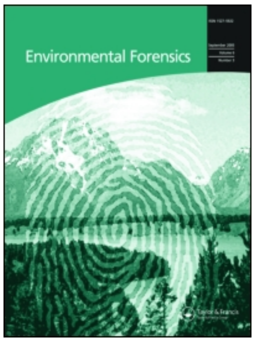

Environmental Forensics

ISSN: 1527-5922 (Print) 1527-5930 (Online) Journal homepage: http://www.tandfonline.com/loi/uenf20

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To cite this article: Sally C. Gamble, Luiza C. Campos & Ruth M. Morgan (2017) Detection of trace peroxide explosives in environmental samples using solid phase extraction and liquid chromatography mass spectrometry, Environmental Forensics, 18:1, 50-61, DOI: 10.1080/15275922.2016.1263901

To link to this article: http://dx.doi.org/10.1080/15275922.2016.1263901

ENVIRONMENTAL FORENSICS
2017, VOL. 18, NO. 1, 50–61
http://dx.doi.org/10.1080/15275922.2016.1263901

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Detection of trace peroxide explosives in environmental samples using solid phase extraction and liquid chromatography mass spectrometry

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ABSTRACT

This article presents solid phase extraction (SPE) and liquid chromatography-mass spectrometry (LC-MS) methods for the trace detection of the peroxide explosives triacetone triperoxide (TATP) and hexamethylene triperoxide diamine (HMTD). Furthermore, experimental studies use these methods to explore the efficiency of wastewater treatment plant (WWTP) processes at removing trace levels of peroxide explosives from water samples to assess the application of the developed methods for the detection of explosives in the environment. The principal results of this study showed that the greatest removal of TATP and HMTD from spiked water samples occurred following the biological treatment stage, however, the WWTP processing did not completely remove all of the analytes from the water, suggesting that such chemicals could contaminate downstream river water samples. The toxicity of chemical pollutants is often determined by their concentration, however, even at trace levels, the monitoring of explosives in the natural environment could be extremely informative for the detection of criminal activity as well as long-term effects upon aquatic life. These findings also have significant implications for crime prevention and disruption approaches that can use this type of data as intelligence to guide investigations regarding the source and attribution of detected explosives.

KEYWORDS
Peroxide; explosives; wastewater; removal; intelligence; forensic

Introduction

The use of organic peroxides to make homemade explosives (HMEs) is becoming increasingly popular among terrorists due to the precursor materials being relatively inexpensive and easy to acquire (Widmer et al., 2002). Peroxide explosives are organic compounds that contain at least one peroxide functional group (R-O-O-R) and are usually cyclic in shape. Such compounds are capable of producing an explosive reaction similar in magnitude to that of a high explosive.

Common chemicals used for the manufacture of homemade peroxide explosives include hydrogen peroxide, acetone, and hexamine. These chemicals, as well as the resulting explosives, may enter the household wastewater system during the manufacturing process in several ways; for example, sinks, bathtubs, and toilets are often used in the manufacture, storage, and disposal of chemicals and explosive materials.

The peroxide explosives triacetone triperoxide (TATP) and hexamethylene triperoxide diamine (HMTD) were both used in the initiation mechanisms of the improvised explosive devices (IEDs) that were responsible for the London 7/7 terrorist bombings and the unsuccessful detonation of further coordinated attacks in London two weeks later (Lefkowitz, 2008; BBC News, 2011). The increased use of IEDs in terror attacks (Widmer et al., 2002; King et al., 2013) means that there is a greater possibility of finding TATP and HMTD compounds in the environment. In addition, the identification of these compounds offers the possibility of discovering the locations where such HMEs are being manufactured through the urban wastewater system. The identification and quantification of these explosives and precursor chemicals in environmental samples can provide information regarding their spatial and temporal distribution within the wastewater system, and thus provide data that can offer insights to their provenance. This is an approach similar to that of “sewage epidemiology” used to back-calculate drug consumption per population (Zuccato et al., 2008;

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Loos et al., 2009; Karolak et al., 2010; Postigo et al., 2010; Valcárcel et al., 2010; Baker and Kasprzyk-Hordern, 2011; Irvine et al., 2011; van Nuijs et al., 2011; Thomas et al., 2012; Lai et al., 2013).

The development of methods for the analysis of peroxide explosives and evaluation of their removal from the environment by wastewater treatment plants (WWTPs) will contribute to the estimation of the extent to which a wastewater analysis approach may be suitable for the detection of trace explosives. The information gathered could offer additional intelligence concerning geographical hotspots where peroxide explosives have been detected in urban wastewater systems, and ultimately the original source of their entry into the wastewater system.

The detection of trace military explosives in seawater, lakes, rivers, soil, and industrial wastewater has been carried out on a worldwide scale following concerns regarding their presence in the natural environment from munitions factories used in World Wars I and II (Darrach et al., 1998; Furton et al., 2000; Psillakis and Kalogerakis, 2001; Monteil-Rivera et al., 2004; Pan et al., 2006; Guan et al., 2007; Ahmad et al., 2008; Ochsenbein et al., 2008; Barreto-Rodrigues et al., 2009; Babaee and Beiraghi, 2010; Schramm et al., 2016). However, in order to monitor explosives throughout the wastewater system and harness any information as an intelligence source, first it is essential to establish working methods for the analysis of peroxide explosives and to understand how they behave in complex environmental matrices (Morgan and Bull, 2007; Chisum and Turvey, 2011).

