RAPID COMMUNICATIONS IN MASS SPECTROMETRY
Rapid Commun. Mass Spectrom. 2009; **23**: 77–84
Published online in Wiley InterScience (www.interscience.wiley.com) DOI: 10.1002/rcm.3857

Liquid chromatography/negative ion atmospheric pressure photoionization mass spectrometry: a highly sensitive method for the analysis of organic explosives

Liguo Song* and John E. Bartmess
Mass Spectrometry Center, Department of Chemistry, University of Tennessee, Knoxville, TN 37996-1600, USA

Received 15 September 2008; Revised 29 October 2008; Accepted 31 October 2008

Gas chromatography/mass spectrometry (GC/MS) is applied to the analysis of volatile and thermally stable compounds, while liquid chromatography/atmospheric pressure chemical ionization mass spectrometry (LC/APCI-MS) and liquid chromatography/electrospray ionization mass spectrometry (LC/ESI-MS) are preferred for the analysis of compounds with solution acid-base chemistry. Because organic explosives are compounds with low polarity and some of them are thermally labile, they have not been very well analyzed by GC/MS, LC/APCI-MS and LC/ESI-MS. Herein, we demonstrate liquid chromatography/negative ion atmospheric pressure photoionization mass spectrometry (LC/NI-APPI-MS) as a novel and highly sensitive method for their analysis. Using LC/NI-APPI-MS, limits of quantification (LOQs) of nitroaromatics and nitramines down to the middle pg range have been achieved in full MS scan mode, which are approximately one order to two orders magnitude lower than those previously reported using GC/MS or LC/APCI-MS. The calibration dynamic ranges achieved by LC/NI-APPI-MS are also wider than those using GC/MS and LC/APCI-MS. The reproducibility of LC/NI-APPI-MS is also very reliable, with the intraday and interday variabilities by coefficient of variation (CV) of 0.2–3.4% and 0.6–1.9% for 2,4,6-trinitrotoluene (2,4,6-TNT).

Large quantities of organic explosives, most of which are nitrated compounds including nitroaromatics, nitramines, and nitroesters, are manufactured worldwide. They are mainly used in industrial processes such as mining, quarrying, road construction and civil engineering, as well as in various types of ammunition, arms, and mines. Their sensitive analysis is required for the forensic analysis of post-blast residues, detection of landmines, and location of unexploded ordnance. Furthermore, since most organic explosives and their degradation compounds are toxic,[1–5] their discharge during manufacture and military operation, leakage from unexploded ordnance, and the subsequent contamination of the environment are likewise a subject of concern, which also requires sensitive analysis.

In the USA, residues of nitroaromatics and nitramines in water, soil or sediment samples are generally analyzed by US Environment Protection Agency (EPA) SW-846 method 8330 using liquid chromatography (LC) with a UV detector. Water samples can be directly analyzed after they have been mixed with acetonitrile or methanol at 1:1 (v/v) ratio and then filtered. However, this method provides an on-column limit of quantification (LOQ) of only approximately 1 ng. When the analyte concentration in the water samples is lower than 10 μg/L, it is necessary to extract the analytes by a salting-out procedure with acetonitrile and sodium chloride. Soil and sediment samples have to be extracted by sonication using acetonitrile for 18 h followed by precipitation of interfering compounds with calcium chloride before analysis. Identification of the organic explosives and their degradation compounds depends on the chromatographic retention time. Therefore, this method is also susceptible to false positive results from co-eluting interferences.

Chromatographic techniques, principally gas chromatography (GC) and LC, are required in the analysis of organic explosives in various matrices for both cleanup and identification purposes. Electron capture detection (ECD)[6–9] and mass spectrometry (MS)[10–13] are usually coupled with GC due to their superior sensitivity and selectivity in the detection of organic explosives. MS offers higher information content than ECD, and its superior selectivity and similar sensitivity in selective ion monitoring (SIM) mode to ECD have been recently demonstrated by Jonsson *et al.*[11] In comparison with GC, LC is generally considered to be more suitable for the analysis of nitramines and nitroesters due to their low vapor pressure and because they are thermally unstable. Although UV is often used as the LC detector in the

**Correspondence to*: L. G. Song, Mass Spectrometry Center, Department of Chemistry, University of Tennessee, Knoxville, TN 37996-1600, USA.
E-mail: song@ion.chem.utk.edu
Contract/grant sponsor: National Science Foundation for the Mass Spectrometry Center of the University of Tennessee; contract/grant number: CHE-0130752.

RCM

analysis of organic explosives,[14] detection by MS has become more popular due to its better sensitivity and selectivity.[15–25]

Ionization methods used in GC/MS for the analysis of organic explosives include electron ionization (EI),[10,11,13] positive ion chemical ionization (PICI),[13] and negative ion chemical ionization (NICI).[10–13] NICI achieved lower LOQs than EI or PICI in comparison studies.[10,11,13] With GC/NICI-MS, 2,4,6-trinitrotoluene (2,4,6-TNT) and RDX (cyclotrimethylene-trinitramine) were detected with LOQs of approximately 200 and 500 pg, respectively.[10] In addition, NICI also gave less fragmentation of all organic explosives, and molecular ions were also observed for nitroaromatics. This allowed GC/NICI-MS in full scan mode to be used to identify some organic explosives without *a priori* knowledge of the sample composition.[10,12,13]

