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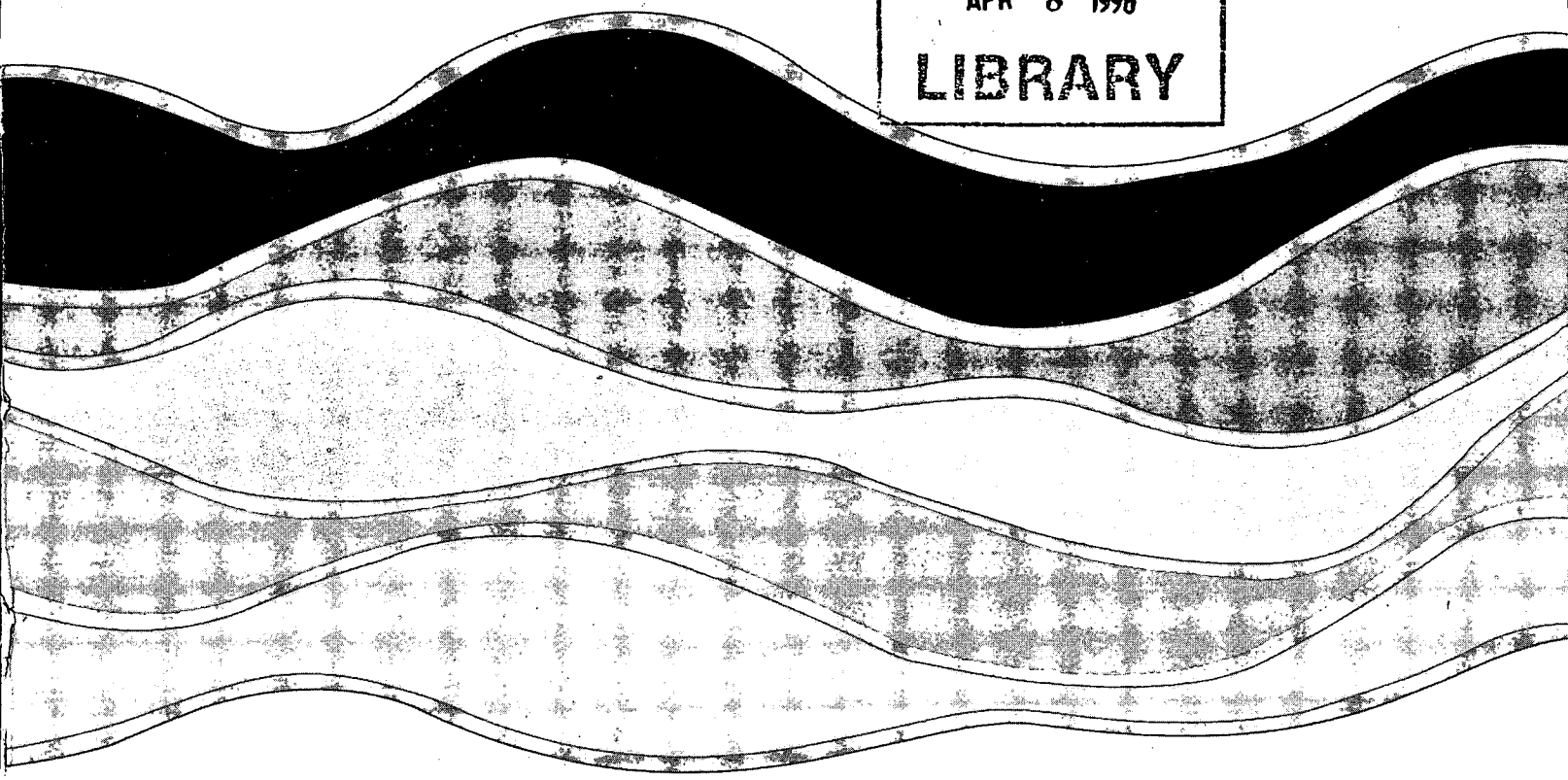

DETERMINATION OF 17β-ESTRADIOL AND ITS METABOLITES IN SEWAGE EFFLUENT

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Determination of 17ß-estradiol and its metabolites in sewage effluent

by

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MANAGEMENT PERSPECTIVE

Among the many chemicals that function as endocrine disruptors, few of them are as potent as the naturally occurring 17ß-estradiol (E_2). Recently, feminization of male fish was observed in rivers downstream of sewage treatment plants and E_2 has been suspected as a major compound responsible for the estrogenic activity in sewage effluents. This manuscript describes the development and application of a chemical method for the determination of E_2 and its metabolites in sewage. The occurrence of these estrogenic compounds as environmental contaminants in some Canadian sewage samples is reported for the first time.

SOMMAIRE À L'INTENTION DE LA DIRECTION

Parmi les nombreux produits chimiques qui agissent comme perturbateurs du système endocrinien, peu d'entre eux sont aussi puissants que le 17β-œstradiol (E_2) naturel. Dernièrement, on a observé une féminisation des poissons mâles fréquentant des cours d'eau en aval de stations d'épuration des eaux d'égout; l'E_2 serait un des principaux composés responsables de l'activité œstrogénique dans les effluents des eaux d'égout. Le présent article décrit l'élaboration et l'application d'une méthode chimique de dosage de l'E_2 et de ses métabolites dans les eaux d'égout. C'est la première fois que l'on signale la présence de ces composés œstrogéniques comme contaminants de l'environnement dans certains échantillons d'eaux d'égout au Canada.

ABSTRACT

A simple and quantitative method designed for the monitoring of 17ß-estradiol (E_2) and its metabolites estrone (E_1) and estriol (E_3) as environmental contaminants in municipal sewage effluents is described. Preconcentration and cleanup of the estrogens were performed by a solid phase extraction technique using a reversed phase, C_{18} cartridge. The estrogens were derivatized with pentafluoropropionic acid anhydride and the products were analyzed by gas chromatography/mass spectrometry. Recoveries of the analytes from spiked distilled water and sewage were better than 87% at fortification levels of 100 and 20 ng/L. With a concentration factor of 5000, the detection limits were 5 ng/L for E_1 and E_2 and 10 ng/L for E_3 in sewage samples. In a brief survey of Canadian wastewater, these estrogens were detected in many raw sewage and effluent samples at ng/L levels.

