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Analytical Method Development And Validation For Determination Of Nitrosamine Impurities N-Nitrosodibutylamine (Ndba) & Nitroso Of N-Dimethyl Erythromycin In Sertraline Hcl Drug Substance By Lc-Ms/Ms

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This work provides a thorough assessment of a method for measuring N-nitrosodibutylamine and N-nitroso of N-dimethyl erythromycin A, two substances with substantial regulatory interest because of their health hazards. The performance characteristics of the method were thoroughly investigated through a series of experiments, including standard solution analysis, quantitation and detection limit determination, linearity assessment, precision testing at various levels, and accuracy evaluation via recovery studies. The findings demonstrate that the technique performs exceptionally well in terms of linearity, accuracy, and precision over a broad range of concentrations. The results indicate that the suggested approach has the potential to accurately quantify these chemicals in a range of sample matrices, making it a useful tool for regulatory compliance and quality assurance in associated industries.

Keywords: linearity, precision, accuracy, technique validation, quantification, limit of detection, the limit of quantitation, N-nitrosodibutylamine, N-nitroso of N-Dimethyl Erythromycin A, and recovery studies

1. INTRODUCTION

One often prescribed drug in the class of selective serotonin reuptake inhibitors (SSRIs) is sertraline hydrochloride (HCl). [1] Major depressive disorder (MDD), obsessive-compulsive disorder (OCD), panic disorder, post-traumatic stress disorder (PTSD), social anxiety disorder, and premenstrual dysphoric disorder (PMDD) are among the psychiatric illnesses for which it is primarily used as an antidepressant. To regulate mood, feelings, and behaviour, serotonin is a neurotransmitter in the brain. Sertraline acts by preventing serotonin from being reabsorbed. Sertraline helps reduce symptoms of anxiety and depression by raising the amount of serotonin

that is accessible in the synaptic clefts between neurons. [2] Due to its therapeutic efficiency and safety profile, sertraline HCl is one of the antidepressants that is given the most frequently in the globe. It can be taken in a variety of forms, such as tablets, capsules, and oral concentrate solutions, giving patients flexible dosing choices to suit their needs.
To ensure the safety and effectiveness of the pharmaceutical product, it is imperative to determine the presence of nitrosamine impurities in sertraline HCl, specifically N-Nitrosodibutylamine (NDBA) and Nitroso of N-Dimethyl Erythromycin A. A family of chemical substances recognized to have the potential to cause cancer in humans is nitrosamines. As a result, regulatory bodies mandate stringent oversight and management of nitrosamine concentrations in medications.[3]

Formation of Impurities:

Pharmaceuticals can contain nitrosamines through a number of different routes, such as: Nitrosation Reactions: In specific circumstances, such as an acidic pH, high temperatures, and the presence of nitrite ions, these reactions take place between secondary amines or their precursors and nitrosating agents, such as nitrous acid or nitrogen oxides. Reaction with Nitrosating chemicals: Nitrosamine can be formed when secondary amines react with nitrosating chemicals found in solvents, raw materials, or manufacturing processes.[4] Contaminated Raw Materials: Nitrosamines may also be included in the finished product due to their presence as contaminants in the beginning materials or excipients utilized in the formulation.

Product Details:

A common selective serotonin reuptake inhibitor (SSRI) used to treat depression, panic disorder, obsessive-compulsive disorder (OCD), and other mental health issues is sertraline HCl.[5] It works by raising the brain's concentration of the neurotransmitter serotonin, which elevates mood, energy, appetite, and sleep quality.

Impurity Details:

N-nitrosodibutylamine, or NDBA, is a recognised nitrosamine contaminant that can arise during the manufacturing or storage of medications that include secondary amines. The International Agency for Research on Cancer has designated NDBA as a potential human carcinogen (IARC).[6]

Another nitrosamine impurity that can be found in medications, especially those made from macrolide antibiotics like erythromycin, is nitroso of N-Dimethyl Erythromycin A. Since this impurity is likewise thought to have the potential to cause cancer, pharmaceutical items must be strictly monitored and controlled for it.

Highly developed analytical techniques, such as high-performance liquid chromatography (HPLC) combined with mass spectrometry (MS), which offer sensitive and selective detection at low levels, are used to quantify these nitrosamine impurities in sertraline HCl. [7]To guarantee patient safety and adherence to quality standards, regulatory bodies impose restrictions on the permissible quantities of nitrosamine contaminants in medications. Thus,

routine monitoring of these contaminants in pharmaceutical goods requires the use of precise and trustworthy analytical techniques.[8]

Malihi Farzad (2022) Nitrosamines are a class of chemicals that are likely carcinogenic to humans. They are typically created when nitrite or other nitrogenous substances combine with secondary and tertiary amines, amides, carbamates, or derivatives of urea. Common nitrosating contaminants, nitrites have been found at parts per million in several excipients. The FDA and EMA guidelines have recognized possible nitrosamine contaminants that may exist in pharmaceutical products. It is essential to have a recognized process in place for screening drug substances and goods as part of the recommendations. Additionally, testing pharmacological items with buffers, salts, and polymeric excipients proved difficult since the existing literature methods could not hold onto early-eluting nitrosamine contaminants long enough to use a converter switch to bypass the salts and excipients from the mass spectrometer ion source. Our lab addressed the aforementioned unmet need by developing an enhanced analytical method employing HPLC/MS/MS that is in line with FDA and EMA regulations. The technique shows good linearity and sensitivity in the range of 0.2–200 ng/mL. Additionally, it was demonstrated that the technique can handle significantly higher dilution percentages of organic solvents. Interested readers can use the procedure as a basis for additional optimization when analyzing the drug ingredients and drug items that they specifically use.[9]

Bharate (2021) A thorough pharmacovigilance process results in a product recall, which is a crucial component of drug control. The discovery of inadequate quantities of carcinogenic contaminants is one of the most important concerns among the many grounds for product recalls. The FDA released guidelines for the pharmaceutical industry addressing the control of nitrosamines in drug products in September 2020 due to the major safety concern raised by the genotoxic and carcinogenic potential of N-nitrosamines. More than 1400 product lots have been taken off the market, according to the FDA database, because they contain carcinogenic N-nitrosamine impurities at levels higher than the recommended daily intake of 26.5 ng. The following medications were found in recalled products: metformin, ranitidine, nizatidine, valsartan, and irbesartan. This viewpoint offers a critical analysis of these product recalls, focusing on the mechanism and source of N-nitrosamine production in these items.[10]

