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Research Article

Synthesis and Quantitation of Genotoxic impurity 5-cyano-2-((4-fluorophenyl) (hydroxy) benzyl 4-methyl benzene sulfonate in Escitalopram Oxalate by RP-UPLC

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Abstract

The objective of the research work is to synthesize potential genotoxic impurity 5-cyano-2-((4-fluorophenyl) (hydroxyl) benzyl 4-methyl benzene sulfonate and to develop suitable UPLC method to quantify the above genotoxic impurity in Escitalopram Oxalate at 15 ppm level. The above genotoxic impurity was synthesized by regio selective tosyaltion of Diol compound, under controlled temperature conditions at 0-5°C with TsCl/pyridine/chloroform and characterized. A new UPLC method was developed by using UPLC BEH Shield RP18 100 x 2.1 mm, 1.7µ column. The mobile phase used is the mixture of 0.05% ortho phosphoric acid and acetonitrile in the ratio of 8:2 (v/v) as solvent-A and 3:7 (v/v) as solvent-B at a flow rate of 0.4 mL/minute. Detector wavelength monitored at 228nm and column temperature was maintained at 27°C. The UPLC method was validated as per International conference on harmonization guidelines. This method is proven as highly sensitive with a detection limit of 5ppm and quantification limit of 15 ppm. Regression analysis showed that the correlation coefficient value of 0.99999. The accuracy of the method was established based on the recovery obtained for 5-cyano-2-((4-fluorophenyl) (hydroxyl) benzyl 4-methyl benzene sulfonate. The present research work provided the route of synthesis as well as an advanced analytical methodology to quantify the above critical Genotoxic impurity known as 5-cyano-2-((4-fluorophenyl) (hydroxyl) benzyl 4-methyl benzene sulfonate in Escitalopram oxalate.

Keywords: Genotoxic impurity; Ultra performance liquid chromatography (UPLC); 5-cyano-2-((4-fluorophenyl) (hydroxyl) benzyl 4-methyl benzene sulfonate; Escitalopram Oxalate; Method validation.

1. Introduction

Escitalopram oxalate is an antidepressant belonging to the class of selective serotonin reuptake inhibitors (SSRIs). Escitalopram is used to treat anxiety in adults and major depressive disorder in adults and adolescents who are at least 12 years old.

Escitalopram is the pure enantiomer (single isomer) of the racemic bicyclic phthalate derivative citalopram. Escitalopram oxalate is designated S-(+)-1-[3(dimethyl-amino) propyl]-1-(p-fluoro phenyl)-5-phthalancarbonitrile oxalate.

Figure 1: Chemical structure of Escitalopram oxalate

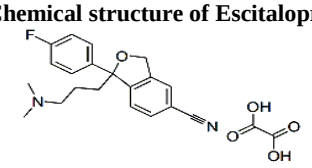
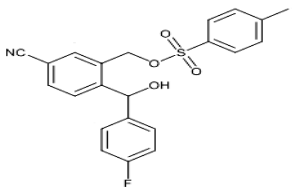


Figure 2: Chemical structure of (5-cyano-2-((4-fluorophenyl) (hydroxyl) benzyl 4-methyl benzene sulfonate):



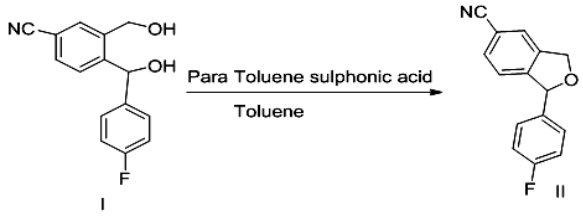
5-cyano-2-((4-fluorophenyl) (hydroxyl) benzyl 4-methyl benzene sulfonate impurity which will arise during the synthesis of Escitalopram oxalate. This impurity was confirmed as a potential genotoxic impurity [1-6] based on the available literature and by the assessment through Ames, Derek, and Toxnet [7]. Tosylates are considered as potential alkylating agents [8] and imparts genotoxicity, carcinogenicity in bacterial and mammalian cell systems. Sulfonate impurities comprise the most investigated group of genotoxic impurities (GIs)[9]. Initially in 2007, sulfonate impurities raised major concern when over a period of three months (March to May 2007), several thousand HIV patients in Europe were exposed to ViraceptR (nelfinavir mesylate) tablets containing the contaminant ethyl methane sulfonate. The presence of trace level impurity in drug substance or drug product is of genotoxicity concern and has severe harmful effects on human health. The control of such genotoxic impurities are closely monitored by regulatory agencies and such impurities should be controlled at TTC level in drug substance or preferably in its intermediate stages itself. Literature survey reveals that there was no method published for the quantification of 5-cyano-2-((4-fluorophenyl) (hydroxyl) benzyl 4-methyl benzene sulfonate impurity and also it is very difficult to achieve the low level quantification for this impurity using HPLC or GC. The concentration limit of the genotoxic impurity has been calculated based on the TTC and maximum daily dose [10]. So the impurity must to be controlled at below 58.8 ppm by considering maximum daily dose for Escitalopram oxalate is 25.5 mg and 1.5µg/day TTC[11] [=1.5 (µg/day)/Dose (g/day)]. To achieve better sensitivity, high resolution and less run time, UPLC was chosen for the development and quantification of 5-cyano-2-((4-fluorophenyl) (hydroxyl) benzyl 4-methyl benzene sulfonate in [L-(-)-Di-p-toluoyl tartaric acid salt of 1-[3-(amino) propyl]-1-(4-fluophenyl)-1, 3-dihydro-5-isobenzofurancarbonitrile] which is the key starting material for Escitaloptam oxalate.

