Application Note

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Improved LC/MS Performance to Determine Polar Nitrosamines Using the Agilent 1260 Infinity II Hybrid Multisampler

Suitable for Agilent
1260 Infinity III LC

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Abstract

This application note demonstrates the use of the Agilent 1260 Infinity II Hybrid Multisampler for the analysis of polar nitrosamines injected from organic solvents of high elution strength. The 1260 Infinity II Hybrid Multisampler is used under optimized feed speed conditions to trap and enrich the polar nitrosamines on the column. This avoids breakthrough and provides better peak shapes with more reliable quantification and improved detection limits.

Introduction

With the chemical synthesis of active pharmaceutical ingredients (APIs), impurities occur during chemical reactions. Genotoxic impurities (GTIs) are especially important because of their carcinogenic potential. To control the amount of GTIs, the U.S. Food and Drug Administration and the European Medicine Agency released guidelines for the maximum intake of GTIs with the daily dose of a drug depending upon the duration of application. According to these guidelines, the exposure to an individual genotoxic impurity must be below 1.5 µg/day for an application time longer than ten years.[1] In 2018, the drug Diovan was withdrawn from the market due to an increased amount of the nitrosamine impurity N-nitrosodimethylamine (NDMA) in the included API valsartan.[2,3] This application note describes the results achieved by the Agilent 1260 Infinity II hybrid injector for the analysis of nitrosamines injected from high organic solvents. The peak performance, like area precision, linearity, and sensitivity, will be discussed.

Experimental

Instrumentation

- Agilent 1290 Infinity II High Speed Pump (G7120A)
- Agilent 1260 Infinity II Hybrid Multisampler (G7167C)
- Agilent 1290 Infinity II Multicolumn Thermostat (G7116B)
- Agilent Ultivo LC/TQ (G6465B)

Software

- Agilent MassHunter Acquisition software for Ultivo LC/TQ v1.2
- Agilent MassHunter Qualitative Analysis software v10.2
- Agilent MassHunter Quantitative Analysis software v10.2

Column

- Agilent ZORBAX Eclipse Plus C18, RRHD, 2.1 × 100 mm, 1.8 µm

HPLC method

Table 1. HPLC method.

Parameter	Value
Flow Rate	0.35 mL/min
Solvent A	Water + 0.1% formic acid
Solvent B	Methanol + 0.1% formic acid
Gradient 1	Time (min) %B 0 2 5 95 6 95 Stop time: 6 min, post time: 3 min
Column Temperature	45 °C
Flow Through Injection	Draw speed: 100 µL/min, wait time: 1.2 sec
Feed Injection	Feed speed 40 µL/min, flush out volume 6 µL, inner wash with Solvent A (S2)
Injection Volume	1, 3, 10 µL
Needle Wash	3 s Solvent B (S1)

MS method

Table 2. MS method.

Parameter	Value
Time Filter	0.03 min
MRM Conditions	See Table 3
Agilent APCI Source	
Gas Temperature	220 °C
Gas Flow	4 L/min
Vaporizer Temperature	325 °C
Nebulizer Pressure	35 psi
Capillary Voltage	2,500 V, positive
Corona Current	5 µA

Standard

2,000 µg/mL, each nitrosamine in MeOH (Table 3).

Calibration

- An LC/MS standard mixture was diluted to a stock solution of 1 mg/L in methanol.
- Calibration curves were created for 0.2, 1, 2, 10, 20, and 100 ng/mL.

Table 3. MRM conditions for the measurement of nitrosamines.

