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Recovery and Detection of Urea Nitrate in Traces*

ABSTRACT: Urea nitrate (uronium nitrate, UN) is a powerful improvised explosive, frequently used by terrorists in the Israeli arena. It was also used in the first World Trade Center bombing in New York in February 1993. It is difficult to identify UN in postexplosion debris, because in the presence of water it readily decomposes to its original components, urea and nitric acid. A method for the recovery and detection of urea nitrate in traces is described. The residues are extracted with hot acetone, and the extract chromatographed on chromosorb G-HP. The eluent is analyzed by liquid chromatography mass spectrometry using atmospheric pressure chemical ionization (APCI). By applying this technique, we were able to identify urea nitrate in actual exhibits. It was found that UN can be also formed during the analytical procedure, by certain combinations of urea, nitrate ions, and a source of protons and, hence, the presence of the characteristic adduct ion does not necessarily indicate an "authentic" urea nitrate. Several solutions are suggested to overcome this ambiguity.

KEYWORDS: forensic science, improvised explosives, postexplosion, urea nitrate, chromatography, recovery, liquid chromatography/mass spectrometry, atmospheric pressure chemical ionization

Urea nitrate (UN) is a powerful improvised explosive (1). Even nonprofessionals can easily prepare large amounts of this material from urea and nitric acid in "back-yard" facilities. It is assumed that UN has been used in improvised mines, which destroyed three Israeli Merkava tanks, and in suicide bombers belts, which caused the loss of well over 100 lives in Israel during the year 2001–2004. In the United States, UN was used as the main charge in the first bombing of the World Trade Center in February1993 (2).

Unlike most common explosives, urea nitrate is a salt. It is composed of uronium cation and nitrate anion. Crystallographic measurements show that the proton is attached to the carbonylic oxygen rather than to the amidic nitrogen (3,4) giving rise to a structure of uronium nitrate (Fig.1).

Several techniques have been suggested for the analysis of UN; among them are color tests, chromatographic and spectroscopic methods. Colorimetric techniques involve the Griess test for the detection of nitrate anion (after its reduction to nitrite) (5), and the newly reported technique for detecting uronium salts with *p*-dimethylaminocinnamaldehyde ("UN-1" reagent) (6). Chromatographic methods suitable for the analysis of salts are ion chromatography (IC) (7) and capillary electrophoresis (CE) (8). By these techniques, the anions are detected by one measurement, whereas the cations are detected by the measurement that follows, using suitable columns.

Identification of urea and nitrate components in the residues, by IC or CE, does not necessarily imply to the presence of urea nitrate! For instance, many inorganic fertilizers contain mixtures of, for example, urea and potassium nitrate (9). It is, therefore, required to unequivocally identify the intact salt molecule. In powder exhibits, this can be accomplished by spectroscopic methods such as X-ray diffraction (XRD) (4), or infra-red (IR) spectroscopy (10–12). Nevertheless, XRD and IR might not always be suitable for the analysis of postblast samples where trace amounts of amorphous residues are dealt with, accompanied by large amounts of impurities.

One of us (T.T.) succeeded recently in identifying urea nitrate by mass spectrometry (MS), using electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) (13). In this work, we examined the solubility of UN in several potential extraction solvents and its compatibility with compounds likely to be found in postexplosion debris. A clean-up procedure for UN in acetone extracts was tried using various adsorbents. Finally, the identification of urea nitrate was carried out by high performance liquid chromatography (HPLC)/APCI/MS. By applying this technique, urea nitrate could be identified in residues from postblast cases. Potential interferences to unequivocal determination of urea nitrate have also been studied.

Materials and Methods

Materials

Acetone, acetonitrile, methanol, ethanol, ethyl acetate, dichloromethane dimethyl formamide (DMF), and urea were purchased from Bio-Lab, Israel; dimethyl sulfoxide (DMSO) from Fluka Chemie, Buchs, Switzerland; hexane, potassium carbonate, sodium bicarbonate, sodium sulfate were purchased from Frutarom, Israel; ferric nitrate, ferrous bromide, kaolin light, potassium nitrate, and sodium silicate from BDH, London, England; and sulfur from Finkelman, Israel. Magnesium sulfate anhydrous AR was purchased from Mallinckrodt, Paris, KY; potassium hydrogen sulfate from Riedel De HaenAG, Seelze, Germany; ammonium sulfate from J. T. Baker, Phillipsburg, NJ; ammonium nitrate and sodium dihydrogen phosphate from Merck, Darmstadt, Germany. Water HPLC grade from Biolab, Israel and methanol HPLC grade from Merck, Darmstadt, Germany. Amberlite XAD-4, Amberlite XAD-7 and Celite 100/120 mesh were purchased from Supelco, Bellefonte,

