二重誘導体化-スターバー抽出-加熱脱着-GC/MS法を用いた河川水中エストラジオールの分析

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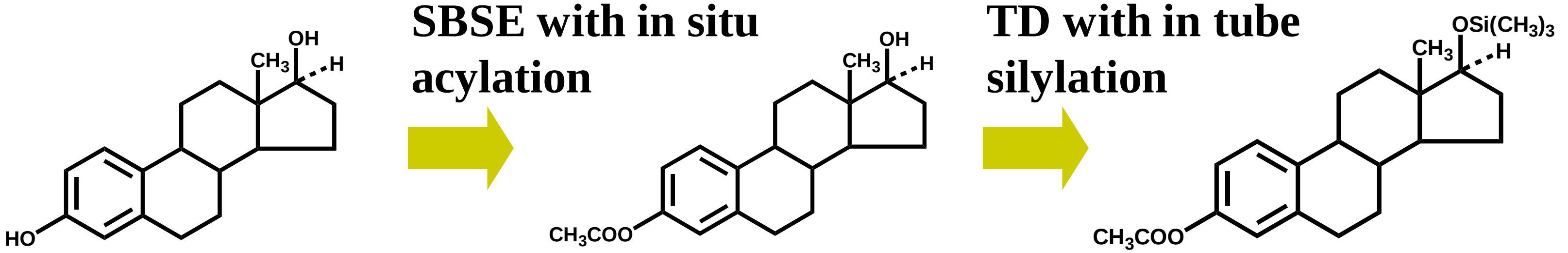
薬業健保会館

Introduction

- A novel method for the trace analysis of 17β-estradiol (E2) in water sample was developed, which involved stir bar sorptive extraction (SBSE) with *in situ* acylation followed by thermal desorption (TD) with quartz wool assisted (QWA) *in tube* silylation and gas chromatography-mass spectrometry (GC-MS).

Strategy

- E2 has both an aromatic and an aliphatic hydroxyl group. In this study, the aromatic hydroxyl group was derivatized by the SBSE with *in situ* acylation. It was expected that the recovery of E2 by SBSE was increased. On the other hand, remaining the aliphatic hydroxyl group was derivatized by TD with *in tube* silylation. It was expected that the volatility of E2 was improved, and the high sensitivity GC-MS analysis was achieved.



