ELSEVIER

Journal of Chromatography A, 923 (2001) 195–204

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

# Analysis of estrogens in river water and effluents using solid-phase extraction and gas chromatography–negative chemical ionisation mass spectrometry of the pentafluorobenzoyl derivatives

Xiao-Yao Xiao[a], David V. McCalley[a,*], James McEvoy[b,1]

[a]*University of the West of England, Faculty of Applied Sciences, Frenchay Campus, Coldharbour Lane, Bristol BS16 1QY, UK*
[b]*Environment Agency, Thames Region, Kings Meadow Road, Reading RG1 8DQ, UK*

Received 7 February 2001; received in revised form 8 May 2001; accepted 9 May 2001

**Abstract**

A procedure was developed for the analysis of estrogens in environmental water and effluents. Samples were extracted by passing through polymer-impregnated solid-phase extraction discs or $C_{18}$ cartridges, followed by gas chromatography–negative chemical ionisation mass spectrometry of the pentafluorobenzoyl derivatives. The derivatives were stable and gave diagnostic negative molecular ions as the base peak for each of the major estrogens studied. The absolute recovery of estrogens spiked into clean groundwater using the disc procedure was 84–116% at the 10 ng $l^{-1}$ level (calculation not based on use of internal standards). Using doubly deuterated estradiol as internal standard added prior to extraction, the % relative standard deviation of estrogen extraction and analysis in spiked groundwater at the 10 ng $l^{-1}$ level was 2.6–9.8%. Detection limits were 0.2 ng $l^{-1}$ or below for the major estrogens, based on a 2.5 litre sample. The most abundant estrogen was estrone, with concentrations over the range 6.4–29 ng $l^{-1}$ in effluents, and 0.2 to 17 ng $l^{-1}$ in water from the River Thames.


*Keywords:* Water analysis; Environmental analysis; Estrogens; Steroids

## 1. Introduction

There has long been concern over the environmental impact of the discharge of sewage effluents to water courses. In addition to the microbiological risks of sewage and other effects such as heavy metal pollution, attention in recent years has also been focussed on substances in sewage which have endocrine disrupting properties. It is possible that these substances may be linked with the supposed decline of sperm counts in adult males, the increasing incidence of breast cancer and testicular cancer, and the decline in the age at which the onset of puberty occurs [1]. Although these issues have been of much public concern, links with endocrine disrupters have remained speculative, due to the lack of sufficient research in these areas. Nevertheless, it has been shown that male fish held in cages exposed to sewage effluent undergo feminisation effects, and indeed that rivers which receive significant amounts of sewage effluent contain numbers of ''intersex'' (part male, part female) fish. It appears that effects of

*Corresponding author. Fax: +44-11-7344-2904.
*E-mail address:* david.mccalley@uwe.ac.uk (D.V. McCalley).
[1]Present address: Postgraduate Research Institute for Sedimentology, University of Reading, Whiteknights, P.O. Box 227, Reading RG6 6AB.

0021-9673/01/$ – see front matter 
PII: S0021-9673(01)00955-4

effluent on fish may be time dependent, thus fish exposed to quite dilute effluents, but for a long period, may show feminisation effects [2]. Chemical fractionation combined with biological screening methods have indicated that natural estrogens (and possibly estrogens derived from contraceptive pills) are largely responsible for these feminisation effects, rather than other chemical pollutants [2,3]. In one investigation, estradiol and estrone were found in all effluents studied at concentrations from 1 to 80 ng $l^{-1}$ although ethynylestradiol levels were usually below the limit of detection of the method (approximately 0.2 ng $l^{-1}$ based on a 20 litre spiked sample of tap water) [3]. Methods for chemical analysis were complex involving solid-phase extraction (SPE) using $C_{18}$ cartridges, further clean-up using a gradient HPLC method with $C_{18}$ column, followed by GC–MS of free and silylated derivatives, using electron impact ionisation (EI). De Aldà [4] recently proposed a method including SPE of 500 ml of sample using $C_{18}$ cartridges followed by HPLC-diode array detection (DAD) and HPLC–MS in series. Electrospray ionisation (ESI) in the negative ion mode was used for the MS detection of estrogens. Detection limits for DAD of estrogens were reported as 50 ng $l^{-1}$ and for ESI–MS 50, 250, 500 and 100 ng $l^{-1}$ for estriol, estradiol, ethynylestradiol and estrone respectively, based on the use of 500 ml samples. Recovery studies of the method were reported at the 10 μg $l^{-1}$ level. These levels seem somewhat high considering the levels of estrogens in effluents reported by other groups. Laganà et al. [5] used a graphitised non-porous carbon black SPE cartridge to prepare samples, followed by LC–tandem mass spectrometry (LC–MS–MS) using atmospheric pressure chemical ionisation (APCI,) which gave limits of quantitation of about 1 ng $l^{-1}$ for estrogens based on 1 litre samples. Recoveries of estrone, estradiol, ethynylestradiol and estriol were 84–95% when sewage effluents were spiked at the 20 ng $l^{-1}$ and 100 ng $l^{-1}$ level. Kelly proposed a method for the determination of three steroids, estrone, estradiol and ethynylestradiol using $C_{18}$ SPE discs and GC–MS or GC–MSMS, using EI [6]. It was claimed that since effluents and environmental waters often have loadings of particulate and colloidal material that SPE cartridges become blocked; furthermore that cartridges often give rise to contaminants leached from the plastic container walls, that masked steroid peaks. The method demonstrated good recovery of steroids spiked into clean river water at levels in the 20–25 ng $l^{-1}$ range. Belfroid et al. [7] reported a method in which 1 litre samples were extracted with SDB-XC SPE discs followed by further clean up using $C_{18}$ and $NH_2$ SPE columns. Steroids were determined by GC–MS–MS after silylation. Limits of detection of 0.1 ng $l^{-1}$ in surface water and 0.1–2.4 ng $l^{-1}$ in effluent were claimed. Concentrations of estrone in treated effluent up to 47 ng $l^{-1}$ were reported with estradiol up to 12 ng $l^{-1}$. Levels of ethynylestradiol were in most cases below the detection limit. Other estrogens in surface waters were mostly below the detection limit, but in a few samples, concentrations of a few ng $l^{-1}$ were reported. Effluent and surface waters treated by enzymatic hydrolysis did not show increased levels of free estrogens, indicating that conjugated estrogens were not present in significant concentrations. Mol et al. [8] proposed a generic GC–MS method for hydroxyl containing endocrine disrupters (including estradiol and ethynylestradiol) in which the compounds were derivatised with MTBSTFA [*N*-methyl-*N*-(*tert*.-butyldimethyltrifluoroacetamide)] followed by GC–MS. Samples were extracted using SPE columns or discs, or liquid–liquid extraction. It was claimed that the *tert*.-butyldimethylsilyl derivatives were less susceptible to hydrolysis than conventional silyl derivatives. For surface waters using a concentration factor of 500, limits of detection of 300 and 50 ng/l were reported for estradiol and ethynylestradiol, respectively, using EI. Again, these detection limits seem a little high for application of the method to determination of estrogens in river waters.

