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A Useful UFLC-MS Technique for Analysing N-Nitroso Vortioxetine Is a Nitrosamine Drug Substance Related Impurities (NDSRIs) in Vortioxetine Hydrobromide Drug Substance Employing a Low-Cost Single Quadrupole Mass Spectrometer

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Abstract

Nitrosamine Drug Substance Related Impurities (NDSRIs) are commonly examined using High Performance Liquid Chromatography (HPLC) in combination with Mass Spectrometry (MS) detection. Due to the great sensitivity needed, high-resolution MS or MS/MS is commonly used. However, it can be difficult to use this kind of strategy for routine analysis at a supply site. Here, we used a low-cost single quadrupole MS instrument to develop and validate a practical, reliable, and user-friendly method for analysing NDSRIs. As an antidepressant drug used to treat Major Depressive Disorder (MDD) in adults, we have utilized 1-{2-[(2,4-dimethylphenyl) thio] phenyl}-4-nitrosopiperazine (N-Nitroso Vortioxetine) in Vortioxetine Hydrobromide API. The limits of detection and quantification for N-Nitroso Vortioxetine, which may be present in Vortioxetine Hydrobromide API, were 0.660 ppm and 1.995 ppm, respectively. We designed and validated this in accordance with the criteria of the International Harmonization Conference (ICH Q2R2). With a percentage RSD of 1.07 percent, the precision for N-Nitroso Vortioxetine is established. The correctness of the procedure was ensured by the linearity study's correlation coefficient of 0.99964 and the percentage recovery of the spiked impurity in the drug material obtained, which fell within LOQ and 150% for a 1.995 ppm to 30 ppm level. The N-Nitroso Vortioxetine impurity in Vortioxetine Hydrobromide API can be found by adapting the method.

Keywords

Ultra-Fast Liquid Chromatography with Mass Spectrometer (UFLC-MS), Nitrosamine Drug Substance Related Impurities (NDSRIs), N-Nitroso Vortioxetine, Vortioxetine Hydrobromide

1. Introduction

According to the ICH M7 recommendation, nitrosamines are a cohort of concern because the majority of them are mutagens and several have been shown in animal tests to be strong carcinogens. Numerous medicinal substances and products include nitrosamines, which can cause cancer in animals [1]. In 2018, an unacceptable amount of N-Nitroso Dimethylamine (NDMA) was discovered in a drug substance called valsartan [2]. Since then, it has been discovered that nitrosamine levels in numerous additional pharmaceutical drugs, including Sartans, exceed the permissible ingestion limit and necessitate their recall [3].

Regulatory organizations and pharmaceutical companies have initially concentrated on identifying and managing common nitrosamines (like NDMA) in medicinal goods. However, as knowledge of nitrosamine production has advanced, the extent and quantity of potential nitrosamine contamination in pharmaceutical goods has now broadened to encompass all Nitrosamine Drug Substance Related Impurities (NDSRIs). [4]-[9]. Nitrosamine Drug Substance-Related Impurities (NDSRIs) are nitrosamine impurities caused by the presence of secondary or tertiary amine functional groups that can react with nitrosating agents under specific circumstances. Nitrosamine Drug Substance-Related Impurities (NDSRIs) can be formed from the Active Pharmaceutical Ingredient (API) or its structural fragments [10]-[12]. Since most NDSRIs lack published toxicity data, a read-across approach is frequently used to establish the Acceptable Intake (AI) limit. Several regulatory bodies recently released a novel method for predicting AI limits utilizing the Carcinogenic Potency Categorization Approach (CPCA). [13]-[15]. N-nitrosamines that have a carbon atom on both sides of the N-Nitroso group and in which the carbon is not directly double-bonded to a heteroatom are covered by the Carcinogenic Potency Categorization Approach (N-Nitrosamides, N-Nitrosoureas, N-Nitrosoguanidines, and other related structures are excluded). Additionally, N-Nitrosamines having an N-Nitroso group enclosed in an aromatic ring (such as nitrosated indole) are not classified using the potency categorization method. The group with the highest anticipated carcinogenic potency (*i.e.*, the group with the lowest numerical potency category) determines the AI for the entire molecule in N-nitrosamines that contain two N-Nitroso groups.

Figure 1 shows how the α- and β-carbons are defined in relation to the N-Nitroso group [16] [17].

