 DOI: 10.18231/2394-2797.2016.0007

A validated UV-Visible Spectrophotometric method for Levosulpiride in bulk and Pharmaceutical formulation

Prasanth SS[1,*], Akhila V[2], Snijarukku PG[3], Fathimath Suhana T[4]

[1]Professor, [2,3,4]Research Scholar, Dept. of Pharmaceutical Analysis, Al-Shifa College of Pharmacy, Kizhattoor, Kerala

***Corresponding Author:**
Email: nakulprasanth@gmail.com

Abstract

The Levosulpiride in bulk and Pharmaceutical formulation was estimated by a validated UV-Visible spectrophotometer method. The λ_{max} obtained for Levosulpiride was 288.1nm in 0.1 N HCl. The drug shows linearity in a concentration range of 6-36µg/ml. The correlation coefficient for standard graph was found to be 0.999. The assay percentage of marketed formulation by the proposed method was good with label claim. The accuracy of the method was checked by recovery experiment performed at three different levels 80%, 100%, 120% and the percentage recovery was in the range 98.00-102.00%. The % RSD found was low which in turn is an indication of the accuracy and reproducibility of the method. Precision study of the method carried out as intra-day, inter-day variations and repeatability which was in good agreement with %RSD. The proposed method was found to be rugged and robust. Hence the above method can be applied for routine analysis of Levosulpiride in bulk and in pharmaceutical dosage form.

Keywords: Area under Curve, Levosulpiride, Validation

Introduction

Levosulpiride is an anti-psychotic Levo-isomer of sulpiride. It can be used for the treatment of Schizophrenia, anxiety disorders and peptic ulcers[1]. It is chemically 5-(aminosulfonyl) -N-[(1-ethyl-2-pyrrolidinyl)methyl]-2-methoxybenzamide (Fig. 1). It is listed in The Merck Index.[(2)] It is not official in any pharmacopoeia.

Levosulpiride, act as a D_2-dopamine antagonist which at low doses increases the dopaminergic neurotransmission, by blocking the dopamine auto receptors which inhibits the pre-synaptic dopamine synthesis and release of dopamine.[(3)] Commonly prescribed to patients with psychosis, depression and functional dyspepsia. Racemic activity was studied by Tonini et *al* and proved Levo form has more activity. Lozano *et al* conducted Toxicity study of Levosulpiride.[(5)] Silambaresan *et al* developed UV Spectrophotometric method and RP-HPLC method for estimation of Levosulpiride in bulk drug and in formulation.[(6)] The aim of this study is to develop simple, sensitive, precise, cost effective and specific analytical methods for the estimation of Levosulpiride in bulk and formulation

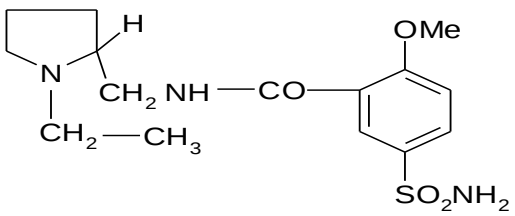


Fig. 1: Chemical structure of Levosulpiride

Materials and Methods

A gift sample of Levosulpiride (pure sample) was obtained from Sun Pharmaceuticals, Jammu. The chemicals with an analytical grade of Hydrochloric acid and Sodium hydroxide and Methanol are used. Shimadzu 1700 UV-Visible spectrophotometer with UV Probe software was used through- out the study.

Quartz cell having path length of 10mm was used as sample holder. The Tablets were procured from local pharmacy. Approximately 100 mg of pure sample was accurately weighed and dissolved in 100 ml of their respective solvents(1 mg/ml). A Levosulpiride stock solution with concentration of 100 µg/ml was made in the respective solvents. In case of formulations twenty tablets were accurately weighed and powdered and then 100 mg of Levosulpiride equivalent was taken for the study. For preparation of different concentrations, aliquots of stock solutions were transferred in to a series of 10ml standard flasks and volumes were made with respective solvents. Five different concentrations were prepared in the range of 20-100µg/ml of Levosulpiride in 0.1 N HCl. The solution were scanned in the spectrum mode from 400 nm to 200nm wavelength range. This solution showed a peak in 280-300nm range. The method was applied for the sample solution of unknown concentration and peak was recorded.

