Chapter Four

Extraction of Target Analytes from River Water

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4.1 Aims and Objectives

This chapter explores the challenges presented by the sample matrix itself, (i.e. the river water) which is not only highly complex in terms of chemical composition but contains analytes and interferences at varying and trace concentrations. The main challenge presented at this stage is that of sample clean-up and pre-analysis concentrating of analytes so that detectability is within the limits of detection of the analytical instrumentation.

A method has been developed for the extraction of the targeted analytes listed in Table 1.2 from simulated aqueous samples and river water samples. The method is based on the use of automated Solid Phase Extraction (SPE) with a Gilson ASPEC XL4 instrument.

A sample is introduced to an SPE cartridge or column which is packed with an appropriate sorbent, the stationary phase. The mobile phase solvent flows through the column and by choosing the correct combination of mobile and stationary phase, the sample will be held or released from the column.

There are several benefits of using SPE over other extraction techniques; these include lower overall user costs due to lower solvent and reagent consumption; greater recoveries achieved through minimal sample transfer with no cross contamination so achieving better accuracy and increased safety as there is minimal exposure of the operators to samples and solvents with less glassware used and faster extraction protocols meaning there is minimal sample evaporation. SPE can also remove interferences that cause matrix effects and also reduce ion suppression in mass spectroscopic analysis, thereby enabling more sensitive, robust analysis and introducing the analyte in a solvent compatible with the liquid chromatography.

4.2 The Basis of Solid Phase Extraction

There are two general strategies for cleaning and isolating samples. Firstly to introduce the sample to the column and let the analyte of interest pass through the column unretained while the matrix interferences are adsorbed to the column. The second and more commonly used method is to introduce the sample to the cartridge and retain the analyte of interest by adsorption to the column and to then remove interferences. The second strategy is used when the analytes of interest are at trace or low concentrations and samples are very dilute. It is also used when the analytes of interest are non-polar or slightly basic in nature and extraction is based on reverse phase chemistry [1]. For this reason the second method protocols were used in this study.

After filtering the samples to remove particulates, the SPE extraction technique followed the four stage protocol of Column Conditioning, Sample Loading, Washing/Eluting Interferences; Sample Elution (see Figure 4.1).

Column conditioning is required to activate the sorbent prior to the introduction of the sample. For reverse phase extraction adsorption media is conditioned with a water-miscible organic solvent such as methanol, followed by water or an aqueous buffer. Methanol wets the surface of the sorbent and penetrates bonded alkyl phases, allowing water to wet the silica surface efficiently [2].

Sample Loading is when the sample is introduced to the cartridge. The sample loading flow rate can affect the retention of analytes to the sorbent. The Wash Stage retains the compounds of interest whilst washing off unwanted or un-retained interferences. The final stage is to elute the analytes from the cartridge in the smallest volume that achieves this.

