LC-MS in Drug Discovery

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What is LC-MS?

(LC) Liquid Chromatography:

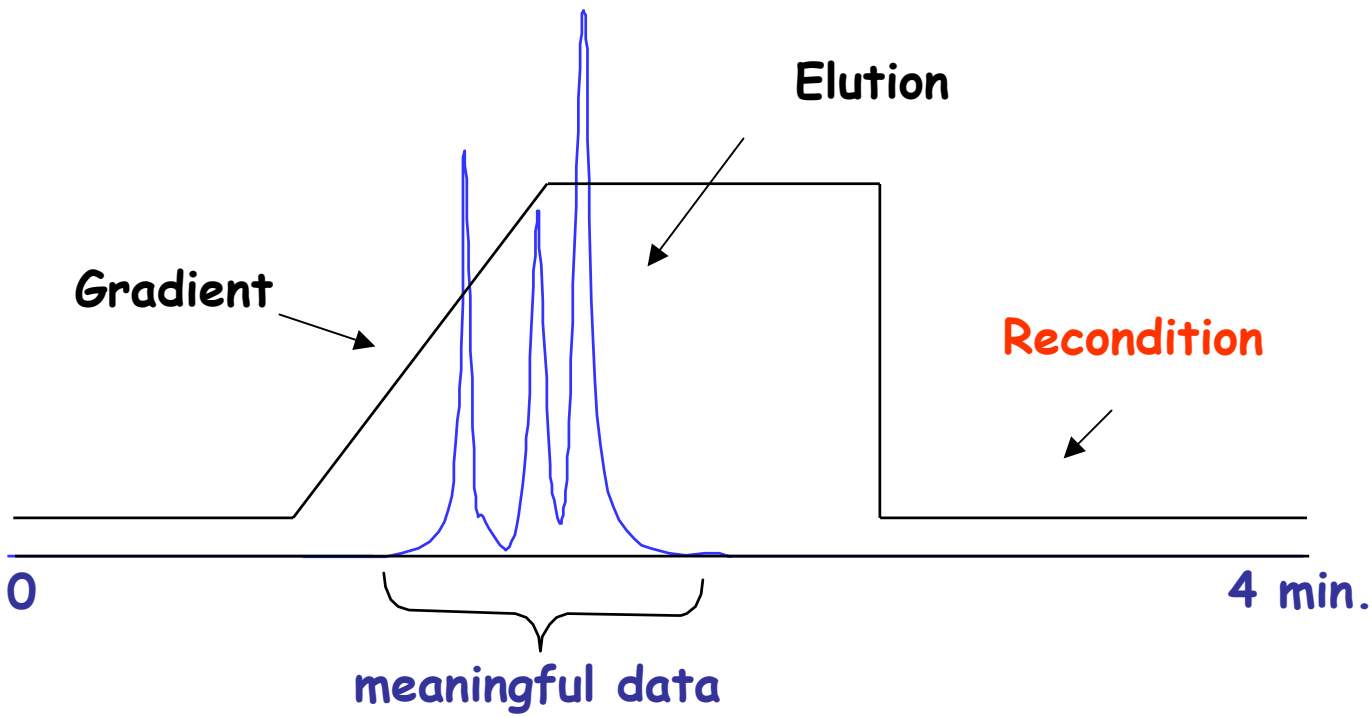
Chromatography is a separations method that relies on differences in partitioning behavior between a flowing mobile phase and a stationary phase to separate the the components in a mixture.

A column holds the stationary phase and the mobile phase carries the sample through it.

Sample components that partition strongly into the stationary phase spend a greater amount of time in the column and are separated from components that stay predominantly in the mobile phase and pass through the column faster.

(www.chem.vt.edu/chem-ed/ac-meths.html)

HPLC Analysis



What is LC-MS?

(MS) Mass Spectrometry:

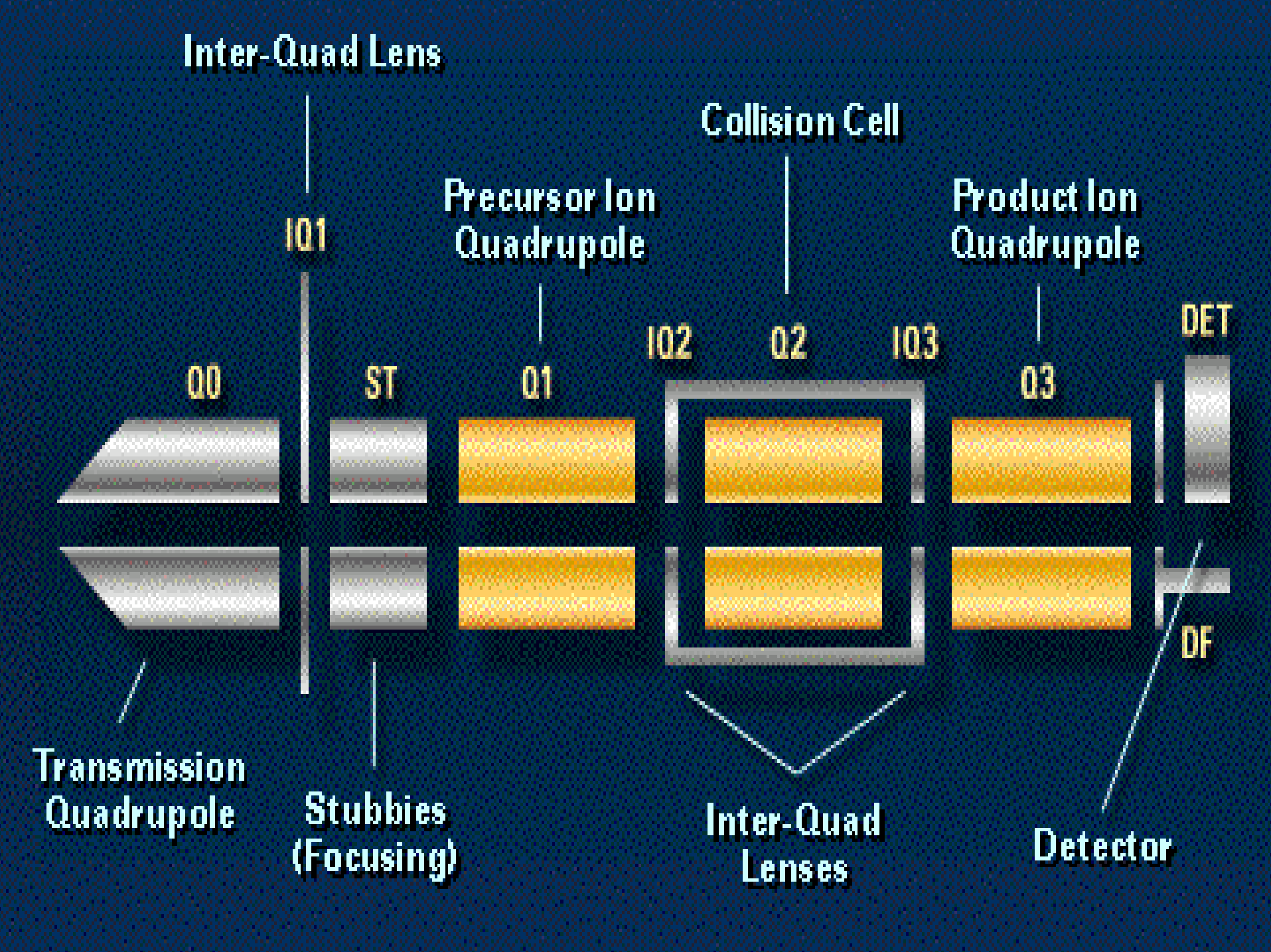
Mass spectrometers use the difference in mass-to-charge ratio (m/z) of ionized compounds to separate them from each other.

Compounds have distinctive fragmentation patterns that provide structural information to specifically detect compounds.

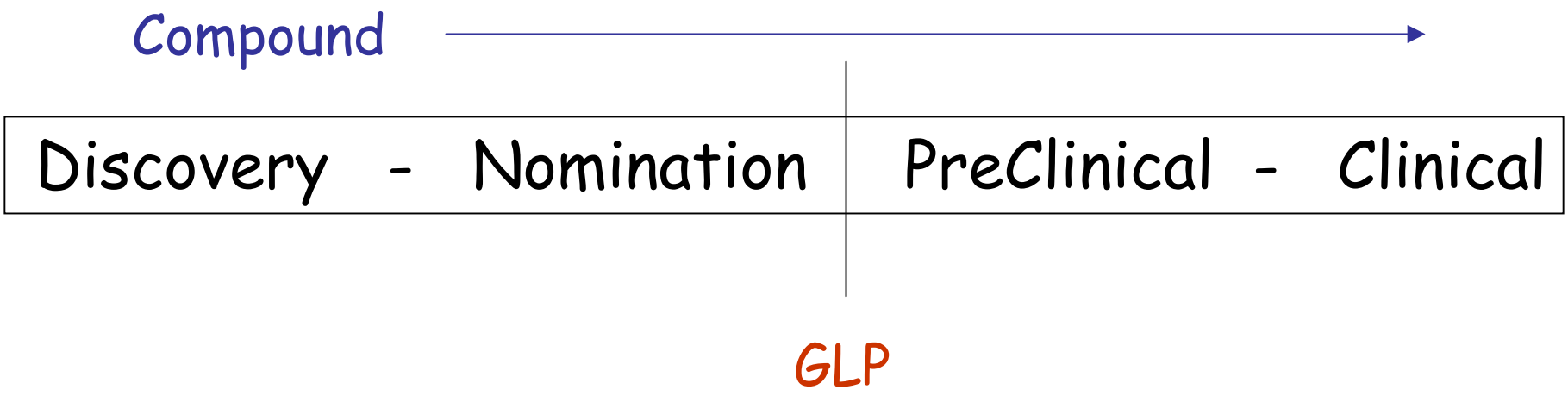
(www.chem.vt.edu/chem-ed/ac-meths.html)

Types of Mass Spectrometers

- **LC-MS** (single quadrupole)
- **LC-MS/MS** (triple quadrupoles)
- **LC-Q** (ion traps, linear ion traps)
- **LC-Q-TRAPS** (quadrupole linear ion traps)
- **LC-TOF-MS** (time-of-flight)
- **MALDI-TOF-MS**
- **Q-TOF-MS** (quadrupole time-of-flight)
- **FT-MS** (Fourier Transform)
- Others


Inter-Quad Lens
IQ1
Collision Cell
Precursor Ion Quadrupole
Product Ion Quadrupole
DET
IQ2
Q2
IQ3
Q0
ST
Q1
Q3
DF
Transmission Quadrupole
Stubbies (Focusing)
Inter-Quad Lenses
Detector

Stages of Drug Discovery and Development



Factors for Consideration

- Bioanalytical Method Requirements
- Application of Technologies
- Risk Assessment

Drug Discovery Programs at BMS

Wallingford, CT:

Virology: HIV (AIDS), Hepatitis C,

CNS: Anxiety, Depression, Alzheimer's, Migraine

Lawrenceville, NJ:

Oncology: Cancer

Immunology: Rheumatoid Arthritis, Asthma

Hopewell, NJ:

Cardiovascular: Thrombosis, Atherosclerosis

Metabolic Diseases: Diabetes, Obesity

Bioanalytical Research Staffing

- Personnel: 24 scientists
- Location: WFD (6), LVL (12), HPW (6)
- Mass Spectrometers : 24 MS/MS
- Full Phase Programs: 31
- Early Phase Programs: 27
- Research Initiatives
- Samples 1-4Q 2003: ~80,000
- Samples 1-4Q 2004: ~135,000

The Gilbert Bioanalytical Chronicles

*"The challenges of bioanalysis stem from the need to **accurately and reproducibly** measure part per million to part per trillion quantities of analyte in complex biological matrices, full of potentially interfering endogenous or drug-related substances."*

John Gilbert *et al.*, "High Performance Liquid Chromatography with Atmospheric Pressure Ionization Tandem Mass Spectrometry as a Tool in Quantitative Bioanalytical Chemistry," in *Biochemical and Biotechnological Applications of Electrospray Ionization Mass Spectrometry,* American Chemical Society (1995)

Relative Amounts

1.0 gram

0.001 gram (milligram)

0.000001 gram (microgram)

0.000000001 gram (nanogram)

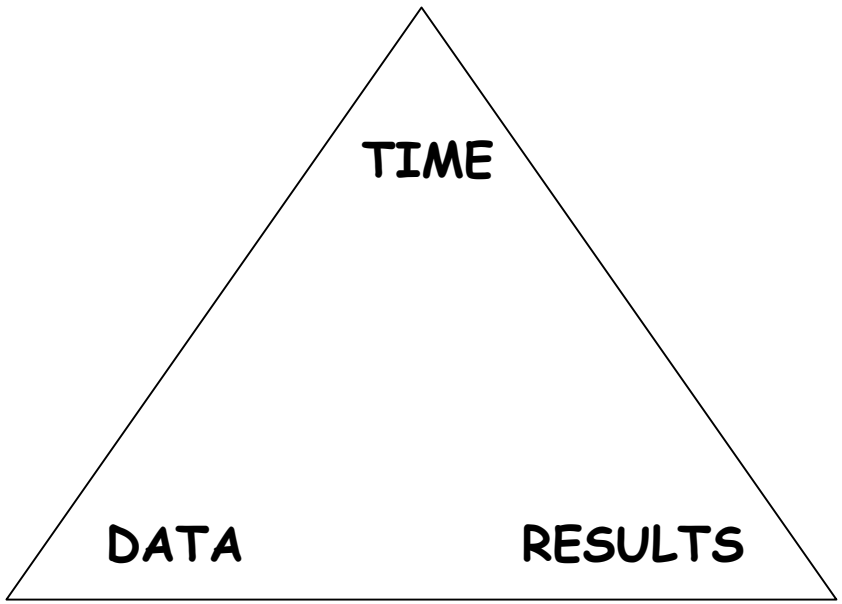
0.000000000001 gram (picogram)

Outline

- Strategies and Work Flow Processes
- Drug Discovery Process
- Development and Implementation of Multiple Component LC-MS-based Bioanalytical Methods
- Examples, Questions, Comments

