



# UNKNOWN SCREENING USING UPLC/TOF MS

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# *TOPICS*

- Introduction
  - General unknown screening (GUS) at AIT
  - Technologies for GUS
- Technical Aspects of our method
  - Intro to TOF-MS
  - Developing the method
  - Running the method
    - QC
    - Extractions
    - Calibration
  - Side by side comparison with GC/MS
- Results Interpretation
- Case Studies

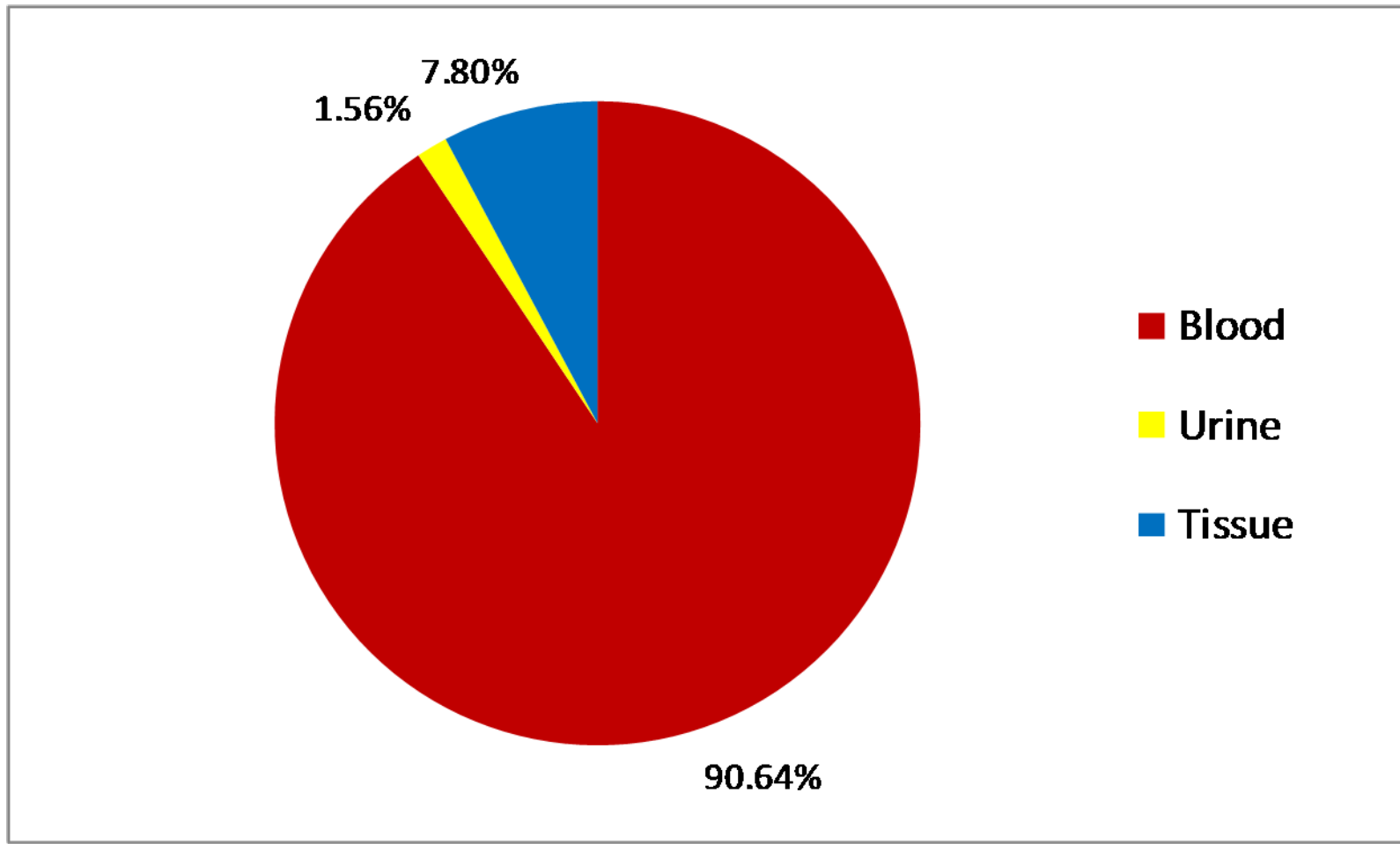


# OUR APPLICATIONS FOR BROAD SPECTRUM TESTING

- Postmortem toxicology
- Drug facilitated sexual assault
- Suspected poisoning with unknown substances
- Crime scene evidence
- Compliance/non-compliance – prescription meds
- Impairment/Human Performance toxicology
- Therapeutic Drug Monitoring/Management
- Meconium testing



# OUR TESTING MIX – Q1 & Q2 2008



# TECHNOLOGIES FOR COMPREHENSIVE SCREENS

- Immunoassay arrays
  - No sample pretreatment, automatable
  - Limited tests available, requires confirmation
- GC/MS
  - Library match using mass data
  - Extensive sample prep, limited use for non-volatile analytes
- HPLC (Remedi system)
  - Potentially larger library because of LC
  - Vendor discontinuing service, requires confirmation
- LC-MS/MS
  - “Crash & shoot” or “dilute & shoot” often possible
  - Quadrupole, ion trap, time of flight (and combinations) all in use
  - Expensive equipment, technically complex

