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Improved determination of femtogram-level organic explosives in multiple matrices using dual-sorbent solid phase extraction and liquid chromatography-high resolution accurate mass spectrometry

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ABSTRACT

Identification and trace quantification of multiple explosives residues, their precursors and transformation products in complex samples remains very challenging. For solid phase extraction (SPE) and liquid chromatography-high resolution accurate mass spectrometry-based methods (LC-HRMS), interferences from co-extracted matrix components can significantly affect recovery during extraction and/or detector signal. The aim of this work was to develop a new, improved and more generalisable extraction approach to trace explosives analysis in a range of matrices using dual-sorbent SPE with LC-HRMS. Recoveries of 44 organic explosives from model solutions were optimised and compared for seven different sorbents (Oasis HLB, HyperSep Retain PEP and Isolute ENV+, HyperSep SAX, HyperSep NH2, Strata Alumina-N and Bond Elut CN). On average, Oasis HLB and Isolute ENV+ yielded the best recoveries (> 80%). For three sorbents, mean recoveries remained ≤1%, which made them potentially suitable for matrix removal when used in series with more analyte-selective sorbents. To evaluate matrix effects, a range of aqueous (river- and wastewater), solid (soil), dirty (road sign swabs), oily (oven hood swabs) and biological (dried blood) samples were selected based on complexity and forensic relevance. With the exception of river water, matrix effects were lowest using dual-sorbent SPE, with little/no compromise in recovery. Quantitative method performance assessment is presented for 14 selected explosives, representative of different classes, molecular weights and volatilities, and across three different matrices (i.e. untreated wastewater, cooking oil residues and dried blood). Limits of detection improved by ~10-fold over a single sorbent approach, enabling low fg sensitivity in many cases. Finally, application of the method to untreated wastewater enabled detection of new explosives traces for the first time, which could be used to help identify clandestine manufacture or sources of environmental toxicity. This approach offered a versatile solution to sample preparation for robust and highly sensitive detection/quantification of large numbers of explosives residues in a range of complex sample types.

1. Introduction

Targeted detection and monitoring of explosives, their precursors and transformation products in different matrices has been common practice for many years using a variety of different techniques. In forensic science, sample types vary widely and, as such, the development of sensitive, flexible and high-assurance analytical approaches for potentially large numbers of explosives in such a diversity of inhomogeneous and inconsistent matrices, in which recovery can easily vary within the same broad sample type, is critical to forensic laboratories and law enforcement agencies [1,2]. New and emerging threats posed by homemade explosives (HMEs) now exist and, as a result, research activity has increased in the area of explosives detection for security, military and counter-terrorism applications [3,4].

Explosives screening in complex mixtures has traditionally been performed using combinations of gas chromatography (GC) [5,6] and

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liquid chromatography (LC) [7,8] and, more recently, GC or LC coupled to mass spectrometry (MS) [9], with high resolution accurate mass spectrometry (HRMS) gaining particular interest in forensic explosives analysis [10–12]. Unlike tandem mass spectrometry (MS/MS), its ability to perform full-scan acquisition at high resolution (up to 140,000 full-width half-maximum) and mass accuracy (generally < 5 ppm) enables targeted, untargeted and suspect screening to be performed simultaneously, with the added capability for retrospective data mining to identify new compounds as needed. This makes it suitable for the broad screening of thousands of ionisable compounds in a sample [13] and it has already been successfully applied to screening of pharmaceuticals, illicit drugs and explosives in very complex matrices, such as wastewater [14–16]. Like LC-MS/MS, however, methods employing LC-HRMS are still subject to ion suppression/enhancement, which is matrix dependent and can significantly limit reliable quantitation and method generalisability across sample types [9,17–19].

The potential for false negatives as a result of matrix effects is clearly undesirable for high sensitivity forensic explosives analysis [20] and, whilst stable isotope-labelled internal standards can be used to compensate for them [21], this is not always possible since such standards do not exist for many explosives and/or their precursors and transformation products. An alternative way to minimise matrix effects, whilst also providing analyte enrichment for reliable trace analysis [22], is through development of more effective clean-up procedures. Today, solid phase extraction (SPE) is one of the most widely used of these techniques as it is simple, exhaustive, uses smaller volumes of less toxic solvents in comparison to other techniques and a wide range of sorbent chemistries are commercially available [23]. Polymeric sorbents composed of styrene and/or (alkyl)vinylbenzene modified with additional functional groups for increased selectivity have been shown to most successfully recover these target analytes from a range of matrices, including aqueous samples [22,24,25], soil and sediment [24] and motor oil [26,27]. Specifically, polymeric reversed-phase sorbents, such as “hydrophilic-lipophilic balanced” (HLB) sorbents containing modified styrene or divinylbenzene copolymers, have been found to give the highest recoveries for a broad range of analytes in comparison to other commercially available sorbents [27–29]. This was most notably demonstrated recently by Rapp-Wright et al. who compared 34 commercially available SPE sorbents for the recovery of a mixture of 18 organic explosives of varying polarities and vapour pressures, including nitrate esters, peroxides, nitramines and nitroaromatics. Of those tested, HLB type chemistries were again found to be the most suitable, yielding recoveries above 83% for all analytes on one particular commercial cartridge (Waters Oasis HLB), and a fully optimised SPE method was developed for quantitative wastewater analysis [16]. Recently, the US Federal Bureau of Investigation (FBI) developed an SPE procedure for 12 trace nitro-organic explosives in soils, including nitramines, nitroaromatics, nitroalkanes and nitroesters [30]. Three commercial copolymeric SPE sorbents were investigated and instrumental analysis was carried out using GC coupled to electrochemical detection (ECD). Despite obtaining the highest recoveries with Oasis HLB sorbents from soil overall, regular blockages occurred and Bond Elut NEXUS was eventually selected instead. Furthermore, recovery varied generally across different soil types (sand, potting soil and oil-contaminated topsoil) using the optimised method, which was likely due to different complexity and organic content. Therefore, even within the same sample type, SPE methods are not always broadly applicable and matrix effects are still a problem.

A multi-modal SPE approach, in which sorbents of different chemistries are used in series to eliminate matrix effects whilst also concentrating analytes of interest, offers a potential solution and has, to date, not been investigated systematically. The aim of this work was therefore to explore the value of dual-sorbent SPE of explosives in complex matrices in more detail. Herein, we characterised a range of SPE sorbents and combinations for the efficient removal of matrix and recovery/concentration of multiple organic explosives and their related compounds from diverse, challenging and forensically relevant sample types. Together with LC-HRMS, this could enable increased assurance and robustness at higher sensitivity and offer a more flexible solution to matrix diversity in forensic explosives analysis.

2. Experimental

2.1. Reagents and materials

HPLC grade methanol, acetonitrile, ethanol and formic acid (> 95% purity) were purchased from Fisher Scientific (Loughborough, UK) and ultrapure water was supplied by a Millipore Synergy-UV water purification system at 18.2 MΩ cm (Millipore, Bedford, USA). Ammonium acetate (> 99% purity) and ammonium chloride (> 99% purity) were sourced from Sigma-Aldrich (Gillingham, Dorset, UK).