To date there has been no published research that outlines the detection of precursor chemicals and/or the resulting peroxide explosives in the urban wastewater system, and certainly not in the context of crime prevention through environmental surveillance for counter-terrorism applications. There is also limited published research regarding suitable methodologies for the extraction, detection, and quantification of peroxide explosives and their precursor chemicals (Crowson and Beardah, 2001; Widmer et al., 2002; Xu et al., 2004; Song-im et al., 2012). Recent advances include methods for the direct detection of peroxide explosives from ambient surfaces without the need for any sample preparation (Cotte-Rodríguez et al., 2008; Rowell et al., 2012; Cooks et al., 2006), which is a very useful technique for in situ trace analysis at airports, for example; however, these techniques would not be suitable for wastewater analysis purposes where a cleanup and preconcentration stage would be essential.

The role of forensic science in the prevention and disruption of terrorist activities is an area that is attracting increased attention. The potential value of forensic intelligence for the detection and reconstruction of crime events has been well discussed (Fraser, 2000; Briody, 2004; Bradbury and Feist, 2005; Ribaux et al., 2006, 2010; Rossy and Ribaux, 2013). However, the harnessing of forensic intelligence to aid in the identification of surveillance targets has not been fully explored in the published literature. A key issue for being able to use forensic intelligence in this manner is the establishment of suitable frameworks that enable the interpretation and implementation of that intelligence in a robust manner that enables the weight and significance of the analysis to be used effectively and appropriately in a given investigation.

The data generated from targeted analyses of wastewater have the potential to be fed into an intelligence-gathering network, which contributes to narrowing down and identifying geographical hotspots where illegal HME manufacturing is occurring. Intelligence of this type can be used to direct investigations and counter-terrorism operations to assist in the prevention and disruption of criminal activities such as the assembly of IEDs. In order to address this potential, the aims of this study were to develop working methods for the extraction and analysis of HMTD, TATP, and a precursor chemical, hexamine, and to investigate their removal at various stages of the wastewater treatment process using a laboratory-based batch reactor.

Materials and methods

A simulated WWTP was designed and set up in the laboratory in order to process spiked water samples and measure the amount of analytes present before the WWTP process and following each of the processing stages, using solid phase extraction (SPE) and liquid chromatography-mass spectrometry (LC-MS). See Figure 1.

Sampling

Thames river water samples were collected on three separate occasions in June, July, and October 2013 in order to replicate the experiment with three different sets of water samples at different times of the year. Four 1 L Nalgene bottles were acid washed in 2% hydrochloric acid for 24 hr, and rinsed with distilled water and deionized water prior to sample collection. Immediately before collection, the bottles were rinsed in the river water itself at the location by Battersea Bridge in London, UK. The river water samples were tested ($n = 3$) for their pH, temperature, dissolved oxygen, turbidity, and conductivity at the time of collection using water chemistry meters with attachable probes. The oxygen content was also measured following five days of incubation in a water bath at 20°C in order to calculate the biochemical oxygen demand $(BOD)_5$ of the

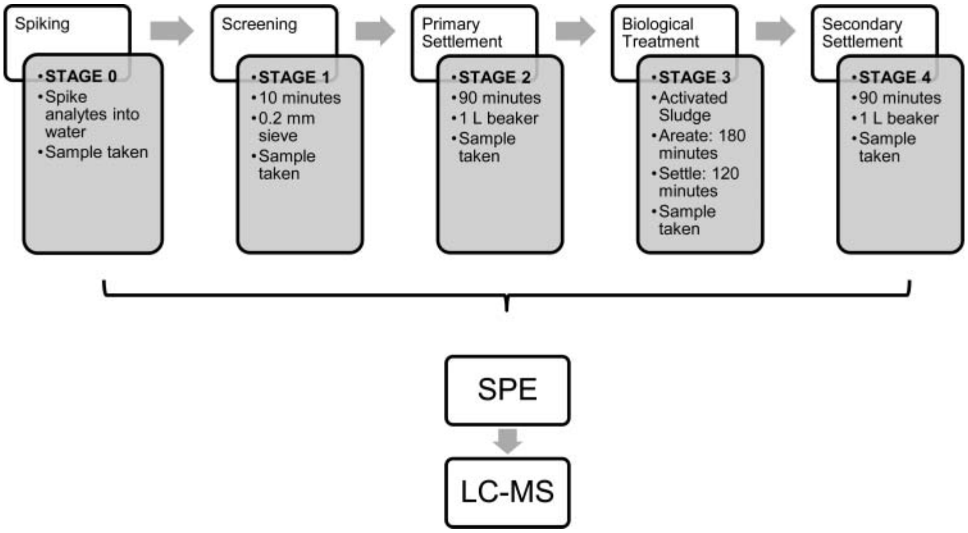

Figure 1. Laboratory WWTP batch processor and analytical methods.

water samples. These measurements give a good indication of the quality of the river water being analyzed and indicated that these samples were a suitable proxy for wastewater samples, based upon data obtained from Thames Water Utilities Ltd. (Thames Water) concerning the quality of the final effluent from Mogden WWTP throughout 2013, which reported BOD_5 values between 3.18 and 11.49 mg/L.

The river water samples were stored at 4°C for up to 24 hr or longer at −20°C in order to preserve the water composition, as described by Baker and Kasprzyk-Hordern (2011); however, it is noted that the samples were not acidified due to the potential for acid hydrolysis of the analytes (Pachman and Matyáš, 2011).

