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The Sensitive Lc-Msms Method & Validation Procedure To Determination Of The N-Nitroso Isavuconazonium

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Abstract

Background and purpose: *The Isavuconazonium sulfate is the Active pharmaceutical ingredient (API) which is used for treatment for the Fungal infections, N-Nitroso Isavuconazonium sulfate(NNIS) is the Nitroso drug substance related impurity (NDSRI) can cause the cancer even the presence of very minute level in the API, thus the determination technique is required to know the amount of impurity is present in the pharmaceutical product which may form during the manufacturing process of the API. The trace level determination of this impurity is difficult due the similar structure of the Impurity as well as API except Nitroso group. We have developed method it explains the ppb level determination of N-Nitroso Isavuconazonium sulfate in Isavuconazonium sulfate drug substance by LC-MSMS with suitable precision ,accuracy, LOD (Limit of Detection) & LOQ (Limit of Quantification), Solution stability by using Sciex-4500 (Q-trap) LC-MSMS instrument.* ***Experimental approach****: The Required separations were achieved in a Kinetix® F5 100A°(Part No: OOF-4723-EO) HPLC Column with 150mm *4.6mm and 2.6µm . The LC-MS system operated flow rate of 0.5 mL/min for 20 minutes. When determining the N-Nitrosamine Isavuconazonium sulfate impurity (NNIS) of commercially manufactured batches of Isavuconazonium sulfate drug substances, this established approach may be helpful for identification and quantification of N-Nitrasamine impurity.* ***Key results****: The specification limit for This N-Nitroso isavuconazonium sulfate impurity (NNIS) is 48ppb, the developed method is capable to detect from 1.32ppb (LOD) & able to Quantify from 4.0ppb(LOQ)* ***Conclusion****: The well-known LCMS technique was developed to be Precise, selective, accurate & Specific.*

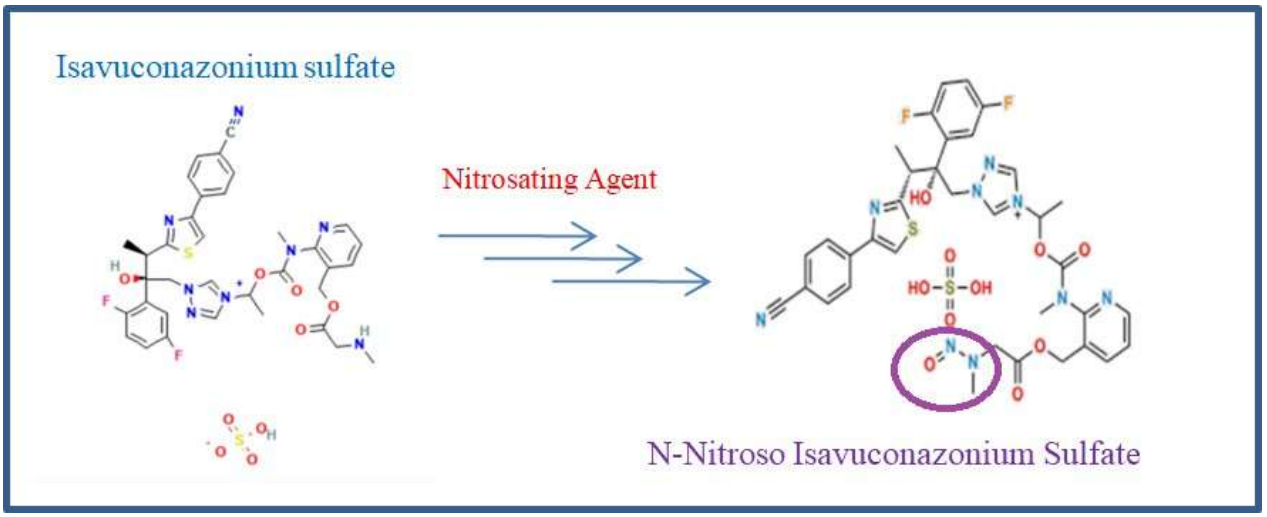
Keywords: *Isavuconazonium sulfate, N-Nitroso Isavuconazonium sulfate (NNIS), LOQ, LOD & Sciex-4500*

