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Application Note 271

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# Quantitative Analysis of Nitrogen Mustard Hydrolysis Products as Ethanolamines

## INTRODUCTION

Ethanolamines have been used as bio- and environmental markers for nitrogen mustards (HN1, HN2, and HN3), which are listed on the Chemical Weapons Convention Schedule of Chemicals[1] to monitor potential exposures. 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 (MDEA), *N*-ethyldiethanolamine (EDEA), and triethanolamine (TEA).[3]

Over half a million tons of ethanolamines are produced annually and used as emulsifying agents, detergents, ingredients in bactericides and cosmetics, and also in the pesticide manufacturing process.[4] Inefficient removal and/or inappropriate disposal of ethanolamimes may cause adverse effects to the environment.

To monitor human and environmental exposure to nitrogen mustard, and also the removal of ethanolamines from industrial discharged waste, a quantitative analytical method is desired.

Reported methods for ethanolamines analysis include GC or LC separation with MS detection.[8] The GC-MS methods involve labor-intensive derivatization which limits throughput, and reported LC methods usually suffer from poor retention and chromatographic separation with reversed-phase (RP) columns. A fast LC-MS/MS method reported the total separation of MDEA, EDEA, and TEA.[6] However, the estimated retention factor (k) for the first-eluted TEA was less than one, making the method subject to possible interference from sample matrices, which was confirmed in the same report.

This study reports a rapid separation liquid chromatography (RSLC) tandem mass spectrometric (MS/MS) method for quantitative analysis of ethanolamines in environmental water samples. An Acclaim® Trinity™ P1 Mixed-Mode column featuring reversed-phase, anion-exchange, and cation-exchange retention mechanisms was used to provide retention and resolution for all analytes within 5 min. The MS detector was operated in multiple reaction monitoring (MRM) mode, and an isotope labeled internal standard (IStd) was used to provide selective and sensitive detection and to ensure quantification accuracy.

## EQUIPMENT

Dionex UltiMate® 3000 RSLC system including:

- DGP-3600RS dual gradient pump
- WPS-3000TRS autosampler
- TCC-3200RS column oven

## CONDITIONS

| | |
|---|---|
| Column: | Acclaim Trinity P1 (2.1 × 100 mm, 3 µm, P/N 071389) |
| Mobile Phase: | Isocratic, 90% Acetonitrile; 5% DI water; 5% Ammonium formate 100 mM, pH 3.7 |
| Flow Rate: | 0.6 mL/min |
| Temperature: | 20 °C |
| Inj. Volume: | 20 µL |

## MASS SPECTROMETRIC CONDITIONS

| | |
|---|---|
| System: | Triple quadropole mass spectrometer with ESI |
| Curtain Gas (CUR): | 15 psi |
| Collision Gas (CAD): | Medium |
| IonSpray Voltage (IS): | 4500 V |
| Temperature (TEM): | 700 °C |
| Ion Source Gas 1 (GS1): | 50 psi |
| Ion Source Gas 2 (GS2): | 20 psi |
| Ihe: | On |
| Acquisition Mode: | Multiple reaction monitoring (MRM); refer to Table 1 for details on MRM scan parameters |
| Software: | DCMS$^{Link™}$,a Chromeleon®-based software module providing the interface for controlling a wide range of Dionex chromatography instruments from different mass spectrometer software platforms. |