Keys to Developing Successful Screening Strategies

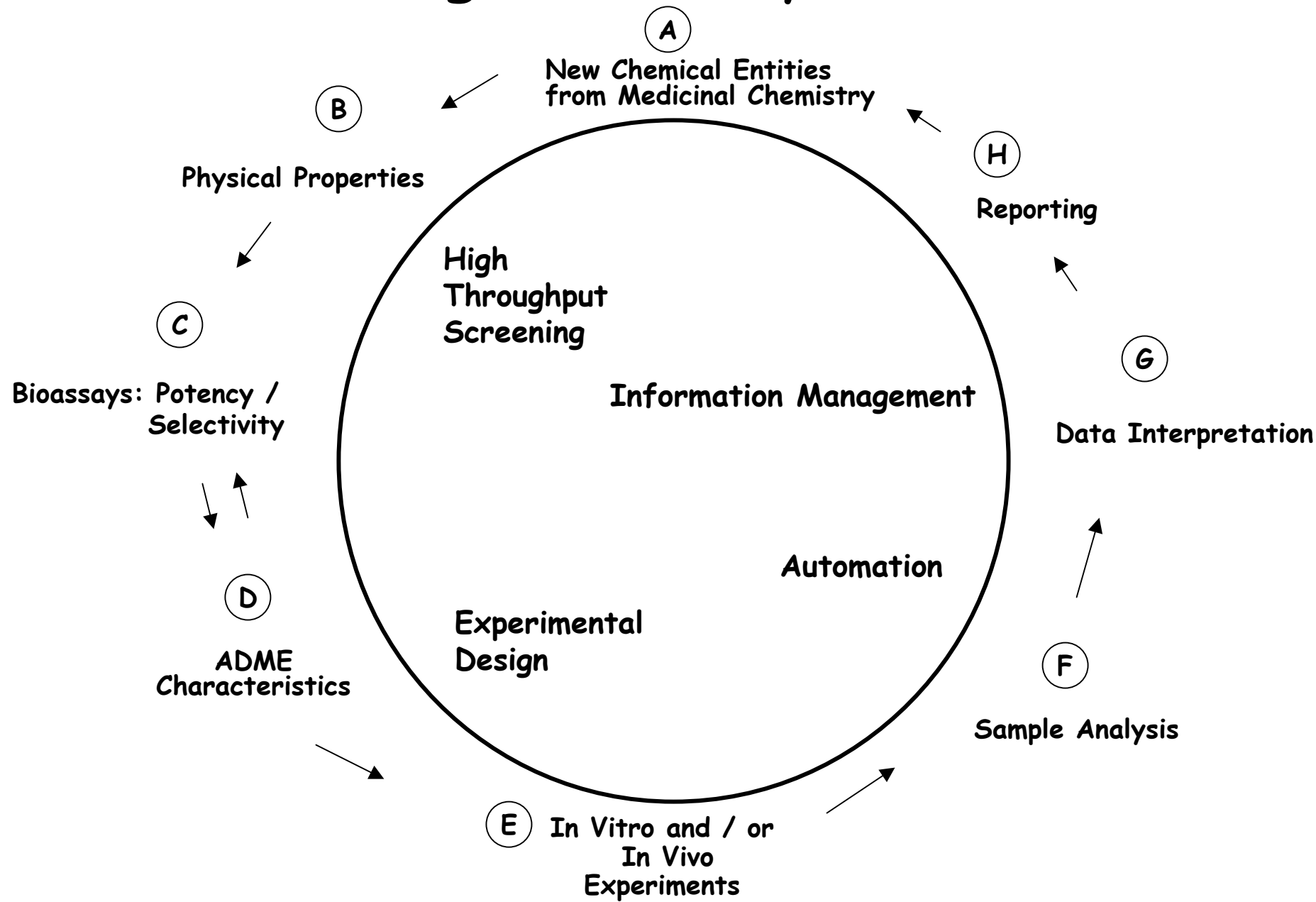
the **TIME** required to generate data



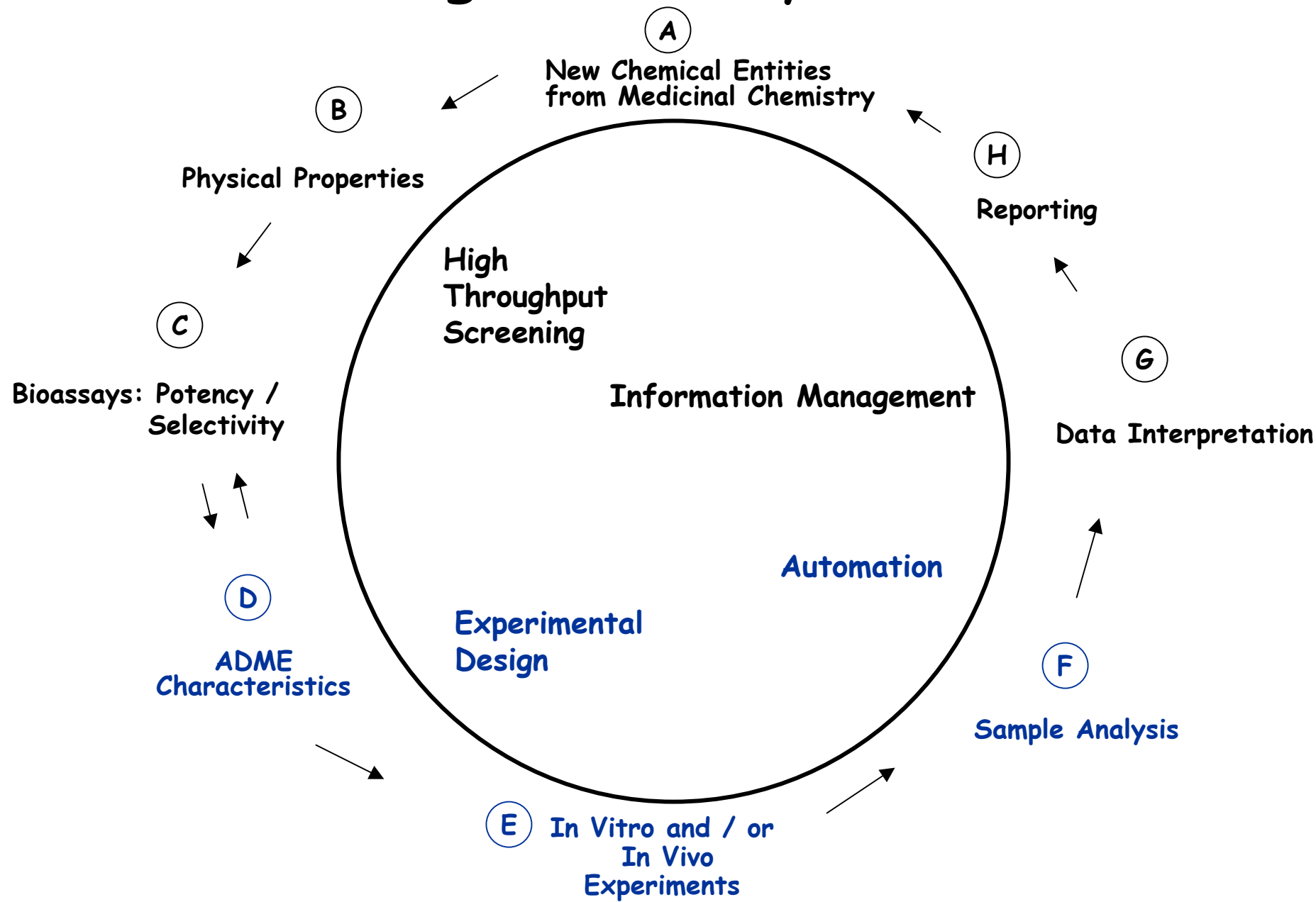
the quality of **DATA** required to generate meaningful results

the type of **RESULTS** that are requested in the screen

Drug Discovery Process



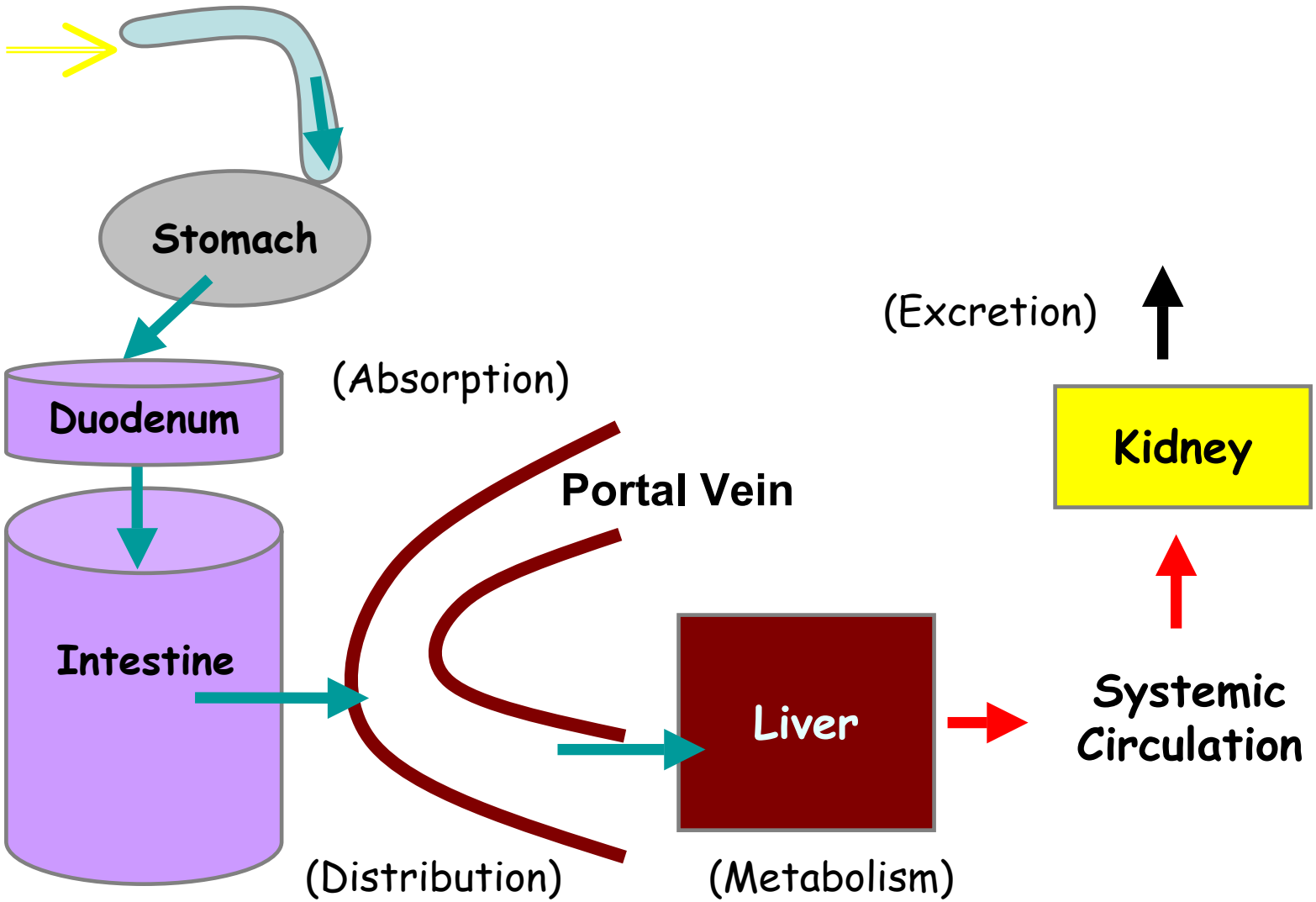
Drug Discovery Process



Relationship of Drug Metabolism with Medicinal Chemistry in Early Drug Discovery

- Add information to compliment HTS data
- Assist in the selection of compounds based upon their ADME characteristics for further evaluation
- Provide direction to medicinal chemists for the design of their next compound in a series
- Identify trends in the architecture of a class of compounds that correlates with a response in an ADME assay (e.g. Potency with P450 inhibition)

"Absorption, Distribution, Metabolism, Excretion"



Desirable ADME Characteristics

- **Absorption**
  - good solubility and permeability
- **Distribution**
  - good exposure at the target, minimal elsewhere
  - acceptable protein binding: estimate "free concentration"
- **Metabolism**
  - minimal first pass effect
  - metabolism by two or more CYP (not 2D6) to few metabolites
  - minimal potential to inhibit or to induce
- **Excretion**
  - balance between metabolism and excretion of parent drug

Experiments to Assess ADME Characteristics

- **Absorption**
  - Caco-2 cells, PAMPA, PgP-transport
  - *in vivo* PK profiling

- **Distribution**
  - *in vitro* protein binding, *in vivo* tissue distribution studies

- **Metabolism**
  - Metabolic stability in microsomes, S9 fractions, hepatocytes
  - P450 Inhibition: microsomes and/or rCYPs, co-administration
  - P450 Induction: Gene Chips, PXR, multiple dosing studies
  - Metabolite characterization

- **Excretion**
  - Quantitation of drugs and metabolites in biological fluids

Bioanalytical Research's Goal

To generate accurate and precise data in the quantitative analysis of drug discovery candidates in samples from *in vivo* and *in vitro* studies that support their biological characterization and optimization as potential development candidates.

BAR Responsibilities

- Rapidly develop and implement multiple component bioanalytical methods using LC-MS/MS-based detection.
  - Analyte and Internal Standard
  - Analyte(s) and Internal Standard(s)
    - Co-Administration Studies, Pro-Drugs
  - Parent Compound, Metabolite(s), IS
  - Parent Compound, Distinct Equilibrium Forms

BAR Responsibilities

- Analyze samples from *in vivo* studies:
  - Early Exposure (Biology), Coarse PK, Full PK, PK/PD, Tissue Distribution, "Bioequivalence", TK (CV Telemetry, Pre-ECN, ECN) …
  - Co-Administration: Screening (N-in-one), P450 Inhibition, Marker Compounds, Stable Label…
  - All routes of administration (IV, PO, IP, SQ, IN…)
  - All types of formulations by Pharmaceutics.

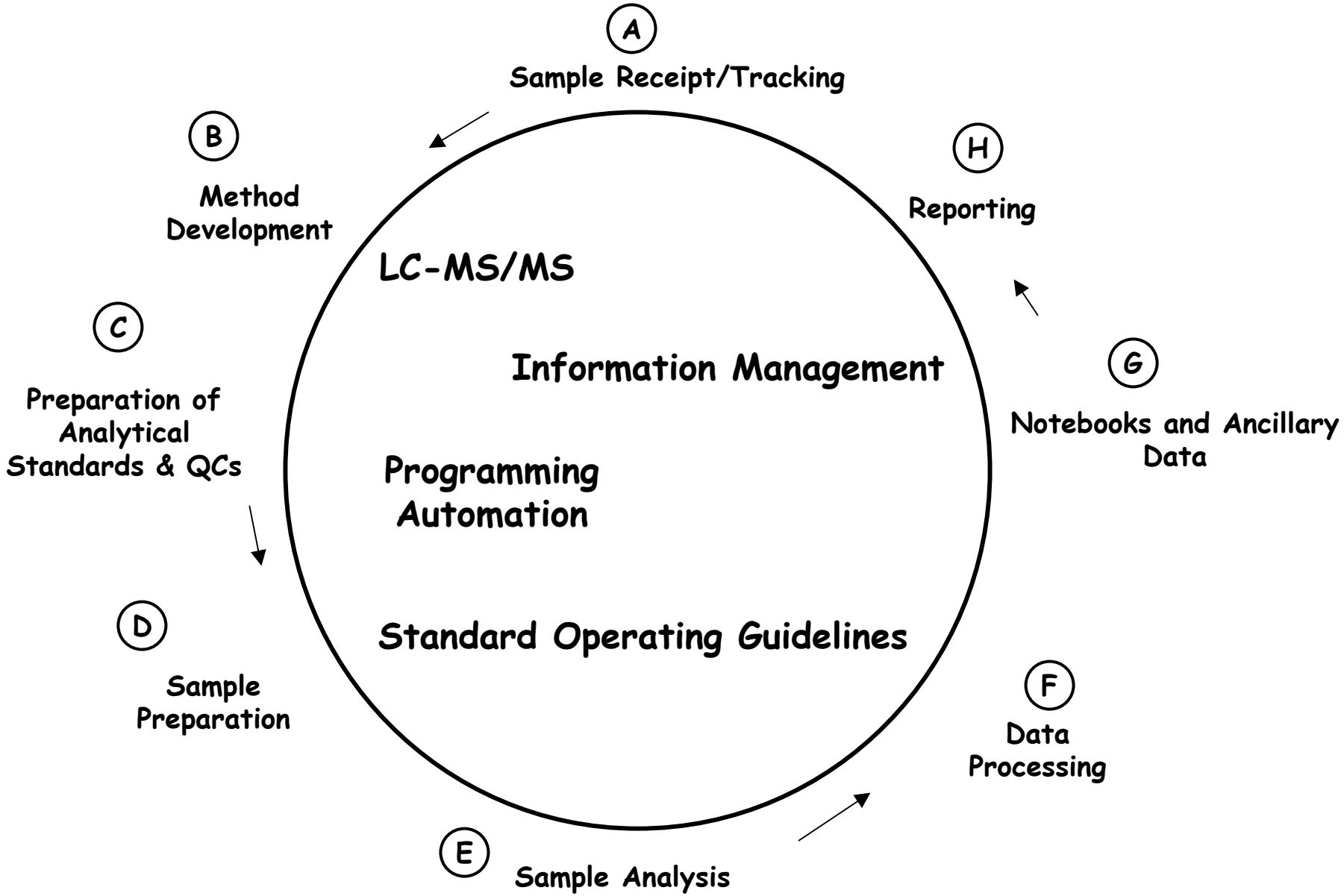
BAR Responsibilities

- Analyze samples from *in vitro* studies:
  - serum protein binding, tissue binding, serum/plasma/chemical stability…
  - intrinsic clearance, P450 inhibition, pgP, PAMPA, Caco-2…
  - biological assays, heart/liver perfusion…

BAR Responsibilities

- Analyze samples in all types of species and biological matrices:
  - mice, rats, marmoset, guinea pig, rabbit, dogs, monkeys, chimp, human…
  - blood, plasma, serum, urine, bile, CSF, SV, brain, lung, heart, liver, GI tract, kidney, muscle, tumor, adipose tissue…
  - microsomes, hepatocytes, S9 fractions, rCYP, incubation systems, mixed matrices, buffers, dosing solutions…

Bioanalytical Work Flow



Uses of Mass Spectrometry in Drug Discovery

- **Qualitative Analysis**

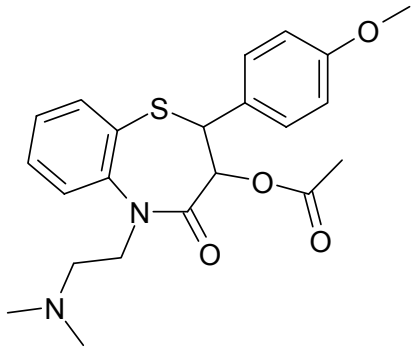
  Elucidation of the structural characteristics of various substances in different matrices:

  What is in the sample?

