A Method for Analysis of MDPV, Pyrovalerone, and Naphryrone "Bath Salts" by GC-Ion Trap MS/MS

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Bath Salts

- MDPV is a psychoactive drug with stimulant properties which act as a norepinephrine-dopamine reuptake inhibitor. In 2010 it was sold as a legal drug alternative and marketed in the US as “bath salts” – copy-cat cocaine, synthetic speed.
- Naphyrone also known as O-2482 or NRG-1 is a drug derived from Pyrovalerone that acts as a triple reuptake inhibitor producing stimulant effects and has been reported as a novel designer drug.
- Pyrovalerone has surfaced in head shops in the United Kingdom first and now in America, being sold as bath salts. Pyrovalerone is a Schedule V controlled substance in the United States (U.S.), and is the only stimulant in that category. In Britain, it is in the Class C category and In Australia it is a Schedule 4 substance. It is closely related on a structural level to a number of other stimulants, such as MDPV

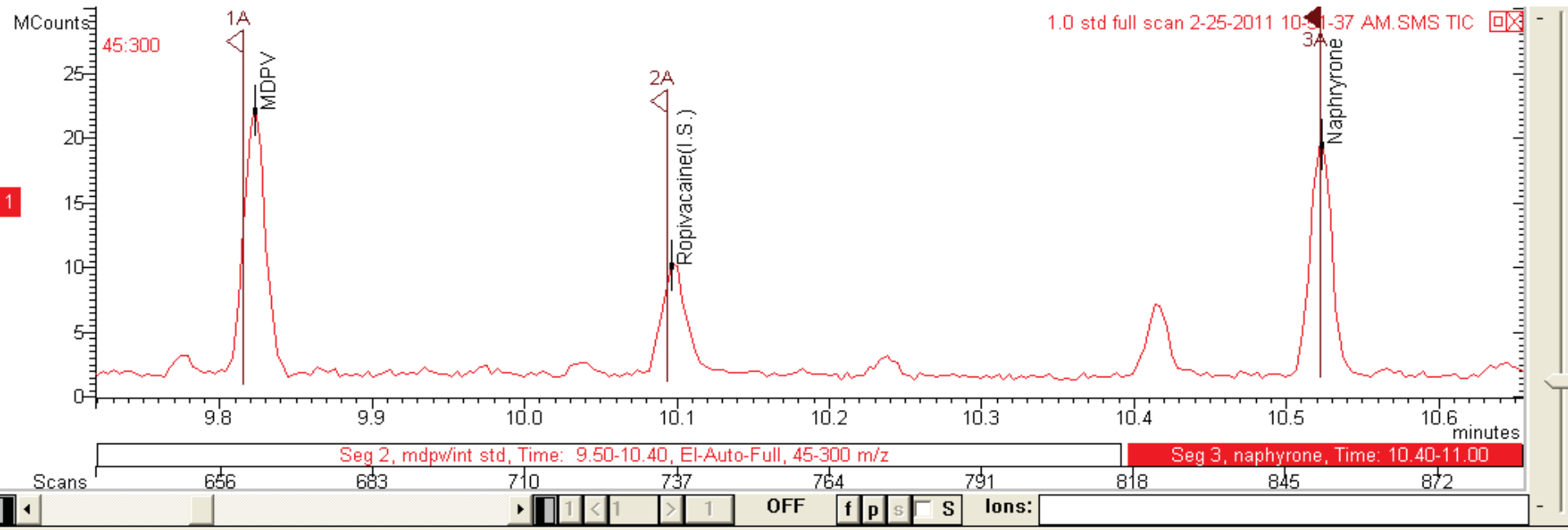
Bath Salts

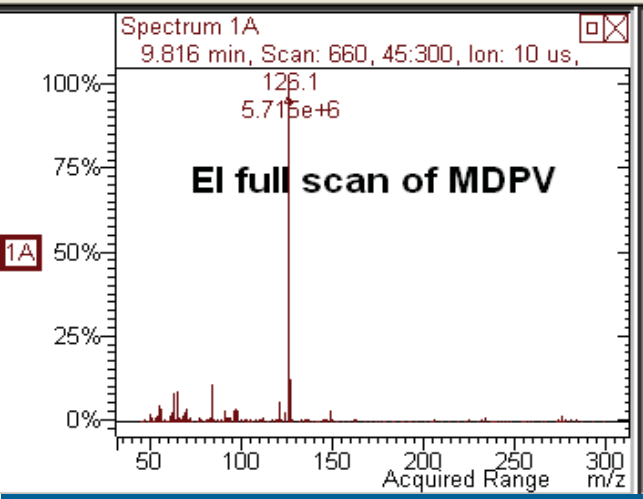
- All compounds were found to have a characteristic precursor ion of 126 m/z in EI, but during the collision induced dissociation (CID) each generated a unique, full scan product ion spectrum.
- The specific product ion spectrum along with retention times were used for compound identification and quantification.
- The EI/MS/MS product ions monitored were 84,124 and 97 for Pyrovalerone, MDPV and Naphyrone; 84, 98 and 56 for the internal standard.
- The CI/MS/MS product ions monitored were 135/175 MDPV, 126 for the IS, and 211/141 for Naphyrone, 175/105 for Pyrovalerone.

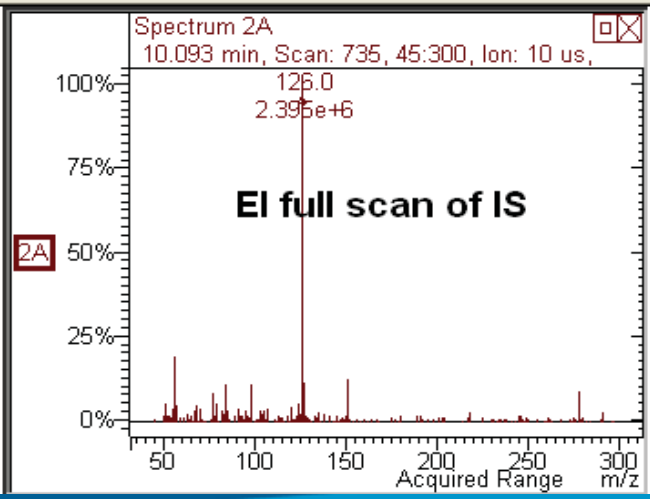
Which MS Solution? Basic questions:

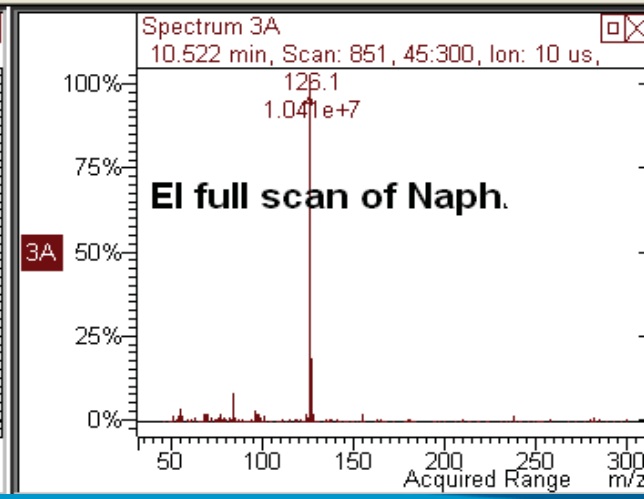
- Target analysis only?
  - Scan or SIM or MS/MS
- Analysis of unknowns?
  - Scan with Quad, IT or TOF
- Matrix?
  - Clean
    - Full Scan with Quad or Ion Trap
  - Complex (heavy)
    - Deconvolution, MS/MS, Q-TOF

The Challenge of Full Scan or SIM









Objectives:

- Confident identification and quantitation of Methylenedioxypyrovalerone (MDPV), Naphthylpyrovalerone (Naphyrone), Pyrovalerone in blood, urine, vitreous, gastric, and tissue matrices using GC-MS/MS methodology.
- Several techniques were tried to find the optimum method.
- EI scan, EI/MS/MS, CI/MS/MS, CI/MS/MS/MS

Many Changes in Ion Traps Since 1990

- Control of Space Charging (Correct use of AGC and AM)
- Better Ion Trap Temperature Control
- Constant Helium Pressure for Internal Ionization
- Elimination of Mass Calibration Errors
- Improved Electrode Geometry
- Improved Auto-Tune (and Use of Multi-Segment Scans)
- SIS Sensitivity (Increased Storage of Analyte Ions)
- MS/MS Sensitivity and Selectivity
- SilChrom Inert Electrodes
- Faster, Higher Resolution Scan Function (240)
- Wider Mass Range (240)

Simple and Reliable Hardware

With just a few pieces, electrodes and spacers are easy to clean and assemble.

