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Development and Validation of UV Spectrophotometric Method for Simultaneous Estimation of Pantoprazole and Levosulpiride in Pharmaceutical Dosage form

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ABSTRACT

The present manuscript describes simple, sensitive, rapid, accurate, precise and economical derivative spectroscopic method for the simultaneous determination of Pantoprazole and Levosulpiride in pharmaceutical dosage form. In this study a first-derivative spectroscopic method was used for simultaneous determination of pantoprazole and levosulpiride using the zero-crossing technique. The measurements were carried out at wavelengths of 269 and 249 nm for Pantoprazole and Levosulpiride respectively. The method was found to be linear (r2>0.9929) in the range of 10-50 µg/ml for Pantoprazole at 269 (ZCP of Levosulpiride) nm. The linear correlation was obtained (r2>0.9948) in the range of 10-50 µg/ml for Levosulpiride at 249 (ZCP of Pantoprazole) nm. The limit of determination was 0.69 and 0.58 µg/ml for pantoprazole and levosulpiride respectively. The limit of quantification was 2.06 and 1.69 µg/ml. The method was successfully applied for simultaneous determination of Pantoprazole and Levosulpiride in pharmaceutical dosage form.

Keywords: Pantoprazole, Levosulpiride, Derivative spectroscopic method, Zero-crossing point.

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INTRODUCTION

Pantoprazole is chemically 6-(difluoromethoxy)-2-{[(3, 4-dimethoxypyridin-2yl) methane] sulfinyl}-1H-1, 3-benzodiazole (Figure 1) is a well known antiulcer agent[1]. It is official in Indian Pharmacopoeia (IP) and British Pharmacopoeia. IP[2] and BP[3] describe liquid chromatography method for its estimation. Literature survey reveals HPLC[4] and UV[5] method for estimation of Pantoprazole alone. Literature survey also reveals HPLC[6] and UV spectrophotometry[7] methods for determination of Pantoprazole with other drugs in combination. Levosulpiride is chemically N-[(1-ethylpyrrolidin-2-yl) methyl]-2-methoxy-5-sulfamoylbenzamide[8] (Figure 2). Levosulpiride is not official in any Pharmacopoeia. Literature survey reveals HPLC[9, 10] and UV[11] method for determination of Levosulpiride alone. Literature survey also reveals HPLC[12, 13], UV spectrophotometry[14] and LC-MS[15] method for the determination of Levosulpiride with other drugs combination. The combination of these two drugs is not official in any pharmacopoeia; hence no official method is available for the simultaneous estimation of Pantoprazole and Levosulpiride in their combined dosage forms. Literature survey does not reveal any simple spectrophotometric method for simultaneous estimation of Pantoprazole and Levosulpiride in combined dosage forms. The present communication describes simple, sensitive, rapid, accurate, precise and cost effective spectrophotometric method based on derivative spectroscopic method for simultaneous estimation of both drugs in bulk and combined dosage forms.

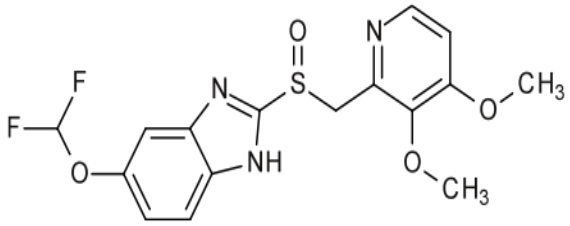

Figure1: Structure of Pantoprazole

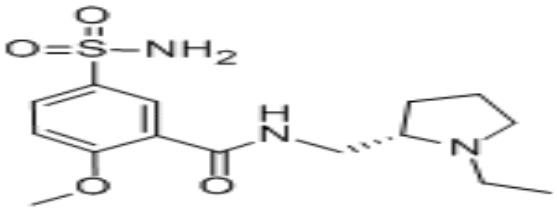

Figure2: Structure of Levosulpiride

MATERIALS AND METHODS

Apparatus

UV double beam spectrophotometer (Shimadzu model 1800) was employed with a spectral band width of 1nm and a wavelength accuracy of 0.3 nm (with automatic wavelength correction with a pair of 1 cm matched quartz cells).

Reagents and Materials

Pantoprazole and Levosulpiride bulk powder were kindly gifted by Torrent Research Centre, Gandhinagar, Gujarat, India, respectively. Methanol (AR Grade, S. D. Fine Chemicals Ltd., Mumbai, India) and Whatman filter paper no. 41 (Millipore, USA) were used in the study.