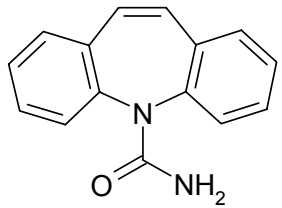
- **Quantitative Analysis**

  Determination of the concentration of various substances in different matrices:

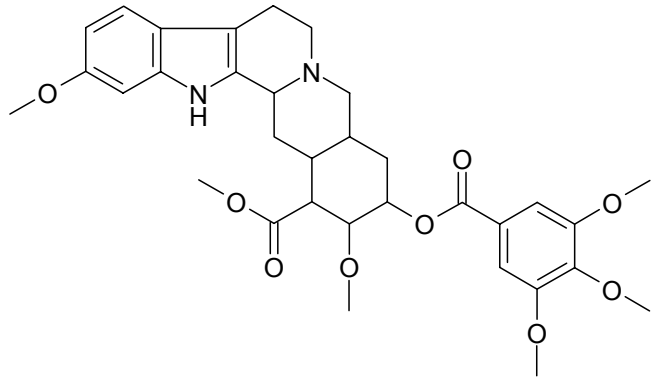
  How much is in the sample?



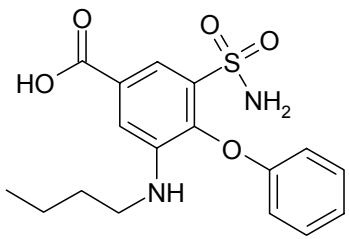
Diltiazem
$C_{22}H_{26}N_2O_4S$
MW = 414.2 Da.



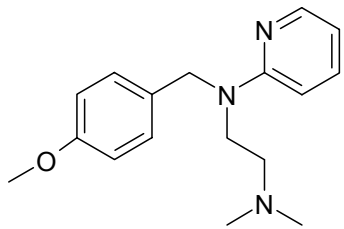
Carbamazepine
$C_{15}H_{12}N_2O$
MW = 236.1 Da.



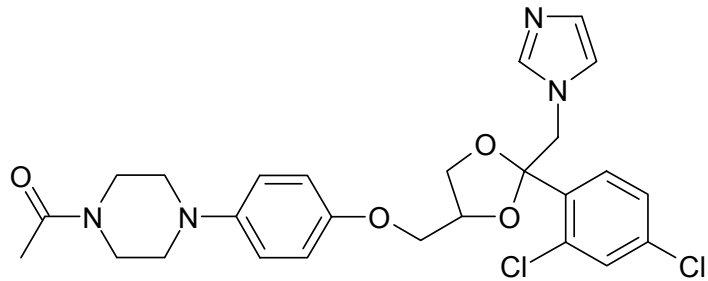
Reserpine
$C_{33}H_{40}N_2O_9$
MW = 608.3 Da.



Bumetanide
$C_{17}H_{20}N_2O_5S$
MW = 364.1 Da.



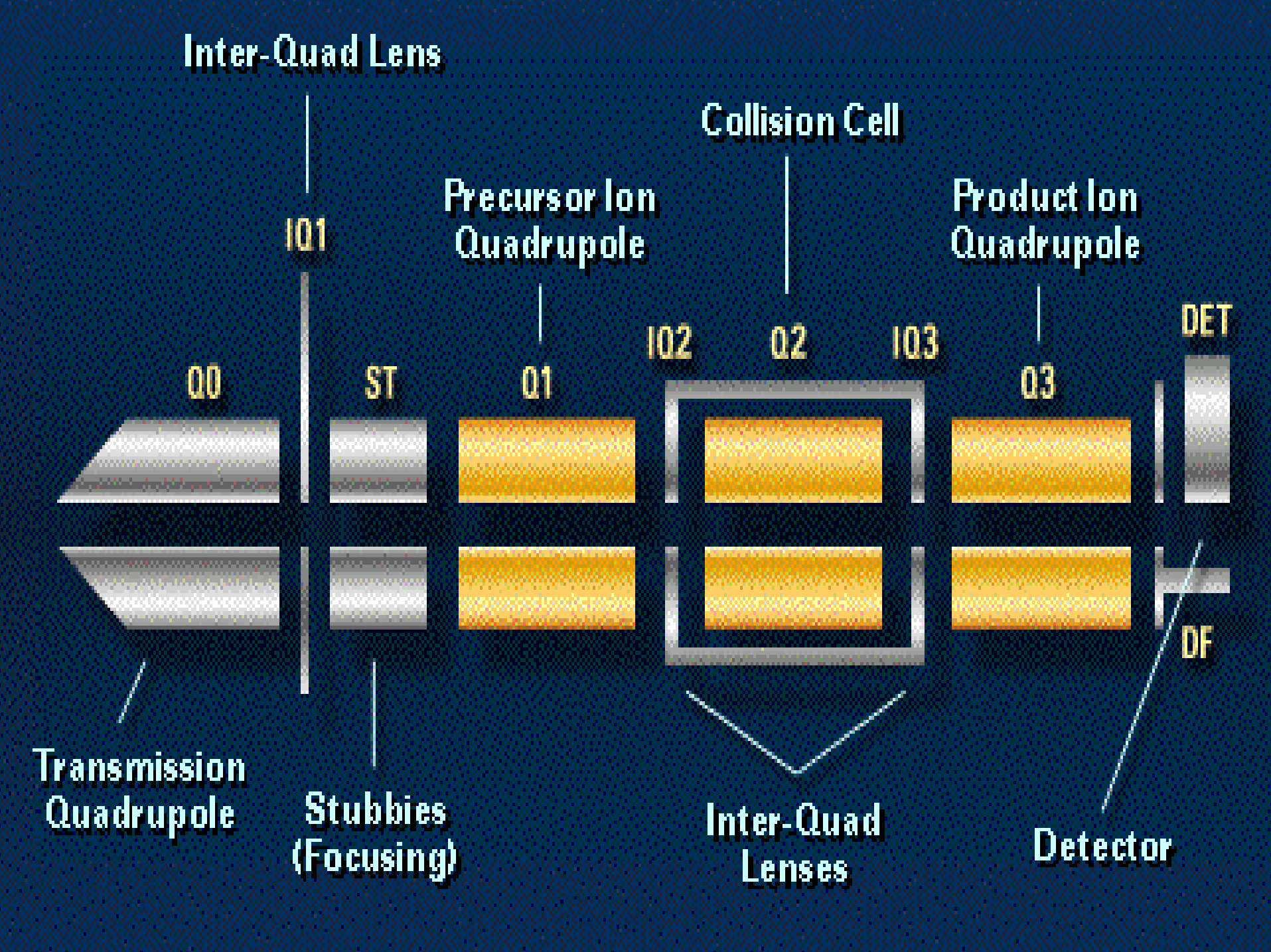
Pyrilamine
$C_{17}H_{23}N_3O$
MW = 285.2 Da.



Ketoconazole (ISTD)
$C_{26}H_{28}N_4O_4Cl_2$
MW = 530.1 Da.

Types of MS Detection Methods

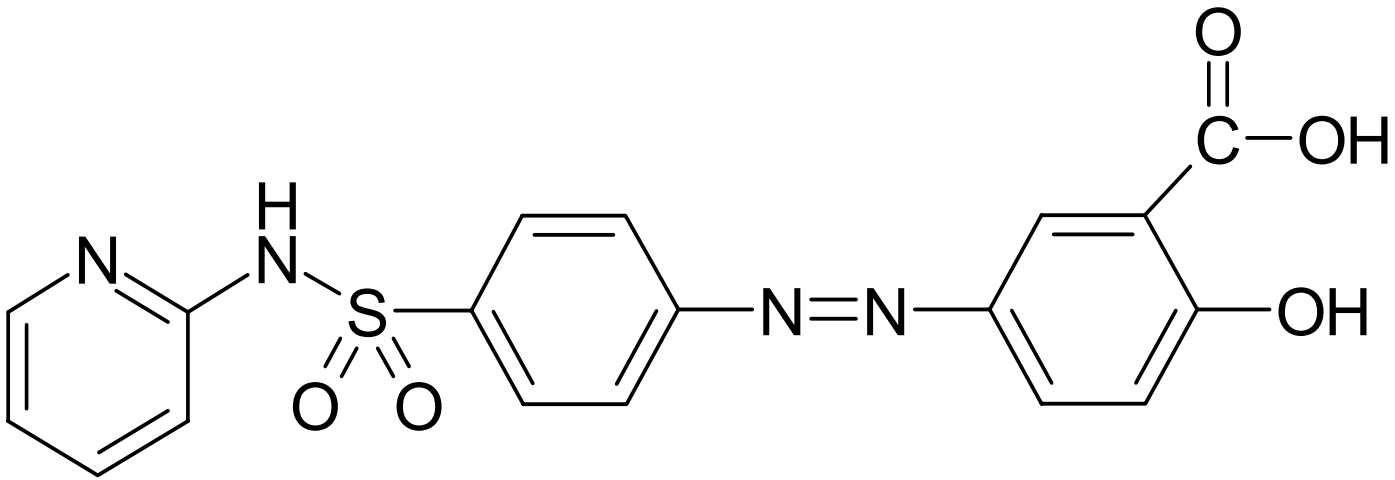
- Full Scan Spectra
- Selected Ion Monitoring
- Product Ion Spectra
- Neutral Loss Scans
- Selected Reaction Monitoring
- Accurate Mass Measurements
- Others


Inter-Quad Lens
IQ1
Collision Cell
Precursor Ion Quadrupole
Product Ion Quadrupole
DET
IQ2
Q2
IQ3
Q0
ST
Q1
Q3
DF
Transmission Quadrupole
Stubbies (Focusing)
Inter-Quad Lenses
Detector

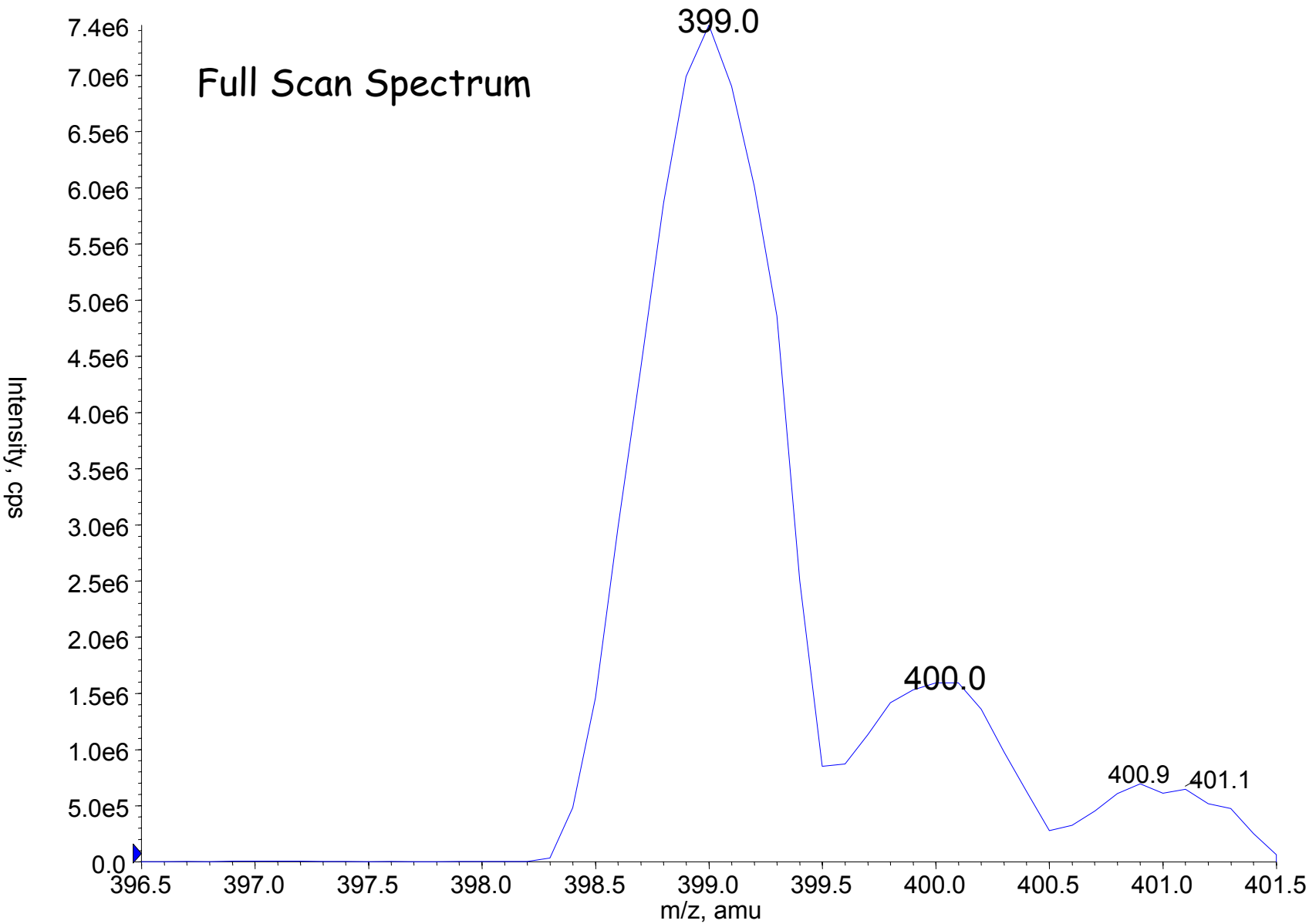
Sulfasalazine

$C_{18}H_{14}N_4O_5S$

MW 398.40

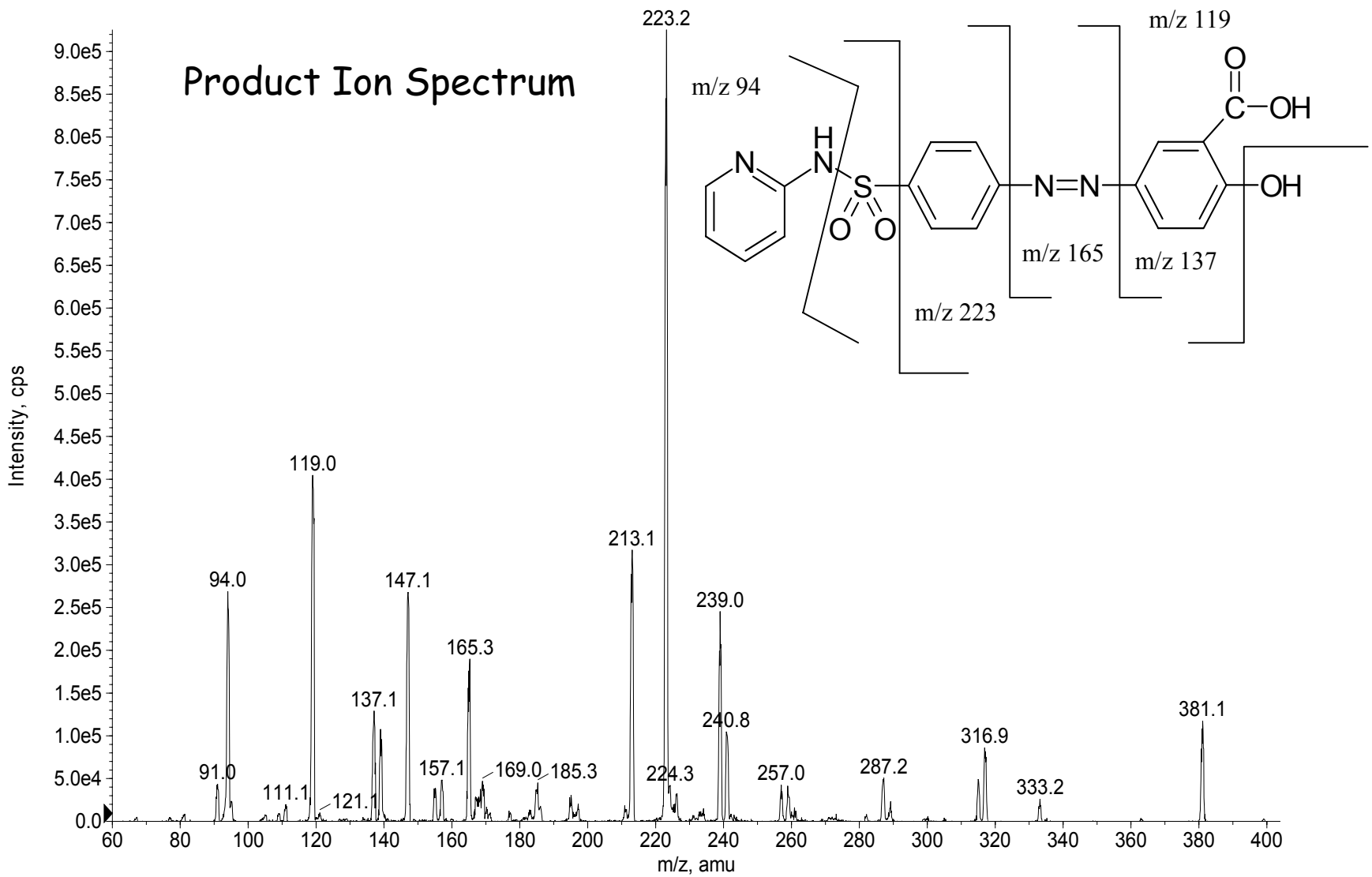


+Q1: 5 MCA scans from sulfasalazine_FinalQ1_Pos.wiff Max. 7.4e6 cps.



