Analysis of Estrone and 17β-Estradiol in Ground Water by GC-NCI-MS/MS

[1] Melissa Churley, [1]Anthony Macherone, [2]Robert White
[1] Agilent Technologies, Inc. 5301 Stevens Creek Boulevard Santa Clara, CA 95051 USA; [2] MPI Research, Inc. 3058 Research Drive State College, PA 16801 USA

ASMS 2011
WP 316

Agilent Technologies

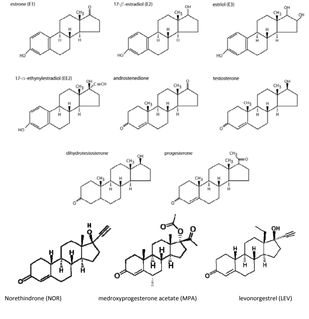
Introduction

Exposure to estrogens has been demonstrated to affect aquatic species reproduction and induce feminization in male fish. One study illustrates reversible sex change in black porgie, a protandrous hermaphrodite, upon exposure to 17β-estradiol. Previously, we demonstrated a GC-NCI-MS/MS method for the analysis of estradiol and estrone. In light of governmental identification of these estrogens as emerging environmental contaminants, we propose that the same analytical methodology may be applied for the analysis of these contaminants in the environment. An attractive aspect of this application may be that only 400 uL of sample is required to measure levels as low as 0.3 pg/mL or 0.6 fg on column. This study evaluates GC-NCI-MS/MS to analyze environmental contaminants in ground water.

Expanded Scope

Since the submission of this abstract, the authors were approached by the United State Environmental Protection Agency to undertake a proof of principle study that builds upon the work reported in the abstract for the analysis of estrone (E1) and estradiol (E2) in ground water. The scope of the project was subsequently expanded to include not only E1 and E2 but also two other estrogens, three androgens and four progestins potentially found in waste-water effluent. Key factors to the proof of principle study are sensitivity, selectivity, robustness and the potential to minimize sample extraction volumes from 500 mL to 20 mL or less.

Target Compounds



Experimental

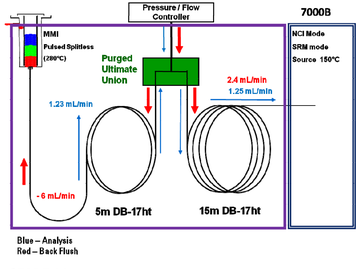
Derivatization was carried out to produce either the pentafluorobenzoyl (PFB) ester or PFB oxime and *in situ* trimethylsilyl ether, -OTMS, at C-17 and/or C-16 (E3). Calibration levels ranged from 0.05 pg/mL to 10 ng/mL. ISTD TEST-D5 was added at 10 pg/mL. Extracted 20 mL and 500 mL wastewater samples were obtained from the EPA.

Analysis was performed in SRM mode on an Agilent 7890A Gas Chromatograph coupled to a 7000B Triple Quadrupole Mass Spectrometer equipped with an 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.

GC configuration showing back flush flows

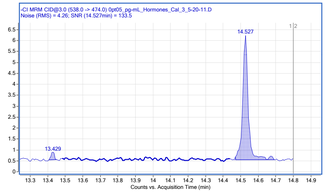


SRM Table

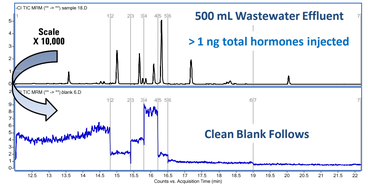
Compound	Time Segment	Retention Time	Precursor	Product	CE
E2	1	14.532	538	474	3
DHT	1	14.502, 14.605	537	507	5
TEST	2	14.993	535	505	5
NOR	3	15.659	545	515	5
EE2	3	15.735	562	498	1
E3	4	15.827	626	562	7
E1	4	16.06	464	400	1
LEV	5	16.303	559	529	5
AND	5	16.282	461	431	1
MPA	6	18.237, 18.330	561	531	7
PROG	7	20.034	684	654	7
TEST-D5	2	14.974	540	510	5

Results

E2: 0.05 pg/mL (1.7 fg on column)
RMS S/N 133:1 0.5 min noise



Use of column back flush eliminates carryover

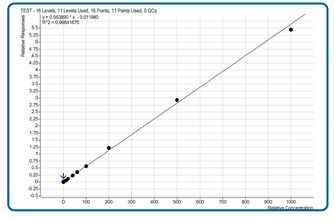


Back flushing the column removes adsorbed components and leads to better RT stability and decreased cycle time. It may be performed post run or concurrently, after the last peak of interest passes the purged union, for the lowest possible cycle time. Using this simple technique, sample carryover for even the dirtiest matrices may be eliminated as shown above. This leads to higher sample throughput and cost savings.

