Non-targeted Screening of Biological Samples for Environmental Contaminants

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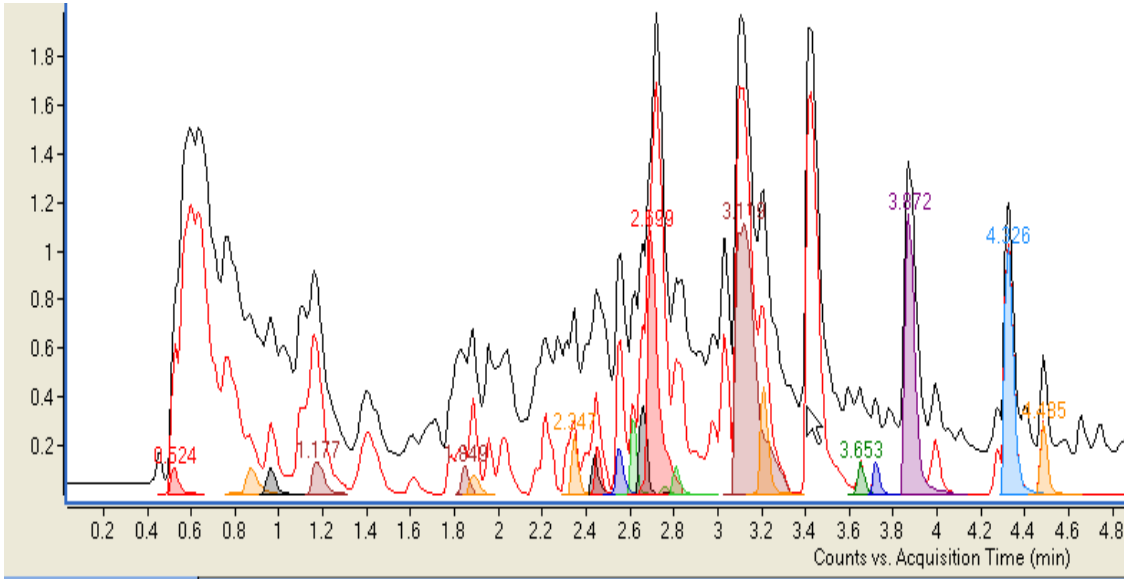
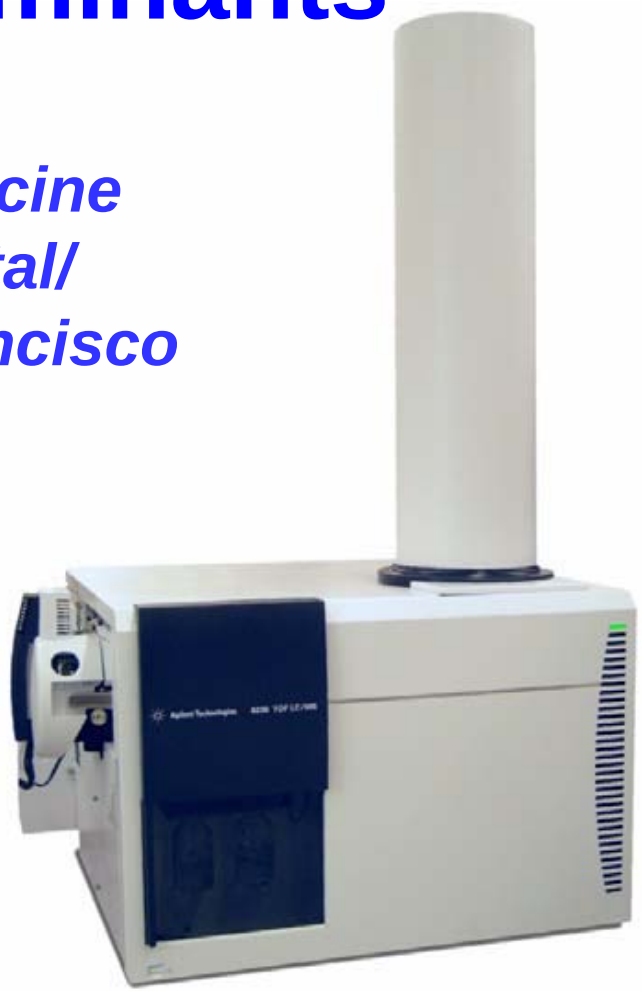




Outline

Rationale for Non-Targeted Analysis

Time-of-Flight Mass Spectrometry (TOF MS)

TOF Analysis of Environmental Pollutants

TOF Analysis of Environmental Toxins in Biological Matrices

Rationale for Non-Targeted Analysis

- **There are more than 3000 industrial chemicals produced and imported in over 1 million pounds/year**
- **Only about 300 chemicals are being biomonitored by TARGETED analysis**
- **Targeted analysis require reference standards for each target analyte; costly and time consuming**
- **More rational approach: non-targeted screening of environmental toxins followed by targeted quantification of analytes that are found in subjects at high frequency**
- **Required analytical platform: Time-of-Flight Mass Spectrometer (TOF MS)**

What is TOF Mass Spectrometry?

