analytical chemistry

Article

pubs.acs.org/ac

Sensitive Analysis of Nitroguanidine in Aqueous and Soil Matrices by LC-MS

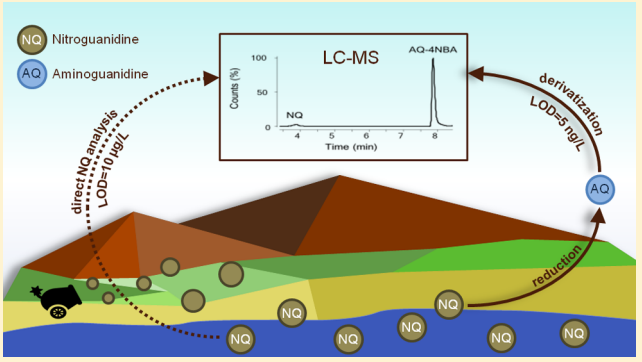
Anna Voloshenko Rossin,[§] Sergey Sladkevich,[§] Guy Gasser,[§] Artem Melman,[†] and Ovadia Lev*[,§]

[§]The Casali Center, The Institute of Chemistry, The Hebrew University of Jerusalem, Edmond J. Safra Campus, Jerusalem 91904, Israel

[†]Department of Chemistry and Biomolecular Science, Clarkson University, 8 Clarkson Ave., Potsdam, New York 13699-5810, United States

Supporting Information

ABSTRACT: Nitroguanidine, a widely used nitramine explosive, is an environmental contaminant that is refractory, persistent, highly mobile in soils and aquifers, and yet under-researched. Nitroguanidine determination in water and soil poses an analytical challenge due its high hydrophilicity, low volatility, charge neutrality over a wide pH range, and low proton affinity which results in low electrospray interface (ESI)-MS sensitivity. A sensitive method for the determination of nitroguanidine in aqueous and soil matrices was developed. The method is based on reduction by zinc in acidic solution, hydrophobization by derivatization, preconcentration on C18 cartridge, and LC-MS quantification. The demonstrated limit of detection (LOD) reaches 5 ng/L and 22 ng/g in water and soil, respectively. Analysis of a contaminated site demonstrates that it is possible to map a contamination plume that extends over 1 km from the source of the contamination.



Nitroguanidine (NQ) is a nitramine explosive, which is often used in combination with 1,3,5-trinitroperhydro-1,3,5-triazine (RDX), nitrocellulose, or nitroglycerin as a double- or triple-based propellant. It was demonstrated that NQ can be used as an ingredient in an insensitive munitions formulation (IMX-101) aimed to replace 2,4,6-trinitrotoluene (TNT) with safer energetic materials that are more stable against detonation stimuli.[1−3] Thus, NQ is an intensively used material that can be found in the areas adjacent to military facilities, where live-fire exercises are held.[4] It is produced in large quantities; the annual production of nitroguanidine was estimated at 8600 metric tons in 2016.[5]

NQ is highly soluble in water (4.4 g/L at 25 °C)[6] and has a low soil−water partition coefficient of 0.15−0.43 L/kg.[7,8] In addition, NQ is not charged between pH −0.5 and 12.2,[9,10] and as such, it is highly mobile in aqueous systems and soils. Although the reduction of NQ can yield nitrosoguanidine, which was shown to be carcinogenic in rats,[11] it is classified by the USEPA as a group D substance, i.e., a compound that is not classifiable as a human carcinogen.[12]

The research on NQ stability in water is rather limited. The reported products of abiotic photocatalytic NQ degradation are guanidine, hydroxyguanidine, cyanoguanidine, urea, ammonia, and nitrosoguanidine.[7,13] The biodegradation of NQ in aerobic conditions results in formation of nitrourea,[14,15] which can further decompose to NH_3, N_2O, and CO_2.[15] The reported degradation time scale varies from several hours to 85 days.[7,14−18] Thus, NQ can persist in the environment, especially in groundwater sources, where the absence of photolysis and anoxic conditions can hinder NQ degradation.

Thus, the large production rate and its high mobility make this compound a potential risk to underground water resources. The low retardation in the ground implies that it can also serve as a potential indicator for propagation of aquifer pollution plumes created by the military industry.

However, NQ cannot be easily extracted from water, and thus, the sensitivity of all of the current analytical techniques is rather limited (Table 1). Most of these methods are based on HPLC-UV/vis analysis, which has low sensitivity with a limit of detection (LOD) of 100 μg/L or higher for water samples, with the exception of the work of Walsh[19] reporting a LOD of 5 μg/L. The specificity of HPLC with UV detection is inferior to mass spectrometric analysis. A preconcentration by rotary evaporation followed by electrochemical analysis was proposed by Manning and Maskarinec,[20] but despite the inherent sensitivity of electrochemical techniques, the LOD for NQ by this technique is as high as 40 μg/L. The most remarkable performance reported to date was published in this journal by Mu et al.[21] Reverse phase UPLC (using methanol−water with 1 mM ammonium acetate) coupled with 4000Q TRAP triple