Compound	Formula	Mass	*m/z*	Retention Time (min)	Fragmentor (V)	Fragment Ion (*m/z*)	Collision Energy (eV)	Fragment Ion (*m/z*)	Collision Energy (eV)
N-Nitrosodimethylamine (NDMA)	$C_2H_6N_2O$	74.1	75.1	1.333	112	43	16	47	8
Nitrosomorpholine (NMOR)	$C_4H_8N_2O_2$	116.1	117.1	1.592	102	87.1	12	45	20
N-Nitrosomethylethylamine (NMEA)	$C_3H_8N_2O$	88.1	89.1	1.810	97	61	12	43.1	12
1-Nitrosopyrrolidine (NPYR)	$C_4H_8N_2O$	100.1	101.1	1.818	102	55.1	20	41	28
N-Nitrosodiethylamine (NDEA)	$C_4H_{10}N_2O$	102.1	103.1	2.384	83	75	8	47	20
Nitrosopiperidine (NPIP)	$C_5H_{10}N_2O$	114.1	115.1	2.542	102	69.1	16	41	24
N-Nitroso-n-propylamine (NDPA)	$C_6H_{14}N_2O$	130.1	131.1	3.414	88	43	12	89	8
N-Nitroso-n-dibutylamine (NDBA)	$C_8H_{18}N_2O$	158.1	159.2	4.091	88	57	12	103	8

Sample preparation

An 80 mg sample of valsartan was dissolved in 2 mL of methanol according to the recommended daily dose and then spiked with the nitrosamine mix. The final concentration of each nitrosamine was 50 ng/mL, according to 1 µg per daily dose.

Solvent and chemicals

- LC/MS grade solvents:
  - Agilent InfinityLab Water for LC/MS (5191-5121)
  - Agilent InfinityLab Methanol for LC/MS (5191-5111)
- Chemicals were purchased from VWR, Germany

Results and discussion

For the measurement of nitrosamines, the API sample is typically extracted with an organic solvent like pure methanol or aqueous methanol. The high content of organic solvent in such a sample can negatively affect the chromatographic performance of early-eluting analytes.

Calibration curves were created for the determination of typical peak parameters for a group of nitrosamines with the classical flow through injection mode, in comparison to the feed injection mode. The calibration curves were measured for a concentration range of 0.2 to 100 ppb (ng/mL) for injection volumes of 1, 3, and 10 µL with 10 repeats for every calibration point. For a visual comparison, the complete group of the eight measured nitrosamines is displayed at the 100 ppb level for all injection volumes (see Appendix). For an injection volume of 1 µL, the resulting peaks obtained from both injection modes showed comparable peak shapes (Appendix, Figures A1 and B1). An injection volume of 3 µL already showed a splitting for the early-eluting peaks of N-nitrosodimethylamine (NDMA) and nitrosomorpholine (NMOR) for flow through injection, while retaining the peak shape for feed injection (Appendix A2 and B2). In the case of an injection of 10 µL using flow through mode, seven of eight peaks over the whole elution range are affected (Appendix A3). All of them suffer from excessive peak broadening, and, in the case of the first eluting NDMA, a breakthrough with the solvent front. In the case of the feed injection, the early-eluting compounds showed a moderated broadening, which is still in a range where a peak can be integrated automatically.

To display the peak performance, the peak area RSDs (%) were calculated for the 10 ppb calibration point (Figure 1). The RSDs obtained for feed injection were typically below 3%, except for NMOR and NMEA, which were below 4%. The peak area RSDs obtained for the experiment with flow through injection showed higher values for all early-eluting nitrosamines between 8 and 14% RSD. The RSD values declined for the later-eluting nitrosamine compounds, which are less affected by the effect of the polar sample solvent.

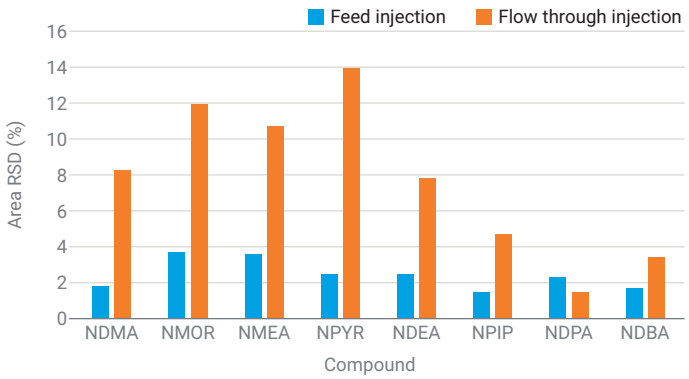

Figure 1. Area RSD obtained for a 10 µL feed injection and flow through injection at an individual nitrosamine concentration of 10 ppb (N = 10).

