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DEVELOPMENT OF STABILITY INDICATING RP-HPLC METHOD FOR DETERMINATION OF LEVOSULPIRIDE HYDROCHLORIDE IN BULK AND PHARMACEUTICAL DOSAGE FORM

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Abstract
A rapid, specific and sensitive stability indicating reverse phase high performance liquid chromatographic method has been developed and validated for analysis of levosulpiride hydrochloride in both bulk and pharmaceutical dosage form. An isocratic stability indicating reversed-phase liquid chromatographic determination was developed for the quantitative determination of levosulpiride in the pharmaceutical dosage form. A sunfire C-18, 4.5µm column with mobile phase containing methanol-water (10:90, v/v) was used. The flow rate was 1.0 mL min^{-1} and effluents were monitored at 232 nm. The retention time of Levosulpiride was 5.5 min. Levosulpiride stock solutions were subjected to acid and alkali hydrolysis, chemical oxidation, wet hydrolysis, dry heat degradation and sun light degradation. The degraded product peaks were well resolved from the pure drug peak with significant difference in their retention time values. Stressed samples were assayed using developed LC method. The proposed method was validated with respect to linearity, accuracy, precision and robustness. The method was successfully applied to the estimation of Levosulpiride in tablet dosage forms. The proposed study describes stability indicating LC method for the estimation of Levosulpiride in bulk and their pharmaceutical dosage form. The method is suitable for the routine analysis of Levosulpiride in tablets.

***Keywords*:** Levosulpiride, Forced degradation, Reversed phase liquid chromatography, Validation.

1. Introduction

Levosulpiride is a levo – enantiomer of racemic sulpiride belonging to the substituted benzamide group (Figure 1). It is a typical neuroleptic drug with sulpiride and inhibits dopaminergic D_2 receptors at the trigger zone both in central nervous system and in the gastrointestinal tract. Developed as an anti – emetic drug, sulpiride soon generated the interest for its antipsychotic properties and low potential to cause extra pyramidal side effect[1]. At low doses, sulpiride acts on pre-synaptic D_2 receptors and increase dopamine turn over in dopamine terminal area[2], this effect produces a behavioral and generalized motor, mental arousal, which is therapeutically useful in depressed patients. At high doses, sulpiride exert its D_2 receptor blocking activity at both pre-synaptic and post-synaptic D_2 receptor sites, eliciting an antipsychotic effect. Levosulpiride acts on central nervous at lower doses than needed with sulpiride. Therefore, it is safe to use[3, 4]. Levosulpiride is a basic drug and has a low bioavailability[5]. Therefore, development of more effective analysis method is demanded for routine analysis in pharmaceutical dosage form.

Several methods has been described in the literature, including UV – visible spectroscopy[6], gas chromatography[7], high performance liquid chromatography with ultraviolet, fluorescence[8,9] or mass spectrometric detection[10] and chiral HPLC method[11]. There has been published method for estimation of levosulpiride in human plasma by HPLC method[12]. Specially, stability indicting RP- HPLC method is routinely used for analysis of levosulpiride in pharmaceutical dosage form as per ICH guidelines[13, 14, 15, 16, 17].

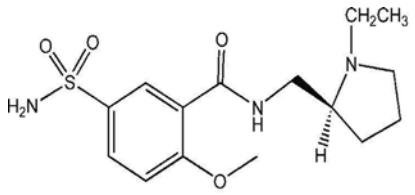

Figure 1: Chemical structure of levosulpiride

2. Experimental

2.1 Apparatus: The liquid chromatographic system of waters (Calcutta, India) containing 515 HPLC isocratic pump, variable wavelength programmable 2998 photodiode array detector and rheodyne injector with 20 µl fixed loop was

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used. A SunFire C_{18} column with 250×4.6 mm i.d. and 5 µm particle size was used as stationary phase.

2.2 Reagents and materials: Analytically pure levosulpiride (LSP) was procured as gratis sample from Sun Pharmaceutical Pvt. Ltd., (Baroda, India). Methanol, water (E. Merck, Mumbai, India) was of LC grade and used for the preparation of mobile phase. Tablet formulation A (Volapride - 25 (25mg), Mankind pharma Ltd., New Delhi, India) containing labelled amount of 25 mg of levosulpiride and tablet formulation 'B' (Nexipride – 50 (50mg), Sun pharmaceutical PVT Ltd., Sikkim, India) containing labelled amount of 50 mg of levosulpiride tablets were purchased from local market.