- analytical technique based on separation of molecules (ions) according to their charge-to-mass ratio (MW)
- involves ionization of molecules and sorting them out according to their mass
- several types according to method of ionization or detection- ESI/APCI TOF-MS, ESI/APCI QTOF-MS, MALDI- TOF, OrbiTrap
- old technique- first mass spectrometers with time-of-flight analyzers appeared in 1950's
- application into biological matrices only started in the last decade
- usually used in tandem with chromatography (LC or GC)

LC- Mass Spectrometry

Ionization

Ion Source

Form ions
(charged molecules)

Mass Sorting (filtering)

Mass Analyzer

Sort Ions by Mass (m/z)

Detection

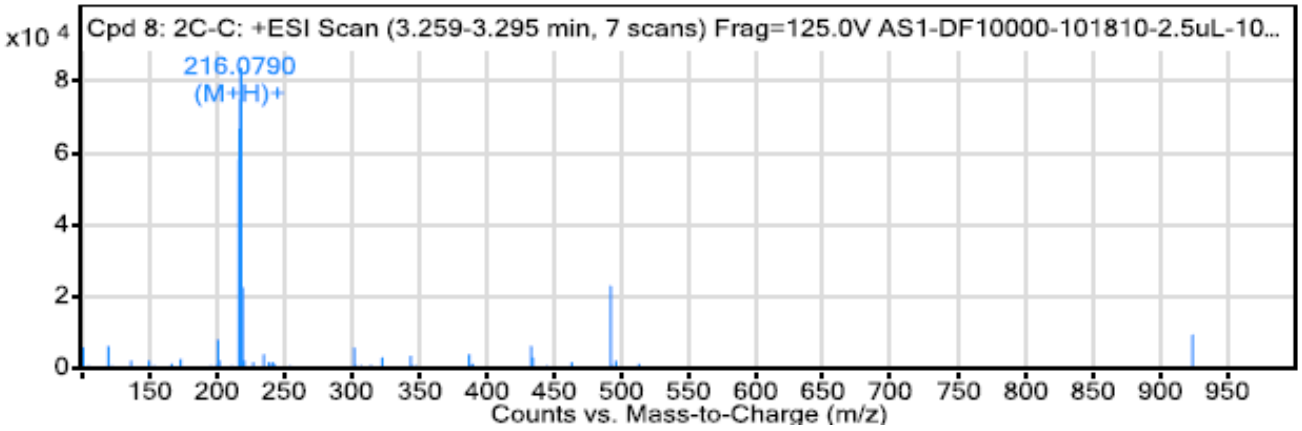
Ion Detector

Detect ions

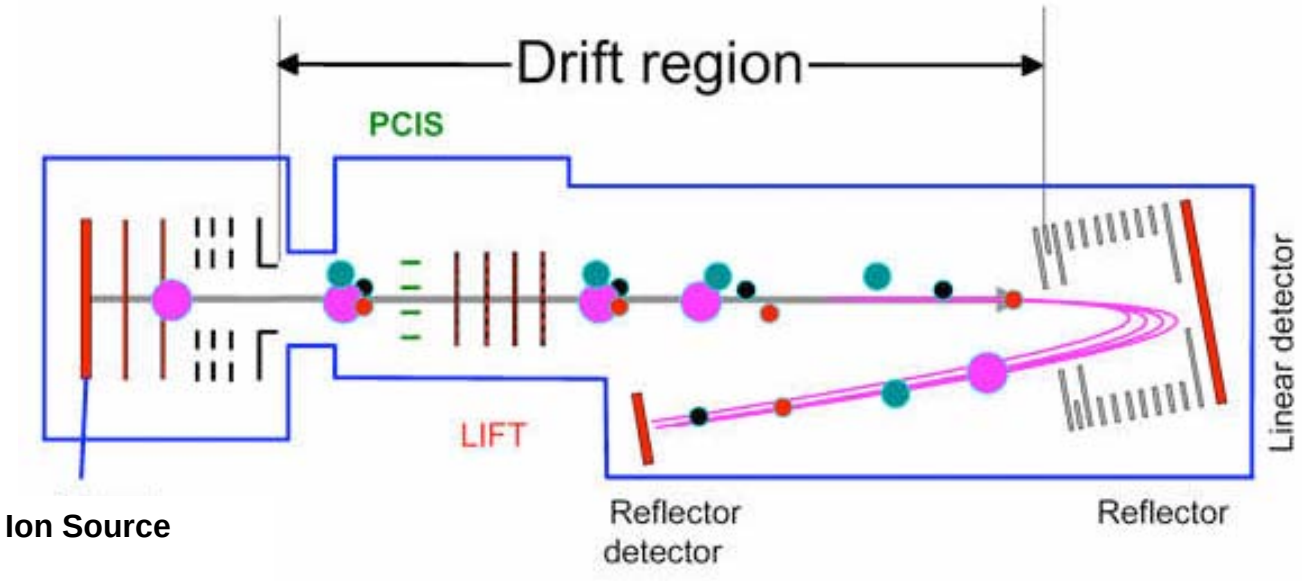
Inlet

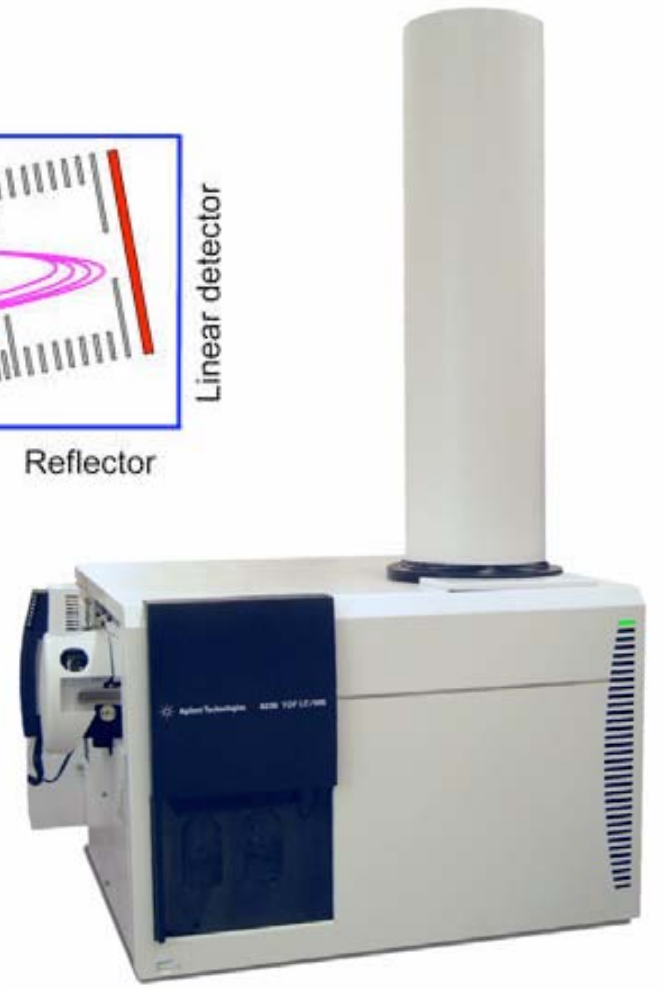
- **Solid**
- **Liquid**
- **Vapor**

HPLC/ GC



Time-of-Flight Mass Spectrometry



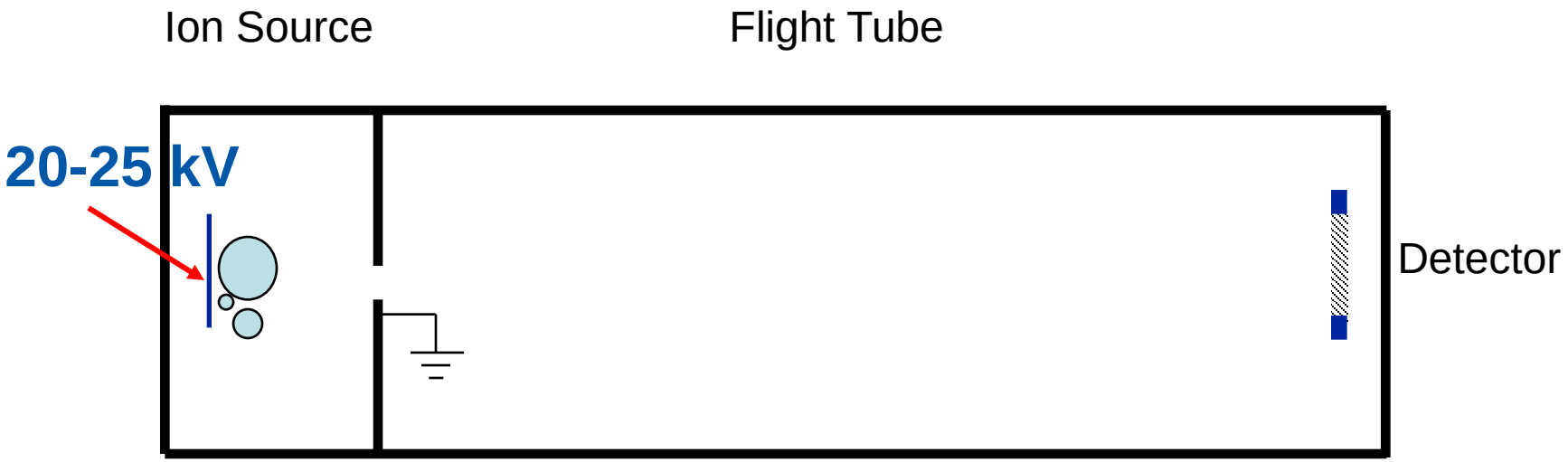


