



Innovation Applied

Analysis of Benzodiazepines in urine using the
Thermo Scientific Exactive Mass Spectrometer

Kent Johnson
Fortes lab, Wilsonville Oregon

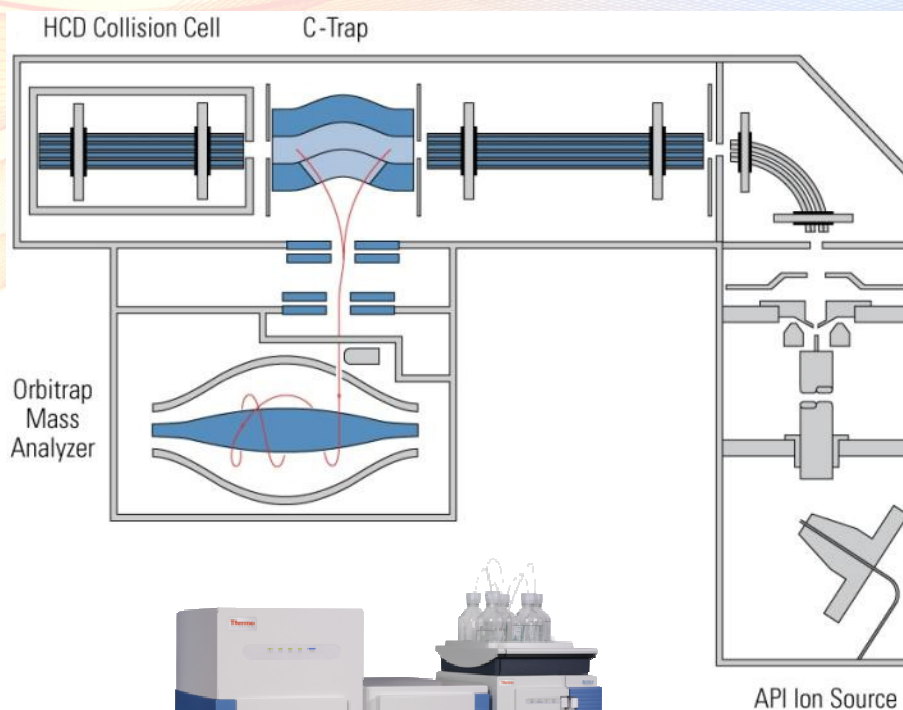
Forensic Toxicology use Only

Goal

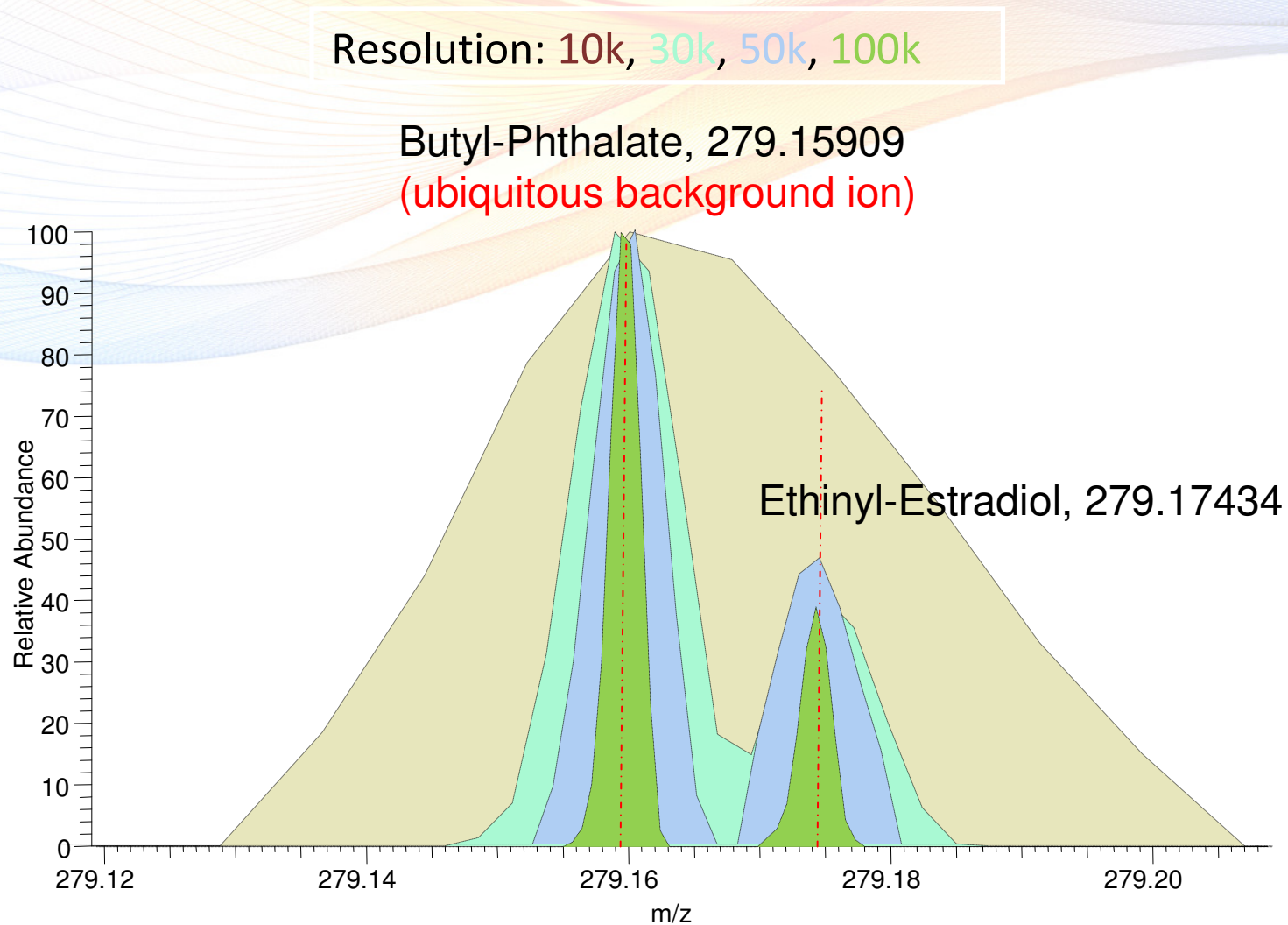
- Development of a fast and easy to use method for analysis of Benzodiazepines in urine
- LOQ, 100ng/mL or better
- Method validation using QC's and real samples

