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A Robust LC-MS/MS Method for Sensitive and Accurate Determination of *N*-Nitroso Fluoxetine in Fluoxetine Oral Solution

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Abstract:

The determination of nitrosamine impurities in pharmaceuticals is critical due to their genotoxic and carcinogenic potential. *N*-Nitroso Fluoxetine is a potential nitrosamine impurity that may form during drug-substance storage and drug-product manufacturing, necessitating accurate monitoring in Fluoxetine formulations. A highly sensitive and selective LC-MS/MS method was developed and fully validated for the quantification of *N*-Nitroso Fluoxetine in Fluoxetine Oral Solution USP, 20 mg/5 mL, in accordance with regulatory guidelines. Chromatographic separation was achieved using a Gemini 3 μ m NX-C18 column (4.6 $\times$ 150 mm) with a gradient elution program employing 0.1% formic acid in water (Phase A) and 0.1% formic acid in acetonitrile (Phase B) at a flow rate of 0.5 mL/min. The method demonstrated excellent linearity over the concentration range Limit of Quantification (LOQ) 200% of the specification (0.398–2.654 ppm, $r^2 = 0.999$), high sensitivity (Limit of Detection (LOD) = 0.152 ppm, LOQ = 0.398 ppm), and robust accuracy (recoveries 107–110%) and precision (%RSD $\leq$ 3.3%). Specificity, solution stability, and filter compatibility were confirmed, with no interference from diluent, placebo, or matrix components. The method was successfully applied to commercial Fluoxetine drug product, providing a reliable, reproducible, and regulatory-compliant tool for routine quality control and trace-level nitrosamine monitoring.

Keywords: LC-MS/MS, N-Nitroso Fluoxetine, Fluoxetine, Placebo, Nitrosamine

1. Introduction:

Nitrosamine formation in drug substances and drug products can occur during manufacturing, formulation, or storage, particularly in the presence of nitrite sources and favorable environmental conditions [1]. In recent years, nitrosamine impurities have received considerable attention in the pharmaceutical industry because contamination of medicinal products with nitrosamines remains a major global public health concern [2]. Nitrosamine impurities are considered highly concerning due to their genotoxic and carcinogenic potential, even at trace levels [3]. Among nitrosamine impurities, nitrosamine drug substance-related impurities (NDSRIs) have emerged as a particularly critical concern in the pharmaceutical field [4]. According to regulatory guidance, several drug products have recently been reported to contain NDSRIs, highlighting the importance of controlling these impurities in both drug substances and finished drug products [5]. Fluoxetine, a widely prescribed antidepressant belonging to the class of selective serotonin reuptake inhibitors (SSRIs), is susceptible to the formation of N-nitroso fluoxetine [6]. Therefore, the development and validation of sensitive and selective analytical methodologies suitable for routine quality control and regulatory compliance have become essential for the quantification of nitrosamines [7,8].

Recent advances in LC–MS/MS-based analytical techniques have significantly improved the detection and quantification of nitrosamine impurities in pharmaceutical products [9,10]. In response to the growing concern regarding nitrosamine contamination, regulatory agencies and pharmacopeial bodies have established stringent expectations and guidance documents for nitrosamine control in pharmaceutical products [11–16]. In parallel, several analytical and validation studies have demonstrated the applicability of LC–MS/MS methods for trace-level determination of nitrosamines and related genotoxic impurities [17–20]. Furthermore, international organizations and health authorities continue to emphasize the importance of risk

assessment, analytical control, and mitigation strategies for nitrosamine impurities in medicines [21–24].

Analytical method validation in accordance with ICH Q2 (R2) and other international quality standards is essential to ensure reliability, reproducibility, sensitivity, and robustness of analytical procedures used for nitrosamine determination [16,25]. Recent studies have also investigated nitrosamine formation pathways, selective detection of NDSRIs, and analytical strategies for pharmaceutical formulations [26,27]. In addition, regulatory agencies such as the Therapeutic Goods Administration (TGA) and other international authorities have reinforced expectations for nitrosamine monitoring and risk management in medicinal products [28]. Advanced analytical technologies and highly sensitive LC–MS/MS approaches have further enhanced pharmaceutical safety evaluation and trace impurity profiling [29,30].

Nitrosamine impurities are considered potentially carcinogenic and pose significant toxicological risks to patient safety [31,32]. Therefore, robust LC–MS/MS methods are increasingly being developed and validated for the determination of nitrosamines and other genotoxic impurities in pharmaceutical products [33]. Recent investigations have also highlighted the occurrence of nitrosamine impurities in SSRI drug products and the associated analytical and regulatory challenges [34]. Moreover, continuous developments in analytical method validation, chromatographic techniques, and nitrosamine monitoring approaches have improved the reliability and sensitivity of impurity profiling methods [35,36]. Quality assurance standards and comprehensive reviews further support the implementation of validated analytical procedures for nitrosamine analysis in pharmaceutical laboratories [37,38]. In the present study, a sensitive, selective, and robust LC–MS/MS method was developed and validated for the quantification of N-nitroso fluoxetine in Fluoxetine Oral Solution USP. To the best of our knowledge, there are no literature reports describing the development and validation of a highly sensitive LC–MS/MS method specifically for the determination of N-

nitroso fluoxetine in Fluoxetine Oral Solution. Therefore, this study aims to address this gap by establishing a reliable analytical method capable of quantifying trace levels of N-nitroso fluoxetine with high accuracy and precision.

2. Result and Discussion:

2.1. Chemicals and Reagents:

All solvents, including methanol, acetonitrile, water, and formic acid (LC–MS grade), were purchased from Sigma-Aldrich, Honeywell, and Fisher Scientific. The reference standard of N-nitroso fluoxetine was procured from Cayman Chemical. All reagents were used as received without further purification. Fluoxetine Oral Solution USP (20 mg/5 mL) and placebo were supplied by ANI Pharmaceuticals and used as received. All glassware and analytical apparatus were thoroughly cleaned and dried prior to use to prevent contamination. LC–MS/MS analysis was performed using a Shimadzu LCMS-8060RX system.

