Toxicology Testing Overview

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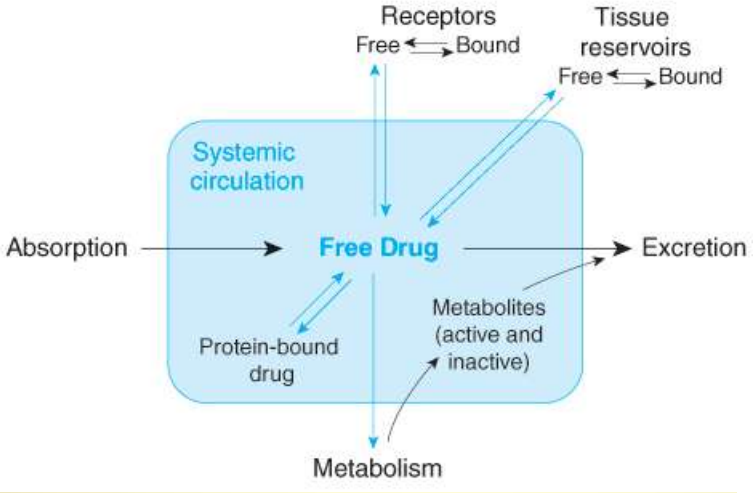
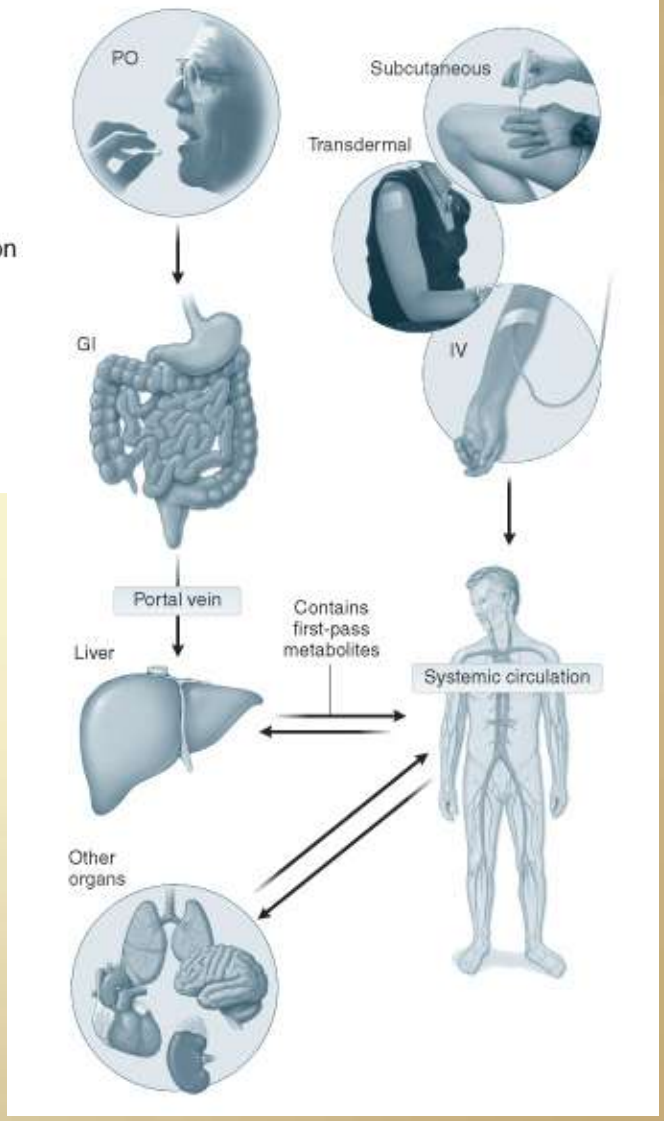
hodsdon.com/wiki

Drug 'Lifecycle' in Humans

- Absorption
  - Ingestion, injection, inhalation, etc.
- Distribution
- Metabolism
  - Metabolites usually inactive
  - Determines length of efficacy
- Excretion
  - What goes in must come out
  - Usually in the urine
  - Also in feces or breath
  - Most often modified
  - Can be quite delayed





Choice of Specimen

Blood

- Generally target active compounds
- Correlates well with current clinical effect
- Quantitation useful (just like therapeutic drug monitoring)
- Not that useful for detecting past use
- Obviously, more invasive
- Hard to adulterate

Urine

- Generally target inactive metabolites
- Not specific for current use
- Renal concentrating ability increases sensitivity
- Detectable for days after use (variable)
- Quantitation varies with hydration status; hence, generally not useful
- Less invasive
- Easier to adulterate

Testing Methods

Immunoassays

- Used for screening
- Testing is generally automated and results are available rapidly
- Can be instrument-based (lab) or simple 'dipstick' (bedside)
- Sensitivity and specificity vary greatly (dipsticks generally perform poorly)
- Less expensive
- Commercially available but dominated by workplace drug testing needs, not clinical

Confirmation

- Includes HPLC, TLC, GC/MS, LC/MS/MS (defined in handout)
- Generally used to confirm screening results, but can be ordered directly
- Often relies on more complicated and expensive equipment (but reagent costs can be less)
- Almost always labor-intensive with turn-around times taking days to weeks
- Sensitivity as good or better than immunoassays
- Specificity is much better; can often provide list of exact compounds

Enzyme-Multiplied Immunoassay Technique (EMIT)

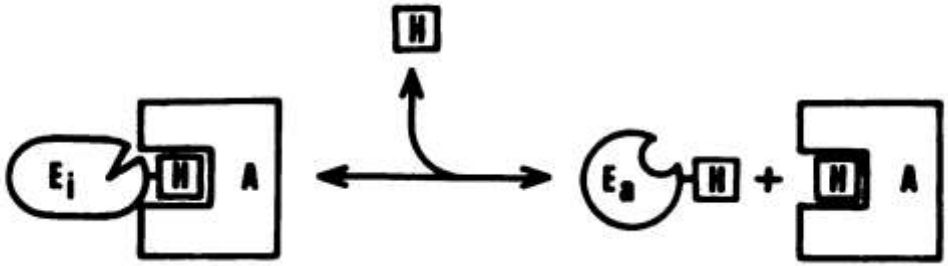


Fig. 1. Principle of Homogeneous EIA

A, antibody; E_a, active enzyme; E_i, inhibited enzyme; *H*, hapten

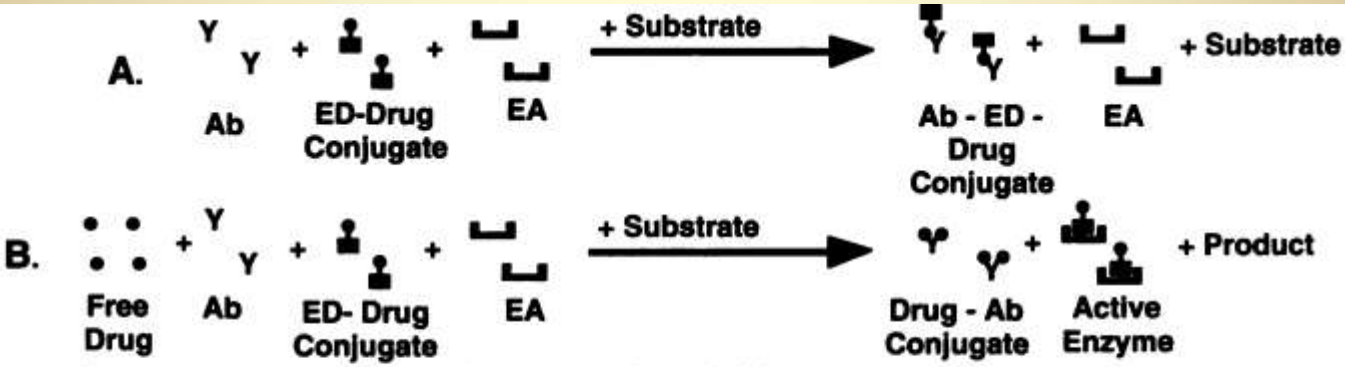


Fig. 1. Principle of the CEDIA (cloned enzyme donor immunoassay) methodology.

(*A*) In the absence of free drug or drug metabolite in a urine specimen, formation of a complete tetrameric β-galactosidase enzyme is inhibited, and no product is generated after addition of substrate to the reaction mixture. (*B*) Drug or drug metabolite in a urine specimen competes with the enzyme donor (ED)–drug conjugate for anti-drug antibody (Ab) binding sites; complete, active β-galactosidase molecules are formed in proportion to the amount of drug or drug metabolite present, and the conversion of substrate to product is also proportional to the drug concentration in the specimen. EA, enzyme acceptor.

Importance of cross-reactivity and matrix effects!

Detection Thresholds

- In most analyzers, antibody reactivity results in a proportional spectrophotometric signal.
  - UV/Vis absorption
  - Fluorescence
  - Chemiluminescence
- Each assay is calibrated using a single drug solution at a fixed concentration (e.g. 300 ng/ml morphine).
  - Reactivity greater (less) than this threshold is considered positive.
  - Reactivity less (greater) than this threshold is considered negative.
  - Note that signal quickly becomes non-linear at concentrations above the detection threshold.
- Many factors alter the reactivity of clinical samples.
  - Variable reactivity of similar drugs (e.g. codeine, hydrocodone, oxycodone, etc.).
  - Conjugated metabolites generally have poorer reactivity (e.g. glucuronidated opiates).
  - Unrelated cross-reacting drugs can add (or subtract) to the final signal.
  - Matrix effects: although clinical samples are diluted (1:20 – 1:40) into standard solutions, variations in urine components can affect final signal.
    - pH, salt concentration, protein, enzyme inhibitors, chromatographic molecules, etc.

Detection Thresholds

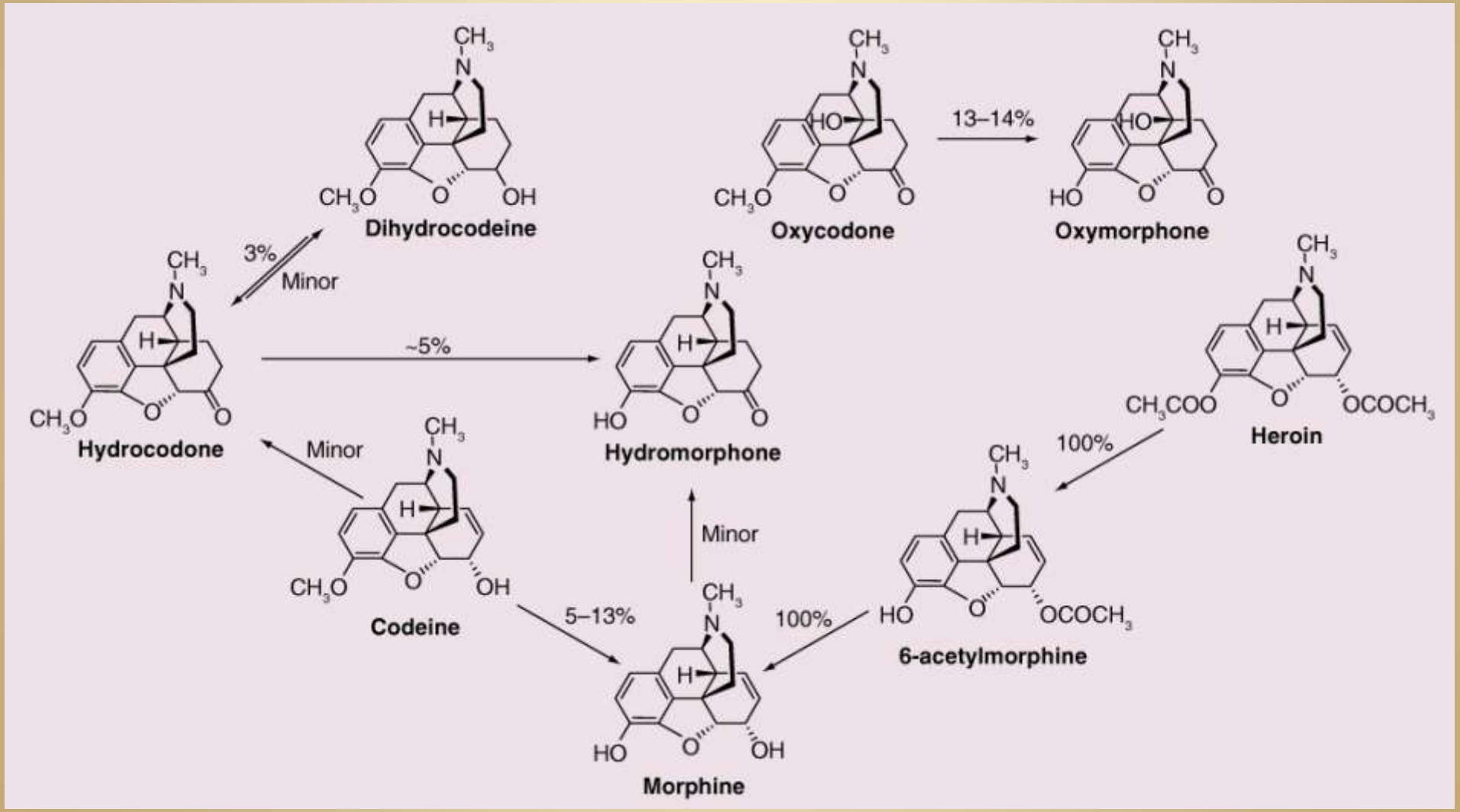
Assay	Calibrator
Cocaine	300 ng/ml benzoylecognine (BZE)
Opiates	300 ng/ml morphine
Oxycodone	100 ng/ml oxycodone
Methadone	300 ng/ml methadone
Barbiturates	200 ng/ml secobarbital
Benzodiazepines	200 ng/ml oxazepam
Amphetamines	1000 ng/ml methamphetamine
Phencyclidine	25 ng/ml phencyclidine (PCP)
Cannabinoids	50 ng/ml 11-nor-Δ9-THC-9-carboxylic acid

Opiate Detection Limits

Opiate	Minimum Detection Limit (in water)
Morphine	300 ng/ml (calibrator)
Codiene	12.5 ng/ml
Hydrocodone	400 ng/ml
Hydromorphone	700 ng/ml
Meperidine	160,000 ng/ml
Oxycodone	26,000 ng/ml
Oxymorphone (estimated)	(>100,000 ng/ml)
Morphine-3-glucuronide	1050 ng/ml

- Also need to consider
  - Dosage / Dosing Interval
  - Metabolism
  - Urine Concentration

Opiate Metabolism



Opiate Metabolism Profiles

1. ***Pharmaceutical Morphine:*** Morphine and *sometimes* oxymorphone when the morphine is very high.
2. ***Codeine:*** Codeine, morphine and norcodeine/norhydrocodone (can't distinguish these two in our GC/MS method due to derivatization).
3. ***Hydrocodone:*** Hydrocodone, hydromorphone and norhydrocodone/norcodeine.
4. ***Hydromorphone:*** Hydromorphone.
5. ***Oxycodone:*** Oxycodone and oxymorphone.
6. ***Heroin Use:*** 6-monoacetylmorphine (6-MAM), morphine, codeine, and *sometimes* oxymorphone (same as with morphine).
7. ***Poppy seeds:*** Morphine and codeine. Difficult to distinguish past heroin use from poppy seed ingestion. NIDA recommends the following criteria.
   - If total morphine > 5000 ng/ml (maybe 10,000 ng/ml better), or codeine > 300 ng/ml,
   - or the morphine:codeine ratio < 2,
   - or total morphine > 1000 without any codeine present,
   - or the presence of any 6-MAM (specific for heroin),
   - then poppy seed ingestion is ***NOT likely*** the sole source of the urine opiates.

