

### Highly Sensitive Analysis of Estradiols Using NCI-GC/MS

17β-estradiol is a female hormone listed as an endocrine disruptor by Japanese Environment Agency. The 17β-estradiol in rivers and other water areas are determined using the ELISA method or the GC/MS (EI) method the ELISA method achieves high sensitivity, but it is suggested that the analysis can be affected by foreign substances. The GC/MS method is not capable of detecting trace concentrations (ppt or lower level) of 17β-estradiol.

The procedure manuals for inspecting mandatory monitoring items for water environmental preservation issued in December 1999 by the Japanese Water Quality Bureau of Environment Agency includes 17β-estradiol as a substance to be monitored. The manual prescribes two derivatization methods; (1) the methyl derivatization and (2) the pentafluorobenzyl (PFB) derivatization methods. This Application News introduces the PFB derivatization method.

The PFB derivatization method derivatizes estradiols into compounds detectable by negative ion chemical ionization (NCI). This increases both the sensitivity and selectivity, and satisfies the minimum detection limits for water quality samples (0.1μg/L), and for sediments and biological samples (0.01μg/kg). Thus

the PFB derivatization method is attracting attention as a new analysis method that achieves analyses with extremely high sensitivity.

#### [Analytical Overview]

Water samples are spiked with surrogate substances and extracted by solid phase extraction. The collected target substances are eluted with methanol and concentrated to dryness. Then the phenol OH group is derivatized with PFB, and the alcohol OH group is derivatized with trimethylsilyl (TMS) for NCI-GC/MS analysis (see Fig. 1 and 2).

Sediments and biological samples are spiked with surrogate substances and extracted using a methanol:acetic acid buffer solution (pH 5), and then dissolved in purified water. Further the sediments samples extracted by solid phase extraction. The biological samples are extracted with dichloromethane and then concentrated. The obtained extracts are cleaned up by column chromatography and so on, derivatized as water samples and then analyzed by NCI-GC/MS (see Fig. 3).

Table 1 Analytical conditions

<b>-GC-</b>	
Column	: DB-5H (30m×0.25mm I.D. df = 0.1μm)
Column Temp.	: 50°C(2 min)-20°C/min-250°C -5°C/min-300°C(5min)
Carrier Gas	: He 250kPa(1min)-120kPa
Injector Temp.	: 280°C
Injection Method	: Splitless (Sampling Time = 1min)
Injection Volume	: 2μL
<b>-GCMS-</b>	
Interface Temp.	: 280°C
Ionization Method	: NCI (i-C <sub>4</sub> H <sub>10</sub> 60kPa)
Scan Range	: m/z 29 - 700
Scan Interval	: 0.5sec
SIM	: 0.2sec
SIM Monitoring Ion	: m/z 343.30, 269.15, 367.30, 431.35, 347.30

