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Development and Validation of Analytical Methods for Simultaneous Estimation of Pantoprazole Sodium and Levosulpiride in Bulk and their combined dosage form

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ABSTRACT

A simple, precise, accurate, rapid and economical spectrophotometric method have been developed for simultaneous estimation of Pantoprazole sodium and Levosulpiride in pure and in combined capsule dosage form. Method-1 simultaneous equations and Method-2 Q-absorbance Ratio method by using 287 nm and 231 nm as absorbance maxima (λ max)for Pantoprazole sodium and Levosulpiride respectively and 248 nm (isoabsorptive point). A methanol was used as Solvent. Linearity was observed in the concentration range of 5-30 µg/ml for Pantoprazole sodium and 5-30 µg/ml for Levosulpiride. The method was validated statistically and recovery study was performed to confirm the accuracy of the method.

Keywords: Pantoprazole sodium, Levosulpiride , UV Spectrophotometric, Simultaneous equation, Q-Absorbance Ratio, Isoabsorptive Point.

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INTRODUCTION

Pantoprazole sodium (PANTO) is chemically, sodium 5-(Difluoromethoxy)-2-[[(3,4-dimethoxy2 -pyridinyl)methyl] sulfinyl]-1H-benzimidazole (Figure 1). PANTO is class of "proton pump inhibitor" that inhibits gastric acid secretion through inhibition of K^+/H^+ ATPase in gastricparietal cells. It is used for short term treatment of erosion and ulceration of the esophagus caused by gastro-esophageal reflux disease (GERD), peptic ulcer, NSAID-associated ulceration and Zollinger-Ellision syndrome. PANTO is official in IP and USP. IP and USP describe HPLC method for its estimation. The review of literature revealed that various analytical methods involving Spectrophotometry, HPLC and HPTLC have been reported for PANTO in pharmaceutical dosage forms and biological fluids individually or in combination with other drugs.

Levosulpiride (LEVO), a purified levo-isomer of sulpirideis chemically 5- (amino sulfonly)-N-[(1-ethyl-2-pyrrolidinyl) methyl]-2-methoxy benzamide (Figure 2).It is a D_2 dopamine receptor antagonist and indicated intreatment of psychosis, depression, functional dyspepsiaas well as used for prokinetic activity and used with someother drugs in combination therapy. LEVO is not official in Indian pharmacopoeia (IP), UnitedState Pharmacopoeia (USP) and British Pharmacopoeia (BP), European Pharmacopoeia (EU) but it is listed in Merck Index, Martindale and Complete Drug Reference. Literature survey revealed that various colorimetric, Spectrofluorimetric, UV spectroscopy and Chromatographic methods have been reported for quantitative estimation of LEVO in pharmaceutical dosage forms and biological fluids individually or in combination with other drugs.

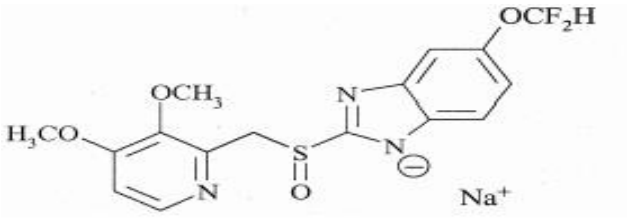

Figure 1: pantoprazole sodium

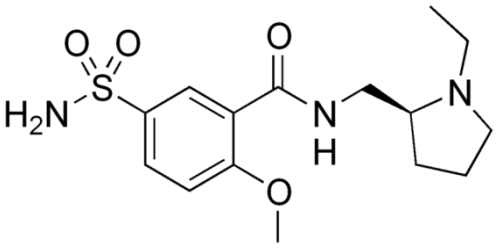

Figure 2: levosulpiride

To the best of our knowledge, there is no published Spectrophotometric method for this combination. So, the present paper describes a simple, accurate and precise method for simultaneous estimation of LEVO and PANTO in combined Capsule dosage form by Method-1 simultaneous equations and Method-2 Q-absorbance Ratio method. The developed method was validated in accordance with ICH Guidelines and successfully employed for the assay of LEVO and PANTO in their combined dosage form.

