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# Synthesis and use of pentadeuteroethyl ethofumesate as an internal standard for the determination of ethofumesate and its metabolites in water by gas chromatography–mass spectrometry

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**Abstract**

Pentadeuteroethyl ethofumesate (ETO-$d_5$) was synthesized starting by acid hydrolysis of ethofumesate to 2,3-dihydro-2-hydroxy-3,3-dimethylbenzofuran-5-yl methanesulfonate (ETO-OH) and successive ethylation with iodoethane-$d_5$. This deuterated compound was used as an internal standard for the determination by gas chromatography–mass spectrometry in the selected-ion monitoring mode of ethofumesate (ETO) and its chief metabolites, ETO-OH and 2,3-dihydro-2-oxo-3,3-dimethylbenzofuran-5-yl methanesulfonate (ETO-K). The recovery of these substances by solid-phase extraction from drinking water was comparable to those with $C_{18}$-bonded silica and Carbopack B phases. The recoveries of ETO and ETO-K were virtually complete, compared with 66% extraction for ETO-OH.

## 1. Introduction

Mass spectrometry is important in the analysis of pesticides and has been proposed as a validating technique on account of its high sensitivity in determining compounds on the basis of their chemico-physical characteristics [1]. GC–MS and LC–MS in the selected-ion monitoring (SIM) mode permit compounds labelled with stable isotopes to be used as internal standards (I.S.), providing good accuracy and reproducibility. The choice of a suitable I.S. is always difficult in pesticide analysis, not only for MS.

We were interested in the MS of ethofumesate (ETO) (**I**) (Fig. 1), a pesticide [2,3] widely used in Europe [1], and its chief metabolites [4] 2,3-dihydro-2-hydroxy-3,3-dimethylbenzofuran-5-yl methanesulfonate (ETO-OH) (**II**) and 2,3-dihydro-2-oxo-3,3-dimethylbenzofuran-5-yl methanesulfonate (ETO-K) (**III**) [5,6], as part of a more extended research programme including other pesticides [7]. A library search [8] found only five reports considering ethofumesate analysis, three dealing with HPLC analysis [9–11] and two with GC [12,13]. Two studies considered water analysis, one by GC–MS [12] and the other by HPLC [9]. Legrand et al. [12] measured

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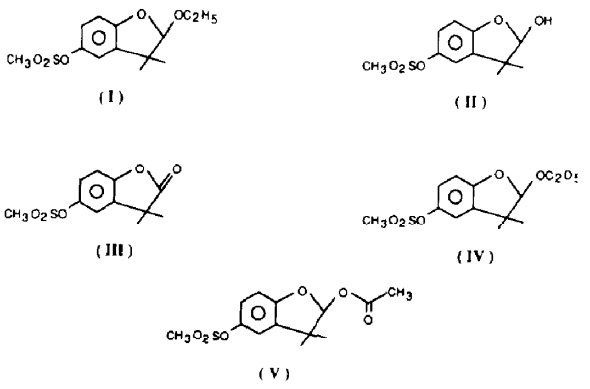


Fig. 1. Formulae of the compounds analysed.

this and 37 other pesticides in French surface and ground waters at or below a concentration of 100 ng $l^{-1}$ and reported a recovery of around 80% for ETO, with liquid–liquid extraction (LLE). They used a moving-needle injector and an MS detector in the SIM mode employing electron ionization (EI) without the use of an I.S. No data were reported regarding reproductibility, linearity and limit of detection.

We present here the synthesis and use of deuterated ethofumesate (**IV**) as an I.S., with the aim of improving the accuracy and reproducibility of analysis. We preferred to use solid-phase extraction (SPE) to recover the compound from water, comparing different extractive materials (Carbopack B and $C_{18}$-bonded silica), as SPE may offer advantages over LLE [14,15]. The simultaneous extraction and determination of the metabolites of ETO, ETO-OH and ETO-K by GC–MS-SIM were considered using the same I.S.

