

Quantitative IC-MS/MS Analysis of Nitrogen Mustard Hydrolysis Products as Ethanolamines in Water Samples

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INTRODUCTION

Ethanolamines have been used as bio- and environmental markers to measure potential exposure to nitrogen mustards (HN1, HN2, and HN3 listed on the Chemical Weapons Convention Schedule of Chemicals¹). Direct quantification of exposure to HN1, HN2, and HN3 is difficult due to their reactivity, extent of metabolism, and short half-life.² Nitrogen mustards readily react with biomolecules and are found in urine as the hydrolysis products: N-methyldiethanolamine, N-ethyldiethanolamine, and triethanolamine.³ Ethanolamines are produced by heating ethylene oxide with concentrated aqueous ammonia under pressure and separating the products by fractional distillation. Over half a million tons of ethanolamines are manufactured every year. Ethanolamines have a wide range of both industrial and domestic uses, including in the manufacture of pesticides, emulsifying agents, detergents, bactericides, and cosmetics.⁴ A quantitative analytical method is necessary to monitor the removal of ethanolamines from industrial-discharged wastewater and to determine the extent of human and environmental exposure to nitrogen mustards.

Reported methods for ethanolamines analysis include GC or LC separation with MS detection.^{5,6} GC-MS methods involve labor-intensive derivatization which limits throughput. Reported LC methods demonstrate poor retention and chromatographic separation with reversed-phase (RP) columns.

This poster describes an ion chromatography tandem mass spectrometric (IC-MS/MS) method for quantitative analysis of ethanolamines in water samples. Ion-exchange columns were used to overcome the poor retention of RP columns, and tandem mass spectrometry with isotope labeled internal standard (IStd) was used to provide selective and sensitive detection and ensure quantification accuracy.

EXPERIMENTAL

Chromatographic Conditions

System:	ICS-2000 RFIC™ system	
Columns:	IonPac® CS15 (250 × 2 mm) cation-exchange column with CG15 guard CR-CTC continuously regenerated cation-trap column (2 mm)	
Temperature:	40 °C	
Mobile Phase:	Methanesulfonic acid generated from EGC II MSA cartridge	
	Time /min	MSA concentration
	-4.0	2 mM
	8.0	2 mM
	18.0	30 mM
	22.0	30 mM
Flow Rate:	0.40 mL/min	
Injection:	20 µL	
Detection:	Conductivity and TSQ Quantum Access™ mass spectrometer	

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Mass Spectrometric Conditions

Interface:	Electrospray Ionization (ESI) positive polarity
Desolvation Solvent:	0.2 mL/min isopropanol delivered by AXP-MS pump
Scan Mode:	Selected Reaction Monitoring (SRM) (See Table 1 for details)
Spray Voltage:	4000 V
Sheath Gas:	60 arbitrary units
Auxiliary Gas:	15 arbitrary units
Capillary Temp.:	300 °C

Chemicals and Standards

Diethanolamine	Fluka: 31589	CAS: 111-42-2
N-methyldiethanolamine	Aldrich: 471828	CAS: 105-59-9
N-ethyldiethanolamine	Aldrich: 112062	CAS: 139-87-7
Triethanolamine	Fluka: 90279	CAS: 102-71-6
Diethanolamine-d ₈	C/D/N Isotopes: D-5308	CAS: 103691-51-6

All chemicals were dissolved in deionized (DI) water to prepare individual primary stock solutions at 1000 µg/mL (ppm). Working stock solutions were prepared for each analyte by diluting primary stock solutions in deionized water to 10 ppm, 1 ppm, and 100 ppb to prepare calibration standards. A working stock solution for the internal standard was prepared at 1 ppm in deionized water for preparation of calibration standards and to spike unknown samples.

Calibration standards were prepared in deionized water at 7 levels: 1 ppb, 5 ppb, 10 ppb, 20 ppb, 50 ppb, 100 ppb, and 200 ppb with internal standard at 20 ppb in each calibrant.

