Title: SUSPECT SCREENING AND QUANTIFICATION OF TRACE ORGANIC EXPLOSIVES IN WASTEWATER USING SOLID PHASE EXTRACTION AND LIQUID CHROMATOGRAPHY-HIGH RESOLUTION ACCURATE MASS SPECTROMETRY

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SUSPECT SCREENING AND QUANTIFICATION OF TRACE ORGANIC EXPLOSIVES IN WASTEWATER USING SOLID PHASE EXTRACTION AND LIQUID CHROMATOGRAPHY-HIGH RESOLUTION ACCURATE MASS SPECTROMETRY

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Graphical Abstract

Highlights

- 34 sorbents tested for SPE of multiple classes of trace explosives
- Recoveries between 56-124 % achieved using mixed polarity polymeric sorbents
- Determination of 29 explosives possible at the ng-µg L^{-1} level in wastewater matrix
- 2,4-dinitrotoluene detected in London composite wastewater samples (≤303 ng L^{-1})
- Both suspect screening and quantitative analysis possible with LC-HRMS

Abstract

The first comprehensive assessment of 34 solid phase extraction sorbents is presented for organic explosive residues in wastewater prior to analysis with liquid chromatography-high resolution accurate mass spectrometry (LC-HRMS). A total of 18 explosives were selected including nitramines, nitrate esters, nitroaromatics and organic peroxides. Three polymeric divinylbenzene-based sorbents were found to be most suitable and one co-polymerised with n-vinyl pyrrolidone offered satisfactory recoveries for 14 compounds in fortified wastewater (77-124 %). Limits of detection in matrix ranged from 0.026-23 µg L^{-1} with $R^2 \geq 0.98$ for most compounds. The method was applied to eight 24-hour composite wastewater samples from a London wastewater works and one compound, 2,4-dinitrotoluene, was determined over five days between 332-468 g day^{-1} (225-303 ng L^{-1}). To further exploit the suspect screening capability, 17 additional explosives, precursors and transformation products were screened in spiked wastewater samples. Of these, 14 were detected with recoveries from 62-92 %, highlighting the broad applicability of the method. To our knowledge, this represents the first screen of explosives-related compounds in wastewater from a major European city. This method also allows post-analysis detection of new or emerging compounds using full-scan HRMS datasets to potentially identify and locate illegal manufacture of explosives via wastewater analysis.

Keywords: Sample preparation • Micro-pollutants • Sewage epidemiology • Energetic materials • 2,4-dinitrotoluene

1. Introduction

In recent years, sewage epidemiology has been successfully applied to estimations of community pharmaceuticals and illicit drugs usage across several cities [1]. In the same way, the detection and monitoring of explosive residues, their precursors and degradation products in wastewater could represent useful intelligence for policing and security services regarding clandestine activity in a specific location.

To date, a number of studies have focused on the presence of toxic nitroaromatic and nitramine explosive compounds in industrial wastewaters and receiving waters such as rivers, lakes, seawater and groundwater to evaluate environmental and human exposure and subsequent health hazards. 2,6-dinitrotoluene (2,6-DNT) was detected at up to 6 µg L^{-1} in a mixture of industrial effluent and raw domestic sewage from Finland and trinitrotoluene (TNT) was measured at high mg L^{-1} concentrations in wastewater effluent sourced from a Brazilian TNT manufacturing plant [2, 3]. Other explosives detected in surface waters include octahydro-1,3,5,7-tetranitro-1,3,5,7-triazine (HMX) and cyclotrimethylene-trinitramine (RDX) at low µg L^{-1} concentrations in groundwater from a military base and HMX, RDX and pentaerythritol tetranitrate (PETN) at sub ng L^{-1} concentrations in lake and river water used for military activities [2, 4, 5]. The detection of a broad range of such chemically unstable compounds in highly complex matrices at trace concentrations has proven to be extremely difficult, especially for influent wastewaters.

Organic explosives in aqueous samples are generally analysed using gas chromatography (GC) or liquid chromatography (LC) with ultra-violet (UV) and/or mass spectrometry (MS) detection [6, 7]. More recently, the high resolving power (up to 100,000) and mass accuracy provided by high resolution accurate mass spectrometry (HRMS) has been shown to provide high selectivity and sensitivity even in highly complex environmental

matrices for targeted, suspect screening and untargeted analysis [8]. Although sensitivities have greatly improved using HRMS, more complex matrices such as wastewater still result in ion suppression. More focus is needed on improving sample pre-treatment and clean-up. Prior to explosive analysis, several extraction and pre-concentration techniques have been applied to wastewater effluent, seawater, surface waters, groundwater and drinking water and include salting-out solvent extraction [9], solid phase extraction (SPE) [4, 10-14] solid phase micro-extraction [5, 15] dispersive liquid-liquid micro-extraction [16, 17] and direct ultra-sound assisted dispersive liquid-liquid micro-extraction [18]. Overall, SPE is the most commonly applied technique for aqueous sample extraction given its simplicity, concentration/cleanup ability and low solvent consumption [19]. Previously used SPE sorbents for explosives have mainly been composed of styrene and/or (alkyl)vinylbenzene and are often with additional polar or ion exchange functionalities for added selectivity [4, 14, 20-25]. Silica and graphitic carbon have also been successfully applied [13, 26]. Most extraction methods focused on a very limited number and range of explosive chemistries, and, in terms of sample complexity, most have been applied to surface and ground waters with very few tackling more complex wastewater matrices.

For the first time, a method for trace detection of multiple classes of explosives in raw wastewater using SPE, high performance liquid chromatography (HPLC) and HRMS is presented. The main objectives were to develop a broadly applicable SPE method based on the extraction of 18 high-order organic explosives, including nitramines, nitrate esters, nitroaromatics and peroxides (Table 1) at the ng L^{-1} concentration range; and to exploit the capability of HRMS further via application to suspect screening of several additional explosives in wastewater influent sampled from one of the largest wastewater treatment plants (WWTP) in the EU, based in London, UK. With continual monitoring of explosive components in influent wastewater, deviations from any measured background concentration

could produce intelligence on the catchment area in which explosives may be illegally manufactured.

