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A new LC-MS Method for the Determination of p-Chloroaniline and (S)-5-Chloro-α-(cyclopropylethynyl)-2- Amino-α- (trifluoromethyl) Benzene Methanol in Efavirenz Bulk Form

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Authors' contributions

This work was carried out in collaboration among all authors. Author IPM has generated the research activity and prepared the manuscript. Authors ND and GS were given guidance and supervision to carry out this research work. All authors read and approved the final manuscript.

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ABSTRACT

The main objective of the present research study is to develop and validate a sensitive, specific, accurate and precise LC-MS method for the determination of p-Chloroaniline and (S)-5-Chloro-α-(cyclopropylethynyl)-2- amino-α- (trifluoromethyl) benzene methanol in Efavirenz bulk form. The effective separation of p-Chloroaniline and (S)-5-Chloro-α-(cyclopropylethynyl)-2- amino-α-(trifluoromethyl) benzene methanol were achieved by using Hypersil BDS (C18, 100 x 4.6 mm, 3 µm) column and a solvent system of Buffer (0.1% Formic acid in water): Methanol (30:70 v/v) with a flow rate of 0.4 ml/min. The p-Chloroaniline and (S)-5-Chloro-α-(cyclopropylethynyl)-2- amino-α-(trifluoromethyl) benzene methanol were monitored on mass spectrometer coupled with atmospheric pressure chemical ionization, positive polarity mode and quadrapole mass analyzer.

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The Retention time of p-Chloroaniline, (S)-5-Chloro-α-(cyclopropylethynyl)-2- amino-α-(trifluoromethyl) benzene methanol and Efavirenz were found at 5.7min, 7.6min and 11.1min resepectively. The detection limit and quantification limit were observed at 0.25ppm and 0.75 ppm respectively for both p-Chloroaniline and (S)-5-Chloro-α-(cyclopropylethynyl)-2- amino-α-(trifluoromethyl) benzene methanol. Those analytes were linear in the concentration ranges from 0.75ppm to 3.75ppm and the percentage relative standard deviation of six replicates of same concentrations of both the analytes were less than 10%. Hence this method was effective in separation and determination of p-Chloroaniline and (S)-5-Chloro-α-(cyclopropylethynyl)-2- amino-α- (trifluoromethyl) benzene methanol in Efavirenz.

Keywords: p-Chloroaniline; (S)-5-Chloro-α-(cyclopropylethynyl)-2- amino-α- (trifluoromethyl) benzene methanol; 01% Formic acid in water; atmospheric pressure chemical ionization; quadrapole mass analyzer.

1. INTRODUCTION

Efavirez is an anti-viral agent used to treat Hepatitis -B and human immune virus diseases[1,2] alone or in combination with other antiviral agents. It mainly acts by inhibiting the DNA polymerase non competitively results in ceases of replication of viral DNA (1,2). The IUPAC name of Efavirez is4S)-6-chloro-4-(2-cyclopropylethynyl)-4-(trifluoromethyl)-1H-3,1-benzoxazin-2-one. p-Chloroaniline (PCA) and (S)-5-Chloro-α-(cyclopropylethynyl)-2- amino-α-(trifluoromethyl) benzene methanol (Impurity-1) are major process impurities in the synthesis of Efavirez. Hence there is a possibility to presence of those impurities in the Efavirez drug substance. To identify and quantify the PCA and impurity-1 in Efavirez, an appropriate and proper analytical method should be needed. The chemical structure of Efavirez PCA and impurity-1 were shown in Fig. 1.

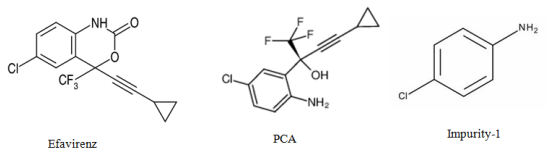

Fig. 1. chemical structure of Efavirez PCA and impurity-1

An in depth literature review reveals that many UV-visible[3,4], HPLC[5], UPLC[6] and few RP-HPLC[7-15] Methods were reported for estimation of Efavirenz in bulk, dosage form and biological samples. With the reported methods PCA and Impurity-1 in Efavirenz could not be estimated. Several attempts were done to develop a specific and sensitive LC-MS method for estimation of PCA and Impurity-1 in Efavirenz.

