RESEARCH PAPER

Integrated Analytical Strategy for Determination of Nitrite and N-Nitrosoquinapril in Pharmaceutical Systems Using HPLC and LC–MS/MS

Prakash Ashok Deshinge[1,2*], Srinivasarao Vaddadi[2], P. R. Upadhyay[2], Nandkumar Bhilare[2], Sureshkumar Ramdoss[2], Gautham Shenoy[1], Lakshmi Narasimham[2], Krishnamurthy Bhat[2,3*]

[1]Manipal Academy of Higher Education, Manipal, Karnataka, India

[2]Lupin Limited, Pune, Maharashtra, India

[3]School of Pharmaceutical Sciences, Manipal University, Jaipur, India

Corresponding authors: Prakash Ashok Deshinge | Email: prakashdeshinge@lupin.com; Krishnamurthy Bhat | Email: km.bhat@manipal.edu

ABSTRACT

Nitrosamine impurities and their precursors, particularly nitrite, have emerged as critical quality concerns in pharmaceutical products due to their potential carcinogenicity and increasing regulatory scrutiny. In this study, a comprehensive analytical strategy was developed and validated for the determination of nitrite and N-nitrosoquinapril, a drug substance–related nitrosamine impurity (NDSRI), in pharmaceutical systems. Nitrite was quantified using a sensitive high-performance liquid chromatography (HPLC) method based on pre-column derivatization via the Griess reaction, forming a stable azo dye detected at 520 nm. N-nitrosoquinapril was determined using a selective liquid chromatography–tandem mass spectrometry (LC–MS/MS) method operated in multiple reaction monitoring (MRM) mode.

Both methods were validated as per ICH Q2(R2) guidelines. The HPLC method demonstrated linearity over 0.005–0.201 ppm ($R^2 > 0.999$), while the LC–MS/MS method showed excellent linearity from LOQ to above 200% specification level. The methods exhibited satisfactory precision, accuracy, and specificity without matrix interference.

The study introduces an integrated approach for simultaneous evaluation of nitrite precursors and NDSRIs, offering a practical strategy for nitrosamine risk assessment and regulatory compliance in pharmaceutical products.

KEYWORDS

Nitrosamine impurities; Nitrite determination; N-Nitrosoquinapril; NDSRI; HPLC; LC–MS/MS; Griess derivatization; Pharmaceutical quality control

HIGHLIGHTS

- Developed a sensitive HPLC method based on Griess derivatization for trace-level determination of nitrite in pharmaceutical excipients.
- Established a selective LC–MS/MS method for quantification of N-nitrosoquinapril as a drug substance–related nitrosamine impurity (NDSRI).
- Demonstrated excellent method performance with high linearity ($R^2 > 0.999$), accuracy, and precision in accordance with ICH Q2(R2) guidelines.
- Introduced an integrated analytical approach for simultaneous evaluation of nitrite precursors and nitrosamine impurities.
- Provided a practical and reliable strategy for nitrosamine risk assessment and regulatory compliance in pharmaceutical products.

How to cite this article: Deshinge PA, Vaddadi S, Upadhyay PR, Bhilare N, Ramdoss S, Shenoy G, Narasimham L, Bhat K. Integrated Analytical Strategy for Determination of Nitrite and N-Nitrosoquinapril in Pharmaceutical Systems Using HPLC and LC–MS/MS. Int J Drug Deliv Technol. 2026;16(54s): 1314-1319. DOI: 10.25258/ijddt.16.54s.116

Source of support: Nil.

Conflict of interest: None.

INTRODUCTION

Nitrosamine impurities have emerged as a major safety concern in pharmaceutical products due to their potential carcinogenicity and mutagenicity. Regulatory agencies such as the United States Food and Drug Administration (US FDA) and the European Medicines Agency (EMA) have therefore established stringent guidelines for their control in drug substances and drug products [1,3]. These impurities may arise from multiple sources, including raw materials,

manufacturing processes, degradation pathways, and interactions between drug substances and excipients.

