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RESEARCH ARTICLE

RP HPLC-Based Method Development, Validation and Stability Indicating Assay of Levosulpiride in Bulk Drug Using Analytical Quality by Design Approach

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Abstract

Objectives: This study aimed at the methodical design and validation of a Reversed-Phase High-Performance Liquid Chromatographic method (RP-HPLC) for the estimation of Levosulpiride in bulk form using analytical quality by design approach. **Methods**: Using custom design, the Critical Method Parameters were methodically optimized. Peak area, retention time, and peak tailing were assessed for flow rate, buffer, and column length as the influencing elements for Critical Analytical Attributes and using an Agilent C8 column in isocratic elution mode with mobile phase NaH_2PO_4 buffer and methanol (50:50% v/v) and flow rate at 1ml/min. **Findings**: Chromatographic separation was accomplished on the Agilent C8 (150×4.6 mm, 5m) column. The optimized and predicted data from JMP PRO 14 software consisted of mobile phase NaH_2PO_4(50%): Methanol (50%), pumped at a flow rate of 1 ml/min which gave the higher desirability function of 77%. LOD and LOQ values were 0.06 g/ml and 0.20 g/ml respectively and models were found to be significant ($p < 0.05$). The validation parameter findings were within the permitted range. Forcefully testing the drug's stability under various stress situations revealed considerable degradation in the presence of heat. **Novelty**: The decrease in the retention and run time shows that the method is simple, accurate, precise and economical for the estimation of Levosulpiride that can be adopted in regular quality control tests in industries.

Keywords: Analytical quality by design; Custom design; Desirability function; Levosulpiride; Reversed Phase Liquid Chromatography

1 Introduction

The concept of "Quality by Design" (QbD) is described as an approach that includes improving scientific understanding of critical processes and product qualities, designing controls and tests based on the scientific limits of understanding during the develop ment phase and utilizing the knowledge obtained during the life-cycle of the product to work on a constant improvement environment[(1)]. QbD explains a pharmaceutical development approach referring to formulation design, development and manufacturing processes to maintain the recommended product quality[(1)]. Guidelines as well as mathematical models are used to ensure the establishment and use of the knowledge on the subject in an independent and integrated way.

Levosulpride is chemically represented as N-[[(2S)-1-Ethylpyrrolidin-2-yl] methyl]-2-methoxy-5-sulfamoyl benzamide, having the molecular formula of $C_{15}H_{23}N_3O_4S$ and molecular weight of 341.43 g/mol. Levosulpride is an antiemetic, antidyspeptic and antipsychotic drug, that functions mainly as a dopamine D2 antagonist and is more selective than most other neuroleptics that block both dopamine D1 and D2 receptors but it lacks effects on norepinephrine, acetylcholine, serotonin, histamine, or gamma-aminobutyric acid (GABA) receptors.

Levosulpiride is available in combination in a capsule dosage form that have been used to treat patients with gastroesophageal reflux syndrome (GERD), to manage psychological problems as well as to reduce the secretion of acid in stressed condition[(2)]. Based on the review of existing literature, levosulpiride is estimated using UV, HPLC and HPTLC methods either alone or in combination with other drugs. However, no other methods have been reported for the estimation of this drug in bulk form[(3)]. The present work is an attempt to QbD approach to develop, validate and stabilize indicating RP-HPLC method development for levosulpiride.

2 Methodology

Material and methods

2.1 Chemicals

Acetonitrile, NaH_2PO_4, Na_2HPO_4, K_2HPO_4, KH_2PO_4, HCl, NaOH, HPLC grade water, and methanol were procured from Merck India Pvt. Ltd, Mumbai, India and from SD Fine-Chem Limited, India. Levosulpiride was obtained from Sunlight Sciences, Hyderabad, India.

2.2 Chemicals

UV/Visible detector Model No. (2489) equipment was utilized. A Waters HPLC 2695 system was used to conduct the HPLC study. Empower 3 is the program used for developing and validating HPLC methods. To enhance the performance of the approach by choosing components and evaluating for responses that produced the ideal chromatographic conditions, Custom Design was used with the aid of the JMP PRO 14 program[(4)].

2.3 Preparation of solutions

2.3.1 Mobile Phase Preparation

50ml (50%) of NaH_2PO_4 buffer and 50 ml of Methanol (50%) were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through a 0.45 μm filter under vacuum filtration.

2.3.2 Preparation of buffer (0.1M NaH_2PO_4 buffer)

1.998g of NaH_2PO_4 was weighed and added into a 1000ml volumetric flask which was dissolved and diluted with water this solution was filtered by using a 0.45-micron membrane filter and sonicated for 10 min.

