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Application News

No. GCMS-1602

Gas Chromatograph Mass Spectrometer

Determination of N-Nitrosamines by USEPA Method 521 using Triple Quadrupole Gas Chromatography Mass Spectrometry

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■ Introduction

N-nitrosamines form during chlorination of finished drinking water. These compounds are classified as possible human carcinogens by the International Agency for Research on Cancer (IARC), and were detected in about 25% of the drinking water systems monitored in the Unregulated Contaminant Monitoring Rule 2 (UCMR2)[1].

Detection and quantitation of N-nitrosamines for contaminant monitoring requires using USEPA Method 521 (2004)[2] employing tandem MS with chemical ionization (CI). Method 521 was originally developed using ion trap MS, and all of the validation data presented in the method are based on this technique. Unfortunately, ion trap mass spectrometers are no longer available. A new method is needed.

This paper describes development of a Multiple Reaction Monitoring (MRM) instrument method using a commercially available triple quadrupole GC/MS/MS for detection and quantitation of N-nitrosamines after extraction as described by USEPA Method 521. GC/MS/MS in MRM mode produces significant improvements in selectivity and specificity, as well as dramatically lower detection limits than single quadrupole GC/MS, especially in complex matrices producing background interferences. In addition, this paper presents final instrument configuration and operating conditions, as well as instrument validation results, including estimated MDLs, and precision & accuracy as evaluated using standards at various concentration levels. Method 521 tandem MS with CI results are compared to the electron impact ionization triple quadrupole results.

■ Objective

The objective of this study was to develop a simpler determination step than that described in the existing EPA 521 method and the various other N-nitrosamine experiments reported elsewhere[3,4]. Because Method 521 is a drinking water method, there is very little modification allowed. Even though Section 6.11.3 of the method states: "The tandem mass spectrometer may be either a triple quadrupole or an ion trap", the same section also states "during the method development, only ion trap spectrometers were used". Changing a detector is not allowed unless there is sufficient data to support the change. Unlike the flexibility allowed with 40 CFR Part 136.6 for wastewater, modifications of drinking water methods must go through a full ATP evaluation; since drinking water regulations are national standards, single laboratory validations are not permitted[5]. This paper describes a "proof of concept" and demonstrates that the triple quadrupole MS/MS detection technique provides data that are as "equally effective" as tandem ion trap MS/MS.

■ Discussion

Preliminary work for a new ASTM method to replace the ion trap tandem GCMS instrument described in EPA method 521 with a newer triple quadrupole MS/MS technology is presented. The proposed method does not change, or modify, any sample collection, preservation, extraction, or concentration of extracts. Quality control measures specific to Large Volume Injection (LVI), CI, and ion trap MS/MS (Table 2, Table 2b, Table 3 and Table 5 in Method 521) need to be eliminated or modified. For the method to be an acceptable alternative to Method 521, all performance QA/QC must be equal or better than the existing method. Again, the intent was to create a determination step simpler than the techniques described in the method; therefore, this method uses 70 Electron Volt EI ionization, and simple syringe injection of 2 micro-liters of sample. Eliminating CI and LVI simplifies the method.

Description of the Technology Modification to Method 521

The Ion Trap Detector required by Method 521 stores ions electronically, manipulating them with a series of timed events. The ions in the trap react with any neutral molecules present for a collision-induced ionization (CID). The product ion is measured. Triple quadrupole mass spectrometry is the linking together of two quadrupoles spatially. Ions travel continuously from the EI source, through the quadrupoles, and to the detector. At any given time, a quadrupole allows only a selected ion to pass; all other ions collide with the poles never reaching the detector. In Selected Reaction Monitoring (SRM), a predefined precursor ion is selected in the first quadrupole (Q1). The collision cell (CID) fragments the ion further, and the second quadrupole (Q3) quadrupole selects a single product ion. Multiple Reaction Monitoring (MRM) is the application of SRM to multiple product ions from one or more precursor ions. In this study, the analysis of a series of transitions (precursor/product ion pairs) provides quantitative and qualitative data. The MRM technique eliminates matrix interference, making it highly selective and extremely sensitive. Faster scanning analyzers, such as the Shimadzu GCMS-TQ8040 are also capable of identification of non-targeted analytes by running full scan mode and MRM mode in a single method.