## 2. Experimental

### 2.1. Chemicals and materials

Ethofumesate was purchased from Riedel-de Haën (Seelze, Germany). [$^2H_5$]Iodoethane (iodoethane-$d_5$), silver nitrite and all other synthesis reagents were obtained from Aldrich (Milwaukee, WI, USA). Preparative and analytical TLC (Kieselgel 60 $F_{254}$) and silica (Kieselgel 60, 70–230 mesh ASTM, 0.062–0.21 mm) were purchased from Merck (Darmstadt, Germany). $C_{18}$-bonded silica phase (Bondesil $C_{18}$, 40 $\mu$m) was purchased from Analytichem International (Harbor City, CA, USA). Carbopack B (120–400 mesh, 0.037–0.125 mm) was purchased from Supelco (Bellefonte, PA, USA). The solvents used for synthesis and residue analysis were obtained from Merck. Other reagents for extraction were ascorbic acid (Carlo Erba, Milan, Italy) and trifluoroacetic acid (Merck); acetic anhydride and pyridine for derivatization were purchased from Aldrich.

### 2.2. Instrumental analysis

Direct inlet system (DIS) MS was performed on a VG TS 250 instrument by EI at 70 eV. For GC–MS analysis we used a Hewlett-Packard HP 5890 Series II gas chromatograph coupled with an HP 5971 mass-selective detector at 70 eV. The chromatographic columns were OV-1701 (10 m × 0.25 mm I.D.) and SE 52 (15 m × 0.25 mm I.D., both with a film thickness of 0.25 $\mu$m, from Mega (Legnano, Italy). The injector temperature was 240°C. The oven temperature was programmed from 120 to 180°C at 10°C/min, then to 200°C at 5°C/min and finally to 270°C at 10°C/min. The carrier gas was helium, the head pressure 30 kPa and the source temperature 180°C.

Three ions for each pesticide and metabolite were chosen for screening analysis by SIM (Table 1).

The 300 MHz $^1H$ NMR spectrum was recorded on a Bruker ACE-300 spectrometer in deuterochloroform using tetramethylsilane as an internal standard; chemical shifts are in $\delta$ (ppm) and the signals are reported as s (singlet), bs (broad singlet), d (doublet) and m (multiplet).

### 2.3. Synthesis of ETO-OH and pentadeuteroethylethofumesate (I.S.)

Pentadeuteroethylethofumesate (**IV**) was obtained from ethofumesate (**I**) by acidic hydrolysis to 2,3-dihydro-2-hydroxy-3,3-dimethylbenzofuran-5-yl methansulfonate (**II**) and further ethylation with iodoethane-$d_5$ [16] (Fig. 2).

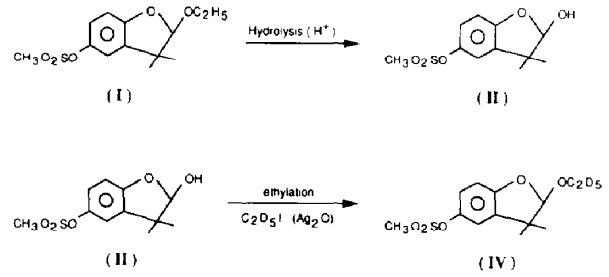


Fig. 2. Scheme of synthesis of pentadeuteroethyl ethofumesate (IV).

### 2.4. 2,3-Dihydro-2-hydroxy- 3,3-dimethyl benzofuran-5-yl methansulfonate (II)

A 100-mg amount of ethofumesate (I) was dissolved in 10 ml of acetone and added to 10 ml of water acidified to pH 1 with sulfuric acid. The acidic solution was stirred, heating at reflux for 16 h to complete reaction, analysed by GC–MS, then cooled to room temperature. After evaporation of the acetone under reduced pressure, the aqueous residue was extracted three times with 20 ml of ethyl acetate; the organic phase was washed twice with 20 ml of water and dried over anhydrous sodium sulfate. After evaporation of the organic solvent under reduced pressure, the crude product was purified on a silica gel column (Kieselgel 60, 70–230 mesh ASTM) and eluted with hexane–ethyl acetate (7:3), obtaining 83 mg of pure product (II) (yield 92%), m.p. 69–71°C. The product was identified by DIS-MS and its purity was ascertained by GC–MS and TLC (Kieselgel 60 $F_{254}$), eluting with hexane–ethyl acetate (7:3).