2. Experimental

2.1. Reagents, chemicals and consumables

HPLC grade methanol, acetonitrile, ethyl acetate, isopropyl alcohol, dichloromethane and dimethyldichlorosilane were purchased from Fisher Scientific (Loughborough, UK). For optimisation of the analytical method for a set of n=18 prioritised explosives, standard solutions of hexahydro-1,3,5-trinitroso-1,3,5-triazine (R-salt, 99.8 %), HMX (99.1 %), RDX (98.6 %), nitrobenzene (NB, 99.8 %), nitroglycerine (NG, 99.4 %), 3,4-dinitrotoluene (3,4-DNT, 100 %), 2,4-dinitrotoluene (2,4-DNT, 100 %), 2,6-DNT (100 %), 2-nitrotoluene (2-NT, 99 %), 3-nitrotoluene (3-NT, 98.7 %), 4-nitrotoluene (4-NT, 99.2 %), erythritol tetranitrate (ETN, 99.9 %), tetryl (98.6 %), TNT (100 %) and PETN (99.4 %) at 1000 mg L^{-1}, and triacetone triperoxide (TATP, 99.1 %), hexamethylene triperoxide diamine (HMTD, 89.1 %) and ethylene glycol dinitrate (EGDN, 96.2 %) at 100 mg L^{-1} were sourced from Accustandard (New Haven, CT, USA). For testing of the developed method for semi-targeted analysis, a second set of explosives, precursors and transformation products were selected (n=17). These included 1,3,5-trinitrobenzene (TNB, 100 %) at 2000 mg L^{-1}, 1,2-dinitrobenzene (1,2-DNB, 100 %), 1,3-dinitrobenzene (1,3-DNB, 97.0 %), 4-amino-2,6-dinitrotoluene (4-Am-2,6-DNT, 100 %), 2-amino-4,6-dinitrotoluene (2-Am-4,6-DNT, 100 %), diphenylamine (DPA, 100 %) at 1000 mg L^{-1}, and nitroguanidine (NQ, 100 %), 2,6-diamino-4-nitrotoluene (2,6-DA-4-NT, 99.7 %), 2,4-diamino-6-nitrotoluene (2,4-DA-6-NT, 100 %), 1,3-dinitroglycerin (1,3-DNG, 99.6 %), 1,2-dinitroglycerin (1,2-DNG, 99.3 %), picric acid (PA, 99.1 %), triethylene glycol dinitrate (TEGDN, 97.4 %), 3,5-dinitroaniline (3,5-DNA, 99.3 %) at 100 mg L^{-1} were also sourced from Accustandard . 2,3-dimethyl-2,3-dinitrobutane (DMDNB, 98 %) was purchased

from Sigma-Aldrich (Gillingham, Dorset, UK) and 1,3-dimethyl-1,3-diphenylurea (DMDPU) and 1,3-diethyl-1,3-diphenylurea (DEDPU) were prepared in methanol at 1000 mg L^{-1} and obtained from the UK Forensics Explosives Laboratory (FEL). Working solutions were prepared in HPLC grade methanol for each stock solution at 10 mg L^{-1} and 100 μg L^{-1} and stored in the dark at -20 °C.

Ultrapure water was supplied by a Millipore Synergy-UV water purification system at 18.2 MΩ cm (Millipore, Bedford, USA). Ammonium acetate (>99 % purity), ammonium chloride (>99 % purity) and hydrochloric acid solution (37 % w/v) were purchased from Sigma-Aldrich .

2.2. Sample collection

Influent wastewater was collected from a wastewater facility located in the greater London (population equivalent =3.5 million). Eight 24-hour composites were sampled between the 8^{th}-16^{th} March, 2016 with collection occurring on the following day. Samples were collected at this time to align with our annual inter-city illicit drug comparison study [1]. For method performance evaluation, 5-6 grab samples were taken over a week on separate days in September 2016 and composited to make a representative matrix. At the laboratory, samples were acidified to pH 2 using HCl (37 % w/v) and stored at -20 °C. Nalgene sample bottles were washed in triplicate with methanol and water separately. Glassware washing and silanisation procedures are described in the supplemental information (SI).

2.3. Sample preparation

Influent wastewater samples were defrosted and filtered under vacuum using GF/F glass microfiber filters (Whatman, Buckinghamshire, UK). Samples were then split into 100 mL aliquots for extraction and fortified where necessary. Recovery studies in influent wastewater

were carried out by spiking 250 µL of a 10 mg L^{-1} standard mix into the 100 mL sample in order to yield an expected final concentration of 1 mg L^{-1} in the sample extract. A SPE manifold (Phenomenex, Macclesfield, Cheshire, UK) was used under vacuum at a pressure of ≤20 kPa. A total of 34 commercially available SPE sorbents were evaluated in triplicate using the manufacturer's guidelines (for details of sorbents, manufacturers and procedures, see Table S1). Following sub-selection of three suitable sorbents, sample/elution volume, elution solvent type, pH of the sample/elution solvent and inclusion/exclusion of an evaporation step were systematically optimised. For application to wastewater profiling, the optimised SPE method utilised 6 mLx200 mg barrel-type Oasis HLB cartridges (Waters Corp., Hertfordshire, UK) which were conditioned with methanol (5 mL) and washed with ultrapure water (10 mL) before loading of the 100 mL acidified samples at 5-10 mL min^{-1}. Cartridges were washed with ultrapure water (5 mL), dried under vacuum for 10 min, eluted with 2.5 mL acetonitrile and extracts transferred to septum capped crimped vials (2 mL, Agilent Technologies, Cheshire, UK). Samples were stored at -20 °C until analysis.

2.4. Instrumentation and optimised conditions

For the determination of SPE recovery from standards prepared in ultra-pure water, analytes bearing a UV chromophore (nitrate esters, nitramine, nitrosamine and nitroaromatic compounds) were determined using an Agilent 1100 series LC system coupled to a diode array detector set at 210 nm (Agilent Technologies). Separations were performed using an ACE C_{18}-AR column (150x2.1 mm, 3 µm, Advanced Chromatography Technologies Ltd., Reading, Berkshire, UK) maintained at 20 °C. Mobile phases were 90:10 (v/v) 8 mM ammonium acetate in water/methanol (A) and 10:90 (v/v) 8 mM ammonium acetate in water/methanol (B). Binary gradient elution was carried out at a flow rate of 0.15 mL min^{-1} over 40 min. Mobile phase was set at 40 % B at 0 min and raised to 100 % B over 30 min.

This was held for a further 10 min before returning to initial conditions and equilibrating for 34.5 min (total run time =75 min). A sample injection volume of 1 μL was used.

