SHIMADZU
Excellence in Science

Analysis of trace amount of 17-β-Estradiol and its metabolites in aqueous samples using online-SPE and accurate MS^n analysis

ASMS 2012 MP21 - 527

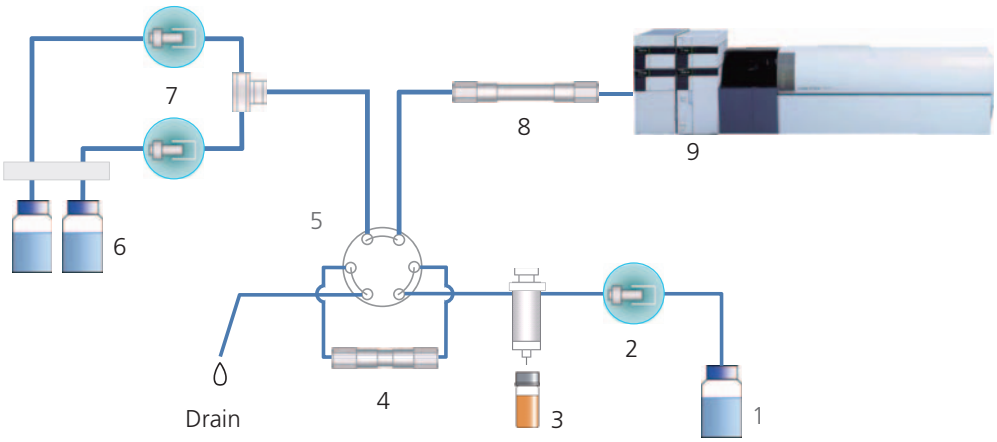
Tetsuo Tanigawa, Tairo Ogura, Ichiro Hirano, Junko Iida
Shimadzu corporation, Kyoto , JAPAN

SHIMADZU
Excellence in Science

Analysis of trace amount of 17-β-Estradiol and its metabolites in aqueous samples using online-SPE and accurate MS^n analysis

Introduction

It has been suggested that many of hormone active compounds, such as naturally occurring estrogen, may be present in environmental water. These may pose a potential health risk as these compounds can act as endocrine disrupting chemicals. It is known that most estrogens, such as 17-β-estradiol (E2), can be metabolized to conjugated forms which have low activity and subsequently excreted, however, it is also known that some conjugated estrogens can return to a highly active free form through de-conjugation. Moreover, treated wastewater has been suggested to contain unknown estrogenic metabolites that as yet have not been fully characterized.
For these reasons the qualitative detection of estrogen-related compounds, such as conjugates in aqueous samples, has great importance. In this study we have developed a qualitative analytical system with online solid phase extraction (online-SPE) and accurate MS^n analysis.

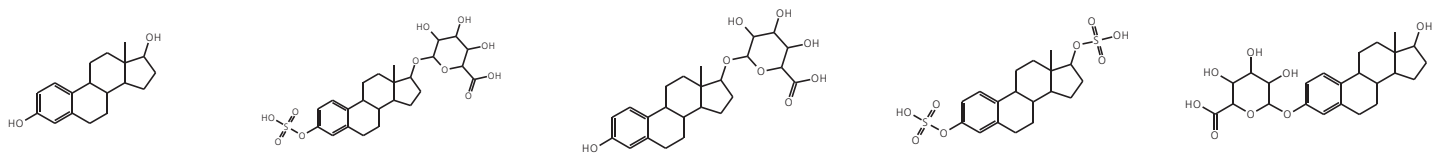


1. Reservoir for online-SPE
2. Pump for online-SPE
3. SIL-10AP with 5 mL syringe
4. Preparative SPE column
5. Flow Change Valve (SPE column)
6. Reservoirs for analysis
7. Pumps for analysis
8. Analytical column
9. LCMS-IT-TOF

Fig. 1 Flow Diagram of online-SPE LCMS-IT-TOF system.

Materials & Methods

Samples were measured by an electrospray ion-trap time-of-flight mass spectrometer (LCMS-IT-TOF, Shimadzu Corporation, Kyoto, Japan) coupled to online-SPE LC system (Nexera series, Shimadzu Co.). Analytical conditions were as follows, analytical column: Shimpack XR-ODS II, C18, 75 × 2 mm, 2.2 µm (Shimadzu Co.); preparative column: MAYI-ODS, 10 × 2 mm (Shimadzu Co.); flow rate: 0.25 mL/min for analytical pump, 2 mL/min for sample loading pump; column temperature: 40°C; mobile phase A and sample loading solvent: water containing 10 mM ammonium acetate; mobile phase B: methanol. 5mL of water sample, filtered by 0.45 µm filter prior to analysis, was injected by SIL-10AP (Shimadzu Co.).