RÉSUMÉ

Le présent article décrit une méthode quantitative simple de la surveillance du 17β-œstradiol (E_2) et de ses métabolites, l'œstrone (E_1) et l'œstriol (E_3), comme contaminants de l'environnement dans les effluents des eaux d'égouts urbains. Les chercheurs ont effectué une préconcentration et une épuration des œstrogènes par extraction en phase solide au moyen d'une cartouche de C_{18} en phase inversée. On a préparé des dérivés des œstrogènes au moyen de l'anhydride de l'acide pentafluoropropionique, et les produits obtenus ont été analysés par chromatographie en phase gazeuse et spectrométrie de masse. La récupération des substances à analyser à partir d'échantillons enrichis d'eau distillée et d'eaux d'égout était supérieure à 87 % pour des taux d'enrichissement de 100 et 200 ng/L. Avec un facteur de concentration de 5 000, les seuils de détection étaient de 5 ng/L pour E_1 et E_2, et de 10 ng/L pour E_3 dans des échantillons d'eaux d'égout. Dans le cadre d'une brève étude des eaux d'égout au Canada, ces œstrogènes ont été décelés dans de nombreux échantillons d'eaux d'égout et d'effluents non traités à des concentrations de l'ordre du ng/L.

INTRODUCTION

It is now well known that many synthetic chemicals can mimic the function of 17ß-estradiol (E_2), a naturally occurring estrogen, causing disruption of the endocrine system [1]. These chemicals have been hypothetically linked to the increase incidence of certain estrogen-dependent cancers in humans, falling sperm counts in men, feminization of fish, and abnormality in the reproductive organs of wildlife [2-4]. Many of these endocrine disruptors occur as environmental contaminants, with examples including organochlorine insecticides such as DDT as well as its metabolites and derivatives, kepone, some hydroxylated polychlorinated biphenyls, chlorinated dioxins, alkylphenols such as bisphenol A, 4-nonylphenol and 4-*tert*-octylphenol, etc. [5-7]. However, using data developed from MCF-7 (a human breast cancer cell) bioassay [8] or rainbow trout *in vitro* hepatocyte bioassay [9,10], these xenobiotic compounds are four to six orders of magnitude weaker than E_2 in relative estrogen potency.

E_2 is the principal hormone that regulates cell proliferation and is responsible for the development and maintenance of the female reproductive system and sex characteristics. In humans, E_2 is metabolized to estrone (E_1) and estriol (E_3) in both conjugated and non-conjugated forms. These steroids are commonly found in human excreta, and have also been reported at higher levels in urine, serum and amniotic fluid samples of pregnant women [11]. The synthetic estrogen, 17α-ethynylestradiol (EE_2), is the major ingredient in many oral contraceptive formulations that have been used since 1960. Structures of these steroids are shown in Figure 1.

Naturally occurring estrogens enter the environment as excretion by humans, domestic and farm animals, and wildlife. While the levels of these steroids in sewage and environmental samples were very low, it has been demonstrated that E_2 and EE_2 at concentrations as small as 1 ng/L can induce the synthesis of vitellogenin (VG) in male fish [12]. VG is a protein precursor of the egg yolk and is normally produced in large quantities by mature female fish. The observation of elevated levels of VG in male fish collected in rivers downstream of sewage treatment plants has led to the belief that estrogenic compounds are present in sewage. Although conclusive evidence is still unfolding, E_2 and EE_2 have been suspected to be a major contributor to the estrogenic activities in sewage.

Very few analytical methods for the determination of E_2 in environmental samples have been published. Previously, a radioimmunoassay technique was developed for the determination of E_2 and testosterone in sewage [13]. Identification of estrogenic compounds in sewage effluent by gas chromatography/mass spectrometry was also reported in a British study [14]. To date, no data for the occurrence of estrogens in the Canadian aquatic environment are available. In this work, we present a simple method for the determination of E_2 and its metabolites E_1 and E_3 in sewage effluent. The present study is a continuation of our current research in new analytical methods for the detection of other estrogenic compounds such as nonylphenol [15], nonylphenol ethoxylates [16] and carboxylates [17] in sewage effluent and sludge samples.

EXPERIMENTAL

Reagents and chemicals

(a) Solvents.-- Distilled-in-glass grade organic solvents, supplied by Burdick and Jackson, were used without further purification.

(b) Estrogens and anhydride.-- E_1, E_2, E_3, EE_2, and pentafluoropropionic acid anhydride (PFPA) were obtained from Sigma-Aldrich-Supelco Canada Ltd. (Oakville, ON, Canada). Stock solutions of each individual estrogen, at 1000 µg/mL, were prepared in acetone. Mixtures of the estrogens, at 100, 10, and 1 µg/mL, were also prepared in acetone and stored at -20°C in the dark.

(c) Extraction apparatus.-- A home-made system consisted of a 12-port solid phase extraction (SPE) vacuum manifold (Supelco Visiprep DL 5-7044), a 20-L stainless steel tank, and a vacuum pump was used. A 6 mL, reversed phase SPE cartridge containing 1 g of a C_{18} sorbent (ENVI-18, Supelco, 5-5706) was used to extract a sewage sample.

Collection of sewage samples

Grab or 24-hr composite influent, primary, and final effluent samples from various municipal sewage treatment plants were collected. For the samples that were extracted within 48 hr after collection, they were kept at 4°C and no preservative was added. For the other samples, they were preserved with 1% formaldehyde and kept at 4°C in the dark until extraction. *Caution*: Due to the presence of bacteria, virus, and parasite that may pose a health hazard to workers, protective equipment must be used to handle sewage samples as a safety precaution.