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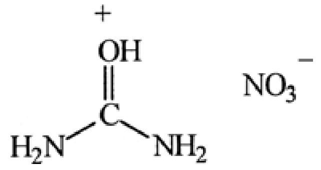


FIG. 1—*The ionic structure of urea nitrate.*

PA; Florisil 60–100 mesh and Silica gel 0.06–0.2 mm from Merck, and Chromosorb G-H.P 100/120 mesh from Johns Manville, Greenwood Plaza, Denver, CO. Terra Rosa soil (pH7, containing quartz [silicon oxide] and calcite (calcium carbonate) as determined by IR and XRD measurements), was collected from the Police Headquarters yard in Jerusalem. Urea nitrate was prepared according to (3), urea sulfate according to (14), and UN-1 reagent according to (6).

Instrumentation

An LCQ_{DUO} mass spectrometer (Thermo-Finnigan, San Jose, CA) with APCI interface, coupled with a Thermo-Separation liquid chromatograph (P4000) was used.

Vaporizer temperature was 180°C, heated capillary temperature was 70°C, discharge current 10 μA, and sheath and auxiliary gas flow rates were 50 and 0 arbitrary units, respectively. Instrument control and data processing were performed using Xcalibur software (version 1.1) (Thermo Scientific, San Jose, CA).

The HPLC separation was performed on Gemini C-18 column (150 × 2 mm i.d., 5 μm particle size) purchased from Phenomenex (Torrance, CA). The mobile phase was water with 0.1% formic acid/methanol 95/5 at a flow rate of 0.18 mL/min. Injection volume was 5 μL. Fourier Transform IR spectroscopy was carried out on a Nicolet 460 ESP Protégé using KBr pellets. XRD measurements were carried out on Scintag, Advanced Diffraction System ×2, Cu tube.

Dissolution Tests

The solvents (200 μL) were added to test tubes containing UN (10 mg) and the test tubes were shaken. Additional 200 μL portions of the solvent were added at room temperature, until the crystals were fully dissolved. The compatibility of urea nitrate with the various solvents was tested qualitatively with UN-1 reagent. Negative response would indicate decomposition of urea nitrate in the solvent.

Compatibility of Urea Nitrate with Commonly Encountered Substances

Crystals of UN and various salts (1:10 w/w, respectively) were mixed in a beaker with a wooden stick. Acetone (20 mL) was added and the beaker was heated over a steam bath until the solvent volume was reduced to *c.* 15 mL. The acetone was filtered through filter paper and the filtrate was evaporated to dryness over a steam bath. The residue was tested qualitatively for urea nitrate by UN-1 reagent and by IR spectroscopy.

LC/MS Measurements of Potential Interferences

Search for the presence of the characteristic adduct ion of urea nitrate at m/z 184.7 ($[\text{urea nitrate}+NO_3]^-$) in mixtures of standard urea (or uronium salt) and nitrate salts was carried out. 0.5 mM of a standard compound (or mixtures of compounds) was dissolved in 2 mL of methanol and measured by HPLC/APCI/MS in the negative mode.

Column Chromatography of Urea Nitrate

Columns were prepared using disposable Pasteur pipettes packed with the adsorbent (*c.* 1 cm length). The columns were conditioned by passing acetone, dichloromethane, and hexane in this order. Crystals of urea nitrate (100 mg) were placed on the adsorbent and 2 mL of each, hexane and dichloromethane, was passed and discarded. Ten milliliter of acetone was passed through the column, collected, and evaporated to dryness on a hot plate. The presence of urea nitrate in the dried acetone extract was determined by IR.

Recovery and Detection of Urea Nitrate and Interferences

Standards and mixtures of potential interferences were processed by the following recovery and detection protocol: 0.5 mM of compound (or mixture) was extracted by *c.* 3 mL of boiling acetone. The acetone was decanted and evaporated to dryness. The dried extract was cleaned on Chromosorb G-HP column by passing, first, hexane, followed by dichloromethane, and then eluting with acetone. The acetone was evaporated and the residue re-dissolved in methanol to produce a solution of 1 μg/μL for liquid chromatography/mass spectrometry (LCMS) analysis.

