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# Determination of Steroids and Coprostanol in Water, Effluent and Mussel Using Gas Chromatography/Mass Spectrometry

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## ABSTRACT

A method was developed for the determination of steroids, including estradiol-17β, estriol, estrone, hydrocortisone, testosterone and coprostanol, in surface water, effluent and mussels. The method is based on the derivatization of steroids containing hydroxyl groups with pentafluorobenzyl bromide (PFB-Br), followed by gas chromatographic/mass spectrometric (GC/MS) determination. Estradiol-17β and estrone were successfully derivatized by this method, whereas coprostanol (a sterol compound) and testosterone showed no spectra of the derivatized form, and were therefore determined as free compounds. The derivatization of estriol and hydrocortisone could not be confirmed under these experimental conditions. Also, the free forms of the two steroid compounds were found to be difficult to chromatograph by this method. Surface water and effluent were extracted by liquid-liquid extraction using dichloromethane, while mussels were extracted using a microwave extraction system. The method detection limits of estradiol-17β, estrone, testosterone and coprostanol were 2 ppt for surface water and effluent, and 3 ng/g for mussel. Recoveries in spiked reagent water and mussel for the same chemicals ranged from 56 to 87% and 21 to 48%, respectively.

## INTRODUCTION

Natural and synthetic hormone residues in sewage wastes may impact on marine animals downstream (Desbrow et al., 1998; Routledge et al., 1998). Routledge et al., 1998 have reported the occurrence of certain natural and synthetic steroidal estrogens in the final effluent of sewage treatment plants (STP). They have detected estradiol-17β and estrone at nanogram-per-litre levels. They have indicated that estrogen levels are sufficient to account for the vitellogenin synthesis observed in caged male fish placed downstream of certain STP effluent discharges in British rivers.

The objective of this work is to develop a GC/MS method for the determination of five steroids (estradiol-17β, estrone, estriol, hydrocortisone, and testosterone) and one sterol compound (coprostanol) in water, effluent and mussel samples.

**Table 1 Trivial and systematic names of steroids and sterol**

| Trivial name | Systematic name |
|---|---|
| Estradiol-17β | Estra-1,3,5,(10)-triene-3,17β-diol |
| Estrone | 3-Hydroxyestra-1,3,5(10)-trien-17-one |
| Estriol | Estra-1,3,5,(10)-triene-3,16α-17β-triol |
| Hydrocortisone | 11β,17α,21-Trihydroxypregn-4-ene-3,20-dione |
| Testosterone | 17β-Hydroxyandrost-4-ene-3-one |
| Coprostanol | 5β(H)-Cholestan-3β-ol |

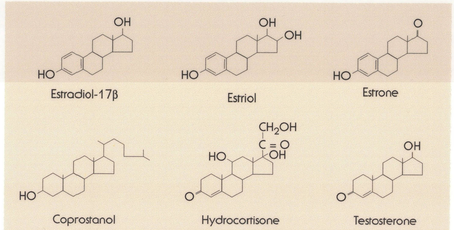


**Figure 1 Chemical structures of steroids and coprostanol compounds**

## ANALYTICAL METHOD

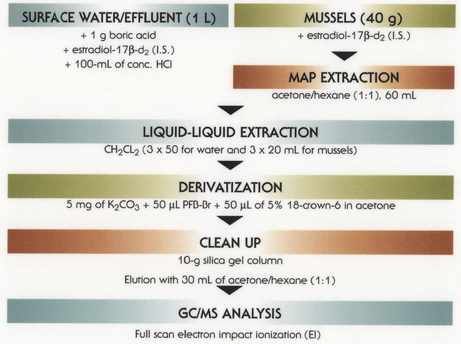



**Figure 2 Chemical structures of derivatized estradiol-17β estrone**

## RESULTS AND DISCUSSION

The EI mass spectra of model compounds were evaluated to determine whether molecular weight information on PFB-Br derivatives of steroids and sterols could be obtained. As can be seen, not all the compounds examined in this study showed the derivatized molecular ion ($M^+$) in the EI spectra. Coprostanol and testosterone behave differently. As neither showed the derivatized molecular ion spectra, they were determined as free compounds. Estriol and hydrocortisone, on the other hand, showed no molecular ions in the EI spectra. No explanation can be advanced to account for the loss of estriol and hydrocortisone, but most likely these two compounds were either adsorbed or decomposed before they could reach the ionization source.

The occurrence of derivatization was identified by $(M\text{-PFB-Br})^+$. All mass spectra of estradiol-17β and estrone contained significant peaks at m/z 181, which corresponded to PFB-Br. Testosterone was identified by $M^+$; coprostanol was indicated by m/z 215, 355 and 373. The EI mass spectra of estriol and hydrocortisone could not be confirmed for both samples and model compounds. By comparing the retention times and the resulting EI mass spectra of steroids and sterols with those of the model compounds, we were able to identify estradiol-17β, estrone and coprostanol in field samples. The sources of these chemicals may be attributed to industrial and domestic waste (Desbrow et al. 1998, Routledge et al. 1998).