INTRODUCTION

Isavuconazonium sulfate is a second-generation triazole antifungal approved by the FDA on March 6, 2015, for treating invasive aspergillosis & invasive mucormycosis, distributed by Astellas under the brand name Cresemba1. It is Isavuconazole prodrug form, the active substance & it is in oral and parenteral formulations[1]. Due to the low solubility in water of isavuconazole on its own, the isavuconazonium formulation is suitable as it has high solubility in water and allows for intravenous administration[1]. Isovuconazonium has good oral bioavailability, measurable pharmacokinetics & also the best safety profile[1]. During the manufacturing process of pharmaceutical drugs, unknown impurities are still found in active pharmaceutical ingredients (APIs)[1]. Nori et al. is knowledgeable with regulatory guidelines and has written reviews on nitrosamine contaminants in pharmaceutical items[2].The ICH quality criteria, in particular chapter number ICH-Q2[3], established the need for an analytical method. The quantitative presence of undesirable n-nitrosamine impurities has increased the danger of mutagenic and carcinogenic potential in some approved medicinal products[4].The discovery of N-nitrosodimethylamine (NDMA) in the drug substance Valsartan was reported in June 2018 and prompted a number of regulatory actions, including market recalls and a referral procedure under Article 31 of Directive 2001/83/EC in the European Union (EU), despite the fact that regulatory published reports of N-nitrosamine impurities in drug substances and drug products had previously been published [5,6]. Furthermore, GC-MS techniques cannot identify nitrosamine in compounds containing losartan and irbesartan. The advantages of the developed approach over EMA LC-MS/MS are as follows. For a single study, EMA can detect just two nitrosamines (NDMA and NDEA), although

having the same sensitivity of 0.2 ng/mL[7].In the manufacturing process of Isavuconazonium sulfate and degradation pathway of Isavuconazonium sulfate drug substances, there is a scope of formation of N-Nitroso Isavuconazonium sulfate impurity (NNIS). An Ultra sensitive analytical method is required to determine the content of N-Nitroso Isavuconazonium sulfate impurity to in line with the Authority guidelines. This gap has been discovered as affecting the quality of Isavuconazonium sulfate drug substances, and our research has been initiated with a quantitative determination of the gap in drug substances using the Q-Trap LC-MSMS method.

Fig-1: Probable formation of N-Nitroso Isavuconazonium sulfate impurity from Isavuconazonium sulfate



Experimental

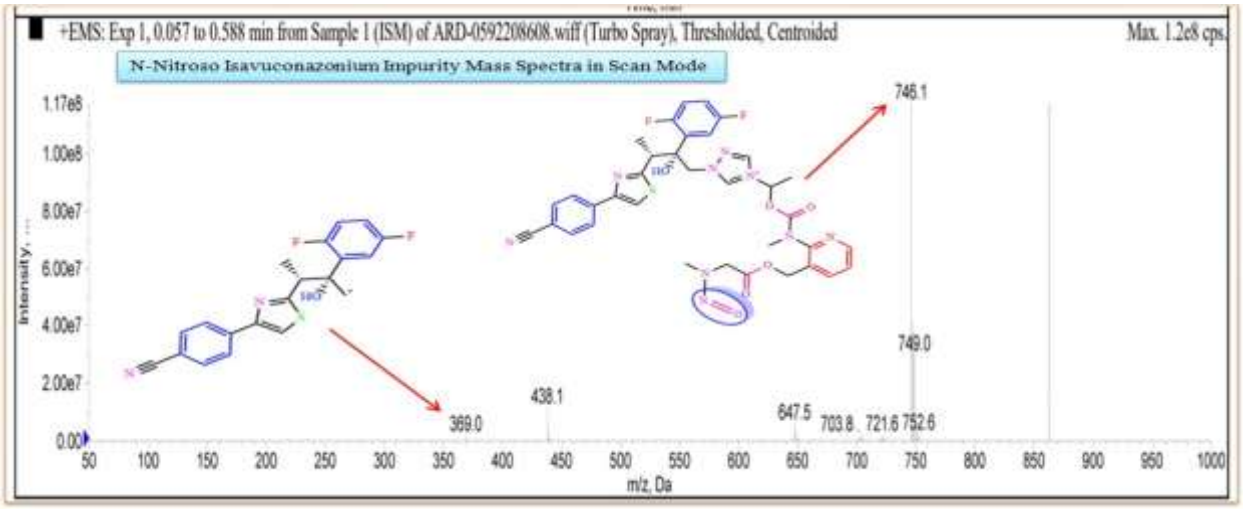
Materials

The method was developed using chemicals and reagents of LC-MS grade. Honeywell provides acetonitrile, whereas Sigma Aldrich provides formic acid.

