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Research Article

Validation and Development of New RpHPLC Methods of Levosulphiride Pellets

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ABSTRACT

A simple, economic, selective, precise, Reverse phase High Performance Liquid Chromatography method for analysis of levosulphride pellets 40%, was developed and validated according to ICH guidelines. The quantification of the drug was carried out using grace smart, 250mm × 4.6mm × 5µm or its equivalent in isocratic mode, with mobile phase compressing of Buffer : Acetonitrile (70:30) The flow rate was 0.8ml/min and the detection was carried out by PDA detector i.e., 237 nm. The retention time for levosulphride pellets was found to be 2.3 min. The percent assay was found to be 98.98%. The method of levosulphride pellets validated for precision, accuracy, linearity range, specificity and robustness.

Key-words: levosulphride pellets, Reverse phase High Performance Liquid Chromatography, Validation.

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Introduction:

Chemically, Levosulpiride is [(S)-(-)-5-(aminosulfonyl)-N-[(1-ethyl-2-pyrrolinyl) methyl]-2 methoxyIbenzamide] (CAS No.23672- 07-3.), a new antipsychotic agent belonging to the substituted benzamide group. Levosulpiride is only a weak D_2 dopamine receptor antagonist. Furthermore, in the D_2 receptor family (which includes D_2, D_3 and D_4 receptors), the affinity of levosulpiride for the D_2 receptor is only 2-3 times greater than that for the D_3 receptor (this contrasts with typical antipsychotics, which are 10-20 times more potent at D_2 than at D_3) [1]. At low doses (50-200 mg/day), levosulpiride preferentially blocks dopamine auto receptors which are located on presynaptic neurons. At these doses, levosulpiride is therapeutic for negative and cognitive symptoms of schizophrenia and for depressive and somatoform disorders. At high doses (400-800 mg/day), levosulpiride blocks both dopamine presynaptic and postsynaptic D_2 receptors and may therefore be effective for the positive symptoms of schizophrenia. Its low incidence of extrapyramidal side effects (EPS) is characteristic of a typical antipsychotic [2,3] .

Material and Methods

Chemicals and Reagents

levosulphride pellets was obtained from hetero pharmaceuticals , hyderabad. Acetonitrile (HPLC grade), Methanol (HPLC grade), Ammonium hydroxide (AR grade) ammonium acetate (AR grade) , tetrabutyl ammonium hydroxide sulphate(AR grade) were of reagent grade.

Instrumentation

A HPLC (LC-2010 (SHIMADZU)) with Waters UV/VIS Detector/PDA detector grace smart, 250mm × 4.6mm × 5µm was used. A Rheodyne injector with a 10 µl loop was used for the injection of sample. The HPLC system was equipped with Empower2 software for data processing.

Chromatographic Condition

The mobile phase containing Buffer: Acetonitrile (70:30) was found to resolve of levosulphride pellets. 1N ammonium Hydroxide solution was used for pH adjustment of buffer. The mobile phase was filtered on a 0.45 nylon membrane filter and then ultrasonicated for 30 min. The flow rate was set to 0.8ml/min. The drug shows good absorbance at 237 nm, which was selected as wavelength for further analysis. All determinations were performed at constant column temperature (45^0C).

Preparation of buffer

Buffer Preparation: Accurately weighed and transfer 3.85gm of ammonium acetate, and1.1gm tetrabutyl ammonium hydrogen sulphate in 1000ml of water into a beaker and mix. Adjust the pH to 7.9 ±0.05 with 1N ammonium Hydroxide solution and dilute to 1:1 with water and mix. Filter the solution through 0.45µm nylon filter paper.

Preparation of Mobile Phase

Preparedly filtered and degassed mixture of buffer and Acetonitrile in the ratio of 70:30 v/v .

Diluent solution

Prepared a degassed mixture of water and Acetonitrile in the ratio 50: 50 %v/v.

Preparation of Standard solution

Weigh down 75mg's of Levosulphiride pellets in 100ml volumetric flask and add about 70ml diluent and sonicate to dissolve. Make upto the mark with diluent and mix. Take 5ml of above solution in 50ml volumetric flask and and make upto the mark with diluents.

Preparation of Sample solution

188mg levosulphiride pellets sample equlivalent to 75mg levosulphiride into a 100ml volumetric flask. Add 70ml diluents and sonicated for 20 minutes with intermittent shaking and the solution was made up to volume with diluent and filtered through 0.45µ membrane.

Placebo Preparation:

188mg placebo pellets equivalent to 75mg of levosulphride pellets into a 100 ml vf. Add 70 ml diluent and sonicate for 20 minswith intermitent shaking. Dilute to volume with diluent and mix.filter the solution through 0.45µ membrane filter.

Method Validation[4]

1) Calibration Curve of levosulphride pellets

The linearity of levosulphride pellets responses at concentrations in the range of 20 to 80 ppm[5,6] was determined by preparing and injecting standard solutions (40µg/ml).

