RESEARCH PAPER

Development and Validation of Stability Indicating Rp-Hplc Method for Quantitative Estimation of Levosulpiride in Pharmaceutical Dosage Form

Ammulu A[1] and Madana Gopal N[2*]

[1]*M. Pharmacy, Department of Pharmaceutical Analysis, Santhiram College of Pharmacy, NH-40, Nandyal-518112, Andhra Pradesh, India*

[2]*Department of Pharmaceutical Analysis, Santhiram College of Pharmacy, NH-40, Nandyal-518112, Andhra Pradesh, India*

***Corresponding author:** N. Madana Gopal, Department of Pharmaceutical Analysis, Santhiram College of Pharmacy, NH-40, Nandyal-518501, Andhra Pradesh, India*

madanapharma@gmail.com

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ABSTRACT

Levosulpiride in pharmaceutical dosage forms was determined using a developed and confirmed RP-HPLC approach that is both reliable and stable. Chromatographic separation was performed on a C18 column (4.6 mm × 250 mm, 5 µm) with a mobile phase of methanol and 0.1% OPA (90:10 v/v) flowing at 0.9 mL/min. Levosulpiride eluted 2.7 minutes after detection at 230 nm using a UV-PDA detector. The approach showed good linearity in the concentration range of 5-30 µg/mL, with an R2 of 0.9992. Validation criteria such as accuracy, precision, robustness, and forced degradation were evaluated in compliance with the standards of ICH Q2(R1). Levosulpiride in pharmaceutical dosage forms was quantitatively measured using the approved method.

Keywords: Levosulpiride, RP-HPLC, Validation, Methanol, Orthophosphoric acid.

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INTRODUCTION

Levosulpiride is a selective dopamine D_2 receptor antagonist that is derived from substituted benzamide. The chemical name for it is N-[(2S)-1-ethylpyrrolidin-2-yl]methyl]-2-methoxy-5-sulfamoylbenzamide. It dissolves in organic solvents like methanol and dimethyl sulfoxide, but it is mostly insoluble in water. Levosulpiride has an empirical formula of $C_{15}H_{23}N_3O_4S$ and a molecular weight of 341.43 g/mol. [1]

Clinically, levosulpiride is used to treat irritable bowel syndrome (IBS), depression, and schizophrenia. Levosulpiride exhibits 40–50% plasma protein binding and intermediate oral bioavailability in terms of pharmacokinetics. It is primarily eliminated by the kidneys and has a half-life of six to eight hours. Levosulpiride has a long-lasting therapeutic effect and is well tolerated for gastrointestinal and mental disorders.

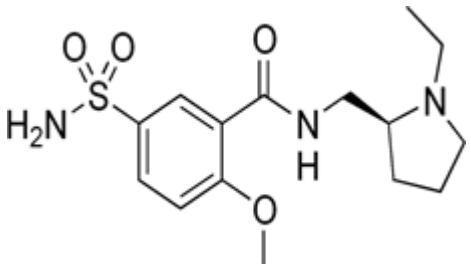

Figure1: Chemical structure of Levosulpiride

Carlota et al. [1] reported the development, validation and stability indicating RP-HPLC assay of Levosulpiride in Bulk drug using an analytical Quality by Design Approach. The chromatographic separation was carried out an Agilent C_8 column with sodium dihydrogen phosphate buffer and methanol (50:50v/v) as the mobile phase. The flow rate was set to 1.0ml/min and the Levosulpiride elution point is 2.014min under the optimized condition. Rana and Raj [2] developed and validated a high- performance liquid chromatography method for Levosulpiride and its Intermediate in Synthetic mixture. Their method utilized water, methanol and acetonitrile (70:15:15) as the mobile phase and separation was achieved using a C_{18} column. Silambarasan et al. [3]

*Author for Correspondence: madanapharma@gmail.com

developed UV Spectrophotometry and RP-HPLC Methods for the estimation of Levosulpiride in Bulk and in Tablet formulation. The optimized RP-HPLC method utilized a mobile phase of methanol and 25Mm phosphate buffer (15:85v/v) adjusted to PH of 3.5. under these conditions, the medication eluted 5.08min with a flow rate of 0.8ml/min. Chopde et al. [4] described a Bio-analytical (RP-HPLC) method development and validation for Levosulpiride from human plasma. Chromatographic separation was achieved on a waters bridge C_{18} column using ammonium formate buffer and methanol (28.19:71.81v/v) as a mobile phase. The analyte was eluted at 5.5min.