MATERIAL AND METHOD

Instrument

Double beam UV-visible spectrophotometer (Shimadzu, model UV1800) having two matched quartz cells with 1 cm light path, Sonicator, Weighing balance.

Reagents and Chemicals

Reference Standards of pantoprazole sodium and levosulpiride were obtained as gift samples from the Zydus Cadila Pharmaceutical Ltd. The drug sample (capsule) PANTO-LEVO manufactured by sun pharma. were procured from market. All other reagents were of analytical grade for Spectrophotometric method.

Procedure

Preparation of standard stock solution of PANTO:

Accurately weighed quantity of PANTO 1 0 mg was transferred into 10 ml volumetric flask, dissolved and diluted up to mark with methanol.This will give a stock solution having strength of 1000µg/ml.

100µg/ml of PANTO solution was prepared by diluting 1 ml of stock solution to 10 ml with methanol.

Preparation of working standard solution of PANTO:

5, 10, 15, 20, 25, 30 µg/ml of PANTO solution was prepared by diluting 0.5, 1, 1.5, 2, 2.5, 3 ml of stock solution (100µg/ml) to 10 ml with methanol.

Preparation of standard stock solution of LEVO:

Accurately weighed quantity of LEVO 1 0 mg was transferred into 10 ml volumetric flask, dissolved and diluted up to mark with methanol.This will give a stock solution having strength of 1000µg/ml.

100µg/ml of LEVO solution was prepared by diluting 1 ml of stock solution to 10 ml with methanol.

Preparation of working standard solution of LEVO:

5, 10, 15, 20, 25, 30 µg/ml of LEVO solution was prepared by diluting 0.5, 1, 1.5, 2, 2.5, 3 ml of stock solution (100µg/ml) to 10 ml with methanol.

Determination of wavelength for measurement

1.5 ml of working standard solution of PANTO and LEVO (100µg/ml) was diluted to 10 ml with methanol to get 15 µg/ml of PANTO and LEVO.Each solution was scanned between 200-400 nm.Wavelengths were selected from the overlain spectra of PANTO and LEVO

(Figure 3). The λmax of Pantoprazole sodium and levosulpiride is 287 nm and 231 nm and isoabsorptive point is 248 nm respectively. The absorbance of resulting solution were measured at their respective λmax and plotted a calibration curve to get linearity and regression equation.

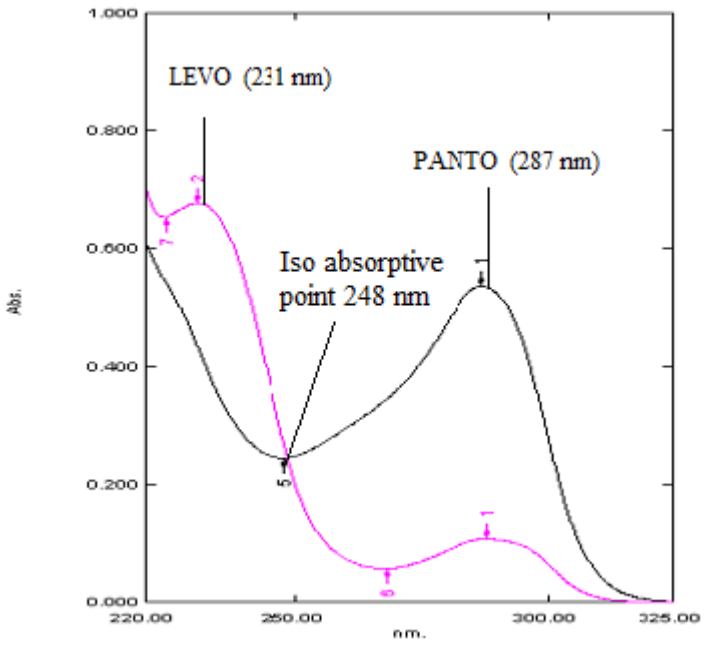

Figure 3: Overlain spectra of pantoprazole sodium (15µg/ml) and levosulpiride (15µg/ml)

