

## Technical Report

# Development of a Spectrum Library of Endocrine Disruptors by LCMS-IT-TOF

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### Abstract:

Endocrine disruptors upset the hormone balance in organisms by binding to hormone receptors and causing damage or harmful effects to the organism. The effects of chemical substances that bind to female hormone (estrogen) receptors to the organism are of particular concern, and the need to analyze for these kinds of chemical substances has increased in recent years. This Technical Report describes a spectral library of endocrine disruptors with estrogenic activity that has been developed using the LCMS-IT-TOF mass spectrometer, which is capable of accurate mass  $MS^n$  measurements.

**Keywords:** LCMS-IT-TOF, estrogenic activity, environmental hormone, spectral library, endocrine disruptor

## 1. Introduction

In our daily lives, we benefit from the use of a large number of chemical substances. However, some chemical substances present in our environment bind with hormone receptors in organisms and upset the hormone balance in the organism (endocrine disruptors). Endocrine disruptors (i.e. environmental hormones) are defined as "exogenous chemical substances in the environment that cause damage or harmful effects to the organism through their effect on the endocrine system." Among endocrine disruptors, the effects of chemical substances that bind to female hormone (estrogen) receptors to the organism have been of particular concern in recent years, which has led to new requirements to analyze chemical substances with estrogenic activity.

This Technical Report describes a spectral library of endocrine disruptors with estrogenic activity that has been developed using the LCMS-IT-TOF mass spectrometer, which is capable of accurate mass  $MS^n$  measurements. This spectral library allows for easy and highly accurate identification of endocrine disruptors with estrogenic activity. This spectral library also contains data on the estrogenic activity of endocrine disruptors, allowing the user to verify data on the estrogenic activity of an identified endocrine disruptor.

## 2. LCMS-IT-TOF

LCMS-IT-TOF (Fig. 1) is a high-performance liquid chromatograph mass spectrometer that combines a high-performance liquid chromatograph with an ion trap (IT) mass spectrometer and time of flight (TOF) mass spectrometer. The LCMS-IT-TOF combines the  $MS^n$  capabilities of IT with the high-resolution and accurate mass measurement capabilities of TOF to enable accurate mass analysis to  $MS^n$ , an analysis that isn't possible with conventional LC-MS/MS. This hybrid mass spectrometer designed for structural analysis uses state-of-the-art high-speed spectral measurement performance and positive/negative ion switching to increase greatly the amount of data acquired in a single measurement, and provide structural analysis with higher reliability. The spectral library is populated with  $MS^n$  measurement data, which can be searched using precursor data or accurate mass spectral data, enabling easy and highly accurate identification of chemical substances.



Fig. 1 LCMS-IT-TOF High-Performance Liquid Chromatograph Mass Spectrometer

### 3. Estrogenic Activity Data

This library contains estrogenic activity data (hER $\alpha$ ) on 75 compounds evaluated by the National Institute for Environmental Studies (NIES), and also includes links to an NIES database compiling estrogenic activity data on each of these compounds. Estrogenic activity testing was performed using a yeast two-hybrid system that utilizes a recombinant yeast (strain Y190) transformed with an expression plasmid containing the human estrogen receptor gene and coactivator (TIF2) and a reporter plasmid that expresses  $\beta$ -galactosidase. For more details of estrogenic activity testing, refer to the NIES website.

(<http://www.nies.go.jp/archiv-edc/estrogen/index.html>).

### 4. Endocrine Disruptor Spectral Library

The endocrine disruptor spectral library contains 194 MS<sup>n</sup> spectra for 75 compounds. Table 1 shows the number of MS<sup>n</sup> spectra registered for each of the 75 compounds. Of these 75 compounds, the library contains MS<sup>2</sup> spectra for 31 compounds and MS<sup>3</sup> spectra for 44 compounds. Fig. 2 shows the MS<sup>n</sup> spectra for tetrachlorobisphenol A. MS<sup>3</sup> spectra enable highly accurate compound identification. In addition to MS<sup>n</sup> spectra data, the spectral library contains other data, including CAS number, theoretical molecular weight, and compositional formula, as shown in Fig. 3. The spectral library also contains estrogenic activity data as evaluated by the NIES, and links to the NIES database.