Method: We can use area under curve (AUC) method using UV Visible spectrophotometer if the peak obtained for a standard solution is broad or not sharp. The area under the peak is calculated in means of square millimeter and plotted as a function of concentration. For that two wavelength are selected λ_1 and λ_2. Area calculation software calculates the area under the curve at the selected wavelength range. The area for different concentrations of standard are found out with repeated scanning and plotted against corresponding concentration in order to get a linear graph. A calibration curve was constructed by plotting concentration(20-100µg/ mL) versus Area Under curve in the selected wavelength range.[7]

Validation of the proposed method: The proposed method was validated in terms of linearity, accuracy, precision and ruggedness.

Linearity study: Stock Levosulpiride solution in 0.1N HCl were transferred into series of 10 ml volumetric flasks and the volume was made up to the mark with water to get concentrations 20,40,60,80,100µg/ml, respectively. The solutions were scanned on spectrometer in the UV range 200-400 nm. The two wavelengths 220 and 288 nm was selected for the determination of Area under Curve (Fig. 2). The calibration plot was constructed as a function of Area under Curve vs. Concentration.

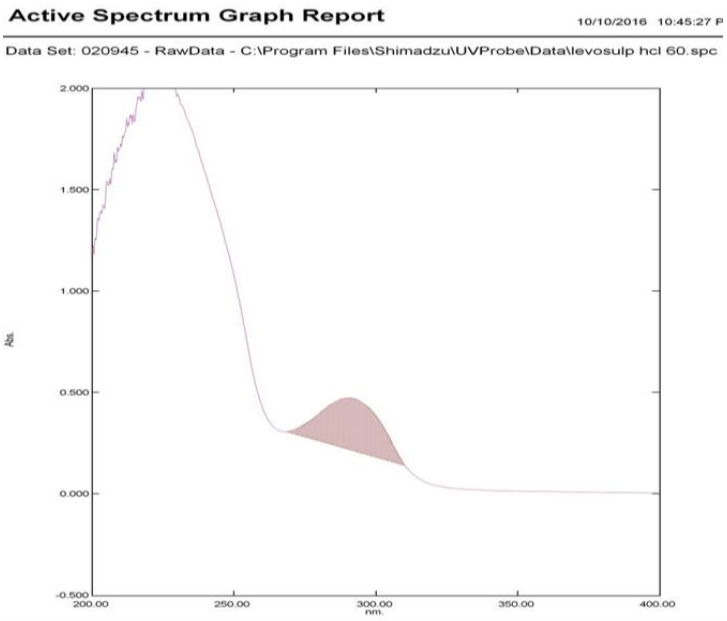


Fig. 2: Area under Curve Spectrum of Levosulpiride in 0.1 N HCl

Accuracy: A known amount of standard stock solution was mixed with the pre -analysed sample at different levels (80%, 100%, 120%). The solutions were reanalyzed using the proposed method.

Precision: The precision was checked for inter-day and intra-day variation. Intra-day precision was determined by analyzing the 60, 80 and 100µg/ml of drug solutions for three times on the same day Inter-day precision of the proposed method was checked by analyzing the 60, 80 and 100µg/ml of Levosulpiride for three different days.

Sensitivity: The sensitivity of the proposed method for the measurements of Levosulpiride was estimated by using Limit Of Detection(LOD) and Limit of Quantification (LOQ). The LOQ and LOD were calculated using equation LOD=3.3 x N/B and LOQ=10xN/B, where N is standard deviation of the peak areas of the drugs(n=3), taken as a measure of noise, and B is the slope of the standard curve.

Repeatability: On analyzing 40µg/ml concentration of Levosulpiride solution for six times repeatability of this method was established.

Ruggedness: The determination of Ruggedness of the proposed method was studied by analyzing 40µg/ml of drug by two analysts under the similar environmental and operational conditions.

Determination of Levosulpiride in Bulk

100mg of Levosulpiride was weighed accurately and transfer to a 100 ml volumetric flask, diluted the mixture with 0.1 N HCl. The whole solution was filtered through a whatmann filter paper no: 42 From the filtrate, make up a final solution of concentration of 60µg/ml. The resulting solution was scanned on a spectrophotometer in the UV range200-400nm. The concentrations of the drug were calculated from linear regression equations.