2. MATERIALS AND METHODS

The PCA, Impurity-1 and Efavirenz pure forms were obtained as a gift sample from Fortune Pharma, Hyderabad upon request. All solvents of HPLC grade were purchased from local distributor of Sigma Aldrich Limited, India. All the solvents were filtered with the support of 0.22µm filters earlier to introduce into the LC-MS system.

2.1 Optimized LC-MS/MS Method Conditions

The method was developed on Agilent, Model: 1290 mass spectrometer coupled with Atmospheric pressure chemical ionization (APCI) and Quadrupole mass analyzer. The effective separation of PCA and impurity -1 were achieved by using Hypersil BDS (C18, 100 x 4.6 mm, 3 µm) column and a solvent system of Buffer (0.1% Formic acid in water): Methanol (30:70 v/v) with a flow rate of 0.4 ml/min. 0.1% Formic acid in water used as diluents to prepare the different levels of standard and sample solution. The PCA and impurity -1 were monitored on mass spectrometer coupled with APCI, positive polarity mode and quadrapole mass analyzer. An ambient and 20ºC temperature was maintained in the auto sampling system and column respectively. MS parameters were described in the Table-1.

2.2 Preparation of PCA and Impurity -1 Standard Stock Solution (100 ppm)

Accurately weighed 5 mg of each PCA and IMPURITY-1 standard substances were diluted to10ml with diluent. 0.1ml of the obtained solution further diluted to 25ml to to obtain a solutuion of 100ppm.

Table 1. MS parameters

Ionization mode	APCI
Acquisition mode	SIM
Polarity Mode	Positive
Ch1	128.05 [M+H] +
Ch2	289.90 [(M+H] +
Interface	4.5 KV
Detector	1.92 KV
DL temp.	300ºC
Heat Block	250ºC
Nebulizing Gas Flow	3.0 L/min.
APCI Temperature	230ºC

2.3 Preparation of Standard Solution of PCA and Impurity -1 (2.5 ppm)

0.5 ml of the above standard stock solution was further diluted to 20 ml to get of 2.5 ppm concentration.

2.4 Preparation of Test Solution

Weigh accurately and transfer about 100 mg of test sample into a 5 mL volumetric flask dilute to the volume with diluent and mix well.

2.5 Method Validation

2.5.1 System suitability test

The system suitability of the current method was done by injecting standard solution PCA and impurity -1 for six times into LC-MS system. At the end % RSD was calculated for the peak areas of PCA and impurity -1 of the obtained chromatograms. As per ICH guidelines, the % RSD should be less than or equal to 10 for six replicates.

2.5.2 Linearity

Linearity study was performed for both PCA and impurity -1 were in the range of 0.75ppm (LOQ level) to 3.75(150% level). Each linearity level solution was injected in thrice and chromatograms were recorded. The regression coefficient (R^2) value was determined from the linear graph plotted between concentration levels versus their average peak areas.

2.5.3 Accuracy

The accuracy of the present method was validated through the recovery studies at QL level and 100% level standard solution concentrations specified in linearity. To the each specified level a known amount of test sample was spiked and the produced each spiked level solutions was introduced three times into LC-MS system. At the each level concentration the percent recovery of both PCA and impurity -1 in spiked solutions were calculated.

2.5.4 Precision

The system precision and precision at QL of the current method was done by injecting both the standard solution and QL solution for six times into LC-MS system. At the end % RSD was calculated for the peak areas of PCA and impurity -1 of the obtained chromatograms.

2.5.5 Sensitivity

Signal to noise ratio(S/N) method used to determine the detection limit (DL) and quantification limit (QL) of the analytical method. DL solution prepared from standard stock solution in such a way to get signal to noise ratio (S/N) around 3:1. The QL solution was prepared to get S/N ratio about 10:1.

2.5.6 Specificity

The specificity of the method was validated by injecting blank solution followed by standard solution, test solution and test solution blended with standard solution subsequently. Observation was done to check the interference at the retention times of PCA and impurity due to bank and other substances in test solution.

3. RESULTS AND DISCSSION

3.1 Method Optimization

The LC-MS method with optimized conditions such as Hypersil BDS (C18, 100 x 4.6 mm, 3

µm) column and a solvent system of Buffer (0.1% Formic acid in water): Methanol (30:70 v/v) with a flow rate of 0.4 ml/min was effectively separate the PCA and impurity-1 with retention time of 5.7min and 7.6min respectively (Fig. 2).