Recently, we have proposed a relatively simple procedure for the quantitative analysis of estrogens in human urine, using gas chromatography–negative chemical ionisation mass spectrometry of the pentafluorobenzoyl derivatives [9]. This method proved sufficiently sensitive and specific for the determination of estrogens in normal urine using only a simple solvent extraction step. In the present work, we have studied the possibility of application of this method, in conjunction with relatively simple sample

extraction procedures, to the determination of the much lower levels of estrogens present in river waters and effluents.

## 2. Experimental

### 2.1. Gas chromatography–mass spectrometry

GC–MS analysis was carried out using a Hewlett-Packard 5890 Series II gas chromatograph coupled to a 5985B quadrupole MS system (Hewlett-Packard, Palo Alto, CA, USA). Manual injections were made using the splitless technique (0.75 min splitless period) on to an HP-5MS capillary column (15 m×0.25 mm I.D., 0.25 μm film thickness, 5% diphenyl–95% dimethylsiloxane phase) interfaced directly into the ion source. The GC oven temperature was maintained at 65°C for 1 min and then programmed to 220°C at 40°C per min, then to 255°C at 5°C per min and finally to 330°C at 20°C per min and maintained at 330°C. The injector and transfer lines were at 330°C. Source and quadrupole temperatures were 200°C and 100°C, respectively. Methane (99.99%) was used as the reagent gas in the negative ion mode with source pressure of 160 Pa.

### 2.2. Reagents and materials

All solvents were analytical grade from Sigma–Aldrich. Estrone (I), 17β-estradiol (II), estriol (III), ethynylestradiol (IV), doubly deuterium-labelled [2,4-$^{2}H_{2}$]17β-estradiol (II-$d_{2}$), 2-hydroxyestrone, 4-hydroxyestrone, 2-hydroxyestradiol, triethylamine (TEA) and pentafluorobenzoyl chloride (PFBO) were also purchased from Sigma–Aldrich. Empore (3M) extraction discs, 47 mm SDB-XC or $C_{18}$ and filter aid 400 beads were obtained from Varian (Walton-on-Thames, UK). Glass microfibre filters (GF/C) were obtained from Whatman (Maidstone, UK). Bond-Elut $C_{18}$ SPE cartridges (5 g sorbent mass) were obtained from Phenomenex (Macclesfield, UK).

### 2.3. Preparation of standards

The aqueous solubility of the target estrogens estrone (12.4 mg $l^{-1}$), ethynylestradiol (4.8 mg $l^{-1}$), estradiol (13.0 mg $l^{-1}$) and estriol (13.3 mg $l^{-1}$) is low. Thus, separate stock standards of estrogens were made up at a level of 100 mg $l^{-1}$ in methanol–acetonitrile (1:1) and stored at −20°C prior to use. From these standards, working standards containing estrogens at a concentration of 0.5–1.0 mg $l^{-1}$ were prepared in the same solvent and stored at −20°C. Calibration standards were prepared by spiking appropriate amounts of the working standard into 2.5 litre samples of clean groundwater (obtained from Rickford, near Bristol, UK) to achieve concentrations of 0, 0.8, 1.6, 8, 40 and 80 ng $l^{-1}$. A fixed amount of internal standard (deuterated estradiol, II-$d_{2}$) to give a concentration of 8 ng $l^{-1}$was added to each calibration sample. River water samples were taken from the Thames in June/July 2000 and from some treatment plants in October/November 1999 and the internal standard at this same concentration was added immediately after sample collection.

