



# Use of a High Resolution Accurate Mass Spectrometer to Expand the Scope of EPA Methods for Emerging Contaminants (EPA 539 and 537)

**October 2015**

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# Why Use Another Toy?



- We don't know what we don't know....
- At trace levels there is always a possibility of **false positives and false negatives**, because the existing methods don't require the same level of certainty as European LC-MS-MS methods.
- The Orbitrap has resolution of  $>70,000$ , allowing one to also watch for false positives and false negatives.
- It gives the ability to look for unknown unknowns.

# Evaluation of the Oribtrap for EPA 539 (Hormones) and 537 (PFCs)



- **Compare performance in Parallel Reaction Monitoring (PRM) mode to existing EPA methods**
- **Determine sensitivity**
- **Assess performance in full mass scan mode**
- **Look for additional library compounds**
- **Look for unknown unknowns**

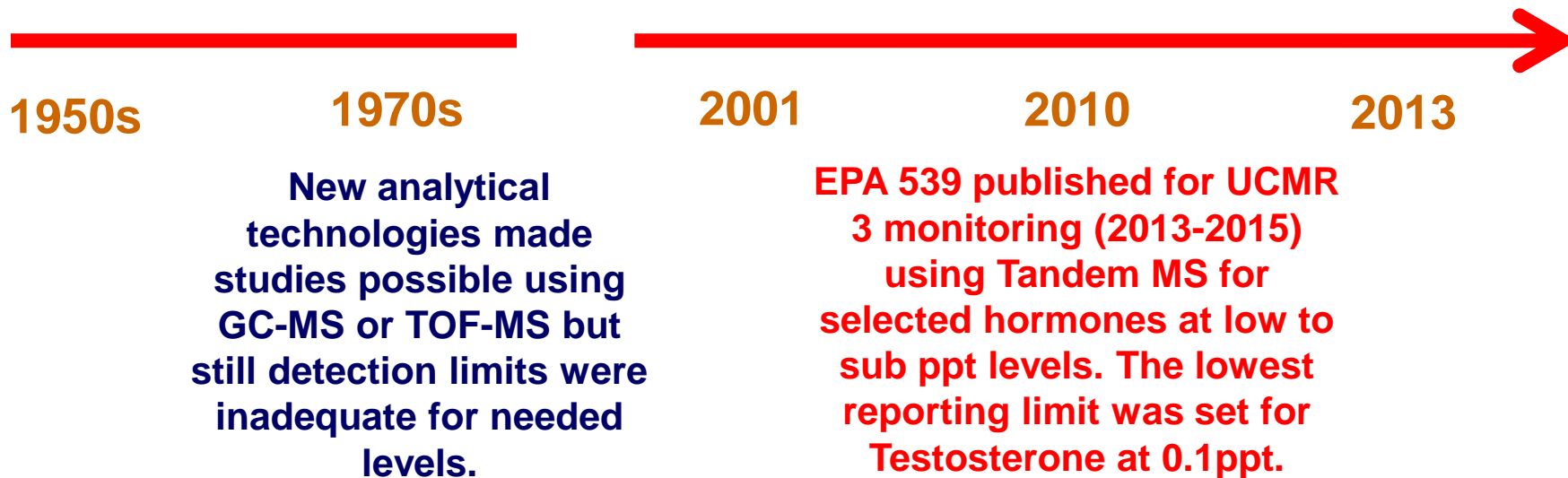
# Hormones in Water are NOT a New Concern



As the TOF-MS and GC-MS concepts were introduced at Harvard, scientists were already speculating on fate and transport of hormones in water streams.

**USGS; ES&T Article: Pharmaceuticals, Hormones, and Other Organic Wastewater Contaminants in U.S. Streams, 1999-2000 - some at low ppt levels, using LCMS as SIM for two of their 5 methods**

**Directive 2013/39/EU— Estrone, Estradiol and Ethynylestradiol at 1.0, 0.3 and 0.03 ppt in water streams such as effluents**

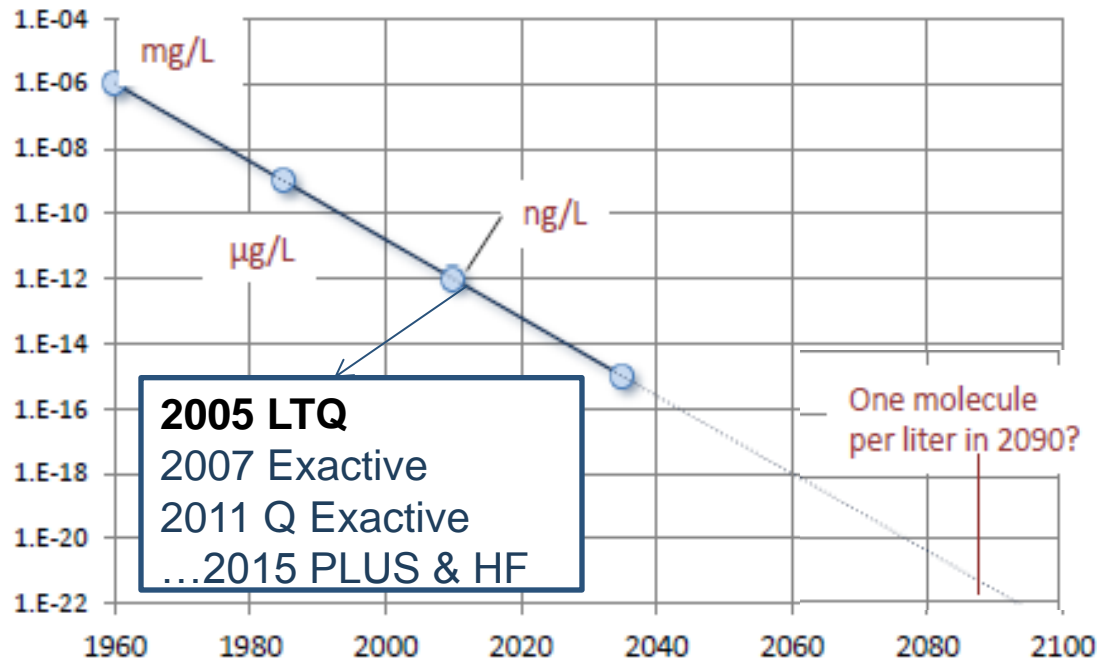


# 2011 Q Exactive: a Hybrid HRAM is Added to the Orbi Exactive Family



- The fastest-growing area of technology in water is analytical technology

### History of Detection Limits



Moore's Law:  
No. of transistors on  
A microchip doubles  
Every 2 years

A New Law:  
Detection Limit for TOrCs  
doubles (drops 2-fold)  
every 2.5 years

# EPA 539 Summary - Mass Spectrometer Requirements



**Summary :** A 1 Liter dechlorinated sample with Omadine is extracted with SPE Octadecyl (C-18) functional group after adding surrogates. The SPE eluent is concentrated to dryness, and diluted to 1 ml with 50/50% MeOH/H<sub>2</sub>O. **An aliquot is injected to the LC-MS/MS after adding IS and quantified against the IS.**

**3.16 MULTIPLE REACTION MONITORING (MRM)** – A mass spectrometric technique in which a precursor ion is isolated, then fragmented into a product ion(s). **Quantitation is accomplished by monitoring a specific product ion.** MS parameters must be optimized for each precursor ion (Sect. 3.17) and product ion (Sect. 3.19).

**6.12.3 ELECTROSPRAY IONIZATION-TANDEM MASS SPECTROMETER (ESI-MS/MS)** – The MS must be capable of **rapid switching between negative ion and positive ion electrospray ionization modes.** The system must be capable of performing MS/MS to produce unique product ions for the method analytes within specified retention time segments. At least 10 – 15 scans across the chromatographic peak are needed to ensure adequate precision.

