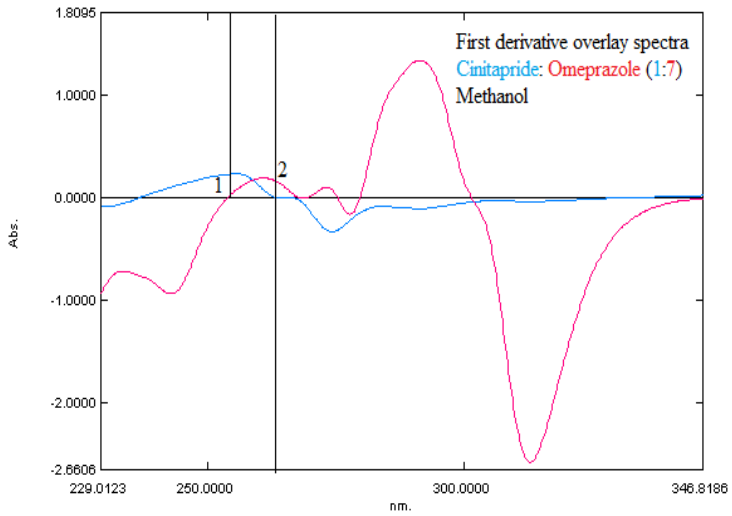

Figure.3

Overlain First order spectra of CNT (Blue) and OMZ (Red) in 1:7 ratios, respectively. (1) and (2) Zero crossing wavelength of OMZ and CNT, respectively.

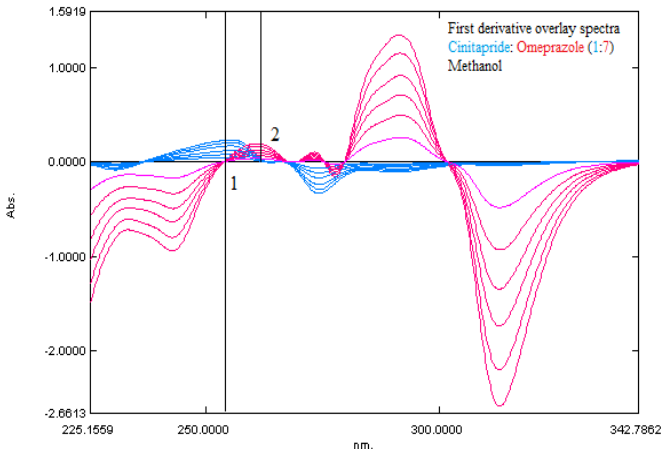

Figure.4

Overlain linear first order spectra of CNT (Blue) and OMZ (Red) in 1:7 ratios. (1) and (2) Zero crossing wavelength of OMZ and CNT, respectively.

Linearity

This method obeyed beer's law in the concentration range (CNT: 1-6 µg/ml, OMZ: 7-42 µg/ml) (table 1) with correlation coefficient (r^2) of 0.9992 and 0.9995 for CNT and OMZ, respectively (fig 5).

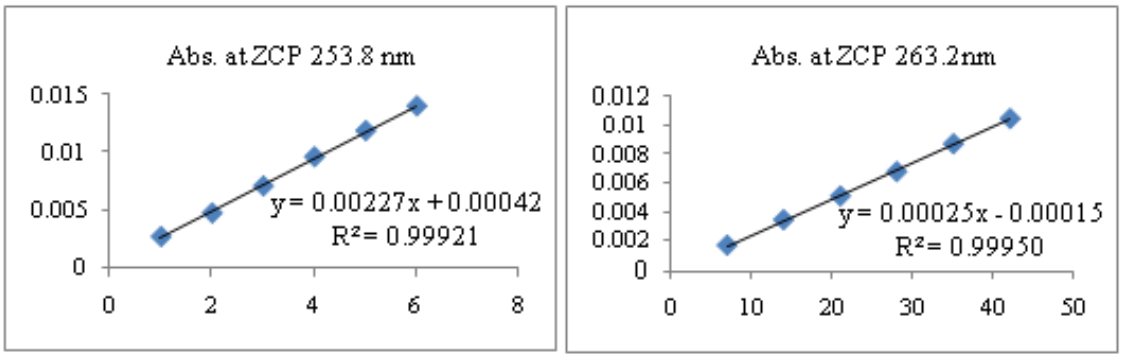

Figure 5
Calibration graph of CNT and OMZ at 253.8 nm and 263.2 nm, respectively

Table 1
Linearity data for CNT and OMZ* (n=6)

