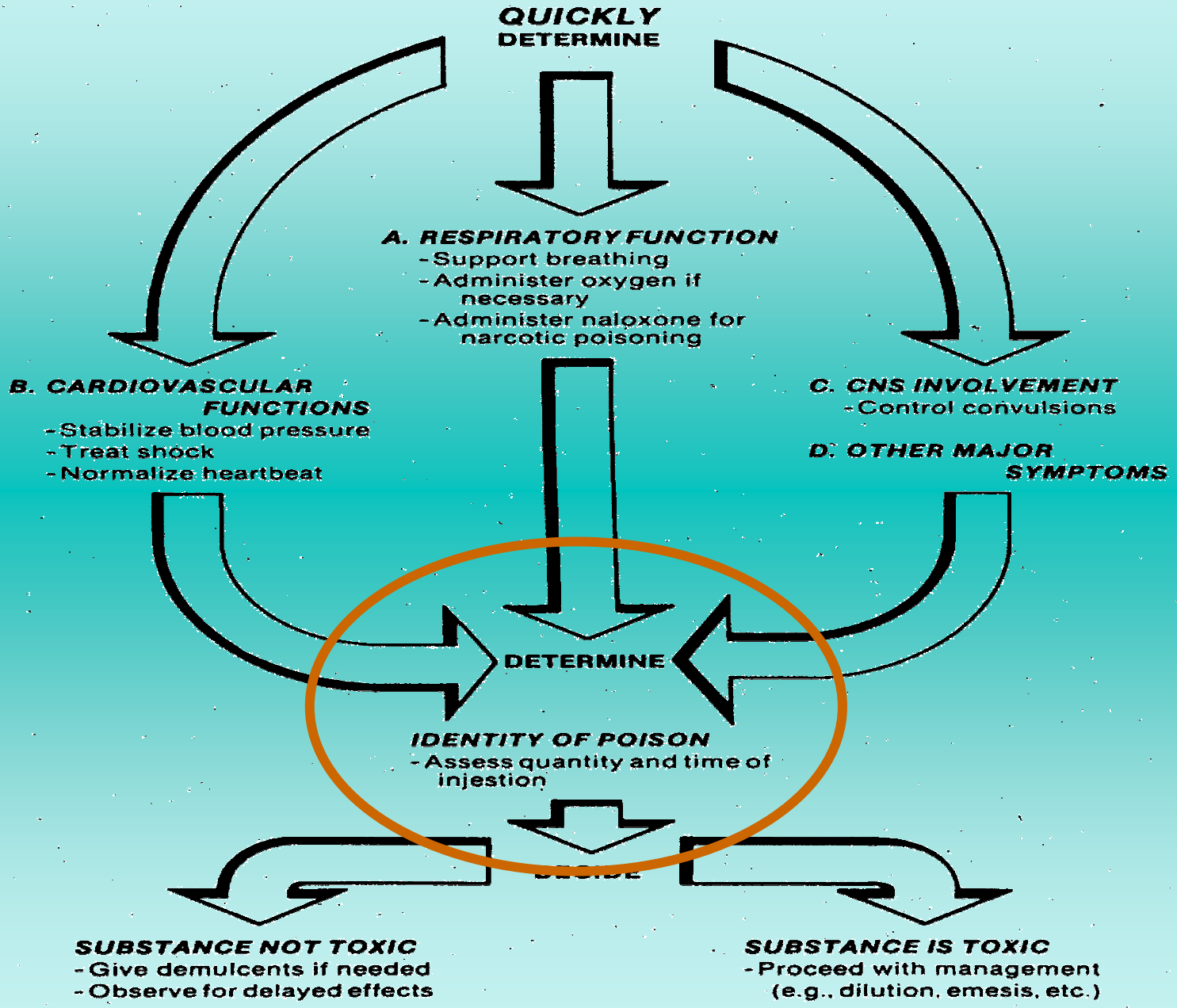
Toxikologische Analytik – Neue analytische Verfahren

Rainer Schmid

Sect. Biopharmaceutical & Toxicological Analysis
Inst. for Medical & Chemical Labdiagnostics (KIMCL)
General Hospital – Medical University of Vienna

PRINCIPLES IN MANAGEMENT OF THE POISONED PATIENT



Major causes of poisoning (in the United States)

Plants
Soaps,detergents,cleaners
Household disinfectants.
deodorizers
Household bleach
Perfume,cologne, toilet water
Cosmetic lotions, creams
Glues, adhesives
Paint
Insecticides (excluding mothballs)
Rodenticides
Corrosive acids, alkalies
Vitamins, minerals

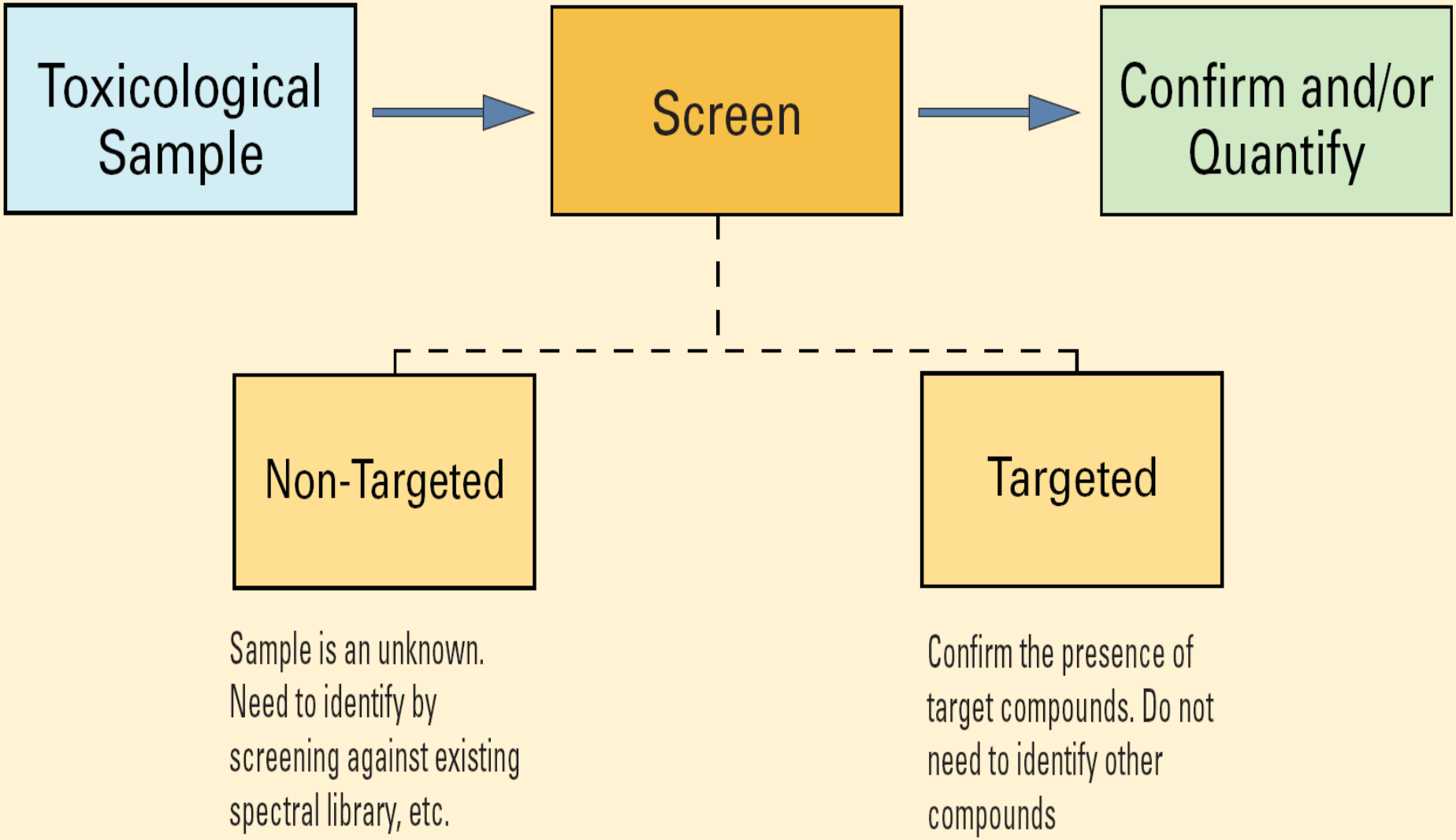
Miscellaneous internal and external medicines:

Aspirin
Miscellaneous analgesics
Antihistamines, cold medications
Antiseptic medications
Psychopharmacologic agents
Cough medicines
Hormones
infernal antibiotics