Recovery of Urea Nitrate from "Natural" Matrices

Samples of crystalline UN were mixed at various ratios with Terra Rosa soil (50 g), wet Terra Rosa soil (50 g soil wetted uniformly with 10 mL tap water), polystyrene chips, and nails made of iron and zinc. The mixtures were extracted once with 50 mL boiling acetone. The acetone was filtered, evaporated on a hot plate, and the residue was passed on Chromosorb G-HP. In the case of wet soil, the filtered acetone was first dried on magnesium sulfate and then evaporated.

Results and Discussion

Dissolution Tests

In postblast analysis, when no unexploded material is observed, the recovery of explosive-related compounds usually involves initial extraction with hot acetone, for the recovery of organic explosives, followed by extraction with hot water, for the recovery of explosive-related salts (15–17) .The dried aqueous extract is analyzed by the aforementioned techniques. We noticed that during the aqueous extraction and evaporation, the characteristic IR-absorption bands of UN at 1705 and 1678 cm^{-1} and the three bands near 1050 cm^{-1} (10–12) (Fig. 2*a*) disappear, and characteristic absorption bands of urea (1657 cm^{-1}) and nitrate (1380 cm^{-1}) appear instead (Fig. 2*b*). No urea nitrate could also be noticed by XRD spectroscopy of the dried aqueous extract. It is obvious that urea nitrate was hydrolyzed by hot water to urea and nitrate ion. It was, thus, necessary to try extracting with solvents other than water. Solid UN was shaken in the following solvents at room temperature: water, acetone, methanol, ethanol, acetonitrile, ethyl acetate, DMSO, DMF, and a mixture of acetone:water (1:1). The presence of urea nitrate in each solvent system was tested by the reaction with UN-1 reagent.

The results of the solubility trials and solvent compatibility of urea nitrate are presented in Table 1. Acetone, methanol, ethanol, acetonitrile, DMSO, and DMF were found capable of dissolving urea nitrate and could be considered for its extraction from

