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Development and Validation of UV Spectroscopic and RP-HPLC method for Simultaneous Estimation of Levosulpiride and Rabeprazole Sodium in bulk and tablet dosage form

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ABSTRACT:

Three simple, rapid, accurate, precise and cost-effective UV spectrophotometric methods and RP – HPLC have been developed and validated for simultaneous estimation of Levosulpiride and Rabeprazole Sodium in tablet dosage form. Method I is estimation using simultaneous equation method at 232 nm (λmax of Levosulpiride) and 284 nm (λmax of Rabeprazole Sodium). Method II is 1st order derivative method utilize absorbance measurement at 247 nm for Levosulpiride and 291.60 nm for Rabeprazole Sodium. Method III is RP-HPLC method for simultaneous estimation of Levosulpiride and Rabeprazole Sodium separation was achieved on a Phenomenexluna ODS C18 (250mm X 4.6 mm i.d., 5 µm particle size) with an mobile phase acetonitrile: 50 mM phosphate buffer pH 5 (adjusted with Sodium hydroxide) in the ratio of 55:45 v/v. The mobile phase at a flow rate of 1.0 ml/min, Injection volume 20µl and detection wavelength was kept at 288 nm. The retention time Levosulpiride and Rabeprazole Sodium was 2.31±0.1min and 3.85 ±0.1min, respectively. The linearity lies between 5-30 µg/ml for Levosulpiride and 2-12 µg/ml for Rabeprazole Sodium.The proposed conditions were successfully applied for the simultaneous estimation of both drugs in commercial tablet preparation and were validated according to ICH guidelines.

KEYWORDS:

Levosulpiride, Rabeprazole Sodium, UV spectrophotometry, Simultaneous equation method, Derivative method, RP-HPLC.

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INTRODUCTION:

Levosulpiride is a substituted benzamide derivative and a selective dopamine D2 antagonist with antipsychotic and antidepressant activity. Chemically Levosulpiride is 5- (aminosulfonyl) -N- [(1-ethyl- 2-pyrrolidinyl) methyl]- 2-methoxy benzamide.

Rabeprazole Sodium is an antiulcer drug in the class of proton pump inhibitors. It inhibits the H+, K+ ATPase of the coating gastric cells. Chemically Rabeprazole is 2-({[4-(3-methoxypropoxy)-3- methylpyridin-2-yl] methane} sulfinyl)-1H-1, 3-benzodiazole.

Levosulpiride and Rabeprazole Sodium in combine tablet dosage form is available in the market. Levosulpiride and Rabeprazole Sodium combination have been reported to be effective in treatment of psychic patient and to suppress acid secretion in stress condition. The literature survey reveals that UV spectrophotometry and RP-HPLC methods have been developed for Levosulpiride and Rabeprazole Sodium in single dosage forms. However, no methods are yet reported for simultaneous estimation of Levosulpiride and Rabeprazole Sodium by simple spectrophotometry method in pharmaceutical preparation.

Hence, main purpose of this study was to develop a simple, accurate, reproducible, rapid and economical method for simultaneous estimation of Levosulpiride and Rabeprazole sodium in tablet formulation and validating of method as per ICH norm.

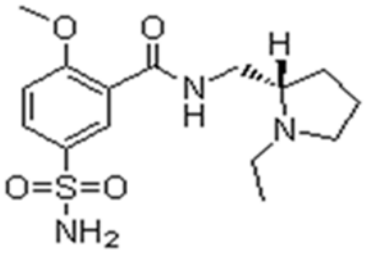

Figure 1 chemical structure of Levosulpiride

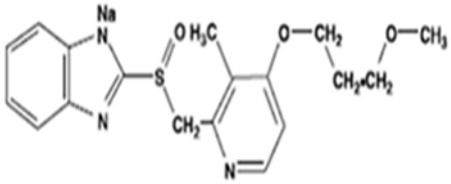

Figure 2 chemical structure of Rabeprazole

UV SPECTROSCOPY

MATERIAL AND METHOD

Instrument used in current research was UV/Visible spectrophotometer with resolution of 1 nm and 0.5 mm slit width (model UV – 1800, shimanzu, Japan) connected to a HP computer system loaded with UV – probe 2.21 software. Standard gifts sample of LEVO and RABE were supplied by Metro-chem API Pvt. Ltd Ahmedabad, India. LEVO and RABE combination tablet (Rabonic Plus,75 mg LEVO and 25 mg RABE , manufactured by Eris Life sciences Pvt. ltd. Ahmedabad, Gujarat) were purchased from local pharmacy. Methanol of analytical grade was used as solvent and purchased from sigma Aldrich, India.