Preparation of standard solutions

An accurately weighed standard Pantoprazole and Levosulpiride powder (10 mg) were weighed and transferred to 100 ml separate volumetric flasks and dissolved in methanol. The flasks were shaken and volumes were made up to mark with methanol to give a solution containing 100 µg/ml of each Pantoprazole and Levosulpiride.

Determination of the zero crossing points

The standard solutions of Pantoprazole (16 µg/ml) and Levosulpiride (30 µg/ml) were scanned separately in the UV range of 200-400 nm. The zero order spectra thus obtained was then processed to obtain first derivative spectrum. It appeared that Pantoprazole showed zero crossing at 249 nm while Levosulpiride showed zero crossing at 269 nm. At 249 nm Pantoprazole showed zero absorbance and Levosulpiride showed reasonable absorbance, while at 269 nm Levosulpiride showed zero absorbance and Pantoprazole showed reasonable absorbance so these two wavelengths were selected for further measurement.

Validation of the proposed method

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines[16].

Linearity (calibration curve)

To check linearity of the method, working standard solution having concentration in range of 10-15 µg/ml for Pantoprazole and 10-50 µg/ml for Levosulpiride were prepared from the standard stock solutions of both drugs. Aliquots of standard solution of Pantoprazole (1.0, 2.0, 3.0, 4.0, 5.0 ml) and Levosulpiride (1.0, 2.0, 3.0, 4.0, 5.0 ml) of standard stock solutions of both drug were transferred separately to a series of 10 ml volumetric flasks and diluted to mark with methanol, and first-derivative absorbance (D1) were measured at 269 nm for Pantoprazole and 249 nm for Levosulpiride. The calibration curves were constructed by plotting absorbance vs. concentration.

Method precision (repeatability)

The precision of the instrument was checked by repeated scanning and measuring the absorbance of solution of (n = 6) Pantoprazole (10 µg/ml) and DIC (10 µg/ml) without changing the parameters of First Derivative Method.

Intermediate precision (reproducibility)

The intraday and interday precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentrations of standard solutions of Pantoprazole and Levosulpiride (10, 20, 30

µg/ml for Pantoprazole and 10, 20, 30 µg/ml for Levosulpiride). The result was reported in terms of relative standard deviation (% RSD).

Accuracy (recovery study)

The accuracy of the method was determined by calculating recovery of Pantoprazole and Levosulpiride by the standard addition method. Known amounts of standard solutions of Pantoprazole and Levosulpiride were added at 50, 100 and 150 % level to prequantified sample solutions of Pantoprazole and Levosulpiride (10 µg/ml for Pantoprazole and 10 µg/ml for Levosulpiride). The amounts of Pantoprazole and Levosulpiride were estimated by applying obtained values to the regression equation of the calibration curve.

Limit of detection and Limit of quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations designated by International Conference on Harmonization (ICH) guidelines18.

$$LOD = 3.3 \times \sigma/S$$

$$LOQ = 10 \times \sigma/S$$

Where, σ = the standard deviation of the response and S = slope of the calibration curve

Analysis of drug formulation

Commercial Pantocid-L capsule containing 40mg of Pantoprazole and 75mg of Levosulpiride were purchased from local market. Twenty tablets were weighed accurately and finely powdered. Tablet powder equivalent to 16 mg Pantoprazole and 30 mg of Levosulpiride was taken and dissolve in 100 ml methanol and sonicated for 15 minutes. From this solution prepare working solutions.

RESULTS AND DISCUSSION

In derivative spectroscopic method, the primary requirement for developing a method for analysis is that the entire spectra should follow the beer's law at all the wavelength, which was fulfilled in case of both these drugs. The two wavelengths were used for the analysis of the drugs were 249 nm (ZCP of Pantoprazole) and 269 nm (ZCP of Levosulpiride) at which the calibration curves were prepared for both the drugs. The first derivative overlain UV absorption spectrum of Pantoprazole (at 249 nm) and Levosulpiride (at 269 nm) is shown in Figure 7.

