Application Note: 10083

Determination of Estrone and Estradiol using GC-MS/MS in Negative Chemical Ionization Mode (NCI)

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Key Words

- GC-MS/MS
- Ion Trap PolarisQ
- Hormones
- Estrone
- Human Plasma
- Negative Ion
- Chemical Ionization

Overview

Purpose: Develop a method to determine low concentrations (pg/µL) of estrone and estradiol in a biological matrix.
Method: Analyze a spiked matrix using NCI MS/MS to increase selectivity. Use derivatizing reagents to increase sensitivity of compounds in NCI.
Results: Method developed is linear and capable of detecting 1 pg/µL concentration in split mode, or 500 pg/mL concentration from the biological matrix.

Introduction

Estrone and estradiol are prescribed in hormone replacement therapy for alleviation of many of the symptoms of menopause such as hot flashes and night sweats. Estradiol is thought to balance lipoproteins in the blood to induce a cardio-protective effect. Unfortunately estrone, a major component of most hormone therapies, and the metabolic product of both orally prescribed estrone and estradiol, has been implicated in cancers induced by hormone medicated therapies. Ultimately, with this delicate balance of benefits and dangers inherent in orally prescribed estrogens, the need arises for monitoring the levels of estrone, and estradiol in patients.

Estrogen levels in the body can be determined in a variety of ways. It is excreted in both the urine and feces, and is found in the blood stream of all adults. All three of these, urine, feces and blood, are complex matrices that can interfere with the hormone signal in chemical analysis. Because of this, an analysis with a large amount of selectivity is required. NCI combined with MS/MS can give the high amount of selectivity needed in these biological matrices.

This project will endeavor to find a derivatizing reagent capable of relinquishing the charge to the compounds of interest in negative chemical ionization mode. It is an unfortunate reality that derivatizing reagents capable of working in an NCI environment, were originally developed for the ECD. Although there is an enhanced response in negative chemical ionization systems with these derivatives, they also tend to chemically disassociate from the compound, taking the charge with it, and giving the user no confirmatory data.

Instrument Parameters

POLARISQ ION TRAP

Source temperature:	275°C
Ionization mode:	Negative Chemical Ionization
Reagent gas:	Methane
Gas flow:	1.1 mL/min
AGC:	25
Injection waveforms:	On
Trap pressure:	5.4 mtorr
MS/MS parameters:	See Table 1

TRACE GC

Column:	Rtx™200, 0.25mm x 30m, 0.25µm
Oven:	110°C, 2.5 min, 20°C/min, 320°C for 6 min
Split injection Injector:	Temperature: 255°C
Split flow:	20 mL/min
Constant flow of 1.0 mL/min	

AS 2000

Injection volume:	2 µL

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Estrone derivative

PRECURSOR ION	WIDTH	ISOLATION TIME	COLLISION ENERGY	COLLISION TIME	Q
269 amu	3 amu	16 msec	2.75 volts	30 msec	0.3

Estradiol derivative

PRECURSOR ION	WIDTH	ISOLATION TIME	COLLISION ENERGY	COLLISION TIME	Q
467 amu	1 amu	16 msec	2.00 volts	30 msec	0.3

Table 1. MS/MS parameters for estrone and estradiol

Method

Urine Extraction

Initially a liquid/liquid extraction was performed with 2 mL urine, 5 mL methyl tert-butyl ether, and 60 µL of 17M KOH. The organic layer was removed, and evaporated to dryness with nitrogen. The sample was dissolved in 1 mL of HPLC grade water, and drawn through an LC18 solid phase extraction tube. The dichloromethane eluent was collected from the tube, evaporated to dryness with nitrogen, and redissolved in 1 mL of dichloromethane.

Extractive Alkylation

The 1 mL extracted urine was added to 1 mL tetrabutylammonium hydrogen sulfate, 50 µL of pentafluorobenzylbromide, and approximately 1 mL of 0.2M KOH to obtain a pH of between 8 and 10. The mixture was shaken for 20 minutes, then allowed to separate. The organic layer was collected, evaporated to dryness with nitrogen, and dissolved in 0.5 mL benzene.

Acylation of Estradiol

The benzene extract was added to 100 µL of 5% triethylamine/benzene(v:v) solution, and 50 µL heptafluorobutyric acid anhydride. Mixture was heated at 65°C for 15 minutes, and then the reaction was quenched with 1 mL of 5% ammonia solution. After the emulsion was allowed to settle out, the benzene layer was collected, evaporated to dryness with nitrogen, and redissolved in 1 mL toluene/dichloromethane (7:3) mix for analysis.

