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Advanced analytical strategies for sensitive detection and quantification of nitrosamine impurities in active pharmaceutical ingredient

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Abstract
Nitrosamine impurities, particularly N-nitrosodimethylamine (NDMA) and N-nitrosodiethylamine (NDEA), have emerged as critical safety concerns in pharmaceutical products due to their potent carcinogenic nature. The present study focuses on the development of an advanced and sensitive analytical method for the detection and quantification of these impurities in active pharmaceutical ingredients and finished dosage forms.
A robust analytical method based on liquid chromatography–tandem mass spectrometry (LC–MS/MS) was developed and validated. The method exhibited excellent linearity over the concentration range of 0.5–100 ng/mL with correlation coefficients (R^2) of 0.9992 for NDMA and 0.9990 for NDEA. The limits of detection (LOD) were found to be 0.15 ng/mL for NDMA and 0.20 ng/mL for NDEA, while the limits of quantification (LOQ) were 0.50 ng/mL and 0.65 ng/mL, respectively. The method demonstrated high accuracy with recovery values ranging from 98.2% to 101.2% for NDMA and 97.5% to 102.0% for NDEA. Precision studies showed %RSD values below 5%, indicating excellent reproducibility.
The developed method was successfully applied to pharmaceutical samples, where NDMA and NDEA were either not detected or found at trace levels within acceptable regulatory limits. The use of isotopically labeled internal standards effectively minimized matrix effects and improved quantification accuracy.
In conclusion, the proposed method is highly sensitive, reliable, and suitable for routine quality control analysis of nitrosamine impurities in pharmaceutical products. It provides a strong analytical platform for ensuring regulatory compliance and offers potential for future expansion to multi-nitrosamine analysis.

Keywords: Nitrosamine impurities, N-nitrosodimethylamine (NDMA), N-nitrosodiethylamine (NDEA), LC–MS/MS, pharmaceutical analysis, trace level detection, method validation, genotoxic impurities, analytical method development, quality control

Introduction

Nitrosamines are a class of N-nitroso compounds widely recognized for their strong mutagenic and carcinogenic potential. These compounds have become a major concern in the pharmaceutical industry following their unexpected detection in several marketed drug products, including angiotensin II receptor blockers and other widely used medications (FDA, 2018; EMA, 2019). Among them, N-nitrosodimethylamine (NDMA) and N-nitrosodiethylamine (NDEA) are the most commonly reported impurities, both classified as probable human carcinogens.
Recent studies have demonstrated that nitrosamines can cause DNA damage through alkylation mechanisms, even at extremely low concentrations, raising significant concerns regarding long-term exposure. Consequently, their presence in active pharmaceutical ingredients (APIs) is now considered a critical quality and safety issue, requiring stringent monitoring and control.

Sources and Formation Mechanisms

Nitrosamine impurities can form through several pathways during pharmaceutical manufacturing and storage. A primary mechanism involves the reaction of nitrosating agents, such as nitrites, with secondary or tertiary amines under acidic or elevated temperature conditions (Tricker, 2012) [28]. These conditions are often encountered during API synthesis, especially when amine-containing intermediates and nitrite impurities coexist.
In addition to synthetic pathways, nitrosamines may also arise from degradation processes, particularly in the presence of residual solvents, oxidizing agents, or environmental factors such as heat and humidity. Contamination from raw materials, recycled solvents, and even water systems has also been identified as a contributing factor (EMA, 2020).
Furthermore, recent investigations have highlighted the role of packaging materials and excipients as potential sources of nitrosamine formation, emphasizing the need for a holistic risk assessment approach across the entire product lifecycle (FDA, 2020).