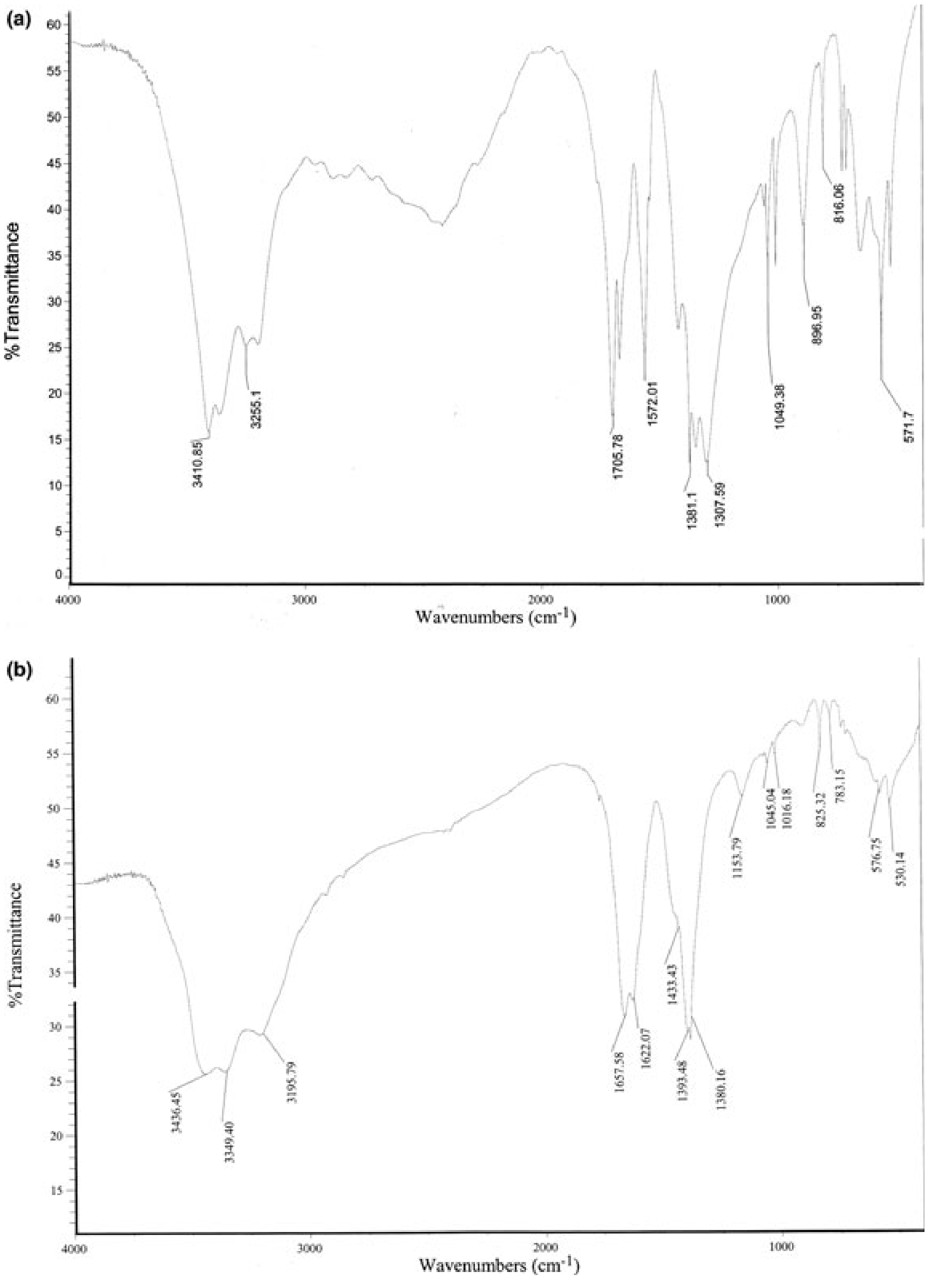


FIG. 2—*Infra red spectrum of urea nitrate (a) and urea nitrate after hot aqueous extraction (b).*

TABLE 1—*Solubility and compatibility of urea nitrate with various solvents.*

Solvent	Solubility (mg/mL)	Reaction with UN-1
Deionized water	50	Negative
Acetone	10	Positive
Methanol	25	Positive
Ethanol	25	Positive
Acetonitrile	2.5	Positive
Ethyl acetate	*	–
Acetone:water(1:1)	50	Positive
DMSO	50	Positive
DMF	50	Positive

DMSO, dimethyl sulfoxide; DMF, dimethyl formamide.
*Insoluble.

TABLE 2—*Urea nitrate detectability in hot acetone in the presence of other compounds.*

Compound	Reaction with UN-1	Presence of Urea Nitrate by IR*
Potassium carbonate	Negative	–
Sodium bicarbonate	Negative	–
Sodium sulfate	Positive	+
Kaolin	Positive	+
Sodium silicate	Positive	–
Ferric nitrate	Positive	–
Ferrous bromide	†	–
Sulfur	Positive	+
Potassium nitrate	Positive	+

*Earlier measurements showed that indicative IR absorption bands of urea nitrate in a mixture with urea and ammonium nitrate are observed only if the amount of urea nitrate exceeds 30%.

†Impossible to observe the color reaction because of the brown color of ferrous bromide.

explosion debris. Acetone was selected, as it is used anyway in the normal extraction procedure for organic explosives. Urea nitrate does not decompose in boiling acetone, which renders it a feasible extraction solvent.

Mixtures of urea with ammonium nitrate are quite common in many fertilizers. To check if such mixtures can be confused with urea nitrate by their IR spectrum, we also examined urea/ammonium nitrate (1:1)/urea nitrate mixtures at various molar ratios.

Urea/ammonium nitrate and urea nitrate (2 mM) were dissolved in acetone. Appropriate volumes of each solution were mixed to produce mixtures of 10%, 20%, 30%, and 40% urea nitrate in urea/ammonium nitrate. The solvent was evaporated to dryness and IR spectrum was recorded.

We noticed that the characteristic absorption band of urea nitrate at 1705 cm^{-1} is not observed at UN molar ratio lower than 40%. The characteristic absorption bands near 1050 cm^{-1} are noticed in the IR spectrum at molar ratio higher than 30% of urea nitrate.

Compatibility of Urea Nitrate with Commonly Encountered Substances

Inorganic compounds from soils, structure materials, and metallic fragments are likely to be found in postexplosion debris. Acetone extraction of urea nitrate in the presence of various salts was studied. Compounds containing anions normally found in soils (carbonate, sulfate, and silicate), bicarbonate, kaolin, and iron salts (ferric nitrate and ferrous bromide) and smokeless powder ingredients (sulfur and potassium nitrate) were mixed with urea nitrate, and the mixtures extracted with hot acetone. The results of these tests, which are summarized in Table 2, suggest an explanation for the poor recovery of unchanged urea nitrate from exhibits containing metal fragments, or from carbonate-rich soils. Active metals such as zinc and iron probably reduce the nitrate anion to nitrite and lower valence nitrogen species, while carbonates simply neutralize the acid moiety of UN thus converting it to urea.

