

Development and Validation of an RP-HPLC Method for Quantitative Estimation of Eslicarbazepine Acetate in Bulk Drug and Tablets

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Singh, *et al.*: HPLC Method Development and Validation of Eslicarbazepine Acetate

A convenient, simple, accurate, precise and reproducible RP-HPLC method was developed and validated for the estimation of eslicarbazepine acetate in bulk drug and tablet dosage form. Objective was achieved under optimised chromatographic conditions on Dionex RP-HPLC system with Dionex C18 column (250×4.6 mm, 5 µm particle size) using mobile phase composed of methanol and ammonium acetate (0.005 M) in the ratio of 70:30 v/v. The separation was achieved using an isocratic elution method with a flow rate of 1.0 ml/min at room temperature. The effluent was monitored at 230 nm using diode array detector. The retention time of eslicarbazepine acetate is found to be 4.9 min and the standard calibration plot was linear over a concentration range of 10-90 µg/ml with $r^2=0.9995$. The limit of detection and quantification were found to be 3.144 and 9.52 µg/ml, respectively. The amount of eslicarbazepine acetate in bulk and tablet dosage form was found to be 99.19 and 97.88%, respectively. The method was validated statistically using the percent relative standard deviation and the values are found to be within the limits. The recovery studies were performed and the percentage recoveries were found to be $98.33 \pm 0.5\%$.

Keywords: Eslicarbazepine acetate, RP-HPLC, ICH guidelines, method validation

Eslicarbazepine acetate chemically known as (S)-10-acetoxy-10,11-dihydro-5H-dibenz[b,f]azepine-5-carboxamide (fig. 1), is a new broad spectrum antiepileptic drug. Eslicarbazepine acetate is effective for the treatment of partial and generalised tonic-clonic seizures as a single drug or as an adjuvant with other antiepileptic drug^[1-6]. Literature survey reveals that no pharmacopoeial method is available in Indian Pharmacopoeia, British Pharmacopoeia and United States Pharmacopoeia. However some high-performance liquid chromatography (HPLC) methods have been reported for the quantification of eslicarbazepine acetate in pharmaceutical dosage forms^[7-10]. Present study involves development of a convenient, rapid and user friendly reversed-phase (RP)-HPLC method with a simple and easily available mobile phase for quantitative estimation of eslicarbazepine acetate in bulk drug and tablet dosage form. The optimised method was developed and validated as per International Conference on Harmonisation (ICH) guidelines^[11].

Sample and working standards of eslicarbazepine acetate were obtained from Sun Pharma Pvt. Ltd.,

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Mumbai, HPLC grade methanol was purchased from E. Merck, Mumbai and ammonium acetate of analytical reagent grade was purchased from Sisco Research Laboratories, Mumbai. The liquid chromatography was performed on Dionex UHPLC ultimate 3000 RS containing pump, autosampler, column compartment and Diode array detector. Analysis was performed using Dionex octadecylsilane column with dimensions 250×4.6 mm and particle size 5 µm. The data acquisition was achieved through Chromoleon software.

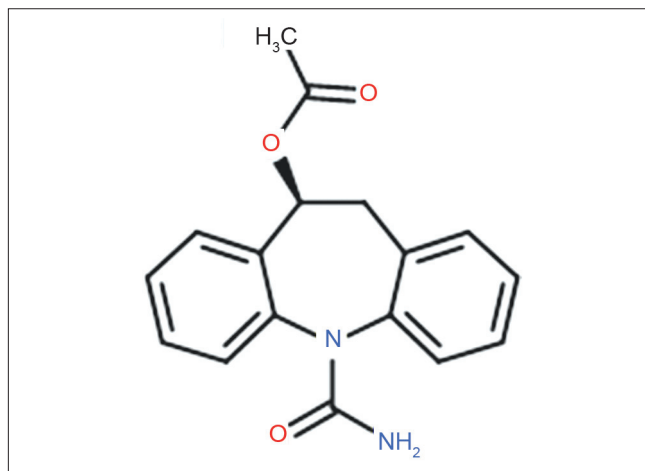


Fig. 1: Chemical structure of eslicarbazepine acetate.

