

Development and Validation of a Sensitive GC-MS Method for Determination of Nitrosamine Impurities in Foscarnet Sodium Injection

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Abstract:

Nitrosamine impurities are an important pharmaceutical quality concern because several members of this chemical class possess mutagenic and carcinogenic potential. The present study focused exclusively on the GC-MS component of the Ph.D. work and evaluated selected nitrosamine impurities in Foscarnet Sodium Injection. The investigated nitrosamines were N-nitrosodimethylamine (NDMA), N-nitrosodiethylamine (NDEA), N-nitrosodiisopropylamine (NDIPA), and N-nitrosodibutylamine (NDBA). A DB-1701 column (30 m × 0.25 mm, 1 µm) was used with helium carrier gas at 1.0 mL/min, an injector temperature of 250°C, electron-impact ionization, 2.0 µL injection volume, and a total run time of 12.33 min. Method validation included specificity, linearity, accuracy, precision, LOD, LOQ, robustness, and system suitability. No interfering peaks were observed in solvent blank and placebo, while clear peaks were obtained for NDMA, NDEA, NDIPA, and NDBA. The GC-MS calibration range was 5–100 ng/mL. Representative NDMA linearity gave $y = 2487x + 215$ with $r^2 = 0.9989$. The reported LOD was 1–3 ng/mL and LOQ was 5 ng/mL. NDMA recovery at 50%, 100%, and 150% levels was 94.0%, 95.5%, and 96.7%, with %RSD values of 7.8%, 6.2%, and 5.4%, respectively. Repeatability was within the stated acceptance limit and no significant variation was observed under deliberate parameter changes. The developed GC-MS method therefore demonstrated sensitivity and selectivity suitable for trace-level nitrosamine analysis in Foscarnet Sodium Injection.

Keywords: Foscarnet Sodium Injection; GC-MS; nitrosamine impurities; NDMA; NDEA; NDIPA; NDBA; SIM mode; method validation; ICH M7.

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1. Introduction

Impurities in pharmaceutical products are unwanted chemicals that may influence product efficacy and safety even when present in small quantities. Impurity profiling therefore

involves both identification and quantitative assessment of impurities associated with the active pharmaceutical ingredient, formulation, manufacturing process, environment, and product aging^{1,2}.

Nitrosamines are organic compounds containing a nitroso group (-NO) bonded to nitrogen, generally represented as $R_2N-N=O$. The Ph.D. study identified nitrosamines as a major contemporary impurity concern because they may be mutagenic and carcinogenic and may enter drug substances or drug products through reagents, catalysts, solvents, raw materials, process formation, direct introduction, degradation, or cross-contamination^{3,4}.

Regulatory and scientific attention to nitrosamines increased following their detection in pharmaceutical products. The study framework referred to ICH M7, the U.S. FDA guidance on control of nitrosamine impurities in human drugs, USP <1469>, WHO guidance, EMA guidance, TGA guidance, and Health Canada recommendations^{3,5,6}.

Gas chromatography coupled with mass spectrometry is particularly useful for volatile and semi-volatile nitrosamines because chromatographic separation can be combined with mass-selective detection. Published GC-MS approaches included in the source material also support trace-level determination of nitrosamines in pharmaceutical matrices^{7,8,9}.

Foscarnet Sodium Injection is a parenteral antiviral product. The broader Ph.D. project investigated both elemental and nitrosamine impurities; however, the present manuscript deliberately excludes the ICP-MS elemental-impurity component and reports only the GC-MS nitrosamine method-development and validation work¹⁰.

2. Research Rationale

The source study identified a need for a sensitive and selective procedure capable of detecting nitrosamine impurities at very low concentrations in Foscarnet Sodium Injection. The analytical strategy therefore emphasized selective SIM-mode detection, low ng/mL sensitivity, a short chromatographic run time, and validation of specificity, linearity, accuracy, precision, LOD, LOQ, robustness, and system suitability. The selected nitrosamines for the GC-MS specificity study were NDMA, NDEA, NDIPA, and NDBA.

