Identification of *N*-Nitrosamines in Treated Drinking Water Using Nanoelectrospray Ionization High-Field Asymmetric Waveform Ion Mobility Spectrometry with Quadrupole Time-of-Flight Mass Spectrometry

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Abstract

We report a nanoelectrospray ionization (nESI) with high-field asymmetric waveform ion mobility spectrometry (FAIMS) and tandem mass spectrometry (MS–MS) method for determination of small molecules of *m/z* 50 to 200 and its potential application in environmental analysis. Integration of nESI with FAIMS and MS–MS combines the advantages of these three techniques into one method. The nESI provides efficient sample introduction and ionization and allows for collection of multiple data from only microliters of samples. The FAIMS provides rapid separation, reduces or eliminates background interference, and improves the signal-to-noise ratio as much as 10-fold over nESI-MS–MS. The tandem quadrupole time-of-flight MS detection provides accurate mass and mass spectral measurements for structural identification. Characteristics of FAIMS compensation voltage (CV) spectra of seven nitrosamines, *N*-nitrosodimethylamine (NDMA), *N*-nitrosomethylethylamine (NMEA), *N*-nitrosodiethylamine (NDEA), *N*-nitrosodi-*n*-propylamine (NDPA), *N*-nitrosodi-*n*-butylamine (NDBA), *N*-nitrosopiperidine (NPip), and *N*-nitrosopyrrolidine (NPyr), were analyzed. The optimal CV of the nitrosamines (at DV –4000 V) were: –1.6 V, NDBA; 2.6 V, NDPA; 6.6 V, NPip; 8.8 V, NDEA; 13.2 V, NPyr; 14.4 V, NMEA; and 19.4 V, NDMA. Fragmentation patterns of the seven nitrosamines in the nESI-FAIMS-MS–MS were also obtained. The specific CV and MS–MS spectra resulted in positive identification of NPyr and NPip in a treated water sample, demonstrating the potential application of this technique in environmental analysis.

Introduction

Nanoelectrospray ionization (nESI) is well established in mass spectrometry (MS) analysis of proteins because it provides efficient sample introduction and ionization. In nESI, as little as 1 µL of a sample can sustain the spray for approximately 1 h, which enables us to collect multiple data from a small volume of a sample. This is a useful feature for environmental analysis when extracts of samples are limited. However, nESI is rarely used for environmental analysis of contaminants of low molecular weight (*m/z* less than 200). This is presumably because mass spectrometric detection of small molecules such as nitrosamines of *m/z* ranging from 50 to 200 often encounters severe background interference (chemical noise) and chromatographic separation may not be sufficient to eliminate such interference. Here, we attempt to develop an alternative method integrating nanoelectrospray ionization with a gas phase ion separation technique and tandem mass spectrometry detection and to demonstrate its potential application to environmental analysis using nitrosamines in drinking water as an example.

The gas phase ion separation technique of our interest is based on high-field asymmetric waveform ion mobility spectrometry (FAIMS) which separates ions by varying the compensation voltage (CV) at a constant dispersion voltage (DV) (1). Small differences in ion structure and mobility can be differentiated using different CVs. This technique is easily coupled to mass spectrometers with atmospheric pressure sources because it separates gas phase ions and operates at atmospheric pressure and room temperature. It has been demonstrated that the FAIMS technique is able to separate small ions of low *m/z* ratios and even small isomer ions with the same *m/z*, which cannot be separated by MS. The common chromatographic separations, such as liquid chromatography and gas chromatography, are often unable to eliminate chemical noise of low *m/z* for mass detectors. Thus, FAIMS is a useful alternative separation technique than mass separation and chromatographic separation. The FAIMS technique has been demonstrated to reduce chemical noise in ESI-MS detection (2). Here, we take advantage of nESI and FAIMS to demonstrate nESI-FAIMS-MS–MS for environmental applications.