Among these, nitrite impurities present in excipients are recognized as critical precursors for nitrosamine formation. Under favorable conditions, nitrites react with secondary or tertiary amines to form nitrosamines, particularly during processing or storage [1]. Hence, accurate quantification of nitrite at trace levels is essential for effective risk assessment and mitigation strategies in pharmaceutical development.

Various analytical techniques, such as spectrophotometry, ion chromatography, and high-performance liquid chromatography (HPLC), have been reported for nitrite determination [19–23]. Among these, derivatization-based methods employing the Griess reaction are widely used due to their sensitivity and selectivity. This reaction involves diazotization of nitrite followed by coupling with aromatic amines to form a colored azo dye, which can be detected spectrophotometrically or chromatographically [14–18]. However, interference from complex excipient matrices necessitates careful optimization and validation to ensure method specificity and reliability.

In parallel, nitrosamine drug substance-related impurities (NDSRIs) have gained increasing attention. These impurities are structurally related to the parent drug and may form during synthesis, degradation, or storage. N-Nitroso Quinapril is one such impurity reported in angiotensin-converting enzyme (ACE) inhibitors, requiring sensitive and selective analytical methods for its determination [11–13].

Advanced analytical techniques such as liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) are widely employed for nitrosamine analysis due to their high sensitivity, selectivity, and capability for trace-level detection [4–10]. These techniques provide reliable quantification even in complex pharmaceutical matrices.

According to ICH guidelines, analytical methods for impurity determination must be validated for parameters including specificity, precision, accuracy, linearity, and detection limits to ensure reliability and regulatory compliance [2,25].

Therefore, the present study aims to develop and validate (i) a sensitive HPLC method based on Griess reaction derivatization for quantification of nitrite in magnesium carbonate, and (ii) a selective LC–MS/MS method for determination of N-nitrosoquinapril. The proposed methods are intended to support routine quality control and facilitate effective management of nitrosamine related risks in pharmaceutical products.

MATERIALS AND METHODS

Chemicals and Reagents

All chemicals and reagents used in the present study were of analytical or chromatographic grade. Sodium nitrite standard (Batch No. BCCH3405) was used as a reference standard. Tetrabutylammonium hydroxide, sulphuric acid, sulphanilic acid, and 1-naphthylamine were obtained in HPLC grade and used as received. Methanol, acetonitrile, glacial acetic acid, and HPLC grade water were used throughout the analysis. Sodium hydroxide pellets (AR grade) were employed for pH adjustment.

For LC–MS/MS analysis, LC–MS grade methanol and acetonitrile, along with formic acid and Milli-Q water, were used. N-Nitroso Quinapril standard was used as the impurity reference standard.

Instrumentation

The analysis was performed using a high-performance liquid chromatography (HPLC) system equipped with a UV–Visible detector. An LC–MS/MS system with an electrospray ionization (ESI) source was used for confirmation of impurities.

The study also utilized an analytical weighing balance, sonicator, pH meter, magnetic stirrer, centrifuge (5000 rpm), and analytical mill for sample preparation and processing.

Determination of Nitrite by HPLC

Principle

Nitrite was determined using a pre-column derivatization method based on the Griess reaction. Under acidic conditions, nitrite reacts with sulphanilic acid to form a diazonium ion, which further couples with 1-naphthylamine to produce a colored azo dye detectable at 520 nm.

Chromatographic Conditions and Mobile Phase Preparation

Chromatographic separation was performed on an Inertsil ODS 3V column (4.6 × 250 mm, 5 µm) with detection at 520 nm. The mobile phase was delivered at a flow rate of 1.0 mL/min with an injection volume of 50 µL. The column oven and autosampler temperatures were maintained at 30°C and 20°C, respectively. The total run time was 35 min, and the analyte eluted at approximately 20 min. The mobile phase consisted of two components: mobile phase A and B. Mobile phase A was prepared by dissolving 6.5 mL

tetrabutylammonium hydroxide (20% w/w) in 1000 mL water and adjusting the pH to 2.50 ± 0.05 with concentrated sulphuric acid, followed by mixing and sonication. Mobile phase B was prepared by mixing buffer, methanol, and acetonitrile in the ratio 400:100:500 (v/v/v), followed by filtration and sonication prior to use.