Anticipated Turn-around Times for a Full-Service Laboratory

	Anticipated Turnaround Time'
Quantitative Tests (Serum)	
Acetaminophen	**30 min - 45 min**
Carbamazepine	**45 min - 1 hr**
Cholinesterase	**1 hr - 2 hr**
Cyclosporine	**1 hr - 1.5 hr**
Digoxin	**1 hr - 1.5 hr**
Ethanol	**15 min - 30 min**
Ethylene Glycol	**1 hr - 2 hr**
Isopropanol	**20 min - 45 min**
Lidocaine	**45 min - 1 hr**
MEGX	**45 min - 1 hr**
Methanol	**20 min - 45 min**
Procainamide & NAPA	**1 hr - 2 hr**
Phenobarbital	**45 min - 1 hr**
Phenytoin	**45 min - 1 hr**
Quinidine	**45 min - 1 hr**
Salicylates	**30 min - 45 min**
Theophylline	**45 min - 1 hr**
Valproic Acid	**45 min - 1 hr**
Qualitative Tests (Urine)	
Comp Drug Screen3	**2hr -4hr**
Stimuilant Panel	**1 hr - 2.5 hr**

Typical Screening Workflow



Relative Specificity, Sensitivity, and Speed of Processing of Different Laboratory Techniques

Laboratory Technique	*Specificitv*	*Sensitivitv*	*Speed*
Spectrophotometry	+	++	+++
Immunology	+(+)	++	++++
Thin-layer (TLC) Chromatography	++	++	++
High-performance liquid (HPLC) Chromatography	+++	++	+ - +++
Gas-liquid (GLC) Chromatography	+++	++	+
Gas chromatography-mass-spectrometry	++++	++++	+
Liquid chromatography-mass-spectrometry	++++	++++	+++

Causes of false immunological screening...

- ***Screen test does not seek the drug***
  structural dissimilarity from drug class prototype e.g. fentanyl
- ***too broad antibody specificity***
  - group test e.g. benzodiazepines
- **cross reactivity with *other compound of different class***
- ***Toxicokinetic characteristics***
  large volume of distribution
  Short elimination half-life

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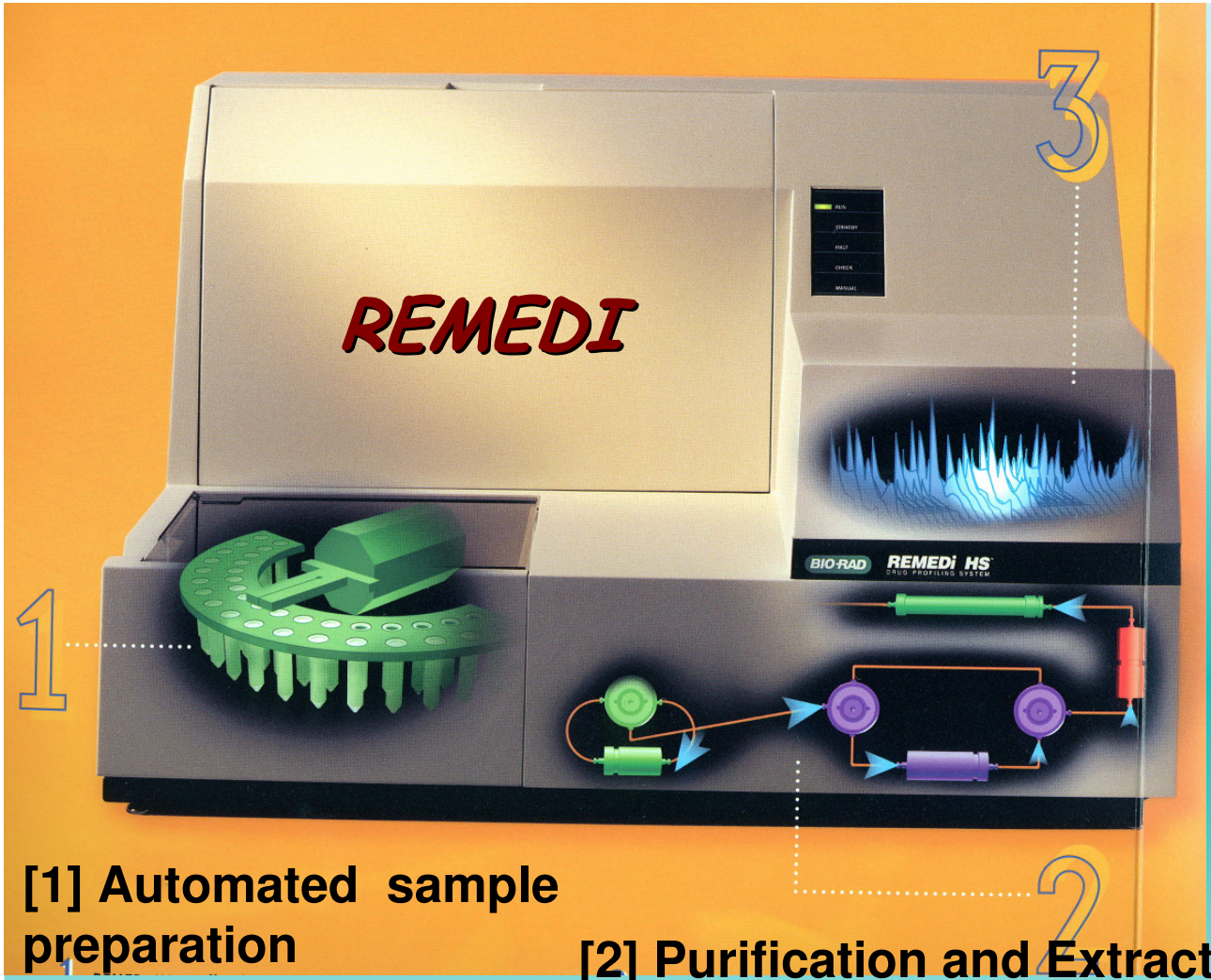


[1] Automated sample preparation

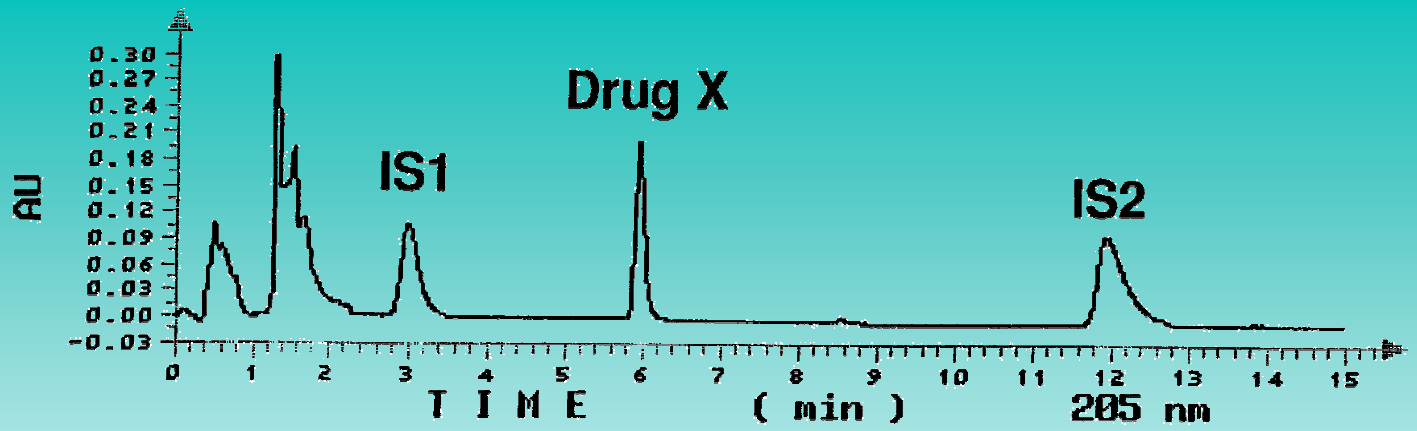
[2] Purification and Extraction Cartridges remove impurities on-line, Separation Cartridges 1and 2 separate drugs of diverse classes into discrete peaks.

[3] Scanning UV detector, resulting in a unique spectral fingerprint for each unknown peak.

[4] "Expert System" comparing retention time data and five distinct spectral characteristics or each unknown peak to an extensive library of drugs and metabolites.