Chromatographic Separation of Urea Nitrate

Acetone extracts of postblast debris usually contain, besides traces of explosives, huge amounts of irrelevant material (e.g., oily compounds and phthalate esters), which are a great nuisance to the separation and identification process. Therefore, it is important to develop an effective clean-up procedure. Various adsorbents (silica gel, florisil, Amberlite XAD-4, Amberlite XAD-7, celite, and chromosorb G-HP) were tested for their ability to pass irrelevant materials by elution with hexane and dichloromethane, followed by elution with acetone to pass intact UN. Urea nitrate decomposed upon passing on silica gel, florisil, and celite, while only minute decomposition was noticed on Amberlite XAD-4, Amberlite XAD-7, and Chromosorb G-HP. Of the above list, minimum decomposition occurred on Chromosorb G-HP, which was selected as a suitable adsorbent for the clean-up procedure.

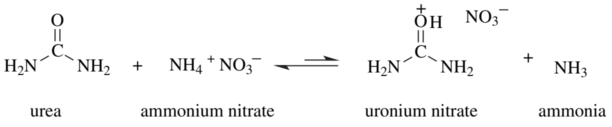
Recovery and Detection of Urea Nitrate

LC/MS measurements of standard urea nitrate, after acetone extraction and column chromatography on Chromosorb G-HP, are shown in Fig. 3. The ion chromatogram of m/z 184.7 is shown in Fig. 3*a*. The full scan mass spectrum of the chromatographic peak at 4.4 min in APCI at the negative mode is shown in Fig. 3*b*. Two adduct ions of urea nitrate were characterized at m/z 184.7 $[UN + NO_3]^-$ (ion a) and at m/z 247.5 $[UN + HNO_3 + NO_3]^-$ (ion b) (13). The MS/MS-collision induced dissociation (CID) of ion **a** is shown in Fig. 3*c*, and of ion **b** is shown in Fig. 3*d*. It is important to note that the two adduct ions at m/z 184.6 and 247.5 appear at about 0.5 dalton lower than their accurate masses, possibly because they are "fragile ions" (13,18). We, therefore conclude that urea nitrate can be detected following the aforementioned protocol.

Potential Interferences to the Identification of Urea Nitrate by Mass Spectrometry

Pure standards and mixtures of potential interferences were measured before and after processing by the aforementioned protocol. Table 3 summarizes the results of the measurements of these mixtures by LC/MS. It can be seen that the following mixtures, before and after clean-up, can also produce the characteristic adduct ion of urea nitrate at m/z 184.7 $[UN + NO_3]^-$ (ion a) with a retention time similar to that obtained from urea nitrate: urea/ammonium nitrate, urea/potassium nitrate/potassium hydrogen sulfate, and uronium sulfate/potassium nitrate. Other mixtures such as urea/potassium nitrate, urea/potassium nitrate/ammonium sulfate, urea/potassium nitrate/sodium hydrogen carbonate, and urea/potassium nitrate/sodium dihydrogen phosphate, did not produce this ion.

At this stage, the described analytical procedure does not enable us to determine whether urea nitrate in the debris, which is indicated by the characteristic adduct ion a, is authentic namely, whether it was one of the explosive components, or whether it was formed during the analytical process by a reaction between urea, nitrate salt, and an acid or acidic salt. In other words, certain combinations that involve urea, nitrates, and proton sources are equivalent to the presence of urea nitrate. We assume an equilibrium between urea and ammonium nitrate to form uronium nitrate and ammonia.



Because urea is a much weaker base than ammonia, the amount of uronium ion in the mixture is very small, but can be detected by, e.g., UN-1 color test. It must be stressed, however, that apart from urea/ammonium nitrate, the other "nuisance" combinations are quite rare and unlikely to be encountered in a common environment. Hence, it is suggested that whenever ion **a** is encountered, a test for the presence of ammonium cation should also be carried out (e.g., Nessler color test). If positive, the authenticity of urea nitrate in the sample is not unequivocal. If ammonium cations are not detected, the likelihood of authentic UN is much higher.