2.3.3 Mobile Phase Preparation

In a 25ml volumetric flask, 25 mg of Levosulpiride (API) was placed and 5ml of diluent (Mobile phase) was added which was followed by sonication for 30mins and preparation was completed by adjusting the final volume with diluent[(5)].

2.4 Method development - Optimized chromatographic conditions

To optimize the final method, initial trials are required. Chromatographic separation was carried out at 25°C using an Agilent C8 (150, 4.6 mm, 5 μm) column. As the mobile phase, 0.1N NaH_2PO_4 and methanol were mixed 50:50 (v/v) and pumped at a flow rate of 1 ml/min. At 254 nm, the UV detector was set.

2.5 Experimental design

The method was optimized using a Custom design. Three factors, namely, buffer (NaH_2PO_4) concentration, flow rate, and column length were chosen and optimized. Different ranges of three parameters 40–60% NaH_2PO_4, flow rate of 0.5–1.5 ml/min, and column lengths of 150 and 250 were taken as shown in Table 1. JMP PRO 14 was utilized to generate and analyze the study design consisting of 12 runs, as depicted in Table 2 [6,7].

Table 1. Design Summary of Custom Design

CMPs	Unit	Role	Lower limit	Higher limit
Flow rate	ml/min	Continuous	0.5	1.5
Column length	–	Categorical	150	250
Buffer (NaH_2PO_4)	–	Continuous	40	60

CQA: Retention time, Theoretical plates, Peak area Runs: 12.

Table 2. M**ethod runs by custom design**

S.No	Factors			Responses		
	Flow rate	Column length	Buffer	Retention time	Peak area	Plate count
1	1.5	250	40	2.3	3668988	8909
2	1.5	250	60	2.1	4013444	8923
3	0.5	150	40	2	3325511	9100
4	1.5	250	40	2.2	3667277	8997
5	0.5	150	40	2	3207890	9120
s	1.5	150	60	1.8	3680000	8908
7	0.5	150	60	2	3496009	9084
8	0.5	250	40	2.3	3546000	9213
9	1.5	150	60	1.8	3599900	8989
10	0.5	250	60	2.1	4109000	9210
11	1.5	150	40	2	3467000	8900
12	0.5	250	60	2.1	3890000	9180

2.6 Method validation

It is a crucial process in the pharmaceutical field to ensure that a particular method is suitable for its intended purpose and produces reliable and accurate results. It involves a series of tests and assessments to confirm the performance, robustness, and reliability of the analytical method.

2.6.1 Linearity

Levosulpiride solutions with concentrations ranging from 50 to 150 g/ml were injected. The graph indicates that the R^2 value was determined to be 1, which means that Levosulpiride passes linearity over the range of 50% to 150% [7].

2.6.2 Accuracy

To check the accuracy, we added a known amount of the drug to the samples at three different levels: 50%, 100%, and 150%. Then, we analyzed these samples using the optimized method. The % recovery was calculated by comparing the measured amounts to the expected amounts [8].

2.6.3 Precision

Drawing from the stock solution, 5ml was pipetted out and diluted, which gives 100μg/ml, 6 vials were prepared and injected each time. Acceptance criteria should be between 97-103%. % Relative Standard Deviation should not be more than 2% between 6 replicas.

2.6.4 Limits of detection (LOD) and limits of quantitation (LOQ)

LOD is the lowest concentration level where the peak area is three times higher than the baseline noise which also helps to identify the smallest amount of the substance that can be reliably detected. LOQ is the lowest concentration level where the peak area has a signal-to-noise ratio higher than ten. It indicates the smallest amount of the substance that can be accurately quantified or measured with confidence [(9)].

2.6.5 Robustness

Robustness studies were performed by changing method parameters in the system such as Temperature (± 2°C), Flow rate (± 10 °C), pH (±0.2) and Composition of mobile phase (±5%).

2.7 System suitability

System suitability parameters pass for Tailing which is less than 2.0, acceptable peak area, Rt, plate count was found to be more than 2500, % RSD was found to be less than 2%. The number of USP plate count was found to be 9247, USP tailing factor 1.73, % RSD was found to be 0.2% and retention time was found to be 2.014 mins [(10)].

2.8 Forced degradation studies

2.8.1 Acid stress

5 ml of levosulpride was pipetted out from the stock solution and diluted to 50 ml. 1 ml of the solution was withdrawn and transferred into a 10 ml volumetric flask, followed by 5 ml of adequately diluted 0.1 N HCL, and sonicated for 30 minutes. The above solution was poured into an HPLC vial and then injected into the HPLC system [(11,12)].