Mass spectrum: *m/z* [relative abundance (%)]: 258 ($M^+$, 36%); 229 (38%); 201 (14%); 179 (100%); 161 (36%); 137 (45%); 105 (33%); 79 (56%).

$^1$H NMR ($\delta$): 1.30 and 1.35 (2s, 6H, $2CH_3$); 3.13 (s, 3H, $SO_2CH_3$); 3.25 (bs, 1H, OH); 5.58 (d, 1H of CH); from 6.80 and 7.03 (m, 3H Ar).

### 2.5. Pentadeuteroethylethofumesate (IV)

A 140-mg amount of product II was dissolved in 15 ml of acetone and 500 mg of silver(I) oxide, previously prepared from silver nitrite with sodium hydroxide solution and dried in an oven at 110°C. Then 400 $\mu$l of iodoethane-$d_5$ were added and the black suspension was heated at reflux for 6 h with vigorous stirring, until complete disappearance of II, confirmed by GC–MS, then cooled to room temperature. After evaporation of the solvent under reduced pressure, the pale oil obtained was dissolved in ethyl acetate, the solution was washed twice with 20 ml of distilled water and then dried over anhydrous sodium sulfate. Then the ethyl acetate was removed under reduced pressure and ETO-$d_5$ (IV) was purified on a silica column (Kieselgel 60, 70–230 mesh ASTM) and by preparative TLC (Kieselgel 60 $F_{254}$) and eluted with hexane–ethyl acetate (7:3), obtaining 73 mg (47% yield) of pure ETO-$d_5$ (IV). The product was identified by DIS-MS and its purity was evaluated by GC–MS and TLC (Kieselgel 60 $F_{254}$), eluting with hexane–ethyl acetate (7:3). The mass spectrum of compound (IV) is reported in Fig. 3B.

Mass spectrum: *m/z* [relative abundance (%)]: 291 ($M^+$, 38%); 241 (7%); 212 (100%); 180 (20%); 161 (43%); 138 (20%); 105 (18%); 79 (45%).

### 2.6. Derivatization of ETO-OH

ETO-OH was derivatized by acetylation [17], dissolving in the solvent–reagent mixture acetic anhydride–pyridine (4:1) for 60 min at room temperature to obtain the acetylated derivative ETO-OAc (V). After this time the sample was dried under a stream of nitrogen and dissolved in ethyl acetate for analysis.

### 2.7. Calibration graphs

Calibration graphs were plotted by injecting 1 $\mu$l of a solution of ETO and its metabolites (ETO-OH and ETO-K) at six different concentrations (0, 25, 50, 100, 500 and 1000 pg $\mu l^{-1}$) obtained by successive dilutions from a solution at 2 ng $\mu l^{-1}$ concentration, against a fixed concentration of the labelled I.S. (200 pg $\mu l^{-1}$). Calculations were made on the basis of the compound/I.S. peak-area ratios using the ions at *m/z* reported in Table 1 and Fig. 6.

### 2.8. Recovery

Recovery studies of ETO, ETO-OH and ETO-K were made by extracting drinking water solutions of the compounds, at different concentrations and in different volumes, by SPE in quadruplicate. After elution, the sample was dried under a stream of nitrogen and derivatized as reported below to obtain acetylated ETO-OH. After dissolution of the sample in ethyl acetate, to obtain a theoretical concentration of 250 pg $\mu l^{-1}$, ETO-$d_5$ (I.S.) was added to each to achieve a final concentration of 200 pg $\mu l^{-1}$ for GC–MS analysis by injection of 1 $\mu l$ of final solution.

