Application Brief

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High-Throughput Analysis of Nitrosamines Using RapidFire Coupled to Ultivo Triple Quadrupole

Introduction

Several highly sensitive quantitative methods have been developed for the analysis of nitrosamines using mass spectrometry.[1] However, these methods rely on chromatographic separations that take several minutes per sample. Rapid, robust screening and quantitation of impurities is an essential analytical tool for a wide variety of laboratories. High-throughput environments must be able to perform these analyses in a way that ensures productivity, minimizes costs, and eliminates backlog. The Agilent RapidFire High-Throughput LC/MS system allows for fast analysis of samples without compromising analytical fidelity.

This work explores the simultaneous quantitation of a panel of nitrosamines in less than 15 seconds per injection. An existing U.S. Food and Drug Administration (US FDA) analytical method[2] was reproduced and then additional nitrosamines were added to assess feasibility and ease of expanding the panel.

Experimental

Instrumentation consisted of a RapidFire System coupled to an Ultivo Triple Quadrupole LC/MS. Online solid phase extraction (SPE) was performed by the RapidFire system using a graphitic carbon cartridge to separate target analytes from salts and any other interferences present in the samples.

Nitrosamine standards, LC/MS grade methanol, and formic acid were purchased from Sigma-Aldrich, St. Louis, MO, USA.

The automated trap, wash, and elute cycle was optimized to achieve an analysis time of less than 15 seconds per injection.

RapidFire conditions

Buffer A: Water + 0.1% formic acid

Buffer B: Methanol + 0.1% formic acid

SPE Cartridge: Graphitic carbon, Type D (G9206A)

State	Time (ms)
Aspirate	600
Load/Wash	2,000
Elution	7,000
Re-Equilibrate	2,000

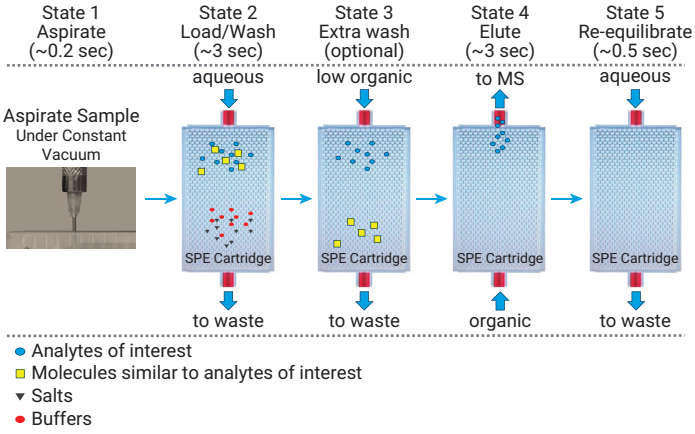
Ultivo conditions

Parameter	Value
Ion Mode	APCI
Polarity	Positive
Drying Gas Temp	300 °C
Drying Gas Flow	6 L/min

Parameter	Value
Nebulizer	55 psi
APCI Heater	350 °C
APCI Needle	4 µA
Capillary Voltage	3,000 V

Compound	Precursor	Product	Dwell (ms)	Fragmentor (V)	CE (V)
NDBA	159.1	57.2	10	70	7
NPIP	115.1	69.1	10	85	9
NPyR	101.1	55.1	10	55	18
NMEA	89.1	61.1	10	75	10

RapidFire analysis in five steps



Results and discussion

Injections were made at a rate of approximately 12.5 seconds per sample while data was acquired by triple quadrupole mass spectrometry. Figure 1 shows 72 injections, including several blank injections, acquired in under 15 minutes.

Triplicate injections of each calibrator demonstrated excellent reproducibility. Coefficients of variation range from 4.5 to 9.0% for NPIP (Figure 1) and are representative of all analytes. Excellent linearity is also observed, with R^2 ranging from 0.997 to 0.999 (Figure 2).

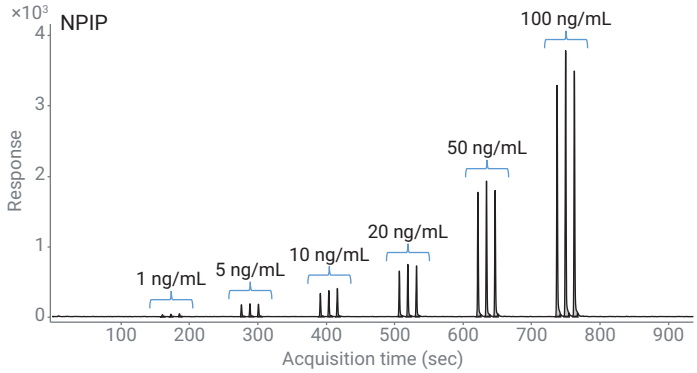


Figure 1. Triplicate injections of a 6-point calibration curve for NPIP.