For the determination of TATP and HMTD recovery in ultra-pure water, shorter 12-min separations were performed on a Waters Sunfire C_{18} column (150x2.1 mm, 3.5 μm) at a temperature of 44 °C. Separations were carried out using an Accela LC system coupled to a HTS-A5 autosampler (Thermo Fisher Scientific, San Jose, CA, USA). A gradient elution profile was performed at a flow rate of 0.3 mL min^{-1}. Mobile phases were 90:10 (v/v) 0.2 mM ammonium chloride in water/methanol (A) and 10:90 (v/v) 0.2 mM ammonium chloride in water/methanol (B). Mobile phase was set at 20 % B at 0 min and raised to 100 % B over 4 min and then held at 100 % for a further 2 min. Re-equilibration time was 5 min. Peroxide detection was performed using HRMS via atmospheric pressure chemical ionisation (APCI) and operated in positive ion mode only. For application of the final method to all wastewater extracts, an optimised LC-HRMS method was utilised for separation and confirmatory detection of all compounds. Separations were performed again on the ACE C_{18}-AR column, but with inclusion of a 1 cm guard column and the oven maintained at 20 °C. A binary gradient was used over 39 min at a flow rate of 0.3 mL min^{-1}: 40 % B at 0 min; a linear ramp to 95 % B over 15 min; to 100 % B over 0.50 min; 100 % B for 5.5 min; to 40 % B over 0.50 min. Re-equilibration time was 17.5 min. Detection was performed using APCI-HRMS and operated in negative ion mode and positive ion mode in separate runs. Where HRMS was used, a 5 μL sample injection volume was the minimum allowed. Samples were maintained at 10 °C during the analysis.

For HRMS, an Exactive^{TM} instrument (Thermo Fisher Scientific) equipped with a heated APCI source was utilised. Nitrogen was used as the nebulising and desolvation gas within the ionisation source and the collision cell. Separate runs in each ionisation polarity mode were used for all analytes using full-scan high resolution at 50,000 FWHM with ranges

between m/z 50–400 and m/z 60–625 for positive and negative ion mode, respectively. Optimised ionisation and mass spectrometric conditions are shown in Table S2 of the supplemental information. All acquired data was processed using Xcalibur v 2.0 software (Thermo Fisher Scientific).

2.5. Analytical method performance in influent wastewater

The analytical performance of the entire method was evaluated in matrix and with respect to linearity, range, accuracy, precision, limits of detection (LOD), lower limits of quantification (LLOQ), recovery and matrix effects according to the ICH guidelines for the validation of analytical procedures [27]. Peak height was used for quantification, as several isobaric analytes existed, such as dinitrotoluene and nitrotoluene-based compounds, which were not fully resolved chromatographically. For TATP, its conformer at m/z 89.0954 was only partially resolved. Signal reproducibility for both peak area and peak height was assessed and were found not to be significantly different following injection of replicate matrix-matched standards (n=6). For linearity and range assessment, and since no previous occurrence data exists, coefficients of determination (R^2) were assessed for all compounds across the same range and concentration intervals. Since no data exist on explosives occurrence in raw wastewater, previous inter-laboratory comparison guidelines for drug detection in wastewater were used to inform the selected concentration levels in this first instance [1]. Wastewater was spiked between 1.25 ng L^{-1} to 25 µg L^{-1} (N=11) and subjected to the entire extraction and analysis procedure. Range was then subsequently defined for those concentration intervals where $R^2 \geq 0.98$ and $N \geq 5$ [27]. Where $R^2 \leq 0.98$ or where $N \leq 5$, compounds were retained in the method for qualitative screening purposes only. The upper limit of this range was selected, as anything beyond this value was not considered realistic for trace explosive occurrence in the wastewater system. Accuracy (trueness) was measured by determination of a spiked sample

(background corrected) at either 2.5 or 25 μg L^{-1} (n=6) from a matrix-matched calibration curve and the result expressed as a mean percentage ±%CV. Detection and quantification limits were determined for all analytes as three and ten times the standard deviation of the response at low-level concentration (at 250 ng L^{-1} or 25 μg L^{-1} for n=3) divided by the slope of the calibration curve. Calculated LODs were checked by measurement at the same concentration (n=3). The changing consistency of influent wastewater was previously shown to affect retention time [28]. Retention time precision was performed for n=6 wastewater samples (spiked at 25 μg L^{-1}) to account for any shift. As stated above, percentage recoveries during SPE method development were determined without matrix by extracting 25 μg L^{-1} standards in ultrapure water and comparison to a 1 mg L^{-1} standard. However, for method performance assessment, recovery and precision were determined in the representative wastewater matrix again via fortification with 25 μg L^{-1} (n=6) and comparison to 1 a mg L^{-1} matrix-matched standards (the theoretical 100 % recovery concentration, for n=3 to allow a standard deviation to be calculated). To determine the matrix effect, ion suppression/enhancement was assessed using matrix-matched standards at 1 mg L^{-1} in influent wastewater extracts (n=3) compared to a 1 mg L^{-1} standard in acetonitrile. Background correction was performed for any native compounds present in the wastewater as appropriate.

2.6. Targeted analysis and suspect screening of additional analytes

Targeted analysis was performed and any identified analytes confirmed using analyte retention time (relative to a matrix-matched standard), as well as the m/z of the most intense ion to within 5 ppm of calculated m/z and its isotopic ratio profile, all within TraceFinder software version 3.1 (Thermo Fisher Scientific). Standard addition quantification (N=4) was performed for each influent wastewater samples separately for added accuracy. Each sample

was split into four 100 mL aliquots and spiked at 0, 100, 250 and 500 ng L^{-1} with all compounds. For quality control purposes, acetonitrile was run between each sample set and no carry over was observed. To further test the applicability of the suspect screening approach, four 100 mL aliquots of a composite wastewater sample (i.e. an equal v/v mixture of samples collected on 15/03/16 and 16/03/16) were spiked with n=17 additional explosives, precursors and transformation products not originally included in method development at concentrations ≥500 ng L^{-1}(> LLOQ). Method recoveries for these additional analytes were then evaluated as described in section 2.5. LC-HRMS analysis data were also mined for the presence of these additional compounds in wastewater sample extracts using TraceFinder.

