LECTURE 6

High Resolution Accurate Mass Spectrometry (HRAMS)

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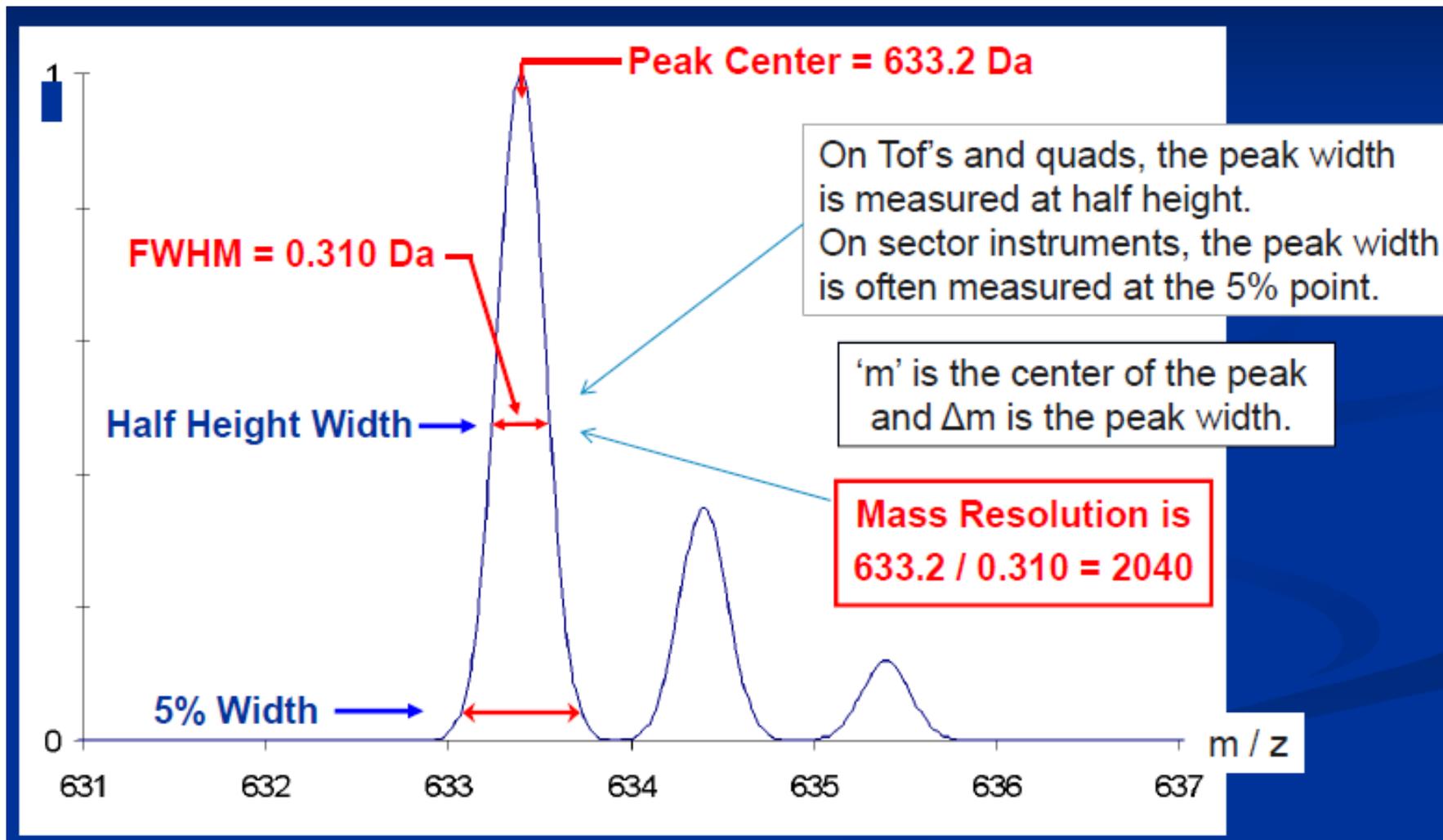**Emeritus Professor, Analytical Toxicology**

