Validated GC–APCI–MS/MS Method for Sensitive Determination of N-Nitrosodimethylamine in Valsartan Hydrochloride

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DOI: https://doi.org/10.51244/IJRSI.2026.1306000351

Received: 19 June 2026; Accepted: 24 June 2026; Published: 10 July 2026

ABSTRACT

The detection of trace-level nitrosamine impurities remains a critical challenge in pharmaceutical analysis, requiring highly selective and reproducible chromatographic and spectroscopic techniques. In this study, we present a validated gas chromatography–atmospheric pressure chemical ionization tandem mass spectrometry (GC–APCI–MS/MS) method for the quantification of N-nitrosodimethylamine (NDMA) in valsartan hydrochloride. The method eliminates pre-concentration steps, enabling rapid high-throughput analysis with a runtime of ~12 minutes. Validation was performed in accordance with ICH Q2(R1) guidelines. The method demonstrated excellent linearity ($R^2 = 0.99985$), low detection limit (LOD = 0.045 ppm), quantitation limit (LOQ = 0.090 ppm), and high recovery (90.4–97.5%). Compared with conventional electron ionization (EI) approaches, APCI provided enhanced selectivity and reduced matrix interference. The robustness of the method was confirmed through precision studies across multiple concentration levels, with %RSD values well within acceptance criteria. This work highlights the analytical advantages of GC–APCI–MS/MS for nitrosamine determination and establishes a reliable platform for pharmaceutical quality control, with potential extension to environmental and food safety applications.

Keywords: Gas Chromatography, Genotoxic Impurities, Mass Spectrometry, NDMA, Valsartan Hydrochloride

INTRODUCTION

Impurity control is a crucial aspect of the pharmaceutical industry that ensures the safety and efficacy of medications. In recent years, the International Council for Harmonization (ICH) has introduced M7 (R1) guidelines. These guidelines focus on the assessment and control of DNA-reactive (mutagenic) impurities in pharmaceuticals to minimize potential carcinogenic risks. Consequently, the management of genotoxic impurities has become a critical element in the drug development process. This includes stages such as research and development, new drug applications, and batch quality control during the drug production. By adhering to these guidelines, the industry aims to protect patients from harmful impurities and maintain high pharmaceutical quality standards. [1-5].

Genotoxic compounds are substances that can bind to DNA and cause mutations. These mutations can potentially lead to severe consequences, such as congenital malformations and cancer. By interfering with the genetic material of cells, genotoxic compounds pose a significant risk to human health, underscoring the importance of their careful assessment and control in pharmaceutical development and production.[6] Genotoxic impurities can be introduced during drug production through various means, such as the use of reactants and solvents, chemical degradation, and side reactions. N-nitrosamines (NAs) are a well-known group of genotoxic chemicals. These harmful compounds are often formed when nitrosating agents, such as nitrogen oxides or nitrites, react with secondary or tertiary amines. The presence of these impurities underscores the need for stringent control and monitoring to ensure the safety and quality of pharmaceutical products. [7] N-nitrosamines (NAs) have been identified in several widely used drugs, including valsartan, metformin, and ranitidine. [8–10] In July 2018, an

incident involving valsartan produced by Zhejiang Huahai Pharm Ltd. revealed contamination with N-nitrosodimethylamine (NDMA), prompting a global recall of the affected batches. [8, 11–13] Besides NDMA, other NAs like N-nitrosodiethylamine (NDEA), N-nitroso-N-methylaminobutyric acid (NMBA), N-nitrosodiisopropylamine (NDIPA), N-nitrosoethylisopropylamine (NEIPA), and N-nitrosodibutylamine (NDBA) have been classified as genotoxic impurities in active pharmaceutical ingredients (APIs) and their formulations. 15. Therefore, it is essential to upgrade sensitive analytical methods to detect various NAs in the pharmaceutical industry. [13, 16]

Recent advancements have enabled the determination of NAs in food, water, and drugs using techniques such as liquid chromatography (LC), gas chromatography (GC), gas–liquid chromatography (GLC), and non-chromatographic methods. [8, 11, 12, 17–20] LC and GC have also been combined with mass spectrometry (MS), tandem mass spectrometry (MS/MS), fluorescence spectroscopy, and UV spectroscopy to enhance their detection capabilities. [15, 21–26.]Generally, sample pre-concentration is required before instrument injection, employing methods such as solid-phase extraction (SPE), [23] solid-phase microextraction (SPME), [27] micro-SPE, [21] dispersive liquid–liquid microextraction, [22] microwave-assisted extraction, [28] needle trap device, [29] and QuEChERS extraction. [30] However, some no-sample-preparation methods have been reported. [12, 24]

For instance, Amelin et al. utilized microwave-assisted extraction-GLC-MS and liquid-liquid extraction-ultra performance LC-MS to determine NAs in food, achieving a limit of quantification (LOQ) for NDMA in beer as low as 2 ng/ml. [23, 28] Lashgari et al. analyzed NAs in wastewater and swimming pool water using micro-SPE-GC-EI-MS/MS, with an LOQ for NDMA of 1.157 ng/ml 21. Liu et al. developed a sensitive and stable method for determining NAs in Sartan substances using GC-MS/MS without any pre-concentration, achieving an LOQ for NDMA of 3.0 ng/ml. [24]

In this study, a novel methodology for the trace determination of NAs in Valsartan Hydrochloride was developed using Gas Chromatography with atmospheric-pressure chemical ionization tandem mass spectrometry (GC-APCI-MS/MS). By employing multiple-reaction monitoring (MRM) scanning in the positive ionization mode, this method enhances analytical selectivity and sensitivity while reducing interference and noise in the second quadruple detector. [31–35] The European Pharmacopoeia (Ph.Eur Eur.) Supplement 10.6, Chapter 2.5.42. HPLC APCI-MS/MS in the MRM mode is recommended for the quantification of NAs. This approach has been optimized and validated for system suitability, selectivity, linearity, range, accuracy, precision, sensitivity, solution stability, and robustness.