2.2. Chromatographic and Mass Spectrometric Conditions

Chromatographic separation of N-nitroso fluoxetine was achieved using a Gemini 3 μm NX-C18 column (4.6 $\times$ 150 mm; Phenomenex). The column oven temperature was maintained at 35°C, while the autosampler was kept at 5°C to ensure sample stability. The mobile phase consisted of 0.1% (v/v) formic acid in water as Mobile Phase A and 0.1% (v/v) formic acid in acetonitrile as Mobile Phase B. A gradient elution program was employed at a flow rate of 0.5 mL/min with a total run time of 20 minutes. The initial composition of 55% Mobile Phase A and 45% Mobile Phase B was held until 3.0 minutes, followed by a linear change to 10% Mobile Phase A and 90% Mobile Phase B at 12.0 minutes. This composition was maintained until 16.0 minutes, after which the system was returned to initial conditions at 16.2 minutes and equilibrated until 20.0 minutes. The injection volume was 20 μL . Under these optimized conditions, the retention time of N-nitroso fluoxetine was approximately 13.2 minutes.

A divert valve program was applied to direct the column effluent to the mass spectrometer only during the analyte elution window. Mass spectrometric data acquisition was initiated at 12.80 minutes and terminated at 16.00 minutes, thereby minimizing contamination and improving method robustness. System suitability was evaluated by injecting six replicate injections of the working standard solution. The %RSD of the ion count (peak area) for N-nitroso fluoxetine was required to be not more than (NMT) 15.0%. The signal-to-noise ratio obtained from the sensitivity solution was required to be NLT 10. Additionally, the %RSD from six replicate injections, including bracketing standards, was required to meet the acceptance criterion of NMT 15.0%.

Detection was performed using a triple quadrupole mass spectrometer equipped with an electrospray ionization (ESI) source operating in positive ion mode. Quantification was carried out using multiple reaction monitoring (MRM) with a precursor ion at m/z 339.10 and a product ion at m/z 177.25. The dwell time was set at 100 ms, with Q1 pre-bias of -18.0 V, collision energy of -10.0 V, and Q3 pre-bias of -34.0 V. The optimized source parameters included an interface temperature of 250°C , interface voltage of 4.00 kV, desolvation line temperature of 220°C , and heat block temperature of 350°C . The nebulizing gas flow was maintained at 5 L/min, while both drying gas and heating gas flows were set at 10 L/min. These optimized chromatographic and mass spectrometric conditions provided adequate sensitivity, selectivity, and reproducibility for the quantification of N-nitroso fluoxetine in pharmaceutical samples.

2.3. Preparation of Solutions

2.3.1. Preparation of Mobile Phase A

Formic acid (1.0 mL) was transferred into a 1000 mL volumetric flask and diluted to volume with water. The solution was mixed thoroughly and sonicated to degas.

2.3.2. Preparation of Mobile Phase B

Formic acid (1.0 mL) was transferred into a 1000 mL volumetric flask and diluted to volume with ACN. The solution was mixed thoroughly and sonicated to degas.

2.3.3. Preparation of Diluent

Methanol (900 mL) and water (100 mL) were transferred into a 1000 mL volumetric flask, mixed well, and sonicated to degas.

2.3.4. Preparation of Standard Solutions

2.3.4.1. Standard Stock Solution (Stock 1)

Approximately 2.8 mg of N-nitroso fluoxetine reference standard was accurately weighed and transferred into a polypropylene tube. Methanol (20 mL) was added, and the solution was vortex-mixed for 30 seconds to ensure complete dissolution. The resulting concentration was approximately 62,619 ppm with respect to the sample concentration.

2.3.4.2. Standard Stock Solution (Stock 2)

An aliquot of 0.2 mL of Stock 1 was transferred into a 20 mL volumetric flask and diluted to volume with diluent to obtain a concentration of approximately 626.19 ppm with respect to the sample concentration.

2.3.4.3. Standard Stock Solution (Stock 3)

An aliquot of 0.4 mL of Stock 2 was transferred into a 20 mL volumetric flask and diluted to volume with diluent to obtain a concentration of approximately 12.52 ppm with respect to the sample concentration.

2.3.4.4. Preparation of Working Standard Solution

An aliquot of 1.0 mL of Stock 3 was transferred into a 10 mL volumetric flask and diluted to volume with diluent. The solution was vortex-mixed for 30 seconds to obtain a final concentration of approximately 1.25 ppm with respect to the sample concentration.

2.3.5. Preparation of Sensitivity Solution

An aliquot of 3.0 mL of the working standard solution was transferred into a polypropylene tube, and 7.0 mL of diluent was added. The solution was vortex-mixed for 30 seconds to obtain a concentration of approximately 0.375 ppm with respect to the sample concentration.

2.3.6. Preparation of Placebo Solution

Approximately 5000 mg of placebo was accurately weighed and transferred into a 10 mL volumetric flask. Diluent (3.0 mL) was added, and the mixture was vortex-mixed for 30 seconds. The solution was diluted to volume with diluent and mixed thoroughly. The resulting solution was filtered through a 0.22 μ m nylon membrane filter, discarding the first 2 mL of filtrate.

2.3.7. Preparation of Sample Solution

Approximately 5000 mg of sample was accurately weighed and transferred into a 10 mL volumetric flask. Diluent (3.0 mL) was added, and the mixture was vortex-mixed for 30 seconds. The solution was diluted to volume with diluent and mixed thoroughly. The final solution was filtered through a 0.22 μ m nylon membrane filter, discarding the first 2 mL of filtrate. The resulting sample concentration was approximately 1789 μ g/mL of fluoxetine.

2.4. Method Validation

The analytical procedure for method validation was performed according to ICH guideline Q2(R2). Therefore, the method for the determination of N-Nitroso Fluoxetine in the Fluoxetine drug product was validated. The mass spectrum of N-Nitroso Fluoxetine is depicted in Fig. 1.

