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METHOD DEVELOPMENT AND METHOD VALIDATION FOR TRACE LEVEL QUANTIFICATION OF TWO POTENTIAL GENOTOXIC IMPURITIES IN BAZEDOXIFENE ACETATE DRUG SUBSTANCE BY USING LC-MS (SIR MODE) SELECTIVE ION RECORDING WITH SHORT RUNTIME ANALYSIS

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Keywords:

LC-MS/Selective ion recording (SIR Mode), 1-Benzyloxy-4-nitrobenzene, 4-benzyloxy-2-bromopropiophenone, Bazedoxifene acetate, TTC (Threshold of toxicological concern), MDD (Maximum daily dose)

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ABSTRACT: Bazedoxifene acetate represents a promising new treatment for osteoporosis. LC-MS SIR (Selective ion recording) mode, the shortest runtime method, was developed and validated for the trace level quantification of two potential genotoxic impurities, i.e., Impurity-A (1 – Benzyloxy – 4 - nitrobenzene) and Impurity – B (4-benzyloxy-2-bromopropiophenone) in Bazedoxifene acetate drug substance. The concentration limits of both genotoxic impurities were calculated a limit of 75ppm based on the concept of TTC (threshold of toxicological concern) and MDD (maximum daily dosage, which is 20mg/day for Bazedoxifene acetate drug substance). The mobile phase used for the method was 10mM Ammonium acetate and acetonitrile in the ratio of (30:70) v/v with isocratic mode elution at flow rate 0.6mL/min, stationary phase used as X-Bridge C18 (50x4.6) mm, 3.5µm and run time was 5 minutes. The Impurities were quantified by using MS detector SIR mode (Selective ion recording) at m/z of 230.12 and 319.01 for Impurity-A and Impurity-B, respectively. The detection limit (DL) was 1.4 ppm for both Impurity-A and Impurity-B. The quantification limit (QL) for Impurity-A was 3.0ppm, and Impurity-B was 3.1ppm, respectively. The method was found to be linear, from 3.0 ppm to 111.0 ppm for Impurity-A and 3.1 ppm to 112.4 ppm for Impurity-B. The method was found to be Specific, Precise, Linear, and Accurate.

INTRODUCTION: Bazedoxifene acetate Chemical name was 1 - [[4 - [2 - (Hexahydro - 1H –azepin – 1 - yl) ethoxy] phenyl] methyl] – 2 - (4-hydroxyphenyl) – 3 – methyl - 1H - indol-5-ol monoacetate (salt).

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Bazedoxifene is a selective estrogen receptor modulator (SERM) used to treat moderate to severe vasomotor symptoms in menopause and osteoporosis alone or in combination with conjugated estrogens.

Impurity-A (1-Benzyloxy-4-nitrobenzene) and Impurity-B (4-benzyloxy-2-bromopropiophenone) will arise during synthesis of Bazedoxifene Acetate drug substance. ROS of Bazedoxifene Acetate is shown in **Fig. 2**. The structure and chemical names of Impurity-A and Impurity-B are shown in Fig. 1. Impurity-A and Impurity-B are confirmed as

potential genotoxic impurities based on the available literature [1-5]. The trace level impurities of Impurity-A and Impurity-B present in the Bazedoxifene drug substance or drug product may cause severe harmful effects on human health. The concentration limit of genotoxic impurities, Impurity-A, and Impurity-B was calculated based on TTC [1-5] and maximum daily dose [1-8]. Impurity-A and Impurity-B each must to be controlled at below 75ppm.

The literature survey review shows some research work related to Bazedoxifene Acetate 6-9 related substances using high-performance liquid chromatographic methods. Based on the literature survey analytical method not available for accurately quantifying Impurity-A and Impurity-B at ppm levels. This work aims to develop and validate a highly Sensitive, accurate, and selective LC-MS SIR mode method developed and validated [9] for the determination of trace level quantification of Impurity-A and Impurity-B in Bazedoxifene Acetate drug substance.

Quantitative structure-activity relationship (QSAR) analysis was carried out for all the raw materials, reagents, intermediates, impurities, and reagents used in the process of Bazedoxifene Acetate drug substance to identify the Mutagenic impurities. We have found that Impurities A and B are mutagenic due to certain electrophilic moieties within a chemical structure. Both compounds are mutagenic and DNA-reactive, *i.e.*, Impurity-A is the intermediate of Bazedoxifene Acetate, and Impurity-B is process impurity due to hydrolysis of the intermediate. Following illustration below shows.

