Optimising Micropollutants Extraction for Analysis of Water Samples: Comparison of Different Solid Phase Materials and Liquid-Liquid Extraction

Leusch, F.D.L.[1], Prochazka, E.[1], Tan, B.L.L.[2], Carswell, S.[2], Neale P.[3] and Escher, B.I.[3]
[1] Smart Water Research Centre, Griffith University, Southport, Queensland
[2] Queensland Health Forensic and Scientific Services, Coopers Plains, Quensland
[3] The University of Queensland, National Research Centre for Environmental Toxicology (Entox), Coopers Plains, Queensland

Summary

Bioanalytical tools are widely applied for water quality monitoring. Typically, the water sample must be enriched prior to application in a bioassay. The aim of this study was to determine the extraction recovery of a wide variety of common environmental micropollutants (eg, pesticides, pharmaceuticals, hormones and industrial compounds) using a variety of solid phase extraction (SPE) materials. Commonly used liquid-liquid extraction (LLE) techniques were also compared. The results show that hydrophilic-lipophilic balanced (HLB) SPE cartridges in particular provide good recovery of a wide range of compounds. A method combining an Oasis HLB cartridge with a Supelco coconut charcoal cartridge produced an average extraction efficiency of 89-93% with more than 92-95% of the compounds recovered from spiked drinking and river water samples. The method is a useful wide-spectrum method to extract micropollutants from water samples for bioanalytical screening.

Keywords
Gas Chromatography-Mass Spectrometry (GC-MS), Hydrophilic-Lipophilic Balanced (HLB), Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS), Liquid-Liquid Extraction (LLE), Solid Phase Extraction (SPE).

Introduction

The increasing contamination of freshwater with thousands of natural and man-made chemical compounds is one of the key environmental issues facing humanity in the 21st century (Schwarzenbach *et al.*, 2006). One of the key challenges in addressing the issue of micropollutants in water is to develop the tools necessary to assess the presence and impact of these compounds on aquatic life and human health, a challenge that current chemical analytical methods can only meet to a limited extent. Combining chemical analysis with bioanalytical screening is fast becoming a well recognised approach to overcome some of the limitations of chemical analysis alone (Escher and Leusch, 2012). There are several advantages to bioassay analysis including the detection of non-target biologically active compounds and integration (to a certain extent) of mixture toxicity.

Bioanalytical tools can detect all biologically active compounds in a water sample, but only if those compounds are successfully extracted from the water phase and recovered during sample concentration. Previous studies have looked at the extraction efficiency of different methods, but are usually focused on specific classes of compounds (such as pharmaceuticals, Escher *et al.*, 2005) or a particular bioassay endpoint (such as estrogenic activity, Leusch *et al*., 2006).

In this study, we looked at the recovery efficiency of various solid phase extraction material and liquid-liquid extraction for a wide range of micropollutants. This understanding is critical to our appreciation of bioanalytical results.

Materials and Methods

The project was carried out in two stages: stage 1 was designed to compare the recovery of different solid phase and liquid-liquid extraction techniques to allow selection of an optimal method; and stage 2 was designed to test the influence of a natural matrix (in this case river water) on the extraction efficiency of the selected method. The water samples were spiked with a wide variety of pesticides, pharmaceuticals, hormones and industrial compounds to determine the recovery efficiency of the method for compounds with a wide range of physico-chemical properties (Figure 1).

