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A robust analytical method for the determination of pesticide residues in wastewater

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Much research has been carried out on the analysis of chemical residue pollutants in the aquatic environment including drinking water, lakes, rivers, ground water, estuaries and coastal zones. However, few studies report the analysis of wastewater for the presence of chemical pollutants, including pesticides, even though this is an important issue because wastewater can be a major point source input of pollutants to the environment. The aim of this research was to develop an analytical method for the detection and confirmation of 13 pesticide residues in wastewater. All 13 pesticides are included on the EU priority pesticides list outlined the Water Framework Directive (2000/60/EC). Pesticides were extracted from wastewater using solid-phase extraction (SPE) on polymeric cartridges containing hydrophilic and lipophilic functional groups capable of retaining pesticides with diverse physico-chemical properties. The pesticides were eluted with organic solvent and concentrated by evaporation prior to analysis by liquid-chromatography coupled to tandem mass spectrometry (LC-MS/MS) operated in electrospray ionization (ESI) mode. LC-MS/MS runs both positive and negative modes were carried out for each sample. Recovery of the 13 pesticides was typically greater than 80%. All 13 pesticides were found to be linear over the concentration range 1 to 100 ng mL^{-1} with linear regression values (R^2) typically greater than 0.99. The limit of detection (LOD) of the method was 1 ng mL^{-1}, except for chlorpyrifos (5 ng mL^{-1}). 11 isotopically labelled internal standards were included in the method to improve accuracy and precision. The final method was used to analyse wastewater samples collected from seven WWTPs over a period of four months. Several pesticides were found to be present in the samples tested at each WWTP. A total of 204 samples were collected from 68 sampling events between 2011 and 2012. Exceedances were detected at each of the seven sites in this study, with diuron, atrazine and simazine most frequently occurring.

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Introduction

Pesticides, including herbicides, fungicides, insecticides and bactericides, are extensively used in household, industrial and agricultural applications to prevent, destroy, repel or mitigate against a wide array of pests on plants. They are particularly important in agriculture as they are the most cost-effective means of pest and weed control, thereby increasing the growing yield of crops. They are also widely used by homeowners, industry and government to prevent or control the growth of weeds. While there are many benefits to using pesticides, there are also a number of drawbacks including their potential toxicity to humans, animals and the wider environment[1]. In addition, the overuse, mishandling, poor storage and leaching of pesticides into groundwater and surface waters can lead to pollution as they can persist for long periods of time. There is currently a range of existing or impending EU measures to prevent pollution of water sources.[2–4] However, these measures have still to be fully implemented and their impact on the reduction of priority substance emissions has therefore not yet been thoroughly evaluated. Water sources included in the EU measures are lakes, rivers, ground water estuaries and coastal zones that are vulnerable to changes induced by human activities.

Modern wastewater treatment plants (WWTPs) are designed to remove bulk contaminants in wastewater, including bacteria and chemicals, prior to release back into water systems but are not capable of removing all chemical contaminants, including pesticides. Wastewater treatment directly affects the receiving water bodies and therefore the environment. It is also important that the WWTPs do not operate above their capacity to ensure the effective removal of pollutants before outflow into the effluent stream. If WWTPs operate above capacity, the excess influent will be diverted to a storm-water overflow where it can then be released, untreated, back into the effluent stream. The return of this effluent to the environment can result in water bodies becoming contaminated with pesticide residues, thereby preventing the achievement of the water quality standards established in the WFD. The main piece of legislation in Europe governing urban wastewater treatment is Council Directive 91/271/EEC which was adopted on 21 May 1991.[5] This

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directive aims to counter the adverse effects of urban and industrial wastewater discharges through planning, regulating, monitoring and reporting on these discharges. In addition, the EU Water Framework Directive (WFD) has listed a number of priority and hazardous substances, with environmental quality standards (EQSs; maximum allowable concentrations) put place to limit the occurrence of these pollutants in the environment.[6] For the purpose of this study, the focus was on 13 priority pesticides included in Annex X of the WFD (2000/60/EC).[7]

