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Research Article

NEW ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF ESLICARBAZEPINE ACETATE BY RP-HPLC METHOD

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ABSTRACT

A simple, more rapid and more accurately approachable method has been developed to quantify Eslicarbazepine acetate. The following study explicates that the analyte component separation was achieved on a C18, 5µm Waters column (150 mm × 4.6 mm) using a mobile phase of Methanol - Potassium dihydrogen phosphate solution (60:40, v/v) containing O-phosphoric acid to adjust to pH-3, having a flow rate of 1.0 ml/min. The UV detector was operated at 230 nm. The method was validated for specificity, linearity, precision and accuracy, limit of detection and limit of quantification. The degree of linearity of the calibration curves, the percent recoveries, limit of detection and quantification for the HPLC method were determined. By using this approach the precision and accuracy of the quantitative method were considerably improved. The method was found to be simple, specific, precise, accurate, and reproducible. The method was validated as per the ICH guidelines. The method was successfully applied for routine analysis of Eslicarbazepine acetate in pure drug from.

Keywords: Eslicarbazepine acetate (ESL), RP-HPLC, UV-Detection, precise, 230 nm.

INTRODUCTION

Eslicarbazepine acetate (ESL) is an anti-epileptic drug. Epilepsy is a chronic disease that necessitates long-term treatment. The WHO predicted that around 50 million people[1] in the world contain epilepsy at any one time, which is approximately 1% of the world population. Antiepileptic drugs (AEDs) are among the most commonly prescribed centrally active agents. In a recent survey of 471,873 persons carried out in Denmark, 5,426 were found to be receiving AEDs, which corresponds to a prevalence of 1.1% [2]. The use of these drugs also increases with increasing age. The U.S. study of 10,168 elderly nursing home residents revealed that 1,132 (11.1%) were prescribed AEDs, and in 19% of these, the indication was unrelated to seizures or epilepsy [3]. AED's are widely used to treat conditions other than epilepsy, including migraine, neuropathic pain, bipolar disorder, anxiety, and many other disorders [4].

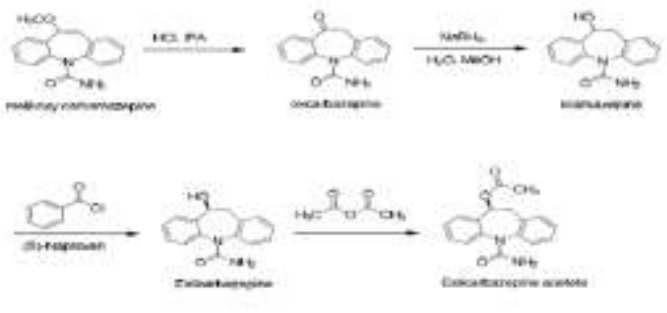
Eslicarbazepine acetate (ESL) is one of the newest AED's [5].The chemical name [6] of Eslicarbazepine acetate (ESL) is (S)-(-)-10-Acetoxy-10, 11-dihydro-5H-dibenz [b, f] azepine-5-carboxamideESL was previously known as BIA 2-093, is a novel chiral drug presently completing phase III clinical trials, as add-on therapy in refractory partial epilepsy, and undergoing phase II clinical trials, as monotherapy in partial epilepsy and in bipolar disorder (Almeida and Soares-da-Silva, 2007).

ESL is a prodrug that is activated to Eslicarbazepine (S-licarbazepine), an active metabolite of oxcarbazepine. It was developed by Bial and marketed as Zebinix or Exalief by Eisai Co. in Europe and as StedESL by Sepracor in America. ESL is chemically related to CBZ and OXC (Benes *et al.*, 1999). In pharmacokinetic studies in humans, ESL was quickly and broadly metabolized to Eslicarbazepine (S-licarbazepine), which is accountable for pharmacological activity. Since polytherapy is commonly employed in the treatment of epilepsy, there are also methods to quantify simultaneously various AED's [7–9].

ESL allocate with carbamazepine and oxcarbazepine the dibenzazepine nucleus bearing the 5-carboxamide substitute, but is structurally different at the 10, 11-position. This molecular variation results in variation in metabolism, avoiding the formation of toxic epoxide metabolites such as carbamazepine-10, 11-epoxide. In pharmacokinetic studies in humans, ESL was quickly and broadly metabolized to Eslicarbazepine (S-licarbazepine), which is accountable for pharmacological activity. ESL was tested in patients with refractory partial-onset seizures and was created to be effective and well tolerated [10-15].