Extraction of sewage samples

Prior to extraction, each sewage sample (1 L) was vacuum filtered through a 47 mm Whatman GF/C filter with a pore size of 1.2 μm, using an all-glass funnel support assembly (Kontes Ultra-Ware, KT-953825-0000). In this process, a filter aid such as Celite 545 was used to minimize plugging of the filter. In preparation for extraction, each C_{18} tube was conditioned with 5 mL of acetone, 5 mL of methanol, and followed by 10 mL of water on a SPE manifold as per manufacturer's instruction. The filtered sample was then applied to the extraction tube via a siphon tubing and an adaptor (Supelco, 5-7275). An average flow rate of ca. 10 mL/min was maintained by adjusting the vacuum to ca. -15 in Hg. As a safety precaution, the vacuum should not exceed -20 in Hg. When the extraction was completed, the tube was dried under vacuum for 5 min. A 10 mL aliquot of an acetone/water mixture (1:4, v/v), in two equal fractions, was used to rinse the tube and the rinsing was discarded. The estrogens were removed from the C_{18} tube by eluting it with 10 mL of acetone. As the acetone extract contained a small amount of water, it was gently evaporated down to ca. 100μL with nitrogen and a water bath at 40°C. The estrogens were then back extracted into four 1 mL aliquots of ethyl acetate and the latter was dried by passing through a disposable pipet filled with 5 cm of anhydrous sodium sulfate.

Derivatization of estrogens

After the ethyl acetate extract was evaporated to 100 μL in a centrifuge tube, the sample was reacted with 50 μL of PFPA at ambient temperature (22°C) for 20 min. At

the end of reaction, 2 mL of petroleum ether (b.p. 30-60°C) and 3 mL of 1% K_2CO_3 were added and the reaction products were partitioned into the organic layer by vortexing for 1 min. After phase separation (centrifugation was required for most sewage samples to break the emulsion), the upper layer was removed and passed through a disposable pipet filled with anhydrous sodium sulfate. The extraction was repeated twice and the combined petroleum ether fraction was evaporated just to dryness. The residue was redissolved in 200 µL of isooctane for GC/MS analysis. For a calibration standard, a mixture of these estrogens (50 ng each) was derivatized and worked up as described above.

Analysis of estrogen derivatives by gas chromatography/mass spectrometry

A Hewlett-Packard 5890 Series II GC equipped with a 5972 Mass Selective Detector and a 30 m x 0.25 mm id x 0.25 µm film thickness HP-5-MS column was used for the quantitation of estrogens. GC conditions were as follows: injection port, 250°C, interface, 280°C, initial oven temperature, 70°C with a 1 min hold, programming rates, 30°C/min (from 70 to 180°C) and 5°C/min (from 180 to 290°C). Carrier gas (helium) linear velocity was held constant at 36.9 cm/sec. Splitless injections (1 µL) were made by a HP7673 autosampler with a splitless time of 1 min. The electron energy and electron multiplier voltage were 70 eV and 400 V above autotune value, respectively. Full scan mass spectral data were collected from m/z 50 to 600. For the quantification of estrogens in a sample extract, the following quantitation and confirmation ions, respectively, were used in selected ion monitoring work: m/z 372 and 416 (E_1), m/z 564 and 401 (E_2), m/z 563 and 399 (E_3), and m/z 359 and 306 (EE_2).

RESULTS AND DISCUSSION

Extraction of estrogens from sewage

Estrogens and other steroidal hormones in plasma and urine of a small sample size (i.e. a few mL) have been successfully extracted by solvents such as ethyl acetate [18], as well as a mixture of diethyl ether and dichloromethane [19]. Extraction of estrogens and estrogen conjugates in late pregnancy fluids has been demonstrated by means of a graphitized carbon black cartridge [11]. Our extraction procedure was based on a previously published solid phase extraction method for estrogen and testosterone in sewage using a C_{18} column [13]. Rather than centrifugation, the suspended particulates in the sewage sample were removed by vacuum filtration through a layer of Celite and a glass fiber filter of 1.2 μm pore size. However, this process should only been performed immediately before the preconcentration step, otherwise, reduction in flow rates or even plugging of the adsorption media may still occur if the filtered sample was stored for an extended period of time.

Prior to the elution of the estrogens, the C_{18} cartridge was washed, to waste, with 10 mL of a 1:4 mixture (v/v) of acetone and water. This was a very useful cleanup step as it removed coextractives of a polarity much higher than the estrogens. Its effectiveness was best illustrated in the extraction of some sewage samples with known inputs from the textile industry. After the dark-colored dyes were removed by this clean-up step, the fraction containing the estrogens was nearly colorless.

In our experience, there was always a small amount (100 to 200 µL) of water remaining on the C_{18} cartridge even if the cartridge was dried under vacuum for 30 to 60 min. The water, removed alongside the estrogen in the acetone elution step, must be removed before chemical derivatization. Rather than using a prolonged nitrogen evaporation step that caused loss of the analytes to eliminate the residual water, the evaporation was stopped when the volume was ca.100 µL or when the solvent was almost entirely water. The estrogens were then back extracted into ethyl acetate which was subsequently evaporated to ca.100 µL for derivatization. The presence of a small volume of ethyl acetate was necessary as it kept the sewage extract in solution and it was also miscible with PFPA for an efficient derivatization.

Derivatization and GC/MS analysis

Several derivatization procedures have been published for the GC determination of estrogens. For example, the formation of trimethylsilyl (TMS) [18, 20, 21] and *tert*-butyldimethylsilyl derivatives of E_1, E_2, and E_3 [21], dimethylethylsilyl derivatives of EE_2 and other steroids used in oral contraceptive formulations [22], as well as pentafluoropropionyl (PFP) derivatives of testosterone acetate [19] has been demonstrated. In the beginning of this study, we have evaluated several derivatives of the estrogens regarding the ease of formation, stability of products, as well as their chromatographic and mass spectral properties. In addition to the TMS and PFP derivatives, the formation of acetyl and the heptafluorobutyryl (HFB) derivatives have also been studied. The PFP derivatives were selected for this work since there were very little side products and they were stable for weeks at -20°C (so that sample extracts could be

saved for further analysis) and were formed in the shortest time under the least vigorous condition as it required only 20 min of reaction at room temperature. Pentafluoroacylation of the estrogens was also reproducible. For six replicate reactions, the coefficient of variations for the yields of the products varied between 3.8 and 6.6%.