3. Aim and Objectives

The aim of the study was to develop and validate a sensitive GC-MS method for determination of selected nitrosamine impurities in Foscarnet Sodium Injection. The objectives were to establish selective detection of the target nitrosamines, optimize GC-MS conditions, establish a calibration range suitable for trace analysis, assess specificity and linearity, determine LOD and LOQ, evaluate recovery and precision, examine robustness under deliberate analytical variations, and demonstrate applicability to the Foscarnet Sodium Injection matrix.

4. Materials and Methods

5.

Nitrosamine Impurities Considered

The summarized seven nitrosamine impurities and their corresponding amine precursors and acceptable intake limits. The GC-MS specificity and validation work specifically focused on NDMA, NDEA, NDIPA, and NDBA.

Table 1. Types of nitrosamines and acceptable intake limits reported

Sr. No.	Amine	Corresponding nitrosamine impurity	AI limit (ng/day)
1	Dimethylamine	N-nitrosodimethylamine (NDMA)	96.00
2	Diethylamine	N-nitrosodiethylamine (NDEA)	26.50
3	Dipropylamine	N-nitrosodipropylamine (NDPA)	96.00
4	Diisopropylamine	N-nitrosodiisopropylamine (NDIPA)	26.50
5	Dibutylamine	N-nitrosodibutylamine (NDBA)	96.00
6	Ethylmethylaniline	N-nitrosomethylethylaniline (NMEA)	26.50
7	4-(methylaniline)butanoic acid	N-nitroso-N-methyl-4-aminobutyric acid (NMBA)	96.00

GC-MS Conditions

The optimized GC-MS conditions were taken directly from the experimental presentation. Chromatographic separation used a DB-1701 capillary column. Helium served as the carrier gas and electron impact was used as the ion source. Dichloromethane was used as the diluent.

Table 2. GC-MS instrumental conditions reported

Parameter	Condition
Column	DB 1701 (30 m × 0.25 mm, 1 µm)
Liner	4 mm split liner or equivalent
Injector temperature	250°C
Carrier gas	Helium
Column flow	1.0 mL/minute
Split ratio	Pulsed Split less 12.285 psi until 0.5 min
Run time	12.33 minutes
Diluent	Dichloromethane
Sample concentration	1 mL
He quench gas	2.25 mL/min
N ₂ collision gas	1.5 mL/min
Injection volume	2.0 µL
Ion source	Electron Impact
MS source temperature	250°C
MS transfer line temperature	250°C
MS Quad	150°C

Method Validation Design

Method validation study was designed to demonstrate suitability, reliability, and regulatory compliance of the developed analytical procedure. The validation framework used specificity, linearity, accuracy, precision, LOD/LOQ, robustness, and system suitability.

Table 3. GC-MS validation design and acceptance criteria reported

Parameter	Source-study procedure / criterion
Specificity	Solvent blank, placebo extract, individual nitrosamine standards and spiked samples; no interfering peaks at analyte retention times.
Linearity	Calibration solutions at 5, 10, 25, 50, 75 and 100 ng/mL; $r^2 \geq 0.99$.
Accuracy	Spiking at 50%, 100% and 150% of specification level; mean recovery 90–110%; %RSD $\leq 10\%$ for GC-MS.
Precision	Repeatability using six independent preparations; intermediate precision on different days/analysts/instruments; GC-MS %RSD $\leq 10\%$.
LOD	Signal-to-noise approximately 3:1.
LOQ	Signal-to-noise approximately 10:1.
Robustness	Carrier-gas flow $\pm 10\%$, oven temperature $\pm 5^\circ\text{C}$, collision-gas flow ± 0.5 mL/min; no significant change in retention time or response.
System suitability	GC-MS %RSD $\leq 10\%$ in validation summary; resolution > 2 for nitrosamines in the method-validation slide.

Specificity Procedure

For GC-MS specificity, a solvent blank was injected to check background interference. A placebo extract prepared using the same liquid-liquid extraction procedure was analyzed, followed by individual nitrosamine standards and spiked sample solutions. Retention times and monitored SIM ions were compared. The acceptance criterion of detectable interfering signal in blank and placebo with clear analyte resolution and consistent retention times.