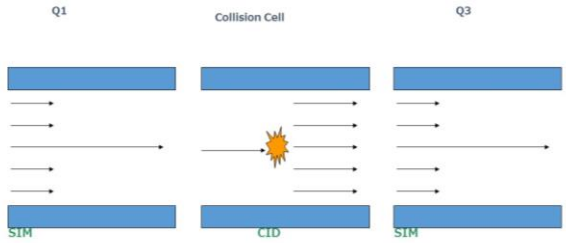

Figure 1: Schematic of a Triple Quadrupole Mass Analyzer; precursor ions collide with a gas in the collision cell to create product ions

Figure 2: Shimadzu GCMS-TQ8040

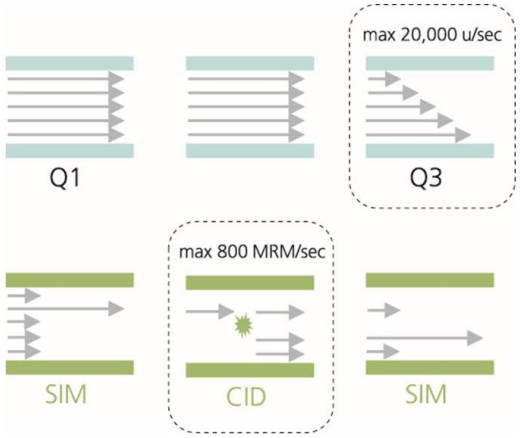

Figure 3: Schematic of a Triple Quadrupole in Full Scan Mode (top) and MRM Mode (bottom)

■ Experimental

This study was conducted using a Shimadzu GCMS-TQ8040 (Figure 2) configured with a Restek capillary column designed specifically for the analysis of N-Nitrosamines. The GC was operated in constant linear velocity mode, providing the best chromatographic resolution, symmetrical peak shapes, and enhanced sensitivity for complete separation of all target analytes from each other and from the solvent peak (Figure 4). A commercial mixture of seven N-nitrosamine compounds, one internal standard, and one surrogate was used to prepare calibration curves ranging from 0.5 – 50 ng/L. The standards were prepared in methylene chloride. Chromatographic conditions were established and the MRM method was optimized for each component. The instrument operating conditions are shown in Table 1.

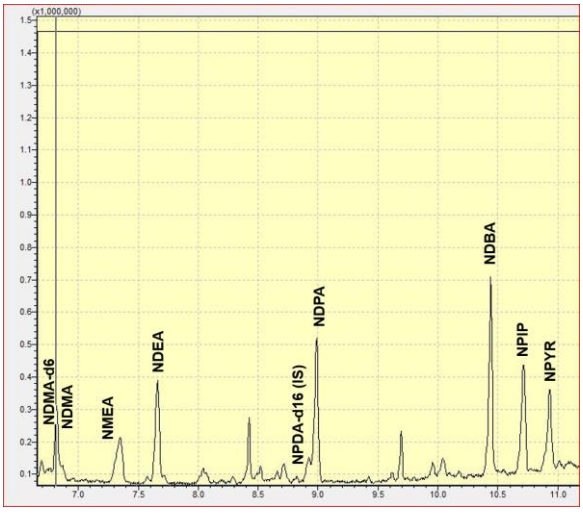

Figure 4: TIC of Scan Data for N-Nitrosamines and Internal Standard NDPA in GC-MS Scan Mode

