



Detection of the improvised explosives ammonium nitrate (AN) and urea nitrate (UN) using non-aqueous solvents with electrospray ionization and MS/MS detection

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ABSTRACT

In this study methods for the detection of trace levels of the improvised explosives urea nitrate and ammonium nitrate were developed using electrospray ionization with infusion. By using a non-aqueous solvent mixture containing 95% acetone with 5% 2-methoxyethanol we were able to preserve the urea and ammonium nitrate ion pairs and discriminate between these and other similar salts. Negative ion electrospray ionization was used for urea nitrate detection and positive ion electrospray ionization was used for ammonium nitrate. Two specific adduct ions were detected for each explosive with ammonium nitrate producing m/z 178 $[2AN+NH_4]^+$ and m/z 258 ions $[3AN+NH_4]^+$ while urea nitrate produced m/z 185 $[UN+NO_3]^-$ and m/z 248 $[UN+HNO_3+NO_3]^-$. The specificity of the analysis was examined by mixing the different explosives with various salts and interferences. Adduct ions formed in the gas phase were found to be useful in distinguishing between ion pairs and mixed salts. Overall the method demonstrates the sensitive detection of both explosives, and more specifically the potential to determine intact urea nitrate.

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1. Introduction

Ammonium nitrate and urea nitrate are salts of nitric acid that are used in improvised explosive devices (IEDs). Ammonium nitrate (AN) is a common fertilizer and was first used as a blasting agent in 1867. Over the years, AN has become the most widely used mining explosive and is typically mixed with 6% fuel oil prior to application [1]. Ammonium nitrate is also routinely used in improvised explosive devices, and has garnered much attention for its use in the Middle East. In the United States, it was most notably used to destroy the Alfred P. Murrah Federal Building in 1995 [2].

Urea nitrate (UN) was mostly unknown to the general public until it was used in the first World Trade Center bombing in 1993. This explosive has become an alternative to ammonium nitrate (AN) in improvised devices. Both urea nitrate and ammonium nitrate are easily obtained or synthesized from largely unregulated materials, and have been employed in bombing incidents around the world. AN (with PETN) was also used in the Delhi bombing in 2011 [3], and UN has been used by Palestinian separatists against the Israeli military [4]. The import of urea fertilizer was banned by the Peruvian government in 1992 because UN was a favorite explosive of the Shining Path guerilla organization [5], and the governments of Pakistan and Afghanistan have banned the use

of AN due its diversion from farming to production of ANFO [6]. UN is also a popular topic of discussion on “citizen scientist” [7,8] websites due to its easily-sourced ingredients and ease of manufacture.

AN may be identified by positive ionization mode electrospray ionization-mass spectrometry (ESI-MS) [9]. Ammonium and nitrate ions may be detected by ion chromatography (IC) [10] or color test reagents such as the diphenylamine test for nitrate ion or the Nessler reagent for ammonium ion, but no color test for the AN ion pair exists. UN may be presumptively identified using *para*-dimethylaminocinnamaldehyde (*p*-DMAC) [11]. UN may also be detected using X-ray diffraction [12], infrared spectroscopy [13], voltammetry [14], in positive ionization mode ESI-MS when complexed with crown ethers [15], and by LC-UV/fluorescence using xanthidrol as a fluorophore [16]. Almog expanded on the use of xanthidrol for the detection of UN by a further reaction with alcohol to form a compound amenable to GC/MS analysis [17]. Residual amounts of ammonium nitrate [9] and urea nitrate [18] may be quickly and selectively determined by electrospray ionization-mass spectrometry (ESI-MS). A technique for the detection of both AN and UN in positive ESI mode using infusion with crown ethers has also recently been published by this group [19]. Photodissociation followed by laser induced fluorescence has also been used for both compounds [20].

This work was built upon the previous ESI-MS work performed by Tamiri and coworkers to demonstrate the effects of common interferences on the detection of AN and UN, and also to address the observation that under certain conditions, a spurious urea

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nitrate signal is generated as an artifact of ionization conditions in samples that contain a mixture of urea and nitrate [15,18].

Following the manufacture or use of an IED, AN and UN may be present as residue on the hands or clothing of individuals, or on surfaces they may have touched. Detection is performed on samples collected by swabbing or extracting cuttings from these surfaces. However, an individual suspected of handling AN or UN may have other potentially interfering substances such as soil, sweat, food products, grease, or oil on their skin or clothes. Any technique used for rapid identification must be able to detect AN or UN in the presence of such interferences. Unfortunately both of these salts undergo a metathetical reaction in polar protic solvents and can freely combine with other salts. Furthermore, urea nitrate decomposes to neutral urea and nitric acid in water. Because urea, nitrate, and ammonium ion are present in the environment, their presence in a sample cannot be taken to mean that an explosive was originally present particularly in situations in which the explosive was present in trace quantities. In order to positively determine the presence of these salts, a technique such as FTIR is typically used; however, this procedure lacks sensitivity. New techniques are needed that are capable of specific detection of trace amounts of these explosives. It was the goal of this project to develop such a procedure using non-aqueous methods capable of detecting trace levels of these salts.