2.3 Preparation of mobile phase and stock solution: Mobile phase was prepared by 90 ml of water was mixed with 10 ml of methanol. The solution was filtered with Whatman filter paper No. 42 (0.45 µm). The solution was sonicated for 15 min for degassing prior to use.

Stock solutions were prepared by accurately weighing 10 mg of LSP and transferring to 10 ml volumetric flasks containing 3 ml of methanol. The flasks were sonicated for 10 minutes to dissolve the solids. Volumes were made up to the mark with methanol, which gave 1000 µg/ml. Aliquots from the stock solutions were appropriately diluted with mobile phase to obtain working standards of 100 µg/ml of drug.

2.4 Chromatographic conditions: A reversed phase C_{18} column (SunFire) equilibrated with mobile phase comprising of methanol: water (10:90) was used. Mobile phase flow rate was maintained at 1 ml/ min and effluent was monitored at 232 nm. A 20 µL of sample was injected using a fixed loop, and the total run time was 10 min. All the chromatographic separations were carried out at controlled room temperature (25 ± 2 ^{o}C).

2. 5 Calibration curves for LSP: Appropriate aliquots of LSP working standard solution was taken in different 10 ml volumetric flasks. The volume was made up to the mark with mobile phase to obtain final concentrations of 0.1, 0.5, 1, 5, 10 and 20 µg/ml of LSP, respectively. The solutions were injected using a 20 µL fixed loop system and chromatograms were recorded. Calibration curves were constructed by plotting peak area versus concentrations of the drug and regression equations was computed for LSP.

2. 6 Analysis of Marketed Formulations: Twenty tablets were weighed accurately and finely powdered. Tablet powder equivalent to 25 mg for tablet 'A' and tablet 'B' of LSP were taken in 25 ml volumetric flask. A few ml of methanol was added to the above flask and the flask was sonicated for 15 minutes. The solution was filtered in another 25ml volumetric flask using Whatman filter paper No. 42 and volume was made up to the mark with the same solvent.

Appropriate volume of the aliquot was transferred to a 10 ml volumetric flask and the volume was made up to the mark with the mobile phase to obtain a solution containing 5 µg/ml of LSP. The solution was sonicated for 10 min. It was injected as per the above chromatographic conditions and peak area was recorded. The quantifications were carried out by keeping these values to the linear equation of calibration curve.

2.7 Validation: The method was validated for accuracy, precision, specificity, detection limit, quantitation limit and robustness.

2.7.1 Accuracy: The accuracy of the method was determined by calculating recoveries of LSP by method of standard additions. Known amount of LSP (0, 2.5, 5, 7.5 µg/ml) was added to a pre quantified sample solutions and the amount of LSP was estimated by measuring the peak area and by fitting these values to the straight-line equation of calibration curve.

2.7.2 Precision: The instrument precision was evaluated by injecting the solution containing LSP (5 µg/ml) three times repeatedly and peak area was measured. The results are reported in terms of % relative standard deviation. The intra-day and inter-day precision study of LSP was carried out by estimating the corresponding responses 3 times on the same day and on 3 different days (first, second and third day) for 3 different concentrations of LSP (0.1, 5, 20 µg/ml) and the results are reported in terms of % relative standard deviation (RSD).

2.7.3 Specificity: The specificity was estimated by spiking commonly used excipients (starch, talc and magnesium stearate) into a pre weighed quantity of drug. The chromatogram was taken by appropriate dilutions and the quantities of drugs were determined.

2.7.4 Limit of detection and quantification: The detection limit is defined as the lowest concentration of an analyte that can reliably be differentiated from background levels. Limit of quantification of an individual analytical procedure is the lowest amount of analyte that can be quantitatively determined with suitable precision and accuracy. LOD and LOQ were calculated using following equation as per ICH guidelines. LOD = 3.3 ×σ /S and LOQ = 10 ×σ

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

2.7.5 Robustness: Robustness of the method was studied by deliberately changing the experimental conditions like flow rate, percentage of organic phase, and also by observing the stability of the sample solution at 25 ± 2° for 24 h. The sample solution was assayed at every 2 h interval up to 24 h.

2.8 Forced degradation study: Stress degradation study using acid and alkali hydrolysis, chemical oxidation, wet hydrolysis exposure to sun light and dry heat degradation was carried out and interference of the degradation products was investigated. LSP was weighed (10 mg) and transferred to 10 ml volumetric flasks and expose to different stress conditions.