SPE was carried out in an all-glass apparatus [15,18] with Carbopack B and $C_{18}$ phases. The extraction procedure for the different phases were as follows.

(i) The $C_{18}$ phase (500 mg) was washed with 10 ml of ethyl acetate, activated with 10 ml of methanol and eluted with 10 ml of ethyl acetate.

(ii) Carbopack B (400 mg) was washed with 10 ml of methylene chloride–methanol (8:2), activated with 5 ml of methanol and 20 ml of ascorbic acid solution (10 mg $ml^{-1}$ in 0.01 *M* HCl). Elution was effected with 10 ml of methylene chloride–methanol (8:2) for the neutral and basic fractions, or 10 ml of methylene chloride–methanol (8:2) containing trifluoroacetic acid (0.2%, v/v) for the acidic fraction [19].

## 3. Results

### 3.1. GC–MS analysis

The mass spectrum of unlabelled ETO, shown in Fig. 3A, shows the molecular ion at *m/z* 286. Loss of $CH_3SO_2^{\cdot}$ gives the ion at *m/z* 207, which by further elimination of ethylene produces the ion at *m/z* 179. This gives rise to the ion at *m/z* 161 through loss of water. The ion at *m/z* 79 corresponds to the $CH_3SO_2^{+\cdot}$ ion.

The mass spectrum of ETO-$d_5$ (Fig. 3B) differs from that of the unlabelled compound in the ions with the ethyl chain or derived from its fragmentation. Thus, the molecular ion is at *m/z* 291; at *m/z* 212 there is the ion corresponding to the ion at *m/z* 207 of ETO. Similarly, the ion at *m/z* 180, due to the loss of ethylene-$d_4$, is shifted 1 u compared with the ion at *m/z* 179 of

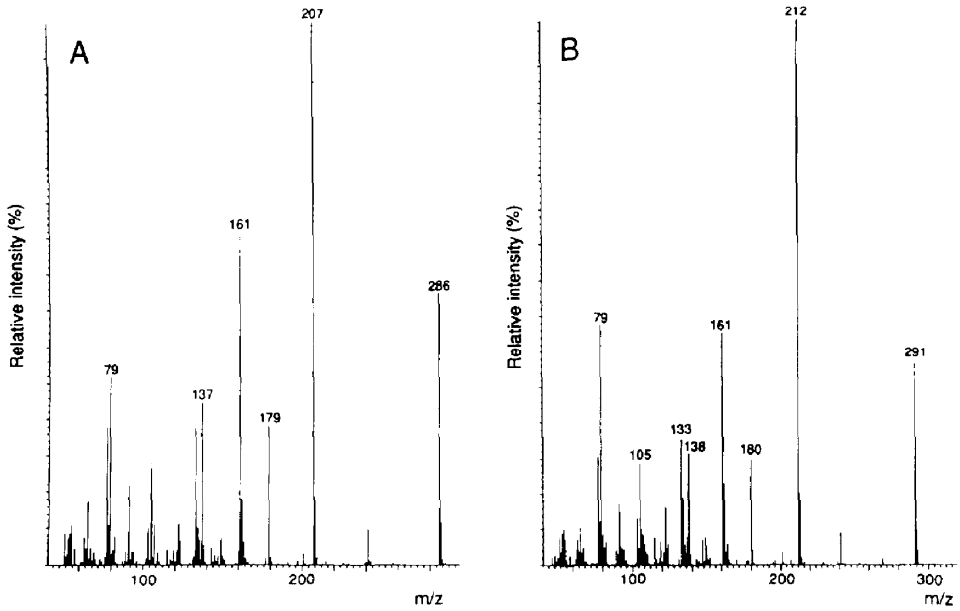


Fig. 3. Mass spectra of (A) ETO and (B) its pentadeuterated derivative ETO-$d_5$.

ethofumesate, on account of the presence of one deuterium.

