METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF LEVOSULPIRIDE AND RABEPRAZOLE SODIUM: A NEW ANALYTICAL Q-ABSORBANCE RATIO APPROACH

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ABSTRACT

Objective: The aim of the study was to develop a precise, accurate, and rapid ultraviolet spectrophotometric method for simultaneous estimation of levosulpiride (LEVO) and rabeprazole sodium (RBS) in the binary mixture and to validate the method as per ICH guidelines.

Method: Estimation of LEVO and RBS was performed by Q-absorbance method. Analysis was performed using the ratio of absorbance at two selected wavelengths, one at iso-absorptive point and other is the absorbance maxima of any one of the components. Single scan spectrum and the overlain spectrum conclude that absorbance maxima of LEVO and RBS are 228 and 291.8 nm, respectively, with a coinciding iso-absorptive point at 255 nm. Method uses a ratio of absorbance for assay at 255 and the second wavelength is 291.8 nm, λ_{max} for RBS. It is also applicable at 228 nm, as the second wavelength.

Results: Linearity of LEVO and RBS was found to be 25-125 and 4-36 µg/ml, respectively. The accuracy of the LEVO and RBS was found 99.26% and 99.51%, respectively and Sandell's sensitivity ranged between 0.0238 and 0.594 µg/cm². Assay of LEVO (75 mg) and RBS (20 mg) in capsule dosage form was found 99.5% and 98.69% w/w, respectively.

Conclusions: The developed method for the estimation of LEVO and RBS in binary mixture were found to be simple, accurate, robust, and reproducible. No interference of excipients and the degraded product was found during the estimation. Therefore, the method can be successfully applied for routine quality control analysis.

Keywords: Levosulpiride, Rabeprazole sodium, Q-absorbance, ICH, Validation.

INTRODUCTION

Levosulpiride (LEVO) is substituted benzamide antipsychotics. LEVO is an atypical antipsychotic agent that blocks the presynaptic dopaminergic D2 receptors. Like its parent compound, LEVO shows antagonism at D_3 and D_2 receptors. LEVO is used in the treatment of the psychosis, anxiety disorder, premature ejaculation (not recommended in some countries), vertigo and irritable bowel syndrome. It is chemically known as-[[(2*S*)-1-ethylpyrrolidin-2-yl] methyl]-2-methoxy-5-sulfamoyl benzamide (Fig. 1). It is soluble in methanol, ethanol, and DMSO.

Rabeprazole sodium (RBS) is a proton pump inhibitor. It is widely used as an antiulcer drug. It falls into the category of proton pump inhibitor. It is used in the treatment of gastric ulcer, peptic ulcer, ulcerative gastroesophageal reflux disease, duodenal ulcer Zollinger-Ellison syndrome, and *Helicobacter pylori* eradication in combination with amoxicillin. Chemically, it is 2-([4-(3-methoxypropoxy)-3-methylpyridin-2-yl] methylsulfinyl)-1H-benzo[d]imidazole (Fig. 2). It is freely soluble in water; soluble in methanol, ethanol and ethyl acetate [1-3].

RBS can be estimated by liquid chromatography method which is official in Indian Pharmacopoeia (2007) [4]. There is no official method reported for the estimation in combination for both the selected drugs. There is some chromatographic method for estimation of single and both combination of rabeprazole and LEVO [5-7]. Some of the methods are available on combinations with itopride and mosapride but not with suggested drug combinations [8,9]. There is no method for selected combination has been developed by Q absorbance ratio. Hence, there is scope for the absorption ratio method is best suited for simultaneous estimation of RBS and LEVO in combined capsule dosage form by ultraviolet (UV) spectrometry. Q absorbance ratio method using UV-visible spectrophotometry depends on the property that for a substance, which obeys beer's law at all wavelength, the ratio of absorbance at any two wavelengths is a constant value independent of concentration or path length. The ratio of the two different wavelengths absorbance is known as Q value [10,11].

METHODS

Instruments
The spectrophotometric estimation was performed on Shimadzu UV-visible double beam spectrophotometer (Model-1800). Weighing of the chemicals was done on electronic analytical balance.

Chemical and reagents
The drug samples of LEVO and rabeprazole were obtained from Mankind Pharma, Baddi H.P. Marketed formulation, Rabekind Plus (Capsules) was procured from the market. All the other chemicals and reagents were of analytical grades.

Preparation of standard stock solution of LEVO (1000 µg/ml)
Accurately weighed LEVO 100 mg was dissolved in sodium hydroxide (0.1 M) up to 100 ml in a volumetric flask to produce 1000 µmg/ml of LEVO.