Received: June 20, 2017
Accepted: July 19, 2017
Published: July 19, 2017

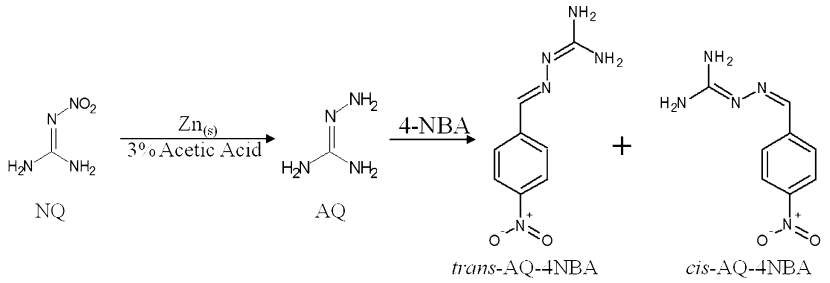
DOI: 10.1021/acs.analchem.7b02364
Anal. Chem. XXXX, XXX, XXX−XXX

Table 1. Reported Methods for Analysis of NQ

method of analysis and detection	column type	LOD water [μg/L]	LOD soil [μg/g]	comments	reference
HPLC-UV	NP: neutral alumina	800		254 nm	22
HPLC-UV	RP: ODS	100		263 nm	23
HPLC-UV	RP: C8	100		254 nm	18
HPLC-EC	RP: C18	40		preconcentration by rotary evaporation	20
HPLC-UV	mixed mode: C18/cation exchange	5	0.5	263 nm	19
HPLC-UV	RP: Hypercarb			286 nm[a]	24
LC-ESI (+)-MS	RP column 1: Synergi hydroRP; RP column 2: Pinnacle II biphenyl	9			25
UFLC-ESI (−)-MS/MS	RP: Knietex C18	0.7	2000		21
HPLC-UV	Synergi 4 μm Polar-RP 80		0.95−1.74	263 nm two-stage soil extraction	26

[a]LOD was not established; the method was used for dissolution study only.

Scheme 1. Schematic Illustration of NQ Reduction Followed by Derivatization with 4-NBA[a]



[a]Below pH 3 only the trans-isomer is obtained.

quadrupole mass spectrometry was used to analyze NQ and other nitro compounds. The authors reported a LOD of 0.7 μg/L for water samples. This, however, may be regarded as too high since, for comparison, the allowable level of any single pesticide in the EU drinking water is 0.1 μg/L. The situation is even more complicated, since NQ is eluted very close to the unretained peak, and thus, analysis of high ionic strength water is expected to result in ion suppression.

In this research, we present a new methodology for determination of NQ based on reduction of NQ to aminoguanidine (AQ) by hydrogenation on zinc under acidic conditions, followed by derivatization by 4-nitrobenzaldehyde (4-NBA) to give a Schiff base 2-[(4-nitrophenyl)methylene]-hydrazinecarboximidamide (AQ-4NBA, Scheme 1). The latter can be preconcentrated on a C18 SPE cartridge prior to LC-MS analysis.

EXPERIMENTAL SECTION

Materials. The following solvents and chemicals were purchased and used without further purification. Methanol (HPLC grade), acetonitrile (HPLC grade), formic acid, acetic acid glacial, ethanol absolute (dehydrated) ULC/MS, and sodium hydroxide were supplied by Bio-Lab LTD (Israel). 4-Nitrobenzaldehyde 99% (4-NBA) was obtained from Alfa Aesar; zinc powder was supplied by Fisher Scientific, and aminoguanidine hydrochloride (≥98%), acridine (≥97%), nitroguanidine, and semicarbazide hydrochloride (SEM) (≥99%) were obtained from Sigma-Aldrich (Israel).

^{15}N-labeled nitroguanidine was synthesized in our laboratory according to ref 27. The identity and purity (>99.5%) of the product was confirmed using high resolution mass spectrometry. The synthesis of AQ-4NBA was carried out according to ref 28. The identity of the product was confirmed using high resolution mass spectrometry and ^{1}H NMR spectroscopy. Stock solutions (1 mg/mL) of NQ and ^{15}N-labeled NQ were prepared by dissolving the compounds in ethanol/water 80:20 (v/v). Stock standard solution of AQ (1 mg/mL) was prepared by dissolving the compound in HPLC-grade methanol. Working solutions of the standards were prepared by dilution of the respective stock solutions with water. All solutions were freshly prepared or kept refrigerated for less than 1 week prior to the analysis.

Solid-phase extraction (SPE) cartridges (500 mg, 6 mL) Strata C18-E (55 μm, 70A) were purchased from Phenomenex. The nitrogen used for drying the solid-phase cartridges and for evaporation of solvents was of 99.995% purity from Maxima, Israel. Ultrapure water was obtained by a Millipore laboratory water purification system.

Instrumentation. LC-MS-MS quantifications were performed using an Agilent 1200 high performance liquid chromatograph (HPLC) coupled via an electrospray interface (ESI) to an Agilent 6410A triple quadrupole MS (QQQ-MS). Agilent 1100 HPLC coupled via an ESI to an Agilent 6520A QTOF MS was used for conclusive identifications.

^{1}H NMR spectra of samples in D_2O were collected on a Bruker Avance-400 (9.4 T) spectrometer. The measurements were performed using a single pulse sequence with rf pulse duration of 10 μs and recycling time of 9−60 s.