Mass scan data

Ion Source is selected as ESI (+ve Mode) the ESI Scan of N-Nitroso Isavuconazonium sulfate was obtained with Sciex-4500 Q-Trap LC-MSMS. The scan data is shown in the Fig-2.

Fig-2: ESI +ve scan of N-Nitroso Isavuconazonium sulfate



Mobile phase- A: 0.1% Formic acid in Milli-Q-Water (1ml of Formic acid in 1000mL of Water)
Mobile Phase- B : Acetonitrile : Milli-Q-Water (900:100 v/v)
Diluent: Acetonitrile: Milli-Q-Water (50:50 v/v),

Test sample preparation:

Weigh accurately test sample (500 mg) into a 10 mL volumetric flask, add 5 mL of diluent, mix well, make it up to the mark with diluent, and filter the solution using a 0.45µm syringe filter.

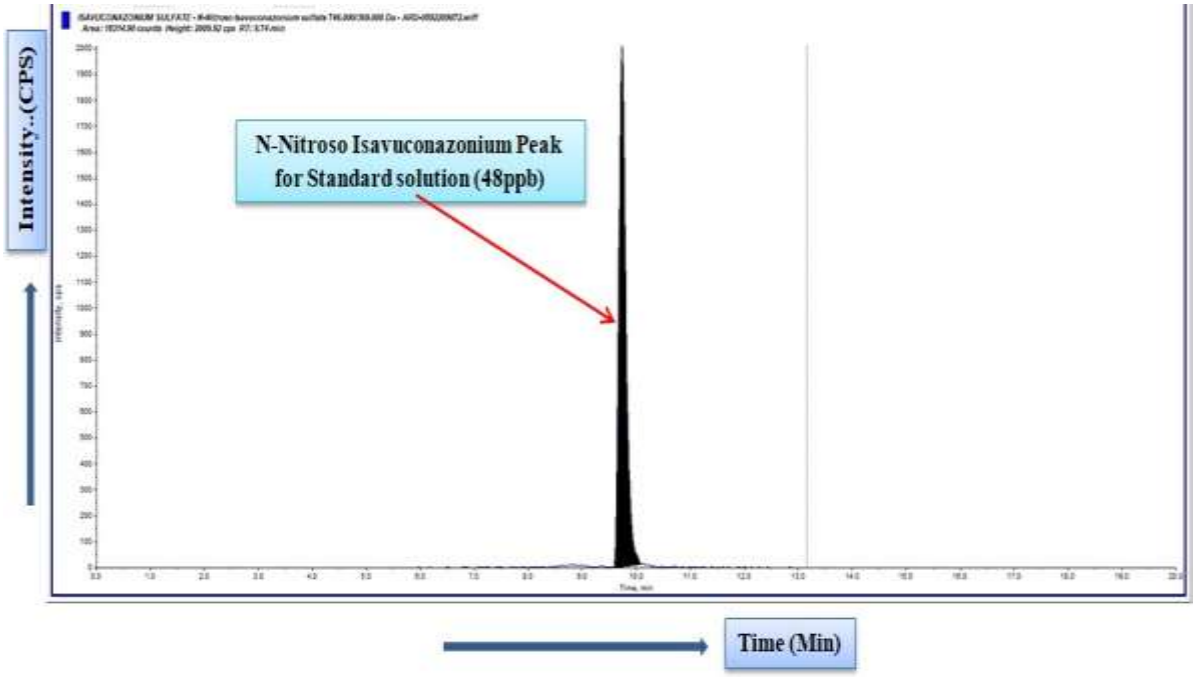
N-Nitroso Isavuconazonium sulfate stock solution preparation:

Weigh NNIS (10.00 mg) into a volumetric flask (10mL), add diluent (5mL) for dissolving as well as making up to the mark with diluent, 0.1 mL of this solution transferred into a volumetric flask (10mL), pour 5 mL of diluent to dissolve and make up to the mark with diluent.