DAU Performance Summary

1) Opiates
   - Detects multiple opiates with varying sensitivity.
   - Poor sensitivity for oxycodone and meperidine
   - Does not detect methadone, propoxyphene or fentanyl at all
   - Fluoroquinolones reported to cross-react.

2) Methadone
   - Good sensitivity and specificity
   - Does not detect any opiate

3) Oxycodone
   - Very sensitive and specific

4) Benzodiazepines
   - Poor sensitivity for Ativan (lorazepam) and Xanax (alprazolam)
   - Good sensitivity for older benzodiazepines that are primarily metabolized to oxazepam
   - See hodsdon.com/wiki for more details, including metabolism pathways.
   - Oxaprozin (Daypro) reported to cross-react (rare)

1) Barbiturates
   - Good sensitivity and specificity

6) Cocaine (BZE)
   - Good sensitivity and specificity

7) Amphetamines
   - Depending on the assay, many drugs cross-react.
   - Anti-histamines most common at YNHH

8) Phencyclidine
   - Common cross-reactivity with dextromethorphan (metabolite)

9) Cannabinoids
   - Sensitivity to inactive metabolites vary; sometimes positive for weeks to months
   - Good specificity

How long should it be positive?

- Very common question, but not easy to answer.
- Two categories of research studies:
  - Drug administered to healthy, non-abusing volunteers and urine samples monitored (often less than 24 hours).
  - Daily urine samples collected from an inpatient drug rehabilitation facility (days to weeks).
  - Summarized for each drug in handout.
- Major difference is the accumulation of inactive metabolites in tissue (especially if use is much more frequent than elimination half-life, $t_{1/2}$).
- Also affected by all the previous considerations of variable urine concentration, reactivity of metabolites, and detection thresholds.

Difficulty with the Interpretation of Urine Drug Concentrations

- Though they generally trend towards lower values, the absolute concentrations of excreted compounds can vary and identification of "new use" is problematic.

- As the figure demonstrates, improved performance can be provided by normalizing drug levels to urine Cr.

- However, this is not common practice and firm guidelines have not been established.

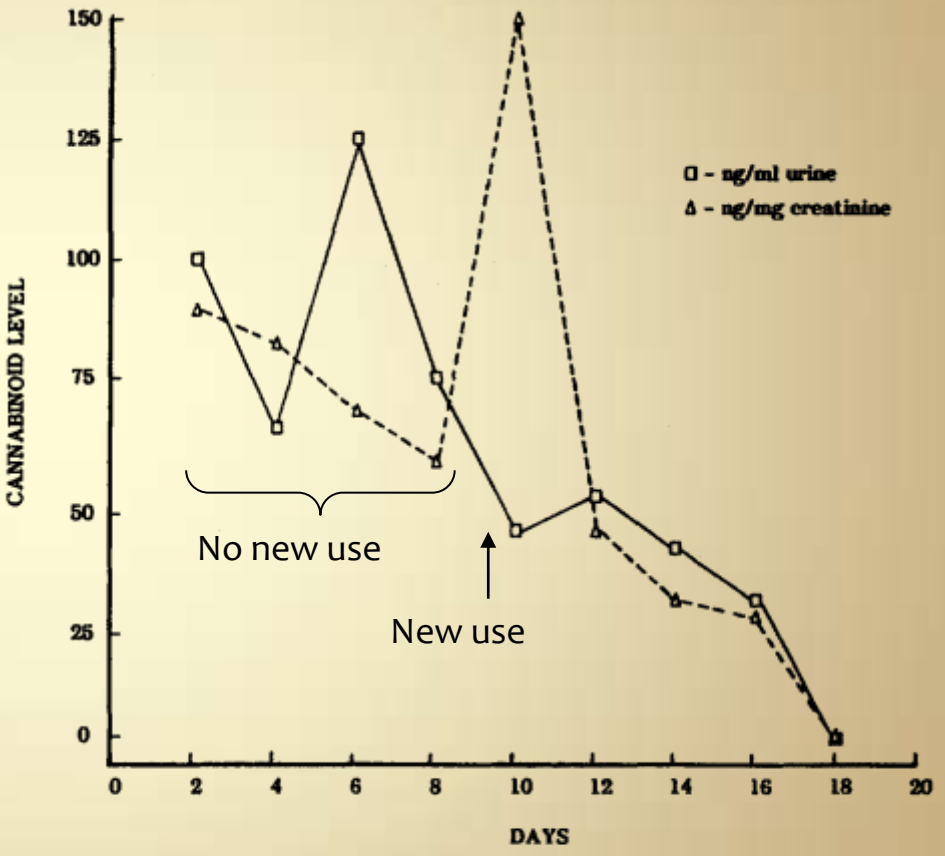


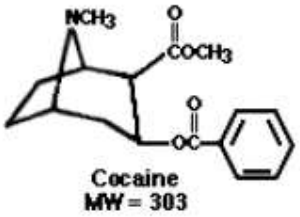
Figure 1. Urinary cannabinoid levels of specimens taken on alternate days after last marijuana use. □ - concentration of THC metabolite in ng/ml urine. Δ - THC metabolite concentration divided by the creatinine concentration expressed in ng metabolite/mg creatinine.

From NIDA Monograph: Urine Testing for Drugs of Abuse

Current versus Past Use

- 6-monoacetylmorphine is highly specific for acute heroin use.
- Unconjugated opiates can generally be detected for up to 12 hours after use.
- In contrast, their conjugated counterparts can be detectable for days.
- Similarly, ‘cocaine’ (the actual parent compound) can be detected for 6 – 12 hours after use.
- The inactive cocaine metabolite, benzoylecognine, is detectable for days to weeks.

Cocaine GC/MS



Cocaine
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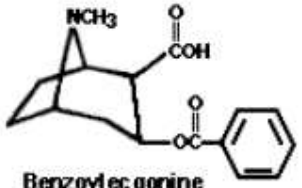

Benzoylecgonine
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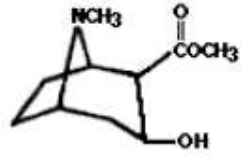

Ecgonine methyl ester
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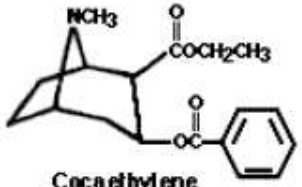

Cocaethylene
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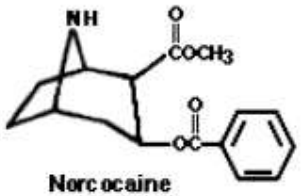

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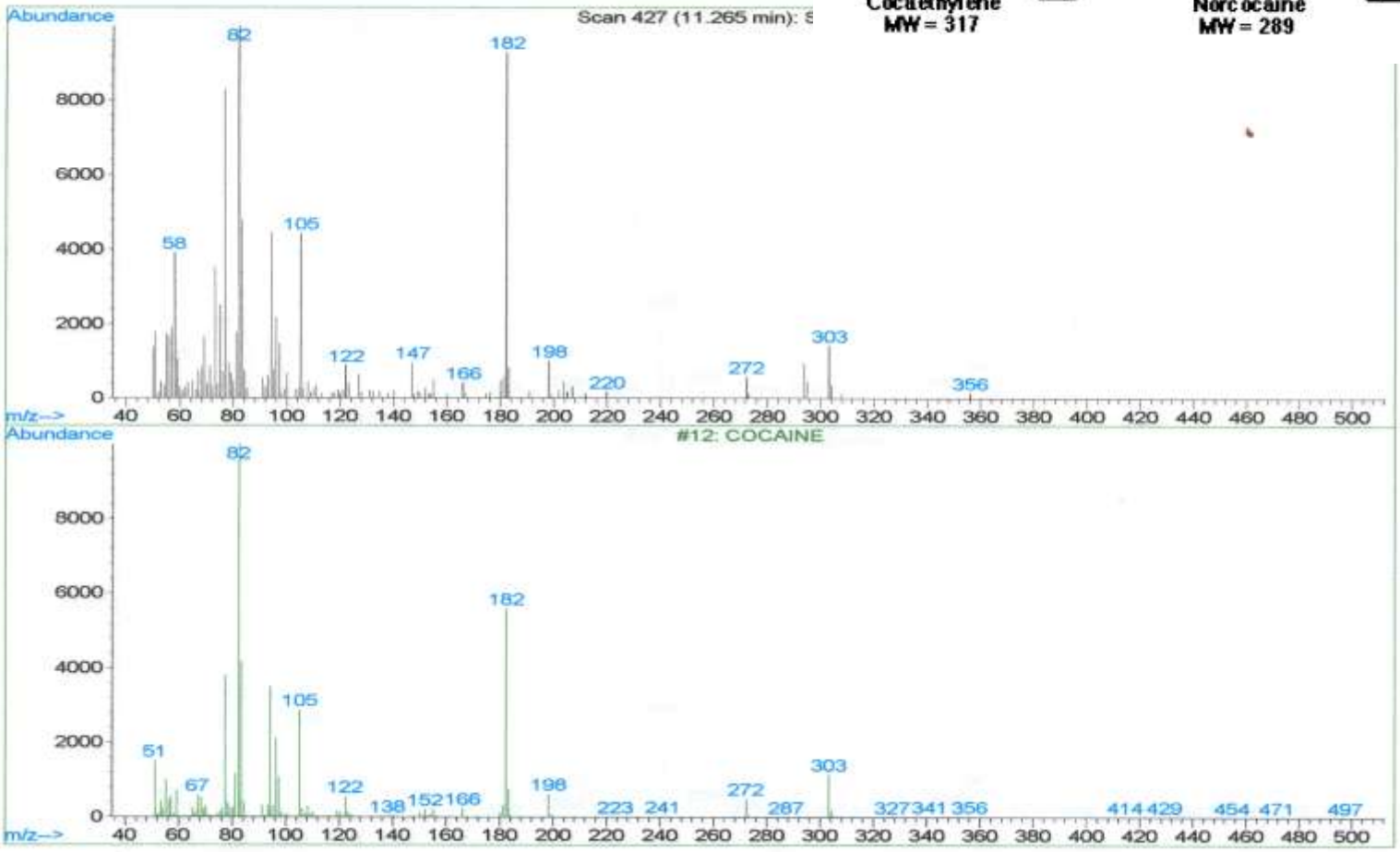
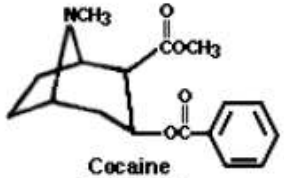


Benzoylecognine



Cocaine
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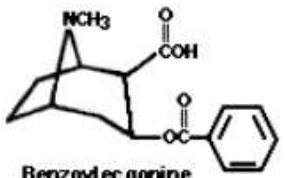

Benzoylecgonine
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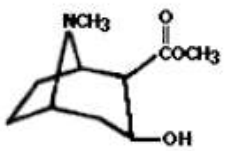

Ecgonine methyl ester
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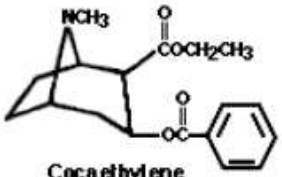

Cocaethylene
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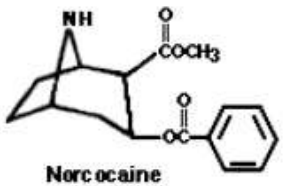