**The Orbitrap™ Technology meets these requirements**

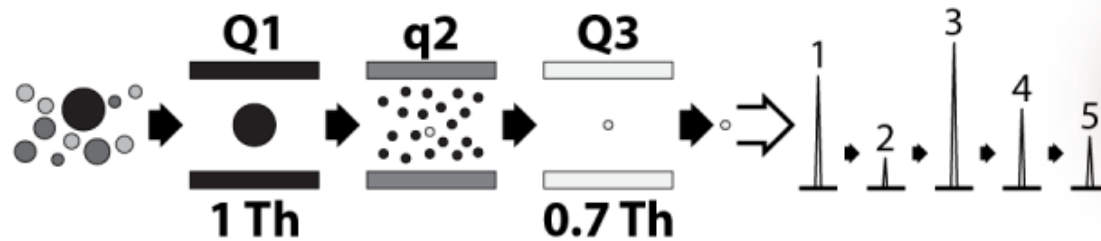
# EPA 539 Is Written For LC-MS/MS so the QE PRM Mode Is Truest To The Method



## Quadrupole-equipped HR/AM instruments

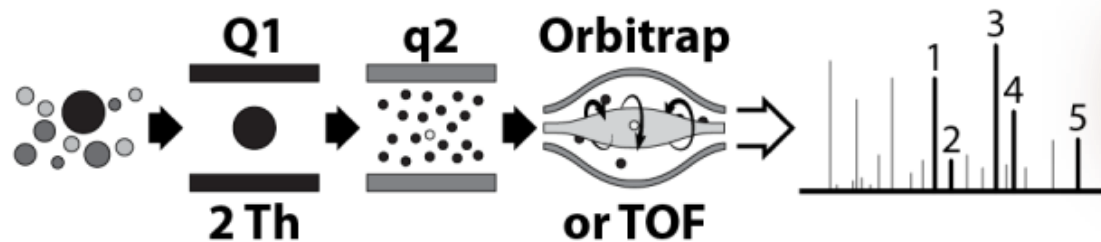
HR/AM analyzer permits parallel detection of all target product ions in one concerted high resolution mass analysis

### A SRM



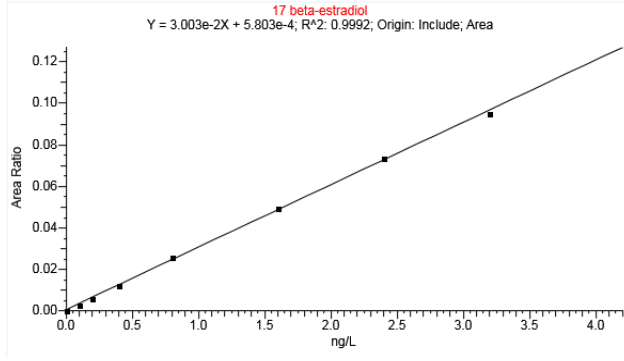
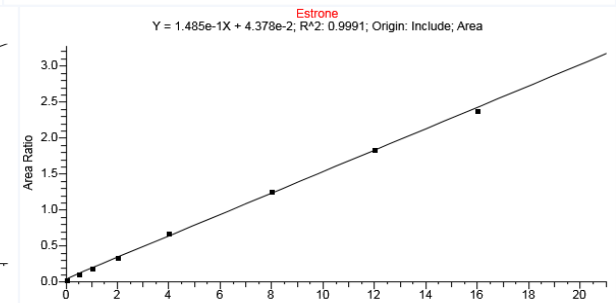
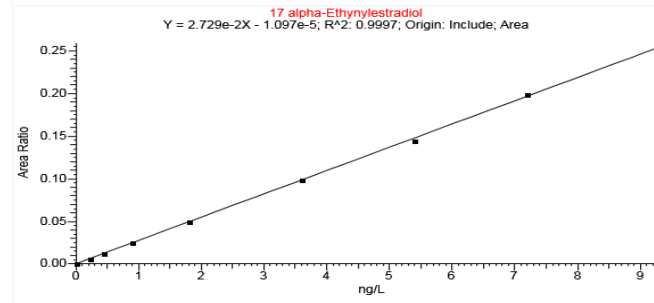
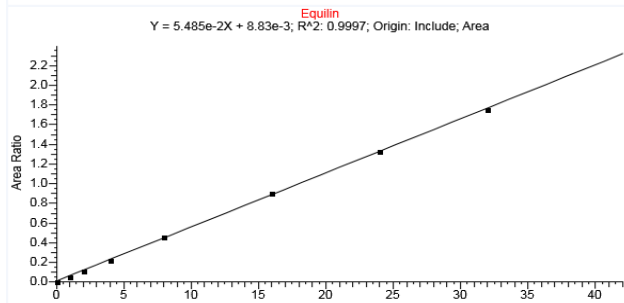
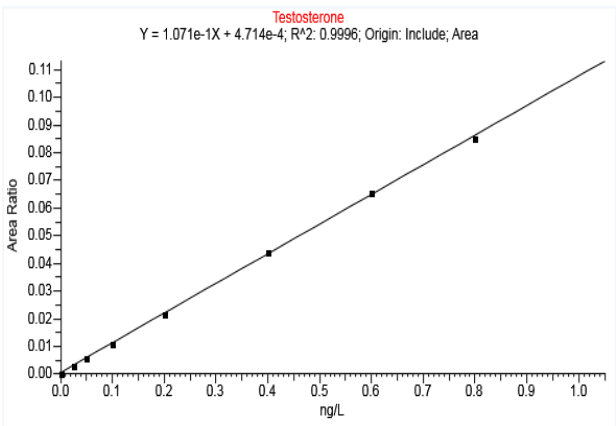
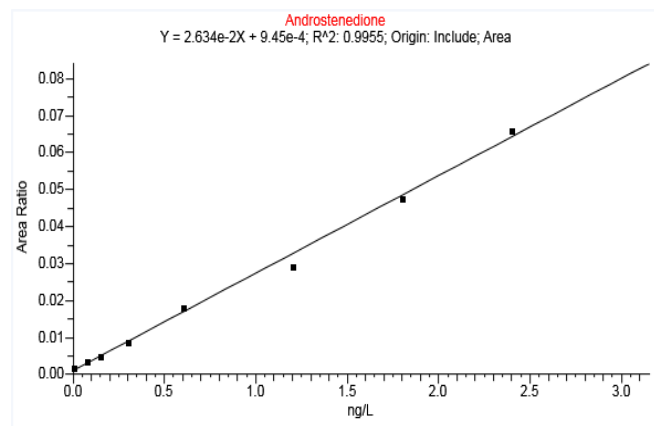
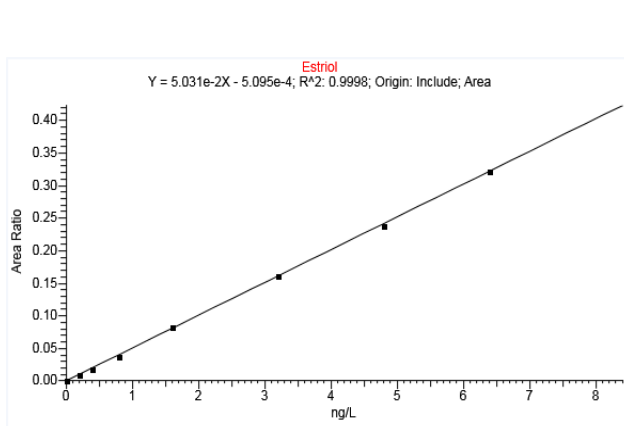
*Serial* monitoring

### B PRM



*Parallel* monitoring

# All Calibration Coefficients > 0.99 and Good Dynamic Range and Linearity



▲

**EE2 range 0.225 – 7.2ppt**



# An Example of a Calibration for EE2 Using TraceFinder 3.2

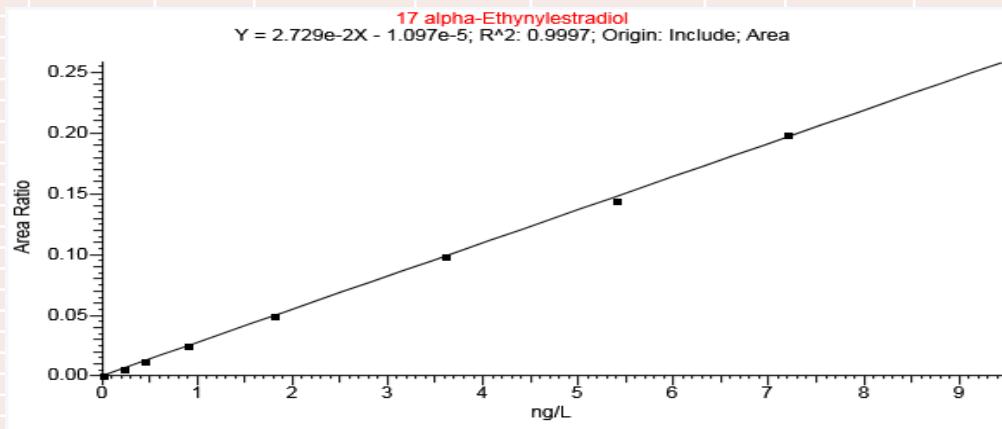


## Compound Calibration Report

Lab Name: EEA  
 Instrument: Q EXACTIVE  
 User: Thermo  
 Batch: 021215-539-PRM-IDOC-LINEAR

Method: 021215-539-PRM-IDOC-LINEAR\_EPA-539-PRM  
 EPA-539-PRM  
 Cali File: 021215-539-PRM-IDOC-LINEAR.calx