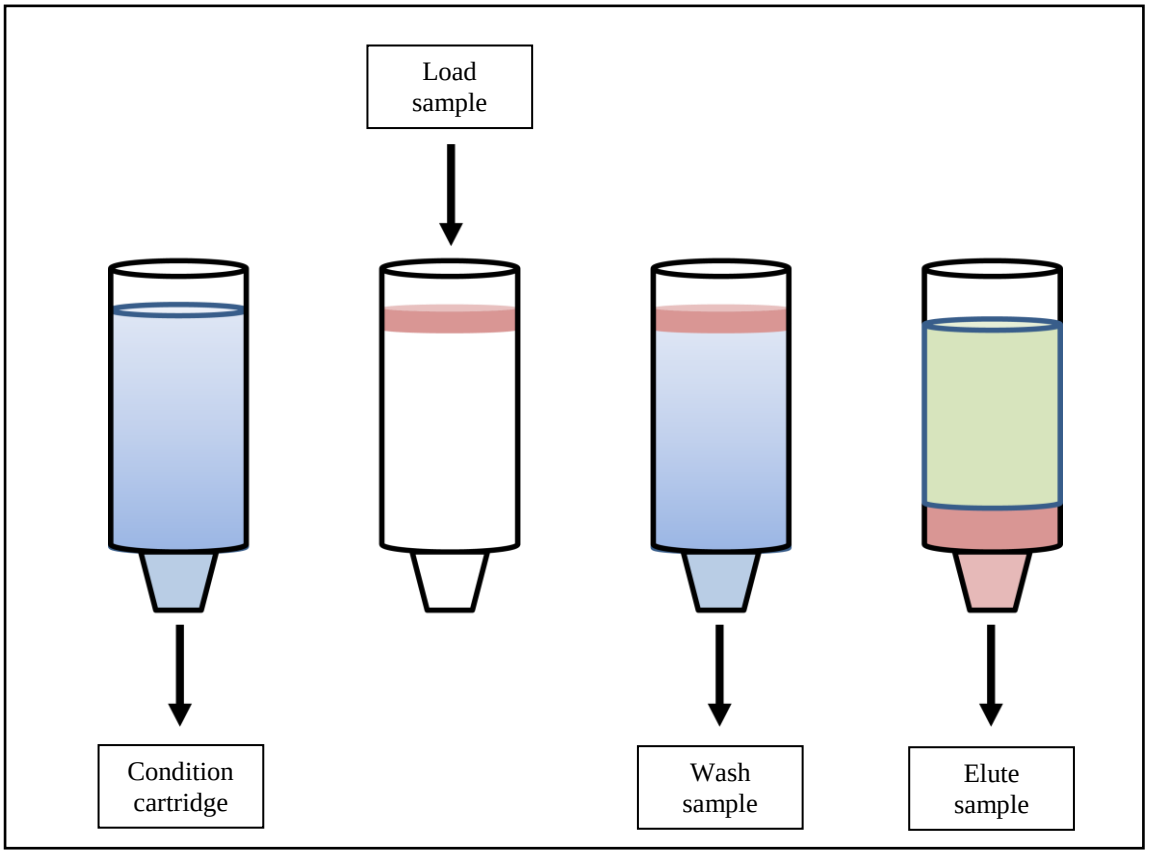

Figure 4.1 Illustration of the elution protocol for SPE

4.2.1 The Gilson ASPEC XL4 Automatic SPE system

This system is an automated 4-port system that can be programmed to perform the steps required for extraction under significantly more repeatable conditions than a manual system due to the automated sampling syringe pump. The instrument consists of the 4-port syringe pump, the cartridge holder section/needle valve assembly and the computer control. The four stage pump system has the ability to extract four individual samples at the same time or it can extract one large volume sample into either one, two, three or four cartridges. It operates under positive pressure, ie. the sample needle valves form an airtight seal at the top of the cartridge and can force solvent through the cartridge. Figure 4.2 is a photograph of the instrument used in this study.

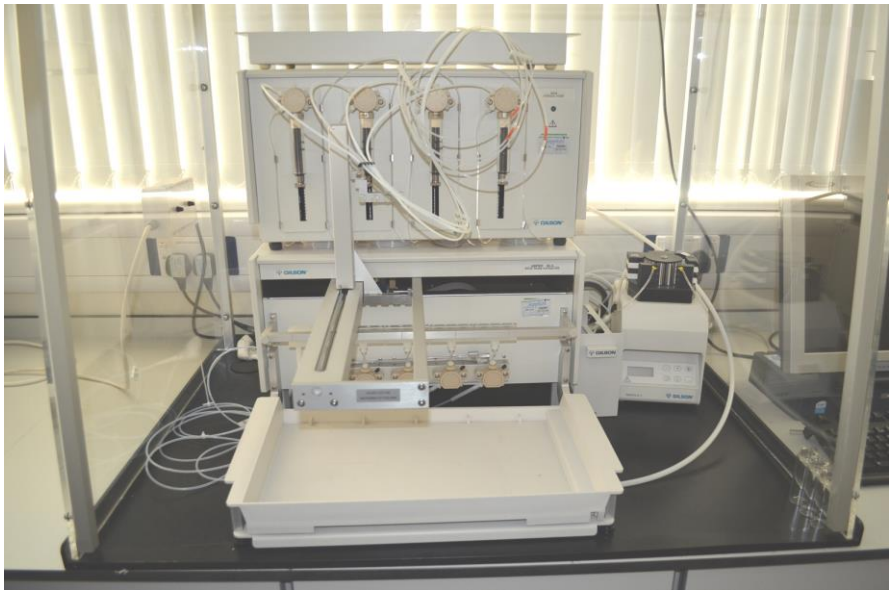

Figure 4.2 Photograph of the ASPEC XL4 automated Solid Phase Extraction system

The 4-port syringe pump draws the required volume of sample or solvent from its particular reservoir via a switching pump and dispenses it through the 4-way needle valves to the required target accurately and safely and is controlled via a specific computer controlled sequence. After extraction, samples were evaporated to dryness under an inert drying gas, nitrogen, before being reconstituted in a solvent that was the initial mobile phase solution used in the chromatographic separation process.