SBSE with in situ acylation

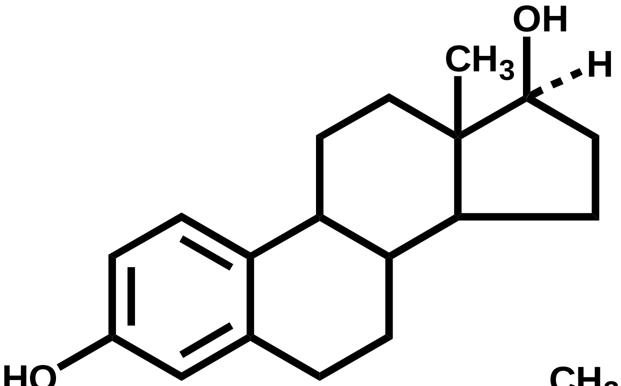
TD with in tube silylation

E2

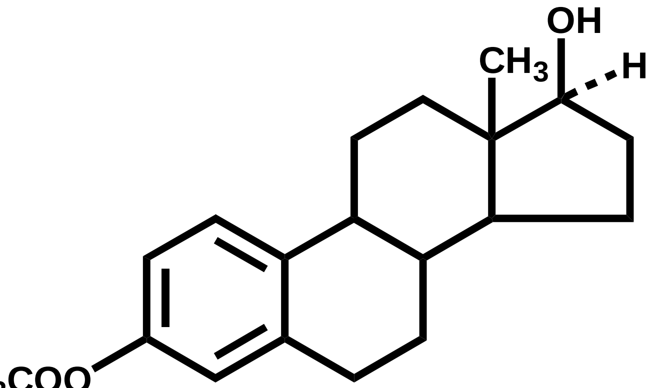
Acyl derivative of E2

Dual derivative of E2

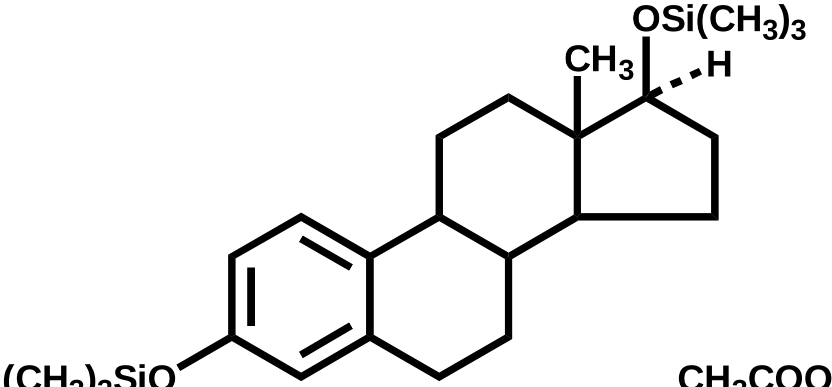
Chemical structures



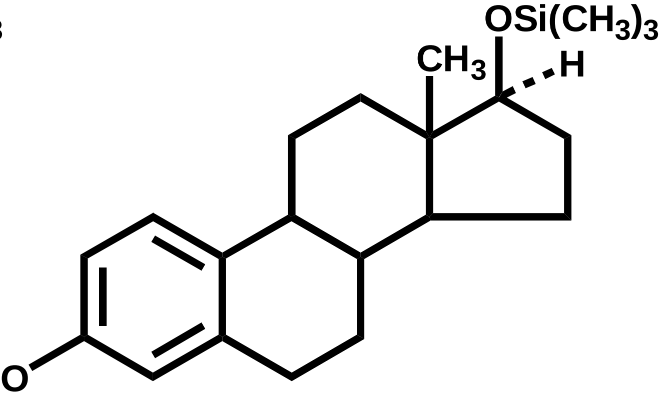
E2



Acyl derivative of E2



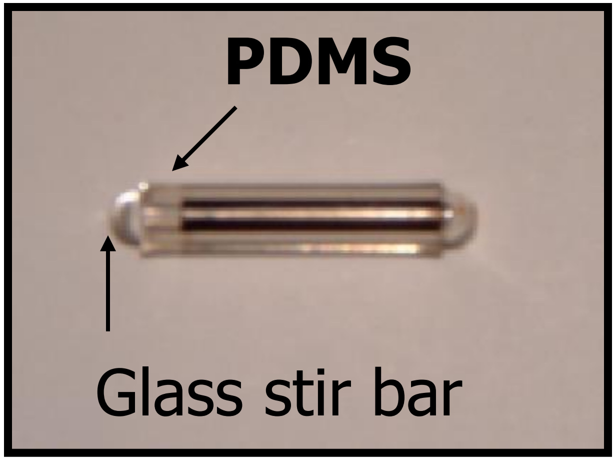
Silyl derivative of E2



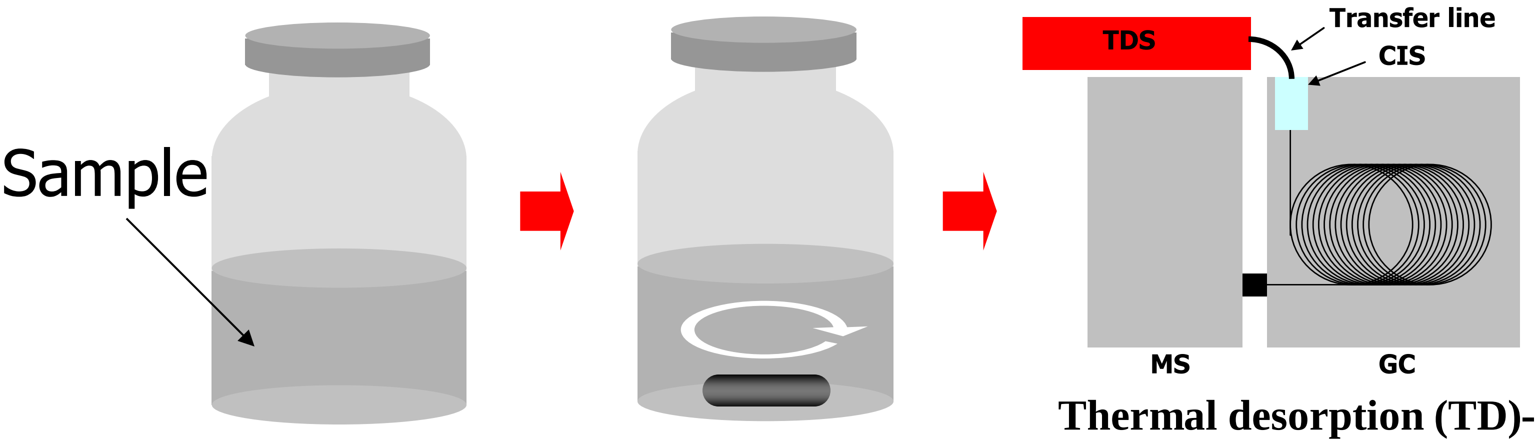
Dual derivative of E2

Hoshi University

Stir bar sorptive extraction



- **Glass stir bar coated with polydimethylsiloxane (PDMS)**
- **Liquid-liquid extraction**
- **Extraction, Concentration and Purification**