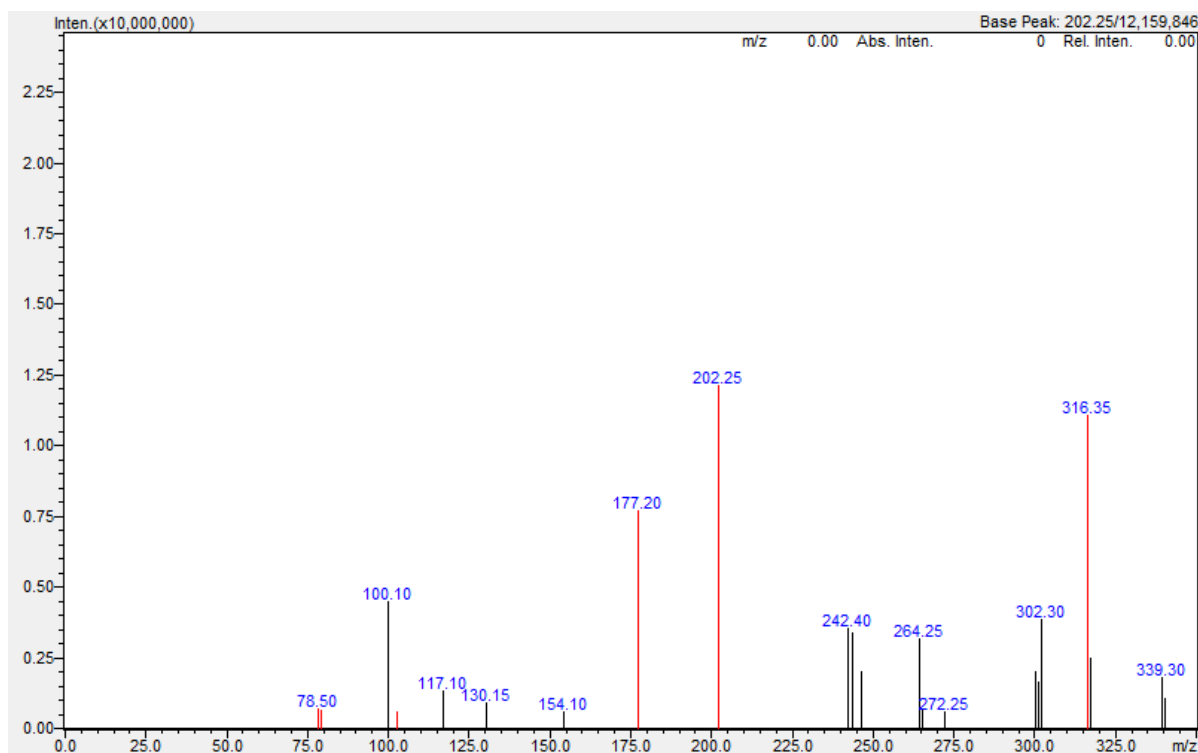


Fig. 1. Mass spectrum of N-Nitroso Fluoxetine.

2.4.1. System Precision

System precision and sensitivity were evaluated using replicate injections of the working standard solution and a sensitivity solution prepared according to the established method. System precision was assessed by injecting six replicate preparations of the working standard solution. The ion count (peak area) for N-nitroso fluoxetine was recorded for each injection, and the percentage relative standard deviation (%RSD) was calculated. The calculated %RSD was 2.5%. This value is well within the predefined acceptance criterion of NMT 15.0%, demonstrating excellent repeatability of detector response and stable instrument performance during analysis. The low variability confirms the reliability of the method for quantitative determination. Sensitivity of the system was evaluated by injecting the sensitivity solution and determining the signal-to-noise (S/N) ratio for the *N*-nitroso fluoxetine peak. The observed S/N ratio was 159, which significantly exceeds the minimum required limit of NLT10. This high S/N value demonstrates the superior

sensitivity of the developed LC-MS/MS method and confirms its capability to detect trace levels of N-nitroso fluoxetine with high confidence. The %RSD of peak area complied with the requirement of NMT 15.0%, and the S/N ratio satisfied the requirement of NLT 10. Therefore, the system was found to be precise, sensitive, and suitable for routine quantitative analysis of N-nitroso fluoxetine in pharmaceutical samples. The chromatogram of the sensitivity solution is shown in Fig. 2.

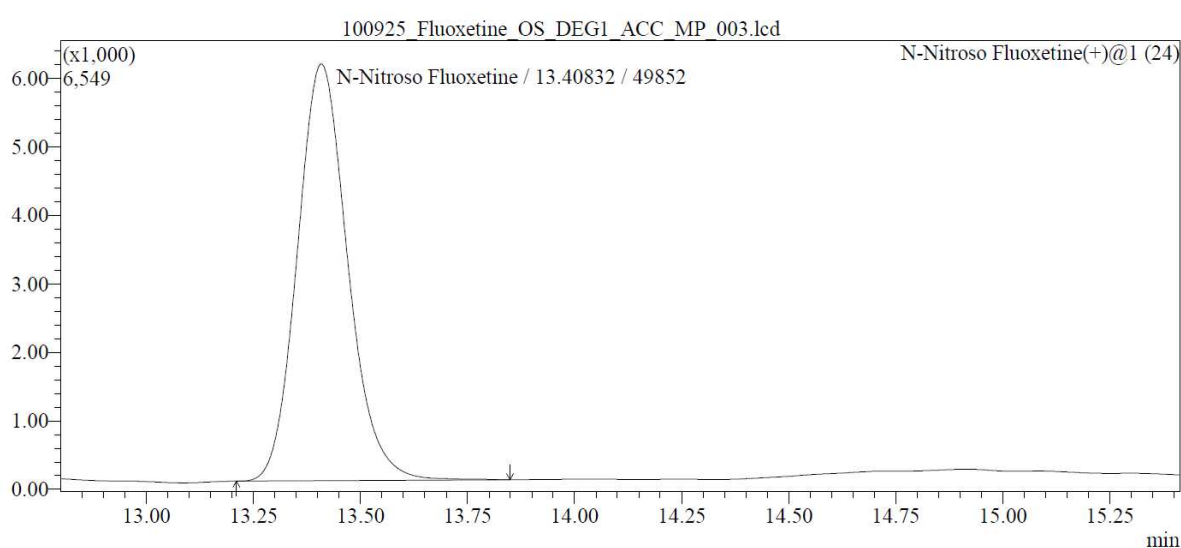


Fig. 2. Chromatogram of the sensitivity solution.

2.4.2. Specificity

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Specificity was evaluated to demonstrate the ability of the method to unequivocally assess N-nitroso fluoxetine in the presence of potential interferences, including diluent, placebo components, and sample matrix constituents. Blank (diluent), standard, placebo, sample, and spiked sample solutions were prepared according to the established analytical procedure and injected into the LC-MS/MS system. The chromatograms were examined to assess any potential interference at the retention time of N-nitroso fluoxetine. The acceptance criterion for specificity required that no interference be observed from the

blank or placebo at the retention time corresponding to the N-nitroso fluoxetine peak. The chromatographic results demonstrated that neither the blank nor the placebo solution exhibited any detectable peaks at the retention time of N-nitroso fluoxetine. Furthermore, the sample and spiked sample chromatograms confirmed clear and well-resolved detection of the analyte without co-eluting impurities or matrix interference. These findings confirm that the method is capable of selectively detecting and quantifying N-nitroso fluoxetine in the presence of formulation excipients and other potential impurities. Based on these results, the method meets the predefined acceptance criteria for specificity and is considered specific for the determination of N-nitroso fluoxetine in pharmaceutical samples. The chromatogram of the blank, placebo, standard solution, sample and spike sample are depicted in Fig. 3-7.