Table 1: GCMS-TQ8040 Operating Conditions

Instrument	**GCMS-TQ8040**
Column	Stabilwax-MS (Restek PN 10673), 30 m x 0.25 mm x 0.25 um df
Oven Program	50°C, hold 2.0 minutes, 15°C/minute to 130°C, 20°C/minute to 220°C, hold 4.0 minutes
Injector	Pulse splitless (300kPa for 1.0 mimute) 200°C Single taper w/wool, 3.5 mm ID x 5.0 x 95 (Restek PN 23336.5) Injection volume, 2.0 uL
Carrier Gas Column Carrier Gas	Helium Constant linear velocity mode, 40.0cm/sec Total Flow 50.0 mL/min, Column Flow = 1.22 mL/min Purge Flow 3.0 mL/min
Interface Temperature	220°C
Mass Spectrometer	
Ion Source Temperature	200°C
MS Operating Mode	Acquisition Mode, MRM CID gas, Argon (200 kPa) Solvent cut time, 6.5 minutes Detector voltage set relative to tune + 0.2 kV Threshold = 0.0 Ionization type, EI Electron Voltage, 70 eV Event time, 0.3 sec
Analysis Time	**15.83 minutes**
GC Cycle Time	20.0 minutes

Calibration

A 7-point calibration curve of 0.5 to 50 ng/L was analyzed using the conditions described in Table 1. The curves of all nine components were evaluated using linear regression and %RSD of the calculated response factors. Table 2 lists calibration data and the abbreviations used for the remainder of this text. Linear calibration curves with internal standards (IS) were established for seven N-nitrosamines as shown in Figure 5. The linearity with correlation coefficient (R^2) greater than 0.999 across the calibration range of 0.5 ppb – 50.0 ppb was obtained. A MRM chromatogram from a mid-point standard is shown in Figure 6.

Table 2: Calibration Data and Abbreviations

Component	**Abbreviation**	**Retention Time (min)**	**% RSD or r^2**
N-Nitrosodi-n-propylamine (IS	NDPA-IS	8.921	IS
N-Nitrosodimethylamine d-6 (SURR)	NDMA (SURR)	6.808	Surr
N-Nitrosodimethylamine	NDMA	6.818	0.9997
N-Nitrosomethylethylamine	NMEA	7.350	0.9998
N-Nitrosodiethylamine	NDEA	7.660	0.9998
N-Nitrosodi-n-propylamine	NDPA	8.986	0.9999
N-Nitrosodi-n-butylamine	NDBA	10.438	0.9999
N-Nitrosopiperidine	NPIP	10.707	0.9998
N-Nitrosopyrrolidine	NPYR	10.929	0.9999

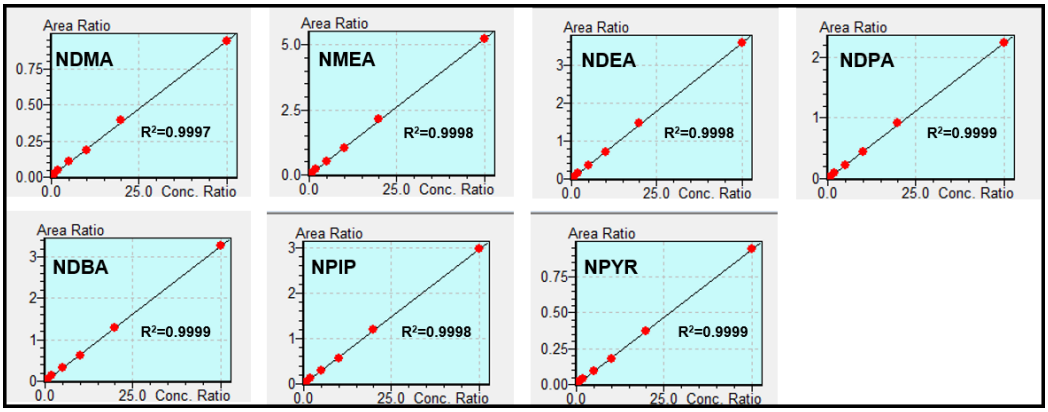

Figure 5: MRM Calibration Curves of seven N-nitrosamines from 0.5, 1.0, 2.0, 5.0, 10.0, 20.0, and 50.0 ppb with IS.