Method-1 (Simultaneous Equation Method)

The Simultaneous Equation Method of analysis based on the absorption of the drugs pantoprazole sodium and levosulpiride at their λmax. Two wavelength selected for the development of Simultaneous Equation are 287 nm (λ_1) and 231 nm (λ_2). absoptivities of both the drugs at both the wavelengths were determined.The equations obtained for the estimation of concentration were,

$$Cx = \frac{A_2 ay_1 - A_1 ay_2}{ax_2 ay_1 \quad ax_1 ay_2}$$

$$Cy = \frac{A_1 ax_2 - A_2 ax_1}{ax_2 ay_1 \quad ax_1 ay_2}$$

Where A_1 and A_2 are absorbance of Sample solution at 287 and 231 nm respectively.

ax_1= Absorptivity of Pantoprazole sodium at 287 nm

ax_2 = Absorptivity of Pantoprazole sodium at 231 nm

ay_1 = Absorptivity of Levosulpiride at 287 nm

ay_2= Absorptivity of Levosulpiride at 231 nm

C_X and C_Y are concentration of Pantoprazole sodium and Levosulpiride in sample solution. (Table-1)

Method-2 (Q-Absorbance OR Absorbance Ratio Method)

The absorbance ratio method of analysis is based on the absorbance at two selected wavelengths; one is an isoabsorptive point and the other being the wavelength of maximum absorption of one of the two components. From overlain spectra (Figure-3) wavelength 248 nm (isoabsorptive point) and 287 nm (λ max of pantoprazole sodium) are selected for Q-Absorbance equation (3 & 4).

$Cx = (Qm-Qy) \times A_1/(Qx-Qy) \times ax_1$ (3)

$Cy = (Qm-Qx) \times A_1/(Qy-Qx) \times ay_1$ (4)

Where A1 and A2 are absorbance of sample solution at 248 nm and 287 nm respectively.

$ax_1 = ay_1$=Absorptivity of isoabsorptive point at 248 nm,

ax_2= Absorptivity of pantoprazole sodium at 287 nm,

ay_2= Absorptivity of levosulpiride at 287 nm

C_X and C_Y are concentration of pantoprazole sodium and levosulpiride in sample solution.

(Table 2)

Sample preparation

Twenty capsule were accurately weighed, and contents were removed. Average weight of the content per capsule was calculated. The contents of a capsule were reduce to fine powder. A quantity of capsule powder equivalent to 40mg of pantoprazole sodium and 75mg of levosulpiride was transferred to 100ml volumetric flask and dissolved in methanol with sonicated for 20 min, was then filtered through Whatman filter paper. The Aliquot portion of filtrate was further diluted to get a final concentration of about 12µg/ml pantoprazole sodium and 22.5 µg/ml of levosulpiride. For Method-1(simultaneous equation method)The absorbance of sample solution was measured at 287nm and 231nm in 1cm cell against the blank. For Method-2(Q-absorbance method). The absorbance of sample solution was measured at 287nm and 248nm in 1cm cell against the blank. The content of pantoprazole sodium and levosulpiride in a capsule was calculated by the simultaneous equation method and Q-absorption method.

Validation of the Method according to ICH Guidelines

Validation of the method was done according to ICH guidelines for Simultaneous Equation method.

Linearity

The linearity of the method is its ability to elicit test results that are directly proportional to the concentration of the analyte in the samples. PANTO was linear with the concentration range of 5-30 µg/ml at 287 nm. LEVO showed the linearity in the range of 5-30 µg/ml at 231 nm.

Precision(repeatability)

The repeatability of the method was confirmed by the analysis of formulation was repeated for 6 times with the same concentration.

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 3 different concentrations of standard solutions of PANTO and LEVO.

Accuracy (recovery study):

To check the accuracy of the proposed methods, recovery studies carried out at 50%, 100%, and 150% of the test concentration as per ICH Guideline. The recovery study was performed three times at each level.

(Table 3)

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) using the following equations designated by International Conference on Harmonization (ICH) guidelines.