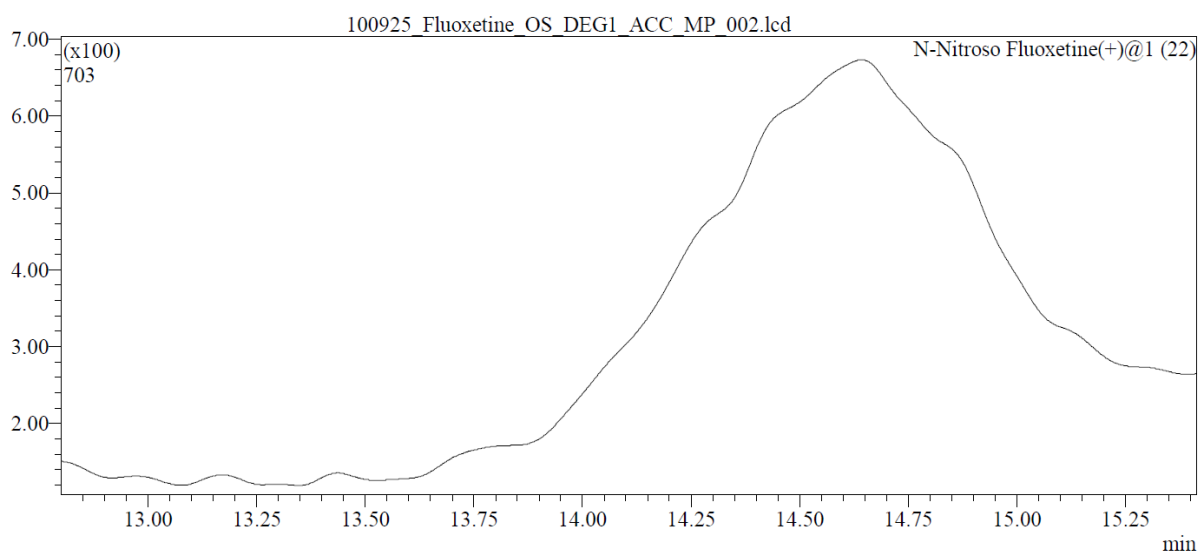


Fig. 3. Chromatogram of the blank solution.

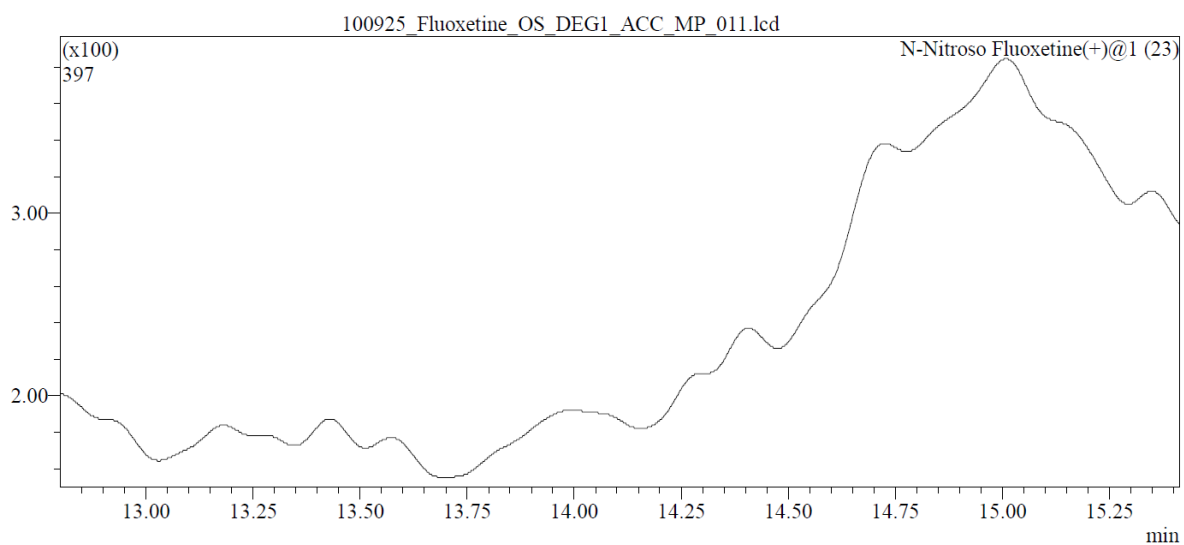


Fig. 4. Chromatogram of the placebo solution.

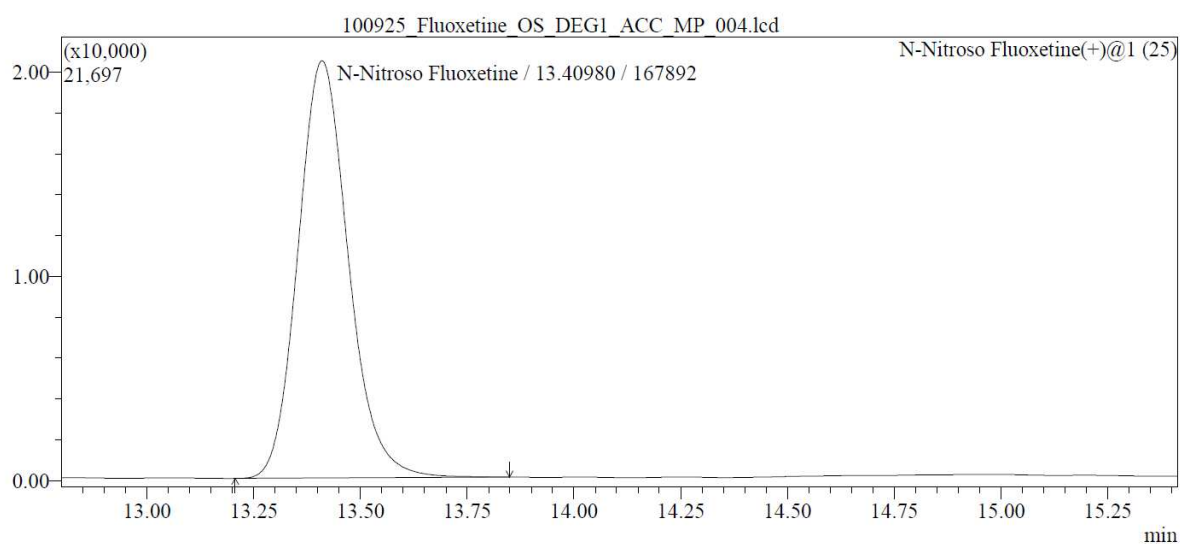


Fig. 5. Chromatogram of the N-Nitroso Fluoxetine standard solution.

