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Journal of Chromatography A, 844 (1999) 87–95

JOURNAL OF CHROMATOGRAPHY A

Sensitive determination of RDX, nitroso-RDX metabolites, and other munitions in ground water by solid-phase extraction and isotope dilution liquid chromatography–atmospheric pressure chemical ionization mass spectrometry

D.A. Cassada, S.J. Monson, D.D. Snow*, R.F. Spalding

Water Sciences Laboratory, School of Natural Resource Sciences, University of Nebraska, Lincoln, NE 68583-0844, USA

Received 28 October 1998; received in revised form 1 March 1999; accepted 4 March 1999

Abstract

Recent improvements in the LC–MS interface have increased the sensitivity and selectivity of this instrument in the analysis of polar and thermally-labile aqueous constituents. Determination of RDX, nitroso-RDX metabolites, and other munitions was enhanced using LC–MS with solid-phase extraction, $^{15}N_3$-RDX internal standard, and electrospray ionization (ESI) in negative ion mode. ESI produced a five-fold increase in detector response over atmospheric pressure chemical ionization (APCI) for the nitramine compounds, while the more energetic APCI produced more than twenty times the ESI response for nitroaromatics. Method detection limits in ESI for nitramines varied from 0.03 $\mu g\,l^{-1}$ for MNX to 0.05 $\mu g\,l^{-1}$ for RDX.

Keywords: Explosives; Nitramines; Nitroaromatics; 1,3,5-Trinitro-1,3,5-triazacyclohexane; RDX

1. Introduction

Explosive compounds have been manufactured, stored, tested and used at formerly-used defense sites throughout the United States [1] and military sites in Europe [2] over the past century. Many of these sites have RDX (1,3,5-trinitro-1,3,5-triazacyclohexane) contaminated soils and ground water resulting from crude handling, storage and waste disposal practices. Recent closures of military bases throughout the U.S. will accelerate cleanup and remediation of these and other sites. Assessment of the status of these sites generally includes numerous analyses of samples for munitions residues and TNT degradates, but lack information of nitramine degradation products. Degradation studies of RDX often lack information on the metabolic products due to the absence of analytical methods for determination of these compounds. It is clear that there is a need for sensitive and reliable methods for determination of RDX and its degradates.

Currently accepted analytical methods for the determination of trace levels of munitions residues in water rely almost exclusively on liquid or gas chromatography. Generally, gas chromatography with either electron capture or mass spectrometry has been a more sensitive technique, but reproducibility suffers especially for thermally labile compounds such as RDX and HMX (1,3,5,7-tetranitro-1,3,5,7-tetrazacyclooctane) [3–5]. High-performance liquid

*Corresponding author.

0021-9673/99/$ – see front matter
PII: S0021-9673(99)00310-6

chromatography with ultraviolet detection (HPLC–UV), using either solvent extraction or solid-phase extraction as a preconcentration step, is the standard method for munitions analysis [6–9]. However, the response of the ultraviolet spectrophotometric detector is dependent on each compound's molar absorptivity and is susceptible to interferences produced by co-eluting compounds. This limits the selectivity and sensitivity of this analytical method. Although the introduction of the photodiode array has improved the specificity of UV detection for munitions analysis [7,10], the method is still susceptible to interferences especially at trace levels.

Until recently, the sensitivity and selectivity of the mass spectrometer as a chromatographic detector was restricted to gas chromatography due to the difficulties associated with developing a suitable LC–MS interface. Improvements in ionization techniques have allowed the mass spectrometer to become more widely used in liquid chromatography, thus overcoming substantial problems with interferences at trace levels. The evolution of the LC–MS interface has also allowed development of sensitive and selective environmental methods for a variety of polar and thermally-labile compounds including explosives [11] and pesticide metabolites (see, e.g. Ref. [12]).

