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A REVIEW ON ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE DETERMINATION OF N-NITROSODIMETHYLAMINE IN CIPROFLOXACIN TABLETS USING GC-MS/MS

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ABSTRACT

N-Nitrosodimethylamine (NDMA) is a highly potent genotoxic impurity that has raised serious safety and regulatory concerns due to its carcinogenic potential. The discovery of NDMA contamination in various pharmaceutical products has necessitated the development of highly sensitive and selective analytical methods for its detection and quantification. Ciprofloxacin, a widely prescribed fluoroquinolone antibiotic, is considered at risk of nitrosamine contamination owing to its synthetic route, raw materials, and formulation processes. This review provides a comprehensive overview of analytical method development and validation strategies for the determination of NDMA in Ciprofloxacin Tablets USP 750 mg, with a specific focus on gas chromatography–tandem mass spectrometry (GC-MS/MS). The article discusses NDMA chemistry, toxicological significance, regulatory expectations, sources of NDMA formation, analytical challenges, sample preparation techniques, chromatographic and mass spectrometric conditions, and validation parameters in accordance with ICH guidelines. Recent advancements, limitations, and future perspectives in NDMA analysis are also highlighted.

KEYWORDS: N-Nitrosodimethylamine, Ciprofloxacin tablets, Nitrosamine impurities, GC-MS/MS, Method development, Method validation.

1. INTRODUCTION

Pharmaceutical impurities have a profound impact on drug safety, quality, and regulatory compliance. Among these, nitrosamine impurities have emerged as a critical concern following their detection in several drug products across multiple therapeutic classes. N-Nitrosodimethylamine (NDMA) is one of the most frequently reported nitrosamines due to its high carcinogenicity and low acceptable intake limits.

Ciprofloxacin is a second-generation fluoroquinolone antibiotic extensively used for the treatment of urinary tract infections, respiratory tract infections, gastrointestinal infections, and systemic bacterial diseases. Given its widespread use and long-term administration in some patient populations, ensuring the absence of genotoxic impurities such as NDMA in ciprofloxacin tablets is of paramount importance.

The detection of NDMA at trace levels (ng/g or ppb) in complex pharmaceutical matrices presents significant analytical challenges. Gas chromatography coupled with tandem mass spectrometry (GC-MS/MS) has emerged as one of the most reliable techniques for NDMA analysis due to its high sensitivity, selectivity, and robustness. This review critically evaluates the analytical approaches employed for NDMA estimation in ciprofloxacin tablets, emphasizing method development and validation using

GC-MS/MS.

Pharmaceutical quality assurance has undergone increasing scrutiny in recent years due to the detection of nitrosamine impurities such as N-nitrosodimethylamine (NDMA) in approved drug products. NDMA is a low-molecular-weight nitrosamine that has been identified as a potent genotoxic compound capable of inducing tumors in multiple organ systems at low exposure levels. Its occurrence in drugs such as angiotensin receptor blockers (ARBs), ranitidine, and metformin led global regulatory agencies to issue safety alerts, product recalls, and guidance on testing and control strategies.

Ciprofloxacin, a second-generation fluoroquinolone antibiotic, is widely prescribed for combatting both Gram-negative and Gram-positive bacterial infections. Ciprofloxacin tablets are included on the World Health Organization's Model List of Essential Medicines and are administered worldwide. Because of its global utilization and long-term dosing in some therapies, ensuring the absence of genotoxic impurities such as NDMA is critical for patient safety and regulatory compliance.

Analytical method development for trace-level impurities requires techniques that combine sensitivity, selectivity, and robustness. Gas chromatography coupled with tandem mass spectrometry (GC-MS/MS) has emerged as a gold standard for volatile and semi-volatile nitrosamines, including NDMA, primarily because of its ability to differentiate target analytes from complex pharmaceutical matrices.

This review comprehensively explores the development and validation of GC-MS/MS methods for NDMA determination in ciprofloxacin tablets, highlights regulatory expectations, discusses analytical challenges, and maps recent advances and future perspectives.

2. N-Nitrosodimethylamine: Chemical and Toxicological Overview

NDMA is a small, volatile, and polar nitrosamine compound with the molecular formula $C_2H_6N_2O$. It is formed through nitrosation reactions involving secondary amines and nitrosating agents such as nitrites under acidic or high-temperature conditions.