For the initial evaluation of SPE sorbent performance n = 44 explosive reference materials were purchased. Standard solutions at (a) 1000 mg L^{-1} (purity given in parenthesis for each compound) of each of 1,3,5-trinitrobenzene (1,3,5-TNB, 97.5%), 3,4-dinitrotoluene (3,4-DNT, 100.0%), 2,6-dinitrotoluene (2,6-DNT, 100.0%), 2,4-dinitrotoluene (2,4-DNT, 100.0%), 4-nitrotoluene (4-NT, 99.2%), 2-nitrotoluene (2-NT, 99%), 3-nitrotoluene (3-NT, 98.7%), 1,2-dinitrobenzene (1,2-DNB, 100.0%), 1,3-dinitrobenzene (1,3-DNB, 97.0%), nitrobenzene (NB, 99.8%), nitroglycerin (NG, 99.4%), nitroguanidine (NQ, 100%), picric acid (PA, 99.1%), picramic acid (100.0%), pentaerythritol tetranitrate (PETN, 99.4%), tetryl (99.6%), 2,4,6-trinitrotoluene (TNT, 100.0%), HMX (99.1%), RDX (98.6%), erythritol tetranitrate (ETN, 99.9%) and 3,5-dinitroaniline (3,5-DNA, 100.0%); (b) 100 mg L^{-1} of each of 4-amino-2,6-dinitrotoluene (4-Am-2,6-DNT, 100.0%), 2-amino-4,6-dinitrotoluene (2-Am-4,6-DNT, 100.0%), 2,6-diamino-4-nitrotoluene (2,6-DA-4-NT, 99.7%), 2,4-diamino-6-nitrotoluene (2,4-DA-6-NT, 99.0%), 1,3-dinitroglycerin (1,3-DNG, 99.3%), 1,2-dinitroglycerin (1,2-DNG, 98.6%), trimethylolethane trinitrate (TMETN, 98.5%), hexanitrodiphenylamine (HND, 97.9%), nitromethane (NM, 100.0%), 1,2-diaminopropane (1,2-DAP, 99.8%), hexamethylene triperoxide diamine (HMTD, 100.0%), triethylene glycol dinitrate (TEGDN, 97.4%), triacetone triperoxide (TATP, 99.1%), PYX (98.3%), hexahydro-1,3,5-trinitroso-1,3,5-triazine (R-salt, 99.8%), 2-nitroglycerin (2-MNG, 99.0%), 1-nitroglycerin (1-MNG, 99.8%) and diethylene glycol dinitrate (DEGDN, 99.9%); and (c) 40 mg L^{-1} of 1,3,5-triamino-2,4,6-trinitrobenzene (TATB, 99.6%) were purchased from Accustandard (New Haven, CT, USA). Propylene glycol dinitrate (PGDN, 99%) and ethylene glycol dinitrate (EGDN, 99%) at 1000 mg L^{-1} were sourced from Thames Restek (Saunderton, Buckinghamshire, UK). 2,3-dimethyl-2,3-dinitrobutane (DMDNB, 98%) and diphenylamine (DPA, > 99%) were obtained from Sigma Aldrich (Gillingham, Dorset, UK). Solutions at 1000 mg L^{-1} of 1,3-dimethyl-1,3-diphenylurea (DMDPU), 1,3-diethyl-1,3-diphenylurea (DEDPU) and diacetone diperoxide (DADP) were prepared in methanol from materials provided by the Forensic Explosives Laboratory (FEL, Dstl, Fort Halstead, Kent, UK). Mixed working solutions at 50 or 5 mg L^{-1}, depending on the starting concentration and mode of detection, were prepared in HPLC grade methanol from each stock solution on the day of use and stored in the dark at −20 °C.

2.2. Matrix selection, collection and preparation

Six sample types were selected either based on those determined by FEL to be forensically relevant (i.e. a priority for forensic casework) or as examples of those with a high degree of complexity, thereby potentially posing a significant challenge to method performance. All Nalgene bottles used (500 or 250 mL) were first washed with methanol then water in triplicate. All sampling and pre-treatment procedures for each sample type are given in detail in the supplementary information (SI).

The chosen sample types were river water, untreated wastewater, soil and swabbed samples of cooking oil residue, dirt residue and

Table 1
SPE recoveries (n = 6 replicates for Oasis HLB, otherwise n = 3) for 44 explosives from model solutions fortified at 250 μg L^{-1} (HPLC-UV) or 25 μg L^{-1} (HPLC-HRMS).

Analyte	Recovery ± standard deviation (%)						
	Oasis HLB	Isolute ENV+	HyperSep Retain PEP	HyperSep SAX	HyperSep NH2	Strata Alumina-N	Bond Elut CN
HMX	96 ± 13	104 ± 7	100 ± 13	nd	nd	nd	nd
RDX	97 ± 13	105 ± 5	100 ± 13	nd	nd	nd	nd
NB	88 ± 11	95 ± 5	86 ± 10	nd	nd	nd	1 ± 1
NG	99 ± 13	105 ± 9	98 ± 12	nd	nd	nd	nd
1,3,5-TNB	98 ± 13	90 ± 4	100 ± 11	nd	nd	nd	nd
3,4-DNT	98 ± 12	99 ± 1	94 ± 5	nd	nd	nd	27 ± 10
4-NT	86 ± 11	88 ± 6	80 ± 8	nd	nd	nd	12 ± 5
2,6-DNT	97 ± 12	99 ± 1	94 ± 8	nd	nd	nd	12 ± 5
PETN	96 ± 13	105 ± 7	97 ± 12	nd	nd	nd	13 ± 1
DPA	80 ± 7	64 ± 5	73 ± 9	2 ± 3	nd	nd	70 ± 17
DEDPU	92 ± 10	65 ± 4	85 ± 5	3 ± 3	nd	nd	85 ± 18
DMDNB	99 ± 13	109 ± 6	90 ± 12	nd	nd	nd	7 ± 2
1,2-DNB	99 ± 12	104 ± 6	92 ± 12	nd	nd	nd	14 ± 4
2-NT	83 ± 12	93 ± 6	83 ± 14	nd	nd	nd	21 ± 6
3-NT	87 ± 13	91 ± 7	83 ± 14	nd	nd	nd	24 ± 6
TNT	97 ± 12	99 ± 6	87 ± 16	nd	nd	nd	4 ± 1
DMDPU	98 ± 11	71 ± 4	88 ± 11	nd	nd	nd	130 ± 24
1,3-DNB	98 ± 3	93 ± 11	100 ± 0	nd	nd	nd	nd
4-Am-2,6-DNT	97 ± 4	98 ± 13	99 ± 1	nd	nd	nd	23 ± 10
2,4-DNT	97 ± 8	89 ± 10	100 ± 13	nd	nd	nd	10 ± 5
Tetryl	100 ± 6	226 ± 29	107 ± 17	nd	nd	nd	29 ± 14
2-Am-4,6-DNT	95 ± 5	103 ± 5	96 ± 1	nd	nd	nd	21 ± 3
ETN	91 ± 6	120 ± 7	95 ± 3	nd	nd	nd	20 ± 3
R-Salt	95 ± 4	106 ± 14	93 ± 19	nd	nd	nd	nd
1,2-DNG	83 ± 17	92 ± 9	97 ± 20	nd	nd	nd	nd
2,6-DA-4-NT	94 ± 5	101 ± 2	92 ± 1	nd	nd	nd	nd
2,4-DA-6-NT	91 ± 5	95 ± 4	84 ± 1	nd	nd	nd	nd
3,5-DNA	96 ± 5	100 ± 2	99 ± 2	nd	nd	nd	nd
EGDN	97 ± 6	89 ± 4	98 ± 3	nd	nd	nd	nd
1,3-DNG	97 ± 4	97 ± 10	94 ± 1	nd	nd	nd	nd
NQ	nd	nd	2 ± 1	nd	nd	nd	nt
DEGDN	132 ± 53	154 ± 78	140 ± 81	nd	nd	nd	nt
HMTD	89 ± 22	81 ± 8	97 ± 10	nd	nd	nd	nt
TATP	100 ± 22	83 ± 3	115 ± 11	nd	nd	nd	nt
DADP	68 ± 12	62 ± 11	82 ± 5	nd	nd	nd	nt
TEGDN	93 ± 18	83 ± 17	109 ± 10	nd	nd	nd	nt
PYX	nd	78 ± 46	15 ± 3	7 ± 1	41 ± 17	11 ± 2	nt
TATB	88 ± 25	26 ± 2	40 ± 5	1 ± 0	1 ± 0	1 ± 0	nt
TMETN	124 ± 49	131 ± 10	131 ± 11	nd	nd	nd	nt
Picramic Acid	15 ± 10	24 ± 1	4 ± 2	nd	1 ± 1	nd	nt
HND	nd	80 ± 4	7 ± 1	nd	4 ± 2	4 ± 1	nt
PGDN	101 ± 13	124 ± 7	128 ± 12	nd	nd	nd	nt
NM	nd	nd	nd	nd	nd	nd	nt
Picric Acid	4 ± 2	54 ± 13	7 ± 3	nd	1 ± 1	nd	nt

nd – not detected.
nt – not tested.