Time of flight of ions measured

TOF of ion is proportional to its m/z

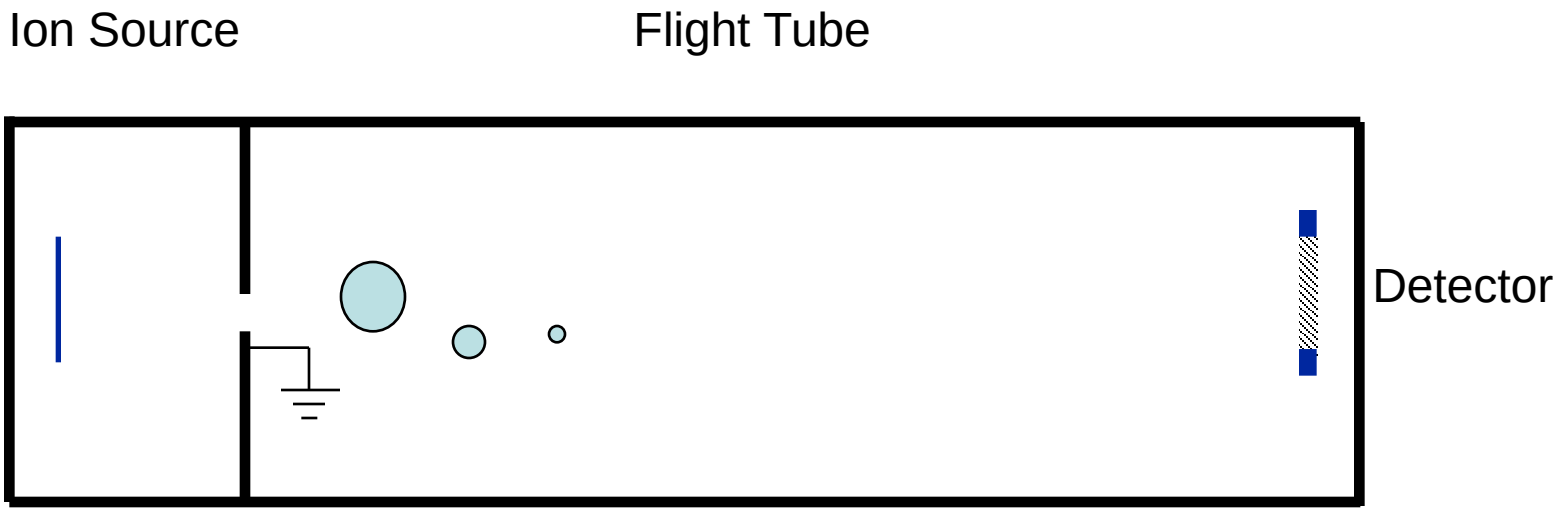
High mass accuracy in the sub- 2 ppm allows for unambiguous assignment of formula to measured MWs

Time-of-flight Mass Analyzer



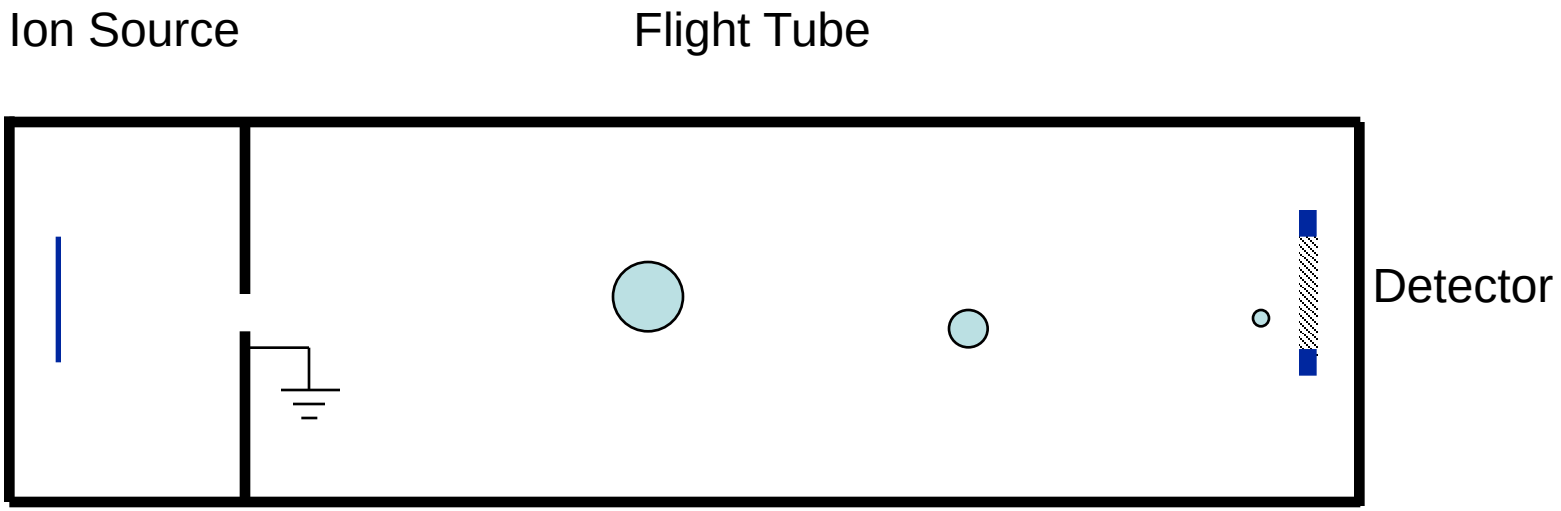
Principle: If ions are accelerated with the same potential at a fixed point and a fixed initial time and are allowed to drift, the ions will separate according to their mass to charge ratios.