Norcocaine
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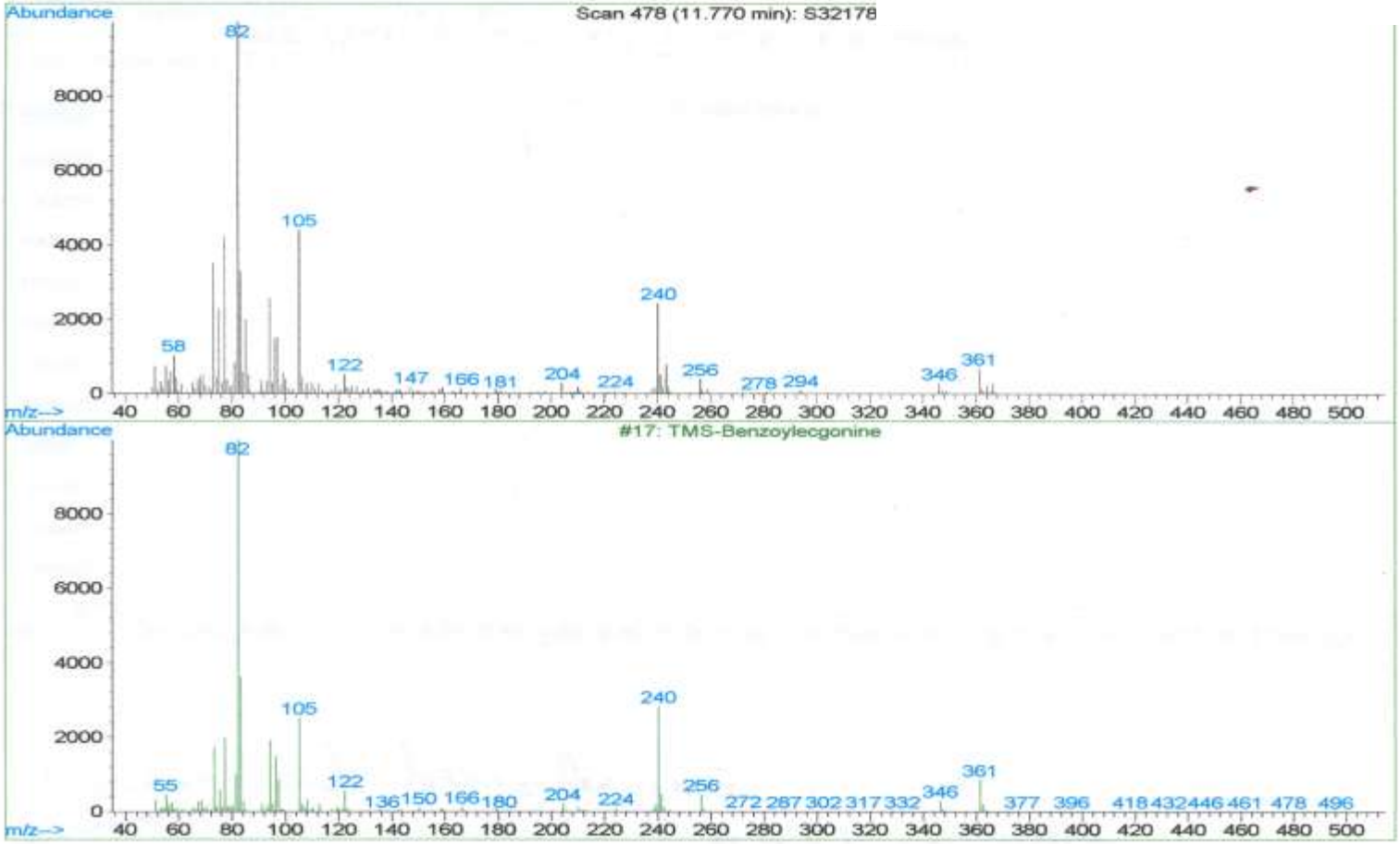
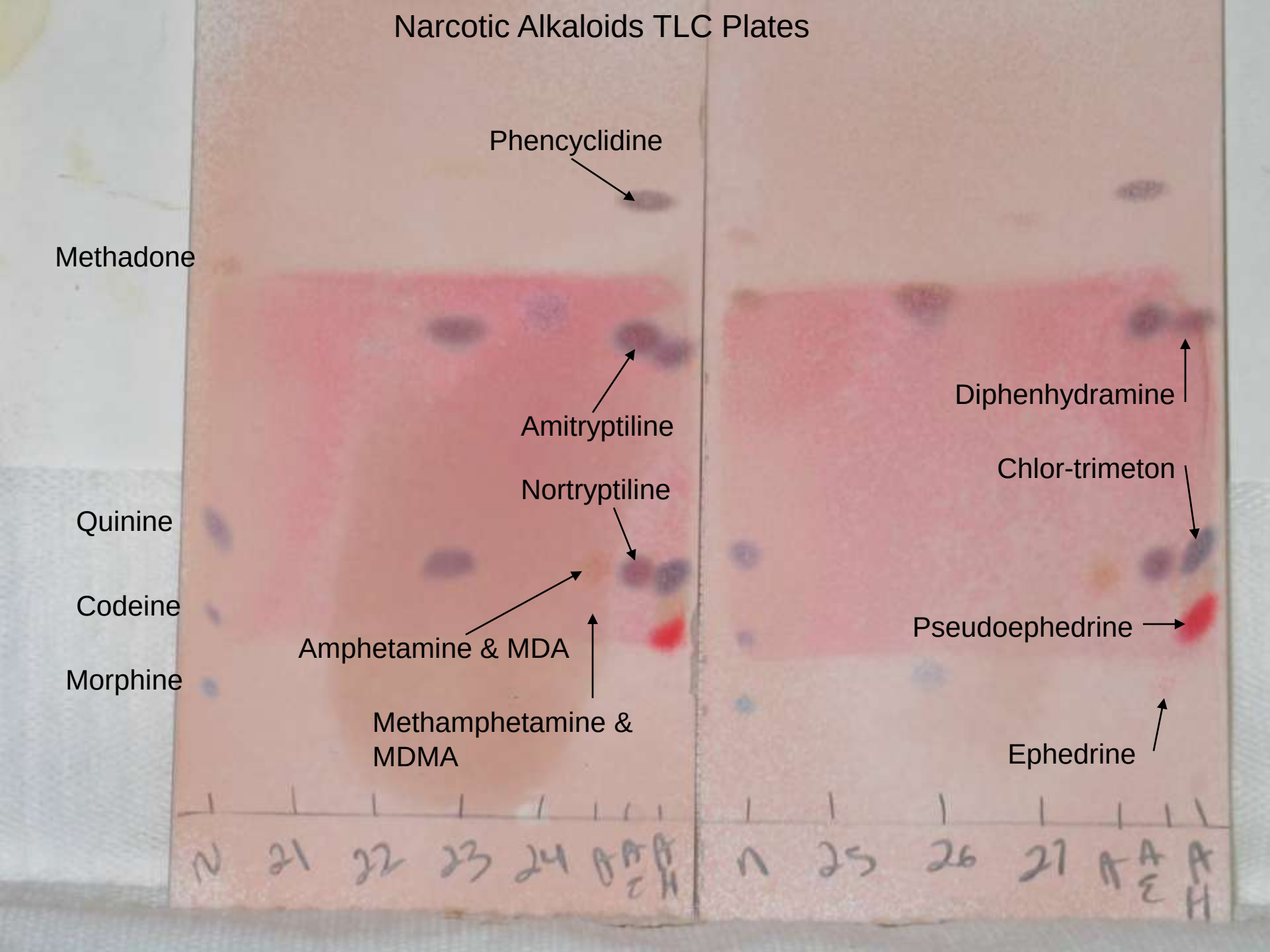


Narcotic Alkaloids TLC Plates



Finding Unexpected Drugs

- **Urine Thin Layer Chromatography (TLC):** used for confirmation of DAU and also to "screen" for about 30 other drugs (when present in high or overdose concentrations).

- **Urine Gas Chromatography/Mass Spectrometry (GC/MS):** mainly for confirmation of DAU, also good for opiates, methadone, meperidine, "free" cocaine, PCP, and dextromethorphan.

- **HPLC of serum or urine by "TCA method":** good for a variety of drugs, especially TCAs, SSRIs, beta blockers, Ca-channel blockers, and benzodiazepines.

- Other specific drug assays intended for routine therapeutic drug monitoring.

Adulteration

- I don't know of any substance a person can ingest safely (i.e. non-toxic) that can 'mask' or interfere with drug screening immunoassays.
  - Except for a diuretic taken with lots and lots of water.
  - http://www.erowid.org/psychoactives/testing/testing.shtml
- However, there are LOTS of things one can add to a urine sample (*ex vivo*) that do interfere.
  - Strong acid, base, detergents (bathroom soap is #1), or any potent protein denaturant (after a 1:40 dilution).
  - Can also buy "clean urine" online.
- Basis for detection of an adulterated specimen is either to
  - test directly for the adulterant or
  - test if chemical characteristics of urine exceed physiologic limitations

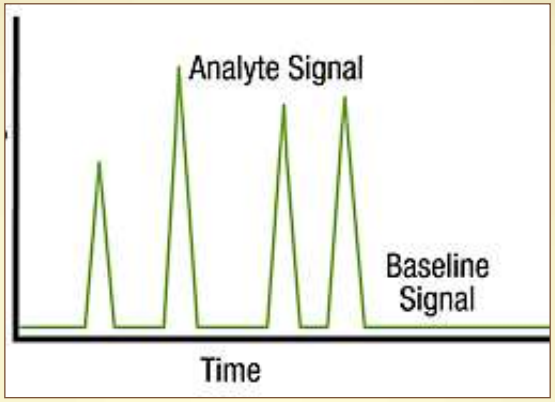
Specimen Validity Testing

1. Temperature measured immediately after void
   - Should be between 32 – 38 °C (90 – 100 ° F)
   - Detects substitution with another urine sample
2. 3 < pH < 11
   - Detects acids, bases, and detergents (often change pH)
3. Concentration
   - A urine Cr < 2 mg/dL and a specific gravity < 1.001 is considered ***inconsistent*** with human urine.
   - A urine Cr < 20 mg/dL and a specific gravity < 1.003 is considered overly ***dilute***. May want to request a second, hopefully more concentrated, sample.
4. Specific additives monitored in forensic settings
   - Nitrites, Chromium, Peroxidase, Halogens, Glutarldehyde

Chromatographic Techniques are Modular

Sample Preparation → Chromatography → Detection

- cell lysis
- protein precipitation
- filtration
- concentration
- liquid-liquid extraction
- solid phase extraction
- hydrolysis
- derivitazation



- gas chromatography
  - packed column
  - capillary
- liquid chromatography
  - reverse phase
  - normal phase
  - ion-exchange
  - affinity
  - size exclusion
- thin layer/paper

- UV/Vis absorption
- infrared absorption
- flame ionization
- electrochemical
- fluorescence
- light scattering
  - nephelometry
  - turbidometry
- **Mass Spectrometry**

Specificity is Also Modular

Sample Prep

Chromatography

Detection

Cumulative Specificity of Method

Potential Preparative Steps

- Cell lysis or tissue homogenation
- Removal of Soluble Protein
  - precipitation
  - filtration
- Extraction
  - single step liquid-liquid extraction
  - multiple step liquid-liquid extraction (“back-extraction”)
  - solid phase extraction
- Chemical Modification
  - derivatization for increased volatility
  - chemical or enzymatic hydrolysis of glucuronide
- Concentration
  - evaporation

Protein Precipitation

- Generally performed chemically:
  - Organic solvents (acetone, acetonitrile, methanol)
  - Zinc sulfate in methanol (pH 7)
  - 5-sulfosalicylic acid in methanol (pH 1-2)
  - Perchloric or trichloroacetic acid
  - Sodium tungstate in sulfuric acid
  - Ammonium sulfate in HCl, heated
- Many lyse RBCs and can be used for whole blood analysis (e.g. zinc sulfate in methanol for immunosuppressants).
- Major concern is potential "trapping" of protein-bound drug in the precipitate.
  - Needs to be tested for empirically, i.e. hard to predict.
  - Can sometimes be extracted by washing the precipitate, but not always reproducible. Use of an internal standard can help.

Protein Filtration

- Uses a nitrocellulose (or equivalent) filter to specifically retain proteins greater than a given size (generally 3000 daltons). Most commonly, the solution is "pushed" through the filter using centrifugation.
- Separates "protein-free" from "protein-bound" drug. Used to get "free" drug concentrations.
- Note that the ratio of free/bound drug is NOT changed during the procedure (as long as you don't do a wash step); therefore, measured concentration of free drug is accurate.
- Generally requires about 1 ml of plasma/serum to get about 0.1 ml of protein-free filtrate.

Organic Extraction

- Most "drugs" are hydrophobic in nature and have greater solubility in organic phases than in aqueous solution.
  - Note that a majority (but not all) of the physiologic components of plasma and urine (protein, carbohydrates, electrolytes) are highly water soluble and are not extracted into an organic phase.
- The "organic phase" can be another liquid (e.g. hexane) or a solid, generally in the form of a column packed with polymer-coated beads.
- Organic extraction can be selective because the aqueous solubility of many drugs are pH dependent.

Sample Preparation for GC/MS

Step 1: "Hydrolysis"

- add β-glucuronidase to 1.0 ml of patient urine
- incubate at 65 °C for 3 hours
- centrifuge to remove debris after cooling to room temperature

Step 2: "Extraction"

- add 10 µl of internal standard solution to each sample.
- add 0.5 of 100 mM phosphate buffer at pH 6.0 (check pH and adjust)
- run each sample over a pre-washed solid-phase extraction column
- wash column extensively with aqueous phosphate buffer
- acidify with 0.1 N HCl and elute with methanol

Step 3: "Concentration"

- evaporate methanol by blowing dry nitrogen over sample

Step 4: "Derivatization"

- add 75 µl of MSTFA and heat at 70 °C for 20 minutes
- volume remaining after derivatization is transferred to autosampler for injection

Chromatographic Components

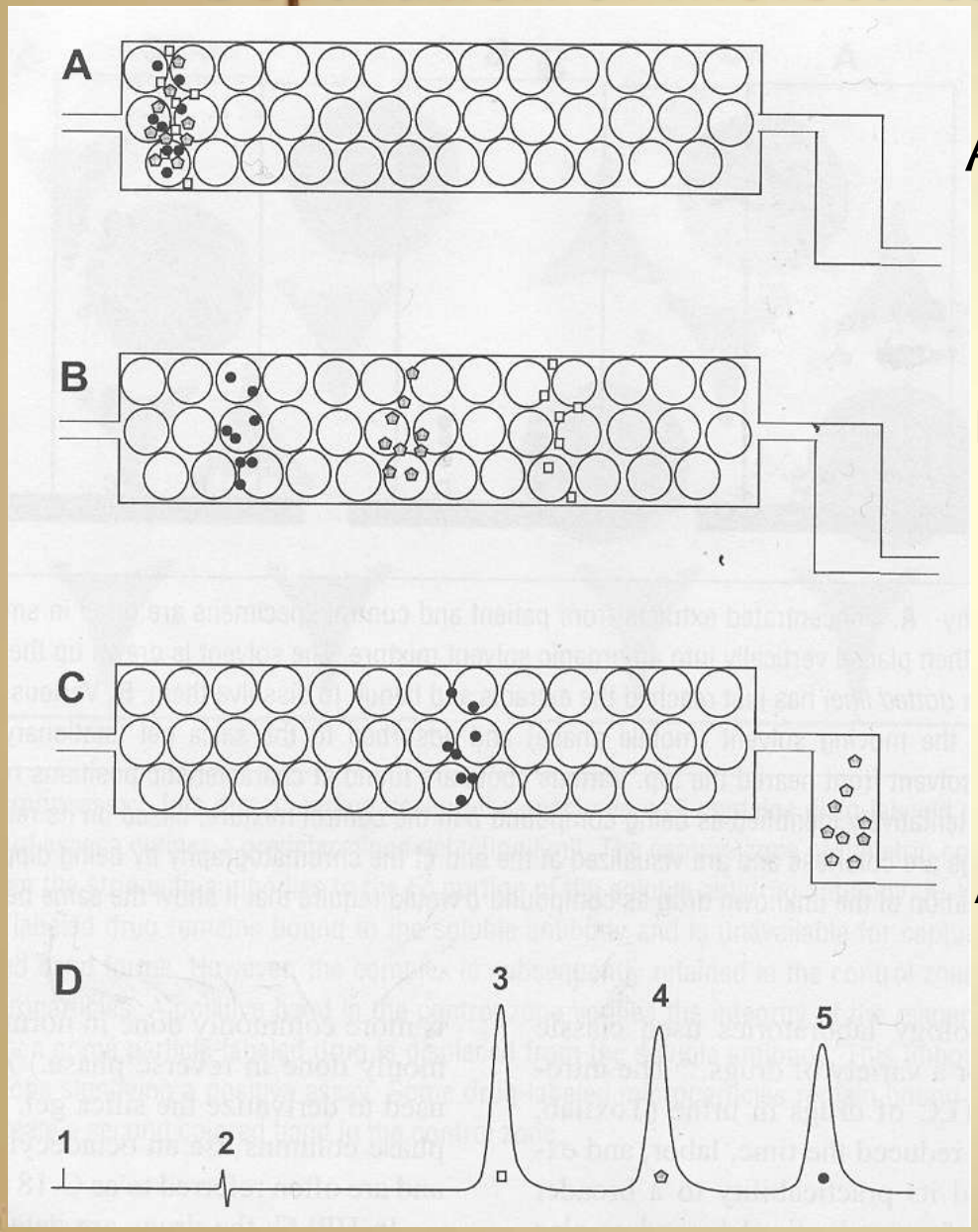
Each flavor of chromatography is best understood by breaking it down into modular components:

1) Sample "loading"
2) The "mobile phase" during separation.
3) The "stationary phase" during separation.