5-cyano-2-((4-fluorophenyl) (hydroxyl) benzyl 4-methyl benzene sulfonate is an insitu intermediate forms during the cyclisation of Diol intermediate as per scheme1. During the synthesis of Escitalopram Oxalate, the first step involves conversion of Diol intermediate into cyclic intermediate (II) utilizing *p*-TsOH. Inherently this transformation involves two steps

a) Reaction of a primary alcohol with *p*-TsOH to form 5-cyano-2-((4-fluorophenyl) (hydroxyl) benzyl 4-methyl benzene sulfonate**.**

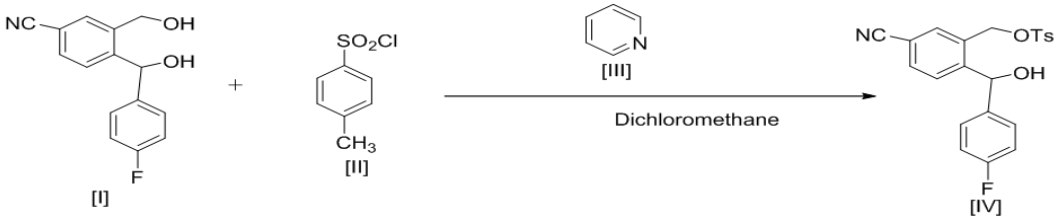
b) Intermolecular nucleophilic attack of secondary alcohol [12] with Tosyl ester to form cyclic intermediate compound (II). The transformation of step-a to step-b was found to be very instantaneous due to labile nature of Tosyl moiety and high nucleophilic nature of secondary alcohol.

Figure 3: Reaction of a primary alcohol with p-TsOH



Attempts to isolate the 5-cyano-2-((4-fluorophenyl) (hydroxyl) benzyl 4-methyl benzene sulfonate were unsuccessful [13] using P-Toluene sulphonic acid due to instantaneous transformation of diol (I) in to cyclic ether. But as per the literature it has been reported that the 1, 2-diols can undergo regioselective tosylation in presence of P-TsCl/Pyridine /Chloroform under lower temperatures.

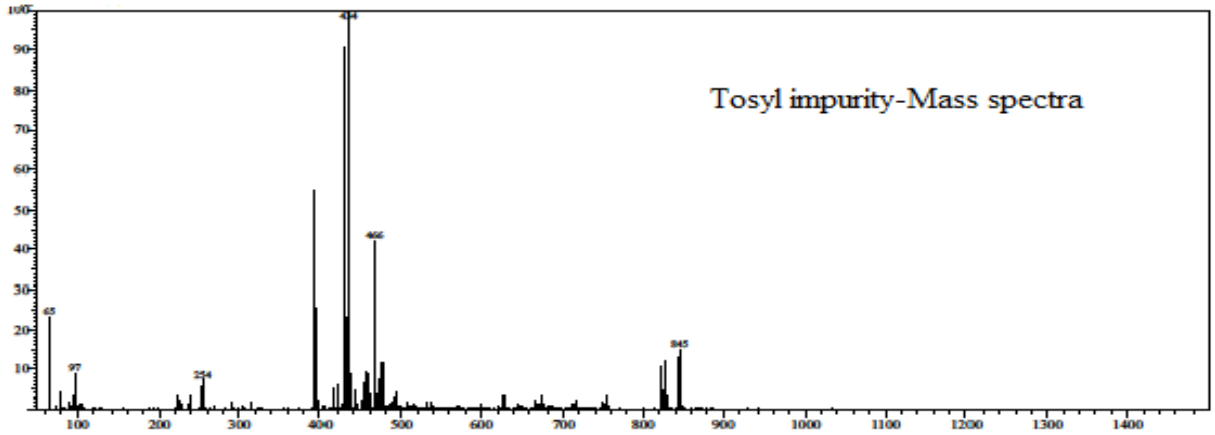
Figure 4: Synthetic scheme of 5-cyano-2-((4-fluorophenyl) (hydroxyl) benzyl 4-methyl benzene sulfonate:



Based on the above literature evidence attempt was performed for regio selective tosyaltion of Diol compound, under controlled temperature at 0-5°C with TsCl/pyridine/chloroform. Where the desired 1-tosylate product was formed as the major product. During the reaction monitoring, it was observed that, the reaction was slowed down and many other impurities were started appearing in TLC apart from the desired impurity (Mono tosyl) and the starting material (diol). Upon the termination of the reaction, 45% of the desired 1-tosylate product was isolated from the reaction crude by using flash column chromatography was characterized and confirmed by ^{1}H NMR, Mass and IR [14].