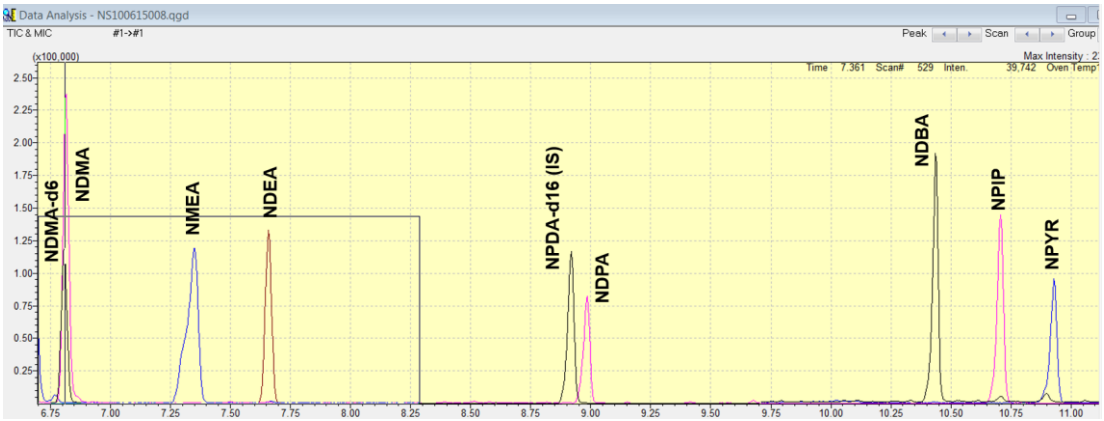

Figure 6: Mid-Point Standard MRM Chromatogram for 20 ng/L Calibration Standard

MRM Method Development

MRM transitions were monitored for each component. Quantitative and qualitative transitions were selected to provide maximum sensitivity and as independent confirmation of the compounds' identity. Even at the standard 70 eV the Ion Shield High Efficiency Source provided sufficient abundance and transmission of ions into the quadrupoles. EI is a suitable alternative to the CI procedure required by Method 521.

Method settings were made to provide enough sensitivity to easily detect and quantify the target analytes at concentrations equal to or better than Method 521. MRM transitions and collision energies (CE) for each compound are shown in Table 3.

Table 3: GCMS-TQ8040 MRM Transitions and Collision Energies Compared to Product Ions Given in Method 521

Component	Quantitative			Qualitative			Method 521
	Precursor	Product	CE (V)	Precursor	Product	CE (V)	Product Ion
NDPA-IS	78.00	50.10	6	144.00	50.10	15	97 (97)
NDMA - (SURR)	80.00	50.10	6	80.00	46.10	18	46(59)
NDMA	74.00	44.10	6	74.00	42.10	15	43 (56)
NMEA	88.00	71.10	6	88.00	73.10	6	61 (61)
NDEA	102.00	85.10	6	102.00	56.10	15	75 (75)
NDPA	130.00	113.20	6	130.00	88.10	6	89 (89)
NDBA	116.00	99.10	6	158.15	99.1	9	57 (103)
NPIP	114.00	84.10	9	114.00	97.10	6	55 (55)
NPYR	100.00	55.10	9	100.00	68.10	9	69 (69)

Method 521 product ions are based on methanol as the ionization gas. Values in parentheses are for an acetonitrile ionization gas. The proposed method monitors two transitions per analyte. The most sensitive transition was chosen for quantitative analysis. The other transition is used as qualitative verification of the identity of each peak.

Instrument Detection Limit

An instrument detection limit (IDL) study was made using eight replicate injections at 0.5, 1.0 and 1.25 ng/L standards. These estimated Method Detection Limit (MDL) results were compared to Method 521 detection limits and are shown in Table 4.

Table 4: Method Detection Limits

Component	IDL ng/L			
	0.5 ng/L standard	1.0 ng/L standard	1.25 ng/L standard	Method 521 MDL (ng/L)
NDMA	0.14	0.22	0.16	0.28
NMEA	0.06	0.11	0.09	0.28
NDEA	0.08	0.05	0.10	0.26
NDPA	0.07	0.07	0.11	0.32
NDBA	0.10	0.08	0.19	0.36
NPIP	0.07	0.06	0.05	0.66
NPYR	0.12	0.14	0.22	0.35

The IDL study for the triple quadrupole method was made using un-extracted standard solutions. Detection limits for extracted samples will be slightly higher. The data indicates that detection limits in extracts should be essentially equivalent to Method 521 MDLs.