Separation of individual sample components is always based on their *relative affinity* for the *mobile* versus the *stationary* phases.

Because some molecules have higher affinity for the stationary phase, they will pass through the column *slower* than others and, therefore, will be separated from each other (we say they have been "retained" by the column).

Separation of Molecules by Chromatography



After injection, all molecules start out overlapping.

Due to varying relative affinity for the stationary versus the mobile phases, individual molecules begin to separate.

As the different molecules elute off of the column, they are detected as resolved “peaks”.

Type of Chromatography	Sample Loading	Mobile Phase	Stationary Phase
Gas Chromatography (GC)	Heating	Variety of Gases	Capillary column with a narrow hydrophobic coating.
Liquid Chromatography (LC)	Injection	Variety of Liquids (gradients also possible)	Column packed with small beads coated with a variety of chemical substituents.
Thin Layer Chromatography (TLC)	“Spotting”, followed by evaporation of the liquid.	Liquid driven by capillary action.	Paper or thin layer of silica plated on glass.

Relative Retention Times

- During separation, the *absolute* rates/times for movement of molecules are not always reproducible.
- For example, columns can get dirty, decreasing the amount of stationary phase available for interaction with molecules. This is equivalent to shortening the length of the column. There can also be variability in the timing of injection.
- However, such perturbations should affect the rates of ***all*** molecules in the same way. Therefore, their ***relative*** rates/times are usually highly reproducible (selection of an appropriate *internal standard* is critical for this reason).
- The "*relative retention time*" (RRT) is defined as the detection time for a individual peak divided by the detection time for a known *internal standard*. RRTs are characteristic and reproducible (but not always 100% specific) identifiers of individual molecules.
- Note that sometimes a "corrected retention time" is reported where the absolute retention time of an analyte is adjusted according to the variation in the retention time of the internal standard compared to a control sample:

$$CRT_{analyte} = RT_{analyte} * (RT_{std} / RT_{std,control})$$

Quantification of Drug Concentrations

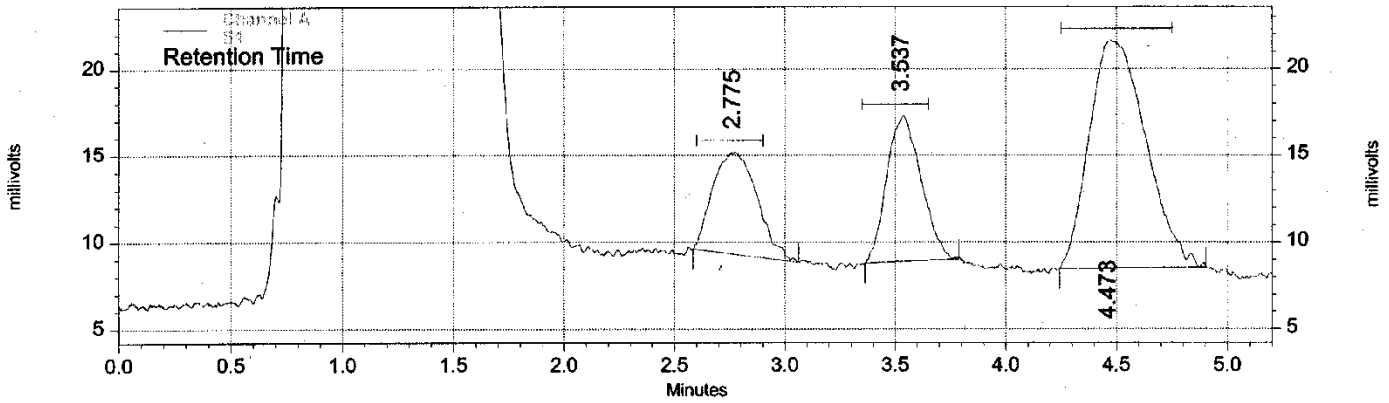
- Peak "area" generally correlates with the amount of drug loaded onto a column and, thus, the original drug concentration.
- However, there can be sample-to-sample variation due to extraction efficiency, loading volumes, detection efficiency, etc.
- Again, the *internal standard* is utilized to correct for variations.
  - Similar to the relative retention time, a *relative peak intensity* is defined and related to drug concentration.
  - Variations in the peak area are not always similar for all molecules (e.g. some molecules may have a lower extraction efficiency due to proteinuria and others may not; it is important that the internal standard shares the same *susceptibility* to interferences or variations in efficiency as the analyte).
  - Therefore, the internal standard is chosen to be chemically similar to the analyte of interest to best correct for variations.
  - However, adequate similarity is *not* easy to predict or establish.
  - Extensive validation is ultimately necessary to satisfy the rigor of your assay. Need to test all anticipated interferences and also un-expected variabilities using "real" patient samples, either with a large correlation study, with a gold standard method and/or with addition/recovery studies.

Protocol for Quantification of Analyte Concentration Based Upon a Calibration Curve

- A known amount of an internal standard is added to every sample (including controls and calibrators) before any other preparative step.
- All samples are brought through the identical preparative steps, separated by a chromatographic method and quantitatively detected.
- The relative peak intensities are measured for a series of calibrators with a fixed amount of internal standard and varying amounts of a known analyte. These relative peak intensities are fit to an equation, generally linear, to define a *calibration curve*.
- Similarly, the relative peak intensities of the unknown samples are calculated and related to the *calibration curve* to quantify the concentration of the analyte (drug) in the original clinical sample.

Amiodarone Report

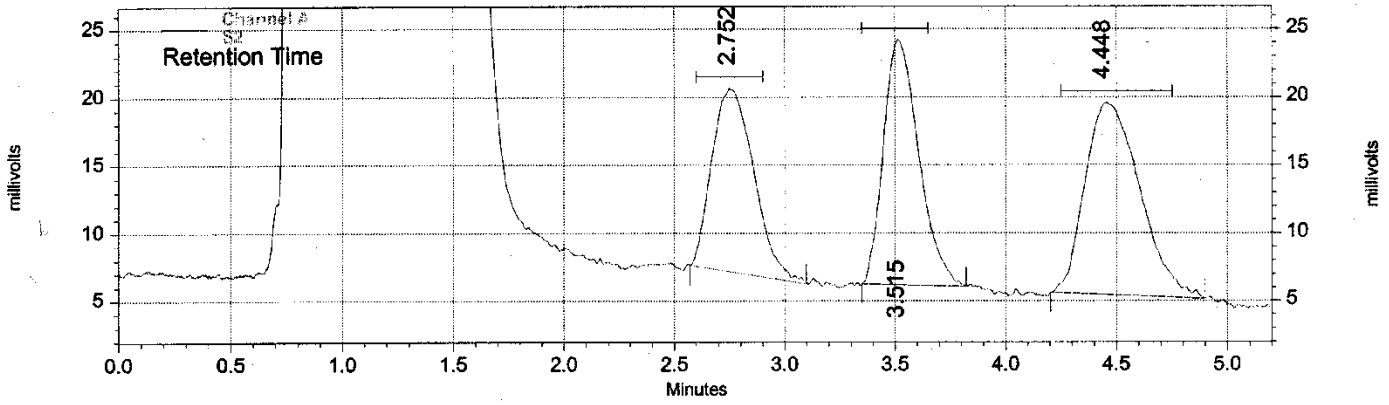
Sample ID: S1
Filename: C:\EZChrom Elite\DATA\012604A02
Method: C:\EZChrom Elite\METHODS\amiodarone.met
Acquired: 1/26/04 11:45:45 AM
Printed: 1/26/04 11:52:13 AM



Name	Retention Time	Area	Concentration	Units
Amiodarone	2.77	8169	0.50 CAL	mg/l
Desethyl	3.54	9061	0.50 CAL	mg/l
IS	4.47	23261	1.00 CAL	mg/l
Totals		40491	2.00 CAL	

Amiodarone Report

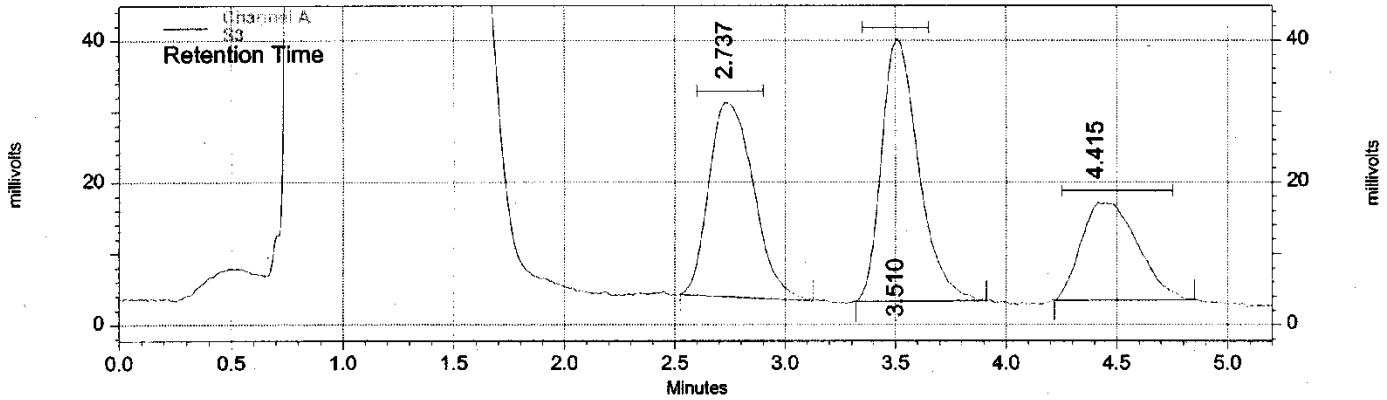
Sample ID: S2
Filename: C:\EZChrom Elite\DATA\012604A03
Method: C:\EZChrom Elite\METHODS\amiodarone.met
Acquired: 1/26/04 11:52:15 AM
Printed: 1/26/04 11:58:43 AM



Name	Retention Time	Area	Concentration	Units
Amiodarone	2.75	17861	1.00 CAL	mg/l
Desethyl	3.52	20046	1.00 CAL	mg/l
IS	4.45	24280	1.00 CAL	mg/l
Totals		62187	3.00 CAL	

Amiodarone Report

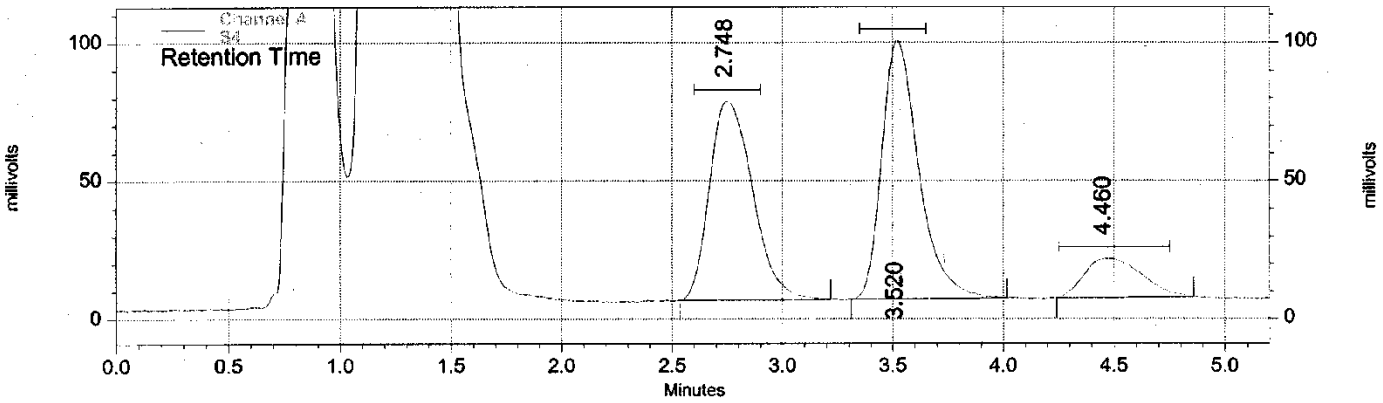
Sample ID: S3
Filename: C:\EZChrom Elite\DATA\012604A04
Method: C:\EZChrom Elite\METHODS\amiodarone.met
Acquired: 1/26/04 11:58:45 AM
Printed: 1/26/04 12:05:13 PM



Name	Retention Time	Area	Concentration	Units
Amiodarone	2.74	38281	2.00 CAL	mg/l
Desethyl	3.51	42913	2.00 CAL	mg/l
IS	4.41	24098	1.00 CAL	mg/l
Totals		105292	5.00 CAL	

Amiodarone Report

Sample ID: S4
Filename: C:\EZChrom Elite\DATA\012604A05
Method: C:\EZChrom Elite\METHODS\amiodarone.met
Acquired: 1/26/04 12:05:15 PM
Printed: 1/26/04 12:11:46 PM



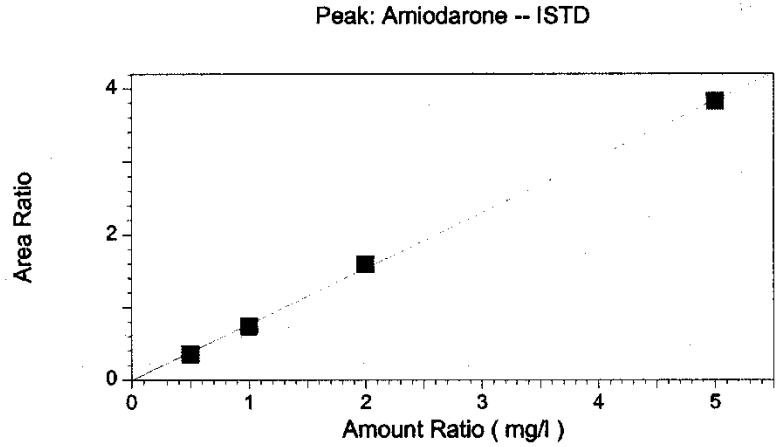
Name	Retention Time	Area	Concentration	Units
Amiodarone	2.75	98285	5.00 CAL	mg/l
Desethyl	3.52	108699	5.00 CAL	mg/l
IS	4.46	25698	1.00 CAL	mg/l
Totals		232682	11.00 CAL	

