Advanced International Journal for Research (AIJFR)
E-ISSN: 3048-7641 ● Website: www.aijfr.com ● Email: editor@aijfr.com

Ultra-Sensitive Determination of Nitrosamine Genotoxic Impurities in Atomoxetine Hydrochloride Using Headspace GC-MS/MS

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Abstract

Nitrosamine impurities such as N-nitrosodimethylamine (NDMA) and N-nitrosodiethylamine (NDEA) have recently attracted intensified regulatory attention owing to their classification as probable human carcinogens and their detection in several pharmaceutical products. Consequently, it is crucial to make sure that these impurities are not present in drug ingredients. The present research has developed and validated a gas chromatography-tandem mass spectrometry (GC-MS/MS) method for the detection of NDMA and NDEA at the trace level in Atomoxetine hydrochloride, a selective norepinephrine reuptake inhibitor used to treat attention-deficit hyperactivity disorder (ADHD). To achieve high sensitivity and selectivity, the method leveraged headspace sampling in conjugation with multiple reaction monitoring (MRM) transitions (NDMA: 74.1 → 44.2; NDEA: 102.2 → 85.1). Excellent specificity has been established by validation, and analyte retention times demonstrated no interference. For both analytes, the technique exhibited a limit of detection (LOD) of 0.01 ppm and a limit of quantification (LOQ) of 0.03 ppm. Precision studies revealed % RSD values of 4.2% and 5.7% respectively, for NDMA and NDEA, whereas recovery studies at the LOQ level demonstrated accuracy of 99% for NDMA and 105% for NDEA. It has been confirmed that NDMA and NDEA were not detected at or above the method's quantitation limits while the validated method was implemented to three successive batches of Atomoxetine Hydrochloride. In order to ensure compliance with international regulatory guidelines for the genotoxic impurities, the results illustrate that the developed GC-MS/MS method is a robust, sensitive, and reliable technique for the routine monitoring of nitrosamine impurities in Atomoxetine hydrochloride.

Keywords: NDMA, NDEA, Atomoxetine hydrochloride, Nitrosamines, GC-MS/MS, Genotoxic impurities.

1. Introduction

The therapeutic characteristics of the active pharmaceutical ingredient (API), as well as the control and elimination of potential impurities in drug ingredients and final products, are significant for the safety and effectiveness of pharmaceutical products. Impurities in pharmaceuticals can originate from multiple sources, including raw materials, intermediates, degradation processes, or even during transportation and

storage (Sigonya, Mokhothu et al. 2023). Considering the fact that most impurities are harmless, a particular category of impurities known as genotoxic impurities (GTIs) has highlighted significant regulatory implications concerning their tendency to interact with DNA and accumulate mutations that could eventually result in cancer (Risk 2018, Alsayadi, Dogra et al. 2025).

Nitrosamines represent one of the most important classes of GTIs precisely due to their reported carcinogenicity(Mirvish 1995, Humans 2010, Lungu-Mitea, Stein Aslund et al. 2025). The existence of trace amounts of nitrosamines in food, drinking water, and pharmaceuticals has been extensively researched for decades(Lijinsky 1992, Chao Huanga and Oub 2024). Nitrosamines are frequently generated when secondary or tertiary amines react with nitrosating agents(Zhao, Hu et al. 2021). Nitrosamines, especially NDMA and NDEA, have been categorized as probable human carcinogens (Group 2A) according to the International Agency for Research on Cancer (Loomis, Huang et al. 2014). The chemical structures of NDMA and NDEA are illustrated in Figure 1.

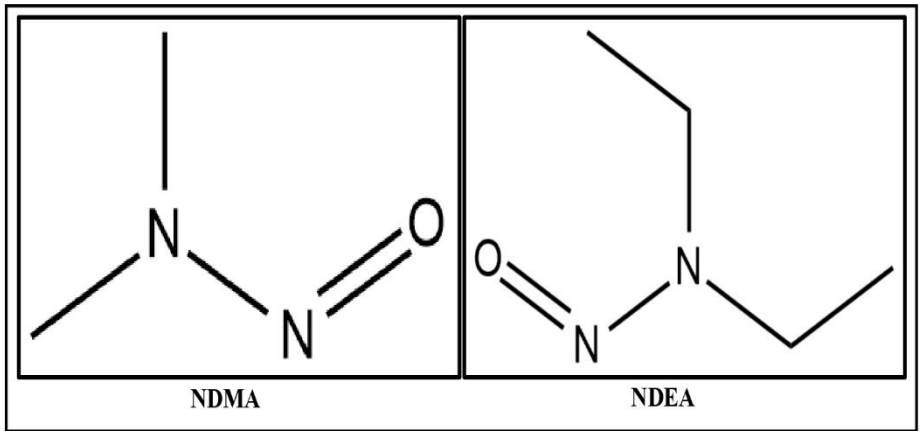

Figure 1: Chemical structure of NDMA and NDEA

These compounds are known to undergo metabolic activation to form highly reactive alkylating species capable of causing DNA damage(Michejda, Kroeger-Koepke et al. 1981). Because of these, even extremely low levels of nitrosamines are considered unacceptable in pharmaceutical products intended for long-term use(FDA 2021, Sedlo, Kolonić et al. 2021, Costa 2023).