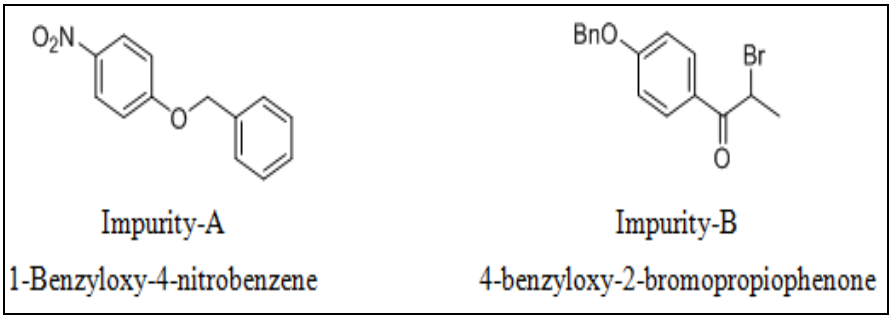

FIG. 1: STRUCTURES OF MUTAGENIC IMPURITIES

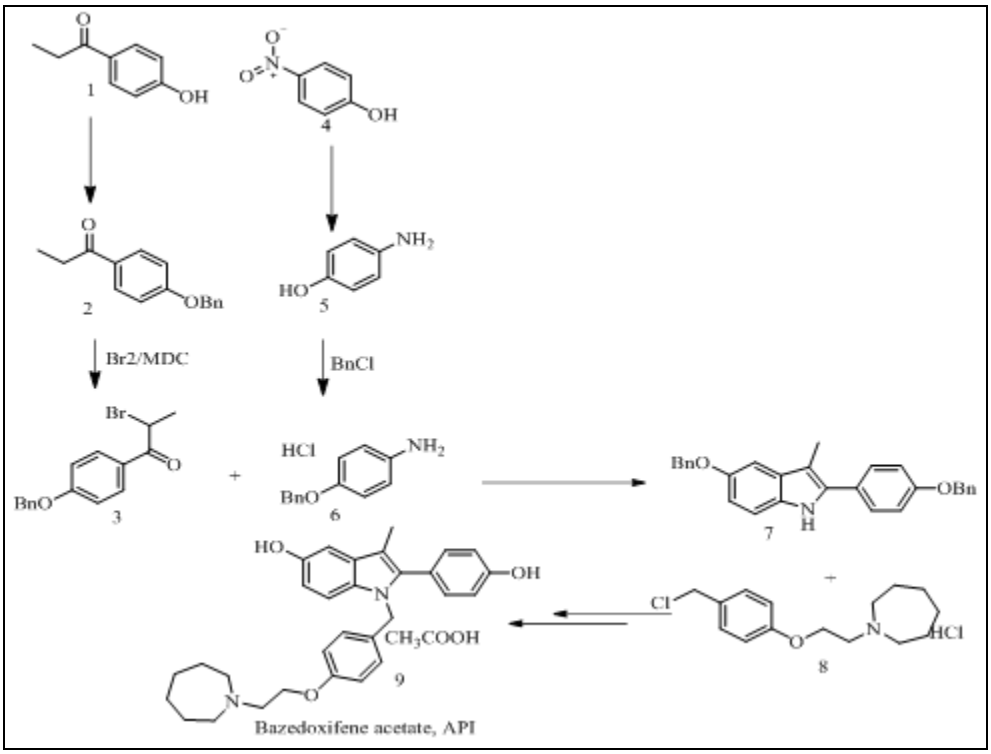

FIG. 2: SYNTHETIC ROUTE OF BAZEDOXIFENE ACETATE DRUG SUBSTANCE

Generation of Impurity-A (1-Benzyloxy-4-nitrobenzene): Impurity-A will generate due to compound-4 & Benzyl chloride reaction conditions. There is a probability of containing a trace level of Compound-4 in Compound-5 as per synthetic route and there is a favorable reaction condition for formation of Impurity-A & It will generate in the preparation of Compound-6 from Compound-5. Aromatic nitro compounds are known as mutagenic compounds. It also supports mutagenic assessment. The overall call is Positive due to reasonable alerting in DEREK and Case ultra-assessment.

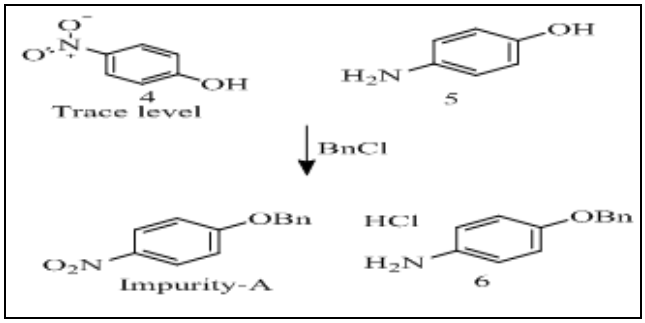

FIG. 3: SCHEMATIC REPRESENTATION FOR IMPURITY-A FORMATION

Generation of Impurity-B (4-benzyloxy-2-bromopropiophenone): The process for the preparation of compound-7 consists of Compound-3 as an intermediate *i.e.*, Impurity-B. It will carry over impurity to Bazedoxifene acetate. Based on mutagenic assessment, Overall call is Positive due to reasonable alerting in DEREK and a possible alkylating reaction at the carbon of the alpha-position of carbonyl group.