REMEDI: Bestimmung der relativen Retentionszeit

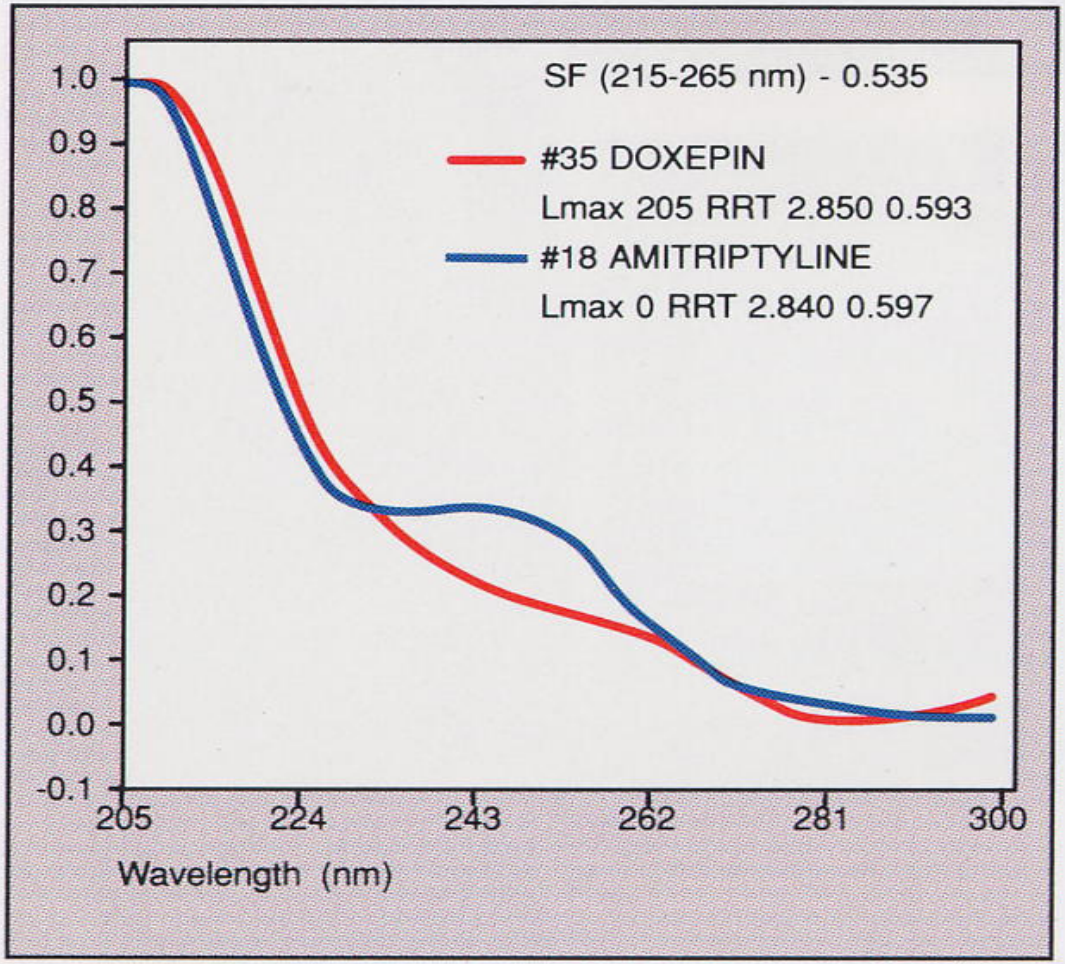
RRT 1: RT of a peak relative to the first internal standard.
RRT 2: RT of a peak relative to the second internal standard.



$$\frac{RT_x - dt}{RT_{I.S.} - dt}$$

$RRT1_x \approx 2.0$

$RRT2_x \approx 0.5$



Drug spectra are compared to a known library of candidates stored in memory.

Derzeit ist eine Spektrenbibliothek von ca. 1000 pharmazeutischen Verbindungen verfuegbar