Reagent, Standard, and Sample Preparation

Reagent A was prepared by dissolving 250 mg of sulphanilic acid in water and diluting to 25 mL, while Reagent B was prepared by dissolving 25 mg of 1-naphthylamine in glacial acetic acid and diluting to 25 mL. A stock solution of sodium nitrite was prepared in methanol and serially diluted to obtain a working concentration of 0.015 µg/mL. Derivatization of the standard solution was carried out using Reagent A, Reagent B, sodium hydroxide, and methanol, followed by stirring for 60 min. For sample preparation, approximately 200 mg of magnesium carbonate was transferred into a 100 mL volumetric flask, and methanol, Reagent A, Reagent B, water, sodium hydroxide, and methanol were added sequentially. The resulting solution was stirred for 60 min to complete derivatization prior to injection.

Determination of N-Nitroso Quinapril by LC–MS/MS

N-Nitroso Quinapril was determined using an Ascentis Express C18 column (50 × 4.6 mm, 2.7 µm) with a flow rate of 0.6 mL/min and an injection volume of 5 µL. The column and autosampler temperatures were maintained at 40°C and 5°C, respectively. The mobile phase consisted of water containing 0.1% formic acid (mobile phase A) and methanol containing 0.1% formic acid (mobile phase B). Mass spectrometric detection was carried out using electrospray ionization in positive mode under multiple reaction monitoring (MRM). The capillary voltage and cone voltage were set at 3.1 kV and 26 V, respectively, with a source temperature of 150°C, desolvation temperature of 400°C, and desolvation gas flow of 600 L/hr. The diluent used was water:acetonitrile (20:80, v/v). The standard solution was prepared at a concentration of 3.0 ng/mL N-Nitroso Quinapril, while the test solution contained Quinapril at 13.333 mg/mL.

Method Validation

The developed methods were validated in ordance with ICH Q2(R2) guidelines. Validation parameters included specificity, system precision, method precision, accuracy (at LOQ, 100%, and 150% levels), linearity, limit of detection (LOD), and limit of quantitation (LOQ). The method demonstrated acceptable performance with linearity ($R^2 \geq 0.99$), precision (%RSD ≤ 15–20), and accuracy (recovery within 70–130%). Specificity was confirmed by the absence of interference at the retention time of the analyte.

RESULTS AND DISCUSSION

Method Development and Optimization

Optimization of Derivatization Time

The effect of derivatization time on analytical response was evaluated at immediate injection, 30 min, and 60 min. The response increased with reaction time, indicating completion of diazotization and coupling reactions. Comparable responses were observed at 30 and 60 min, whereas lower response was obtained for immediate injection. However, 60 min was selected as the optimized condition to ensure complete reaction and improved method robustness. This observation is consistent with the kinetics of the Griess reaction, which requires sufficient time for stable azo dye formation [14–18].

Optimization of Reaction Steps

Derivatization was evaluated using sequential (diazotization followed by coupling) and simultaneous approaches. The simultaneous process showed slightly higher response compared to the sequential approach, indicating efficient in situ azo dye formation without intermediate handling. Therefore, the simultaneous approach was selected due to its simplicity and improved sensitivity.

Optimization of Reagent Volume

Method robustness was evaluated by varying reagent volumes (±2%). No significant variation in peak area was observed, demonstrating that the method is insensitive to small changes in reagent concentration. This confirms that the selected reagent volumes are adequate for complete reaction and ensure reproducibility during routine analysis.

Optimization of Sample Weight

The effect of sample weight was studied using 150 mg, 200 mg, and 250 mg. The calculated nitrite content showed minimal variation across this range, indicating that the method is not affected by minor variations in sample quantity. A sample weight of 200 mg was selected as the optimized condition.

Method Validation

The developed methods were validated as per ICH guidelines [2,25] to establish their suitability for the intended application.

System Precision

System precision was evaluated using six replicate injections of the standard solution. The %RSD values were within acceptable

limits (≤15%), indicating adequate system performance and stability.

Method Precision

Method precision was assessed using six independent sample preparations. The %RSD values were within acceptance criteria (≤20%), demonstrating good repeatability. For the LC–MS/MS method, the %RSD for N-Nitroso Quinapril was below 5%, indicating high precision suitable for trace-level analysis [5,8–10].