This paper describes the development of a sensitive, selective and relatively simple method for the determination of munitions residues and RDX-metabolites in water using atmospheric pressure ionization (API) electrospray ionization (ESI) LC–MS. Munitions compounds and RDX metabolites detected and quantified are RDX, HMX, mononitroso-RDX (1-nitroso-3,5-dinitro-1,3,5-triazacyclohexane), trinitroso-RDX (1,3,5-trinitroso-1,3,5-triazacyclohexane), TNT (2,4,6-trinitrotoluene), TNB (1,3,5-trinitrobenzene), 2ADNT (2-amino-4,6-dinitrotoluene), DNT (2,4-dinitrotoluene) and DNB (1,3-dinitrobenzene). Instrumental sensitivity using the electrospray ionization (ESI) source is also compared to the atmospheric pressure chemical ionization (APCI) source. The method selectivity and accuracy for the RDX compound is enhanced through the use of isotope dilution and ion trap mass spectrometry. The need for the selectivity of a mass spectrometer detector for determination of RDX and its degradation products is also demonstrated by the difficulty in chromatographically resolving and detecting these compounds at trace levels.

2. Experimental

2.1. Reagents and standards

Munitions standards were obtained as a commercial mixture available for EPA Method 8330 [9] from Supelco (Bellefonte, PA). Ring-labeled $^{15}N_3$-RDX (internal standard) and the RDX metabolites mononitroso-RDX (MNX) and trinitroso-RDX (TNX) (Fig. 1) were obtained from SRI International (Menlo Park, CA). A surrogate compound, 4-nitrobenzamide (4-NBA), was obtained from Acros Organics (Geel, Belgium). Methanol, isopropanol and acetone (Optima grade) and ammonium hydroxide (Reagent grade) were obtained from Fisher Scientific (Pittsburgh, PA). Ammonium formate (Baker grade) was obtained from J.T. Baker (Phillipsburg, NJ). All HPLC solvents were filtered through 0.5 μm Millicup filters (Millipore) before use and degassed with a Waters in-line degasser. Liquid nitrogen and

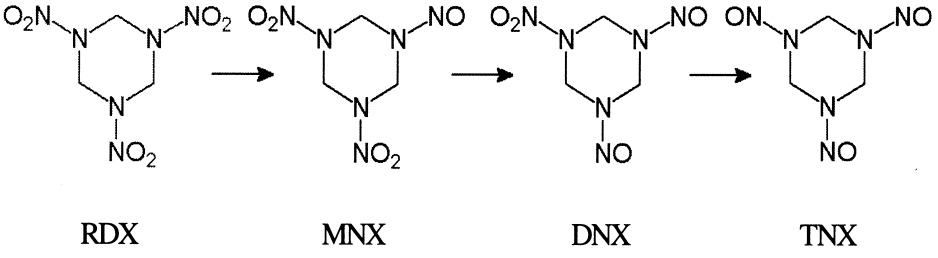

Fig. 1. Structures and degradation scheme for RDX, mononitroso-RDX, dinitroso-RDX and trinitroso-RDX metabolites. A purified standard for the dinitroso-RDX (DNX) is currently unavailable.

helium (Ultra-Pure Carrier grade) were obtained from Air Products (Allentown, PA).

Stock solutions of ring-labeled $^{15}N_3$-RDX, calibration standards, and spiking solutions were prepared in 12-ml amber silanized vials (National Scientific, Lawrenceville, GA). Porapak R_{DX} solid-phase extraction (SPE) cartridges (500 mg Sep-Pak, Waters Corporation, Milford, MA) were used to extract munitions from the water samples.

2.2. Extraction procedure

Ground water samples were collected in pre-cleaned and combusted (500°C) 1-l amber bottles, transported to the analytical laboratory in ice-filled coolers, and stored at 4°C. Fortified reagent water was prepared by spiking a known concentration of munitions into organic-free distilled deionized water (Nanopure, Barnstead/Thermolyne, Dubuque, IA). The SPE cartridges were conditioned immediately prior to sample extraction with successive 10-ml applications of acetone, methanol, and organic-free water. For the extraction, a 400 ml aliquot of sample was transferred to a separate container, weighed and fortified with ring-labeled $^{15}N_3$-RDX and 4-NBA at concentrations of approximately 1 $\mu g\,l^{-1}$ each. The sample was then shaken and completely vacuum-siphoned at a flow-rate of ~10 ml min^{-1} through the SPE cartridge, which was then allowed to dry for 3–5 min. The munitions residues were then eluted from the SPE cartridge with 2.5 ml of acetone into a small test tube (10×75 mm). The eluate was concentrated under a stream of dry nitrogen to a final volume of 200 μl, consisting of mostly residual water, and transferred to an HPLC autosampler vial equipped with a glass insert (Kimble, Vineland, NJ).