Preparation of standard stock solution of RBS (100 µg/ml)
Accurately weighed RBS 25 mg was dissolved sodium hydroxide (0.1 M) up to 25 ml in a volumetric flask. Aliquots of 10 ml of the stock solution were diluted up to 100 ml from the same to produce 100 µmg/ml of RBS.

Selection of wavelength

Aliquots of any one concentration of LEVO and RBS were scanned between 200 and 400 nm against 0.1 M sodium hydroxide as blank. The absorbance maxima of LEVO and RBS were found to be 228 and 291.8 nm, respectivelyv (Figs. 3 and 4). The overlain spectrum was obtained by combining both the scan. The isosbestic point was obtained at 255 nm (Fig. 5).

llPreparation of calibration curves

Calibration curve of LEVO

Aliquots of LEVO in the concentration range of 25-125 µmg/ml were obtained by transferring 50 ml of LEVO stock solution (1000 ppm) to the 100 ml volumetric flasks and volume were made up of the solvent 0.1 M sodium hydroxide. They were further diluted up to in series up to 10 ml in volumetric using the same solvent. The absorbance of the solutions was measured at 255 (isobestic) and 291.8 nm (second λ_{max}) against the solvent system as blank and calibration curves were plotted (Figs. 6 and 7).

Calibration curve of RBS

Aliquots of RBS in the concentration range of 4-36 µg/ml were obtained by transferring required volume of standard stock of RBS (100 ppm) to the series of 10 ml volumetric flasks, and then volumes in each volumetric flask were made up with solvent 0.1 M sodium hydroxide. The absorbance of the solutions was measured at 255 (isobestic) and 291.8 nm (self) against the solvent system as blank and calibration curves were plotted (Figs. 8 and 9).

Preparation of synthetic mixtures of rabeprazole and LEVO

The synthetic mixture of LEVO and RBS was prepared in a ratio of 15:4. They are accurately weighed 25 mg and 6.6 mg, respectively, and were transferred to 25 ml volumetric flask, and 15 ml of the same solvent system was added. Common excipients, such as 8% starch, 7% magnesium stearate and 84% lactose, 1% talc, cellulose acetate phthalate (for 1000 µg/ml) which were used in the formulation, were added to this mixture and sonicated for 20 minutes. Drug sample was prepared using basic knowledge of excipients available in text books [12]. This solution was filtered through the Whatman filter paper and residues were washed with the solvent system. The filtrate and washings were combined, and volume was made up to the 25 ml with the solvent system. Then, 2.5 ml of the solution was diluted to 10 ml with the solvent system. The decision of this ratio of drugs in the synthetic mixture was based on the dosage strength of combination, which is available in the market. This combination has been approved by CDSCO, India, as capsule dosage form. It is marketed under the brand name "Rabekind," containing LEVO (75 mg) with rabeprazole (20 mg).

Calibration curves of LEVO and rabeprazole in synthetic mixture

The synthetic mixture (1.2, 1.8, 2.4, 3.0, 3.6, 4.2, 4.8, 5.4 ml) was transferred to a series of 10 ml volumetric flasks separately, and volume was made up to the mark with the solvent system. The absorbance of these solutions was measured at 255 and 291.8 nm and calibration curves were plotted (Figs. 10 and 11).

Introduction to Q-absorbance ratio method

In absorption ratio method, the absorbance of both the drugs was calculated at two selected wavelengths among which λ_1 is the wavelength of isosbestic point (where both drugs show the same absorbance) and λ_2 is the λ max of either drug among the drugs to be analyzed. From the overlain spectra, wavelength 255 nm (λ_1, i.e., an isosbestic point) and 291.8 nm (λ_2, i.e., absorbance maxima of rabeprazole) were selected for analysis. The concentration of the individual drug is determined by equations (1 and 2) written below [10,11].

$$C_x = \frac{Q_m - Q_y}{Q_x - Q_y} \times \frac{A_1}{a_{x1}} \tag{1}$$

C_x: Concentration of RBS.

$$C_y = \frac{Q_m - Q_x}{Q_y - Q_x} \times \frac{A_1}{a_{y1}} \tag{2}$$

C_y: Concentration of LEVO.

Where, $Q_m = \dfrac{A_2}{A_1}$ - A_1 is absorbance of sample at λ_1 (isosbestic point),

A_2 is absorbance of sample at λ_2 (λ_{max} RBS),

$Q_x = \dfrac{a_{x2}}{a_{x1}}$ - a_{x1}, a_{x2}: Absorptivities of RBS at λ_1 and λ_2 respectively,

$Q_y = \dfrac{a_{y2}}{a_{y1}}$ - a_{y1}, a_{y2}: Absorptivities of LEVO at λ_1 and λ_2, respectively.