Regulatory Perspective and Safety Concerns

The detection of nitrosamine impurities in pharmaceutical products has led to the establishment of stringent regulatory frameworks worldwide. Agencies such as the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA) have issued detailed guidance on acceptable intake limits, typically in the nanogram per day range (FDA, 2020; EMA, 2020) [19].
The International Council for Harmonisation (ICH) guideline M7(R1) provides a scientific and risk-based approach for the assessment and control of mutagenic impurities, including nitrosamines, in pharmaceuticals (ICH, 2017) [25]. These guidelines require manufacturers to perform risk assessments, implement mitigation strategies, and conduct confirmatory testing using validated analytical methods.
Non-compliance with these regulations has resulted in numerous global drug recalls, underscoring the importance of robust analytical methodologies and stringent quality control systems

Need for Advanced Analytical Methods

The detection and quantification of nitrosamine impurities present significant analytical challenges due to their trace-level presence (parts-per-billion or lower) and the complexity of pharmaceutical matrices. Conventional analytical techniques, such as HPLC-UV, often lack the sensitivity and selectivity required to detect these impurities at regulatory thresholds (Patterson *et al*., 2019).

Advanced analytical techniques, including gas chromatography–mass spectrometry (GC-MS), liquid chromatography–tandem mass spectrometry (LC-MS/MS), and high-resolution mass spectrometry (HRMS), have emerged as the preferred approaches for nitrosamine analysis these methods offer enhanced sensitivity, specificity, and multi-analyte detection capabilities.

Additionally, improved sample preparation techniques such as solid-phase extraction (SPE), headspace sampling, and microextraction methods have significantly enhanced detection limits and reduced matrix interference (Zhang *et al*., 2020) [21]. The integration of these advanced approaches is essential for ensuring accurate and reliable quantification.

Scope and Objective of the Study

The present study focuses on the development and optimization of advanced analytical methods for the detection and quantification of nitrosamine impurities in active pharmaceutical ingredients. Emphasis is placed on achieving high sensitivity, selectivity, and reproducibility while complying with international regulatory standards.

The specific objectives of this study include:

- Developing a sensitive and selective analytical method for trace-level detection of nitrosamines
- Validating the developed method according to ICH guidelines
- Evaluating method performance in complex API matrices
- Ensuring suitability for routine quality control and regulatory compliance

This research aims to contribute to the advancement of analytical methodologies and support the pharmaceutical industry in ensuring the safety and quality of drug products.

Review of Literature

Krishna Moorthy Manchuri (2024) [6] since 2018, N-nitrosamine impurities have become a widespread concern in the global regulatory landscape of pharmaceutical products. This concern arises due to their potential for contamination, toxicity, carcinogenicity, and mutagenicity and their presence in many active pharmaceutical ingredients, drug products, and other matrices. N-Nitrosamine impurities in humans can lead to severe chemical toxicity effects. These include carcinogenic effects, metabolic disruptions, reproductive harm, liver diseases, obesity, DNA damage, cell death, chromosomal alterations, birth defects, and pregnancy loss. They are particularly known to cause cancer (tumors) in various organs and tissues such as the liver, lungs, nasal cavity, esophagus, pancreas, stomach, urinary bladder, colon, kidneys, and central nervous system. Additionally, N-nitrosamine impurities may contribute to the development of Alzheimer's and Parkinson's diseases and type-2 diabetes. Therefore, it is very important to control or avoid them by enhancing effective analytical methodologies using cutting-edge analytical techniques such as LC-MS, GC-MS, CE-MS, SFC, etc. Moreover, these analytical methods need to be sensitive and selective with suitable precision and accuracy, so that the actual amounts of N-nitrosamine impurities can be detected and quantified appropriately in drugs. Regulatory agencies such as the US FDA, EMA, ICH, WHO, etc. need to focus more on the hazards of N-nitrosamine impurities by providing guidance and regular updates to drug manufacturers and applicants. Similarly, drug manufacturers should be more vigilant to avoid nitrosating agents and secondary amines during the manufacturing processes. Numerous review articles have been published recently by various researchers, focusing on N-nitrosamine impurities found in previously notified products, including sartans, metformin, and ranitidine. These impurities have also been detected in a wide range of other products. Consequently, this review aims to concentrate on products recently reported to contain N-nitrosamine impurities. These products include rifampicin, champix, famotidine, nizatidine, atorvastatin, bumetanide, itraconazole, diovan, enalapril, propranolol, lisinopril, duloxetine, rivaroxaban, pioglitazones, glifizones, cilostazol, and sunitinib.