2.8.2 Base stress

5 ml of levosulpride was pipetted out from the stock solution and diluted to 50 ml. 1ml of the solution was withdrawn and transferred into a 10ml volumetric flask and 5 ml of 0.1N NaOH was added, mixed well, and diluted to the correct volume with diluent. The mixture was sonicated for 30 minutes. The above solution was poured into the HPLC vial and then injected into the HPLC system [(13,14)].

2.8.3 Hydrolysis

5 ml of levosulpride was pipetted out from the stock solution and diluted to 50 ml. 1 ml of the solution was withdrawn and transferred into 10ml volumetric flask, followed by 5 ml of water that had been diluted to the appropriate level. The mixture was sonicated for 30 minutes. The above solution was poured into an HPLC vial and then injected into the HPLC system [(15)].

2.8.4 Peroxide

5 ml of levosulpride was pipetted out from the stock solution and diluted to 50 ml. 1 ml of the solution was withdrawn and transferred into a 10 ml volumetric flask. 5 ml of 1% H_2O_2 was added, mixed well, and diluted to the correct volume with diluent. The mixture was sonicated for 30 minutes and the solution was poured into an HPLC vial and injected into the HPLC [(16,17)].

2.8.5 Heat

5 ml of levosulpride was pipetted out from the stock solution and diluted to 50 ml. 1ml of the solution was withdrawn and transferred into a 10ml volumetric flask filled to the appropriate level with a diluent that has been heated at 60 °C for 30 minutes, poured into HPLC vial and injected into the HPLC apparatus [(18,19)].

2.8.6 Sunlight

5 ml of levosulpride was pipetted out from the stock solution and diluted to 50 ml. 1 ml of the solution was withdrawn and transferred into a 10 ml volumetric flask that had been correctly diluted with a solution that had been exposed to sunlight for six days. The solution was poured into the HPLC vial and injected into the HPLC apparatus [(20,20)].

3 Results and Discussion

Such analytical methods are, in fact, an indicator of a quality product and the robustness of that product for the duration of the lifecycle of that product. The main goal of any HPLC method is to separate and quantitate analyte(s) of interest from any impurity and/or excipients. Initially, it is important to establish the critical quality attributes (CQA) of a system that may impact the quality of the analytical method[21]. Development of Analytical RP-HPLC Method with Design Space and Control Strategy determination by optimization study all the computations for the current optimization study and statistical analysis were performed using Design Expert® software (Design Expert trial version). State-Ease Inc., Minneapolis, MN, USA.

3.1 Design of experiment by custom design

3.1.1 Effect Summary

In Figure 1, it is evident that the method is influenced by factors such as flow rate, column length, and buffer concentration. A bar representing the flow rate, column length, and buffer crosses the blue vertical line to indicate significance. The interplay of these variables is crucial in understanding and optimizing the overall process.

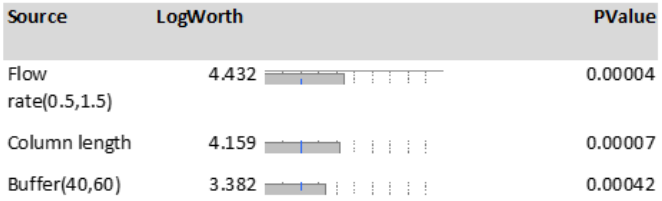

Fig 1. Effect summary

3.2 Model fit of specific HPLC technique responses

To evaluate model fit, data from all 12 runs were incorporated into the design which increases the robustness of the method. In accordance with Figure 2, at a significance threshold of $p<0.05$, $R^2 = 0.91$ and $p = 0.0002$ for response retention time, $R^2 = 0.91$ and $p = 0.0002$ for response peak area, and $R^2 = 0.90$ and $p = 0.0002$ for response tailing are all statistically significant. [Figure 2] The high R^2 values and low p values suggest that the model being evaluated is a good fit for the data which is comparable with Ahmad et. al and Chhalotiya et al. studies[15,22].

3.3 Statistical optimization of selected responses of the HPLC method

Numerical optimization: The prediction profiler displays a continuous correlation between various parameters and multiple responses, providing a thorough picture of the variability that can exist within the extreme results. Figure 3 shows that the greatest global desirability value of 77.49% provides the chance of achieving the desired goal for all three responses, which do not vary too much and therefore considered to be the best method indicating the validity of the model [Figures 3, 4 and 5 Figure 6 and Table 3].