dried blood. River water grab samples (n = 6) were taken from the River Thames in Central London at the South Bank on two separate mornings (December 2016 and March 2017). Influent wastewater represented the most complex wastewater type in this case. The identification of explosives residue via wastewater-based epidemiology was considered a priority here to potentially identify clandestine explosives manufacture in a city. Six time-proportional (30 min sampling frequency), 24-h composite influent wastewater samples from a major London wastewater treatment plant (population equivalent: 3.5 million) were taken between the 8th −16th March 2016. On each day, samples were collected from the day before and transported back to the laboratory in cooler bags. Samples were collected at this time to align with an annual inter-city illicit drug comparison study [31,32]. Characterised topsoil was purchased from Springbridge Direct Ltd. (Uxbridge, UK) and stored at 4 °C in Nalgene bottles until analysis. The soil had the following properties: pH (100 g L^{-1}) 5.5–6.0; particle size distribution of 0–12 mm; and a density of 200–250 g L^{-1}, and, as compost, was primarily made up of organic material. With regards to the swabbed samples, defibrinated equine blood (VWR International Ltd, Leicestershire, UK) or pooled whole human blood from five volunteers (0.5 mL) was pipetted onto a glass microscope slide (Thermo Fisher, Paisley, UK) and left to dry on a hotplate set to 40 °C. Cotton swabs (Sainsbury's, London, UK) of this dried blood, residential oven hoods (cooking oil residue) and urban road signs (dirt residue) were collected and prepared for extraction according to the FEL Standard Operating Procedure for the Use and Extraction of Swabs.

2.3. Solid phase extraction (SPE)

For all SPE work, a 12-port SPE manifold (Phenomenex, Macclesfield, Cheshire, UK) was used under vacuum at a pressure of ≤20 kPa. Oasis HLB (Waters Corp., Hertfordshire, UK), Isolute ENV+ (Biotage, Uppsala, Sweden), HyperSep Retain PEP, HyperSep SAX and HyperSep NH2 (Thermo Fisher, Paisley, UK), Bond Elut CN (Agilent Technologies, Cheshire, UK) and Strata Alumina-N (Phenomenex,

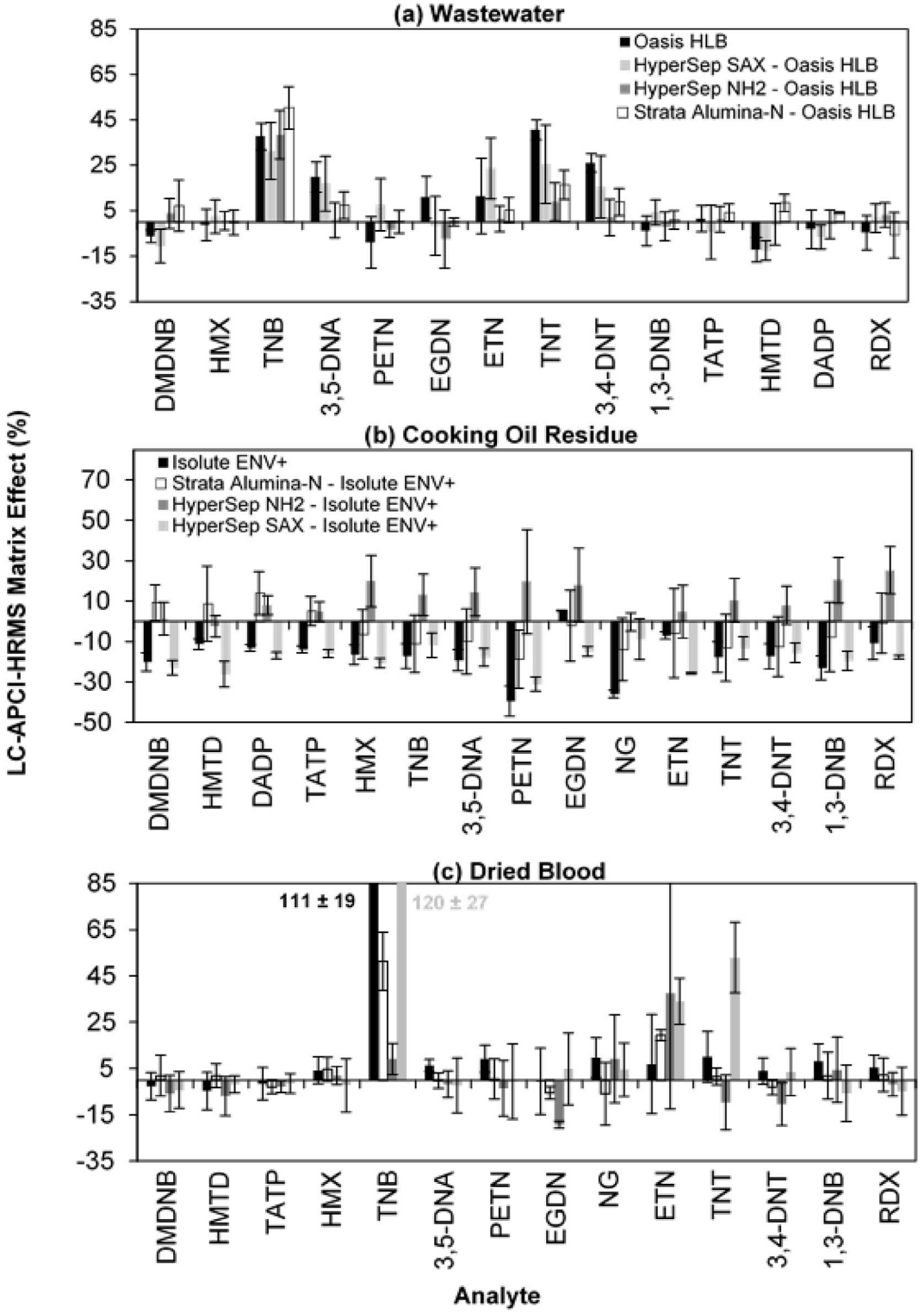


Fig. 1. LC-APCI-HRMS matrix effects for a selection of probe explosives in three different matrices after single- and dual-sorbent SPE. Error bars represent the standard deviation of triplicate extraction experiments. The key for wastewater is given in (a) and for the other two, the key is given in (b). For soil, dirt residue and river water data, see Fig. S1.

Cheshire, UK) cartridges were supplied by the respective manufacturers (see Table S1 of supplementary information for additional details). Based on the conclusion by Rapp-Wright that pH did not affect the recoveries of explosives in SPE [16], and besides acidification of wastewater samples to minimise bacterial activity only, other sample types were not pH adjusted before SPE.