High peak area RSDs influence other important parameters like linearity. For all calibration curves, the R^2 value was determined (Figure 2). The R^2 values obtained for calibration curves measured with feed injection are > 0.999. The R^2 values obtained for the calibration curves measured with flow through injections were typically > 0.990 and < 0.998.

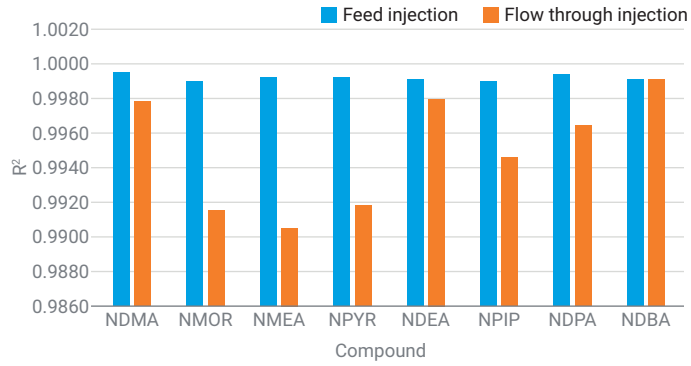

Figure 2. Linearity R^2 values obtained for calibration curves measured with feed injection and flow through injection for 10 µL injection volume.

From the measured calibration points, the limit of quantification (LOQ) for the individual nitrosamines was calculated from the lowest accessible calibration concentration to a signal-to-noise ratio of 10. In the case of the highest polar nitrosamine (NDMA), an LOQ of 10 ppb was determined for flow through injection. This is due to the strong peak broadening and a breakthrough of this compound (Appendix, Figure A3). In comparison, the LOQ obtained for NDMA with feed injections was below 2 ppb (Figure 3). The early-eluting nitrosamines generally showed a higher LOQ for flow through injection compared to feed injection by a factor of 3 to 5. The latest eluting nitrosamines, NDPA and NDBA, showed comparable LOQs for flow through and feed injection due to their lower polarity and better retention behavior.

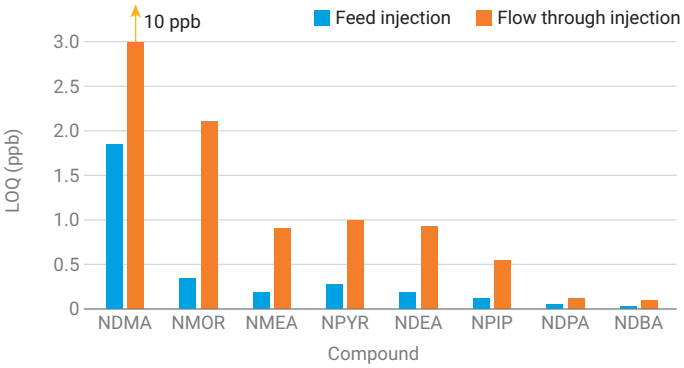

Figure 3. LOQ is determined from the calibration points measured with flow through injection and feed injection for 10 µL injection volume.

For the determination of the recovery from an API sample, valsartan was spiked with a mixture of nitrosamines to receive 1 µg in a daily dose. This was dissolved in methanol to give a final concentration of 50 ng/mL (50 ppb). The typical recoveries for feed injection and flow through injection were between the accepted limits of 70 and 120% of the expected value (Figure 4). Due to the strong peak broadening (or even breakthrough in case of the early-eluting nitrosamines with flow through injection), their recovery is compromised.