Compound Name: 17 alpha-Ethynylestradiol



Linear  
 Pass

Level	Std Amount	Std Area	IS Amount	IS Area	Resp factor/ ratio	Calc Amount	Units	% CV	% RSD
0	0	419	100	9244925	0	0.002	ng/L	N/A	N/A
ICAL-1	0.225	63224	100	10063239	0.006	0.231	ng/L	N/A	N/A
ICAL-2	0.45	110807	100	9324158	0.012	0.436	ng/L	N/A	N/A
ICAL-3	0.9	243598	100	9755408	0.025	0.915	ng/L	N/A	N/A
ICAL-4	1.8	491433	100	9920757	0.05	1.815	ng/L	N/A	N/A
ICAL-5	3.6	942018	100	9557799	0.099	3.612	ng/L	N/A	N/A
ICAL-6	5.4	1415265	100	9788761	0.145	5.298	ng/L	N/A	N/A
ICAL-7	7.2	1850842	100	9334500	0.198	7.266	ng/L	N/A	N/A

# Determination of Minimum Reporting Limit (MRL) using LCMRL calculation



## Enter Test Data

**NB – Calculator only works with 32 bit computers.**

Contaminant - 17 alpha-Ethynylestradiol

Method - EPA 539

Units for all measurements - Nanograms/Liter (ng/L)

**\*\*Important\*\* Please do not truncate or round any of the results. If possible, results should have a minimum of 3 significant digits.**

Use the buttons below to add a concentration (column) or measured value (row). To remove a row or column check the box next to it and click the Remove button.

Add Concentration

Add Measurement

Remove



	Concentrations							
	<input type="checkbox"/> Conc. 1	<input type="checkbox"/> Conc. 2	<input type="checkbox"/> Conc. 3	<input type="checkbox"/> Conc. 4	<input type="checkbox"/> Conc. 5	<input type="checkbox"/> Conc. 6	<input type="checkbox"/> Conc. 7	<input type="checkbox"/> Conc. 8
	0.225	0.45	0.9	1.8	3.6	5.4	7.2	0
Measured Concentrations								
<input type="checkbox"/> Value 1	0.184	0.347	0.684	1.361	2.657	4.469	6.07	0.003
<input type="checkbox"/> Value 2	0.184	0.35	0.728	1.365	2.819	4.551	5.814	0.000
<input type="checkbox"/> Value 3	0.202	0.36	0.705	1.363	2.66	4.323	6.046	0.000
<input type="checkbox"/> Value 4	0.174	0.4	0.534	1.362	2.987	4.207	5.563	0.000

[http://water.epa.gov/scitech/drinkingwater/labcert/analyticalmethods\\_ogwdw.cfm](http://water.epa.gov/scitech/drinkingwater/labcert/analyticalmethods_ogwdw.cfm)

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Next >>

The LCMRL is defined as the lowest spiking concentration at which recovery of between 50 and 150 percent is expected 99 percent of the time by a single analyst. The procedure requires, at a minimum, four replicates at each of seven fortification levels. Four laboratory reagent blanks should also be included. All must be processed through the entire method procedure

# Evaluating the QE in Parallel Reaction Mode (PRM) for EPA 539



## ➤ Initial Demonstration of capability (IDC) from the method:

- 1. DEMONSTRATION OF LOW SYSTEM BACKGROUND <math>< 1/3 \text{ MRL}</math>
- 2. DEMONSTRATION OF PRECISION – 4-7 LFB's RSD <math>\leq 20\%</math>
- 3. DEMONSTRATION OF ACCURACY – LFB's recovery diff. <math>\leq 30\%</math> of T.V.
- 4. MINIMUM REPORTING LEVEL (MRL) CONFIRMATION or doing **LCMRL**

## ➤ Targeted compounds and their MRLs:

Analyte	DL Fortified Concentration (ng/L)	DL (ng/L)	<b>LCMRL (ng/L)</b>
Estriol	1.10	0.24	<b>0.28</b>
Estrone	1.05	0.19	<b>4.0</b>
17 $\beta$ -Estradiol	1.30	0.39	<b>0.32</b>
17 $\alpha$ -Ethinylestradiol	1.75	0.33	<b>1.3</b>
Androstenedione	0.50	0.20	<b>0.37</b>
Testosterone	0.50	0.04	<b>0.062</b>
Equilin	1.25	2.94	<b>3.0</b>

# LCMRL < 0.19 ppt for EE2. It Could Not Be Calculated Because It Needed a Lower Point



## Test Results

Results for: 17 alpha-Ethynylestradiol

Number of true concentrations: 8

Total number of true measurements: 32

### Results

Adj. R squared :

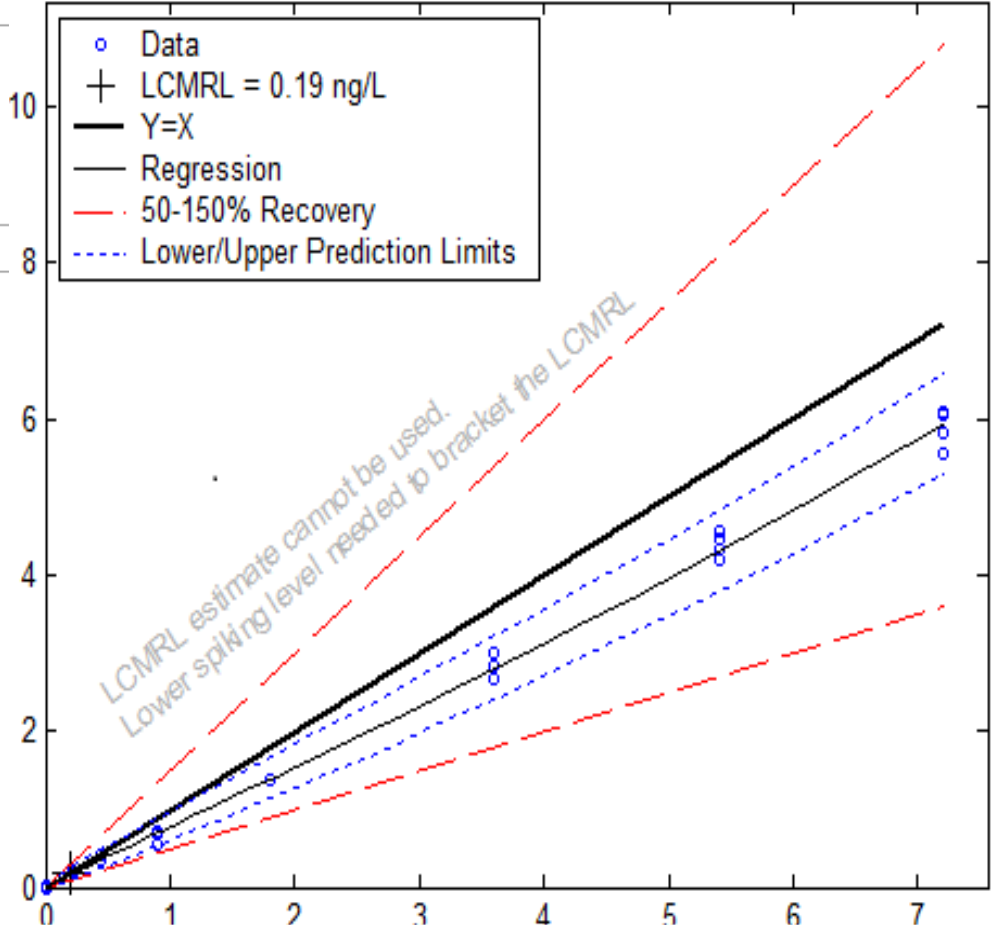
The LCMRL cannot be determined due to the following reason:  
Lower spiking level needed to bracket the LCMRL