Adverse drug reactions

The most common side effects observed regardless of amount or frequency of dosage were dizziness, somnolence, headache, nausea, diplopia and vertigo.[16,17,18,19] The main adverse events leading to discontinuation of the medication in clinical trials included dizziness, abnormal coordination, and nausea.[17,18,19] These side effects appear to be dose-dependent. Skin rash has been reported in approximately 3% patients treated with ESL in the preclinical trials.[17,18,19] Electrocardiographic changes consisting of a mild prolongation of the PR interval has been observed in patient receiving ESL.[20] ESL should not be used in patients with 2nd and 3rd degree heart block, and used with caution when co-administered with other drugs that can prolong the PR interval[20].



A brief synthetic scheme of Eslicarbazepine acetate

MATERIALS AND METHOD

Optimized chromatographic conditions for the determination of ESL by HPLC:

Column: WATERS C_{18} 150 mm x 4.6 mm x 3.5µm.
Flow rate: 1.0 ml/min
Column temperature: 24° C
Materials required: Acetonitrile, methanol, Water, Buffers, and Eslicarbazepine acetate.
Injection volume: 20.0µL
Diluent: Methanol
Preparation of Mobile phase: A mixture of 0.01M KH_2PO_4 of pH-3 and Methanol in the ratio of 60:40 (v/v).

Preparation of Stock Solutions:

An ESL stock solution (1 mg/ml) was prepared by dissolving a 25 mg of Eslicarbazepine acetate in a 25ml volumetric flask separately and dissolved in Methanol and the volume was made up to the mark with the Methanol. From the above 1mg/ml solution, six dilutions in between 10-60 µg/ml of Eslicarbazepine acetate were made with Methanol by pipetting out 0.1-0.6 ml from the 1mg/ml solution.

Preparation of Sample Solution:

25 mg powder of Eslicarbazepine acetate was weighed. The powder was accurately transferred to 25ml volumetric flask containing 20 ml of the Methanol and sonicated for 5-10 min. The above solution was carefully filtered through Whatmann filter paper (No. 41) only if solution remains unclear and then the volume was made up to the mark with Methanol.

METHOD DEVELOPMENT

Selection of wavelength:

ESL standard solutions were prepared in diluent at a concentration range of 10µg-60µg/ml and scanned in UV detector; all the solutions of ESL were having UV maxima at around 230 nm.

Hence detection at 230 nm was selected for method development purpose.

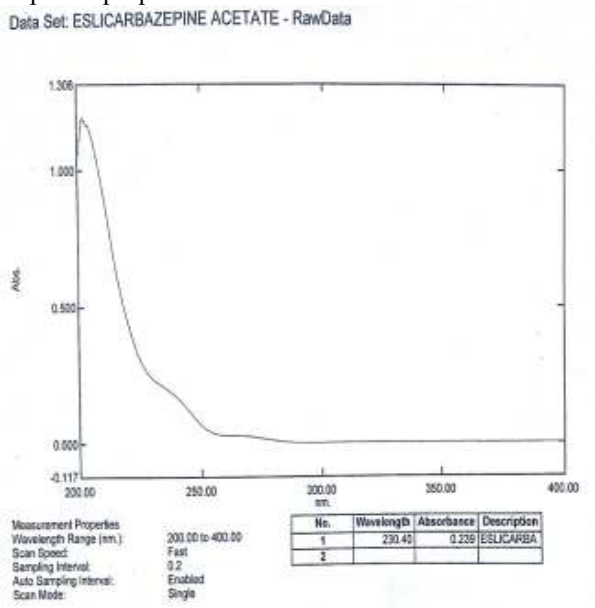


Figure: UV-Spectrum of Eslicarbazepine acetate

Method development approach for the selection of suitable column and mobile phase:

The main aim of the chromatographic method was to separate critical closely eluting compounds of ESL and to elute ESL as a symmetrical peak. ESL spiked solutions were subjected to separation by reversed-phase Zorbax SBC18 column, 150 x 4.6mm x 3.5µm and carried out the analysis with the above conditions. The successful separation was observed with resolution greater than 2.0.