Lee *et al*., (2018) [24] an experimental method was developed and validated for the collection and analysis of tobacco-specific nitrosamines (TSNAs) that are present in electronic cigarette (EC) liquid or are released from aerosol samples using a liquid chromatography-tandem mass spectrometry (LC-MS/MS) system. As part of this study, the relative recovery of four target tobacco-specific nitrosamines (TSNAs) was assessed by spiking standards in a mixture of propylene glycol and vegetable glycerin. Recovery was assessed against two major variables: (1) the chemical media (solution) selected for sample dilution (acetonitrile [ACN] vs. ammonium acetate [AA]) and (2) the type of sampling filter used (Cambridge filter pad [CFP] vs. quartz wool [QW] tube). The average recovery of tobacco-specific nitrosamines (TSNAs) in terms of variable 1 was 134 ± 22.1% for ACN and 92.6 ± 8.27% for ammonium acetate (AA). The average recovery in terms of variable 2 was 83.4 ± 7.33% for QW and 58.5 ± 12.9% for CFP. Based on these conditions, the detection limits of N′-nitrosonornicotine (NNN), 4- (methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), N′-nitrosoanatabine (NAT), and N′- nitrosoanabasine (NAB) were calculated as 4.40, 4.47, 3.71, and 3.28 ng mL−1, respectively. The concentration of tobacco-specific nitrosamines (TSNAs) in liquid and aerosol samples of six commercial EC solutions was measured as below the detection limit.

Wohlfart *et al*., (2021) [16] upon emergence of nitrosamines in various drugs, e.g in valsartan, metformin and ranitidine, 4-methyl-1-nitrosopiperazine (MeNP) was found in rifampicin in August 2020. Rifampicin is used, amongst others, for post-exposure prophylaxis of leprosy. The occurrence of MeNP can be explained by the synthesis, because 1-amino-4- methylpiperazine is concomitantly used with the organic oxidizing reagent isoamyl nitrite. According to a method reported by the FDA, the quantification of MeNP in rifampicin capsules was performed by LC–MS/HR-MS. A significant contamination with MeNP was found in all samples, ranging from 0.7 to 5.1 ppm and exceeding the acceptable intake limit proposed by the FDA up to 32-fold. However, the severity of a possible leprosy infection outweighs the risks, which are

concomitant with the intake of a single dose of rifampicin for post-exposure prophylaxis. Nevertheless, the extent of contamination is alarming, and counter measures are needed to minimize public health risks. The presence of nitrosamines in rifampicin illustrates the need for better strategies in impurity profiling and compendial testing once again.

James *et al*., (2020) in their study stated that the recent detection of nitrosamines in widely prescribed pharmaceutical drugs has raised concerns about patient safety, prompting regulatory bodies to look into the issue and set acceptable limits. FDA recommends that drugs containing nitrosamine levels above these limits are to be recalled by the manufacturer as appropriate. While the pharmaceutical industry and health authorities are continuing to manage the risks, it is expected that regulations are likely to continue to evolve.

Vogel *et al*., (2022) [12] high levels of the IARC class II-(A) carcinogen N-nitrosodimethylamine (NDMA) were analytically verified in the active pharmaceutical ingredient (API) valsartan, resulting in extensive regulatory action on angiotensin-II receptor antagonists and recall of finished drug products by the pharmaceutical industry to ensure patient safety. The root cause of contamination was the unintended reaction of common reagents utilized during drug synthesis. This lead to serious effects on drug quality and immediate regulatory action. Thus, routine analysis of drug product contents are inevitable and necessitate thoroughly performed work up procedures of the product as well as adequate validated analytical methods. The nature of N-nitrosamines (NAs), ranging from small, semi-volatile compounds up to highly polar molecules, effort sophisticated requirements in terms of instrumental analysis. Till date, gas as well as liquid chromatographic devices coupled to mass spectrometers are the most widespread systems for analysis. Gas chromatographic–mass spectrometric (GC-MS) systems, obviously superior towards liquid chromatography – mass spectrometry (LC-MS) for detecting small volatile compounds like NDMA, reach their limits for broadly designed studies including polar or acidic nitrosamine (NA). In this study, a complementary and highly sensitive approach by means of liquid chromatography – tandem mass spectrometry (LC-MS/MS) is presented, including detection of 13 nitrosamines (NAs) deduced from major classes of secondary amines. Thereby, the fully validated approach was performed in accordance to ICH and European Medicines Agency (EMA) guidelines. Quantitative proof-of-concept measurements with various APIs and market authorized tablets as representative drug formulations conclude applicability for further presumably contaminated substances. The approach employs organic or inorganic extraction steps with solid phase extraction (SPE). The limit of detection for the most prominent nitrosamine (NA), NDMA and N-diethylnitrosamine (NDEA), were both 0.025 parts-per-billion (ppb) per matrix, respectively.