2.3.1. Procedures for SPE of liquid samples

Waters Oasis HLB (n = 6 replicates) and an additional six commercially available sorbents (n = 3 replicates of each) were evaluated with a standard mix of explosives (50 or 5 mg L^{-1}) in ultrapure water, all using a previously optimised SPE method [16] (see SI for full details). This same method was also used for river water and wastewater.

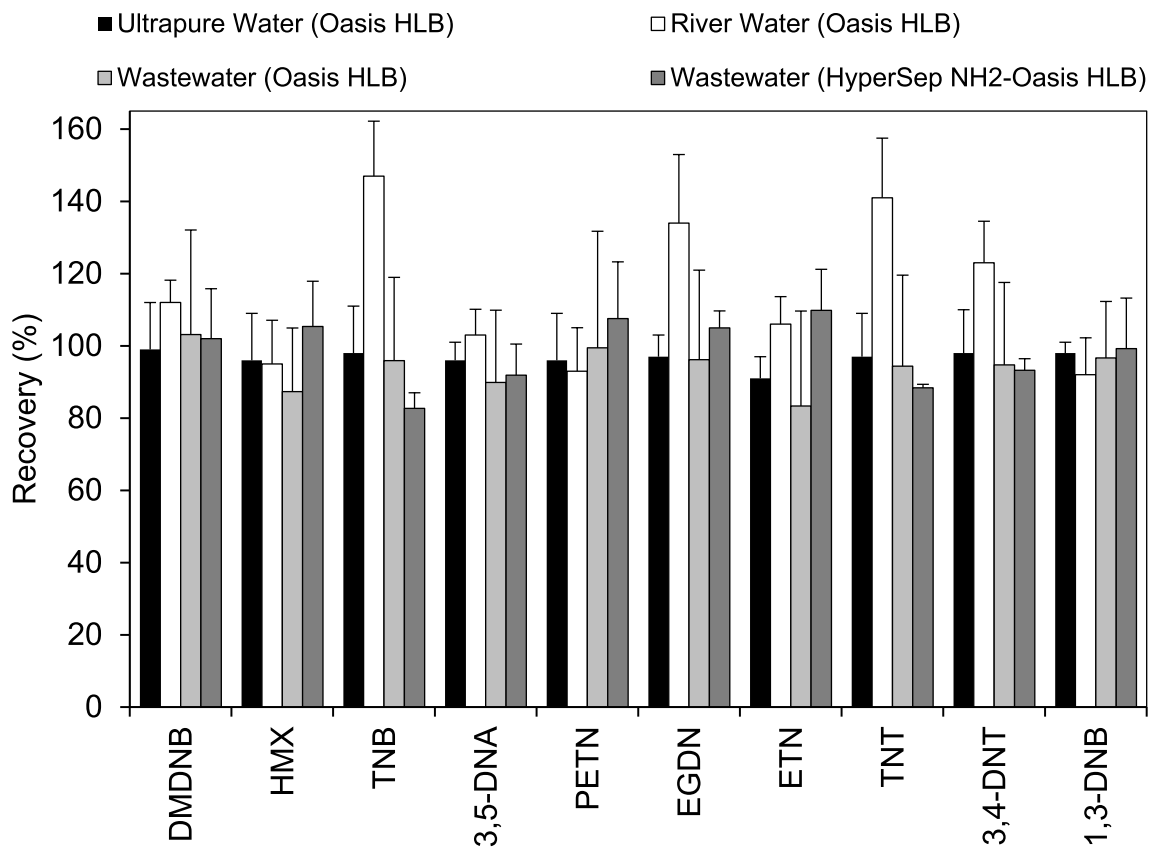


Fig. 2. Recoveries for a selection of 10 chemically diverse explosives from river water using single sorbent SPE; and wastewater after both single and dual-sorbent SPE. Error bars represent the standard deviations of triplicate recovery experiments.

For experiments where combined cartridges were used, two sorbents were connected in series with matrix removal sorbents configured first in the line of sample flow followed by the analyte-selective sorbent second. Most cartridges were conditioned with the same solution as the selective extraction cartridge. However, for anion exchange sorbent combinations (HyperSep-NH2 or HyperSep SAX), water containing 0.1% formic acid was used in the conditioning step. The addition of formic acid is required for anion exchange sorbents and, since not yet in use by FEL, this is a deviation from standard protocol. The sample was then loaded onto the dual-cartridge set-up. After loading, the matrix removal sorbent was discarded and the selective extraction sorbent washed with ultrapure water, dried and eluted as above.

2.3.2. Procedures for SPE of swab and soil extracts

The 20 mL extracts from matrix-contaminated swabs and soils were further treated according to the standard procedure used by FEL. Isolute ENV+ cartridges (100 mg, 6 mL) were conditioned with 1 mL ethanol:water (50:50 v/v), or 1 mL ethanol:water (50:50 v/v) containing 0.1% formic acid, and the 20 mL samples loaded at a rate of 1–2 mL min^{-1}. Other solvents were not considered since the developed method was to be compliant with standard routine procedures currently in use at FEL. Cartridges were eluted in 1 mL acetonitrile and extracts transferred to 2 mL septum capped crimped vials and stored at – 20 °C until analysis.

2.4. Instrumentation

Discussion of the development of the LC-UV and LC-HRMS methods used is not within the scope of this work and were previously optimised in-house (further details can be found in the SI). For the analysis of most UV-active analytes, an Agilent 1100 series LC instrument coupled to a diode array detector set at 210 nm and 254 nm was used during the initial optimisation stages of method development where no matrix was included (Agilent Technologies, Cheshire, UK). After trialling a number of different stationary phases with varying degrees of polarity (e.g., C_{18}, pentafluorophenyl, (bi)phenyl, etc.), an ACE C_{18}-AR (150 × 2.1 mm, 3.0 µm, Advanced Chromatography Technologies Ltd., Reading, UK) was chosen for the separations and was configured with a 1 cm ACE C_{18}-AR guard column (Hichrom Ltd, Reading, UK). This uses a C_{18} chain with an integral phenyl ring. Stationary phases with aromatic character were considered most beneficial as they added dipole-induced dipole and π-π interactions in addition to van der Waals interactions enabled by C_{18}. This column aided separation of nitroaromatics in particular and likely based on the respective location of the nitro group(s) on the phenyl ring. Amide-type stationary phases were not evaluated here, though they may offer some alternative selectivity based on fewer interaction mechanisms. The column oven was set to 20 °C and a 5 µL sample injection volume was used throughout. Binary gradient elution at 0.15 mL min^{-1} using 8 mM ammonium acetate in water:methanol 90:10 (v/v) (mobile phase A) and 8 mM ammonium acetate in water:methanol 10:90 (v/v) (mobile phase B) was carried out over 40 min. Initial mobile phase composition was 40% B, raised to 100% B over 30 min and then held for 10 min before returning to 40% B and equilibrating for 34.5 min (total run time = 75 min).