Toxicologically, NDMA is classified as a probable human carcinogen (Group 2A) by the International Agency for Research on Cancer (IARC). It has been shown to cause liver toxicity, mutagenicity, and tumor formation in animal studies at extremely low exposure levels. Due to its genotoxic nature, NDMA does not have a safe threshold, necessitating strict regulatory limits.

Chemical Properties

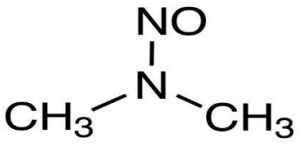

Fig. 1: N-Nitrosodimethylamine.

N-Nitrosodimethylamine (NDMA) is a semi-volatile organic compound with the chemical formula $C_2H_6N_2O$ and a molecular weight of 74.08 g/mol. It belongs to the N-nitrosamine class, formed by the nitrosation of secondary amines in the presence of nitrosating agents (e.g., nitrites) under acidic or elevated temperature conditions. NDMA is soluble in water, organic solvents such as methanol and dichloromethane, and exhibits moderate volatility—making it amenable to headspace gas chromatography techniques.

Toxicological Significance

NDMA is classified by the International Agency for Research on Cancer (IARC) as a Group 2A carcinogen (probable human carcinogen). Extensive animal studies have demonstrated hepatic carcinogenicity at microgram-level exposures, and NDMA has been shown to induce DNA alkylation, contributing to mutagenesis. Its genotoxicity is linked to metabolic activation in the liver via cytochrome P450 enzymes. Acute and chronic exposure may also lead to liver toxicity, reproductive toxicity, and systemic adverse effects.

Because NDMA exhibits no clear threshold below which genotoxic effects are absent, regulatory bodies have set extremely low acceptable intake (AI) levels for NDMA in pharmaceuticals, typically in the low nanogram per day range.

3. Regulatory Landscape for NDMA in Pharmaceuticals

Global regulatory authorities have issued stringent guidelines to control nitrosamine impurities in pharmaceuticals:

* US FDA: Mandates risk assessment, confirmatory testing, and reporting of NDMA levels.
* EMA: Requires manufacturers to identify and mitigate nitrosamine risks.
* ICH M7: Provides guidance on the assessment and control of DNA-reactive impurities.

The acceptable intake (AI) limit for NDMA is generally set at 96 ng/day, though lower limits may apply depending on treatment duration and patient exposure. Analytical methods must therefore achieve detection limits well below this threshold.

Global regulatory agencies have issued guidance and mandatory requirements for the control and testing of NDMA and other nitrosamines in drugs:

FDA (USA): Issued guidance on risk assessment and control strategies for nitrosamine impurities. It established acceptable intake limits and recommended validated analytical testing for NDMA in active pharmaceutical ingredients (APIs) and finished products.

EMA (European Union): Released a detailed guideline requiring manufacturers to evaluate potential nitrosamine formation pathways and implement control strategies. It emphasizes risk assessment and requires appropriate analytical methods with defined performance characteristics.

ICH Q3A/B: Covers impurity evaluation and reporting limits in pharmaceuticals but has been supplemented by specific nitrosamine guidance due to the unique toxicological profiles of these compounds.

USP General Chapter <1469> (United States Pharmacopeia): Provides procedures for nitrosamine detection, including method recommendations and performance criteria.

Regulatory expectations include

- Comprehensive risk assessment throughout synthesis and formulation.
- Use of validated methods capable of detecting NDMA well below accepted intake limits (often ≤100 ng/day equivalent).
- Demonstration of method specificity, sensitivity, accuracy, precision, robustness, and stability.

4. Sources of NDMA in Ciprofloxacin Tablets

NDMA contamination in ciprofloxacin tablets may arise from several sources:

* Use of dimethylamine or secondary amines in synthesis
* Presence of nitrite impurities in raw materials
* Solvent degradation during manufacturing
* Cross-contamination during production
* Packaging and storage conditions

Understanding these sources is essential for both analytical method development and risk mitigation.