EZChrom *Elite*

Calibration Report

Method: C:\EZChrom Elite\METHODS\amiodarone.met
Print Time: 1/26/04 12:11:42 PM
User: System
Instrument: applied biosytem

Amiodarone (applied biosytem)
Average RF: 0.749301 RF StDev: 0.0393945 RF %RSD: 5.2575
Scaling: None LSQ Weighting: None Force Through Zero: On
Replicate Mode: Replace
Fit Type: Linear
y = 0.767319x + 0
Goodness of fit (r^2): 0.999296



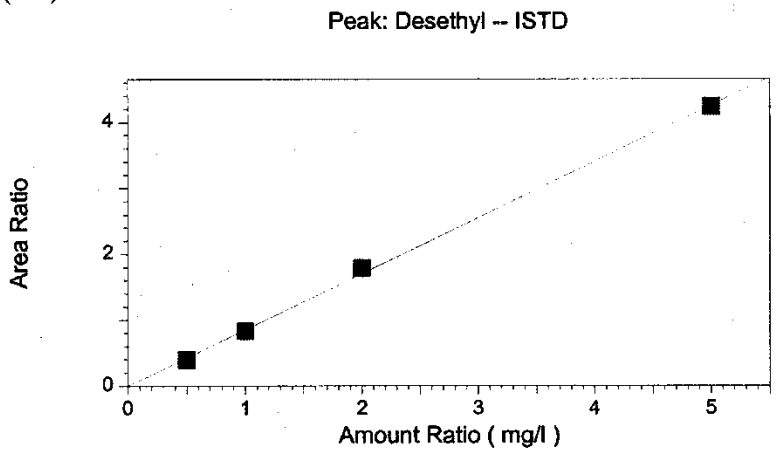
	Level 1	Level 2	Level 3	Level 4
Area Ratio	0.351189	0.735626	1.58856	3.82462
Amount Ratio	0.5	1	2	5
RF	0.702377	0.735626	0.794278	0.764923
Last Area Ratio				
Rep StDev				
Rep %RSD				
Rep Area Ratio 1	0.351189	0.735626	1.58856	3.82462

EZChrom *Elite*

Calibration Report

Method: C:\EZChrom Elite\METHODS\amiodarone.met
Print Time: 1/26/04 12:11:43 PM
User: System
Instrument: applied biosytem

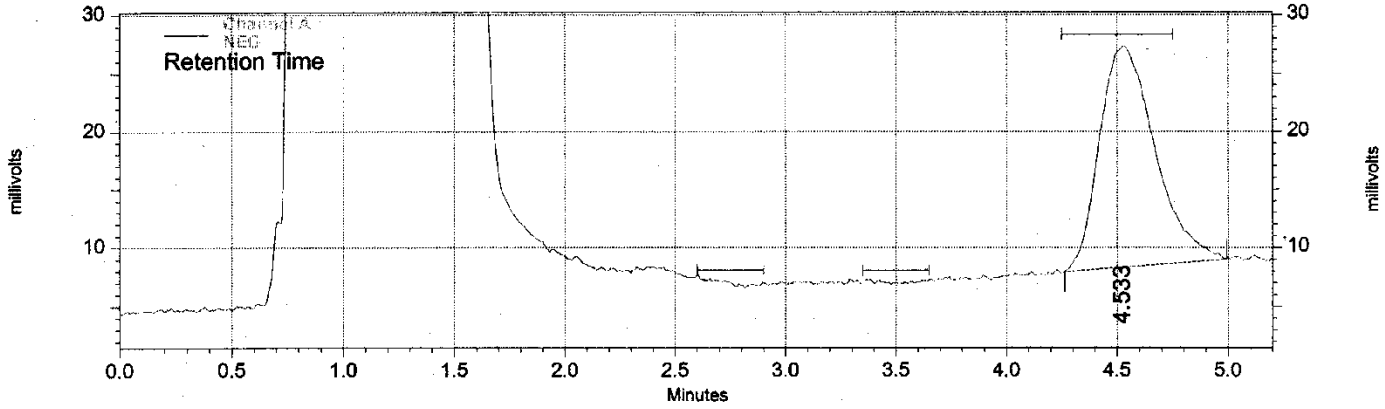
Desethyl (applied biosytem)
Average RF: 0.835262 RF StDev: 0.0462009 RF %RSD: 5.5313
Scaling: None LSQ Weighting: None Force Through Zero: On
Replicate Mode: Replace
Fit Type: Linear
y = 0.850619x + 0
Goodness of fit (r^2): 0.999008



	Level 1	Level 2	Level 3	Level 4
Area Ratio	0.389536	0.825618	1.78077	4.22986
Amount Ratio	0.5	1	2	5
RF	0.779072	0.825618	0.890385	0.845972
Last Area Ratio				
Rep StDev				
Rep %RSD				
Rep Area Ratio 1	0.389536	0.825618	1.78077	4.22986

Amiodarone Report

Sample ID: NEG
Filename: C:\EZChrom Elite\DATA\012604A23
Method: C:\EZChrom Elite\METHODS\amiodarone.met
Acquired: 1/26/04 2:30:41 PM
Printed: 1/26/04 2:37:25 PM

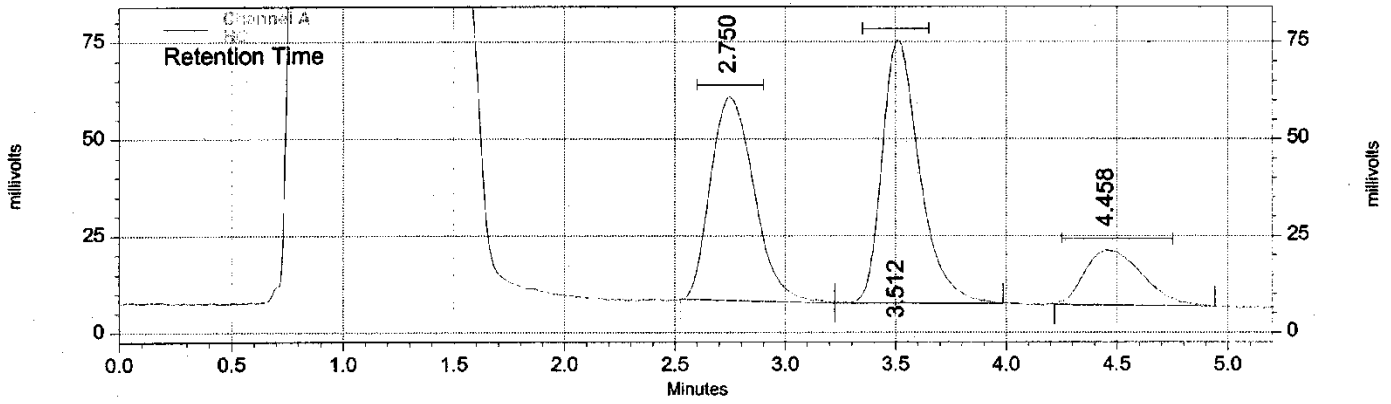


Name	Retention Time	Area	Concentration	Units
Amiodarone			0.00 BDL	mg/l
Desethyl			0.00 BDL	mg/l
IS	4.53	32979	1.00	mg/l
Totals				
		32979	1.00	

Amiodarone Report

Sample ID: HC
Filename: C:\EZChrom Elite\DATA\012604A07
Method: C:\EZChrom Elite\METHODS\amiodarone.met
Acquired: 1/26/04 12:18:13 PM
Printed: 1/26/04 12:24:40 PM

Rel. Ret. Time for Amiodarone = 2.75 / 4.46 = 0.62

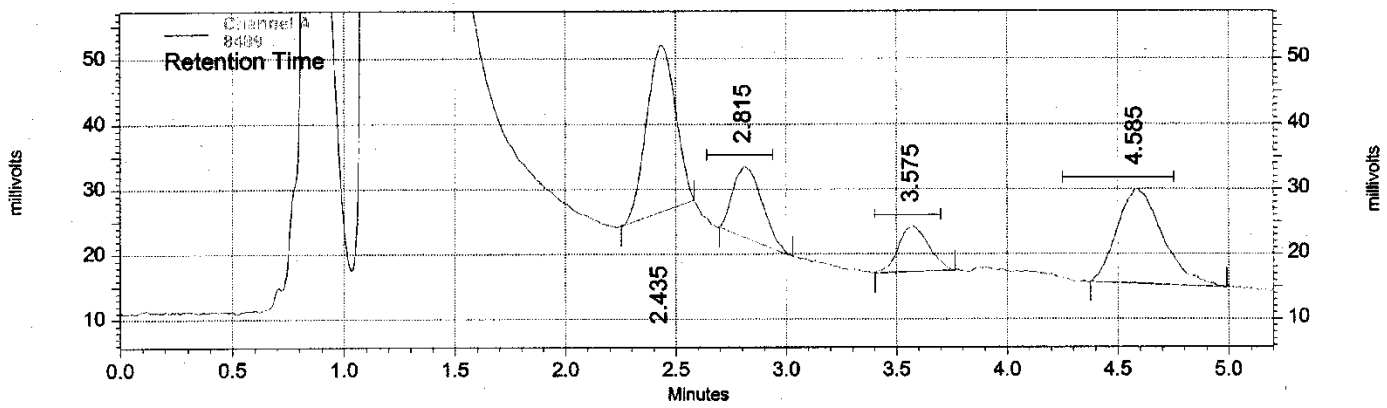


Name	Retention Time	Area	Concentration	Units
Amiodarone	2.75	72256	3.69	mg/l
Desethyl	3.51	80112	3.69	mg/l
IS	4.46	25530	1.00	mg/l
Totals		177898	8.38	

Amiodarone Report

Sample ID: 8489
Filename: C:\EZChrom Elite\DATA\012604A100
Method: C:\EZChrom Elite\METHODS\amiodarone.met
Acquired: 1/26/04 2:59:04 PM
Printed: 1/26/04 3:04:41 PM

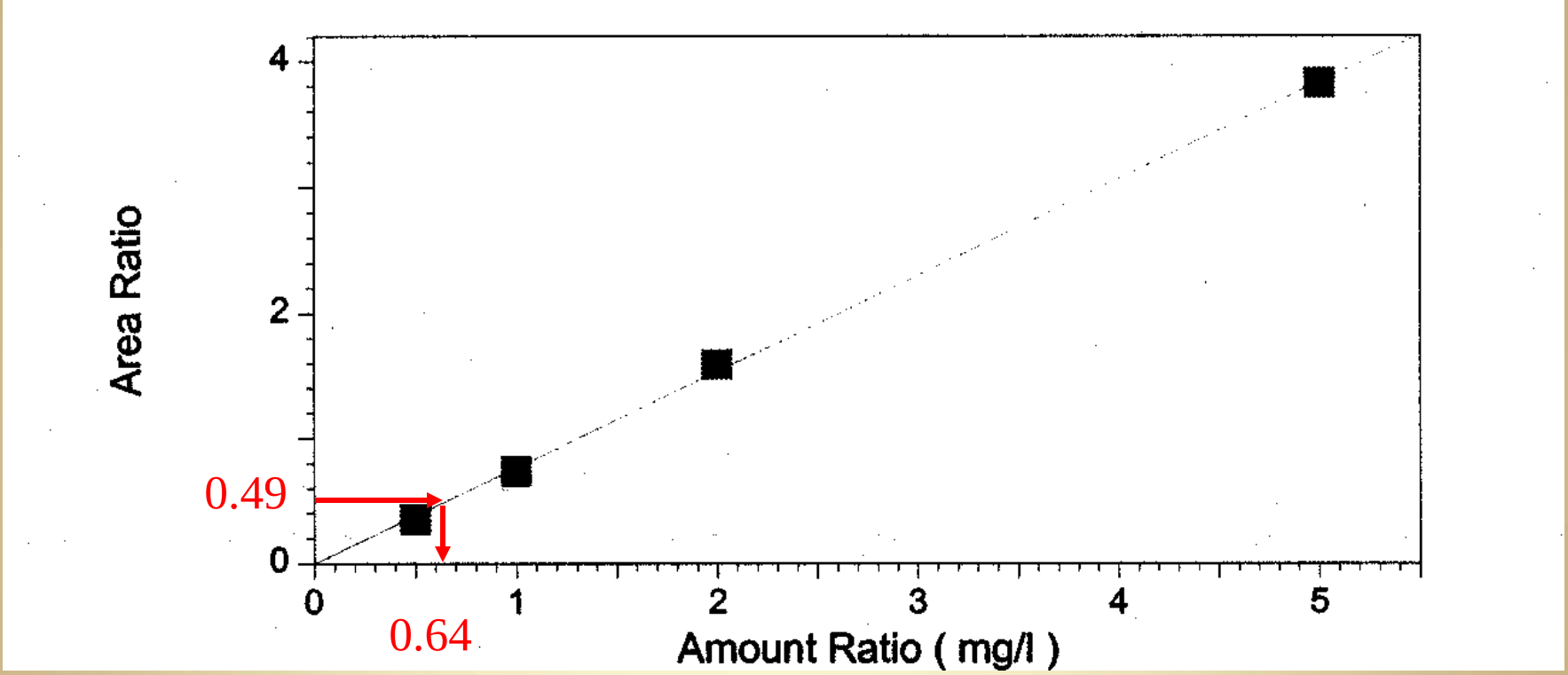
Rel. Ret. Time for Amiodarone = 2.82 / 4.59 = 0.61; agrees with standard and therefore confirms identity…



Name	Retention Time	Area	Concentration	Units
Amiodarone	2.82	10129	0.64	mg/l
Desethyl	3.58	6577	0.38	mg/l
IS	4.59	20579	1.00	mg/l
Totals		37285	2.02	

Rel. Peak Int. for Amiodarone = 10,129 / 20,579 = 0.49
Now look on curve…

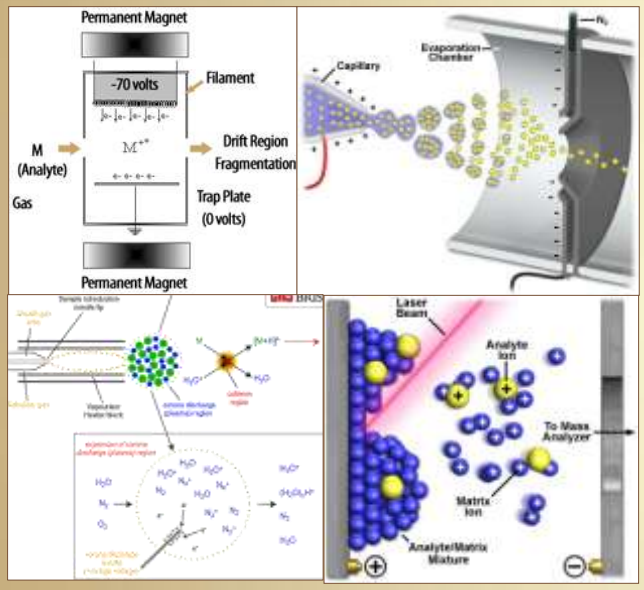
Calibration curve for Amiodarone



Although, the computer fits the measured relative peak intensity to this curve mathematically, visual inspection clearly identifies the correct concentration of amiodarone.