DL: 0.10 ng/L

Critical Level : 0.051 ng/L

**0.05ppt as critical level**

17 alpha-Ethynylestradiol-LCMRL Plot



# Area and Height of the Lowest CAL Std for EE2 – Using Trace Finder 3.2



## Can Directive 2013/39/EU be met at 0.03ppt based on Area & Height ?

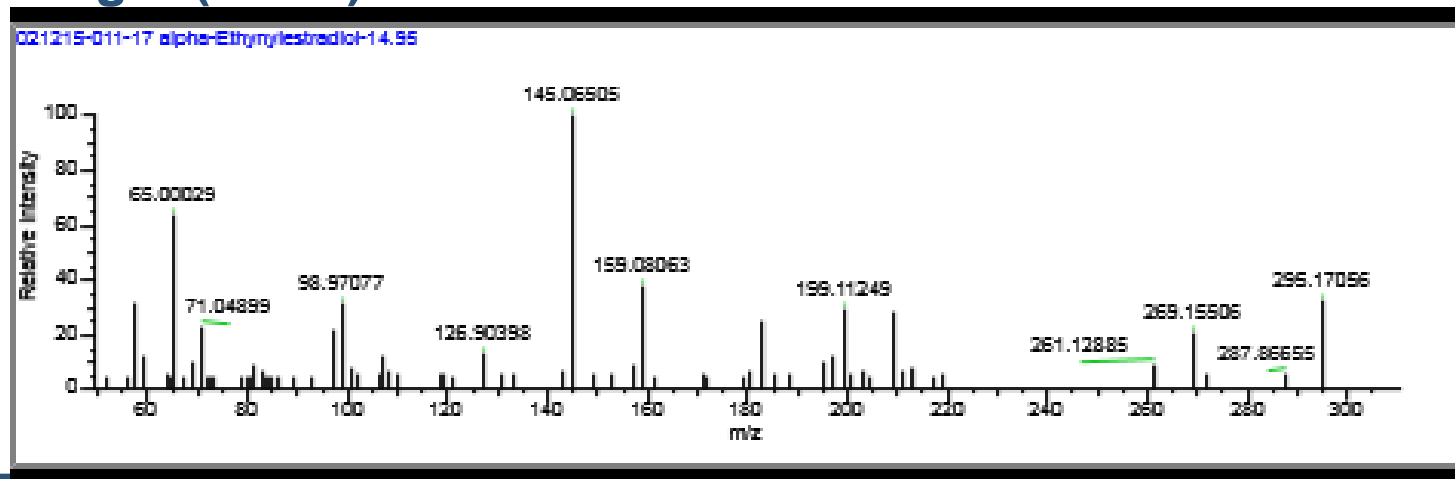
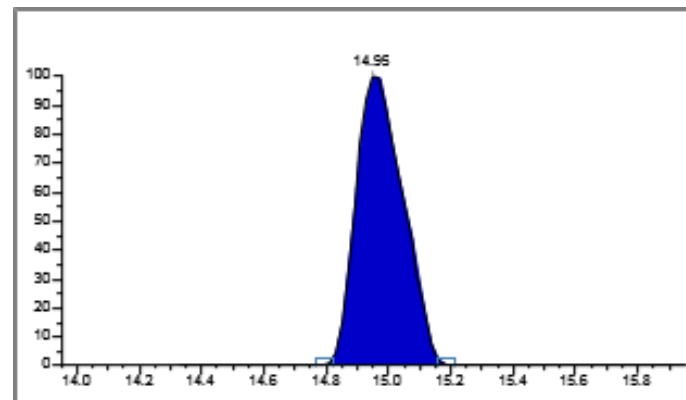
Compound Name: 17 alpha-Ethynylestradiol

Sample Conc: 0.225 ng/L

Retention time: 14.96

Area (Quan): 61535

Height (Quan): 5855



# HRAM is More Sensitive than the Published LCMRL for EPA539



Extraction:	Concentrated <b>500 ml</b> of solutions to final 1 ml - per EPA 539 preservatives/extraction procedure		
Instrument:	Q- Exactive, hybrid quadrupole Mass Spectrometry HRAM, Resolution for collected data : <b>70000 (mz 200 FWHM)</b> AGC 2e5 Max IT 200 ms Isolation window 1.0 m/z		
Scans/peak	collected >10-15 scans per peaks		
HPLC:	Thermo RS Ultimate UHPLC 3000, binary pump, autosampler and column heater with 100 ul sample loop		
Column:	Acclaim Polar Advantage II, 2.1x150 mm, 3 um, 120A		
Eluents:	A) 1 mM Ammonium Fluoride B) 50%/50% ACN/MeOH	Gradient flow at 0.3ml/min with a 21.4 minutes run.	
Injection volume:	50 ul		



Method 539 UCMR3 Analyte	UCMR MRL (ng/L)	EPA 539 published LCMRL (ng/L)	Q Exactive HRAM 70000 Res. LCMRL (ng/L)	QE-LCMRL Calc -DL (ng/L)
17 $\alpha$ -ethynylestradiol	0.9	1.3	<b>Critical level 0.05</b>	0.1
17 $\beta$ -estradiol	0.4	0.32	0.17	0.047
equilin	4	0.28	<b>Critical level 0.23</b>	0.48
estriol	0.8	3	0.27	0.2
estrone	2	4	0.84	0.48
testosterone	0.1	0.062	0.033	0.027
androstenedione	0.3	0.37	0.19	0.08

# Precision and Accuracy for LFB (10xMRL) and a Spiked Sample at MRL



## QE-HRAM-MS2 70000 resolution PRECISION AND ACCURACY IN FORTIFIED REAGENT WATER (n=4)

Analyte	Fortified Concentration (ng/L)	Avg. %Recovery	%RSD
17 $\alpha$ -ethynylestradiol	7.2	82%	4
17 $\beta$ -estradiol	3.2	84%	3
equilin	32	81%	3
estriol	6.4	100%	4
estrone	16	83%	4
testosterone	0.8	87%	5
4-androstene-3,17-dione	2.4	85%	8

## QE-HRAM-MS2 70000 resolution PRECISION AND ACCURACY IN Sample 20150108002 (2) Low Level spike

Analyte	Fortified Concentration (ng/L)	Avg. %Recovery	%RSD
17 $\alpha$ -ethynylestradiol	0.72	95%	2.3
17 $\beta$ -estradiol	0.32	87%	0.6
equilin	3.2	92%	8.2
estriol	0.64	101%	4.0
estrone	1.6	95%	3.4
testosterone	0.08	99%	0.1
4-androstene-3,17-dione	0.24	118%	0.1

# Continued: Spiked Field Samples at Reporting Levels. Samples Had No Hits.



## Spike recovery at the MRL is a truer measure of performance than the LCMRL.

QE-HRAM-MS2 70000 resolution PRECISION AND ACCURACY IN Sample 20150108002 (2) Low Level spike

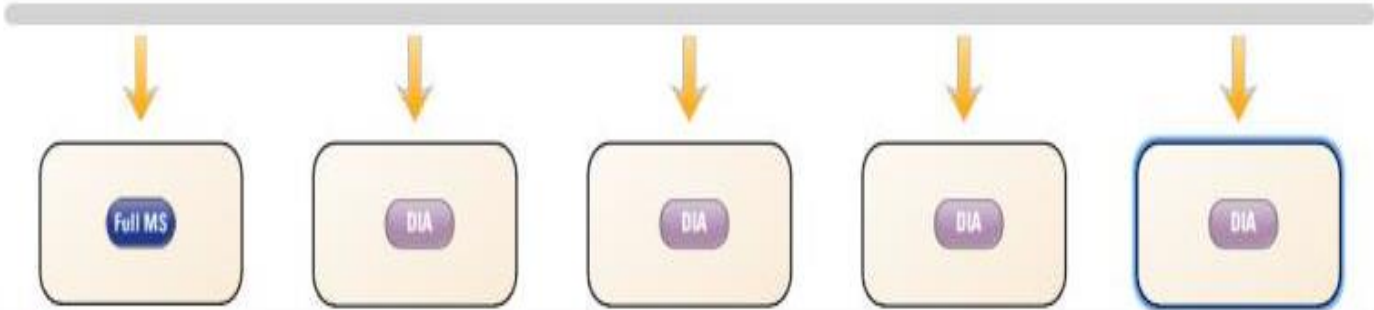
Analyte	Fortified Concentration (ng/L)	Avg. %Recovery	%RSD
17 $\alpha$ -ethynylestradiol	0.72	95%	2.3
17 $\beta$ -estradiol	0.32	87%	0.6
equilin	3.2	92%	8.2
estriol	0.64	101%	4.0
estrone	1.6	95%	3.4
testosterone	0.08	99%	0.1
4-androstene-3,17-dione	0.24	118%	0.1

QE-HRAM-MS2 70000 resolution PRECISION AND ACCURACY IN Sample 201501090001 (2) Low Level spike

Analyte	Fortified Concentration (ng/L)	Avg. %Recovery	%RSD
17 $\alpha$ -ethynylestradiol	0.72	97%	16
17 $\beta$ -estradiol	0.32	95%	13
equilin	3.2	92%	11
estriol	0.64	107%	13
estrone	1.6	95%	14
testosterone	0.08	97%	19
4-androstene-3,17-dione	0.24	104%	20

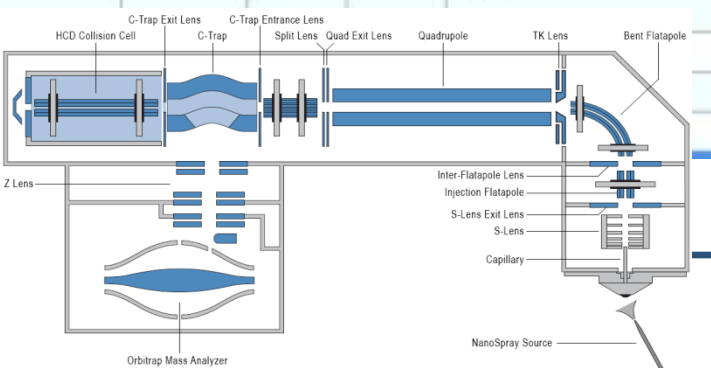


# Look For Non Targeted And Unknowns While Simultaneously Quantifying 539



File	Edit	Help	Mass [m/z]	Formula [M]	Species	CS [z]	Polarity	Start [min]	End [min]	(N)CE	MSX ID
1			125.00000				Negative				
2			175.00000				Negative				
3			225.00000				Negative				
4			275.00000				Negative				
5			325.00000				Negative				
6			375.00000				Negative				
7			287.16527	C18H24O3	-H	1	Negative			75	Estriol
8			425.00000				Negative				
9			475.00000				Negative				
10			700.00000				Negative				
11			900.00000				Negative				

**DIA # 1: 50 amu isolation window, loop 6**  
**DIA # 2: Estriol with isolation window 1 amu, NCE 75**  
**Loop 1**  
**DIA # 3: 50 amu isolation window loop 1**  
**DIA # 4 : 200 amu isolation window loop 2**



Why add a PRM for estriol?

