

# Direct Determination of N-Nitrosodiethanolamine (NDELA) in Ethanolamines by LC-MS-MS

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## Abstract

A sensitive and direct method for quantification of N-nitrosodiethanolamine (NDELA) in ethanolamine has been developed. The sample was dissolved in water and directly injected to high performance liquid chromatography with mass spectrometry to quantify NDELA. The eluent was transferred to mass spectrometer and ionized by heated electrospray positive ionization. The analyte was quantified in parts per billion level by using multiple reaction monitoring (MRM) mode. The MRM transition was  $m/z$  135.0 to 74.1 and confirmed by  $m/z$  104.0. To avoid ion suppression and better sensitivity for quantification, cation exchange column was used. A linearity coefficient of  $R^2 > 0.995$  was observed in calibration over a wide range of concentration 1 ppb to 10 ppb of NDELA. The method was validated by linearity, accuracy and precision studies. The results show that recoveries were in between 97% to 106%.

**Keywords:** N-nitrosodiethanolamine (NDELA); LCMS; MRM; Method validation; Ethanolamine

## Introduction

Ethanolamines (monoethanolamine (MEA), diethanolamine (DEA) and triethanolamine (TEA)) are a group of chemicals combine the properties of both amine and alcohol. These ethanolamines are bifunctional compounds allowing for wide spread use in the chemical industry as a components in detergents, pharmaceuticals and cosmetics [1,2]. Ethanolamines are hydrophilic in nature and most of the alcohols making them ideal for use as surfactants, either alone or esterified. N-nitrosodiethanolamine (NDELA, Figure 1) is formed by the action of nitrosating agents such as nitrites and nitrogen oxides on diethanolamine and triethanolamine [3]. The rate of formation of NDELA in ethanolamines is pH, temperature and time-dependent. Tertiary amines nitrosate at a slower rate than secondary amines because the reaction involves a nitrosative dealkylation rate-limiting step to yield a secondary amine which is then available for further nitrosation [4].

Ethanolamines are mainly used in personal-care products, detergents and one of the potential health contaminant is NDELA. N-nitrosodiethanolamine has been recognized as a class of hazardous compounds and considered as more toxic in more animal species than any other category of chemical carcinogen. It has been proven that, NDELA can induce cancer in experimental animals [5,6]. As considering the toxicity of NDELA, in parts per billion level quantitation is very critical to qualify ethanolamines used for personal-care products. There are a great number of scientific papers reported in the literature for qualitative and quantitative estimation of NDELA in different matrixes [7-9]. In general, these quantification methods recommend the multi-step extraction of nitrosamines from the matrix by extraction

methods, including distillation (steam, vacuum, or atmospheric), solvent extraction, solid-phase extraction, autoclave extraction, and supercritical fluid extraction [10]. Most of the methods like colorimetry, spectrophotometry, polarography, capillary electro-chromatography, micellar electro-kinetic capillary chromatography, gas chromatography with flame ionization detection, nitrogen phosphorous detection, thermal energy detection, nitrogen chemiluminescence detection, mass spectrometry detection, high-performance liquid chromatography with thermal energy analyzer, mass spectrometry and fluorescence detection, comprise two or more clean-up steps derivatizations, tedious sample preparation etc. [11,12].

The aim of the work was to develop a simple liquid chromatography mass spectrometry method for direct estimation of NDELA from ethanolamines matrix at <10 ppb level. The required sensitivity for the method was <1 ppb level for neat NDELA standard prepared in water. The required sensitivity was achieved by using multiple reaction monitoring (MRM) mode in LC-MS. The ion suppression due to amine matrix in electrospray positive ionization mode (ESI +Ve) was overcome by using cation exchange columns, switch over valve and followed by washing with acidic aqueous solutions. This method is simple to perform and provides an accurate and precise quantitative results for the measurement of NDELA in ethanolamines.

## Materials and Methods

### Chemicals and reagents

N-nitrosodiethanolamine was purchased from Sigma-Aldrich, monoethanolamine, diethanolamine and triethanolamine were received

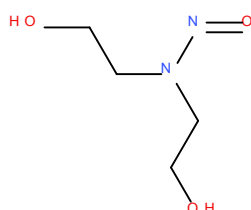


Figure 1: N-nitrosodiethanolamine.

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from in house synthesized SABIC technology center Riyadh. The purity of standard NDELA was greater than or equal to 90%. Water, acetonitrile, methanol and formic acid (LCMS grade) were purchased from Fisher Scientific.