Parr (2019) The July 2018 pharmaceutical product batch recalls involving valsartan sparked a conversation about potential N-nitroso dimethylamine (NDMA) contaminations. It seemed that the synthesis of the active pharmaceutical ingredient (API) from reagent nitrite and solvent dimethylformamide (DMF) produced NDMA. Since then, the topic of NDMA as an API impurity has been expanded to include other medications. Scientific literature has already described NDMA as a drug contaminant in several other medications several years prior, highlighting the obvious risk. As of right now, there is no pharmacopeia testing for NDMA, and the few publications of methods for determining it in medications are scarce. This study addresses the possible usefulness of nitrosamines (NAs) for drug investigations and summarises key characteristics of NA analyses, with a particular emphasis on NDMA. Because of its great selectivity and low detection levels, GC–MS or GC–MS/MS is used in

most recent articles. High nitrosamine selectivity is another benefit of GC-TEA. However, this combo is quite hard to come by right now. As an alternative, NA analysis also uses LC-MS/MS.[11]

Khorolskiy M (2022) Genotoxic nitrosamine impurity levels have been regulated and controlled since 2018 and are now required as a quality and safety feature for a number of medications. The primary problems with nitrosamine determination in pharmaceuticals are the constantly growing list of banned chemicals and the low sensitivity of the current technologies. The lack of chromophores in their structure and the low safe daily dose of these contaminants are the causes. The development and validation of a method utilizing high-performance liquid chromatography with mass spectrometry detection for the identification of nitrosamine impurities (controlled by the regulatory bodies) in Valsartan, Losartan, and Irbesartan. This investigation used an Agilent Infinity II chromatographic system with atmospheric pressure chemical ionization and a mass spectrometric detector (MSD 6460 Triple Quad). The best conditions for chromatographic separation (gradient parameters, mobile phase composition) and mass spectrometric detection parameters were chosen throughout the method's development. The method's maximal sensitivity with respect to the nitrosamines under study was obtained through the use of chemical ionization, and the matrix effect was eliminated through the optimization of mass spectrometric detection settings. One key benefit of nitrosamine's sophisticated determination approach is its ability to drastically cut down on analysis time by eliminating the need for further purification and concentration steps. The resulting procedure was verified in terms of accuracy, precision, LOQ, linearity, specificity, and LOD. The final technique satisfied all requirements for approval and may be applied to regular quality monitoring of pharmaceuticals like as valsartan, losartan, and irbesartan [12]

Vadariya, (2024). The pharmaceutical industry has paid close attention to nitrosamines and N-nitroso impurities (NDSRi) because of the possible health hazards they provide, including the possibility of cancer. Consequently, there is a growing need for analytical techniques that can recognize and measure these contaminants in drug substances and drug products. The analytical methods that have become indispensable for the thorough evaluation of nitrosamines and N-nitroso contaminants in pharmaceutical formulations are succinctly summarized in this study. Regarding various regulatory bodies, nitrosamine impurities in pharmaceutical products are a major issue to avoid patients from being exposed to carcinogenic and mutagenic consequences. The most difficult task is confirming its existence using extremely precise and sensitive analytical techniques with a ppb-level lower limit of quantification. Furthermore, a lot of attention has been focused on the overestimation of nitrosamines brought on by artificial nitrosamine synthesis during sample preparation and injection. Numerous analytical techniques have been described for the quantification of nitrosamine impurities in active pharmaceutical substances (Drug substance) and medicinal products (Drug product) at the interim limit (numbers) criteria as preventive measures. In this review, we thoroughly examine the documented methods for nitrosamine measurement in pharmaceuticals, including chromatographic conditions and detection sensitivity, as well as gas and liquid chromatographic methods. In summary, this study highlights how important it is to have trustworthy analytical methods for identifying and quantifying nitrosamine and N-

nitroso contaminants in medications. It provides an overview of the advancements made in this field and opinions on the challenges and opportunities for further research and development. By implementing these measures, the pharmaceutical industry can ensure the safety and quality of drug goods, thus safeguarding the public's health. [13]

Xie B. (2022) The third N-nitrosamine impurity identified in sartans is N-nitroso-N-methyl-4-aminobutyric acid (NMBA). Here, the quantitative measurement of NMBA in four sartan compounds has been achieved through the development of a sensitive and stable LC-MS/MS method with multiple reactions monitoring mode. NMBA and sartan compounds were successfully separated using a C18 column and gradient elution techniques. The internal standard method and mass spectrometry approach of the atmospheric pressure chemical ionization source were chosen as the quantitative analysis method of NMBA. The LC-MS/MS analysis method that was suggested was then verified with respect to its specificity, sensitivity, linearity, accuracy, precision, and stability. The limit of quantification was 3 ng/mL, and good linearity with a correlation coefficient > 0.99 was obtained at the NMBA concentration of 3-45 ng/mL. In addition, there was a range of 89.9% to 115.7% in the NMBA recoveries in four sartan compounds. The relative standard deviation values within and between days were both less than 5.0%. In conclusion, the liquid chromatography–tandem mass spectrometry method for NMBA measurement that was developed demonstrated good sensitivity, high accuracy, and precision. These qualities will be very helpful for the quantitative analysis of NMBA in sartan products. [14]

2. MATERIALS AND METHOD

SAMPLE DETAILS

Name: Sertraline HCL

REAGENTS & MATERIALS

Formic acid

Make: Rankem

Grade: AR

Batch No./Lot No.: R133F23

Ammonium hydrogen carbonate

Make: Lobachemie

Grade: AR

Batch No./Lot No.: L389282106

Methanol

Make: Duksan

Grade: LCMS

Water

Make: In-house

Grade: HPLC

Preparation of Solutions and Reagents

Mobile Phase Preparation

Mobile phase-A (0.5% Formic acid in water v/v)

Add 5ml of Formic acid into a 1000mL reagent bottle containing 1000mL ultra-pure water.

Mix well and sonicate.

Mobile Phase-B (0.5% Formic acid in Methanol v/v)

Add 5ml of Formic acid into a 1000mL reagent bottle containing 1000mL methanol.

Mix well and sonicate.

Diluent Solution: (Methanol: 50mM Ammonium Hydrogen Carbonate; 80:20% v/v)

50mM Ammonium Hydrogen Carbonate Solution:

Weigh about 2.0gm of Ammonium Hydrogen Carbonate into a 500mL reagent bottle containing 500mL water.

Mix well and sonicate.

Transfer 800 mL of methanol & 200 ml of 50mM Ammonium Hydrogen Carbonate Solution into a 1000ml reagent bottle using a measuring cylinder.

Mix well and sonicate.

Auto sampler Rinsing Solution (Methanol: water; 50:50 v/v)

Transfer 500mL of Methanol & 500ml of water into an appropriate reagent bottle using a measuring cylinder.

Mix well and sonicate.

Preparation of Standard & Sample Solution

Standard Stock Solution-1 (NDBA)

Accurately weigh about 5.0 mg of working standard into a 10 ml volumetric flask.