Results and Discussion

Selectivity was greatly enhanced with the use of MS/MS (Figures 1 and 3). The ability to determine the presence of estrone with full scan MS, and the comparison using MS/MS is dramatically displayed in Figures 2 and 4. In the full scan spectrum, the base peak, 269, of the estrone derivative is lost among all the matrix ions. With MS/MS, all the extraneous ions are removed using isolation waveforms, allowing for a clean product spectrum of the estrone derivative.

Negative CI MS - Juvenile urine spiked at 20 pg/µL

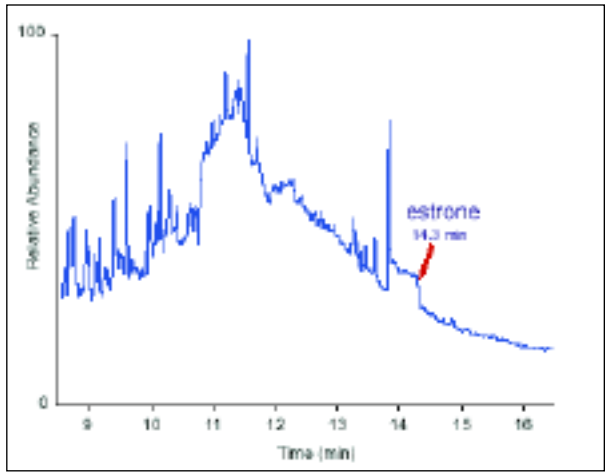

Figure 1: TIC of derivatized estrone in Full Scan Negative CI

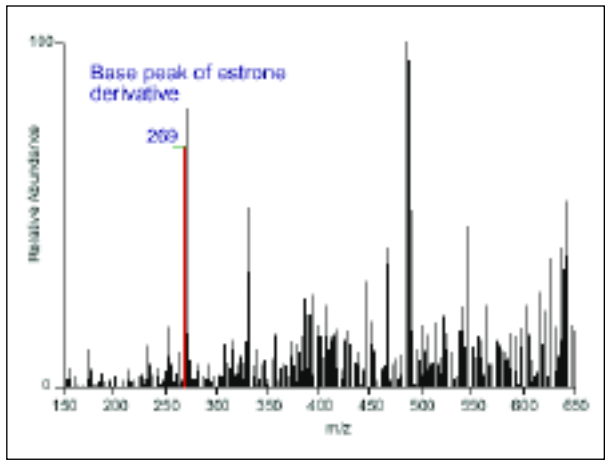

Figure 2: Background subtracted Negative CI spectrum of estrone in Full Scan showing high chemical background levels.