In this paper, an alternative procedure for rapid detection of urea and ammonium nitrate via infusion based ESI-MS analysis of AN and UN has been developed that increases sample stability and does not require the use of crown ethers. The specificity of the procedure was examined by testing against potential interferences that can be seen in environments where samples would be taken. Furthermore, the method can be used to differentiate urea nitrate from the mass spectral signal generated by mixtures of urea and nitrate salts using collision-induced dissociation in non-aqueous solvents.

2. Experimental

2.1. Materials

Urea nitrate was acquired from law enforcement sources and its structure confirmed by FT-IR. Ammonium nitrate, potassium nitrate, and 2-methoxyethanol (methyl cellosolve) were purchased from Sigma-Aldrich. Urea standards were purchased from Beckman Coulter. Formic acid and methanol (both Optima LC/MS grade) were purchased from Fisher. Acetone (reagent grade) was purchased from Pharmco-AAPER. *para*-dimethylaminocinnamaldehyde (p-DMAC) was purchased from Acros Organics. Standard and sample solutions were prepared in 95% acetone/5% 2-methoxyethanol.

2.2. Instrumentation

A Quattro Micro triple-quadrupole mass spectrometer (Waters Corporation, Milford, MA) with ESI interface was used. Samples were introduced using a 250 μ L syringe (Hamilton Company, Reno, NV) at a flow rate of 75 μ L per minute. A mobile phase/ionization promoter of 0.05% concentrated formic acid in methanol was infused at 25 μ L per minute. Sample and mobile phase were mixed at a T-fitting and introduced directly into the spray chamber. Conditions for negative ESI: capillary voltage, 4.0 kV; cone voltage, -40 V, source temperature 100 $^{\circ}$ C and desolvation temperature 100 $^{\circ}$ C. Nitrogen desolvation gas flow was 100 l/h; cone gas flow was turned off. For positive ESI all conditions were the same except for the cone voltage, which was +40 V. For MS/MS analysis, conditions were the same with argon as the collision gas.

3. Results and discussion

3.1. Choice of solvent

In aqueous and protic solvents, ammonium nitrate dissociates into its constituent anion and cation; however, the UN molecule undergoes a decomposition reaction to yield urea and nitric acid. Because these ions are so common in the environment, the presence of neutral urea, ammonium or nitrate in a sample cannot be taken to mean that ammonium nitrate or urea nitrate was present originally. To prove the presence of these salts, it is necessary to capture the intact ion pair during the analysis. To do this the sample must be solubilized in a non-aqueous solvent and directly sprayed into the mass spectrometer.

To do this it was first necessary to find a solvent in which UN was soluble but would not dissociate. Approximately 2 mg of UN was added to 1 mL of the chosen solvent and dissolution of the solid UN was observed. Next, 50 μ L of p-DMAC color test reagent were added. Development of a red color confirmed that the uronium (protonated urea) ion was present and the solvent did not dissociate the UN ion pair. The results of the UN solvent tests are summarized in Table 1. The solvents that did not dissociate the UN ion pair were further evaluated for their ability to dissolve AN. AN was added by 1 mg amounts to 1 mL of the solvents of interest and visually checked for dissolution. The results for AN are shown in Table 2.

The solvent chosen after evaluation was a mixture of 95% acetone with 5% 2-methoxyethanol (methyl cellosolve, ethylene glycol monomethyl ether), which enhanced the dissolution of AN [21]. While both compounds showed the greatest solubility in dimethylformamide (DMF), this solvent was rejected because of its potential adverse health effects [22].

Table 1

Response of UN to dissolution in different solvents. A positive result indicates the development of a red color with p-DMAC reagent.

Solvent	Result
n-Propanol	Positive
Isopropyl alcohol	Strong positive, fades
Dimethylformamide	Strong positive
Ethyl acetate	Positive
n-Butyl chloride	UN did not dissolve
Acetonitrile (ACN)	Positive
Deionized water	Negative
Dichloromethane	Positive
Acetone	Positive
1-Decanol	UN did not dissolve
Ethanol	Positive, fades
95% Acetone/5% 2-methoxyethanol	Positive
Methanol	Positive, fades quickly

Table 2

Response of AN to dissolution in different solvents.