The pre-aligned electron multiplier makes replacement quick and easy when needed.

•The 220-MS does not require frequent cleaning since it does not have lenses and other components of an external ion source. Occasional cleaning is easy because the analyzer can be removed from the MS without tools in less than two minutes.

Dual filaments allow simple and fast switch over for better productivity.

Holds tune for extended periods (months) of time!

For Forensic Use.

Internal Ionization Ion Trap MS/MS Features

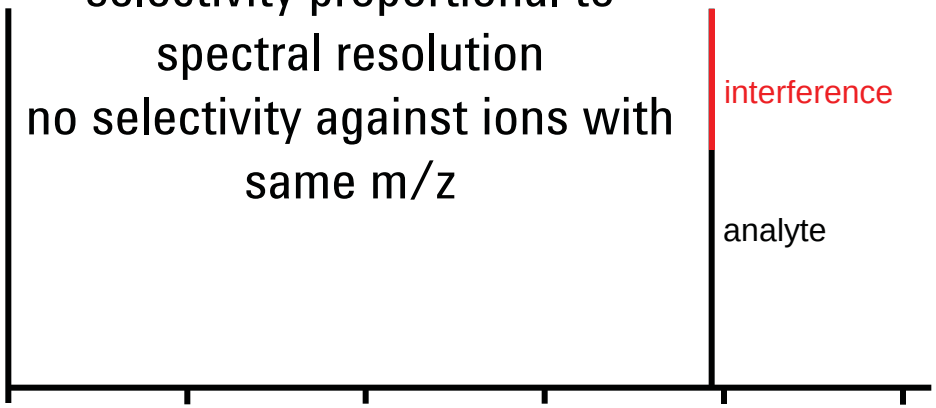
- PCI-MS/MS
  - Great sensitivity and selectivity for MS/MS precursor
  - Liquid reagents are a real and significant benefit to low pressure, internal ionization PCI
- Product Ion Spectrum – Maximum Information Content
  - All product ions from the analyte of interest
  - Also product ions from isobaric interference with precursor ion
- MS/MS – Always More Selective Than SIM
  - But not necessarily more sensitive than SIM
  - Critical question: how bad is the chemical noise from matrix?

Why MS/MS?
Greater Selectivity Than SIM or Scan

EI-SIM

selectivity proportional to spectral resolution

no selectivity against ions with same m/z

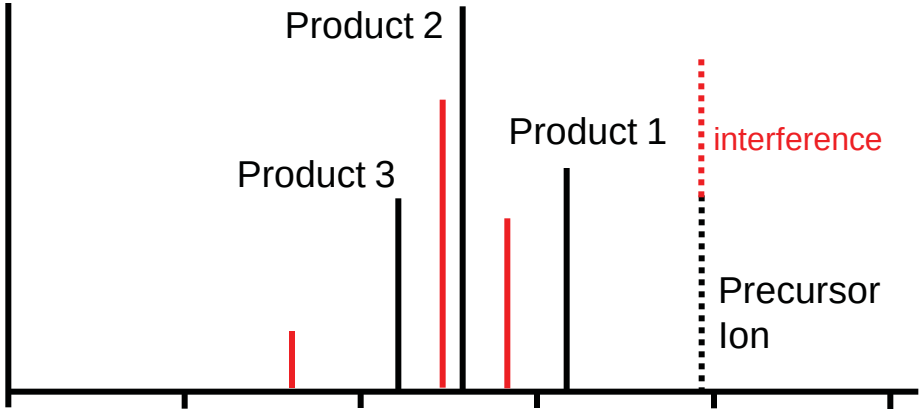


unit mass resolution

EI-MS/MS

Precursor selectivity same as SIM

High probability that at least one product ion will be a unique dissociation product of the precursor BUT not the interference



The precursor ion should ***NOT*** be used for ion ratios or quantitation since the interferences will be the same as the SIM ion

New Agilent 7890A/220 and 7890A/240 Systems

Internal EI/CI

MS/MS

For Forensic Use.

GC-MS/MS Conditions

GC:
Injector -250 C

GC-Column – DB-5 phase , 30 m x 0.25 x 0.5

Oven Program:
70 °C/min. hold for 1.0 min.
25 °C/min to 310 °C hold for 4.4 min.

Injection Volume 0.50 uL, splitless.

Column flow 1.3 mL/min.

MS:
Transferline 310 °C.

Trap 210 °C.

All compounds were found to have a predominate ion at m/z 126 to use as the Precursor ion. The MS/MS CID voltages were optimized using the Automated Method Development (AMD) tool in the acquisition software.

EI MS/MS Mode: MRM Transitions

Name	Precursor	Product	Collision Energy
MDPV	126	84,124	0.50 Volts
Naphyrone	126	84, 124	0.50 Volts
Ropivacaine (IS)	126	84, 98	0.50 Volts

Sample Preparation

Calibration samples were prepared in comparable matrix with Pyrovalerone, MDPV, Naphyrone, internal standard (Ropivacaine) and extracted with a basic liquid-liquid extraction along with unknown samples and spiked controls. (blood and urine matrix)

Normal Dilutions were done, Blood diluted three-fold, liver four-fold, and urine ten-fold.

Calibrator	Working Standard	Negative Matrix
50 ng/mL	0.010 mL	3 mL
100 ng/mL	0.020 mL	3 mL
250 ng/mL	0.050 mL	3 mL
500 ng/mL	0.100 mL	3 mL
1000 ng/mL	0.200 mL	3 mL

1.In culture tubes pipet 3.0 mL of each sample and control. Add 50 uL of the working internal standard and 2 mL of pH 9.8 Carbonate Buffer. Add 2 drops of NH4OH and vortex gently to mix.

2. Add 7.0 mL of n-butyl chloride to each tube and rotate all tubes for at least 10 minutes.

3.Centrifuge all tubes at 3000 RPM for 10 minutes.

4. Transfer organic layer to a second culture tube and add 2 drops of 0.1% methanolic HCL and evaporate to dryness at 37 C with nitrogen.

5. Reconstitute dried extracts with 200 uL of ethyl acetate and transfer to ALS vial.

6. Inject 0.5 uL into GC/MS/MS for analysis.

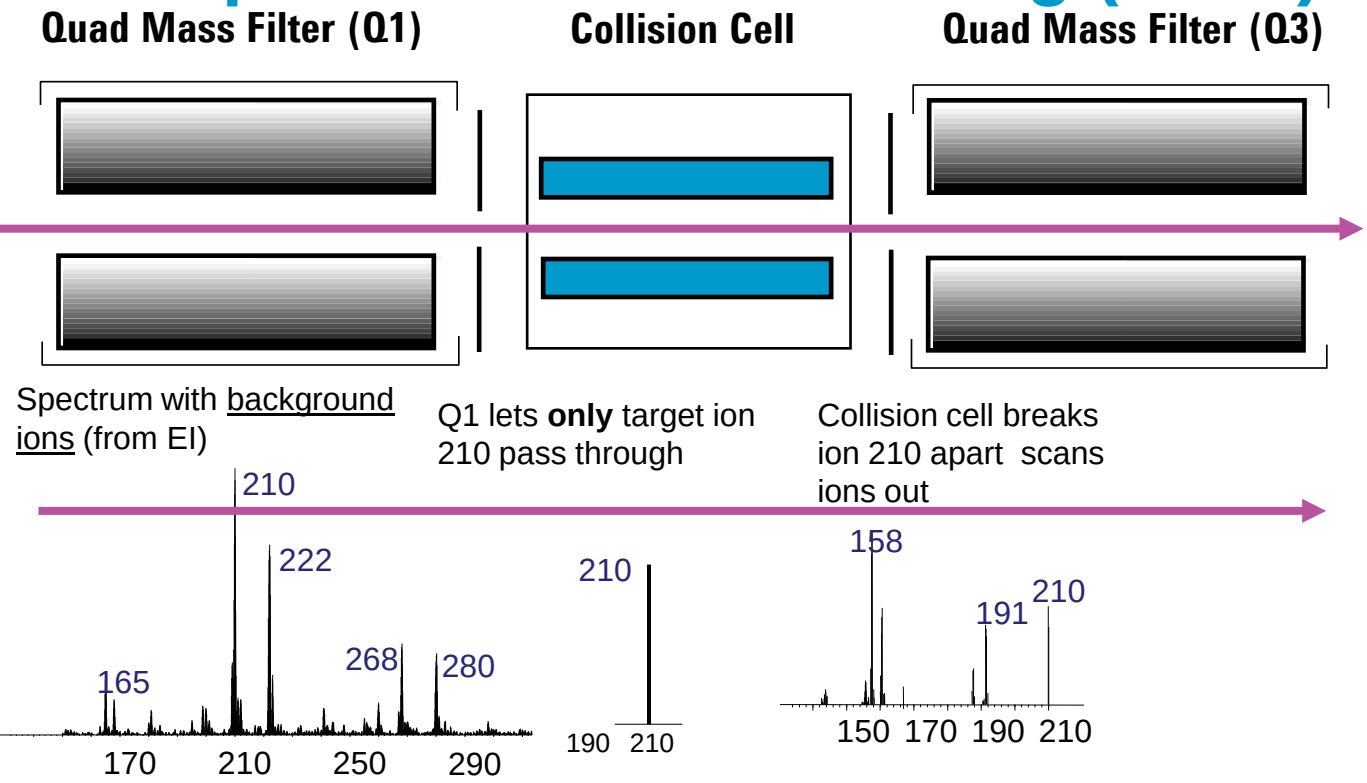
MS/MS

- Why MS/MS
- Steps in the MS/MS process
- Examples of EI/MS/MS and CI/MS/MS with real world samples