The CPCA gives applicants a uniform method to determine the AI limitations, which vary from 18 ng/day for Category 1 to 1500 ng/day for Category 4 and 5,

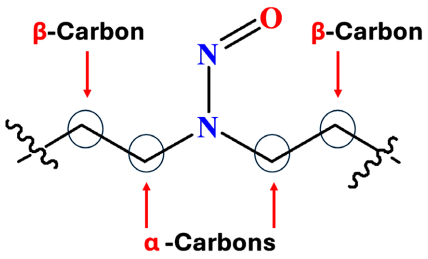


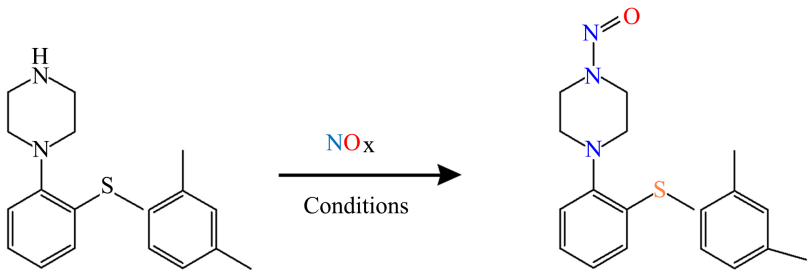
Figure 1. *α*- and *β*-carbons on an *N*-nitrosamine.

however it is still a work in progress. The low AI limits necessitate highly sensitive and precise analytical techniques for detection and quantification, particularly for Category 1 and 2 NDSRIs. [18] Supporting the development of analytical methods and routine testing of these trace-level contaminants is a major problem for pharmaceutical companies. Due to their high sensitivity requirements, the majority of NDSRIs are non-volatile and are nearly always studied utilizing High Performance Liquid Chromatography (HPLC) with Mass Spectrometer (MS) detection [18]-[25]. Methods based on HRMS or MS/MS are impractical for routine analysis at a supply site because of their high instrument cost, frequent and involved maintenance activities, drifting performance, and required analyst training, even though LC-MS has been shown to be a useful tool for NDSRI analysis. Additionally, regular release and/or stability testing of the drug product may be necessary. This type of testing frequently entails thousands of samples, particularly for high-volume pharmaceutical products for common conditions like diabetes or hypertension. It is necessary to have a method that is resilient, practical, high-throughput, and easy to use on a low-cost device.

Common Chromatography Data Systems (CDS) that are already in use in supply laboratories like Labsolution can frequently be used to control UFLC connected to single quadrupole mass spectrometry detection, which is less expensive and simpler to use. The likelihood of successful routine testing is increased and the work and time required to introduce a new NDSRI method to a supply laboratory in a GMP setting is greatly decreased by using already-existing instruments and software platforms. Since HRMS is significantly more sensitive and specific than single quadrupole detectors, liquid chromatography separation optimization is essential to provide a technique that can provide the low-level detection needed for NDSRI analysis.

To develop an analytical method on UFLC-MS technique for 1-{2-[(2,4-dimethylphenyl) thio] phenyl}-4-nitrosopiperazine as a N-Nitroso Vortioxetine related NDSRI is discussed here. With regard to 2 mg/mL Vortioxetine HBr, the optimized method attains a limit of quantitation of 0.660 ppm to 1.995 ppm. After the chromatography and MS settings were completely tuned, we showed that a single quadrupole mass spectrometry detector could provide the necessary specificity and sensitivity. This strategy serves as an example of a methodical approach to method development that can be applied to the creation of different NDSRIs. As seen in **Figure 2**, the chemical name for Vortioxetine Hydrobromide is 1-[2-(2,4-

Dimethylphenylsulfanyl) phenyl] piperazine, which has a secondary amine functional group in a six-membered piperazine that causes the synthesis of N-Nitroso Vortioxetine when acidic circumstances [x] are present.

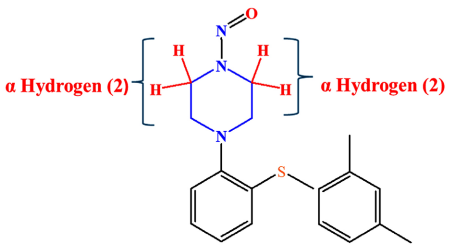


1-[2-(2,4-Dimethylphenylsulfanyl) phenyl]piperazine

1-{2-[(2,4-dimethylphenyl) thio] phenyl}-4-nitrosopiperazine

Figure 2. Possible reaction scheme for formation of N-Nitroso vortioxetine from vortioxetine.

N-Nitroso vortioxetine's risk has been determined using the CPCA technique, and its potency score for carcinogenicity is 3. The prediction's specifics are displayed in **Figure 3**.



CPCA Interpretation	Score Value
α Hydrogen 2,2	1
Nitroso group in 6 membered ring having individual deactivating feature	+2
Activating Feature Scores	0
Potency Score	α-Hydrogen Score + Deactivating Feature Scores + Activating Feature Scores
Potency Score	1+2+0=3
Potency Category	3
Acceptable Intake (AI)	400 ng/day
Risk Category	Medium
Maximum Daily Dose (MDD) for Vortioxetine Hydrobromide	20 mg/day
Target level for N-Nitroso Vortioxetine : AI/MDD	400/20
Target level for N-Nitroso Vortioxetine is 20 ppm	

Figure 3. Carcinogenic potency categorization approach for N-Nitroso vortioxetine.

