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Short communication

Analysis of steroids in environmental water samples using solid-phase extraction and ion-trap gas chromatography–mass spectrometry and gas chromatography–tandem mass spectrometry

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Abstract

This paper describes an improved method for the extraction and determination of three steroids, oestrone, 17β-oestradiol, and the synthetic contraceptive steroid 17α-ethinyloestradiol in aqueous matrices. Samples of wastewater and environmental water were spiked with internal standards, comprising isotopically labelled analogues of the steroids to be determined. The samples were extracted using solid-phase extraction disks and the extracts were then derivatized to form *tert*.-butyldimethylsilyl derivatives. The derivatised steroids were determined in the final extracts by GC–MS or GC–MS–MS allowing an operational detection limit for each steroid in effluent samples of 1 ng l^{-1}. Crown copyright © 2000 Published by Elsevier Science B.V. All rights reserved.

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1. Introduction

Previous work had established that the oestrogenic activity found in predominantly domestic sewage effluents could be solely attributed to the presence of three oestrogens [1]. These were the naturally occurring steroids oestrone and 17β-oestradiol, and the synthetic contraceptive steroid 17α-ethinyloestradiol.

Collaborative studies undertaken between the Water Research Centre (WRc) and CEFAS have established and validated a method for the determination of these three steroids in filtered freshwater from the River Thames [2]. However, when this method was applied subsequently to the analysis of wastewater and reservoir water samples, poor recoveries were obtained for the surrogate standards, generally below 2%. These types of environmental waters and effluents often had a very high particulate loading, and could also contain high concentrations of colloidal material (possibly due to the presence of polysaccharides derived from the breakdown of algae). As a result of this difficult matrix, the solid-phase extraction (SPE) cartridges rapidly became clogged with colloidal and particulate material, so extraction of each sample could require the use of multiple SPE cartridges for extraction purposes. These cartridges also proved difficult to elute, and often contained additional contaminants, leached from the plastic walls, that masked the steroid peaks. It was therefore decided to investigate the use of SPE disks as an alternative extraction procedure. The disks offered the advantage of larger water/extrac-

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tant surface areas and low levels of contamination. This investigation demonstrated that they provide a viable technique for the analysis of water samples with a high particulate loading, and the technique proved applicable to a wide range of environmental water samples. The concentrations of suspended solids were not measured in the samples collected for steroid analysis. Wastewater from the treatment works was analysed for suspended solids by Essex and Suffolk Water, and the average level of suspended solids in wastewater from the treatment works during this period, was 16 mg l^{-1}. The effectiveness of SPE disks with depth filter had previously been demonstrated with samples containing up to 500 mg l^{-1} of suspended solids. [3]

2. Experimental

Water samples were collected in previously silanised 2.5-l glass Winchester bottles. These samples were stored at 4°C prior to extraction. Extractions were always completed within a week of sample collection, as longer-term storage resulted in the degradation of 17β-oestradiol to oestrone. The method was validated by the analysis of spiked samples of river water, and of water from a reverse osmosis system at the laboratory. Commercially available standards of oestrone, 17β-oestradiol, and 17α-ethinyloestradiol (Sigma, Poole, UK) were added to 20 l of water, and the water was thoroughly mixed using a magnetic stirrer for 20 min prior to sampling. The water was run through PTFE tubing to a PTFE tap and the 2.5-l sample was collected at this point. Prior to extraction, internal standards were added for the purpose of peak identification and quantification. Response factors for the oestrogens were generated by the use of spiked blanks. The internal standards used were the isotopically labelled analogues of the steroids to be determined, [$^2H_4$]oestrone, [$^2H_4$]17β-oestradiol and [$^2H_4$]17α-ethinyloestradiol (Univar, Croydon, UK). Aqueous samples were extracted using 90 mm C_{18} disks (Varian, Walton-on-Thames, UK) with 40–60 g of depth filter (Empore filter aid 400, Varian). The depth filter was added after location of the disk in the extraction manifold, and underwent the same procedure. Prior to extraction the disks were washed with a methanol–water (85:15, v/v) mixture to clean the disk, and to remove any potentially interfering compounds. Following the wash the disks were conditioned, first by passage of methanol and then of water. The water samples were transferred to a 3M Empore extraction manifold (Varian) and drawn through the disks under vacuum. Each disk was then allowed to dry prior to elution of the steroids. The disk was washed with 50 ml of methanol–water (45:50) to remove any interfering compounds, and the wash mixture discarded. The steroids were eluted with two 30-ml aliquots of methanol–water (85:15), and the aliquots collected and combined in a silanised glass tube. The extract was concentrated under a stream of dry nitrogen gas using a Turbovap evaporator (Zymark, Warrington, UK) at 40°C, to a volume of approximately 200 μl. The residue was quantitatively transferred in methanol to a Reacti-Vial (Pierce & Warriner, Chester, UK) and reduced to dryness under nitrogen in the Turbovap unit. The steroid extract was then derivatized using 100 μl of silylation grade acetonitrile and 100 μl of *N*-methyl-*N*-*tert*.-butyldimethylsilyltrifluoroacetamide (MTBSTFA), containing 1% *tert*.-butyldimethylchlorosilane (TBDMCS) (Pierce & Warriner) to form *tert*.-butyldimethylsilyl derivatives. The products formed by the reaction of MTBSTFA containing 1% TBDMS are shown in Fig. 1. They were all mono-substituted derivatives due to steric hindrance. The advantage of these derivatives over the conventional trimethylsilyl (TMS) derivatives were that they had rapid reaction times and that they were more stable against hydrolytic cleavage than the comparable TMS derivatives.