### Instrument and Chromatographic conditions

The method development for quantification of NDELA was performed by using an Agilent 1290 Infinity LC system and Agilent 6460 Triple Quadrupole LC/MS system with Agilent Jet Stream source. The Agilent Mass Hunter Workstation was used to capture and analyze the data. LC/MS parameters used are depicted in Table 1. During the method optimization, LC System with reverse phase chromatographic conditions with C18 column were also used and later changed to Dionex IonPac CS 18 cation exchange column. The mass spectrometry analysis was performed in electrospray positive ionization mode with spray voltage. Nitrogen was used as the sheath, auxiliary and ion sweep gas. The system was operated in selected multiple reaction monitoring (MRM) mode with argon as the collision gas with collision energy of 5V.

### Preparation of calibration standards

Stock solution of NDELA (1000 µg/mL) was prepared in water and stored in a refrigerator at 5°C. The purity factor of 90% was applied to calculate the final concentration of the stock solution. From the stock solution of NDELA, the working standards (0.8 to 8 ppb.) were prepared by further dilution in water. 100 µL of this calibration standards were injected to LCMS to generate calibration curve.

### Sample preparation

Ethanolamines (MEA, DEA and TEA) was prepared by dissolving in water. 2 g of samples were accurately weighed into 10 mL of standards flask and made up to the mark with water. The samples were vortexed mixed for 1 min and filtered through 0.45 µm syringe filter before injection to LCMS.

### Method Validation

The method was validated as per standard LCMS testing guidelines.

LC conditions	
Column	Dionex IonPac CS18 cation exchange column
Column temperature	40°C
Mobile phase A:	0.02% Formic acid in water
Mobile phase B:	20 mmol Methane sulfonic acid in water
Flow-rate	1 mL/min
Gradient Time (min)	% A %B
0	100
5	100
7	100
31	100
32	100
45	100
Injection volumes	100 µL
Agilent 6460 Triple Quadrupole source conditions	
Gas heater	350°C
Gas flow	7 L/min
Nebulizer pressure	45 psi
Sheath gas heater	350°C
Sheath gas flow	11 L/min
Vcap	4,000 V
Nozzle voltage	1000 V
Delta EMV	300 V

Table 1: LC/MS conditions.

The quantification method is fully validated for, linearity, accuracy, precision, recovery and matrix effects.

### Linearity

The linearity of different concentration levels for this quantification method was validated by using calibration curves as described in the results and discussion section. The calibration curves were generated by plotting the peak area of NDELA to the concentration in ppb. Linear equation was used to calculate the concentration of NDELA from calibration curve. The limit of quantification (LOQ) was defined as the lowest concentration of analyte where the signal-to-noise ratio (S/N) of NDELA at the LOQ exceeds the minimum requirement.

### Accuracy and precision

Accuracy of the measurement was assessed by performing a recovery experiments by spiking studies. 2 g of samples (MEA, DEA and TEA) were accurately weighed into the 10 mL of standards flask, 2 ppb level of NDELA was also spiked into the ethanolamine and finally made up to the mark with water. The samples were vortexed mixed for 1 min and filtered through 0.45 µm syringe filter before injection to LCMS. Three level spiking studies were also conducted for different matrixes. Precision study was conducted using 2 ppb level spiked sample by injecting repeatedly >15 times and the data was evaluated. The mean and standard deviations were determined over the validation period and the precision was calculated.

### Matrix Interference and ion suppression

To evaluate the matrix effects (MEA, DEA and TEA) a post column infusion experiment was performed using direct infusion pump. The out let of the syringe from infusion pump was directly inserted into the mass spectrometer. A standard NDELA solution and spiked ethanolamines were separately infused into the eluent stream at a flow rate of 50 µL/min. The signals of the corresponding MRM transitions of the analyte were recorded. The ethanolamine that interfered with the ionization of NDELA analyte would lead to a depression of the MRM signal which would represent matrix effects.

## Results and Discussions

### Chromatography conditions and MRM analysis

The liquid chromatographic parameters were optimized to maximize sensitivity of the analyte. Initially, the LC MS analysis was carried out with Eclipse XDB C-18, 5 µm, 4.6 × 150 mm column to get the sensitivity of the method and mass transitions to optimize the MS parameters. Various mobile phase compositions, flow rates, MS conditions and profiles were evaluated. The required sensitivity of the method (0.87 ppb of neat NDELA in water) was achieved by separation performed at a flow rate of 1 mL/min with elution of 0.02% HCOOH in water. The optimized mass spectrometer parameters were mentioned in Table 1. The TIC chromatogram for the blank (water) and lowest standard is depicted in Figure 2. There was no interference from blank and signal to noise ratio was >10. The MRM transition was m/z 135.0 to 74.1 and confirmed by m/z 104.0. Further the MRM conditions were optimized (Table 1) to get complete transition of precursor ion (m/z 135.0) to product ions (Figures 3 and 4). This achieved the signal to noise ratio far >10, with this conditions the linearity and quantification of NDELA was performed.