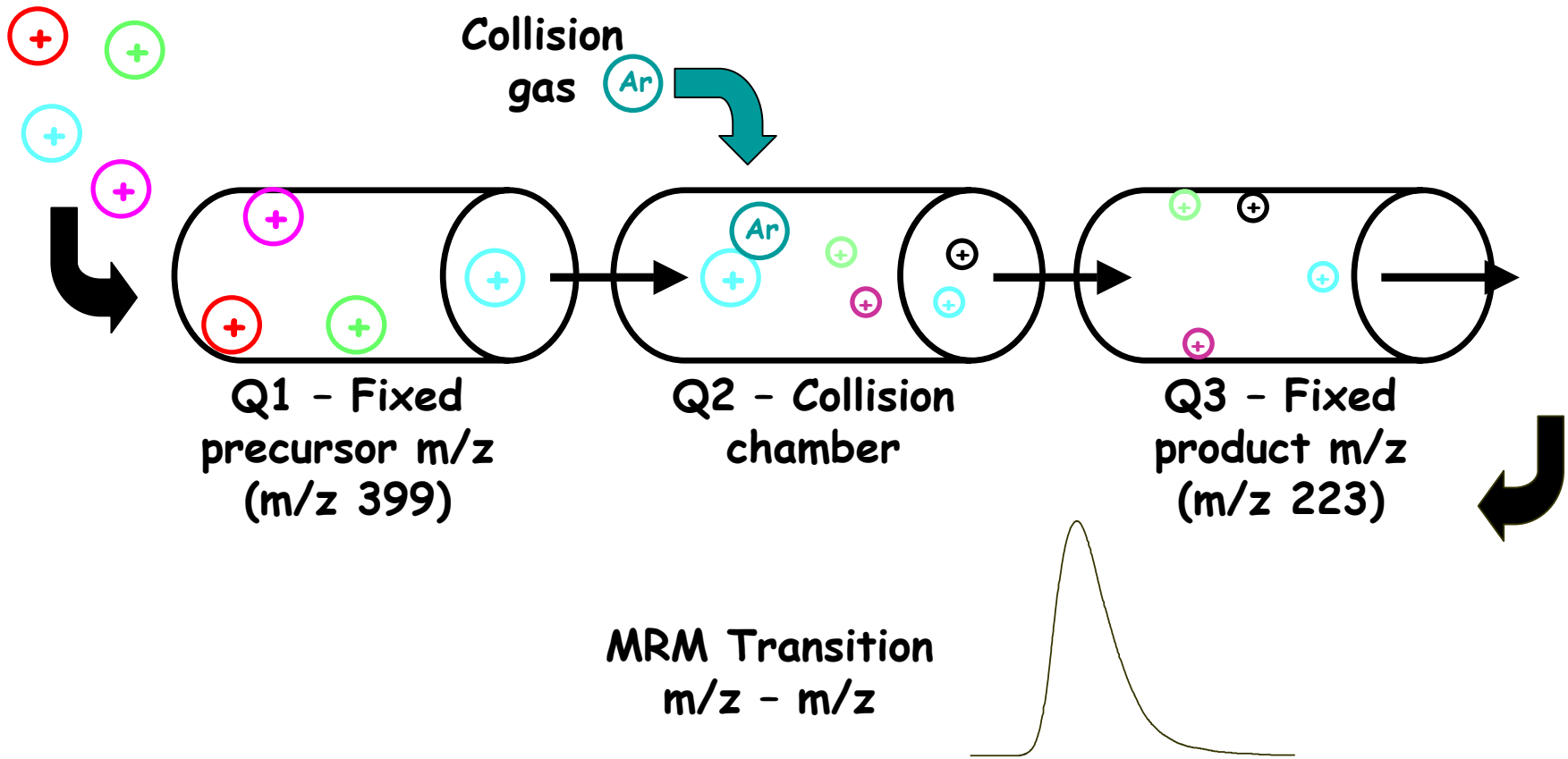
+Product (399.0): Experiment 3, 0.345 to 1.047 min from 049-IS Sulfasalazine MSMS.wiff

Max. 9.3e5 cps.



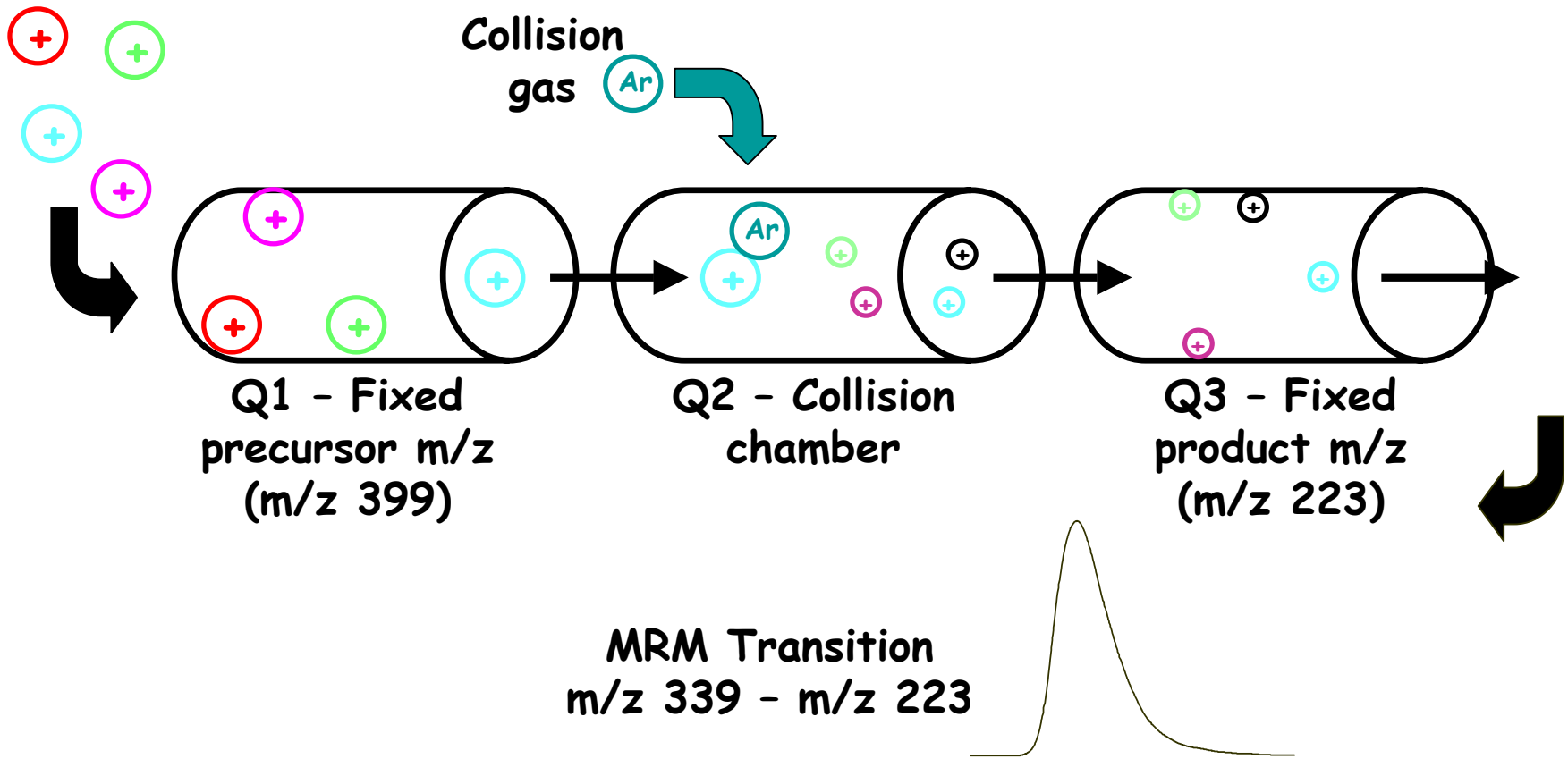
Multiple Reaction Monitoring

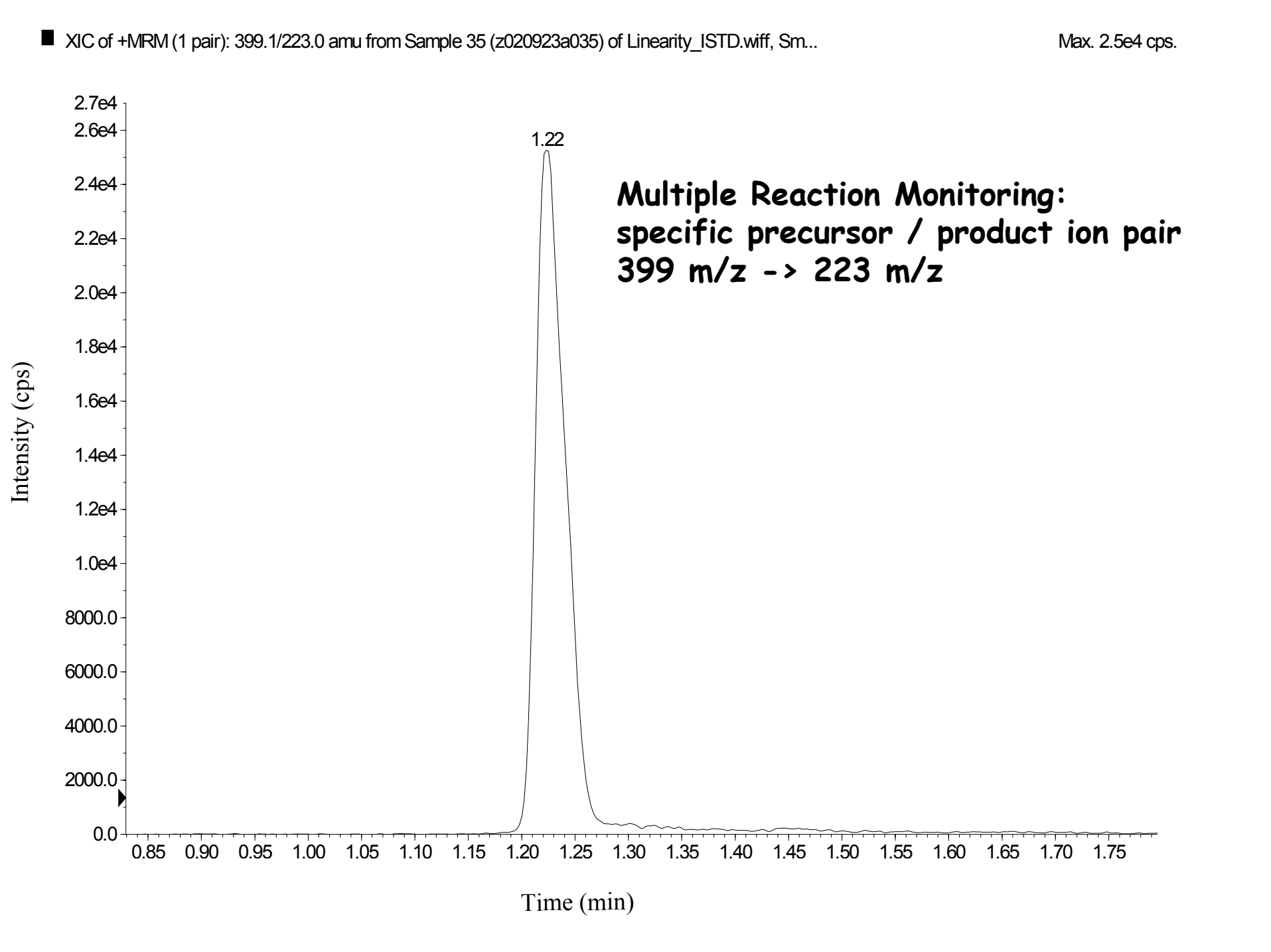
+ ES Ionization



Multiple Reaction Monitoring

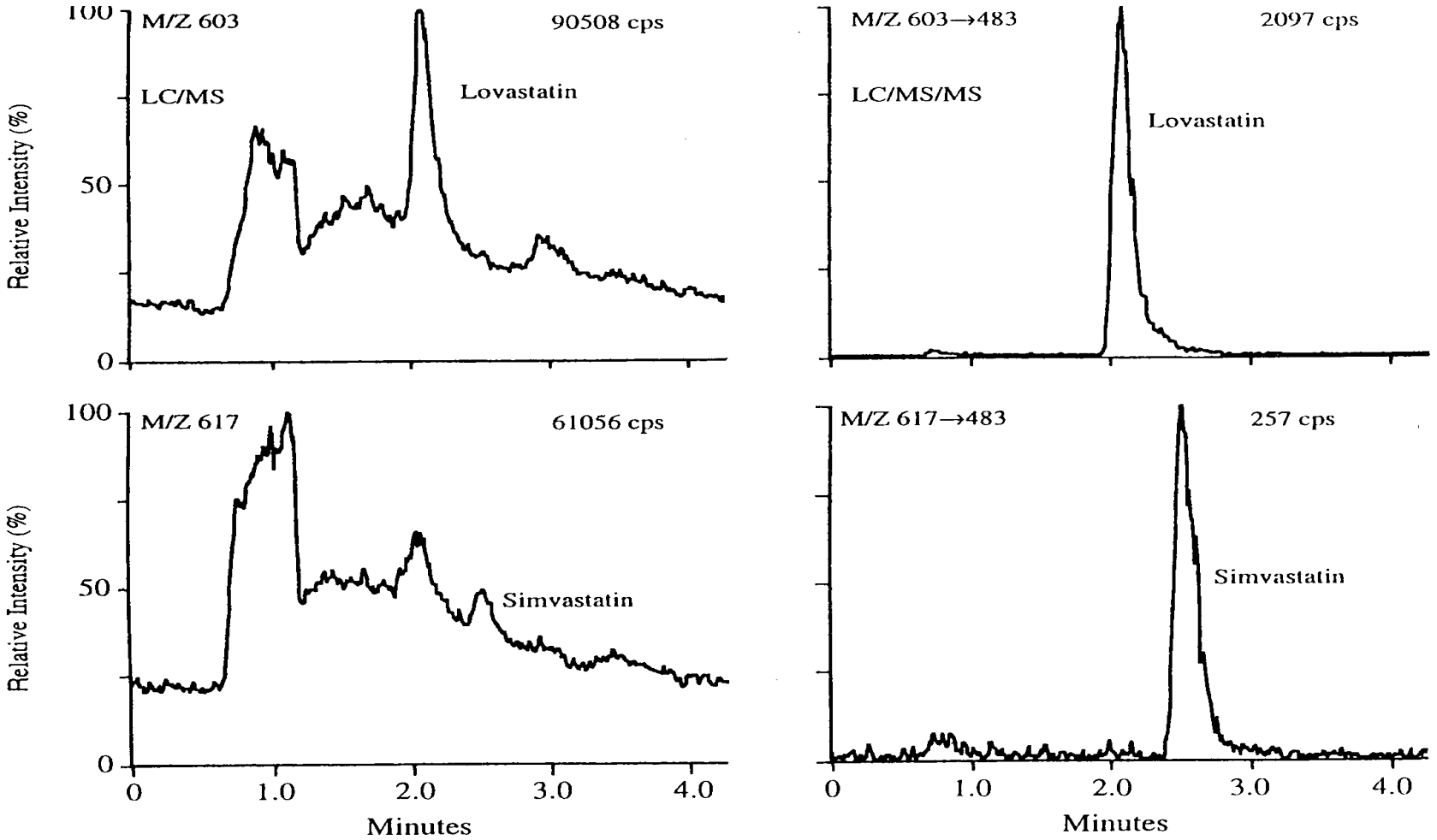
+ ES Ionization




XIC of +MRM (1 pair): 399.1/223.0 amu from Sample 35 (z020923a035) of Linearity_ISTD.wiff, Sm...
Max. 2.5e4 cps.
1.22
Multiple Reaction Monitoring:
specific precursor / product ion pair
399 m/z -> 223 m/z
Intensity (cps)
Time (min)
2.7e4
2.6e4
2.4e4
2.2e4
2.0e4
1.8e4
1.6e4
1.4e4
1.2e4
1.0e4
8000.0
6000.0
4000.0
2000.0
0.0
0.85
0.90
0.95
1.00
1.05
1.10
1.15
1.20
1.25
1.30
1.35
1.40
1.45
1.50
1.55
1.60
1.65
1.70
1.75