3. Results and discussion

3.1. Solid phase extraction (SPE) sorbent selection

Sorbent selection was critical to maximise analytical sensitivity for trace explosives in such a complex matrix. Given that no published works exist for broad scope explosive detection in raw wastewater, a wide range of 34 sorbents of various chemistries were selected for recovery assessment and included both silica and organic polymers. Several of these were grafted or co-polymerised with additional functionalities to enable van der Waals, ion exchange, π-π, dipole-(induced) dipole interactions and hydrogen bonding. All analyte recovery results are presented in Table S3. Compounds with vapour pressures $>10^{-7}$ Torr, i.e. EGDN, NB, NG, 2-NT and 4-NT, yielded little or no recovery relative to less volatile analytes. Peroxide recoveries varied greatly and often in excess of 100 %, but this was not unexpected given the solvent and analyte volatilities and no internal standards were used for compensation. Solvent volatility was a major issue in both extracts and standards used, which may also promote evaporation of solutes, not least for TATP, which is a very volatile analyte (as per the vapour pressures listed in Table 1). Overall, promising results were found for

'mixed-mode' co-polymeric styrene-divinylbenzene (PS-DVB) sorbents which was consistent with their widespread usage in sewage epidemiology for drugs determination [30, 31]. Based on this initial assessment, Oasis HLB (DVB with n-vinylpyrrolidone), Bond Elut ENV (unmodified PS-DVB) and Isolute ENV+ (hydroxylated PS-DVB) were selected for in depth optimisation.

Several additional conditions were systematically optimised including elution solvent type and volume, evaporation conditions, sample volume and pH in standards prepared in ultrapure water at 250 µg L^{-1}. During method development, recovery was determined as described in section 2.5, for a minimum of n=3 replicates (unless otherwise stated) to allow a standard deviation to be calculated. All data are presented in the supplemental information.

3.1.1. Elution and evaporation steps

The polarity of explosives varies greatly, with predicted LogK_{ow} values ranging from -1.71 to 3.64 (Table 1) [19]. Duplicate elution profiles across the three sorbents were first characterised using a protic solvent (methanol) to potentially maximise sensitivity. This was achieved via analysis of 1 mL eluate aliquots (Figure S1). For Oasis HLB, all selected analytes eluted in 2-3 mL. In contrast, nitroaromatic compounds had a strong affinity for the Isolute ENV+ sorbent and were detected up to 8 mL. For Bond Elut ENV, most analytes eluted in 1-4 mL methanol except for 3-NT and 2,6-DNT which required up to 5 mL for elution. It was concluded that 2.5 mL elution volumes offered the best compromise between overall analyte recoveries and concentration factor. The removal of the post-elution evaporation step on recovery was then investigated with respect to recovery and variance. A marginal increase in analyte recoveries was observed upon removal of the evaporation step (Table S4). In particular, recoveries were generally higher and less variable using Oasis HLB and Bond Elut ENV sorbents except for HMX, RDX and PETN. Tetryl recovery decreased

on all three sorbents. Nitroaromatics showed relatively poorer affinity for the Isolute ENV+ sorbent and compounds such as 3-NT, 2,6-DNT and 2,4-DNT were undetectable without the evaporation step. Most importantly, the more volatile peroxide-based analytes, which were previously not observed, were now recovered. Therefore, for explosives, it is recommended that the evaporation step is removed from SPE procedures. Whilst unfortunately this limited the concentration factors possible, this offered the best chances of detection of a broader range of analytes using LC-HRMS. Following this, three solvents (methanol, acetonitrile and ethyl acetate) were compared with respect to analyte recovery and reproducibility (Figure S2). Peroxides were excluded from this stage of optimisation to simplify measurements of other compounds with LC-UV and peroxide recoveries were measured in matrix later in the process. Ethyl acetate yielded the highest recoveries and were often >100 %. This was again likely related to evaporation and resulted in an undetermined concentration error. Interestingly, R-Salt was not detected across all three sorbents using this solvent. Acetonitrile offered a marginally improved recovery over methanol from Oasis HLB and enabled detection of 3-NT, 2,4-DNT and 2,6-DNT using Isolute ENV+. It also has a relatively higher boiling point than methanol, which may serve to further minimise evaporation. Although it did not perform very well with Bond Elut ENV, acetonitrile yielded the best recoveries overall and was chosen as the eluting solvent for the selected analytes (again in 2.5 mL volumes).

3.1.2. Sample pH and volume

Due to biological activity in wastewater, samples are often acidified to preserve stability [37]. The effect of low pH (<2) on recovery was investigated on Oasis HLB which showed no significant difference in analyte recoveries (Figure S3). Furthermore, and now that SPE conditions had been optimised for most explosives, it was considered logical to re-evaluate

the comparative performance of the three cartridge types under these optimised conditions. Recoveries are listed in Table S5. Oasis HLB performed best overall. Based on recoveries yielded for 16 compounds in 10-1000 mL ultrapure water (Figure S4), sample volume was then assessed in matrix using spiked influent wastewater at 100 and 250 mL on Oasis HLB (Figure S5). At 100 mL sample volumes, Oasis HLB yielded the highest recoveries for EGDN, 2-NT and 4-NT, which were not observed for 250 mL sample volumes. To offer the best balance between sensitivity and matrix interference, 100 mL sample volumes were selected. A wash solvent of 5:95 % (v/v) methanol:water (Figure S6) performed poorer than pure water where higher-polarity analytes such as HMTD were lost.

3.2. Analytical method performance in influent wastewater

From the initial 18 analytes selected, method performance assessment for four compounds, *i.e.* NB, NG, 3-NT and ETN, was not possible due to poor recovery and limited sensitivity at concentrations ≤25 μg L^{-1}. Method performance for all explosives in spiked influent wastewater is shown in Table 2.

Retention times varied ≤2.4 %. Coefficients of determination of R^2>0.98 using N≥5 spiked influent sample concentrations were achieved for most compounds and over three orders of magnitude (from low ng-mg L^{-1}) except for 2-NT, TATP and EGDN. That said, these compounds could still be included in the method for qualitative screening purposes. On average, accuracy was determined at 98 ±10 % with the worst being 4-NT at 121 ±14 %. Recoveries ranged from 77-124 % (n=6) with coefficients of variation (% CVs) averaging at 13 % (excluding HMTD which demonstrated the highest variation at 25 %). For LOD and LLOQ, EGDN, 2-NT and PETN were detected from 20 μg L^{-1} but LLOQ lay above the concentration range tested (>25 μg L^{-1}). For the majority of compounds, no background noise was observed, possibly due to the use of APCI over electrospray ionisation which has been

shown to reduce matrix effects [29]. However, due to the complexity of wastewater, ion suppression/enhancement still occurred in the range of -28 to 40 %. Generally, nitramines and nitrate esters (except EGDN) underwent enhancement; peroxides underwent suppression; and no specific trend was observed for nitroaromatic compounds. The mass accuracy was satisfactory from -3.77 ppm for R-salt to +1.41 ppm for HMTD. An attempt was made to increase method sensitivity by injecting larger sample volumes on column (10, 25, 50 µL). However, using ≥10 µL resulted in the splitting of earlier eluting peaks and lower resolution for DNT isomers. Therefore, the injection volume remained at 5 µL. Aqueous extract dilution to enable larger injection volumes was not considered. Extracted ion chromatograms (EICs) for each of the 14 analytes fortified in an influent wastewater sample at 25 µg L^{-1} and analysed by HPLC-HRMS, are shown in Figure 1.