One of the key advantages of the developed method is its short analytical time and the fact that it does not require preconcentration procedures, making it highly convenient for the drug production industry. Additionally, this methodology is instrumental in guiding the efficient and rapid analysis of various NAs in active pharmaceutical ingredients (APIs).

By implementing such advanced analytical techniques, the pharmaceutical industry can more effectively ensure the safety and quality of medications while maintaining compliance with regulatory standards.

MATERIALS AND METHODS

1. Instrumentation

- GC-MS System: Shimadzu GCMS TQ with Electron Impact Ionization (EI) mode
- Column: ZB-WAXplus capillary column (30 m × 0.32 mm × 1.0 μm)
- Balance: Mettler Analytical Balance
- Carrier Gas: Helium (99.999% purity)
- CID Gas: Argon

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2. Reagents and Standards

- Acetonitrile: GC-grade or Analytical Reagent (A.R.) grade (used as diluent)
- Methanol: HPLC grade (used for vial washing)
- NDMA Standard: High-purity analytical grade

3. Sample Preparation

- Blank Solution

Fill the autosampler vial with the diluent.

- Standard Solutions

1. Stock Solution-1 (1,200 µg/mL)

Weigh 12.0 mg NDMA standard into 10 mL volumetric flask

Dilute to volume with diluent

2. Stock Solution-2 (24.0 µg/mL)

Pipette 1.0 mL of Stock-1 into 50 mL flask

Dilute to volume

3. Stock Solution-3 (0.48 µg/mL)

Pipette 1.0 mL of Stock-2 into 50 mL flask

Dilute to volume

4. Standard Solution (9.6 ng/mL)

Pipette 1.0 mL of Stock-3 into 50 mL flask

Dilute to volume

5. System Check Solution (1.44 ng/mL)

Pipette 3.0 mL of Standard Solution into 20 mL flask

Dilute to volume

- Test Solution

Weigh 160.0 mg valsartan sample into 5 mL volumetric flask

Add 2.0 mL diluent, shake for 1 min

Dilute to volume, vortex at 2,750 rpm for 1 min

Let stand 2–3 min with intermittent shaking

Transfer to autosampler vial

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4. Chromatographic Conditions

- Injection Parameters
  - Mode: Splitless
  - Volume: 2.0 µL
  - Injector Temp: 200 °C
  - Split Ratio: 50:1
  - Purge Flow: 3.0 mL/min
  - Injection Pressure: 15 psi
  - Oven Program: 80 °C (hold 3 min) → ramp 20 °C/min → 230 °C (hold 12 min)
- Autosampler Settings
  - Pre-rinse: 3
  - Post-rinse: 10
  - Sample rinse: 3
  - Plunger speed: High
  - Injection dwell time: 0.3 sec

5. MS Parameters

- Ion Source Settings
- Ion Source Temp: 230 °C
- Detector Voltage: +0.40 kV
- Solvent Cut Time: 4.0 min
- Acquisition Mode: MRM

Procedure

1. **Column Equilibration:**
   - The column was equilibrated with the carrier gas under the specified chromatographic conditions.
2. **Instrument Tuning:**
   - Performed tuning of the instrument in 'Normal Mode' using argon gas as the CID gas.
3. **Injection Sequence:**
   - Equal volumes of the prepared solutions were injected into the chromatograph according to the following sequence:

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4. **Data Acquisition:**

- Chromatograms were recorded for each injection.
- System suitability parameters were measured, including retention time, peak area, and resolution.

5. **System Suitability and Optimization:**

- MS parameters were optimized as necessary (excluding m/z values) to meet system suitability criteria. [Table 1A]
- Ensured that the system check solution met the predefined acceptance criteria before proceeding with sample analysis.

Sequence of Injections: [Table 1B]

Table 1: MS Transition Table

Channel	**Precursor Ion [m/z]**	**Product Ion [m/z]**	**Collision Energy [V]**	**Transition**
Ch1	74.0	42.0	16.0	74.0 > 42.0
Ch2	74.0	43.0	11.0	74.0 > 43.0
Ch3	74.0	44.0	6.0	74.0 > 44.0

Sequence of Injections		
Sr No.	**Solution**	**Number of Injections**
1.	Blank Solution	2
2.	System Check Solution	1
3.	Standard Solution	6
4.	Blank Preparation	1
5.	Test Preparation – 1	1
6.	Test Preparation - 2	1

In this validation as the prime interest is anlaysis of n-Nitrosodimethylamine rest all un-relevant peaks are rejected if observed in test sample except peak of n-Nitrosodimethylamine

Retention times:

Retention time of impurity - N-Nitrosodimethylamine – 6.4 min

Calculation:

The area of the TIC of the impurity was used for the calculation.

