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Development and Validation of Spectrophotometric Method for Estimation of Levosulpiride in Bulk and Tablet Dosage Form

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ABSTRACT
A simple, accurate, precise and economical spectrophotometric method has been developed for estimation of Levosulpiride in bulk form as well as marketed formulations. The estimation of Levosulpiride was done at 291.2 nm in pH 6.8 phosphate buffer using UV-Visible double beam spectrophotometer. In the developed method, linearity over the concentration range of 10-100µg/ml of Levosulpiride was observed and was found in agreement of Beer's law. The linear regression was found to be 0.999. The results of analysis have been validated statistically and recovery studies confirmed the accuracy of the proposed method. The precision (intra-day & inter-day) of method was found within limits (RSD < 2%). The sensitivity of the method was assessed by determining limit of detection and limit of quantification. The percentage of Levosulpiride in the marketed formulation (LEFIT-50) was observed to be 99.46%. It could be concluded from the results obtained in the present investigation that the method for estimation of Levosulpiride in pure form and in pharmaceutical dosage form is simple, rapid, accurate, precise and economical and can be used, successfully, in the quality control of pharmaceutical formulations and other routine laboratory analysis.
Keywords: levosulpiride, UV spectrophotometer, beer's law, validation

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INTRODUCTION

Levosulpiride is a substituted benzamide derivative cegorized as atypical antipsychotic and antidepressant. Chemically, it is a levo form of sulpiride having chemical formula 5-(aminosulfonyl) -N- [(1-ethyl- 2-pyrrolidinyl) methyl]- 2-methoxy benzamide. Officially, it is not listed in any Pharmacopoeia, but is included in Martindale: The Complete Drug Reference [1] and Merck Index [2].

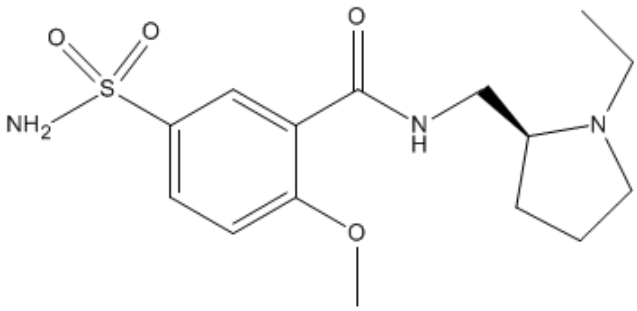

Figure 1. Structure of Levosulpiride

It is reported to have selective antagonistic activity against dopamine D_2 receptors on both central and peripheral levels. Levosulpiride is also claimed to have mood elevating properties [3]. At low doses, levosulpiride increases dopaminergic neurotransmission, primarily by blocking the dopamine autoreceptors, which inhibits the pre-synaptic dopamine synthesis and release of dopamine [4]. Levosulpiride is used in the treatment of psychoses, particularly negative symptoms of schizophrenia, anxiety disorders, dysthymia, vertigo, dyspepsia, irritable bowel syndrome and premature ejaculation. Compared with racemic and dextro-forms, the levo-form of sulpiride has greater central antidopaminergic activity, antiemetic and antidyspeptic effects with lower acute toxicity [5].

RP-HPLC method is used for determination of Levosulpiride in human plasma. HPLC method is used for the estimation of Levosulpiride in bulk as well as marketed formulations. This method has the disadvantage of being time consuming and expensive.

Another method (UV spectrophotometric) of Levosulpiride was developed at 291 nm in 0.1N NaOH, 288.7 nm in Methanol and first order derivative spectrum in Methanol at 282.4 nm with n=1 [6].

The main objective of this research work was to develop & establish a simple, accurate, rapid and economical method for the estimation of Levosulpiride content in marketed tablet formulation and to validate the developed method following International Conference on Harmonization (ICH) guidelines [7].