As illustrated by the chromatogram shown in Figure 2, all PFP derivatives were baseline resolved and their order of elution is E_2, E_3, E_1, and EE_2. It was noted that tailing of the EE_2 derivative occurred as well as its response to the Mass Selective Detector was reduced upon a buildup of contaminants in the splitless liner. Presumably, adsorption took place between this derivative, which has one active OH group, and the polar coextractives such as surfactants which are commonly found in sewage samples. When it happens, the response of EE_2 can easily be restored by replacing the liner and cutting a few cm of the capillary column in the injector end. In contrast, the responses for the PFP derivatives of E_1, E_2, and E_3 were not greatly affected by the cleanliness of the liner.

All hydroxy groups in the naturally occurring, endogenous estrogens, i.e. 3-OH of E_1, 3- and 17-OH of E_2, as well as 3-, 16-, and 17-OH of E_3 reacted readily with PFPA to yield the respective mono-, di-, and tri- PFP derivatives. This was evidenced by the observation of their molecular ions at m/z 416, 564, and 726 (Table 1). The M^+ for the E_3 derivative was observed on a Hewlett-Packard MS Engine as its molecular weight is beyond the upper mass range of the Mass Selective Detector. Under the reaction conditions used, no partially derivatized E_2 and E_3 (i.e. a mono-substituted E_2 and either a mono- or di-substituted E_3) was observed. The absence of such intermediates enables straightforward quantification of the estrogens. In contrast, a mono-substituted derivative for the synthetic estrogen, EE_2, was formed (M^+ at m/z 442). Since mestranol, the 3-

methyl ether derivative of EE_2, did not react with PFPA under such conditions, it suggested that the hydroxy group at the 17-position was protected from being derivatized by the presence of the ethynyl group at the same location.

All PFP derivatives displayed ions at m/z 69 (CF_3^+) and 119 ($C_2F_5^+$) which are characteristic of the pentafluoropropionyl group (Figure 2). While the molecular ions for the E_1 and E_2 derivatives were either the base peak or very strong, those for E_3 and EE_2 were relatively weak. Another ion, m/z 359, was also prominent for all steroidal estrogens. This ion likely resulted from the loss of carbons at the 15, 16, and 17 position of the 5-member ring (ring D), their substituents and a hydrogen from the molecular ion of each derivative. Using the E_2 derivative as an example, the ion m/z 359 or $(M-205)^+$, arisen from the loss of C_3H_5O, C_2F_5CO, and H moieties from M^+. For EE_2, the ion m/z or $(M-83)^+$, was derived from the loss of C_3H_5O, the ethynyl group, and a hydrogen from M^+. The fragmentation pattern of EE_2 also provided direct evidence for the fact that the hydroxy group at the 17-position of this synthetic steroid was not derivatized.

The mass number and % relative abundance of the major ions for these PFP derivatives are listed in Table 1. Their mass spectra are shown in Figure 2. Extracted ion chromatograms of the quantitation and confirmation ions derived from a calibration standard are depicted in Figure 3.

Validation of the method

The accuracy and precision of this procedure have been determined by the analysis of distilled water and sewage effluent samples fortified with the estrogens at environmentally relevant levels (Table 2). At spiking levels of 100 and 20 ng/L, the

precision of this method was very good as the standard deviation was less than 10% for most samples. The recovery of the estrogens, ranging from 87 to 107% in all except one case, was close to quantitative.

Based on a concentration factor of 5,000 (1 L of sample extracted and a final volume of 200 μL), the detection limits for these estrogens in sewage samples were estimated to be 5 ng/L for E_2, E_1, and EE_2 and 10 ng/L for E_3. The detection limits could have been reduced by a factor of 2 to 3 in distilled water samples where interference is minimal. As indicated in the following discussion, this method is adequate for the determination of E_1 and E_3 in most sewage sample as well as E_2 in the raw influent and primary sewage effluent, but may not be sensitive enough for the determination of E_2 in some final effluent. This procedure is not likely to be applicable to the determination of EE_2 in sewage samples since its estimated environmental concentration is much lower than the method detection limit of 5 ng/L.

Application

This method has been applied to the determination of the steroidal estrogens in sewage collected from the sewage treatment plants of a few Canadian cities. E_1, E_2, and E_3 were found in all sewage influent and primary effluents that were extracted within 48-hr after collection. In these samples, the concentrations of E_1 and E_3 were relatively high and they varied from 14 to 109 ng/L and from 53 to 250 ng/L, respectively (Table 3). The level of E_2, the most potent endogenous estrogen, in the influent or primary effluent was much lower with a range from 6 to 15 ng/L. These concentrations are substantially lower than those reported in the work by Shore *et al.* (48-141 ng/L) for raw sewage samples

collected in Israel [13]. Presumably, the discrepancy was caused by differences in weather (i.e. amount of precipitation) and patterns of water usage between the two countries.

The occurrence of E_1, E_2, and E_3 in a primary sewage effluent collected in Burlington is illustrated in Figure 4. The identity of each estrogen was confirmed by the presence of both characteristic ions at the expected retention time (± 0.04 min) and in a similar area ratio (± 35%) as compared to a standard (Figure 3). It was noted that, while E_1 and E_3 were found in the two preserved primary effluent samples, E_2 was not detected in those sample. Therefore the stability of E_2 and other estrogens in preserved sewage should be investigated.

As indicated in Table 3, the concentrations of the estrogens were substantially lower in the final effluents. In fact, in all cases E_2 was below the detection limit of 5 ng/L. There was a 4-fold or more decrease in E_1 and E_3 levels between the primary and the final effluents collected on the same day and at the same sewage treatment plant. The reduction is probably due to degradation of E_2 and its metabolites by activated sludge in the secondary sewage treatment processes [23]. Their concentrations found in the final effluents varied from 5 to 19 ng/L for E_1 and from < 10 ng/L to 34 ng/L for E_3. None of the sewage effluent tested had any detectable amounts of EE_2.