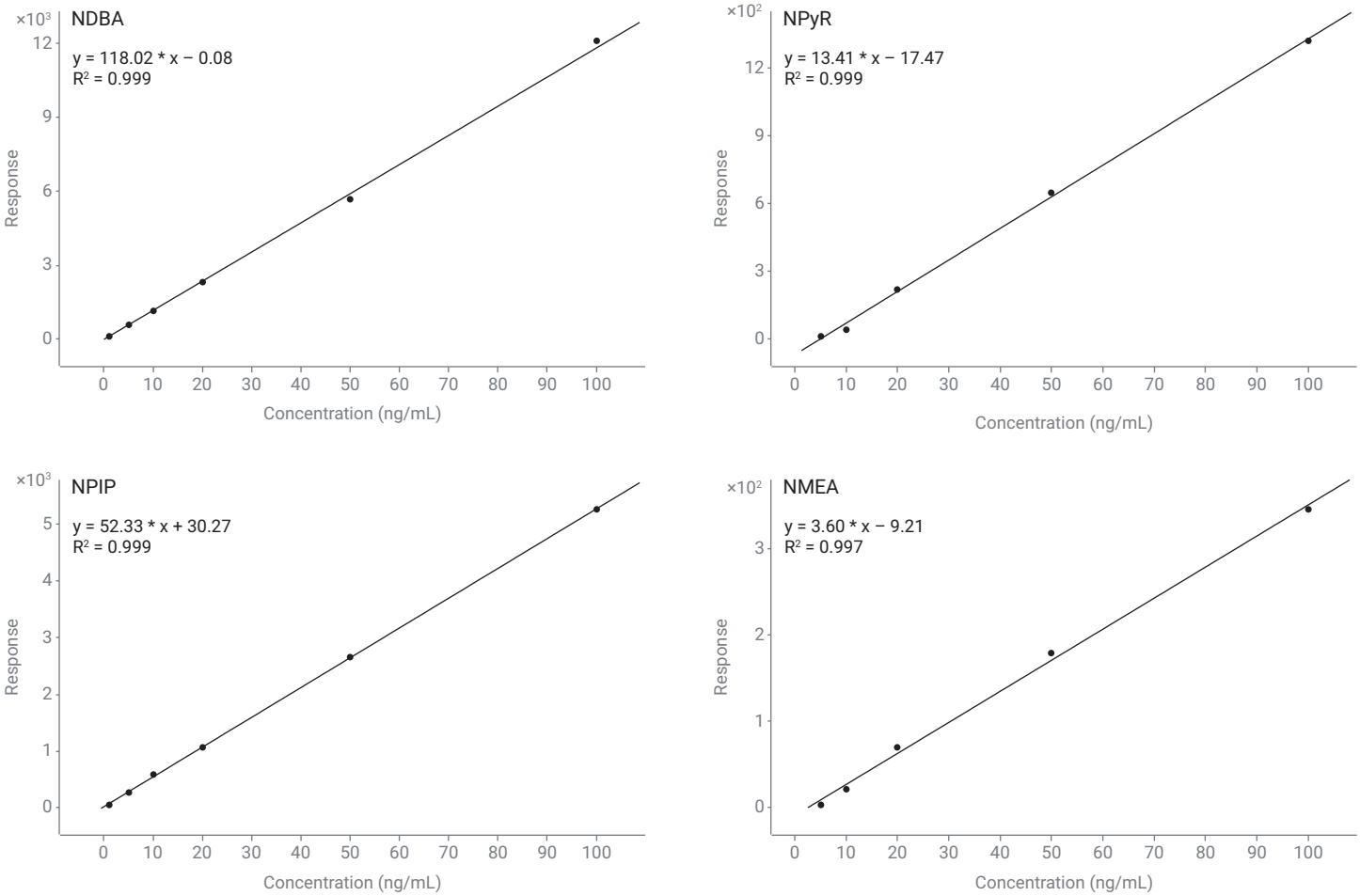


Figure 2. Calibration from 1 to 100 ng/mL for NDBA and NPIP. Calibration from 5 to 100 ng/mL for NPyR and NMEA.

Conclusion

The US FDA's method for rapid analysis of nitrosamine impurities has been replicated with consistent results. Further proof-of-concept work demonstrates the simplicity of adding additional nitrosamine analytes.

References

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2. Development and validation of a RapidFire-MS/MS method for screening of nitrosamine carcinogen impurities…in ARB drugs. (2019, July 24). FDA. Retrieved April 15, 2020. https://www.fda.gov/media/125477/download

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