Preparation of standard stock solution

An accurately weighed standard powder of 50 mg of LEVO and RABE were transferred in 50 ml volumetric flask separately, dissolved and diluted up to the mark with methanol AR grade, to get final concentration 1000 µg/ml of LEVO and RABE. From the above stock solution,100 µg/mlwas prepared by diluting 1 ml of stock solution to 10 ml with methanol.

From this standard stock solution, different aliquots were transferred into 10 ml volumetric flask and volume was made up to the mark with Methanol. This solution was used as a working standard solution (WSS).

Selection of analytical wavelength

5 µg/ml solution of LEVO was prepared in methanol and spectrum was recorded between 200-400 nm.Similarly 2 µg/ml solutions of RABE was prepared in methanol and spectrum was recorded between 200-400 nm. The overlain spectrum of both drug wererecorded.(Figure 3)

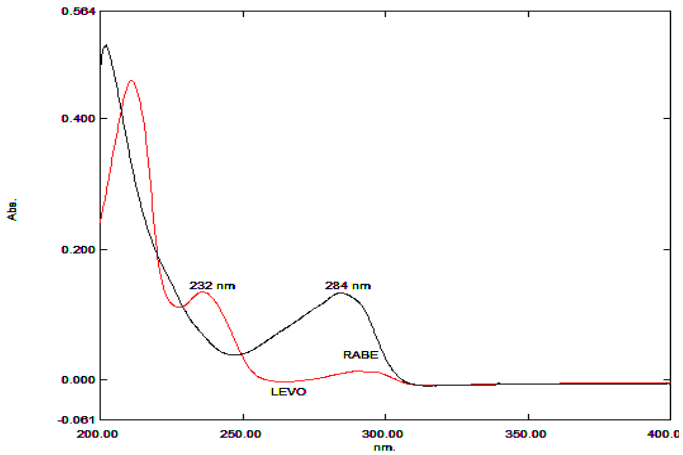

Figure 3 Overlain spectra of Levosulpiride(5 µg/ml) and Rabeprazole Sodium (2 µg/ml) in methanol

Preparation for calibration curve

For construction of calibration curve, two series of different concentration in range of 5-30µg/ml for LEVO and 2-12µg/ml for RABE were prepared in Methanol from stock solution. These solutions were scanned in range of 200-400 nm and absorbances were measured at selective wavelength and calibration curve were plotted for absorbance vs. concentration.

RP- HPLC

Selection of detection wavelength

In the present study individual drug solutions of 10µg/ml was prepared in methanol. These drug solutions were scanned in the UV region of 200-400 nm and the overlain spectrums were recorded. Overlain spectra showed an appreciable absorbance at 288 nm.Hence it was selected as the wavelength for detection.

Selection of Mobile phase

Optimization can be started only after a reasonable chromatogram has been obtained. A reasonable chromatogram means that more or less symmetrical peak on the chromatogram detects all the compounds. By slight change of the mobile phase composition, the position of the peaks can be predicted within the range of investigated changes. An optimized chromatogram is the one in which all

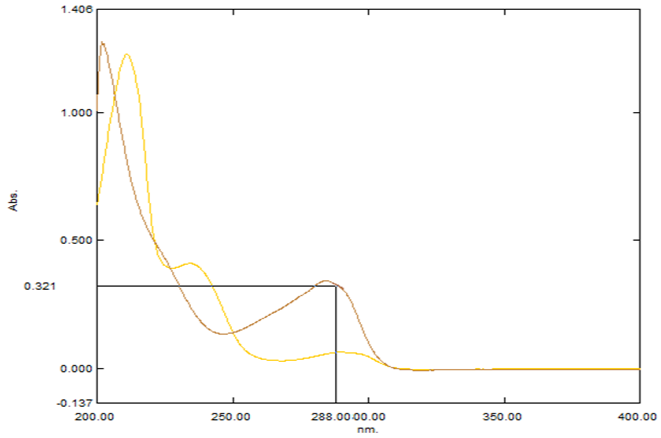

Figure 4 Overlain spectra of Levosulpiride and Rabeprazole Sodium (10(µg/ml)

