



*Innovation Applied*

Analysis of ETG, ETS using the  
Thermo Scientific Exactive Mass Spectrometer

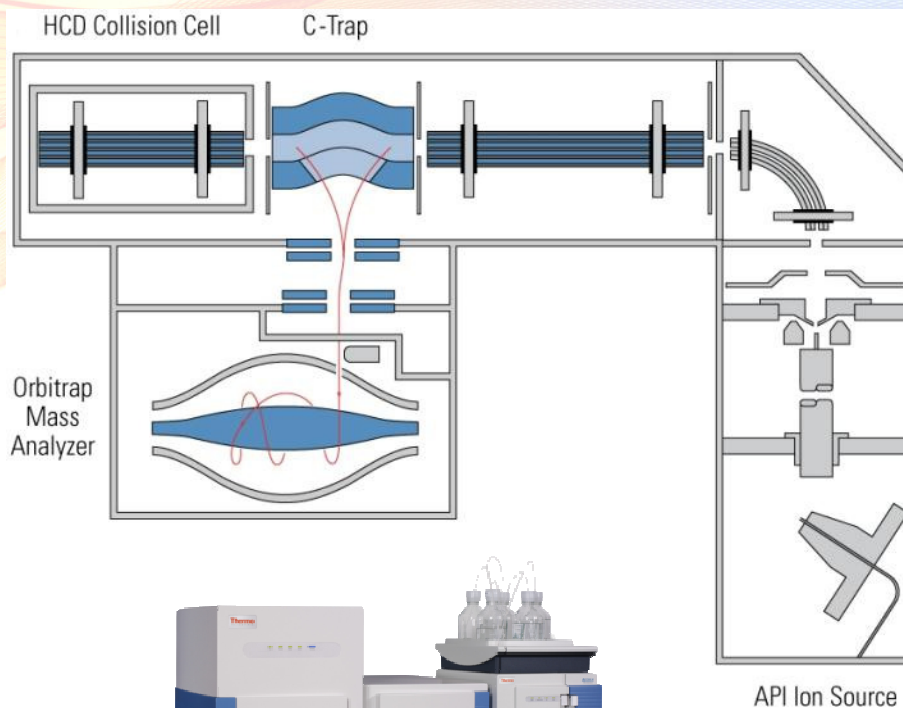
Kent Johnson  
Fortes lab, Portland Oregon

Forensic Toxicology use Only

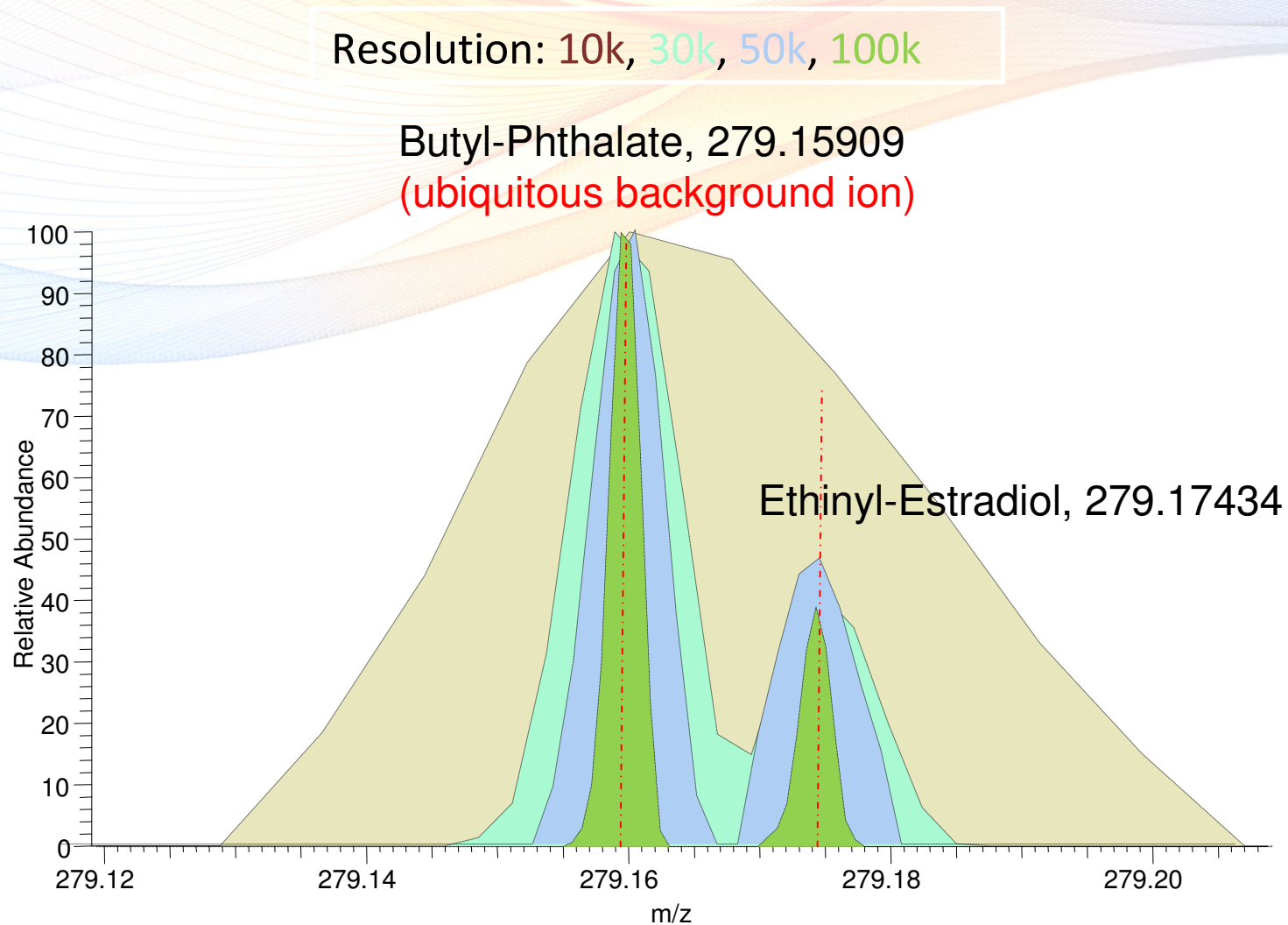
# Goal

- Development of an easy to use, interference free, less than 4 min analysis method for ETG and ETS in urine
- LOQ, 100ng/mL or better
- Method validation using QC's and real samples

- Resolution  
100,000 at 1 scan per second  
10,000 at 10 scans per second
- Mass accuracy  
Sub ppm
- Sensitivity  
500 fg Buspirone with S/N >10:1
- Dynamic range  
>10,000 within a spectrum
- Scan speed  
Up to 10 scans per second
- Mass range  
m/z 50 - 4000
- Polarity switching  
One positive and one negative scan < 1 second (25K Resolution)



# Ethinyl-Estradiol at Different Mass Resolutions



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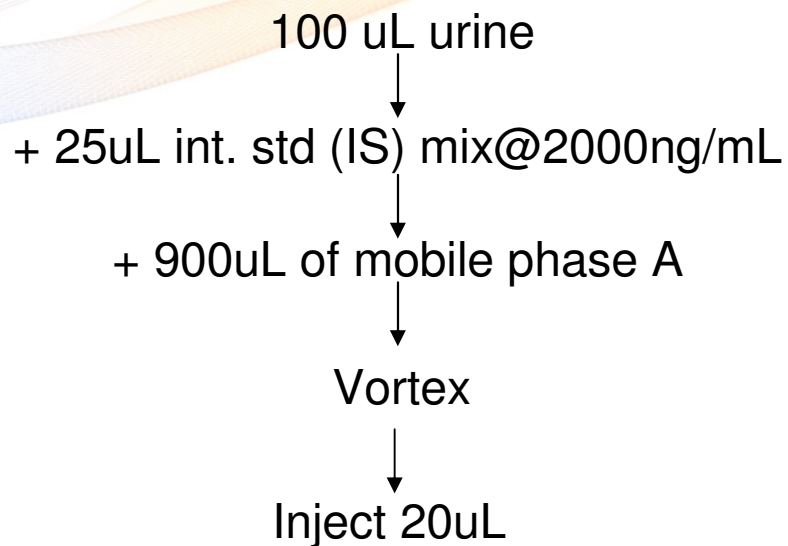
## ETG and ETS in Urine



# Standards, QC and sample preparation

## ETG and ETS

### Sample preparation method



Note: IS are ETG-D5 and ETS-D5  
50 -100 x dilution with mobile phase recommended

# LC/MS Method

- LC method
  - Thermo Hypersil Gold 50 x 2.5 mm, 5u
  - Mobile Phase A: 5mM Dihexylammonium acetate in LC DI H2O
  - Mobile Phase B: 5mM Dihexylammonium acetate in CAN
  - Mobile Phase C: ACN/IPA/Acetone = 45/45/10 v/v/v
  - Column Temp: 50 Deg C
- Exactive MS method
  - HESI source
  - Full scan MS acquisition @ 100,000 Resolution
  - External mass calibration
  - Extract chromatogram for drugs of interest (m/z)@ better than 5ppm mass accuracy
  - Confirm the identity using mass accuracy and RT
  - Quantitate using peak area in the chromatogram