In 2018, NDMA had been detected in several batches of angiotensin II receptor blockers, commonly preferred to as sartans, which prompted this instance of nitrosamine contamination in pharmaceuticals to the spotlight throughout the globe(Pottegård, Kristensen et al. 2018, Schmidtsdorff and Schmidt 2019). This triggered global research into the source and management of nitrosamine impurities and eventually led to extensive product recalls (Alsayadi, Dogra et al. 2025). The subsequent detection of NDMA and related nitrosamines in additional commonly used medications, including metformin, ranitidine, and nizatidine, emphasized the critical need for sensitive analytical techniques that can identify these contaminants at low levels(Manchuri, Shaik et al. 2024, Sampaio 2024).

The World Health Organization (WHO), the European Medicines Agency (EMA), and the U.S. Food and Drug Administration (FDA) all established stringent guidelines designed to minimize the risk of nitrosamines in pharmaceuticals in response to these assessments (FDA 2021, Sedlo, Kolonić et al. 2021,

Costa 2023). The International Conference for Harmonization (ICH) M7 guideline establishes extremely low acceptable intake (AI) concentrations based on lifetime cancer risk and offers a framework for the evaluation and management of genotoxic impurities, such as nitrosamines(Risk 2018). The AI is set at 96ng/day for NDMA and significantly lower at 26.5ng/day for NDEA(Roberts, Lennard et al. 2021). The development of extremely sensitive analytical techniques for monitoring is required since these limits correlate to concentrations in therapeutic products as low as parts per billion.

Atomoxetine hydrochloride, a selective norepinephrine reuptake inhibitor, is commonly used to treat attention-deficit hyperactivity disorder (ADHD). Atomoxetine, that exhibits a distinct mode of action from stimulant medications, is frequently used as a substitute for individuals who are unable to take stimulant therapy. Chemically, Atomoxetine has a secondary amine functional group that poses a structural possibility for the synthesis of nitrosamine in the presence of favorable circumstances, such as exposure to nitrites, certain solvents, or storage-related degradation pathways(Alsayadi, Dogra et al. 2025). Recognizing Atomoxetine's medicinal significance, it is imperative for ensuring that it is free of nitrosamine contamination for patient safety and regulatory compliance (Lungu-Mitea, Stein Aslund et al. 2025). Figure 2 symbolizes the structure of Atomoxetine hydrochloride.