The Reacti-Vial was capped and sonicated for 1 min, left to stand for 15 min, sonicated for a further minute, and then allowed to stand for 15 min. The mixture was again taken to dryness under nitrogen, reconstituted with 50 μl of diethylether, and sonicated to ensure that the residue was completely redissolved before being transferred using a microliter syringe to a silanised autosampler vial. Samples that contained a lot of coextractives were dissolved in up to 1 ml of diethylether, and sonicated for a longer period to allow dissolution of the dried derivatized mixture. They were then concentrated back down to 50 μl using the Turbovap.

The derivatised steroids were determined in the final extracts by GC–MS or GC–MS–MS using a

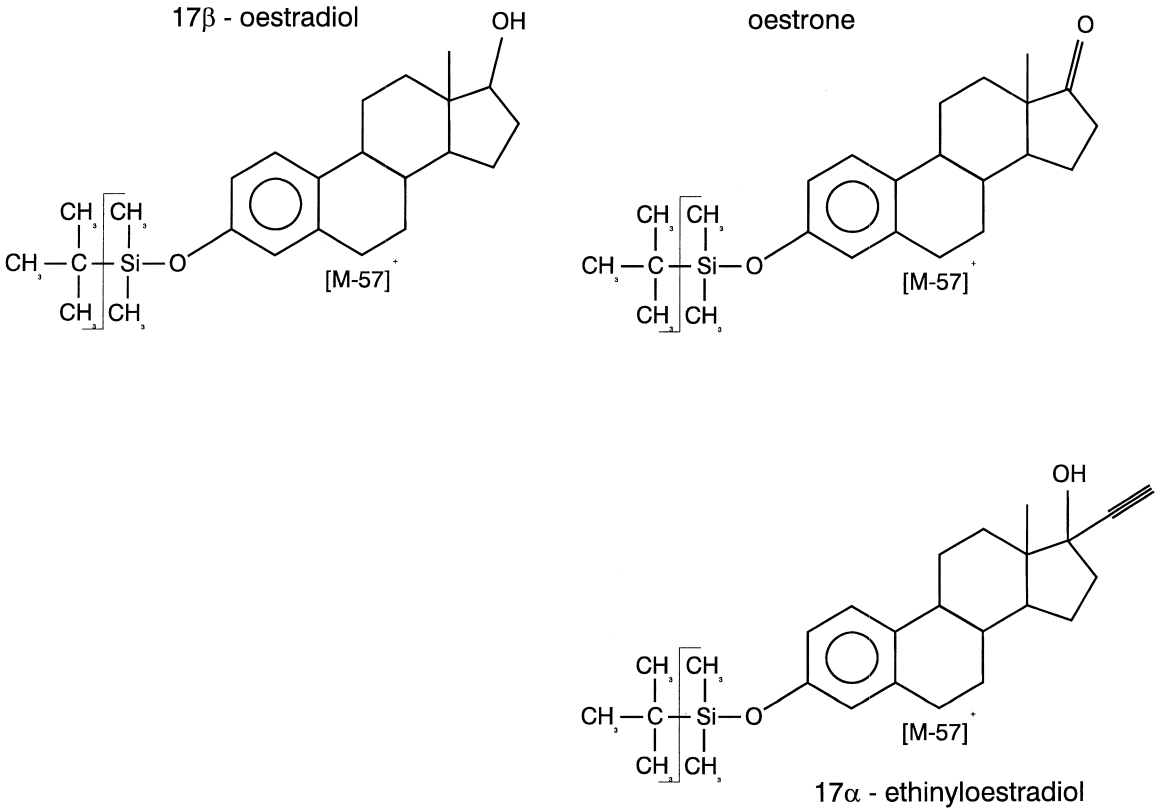

Fig. 1. Structures to show products formed during derivatization and ions formed during GC–MS–MS.