Preparation of Standard Solution (48 ppb):

(0.024 mL) of the above N-Nitroso Isavuconazonium sulfate stock solution into a volumetric flask (100 mL), poured 50 mL of diluent, mix well, and make to mark with diluent. This mixture contains 48 ppb of N-Nitroso Isavuconazonium sulfate concerning a test sample concentration as 50 mg/mL. The typical Standard solution chromatogram is shown in Fig-3.

Fig-3: The typical Standard solution chromatogram



RESULTS AND DISCUSSION

Method development:

The process is holistically developed using a variety of stationery phase columns that contain ascents express, PFP, amino, C18, and C8 before it is frozen. These columns are examined using a variety of mobile-phase solutions, including ammonium acetate, Ammonium format, acetic acid and methanol. Ultimately, Kinetix® F5 100A°(Part No: OOF-4723-EO) (150mm, X 4.6mm & 2.6µm) with Formic acid and Acetonitrile with a 20-minute run period yielded the final chromatographic parameters. ESI in +ve MRM mode was used to optimize the mass chromatography.

Chromatographic conditions:

Sample cooler temperature	: 5°C
Column	: Kinetix® F5 100A° (150mm, X 4.6mm & 2.6µm)
Flow rate	: 0.6mL/min
Column oven temperature	: 40°C
Injection volume	: 20µL
Run time	: 20min

Diluent : Acetonitrile : Milli-Q-Water (50:50 v/v)

Mobile phase-A : (1000µL) of formic acid in 1Liter of Milli-Q-water

Mobile phase-B : Acetonitrile : Milli-Q-Water (900:100 v/v)

Gradient : Gradient programme shown in Table-1

Table-1: Gradient Programme

Time(min)	Mobile phase-A(%)	Mobile phase-B(%)
0.00 min	60.0%	40.0%
2.00 min	60.0%	40.0%
7.00 min	10.0%	90.0%
12.00 min	0.0%	100.0%
15.00 min	0.0%	100.0%
15.50 min	60.0%	40.0%
20.00 min	60.0%	40.0%

Mass analyzer parameters

Ion Source : ESI (Electro spray ionization)
Q1 Mass (Da) : 745.80 m/z (Product ion)
Polarity : Positive
Scan Type : MRM
Q1 Mass (Da) : 745.80 m/z (Product ion)
Q3 Mass (Da) : 377.00 m/z (Daughter ion)
Resolution : Unit
Ion Source gas-1 55
Ion Source gas-2 50
Temperature (°C) 500
Ion Spray voltage (IS) 4500
Polarity Dwell time (ms) :200
Entrance Potential (EP) 10
Collision gas (CAD) : Medium
Collision cell exit potential (CXP): 10.0
Schematic representation for daughter ions for N-Nitroso Isavuconazonium sulfate is shown in Fig-4

Method Validation:

This recognized approach was verified in accordance with ICH-Q2R1 requirements.

System Suitability:

N-Nitroso Isavuconazonium sulfate standard solution (48 ppb) was introduced into LC-MS as part of the investigation, and the findings are mentioned in Table-2