Exactive Benchtop LC-MS

- 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

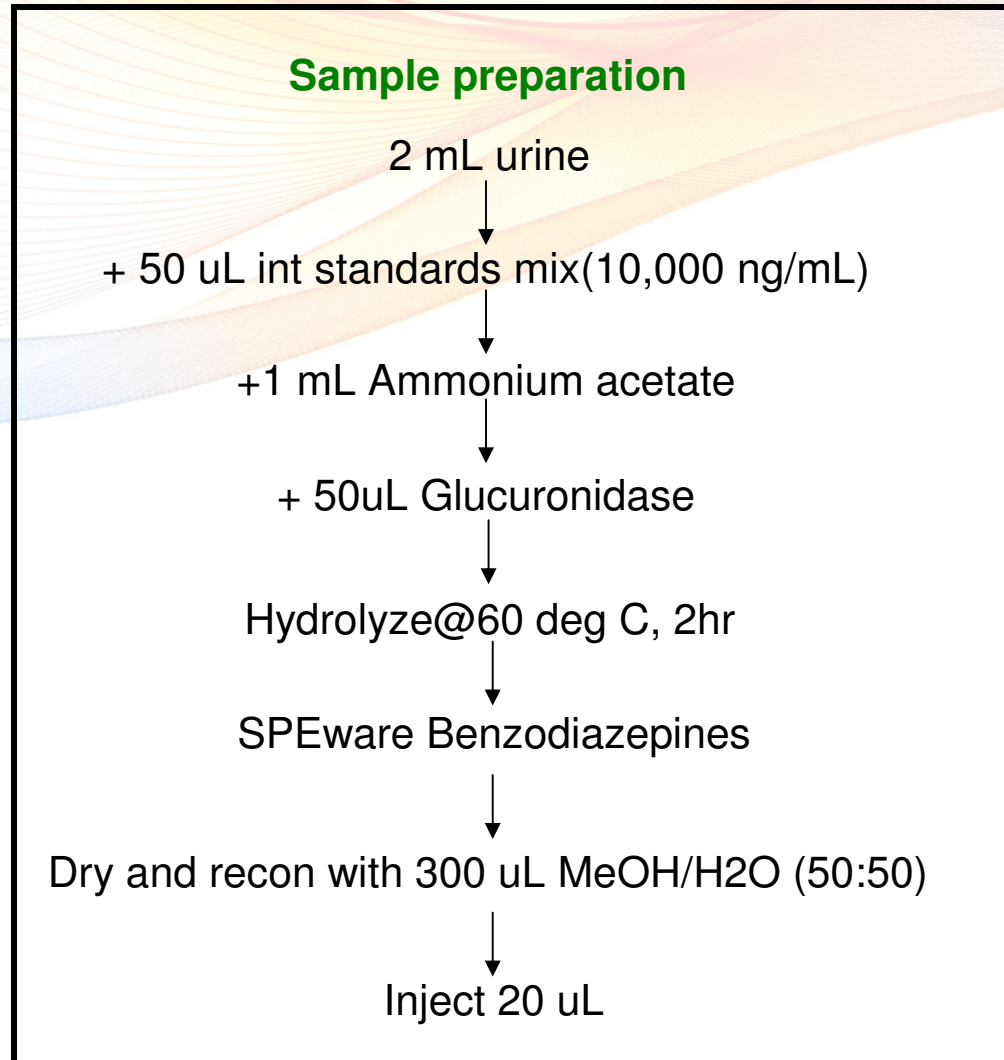


Forensic Toxicology use Only



Benzodiazepines in Urine

Standards, QC and sample preparation



Forensic Toxicology use Only

LCMS Conditions

- **HPLC method**

- 50 x 2.5 mm Thermo Hypersil PFP 5u
- Mobile Phase A 20mM Ammonium Acetate in H₂O with 0.1% Formic Acid
- Mobile Phase B 0.1% Formic Acid in ACN

