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Simultaneous determination of Itopride Hydrochloride and Lansoprazole in Synthetic Mixture using Spectrophotometric technique (First order Derivative Method)

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ABSTRACT

A simple, accurate and precise spectroscopic method was developed for simultaneous estimation of Itopride Hydrochloride and Lansoprazole in synthetic mixture using first order derivative zero-crossing method. Itopride Hydrochloride showed zero crossing point at 278.12nmwhile Lansoprazole showed zero crossing point at 244.58nm. The dA/dλ was measured at 244.12 nm for Itopride Hydrochloride and 278.12nmfor Lansoprazole and calibration curves were plotted as dA/dλ versus concentration, respectively. The method was found to be linear (r2>0.999) in the range of 5-25µg/ml for Itopride Hydrochloride at 244.58nm. The linear correlation was obtained (r2>0.996) in the range of 5-25 µg/ml for Lansoprazole at 278.12 nm. The limit of determination was 0.155µg/ml and 0.059µg/ml for Itopride Hydrochloride and Lansoprazole, respectively. The limit of quantification was 0.472µg/ml and 0.179µg/ml for Itopride Hydrochloride and Lansoprazole respectively. The accuracy of these method were evaluated by recovery studies and good recovery result were obtained greater than 99% shows first order derivation zero crossing. The method was successfully applied for simultaneous determination of Itopride Hydrochloride and Lansoprazole in binary mixture.

Keywords: Itopride Hydrochloride, Lansoprazole, First Derivative Method, Spectroscopic method

INTRODUCTION

Itopride has anticholinesterase (AchE) activity as well as dopamine D2 receptor antagonistic activity and is being used for the symptomatic treatment of various gastrointestinal motility disorders. It is well established that M3 receptors exist on the smooth muscle layer throughout the gut and acetylcholine (ACh) released from enteric nerve endings stimulates the contraction of smooth muscle through M3 receptors. Itopride, by virtue of its dopamine D2 receptor antagonism, removes the inhibitory effects on Ach release. It also inhibits the enzyme AchE which prevents the degradation of Ach. The net effect is an increase in ACh concentration, which in turn, promotes gastric motility, increases the lower esophageal sphincter pressure, accelerates gastric emptying and improves gastro-duodenal coordination. This dual mode of action of Itopride is unique and different from the actions of other prokinetic agents available in the market. Chemically N-[[4-[2-(Dimethyl amino) ethoxy] phenyl] methyl]-3, 4-dimethoxy benzamide hydrochloride. Itopride Hydrochloride is white amorphous powder having molecular weight 394.93 g/mol.[1]

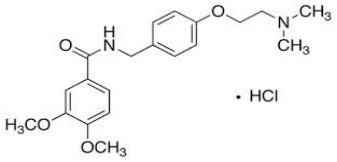

FIG. 1 CHEMICAL STRUCTURE OF ITOPRIDE HYDROCHLORIDE

Lansoprazole belongs to a class of antisecretory compounds, the substituted benzimidazoles, that do not exhibit anticholinergic or histamine H2-receptor

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antagonist properties, but rather suppress gastric acid secretion by specific inhibition of the (H+,K+)-ATPase enzyme system at the secretory surface of the gastric parietal cell. Because this enzyme system is regarded as the acid (proton) pump within the parietal cell, Lansoprazole has been characterized as a gastric acid-pump inhibitor, in that it blocks the final step of acid production. This effect is dose-related and leads to inhibition of both basal and stimulated gastric acid secretion irrespective of the stimulus**.** Chemically 2-([3-methyl-4-(2, 2, 2-trifluoroethoxy) pyridin-2-yl] methylsulfinyl)-1 H-benzimidazole. Lansoprazole is White to brownish-white odorless crystalline powder having molecular weight 369.37 g/mol. [2,3]

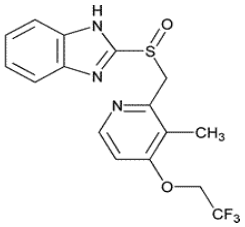

FIG. 2 CHEMICAL STRUCTURE OF LANSOPRAZOLE

The review of literature regarding quantitative analysis of Itopride Hydrochloride and Lansoprazole revealed that no Simultaneous Equation method attempt was made to develop analytical methods for Itopride Hydrochloride and Lansoprazole. Some spectrometric methods and chromatographic methods have been reported for the estimation of the individual and combination of drugs[4-11].The focus of the present study was to develop and validate a rapid, stable, specific, and economic Spectroscopic method for the estimation of Itopride Hydrochloride and Lansoprazole in Synthetic Mixture.