With a focus on practical application in routine quality control, this study attempts to develop and test a sensitive, reproducible UFLC-MS method for assessing N-Nitroso-Vortioxetine in Vortioxetine hydrobromide medicinal product with a target concentration 20 ppm. In addition to improving the safety and quality of pharmaceutical products, the trace-level analytical method optimization will give manufacturers and regulatory bodies crucial tools for dealing with nitrosamine contaminants.

2. Experimental

2.1. Material

Vortioxetine Hydrobromide sample, N-Nitroso Vortioxetine Impurities was obtained from Megafine Pharma (P) Ltd., Nashik.

2.2. Chemicals and Reagents

Ammonium Formate, Formic Acid Methanol, Ammonia, and Milli-Q Water are used for the solution preparation (all solvents/chemicals used are LCMS grade).

2.3. Instrumentation

Utilizing a single quadrupole mass spectrometer for ultra-fast liquid chromatography 2020 Nexera LCMS Make: Shimadzu has a column oven, automated sampler, degasser, and binary gradient pump. A single quadrupole mass spectrometer and LC Solution software are used to hyphenate this LC system. The analytical balance is manufactured by Metler Toledo.

2.4. Chromatographic Conditions

LC column used for the analysis was ACQUITY CSH™ 1.7 µ Phenyl-Hexyl, 100 mm length 2.1 mm internal diameter. Mobile Phase A and B used for elution were 10 mmol/L Ammonium Formate in 0.1% Formic Acid in water Methanol and Water (9:1 v/v) respectively. The injection volume was optimized to 20 µL. Elution was obtained at a flow rate 0.4 mL/min in a gradient mode: 5% Solvents (0.01 - 2 min.), 30% (2 - 4 min.), 65% (4 - 6 min.), 65% (6 - 11 min.), 75 (11 - 14 min.), 75% (14 - 16 min.), 5% (16 - 17 min.). 5% (17 - 20 min.) Column oven temperature 40˚C with autosampler temperature 25˚C.

The mass spectrometer was equipped with Electron Spray Ionization (ESI) source with DL temperature 250˚C or as per tuning file, Acquisition Mode: SIM Mode CH1 = 328 (m/z), Interface Temperature: 350˚C, Nebulizing Gas Flow: 1.5 L/min, Drying Gas: 10.00 L/min, Heat Block: 200˚C, Detector Voltage: 1.65 kV, MS Program: Cut Off, 0.01 min FCV2 (0), 5.00 min. FCV2 (1), 13 min, FCV2 (0).

2.5. Preparation of Solutions

All preparations were made using Milli-Q water as a diluent. After weighing and transferring 50.0 mg of test sample into 25 mL volumetric flask, dissolve and dilute to the volume with diluent. Filtered the solution through 0.22-µ PVDF filter syringe and collected the clear filtrate in HPLC vial. The test solution preparation will contain about 2.0 mg/mL of Vortioxetine. For N-Nitroso Vortioxetine standard solution was prepared with a concentration of 0.04 µg/mL of N-Nitroso Vortioxetine with respect to test concentration.

3. Method Optimization

Using an ACQUITY CSHTM 2.1 µm Phenyl-Hexyl, 100 mm length, and 1.7 micron, LC column, a reliable and selective UFLC-MS technique was created for the

analysis of N-Nitroso Vortioxetine impurity, specifically targeting N-Nitroso Vortioxetine in SIM Mode for m/z 328 positive mode. The Phenyl-hexyl stationary phase facilitates π-π interactions between the stationary phase and electron-rich analytes, which is why this column was selected. It is hence very useful for nitrosamine analysis. [23] Methanol, 0.1% formic acid, and 10 mM ammonium acetate in water (Mobile Phase A) made up the mobile phase. The 900 mL, 100 mL, and 10 mL v/v/v ammonia solution (Mobile Phase B) of purified water was designed to enhance ionization in the positive ESI mode and guarantee a favourable peak shape for. N-Nitroso Vortioxetine was successfully separated using a gradient elution, which eluted at around 13 minutes. The diverter valve FCV2 was set to position 0 at 0.01 minutes to direct the initial flow to waste. At 5.00 minutes, the valve was switched to position 1 to allow flow to the mass spectrometer for analyte detection. Finally, at 13.00 minutes, the valve was returned to position 0 to divert late-eluting contaminants to waste. The valve permitted analytes, such as N-Nitroso vortioxetine, to enter the mass spectrometer for measurement between 13 and 20 minutes. In order to allow for enough separation, MS detection, and re-equilibration during each cycle, the total chromatographic run duration was kept at twenty minutes. N-Nitroso Vortioxetine was reliably detected in Vortioxetine samples using this approach, which also offered good sensitivity and decreased MS contamination.

4. Results and Discussion

4.1. Method Validation

According to the recommendations of the International Conference on Harmonization (ICH), the developed analytical method was found to be more reliable as it satisfied the system appropriateness criteria during the method validation and batch analysis. Specificity, precision, limit of detection, limit of quantitation, linearity, accuracy, stability of the solution, and robustness were all considered in the validation of the analytical method [26] [27].