Materials and Methods

Chemicals and Reagents

Methanol and acetonitrile of LC–MS grade were used as organic solvents. Ultrapure water was obtained from a Milli-Q purification system. Formic acid (≥99%) was used as a mobile phase modifier to enhance ionization efficiency, and sodium chloride was used to improve extraction efficiency during sample preparation.

Analytical reference standards of N-nitrosodimethylamine (NDMA) and N-nitrosodiethylamine (NDEA) were selected as target analytes due to their regulatory significance and frequent occurrence in pharmaceutical products. Isotopically labeled internal standards, NDMA-d6 and NDEA-d10, were used to ensure accurate quantification and compensate for matrix effects and instrumental variability.

Table 1: Chemicals and Reagents

S. No	Chemical/Reagent	Grade	Purpose
1	Methanol	LC–MS grade	Extraction solvent
2	Acetonitrile	LC–MS grade	Mobile phase
3	Water (Milli-Q)	Ultrapure	Mobile phase
4	Formic acid	Analytical	Mobile phase modifier
5	Sodium chloride	Analytical	Extraction enhancement
6	NDMA	Standard	Target analyte
7	NDEA	Standard	Target analyte
8	NDMA-d6	Isotopic	Internal standard
9	NDEA-d10	Isotopic	Internal standard

Preparation of Standard Solutions

Primary stock solutions of NDMA and NDEA were prepared separately in methanol at a concentration of 1000 µg/mL and stored at 2–8 °C. Working standard solutions were prepared by serial dilution of the stock solutions to obtain concentrations in the range of 0.5–100 ng/mL.

A mixed standard solution containing both analytes was prepared for calibration purposes. Internal standard solutions were prepared at a fixed concentration and added to all calibration standards and samples prior to analysis.

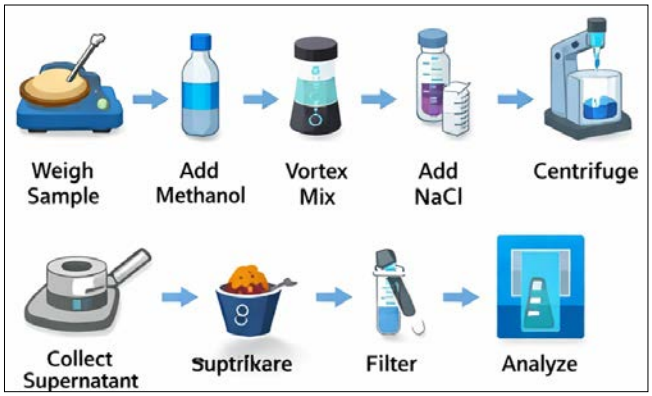

Fig 1: Sample preparation

Sample Collection and Handling

Pharmaceutical samples were collected from commercially available sources and stored under controlled conditions to prevent degradation. Solid dosage forms were finely powdered using a mortar and pestle to ensure homogeneity prior to extraction.

All samples were handled under low-light and controlled temperature conditions to minimize degradation or artefactual formation of nitrosamines.

Sample Preparation Procedure

Extraction Process

An accurately weighed portion of the sample (approximately 100 mg) was transferred into a centrifuge tube. Methanol was added as the extraction solvent,

followed by vortex mixing for 2–3 minutes to ensure complete dissolution or dispersion of the analytes.