IR Spectra: *v* (cm^{-1}) = 3055.06, 1732.72, 1602.77, 1421.46 1265.59, 1177.46 and 739.02.
Mass **:** 434 (M+23, Na adduct)

Figure 5: Mass spectrum of 5-cyano-2-((4-fluorophenyl) (hydroxyl) benzyl 4-methyl benzene sulfonate



^{1}H NMR: (400 MHz, $CDCl_3$)

Figure 6: NMR spectrum of 5-cyano-2-((4-fluorophenyl) (hydroxyl) benzyl 4-methyl benzene sulfonate

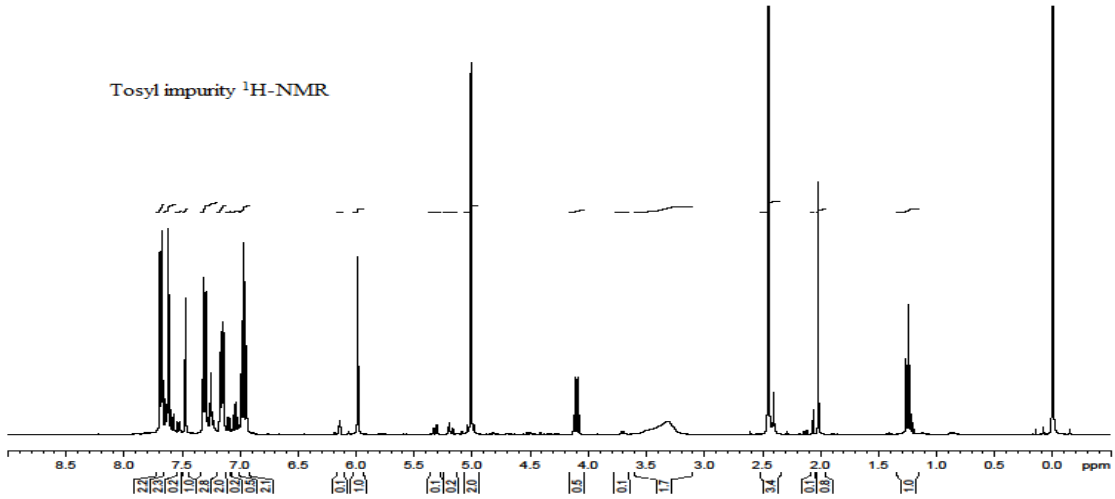


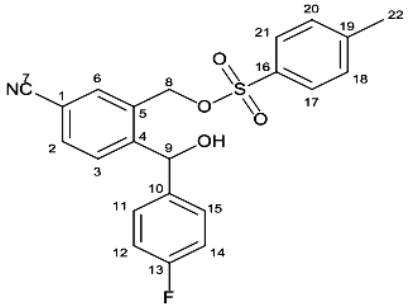
Table 1: NMR assignments of 5-cyano-2-((4-fluorophenyl) hydroxyl) benzyl 4-methyl benzene sulfonate

Assignments	Number of Protons	Multiplicity*	^{1}H (δ ppm)
2,3,6,11,12,14,15,17,18, 20 & 21	11H	m	6.9-7.7
8	2H	s	5.0
9	1H	s	6.0
22	3H	s	2.5
-OH	1H	b	3.3

*****s-singlet, t-triplet, d-doublet, m- multiplet.

NMR assignments for 5-cyano-2-((4-fluorophenyl) (hydroxyl) benzyl 4-methyl benzene sulfonate):

Figure 7: NMR assignments for 5-cyano-2-((4-fluorophenyl) (hydroxyl) benzyl 4-methyl benzene sulfonate):



2. Materials & Methods

2.1 Chemicals and Reagents

Samples of Escitalopram oxalate and 1-(4-fluoro phenyl)-1, 3-dihydro isobenzo furan-5-carbonitrile are received from API unit-4 of Dr. Reddys Laboratories, Hyderabad. 5-cyano-2-((4-fluorophenyl) (hydroxyl) benzyl 4-methyl benzene sulfonate is prepared in-house. HPLC grade Acetonitrile was purchased from Merck, Mumbai, India. Ortho phosphoric acid was purchased from Sigma Aldrich, Mumbai, India. High pure water was prepared by using Millipore Milli Q plus purification system (Millipore, USA).

2.2 Equipment:

The Instrument used for Method development [15] and Method validation [16] is Acquity UPLC H-class system equipped with PDA detector, Quaternary solvent manager and Sample manager-FTN (Waters India private limited). The data were collected and processed by using Empower-3 software.