Dissolve and dilute to volume with methanol and mix.

Transfer 0.130 ml of the standard stock solution into a 100 ml volumetric flask & dilute up to the mark with diluent and mix.

Concentration: NDBA: 0.65 ppm

Acceptance Criteria

The Relative Standard Deviation (RSD) of the response area of NDBA & N-Nitroso of N-Dimethyl Erythromycin A obtained from six replicate injections of Standard Solution should not be more than 15.0 %.

The Signal to Noise ratio at the Sensitivity/LOD level must be greater than or equal to 3:1.

Linearity and Range

Standard Stock Solution-1 (NDBA)

Accurately weigh 5.008 mg of working standard into a 10 ml volumetric flask.

Dissolve and dilute to volume with methanol and mix.

Transfer 0.130 ml of standard stock solution into a 100 ml volumetric flask and dilute up to the mark with diluent and mix.

Standard Stock Solution-2 (N-Nitroso of N-Dimethyl Erythromycin A)

Accurately weigh 5.047 mg of working standard into a 10 ml volumetric flask.

Dissolve and dilute to volume with methanol and mix.

Transfer 0.75 ml of standard stock solution into a 10 ml volumetric flask and dilute up to the mark with diluent and mix.

Acceptance Criteria

Relative Standard Deviation (RSD) of the response area of (NDBA & Nitroso of N-Dimethyl Erythromycin A) obtained from six replicate injections of Standard Solution should not exceed 15.0%.

The squared correlation coefficient (r^2) should not be less than 0.990.

The signal to Noise ratio at the LOD level must be greater than or equal to 3:1.

Precision at Different Levels

Standard Stock Solution-1 (NDBA)

Accurately weigh 5.118 mg of working standard into a 10 ml volumetric flask.

Dissolve and dilute to volume with methanol and mix.

Transfer 0.130 ml of standard stock solution into a 100 ml volumetric flask and dilute up to the mark with diluent and mix.

Standard Stock Solution-2 (N-Nitroso of N-Dimethyl Erythromycin A)

Accurately weigh 4.962 mg of working standard into a 10 ml volumetric flask.

Dissolve and dilute to volume with methanol and mix.

Transfer 0.75 ml of standard stock solution into a 10 ml volumetric flask and dilute up to the mark with diluent and mix.

Acceptance Criteria

RSD of response area of NDBA & N-Nitroso of N-Dimethyl Erythromycin A obtained from six replicate injections of Standard Solution should not exceed 15.0%.

Signal to Noise ratio at LOD level must be greater than or equal to 3:1.

System Precision

Preparation of Standard Solution

Preparation of Standard Solution, Sensitivity solution/LOD Solution details refer Method Precision Experiment.

Acceptance Criteria

The Relative Standard Deviation (RSD) of the response area of N-Nitrosodibutylamine (NDBA) & N-Nitroso of N-Dimethyl Erythromycin A obtained from six replicate injections of Standard Solution should not be more than 15.0%.

The Signal to Noise ratio at the LOD level must be greater than or equal to 3:1.

Method Precision

Preparation of Standard and Sample Solutions

Standard Stock Solution-1 (NDBA)

Accurately weighed 5.018 mg of working standard into a 10 ml volumetric flask. Dissolved and diluted to volume with methanol and mixed. Transferred 0.130 ml of standard stock solution into a 100 ml volumetric flask & diluted up to the mark with diluent and mixed.

Standard Stock Solution-2 (N-Nitroso of N-Dimethyl Erythromycin A)

Accurately weighed 5.044 mg of working standard into a 10 ml volumetric flask. Dissolved and diluted to volume with methanol and mixed. Transferred 0.75 ml of standard stock solution into a 10 ml volumetric flask & diluted up to the mark with diluent and mixed.

Standard Stock Solution-3:

Transferred 0.5 ml each of standard stock solution-1 & standard stock solution-2 into a 20 ml volumetric flask & diluted up to the mark with diluent and mixed.

Sensitivity/LOD Solution:

Transferred 1 ml of standard stock solution-3 into a 100 ml volumetric flask & diluted up to the mark with diluent and mixed.

Standard Solution at 100%

Transferred 10.0 mL of Standard Stock Solution-3 into a 50 mL volumetric flask and diluted to volume and mixed.

3. RESULTS

Table 1 Results of Standard Solution and S/N of LOD Solution

Sr. No.	Area Response (N-Nitrosodibutylamine)	Area Response (N-Nitroso of N-Dimethyl Erythromycin A)
1	183190	211823
2	178179	203553
3	163531	190100
4	173612	195132
5	171197	189986
6	169762	196895
Average	173245.2	197914.8
%RSD	3.95	4.28
LOD Solution S/N	9	261

Table 1 displays the area response of N-Nitrosodibutylamine and N-Nitroso of N-Dimethyl Erythromycin A for six standard solutions, along with their average and %RSD. Additionally, it includes the signal-to-noise ratio (S/N) of the Limit of Detection (LOD) solution, indicating its sensitivity.

Table 2 Results of Determination of LOD_LOQ

Sr. No.	Sample Details	N-Nitrosodibutylamine		N-Nitroso of N-Dimethyl Erythromycin A	
		Area Response	S/N	Area Response	S/N
1.	Standard Solution – 5%	11563	48	8221	750
2.	Standard Solution – 10%	17939	23	16590	903
3.	Standard Solution – 15%	24138	69	23882	1923

Table 2 presents the results of determining the Limit of Detection (LOD) and Limit of Quantitation (LOQ) for N-Nitrosodibutylamine and N-Nitroso of N-Dimethyl Erythromycin A. It includes the area response and signal-to-noise ratio (S/N) for each sample at different concentration levels (5%, 10%, and 15%) of the standard solutions.

Table 3 Results of Linearity

N-Nitrosodibutylamine							
Sr. No.	**LINEARITY LEVEL**						
	LOQ (10%)	**50%**	**75%**	**100%**	**125%**	**150%**	**200%**
	Concentration (ppm)						
	0.0003252	**0.0016258**	**0.0024387**	**0.0032516**	**0.0040645**	**0.0048774**	**0.0065032**
	Area Response						
1	12780	62539	92063	119353	142318	177351	236290
2	13262	62670	94356	110184	143559	176916	239942
Average	13021.0	62604.5	93209.5	114768.5	142938.5	177133.5	238116.0
			Slope			35899094.2695	
			Intercept			1858.6643	
			Y-Intercept			1.6195	
			Correlation Coefficient (r)			0.9990	

Coefficient of determination (r^2)	0.9980
Residual sum of squares	66201712.7189

Table-3 illustrates the linearity results for N-Nitrosodibutylamine, displaying the concentration levels ranging from the Limit of Quantitation (LOQ) to 200%. The corresponding area responses are recorded alongside the calculated slope, intercept, correlation coefficient (r), coefficient of determination (r^2), and residual sum of squares, demonstrating a highly linear relationship with a coefficient of determination (r^2) of 0.9980

Table-4 Results of Precision at different levels.