Plasma Extract SIM: m/z 603

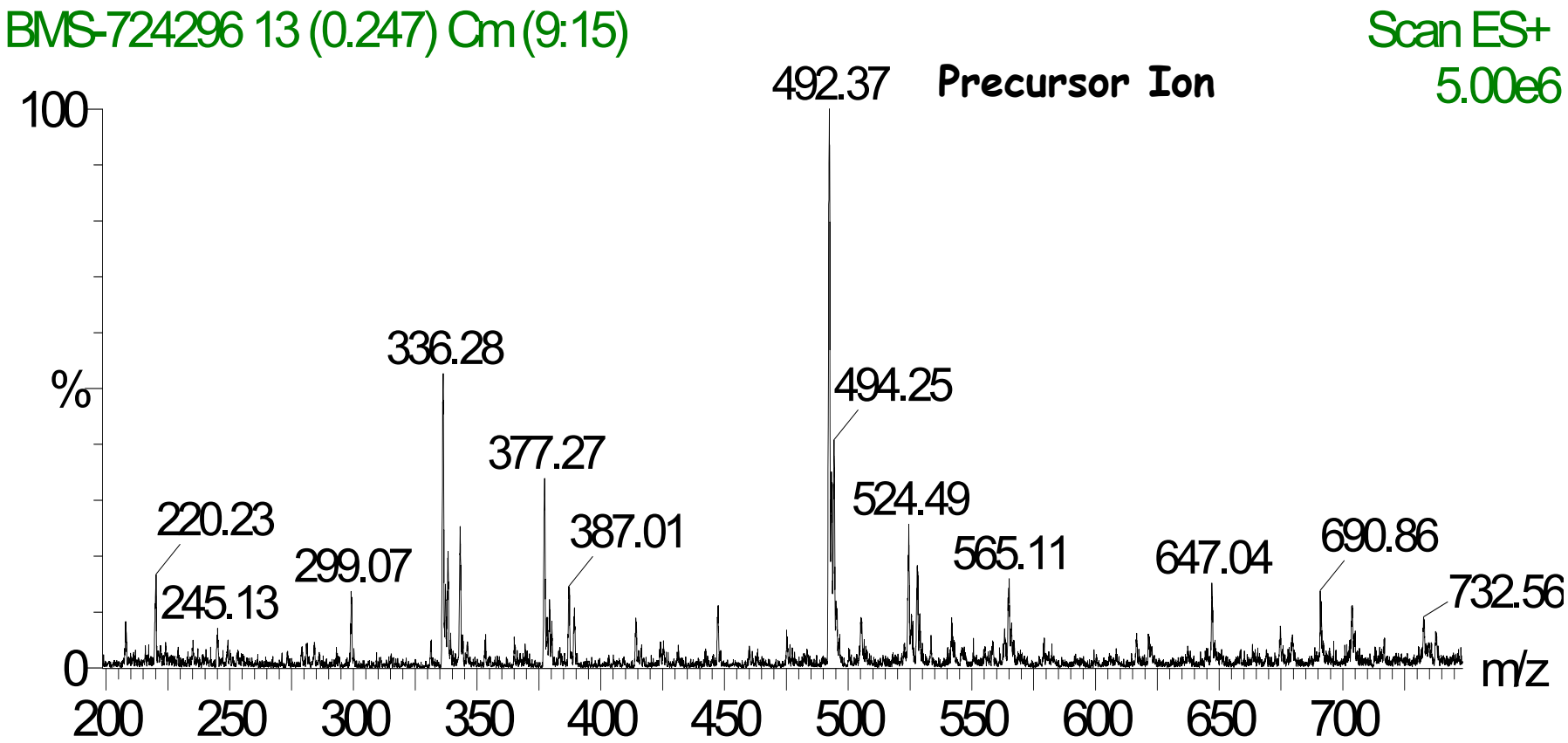
Plasma Extract MRM: m/z 603-> m/z 483



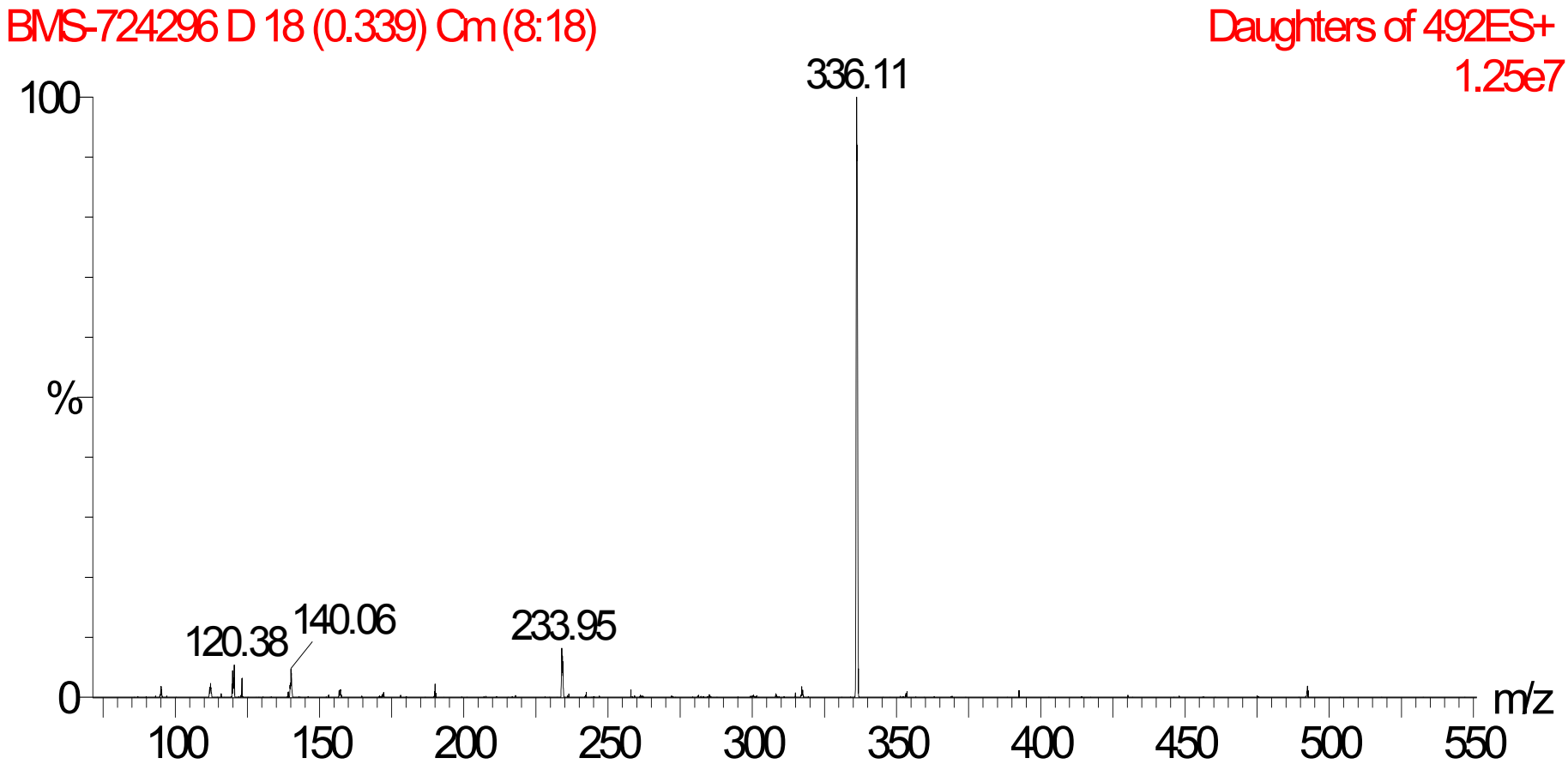
Plasma Extract SIM: m/z 617

Plasma Extract MRM: m/z 617-> m/z 483

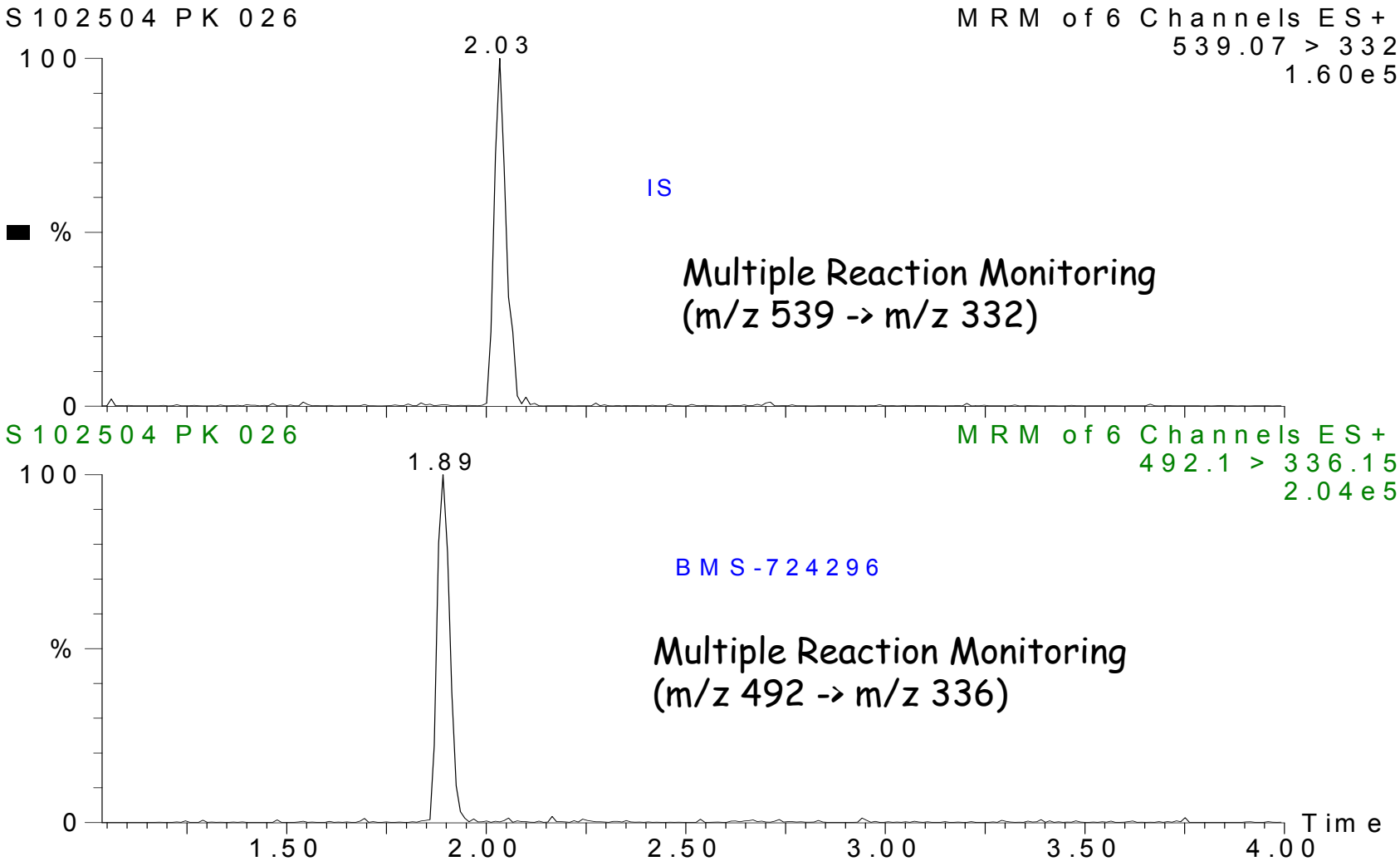
Full Scan Spectrum



Product Ion Spectrum

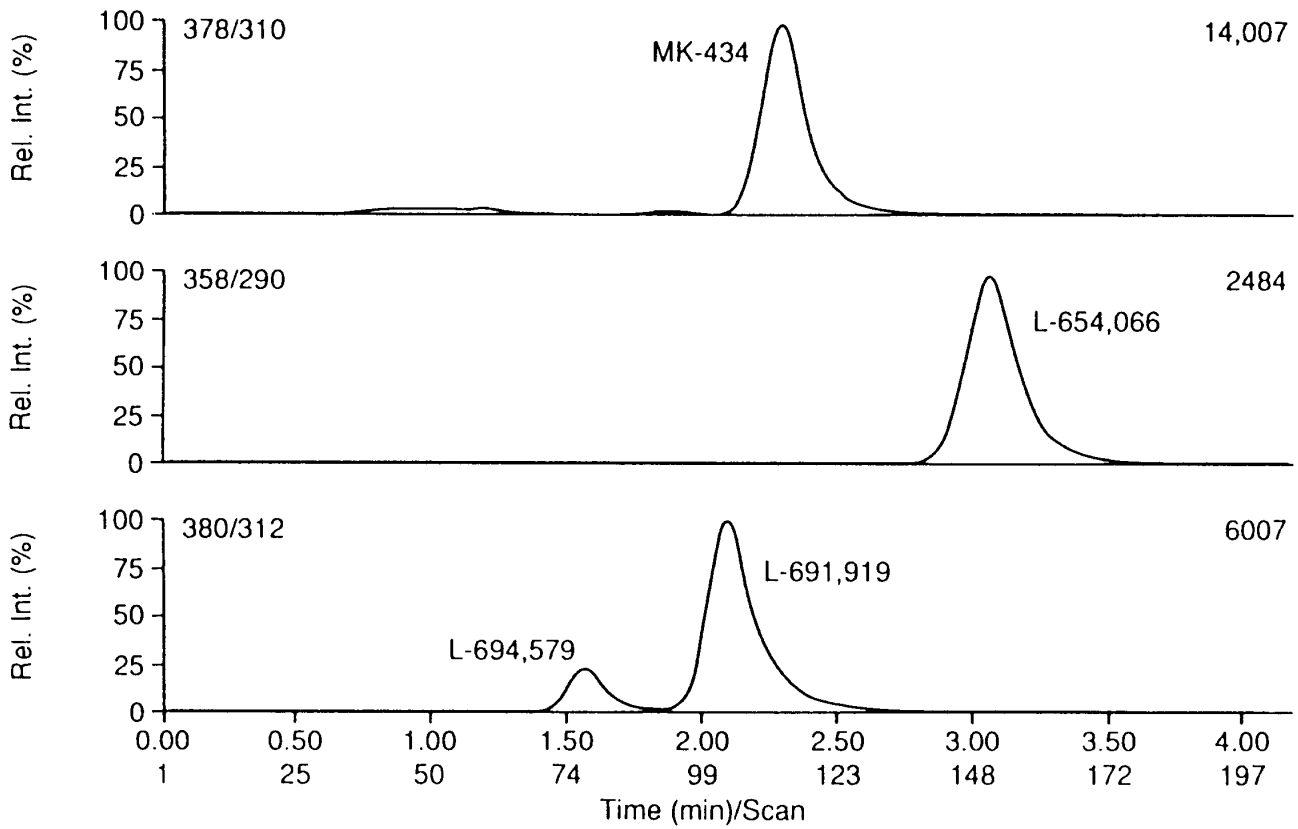


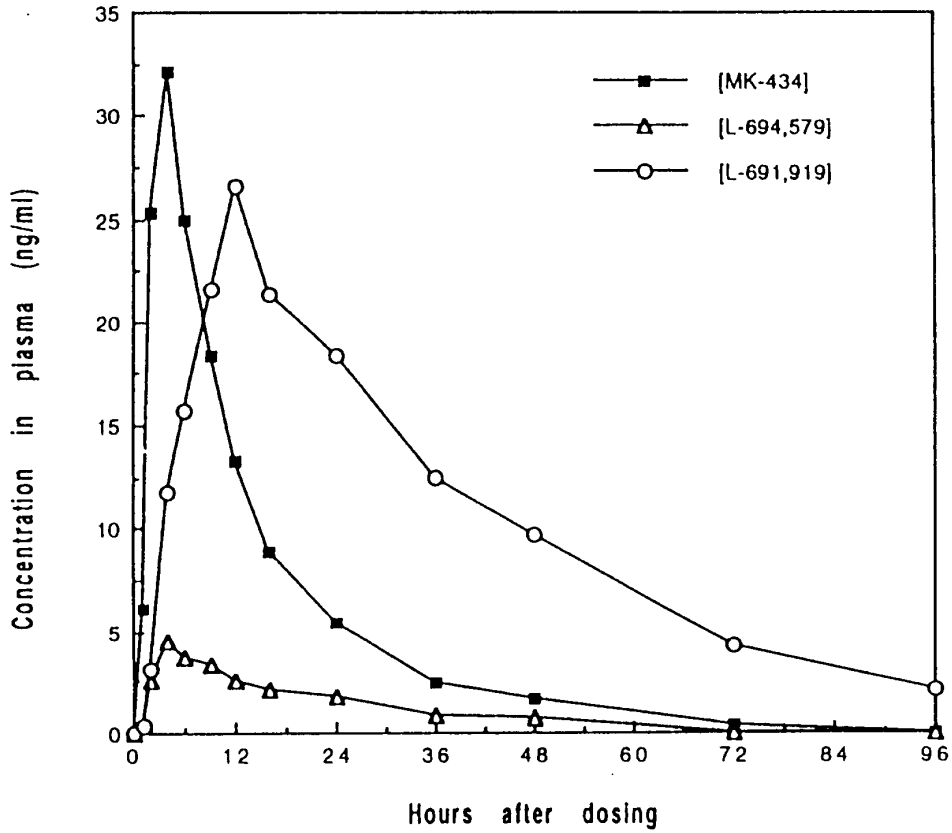
Multiple Reaction Monitoring (m/z 492 -> m/z 336)