The elution of eslicarbazepine acetate was obtained by running HPLC in isocratic mode using methanol and 0.005 M ammonium acetate in a ratio of 70:30 v/v, flow rate was maintained at 1.0 ml/min with run time of 10 min. The retention time for eslicarbazepine acetate was obtained at 4.9 min and detection was performed at 230 nm. Mobile phase was previously filtered through Whatmann filter paper no 41.

Stock solution (1000 ppm) of eslicarbazepine acetate was prepared by dissolving 100 mg of eslicarbazepine acetate standard in methanol in a 100 ml volumetric flask, the volume was made up to the mark with methanol. This stock solution was further diluted to obtain desired concentrations.

Twenty tablets of eslicarbazepine acetate (labelled claim 400 mg of eslicarbazepine acetate) were weighed and average weight was calculated. The tablets were crushed to get homogenous powder and a quantity equivalent to 100 mg was weighed in a 100 ml volumetric flask. The powder was then allowed to dissolve in methanol by sonication. Make up the volume to the mark with methanol and filter the solution through 0.2 μm filters. The filtrate was diluted to obtain desired concentrations. Appropriate dilutions of eslicarbazepine acetate were prepared from the stock solution, which were scanned over a range of 200-400 nm. The maximum absorbance was found at 230 nm.

The analytical method was optimised by varying the ratio of mobile phase. A 70:30 ratio of methanol and 0.005 M ammonium acetate was found to give the

best system suitability parameters. The criteria for the selection of mobile phase and its composition were symmetry, shape and retention time of the peak. The selection of buffer is due to its slightly neutral pH, which is safe for column life and suitable for analyte stability, whereas methanol is readily available solvent. Considering the already proposed methods in literature, the advantages of this new proposed method are rapid results (retention time 4.9 min), quick analysis time (run time 10 min), economic mobile phase, user friendly and convenient approach. All these key features proposed that this method can be considered as advantageous over other methods (fig. 2).

The developed analytical method was further subjected to validation in accordance to the ICH guidelines. The parameters evaluated were system suitability, linearity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ), robustness and specificity.

System suitability was evaluated by replicate injections of 50 ppm standard solution of eslicarbazepine acetate. The parameters, such as tailing factor, percent relative standard deviation (RSD) and theoretical plates, were studied and found satisfactory (Table.1).

Precision of an analytical procedure is referred to as degree of scatterness between a series of observations obtained from multiple sampling of same homogenous sample in given conditions. The term intraday precision (repeatability) refers to the use of analytical procedure within same laboratory conditions over a short period of time by same

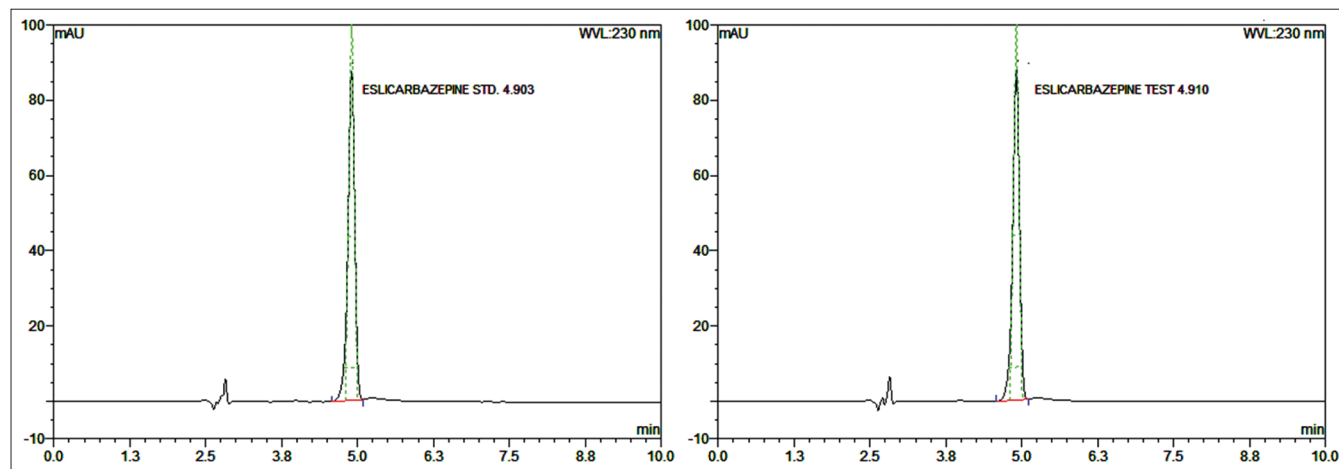


Fig. 2: Chromatograms of eslicarbazepine acetate. Chromatograms of eslicarbazepine acetate standard (a) and test (b).