The concentration of N-nitrosodimethylamine was calculated in ppm, and the results were reported on an average basis using the following formula:

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$$\frac{At}{As} \times \frac{Ws}{10} \times \frac{1}{50} \times \frac{1}{50} \times \frac{5}{Wt} \times \frac{P}{100} \times 10^6$$

Where,

At = Area of the N-nitrosodimethylamine impurity in the test chromatogram.

As = Average area of N-nitrosodimethylamine impurity from standard chromatograms.

Ws = Weight of N-Nitrosodimethylamine impurity standard in mg

Wt = weight of test in mg

P = Percent purity of the N-nitrosodimethylamine impurity standard.

Validation Protocol and Statistical Analysis

Method Validation is a critical process in analytical chemistry, and we meticulously performed this validation to ensure that the method is suitable and reliable for its intended purpose. We systematically examined and demonstrated the method's performance characteristics, such as accuracy, precision, specificity, linearity, range, detection limit, quantitation limit, robustness, and stability. These parameters were analyzed following the ICH Q2(R1) "Validation of Analytical Procedures: Text and Methodology" guidelines, ensuring that our validation process meets international standards.

- System Suitability [Figure 1A]

Inject blank (×2), system check (×1), standard (×6), test prep (×2)

NDMA peak must be visually detected in system check

%RSD of NDMA peak area in standard injections ≤ 15%

- Specificity

Confirm no interference in blank, standard, and spiked test solutions

NDMA retention time: 6.4 min

- LOD & LOQ [Figure 1B, 1C]

LOD: 0.045 ppm

LOQ: 0.090 ppm

Established via visual detection and recovery studies

- Precision

System Precision: %RSD = 1.41

Repeatability: %RSD = 0.42

Intermediate Precision: Not detected across replicates

Precision at LOQ, 100%, 200%: RSD = 2.76, 1.41, 0.76 respectively

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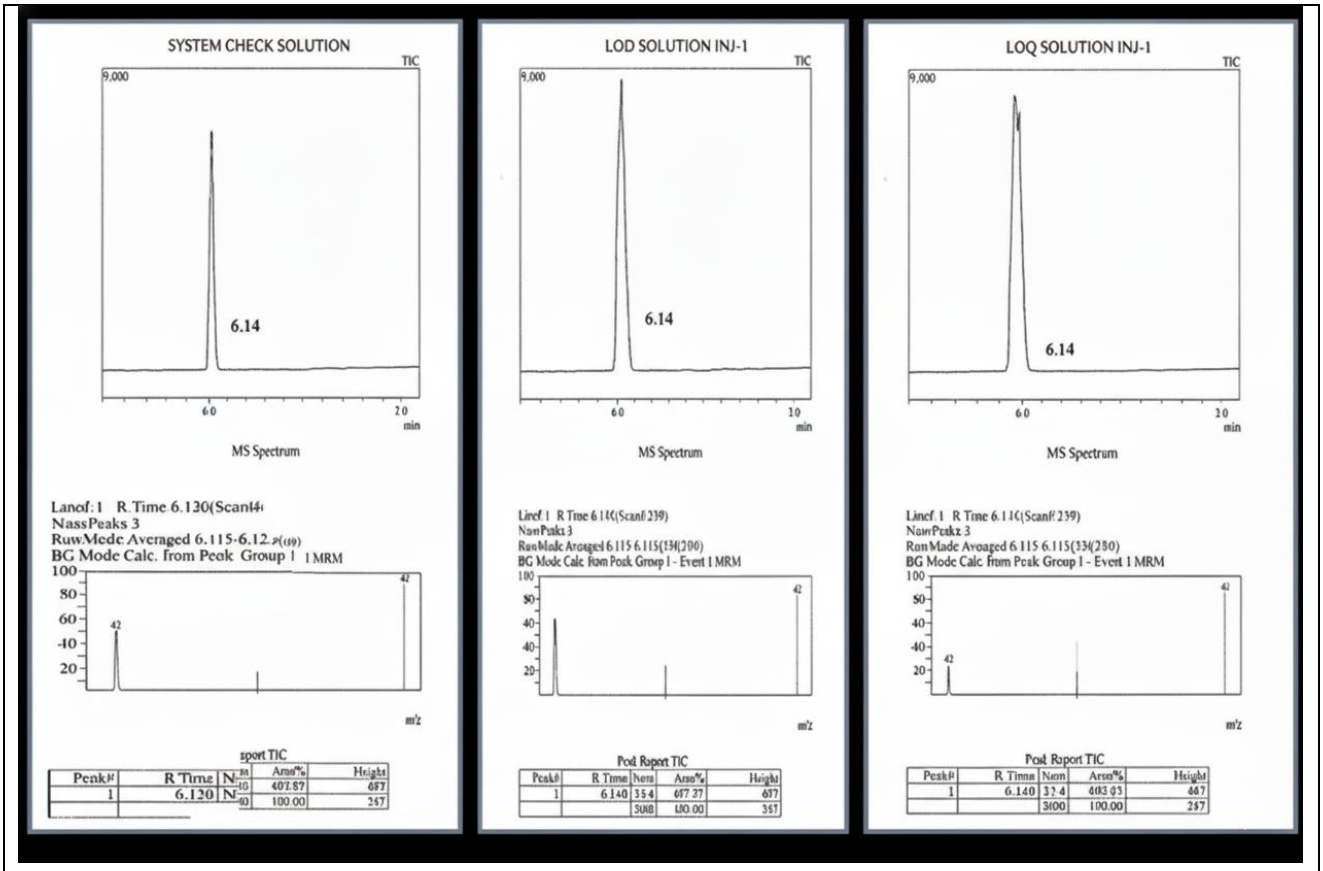