Material and Methods / Experimental Details / Methodology:

MATERIALS AND METHODS: Solvent Acetonitrile (LC-MS grade) was used for the experimentation procured from Fisher chemicals. Ammonium acetate (LC-MS grade) reagent used for mobile phase preparation procured from Sigma Aldrich. . Purified water was collected from Mill-Q plus water purification system. Standards of Impurity-A (1-Benzyloxy-4-nitrobenzene) CAS No: 1145-76-2 and Impurity-B (4-benzyloxy-2-Bromo-propiophenone) CAS No: 35081-45-9 procured from TCI Chemicals. The mass spectra of Impurity-A and Impurity-B are shown in **Fig. 4** and **Fig. 5,** respectively. Mass spectra were obtained by using waters UPLC H-Class connected to XEVO TQ MS/MS detector connected with Electrospray ionization (ESI) source positive mode.

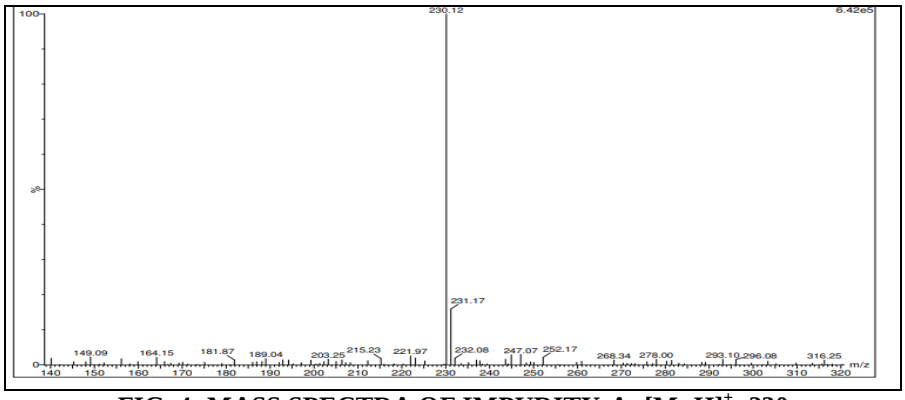

FIG. 4: MASS SPECTRA OF IMPURITY-A: $[M+H]^+$: 230

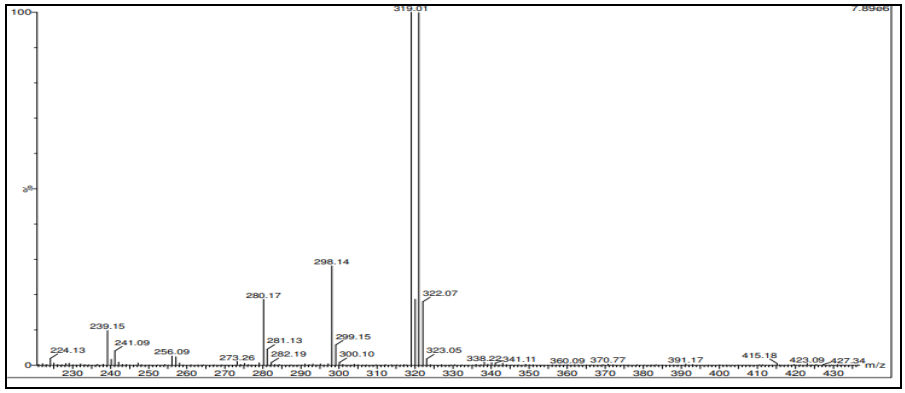

FIG. 5: MASS SPECTRA OF IMPURITY-B: [M+H]+: 319

METHODOLOGY:

Mobile Phase used for this method as 10mM Ammonium Acetate in Water: Acetonitrile (30:70) % v/v and diluent used for samples and standard preparation - Acetonitrile: Water (70:30) % v/v.

Preparation of Standard Solution: Weighed about 38mg of Impurity-A and Impurity-B, transferred in a 50 mL volumetric flask, and dissolved with diluents (Stock-1).

Pipette out 1.0mL from Stock-1 into 100mLvolumetric flask and makeup to the mark with diluents (Stock-2). Further transferred 5.0mL of stock-2 solution into 100mL volumetric flask and make up to the mark with diluents (Standard solution).

Preparation of Sample Solution: Weighed about 200mg of Bazedoxifene Acetate drug substance sample transferred into 20 mL volumetric flask, dissolved, and makeup to the mark with diluent.

LC-MS Operating Conditions: Experimentation performed by using Water's Acquity UPLC H-Class connected to XevoTQ MS/MS detector with ESI Source (Electron spray ionization). The stationary phase was used as X-Bridge C18 (50x4.6mm), 3.5µm column to separate the Impurity-A, Impurity-B and Bazedoxifene Acetate. The chromatographic method developed in the isocratic mode of elution with Mobile phase 10mM Ammonium acetate: Acetonitrile (30:70) %v/v with a flow rate of 0.6mL/min and a runtime of 5 minutes.Column oven temperature was maintained at 35°C and autosampler temperature was maintained at 5°C with an injection volume of 10µL. A triple quadrupole MS equipped with a positive electron spray ionization (ESI) source was used in the SIR (Selective ion recording) mode. The equipment was set with a Capillary voltage 3.0kV, Source temperature 150°C, Desolvation temperature 500°C, Desolvation Gas flow 1000L/hr, and Cone gas flow 50L/hr.