Single crystal X-ray analysis for the synthesized AQ-4NBA was performed on a Bruker SMART APEX CCD diffractometer using graphite monochromatized Mo Kα radiation (λ = 0.71073 Å). The structure was solved by direct methods and

DOI: 10.1021/acs.analchem.7b02364
Anal. Chem. XXXX, XXX, XXX−XXX

Table 2. LC-MS/MS Parameters

compound	retention time (min)	precursor ion	product ions (m/z) quantifier	product ions (m/z) qualifier	collision energy (eV)	fragmentation voltage (V)
AQ-4NBA	7.8	208.3	191.3	162.3	15 20	110
				149.3	15	
^{15}N-labeled AQ-4NBA	7.8	212.3	194.3	150.2	15 15	120
acridine	7.4	180	178	151	40 55	130
SEM	8.7	209.2	192	149	10 15	120
				103	20	

refined by full matrix least-squares on F^2. Details of single crystal X-ray studies are given in Table S1.

Sampling and Storage. Water samples were collected in amber glass bottles from several water wells in the vicinity of an abandoned industrial site in Israel. The water samples were transported and kept under refrigeration (2−4 °C). Extraction was conducted within 120 h of sampling. Extracts were stored for less than 1 week in amber glass vials in a refrigerator.

Soil samples were collected at different depths of a secondary effluent recharge of Soil Aquifer Treatment (SAT) plant managing domestic wastewater in the Tel-Aviv region (Israel). Different depths of the SAT are characterized by different lithology and water and organics content. In addition to the analysis of soils prior to NQ enrichment, we have carried out analysis of the effluent collected from the relevant wastewater treatment facility to show that the water treatment system and the soil do not contain any NQ or AQ.

Analytical Procedure of NQ Analysis in Water Samples. *Reduction of NQ.* NQ was reduced to AQ by zinc powder in acidic solution. Glacial acetic acid (0.3 mL), zinc powder (200 mg), and ^{15}N-labeled-NQ internal standard (10 μL of the 1 mg/L) were added to 10 mL of water sample. The mixture was stirred for 20 min at room temperature and then filtered through a 0.45 μm PVDF syringe filter (Millex-HV, Merck-Millipore).

Derivatization of AQ. The AQ solution obtained in the previous step was reacted with 0.2 mL of 4-NBA (10 mg/mL) to form AQ-4NBA. SEM was added as a surrogate standard for the derivatization process. The mixture was shaken for 3 h, and then, the derivate was extracted by SPE using a C18 cartridge. The cartridge was preconditioned with 6 mL of methanol and 6 mL of water consecutively. The sample was loaded into the cartridge and left to percolate by gravity followed by the cartridge drying step under vacuum for 2 min. Analyte (AQ-4NBA) was subsequently eluted with 6 mL of methanol, and the obtained extract was concentrated to a final volume of 0.5 mL by gentle nitrogen flow at 55 °C. The final sample was filtered through a 0.22 μm PVDF syringe filter (Millex-GV, Merck-Millipore). Five μL of a 5 mg/L acridine solution was added as an instrument internal standard.

LC analyses were performed on an Agilent ZORBAX Eclipse Plus C18 (2.1 mm ID, 100 mm length, and 3.5 μm particle size). Column temperature was set at 25 °C. The mobile phase consisted of 10% acetonitrile, 90% H_2O, and 0.1% formic acid. The eluent composition was as follows: initial conditions, 10% aetonitrile fed at 0.2 mL/min for 1 min. At t = 10 min, the flow rate was increased to 0.3 mL/min, and the composition was ramped to 100% acetonitrile, maintaining a flow rate of 0.3 mL/min until t = 25 min. Finally, the composition of the eluent was changed gradually to initial conditions at t = 17 min and remained until t = 25 min for stabilization of the column before the next injection. 1.5 min was set for post run at initial conditions. Injection volume was 15 μL, unless otherwise specified. The MS parameters are presented in Table 2. Quantification was carried out with isotopically labeled internal standard and by multipoint calibrations with good linear correlation (R^2 = 0.9999 in the range of 1−500 μg/L).

Extraction of NQ from Soil. The analysis of NQ in soils was carried out on the basis of the soil extraction protocol reported in ref 19, using the calibration curve with ^{15}N-labeled NQ as internal standard. Briefly, 5 g of the examined soil was weighed into a 50 mL centrifuge tube, and 2 mL of deionized water was then added. The sample was shaken in the dark at room temperature. After 20 h, the sample was transferred to a round-bottom flask for lyophilization. After the sample was completely dried, 10 mL of deionized water was added to each sample and the mixture was transferred to an ultrasonic water bath (GT Sonic, 40 kHz) maintained at less than 25 °C for 2 h. The extracted sample was then centrifuged and filtered through a 0.45 μm PVDF membrane filter. NQ content in obtained water extracts was estimated according to the analytical procedure of NQ analysis in water samples described above. Soils were analyzed in triplicates.

RESULTS AND DISCUSSION

Preliminary Studies. Preliminary studies were carried out to examine the possibility of direct ESI-MS analysis of NQ. The obtained LOD of NQ was higher than 5 μg/L. The attempts to extract NQ from water in a wide pH range using various commercial SPE cartridges did not provide reasonable recovery (additional details are specified in the Supporting Information).