MATERIALS AND METHODOLOGY

Apparatus

A double beam UV/Visible spectrophotometer (Shimadzu model 2450, Japan) with spectral width of 2nm, 1 cm quartz cells was used to measure absorbance of all the solutions. Spectra were automatically obtained by UV-Probe system software.

Reference samples

ITO and LAN reference standard are kindly supply by Cipla pharmaceuticals, Ankleswar and Galpha Laboratories, Ankleswar as a gift sample respectively.

Materials and reagents

Methanol AR grade (RANKEM)

FIRST DERIVATIVE CONDITIONS

Mode: Spectrum
Scan speed: Fast
Wavelength range: 200-400 nm
Derivative order: first
Scaling factor: 1

Preparation of Standard Solution and Synthetic Mixture

Preparation of stock solution of Itopride Hydrochloride: An accurately weighed quantity equivalent to 10mg of Itopride Hydrochloride was transferred to 100 ml volumetric flask made up to the mark with the methanolto obtain standard solution having concentration of ITO (100µg/ml).

Preparation of standard stock solution of Lansoprazole: An accurately weighed quantity of LAN (10 mg) was transferred to a separate 100 ml volumetric flask and dissolved and diluted to the mark with methanol to obtain standard solution having concentration of LAN (100µg/ml).

Preparation of Standard Mixture Solution (ITO+ LAN): 1 ml of working standard stock solution of ITO (100µg/ml) and 0.3ml of standard Stock solution of LAN (100µg/ml) were pipetted out into 10ml volumetric flask and volume was adjusted to the mark with methanol to get 10µg/ml of ITO and 3µg/ml of LAN.

Preparation of Test Solution

The preparation of synthetic mixture was as per patent:
Itopride Hydrochloride…….100mg
Lansoprazole……30mg
Excipients……qs.

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40mg Synthetic mixture was taken in 25ml methanol and sonicate for 15 min and make up to 100ml with methanol, filter it and take filtrate in which the concentration of ITO was 100µg/ml and LAN was 30µg/ml. From this solution, take 1ml and diluted with10ml Methanol to make the concentration 10µg/ml of ITO and 3µg/ml of LAN.

Procedure

Selection of wavelength and method development for determination of Itopride Hydrochloride and Lansoprazole

The standard solution of ITO and LAN were scanned separately between 200-400nm, and zero-order spectra were not showed overlapping peaks.