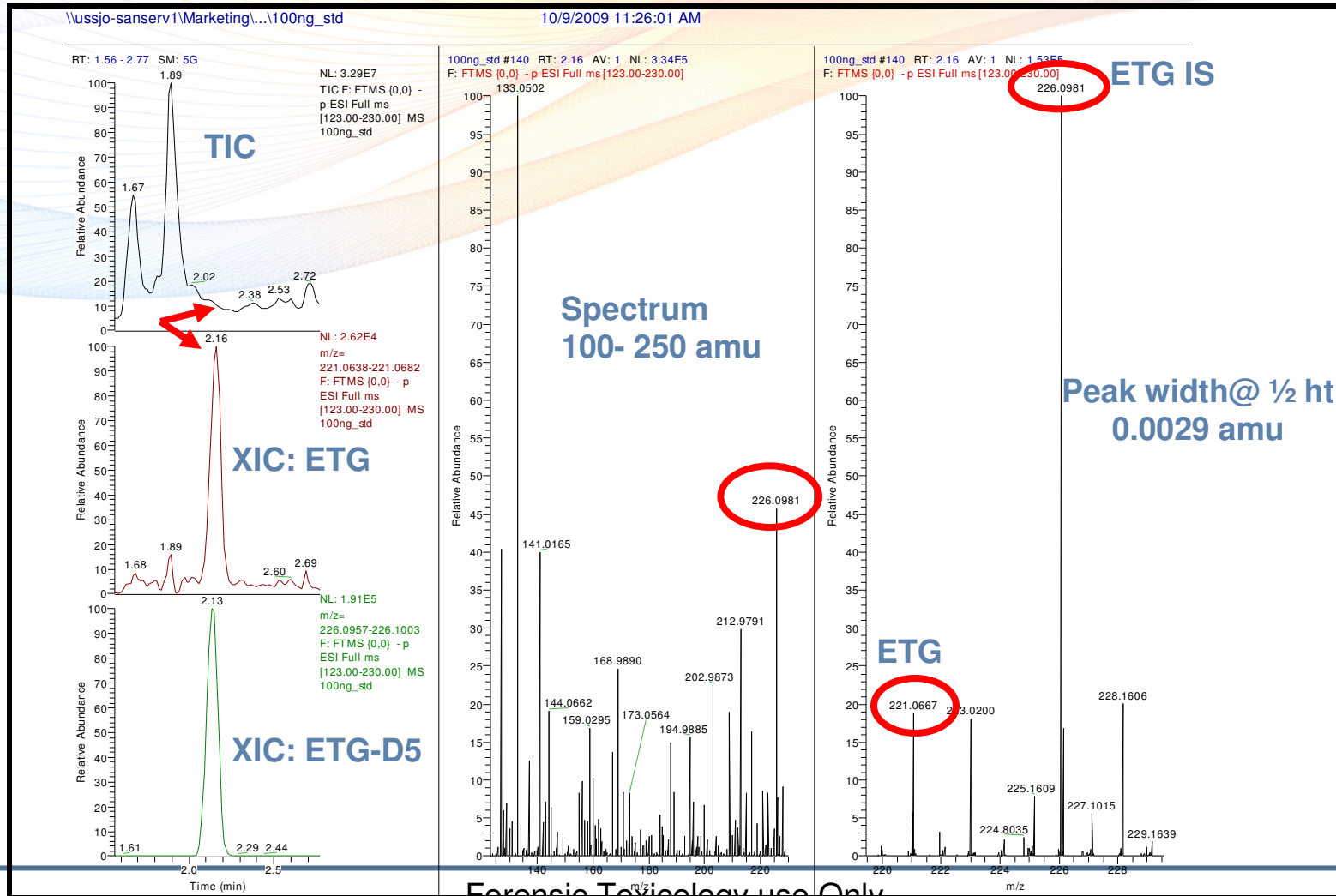
# ETG-ETS LC gradient

Time (min)	%A	%B	%C	Flow (mL/min)
0	95	5	0	0.8
0.1	95	5	0	0.8
3.0	64	36	0	0.8
3.01	0	100	0	0.8
3.5	0	100	0	0.8
3.51	0	0	100	0.8
4.0	0	0	100	0.8
4.01	95	5	0	0.8
5.0	95	5	0	0.8



# 100 ng/mL EtG standard in urine

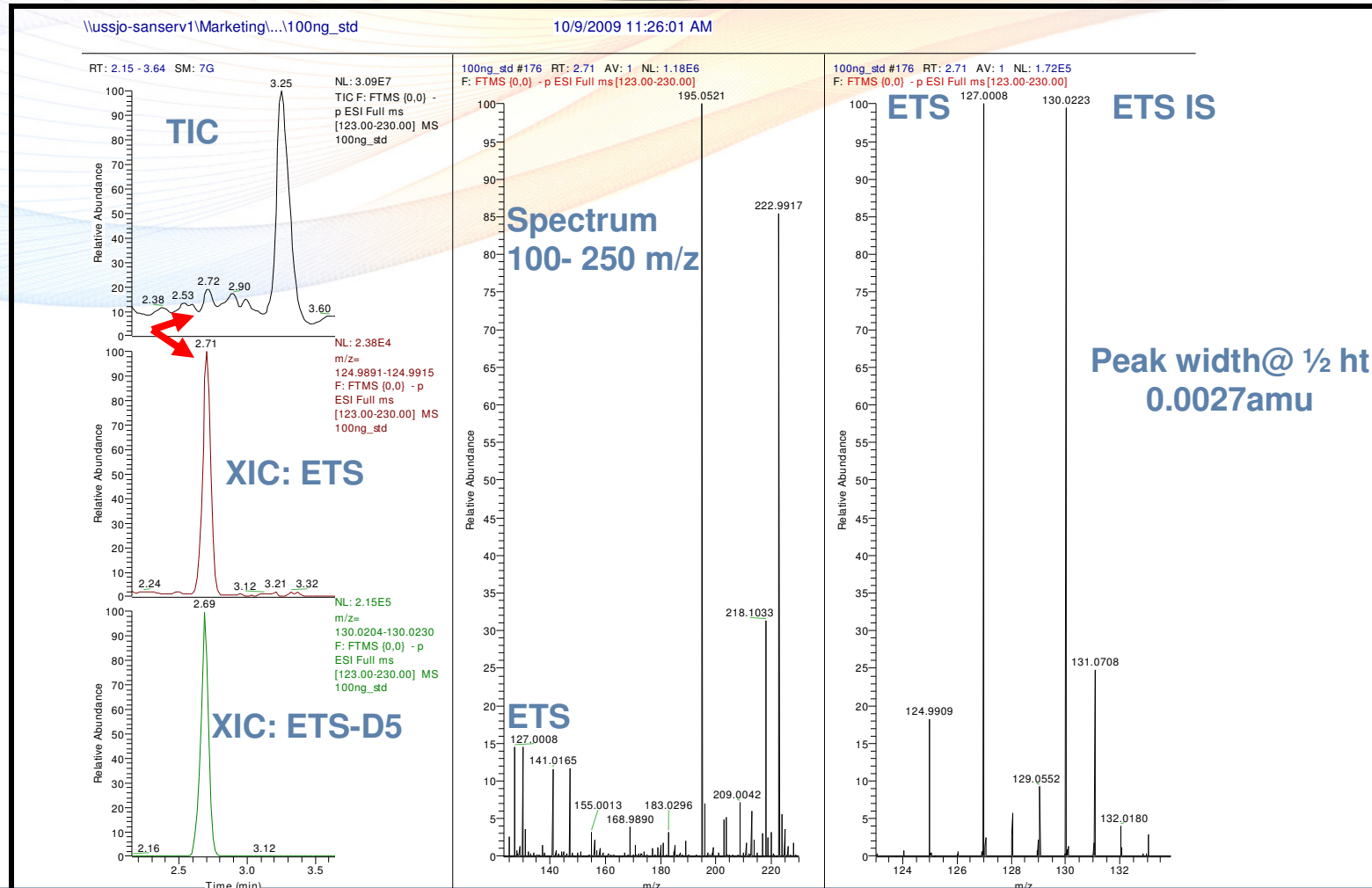
Very High Specificity



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# 100 ng/mL EtS standard in urine