Ionization methods used in LC/MS for the analysis of organic explosives have included negative ion electrospray ionization (NI-ESI)[15,17–19,21,23–25] and negative ion atmospheric pressure chemical ionization (NI-APCI).[15,16,20–22] NI-ESI was very effective in the ionization of nitramines and nitroesters as their adducts with anions such as chloride, nitrate, formate and acetate. With LC/NI-ESI-MS, RDX was detected with a LOQ of approximately 25 pg by selected reaction monitoring (SRM).[23] Although NI-ESI could also ionize some nitroaromatics,[15,24] e.g. 2,4,6-TNT, as their $[M–H]^-$ ions, the ionization was much less effective than for nitramines and nitroesters. On the other hand, NI-APCI could effectively ionize not only nitramines and nitroesters as their adducts with anions, but also nitroaromatics. However, the NI-APCI spectra of nitroaromatics usually included not only molecular ions and deprotonated molecular ions, but also fragment ions.[15,20,21] With LC/NI-APCI-MS in full scan mode, 2,4,6-TNT and RDX were detected with LOQs of 1.1 and 6.7 ng, respectively.[15]

Atmospheric pressure photoionization (APPI) was developed in 2000 as a complement to LC/ESI-MS and LC/APCI-MS.[26] APPI used a fundamentally different ionization process[26,27] from ESI and APCI. NI-APPI was initially studied through extensive investigation of the ionization mechanism by Kostiainen and co-workers[28,29] and Traldi and co-workers.[30,31] Since then, a few applications where LC/NI-APPI-MS is superior to LC/NI-ESI-MS and/or LC/NI-APCI-MS have been reported,[32–38] with most of them related to acidic analytes.[34–38] To our knowledge, polybrominated diphenyl ethers (PBDEs) containing more than five bromines[32] have been the only low-polarity analytes analyzed by LC/NI-APPI-MS.

In our recent studies on the ionization mechanisms of NI-APPI,[39,40] we have demonstrated that NI-APPI is highly efficient in the ionization of low-polarity compounds, i.e. fullerenes, perfluorinated compounds, pentafluorobenzyl derivatives, nitroaromatics, and nitramines. In the present study, we further demonstrate LC/NI-APPI-MS as a novel and highly sensitive method for the quantification of low-polarity compounds by using organic explosives as an example. For the simplicity of the demonstration, the sample preparation and LC separation procedures described in US EPA SW-846 method 8330 were followed. However, NI-APPI-MS in full scan MS mode rather than UV was used for the detection of the organic explosives.

EXPERIMENTAL

Reagents

Toluene, methanol, and water were HPLC grade and purchased from Fisher Scientific (Suwanee, GA, USA). Calcium chloride and HPLC grade methylene chloride were purchased from Aldrich Chemical (St. Louis, MO, USA). 1,3-Dinitrobenzene (1,3-DNB), 2,4-dinitrotoluene (2,4-DNT), 2,6-dinitrotoluene (2,6-DNT), 1,3,5-trinitrobenzene(1,3,5-TNB), 4-amino-2,6-dinitrotoluene (4-amino-2,6-DNT), 2-amino-4,6-dinitrotoluene (2-amino-4,6-DNT), 2,4,6-trinitrotoluene (2,4,6-TNT), tetryl (2,4,6-trinitrophenyl-N-methylnitramine), RDX (cyclotrimethylene-trinitramine), and HMX (1,3,5,7-tetranitro-1,3,5,7-tetraocane) were purchased from AccuStandard (New Haven, CT, USA). The structures of the studied compounds are shown in Fig. 1. Their thermochemical data is given in Table 1. The thermochemical data of oxygen and HO_2 are also included in Table 1, as the existence of oxygen in an APPI source plays a key role in NI-APPI.[28,39,40] Stock solutions of individual organic explosives were prepared at a concentration of 1 mg/mL in methanol and stored in refrigerator at 4°C in the dark. Working solutions of organic explosives were prepared fresh every day by mixing the individual stock solutions together and then diluting the mixture with methanol to the desired concentration. The solutions were then further diluted by 50% (v/v) with 5 g/L aqueous calcium chloride solution immediately before use.

Apparatus

A Ultimate fully integrated micro-, capillary-, and nano-HPLC system (Dionex/LC Packings, Sunnyvale, CA, USA) was used for the separation. It was coupled online to an QSTAR XL triple quadrupole time-of-flight (QTOF) mass spectrometer (Applied Biosystems/MDS Sciex, Concord, Ontario, Canada) through an PhotoSpray source (Applied Biosystems/MDS Sciex) with a 10 eV krypton discharge lamp.

LC/NI-APPI-MS

The LC separation used a 218TP52 C18 column (25 mm × 2.1 mm; Grace Vydac, Deerfield, IL, USA) with an isocratic elution using solvent A and solvent B at a 1:1 ratio. For the separation of nitroaromatics, solvent A and solvent B were, respectively, water and methanol. However, for the separation of nitramines, solvents A and B were water and a mixture of methanol and methylene chloride (98:2, v/v), respectively. The LC column flow rate was 200 μL/min. The separation times for the nitroaromatics and nitramines were 10 and 7 min, respectively. The LC system was connected to a Valco valve (VICI, Houston, TX, USA) which was integrated with the QSTAR before it was connected to the PhotoSpray source. The Valco valve was used to divert the effluents from the LC separation for the initial 2.5 min to avoid contamination of the QSTAR ion source by inorganic salts from the samples. HPLC grade toluene was used as the dopant and was delivered with a 1050 series HPLC system (Agilent, Foster City, CA, USA) to the PhotoSpray source at a