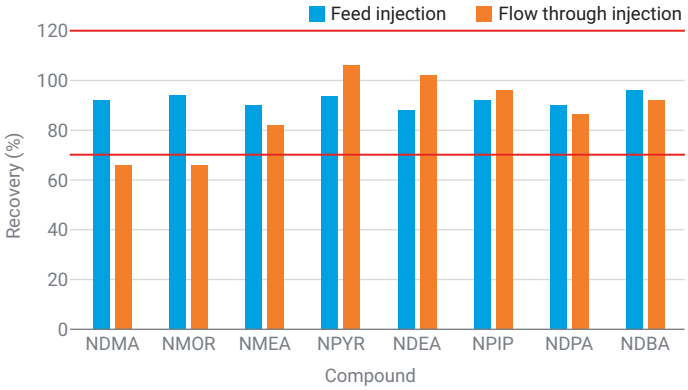

Figure 4. Recoveries obtained for a 50 ppb spike of valsartan for feed injection and flow through injection mode. The red lines are the acceptance limits at 70 and 120% of the expected concentration.

Conclusion

This application note describes the comparative measurement of nitrosamines using the Agilent 1260 Infinity II Hybrid Multisampler in feed injection mode and classical flow through injection mode. With the application of an optimized feed injection method, highly polar nitrosamines in a solvent of high eluting strength can be separated with good peak shapes. With classical flow through injection, massive peak broadening or even breakthrough were observed. Peak area RSD and LOQs are typically lower by a factor of 3 to 5 with feed injection.

References

1. European Medicine Agency (EMA), Committee for Human Medicinal Products. CH Guideline M7(R1) on Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk, EMA/CHMP/ICH/83812/2013. European Medicine Agency, August **2015**.
2. Masada, S.; Tsuji, G.; Arai, R.; Uchiyama, N.; Demizu, Y.; Tsutsumi, T.; Abe, Y.; Akiyama, H.; Hakamatsuka, T.; Izutsu, K.; *et al.* Rapid and Efficient High-Performance Liquid Chromatography Analysis of N-Nitrosodimethylamine Impurity in Valsartan Drug Substance and Its Products. *Sci. Rep.* **2019**, *9(1)*, 11852.
3. U.S. Food and Drug Administration, Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS) Method for the Determination of Six Nitrosamine Impurities in ARB Drugs, May 21, **2019**.

Appendix

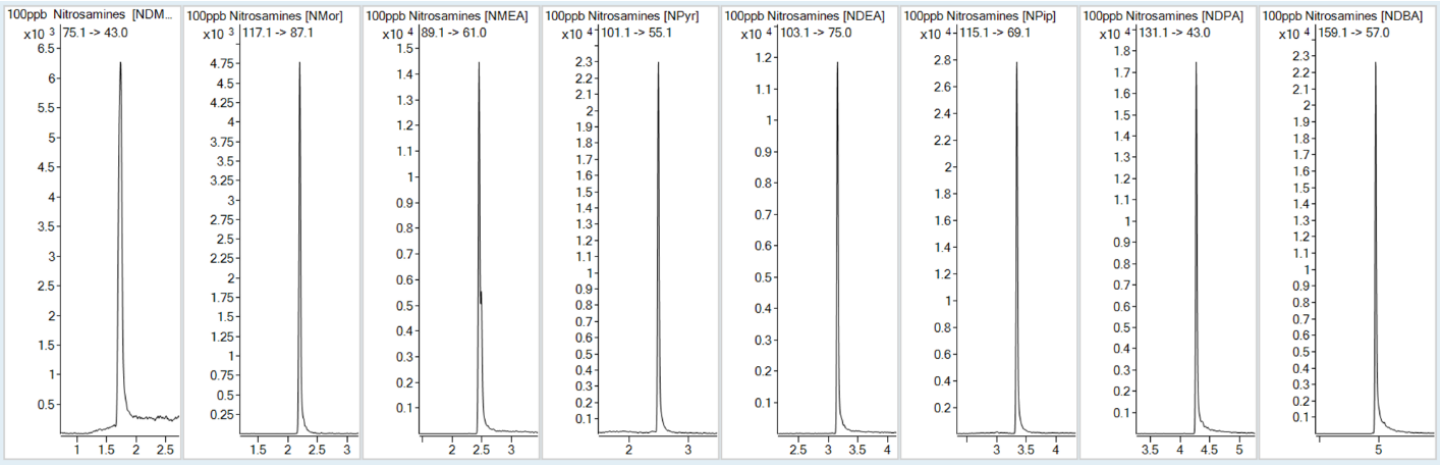

Figure A1. Flow through injection, 1 µL.