the peaks are symmetrical and are well separated in less run time. The mobile phase was selected on the basis of best separation, peak purity index, peak symmetry, theoretical plate etc. So, numbers of trial were taken. After number of trial Acetonitrile: 50mM Phosphate buffer (pH 5) (55:45v/v) was selected.

Method I(simultaneous equation method)

Two wavelengths selected for the method are 232nm(λ1) and 284 nm(λ2) that are absorbance maxima of LEVO and RABE respectively in Methanol .Standard stock solution(s) of 100 µg/ml each of LEVO and RABE werepreparedseparately in Methanol. The stock solutions of both the drugs were further diluted separately with to get a series of standard solutions of 5-30 µg /ml of LEVO and 2-12µg /ml of RABE. The absorbance was measured at the selected wavelengths and absorptivities (A 1%, 1 cm) for both the drugs were determined as mean of three independent determinations. Concentrations in the sample were obtained by using following equations:

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

Cx and Cy = Concentration of RABE and LEVO respectively (gm/100 ml)
ax1 and ax2= Absorptivity of RABE at λ1 and λ2 respectively
ay1 and ay2= Absorptivity of LEVO at λ1 and λ2 respectively
A1 and A2= Absorbance of test at λ1 and λ2 respectively

Method II (1st order derivative method)

Zero Crossing 1st Derivative spectrophotometric method was developed for simultaneous estimation of LEVO and RABE in their binary mixture. In this method the absorbance at 247 nm (Zero crossing point of RABE) of the 1st derivative spectra of the binary mixture containing LEVO and RABE were measured for the estimation of LEVO. Similarly the absorbance at 291.60 nm (Zero crossing point of LEVO) of the 1st derivative spectra of the binary mixture containing LEVO and RABE were for the estimation of RABE. Linearity was observed over concentration range of 5-30 µg/ml for LEVO and 2-12µg/ml for RABE. The proposed Zero Crossing 1st Derivative method is found to be simple, specific, accurate, precise, robust, rapid and economical. Binary mixture and Formulations were successfully analyzed using the developed method.

Method III (RP -HPLC)

RP –HPLC method was developed for simultaneous estimation of LEVO and RABE in their binary mixture. In this method the absorbance at 288 nm selected for both. Optimized chromatographic condition and mobile phase selected Acetonitrile: 50 mM Phosphate buffer with adjusting pH 5. Flow rate is 1.0 ml/min and run time is 6 minute.

Procedure for Analysis of Tablet Formulation

To determine the content of Levosulpiride and Rabeprazole Sodium simultaneously in tablets (label claim: 75 mg Levosulpiride and 20 mg Rabeprazole Sodium, Enteric coated); twenty tablets were weighed and average weight was calculated. The tablets were crushed to obtain fine powder. Tablet powder equivalent to 75 mg of Levosulpiride was transferred to 50 ml volumetric flask and diluted to 50 ml with methanol. Sonication for 15 min .It was mixed and filtered the resulting solution with what Mann filter paper. 3.5 ml of resulting solution was taken and diluted to 50 ml with methanol mixed properly. The resulting solution appropriate diluted with methanol to obtain 15µg/ml of Levosulpiride and 4 µg/ml of Rabeprazole Sodium. The concentration of both Levosulpiride and Rabeprazole Sodium were determined by measuring the absorbance of the sample at 232.0 nm, 284.0 nm .In HPLC method concentration of both Levosulpiride and Rabeprazole Sodium were determined at 288 nm and dilution were made by using mobile phase as diluents .The results of the tablet analysis were calculated against the calibration curve in quantitation mode.