2.3. Instrumental

All analyses were performed using high-performance liquid chromatography separation with ultraviolet and ion trap mass spectrometry detection (HPLC–UV–MS). The HPLC consisted of a model 717+ autosampler, a model 600S gradient controller, and a model 486 UV detector (Waters Corporation, Milford, MA). The mass analyzer was a model LCQ ion trap mass spectrometer (Finnigan Corp., San Jose, CA) operated in ESI or APCI negative ion mode while scanning from 400–100 amu. Chromatographic separation was achieved with a Kromasil C8 reversed-phase column (250×2 mm, Eka Nobel, Bohus, Sweden) using an isocratic mobile phase of methanol–water–ammonium formate (50:48:2), or isopropanol–water–ammonium formate (20:78:2) at a flow-rate of 0.2 ml min^{-1} and a column temperature of either 30 or 32°C. The concentration of the ammonium formate solution was 0.5 *M* and was adjusted to a pH of 8 with 10% ammonium hydroxide. Liquid nitrogen was used as a source of nitrogen gas at a maintained pressure of 550 kPa (80 p.s.i.). Instrumental conditions of the LCQ are listed in Table 1. In addition, the APCI source discharge current was set at 5.00 μA and the method utilized source collision induced dissociation (CID) in the first octapole region with a 1 V collision energy.

3. Results and discussion

3.1. Mass spectrometry

Instrumental tests with standard solutions in positive ion detection mode produced little or no response, presumably because of the low proton affinity for the nitroaromatic and nitramine compounds. Previous work has shown that these compounds have a greater tendency to form negative ions due to the charge stabilizing electronegative nitro groups, and that nitramine compounds predominantly form adducts rather than molecular ions in ESI [11,13]. Negative ion detection was initially poor using the standard methanol–water LC column mobile phase, prompting the search for a suitable buffer solution which would promote molecular ion or adduct ion formation.

Several volatile buffers, including ammonium nitrate, ammonium acetate, ammonium formate, formic acid, acetic acid, and trifluoroacetic acid, were tested and evaluated for the ability to reproducibly promote negative ion formation in the electrospray ion source. While adduct ion formation was observed in several of these solutions, the ammonium formate buffer produced more stable negative ion adducts with the lowest background ion formation. Since our methodology was primarily focused on detection of the nitramine compounds,

Table 1
Source parameters for LCQ Ion Trap Mass Spectrometer for both electrospray and atmospheric pressure chemical ionization (APCI) configurations[a]

Ionization mode Mobile phase/buffer	Electrospray M/W/AF8	Electrospray I/W/AF8	APCI M/W/AF8
Sheath gas flow-rate	50	45	21
Auxillary gas flow-rate	12	9	1
Spray voltage (kV)	3.80	5.00	NA
Spray current (μA)	5–20	12–25	NA
Capillary temperature (°C)	150	150	150
Capillary voltage (V)	26.00	−4.00	−4.00
Tube lens Offset (V)	25.00	15.00	25.00
Octapole 1 offset (V)	3.00	2.75	1.50
Lens voltage (V)	3.50	24.00	14.00
Octapole 2 offset (V)	7.50	6.50	6.50
Octapole RF amplitude (Vp/p)	800	800	400

[a] Mobile phase/buffer composition as follows: 'M/W/AF8' – methanol–water–ammonium formate at pH=8 (50:48:2) and 'I/W/AF8' – isopropanol–water–ammonium formate at pH=8 (20:78:2).

additional work was directed toward the formation of stable adduct anions. Optimization of adduct ion formation is highly dependent on interface geometry, source gas pressure and temperature, and thus can vary between different instruments under similar conditions.

3.2. Ion stability

Chromatographic separation and quantitation was initially achieved using the column manufacturer's recommended methanol–water mobile phase amended with the basic (pH=8) ammonium formate buffer to promote ion formation in the source. Persistent ions were the formate adducts $(M+CHO_2)^-$ for nitramine compounds, while two nitroaromatic compounds (TNT and TNB) formed methoxy adduct ions $(M+CH_3O)^-$. However, variability in calibration results suggested that adduct ion formation was not as stable as desired for accurate and consistent quantitation in the methanol matrix, especially for the nitroaromatic compounds. Methanol was then replaced with isopropanol since it was a better solvent for negative-ion formation in previous electrospray analytical studies [13–15]. The UV and ion chromatograms using isopropronol–ammonium formate mobile phase are shown in Fig. 2.