**Table 2 Characteristic EI spectra of free and derivatized compounds**

| Analyte | MW | m/z | | | | | |
|---|---|---|---|---|---|---|---|
| | | Underivatized | Derivatized/PFB-Br | | Others | | |
| Estradiol-17β | 272.4 | 272 | 181 | **452*** | - | - | - |
| Estrone | 270.4 | 270 | **450*** | 181 | - | - | - |
| Estriol | 288.4 | - | - | - | - | - | - |
| Hydrocortisone | 362.5 | - | - | - | - | - | - |
| Testosterone | 288.4 | **286*/287*** | - | - | 124 | 148 | 244 |
| Coprostanol | 388.7 | - | - | - | **215*** | 355 | 373 |
| Estradiol-17β-$d_2$ | 274.4 | 274 | **454*** | 181 | - | - | - |

*Used for quantitation.

**Table 3 QA/QC study of selected steroids**

| Compound | Blank | | MDL | | Recovery (%) | |
|---|---|---|---|---|---|---|
| | Water (ppt) | Mussel (ng/g) | Water (ppt) | Mussel (ng/g) | Water (n = 2) | Mussel (n = 5) |
| Estradiol-17β | ND | ND | 2 | 3 | 87 ± 13 | 21 ± 10 |
| Estriol | NC | NC | NC | NC | NC | NC |
| Hydrocortisone | NC | NC | NC | NC | NC | NC |
| Coprostanol | ND | ND | 2 | 3 | 61 ± 12 | 48 ± 16 |
| Estrone | ND | ND | 2 | 3 | 78 ± 16 | 30 ± 8 |
| Testosterone | ND | ND | 2 | 3 | 56 ± 22 | 25 ± 12 |

ND: not detected.
NC: analyzed but could not be confirmed.

**Table 4 Results of GC/MS analysis of environmental samples taken from the St. Lawrence River**

| Compound | Concentration in environmental matrixes | | |
|---|---|---|---|
| | Surface water (ppb) | Effluent (ppb) | Mussels (ng/g) |
| Estradiol-17β | 0.038 | < MDL | < MDL |
| Estriol | NA | NA | NA |
| Hydrocortisone | NA | NA | NA |
| Coprostanol | 0.067 | 14.667 | 32252 |
| Estrone | 0.006 | < MDL | < MDL |
| Testosterone | < MDL | < MDL | < MDL |

MDL: method detection limit.
NA: not applicable.

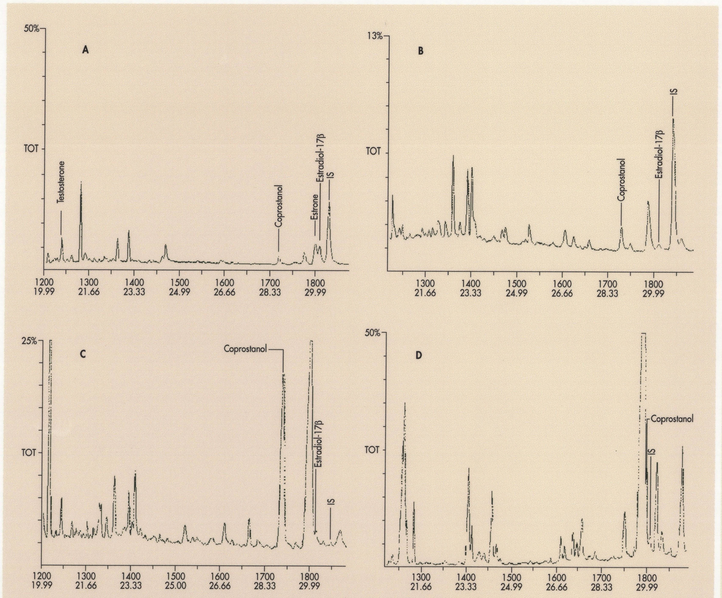


**Figure 3 Reconstructed EI ion chromatograms of field samples and model compounds: A) model compounds, B) water, C) effluent and D) mussel**

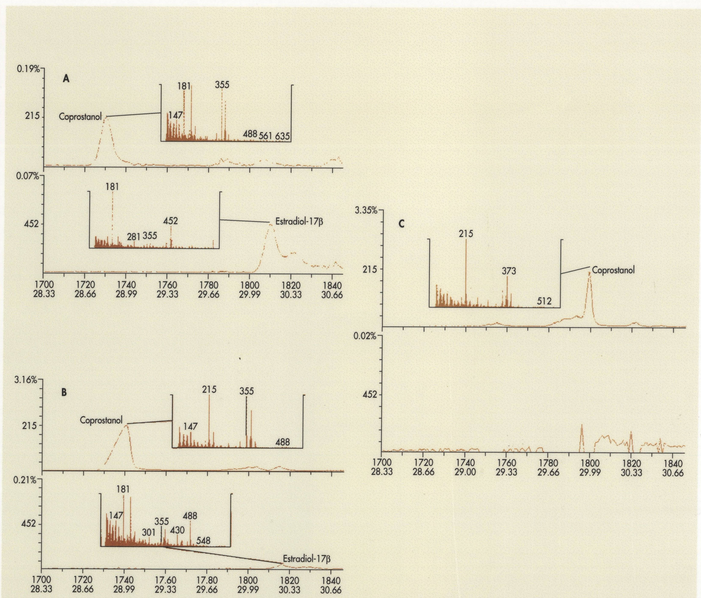


**Figure 4 EI ion trap mass spectra of steroids and sterols found in field samples: A) water, B) effluent and C) mussel**

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