For all other analytes, and for evaluation of recovery and ion suppression in river- and wastewater extracts, analysis was carried out using an optimised LC-HRMS method on an Accela HPLC system coupled to an Exactive™ instrument (Thermo Fisher Scientific, San Jose, CA, USA) equipped with a heated atmospheric pressure chemical ionisation source (APCI), and operated in both positive (*m/z* 50–400) and negative modes (*m/z* 60–625) using full-scan high resolution (50,000 FWHM). MS conditions were optimised using both ESI and APCI, but APCI enabled the determination of more compounds by comparison and outweighed the higher signal intensity seen with ESI for the

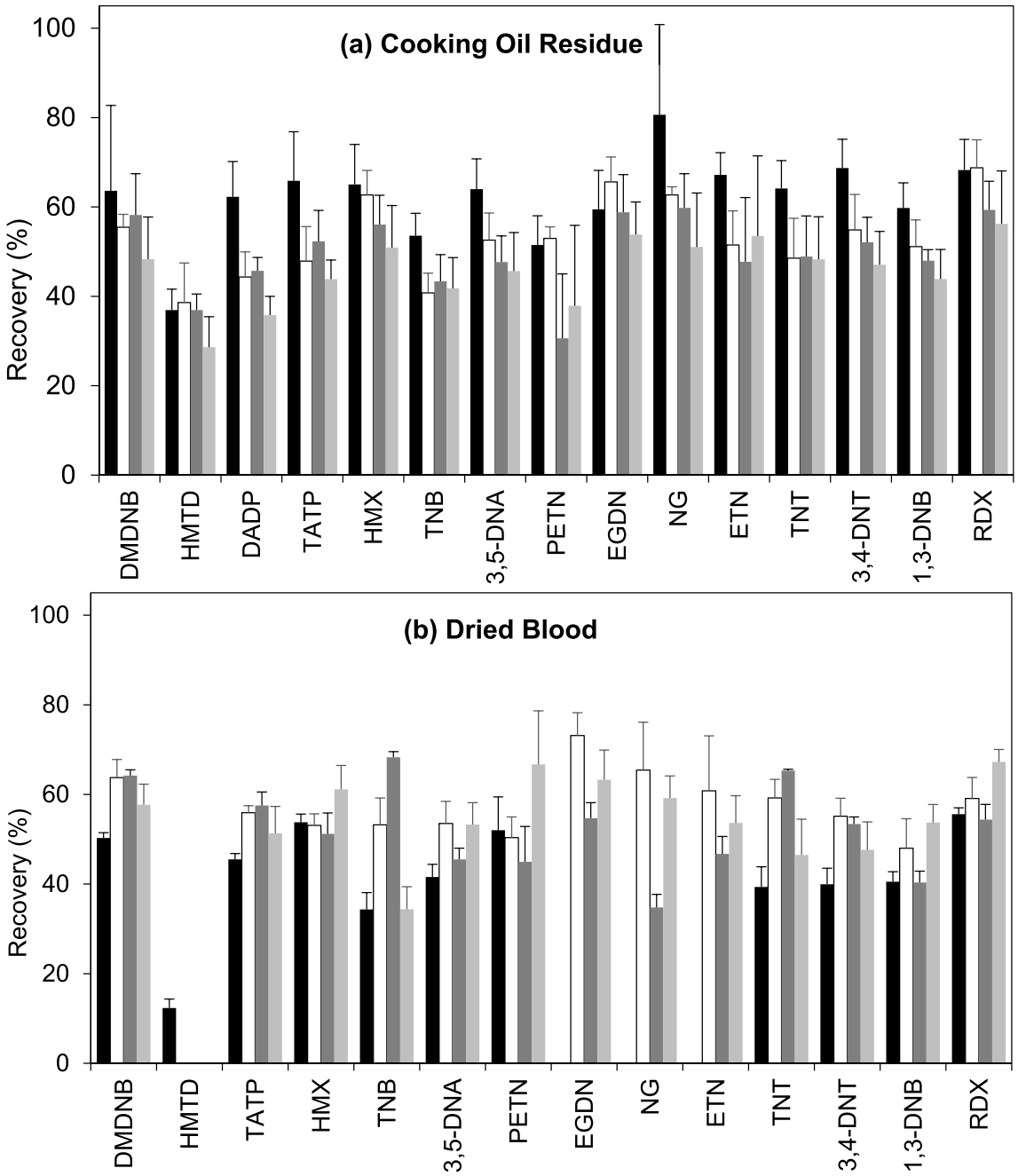


Fig. 3. Recoveries for a selection of n = 14 chemically diverse explosives from swabs of a) cooking oil residues and b) dried human blood using single- and dual-sorbent SPE combinations. Error bars represent the standard deviations of triplicate recovery experiments. For soil and dirt residue data, see Fig. S2.

compounds it could detect. This paper was focussed on the development of a broadly applicable sample preparation approach that could be adopted by practicing laboratories and the observation of more false negatives with ESI in general tipped the balance in favour of APCI as the preferred ionisation technique here (data not shown). The same C_{18}-AR column was used for the separations, temperature was maintained at 20 °C and injection volume was 5 µL. Binary gradient elution at 0.3 mL min^{-1} using 0.2 mM ammonium chloride in water:methanol 90:10 (v/v) (mobile phase C, apparent pH 7.5) and 0.2 mM ammonium chloride in water:methanol 10:90 (v/v) (mobile phase D, apparent pH 7.5) was carried out over 39 min according to the following programme: 40% D at 0 min; linear ramp to 95% D over 15 min; to 100% D over 0.50 min; hold at 100% D for 5.5 min; return to 40% D over 0.50 min; re-equilibration for 17.5 min. All analytes could be separated and detected using LC-HRMS under these conditions. Samples were kept at 10 °C throughout the analysis. The nebulising and desolvation gas in the ionisation source and collision cell was nitrogen and optimised conditions are given in Table S2 of the supplementary information. All data was processed using Thermo Xcalibur v 2.0 software.

2.5. Determination of recovery and MS detection matrix effect

Analyte recoveries were expressed as the percentage of the ratio of the measured analyte peak area in the extract by the analyte peak area in the corresponding matrix-matched standard at the theoretical 100% recovery concentration. Although the majority of these compounds were not ionisable in solution, the apparent pH of the mobile phase was maintained at 7.5 in order to leave flexibility for suspect screening of new acidic or basic compounds in future applications as needed. See SI for specific information about how recoveries from each sample type

were determined.

To determine the MS detection matrix effect, percentage ion suppression/enhancement was calculated using peak areas in matrix-matched standards prepared in reconstituted soil, swab (of dried blood, cooking oil and dirt residue, separately), river- and influent wastewater extracts (n = 3) and comparison to a 1 mg L^{-1} standard of all analytes in acetonitrile (see formula below). Background correction for compounds present in the blank samples was performed where necessary.

$$\%\ Matrix\ Effect\ = \frac{Analyte\ Peak\ Area (in\ a\ matrix - matched\ standard) - Analyte\ Peak\ Area\ (unfortified\ sample\ matrix\ extract)}{Analyte\ Peak\ Area\ (standard)} \times 100$$

2.6. Method performance assessment in untreated wastewater, cooking oil residue and dried blood

The ICH Harmonised Tripartite Guidelines for the validation of analytical procedures were used for assessment of method performance [33]. Using the optimal SPE cartridge combinations, limits of detection, linearity and range were evaluated separately across three selected matrices (untreated wastewater and contaminated swabs containing cooking oil residue and dried blood) as examples of environmental, chemical and biological sample types. Linearity of the method in 24-h composite wastewater samples was assessed by coefficients of determination (R^2) over the range of 0.625 ng L^{-1} to 50 µg L^{-1} (N ≥ 6). For oil and dried blood, swabs of each were spiked over the range 0.005 ng–2 µg on swab (the variance associated with the swab uptake process from the surface was therefore excluded here and beyond the scope of this work). LOD was obtained by calculation of three times the standard deviation of the response (at the lowest concentration at which the analyte could be seen with a S/N ≥ 10) divided by the slope of the calibration curve (n ≥ 5). Matrix-matched calibration using pooled samples as 'representative matrices' was carried out and, where any analytes were present, the background was subtracted before application to quantification in real samples. For method precision, and for comparison across all SPE cartridge combinations tested, the standard deviations of both the recoveries and matrix effects from Section 2.5 above and across all matrices were calculated and compared for statistical significance.