There is a continued demand for more sensitive and reliable methods that will detect residue violations, identify usage patterns of products and provide more quantitative results for exposure and risk assessment. LC-MS/MS is the most effective means of meeting all of these needs. LC-MS/MS enables the determination of pesticides with a wide range of molecular masses as well as polar, non-volatile and thermally labile analytes without derivatization. However, for the analysis of complex matrices, an appropriate sample preparation step is necessary to minimize matrix effects in the ionisation process and improve the accuracy of a method.[8–10] The inclusion of isotopically labelled internal standards into a method can also help to correct for matrix effects as well as loss of analytes during sample preparation.[11] Solid-phase extraction (SPE) is one of the most widely used sample preparation techniques in chemical residue analysis, including pesticide analysis. Advantages of SPE include speed, selectivity, applicability to polar compounds, the ability to concentrate pesticide residues which are present in samples at low levels, and cleanliness of the final extract. Unlike traditional SPE that uses silica-based sorbent, polymeric-SPE utilizes a cross-linked polystyrene–divinylbenzene (PS/DVB) sorbent that also has hydrophobic properties but with a much higher capacity than silica sorbents. In addition, the PS/DVB can be bonded with hydrophilic and/or ion-exchange functional groups to allow for the extraction of a wider range of compounds. One such functional group is *N*-vinylpyrrolidone, which together with the PS/DVB backbone, allows the SPE sorbent to retain polar, non-polar and charged compounds.

The aim of this research was to develop an analytical method capable of detecting and confirming a selection of 13 pesticide residues (included in the WFD), representative of the main groups of pesticides listed included in the WFD (organochlorine, organophosphorous, triazine, phenyl ureas) in wastewater effluent samples. The developed analytical method was then used to screen for the presence of pesticides in wastewater effluent samples taken from seven WWTPs over a four-month period. Since no maximum allowable concentrations (AA MAC) have been defined for pesticides in wastewater, the EQS limits outlined in the WFD for surface waters were used as the target concentration when developing the method and analysing real samples.

Experimental

Reagents, chemicals and apparatus

HPLC grade acetonitrile (MeCN) and diethyl ether, pesticide grade methanol (MeOH), GC grade dimethyl sulfoxide (DMSO) and LC-MS grade water (H_2O) were obtained from Fisher Scientific (Dublin, Ireland). Reagent grade ammonium formate and LC-MS grade acetic acid (HAc), formic acid (FA) and 2-propanol (IPA) were obtained from Sigma-Aldrich (Arklow, Ireland). Analar grade ammonium acetate was obtained from BDH (VWR, Dublin, Ireland). A Yellowline TTS2 vortex mixer (IKA-Werke GmbH & Co. KG, Staufen, Germany), Elma Ultrasonic LC20H sonicator (Singen, Germany), Zymark Turbovap LV (Hopkinton MA, USA) and a Dispensette III 0.5–5 mL bottle-top dispenser (BRAND GMBH + CO KG, Wertheim, Germany) were used during sample preparation.

Standard solutions

All standards used in the study were of the highest available purity. Alachlor, chlorfenvinphos, chlorpyrifos, diuron, epoxiconazole, fenitrothion, malathion, mecoprop, pentachlorophenol, pirimiphos-methyl and simazine, were obtained from Sigma-Aldrich (Arklow, Ireland). Atrazine and isoproturon were purchased from ChemService (West Chester, PA, USA).

The internal standards chlorfenvinphos-D_{10}, chlorpyrifos-D_{10}, fenitrothion-D_6, malathion-D_6, mecoprop-D_3, phentachlorophenol-$^{13}C_6$ and pirimiphos-methyl-D_6 were obtained from Dr Ehrenstorfer GmbH (Augsburg, Germany). Alachlor-D_{13}, atrazine-D_5, diuron-D_6 and simazine-D_{10} were obtained from Sigma-Aldrich (Arklow, Ireland).

Individual stock solutions (2000 μg mL^{-1}) were prepared by accurately weighing 20 mg of standard into a 10 mL volumetric flask. Depending on the specific solubility properties, the compounds were dissolved and diluted to volume with MeCN, MeOH, diethyl ether or DMSO. A mixed stock solution (100 μg mL^{-1}) was prepared by transferring 500 μL of each individual stock solution into a 10 mL volumetric flask and diluted to volume with MeCN. QC spiking solution 1 (1000 ng mL^{-1}) was prepared by transferring 100 μL of the mixed stock solution into a 10 mL volumetric flask and diluted to volume with MeCN. QC spiking solution 2 (100 ng mL^{-1}) was prepared by transferring 1 mL of QC spiking solution 1 into a 10 mL volumetric flask and diluting to volume with MeCN. Calibration curve solutions (1, 2, 5, 10, 25, 50 and 100 ng mL^{-1}) were prepared by transferring 10, 20, 50, 100, 250, 500 and 1000 μL of the QC spiking solution 1 (100 ng mL^{-1}) into 10 mL volumetric flasks and diluting to the mark with MeCN. 50 μL of the 2000 ng mL^{-1} mixed internal standard stock solution was added to each volumetric flask, which gives an internal standard concentration of 10 ng mL^{-1} and is equal to the concentration of internal standard in the final sample extract.