To study the importance of the method with respect to mobile phase pH, various pH's of the buffer were prepared from pH=2.0 to 7.0.

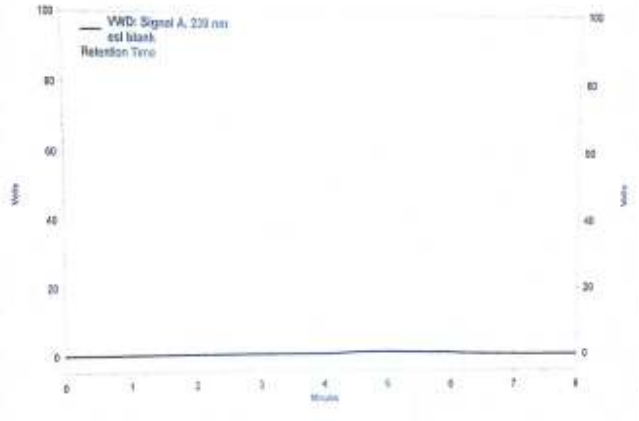


Figure: Typical blank chromatogram of method development

VALIDATION PARAMETERS

Accuracy:

The accuracy of an analytical method is the degree of closeness between the true value of analytes in the sample and the value determined by the method and is sometimes called trueness [21]. Accuracy can be measured by analyzing samples with known concentrations and comparing the measured values with the true values. According to FDA [22] the accuracy for bio-analysis should be determined by a minimum of five determinations for at least three concentrations (low, medium and high) in the range of expected concentrations.

% Active/impurity content	Acceptable mean recovery
≥ 10	98 –102%
≥ 1	90 –110%
0.1 – 1	80 – 120%
< 0.1	75 – 125%

Table: Acceptance Criteria for Accuracy

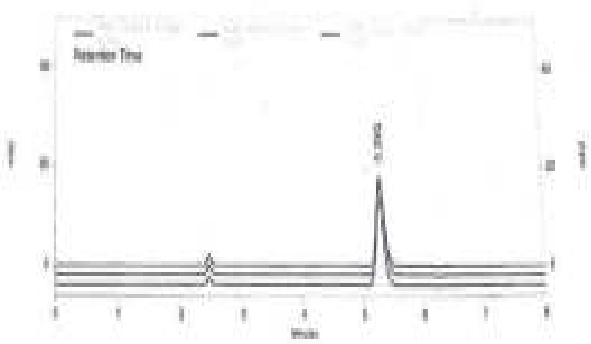


Fig: Accuracy of Eslicarbazepine acetate at 80%

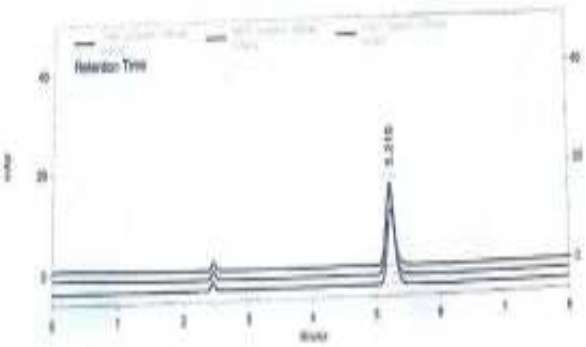


Fig: Accuracy of Eslicarbazepine acetate at 100%

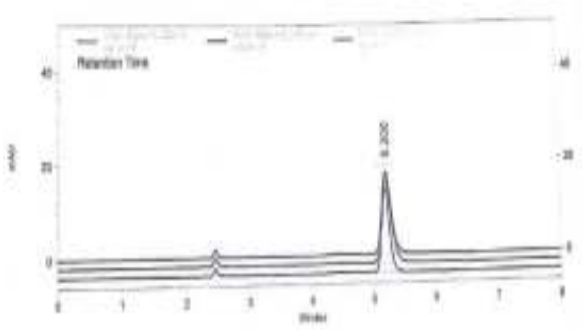


Fig: Accuracy of Eslicarbazepine acetate at 120%

Precision:

The precision of an analytical method is the closeness of a series of individual measurements of an analyte when the analytical procedure is applied repeatedly to multiple aliquots of a single homogeneous volume of biological matrix [22]. The precision is calculated as coefficient of variation (C.V.) i.e., relative standard deviation (RSD). The measured RSD can be subdivided into three categories: repeatability (intra-day precision), intermediate precision (inter-day precision) and reproducibility (between laboratories precision) [22, 21, 23]. Repeatability should be tested by the analysis of a minimum of five determinations at three different concentrations (low, medium and high) in the range of expected concentrations, according to FDA [22].