3.3. Application to influent wastewater analysis

The method was applied to the determination of explosive residues in eight 24-h composite influent samples. 2,4-DNT was the only explosive-related component detected and in five out of the eight samples. Identification was confirmed via retention time, accurate mass and isotope ratio profiles to those produced from a spiked wastewater sample (Figure 2). The daily mass load of 2,4-DNT entering the WWTP was estimated by multiplying its measured concentration in influent by the corresponding daily influent flow rates through the WWTP. The daily mass load (Table 3), reached maximum loads midweek (between 332-468 g day^{-1}) before returning to detectable levels albeit just below the LLOQ (<48 ng L^{-1}). Another isomer, 2,6-DNT, was previously determined in Finnish municipal wastewater up to 5.9 µg L^{-1} (≤ 454 g day^{-1}), but sources of input were not known [3]. On the other hand, 2,4-DNT is commercially available as a purified isomer or as a component of DNT mixtures (~80 % 2,4-DNT, ~20 % 2,6-DNT) and is mainly used as an intermediate in the production of

polyurethane foams for furniture, bedding and automobile and airline seating and as cross-linking agents in the non-ferrous metals industry. Although not currently in the EU, 2,4-DNT is also used in automobile airbags, azo dyes and pigments [30]. In the field of forensic science, DNT is utilised in explosives and ammunition manufacture as a gelatinising-plasticising and stabilising agent and is often found as a by-product in TNT explosives (<1 %) and propellant formulations (<10 %) [31].

In a recent report prepared for the European Chemicals Agency, the amount of DNT in explosives and ammunition represented <1 % of the total amount of DNT used in Europe [30]. With the estimated total production weight of 'technical grade' DNT (80 % 2,4-DNT) between 540,000-810,000 tonnes per year from France, Germany, Hungary and Poland, the European Chemicals Bureau indicated regional releases of 2,4-DNT at 0.53 kg day^{-1} to wastewaters and continental releases at 0.34 kg day^{-1} [32]. Although 2,4-DNT is not produced in the UK, residues measured in this study fall between the EU regional and continental values reported which suggests import and substantial use within the UK. Reported UK uses of 2,4-DNT are in the non-ferrous metal industry particularly for aluminium smelting and blast furnace linings [30]. However, aluminium smelting facilities are based mainly in Scotland and the West Midlands. 2,4-DNT was previously detected in groundwater collected near a former TNT manufacturing plant along with lower amounts of TNT and its main transformation products [33]. In our study, only 2,4-DNT was detected and, in the absence of other nitrotoluene-based compounds, further suggests that it was unlikely to come from an explosives-related source alone. Furthermore, commercial blasting explosives are rarely used in and around Central London for demolition activity and are predominantly NG/EGDN-based. Finally, explosives-related compounds, including 2,4-DNT, have been shown to accumulate in natural flora and exert unwanted ecological effects [34-37]. Removal of 2,6-DNT following mechanical and activated sludge treatment has been

shown [3]. Therefore, if 2,4-DNT undergoes similar removal efficiency in the London treatment works (which incorporates the same processes), receiving waters are likely to be less impacted. Overall, and aside from 2,4-DNT, occurrence of other explosives in wastewater from London at the ng L^{-1} concentration level was minimal.

3.4. Suspect screening of additional compounds

HRMS is one of the most promising emerging tools for retrospective detection of additional, new or emerging compounds and the broader potential for detection of explosives-related compounds was assessed further here. An additional 17 explosives-related analytes were spiked into four 100 mL aliquots of composite wastewater and the optimised screening method applied. Some of these compounds included a number of TNT degradation products transformed via photolysis and biotic and abiotic processes e.g. 4-Am-2,6-DNT, 2-Am-4,6-DNT, 2,6-DA-4-NT, 2,4-DA-6-NT, 1,2-DNB, 1,3-DNB, TNB [38]. None of the additional compounds were observed in the composite wastewater samples.

Obviously, one potential limitation of the method is the selectivity of the SPE method for broad application to so many compounds. The recoveries of the 17 extra analytes were determined and are presented in Table 4. The method performed well with 14 recovered at ≥62 %. Performance for DMDNB was variable and was recovered in only two of the spiked samples at 56 %. This was likely due to its presence at concentrations close to its LOD i.e. ~5 µg L^{-1}. PA and NQ were not detected in the spiked sample extracts but showed a high response at the theoretical 100 % recovery concentration in a matrix-matched standard. It is possible that PA and NQ eluted during the wash step (calculated logD_{ow} at pH 7 for PA = -1.4 and -1.0 for NQ). Although TNT degradation products were not detected in the unfortified extracts analysed earlier, the extraction method had a high efficiency for these compounds and if they were present in the sample they would likely be detected alongside

2.4-DNT. This result strengthens the reasoning that the 2,4-DNT detected in the wastewater extracts may not be sourced directly from TNT explosives.

4. Conclusions

A flexible, high resolution analytical method for 14 prioritised organic explosives in influent wastewater samples was developed, performance tested and applied for the first time. Of these, the method could be used quantitatively for 10 compounds. PS-DVB-type sorbents performed best for SPE of raw wastewater, with an HLB-type sorbent selected as the best of 34 cartridge types tested. Several factors were important particularly the exclusion of an evaporation step as well as careful optimisation of the sample volume, wash step and elution volume/solvent in order to achieve sensitivity in the ng L^{-1} to µg L^{-1} concentration range in influent wastewaters. The compounds NB, NG, 3-NT and ETN were not detected due to instrumental method matrix interference, despite the use of APCI. The method was applied to 24-h composite influent from a wastewater works in London in which one analyte, 2,4-DNT, was detected over five consecutive days. Furthermore, the application of this broad extraction method with LC-HRMS facilitates retrospective data-mining for identification of new and emerging compounds if necessary.