Internal standard stock solutions (1000 μg mL^{-1}) of powdered standards were prepared by accurately weighing 10 mg of standard into a 10 mL volumetric flask and diluting to volume with MeOH. Additional standards were purchased in liquid form at concentrations of 100 μg mL^{-1}. A mixed internal standard stock solution (2000 ng mL^{-1}) was prepared by transferring 20 μL of the 1000 μg mL^{-1} stock solutions and 200 μL of the 100 μg mL^{-1} stock solutions into a 10 mL volumetric flask and diluting to volume with MeCN. A working internal standard spiking solution (200 ng mL^{-1}) was prepared by

transferring 1 mL of the mixed internal standard solutions into a 10 mL volumetric flask and diluting to volume with MeCN. All standards were stored at −20 °C before use.

Sampling plan

In order to ensure the sampling results for priority pesticides in wastewater were as comprehensive and representative as possible, seven WWTPs were chosen as sampling locations, Table 1. Different treatment processes are employed at the respective plants and each serves varying levels of domestic, industrial and agricultural inputs. The sampling plan involved collecting samples on a monthly basis over a four-month period and a period of high intensity sampling over three days during 1 week. Effluent samples of the same volume were collected at the same location at each sampling time point and strictly employed SOPs were applied to all samples.

When designing the sampling plan for this study the half-life values of the respective pesticides were considered. Pesticides such as alachlor, pentachlorophenol and epoxiconazole were unlikely to be detected due to their short half-lives in surface water (<7 days) whereas pesticides such as atrazine, diuron and chlorfenvinphos are more persistent in the environment (>6 months) and are therefore more likely to be detected. These compounds were selected and included in the study based on legislation at the time of monitoring.

Wastewater sample collection

Shatterproof amber glass bottles were silanised to minimise adsorbance of analytes to the bottle walls. The glassware was initially cleaned with detergent, acetone, and rinsed with deionised water. A solution of 10% (v/v) dichloro-dimethylsilane in toluene was used to rinse the inside of the bottle, which was finally rinsed with toluene and methanol. Wastewater samples were collected in a plastic bucket from the effluent stream. The bucket was rinsed three times with the effluent, then refilled and used to rinse the glass bottles three times. The bottles were then filled to capacity and stored in a container during transport to the lab.[12]

Table 1 Overview of the WWTPs in this study. NR = nutrient removal. This information was gathered from the Environmental Protection Agency (EPA) wastewater licence applications of the respective WWTPs and from the Urban Wastewater Report, 2007

Site code	Treatment level	Plant population equivalent
1	Secondary	15 000
2	Secondary	20 000
3	Secondary	6415
4	Secondary	15 000
5	Secondary, NR	12 960
6	Secondary, NR	12 000
7	None	0

Sample preparation

Three 1 L shatterproof amber bottles containing wastewater samples from each sample location were received in the lab. From each bottle an aliquot (500 mL) was filtered to remove any solid particulates and transferred into a clean 500 mL labelled glass bottle. The remaining unfiltered water (500 mL) was transferred to a second 500 mL labelled glass bottle. Samples were fortified with internal standard (50 μL), shaken and let to stand for 15 min. This gives a concentration of 0.02 ng mL^{-1} in the wastewater sample, which is equivalent to 10 ng mL^{-1} in the final extract (1 mL). Four 500 mL glass bottles were filled with deionised water and used for QC samples. Two samples were fortified with 50 μL of QC spiking solution 1 and two were fortified with QC spiking solution 2, which gives concentrations of 0.1 and 0.01 ng mL^{-1} (50 and 5 ng mL^{-1} in final extract). All four QC samples were fortified with 50 μL of internal standard.

Polymeric Strata-X solid phase extraction (SPE) cartridges (500 mg/6 mL; Phenomenex, Macclesfield, UK) were conditioned with MeOH (2 × 3 mL) followed by H_2O (2 × 3 mL). The wastewater samples were then applied to the SPE cartridges using Chromabond® tubing adapters (Machery Nagel, Düren, Germany) to transfer the water from the 500 mL glass bottles to the SPE cartridges. The SPE cartridges were rinsed with H_2O (2 mL) and dried under vacuum. The pesticides were eluted using IPA (2 mL) followed by MeCN (2 mL) and collected in glass tubes. The 4 mL solvent was evaporated under N_2 using a Turbovap apparatus and the sample was reconstituted in MeCN (1 mL). The sample extract was sonicated (10 min) and vortexed (1 min) before an aliquot (500 μL) was transferred to 0.45 μm Mini Uni-prep PTFE filter vials (Whatman, Maidstone, UK) and analysed by LC-MS/MS.