- **Exactive MS method**

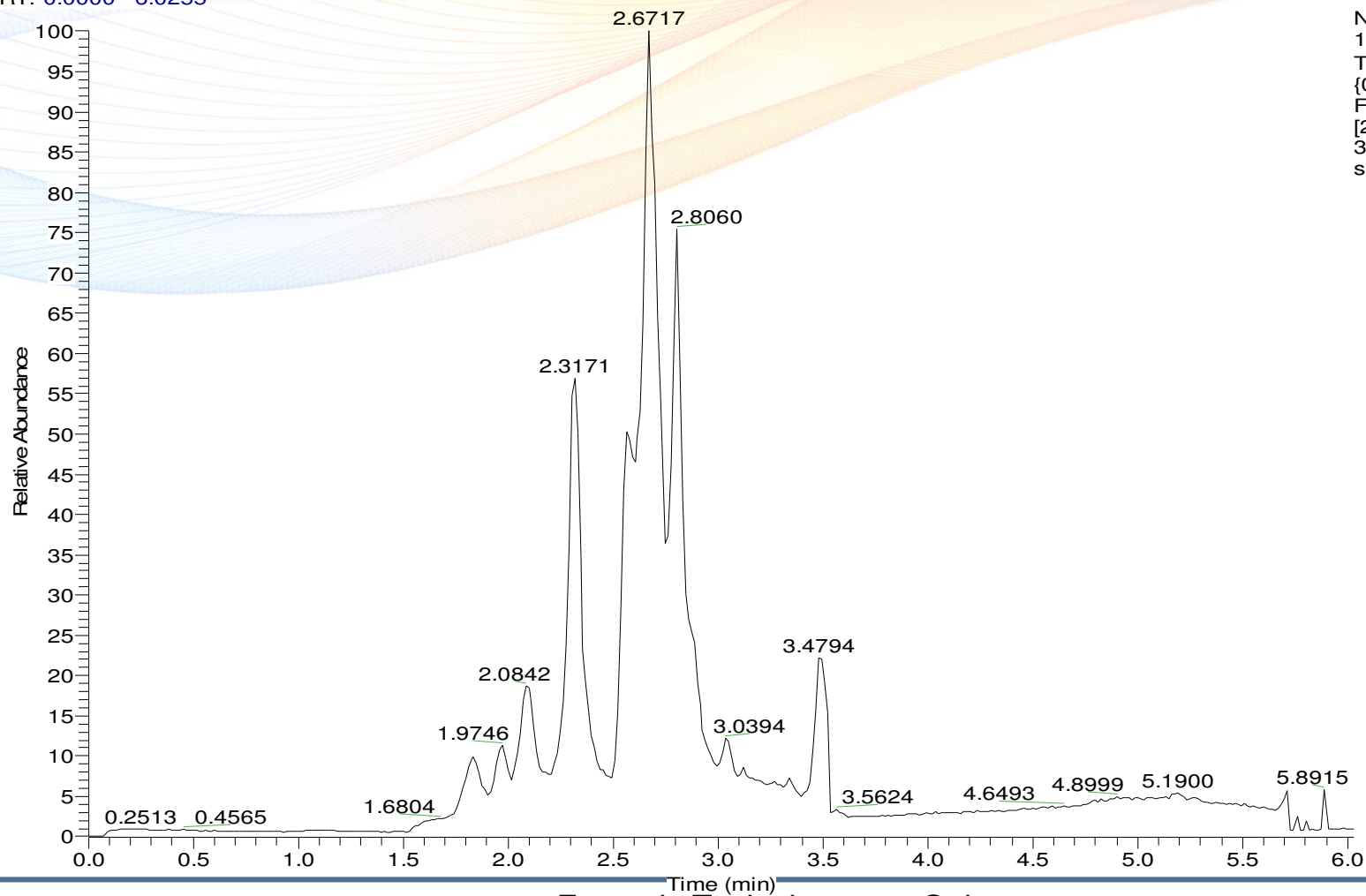
- HESI source
- Full scan MS acquisition @ 100,000 Resolution
- External mass calibration
- Extract chromatogram for drugs of interest (m/z)@ better than 5ppm mass accuracy
- Confirm the identity using mass accuracy and RT
- Quantitate using peak area in the chromatogram

10 ng/mL std mix in urine

Total Ion Chromatogram (TIC)

\\ussjo-sanserv1\Marketing\...\std10

RT: 0.0000 - 6.0255



NL:
1.29E8
TIC F: FTMS
{0,0} + p ESI
Full ms
[250.00-
370.00] MS
std10

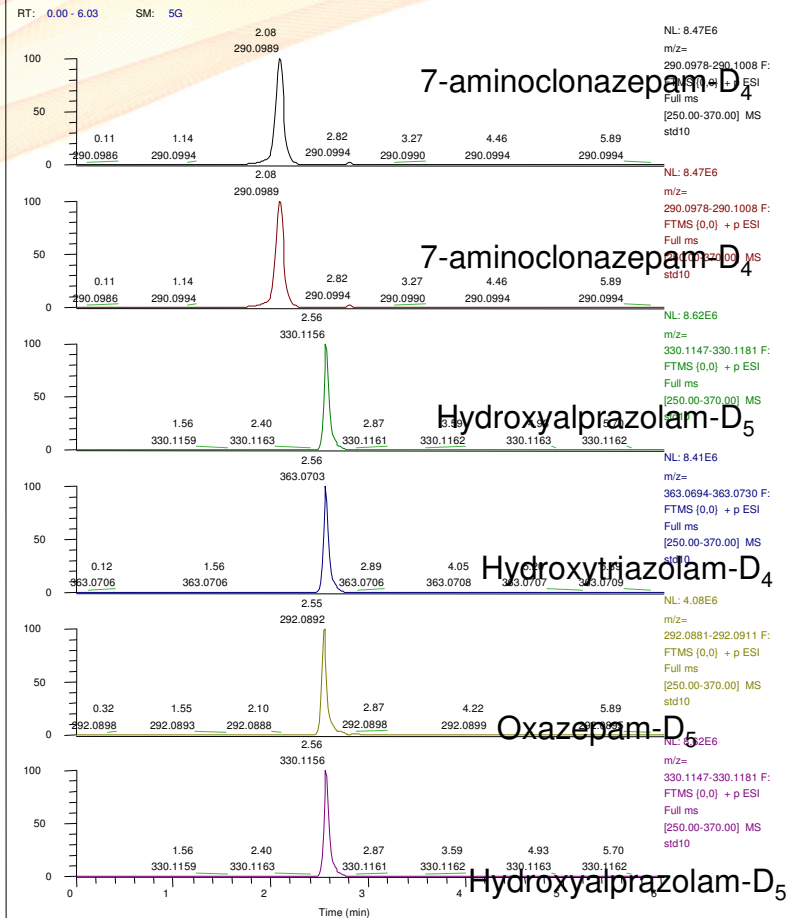
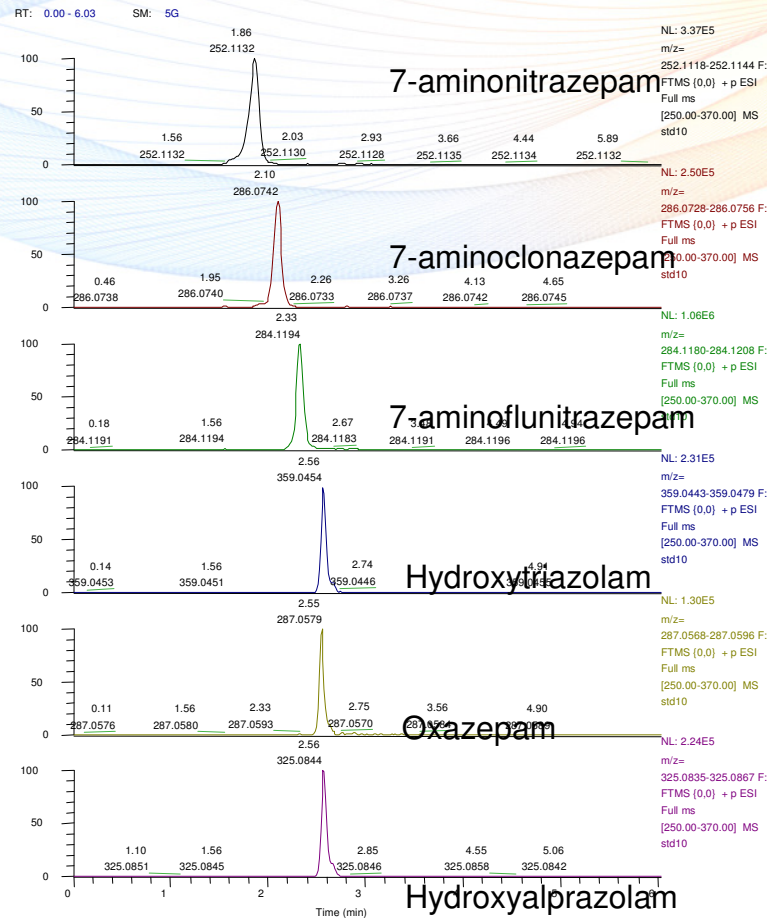
Forensic Toxicology use Only