**Table 1. MRM Scan Parameters of Studied Analytes**

| Peak No. | Analyte | ID | Q1 (*m/z*) | Q3 (*m/z*) | Time (ms) | DP (V) | CE (V) | CXP (V) | $t_R$ (min) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | *N*-ethyldiethanolamine | EDEA-1 | 134.1 | 116.0 | 75 | 51 | 21 | 8 | 1.8 |
| | | EDEA-2 | 134.1 | 72.0 | 25 | 51 | 25 | 4 | |
| 2 | *N*-methyldiethanolamine | MDEA-1 | 120.1 | 102.0 | 75 | 46 | 19 | 8 | 2.3 |
| | | MDEA-2 | 120.1 | 58.0 | 25 | 46 | 27 | 4 | |
| 3 | Triethanolamine | TEA-1 | 150.0 | 132.0 | 75 | 61 | 19 | 10 | 2.7 |
| | | TEA-2 | 150.0 | 88.0 | 25 | 61 | 23 | 6 | |
| 4 | Diethanolamine | DEA-1 | 106.1 | 88.0 | 350 | 66 | 19 | 6 | 3.6 |
| | | DEA-2 | 106.1 | 70.0 | 50 | 66 | 21 | 4 | |
| 5 | Diethanolamine-$d_8$ | DEA-IS | 114.1 | 78.0 | 100 | 53 | 24 | 6 | 3.6 |
| 6 | Ethanolamine | EA-1 | 62.0 | 44.1 | 350 | 46 | 15 | 6 | 4.0 |
| | | EA-2 | 62.0 | 45.0 | 50 | 46 | 19 | 6 | |

The 1st MRM of each analyte was used for quantiation, and the 2nd MRM was used for confirmation only.

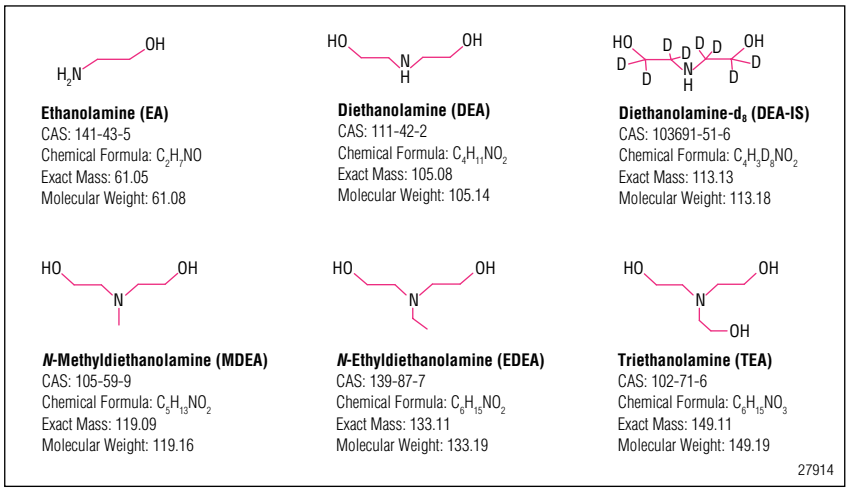


*Figure 1. Chemical structures of studied compounds.*

## *PREPARATION OF SOLUTIONS AND REAGENTS*

### Chemical and Reagents

Standards of studied analytes were purchased from Sigma-Aldrich: ethanolamine (EA, CAS: 141-43-5, Aldrich: 411000), diethanolamine (DEA, CAS: 111-42-2, Fluka: 31589), *N*-methyldiethanolamine (MDEA, CAS: 105-59-9, Aldrich: 471828), *N*-ethyldiethanolamine (EDEA, CAS: 139-87-7 Aldrich: 112062), triethanolamine (TEA, CAS: 102-71-6, Fluka: 90279). Isotope labeled internal standard (IStd) diethanolamine-$d_8$ (DEA-IS) was purchased from C/D/N Isotopes (CAS: 103691-51-6, D-5308). Figure 1 shows the chemical structures and related information.

Ammonium formate was purchased from Aldrich (516961). Acetonitrile was obtained from Burdick & Jackson (HPLC grade, AH015-4). Deionized water (18.2 MΩ-cm resistance) used in this study was obtained from a Millipore water station.

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 DI water to 1 ppm, 100 ppb, 10 ppb, and 1 ppb to prepare calibration standards. A working stock solution for the internal standard was prepared at 100 ppb in deionized water for the preparation of calibration standards and to spike unknown samples. Calibration standards were prepared in DI water at 8 levels: 0.05 ppb, 0.1 ppb, 0.5 ppb, 1 ppb, 2 ppb, 5 ppb, 10 ppb, and 20 ppb. Each level contains all five target analytes with internal standard spiked at 1 ppb.