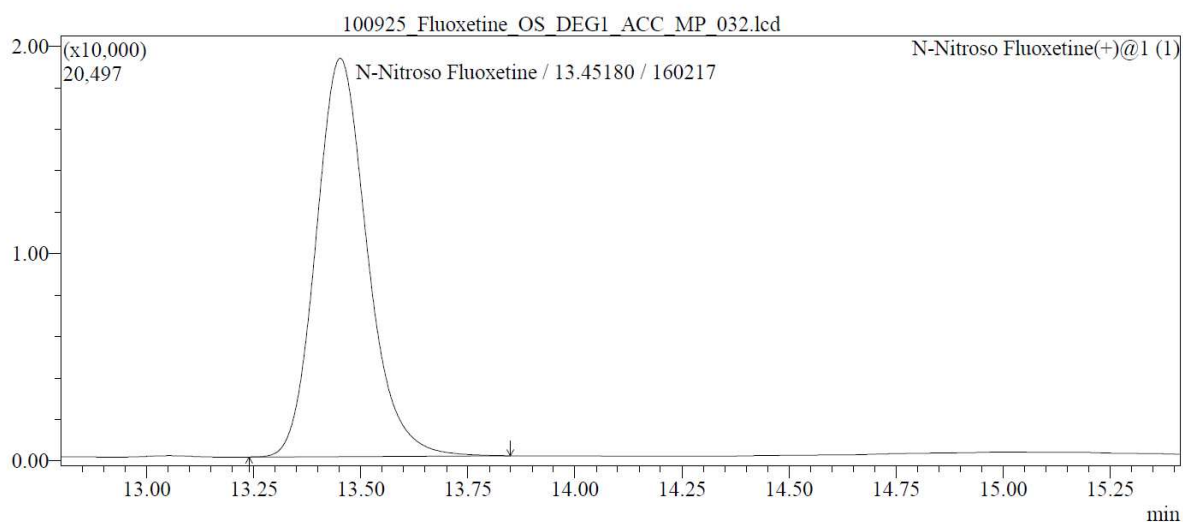


Fig. 6. Chromatogram of Fluoxetine Oral Solution USP (20 mg/5 mL).

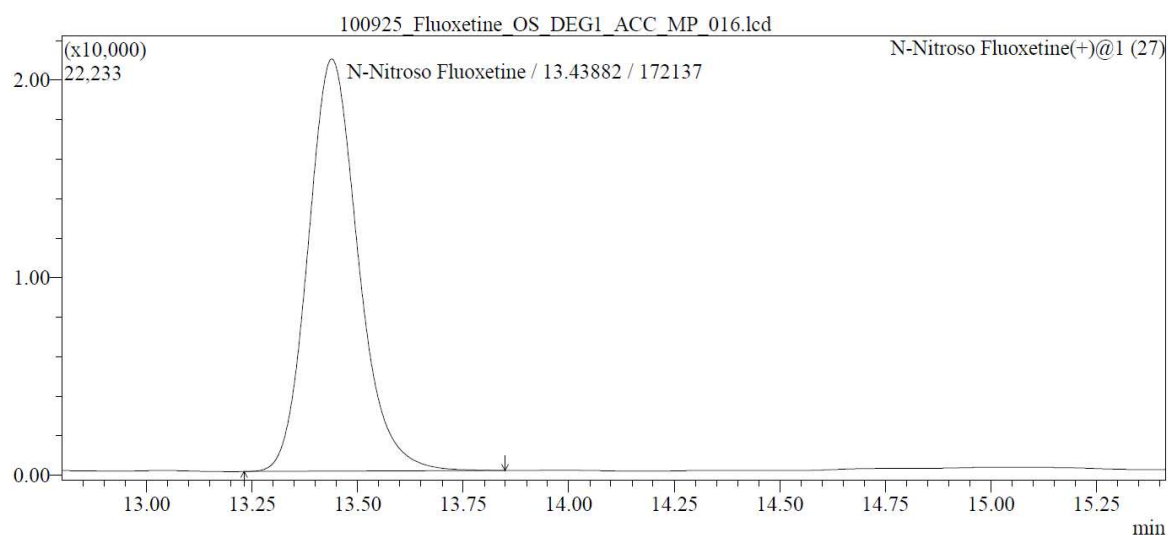


Fig. 7. Chromatogram of the spiked sample solution.

2.4.3. Precision

2.4.3.1. Method Precision

Method precision was evaluated to determine the repeatability of the analytical procedure under normal operating conditions. Six individual sample preparations of Fluoxetine Oral

Solution USP (20 mg/5 mL) were prepared by spiking N-nitroso fluoxetine at the specification level. The corresponding chromatograms are shown in Fig. 8. Each preparation was processed according to the established test method and injected into the LC–MS/MS system. The content of N-nitroso fluoxetine (in ppm) was calculated for each sample preparation. The acceptance criterion for method precision required that the %RSD of N-nitroso fluoxetine content from six sample preparations should be NMT 15.0%. The measured N-nitroso fluoxetine content ranged from 1.498 to 1.548 ppm, with a mean value of 1.521 ppm. The calculated %RSD was 1.2%. The retention times were consistent (approximately 13.44 minutes), confirming chromatographic stability throughout the analysis. The low %RSD value indicates excellent repeatability of the method and demonstrates that the sample preparation procedure and instrument performance are reliable and consistent.