4.3 Method Development Rationale

To develop a suitable extraction method for optimum recovery of the target analytes an SPE sorbent that is the same in terms of polarity as the analytical UPLC column was used. Since the UPLC column used in this study was a C18 non-polar column, SPE cartridges of similar chemistry were trialled. These were Waters Oasis HLB, a hydrophilic-lipophilic-balanced reversed-phase sorbent for acids, bases and neutrals. Waters Oasis MCX **m**ixed-mode **c**ation e**x**change sorbent for bases and Chromabond C18ec is a silica based non-polar sorbent for non polar or slightly basics.

In order to discover the optimised parameters which achieve a desirable extraction of the target analytes (e.g. high analyte recovery), one litre samples of ultrapure water were spiked with a standard mixture of the seventeen analytes each at a concentration of 500 μg l^{-1} to create a simulated matrix.

All seventeen compounds including the native pesticides, their metabolites, plus internal and external standards, were tested with the intention of recovery of all compounds using a single set of conditions.

The optimised method from this simulated study was then applied to extracting the analytes of interest from the river water samples later in the study.

Sorbent types, solvent volumes, conditioning and washing procedures along with flow rates was based on work previously undertaken by Tran [3], Nogueira [4], Ayano [5] along with sorbent manufacturers' standard procedures.

4.4 Sorbent Chemistry

Three different types of sorbent cartridges were considered in this research, namely

- The 'Oasis HLB' Cartridge (Waters UK), (60mg) P/N-186003365
- The 'Oasis MCX' Cartridge (Waters UK) (60mg) P/N-186000253
- The 'Chromabond C18 ec' cartridge (Anachem UK) (200mg) P/N-730012

4.4.1 Reverse Phase Sorbents

Reverse phase sorbents operate effectively when the analyte(s) under investigation have moderate to low polarity and separation is based on hydrophobicity. The sample matrix should be aqueous and as the sorbent surface polarity is low to medium, the solvent polarity range is medium to high [6].

4.4.2 Ion Exchange Sorbents

Ion exchange sorbents separate analytes based on electrostatic interactions between the analyte of interest and charged groups on the stationary phase. Compounds that are ionic or ionizable can be isolated using ion-exchange SPE. The mode is orthogonal to reverse-phase mode and can provide a powerful selective second dimension. Cation exchange uses the negatively charged surface of the stationary phase to attract the positively charged analyte, the cation.

4.4.3 The Oasis HLB cartridge

The Oasis HLB 60 mg Hydrophillic-Lipophillic cartridge is a balanced reverse-phase sorbent made of two monomers; a hydrophilic *N*-vinylpyrrolidone for reverse-phase retention and a lipophillic divinylbenzene for polar interaction. Figure 4.3 shows the interactions that occur with this sorbent [7].