Then, an alternative approach involving functionalization of the NQ in the aqueous solution was pursued to attain better affinity to SPE cartridges (further details on the unsuccessful protocols can be found in the Supporting Information). We decided to reduce NQ to AQ which has higher MS visibility and is more amenable for derivatization. Different reduction approaches were tested (further details on the unsuccessful reduction protocols can be found in the Supporting Information). By far, the most successful approach involved the reduction of NQ by Zn in acidic solution.[29] Parametric dependence of this method is presented below. Preconcentration of the protonated AQ on a cation exchange SPE cartridge failed due to the presence of Zn^{2+}, and a protocol involving derivatization by a Schiff base reagent and SPE preconcentra-

DOI: 10.1021/acs.analchem.7b02364
Anal. Chem. XXXX, XXX, XXX−XXX

tion for sensitive determination of the AQ product was developed.

Method Optimization. The optimized method of analysis is detailed in the Experimental Section, and only pertinent parametric dependencies are discussed here.

Reduction Step. NQ reduction was carried out by addition of 20 mg/mL zinc powder and 3% (v/v) acetic acid. Synthesis of amino compounds by reduction of the respective nitro compounds on zinc under acidic conditions was discussed by Gowda et al.[30] who showed that reduction time (using 90% acetic acid and 78.5 mg/mL zinc) of 20 different nitro-compounds ranged between 2 and 20 min, though NQ reduction was not studied. The dependence of the observed relative LC-MS signal of AQ-4NBA on reaction time is depicted in Figure 1. A monotonous increase of the yield with

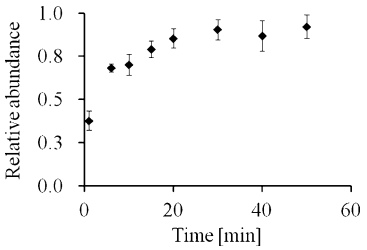


Figure 1. Dependence of the MS response level of AQ-4NBA on the reduction time of NQ by zinc (3.5 μg/L NQ was used). Bars delineate standard deviation of triplicates.

time of reaction was observed, and 20 min was selected as an optimal reaction time for the reduction process. Note that the initial reduction step is rather rapid and almost 50% of the reduction is completed within less than 1 min, which may be used when rapid analysis is desirable.

Zinc Cleanup. Comparative tests were made in order to determine whether the production of Zn(II) during the reduction step interferes with the analysis of NQ. The zinc removal process (by increasing pH with sodium hydroxide resulting in precipitation of zinc hydroxide[31]) did not change the LOD or the recovery, but two chromatographic peaks (at retention time t_r = 5.7 and 7.8 min), instead of a single one at 7.8 min, were obtained. These two peaks with identical m/z ($[M + H]^+$ = 208.0829) and MS/MS fragmentation were assigned to cis- and trans-isomers of AQ-4NBA product. In order to clarify the nature of these isomers, AQ-4NBA was synthesized separately and LC-MS analysis of the product as a function of pH was performed. At pH 3, we observed a peak (t_r = 7.8 min), constituting 100% of the material, and at pH higher than 5, the two peaks were observed at the ratio of ca. 2:3 (Figure 2).

NMR studies were carried out in order to glean insight into the identity of the two chromatographic peaks. However, ^{1}H NMR of the synthesized AQ-4NBA in D_2O revealed only a spectrum of the trans-isomer (Figure S1). To synthesize cis-AQ-4NBA, we used UV irradiation (300 W Xe lamp, Oriel) of a solution of the synthesized AQ-4NBA in D_2O. The ^{1}H NMR spectrum of irradiated sample revealed a mixture of trans- (major) and cis- (minor) isomers of AQ-4NBA in 1.0:0.43 ratio. The LC-MS of the solution soon after the irradiation revealed two chromatographic peaks both having the same mass and retention times that were observed in the pH study (vide supra, Figure 2).

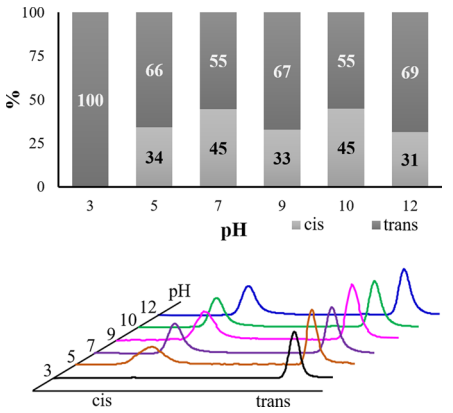


Figure 2. Dependence of the relative response of the trans- (dark shade) and cis-isomers. Bottom frame depicts chromatograms of the two isomers of AQ-4NBA at different pH values. The cis-isomer is the first eluting peak.

The observed ratio between the two chromatographic peaks was similar to the ratio between the trans- and cis-isomers of AQ-4NBA in the ^{1}H NMR study in D_2O. The ^{1}H NMR of the trans-isomer showed two apparent doublets at 7.89 and 8.27 ppm and a singlet at 8.12 ppm, which were attributed to the hydrogen atoms on C5, C3, C2, C6, and C7, respectively (Figure S2). ^{1}H NMR after the irradiation showed two additional apparent doublets at 7.77 and 8.36 ppm and a singlet at 7.82 ppm which were attributed to the hydrogens on C5, C3, C2, C6, and C7 atoms, respectively (Figure S2). On the basis of these observations, the first chromatographic peak was attributed to the cis-isomer and the second peak to the trans-isomer (Figure S3, Scheme 1). Single crystal X-ray analysis was carried out in order to get additional information about isomer structure of the synthesized AQ-4NBA crystals. Figure S4 represents the structure of the molecule (as AQ-4NBA acetate), confirming the formation of trans-isomer in the reaction between AQ and 4-NBA. The presence of dissolved zinc at pH 3 did not change the method performance, and therefore, we could analyze the AQ-4NBA at pH 3 without zinc removal.