Recovery of Urea Nitrate from "Natural" Matrices

The efficacy of the recovery method was tested with various matrices. The results of these tests are summarized in Table 4. Surprisingly, the recovery of urea nitrate from a wet soil matrix was higher than that from a dry soil. A possible explanation for this observation could be the higher solubility of UN in acetone–water than in pure acetone. However, this result will be investigated in a further study. Urea nitrate could also be recovered from mixtures with polystyrene chips and with iron–zinc nails.

Real Case Analysis

A metal fragment from a rocket fired from the Gaza Strip was submitted to the laboratory. No crystals were observed on the metal fragment. Acetone extraction of the fragment yielded a brownish

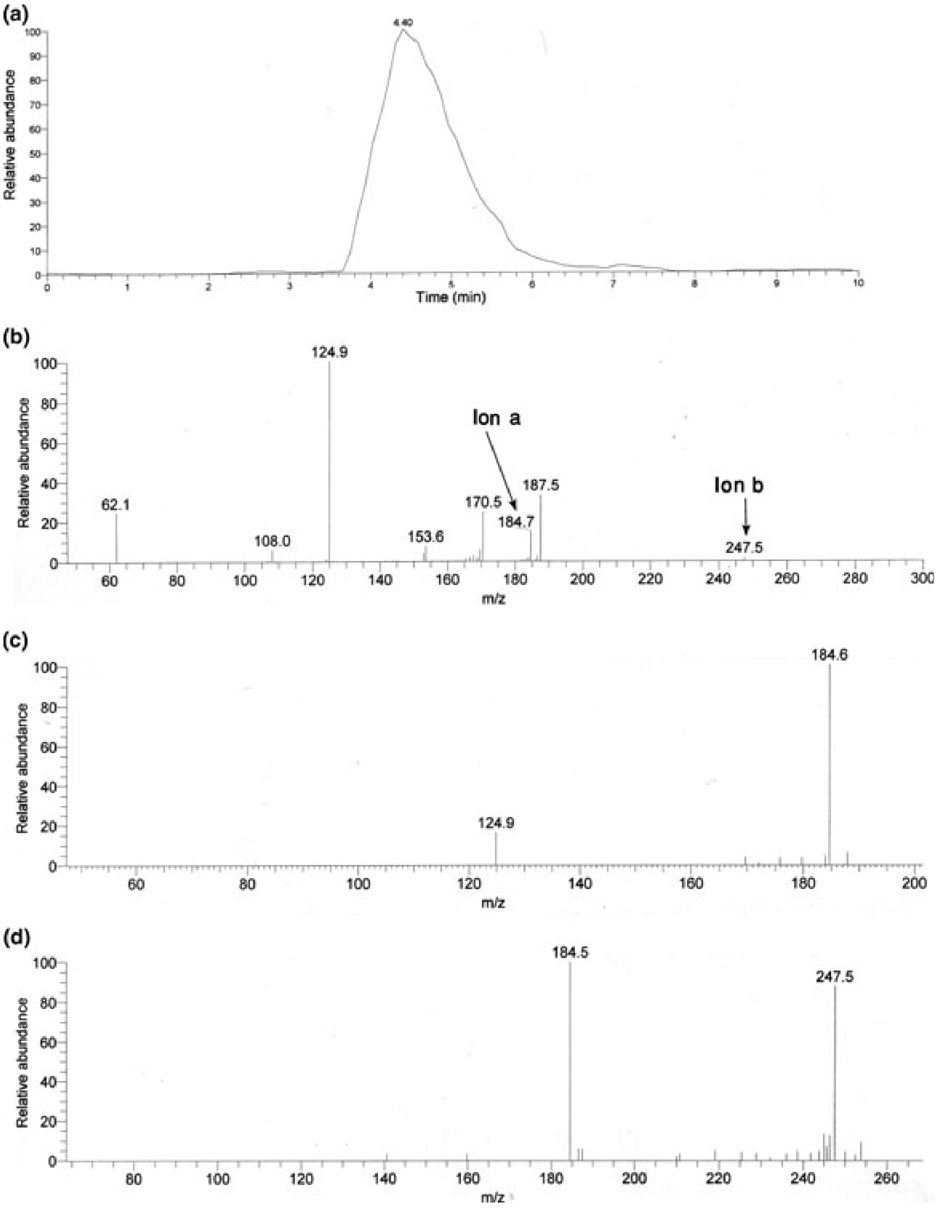


FIG. 3—*LCMS measurements of standard urea nitrate after clean-up on Chromosorb G-HP. (a) Smoothed mass chromatogram of the ion at m/z 184.7, (b) full scan mass spectrum in negative-ion atmospheric pressure chemical ionization (APCI) of the chromatographic peak at 4.4 min, (c) MS/MS-collision induced dissociation (CID) spectrum of the ion at m/z 184.7 at a window width 20 amu, (d) MS/MS-CID spectrum of the ion at m/z 247.5 at a window width 30 amu.*

solid material. In the Griess reaction, the dried extract produced the typical pink–red color, only after reduction with zinc, indicating the presence of nitrate.