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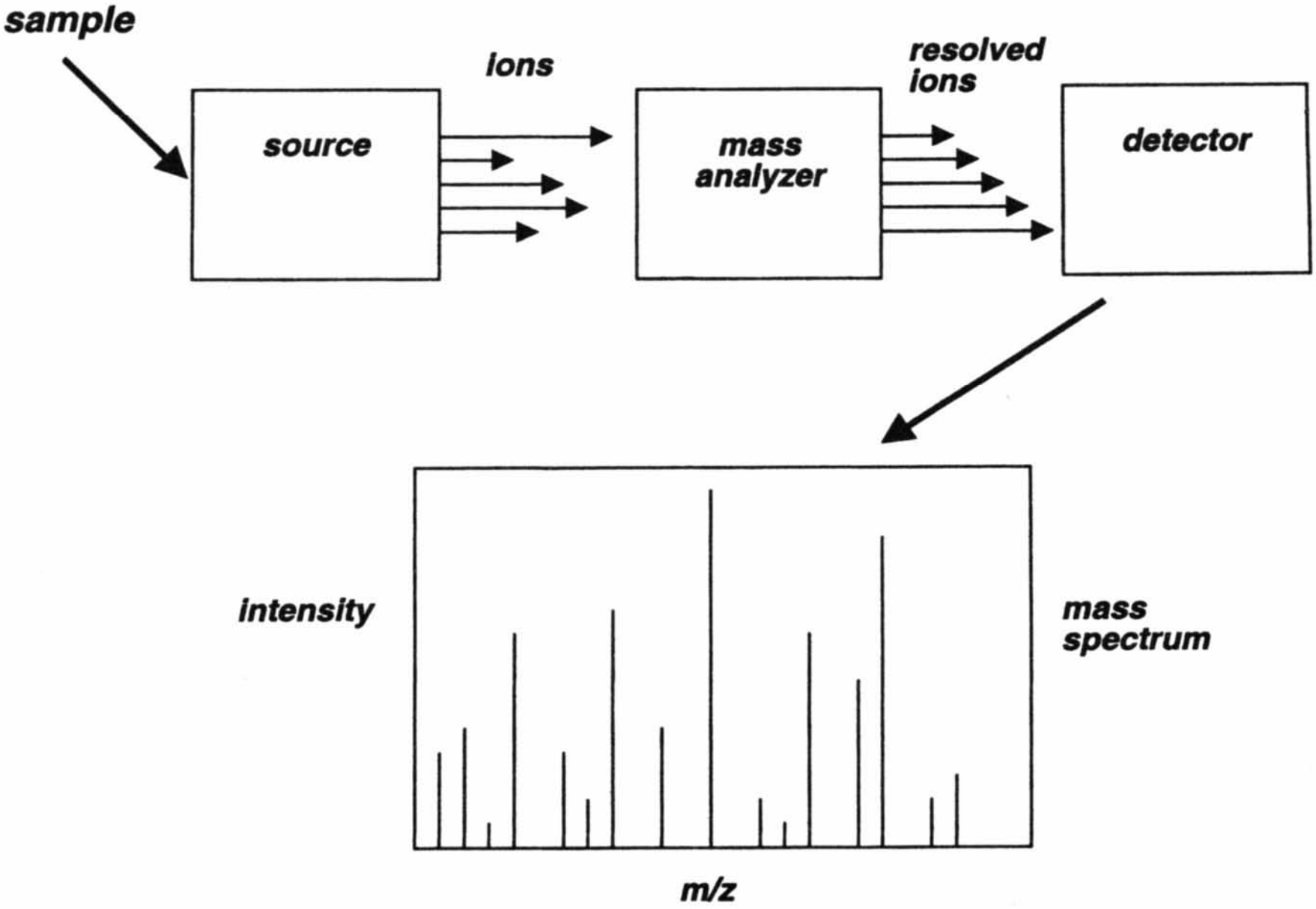
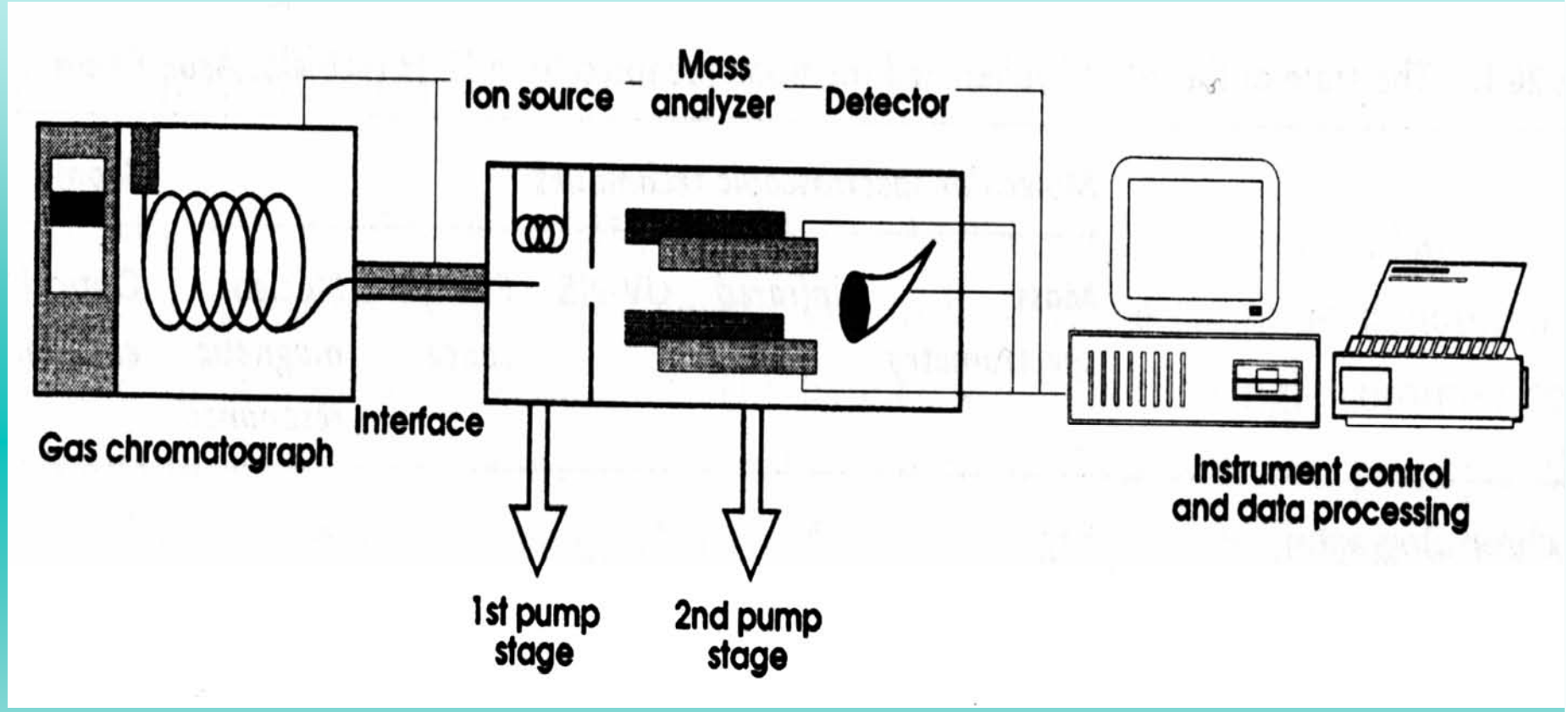
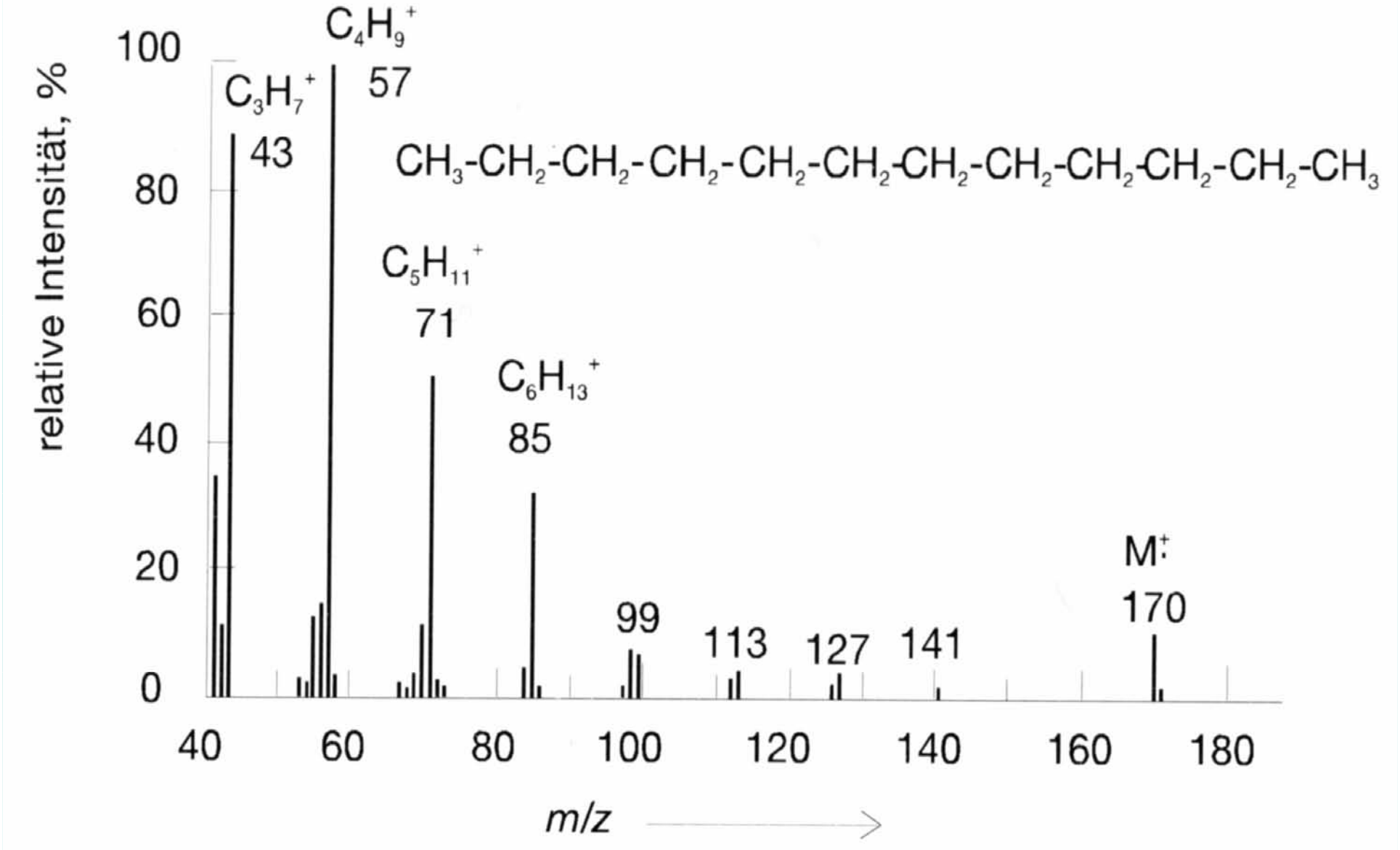
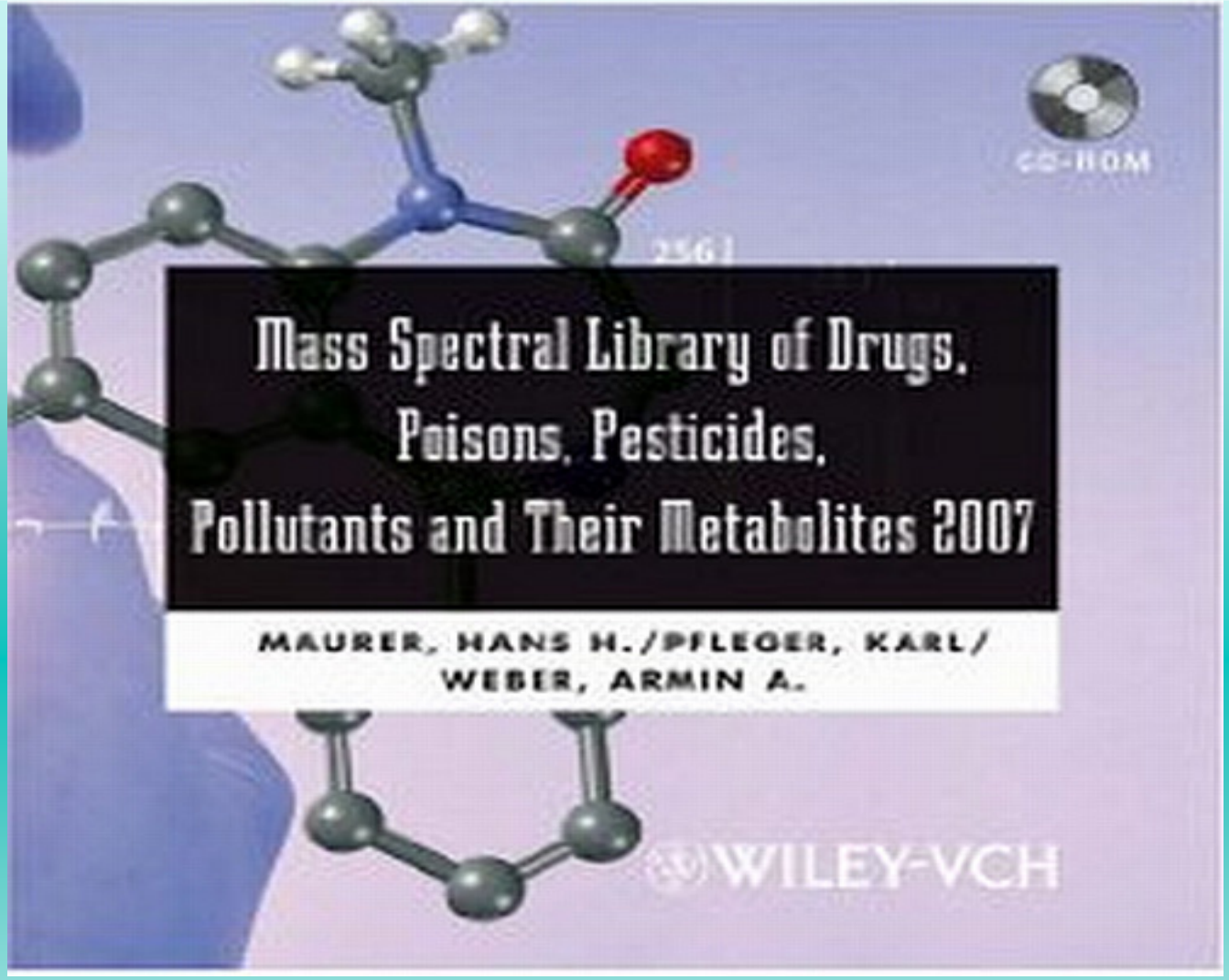
Liquid chromatography-mass-spectrometry	++++	++++	+++