### 2.4. Solid-phase extraction

Prior to use, all glassware was deactivated with a 10% dimethyldichlorosilane solution in dichloromethane and rinsed twice with dichloromethane. Samples were passed through a 47 mm vacuum extraction manifold containing in sequence a 10 mm layer of glass beads (Filter Aid 400 beads, 40 μm diameter), a glass microfibre filter (GF/C) followed by an SDB-XC extraction disc. In order to activate the discs, the assembly was washed with 20 ml each of methanol, methanol–acetonitrile (1:1), methanol–water (4:1), methanol–water (2:3) and water. After the sample was passed through, the discs were washed with 20 ml of methanol–water (2:3), discarding the eluent. The estrogens were eluted with 2×5 ml of methanol–water (4:1) and 2×5 ml of methanol–acetonitrile (1:1). For the analysis of sewage effluents, $C_{18}$-ODS cartridges were activated by washing with methanol followed by distilled water. Estrogens were eluted from the cartridge by washing with 20 ml methanol and 20 ml methanol–dichloromethane (1:1) as described in a standard procedure [2], although we did not use a further HPLC clean-up stage. The estrogen extracts were

collected, combined and blown to dryness using a stream of oxygen-free nitrogen. The residue was transferred quantitatively with dichloromethane–methanol (1:1) to a small screw-capped glass vial and blown to dryness prior to derivatisation.

### *2.5. Derivatisation*

PFBO esters were made by adding 75 μl of acetonitrile, 40 μl of 5% TEA in acetonitrile and 25 μl pentafluorobenzoyl chloride to the dried sample and heating for 3 h at 80°C. These optimum conditions for derivatisation were deduced previously [9]. The dried sample was taken up in dichloromethane (typically 0.5–1.0 ml) and injected into the GC–MS.

## 3. Results and discussion

Recently, we investigated the preparation of derivatives of estrogens suitable for use in capillary GC–negative chemical ionisation mass spectrometry (GC–NCI–MS). NCI–MS is a very selective detection technique since only electron-capturing species should give rise to an analytical signal. This increase in selectivity should allow the use of simpler sample preparation techniques. The PFBO derivatives gave the diagnostic negative molecular ions as the base peak in each case, and compared favorably in this respect with other derivatives studied for negative ion MS including heptafluorobutyl, trifluoroacetyl, pentadecafluoroocatanoyl and perfluorotolyl derivatives [9]. PFBO derivatives also gave good stability. Quantitation was based on single ion monitoring (SIM) of the negative molecular ions at $m/z$ 464 for I, 660 for II, 662 for II–$d_2$, 490 for IV and 870 for III. In the case of the three naturally occurring estrogens (I–III), complete derivatisation of all free –OH groups was achieved. Thus, mono-PFBO derivatives were formed for estrone, di-PFBO derivatives for the estradiols, and tri-PFBO derivatives for estriol. However, the synthetic estrogen ethynylestradiol was unique in forming only a mono-PFBO derivative. The NCI mass spectra of the derivatives are shown in Fig. 1. The simplicity of the spectra, with very few fragments shown especially for estrone is an advantage in terms of the sensitivity achievable when single ion monitoring is used for identification and quantitation, in conjunction with the retention time of the compound. However, a disadvantage of the spectral simplicity is that if another ion is monitored for confirmation purposes (e.g. $m/z$ 446 for estrone) sensitivity drops considerably. Nevertheless, similar problems are likely to be obtained with alternative approaches like LC–MS, which also tends to generate rather undiagnostic spectra.

SPE discs appear to be a convenient means of extraction of estrogens from large volumes of water. Previously, Kelly reported good results for the extraction of estrone, estradiol and ethynylestradiol using PTFE SPE discs impregnated with $C_{18}$ particles [6]. In the light of the large concentrations of estriol found in the urine of pregnant women (levels are significant in normal urine, and may increase several thousand times during pregnancy [9]), we investigated $C_{18}$ discs for the extraction of estriol in addition to the other three sterols.

A glass fibre filter paper and glass beads were placed on top of the extraction discs in the extraction apparatus in each case to prevent clogging with particulate matter. It has been shown recently that it is possible for estrogens to become adsorbed to suspended particulate matter. Due to its small size and high organic content, association with particulates is more likely than with bed sediments [10]. In our procedure, filtered particulates and estrogens adsorbed to the extraction disc are ultimately washed with organic solvents. However, it is questionable whether merely rinsing particulates with organic solvent will quantitatively desorb the analytes from these particulates, and further studies are necessary in this area. We found $C_{18}$ discs gave poor recoveries of estriol (results not shown), a compound not included in the previous study [6]. Due to the presence of three –OH groups, this estrogen is expected to be less hydrophobic and thus should have a low retention on the small amount of $C_{18}$ material contained within these discs.