Table 1 List of Compounds Registered in Spectral Library

Compound Name	MS <sup>n</sup> Number Included
1,1-Bis(4-hydroxyphenyl)-propane	2
11-KetoTestosterone	3
16 $\alpha$ -hydroxyestrone	3
17 $\alpha$ -Estradiol	3
17 $\beta$ -Estradiol	3
2-(1-Adamantyl)-4-methylphenol	2
2,2',4,4'-Tetrahydroxybenzophenone	3
2,4-Dihydroxybenzophenone	3
2-Ethylhexyl p-Hydroxybenzoate	3
2-Hydroxy-9-fluorenone	2
4-(1-Adamantyl)phenol	3
4,4'-(1,3-Adamantane-diyl)diphenol	2
4,4'-Cyclohexylyden bisphenol	3
4,4'-methylenebisphenol (Bisphenol F)	3
4,4'-thiodiphenol	2
4-cyclohexylphenol	2
4-cyclopentylphenol	2
4-Dodecylphenol	2
4-hydroxybenzoic Acid Isobutyl Ester	3
4-hydroxybenzoic Acid n-Amyl Ester	3
4-hydroxybenzoic Acid n-Hexyl Ester	3
4-phenylphenol	2
4-sec-Butylphenol	2
5 $\alpha$ -dihydrotestosterone	2
6-hydroxy-1,2,3,4-tetrahydronaphthalene	2
6-Hydroxyflavanone	3
7-Hydroxyflavanone	3
Anthrarobin	3
Apigenin	3
Benzyl 4-hydroxybenzoate	3
Benzyl n-Butyl Phthalate	3
Biochanin A	3
Bisphenol A	2
Bisphenol B	3
Bisphenol E	2
Butamifos	2
Butyl-p-hydroxybenzoate	3
Coumestrol	2

Compound Name	MS <sup>n</sup> Number Included
Crysin	3
Cyasterone	3
Dienestrol	3
Diethylstilbestrol(DES)	3
Equol	2
Estriol	3
Estrone	2
Ethinylestradiol	3
Fluazifop-butyl	3
Genistein	3
hexestrol	3
isopropyl 4-hydroxybenzoate	3
Kaempferol	3
Methyltrienolone	3
Mibolerone	3
Murisuterone A	3
Octafluoro-4,4'-biphenol	3
p-Benzylphenol	2
p-Hydroxybenzophenone	2
p-n-butylphenol	2
p-n-Heptylphenol	2
p-n-Hexylphenol	2
p-n-Nonylphenol	2
p-n-Octylphenol	2
p-Nonylphenol	2
p-n-Pentylphenol	2
propyl p-hydroxybenzoate	2
p-t-Octylphenol	2
p-t-Pentylphenol	2
Tebufenozide	3
Tetrachlorobisphenol A	3
TetramethylbisphenolA	3
Zearalenone	3
$\beta$ -Ecdysterone	2
$\beta$ -Estradiol 17-( $\beta$ -D-Glucuronide)	3
$\beta$ -Estradiol 3-( $\beta$ -D-Glucuronide)	3
$\beta$ -Estradiol 3-Sulfate	3