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HRMS Contents

- Fundamentals
- Mass Defects
- Isotopic patterns
- Mass axis calibration
- Types of HRMS systems
- Qual/Quan Analysis
- Data mining processes
- Future directions

Definition of Mass Resolution

Background Definitions

- Quadrupoles are common and do not provide high mass resolution
  - Peak width at half hight usually about 0.5-0.7 Da
  - This provides 'unit mass resolution which provides nominal mass accuracy; e.g. m/z 301 vs. m/z 302
- HRMS provides high selectivity with accurate mass determination
  - Measures small mass differences; often 0.001 Da
- HRMS provides refined mass resolution
  - Beneficial when coupled with accurate mass measurement
  - High accuracy when coupled with careful operation and calibration of mass scale.

Information provided by HRMS?

- Extracted ion current of accurate masses provides unparalleled selectivity
  - Extract an exact mass from the full-scan HRMS acquisition
- Qual/Quan
  - Qualitative analysis: what is this chemical?
    - Provides reliable elemental compositions
  - Quantitative analysis: How much of this chemical is present?
    - When/how does HRMS replace triple quad data?

Definition of Terms

- Mass defect
- Isotopes
  - Isotope ratios
- Monoisotopic, exact, ppm, mDa., etc.
- Resolving power vs. resolution?
- Calibration
  - Internal, external, lock mass…
- Mass measurement
  - Centroiding vs. mass peak apex

Mass Units

- Unified atomic mass unit: "u"
  - 1/12th of mass of carbon ^{12}C.
  - This "u" is also known as a dalton (Da) or thomson (Th)
    - Notice in each case the name is lower case and the abbreviation is capitalized
- Previously the definition was based upon oxygen
  - E.g. 1/16th of the mass of ^{16}O
- The use of the term 'amu' is no longer accepted
  - Some manufacturers still incorrectly use this term for the mass axis scale.

Mass Terminology

- **Elemental Composition:**
  - **Molecular formula showing the exact number of atoms of each element in the molecule.**

- **Nominal Molecular Weight: 291**
  - **Sum of nominal masses of the elements**

- **Average Molecular Weight: 291.3654 (Do not use this value for mass spectrometry)**
  - **Calculated using the average atomic weight of each element present in a compound**

- **Exact Mass: 291.0925**
  - **Exact molecular mass of the structure, where atomic masses of each atom are based on the most common isotope for the element.**

Atoms, Isotopes, Molecules and Molecular Weights

Exact Masses and Natural Isotopic Abundance Ratios

Element	Symbol	Exact Mass*	Abundance	X + 1 Factor†	X + 2 Factor†
Hydrogen	H	1.007825	99.99		
	[D or ^{2}H]	2.014102	0.01		
Carbon	^{12}C	12.000000	98.9		
	^{13}C	13.003354	1.1	$1.1n_C$	$0.0060n_C^2$
Nitrogen	^{14}N	14.003074	99.6		
	^{15}N	15.000108	0.4	$0.37n_N$	
Oxygen	^{16}O	15.994915	99.76		
	^{17}O	16.999133	0.04	$0.04n_O$	
	^{18}O	17.999160	0.20		$0.20n_O$
Fluorine	F	18.998405	100		
Silicon	^{28}Si	27.976927	92.2		
	^{29}Si	28.976491	4.7	$5.1n_{Si}$	
	^{30}Si	29.973761	3.1		$3.4n_{Si}$
Phosphorus	P	30.973763	100		
Sulfur	^{32}S	31.972074	95.02		
	^{33}S	32.971461	0.76	$0.8n_S$	
	^{34}S	33.967865	4.22		$4.4n_S$
Chlorine	^{35}Cl	34.968855	75.77		
	^{37}Cl	36.965896	24.23		$32.5n_{Cl}$
Bromine	^{79}Br	78.918348	50.5		
	^{81}Br	80.916344	49.5		$98.0n_{Br}$
Iodine	I	12.904352	100		

*Data reported by Konig, L. A., Mattauch, J. H. E., and Wapstra, A. H. (1962), *Nucl. Phys.*, 31:18.