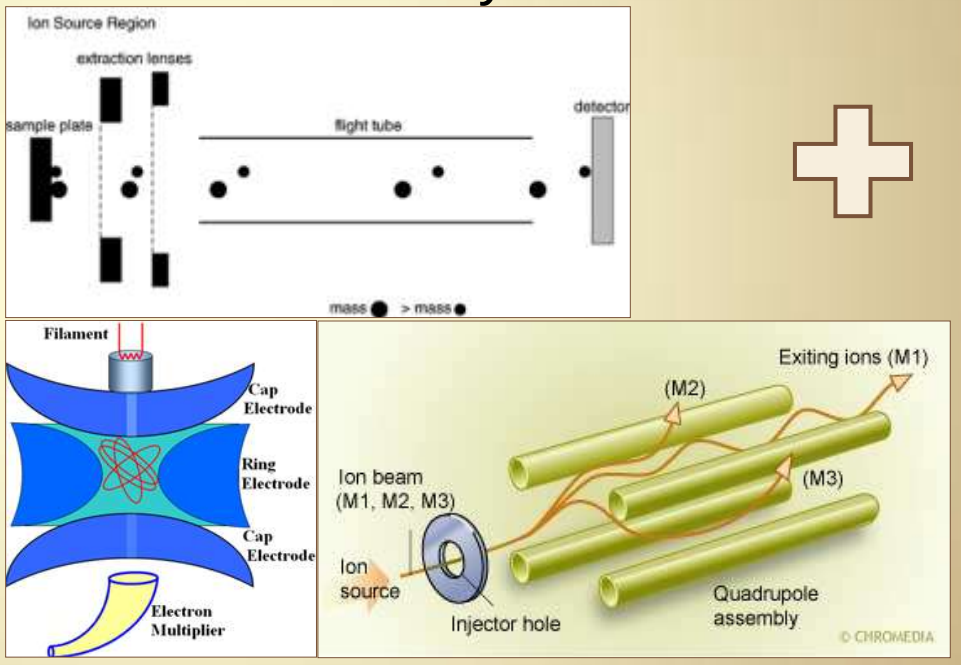
Modern Mass Spectrometers are also *Modular*

Molecular Ionization Method/Device



1. Electron Ionization (EI)
2. Chemical Ionization (CI)
   - APCI
3. ***Electrospray***

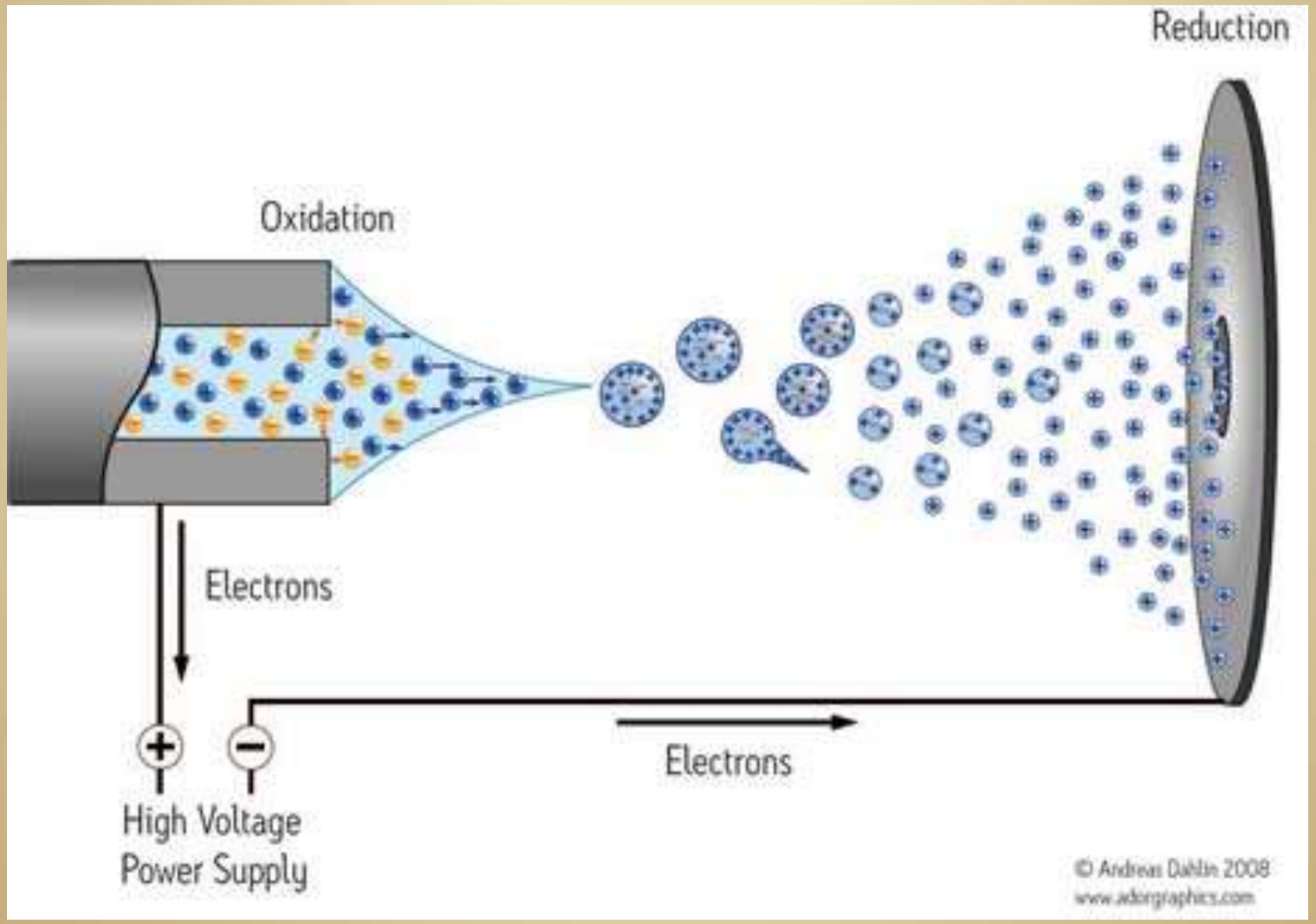
Sequential Array of One or More Mass Analyzers



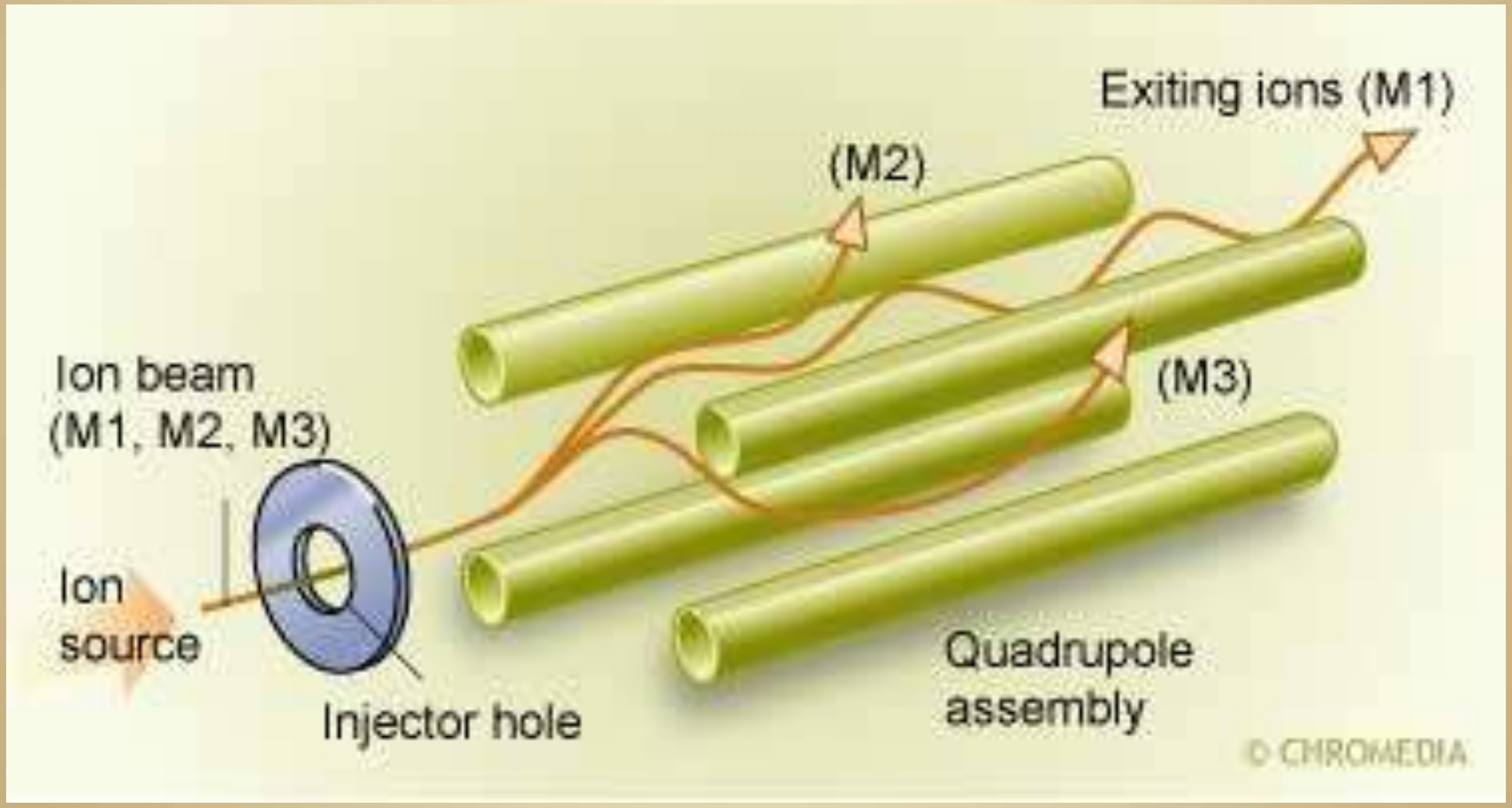
1. Quadrupole
2. Ion Trap
3. Time-of-Flight (TOF)
4. *Molecular*

Non-specific Ion Detector

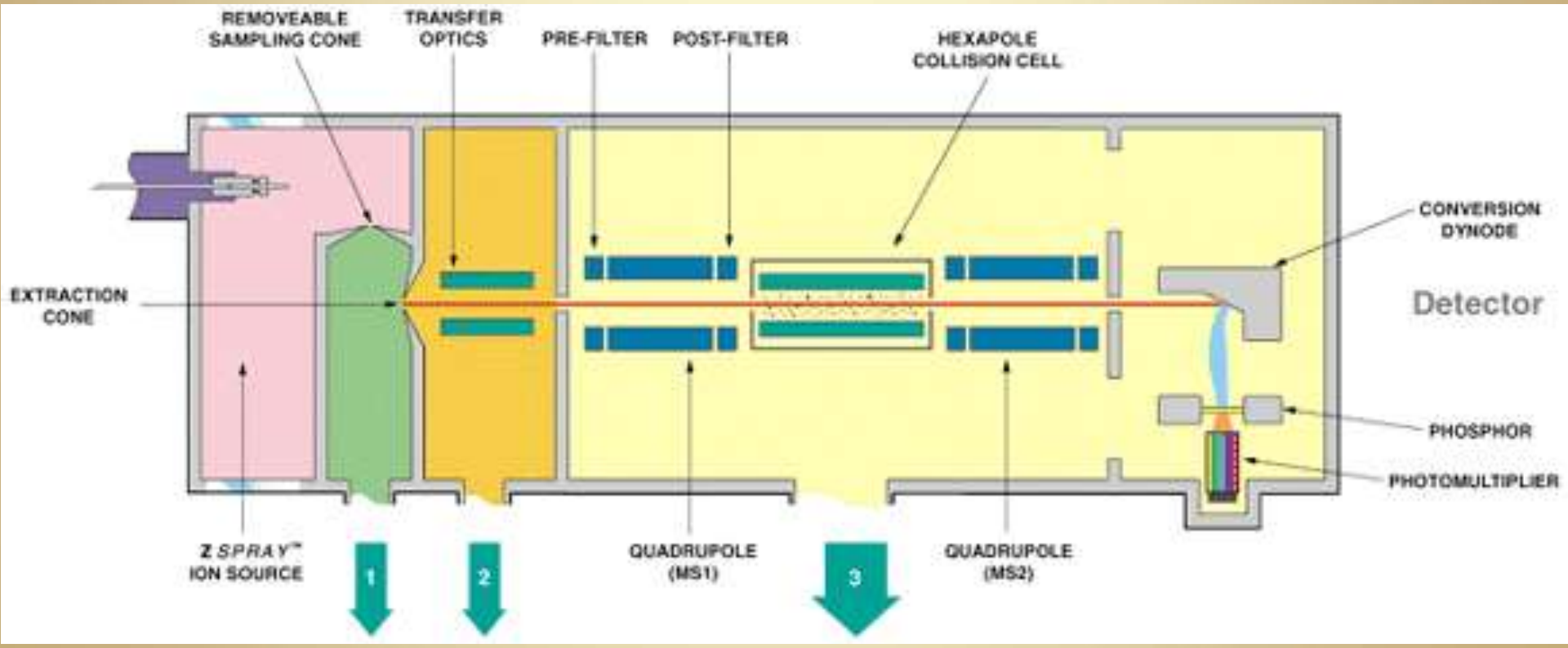
Electrospray Ionization (ESI)



