Application Note

Pharmaceutical

Enhanced Data Quality in LC/MS/MS Analysis of Nitrosamines

Using the Agilent InfinityLab quick change solvent purifier significantly reduces background noise and enables reliable detection of trace-level nitrosamines in complex pharmaceutical matrices

Authors

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Abstract

Nitrosamine analysis by liquid chromatography/tandem mass spectrometry (LC/MS/MS) requires exceptional sensitivity and selectivity to detect trace-level contaminants in complex matrices. This application note demonstrates how integration of an Agilent InfinityLab quick change solvent purifier enhances data quality in LC/MS/MS analysis of nitrosamines, specifically N-nitrosodimethylamine (NDMA) and N-nitrosodibutylamine (NDBA). An Agilent 1290 Infinity II liquid chromatography (LC) system coupled with an Agilent 6475 triple quadrupole mass spectrometer (MS) and an integrated Agilent quick change solvent purifier was employed for analysis. Installation of the solvent purifier significantly reduced background noise and enabled detection of 0.1 ng/mL NDMA, which was undetectable without purification due to high baseline interference. Additionally, the solvent purifier effectively eliminated NDBA interference peaks originating from the mobile phase and system, improving quantitative accuracy. These results demonstrate that solvent purification is essential for achieving reliable, low-level nitrosamine detection in LC/MS/MS applications.

Introduction

Nitrosamines represent a critical class of contaminants requiring sensitive detection and quantification in pharmaceutical applications. N-nitrosodimethylamine (NDMA) and N-nitrosodibutylamine (NDBA) are particularly concerning due to their potential genotoxic and carcinogenic properties, necessitating robust analytical methods capable of detecting these compounds at extremely low concentrations. LC/MS/MS has emerged as the gold standard for nitrosamine analysis, offering superior selectivity and sensitivity compared to conventional approaches.

However, a significant analytical challenge persists in background noise and interference peaks originating from mobile phase impurities and system contamination can substantially compromise data quality, particularly when quantifying analytes at trace levels. These background signals not only obscure low-abundance target compounds but also introduce systematic errors in quantitative measurements, potentially leading to false negatives or inaccurate results that undermine regulatory compliance and risk assessment.

While solvent purification has traditionally been employed in high-performance liquid chromatography (HPLC) to eliminate chemical impurities responsible for "ghost peaks," its application to LC/MS/MS systems for reducing background noise and removing solvent- or system-derived interference peaks remains underexplored. The integration of a solvent purifier into the LC/MS/MS workflow, positioned between the pump and autosampler. presents a straightforward yet potentially transformative approach to enhancing analytical performance. This application note describes how the incorporation of a solvent purifier into an LC/MS/MS method significantly improves data quality in nitrosamine analysis by reducing baseline background and eliminating interference peaks, thereby enabling reliable detection and quantification of these critical contaminants at clinically and regulatory-relevant concentrations.

Experimental

Instrumentation

The instrumentation and consumables used for this analysis are outlined in Table 1.

Table 1. Instrumentation and consumables used in analysis.

Product	Part Number
Agilent 1290 Infinity II LC system	–
Agilent 6475 triple quadrupole mass spectrometer	–
Agilent InfinityLab Poroshell 120 EC-C18 column, 3.0 × 100 mm	695575-302
Agilent Infinitylab quick change solvent purifier, 3.0 × 75 mm, installed between pump and autosampler	5067-1621

LC/MS conditions

Table 2. LC/MS conditions.

Parameter	Value
Mobile Phase	A) 0.1% formic acid in water B) 0.1% formic acid in methanol
Column Temperature	40 °C
Flow Rate	0.5 mL/min
Injection Volume	20 µL
Sample Concentration	0.1 ng/mL in 50% methanol
Gradient Program	Time (min) %A %B 0 95 5 3 95 5 7 40 60 10 25 75 13 20 80 16 10 90 17 10 90 17.1 95 5

Multiple reaction monitoring (MRM) parameters: Complete LC gradient and multiple MRM conditions are provided in Table 3. MRM transitions were monitored in positive ion mode using atmospheric pressure chemical ionization (APCI).