TABLE 1: IMPURITY-A AND IMPURITY-B SIR MODE CONDITIONS

S. no.	Analyte	Parent(m/z)	Dwell(s)	Cone voltage (eV)
1	Impurity-A	230.13	0.161	24
2	Impurity-B	319.19	0.161	24

Method Validation: The method validation study was conducted as per ICH Q2 (R2) guideline for the optimized LC-MS method, and the study was performed for specificity, precision, the limit of detection (LOD), Limit of quantification (LOQ), Linearity and Accuracy parameters. The linearity study was conducted by preparing and analyzing six different levels of concentration in the range of 3.0 ppm to 110.9 ppm for Impurity-A and 3.1 ppm to 112.4 ppm for Impurity-B. The values of Slope, Y-intercept, and Correlation coefficient were reported from the linearity study. The limit of detection (LOD) and quantification was established based on the signal-to-noise ratio method. The detection Limit (DL) value for Impurity-A was 1.4ppm, and Impurity-B was 1.4ppm. The quantification Limit (QL) for Impurity-A was 3.0 ppm and for Impurity-B was 3.1 ppm. Performed Precision at LOQ and Accuracy at LOQ to prove LOQ concentration was Accurate and precise. Method precision was conducted by preparing a 100% level spiked sample solution of Bazedoxifene Acetate drug substance containing concentration 75pp meached Impurity-A and Impurity-B and reported the %Relative standard deviation (%RSD) of the content of Impurity-A and Impurity-B. The method's accuracy was proved by adding known amounts of Impurity-A and Impurity-B to the Bazedoxifene Acetate drug substance sample at 50%, 100%, and 150% levels and calculated % recovery.

RESULTS AND DISCUSSION:

Method Development and Optimization: The LC-MS method's aim in this study is to develop a specific, sensitive, precise and Accurate analytical method for quantifying Impurity-A and Impurity-B in Bazedoxifene Acetate drug substance with a short runtime method. Method development was initiated using various acidic mobile phases (such as formic acid, trifluoroacetic acid, diflouoroacetic acid, ammoniumformate) and basic mobile phases (Ammonia solution, Ammonium bicarbonate) mixed with organic modifiers such as Acetonitrile and methanol isocratic mode elution have been tested. In acidic and basic mobile phase conditions

Gaussian curve peak shape was not observed. So, the experiment was conducted using 10 mM ammonium acetate at neutral pH using the C18 stationary phase. Finally, separation of Impurity-A, Impurity-B, and Bazedoxifene Acetate drug substance was achieved by using stationary phase as X-Bridge C18(50x4.6) mm, 3.5µm (Make: Waters). Gaussian curve peak shapes were observed in 10mM Ammonium acetate(Mobile phase-A) and Acetonitrile (Mobilephase-B) in the ratio of (30:70)v/v in the isocratic mode elution with a flow rate of 0.6 mL/min and very short runtime as 5minutes. Impurity-A, Impurity-B and Bazedofiene drug substance peaks are eluted within 5 minutes of runtime.

Optimization of MS/MS Conditions: MS Conditions optimization started by using an electron spray ionization (ESI) source in positive mode. SIR (Single ion recording) mode shows high sensitivity due to parent ion response. Injected standard solution of Impurity-A, observed it's $[M+H]^+$ at m/z 230.13, showed a significant response at Cone voltage 24V. Similarly injected, a standard solution of Impurity-B observed its $[M+H]^+$ at m/z of 319.19 showed a significant response at Cone voltage 24V. So, SIR (Selective ion recording) mode was highly sensitive and specific for quantification of trace level detection of Impurity-A and Impurity-B in Bazedoxifene acetate drug substance.

Validation Results of the Method: The LC-MS method for trace level quantification of Impurity-A and Impurity-B in Bazedoxifene Acetate drug substance was validated per ICH Q2 (R2) guidelines. The method was evaluated for its specificity, Sensitivity, LOD (limit of detection), LOQ (Limit of quantification), Linearity, Accuracy, and Precision

Specificity: Specificity is the ability to measure accurately and specifically the analyte of interest in the presence of other components that may be expected to be present in the sample matrix.

Specificity was performed by injecting the individual impurity standards Impurity-A and Impurity-B, each at about 75ppm level concerning 20mg/mL analyte concentration, Bazedoxifene Acetate drug substance at 0.5mg/mL, spiked sample solution of Bazedoxifene Acetate drug substance containing Impurity-A and Impurity-B **Table 2.**

TABLE 2: SPECIFICITY TIC: TOTAL ION CHROMATOGRAM, PDA: PHOTODIODE ARRAY DETECTOR

S. no.	Component name	Retention time (min)
1	Impurity-A	2.03 (From TIC)
2	Impurity-B	2.53 (From TIC)
3	Bazedoxifene Acetate	1.19 (From PDA detector)