Sr. No	Concentration (CNT:OMZ) (µg/ml)	Absorbance (253.8 nm)±SD*	Absorbance (263.2 nm) ±SD*
1	1:7	0.0028±0.000150	0.00165±0.00010
2	2:14	0.00481±0.00133	0.00341±0.00133
3	3:21	0.00713±0.00015	0.00506±0.00012
4	4:28	0.00963±0.00024	0.00676±0.00013
5	5:35	0.01186±0.00025	0.0087±0.000120
6	6:42	0.01398±0.00024	0.01045±0.00016

Precision

The obtained intra-day and inter-day results are presented in table 2 and 3 respectively. The %RSD value was found to be less than ± 1.0 indicated that the method is precise.

Table 2
Intraday precision (Repeatability) *(n=3)

Conc. (µg/ml) (CNT : OMZ)	Absorbance (253.8 nm)	Avg. ± SD (253.8 nm) *	% RSD	Absorbance (263.2 nm)	Avg. ± SD (263.2nm) *	% RSD
2:14	0.00406 0.00403 0.00403	0.00404±0.00002	0.4287	0.00259 0.00266 0.00261	0.00261±0.00003	0.9029
4:28	0.0081 0.00814 0.00803	0.00809±0.00006	0.6882	0.00438 0.00436 0.00431	0.00437±0.00004	0.8288
6:42	0.01228 0.01235 0.01212	0.01225±0.00011	0.9624	0.00736 0.00745 0.00735	0.00738±0.00006	0.7456

Table 3
Interday precision (Intermediate precision) *(n=3)

Conc. (µg/ml) (CNT: OMZ)	Absorbance (253.8 nm)	Avg. ± SD (253.8 nm) *	% RSD	Absorbance (263.2 nm)	Avg. ± SD (263.2nm) *	% RSD
2:14	0.00391	0.00391±0.00002	0.3903	0.00229	0.00227±0.00002	0.6719
	0.00393			0.00227		
	0.0039			0.00226		
4:28	0.00811	0.00814±0.00005	0.6057	0.00461	0.00457±0.00004	0.7673
	0.00812			0.00458		
	0.0082			0.00454		
6:42	0.01216	0.0205±0.00011	0.9138	0.00681	0.00685±0.00004	0.5897
	0.01206			0.00686		
	0.01194			0.00689		

Ruggedness and Robustness

The obtained Ruggedness and Robustness results are presented in table 4.No significant changes in the spectrums were observed, proving that the developed method is rugged and robust.

Table: 4
Ruggedness and Robustness * (n=3)

Conc. (µg/ml) (CNT : OMZ)	Change in Condition	Avg. ± SD* (µg/ml) CNT	%RSD* CNT	Avg. ± SD* (µg/ml) OMZ	% RSD* OMZ
6+42	10†	0.0129±0.00002	0.1604	0.0082±0.00007	0.8064
	50†	0.0135±0.00001	0.7583	0.0086±0.00004	0.4168
	100†	0.0133±0.00010	0.7129	0.0085±0.00007	0.8265
	1#	0.0120±0.00004	0.3307	0.0072±0.00039	0.5484
	2#	0.0126±0.00001	0.1579	0.0006±0.00002	0.2894
	UV 2450‡	0.0132±0.00004	0.3018	0.0087±0.00004	0.4025
	UV 1800‡	0.0129±0.00002	0.1180	0.0086±0.00003	0.3719

† Volumetric flask, # Analyst, ‡ UV-Vis Spectrophotometer model

Accuracy

The % recovery values are tabulated in Table 5. Percentage recovery for CNT and OMZ by this method was found in the range of 98.58 to 101.83% and 99.73 to 102.38%, respectively, value of %RSD within the limit indicated that the method is accurate and percentage recovery shows that there is no interference from the excepients.