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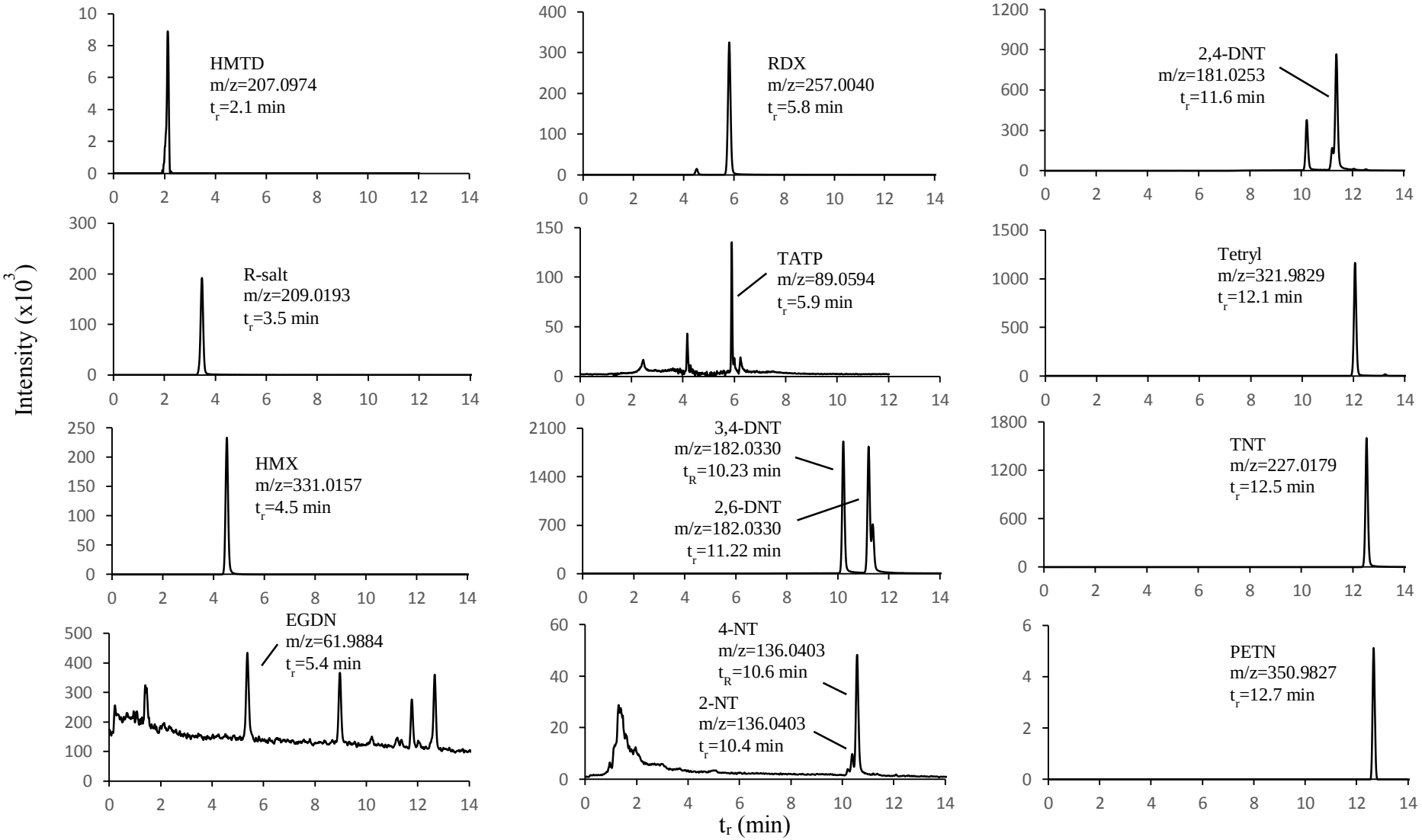


Figure 1. Extracted ion chromatograms of a 25 μg L^{-1} spiked influent wastewater sample containing 14 prioritised explosives.

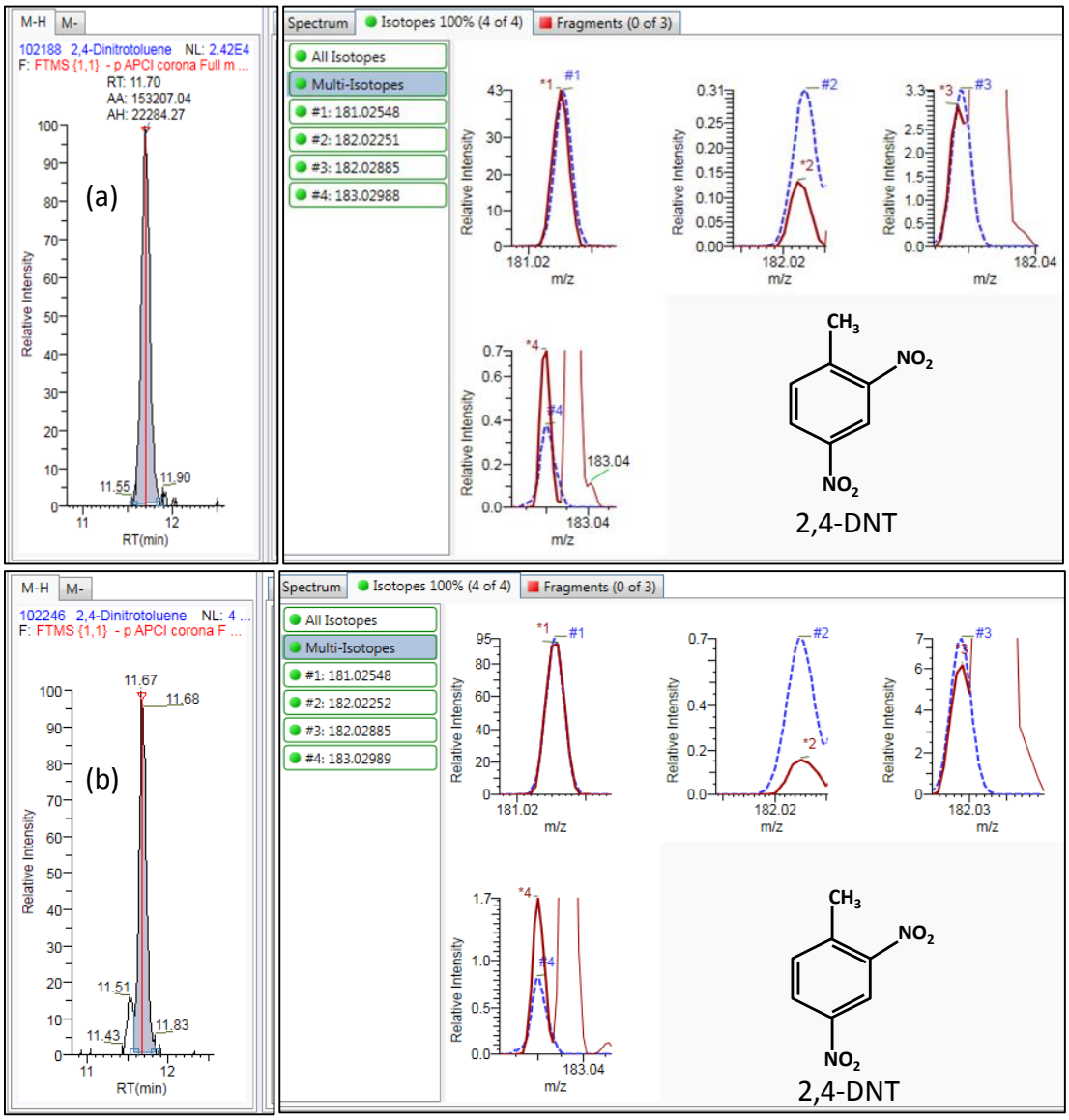
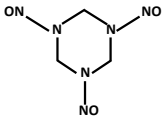
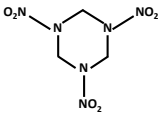
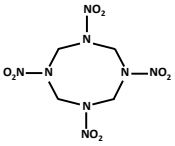
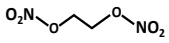
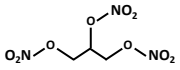
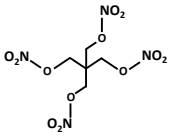
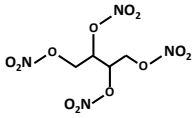
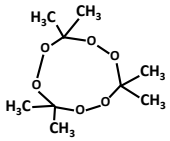
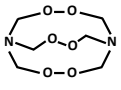
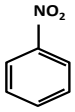
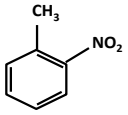
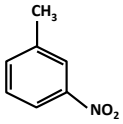
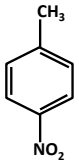
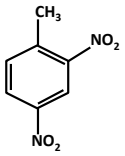
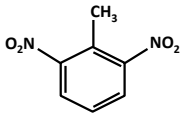
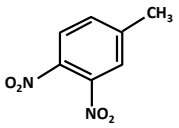
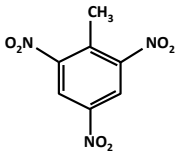
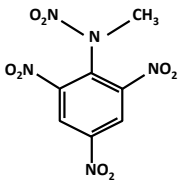