Examples of the Use of Multiple Component LC-MS/MS-based Bioanalytical Methods in Drug Discovery

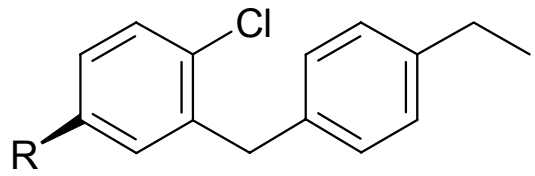
- Determination of Drugs and Metabolites
- Co-Administration Studies


Rel. Int. (%)
100
75
50
25
0
378/310
MK-434
14,007
358/290
L-654,066
2484
380/312
L-694,579
L-691,919
6007
0.00
0.50
1.00
1.50
2.00
2.50
3.00
3.50
4.00
1
25
50
74
99
123
148
172
197
Time (min)/Scan

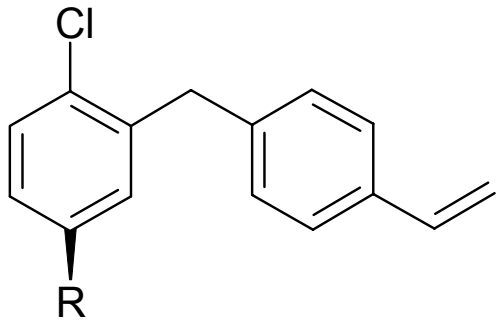

35
30
25
20
15
10
5
0
0
12
24
36
48
60
72
84
96
Concentration in plasma (ng/ml)
Hours after dosing
[MK-434]
[L-694,579]
[L-691,919]

Bioanalytical Method for Determination of BMS-xxxxx and Metabolites in Biological Fluids to Support the Diabetes Program

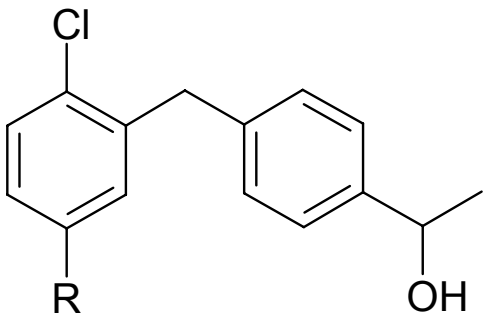
Structures



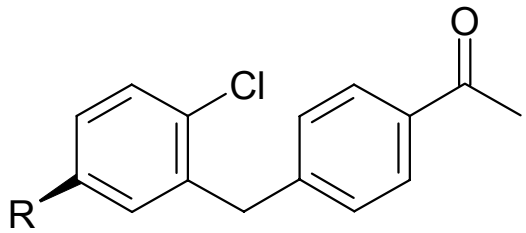
Parent Compound
MW=392.88



Styrene
Metabolite
MW=390.87



Alcohol
Metabolite
MW=408.88



Ketone
Metabolite
MW=406.88

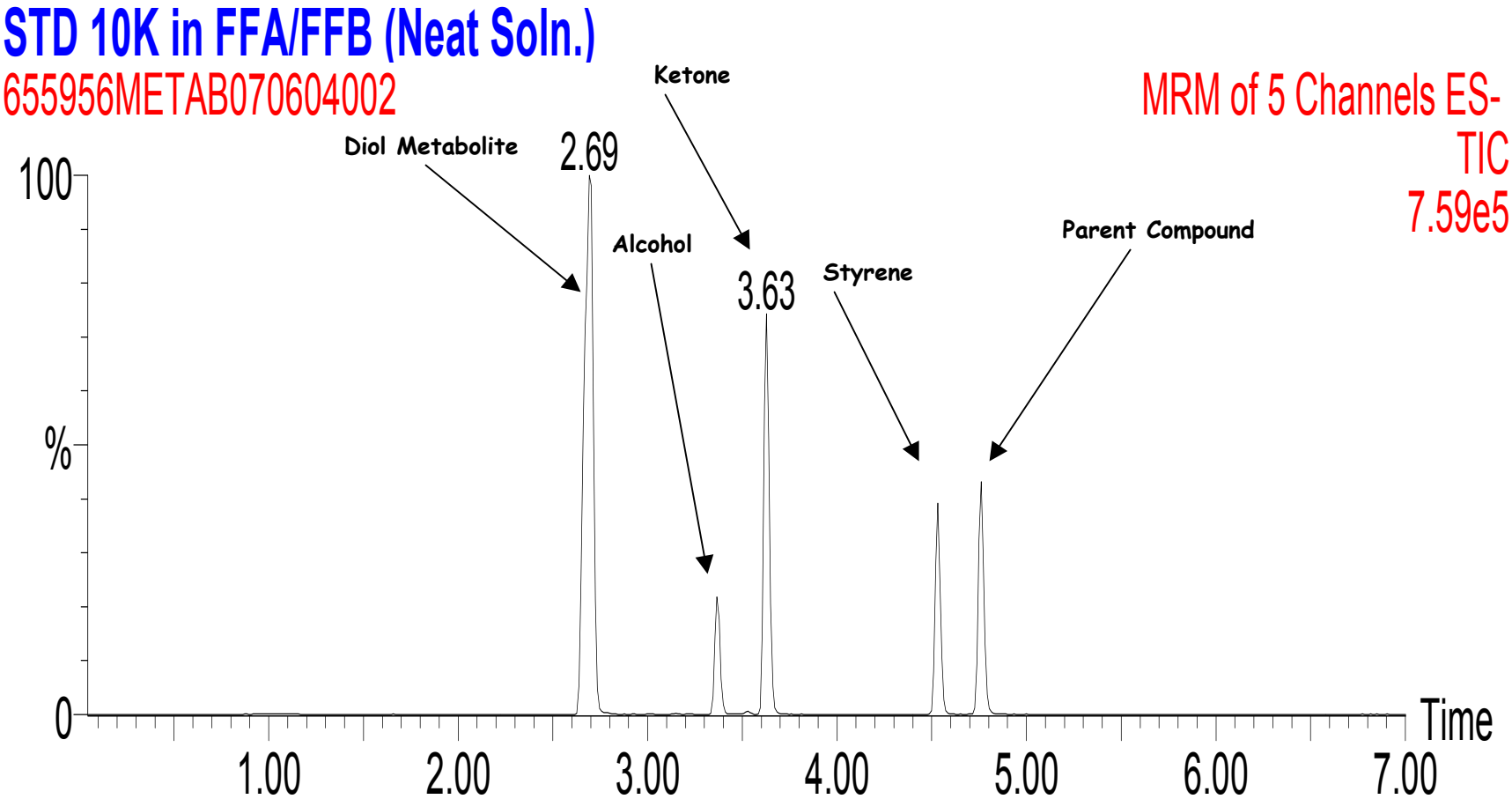
Multiple Component Bioanalytical Method

➢Mass Spectrometry:

Negative Ion Electrospray
Multiple Reaction Monitoring

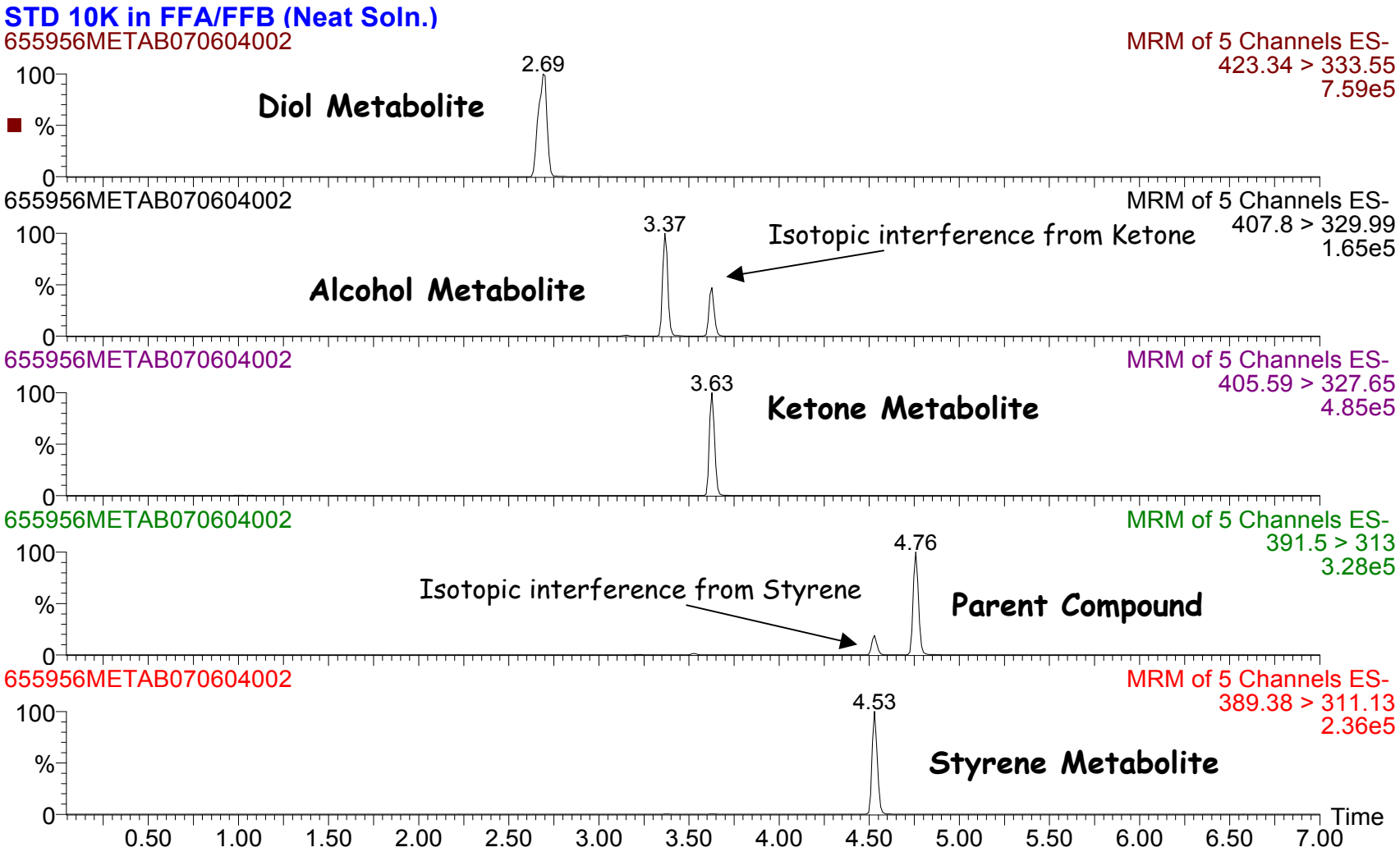
	Function	Reaction	Dwell(secs)*	Cone Volt.	Col.Energy
Parent	2	391.5 > 313.1	0.2	45	15
Styrene Metabolite	2	389.4 > 311.1	0.2	45	15
Ketone Metabolite	1	405.6 > 327.7	0.1	40	15
Alcohol Metabolite	1	407.8 > 329.9	0.1	40	15
Diol Metabolite	1	423.3 > 333.5	0.1	45	20
Internal Standard	1	379.5 > 289.21	0.1	45	30

Chromatography Requirements



Due to isotopic interferences there was a need for chromatographic separation of the parent compound and the metabolites.