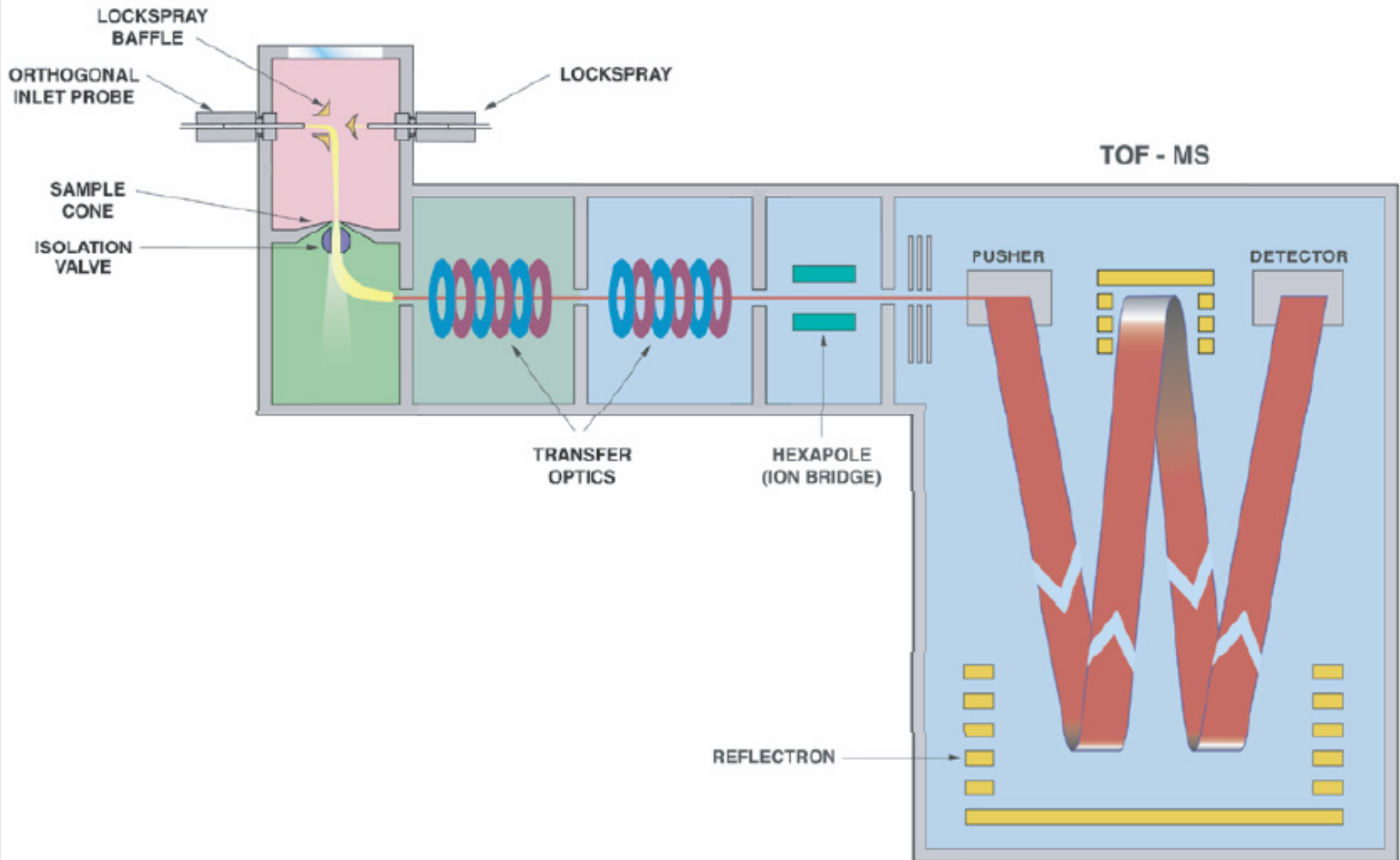


# TIME OF FLIGHT MASS ANALYZER

- Very accurate clock!
- Ions formed in an ion source are accelerated to a high velocity by an electric field into a long ‘drift tube’
- The ions pass along the tube until they reach a detector
- The time taken for an ion to traverse the analyzer is inversely proportional to its velocity, hence proportional to its mass
- Each  $m/z$  value has its characteristic time-of-flight from the source to the detector.



# TIME OF FLIGHT MASS SPECTROMETER



# TOF ALLOWS HIGH RESOLUTION

$C_{20}H_9^+$   
 $C_{19}H_7N^+$   
 $C_{13}H_{19}N_3O_2^+$

3 different compounds  
Same nominal mass  
Low resolution



$C_{20}H_9^+$

$C_{19}H_7N^+$

$C_{13}H_{19}N_3O_2^+$

3 different compounds  
3 different exact masses  
High resolution, high accuracy





# LIBRARY CREATION

- Acquire reference standard from supplier
- Dissolve reference standard in the appropriate solvent
- Dilute solution to 1  $\mu\text{g}/\text{mL}$
- Inject reference standard on UPLC/TOFMS
- Calculate exact  $[\text{M}+\text{H}]^+$
- Extract theoretical exact  $[\text{M}+\text{H}]^+$  out of the TIC
- Record the retention time of the analyte
- Make a new entry in the method and add the appropriate information (i.e. analyte name, aliases, exact  $[\text{M}+\text{H}]^+$ , retention time, along with any integration and tolerance parameters.)



## Partial Analyte Library – entire library contains >300 compounds

**Anesthetics** - Lidocaine, Procaine, Midazolam, Ketamine, Bupivacaine, Mepivacaine, Ropivacaine, Pramoxine, Thiopental, Methohexital

**Antibiotics** – Azithromycin, Piperacillin, Chloramphenicol, Ciprofloxacin, Ceftriaxone, Tilmicosin, Clindamycin

**Anticonvulsants** – Primidone, Valproic Acid, Lamotrigine, Zonisamide, Phenytoin, Levetiracetam, Gabapentin, Felbamate, Carbamazepine, Topiramate

**Antidepressants** – Fluoxetine, Sertraline, Amitriptyline, Bupropion, Escitalopram/Citalopram, Venlafaxine, Duloxetine, Fluvoxamine, Phenelzine, Trazodone

**Antipsychotics** – Olanzapine, Risperidone, Paliperidone, Aripiprazole, Quetiapine, Chlorpromazine, Haloperidol, Loxapine, Pimozide, Ziprasidone

