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Ultra-Fast Analysis of Nitrosamines Using SPE-QQQ

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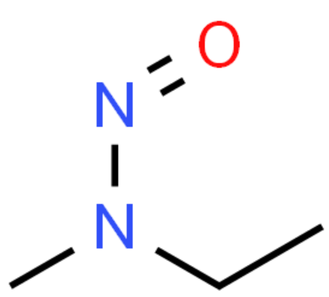
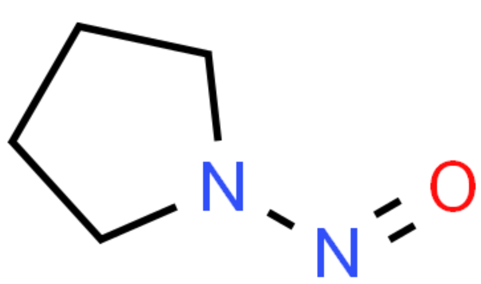
Introduction

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 use of Solid Phase Extraction Triple Quadrupole Mass Spectrometry (SPE-QQQ) allows for the ultra-fast analysis of samples without compromising analytical fidelity.

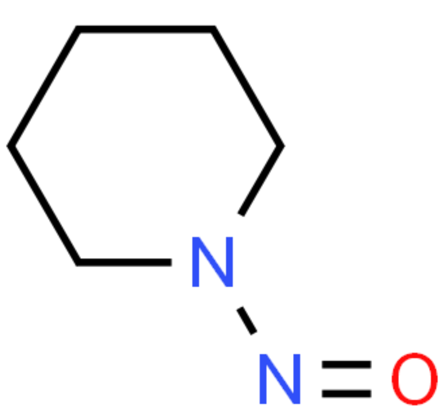
This work explores the simultaneous quantitation of a panel of nitrosamines in less than 15 seconds per injection. An existing U.S. Food and Drug Administration (US FDA) analytical method[2] was reproduced and then additional nitrosamines were added to assess feasibility and ease of expanding the panel.



N-Nitrosoethylmethylamine (NMEA)



N-Nitrosopyrrolidine (NPyR)



N-Nitrosopiperidine (NPIP)

https://www.chemspider.com/

Figure 1. Three nitrosamines studied as proof-of-concept additions to FDA's RapidFire method for the analysis of nitrosamine impurities.

Experimental

Instrumentation

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

Figure 2. Agilent RapidFire 400 High-Throughput Mass Spectrometry System coupled to an Agilent Ultivo triple quadrupole LC/MS

Chemicals and Reagents

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

Method

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

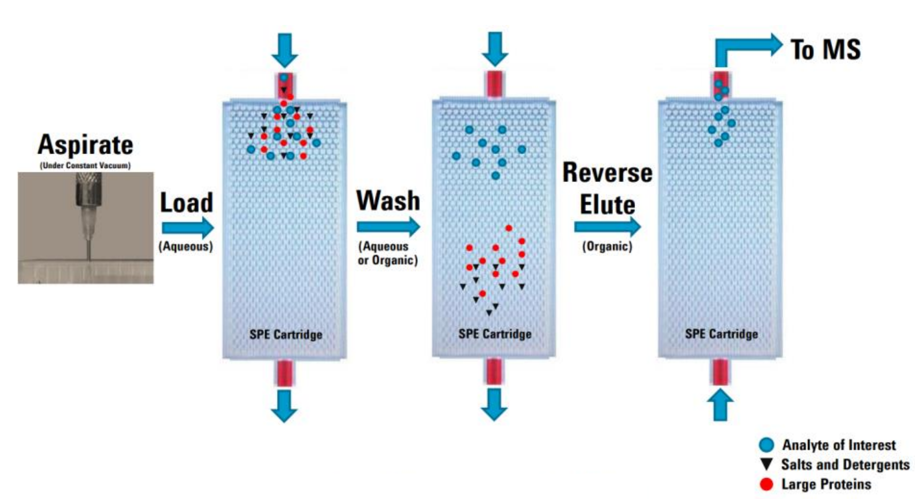


Figure 3. RapidFire injection cycle.

Instrument Settings

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	2000
Elution	7000
Re-Equilibrate	2000

Ultivo Conditions

Parameter	Value
Ion Mode	APCI
Polarity	Positive
Drying Gas Temp	300 °C
Drying Gas Flow	6 L/min
Nebulizer	55 psi
APCI Heater	350 °C
APCI Needle	4 μA
Capillary Voltage	3000 V

Results and Discussion

Ultra-Fast Data Acquisition

Injections were made at a rate of approximately 12.5 seconds per sample while data was acquired by triple quadrupole mass spectrometry. Figure 4 shows 72 injections acquired in under 15 minutes; several blanks were run between calibration levels to assess carryover.