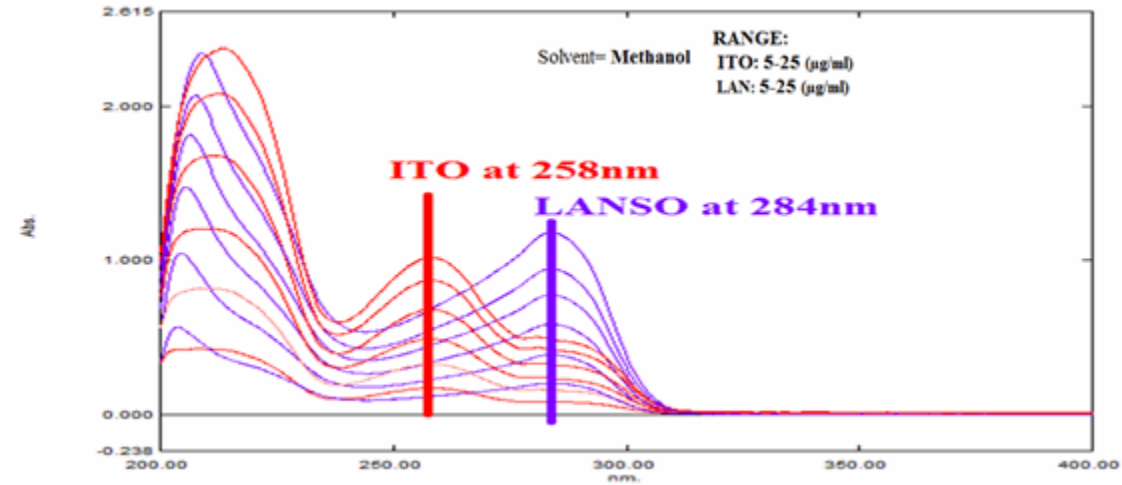

Figure 3 OVERLAINZERO ORDERSPECTRA OFITO AND LAN (10:3) RATIOS, RESPECTIVELY

Thus obtained spectra were then processed to obtain first-derivative spectra.

First order derivative spectrum for ITO showed zero crossing point: 244.58nm. The wavelength selected for estimation of ITO was 244.58 nm because it showed r2>0.999at this wavelength in mixture.

First order derivative spectrum for LAN showed zero crossing point: 278.12 nm. The wavelength selected for estimation of LAN was278.12 nm because it showed r2>0.996 at this wavelength in mixture (Figure 3)

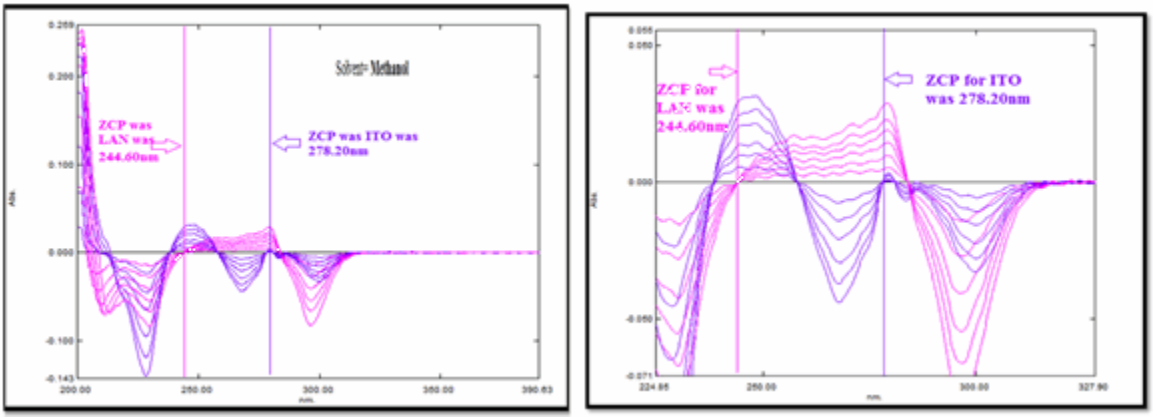

Figure 4 Overlain linear first order spectra of ITO and LANSO in10:3 ratio

Calibration curves for Itopride Hydrochloride:

This series consisted of six concentrations of standard ITO solution ranging from 5 to 30µg/ml. The solutions were prepared by pipetting out Standard ITO stock solution (100µg/ml). Then pipetting out (0.5ml, 1.0ml, 1.5ml, 2.0ml, and 2.5ml)

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was transferred into a series of 10 ml volumetric flask and volume was adjusted up to mark with methanol. A zero order derivative spectrum of the resulting solution was recorded and processed to first derivative spectra, measured the absorbance at 244.60 nm against are agent blank solution (Methanol). Calibration curve was prepared by plotting absorbance versus respective concentration of ITO.

Calibration curves for Itopride Hydrochloride:

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This series consisted of five concentrations of standard LAN solution ranging from 5 to 30µg/ml. The solutions were prepared by pipetting out Standard LAN stock solution (0.5ml, 1.0ml, 1.5ml, 2.0ml, and 2.5ml) was transferred into a series of 10 ml volumetric flask and volume was adjusted up to mark with methanol. A zero order derivative spectrum of the resulting solution was recorded and processed to first derivative spectra, measured the absorbance at 278.12 nm against a reagent blank solution (Methanol). Calibration curve was prepared by plotting absorbance versus respective concentration of LAN.