Liquid chromatography

The HPLC system consisted of an Agilent 1100 series LC (Santa Clara, CA, USA) including a binary pump, a vacuum degasser, column oven and a temperature controlled auto-sampler. Reversed-phase separation of analytes was performed at 40 °C on a XBridge C_{18} column (150 mm × 4.6 mm I.D., 3.5 μm particles) protected by a C_{18} guard column (20 mm × 4.6 mm I.D.), both from Waters (Wexford, Ireland). The final mobile phase conditions included 1 mM ammonium formate and 0.01% HAc in both (A) H_2O : MeCN (90 : 10, v/v) and (B) MeCN. The sample injection volume was 20 μL and gradient elution was performed at 0.3 mL min^{-1}. There were two separate gradients for ESI^+ and ESI^-. The ESI^+ gradient was 0–3 min 50–100% B, 3–23 min 100% B, 23–25 min 100–50% B and 25–35 min 50% B. The ESI^- gradient was 0–4 min 0% B, 4–8 min 0–100% B, 8–20 min 100% B, 20–22 min 100–0% B and 22–30 min 0% B.

Mass spectrometry

The MS system consisted of an Applied Biosystems API 3000 tandem quadrupole MS instrument (Foster City, CA, USA) with an electrospray ion (ESI) interface and divert valve placed between the column and source. The mass spectrometer was

fully controlled by Analyst software version 1.5. A syringe pump (Harvard Apparatus model 33, Holliston, MA, USA) connected to the interface was used for tuning purposes. MS analysis was performed by atmospheric pressure electrospray ionisation (ESI) in positive (ESI^+) and negative (ESI^-) modes applied in sequential injections using different LC conditions. The eluent flow was diverted to waste during the LC equilibration step prior to the next chromatographic sequence to minimise source contamination. Zero air was used for nebulisation and desolvation gas. Nitrogen was used for exhaust and collision gas. The source temperature was set at 450 °C and the ion-spray voltage was set at 5500 V and −4200 V for ESI^+ and ESI^-, respectively. The analytes were detected by tandem MS using the multiple reaction monitoring (MRM) function of two transitions with a dwell time of 100, 150 or 300 ms, except pentachlorophenol which was detected by selected ion monitoring mode (SIM). The MS acquisition was divided into three time periods to enhance target analyte sensitivity. ESI^+ was divided into 0–12.6 min, 12.6–18.0 min, and 18.0–23 min and ESI^- was divided into 0–10 min, 10–16.0 min, and 16.0–20 min. The MS conditions were optimised by tuning the analyte-specific parameters, including de-clustering potential, focusing potential, collision energy, and collision cell exit potential. This optimisation was carried out by infusion (10 μL min^{-1}) of a 500 ng mL^{-1} standard solution of each analyte and by monitoring the two most abundant fragment ions produced from the molecular ion. The entrance potential was set at 10 and –10 V for ESI^+ and ESI^-, respectively. A summary of the retention times, monitored ions and optimised MS parameters obtained for each analyte is reported in Table 2. The acceptance criteria for MRM ratios between quantitative and confirmatory ion was +/−20%. For isoproturon and epoxiconazole external calibration was employed.

Table 2 Summary of the retention times, diagnostic ions and the MS/MS operating conditions for the 13 pesticides and 11 internal standards included in the study. Q is quantitative ion and C is confirmatory ion