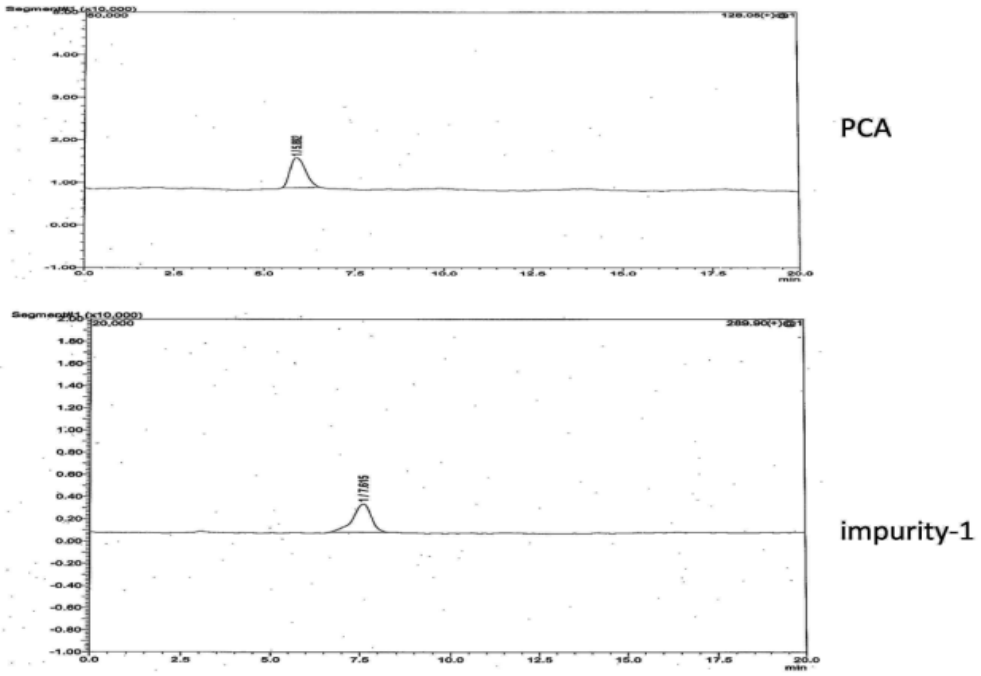

Fig. 2. LC-MS Chromatogram of standard solution

Table 2. System suitability results of PCA and impurity-1

S.NO	Peak area of PCA	Peak area of Impurity-1
Injection-1	208427.00	90729.00
Injection-2	200692.00	86861.00
Injection-3	205298.00	84240.00
Injection-4	201178.00	86035.00
Injection-5	208315.00	81677.00
Injection-6	207804.00	85325.00
Average	205285.7	85811.2
SD	3559.8	3004.4
%RSD	1.7	3.5

Table 3. Linearity for DPP

Level	Concentration(ppm)	Average peak area of PCA	Average peak area of Impurity-1
Level-1	0.75	63014.0	24325.5
Level-2	1.25	79728.5	31776.5
Level-3	1.85	122100.0	43262.5
Level-4	2.50	190489.0	68589.0
Level-5	3.10	231524.0	85787.5
Level-6	3.75	282478.0	103850.0
Correlation Coefficient		0.994	0.992