- **Easy sample preparation**



Thermal desorption (TD)-GC-MS

SBSE with in situ acylation

- **Phenolic hydroxyl group** **(High polarity and low volatility)**

Low sensitivity

- **Derivatization in sample solution (*in situ*)**

- **Acyl derivative by acetic acid anhydride**

(Low polarity and high volatility)

●Merits of SBSE

・Low polarity → High recovery

・High volatility → Improvement of TD efficiency

●Merits of GC/MS

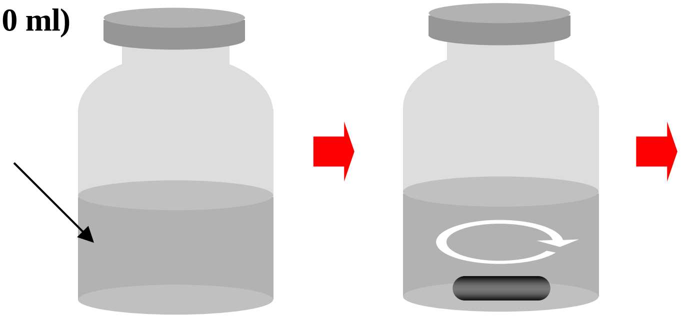
・Low polarity

・High volatility

} Improvement of peak form

} High sensitivity

Extraction time profile



10 ng ml^{-1} standard solution (10 ml)

1M K_2CO_3 (1 ml)

Acetic acid anhydride (100 µl)