Sr. No.	**Area Response (N-Nitrosodibutylamine)**		**Area Response (N-Nitroso of N-Dimethyl Erythromycin A)**	
	100 %	**150 %**	**100 %**	**150 %**
1.	177963	264843	271092	428223
2.	181299	268773	278830	450626
3.	177033	270928	284910	468084
4.	176589	261800	271598	463011
5.	175054	269559	274556	448253
6.	180903	267093	274757	465274
Average	178140.2	297166.0	275957.2	453911.8
%RSD	1.39	1.26	1.88	3.29

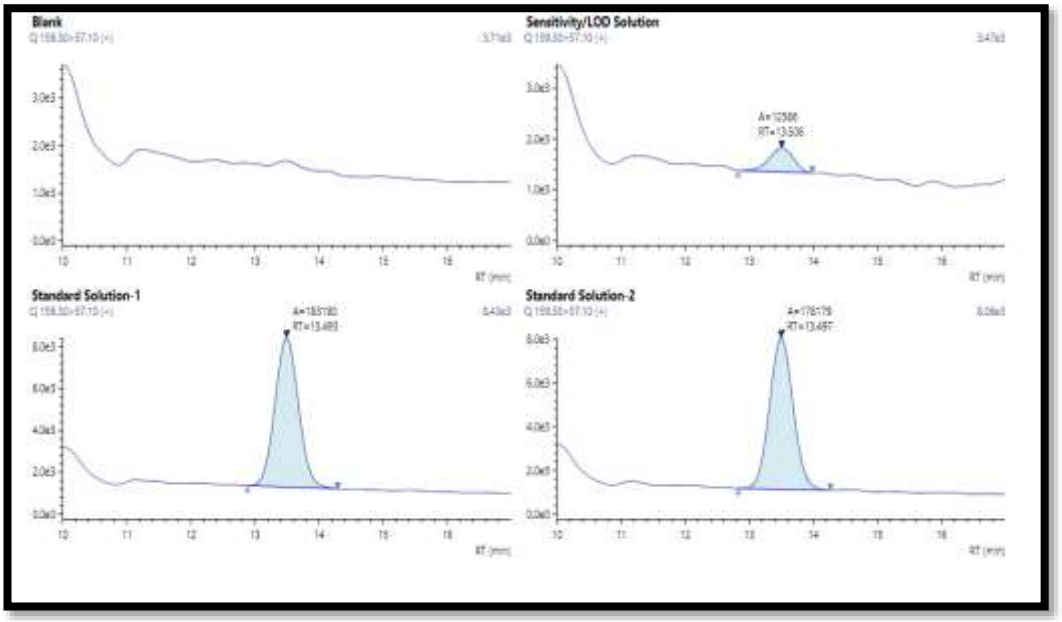
Table-4 presents the precision results at different levels for both N-Nitrosodibutylamine and N-Nitroso of N-Dimethyl Erythromycin A. The data includes area responses at 100% and 150% levels, showing average responses and percentage relative standard deviation (%RSD) for each compound. The %RSD values indicate good precision, with values ranging from 1.26% to 3.29%.

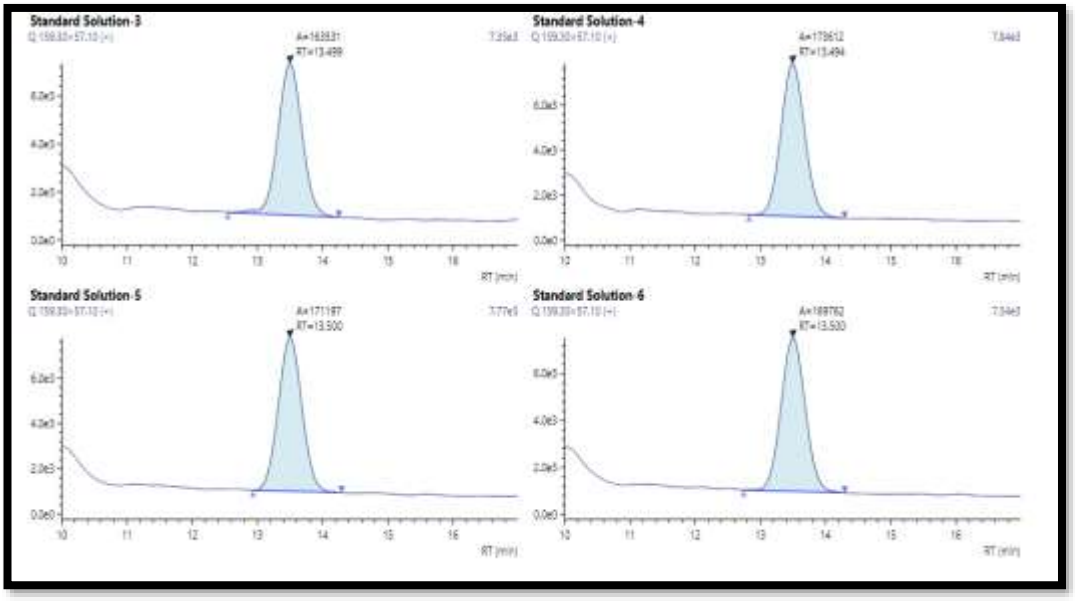
Table 5 Results of Accuracy (Recovery)