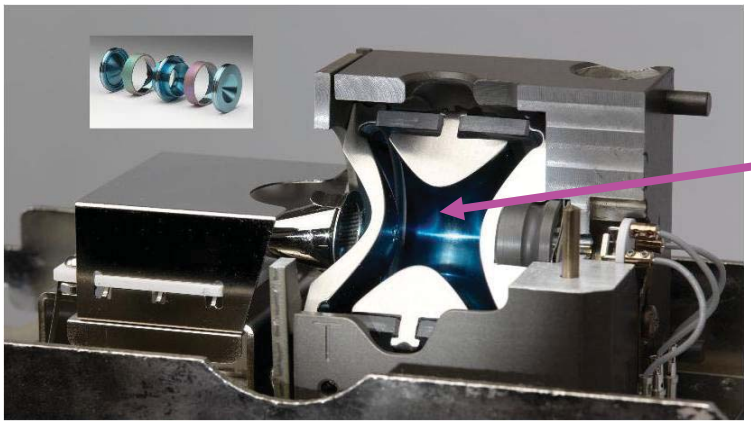
Steps in the MS/MS Process

1) Ionize Analytes using EI or CI
2) Isolate Parent Ions
    1) This process is like Selected Ion Monitoring (SIM) or Selected Ion Storage (SIS)
3) Dissociate Parent Ions
    1) Collision Induced Dissociation (CID) - collisions with inert atoms (Nitrogen or He)
    2) User must determine collision energy experimentally
4) Mass-Analyze Product Ions
    1) This is same process used in collecting a full-scan mass spectrum

Multiple Reaction Monitoring (MRM)



Ionize
SIM Ion 210
CID Ion 210
SIM of Product ions(s)



Ionize
Isolate Ion 210
CID Ion 210
Full Scan

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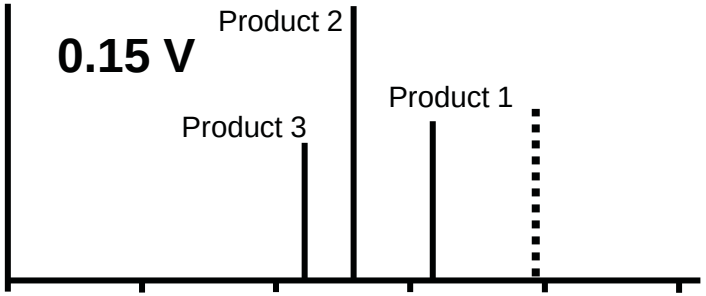
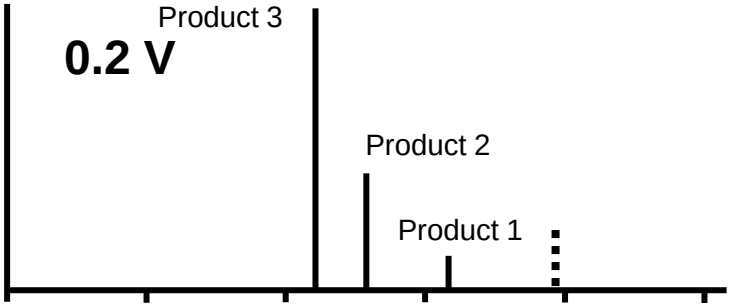
Ion Trap - Use of automated methods development (AMD)