TD-GC-MS

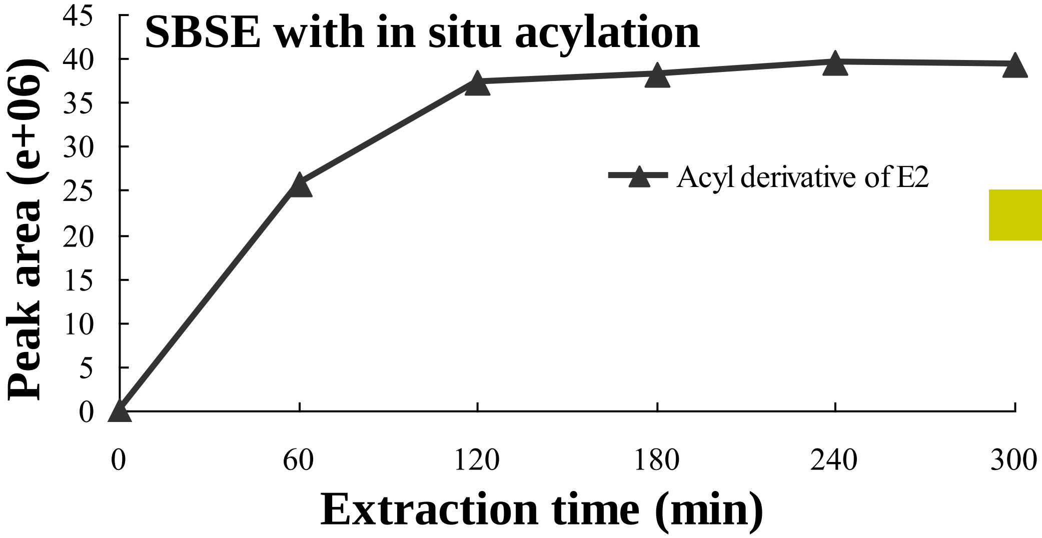
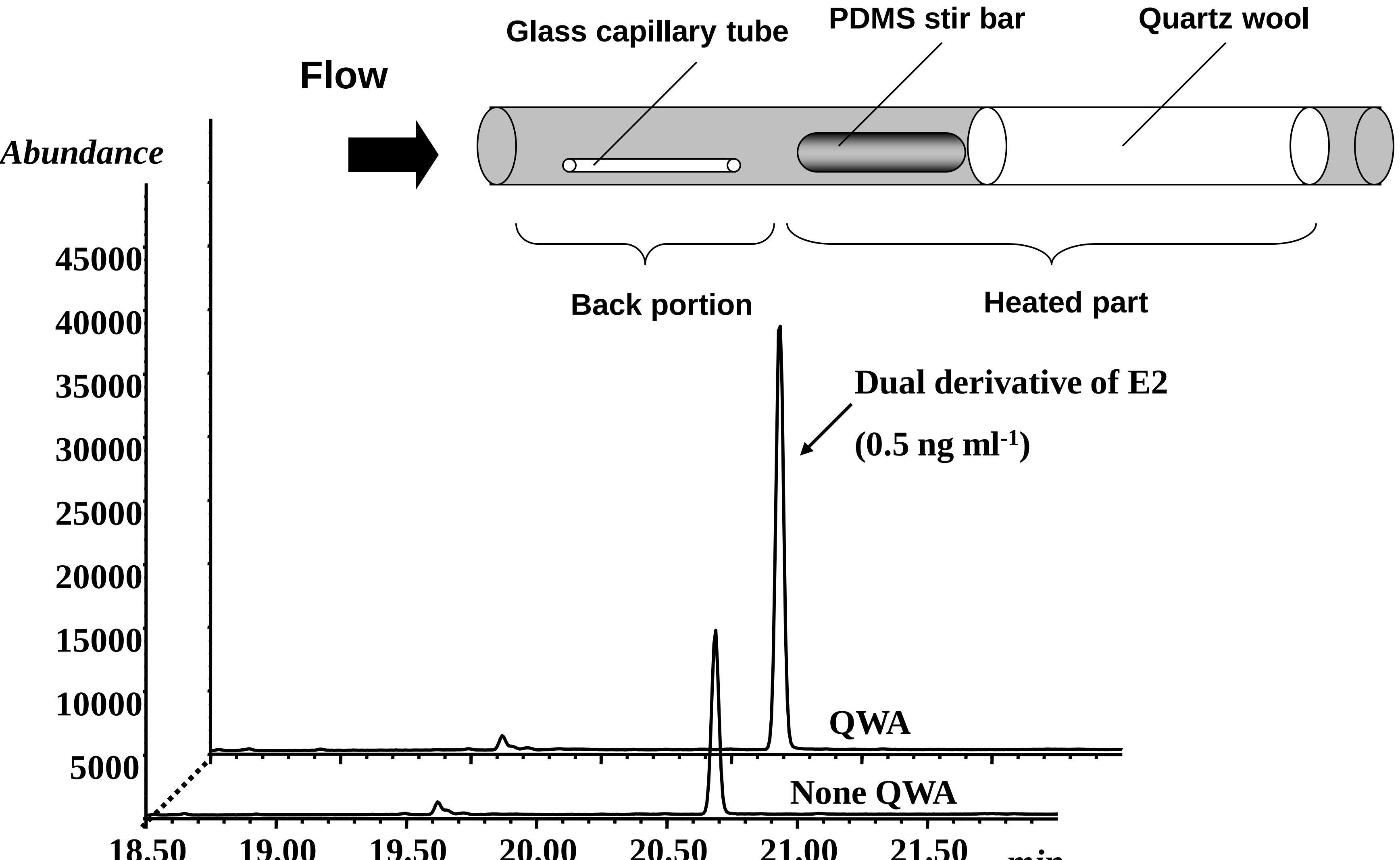