Analysis of *N*-nitrosodimethylamine (NDMA), *N*-nitro-

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somethylethylamine (NMEA), *N*-nitrosodiethylamine (NDEA), *N*-nitrosodi-*n*-propylamine (NDPA), *N*-nitrosodi-*n*-butylamine (NDBA), and *N*-nitrosopiperidine (NPip) in drinking water has become more important, because they have been included in the Unregulated Contaminant Monitoring Rule-2 by the United States Environmental Protection Agency (U.S. EPA) (3). In addition, nitrosamines are known as rodent carcinogens and most are considered as probable human carcinogens by the International Agency for Research on Cancer (4) and the U.S. EPA (5). The common analytical methods include gas chromatography–mass spectrometry (GC–MS) and liquid chromatography (LC)–MS–MS combined with solid-phase extraction (SPE) (6-9). However, no study has demonstrated the application of nESI-FAIMS-MS–MS to environmental analysis. Thus, trace analysis of nitrosamines in water provides a new example of the application of nESI-FAIMS-MS–MS.

Experimental

Chemicals

A nitrosamine standard mixture containing 10 µg/mL each of *N*-nitrosodimethylamine (NDMA), *N*-nitrosomethylethylamine (NMEA), *N*-nitrosodiethylamine (NDEA), *N*-nitrosodi-*n*-propylamine (NDPA), *N*-nitrosopyrrolidine (NPyr), *N*-nitrosopiperidine (NPip), *N*-nitrosodi-*n*-butylamine (NDBA) was purchased from Sigma-Aldrich (Oakville, ON, Canada). Methanol (AnalR grade) and dichloromethane (Omni-Solv grade) were purchased from VWR International (Mississauga, ON, Canada). These standards were diluted with methanol to make working solutions. Ammonium acetate (ACS reagent grade) and L-ascorbic acid (analytical grade) were supplied by Sigma-Aldrich. All other chemicals were of analytical grade and obtained from Fisher

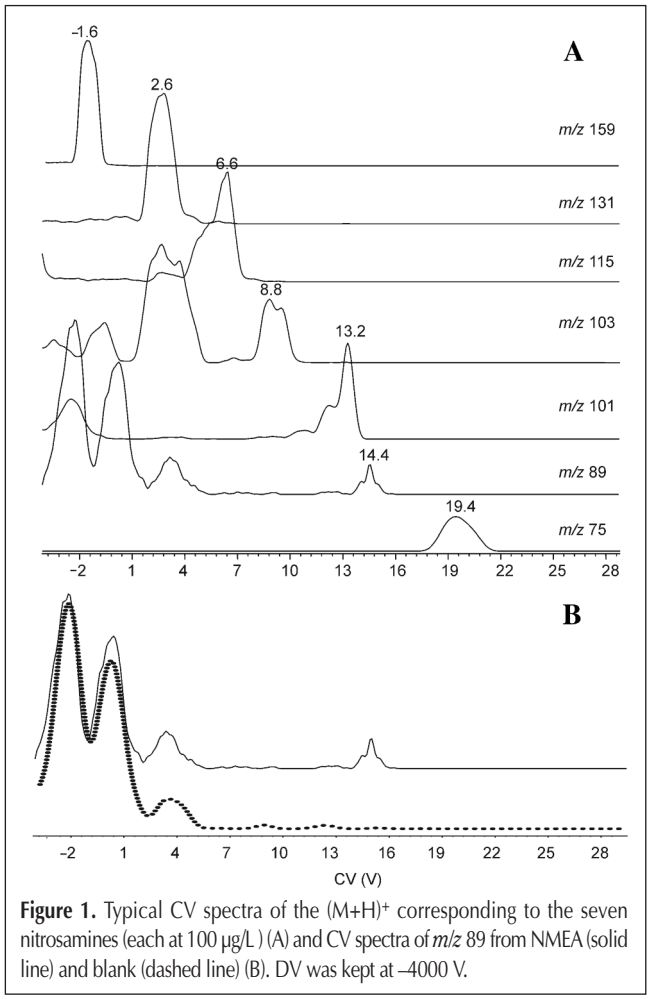

Figure 1. Typical CV spectra of the $(M+H)^+$ corresponding to the seven nitrosamines (each at 100 µg/L) (A) and CV spectra of *m/z* 89 from NMEA (solid line) and blank (dashed line) (B). DV was kept at –4000 V.

