Use of GC-MS Triple Quadrupole Instrumentation for Nitrosamine Analysis

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Overview

Purpose: The following methodology explores an alternative approach to nitrosamine analysis using GC-MS triple quadrupole.

Methods: Various instrument parameters were optimized to obtain the highest detector response to various nitrosamine compounds. The unique functionality of Auto Select Reaction Monitoring (SRM) was used to determine preferred precursor / product ion pairs suitable for two different electron energy ionization settings.

Results: Lower electron ionization energy settings yielded softer ionization of the target compounds and alternate ion intensities with more prevalent higher molecular weight fragments. Optimization of the SRM transitions at 40 eV ionization energy setting with optimized collision energy produced an IDL equivalent to current U.S. EPA 521 methodology.

Introduction

"Classical" nitrosamine analysis was performed for many years by gas chromatography using a thermal energy analyzer (TEA) as detector. Today mass spectrometric methods have increasingly replaced the TEA detector methodology. U.S. EPA method 521 by Munch and Bassett from 2004[1] provided a suitable GC-MS method based on chemical ionization (CI) using an ion trap mass spectrometer with internal ionization, in contrast to standard quadrupole or ion trap mass spectrometers using a dedicated (external) ion source design. Analysis also required a large sample volume injection (10 – 20 µL) to obtain the desired sensitivity requirements. At present current developments in GC-MS triple quadrupole technology deliver enhanced sensitivity and selectivity for small molecule mass range compounds and allow the detection of nitrosamines at low concentration levels even in complex matrix samples. This is made possible by using a much simpler and standard approach with the regular electron impact ionization (EI) for a very straightforward method that enables low concentration nitrosamine analysis. The following methodology explores an alternative approach to nitrosamine analysis using GC-MS/MS.

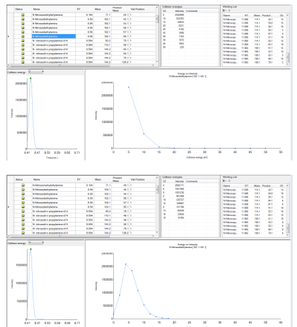
Methods

Method Development – Transition Determination

Compounds with labile chemical bonds require additional consideration when undergoing analysis by GC/-MS/MS. Due to their labile nature standard 70 eV EI fragmentation produces a mass spectrum that exhibits numerous low molecular weight fragments. The absence of high molecular weight fragments limits the availability of precursor / product ion pairs with significant and reproducible intensities to support low concentration analysis.

A mix of 7 nitrosamines, 1 surrogate and 1 deuterated internal standards at 500 pcg on column concentration diluted in methylene chloride was used for method development. Using Auto SRM precursor / product ion pairs were developed and optimized for each nitrosamine compound. Initial product ions were selected based on optimal intensity from varying collision energies from 5V with subsequent 5V increment increases. After selecting the highest intensity precursor / product ion pairs and optimal collision energies another round of targeted collision energy optimization was completed using 2v increments bracketing the initial collision energy voltage.

FIGURE 1. The displays of the optimization experiments using Auto SRM.



Additional precursor / product ion pair optimization was completed at 40 eV ionization energy to evaluate the effect of softer ionization on ion intensities and to attempt to increase method selectivity.

A Thermo Scientific™ TriPlus RSH™ Autosampler coupled to a Thermo Scientific™ TRACE™ 1310 Gas Chromatograph, equipped with a split/splitless injection module and single tapered liner, provided 1.0 µL sample introduction into the Thermo Scientific™ TSQ™ Duo Triple Quadrupole GC-MS/MS. Chromatographic separation was achieved using a TG-WAX MS, column with 0.25 mm internal diameter, 30 meter length and 0.5 µm film thickness. The detection of the Nitrosamine compounds was performed using the TSQ Duo triple quadrupole mass spectrometer system operated in MS/MS mode. Thermo Scientific™ TraceFinder™ (3.2) software provided automated acquisition and processing of all data, including quantitation and response factor calculations. Extended operating parameters are available in Figure 2.

TABLE 1. Extended operating parameters for GC-MS/MS analysis.

Trace 1310 GC Parameters	
Inlet Module and Mode:	iC S/SL, Splitless for 1 min / Surge @ 300 kpa
Inlet Temperature (°C):	250
Injection Volume (mL):	1.0
Carrier Gas, (mL/min):	He, 1.0
GC Oven Temperature Program	
Initial Temperature:	45°C Hold for 3.0 min
Ramp 1 (°C):	45°C to 130°C @ 25 °C/min
Ramp 2 (°C):	130°C to 280°C @ 12 °C/min, Hold for 1.0 min
Runtime (min.):	14.7
TSQ Duo Mass Spectrometer Parameters	
Acquisition Mode:	Timed SRM
Transfer line (°C):	250
Ionization type:	EI
Ion source(°C):	220
Electron voltage (eV) :	70 / 40

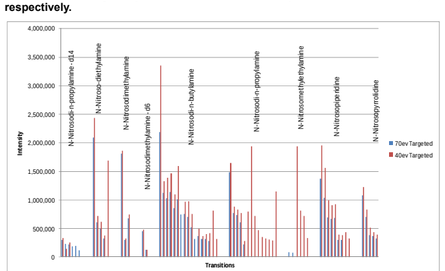
Final method parameters were evaluated using in solvent spiked standards at 6 concentration levels and replicate evaluation at 1 pcg on column to estimate an instrument detection limit (IDL).