METHOD VALIDATION

The proposed methods were validated accordance to ICH Q2 (R1) guidelines for linearity, precision, accuracy, limit of detection, limit of quantification. The results are shown in table 1

Linearity & Range

The standard stock solution containing 25 mg/ml each of LEVO and RABE were further diluted to get linearity conc. of 5 -30 µg/ml LEVO and 2 – 12 µg/ml for RABE Respectively. Calibration curve was plotted by taking absorbanc on Y – axis and conc on X – axis the relation between drug and its absorbance is expressed by the equation Y = MX + C, where 'M' is slope and 'C' is intercept,linear regression eq.as follow.(table 1)

Accuracy

Recovery studies were performed to validate the Accuracy of developed method by adding a 50 %,100 %,150 %. of standard drug in Pre-analyzed sample solution. These results summarized in (table 2)

Precision

Repeatebility

Nine dilution in three replicate were analyzed in same day for repeatability and result were found within acceptable limit (RSD < 2) as shown in table 3.

Intermediate Precision

Nine dilutions in the three Replicate ware analyses on two different day, two analysts for day to day & analyst to analyst variation. All result were fall within acceptable limits (RSD < 2) as shown in (table 3)

Limit of Detection and Quantification

The limit of detection and limit of quantification were estimated from the std. calibration curve. The residual standard deviation of regression line or std. deviation of Y – intercepts of regression lines was used to calculate LOD and LOQ. Here LOD = 3.3*D/S and LOQ = 10*D/S. Where D is the Standard deviation of Y – intercept of regression line S is the slope of calibration curve (table 1).

RESULT AND DISCUSSION

Both, UV spectrophotometric and HPLC methods were found to be simple, accurate, economic and rapid for routine simultaneous estimation ofLevosulpiride and Rabeprazole Sodium, in tablet dosage forms. For UV spectrophotometric method, linearity was obtained in concentration range of 5– 30µg/ml of Levosulpiride and 2- 12 µg/ml of Rabeprazole Sodium, with regression 0.9996 and 0.9997, intercept – 0.0388and – 0.0535 and slope 0.0184 and 0.0366 for Levosulpiride and Rabeprazole Sodium, respectively. Recovery was in the range of 99 – 101 %; the value of standard deviation and % R.S.D. were found to be < 2 %; shows the high precision of the method In HPLC method, HPLC conditions were optimized to obtain, an adequate separation of eluted compounds. Initially, various mobile phase compositions were tried, to separate drugs. Mobile phase and flow rate selection was based on peak parameters (height, tailing, theoretical plates, capacity factor), run time etc. The system with acetonitrile: 50 mM Phosphate buffer pH 5 (55:45v/v) with 1 mL.min-^{1}flow rate. The optimum wavelength for detection was 288 nm at which better detector response for drugs was obtained. The average retention times forLevosulpiride and Rabeprazole Sodium was found to be, 2.317 min and 3.853 min, respectively. The calibration was linear in concentration range of 5– 30µg /mlof Levosulpiride and 2- 12 µg/ml of Rabeprazole Sodium, with regression 0.9996 and 0.9999, intercept – 20.206 and – 6.0893and slope 6.7319 and 37.868 for Levosulpiride and Rabeprazole Sodium, respectively. The low values of % R.S.D. indicate the method is precise and accurate. The mean recoveries were found in the range of 99 – 100 %.