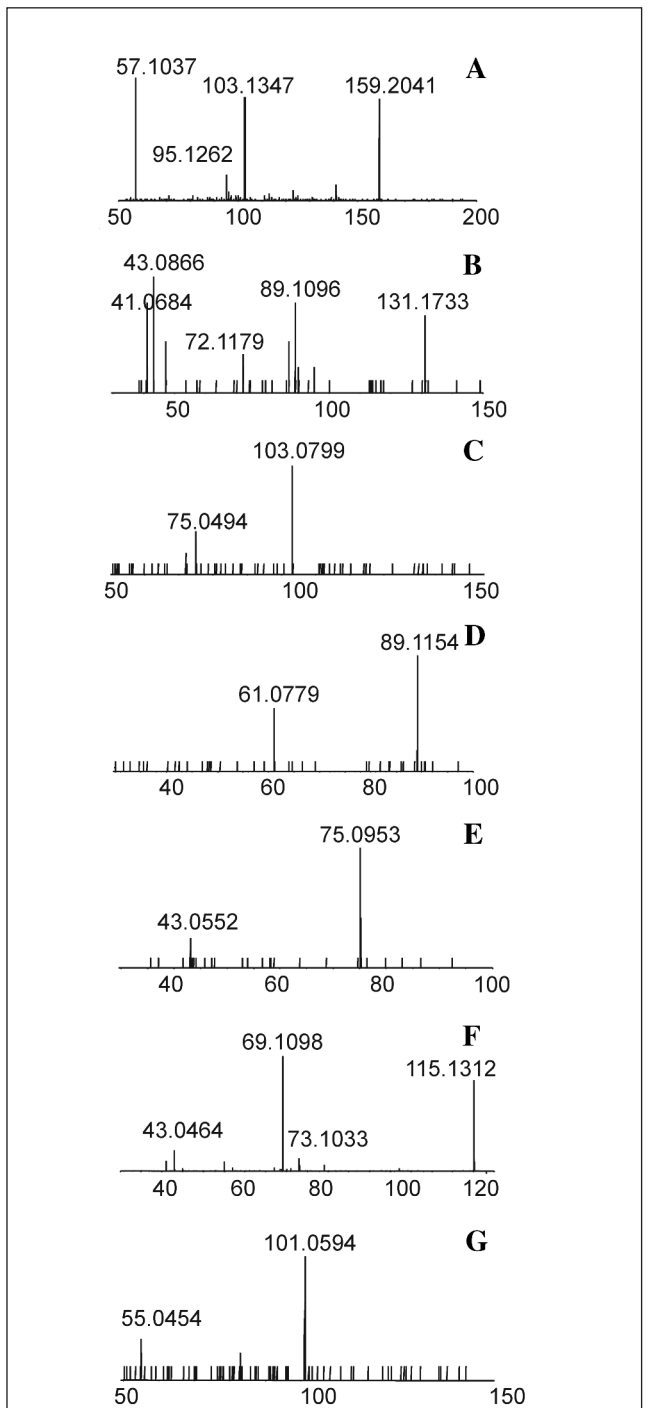

Figure 2. MS–MS spectra of the seven nitrosamines. The concentration of all nitrosamines was 10 µg/L. NDBA (*m/z* 159) (A); NDPA (*m/z* 131) (B); NDEA (*m/z* 103) (C); NMEA (*m/z* 89) (D); NDMA (*m/z* 75) (E); NPip (*m/z* 115) (F); NPyr (*m/z* 101) (G).

Scientific (Nepean, ON, Canada) unless otherwise indicated. The SPE packing materials, Ambersorb 572 (Rohm & Haas; Philadelphia, PA) and LiChrolut EN (Merck; Darmstadt, Germany) were obtained from Sigma-Aldrich and VWR International, respectively.

Sample preparation

Amber glass bottles (1 L) with PTFE-lined caps were used for sample collection. The bottles were pre-cleaned and baked at 180°C for 24 h prior to sample collection. Water was collected from a drinking water distribution system in Alberta, Canada. The disinfectant residual was quenched with ascorbic acid (20 mg/L), and samples were kept at 4°C until extraction. The nitrosamine extraction procedure has been described previously (7,10). Ten SPE cartridges prepared in-house, packed with 350 mg of LiChrolut EN (bottom layer), 500 mg of Ambersorb 572 (middle), and glass wool (top), were loaded onto a 12-port Visiprep DL SPE vacuum manifold (Sigma-Aldrich). Then, 500 mL of sample was passed through the SPE columns at a flow rate 3~5 mL/min. A vacuum (–30 KPa) was applied to completely dry the SPE material after which the nitrosamines were eluted using 15 mL of dichloromethane. The eluants were further concentrated to ca. 200 µL using a Turbovap II concentrator with high purity nitrogen in a 40°C water bath. The extracts were stored at 4°C prior to analysis.