RESULTS AND DISCUSSION

Chromatography

Cation-exchange chromatography was selected for this study to overcome the poor retention reported with standard reversed-phase columns. Significant retention for all target analytes was observed in tests of two cation-exchange columns, i.e. CS15 and CS18 columns. The CS15 column was selected as the analytical column because it provided retention of target analytes and also sufficient separation of the target analytes from major common cations that may introduce interference and contribute to ion suppression, mainly from sodium and potassium. The conductivity trace in Figure 1 shows the chromatographic separation of four target ethanolamines from six commonly seen cations.

Possible errors in the preparation of the IC mobile phase were precluded by the use of RFIC methodology where the eluent is electrolytically generated and can be reproduced with a very high level of precision. To make the IC eluent compatible with MS detection, a CSRS® continuously regenerated suppressor was used to remove MSA from the mobile phase by electrolytically converting the MSA mobile phase to DI water prior to the introduction to the electrospray source.

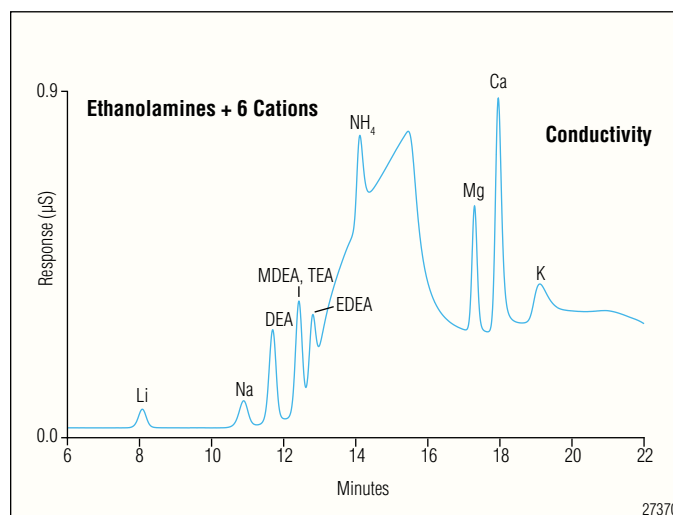


Figure 1. Separation of ethanolamines from common cations.

Mass Spectrometry

Although MSA is removed from the IC eluent before entering the MS detector, the IC eluent as a 100% aqueous mobile phase requires a high source temperature and a higher nebulizer gas flow for efficient ionization. The detrimental effects of a high source temperature may cause target analytes to decompose, thus decreasing sensitivity. To improve the ionization/desolvation process while maintaining sensitivity, a desolvation solvent (isopropanol) was introduced to the IC eluent using a low-volume static mixing tee using an AXP-MS auxiliary pump prior to entering the MS detector.

For each analyte, two SRM transitions were used with the higher responsive SRM as the quantitative transition while the other served as a confirmative transition; details are shown in Table 1.

TABLE 1. SRM SCAN PARAMETERS					
Analyte	Abbreviation	t_r	Q1MS	Q3MS	Collision Energy
Diethanolamine	DEA	11.7	106.1	88.1	10
			106.1	70.1	14
Diethanolamine-d ₈	DEA-IS	11.7	114.1	96.1	11
			114.1	78.1	14
N-Methyldiethanolamine	MDEA	12.4	120.1	102.0	13
			120.1	58.0	18
N-Ethyldiethanolamine	EDEA	12.8	134.1	116.0	13
			134.1	72.0	18
Triethanolamine	TEA	12.4	150.1	132.0	12
			150.1	88.0	17

Method Performance

The chromatography was optimized to retain and separate target analytes from commonly present cations and with the MS detector operating at SRM MS/MS mode. Figure 2 shows the SRM chromatograms of four target ethanolamines and the internal standard.

Method performance was evaluated with respect to selectivity, carryover, calibration, detection limits, precision, accuracy, and matrix effects.

Method selectivity was evaluated against two matrices: DI water and lab-simulated matrix, which contain 20 ppm of sodium (sodium chloride) and potassium (potassium chloride). No interference was observed. Carryover was evaluated by injecting DI water blank after the highest calibration standard (200 ppb). No quantifiable peak was observed.