4.1.1. Specificity

Specificity is the ability to conclusively assess the analyte in the presence of possibly anticipated components. [26]. By verifying that the m/z value of each individual impurity should not affect the m/z value of the others and that no blank interference peak should be detected at the retention time of N-Nitroso vortioxetine, the specificity of the method was verified. The analysis of a blank or unspiked API sample confirmed the absence of interfering peaks at the retention time and m/z of N-Nitroso vortioxetine. Since N-Nitroso Vortioxetine's retention time is listed in **Table 1**, **Figure 4** and **Figure 5** demonstrate the approach's specificity.

Table 1. Retention Times (RT).

Sr. No.	Name of Compound	RT in Minutes	Detector
1	Vortioxetine	7.2	UV
2	N-Nitroso Vortioxetine	13.8	MS

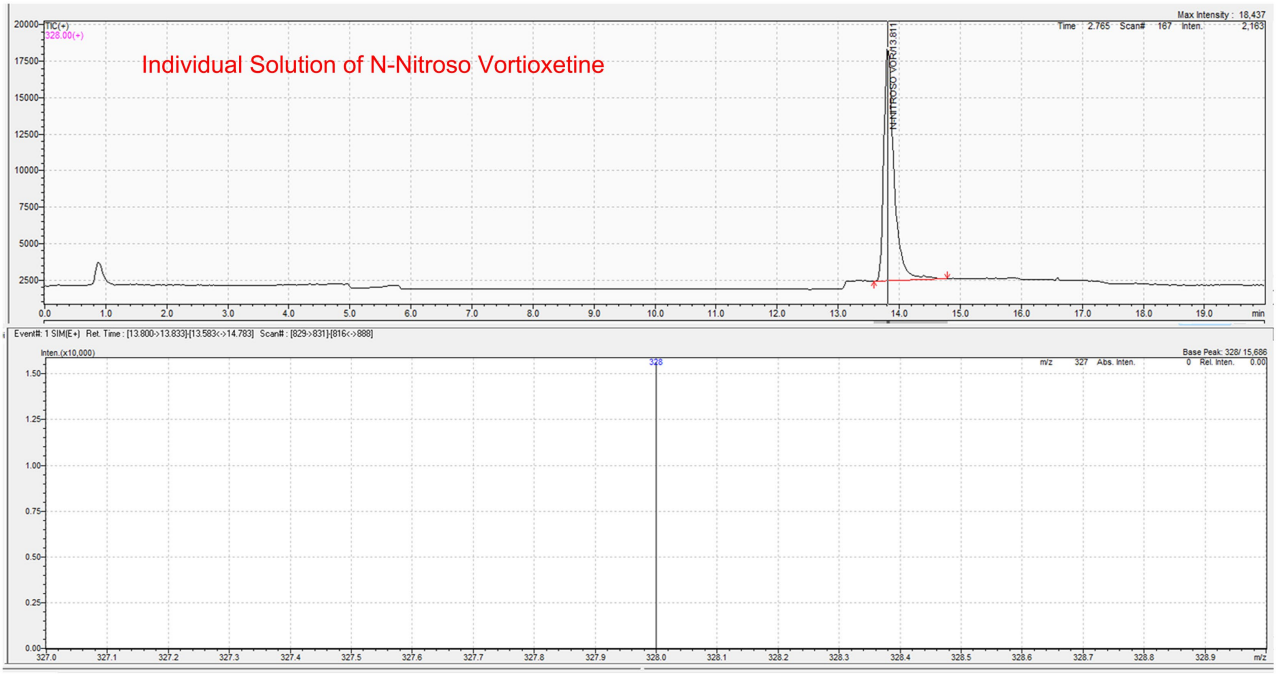


Figure 4. Individual solution of N-Nitroso vortioxetine.