The principle of mass spectrometric analysis


Ion source
Mass analyzer
Detector
Gas chromatograph
Interface
1st pump stage
2nd pump stage
Instrument control and data processing


relative Intensität, %
100
80
60
40
20
0
$C_4H_9^+$
57
$C_3H_7^+$
43
$C_5H_{11}^+$
71
$C_6H_{13}^+$
85
99
113
127
141
$M^{+\cdot}$
170
CH_3-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_3
40
60
80
100
120
140
160
180
m/z



Contains 7840 data sets. Over 3300 data sets are from ***metabolites***, over 2300 from ***acetylated***, over 1000 from ***methylated***, over 700 from ***trimethylsilylated***, over 400 from ***trifluoroacetylated***, over 200 each from ***pentafluoropropionylated*** or ***heptafluorobutyrylated compounds***.

Systematic evaluation of 1-chlorobutane for liquid-liquid extraction of drugs

U. Demme[1*], J. Becker[2], H. Bussemas[3], T. Daldrup[4], F. Erdmann[5], M. Erkens[6], P.X. Iten[7], H. Käferstein[8], K.J. Lusthof[9], H.J. Magerl[10], L.v. Meyer[11], A. Reiter[12], G. Rochholz[13], A. Schmoldt[14], E. Schneider[15], H.W. Schütz[13], T. Stimpfl[16], F. Tarbah[17], J. Teske[18], W. Vycudilik[16], J.P. Weller[18], W. Weinmann[19]

*On behalf of the "Workgroup Extraction" of the GTFCh (Society of Toxicological and Forensic Chemistry, Germany)

Institutes of Forensic Medicine of [1]Jena, [2]Mainz, [4]Duesseldorf, [5]Giessen, [7]Zuerich (CH), [8]Cologne, [10]Wuerzburg, [11]Munich, [12]Luebeck, [13]Kiel, [14]Hamburg, [16]Vienna (A), [18]Hannover, [19]Freiburg and [3]Praxis Labormedizin Dortmund, [5]Clin.-Chem. Central Laboratory, Aachen, [9]Nat. For. Inst., Den Haag (NL), [15]LKA Baden-Wuerttemberg, Stuttgart [17]Dubai Police Dept.

N°	drug	Y°	Ref.
24	Acebutolol	0.05	
25	Adenosin	0	
26	Ajmaline	0.5	
27	Alfentanil	1	
28	Alimemazine	1	
29	Alprazolam	0.95	[43-45]
30	Alprenolol	1	
31	Amantadine	0.5	
32	Amfebutamon	1	
33	Amfepramon	1	
34	Amfetaminil	1	
35	Amiodarone	0.95	
36	Amisulprid	0.6	
37	Amitriptyline	1	[46]
38	Amitriptyline-Oxid	0.1	
39	Amlodipine	1	
40	Amphetamine	0.5	[47-55]
41	Apomorphin	0.8	
42	Aprindin	0.95	
43	Articain	1	
44	Atenolol	0	
45	Atropine	0.6	
46	Azathioprine	0	
47	Azinphos-ethyl	1	
48	Azinphos-methyl	1	
49	Benperidol	1	
50	Benserazid	0	
51	Benzatropin	1	
52	Benzoylecgonine	0	
53	Betaxolol	1	
54	Biperidene	1	
55	Bisacodyl	0.7	
56	Bisoprolol	0.9	
57	Bromazepam	0.9	
58	Bromocriptin	1	
59	Bromophos-ethyl	1	

(cont. : see www.gtfch.org/chlorobutaneextraction.pdf)

Yo is the extraction yield in the organic phase

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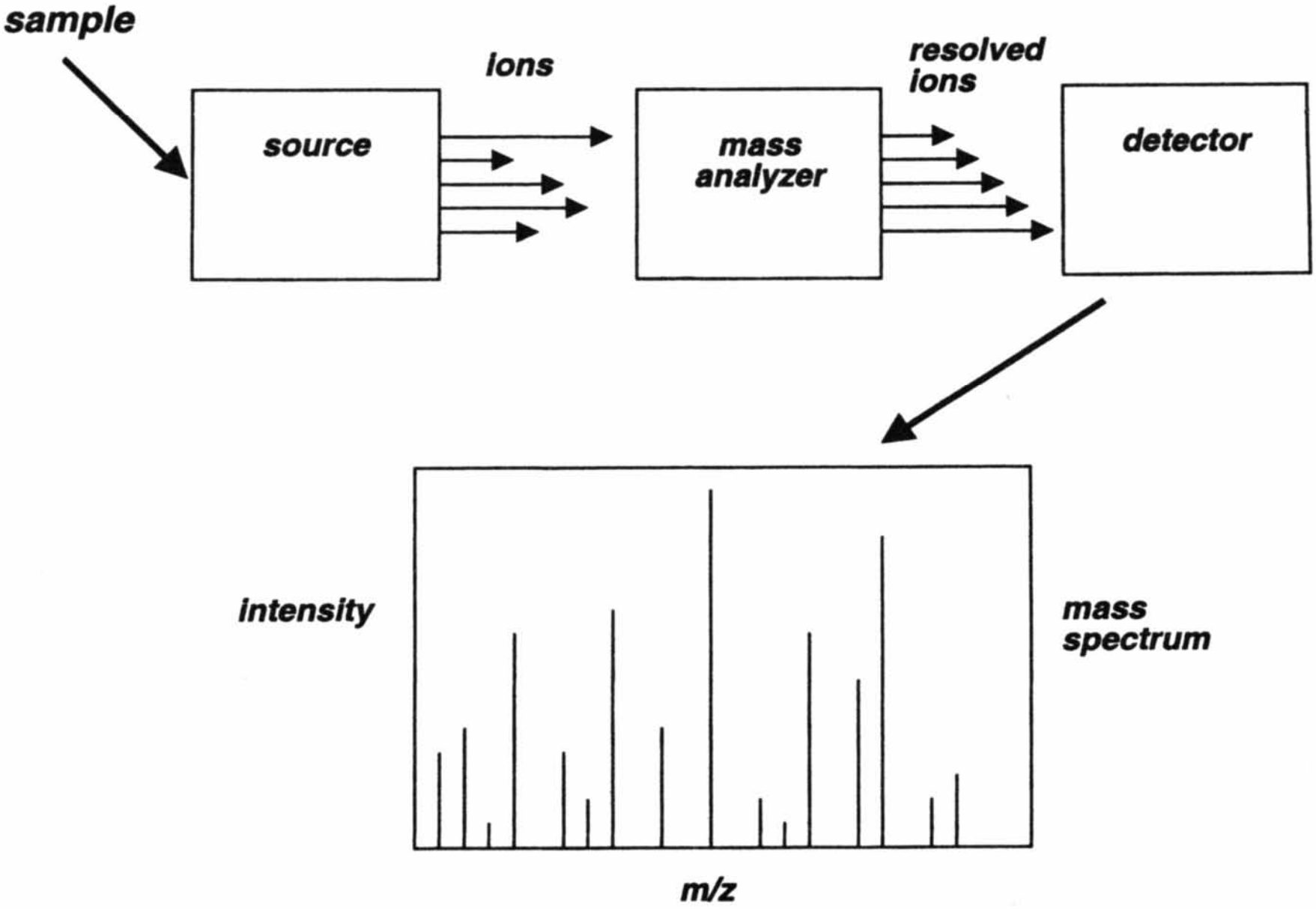
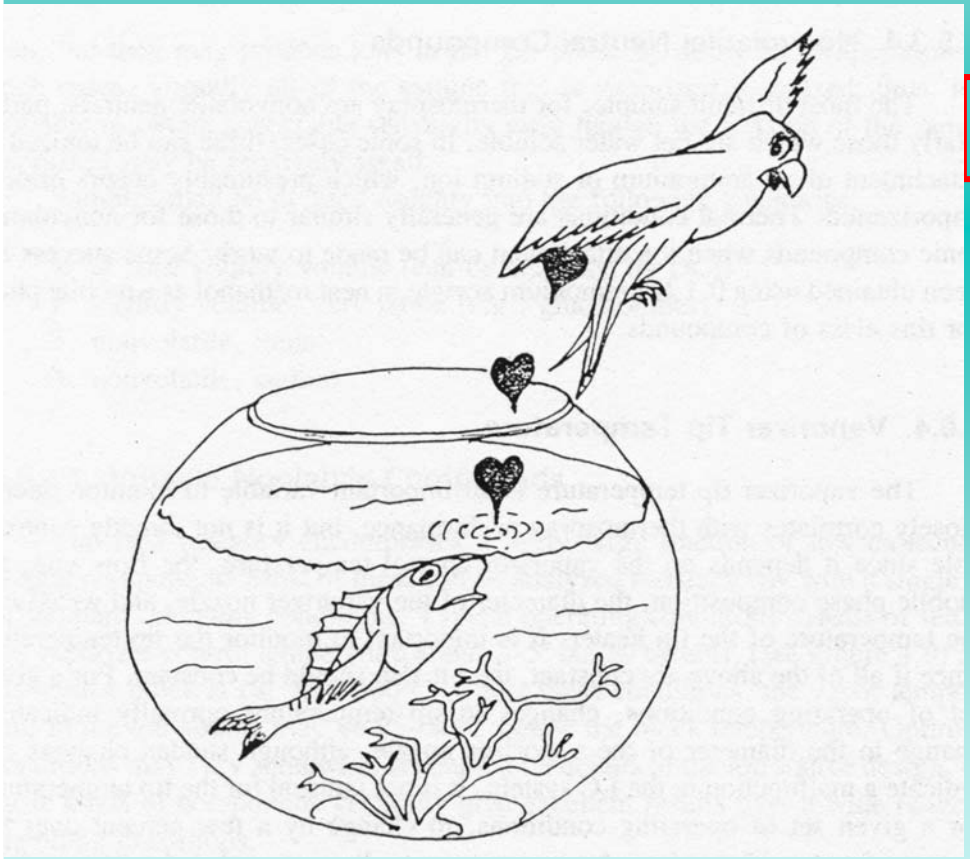