Analyte	ESI mode	RT (min)	Q1 (Da)	Q3 (Da)	Dwell time (ms)	DP (V)	FP (V)	CE (V)	CXP (V)
Simazine D_{10}	+	8.86	212.71	137.02	100	56	190	29	6
Simazine C	+	8.95	202.00	103.89	100	56	180	37	6
Simazine Q	+	8.95	202.00	131.94	100	56	180	29	8
Isoproturon Q	+	9.76	207.08	71.92	100	56	190	31	4
Isoproturon C	+	9.76	207.08	164.97	100	56	190	21	8
Diuron D6	+	9.77	239.03	77.95	100	46	160	35	4
Diuron Cl^{37}	+	9.84	234.92	71.90	100	41	170	37	4
Diuron Q	+	9.85	232.89	71.90	100	41	140	37	4
Atrazine D_5	+	9.96	221.19	178.83	100	51	170	29	10
Atrazine Q	+	10.03	216.20	173.79	100	61	190	25	10
Atrazine C	+	10.03	216.20	67.92	100	61	190	49	4
Epoxiconazole Q	+	11.02	330.13	120.96	100	56	180	29	6
Epoxiconazole C	+	11.02	330.13	100.97	100	56	180	65	6
Malathion D6	+	11.44	355.09	99.98	100	16	100	37	6
Malathion Q	+	11.49	348.02	126.93	100	31	130	23	6
Malathion C	+	11.49	348.02	98.92	100	31	130	37	6
Alachlor D_3	+	11.87	283.03	251.11	100	51	190	13	12
Alachlor Q	+	11.97	270.16	237.89	100	51	180	15	12
Alachlor C	+	11.97	270.16	161.99	100	51	180	29	8
Chlorfenvinphos D_{10}	+	11.97	368.88	100.93	100	51	170	47	6
Chlorfenvinphos Q	+	12.05	358.88	154.90	100	46	150	19	8
Chlorfenvinphos C	+	12.05	358.88	98.92	100	46	150	47	6
Pirimiphos D_6	+	13.00	311.99	163.94	150	51	150	31	8
Fenitrothion D_6	+	13.02	283.94	130.91	150	51	100	33	8
Pirimiphos C	+	13.04	306.03	66.98	150	56	190	61	4
Pirimiphos Q	+	13.04	306.03	164.05	150	56	190	31	10
Fenitrothion C	+	13.05	277.94	78.93	150	71	230	47	4
Fenitrothion Q	+	13.05	277.94	124.81	150	71	230	29	6
Chlorpyrifos D_{10}	+	13.65	359.90	198.81	150	41	160	31	10
Chlorpyrifos Q	+	13.69	349.90	197.81	150	51	190	29	10
Chlorpyrifos C	+	13.69	349.90	96.85	150	51	190	47	6
Mecoprop D_3	−	14.72	216.10	143.98	300	−31	−110	−22	−7
Mecoprop Q	−	14.75	213.20	141.00	300	−56	−180	−22	−7
Mecoprop C	−	14.75	213.20	71.20	300	−56	−180	−16	−1
PCP $^{13}C_2$ 1	−	16.86	268.80	268.80	300	−46	−130	−5	−5
PCP $^{13}C_2$ 2	−	16.86	270.80	270.80	300	−46	−130	−5	−5
PCP $^{13}C_2$ 3	−	16.86	272.83	272.83	300	−41	−120	−5	−5
PCP Q	−	16.94	262.60	262.60	300	−61	−200	−5	−5
PCP C1	−	16.94	264.62	264.62	300	−61	−190	−5	−5
PCP C2	−	16.94	266.65	266.65	300	−61	−190	−5	−5

Results and discussion

Pesticides enter river systems as either point sources or diffuse sources, with point sources being certain locations on the body of water (*e.g.* sewage plants, sewer overflows and losses due to bad management practices of farmers) and diffuse sources which are inputs along the water course (*e.g.* drain-flow, deposition, runoff, drift and contribution through groundwater). This makes pesticides especially relevant to water quality management and the regulation of environmental risk as they impede the achievement of a good water quality status.[12,13] The focus of this work was on wastewater inputs and the development of a method that would be robust and capable of reaching the low limits of detection required by most environmental regulations and legislation.

LC-MS/MS

The pesticides used in this study comprise a wide range of compounds with different physico-chemical properties (see Fig. 1 for the pesticides structures). As a result, the mobile phase composition was carefully optimised to achieve efficient ionisation and separation of all 13 analytes and 11 internal standards. Some of the other isotopically labeled internal standards that were included in the method were evaluated as internal standards for isoproturon and epoxiconazole. However, none were found to be suitable for quantitation purposes (they produced non-linear calibration curves, inconsistent results, or high or low recovery) and ultimately it was decided to use external calibration for these two compounds. Formic acid (0.1 and 1%) was initially evaluated as a mobile phase additive but was found to be unsuitable for the ionisation of all compounds. Acetic acid (0.01, 0.1 and 1%) was found to be a more suitable additive than formic acid but was also unable to ionise all the pesticides. Ammonium acetate and ammonium formate were evaluated as alternative additives and were more successful in forming reproducible ions ($[M + H]^+$, $[M + NH_4]^+$ and $[M - H]^-$) for the analytes. Next, a combination of acetic acid and ammonium acetate was evaluated and was found to give the best overall results. Ultimately, the optimal mobile phase conditions were 1 mM ammonium formate and 0.01% HAc in both (A) H_2O : MeCN (90 : 10, v/v) and (B) MeCN.

Software controlled, automated infusion optimisation steps of standards in the presence of various mobile phases spiked with buffers was carried out using the API3000 Analyst software. The software-generated results indicated the optimised mobile phase for the highest ionisation efficiency of the target analytes as reported in this study. This is a routine software automated infusion step that is carried out with all QqQMS instruments when tuning analyte ionization against various mobile phase compositions and the software optimum value is infusion study recorded by the analyst as reported in this manuscript.