**Cardiovascular** – Verapamil, Propranolol, Mexiletine, Papaverine, Atenolol, Metoprolol, Quinidine, Warfarin, Amiodarone, Furosemide, Diltiazem

**Hallucinogens** – Phencyclidine, Lysergic Acid Diethylamide, Mescaline

**Narcotics** – Alfentanil, Dextromethorphan, Meperidine, Propoxyphene, Buprenorphine, Fentanyl, Sufentanil, Alfentanil, Pentazocine, Tramadol

**NSAIDs** – Ibuprofen, Naproxen, Piroxicam, Fenoprofen, Flurbiprofen, Tolmetin, Diclofenac, Ketoprofen, Sulindac, Indomethacin

**Opiates** – Morphine, Codeine, Diacetylmorphine, 6-Monoacetylmorphine, Hydrocodone, Hydromorphone, Oxycodone, Oxymorphone

**Sedative and Hypnotics** – Orphenadrine, Promethazine, Zolpidem, Flunitrazepam, Methaqualone, Glutethimide, Pentobarbital, Eszopiclone/Zopiclone

**Stimulants** – Amphetamine, Methamphetamine, MDMA, MDA, Methylphenidate, Benzphetamine, Methcathinone, Caffeine, Nicotine, Cotinine

**Miscellaneous** – Tolbutamide, Chlorpropamide, Trihexyphenidyl, Betamethasone, Dexamethasone, Oxymetazoline, Strychnine, Methocarbamol, Metoclopramide, Donepezil