2.3 UPLC Chromatographic conditions:

The separation was achieved on Acquity UPLC BEH Shield RP-18 (2.1 mm length x 100 mm internal diameter, 1.7 µm particle size) column using the mobile phase mixture of 0.05% ortho phosphoric acid at Ph 3.5 (adjusted with triethylamine) and Acetonitrile in the ratio of 8:2 (v/v) as solvent A and 3:7 as solvent B. The flow rate of the mobile phase was 0.4 mL/minute. The column was maintained at ambient temperature and detector wavelength of 228 nm for detection of target impurity. The injection volume is 5µL and sample cooler was maintained at 5°C. Water and acetonitrile in the ratio of 2:8 used as diluent to dissolve the impurities and test samples. The gradient program (time in min/% B) was set as 0.01/40, 2/40, 8/80, 12/80, 12.5/40 and 15/40.

Preparation of Impurity standard solution:

The stock solution of the impurity was prepared at 0.5 mg mL^{-1} in diluent and the stock solution is diluted with diluent to get the concentration of 58 ppm with respect to the test concentration of 5.0 mg mL^{-1}.

Preparation of Test solutions:

Weighed and transferred about 50 mg test samples in 10 ml volumetric flask, dissolved in 5 ml of diluent and made up to the mark with diluent and cyclomix for 1 min to get clear solution.

3. Results and Discussion:

3.1 Optimization of chromatographic conditions

The main objective of the method development is to develop sensitive and selective quantification method 5-cyano-2-((4-fluorophenyl) (hydroxyl) benzyl 4-methyl benzene sulfonate in Escitalopram oxalate. The attempts were made out to find the suitable diluent to dissolve Escitalopram oxalate samples and the target genotoxic impurity. The main challenge is to soluble both Escitalopram and the impurity in a single solvent medium. However Escitalopram oxalate samples are not soluble in acetonitrile, whereas the impurity solution was found to be stable only in acetonitrile. Trials were continued with the different ratios of solvent mixtures, finally test samples and the impurity were found to be soluble in the mixture of acetonitrile and water in the ratio of 8:2 and found to be stable up to 24hrs. The impurity standard solution degradation observed at room temperature and studied the stability at 2-8°c and found satisfactory. To quantify the impurity at very low level, higher concentration of the samples were injected in to the chromatographic system and found that the interferences from the sample matrix. To overcome the sample matrix interferences, continued the method development by varying mobile phase pH and gradient composition. Successfully achieved the separation of the desired impurity from the sample matrix interferences and specified impurities. The same was confirmed by injecting all the specified impurities and by checking the peak purity of the desired impurity by PDA detector. It was found that the purity angle is less than the purity threshold. The desired detection levels also achieved by using finalized the chromatographic conditions and proceeded for method validation.

3.2 Method validation:

3.2.1 Limit of quantification and limit of detection

Diluted the Impurity stock solution with diluent to get the concentration of 15 ppm for LOQ and 5 ppm for LOD and injected into chromatographic system and observed the signal to noise ratio of 9.5 for LOQ solution and 3.0 for LOD solution.