### Sample Preparation

Surface water samples were collected in HDPE plastic bottles and stored under refrigeration at 4 °C until analysis. An aliquot of each water sample was spiked with internal standard at 1 ppb in a 1.5 mL autosampler vial and analyzed directly (filter the surface water samples when necessary, e.g., if suspended particles are observed).

## *RESULTS AND DISCUSSION*

### Chromatography

As shown in Figure 2, all five target analytes were separated to baseline within 4.5 min. A retention factor (k') of 3.3 for the first-eluted EDEA indicated sufficient retention for all analytes and thus ensured the separation of targeted analytes from early eluting species. Different from general RP columns, the Acclaim Trinity P1 Mixed-Mode column features RP and ion exchange mechanisms, thus providing unique selectivity for ionizable organics. For the mixed-mode column, eluent strength is affected by organic modifier composition, buffer type, buffer pH, and buffer concentration. Refer to the column manual for more information on method development and modification. The conditions described in the experimental section were developed to achieve sufficient retention and total resolution for all target analytes with consideration of method throughput. Although the separation was completed within 4.5 min, the total run time was set at 8 min to elute any possible strongly retained species and thus improve method ruggedness.

### Mass Spectrometry

The aim of this study was to develop a selective and sensitive method for the direct analysis of trace level ethanolamines in environmental water samples, therefore, MS/MS instrumentation was selected for its sensitivity and ability to provide trace level detection. In addition, the selectivity of MS/MS instrumentation allows minimal sample preparation and cleanups. The MS/MS instrument was tuned and run in MRM mode. With continuous infusing of individual standards, each target analyte showed a strong protonated molecular ion $[M+H]^+$ in positive ESI mode, and was used as the Q1MS precursor ion for MRM experiments. Product ions were selected using the Compound Optimization option from the instrument operating software. The three most intense MRM transitions were selected as MRM candidates for further selectivity evaluation. The MRM selectivity was evaluated by analyzing individual standards with respect to chromatographic separation and MS/MS detection with MRM candidate transitions.

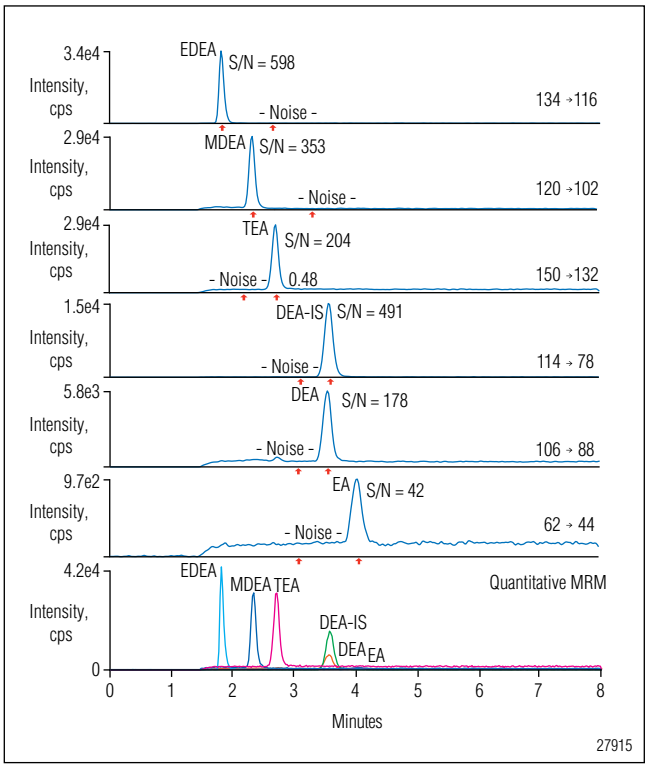


*Figure 2. MRM chromatograms of five ethanolamines by RSLC-MS/MS on an Acclaim Trinity P1 column with 0.5 ppb of each analyte.*