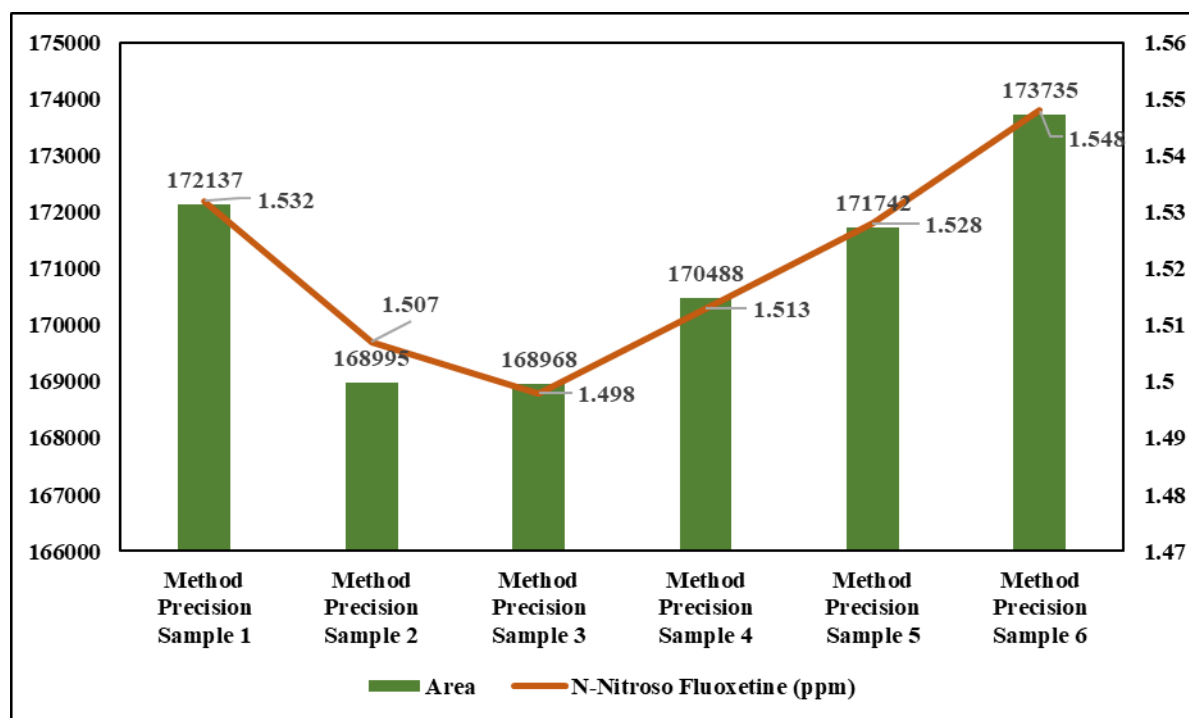


Fig. 8. Method precision results for Fluoxetine Oral Solution USP (20 mg/5 mL) spiked with N-Nitroso Fluoxetine.

2.4.3.2. Intermediate Precision

Intermediate precision was assessed to evaluate the reproducibility of the method under varied conditions, including a different analyst, different day, and different column. Six additional individual sample preparations of Fluoxetine Oral Solution USP (20 mg/5 mL), spiked with N-nitroso fluoxetine at the specification level, were prepared and analysed following the same analytical procedure. The corresponding results are depicted in Fig. 9. The acceptance criteria for intermediate precision were as follows:

- (a) The %RSD of N-nitroso fluoxetine content from six sample preparations should be NMT 15.0%.
- (b) The cumulative %RSD for N-nitroso fluoxetine content from twelve preparations (method precision and intermediate precision combined) should be NMT 15.0%.

The N-nitroso fluoxetine content obtained during intermediate precision analysis ranged from 1.569 to 1.637 ppm, with a mean value of 1.611 ppm and a %RSD of 1.5%. Retention times remained consistent at approximately 13.41–13.43 minutes, demonstrating robustness of the chromatographic conditions.

When the results from method precision and intermediate precision were combined, the overall mean content was 1.566 ppm with a cumulative %RSD of 3.3%, which is well within the acceptance criterion of NMT 15.0%. These results demonstrate that the developed LC–MS/MS method is precise, reproducible, and rugged for the determination of N-nitroso fluoxetine in Fluoxetine Oral Solution USP.

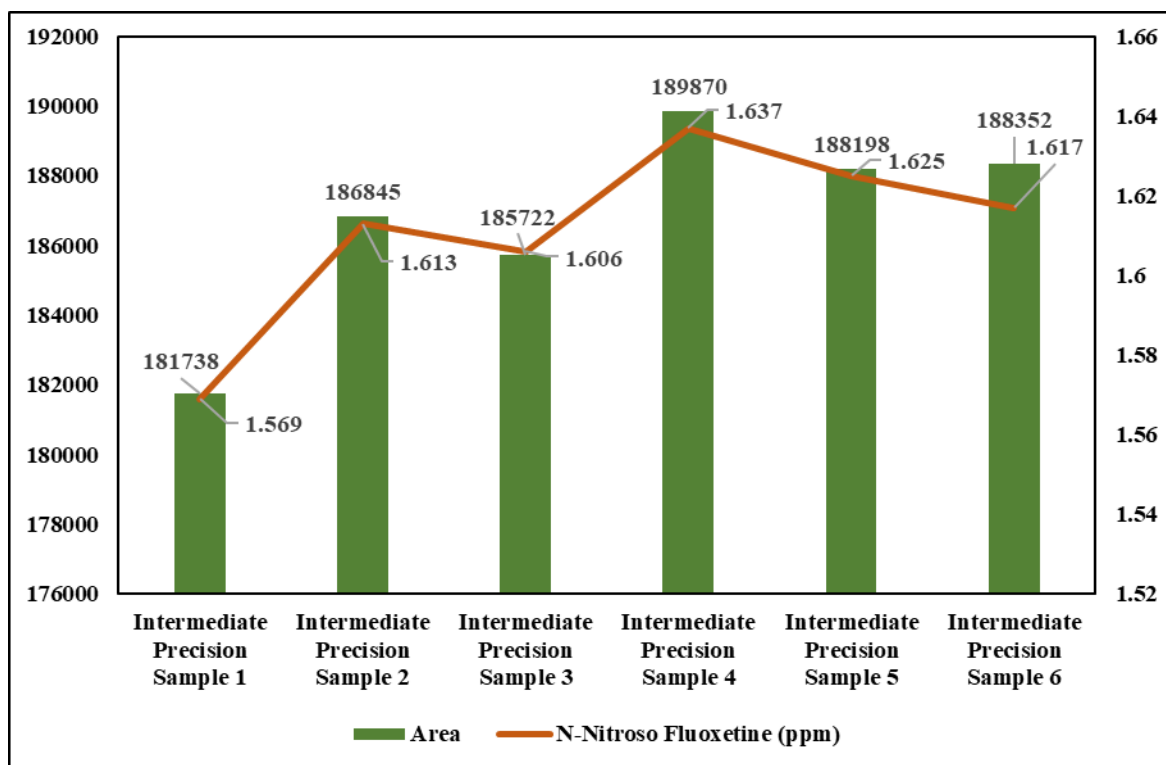


Fig. 9. Intermediate precision results for Fluoxetine Oral Solution USP (20 mg/5 mL) spiked with N-Nitroso Fluoxetine.