ACKNOWLEDGMENTS

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Table 1. **Mass number (m/z) and % relative abundance (in parentheses) of the molecular and major ions for the PFP derivatives of estrogens.**

Estrogen derivative	M^+	Major ions
E_1	416 (100)	372 (82), 359 (52), 306 (37), 119 (56)
E_2	564 (57)	401 (31), 359 (43), 306 (41), 119 (100)
E_3	726 (5)	563 (7), 399 (22), 359 (9), 119 (100)
EE_2	442 (9)	359 (100), 306 (57), 279 (18), 119 (24)

Table 2. Accuracy and precision for the recovery data of estrogens from fortified water and sewage samples (no. of replicates = 6).

	Mean % recovery ± standard deviation			
Matrix	Distilled water		Sewage	
Spiking level (ng/L)	100	20	100	20
E_1	101 ± 3	107 ± 5	96 ± 4[1]	102 ± 8[2]
E_2	95 ± 4	91 ± 5	105 ± 5	98 ± 9
E_3	93 ± 5	98 ± 6	101 ± 8[1]	127 ± 13[2]
EE_2	89 ± 6	102 ± 8	88 ± 8	87 ± 11

[1] A composite sewage final effluent was used for spiking; results were blank subtracted.

[2] A composite sewage final effluent that has previously been extracted by this procedure was used for spiking; results were not corrected as blanks were below the detection limits.

Table 3. **Levels of E_1, E_2, and E_3 (mean ± standard deviation, ng/L) in sewage influent, primary and final effluents. All samples were unpreserved, grab except for those noted below.**

Location	Sewage type	Sampling date	No. of replicates	E_1	E_2	E_3
Burlington	primary	12/16/97	3	26±1	7±0.6	128±10
Burlington	final	12/16/97	3	6±0.5	<5	18±1
Burlington	primary	1/20/98	3	53±4	14±2	220±20
Burlington	final	1/20/98	3	8±1	<5	33±1
Dundas	primary	1/13/98	2	70, 68	9, 8	243, 203
Dundas	final	1/13/98	2	8, 10	<5, <5	<10, <10
Edmonton	primary, preserved[a]	8/20/97	3	109±5	<5	209±14
Edmonton	final, preserved[a]	8/20/97	3	72±2	<5	<10
Guelph	influent, preserved[a]	12/18/97	2	58, 75	<5	164, 158
Guelph	final, preserved[a]	12/18/97	2	18, 16	<5	37, 30
Guelph	influent	1/22/98	3	41±4	15±2	250±32
Guelph	final	1/22/98	3	14±1	<5	30±2
Montreal, north	influent[b]	11/17/97	3	28±6	6±1	72±6
Montreal, south	influent[b]	11/17/97	3	15±2	7±0.7	53±3
Montreal	primary[b]	11/17/97	3	19±3	6±0.7	68±7

[a] With 1% formaldehyde

[b] 24-Hr composite sample

FIGURE CAPTIONS

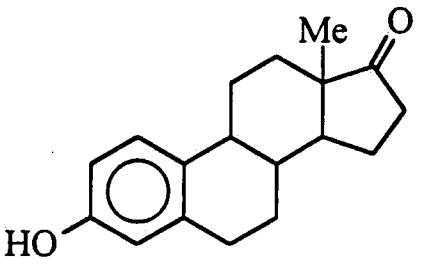
Figure 1. Chemical structures of the estrogens.

Figure 2. Total ion current chromatogram and mass spectra of the PFP derivatives of E_2, E_3, E_1, and EE_2.

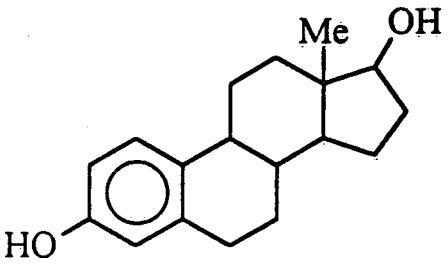
Figure 3. Extracted ion chromatograms for the quantitation and confirmation ions of E_2, E_3, and E_1 from a 200 pg/μL standard.

Figure 4. Extracted ion chromatograms of a derivatized, primary sewage effluent extract indicating the presence of E_2, E_3, and E_1 in the sample.

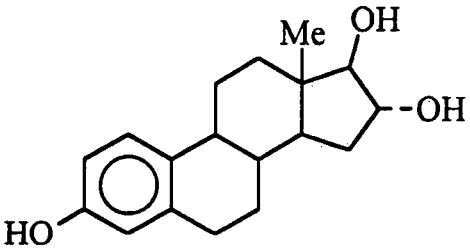
Figure 1



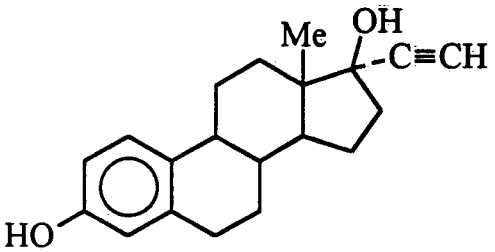
Estrone (E_1)



17ß-Estradiol (E_2)



Estriol (E_3)



17α-ethynylestradiol (EE_2)