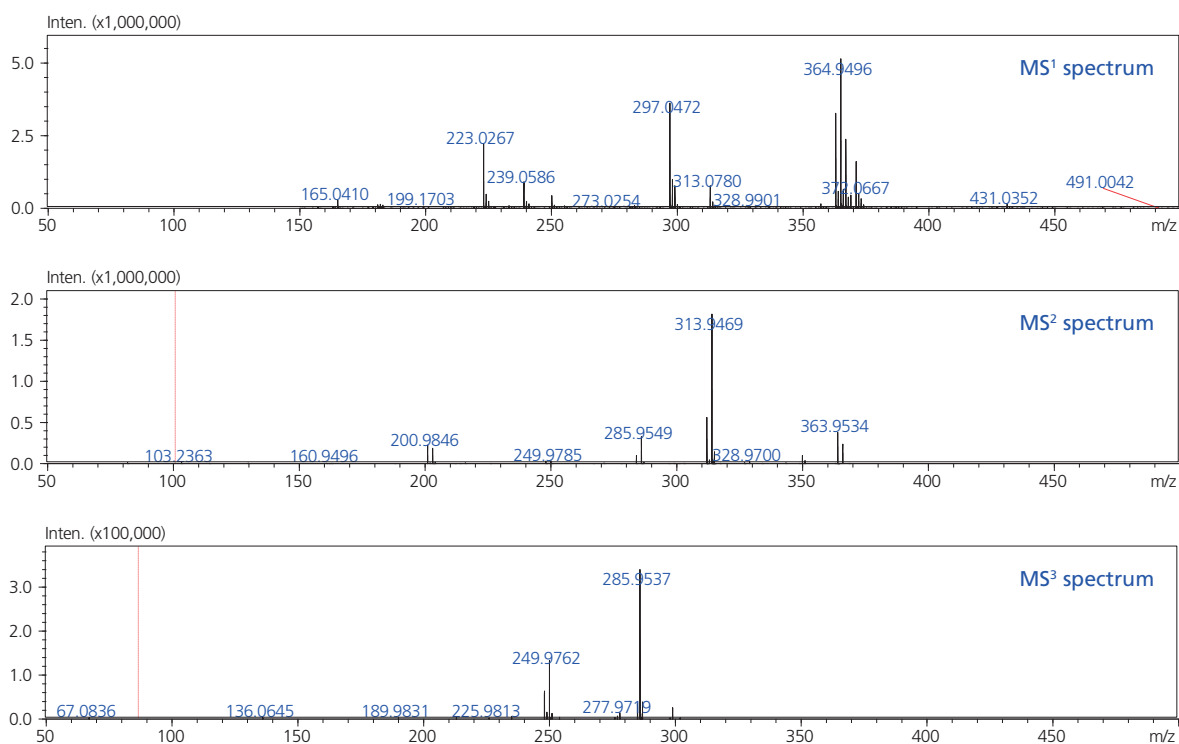


Fig. 2 MS<sup>n</sup> Spectra for Tetrachlorobisphenol A

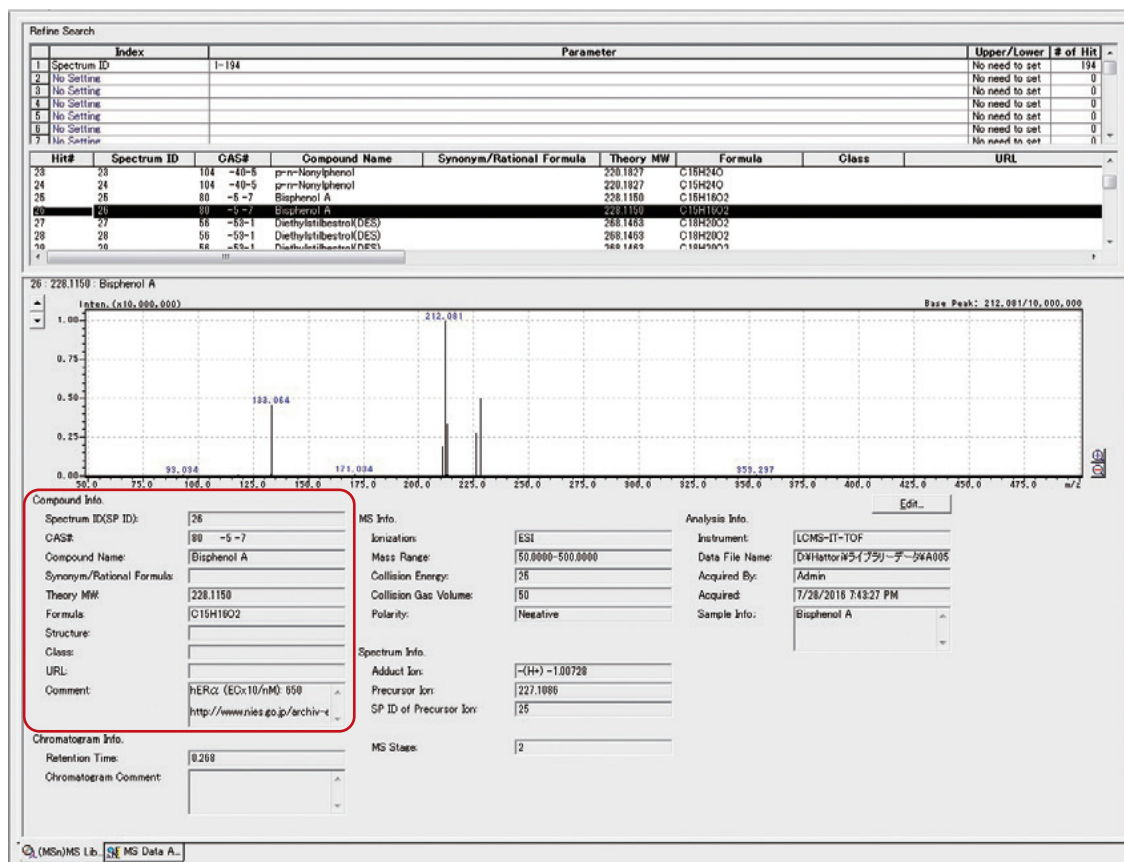


Fig. 3 Library with Endocrine Disruptors Registered

The library search function allows compounds to be identified based on the MS<sup>n</sup> spectral data obtained by user analysis of a chemical substance and MS<sup>n</sup> spectral data contained in the library. Fig. 4 shows the library search results for the identification of tetrachlorobisphenol A based on the MS<sup>3</sup> spectrum of a detected chemical substance. Using the MS<sup>3</sup>