Figure 1

System Suitability	LOD	LOQ

- Linearity [Figure 2]

Range: LOQ to 200%

$R^2 = 0.99985$

Slope = 890,548.29; Intercept = –163.61

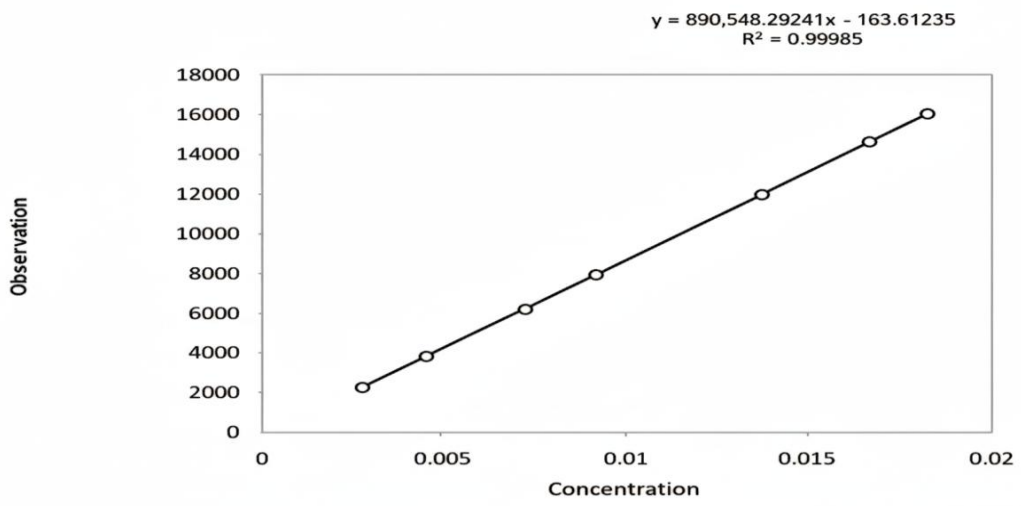

Figure 2: Linearity

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- Accuracy

Recovery at LOQ: 97.5%

Recovery at 100%: 94.7%

Recovery at 150%: 90.4%

RESULTS AND DISCUSSION

The results for all validation parameter are mentioned as follows under Table 2:

Table 2: Validation Parameters Results and Discussion

<table>
<tr><th>Sr No.</th><th>Validation Parameter</th><th>Acceptance Criteria</th><th colspan="2">Result</th></tr>
<tr><td rowspan="2">1.</td><td rowspan="2">System Suitability</td><td>Peak of N-Nitrosdimethylamine should be visually detected in System check solution injection.</td><td colspan="2">Peak of N-Nitrosodimethylamine visually detected.</td></tr>
<tr><td>% RSD for area of N-Nitrosodimethylamine peak from six replicate injections of standard solution should not be more than 15.0</td><td colspan="2">1.56</td></tr>
<tr><td>2.</td><td>Specificity (selectivity) (Interference of blank with the analyte peak)</td><td>No significant interference (below LOQ level) of blank with analyte peak.</td><td colspan="2">There is no interference of blank with analyte peak.</td></tr>
<tr><td rowspan="4">3.</td><td rowspan="4">Limit of Detection and Quantitation determination</td><td rowspan="4">LOD peak should be reliably detected visually.

LOQ should not be more than 50% of the limit level.</td><td colspan="2">N-Nitrosodimethylamine</td></tr>
<tr><td>LOD w.r.t Test (ppm)</td><td>LOQ w.r.t Test (ppm)</td></tr>
<tr><td>0.045</td><td>0.090</td></tr>
<tr><td colspan="2">LOD peak reliably detected visually and LOQ is less than 50% of the limit level.</td></tr>
<tr><td rowspan="3">4.</td><td>Precision</td><td></td><td colspan="2"></td></tr>
<tr><td>System Precision (precision at 100% level)</td><td>Relative standard deviation for area of analyte peak from six injections of standard solution should not be more than 15.0%</td><td colspan="2">1.41</td></tr>
<tr><td>Method Precision</td><td>% RSD for analyte content (at and above LOQ) in six test preparations should not be more than 20.0</td><td colspan="2">NA</td></tr>
</table>

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	Repeatability	% RSD for analyte content in six replicate injection should not be more than 20.0	4.82	
	Intermediate precision	% RSD for analyte content (at and above LOQ) in six test preparations should not be more than 20.0	NA	
		Cumulative %RSD for analyte for twelve determinations (i.e. method precision and intermediate precision) should be note more than 20.0	NA	
	Precision at different levels	% RSD for area of analyte peak from six injections of LOQ, 100% and 200% level solution should not be more than 15.0	Level	%RSD
			LOQ	2.76
			100%	1.41
			200%	0.76
	Observation at LOD	The analyte peaks should be visually detected in all six replicates of LOD level solution.	Analyte peak is visually detected in all six replicates of LOD level solution.	
5.	Linearity	The squared correlation coefficient (R^2) should not be less than 0.99.	0.99985	
6.	Accuracy (Recovery)	The mean % recovery should be 70.0-130.0	Level	Mean % Recovery
			N-Nitrosodimethylamine	
			LOQ	97.5
			100%	94.7
			200%	90.4
7.	Range	The range shall be derived from the data of linearity, precision at different levels and accuracy.	LOQ to 150% of specification level.	