Precision and Accuracy

Eight replicates of 10 ppb and 2.0 ppb were made to determine precision and accuracy. Table 5 and Table 6 list the results of the precision and accuracy studies compared to EPA 521.

Table 5: Precision and Accuracy at 10 ng/L

Component	Precision and Accuracy (10 ng/L)			
	Triple Quad Method		EPA 521	
	% REC	%RSD	%REC	%RSD
NDMA	106	2.1	88.7	3.8
NMEA	101	1.1	86.5	4.5
NDEA	98.9	1.0	87.5	9.1
NDPA	98.7	0.6	97.0	10
NDBA	95.4	1.3	86.4	9.4
NPIP	96.4	1.4	91.8	3.7
NPYR	94.7	1.9	101	5.0

Table 6: Precision and Accuracy at 2.0 ng/L

Component	Precision and Accuracy (2.0 ng/L)			
	Triple Quad Method		EPA 521	
	% REC	%RSD	%REC	%RSD
NDMA	103	2.9	94.7	12
NMEA	97.8	1.3	81.8	9.6
NDEA	96.6	2.2	84.6	9.0
NDPA	95.5	1.5	81.7	8.0
NDBA	99.8	3.0	85.2	16
NPIP	97.5	2.0	98.3	20
NPYR	89.4	4.4	92.6	12

The triple quadrupole method results were on standards, not extracts, so it is expected that the recoveries and precision would be better than what would be obtained on extracted samples. However, the data indicates no problem with the instrumental method and that recovery and precision in extracted samples should be essentially equivalent to results obtained on an ion trap detector.

Surrogate Standard Stability

Surrogate recovery was monitored throughout the entire sequence of calibration and analysis of check standards. These analyses were performed in separate batches over a period of two days. These data are shown in Figure 7. Method 521 requires that surrogate recovery be within 70 - 130 %. The excellent recovery (within 80 -120%) of the surrogate standard throughout the run indicates the triple quad method is rugged and suitable for routine laboratory use.

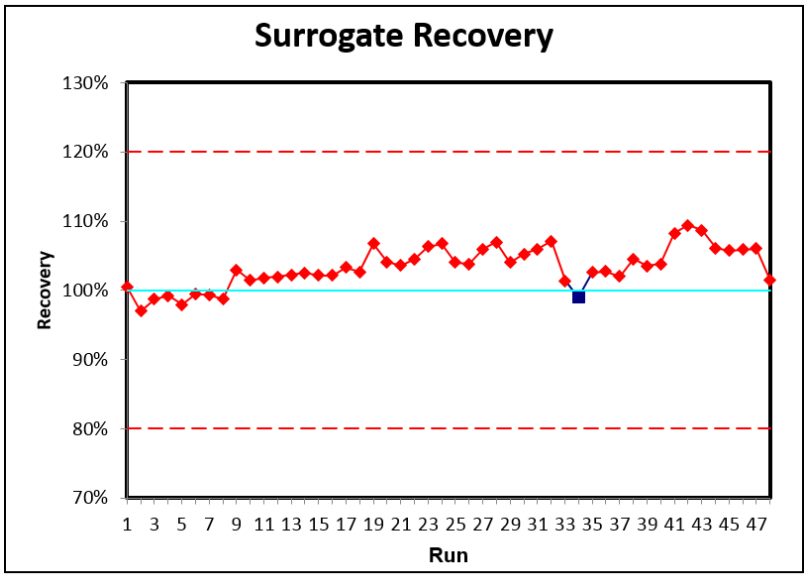

Figure 7: Surrogate Standard Recovery

■ Conclusion

Triple quadrupole analysis with 70 EV EI ionization and 2uL direct injections simplifies detection and quantitation of the N-Nitrosamine compound. Detection limits, precision, and accuracy appear equal to or better than Method 521. Using a triple quadrupole GCMS, such as the Shimadzu GCMS-TQ8040, for N-nitrosamines in drinking water is a viable alternative to EPA Method 521.

This paper evaluated standards only. Our evaluation indicates that triple quadrupole GCMS is a suitable alternative to the ion trap detector described in Method 521.

■ References

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First Edition: January 2016

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