Figure 2. Extracted ion chromatograms confirming 2,4-DNT in (a) an unspiked influent wastewater sample and (b) a 250 µg L^{-1} spiked influent wastewater sample. The left panels show the $[M-H]^-$ ion of 2,4-DNT. The right panel shows the isotopic profile of the $[M-H]^-$ ion relevant to the theoretical isotopic profile.

Table 1. Chemical structure, formula, CAS no. and physicochemical properties of 18 selected explosives.

Class *Name* (Abbreviation)	Structure	Formula	Vapour pressure (atm at 25°C)	LogK_{ow}[a]	CAS No.	Ref.
Nitramines						
Hexahydro-1,3,5-trinitroso-1,3,5-triazine (R-salt)		$C_3H_6N_6O_3$	~10^{-10} - 10^{-12}	-1.18	13980-04-6	[39]
1,3,5-trinitroperhydro-1,3,5-triazine (RDX)		$C_3H_6N_6O_6$	4.85 x 10^{-12}	-1.20	121-82-4	[39]
Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX)		$C_4H_8N_8O_8$	2.37 x 10^{-17}	-1.71	2691-41-0	[39]
Nitrate esters						
Ethylene glycol dinitrate (EGDN)		$C_2H_4N_2O_6$	1.02 x 10^{-4}	1.51	628-96-6	[39]
Nitroglycerin (NG)		$C_3H_5N_3O_9$	6.45 x 10^{-7}	2.41	55-63-0	[39]
Pentaerythritol tetranitrate (PETN)		$C_5H_8N_4O_{12}$	1.07x10^{-11}	3.64	78-11-5	[39]
Erythritol tetranitrate (ETN)		$C_4H_6N_4O_{12}$	3.15 x 10^{-8}	3.21	7297-25-8	[40]
Organic peroxides						
Triacetone triperoxide (TATP)		$C_9H_{18}O_6$	6.31 x 10^{-5}	2.16	17088-37-8	[39]
Hexamethylene triperoxide diamine (HMTD)		$C_6H_{12}N_2O_6$	<3.95 x 10^{-7}	0.13	283-66-9	[41]

Nitroaromatics						
Nitrobenzene (NB)		$C_6H_5NO_2$	$3.95x10^{-4}$ [a]	1.81	98-95-3	
2-nitrotoluene (2-NT)		$C_7H_7NO_2$	$1.89x10^{-4}$	2.45	88-72-2	[42]
3-nitrotoluene (3-NT)		$C_7H_7NO_2$	$\sim10^{-5}$ [b]	2.45	99-08-1	
4-nitrotoluene (4-NT)		$C_7H_7NO_2$	6.43×10^{-5}	2.45	99-99-0	[42]
2,4-dinitrotoluene (2,4-DNT)		$C_7H_6N_2O_4$	4.11×10^{-7}	2.10	121-14-2	[39]
2,6-dinitrotoluene (2,6-DNT)		$C_7H_6N_2O_4$	8.93×10^{-7}	2.10	606-20-2	[39]
3,4-dinitrotoluene (3,4-DNT)		$C_7H_6N_2O_4$	$\sim10^{-7}$ [b]	2.15	610-39-9	
Trinitrotoluene (TNT)		$C_7H_5N_3O_6$	9.15×10^{-9}	1.79	118-96-7	[39]
2,4,6-trinitrophenylmethyl nitramine (Tetryl)		$C_7H_5N_5O_8$	7.41×10^{-12}	1.25	479-45-8	[39]

[a] Predicted by ACD/Labs Percepta software

[b] Estimated value based on vapour pressure of isomers (3-NT and 3,4-DNT) or similarly structured explosives (TNX and RDX used for R-Salt approximation)

Table 2. Analytical method performance data for the selected explosives in influent wastewater matrix[a].