Ion and calibration stability was monitored for 12 h (Fig. 3) for all compounds. The instrument response and stability was improved for all compounds except 2ADNT, which showed a slight decrease in precision in the isopropanol matrix. Precision for TNT was dramatically improved in the isopropanol matrix, and ions for two additional nitroaromatics (DNB and DNT) were detected with a precision of $<\pm 15\%$ (Fig. 3). Calibration stability was greatly improved in subsequent analyses using the isopropanol mobile phase, with the variability in detector response for standards reduced by an average of 9 to 40% during a single run.

Additional instrumental conditions had a marked effect on ion formation, and the use of isopropanol increased the range of operating conditions which could be selected. For example, the effect of the electrospray needle voltage is shown in Fig. 4. The needle voltage had only a minor effect on the nitramide compounds, but markedly increased the detector response for the nitroaromatics, especially the molecular ion of TNT. This is due to the resulting increase in the electrical field strength needed for nitroaromatic/alkoxy cluster ion formation. The increase in needle voltage also increases the spray current from 6 μA at 2.5 kV to 30 to 50 μA at 6.5 kV. Using isopropanol as a mobile phase constituent enables the use of the higher needle voltage settings before corona discharge occurs [13], thus generating more signal for the hard to ionize nitroaromatic clusters. More polar solvents, such as methanol, limit the needle voltage setting to values between 4.0 and 4.5 kV where the spray current