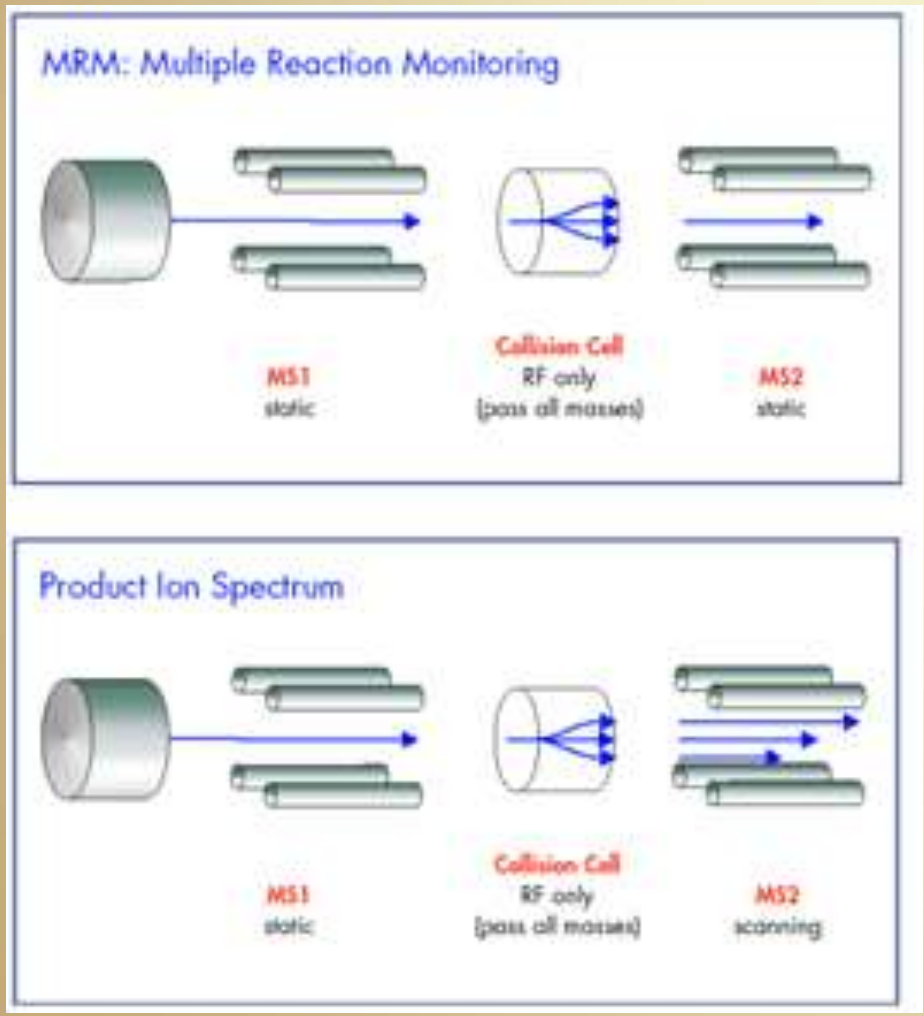
Quadrupole Mass Analyzer

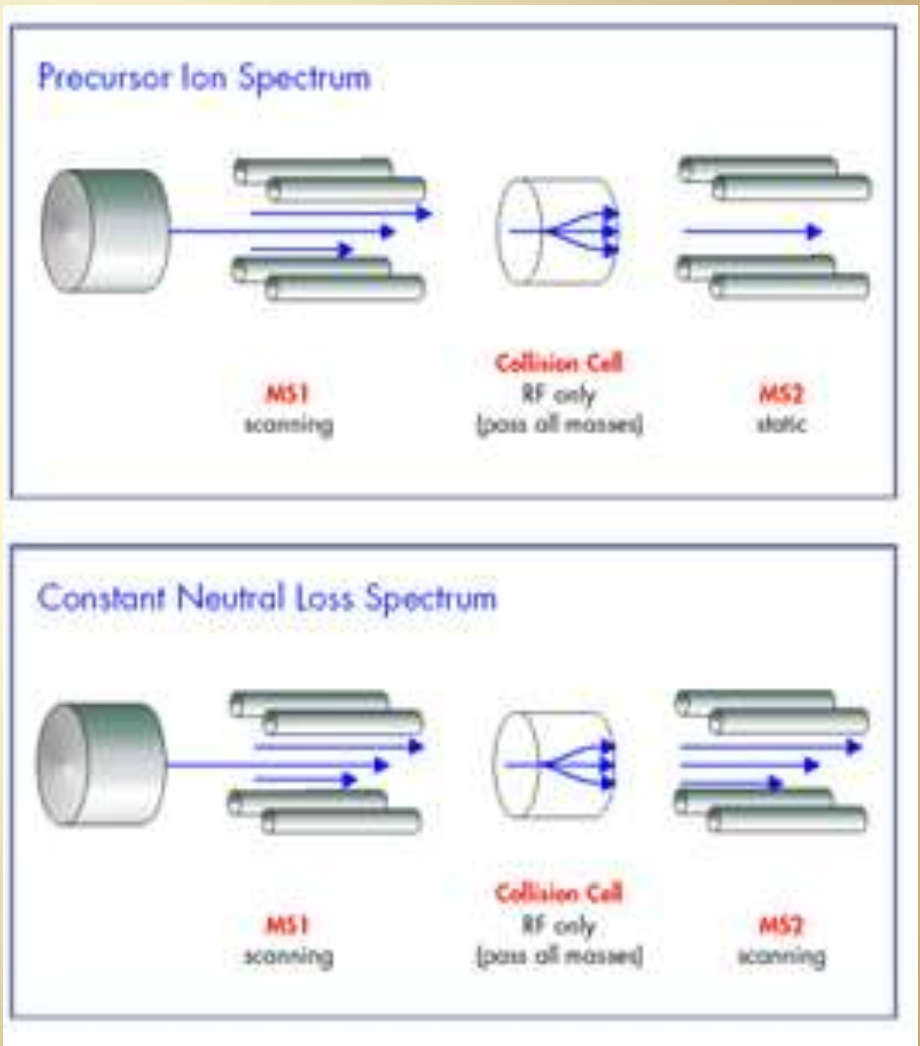


LC-ESI-QQQ "Triple Quad LC/MS/MS"