The two final MRM transitions were selected that showed specific MS peaks with better intensity. It is worth noting that interference was observed for both DEA MRM channels from TEA and EDEA; and this can be explained by the source region fragmentation of TEA and EDEA: $[M\text{-}C_2H_3OH+H]^+$, and $[M\text{-}C_2H_4+H]^+$, respectively, which have the identical *m/z* as the precursor ion of DEA at 106 *m/z*. This observation also indicated that chromatographic separation for EDEA, TEA, and DEA are crucial for quantification accuracy. The scan time for MRM scans was optimized to focus on quantitative MRMs and MRMs with less intensity in order to achieve better signal-to-noise ratio (S/N) for those mentioned MRMs, thus providing balanced overall method performance. The detailed MRM scan parameters are listed in Table 1. The TurboV with ESI ionization source parameters were optimized by a series of runs with varying parameter settings, and the optimum settings are listed in the experimental section.

## Method Performance

As mentioned earlier, the selectivity for this method was evaluated by observing the specific MRM peaks at the specific retention times for each analyte. Although interference was observed in both MRM channels for DEA, ($[M\text{-}C_2H_3OH+H]^+$ from TEA, and $[M\text{-}C_2H_4+H]^+$ from EDEA), as seen in Figure 3, these interference peaks were chromatographically separated and thus did not affect the method selectivity for the accurate quantification of DEA. Carryover was evaluated by injecting a reagent blank (DI water) after the highest calibration standard at 20 ppb. No quantifiable peak was observed at the specific retention time for each analyte thus indicating no observed carryover for this method.

Linearity was evaluated and calibration curves were generated with duplicate assays of eight calibration standards from 0.05 ppb to 20 ppb using isotope labeled DEA-$d_8$ as the internal standard. Linear regression was used to fit all experimental data with 1/x weighting factor. Excellent linearity was achieved from 0.05 ppb (except EA from 0.2 ppb) to 20 ppb with correlation of determination (r) greater than 0.999 for each analyte.

Figure 4 shows the calibration curve for DEA as an example. Run-to-run precision and accuracy was evaluated by seven replicate assays of the 0.5 ppb standard and measured by RSD and %Accuracy (calculated by Observed Amount/Specified Amount × 100%). Method detection limit (MDL) was statistically calculated for each analyte using the standard deviation obtained from the seven replicate analysis of a 0.5 ppb standard following this equation: MDL = s × *t* where s is the standard deviation and *t* is the Student's *t* at 99% confidence interval. Excellent precision was observed with RSDs ranging from 3.26% (MDEA) to 5.49% (TEA). The calculated MDL ranged from 0.050 ppb (MDEA) to 0.092 ppb (TEA).