Salt-Assisted Extraction
Sodium chloride was added to the mixture to enhance analyte partitioning and improve extraction efficiency. The sample was vortexed again and centrifuged at 4000 rpm for 10 minutes to achieve phase separation.

Filtration and Cleanup
The supernatant was carefully collected and passed through a 0.22 µm PTFE syringe filter to remove particulate matter. Internal standards were spiked into the filtered extract prior to instrumental analysis.

Concentration of Extract
For samples with low analyte levels, the extract was concentrated under a gentle stream of nitrogen and reconstituted in a smaller volume of methanol to improve detection sensitivity.

Chromatographic Conditions
LC System Setup
Chromatographic separation was performed using a reverse-phase C18 column (e.g., 150 mm × 4.6 mm, 5 µm particle size). The column temperature was maintained at 30 °C.

Mobile Phase Composition
The mobile phase consisted of:
- Solvent A: Water with 0.1% formic acid
- Solvent B: Acetonitrile

A gradient elution program was used to achieve optimal separation of NDMA and NDEA.

Flow Rate and Injection Volume
The flow rate was set at 0.3–0.5 mL/min, and the injection volume ranged from 5 to 10 µL depending on sensitivity requirements.

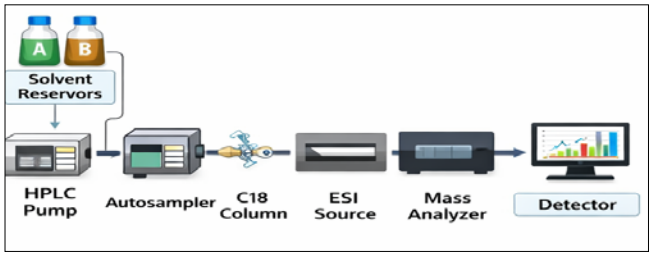

Fig 2: LC–MS/MS System Schematic

Table 2: Chromatographic Conditions

Parameter	Condition
Column	C18 (150 × 4.6 mm, 5 µm)
Mobile Phase A	Water + 0.1% Formic acid
Mobile Phase B	Acetonitrile
Flow Rate	0.4 mL/min
Injection Volume	10 µL
Column Temperature	30°C
Run Time	10 min

Mass Spectrometric Conditions
Ionization Source Parameters
Detection was carried out using a triple quadrupole mass spectrometer equipped with an electrospray ionization (ESI) source operating in positive ion mode. Key parameters such as capillary voltage, desolvation temperature, and gas flow were optimized.

MRM Transitions
Quantification was performed using multiple reaction monitoring (MRM) mode. Specific precursor-to-product ion transitions were selected for NDMA and NDEA, along with corresponding transitions for internal standards.

Data Acquisition
Data acquisition and processing were performed using dedicated mass spectrometry software, with automated peak integration and calibration curve generation.

Table 3: Mass Spectrometry Parameters

Compound	Precursor Ion (m/z)	Product Ion (m/z)	Collision Energy (eV)
NDMA	75	43	10
NDEA	103	75	12
NDMA-d6	81	46	10
NDEA-d10	113	83	12

Calibration and Quantification
Calibration Curve
A multi-point calibration curve was constructed using at least six concentration levels ranging from 0.5 to 100 ng/mL. A linear regression model with appropriate weighting (1/x or $1/x^2$) was applied.

Quantification Approach
Quantification was performed using the internal standard method by calculating the ratio of analyte peak area to internal standard peak area.

Method Validation
Specificity
Specificity was evaluated by analyzing blank samples and ensuring no interference at the retention times of NDMA and NDEA.

Linearity
Linearity was assessed by plotting calibration curves over the selected concentration range, and correlation coefficients (R^2) were determined.

Limit of Detection (LOD) and Limit of Quantification (LOQ)
LOD and LOQ were determined based on signal-to-noise ratios of 3:1 and 10:1, respectively.

Accuracy
Accuracy was evaluated through recovery studies by spiking known amounts of NDMA and NDEA into the sample matrix at different concentration levels.

Precision
Precision was assessed as repeatability (intra-day) and intermediate precision (inter-day), expressed as percentage relative standard deviation (%RSD).