DOI: 10.1002/rcm

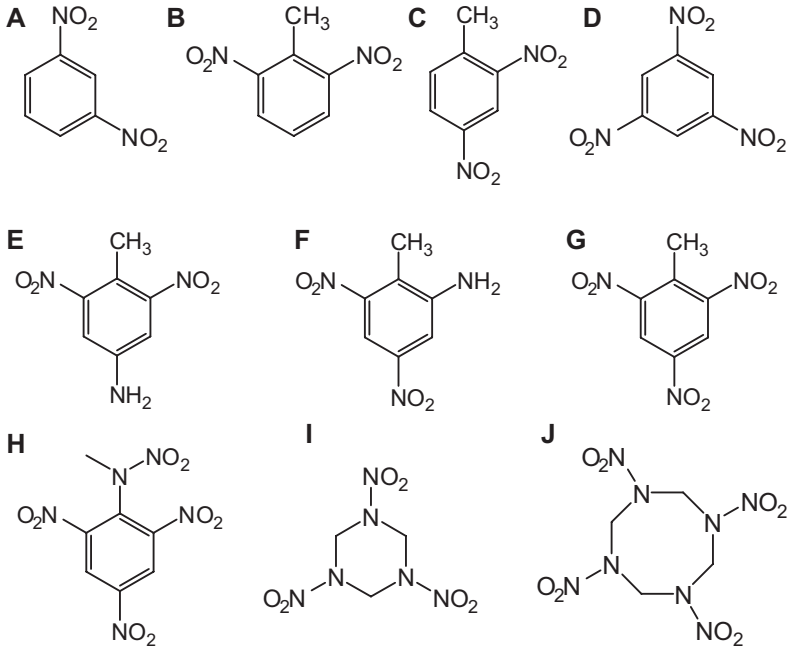

Figure 1. Structures of studied compounds: (A) 1,3-dinitrobenzene; (B) 2,4-dinitrotoluene; (C) 2,6-dinitrotoluene; (D) 1,3,5-trinitrobenzene; (E) 4-amino-2,6-dinitrotoluene; (F) 2-amino-4,6-dinitrotoluene; (G) 2,4,6-trinitrotoluene; (H) tetryl; (I) RDX; and (J) HMX.

flow rate of 200 μL/min. It should be noted that the dopant, i.e. toluene, and the mobile phase, e.g. water/methanol 1:1, are mixed after heated nebulization. Therefore, LC mobile phases which are immiscible with the dopant can still be used. The autosampler of the Ultimate LC system used a 5 μL loop for a full loop injection.

Ultra-high purity (UHP) nitrogen was used as the nebulizer, auxiliary, curtain and lamp gas. For the detection of nitroaromatics, the general controlling parameters and settings for NI-APPI-MS using a TOFMS scan were GS1 (gas 1 or nebulizer gas), 30 arbitrary units (au); GS2 (gas 2 or auxiliary gas), 30 au; CUR (curtain gas), 40 au; IS (ion spray voltage), −1500 V; TEM (temperature), 350°C; DP (declustering potential), −25 V; FP (focusing potential), −80 V; DP2 (declustering potential 2), −12 V; and CAD (collision gas pressure), 3 au. The acquisition mass range was *m/z* 120 to 300 and the acquisition time for one spectrum was 1 s. For the detection of nitramines, the general controlling parameters and settings for NI-APPI-MS were as above except that the IS (ion spray voltage) was −1900 V. The acquisition time for one spectrum was also 1 s but the acquisition mass range was *m/z* 150 to 350.

Table 1. Thermodynamic data for studied compounds[a]

Name	Formula	MW	M−H (*m/z*)	M-NO[b] (*m/z*)	EA (eV)	ΔH_{acid} (kcal/mol)
Oxygen	O_2	31.9898	/	/	0.45[c]	/
/	HO_2	/	31.9898	/	/	353.0[c]
1,3-DNB	$C_6H_4N_2O_4$	168.0171	167.0093	138.0191	1.66[c]	~356[d]
2,4-DNT	$C_7H_6N_2O_4$	182.0328	181.0249	152.0348	~1.6[d]	~328[d]
2,6-DNT	$C_7H_6N_2O_4$	182.0328	181.0249	152.0348	~1.6[d]	~333[d]
1,3,5-TNB	$C_6H_3N_3O_6$	213.0022	211.9944	183.0042	~2.6[d]	~340[d]
2-Amino-4,6-DNT	$C_7H_7N_3O_4$	197.0437	196.0358	167.0457	~1.6[d]	~329[d]
4-Amino-2,6-DNT	$C_7H_7N_3O_4$	197.0437	196.0358	167.0457	~1.6[d]	~334[d]
2,4,6-TNT	$C_7H_5N_3O_6$	227.0178	226.0100	197.0199	~2.5[d]	315.6[c]
Tetryl	$C_7H_5N_5O_8$	287.0138	286.0060	241.0209	~2.5[d]	340.0[c]
RDX	$C_3H_6N_6O_6$	222.0349	/	/	<0[d]	>400[d]
HMX	$C_4H_8N_8O_8$	296.0465	/	/	<0[d]	>400[d]

[a] EA, electron affinity; ΔH_{acid}, gas-phase acidity.
[b] M-NO_2 for tetryl.
[c] Data obtained from Ref. 41.
[d] Data estimated using experimental data for structural analogs, with data from Ref. 41. Uncertainties are estimated as being ±5 kcal/mol for acidities, and ±0.2 eV for electron affinities.

DOI: 10.1002/rcm

RESULTS AND DISCUSSION

LC/NI-APPI-MS conditions

The majority of the studies of the mass spectrometric ionization of nitroaromatics, especially those containing two or more nitro groups, have used the negative ion (NI) mode. This choice can be explained by the high electron affinities (EAs) of the compounds, as shown in Table 1. Basically, the molecules are able to stabilize a thermal electron by collision stabilization to form molecular ions in the NI mode. At the same time, the molecules may be also able to stabilize a thermal electron by bond rupture to form fragment ions. On the other hand, some of the nitroaromatics also possess strong enough gas-phase acidities that a proton can be abstracted from the molecules with an appropriate base, resulting in the formation of $[M–H]^-$ ions. Finally, fragmentation of the above described ions has frequently been observed. These possible ionization mechanisms often compete with each other, and this is not advantageous for sensitive detection. However, such fragmentation is advantageous for identification using full scan mode when there is not *a priori* knowledge of the sample composition. Unfortunately, this advantage may be also limited because isomers of nitroaromatics often have the same fragmentation pattern. Therefore, hyphenated methods using GC or LC with MS are necessary for the analysis of organic explosives.