### Linearity and Quantification

A linear relationship was found between NDELA concentration and peak area throughout the range of 0.87 ppb to 8 ppb. The coefficient

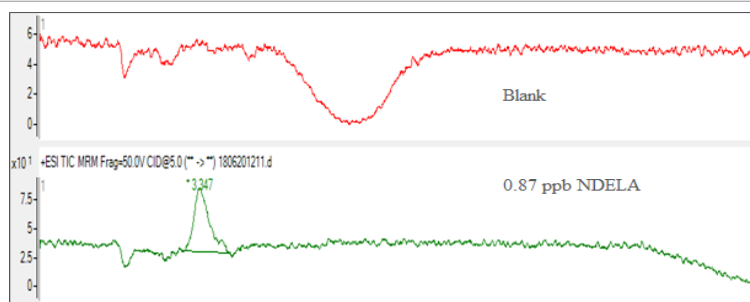


Figure 2: TIC chromatogram of 0.87 ppb NDELA standard in water.

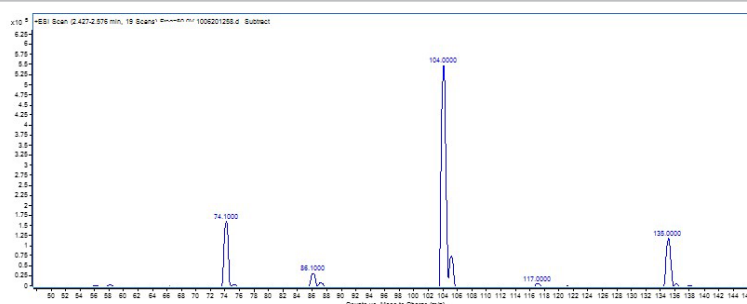


Figure 3: MRM transition of precursor ion m/z 135.0.

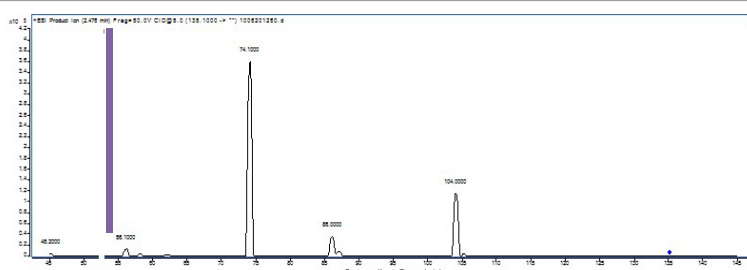


Figure 4: Complete MRM transition of precursor ion m/z 135.0 to product ion.

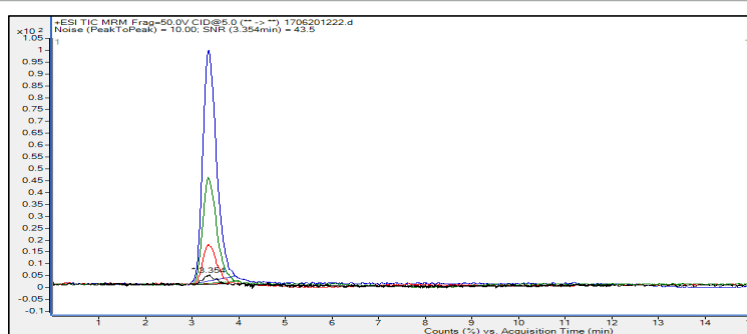


Figure 5: Overlaid TIC chromatogram of NDELA calibration standards.

of correlations  $R^2$  as determined by four point calibration curve was greater than 0.995. The signal to noise (S/N) of NDELA at the lowest limit of quantification (LLOQ) was greater than 100. The overlaid TIC chromatogram of standards and calibration curve generated are depicted in Figures 5 and 6. The sample analysis was performed as per the section materials and methods. The prepared TEA and DEA were injected multiple times to detect the NDELA but the MRM analysis

couldn't show any signal towards selected ions (135.0, 104 and 74.1 (Figure 7). In order to confirm the analysis, spiking studies were carried out in the TEA and DEA matrix at 2 ppb level. The percentage recovery for the analysis of TEA is found 50-60 and DEA was <50. In contrast to expectation, the percentage recovery was very low and this leads to detailed investigation of interaction of the matrixes, method and machine parameters, etc.