Derivatization. The derivatization process of AQ was based on the method described by Beaven et al.[32] Schiff base reactions are frequently used for coupling of amines and aldehydes or ketones in aqueous solutions. We screened different derivatization reagents including 3,4-dihydroxybenzaldehyde, heptaldehyde, 3-pyridinecarboxaldehyde, methyl ethyl ketone, acetone, cyclohexanecarboxaldehyde, and 4-nitrobenzaldehyde. The relative response levels of the different products by direct analysis by LC-QTOF-MS are depicted in Figure 3. The highest responses were obtained after derivatization with either acetone or 4-NBA. We preferred the latter to endow hydrophobicity, since SPE of the 1-(propan-2-ylideneamino)guanidine (acetone derivatization product) had lower recovery compared to the 4-NBA derivatization product.

We optimized the reaction time for derivatization of AQ. For this purpose, we analyzed 3.5 μg/L NQ according to the full procedure, including reduction by zinc and derivatization with 200 mg/L 4-NBA, varying only the reaction time. Figure 4 shows product formation as a function of time. The tests were conducted in triplicates, and their average and standard deviations are presented. Optimal derivatization time under these conditions was 3 h; further increase of the reaction time

DOI: 10.1021/acs.analchem.7b02364
Anal. Chem. XXXX, XXX, XXX−XXX

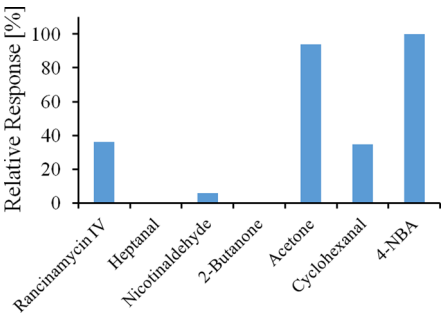


Figure 3. Relative response of AQ after derivatization with different Schiff base reagents.

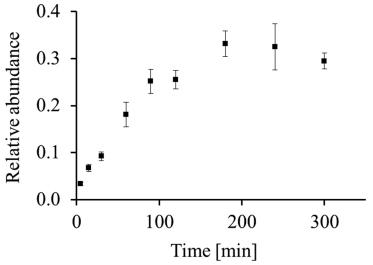


Figure 4. MS signal dependence on derivatization time (for 1.7 μg/L NQ standard). Bars indicate standard deviation of triplicates.

did not improve the obtained signal. However, it was possible to decrease the derivatization time to 30 min by carrying out the derivatization reaction at 70 °C (relative response was 99 ± 7% of the response obtained after 3 h of derivatization at room temperature). This protocol is, however, more complicated and requires an additional heating bath, and therefore, the optimized protocol was based on 3 h of derivatization at room temperature.

Increasing 4-NBA dose can reduce the derivatization time, but the unreacted 4-NBA interferes with the extraction and MS analysis. Therefore, 200 mg/L 4-NBA was chosen as a compromise between lower reaction time and lower analytical interferences of the unreacted reagent.

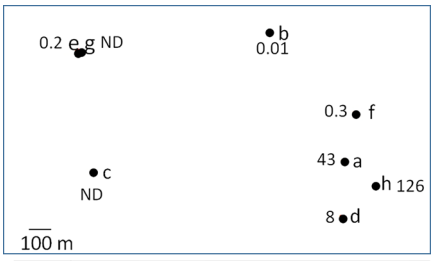
Method Validation. The performance of the proposed method was established by employing assays with sample blanks and spiked samples. Linearity, matrix effects, selectivity, trueness, precision, and detection limit were studied. Quantifications were carried out by multipoint calibration with ^{15}N-labeled NQ as internal standard. An 8-points calibration curve (Figure S5) was prepared in the range of 1−500 μg/L by addition of NQ standards to deionized water and carrying out the full process, including reduction, derivatization, and extraction in triplicates. A good linear correlation was observed with correlation coefficient R^2 = 0.9999. The LOD of the analytical method, calculated at 3 times the noise level, was 10 ng/L for a 15 μL injection or 5 ng/L for a 30 μL injection. Since the injected sample contained over 60% water, the peak maintained its nearly Gaussian shape even for a 30 μL injection. Peak symmetry, computed as the ratio between the front half-width and the back half-width,[33] was 1.6 (the respective calibration curve and the chromatographic peaks shape for a 30 μL injection are depicted in Figure S5). These limits of detection are 70 and 140 times lower than previously reported for 15 and 30 μL per injection, respectively (Table 1).

Recovery Tests. An analysis of the recovery loss at each of the different analytical steps was carried out. The recovery loss at the reduction step was evaluated by 3-points standard addition of AQ to the vial after reduction of NQ (2.5 μg/L) by the optimized zinc reduction protocol. Calculated recovery in triplicate was 97 ± 12%.