The principle of mass spectrometric analysis

Liquid-chomatography – Mass Spectrometry

– a difficult marriage!!



HPLC = Liquid at 1 atm

↓

???

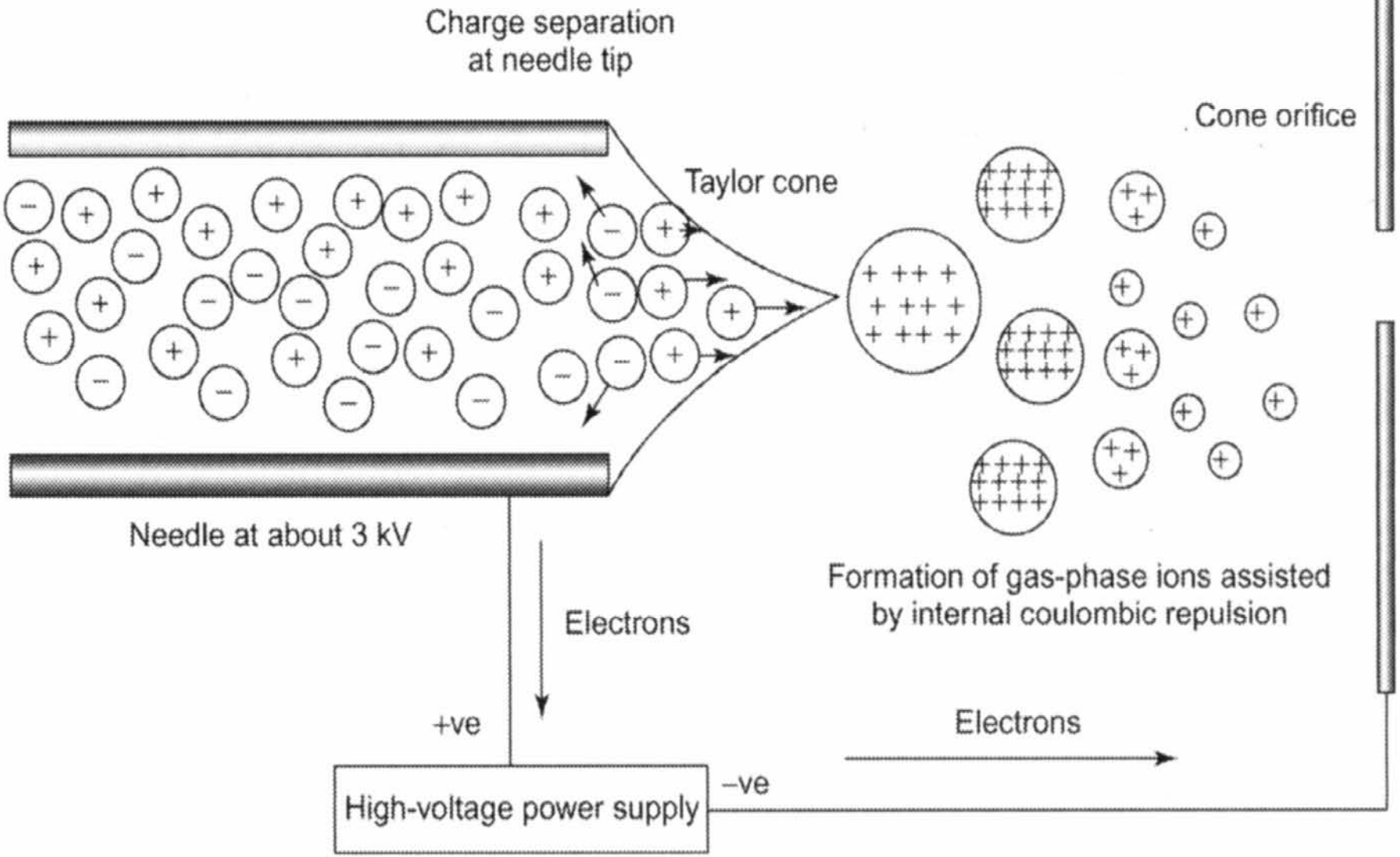
MS = Gas at 10^{-6} atm

Electrostatic spraying of water in the 18th century.