2.4.4. LOD and LOQ

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The analytical sensitivity of the method for N-Nitroso Fluoxetine was established through determination of the LOD and LOQ using the S/N ratio technique. The LOD was determined by serially diluting N-Nitroso Fluoxetine to progressively lower concentrations and injecting each level into the system. The minimum concentration that generated an S/N ratio meeting the predefined criterion of not less than (NLT) 3 was identified as the LOD. An S/N ratio of 53 was achieved at 0.152 ppm, which significantly exceeds the acceptance requirement, thereby confirming adequate detection capability at trace levels. The LOQ was assessed by analyzing six independent replicate injections prepared at the LOQ concentration. Method performance at this level was evaluated against acceptance criteria of %RSD for peak area NMT 15.0 and an S/N ratio NLT 10. The calculated %RSD of 0.9% demonstrates excellent repeatability, and all recorded S/N

ratios were substantially higher than the minimum requirement. The LOQ concentration was established at 0.398 ppm, and the corresponding results are depicted in Fig. 10. Overall, the results verify that the method possesses robust sensitivity and precision, ensuring reliable detection and quantification of N-Nitroso Fluoxetine at low concentration levels.

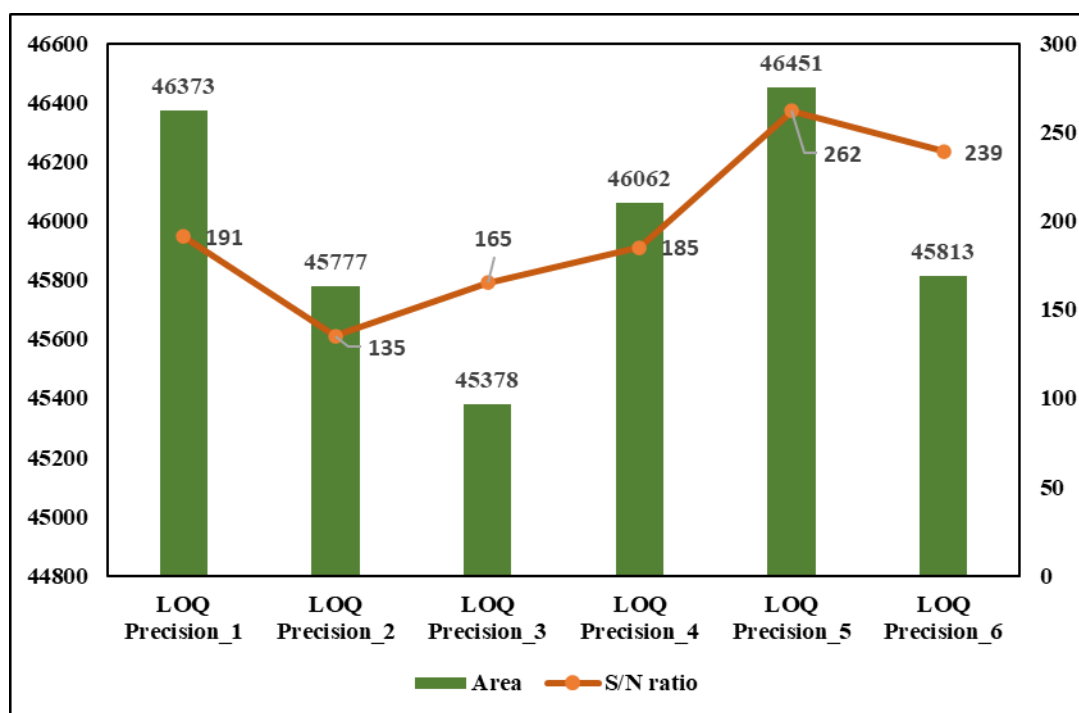


Fig. 10. LOQ Precision of N-Nitroso Fluoxetine.

2.4.5. Accuracy

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Method accuracy was established through recovery experiments performed by spiking known quantities of N-Nitroso Fluoxetine into the sample matrix across a concentration range from the LOQ level up to 200% of the specification limit. The spiked samples were prepared according to the validated analytical procedure and analyzed in replicate, and the results are tabulated in Table 1. The percentage recovery at each level was calculated by comparing the corrected measured concentration with the corresponding amount added. Acceptance criteria were predefined as follows: recovery between 70–130% at the LOQ level; recovery between 80–120% at the 100% and 200% levels; and %RSD for replicate

preparations at each concentration NMT 15.0. At the LOQ level (0.3989 ppm), the mean recovery was 108% with a %RSD of 2.4%. At the 100% level (1.3296 ppm), the mean recovery was 110% with a %RSD of 1.3%. At the 200% level (2.6591 ppm), the mean recovery was 107% with a %RSD of 0.7%. All individual recovery values were within the specified acceptance limits, and the variability among replicates remained well below the maximum allowable limit. These results confirm that the analytical method demonstrates acceptable trueness and consistency across the evaluated concentration range, thereby establishing its accuracy for the quantification of N-Nitroso Fluoxetine.

Table 1. Accuracy Level of N-Nitroso Fluoxetine in LOQ to 200%

Accuracy Level	Amount added in ppm	Corrected amount found in ppm	% Recovery	Mean % Recovery	% RSD
ACC_LOQ -1	0.3989	0.4222	106	108	2.4
ACC_LOQ -2		0.4250	107		
ACC_LOQ -3		0.4410	111		
ACC_100%-1	1.3296	1.4675	110	110	1.3
ACC_100%-2		1.4426	109		
ACC_100%-3		1.4342	108		
ACC_100%-4		1.4492	109		
ACC_100%-5		1.4639	110		
ACC_100%-6		1.4842	112		
ACC_200%-1	2.6591	2.8674	108	107	0.7
ACC_200%-2		2.8464	107		
ACC_200%-3		2.8299	106		

2.4.6. Linearity and Range

The linearity of the analytical procedure for N-Nitroso Fluoxetine was evaluated to confirm that the detector response is directly proportional to analyte concentration across the specified working range. Standard solutions were prepared at multiple concentration levels spanning from the LOQ to 200% of the specification limit. Each level was injected, and the corresponding ion count (area) was recorded and the results are illustrated in Fig. 11. A calibration curve was constructed by plotting concentration (ppm) against ion response (area), and linear regression analysis was performed to determine the correlation coefficient (r^2). The predefined acceptance criterion required an r^2 value of NLT 0.98. The method demonstrated excellent linearity over the evaluated range, with concentration levels of 0.3982 ppm (LOQ), 0.6636 ppm (50%), 1.3272 ppm (100%), 1.9908 ppm (150%), and 2.6544 ppm (200%) showing a consistent and proportional increase in detector response. The calculated correlation coefficient (r^2) was 0.999, exceeding the acceptance requirement. These results confirm that the method exhibits a strong linear relationship between concentration and response throughout the studied range (LOQ to 200% of the specification level), establishing its suitability for accurate quantification of N-Nitroso Fluoxetine within the intended analytical limits.