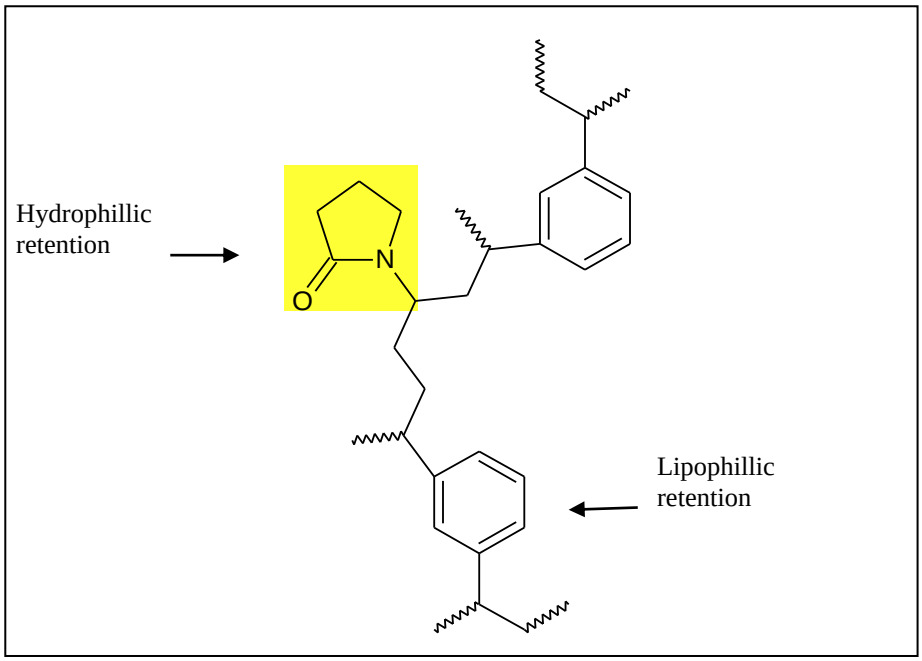

Figure 4.3 The interaction of Oasis HLB sorbent.

4.4.4 The Oasis MCX Cartridge

The Oasis MCX 60 mg mixed-mode cation-exchange reverse phase sorbent enables reverse phase interactions and a strong cation exchange mode. Figure 4.4 shows the interactions that occur within this sorbent. [7].

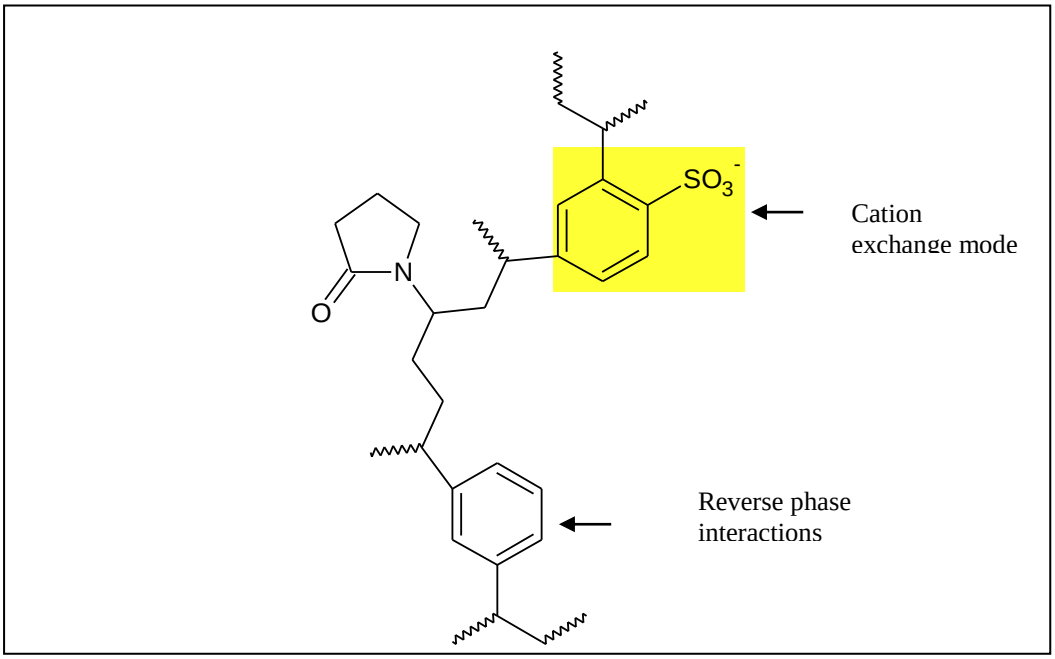

Figure 4.4 The interaction of Oasis MCX sorbent

4.4.5 The Chromabond C18ec Cartridge

The Chromabond C18 ec 200 mg cartridge is an octadecyl modified silica phase endcapped sorbent. Retention is through very non-polar, hydrophobic interactions between the analyte and sorbent. Figure 4.5 shows the interactions that occur within this sorbent [7].