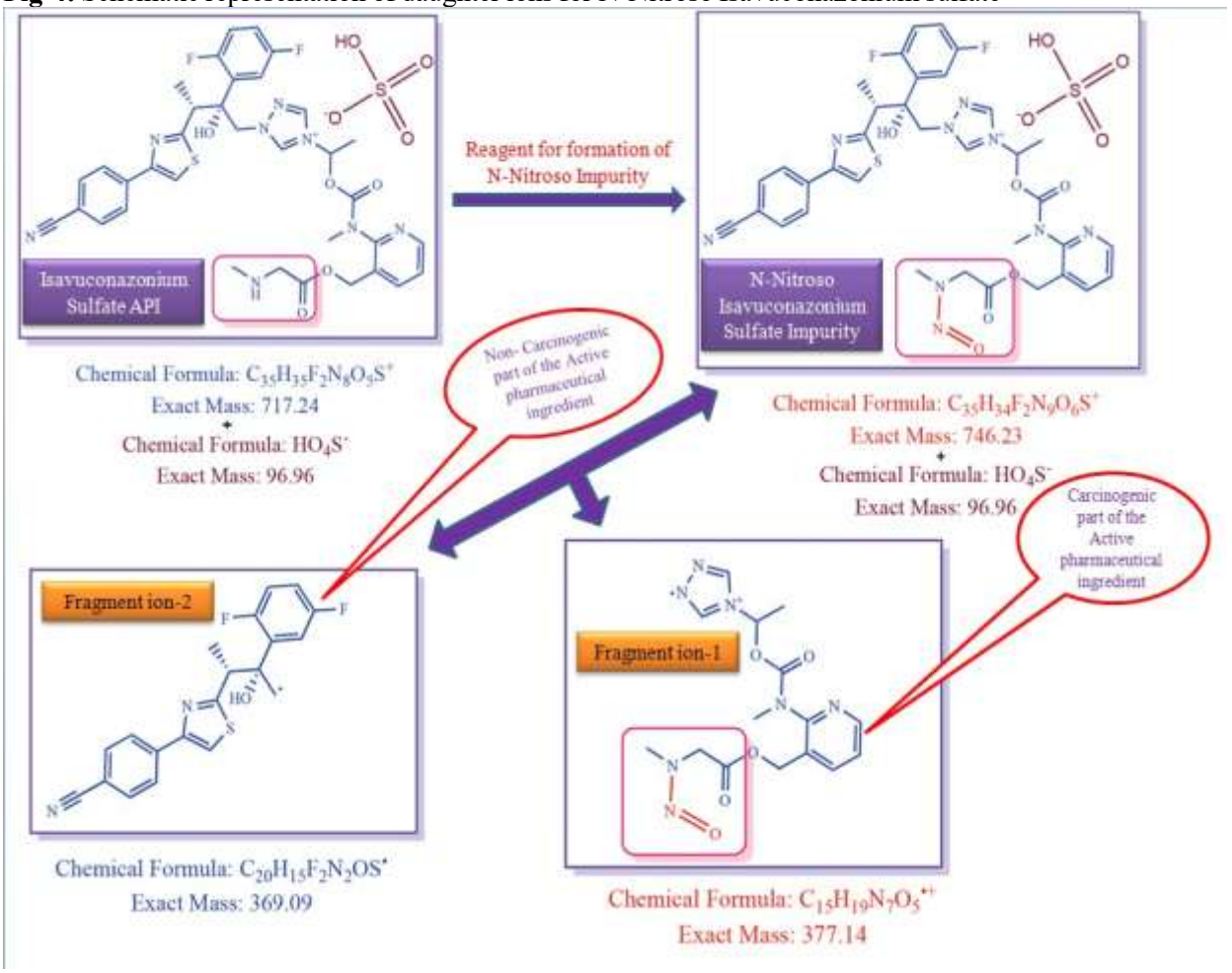
Table-2: The System suitability data

Preparation	Analyte response
Standard solution inj-1	18314.90
Standard solution inj-2	17670.66
Standard solution inj-3	18020.63
Standard solution inj-4	18612.95
Standard solution inj-5	18249.12
Standard solution inj-6	18272.11
Average area	**18190.06**
STDV	**317.21**

% RSD	1.74

The system appropriateness criteria have been verified since the analyte's percentage relative standard deviation (%RSD) area, as determined by six standard solutions, is within the acceptable range.

Fig-4: Schematic representation of daughter ions for N-Nitroso Isavuconazonium sulfate



Specificity:

Specificity testing was performed by administering blank, control, and spiked sample solutions.

The analysis confirmed that there was no interference from the blank solution while the N-Nitroso Isavuconazonium sulfate peak was retained in the spiked sample solution.