MATERIALS AND METHODS:

Materials

The instruments were Shimadzu LC-20AD HPLC, Shimadzu UV-1800ENG240V UV Visible Spectroscopy, Shimadzu ATY224 electronic balance, digital ultrasonic cleaner (SONICA2200MH), hot air oven (VISION LAB EQUIPMENT), and UV Cabinet (MONOQUARTZ).

Chemicals

The chemicals used in this work included Levosulpiride, Methanol-HPLC grade (Merck), Water (HPLC grade, Merck), ortho phosphoric acid (s d fine-chem limited).

RP-HPLC Chromatographic conditions:

The HPLC study was performed using a Shimadzu HPLC system equipped with a solvent reservoir, column oven, and photodiode array (PDA) detector. Chromatographic separation was performed using a C18 column (250 × 4.6 mm i.d., 5 µm particle size). The mobile phase consisted of methanol and 0.1% orthophosphoric acid at a ratio of 90:10 (v/v), which was improved by experimenting with various solvent combinations in compliance with standard procedures. The detection was carried out at 230 nm while maintaining a flow rate of 0.9 mL/min. A standard stock solution was made by dissolving 10 mg of levosulpiride in 10 mL of methanol. The mobile phase was used to further dilute working standard solutions with concentrations ranging from 5 to 30 µg/mL. Prior to analysis, the drug solutions and mobile phase were filtered using a 0.45 µm membrane filter. A 20 µL fixed loop injector was used to inject the samples into the chromatographic equipment. Levosulpiride's peak was detected at a retention time of roughly 2.7 minutes, and the chromatograms were obtained with a total run time of 5 minutes.

Preparation of mobile phase:

Depending on solubility, various mobile phase compositions were tried in order to obtain sharp peaks and good resolution. The standard solution was tested with different compositions of the mobile phase. Because it produced a strong peak with good symmetry within permitted limitations, methanol:0.1% OPA (90:10, v/v) was finally selected. To dissolve the medication stock solution, methanol was utilized. All subsequent dilutions were made using a 90:10 (v/v) mixture of methanol and 0.1% orthophosphoric acid.

Preparation of standard stock solution:

10 mg of levosulpiride API was first meticulously weighed into a 10 mL volumetric flask using an analytical electronic balance. To fully dissolve the medication, a few milliliters of methanol were added as the solvent, and the mixture was agitated for a few minutes. After correcting the volume with methanol and vigorously mixing the combination, a standard stock solution with a concentration of 1000 µg/mL was produced.

Method validation:

The ICHQ2(R1) guidelines were followed in the validation of the developed RP-HPLC technique.

System suitability

To assess the system applicability, six replicate injections of the prepared standard solution at a concentration of 20 µg/mL were injected into the HPLC system under ideal chromatographic conditions. Retention duration, tailing factor, theoretical plates, and peak area were among the metrics that were determined by analyzing the chromatograms.

Linearity:

The linearity range of levosulpiride was found to be 5–30µg/ml. Plotting concentration on the x-axis and peak area on the y-axis produced a graph.

Precision

Intra-day Precision:

At 9 AM, 12 PM, and 4 PM throughout the day, three intraday precision phases were conducted. The stock solution was used to achieve the optimal concentration of 20 µg/mL, which was subsequently injected into the HPLC system six times. Once the corresponding peak responses from the six replicate injections were collected, the percentage relative standard deviation (%RSD) was computed.

Inter-day Precision:

Inter-day precision was done across three days. After the stock solution reached an ideal concentration of 20 µg/mL, six replicate injections were made into the HPLC system over a period of three days. Each peak response was recorded independently, and then the percentage relative standard deviation (%RSD) was calculated.

Accuracy

Levosulpiride tablet powder was used to create a series of 50%, 100%, and 150% solutions, which were then injected into the chromatographic apparatus. The mean percentage of recovery was computed.

Limit of detection and Limit of quantification (LOD & LOQ):

The following formulas were used to calculate the limits of detection (LOD) and quantification (LOQ).

LOD= 3 × standard deviation/ slope

LOQ= 10 × standard deviation/ slope

Robustness:

The optimal concentration (20 µg/mL) was prepared using the standard stock solution. The detection wavelength was

altered to 228 nm, 230 nm, and 232 nm, the column's temperature was set to 25°C, 30°C, and 35°C, and the flow rate was adjusted to 0.8, 0.9, and 1.0 mL/min. The peak reactions were recorded after injection into the HPLC equipment.