Thames Water's Mogden wastewater treatment plant in Twickenham, UK, provided three 1 L samples of mixed liquor suspended solids (MLSS) of approximate concentration 4000 mg/L, containing microorganisms and nonbiodegradable suspended matter, in June, July, and October 2013. Each of these MLSS samples was used in the biological treatment stage of the processing, within five days of their collection, as advised by Thames Water (due to the sample microorganismal content).

Materials

Peroxide standard solutions TATP and HMTD were purchased from Kinesis (St. Neots, UK) in 1 mL ampoules at 0.1 mg/mL. Hexamine and methanol (HPLC grade) were purchased from Sigma Aldrich (Gillingham, UK), and acetonitrile (HPLC grade) and hydrochloric acid (ACS reagent, 37%) were purchased from Fisher Scientific (Gillingham, UK). Deionized water was provided by a PureLab Ultra dispenser from Elga (Marlow, UK). The air pump for activated sludge aeration was purchased from Interpet Aquarium Accessories (Northcote Pet Shop, London, UK); the water quality testing was carried out using a Water Chemistry Meter HQ30d with attachable pH, temperature conductivity, and dissolved oxygen probes (Hach Lange, Salford, UK); and turbidity was measured using a Thermo Orion Aquafast II AQ2010 (Thermo Scientific, Gillingham, UK).

Laboratory WWTP batch reactor

The four main processes used at the WWTPs in Greater London were simulated as accurately as possible in the laboratory, taking into account the process, the timings, and the scale of real WWTPs. The laboratory WWTP setup was operated in batches rather than in a

Table 1. The four wastewater treatment processes recreated to generate water samples for analysis.

Stage	Process	Treatment aim
1	Screening	Remove large materials that could damage the treatment equipment and that cannot be treated such as wood, brick, rags, paper, and plastic.
2	Primary settlement	Minimize the suspended solids content by removing particles that will settle easily by gravity.
3	Biological treatment	Degrade the organic content of the sewage using sludge flocs formed by oxygen/air, and microorganisms, which produce less toxic end products and new biomasses.
4	Secondary settlement	The final treatment stage; further improve the quality of the effluent before it is discharged, e.g., into a river. Residual suspended matter are settled by gravity and recirculated to the activated sludge tank and removed for further treatment/disposal/reuse.

continuous process, making it easier to control the processing variables and to prevent cross-contamination of the analytes between batches. Table 1 details each of the four stages that were used to design the laboratory WWTP setup. Figure 1 describes the process followed to generate the samples for analysis. River water was used as a proxy for contaminated wastewater as the required quantity of real wastewater was not available due to health and safety concerns. This is a limitation to the experimental setup, as it is not assumed that river water would behave in exactly the same manner as wastewater. This study sought to explore the potential for the detection of trace levels of peroxide explosives in complex environmental matrices undergoing common treatment processes.

Spiking

First, 100 mL samples of river water were spiked with either 3 μg/mL of TATP or HMTD or 5 μg/mL hexamine. These levels were chosen based upon the maximum analyte concentration available and the lower limit of detection based upon the performance of the extraction and the instrumental methods. Un-spiked river water samples were used to run a separate control batch to validate that there were no pre-existing detectable levels of the analytes in the river water or MLSS samples. Measurements of the analytes in river water samples over the processing period time (8 hr 10 min), without undergoing any processing, were also taken in order to assess the stability of the analytes in the matrix being tested over the course of the experiment.

Screening

The 100 mL spiked water samples then underwent screening through a 0.2 mm sieve into a glass volumetric flask. Glassware was used wherever possible for the apparatus in the batch WWTP to reduce any potential adsorption of analytes to the apparatus itself. The flow rate of the spiked samples through the sieve was timed to be 10 mL per minute, taking 10 min to complete the process.

Primary settlement

The first of two settlement stages was achieved by pouring the spiked post-screen sample from the volumetric flask into a 1 L glass beaker and leaving it for 90 min to settle the suspended solids by gravity. The top of the beaker was covered with parafilm in order to prevent any evaporation of the analytes from the beaker.

Biological treatment

Following the primary settlement stage the supernatant was removed by pipette and added to the 1 L glass beaker containing a MLSS 4000 mg/L sub-sample. Biological treatment consisted of MLSS containing live microorganism cultures and nonbiodegradable suspended matter, which was aerated with the spiked water sample using an air pump to generate a continuous oxygen supply. The spiked water sample was aerated for 180 min and left to settle for 120 min. Recirculation of the settled matter as activated sludge was not simulated so as to prevent cross-contamination of the analytes between batches. The top of the beaker was covered with parafilm in order to prevent any evaporation of the analytes from the beaker.

Secondary settlement

The supernatant from the biological treatment stage was removed by pipette and added to a second 1 L settlement beaker. The sample was left to settle by gravity for 90 min. The top of the beaker was covered with parafilm in order to prevent any evaporation of the analytes from the beaker.

A 6 mL sub-sample was collected following the initial spiking stage (stage 0) and after each of the water treatment processes (stages 1–4) for solid phase extraction and analysis by liquid chromatography-mass spectrometry; see Figure 1.