Few Product Ions

More Product Ions



Optimum Product Ions



Optimum CE Product Ion Spectrum

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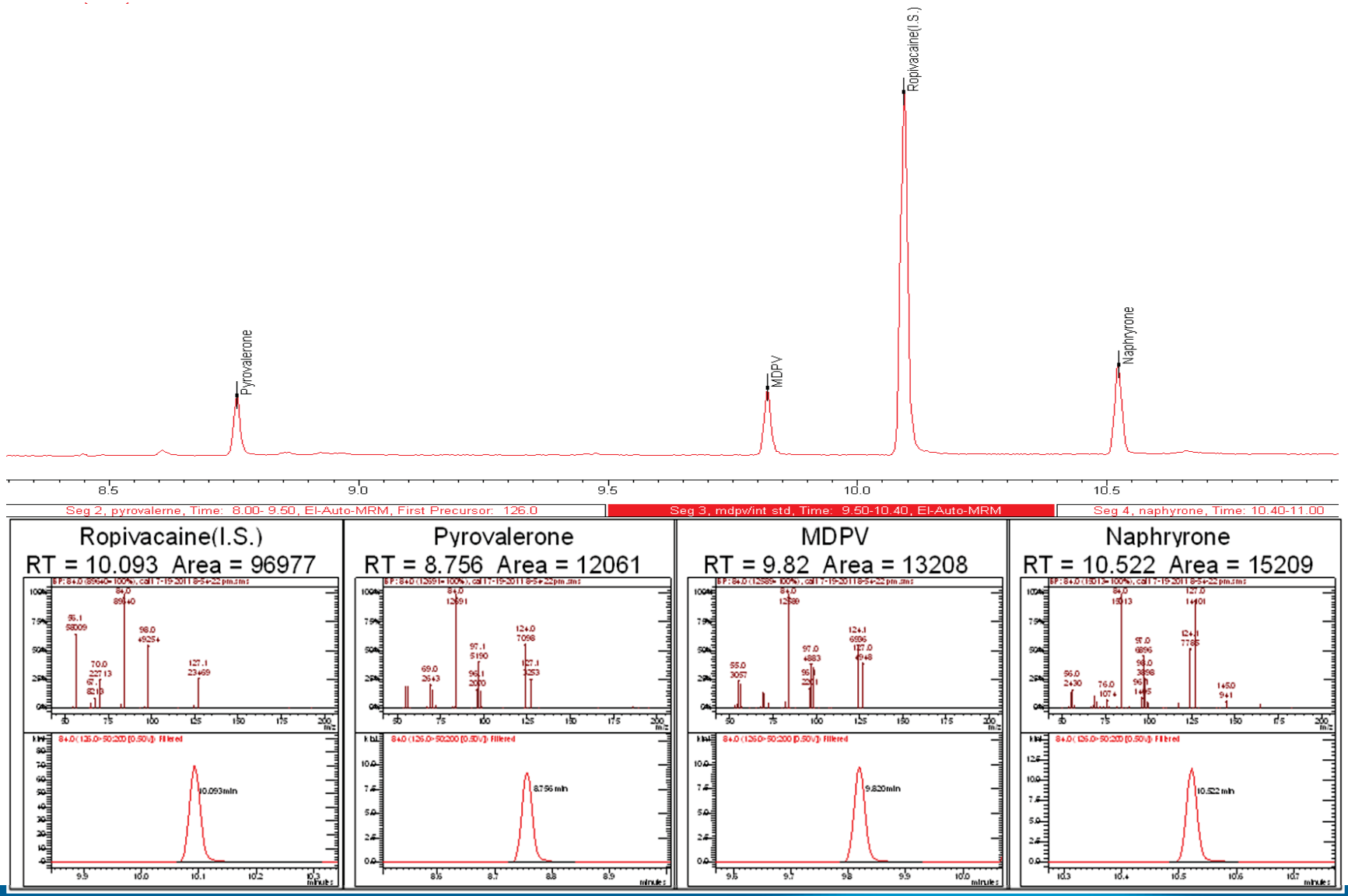
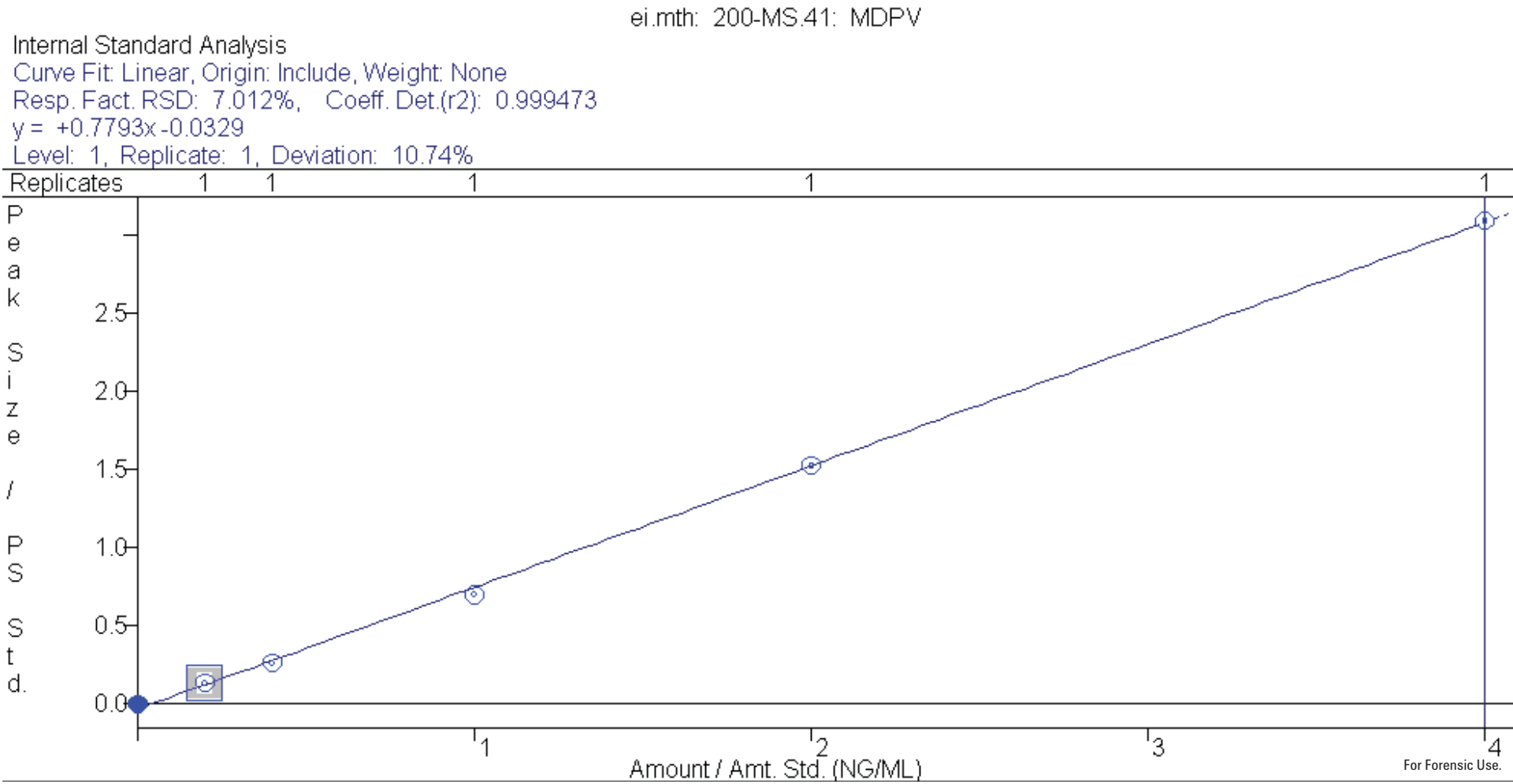
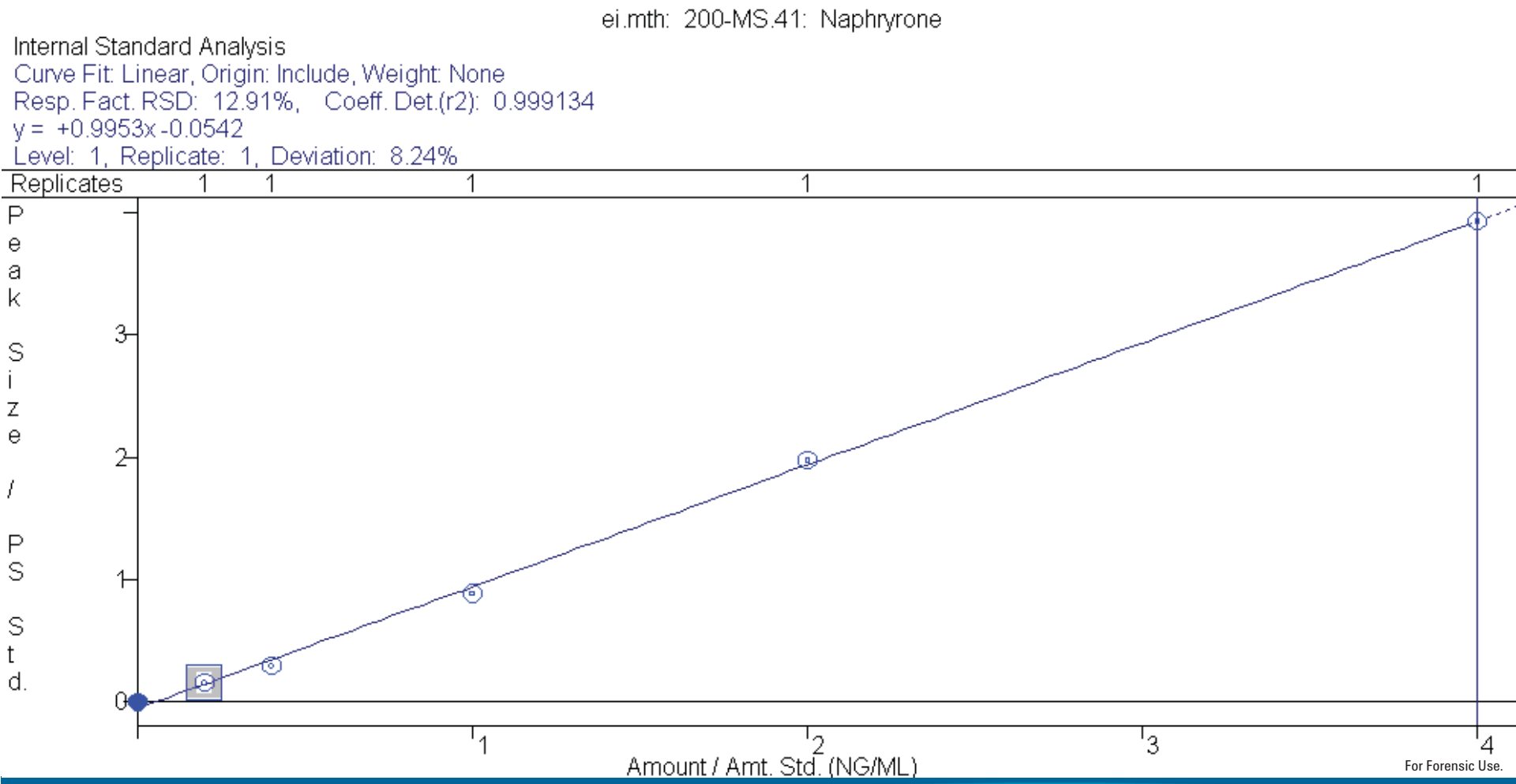
EI/MS/MS TIC for 50 ng/mL more ions for validation