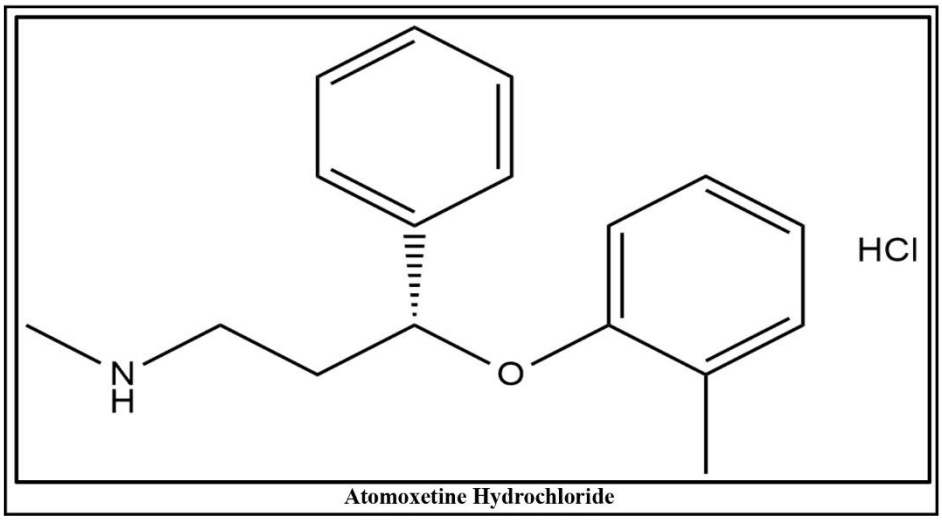

Figure 2: Structural Representation of Atomoxetine Hydrochloride

There are considerable analytical challenges in detecting nitrosamines at the incredibly low concentrations that regulators mandate(Kumar, Andrewsb et al.). The sensitivity and selectivity needed to accomplish trace-level quantification are frequently absent from conventional chromatographic approaches(Chao Huanga and Oub 2024). For the detection of nitrosamines, gas chromatography coupled with tandem mass spectrometry (GC-MS/MS) has become the analytical method of choice. This method assures both sensitivity and specificity, even in complicated pharmaceutical matrices by offering high-resolution separation together with the capability of utilizing multiple reaction monitoring (MRM) to track particular precursor-to-product ion transitions(Bian, Zhang et al. 2021).

Nowwithstanding the substantial regulatory focus on nitrosamine management, there is a scarcity of published information on approved techniques for NDMA and NDEA detection in Atomoxetine

hydrochloride in particular(Alsayadi, Dogra et al. 2025). To facilitate regular quality control and regulatory submission, this gap must be bridged.

Accordingly, the goal of the present investigation was to develop and validate a GC-MS/MS approach for the simultaneous detection of NDMA and NDEA in Atomoxetine hydrochloride. The technique was validated for specificity, accuracy, precision, and sensitivity, and it was intended to accomplish detection at or below regulatory requirements. Ultimately, the verified technique was employed on many Atomoxetine hydrochloride production batches to verify that there were no nitrosamine contaminants present.

2. Materials and Methods

2.1 Chemicals and Samples

Splendid Laboratories provided us with NDMA and NDEA reference standards (1000 ppm solutions in methanol). Dimethyl sulfoxide (DMSO, Honeywell GC-HS grade) was utilized as a diluent. Merck and Honeywell supplied analytical-grade isopropyl alcohol and toluene, respectively. As a gift sample, I received three consecutive batches of Atomoxetine hydrochloride.

2.2 Analytical and Instrumentation Conditions

The analysis was executed utilizing an Agilent 8890 GC system with a 7010B triple quadrupole mass spectrometer with MassHunter software. Chromatographic separation was accomplished using a CP-Wax 52 CB column (60 m × 0.25 mm i.d., .25 µm film thickness). With a split ratio of 5:1, helium was utilized as the carrier gas at a flow rate of 1.0 mL/min. Ion source and transfer line temperature were maintained at 240 ℃, while the injector temperature was fixed at 220 ℃. Data was acquired in MRM mode, analysing NDMA transitions of 74.1 → 44.2 and NDEA transition of 102.2 → 85.1. The oven program was set up to operate for 22 minutes, ramping up to 240 ℃ at a rate of 20 ℃ per minute and holding it there for 9.5 minutes after starting at 70 ℃ and holding it for 4 minutes.

2.3 Preparation of Solutions

In order to prepare working solutions for calibration and validation evaluations, stock solutions of NDMA and NDEA were prepared utilizing specified standards and diluted. After precisely weighing 250 mg of Atomoxetine hydrochloride into a 20 mL headspace vial, 1.0 mL of diluent was added to prepare the test solutions. After being sealed, the vials were examined.

2.4 Method Validation

In accordance with ICH M7 and regulatory standards for genotoxic contaminants, the method was validated. Specificity, precision, accuracy, system suitability, limit of quantification (LOQ), Limit of detection (LOD), and precision were among the validation parameters. Blanks, standards, and spiked samples were all analyzed to determine specificity. LOD and LOQ levels were established in order to determine sensitivity. Replica injections of standards and samples were used to measure precision, whereas recovery assessments at LOQ levels were used to evaluate accuracy.

3. Results and Discussion

Specificity

NDMA and NDEA were clearly separated chromatographically by the method, with retention times of 10.1 and 10.8 minutes, respectively. Neither the blank nor the specificity solutions showed any interfering peaks. The chromatograms of the NDMA and NDEA standard solutions and the specificity mixture solution are presented in Figure 3. The specificity of the approach was confirmed by the unambiguous recognition provided by the preferred MRM transitions.

Sensitivity

For both NDMA and NDEA, the LOD and LOQ were found to be 0.01 ppm and 0.03 ppm, respectively. Analyte peaks were consistently detected with tolerable precision (%RSD ≤ 20%) at these concentrations.

Accuracy and Precision

Recovery studies accomplished at LOQ levels demonstrated recoveries of 105% for NDEA and 99% for NDMA, which are well within the 70-130% acceptable range. With %RSD values of 4.2% for NDMA and 5.7% for NDEA, the approach showed exceptional precision and was well below the acceptable criteria.