Solid phase extraction (SPE)

Solid phase extraction cartridges were used to clean up the samples and extract the analytes for LC-MS analysis. The following four solid phase extraction columns were compared: Bond Abs Elut Nexus 200 mg, 6 mL (Agilent, Stockport, UK), TELOS ENV 200 mg 6 mL and TELOS neo PRP 200 mg 6 mL (both from Kinesis, St. Neots, UK), and Oasis HLB 6cc 200 mg (Waters, Elstree, UK). River water samples were spiked separately at the following concentrations: hexamine 5 μg/mL, TATP 3 μg/mL, and HMTD 3 μg/mL. The average removal efficiencies of each cartridge for each analyte were calculated. The methodologies for the Oasis HLB and Nexus columns were based upon those stated in Song-im et al. (2012) and the methodologies for the TELOS ENV and neo PRP columns were modified from the manufacturers' recommendations (see Table 2).

Instrumental analysis

Conditions were optimized on a Shimzadu LCMS-IT-TOF apparatus in order to establish working parameters for the identification and quantification of TATP, HMTD, and hexamine. Stock solutions of each analyte were diluted to 5 μg/mL in acetonitrile and analyzed independently, varying one parameter at a time. Methods described in Widmer et al. (2002), Xu et al. (2004), and

Table 2. Summary of the methodologies used for each SPE column.

	Oasis HLB	Nexus	TELOS ENV	TELOS neo
Conditioning and equilibration	4 × 1 mL MeOH, 4 × 1 mL water	4 × 1 mL MeOH, 4 × 1 mL water	2 × 2 mL ACN, 2 × 2 mL MeOH, 2 × 2 mL water	6 mL MeOH, 6 mL water
Loading	6 mL sample	6 mL sample	6 mL sample	6 mL sample
Washing	4 mL 60% v/v MeOH/water	4 mL 60% v/v MeOH/water	2 × 2 mL water	6 mL 5% v/v MeOH/water
Elution	4 mL ACN	4 × mL ACN	2 × 2 mL ACN, 1 min soak, 2 × 2 mL ACN	2 × 2 mL ACN,1 min soak, 2 × 2 mL ACN

MeOH: methanol, ACN: acetonitrile.

Crowson and Beardah (2001) were used as a platform to optimize the following parameters: ionization source, injection volume, oven, curved desolvation line and interface temperatures, flow rate, and ion accumulation time.

Results and discussion

Water chemistry

Table 3 shows the results of the water chemistry testing. The BOD_5 of the samples collected fell within the acceptable range determined by the U.K. Environment Agency (2008) and was an indication that the water was of an expected level of pollution for a river in an urban location such as Central London.

Conductivity is another gauge of water quality but also can indicate the geology of the area, depending on the inorganic dissolved solids content, including positive and negative ions (U.S. Environmental Protection Agency, 2012). The higher the concentration of inorganic dissolved solids, which allow the water to pass an electrical current, the higher the conductivity of the water and thus the poorer the water quality.

The pH of the river is also another indicator of the quality of the water and thus how hospitable it is to living organisms. According to the national requirements for chemical parameters, the pH of rivers in the UK should fall between 6.5 and 10.0 (Severn Trent Water, 2004; UK Technical Advisory Group on the Water Framework Directive, 2008); the pH of the river water studied fell comfortably within this range.

The temperature measurements varied according to the time of year, as expected. Temperatures between 3 and 20°C are graded “excellent”; temperatures above this indicate increasingly poor quality and are a sign of unnatural warming as a result of pollution (Thames21, 2016). The average temperatures recorded in June and July were above 20°C, which according to the grading system would be described as “good.”

Table 3. Average ($n = 3$) pH, temperature, dissolved O_2, turbidity, conductivity, and biological oxygen demand measurements of the river water samples collected in 2013.

Month	pH	Temp. °C	Dissolved O_2 ppm	Turbidity NTU	Conductivity (μS/cm)	BOD_5 (mg/L)
June	8.57	20.12	8.5	240	669	12.83
July	8.93	21.65	10.3	200	662	12.02
October	8.04	13.02	10.2	50	654	13.77

The amount of dissolved oxygen available for the river life to use also gives an indication of the health of a river. The average amounts of dissolved oxygen measured ranged between 8 and 10 ppm, both of which values are graded as “excellent” (Thames21, 2016). Levels below 5 ppm can be stressful for aquatic life and lower than 3 ppm are too low for fish populations to survive (Oram, 2014).

Finally, the average measurements of the turbidity of the river water samples ranged between 50 and 240 nephelometric turbidity units (NTU), indicating a relatively large variation in the amount of particulate matter suspended in the samples collected. A high level of turbidity is expected in Thames water because of the silt and sediment in the riverbed, and will vary depending upon the time of year and when the sample is taken in relation to the Thames’s tidal cycle.

The overall quality of the river water used in this study was taken into account during the method development of the extraction and detection components of the experimental approach. Recent data published on the water quality of the Thames in 2014 (Thames21, 2016) showed that the temperature ranged from 7 to 25°C with a modal average of 18°C, dissolved oxygen levels ranged from 2 to 12 ppm with a modal average of 10 ppm, pH ranged from 6 to 8 with a modal average of pH 8, and the turbidity ranged from 12 to 240 NTU with a modal average of 14 NTU.