Figure 8: LOD chromatogram

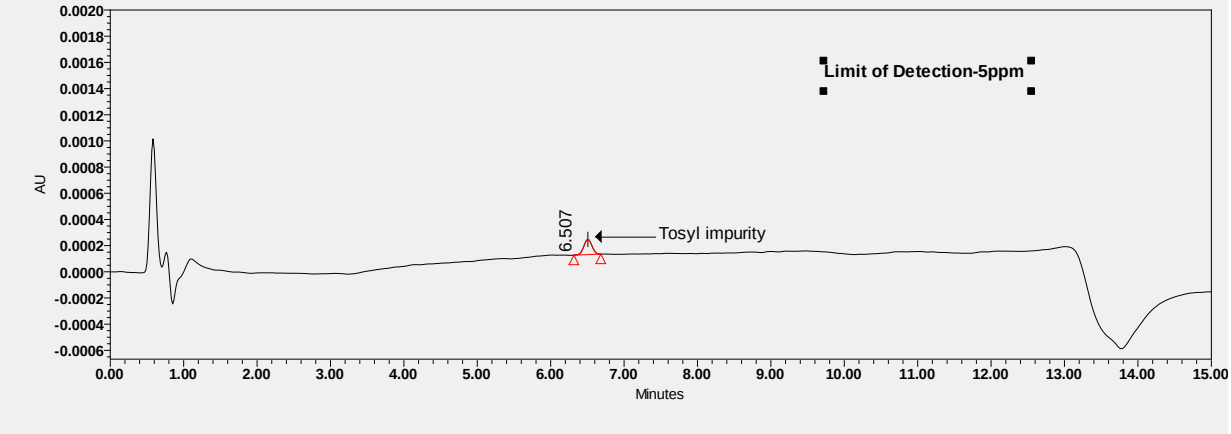


Figure 9: LOQ chromatogram

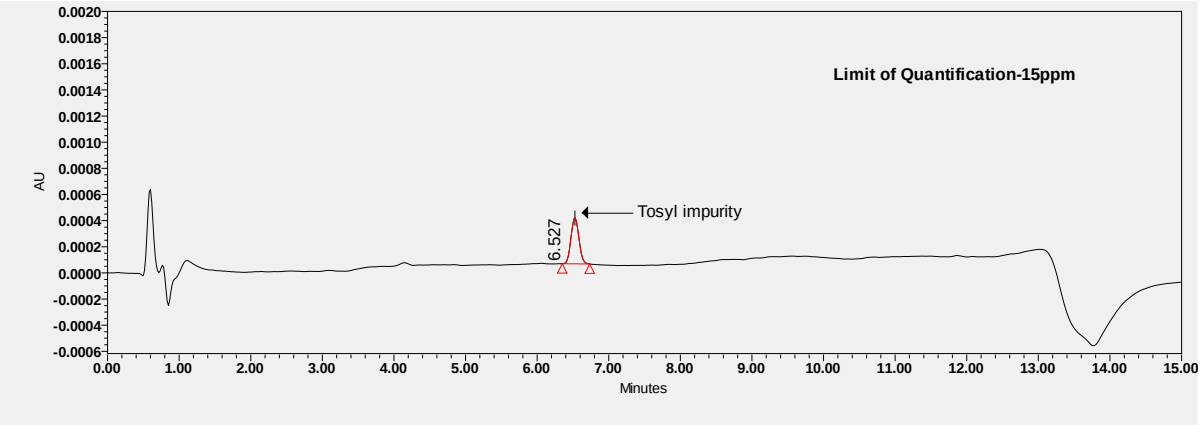


Table-II: Limit of detection and Limit of quantification results table

Parameter	Concentration in ppm	Signal/Noise
Limit of detection	5	3.0
Limit of quantification	15	9.5

ppm- parts per million

3.2.2 Linearity:

The linearity [16] of an analytical test procedure is its ability to obtain test results within a given range, which is directly proportional to the concentration of the analyte in the sample. Linear calibration plot for 5-cyano-2-((4-fluorophenyl) (hydroxyl) benzyl 4-methyl benzene sulfonate impurity was established over the concentration range of LOQ (15 ppm) to 150% (87 ppm) with respect to 58 ppm as 100% standard. The correlation coefficient is found to be 0.99999 which shows excellent correlation between the peak response and the concentration of the impurity.

Table-III: Linearity results table:

Level of Impurity	Conc. in µg/ml	Area of Impurity
LOQ	0.092416	2711
50%	0.173280	5057
75%	0.259920	7552
100%	0.346560	10088
125%	0.433200	12587
150%	0.519840	15016

Correlation coefficient-0.999995(*)
Slope-28924.47
Intercept-43.77
% Y-intercept at 100% level-0.4

Figure 10: Linearity chromatogram

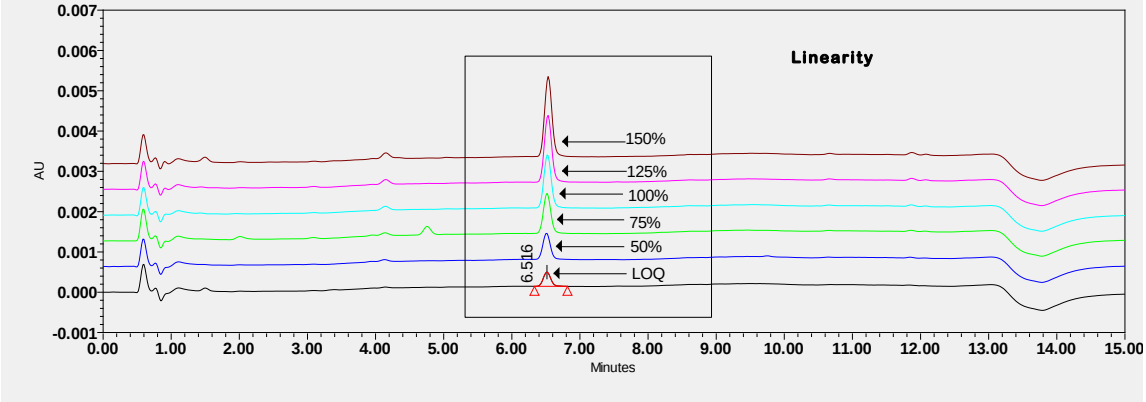
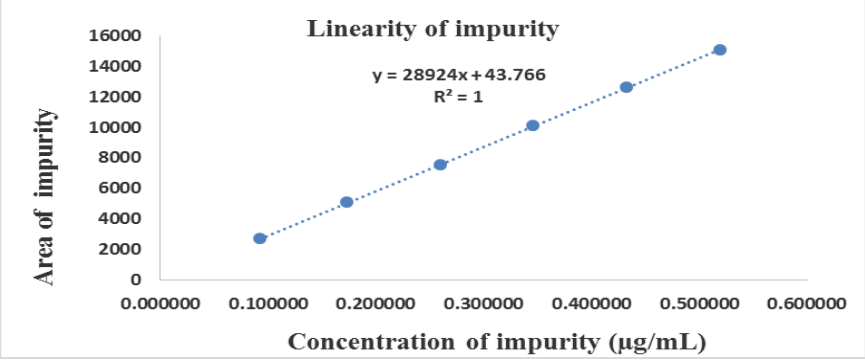