Table 3. LC/MS MRM conditions.

Compound Name	Precursor (*m/z*)	Product (*m/z*)	Fragmentor (V)	CE (V)	Polarity
NDBA	159.2	57.1	90	12	Positive
NDBA	159.2	41.1	90	16	Positive
NDMA	75.1	58	80	10	Positive
NDMA	75.1	43.1	80	16	Positive

Data acquisition

Analyses were performed with and without the solvent purifier installed in the LC/MS system to evaluate the impact on background signal reduction and interference peak elimination. Blank injections and 0.1 ng/mL standards were analyzed under both conditions to assess baseline noise levels and contamination from the mobile phase and laboratory water systems.

Results and discussion

Installation of an Agilent InfinityLab quick change solvent purifier (3.0 × 75 mm) between the pump and autosampler significantly improved data quality in LC/MS/MS analysis of nitrosamines. The purifier effectively reduced background signal and eliminated interference peaks originating from the mobile phase and laboratory water systems.

Detection of N-nitrosodimethylamine (NDMA)

Analysis of 0.1 ng/mL NDMA revealed a critical limitation in sensitivity without solvent purification. Without the solvent purifier installed, NDMA could not be detected due to excessive background noise in the baseline. Installation of the solvent purifier reduced the baseline by approximately seven-fold, enabling reliable detection of NDMA at 0.1 ng/mL.

The purifier effectively removed contaminating NDMA that originated from the pure water system in the laboratory, allowing the 0.1 ng/mL standard to be clearly resolved from background (*m/z* 75.1 → 43.1, retention time approximately 3.8 minutes).

Figure 1 illustrates the dramatic improvement in baseline quality and analyte detectability. Without the solvent purifier, elevated baseline noise completely obscured the NDMA signal, rendering quantification impossible at this concentration level. With the purifier installed, the reduced baseline enabled clear detection and integration of the NDMA peak, demonstrating the essential role of solvent purification in achieving the sensitivity required for trace-level nitrosamine analysis.