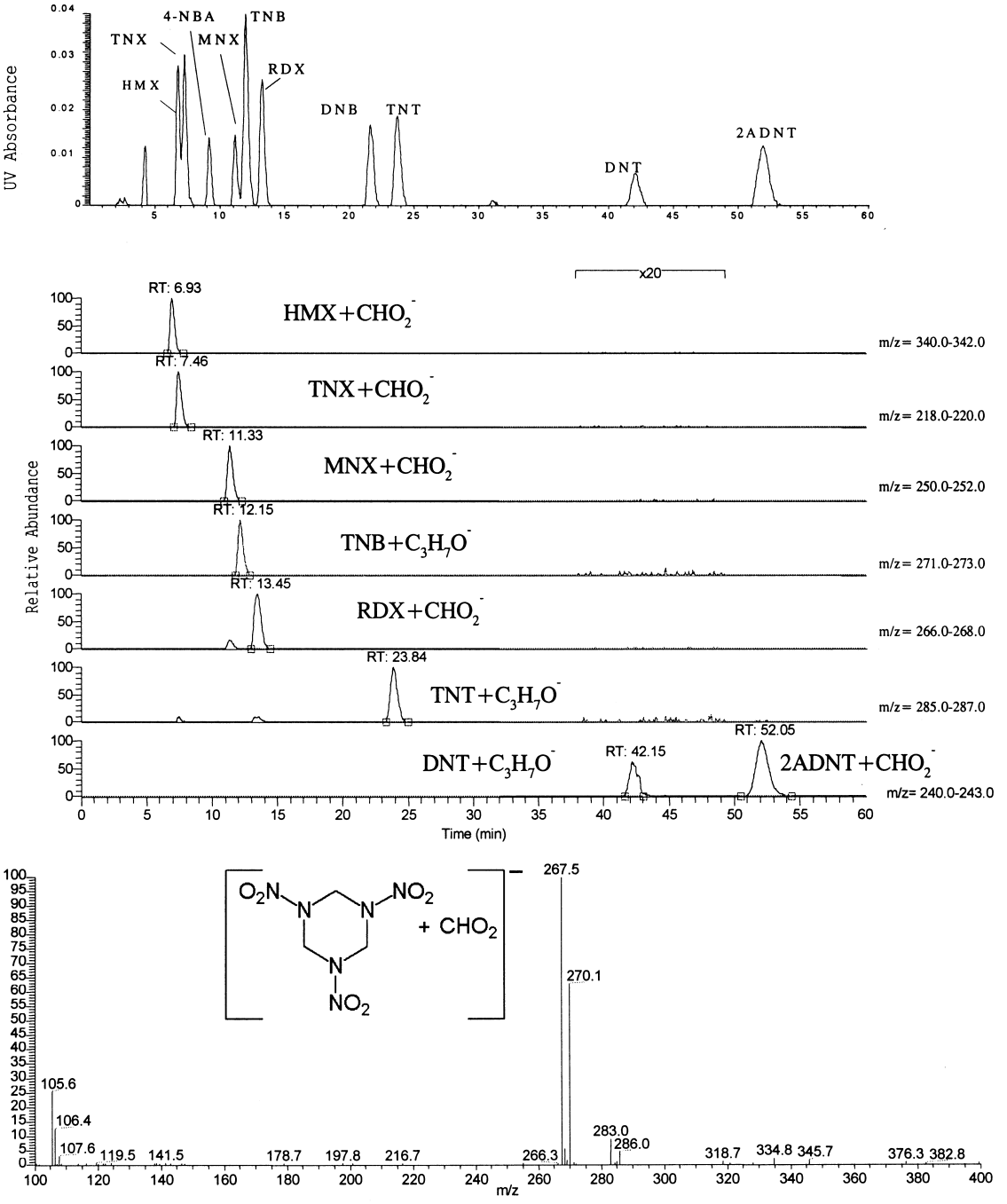

Fig. 2. UV chromatogram (top), ion chromatograms for HMX, TNX, MNX, TNB, RDX, TNT, DNT, and 2ADNT adduct ions, and mass spectrum of RDX and $^{15}N_3$-RDX adduct ions (bottom) from mid-range calibration standard.

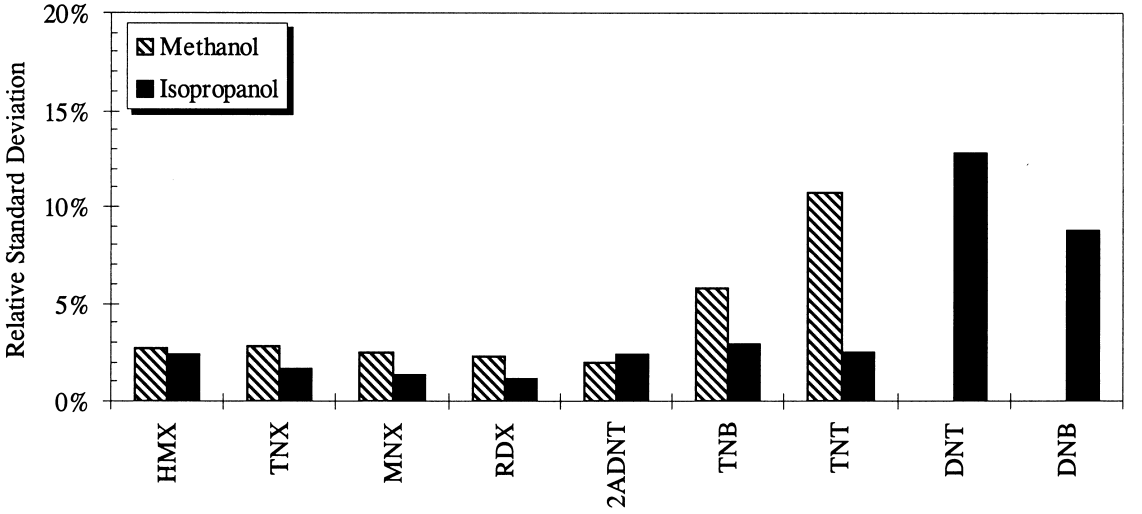

Fig. 3. Calibration stability as relative standard deviation of the instrument response for the mid-range standard over a 12-h period using ammonium formate buffer with either methanol or isopropanol in the mobile phase. Nitramine compounds were detected as formate adduct ions, and nitroaromatics as methoxy adduct ions in methanol or propoxy adduct ions in isopropanol. Adduct ions for 1,3-dinitrobenzene (DBN) and 2,4-dinitrotoluene (DNT) were only detected using isopropanol–ammonium formate buffer.

climbs to over 100 μA. The more easily ionized nitramine/formate clusters do not benefit from the increased needle voltage and subsequently larger electrical field.