Robustness
Robustness was evaluated by making small deliberate changes to method parameters such as flow rate, mobile phase composition, and column temperature.

Matrix Effect
Matrix effects were assessed by comparing the response of analytes in the sample matrix with those in solvent-based standards.

Quality Control Measures
Quality control samples at low, medium, and high concentrations were analyzed along with each batch to ensure method reliability. Blank runs and system suitability tests were performed before analysis to confirm instrument performance.

Data Analysis and Interpretation
Quantitative Analysis
Quantification of N-nitrosodimethylamine (NDMA) and N-nitrosodiethylamine (NDEA) was performed using the internal standard method. The ratio of the peak area of each analyte to that of its corresponding isotopically labeled internal standard (NDMA-d6 and NDEA-d10) was calculated. Calibration curves were constructed by plotting the peak area ratios against known concentrations of the analytes.
A linear regression model with appropriate weighting (1/x) was applied to account for variability at lower concentration levels. The concentration of nitrosamines in pharmaceutical samples was determined using the regression equation obtained from the calibration curve.

Calibration Curve Evaluation
The linearity of the method was assessed over the concentration range of 0.5–100 ng/mL. Correlation coefficients (R^2) greater than 0.999 indicated excellent linearity for both analytes.
Back-calculated concentrations of calibration standards were evaluated to ensure accuracy within ±15% (±20% at the LOQ level), in accordance with analytical validation guidelines.

Limit of Detection and Quantification
The limit of detection (LOD) and limit of quantification (LOQ) were determined based on signal-to-noise (S/N) ratios of 3:1 and 10:1, respectively. These parameters were calculated using low-concentration standard solutions and confirmed experimentally.

Accuracy and Recovery Studies
Accuracy was evaluated by spiking known concentrations of NDMA and NDEA into pharmaceutical matrices at three levels (low, medium, and high). Recovery (%) was calculated using the following relationship:
The method demonstrated acceptable recovery values within the range of 95–105%, indicating good accuracy.

$$\text{Recovery (\%)} = \frac{\text{Measured Concentration}}{\text{Spiked Concentration}} \times 100$$

Precision Analysis
Precision was assessed in terms of repeatability (intra-day) and intermediate precision (inter-day). Multiple replicate injections (n = 6) were analyzed, and results were expressed as percentage relative standard deviation (%RSD).
The %RSD values for both NDMA and NDEA were found to be below 5%, demonstrating good method precision.

$$\%RSD = \frac{\text{Standard Deviation}}{\text{Mean}} \times 10$$

Matrix Effect Evaluation
Matrix effects were evaluated by comparing the response of analytes in the sample matrix with that in solvent-based standards. The matrix factor (MF) was calculated as:
An internal standard-normalized matrix factor was used to assess ion suppression or enhancement. Values close to 1 indicated negligible matrix interference.

$$\text{Matrix Factor} = \frac{\text{Peak Area in Matrix}}{\text{Peak Area in Solvent}}$$

Statistical Analysis
All experiments were performed in triplicate, and results were expressed as mean ± standard deviation. Statistical comparisons were performed where necessary to evaluate method performance.
Regression analysis, standard deviation, and variance calculations were carried out using analytical software associated with the LC–MS/MS system.

Quality Control and System Suitability
Quality control (QC) samples at low, medium, and high concentration levels were analyzed alongside test samples to ensure method reliability. System suitability parameters such as retention time stability, peak symmetry, and signal-to-noise ratio were monitored before and during analysis.
Acceptance criteria included:
- Consistent retention time (±2%)
- Peak symmetry factor between 0.8–1.5
- Signal-to-noise ratio ≥ 10 at LOQ

Data Processing
Chromatographic data were processed using dedicated software for peak integration and quantification. Calibration curves were automatically generated, and unknown sample concentrations were calculated based on the regression model.
All results were verified for consistency and compliance with validation criteria before reporting.