Linearity Procedure

Nitrosamine calibration solutions were prepared at 5, 10, 25, 50, 75, and 100 ng/mL. The reported a representative NDMA regression equation of $y = 2487x + 215$ with $r^2 = 0.9989$.

Accuracy and Precision

Accuracy was evaluated by spiking at 50%, 100%, and 150% levels and analyzing each level in triplicate. Precision was assessed using repeatability and intermediate-precision concepts. The detailed numerical recovery was for NDMA; therefore, no unreported analyte-specific recovery values were noted for NDEA, NDIPA, or NDBA.

LOD, LOQ, Robustness and Data Analysis

LOD and LOQ were determined using signal-to-noise ratios of approximately 3:1 and 10:1, respectively. Robustness included deliberate variation in carrier-gas flow, oven temperature, and collision-gas flow. GC-MS quantitative analysis was performed using MassHunter GC-MS Quantitative Analysis Software and statistical analysis was performed using Microsoft Excel.

6. Results

Specificity

No interfering peaks were observed in the solvent blank and placebo. Clear peaks were obtained for NDMA, NDEA, NDIPA, and NDBA. These observations supported selective detection using SIM mode.

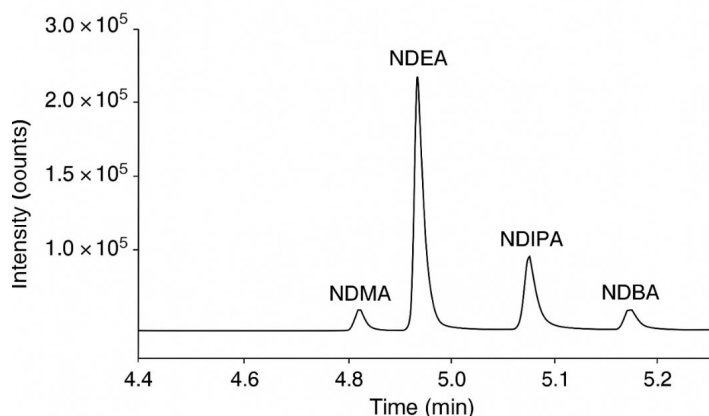
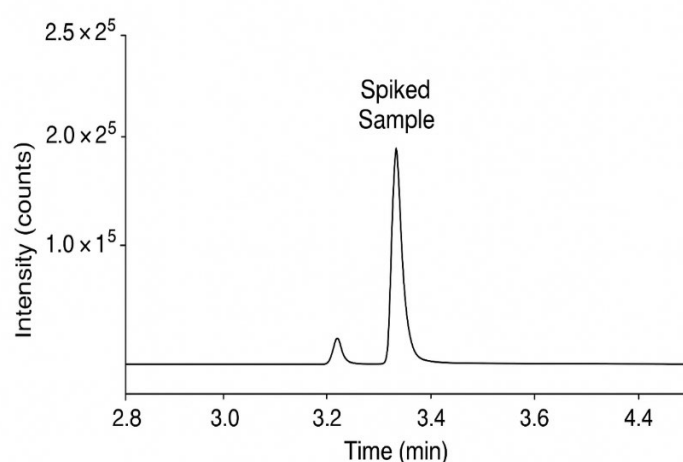


Figure 1: GC-MS SIM chromatogram of nitrosamine standards (NDMA, NDEA, NDIPA, and NDBA with RT labels)



Blank GC-MS' Arits Chromatogram of nitrosamine

Figure 2: GC-MS blank vs spiked sample chromatograms

Linearity

The GC-MS linearity range was 5–100 ng/mL. Representative NDMA data demonstrated a proportional increase in peak area with concentration. The reported regression equation was $y = 2487x + 215$ and the correlation coefficient was $r^2 = 0.9989$.