†Assume X = 100%; X represents the relative intensity of the first peak in a cluster of isotope peaks.

The factor is multiplied by the number (n) of atoms present to determine the magnitude of the abundance contribution for a given isotope. For example, the contribution at X + 1 from ^{15}N for an ion containing three nitrogens would be 0.37 × 3 = 1.11 relative to 100 at X.

$C_{15}H_{17}NO_3S$

- **Atomic weight of Carbon = 12**

- **Atomic weight of Carbon = 13**

- **Atomic weight of Hydrogen = 1**

- **Atomic weight of Deuterium = 2**

- **Atomic weight of Nitrogen = 14**

- **Atomic weight of Oxygen = 16**

- **Atomic weight of Sulfur = 32**
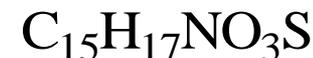
C15 = 180

H17 = 17

N 1 = 14

O 3 = 48

S 1 = 32

MW = 291

But is this the correct mass?

Introduction to Mass Spectrometry 2nd Edition, Raven Press, 1985

Nominal vs. Exact Masses for MW Determination

	Nominal Masses	Exact Masses		'Actual' Masses

$$C_{15}H_{17}NO_3S$$

Nominal Masses

- Atomic weight of Hydrogen = 1
- Atomic weight of Deuterium = 2
- Atomic weight of Carbon = 12
- Atomic weight of Carbon = 13
- Atomic weight of Nitrogen = 14
- Atomic weight of Oxygen = 16
- Atomic weight of Sulfur = 32

Exact Masses

H17 = 17

C15 = 180

N 1 = 14

O 3 = 48

S 1 = 32

MW = 291 (Nominal)

'Actual' Masses

- Atomic weight of Hydrogen = 1.0078
- Atomic weight of Deuterium = 2.0141
- Atomic weight of Carbon = 12.0000
- Atomic weight of Carbon = 13.0033
- Atomic weight of Nitrogen = 14.0030
- Atomic weight of Oxygen = 15.9949
- Atomic weight of Sulfur = 31.9720
- **Exact MW = 291.0925**

Exact mass

- **Exact Mass: 291.0925** $C_{15}H_{17}NO_3S$
  - Exact molecular mass of the structure, where atomic masses of each atom are based on the most common isotope for the element.

Definitions of Small Mass Differences

- PPM: Parts-per-million
  - A relative measure analogous to percent
    - $\dfrac{\text{Measured mass} - \text{Theoretical mass}}{\text{Theoretical}} \times 10^6 = \text{ppm}$

- Millimass units (mmu or mDa)
  - An absolute measure
  - 1 mmu = 0.001 u

Relationship Between ppm and mmu

m/z	ppm	u
200	2	0.0004
	5	0.001
	10	0.002
600	2	0.001
	5	0.003
	10	0.006
1000	2	0.002
	5	0.005
	10	0.010

Using Accepted Mass Terminology

- Resolving power vs. Mass resolution
  - **Mass Resolving Power** is defined as m/dm
    - Where m designates the mass and dm the peak width necessary for separation at mass m. A specific m/z value and also the method like 10% valley or 50% valley or full width at half maximum (FWHM) must be given.
    - **Mass Resolution** usually refers to the ability of separating two narrow mass spectral peaks
- Refer to good reference books
  - IUPAC Gold Book
  - ASMS definition of terms
  - MS terms Wiki
  - Mass Spec Desk Reference by O. David Sparkman

Representative Examples of Resolving Power

Type	Resolving Power (FWHM)
FT-ICR-MS	1,000,000
FT-Orbitrap	100,000
High-Res-TOF	60,000
TOF	10,000
Quadrupole / IonTrap in UltraZoom mode	10,000
Quadrupole / Iontrap	1,000

Mass Defect

- ^{12}C is the only isotope with an integer exact mass: 12.00000.
  - Used to set mass scale for all other elements, isotopes

- All others have a "Mass Defect".
  - Difference between actual exact mass and integer mass

Mass Defect Details

- Positive mass defect: Hydrogen 1.<u>0078</u>
  - gain almost 0.008 Da per hydrogen
  - typically, many hydrogen present: 40 hydrogen in molecule yield positive mass defect of about +0.3 Da.