Table 3. Final optimized HPLC chromatographic conditions

S. No	Parameters	Results
1	Linearity	R^2=1
2	Accuracy	RSD%=0.3
3	Precision	RSD%=0.11
4	LOD&LOQ	0.06&0.20
5	Retention time	2.014
6	Tailing factor	1.73
7	Plate count	9247

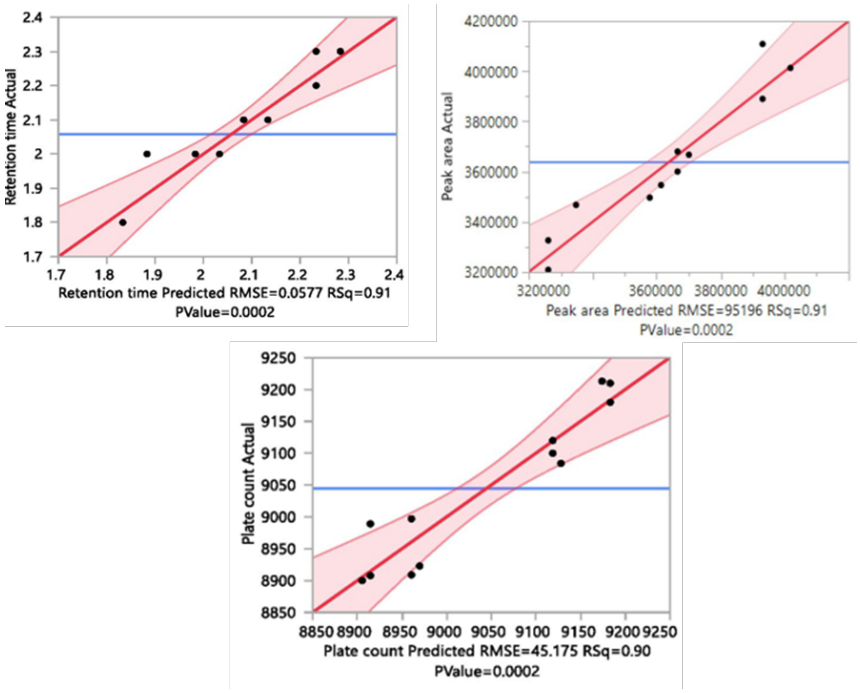

Fig 2. Actual vs Predicted plot for selected responses (retention time, peak area, tailing)

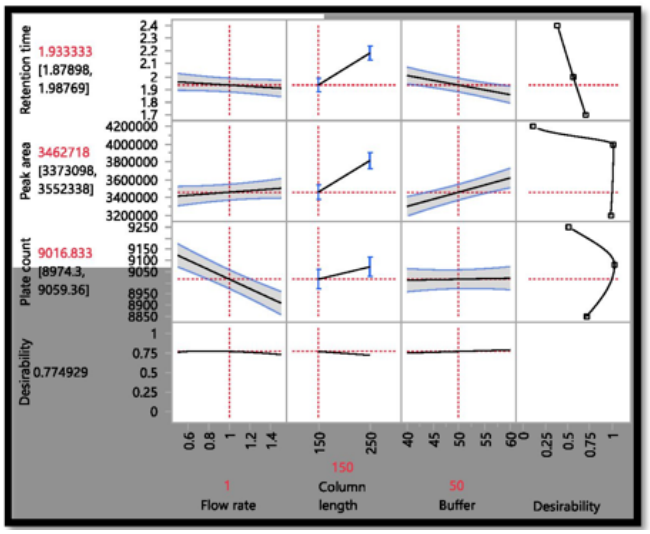

Fig 3. Prediction profiler with maximized desirability

3.4 Method validation

To develop an effective RP-HPLC method, various mobile phases such as methanol, acetonitrile, HPLC water and phosphate buffer at various proportions with different flow rates to obtain a suitable RP-HPLC method, ensuring accurate and efficient separation of components in the sample. After testing various combinations of mobile phases with different ratios, a distinct peak was not found, and the retention time was longer than desired. Finally, sodium dihydrogen phosphate buffer and methanol were tried using at 50:50v/v and got a well-defined peak at 2 mins with a flow rate of 1ml/min, and detected it at 254nm and this method approach works well across a range of concentrations from 50 to 150 micrograms per milliliter. The correlation coefficient shows 1 which shows a strong indication between concentration and the method's response. In accuracy, the