The ratio of absorbance of calibration curves at different two wavelengths was calculated as below. These ratio values are used in equations to calculate the Q_m values to determine the assay values. The values are given in Tables 1 and 2.

Calculation of Q ratio equation values

Absorptivities from the standard curve data are used to determine the absorptivities, as well as the ratio, are used for determination of Q_m values. The calculation is given in Tables 3 and 4.

Table 1: Ratio of absorbance of LEVO

S.No.	Concentration (ppm)	Absorbance in nm		Ratio of absorbance
		255	291.8	
1.	25	0.291	0.188	1.547
2.	35	0.452	0.289	1.564
3.	45	0.489	0.310	1.577
4.	55	0.616	0.390	1.579
5.	65	0.743	0.465	1.598
6.	75	0.862	0.541	1.593
7.	85	0.938	0.590	1.589
8.	95	1.093	0.695	1.572
9.	105	1.303	0.822	1.585
10.	115	1.487	0.949	1.567
11	125	1.539	0.979	1.571

LEVO: Levosulpiride

Table 2: Ratio of absorbance of RBS

S.No.	Concentration (ppm)	Absorbance in nm		Ratio of absorbance
		255	291.8	
1.	4	0.021	0.096	0.218
2.	8	0.070	0.238	0.293
3.	12	0.111	0.392	0.285
4.	16	0.124	0.442	0.259
5.	20	0.189	0.667	0.283
6.	24	0.243	0.825	0.294
7.	28	0.290	0.935	0.305

RBS: Rabeprazole sodium

Table 3: Value of absorptivities

a_{x1}	a_{x2}	a_{y1}	a_{y2}
12.896	41.887	9.776	6.252

Table 4: Values of Q ratio

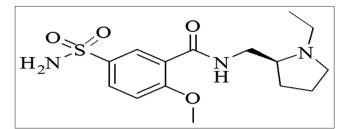| Q_x	Q_y	Q_m
3.24	0.639	1.295

Fig. 1: Chemical structure of levosulpiride

Fig. 2: Chemical structure of rabeprazole sodium

Fig. 3: Scan for determination of l$_{max}$ of levosulpiride

Fig. 4: Scan for determination of l$_{max}$ of rabeprazole sodium

Fig. 5: Overlain spectra of levosulpiride and rabeprazole sodium

Fig.6: Calibration curve for levosulpiride at 255 nm (isobestic point)

Validation of the developed method according to ICH guidelines

Following parameters were taken into consideration for validation of proposed methods [13-15].

1. Linearity: Linearity range is actually made of series of dilutions which are abiding the Beer Lambert's law. The intercept must be close to zero for the better linear following of dilution at selected wavelength. The result for linearity with respective R^2 values and slope parameters for the active pharmaceutical ingredients (API) are given in Table 5.

2. Range:
   a. Working range: It begins from limit of quantification to the maximum concentration used for the development of the analytical method. In this case, it is found to be 13.15-155 mg/ml and 3.17-36 mg/ml for LEVO and RBS, respectively.
   b. Linearity range: It is the interval in which the response is directly proportional to the concentration between the upper and lower levels including the level (which is generally ±5% of the intercept having a slope equal to zero). In this case, it is equal to 25-155 µg/ml and 4-36 µg/ml for LEVO and RBS respectively.
   c. Target range: It is that concentration which is 80%, 100%, and 120% of the target concentration. In this case, these are equal to 72.0, 90.0, and 108.0 µg/ml for LEVO and 16.0, 20.0, and 24.0 µg/ml for RBS.
   d. Target concentration: It is defined as the concentration, which is equal to the midpoint of linearity range. It is equal [(70+5)/2]=37.5 mg/ml. The results for range studies are shown in Table 6.

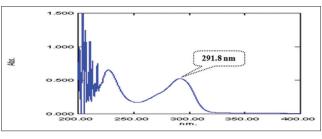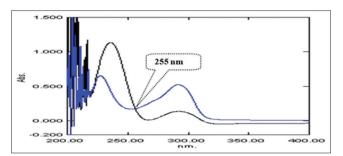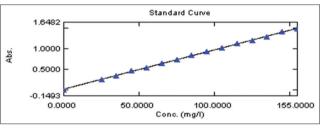3. Accuracy: The results of analysis are obtained in three group containing three replicate experiments with API and capsule dosage form, had good agreement with the labeled amount of the drug.
   a. Method: In nine different 10 ml volumetric flasks, 1 ml of the pre-analyzed mixture solution (100 µg/ml) was taken and added ml of standard solution of bulk (API) mixture (100 µg/ml), and the volume was 0.12, 0.16, 1.9 ml added up to 10 ml with solvent system. The results are shown in Table 7.