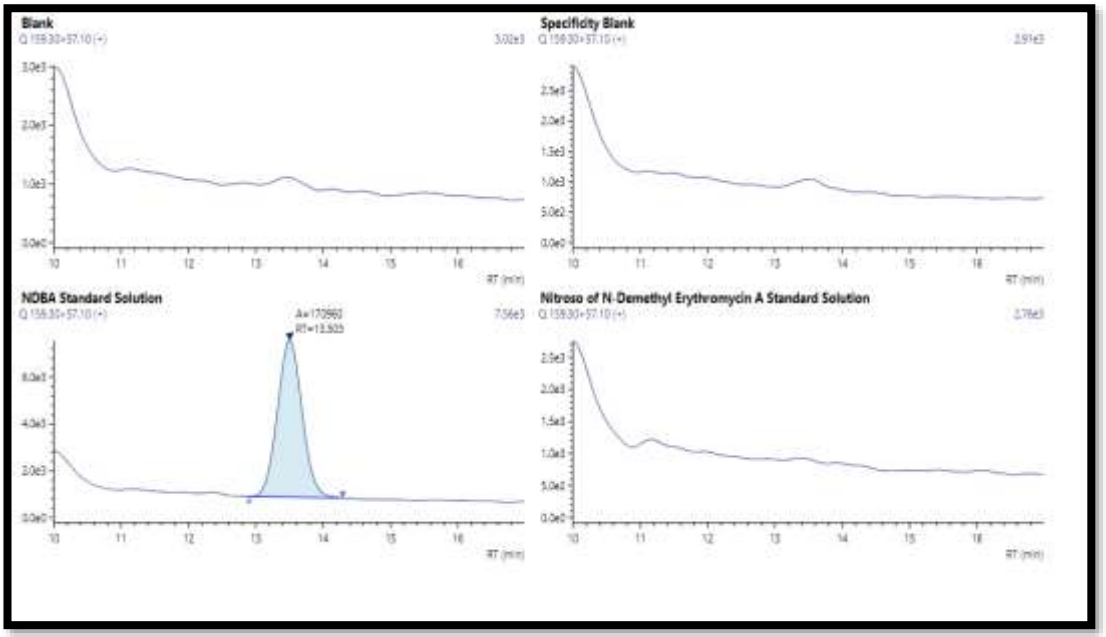
N-Nitrosodibutylamine					
Concentration Level	**Amount added (w.r.t test conc. in ppm)**	**Corrected Amount Recovered (w.r.t test conc. in ppm)**	**% Recovery**	**% Mean Recovery**	**%RSD**
LOQ	0.01298	0.01	94.99	94.34	3.90
	0.01295	0.01	90.38		
	0.01297	0.01	97.65		
50%	0.06485	0.06	88.33	91.33	2.98
	0.06506	0.06	92.04		
	0.06496	0.06	93.63		
100%	0.12986	0.12	91.59	90.52	2.75
	0.12975	0.11	87.67		
	0.13017	0.12	92.30		
150%	0.19460	0.17	88.02	89.06	1.16
	0.19468	0.17	89.08		
	0.19482	0.18	90.09		

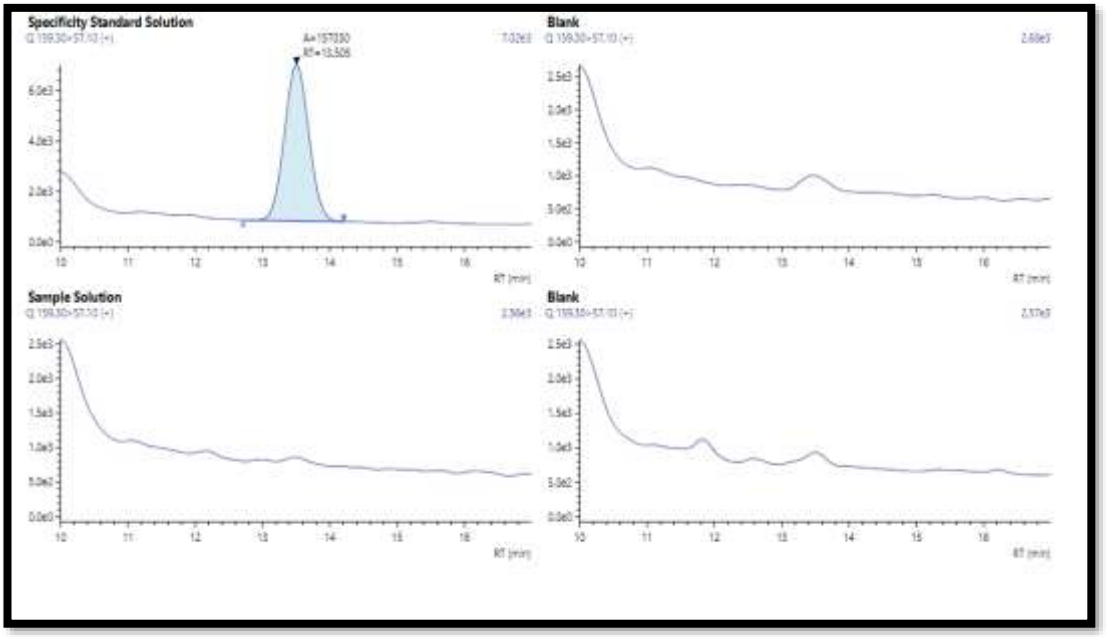
Table 5 displays the accuracy results (recovery) for N-Nitrosodibutylamine at different concentration levels. It includes the amount added and the corrected amount recovered, both in parts per million (ppm), along with the percentage recovery. The % Mean Recovery and %RSD values indicate the overall recovery performance and its variability, showing satisfactory mean recoveries ranging from 89.06% to 94.34%, with %RSD values generally below 4%.

