Approaching The Ultimate Limits of Detection For Endocrine Disruptors in Wastewater Effluent Using GC-NCI-MS/MS

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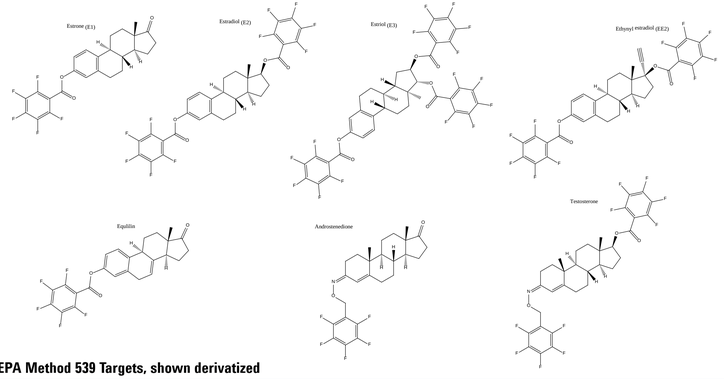
Objective

To efficiently evaluate our wastewaters, an integrated approach combining bioassays with targeted chemical analysis needs to be implemented. High-throughput, robust and sensitive analytical methods are required.

Described herein is the development of a rugged GC-NCI-MS/MS analytical method designed for detecting trace levels of Endocrine Disrupting Chemicals (EDCs) in decreased sample volumes (< 500mL) of wastewater effluent.

EDC analysis by GC-NCI-MS/MS appears to be a plausible alternative to LC-MS/MS analysis based in part on the potential advantage to use smaller sample volumes, thus lowering testing costs, and achieving similar or lower detection levels. In fact, estimated MDLs for estrogens and androgens are at least two orders of magnitude lower than those determined by LC/MS/MS.

This work centers on EPA Method 539, currently an LC-ESI-MS/MS method (with derivatization required), in an effort to evaluate the GC-NCI/MS/MS approach.



EPA Method 539 Targets, shown derivatized

Experimental

Analysis was performed using an Agilent 7890A Gas Chromatograph coupled to a 7000B Triple Quadrupole Mass Spectrometer equipped with a Multi Mode Inlet and Purged Ultimate Union used for back flushing the column. The union was placed between two DB-17ht columns of dimensions 5m x 0.25mm x 0.15µm and 15m x 0.25mm x 0.15 µm. The GC was programmed to reach 330°C in constant flow mode and 2 µL was injected. Back flush was accomplished by lowering the inlet head pressure for 1 minute post run.

Negative chemical ionization was performed with 40% ammonia reagent gas and a source temperature of 150°C.The MS source temperature was 310°C. An EM gain of 100 was used for this analysis. Derivatization was carried out to produce either the pentafluorobenzoyl (PFB) ester or PFB oxime at C-3. The PFB ester was formed at C-17 in some cases and also at C-16 in the case of E3. Calibration was performed using non-extracted standards with levels ranging from 0.05 pg/mL to 10 ng/ml. ISTDs Estradiol-D3, Testosterone-D5 and Estrone-D4 were added at 10 pg/ml.

Extracted 20 ml and 500 ml wastewater samples were obtained from the US EPA.

Experimental

Derivatization Procedure

1. Add 0.5 mL 1% pyridine in ethyl acetate to dried sample
2. Add 50 µL 10% pentafluorobenzoyl chloride in ethyl acetate
3. Cap and vortex 1 minute, heat 30 min at 60°C
4. Evaporate to dryness at 60°C under gentle N_2 stream
5. Add 1 mL 0.5M aqueous sodium bicarbonate, vortex
6. Cap and react 10 minutes at room temperature
7. Centrifuge 5 min
8. Transfer organic layer to a second tube for next steps
9. Dry at 60°C under gentle nitrogen
   a. If estrogens only, go to step 14
10. Add 0.5 mL isooctane
11. Add 100 µL 0.1% (wt/v) PFB-hydroxylamine hydrochloride in pyridine
12. Cap and vortex 1 minute, heat at 60°C for 5 min
13. Dry at 60°C under gentle nitrogen
14. Add 50 uL isooctane for injection

Notes about derivatization chemistry

All estrogens react with pentafluorobenzoyl chloride at R positions where R = OH to form the corresponding ester.