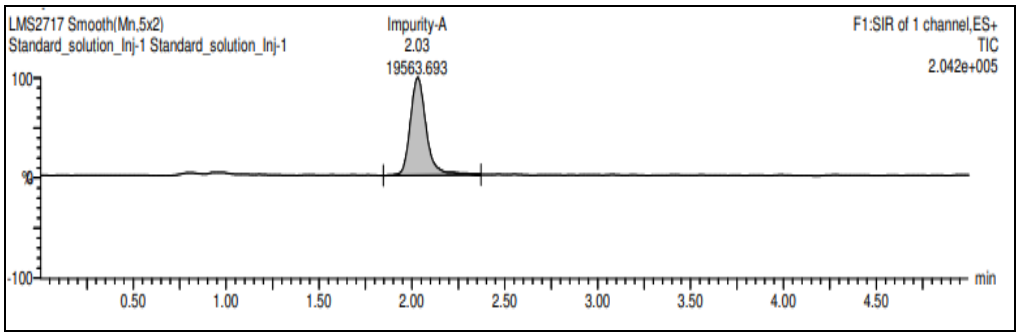

FIG. 6: IMPURITY-A TIC CHROMATOGRAM OF STANDARD (SIR MODE AT M/Z: 230)

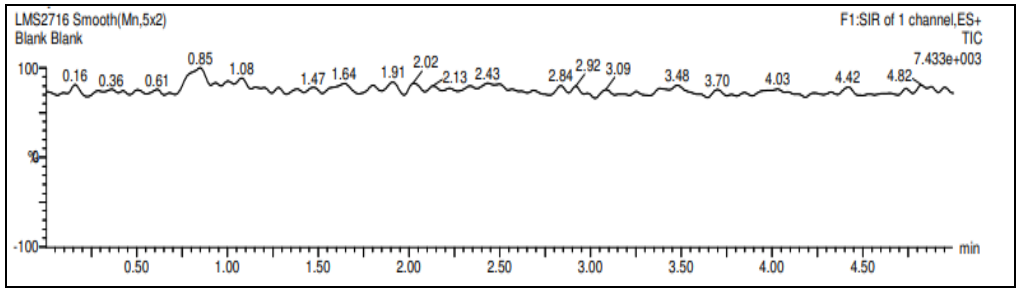

FIG. 7: IMPURITY-A TIC CHROMATOGRAM OF BLANK (SIR MODE AT M/Z: 230)

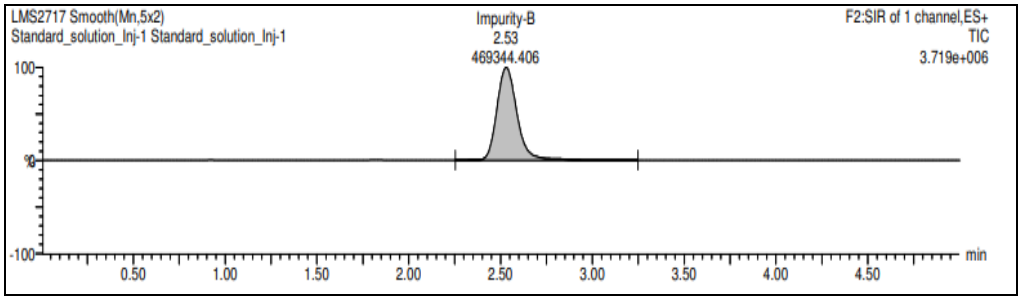

FIG. 8: IMPURITY-B TIC CHROMATOGRAM OF STANDARD (SIR MODE AT M/Z: 319)

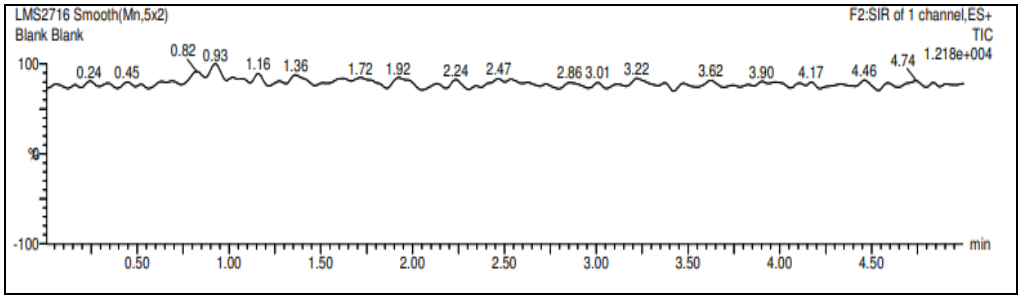

FIG. 9: IMPURITY-B TIC CHROMATOGRAM OF BLANK (SIR MODE AT M/Z: 319)

Sensitivity: The Limit of detection (LOD) **Fig. 4** and the Limit of quantification (LOQ) were determined from the signal to noise ratio (S/N) method. Prepared and injected a series of diluted solutions from individual standard solutions. Based on S/N ratios of diluted solutions, LOD and LOQ concentrations of Impurity-A and Impurity-B were reported. The LOD and LOQ concentrations of Impurity-A and Impurity-B are reported in **Tables 3** and **4,** respectively.