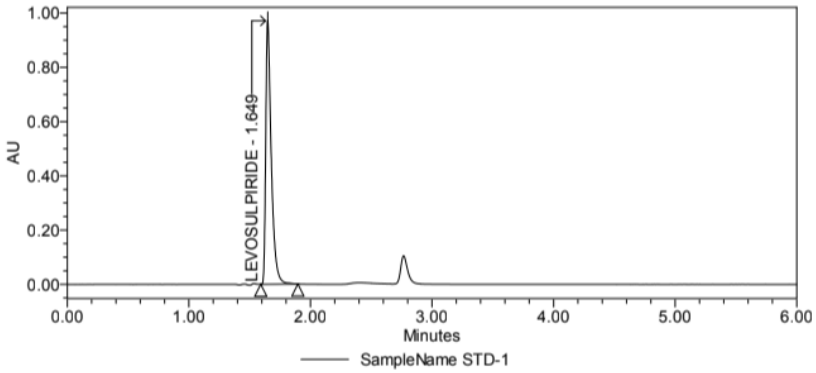

Fig 4. Chromatogram of the optimized method (standard chromatogram)

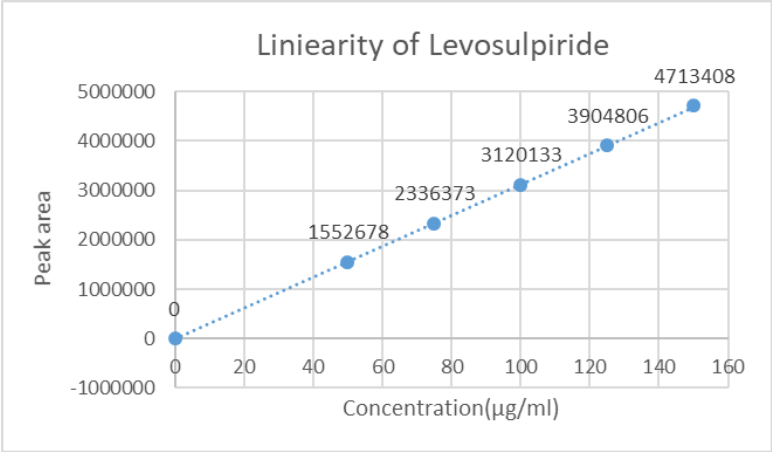

Fig 5. Linearity curve of Levosulpiride

measured values fall within the accepted range where it also shows low variability in measurements. The % RSD values are all < 2 %. The LOD and LOQ were found to be 0.06μg/ml and 0.20μg/ml. Minor variations in experimental conditions, such as flow rate, temperature, etc. These variations' %RSD values are less than 2 per cent overall, which has no discernible impact on the outcomes. The investigation conducted by Ahmad et al. revealed that the LOD and LOQ values were higher and the values were 0.18μg/ml and 0.5506μg/ml[15]. The plate count was found to be more than 2500 showing efficient separation in the chromatographic process and the tailing factor less than 2 signifies symmetrical peak shapes, further confirming the reliability as well as precision of the method[15,22]. The result of the method validation parameters is shown in Table 4.

Table 4. Results of Validation Parameter

Chromatographic condition	Value
Mobile phase	NaH2PO4 (50%): Methanol (50%)
Flow rate	1ml/min
Temperature of the column	25°C

3.5 Forced degradation studies

The stability of levosulpride was tested by intentionally subjecting it to different harsh conditions like acidity, basic conditions, peroxide exposure, heat, light exposure, and water exposure. Among these, the drug underwent noticeable degradation under high-temperature conditions, as shown in **Figure 6**. The detailed outcomes of these forced degradation studies are summarized in Table 5. Likewise, degradation peaks of levosulpride were also notable and comparable for acidic, alkaline, peroxide and heat

with the various studies [15,23,24].

Table 5. Results of forced degradation studies

Stress Conditions	Sample weight (mg)	% Assay	% Degraded
Acid stress	100	90.88	9.12
Base stress	100	94.19	5.81
Peroxide stress	100	96.12	3.33
Heat stress	100	88.09	11.91
Photo stress	100	92.42	7.58
Hydrolysis stress	100	99.51	0.49

4 Conclusion

A simple, rapid, reliable, robust as well as optimized reversed-phase high-performance liquid chromatographic method for the estimation of levosulpride was successfully developed and validated as per the International Conference on Harmonization guidelines Q2 (R1). The QbD technique was used to optimise the wavelength, flow rate, and percentage of the mobile phase. Therefore, this sensitive, accurate and stability-indicating method can be adopted with a high degree of practical utility.

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