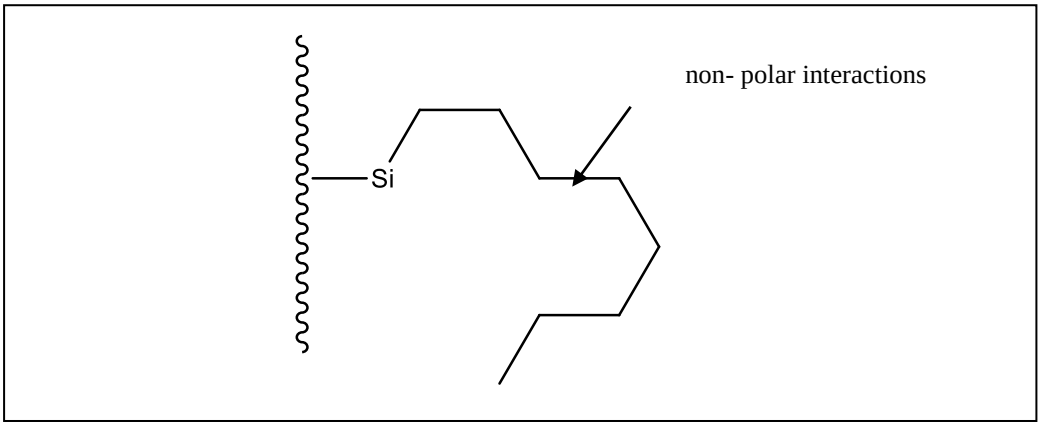

Figure 4.5 The interaction of Chromabond C18 sorbent

4.5 Method

The Gilson ASPEC 4XL was used for all the automated sequence processing conditions. Prior to extraction, the instrument solvent lines, syringe lines, syringe cylinders, needle lines, needles and solvent ports were flushed and rinsed with a water/methanol 50/50 solution.

Solvent reservoirs were used to hold the required solvents that were delivered to the cartridges for extraction. Prior to processing all reservoir / solvents ports are primed twice with 2 cm^3 of with the same solvent it delivers.

The conditioning phase was a two-step process, firstly 100% methanol was passed through the column followed by a water / methanol (98% / 2%) solution. Samples were loaded onto the cartridge and then washed with a water / methanol (98% / 2%) solution before elution in a methanol / ammonium hydroxide (95% / 5%) solution.

Extracted samples were collected in silanised glass tubes before evaporating to dryness with nitrogen in a Turbvap evaporator [8] and reconstituted in 1.0 cm^3 of 80/20 water/acetontrile solution. This solution is the same as the initial mobile phase condition. Each extracted sample is then filtered through a 0.2 µm PTFE (Whatman Puradisc 13mm) to remove any particulate matter.

Table 4.1 details the volumes and flow rates of the process sequence that were used for the first trial runs.

Table 4.1 SPE process sequence developed for the extraction of target analytes

Step	**Solution**	**Volume (cm^3)**	**Flow rate (cm^3 min^{-1})**
Condition 1	methanol 100%	5	3
Condition 2	water/methanol (98%/2%)	5	3
Load	(from sample reservoir)	100	5
Wash	water/methanol (98%/2%)	5	6
Elute	methanol/NH_4OH (95%/5%)	3	6

A standard solution was prepared from the analytical standards prepared for the chromatography and mass spectrometry method development sections, so that each of the seventeen compounds listed in Table 1.2 were at a concentration of 500 ug l^{-1}.

One litre of ultrapure water was adjusted to pH=3.6 with hydrochloric acid (Sigma Aldrich). The acidified water was spiked with 1 cm^3 of the standard solution prepared above and

vacuum filtered through a 0.7 µm glass fibre filter GF/F (Whatman UK). This produced a 'working extraction sample' (simulated matrix) with each of the seventeen compounds having a concentration of 0.5 µg l^{-1}

The one litre sample was processed using the conditions outlined in Table 4.1 and passed through six Oasis HLB cartridges in order to study the recovery of the target analytes. Two further identical one litre volume working extraction samples were prepared and each processed in the same way through six Oasis MCX and six Chromabond C18 cartridges.

The collected extracts were then evaporated to dryness with nitrogen and then reconstituted in a 1 cm^3 solution of water / acetonitrile (80%/20%), representing the initial mobile phase used in the chromatography. The extracted solution was then filtered through a 0.2 µm PTFE (Whatman Puradisc 13 mm) to remove any particulate matter.

Each extract was then analysed by the chromatographic and mass spectrometers procedures developed in the preceding chapters.

4.6 Method Validation

A series of experiments were performed to test the suitability of each cartridge Oasis HLB, Oasis MCX and Chromabond C18 to extract the analytes under investigation using the method in Table 4.1.

A mixture of all seventeen analytes was prepared at a concentration of 500 µg l^{-1} in HQ water to give a matrix sample, 100 cm^3 of this matrix water was extracted through six cartridges of each of the three sorbents. The extracted samples were evaporated to dryness and

reconstituted to 1 cm^3 in a solution of mobile phase of initial condition (80/20 water/acetonitrile).

