Innovative Screening Workflows in Liquid Phase Mass Spectrometry

Dr Shaun Bilsborough

The Measure of Confidence

Relative Applicability of LC/MS Techniques
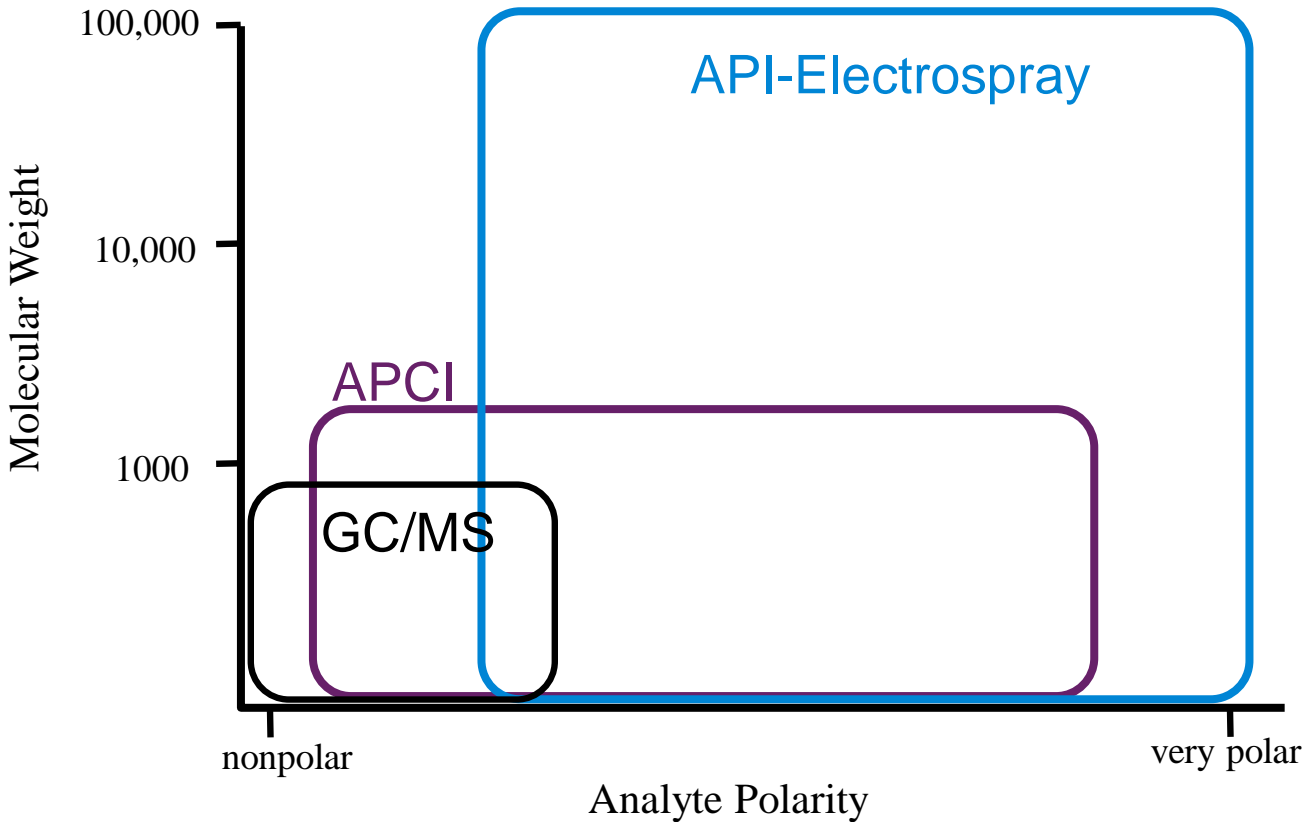
Guiding Principles in MS Analyzer Selection

The correct choice of analyzer depends on whether one is "LOOKING for **UNKNOWNS**" or whether one is "CONFIRMING & MEASURING **KNOWNS**".