The APCI source was tested with standard solutions of the munition compounds to determine whether it produced stable ionization more efficiently than ESI. The APCI results (Fig. 5) indicate a 40% decrease in RDX sensitivity when compared to electrospray ionization. Decreased sensitivity for the

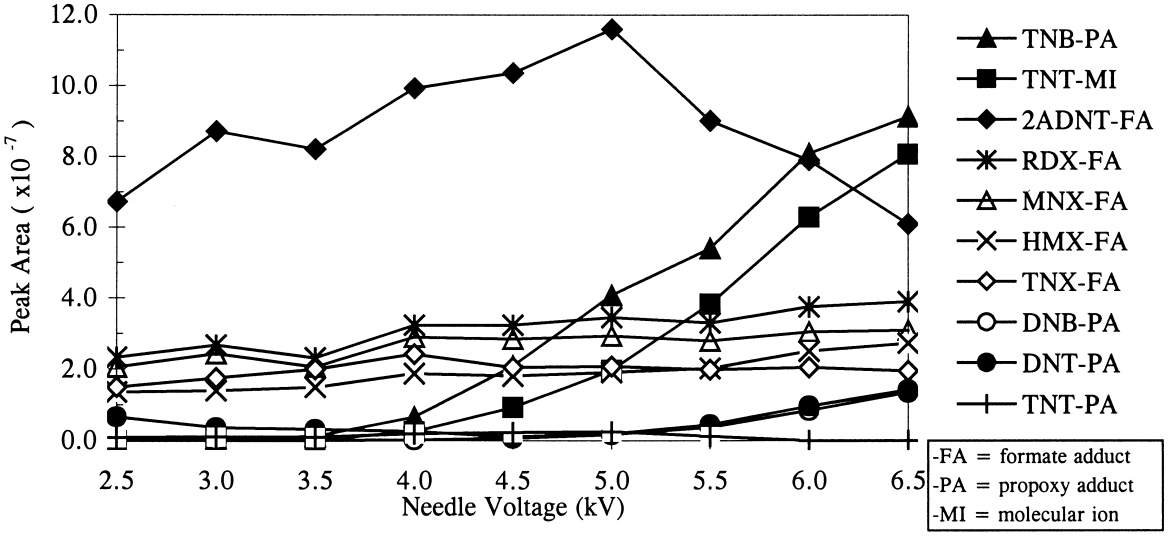

Fig. 4. Detector response and the effect of needle voltage on ion formation in electrospray source using isopropanol–ammonium formate buffer. No formate adduct ions were detected for nitroaromatics.