Chromatograms 10 ng/mL standard extracted @ 5 ppm

Analyte

Highly specific detection

Internal standard



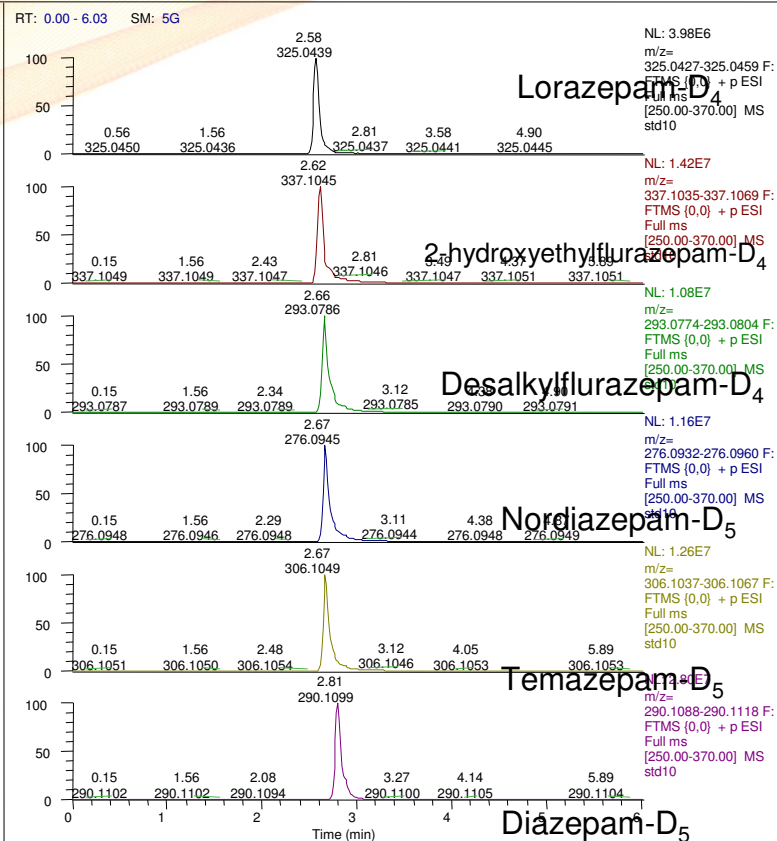
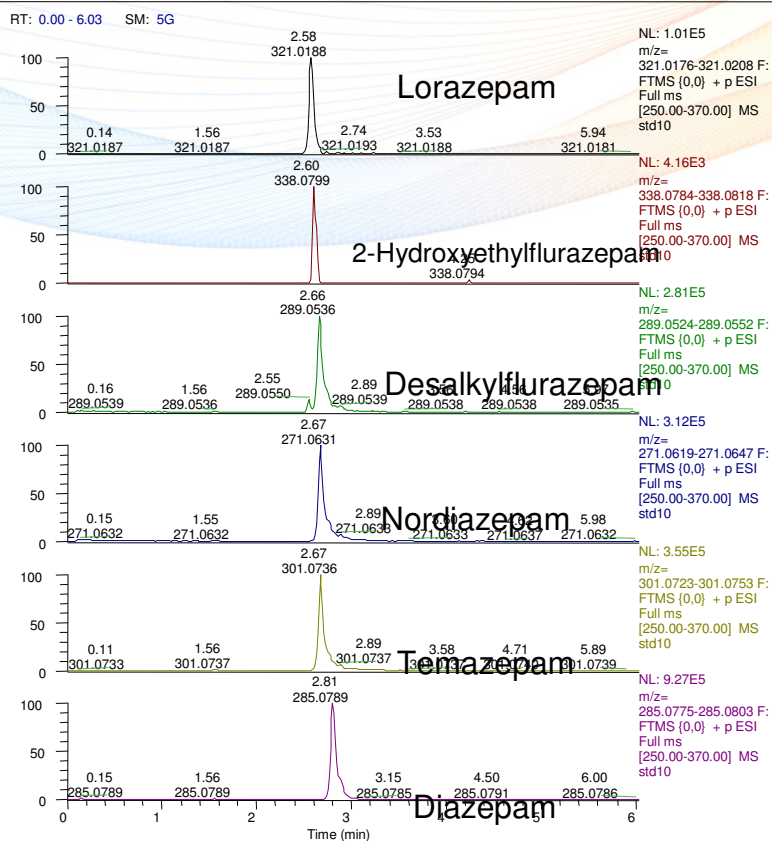
Forensic Toxicology use Only