Fig. Extraction time profiles of E2 by SBSE without derivatization and acyl derivative of E2 obtained by SBSE with in situ acylation

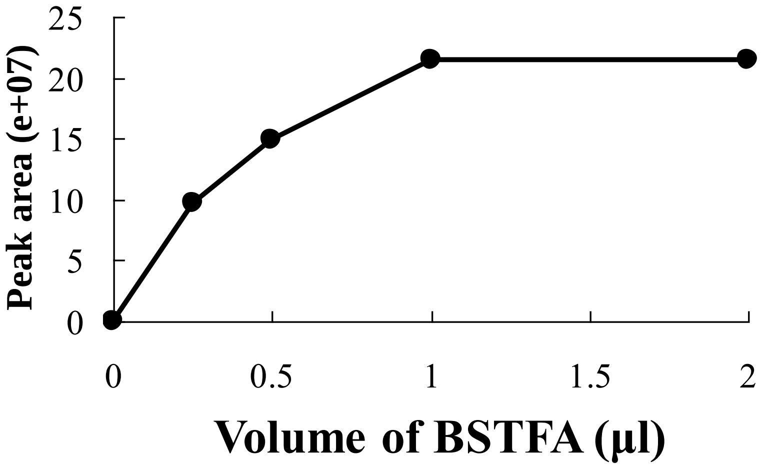
Optimum extraction time

120 min

TD with QWA in tube silylation



In tube derivatization reagent

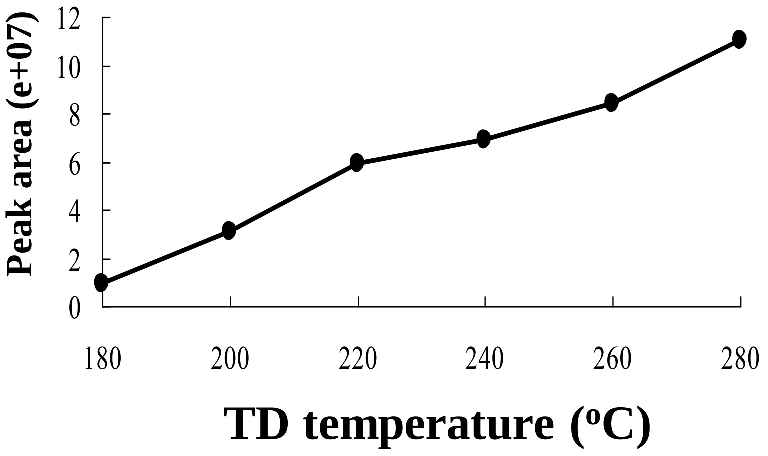


Optimal volume of BSTFA

1.0 µl

Fig. Optimum volume of BSTFA for TD with QWA in tube silylation
A PDMS-coated stir bar and acylation reagents were added to 10 ml of standard solution (10 ng ml^{-1}) and stirring was performed. Then, a glass capillary tube filled with BSTFA as silylation reagent was inserted into the back portion of the glass TD tube with quartz wool. The TD tube was placed inside the TD system where the stir bar was thermally desorbed and subjected to GC-MS thereafter. The optimum volume of BSTFA was examined in the range of 0 to 2.0 µl.