Electron capture negative chemical ionization mode

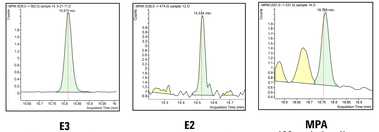
The use of negative chemical ionization, or NCI, allows for lower detection limits when compared with EI mode. It is a highly selective process in which electron capture takes place for halogenated derivatives. This allows for relatively higher selectivity of the analyte versus matrix interferences and ions that would be observed in EI mode. Relatively less fragmentation of the molecular ion compared to EI fragmentation results in increased sensitivity.

Testosterone 5 pg/mL – 10,000 pg/mL R^2 0.998



SRM profiles for hormones in wastewater

Extract of 20 mL:

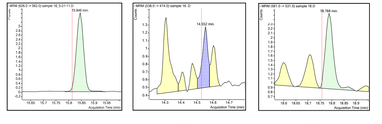


E3
100 pg/mL spike

E2
non-spiked sample

MPA
100 pg/mL spike

Extract of 500 mL:



E3
2500 pg/mL spike

E2
non-spiked sample

MPA
2500 pg/mL spike

Extracts of 20 mL samples yielded chromatographic peaks that had stable retention times with respect to the calibration standards and, in some cases, superior chromatography versus the extracts of 500 mL samples.

Results and Discussion

0.5 pg/mL Precision

E2 and E1 extracted from laboratory water at 0.5 pg/mL yielded % RSDs of 6.5 and 8.2, respectively, for five injections.

	E2 Peak Area	E1 Peak Area
	67.5	202.1
	62.2	164.8
	72.3	176.3
	64.2	175.8
	62.2	169.1
Average	65.7	177.6
STDEV	4.3	14.5
%RSD	6.5	8.2

Hormones in extracted wastewater effluent

In a blind study and first attempt to quantify EPA samples extracted from 20 mL and 500 mL wastewater effluent using this method, values were obtained as shown in the table below. Both spiked and non-spiked samples were run. Blue and red indicate values below or above the curve fit limit, respectively.

Calculated spike amounts based on the difference between resultant non-spiked and spiked values are compared with actual spike amounts provided by the EPA. Recoveries for six hormones in this first trial were within 28%, except in the case of E3 determined in the 20 mL sample.

Recovery in EPA wastewater samples (pg/mL)

	20 mL Extr. Effluent	20 mL Effluent - Spiked	Calculated Spike Amt.	Actual Spike Amt.
DHT	29.8	133	103	100
TEST	2.0	106	104	100
NOR	4.9	92.4	87.5	100
EE2	8.1	111	103	100
E3	19.1	282	263	100
LEV	17.6	121	103	100

	500 mL Extr. Effluent	500 mL Effl. - Spiked	Calculated Spike Amt.	Actual Spike Amt.
DHT	390	2445	2055	2500
TEST	19.1	2104	2085	2500
NOR	(chrom)	1699	N/A	2500
EE2	83.3	1887	1804	2500
E3	92.2	2357	2265	2500
LEV	29.5	2597	2568	2500

Comparison with Current Method

Currently employed for this analysis is LC-MS/MS wherein 500 mL wastewater extracts are used and 20 µL injections are made. Herein we have presented compelling evidence that the same detection limits or better may be achieved using GC-NCI-MS/MS with 20 mL extracts and 2 µL injections.

Conclusions

A sensitive method for the determination of hormones extracted from water has been successfully applied to environmental samples.

Proof of principle has been established in terms of sensitivity, selectivity, and robustness.

The results demonstrate the potential to decrease environmental sample extraction volumes from 500 mL to 20 mL.

Acknowledgements

The authors would like to gratefully acknowledge the United States Environmental Protection Agency and especially Shoji Nakayama, Ruth Marfil-Vega and Marc Mills for providing standards and prepared samples for the study presented herein.

The United States Environmental Protection Agency through its Office of Research and Development contributed to the research described here. Mention of trade names or commercial products does not constitute endorsement or recommendation for use by USEPA.

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