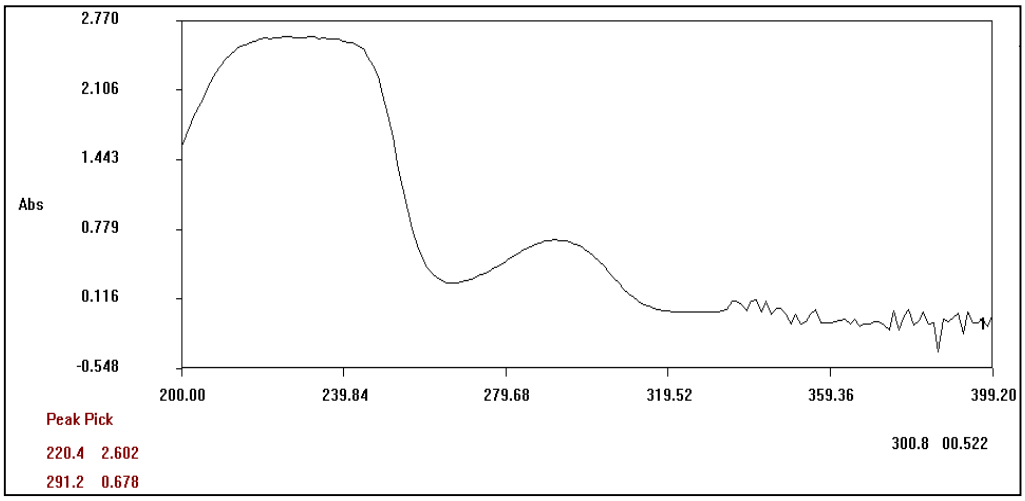

Figure 2. UV Spectrum of Levosulpiride

MATERIALS AND METHODS

Instrumentation

A double beam Systronics UV-Visible Spectrophotometer, model UV-2201 (India) with a spectral bandwidth of 1nm, wavelength accuracy of ±0.5nm and a pair of 1cm quartz cells were used to measure absorbance of the resulting solution.

Materials

Standard sample of Levosulpiride was kindly gifted by Shagun Pharmaceuticals, Mumbai (India). The marketed Levosulpiride tablets (LEFIT-50) containing 50 mg of Levosulpiride and manufactured by Avenue Remedies (India) Pvt. Ltd., were used for the analysis. All other chemicals used in study were of analytical grade.

Preparation of stock solution

Accurately weighed 10 mg of Levosulpiride standard was transferred into 10 ml volumetric flask. Then dissolved in some amount of pH 6.8 phosphate buffer. The solution was sonicated for 1 minute in bath sonicator and diluted up to the mark with 6.8 pH phosphate buffer to have a stock solution. From this, 1ml was taken and diluted further to have the working solution with a concentration of 100µg/ml.

Preparation of sample stock solution

To measure the Levosulpiride content of tablet (label claim 50 mg Levosulpiride per tablet), twenty tablets were weighed and their mean weight was determined. The tablets were crushed and a weight of the powder equivalent to 100 mg of Levosulpiride was transferred to a 10 ml volumetric flask containing 5 ml phosphate buffer and the mixture was sonicated for

1 minute and made up the volume with phosphate buffer (stock solution). The solution was filtered and 1 ml of filtered solution was diluted tenfold to furnish a concentration of 100µg/ml.

VALIDATION OF PROPOSED METHODS

The method was validated in terms of linearity, precision, specificity Limit of Detection and Limit of Quantification.

Linearity and range

The linearity was determined by analyzing 5 independent levels of calibration curve in the range of 10-100 µg/ml. Absorbance of each solution against 6.8 pH phosphate buffer was recorded at 291.2 nm. The calibration curve of absorbance vs. concentration was plotted and correlation co-efficient and regression equation for Levosulpiride were determined.