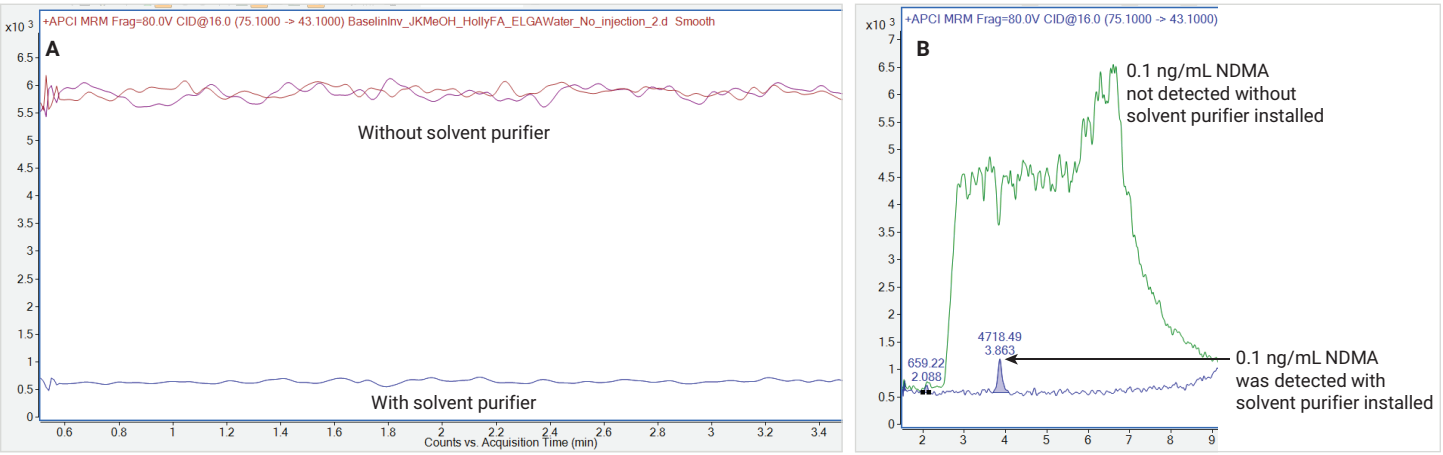

Figure 1. Extracted ion chromatograms (EICs) for N-nitrosodimethylamine (NDMA) at *m/z* 75.1 → 43.1 demonstrating the impact of solvent purification on baseline noise and analyte detection. (A) Analysis without solvent purifier showing elevated baseline that obscures the 0.1 ng/mL NDMA signal; (B) analysis with solvent purifier installed showing reduced baseline and clear detection of 0.1 ng/mL NDMA at retention time 3.8 minutes.

Elimination of N-nitrosodibutylamine (NDBA) interference

Analysis of 0.1 ng/mL NDBA demonstrated that the solvent purifier removed interference peaks caused by mobile phase or system contamination. Blank injections without the solvent purifier showed detectable NDBA signal (*m/z* 159.2 → 41.1, retention time approximately 11.2 minutes), indicating background contamination. Installation of the solvent purifier eliminated these interference peaks in blank runs, while maintaining detection of the 0.1 ng/mL NDBA standard.

Figure 2 illustrates that the presence of NDBA signal in blank runs without purification leads to systematic overestimation of sample concentrations and compromises quantitative accuracy. In contrast, Figure 3 demonstrates that after removing this contamination at the source with a solvent purifier, the detected signal originates exclusively from the analyte of interest.

Practical implications

These results demonstrate that solvent purification is essential for achieving low detection limits and accurate quantification in nitrosamine analysis, particularly when analyzing compounds at trace concentrations where background contamination would otherwise compromise analytical performance. The straightforward integration of the solvent purifier between the pump and autosampler requires minimal modification to existing LC/MS/MS workflows while delivering substantial improvements in data quality.

For laboratories conducting routine nitrosamine analysis in pharmaceutical quality control, the solvent purifier offers a practical solution to persistent challenges with baseline noise and interference peaks. By addressing contamination from laboratory water systems and mobile phase components, the purifier enables more confident detection at regulatory-relevant concentrations and reduces the risk of false negatives that could have significant compliance implications.