Figure 11: Linearity graph



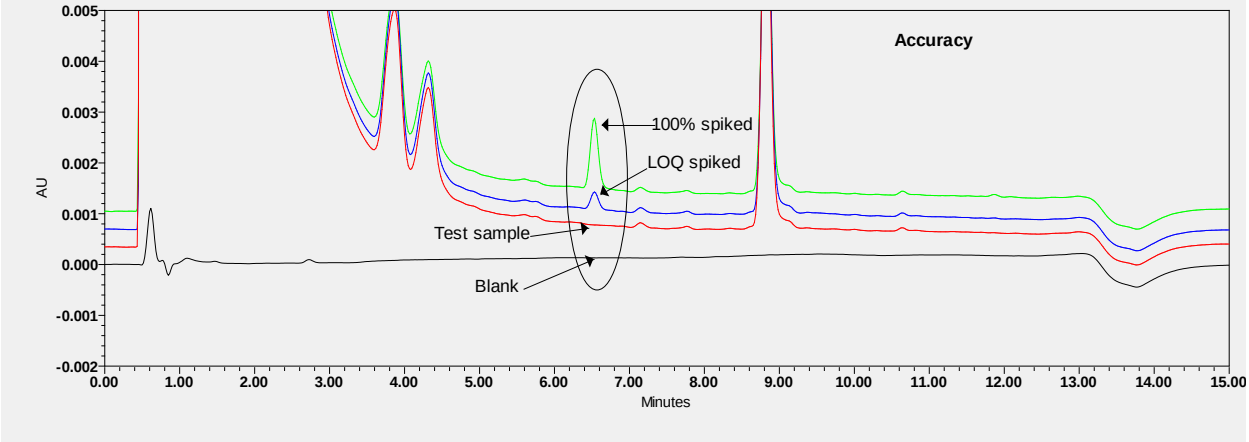
Accuracy:

The accuracy [16] of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the expected value found. The accuracy of the method was established by preparing test sample solution duplicate and the impurity spiked solutions at LOQ level (15 ppm) and 100% level (58ppm) triplicate. Injected the above solutions into the chromatographic system and calculated the % Recovery of the impurity from the spiked solutions against unspiked test solutions. The % recoveries are between 80% to 120%. At such low levels of impurity these recoveries are satisfactory.

Table-IV: Accuracy results table

Preparation	Amount of Impurity Spiked (ppm)	Amount of Impurity Spiked (ppm)	% Recovery
LOQ spiked-1	15.35	12.58	82
LOQ spiked-2	15.42	12.68	82
LOQ spiked-3	15.62	12.69	81
100% spiked-1	57.30	53.51	93
100% spiked-2	57.42	54.02	94
100% spiked-3	58.20	53.70	92
100% spiked-4	57.92	57.67	99
100% spiked-5	57.55	59.32	103
100% spiked-6	58.25	56.39	97

Figure 12: Accuracy



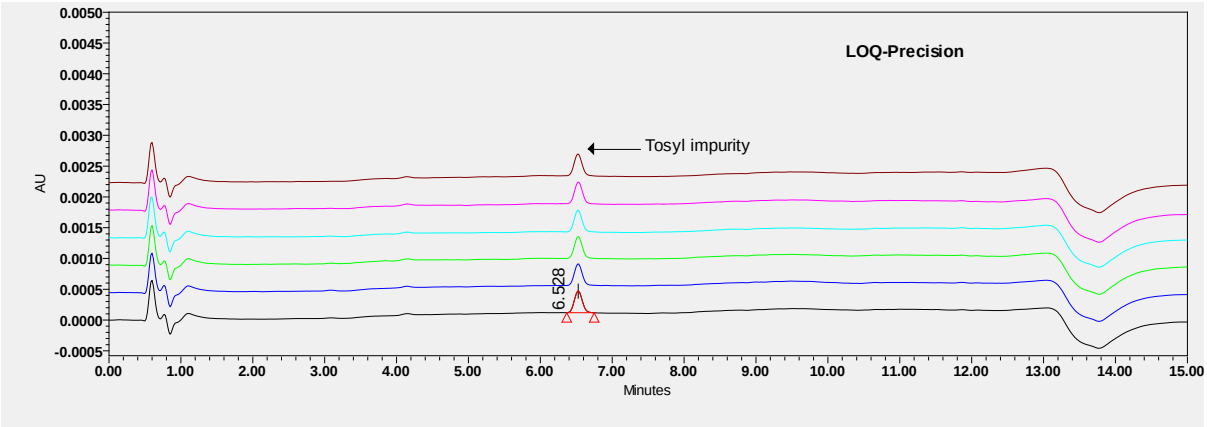
Precision:

The precision [16] of an analytical procedure expresses the closeness of agreement between a series of measurements from multiple sampling of the homogeneous sample under prescribed conditions.