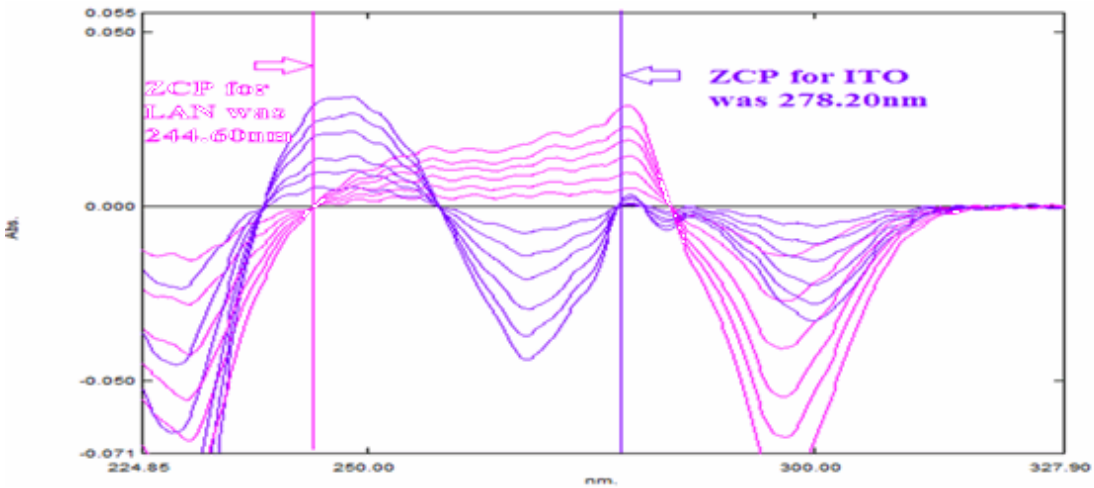

Figure 5 Overlain linear first order spectra of ITO and LANSO in10:3 ratio

RESULTS AND DISCUSSION

Validation Parameters [12]

Linearity

Five point calibration curves were obtained in the concentration range of 5-25µg/ml for Itopride Hydrochloride and 5-25µg/ml for Lansoprazole. The response of drug was found to be linear in investigation range and the regression equations was found to be y = 0.001x - 0.0004 for ITO

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(n=5) and y = 0.0003x + 0.0002for LAN (n=5), with the correlation coefficient 0.999and 0.996 (n=5) respectively, is listed in Table 1.

Itopride Hydrochloride	Absorbance (n=6)	Lansoprazole	Absorbance (n=6)
5	0.005	5	0.005
10	0.010	10	0.009
15	0.015	15	0.014
20	0.020	20	0.018
25	0.025	25	0.022