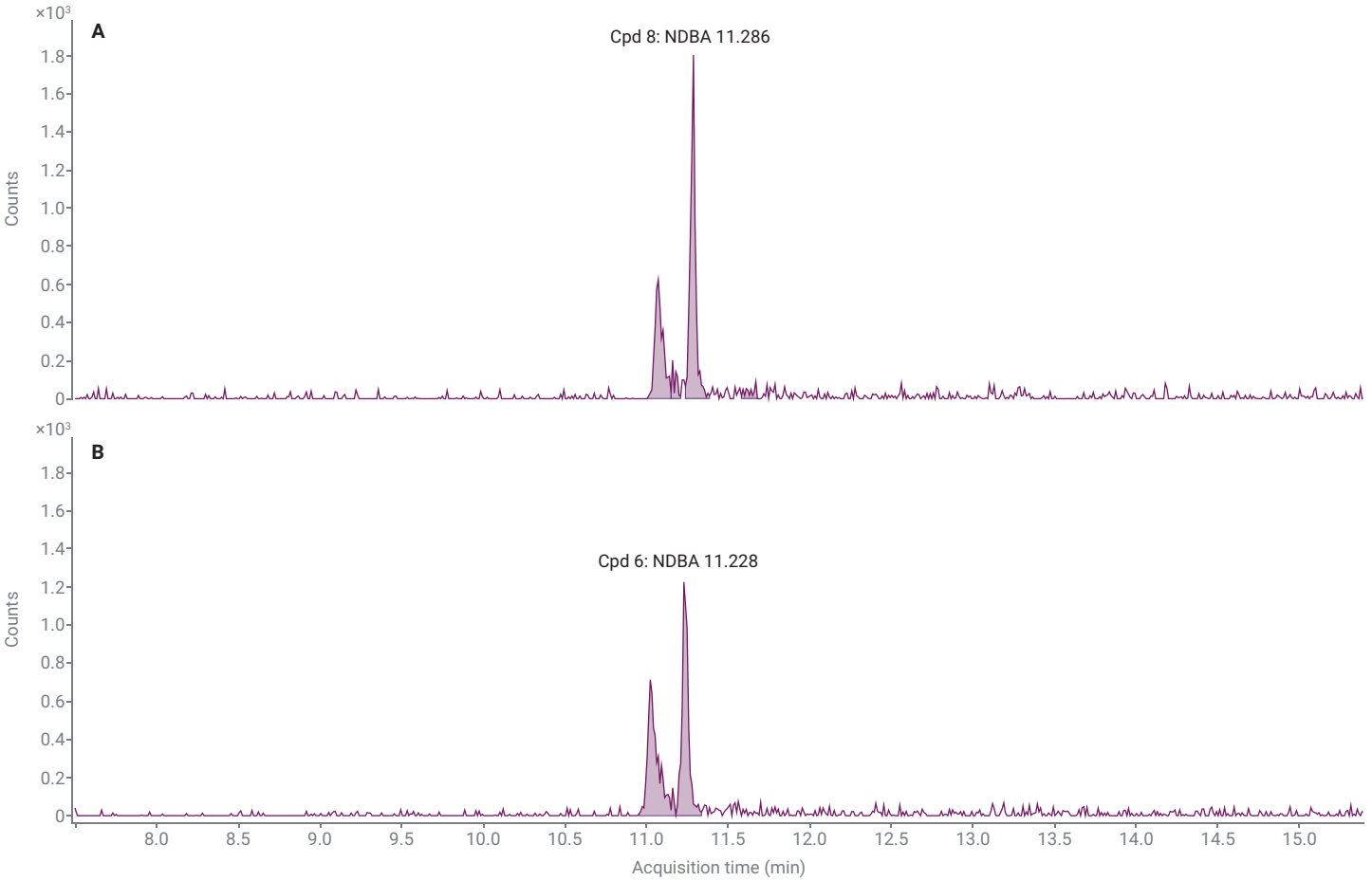

Figure 2. Extracted ion chromatograms (EICs) for N-nitrosodibutylamine (NDBA) at *m/z* 159.2 → 41.1 demonstrating elimination of interference peaks by solvent purification. (A) 0.1 ng/mL NDBA standard injection without solvent purifier at a retention of approximately 11.2 minutes; (B) No injection without solvent purifier showing contamination of solvent and system.