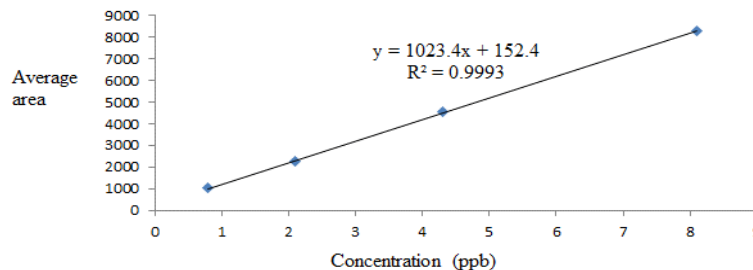


Figure 6: Calibration curve for NDELA standards.

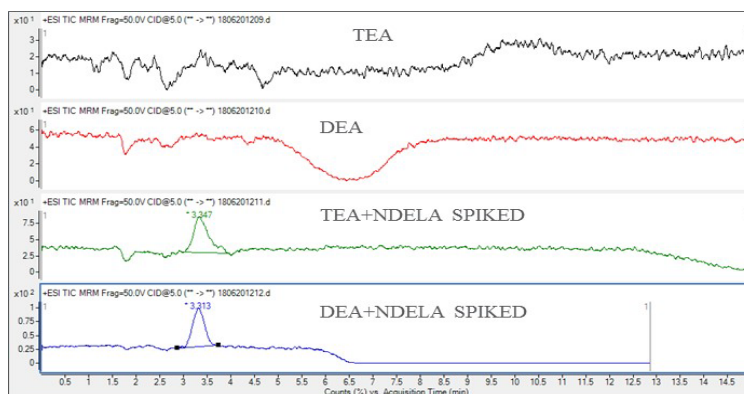


Figure 7: NDELA quantification and spiking studies in TEA and DEA.

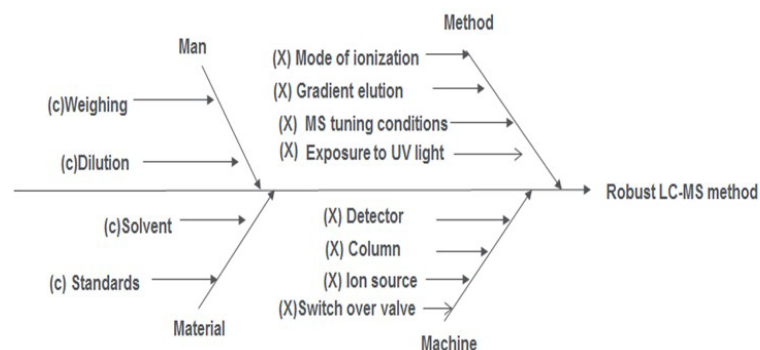


Figure 8: Cause effect diagram for LCMS analysis.

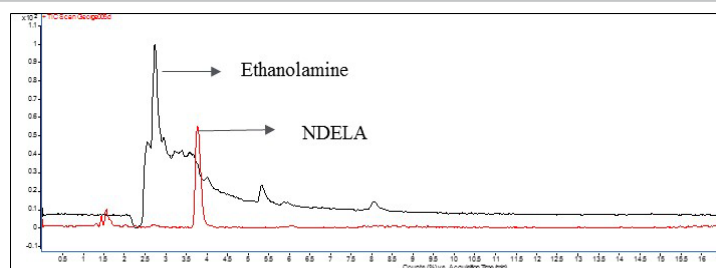


Figure 9: Co-elution of NDELA with ethanolamine.

### Matrix interference and ion suppression

A cause effect diagram (Figure 8) was used to evaluate the possible matrix interference and ion suppression during the quantification of NDELA. We have ruled out interference from common endogenous compounds that can potentially cause ion suppression. The main

cause for the low recovery due to ion suppression was found to be the analytical column and elution mode used in HPLC and MS detector respectively.

From the low level percentage recovery of NDELA, it was clear that conventional reverse phase HPLC column was leading to co-elution of