Calibration curves were generated by analyzing the calibration standards from 1 ppb to 200 ppb using internal calibration. $1/x$ was used as the weighting factor to achieve better accuracy for lower-level quantification. Excellent coefficients of determination (r^2) were achieved for all analytes: 0.9997 for DEA (5 ppb to 200 ppb), 0.9990 for MDEA (1 ppb to 200 ppb), 0.9997 for TEA (5 ppb to 200 ppb), and 0.9997 for EDEA (1 ppb to 200 ppb). Figure 3 shows the calibration curve for TEA, and the insert shows the calibration curve at lower levels.

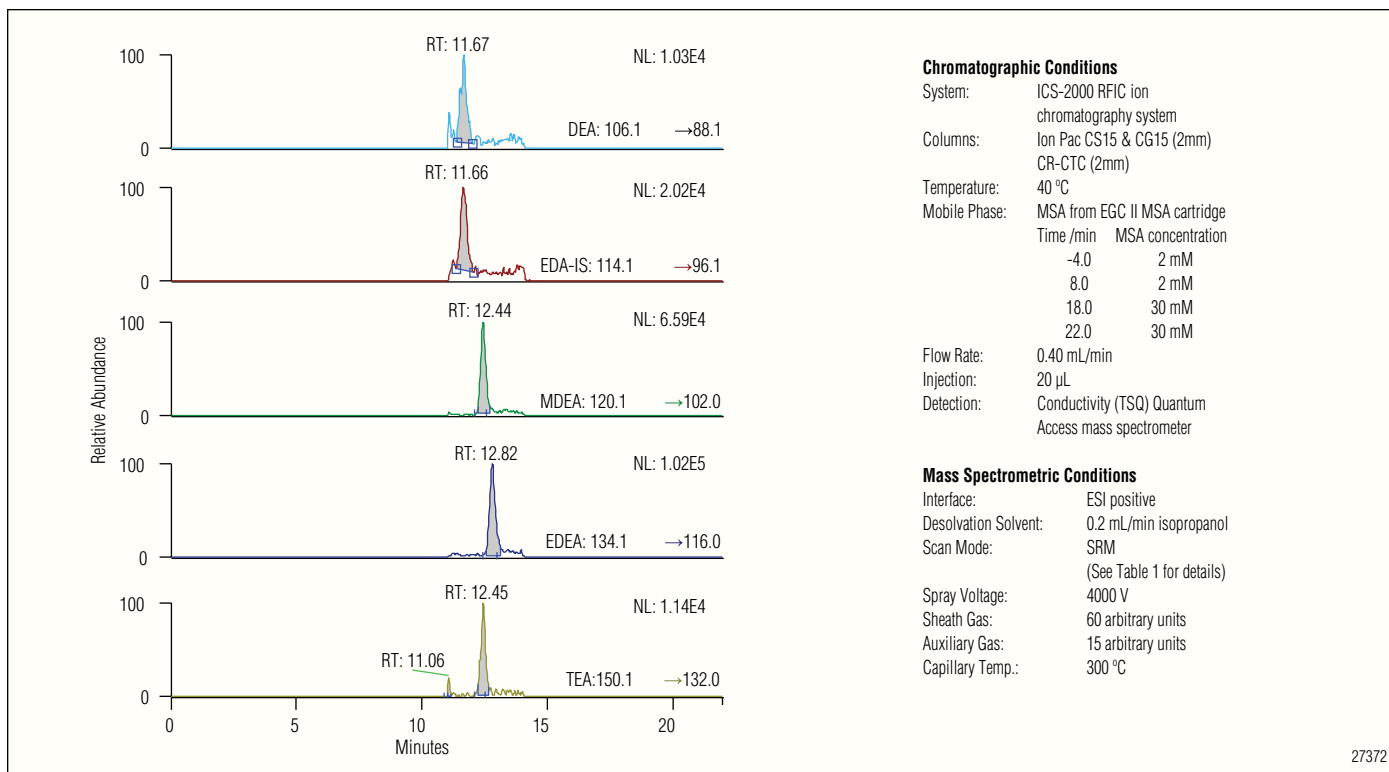
Method detection limits (MDL) were evaluated by seven replicate injections of a mixed standard solution at 5 ppb, and calculated by $MDL = s \times t$, where s is the standard deviation and t is the Student's t at 99% confidence interval. Results are shown in Table 2. Precision and accuracy were also measured by seven replicate injections of a mixed standard solution at 5 ppb and addressed as %RSD and %deviation (see Table 2).