Table 5
Recovery data of proposed method *(n=3)

Formulation (BURPEX) (CNT+OMZ) (µg/ml)	(API %) CNT+OMZ (µg/ml)	Amt. Recovered (CNT)±S.D. (µg/ml) *	%RSD CNT	%Recovery CNT	Amt. Recovered (OMZ)±S.D. (µg/ml) *	%RSD OMZ	%Recovery OMZ
2+14	(80)1.6+11.2	3.66±0.0508	1.38	101.72	25.13±0.2309	0.91	99.73
	(100)2.0+14.0	4.07±0.0672	1.65	101.83	27.93±0.2309	0.82	99.76
	(120)2.4+16.8	4.33±0.0508	1.17	98.58	31.53±0.4618	1.46	102.38

LOD and LOQ

The LOD for CNT and OMZ was conformed to be 0.06µg/ml and 0.33µg/ml, respectively. The LOQ for CNT and OMZ was conformed to be 0.18µg/ml and 1.00µg/m, respectively. The obtained LOD and LOQ results are presented in table 6.

Table: 6
LOD and LOQ *(n=10)

Conc. (µg/ml) (CNT : OMZ) (µg/ml)	Absorbance (253.8 nm)	Avg. ± SD (253.8 nm) * CNT	% RSD* CNT	Absorbance (263.2 nm)	Avg. ± SD (263.2nm) * OMZ	% RSD* OMZ
2:14	0.0042	0.00422±0.00004	0.9759	0.00251	0.00253±0.00003	0.9883
	0.00423			0.00254		
	0.00424			0.00255		
	0.00428			0.00252		
	0.00423			0.0025		
	0.00426			0.00252		
	0.00413			0.00255		
	0.00424			0.00256		
	0.00421			0.00252		
	0.00427			0.00258		
LOD(µg/ml)	0.06			0.33		
LOQ(µg/ml)	0.18			1.00		

Assay

The results from the analysis of commercial formulation containing cinitapride (3 mg) and omeprazole (20 mg) in combination are presented in table in 7.The percent assay shows that there is no interference from excipients and the proposed method can successfully applied to analysis of commercial formulation containing CNT and OMZ. The % assay values are tabulated in Table 7.

Table 7
Analysis of commercial formulation * (n=3)

Sr. No.	Formulation (BURPEX) (CNT+OMZ)	Absorbance (253.8 nm)	% Assay CNT±SD*	Absorbance (263.2 nm)	% Assay OMZ±SD*
1		0.00719		0.00479	
2	3:20	0.00711	98.82±0.58	0.00484	99.66±0.80
3		0.00715		0.00487	

The validation result is summarized in Table 8.

Table 8
Summary of validation parameters for proposed method

PARAMETERS	First-derivative UV Spectrometry	
	Cinitapride	Omeprazole
Concentration range(µg/ml)	1-6	7-42
Regression equation	y = 0.00227x +0.00042	y = 0.00025x -0.00015
Correlation Coefficient(r^2)	0.9992	0.9995
Accuracy(%Recovery) (n=3)	100.71	100.62
Intra-day Precision (%RSD) (n=3)	0.42-0.96	0.74-0.90
Inter-day precision (%RSD) (n=3)	0.39-0.91	0.58-0.76
LOD(µg/ml)	0.06	0.33
LOQ(µg/ml)	0.18	1.00
Ruggedness and Robustness	0.11-0.75	0.28-0.82
% Assay	98.82	99.66

CONCLUSION

All the parameters for two substances met the criteria of the ICH guidelines for the method validation and found to be suitable for routine quantitative analysis in pharmaceutical dosage forms. The result of linearity, accuracy, precision proved to be within limits with lower limits of detection and quantification. Ruggedness and Robustness of method was conformed as no significant were observed on analysis by subjecting the method to slight change in the method condition. Assay results obtained by proposed method are in fair agreement. Thus, the proposed method is simple, accurate, precise, linear across the analytical range & robust and can be employed for routine quantitative analysis in pharmaceutical dosage form.

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