# Estriol Problematic In Full-MS (70K) Method. No Signal for Cal1&2 (0.2 & 0.4ppt) Stds



Thermo TraceFinder EFS LC

File View Tools Help

Real time status | User: Thermo | ?

Analysis

Data Review - 031515-DIA-10-30-60-NCE-NEG\*

Flags	Compound	Expected RT	Compound Type
1	13C2-Ethynylestradiol	13.25,16.93	Internal Standard
2	13C6-Estradiol	12.29	Internal Standard
3	17 alpha-Ethynylestradiol	13.25	Target Compound
4	17 beta-estradiol	12.29	Target Compound
5	Androstenedione	8.95	Target Compound
6	Equilin	10.05,10.77	Target Compound
7	Estriol	6.44	Target Compound
8	Estriol-d2	6.36	Internal Standard
9	Estrone	10.77	Target Compound
10	Ethynylestradiol-d4	13.19	Surrogate
11	Testosterone	10.01	Target Compound
12	Testosterone-d3	10.01	Internal Standard

Acc	Flags	Flag Details	Status	Filename	Sample Type	Level	Sample ID	Sample Name	Comments	Height	Area	Expected RT	Actual RT	RT Delta	Formula
1	<<CF			031515-001 Unknown	SOLVENT3	SOLVENT3				N/F	N/F	6.44	N/F		
2	<<CF			031515-002 Cal Std	0	0				N/F	N/F	6.44	N/F		
3	<<CF			031515-003 Cal Std	ICAL-1	ICAL-1				N/F	N/F	6.44	N/F		
4	<<CF			031515-004 Cal Std	ICAL-2	ICAL-2				N/F	N/F	6.44	N/F		
5				031515-005 Cal Std	ICAL-3	ICAL-3				937	6569	6.44	6.28	-0.16	
6				031515-006 Cal Std	ICAL-4	ICAL-4				4693	35328	6.44	6.25	-0.19	
7				031515-007 Cal Std	ICAL-5	ICAL-5				9393	77038	6.44	6.27	-0.17	
8				031515-008 Cal Std	ICAL-6	ICAL-6				13686	120166	6.44	6.28	-0.16	
9				031515-009 Cal Std	ICAL-7	ICAL-7				18737	168055	6.44	6.25	-0.19	
10				031515-010 Chk Std	MRL_CHK	CAL-2.5				N/A	N/A	6.44	N/A		
11				031515-011 Chk Std	QCS	QCS				N/A	N/A	6.44	N/A		
12				031515-012 Unknown	MBLK	MBLK				N/A	N/A	6.44	N/A		
13				031515-013 Unknown	2.5-1	2.5-1				N/A	N/A	6.44	N/A		

Compound Details

Quan Peak

Estriol RT: 6.28 | 031515-005

RT: 6.28  
AA: 6568.79  
A4: 937.43  
SN: INF

Apex RT: 6.28  
Area: 6569

m/z: 171.08080

ICAL-3 = MRL

Calibration Curve

Estriol

$Y = -3.292e-3X^2 + 8.449e-2X - 4.281e-2$ ;  $R^2: 0.9941$ ; Origin: Ignore; W: 1X; Area

Area Ratio

ng/L

Spectra

MS-Data

Relative Intensity

m/z

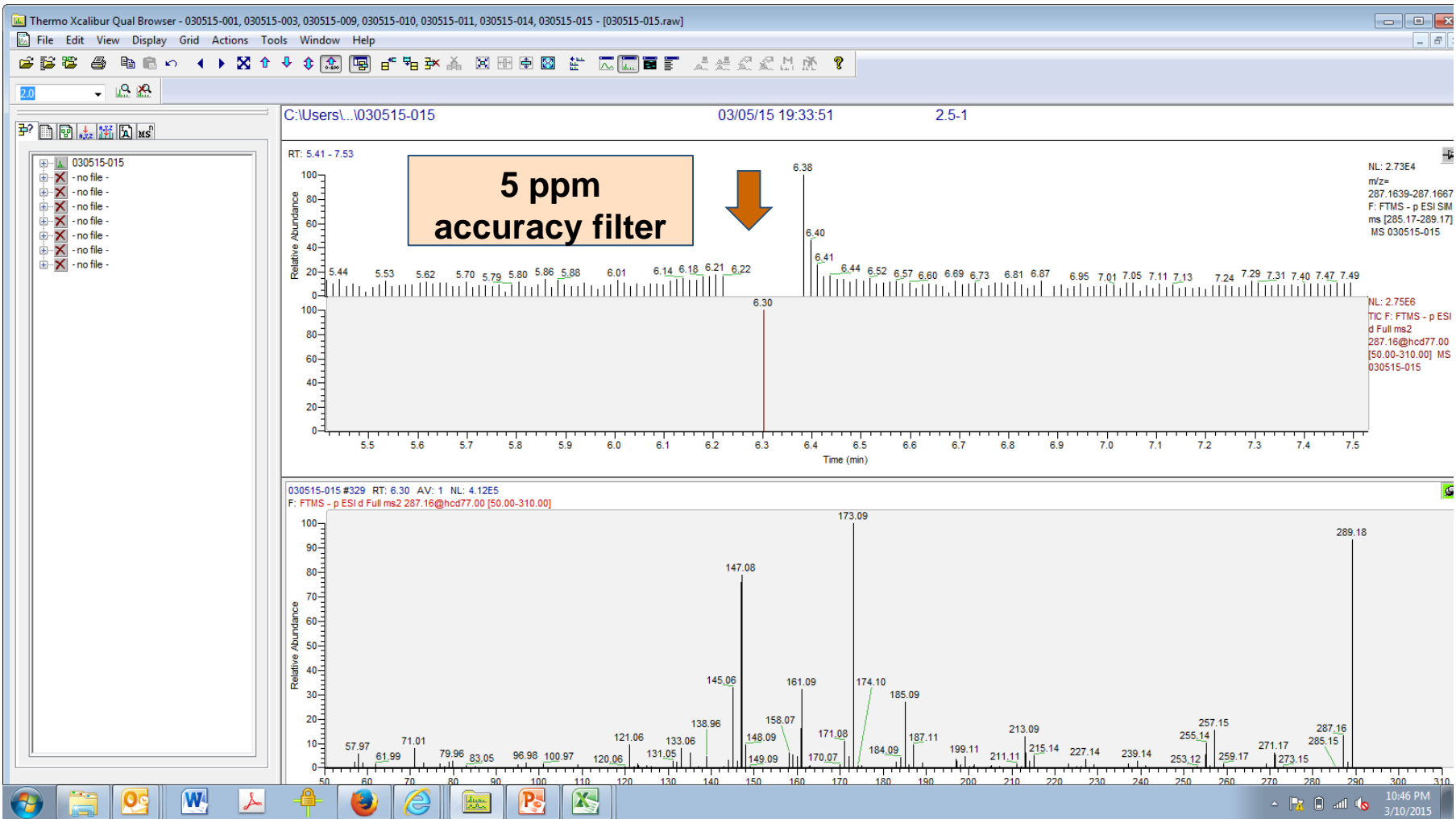
(171.079666137695, 2702.9716796875)