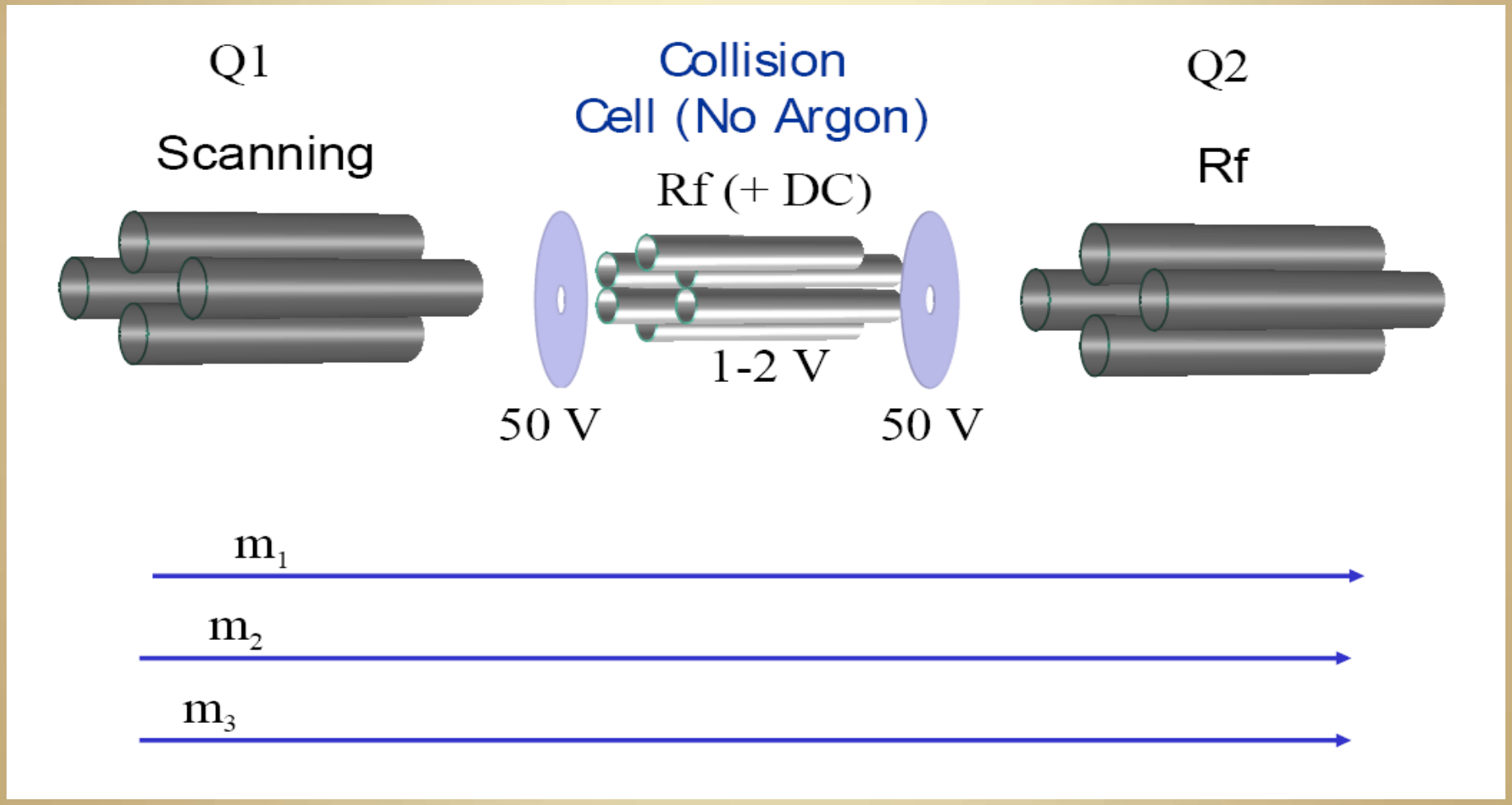


Multiple "Modes" for QQQ MS

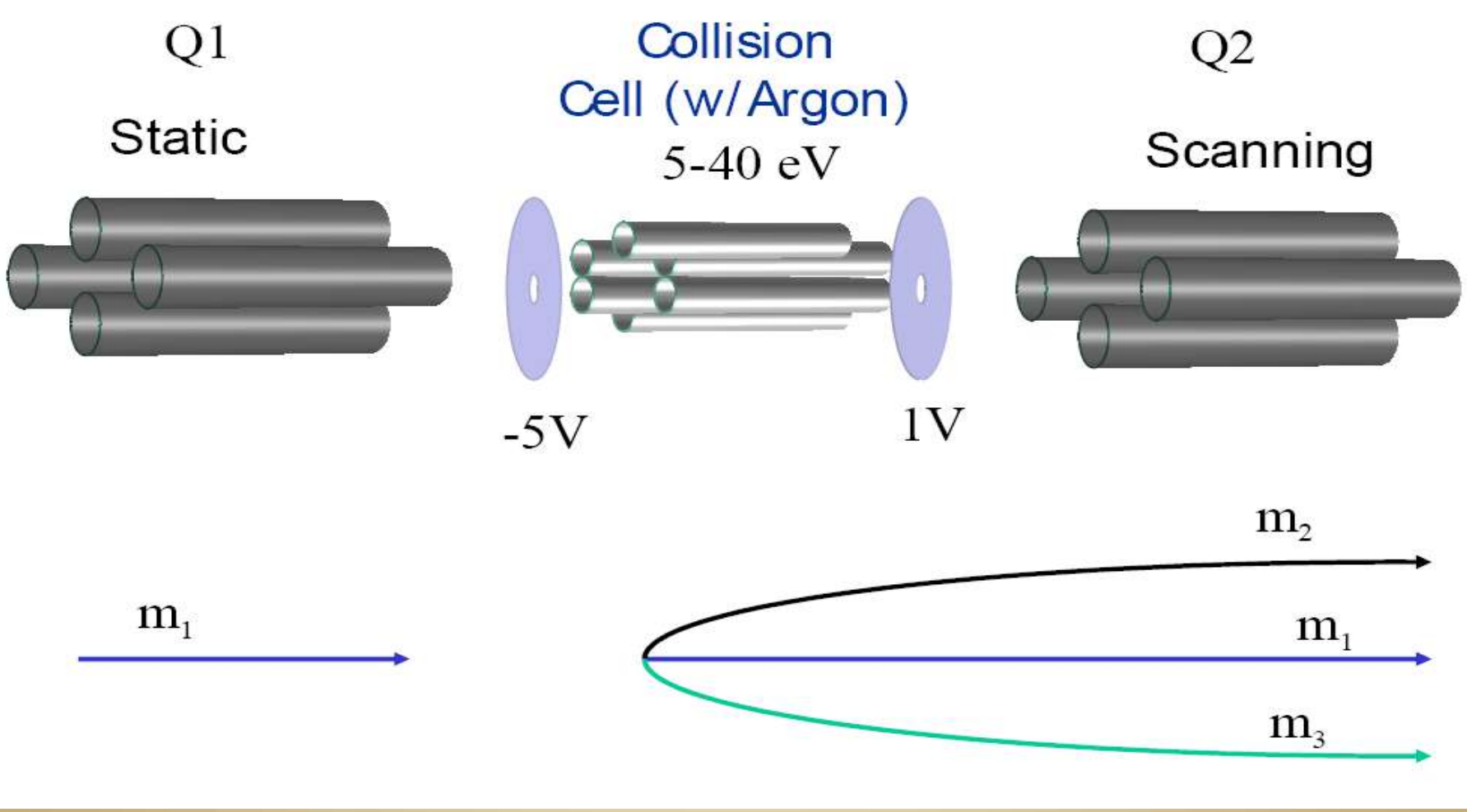




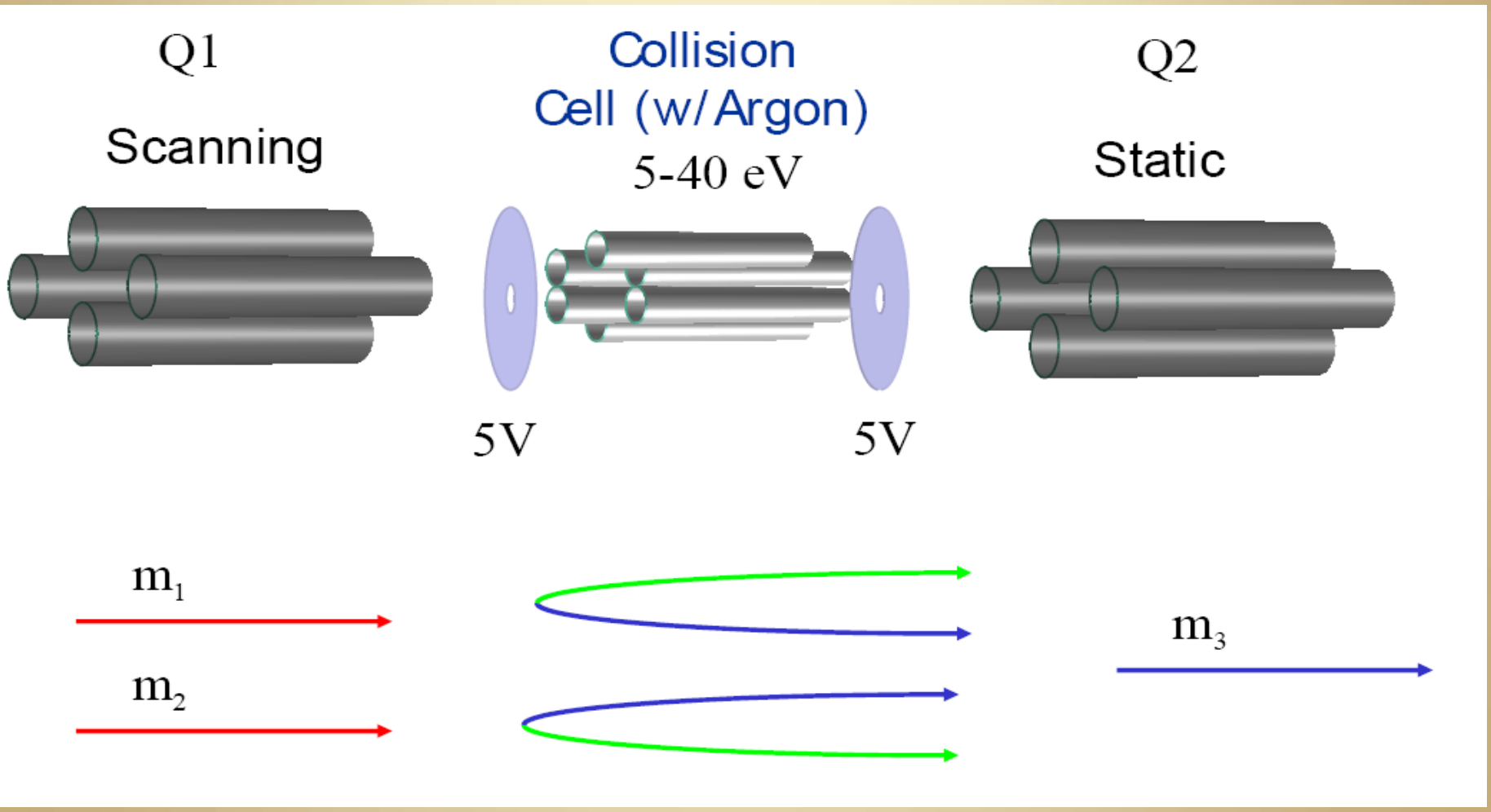
MS1 Scan (same as single quad)



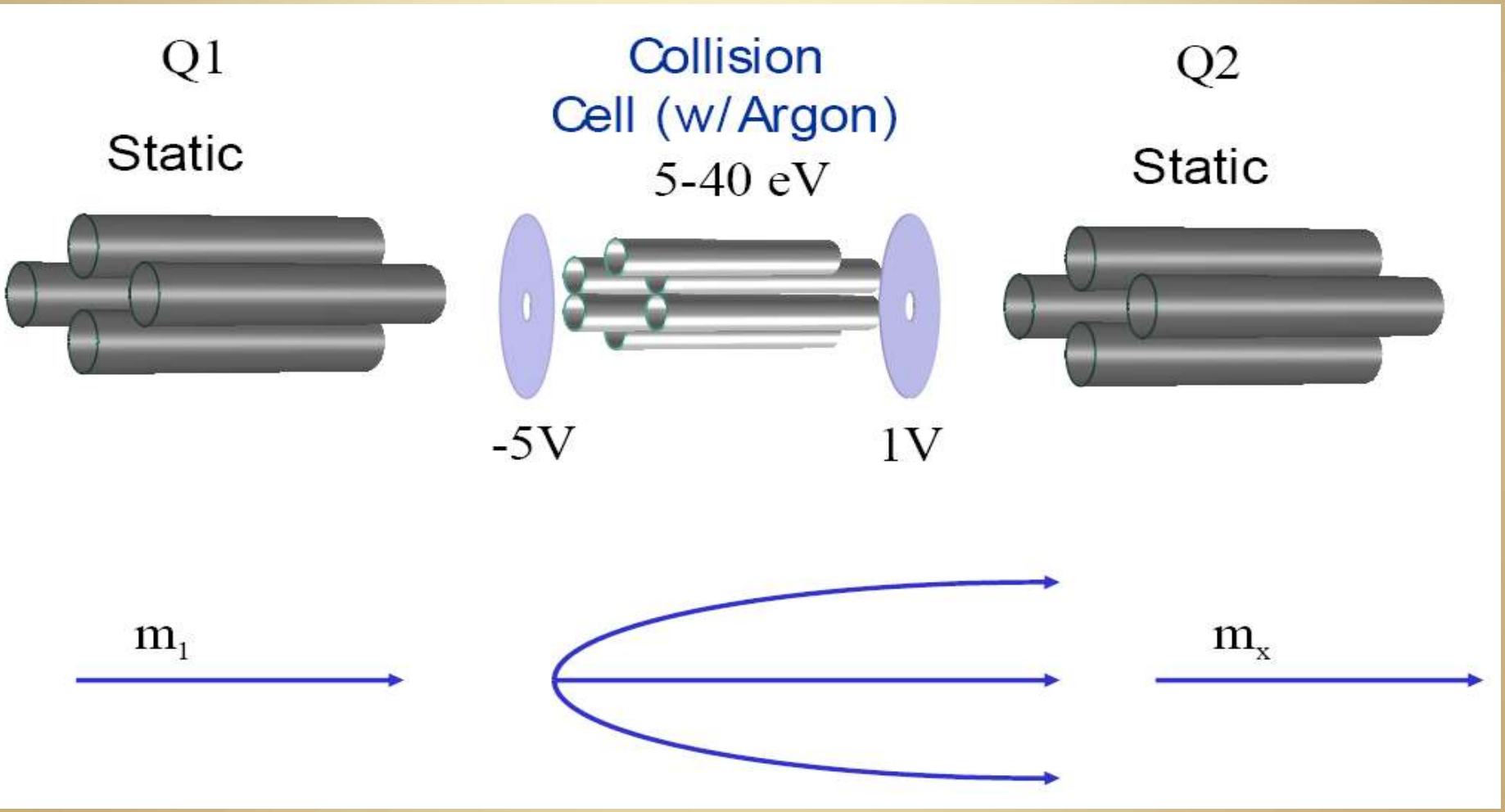
Product Ion Scan



Precursor Ion Scan

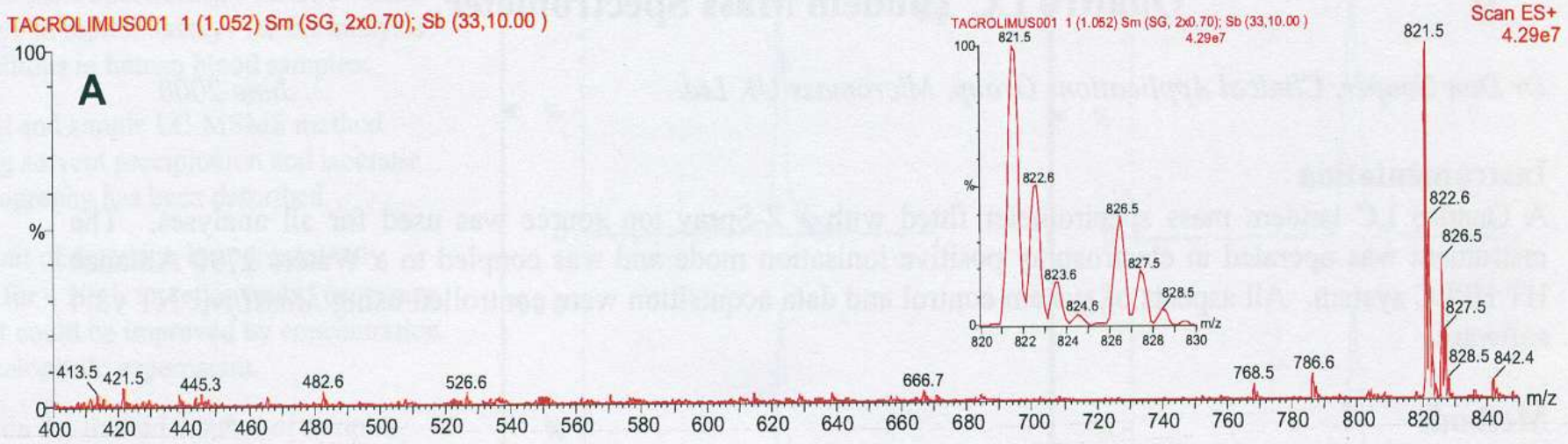


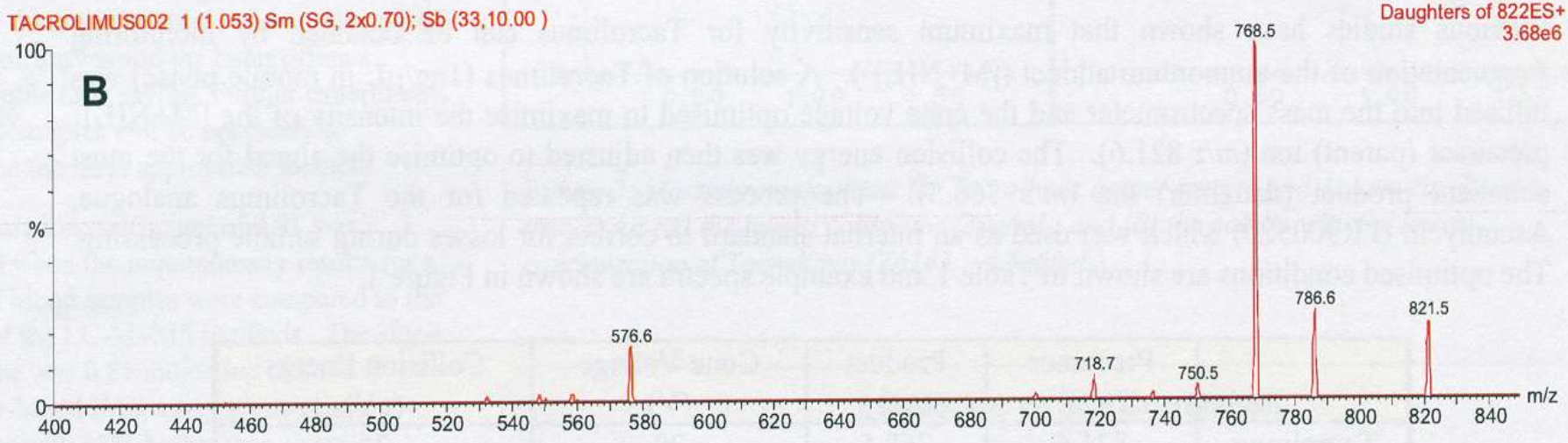
Multiple/Selected Reaction Monitoring (MRM or SRM) Scan



Tacrolimus:

MS1 (A) and Product (B) Ion Scans





Chromatographic Traces for Tacrolimus and Ascomycin MRMs

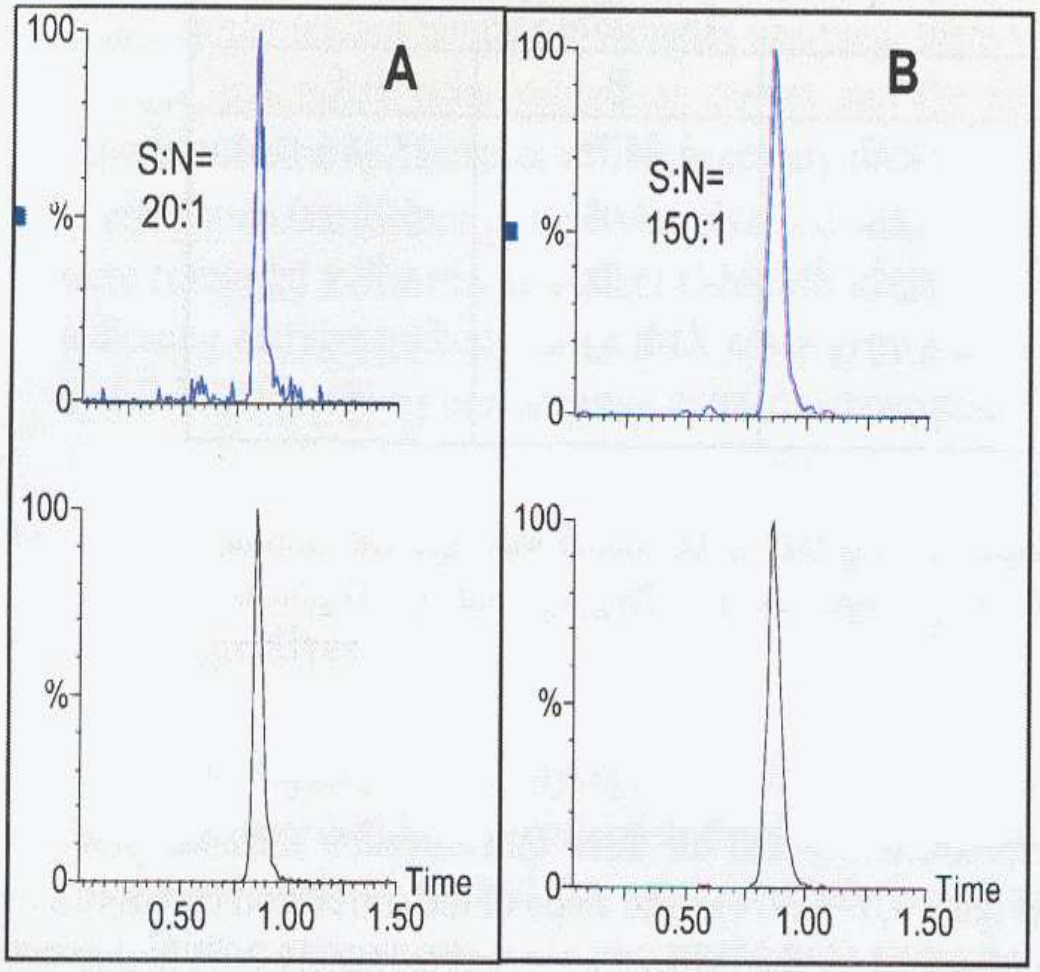


Figure 2. *Mass chromatograms for Tacrolimus (upper traces) and Ascomycin (lower traces) for (A) the lowest calibrator (3ng/mL) and (B) the patient with the lowest concentration of Tacrolimus (Z6143, ~4.5ng/mL)*

The Near Future

- Rapid "dilute and shoot" LC/MS/MS methods will replace immunoassays for detecting drugs of abuse in urine.
- Advantages
  - Lower Reagent Cost
  - More sensitive and more specific
  - Molecule-specific reporting, not "class reactivity"
  - Option for a broad "screen"
  - Confirmation testing no longer required
- Disadvantages
  - Expensive and complex instrumentation
  - Possible issues with turn-around times