Solvent	Result
n-Propanol	Insoluble
Isopropyl alcohol	Insoluble
Dimethylformamide	Very soluble
Ethyl acetate	Insoluble
Acetonitrile (ACN)	Insoluble
Dichloromethane	Insoluble
Acetone	Soluble
Ethanol	Slightly soluble
95% Acetone/5% 2-methoxyethanol	Soluble (more so than acetone)

3.2. Electrospray ionization of urea and ammonium nitrate

Next, urea, ammonium nitrate, and synthesized urea nitrate were dissolved in 95% acetone/5% 2-methoxyethanol and run by themselves in both positive and negative ESI modes. Best results for ammonium nitrate were obtained in the positive ion mode and for urea nitrate, the negative ion mode. Fig. 1 illustrates the spectrum of AN obtained in positive ESI mode. Two adducts of AN are present in the spectrum: m/z 178 $[2\text{AN} + \text{NH}_4]^+$ and m/z 258 $[3\text{AN} + \text{NH}_4]^+$. The spectrum of UN obtained in negative ESI mode also contained two adducts, m/z 185 $[\text{UN} + \text{NO}_3]^-$ and m/z 248 $[\text{UN} + \text{HNO}_3 + \text{NO}_3]^-$, Fig. 2.

Detection limits for AN and UN were determined using the infusion procedure described above. The detection limit for AN was 30 ppm and the linear range of the calibration curve was from 60–400 ppm. For UN, the detection limit using this technique was 50 ppm and the linear range of the calibration curve was 75–600 ppm.

3.3. Differentiation of UN from mixtures of urea and nitrate salts

Tamiri [15,18] noted the presence of m/z 185 and m/z 248 ions in solutions of urea and ammonium nitrate in methanol under the conditions of the electrospray process and suggested that the mass

spectral data had the potential to offer a way to determine the presence of true UN from the spurious formation of the m/z 185 signal in samples containing mixtures of urea, nitrate ion, and a proton source. As a result, the potential for the spurious formation of both AN and UN from certain mixtures of ionic salts under electrospray conditions and the ability of the true and false peaks to be differentiated was examined. While this would remain a possibility for a mixture of nitrate and ammonium salts, we believed that higher specificity would be possible in the determination of urea nitrate due to the necessity of the transfer of a proton to the urea molecule during its formation.

For this experiment, negative electrospray ionization coupled to a triple quadrupole mass spectrometer was used in combination with collision induced dissociation to simultaneously determine mixtures of urea and ammonium nitrate. The concentration of one compound was held constant at 10 mM while the other was added at 1 mM, 5 mM, and 10 mM amounts. In the first experiment, the concentration of urea in the samples was held constant at 10 mM and ammonium nitrate was added in concentrations of 1 mM, 5 mM, and 10 mM (Fig. 3).

For all mixtures a peak of varying size at m/z 185 was present, showing the formation of the $[\text{UN} + \text{NO}_3]^-$ adduct under electrospray conditions. False UN spectra, however, can be distinguished from true

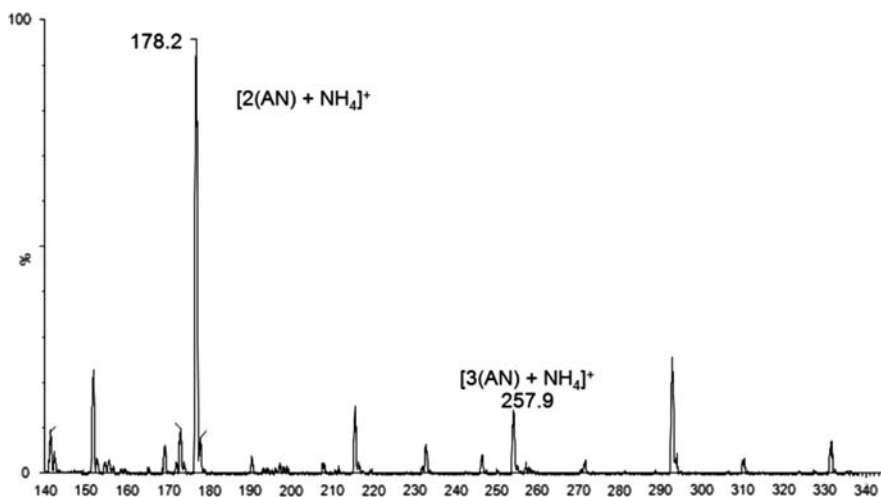


Fig. 1. Positive ESI mass spectrum of 10 mM ammonium nitrate (AN) standard.