ThermoQuest GCQ benchtop ion-trap instrument (ThermoQuest, Hemel Hempstead, UK). The GC was fitted with a split/splitless injector which was operated in the splitless mode, with the split valve being opened to purge the injector 2 min after injection. The injector temperature was held at 200°C. The GC column used was a 30 m×0.25 mm I.D. crosslinked fused-silica capillary column coated with 5% phenyl methylsilicone fluid (0.25 μm film thickness). The carrier gas was helium at a linear flow-rate of 40 cm s^{-1}. The 2 μl injection was made at a column temperature of 30°C. Following injection, the oven temperature was held at 30°C for 1 min, and subsequently raised at 10°C min^{-1} to a final temperature of 300°C, which was then held for 10 min, giving a total run time of 38 min. The GC column was directly coupled, via a transfer line heated to 275°C, to the ion source of the ion-trap mass spectrometer. This was interfaced to and controlled by a data system using GCQ 2.2 software

Table 1
Dissociations monitored during steroid analysis using selected reaction monitoring

Compound to be determined	Dissociation monitored by GC–MS–MS
Oestrone	384 → 327
[$^2H_4$]Oestrone	388 → 331
17β-Oestradiol	386 → 329
[$^2H_4$]17β-Oestradiol	390 → 333
17α-Ethinyloestradiol	410 → 353
[$^2H_4$]17α-Ethinyloestradiol	414 → 357