- Negative mass defect: Cl, Br, S, O
  - lose 0.031 Da per chlorine
  - Factor in HRMS of environmental analytes

Mass Defect: Positive and Negative

- **Difference between the exact mass and the integer mass of the nuclide (the element's atoms)**
  - **Mass defect of an ion is the sum of all the mass defects within that ion's nuclides (atoms).**
- **Carbon-12 is the reference element and has no mass defect: C=12.0000**
  - **Masses of the other nuclides all have a mass defect relative to carbon**
  - **Positive mass defect: atomic weight greater than nominal mass**
    - **H,N**
  - **Negative mass defect: atomic weight less than nominal mass**
    - **F,Cl,Br,I,O,Si,P,Na,K**
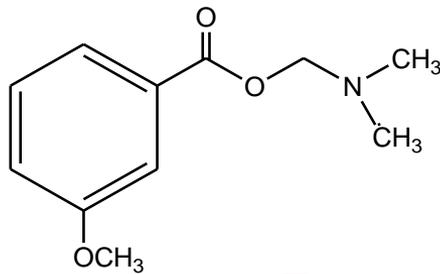
$C_{12}H_{18}NO_3$
Exact Mass: 224.1287

Pos Mass Defect
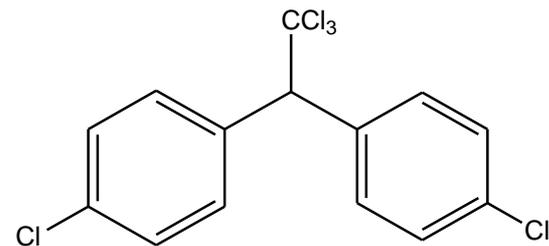
$C_{14}H_9Cl_5$
Exact Mass: 351.9147