Much improved recoveries for estriol were obtained on SDB-XC discs which contain embedded particles of poly(divinylbenzene–co-*N*-vinylpyrrolidinone), which is a more hydrophobic surface. Furthermore, no losses of any of the estrogens were obtained by washing the discs after sample extraction

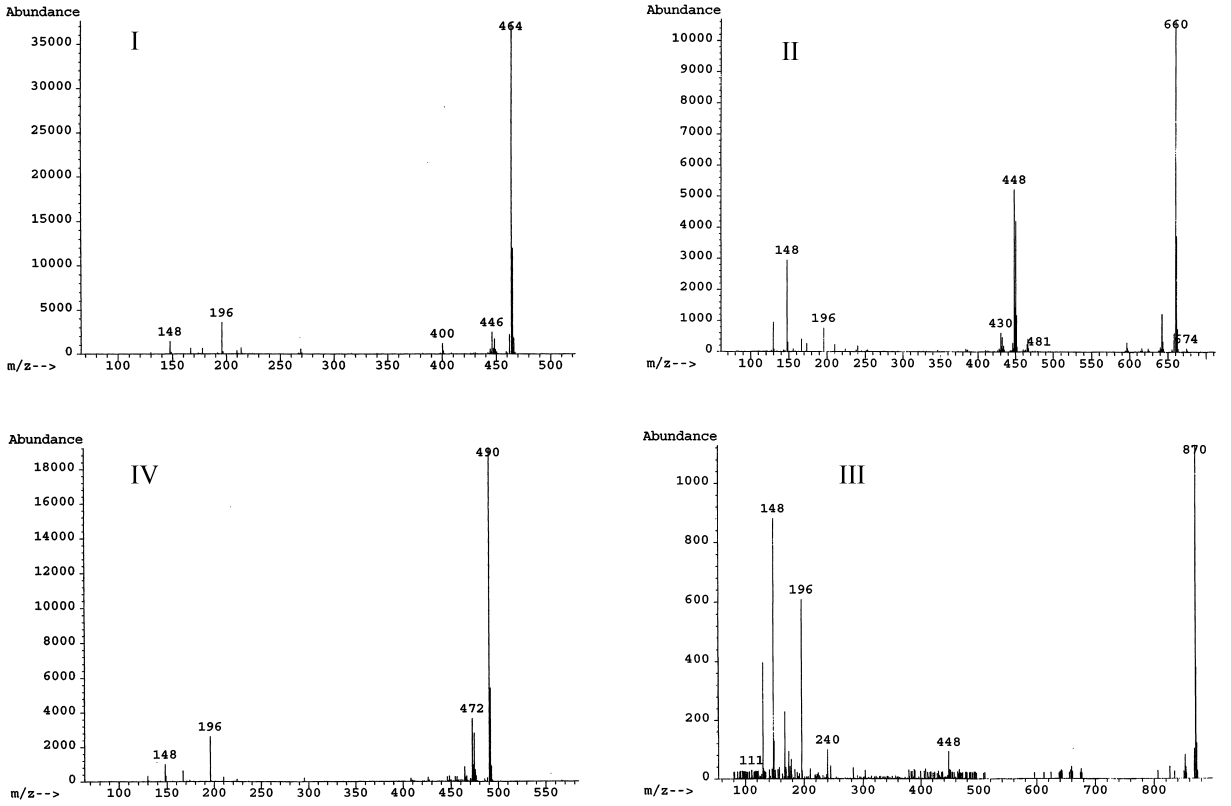


Fig. 1. NCI mass spectra of perfluorobenzo derivatives of estrone (I), ethynylestradiol (IV), estradiol (II) and estriol (III).

with 20 ml of methanol–water (2:3, results not shown) which allows removal of less hydrophobic compounds which could cause interference in the subsequent GC stage. Table 1 shows the calibration lines obtained by spiking clean ground water with each of the four target estrogens in the concentration range 0–80 ng $l^{-1}$. Each sample was taken through the disc extraction procedure (see Experimental) and the results quoted are based on use of the internal standard which was added to each sample at a concentration of 8 ng $l^{-1}$. The results indicate good linearity of the calibration. In our previous studies involving urine, we used a hydrolysis step to release estrogens from conjugates before analysis [9]. However, this step was omitted in the present work, since other studies have indicated that cleavage of estrogen conjugates is likely to occur during sewage treatment [7,11].

Table 1
Calibration lines of PFB-estrogens

| Compound | Line | Correlation coefficient |
|---|---|---|
| Estrone (I) | $y=0.1026x+0.1283$ | 0.9988 |
| Estradiol (II) | $y=0.1095x+0.1242$ | 0.9998 |
| Estriol (III) | $y=0.0166x-0.0048$ | 0.9992 |
| Ethylnylestradiol (IV) | $y=0.0425x-0.0010$ | 0.9991 |

The calibration lines are based on a single extractions of 2.5 litre of a standard at each of 6 levels, followed by derivatisation and average of triplicate injection of the standard at each level (see Experimental).