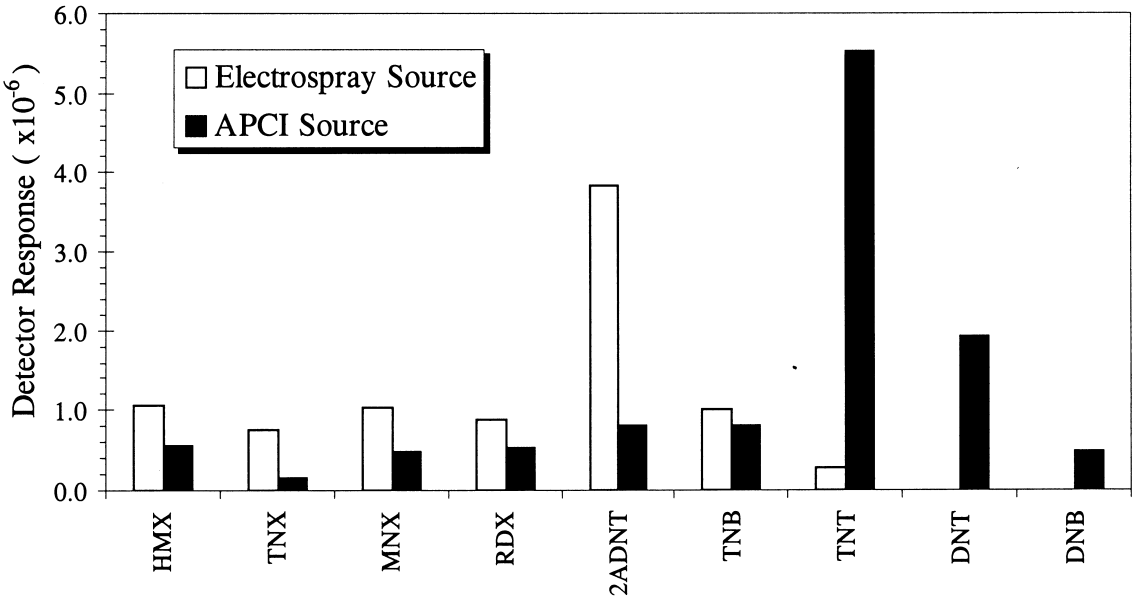

Fig. 5. Electrospray versus APCI source ion intensity for 700 ng standard using methanol–ammonium formate mobile phase. Nitramine compounds were detected as formate adduct ions in both APCI and electrospray. Nitroaromatics were detected as methoxy adducts in electrospray and as the molecular ion in APCI. 2,4-Dinitrotoluene (DNT) and 1,3-dinitrobenzene (DNB) were not detected using electrospray source with the methanol–ammonium formate buffer.

other nitramine adduct ions was also observed. In contrast to ESI, the nitroaromatics response in APCI show a dramatic increase in sensitivity for the molecular ion species [M-H]. Except for TNB, the nitroaromatic response for the molecular ion species is significantly greater than the ESI sensitivity for the alkoxy adduct. The sensitivity of TNT increased by a factor of 20 and both DNT and DNB were easily detected in the methanol/formate mobile phase with APCI but are not detected using electrospray. This observation is consistent with previous comparisons between APCI and electrospray ionization for explosives [16]. The electrospray source was thus determined to be the most sensitive for the nitramines and also provided sufficient response for determination of several nitroaromatic compounds for routine analysis.

3.3. Method detection limits

Previous work has shown that solid-phase extraction (SPE) using specially-cleaned divinylbenzene-vinylpyrrolidone copolymer resin (Porapak R_{DX}) is comparable to the more conventional salting-out solvent extraction method for nitramine and nitroaromatic explosive compounds in water [8]. Evaluation of several solvents indicated that elution with acetone produced the best overall recoveries and was more rapidly concentrated by evaporation than acetonitrile. Utilization of the $^{15}N_3$-RDX internal standard also corrects for any variability in recovery of RDX as well as variations in detector response, thus improving the method sensitivity for this compound. Due to lack of fragment ions produced under these instrumental conditions, confirmation relied solely on matching retention times of the corresponding adduct or molecular ion (m/z).

Estimated instrument detection levels (IDLs) in ESI determined from replicate analysis of the lowest standard ranged from 0.004 $\mu g\,l^{-1}$ for RDX to 0.036 $\mu g\,l^{-1}$ for DNB (Table 2). Method detection limits (MDLs) were estimated from extraction and analysis of five portions of fortified reagent water. The MDLs for the nitramine compounds ranged from 0.03 $\mu g\,l^{-1}$ for MNRDX to 0.05 $\mu g\,l^{-1}$ for RDX, while the MDLs for the nitroaromatics were nearly three times higher, ranging from 0.09 $\mu g\,l^{-1}$ for DNB to 0.12 $\mu g\,l^{-1}$ for TNT. Recoveries for all compounds

Table 2
Instrument detection level (IDL=3σ), method detection limits (MDL) and recoveries for determination of munitions compounds in water by LC–MS

Compound	IDL ($\mu g\,l^{-1}$)	MDL ($\mu g\,l^{-1}$)	Recovery (%)	Standard deviation (%)
HMX	0.010	0.037	130	6.1
RDX	0.004	0.047	86	12
MNX	0.005	0.030	90	6.0
TNX	0.009	0.032	84	6.2
TNB	0.013	0.142	71	35
TNT	0.016	0.116	110	23
2ADNT	0.009	0.037	110	7.1
DNB	0.034	0.107	97	24
DNT	0.022	0.092	87	22

ranged from 71% for TNB to 130% for HMX, with recoveries for the primary analytes, RDX and the two nitroso-RDX metabolites, between 84 and 90%. (Table 2). Although method sensitivity for the nitroaromatic compounds may have been improved by analyzing extracts a second time utilizing the APCI source, time constraints would prohibit alternating between ionization modes for routine analysis. Since this method was primarily developed for sensitive detection of nitramines, the slightly diminished sensitivity for nitroaromatics was acceptable.