Accuracy

Accuracy was evaluated at LOQ, 100%, and 150% levels. The recoveries were within 70–130% for both methods, confirming their accuracy. These results demonstrate that the methods are suitable for quantification of tracelevel impurities.

Linearity

The HPLC method for nitrite showed excellent linearity over the range of 0.005–0.201 ppm (R^2 = 0.99968). The LC–MS/MS method for N-Nitroso Quinapril was linear from LOQ to above 200% of the specification limit (R^2 = 0.99995), indicating a strong correlation between concentration and response.

Specificity

Specificity was demonstrated by analyzing blank, placebo, and standard solutions. No interference was observed at the retention times of nitrite and N-Nitroso Quinapril, confirming the selectivity of the methods.

Limit of Detection and Quantitation

The methods showed adequate sensitivity with low LOD and LOQ values, enabling detection and quantification of nitrite and nitrosamine impurities at trace levels. The sensitivity of the LC–MS/MS method is consistent with reported approaches for nitrosamine determination, highlighting the suitability of mass spectrometry for trace analysis [4–10].

Discussion

The developed HPLC method based on Griess reaction derivatization proved to be simple, sensitive, and reliable for the determination of nitrite in excipients. Optimization studies demonstrated that the method is robust and not significantly affected by minor variations in experimental parameters.

The LC–MS/MS method exhibited high selectivity and sensitivity for the determination of N-Nitroso Quinapril, enabling detection at trace levels in line with current regulatory expectations [1,3].

Compared to conventional techniques such as spectrophotometry and ion chromatography, the proposed methods offer improved specificity and are more suitable for complex pharmaceutical matrices [19–23]. The combined use of HPLC and LC–MS/MS provides a comprehensive analytical strategy for monitoring both nitrite (precursor) and nitrosamine impurities, which is critical for effective risk assessment and control.

Furthermore, the applicability of the developed methods extends beyond excipients and drug substances to finished pharmaceutical products. The approach enables monitoring of nitrite levels and detection of N-nitrosoquinapril in drug products during stability studies, facilitating the evaluation of in situ nitrosamine formation under real storage conditions. Thus, the methodology serves as a practical tool for integrated risk assessment and supports regulatory compliance throughout the product lifecycle.

CONCLUSION

In the present study, sensitive and robust analytical methods were successfully developed and validated for the determination of nitrite in excipients and N-nitrosoquinapril as a drug substance-related nitrosamine impurity. The HPLC method based on Griess reaction derivatization demonstrated good selectivity, precision, and robustness for the determination of trace levels of nitrite in magnesium carbonate. Optimization studies confirmed that the method is reliable and unaffected by minor variations in experimental conditions.

The LC–MS/MS method showed excellent sensitivity and specificity for the quantification of N-nitrosoquinapril at trace levels, meeting stringent regulatory requirements for nitrosamine impurity analysis.

Both methods complied with ICH validation requirements and demonstrated satisfactory performance in terms of precision, accuracy, linearity, and specificity. The combined analytical approach provides an effective strategy for simultaneous monitoring of precursor (nitrite) and nitrosamine impurities. Overall, this integrated analytical strategy enables sensitive quantification of nitrite and N-nitrosoquinapril and provides a robust framework for evaluating nitrosamine formation risk. It supports proactive risk assessment, ensures regulatory compliance, and enhances quality assurance of pharmaceutical materials and finished products.

ACKNOWLEDGEMENT

The authors thank Lupin Research Park and Manipal Academy of Higher Education

CONFLICT OF INTEREST

The authors declare no conflict of interest.