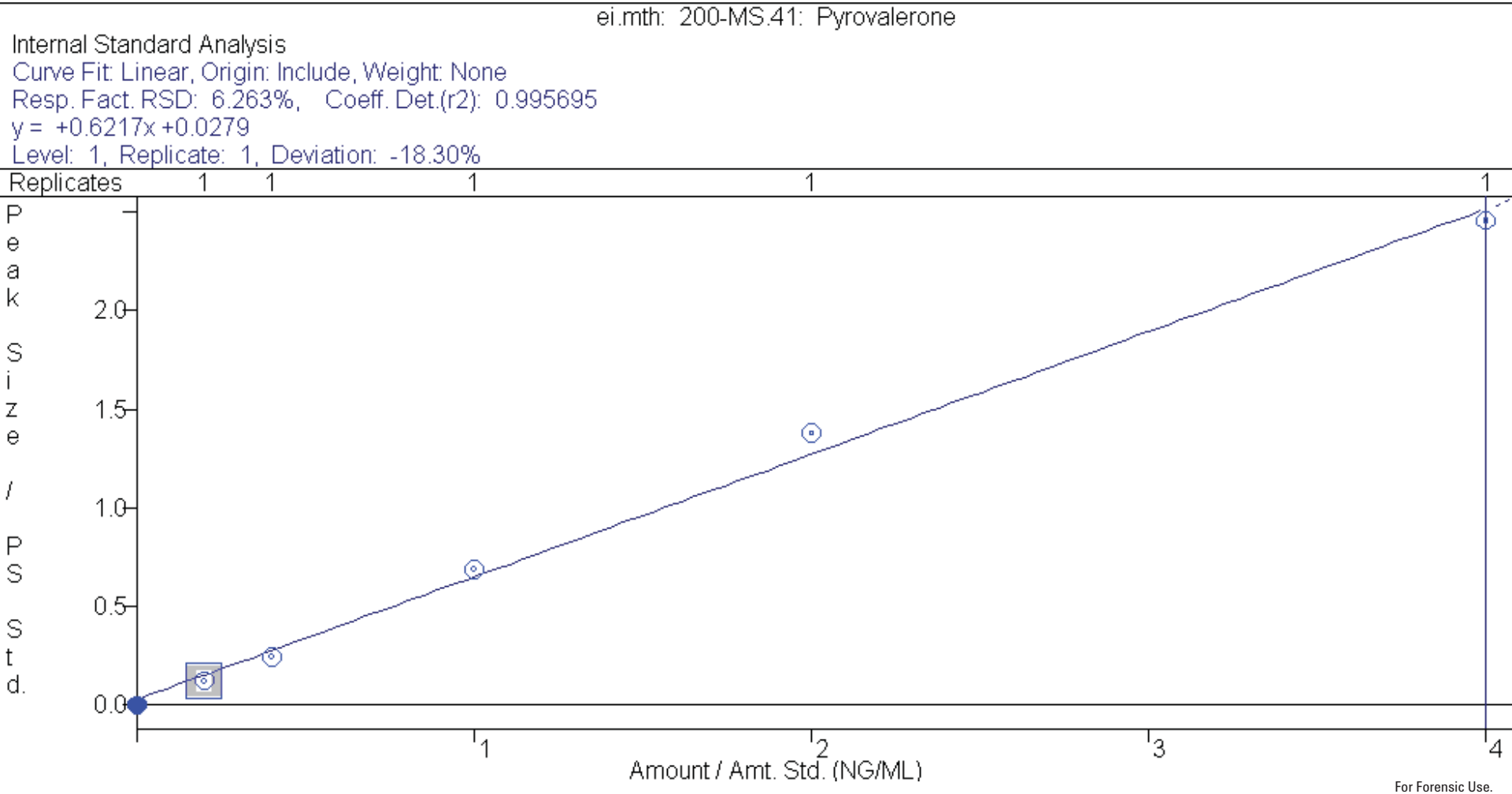
Calibration Curve for MDPV 50.0 ng/mL to the ULOL at 1000 ng/mL.



Calibration Curve for Naphyrone 50.0 ng/mL to the ULOL at 1000 ng/mL.



Calibration Curve for Pyrovalerone 50.0 ng/mL to the ULOL at 1000 ng/mL.



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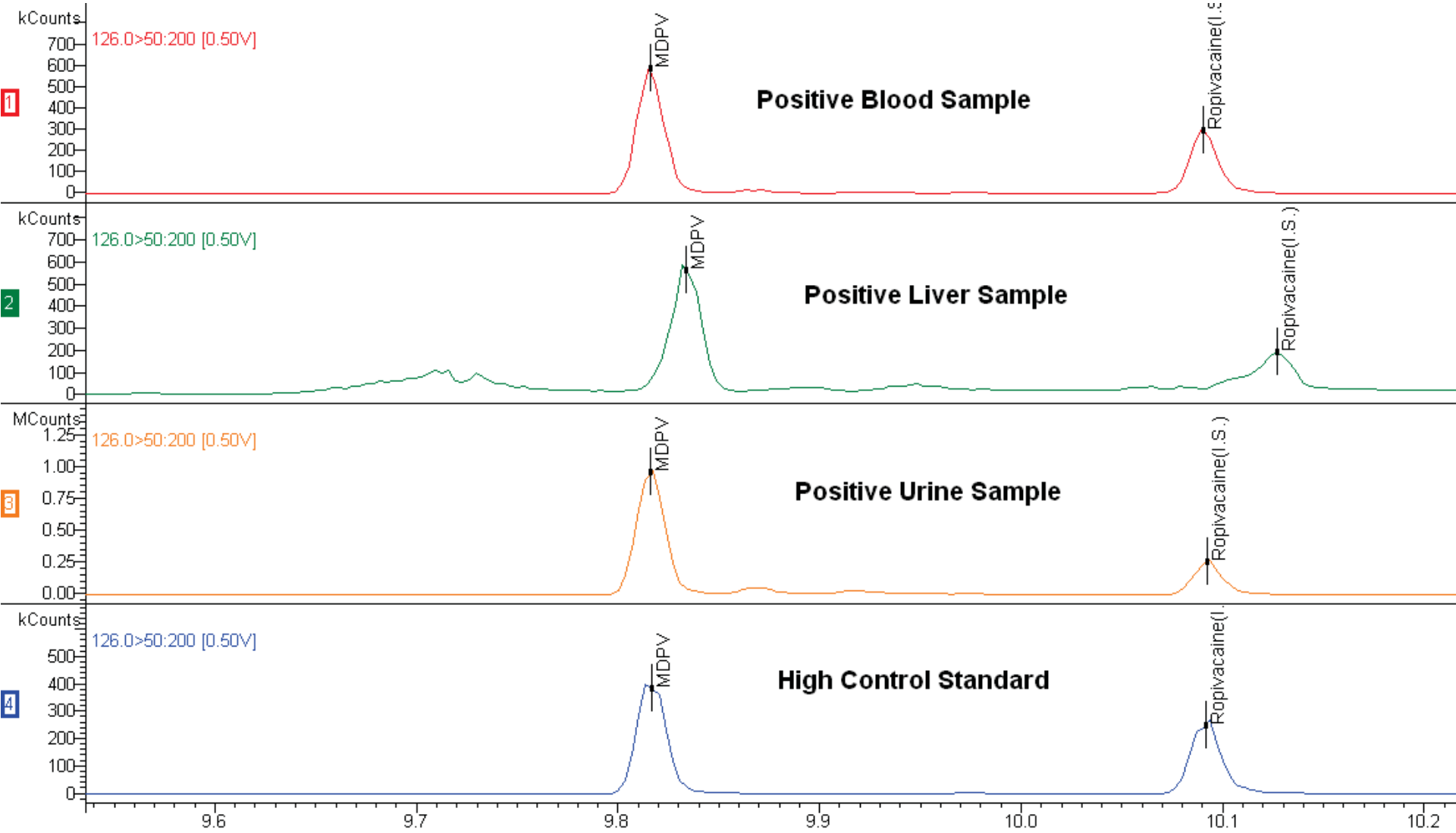
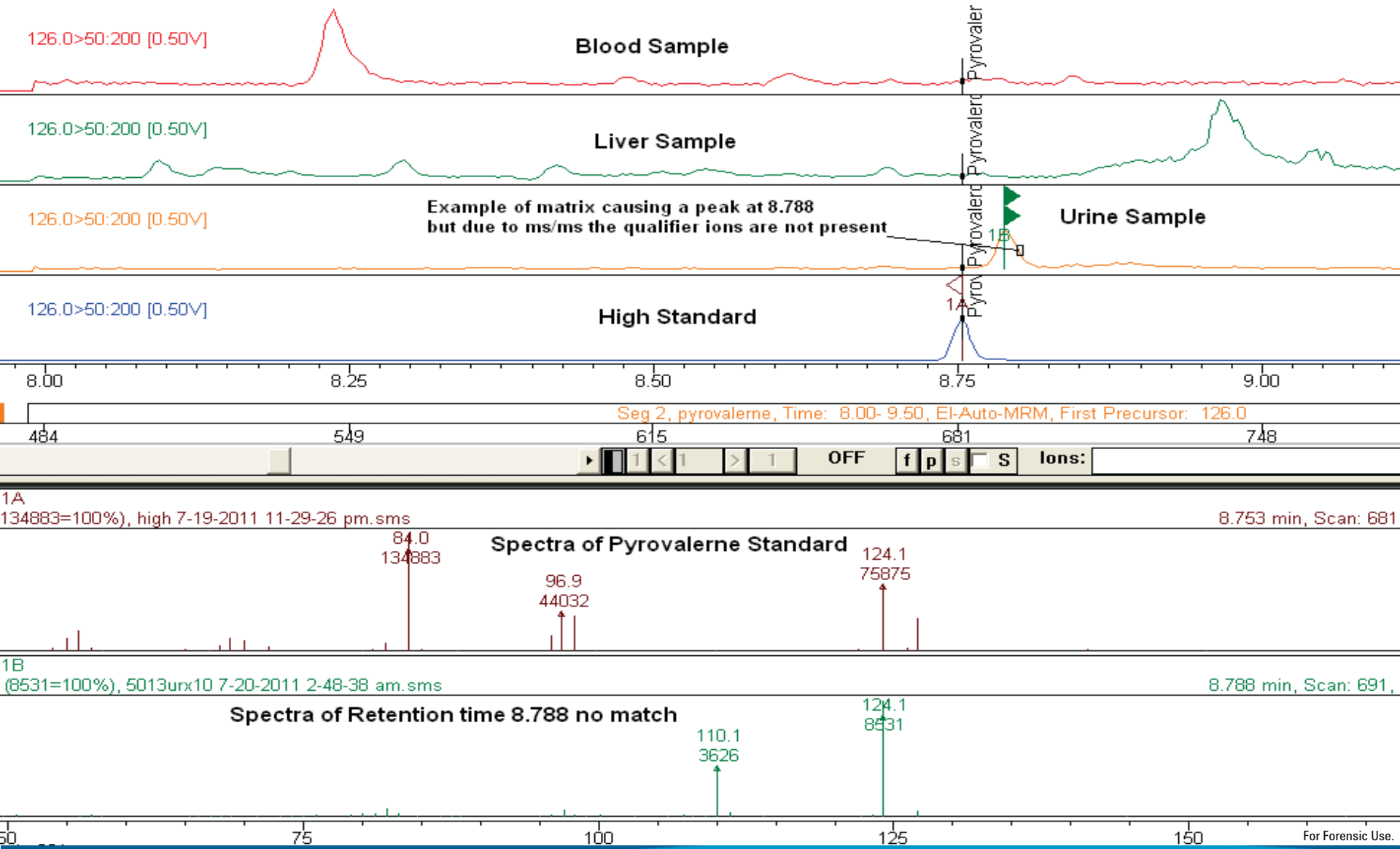
EI/MS/MS Positive Results for MDPV