The correct choice of analyzer depends on the COMPLEXITY of the **MATRIX**.
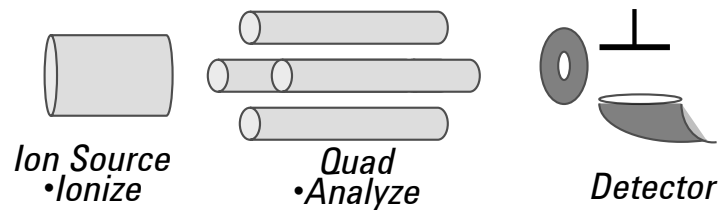
Screening Workflow Solutions

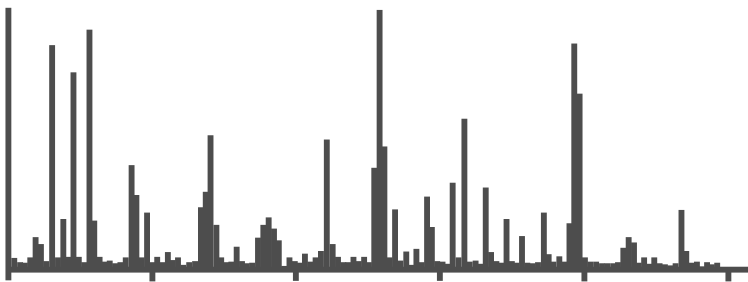| Screening	Target	Suspect	Discovery
SQ or QQQ	Using SIM, MRM and tMRM databases, method can be quickly developed for implementation. Standards required for QQQ method development and quantitation		
TOF	Using PCDLs and an All-Ions workflow Quantitation and Qualification is possible using four points for confirmation (Rt, AccMass, Fragments, IP).	Suspect Screening requires the use of Find by Formula referring to a suspect PCDL where MS/MS spectra are available.	
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Single Quad (SQ)

Selected Ion Monitoring selects a single mass
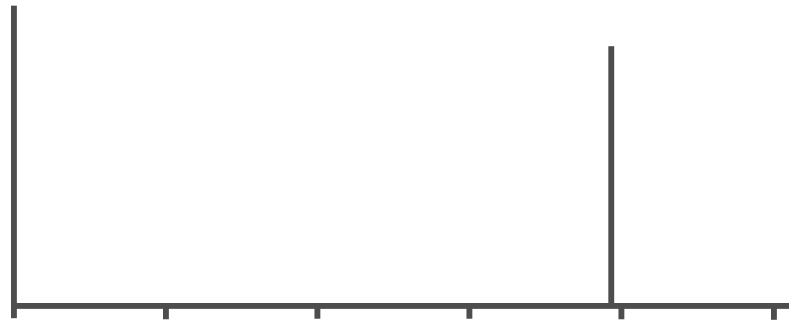Allows sensitive, selective quantitation in relatively clean samples

a single quad instrument...

Ion Source
•Ionize
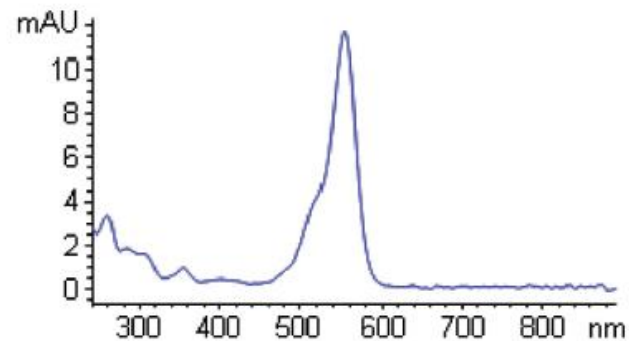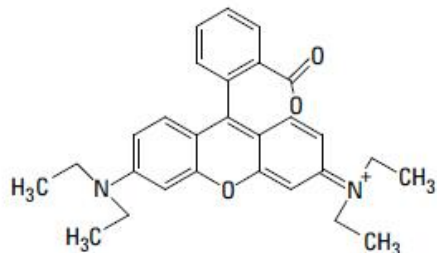
Quad
•Analyze