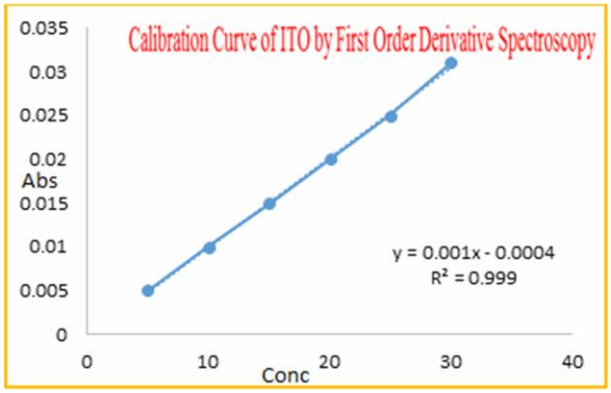

FIG. 6 CALIBRATION CURVE FOR ITO AT 244.58NM

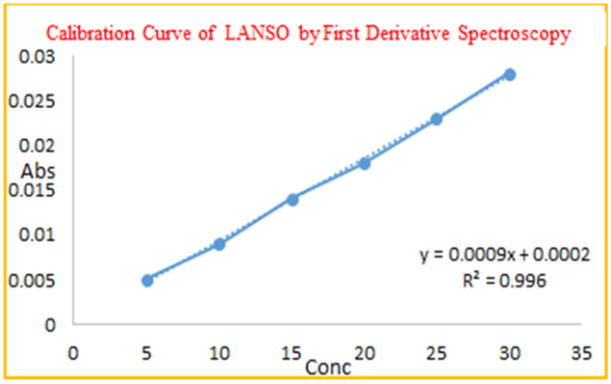

FIG. 7 CALIBRATION CURVE FOR LAN AT 278.12NM

Precision

The precision of the method was evaluated in terms of inter-day and intra-day by carrying out independent assays of three concentrations chosen from range of the standard curves (15, 20 and 25µg/ml of ITO and LAN respectively) and the %RSD of assay (inter-day and intra-day) was calculated. The results of study are shown in Table 2 and 3.

TABLE 2.INTRADAY PRECISION DATA FOR ESTIMATION OF ITOAND LAN *(n=3)

Conc. (µg/ml)		ITO Abs.* ±%RSD ±% RSD Abs.	LAN Abs.*±%RSD
ITO	LAN		
15	15	0.0108 ± 0.03	0.0120 ± 0.63
20	20	0.0132 ± 0.71	0.0128 ± 0.38
25	25	0.0243 ± 0.48	0.0231 ± 0.21

TABLE 3.INTERDAY PRECISION DATA FOR ESTIMATION OF ITOAND LAN *(n=3)

Conc. (µg/ml)		ITO Abs.* ±%RSD ±% RSD Abs.	LAN Abs.*±%RSD
ITO	LAN		
15	15	0.0109 ± 0.09	0.0122 ± 0.81
20	20	0.0134 ± 0.74	0.0131 ± 0.76
25	25	0.0242 ± 0.85	0.0240 ± 0.26

Accuracy

The accuracy of the method was determined by spiking of CIL and VAL to pre quantified sample solutions of ITO (10µg/ml) and LAN (3 µg/ml) in triplicate at three concentration level of 80, 100, 120% of the specified limit. The percentage recoveries of ITO and LAN were calculated and the result is nearer to 100% shown in Table 4 and 5.

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Table 4 Recovery data ofITO*(n=3)

Concentration of ITO from formulation (µg/ml)	Amount of ITO spiked (µg/ml)	Total amount (µg/ml)	Amount found (µg/ml)	% recovery	S.D	% RSD
10	-	10	10.04	100.4%	0.0010	0.25
10	8.0	18	18.1	100.5%	0.0015	0.50
10	10	20	20.06	100.3%	0.001	0.37
10	12	22	22.2	100.9%	0.002	0.70

Table 5 Recovery data ofLAN*(n=3)

Concentration of LAN from formulation (µg/ml)	Amount of LAN spiked (µg/ml)	Total amount (µg/ml)	Amount found (µg/ml)	% recovery	S.D	% RSD
3	-	3.0	3.04	100.6%	0.0010	0.18
3	2.4	5.4	5.44	100.7%	0.0015	0.20
3	3.0	6.0	6.03	100.5%	0.001	0.57
3	3.4	6.4	6.45	100.7%	0.002	0.17

Limit of Detection and Limit of Quantification

The limit of detection (LOD) and limit of quantitation (LOQ) of the method were evaluated by standard deviation of response and slope method. LOQ and LOD were calculated by the equation LOD = 3.3 × *N/B* and LOQ = 10 × *N/B*, where "*N*" is standard deviation of the absorbance, and "*B*" is the slope of the corresponding calibration curve. The limit of detection (LOD) were found to be 0.233µg/ml for ITO and 0.706 µg/ml for LAN and respectively and limit of quantitation (LOQ) were found to be 0.162µg/ml for ITO and 0.491 µg/ml for LAN presented in Table 6.