Figures:

Nitrite (HPLC Method)

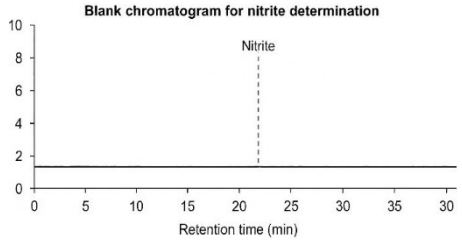

Figure 1a

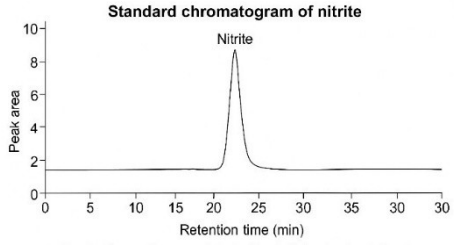

Figure 1b

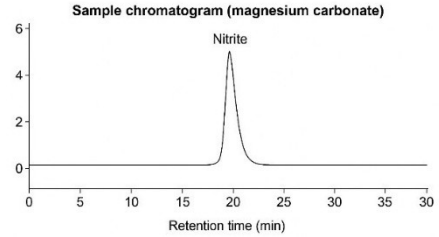

Figure 1c

Figure 1: Representative HPLC chromatograms for nitrite determination using Griess derivatization: (a) blank showing absence of interference, (b) derivatized nitrite standard showing a well-resolved peak at approximately 20 min, and (c) sample chromatogram demonstrating nitrite detection without matrix interference.

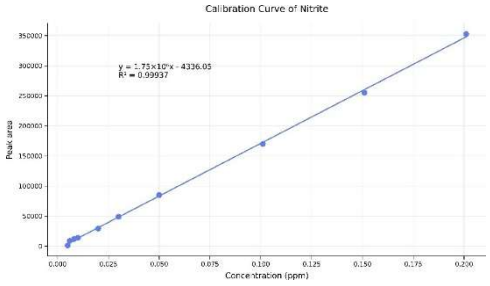

Figure 2: Calibration curve of nitrite obtained using the HPLC method, demonstrating linear response over the concentration range of 0.005–0.201 ppm ($R^2 > 0.999$).

NDSRI (LC–MS/MS Method)

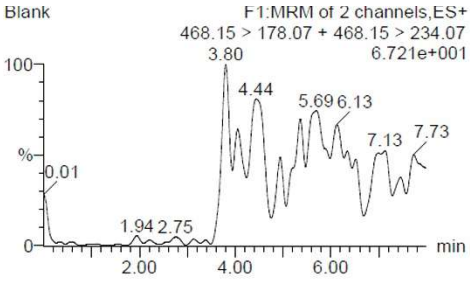

Figure 3a

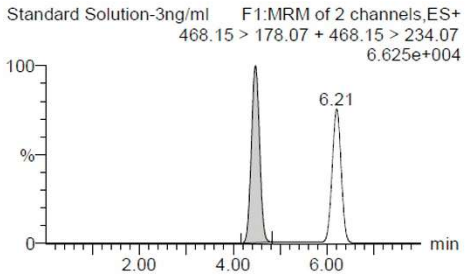

Figure 3b

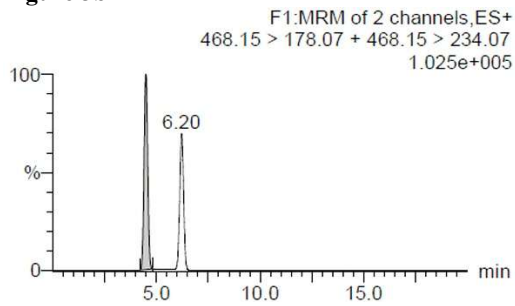

Figure 3c

Figure 3: Representative LC–MS/MS MRM chromatograms for determination of N-nitrosoquinapril: (a) blank sample showing no interference, (b) standard solution showing a sharp peak within the retention time window of 6–7 min, and (c) sample chromatogram confirming detection without matrix interference.

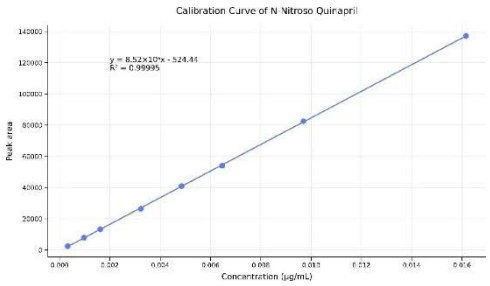

Figure 4: Calibration curve of N-nitrosoquinapril obtained using LC–MS/MS, showing excellent linearity from LOQ to above 200% of the specification level ($R^2 > 0.999$).