Very High Specificity



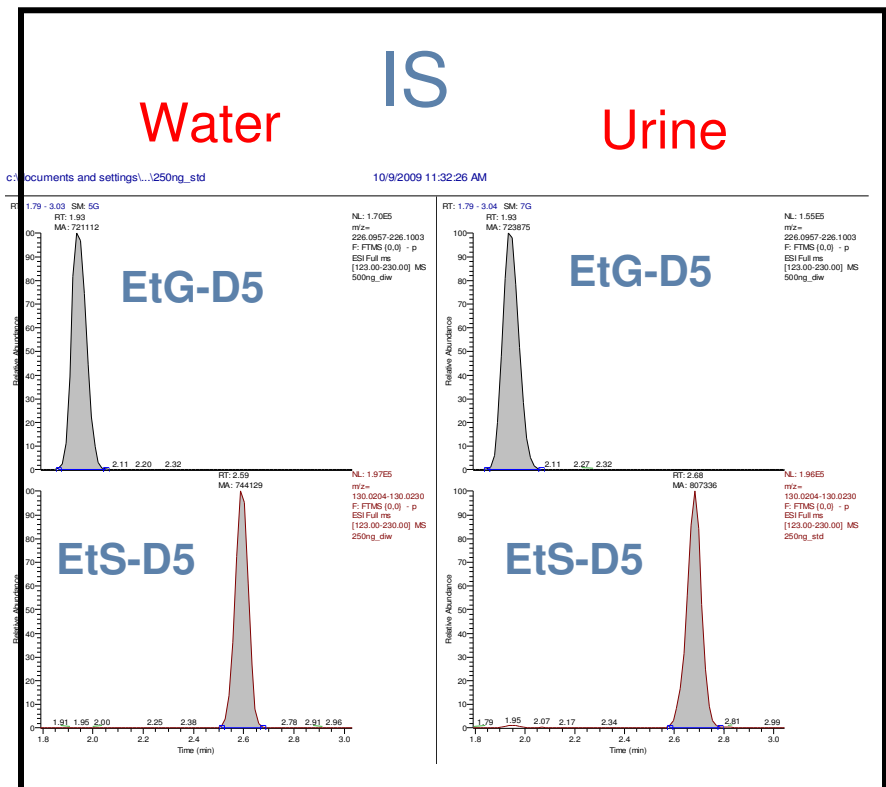
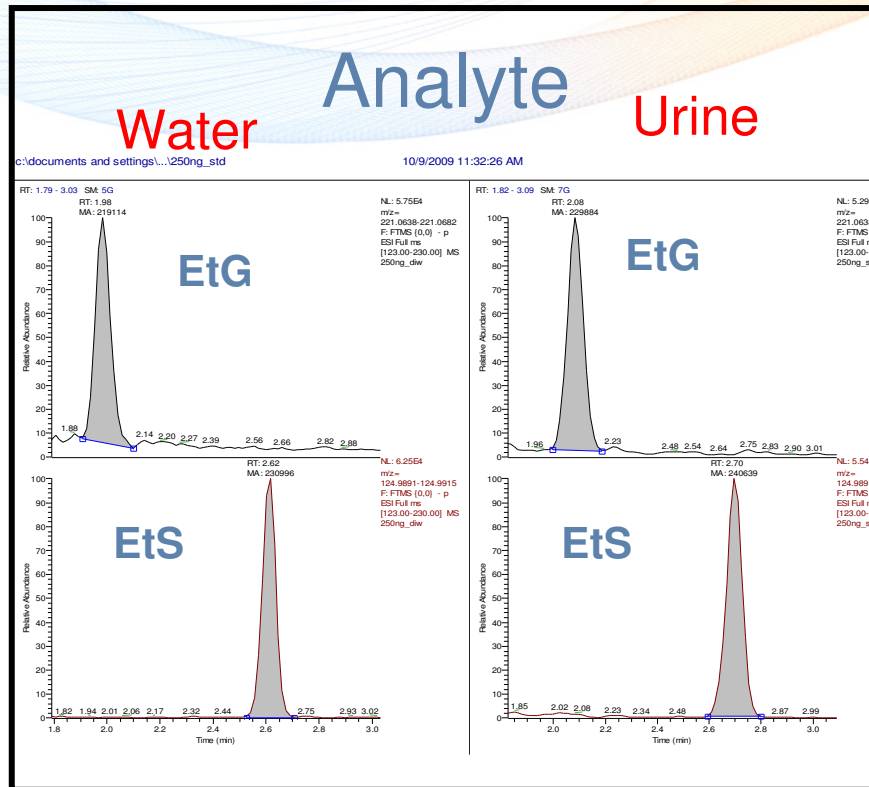
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# Ion Suppression Test - Water vs Urine 250 ng/mL std

Compound	peak area water	peak area urine
ETG	5.75	5.29
ETG D5	17	15.5
ETS	6.25	5.5
ETS D5	19.7	19.6

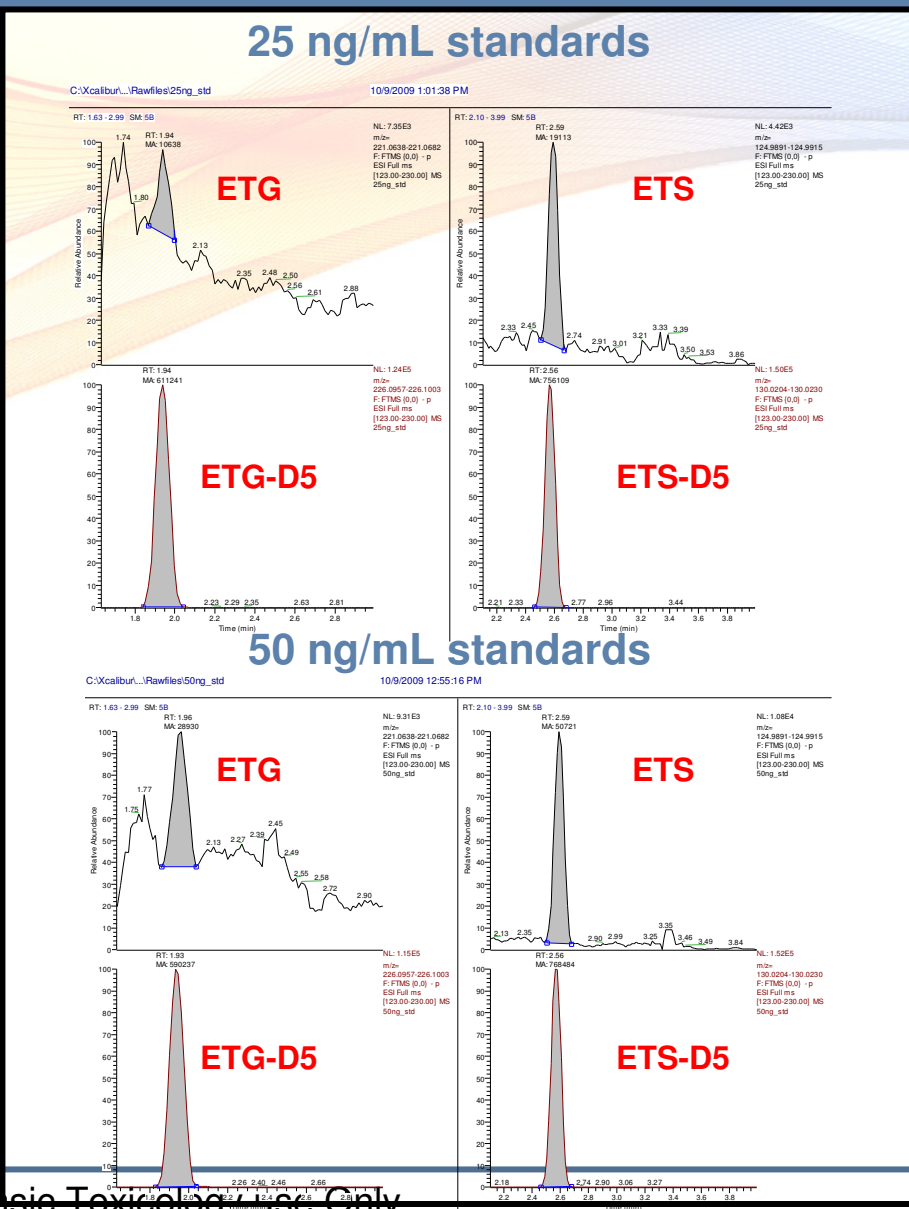
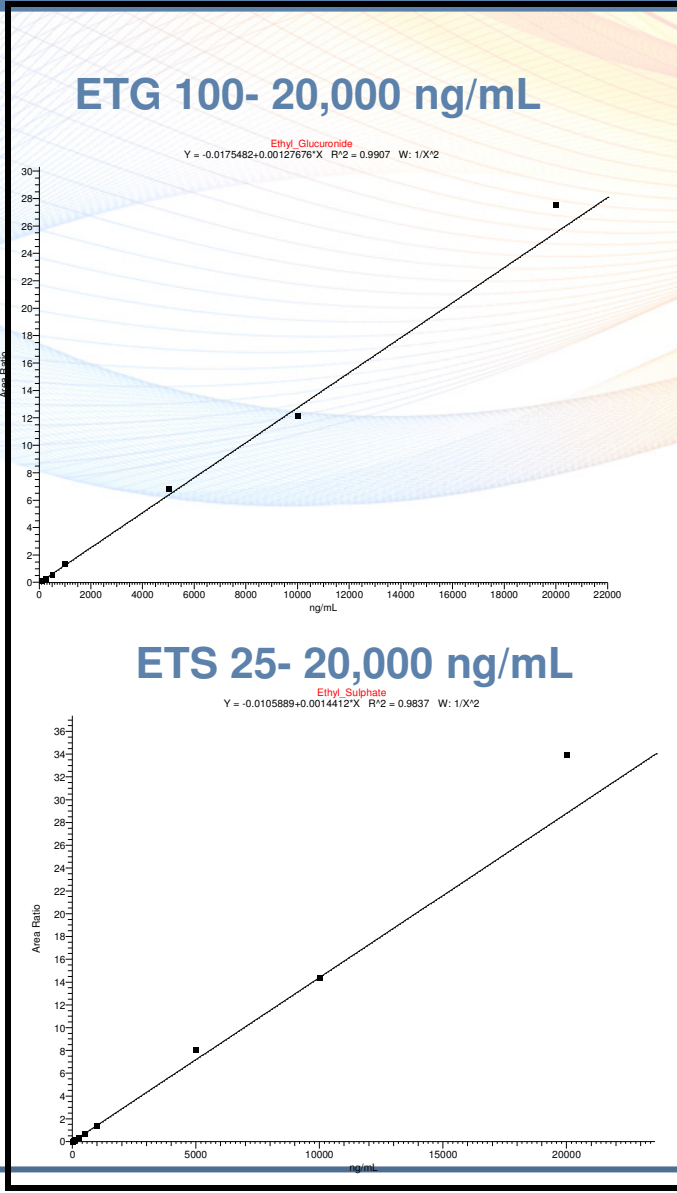
No Ion Suppression  
Peak Areas Similar  
Analyte/IS ratio similar