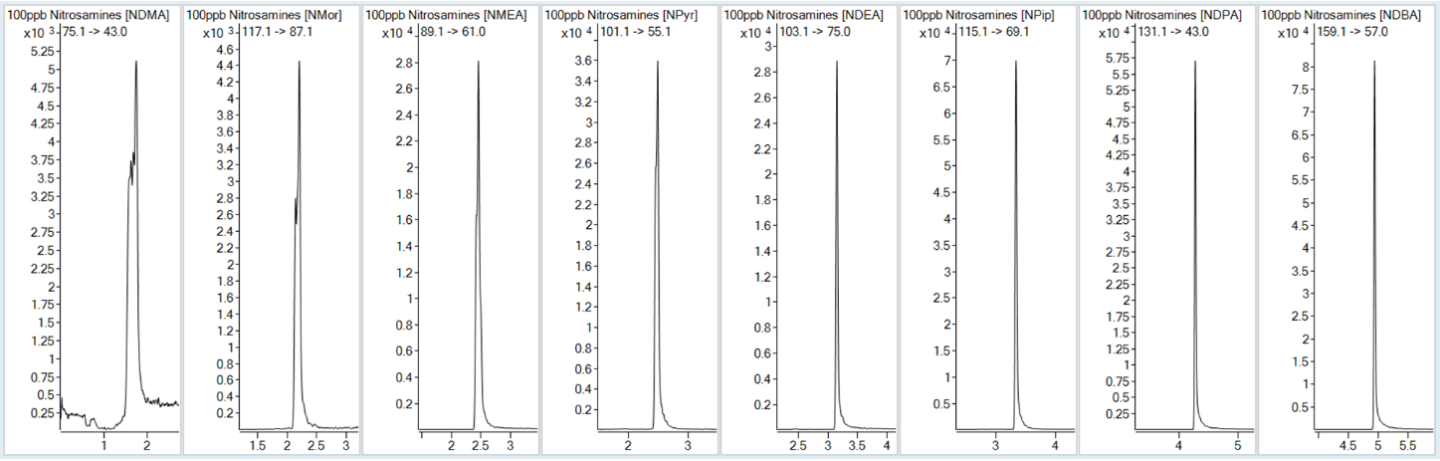

Figure A2. Flow through injection, 3 µL.