The recovery after the derivatization step was evaluated by a 5-point calibration curve for home synthesized AQ-4NBA standard in the range of 25−150 μg/L prepared using blank water, which was subjected beforehand to the full reduction−derivatization process (in order to account for possible matrix effects). To calculate derivatization recovery, 1.8 μg/L AQ standard was passed through an optimized derivatization protocol (with 4-NBA) and analyzed. Calculated recovery in triplicate was 83 ± 7%.

The recovery of the SPE extraction step was evaluated by comparison of the response of 5 μg/L AQ-4NBA that was spiked in distilled water that was previously subjected to the zinc reduction and derivatization protocol. Then, SPE extraction was conducted, and the response was compared to a calibration curve describing the direct response of AQ-4NBA standard in 60:40 methanol/water solution at pH 3. The calibration curve was measured in the range of 25−150 μg/L. Obtained recovery was 91 ± 11%. The overall recovery of the whole process starting with NQ spiked distilled water was 73 ± 14%.

Analysis of a Contaminated Site. In order to examine the applicability of the new method for real water samples, we have analyzed water wells in the vicinity of an industrial site that was contaminated by NQ. Eight water wells at various distances and directions from the focal point of contamination were examined (Figure 5). The analysis was carried out by three different modes: (i) direct injection, i.e., analysis of NQ by direct injection of the sample to HPLC-QQQ-MS without reduction and derivatization and quantification by the calibration curve; (ii) optimized reduction−derivatization, i.e., quanitification of NQ by the method reported here, using the calibration curve



point	NQ	CLO4	TCMA	TCE	1,2-DCE (cis)
g	ND	6	ND	ND	ND
c	ND	2400	ND	ND	ND
b	0.01	2430	ND	ND	ND
f	0.3	8620	14.3	3.1	117.2
e	0.2	2740	ND	ND	ND
d	8	9940	ND	ND	9.0
h	126	26000	3.7	3.7	23.9
a	43	4160	4.6	3.2	30.0

*The concentrations in the table in µg/L; ND – not detected

Figure 5. Scheme of the relative location of the studied water wells (designated by letters a−h) in the vicinity of the examined site. Numbers in the scheme report NQ concentration in the corresponding wells (in μg/L). The concentrations of NQ and other contaminants are delineated in the table.

DOI: 10.1021/acs.analchem.7b02364
Anal. Chem. XXXX, XXX, XXX−XXX

and ^{15}N-labeled NQ as internal standard; (iii) standard addition; i.e., quantification of NQ for the low concentration range was also carried out by reduction−derivatization but with 4 point standard addition calibration. Table 3 summarizes the

Table 3. Comparison of NQ Analyses of Water Samples from the Studied Wells by Direct Injection (Without Reduction and Derivatization, Method (i)), by Optimized Reduction−Derivatization (Method (ii)), and by Standard Addition (Method (iii))[a]

point name	NQ concentration [μg/L]		
	(i) direct water sample injection	(ii) calibration curve	(iii) standard addition
a	43.6 ± 3.0	42.7 ± 2.7	NA
b	ND	0.010 ± 0.004	0.010 ± 0.002
c	ND	ND	ND
d	3.7 ± 0.4[b]	8.1 ± 0.4	8.5 ± 0.8
e	ND	0.15 ± 0.05	0.20 ± 0.07
f	ND	0.31 ± 0.02	0.33 ± 0.04
g	ND	ND	ND
h	81.8 ± 6.5	126.2 ± 3.5	NA

[a]The point names (a−h) designate the sampling well location depicted in Figure 5. ND: not detected; NA: not analyzed. [b]The averaged value is below the LOD.

results of the different analyses. NQ was found only in 3 water wells by direct injection (method (i), above) and in 6 out of the 8 water wells that were analyzed by method (ii). Good fit was observed between method (ii) and method (iii) (Figure S6). The slope of the fit between the results of the two methods was 1.05 (instead of a perfect 1.0) with R^2 = 0.99997. Even for the point with the lowest detectable NQ level (10 ng/L, at well b), the fit between the two methods was good (both methods gave 10 ng/L).

Analysis of the 6 different field samples (that tested positive for NQ) without the reduction step but after derivatization with 4-NBA did not show any false positive, which could theoretically occur due to the presence of AQ in the water samples. It should be noted that, although AQ was tested as a pharmaceutical compound,[34] it did not pass phase III FDA analysis and has never been in use. Our literature survey failed to show any report on the presence of AQ in the environment.

To complete the description of the contamination in the field test site, Figure 5 (and the table inset) depicts the observed concentrations of perchlorate (CLO4), chloroform (TCMA), tetrachloroethylene (TCE), and cis-1,2-dichloroethylene (1,2-DCE) at the same points. The NQ plume was much larger than the plumes of all other organic contaminants, though smaller than the perchlorate plume. This illuminates the risks entailed by NQ due to its high mobility compared to other organic contaminants, as well as its possible application as an indicator for pollution plumes around polluting military industry sites.