LOD and LOQ:

The Signal to noise ratio approach was utilized to determination of LOD & LOQ values as per the ICH Q2R1 guidelines for analytical method validations. The LOD and LOQ values for N-Nitroso Isavuconazonium sulfate are 1.32 ppb & 4 ppb respectively. The % RSD for the peak area of the N-Nitroso Isavuconazonium sulfate for six LOQ preparations is 6.93. Represented data for LOD & LOQ are mentioned in Table-3, and LOQ precision data is tabulated in Table-4

Table-3: LOD & LOQ data

Name of the Impurity	RT	Con (ppb)	LOD		LOQ	
			S/N	Con(ppb)	S/N	Con (ppb)
NNIS	9.74	48	3.18	1.32	10.73	4.0

NNIS-N-Nitroso Isavuconazonium sulfate; RT-Retention time; Con-Concentration; ppb-Parts per billion LOD-Limit of Detection; S/N-Signal to noise ratio; LOQ-Limit of quantification

Table-4: LOQ precision data

Preparation	**Analyte response at the LOQ level**
LOQ Preparation-1	1408.20
LOQ Preparation-2	1418.78
LOQ Preparation-3	1468.61
LOQ Preparation-4	1454.71
LOQ Preparation-5	1422.39
LOQ Preparation-6	1678.32
Average	**1475.17**
Standard Deviation	**102.16**
% RSD	**6.93**

Accuracy at LOQ level

Accuracy was established by injection of a preparation of 4.0 ppb analyte (N-Nitroso Isavuconazonium sulfate) LOQ solution spiked to the test sample in triplicate. The % RSD values obtained were within the acceptance criteria. The results of the accuracy at LOQ level solution analysis are shown in Table-5.

Table-5: Accuracy at LOQ level solution (4.0ppb)

Name of the Impurity	**Ar in LOQ Sol**	**Ar in TS**	**Ar in TS+ LOQ Sol Spike**	**Recovery (%)**
preparation-1	1408.20	0.0	1559.42	110.74
preparation-2	1418.78	0.0	1527.06	107.63
preparation-3	1468.61	0.0	1762.55	120.01
Average	1431.86	0.0	1616.34	112.80
STEDV	32.26	0.0	127.65	6.44
%RSD	2.25	0.0	7.90	5.71

Ar-Analyte response; sol-Solution; TS-Test sample; STEDV-Standard deviation; RSD-Related standard deviation

Accuracy at the Specification level:

Accuracy was established by injection of a preparation of 48 ppb analyte (N-Nitroso Isavuconazonium sulfate) Specification level solution spiked to the test sample in triplicate. The % RSD values obtained were within the acceptance criteria. The results of the accuracy at Specification level solution analysis are shown in Table-6.

Table-6: Accuracy at Specification level solution (48ppb)

Name of the Impurity	Ar in Std Sol	Ar in the TS	Ar in TS + Std Sol Spike	Recovery (%)
preparation-1	18314.90	0.00	17236.83	94.11
preparation-2	17670.66	0.00	17838.77	100.95
preparation-3	18020.63	0.00	18094.38	100.41
Average	18002.06	0.00	17723.33	98.49
STEDV	322.52	0.0	440.28	3.80
%RSD	1.79	0.0	2.48	3.86