This spectrum confirms the identity of the deuterated I.S., and supports the fragmentations indicated for ETO. ETO-$d_5$, determined by SIM, was found to contain about 1% of unlabelled ETO. Hence this deuterated I.S. does not cause significant interference when less than 500 pg are injected. The mass spectrum of ETO-K is reported in Fig. 4A, and Fig. 4B and C show the mass spectra of ETO-OH and ETO-OAc.

Only ETO and ETO-K can be determined by GC–MS using an SE-52 column, so an OV-1701 column was employed for the simultaneous determination of ETO-OH and the other compounds. The sensitivity for ETO-OH was higher with acetylation (Table 1) and, in this way, both columns can be employed to determine ETO and its metabolites together. GC–MS of the mixture before and after acetylation indicated no decomposition of ETO-K and ETO during derivatization.

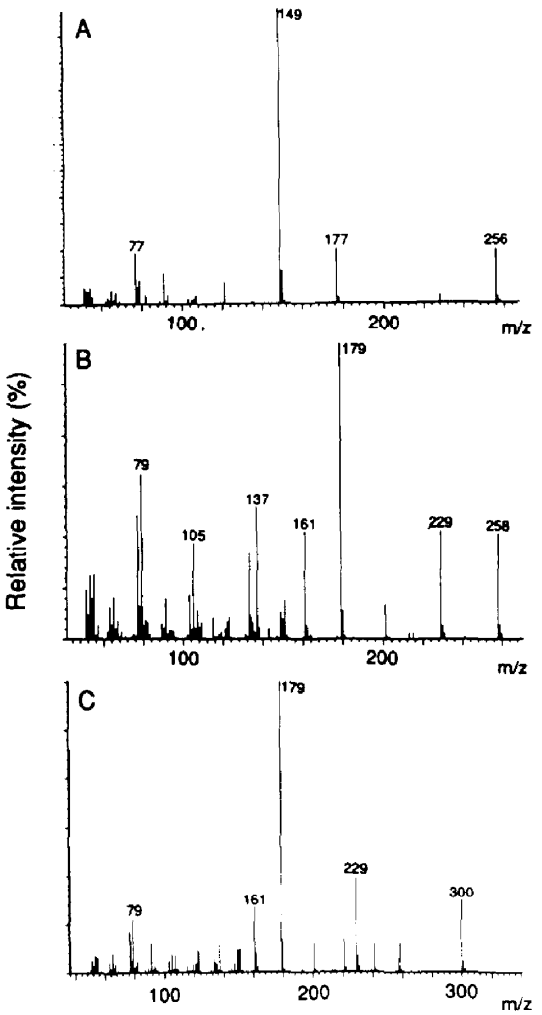


Fig. 4. Mass spectra of (A) ETO-K, (B) ETO-OH and (C) its acetylated derivative, ETO-OAc.

The ions reported in Table 1 were chosen for SIM analysis. For ETO, the ions at $m/z$ 286 (molecular ion) and 161 were selected, the ion at $m/z$ 207 not being considered because of its possible interference with column bleeding. For ETO-$d_5$, the parent ion at $m/z$ 212 was chosen with the molecular ion at $m/z$ 291. For ETO-K, ions at $m/z$ 149 and 177 were chosen, whereas $m/z$ 179 and 229 were selected for both ETO-OH and its acetylated derivative. Examples of SIM chromatograms for ETO, ETO-$d_5$, ETO-K and ETO-OAc are presented in Fig. 5.

The calibration graphs are shown in Fig. 6A and B, with the analytes-to-ETO-$d_5$ (ion at $m/z$ 212) peak-area ratio on the abscissa. Each point is the mean of three injections of the same standards. The correlation coefficients ($r^2$) were >0.999 for ETO and ETO-OH as acetylated derivative, whereas for ETO-K it was lower (0.996).

The instrumental limits of detection (LOD) (Table 1) were at the picogram level for ETO, ETO-K and ETO-OAc, but considerably higher (1000 pg) for underivatized ETO-OH than for its acetylated derivative.