Chromatograms 10 ng/mL standard extracted @ 5 ppm

Analyte

Highly specific detection

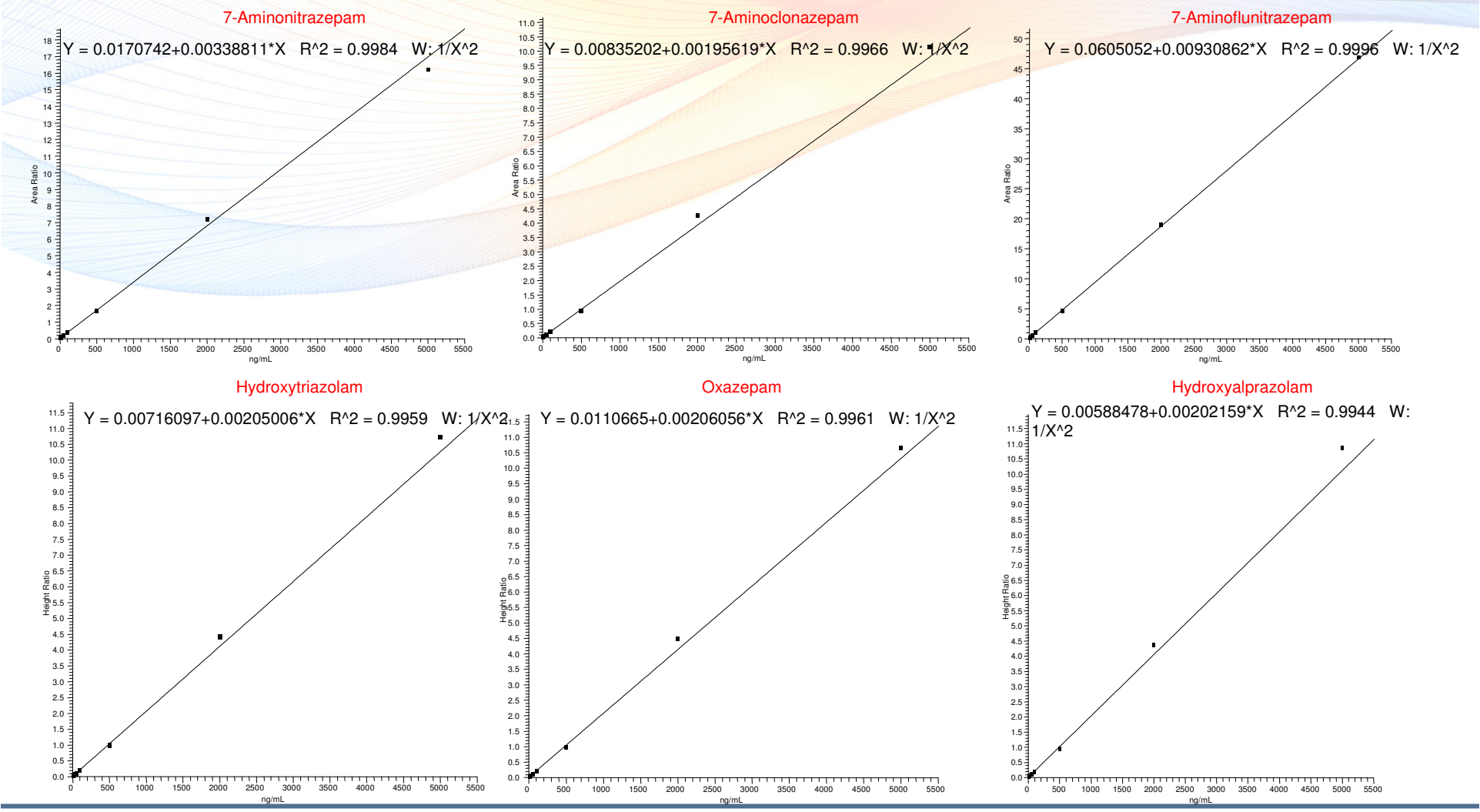
Internal standard



Forensic Toxicology use Only

Calibration curves 10-5000 ng/mL

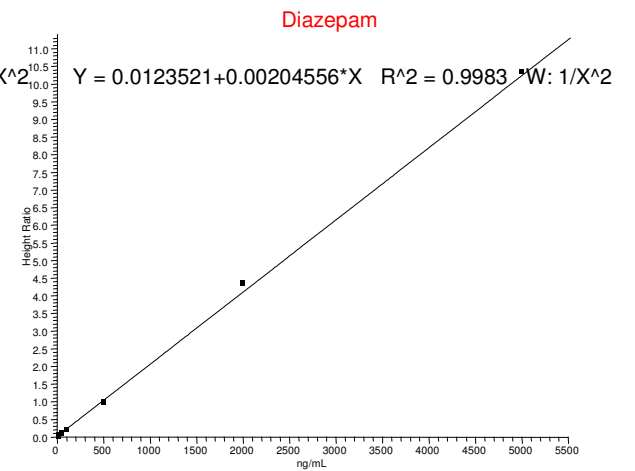
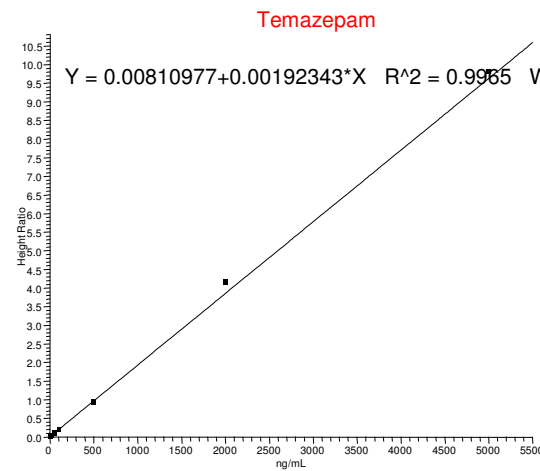
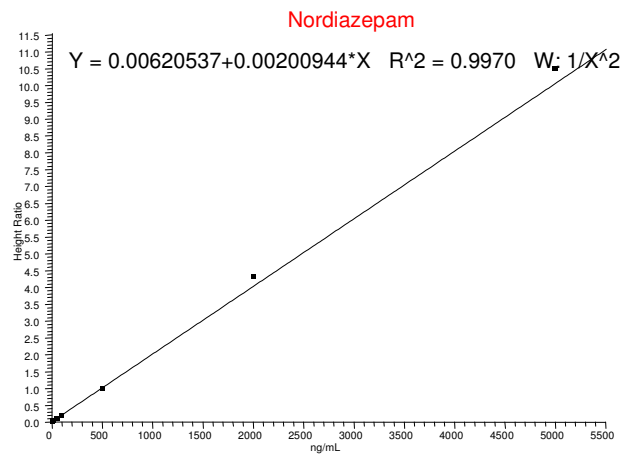
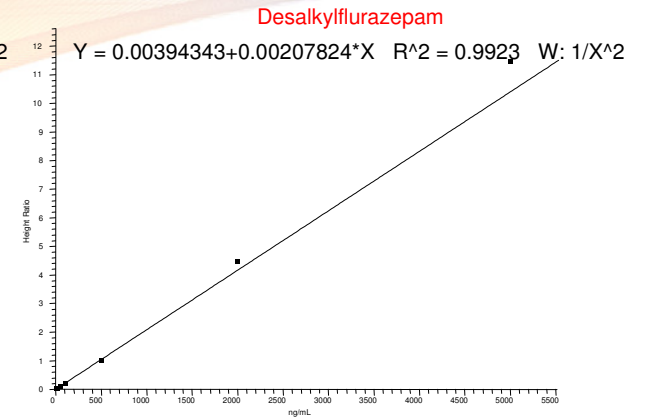
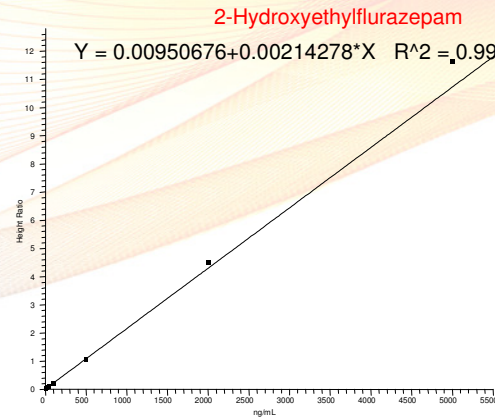
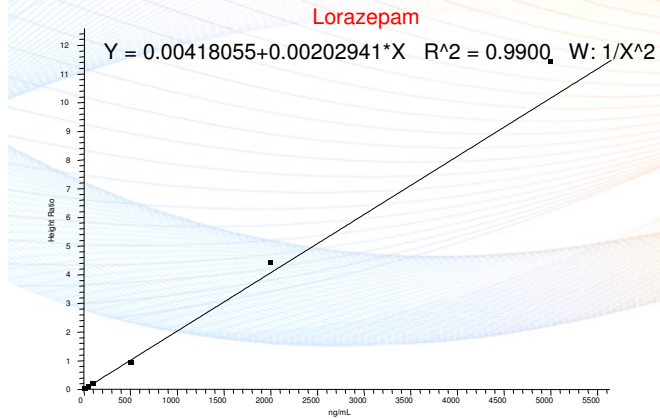
Good linearity for all compounds



Forensic Toxicology use Only

Calibration curves 10-5000 ng/mL

Good linearity for all compounds



Forensic Toxicology use Only

Calibration standards accuracy

Good accuracy for all compounds

7-Aminonitrazepam	
Standard	% Diff
10 ng/mL	-1.51
20 ng/mL	3.65
50 ng/mL	-1.33