Time-of-flight Mass Analyzer



The ions enter the flight tube with the lighter ions travelling faster than the heavier ions to the detector

Time-of-flight Mass Analyzer



The lighter ions strike the detector before the heavier ions.
This “time of flight” (TOF) can be converted to mass

Mass Accuracy is key to utility of TOF

Accuracy is measured as mass error (in ppm)

$$\text{Mass error (ppm)} = \frac{| MW_{meas} - MW_{theo} |}{MW_{theo}} \cdot 1 \times 10^6$$

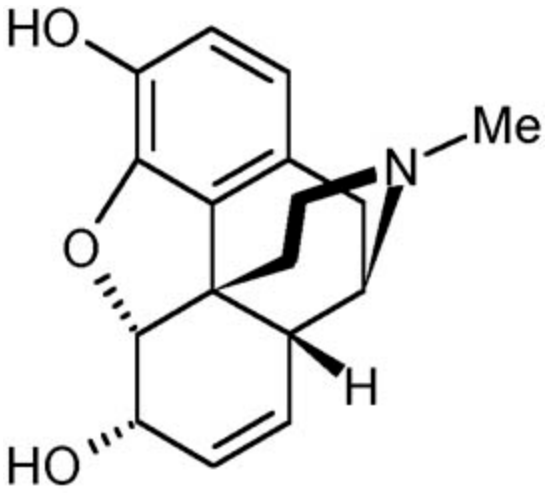
$$= \frac{| 250.0005 - 250.0000 |}{250.0000} \cdot 1 \times 10^6$$

$$= 2 \text{ ppm}$$

2 ppm accuracy translates to:

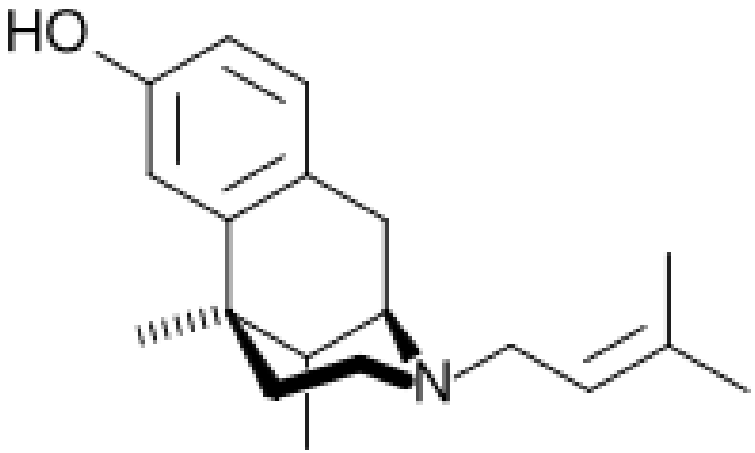
MW	amu error
100	0.0002
200	0.0004
300	0.0006
400	0.0008

Mass Accuracy is key to utility of TOF



Morphine: $C_{17}H_{18}NO_3$

285.1365



Pentazocine: $C_{19}H_{27}NO$

285.2093

High mass accuracy allows unambiguous formula assignment to measured masses

For M/Z = 285.1365 (assuming C,H,O,N only)

At nominal mass resolution	Hundreds of formula
At 10ppm accuracy	5 possible formula
At 5ppm accuracy	$C_{17}H_{18}NO_3$, $C_{15}H_{26}N_4O_2$, $C_{20}H_{16}N_2$
At 3ppm accuracy	$C_{17}H_{18}NO_3$