MS-Reference

Relative Intensity

m/z

# Estriol At Ultra Low Concentrations Shows Selectivity Issues @ 70000 res(TSIM/ddMS2)



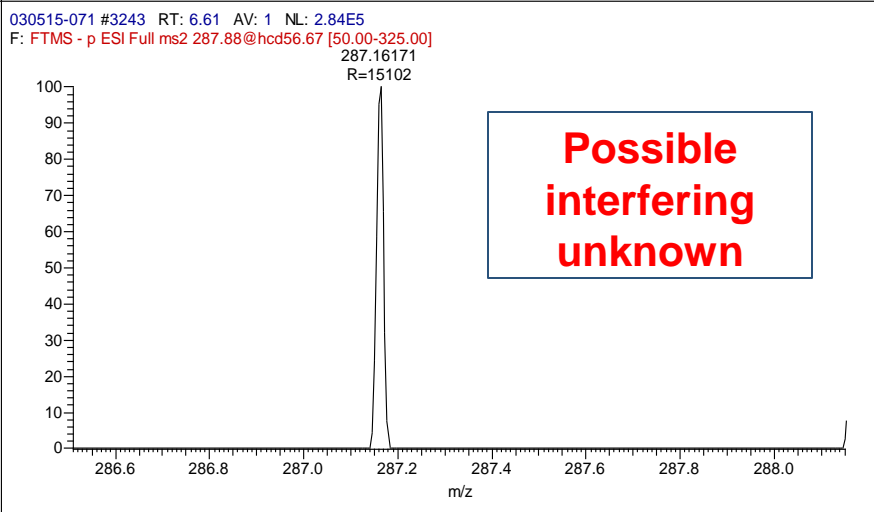
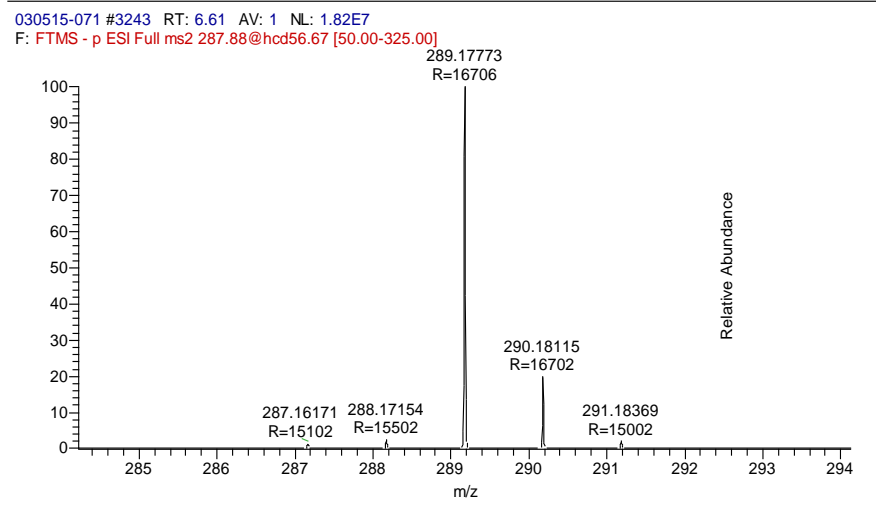
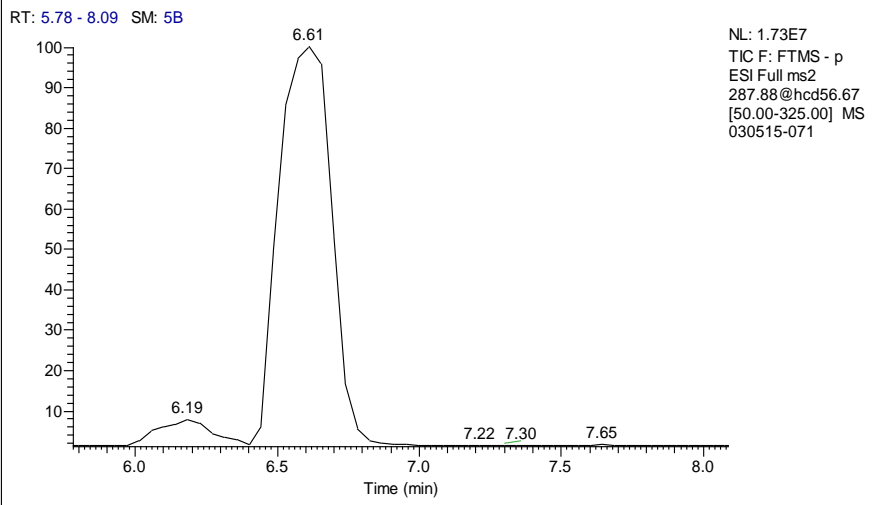
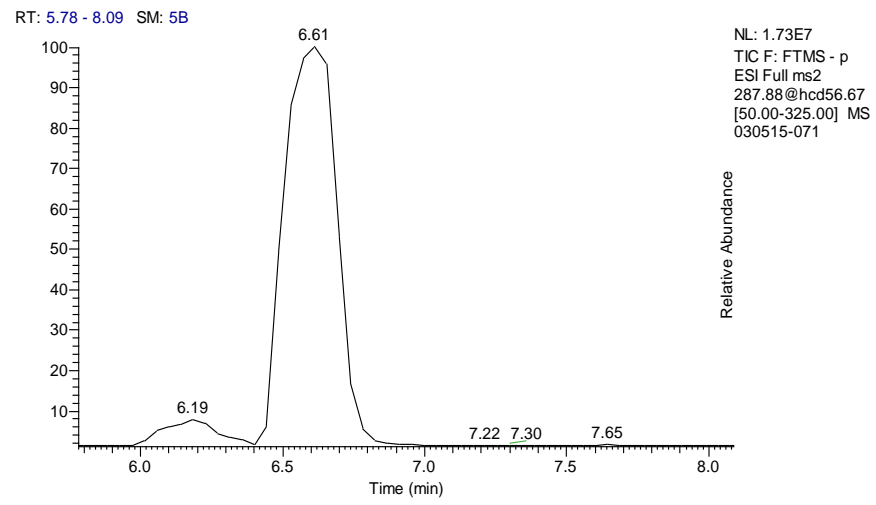
# Internal Std Estriol-d2 Spiked At 10x In DI 289.7773 AMU + Contaminated Interference 287.16171 as a Suspect for Non Selectivity >10ppm For Estriol 287.16528