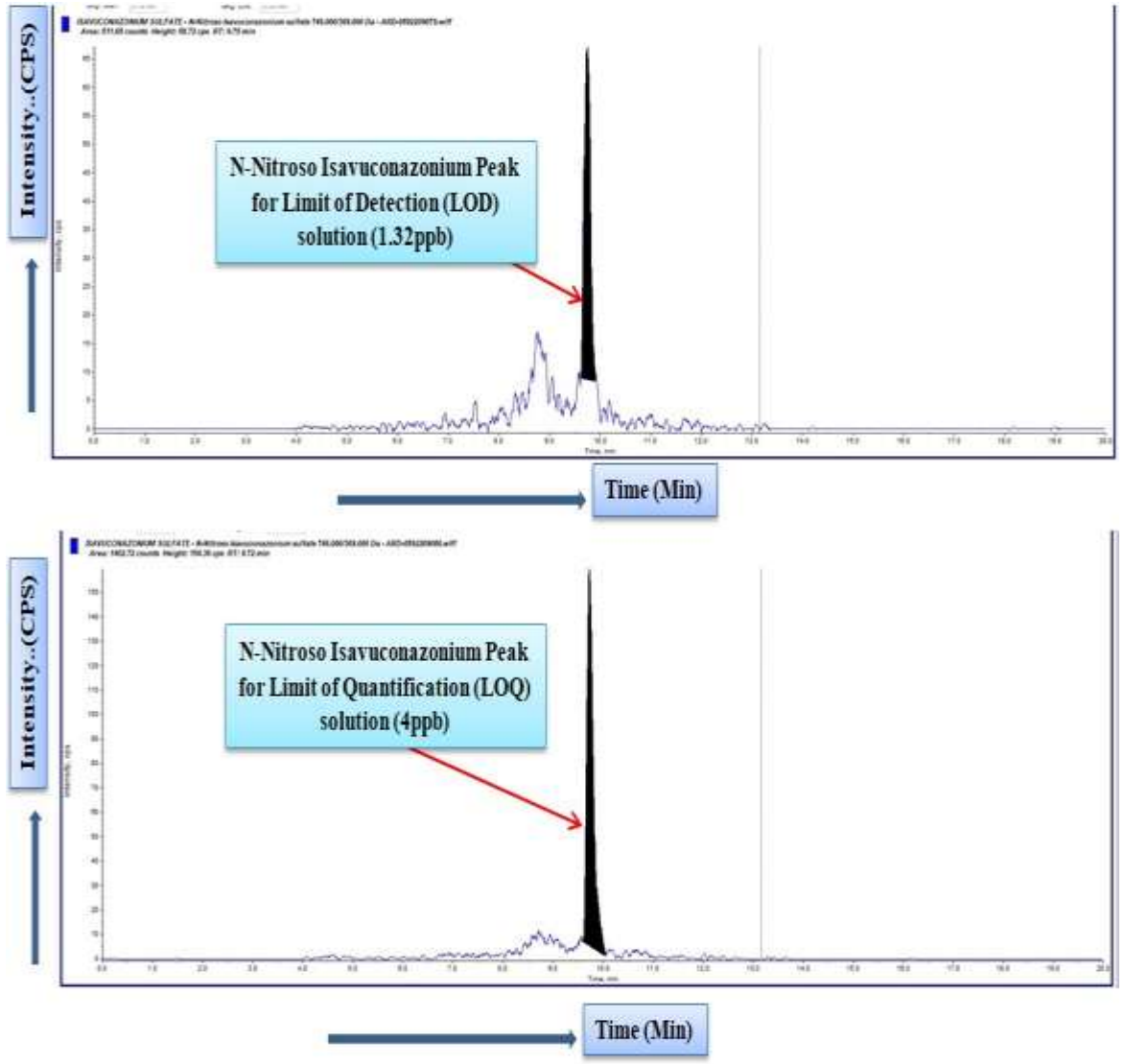
Ar-Analyte response; std- Standard; sol-Solution; TS-Test sample; STEDV-Standard deviation; RSD-Related standard

The above tabulated results of quantitative method evaluation data represents, the recommendations of the standard test The procedure for determining the N-Nitroso Isavuconazonium sulfate in drug substances of Isavuconazonium sulfate is shorter in Table-7

Table-7: Method outcome

Retention time (min)	**LOD (ppb)**	**LOQ (ppb)**
9.74	1.32	4.0

Fig-5: Typical chromatogram for LOD and LOQ



CONCLUSION

Developed LC-MS / MS ultra sensitive method to quantify the trace levels detection of N-Nitroso Isavuconazonium sulfate in the Isavuconazonium sulfate drug substance. As per the data & results, this method is ultra sensitive, specific, accurate and precise. Therefore, this LC-MS/MS method can be used to monitor the N-nitroso Isavuconazonium sulfate and evaluate the quality of the drug substance Isavuconazonium sulfate. This LC-MS quantitative method has practical advantages for the generic drug industry, helping to maintain the quality and safety of drug substances in Isavuconazonium sulfate.

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AUTHORS CONTRIBUTION

All the authors contributed significantly to this manuscript, participated in reviewing/editing, and approved the final draft for publication. The research profile of the authors can be verified from their ORCID IDs, given below:

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Conflict of interest*: The authors declare that they have no conflict of interest in the publication of this article. The authors have no conflicts of interest to report in this work. The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.*

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