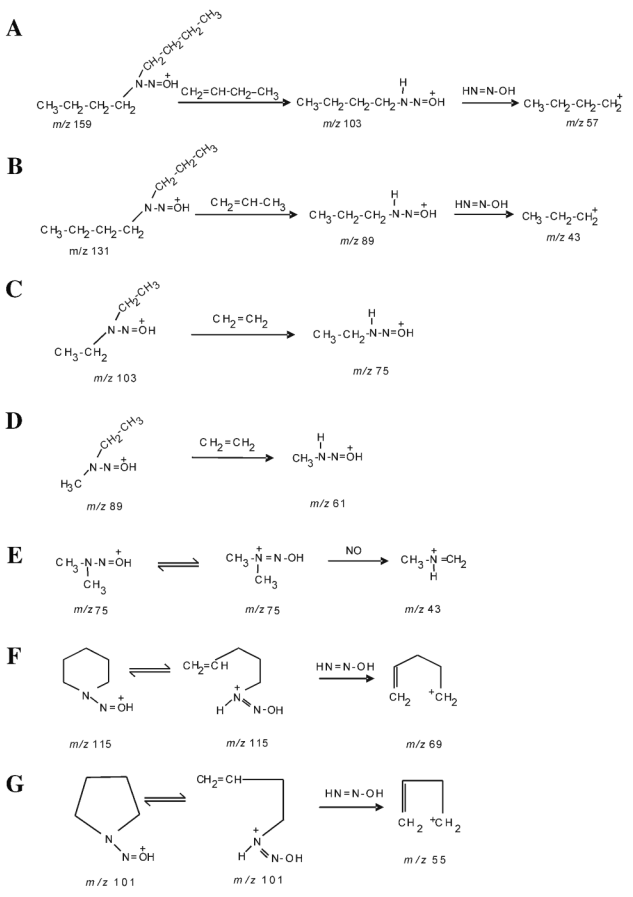

Figure 3. Proposed fragmentation pathways for the seven nitrosamines based on the MS–MS spectra shown in Figure 2.

nESI-FAIMS-QqTOF system

The nESI-FAIMS-QqTOF system consists of an nESI built in-house, a FAIMS unit (Ionalytics Corporation; Ottawa, ON, Canada), and a Qstar pulser (Qq-TOF) mass spectrometer (Applied Biosystems/MDS Sciex; Concord, ON, Canada). The Ionalytics Selectra (FAIMS) electrodes were installed between the nESI source and the Q_0 mass analyzer of the Qstar and were operated at atmospheric pressure and room temperature. The DV of FAIMS was kept at –4000 V. A mixture of 50:50 nitrogen–helium gas was introduced in the region between the curtain plate and the outer plate of the FAIMS electrodes at a combined flow rate of 2.5 L/min. A small fraction of the mixed gas traversed the curtain plate and the outer electrode to provide desolvation of the electrospray ions, while the remainder carried the ions through the electrodes and into the mass spectrometer.

Results and Discussion

Separation of nitrosamines using nESI-FAIMS-QTOFMS

We first examined the spectral characteristics of nitrosamines using non-chromatographic FAIMS separation in the nESI-

Table I. Theoretical and Experimental Mass of the Protonated Molecular Ions of Nitrosamines and the Mass Accuracy Obtained

Compound	MW + H + (amu)	Exp. mass. (amu)	Error (ppm)
NDMA	75.0552	75.0641	118
NMEA	89.0709	89.0653	63
NDEA	103.0866	103.0822	42
NDPA	131.1179	131.1176	1.9
NDBA	159.1492	159.1490	0.9
NPip	115.0866	115.0870	3.9
NPyr	101.0709	101.0770	56