These extracted samples were then analysed by the analytical procedure developed in the preceding chapters.

A sample of the same 500 µg l^{-1} High Quality (HQ) matrix sample was analysed at the same time as the under the same operating conditions. This sample would be used as a standard to test against the extracted sample.

There were 6 replicate measurements for each of the 6 extracted samples per sorbent and 6 replicate measurements for the HQ matrix standard.

4.6.1 Results

Table 4.2 shows the percentage recovery of each extracted analyte (500 µg l^{-1}) against HQ matrix standard of the three sorbent cartridges, Oasis HLB, Oasis MCX and Chromabond C18ec.

Table 4.2 Percentage recovery of target analytes by SPE (500 µg l^{-1})

Analyte	**Average % Recovery** (6 repeat samples)						
	Oasis HLB (6 replicates)			**Oasis MCX** (6 replicates)			**Chromabond C18** (6 replicates)
	% recovery	std. dev.	%RSD	% recovery	std. dev.	%RSD	% recovery
Atrazine	30.4	6.8	7.5	22.1	9.5	12.5	62.2
Propazine	14.6	4.9	5.2	21.1	10.0	12.8	54.8
Isoproturon	83.2	6.2	19.5	62.9	3.2	18.4	97.1
Diuron	68.2	11.2	40.3	64.4	5.1	19.1	81.7
Atr-desisopropyl	9.8	2.2	2.3	17.7	7.5	8.7	0.4
Atr-desethyl	23.1	1.5	1.6	19.7	8.4	9.8	3.4
Simazine	32.2	5.7	6.4	26.5	9.4	12.4	40.0
Cyanazine	31.1	5.1	7.9	37.6	13.1	19.9	66.8
Alachlor	5.6	1.3	1.3	36.1	5.8	6.7	3.4
Chlortoluron	89.9	18.7	18.7	72.1	13.1	12.5	97.4
Linuron	25.8	12.1	16.6	21.6	8.8	17.6	42.0
Atrazine_d_5	30.9	6.5	7.2	22.5	9.2	11.9	63.2
Propazine-2-hydroxy	20.6	13.5	39.1	89.5	6.6	14.6	10.8
Isoproturon_d_6	92.5	5.8	17.5	69.0	3.8	22.1	100.8
Diuron_d_6	90.2	8.1	50.2	84.4	5.5	28.2	91.5
Simazine_d_{10}	36.9	6.3	7.0	30.3	8.9	11.5	38.5
Alachlor_d_{13}	5.7	1.4	1.0	36.8	4.6	5.3	2.9
Avg. of all analytes	40.6			43.2			50.4

The results show that the average recovery for each type of cartridge was 40.6% for Oasis HLB, 43.2% for Oasis MCX and 50.4% for Chromabond C18. Whilst the average recovery for Chromabond C18 is higher than the two Oasis cartridges it does not recover the analyte atrazine desisopropyl (0.4%) and has a low recovery rate for atrazine desethyl (3.4%) and alachlor (3.4%). It also has a low percentage recovery of the deuterated standard alachlor_d_{13} (2.9%). Since the aim of this study was to recover all analytes in a single cartridge, the Chromabond C18 sorbent did not achieve this and so was not considered further in this research.

The two Oasis cartridges were able to recover all analytes in a single cartridge. For the Oasis HLB cartridge greatest recoveries were for chlortoluron (89.9%), diuron_d_6 (90.2%) and isoproturon (92.5%). The Oasis MCX cartridge greatest recovery rates were for chlortoluron (72.1%), diuron_d_6 (84.4%) and propazine-2-hydroxy (89.5%).

The Oasis HLB has a low recovery rate for the metabolite atrazine desisopropyl (9.8%), alachlor (5.6%) and the deuterated standard alachlor_d_{13} (5.7%). The Oasis MCX shows the lowest recovery rates for atrazine desisopropyl (17.7%), atrazine desethyl (19.7%) and propazine (21.1%). The lowest percentage recovery rate for the Oasis MCX cartridge (atrazine desispropyl, 17.7%) was greater than four of the analytes using Oasis HLB (propazine 14.6%; atrazine desisopropyl 9.8%; alachlor 5.6%; alachlor_d_{13}).

Table 4.3 shows the comparative percentage recoveries of the deuterated standards against the non-deuterated standards for both types of cartridge, Oasis HLB and Oasis MCX.