Analyte	Water area	Urine area	Water	Urine
			Ratio	Ratio
ETG	5.75	5.29	0.34	0.34
ETG-IS	17	15.5		
ETS	6.25	5.5	0.32	0.28
ETS-IS	19.7	19.6		



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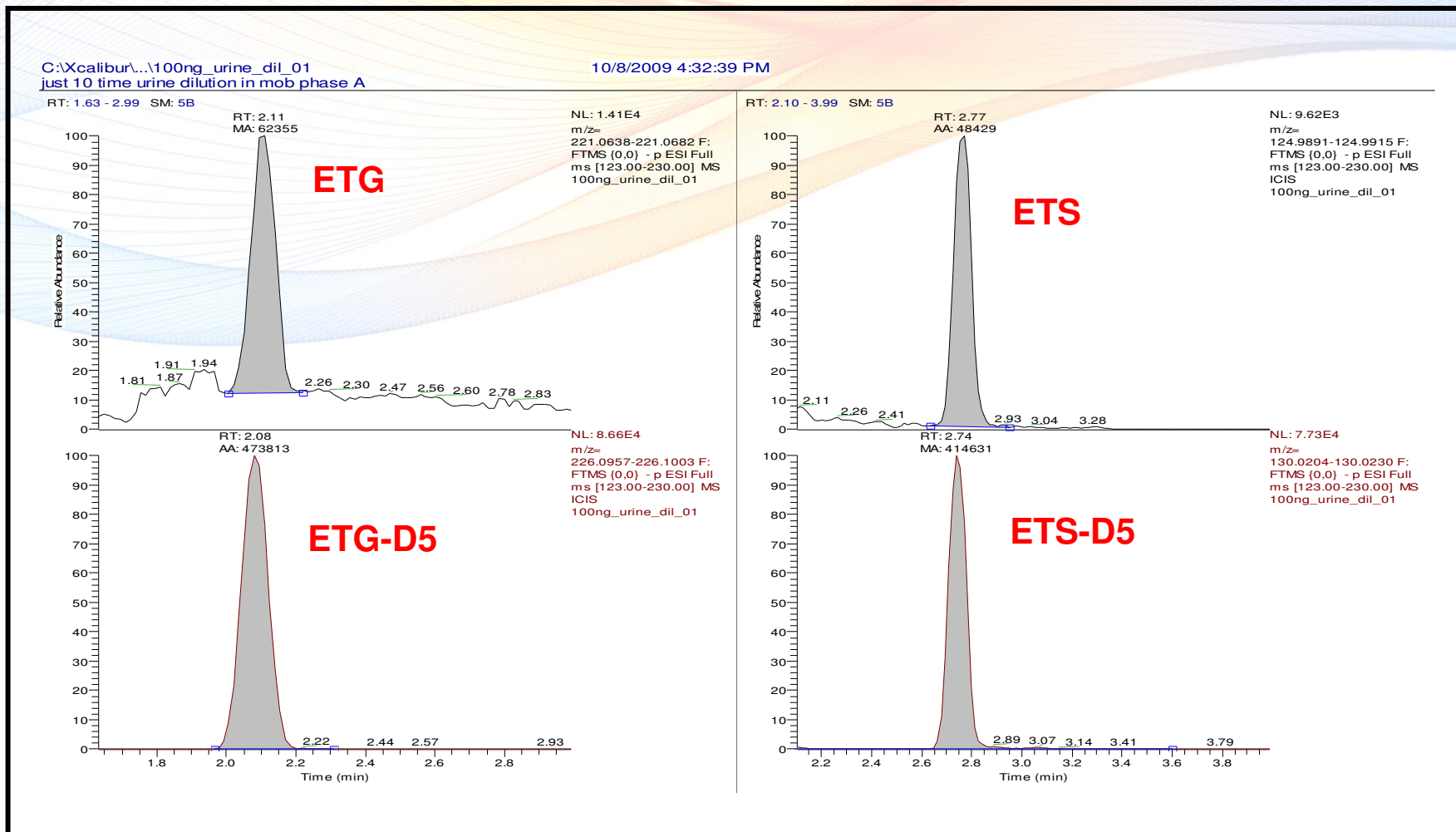
# Calibration curves and LOQ



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# 100 ng/mL, 20 uL standards in urine



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# Urine – Negative control

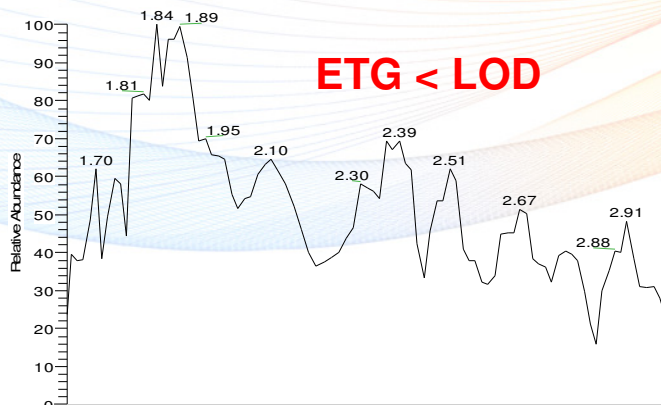
## ETG

## ETS

C:\Xcalibur\...\Neg\_control\_urine\_dil\_01

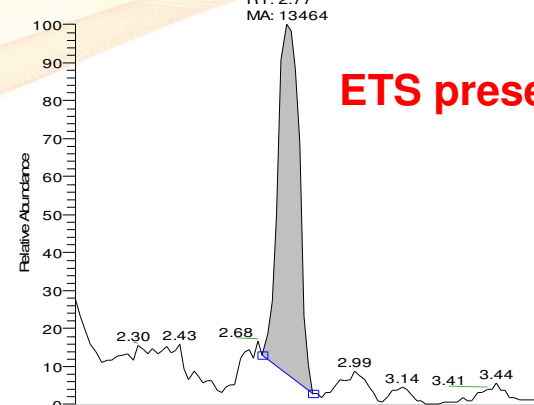
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RT: 1.63 - 2.99 SM: 5B



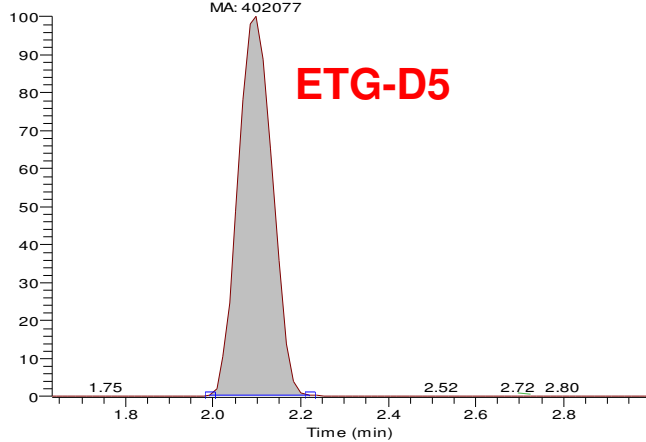
NL: 3.34E3  
m/z=  
221.0638-221.0682 F:  
FTMS (0,0) - p ESI Full  
ms [123.00-230.00] MS  
Neg\_control\_urine\_dil\_0  
1

RT: 2.10 - 3.99 SM: 5B



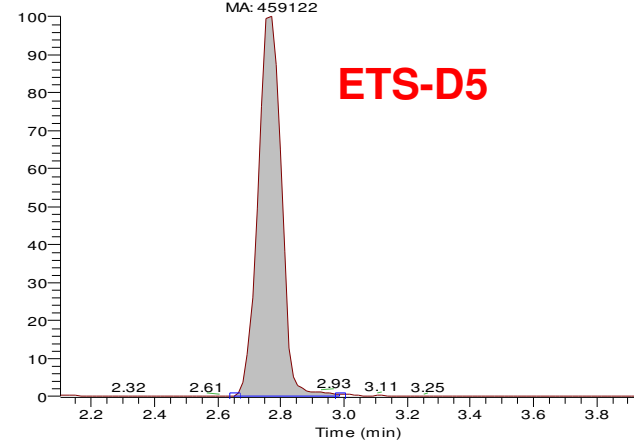
NL: 3.11E3  
m/z=  
124.9891-124.9915 F:  
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1

RT: 2.10  
MA: 402077



NL: 7.40E4  
m/z=  
226.0957-226.1003 F:  
FTMS (0,0) - p ESI Full  
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1