Analyte	t_r±%CV (min) n=6[b]	Adduct	Calculated m/z	Mass error (ppm)	% Accuracy (trueness) ±%CV (%), n=6[b, c]	LOD (µg L^{-1}) n=6[b]	% Recovery ±%CV n=6[b, d]	Linear range[e] (µg L^{-1})	R^2 N≥5[b]	Matrix effect[f] ±%CV n=3[b]
HMTD	2.1±0.5	$[M+CH_3OH-HOOH+H]^+$	207.0976	-1.45	87±20[d]	5	80±25	13-25	0.9958 (N=5)	+40±4
R-salt	3.5±0.5	$[M+Cl]^-$	209.0201	-3.77	92±11	0.059	90±11	0.177-13	0.9935 (N=6)	-16±13
HMX	4.5±0.4	$[M+Cl]^-$	331.0165	-2.30	89±10	0.039	88±15	0.119-13	0.9944 (N=6)	-10±13
EGDN[g]	5.4±2.4	$[NO_3]^-$	61.9884	0.48	nd	20	110±14	nd	nd	+2±9
RDX	5.8±0.3	$[M+Cl]^-$	257.0048	-3.24	95±11	0.040	91±12	0.122-13	0.9941 (N=7)	-16±15
TATP[g]	5.9±0.5	$[C_4H_9O_2]^+$	89.0597[h]	-3.48	102±16	2	102±15	5-25	0.9443 (N=5)	+28±7
3,4-DNT	10.2±0.3	$[M]^-$	182.0333	-1.68	96±14	0.029	115±13	0.088-19	0.9933 (N=8)	+13±10
2-NT[g]	10.4±1.0	$[M-H]^-$	136.0404	-0.75	nd	23	102±12	nd	nd	+17±1
4-NT	10.6±0.2	$[M-H]^-$	136.0404	-0.75	121±14[d]	0.93	93±11	3-25	0.9988 (N=5)	+1±4
2,6-DNT	11.3±0.3	$[M]^-$	182.0333	-1.68	98±12	0.026	116±13	0.078-19	0.9897 (N=8)	+17±9
2,4-DNT	11.6±0.3	$[M-H]^-$	181.0255	-1.00	109±11	0.048	77±14	0.145-25	0.9935 (N=9)	-28±23
Tetryl	12.1±0.2	$[M-NO_2]^-$	241.0215	1.41	102±12	0.049	102±12	0.148-19	0.9979 (N=7)	-22±5
TNT	12.5±0.2	$[M]^-$	227.0184	-2.14	91±12	0.033	124±10	0.099-13	0.9930 (N=7)	+24±12
PETN[g]	12.7±0.5	$[M+Cl]^-$	350.9838	-3.21	nd	23	86±12	nd	nd	-14±12
NB	nd	$[M]^-$	123.0326[i]	0.18[i]	nd	nd	nd	nd	nd	nd
NG	nd	$[M+Cl]^-$	261.9725[i]	-3.56[i]	nd	nd	nd	nd	nd	nd
3-NT	nd	$[M]^-$	137.0482[i]	-0.20[i]	nd	nd	nd	nd	nd	nd
ETN	nd	$[M+Cl]^-$	336.9682[i]	-2.60[i]	nd	nd	nd	nd	nd	nd

[a] as per ICH guidelines [27]; [b] n=replicate number at the same concentration, N=replicate number at different concentrations; [c] at a 2.5 µg L^{-1} spiking level unless otherwise specified; [d] at a 25 µg L^{-1} spiking level. [e] range from LLOQ to the highest concentration with R^2>0.98; [f] defined as HRMS signal suppression (-) or enhancement (+); [g] the method should only be used qualitatively for these analytes in wastewater; [h]most intense ion for TATP (molecular ion at *m/z* 240.1442 was also monitored but sensitivity was poor); [i]m/z and accuracy determined from direct infusion of a 5 µg mL^{-1} methanolic standard; nd – not detected/determined in extracts of wastewater matrix

Table 3. Measured concentrations of 2,4-dinitrotoluene (2,4-DNT) in daily composite samples and the calculated daily load.

Day of sample (Collection date)	Measured concentrations of 2,4-DNT ($ng\ L^{-1}$)	Daily flow of wastewater ($m^3\ day^{-1}$)	Daily load for 2,4-DNT ($g\ day^{-1}$)
Monday (08/03/16)	<LLOQ	1,122,756	<LLOQ
Tuesday (09/03/16)	225	1,735,891	391
Wednesday (10/03/16)	303	1,542,749	468
Thursday (11/03/16)	279	1,190,735	332
Friday (12/03/16)	<LLOQ	1,169,810	<LLOQ
Saturday (13/03/16)	n.d.	1,102,207	-
Monday (15/03/16)	n.d.	1,106,421	-
Tuesday (16/03/16)	n.d.	1,137,212	-

Table 4. Recoveries of 17 additional explosives, precursors and transformation products in spiked composite wastewater samples (N=4, spiked concentration >LLOQ).

Analyte	Concentration (µg L^{-1})	t_r ±%CV (min)	Measured ion	Adduct	Relative recovery ±CV (%)
NQ	0.5	1.4[a]	105.0411	$[M+H]^+$	No recovery
2,6-DA-4-NT	0.5	3.2±0.2	168.0774	$[M+H]^+$	71±9
2,4-DA-6-NT	0.5	3.6±0.5	168.0774	$[M+H]^+$	62±10
1,3-DNG	5.0	4.0±0.2	216.9860	$[M+Cl]^-$	81±14
1,2-DNG	5.0	4.4±0.1	216.9860	$[M+Cl]^-$	92±17
PA	0.5	4.5[a]	227.9888	$[M-H]^-$	No recovery
DMDNB[b]	5.0	7.3±0.5	194.1144	$[M+NH_4]^+$	56±5
TEGDN	5.0	7.6±0.2	258.0942	$[M+NH_4]^+$	86±12
1,2-DNB	0.5	7.9±0.1	168.0170	$[M]^-$	81±9
1,3-DNB	0.5	9.0±0.2	168.0170	$[M]^-$	82±10
3,5-DNA	0.5	9.2±0.2	183.0278	$[M-H]^-$	75±9
TNB	0.5	9.8±0.1	213.0019	$[M]^-$	78±9
4-Am-2,6-DNT	0.5	10.3±0.1	196.0356	$[M-H]^-$	78±9
2-Am-4,6-DNT	0.5	10.6±0.2	196.0356	$[M-H]^-$	76±9
DMDPU	0.5	12.4±0.2	241.1344	$[M+H]^+$	74±6
DPA	0.5	13.9±0.2	170.0971	$[M+H]^+$	65±22
DEDPU	0.5	14.8±0.1	269.1657	$[M+H]^+$	70±11

[a] t_r measured for standard as analyte not detected in wastewater matrix
[b] Analyte detected in only two out of four spiked wastewater extracts (n=2)