EI/MS/MS Negative Results for Pyrovalerone



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The imprecision for both intra and inter-assay analysis was determined to be less than 5 % for all sample types.
The statistical LOQ values for matrices:

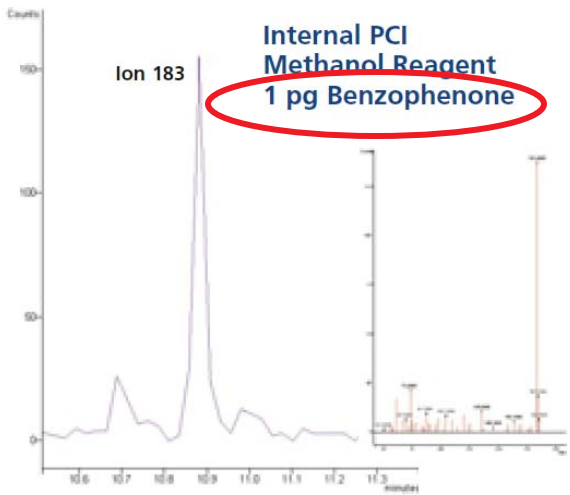
	std dev	std dev		
MDPV	**Inter**	**Intra**		**LOQ ng/mL**
number	**25**	**5**		
blood	2.825	1.073		0.2369
brain	4.63	0.72927		0.2558
liver	2.494	2.583		0.9263
Naphyrone	**Inter**	**Intra**		**LOQ ng/mL**
number	**25**	**5**		
blood	1.943	2.45086		0.2291
brain	2.79107	0.85757		0.0557
liver	1.04588	0.71814		0.6768

Seven replicates were run at 50 ng/mL with CV(method precision) of 5.4% for MDPV and 4.2% for Naphryone in urine extracts.

Internal Ionization Ion Trap – PCI another level of Confirmation

- Internal PCI (Low Reagent Gas Pressure-Liquid CI)
  - High sensitivity
  - Long residence of trapped ions increases opportunity for CI reactions
  - 80 L/s turbo compatible
  - No hardware change from EI to PCI; switch in same run
  - No contamination of source as with methane and isobutane
  - Liquid reagents are a real and significant benefit
    - Safer and cheaper than compressed gases
    - S/N still very high
    - Clever experiments run with deuterated and other specialty reagents
      - Selectivity extended by these reagents

The most sensitive positive CI system



The most sensitive PCI. *Generate full scan PCI results in the single picogram range.*

Unique Liquid CI capability

Safety, Cost, Convenience – No Hardware Changeover from EI to CI

No Requirement for Heating or Purging

Highly Selective Chemical Reactions

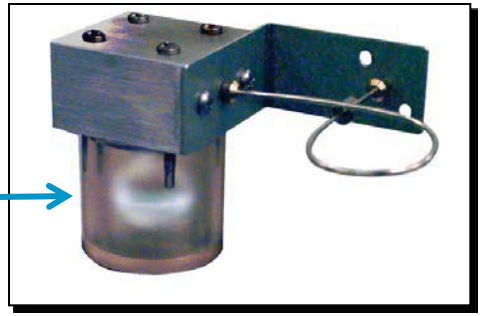
Wide Range of “Hard” to “Soft” Reagents

Selective Reactions with Target Structures

Unique Adduct Ions For MW Confirmation
Predictable Shifts in m/z (M+17, M+29, or M+41)
Numerous Liquid Reagents

ACETONE	METHANOL
DIETHYL ETHER	WATER
ACETONITRILE	CARBON DISULFIDE

Liquid CI

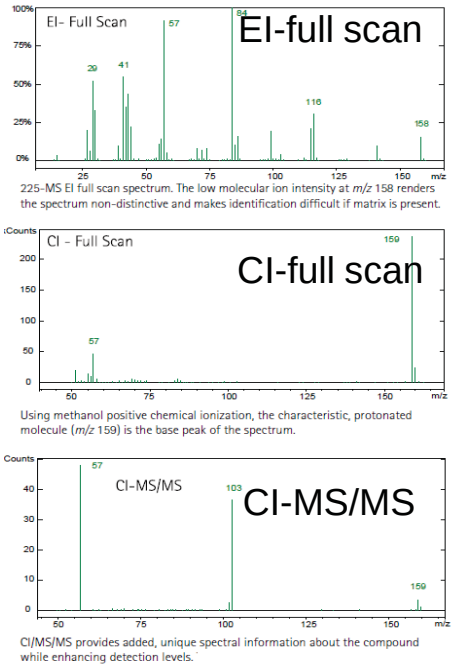


5 – 6 ml Vial with POLYSULFONE SHIELD

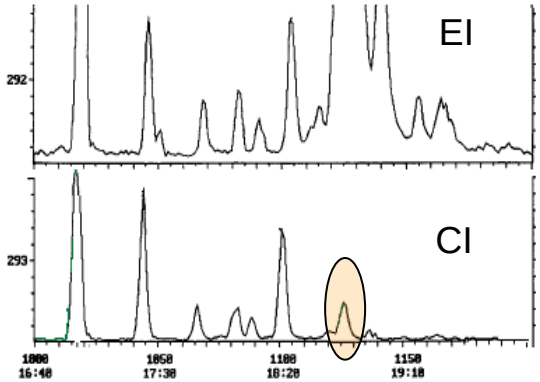
Also traditional gas reagents

Increase Selectivity with CI-MS/MS

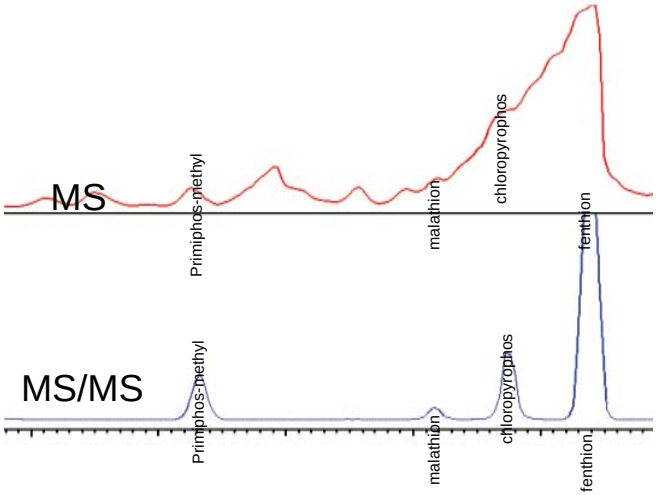
Chemical ionization by nature is a selective ionization technique, ionizing only species amenable to protonation, not generating signal from hydrocarbons and other common background contaminants, thereby reducing the matrix effect. It also “concentrates” the ion intensity into ion M+1, creating the ideal precursor ion for MS/MS, which further reduces the background matrix, increasing the overall detectivity. The ion trap has the unique ability to use the vapor from liquids for chemical ionization.