Figure 2

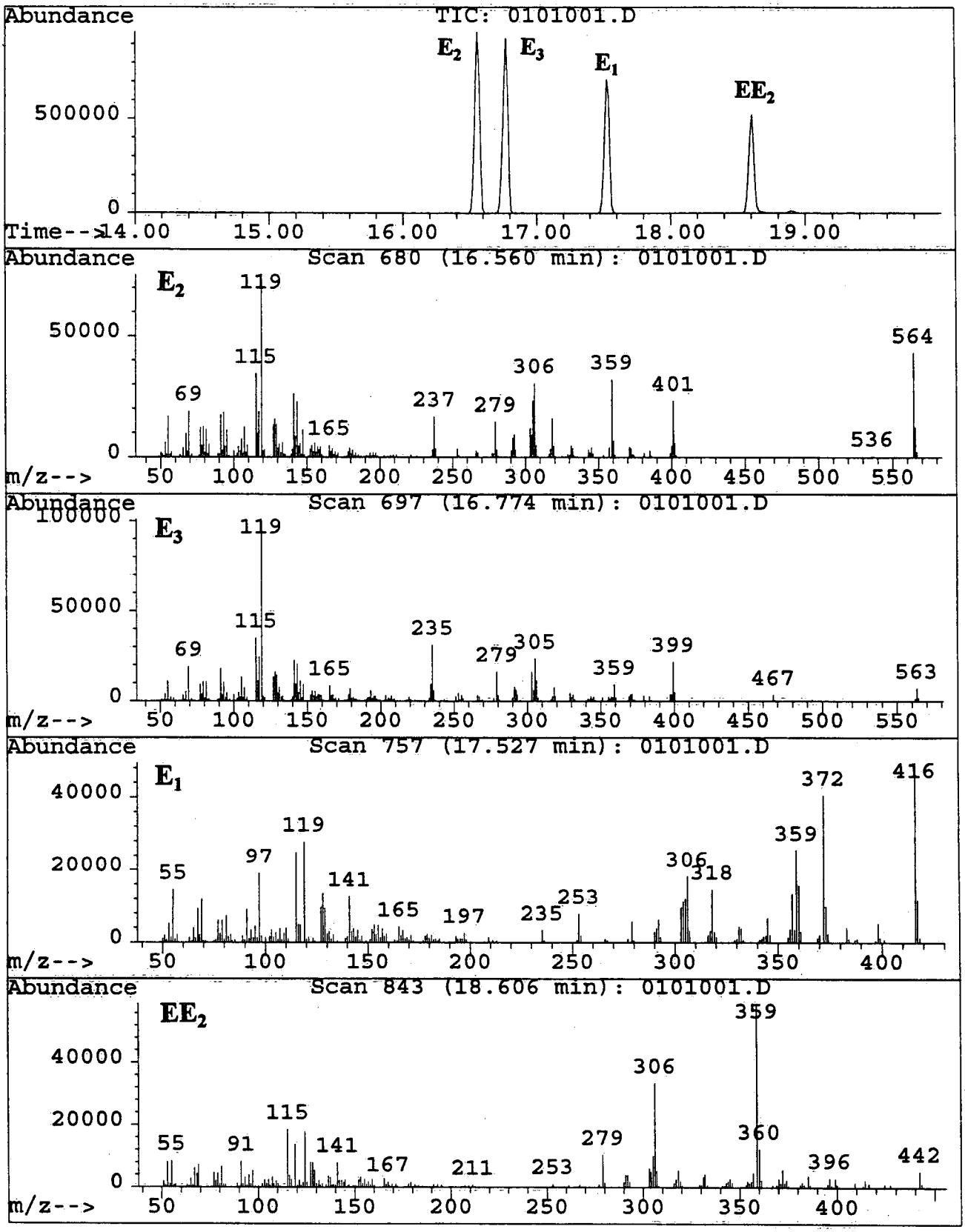

Abundance
TIC: 0101001.D
E2
E3
E1
EE2
500000
0
Time-->14.00
15.00
16.00
17.00
18.00
19.00
Abundance
Scan 680 (16.560 min): 0101001.D
E2
119
50000
115
306
359
564
69
237
279
401
165
536
0
m/z-->
50
100
150
200
250
300
350
400
450
500
550
Abundance
Scan 697 (16.774 min): 0101001.D
100000
E3
119
50000
115
235
69
279
305
399
165
359
467
563
0
m/z-->
50
100
150
200
250
300
350
400
450
500
550
Abundance
Scan 757 (17.527 min): 0101001.D
E1
40000
372
416
119
359
20000
97
306
318
55
141
253
165
197
235
0
m/z-->
50
100
150
200
250
300
350
400
Abundance
Scan 843 (18.606 min): 0101001.D
EE2
359
40000
306
115
20000
55
91
141
279
360
396
442
167
211
253
0
m/z-->
50
100
150
200
250
300
350
400