Table 4.3 Comparing deuterated against non-deuterated analyte percentage recoveries of Oasis HLB and MCX cartridge.

Analyte	**Oasis HLB** (6 replicates)			**Oasis MCX** (6 replicates)		
	% recovery	std dev.	%RSD	% recovery	std. dev.	%RSD
Atrazine	30.4	6.8	7.5	22.1	9.5	12.5
Atrazine_d_5	30.9	6.5	7.2	22.5	9.2	11.9
Isoproturon	83.2	6.2	19.5	62.9	3.2	18.4
Isoproturon_d_6	92.5	5.8	17.5	69.0	3.8	22.1
Diuron	68.2	11.2	40.3	64.4	5.1	19.1
Diuron_d_6	90.2	8.1	50.2	84.4	5.5	28.2
Simazine	32.2	5.7	6.4	26.5	9.4	12.4
Simazine_d_{10}	36.9	6.3	7.0	30.3	8.9	11.5
Alachlor	5.6	1.3	1.3	36.1	5.8	6.7
Alachlor_d_{13}	5.7	1.4	1.0	36.8	4.6	5.3

Since deuterated analytes are identical in shape and only very slightly heavier than their non-deuterated counterpart, atrazine_d_5 is 5 mass units heavier than atrazine, the recovery rates, standard deviation and %RSD of both types of standards should be very similar and is an indication of the SPE reproducibility for the extraction of target analytes.

From Table 4.3, considering the Oasis HLB cartridge, comparing the percentage recoveries between the non-deuterated and deuterated samples, atrazine / atrazine_d_5 (30.4% / 30.9%) and alachlor / alachlor_d_{13} (5.6% / 5.7%) produced very similar percentage recoveries. The comparative standard deviation of the samples was also very similar for atrazine / atrazine_d_5 (6.8 / 6.5) and alachlor / alachlor_d_{13} (1.3 / 1.4) and the comparative %RSD were also very similar for atrazine / atrazine_d_5 (7.5 / 7.2) and alachlor / alachlor_d_{13} (1.3 / 1.0). Simazine and simazine_d_{10} also showed similar results when comparing the percentage recovery (32.2% / 36.9%), standard deviation of (5.7 / 6.3) and %RSD (6.4 / 7.0) although the percentage recovery difference was higher for simazine / simazine-d_{10} than for the atrazine and alachlor samples.

The Oasis MCX cartridge showed a similar pattern to the Oasis HLB cartridge, the comparative percentage recovery for atrazine / atrazine-d_5 (22.1% / 21.5%), standard deviation (9.5 / 9.2) and %RSD (12.5 / 11.9). For alachlor / alachlor_d_{13}, the comparative percentage recovery was (36.1% / 36.8%), standard deviation (5.8 / 4.6) and %RSD (6.7 / 5.3).

Comparing Isoproturon / isoproturon_d_6, the standard deviation and %RSD for both the Oasis cartridges were very similar however the percentage recovery of Oasis HLB had a difference of 9.3% and for the Oasis MCX cartridge, 6.1%. The greatest difference between percentage recovery of non-deuterated to deuterated samples occurred with diuron /diuron_d_6. The Oasis

HLB percentage recovery difference was 22.0% and for the Oasis MCX, 20.0%, the deuterated standard in each producing a greater recovery.

4.7 Conclusions

The Chromabond C18 cartridge was discarded from further study due to its inability to recover the analyte alachlor and the two metabolites atrazine desisopropyl and atrazine desethyl.

The Oasis HLB and Oasis MCX both successfully recovered all 17 compounds in a single cartridge but in comparison to the Oasis MCX cartridge, Oasis HLB had lower percentage recoveries for propazine (14.6%) and atrazine desisopropyl (9.8%) whereas the lowest percentage recovery for any of the 17 compounds for the Oasis MCX cartridge was 17.7% (atrazine desisopropyl). The Oasis HLB also had a very low percentage recovery rate for alachlor (5.6%) and alachlor_d_{13} (5.7%) whilst the Oasis MCX percentage recovered for alachlor was 36.1% and 36.8% for the deuterated standard alachlor_d_{13}.

It was shown that the Oasis MCX cartridge could recover all 17 compounds in a single cartridge which included the deuterated standards of analytes under investigation. It was therefore concluded that the cartridge of choice for solid phase extraction for research into river water was Oasis MCX.

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