TABLE 3: LIMIT OF DETECTION (LOD) RESULTS

S. no.	Component name	S/N Ratio	LOD Concentration
1	Impurity-A	3.1	1.4ppm
2	Impurity-B	3.3	1.4ppm

TABLE 4: LIMIT OF QUANTIFICATION (LOQ) RESULTS

S. no.	Component name	S/N Ratio	LOQ Concentration
1	Impurity-A	10.4	3.0ppm
2	Impurity-B	10.2	3.1ppm

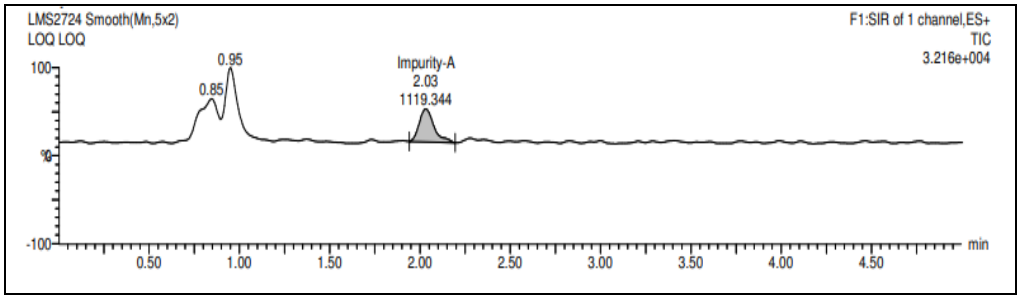

FIG. 10: LOQ (LIMIT OF QUANTIFICATION) SIR CHROMATOGRAM OF IMPURITY-A

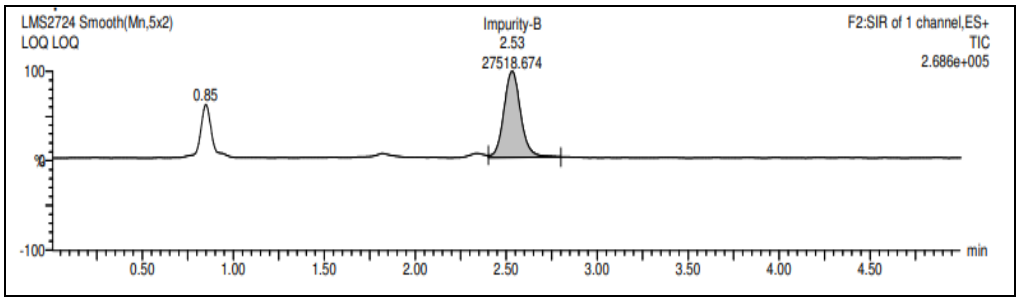

FIG. 11: LOQ (LIMIT OF QUANTIFICATION) SIR CHROMATOGRAM OF IMPURITY-B

Precision at LOQ: Prepared and injected the LOQ standard solution (each Impurity-A and Impurity-B at a concentration of 3.0 ppm and 3.1 ppm, respectively. Recorded the SIR peak areas of each Impurity-A and Impurity-B and calculated the %RSD from six replicate injections Results are tabulated in **Table 5.**

TABLE 5: LOQ PRECISION RESULTS

S. no.	Name of the solution	Impurity-A area	Impurity-B area
1	LOQ Standard solution injection-1	1061.363	27100.52
2	LOQ Standard solution injection-2	1111.561	26740.57
3	LOQ Standard solution injection-3	1191.302	27198.658
4	LOQ Standard solution injection-4	1195.229	26744.25
5	LOQ Standard solution injection-5	1059.282	25935.328
6	LOQ Standard solution injection-6	1137.287	25899.051
7	Mean	1126.004	26603.06283
8	Standard deviation	60.03686	562.5907467
9	%RSD	5.3	2.1

Linearity: Linearity studies were performed for Impurity-A and Impurity-B at different concentrations from QL to 200% (QL, 25, 50,100,150 and 200%) of the specification level concerning the analyte concentration. Plotted a linear graph by taking the SIR peak areas on Y-axis and the corresponding concentration on X-axis. The values of the correlation coefficient, slope, y-intercept and residual sum of squares from the linearity study were reported. For linearity results of Impurity-A, refer to **Table 6,** and for Impurity-B, refer to **Table 7,** respectively.

