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Short communication

A comparison of three analytical techniques for the measurement of steroidal estrogens in environmental water samples

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ABSTRACT

Research into the analysis and monitoring of steroidal estrogens has grown significantly over the last decade, resulting in the emergence of a range of applicable techniques. In this study, three popular techniques, gas chromatography–mass spectrometry (GC–MS), gas chromatography–tandem mass spectrometry (GC–MS–MS) and liquid chromatography–tandem mass spectrometry (LC–MS–MS) for the analysis of three highly potent steroidal estrogens in the aquatic environment have been compared. It has been observed that overall, the three techniques appear comparable in generating similar estrogen concentrations for river and effluent samples. Of the three techniques, the GC–MS technique is the simplest to operate, but fails to detect the estrogens at the lower-end of environmentally relevant concentrations. The tandem MS techniques are more selective than MS, and therefore able to detect lower concentration levels of the three steroidal estrogens of interest. However, the LC–MS–MS technique is more susceptible to matrix interferences for the analysis of samples, resulting in a reduction of the signal-to-noise ratio and a subsequent reduction in reliability and stability compared to GC–MS–MS. With the GC–MS–MS technique offering increased selectivity, the lowest limits of detection, and no false positive identification, it is recommended to be the preferred analytical technique for routine analysis of estrogens in environmental water samples.

1. Introduction

Of current concern worldwide are the so-called endocrine-disrupting chemicals (EDCs) which are broadly defined as chemicals that may interfere with the function of the endocrine system in wildlife and humans. Endocrine disruption has been shown to reduce fish fertility, to be linked to human cancers, and may also affect human fertility [1–4]. A wide diversity of compounds has been found to possess endocrine disrupting properties, including naturally occurring estrogens such as estrone (E1), 17β-estradiol (E2) and 16α-hydroxyestrone, androgens and progestogens [5–7]. In comparison, man-made EDC suspects are more diverse in range and are produced in greater quantities than natural EDCs and include the synthetic steroid 17α-ethynylestradiol (EE2; the contraceptive pill), certain pesticides and industrial chemicals such as bisphenol A and alkylphenols [7,8]. Many of such compounds are classified as priority substances in the EU's Water Framework Directive (2000/60/EC). In terms of estrogenic activity, however, the most important EDCs are E1, E2 and EE2 as they are far more potent than other compounds such as bisphenol A or alkylphenols, and can cause fish feminisation at approximately the ng L^{-1} level [9,10]. Due to uncertainty in their impacts on terrestrial and aerial organisms as a result of lack of data, E1, E2 and EE2 are not yet included in the list of 146 substances with endocrine disruption classification [11], nevertheless, their feminisation effects in invertebrates and fish have been confirmed worldwide. In addition, it is widely recognised that effluent discharges from sewage treatment works (STW) are the main source of EDC inputs to the aquatic environment such as rivers and streams [12,13]. Other sources include animal agriculture, aquaculture and spawning fish [14].

In order to minimise EDC impacts on fish populations, reliable and sensitive analytical methods are needed to detect EDCs in the aquatic environment. The concentrations of EDCs are generally low in aquatic systems, up to 19.4 ng L^{-1} in surface water, although levels as high as 5400 ng L^{-1} have been found in some STW effluents [7]. As a result, water samples are usually concentrated using solid-phase extraction (SPE). A wide variety of analytical techniques have been developed and subsequently optimised for EDC analyses, among which gas chromatography (GC) coupled with mass spectrometry (MS) and tandem MS is the first developed and still widely used [15–19]. A more recent and increasingly popular technique has been the liquid chromatography (LC) coupled with MS or MS–MS

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which does not require sample derivatisation [20–24]. As EDCs are being measured at trace levels, often close to the limit of detection (LOD) of the instruments, there is a need to understand how the different techniques compare in terms of their performance. Only by knowing which technique(s) are most reliable and reproducible, can we appraise relative merits and focus on the optimisation of methodologies.

This study investigates the performance of three analytical techniques including GC–MS, GC–MS–MS and LC–MS–MS, all previously developed and validated for the analysis of emerging contaminants including E1, E2 and EE2 in environmental water samples [17,19,25]. The influence of sample matrix on analytical quality at trace levels is highly important and widely speculated, and is addressed.

2. Experimental

2.1. Chemicals and standard solution

All solvents used (methanol, ethyl acetate, acetone, dichloromethane, hexane and acetonitrile) were of distilled-in-glass grade (purchased from Rathburn Chemicals Ltd., Walkerburn, Scotland). EDC standards including E1, E2 and EE2, together with their deuterated internal standards E2-d_2 were purchased from Sigma, UK. In addition, other internal standards E1-d_4, E2-d_4 and EE2-d_4 were obtained from Qmx Laboratories Ltd., UK, all with an isotopic purity >98%. Separate stock solutions of individual standards (1000 mg L^{-1}) were prepared in methanol, from which working standards (10 mg L^{-1}) of individual compounds and mixtures were prepared. All standards were stored at −18 °C. Ultrapure water was supplied by a Maxima Unit from USF Elga, UK.