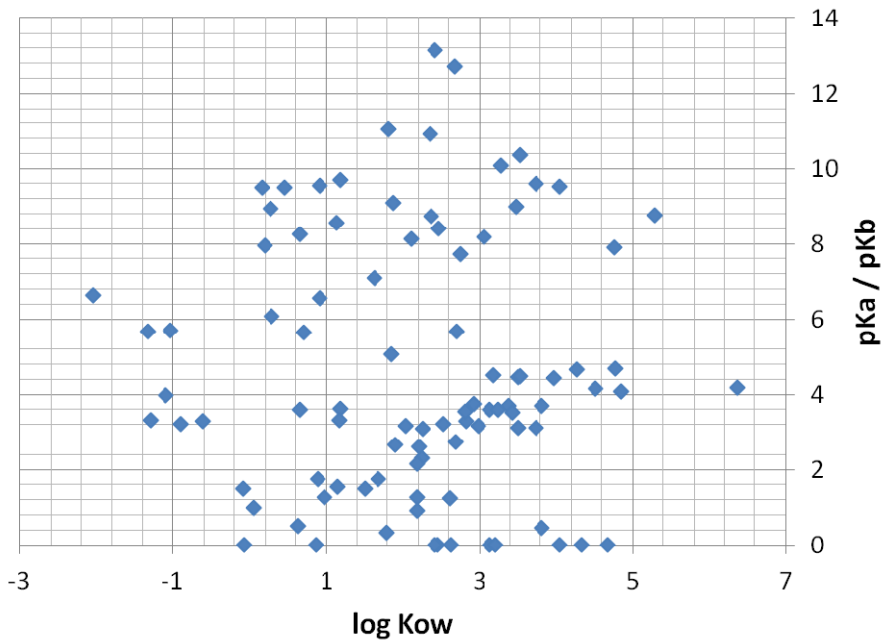

Figure 1. Acid dissociation constant (pKa) *vs.* octanol-water partition coefficient (Kow) for the pesticides spiked in this study, showing the wide range of physico-chemical properties of the spiked compounds.

Stage 1 – Comparison of Different Material and Extraction Methods with Pure Water

In the first stage, ultrapure (reverse osmosis) laboratory water was spiked with 179 pesticides at 1 µg/L and 84 pharmaceuticals and herbicides at 20 ng/L. The pH of the spiked water was adjusted to pH 2 or pH 7, and the samples were extracted in duplicates using eight different extraction methods, six solid phase extraction (Table 1) and two liquid-liquid extractions (LLE).

Table 1. Solid-phase extraction (SPE) cartridges used in this study.

Cartridge	Size (sorbent / cartridge)	Distributor	Catalogue Number
Oasis HLB	200 mg / 6 cc	Waters Corporation, NSW, Australia	WAT106202
Supelco SupelSelect HLB	200 mg / 6 cc	Sigma-Aldrich, NSW, Australia	54183-U
Varian Bond Elut PPL	500 mg / 6 cc	Agilent Technologies, VIC, Australia	12255001
Strata X	500 mg / 6 cc	Phenomenex, NSW, Australia	8B-S100-HCH
Supelco Supelclean Coconut Charcoal	2 g / 6 cc	Sigma-Aldrich, NSW, Australia	57144-U
Varian Bond Elut Carbon	500 mg / 6cc	Agilent Technologies, VIC, Australia	12252201

For the solid phase extraction, the SPE cartridges were pre-conditioned by passing 2× 5 mL of acetone:hexane 50:50, 2 × 5 mL of methanol, and 2× 5 mL of ultrapure water by gravity. One L of the spiked water was then passed by vacuum (up to 2.6 kPa) through the 6 mL cartridges (Table 1). After passing the full one L, the cartridges were air-dried on the manifold for a minimum of 30 min, until visibly dry, and stored at 4ºC until ready for the next step. The cartridges were eluted with 2× 5 mL methanol and 2× 5 mL acetone:hexane 50:50, allowing the solvent to pass through the sorbent bed by gravity first and finished by applying vacuum to pull all the solvent off the cartridge. The 20 mL eluate was pooled and evaporated to dryness at 40ºC under gentle nitrogen stream, reconstituted in 2.5 mL of methanol, and split into three aliquots for the different analysis methods: 1 mL for liquid chromatography – tandem mass spectrometry (LC-MS/MS) analysis; 1 mL (solvent-exchanged into dichloromethane) for gas chromatography – tandem mass spectrometry GC-MS/MS analysis; and 0.5 mL for archiving.

For the liquid-liquid extraction, 500 mL of the spiked water was mixed with 200 mL of either ethyl acetate (EthA) or methyl tert-butyl ether (MTBE) on a shaker for 30 min. The solvent was recovered using a separatory funnel, and the operation repeated twice more with 50 mL of solvent. The pooled 300 mL solvent was evaporated to dryness in a rotary evaporator, reconstituted in 2.5 mL of methanol, and split into three aliquots for the different analysis methods: 1 mL for LC-MS/MS analysis; 1 mL (solvent-exchanged into dichloromethane) for gas chromatography – mass spectrometry (GC-MS) analysis; and 0.5 mL for archiving.