Reproducible and Accurate Results

Triplicate injections of each calibrator demonstrated excellent reproducibility. Coefficients of variation range from 4.5 to 9.0% for NPIP (Figure 4) and are representative of all analytes. Excellent linearity is also observed, with R^2 ranging from 0.997 to 0.999 (Figure 5).

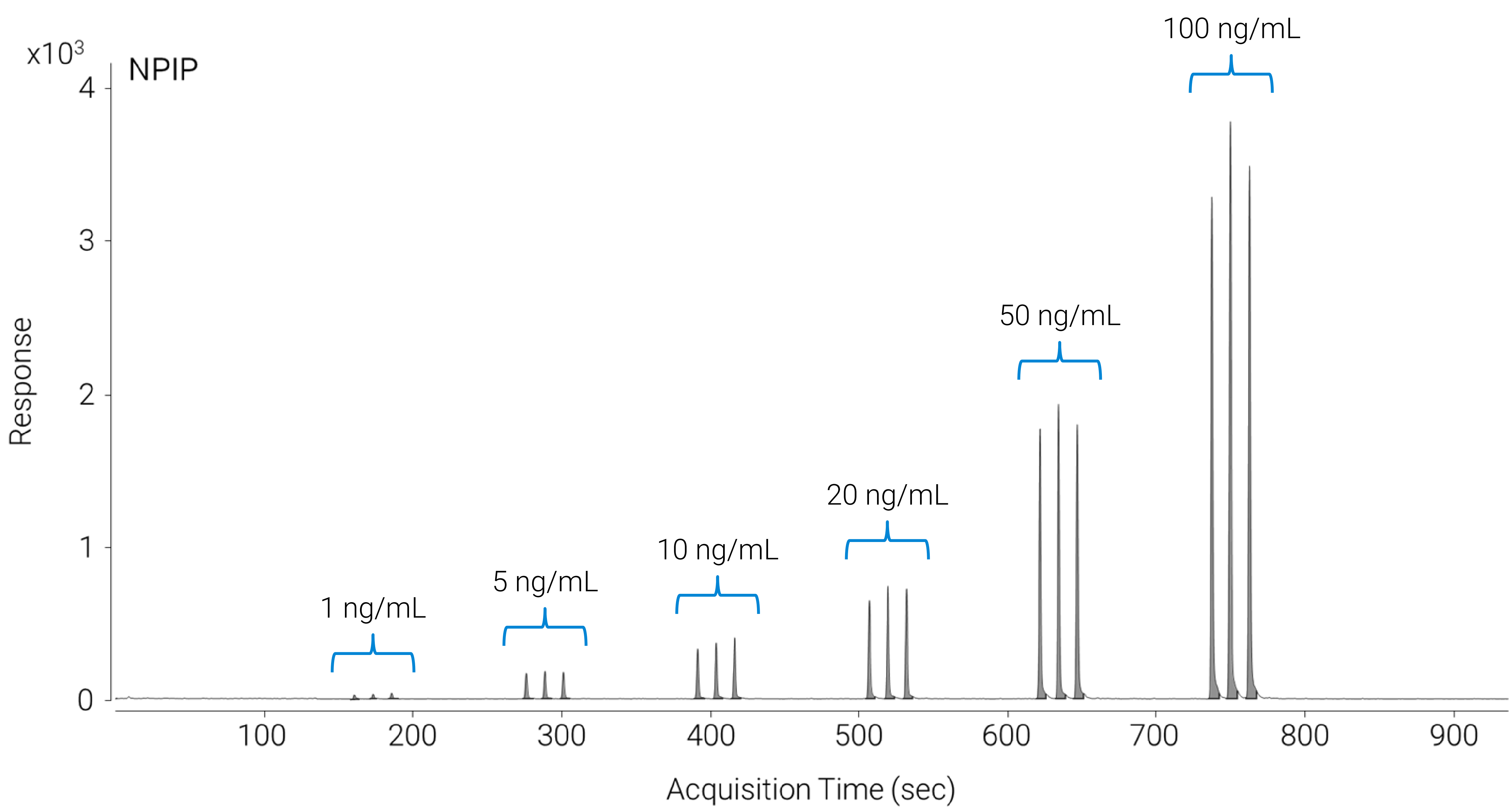


Figure 4. Triplicate injections of a 6-point calibration curve for NPIP. Six blank injections were made between each calibration level to evaluate carryover.