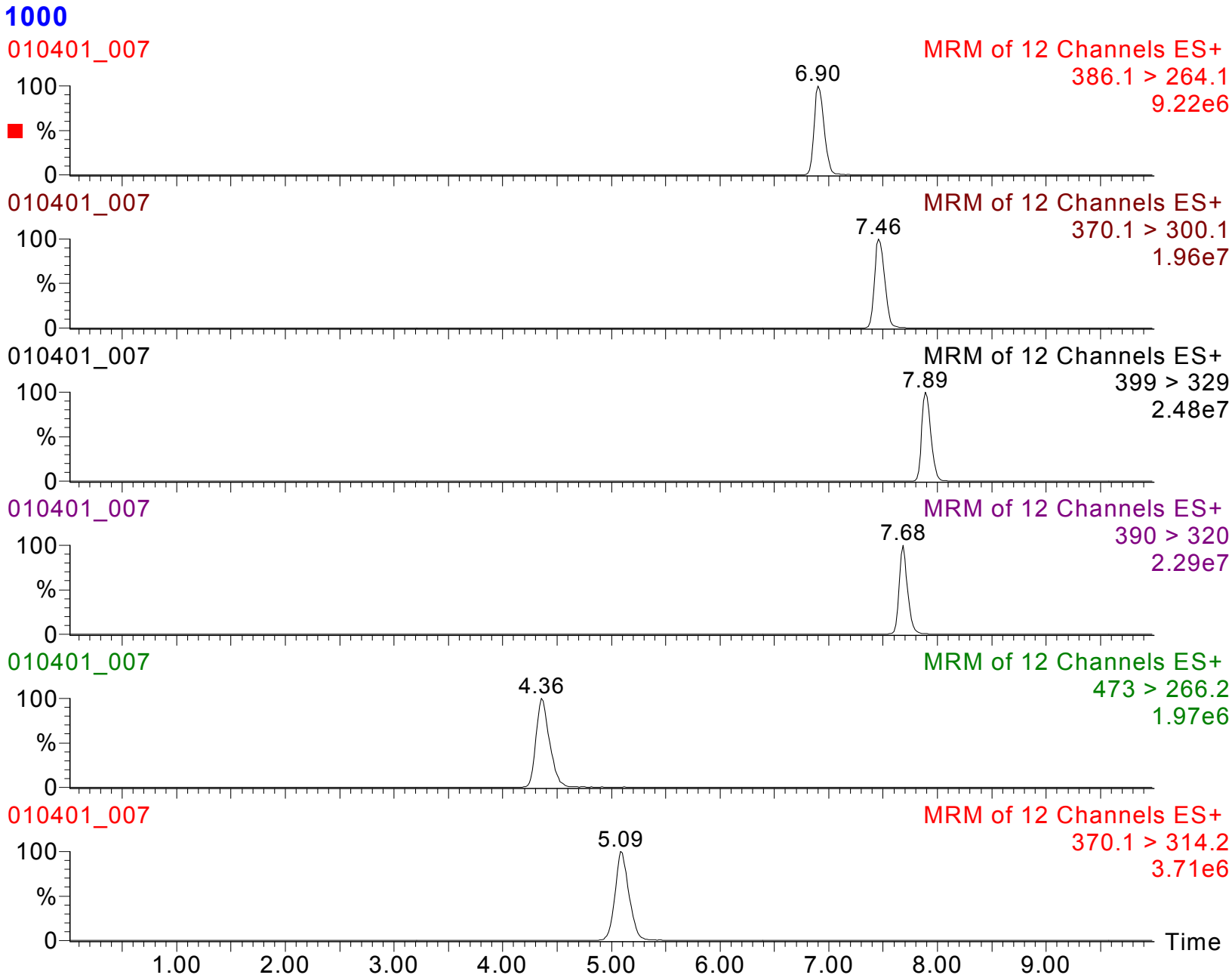
Chromatography Requirements

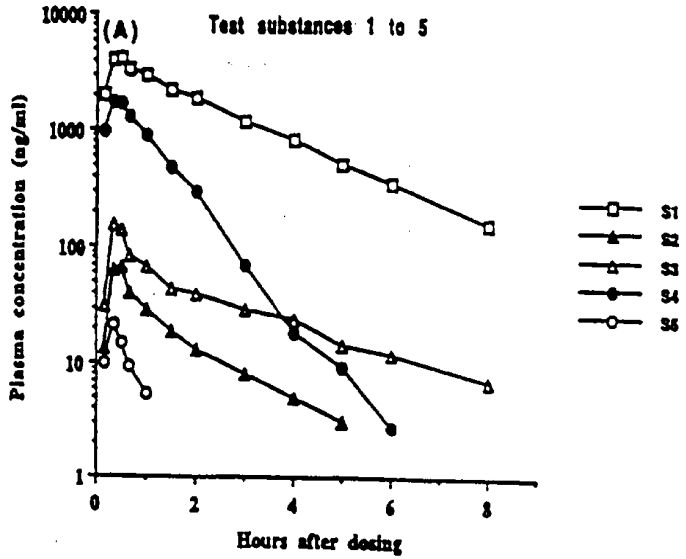


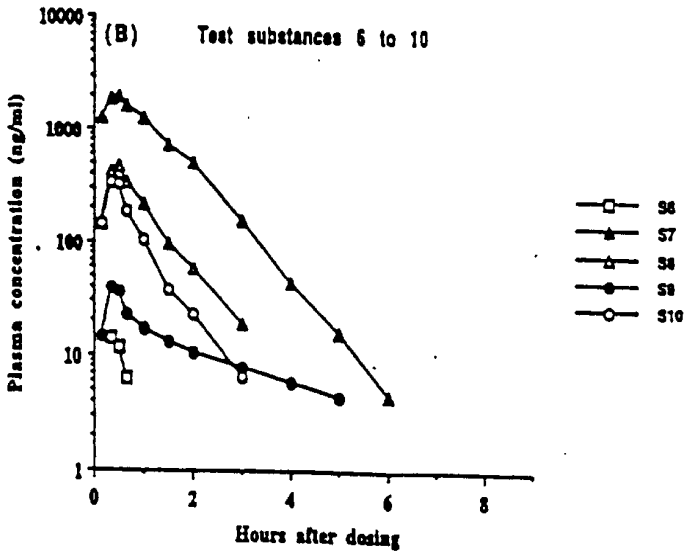
What are "Co-Administration" Studies?

(Cassette Dosing / N-in-One Studies)

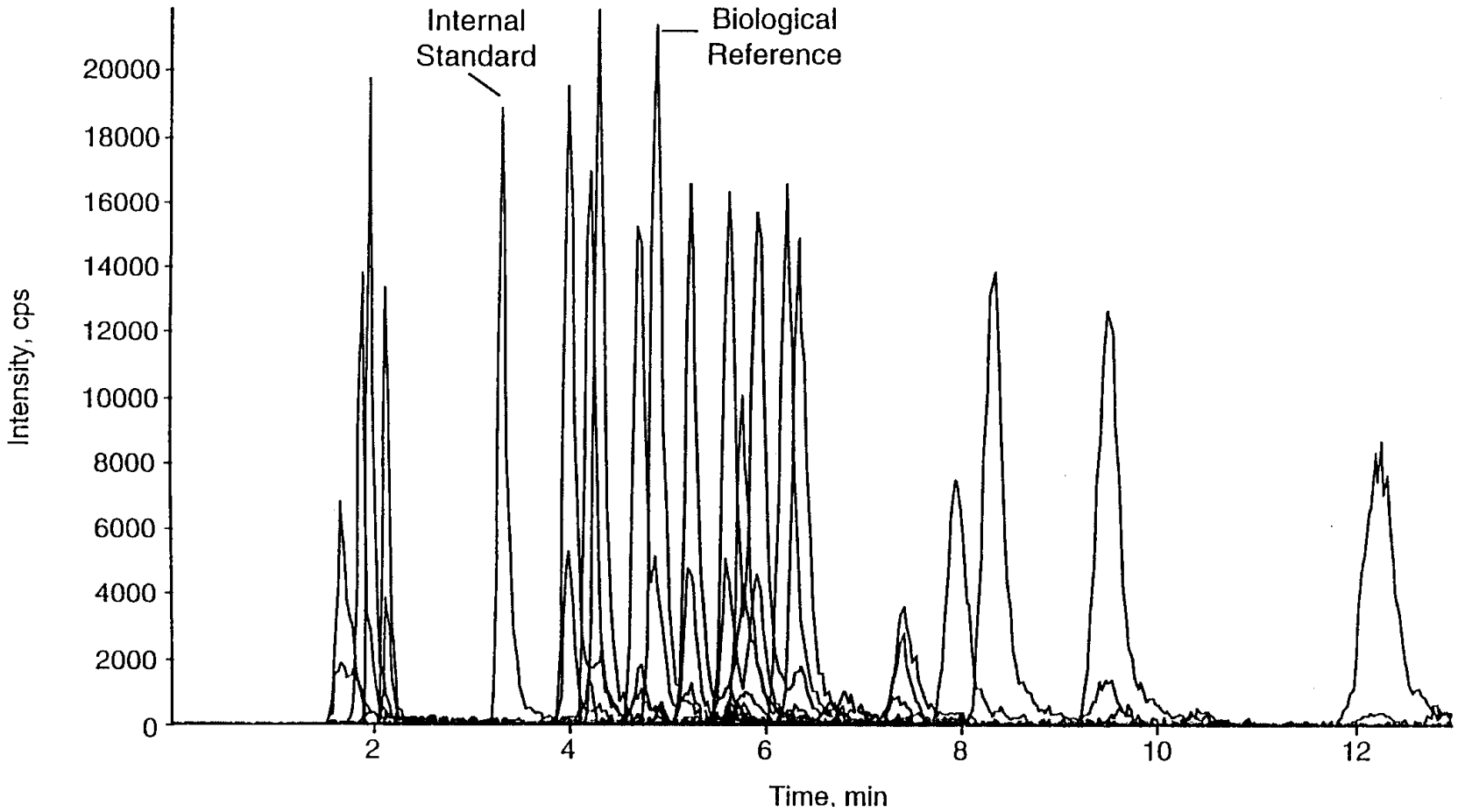
Simultaneous administration of multiple compounds (including a standard compound) to individual animals with the subsequent determination of the concentrations of each compound contained within single test samples.


1000
010401_007
MRM of 12 Channels ES+
386.1 > 264.1
9.22e6
6.90
100
%
0
010401_007
MRM of 12 Channels ES+
370.1 > 300.1
1.96e7
7.46
100
%
0
010401_007
MRM of 12 Channels ES+
399 > 329
2.48e7
7.89
100
%
0
010401_007
MRM of 12 Channels ES+
390 > 320
2.29e7
7.68
100
%
0
010401_007
MRM of 12 Channels ES+
473 > 266.2
1.97e6
4.36
100
%
0
010401_007
MRM of 12 Channels ES+
370.1 > 314.2
3.71e6
5.09
100
%
0
Time
1.00
2.00
3.00
4.00
5.00
6.00
7.00
8.00
9.00


(A)
Test substances 1 to 5
Plasma concentration (ng/ml)
10000
1000
100
10
1
0
2
4
6
8
Hours after dosing
S1
S2
S3
S4
S5

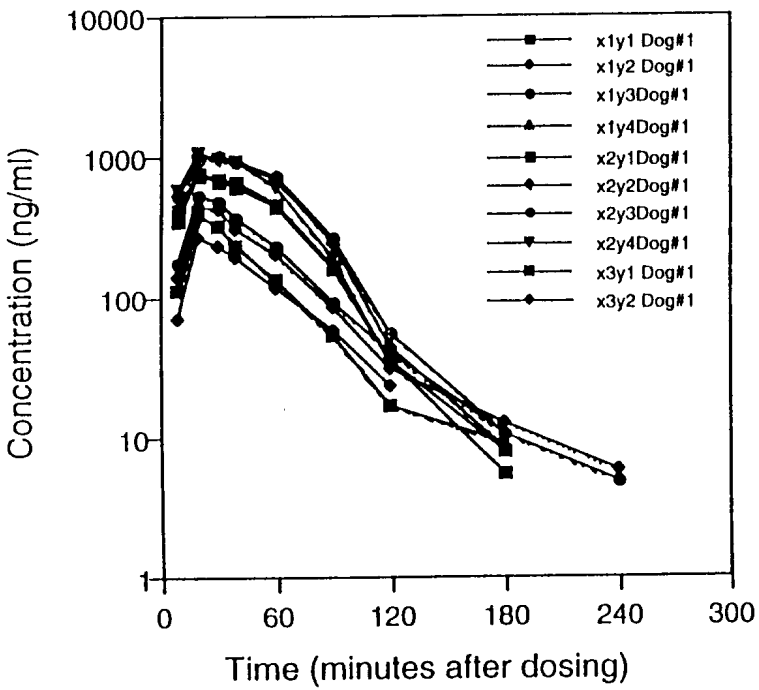

(B)
Test substances 6 to 10
Plasma concentration (ng/ml)
10000
1000
100
10
1
0
2
4
6
8
Hours after dosing
S6
S7
S8
S9
S10

SRM Chromatograms of Plasma Extract from a Dog Dosed with 20 Substances Simultaneously

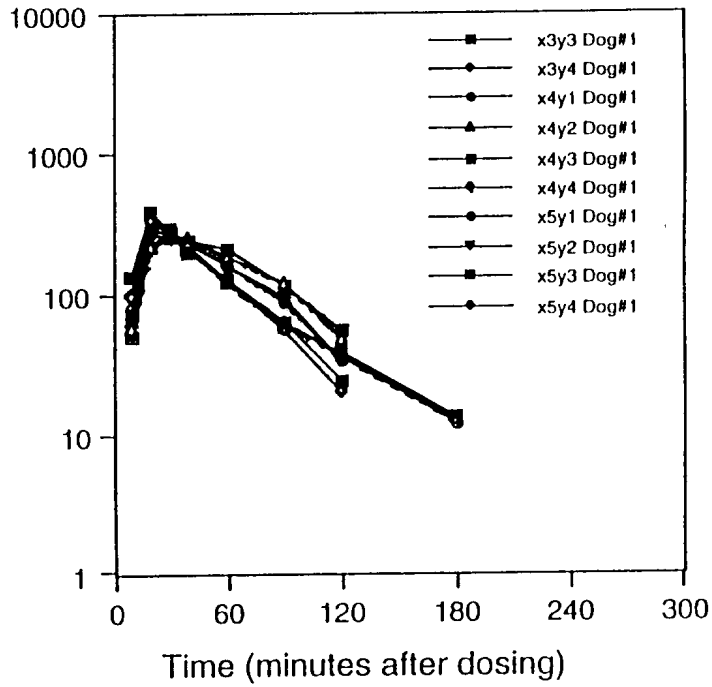


PLASMA CONCENTRATION PROFILES OF 20 COMPOUNDS GIVEN SIMULTANEOUSLY

Substances 1-10



Substances 11-20



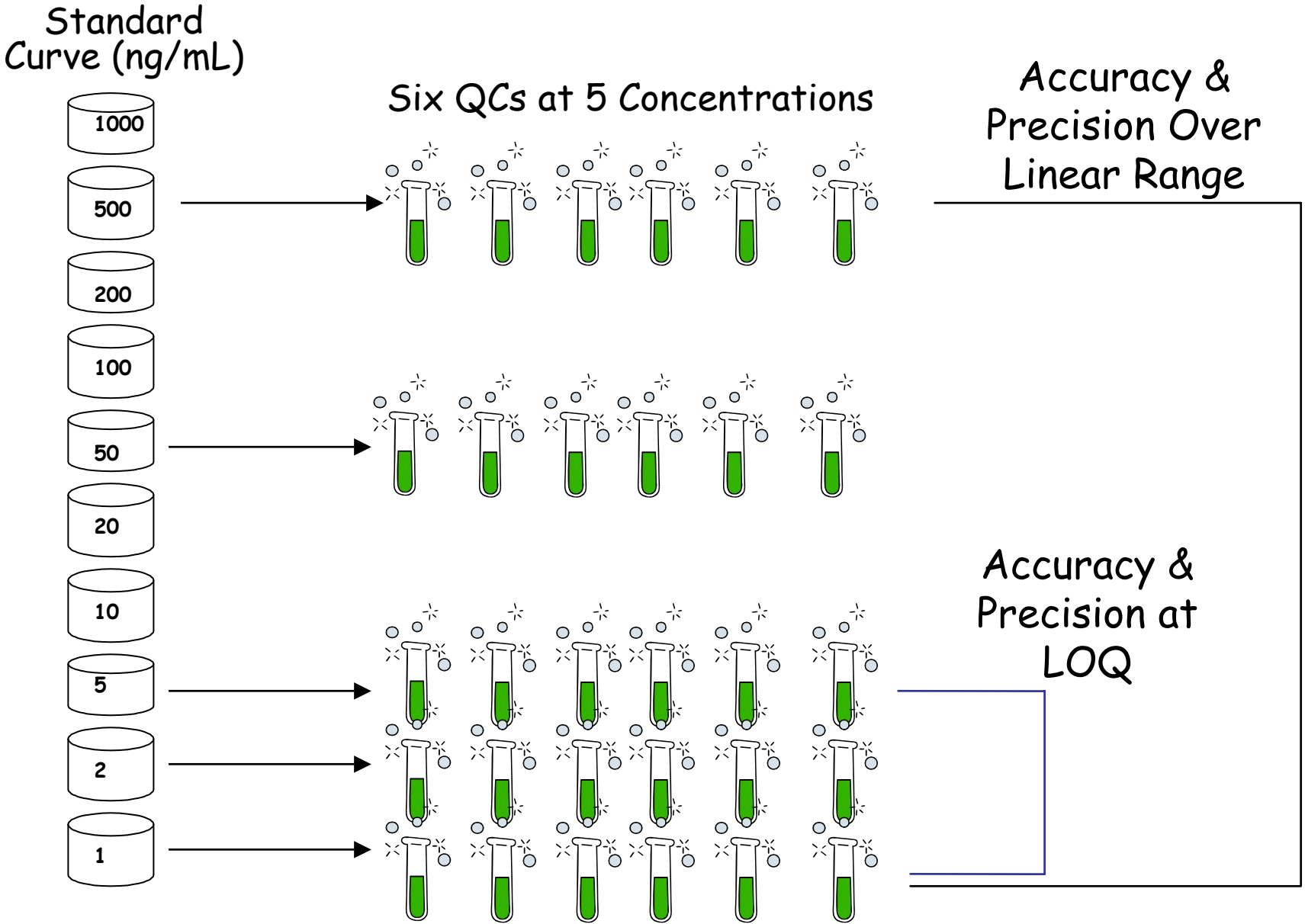
Method Development

- LC-MS/MS-based Methods
  - MS parameters are established for every analyte
  - HPLC conditions required for adequate response, specificity and sensitivity
  - Preparation of biological samples is critical to the integrity and quality of the method
    - Analyte response will differ in different biological extracts
    - Differentiates Bioanalytical from Analytical

Preparation of Standard and Quality Control (QC) Samples

- Known quantities of the compounds to be evaluated are added to blank biological matrix at established concentration ranges
- Internal Standard is added to Standard, QC, and Test samples
- Standard, QC, and Test samples are then processed in an identical manner
- All of the samples are then analyzed and the standard and QC samples are used to assess assay performance in terms of accuracy and precision