100 ng/mL	-0.31
500 ng/mL	-1.94
1000 ng/mL	5.75
5000 ng/mL	-4.32

7-Aminoclonazepam	
Standard	% Diff
10 ng/mL	2.26
20 ng/mL	-1.94
50 ng/mL	-4.23
100 ng/mL	-4.12
500 ng/mL	-4.36
1000 ng/mL	8.66
5000 ng/mL	3.73

7-Aminoflunitrazepam	
Standard	% Diff
10 ng/mL	0.97
20 ng/mL	-1.16
50 ng/mL	-2.67
100 ng/mL	1.52
500 ng/mL	-1.08
1000 ng/mL	1.77
5000 ng/mL	0.65

Hydroxytriazolam	
Standard	% Diff
10 ng/mL	-0.65
20 ng/mL	4.58
50 ng/mL	-4.73
100 ng/mL	-6.40
500 ng/mL	-4.73
1000 ng/mL	7.34
5000 ng/mL	4.59

Oxazepam	
Standard	% Diff
10 ng/mL	1.27
20 ng/mL	0.25
50 ng/mL	-6.01
100 ng/mL	-1.10
500 ng/mL	-6.65
1000 ng/mL	8.83
5000 ng/mL	3.41

Hydroxyalprazolam	
Standard	% Diff
10 ng/mL	0.11
20 ng/mL	2.41
50 ng/mL	-2.15
100 ng/mL	-7.81
500 ng/mL	-7.83
1000 ng/mL	7.77
5000 ng/mL	7.50