Matrix effects were evaluated by comparing quantification results of standards prepared in DI water and prepared in a laboratory-simulated matrix (containing 20 ppm sodium chloride and potassium chloride). No matrix effects were observed for retention times. However, the MDL was slightly affected by the matrix, showing slightly higher MDL for DEA and TEA. The %RSD was also affected—with a much higher variation for TEA.

TABLE 2. PRECISION AND ACCURACY, MDL, AND MATRIX EFFECTS

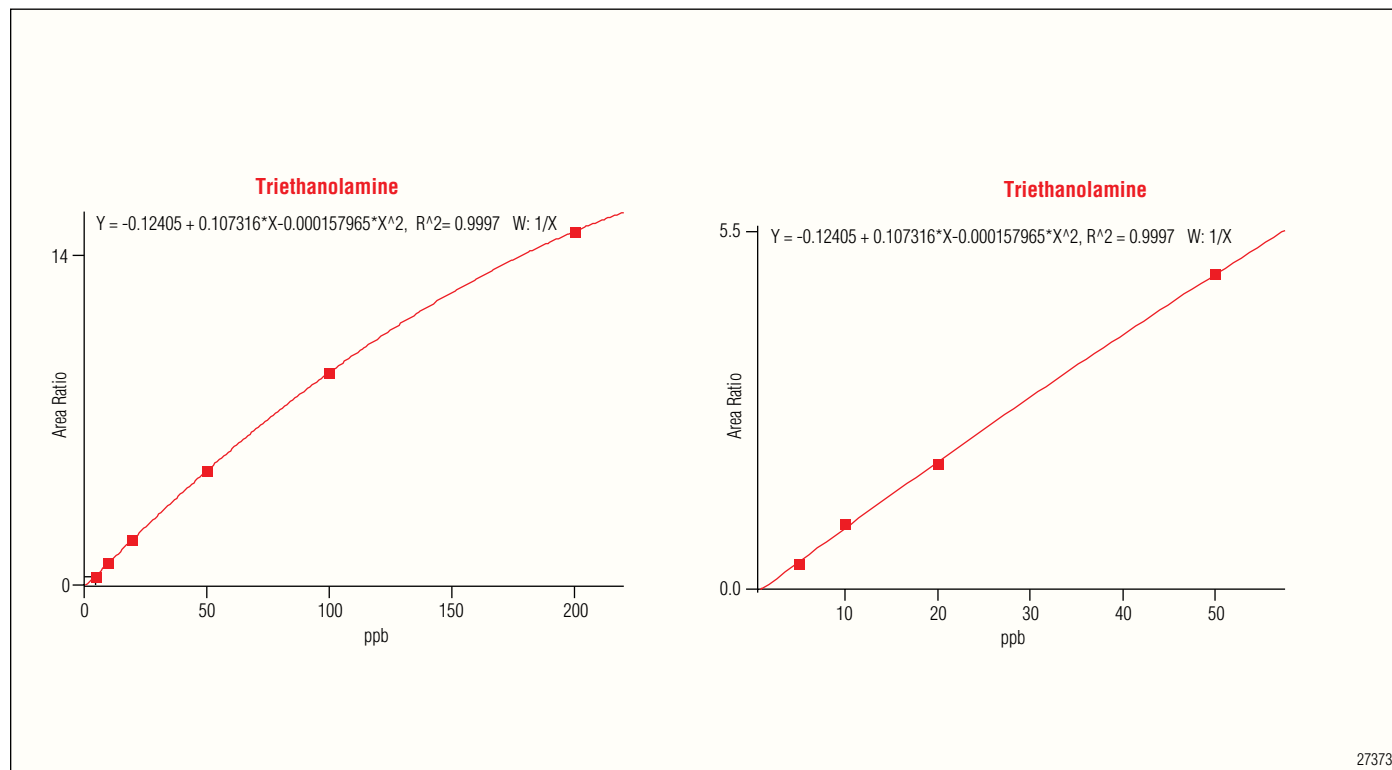
	Analyte	t_R (min)	r^2	Mean*	%Deviation	%RSD	MDL
DI Water	DEA	11.7	0.9997	5.38	7.6	5.65	0.89
	MEDA	12.5	0.9990	5.87	17.4	5.87	0.92
	EDEA	12.9	0.9997	5.25	5.0	6.10	0.96
	TEA	12.5	0.9997	5.84	16.8	3.64	0.57
Matrix	DEA	11.7	0.9999	6.17	23.4	8.80	1.71
	MEDA	12.4	0.9996	4.60	-8.0	4.35	0.63
	EDEA	12.8	0.9979	4.41	-11.8	4.32	0.60
	TEA	12.4	0.9981	4.90	-2.0	10.59	1.63