Neg Mass Defect

DDT

Mass Defect Isotopes
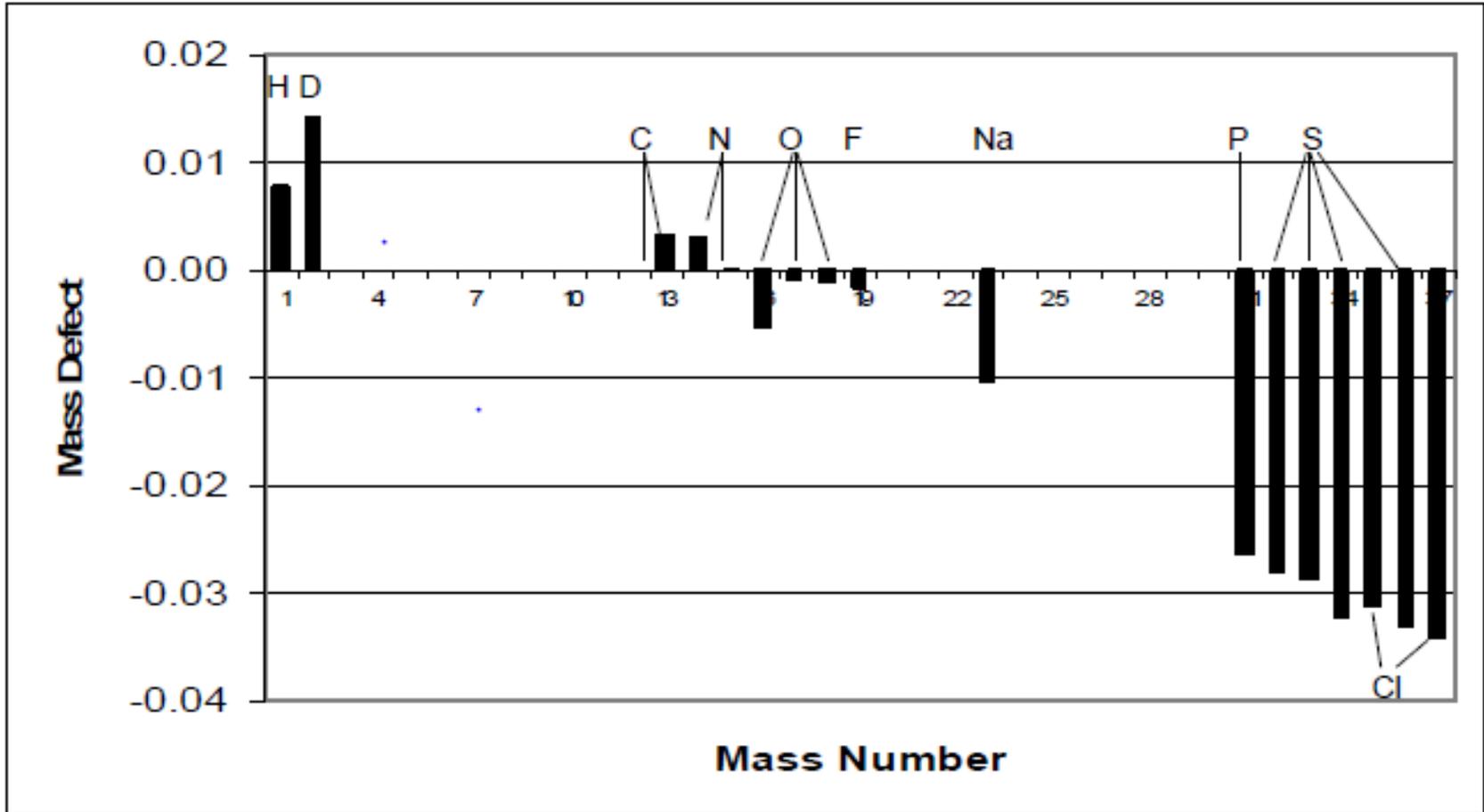
Impact of Mass Defect

- The magnitude of the mass defect depends upon elements present and their abundance
  - Mass defect is an indication of:
    - Low mass defect may indicate halogens (look for their unique isotopic pattern) or rings plus double bonds
      - Unsaturated, few hydrogens

Name	Formula	Nominal	Accurate	Average
Methyl Stearate	$C_{19}H_{38}O_2$	298	298.2872	298.5114
Ubiquitin	$C_{378}H_{630}N_{105}O_{118}S$	8556	8560.6254	8565.873

    - High mass defect may indicate many hydrogens (highly saturated) or very little unsaturation or rings present.