Detector

Full Scan
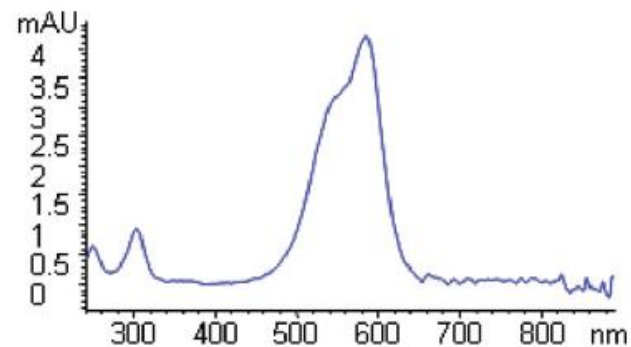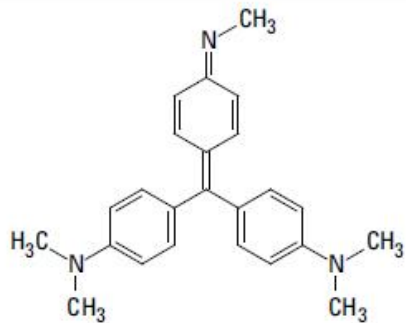
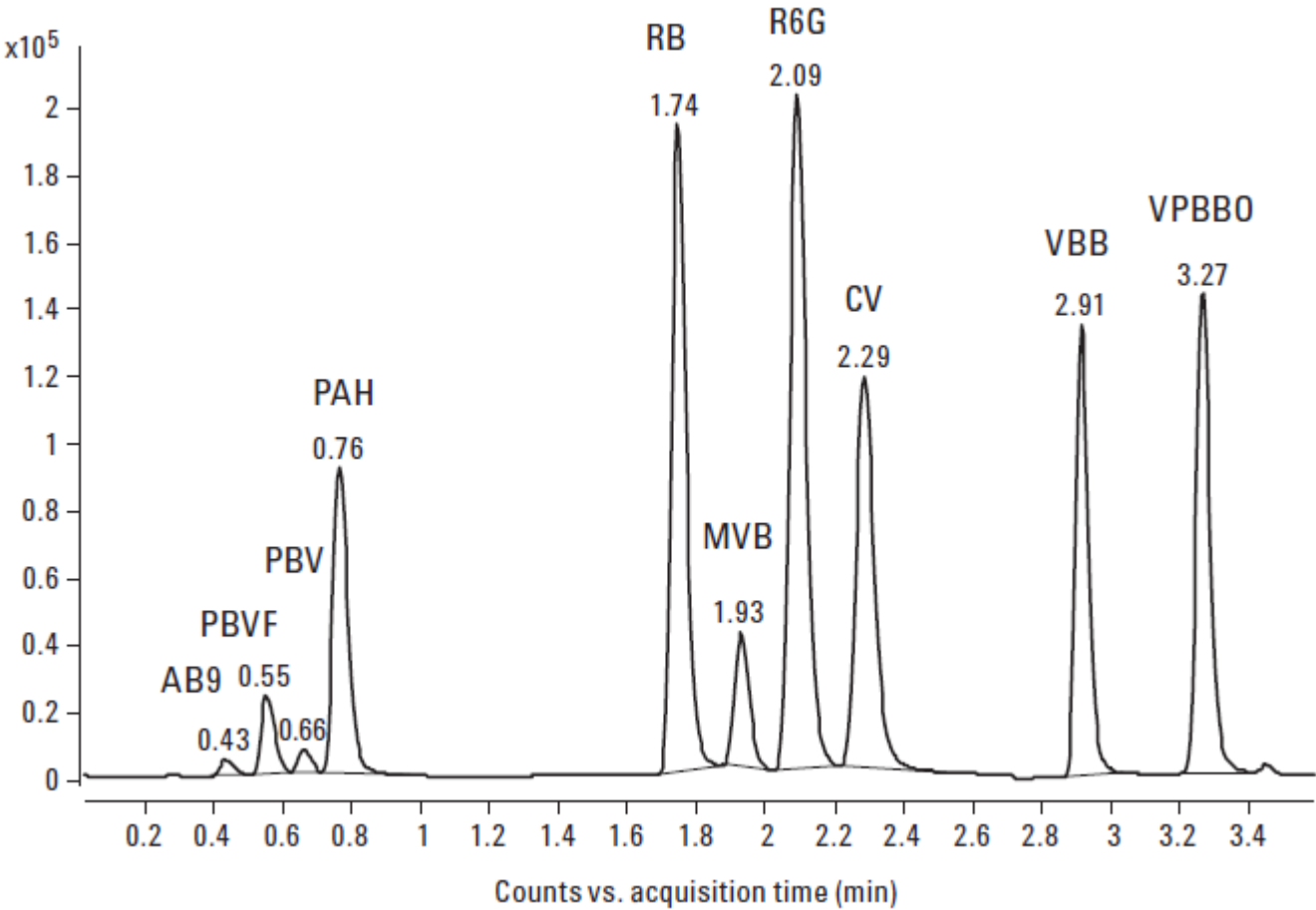## Selected Ion Monitoring (SIM)

LC + DAD

Rhodamine B (RB)