NDMA in ciprofloxacin tablets may arise from multiple pathways:

- Synthesis-Related Formation: Use of dimethylamine precursors or nitrosating agents in the synthesis of ciprofloxacin or intermediates can lead to residual NDMA formation.
- Excipient Contamination: Excipients containing secondary amines or nitrosating impurities (e.g., sodium nitrite) may react under formulation conditions.
- Solvent Impurities: Solvents used during synthesis or formulation may contain nitrosating agents that facilitate NDMA formation.
- Process Conditions: Elevated temperatures and acidic environments during manufacturing can catalyze nitrosation reactions.
- Cross-Contamination: Shared equipment without effective cleaning protocols may transfer nitrosamines from one production batch to another.

Understanding these sources is crucial for analytical strategy and process control.

5. Analytical Challenges in NDMA Determination

The estimation of NDMA in ciprofloxacin tablets is analytically demanding due to:

* Extremely low regulatory limits
* Volatile and thermally stable nature of NDMA
* Matrix interference from API and excipients
* Risk of in-situ NDMA formation during analysis

These challenges necessitate the use of highly selective and sensitive analytical techniques.

Quantifying NDMA in pharmaceutical matrices such as ciprofloxacin tablets presents several key challenges:

- ✓ **Trace-Level Detection:** Regulatory limits for NDMA are extremely low (nanogram per day intake), often requiring detection limits in the parts-per-billion (ppb) or lower range.
- ✓ **Matrix Interference:** Excipients and APIs may co-elute or suppress ionization, complicating quantification.
- ✓ **Artifact Formation:** Sample preparation conditions may inadvertently generate NDMA from precursors, leading to false positives.
- ✓ **Volatility and Stability:** NDMA's volatile nature necessitates careful handling and suitable analytical techniques, such as headspace sampling or optimized GC conditions.
- ✓ **Instrument Requirements:** High sensitivity and selectivity instruments (GC-MS/MS) are required, which demand skilled operators and rigorous quality control.

6. OVERVIEW OF ANALYTICAL TECHNIQUES FOR NDMA ANALYSIS

Several analytical techniques have been reported for NDMA determination:

6.1 LC-MS/MS

While widely used, LC-MS/MS may suffer from matrix suppression and limited sensitivity for volatile nitrosamines.

6.2 GC-MS

GC-MS offers better volatility compatibility but may lack sufficient selectivity at ultra-trace levels.

6.3 GC-MS/MS

GC-MS/MS operating in multiple reaction monitoring (MRM) mode provides superior sensitivity, selectivity, and reproducibility, making it the preferred technique for

NDMA analysis in pharmaceuticals.

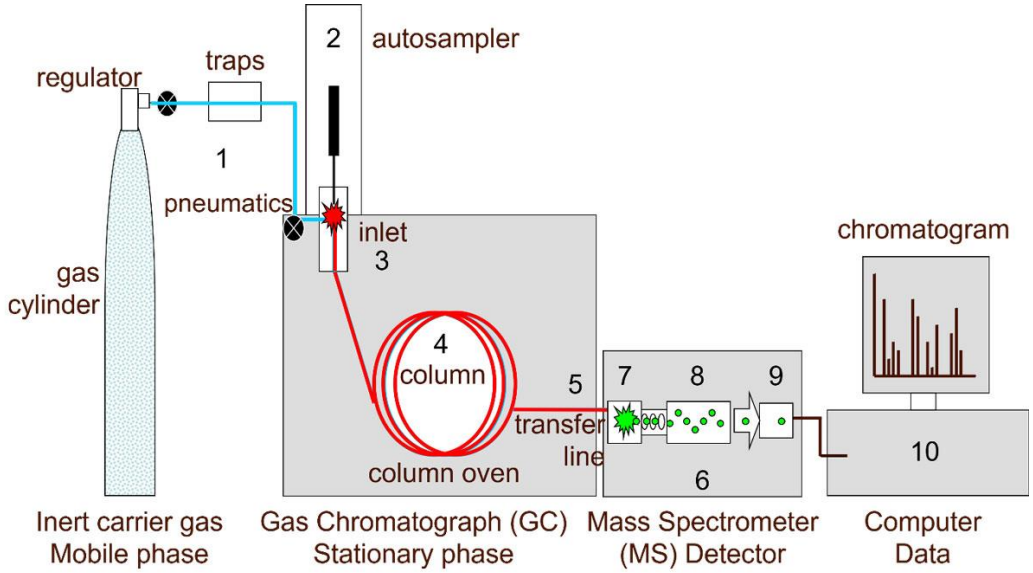

Fig. 2: Schematic Diagram of GC-MS/MS.