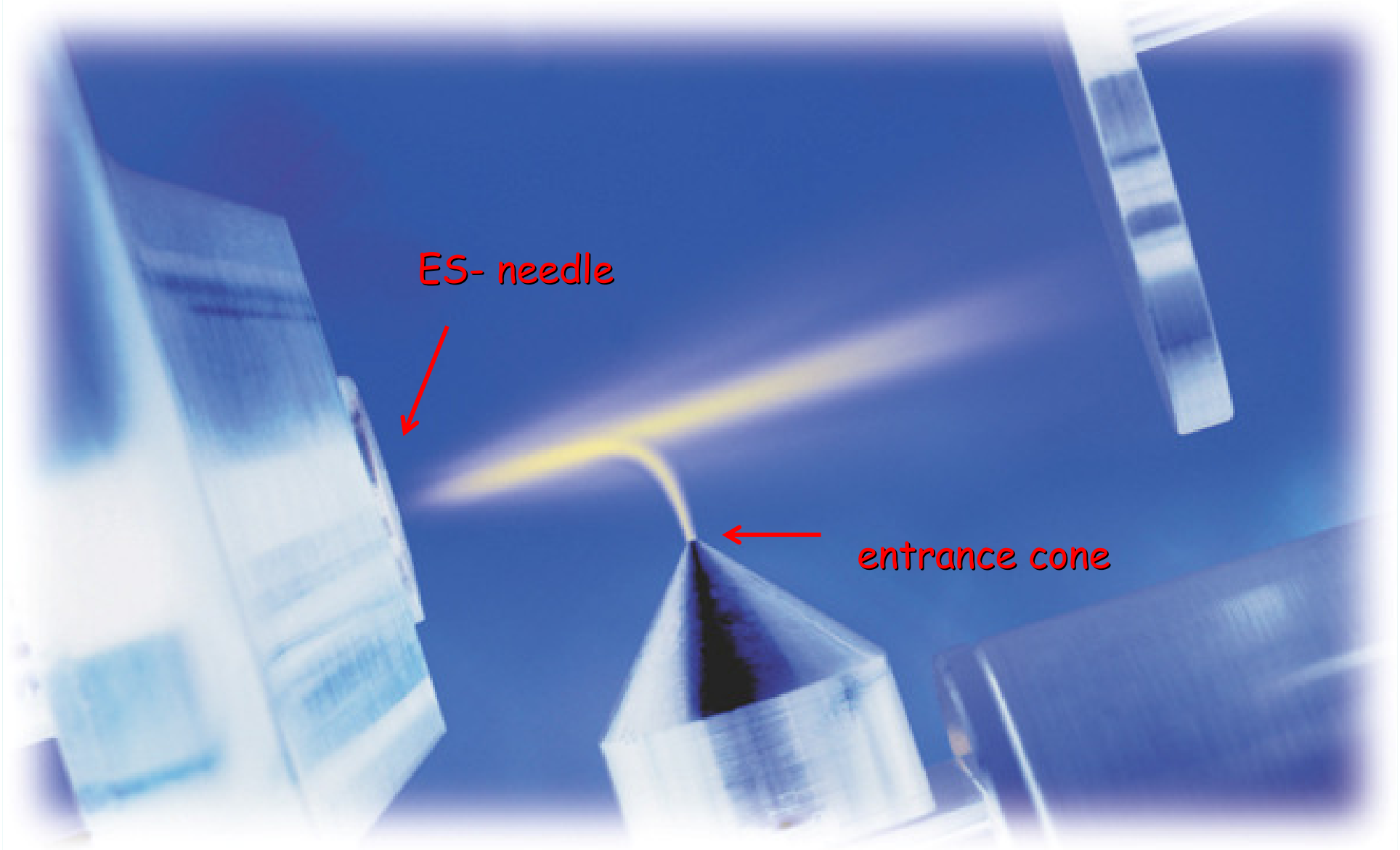
Not a new technique…

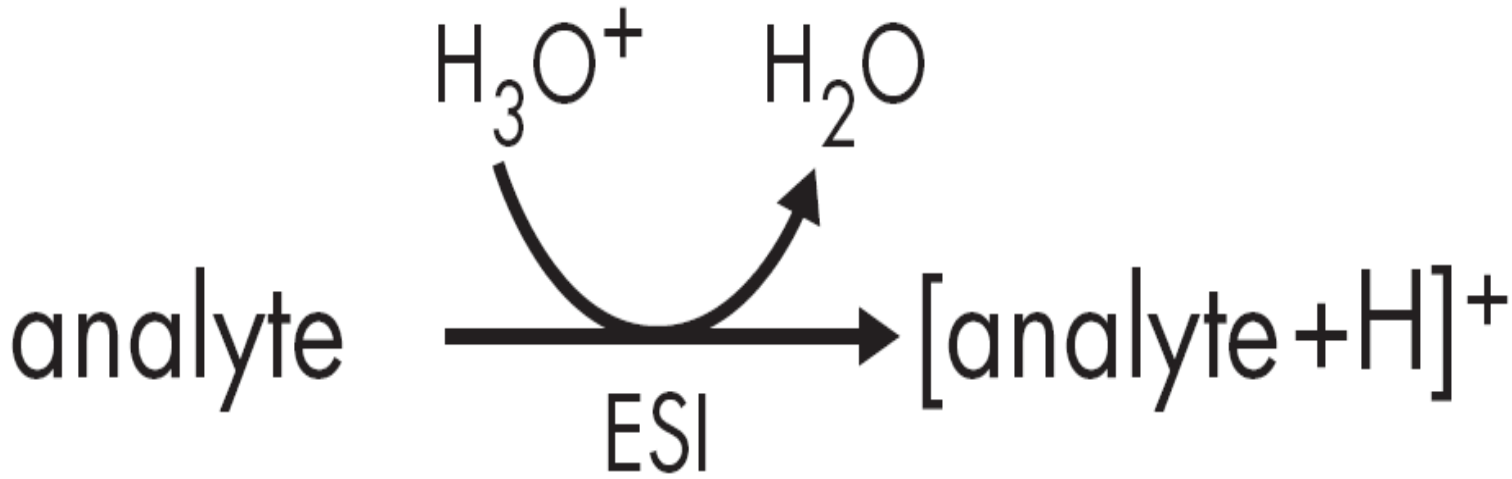
First ionization with an 'ESI' was done more than 200 years ago…

The electrospray process (ESI)



Electro-Spray



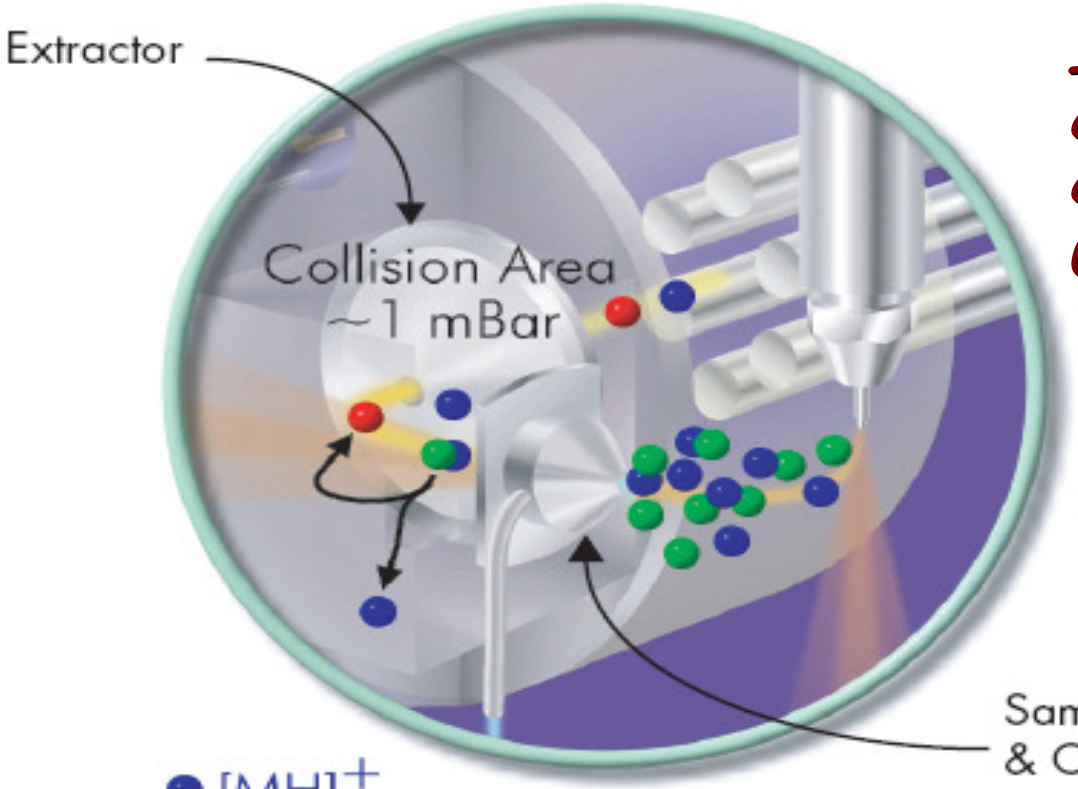


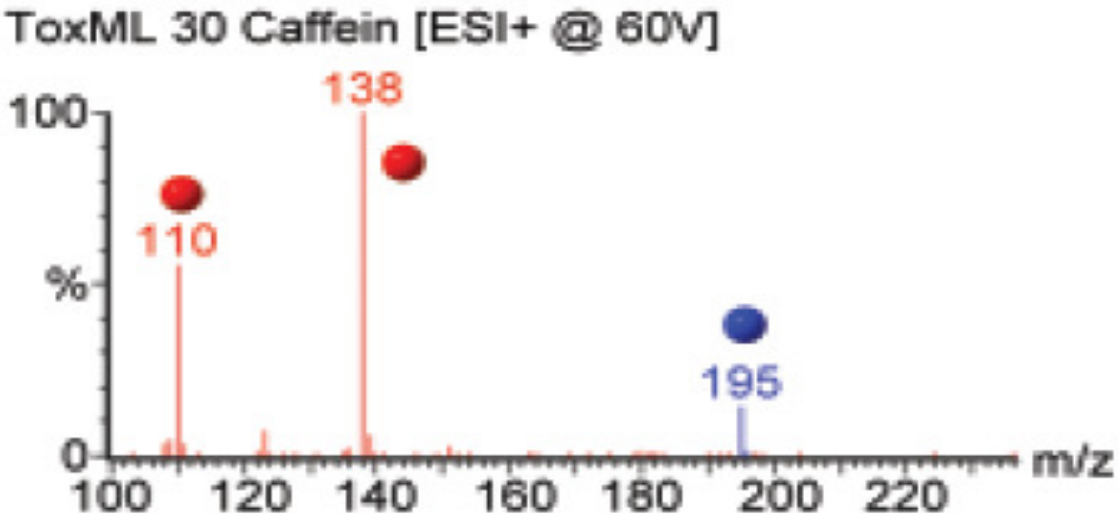
Atmospheric Pressure Ionisation (API) process