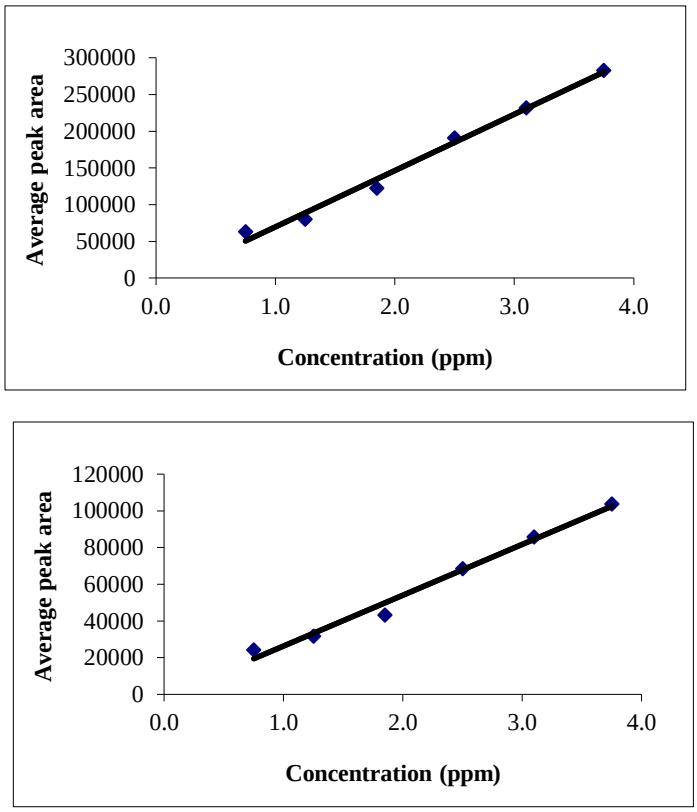

Fig. 3. Linearity curve of PCA and impurity-1

Table 4. Accuracy results of DNP

Accuracy levels	No. of Preparations	% Recovery of PCA	% Recovery of Impurity-1
QL	1	107.1	88.8
	2	104.5	98.3
	3	109.4	93.5
100 %	1	89.8	87.1
	2	92.2	91.9
	3	89.6	86.5

Table 5. Summary of results for system precision and precision at QL level

No. of Injections	Precision of QL solution		System precision	
	PCA	Impurity-1	PCA	Impurity-1
1	64744.00	24527.00	208427.00	90729.00
2	61284.00	24124.00	200692.00	86861.00
3	61545.00	23982.00	205298.00	84240.00
4	66757.00	23711.00	201178.00	86035.00
5	67040.00	22743.00	208315.00	81677.00
6	62478.00	23513.00	207804.00	85325.00
Average	63974.67	23766.67	205285.7	85811.2
SD	2573.4	611.1	3559.8	3004.4
% RSD	4.0	2.6	1.7	3.5

Table 6. DL and QL results of PCA and impurity-1

Parameter	Name of the analyte	Signal to noise ratio(S/N)	Concentration (ppm)
LOD	PCA	4.43	0.25
	Impurity-1	3.58	0.25
LOQ	PCA	13.21	0.75
	Impurity-1	14.54	0.75

3.2 Method Validation

The method was validated according to Q2 guidelines ICH.

3.3 System Suitability

The system suitability parameter of the optimized method was confirmed by calculating the % RSD of the peak areas of the PCA and impurity-1. The % RSD values were found to be 1.7 and 3.5 for PCA and impurity-1 respectively (Table 2).

3.4 Linearity

The regression coefficient (R^2) value for the given series of concentrations were computed to be as 0.994 and 0.992 respectively for PCA and impurity-1(Table 3 and Fig. -3).

3.4.1 Accuracy

The % recovery of PCA and impurity -1 in spiked solutions of QL level and 100% levels were found to be in the range of 88.8 to 109.4 % (Table 4) which were in the acceptance limits about 85 to 100%. Hence the method said to be highly accurate.

3.4.2 Precision

The % RSD values for precision at QL and system precision (100%level) of the PCA and impurity-1 were ≤10. The Table 5 represents the precision results.

3.4.3 Sensitivity

The DL and QL values were observed to be 0.25ppm and 0.75ppm for both PCA and impurity-1, which resembles that the method has sensitive. The DL and QL results of PCA and impurity-1 were represented in Table 6.

3.4.4 Specificity

Interference was not observed at retention time of the PCA and impurity-1 by the blank and impurities. Hence this method was specific to the determination of PCA and impurity-1only.

A specific and sensitive LC-MS method was essential for determination of the process impurities in pure and pharmaceutical dosage form. Till now a single analytical method was not available to estimate PCA and impurity-1 in Efavirenz powder and pharmaceutical tablet dosage form. Even though few LC analytical methods were reported for estimation of Efavirenz and its degradants which were generated by forced degradation studies, a new LC-ms method was required to produces y good sensitive, accuracy and specificity. Hence the developed method has advantages over reported methods in process chemistry department and quality control department to identify and quantify the Phenyl vinyl sulfone impurity in Eletriptan hydrobromide.

4. CONCLUSION

A LC-MS method with simple mobile phase composition was developed for the estimation of PCA and impurity-1 in Efavirenz bulk powder and pharmaceutical tablet dosage form. The validated method has very good sensitive, accurate, and precision. Besides it has the advantage of shorter elution time and the possibility of examination of more number of samples, both of those significantly trim down the analysis time per sample. Thus, this method can rightly appropriate for regular analysis of PCA and impurity-1 in Efavirenz pure and dosage forms in quality control department in the pharmaceutical industry

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by

the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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