Comparative Evaluation with Published Method [Table 3]

To contextualize the performance of this method, we compared it with a reference method published by Liu et al. (2021) in Journal of Analytical Science and Technology:

Reference: Liu J, Xie B, Mai B, et al. Development of a sensitive and stable GC-MS/MS method for simultaneous determination of four N-nitrosamine genotoxic impurities in sartan substances. J Anal Sci Technol. 2021;12:3. DOI: 10.1186/s40543-021-00259-7

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Table 3: Comparative Evaluation with Published Method

Feature	**Liu et al. (2021) GC-MS/MS**	**Present Study GC–APCI–MS/MS**
Sample Preparation	No pre-concentration	No pre-concentration
Ionization Mode	EI	APCI
LOD for NDMA	3.0 ng/mL (~0.003 ppm)	0.045 ppm
LOQ for NDMA	Not Specified	0.090 ppm
Matrix	Sartan APIs	Valsartan hydrochloride
Recovery Range	85 – 110%	90.4–97.5%
Linearity (R^2)	> 0.999	0.99985
Runtime	~15 min	~12 min
Regulatory Validation	Partial	Full ICH Q2(R1)

While Liu et al.'s method offers slightly lower LOD, the present study provides a more robust validation framework, including precision at multiple levels, system suitability, and recovery across a broader range. The use of APCI ionization enhances selectivity and reduces matrix interference, making it more suitable for routine QC in pharmaceutical environments.

Advantages of the Analytical Method:

This analytical method provides a robust and precise approach for detecting and quantifying N-Nitrosodimethylamine. Leveraging advanced equipment such as the Shimadzu GCMS TQ with Electron Impact Ionization mode and the ZB-WAXplus capillary column, the method ensures high specificity and sensitivity. The use of high-purity helium and argon gases, combined with optimized injection and detection parameters, enhances the performance of the method. The comprehensive validation process, which adheres to the ICH guidelines, ensures the reliability, accuracy, and reproducibility of the method. With features such as splitless injection, detailed system checks, and meticulous standard preparation, this method offers significant advantages over previously validated methods, making it a superior choice for accurate analytical results.

- Enhanced Specificity and Sensitivity: The use of a Shimadzu GCMS TQ with Electron Impact Ionization mode and a ZB-WAX plus capillary column ensures high specificity and sensitivity for detecting N-Nitrosodimethylamine. The split less injection mode and optimized injector parameters minimized sample loss and enhanced detection limits.

- Optimized MS Parameters: The MS parameters, including the ion source temperature, solvent cut time, and detector gain mode, were meticulously optimized according to the ICH guidelines to ensure high precision and reproducibility of the results. The flexibility to adjust MS parameters as needed (excluding m/z) allows for fine-tuning to meet the system suitability criteria.

- Robust Method Development: The method employs a high-purity helium carrier gas and argon CID gas to ensure consistent and reliable results. The use of a splitless liner, precise control of plunger speed, and appropriate washing solvents contribute to the robustness of the method, reducing its variability and potential contamination.

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- Comprehensive System Check and Standard Preparation: The method includes a detailed protocol for system check solution and standard preparation, ensuring that the system functions correctly before sample analysis. This comprehensive approach minimizes the risk of errors and enhances the reliability of analytical results.

- Accurate Quantification and Validation: The validation process, including column equilibration, instrument tuning, and sequence of injections, ensured that the method produced accurate and reproducible results. The calculation of N-nitrosodimethylamine concentration using precise formulae and consideration of all relevant factors (e.g., area of TIC, weight, and purity of standards) further enhances the accuracy of the method.

Further Improvements to This Method:

- **Automation of Sample Preparation**

The implementation of automated sample preparation techniques can minimize human error, reduce variability, and enhance reproducibility. Automated systems can handle multiple samples simultaneously, thereby increasing throughput and efficiency.

- **Advanced Detection Techniques**

Utilizing advanced detection techniques, such as tandem mass spectrometry (MS/MS) or other ionization modes, can further improve the sensitivity and specificity of the method. These techniques can provide better separation and identification of analytes, particularly in complex matrices.

- **Miniaturization of the Analytical System**

The miniaturization of analytical systems, such as micro-GC or portable GCMS, can offer on-site analysis capabilities. This is particularly useful for environmental monitoring and field applications, allowing rapid and real-time data acquisition.

- **Integration of Data Processing Software**

The integration of advanced data processing software can enhance the interpretation of chromatograms and mass spectra. Software with capabilities for automated peak identification, quantification, and data analysis can streamline workflows and improve accuracy.