λ max: 554
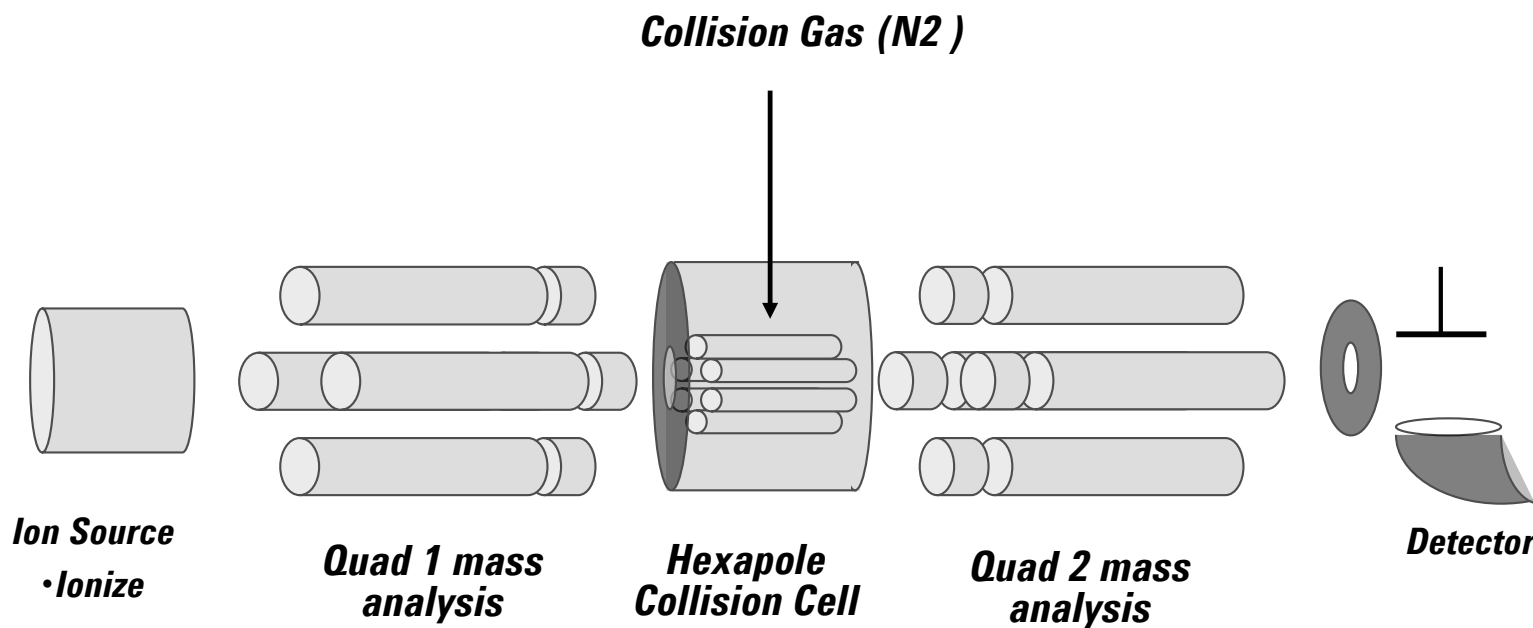
Methyl violet B base (MVB)

λ max: 580

LC/SQ MS + DAD

Screening Workflow Solutions

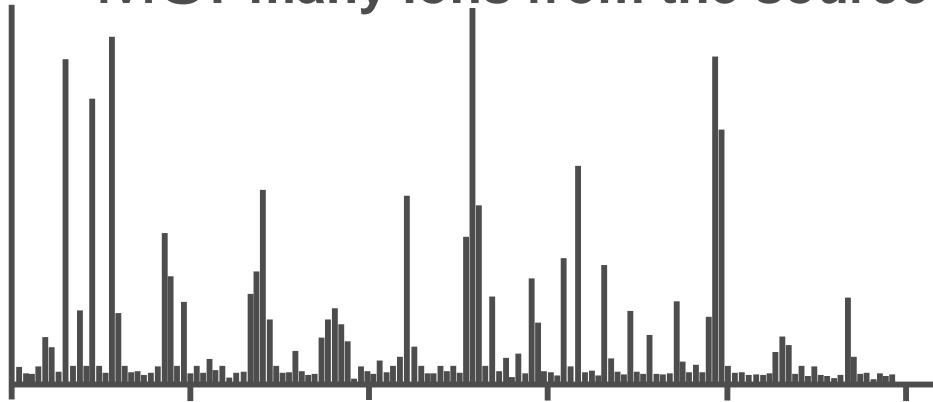| Screening	Target	Suspect	Discovery
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Triple Quad (QQQ)

Quad – Hex - Quad

Collision Gas (N2)

Ion Source
•Ionize

Quad 1 mass
analysis

Hexapole
Collision Cell

Quad 2 mass
analysis

Detector

What is MRM MS/MS using QQQ?