4. Precision:
   a. Repeatability: Repeatability was assessed using: Six-time repetition of target concentration 100% that is (20+90 µg/ml/2=55 µg/ml)
   b. Intra-day precision: In the study of the intraday which was conducted at three different times such as 9 am, 12 pm, 3 pm on the solution having the concentration value 80%, 100% and 120% of the target concentration (n=3)
   c. Interday precision: In the study of the interday which was conducted on the solution having concentration value 80%, 100%, and 120% of the target concentration (n=3), on three different days. The results of precision are shown in Table 6.

5. Limit of detection (LOD): The LOD was determined from the set of 6 calibration curves used to determine method linearity. The detection limit (LOD) may be expressed as:
   LOD=3.3(SD/S)

6. Limit of quantitation (LOQ): The LOQ was determined from the set of 6 calibration curves used to determine method linearity. The detection limit (LOQ) may be expressed as:
   LOQ=10(SD/S)
   Where SD=Standard deviation of the response. It is actually the intercept of the calibration curve, S=Slope of the calibration curve. The results are shown in Table 6.

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Table 5: Optical and regression parameters

Parameters	RBS		LEVO	
	255 nm	291.8 nm	255 nm	291.8 nm
Beer's law limit (µg/ml)	4-36		25-155	
Molar absorptivity (L mole^{-1} cm^{-1})	4.916×10^3	1.597×10^4	5.747×10^3	4.25×10^3
Sandell's sensitivity (mg/cm^2/0.001 absorbance unit)	0.0771	0.0238	0.0802	0.594
Regression equation				
Slope (b)	0.01231	0.04062	0.00960	0.00627
Intercept (a)	0.00586	0.01509	0.01263	0.00037
Correlation coefficient	0.99901	0.99900	0.99932	0.99940

RBS: Rabeprazole sodium, LEVO: Levosulpiride

Table 6: Summary of validation parameters

Validation parameters	LEVO	RBS
Specificity (% interference)	0.209	0.145
Linearity (µg/ml)	25-155	4-36
Range (µg/ml)	13.15-155	3.17-36
Precision** (% RSD)		
Repeatability (n=6)	0.91	0.825
Intraday (n=3)	0.255	0.177
Interday (n=3	0.92	0.720
Accuracy* (% recovery)	99.28	99.51
LOD (µg/ml)*	4.34	1.2
LOQ (µg/ml) *	13.15	3.17

*Acceptance criteria 99-101%, **acceptance criteria: RSD≤2. LOD: Limit of detection, LOQ: Limit of quantitation, RSD: Relative standard deviation, RBS: Rabeprazole sodium, LEVO: Levosulpiride
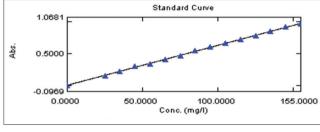
7. Specificity:
    a. Method: For specificity determination, a few excipients such as 8% starch, 7% magnesium stearate and 84% lactose, 1% talc, cellulose acetate phthalate (for 1000 µmg/ml) were added, then after filtration, any change in absorbance are observed. The results are shown in Table 8.
8. Assay procedure in capsule dosage form: 20 capsules were taken and the IP method was followed to determine the average weight.
    a. Method: Above weighed capsule were finally weighed and contents were triturated well. Quantity of powder equivalent to 25 mg of LEVO and 6.66 mg of RBS (ratio is 15:4) was transferred to 25 ml volumetric flask volume was made up to 25 ml with previously sonicated solvent. The solution was filtered and the filtrate, 5 ml was transferred to 25 ml volumetric flask and volume was made up to 25 ml with the solvent system. Then, 3.6, 4.5, 5.4 ml of aliquots were transferred to three 10 ml volumetric flasks and volume were made up to 10 ml with the same solvent. The absorbance was measured at 255 nm and 291.8 nm. Percent capsule claim for LEVO and RBS was determined by above-discussed equation 1 and 2. The results are shown in Table 9.
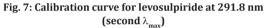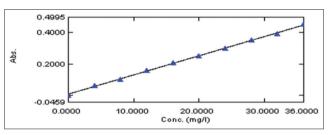
EXPERIMENTAL RESULTS

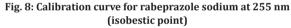Tables 5-9 summarizes the experimental results.