Stage 2 – Performance of the Selected Method with Spiked Drinking and River Water

After selecting a combination of Waters Oasis HLB and Supelco Supelclean coconut charcoal SPE methods based on the results of Stage 1 and those from a previous project (NWC, 2011), the performance of the established method was tested in more relevant environmental matrices such as drinking and river water.

Metropolitan tap water and river water samples were collected. The river water was filtered (Millipore AP20 filter) and half the samples were spiked with 12 endocrine disrupting compounds (hormones and industrial xeno-estrogens) at 50 ng/L, 215 pesticides at 0.8 µg/L, and 88 pharmaceuticals and herbicides at 30 ng/L. The pH of the river water samples was adjusted to pH 2 or pH 7 (only pH 7 for the drinking water), and the samples were extracted in duplicates using the following SPE method.

The SPE cartridges were pre-conditioned separately by passing 2× 5 mL of acetone:hexane 50:50, 2 × 5 mL of methanol, and 2× 5 mL of ultrapure water by gravity. The cartridges were then stacked, with a Waters Oasis HLB cartridge on top of a Supelco Supelclean coconut charcoal cartridge. One L of the spiked and unspiked water samples were passed by vacuum (up to 2.6 kPa) through two cartridges in series. After passing the full one L, the cartridges were separated and air-dried on the manifold for a minimum of 30 min, until visibly dry, and stored at 4ºC until ready for the next step. The cartridges were eluted with 2× 5 mL methanol and 2× 5 mL acetone:hexane 50:50, allowing the solvent to pass through the sorbent bed by gravity first and finished by applying vacuum to pull all the solvent off the cartridge. The two 20 mL eluates were pooled and evaporated to dryness at 40ºC under gentle nitrogen stream, reconstituted in 3 mL of methanol, and split into three aliquots for the different analysis methods: 1 mL for LC-MS/MS analysis; 0.5 mL (solvent-exchanged into dichloromethane) for pesticides GC-MS analysis and 0.5 mL (solvent-exchanged into dichloromethane) for endocrine disrupting compound GC-MS analysis; and 1 mL for archiving.

Chemical Analysis

All samples from Stages 1 and 2 were analysed using standard methods at the NATA-accredited Queensland Health Forensic and Scientific Services (QHFSS) laboratory.

Pesticides were analysed by GC-MS for multi-screening of organochlorine, organophosphorus, synthetic pyrethroid pesticides and some herbicides using a standard protocol (QHFSS Document No 16315: Organochlorine, Organophosphorus and Synthetic Pyrethroid Pesticides, Urea and Triazine Herbicides and PCBs in Water).

Pharmaceuticals and herbicides were analysed by LC-MS/MS using a standard protocol (QHFSS Document No 27701: PPCP in Water, Preparation and Analysis by SPE and LCMSMS).

Endocrine disrupting compounds (only spiked in stage 2) were derivatised with N,N-bis(trimethylsilyl)trifluoroacetamide (BSTFA) + 1% trimethylchlorosilane (TMCS) and analysed by GC-MS using a standard protocol (QHFSS Document No 25391: Determination of Endocrine Disrupting Compounds in Effluent, River and Recycled Water).

Total (and dissolved) organic carbon was measured using a Shimadzu TOC-V CSH total organic carbon analyser at the Smart Water Research Centre.

Results and Discussion

Stage 1 – Comparison of Different Materials and Extraction Methods with Pure Water

The results show that most compounds are well recovered by most of the SPE materials selected for comparison, and confirm the wide retention spectrum of HLB sorbent (Figure 2). The Supelco Supelclean coconut charcoal cartridge retained the least number of compounds and had the lowest median recovery, but had been previously shown to be relatively effective at capturing amines such as NDMA (NWC, 2011). For this reason, we chose to combine an Oasis HLB cartridge with the Supelco coconut charcoal cartridge in Stage 2.

Lowering the pH to 2 resulted in a minor improvement in both median extraction recovery and the number of compounds recovered (Figure 2).

Both LLE techniques yielded an average extraction recovery for the compounds selected in this study that was similar to that of the SPE methods (Figure 2), however the recovery efficiency was significantly more variable between different compounds (as indicated by the larger standard error with the LLE samples, Figure 2, left) and twice as many compounds were not recovered with LLE compared with SPE methods (Figure 2, right). The LLE methods also used significantly more solvent than the SPE methods (600 mL / 1 L *vs.* 20 mL / 1 L) and left an insoluble residue after evaporation. Liquid-liquid extraction can also create emulsions at the interface between the solvent and the water, which can make extraction of some environmental water samples difficult (Wells, 2002).