Multiple Reaction Monitoring
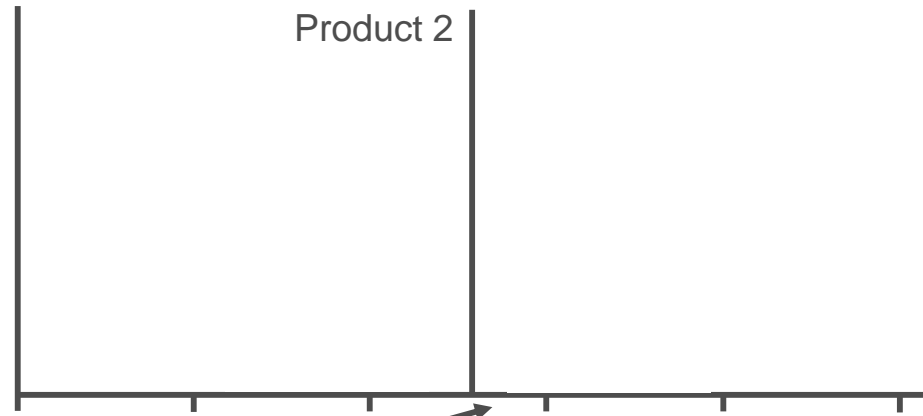

MS: **many ions from the source**

Q3: SIM

Product 2

Q1 SIM | **isolate precursor before CID**
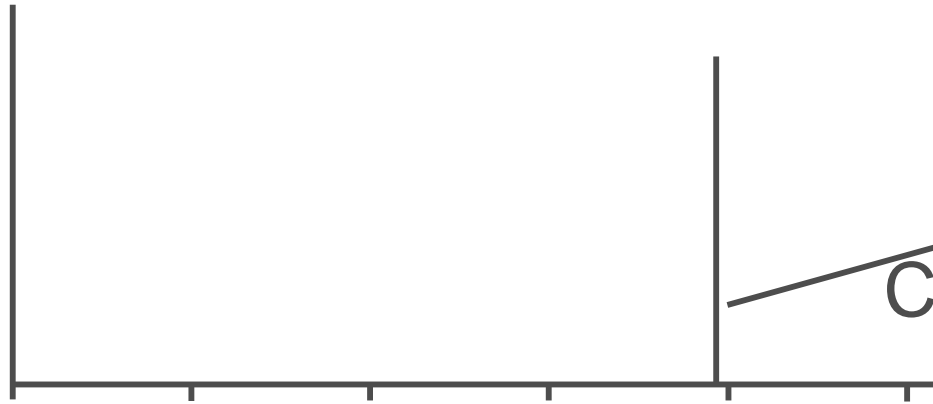
CID + Q2 SIM

Why MS/MS?
Greater Selectivity Than SIM

SIM (Single Quad)
• selectivity proportional to spectral resolution
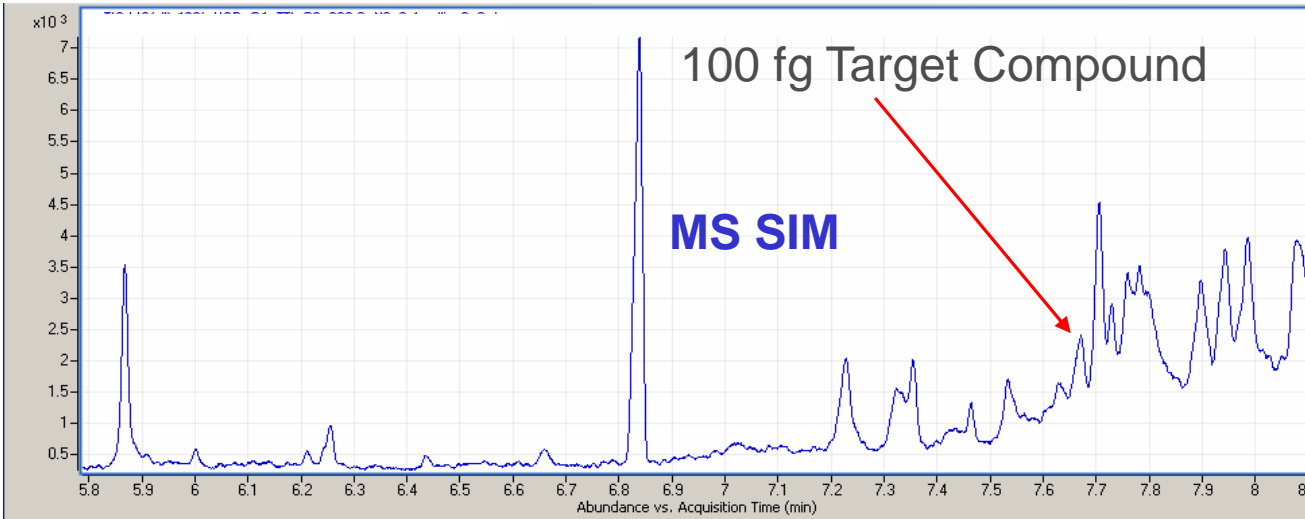• no selectivity against ions with same m/z