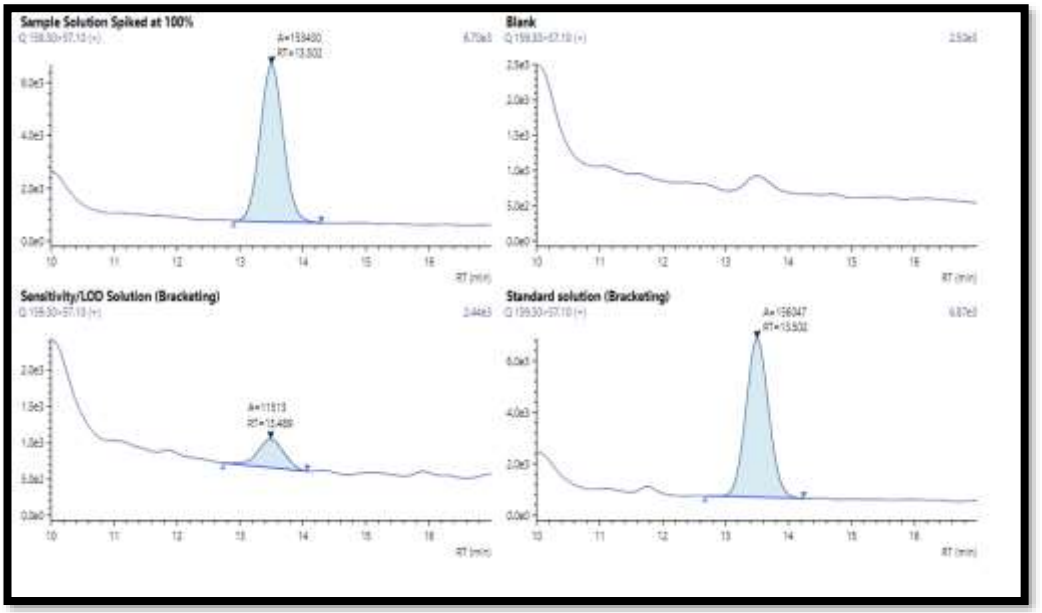
Compound: NDBA

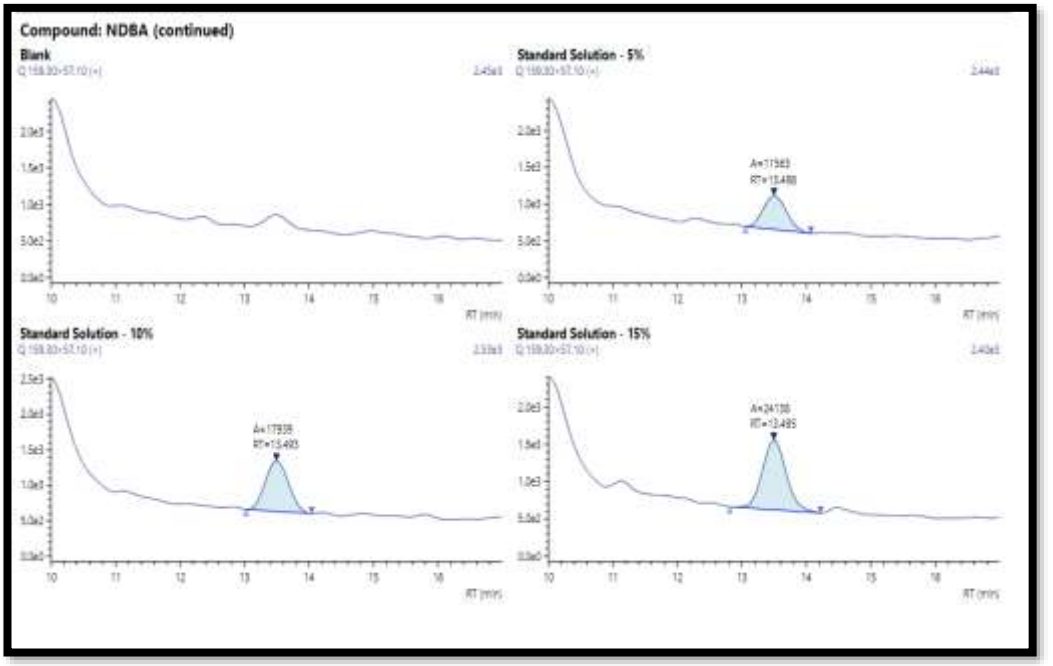

Blank
Sensitivity/LOD Solution
Standard Solution-1
Standard Solution-2


Standard Solution-3
Standard Solution-4
Standard Solution-5
Standard Solution-6

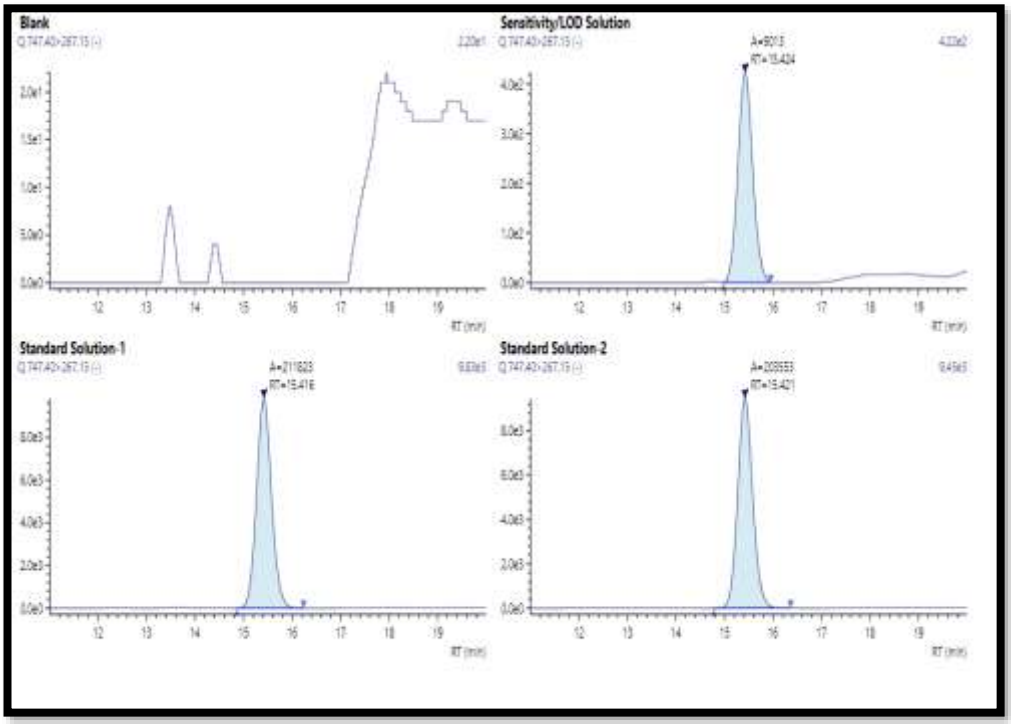

Blank
Specificity Blank
NDBA Standard Solution
Nitroso of N-Demethyl Erythromycin A Standard Solution
RT (min)

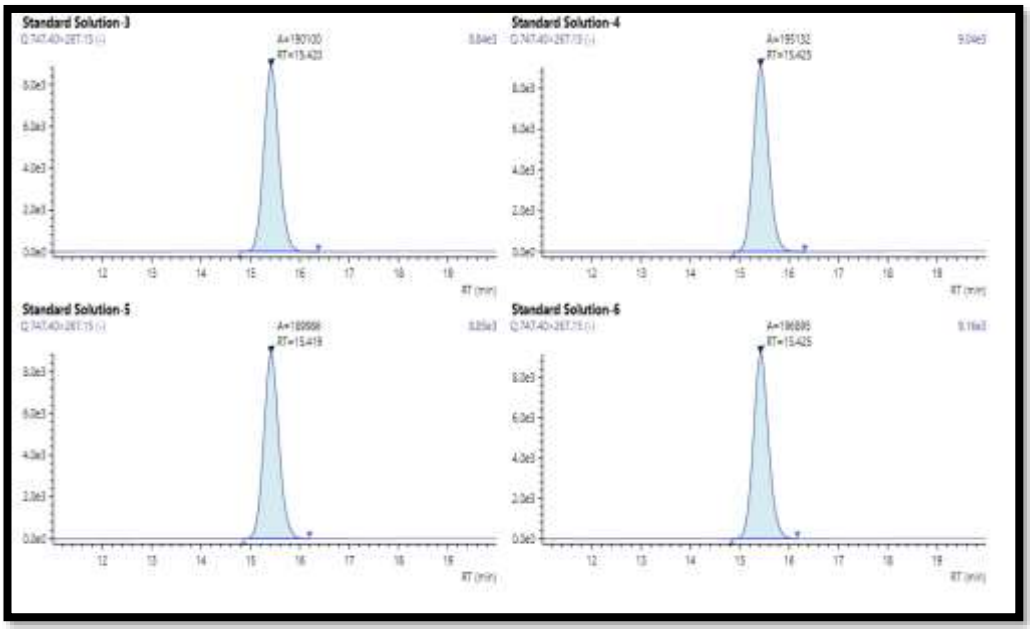

Specificity Standard Solution
Blank
Sample Solution
Blank
RT (min)