The accuracy and precision of the procedure was checked by spiking clean groundwater to give a final concentration of 10 ng $l^{-1}$ for the four estrogens. This level was chosen in the light of previously published results on the likely levels of estrogens in sewage effluents, and the relatively low dilution factors that can be present in such discharges to the River Thames. The complete analytical procedure (SDB-XC disc extraction, derivatisation and GC–NC–MS) was repeated five times. Table 2 shows the

Table 2
Recovery of estrogens at spiking level of 10 ng $l^{-1}$ ($n=5$)

| Estrogen | Recovery | RSD (%) |
|---|---|---|
| Estrone (I) | 84 | 16 |
| Estradiol (II) | 84 | 11 |
| Deuterated estradiol (II-$d_2$) | 84 | 18 |
| Estriol (III) | 92 | 24 |
| Ethylnylestradiol (IV) | 116 | 22 |

recoveries of estrogens taken through the entire procedure when the peak areas were compared to simple standards derivatised and dissolved in dichloromethane (i.e. which had not gone through the extraction procedure). The internal standard was not used to correct for precision or accuracy in any way, and so the recovery of the internal standard II-$d_2$ is also quoted. The recoveries are seen to be acceptable (84–116%) but the precision is rather poor (RSD 11–24%). We found that a significant source of the poor precision was in the GC stage itself, which involved manual splitless injection. Calculations based on the internal standard added to the sample prior to extraction allow its use to improve the precision of the whole procedure (including the GC stage). When used in this way, the RSDs of extraction and GC analysis were 9.6%, 2.6%, 8.3% and 9.8% for estrone, estradiol, estriol and ethynylestradiol, respectively. When making measurements on river water samples, the calibration curve was constructed by spiking ground water samples with estrogens (see above) and samples were measured against this curve. The peak area ratio of estrogen peak area/internal standard peak area was used in this case. In this method, the internal standard can also compensate for losses in the analytical procedure, as long as the behaviour of analytes and internal standards is the same. It might have been preferable to use a deuterated equivalent of the 4 target estrogens, however, these standards are difficult to obtain commercially in pure form, and their use would have added considerably to the method complexity.

A typical extracted ion current profile of a standard spiked into groundwater and extracted using the above procedure is shown in Fig. 2. Fig. 2 shows few co-extracted impurities appearing on the chromatograms under the conditions used. However, the extracted ion current profile for estrone shows some

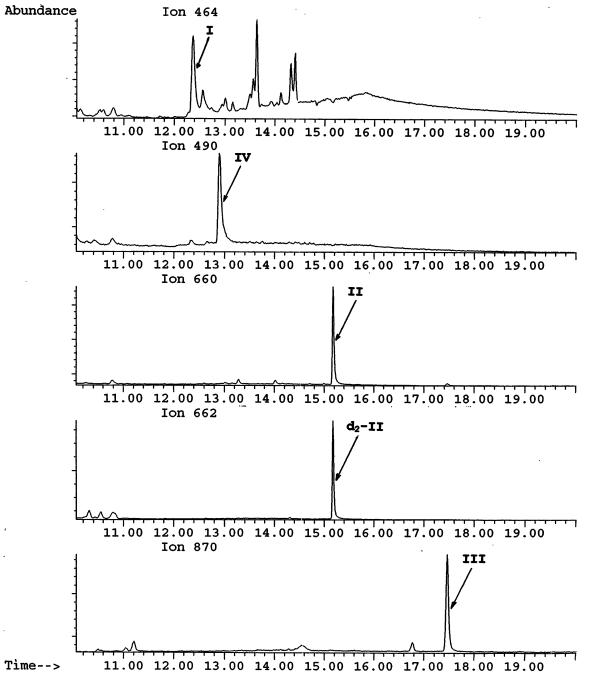


Fig. 2. Analysis of estrone (I), ethynylestradiol (IV), estradiol (II), doubly deuterated estradiol (II-$d_2$) and estriol (III) using single ion monitoring of ions at $m/z=464$, 490, 660, 662 and 870, respectively. Extraction of 2.5 l of groundwater spiked with 10 ng $l^{-1}$ each estrogen using SDB-XC disc procedure. Final volume of derivative solution 0.5 ml (concentration factor 5000). Temperature programme: 65°C 1 min hold, to 220°C at 40°C $min^{-1}$, to 255°C at 5°C $min^{-1}$ and to 330°C at 20°C $min^{-1}$. Column: 15 m methylphenylsilicone. For other conditions, see Experimental.

visualised impurities at higher retention time. Fig. 3 shows a blank sample of unspiked groundwater extracted and derivatised according to the normal procedure. (Note the $y$ axis scale is the same for estrone as in Fig. 2, but for the other estrogens the blank is displayed at considerably higher amplification — see figure legends). On the basis of these results, we estimated detection limit for estrogens spiked into groundwater as approximately 0.2 ng $l^{-1}$ for estrone, 0.03 ng $l^{-1}$ for estradiol, 0.06 ng $l^{-1}$ for estriol and 0.05 ng $l^{-1}$ for ethynylestradiol based on the analyte signal which would generate three times the noise level, with extraction of a 2.5 litre sample of groundwater. The higher detection limit for estrone is due to the visualisation of co-extracted impurities with monitoring at $m/z$ 464. It is likely that less favourable detection limits would be ob-