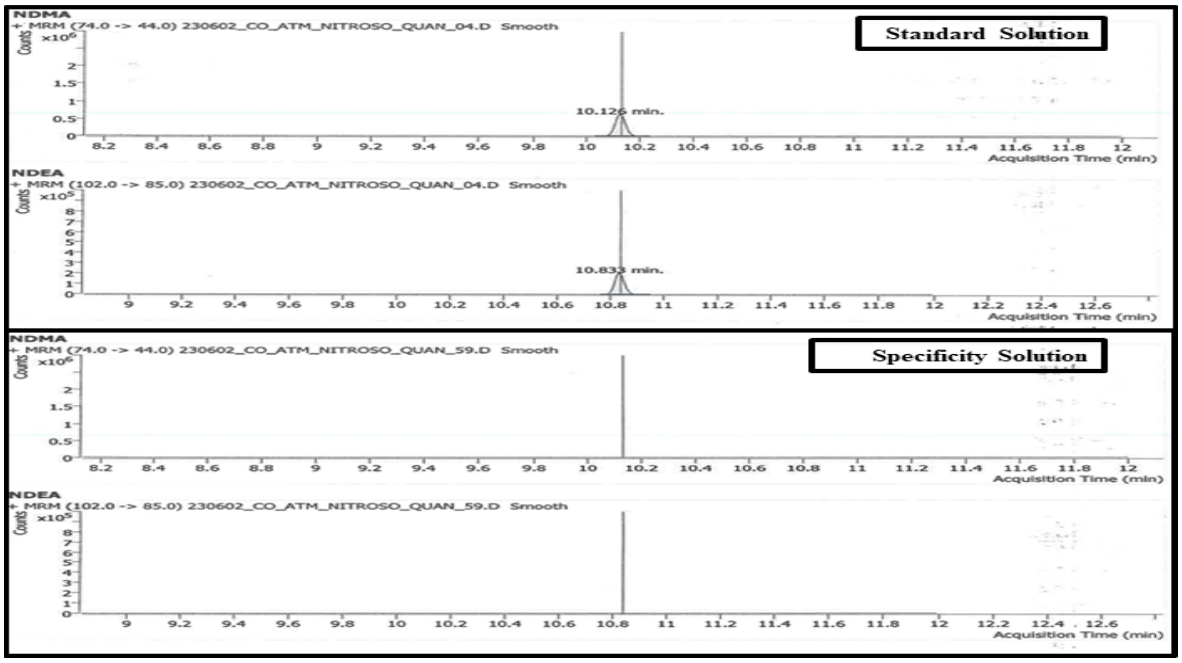

Figure 3: Overlay Chromatogram of Standard Solution and Specificity Mixture Solution

Application to Consecutive Batches

Three successive batches of Atomoxetine hydrochloride were analyzed using the verified method. The absence of NDMA and NDEA in every instance attests to the manufacturing process's resilience and validates compliance with regulatory requirements. Table 1 exhibits the NDMA and NDEA Validation Summary by GC-MS/MS.

Table 1: Validation Summary of NDMA and NDEA by GC-MS/MS

Parameter	NDMA Result	NDEA Result	Acceptance Criteria
Specificity	Peak observed at RT 10.1 min; MRM transition 74.1 → 44.2; no interference from blank or matrix	Peak observed at RT 10.8 min; MRM transition 102.2 → 85.1; no interference from blank or matrix	Peaks should be well resolved; no interference from blank or specificity solution
LOD (ppm)	0.01 ppm (0.0025 µg absolute)	0.01 ppm (0.0025 µg absolute)	Consistent detection
LOQ (ppm)	0.03 ppm (0.0075 µg absolute)	0.03 ppm (0.0075 µg absolute)	%RSD NMT 20%
Precision (%RSD at LOQ)	8.1%	10.3%	NMT 20%
Accuracy (%Recovery at LOQ Spike)	99%	105%	70-130%

4. Conclusion

The detection of NDMA and NDEA in Atomoxetine hydrochloride was accomplished by developing and validating a sensitive and reliable GC-MS/MS approach. The technique produced exceptional precision, high accuracy, and low detection limits. By implementing the approach to consecutive batches, the absence of NDMA and NDEA was verified, ensuring patient safety and regulatory compliance. This proven technique can be used as a model for nitrosamine testing in other pharmaceutical substances and is appropriate for routine quality control analysis of Atomoxetine hydrochloride.

Conflict of interest

The authors disclosed no conflict of interest.

Acknowledgments

We sincerely appreciate the principal and administration of the Arunodaya University for providing us with all the necessary resources to complete this research.

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