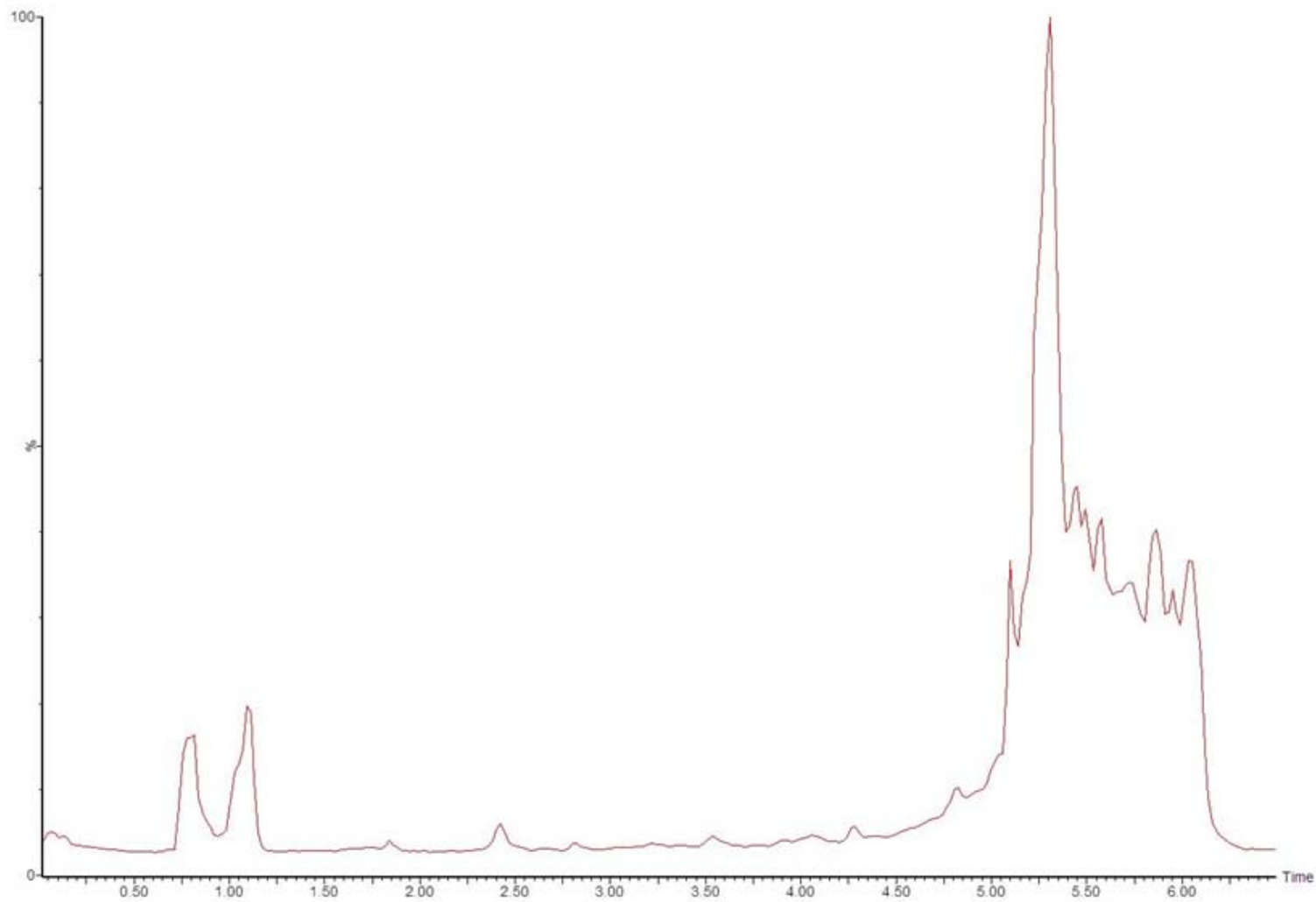


# QUALITY CONTROL

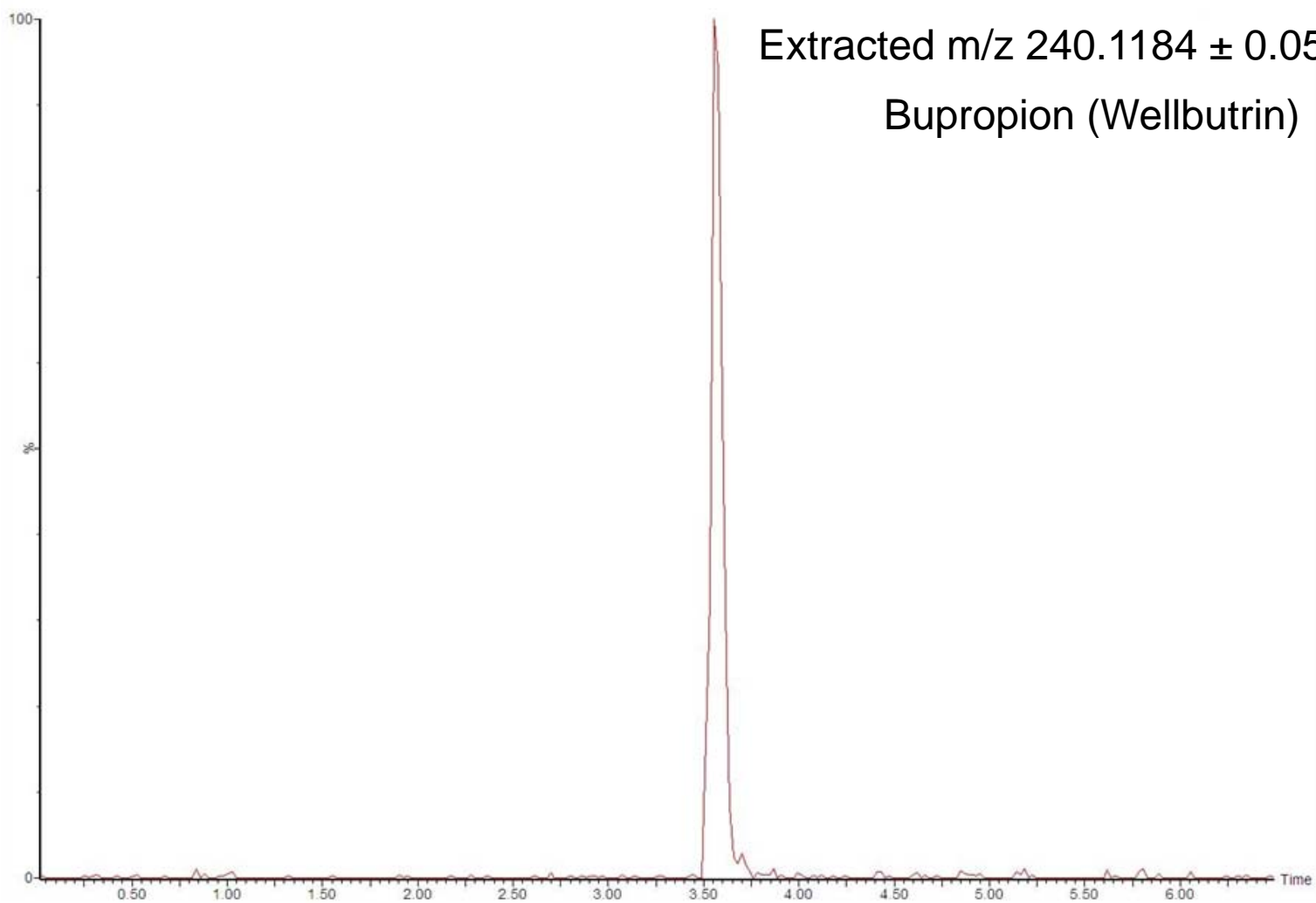
- Each batch of specimens
  - 7 positive quality control samples that include all analytes in custom library – processed exactly like actual specimen
  - 1 negative quality control sample – processed exactly like actual specimens
  - Internal standard (proadifen) added to all specimens
  - Discarded red cells reconstituted with saline
- Daily calibration with sodium formate over entire mass range
- Continual calibration with lockspray feature
  - Every 24 data sets – Leucine-Enkephalin infused
  - Linear time *vs*  $m/z$  relationship allows for single point calibration