Table 4. GC-MS calibration curve summary for NDMA (representative)

Concentration (ng/mL)	Peak Area	%RSD
5	12,450	6.5
10	25,120	5.2
25	61,890	4.3
50	124,500	3.1
75	186,230	2.6
100	248,910	2.1

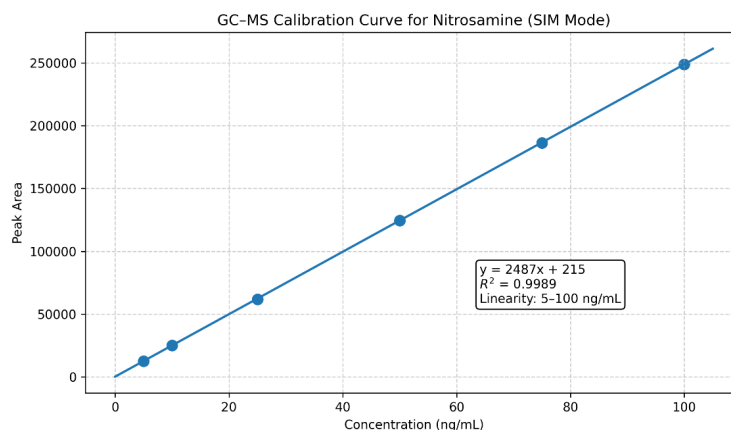


Figure 3: GC-MS Calibration Curve (SIM Mode)

Accuracy

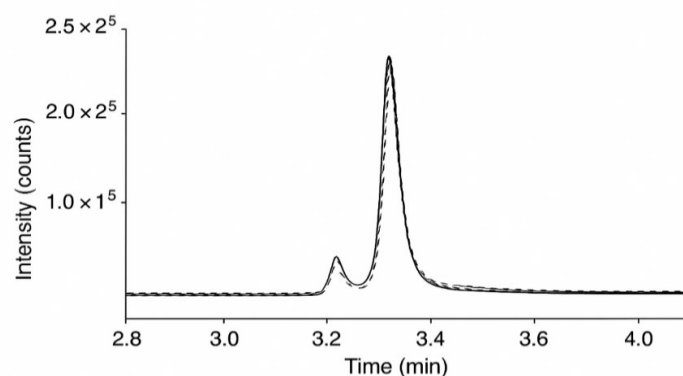
The representative NDMA recovery results ranged from 94.0% to 96.7%. The %RSD decreased from 7.8% at the 50% level to 5.4% at the 150% level. All reported results were within the GC-MS accuracy and precision limits stated in the source validation plan.

Table 5. GC-MS accuracy results for NDMA

Spiking Level	Added (ng/mL)	Found (ng/mL)	% Recovery	%RSD
50%	10	9.4	94.0	7.8
100%	20	19.1	95.5	6.2
150%	30	29.0	96.7	5.4

Precision

GC-MS repeatability was evaluated using overlay chromatograms. The source study reported consistent retention times and peak areas and concluded that repeatability was within acceptable limits.



GC-MS Repeatability Overlay (n=6 injections)

Figure 4: GC-MS Repeatability Overlay (n=6)

LOD and LOQ

The reported GC-MS LOD was 1–3 ng/mL and the LOQ was 5 ng/mL. These values demonstrated low-level sensitivity of the developed procedure for nitrosamine analysis.

Robustness and System Suitability

No significant variation was observed under deliberate parameter changes. The method-validation summary reported GC-MS system-suitability %RSD $\leq 10\%$, while the broader validation summary described robustness within acceptable limits.

Table 6. GC-MS method-validation summary reported in the source study

Validation Parameter	GC-MS Result
Specificity	No interfering peaks observed in solvent blank and placebo; clear peaks for NDMA, NDEA, NDIPA, and NDBA.
Linearity Range	5–100 ng/mL
Calibration Levels	5, 10, 25, 50, 75, 100 ng/mL
Regression Equation (NDMA)	$y = 2487x + 215$
Correlation Coefficient (NDMA)	$r^2 = 0.9989$
Accuracy (% Recovery)	50%: 94.0% (RSD 7.8%); 100%: 95.5% (RSD 6.2%); 150%: 96.7% (RSD 5.4%)
Precision	Repeatability within acceptable limits using overlay chromatograms
LOD	1–3 ng/mL
LOQ	5 ng/mL
Robustness	No significant variation observed under deliberate parameter changes
System Suitability	%RSD $\leq$ 10%
Overall Conclusion	Method suitable for ultra-trace nitrosamine analysis as per ICH M7 and ICH Q2(R2)

Comparative Analysis of Marketed Injectable Formulations

The source presentation also compared Foscarnet Sodium Injection with two marketed injectable formulations. For the nitrosamine portion, NDMA, NDEA, NDIPA, NDBA, and NMBA were reported as not detected in all three evaluated formulations.