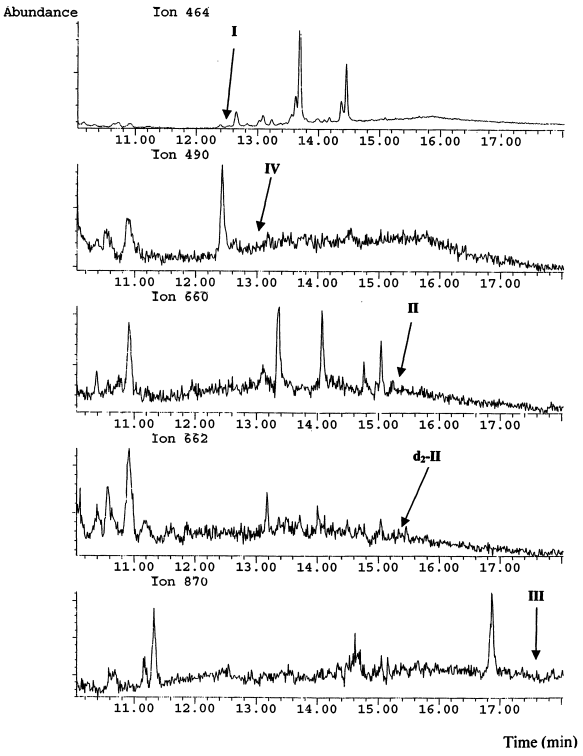


Fig. 3. Analysis of blank groundwater sample. For other conditions see Fig. 2. The *y*-axis expansion factor is approximately the same as in Fig. 2 for estrone, whereas the expansion factor is increased about 13 times for ethynylestradiol, and about 20 times for estradiol, deuterated estradiol, and estriol compared with Fig. 2.

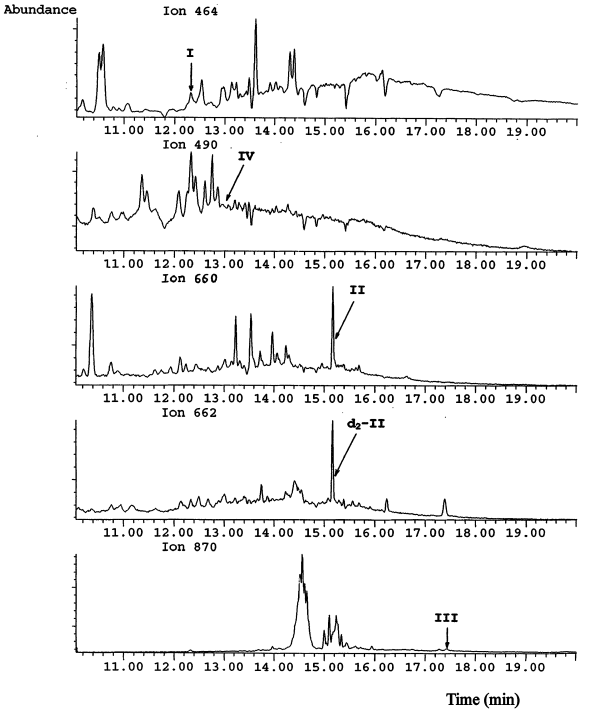


Fig. 4. Analysis of Thames river water 400 m below Crossness (June 2000) using SDB-XC extraction procedure. Concentrations of major estrogens: estrone 4.7, estradiol, 7.0, estriol 1.2 ng $l^{-1}$. For other conditions see Fig. 2.

tained if more complex river waters rather than ground water had been used for this experiment. Table 3 gives estrogen levels in River Thames water sampled in June/July 2000 and Fig. 4 shows extracted ion current profiles of a typical sample. The Reading sample (freshwater containing no estuarine water) was taken from a part of the Thames which is generally unpolluted, and shows low levels of estrogens. The major estrogen found in river water

Table 3
Concentrations of estrogens in river water samples from Thames (ng $l^{-1}$)

| | Estrone | Estradiol | Estriol | Ethynyl estradiol |
|---|---|---|---|---|
| *June 2000* | | | | |
| Thames 400 m below Crossness | 4.7 | 7.0 | 1.2 | n.d. |
| Thames below Mogden | 17 | 7.1 | 2.0 | n.d. |
| Thames above Mogden (Richmond Lock) | 1.7 | 3.3 | n.d. | n.d. |
| *July 2000* | | | | |
| Thames at Reading (freshwater) | 0.2 | 0.5 | 3.1 | n.d. |
| Thames 400 m below Crossness | 10 | n.d. | n.d. | n.d. |
| Thames above Crossness | 8.5 | n.d. | n.d. | n.d. |