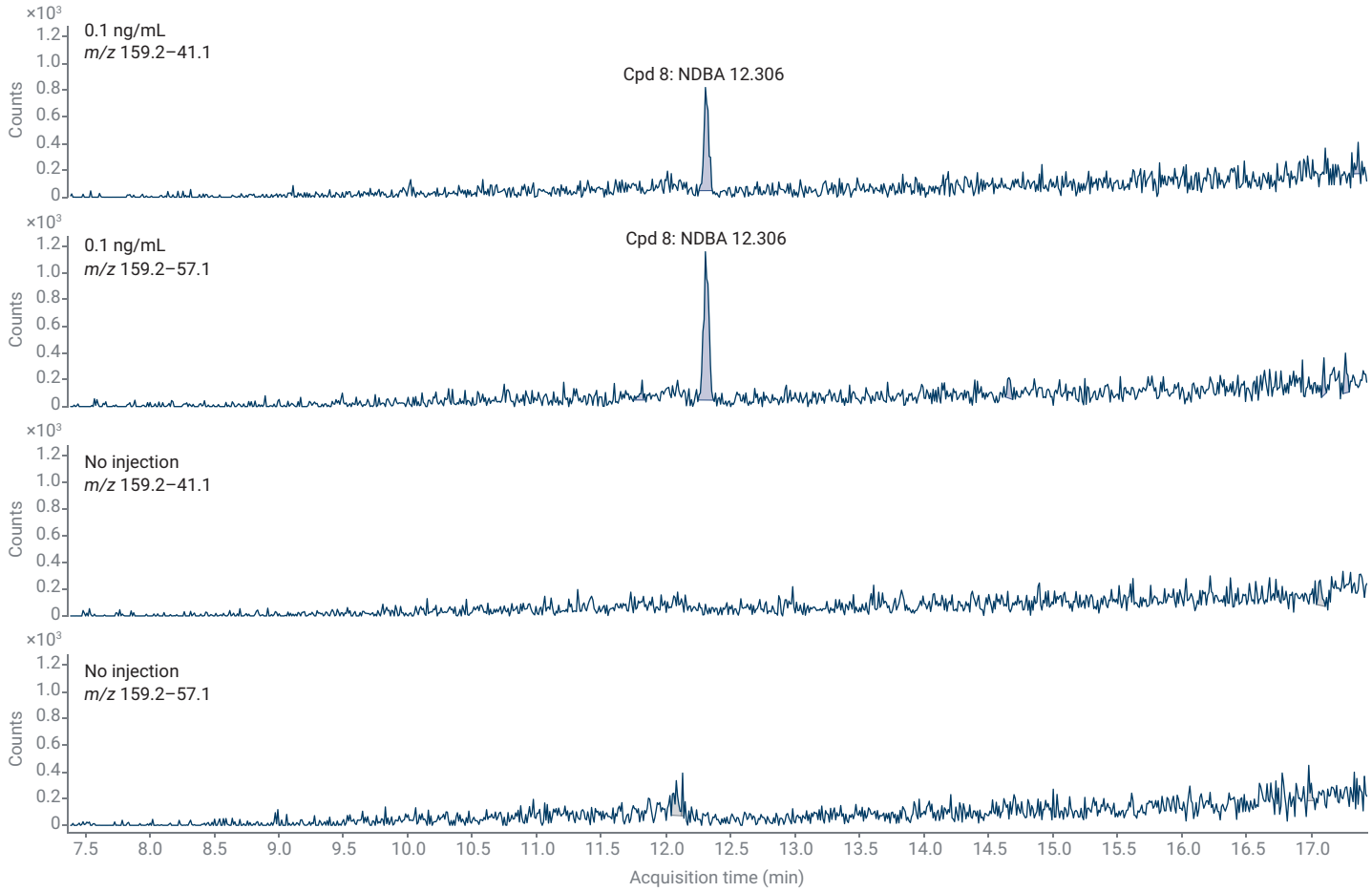

Figure 3. Extracted ion chromatograms (EICs) for N-nitrosodibutylamine (NDBA) at alternative transition *m/z* 159.2 → 41.1 and 159.2 → 57.1 demonstrating improved signal clarity and confirmation of interference removal across multiple detection channels with solvent purifier installation.

Conclusion

This application note shows that adding the Agilent InfinityLab quick change solvent purifier to LC/MS/MS workflows for nitrosamine detection greatly enhances sensitivity and reliability. Background noise was reduced seven-fold, allowing detection of 0.1 ng/mL NDMA; interference peaks from mobile phase contamination were eliminated, improving NDBA quantitation; and signal clarity improved across multiple transitions. Solvent purification offers an efficient and practical solution for trace-level analysis, helping laboratories meet regulatory standards more confidently.

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