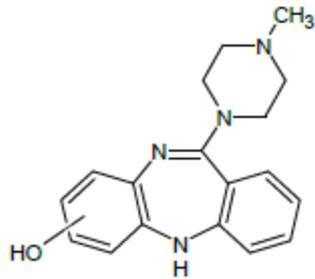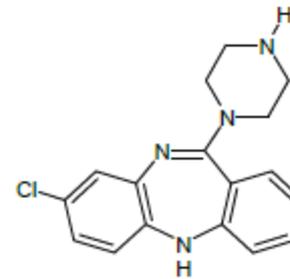# Using Negative Mass Defects to Locate Chemical Alterations
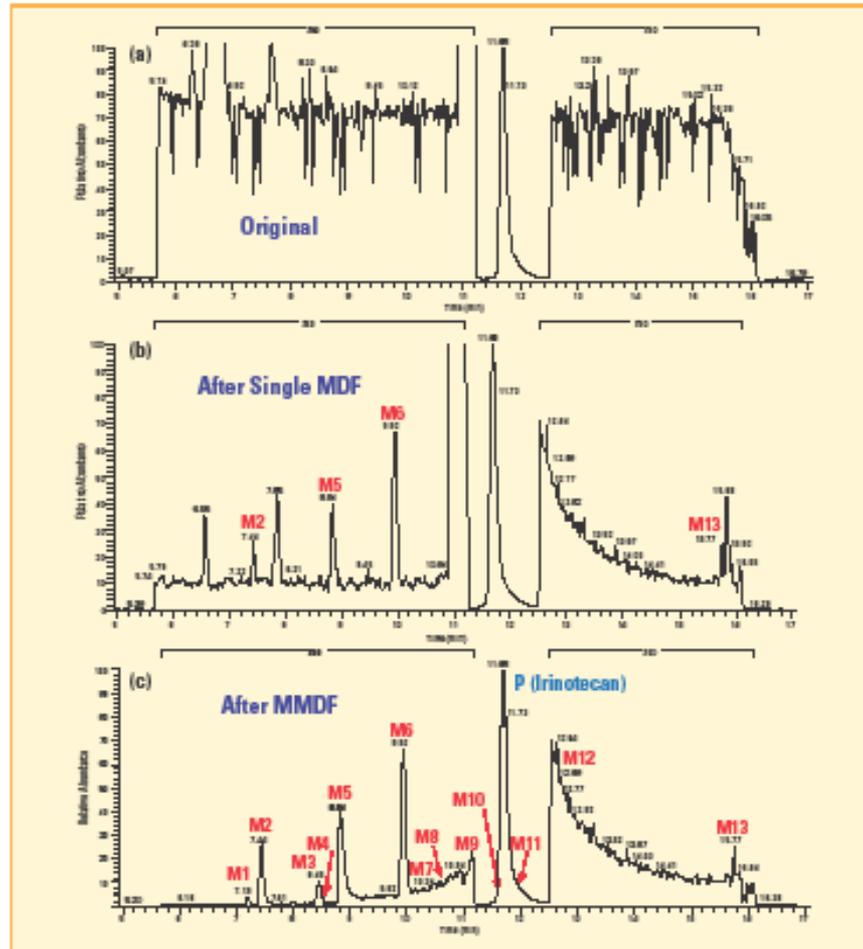
Figure 1: Base peak chromatograms of 10mM Irinotecan rat hepatocyte incubation: (a) Original; (b) After single Mass Defect Filter; (c) After Multiple Mass Defect Filters (MMDF).
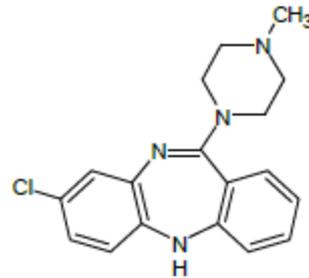
Mass Defect Filtering
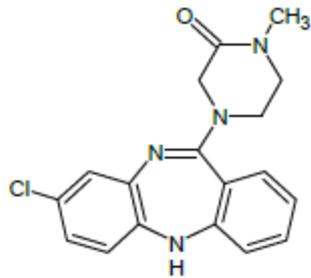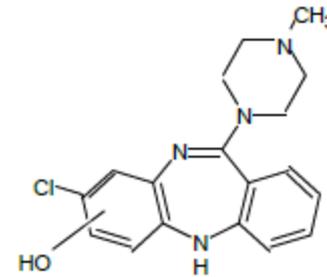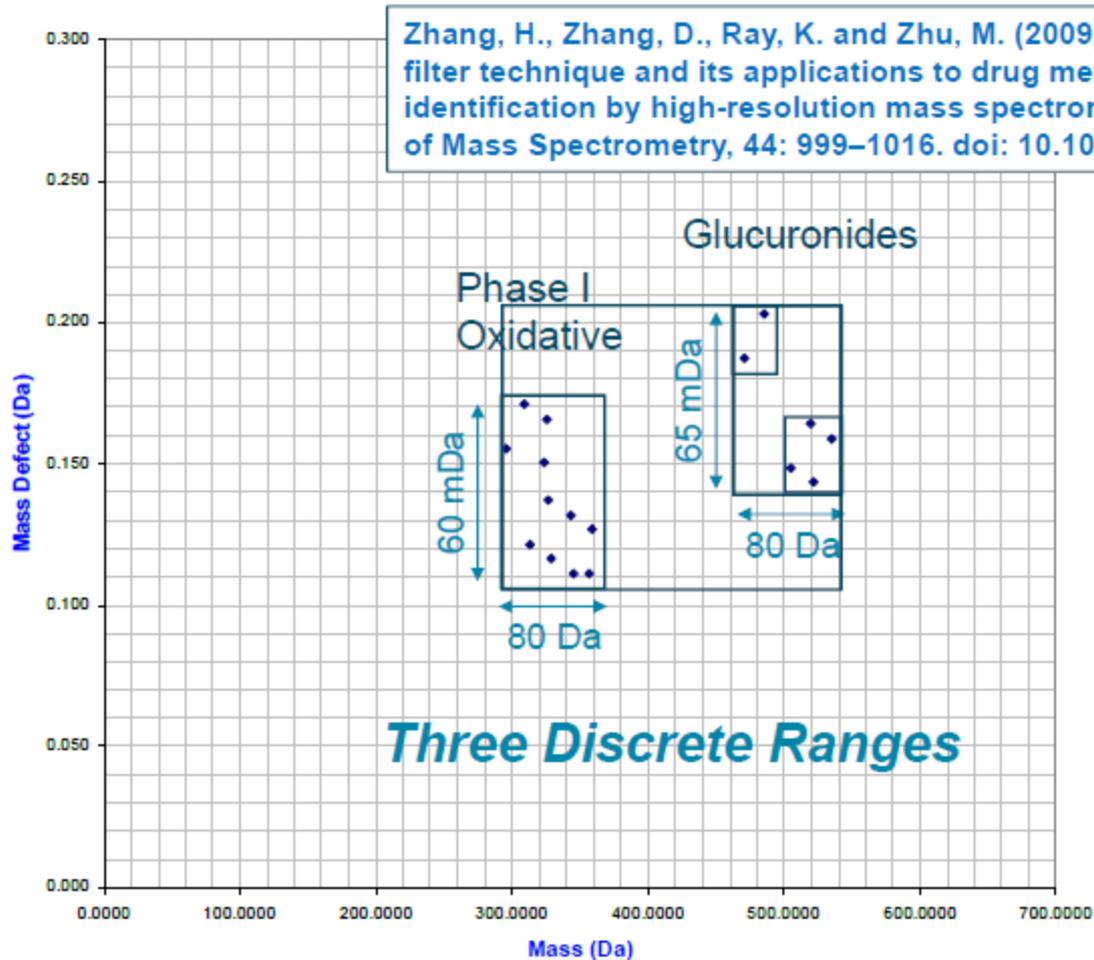