RT: 2.77  
MA: 459122



NL: 8.82E4  
m/z=  
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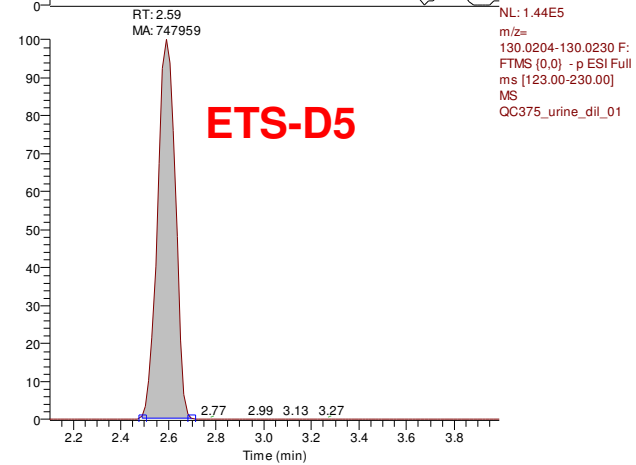
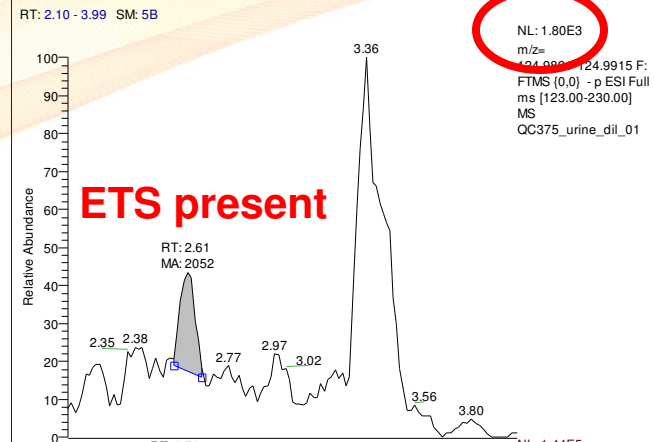
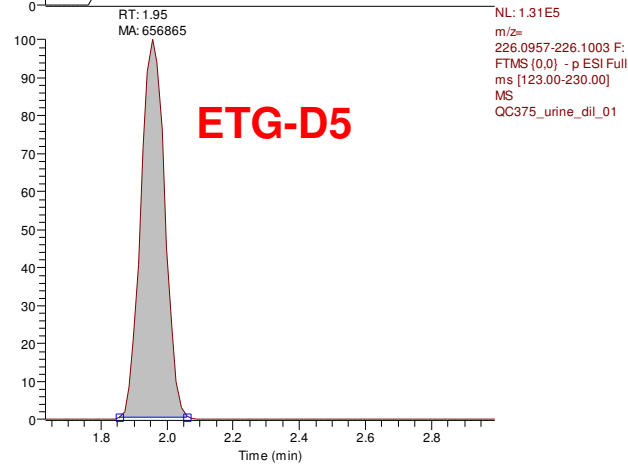
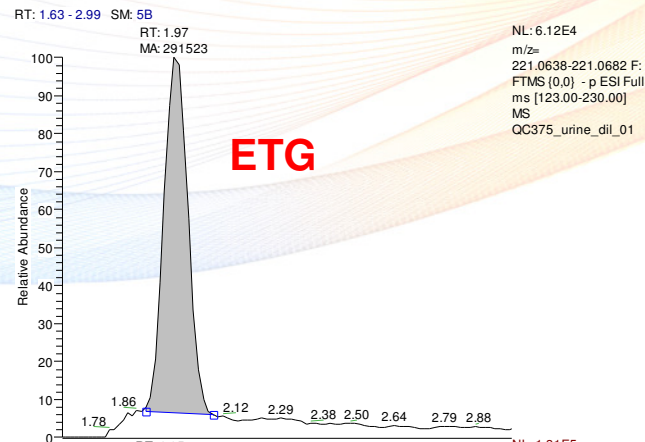
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# QC 375 ng/mL - ETG

## ETG=317 ng/mL, ETS present

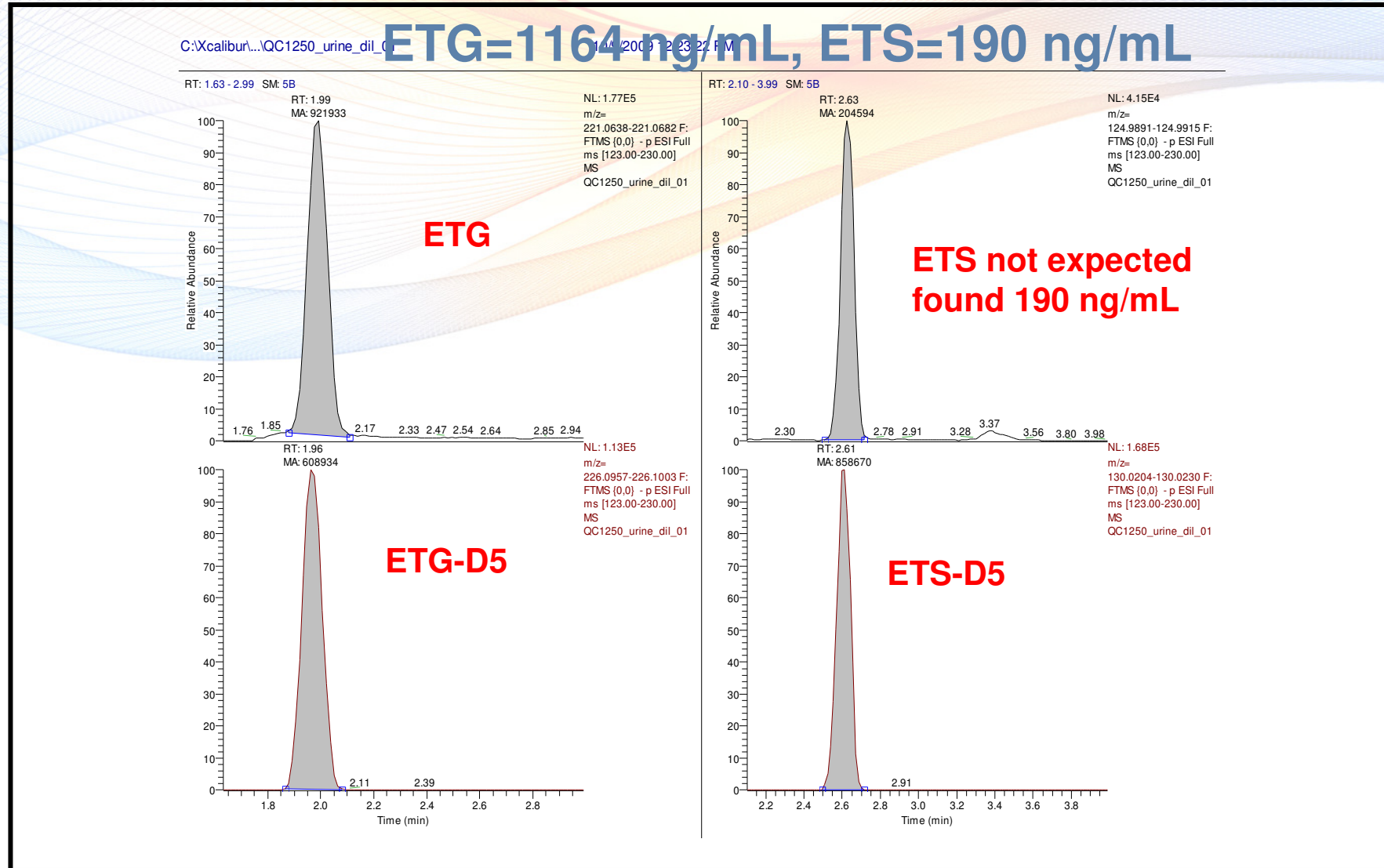
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10/9/2009 12:16:59 PM



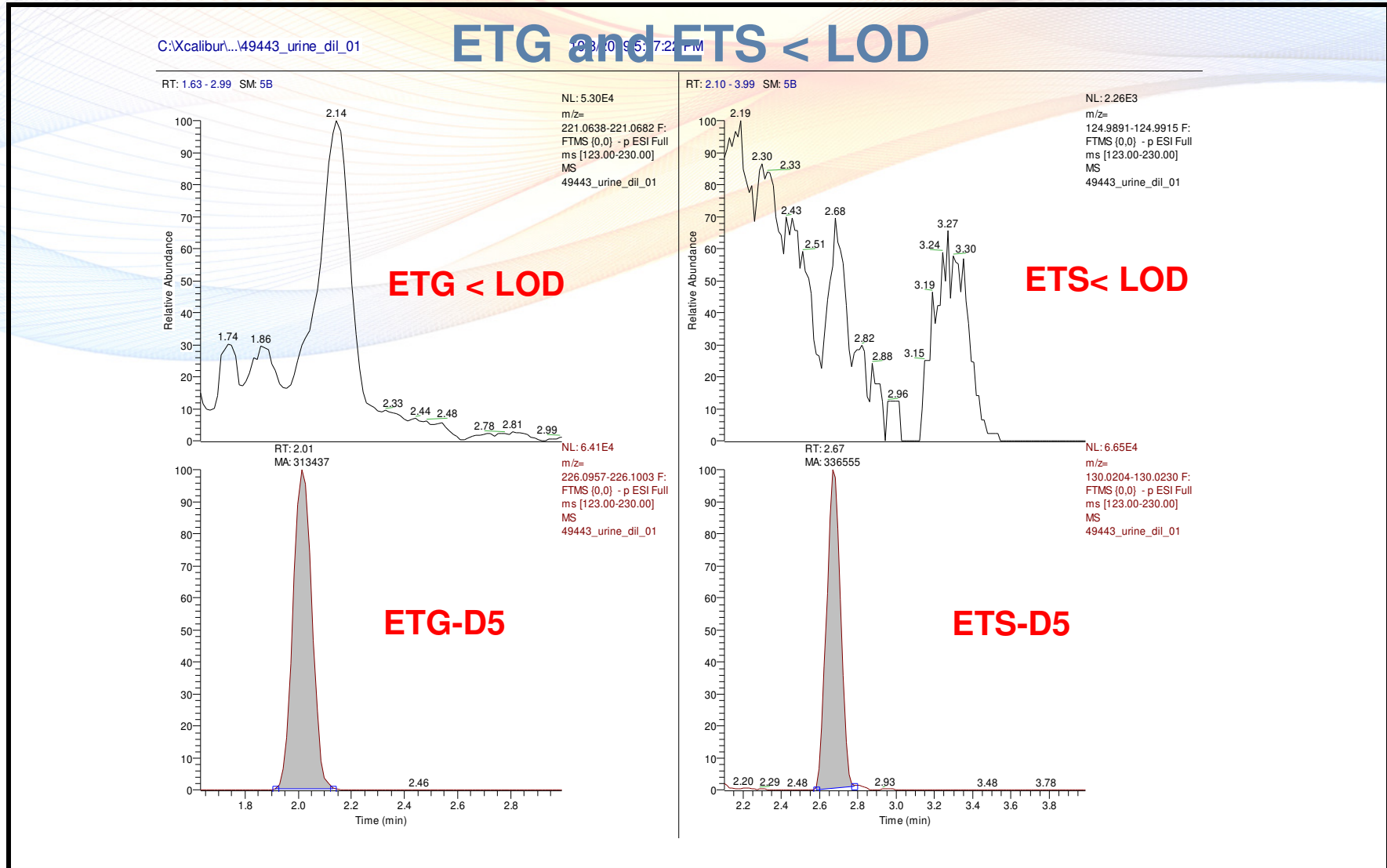
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# QC 1250 ng/mL - ETG