3. Results and discussion

3.1. Sorbent selection for matrix removal and explosives extraction

Based on our previous assessment of recovery of explosives across 34 commercially available SPE cartridges [16], three potential sorbents for high selectivity organic explosives extraction (Oasis HLB, HyperSep Retain PEP and Isolute ENV+) and four for matrix removal (HyperSep SAX, HyperSep NH2, Bond Elut CN and Strata Alumina-N) were initially chosen for recovery assessment (Table 1). Overall, very good analyte recoveries were obtained for the 44 explosive residues prepared in model solutions using all three analyte-selective extraction sorbents. While no statistically different recoveries were observed between these three cartridges, only Oasis HLB and Isolute ENV+ were chosen for further investigation. Firstly, method development work using Oasis HLB for explosives in wastewater has already been published [16] and Isolute ENV+ is currently used in the standard clean-up procedure by FEL for other sample types. Secondly, the use of polymeric divinylbenzene-based sorbents is in line with those used for the extraction of a range of different micropollutant classes from complex samples (e.g. illicit drugs and pharmaceuticals [15,34–36]), enabling potential later expansion of such methods to include additional compound types.

With respect to matrix removal sorbent selection, recoveries > 10% were measured for a number of explosives analytes on Bond Elut CN. Therefore, this sorbent was eliminated from further consideration. With the exception of PYX, the remaining three sorbents showed little or no recovery for any analytes, with an average recovery of ≤1%. All three were taken forward to assess matrix removal performance.

3.2. Evaluation of dual-sorbent SPE for trace explosives in complex sample types

3.2.1. Aqueous matrices

Samples of influent wastewater and river water were used as examples of different aqueous environmental matrices. These were also chosen to potentially aid identification of clandestine explosives manufacture via the sewer network and improve monitoring of environmental exposure to toxicants. Oasis HLB was used as the analyte selective SPE cartridge for these sample types, based on our previous work [16]. Matrix effects were assessed first, and across several combinations, to identify whether there was any advantage to a dual sorbent approach. For river water the lowest matrix effect was generally measured using Oasis HLB alone. For wastewater, matrix effects improved using dual-sorbent SPE, and markedly so using the Hypersep-NH2-Oasis HLB combination (Fig. 1(a)). This difference in matrix effects across the two aqueous matrices highlighted the need for a versatile clean-up procedure that could be chosen based on sample type. Based on the assessment of matrix effects across all combinations of sorbents, it was decided to prioritise assessment of recovery from river water using Oasis HLB alone and from wastewater using both Oasis HLB and the Hypersep NH2-Oasis HLB combination (Fig. 2). Recoveries overall from both sample types and across SPE combinations were acceptable at ≥83%. Significantly higher recoveries (p = 0.019) from river water existed in comparison to model solutions, potentially due to a salting-out effect (the Thames river at London is brackish and tidal) [37]. Recoveries were significantly lower for wastewater than river water using Oasis HLB alone (p = 0.011), but when the dual sorbent combination was examined, no significant difference in recovery existed. No significant difference in recovery existed for explosives in model solutions and wastewater using the dual SPE sorbent approach either. Therefore, at least for wastewater, the Hypersep NH2-Oasis HLB combination showed a clear advantage, both in terms of recovery and matrix effects.

3.2.2. Topsoil

Recoveries and matrix effects for soil are shown in Fig. S1 (c) and Fig. S2 (a) of the SI. Strata Alumina-N coupled with Isolute ENV+ was unsuitable since, although good recoveries were seen for the majority of analytes, ETN, NG and EGDN were not detected in spiked soil extracts using this combination. Generally, average recoveries were highest with the HyperSep SAX – Isolute ENV+ combination, which is mirrored also in the lowest average matrix effects being seen for 9 of the 15 analytes with this approach. A cleaner sample loaded onto the selective extraction sorbent, after initial clean-up by HyperSep SAX, therefore seemed to reduce competitive uptake of interfering matrix components and, as a result, recoveries increased overall. Since a large component of dissolved organic matter in liquid extracts of soil are humic acids [38], it was postulated that the strong anion exchange sorbent would be effective for their removal. Samples were loaded at approximately pH 6.0, therefore the majority of the humic acids would likely have been in their deprotonated state and retained by the first anion exchange matrix removal cartridge. The analytes were not retained by this cartridge, but progressed to the second analyte extraction cartridge.

3.2.3. Cooking oil, dirt and dried blood-contaminated swabs

In contrast to soil, Strata Alumina-N combined with Isolute ENV+ gave the lowest average matrix effects for most analytes and across

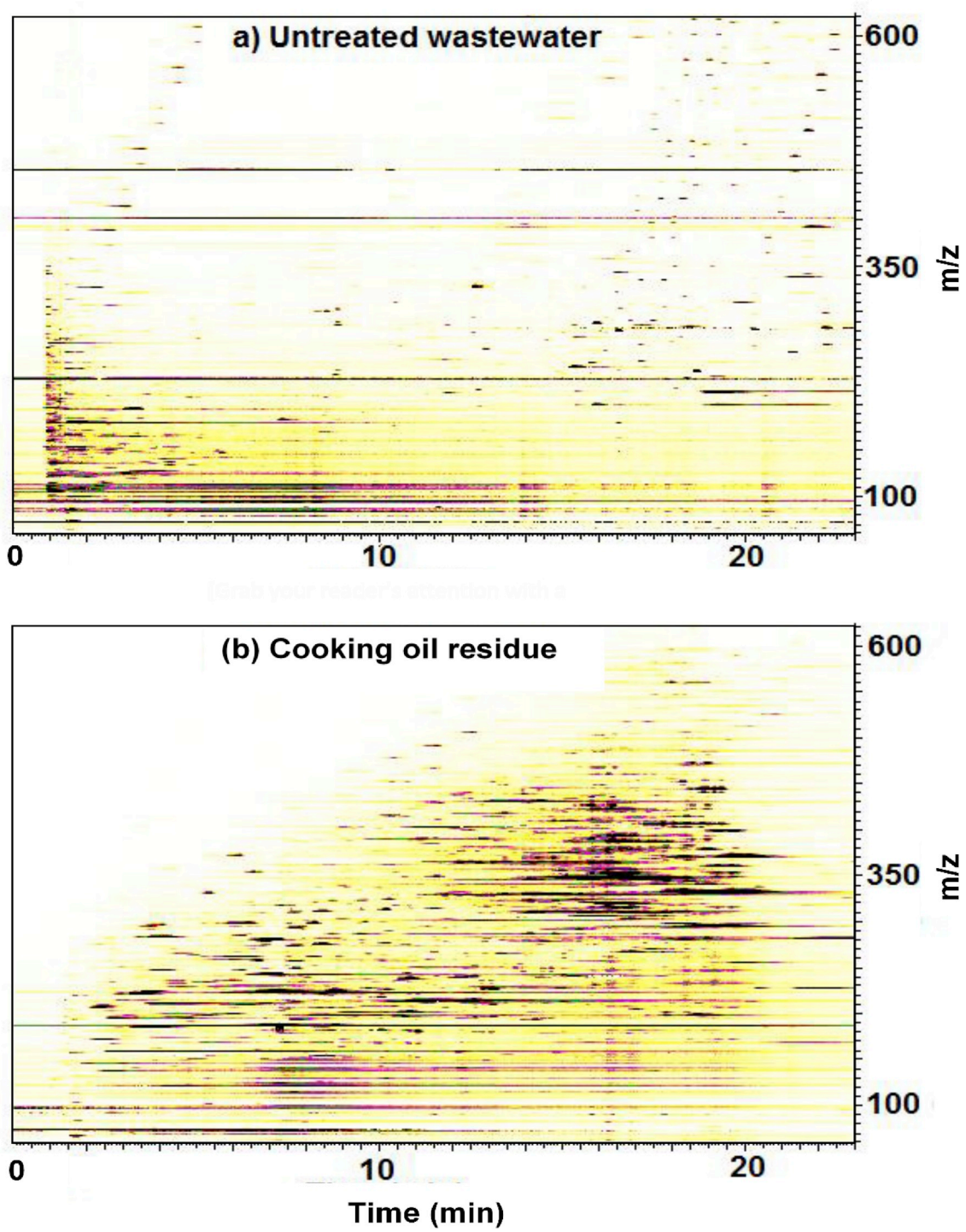


Fig. 4. Example differential LC-HRMS ion plots for untreated wastewater (positive mode) and cooking oil residue (negative mode) showing the additional degree of matrix removal using the optimised dual-SPE approach. Ion plots were generated by subtraction of single-SPE from dual-SPE LC-HRMS ion plots. For all other sample types, and for individual LC-HRMS ion plots of single and dual SPE treated samples, see Figs. S3–S5.