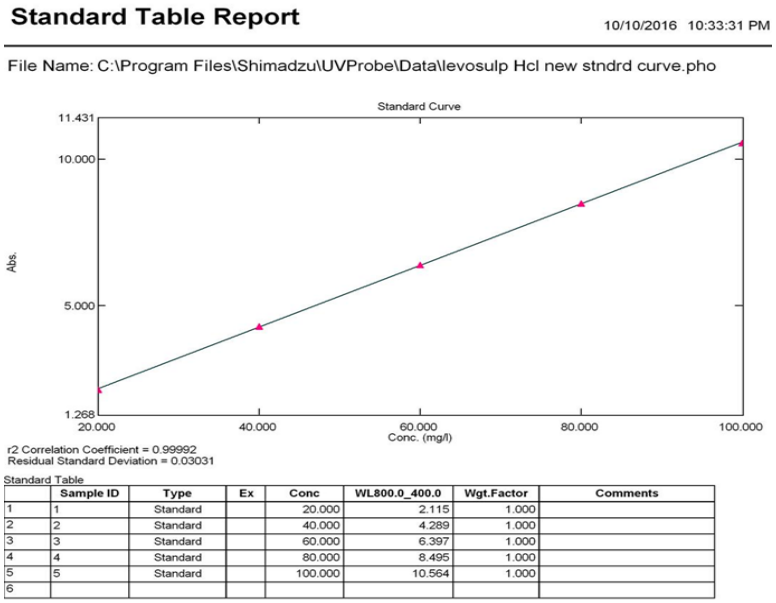
Application of the developed method for pharmaceutical formulation: For analysis of the commericial formulation 100 mg of levosulpiride was

transferred to a 100ml volumetric flask and 50 ml 0.1N HCl was added. After ultrasonication for 15 minutes. The mixture was diluted up to the mark with 0.1 N HCl. The whole solution was filtered through a whatmann filter paper no: 42. From filtrate correct dilution were taken in such a way that the final concentration is 60µg/ml. The concentrations of the drug were calculated from linear regression equations. The resulting solution was scanned on a spectrophotometer in the UV range 200-400nm.

Results and Discussion

Method validation: The developed method was validated in accordance with ICH guidelines. Drug solutions with different concentrations are prepared as per the procedure described in the experiment.

Linearity studies: The linear regression data of Area under curve versus concentration gives a linear relationship at the range of 20-100µg/ml for levosulpiride(Fig.3). Linear regression equation was found to be Y= 0.10552 X+0.04080. (Table 1) with a correlation coefficient of r^2 =0.9992.



	Sample ID	Type	Ex	Conc	WL800.0_400.0	Wgt.Factor	Comments
1	1	Standard		20.000	2.115	1.000	
2	2	Standard		40.000	4.289	1.000	
3	3	Standard		60.000	6.397	1.000	
4	4	Standard		80.000	8.495	1.000	
5	5	Standard		100.000	10.564	1.000	
6							

Fig. 3: Calibration curve of levosulpiride

Accuracy: Recovery studies at 80,100,120% of the pre analysed sample shows that the % recovery was between 99-101%. (Table 2)

Precision: The precision of proposed method expressed in % RSD and the value was found to be less than 2.This indicate that the method is precise and can be used for the determination of both drugs in formulation and shows the reproducibility of the assay. (Table 3)

Sensitivity: The LOD and LOQ were found to be 0.9479 and 2.8724 µg, respectively (Table 4).

Repeatability: Repeatability was determined by analyzing 60 µg/ml concentration of Levosulpiride (6 times) and the % RSD was found to be less than 2 (Table 5).

Ruggedness: Peak Area measurement of six concentrations of drug shows that the % RSD was found to be less than 2% (Table 6).

Determination of Levosulpiride in bulk: The concentrations of the drug was calculated from Area under Curve versus Concentration Linear regression equations. The percentage amount was found in between 98.00% to 102.002% (Table 7).

Application of developed method on formulations: The concentrations of the drug in a marketed formulation(Nexipride) was calculated from Area under Curve versus Concentration Linear regression equations. The percentage amount was found in between 98.00% to 102.002% (Table 8).