TABLE 3—*Detection of adduct ion* ***a*** *by LCMS in mixtures of urea (or uronium salt) with nitrate salts before and after clean-up.*

	Compound	Standards	After Clean-Up
1	Urea/KNO_3	–	–
2	Urea/NH_4NO_3	+	+*
3	Urea/KNO_3/$(NH_4)_2SO_4$	–	–
4	Urea/KNO_3/$KHSO_4$	+*	+*
5	Urea/KNO_3/NaH_2PO_4	–	–
6	Urea/KNO_3/$NaHCO_3$	–	–
7	Uronium sulfate/KNO_3	+*	+*

*Small ion at m/z 184.7.

The dried extract gave a negative response to the UN-1 reagent, suggesting the absence of uronium ion or an amount lower than 10 μg (6). The dried extract gave a negative response to the Nessler reagent indicating either the absence of ammonium cation or its presence at levels below 0.3 μg—the LOD of this test (19).

The IR spectrum of the dried extract (Fig. 4*a*), showed a major absorbance band at 1398 cm^{-1}, indicating nitrate anion, and bands at 1661 and 1619 cm^{-1}, suggesting the presence of urea. The characteristic absorption bands of urea nitrate (1705 and 1678 cm^{-1} and near 1050 cm^{-1}) could not be observed. These results can suggest either the absence of urea nitrate or its presence in the sample in a concentration lower than 30%.

The acetone extract was then chromatographed on Chromosorb G-HP, yielding a light-brown material. HPLC/APCI/MS in the negative mode was performed. The mass-chromatogram of the ion at m/z 184.6 is shown in Fig. 4*b*. The full scan mass spectrum of