Component measured in sample	Precision
≥ 10.0%	2%
1.0 up to 10.0%	≤ 5%
0.1 up to 1.0%	≤ 10%
<0.1%	≤20%

Table: Acceptance Criteria for Precision

Precision may be measured at three levels:

- Repeatability,
- Intermediate precision and
- Reproducibility.

It is normally expressed as RSD %.

Repeatability is the results of a method operated over a short interval of time under the same conditions.

Inter-day precision

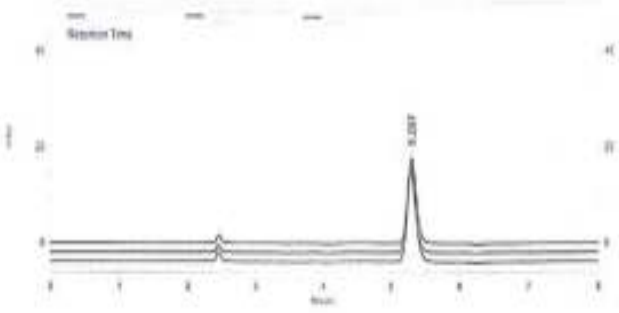


Figure: Chromatogram for precision of ESL Day-1

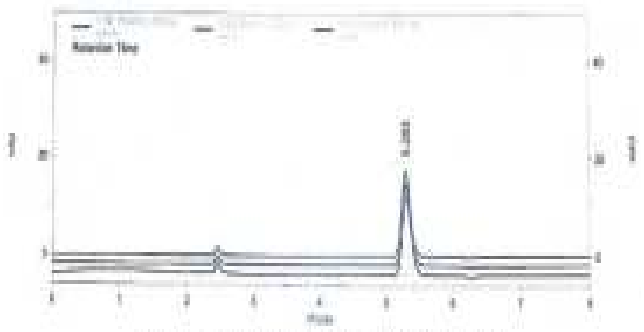


Figure: Chromatogram for precision of ESL Day-2

Intraday precision

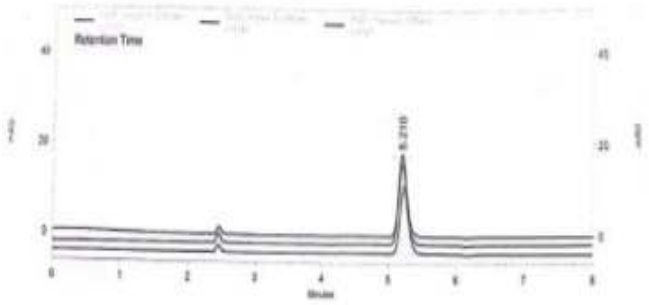


Figure: Chromatogram for precision of ESL Morning

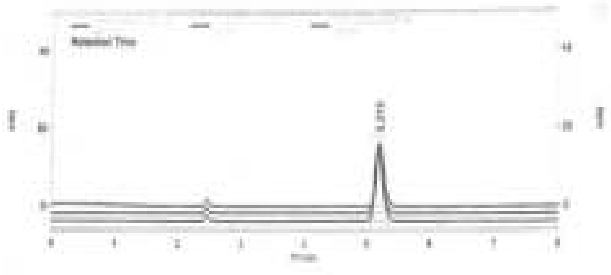


Figure: Chromatogram for precision of ESL Afternoon

Reproducibility is determined by testing the homogeneous samples in different laboratories. It is a measure of precision between laboratories.

Specificity

It is defined as the instrument's ability to measure or identify the analyte without any interference from sample matrix, impurities, precursors or degradation products. Vessman [25] pointed out; specificity refers to a method that gives response for only one single analyte.

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components. Lack of specificity of an individual analytical procedure may be compensated by other supporting analytical procedures.

Linearity:

The linearity of an analytical method is its ability to elicit test results that are directly proportional to the concentration of analytes in samples within a given range or proportional by means of well-defined mathematical transformations. Linearity may be demonstrated directly on the test substance (by dilution of a standard stock solution) and/or by using separate weighing of synthetic mixtures of the test product components, using the proposed procedure.