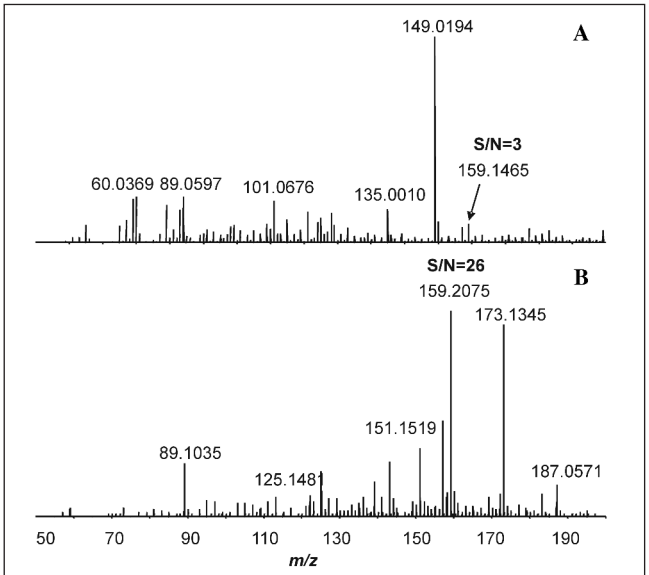

Figure 4. nESI-MS spectra of 100 µg/L NDBA obtained without (A) and with (B) FAIMS.

FAIMS-MS–MS in order to demonstrate the separation of the ions of interest from background chemical noise. For an analyte of interest, a CV spectrum corresponding to the molecular ion was obtained with varying CV while the DV was kept constant (at –4000V). Figure 1 shows the typical CV spectra of the protonated molecular ions corresponding to the 7 nitrosamines (*m/z* 75 for NDMA, *m/z* 89 for NMEA, *m/z* 101 for NPyr, *m/z* 103 for NDEA, *m/z* 115 for NPip, *m/z* 131 for NDPA, and *m/z* 159 for NDBA). The nitrosamines are well-separated based on their characteristic CV values, resembling GC or HPLC separation.

The CV spectra of the selected ions of *m/z* 159 (NDBA), *m/z* 131 (NDPA), *m/z* 115 (NPip), and *m/z* 75 (NDMA) show a single peak, while multiple peaks are detected in the CV spectra of ions at *m/z* 89 (NMEA), *m/z* 101 (NPyr), and *m/z* 103 (NDEA). We proposed that some of these peaks could result from sample matrices. To further identify the CVs specific to NMEA, NPyr, and NDEA, the CV spectrum of *m/z* 89 of a blank solution was obtained (Figure 1B). Three peaks at CVs ranging from –2.0 V to 4 V (dashed line trace in Figure 1B) were detected in the blank. Comparing the four distinct peaks of *m/z* 89 at CV –2.0 V, –0.4 V, 2.6 V, and 14.4 V in the sample with the two peaks in the blank identifies the peak at CV 14.4 V as specific to NMEA. Similarly, the CV peaks specific to *m/z* 103 (NDEA) and *m/z* 101 (NPyr) are identified as 8.8 and 13.2 V, respectively. These results demonstrate that the ions with the same mass-to-charge ratio are differentiated based on the difference in ion mobility and that the FAIMS technique is complementary to chromatographic and mass separations.

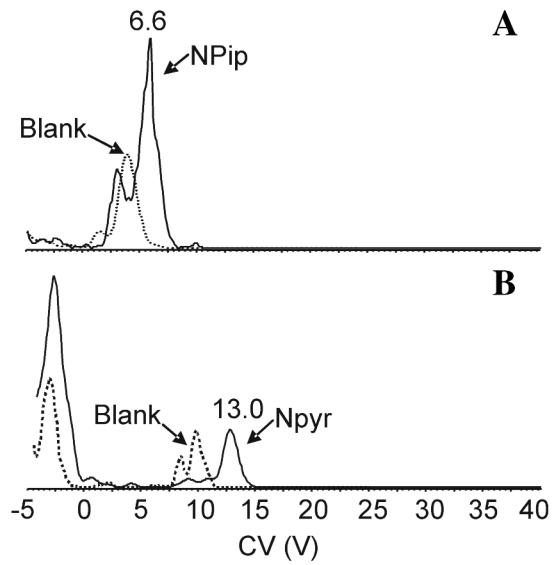

Figure 5. CV spectra of *m/z* 115 and *m/z* 101 obtained from a treated water sample and blanks, identifying NPip (*m/z* 115) and NPyr (*m/z* 101) in the sample.