Negative CI MS/MS - Juvenile urine spiked at 20 pg/µL

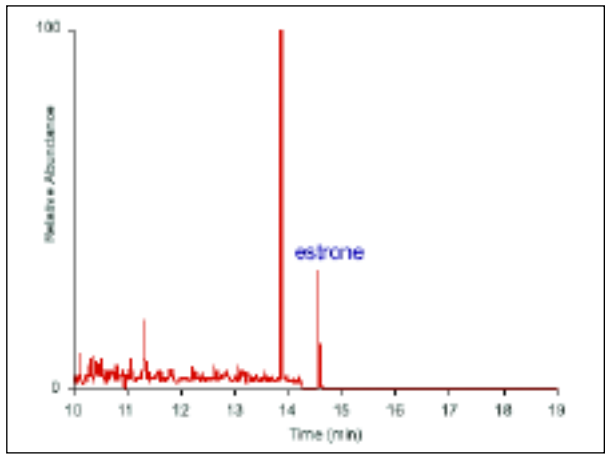

Figure 3: TIC of derivatized estrone in Full Scan Negative CI-MS/MS

Negative CI MS/MS - Juvenile urine spiked at 20 pg/µL

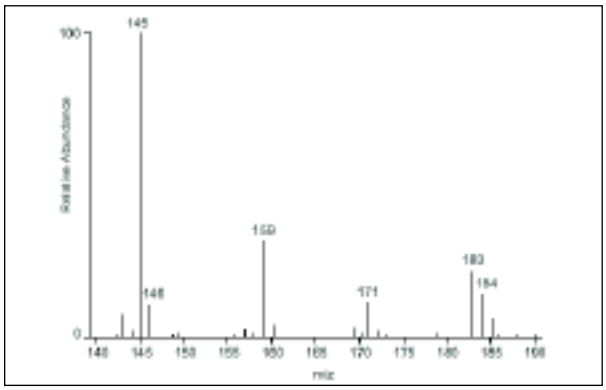

Figure 4: Negative CI MS/MS product ion spectrum of estrone derivative

Estrogen Compounds in Urine

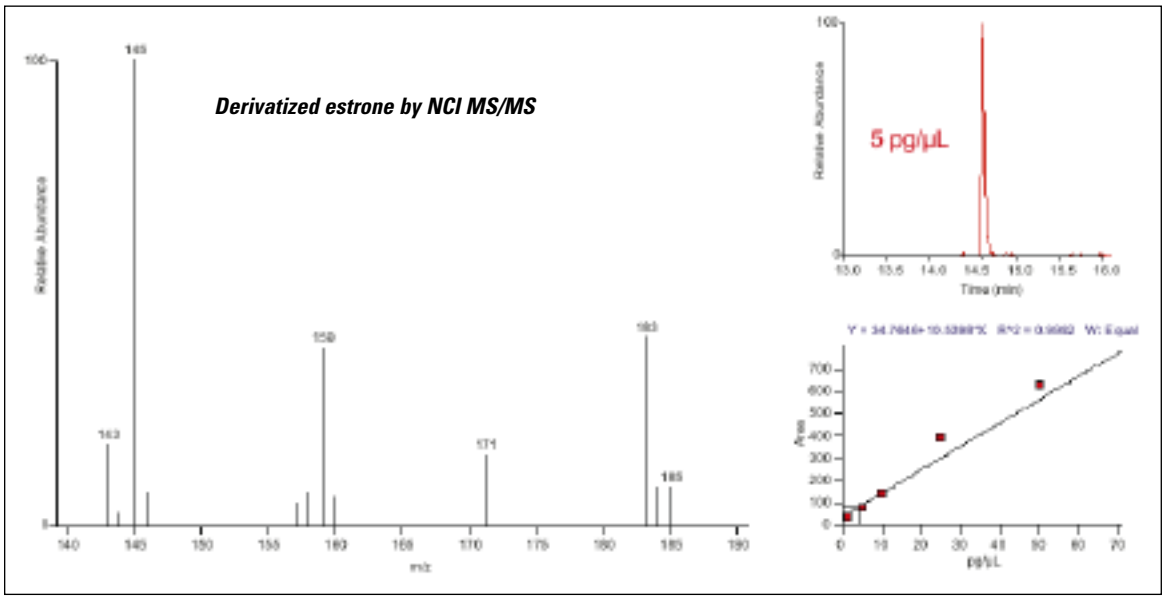

Figure 5. Mass spectrum, typical sensitivity, and linearity for derivatized estrone using Negative CI MS/MS

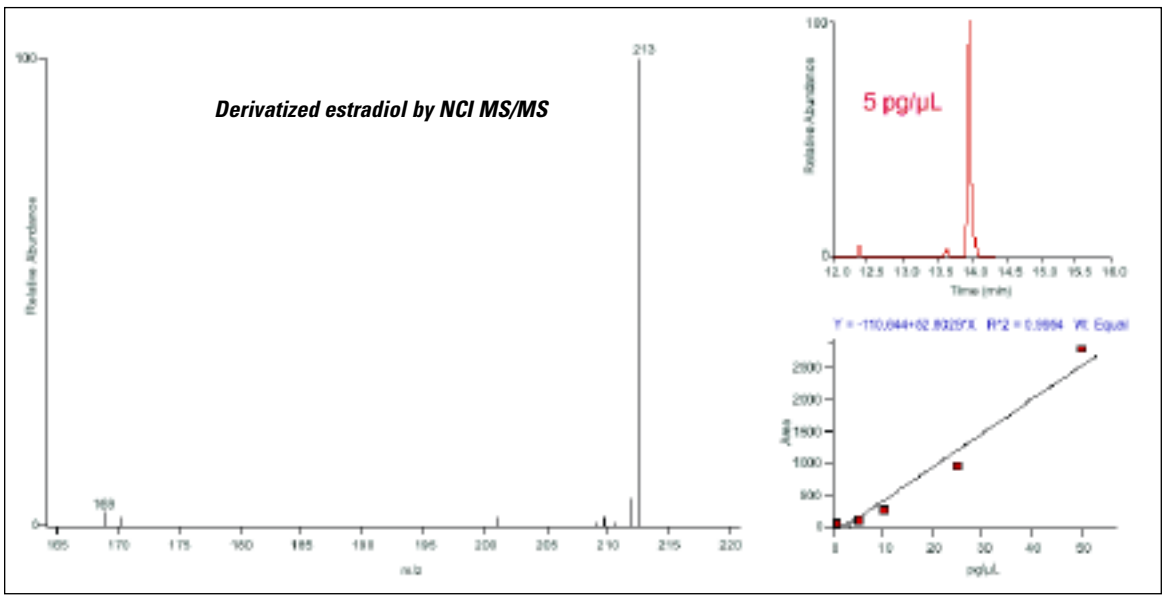

Figure 6. Mass spectrum, typical sensitivity, and linearity for derivatized estradiol using Negative CI MS/MS