MS/MS (QQQ)
• precursor selectivity same as SIM
• high probability that at least one product ion will be a unique fragment of the precursor BUT not the interference
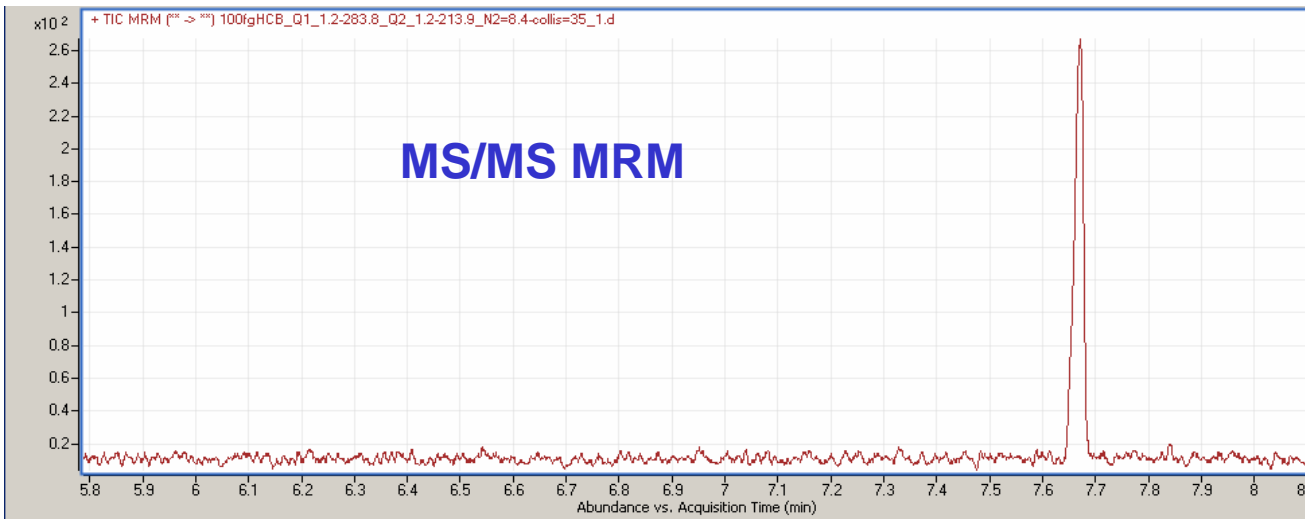

unit mass resolution
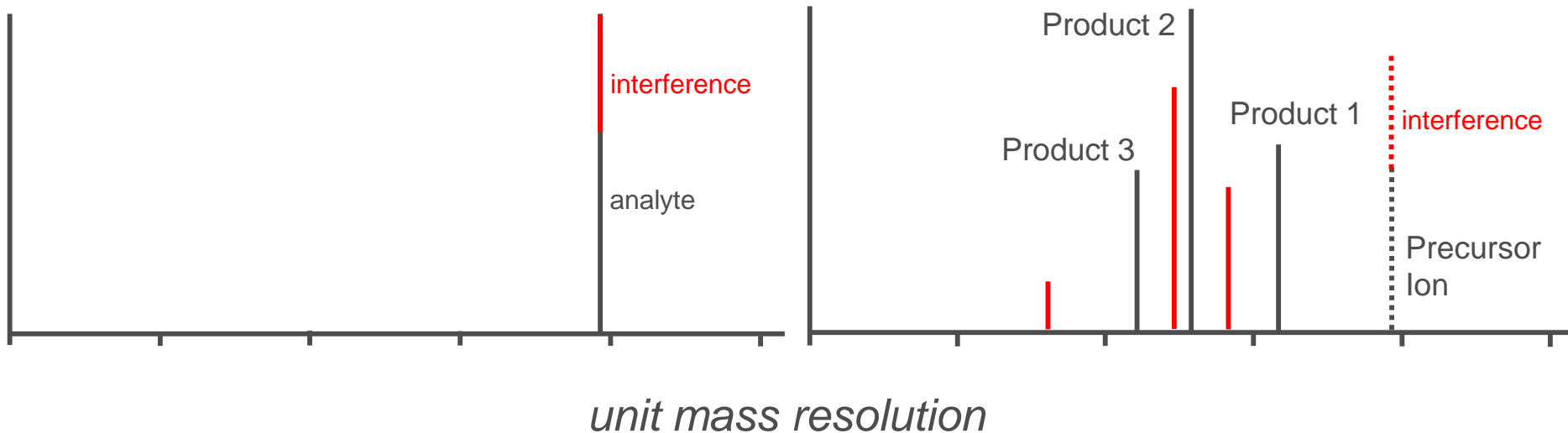
Why MS/MS?
A Picture Is Worth a Thousand Words