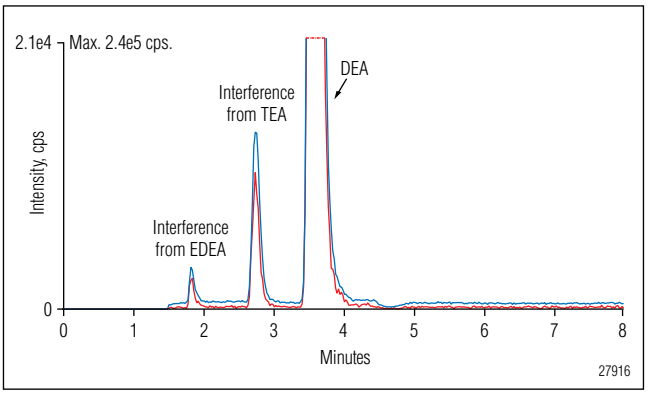


*Figure 3. Chromatographically separated MRM interferences for DEA.*

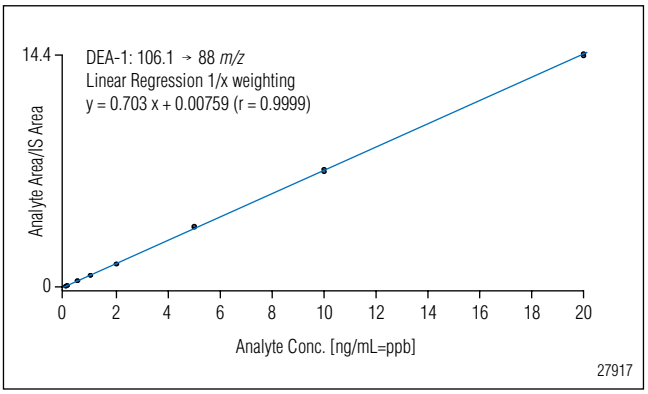


*Figure 4. Calibration curve of DEA (106.1→88.0* m/z*) from 0.05 to 20 ppb using isotope labeled DEA-$d_8$ as internal standard.*

| Table 2. Calibration, RSD, Detection, and Reporting Limits | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Analytes** | **Calibration** | **r** | **Mean[a]** | **Accuracy[a] (%)** | **RSD[a]** | **MDL[a]** | **LLOQ (ppb)** | **S/N at LLOQ** | **LRL[b]** |
| EDEA | y = 4.64x + 0.00242 | 0.9993 | 0.463 | 97.57 | 3.58 | 0.052 | 0.05 | 106.8 | 0.052 |
| MDEA | y = 4.73x - 0.0109 | 0.9996 | 0.484 | 96.77 | 3.26 | 0.050 | 0.05 | 54.5 | 0.050 |
| TEA | y = 4.22x + 0.169 | 0.9994 | 0.530 | 106.0 | 5.49 | 0.092 | 0.05 | 26 | 0.092 |
| DEA | y = 0.703x + 0.00759 | 0.9999 | 0.508 | 101.5 | 3.85 | 0.061 | 0.05 | 24.9 | 0.061 |
| EA | y = 0.217x - 0.00756 | 0.9990 | 0.510 | 101.9 | 5.34 | 0.085 | 0.20 | 13.1 | 0.20 |

All concentrations were in the unit of ppb.

[a] Calculated based on seven replicate assays of a standard at 0.5 ppb.

[b] LRL, Lowest reporting limit: the lowest concentration can be reported by this method, and is the higher concentration between MDL and LLOQ.

The lower limit of quantification (LLOQ) was determined as the lowest calibration standard consistently showing S/N greater than 10. The LLOQ for all analytes were reported as 0.05 ppb, except EA, which was 0.2 ppb. Figure 5 shows the MRM chromatograms of each analyte at LLOQ. The lowest reporting limit (LRL) for each analyte is the lowest concentration that can be reported by this method and was determined as the higher concentration between MDL and LLOQ. The results for method performance evaluations are summarized in Table 2.