References

US Food and Drug Administration. Control of nitrosamine impurities in human drugs: guidance for industry. Silver Spring (MD): FDA; 2024.

International Council for Harmonisation (ICH). Q2(R2): Validation of analytical procedures. Geneva: ICH; 2023.

European Medicines Agency. Assessment report: nitrosamine impurities in human medicinal products. Amsterdam: EMA; 2020.

Shim M, Kim JY, Jung S, Hong M, Han SB, Lee DK. Determination of nitrosamine drug substance-related impurities using LC–MS/MS. Pharmaceuticals. 2025;18(11):1673–1686.

Moser J, Ashworth IW, Harris L, Hillier MC, Nanda KK, Scrivens G. N-nitrosamine formation in pharmaceutical solid drug products: experimental observations. J Pharm Sci. 2023;112(5):1255–1267.

Dakhole MR, Patil SS, Kshirsagar SJ. Nitrosamine impurities in pharmaceutical dosage forms: current challenges. Int J Front Chem Pharm Res. 2023;3(2):45–52.

Cheung J, Dobo K, Zhang S, Nudelman R, Schmidt F, Wenzel J, et al. Evaluation of nitrosamine impurities of ACE inhibitors. Environ Mol Mutagen. 2024;65(3):145–158.

Liu J, Xie B, Mai B, Cai Q, He R, Guo D, et al. Development of a sensitive GC–MS/MS method for simultaneous determination of nitrosamines in pharmaceuticals. J Anal Sci Technol. 2021;12:1–8.

Malihi F, Wang T. An improved analytical method for quantitation of nitrosamine impurities using LC–MS/MS. J Chromatogr Open. 2022;2:100037.

Kosuri E, McMahon T, Smith L. GC–MS/MS method for trace level quantification of nitrosamines in organic solvents. J Pharm Sci. 2022;111(12):3456–3465.

Tandan N, Reddy S. Quinapril. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024.

Brizzolari A, Santi M, Bianchi F. Development of a high-throughput Griess assay for nitrite determination. Molecules. 2021;26(9):2521–2532.

Das A, Roy S, Banerjee S. Advanced Griess reaction-based sensors for nitrite detection. Mater Adv. 2025;6:2145–2158.

Najdenkoska A. Development of HPLC method for analysis of nitrite and nitrate. J Agric Food Environ Sci. 2016;67:33–39.

Tatarczak-Michalewska M, et al. HPLC-DAD determination of nitrite in human saliva. Molecules. 2019;24(9):1754.

Hu W, et al. Direct determination of nitrite by ion chromatography. Fresenius J Anal Chem. 2001;371:1109–1112.

Lin H, et al. Determination of nitrite and nitrate using ion chromatography. Se Pu. 2012;30(4):374–377.

Fang Y, et al. Fluorometric determination of nitrite using HPLC. J Pharmacol Toxicol Methods. 2009;59(3):153–155.

Mirvish SS. Formation of N-nitroso compounds: chemistry, kinetics, and in vivo occurrence. Toxicol Appl Pharmacol. 1995;31(3):325–351.

Mitch WA, Sedlak DL. Formation of N-nitrosodimethylamine (NDMA) from dimethylamine during chlorination. Environ Sci Technol. 2002;36(4):588–595.

Lhasa Limited. Nitrosamine impurity formation guidance and risk assessment strategies. Leeds: Lhasa Ltd.; 2022.

Bharate SS. Critical evaluation of analytical methods for detection of nitrosamine impurities in pharmaceuticals. J Pharm Biomed Anal. 2021;205:114270.

Elder DP, Teasdale A, Lipczynski A. Control of nitrosamine impurities in drug substances and drug products. Org Process Res Dev. 2020;24(10):2211–2228.

Rao RN, Raju AN. LC–MS/MS determination of genotoxic impurities in pharmaceutical products: a review. Biomed Chromatogr. 2019;33(1):e4398.

Teasdale A, et al. Nitrosamines and nitrosatable impurities in pharmaceuticals: sources and mitigation strategies. Regul Toxicol Pharmacol. 2019;108:104450.