DISCUSSIONS

A UV spectrophotometry method was developed and validated for the determination of LEVO and rabeprazole in the binary mixture as per the ICH guidelines. The analytical method showed linearity in the concentration range of 25-155 µg/ml and 4-36 µg/ml for LEVO and RBS, respectively. The developed method showed good precision by % relative standard deviation of 0.255% and 0.177% for LEVO and RBS, respectively. Specificity of the developed analytical method was shown the negligible interference of the excipients. The % interference was 0.209% and 0.145% for LEVO and RBS, respectively. The accuracy of the method was validated by mean percentage recovery which was found to be in the acceptable range of above 99% for both the drugs in combination.

Fig. 7: Calibration curve for levosulpiride at 291.8 nm (second λ_{max})

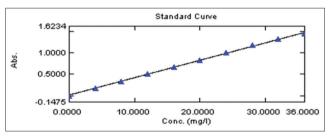**Fig. 8: Calibration curve for rabeprazole sodium at 255 nm (isobestic point)**

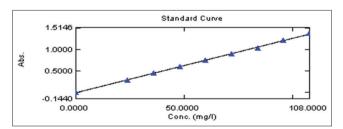**Fig. 9: Calibration curve for rabeprazole sodium at 291.8 nm (second λ_{max})**

Fig. 10: Calibration curve for synthetic mixture at 255 nm

CONCLUSIONS

The proposed method was linear, accurate, precise, and specific as per validation parameters (Table 9) discussed above as per ICH guidelines.

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Table 7: Recovery study (accuracy) in synthetic mixture

S.No.	WL (nm)	Concentration of solution (ppm)	Standard added (ppm)	Absorbance	Amount found (mg)	% Recovery	Mean recovery±SD	% RSD
1*	255	90	4.86	1.18	94.2	99.36	LEVO	
	291.8	24	1.28	1.59	25.1	99.41	99.28±0.1497	0.99
2*	255	90	4.86	1.17	94.1	99.58		
	291.8	24	1.28	1.58	25.0	99.33		
3*	255	90	4.86	1.19	94.3	99.6	RBS	
	291.8	24	1.28	1.59	24.9	99.12	99.51±0.1331	1.00

*Replicated 3 times. RBS: Rabeprazole sodium, LEVO: Levosulpiride, SD: Standard deviation

Table 8: Specificity study for the synthetic mixture (LEVO: RBS=15:4)

Mix	Concentrated (µg/ml) LEVO: RBS	λ_{max} (nm)	Before addition of excipients		After addition of excipients		% Interference
			Absorbance	Concentrated (µg/ml)	Absorbance	Concentrated (µg/ml)	
1	30:8	255	0.5946	29.19	0.5935	29.06	−0.445
		291.8	0.7019	7.41	0.7123	7.43	0.269
2	45:12	255	0.7611	44.1	0.7632	44.3	0.453
		291.8	0.9251	11.5	0.9239	11.3	−1.739
3	60:16	255	0.9020	59.3	0.9053	59.5	0.337
		291.8	1.1120	15.3	1.1153	15.6	1.96
4	75:20	255	1.0445	74.2	1.0491	74.5	0.404
		291.8	1.3025	19.4	1.2785	19.6	1.03
5	90:24	255	1.1656	88.89	1.1695	89.2	0.348
		291.8	1.5092	23.78	1.5391	23.8	0.0841
6	105:28	255	1.3377	104.2	1.3564	104.5	0.287
		291.8	1.7069	27.6	1.6992	27.7	0.362
7	120:32	255	1.5162	119.2	1.5234	119.3	0.083
		291.8	1.9479	31.7	1.9325	31.4	−0.946
Mean	LEVO						0.209
	RBS						0.145

RBS: Rabeprazole sodium, LEVO: Levosulpiride

Table 9: Assay of rabeprazole and LEVO

Formulation	Label claim (milligrams)		Amount found (milligrams)		Percentage assay±SD (n=3)	
	RBS	LEVO	RBS	LEVO	RBS	LEVO
Synthetic mixture	20	75	19.76	74.4	98.25±0.2102	99.2±0.4031
Rabikind plus	20	75	19.76	74.66	98.69±0.1934	99.50±0.3093

RBS: Rabeprazole sodium, LEVO: Levosulpiride, SD: Standard deviation
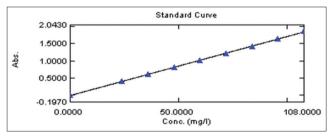

Fig. 11: Calibration curve for synthetic mixture at 291.8 nm

The developed method was evaluated successfully for the content determination of marketed formulation of a combination of LEVO (75 mg) and RBS (20 mg) in capsule dosage form as well self-made synthetic mixture. It can also be used for any other solid dosage form for the same combination.

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