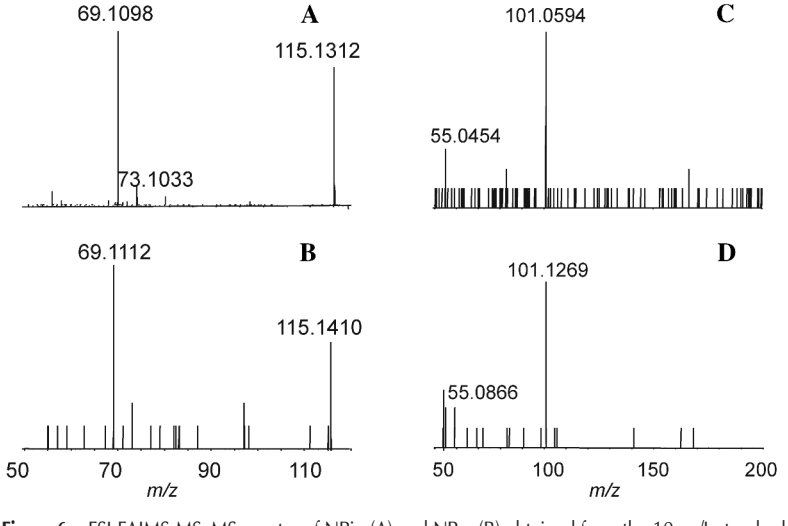

Figure 6. nESI-FAIMS-MS–MS spectra of NPip (A) and NPyr (B) obtained from the 10 µg/L standards (A and C) and the extract of a treated water sample (B and D).

Characteristic fragmentation pathways of nitrosamines in nESI-FAIMS-QqTOF

To assist the identification of nitrosamines in samples, characteristic high resolution tandem mass spectra of these analytes were obtained using the nESI-FAIMS-MS–MS. Figure 2A–2G illustrates typical MS–MS spectra of the seven nitrosamines and Figure 3A-3G their probable fragmentation pathways. The nitrosamines containing a γ-position hydrogen first undergo Mclafferty rearrangement, followed by the fragmentation of a neutral alkene group from the alkyl nitrosamines, as illustrated in Figure 3A–3D. Figure 2A-2D shows the spectra of NDBA, NDPA, NDEA, and NMEA. NDBA (*m/z* 159) loses 1-butene to produce a fragment ion at *m/z* 103, followed by a neutral loss of NH=N-OH to form the ion at *m/z* 57 (Figure 2A). Similarly, NDPA (*m/z* 131) loses a propene residue at first to form an ion with *m/z* of 89 and then loses NH=N-OH to form an ion at *m/z* 43 (Figure 2B), and NDEA and NMEA both lose ethylene to form fragment ions at *m/z* 75 and *m/z* 61, respectively (Figures 2C and 2D). NDMA (Figure 2E and 3E) does not have an available γ-position hydrogen, thus resulting in a different fragmentation pathway from the other nitrosamines that produces a fragment ion of *m/z* 43 ($CH_3N_2^+$) after neutral loss of CH_3OH. The cyclic nitrosamines (i.e., NPip and NPyr) are fragmented through a γ-position hydrogen rearrangement mechanism, followed by a neutral loss of NH=N-OH to yield fragment ions at *m/z* 69 and *m/z* 55, respectively (Figure 2F and 2G). Neutral loss is observed for all nitrosamines.

The nESI-FAIMS-QqTOF also provides accurate mass measurements. Table I summarizes the theoretical masses, experimental masses, and mass accuracy of the seven nitrosamines determined. With external calibration, the mass accuracy of the seven nitrosamines ranges from 0.9 to 118 ppm.

Enhancement of sensitivity

The use of FAIMS as an interface between nESI and the QqTOF provides another advantage: the reduction or elimination of chemical noise, resulting in enhancement of the signal-to-noise (S/N) ratio and lowering of the limit of detection.