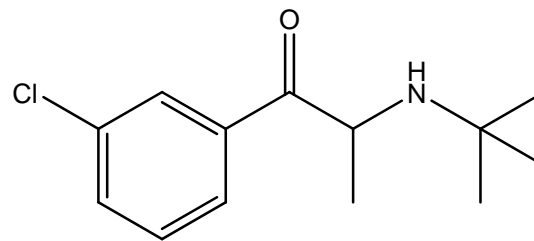
# TOTAL ION CHROMATOGRAM



# EXTRACTED ION CHROMATOGRAM

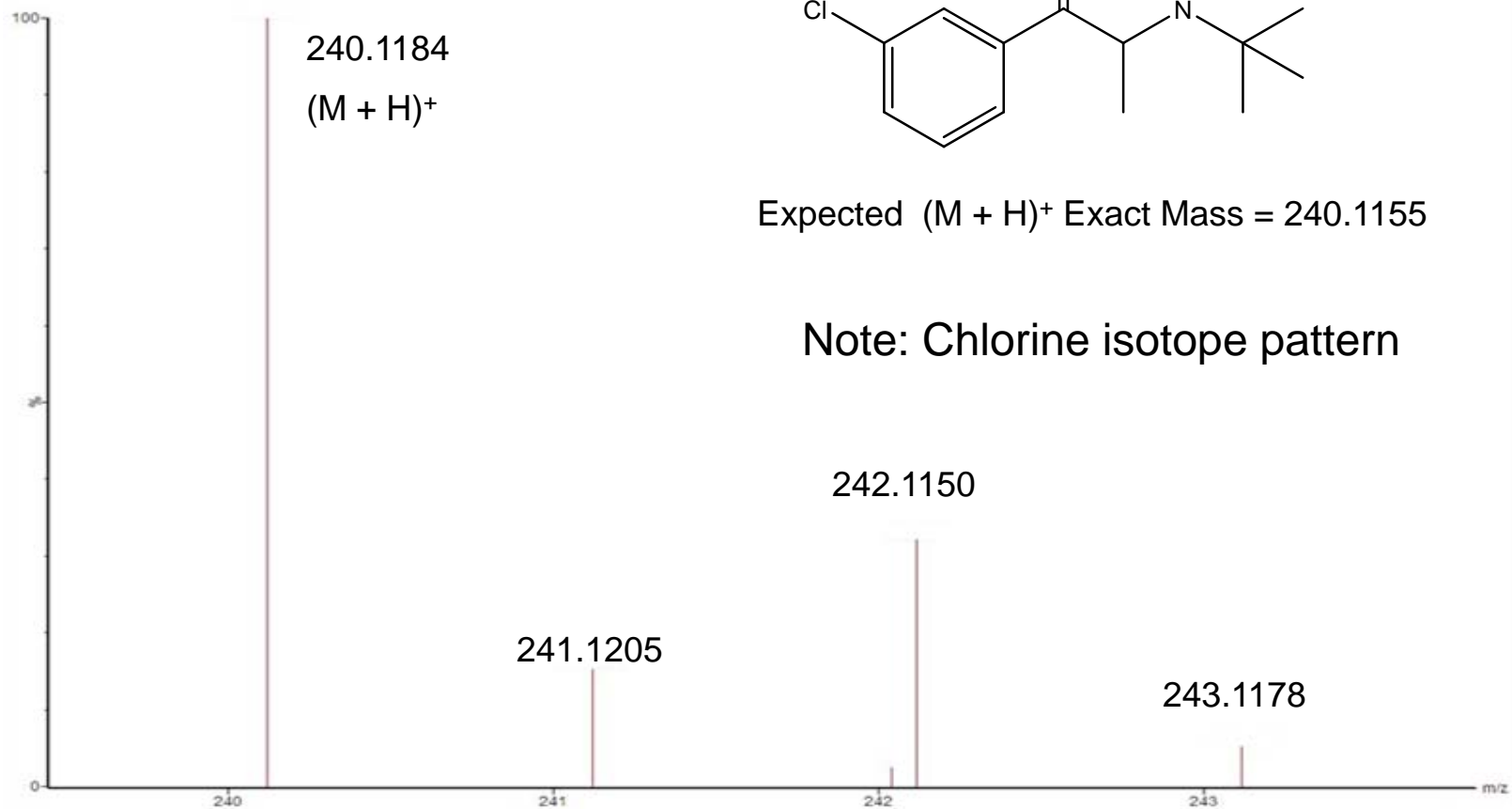


# MASS SPECTRUM



Expected (M + H)<sup>+</sup> Exact Mass = 240.1155

Note: Chlorine isotope pattern



## ‘Old’ Screen Approach

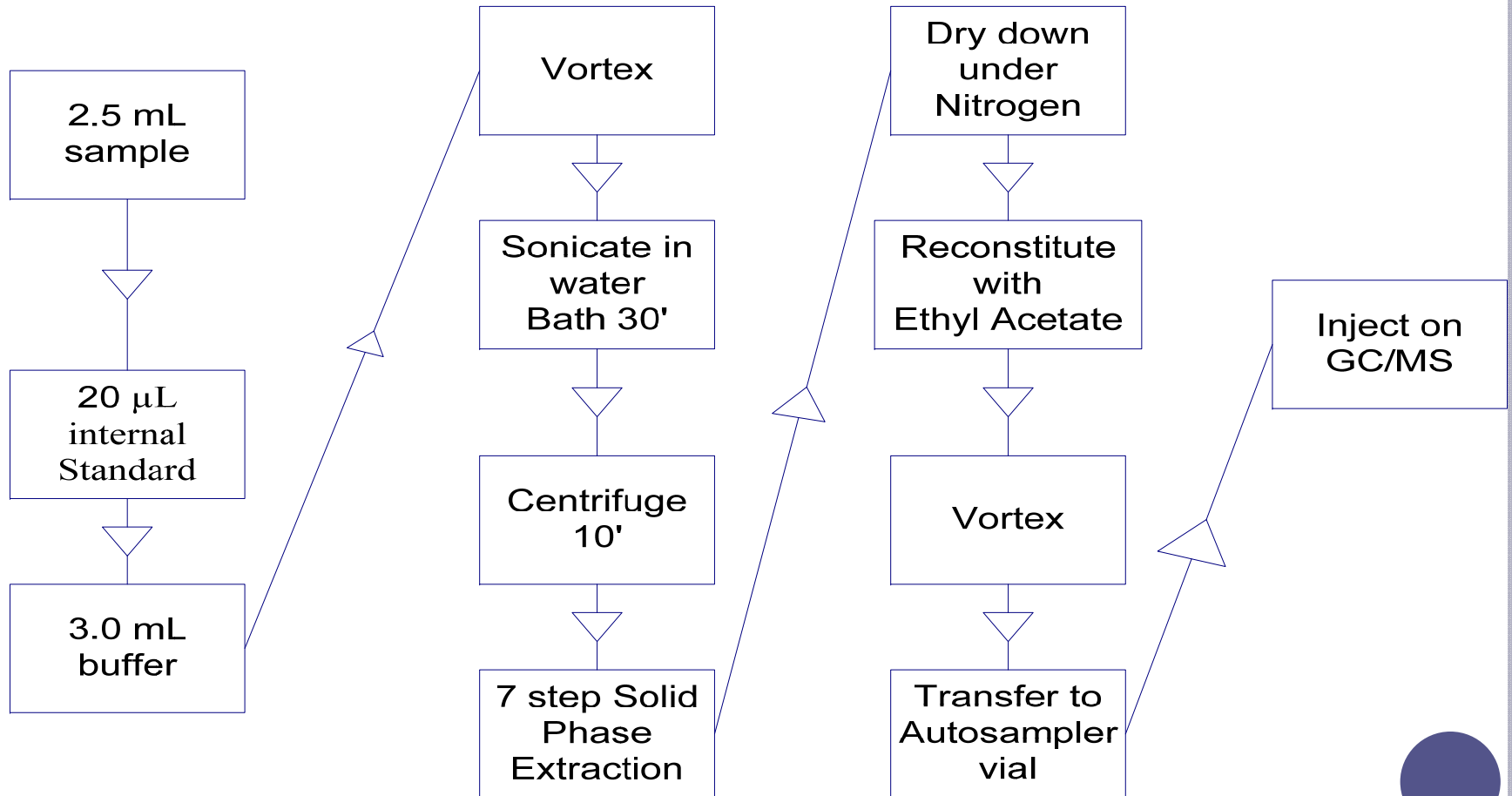
- ELISA screen for 13 drug classes
- GC/MS screen for therapeutics and other drugs of abuse
- Methodology limits # of drugs that can be detected
  - Predominantly fragments (not always molecular ion), sensitivity problems for many analytes