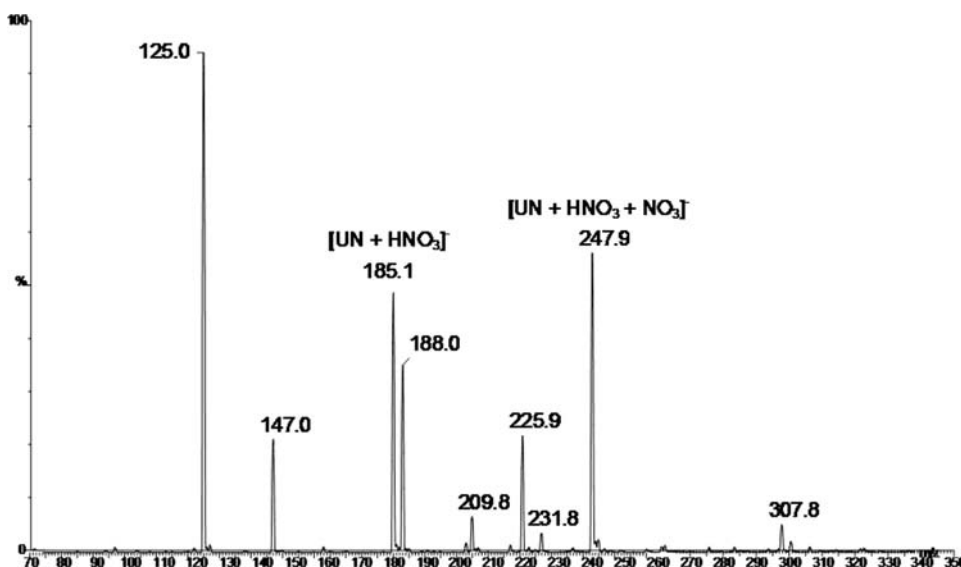


Fig. 2. Negative ESI mass spectrum of 10 mM urea nitrate (UN) standard.

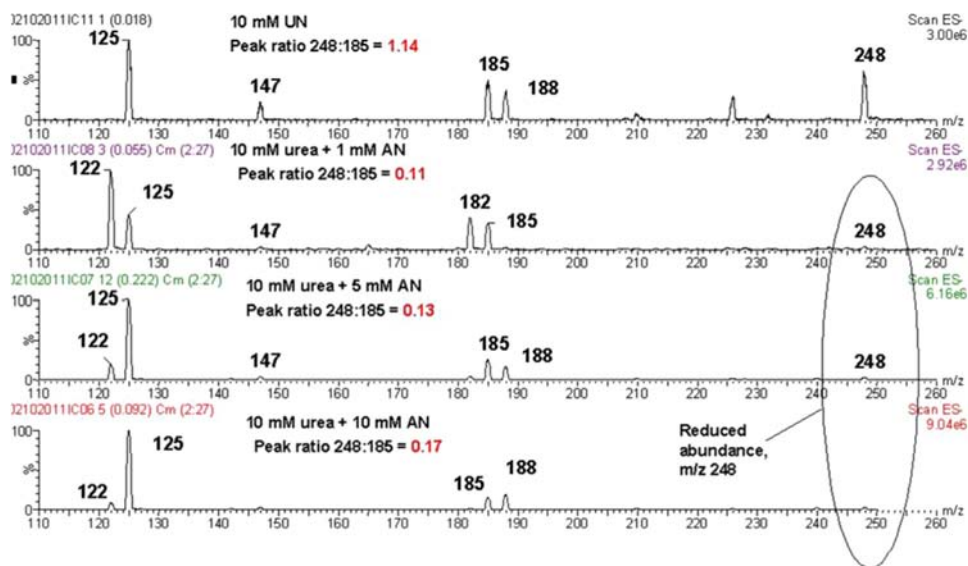


Fig. 3. Comparison of negative ESI mass spectrum of 10 mM UN (top) with negative ESI mass spectra of 10 mM urea with varying amounts of AN. Note the presence of the m/z 182 peak in the mixtures; this peak is not present in a true UN sample. Also note that in true UN the ratio of the 248:185 peaks is greater than 1.0. In the mixtures, the 248:185 ratio is less than 0.2.

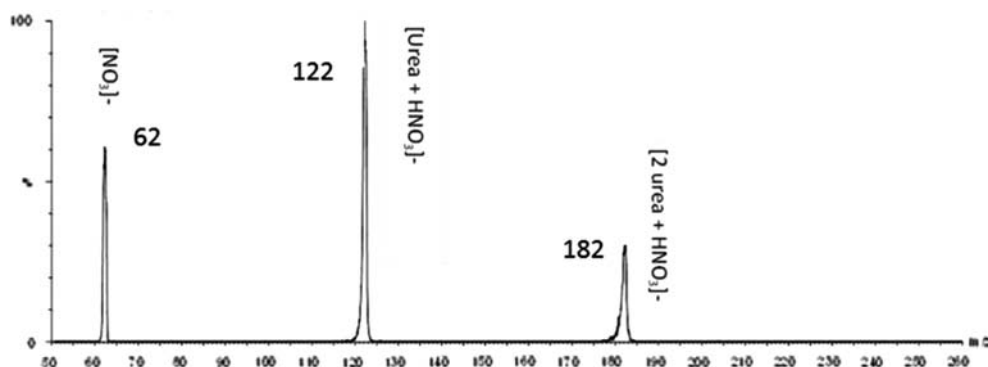


Fig. 4. Collision-induced dissociation mass spectrometry (CD-MS/MS) was performed to determine the identity of the product ions of the m/z 182 peak. Identity and masses are labeled.