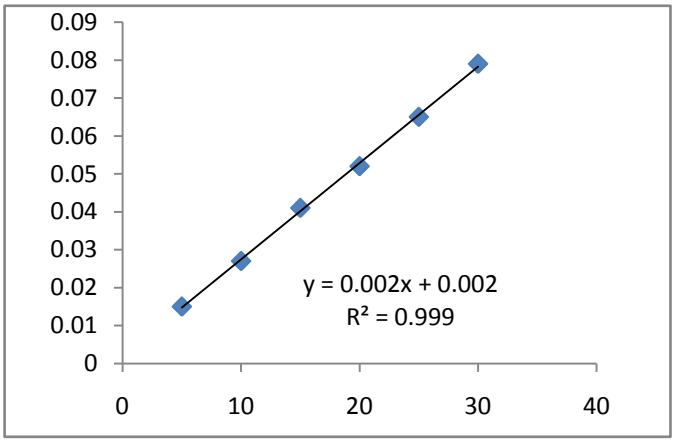

Figure 5 Calibration curve of Rabeprazole Sodium at 232 nm

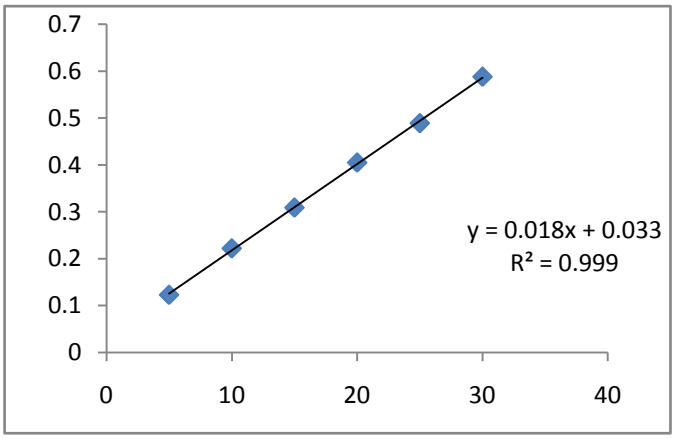

Figure 6 Calibration curve of Levosulpiride at 284 nm

Table 1 Summary of Linear regression analysis and optical characteristics of LEVO ad RABE

Parameter	UV Spectroscopy				RP HPLC	
	Method I		Method II			
	LEVO	RABE	LEVO	RABE	LEVO	RABE
Analytical wavelength(nm)	232	284	247	291.60	288	
Beer's law limit (µg/ml)	5-30	2-12	5-30	2-12	5-30	2-12
Coefficient of Correlation(r^2)	0.9996	0.9997	0.9990	0.9990	0.9990	0.9990
Slope	0.0184	0.0366	0.0009	0.0143	6.731	37.86
Y Intercept	0.0335	0.0534	0.0009	0.0143	20.20	6.089
LOD(µg/ml)	0.053	0.027	1.4	0.707	0.8	0.15
LOQ(µg/ml)	0.163	0.081	4.5	2.1	2.4	0.45

Table 2: Results of Recovery Study

Amount taken			% Recovery*					
LEVO (µg/ml)	RABE (µg/ml)	% Added	Method I		Method II		RP- HPLC	
			LEVO	RABE	LEVO	RABE	LEVO	RABE
10	4	50	99.40	99.50	101	99.50	100.4	100.50
	100	99.45	99.25	100.75	100.50	101.5	100.75	
	150	99.70	99.16	100.90	100.83	100.6	100.16	
	Mean		99.50	99.33	100.88	100.27	100.8	100.47
	SD		0.403	0.216	0.205	0.823	0.130	0.162
	%RSD		0.412	0.219	0.208	0.824	1.21	0.98

*Average value of three determinations, RSD –Relative standard deviation, SD – Standard Deviation

Table 3: Result of Precision Study

	LEVO	RABE	Method I		Method II		RP HPLC	
	(µg/ml)	(µg/ml)	LEVO	RABE	LEVO	RABE	LEVO	RABE
	5	2	0.99	0.94	0.77	1.40	2.0	0.96
Intraday	15	6	0.75	1.47	0.52	0.90	0.99	1.1
***(% RSD)**	30	12	0.45	0.42	0.31	0.64	0.97	0.52
Interday*(%RSD)	5	2	1.43	2.0	1.7	1.7	0.40	0.57
	15	6	0.45	0.60	0.77	1.1	0.40	1.0
	30	12	0.26	0.32	0.44	0.58	0.63	0.38

***Average value of three determinations, RSD –Relative standard deviation**

Table 4: Analysis of marketed formulation by proposed method

		Assay* ± SD		
Drug	Rabonic Plus Label claim mg/tablet	Method I	Method II	RP HPLC
LEVO	**75**	99.86 %±0.0141	109.08 %±0.028	99.97 %±0.141
RABE	**20**	99.66% ±0.0158	100.58% ±0.030	99.95% ±0.158

**Average value of six determinations, SD –standard deviation*

CONCLUSION

All the validation parameters for all the developed methods were studied as per the ICH guidelines. All the methods were found to be simple, accurate, Specific, Selective, Precise and reproducible. Hence, the methods can be used for routine analysis of both the drugs in their combined solid dosage form.

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