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# Urine sample



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# Urine sample

## ETG=312247, ETS=262149 ng/mL

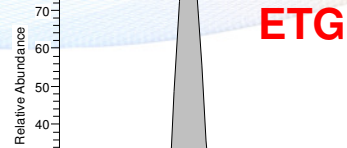
C:\Xcalibur...49644\_urine\_dil\_01

10/8/2009 5:23:45 PM

RT: 1.63 - 2.99 SM: 5B

RT: 2.02  
MA: 115295053

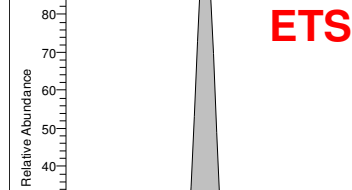
NL: 2.09E7  
m/z=  
221.0638-221.0682 F:  
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MS  
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RT: 2.10 - 3.99 SM: 5B

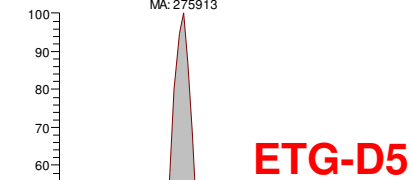
RT: 2.68  
MA: 42796061

NL: 7.24E6  
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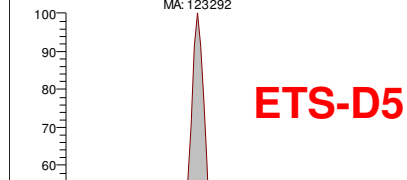
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49644\_urine\_dil\_01



RT: 2.65  
MA: 123292

NL: 2.31E4  
m/z=  
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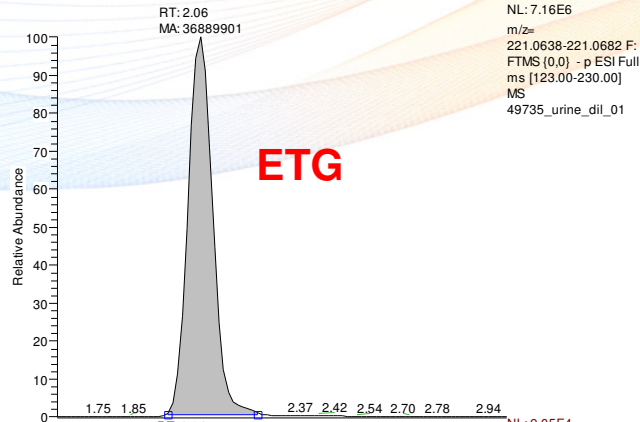
# Urine sample

**ETG=68116, ETS=17789 ng/mL**

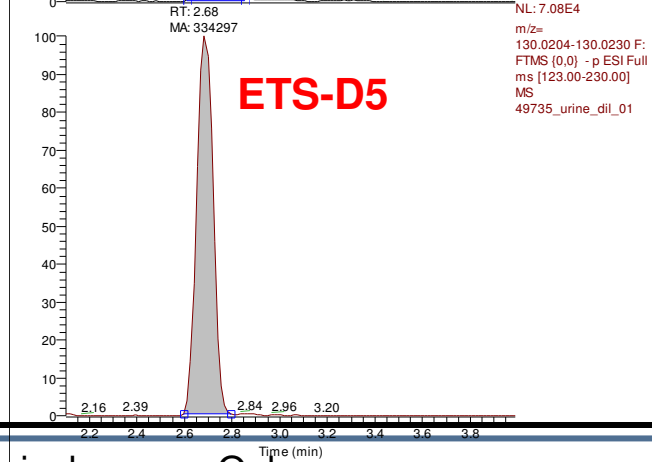
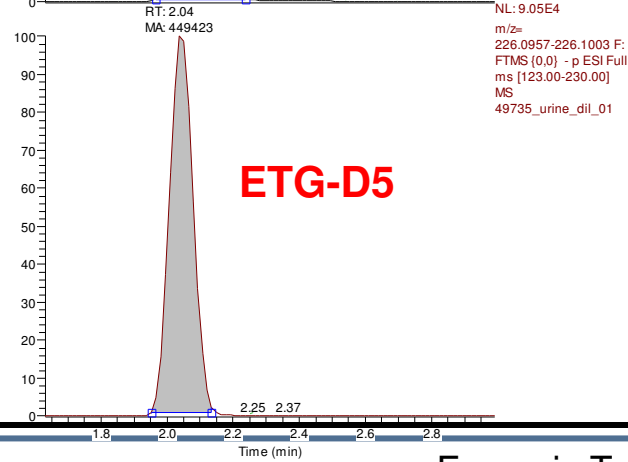
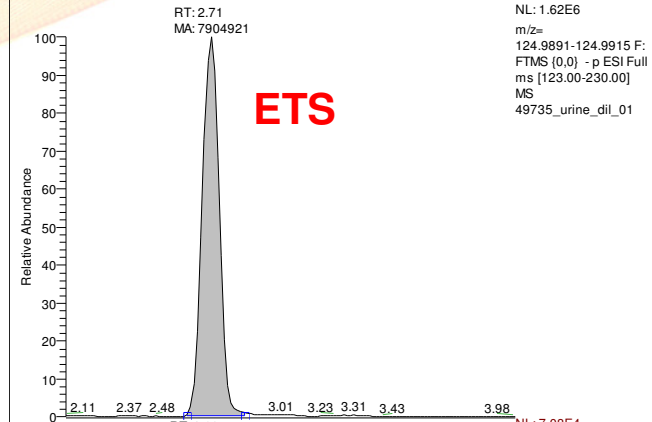
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10/8/2009 5:36:32 PM

RT: 1.63 - 2.99 SM: 5B



RT: 2.10 - 3.99 SM: 5B



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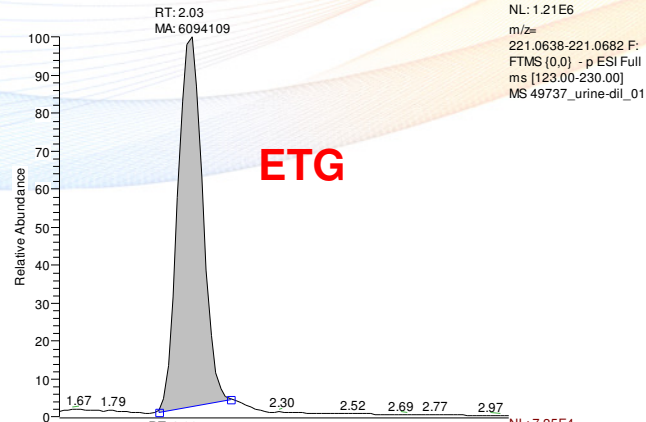
# Urine sample

## ETG=14397, ETS=1176 ng/mL

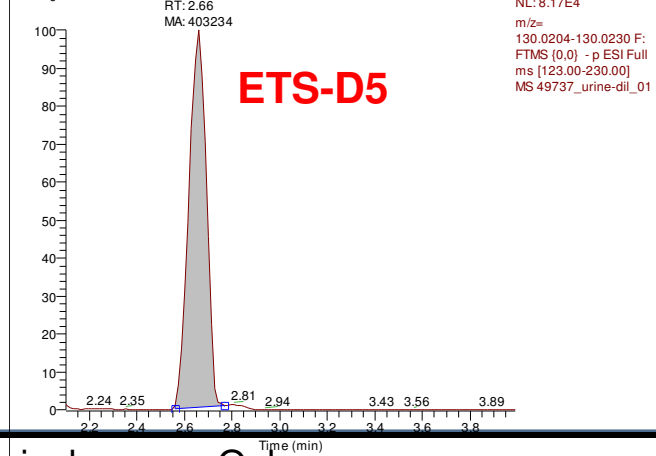
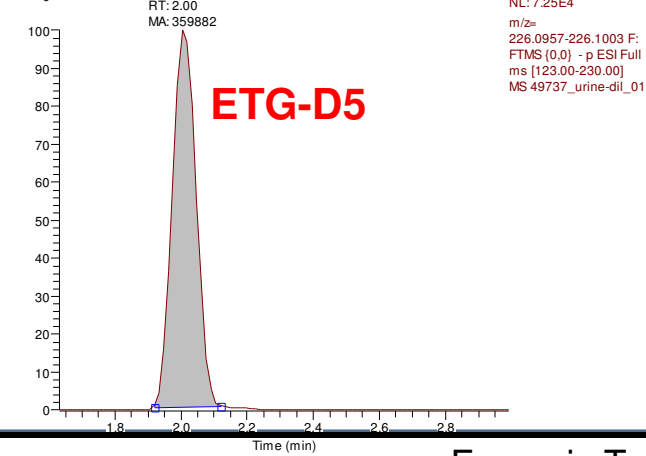
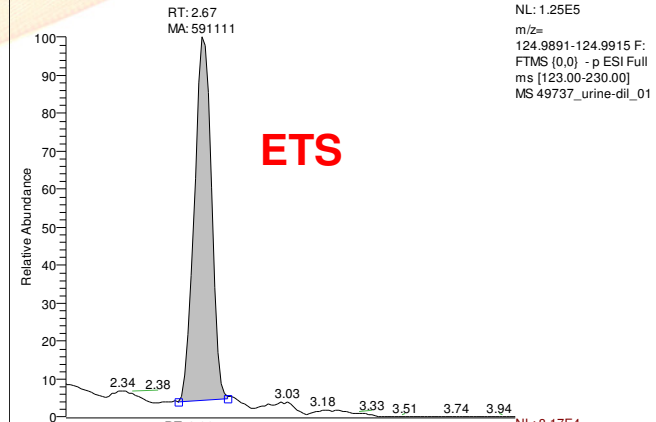
C:\Xcalibur...49737\_urine-dil\_01