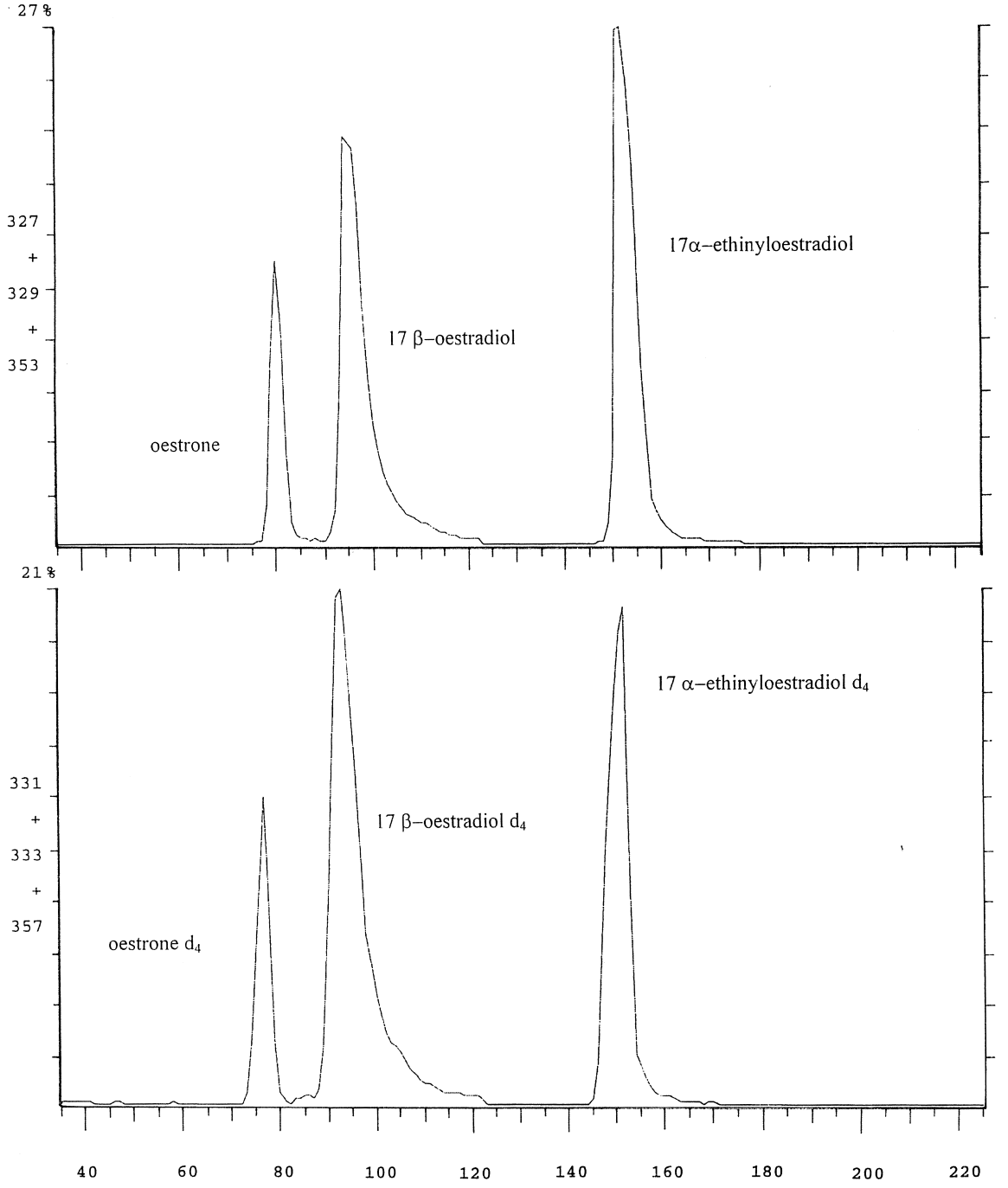

Fig. 2. GC–MS–MS chromatogram of spiked river water.

running under Windows NT. The software controlled the GC–MS instrument as well as data acquisition and storage. For full scan acquisition (GC–MS), the mass spectrometer was operated in the electron-impact ionisation mode at 70 eV, and scanned from 50 to 500 u. A cycle time of 1 s was used to ensure that an adequate number of data points were collected in order to fully define the chromatographic peaks due to the steroids. For dual selected reaction monitoring (GC–MS–MS), the mass spectrometer was used to monitor the dissociations listed in Table 1.

The ions selected for the GC–MS–MS work were the lower mass ions shown in Table 1. The reaction monitored by MS–MS was the $M^+ \rightarrow [M-57]^+$ dissociation, the $[M-57]^+$ ion resulting from the loss of the tertiary butyl group from the molecular ion as shown in Fig. 1.

The cycle time in all cases was 0.5 s. The system performance was optimised with respect to excitation voltage (collision energy), which was generally between 0.6 and 0.85 V, by analysis of a standard solution containing the six derivatised steroid compounds.

3. Results and discussion

A typical GC–MS–MS chromatogram from a sample of spiked river water is shown in Fig. 2.

Peak tailing of 17 β-oestradiol occurred as contaminants accumulated in the injection port liner. The response was restored by replacing the liner and removing a section of the capillary column at the injector end. The problem of peak tailing has also been reported for 17α-ethinyloestradiol [4].

The use of SPE disks yielded extracts free from the interferences which were sometimes seen when using SPE cartridges containing similar sorbents. These interferences were thought to result from the leaching of plasticisers from the cartridge support material during elution. The results from the validation study are given in Table 2.

These results demonstrated that by using SPE disks the extraction method was efficient and reproducible, with analyte recoveries above 90%, and RSDs between 2 and 6%. The concentrations of the three steroids in the procedural blanks were <0.2 ng l^{-1}, allowing an operational detection limit for each steroid in effluent samples of 1 ng l^{-1}. Similar results were obtained for the analysis of spiked water from the River Blackwater, and of spiked reservoir water, with the recoveries of the internal standards generally above 70%.

This method has demonstrated its applicability in a number of studies undertaken within the last 2 years [5]. In conjunction with a yeast oestrogen inducible expression (YES) assay [6], it has been used to study the fate of steroids during ultraviolet light disinfection of treated wastewater at a water recycling plant. The concentrations of steroids determined in 30 treated wastewater samples were within the range of <1–55 ng l^{-1}. The concentration ranges for the individual steroids were oestrone 10–55 ng l^{-1},

Table 2
Concentrations of steroids determined and spiked values from the analysis of reverse osmosis water

Sample	Concentration (ng l^{-1})		
	Oestrone	17β-Oestradiol	17α-Ethinyloestradiol
Spiked concentration	25.5	21.6	26
Sample 1	25	21	25
Sample 2	26	21	26
Sample 3	25	22	24
Sample 4	25	21	23
Sample 5	26	22	23
Blank water	0.06	0.1	0.2
Recovery (%)	99.6	99	92
Mean (ng l^{-1})	25.4	21.4	24
Standard deviation (ng l^{-1})	0.548	0.548	1.41
RSD (%)	2.2	2.6	5.9

17β-oestradiol 2–48 ng l^{-1} and 17α-ethinyloestradiol <1–55 ng l^{-1}.

Further work is being conducted on sewage effluent treatment techniques, with the aim of reducing the quantities of steroids released to the aquatic environment following sewage treatment.

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