2.8.1 Alkali hydrolysis: To the 10 ml volumetric flask, 10 mg of LSP was taken and 2 ml of 0.1 N NaOH was added to perform base hydrolysis. The flask was heated at 80ºC for 1 week and allowed to cool to room temperature. Solution was neutralized with 0.1 N HCl and volume was made up to the mark with methanol. Appropriate aliquots was taken from the above solution and diluted with mobile phase to obtain final concentration of 10µg mL^{-1} of LSP.

2.8.2 Acid hydrolysis: To the 10 ml volumetric flask, 10 mg of LSP was taken and 2 ml of 0.1 N HCl was added to perform acid hydrolysis. The flask was heated at 80ºC for 1 week and allowed to cool to room temperature. Solution was neutralized with 0.1 N NaOH and volume was made up to the mark with methanol. Appropriate aliquot was taken from the above solution and diluted with mobile phase to obtain final concentration of 10µg mL^{-1} of LSP.

2.8.3 Wet hydrolysis: To the 10 ml volumetric flask, 10 mg of LSP was taken and 2 ml of HPLC grade water was added to perform wet hydrolysis. The flask was heated at 80ºC for 1 week and allowed to cool to room temperature and volume was made up to the mark with methanol. Appropriate aliquot was taken from the above solution and diluted with mobile phase to obtain final concentration of 10µg mL^{-1} of LSP.

2.8.4 Oxidative stress degradation: To perform oxidative stress degradation, 10mg of LSP was taken in 10 ml volumetric flask and 2 ml of 3% hydrogen peroxide was added. The mixture was heated in a water bath at 80°C for 2 h. and allowed to cool to room temperature and volume was made up to the mark with methanol. Appropriate aliquot was taken from above solution and diluted with mobile phase to obtain final concentration of 10 µg mL^{-1} of LSP.

2.8.5 Dry heat degradation: Analytically pure 10 mg sample of LSP was exposed in oven at 80°C for 1 week. The solids were allowed to cool and transferred to volumetric flasks (10 ml) and dissolved in few ml of methanol. Volume was made up to the mark with the methanol. Solution was further diluted by mobile phase taking appropriate aliquots in 10 ml volumetric flask to obtain final concentration of 10 µg mL^{-1} of LSP.

2.8.6 To study photolytic (sunlight) degradation: 10 mg of drug were exposed to sunlight for 1 week. The solids were allowed to cool and transferred to volumetric flask (10 ml) and dissolve in few ml of methanol. Volume was made up to the mark with the methanol. Solution was further diluted with the mobile phase taking appropriate aliquots in 10 ml volumetric flask to obtain final concentration of 10 µg mL^{-1} of LSP.

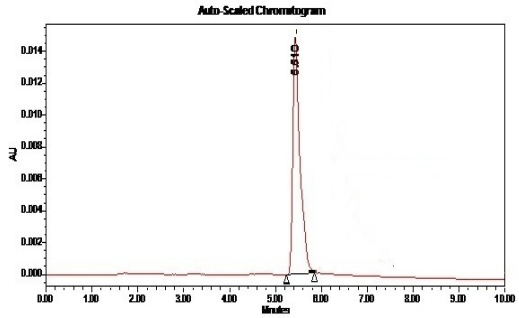
All the reaction solutions were injected in the liquid chromatographic system and chromatograms were recorded.

3. Results and Discussion

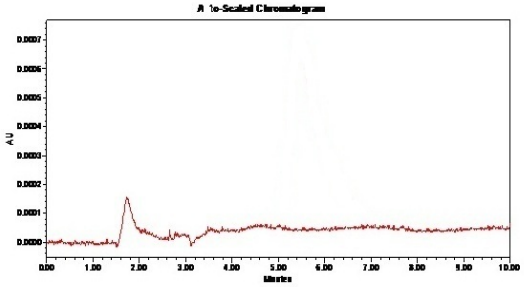
3.1 Optimization of mobile phase: The objective of the method development was to resolve chromatographic peaks for active drug ingredients and degradation products produced under stressed conditions with less asymmetric factor.

Various mixtures containing aqueous buffer, methanol, and acetonitrile were tried as mobile phases in the initial stage of method development. Mixture of methanol: water (90:10, v/v), methanol-water (60:40, v/v), acetonitrile-water (50:50, v/v), were tried as mobile phase but satisfactory resolution of drug and degradation peaks were not achieved.