n.d., not detected

receiving sewage effluent appears to be estrone, which is in agreement generally with results obtained by other workers [7]. While it has been shown that estrone is present at a higher concentration in female urine than estriol, followed by estradiol [12], Ternes and co-workers [11,13] have also shown that while in contact with activated sludge, estradiol is oxidised to estrone (which is then gradually eliminated). These factors explain the generally higher levels of estrone present in treated sewage samples. However, the rate of degradation of estrogens in a treatment plant may depend on a number of factors, including microbial activity, temperature and rainfall, which may lead to considerable temporal and seasonal variability of estrogen concentrations in discharges [2]. Furthermore, it has been shown that the degradation of estrogens in biological treatment plants receiving human waste is much greater than in plants receiving exclusively industrial organic waste, suggesting that there is a build up of estrogen-degrading organisms in plants which regularly receive inputs of these compounds [14]. Ethynylestradiol was not detected in any of our samples, which again is in general agreement with the results obtained by other workers, who have reported the absence of this sterol, or presence at very low levels, in effluents. We failed to detect ethynylestradiol even in the urine of a female taking oral contraception containing this compound [9], and attributed this result to extensive metabolisation of ethynylestradiol [15,16] although it is possible that metabolites which were not measured in our previous study are themselves estrogenic. Samples included river water taken above and below the input of effluent from Mogden sewage treatment plant (STP) in June 2000 (in the upper estuary), and above and below Crossness STP in July 2000 (in the middle of the Thames Estuary). The dilution factors of the effluents entering the Thames can be low, with levels of effluent from Mogden exceeding 50% of the total river flow in the upper estuary, especially with lower summer river flows. Thus the relatively high levels of estrogens reported in the sample below Mogden STP in summer 2000 compared with Richmond Lock upstream of the works are unsurprising. Nevertheless, the estrone concentration measured downstream of Crossness STP in July 2000 was only slightly greater than the upstream value. However, the large Beckton STP is upstream of Crossness in the middle estuary. Furthermore, the Thames is tidal, causing movement of water upstream as well as downstream. A large sampling programme would be necessary to investigate these effects.

While we found extraction discs suitable for river water samples, the recoveries of estrogens extracted from samples of treated sewage effluent was found to be low. It is possible that the large amount of organic material present in effluents exceeds the capacity of the discs, allowing estrogens to pass through unretained. A solution to this problem might be to use 90 mm discs instead of 47 mm discs which would increase sample capacity almost fourfold and would also increase sample processing rates. Other workers have successfully validated $C_{18}$ SPE cartridges for initial estrogen extraction, although this is followed usually by another sample preparation step such as preparative HPLC if conventional EI–MS is used as the detection technique after GC separation [2]. Further studies have validated other types of SPE cartridge for estrogen extraction [17]. For the analysis of sewage effluents, we employed a fairly standard procedure [2] using large volume $C_{18}$ extraction cartridges (5 g sorbent mass), eluting the estrogens with methanol and methanol–dichloromethane (1:1). Processing large liquid volumes was found to be less convenient than using discs; however, estriol was not lost during passage of the sample as with $C_{18}$ discs and sample extracts appeared freer of impurities than when using the disc procedure. Presumably, the much larger sorbent mass and effective length of the separation medium is responsible for these differences. Fig. 5 shows a typical extracted ion current profile from a sample eluted from these $C_{18}$ cartridges, derivatised and injected as before. A more rapid temperature program was utilised since use of cartridges rather than discs seemed to produce a cleaner extract, with fewer co-eluting impurities detected. Thus a shorter cycle time is achievable for the GC–MS analysis. Table 4 shows the concentrations of estrogens detected in effluents from three treatment plants, each of which utilise the activated sludge process. As expected, estrone again appears to be the most significant estrogen. The concentration of some significant metabolites of estrone and and estradiol are also recorded by monitoring of the ions at $m/z$ 674 for 2- and 4- hydroxyestrone and $m/z$ 870 for 2-hydroxyestradiol, since our method is also

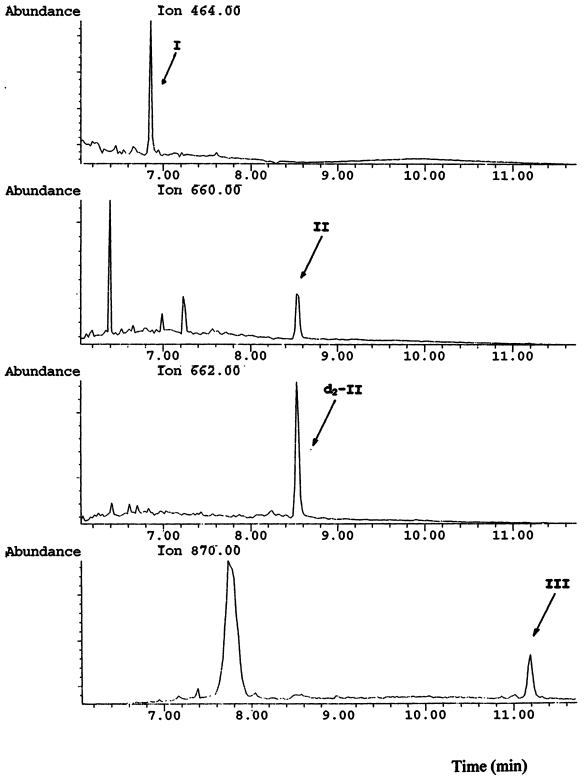


Fig. 5. Analysis of effluent from Beckton sewage treatment plant using Bond-Elut $C_{18}$ extraction cartridge. Concentrations of major estrogens: estrone 29, estradiol, 7.4, estriol 4.0 ng $l^{-1}$. Conditions: as Fig. 2 except temperature programme: 65°C 1 min hold, to 310°C at 50°C $min^{-1}$, hold 2.5 min, to 330°C at 15°C $min^{-1}$.

capable of analysis of these compounds [9]. It should be noted that effluents were sampled in Autumn 1999, whereas the river water samples were taken in Summer 2000. Thus, it is not possible to directly correlate the two groups of values. The high levels of estrone metabolites in some samples suggest that it may also be advisable to monitor these compounds, although to our knowledge, their estrogenic potential has not been reported in the literature.