It is understandable that NI-ESI is not effective in the ionization of nitroaromatics[15,24] due to their limited gas-phase acidities according to Table 1. With NICI[10–13] and NI-APCI,[15,20,21] multiple ionization products have been observed, which is due to their intrinsic ionization processes and will not be discussed in detail here. On the other hand, in our recent studies on the ionization mechanisms of NI-APPI,[39,40] we demonstrated that fewer ionization products were generated from nitroaromatics, which was advantageous for their sensitive detection. Briefly, NI-APPI was initiated by thermal electrons which were ejected from toluene molecules, the dopant, by absorbing photons. Electron capture (EC) and dissociative EC ionization mechanisms were subsequently initiated both for the nitroaromatics and for oxygen from the atmosphere. The formation of $O_2^{-\cdot}$ in the APPI source further initiated a proton transfer ionization mechanism due to its stronger gas-phase basicity than some of the nitroaromatics, as shown in Table 1.

Because of the ionization mechanisms involved in the NI-APPI of nitroaromatics, in order to achieve the best sensitivity when LC/NI-APPI-MS was used for the analysis of nitroaromatics, the LC mobile phases could not contain any molecules that would consume either thermal electrons or $O_2^{-\cdot}$. This, therefore, eliminates the use of halogenated solvents and acidic buffer additives. The LC conditions used in US EPA SW-846 method 8330 met those requirements and therefore they were used without any modification in this study.

NI-ESI[15,17–19,21,23–25] and NI-APCI[15,16,20–22] of nitramines and nitroesters was dependent on the formation of anion adducts and was proven to be superior to NICI.[10–13] Recently, we achieved similar ionization, i.e. anion attachment, with NI-APPI when 1% (v/v) halogenated solvents were used as additives.[39,40] Briefly, NI-APPI was first initiated by thermal electrons which were ejected from toluene molecules, the dopant, by absorbing photons. A dissociative EC ionization mechanism was subsequently initiated for the halogenated solvent additives. The formation of halide anions in the APPI source further initiated anion attachment for nitramines and nitroesters.

Because of the ionization mechanisms involved in the NI-APPI of nitramines, we modified the LC solvents used in the US EPA SW-846 method 8330 for the analysis of nitramines by LC/NI-APPI-MS in this study. While solvent A remains as water, solvent B became a mixture of methanol and methylene chloride (98:2, v/v). Consequently, nitroaromatics and nitramines were analyzed using different LC/NI-APPI-MS methods.

Figures 2 and 3 demonstrate the LC/NI-APPI-MS analysis of nitroaromatics and nitramines, respectively, under optimum conditions. It can be seen that both isomer pairs, i.e. 4-amino-2,6-DNT and 2-amino-4,6-DNT, and 2,6-DNT and 2,4-DNT, were baseline separated, which is important for their identification and quantification. It is noted that other C18 columns, e.g. 218MS52 C18 (Grace Vydac, Deerfield, IL, USA), were tested and showed partial separation of both isomer pairs. The Grace Vydac 218TP52 C18 column was uniquely suitable for this application. However, it was also noted that tetryl and 1,3-DNB, and 2-amino-4,6-DNT and 2,6-DNT, were not baseline separated from each other. Fortunately, their NI-APPI mass spectra are totally different, as will be discussed later.

Optimum NI-APPI-MS conditions for the detection of nitroaromatics and nitramines were determined by infusion experiments as reported previously,[40] so these will not be described in detail here. However, the optimum dopant flow

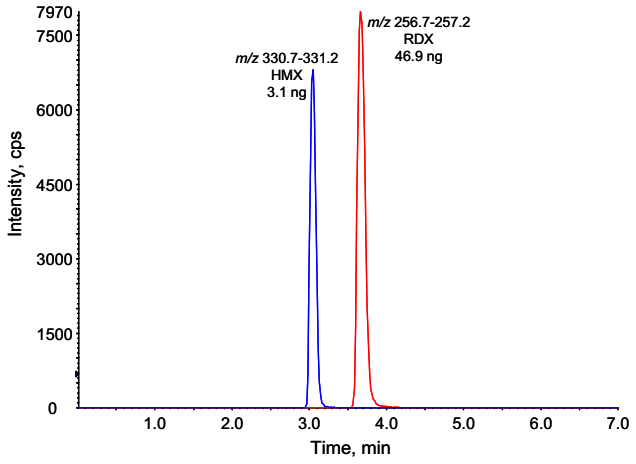

Figure 2. Representative LC/NI-APPI-MS chromatogram of nitramines. The LC/NI-APPI-MS conditions are described in the Experimental section. Although the acquisition used a full scan mode from *m/z* 150 to 350, an extracted ion chromatogram with a mass window of 0.5 *m/z* units was used to demonstrate the separation of the analytes. This figure is available in color online at www.interscience.wiley.com/journal/rcm.