Accuracy

Accuracy of the method was assessed by percentage recovery experiments performed at three different levels, that is, 50, 100, and 150%. Known amounts of standard Levosulpiride solutions were added to the preanalyzed sample solutions; absorbances were recorded and reanalyzed by proposed method. The % recovery was calculated by using formula [8]:

$$\% \, \mathbf{Recovery} = \frac{\mathbf{A} - \mathbf{B}}{\mathbf{C}} \times \mathbf{100}$$

where A is the total amount of drug estimated, B is the amount of drug found on preanalyzed basis, and C is the amount of bulk drug added.

Precision

Intra-day precision was determined by analyzing Levosulpiride (10-100 µg/ml) at three different time points of the same day and inter-day precision was determined by analyzing Levosulpiride (10-100 µg/ml) at three different time points on different days and %RSD was calculated [9,10].

Limit of Detection (LOD) Limit of Quantification (LOQ)

The LOD and LOQ were estimated from the set of 5 calibration curves used to determine method linearity [11, 12].

$$\mathbf{LOD} = \mathbf{3.3} \times \boldsymbol{\sigma}/\mathbf{S} \text{ and } \mathbf{LOQ} = \mathbf{10} \times \boldsymbol{\sigma}/\mathbf{S}$$

where σ is the standard deviation of y-intercepts of regression lines, and S is the slope of the calibration curve.

Table 1. Calibration data of Levosulpiride by UV spectrophotometer

Sr. No.	Concentration (µg/ml)	Absorbance
1.	10	0.048
2.	20	0.098
3.	30	0.151
4.	40	0.207
5.	50	0.269
6.	60	0.320
7.	70	0.378
8.	80	0.442
9.	90	0.502
10.	100	0.554

Table 2. Absorbance of Levosulpiride marketed tablets solution in phosphate buffer pH 6.8

Sr. No.	Concentration (µg/ml)	Absorbance (n=3)
1	10	0.071
2	20	0.119
3	30	0.196
4	40	0.266
5	50	0.331
6	60	0.386
7	70	0.459
8	80	0.531
9	90	0.601
10	100	0.676

Analysis of marketed tablets by UV spectrophotometric method

From the sample stock solution, 1 ml was taken into a 10 ml volumetric flask and diluted upto the mark with phosphate buffer pH 6.8 (i.e. 10 µg/ml).

RESULTS AND DISCUSSION

Linearity

The linearity of Levosulpiride was found to be in the range of 10 -100 µg/ml with correlation co-efficient 0.999. Calibration data with %RSD is shown in **Table 1** and calibration curve is shown in **Figure 3**.

Similarly the linearity of Levosulpiride in the marketed formulation was found to be in the range of 10 -100 µg/ml with correlation co-efficient 0.998. Calibration data with %RSD is shown in **Table 2** and calibration curve is shown in **Figure 4**.