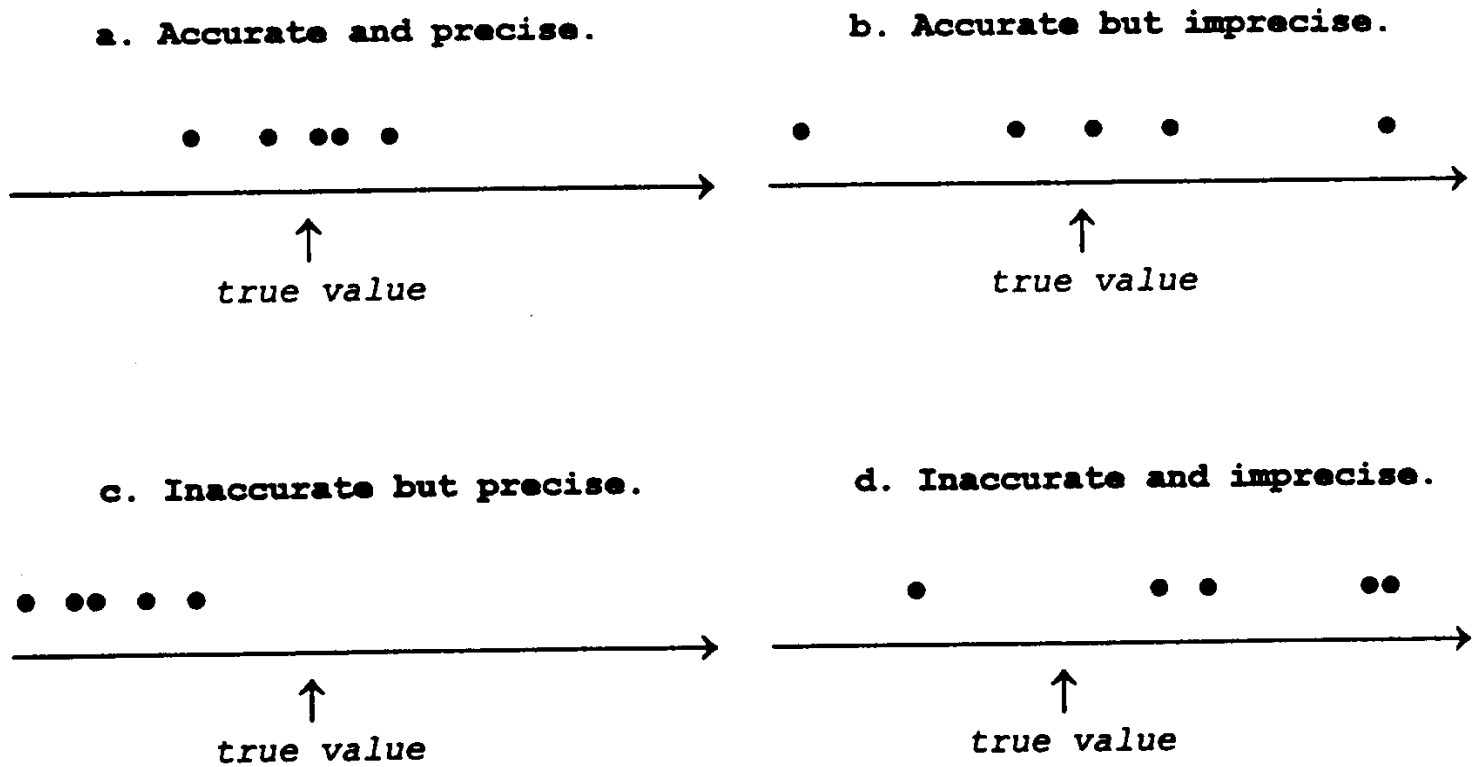
How do we assess variability of our analytical methods in drug discovery?



Assay Integrity

- Determined by Standard and QC results in conjunction with analytical requirements of the study
- Alternative method development is carried out, as needed
  - Modifications to MS parameters, chromatography, sample preparation procedures, Internal Standard selection, assay dynamic range, etc.
- Goal to provide quality data in all analysis

Figure 1-2. Accuracy and precision illustrated by measurements plotted as dots on a number line.



Quality Control Data for 'Compounds A and B'

Compound A (LOQ = 2.5 ng/mL)

	Low (2.5 ng/mL)	Mid (5 ng/mL)	Mid (10 ng/mL)	Mid (50 ng/mL)	High (500 ng/mL)
Mean	2.74	5.32	9.84	50.44	507.75
S.D.	0.43	0.76	1.28	3.04	53.64
%CV	15.69	14.29	13.01	6.03	10.56
%Theoretical	109.6	106.4	98.4	100.9	101.6
n	6	6	6	6	6

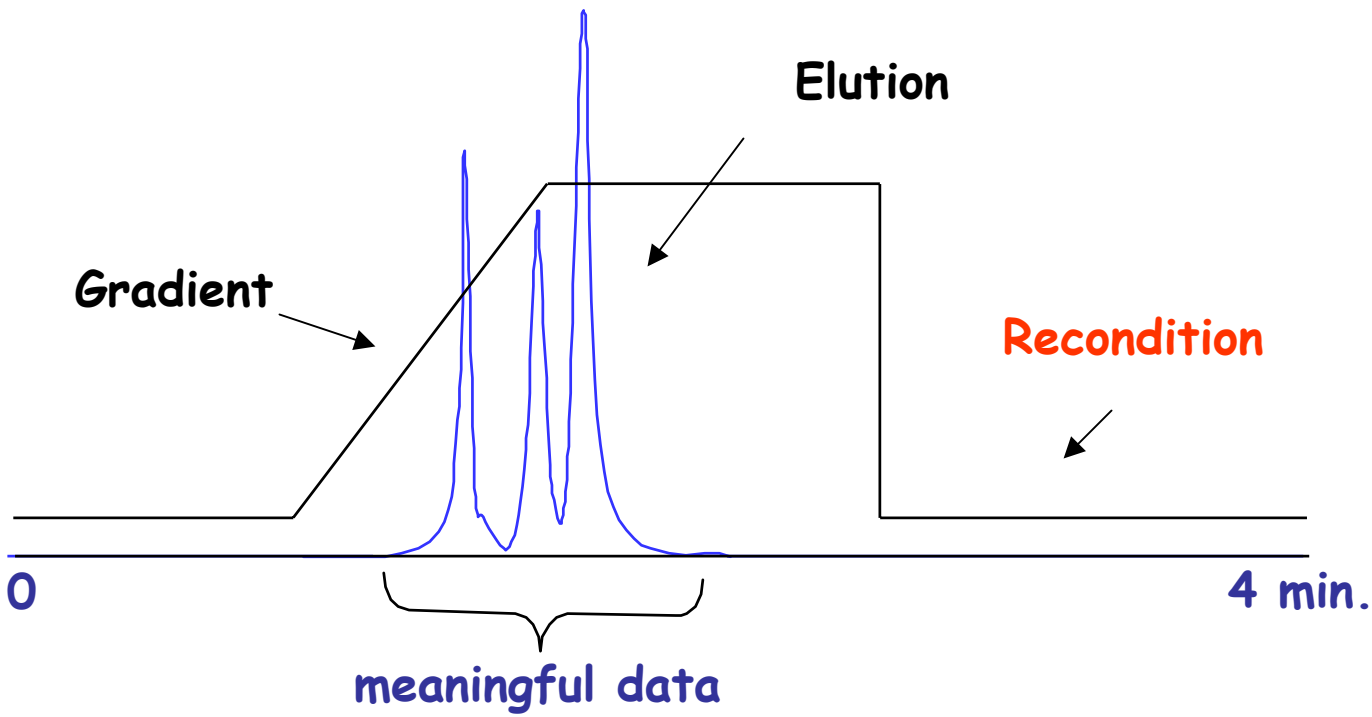
Compound B (LOQ = 5.0 ng/mL)

	Low (2.5 ng/mL)	Mid (5 ng/mL)	Mid (10 ng/mL)	Mid (50 ng/mL)	High (500 ng/mL)
Mean	2.10	4.79	10.03	51.55	529.65
S.D.	1.07	0.82	1.36	1.93	22.99
%CV	50.95	17.12	13.56	3.74	4.34
%Theoretical	84.0	95.8	100.3	103.1	105.9
n	6	6	6	6	6

‘Compound X’ Fails Quality Control Requirements

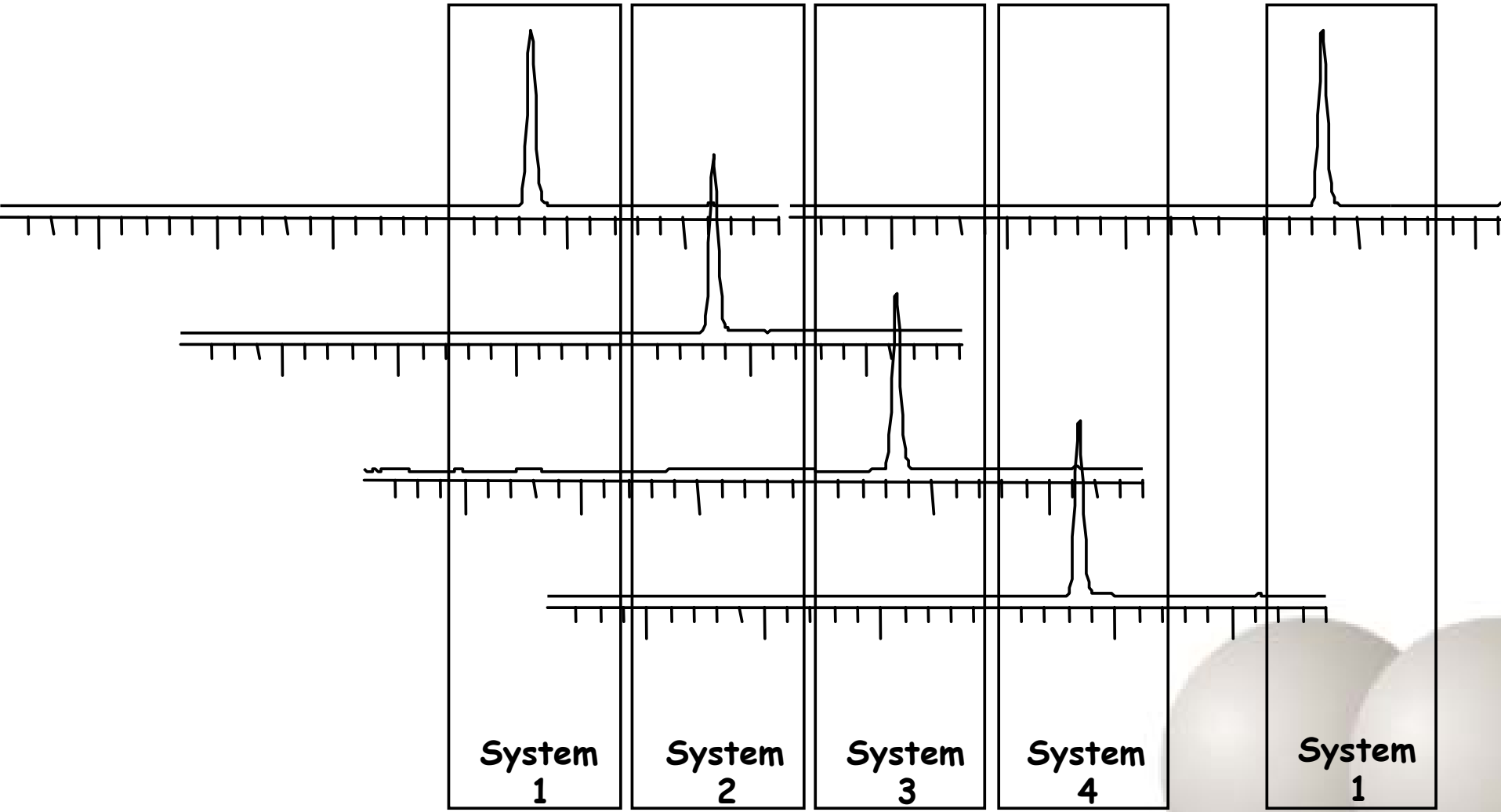
Run Date	Low (1ng/mL)	Mid (2 ng/mL)	Mid (5 ng/mL)	Mid (10 ng/mL)	Mid (50 ng/mL)	High (500 ng/mL)
Mean	-1.31	1.19	3.44	8.29	61.83	543.21
S.D.	1.12	3.31	2.67	3.58	21.15	98.78
%CV	-85.50	278.15	77.62	43.18	34.21	18.18
%Theoretical	-131.0	59.5	68.8	82.9	123.7	108.6
n	6	6	5	4	5	5

HPLC Analysis



Peaks are sent to the MS for only 1/4 of the total run time, leaving the MS *idle* for 3/4 of the time.

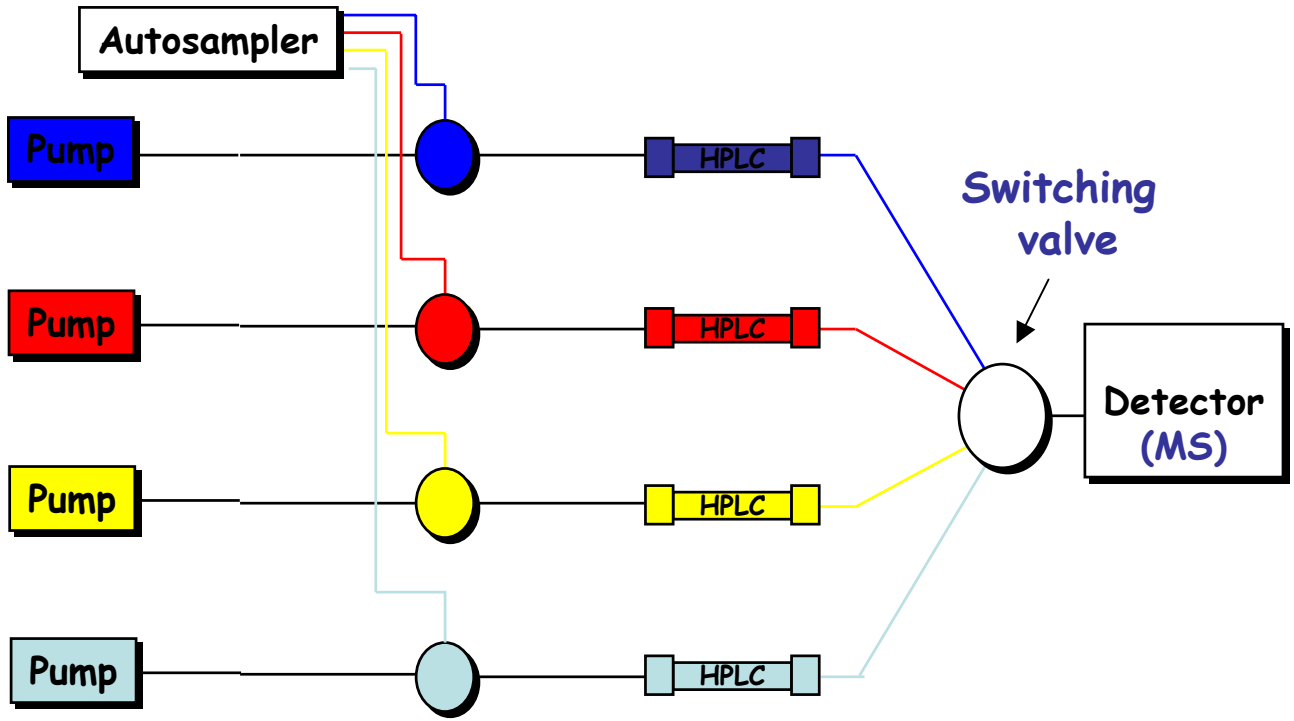
Staggered Parallel Analysis

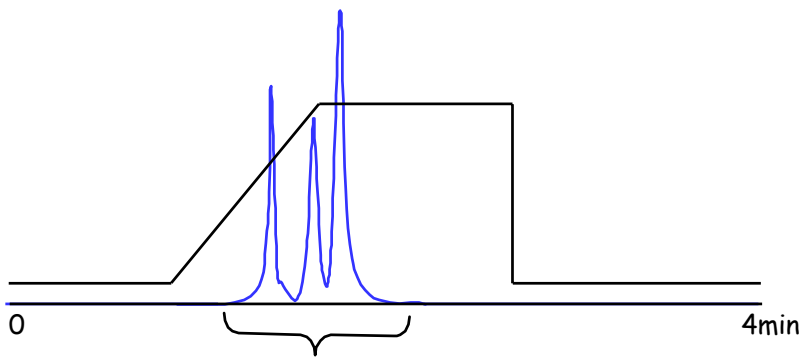


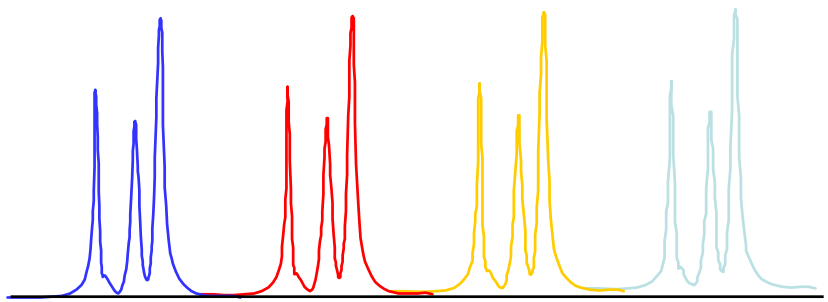
COHESIVE TECHNOLOGIES

Mass spectrometer data collection

Aria LX4: Four HPLC Systems to One MS







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