DOI: 10.1002/rcm

Table 2. Ionization products by LC/NI-APPI-MS and corresponding ionization mechanism

RT (min)	Analyte	Detected ions including **analyte ions** (m/z) with relative abundance greater than 5%	Ionization mechanism
3.051	HMX	**331.0154 (100); 333.0128 (27.6)**	Cl^- attachment
3.884	RDX	**257.0037 (93.6); 259.0011 (25.2); 334.9176 (100)**	Cl^-/anion attachment
4.317	1,3,5-TNB	**183.0042 (51.5); 213.0022 (100)**	EC+dissociative EC[a]
5.117	Tetryl	**181.0249 (14.4); 182.0319 (6.6); 212.0264 (43.6); 213.0175 (7.6); 241.0209 (100)**	Dissociative EC
5.251	1,3-DNB	**138.0191 (12.6); 168.0170 (100); 183.0192 (5.6);** 226.9955 (9.3); 248.9798 (9.1)	EC+dissociative EC +substitution reaction
5.584	2,4,6-TNT	**226.0100 (100)**	Proton transfer
6.117	4-Amino-2,6-DNT	170.9405 (5.7); **196.0358 (100);** 212.0735 (66.9); 212.9050 (5.0); 226.9767 (37.2); 248.9579 (23.1); 264.9269 (7.4)	Proton transfer
6.451	2-Amino-4,6-DNT	**196.0358 (100);** 212.0735 (51.5); 226.9802 (23.7); 248.9652 (11.3)	Proton transfer
6.584	2,6-DNT	**181.0249 (100);** 212.0735 (8.0)	Proton transfer
7.001	2,4-DNT	**181.0249 (100);** 212.7350 (7.3)	Proton transfer

[a] In-source fragmentation is probably also responsible for the fragment ions, which cannot be distinguished from dissociative EC.
Masses shown in **bold** font were assigned to the corresponding analyte; masses in regular font were assigned to the background.

rate was investigated. Most published LC/APPI-MS analyses, including those using LC/NI-APPI-MS,[34–38] have used dopant flow rates less than 20% (v/v), usually 10% (v/v), of the LC column flow rate, possibly by following the pioneer works of LC/APPI-MS of both Bruins and co-workers[26] and Syage and co-workers.[27] While this dopant flow rate may be optimum for LC/PI-APPI-MS, this is not the case for LC/NI-APPI-MS according to our results. As shown in Fig. 4, the NI-APPI-MS signals of most of the analytes increased with the dopant flow rate up to 200 μL/min, which was the LC column flow rate. Higher dopant flow rates than 200 μL/min were not investigated because the combined flow rates from the LC column and the dopant exceeded the maximum flow rate recommended for the PhotoSpray source and would result in incomplete vaporization. These results are easily understood because a higher content of dopant would result in a high content of thermal electrons and $O_2^{-\cdot}$ in the APPI source; and therefore should enhance ionization by NI-APPI.

Ionization products

The ionization products of the individual organic explosives were first determined by infusion experiments as reported previously.[40] However, in the mass range where the ionization products of organic explosives appeared, there were many background ions which could prevent analyte ions from being identified. LC/NI-APPI-MS provided better opportunities to identify the ionization products of each organic explosive.

Using the major distinctive ion of each analyte determined by infusion experiment, i.e. *m/z* 331.0154, 257.0037, 183.0042, 241.0209, 168.0170, 226.0100, 196.0358, 196.0358, 181.0249, and 181.0249 for HMX, RDX, 1,3,5-TNB, tetryl, 1,3-DNB, 2,4,6-TNT, 4-amino-2,6-DNT, 2-amino-4,6-DNT, 2,6-DNT and 2,4-DNT, respectively, an extracted ion chromatogram of each analyte with a mass window of 0.5 *m/z* units was obtained when analyzing 3.1 ng HMX, 46.9 ng RDX, 7.8 ng 1,3,5-TNB, 15.6 ng 1,3-DNB, 1.6 ng 2,4,6-TNT, 1.6 ng 4-amino-2,6-DNT, 1.6 ng 2-amino-4,6-DNT, 7.8 ng 2,6-DNT and 3.1 ng 2,4-DNT. The retention time of each analyte was then determined. The mass spectrum of each analyte at its maximum chromatographic retention time was subsequently obtained and the ions observed are listed in Table 2. The masses of the ions shown in **bold** font were assigned to the corresponding analyte; masses in regular font were assigned to background. The assignment was made based on the extracted ion chromatograms: while ions from one analyte would correlate with chromatographic peaks having the same shape and retention time, background ions would not correlate with any chromatographic peaks.

As shown in Table 2, HMX was ionized exclusively through Cl^- attachment and the ionization product showed the distinctive chloride isotopic pattern, i.e. *m/z* 331.0154 (100%) and 333.0128 (27.6%). However, RDX was ionized not only through Cl^- attachment, i.e. *m/z* 257.0037 (93.6%) and 259.0011 (25.2%), but also through an unknown anion attachment, i.e. *m/z* 334.9176 (100%). This unknown anion had an *m/z* value of 112.8827 and did not show a chloride isotopic pattern but it was not investigated further in this study.

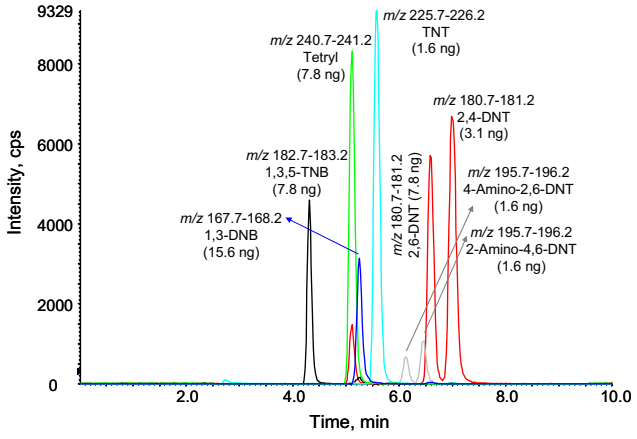

Figure 3. Representative LC/NI-APPI-MS chromatogram of nitroaromatics. The LC/NI-APPI-MS conditions are described in the Experimental section. Although the acquisition used a full scan mode from *m/z* 120 to 300, an extracted ion chromatogram with a mass window of 0.5 *m/z* units was used to demonstrate the separation of the analytes. This figure is available in color online at www.interscience.wiley.com/journal/rcm.