## ‘New’ Screen Approach

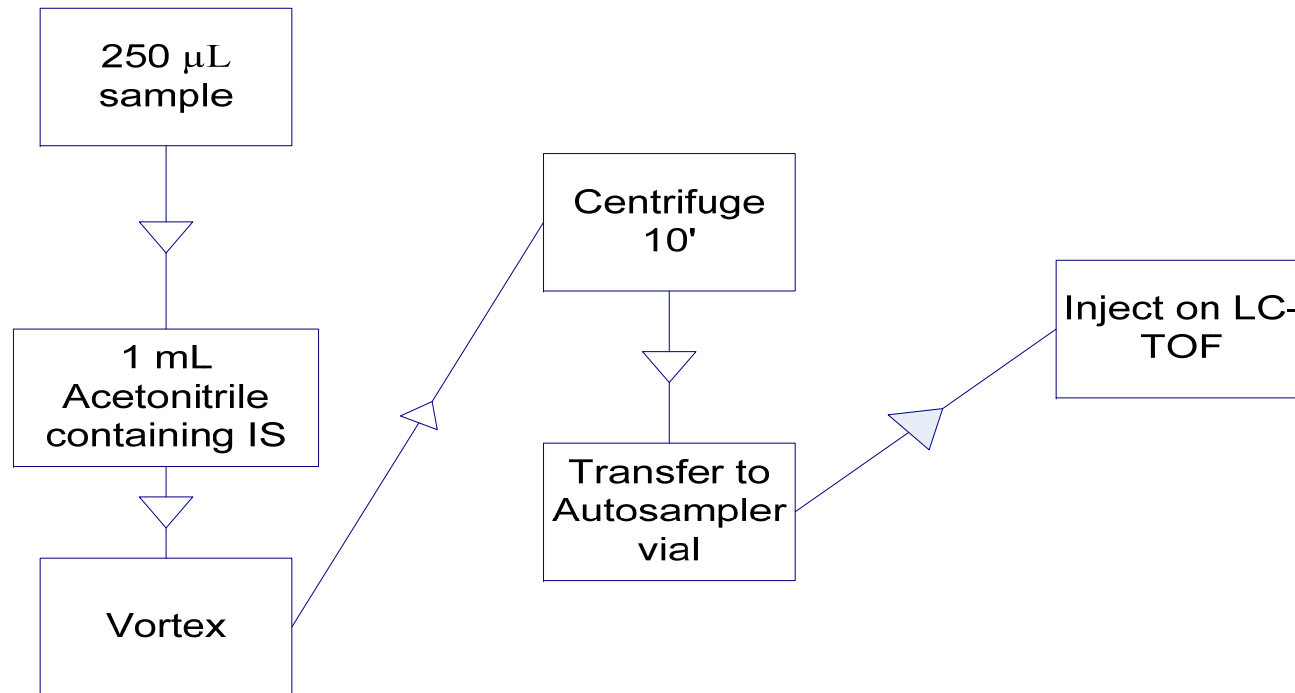
- ELISA screen for 2 drug classes (opiates, cannabinoids)
- UPLC/TOF screen for expanded number of therapeutics, drugs of abuse – ~300 drugs currently in library
- Performed on Waters LCT Premier (UPLC/TOF)



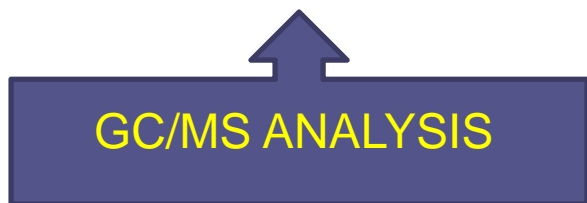
# GC/MS EXTRACTION FLOW CHART



# TOF EXTRACTION FLOW CHART



# Timeline Comparison



# “IN-PRODUCTION” NUMBERS

## - 53 SAMPLE ‘PARALLEL’ BATCH

### TOF Data

- Extraction Time is approximately 1.5 hours
- Run Time (Minutes) on TOF: 9 minutes per sample
- Total Run Time (Minutes) on TOF: 477 minutes
- 477 minutes = 7.95 hours

### GC/MS Data

- Extraction Time is approximately 4 hours
- Run Time (Minutes) on GC/MS: 26 minutes per sample
- Total Run Time (Minutes) on GC/MS: 1,378 minutes
- 1,378 minutes = 22.97 hours



# UPLC-TOF BROAD-SPECTRUM SCREENS

- Savings of 2.5 hours prep time
- Savings of 15 hours run time
- Elimination of 11 of our 13 ELISA tests
  - Opiates, THC still performed by ELISA
- Elimination of labor- and cost-intensive solid phase extraction
- Analytes can be run semi-quantitatively, saving confirmations
  - Acetaminophen