Linear correlation was obtained between absorbance and concentrations of Pantoprazole and Levosulpiride in the concentration ranges of 10-50 µg/ml and 10-50 µg/ml, respectively. The linearity of the calibration curve was validated by the high values of correlation coefficient of

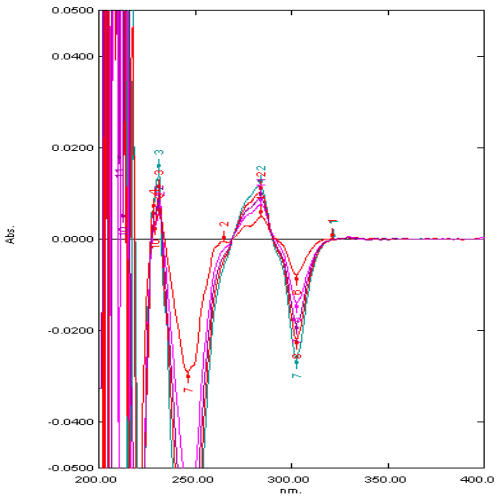

Figure 3 First order derivative spectrum of Pantoprazole

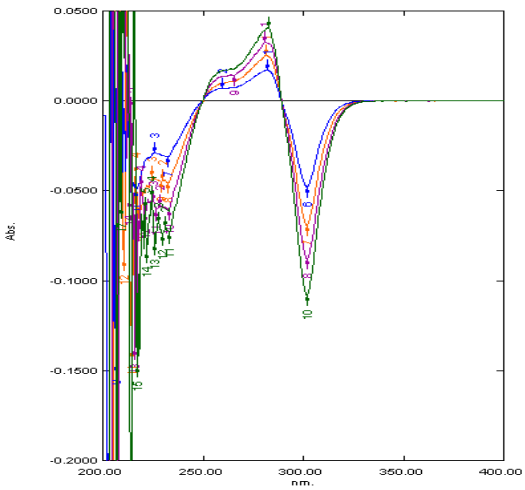

Figure 4 First order derivative spectrum of Levosulpiride

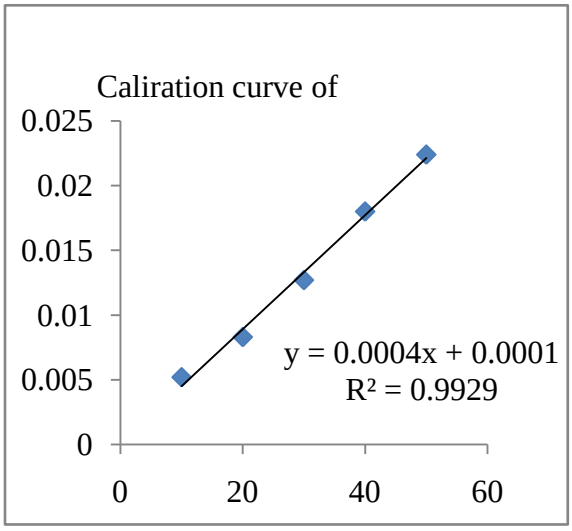

Figure 5 Calibration curve of Pantoprazole

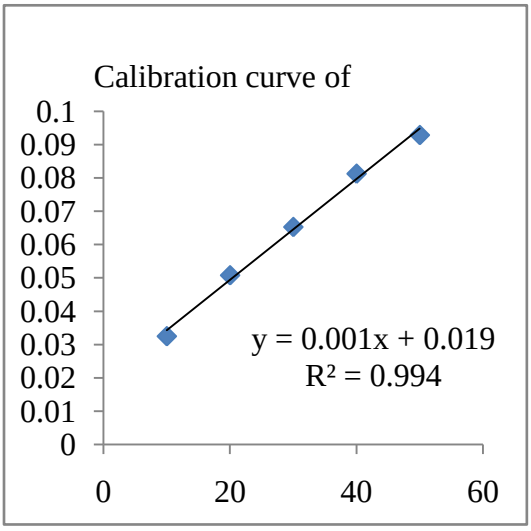

Figure 6 Calibration curve of Levosulpiride

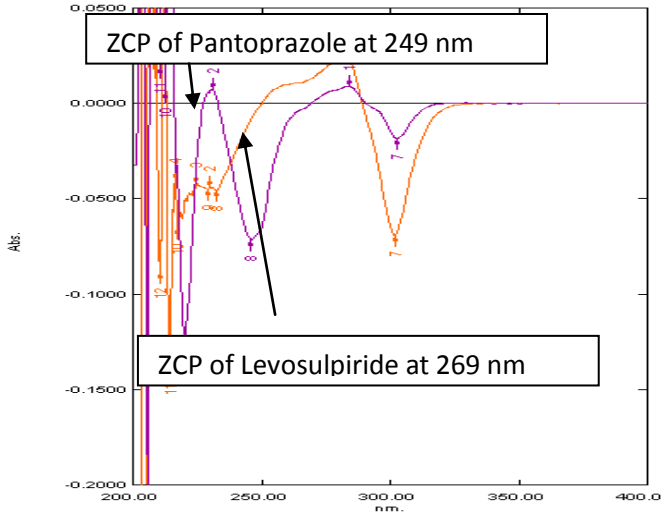

Figure 7: Overlay first order derivative spectrum of pantoprazole and Levosulpiride