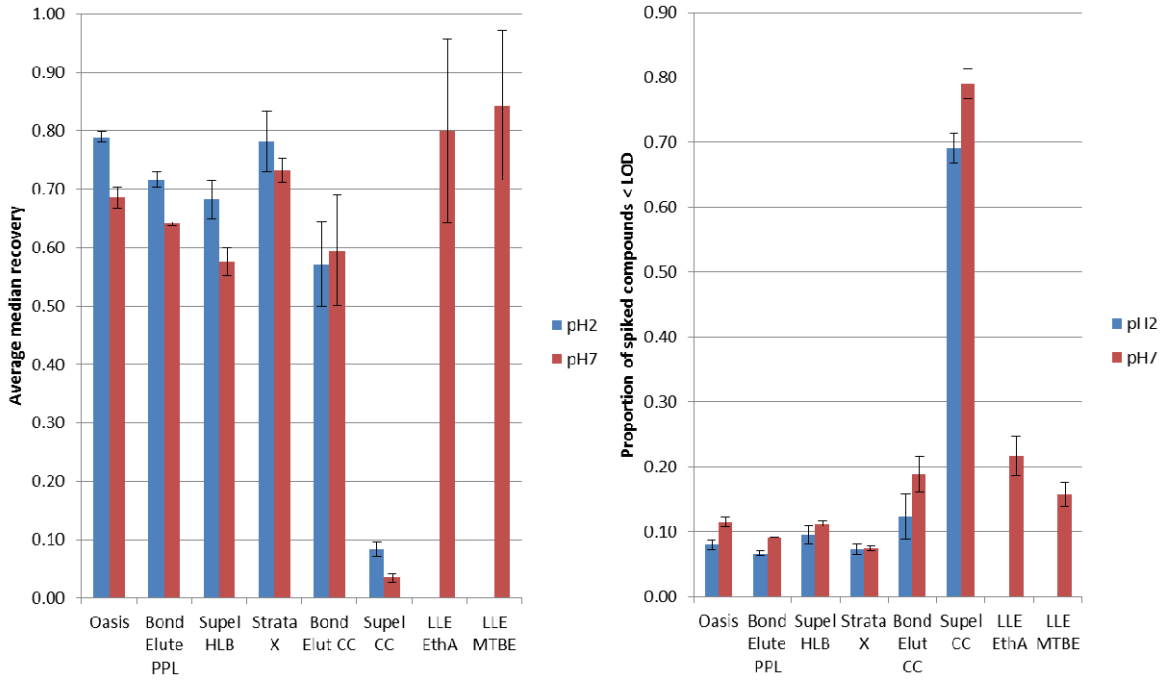

Figure 2. Average median recovery (left) and proportion of spiked compounds below the limit of detection (right) ± standard error for the different solid phase media (Oasis HLB, Bond Elute PPL, Supelco HLB, Strata X, Bond Elute CC and Supelco CC) and liquid extraction methods at pH 2 (blue) and pH 7 (red).

Stage 2 – Performance of the Selected Method with Spiked Drinking and River Water

A few pesticides and pharmaceuticals were detected at low ng/L concentrations in the river water sample (data not shown), and the spike recovery is therefore calculated as (spiked – unspiked) / spiked concentration.

The recovery of the combined Oasis HLB / Supelco CC method was very good, with an average recovery of 89-93% in both river and drinking water (Figure 3). The more complex river water sample did not affect the recovery efficiency, suggesting that the extraction method is sufficiently robust to deal with a moderate level of organic matter (Table 2).