# PARALLEL SPECIMENS COMPARISON

Number	GC/MS and ELISA Results	UPLC/TOFMS Results
11	Acetaminophen, Cyclobenzaprine, Opiates	Acetaminophen, Cyclobenzaprine, <b>Morphine, Citalopram</b>
20	Opiates, Cocaine	<b>Morphine, 6-MAM, Codeine</b> , Cocaine, Benzoyllecgonine
30	Promethazine, Methadone, Barbiturates, Opiates	Promethazine, Methadone, <b>EDDP</b> , Phenobarbital, Morphine, <b>Flurazepam, Desalkylflurazepam, Methocarbamol, Hydroxyzine</b>
49	Lidocaine	Lidocaine, <b>Amiodarone</b>
64	Promethazine, Methadone	Methadone, EDDP
83	Negative	<b>Carbamazepine</b>
87	Fluoxetine	Fluoxetine, Norfluoxetine, <b>Flecainide</b>
107	Ibuprofen, Lamotrigene, Amitriptyline, Nortriptyline, Bupropion, Diphenhydramine, Chlorpheniramine, Promethazine, Methadone	Ibuprofen, Lamotrigene, Amitriptyline, Nortriptyline, Bupropion, Diphenhydramine, Chlorpheniramine, Promethazine, Methadone, <b>Propranolol, Buspirone, Topiramate</b>
110	Negative	<b>Carbamazepine, Carbamazepine-10,11-epoxide</b>
114	Venlafaxine, Norvenlafaxine, Cocaine	Venlafaxine, Norvenlafaxine, Benzoyllecgonine, <b>Topiramate</b>
115	Ibuprofen, Verapamil, Opiates, Benzodiazepines	Ibuprofen, Verapamil, Desmethylverapamil, Diazepam, Nordiazepam, Morphine, Codeine, <b>Sildenafil</b>

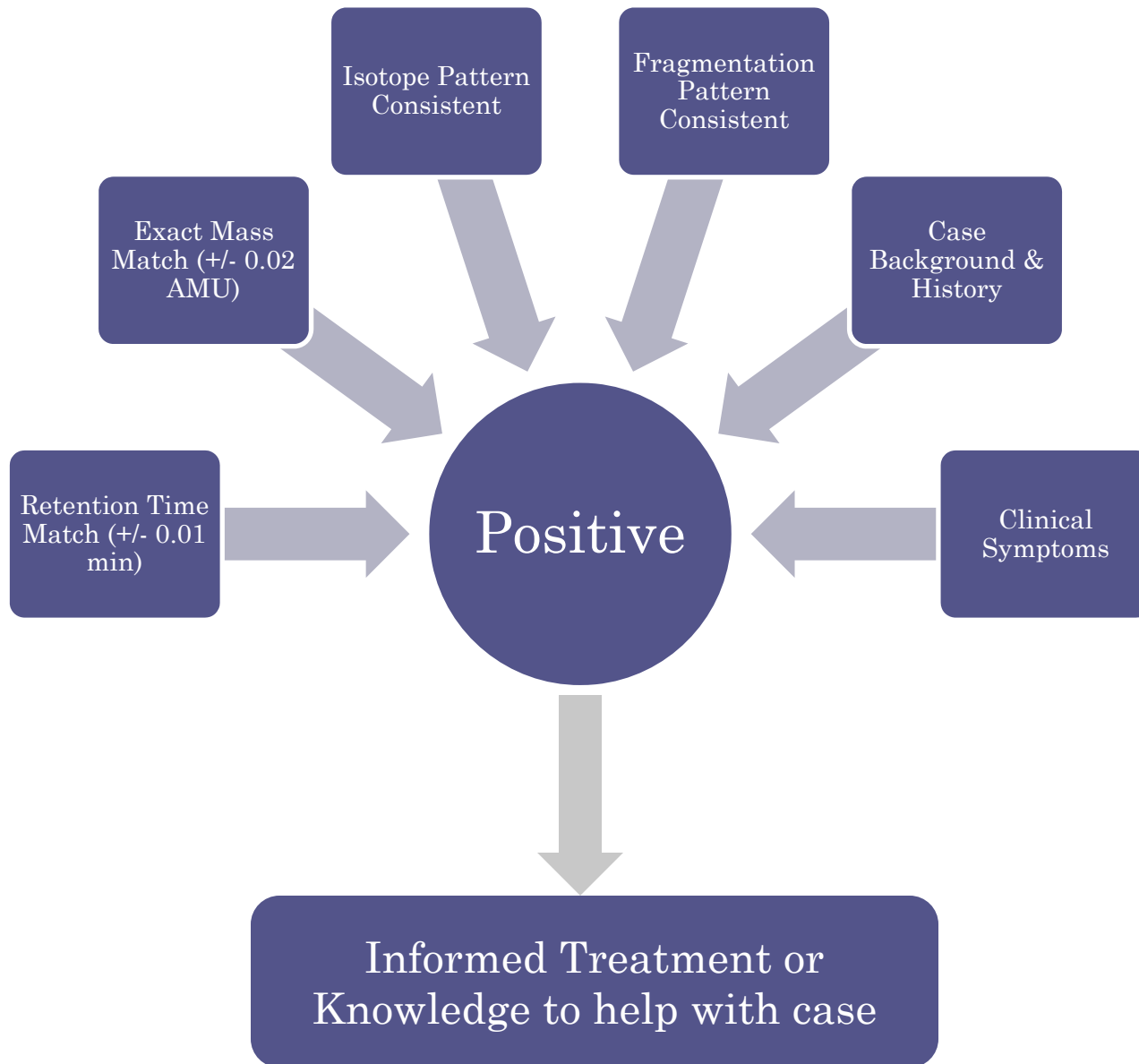


# IF A CONFIRMATORY RUN IS DESIRED...

- Single or tandem quadrupole mass spectrometry is common – with ionization conditions sufficient to create fragmentation – fragmentation pattern provides what is effectively a “chemical fingerprint” pattern for identification
- TOF is a good option
  - Same extraction, different aliquot of sample
  - In-source fragmentation by increasing the cone voltage
  - Allows a spectral match using fragmentation pattern along with accurate mass measurement



# RESULTS INTERPRETATION



# CASE STUDY 1 – DETERMINATION OF AN UNKNOWN ANALYTE

## ○ History

- Male found deceased on a farm outside of a barn. A puncture wound is seen on the decedent's wrist. A syringe is found in close proximity to the victim. The syringe and the deceased's postmortem specimens (blood, urine, vitreous fluid) were all sent to the laboratory for screening.