Linearity is determined by a series of 3 to 6 injections of 5 or more standards whose concentrations span 80–120 percent of the expected concentration range. The response should be directly proportional to the concentrations of the analytes or proportional by means of a well-defined mathematical calculation. A linear regression equation applied to the results should have an intercept not significantly different from 0. If a significant nonzero intercept is obtained, it should be demonstrated that this has no effect on the accuracy of the method.

Linearity of Eslicarbazepine acetate

The linearity of an analytical method is the ability to attain test results which are directly proportional to the concentration of analyte with in the given range.

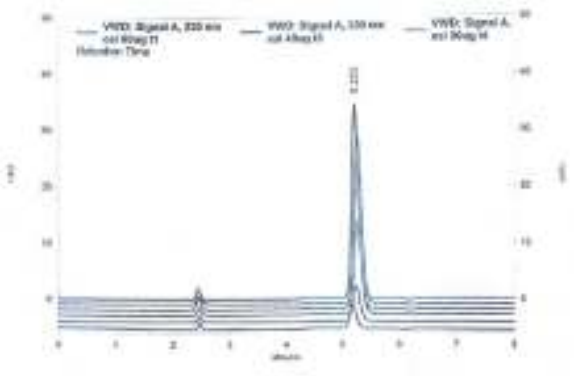
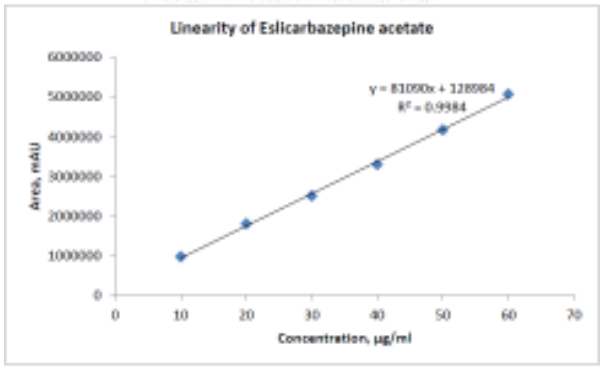


Figure: Chromatogram for linearity of ESL



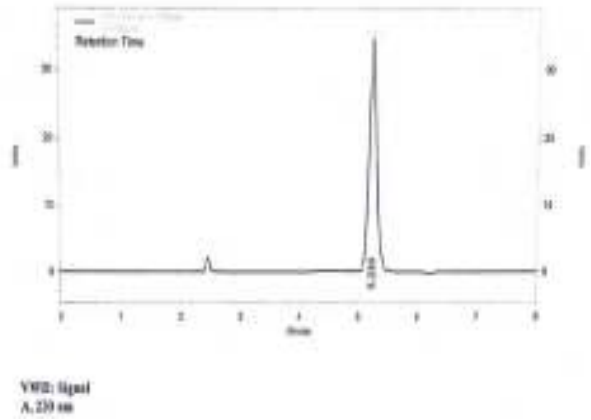
Linearity coefficient (r^2) = 0.9984

System suitability test:

System suitability testing is an integral part of chromatographic method. The tests are based to ensure that the equipment, analytical operations, electronics and samples to be analysed make an integral system and it can be calculated as such.

Tailing factor and theoretical plates for ESL was calculated. System suitability results were tabulated.

Figure: System suitability test



Theoretical plates per column were calculated from the data obtained from the peak using the following expression

$$n = (5.54Vr2)/LWh2$$

Theoretical plates per meter were calculated from the data obtained from the peak.

$$n = (5.54Vr2)/Wh2$$

Where, 'n' is number of theoretical plates per meter, 'Vr' is the distance along the base line between the point of injection and a perpendicular dropped from the maximum of the peak of interest and 'Wh' is the width of the peak of interest at half peak height.

Tailing factor is also known as symmetry factor symmetry factor of peak was calculated from the following expression.

Symmetry factor = a/b

Where, **a** = ½ width of the peak at one twentieth of the peak height,

b = ½ width of the peak at one twentieth of the peak height.

Limit of Quantification (LOQ) and Limit of Detection (LOD):

LOQ and LOD established for all impurities based on the impurities dilution linearity method.