The MS instrument used in the study required 700 ms to switch between positive and negative modes and an additional few seconds to equilibrate. An attempt was made to analyse all the pesticides in a single run however, it was not possible to achieve adequate resolution between all the compounds to allow for fast ESI^+ and ESI^- switching. This is particularly the case for chlorpyrifos (ESI^+) and mecoprop (ESI^-) which eluted close together (baseline peak width of both compounds was 0.6 min). Therefore 2 separate injections were carried out avoiding any co-elution of any ESI^+ and ESI^- compounds and offering the best possible sensitivity. It was therefore found to be unsuitable to allow determination of all the analytes in a single injection. As a result, two injections were required to allow the sensitive determination of the 12 ESI^+ and 2 ESI^- analytes for each sample. All 13 analytes and 11 internal standards eluted within 23 min, with a further 10 min period required for equilibration. A number of faster gradient conditions were investigated but resulted in analytes eluting too early, which led to matrix co-elution with target peaks. Fig. 2 and 3 show examples of total ion current (TIC) chromatograms of a sample extract at 1 μg L^{-1} (corresponds to 0.002 μg L^{-1} in wastewater) for ESI^+ and ESI^- mode, respectively.

Pentachlorophenol (PCP) is a low molecular weight compound that is difficult to fragment. However, due to the large number of chlorine atoms on the molecule it has a very distinctive mass spectrum consisting of a cluster of three peaks that are 2 Da apart. Therefore, it was decided to scan this compound in selected ion monitoring mode (SIM) for PCP whereby three masses were detected, namely 262, 264 and 266 Da, which had very good signal intensity. By analysing the three masses for PCP, selectivity was sufficient for target identification and quantitation. While most pesticides could be detected at 1 ng mL^{-1} in solvent, chlorpyrifos had reduced sensitivity and could only be detected at 5 ng mL^{-1}. The limits of quantitation (LOQ) of the method and surface annual average EQS limits (2008/105/EC and 2013/39/EU), where available, are outlined in Table 3.

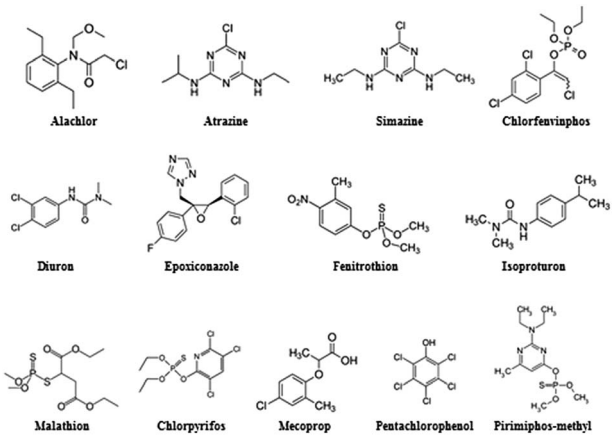

Fig. 1 Structures of the pesticides included in this study.

Analytical performance

Most of the pesticides were found to have a linear response over a range of 2–2000 ng mL^{-1}. Linear regression values (R^2) were typically greater than 0.99. Four compounds, namely epoxiconazole, isoproturon, pirimiphos-methyl and pentachlorophenol, were found to be linear over a range of 2–200 ng mL^{-1}

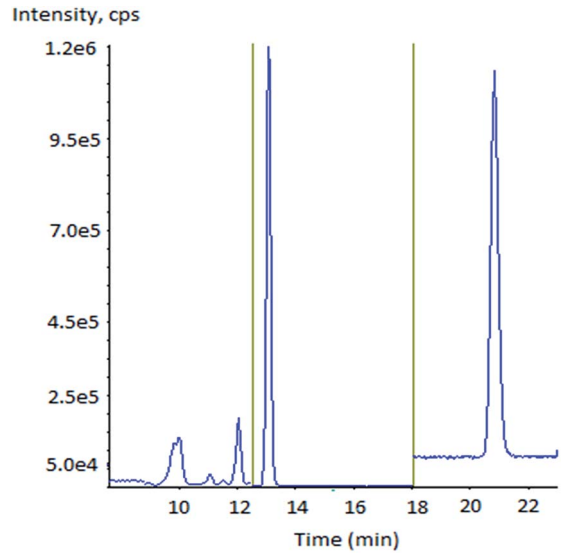

Fig. 2 Total ion current (TIC) of ESI$^+$ pesticides at 1 ng mL^{-1} (equal to 0.002 ng mL^{-1} in wastewater sample).