Solid phase extraction

The results of the four different SPE cartridges tested for extraction of hexamine, HMTD, and TATP are shown in Figure 2. The cartridge with the highest removal of hexamine (82.94% ± 5.6%) and HMTD (109.00% ± 5.2%) was the TELOS ENV cartridge (Kinesis) containing styrene and divinylbenzene

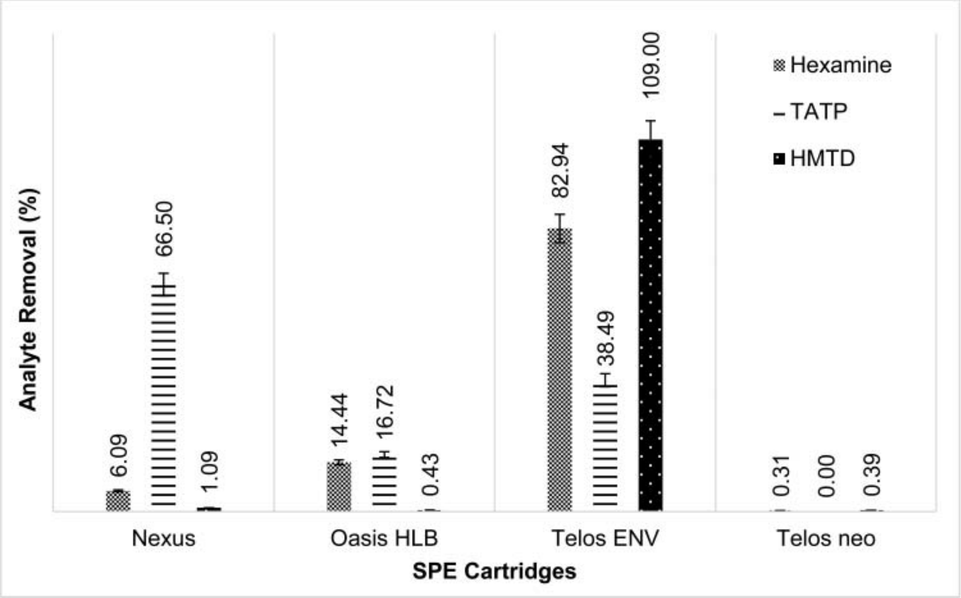

Figure 2. Percentage removal of hexamine, TATP, and HMTD from river water using four different solid phase extraction cartridges.

sorbent material. The Bond Abs Elut Nexus (Agilent) cartridge containing methyl methacrylate and divinylbenzene sorbent material had the highest recoveries of TATP (66.50% ± 3.1%) from the spiked water samples. The Oasis HLB and TELOS neo PRP cartridges both had poor removal of all the analytes tested. The methods developed were based upon individual extraction efficiencies for each analyte rather than for all the analytes in one mix.

LC-MS analysis

Table 4 outlines the optimized LC-MS parameters for each analyte for this study. The results of the optimization studies are shown in Figures 3–8, and the ions used to identify the analytes are shown in Table 5.

Table 4. Optimized parameters for detection of hexamine, TATP, and HMTD using the LCMS-IT-TOF instrument.

	Hexamine	TATP	HMTD
Injection volume (μL)	50	50	50
Column	Kinetex C18 100A 5 μM 100 × 2.1 mm		
Buffer	10 mM ammonium formate (aq) pH 8.22		
Mobile phase (isocratic)	10% buffer 90% methanol		
Flow rate (mL/min)	0.40	0.05	0.05
Oven temperature (°C)	40	20	20
Ion source and polarity	APCI positive mode		
Interface temperature (°C)	150	200	150
CDL and heat block temprature (°C)	100	100	125
Ion accumulation time (milliseconds)	50		
Detector voltage (kV)	1.75		
Total run time (min)	11	11	11
Scan range (*m/z*)	141.0500–141.0504	240.144–240.1444	209.0759–209.0763

The scan range on the LC-MS instrument was set to 0.004 *m/z* to increase the mass accuracy and the confidence that the ions detected were in fact the analytes being studied.

Ionization source

Initial analyses were performed with electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) in both positive and negative modes. The only ionization source and polarity that gave a signal for all three analytes was APCI in positive mode.

Injection volume

All three analytes gave a greater signal-to-noise response with the larger injection volume (50 μL), without compromising the peak shape, enabling greater sensitivity (see Figure 3).

Flow rate

Slower flow rates increased the signal-to-noise ratio for the TATP and HMTD responses, but the opposite was true for hexamine. There was a significant increase in signal for hexamine when the flow rate was 0.4 mL/min compared to 0.05 mL/min favored by the peroxides (see Figure 4).

Column oven temperature

A lower column oven temperature of 20°C gave a higher signal-to-noise ratio for both of the peroxides, which are prone to thermal degradation; it is thought that the higher column oven temperature could increase the dissociation of the HMTD and TATP ions. Hexamine, on the other hand, showed little difference in response to the column oven temperature, but the signal-to-noise

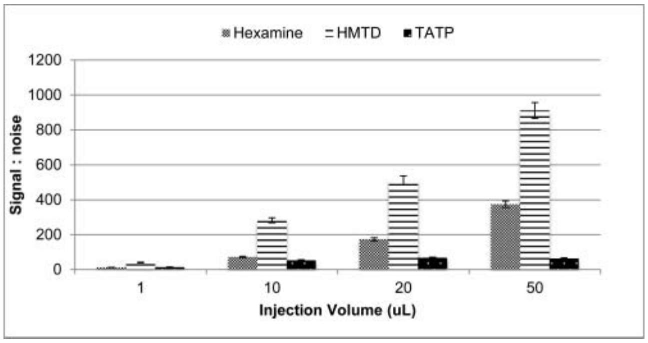

Figure 3. Signal-to-noise ratios for hexamine, HMTD, and TATP detection at varying injection volumes.