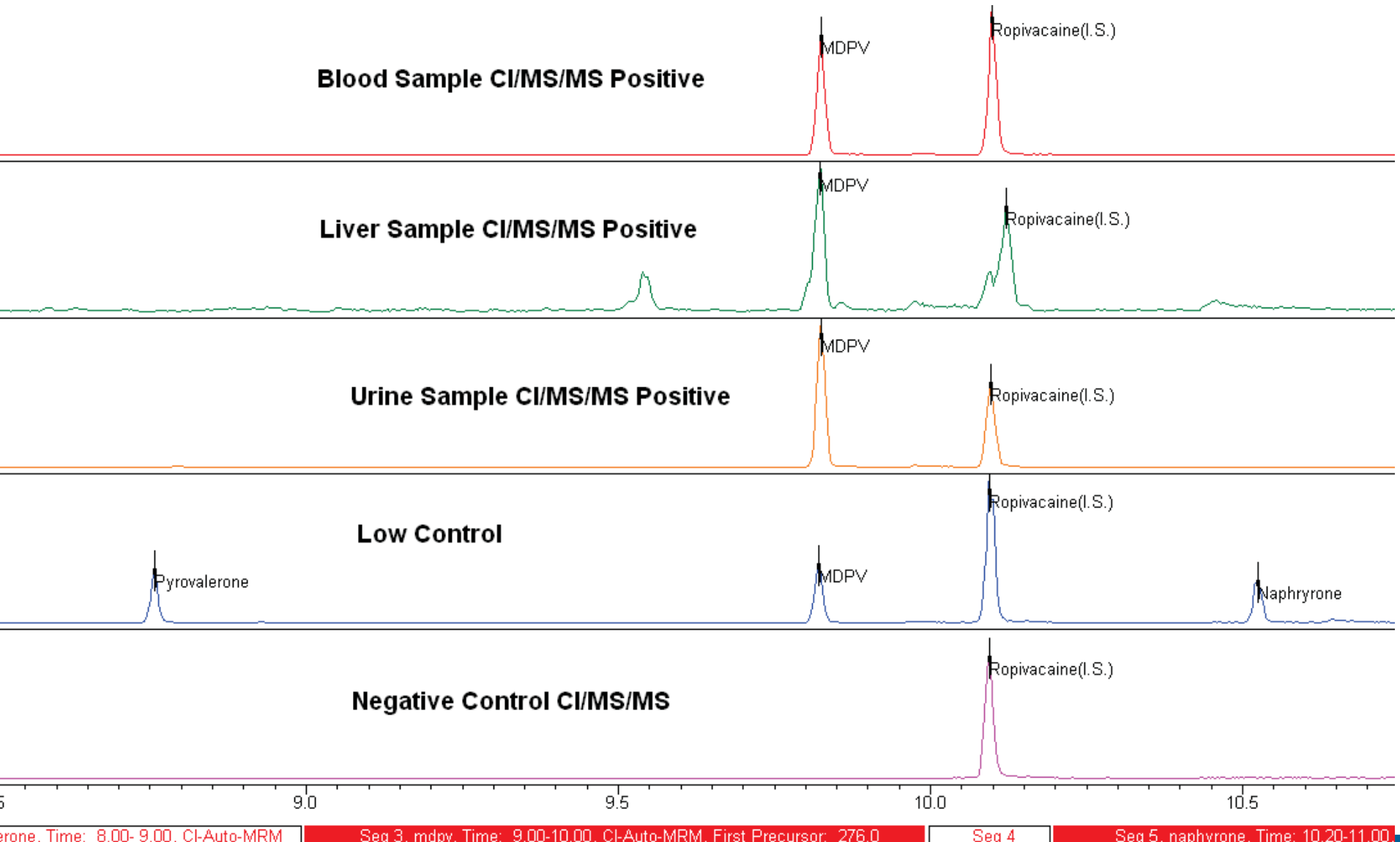
Comparison of EI-Full scan, CI-Full scan and CI-MS/MS



Liquid CI is more selective than EI, making it possible to detect compounds that may be hidden under the matrix



Confirmation of EI/MS/MS Samples by CI/MS/MS



MS^n as a Practical Analytical Tool

- In contrast to multisector instruments, ion traps do not have transmission losses that limit tandem-in-space
- Because of this tandem-in-time approach, sensitivity is maintained even for multiple MS stages
- Ion trap users can use MS^n both to probe fragmentation pathways and to enhance selectivity against matrix interference in sample background

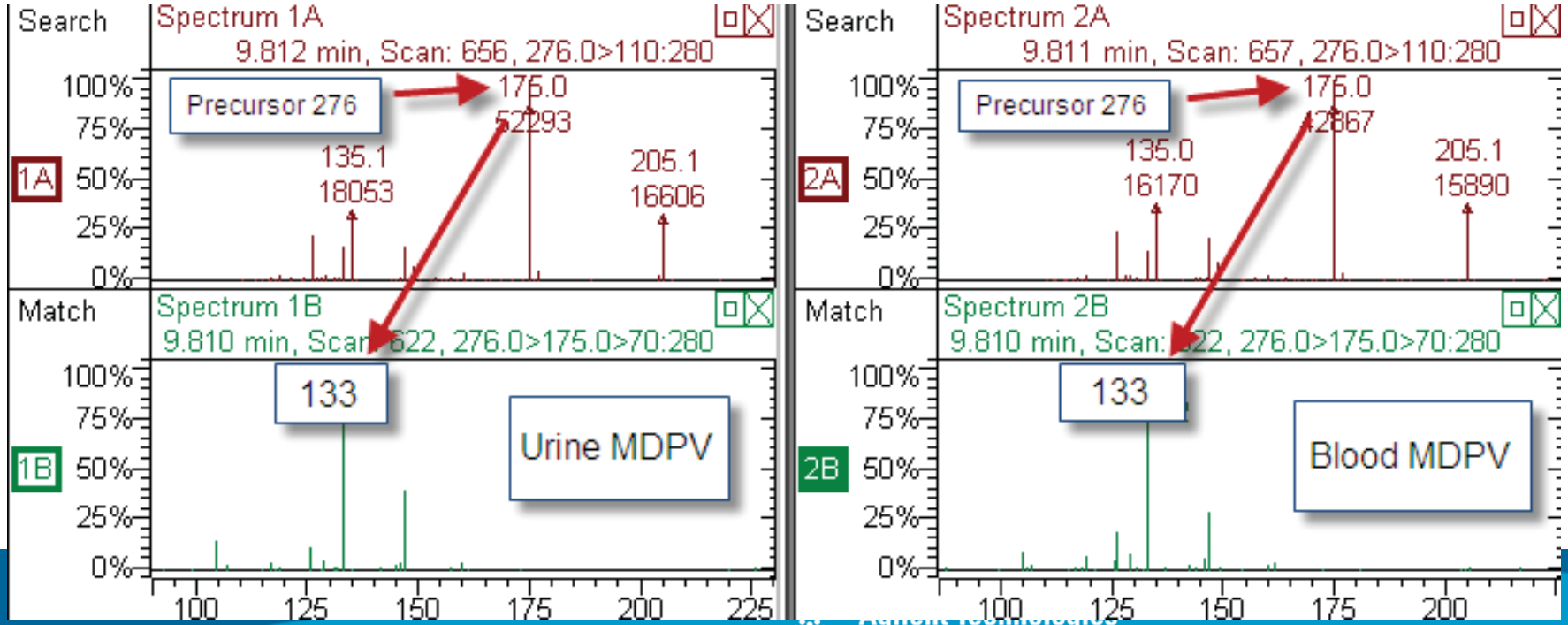
Spectrum of MDPV top is step one of MS^n CI/MS/MS Lower is CI/MS/MS/MS

Shown here is a high control - extracted in both Urine and Blood, comparing the selectivity of using one more step in the MS/MS function available only to Ion Traps to give more selective information

The selection of the M+ ion for the precursor and observing the full scan product ions gives the MS/MS step. In the case of MDPV, MW 275.3 (CI ion 276 M+1) Precursor 276>175 (CI/MS/MS))