To evaluate the improvement in the S/N ratio, the mass spectrum of a 100-µg/L standard solution of NDBA was obtained using the nESI-MS with and without FAIMS. As shown in Figures 4A and 4B, without FAIMS, the ion of NDBA at *m/z* 159 at 100 µg/L was detected with an S/N ratio of 2.8, whereas an S/N of 25.5 was obtained for the same ion when the FAIMS was set at DV = –4000 V and CV = 1.6 V. Using the specific CV 1.6 V effectively reduces chemical noise and enhances the sensitivity for NDBA. The LOD of NDBA is estimated to be 10 µg/L with FAIMS and 100 µg/L without FAIMS.

Analysis of drinking water samples

To demonstrate the potential application of the nESI-FAIMS-QqTOF method for the analysis of nitrosamines in drinking water, extracts of tap water and blank samples were analyzed and a few nitrosamines were identified. To identify nitrosamines in a sample, the CV spectra of the specific $(M+H)^+$ ions corresponding to individual nitrosamines were obtained, and then tandem mass spectra of the specific ions at the specific CVs were also obtained. Figure 5 shows the CV spectra of *m/z* 115 and *m/z* 101 from the sample and blank, showing that the peaks at CV 6.6 V for *m/z* 115 and CV 13.0 V for *m/z* 101 match those of NPip and NPyr, respectively. In addition, the MS–MS spectra of the peaks *m/z* 115 at CV 6.6 and *m/z* 101 at CV 13.0 detected in the sample (Figure 6) also match the spectra of these standards (Figure 1).

Conclusions

We have demonstrated a nESI-FAIMS-QqTOFMS method for the identification of nitrosamines in drinking water. Using compound-dependent CVs, this technique effectively reduces chemical noise resulting in enhanced S/N for small ions at the low *m/z* range. This technique uses FAIMS to provide non-chromatographic separation of gas phase ions. This study demonstrates the use of CV spectra, tandem MS spectra at specific CVs, and accurate mass measurements for identification of analytes. Identification of NPip and NPyr in a water sample demonstrates the potential application of the method for the analysis of environmental contaminants of low molecular weights.

Acknowledgements

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References

1. R. Guevremont. High-field asymmetric waveform ion mobility spectrometry: A new tool for mass spectrometry. *J. Chromatogr. A* **1058:** 3–19 (2004).
2. X. Liu, Y.Y. Zhao, K. Chan, S.E. Hrudey, X. F. Li, and J.J. Li. Analysis of nitrosamines by capillary electrospray-high-field asymmetric waveform ion mobility spectrometry-MS with programmed compensation voltage. *Electrophoresis* **28:** 1327–34 (2007).
3. United States Environmental Protection Agency. Unregulated contaminant monitoring regulation (UCMR) for public water systems revisions. *Fed. Regist.* **70:** (August 22, 2005).
4. International Agency for Research on Cancer. IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans: Some *N*-nitroso compounds. **17:** 365 (1978).
5. United States Environmental Protection Agency. Integrated Risk Information System. Accessed January 9, 2007.
6. J.W. Munch and M.V. Bassett. *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)*; National Exposure Research Laboratory, Office of Research and Development, United States Environmental Protection Agency: Cincinnati, Ohio, 2004; Method 521; available at http://www.epa.gov/nerlcwww/m_521.pdf.
7. J.W.A. Charrois, M.W. Arend, K.L. Froese, and S.E. Hrudey. Detecting N-nitrosamines in drinking water at nanogram per liter levels using ammonia positive chemical ionization. *Environ. Sci. Technol* **38:** 4835–41 (2004).
8. Y.Y. Zhao, J. Boyd, S.E. Hrudey, and X.F. Li. Characterization of new nitrosamines in drinking water using liquid chromatography tandem mass spectrometry. *Enwiron. Sci. Technol.* **40:** 7636–41 (2006).
9. M.H. Plumlee, M. López-Mesas, A. Heidlberger, K.P. Ishida, and M. Reinhard. N-nitrosodimethylamine (NDMA) removal by reverse osmosis and UV treatment and analysis via LC–MS/MS. *Water Res.* **42:** 347–55 (2008).
10. J.W.A. Charrois, J.M. Boyd, K.L. Froese, and S.E. Hrudey. Occurrence of *N*-nitrosamines in Alberta public drinking-water distribution systems. *J. Enwiron. Eng. Sci.* **6:** 103–14 (2007).

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