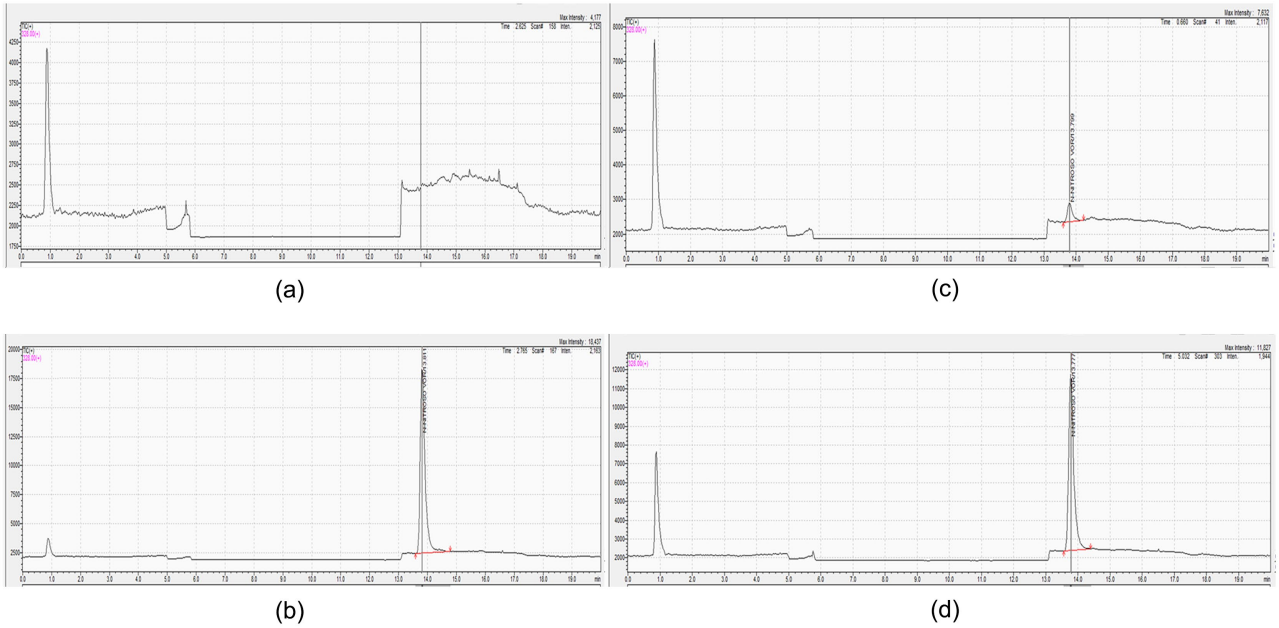


Figure 5. Specificity data of N-Nitroso vortioxetine (a) blank, (b) standard, (c) unspiked test sample and (d) spiked test sample.

4.1.2. Precision

The precision of an analytical method is defined as the degree of agreement (or scatter) between a set of measurements made by repeatedly sampling the same homogenous sample under prescribed conditions [26]. By analyzing six different samples, the system precision for N-Nitroso vortioxetine was shown. The percent relative standard deviation (% RSD) for N-Nitroso vortioxetine was shown to be less than 15.0%. **Table 2** reports the outcome.

Table 2. % RSD for system precision data.

Number of Injections	Area of N-Nitroso Vortioxetine (n = 6)
Run No. 1	179,153
Run No. 2	180,071
Run No. 3	180,458
Run No. 4	182,414
Run No. 5	181,625
Run No. 6	184,585
Mean	181,384
Standard Deviation	1945.466
% RSD	1.07

The N-Nitroso Vortioxetine impurity was spiked at 20 ppm using six injections of standard solution to determine the method's precision. The results are shown in Table 3 below. The percent relative standard deviation (% RSD) for N-Nitroso Vortioxetine was found to be 2.79%.

Table 3. % RSD for method precision data.

Sample Replicate	N-Nitroso Vortioxetine (ppm)
Spiked Preparation-1	21.994
Spiked Preparation-2	21.534
Spiked Preparation-3	21.507
Spiked Preparation-4	22.877
Spiked Preparation-5	21.242
Spiked Preparation-6	21.373
Mean	**21.755**
Std. Dev.	**0.606**
% RSD	**2.79**

4.1.3. Intermediate Precision

Similar steps were taken on different days and with different columns to demonstrate the robustness of this method. The percentage RSD of the results from six test-spiked preparations for the N-Nitroso Vortioxetine impurity was computed, and the results are displayed in Table 4. The total percentage RSD of the intermediate precision and method precision results was then computed. Table 5 presents the tabulated statistics.

Table 4. % RSD for intermediate precision data.

Sample Replicate	N-Nitroso Vortioxetine (ppm)
Spiked Preparation-1	24.684
Spiked Preparation-2	23.983
Spiked Preparation-3	24.176
Spiked Preparation-4	24.949
Spiked Preparation-5	23.748
Spiked Preparation-6	23.777
Mean	**24.220**
Std. Dev.	**0.495**
% RSD	**2.04**

Table 5. Overall percentage relative standard deviation % RSD for Method precision and Intermediate Precision data.

Sample Replicate	N-Nitroso Vortioxetine (ppm)	
Different Day	**Day-1**	**Day-2**
Different Column	**Column-1**	**Column-1**
Spiked Preparation-1	21.994	24.684
Spiked Preparation-2	21.534	23.983
Spiked Preparation-3	21.507	24.176
Spiked Preparation-4	22.877	24.949
Spiked Preparation-5	21.242	23.748
Spiked Preparation-6	21.373	23.777
Overall Mean	**22.987**	
Overall SD	**1.391**	
Overall % RSD	**6.05**	