10/8/2009 5:42:54 PM

RT: 1.63 - 2.99 SM: 5B



RT: 2.10 - 3.99 SM: 5B



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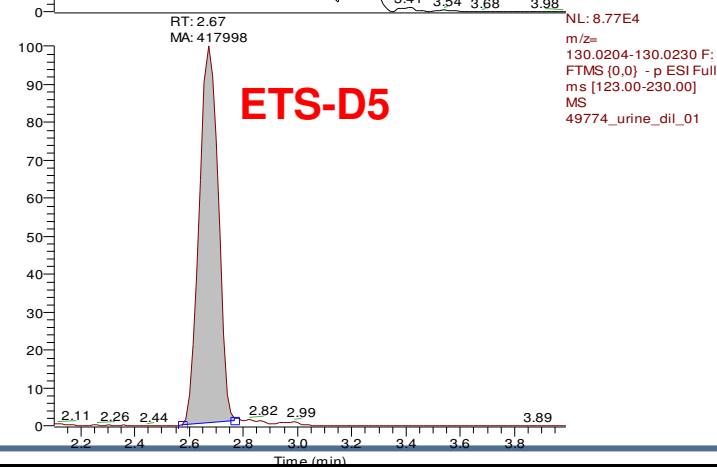
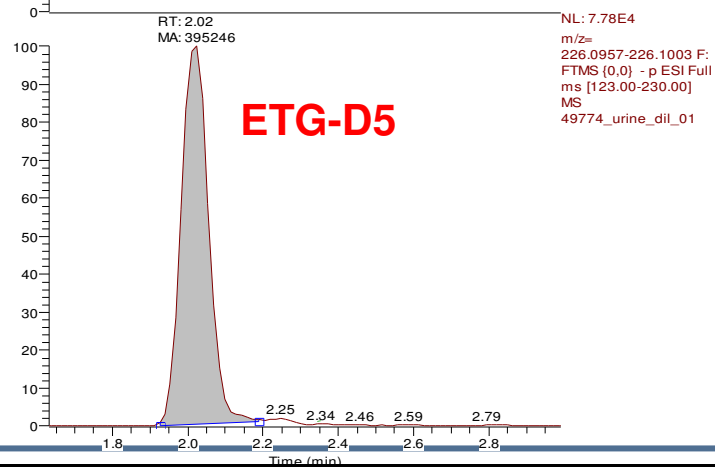
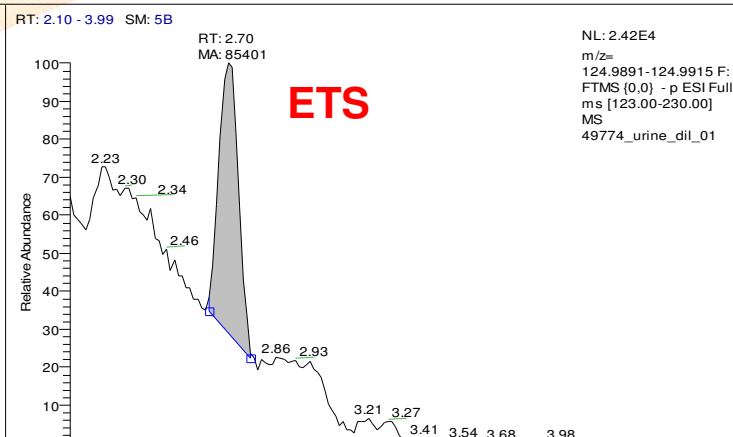
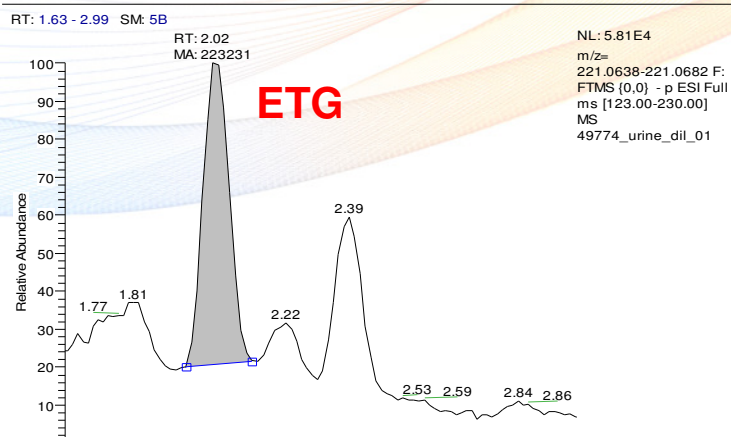
# Urine sample