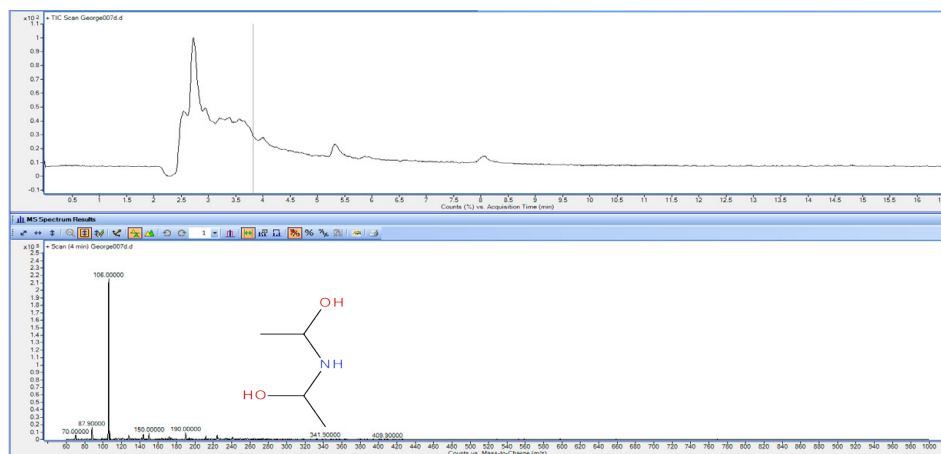


Figure 10: Diethanolamine in ESI +ve ionization mode.

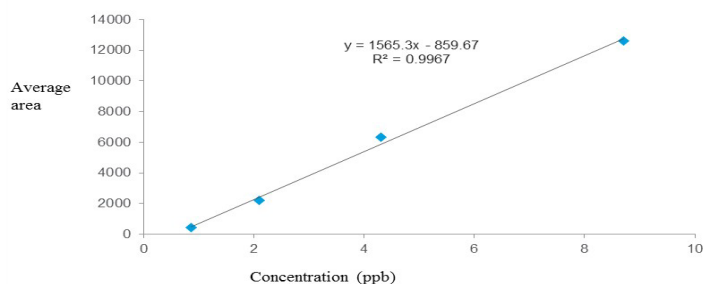


Figure 11: Calibration curve for NDELA standards using cation exchange column.

Analysis result		Spiking studies	
Sample	NDELA(ppb)	Sample	%Recovery
TEA	<10	TEA (10 ppb NDELA)	97.5
DEA	<10	DEA (10 ppb NDELA)	105.7
MEA	<10	MEA (10 ppb NDELA)	105.9

Table 2: Quantification of NDELA and recovery results using cation exchange column.

NDELA with ethanolamine and cause ion suppression (Figure 9). In the TIC chromatogram of Figure 9, it was clear that NDELA is partially coeluted with the huge peak of ethanolamine and the peak retention time from 2.5 minutes to more than 5 minutes were enriched by m/z of 106 which is corresponding to diethanolamine (Figure 10). The co-elution of NDELA with very high concentration of amines can definitely cause ion suppression in ESI positive mode since the ethanolamines are very much susceptible to ionization at positive mode in presence of formic acid. The easy ionization of abundant ethanolamine can reduce the probability of trace level NDELA to get ionized which in turn cause the low level detection and recovery of NDELA in amine matrix. By the usage of cation exchange ion chromatography column enhanced the chromatographic resolution of the NDELA from ethanolamine matrix which in turn reduced the ion suppression. Alternately, the performance of ionization of NDELA was increased by restricting the introduction of amine ions in the MS source by the usage of switch over valves in the MS detector. Also, the column was washed at adequate time using methane sulfonic acid to regenerate the column if any exchange happened. The prepared calibration standards and freshly prepared samples were analyzed with these modifications in the method. The results were validated by spiking of NDELA in matrix to get 10 ppb level of quantification. The calibration curve and analysis results are

depicted in Figure 11 and Table 2. All the samples, the level of NDELA was found to be less than 10 ppb.

### Accuracy and precision

The accuracy of the assay was evaluated by the recovery experiments, resulted recovery of NDELA ranging between 97.5 to 105.9% (Table 2). The precision of the method was evaluated by repeated injection of NDELA spiked in TEA sample for many days. Since the NDELA was <10 ppb in the amines, NDELA spiked in TEA were used as the sample for calculating the precision of the method. The percentage recovery remains constant (97 to 106%) or all the 10 to 15 data points of repeated analysis.

### Conclusion

This article describes the development and validation of a simple direct method for determination of N-nitrosodiethanolamine in ethanolamine by LC-MS. Other methods have used multistep extraction or derivatization steps which are subject to elaborate procedures and additional instrumentation for analysis. This method excludes any prior sample preparation steps and provide direct analysis of NDELA which yields the required sensitivity up to 10 ppb level in ethanolamine matrix. The method is fully validated for specificity of the ions,

sensitivity, accuracy linearity and recovery. The method is accurate, with recovery ranging between 97 to 107%. The matrix interferences and ion suppression were minimized by using cation exchange ion chromatographic columns in HPLC and switch over valves in MS detector. The LC-MS/MS assay was extensively used in our technology centers to determine NDELA levels in various ethanolamine batches.

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