The mobile phase methanol: water (10:90) was found to be satisfactory and gave symmetric peak for LSP. The retention time for proposed method was found to be 5.5 min as shown in Figure 2. The system suitability parameters like theoretical plates per meter and asymmetric factor for LSP were found to be 4805 and 0.79, respectively. The mobile phase flow rate was maintained at 1 mL min^{-1}. The UV spectra of the drug showed that LSP absorbed appreciably at 232 nm, so detection was carried out at 232 nm.



(A)



(B)

Figure 2: (A) Liquid chromatogram of LSP (RT 5.510) (B) Liquid chromatogram of placebo on C_{18} SunFire column using Methanol: Water (10:90, v/v) as the mobile phase

3.2 Validation of the Proposed Methods

3.2.1 Linearity: The calibration curve for LSP was found to be linear in the range of 0.1 - 20 µg mL^{-1} with a correlation coefficient of 0.9985. The standard deviation value of slope and intercept of LSP was found to be 1399.73 and 1499.16, respectively which indicated strong correlation between peak area and concentration. The regression equation of calibration curves was obtained as y = 47101x + 9567.5.

3.2.2 Precision: Instrument precision was determined by performing injection repeatability test and the % RSD value for LSP was found to be 0.89. The intra-day and inter-day precision studies were carried out and the % RSD value was found to be 0.77 – 1.09 and 1.02 – 1.37, respectively. The low RSD values indicate that the method is precise.

3.3.3 Accuracy: The accuracy of the method was determined by calculating recoveries of LSP by method of standard addition. The recoveries found to be 98.85 – 101.78 % for LSP shown in Table 1. The high values indicate that the method is accurate.

Table 1: Accuracy study of proposed LC method

Amount of Sample (µg/ml)	Sets	Amount drug of spiked (µg/ml)	Area (n=3)	Average amount recovered (µg/ml)	% Recovery	Mean % Recovery	% RSD
5	1	0	243213	5.03	99.21	100.53	1.48
	2	0	250345		102.24		
	3	0	245410		100.14		
5	1	2.5	360770	7.44	99.13	98.85	0.90
	2	2.5	362978		100.06		
	3	2.5	356579		97.35		
5	1	5	480344	10.00	99.90	100.07	0.64
	2	5	477899		98.86		
	3	5	484001		101.45		
5	1	7.5	605838	12.59	103.19	101.78	0.59
	2	7.5	598777		100.19		
	3	7.5	602952		101.96		

3.3.4 Limit of detection and limit of quantification: The detection limit and quantitation limit for LSP was 0.1 µg mL-1 and 0.3 µg mL-1, respectively. The above data shows that a nanogram quantity of the drug can be accurately and precisely determined.

3.3.5 Specificity: The specificity study was carried out to check the interference from the excipients used in the formulation by preparing synthetic mixture containing the drug and excipients. The chromatogram showed peaks for the drug without any interfering peak.

3.3.6 Solution stability: The solution stability study showed that LSP was evaluated at room temperature for 24 hr. The relative standard deviation was found below 2.0%. It showed that solution were stable up to 24 hrs at room temperature.

3.3.7 Robustness: The method was found to be robust, as small but deliberate changes in the method parameters have no detrimental effect on the method performance as shown in table 2. The low value of relative standard deviation was indicating that the method was robust.

Table 2: Data derived from robustness of LSP for proposed LC Method

Parameters	Normal Condition	Change in condition	Area (n=3)	% Recovery	Change in % RSD
Flow rate	1.0 ml/min	0.9 ml/min	248961.7	101.65	1.34
		1.1 ml/min	246271.6	100.45	0.95
Mobile phase ratio	Methanol : Water (10:90)	(8:92)	245562.3	100.21	0.89
		(12:88)	243446.3	99.31	1.24

3.4 Forced degradation study: Chromatogram of base hydrolysis performed at 80^{0}C for 1 week reflux showed degradation of LSP with degradation product peak at retention time (RT) 4.44 min and 12.07 min (Figure 3).