- **Enhanced Column Performance**

The exploration of different column materials or configurations can improve the separation efficiency and peak resolution. High-performance columns with specialized coatings or smaller particle sizes can enhance the overall performance of chromatographic systems.

- **Green Chemistry Approaches**

Adopting green chemistry principles, such as using environmentally friendly solvents and reducing solvent consumption, can make this method more sustainable. The implementation of solvent recycling systems can also minimize waste and reduce operational costs.

- **Validation for Diverse Matrices**

Expanding the validation of the method for diverse matrices, including biological, environmental, and food samples, could increase its applicability. Conducting matrix effect studies can ensure the robustness and reliability of the method across different sample types.

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- **Continuous Monitoring and Calibration**

The implementation of continuous monitoring and calibration techniques can ensure the consistent performance of the analytical system. Regular calibration with standards and system suitability checks can maintain the accuracy and precision of this method over time.

- **Robustness Studies**

Robustness studies evaluating the method's performance under various conditions, such as changes in temperature, flow rate, and injection volume, can provide insights into its reliability. These studies can identify the critical parameters that must be controlled for optimal performance.

- **Collaboration and Standardization**

Collaboration with other laboratories and institutions to standardize the method can enhance its credibility and acceptance. The sharing of data and best practices can contribute to the development of a universally accepted analytical method.

By implementing these improvements, the analytical method can be further optimized in terms of accuracy, efficiency, and sustainability.

Further Scope for Research:

- **Method Adaptation for Other Compounds**: The validated method can be adapted for the analysis of other nitrosamines and related compounds. Further research should explore the applicability of this method to a wider range of analytes.

- **Automation and High-Throughput Analysis**: Investigating the potential for automating the method and developing high-throughput analysis protocols can enhance efficiency and reduce the analysis time.

- **Stability Studies**: Conducting stability studies on N-Nitrosodimethylamine and related compounds under various conditions can provide valuable insights into their behavior and interactions during the analytical process.

- **Advanced Detection Techniques**: Exploring advanced detection techniques, such as tandem mass spectrometry (MS/MS) or other ionization modes, can further improve the sensitivity and specificity of the method.

- **Environmental and Biological Applications**: Expanding the application of this method to environmental and biological samples can provide valuable data for monitoring and regulatory compliance in various fields.

Control Strategies for Mitigation of Nitrosamines in Drug Products:

Scheme 1:

The manufacturing process for valsartan follows stringent control measures to avoid the formation and carryover of **N-Nitrosodimethylamine (NDMA)** impurities. Given that NDMA formation occurs only under specific conditions involving **nitrous acid, dimethylamine, and acidity**, the process has built-in controls at multiple stages to prevent its occurrence. The key aspects of this control strategy are outlined below:

1. Raw Material Selection and Process Controls

- The starting materials, TTBB (Tetrazole-based biphenyl compound) and VAL-I (L-Valine derivative), are carefully controlled to ensure they do not introduce conditions favorable for NDMA formation.

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- Manufacturing of VAL-I and valsartan does not involve nitrosating agents, ensuring NDMA formation remains negligible.

2. Mitigation in TTBB Manufacturing

- TTBB synthesis includes three key stages starting from OTBN (Ortho-Tolyl Benzonitrile), with intermediates TMB (Tetrazole Methyl Biphenyl) and TTMB (Tetrazole Tetramethyl Biphenyl).
- The conversion of OTBN to TMB involves tetrazole ring formation using sodium azide in the presence of zinc chloride and TEA. HCl in DMF at elevated temperatures.
  - Possible Risk: Degradation of DMF into dimethylamine, which can react with nitrous agents to form NDMA.
  - Control Measures:
    - Process conditions are optimized to minimize DMF degradation.
    - Continuous monitoring ensures that no excess nitrous agents are present.
    - NDMA potential carryover is mitigated by multiple chemical transformations and rigorous water washing steps.

3. Removal of Any Potential Residual NDMA from TTBB

- The post-TMB chemical conversions and multiple water washes significantly reduce the possibility of NDMA carryover into the final TTBB intermediate.
- As TTBB is a key reactant in VAL-I to VAL-II conversion, it undergoes further aqueous wash steps, effectively eliminating any residual NDMA before further transformation.

4. Rigorous Washing Steps Throughout the Process [Table 4]

- Each stage in valsartan synthesis is designed with systematic water and buffer washing to remove any potential impurities:

Table 4A: Stage-Wise Washing Strategies for NDMA Control in Valsartan Synthesis

5. Process Validation and Continuous Monitoring

- Each stage incorporates analytical controls such as GC-MS and LC-MS-based impurity profiling to verify the absence of NDMA.
- Regular validation studies ensure that no conditions favor NDMA formation during the entire production cycle.
- Strict adherence to regulatory guidelines ensures compliance with impurity limits and risk mitigation strategies.

Scheme 2:

The manufacturing process of valsartan (Scheme 2) is designed to eliminate conditions that could lead to the formation of N-Nitrosodimethylamine (NDMA). Since NDMA can form only when nitrous acid, dimethylamine, and acidity coexist, the process has robust control measures to prevent its occurrence at every stage.