2) Specificity

To demonstrate that diluents and placebo are not interfering with analytic peak. Solutions of levosulphride pellets standard, sample and placebo were prepared individually at 0.040mg/ml concentration. The peak purity of analyte peak should be not less than 0.999[7,8,9].

3) System suitability:

A Standard solution was prepared by using Levosulphride working standards as per test method and was injected Five times into the HPLC system. The system suitability parameters were evaluated from standard chromatograms by calculating the % RSD from five replicate injections for levosulphride pellets **,** retention times and peak areas[10,11].

4) Precision

Precision was measured in terms of repeatability of application and measurement. Repeatability of standard application was carried out using six replicates of the same standard concentration (40µg/mL for standard application)[12-14].

5) Accuracy (%Recovery)

Accuracy (Recovery) of the method was tested by spiking 50, 100 and 150% of levosulphride pellets working standard. The accuracy of the analytical method was established in triplicate across its range according to the assay procedure[15-19].

6) Robustness

The robustness of the proposed method was determined by analysis of aliquots from homogenous lots by differing physical parameters like flow rate, column temperature which may differ but the responses were still within the specified limits[20-25].

7) Limit of Detection and Limit of Quantification

LOD and LOQ were determined from standard deviation and slope method as per ICH guidelines[25-27]

8) Sieve analysis[28-31] :

Weigh about 50gm of test sample. Place the required sieves(14&20)on top plate of instrument. Place previously weighed sample of pellet on top mos sieve. Now lock the both sieve clamp knob by turning it in clockwise direction. Set the desired power level and time for the test by increment and decrement key at the front panel for the power and time respectively. Press start key from the front panel the display shows the elapsed time and set value of the power. After completion of the passed through corresponding sieves and calculate the percentage[32-39].

9)Bulk Density :

Weight accurately 25gm of test sample and transfer onto 50ml graduated cylinder . carefully level the sample without compacting and read the unsetteled apparent volume (v_o) to the graduated unit calculate the bulk density in g/ml by formula m/v_0[40-42].

10) Water Analysis[42-44] :

Fill the burette with karl fisher reagent with the help of rubber bellow. Fill the titration vesselwith 40ml of methonol. Swith on instrument to half opened position and press the start button [45-47]. stop the ehrn the siren is heard. note down the initial burette reading(I) take about 3.0gm of pellets crushing weigh accurately and transfer about 0.2 gm of test sample (w) into the titration vessel. Switcj on the start button note down the final burette reading(f)[48,49]. when sound of siren is heard. Calculation the water content of test sample.

$$\%w/w = \frac{(F-I)*K.F\ FACTOR*100}{W*1000}$$

RESULTS AND DISCUSSION

1) Linearity: A linear relationship of levosulphride pellets across the range (20-80 ppm) of the analytical procedure in triplicate. The range of concentrations was selected based on 20-80 ppm of the test concentration (for assay). Peak area and concentrations were subjected to least square regression analysis to calculate regression equation. The correlation coefficient (r2) was found to be 0.999 and shows good linearity. The data of the calibration curve was given in **Table 1**.

Concentration (ppm)	Average Area	Statistical Analysis	
0	0	Slope	58.76
20	1212	y-Intercept	27.40
30	1840	Correlation Coefficient	0.999
40	2360		
50	2975		
60	3535		
70	4139		
80	4727		

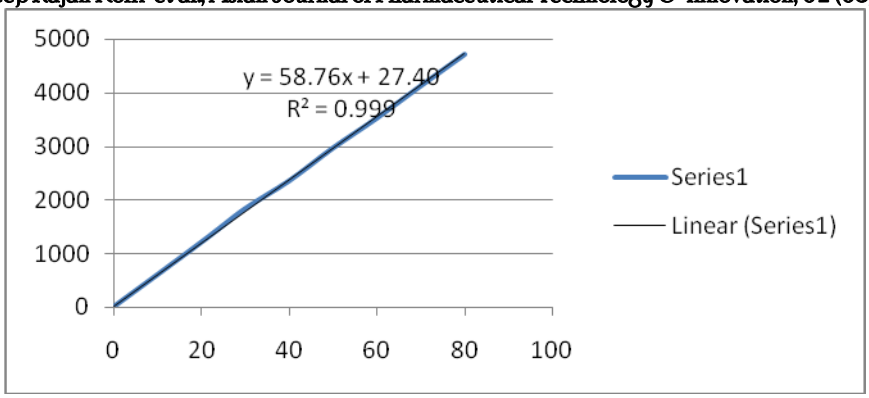

Fig1: Linearity Plot (Concentration Vs Response) of levosulphride

2) Specificity :The specificity of the method was established by the peak purity of levosulphride pellets were assessed by comparing the retention time (TR) of standard levosulphride pellets .Good correlation was obtained between the retention time of standard and sample of levosulphride pellets.