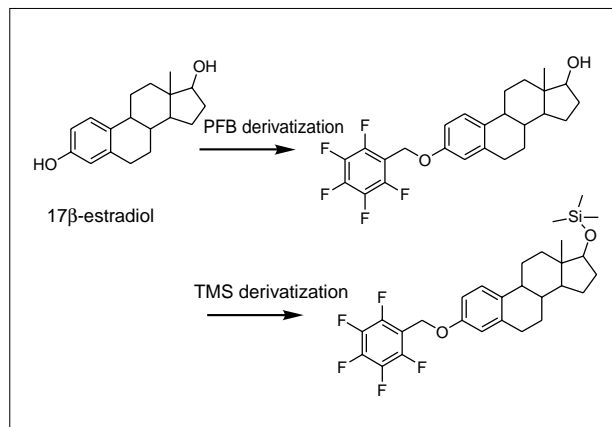


Fig.1 Derivatization of β-Estradiol

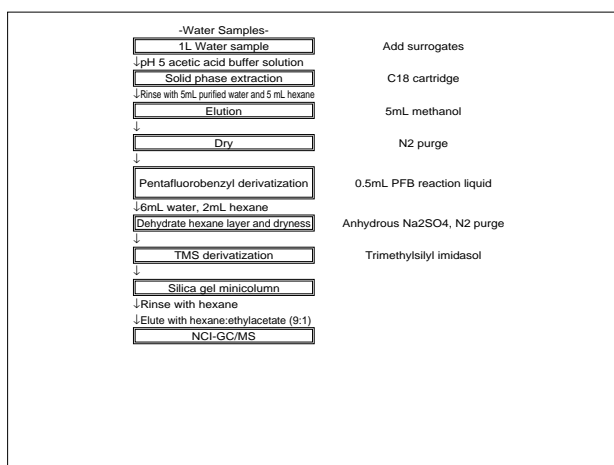


Fig.2 Analysis Method for Water Samples

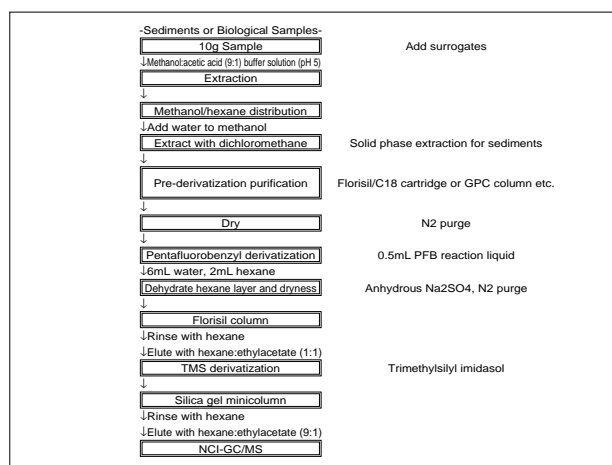


Fig.3 Analysis Method for Sediments and Biological Samples

### Mass Spectra

Five substances – 17 $\alpha$ -estradiol, 17 $\beta$ -estradiol, estron, ethynyl estradiol and estriol – were analyzed and d4-17 $\beta$ -estradiol was added as a surrogate. The mass spectrum obtained by SCAN mode analysis did not detect molecular ions but only ions of M<sup>+</sup>-181 (PFB group). Therefore, SIM analysis was performed using the ions of M<sup>+</sup>-181 as a monitoring ion. (Fig. 4 shows the scan mode TIC and Fig. 5 NCI mass spectrum.)

### SIM Measurement

The SIM measurement with NCI detects standard samples at concentrations down to 5 ng/L (see Fig. 7 for chromatograms for estradiols at 5ng/L). The linearity was also verified for the 5.0 - 1000ng/L range (see Fig. 6).

### Conclusion

Estradiols can be detected to concentrations down to 5ng/L by PFB and TMS derivatization and GC/MS analysis with NCI. As actual samples will be concentrated by 1000 - 10,000 times, they can be measured at ppt levels or lower.