At LOQ level (15 ppm):

Precision was established at LOQ level by diluting stock solution six times individually to get the concentration of 15 ppm and injected each solution once into the chromatographic system. Calculated % Relative standard deviation for the area and retention time and found 1.4 and 0.1 respectively.

Figure 13: Precision at LOQ



At 100% level (58 ppm):

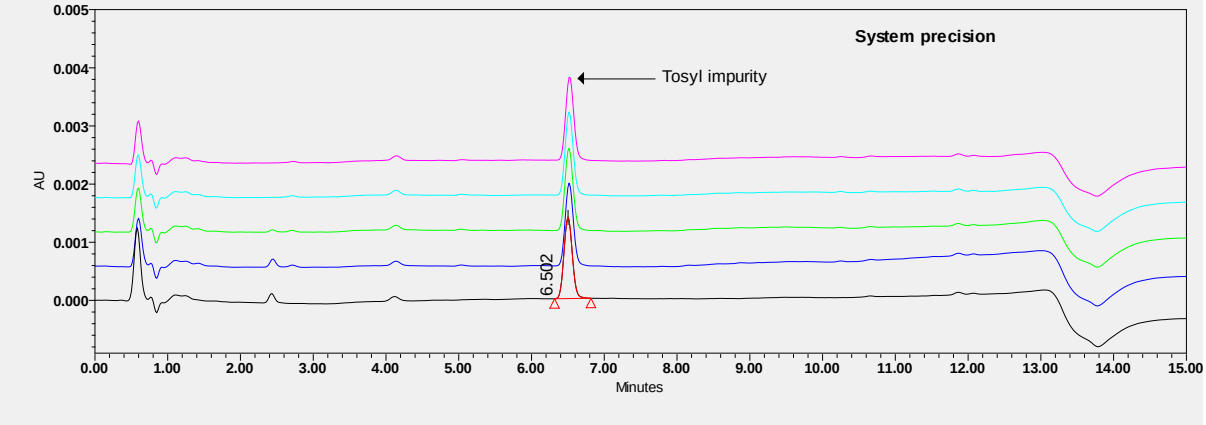
Precision was established at 100% level by diluting stock solution six times individually to get the concentration of 58 ppm and injected each solution once into the chromatographic system. Calculated % Relative standard deviation for the area and retention time and found 0.31 and 0.1 respectively.

Table-V: Precision results table

S. No	LOQ level	100% level
Injection-1	2749	11168
Injection-2	2662	11196
Injection-3	2724	11145
Injection-4	2723	11124
Injection-5	2660	11217
Injection-6	2723	11154
Average	2706.8	11167
Standard devation	36.88	34.19
% RSD	1.4 (*)	0.31 (*)

RSD-Relative standard deviation
LOQ-Limit of quantification

Figure 14: System precision



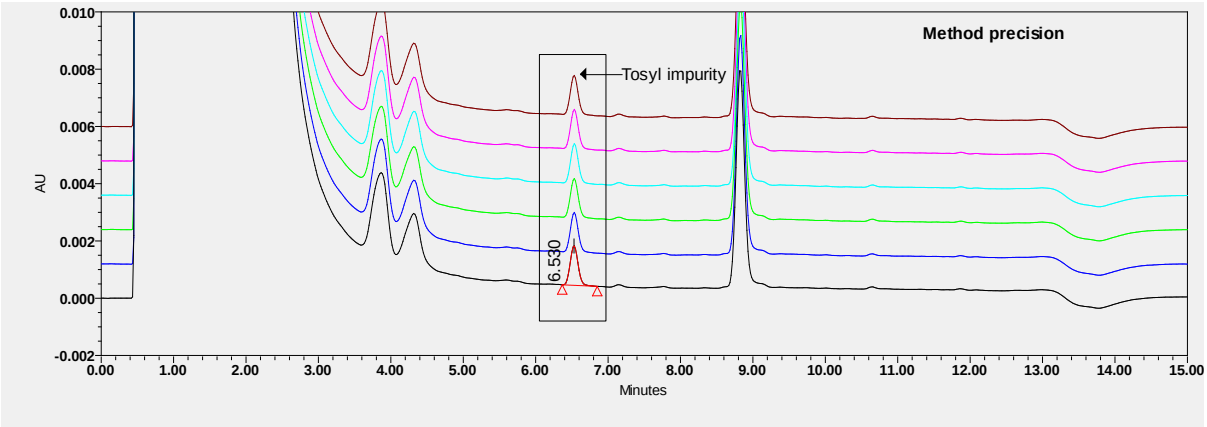
Method precision:

Method precision [16] was established by preparing six individual test solutions spiked with 100% (58ppm) level of impurity and injected into the chromatographic system. Calculated impurity content from each preparation and found % Relative standard deviation for six preparations was 4.3.