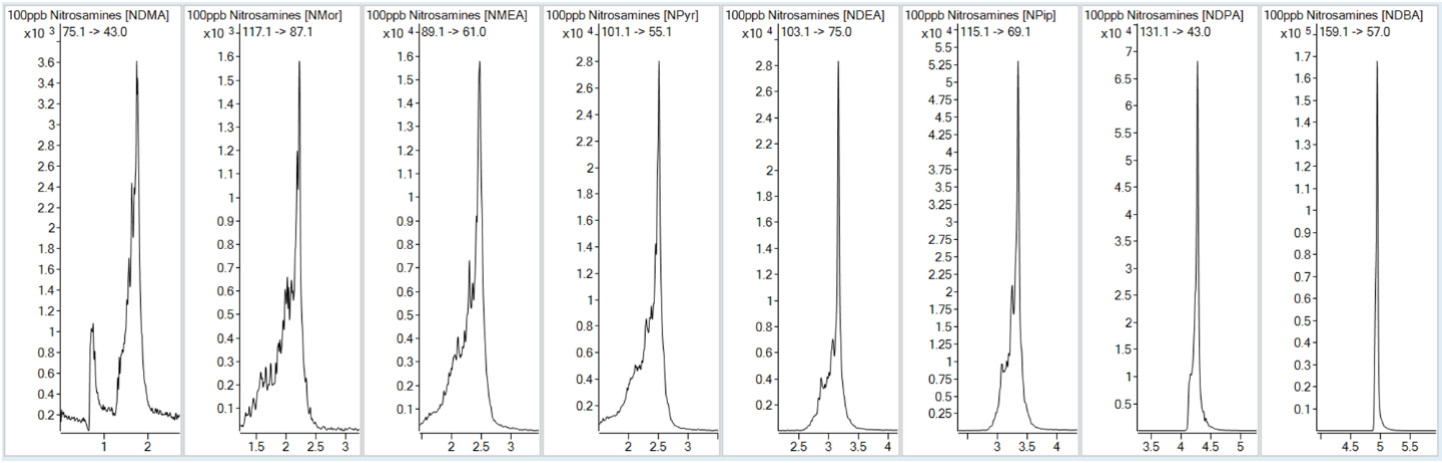

Figure A3. Flow through injection, 10 µL.

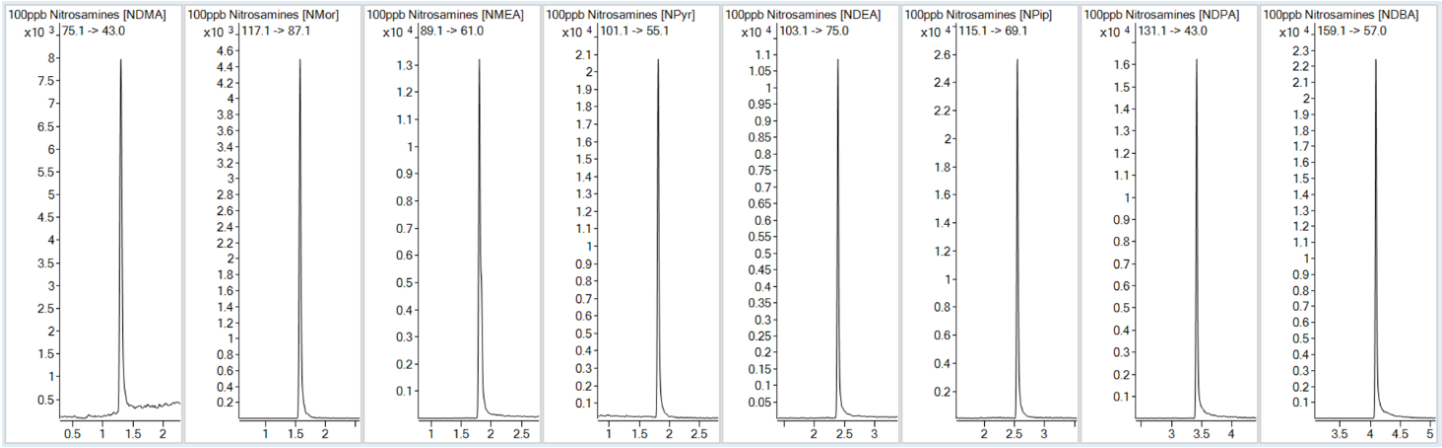

Figure B1. Feed injection, 1 µL.