The chromatogram of acid hydrolysis performed at 80^{0}C for 1 week reflux showed degradation of LSP with degradation product peak at retention time (RT) 4.64 min and 10.96 min (Figure 4). The chromatogram of oxidized LSP with 3% hydrogen peroxide at 80^{0}C for 2 h reflux showed degradation of LSP with degradation product peak at retention time (RT) 5.06 min, 14.0 min, 22.93 min, 30.14 min and 32.34 min (Figure 5). The chromatogram of wet hydrolysis LSP with water at 80^{0}C for 1 week drug was found to be stable and the chromatogram of LSP with dry heat at 80^{0}C for 1 week showed drug was found to be stable. The chromatogram of LSP expose to sun light for 1 week showed drug was found to be stable

The degradation study thereby indicated that LSP was found to be stable to wet hydrolysis, dry heat degradation study, effect of sun light while it was susceptible to base hydrolysis and acid hydrolysis and oxidation (3% hydrogen peroxide) (Table 3). No degradation products from different stress conditions affected determination of LSP.

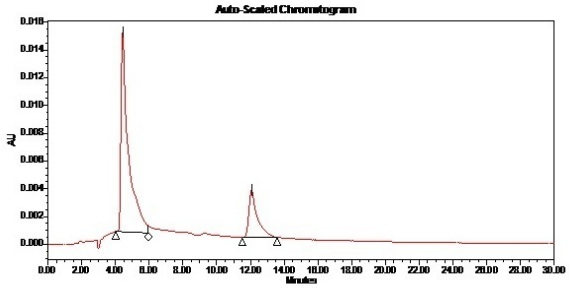


Figure 3: Chromatogram of 0.1M NaOH treated LSP at 80 ^{0}C temperature for 1 week reflux.

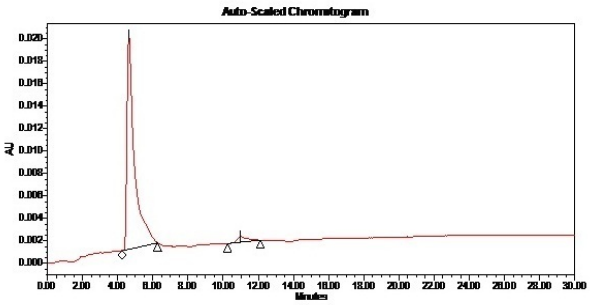


Figure 4: Chromatogram of 0.1M HCl treated LSP at 80 ^{0}C temperature for 1 week reflux.

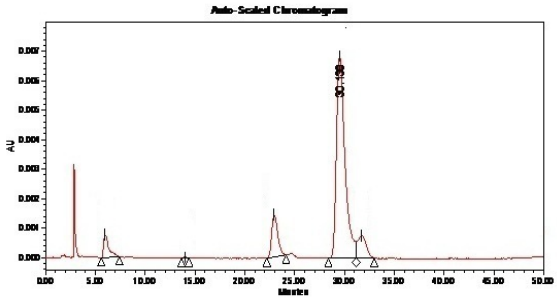


Figure 5: Chromatogram of 3% H_2O_2 treated LSP at 80 ^{0}C temperature for 2 hrs reflux.

Table 3: Data derived from forced degradation study of LSP for proposed LC method

Condition	Time	% Recovery LSP	Retention time of degradation products
0.1 N NaOH[a]	1 week	0	4.43, 12.07
0.1 N HCl[a]	1 week	0	4.64, 10.96
HPLC grade water[a]	1 week	99.92	--
3% H_2O_2[a]	2 hrs	4.03	14.00, 22.93, 30.14, 32.34
Dry heat[a]	1 week	99.51	--
Sun Light	1 week	98.08	--

[a]solutions were heated at 80oC for specified period of time

3.5 Analysis of marketed formulations: The proposed method was successfully applied to the determination of LSP in their tablet dosage form (Tablet A and Tablet B). The % recovery for LSP for tablet 'A' and tablet 'B' was found to be 100.40 ± 1.16 and 98.66 ± 1.09 mean value ± standard deviation of three determinations which was comparable with the corresponding labeled amounts.

Conclusion

Proposed study describes stability indicating LC method for the estimation of LSP in bulk and their pharmaceutical dosage form. The method was validated and found to be simple, sensitive, accurate and precise. Statistical analysis proved that method was repeatable and selective for the analysis of LSP without any interference from the excipients. The method was successfully used for determination of drug in their pharmaceutical formulation. Also the above results indicate the suitability of the method for acid, base, oxidation, dry heat and photolytic degradation study. As the method separates the drugs from its degradation products, it can be used for analysis of stability samples. The method is suitable for the routine analysis of LSP in tablets. In addition, the HPLC procedure can be applied to the analysis of samples obtained during accelerated stability experiments to predict expiration dates of pharmaceuticals.

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