all swabbed sample types. Strata Alumina-N is a neutral sorbent and hence good for the removal of strongly lipophilic compounds, such as fats in cooking oil, black carbon and organic matter in dirt residues and organic biological components in blood. The worst matrix effects were observed for cooking oil residues (Fig. 1 (b)). Strong suppression for all analytes but EGDN was observed when a single-sorbent was used. This could lead to potential false negatives and/or inaccurate quantification, both of which are undesirable in high sensitivity forensic analysis. A paired *t*-test, however, showed matrix effects were significantly lower using a Strata Alumina N - Isolute ENV+ combination ($p = 0.010$, where p is the probability value that the two observations are not significantly different). For dirt (road sign residue) and blood (Fig. 1 (c)), matrix effects in general were lower, with the exception of TNB. Strong signal enhancement was observed for this analyte, regardless of SPE combination. Arguably, suppression would be considered a worse scenario from a qualitative perspective.

Recoveries, on average, from cooking oil (Fig. 3 (a)) and dirt residues were slightly better using a single Isolute ENV+ sorbent. This

Table 2
Method performance for 14 analytes in three complex matrices; untreated wastewater, cooking oil residue and dried blood.

Analyte	Mass Accuracy (δ ppm, n = 6)			Linearity (R^2)[a]			Range[b] (pg on column)			Limit of Detection (LOD)[c] (fg on column)			
	Cooking Oil Residue	Dried Blood	Untreated Wastewater	Cooking Oil Residue	Dried Blood	Untreated Wastewater	Cooking Oil Residue	Dried Blood	Untreated Wastewater	Cooking Oil Residue	Dried Blood	Untreated Wastewater	Published LOD in Untreated Wastewater [20]
DMDNB	−1.39	−0.70	−0.01	0.99	0.99	1.00	50–250	25–250	12.5–125	21,180	33,850	8840	–
HMTD	−0.95	−0.87	−0.38	0.99	0.97	0.99	25–250	25–250	12.5–125	13,100	19,380	7020	25,000
TATP	−2.25	−2.23	−1.96	0.99	0.99	1.00	25–250	25–250	12.5–125	410	20,660	6210	10,000
HMX	−0.84	−0.91	1.19	0.95	1.00	0.98	0.25–250	0.25–250	0.125–125	40	40	20	200
TNB	0.60	−0.54	2.24	0.96	1.00	0.98	0.25–250	0.25–250	1.25–125	90	20	360	–
3,5-DNA	−0.73	−1.68	1.11	0.97	1.00	1.00	0.25–250	0.25–250	0.125–125	30	70	30	–
PETN	−1.10	−3.18	0.03	0.98	0.99	0.99	50–250	25–250	12.5–125	540	25,700	460	4950
EGDN	−0.19	−1.02	1.80	1.00	0.99	1.00	100–500	100–500	25–250	48,750	55,040	12,950	100,000
NG	−0.19	−1.24	1.77	0.99	0.99	0.99	100–500	100–500	50–250	70,270	51,120	31,910	nd
ETN	−0.16	−1.10	1.58	0.98	0.98	0.99	50–500	100–500	50–250	14,540	31,680	41,110	nd
TNT	−0.04	−1.39	2.00	0.97	0.99	0.98	0.25–250	0.25–250	0.125–125	30	150	10	170
3,4-DNT	0.38	−0.80	1.88	0.97	1.00	0.98	0.25–250	0.25–250	1.25–125	130	120	790	150
1,3-DNB	−0.26	−0.08	1.57	0.97	0.99	0.98	0.25–250	0.25–250	0.125–125	10	70	10	–
RDX	−1.44	−2.65	0.45	0.97	0.99	0.98	2.5–250	0.25–250	1.25–125	10	50	30	200

nd Not detected.
- Data not available.
[a] Based on N ≥ 5 concentrations and analysed by dual-SPE and LC-HRMS. Background subtraction was applied to neat samples, where required.
[b] Range expressed for measured calibrants only.
[c] Determined using 3 x standard deviation of the peak area of a lower concentration range matrix-matched standard (n = 3) divided by the slope of the calibration line. Matrix-matched calibration was carried out using pooled samples as 'representative matrices' and, where any analytes were present, the background was substracted before quantification in real samples.

benefit was, however, offset by a marked reduction in matrix effects using a Strata Alumina-N - Isolute ENV+ combination. The poorest recoveries overall were observed for blood (Fig. 3 (b)) and were significantly lower when Isolute ENV+ was used alone compared to any of the dual-SPE approaches ($p < 0.05$ in all cases), presumably due to sorbent capacity exceedance and/or competitive sorption of matrix. EGDN, ETN and NG were only detected in blood using a dual-SPE approach and recovery was again best for the Strata Alumina-N - Isolute ENV+ combination. HMTD was not observed in blood matrices using a dual sorbent combination. Although it was recovered by Isolute ENV + alone, its very low recovery of just 12% is unreliable and likely due to the fact that HMTD does not form gas phase ions easily, mainly due to its instability [39].

Fig. 4 shows one positive mode example (wastewater) and one negative mode example (cooking oil residue) of differential full-scan LC-HRMS data for all ions that were removed specifically by the used dual-sorbent approaches. The data for all six matrices and the comparisons of the full-scan ion plots between single- and dual-sorbent SPE in both positive and negative modes can be seen in Figs. S3–S5 of the SI. The extent of matrix removal differed across matrices, but in all cases it was clear that a large number of potential interferences were substantially reduced or even removed. Oil residue and untreated wastewater were the most complex sample types and the dual-SPE approach was particularly effective for these, removing ions across the entire chromatographic run and m/z range. For topsoil, dried blood and dirt residue, the majority of matrix removed by the dual-SPE approach occurred across a retention time range where most explosives eluted (6–12 min) and also within the particular m/z range in which most of them are detected ($m/z \leq 250$). This explains why ion suppression/enhancement improved as a result of the combined sorbent approach.

3.3. Method performance assessment

Analytical method performance with respect to mass accuracy, linearity, range and limit of detection was evaluated for the subset of 14 analytes using the three most challenging matrices tested (i.e. wastewater, cooking oil residue and dried blood). Results are presented in Table 2. For most analytes, mass inaccuracy was < 2 ppm across all matrices and all lay < 5 ppm. For linearity, coefficients of determination were ≥0.99 in many cases. Linearity assessment here for cooking oil and dried blood did not include the recovery step for explosives from a surface, but represented incremental concentrations spiked directly onto swabs already contaminated in matrix and extracted subsequently. Linearity was particularly good for wastewater, which was pre-spiked with explosives before dual-SPE, and very low LODs at the low fg-pg on column level were observed across all sample types using the dual-SPE approach. In particular, HMX, RDX, 3,5-DNA, TNT and 1,3-DNB yielded excellent LODs between 10 fg-70 pg across all three matrices, with a median value of 435 fg. For wastewater in particular, the dual-SPE approach yielded an approximate 10-fold improvement for the majority of the analytes in comparison to a previously published method using single-sorbent SPE [16]. Increased sensitivity through reduced matrix effects, coupled with little compromise to recovery, highlighted, once again, the advantage of the proposed novel and flexible clean-up procedure in comparison to that currently in use.