regression. The RSD values of Pantoprazole were found to be 1.50% at 269 nm, respectively. The RSD value of Levosulpiride was found to be 1.15% at 249 nm, respectively. Relative standard deviation was less than 2 %, which indicates that proposed method is repeatable. The low RSD values of interday (0.40-1.10% and for Pantoprazole at 269 nm, respectively and 0.36-1.05% for Levosulpiride at 249 nm, respectively) and intraday (0.77-1.50% for Pantoprazole 269 nm, respectively and 0.60-1.35% for Levosulpiride at 249 nm, respectively) variation for Pantoprazole and Levosulpiride, reveal that the proposed method is precise. LOD and LOQ values for Pantoprazole were found to be 0.69 and 2.06 µg/ml and at 269 nm, respectively. LOD and LOQ values for Levosulpiride were found to be 0.58 and 1.69 µg/ml and at 249 nm, respectively. These data show that method is sensitive for the determination of Pantoprazole and Levosulpiride. The regression analysis data and summary of validation parameters for the proposed method is summarized in Table 1.

Table 1: Regression analysis data and summary of validation parameters for the proposed method

Parameters	Pantoprazole	Levosulpiride
Wavelength range (nm)	269	249
Beer's law limit (µg/ml)	10-50 µg/ml	10-50 µg/ml
Regression equation (y = a + bc)	y=0.0004x+0.0001	y=0.0015x+0.0192
Slope (b)	0.0004	0.0015
Intercept (a)	0.0001	0.0192
Correlation Coefficient (R^2)	0.9929	0.9948
Accuracy (Recovery) Level 1	100.44±0.50	98.99±0.88
Level 2	102.75±0.25	99.00±0.50
Level 3	100.46±0.30	100.00±1.05
Method precision (Repeatability) (% RSD, n = 6)	1.13	1.10
Interday (n = 3) (% RSD[a])	0.40-1.10	0.36-1.05
Intraday(n = 3) (% RSD)	0.77-1.50	0.60-1.35
LOD[b] (µg/ml)	0.69	0.58
LOQ[c] (µg/ml)	2.06	1.69
Assay ± S. D[d] (n = 3)	100.2 ± 0.63	99.85 ±1.32

[a]RSD = Relative standard deviation. [b]LOD = Limit of detection. [c]LOQ = Limit of quantification. [d]S. D. is standard deviation

Table 2: Recovery data of proposed method

Drug	Level	Amount taken (µg/ml)	Amount added (%)	% Mean recovery ± S.D. (n = 3)
Pantoprazole	I	10	50	100.44±0.50
	II	10	100	102.75±0.25
	III	10	150	100.46±0.30
Levosulpiride	I	10	50	98.99±0.88
	II	10	100	99.00±0.50
	III	10	150	100.00±1.05

S. D. is Standard deviation and n is number of replicate

Table 3: Analysis of Pantoprazole and Levosulpiride Drug formulation

Drug formulation	Label claim (mg)		Amount found (mg)		% Label claim ± S. D. (n = 3)	
	PANTO	LEVO	PANTO	LEVO	PANTO	LEVO
1	40	75	40.09	74.90	100.23 ± 0.50	99.86 ± 1.11
2	40	75	40.07	74.87	100.17 ± 0.75	99.83 ± 1.53

The recovery experiment was performed by the standard addition method. The mean recoveries were 100.2 ± 0.63 and 99.85 ±1.32 for Pantoprazole and Levosulpiride, respectively (Table 2). The proposed validated method was successfully applied to determine Pantoprazole and Levosulpiride in their combined dosage form. The results obtained for Pantoprazole and Levosulpiride were comparable with the corresponding labeled amounts (Table 3). No interference of the excipients with the absorbance of interest appeared; hence the proposed method is applicable for the routine simultaneous estimation of Pantoprazole and Levosulpiride in pharmaceutical dosage forms.

CONCLUSION

The proposed spectrophotometric method was found to be simple, sensitive, accurate and precise for determination of Pantoprazole and Levosulpiride in pharmaceutical dosage form. The method utilizes easily available and cheap solvent for analysis of Pantoprazole and Levosulpiride hence the method was also economic for estimation of Pantoprazole and Levosulpiride from pharmaceutical dosage form. The common excipients and additives are usually present in the pharmaceutical dosage form do not interfere in the analysis of Pantoprazole and Levosulpiride in method, hence it can be conveniently adopted for routine quality control analysis of the drugs in combined pharmaceutical formulation.

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