Forensic Toxicology use Only

Calibration standards accuracy

Good accuracy for all compounds

Lorazepam	
Standard	% Diff
10 ng/mL	4.34
20 ng/mL	-4.90
50 ng/mL	-5.36
100 ng/mL	-7.07
500 ng/mL	-8.89
1000 ng/mL	9.25
5000 ng/mL	12.64

2-Hydroxyethylflurazepam	
Standard	% Diff
10 ng/mL	2.24
20 ng/mL	-0.58
50 ng/mL	-7.08
100 ng/mL	-5.23
500 ng/mL	-2.65
1000 ng/mL	4.80
5000 ng/mL	8.49

Desalkylflurazepam	
Standard	% Diff
10 ng/mL	5.92
20 ng/mL	-8.08
50 ng/mL	-6.10
100 ng/mL	-6.64
500 ng/mL	-2.40
1000 ng/mL	7.06
5000 ng/mL	10.25

Nordiazepam	
Standard	% Diff
10 ng/mL	1.41
20 ng/mL	0.33
50 ng/mL	-5.37
100 ng/mL	-4.81
500 ng/mL	-3.02
1000 ng/mL	7.15
5000 ng/mL	4.32

Temazepam	
Standard	% Diff
10 ng/mL	4.09
20 ng/mL	-6.44
50 ng/mL	-3.52
100 ng/mL	-1.49
500 ng/mL	-2.94
1000 ng/mL	8.25
5000 ng/mL	2.04

Diazepam	
Standard	% Diff
10 ng/mL	1.81
20 ng/mL	-1.90
50 ng/mL	-3.54
100 ng/mL	-1.33
500 ng/mL	-3.03
1000 ng/mL	6.76
5000 ng/mL	1.21

Unknown urine sample

Found hydroxyalprazolam@461 ng/mL

C:\ **Analyte**

6/22/2009 6

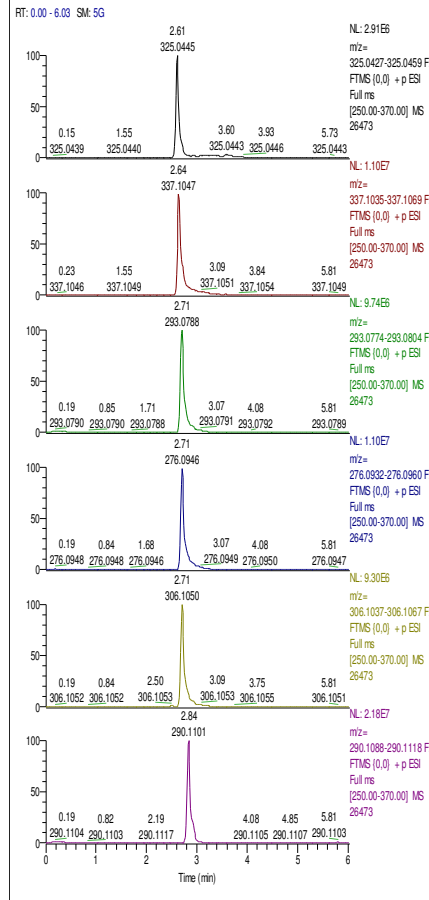
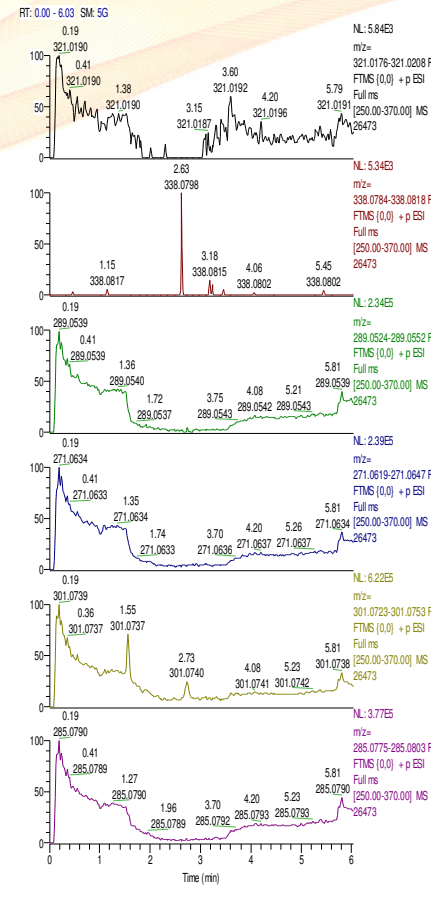
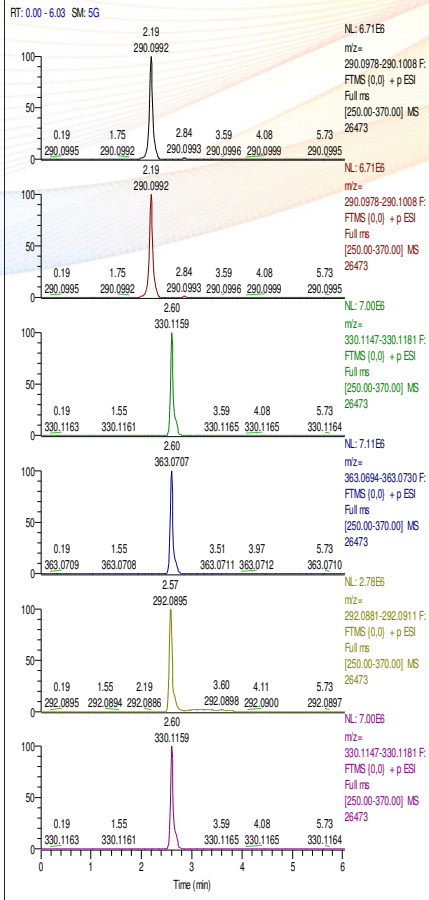
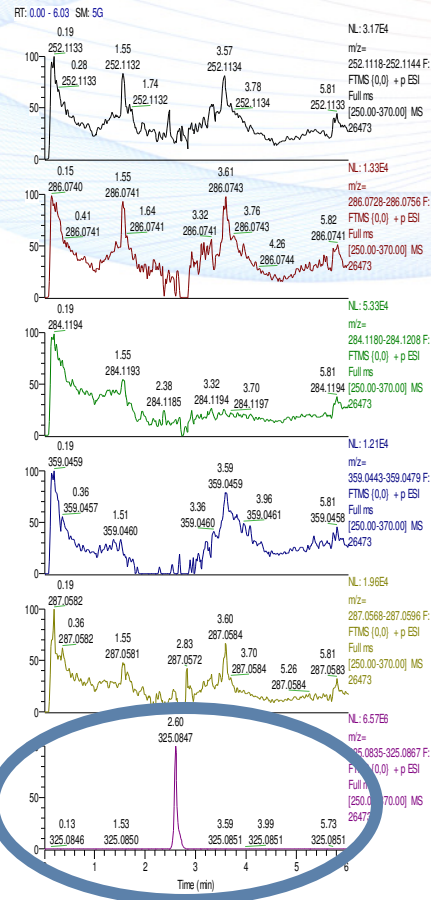
Internal standard