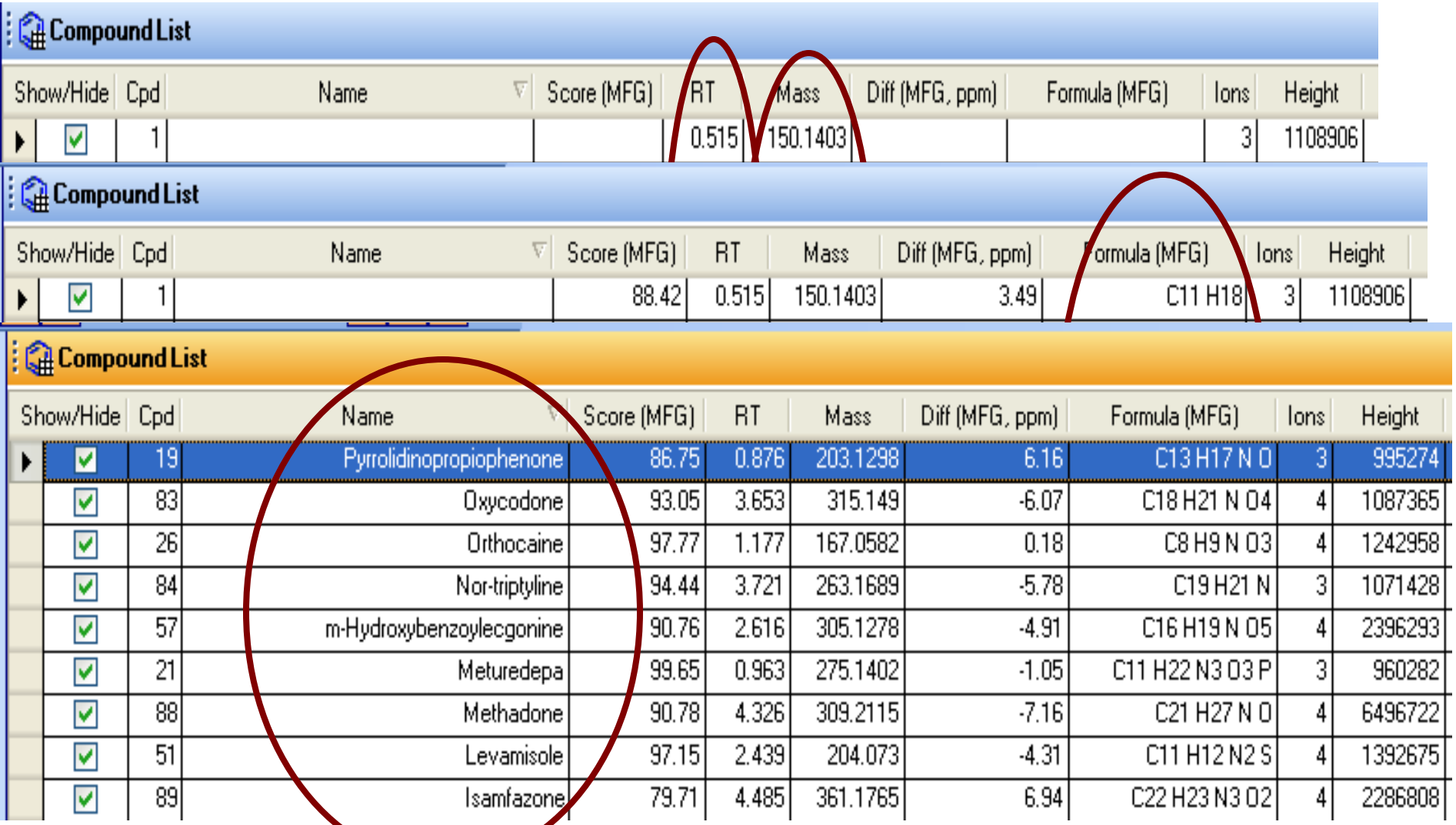
Non- Targeted Qualitative Analysis

Protocol

1. Full scan mass spectra of sample is obtained
2. Algorithm generates all the masses (of chemicals) measured in a sample that eluted at specific retention times (RT)
3. Another algorithm assigns the best formula match to each of the masses measured
4. The generated formula matches are then screened for specific compound matches against a database (usually " home-brewed" → potential Compound Hits in sample
5. To confirm the actual presence of Compound Hits →

   Run a reference standard of the compound hit (if available) and compare the ref std's RT to that of the compound hit

Extracting Information from Non-Targeted Analysis



Compound List

Show/Hide	Cpd	Name	Score (MFG)	RT	Mass	Diff (MFG, ppm)	Formula (MFG)	Ions	Height
☑	1			0.515	150.1403			3	1108906

Compound List

Show/Hide	Cpd	Name	Score (MFG)	RT	Mass	Diff (MFG, ppm)	Formula (MFG)	Ions	Height
☑	1		88.42	0.515	150.1403	3.49	C11 H18	3	1108906