Table 7. Nitrosamine results from comparative analysis of marketed injectable formulations

Parameter	Foscarnet Sodium Injection	Marketed Formulation A	Marketed Formulation B
GC-MS Method	Validated	Validated	Validated
Nitrosamine Evaluation	Performed	Performed	Performed
NDMA	Not Detected	ND	ND
NDEA	Not Detected	ND	ND
NDIPA	Not Detected	ND	ND
NDBA	Not Detected	ND	ND
NMBA	Not Detected	ND	ND

7. Discussion

The developed GC-MS method addressed the requirement for trace-level nitrosamine analysis in a parenteral pharmaceutical matrix. The work demonstrated specificity through the absence of interfering peaks in blank and placebo preparations and the presence of clear target

peaks in standard and spiked preparations. This is important because nitrosamine analysis requires discrimination of low-level analyte signals from matrix-related background.^{7,8,9}

The reported calibration range of 5–100 ng/mL covered the established LOQ and extended to a concentration suitable for evaluating method response over a broad low-level analytical interval. Representative NDMA linearity, with $r^2 = 0.9989$, met the acceptance requirement of $r^2 \geq 0.99$.

The LOD of 1–3 ng/mL and LOQ of 5 ng/mL demonstrated the sensitivity of the method. These performance characteristics are consistent with the purpose of a nitrosamine impurity procedure, where the analytical challenge is detection and quantification at trace concentration.

Accuracy was demonstrated in the available quantitative NDMA dataset by recoveries of 94.0%, 95.5%, and 96.7% at the 50%, 100%, and 150% levels. The corresponding %RSD values remained below the 10% GC-MS precision criterion stated in the validation plan. The precision overlay further showed consistent chromatographic behavior across replicate analyses.

Robustness testing considered small deliberate changes in carrier-gas flow, oven temperature, and collision-gas flow. The validation summary reported no significant variation, supporting the reliability of the method during routine analytical operation.

The comparative marketed-formulation data showed no detected NDMA, NDEA, NDIPA, NDBA, or NMBA in the evaluated products. Because the detailed numerical validation data available are most compatible for NDMA.

The study work are within the framework of ICH M7 and analytical procedure validation guidance. ICH M7 addresses assessment and control of DNA-reactive mutagenic impurities, while the current ICH Q2(R2) framework describes validation characteristics for analytical procedures.

8. Limitations

The principal limitation is that the detailed analyte-specific numerical validation dataset preserved in the source presentation is most complete for NDMA. The study confirms chromatographic specificity for NDMA, NDEA, NDIPA, and NDBA.

9. Conclusion

A sensitive GC-MS method was developed and validated for selected nitrosamine impurities in Foscarnet Sodium Injection. The method employed a DB-1701 column, helium carrier gas, electron-impact ionization, and a 12.33-minute run time. Specificity studies showed no interfering peaks in solvent blank and placebo and clear peaks for NDMA, NDEA, NDIPA, and NDBA. The working calibration range was 5–100 ng/mL, and representative NDMA analysis produced $y = 2487x + 215$ with $r^2 = 0.9989$. The reported LOD was 1–3 ng/mL and LOQ was 5 ng/mL. NDMA recovery was 94.0–96.7%, with %RSD of 5.4–7.8%. Repeatability was acceptable and robustness testing showed no significant variation under deliberate changes. Based on the experimental results, the developed GC-MS procedure was suitable for trace-level nitrosamine impurity analysis in Foscarnet Sodium Injection.

10. Conflict of Interest

The authors declare no conflicts of interest related to the publication of this article.

11. Acknowledgement

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Author Contribution: Krina Thakkar: Conceptualization, data curation, formal analysis, writing – original draft; Foram Patel: investigation, supervision, validation; Parina Dobariya and Kalubha Zala: data curation, formal analysis; Ravikumar Patel: data curation, formal analysis and supervision.

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