TABLE 1: VALIDATION DATA

Parameters	Average	% RSD
Theoretical plates	9434	0.58
Tailing factor	0.945	1.76
Precision (area)		
Inter-day	13.230	0.64
Intra-day	13.35	0.10
Recovery studies		
0% analyte added	97.8	0.57
80% analyte added	98.0	0.35
100% analyte added	98.9	0.78
120% analyte added	98.4	0.78
Robustness (retention time)		
% of Methanol (± 2 units)	4.87	0.71
Flow rate (± 0.1 unit)	4.89	0.51
Column temperature (± 0.2 unit)	4.90	0.71
Assay (%)		
Bulk drug	99.19	0.72
Tablets	97.84	0.72

RSD=Relative standard deviation

analyst and same instrument, whereas interday precision (intermediate precision) refers to the use of analytical procedure in same laboratory conditions on different days by different analysts. Six injections of eslicarbazepine acetate 50 ppm were applied twice after a short interval of time on the same day, the procedure was repeated on two consecutive days by different analysts and %RSD was reported (Table 1).

Accuracy is the closeness of the test results to that of the true value, which can be determined in terms of percent recovery. The recovery study was performed at three levels mainly 80, 100 and 120% by adding known amount of pure drug in to the 50 ppm sample solution. The results of recovery studies was tabulated and found satisfactory (Table 1).

Different concentrations ranging from 10 to 90 ppm were injected in duplicate, out of which five concentrations (10, 30, 50, 70 and 90 ppm) were selected and each concentration was injected in duplicate. The mean peak areas were recorded and the calibration curve was established by plotting concentration on x-axis and peak area on y-axis. The method gives linear response as shown by the regression equation, $Y=0.259X+0.218$, r^2 is obtained 0.9995 where Y is the peak area in mAU and X is the concentration in ppm and r^2 is the correlation coefficient. The results show that the Beer's law is obeyed in the concentration range of 10-90 ppm.

Range is the minimum and maximum concentration of the sample at which the analytical procedure

gives reproducible results. Range can be determined by linearity, accuracy and precision studies. The method was found acceptable across wide range of concentration (10-90 ppm). The LOD is the minimum concentration of the analyte that gives a measurable response, which can be calculated using the formula $LOD=3.3 \times \sigma/S$, where as LOQ is the minimum concentration that can be quantified accurately and precisely and was calculated using the equation, $LOQ=10 \times \sigma/S$, where σ is residual standard deviation of the response and S is the slope of the calibration curve. The LOD and LOQ is calculated as 3.144 and 9.52 $\mu\text{g/ml}$ units, respectively.

The retention time of the sample solution of eslicarbazepine acetate tablet was found to be 4.9 min, which is similar to that of the standard solution of eslicarbazepine acetate. This indicates that there is no drug-excipient interference and the drug is properly resolved by the developed method.

Robustness determines the reproducibility of the test result with small and deliberate variations in the method parameters. The experiment was carried out by slightly changing the ratio of methanol (50 ± 2 ml) in the mobile phase, column oven temperature ($25.0 \pm 0.2^\circ$) and flow rate (1.0 ± 0.1 ml), The effectiveness of the deliberate variations was observed on retention time of peak. The statistical data gives no significant variations in the above parameters indicating that the method is robust (Table 1).

The proposed method was successfully applied for the estimation of eslicarbazepine acetate in bulk drug and tablet dosage form. The assay results were compiled, (Table 1) found satisfactory and show that there is no interference of the tablet matrix with the drug. Low %RSD shows that this method can be easily applied for the estimation of eslicarbazepine acetate in bulk drug and tablet dosage form.

A HPLC method for the quantitative estimation of eslicarbazepine acetate in bulk drug and tablet dosage form has been developed and found to be applicable for the routine analysis of eslicarbazepine acetate in bulk and tablet dosage forms without any interference from the excipients. Statistical results and low %RSD values indicate that the method is precise, accurate, robust, specific and can be used for routine analysis of the drug.

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