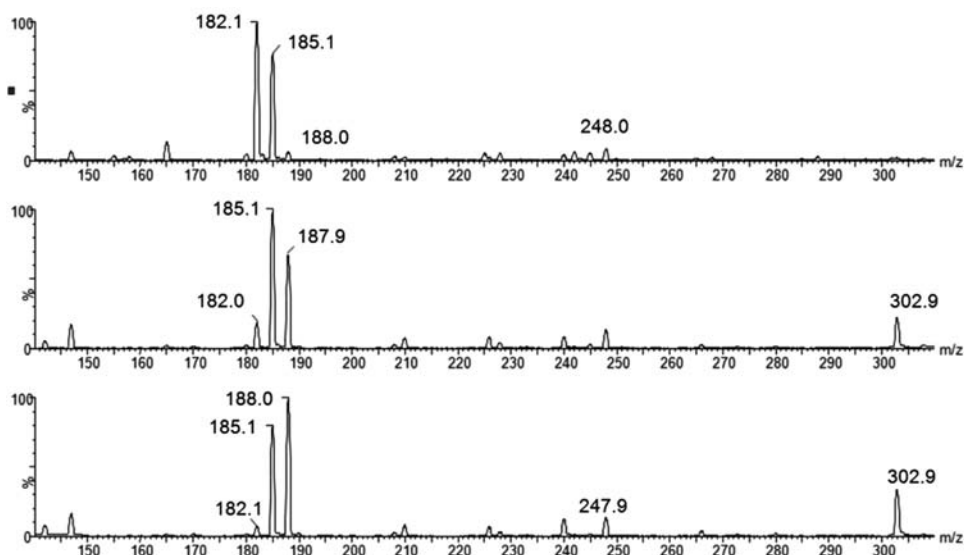


Fig. 5. Closeup of negative ESI spectrum of urea and AN mixtures with urea held constant at 10 mM. Top: 1 mM AN. Middle: 5 mM AN. Bottom: 10 mM AN. The m/z 182 peak corresponds to $[2 \text{ urea} + \text{NO}_3]^-$ and m/z 188 peak corresponds to $[2\text{HNO}_3 + \text{NO}_3]^-$.

urea nitrate by the presence of a peak at m/z 182, which is not present in the spectrum of urea nitrate. Additionally, there are differences in the height of the m/z 248 peak in relation to the m/z 185 peak. The height of the m/z 182 peak varies with the proportions of urea and ammonium nitrate. When subjected to MS/MS analysis,

fragmentation of the m/z 182 peak gives the formula $[2 \text{ urea} + \text{NO}_3]^-$ with a neutral loss of urea followed by loss of the second urea molecule and a proton (Fig. 4).

These experiments demonstrated that all mixtures of urea and ammonium nitrate do contain an m/z 185 peak suggesting the