Table 1
SIM ions chosen for each analyte, in order of relative abundance, and the corresponding limit of detection (LOD)

| Compound | Ions ($m/z$) | LOD (pg) (ion)[a] |
|---|---|---|
| ETO | 161, 286, 179 | 3 pg (286) |
| ETO-K | 149, 177, 256 | 10 pg (149) |
| ETO-OH | 179, 229, 258 | 1 ng (179) |
| ETO-OAc | 179, 229, 300 | 3 pg (179) |
| ETO-$d_5$ | 212, 291, 180 | 1 pg (212) |

[a] In parentheses are the ions ($m/z$) chosen for establishing the LOD.

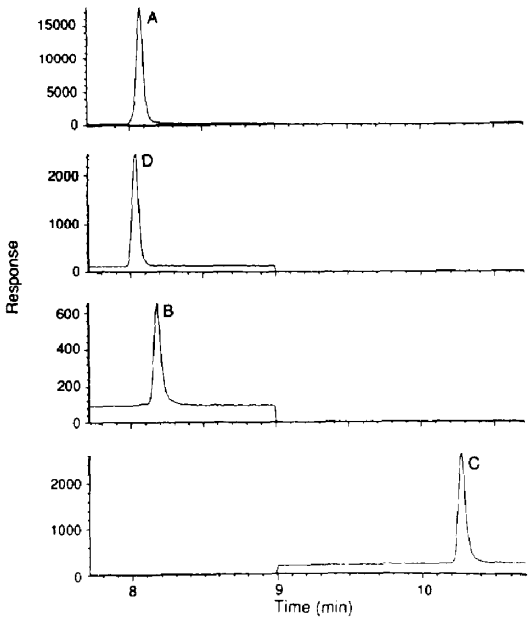


Fig. 5. SIM chromatograms of the compounds analysed, using an OV-1701 column as reported under Experimental. (A) ETO (*m/z* 286); (B) ETO-K (*m/z* 256); (C) ETO-OAc (*m/z* 179); (D) ETO-$d_5$ (*m/z* 212).

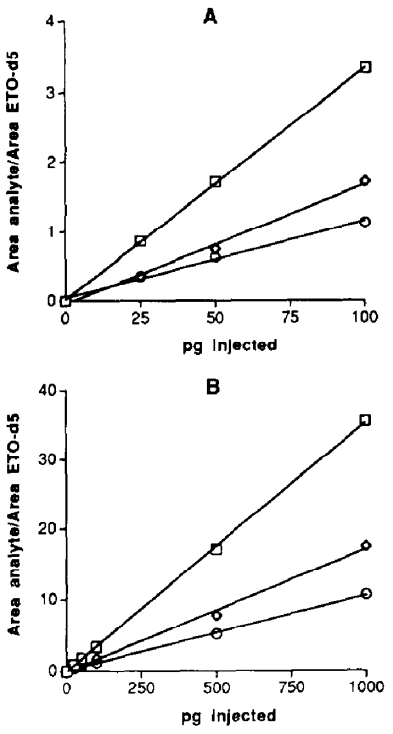


Fig. 6. Calibration graphs for ETO and its metabolites in different amounts with 200 pg of I.S. for each point, using an OV-1701 column. Experimental conditions are reported in the text. (A) Low-range curves (0–100 pg); (B) full-range curves (0–1000 pg). □ = ETO (*m/z* 286), regression equation $y = 0.035x - 0.138$; ◇ = ETO-K (*m/z* 149), regression equation $y = 0.017x - 0.156$; ○ = ETO-OH, after acetylation (*m/z* 179), regression equation $y = 0.010x + 0.038$.

Table 2 reports the day-to-day reproducibility for the ETO calibration graph. The reproducibility was good, as shown by the relative standard deviations, which with the exception of the 100-pg injections (18.4%), were always less than 15%.