Analysis of NQ in Soils. Recovery and LOD of NQ were examined using 4 different soils (sandy, clayey, and sandy clayey) with different organic matter content (0.05−0.36%). The samples were spiked at a level of 5 ng/g and analyzed according to the soil extraction protocol detailed in the Experimental Section, followed by NQ analysis of the water extract by the optimized NQ protocol. Recovery was evaluated by a comparison with the response factor of the calibration curve for NQ in water (Figure S3). LOD for each soil type was calculated at 3 times the noise level obtained by analysis of the

Table 4. LOD and Spike Recovery of NQ in Different Soil Matrices

	soil A	soil B	soil C	soil D
type	sand	clay	sandy−clay	sand
	93% sand, 7% silt	19% sand, 14% silt, 67% clay	60% sand, 10% silt, 30% clay	89% sand, 7% silt, 4% clay
depth	3 m	9 m	16.5 m	18 m
organic matter	0.05%	0.36%	0.2%	0.12%
average ± SD [ng/g]	4.9 ± 1.0	5.3 ± 0.8	4.9 ± 0.7	4.9 ± 0.2
recovery % (n = 3)	97 ± 20	105 ± 16	99 ± 14	98 ± 5
LOD [pg/g]	22	21	20	22

various soil blanks. Table 4 depicts the test results for the different samples. The recovery was in the range of 97.3−105.2% with RSD of triplicates in the range of 5−20%. The LOD of the method was in the range of 20−22 pg/g.

CONCLUSIONS

A highly sensitive method for the determination of NQ, a widely used nitramine explosive, was developed on the basis of reduction, hydrophobization, and SPE preconcentration followed by LC-MS analysis. The method exhibits high recovery and low limit of detection and is applicable for the analysis of water samples as well as soils.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.7b02364.

Preliminary study: detailed unsuccessful protocols for (i) preconcentration, (ii) aqueous derivatization, and (iii) reduction of NQ; ^{1}H NMR spectra of AQ-4NBA before and after irradiation under UV; structures of cis- and trans-isomers of AQ-4NBA; single crystal X-ray structure of trans-AQ-4NBA; crystal data, data collection, and refinement parameters for trans-AQ-4NBA acetate; calibration curve for NQ; comparison of NQ quantification by standard addition method and by calibration using ^{15}N-labeled NQ as internal standard (PDF)

AUTHOR INFORMATION

Corresponding Author

*E-mail: ovadia@mail.huji.ac.il.

ORCID

Sergey Sladkevich: 0000-0002-2486-0078

Ovadia Lev: 0000-0002-3536-2277

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors thank the Scientific Infrastructure Program of the Ministry of Science, Technology and Space and the Ministry of agriculture and rural development for financing this research. The authors thank the Harvey M. Krueger Family Center for Nanoscience and nanotechnology.

DOI: 10.1021/acs.analchem.7b02364
Anal. Chem. XXXX, XXX, XXX−XXX

REFERENCES

(1) Lee, K. E.; Balas-Hummers, W. A.; Di Stasio, A. R.; Patel, C. H.; Samuels, P. J.; Roos, B. D.; Fung, V. Qualification Testing of the Insensitive TNT Replacement Explosive IMX-101. In *Insensitive Munitions and Energetic Materials Technology Symposium*, Munich, Germany, October 11−13, 2010; pp 1−13.

(2) Oxley, J. C.; Smith, J. L.; Donnelly, M. A.; Colizza, K.; Rayome, S. *Propellants, Explos., Pyrotech.* **2016**, *41*, 98−113.

(3) Picatinny Public Affairs. *Army approves safer explosive to replace TNT*; 2010; https://www.army.mil/article/43553/army- (accessed July 10, 2017).

(4) Walsh, M. R.; Walsh, M. E.; Ampleman, G.; Thiboutot, S.; Brochu, S.; Jenkins, T. F. *Propellants, Explos., Pyrotech.* **2012**, *37*, 393−406.

(5) *Nitroguanidine (CAS 556-88-7) Market Research Report 2016*; http://www.businesswire.com/news/home/20161109005957/en/Global-Nitroguanidine-CAS-556-88-7-Market-Research-Report (accessed July 10, 2017).

(6) Yalkowsky, S. H.; He, Y.; Jain, P. *Handbook of Aqueous Solubility Data*, 2nd ed.; CRC Press: Boca Raton, FL, 2010.

(7) Haag, W. R.; Spanggord, R.; Mill, T.; Podoll, R. T.; Chou, T. W.; Tse, D. S.; Harper, J. C. *Environ. Toxicol. Chem.* **1990**, *9*, 1359−1367.

(8) Pennington, J. C.; Jenkins, T. F.; Ampelman, G.; Thiboutot, S.; Brannon, J.; Clausen, J.; Hewitt, A. D.; Brochu, S.; Dubé, P.; Lewis, J.; Ranney, T.; Faucher, D.; Gagnon, A.; Stark, J. A.; Brousseau, P.; Price, C. B.; Lambert, D.; Marois, A.; Bouchard, M.; Walsh, M. E.; Yost, S. L.; Perron, N. M.; Martel, R.; Jean, S.; Taylor, S.; Hayes, C. A; Ballard, J.-M.; Walsh, M. R.; Mirecki, J. E; Downe, S.; Collins, N. H.; Porter, B.; Karn, R. *Distribution and Fate of Energetics on DoD Test and Training Ranges: Interim Report*; ERDC TR-04-4; U. S. Army Corps of Engineers: Washington, DC, 2004.