4.1.4. Limit of Detection and Limit of Quantitation

The detection limit of a certain analytical procedure is the lowest concentration of analyte in a sample that can be recognized but isn't necessarily measured as an exact value [26]. The quantitation limit of a given analytical procedure is the lowest analyte concentration in a sample that can be quantitatively determined with suitable precision and accuracy [26]. For N-Nitroso vortioxetine, the predetermined Limit Of Quantification (LOQ) level and Limit Of Detection (LOD) level were assessed. For N-Nitroso Vortioxetine, approximately 3.3% (*i.e.*, 0.66 ppm w. r. t. test conc.) of the target level was chosen as the LOD, and approximately 10% (*i.e.*, 2 ppm w. r. t. test conc.) of the specified level was chosen as the LOQ. Six

replicate injections of the LOQ solution were used to verify the evaluated Limit Of Quantification (LOQ) level for N-Nitroso vortioxetine. The signal to noise ratio of the anticipated LOQ has been determined to be greater than 10, and the percentage RSD of six replicate injections of the LOQ solution is 2.57%. The data has been shown in Table 6 and Figure 6.

Table 6. LOQ precision and S/N ratio for N-Nitroso vortioxetine.

Injection	N-Nitroso Vortioxetine	
	Area	S/N Ratio
LOQ Level Run-1	19,277	127.93
LOQ Level Run-2	19,074	134.60
LOQ Level Run-3	17,966	118.59
LOQ Level Run-4	18,890	103.91
LOQ Level Run-5	18,369	99.79
LOQ Level Run-6	18,757	91.20
Mean	**18,722**	
SD	**481.149**	
% RSD	**2.57**	

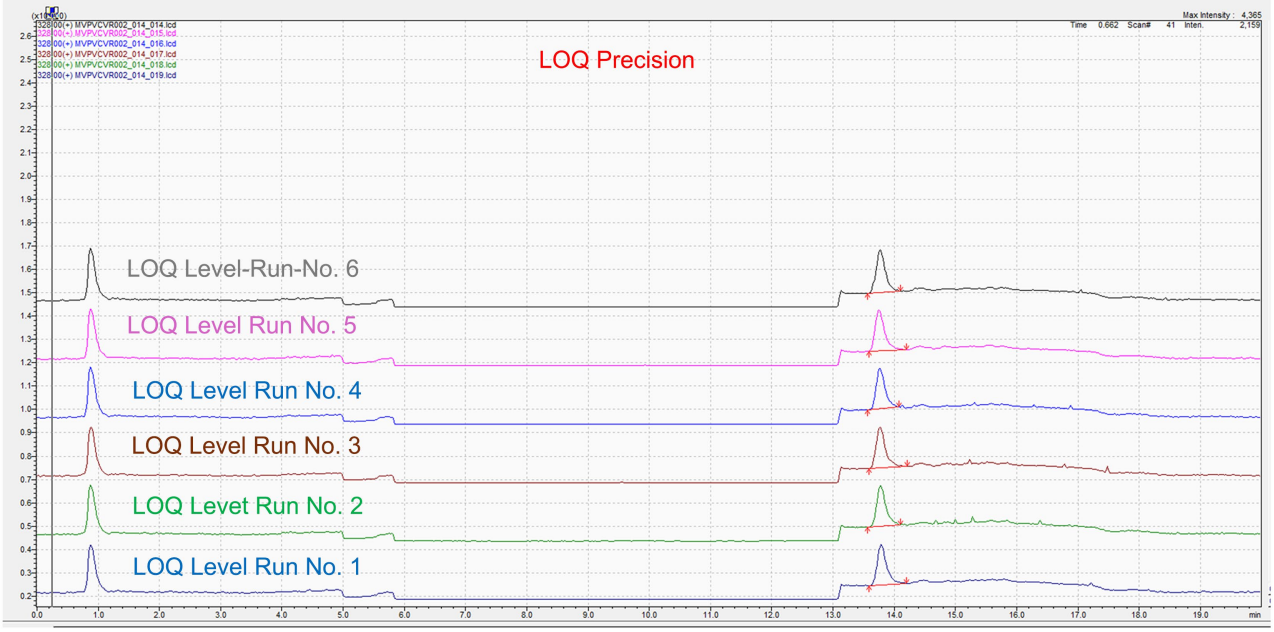


Figure 6. LOQ precision.

4.1.5. Linearity

The capacity of an analytical process to yield test results that are exactly proportionate to the concentration (quantity) of analyte in the sample (within a given range) is known as linearity [26].

Following regression analysis of the data, the correlation coefficient (r) shouldn't be less than 0.99 [26].

Linearity curves were obtained by plotting the peak area of the N-Nitroso Vortioxetine impurity against the equivalent concentration of linearity solution (**Figure 6**). N-Nitroso Vortioxetine impurity was generated as a series of standard solutions with concentrations ranging from LOQ to 150% (10%, 50%, 80%, 100%, 120%, and 150%) of the target concentration (20 µg/mL w. r. t. sample). **Table 7** reports the observed correlation coefficient of the N-Nitroso Vortioxetine impurity, **Figure 7** shows the linear regression data for N-Nitroso Vortioxetine impurity and chromatogram **Figure 8**. Plotting the peak area of the N-Nitroso Vortioxetine impurity against its corresponding concentration of linearity solution produced linearity curves.