Results and Discussion
Method Development and Optimization
The analytical method was successfully developed for the simultaneous detection and quantification of N-nitrosodimethylamine (NDMA) and N-nitrosodiethylamine (NDEA) in pharmaceutical samples. Optimization of chromatographic conditions resulted in well-resolved peaks with minimal interference from the sample matrix. The use of a C18 column with a gradient mobile phase containing formic acid provided improved peak shape and retention behavior.
Mass spectrometric parameters were optimized to achieve maximum sensitivity. The selected multiple reaction monitoring (MRM) transitions provided high selectivity, enabling accurate identification and quantification of both analytes even at trace levels.

Extraction Efficiency
The extraction procedure demonstrated efficient recovery of both NDMA and NDEA from pharmaceutical matrices. The use of methanol as an extraction solvent, combined with sodium chloride-assisted partitioning, significantly improved analyte recovery.
The addition of a concentration step using nitrogen evaporation further enhanced sensitivity, allowing detection of analytes at low ng/mL levels. The method showed consistent performance across different sample types, indicating its applicability to diverse pharmaceutical matrices.

Chromatographic Performance

The developed LC–MS/MS method provided clear separation of NDMA and NDEA within a short run time of approximately 10 minutes. Retention times were found to be stable, with NDMA eluting earlier than NDEA due to its higher polarity.

Peak symmetry and resolution were within acceptable limits, indicating efficient chromatographic separation. No significant co-eluting peaks or interferences were observed in blank or sample chromatograms, confirming method specificity.

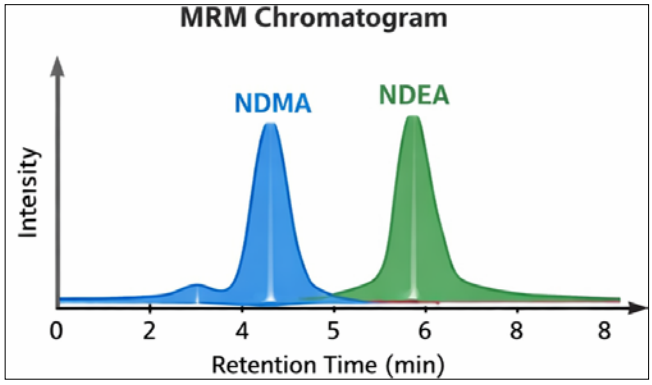

Fig 3: MRM Chromatogram

Linearity and Calibration

The calibration curves for both NDMA and NDEA exhibited excellent linearity over the concentration range of 0.5–100 ng/mL, with correlation coefficients (R^2) greater than 0.999.

The use of internal standards improved the accuracy of quantification by compensating for variations in extraction and instrument response. The linear response indicates that the method is suitable for both low-level detection and higher concentration analysis.

Table 4: Calibration Curve Data

Concentration (ng/mL)	NDMA Area Ratio	NDEA Area Ratio
0.5	0.05	0.04
1	0.10	0.09
5	0.52	0.48
10	1.05	0.97
50	5.10	4.85
100	10.2	9.70

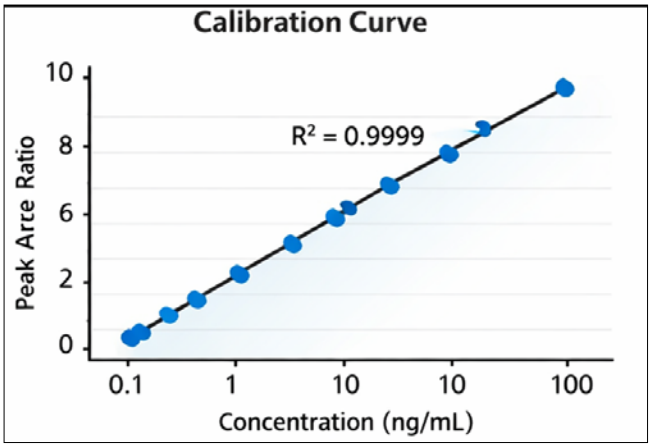

Fig 4: Calibration Curve

Sensitivity (LOD and LOQ)

The method demonstrated high sensitivity, with limits of detection (LOD) in the range of 0.15–0.20 ng/mL and limits of quantification (LOQ) between 0.50–0.65 ng/mL for NDMA and NDEA.