Assay of Marketed Formulation:

The average weight of five levosulpiride tablets was calculated after weighing each of the twenty tablets. Tablet powder equivalent to 10 mg of levosulpiride (31.58 mg) was carefully weighed, placed in a 10 mL volumetric flask, dissolved in methanol, and sonicated for 10 minutes to produce a stock solution of 1000 µg/mL. An aliquot of this solution was diluted to a concentration of 20 µg/mL using the mobile phase (methanol: 0.1% orthophosphoric acid, 90:10 v/v). The chromatographic responses were obtained after the solution was passed through a 0.45 µm nylon membrane filter and fed into the HPLC system six times.

Degradation studies:

1. Acid Degradation

0.2 mL of the sample solution prepared from the stock solution was put into a 10 mL volumetric flask. After adding 1 mL of 0.1 N HCl, the mixture was heated to 50 °C for 15 minutes on a heating mantle before being left to cool to room temperature. The solution was neutralized by adding 1 mL of 0.1 N NaOH after cooling, and the volume was adjusted using the mobile phase. The resulting solution was injected into the HPLC instrument, and the peak reaction was recorded.

2. Base Degradation

0.2 mL of the sample solution from the prepared stock solution was added to a 10 mL volumetric flask. After adding 1 mL of 0.1 NaOH, the mixture was heated to 50 °C for 15 minutes on a heating mantle before being allowed to cool to room temperature. Following cooling, the solution was neutralized with 1 mL of 0.1 N HCl, and the content was adjusted using the mobile phase. After the final solution was added to the HPLC equipment, the peak responses were recorded.

3. Peroxide Degradation

0.2 mL of the sample solution from the prepared stock solution was added to a 10 mL volumetric flask. The mobile phase was used to adjust the volume after 0.1 mL of 3% H2O2 was added. For twelve hours, the solution was left at room temperature. After the final solution was added to the HPLC equipment, the peak responses were recorded.

4. Thermal Degradation

0.2 mL of the sample solution from the prepared stock solution for heat degradation was added to a 10 mL volumetric flask. It was cooled, injected into an HPLC, and the peak responses were noted after adjusting the volume with the mobile phase and maintaining it over a heating mantle at 60°C for 10 minutes.

5. Photolytic degradation method

0.2 mL of the sample solution from the prepared stock solution was added to a 10 mL volumetric flask. It was injected into the HPLC and the peak responses were noted after the volume was adjusted using the mobile phase and it was exposed to a UV cabinet for an hour.

RESULTS AND DISCUSSION

System suitability:

Figure 2 displays the chromatogram obtained at a concentration of 20 µg/mL under optimal circumstances. The method is appropriate for analysis since the system suitability parameters were found to be within acceptable bounds (Table 1).

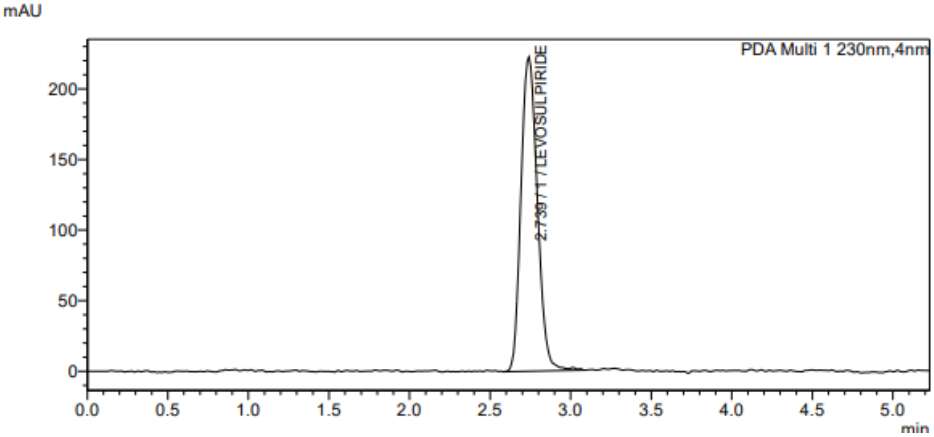

Figure 2: Chromatogram of Levosulpiride under optimized conditions

Table 1: System suitability parameters for the suggested HPLC technique

S. No.	Peak area	Ret Time(min)	Theoritical plate count	Tailing Factor
1	2915864	2.744	3108	1.12
2	2958374	2.750	3145	1.10
3	2906386	2.744	3084	1.12
4	290780	2.745	3121	1.11
5	2929457	2.744	3116	1.12

6	2959829	2.750	3068	1.11
Average	2929618.50	2.750	3106.17	1.26
STDEV	24267.34	0.00	28.92	0.01
%RSD	0.83	0.09	0.93	0.65
Limits	-	-	>2000	<2.0
%RSD	<2.0	<2.0	<2.0	<2.0

Linearity:

The correlation coefficient (R2) was found to be 0.9992, indicating conformity to allowable limits and acceptable linearity within the concentration range under investigation. These results confirm that the approach is suitable for quantitative analysis. The calibration curve is shown in Figure 3 and the corresponding peak area values are shown in Table 2.