DOI: 10.1002/rcm

RCM

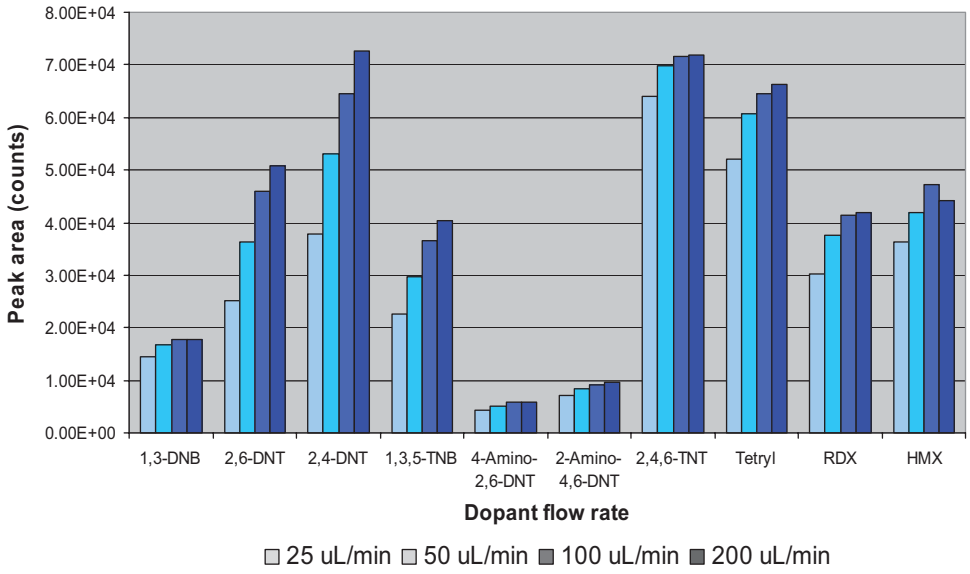

Figure 4. Effect of dopant flow rate on the efficiency of LC/NI-APPI-MS. The LC/NI-APPI-MS conditions are described in the Experimental section. This figure is available in color online at www.interscience.wiley.com/journal/rcm.

Table 3. Comparison of limits of quantifications (LOQs) of organic explosives by LC/NI-APPI-MS, LC/NICI-MS and LC/NI-APCI-MS

	LOQs (ng), S/N ratio ≥10									
Method	1,3-DNB	2,6-DNT	2,4-DNT	1,3,5-TNB	4-Amino-2,6-DNT	2-Amino-4,6-DNT	2,4,6-TNT	Tetryl	RDX	HMX
LC/NI-APPI-MS (this study)	0.361	0.163	0.054	0.228	0.070	0.042	0.029	0.100	0.305	0.038
GC/NICI-MS[10]	/	0.2	0.3	/	/	/	0.2	/	0.5	/
LC/NI-APCI-MS[15]	8.7	/	2.2	5.6	/	/	1.1	5.4	6.7	10.7

Table 4. Comparison of calibration dynamic range of organic explosives by LC/NI-APPI-MS, LC/NICI-MS and LC/NI-APCI-MS

	Calibration dynamic range (ng)									
Method	1,3-DNB	2,6-DNT	2,4-DNT	1,3,5-TNB	4-Amino-2,6-DNT	2-Amino-4,6-DNT	2,4,6-TNT	Tetryl	RDX	HMX
LC/NI-APPI-MS (this study)	0.361–62.5	0.163–7.81	0.054–3.03	0.228–8.31	0.070–12.5	0.042–12.5	0.029–1.56	0.122–7.81	0.732–23.4	0.049–1.56
GC/NICI-MS[10]	/	10-50	10–50	/	/	/	10–50	/	60–140	/
LC/NI-APCI-MS[15]	8.7–200	/	2.2–200	5.6–200	/	/	1.1–200	5.4–200	6.7–200	10.7–200

The ionization of 1,3,5-TNB involved EC, dissociative EC and/or in-source fragmentation mechanisms as both the $M^{-\cdot}$ ion, i.e. *m/z* 213.0022 (100%), and $[M–NO]^-$ ion, i.e. *m/z* 183.0042 (51.5%), were observed. Both dissociative EC and in-source fragmentation could be responsible for the $[M–NO]^-$ ion. However, the ionization of 1,3-DNB involved not only those ionization mechanisms, but also a substitution reaction to generate the $[M–H+O]^-$ ion, i.e. *m/z* 183.0192 (5.6%).

Although the ionization of tetryl involved only dissociative EC and/or in-source fragmentation, a few fragment ions including $[M–NO_2]^-$, *m/z* 241.0209 (100%); $[M–NO_2–NO+2H]^-$, *m/z* 213.0175 (7.6%); $[M–NO_2–NO+H]^-$, *m/z* 212.0264 (43.6%); $[M–2NO_2–CH_3+2H]^-$, *m/z* 182.0319 (6.6%); and $[M–2NO_2–CH_3+H]^-$, *m/z* 181.0249 (14.4%), were observed.

2,4,6-TNT, 4-amino-2,6-DNT, 2-amino-4,6-DNT, 2,6-DNT and 2,4-DNT were ionized exclusively by proton transfer as only an $[M–H]^-$ ion was observed.