Methodology for establishment of LOQ and LOD:

LOD and LOQ are determined by injecting linear solutions from 10µg/mL to 60µg/ml. The calculation method is based on the standard deviation (SD) of the response and the slope (S) of the calibration plot and using the formula;

LOQ = 10 x SD/S and
LOD = 3 x SD/S.

RESULTS AND DISCUSSION

The method that was developed and optimized in HPLC was considered for method validation. The analytical method validation was carried out in accordance with ICH guidelines. The results are discussed in the following section.

Accuracy:

The accuracy of an analytical method is measure of the closeness of test results obtained to the true value.

Table: Accuracy results of Eslicarbazepine acetate

S.no	Area of the % level concentrations, mAU		
	80 %	100 %	120 %
1	2506177	2506620	2506951
2	2506412	2506712	2507018
3	2506881	2507127	2506886
Average	2506493	2506820	2506952
Standard deviation (SD)	363.8823	270.1043	66.00253
%RSD	0.014518	0.010775	0.002633

Precision results:

The precision of the method was also ensured by injecting six individual preparations of ESL. Upon repetitive injections at quantification limit, the peak properties like retention time, area were not influenced. Results have shown negligible variation in measured responses which revealed that the method was repeatable with RSD below %.

Table: Precision results of ESL

S.no	Peak name	Morning	Afternoon	Day - 1	Day - 2
1	Esl	5066719	5066857	5067005	5066491
2	Esl	5066780	5067243	5067462	5066832
3	Esl	5067221	5067192	5067081	5067102
4	Esl	5066883	5066983	5066864	5067344
5	Esl	5066772	5067214	5067241	5067138
6	Esl	5066366	5066992	5067189	5066936
Average		5066790	5067124	5067159	5066955
Standard Deviation (SD)		251.5455	180.9286	173.476	301.946
%RSD		0.004965	0.003571	0.00343	0.005959

Linearity:

Table: Linearity results of Eslicarbazepine acetate

Concentrations in µg/ml	Area, mAU
10	970122
20	1800455
30	2500523
40	3297328
50	4167624
60	5066762
Correlation coefficient (r^2)	0.9984
Slope	81090
Y intercept	y = 81090x + 128984

System suitability test:

System suitability testing is an integral part of chromatographic method. The tests are based to ensure that the equipment, analytical operations, electronics and samples to be analyzed make an integral system and it can be calculated as such.

Table: Results of system suitability

Compound Name	Retention time (R_t), min	Theoretical plates (N)	Capacity Factor	Asymmetry	S/N(6 sigma)
Eslicarbazepine acetate	5.230	8034	-0.01154	1.15408	110.378519

Limit of Quantification (LOQ) and Limit of Detection (LOD):

Table: LOQ and LOD values of ESL

S.no	Product name	LOQ in µg/ml	LOD in µg/ml
1	Eslicarbazepine acetate	5.4362	1.6308

Table: Recovery results of Eslicarbazepine acetate

S.no	Level, %	Label	Amount added	Amount recovered	% Recovery	% RSD
1	80	400	320	400.92	100.23	0.020768
2	100	400	400	401.0	100.25	0.026399
3	120	400	480	401.04	100.256	0.015235

Acceptance criteria: The individual and the mean recovery value should be within 98 to 102%.

Conclusion: The result obtained in this method was within the limit of 98.0% to 102%.

The % RSD is less than 2.0.

SUMMARY AND CONCLUSION

The quick and effective RP-HPLC method developed for quantitative estimation of Eslicarbazepine acetate is accurate, precise, linear, and specific.

Table: Summary of validation results by RP-HPLC

Test parameters	Eslicarbazepine acetate
Precision (%RSD)	0.003959
LOD (µg/mL)	1.6308
LOQ (µg/mL)	5.4362
Linearity (R^2 value)	0.9984
Accuracy (% RSD)	0.014513
Retention time (R_t) min	5.230
Theoretical plates (N)	8034

The proposed method does not require any laborious clean up procedure before measurement. Acceptable results were obtained from validation of the method. This method revealed an excellent performance in terms of sensitivity and speed.

The method is proven as stability-indicating and can be used for routine analysis of production samples and to quantify the samples of ESL in drug substances and pharmaceutical dosage forms. Thus the method was validated as a safe, precise, fast, a much specific approach and accurate.

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