Results

Results – Method Development

Evaluation of compounds in 5v increments did not produce a visible apex for response of the compounds. Using a second round of targeted collision energy optimization allowed determination of the apex response for each nitrosamine compound at the corresponding collision energy ensuring method optimization. Use of softer ionization to increase selectivity through higher-mass precursor selection is well-known, (e.g, the technique is also used for the analysis of dioxins[2]). A comparison of precursor product ion pair intensity found at standard 70 eV and 40 eV ionization energies is shown in Figure 2.

FIGURE 2. Intensity comparison of SRM transitions optimized at 70 and 40 eV, respectively.



The higher intensity fragments and higher abundance of available transitions were produced using targeted CE optimization and softer electron ionization energy of 40 eV. The resulting optimized precursor product ion pairs are optimized at 40 eV ionization energy are displayed in Table 2.

TABLE 2. Optimized precursor production pairs developed using Auto SRM.

Name	RT	Precursor Mass	Product Mass	Collision Energy
N-Nitrosodimethylamine	7.8	74.1	44.1	6
		74.1	42.1	14
		74.1	43.1	12
N-Nitrosodimethylamine-d6	7.8	80.1	50.1	6
		80.1	46.1	14
N-Nitrosomethylethylamine	8.2	88.1	71.1	4
		88.1	42.1	16
		88.1	43.0	8
N-Nitrosodiethylamine	8.5	102.1	85.1	4
		102.1	44.1	12
		57.1	42.1	6
N- nitrosodi-n-propylamine-d14	9.6	78.1	50.1	6
		78.1	46.0	10
		144.2	50.0	10
N-Nitrosodi-n-propylamine	9.7	130.1	113.1	4
		101.1	70.1	6
		70.1	43.1	6
N-Nitrosodi-n-buytlamine	11.3	116.1	99.1	6
		115.1	84.1	6
		158.2	99.1	8
N-Nitrosopiperidine	11.7	114.1	84.0	8
		114.1	41.1	12
		114.1	97.1	6
Nitrosopyrrolidine	12.0	100.1	55.1	6
		100.1	43.1	10
		100.1	70.1	6

Results

Results – Method Evaluation

Under the optimized conditions calibration curves were generated for the concentration range of 1 to 50 pg injected on-column, with six calibration levels in methylene chloride solvent. 10 replicates were analyzed at a concentration of 1 pcg on column with an injection of only 1 µL of solution. The correlation coefficient values for the seven nitrosamine compounds were determined at greater than or equal to 0.999. (Table 3). Replicate injection results were used to calculate IDLs for the seven targeted nitrosamines (Table 3).

TABLE 3. The resulting correlation coefficients with IDL comparison to existing nitrosamine analysis methodology.

Compound	R^2	TSQ Duo IDL	EPA Method 521
N-Nitrosodimethylamine	0.9997	0.33	0.28
N-Nitrosodimethylethylamine	0.9997	0.19	0.28
N-Nitrosodiethylamine	0.9999	0.13	0.26
Nitrosodi-n-propylamine	0.9996	0.26	0.32
Nitrosodi-n-butylamine	0.9998	0.32	0.36
N-Nitrosopiperidine	0.9998	0.28	0.66
N-Nitrosopyrrolidine	0.9990	0.44	0.35

Conclusion

An alternative methodology was explored for the analysis of nitrosamine compounds using GC-MS triple quadrupole.

- SRM transitions for seven nitrosamines were optimized using Auto SRM.
- Transition intensity and abundance were increased using an alternative electron ionization energy of 40 eV.
- Using a lower (1 µL) injection volume similar sensitivity was achieved in comparison to large volumes required for U.S. EPA method 521.
- Method development produced IDL's equivalent to requirements for routine analysis using ion trap instrumentation via U.S. EPA 521 methodology.

Auto SRM allows optimization of collision energies to increase response of target analytes and exploit the sensitivity of the triple quadrupole technique to its full potential. The need for large injection volumes to increase compound concentrations and chemical ionization to reduce fragmentation and increase overall sensitivity are obviated using the TSQ Duo. The increases in sensitivity of current triple quadrupole instrumentation and advanced method development software like Auto SRM provides a prospect for replacement of established ion trap methodologies.

References

1. Munch, J.W., Bassett, M.V. *EPA Method 521 Determination of Nitrosamines in Drinking Water by solid phase extraction and Capillary Column Gas Chromatography with Large Volume Injection and Chemical Ionization Tandem Mass Spectrometry (MS/MS)*, Version 1.0, September 2004.
2. Cristian Cojocariu, C., Silcock, P., Kotz,A. *Validation of GC-MS/MS for Detection and Confirmation of Low-Level Dioxins.* Application Note 10406; Thermo Scientific: Runcorn, U.K.

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