Table 7. Correlation coefficient data.

Name of Compound	Slope	Intercept	Correlation Coefficient
N-Nitroso Vortioxetine	4623704.68	1767.20	0.99964

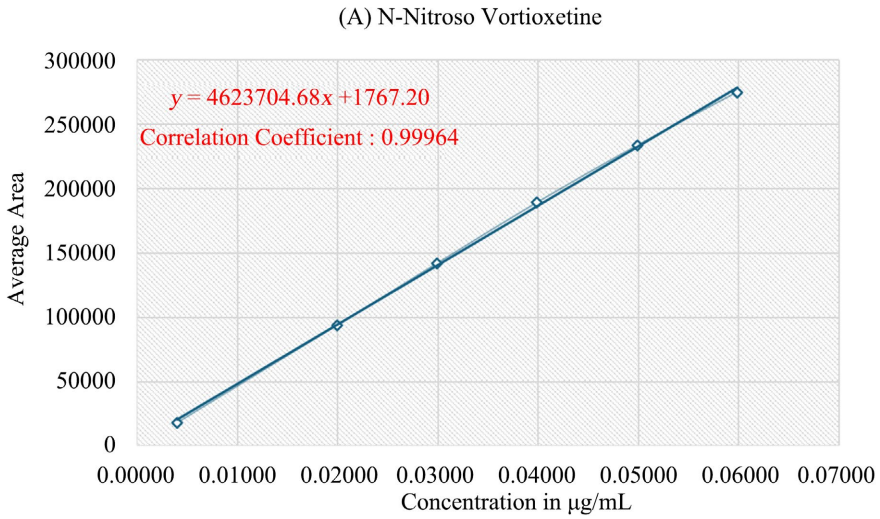


Figure 7. Linearity of N-Nitroso vortioxetine.

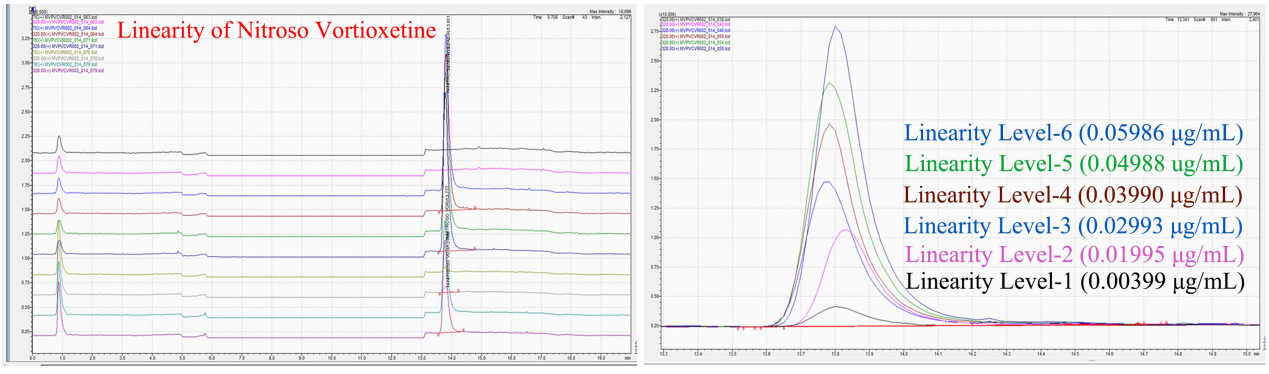


Figure 8. Linearity chromatogram of N-Nitroso vortioxetine.

4.1.6. Accuracy

The correctness of an analytical method is determined by the degree of agreement between the value found and the value that is accepted as either a conventional true value or an acceptable reference value [26]. The recovery percentage should fall between 80% and 120% when known concentrations of N-Nitroso Vortioxetine are spiked in Vortioxetine Hydrobromide API at levels LOQ, 50%, 100%, and 150%. Recovery was used to evaluate the procedure's accuracy (Table 8). Three preparations of each level were made and analyzed in accordance with the methodology; the recovery percentage and mean were calculated. The obtained % Recovery for N-Nitroso Vortioxetine is found well within limit, the % Recovery for levels LOQ, 50%, 100%, and 150% is found 92.42% to 104.54%, based on recovery data, this method highly accurate for determination of N-Nitroso Vortioxetine in Vortioxetine Hydrobromide API.

Table 8. Accuracy of N-Nitroso vortioxetine.