Figure 3

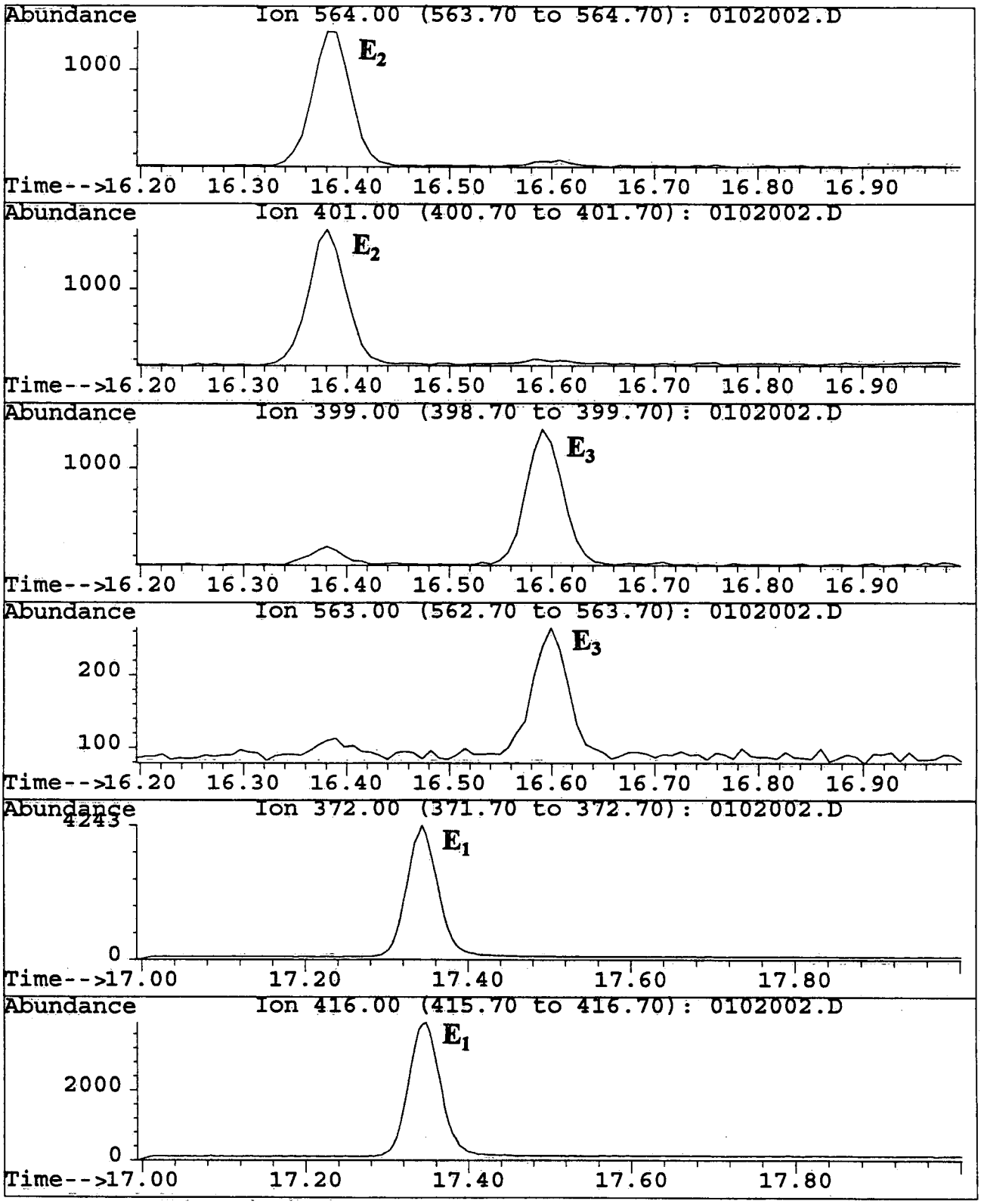
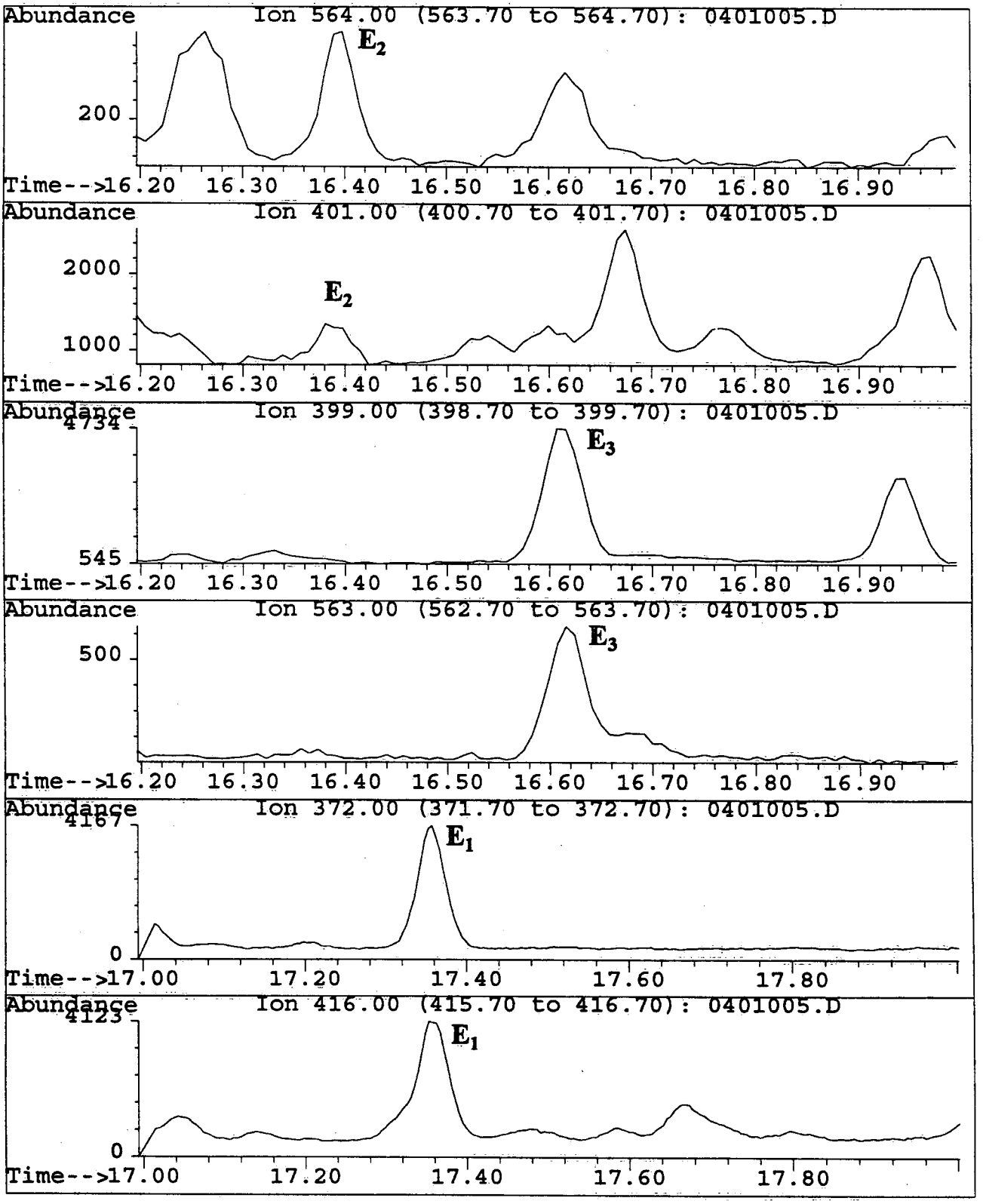
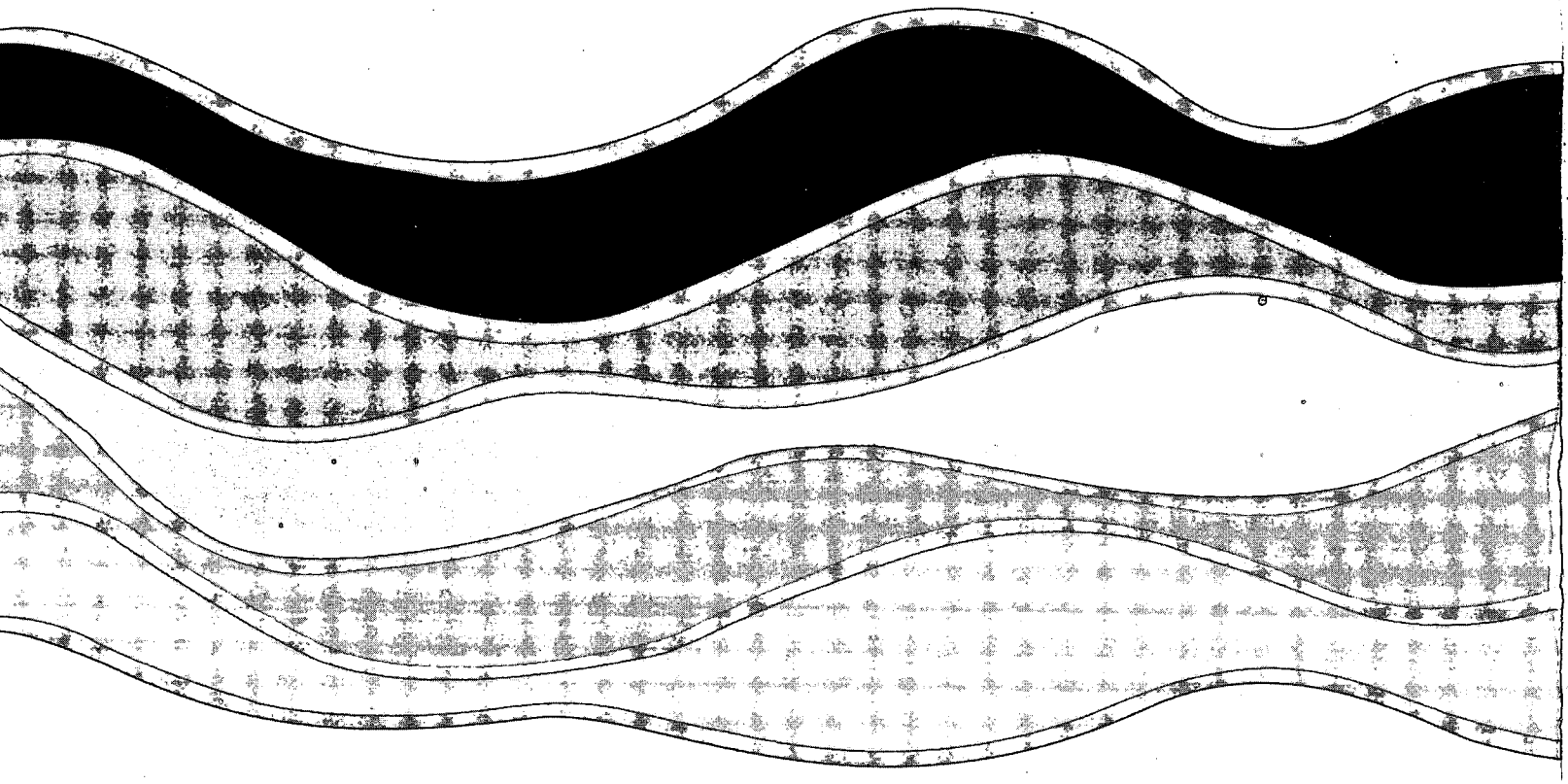