TABLE 6: IMPURITY-A LINEARITY RESULTS

Level	Concentration(ppm)	SIR Peak Area of Impurity-A
LOQ	3.033	1033.362
25%	18.492	5781.153
50%	36.984	10425.469
75%	55.476	13725.743
100%	73.967	18307.592
150%	110.951	26258.244
	Correlation coefficient	0.998
	Slope	0.0043
	Y-Intercept	1171.209055

TABLE 7: IMPURITY-B LINEARITY RESULTS

Level	Concentration (ppm)	SIR Peak Area
LOQ	3.064	26682.041
25%	18.730	146880.859
50%	37.460	259351.125
75%	56.190	327271.500
100%	74.920	497206.563
150%	112.379	789053.313
	Correlation coefficient	0.994
	Slope	0.0001
	Y-Intercept	-1840.129356

Precision:

Method Precision: Prepared the spiked sample solution six times containing each Impurity-A and Impurity-B at specification level at each preparation and injected each once. Calculated the content of each Impurity-A and Impurity-B and reported % RSD for Impurity-A and Impurity-B content from six spiked sample preparations. Results were tabulated in **Table 8.**

TABLE 8: METHOD PRECISION RESULTS

Level	Impurity-A (ppm)	Impurity-B (ppm)
Method Precision Preparation-1	75.690	75.230
Method Precision Preparation-2	75.383	75.773

Method Precision Preparation-3	75.442	75.396
Method Precision Preparation-4	75.175	75.552
Method Precision Preparation-5	75.044	75.150
Method Precision Preparation-6	75.645	75.125
Mean	75.4	75.4
Standard deviation	0.2543	0.2546
%RSD	0.3	0.3

Accuracy: The accuracy of the test method was demonstrated by preparing the un-spiked sample solutions and spiked sample solution with the known concentration of Impurity-A, and Impurity-B at LOQ level, 50%, 100%, and 150% of the specification limit. Calculated the %recovery of Impurity-A and Impurity-B at each level. The mean %recovery of Impurity-A at LOQ, 50%, 100%, and 150% levels were 100.8%, 102.2%, 102.1%, and 101.3%, respectively. The mean %recovery of Impurity-B at LOQ, 50%, 100%, and 150%, was 99.9%, 99.7%, 100.7%, and 100.1%, respectively. Results were tabulated in **Table 9.**

TABLE 9: % RECOVERY OF IMPURITY-A AND IMPURITY-B

	LOQ (3ppm)	50% (37.5ppm)	100% (75ppm)	150% (112.5ppm)
Mean % Recovery for Impurity-A (n=3)	100.8	102.2	102.1	101.3
Mean % Recovery for Impurity-B(n=3)	99.9	99.7	100.7	100.1

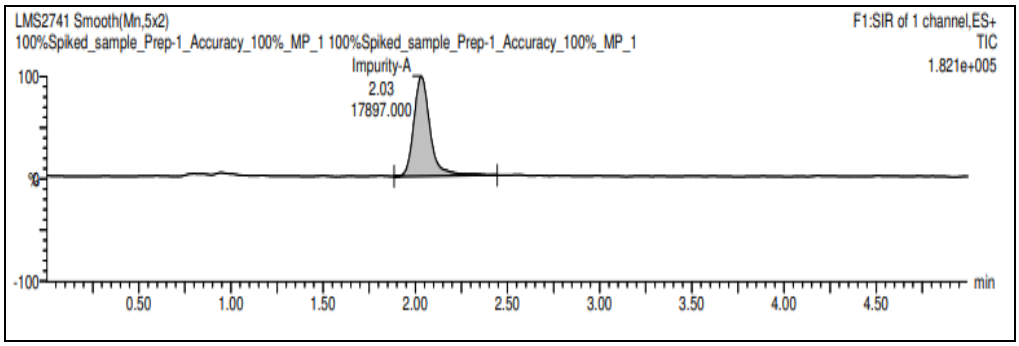

FIG. 12: 100% SPIKED SAMPLE SIR (TIC) CHROMATOGRAM OF IMPURITY-A

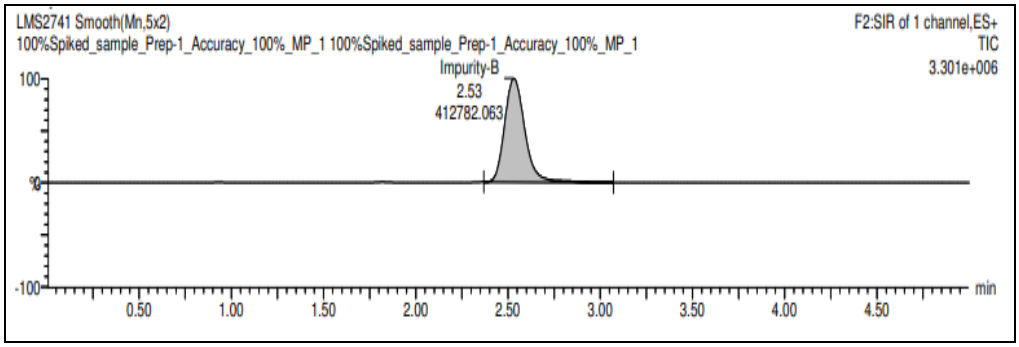

FIG. 13: 100% SPIKED SAMPLE SIR (TIC) CHROMATOGRAM OF IMPURITY-B

CONCLUSION: A sensitive and selective LC-MS/MS SIR quantification method for genotoxic impurities i.e.; 1 – Benzyloxy – 4 - nitrobenzene (Impurity-A) and 4 – benzyloxy – 2 –bromo-propiophenone (Impurity-B) in Bazedoxifene Acetate drug substance developed and validated as per ICH method validation guidelines.