## 4. Conclusions

Gas chromatography with negative chemical ionisation mass spectrometry of the pentafluorobenzoyl derivatives provides a sensitive and specific means for analysis of estrogens in effluents and river waters. The selectivity of the final analysis method allows the use of a relatively simple solid-phase extraction clean-up stage without requiring extra complex and time-consuming steps such as preparative HPLC. Solid-phase extraction discs impregnated with polymeric particles seemed to provide a convenient procedure for the extraction of large volumes of river water samples, giving good recoveries of the major estrogens estrone, estradiol, estriol and ethynylestradiol at the ng $l^{-1}$ level. However, recoveries using discs were low for sewage effluents, possibly due to overloading by the large amount of matrix organic material present. For these samples, extraction using large volume $C_{18}$ cartridges provides an alternative procedure, which although more cumbersome for use in sample processing, also seemed to produce more effective sample clean-up; nevertheless, further comparative work is necessary to investigate the efficacy and robustness of each of these methods in order to establish a routine sample processing method for estrogens in environmental samples.

## Acknowledgements

This research was supported by the Environment Agency (Thames Region). The authors thank Alan

Table 4
Concentration of estrogens in treated effluents

| STP (date) | Estrone | Estradiol | Estriol | Ethynyl estradiol | 4-Hydroxy-estrone | 2-Hydroxy-estrone | 2-Hydroxy-estradiol |
|---|---|---|---|---|---|---|---|
| Deephams (October 1999) | 6.4 | 1.6 | 3.0 | n.d. | 2.8 | 1.7 | 3.6 |
| Crossness (November 1999) | 9.8 | 2.6 | 2.0 | n.d. | n.d. | 14 | n.d. |
| Beckton (November 1999) | 29 | 7.4 | 4.0 | n.d. | 14 | n.d. | 6.3 |

n.d. = not detected.

Millett (UWE, Bristol) for technical assistance and Professor S. Rowland (University of Plymouth) for constructive comments on the draft manuscript.

## References

[1] Anon, Endocrine Disrupting Chemicals, Royal Society, London, 2000.
[2] T.P. Rodgers-Gray, S. Jobling, S. Morris, C. Kelly, S. Kirby, A. Jambaksh, J.E. Harries, M.J. Waldock, J.P. Sumpter, C.R. Tyler, Environ. Sci. Technol. 34 (2000) 1521.
[3] C. Desbrow, E.J. Routledge, G.C. Brighty, J.P. Sumpter, M. Waldock, Environ. Sci. Technol. 32 (1998) 1549.
[4] M.J. López de Aldà, D. Barceló, J. Chromatogr. A 892 (2000) 391.
[5] A. Laganà, A. Bacaloni, G. Fago, A. Marino, Rapid Commun. Mass Spectrom. 14 (2000) 401.
[6] C. Kelly, J. Chromatogr. A 872 (2000) 309.
[7] A.C. Belfroid, A. van der Horst, A.D. Vethaak, A.J. Schaefer, G. B Rijs, J. Wegener, W.P. Cofino, Sci. Tot. Environ. 225 (1999) 101.
[8] H.G. Mol, S. Sunarto, O.M. Steijger, J. Chromatogr. A 879 (2000) 97.
[9] X.-Y. Xiao, D. McCalley, Rapid Commun. Mass Spectrom. 14 (2000) 1991.
[10] K.M. Lai, K.L. Johnson, M.D. Scrimshaw, J.N. Lester, Environ. Sci. Technol. 34 (2000) 3890.
[11] T.A. Ternes, P. Kreckel, J. Mueller, Sci. Tot. Environ. 225 (1999) 91.
[12] H. Adlercreutz, T. Fotsis, C. Bannwart, E. Hamalainen, S. Bloigu, A. Ollus, J. Steroid Biochem. 24 (1986) 289.
[13] T.A. Ternes, M. Stumpf, J. Mueller, K. Haberer, R.D. Wilken, M. Servos, Sci. Tot. Environ. 225 (1999) 81.
[14] A.C. Layton, B.W. Gregory, J.R. Seward, T.W. Scultz, G.S. Sayler, Environ. Sci. Technol. 34 (2000) 3925.
[15] M.L. Orme, D.J. Back, S. Ball, Pharmacol. Ther. 43 (1989) 251.
[16] F.P. Guengerich, Life Sci. 47 (1990) 1981.
[17] M.S. Young, M.F. Early, J. Romano, J. Krol, in: Proc. 3rd Biennial Conference On Monitoring and Measurement in the Environment, Carleton University, Ottawa, Canada, 2000, p. 253.