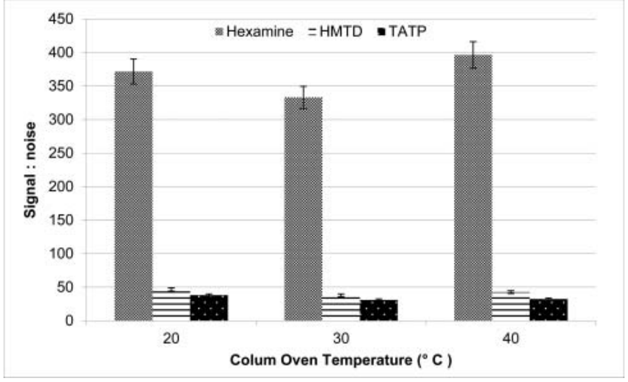

Figure 5. Signal-to-noise ratios for hexamine, HMTD, and TATP detection with varying column oven temperatures.

ratio was slightly increased with a higher temperature of 40°C (see Figure 5).

Curved desolvation line and heat block temperature

Both hexamine and TATP responses had a higher signal-to-noise ratio when the curved desolvation line (CDL) and heat block temperature was set to 100°C; HMTD had a slightly higher signal-to-noise ratio at 125°C (see Figure 6).

Interface temperature

When the interface temperature was set to 150°C hexamine and HMTD had higher signal-to-noise responses, which then decreased again as the temperature increased further. For TATP the optimal interface temperature was slightly higher, at 200°C, and as was seen for the other analytes, the response then decreased significantly as the interface temperature increased up to 300°C (see Figure 7).

Ion accumulation time

All three analytes' signal-to-noise responses were greatest with the ion accumulation time set to 50 milliseconds. This was the shortest amount of time tested, indicating that a longer accumulation time may be overloading the ion trap (see Figure 8).

The ion identified for the detection of hexamine with the mass-to-charge ratio (*m*/*z*) of 141.502 was the molecular ion plus hydrogen, $[M+H]^+$. The characteristic ions for the identification of TATP and HMTD are consistent with those stated in the published literature (Crowson and Beardah, 2001; Widmer et al., 2002; Xu et al., 2004); these are the ammonium adduct 240.1442 *m*/*z* for TATP, formed with ammonium from the buffer in the mobile phase, and the molecular ion plus hydrogen, $[M+H]^+$ (209.0761 *m*/*z*) for HMTD. Further optimization of the instrument conditions and implementation of a mobile phase gradient could further lower the limits of detection, as well as testing methods on different LC-MS systems, including an instrument with a triple quadrupole. However, for this study the sensitivity was satisfactory; the lower limits of detection of the instrument were 35.77, 252, and 44.10 ng/mL for hexamine, TATP, and HMTD respectively. Calibration curves were run with each analysis, and linearity was excellent with $r^2 > 0.998$ for all analytes.

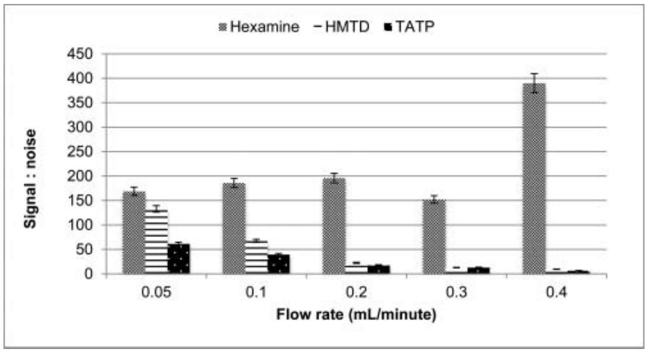

Figure 4. Signal-to-noise ratios for hexamine, HMTD, and TATP detection at varying flow rates.

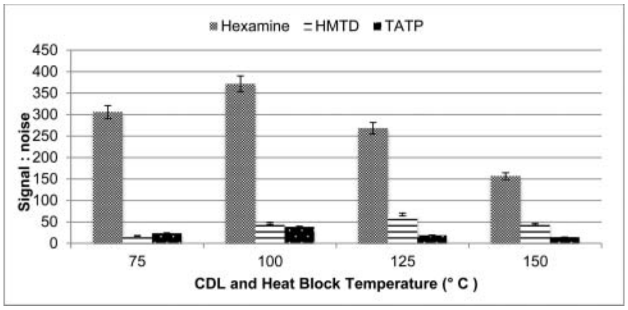

Figure 6. Signal-to-noise ratios for hexamine, HMTD, and TATP detection at varying CDL and heat block temperatures.

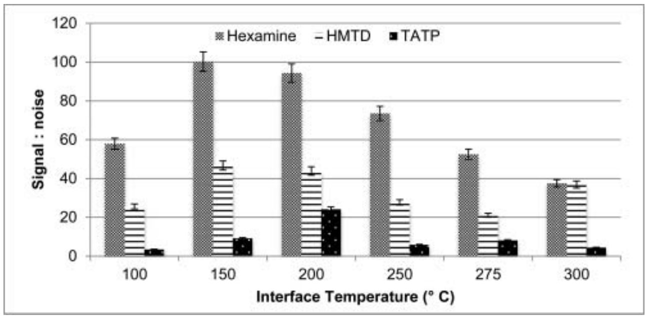

Figure 7. Signal-to-noise ratios for hexamine, HMTD, and TATP detection at varying interface temperatures.