100 fg Target Compound

MS SIM

Single Quad SIM of single m/z

MS/MS MRM

MS/MS QQQ MRM of single transition

Single peak on flat baseline

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Screening Workflow Solutions

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TOF and QTOF

Why Accurate Mass?

MS

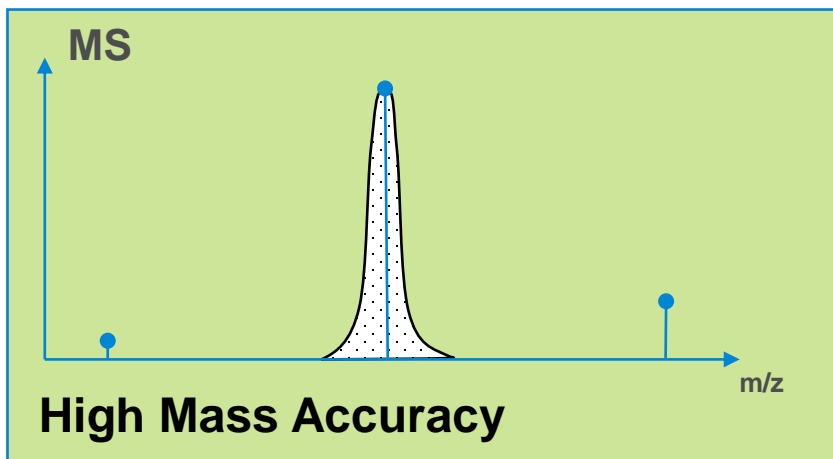**Low Mass Accuracy**

m/z
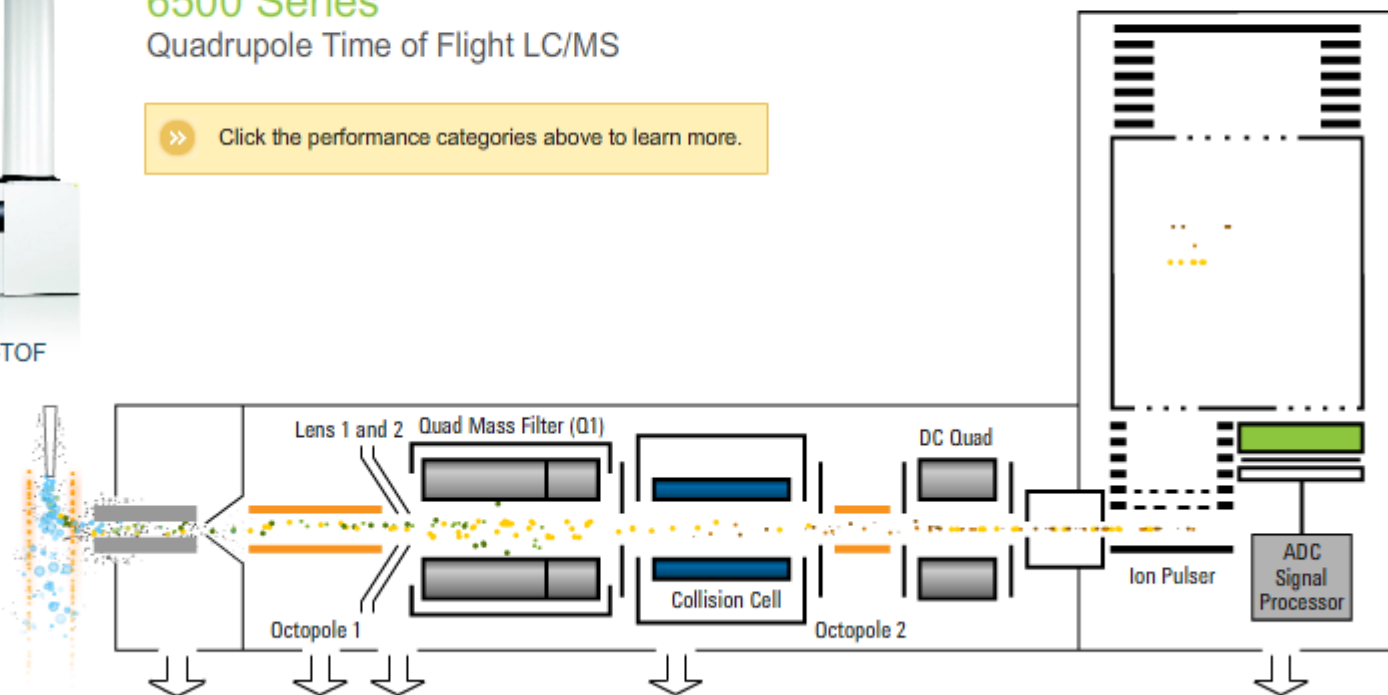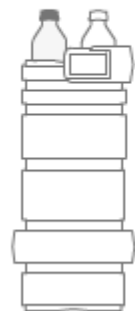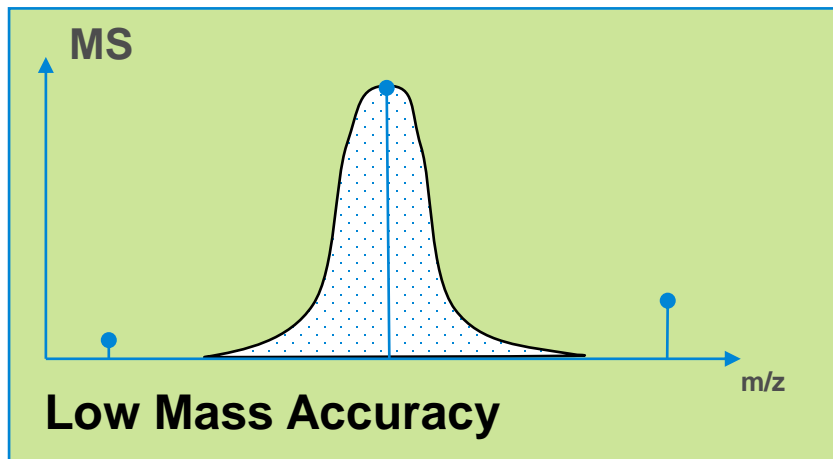
Unit accuracy instrument
e.g. ion trap, QQQ, Q
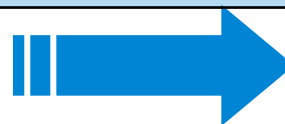
C12 H14 N9 Cl
C13 H23 N3 P S Cl
C14 H24 N2 S2 Cl
C14 H22 N O3 S Cl
C14 H16 N6 O Cl
C15 H25 O P S Cl
C16 H19 N3 P Cl
C17 H20 N2 S Cl
C18 H21 O P Cl

MS

High Mass Accuracy

m/z

High mass accuracy
instrument
e.g. QTOF, TOF

C13 H23 N3 P S Cl
C14 H24 N2 S2 Cl
C14 H22 N O3 S Cl
C14 H16 N6 O Cl
C15 H25 O P S Cl
C16 H19 N3 P Cl
C17 H20 N2 S Cl
C18 H21 O P Cl

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Molecular Formula Generation (MFG) Algorithm Uses All Available Information

PCDLs by Compounds & Spectra

Accurate Mass (AM) LC/MS PCDL	Market	Compounds	Compounds with MS/MS Spectra	Total number of Spectra
Extractables and Leachables	Pharma, BioPharma, Food Safety	>1,000	>350	>1300
Broecker, Herre & Pragst Forensic Toxicology	Forensic Toxicology	>9,200	>3,900	>13,500
Pesticides	Food Safety / Environmental	1,750	>825	>2,700
Veterinary Drugs	Food Safety	>2,150	>1,525	>5,200
Mycotoxins	Food Safety	>450	>300	>1,350
Water Contaminants	Environmental	>1,400	>1,050	~3,900
METLIN	Metabolomics / Lipidomics	>79,600*	>9,450	>32,000

* Plus 168k theoretical

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