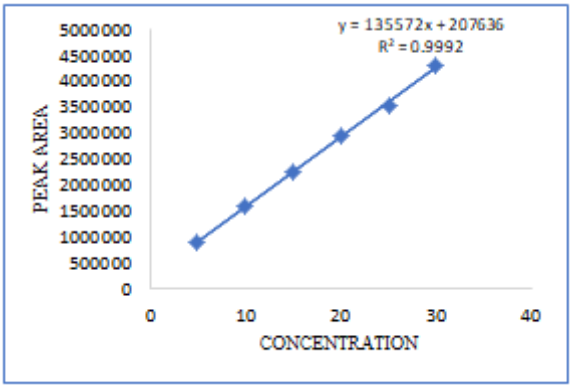

Figure:3 Linearity graph of Levosulpiride

Table 2: Levosulpiride Linearity

S. No	Linearity level	Conc. (µg/ml)	Peak area
1	25%	5	876724
2	50%	10	1576490
3	75%	15	2242572
4	100%	20	2945598
5	125%	25	3531084
6	150%	30	4308369

Precision:

The method's precision and adherence to permissible limits were demonstrated by the %RSD being less than 2%. Tables 3 and 4 give the precision results.

Table 3: Inter day precision

S. No	Day 1	Day 2	Day 3
1	2915864	2959609	2977393
2	2958374	2981763	2925683
3	2906386	2955949	2913409
4	2907801	2964842	2908929
5	2929457	2964842	2904861
6	2959829	2952358	2902586
Average	2929618.50	2961726.00	2922143.50
STDEV	24267.34	10691.21	28274.12
% RSD	0.83	0.36	0.97
Limits	**% RSD: <2.0**	**% RSD: <2.0**	**% RSD: <2.0**

Table: 4 Intra- day Precision

S. No	9:00 AM	1:00 PM	5:00 PM
1	2984754	2936762	2937704
2	2955063	2945854	2882134
3	2986042	2930482	2957691
4	2963877	2930482	2902586
5	2983068	2941396	2932789
6	2927057	2928828	2964082

Average	2966643.50	2935634.00	2929497.67
STDEV	23164.23	6904.05	31738.53
% RSD	0.78	0.24	1.08
Limits	**% RSD <2.0**	**% RSD <2.0**	**% RSD <2.0**

Accuracy

The percentage recovery was determined to be within the acceptable range of 98–102%, demonstrating the devised method's good accuracy. Table 5 displays the findings.

Table 5: Accuracy displaying recovery percentage

S. No.	Levels	Conc taken (µg/ml)	Conc added (µg/ml)	Peak Area	Conc recovered (µg/ml)	% Recovery	Average Recovery
1.	**50%**	20	10	4278426	10.0268	100.2678	**99.42**
2.		20	10	4253242	9.8410	98.4101	
3.		20	10	4268960	9.9570	99.5695	
4.	**100%**	20	20	5662260	20.2341	101.1707	**100.77**
5.		20	20	5671925	20.3054	101.5272	
6.		20	20	5619944	19.9220	99.6101	
7.	**150%**	20	30	6973635	29.9071	99.6902	**99.27**
8.		20	30	6918482	29.5002	98.3341	
9.		20	30	6977739	29.9373	99.7911	

LOD & LOQ

Levosulpiride can be detected and quantified at low concentration levels using the established approach, as evidenced by the LOD and LOQ of 0.590 µg/mL and 1.789 µg/mL, respectively. Table 6 shows the outcomes.

Table 6: HPLC Method LOD and LOQ

Parameters	Slope from Linearity	SD of peak from system suitability
	135572	24267.34
LOD = 3.3 x SD/Slope	0.590µg/ml	
LOQ = 10 x SD/Slope	1.789µg/ml	

Robustness

In order to verify robustness, the flow rate, wavelength, and column oven temperature were all varied by ±0.1 mL/min, ±2 nm, and ±5°C, respectively. The observed values were found to be within acceptable ranges and the parameters are displayed in Table 7.