(9) DeVries, J. E.; Gantz, E. S. C. *J. Am. Chem. Soc.* **1954**, *76*, 1008−1010.

(10) Bonner, T. G.; Lockhart, J. C. *J. Chem. Soc.* **1958**, 3858−3861.

(11) Bower, M. E.; Hartley, W. R.; Roberts, W. C. In *Drinking Water Health Advisory: Munitions*; Roberts, W. C.; Hartley, W. R., Eds.; CRC Press, Lewis Publishers: Boca Raton, FL, 1992; pp 201−246.

(12) U. S. Environmental Protection Agency. *Nitroguanidine (CAS 556-88-7). Integrated Risk Information System (IRIS). Chemical Assessment Summary*. https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0402_summary.pdf (accessed July 10, 2017).

(13) Dickinson Burrows, W.; Schmidt, M. O.; Chyrek, R. H.; Noss, C. I. *Photochemistry of Aqueous Nitroguanidine*; Technical Report 8808; U. S. Army, Toxic and Hazardous Materials Agency: Aberdeen, MD, 1988.

(14) Richard, T.; Weidhaas, J. *J. Hazard. Mater.* **2014**, *280*, 372−379.

(15) Perreault, N. N.; Halasz, A.; Manno, D.; Thiboutot, S.; Ampleman, G.; Hawari, J. *Environ. Sci. Technol.* **2012**, *46*, 6035−6040.

(16) Mirecki, J. E.; Porter, B.; Weiss, C. A., Jr. *Environmental Transport and Fate Process Descriptors for Propellant Compounds*; ERDC/EL TR-06-7; U. S. Army Corps of Engineers: Washington, DC, 2006.

(17) Mulherin, N. D.; Jenkins, T. F.; Walsh, M. E. *Stability of Nitroguanidine in Moist, Unsaturated Soils*; ERDC/CRREL TR-05-2; U. S. Army Corps of Engineers: Washington, DC, 2005.

(18) Kaplan, D. L.; Kaplan, A. M. *Degradation of Nitroguanidine in Soils*; Technical Report TR-85/047; U. S. Army, Natick Research and Development Command: Natick, MA, 1985.

(19) Walsh, M. E. *Analytical Methods for Determining Nitroguanidine in Soil and Water*; Special Report 89-35; U. S. Army Corps of Engineers: Washington, DC, 1989.

(20) Manning, D. L.; Maskarinec, M. *Analysis of Nitroguanidine in Aqueous Solutions by HPLC with Electrochemical Detection and Voltammetry*; ORNL/TM-10295; U. S. Army Toxic and Hazardous Materials Agency: Aberdeen Proving Ground, MD, 1987.

(21) Mu, R.; Shi, H.; Yuan, Y.; Karnjanapiboonwong, A.; Burken, J. G.; Ma, Y. *Anal. Chem.* **2012**, *84*, 3427−3432.

(22) Bissett, F. H.; Levasseur, L. A. *Analytical Methods for Nitroguanidine and Characterization of its Degradation Products*; Technical Report TR-76/47; U. S. Army, Natick Research and Development Command: Natick, MA, 1976.

(23) Kenyon, K. F. *A Data Base Assessment of Environmental Fate Aspects of Nitroguanidine*; U. S. Army Medical Bioengineering Research and Development Laboratory: Frederick, MD, 1982.

(24) Braida, W.; Wazne, M.; Pavlov, J.; Kalomoiri, A.; Koutsospyros, A.; Balok, V. In *Proceedings of Protection and Restoration of the Environment XI*, Thessaloniki, Greece, July 3−6, 2012; pp 1634−1642.

(25) Russell, A. L.; Seiter, J. M.; Coleman, J. G.; Winstead, B.; Bednar, A. J. *Talanta* **2014**, *128*, 524−530.

(26) Felt, D.; Gurtowski, L.; Nestler, C. C.; Johnson, J.; Larson, S. *Chemosphere* **2016**, *165*, 18−26.

(27) Bulusu, S.; Dudley, R. L.; Autera, J. R. *Magn. Reson. Chem.* **1987**, *25*, 234−238.

(28) Ring, J. R.; Zheng, F.; Haubner, A. J.; Littleton, J. M.; Crooks, P. A. *Bioorg. Med. Chem.* **2013**, *21*, 1764−1774.

(29) Shriner, R. L.; Neumann, F. W. *Org. Synth.* **1946**, *26*, 7−10.

(30) Gowda, D. C.; Mahesh, B.; Gowda, S. *Indian J. Chem., Sect. B* **2001**, *40B*, 75−77.

(31) Albrecht, T. W. J.; Addai-Mensah, J.; Fornasiero, D. In *Chemeca 2011: Engineering a Better World*, Sydney, Australia, September 18−21, 2011, pp 2100−2110.

(32) Beaven, M. A.; Gordon, J. W.; Jacobsen, S.; Severs, W. B. *J. Pharmacol. Exp. Ther.* **1969**, *165*, 14−22.

(33) Dolan, J. W. *LC GC Eur.* **2003**, *16*, 610−613.

(34) Bolton, W. K.; Abdel-Rahman, E. *Expert Opin. Invest. Drugs* **2002**, *11*, 565−574.

DOI: 10.1021/acs.analchem.7b02364
Anal. Chem. XXXX, XXX, XXX−XXX