Compound List

Show/Hide	Cpd	Name	Score (MFG)	RT	Mass	Diff (MFG, ppm)	Formula (MFG)	Ions	Height
☑	19	Pyrrolidinopropiophenone	86.75	0.876	203.1298	6.16	C13 H17 N O	3	995274
☑	83	Oxycodone	93.05	3.653	315.149	-6.07	C18 H21 N O4	4	1087365
☑	26	Orthocaine	97.77	1.177	167.0582	0.18	C8 H9 N O3	4	1242958
☑	84	Nor-triptyline	94.44	3.721	263.1689	-5.78	C19 H21 N	3	1071428
☑	57	m-Hydroxybenzoylecgonine	90.76	2.616	305.1278	-4.91	C16 H19 N O5	4	2396293
☑	21	Meturedepa	99.65	0.963	275.1402	-1.05	C11 H22 N3 O3 P	3	960282
☑	88	Methadone	90.78	4.326	309.2115	-7.16	C21 H27 N O	4	6496722
☑	51	Levamisole	97.15	2.439	204.073	-4.31	C11 H12 N2 S	4	1392675
☑	89	Isamfazone	79.71	4.485	361.1765	6.94	C22 H23 N3 O2	4	2286808

What can be analyzed using the TOF?

Targeted and Non-Targeted Screening of Small Molecules
Small Molecules: 75- 3000 amu (100-1000 amu)

- **Most Organic Drugs and metabolites, Herbal and Food Supplements' bioactive compounds and adulterants, Small molecule biomarkers, Non- proteinaceous hormones, Small peptides, Steroids, Fatty acids, Fat and Cholesterol derivatives, Nucleotides and derivatives, Sugars and small oligosaccharides (~10mer), Pesticides, Small Organic Environmental Pollutants**

Samples: Serum, Urine, CSF, Breast milk, Other Body Fluids (Saliva, Sweat etc.), Tissue extracts, Plant extracts, Cell lysates, Pills
Qualitative Data, Semi-quantitative, Quantitative
TAT for LC-MS/TOF run: 8-12 min Sample Prep: 30-60min
What can't be analyzed:

- **Proteins, Nucleic acids, Polysaccharides, Polymers, Metals, Gaseous compounds, Inorganic salts**

Quadrupole Time-of-Flight Mass Spectrometry (QTOF)

- Addition of a quadrupole in front of the TOF allows for fragmentation of parent ions
- Allows measurement of accurate masses of both parent and daughter (fragment) ions
- Allows structure elucidation of unknowns

(Q)TOF Analysis of Environmental Pollutants

Since 2001 there have been attempts in the use of (Q) TOF- MS in the non-targeted analysis of environmental pollutants

- **Screening and identification of unknown contaminants in water with liquid chromatography and quadrupole-orthogonal acceleration- time-of-flight tandem mass spectrometry (***Bobeldjik et al, 2001***)**
- **Searching for non-target chlorinated pesticides in food by LC-TOF mass spectrometry (***Garcia-Reyes et la, 2005***)**
- **Application of LC/ Quadrupole-Linear Ion Trap MS and TOF mass spectrometry to the determination of pharmaceuticals and related contaminants in wastewater (***Bueno et al, 2007***)**
- **Accurate mass screening and identification of emerging contaminants in environmental samples by LC- hybrid linear ion trap Orbitrap mass spectrometry (***Hogenboom et al, 2009***)**