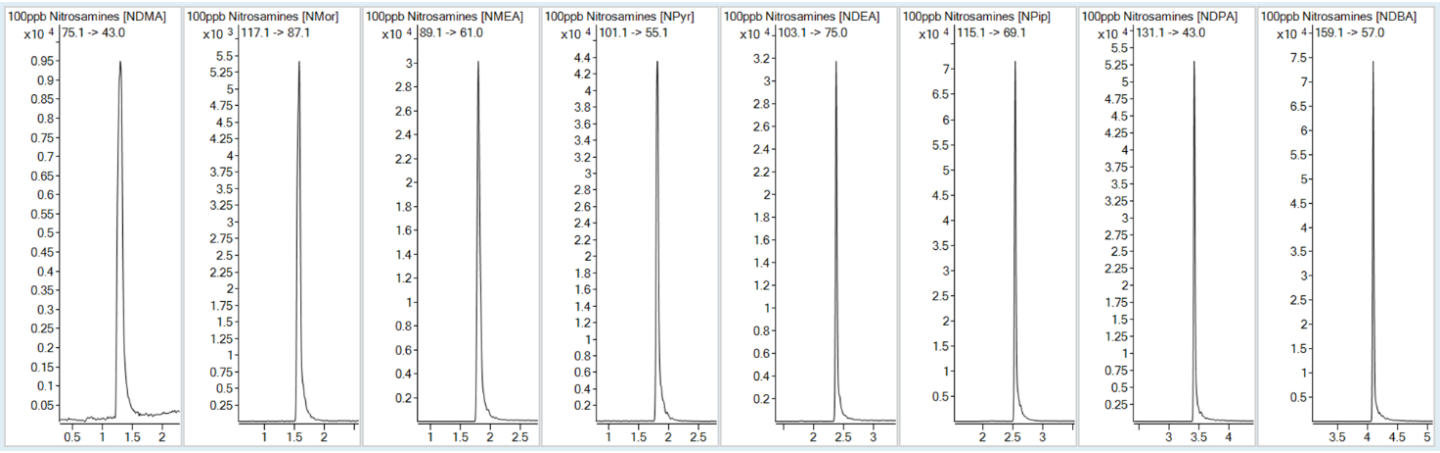

Figure B2. Feed injection, 3 µL.

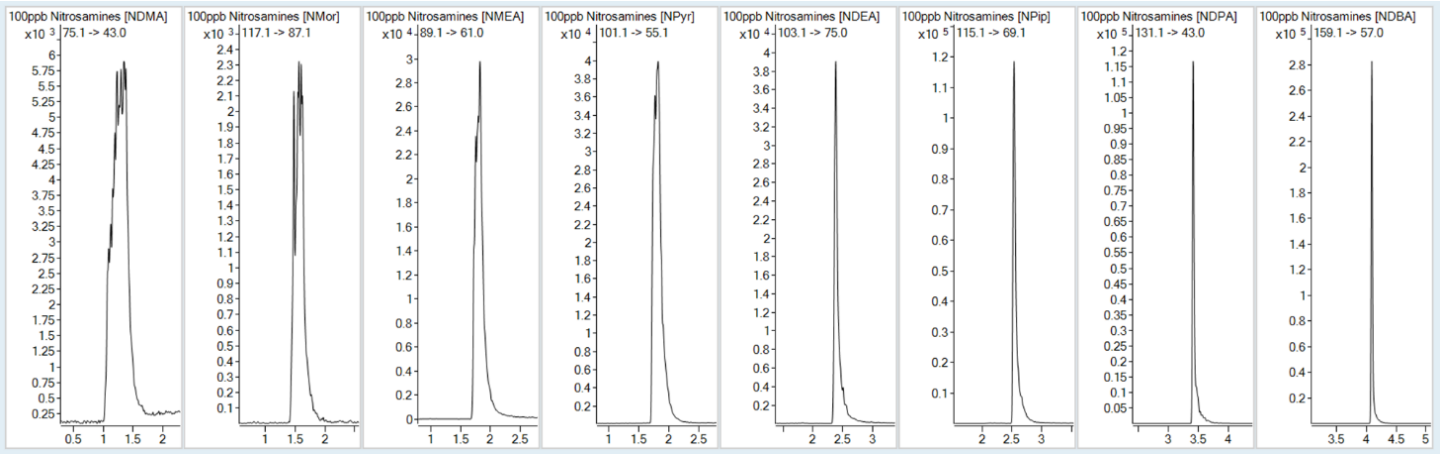

Figure B3. Feed injection, 10 µL.

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