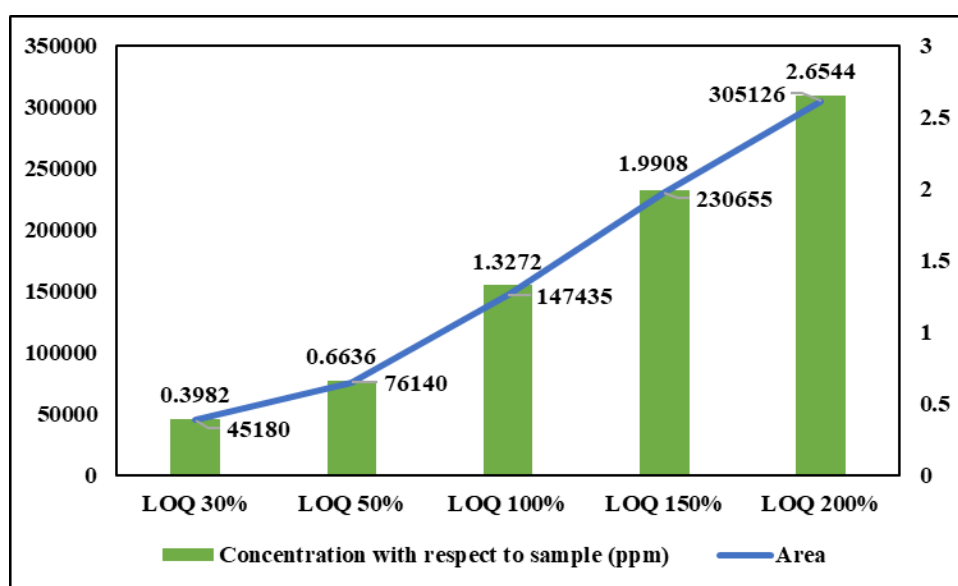


Fig. 11. Linearity and Range of N-Nitroso Fluoxetine (LOQ to 200%).

2.4.7. Solution Stability

The stability of the N-Nitroso Fluoxetine standard and spiked sample solutions was assessed under refrigerated conditions (5°C) over specified time intervals. Freshly prepared standard and sample solutions were analyzed initially and subsequently reinjected at defined time points to evaluate any changes in response and content over time. For the standard solution, injections were performed at initial, 6 hours, and 13 hours. The percentage relative difference in peak area at each time point was calculated with respect to the initial result. The observed relative differences were 0.5% at 6 hours and 5.3% at 13 hours, both well within the acceptance criterion of NMT 25.0%. For the spiked sample solution, analyses were conducted at initial, 3 hours, 8 hours, and 30 hours. The percentage relative difference in content (ppm) at each interval was determined against the initial value. The calculated differences were 1.6%, 3.0%, and 1.0%, respectively, all complying with the predefined limit of NMT 25.0%. Since all observed variations remained significantly below the specified acceptance threshold, the results confirm that both the standard and sample solutions are stable at 5°C for the evaluated duration. The solution stability data for the standard and sample solutions at different time intervals are presented in Table 2.

Table 2. Solution Stability Data for Standard and Sample Solutions at Different Time Intervals.

Name	Time interval	Relative difference in content
Standard Solution (N-Nitroso Fluoxetine)	6 hours	0.5
	13 hours	5.3
Sample Solution (N-Nitroso Fluoxetine)	3 hours	1.6
	8 hours	3.0
	30 hours	1.0

2.4.8. Filter Study

A filter study was carried out to evaluate the effect of filtration on the determination of N-Nitroso Fluoxetine in Fluoxetine Oral Solution USP, 20 mg/5 mL at the specification level. The spiked sample solution was filtered through a 0.22 μ m nylon filter, and different volumes of the initial filtrate (2 mL, 4 mL, 6 mL, and 8 mL) were discarded before analysis. The corresponding results are presented in Table 3. Each filtered sample was injected and compared with an unfiltered centrifuged sample, which was used as the control. The content of N-Nitroso Fluoxetine in the centrifuged sample was found to be 1.422 ppm, while the filtered samples showed results ranging from 1.433 ppm to 1.447 ppm. The percentage relative difference between the filtered and centrifuged samples ranged from 0.8% to 1.8%, which is well within the acceptance criterion of NMT 10.0%. Based on these results, filtration through a 0.22 μ m nylon filter does not significantly affect the determination of N-Nitroso Fluoxetine. Therefore, the sample solution can be filtered by discarding the first 2 mL of the filtrate prior to analysis.

Table 3. Filter Study Results Using a 0.22 μ m Nylon Filter

Name	Content (ppm)	Relative Difference
Centrifuge Sample	1.422	NA
0.22 μ m Nylon 2 mL discard	1.447	1.8
0.22 μ m Nylon 4 mL discard	1.433	0.8
0.22 μ m Nylon 6 mL discard	1.443	1.5
0.22 μ m Nylon 8 mL discard	1.433	0.8

3. Conclusion

In Conclusion the developed LC–MS/MS method for the quantification of N-Nitroso Fluoxetine in Fluoxetine Oral Solution USP, 20 mg/5 mL, has been fully validated according to regulatory guidelines and demonstrates suitability for routine pharmaceutical analysis. System suitability testing confirmed excellent instrument performance, with high sensitivity ($S/N = 159$) and system precision ($\%RSD = 2.5\%$) within acceptance limits. The method is highly specific, showing no interference from diluent, placebo, or matrix components. Method and intermediate precision were robust ($\%RSD \leq 3.3\%$), and linearity was excellent across the working range (0.398–2.654 ppm, $r^2 = 0.999$). Accuracy was confirmed with recoveries between 107–110%, while LOD (0.152 ppm) and LOQ (0.398 ppm) demonstrated adequate sensitivity for trace-level detection. Solution stability and filter compatibility studies further verified method reliability. Overall, the method is precise, accurate, robust, and regulatory-compliant, suitable for the quantitative determination of N-Nitroso Fluoxetine in pharmaceutical formulations.

Author Contribution statement

Rajamani dhanasekar : Methodology, Conceptualization, Investigation, Software, Formal analysis, Data curation, Writing – original draft.

Rajendren S. D: Conceptualization, Supervision, Validation, Writing – review & editing.

Velmurugan Vadivel: Conceptualization, Supervision, Validation, Writing – review & editing.

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Consent to Publish declaration : Not Applicable

Funding Declaration: Nil

Consent to Participate declaration: Not Applicable

Ethics declaration: Not applicable.

Conflicts of Interest : The authors declare no conflicts of interest.

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