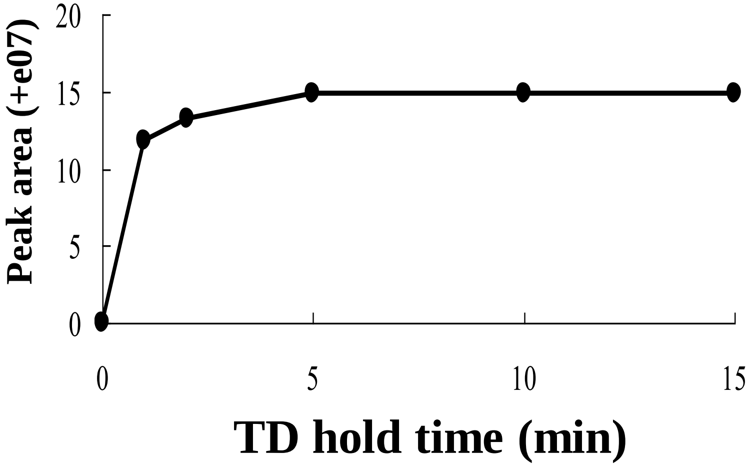
TD temperature condition



The dual derivative of E2 did not reach equilibrium even after the temperature reached approximately 280 °C.

Fig. Optimum TD temperature for analysis of E2 by dual derivatization method
A PDMS-coated stir bar and acylation reagents were added to 10 ml of standard solution (10 ng ml^{-1}) and stirring was performed. Then, a glass capillary tube filled with BSTFA as silylation reagent was inserted into the back portion of the glass TD tube with quartz wool. The TD tube was placed inside the TD system where the stir bar was thermally desorbed and subjected to GC-MS thereafter. The TD hold time was set at 5 min, and the optimum TD temperature was examined in the range of 180 to 280 °C.

TD hold time condition



Optimum TD temperature

280 ^{o}C

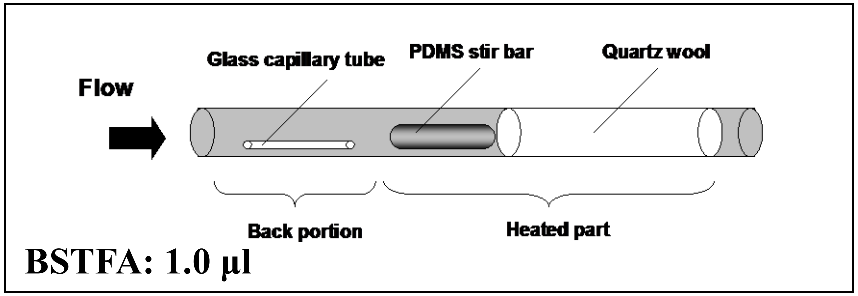
Optimum TD hold time

5 min

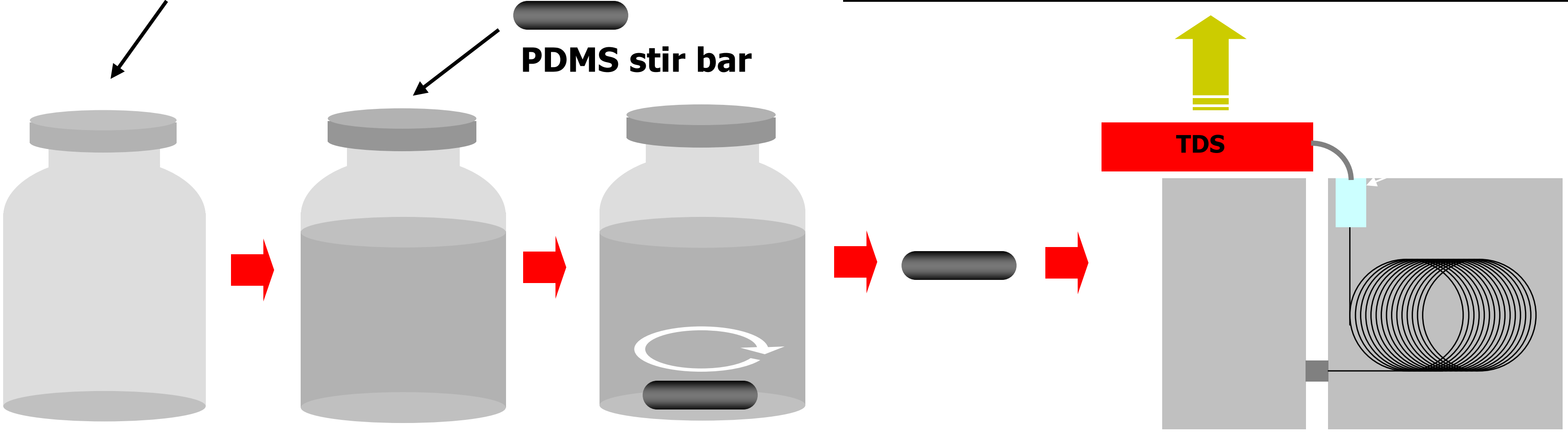
Fig. Optimum TD hold time for analysis of E2 by dual derivatization method A PDMS-coated stir bar and acylation reagents were added to 10 ml of standard solution (10 ng ml^{-1}) and stirring was performed. Then, a glass capillary tube filled with BSTFA as silylation reagent was inserted into the back portion of the glass TD tube with quartz wool. The TD tube was placed inside the TD system where the stir bar was thermally desorbed and subjected to GC-MS thereafter. The TD temperature was set at 280 ^{o}C, and the optimum TD hold time was examined in the range of 0 to 15 min.