LOQ, calibration dynamic range and reproducibility

This study was to demonstrate the use of LC/NI-APPI-MS as a novel and highly sensitive method in the analysis of low-polarity compounds by using organic explosives as an example. For the simplicity of demonstration, the sample preparation and LC separation procedures described in US EPA SW-846 method 8330 were followed. Analytes were

DOI: 10.1002/rcm

Table 5. Reproducibility of the LC/NI-APPI-MS method for the quantification of 2,4,6-TNT

Loading amount (pg)		48.8	97.7	195.3	390.6	781.3	1562.5
Day 1, n = 3	Calculated mean	51.9	93.8	185.7	393.0	816.3	1540.0
	RSD (%)	3.4	0.7	2.7	1.2	0.2	1.3
Day 2, n = 3	Calculated mean	53.6	92.6	182.0	391.0	813.0	1560.0
	RSD (%)	2.1	1.3	1.5	1.4	0.4	0.6
Day 3, n = 3	Calculated mean	53.7	92.1	181.3	396.7	806.0	1560.0
	RSD (%)	3.0	2.9	1.9	1.5	0.6	1.3
Overall	Calculated mean	53.1	92.8	183.0	393.6	811.8	1553.3
	RSD (%)	1.9	1.0	1.3	0.7	0.6	0.7

Table 6. Reproducibility of the LC/NI-APPI-MS method for the quantification of organic explosives

Analytes		1,3-DNB	2,6-DNT	2,4-DNT	1,3,5-TNB	4-Amino-2,6-DNT	2-Amino-4,6-DNT	2,4,6-TNT	Tetryl	RDX	HMX
Loading amount (pg)		977.0	488.0	195.3	488.3	97.7	97.7	97.7	488.0	2930.0	195.0
Day 1, n = 3	Calculated mean	913.0	487.0	191.0	486.0	98.0	94.2	93.8	477.3	2720.0	187.0
	RSD (%)	3.5	6.1	3.4	1.9	8.0	1.5	0.7	4.4	0.7	3.5
Day 2, n = 3	Calculated mean	890.3	473.7	187.3	485.0	97.0	98.2	92.6	468.3	2546.7	171.3
	RSD (%)	6.2	1.2	2.4	5.7	9.2	1.6	1.3	1.2	0.2	1.9
Day 3, n = 3	Calculated mean	865.0	470.0	188.0	493.3	102.0	102.5	92.1	474.3	2526.7	171.0
	RSD (%)	5.1	3.6	1.6	6.0	10.3	8.0	2.9	0.5	2.4	2.5
Overall	Calculated mean	889.4	476.9	188.7	488.2	99.0	98.3	92.8	473.3	2597.8	176.4
	RSD (%)	2.7	1.9	0.9	0.9	2.7	4.3	1.0	1.0	4.1	5.2

detected by NI-APPI-MS in TOFMS mode using a QTOF mass spectrometer. Quantification was accomplished using the extracted ion chromatograms of individual analytes. The mass window for HMX, RDX, 1,3,5-TNB, tetryl, 1,3-DNB, 2,4,6-TNT, 4-amino-2,6-DNT, 2-amino-4,6-DNT, 2,6-DNT and 2,4-DNT were respectively *m/z* 330.7–331.2, 256.7–257.2, 182.7–183.2, 240.7–241.2, 167.2–168.2, 225.7–226.2, 195.7–196.2, 195.7–196.2, 180.7–181.2, and 180.7–181.2. It is noted that the $M^{-\bullet}$ ion at *m/z* 212.7–213.2 was not used to quantify 1,3,5-TNB although this ion was more intense than the $[M–NO]^-$ ion at *m/z* 182.7–183.2 actually used. This was because a major background ion at *m/z* 212.07 with an isotopic ion at *m/z* 213.07 could interfere with quantification using the $M^{-\bullet}$ ion. It is also noted that tetryl was detected using both *m/z* 240.7–241.2 and 180.7–181.2, as shown in Fig. 3. However, the quantification was not affected because tetryl, 2,6-DNT and 2,4-DNT were baseline separated. 1,3-DNB was also detected by both *m/z* 167.7–168.2 and 182.7–183.2 but again the quantification was not affected as 1,3-DNB was baseline separated from 1,3,5-TNB. Quantification of the simulated aqueous samples was accomplished three times on each of three separate days. The peak area of an individual analyte was then used to create a calibration curve using a power regression with ln*x* weighting.

LC/NI-APPI-MS demonstrated high sensitivity in the analysis of organic explosives. As shown in Table 3, LOQs (signal-to-noise (S/N) ratio $\geq$10) down to the middle pg range have been achieved, which are up to one or two orders of magnitude lower than the lowest published values by GC/NICI-MS and LC/NI-APCI-MS, respectively. LC/NI-APPI-MS also demonstrated a wider calibration dynamic range than GC/NICI-MS and LC/NI-APCI-MS, and this is shown in Table 4.

The reproducibility of LC/NI-APPI-MS was also found to be very good, as shown in Table 5 using 2,4,6-TNT as an example with the intraday and interday variability by coefficient of variation (CV) being 0.2–3.4% and 0.6–1.9%. The good reproducibility of LC/NI-APPI-MS is also demonstrated in Table 6 for all the analytes at a concentration of twice the corresponding LOQs with the intraday and interday variability by CV of 0.2–6.1% and 0.9–5.2%.

CONCLUSIONS

This study has demonstrated that LC/NI-APPI-MS is a novel and highly sensitive method in the analysis of thermally labile and/or low-polarity compounds, whose analysis is challenging. LC/NI-APPI-MS showed good sensitivity in the quantification of organic explosives with LOQs up to one to two orders magnitude lower than those obtained by GC/NICI-MS and LC/NI-APCI-MS, respectively. In order to enhance the sensitivity, the LC/NI-APPI-MS conditions were optimized according to the ionization mechanisms involved in the ionization of the analytes. A dopant flow rate comparable with the LC column flow rate was found to be optimal for LC/NI-APPI-MS, which is different from what has previously been reported. Future work should focus on the coupling of NI-APPI-MS with better LC separation, e.g. LC separation using a porous graphite carbon (PGC) column where as many as 21 organic explosive-related compounds can be chromatographically separated in a single run.[15,16,20]

DOI: 10.1002/rcm

Acknowledgements

The authors greatly appreciate financial support from the National Science Foundation for the Mass Spectrometry Center of the University of Tennessee (CHE-0130752).