3.4. Application to untreated wastewater

Although the method was validated for wastewater, cooking oil residues and dried blood, it was not applied to real samples of dried blood or cooking oil as this would have required access to forensic casework samples, which was not permitted. The optimised method was applied to an untreated 24-h composite wastewater sample. Clear signals for $[M]^-$ were detected for TNT, 3,4-DNT and 1,3-DNB (Fig. 5), but each were present at intensities below their limits of quantification of 6 ng L^{-1} (defined as 10 times the standard deviation of the response of a low-level standard divided by the slope of the calibration curve). Identification was performed by matching retention time (all < 2.5%) and accurate mass (< 5 ppm) relative to spiked wastewater samples. Retention times all lay within 1.0% of their corresponding reference values. For both TNT and 3,4-DNT, very low intensity $[M-H]^-$ ions were also observed, but these were very close to the LOD. No additional qualifier ion was detected for 1,3-DNB. HRMS signals for $[M]^-$, $[M-H]^-$ and $[M-OH]^-$ and matching retention data also allowed

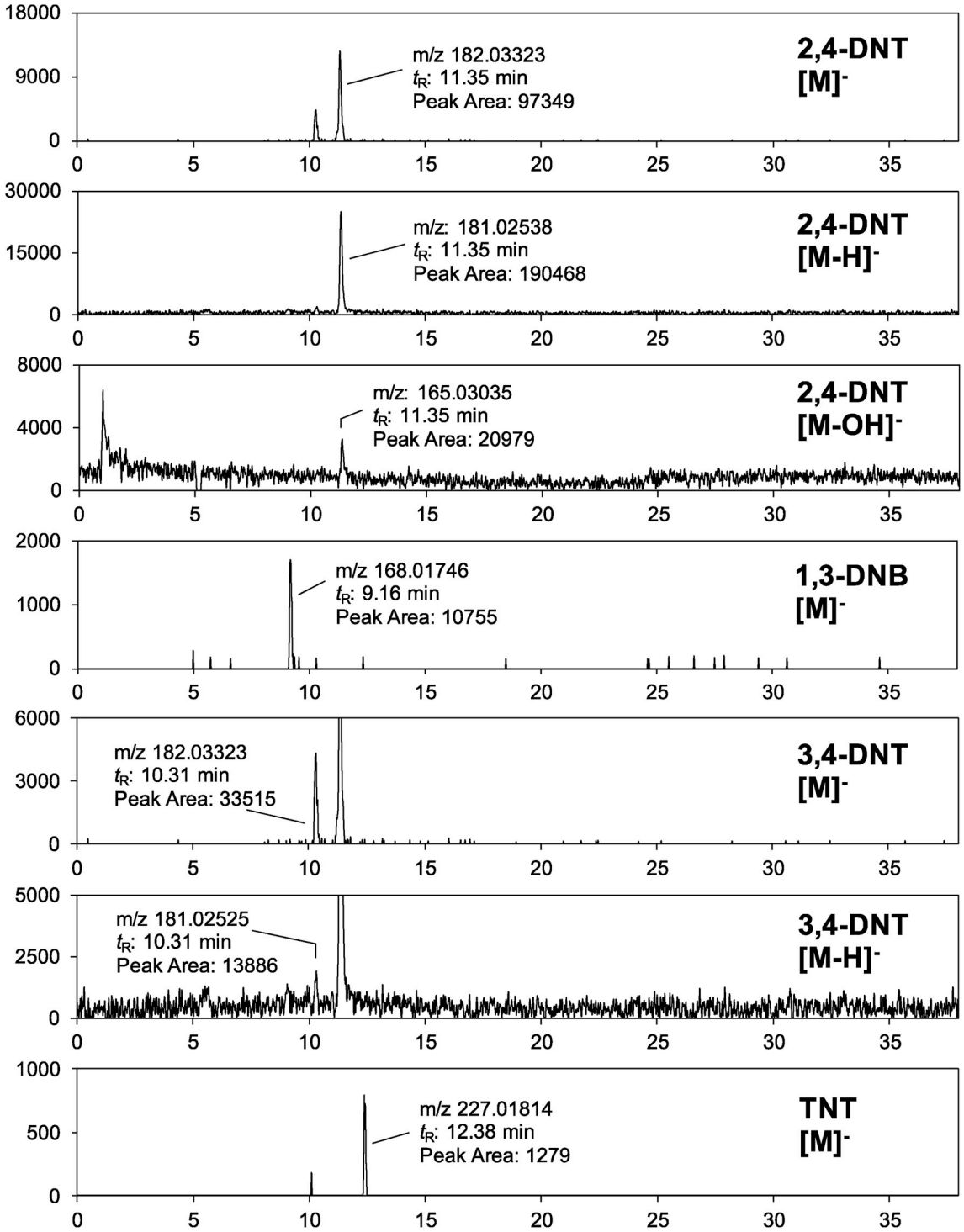


Fig. 5. Extracted ion chromatograms corresponding to *m/z* of 2,4-DNT, 1,3-DNB, 3,4-DNT and TNT in unspiked influent wastewater (parent ion $[M]^-$ is shown for each and followed by any qualifying ion signals). The peak area for the $[M - H]^-$ qualifying ion for TNT (*m/z* 226.01030) was 331 and the data is not shown.

identification of 2,4-DNT (which was quantified previously in Ref. [16] at 279 ng L^{-1} or 332 g day^{-1} entering the treatment works from its 3.5 million population equivalent catchment). The increased sensitivity offered here by the dual-SPE approach therefore enabled detection of more nitrotoluene-based explosives in municipal wastewater of a major capital city for the first time. Determination of relative occurrences of explosive residues in wastewaters from other major cities is now important, in order to characterise background thresholds and possibly identify illegal manufacture of explosives, as well as the potential for environmental toxicity after treatment.

4. Conclusion

The effectiveness of dual-sorbent SPE for the selective extraction of multiple classes of explosives, as well as enhanced removal of matrix interferences from a range of different sample types, was demonstrated for the first time. This approach was particularly effective for very complex samples and especially oil, blood, topsoil and untreated wastewater. One combination was not found to be universally applicable to all sample types, but was mostly dependent on the matrix removal sorbent used. The dual-sorbent SPE approach resulted in a decrease in

overall HRMS matrix effects, reducing the likelihood of false negatives and improving quantitative precision and accuracy. Excellent detection limits for all compounds were achieved across three different and highly complex matrices (dried blood, cooking oil residues and wastewater) with a median of 435 fg. Finally, as a real application, four nitroaromatic based explosive residues were detected at the low-sub ng L^{-1} concentration level in untreated London wastewater for the first time (TNT, 1,3-DNB, 3,4-DNT and 2,4-DNT). The dual-sorbent SPE approach presented herein represents a more widely applicable sample preparation methodology for high-assurance and selective detection of explosives in complex sample types. Using full-scan LC-HRMS for analysis, post-hoc data mining is also possible for new/suspect compound identification (e.g. 2,4-DNT shown here) and represents a much more flexible and powerful approach to forensic investigation of trace explosives occurrence.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.talanta.2019.05.047.

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