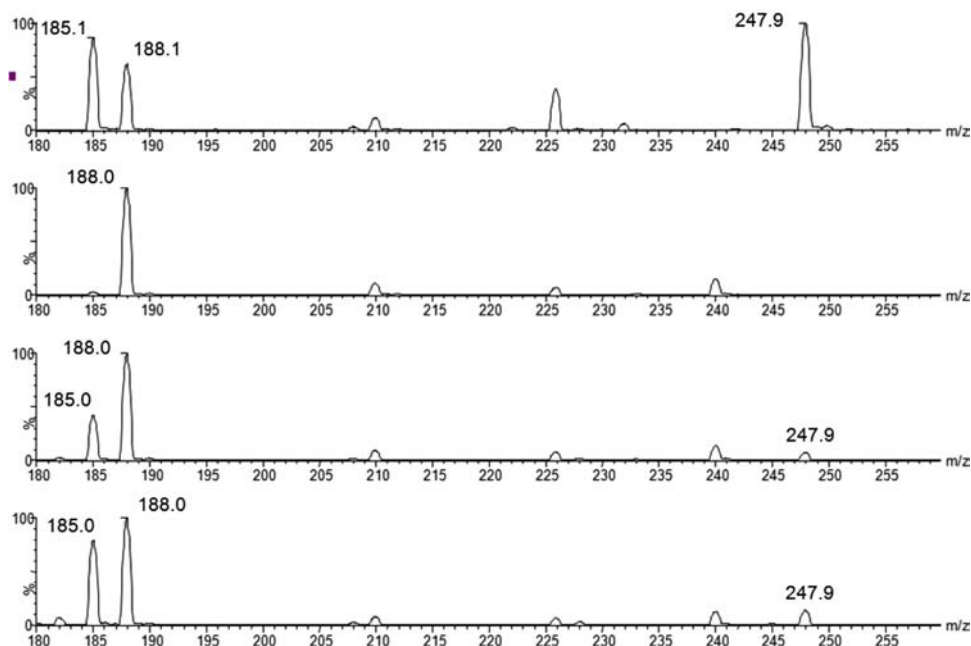


Fig. 6. Closeup of negative ESI spectrum of urea and AN mixtures with AN held constant at 10 mM. Top: 10 mM UN standard for comparison. Second: 1 mM urea. Third: 5 mM urea. Bottom: 10 mM urea. Note that m/z 185 peak increases in abundance as urea concentration increases but m/z 248 peak remains insignificant.

Table 3

CID-MS peak data and attribution.

Ion m/z	Urea+AN mixture	Urea nitrate	Product ions
182, $[2 \text{ urea} + \text{NO}_3]^-$	Present	Absent	122, 62
185, $[\text{UN} + \text{NO}_3]^-$	Present	Present	125, 122, 162
188, $[2 \text{ HNO}_3 + \text{NO}_3]^-$	Present	Present	125, 62
242, $[3 \text{ urea} + \text{NO}_3]^-$	Present	Minimally present	182, 122, 62
248, $[\text{UN} + \text{HNO}_3 + \text{NO}_3]^-$	Present	Present	188, 185, 125
Peak ratio 248:185	≤ 1.0	> 1.0	

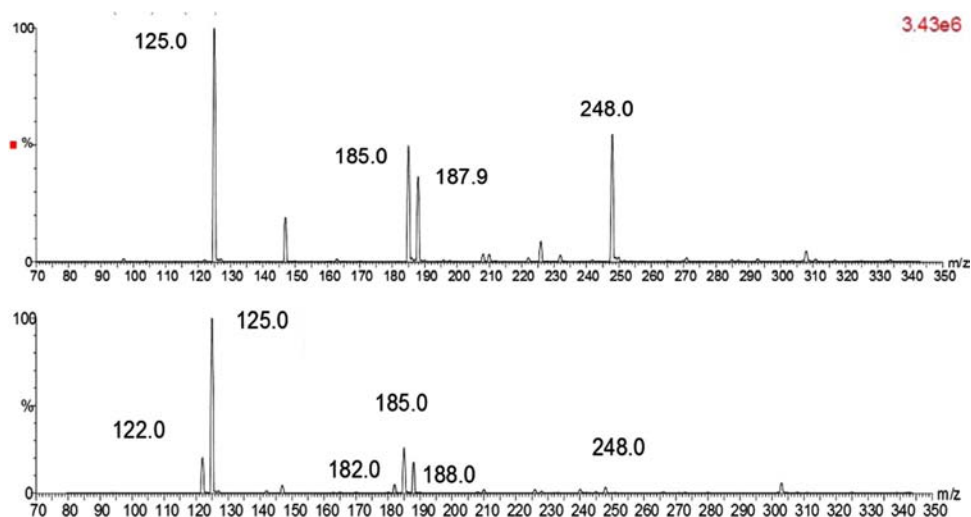


Fig. 7. Direct comparison of UN spectrum (top) with spectrum of urea plus AN (bottom). The m/z 182 ion is not present and the m/z 242 ion is present but negligible in the UN spectrum.

presence of the $[\text{UN}+\text{NO}_3]^-$ cluster. However, the presence of urea interference can be inferred by a strong m/z 182 peak with a relatively reduced m/z 248 ion $[\text{UN}+\text{HNO}_3+\text{NO}_3]^-$. This contrasts with the obvious presence of the m/z 248 peak in true urea nitrate samples. A simple ratio of peak abundances (248:185) for each sample shows a much smaller value for mixtures of urea and ammonium nitrate when compared to true urea nitrate. Furthermore, as the concentration of ammonium nitrate increases relative to the concentration of urea, the abundance of the m/z 182 peak decreases (Figs. 3 and 5).

Following this study, standards with 10 mM ammonium nitrate and varying concentrations of urea (1, 5, and 10 mM) were run and compared to the urea nitrate standard (Fig. 6). A similar result was obtained with the abundance of the m/z 185 peak increasing as urea concentration rose and again the ratio of m/z 248: m/z 185 varied with the mixture ratio.