Results and Discussion

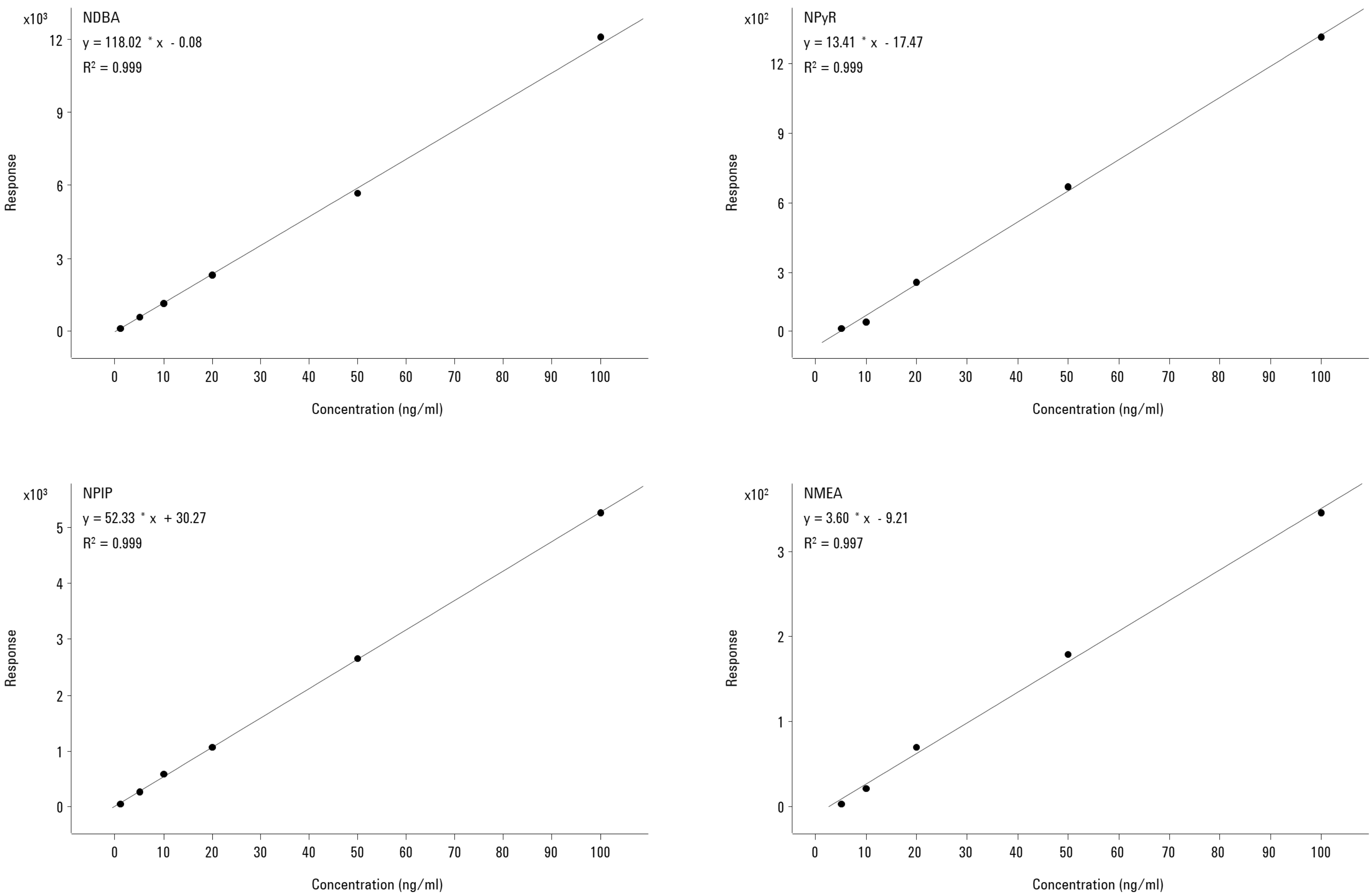


Figure 5. Calibration from 1-100 ng/mL for NDBA and NPIP. Calibration from 5-100 ng/mL for NPyR and NMEA.

Conclusions

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, without any significant method development.

References

[1] FDA Updates and Press Announcements on Angiotensin II Receptor Blocker (ARB) Recalls. (2019, November 13). FDA. Retrieved April 15, 2020. https://www.fda.gov/drugs/drug-safety-and-availability/fda-updates-and-press-announcements-angiotensin-ii-receptor-blocker-arb-recalls-valsartan-losartan

[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

[3] Information about Nitrosamine Impurities in Medications. (2020, February 3). FDA. Retrieved April 15, 2020. https://www.fda.gov/drugs/drug-safety-and-availability/information-about-nitrosamine-impurities-medications

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