spectrum allows for identification with higher accuracy compared to MS/MS analysis. In addition, once a compound is identified, data on that compound contained in the library enables quick verification of the estrogenic activity data on the compound as an endocrine disruptor, and other details about the compound as a chemical substance.

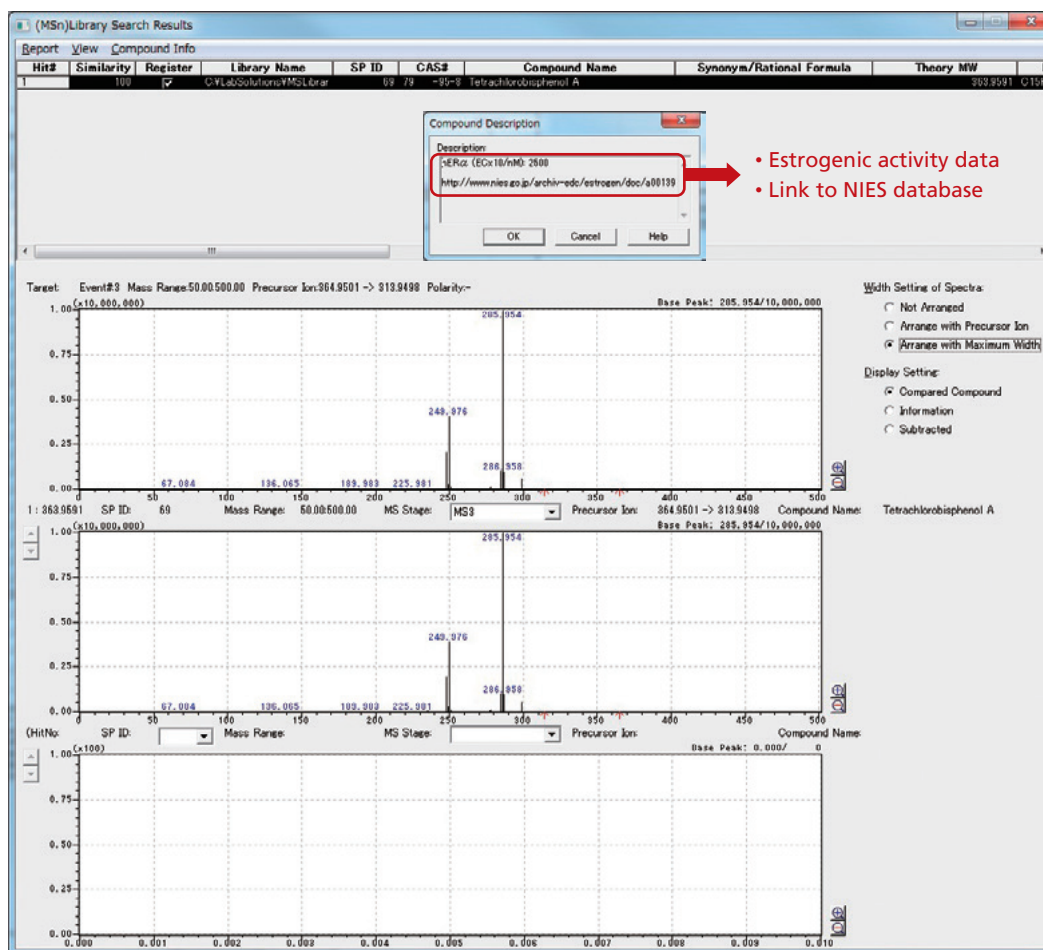


Fig. 4 Library Search Results

## 5. Conclusions

Using LCMS-IT-TOF, which is capable of high-resolution and accurate mass MS<sup>n</sup> measurement, together with a spectral library of endocrine disruptors with estrogenic activity, allows for easy and highly accurate identification of endocrine disruptors with estrogenic activity. Upon compound identification, data on that compound contained in the library also enables quick verification of the estrogenic activity data on the compound as an endocrine disruptor and other details about the compound as a chemical substance.

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