Table 1: Multiple Analytical Approaches Have Been Applied To NDMA Determination.

Technique	Principle	Pros	Cons
GC-MS	Separates volatile analytes by gas chromatography, detects by mass spectrometry	Good separation, established technology	Limited sensitivity for ultra-trace levels
GC-MS/MS	Tandem mass spectrometry with multiple reaction monitoring (MRM)	Superior selectivity & sensitivity	Costly, requires expertise
LC-MS/MS	Liquid chromatography coupled to tandem MS	Applicable to non-volatile analytes	Often lower sensitivity for volatile nitrosamines
Headspace GC-MS(/MS)	Volatile compounds measured from headspace	Minimizes matrix	Optimization required to avoid artifact formation
SPE + GC-MS/MS	Solid-phase cleanup before GC	Reduces matrix interference	Additional prep time

Discussion: GC-MS/MS stands out as the preferred method for volatile nitrosamines like NDMA due to its ability to isolate target compounds from complex matrices using MRM, greatly improving signal-to-noise ratios compared to single-quadrupole GC-MS. LC-MS/MS can be used for less volatile nitrosamines or when GC analyzability is an issue, but its applicability for NDMA in ciprofloxacin is limited due to NDMA's volatility and low molecular weight.

7. REVIEW OF LITERATURE OF NDMA

Table-2: Reported GC-MS/MS Methods for NDMA in Pharmaceuticals.

Reference	Drug Matrix	Sample Prep	Detection	LOD/LOQ	Key Findings
Lim et al., 2020	Sartans	Headspace	GC-MS/MS	<0.5 ng/mL	Effective for NDMA/NDEA
Zou et al., 2023	Metformin HCl	SPE + GC-MS/MS	GC-MS/MS	<1 ng/mL	High recovery using SPE
Derringer et al., 2020	Ranitidine	HS-GC-MS/MS	GC-MS/MS	~0.2 ng/mL	Differentiated NDMA from matrix
Zhao et al., 2021	Sartan API	Liquid extraction + GC-MS/MS	GC-MS/MS	~0.3 ng/mL	Validated protocol
Mohanareddy et al., 2025	Zidovudine	SPE + GC-MS/MS	GC-MS/MS	~0.8 ng/mL	Good precision & accuracy

Discussion: Across multiple drug matrices, GC-MS/MS has reliably achieved ultra-trace detection of NDMA (often <1 ng/mL), demonstrating its general applicability. Sample preparation varied from direct headspace techniques to SPE cleanup, indicating flexibility based on matrix complexity.

8. GC-MS/MS METHOD DEVELOPMENT FOR NDMA IN CIPROFLOXACIN TABLETS

8.1 Sample Preparation

Sample preparation is critical to minimize matrix interference and avoid artificial NDMA formation. Common approaches include:

* Solvent extraction using methanol or dichloromethane
* Solid-phase extraction (SPE) for cleanup
* Filtration and concentration steps

The choice of extraction solvent and conditions significantly affects recovery and reproducibility.

8.2 Chromatographic Conditions

Optimized GC conditions typically include:

* Capillary columns such as DB-624 or equivalent
* Helium as carrier gas
* Temperature-programmed oven conditions

Proper chromatographic separation ensures minimal interference and accurate quantification.

8.3 Mass Spectrometric Detection

Electron impact ionization is commonly used for NDMA analysis. In GC-MS/MS, specific precursor-to-product ion transitions are monitored, significantly enhancing selectivity and lowering detection limits.

9. METHOD VALIDATION ACCORDING TO ICH GUIDELINES

Analytical methods must be validated as per ICH Q2(R1) guidelines.

9.1 Specificity

Ability to unequivocally assess NDMA in the presence of ciprofloxacin and excipients.

Ability of the method to unequivocally assess NDMA in the presence of ciprofloxacin and excipients. Verified through:

- ✓ Blank matrix analysis
- ✓ Spiked matrix preparations
- ✓ Confirmation via multiple MRM transitions

9.2 Linearity

Typically demonstrated over a low ng/mL concentration range.