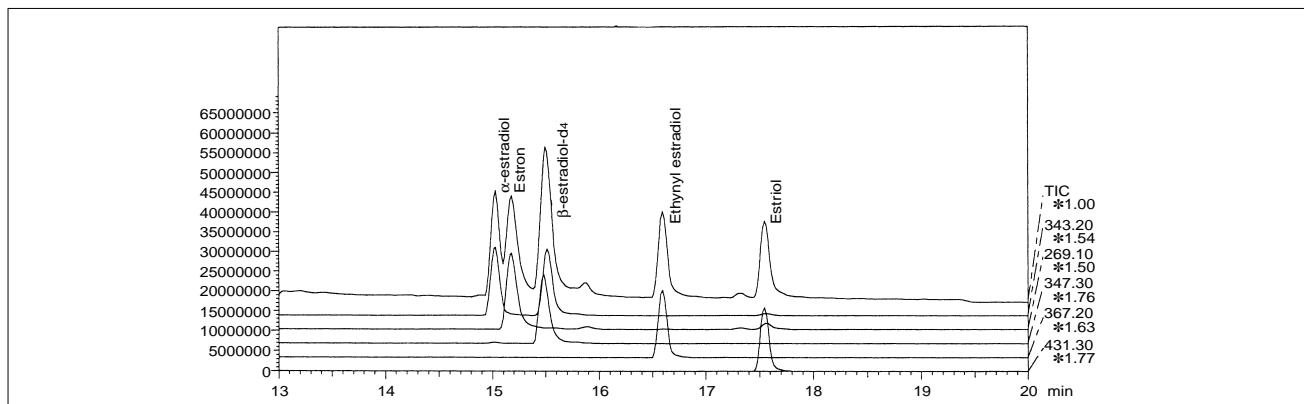


Fig.4 TIC of Estradiols with NCI

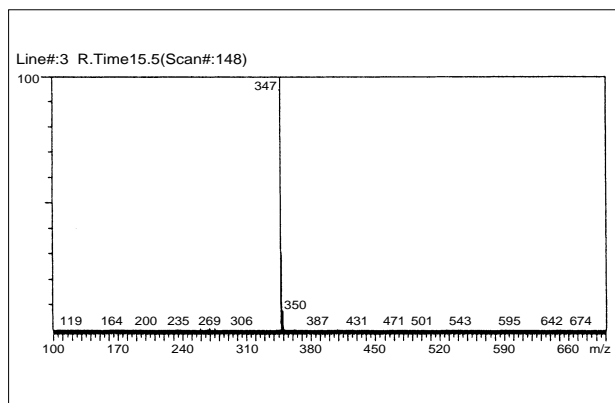


Fig.5 NCI Mass Spectrum of  $\beta$ -estradiol-d4

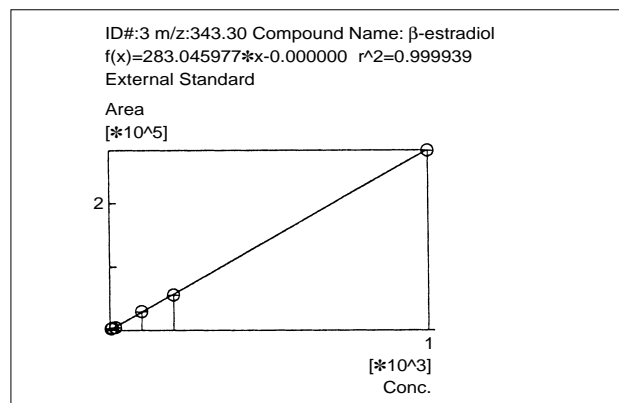


Fig.6 NCI Calibration Curve for  $\beta$ -estradiol

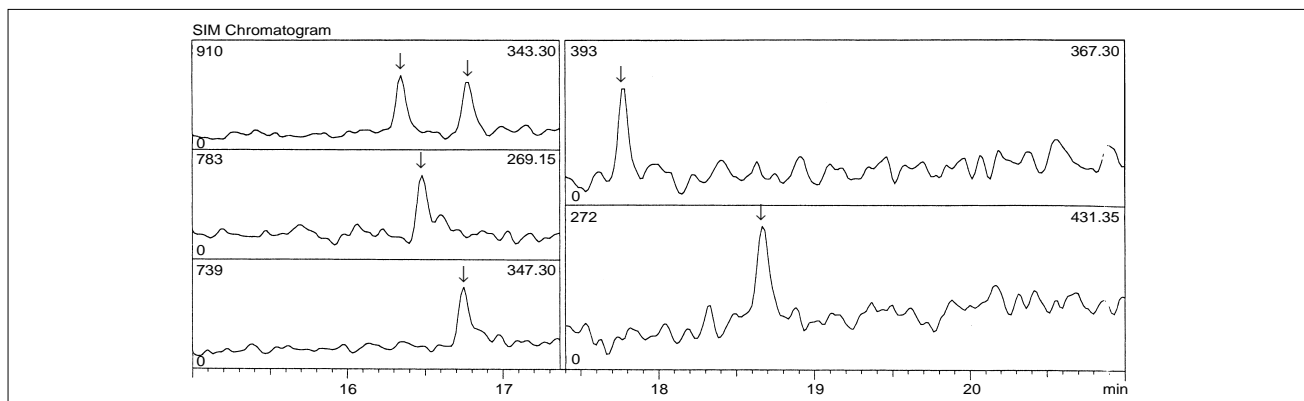


Fig.7 SIM Chromatograms of Estradiols (5ng/L) with NCI



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