Collision-induced dissociation permitted the identification of peaks for m/z 182, 185, 188, 242, and 248 in the mass spectrum of urea+ammonium nitrate. A summary of the CID-MS data of the urea+ammonium nitrate mixture and of true urea nitrate is shown in Table 3. Note that the m/z 182 is not present and the m/z 242 ion is present only in vanishingly small amounts in the mass spectrum of an uncontaminated urea nitrate standard (Fig. 7) when compared to the urea/AN mixture.

To determine whether the same compounds would form in the presence of a nitrate compound that was not a source of acidic protons, a sample with urea and potassium nitrate was prepared and analyzed (Fig. 8).

In the absence of a source of acidic protons (bottom), neither the m/z 185 nor the m/z 248 peak are present.

The ability to detect AN and UN on vehicles, clothing, or skin that may be contaminated with other substances such as fuels, oils, solvents, or other compounds is important in the attempt to prevent a bombing or identify a person of interest who may still be in the vicinity after an incident. Therefore compounds were chosen that could reasonably be expected to be found on individuals or vehicles

suspected of handling or transporting AN or UN. These compounds were added to AN and UN standards in solution and their effects on the target compounds determined under the same analysis

Table 4

Analysis of AN with interfering compounds (1 mL 5 mM AN standard, 5 μL interferent.)—indicates complete suppression of the ion.

Interferent	Relative change in peak height m/z 178	Relative change in peak height m/z 258
5 mM AN standard (reference)	100	100
5 μL Citgo premium gasoline	118	186
5 μL Mazola corn oil	40	63
5 μL WD-40	44	72
5 μL diesel fuel (unknown origin)	50	137
5 μL Shell diesel fuel	58	81
5 μL Carbonell olive oil	45	63
5 μL absolute ethanol (Pharmco-AAPER, lot AS4001)	75	111
5 μL Assured (Wal-Mart brand) hand sanitizer, (62% EtOH)	76	134
5 μL methanol (Fisher Scientific, lot 100970)	105	191
5 μL isopropanol (Acros, lot B0511904)	107	195
5 μL BreakFree CLP weapons lubricant	105	195
5 μL Sweet's 7.62 solvent weapons cleaner	28	—
5 μL Shooter's choice FP-10 lubricant	28	28
5 μL Gatorade perform O2, orange flavor	29	36
5 μL Diet Pepsi	108	183
5 μL Windex glass cleaner	28	41
2 μL Windex glass cleaner	73	109
2 μL Great value (Wal-Mart brand) sodium hypochlorite bleach	11	—

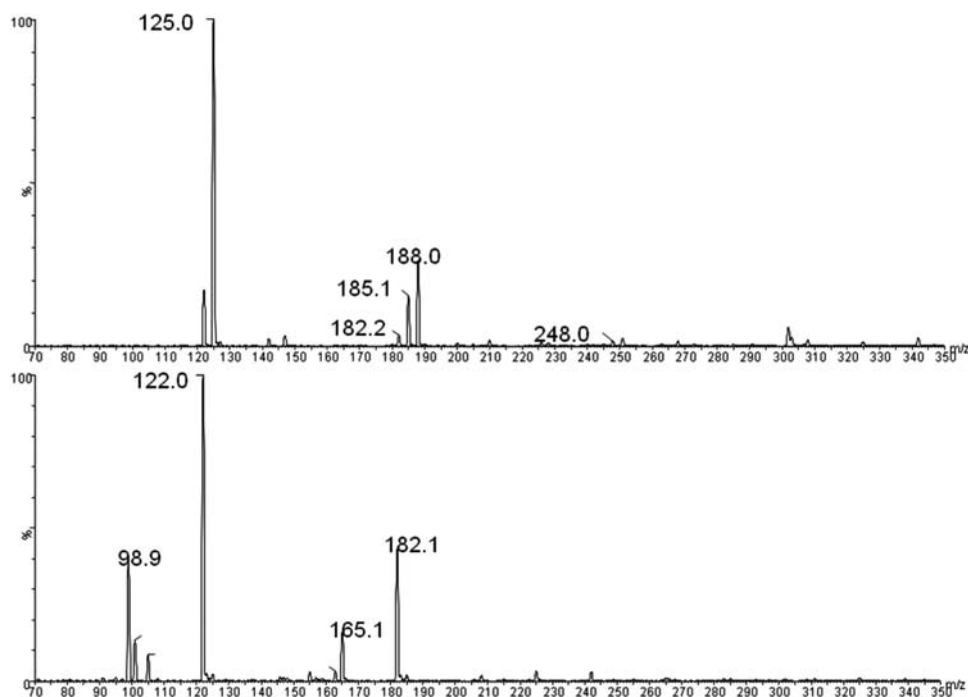


Fig. 8. Top: 10 mM urea+10 mM AN. Bottom: 10 mM urea+10 mM KNO₃. Without a proton source, no UN signal is generated.