WWTP batch processing

Analysis of the spiked river water that did not undergo any processing showed no significant change in concentration over the 8 hr and 10 min of the experiment. The results of the control experiment were that all of the samples tested throughout the WWTP were below the limit of detection, indicating that there were no detectable traces of the analytes present in either the river water samples taken or the MLSS samples. Figures 9–11 show the average concentration ($n = 3$) of the analytes in the spiked water samples directly following spiking and throughout the wastewater treatment processing in the laboratory setup.

It is important to note that there is no legitimate reason for TATP and HMTD to be in river water, unlike their commonly found precursors such as hydrogen peroxide, acetone, and hexamine, which are found legitimately in substances such as drain cleaner, hair dye, nail varnish remover, and commercial solvents. Control samples (river water only) were also subjected to the wastewater treatment batch processing, and none of the analytes were detected in any of the samples. The long-term stability of explosives in the environment is thus far unknown, therefore it is not currently possible to establish how long peroxides that are detected, such as TATP and HMTD, will have been present.

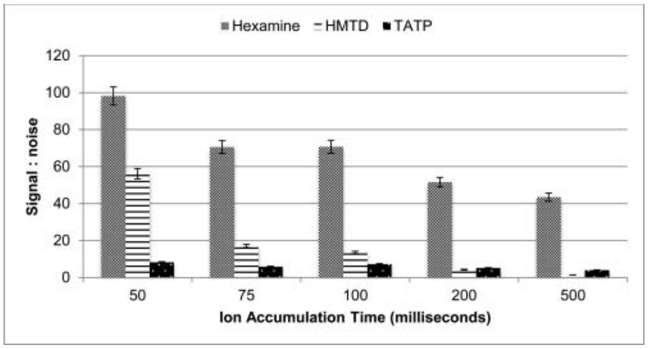

Figure 8. Signal-to-noise ratios for hexamine, HMTD, and TATP detection at varying ion accumulation times.

Table 5. Summary of the ions and retention times used to identify the analytes in this study.

Analyte	Ion (*m/z*)	Proposed formula	Retention time (min)
Hexamine	141.0502	$[M+H]^+$	2.80
TATP	240.1442	$[M+NH_4]^+$	6.33
HMTD	209.0761	$[M+H]^+$	6.02

WWTP treatment efficiency

The greatest percentage removal of all three analytes occurred following the biological treatment stage; 24.12% of hexamine, 44.64% of TATP, and 54.50% of HMTD were removed at this stage of the treatment process. Similar studies regarding the elimination of illicit drugs by wastewater treatment reported that in general $> 50\%$ of the organic compounds were removed, however the individual stages were not analyzed (Metcalfe et al., 2010). This reported removal could be due to bioremediation facilitated by the mixture of naturally occurring microorganisms contained in the activated sludge to breakdown toxic components of the wastewater. The stability of the analytes in wastewater is also a key characteristic to take into account, however the common problems associated with the instability of the peroxy group contained in TATP and HMTD are reduced significantly when they are diluted into a liquid phase, as was the case in this study. Further removal occurred during settling, which allowed any suspended solids containing explosive compounds to be separated from the liquid phase by gravity. Future studies concerning long-term stability and analysis of settled material and sludge would be beneficial to ascertain whether the analytes were still present in their original form or if they had been degraded.

Since the wastewater treatment processing did not remove all of the spiked analytes following the processing, it may be possible to use effluent to detect these explosives in

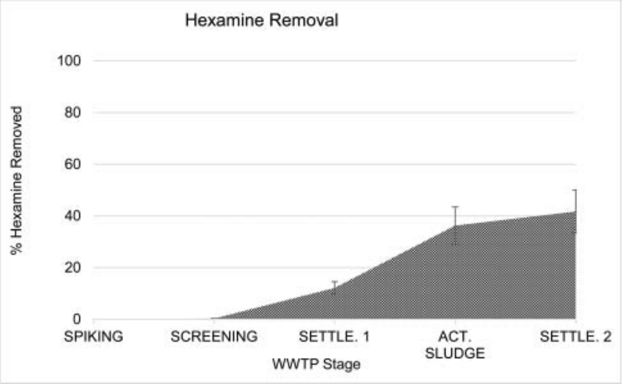

Figure 9. Hexamine removal (%) following spiking and following each wastewater treatment process (WWTP) in spiked water samples. CV (coefficient of variation) between 5.1 and 10.3%. Initial concentration was 5 μg/mL.

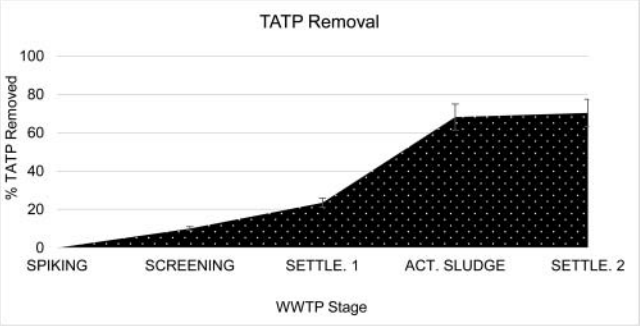

Figure 10. TATP removal (%) following spiking and following each wastewater treatment process (WWTP) in spiked water samples. CV between 5.4 and 12.3%. Initial concentration was 3 μg/mL.

the environment; however, in a real-life scenario the analytes could be at significantly lower levels. Sampling for the wastewater analysis of drugs and pollutants in previous studies (Karolak et al., 2010; Postigo et al., 2010; Irvine et al., 2011; van Nuijs et al., 2011; Thomas et al., 2012; Lai et al., 2013) has taken place from influent samples from various WWTPs worldwide, however, the dilution factor encountered by potential analytes even at this stage would make the detection process from WWTP samples much more complex. Since it is viable to collect wastewater samples at the inlet pipe of wastewater treatment plants, this sampling location would be feasible both operationally and in line with the optimum sampling methodology. However, with low levels of contamination likely to be released from a manufacturing location, it would be greatly beneficial to be able to collect samples as close to the source as possible.