Table 6 LOD and LOQ data of ITO and LAN*(n=10)

Conc. (µg/ml)		Avg.abs* ± SD (258.00nm) ITO	% RSD	Avg.abs*±SD (284.00nm) LAN	% RSD
ITO	LAN				
10 2	3	0.616±0.0017	0.27	0.5577±0.0011	0.20
LOD (µg/ml)		0.155		0.472	
LOQ (µg/ml)		0.059		0.179	

Robustness and Ruggedness

Robustness and Ruggedness of the method was determined by subjecting the method to slight change in the method condition, individually, the :

Different stock solution preparation

Change in instrument (UV-Vis Spectrophotometer model 1800 and 2450).

Three replicates were made for the same concentration

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% RSD was calculated mentioned in **Table No.7**

Condition	Conc. (µg/ml)	Different Instrument		Different Stock Solution Preparation	
		UV-2450	UV-1800	Stock-1*	Stock-2*
ITO Mean (n=3) ± % RSD	15	0.004 ± 0.24	0.003 ± 0.32	0.006 ± 0.16	0.005 ± 0.88
	20	0.006 ± 0.24	0.005 ± 0.38	0.007 ± 0.28	0.006 ± 0.14
	25	0.008 ± 0.18	0.007 ± 0.19	0.009 ± 0.57	0.008 ± 0.57
LANSO Mean(n=3) ± %RSD	15	0.003 ± 0.48	0.004 ± 0.18	0.005 ± 0.18	0.003 ± 0.63
	20	0.005 ± 0.38	0.006 ± 0.79	0.006 ± 0.24	0.005 ± 0.11
	25	0.007 ± 0.82	0.008 ± 0.11	0.008 ± 0.11	0.007 ± 0.12

*Stock-1:- 10 mg dissolve in 100 ml Methanol
*Stock-2:- 50 mg dissolve in 250 ml Methanol

APPLICATION OFTHEPROPOSED METHOD FOR ANALYSISOF CIL AND VALIN SYNTHETIC MIXTURE

A zero order spectrum of the sample solution containing 10µg/ml of ITO and 3µg/ml of LAN was recorded and the absorbance at 244.58nm and 278.12nm were noted for estimation of ITO and LAN, respectively. The concentration of ITO and LAN in mixture was determined using the corresponding calibration graph. The results from the analysis of synthetic mixture containing Itopride Hydrochloride(100mg) and Lansoprazole (30mg) in combination are presented in Table 8.The present assay shows that there is no interference from excipients and the proposed method can successfully applied to analysis of commercial formulation containing ITO and LAN. The % assay values are tabulated in **Table 8**.

Drugs	% Assay ± SD	% RSD(n=3)
Itopride Hydrochloride	100.4 ± 0.00070	0.25
Lansoprazole	100.66 ± 0.00005	0.18

Summary Table

TABLE.9 SUMMARY OF VALIDATION PARAMETERS

SR.NO.	PARAMETER	Itopride Hydrochloride	Lansoprazole
1	Zero crossing point(ZCP)	278.20nm	244.60nm
2	Linearity (µg/ml) (n=6)	5-30 µg/ml	5-30 µg/ml
3	Regression equation	y = 0.001x + 0.0004	y = 0.0009x + 0.0002
4	Correlation coefficient (r^2)	0.999	0.996
5	Accuracy (%Recovery)(n=3)	100.58%	100.67%
6	LOD (µg/ml) (n=10)	0.155	0.059
7	LOQ (µg/ml) (n=10)	0.472	0.179
8	Precision Intra-day (%RSD)(n=3) Inter-day (%RSD)(n=3)	 0.03 - 0.48 0.09 - 0.85	 0.21 - 0.48 0.26 - 0.63
9	Assay	100.4%	100.66%
10	Robustness(%RSD)	0.18 - 0.88	0.11 - 0.82

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CONCLUSION

The result of all parameters like linearity, precision, LOD, LOQ and Accuracy fall within the limit as per ICH guideline. Method was found to be simple, accurate, precise and easy to reproducible.
All the parameters are validated as per ICH guidelines.

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