Table-VI: Method precision results table

Preparation	Impurity content in ppm
100% spiked Preperation-1	53.51
100% spiked Preperation-2	54.06
100% spiked Preperation-3	53.70
100% spiked Preperation-4	57.67
100% spiked Preperation-5	59.32
100% spiked Preperation-6	56.39
Average	55.79
Standard devation	2.41
% RSD	4.3(*)

Figure 15: Method precision



Intermediate precision:

Intermediate Precision [16] is to cover the various influences within a laboratory, i.e. conducting analyses on two different days by different laboratory staff members, with different equipment (if available), etc. This study was performed by second analyst with different system, chromatographic column, reagents and chemicals by using the same chromatographic conditions.

Intermediate precision was established by preparing six individual test solutions spiked with 100% (58ppm) level of impurity and injected into the chromatographic system. Calculated impurity content from each preparation and found % Relative standard deviation for six preparations was 1.89.

Table-VII: Intermediate precision results table

Preparation	Impurity content in ppm
100% spiked Preperation-1	55.62
100% spiked Preperation-2	58.25
100% spiked Preperation-3	56.72
100% spiked Preperation-4	55.64
100% spiked Preperation-5	56.52
100% spiked Preperation-6	57.50
Average	56.71
Standard devation	1.04
% RSD	1.89(*)

4. Conclusion

The present research study emerges with detailed synthetic process of potential genotoxic impurity 5-cyano-2-((4-fluorophenyl) (hydroxyl) benzyl 4-methyl benzene sulfonate and developed an accurate, precise and sensitive quantification method by UPLC for quantification of 5-cyano-2-((4-fluorophenyl) (hydroxyl) benzyl 4-methyl benzene sulfonate in Escitalopram oxalate. The described method is highly sensitive, accurate and precise for quantification of Genotoxic impurity in Escitalopram oxalate and can be used for routine testing.

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References

[1]. Genotoxic and Carcinogenic Impurities in Drug Substances and Products: Recommended approaches (2008) US Department of Health and Human Services, Food and Drug administration, Centre for Drug Evaluation and Research CDER USA.

[2]. Liu DQ, Sun M, Kord AS. Recent advances in trace analysis of pharmaceutical genotoxic impurities. *J Pharm Biomed Anal* 2010; 51: 999-1014.

[3]. Yuabova, Holschlag ZY, Rodriguez DR, Qin SA, Papov C, et al. Genotoxic Impurities: A quantitative Approach. *J Liq Chromatogr Relat Technol* 2008; 31: 2318-2330.

[4]. Guideline on Impurities in New Drug Substances, Q3A (R2), ICH.

[5]. Muller L, Mauthe RJ, Riley CM, Andino MM, Antonis DD, et al. A rationale for determining, testing, and controlling specific impurities in pharmaceuticals that possess potential for genotoxicity. *Regul Toxicol Pharmacol* 2006; 44: 198-211.

[6]. IARC Monographs Program on the Evaluation of Carcinogenic Risks to Humans for ethyl methane-sulphonate 7 (1974) 245.

[7]. Dobo KL, Greene N, Cyr MO, Caron S, Ku WW. The application of structure-based assessment to support safety and chemistry diligence to manage genotoxic impurities in active pharmaceutical ingredients during drug development. *Regul Toxicol Pharmacol* 2006; 44: 282-293.

[8]. Kim C, Keum JE, Yu SH, Ko SY. Regioselective Mitsunobu-tosylation of 1, 2-diols. *Bulletin of the Korean Chemical Society*. 2009 Jul 20; 30(7):1671-4.

[9]. Jouyban A, Parsa H. Genotoxic impurities in pharmaceuticals. *InToxicity and Drug Testing* 2012.

[10]. ICH M7, Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk, Current Step 4 version dated 23 June 2014.

[11]. Edward JD. An impact analysis of the application of the threshold of toxicological concern concept to pharmaceuticals. *J. Regul. Toxicol. Pharmacol*, 2007; 49: 107-124.

[12]. Kochi JK, Hammond GS. Benzyl tosylates. I. Preparation and properties. *Journal of the American Chemical Society*. 1953 Jul; 75(14):3443-4.

[13]. Ding R, He Y, Wang X, Xu J, Chen Y, Feng M, Qi C. Treatment of alcohols with tosyl chloride does not always lead to the formation of tosylates. *Molecules.* 2011 Jul 1; 16(7):5665-73.

[14]. Lohr LL, Jensen AJ, Sharp TR. NMR characterization of impurities. *Separation Science and Technology.* 2004 Dec 31; 5:301-39.

[15]. Naga Malleswararao CS, Suryanaryana MV, Krishna K, Mukkanti K. Stability indicating uplc method for the rapid separation of related components of gemcitibine hydrochloride. *Journal of Liquid Chromatography & Related Technologies.* 2012 Jan 1; 35(18):2511-23.

[16]. ICH, Q3A (R2) Guideline on Impurities in New Drug Substances, 2006. IFPMA, Geneva