Passes lie detector test for alcohol but drinks 1 bottle of Nyquil every night

## ETG=459.9 ETS=160.8 ng/mL

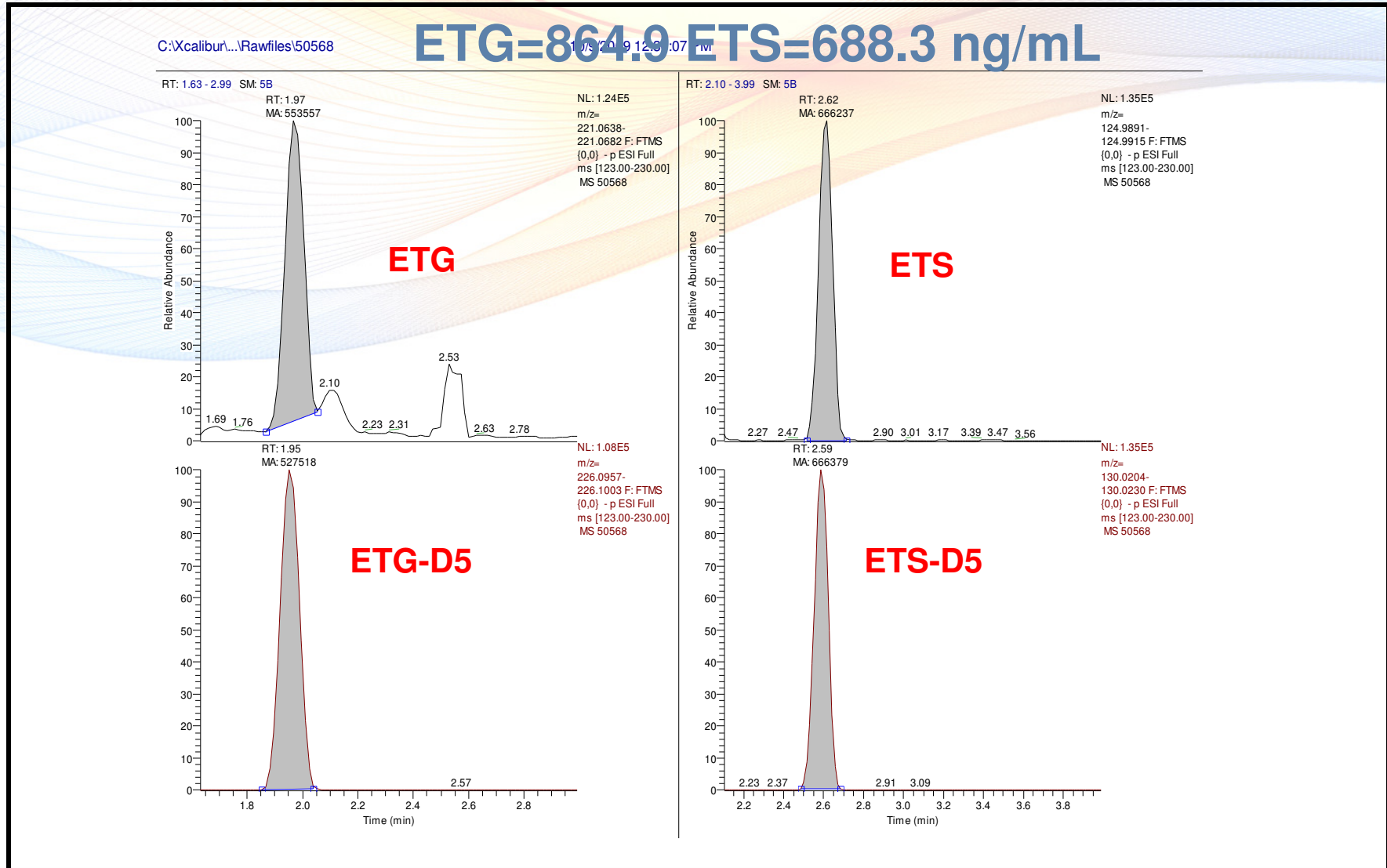
C:\Xcalibur...\49774\_urine\_dil\_01

10/8/2009 5:49:17 PM



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# Urine sample



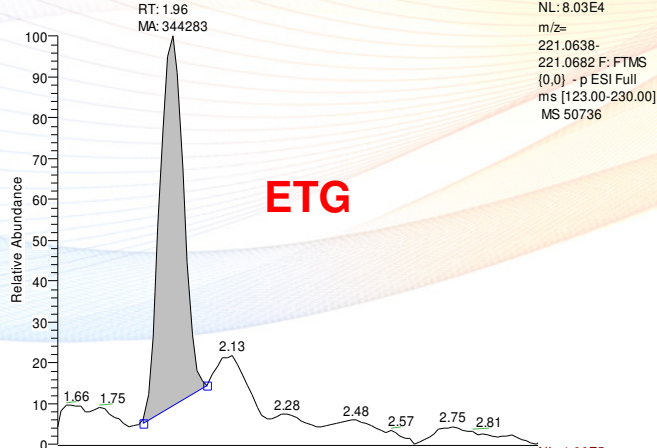
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# Urine sample

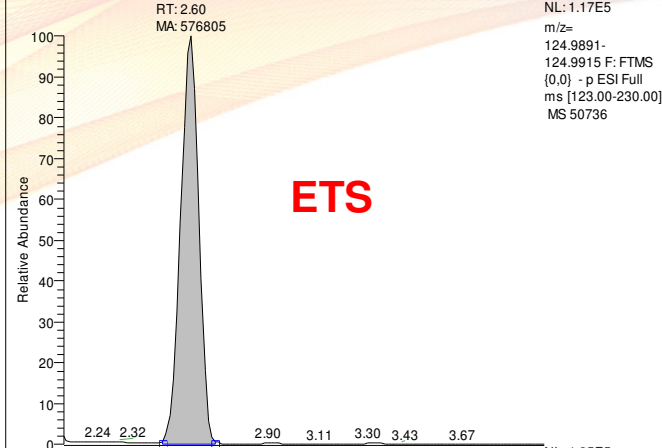
**ETG=559.2 ETS=616.4 ng/mL**

C:\Xcalibur\...Rawfiles\50736

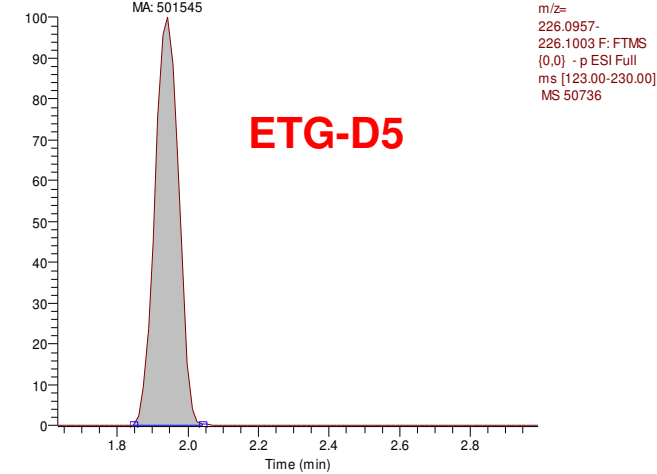
RT: 1.63 - 2.99 SM: 5B



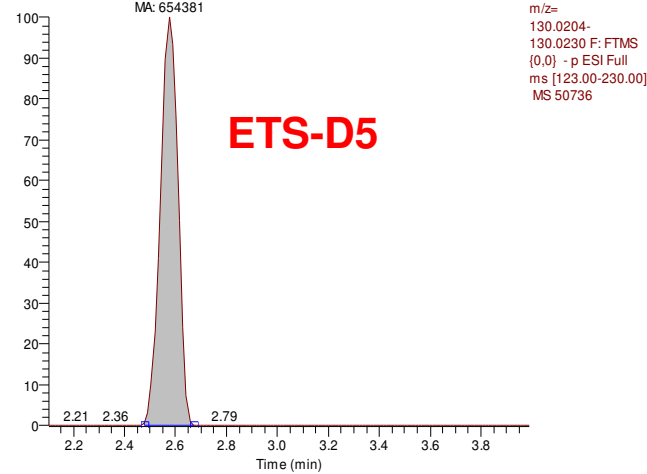
RT: 2.10 - 3.99 SM: 5B



RT: 1.94 MA: 501545



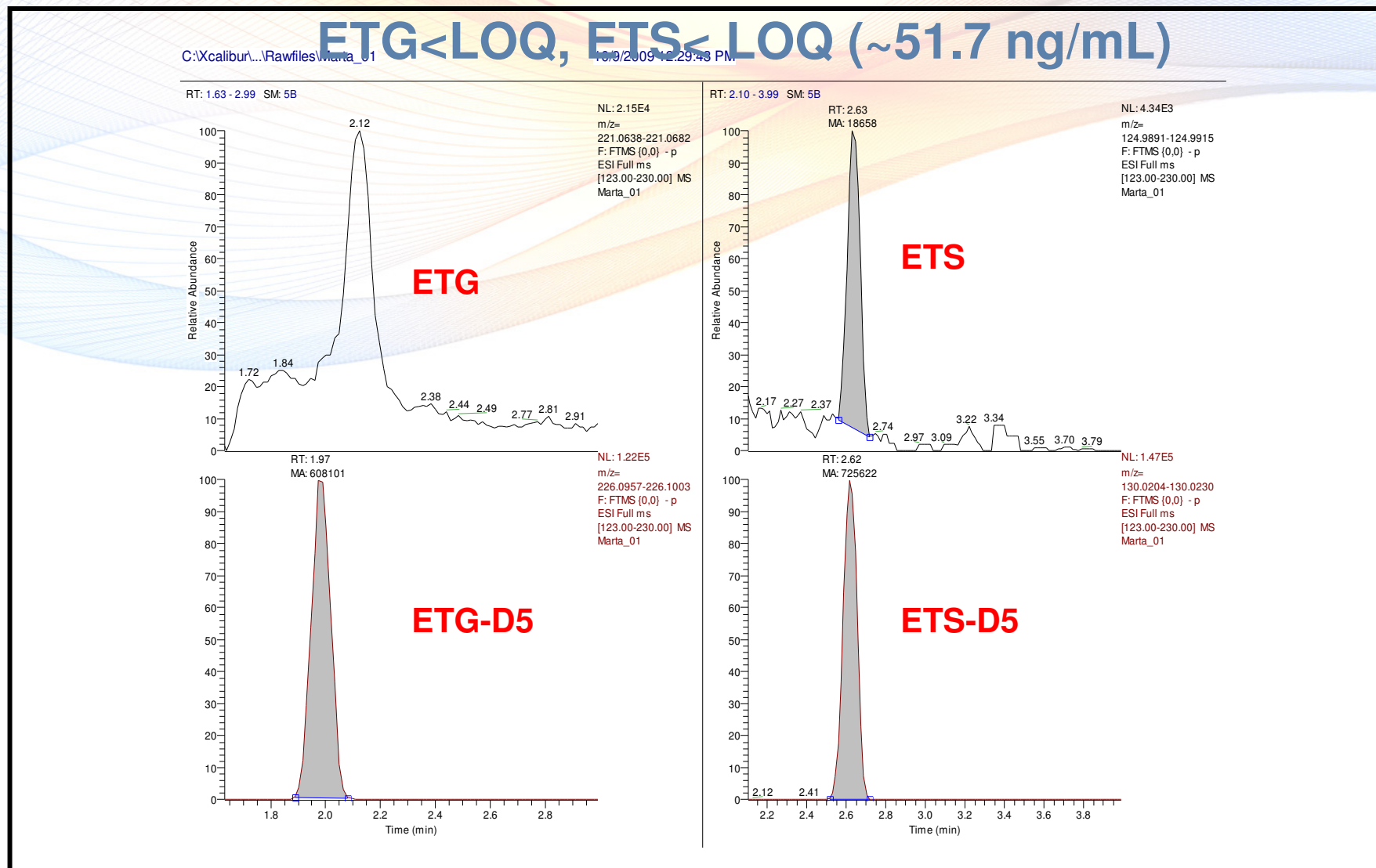
RT: 2.57 MA: 654381



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# Urine sample



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# Conclusions

- Assay based on Exactive ultra high resolution MS coupled to LC system is
  - Well suited for accurate analysis of ETG and ETS
  - Analysis time of 4 min for ETG and ETS
  - ETG LOQ = 100ng/mL, LOD = 25ng/mL
  - ETS LOQ = 25ng/mL, LOD = 10ng/mL
- Exactive with a resolution of 100,000 offers  $\geq$  SRM selectivity (SRM data comparison not shown)
- Very easy for method development and routine operation
- Can be multiplexed for even higher throughput

# Acknowledgements

- Kent Johnson, Fortes Lab Portland, Oregon
- Marta Kozak, Thermo Fisher Scientific
- Perkin Elmer (Steroid kit)
- LipoMed (EtS/EtS D5)
- Cerilliant (EtG/EtG D5)
- Thermo Scientific; Instrument, Technical, and application support
- TCI - Dihexylammonium acetate