C:\Documents and

Analyte

6/22/2009 6:47:33

Internal standard

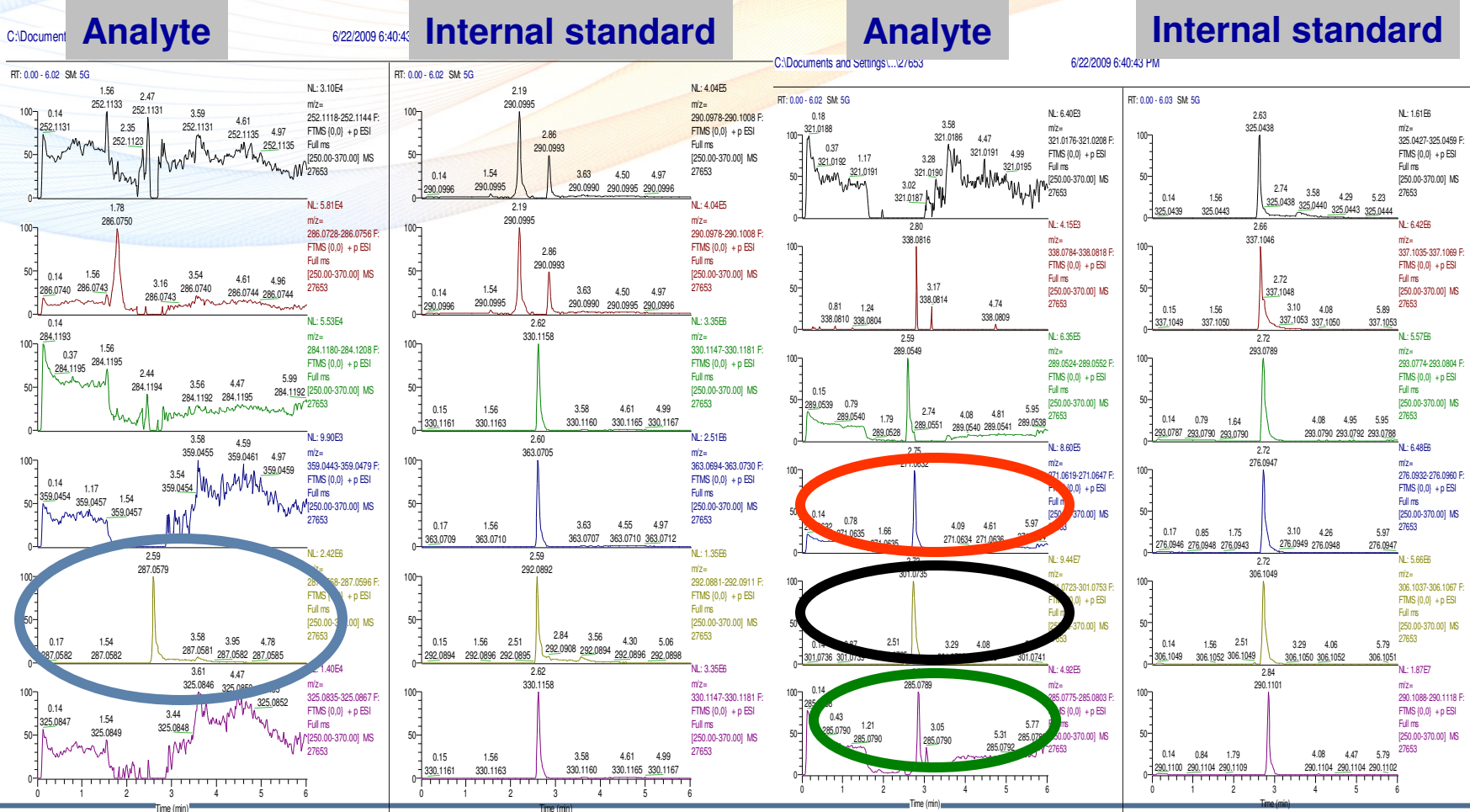


Forensic Toxicology use Only

Unknown urine sample

Found

Oxazepam@19.9 ng/mL; Nordiazepam@62.8ng/mL; Temazepam@ 8640 ng/mL; Diazepam<10ng/mL



Forensic Toxicology use Only

Conclusions

- Assay based on Exactive ultra high resolution MS coupled to LC system is
 - Well suited for accurate analysis of Benzodiazepines
 - Analysis time of 6 minutes for Benzodiazepines
 - Benzodiazepines LOQ = 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 and References

- Marta Kozak, Thermo Fisher Scientific
- Thermo Scientific; Technical, and application support
- TCI - Dihexammonium acetate
- SpeWare - Solid phase extraction columns and procedure for Benzodiazepines