Abundance
Ion 564.00 (563.70 to 564.70): 0102002.D
1000
E2
Time-->16.20 16.30 16.40 16.50 16.60 16.70 16.80 16.90
Abundance
Ion 401.00 (400.70 to 401.70): 0102002.D
1000
E2
Time-->16.20 16.30 16.40 16.50 16.60 16.70 16.80 16.90
Abundance
Ion 399.00 (398.70 to 399.70): 0102002.D
1000
E3
Time-->16.20 16.30 16.40 16.50 16.60 16.70 16.80 16.90
Abundance
Ion 563.00 (562.70 to 563.70): 0102002.D
200
100
E3
Time-->16.20 16.30 16.40 16.50 16.60 16.70 16.80 16.90
Abundance
Ion 372.00 (371.70 to 372.70): 0102002.D
4243
0
E1
Time-->17.00 17.20 17.40 17.60 17.80
Abundance
Ion 416.00 (415.70 to 416.70): 0102002.D
2000
0
E1
Time-->17.00 17.20 17.40 17.60 17.80

Figure 4


Abundance
Ion 564.00 (563.70 to 564.70): 0401005.D
E2
200
Time-->16.20 16.30 16.40 16.50 16.60 16.70 16.80 16.90
Abundance
Ion 401.00 (400.70 to 401.70): 0401005.D
2000
1000
E2
Time-->16.20 16.30 16.40 16.50 16.60 16.70 16.80 16.90
Abundance
Ion 399.00 (398.70 to 399.70): 0401005.D
4734
545
E3
Time-->16.20 16.30 16.40 16.50 16.60 16.70 16.80 16.90
Abundance
Ion 563.00 (562.70 to 563.70): 0401005.D
500
E3
Time-->16.20 16.30 16.40 16.50 16.60 16.70 16.80 16.90
Abundance
Ion 372.00 (371.70 to 372.70): 0401005.D
4167
0
E1
Time-->17.00 17.20 17.40 17.60 17.80
Abundance
Ion 416.00 (415.70 to 416.70): 0401005.D
4123
0
E1
Time-->17.00 17.20 17.40 17.60 17.80



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Environment Canada Environnement Canada

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