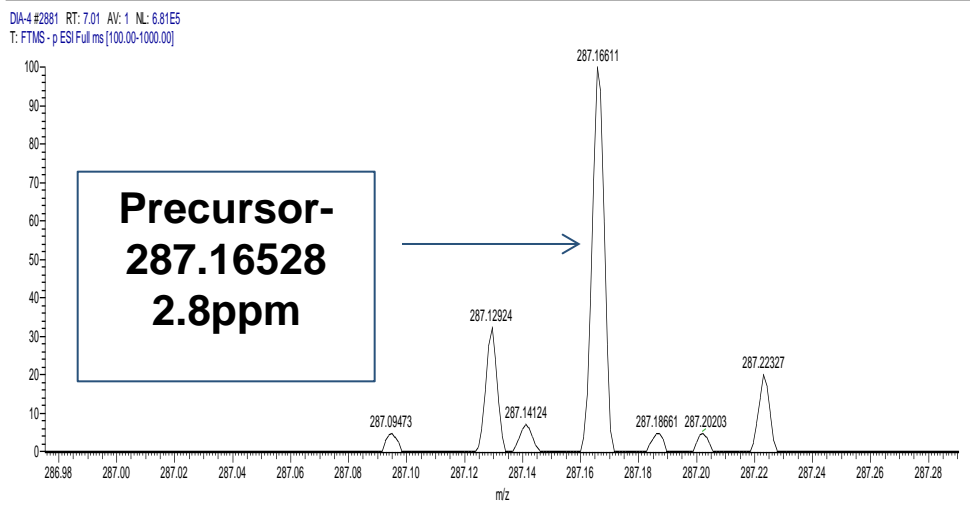
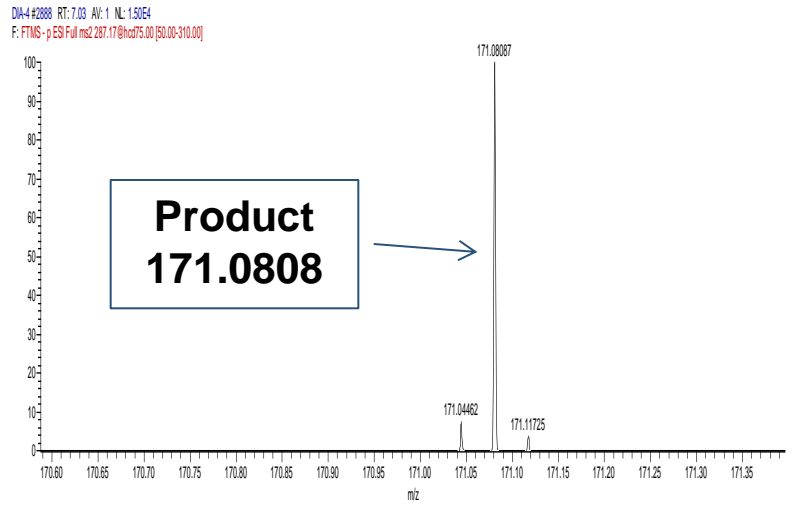
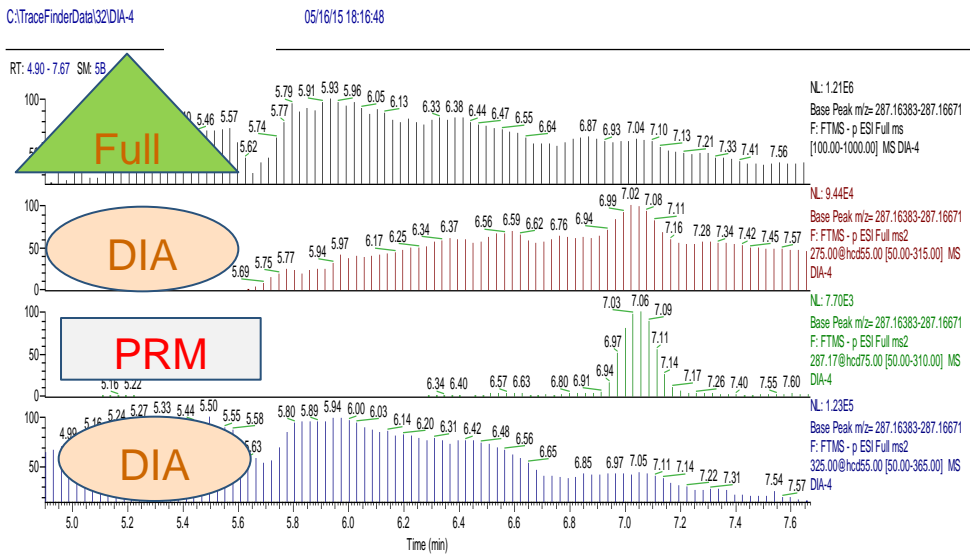
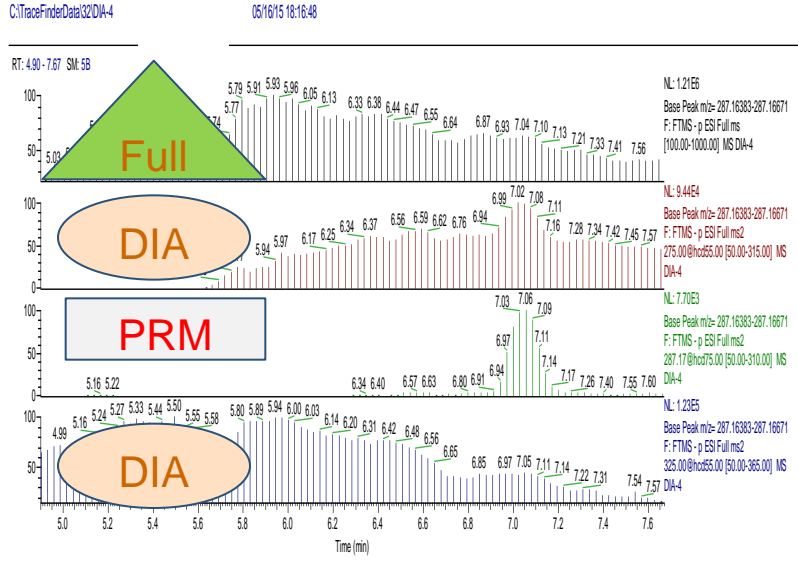
C:\TraceFinderData\...Data\030515-071

03/25/15 20:59:58

IS



# Full MS Does Not See 287.16528. DIA/PRM Sees Precursors In MS2 + Product Ion: Spiked Sample At MRL



# Estriol Lowest Standard Using Trace Finder

## 3.2 Spectral Library Match / Identified



Thermo TraceFinder EFS LC

File View Tools Help

Real time status | User: Thermo

Analysis

Data Review - 051815-DIA-PRM\*

Batch View

- Samples
- Reference Sample
- Threshold Samples
- Data Review
- Sample View
- Compound View
- Comparative View
- Qualitative View

Report View

- Local Method
- Acquisition
- Quantitation
- Processing
- Compounds
- QAQC
- Groups
- Reports

Acquisition

Analysis

Method Development

Compounds

Flags	Compound	Expected RT	Compound Type
5	Equilin	10.05,13.12	Target Compound
6	Estriol	7.55	Target Compound
7	Estriol-d2	7.61	Internal Standard
8	Estrone	13.60	Target Compound

Sample Results

Act	Flags	Flag Details	Status	Filename	Sample Type	Level	Sample ID	Sample Name	Theoretical Amt	Calculated Amt	Comments	Height	Area	Expected RT	Act
1	1	<<,CF	●	051815-001 Unknown	SOLVENT1			SOLVENT1	N/A	N/F		N/F	N/F	7.55	N/
2	2	<<,CF	●	051815-002 Unknown		0	0		N/A	N/F		N/F	N/F	7.55	N/
3	3		●	051815-003 Cal Std	ICAL-1		ICAL-1	ICAL-1	0.200	0.187		5193	43085	7.55	7.5

Compound Details

Quan Peak

Estriol RT: 7.54 | 051815-003 1/1

RT: 7.54  
AA: 43085.06  
AH: 5192.89  
SN: INF

Relative Intensity

Apex RT: 7.54  
Area: 43085

Calibration Curve

Estriol

$Y = -7.561e-6X^2 + 3.514e-3X - 3.082e-5$ ;  $R^2: 0.9998$ ; Origin: Ignore; W: 1/X; Area

Area Ratio

ng/L

Library Match

#1: Estriol 77

051815-003 # 2996 RT: 7.54  
F: FTMS - p ESI Full ms2 287.17@hcd75.00 [50.00-310.00]

Relative Intensity

m/z

# Full-ms/DIA Gives The Same Result As PRM Experiment. Precision/Accuracy Of LFB And Matrix Spikes Matched Very Closely To PRM Experiment.



Extraction:	Concentrated 500 ml of solutions to final 1 ml - per EPA 539 preservatives/extraction procedure					
Instrument:	Q- Exactive, hybrid quadrupole Mass Spectrometry HRAM,				Q- Exactive, hybrid quadrupole Mass Spectrometry HRAM,	
	Resolution for collected data : 70000 (mz 200 FWHM)				Full-MS - Resolution for collected data : 70000 (mz @200 FWHM)	
	Isolation window 1.0 m/z				DIA experiment one 100-520 60 amu isolation window, loop 7	
					DIA experiment #2 510-1100 200 amu isolation window, loop 2	
HPLC:	Thermo RS Ultimate UHPLC 3000,, binary pump, autosampler and column heater with 100 ul sample loop				Step NCE 35, 80	
Column:	Acclaim Polar Advantage II, 2.1x150 mm, 3 um, 120A					
Eluents:	A) 1 mM Ammonium Fluoride B) 50%/50% ACN/MeoH					
	Gradient flow at 0.3ml/min with a 21.4 minutes run.			Injection volume:	50 ul	

Method 539 UCMR3 Analyte	UCMR MRL (ng/L)	EPA 539 published LCMRL (ng/L)	PRM Q Exactive HRAM 70000 Res. LCMRL (ng/L)	PRM QE-LCMRL Calc -DL (ng/L)	FULL-MS/DIA(Estriol PRM) Q Exactive HRAM 70000 Res. LCMRL (ng/L)	FULL-MS/DIA(Estriol PRM) QE-LCMRL Calc -DL (ng/L)
17 $\alpha$ -ethynylestradiol	0.9	1.3	Critical level 0.05	0.1	Critical level 0.05	0.1
17 $\beta$ -estradiol	0.4	0.32	0.17	0.047	0.16	0.12
equilin	4	0.28	Critical level 0.23	0.48	Critical level 0.23	0.44
estriol	0.8	3	0.27	0.2	Critical level 0.046	0.068
estrone	2	4	0.84	0.48	0.7	0.45
testosterone	0.1	0.062	0.033	0.027	NA	NA
androstene-dione	0.3	0.37	0.19	0.08	NA	NA