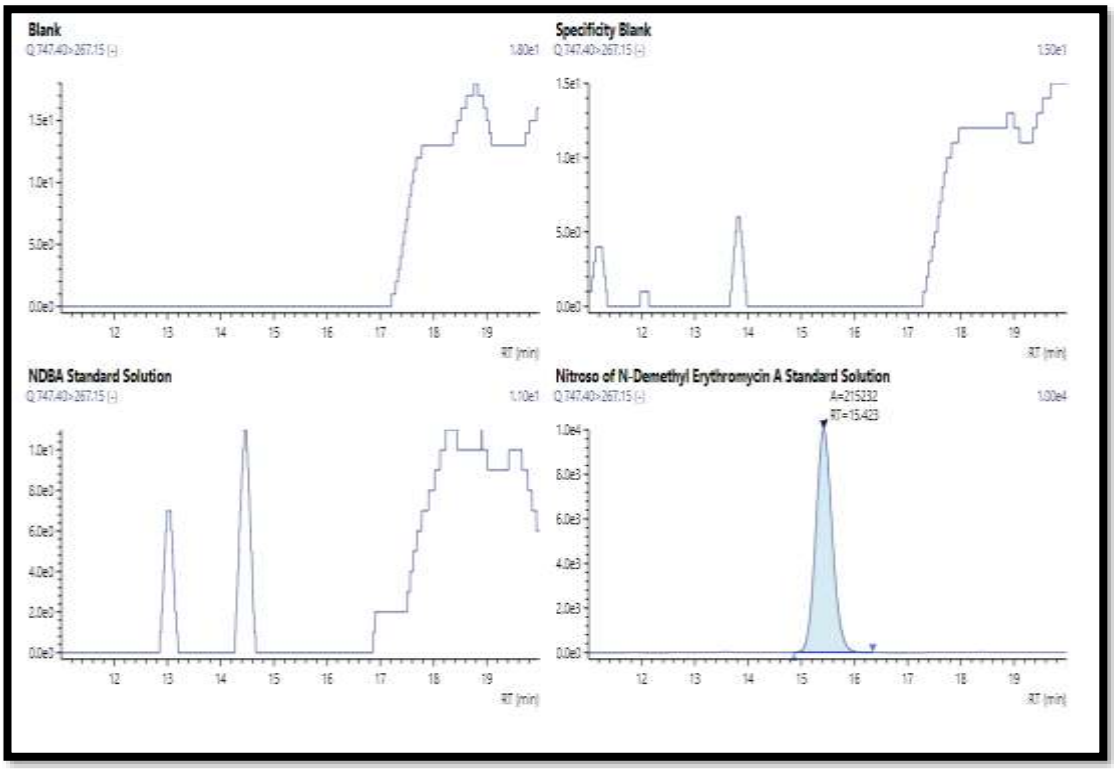

Sample Solution Spiked at 100%
Blank
Sensitivity/LOD Solution (Bracketing)
Standard solution (Bracketing)
RT (min)

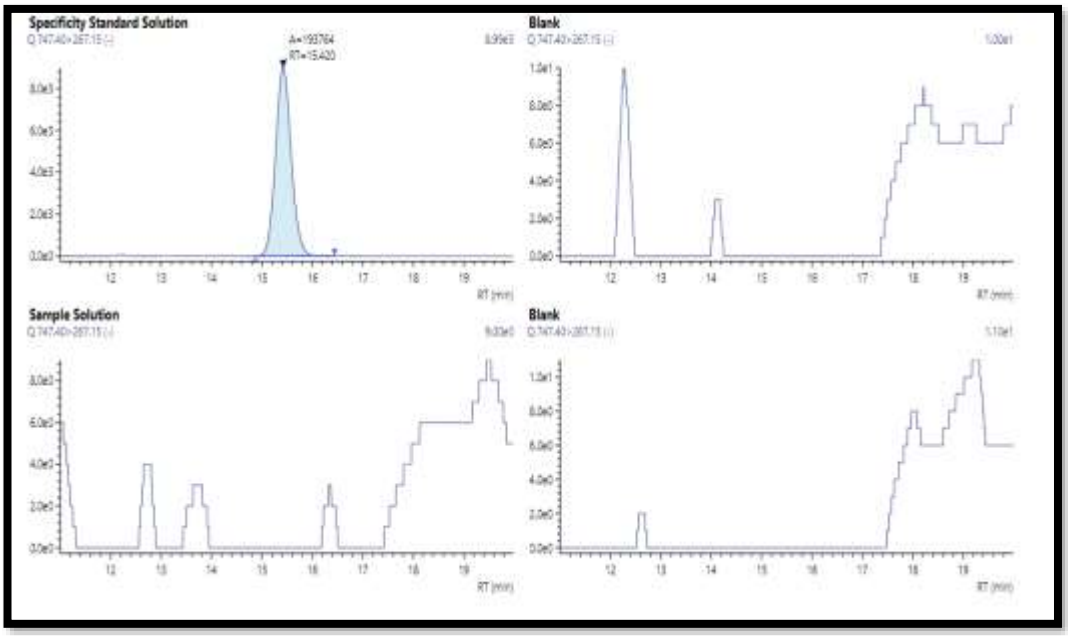

Compound: NDBA (continued)
Blank
Standard Solution - 5%
Standard Solution - 10%
Standard Solution - 15%
RT (min)