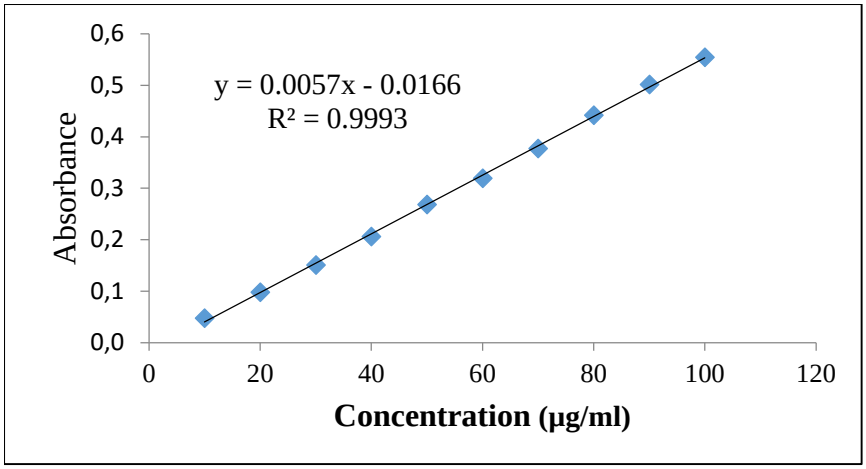

Figure 3. Calibration Curve of Levosulpiride by UV Spectrophotometer

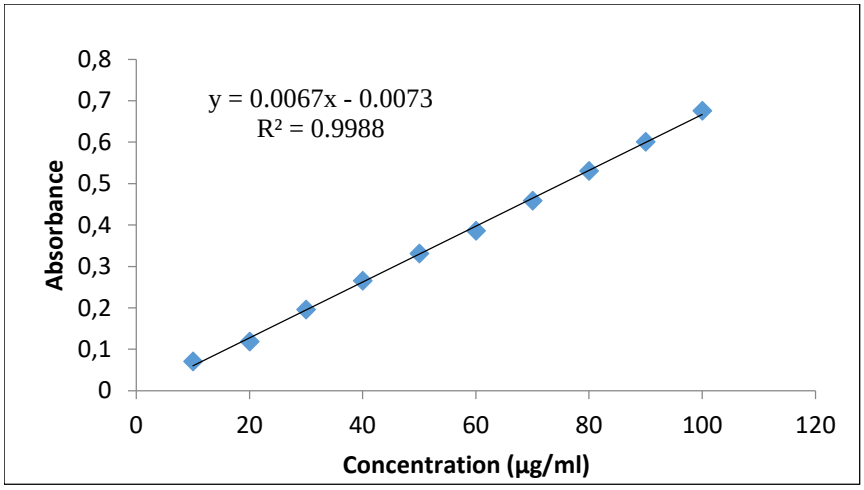

Figure 4. Calibration Curve of marketed tablets of Levosulpiride

Accuracy

Accuracy of the method was checked by the recovery studies at three different levels, that is, 50%, 100%, and 150%. The mean % recovery for Levosulpiride was found to be 100.15%. The results obtained are shown in **Table 3**.

Precision

Intra-day precision

%RSD was found to be in the range of 0.23-0.33.

Inter-day precision

%RSD was found to be in the range of 0.17-0.32.

Table 3. Results of recovery studies

Recovery Level	Initial Concentration	Concentration of standard	% Recovery (n=3)
50%	20	10	100.46
100%	20	20	100.12
150%	20	30	99.89
Mean			100.15

Table 4. Results of Intra-day precision

Concentration (µg/ml)	Absorbance mean	%RSD
30	0.465	0.33
50	0.494	0.23
70	0.582	0.26

Table 5. Results of Inter-day precision

Concentration (µg/ml)	Absorbance mean	% RSD
30	0.463	0.32
50	0.494	0.30
70	0.580	0.17

The results indicate acceptable accuracy and precision of the proposed methods for the analysis of the drug.

LOD & LOQ

The sensitivity of method was assessed by determining LOD and LOQ. For Levosulpiride, LOD and LOQ were found to be 1.27µg/ml and 3.86µg/ml, respectively.

Analysis of marketed tablets by UV spectrophotometric method

The percentage of Levosulpiride in the marketed formulation (LEFIT-50) was calculated using the calibration curve of drug. The result of percentage assay is given in the table below.

CONCLUSION

The proposed spectrophotometric method was found to be simple, sensitive, accurate, precise, reproducible and economical which can be used for the routine simultaneous estimation of Levosulpiride in bulk form and marketed formulations.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests.

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Table 6. Estimation of Levosulpiride in marketed tablets

Marketed Brand	Label Claim	Assay (% of label claim*)± % RSD
LEFIT-50	50 mg	99.46 ± 0.66

**Average of three estimations*

Table 7. Summary of Validation parameters

Validation Parameters	Results
Absorption maxima (nm)	291.2
Linearity range (µg/ml)	10-100
Standard Regression Equation	Y= 0.005x-0.016
Slope (m)	0.005
Y – intercept (c)	0.016
Correlation Co-efficient (R^2)	0.999
% Recovery	100.15
LOD (µg/ml)	1.27
LOQ (µg/ml)	3.86
Precision (%RSD)	
Intra-day (n=3)	0.273
Inter-day (n=3)	0.263

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