# Using Full-MS data with Sieve Software for Site Specific Emerging Contaminants



## FrameTable: 209 Items

CompID	ID	CompMW	Formula	MZ	Time	HitCount				
10	10	211.1428815	C9H24N1[32]S2	210.1356049	5.512476921	1				
DetailTable: 1 Items										
CSID	Name	Formula	SMILES	MW	AvgMass	MonoisotopicMass	SearchMass	DeltaPPM	Adduct	
14620	<b>Atraton</b>	C_{9}H_{17}N_{5}O	CC=N=c/1\[nH]/c(=N/C(C)C)/[nH]c(n1)OC	211.2642	211.2642	211.143311	211.1428815	<b>2.03436459</b>	comp	
11	11	232.016676	C1H5O9N5	231.0093994	7.780558586	1				
DetailTable: 1 Items										
CSID	Name	Formula	SMILES	MW	AvgMass	MonoisotopicMass	SearchMass	DeltaPPM	Adduct	
3008	<b>Diuron</b>	C_{9}H_{10}Cl_{2}N_{2}O	CN(C)C(=N)c1ccc(c(c1)Cl)Cl/O	233.0945	233.0945	232.017014	232.016676	<b>1.45673122</b>	comp	
12	12	234.1254712		233.1181946	5.222514153	2				
DetailTable: 2 Items										
CSID	Name	Formula	SMILES	MW	AvgMass	MonoisotopicMass	SearchMass	DeltaPPM	Adduct	
4E+06	<b>Stiripentol</b>	C_{14}H_{18}O_{3}	CC(C)(C)C/C=C/c1cc2c(c1)OCO2O	234.29092	234.2909	234.125595	234.1254712	<b>0.528861389</b>	comp	



# 537 Produces Similar Results: Better than Traditional LC-MS-MS



**Method more than meets UCMR3 sensitivities.**

PRM Mode	LCMRL (ng/L)	Full-MS, vDIA	LCMRL (ng/L)	Method 537	LCMRL (ng/L)	UCMR3	MRL
PFBS	4.5	PFBS	<2.5	PFBS	3.7	PFBS	90
PFDA	<2.5	PFDA	3.2	PFDA	3.8	PFDA	not included
PFDoA	<2.5	PFDoA	2.9	PFDoA	3.5	PFDoA	not included
PFHpA	3	PFHpA	<2.5	PFHpA	3.8	PFHpA	10
PFHxA	<2.5	PFHxA	<2.5	PFHxA	2.9	PFHxA	not included
PFHxS	<2.5	PFHxS	<2.5	PFHxS	8	PFHxS	30
PFNA	3.4	PFNA	3.1	PFNA	5.5	PFNA	20
PFOA	<2.5	PFOA	3.7	PFOA	5.1	PFOA	20
PFOS	<2.5	PFOS	<2.5	PFOS	6.5	PFOS	40
PFTA	<2.5	PFTA	<2.5	PFTA	4.7	PFTA	not included
PFTTrDA	2.5	PFTTrDA	5	PFTTrDA	3.8	PFTTrDA	not included
PFUnA	<2.5	PFUnA	2.8	PFUnA	6.9	PFUnA	not included

# We Can Take the Same Approach with Method 537 (PFCs) Extracts



- Analyzed 5 UCMR3 samples for PFCs on the HRAM.
- Compared to library of ~75 PFCs and related compounds
- All 5 had detects for PFHpS
- In addition we found....

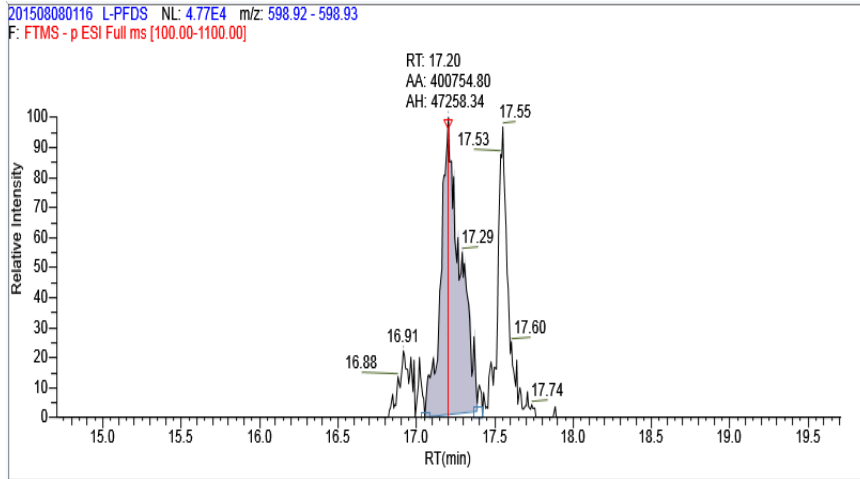
**All have mass errors of < 3 ppm so highly accurate identification**

- 6:2 diPAP – 5 out of 5
- PFDS – 3 out of 5 (063, 116, 355)
- 10:2 FTOH – 1 out of 5 (116)
- 8:2 FTA - 1 out of 5 (348)
- 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoate – 1 out of 5 (116)
- PFDOA – 1 out of 5 (355)
- PFHxDA – 1 out of 5 (355)

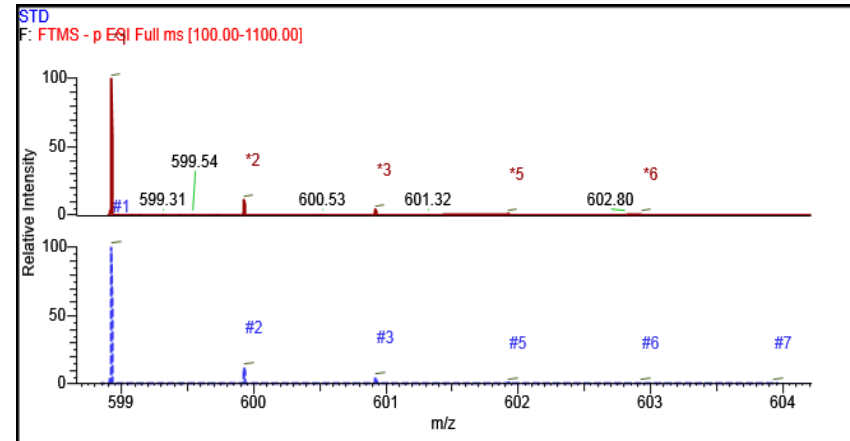
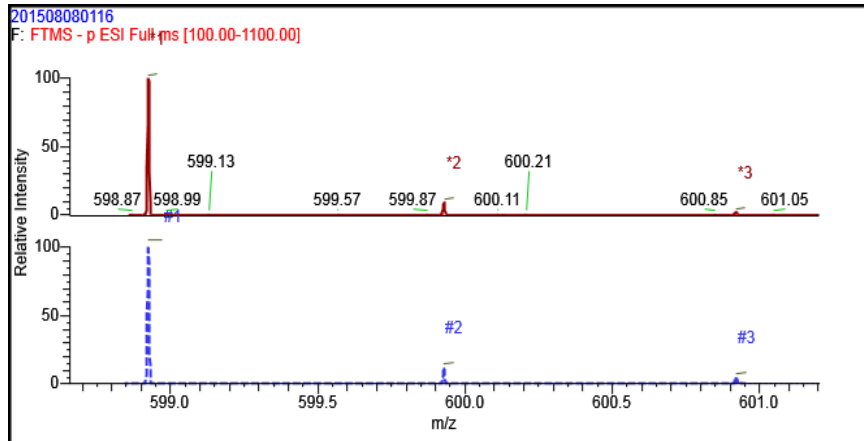
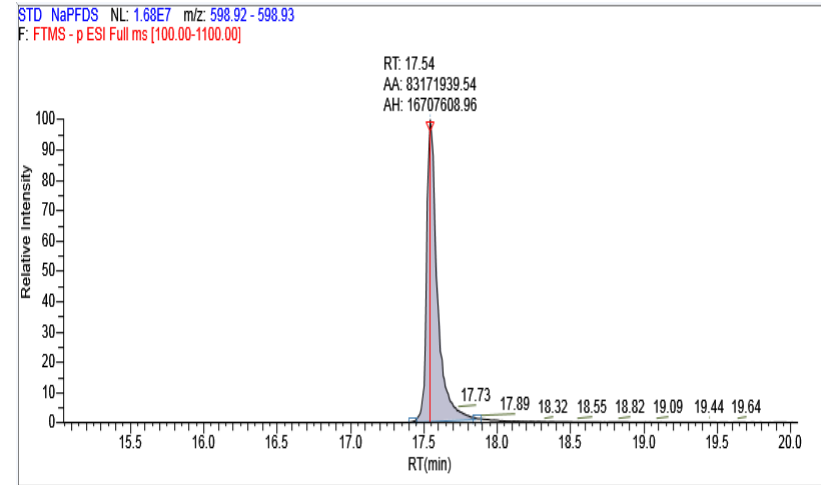
# But Are Concentrations Significant?



## Sample 116 PFDS 400K area



## PFDS 80 ng/L unbranched standard 80E6 area



# Summary and Questions



- The HRAM performance has better sensitivity than LC-MS/MS for EPA 539 and EPA 537 with additional confirmation such as product ions and a spectral library match.
- Further the same injection can be used for identification of non-target and unknowns simultaneously, using built in library, Sieve software, and/or looking for formation and degradation products using Compound discovery software.

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