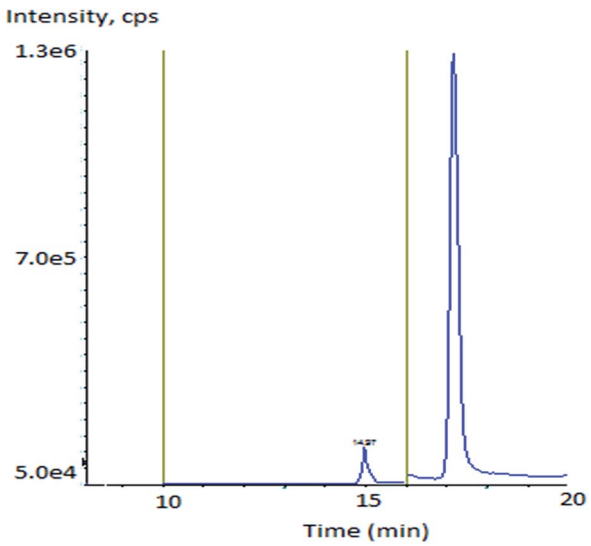

Fig. 3 Total ion current (TIC) of ESI$^-$ pesticides at 1 ng mL^{-1} (equal to 0.002 ng mL^{-1} in water sample).

and 500–2000 ng mL^{-1} but not over the entire 2–2000 ng mL^{-1} range. For these pesticides, initial analysis was carried out using the lower calibration range. However, if the concentration exceeded 200 ng mL^{-1}, the higher calibration range was used for final quantitation. For pesticides that had an isotopically labelled internal standard, calibration curves were plotted using the response of the analyte divided by the internal standard response. No weighting function was used for the calibration curves.

To assess ion suppression, the target compound standards and corresponding internal standards were spiked into 'analyte free' waste water matrix and following SPE clean-up of the extracted analytes, recoveries were determined by LC-MS/MS (Table 4). Eleven analytes were quantified using their corresponding internal standard calibration plots. Two of the analytes (isoproturon and epoxiconazole) were also spiked into 'analyte free' waste water and gave comparable calibration curve slopes and acceptable recoveries (109% and 95% respectively) following SPE clean-up using external calibration curves for verification. This constituted a reasonable representation of matrix effects for all the target compounds. Once the recoveries were established and were repeatable, the method was deemed to have acceptable accuracy.

Table 3 Limit of quantitation (LOD) of each pesticide included in the LC-MS/MS method and the corresponding surface Annual Average EQS limit

Pesticide	LOD (ng mL^{-1})	EQS (ng mL^{-1})
Alachlor	2	300
Atrazine	2	600
Chlorfenvinphos	2	100
Chlorpyrifos	10	30
Diuron	2	200
Epoxiconazole	2	—
Fenitrothion	2	—
Isoproturon	2	—
Malathion	2	—
Mecoprop	2	—
Pentachlorophenol	2	—
Pirimiphos methyl	2	—
Simazine	2	1000

Application to real samples

The levels of chemical residues present in various water sources are most commonly judged against environmental quality standards (EQSs) set by the EU, although they can also vary among different countries. These standards dictate the maximum allowable concentrations (MAC EQS) or range of concentrations (Annual Average or AA EQS) of specific pollutants to ensure compliance with EC guidelines. Directive 2008/105/EC defines the 33 latest EQS values for surface waters across Europe. Table 3 lists the EQS values of the individual pesticides included in this study, where available. It was found that all samples contained trace amounts of different priority pesticides. Pesticide concentrations were found to exceed EQS limits for one of the 13 pesticides (diuron) on two occasions at the same WWTP (Site 2). Seven WWTPs were surveyed as part of this sampling plan. Each plant catered for different population sizes, and types and sources of influent. One of the main aims of this research was to identify current gaps in knowledge in the area of water monitoring, primarily in wastewater samples. Results are shown in Table 5.

Five of the pesticides included in the analytical method, including atrazine, diuron, epoxiconazole, mecoprop and simazine, were found to be present in the wastewater samples tested. Most wastewater samples were found to be positive for at least two pesticides, except for samples taken from Site 7 which were found to contain one pesticide (diuron) on two occasions. The remaining eight pesticides included in the method were not detected in any of the samples taken during the four-month

Table 4 Linear range and average recoveries of each pesticide (50 μg L^{-1}) spiked into into 'analyte free' waste water following SPE clean-up. N/A = a deuterated internal standard was not commercially available at the time of the study

Analyte	Internal standard	MS polarity	Average recovery (%) ($n = 3$)*	Linearity (R^2) 1–1000 μg L^{-1}
Alachlor	Alachlor-D_{13}	ESI^+	90	0.997
Atrazine	Atrazine-D_5	ESI^+	99	0.998
Chlorfenvinphos	Chlorfenvinphos-D_{10}	ESI^+	98	0.995
Chlorpyrifos	Chlpyrifos-D_{10}	ESI^+	84	0.999
Diuron	Diuron-D_6	ESI^+	95	0.998
Epoxiconazole	N/A	ESI^+	95	0.999 (1–100 μg L^{-1})
Fenitrothion	Fenitrothion-D_6	ESI^+	71	0.999
Isoproturon	N/A	ESI^+	109	0.998 (1–100 μg L^{-1})
Malathion	Malathion-D_6	ESI^+	86	0.999
Mecoprop	Mecoprop-D_3	ESI^-	138	0.995
Pentachlorophenol	Pentachlorophenol-$^{13}C_6$	ESI^-	145	0.994 (1–100 μg L^{-1})
Pirimiphos-methyl	Pirimiphos-methyl-D_6	ESI^+	127	0.986
Simazine	Simazine-D_{10}	ESI^+	98	0.999