Demonstrated by analyzing calibration standards over a defined range, often down to low ppb concentrations. Linearity typically requires:

- ➢ Correlation coefficient (r^2) ≥0.99
- ➢ Consistent response across calibration levels

9.3 Accuracy

Evaluated through recovery studies at multiple spiking levels.

Assessed by recovery studies at multiple spiking levels (e.g., 1 ng/g, 5 ng/g, 10 ng/g). Acceptable recovery: 80–120% (varies by guideline and matrix).

9.4 Precision

Includes repeatability and intermediate precision.

Evaluated as

- ➢ Repeatability (intra-day): Same day analyses
- ➢ Intermediate precision (inter-day): Across days, analysts

Precision usually expressed as %RSD ≤15%.

9.5 LOD and LOQ

Critical parameters ensuring compliance with regulatory limits.

Limits defined using signal-to-noise ratios (LOD ~3:1, LOQ ~10:1) or statistical approaches. Methods typically achieve LOQs <1 ng/g.

9.6 Robustness

Assesses method reliability under small variations in conditions.

Assessed by deliberate small changes in method conditions (e.g., temperature, flow rate) to confirm method stability.

Validated GC-MS/MS methods consistently demonstrate LOQs well below regulatory thresholds.

10. Applications in Quality Control and Regulatory Compliance

GC-MS/MS methods are increasingly used for:

* Routine quality control testing of finished ciprofloxacin batches
* Stability studies to assess NDMA formation over time
* Regulatory submissions by pharmaceutical manufacturers
* Risk assessment and mitigation strategies required by regulators

Their robustness and sensitivity make them suitable for long-term monitoring of NDMA in ciprofloxacin tablets.

These tests ensure that NDMA does not exceed acceptable intake limits and that the product meets safety criteria before market release.

11. Recent Advances and Emerging Trends

Recent developments include:

- ❖ Automated sample preparation: SPE automation and robotic headspace systems reduce variability and increase throughput.
- ❖ Microextraction techniques: Headspace solid-phase microextraction (HS-SPME) improves detection without solvents.
- ❖ High-resolution MS (HRMS): Provides exact mass confirmation, beneficial in complex matrices.
- ❖ Green analytical techniques: Minimize solvent use and waste.
- ❖ AI-Assisted data processing: Improves peak recognition and quantification reliability.

These advancements aim to improve throughput, accuracy, and sustainability.

12. Limitations and Challenges

Despite its advantages, GC-MS/MS has limitations:

* High instrument costs remain high, limiting accessibility.
* Requirement for skilled personnel
* Potential for contamination if not properly controlled
* Expertise requirements for GC-MS/MS operation and data interpretation.
* Matrix complexity can still pose interference challenges.
* Artifact generation risks if sample prep conditions are not carefully controlled.

Continuous method optimization is necessary to overcome these challenges.

13. Future Perspectives

Future research should focus on:

* Preventive strategies for NDMA formation
* Standardized analytical protocols mandated across pharmacopeias.
* Integration of analytical and manufacturing controls
* Real-time monitoring technologies during manufacturing.
* Expanded green analytical chemistry principles to improve sustainability.

Such approaches will enhance pharmaceutical safety and regulatory compliance.

14. CONCLUSION

The determination of NDMA in Ciprofloxacin Tablets USP 750 mg is a critical requirement driven by patient safety and regulatory expectations. GC-MS/MS has emerged as a powerful analytical tool due to its exceptional sensitivity and selectivity for trace-level NDMA analysis. Comprehensive method development and rigorous validation are essential to ensure accurate, reliable, and reproducible results. This review highlights the importance of GC-MS/MS-based analytical methodologies in safeguarding pharmaceutical quality and underscores the need for continuous advancement in impurity analysis.

NDMA contamination in pharmaceuticals poses significant risks to public health and regulatory compliance. GC-MS/MS has proven to be a powerful analytical technique for the trace determination of NDMA in ciprofloxacin tablets due to its high sensitivity, selectivity, and adaptability to volatile analytes. Careful method development, rigorous validation per ICH guidelines, and appropriate application in quality control are paramount to ensure patient safety. Although challenges remain, continued advancements in analytical science will facilitate more efficient, accurate, and sustainable testing strategies.

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Conflicts of Interest

The authors declare no conflicts of interest.

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