Table 7: HPLC method Robustness

Parameter	Condition	Condition	RT	Peak area	Theoretical plates	% Assay
Flow (ml/min) min±0.1 ml	Less flow	0.8ml/min	3.078	2592362	3280	87.98
	Optimized	0.9 ml/min	2.747	2945598	3085	99.97
	More flow	1.0 ml/min	2.482	2751382	2769	93.38
Temp (°C) min±5°C	Less temp	25°C	2.752	2986739	3186	101.37
	Optimized	30°C	2.747	2945598	3085	99.97
	More temp	35°C	2.739	2998557	3200	101.77
Wave length (nm) min±2 nm	Low WL	228 nm	2.749	3046001	3005	103.38
	Optimized	230 nm	2.747	2945598	3085	99.97
	High WL	232 nm	2.749	2943689	2933	99.91

Assay

The method yields accurate and consistent findings within acceptable bounds, as seen by the average % assay of 99.58% with a %RSD of 0.31.

Degradation studies

Acidic, basic, oxidative, thermal, and photolytic conditions were used in forced degradation investigations. The analyte retention time showed no interference, supporting the method's stability-indicating character. Table 8 displays the outcomes.

Table 8: Degradation studies

S. No	Condition	Peak Area	% Assay	% Degradation
1	Acid	2630326	89.2	10.7%

2	Base	2710145	91.9	8.0%
3	Peroxide	2670313	90.6	9.3%
4	Thermal	2653320	90	9.9%
5	Photolytic	2679935	90.9	9.0%

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REFERENCES

1. Carlota RR, Nayak A, Reddy YV, et al. RP HPLC-Based Method Development, Validation and Stability Indicating Assay of Levosulpiride in Bulk Drug Using Analytical Quality by Design Approach. *Indian Journal of Science and Technology*. 2023;16(46):4436–4444. DOI: 10.17485/IJST/v16i46.2995.
2. Rana MA, Raj HA. Development and Validation of High Performance Liquid Chromatography Method for Levosulpiride and Its Intermediate in Synthetic Mixture. *Asian Journal of Pharmaceutical Technology*. 2015;5(2):97–106. DOI: 10.5958/2231-5713.2015.00015X.
3. Silambarasan SP, Anandakumar K, Venkatalakshmi R, et al. Development of UV Spectrophotometry and RP-HPLC Methods for the Estimation of Levosulpiride in Bulk and Tablet Formulation. *Asian Journal of Research in Chemistry*. 2010;3(3):542–546.
4. Chopde A, Anmulwad B, Bhandari A, et al. Bio-analytical (RP-HPLC) Method Development and Validation for Levosulpiride in Human Plasma. *International Journal of Pharmaceutical Sciences*. 2025;3(7):1886–1896. DOI: 10.5281/zenodo.15878983.
5. Jin SE, Ban E, Kim YB, et al. Development of HPLC Method for the Determination of Levosulpiride in Human Plasma. *Journal of Pharmaceutical and Biomedical Analysis*. 2004;35(4):929–936. DOI: 10.1016/j.jpba.2004.02.024.
6. Cho HY, Lee YB. Improvement and Validation of a Liquid Chromatographic Determination of Levosulpiride in Human Serum and Urine. *Journal of Chromatography B*. 2003;796(2):243–251. DOI: 10.1016/j.jchromb.2003.07.009.
7. Vani D, Fatima T. Analytical Method Development and Validation of Levosulpiride and Pantoprazole by Using Reverse Phase HPLC Method. *International Journal of Pharmacy and Analytical Research*. 2021;10(2):101–106. DOI: 10.61096/ijpar.v10.iss2.2021.101-106.
8. Dudhagaonkar T, Lade P, Chopade V, et al. HPLC Method Development and Validation of Rabeprazole and Levosulpiride in Its Bulk and Dosage Form. *International Journal of Pharmaceutical Sciences and Drug Research*. 2022;14(4):378–384. DOI: 10.25004/IJPSDR.2022.140403.
9. Ahmad S, Khairnar M, Bakhshi AR, et al. QbD Approach to Develop Stability Indicating RP-HPLC Method for Levosulpiride and Ilaprazole. *International Journal of Health Sciences*. 2022;6(S5):7413–7429. DOI: 10.53730/ijhs.v6nS5.11625.
10. Hashim M, Ahmad L, Khan A, et al. Development and Validation of a Reversed-Phase HPLC-UV Method for Simultaneous Determination of Levosulpiride and Omeprazole in Human Plasma: Applicability for Evaluation of Pharmacokinetic Drug-Drug Interactions. *PLOS ONE*. 2024;19(8):1–17. DOI: 10.1371/journal.pone.0309453.