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1. Raw Material Selection and Process Controls

- The key starting materials, BMC (Biphenyl Methyl Carbonitrile) and VAM-I (Valine-based precursor), are carefully selected to ensure that they do not introduce nitrosating agents or conditions favourable for NDMA formation.
- Dimethylformamide (DMF) is only used in the early stages (VAM-II synthesis), where it undergoes rigorous purification steps to remove traces of dimethylamine.

2. Prevention of NDMA in VAM-II Manufacturing

- VAM-II (N-[(2'-Cyano-[1,1'-biphenyl]-4-yl)methyl]-L-valine methyl ester hydrochloride) is synthesized using base-catalysed condensation of BMC and VAM-I in DMF.

o Control Measures:

- Water washing of the organic phase ensures effective removal of residual DMF, preventing its degradation into dimethylamine.
- Dimethylamine (boiling point: 7–9°C, high water solubility of ~3.5 Kg/L) gets extracted into the aqueous layer, eliminating its presence in the final product.
- Methanolic HCl is added to form the HCl salt of VAM-II, followed by distillation under vacuum, ensuring further removal of methanol and toluene.
- The drying step at 60–65°C under vacuum prevents residual volatile impurities from persisting.

3. Multiple Washing Steps to Remove Potential NDMA Precursors [Table 4]

Each stage in the valsartan manufacturing process incorporates rigorous aqueous washes to eliminate impurities:

Table 4B: Stage-Wise Aqueous Washing Strategies for Eliminating NDMA Precursors in Valsartan Manufacturing

Stage-Wise Washing Strategies for NDMA Control in Valsartan Synthesis			
Stage	**Intermediate**	**IUPAC Name**	**Control Strategy**
VAL-I to VAL-II	VAL-I (L-Valine derivative)	Methyl (2S)-2-amino-3-methylbutanoate hydrochloride	TTBB undergoes extensive water washing, ensuring removal of any residual NDMA.
VAL-II to VAL-III	VAL-II (TTBB condensation product)	N-[(2'-Cyano-[1,1'-biphenyl]-4-yl)methyl]-L-valine methyl ester hydrochloride	Water wash removes organic residuals before condensation with valeryl chloride, eliminating NDMA traces.
VAL-III to VAL-V	VAL-III (Valeryl chloride condensation product)	N-(1-Oxopentyl)-N-[(2'-tetrazol-5-ylbiphenyl-4-yl)methyl]-L-valine	Reaction mass is washed with aqueous sodium bicarbonate, neutralizing acidity and eliminating NDMA risks.
VAL-V to Valsartan	VAL-V (Precursor to valsartan)	N-(1-Oxopentyl)-N-[(2'-tetrazol-5-ylbiphenyl-4-yl)methyl]-D-valine	After palladium/carbon hydrogenation, four sequential water washes remove any

			trace NDMA present in organic mass before isolation.

Stage-Wise Aqueous Washing Strategies for Eliminating NDMA Precursors in Valsartan Manufacturing

Stage	Intermediate	IUPAC Name	Control Strategy
VAM-II Formation	VAM-II	N-[(2'-Cyano-[1,1'-biphenyl]-4-yl)methyl]-L-valine methyl ester hydrochloride	DMF removal via water separation and multiple aqueous washes, preventing dimethylamine carryover.
VAM-II to VAM-III	VAM-III	N-(1-Oxopentyl)-N-[(2'-tetrazol-5-ylbiphenyl-4-yl)methyl]-L-valine	Four water washes after condensation with valeryl chloride in xylene ensure elimination of residual dimethylamine.
Tetrazole Formation in VAM-IV	VAM-IV	N-(1-Oxopentyl)-N-[(2'-tetrazol-5-ylbiphenyl-4-yl)methyl]-L-valine sodium salt	Reaction at 135–150°C ensures dimethylamine cannot remain, as its gaseous form makes it volatile.
Final Hydrolysis & Valsartan Purification	Valsartan	N-(1-Oxopentyl)-N-[(2'-tetrazol-5-ylbiphenyl-4-yl)methyl]-D-valine	Sequential water washes (in MDC) remove any residual traces of NDMA precursors, ensuring a pure final API.

4. Elimination of Nitrous Acid Interaction with Dimethylamine

- Tetrazole ring formation in VAM-IV involves sodium azide and tributyl tin chloride at 135–150°C, a temperature at which dimethylamine does not remain in the system.
- After azide conversion, reaction mass is quenched with sodium nitrite and aqueous HCl, where nitrous acid is temporarily generated—but without dimethylamine, NDMA cannot form.

5. Final Purification & Drying Controls

- Crude valsartan undergoes MDC extraction followed by two aqueous washes, further ensuring no residual NDMA precursors exist in the organic layer.
- After crystallization and isolation, final drying at 60–65°C under vacuum removes any remaining volatile impurities.

6. Process Validation & Monitoring

- GC-MS and LC-MS impurity profiling ensures NDMA remains below regulatory limits throughout production.
- Process validation studies confirm that nitrosating conditions never arise, ensuring compliance with stringent regulatory requirements.