Table 1: Linearity studies

Concentration µg/mL	Area,[a] mean± SD (n=6)	%RSD
20	2.115±0.0148	0.28
40	4.289±0.0101	0.10
60	6.397±0.0458	0.37
80	8.495±0.0438	0.20
100	10.564±0.0622	0.20

(n=no. of estimations)

Table 2: Recovery studies

Drug	Initial amount(µg/mL)	Amount added (µg/mL)	Amount recovered(µg/mL, n=3)	% recovered	%RSD
Levosulpiride	40	32	31.9351	99.7972	0.6526
	40	40	40.2342	100.5855	0.4855
	40	48	48.0809	100.168	0.8570

(n=no. of estimations)

Table 3: Results of Precision studies

Component	Concentration (µg/mL)	Intra-day precision[a] (n=3)		Inter-day precision[a] (n=3)	
		Amt. found	% RSD	Amt. found	% RSD
Levosulpiride	40	40.0461	0.4860	39.9351	0.6526
	60	60.0809	0.8570	60.1208	0.2526
	80	80.2127	0.4192	80.1387	0.6109

[a]Average of three estimation

Table 4: Sensitivity Studies

LOD(µg/mL)	LOQ(µg/mL)
0.9479	2.8724

Table 5: Repeatability Studies

Sample	Amount taken(µg/mL) (n=6)	Amount found in [a]%	% RSD
Levosulpiride	60	99.33±0.24	0.26

[a]Average of six estimation

Table 6: Ruggedness study

Component	Amount taken(µg/mL) (n=6)	Amount found [a](%)		% RSD	
		Analyst I ±SD	Analyst II ±SD	Analyst I	Analyst II
Levosulpiride	60	100±0.5083	100±0.7413	0.5047	0.7372

[a]Average of six estimation

Table 7: Analysis of Levosulpiride in bulk

Concentration in µg/mL	Amount found(µg)	Amount found in %
60	59.8578	99.4076
	59.5844	98.2682
	59.8891	99.5378
	59.7953	99.1471
	59.8656	99.4401
	59.7406	98.9193
mean ± SD	59.7888±0.4746	99.1200±0.4746
%RSD	0.4788	0.4788

Table 8: Analysis of Levosulpiride in formulation (Nexipride100mg, sun pharma)

Concentration(µg/mL)	Amount found(µg)	Amount found (%)
60	59.8578	99.4076
	59.7797	99.0820
	59.8109	99.2122
	59.8422	99.3424
	59.8344	99.3099
	59.8188	99.2488
mean ± SD	59.8240 ±0.110	99.2665 ±0.1140
%RSD	0.1149	0.1149

Conclusion

The UV Spectrophotometric method is simple, precise, sensitive and reproducible. The Levosulpiride in formulation have been quantified by this method. The validation method reveals that, it is an appropriate and accurate quantification technique which being used

for routine quality control of compound in bulk and in formulations.

Acknowledgements

The Authors are grateful for providing pure Levosulpiride as gift sample from SUN PHARMA, Jammu.

Conflict of interest: Nil

Source of support: Nil

References

1. Website: www.druginfosys.com/drug aspx/Levosulpiride over view.
2. The Merck Index An Encyclopedia of Chemicals, Drug and Biologicals, Merck & Co. Inc. 14th edition, 2006;1542.
3. Mucci A, Nolfe G, Maj M. Levosulpiride: A review of its clinical use in psychiatry. Pharmacol Res 1995;31:95-101.
4. Tonini M, Cipollina L, Pouzzie, Crema F, Corazza G, De Ponti F. Clinical implications of enteric and central D2 receptor blockade by antidopaminergic gastrointestinal prokinetics, Alimentary Pharmacology and Therapeutics,2004;19:379-90.
5. Lozano R, Peralta Concha M ,Montealegre A, de Leon A, Ortiz Villalba J, Ensteban H, et al. Effectiveness and safety of levosulpiride in the treatment of dysmotility-like functional dyspepsia. Therapeutics and clinical Risk Management 2007;3:149-155.
6. Silambarasan S P, Anandakumar K, Venkatalakshmi R, Sasikal C. Development of UV Spectrophotometry and RP-HPLC methods for the estimation of Levosulpiride in bulk and in tablet formulation. Asian J Res Chem 2010;03(3);542.
7. Pritam S. Jain, Harshal P. Chaudhari, Pankaj R. Bari, Sanjay J. Surana. Area under curve method development and validation of Rizatriptan benzoate. International journal of pharmaceutical chemistry and analysis 2016;3(1):13-17.