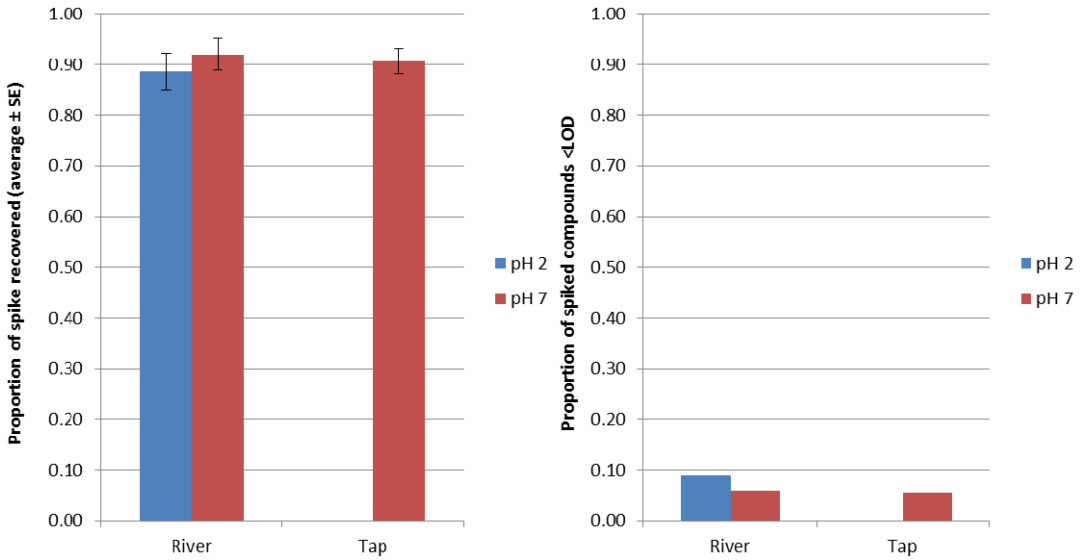

Figure 3. Average recovery ± standard error (left) and proportion of spiked compounds below the limit of detection (right) for compounds spiked in river and tap water at pH 2 (blue) and pH 7 (red) using a tandem Oasis HLB / Supelco CC solid phase extraction cartridge.

Table 2. Total organic carbon in the water samples used in this study.

Sample Type	Total Organic Carbon (TOC)
Ultrapure laboratory water	0.15 mg/L
Tap water	2.05 mg/L
River water (filtered)	8.31 mg/L

Conclusions

The use of a combined Oasis HLB and Supelco coconut charcoal cartridges in series results in good recoveries of a wide spectrum of micropollutants even in environmental water samples. This extraction technique provides a sound method for extraction and concentration of water samples for bioassay analysis. Further work will develop a empirical-based model to predict the recovery of compounds of different chemistry in the various SPE sorbents tested in this study.

Acknowledgments

This project was supported by the Urban Water Security Research Alliance.
We thank Dr Nicole Knight, Steve Carter, Vince Alberts for laboratory support.

References

Escher, B.I. and Leusch, F.D.L. (2012). Bioanalytical tools in water quality assessment. With contributions by Chapman, H.F. and Poulsen, A. IWA Publishing, London, UK.

Escher, B.I., Bramaz, N., Maurer, M., Richter, M., Sutter, D., von Kanel, C. and Zschokke, M. (2005). Screening test battery for pharmaceuticals in urine and wastewater. Environ. Toxicol. Chem. 24(3): 750-758.

Leusch, F.D.L., van den Heuvel, M.R., Chapman, H.F., Gooneratne, S.R., Eriksson, A.M.E. and Tremblay, L.A. (2006). Development of methods for extraction and *in vitro* quantification of estrogenic and androgenic activity of wastewater samples. Comp. Biochem. Physiol. C 143(1): 117-126.

NWC (2011) A national approach to health risk assessment, risk communication and management of chemical hazards from recycled water. Chapman, H.F., Leusch, F.D.L., Prochazka, E., Cumming, J., Ross, V., Humpage, A., Froscio, S., Laingam, S., Khan, S.J., Trinh, T. and McDonald, J.A. (eds). Waterlines Report 48, National Water Commission, Canberra, ACT, Australia.

Schwazenbach, R.P., Escher, B.I., Fenner, K., Hofstetter, T.B., Johnson, C.A., von Gunten, U. and Wehrli, B. (2006). The challenge of micropollutants in aquatic systems. Science 313(5790): 1072-1077.

Wells, M.J.M. (2002). Principles of extraction and the extraction of semi-volatile organics from liquids. In: Sample preparation techniques in analytical chemistry, Mitra, S. (ed). John Wiley and Sons, Hoboken, New Jersey, USA, pp. 37-138.