## ○ Results

- The syringe was rinsed with Methanol:DI Water. The syringe rinse and postmortem specimens were run according to the outlined screening method.
- Urine specimen was negative on immunoassay array
- Blood specimens were negative for all drugs of abuse and therapeutic drugs contained within the accurate mass library BUT an unknown analyte peak at 3.57 minutes with a mass of 869.57 was observed in both specimens.



# CASE STUDY 1 – DETERMINATION OF AN UNKNOWN ANALYTE

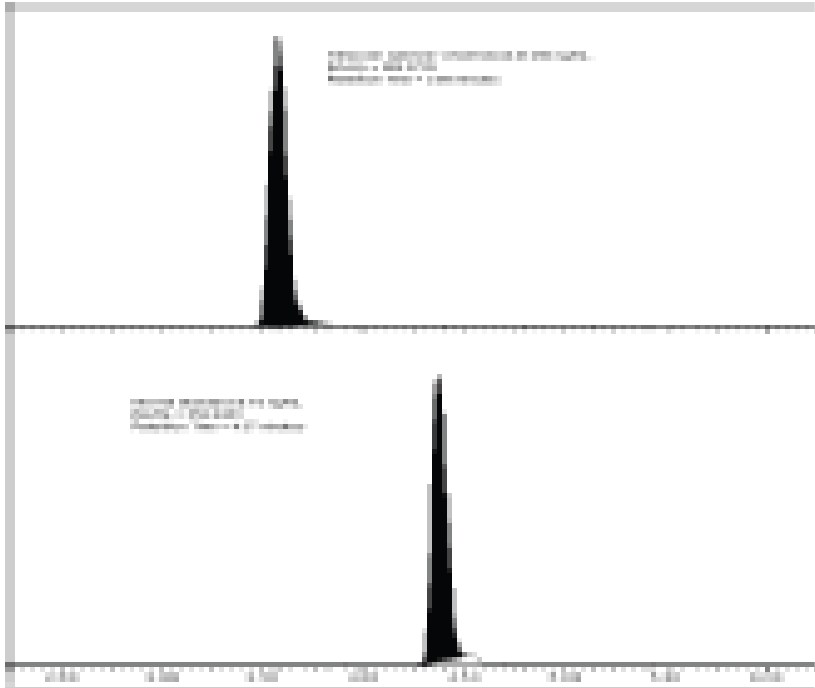
## ○ Investigation

- Research was conducted to find a drug or chemical that had a mass equal to 869.57 Daltons.
- It was found that a macrolide antibiotic drug for use in cattle, sheep, and goats (Tilmicosin, trade name Micotil®) fit the requirement.
- A reference standard was obtained from a supplier and spiked in blood.



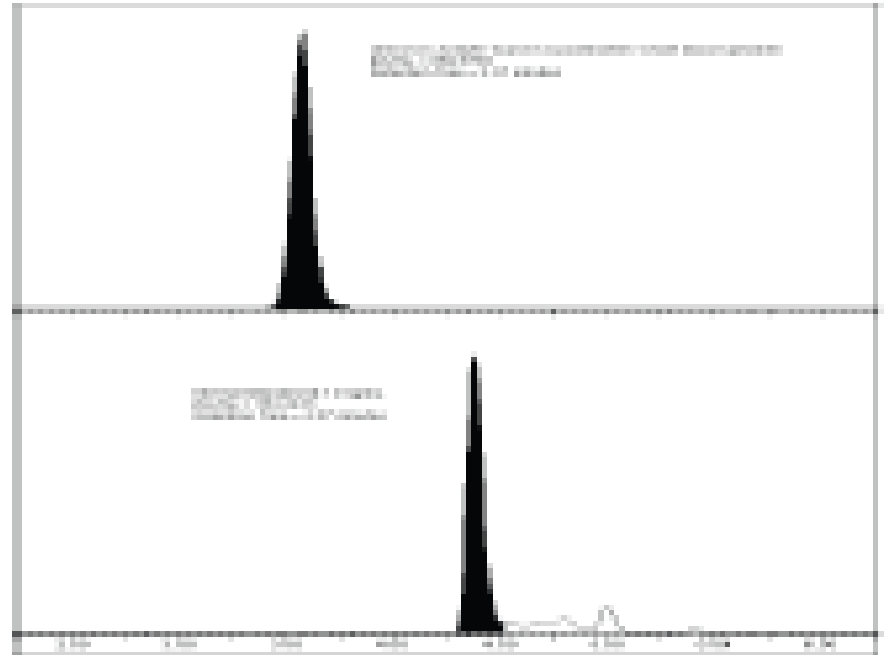
# IT'S A MATCH!

Figure 3



Reference Standard

Figure 5



Whole Blood



# CASE STUDY #2 – “EYE SEE CLEARLY NOW” – IDENTIFICATION OF VISINE (TETRAHYDROZOLINE)

## ○ History

- Two siblings (infant and toddler) seen in the emergency room
- Case history and initial investigation led to the suspicion of ingestion of Visine (tetrahydrozoline)

## ○ Results

- Urine specimen submitted to lab for identification of drugs and other chemical substances – including tetrahydrozoline
- Screening analysis negative for drugs of abuse
- Screening analysis positive for tetrahydrozoline – confirmed with standard

## ○ Investigation

- Litigation still pending