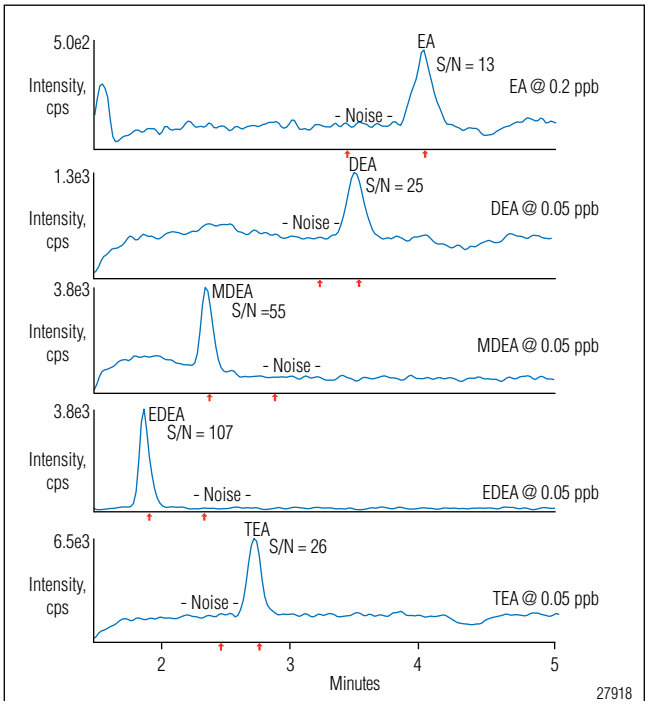


*Figure 5. MRM chromatograms of five ethanolamines at LLOQ.*

### Analysis of Water Samples

This method was used to analyze local municipal water samples, a local creek water sample, and a Nevada lake water sample. Following the procedures in the Experimental section, these samples were spiked with the internal standard and analyzed directly. None of the tested samples showed concentrations of target analytes above the lowest reporting limit, and thus the local creek water and the Nevada lake water were used as blank matrices to evaluate the method recovery. Each matrix was spiked with ethanolamines at three levels: 0.5 ppb, 5 ppb, and 20 ppb with three replicates at each level, and the internal standard was spiked at 1 ppb.

As summarized in Table 3, consistent recoveries were observed for most analytes at different levels in two different matrices. However, differences in concentration and matrices showed significant effects on the recovery of EA: 37.8% for 0.5 ppb vs 61.9% for 5 ppb and 70.0% for 20 ppb in Matrix A; not detected for 0.5 ppb vs 20.2% for 5 ppb and 22.8% for 20 ppb in Matrix B.

Higher recovery was observed for samples spiked at higher levels, and prepared in Matrix A. It is worth noting that the recovery for DEA was observed near 100%, indicating the benefit of using isotope labeled analogues as an internal standard correcting the matrix effect on that specific analyte. The deviation of recoveries from 100% indicates the different extents of matrix effects on each analyte, i.e., significant relative signal enhancement for EDEA and MDEA.

It was also noticed that EA exhibited short-term instability, although the samples were placed in the thermally controlled autosampler at 10 °C and sheltered from light. The duplicate assays of a batch of samples run on the following day of the sample preparation showed no detectable EA, suggesting immediate analysis after sample preparation is required. The stability of prepared sample in target matrices should also be evaluated to avoid degradation.

**Table 3. Recoveries of Ethanolamines in Two Water Matrices**

| Analyte | Matrix A: Local Creek Water | | | | | | Matrix B: Nevada Lake Water | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0.5 ppb | | 5 ppb | | 20 ppb | | 0.5 ppb | | 5 ppb | | 20 ppb | |
| | % RVY[a] | % RSD | % RVY[a] | % RSD | % RVY[a] | % RSD | % RVY[a] | % RSD | % RVY[a] | % RSD | % RVY[a] | % RSD |
| EDEA | 136.0 | 0.78 | 135.6 | 1.81 | 131.3 | 1.34 | 133.8 | 1.76 | 123.9 | 1.03 | 118.3 | 1.71 |
| MDEA | 145.9 | 1.87 | 137.2 | 1.83 | 142.2 | 2.34 | 140.7 | 0.70 | 127.5 | 1.97 | 127.8 | 2.01 |
| TEA | 83.9 | 2.08 | 86.8 | 1.83 | 95.0 | 1.58 | 79.3 | 4.92 | 85.4 | 0.81 | 93.7 | 0.82 |
| DEA | 98.5 | 1.54 | 101.8 | 0.52 | 100.2 | 0.29 | 103.3 | 3.89 | 100.5 | 0.83 | 101.8 | 0.28 |
| EA | 37.8 | 1.83 | 61.9 | 1.66 | 70.0 | 0.71 | N/A | N/A | 20.2 | 8.65 | 22.8 | 1.08 |

[a]Recovery, mean of three replicate recoveries calculated by Observed Amount /Specified Amount × 100%.

## CONCLUSION

An RSLC-MS/MS method for quantitative analysis of five ethanolamines was developed and described. By using a mixed-mode analytical column and selective MRM MS/MS detection, this method showed significant improvements over previously reported methods with minimum sample preparation, total chromatographic resolution, capability of sub-ppb level quantification, and high throughput. Application of this method to the analysis of surface waters was demonstrated and showed no quantifiable amounts above the LRLs. Matrix effects and recovery were evaluated using two surface water matrices and the results indicated better quantitation accuracy for DEA by using an isotope labeled analogue as an internal standard.

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