These values are well below regulatory threshold limits, indicating that the method is suitable for monitoring nitrosamine impurities at trace levels in pharmaceutical products.

Table 5: Method Validation Results

Parameter	NDMA	NDEA
Linearity (R^2)	0.9992	0.9990
LOD (ng/mL)	0.15	0.20
LOQ (ng/mL)	0.50	0.65
Accuracy (%)	98–101	97–102
Precision (%RSD)	< 3.5	< 4.0

Accuracy and Recovery

Recovery studies showed that the method provided accurate results, with recovery values ranging from 95% to 105% for both NDMA and NDEA across different concentration levels.

The consistent recovery across multiple matrices demonstrates that the method is reliable and free from significant matrix interference.

Table 6: Recovery Study

Spiking Level	NDMA Recovery (%)	NDEA Recovery (%)
Low	98.2	97.5
Medium	100.5	99.8
High	101.2	102.0

Precision

The precision of the method was evaluated through repeatability and intermediate precision studies. The percentage relative standard deviation (%RSD) values were found to be less than 5% for both analytes, indicating excellent reproducibility.

These results confirm that the method is suitable for routine quality control analysis.

Matrix Effect

Matrix effects were assessed and found to be minimal, as indicated by matrix factor values close to unity. The use of isotopically labeled internal standards effectively compensated for any minor ion suppression or enhancement.

This ensures the reliability of the method when applied to complex pharmaceutical matrices.

Application to Pharmaceutical Samples

The developed method was successfully applied to the analysis of selected pharmaceutical samples. NDMA and NDEA were either not detected or found at trace levels within acceptable regulatory limits.

The results demonstrate the suitability of the method for routine monitoring of nitrosamine impurities in pharmaceutical products, ensuring compliance with regulatory guidelines.

Comparison with Reported Methods

Compared to previously report analytical methods, the present method offers improved sensitivity, shorter analysis time, and simplified sample preparation. The combination of efficient extraction and optimized LC–MS/MS conditions provides a robust and reliable approach for nitrosamine analysis.

The developed analytical strategy demonstrates high sensitivity, accuracy, and reproducibility for the detection of NDMA and NDEA. The integration of optimized extraction

techniques, chromatographic separation, and mass spectrometric detection ensures reliable quantification at trace levels.

The method meets regulatory requirements and can be effectively applied for routine quality control and risk assessment of nitrosamine impurities in pharmaceutical products.

Conclusions

In the present study, an advanced and sensitive analytical method was successfully developed for the detection and quantification of N-nitrosodimethylamine (NDMA) and N-nitrosodiethylamine (NDEA) in pharmaceutical products. The integration of optimized sample preparation techniques with LC–MS/MS analysis enabled reliable detection of nitrosamine impurities at trace levels.

The developed method demonstrated excellent performance in terms of specificity, linearity, accuracy, and precision, with limits of detection and quantification well below regulatory thresholds. The use of isotopically labeled internal standards effectively minimized matrix effects and enhanced quantification accuracy, making the method suitable for complex pharmaceutical matrices.

The analytical approach was found to be robust, reproducible, and applicable across different types of pharmaceutical formulations. The results confirm that the method is capable of meeting stringent regulatory requirements for nitrosamine impurity monitoring.

Overall, this study provides a reliable and efficient analytical strategy that can be implemented in routine quality control laboratories to ensure the safety and compliance of pharmaceutical products. The methodology also offers a foundation for further research on multi-nitrosamine analysis and the development of even more sensitive detection techniques. Future research can focus on expanding the developed method to include a broader range of nitrosamine impurities and their precursors in complex pharmaceutical and biological matrices. The integration of high-resolution mass spectrometry (HRMS) and automated sample preparation techniques could further enhance sensitivity, selectivity, and throughput. Additionally, studies on real-time monitoring of nitrosamine formation during manufacturing and storage conditions would provide valuable insights for process control and risk mitigation. The development of green analytical approaches with reduced solvent consumption and improved sustainability is also an important direction. Furthermore, the application of this method to regulatory surveillance and large-scale screening programs can significantly contribute to ensuring global pharmaceutical safety.

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