Mass Defect in Metabolism

308.1637 Da

312.1141 Da

326.1298 Da

340.1091 Da

342.1247 Da

Types of Metabolites May be Distinguished

Mass Defect Distribution – Clozapine Metabolites

Zhang, H., Zhang, D., Ray, K. and Zhu, M. (2009), Mass defect filter technique and its applications to drug metabolite identification by high-resolution mass spectrometry. Journal of Mass Spectrometry, 44: 999–1016. doi: 10.1002/jms.1610

Glucuronides

Phase I Oxidative

65 mDa

60 mDa

80 Da

80 Da

Three Discrete Ranges

Real-Time Multiple Mass Defect Triggered IDA (Information-Dependent Acquisition)

- Looks at TOF MS scan to identify ions matching the specified mass defect ranges

- Gives priority to matching ions over other ions, even if they are more intense

- Two modes
  - Non-exclusive mode: Priority given to matching ions. Other ions are still triggered if no matching ions take priority
  - Exclusive mode: Only matching ions are triggered. Other ions ignored even if there are no matching ions

- Significantly improves MS/MS triggering of metabolites in difficult matrices e.g. bile and protein precipitated plasma

- Marginal advantage for high concentrations in clean matrix e.g. 10 µM incubation in microsomes

- Best results obtained when MDF and DBS combined