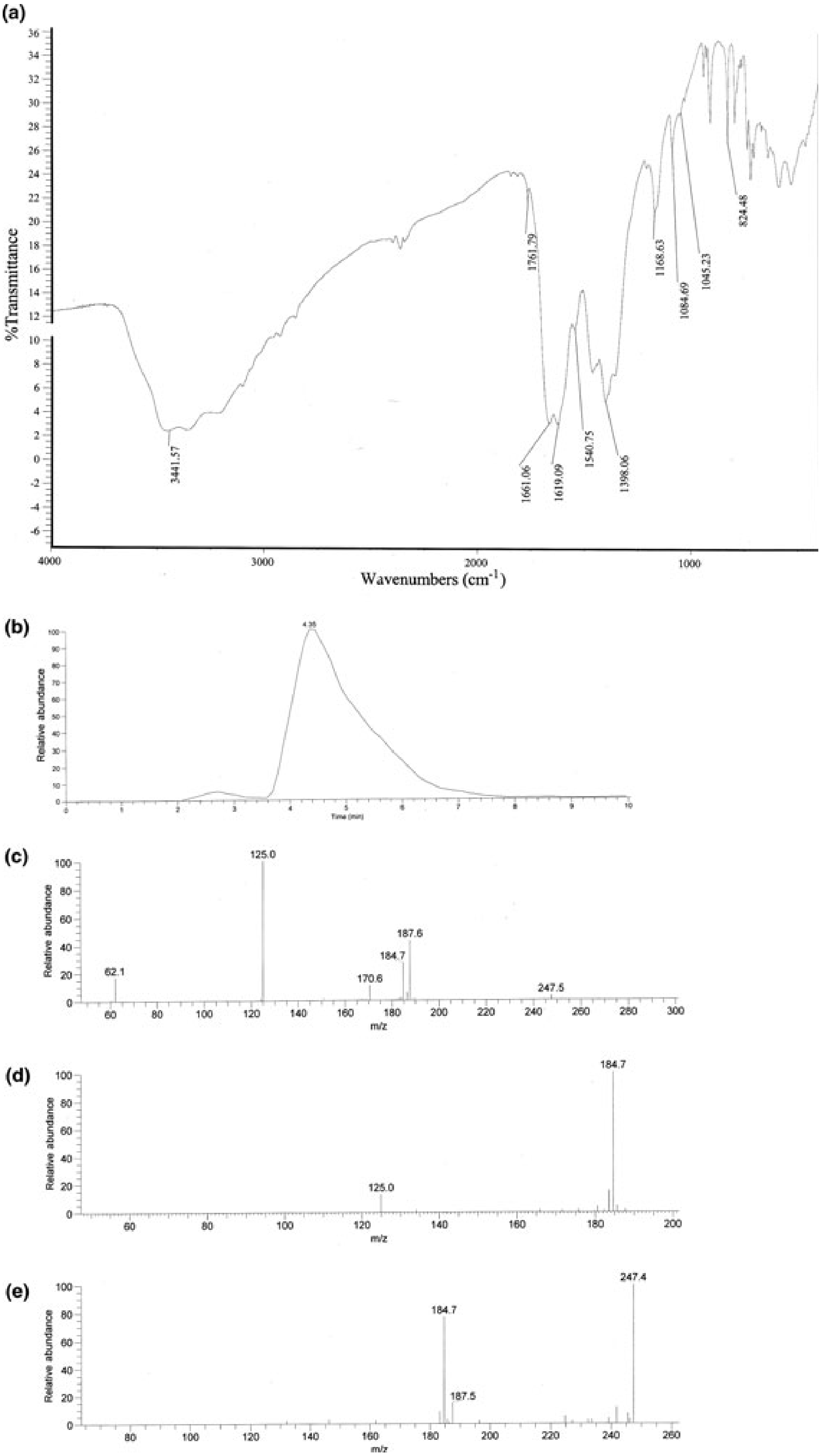


FIG. 4—*Measurements of an acetone extract from a rocket fragment after clean-up on Chromosorb G-HP. (a) Infra red (IR) spectrum of the acetone extract, (b) smoothed mass chromatogram of the ion at m/z 184.7, (c) full scan mass spectrum in negative-ion atmospheric pressure chemical ionization (APCI) of the chromatographic peak at 4.35 min, (d) MS/MS-collision induced dissociation (CID) spectrum of the ion at m/z 184.7 at a window width 20 amu, (e) MS/MS-CID spectrum of the ion at m/z 247.5 at a window width 30 amu.*

TABLE 4—*Recovery of urea nitrate from "natural" matrices.*

Matrix	Amount of Urea Nitrate (mg)	Amount of Dry Residue (mg)	Reaction with UN-1	LCMS Analysis for Urea Nitrate
Dry soil (50 g)	100	2.6	Negative	Not detected
Dry soil (50 g)	50	–		
Dry soil (50 g)	10	–		
Wet soil (50 g) + 10 mL water	100	6.7	Negative	Detected
Wet soil (50 g) + 10 mL water	50	2.5	Negative	Detected
Wet soil (50 g) + 10 mL water	10	0.4	Negative	Detected
Polystyrene chips (5 g)	10	4.9	Positive	Detected
Nails (56 g)	100	40	Positive	Detected

the chromatographic peak at 4.35 is shown in Fig. 4*c*. The MS/MS-CID spectrum of the ion at m/z 184.6 is shown in Fig. 4*d* and of the ion at m/z 247.4 is shown in Fig. 4*e*.

The ions at m/z 184.6 and 247.4 in the full scan mass spectrum, and the fragmentation of the ions at m/z 184.6 and 247.4 in the MS/MS-CID measurements indicate the presence of urea nitrate in the extract.

Conclusions

1. It is difficult to identify urea nitrate in traces because of its instability under various conditions, including contact with carbonates, soils and structural materials, and particularly, hydrolysis during hot aqueous extraction and clean-up.
2. A technique for identifying urea nitrate in traces was demonstrated. It involves extraction with hot acetone, clean-up on Chromosorb G-HP column and analysis by HPLC/APCI/MS. Corroborative techniques are IR spectroscopy and "UN-1" color test.
3. Urea nitrate can be formed also during the analytical process by reaction between urea, nitrate salts and acids or acidic salts. Consequently, the detection of the characteristic adduct ion by mass spectrometry does not necessarily prove the presence of "authentic" urea nitrate in the sample.
4. A study for optimizing the analytical protocol is underway.
5. A further study is required to find ways to distinguish between "authentic" urea nitrate and an artifact formed during the analytical process.

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