3.4. Sample analysis

Nearly 100 ground water samples were collected off-site from the Cornhusker Army Ammunition Plant near Grand Island, Nebraska from 1997 to 1998 and analyzed for munitions using the method for mass spectrometric detection. Recovery of the 4-NBA surrogate compound averaged 96±26% in these samples. Profiles of HMX, RDX, mononitroso-RDX and trinitroso-RDX with depth in the ground water indicates that concentrations ranged from below detection up to 80 $\mu g\,l^{-1}$ (Fig. 6). The appearance of a peak in some of the samples with a retention time between the MNX and TNX metabolites, and a mass corresponding to the dinitroso-RDX formate adduct ion $(DNX+CHO_2)^-$; $m/z=235$), suggests the occurrence of this metabolite (Fig. 1). However, since a purified standard for dinitroso-RDX is currently unavailable, this metabolite could not be confirmed nor quantified. The USEPA accepts 2 $\mu g\,l^{-1}$ of RDX as the maximum contaminant level (MCL) in potable waters. Confirmation of the concentrations and identity of these compounds, including two RDX metabolites, was possible through the use of mass spectrometric detection.

4. Conclusions

LC–MS with API electrospray provides sensitive and selective determination of nitramine munitions and degradation products and is sufficiently sensitive for routine determination of nitroaromatics in ground water. Atmospheric pressure chemical ionization (APCI) produces a much greater signal for nitroaromatics, but is not suitable for ionization of nitramine compounds such as RDX and nitroso-RDX metabolites. Modification of the mobile phase composition enhanced sensitivity and stability of both nitramine and nitroaromatic compounds using the electrospray source. Method detection limits indicate that nitramine and nitroaromatic munitions residues, including RDX metabolites, can be routinely determined in ground water at sub-ppb levels by electrospray LC–MS after preconcentration by solid-phase extraction.

Acknowledgements

Support for this work was provided by the Nebraska Research Initiative and the Central Platte Natural Resources District. This manuscript has been assigned Journal Series No. 12527, Agricultural Research Division, University of Nebraska.

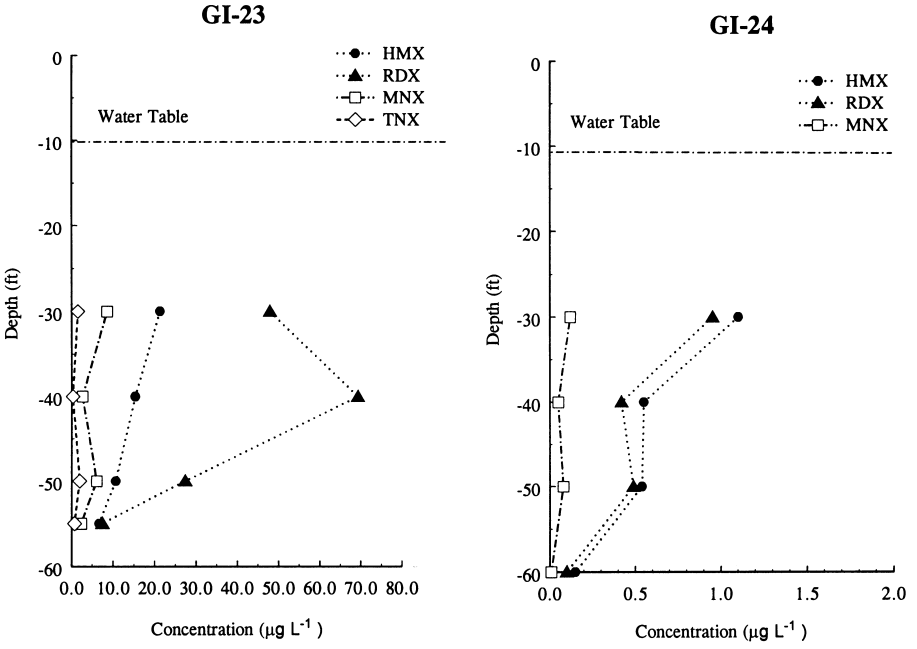

Fig. 6. Concentrations of HMX, RDX, MNX and TNX at two locations in ground water samples collected downgradient of the Cornhusker Army Ammunition Plant. TNX was not detected in samples collected at location GI-24.

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