17β-estradiol (E2)
$C_{18}H_{24}O_2$

17β-estradiol 3-sulfate-17-(beta-D-glucuronide) (E2-3S-17G)
$C_{24}H_{32}O_{11}S$

17β-Estradiol 17-(β-D-glucuronide) (E2-17G)
$C_{24}H_{32}O_8$

17beta-Estradiol-3,17-disulfate (E2-3S-17S)
$C_{18}H_{24}O_8S_2$

17β-Estradiol 3-(β-D-glucuronide)(E2-3G)
$C_{24}H_{32}O$

Fig. 2 Structure of 17β-Estradiol and its conjugates.

Analysis of trace amount of 17-β-Estradiol and its metabolites in aqueous samples using online-SPE and accurate MS^n analysis

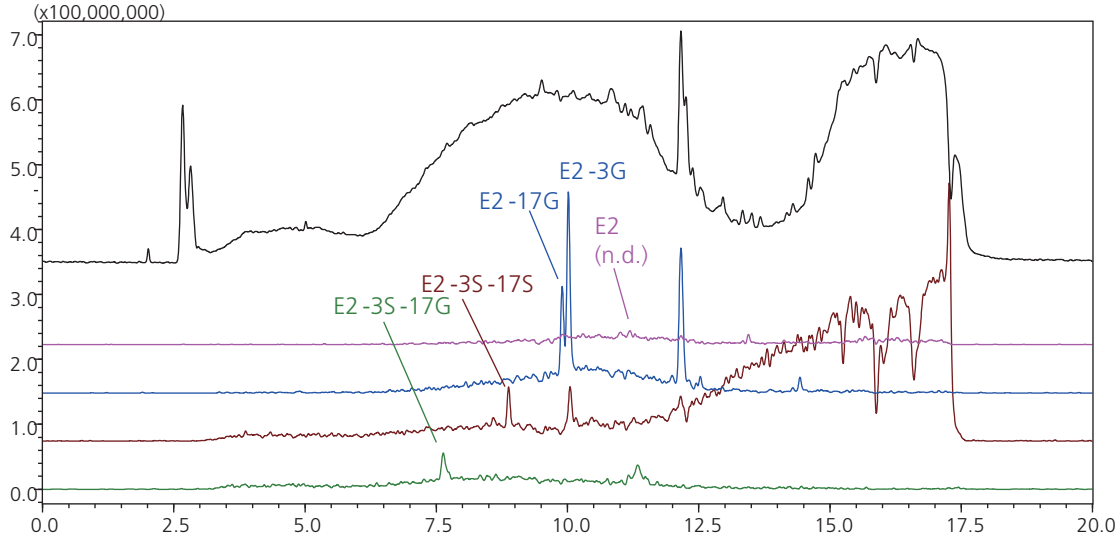
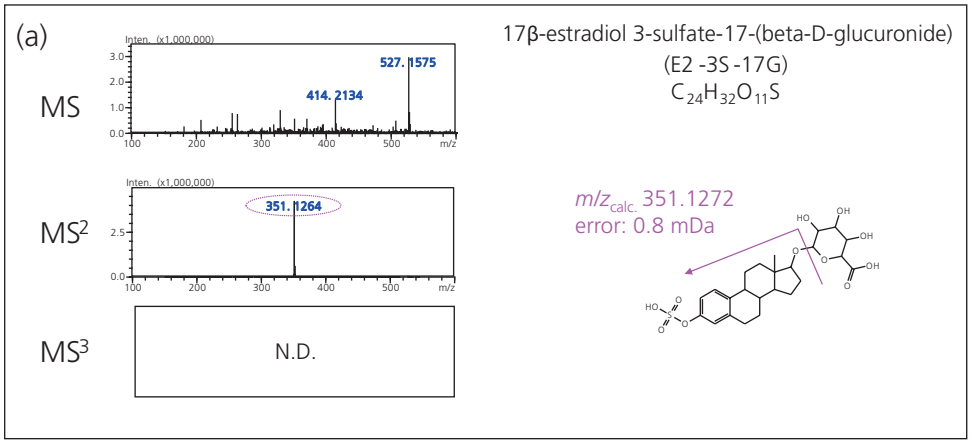
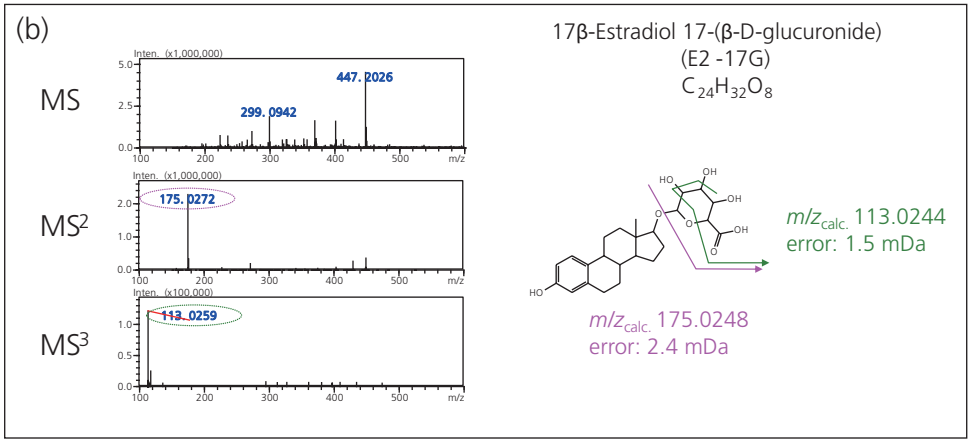