$LOD = 3.3 \times \sigma/S$

$LOQ = 10 \times \sigma/S$

Where,

σ = the standard deviation of the response and

S = slope of the calibration curve.

RESULTS AND DISCUSSION

Simultaneous equation method the linearity is observed in the concentration range of 5 – 30 µg/mL with co-efficient of correlation, (r^2) = 0.999 for PANTO at 287 nm and 231 nm and (r^2) = 0.998 and 0.997 for LEVO at 287 nm and 231 nm, respectively.

Q-absorbance method the linearity is observed in the concentration range of 5 – 30 µg/mL with co-efficient of correlation, (r^2) = 0.999 for PANTO at 287 nm and 231 nm and (r^2) = 0.999 for isoabsorptive point at 248 nm, respectively (Table 1 to 4).

The result of the analysis of pharmaceutical formulation by the proposed method is highly reproducible and reliable and it is in good agreement with the label claim of the drug. The method can be used for the routine analysis of the PANTO and LEVO in combined dosage form without any interference of the excipients.

Table 1: Optical Characteristic: (simultaneous equation method)

Parameters	Pantoprazole sodium		Levosulpiride	
Wavelength (nm)	287	231	287	231
Beer's law limit(µg /ml)	5-30	5-30	5-30	5-30
Regression eq. (y = a + bc)	y = 0.178x - 0.002	y = 0.151x - 0.038	y = 0.036x - 0.006	y = 0.231x 0.048
Slope (b)	0.178	0.151	0.036	0.230
Intercept (a)	-0.002	-0.038	-0.006	-0.048
Correlation coefficient (r^2)	0.999	0.999	0.998	0.997
LOD (µg/ml)	0.20	0.14	0.60	0.42
LOQ (µg /ml)	0.62	0.44	1.82	1.27

Table 2: Optical Characteristic: (Q-Absorbance Ratio method)

Parameters	Pantoprazole sodium	Levosulpiride	isoabsorptive point
Wavelength (nm)	287	287	248
Beer's law limit (µg /ml)	5-30	5-30	5-30
Regression equation (y = a + bc)	y = 0.178x -0.002	y = 0.036x - 0.006	y = 0.083x - 0.009
Slope (b)	0.178	0.036	0.083
Intercept (a)	-0.002	-0.006	-0.009
Correlation coefficient (r^2)	0.999	0.998	0.999
LOD (µg/ml)	0.20	0.60	0.11
LOQ (µg /ml)	0.62	1.82	0.34

Table 3: Results of the recovery studies

Method	Recovery Level	% Recovery	SD(n=6)	% Recovery	SD(n=6)
		Pantoprazole sodium		Levosulpiride	
simultaneous	50%	98.94	±1.49	97.49	±0.45
Equation	100%	98.85	±0.43	98.85	±0.43
	150%	99.50	±0.60	99.50	±0.60
Q-Absorbance	50%	98.41	±0.53	99.17	±0.14
Ratio	100%	99.19	±0.82	99.95	±0.57
	150%	99.50	±0.50	97.49	±0.26

SD = Standard deviation

Table 4 : Assay Results of Marketed Formulation

Drugs	Simultaneous equation Method %Assay ± SD (n=6)	Q-Absorbance method
Pantoprazole sodium(40mg)	99.8% ± 0.45	100.97 ± 0.56
Levosulpiride (75 mg)	101.0% ± 0.29	99.76%±0.71

CONCLUSION

The proposed methods are simple, rapid and validated in terms of linearity, precision, accuracy, reproducibility, and can be used successfully for routine simultaneous estimation of Pantoprazole sodium and Levosulpiride in pure and capsule dosage forms.