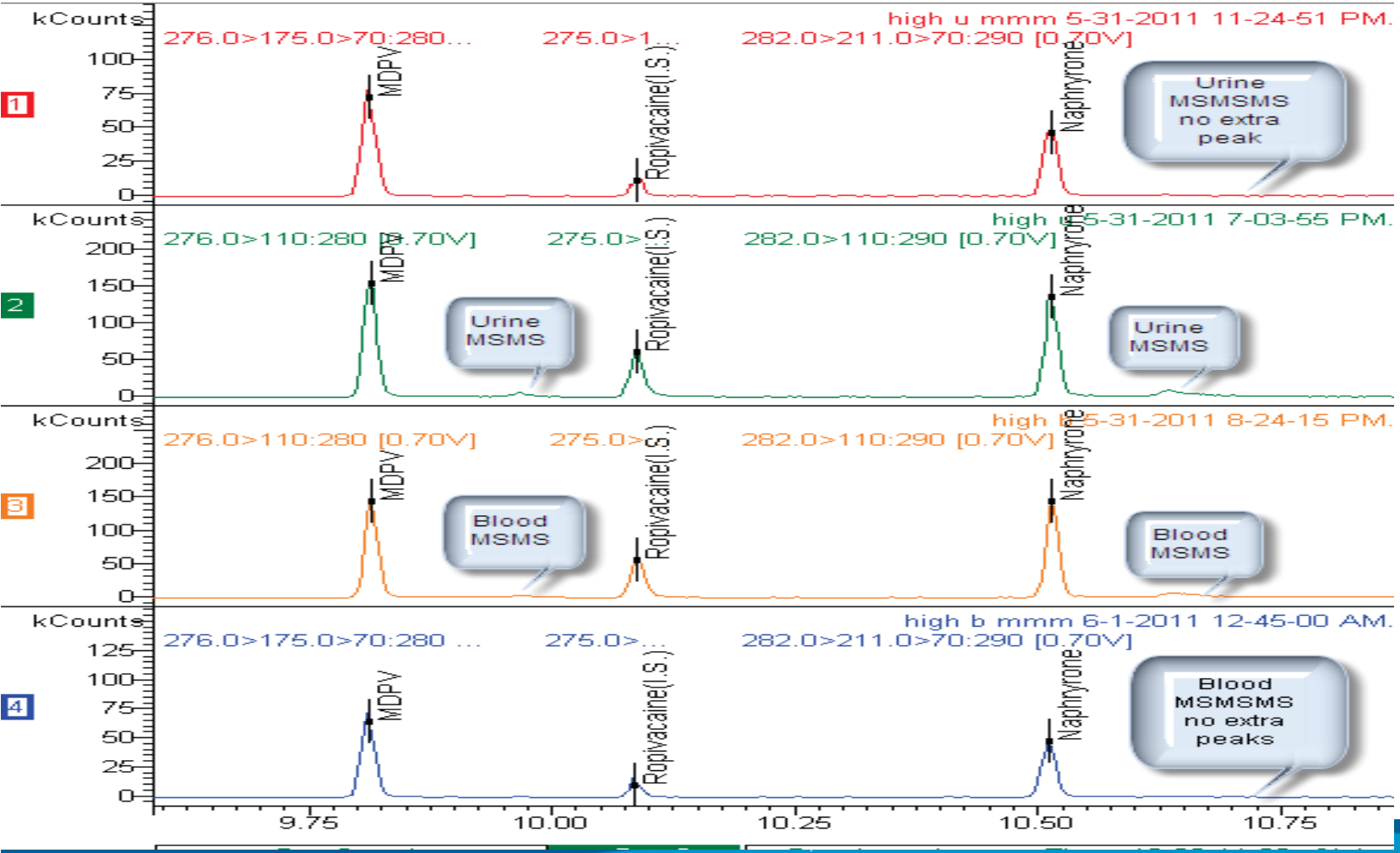
We then select a significant higher mass from the product ions and use this as a new precursor to produce again full scan product ions.

In this case we used the M+ ion 276 for MDPV which gave a significant 175 ion to use as the precursor in the MS/MS/MS step. - 276>175>133 (CI/MS/MS/MS



Use of CI/MS/MS/MS to further eliminate false positives / matrix issues

CI delivers unique, intense precursor ions, the M+1 ions which is 276 for MDVP and 282 for Naphyrone.

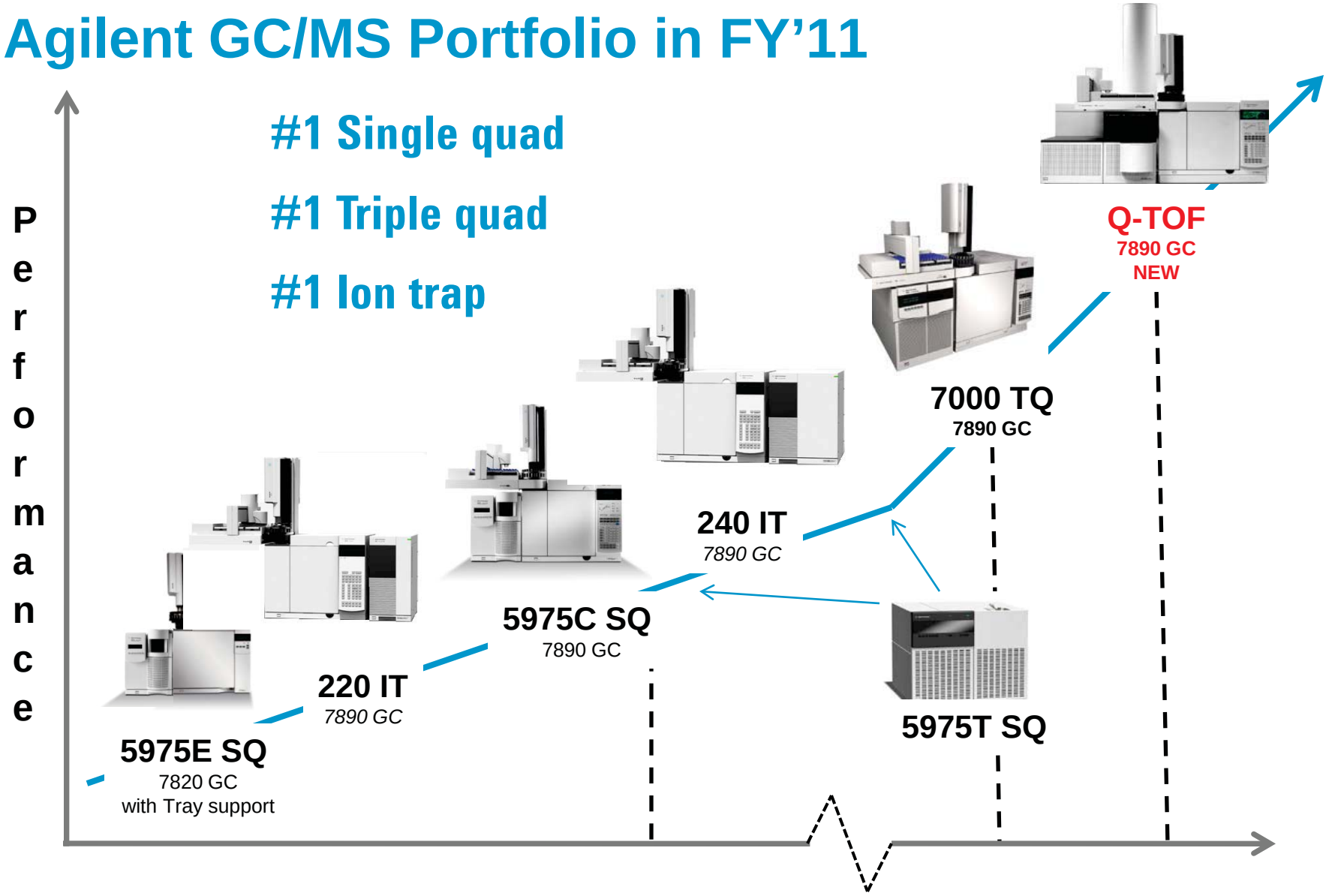


Conclusions

- Herein is presented a sensitive, selective, and robust method to determine Pyrovalerone, MDPV and Naphyrone using Ropivacaine as an internal standard.
- The method allows for “Bath Salts” to be identified in blood, urine, vitreous, gastric, brain and liver tissue matrix by their unique precursor product ion spectrum and retention times.
- Matrix interference was mitigated through the MS/MS processes.
- Two levels of positive controls were used in conjunction with negative controls to assure accurate quantification and rule out false negatives in the unknown biological samples.
- Low nanogram/mL detection limits were observed for Pyrovalerone, MDPV and Naphyrone in the various sample matrices.

- Thanks to Joe Crifasi, Christopher Long, and the group at the St. Louis University Forensic Toxicology Laboratory

Agilent GC/MS Portfolio in FY'11



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