Table 5 Results of the analysis of real wastewater samples from seven WWTPs ($n = 3$) and limits of quantitation of the LC-MS/MS method and the corresponding surface annual average EQS limits. LOD = 2 ng mL^{-1}

			Month 1		Month 2		Month 3		Month 4	
WWTP	Analyte	EQS (ng mL^{-1})	Mean (ng mL^{-1})	RSD	Mean (ng mL^{-1})	RSD	Mean (ng mL^{-1})	RSD	Mean (ng mL^{-1})	RSD
Site 1	Atrazine	600	9	6	407	2	62	6	41	2
	Diuron	200	83	22	375	1	65	3	977	5
	Simazine	1000	30	8	85	6	37	15	38	9
	Mecoprop	—	—	—	446	8	—	—	—	—
Site 2	Atrazine	600	14	4	4	7	15	5	3	4
	Diuron	200	87	7	81	5	81	6	164	4
	Simazine	1000	43	11	45	8	16	4	53	9
	Mecoprop	—	—	—	—	—	56	51	311	5
Site 3	Atrazine	600	7	4	31	3	9	8	5	6
	Diuron	200	24	10	31	9	51.	7	42	1
	Simazine	1000	5	20	—	—	—	—	4	—
Site 4	Atrazine	600	12	3	11	4	9	4	7.0	3
	Diuron	200	36	5	49	5	56	5	168	5
	Simazine	1000	7	10	15	4	19	20	191	8
Site 5	Atrazine	600	8	3	9	2	5	50	9	6
	Diuron	200	34	6	82	3	37	49	49	6
	Epoxiconazole	—	—	—	2 (<LOD)	5	—	—	—	—
	Simazine	1000	56	9	10	3	5	54	10	9
Site 6	Atrazine	600	37	4	15	23	6	7	27	3
	Diuron	200	72	5	26	50	48	4	38	1
	Epoxiconazole	—	—	—	2 (<LOD)	31	1 (<LOD)	14	—	—
	Simazine	1000	20	5	10	18	123	78	25	16
Site 7	Diuron	200	50	10	47	78	20	13	22	3
	Simazine	1000	38	18	—	—	—	—	7	12

testing period. Two of the five pesticides that tested positive have EQS limits, namely atrazine and simazine, while the other three pesticides detected do not have any EQS limits. The majority of the positive samples contained pesticide residues below their corresponding EQS limits. However, two samples taken at the Site 1 were found to contain diuron above the EQS (200 ng mL^{-1}) threshold, namely on month 2 and 4 at concentrations of 374.7 and 977 ng mL^{-1}, respectively. The latter concentration was also the highest concentration of pesticide found in the samples tested at the seven WWTPs throughout the study. Diuron was the only pesticide that tested positive at all WWTPs and in all the samples analysed. Simazine was found to be present at all WWTPs but not on each day tested. However, simazine does not have an EQS limit. Atrazine was found to be present in all the samples analysed at six of the seven WWTPs, but never exceeded the EQS limit (600 ng mL^{-1}). Mecoprop and epoxiconazole were both detected at two WWTPs but these don't have any established EQS limits. Mecoprop was detected at Site 1 on month 2 (446 ng mL^{-1}) and Site 2 on month 3 (56.1 ng mL^{-1}) and 4 (311.3 ng mL^{-1}). Epoxiconazole

was detected below the LOD of 2 ng mL^{-1} at Site 5 on month 2 (1.9 ng mL^{-1}) and Site 6 on month 3 (1.6 ng mL^{-1}) and 4 (1.4 ng mL^{-1}).

Conclusions

As legislation constantly evolves and adapts with the latest knowledge on the potentially harmful effects of substances such as pesticides to both humans and the environment, it is necessary for analytical methods to do the same. The importance of high-quality, robust methods for the analysis of pesticides in complex environmental matrices like wastewater is paramount for the successful monitoring of these pollutants in our environment. In this study, such a method has been achieved and applied to real samples.

The final method was used to analyse wastewater samples collected from seven WWTPs over a period of four months. Several pesticides were found to be present in the samples tested at each WWTP. A total of 204 samples were collected from 68 sampling events. Exceedances were detected at each of the seven sites in this study, with diuron, atrazine and simazine most frequently occurring. This type of study gives a greater understanding of our environment and allows for more targeted monitoring moving forward. The techniques developed here allow for monitoring at the low limits of detection necessary to assess the risk of these pesticides to the greater environment.

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