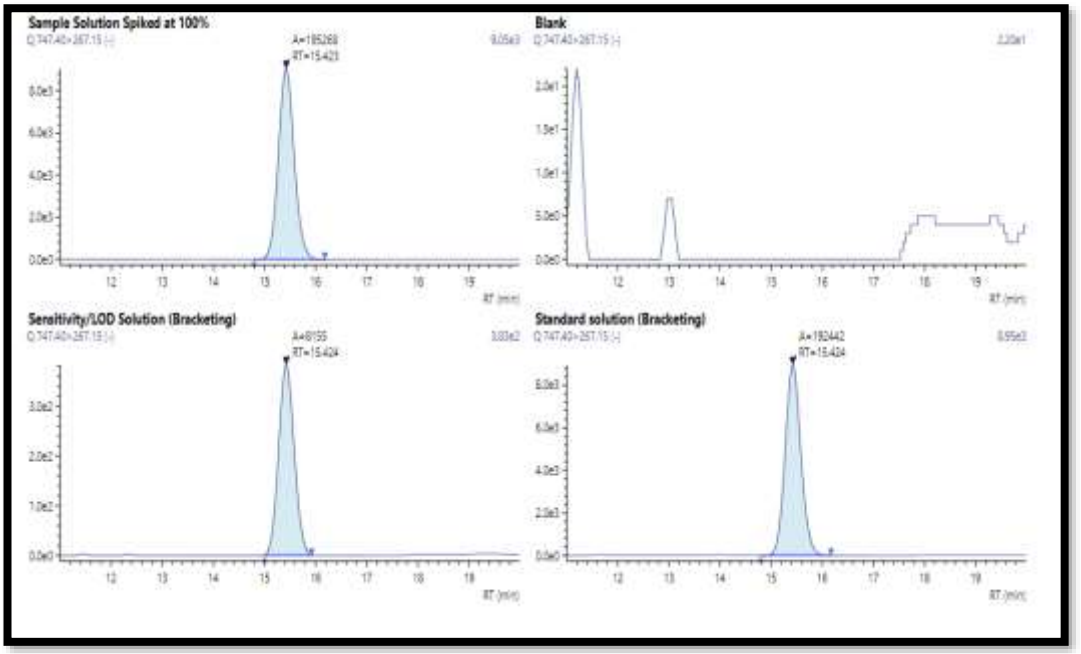
Compound: Nitroso of N-Demethyl Erythromycin A

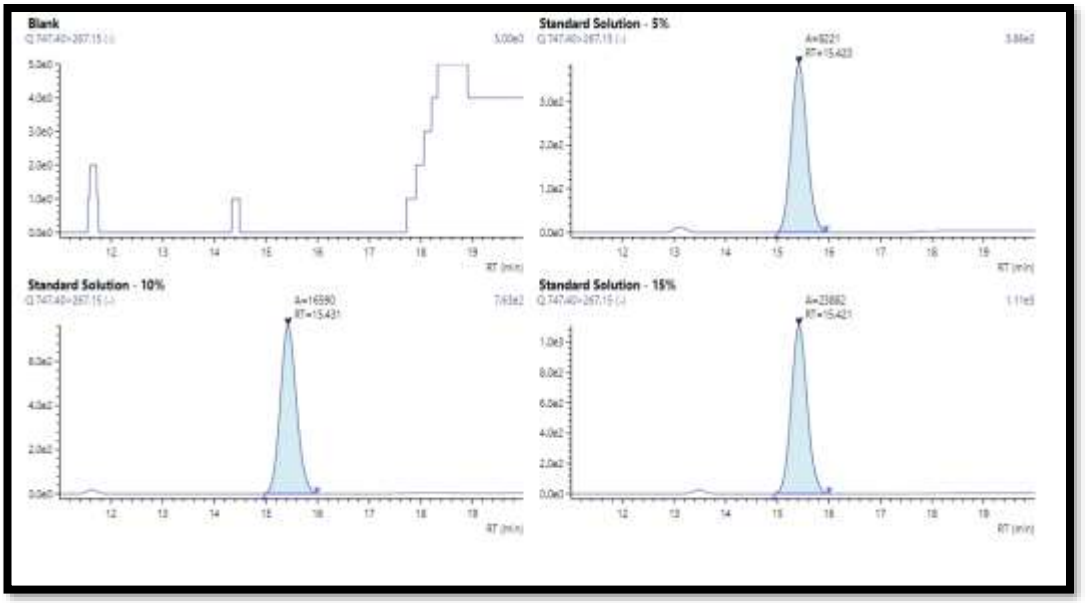





Blank
Specificity Blank
NDBA Standard Solution
Nitroso of N-Demethyl Erythromycin A Standard Solution
A=215232
RT=15.423
RT (min)


Specificity Standard Solution
A=193764
RT=15.420
Blank
Sample Solution
Blank
RT (min)


Sample Solution Spiked at 100%
Blank
Sensitivity/LOD Solution (Bracketing)
Standard solution (Bracketing)


Blank
Standard Solution - 5%
Standard Solution - 10%
Standard Solution - 15%

4. DISCUSSIONS

The analytical results presented across five tables offer a thorough assessment of the performance characteristics for N-Nitrosodibutylamine and N-Nitroso of N-Dimethyl Erythromycin A. [15]Table-1 outlines the standard solution's area responses and the signal-to-noise ratio (S/N) of the Limit of Detection (LOD) solution, setting the groundwork for subsequent analyses. Table 2 delves into the determination of the LOD and Limit of Quantitation (LOQ) across different concentration levels, providing crucial insights into sensitivity thresholds. [16]The linearity of N-Nitrosodibutylamine is meticulously evaluated in Table 3, showcasing a highly linear relationship across various concentration levels. Table 4 elucidates precision levels at different concentrations, demonstrating consistently good precision for both compounds. [17]Finally, Table 5 meticulously examines the accuracy (recovery) of N-Nitrosodibutylamine, revealing satisfactory mean recoveries across diverse concentration levels. Together, these tables furnish a comprehensive understanding of the analytical performance of the studied compounds, which is vital for method validation and quality assurance in analytical chemistry.

5. CONCLUSIONS

The comprehensive analysis presented in the five tables provides valuable insights into the analytical performance of N-Nitrosodibutylamine and N-Nitroso of N-Dimethyl Erythromycin A. The determination of standard solution responses, sensitivity limits, linearity, precision at various levels, and accuracy/recovery rates collectively underscore the robustness and reliability of the analytical method employed. [18]The method demonstrates adequate sensitivity, linearity, precision, and accuracy across a range of concentrations, indicating its suitability for quantitative analysis of the target compounds. These findings affirm the method's validity and efficacy, making it a dependable tool for quality control and regulatory compliance in relevant analytical applications. Continuing the assessment, the observed %RSD values for precision at different levels indicate good reproducibility and consistency in the measurements, bolstering confidence in the method's reliability. Additionally, the high correlation coefficient (r) and coefficient of determination (r^2) obtained from the linearity study signify a strong linear relationship between concentration and response, further validating the method's accuracy and suitability for quantitative analysis. Moreover, the satisfactory recovery rates obtained at various concentration levels demonstrate the method's ability to accurately quantify N-Nitrosodibutylamine and N-Nitroso of N-Dimethyl Erythromycin A in complex matrices, enhancing its applicability in real-world sample analysis scenarios. These combined findings highlight the method's robustness, precision, and accuracy, making it a valuable tool for quality assurance and regulatory compliance in industries where these compounds are of concern.

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