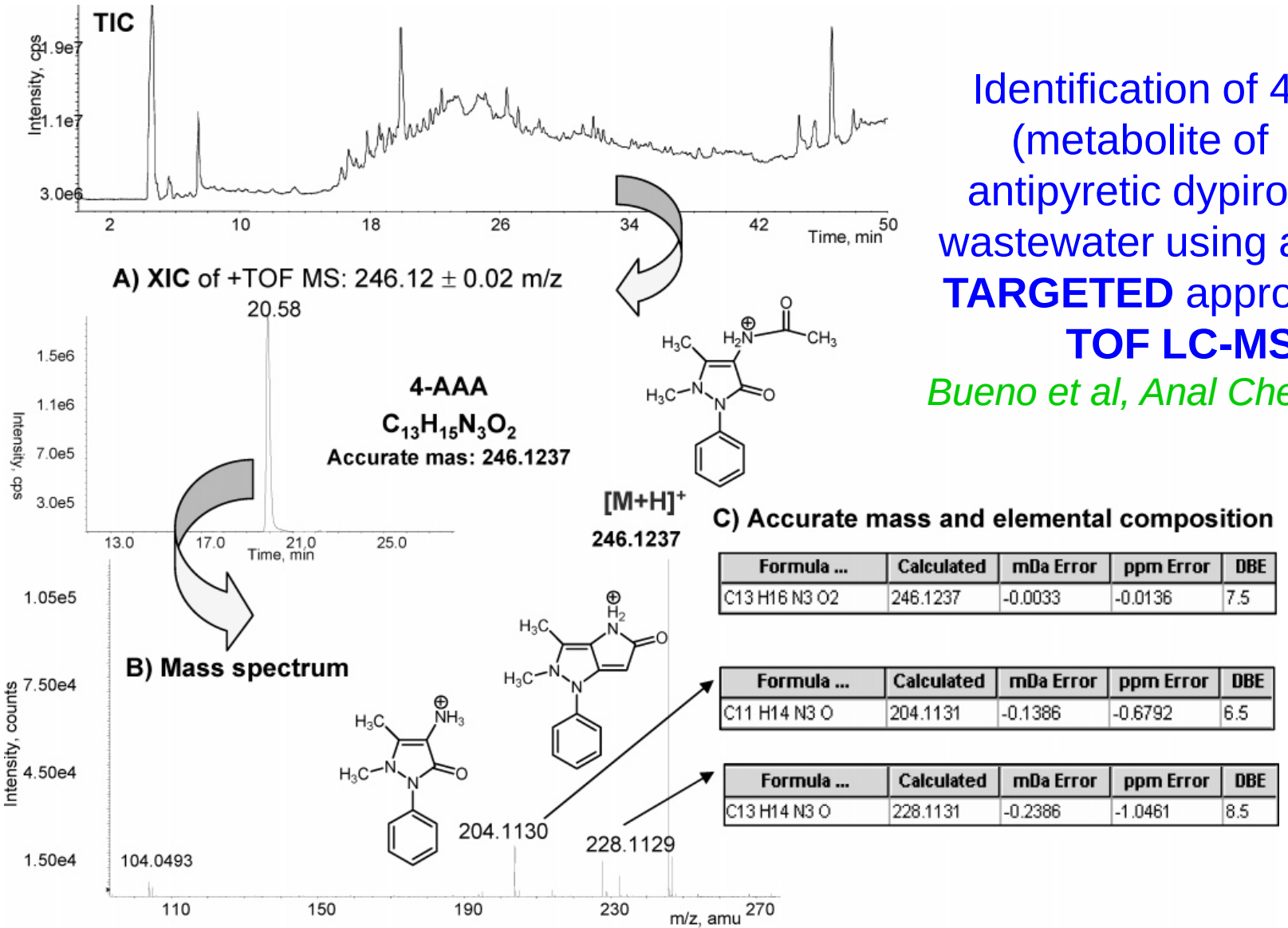
(Q)TOF Analysis of Environmental Pollutants

Determination of pharmaceuticals and related contaminants in wastewater using LC-TOF/MS

Bueno et al, Anal Chem, 2007

compound	positive samples	range (ng/L)	mean (ng/L)
paraxanthine	11	131–80875	15001
biphenylol	11	2504–18900	7662
4-aaa	14	2109–25030	7260
caffeine	15	262–24658	5753
ofloxacin	14	217–13426	4422
hydrochlorothiazide	17	872–14857	3683
4-FAA	14	40–10114	3386
ibuprofen	15	42–10639	2567
gemfibrozil	17	2–28571	2337
4-AA	11	131–9286	2098
naproxen	14	359–4200	1840
atenolol	16	275–4850	1720
ketorolac	14	13–4070	1289
chlorophene	8	14–2850	1279
nicotine	8	92–7822	1201
paroxethine	1	-	1141
4-MAA	15	9–9253	1051
furosemide	15	155–2957	1050
codeine	15	297–4826	1039

(Q)TOF Analysis of Environmental Pollutants



C) Accurate mass and elemental composition

Formula ...	Calculated	mDa Error	ppm Error	DBE
C13 H16 N3 O2	246.1237	-0.0033	-0.0136	7.5

Formula ...	Calculated	mDa Error	ppm Error	DBE
C11 H14 N3 O	204.1131	-0.1386	-0.6792	6.5

Formula ...	Calculated	mDa Error	ppm Error	DBE
C13 H14 N3 O	228.1131	-0.2386	-1.0461	8.5

Identification of 4-AAA (metabolite of the antipyretic dypirone) in wastewater using a **NON-TARGETED** approach by **TOF LC-MS**

Bueno et al, Anal Chem, 2007

Non-Targeted Analysis of Environmental Toxins in Biological Matrices

New frontiers in expanding our understanding of chemicals in pregnant women (T Woodruff, A Zota, R Gerona)

Rationale: No non-targeted analysis of environmental toxins in biological samples has been reported so far

Objective: To use unbiased interrogation methods to identify previously unmeasured environmental chemicals in the serum of pregnant women.

Methods: TOF LC-MS

Funding Source: Passport Foundation, Science Innovation Fund

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