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REFERANCES

1. Tripathi KD, Essentials of Medical Pharmacology, 6th edn, Jaypee Brothers Publisers, New delhi, 2008, 632-648.
2. Florey Klaus, Analytical Profile of Drug Substances, vol.17, Academic Press, 607.
3. Florey Klaus, Analytical Profile of Drug Substances, vol.29, Academic Press, 213.
4. The merck Index, An Encyclopedia of Chemical Drug and Biologicals, 13th edn, Merck Research Laboratories, 1256.
5. Beckett AH, Stenlake JB. Uv-visible spectrophotometry practical pharmaceutical chemistry; 4th end; C.B.S. Publishers, Delhi, 2001, 285-297.
6. Skoog DA, Introduction to uv spectroscopy in, principle of instrumental analysis, 5th edn, Thomson brooks/cole publication, 301:893-934.
7. Ashutoshkar, Pharmaceutical Drug Analysis, Minerva Press, 2001, 369-390, 565-591.
8. Gupta KR, Chawla RB, Wadodka SG. Spectrophotometric methods for simultaneous estimation of pantoprazole and itopride hydrochloride in capsules. The EJC 2010; 2(2): 181-188.
9. Savaliya PR, Bangoriya UV, Kakadia AK. Development and Validation Of Spectrophotometric Method For Simultaneous Estimation of Pantoprazole Sodium And Ondansetron Hydrochloridein Combined Dosage Form. IRPA 2012.
10. Pandya J, Solanki S, Patel M. Development and Validation of Differential Spectrophotometric method for Determination of Pantoprazole in Tablet Dosage Form. JPSBR 2012; 2(1): 02-04.
11. Reddy P, Reddy B, Reddy K. Development and Validation of RP-HPLC for the Pantoprazole Sodium Sesquihydrate in Pharmaceutical dosage forms and Human Plasma. IJCR 2009; 1(2): 195-198.
12. Khan,FA, Gadewar CK, Bakal RL, Chandewar AV. Method development and validation of simultaneous estimation of Pantoprazole Sodium and naproxen in capsule dosage forms by RP-HPLC. IJPR 2011; 3(5): 8-14.
13. Chaudhary A, Sahoo S, Murthy P, Samanta T and Mohanty D. RP-HPLC method for the

estimation of pantoprazole sodium in bulk and in dosage forms. IJCAS 2011; 2(5): 34-36.

14. Konari S and Vipin P. Development and Validation of RP-HPLC and Spectrophotometric Method for Simultaneous Estimation of Domperidone and Pantoprazole in Pharmaceutical Dosage Forms. IJCAS 2011; 2(8): 133-135.
15. Bhalodiya HA, Boda JM, Patel PB. Application Of Absorbance Ratio Method For Simultaneous Spectroscopic Estimation Of Rabeprazole Sodium And Levosulpiride In Combined Pharmaceutical Formulation. IRPA 2012; 12: 320.
16. Pekamwar SS, Kalyankar TM, Attar MS. Simultaneous Estimation of Rabeprazole Sodium and Levosulpiride by UV-spectrophotometry in Tablet Formulation. DPC. School of Pharmacy. S.R.T.M. University. Nanded.
17. Agrawal YP, Gautam SP, Verma A, Agrawal MY and Gupta AK. Simultaneous estimation of esomeprazole and levosulpiride in solid dosage form. PRL Der Pharmacia Sinica 2012; 3(3): 337-342.
18. Brahmbhatt DD, Patel MB, Patel JC. Development and validation for Estimation of Levosulpiride by Area Under Curve and Difference Spectrophotometric Method. IJCR 2012; 4(3): 945-950.
19. Sirisha A, Ravi Kumar A. Method development and validation of simultaneous estimation of levosulpiride and rabeprazole in bulk and pharmaceutical dosage form by RP-HPLC. IRJPSI 2012; 2(4): 49-55.
20. Jin SE, Ban E, Kim YB, Kim CK.Development of HPLC method for the determination of levosulpiride in human plasma. JPBA 2004; 35(4): 929-936.
21. Agarwal N. Development and validation of stability indicating RP-HPLC method for simultaneous estimation of levosulpiride and rabeprazole sodium. IJPBS 2012; 3(4): 718 – 726.
22. Walash M, Sharaf E-Din M, El-Enany N, Eid M, Shalan Sh. First derivative synchronous fluorescence spectroscopy for the simultaneous determination of sulpiride and mebeverine hydrochloride in their combined tablets and application to real human plasma. 2010; 20(6): 1275-85.