Method

Sample (10 ml)
Surrogate standard
1 M K_2CO_3 (1 ml)
Acetic acid anhydride (100 µl)



BSTFA: 1.0 µl



1000 rpm, 120 min

Surrogate standard

Estradiol-$^{13}C_4$

TD and GC-MS conditions

TDS conditions

Flow Mode : Splitless

Initial Temp : 20 ℃

Transfer Temp: 300 ℃

Ramp Rate : 60 ℃/min

Final Temp : 280 ℃

Final Time : 5 min

CIS conditions

Initial Temp : －150 ℃

Ramp Rate : 12 ℃/s

Final Temp : 300 ℃

Final Time : 10 min

GC-MS conditions

Flow gas : Helium (99.9999 %)

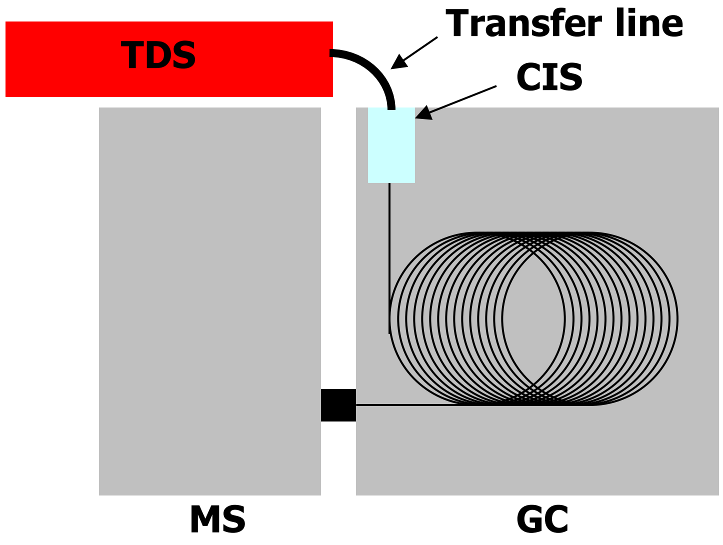
Flow rate : 1.2 mL/min

Injection : splitless

Column : DB-5ms (0.25 mm X 30 m, 0.5 µm)

Oven Temp : 60 ℃ - 15 ℃/min - 300 ℃ (4 min)

Ionization : EI (70 eV)



Validation

Table Figures of merit of SBSE methods

Compound	Derivatization	LOD[a] (pg ml^{-1})	LOQ[b] (pg ml^{-1})	Correlation coefficient (*r*)
E2	none	100	500	0.999 (500-10000)[c]
Acyl derivative of E2	in situ derivatization	2	10	0.999 (10-10000)
Silyl derivative of E2	in tube derivatization	5	20	0.999 (20-10000)
Dual derivative of E2	dual derivatization	0.5	2	0.999 (2-10000)

[a] LOD : limit of detection (S/N = 3)

[b] LOQ : limit of quantification (S/N > 10)

[c] Values in parentheses are the linear ranges of the calibration curves (pg ml^{-1}).