REFERENCES

1. Symons ZC, Bruce NC. *Nat. Prod. Rep.* 2006; **23**: 845.
2. Lewis TA, Newcombe DA, Crawford RL. *J. Environ. Manag.* 2004; **70**: 291.
3. Ye J, Singh A, Ward OP. *World J. Microb. Biotechnol.* 2004; **20**: 117.
4. Snellinx Z, Nepovim A, Taghavi S, Vangronsveld J, Vanek T, van der Lelie D. *Environ. Sci. Pollut. Res.* 2002; **9**: 48.
5. Hawari J, Shen CF, Guiot SR, Greer CW, Rho D, Sunahara G, Ampleman G, Thiboutot S. *Water Sci. Technol.* 2000; **42**: 385.
6. Calderara S, Gardebas D, Martinez F. *Forensic Sci. Int.* 2003; **137**: 6.
7. Monteil-Rivera F, Beaulieu C, Hawari J. *J. Chromatogr. A* 2005; **1066**: 177.
8. Pan XP, Zhang BH, Cobb GP. *Talanta* 2005; **67**: 816.
9. Waddell R, Dale DE, Monagle M, Smith SA. *J. Chromatogr. A* 2005; **1062**: 125.
10. Calderara S, Gardebas D, Martinez F, Khong SP. *J. Forensic Sci.* 2004; **49**: 1005.
11. Jonsson S, Gustavsson L, van Bavel B. *J. Chromatogr. A* 2007; **1164**: 65.
12. Sigman ME, Ma CY. *Anal. Chem.* 1999; **71**: 4119.
13. Sigman ME, Ma CY. *J. Forensic Sci.* 2001; **46**: 6.
14. Gaurav D, Malik AK, Rai PK. *Crit. Rev. Anal. Chem.* 2007; **37**: 227.
15. Tachon R, Pichon V, Le Borgne MB, Minet JJ. *J. Chromatogr. A* 2007; **1154**: 174.
16. Crescenzi C, Albinana J, Carlsson H, Holmgren E, Batlle R. *J. Chromatogr. A* 2007; **1153**: 186.
17. Pan XP, Zhang BH, Tian K, Jones LE, Liu J, Anderson TA, Wang JS, Cobb GP. *Rapid Commun. Mass Spectrom.* 2006; **20**: 2222.
18. Pan XP, Tian K, Jones LE, Cobb GP. *Talanta* 2006; **70**: 455.
19. Mathis JA, McCord BR. *Rapid Commun. Mass Spectrom.* 2005; **19**: 99.
20. Holmgren E, Carlsson H, Goede P, Crescenzi C. *J. Chromatogr. A* 2005; **1099**: 127.
21. Xu XM, van de Craats AM, de Bruyn P. *J. Forensic Sci.* 2004; **49**: 1171.
22. Zhao XM, Yinon J. *J. Chromatogr. A* 2002; **946**: 125.
23. Beller HR, Tiemeier K. *Environ. Sci. Technol.* 2002; **36**: 2060.
24. Garofolo F, Longo A, Migliozzi V, Tallarico C. *Rapid Commun. Mass Spectrom.* 1996; **10**: 1273.
25. Casetta B, Garofolo F. *Org. Mass Spectrom.* 1994; **29**: 517.
26. Robb DB, Covey TR, Bruins AP. *Anal. Chem.* 2000; **72**: 3653.
27. Hanold KA, Fischer SM, Cormia PH, Miller CE, Syage JA. *Anal. Chem.* 2004; **76**: 2842.
28. Kauppila TJ, Kotiaho T, Kostiainen R, Bruins AP. *J. Am. Soc. Mass Spectrom.* 2004; **15**: 203.
29. Kauppila TJ, Kuuranne T, Meurer EC, Eberlin MN, Kotiaho T, Kostiainen R. *Anal. Chem.* 2002; **74**: 5470.
30. Basso E, Marotta E, Seraglia R, Tubaro M, Traldi P. *J. Mass Spectrom.* 2003; **38**: 1113.
31. Tubaro M, Marotta E, Seraglia R, Traldi P. *Rapid Commun. Mass Spectrom.* 2003; **17**: 2423.
32. Debrauwer L, Riu A, Jouahri M, Rathahao E, Jouanin I, Antignac JP, Cariou R, Le Bizec B, Zalko D. *J. Chromatogr. A* 2005; **1082**: 98.
33. van Leeuwen SM, Hendriksen L, Karst U. *J. Chromatogr. A* 2004; **1058**: 107.
34. Takino M, Daishima S, Nakahara T. *Rapid Commun. Mass Spectrom.* 2003; **17**: 383.
35. Takino M, Daishima S, Nakahara T. *Rapid Commun. Mass Spectrom.* 2003; **17**: 1965.
36. Takino M, Daishima S, Nakahara T. *J. Chromatogr. A* 2003; **1011**: 67.
37. Yoshioka N, Akiyama Y, Teranishi K. *J. Chromatogr. A* 2004; **1022**: 145.
38. Hakala KS, Laitinen L, Kaukonen AM, Hirvonen J, Kostiainen R, Kotiaho T. *Anal. Chem.* 2003; **75**: 5969.
39. Song LG, Wellman AD, Yao HF, Adcock J. *Rapid Commun. Mass Spectrom.* 2007; **21**: 1343.
40. Song LG, Wellman AD, Yao HF, Bartmess JE. *J. Am. Soc. Mass Spectrom.* 2007; **18**: 1789.
41. Bartmess JE. Negative ion energetics data. In NIST Chemistry WebBook, NIST Standard Reference Database Number 69, National Institute of Standards and Technology: Gaithersburg, MD, 2005. Available: http://webbook.nist.gov.

DOI: 10.1002/rcm