2.2. Sampling and sample treatment

Water samples (in triplicate) were collected in pre-cleaned Winchester amber-glass bottles (2.5 L) from four sites (sites 1–4) along the River Ray, and at one control site (site 5) on the River Ock, Swindon, UK. Site 1 is approximately 3.5 km upstream from the effluent of Rodbourne STW (adjacent to site 2). Sites 3 and 4 are 1.7 and 8.3 km downstream of the effluent, respectively. Sodium azide (10 mL, 2 M) was added to each sample as a general biocide to eliminate bacteria and thus minimise biodegradation during sample storage and processing. Samples were refrigerated at 4 °C until filtration and extraction. Each sample was filtered under vacuum using pre-ashed glass fibre filters (Whatman, GF/F). The filtrates were subsequently spiked with 100 ng of the internal standards.

2.3. SPE

The target compounds were extracted from the filtered water samples using SPE. Oasis® SPE cartridges (0.2 g HLB, Waters) were conditioned with 5 mL of ethyl acetate to remove residual bonding agents, followed by 5 mL of methanol which was drawn through the cartridges under a low vacuum to ensure that the sorbents were soaked in methanol for 5 min. Ultrapure water (3× 5 mL) was then passed through the cartridges at a rate of approximately 1–2 mL min^{-1}. Water samples (2 L) were then extracted at approximately 10 mL min^{-1}, as this has been shown to be optimal [18]. The SPE cartridges were subsequently dried under vacuum and the extracts eluted from the sorbents into 20 mL vials with 10 mL of methanol at a flow rate of 1 mL min^{-1}. The solvent was then blown down to 100 μL under a gentle N_2 flow, and transferred to 300 μL microvials ready for analysis.

2.4. Derivatisation

In the case of GC analyses of EDCs, the target compounds need to be derivatised to produce less polar derivatives. This enhances chromatographic performance by improving peak shape, reduces tailing and provides a better baseline. Briefly, the extracts were transferred into 3 mL reaction vials and were evaporated to dryness under a gentle stream of nitrogen. The dry residues were then derivatised by the addition of 50 μL each of pyridine (dried with KOH solid) and *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA), which were heated in a heating block at 60–70 °C for 30 min following a previously optimised method [18]. The derivatives were cooled to room temperature, evaporated under a gentle stream of nitrogen to dryness, reconstituted in 100 μL of hexane and transferred to 300 μL microvials ready for analysis by GC–MS and GC–MS–MS.

2.5. Sample analyses

2.5.1. LC–MS–MS

The untreated extracts in methanol were analysed using a Waters 2695 HPLC separations module (Waters, Milford, MA, USA) fitted with a Waters Symmetry C_{18} column (4.6 mm × 75 mm, particle size 3.5 μm). The mobile phase comprised of eluent A (0.1% formic acid in ultrapure water), solvent B (acetonitrile) and eluent C (methanol). The flow rate was 0.2 mL min^{-1} and the elution started with 90% eluent A:10% eluent B, a 25 min gradient to 80% of eluent B, then a 3 min gradient to 100% eluent B, followed by an 8 min gradient to 100% of eluent C. This was held for 10 min and then returned back to the initial conditions within 4 min. The system re-equilibration time was 10 min and the sample injection volume was 10 μL. The MS–MS analyses were completed with a Micromass Quattro triple-quadrupole mass spectrometer equipped with a Z-spray electrospray interface. The analyses were in negative ion mode. The parameters for the analyses were: electrospray source block and desolvation temperature 100 and 300 °C, respectively; capillary and cone voltages 3.0 kV and 30 V, respectively; argon collision gas 3.6×10^{-3} mbar; cone nitrogen gas flow and desolvation gas: 25 and 550 L h^{-1}, respectively. Following the selection of the precursor ions, product ions were obtained at optimum collision energies and were selected according to the fragmentation that produced a useful abundance of fragment ions. The optimal collision energy, cone voltage and transitions chosen for the multi-

Table 1
Retention times (RT) and ions used for the analysis of E1, E2 and EE2.

Compound	LC–MS–MS			GC–MS–MS			GC–MS		
	RT (min)	Precursor ion (*m*/*z*)	Product ion (*m*/*z*)	RT (min)	Precursor ion (*m*/*z*)	Product ion (*m*/*z*)	RT (min)	Quantitative ion (*m*/*z*)	Confirmation ion (*m*/*z*)
E1-d_4							14.44	346	257 (100%), 285 (30%)
E1	19.50	269	183	18.10	342	257 (100%), 327 (10%)	14.51	342	257 (100%, 218 (20%)
E2-d_4							17.72	289	420 (100%), 330 (35%)
E2-d_2	17.75	273	186	18.50	418	287 (100%), 233 (75%)			
E2	17.70	271	145	18.50	416	285 (100%), 243 (28%)	18.08	285	416 (100%), 326 (40%)
EE2-d_4							19.29	289	430 (100%)
EE2	19.05	295	145	19.70	425	193 (100%), 231 (70%)	19.42	285	425 (100%), 232 (30%)