Initial choices for derivatives were a series of anhydrides, due to their ease of use and complete reaction. Trifluoroacetic acid anhydride, pentafluoropropionic acid anhydride, and heptafluorobutyric acid anhydride, all reacted quantitatively with the estrogens, but elicited only an ion indicative of the derivative in NCI. Pentafluorophenyldimethylsilyl chloride (flophemesyl) was also tried and showed promising results, but was not pursued due to the amount of work required to optimize conditions. Pentafluorobenzylbromide reacted completely with the phenolic group common to both compounds, and gave an ion of M-1 for each. Unfortunately, the hydroxyl group still present on estradiol caused its peak to tail chromatographically, and so a subsequent derivatization step with HFBA was added (Figure 7).

This method was found to be linear within a range of 1 pg/µL to 100 pg/µL concentration split, or 500 pg/mL to 10 ng/mL concentration in urine (Figures 5 and 6).

Finally, additional increase in sensitivity was achieved by optimizing trap conditions (Part No. 120053-0001, Table 1, Table 2)[1].

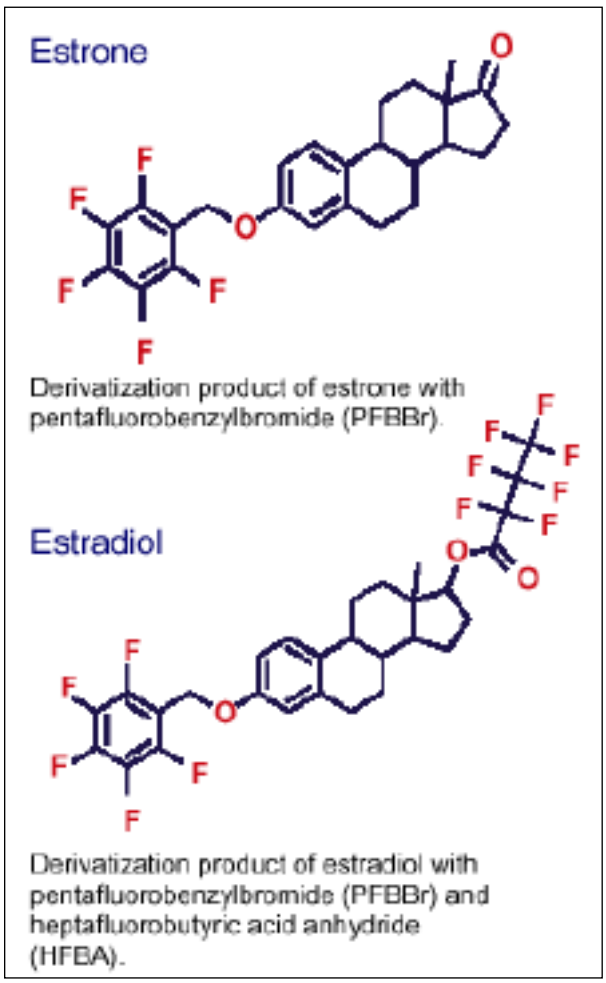

Figure 7: Derivatization scheme

Estrone derivative

TRAP PRESSURE	AVERAGE COUNTS	%RSD (N=3)	% INCREASE
1 mtorr	7388	23%	NA
5.4 mtorr	14089	9%	264%

Estradiol derivative

TRAP PRESSURE	AVERAGE COUNTS	%RSD (N=3)	% INCREASE
1 mtorr	23745	24%	NA
5.4 mtorr	62612	10%	191%

Table 2: MS/MS parameters for estrone and estradiol

Conclusion

- Choice of PFBBr with subsequent derivatization with HFBA for the hydroxyl function of estradiol, yields a compound that retains the charge on the estrogen core after ionization, allowing for confirmation.
- Method is linear within a range of 500 pg/mL to 500 ng/mL concentratration back to urine.
- Optimization of trap conditions resulted in a two-fold increase in sensitivity.

References

Grabic R., Novak J., Pacakova V.; "Optimi-zation of a GC-MS/MS Method for the Analysis of PCDDs and PCDFs in Human and Fish Tissue"; J. High Resol. Chromatogr. 23, 595-599 No. 10, 2000)

Acknowledgement

We would like to thank Ari Tutko for his contribution to this work.

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