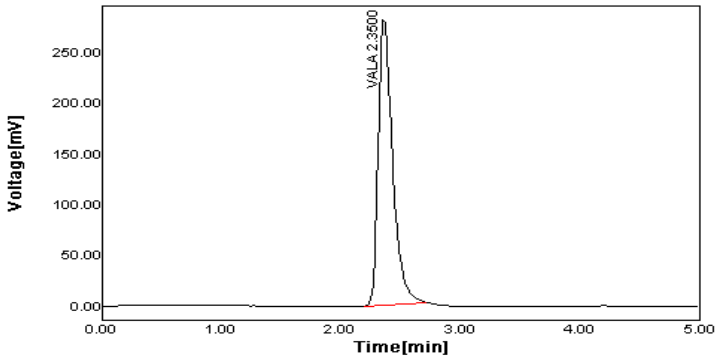

Fig2: Got a peak for std at an RT of 2.3

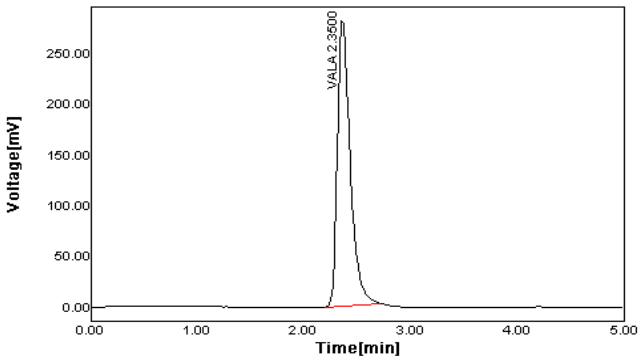

Fig3: Got a peak for sample at RT of 2.3

3) Precision : Precision studies were carried out in terms of repeatability. Six determinations of 100 % concentration at 24 µg/mL level and the data given in a Table. The % RSD was found to be below 2 and fulfilled the ICH guidelines criteria. **Table 2**.

TABLE 2: Data of Precision

	Injection	Peak Areas of levosulphride	%Assay
Concentration 40ppm	1	2350.3960	98.81
	2	2394.8147	100.68
	3	2369.9285	99.62
	4	2372.5720	99.64
	5	2366.0735	99.67

Statistical Analysis	Mean	2370.757	98.68
	SD	15.96292	0.663
	% RSD	0.67	0.67

4) Accuracy: The recovery of the added standard to the drug product sample was calculated and it was found to be 97.70-101.5%, whichindicates a good accuracy of the method to that of the labeled claim. The obtained recovery results were given in **Table 3**.

TABLE 3: Data of Accuracy

Concentration % of spiked level	Amount added (ppm)	Amount found (ppm)	% Recovery	Statistical Analysis of % Recovery	
50% injection 1	20	20.15	100.79	**MEAN**	100.79
50% injection 2	20	20.17	100.88		
50% injection 3	20	20.14	100.71	**%RSD**	0.07
100 % injection 1	40	39.53	98.81	**MEAN**	99.68
100 % injection 2	40	40.28	100.68		
100% injection 3	40	39.82	99.55	**%RSD**	0.94
150% injection 1	60	60.34	100.76	**MEAN**	99.44
150% injection 2	60	59.38	98.966		
150% injection 3	60	59.28	98.70	**%RSD**	0.97

5) Lod And Loq : LOD and LOQ were calculated form the average slope and standard deviation form the calibration curve. LOD and LOQ were found to be 0.1920 g/mL and 0.5813 g/mL respectively, indicating high sensitivity of the method

6) Bulk density :

Bulk density = $\frac{weight\ of\ sample}{volume\ of\ sample}$

Weight taken = 25gm
Volume unsettled = 30 ml

Bulk density = 25/30

0.83 gm/ml

7) Water Analysis:

% w/w = $\frac{(F-1)*K.F\ FACTOR*100}{W*1000}$

= (28.8-27.4)*2*100/0.2*1000

= 1.4 %

8) Sieve Analysis:

% passed through #14 = 100- $\frac{weight\ of\ sample\ retained\ on\ \#14*100}{weight\ of\ sample\ taken}$

2gm/50 gm =0.04

% Retained on #20 = 100- $\frac{weight\ of\ sample\ retained\ on\ \#20*100}{weight\ of\ sample\ taken}$

1gm/50 gm =0.02

9) LIMIT OF DETECTION AND LIMIT OF QUANTITATION (LOD and LOQ):

From the linearity plot the LOD and LOQ are calculated:

$$LOD = \frac{3.3\ \sigma}{S}$$

$$\frac{3.3 * 26.20098}{58.76}$$

$$= 1.47$$

$$LOQ = \frac{10\ \sigma}{S}$$

$$\frac{10 * 26.20098}{58.76}$$

$$= 4.45$$

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