Fig. 3 Chromatogram of river water containing E2 and its conjugates (2 ppt each)

Results





Analysis of trace amount of 17-β-Estradiol and its metabolites in aqueous samples using online-SPE and accurate MS^n analysis

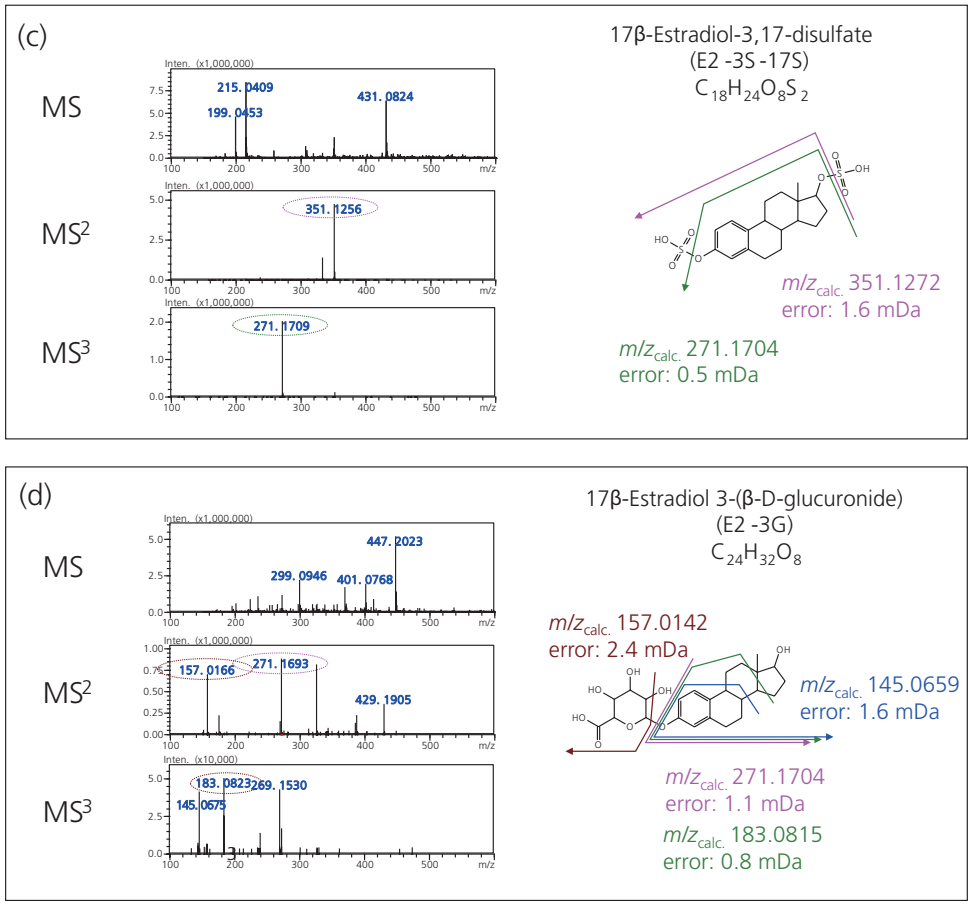


Fig. 4 Accurate MS^n analysis of 17β-estradiol conjugates (a-d).

Table 1 Mass accuracy and peak intensity (S/N) of 17β-estradiol conjugates.

Compound	R.T.	*m/z* obs.	*m/z* calc.	Error (mDa)	S/N at 2ppt
E2	11.18*	271.1702*	271.1704	0.2*	N.D
E2-3S,17G	7.57	527.1575	527.1593	2.9	11.2
E2-3S,17S	8.80	431.0824	431.0840	1.6	9.8
E2-17G	9.86	447.2026	447.2024	0.2	18.6
E2-3G	9.98	447.2023	447.2024	0.1	37.9

*at 10 ppt

Conclusions

- Trace amounts of E2 conjugates E2-3G, E2-17G, E2-3S-17S, and E2-3S-17G were detected in aqueous samples by using online-SPE and accurate MS^n analysis.
- Excellent mass accuracy and comprehensive MS^n data enabled confident assignment to conjugate structures.
- Through techniques developed in this study it is expected to apply these methods to identify other compounds and respective metabolites caused by reduction or oxidation formed by microbes in the natural environment.

First Edition: May, 2012

Shimadzu Corporation
www.shimadzu.com/an/