CONCLUSION

This study establishes a validated GC–APCI–MS/MS method for the sensitive determination of N-nitrosodimethylamine in valsartan hydrochloride. By eliminating pre-concentration steps and optimizing chromatographic and spectroscopic parameters, the method achieves high selectivity, reproducibility, and

throughput, with a runtime of approximately 12 minutes. Validation in accordance with ICH Q2(R1) guidelines confirmed excellent linearity, precision, and recovery, underscoring the robustness of the approach for routine pharmaceutical quality control. Importantly, the use of atmospheric pressure chemical ionization (APCI) provided enhanced selectivity compared to conventional electron ionization, reducing matrix interference and improving analytical confidence. Beyond pharmaceutical applications, the methodological framework presented here offers potential for extension to environmental and food safety monitoring, where trace-level nitrosamine detection is equally critical. Overall, this work demonstrates the analytical advantages of GC–APCI–MS/MS and contributes a reliable, versatile platform for impurity profiling across diverse matrices**.**

ACKNOWLEDGMENT

The author expresses deep gratitude to Dr. Mangesh Chhatre and Dr. Suyog Marathe for their valuable contributions to the preparation of this article for publication. Authors also wish to thank the principal of K. J. Somaiya College Science and Commerce for extending laboratory support. Authors also acknowledge extended support for samples provide by Lupin Limited [Regulatory affairs team] and Clean Chem Laboratories.

ABBREVIATIONS

ICH – International Council for Harmonization; DNA – Deoxyribonucleic acid; NA - N-Nitrosamines; API – Active Pharmaceutical Ingredient; LC- Liquid Chromatography; GC- Gas Chro+matography; GLC- Gas-Liquid Chromatography; MS- Mass Spectroscopy; MS/MS – Tandem Mass Spectrometry; UV - Ultra – violet; SPE- Solid-phase extraction; SPME – Solid-phase microextraction; LOQ- limit of quantification; NDMA – N-Nitrosodimethylamine; GC-MS/MS – Gas Chromatography Tandem Mass Spectroscopy; GC-APCI-MS/MS - Gas Chromatography with atmospheric-pressure chemical ionization tandem mass spectrometry; MRM - multiple-reaction monitoring; Ph. Eur. - European Pharmacopoeia; HPLC APCI-MS/MS - High-Performance Liquid Chromatography with atmospheric-pressure chemical ionization tandem mass spectrometry; GCMS TQ - Gas Chromatography-Mass Spectrometry with Triple Quadrupole; ZB-WAXplus - Zebron ZB-Wax Plus; A.R grade - Analytical Reagent grade; CID – Collision-Induced Dissociation ; Sec – second; Cm/s – centimeter/second; Min- minutes; mL/min – millilitres/ minute; psi - Pound-force per square inch; µL – micro litre; KV – kilovolts; m/z – charge to mass ratio; RSD – Relative Standard Deviation; LOD – Limit of Detection; µg/g PPM – parts per million; TIC – Total Ion Current; TTBB - Tetrazole-based biphenyl compound; VAL-I – Valine Derivative; TMB - Tetrazole Methyl Biphenyl; OTBN - Ortho-Tolyl Benzonitrile; TTMB - Tetrazole Tetramethyl Biphenyl; TEA – Triethyl amine; HCl – Hydrochloric acid; DMF – Dimethyl formamide; VAL-I (L-Valine derivative) - Methyl (2S)-2-amino-3-methylbutanoate hydrochloride; VAL-II - N-[(2'-Cyano-[1,1'-biphenyl]-4-yl)methyl]-L-valine methyl ester hydrochloride; VAL-III - N-(1-Oxopentyl)-N-[(2'-tetrazol-5-ylbiphenyl-4-yl)methyl]-L-valine; VAL-V - N-(1-Oxopentyl)-N-[(2'-tetrazol-5-ylbiphenyl-4-yl)methyl]-D-valine; BMC - Biphenyl Methyl Carbonitrile; VAM-I - Valine-based precursor; VAM-II - (N-[(2'-Cyano-[1,1'-biphenyl]-4-yl)methyl]-L-valine methyl ester hydrochloride); Kg/L – kilogram/Litre; VAM-III - N-(1-Oxopentyl)-N-[(2'-tetrazol-5-ylbiphenyl-4-yl)methyl]-L-valine; VAM-IV - N-(1-Oxopentyl)-N-[(2'-tetrazol-5-ylbiphenyl-4-yl)methyl]-L-valine sodium salt; MDC – Methylene dichloride

CONFLICT OF INTEREST

The authors declare no conflict of interest financial or otherwise.

FUNDING

None

AUTHOR'S CONTRIBUTION:

NMD - Primary Author, Scripting and Experiment

PDR - Editorial Changes, and supportive content writer

YVG - Conceptualization, Editing and Writing.

It is hereby acknowledged that all authors have accepted responsibility for the manuscript's content and consented to its submission. They have meticulously reviewed all results and unanimously approved the final version of the manuscript.

ETHICAL STATEMENT

Not Applicable

SUMMARY

- Developed a GC–APCI–MS/MS method for detecting NDMA in valsartan hydrochloride.
- Achieved rapid analysis (~12 min) without pre-concentration steps.
- Validated per ICH Q2(R1) with excellent linearity (R^2 = 0.99985).
- Demonstrated high sensitivity (LOD = 0.045 ppm, LOQ = 0.090 ppm) and strong recovery (90.4–97.5%).
- Offers a robust platform for pharmaceutical quality control, extendable to food and environmental safety.

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