Recovery Concentration (µg/mL)	% Recovery
	N-Nitroso Vortioxetine
2 µg/mL	104.54
2 µg/mL	98.92
2 µg/mL	92.42
10 µg/mL	104.93
10 µg/mL	96.35
10 µg/mL	97.08
20 µg/mL	104.86
20 µg/mL	101.65
20 µg/mL	101.57
30 µg/mL	102.16
30 µg/mL	98.03
30 µg/mL	98.42

4.1.7. Solution Stability

The standard solution, test solution (Unspiked), and test solution (spiked) with the N-Nitroso Vortioxetine impurity at the specification level were used to illustrate the solution stability. Freshly made reference solution, test solution (Unspiked), and test solution (spiked) containing N-Nitroso Vortioxetine were administered at 6, 12, and 24 hour intervals. N-Nitroso vortioxetine's cumulative percentage RSD in standard solution, test solution (Unspiked), and test solution (spiked) is less than 15%, shows that the standard solution, test solution (Unspiked), and test

solution (spiked) are stable for analysis and research for up to 24 hours at room temperature (25˚C), as indicated by the data in Table 9.

Table 9. Accuracy of N-Nitroso vortioxetine.

Interval	Cumulative % RSD for area of N-Nitroso Vortioxetine		
	Standard Solution	Test Solution Unspiked	Test Solution Spiked
Fresh	-	-	-
After 06 Hrs	2.91	10.07	2.20
After 12 Hrs	2.24	7.53	2.43
After 24 Hrs	2.60	6.18	3.07

4.1.8. Robustness

By purposefully altering the experimental parameters, such as the flow rate percentage (Flow = 0.360 mL/min & 0.440 mL/min) and the column oven temperature ±3˚C (37˚C & 43˚C), the method's robustness is confirmed. The N-Nitroso Vortioxetine impurity was spiked at the specification level in the test sample to illustrate the robustness technique. Each condition's system applicability was assessed, and three test samples that were tainted with N-Nitroso Vortioxetine impurity at the specification level were examined. By computing the overall percentage RSD, the findings are compared with the technique precision results. The overall percentage relative standard deviation of results (content of N-Nitroso Vortioxetine in ppm) from nine different test solution (spiked) preparations of method precision and robustness is found to be 3.74% to 7.06%, while the percentage relative standard deviation of results (content of N-Nitroso Vortioxetine in ppm) from three different sample preparations is found to be 1.49% to 3.01%. Details of robustness data are displayed below Table 10 and Table 11.

Table 10. Robustness data for deliberately changing parameter.

Injection Number.	**N-Nitroso Vortioxetine in ppm**			
	Flow Rate 0.360 mL/min	**Flow Rate 0.440 mL/min**	**Column Oven Temp. 37˚C**	**Column Oven Temp. 43˚C**
Spiked Test Sample-1	24.872	25.205	24.979	23.616
Spiked Test Sample-2	24.272	24.453	24.452	22.768
Spiked Test Sample-3	24.414	24.741	23.534	22.569
Mean	24.519	24.800	24.322	22.984
SD	0.314	0.379	0.731	0.556
% RSD	1.28	1.53	3.01	2.42

Table 11. Overall comparison with method precision and robustness data for deliberately changing parameters.

Injection Run No.	N-Nitroso Vortioxetine in ppm				
	Method precision	Deliberate change in Method Parameters			
		Flow Rate 0.360 mL/min	Flow Rate 0.440 mL/min	Column Oven Temp. 37˚C	Column Oven Temp. 43˚C
1	21.994	24.872	25.205	24.979	23.616
2	21.534	24.272	24.453	24.452	22.768
3	21.507	24.414	24.741	23.534	22.569
4	22.877	-	-	-	-
5	21.242	-	-	-	-
6	21.373	-	-	-	-
Mean	21.755	24.519	24.800	24.322	22.984
SD	0.606	0.314	0.379	0.731	0.556
% RSD	2.79	1.28	1.53	3.01	2.42
Overall Mean		22.676	22.770	22.610	22.164
Overall SD		1.471	1.607	1.418	0.828
Overall % RSD		6.49	7.06	6.27	3.74

4.1.9. Application of Method

In order to identify the N-Nitroso Vortioxetine impurity in Vortioxetine Hydrobromide API, this method is more precise and highly specific, according to the drug substance's investigation. Table 12 displays the sample batch data.

Table 12. Sample batch data.

Sample Replicate	N-Nitroso Vortioxetine (ppm)
Batch No-1	ND
Batch No-2	ND
Batch No-3	ND

5. Conclusion

The N-Nitroso Vortioxetine in Vortioxetine Hydrobromide API is separated and successfully detected using a single Quadrupole Ultra-fast liquid chromatography with mass spectrometer approach that is sensitive, specific, linear, accurate, and efficient. Based on the findings of all the data generated, we can infer that the current technique can be helpful for recognizing and quantifying the N-Nitroso Vortioxetine in Vortioxetine Hydrobromide API. This method has been validated as being more precise, linear, accurate, and robust. The procedure is therefore considered appropriate for regular N-Nitroso Vortioxetine.

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Conflicts of Interest

The authors declare no conflicts of interest.

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