The in situ acylation method exhibited approximately 50-fold higher sensitivity than the method without derivatization. Moreover, the QWA in tube silylation method exhibited approximately 25-fold higher sensitivity than the method without derivatization. Furthermore, the dual derivatization method exhibited approximately 5-fold higher sensitivity than the in situ acylation method, 10-fold higher sensitivity than the in tube silylation method, and 250-fold higher sensitivity than the method without derivatization.

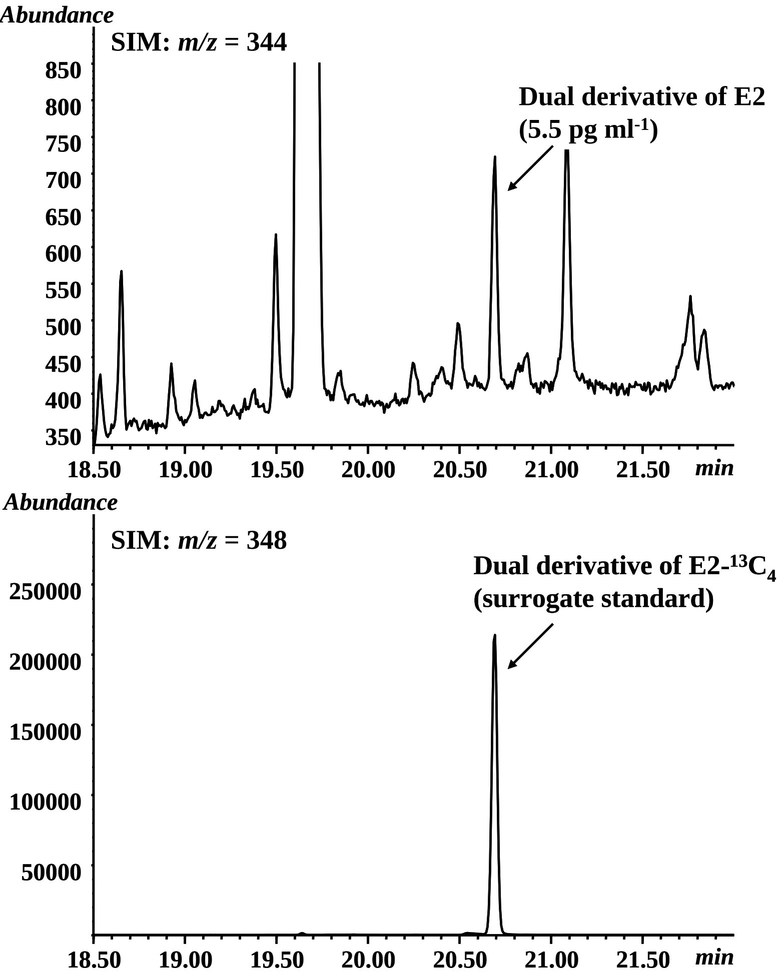
Recovery test

Table Recovery test (n = 6)

Compound	Amount spiked			
	0.05 ng ml^{-1}		1.0 ng ml^{-1}	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
E2	93.1	1.4	98.4	0.8

- Therefore, the method is applicable to the precise determination of trace amounts of E2 in river water samples.

Application



We measured the concentrations of E2 in two water samples collected from Tama River, Japan. Typical chromatograms of the river water samples (Point A) are shown. E2 from Points A and B was detected at concentrations of 5.5 and 4.7 pg ml^{-1}, respectively.

In the present study, the dual derivatization method enabled the successful determination of trace amounts of E2 in a small volume of water sample (10 ml) with only one PDMS stir bar.

Fig. SIM chromatograms of dual derivatives of E2 and E2-$^{13}C_4$ in river water sample (A).

Conclusions

- Determination of E2 in river water samples by SBSE with *in situ* acylation and TD with QWA in tube silylation followed by GC/MS

↓

- Acyl derivatives by using acetic acid anhydride
- Silyl derivative by using BSTFA

→ sub pg/ml (ppt)

- This simple, accurate and highly sensitive method is expected to have potential applications in various water samples.