*Mean is calculated as the average of the observed amount of seven replicate injections.



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Figure 2. SRM chromatograms of ethanolamines at 10 ppb.



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Figure 3. Calibration curve for TEA using DEA-d₃ as the internal standard.

Analysis of Water Samples

A lab-simulated matrix was used because no ethanolamines were found in the available municipal drinking water sources. This matrix contains 20 ppm of sodium chloride and potassium chloride; the most commonly seen cations contributing to interference and ion suppression for IC-MS/MS quantification. As shown in the SRM chromatogram of ethanolamines at 5 ppb in matrix (Figure 4), the retention time, selectivity, and sensitivity were well-maintained. Recovery was calculated by:

$$\text{Observed Amount / Specified Amount} \times 100\%$$

and ranged from 89.2% (EDEA) to 123.4% (DEA).

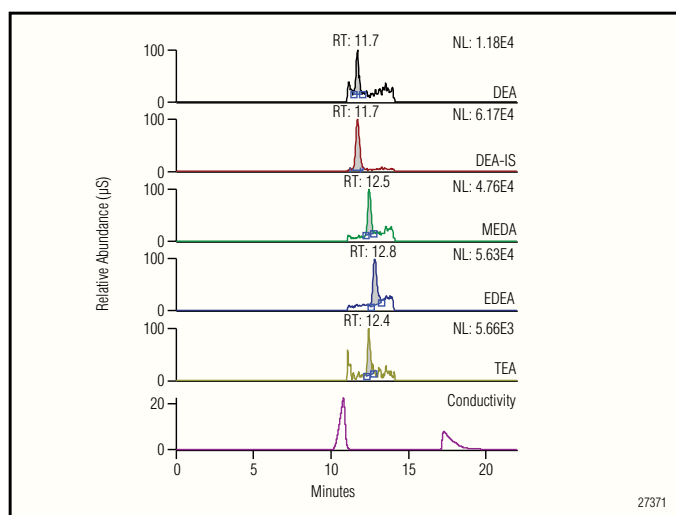


Figure 4. SRM chromatograms of ethanolamines at 5 ppb in simulated matrix (Refer to Figure 2 for instrument conditions).

CONCLUSION

An IC-MS/MS method with direct injection and without sample preparation was developed for quantitative analysis of nitrogen mustard hydrolysis products as ethanolamines. Ion chromatography was selected to retain and separate target analytes from commonly present cations, and MS/MS was used to ensure selective and sensitive detection with a stable isotope-labeled internal standard for quantification. Excellent calibration was achieved for each analyte, with ethanolamines quantified from low ppb to 200 ppb with $r^2 > 0.99$ for each analyte.

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