In all cases (estrogen and androgen) where the 17 position is a keto moiety, pentafluorobenzyl hydroxylamine either reacts poorly or not at all.

Using this derivatization procedure, all estrogens have a predictable SRM transition M -> (M-64).

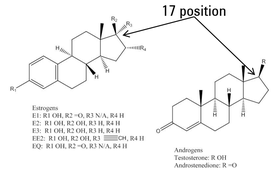


Table of SRM transitions

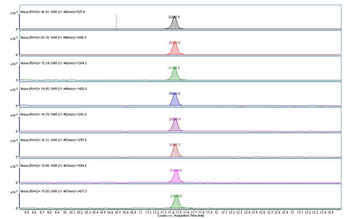
Time Segment	Compound	Precursor Ion	Product Ion	dwell	Collision Energy
1	EE2	490.5	426.5	60	6
1	E1	464.4	400.4	60	6
1	EQ	462.4	398.4	60	4
1	EQ	462.4	370.4	60	8
1	ASD	461.5	431.5	60	6
2	Testosterone	677.6	657.6	100	4
2	Testosterone	677.6	627.6	100	6
2	E2	660.5	596.5	100	8
3	E3	870.6	806.6	150	6
3	E3	870.6	167.6	150	10

Results and Discussion

Results

The previously reported limit of detection for E2 was again achieved in our laboratory (Churley and Macherone, 2010.; Macherone, 2012). Figure 1 illustrates the S/N ration for 8 replicate injections of E2.

Figure 1. Eight replicate injections of E2.



The instrument detection limit (IDL) can be determined by the equation:

IDL = (t_α)(%RSD)(amount of standard)/100

where t_α is a statistical confidence factor found in the Student t- distribution table. With 99% confidence, the t_α value for 8-1 degrees of freedom is 2.998. Substituting this into equation 1 gives:

IDL = (2.998)(3.0%)(10 pg/ml)/100 = 0.9 pg/ml E2

Table 1 illustrates the raw data for the IDL determination of estradiol (E2) and Table 2 illustrates the area precision for a 2 µl injection of a 0.5 pg/ml E2 standard.

Table 1. Raw E2 data for IDL determination

Estradiol		
Inection #	S/N	Area
1	483	20005
2	509	20515
3	405	20722
4	548	21139
5	420	21368
6	352	21525
7	399	21614
8	410	21894
Average	441	21098
St Dev		636
% RSD		3.0

Table 2. Area precision for E2 at 0.5 pg/ml (2 µl injection = 1 fg on column)

Name	E2 Area
0p5_01.D	684
0p5_02.D	620
0p5_03.D	723
0p5_04.D	641
0p5_05.D	620
Average	658
St Dev	45
% RSD	6.8

Conclusions

The accurate and sensitive measurement of steroidal analogs is an important requirement to monitor the fate and transport of steroidal analogues in the environment. This poster outlines a procedure that modifies steroidal analogs such that they become amenable to electron capture negative chemical ionization mechanisms and provides GC/MS/MS conditions required for a highly sensitive and robust analytical method with IDL on the order of 1 pg/ml or less. Moreover, the procedures described herein are amenable to matrices other than waste water effluent, soil and bio-solids and can be extended to biological sources such as serum or plasma for measurement of the exposome. In addition to E2 data, preliminary data for 17a-ethinylestradiol (EE2) in wastewater show that calibration is possible using a set of standards containing from 0.2 to 200 pg per vial, where R^2 = 0.99. Based on a 20 mL sample volume, this would be equivalent to 0.01 to 10 ng/L. An extracted 20 mL wastewater sample was quantified to contain EE2 at 17 ng/L (ppt) with adequate GC peak separation.

References

A. Macherone (2012),
Agilent Application Note 5990-9478EN
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