Table 5

Analysis of UN with interfering compounds (1 mL 5 mM UN standard, interferent concentration as noted).

Interferent	Relative change in peak height m/z 185	Relative change in peak height m/z 248
1 mL 5 mM UN standard (reference)	100	100
5.0 μ L Citgo premium gasoline	93	65
5.0 μ L Mazola corn oil	93	80
5.0 μ L WD-40	92	87
5.0 μ L diesel fuel (unknown origin)	59	63
5.0 μ L Shell diesel fuel	81	111
5.0 μ L Carbonell olive oil	90	84
5.0 μ L absolute ethanol (Pharmco-AAPER, lot AS4001)	91	79
2.5 μ L Assured (Wal-Mart brand) hand sanitizer, (62% EtOH)	119	169
2.5 μ L methanol (Fisher Scientific, lot 100970)	57	83
5.0 μ L isopropanol (Acros, lot B0511904)	63	51
5.0 μ L BreakFree CLP weapons lubricant	34	16
5 μ L Sweet's 7.62 solvent weapons cleaner	14	–
5.0 μ L Shooter's choice FP-10 lubricant	81	96
2.5 μ L Gatorade perform O2, orange flavor	80	46
5 μ L Diet Pepsi	41	44
2.5 μ L Windex glass cleaner	88	57
2.0 μ L Great value (Wal-Mart brand) sodium hypochlorite bleach	38	15

conditions as the standards. The amounts chosen were enough to show an effect (to a maximum of 5 μ L or 5 mg of potential interferent per 1 mL of 5 mM AN standard [0.40 mg/mL] or 5 mM UN [0.62 mg/mL]) without completely overwhelming the signal. Representative results for each group of interferents are presented and some qualitative conclusions drawn. The interferents are listed in Table 4 for AN and Table 5 for UN. They include aqueous based liquids, solvents and cleaning supplies, fuels and lubricants. Five μ L of each interferent were added to 1 mL of 5 mM AN standard and analyzed by the infusion method described previously. The peak abundances of the samples with interferents were normalized to the abundance of the m/z 178 and m/z 258 peaks of the AN standard. With all interferences, the m/z 178 peak remained detectable, although in some cases its abundance was significantly suppressed. In two cases the m/z 258 ion was completely suppressed.

Both AN and UN decomposed in aqueous-based liquids. The effect was more pronounced for UN. Other ions present in the adulterating compound can also overwhelm any signal attributable to UN. While AN is more stable, aqueous liquids have a deleterious effect on the mass spectrum. Alcohol-based ammonia formulated window cleaners such as Windex show a slight effect on the UN signal at the time of addition and with the passage of time UN is slowly degraded due to the methanol in the glass cleaner. Addition of bleach results in the suppression of the UN signal and an enhancement of the m/z 147 peak as the $[\text{NaNO}_3 + \text{NO}_3]^-$ cluster is formed. Also enhanced is the m/z 168 peak, which may be the $[\text{CaNO}_3 + \text{NO}_3]^-$ cluster. Common motor fuels show minimal interference with the detection of UN and oils such as corn oil or canola oil do not interfere with the detection of UN. Petroleum-based weapons cleaning compounds show an effect on AN similar to fuels, reducing the peak abundance. Both compounds were

severely affected by Sweet's 7.62 solvent. This material contains 5% ammonia and results in a loss of signal for both compounds.

4. Conclusions

In this paper a method for the determination of urea and ammonium nitrate by non-aqueous electrospray MS/MS is described. Examination of the mass spectral data permits the differentiation of true urea nitrate from samples containing mixtures of urea, a nitrate salt, and an acidic proton source. Even though both contain a peak at m/z 185 corresponding to the $[\text{urea nitrate} + \text{NO}_3]^-$ cluster, the AN/urea mixture also shows peaks at m/z 182 and m/z 242 due to cluster ions containing urea that are not present in a mass spectrum of true urea nitrate. Additionally, the m/z 248 peak corresponding to $[\text{urea nitrate} + \text{HNO}_3 + \text{NO}_3]^-$ will be substantially reduced in a mixed sample. Taking a simple ratio of the m/z 248:185 peaks also aids in the determination of whether a sample contains urea nitrate. For true UN samples the ratio is greater than 1 and for mixtures, the ratio is approximately 0.2. If the mixture does not have an acidic proton source present, the m/z 185 ion is not produced. Interfering compounds show a wide variety of influences on urea nitrate. Oils and fuels have minimal effects, while aqueous liquids and cleaning products have detrimental and complicating effects on the mass spectrum. Overall this procedure provides a rapid method for quickly screening for the presence of these improvised explosives on a variety of surfaces.

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