The results presented in this article offer insight into one part of the journey undertaken by trace explosives in the wastewater system and demonstrate that the approach for using their detection levels as forensic intelligence is a viable one. There is added significance to finding peroxide explosives themselves in a wastewater system since they are not legitimately observed in the environment, unlike the chemicals that are often used to synthesize HMEs. The detection of precursor chemicals in a wastewater system does however need to be considered within the context of a specific investigation with particular focus on the quantity detected. The implications for using environmental analyses to inform a counter-terrorism investigation are considerable. Instead of relying upon unrelated chance encounters, investigators will be able to use forensic analyses and geographic information systems in order to make informed decisions based upon empirical data regarding where to target their operations in a proactive and preventive approach. This proposed approach to examine the natural environment, which is often used to monitor other types of pollutants, has the potential to be applied to the security domain, utilizing the incomplete removal of peroxide explosives from water samples while being mindful of the implications to the natural aquatic environment.

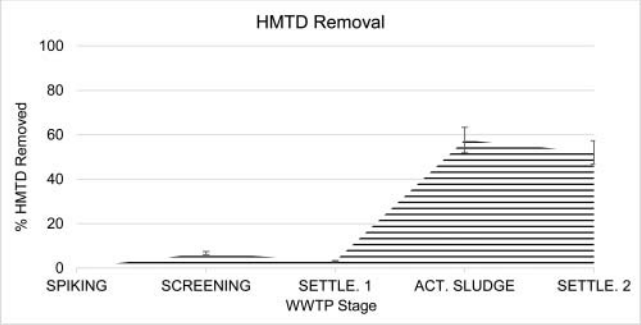

Figure 11. HMTD removal (%) following spiking and following each wastewater treatment process (WWTP) in spiked water samples. CV between 3.8 and 12.3%. Initial concentration was 3 μg/mL.

Overview and implications

This study sought to investigate the spatial and temporal distribution of trace peroxide explosives throughout the wastewater treatment process as a proof of principle study for the use of water analysis for trace explosives detection. The main findings were:

- Conditions for solid phase extraction and liquid chromatography-mass spectrometry were developed for the successful identification and quantification of trace peroxide explosives in river water samples, although further optimization and comparison of LC-MS instruments are required for increased sensitivity.
- The SPE cartridge that gave the best recoveries for HMTD was the TELOS ENV, and the Nexus was the best at extracting TATP in individual samples on this occasion. Further testing of the optimum extraction conditions for the analytes in the same sample is required in order to screen for multiple peroxide-related compounds using one SPE method.
- Even though the treatment processes employed in this study significantly reduced the quantity of peroxide explosives in water, the explosives were not completely removed, and in theory these chemicals could be present in the effluent that is discharged back into the environment if they have not already been degraded in the wastewater treatment process.
- Influent wastewater from the inlet pipe at a WWTP would appear to be the most appropriate and representative sample (from within the WWTP) to monitor activities that occur at the population level, as is seen in other wastewater analysis studies for both illegal and prescribed drugs.

- The potential to use detected levels of explosives from wastewater samples in order to locate their origin has been established using an empirically generated data set. Further studies into the behaviors of explosives in the environment are required, in particular the effect of bioremediation on such compounds in wastewater.
- Although the occurrence of peroxide explosives in wastewater is hopefully rare, the effect these compounds could have upon human and aquatic biosystems is not known, and further investigation is required.
- Given the instability of organic peroxide compounds, it is suggested that there is value in determining the long-term natural decay of these substances in different water matrices including wastewater and river water. While the thermal stability of HMTD and TATP in their solid state has been investigated previously (Agrawal and Hodgson, 2007; Hiyoshi et al., 2007), their integrity in natural water bodies has not yet been examined.

The findings from this study demonstrate that the potential exists to harness the power of wastewater analysis as a form of intelligence that can inform criminal investigations. In forensic science, there is a general focus on the detection of a crime after the event, identifying the "who," "when," "where," and "how"; this study highlights the potential for forensic evidence from wastewater analysis for environmental surveillance to also have a part to play in the prevention of crimes.

Acknowledgment

Many thanks to the staff at Thames Water UK Ltd., in particular Luke Robinson, David Furness, Zak Espi-Castillo and Emily Goren. For the use of their laboratory and analytical equipment, the authors extend their gratitude to Ms Janet Hope (UCL, Department of Geography) and Dr Carolyn Hyde (UCL, Wolfson Institute of Biomedical Research). The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript, and all authors contributed equally. The authors declare no competing financial interest.

Funding

The authors acknowledge the funding for this research from the Engineering and Physical Sciences Research Council (EPSRC), grant code: EP/G037264/1.

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