### 3.2. Water recovery

The water extraction recoveries for ETO using Carbopack B under different conditions are reported in Table 3. Carbopack B provided high recoveries in the neutral fraction, but ETO was not found in the acidic fraction. The recovery was always higher than 80% with this SPE procedure, studying different concentrations and water volumes, and was complete (103%) at a concentration of 0.1 $\mu g\ l^{-1}$, which is the limit set by many European regulations regarding pesticides and related compounds. The standard deviation was low even when the recovery was measured in a day-to-day evaluation (Table 3).

The recovery was also good with Carbopack B for metabolites (Table 4), particularly ETO-K, but was less complete for ETO-OH.

Table 2
Day-to-day reproducibility for GC–MS analysis of the ETO calibration samples

| Day | ETO concentration (pg/μl) | | | | |
|---|---|---|---|---|---|
| | 25 | 50 | 100 | 500 | 1000 |
| 1 | 3.06 | 5.44 | 10.70 | 47.19 | 94.25 |
| 2 | 2.42 | 4.26 | 7.80 | 36.52 | 74.24 |
| 3 | 2.52 | 4.33 | 7.96 | 36.98 | 73.59 |
| Mean | 2.66 | 4.67 | 8.82 | 40.22 | 80.69 |
| R.S.D. (%) | 12.7 | 14.1 | 18.4 | 14.9 | 14.5 |

Each value represent the ratio of the ETO peak area (*m/z* 161) to the ETO-$d_5$ peak area (*m/z* 212), and is the mean of three injections of the same solution, using an OV-1701 column, as described under Experimental.

Table 3
Recovery (±S.D.) by Carbopack B extraction of ethofumesate, with different procedures, and day-to-day reproducibility

| Extraction | $n^a$ | Recovery (%) | |
|---|---|---|---|
| | | Cpb N[b] | Cpb A[c] |
| 50 ml, 100 μg/l | 4 | 81.2 ± 6.3 | 0 |
| 500 ml, 1 μg/l | 4 | 82.7 ± 5.9 | 0 |
| 500 ml, 0.1 μg/l | 4 | 103.1 ± 3.7 | 0 |
| Day-to-day[d] | 10 | 101.2 ± 8.5 | 0 |

Extraction procedure and analysis by GC–MS, using an OV-1701 column, were as described under Experimental.
[a] No. of replicates.
[b] Carbopack B neutral fraction.
[c] Carbopack B acidic fraction.
[d] Samples consisting of extractions of 500-ml portions of water spiked with 0.1 μg/l, on three different days.

Comparable results were obtained with SPE using the $C_{18}$ phase (Table 4) and the standard deviations were generally lower than 10%, except for ETO-OH extraction with Carbopack B (18%).

## 4. Conclusions

ETO-$d_5$ can be easily and quickly prepared and conveniently used in the determination of ETO by GC–MS-SIM, increasing the accuracy of the method. This pesticide can be completely extracted from water samples with the SPE procedure, using Carbopack B, improving the previously reported recovery by LLE [12].

Table 4
Recovery of extraction (±S.D.) for ETO and metabolites from 500 ml of mineral water spiked with 0.1 μg/l, using $C_{18}$-bonded silica and Carbopack B

| Analyte | Recovery (%) | |
|---|---|---|
| | Cpb N[a] | C-18[b] |
| ETO | 103 ± 3 | 94 ± 5 |
| ETO-K | 109 ± 8 | 95 ± 10 |
| ETO-OH | 66 ± 18 | 66 ± 11 |

[a] Carbopack B neutral fraction; No. of replicates = 4.
[b] $C_{18}$-bonded silica; No. of replicates = 4.

These results, and the sensitivity and accuracy of the instrumental technique, show that this procedure, using the deuterated I.S., can be successfully employed for the determination of ethofumesate in water as required by European regulations.

A further application of this I.S. and the analytical procedure is the determination of two major ETO degradation products, ETO-OH and the related ETO-K. ETO and its chief degradation products (ETO-OH and ETO-K) can alternatively be extracted by SPE using $C_{18}$, achieving a comparable performance. These results could be usefully exploited for multi-residue or on-line water analysis.

## Acknowledgement

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