The method can be suitable for trace-level quantification of these two potential genotoxic impurities in the Bazedoxifene Acetate drug substance.

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CONFLICTS OF INTEREST: The authors declare that there is no conflict of interest regarding the publication of this paper.

REFERENCES:

1. US Food and drug administration: M7(R1) on assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk guidance for industry 2018.
2. ICH: Assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk M7 (R1) 2017.
3. Pounikar AR, Umekar MJ and Gupta KR: "Genotoxic impurities-an important regulatory aspect. Asian Journal of Pharmaceutical and Clinical Research 2020; 13(6): 10-25, doi:10.22159/ajpcr.2020.v13i6.37370.
4. Ashok B. Patel, Ashish H. Asnani, Amitkumar J. Vyas, Nilesh K. Patel, Ajay I. Patel and Arvind N. Lumbhani: A Brief review on genotoxic impurities in Pharmaceuticals; Asian J of Pharmaceutical Research 2021; 11(3): 7-9.
5. Shaikh and Tabrez: Genotoxic Impurities and Its Risk Assessment in Drug Compounds 2018; 23. DOI: 10.32474/DDIPIJ.2018.02.000143, Available at SSRN: https://ssrn.com/abstract=3958587.
6. Masato Okano, Mitsuhiko Sato and Shinji Kageyama: Detection of bazedoxifene, a selective estrogen receptor modulator, in human urine by liquid chromatography-tandem mass spectrometry; Drug testing and Analysis 2022; https://doi.org/10.1002/dta.3225.
7. Karri Visweswara Rao, Kesareddy Padmaja Reddy, Kondapaturu Siva Kumari and Mudigonda Srinivas: A Stability-Indicating HPLC Method for the Determination of Bazedoxifene Acetate and its Related Substances in Active Pharmaceutical Ingredient Journal of Chromatographic Science 2013; 51(3): 215–221, https://doi.org/10.1093/chromsci/bms130.
8. Gandra Smitha, Sheelam Sharath C. Reddy, Maddela Rasagna, Reddymalla Priyanka, Vasudha Bakshi and Raju Jukanti: Bazedoxifene acetate quantification in rat serum with the aid of RP-HPLC: Method development and validation; World J of Pharmaceutical Sciences; Received: 10-10-2015/ Revised: 18-11-2015 / Accepted: 25-11-2015.
9. Ji-Sun Yeun, Hye-Su Kan, Minyu Lee, Namsick Kim, Tae-Young Oh, Seung-Kwan Nam, Yoon Seok Choi, In Sun Kwon and Jang Hee Hong: Pharmacokinetic comparison of two bazedoxifene acetate 20 mg tablet formulations in healthy Korean male volunteers; Translational and Clinical Pharmacology; https://doi.org/10.12793/tcp.2020.28.e7.
10. Shuhong, Liae, Lixin, Dongae, Keqin, Tangae, Zhenwei, Lanbcd, Rong, Liue, Yuanjian, Wange, Ruixun, Wange and Huaqing Lin: Simultaneous and trace level quantification of two potential genotoxic impurities in valsartan drug substance using. UPLC-MS/MS 2022; 114630.
11. Xin-yin, Cai Zi-jia, Zhang Jing-lin, Xiong Meng, Yang and Zheng-tao Wang: Experimental and molecular docking studies of estrogen-like and anti-osteoporosis activity of compounds in Fructus Psoraleae. Journal of Ethnopharmacology 2021; 276(10): 114044.
12. Tina Trdan Lušin, Tihomir Tomašić, Jurij Trontelj, Aleš Mrhar and Lucija Peterlin-Mašič: *In-vitro* bioactivation of bazedoxifene and 2-(4-hydroxyphenyl)-3-methyl-1H-indol-5-ol in human liver microsomes; Chemico-Biological Interactions 2012; 197(15) 8-15. https://doi.org/10.1016/j.cbi.2012.03.001.
13. LiShen, Syed Ahmad, SeongHee Park, William DeMaio, Aram Oganesian, Theresa Hultin, JoAnn Scatina, Peter Bungay and Appavu Chandrasekaran: *In-vitro* Metabolism, Permeability and Efflux of Bazedoxifene in Humans; Drug Metabolism and Disposition September 2010; 38(9): 1471-1479; DOI: https://doi.org/10.1124/dmd.109.030999.
14. Yuyuan Chen, Song Wu and Qingyun Yang: Development and Validation of LC-MS/MS for Analyzing Potential Genotoxic Impurities in Pantoprazole Starting Materials; J Anal Methods Chem 2020; 2020: 6597363. Published online 2020 Mar 9. doi: 10.1155/2020/6597363.
15. Chandramohan Alluri, Kiran Kumar Naramsetti a and G. Veera Raghava Sharma: Sensitive and selective analytical method for the quantification of two potential genotoxic impurities in azilsartan drug substance by using LC-MS/MS with Multiple Reaction Monitoring (MRM mode); Journal of Pharmaceutical Research International DOI: 10.9734/JPRI/2021/v33i63A36047.

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