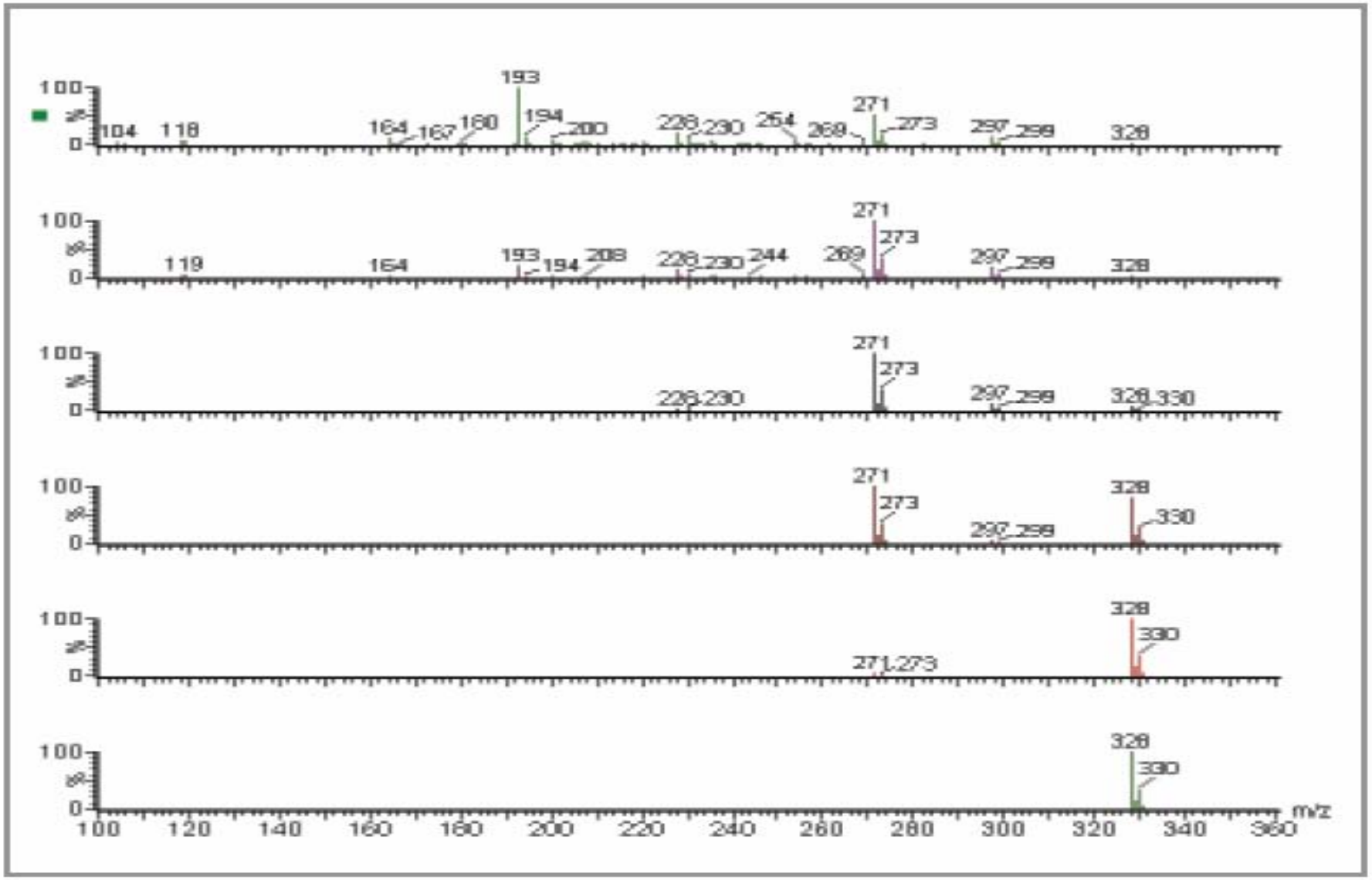
This ***soft ionisation process*** leads to cations in positive ion mode and anions in negative ion mode which are generally stable.

The molecular ions can be fragmented in the ***source region*** of LC/MS instruments

InSource-CID fragmentation of the moleculer ion (m/z 195) of caffeine at 60V in the (Quattro micro) API ion source

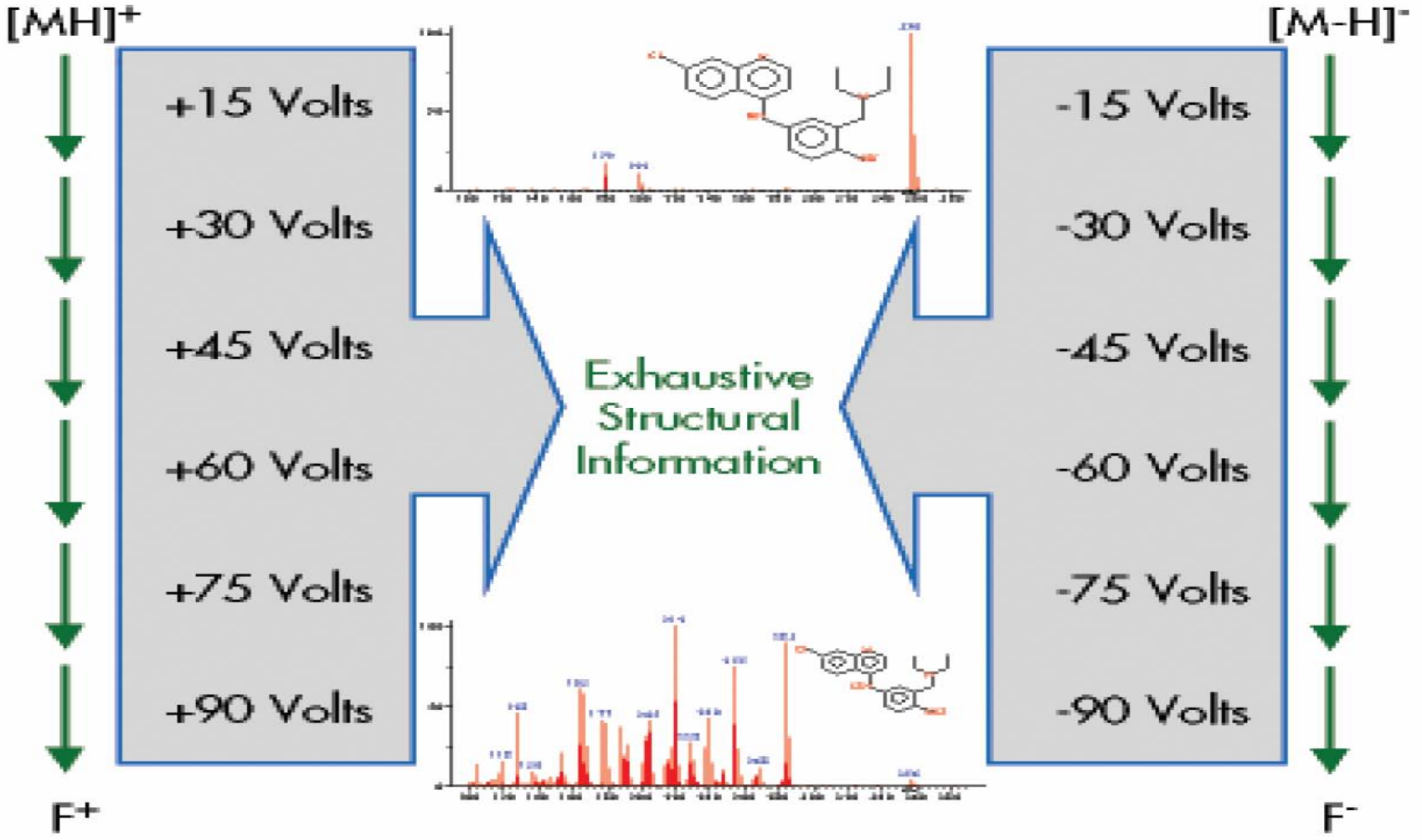




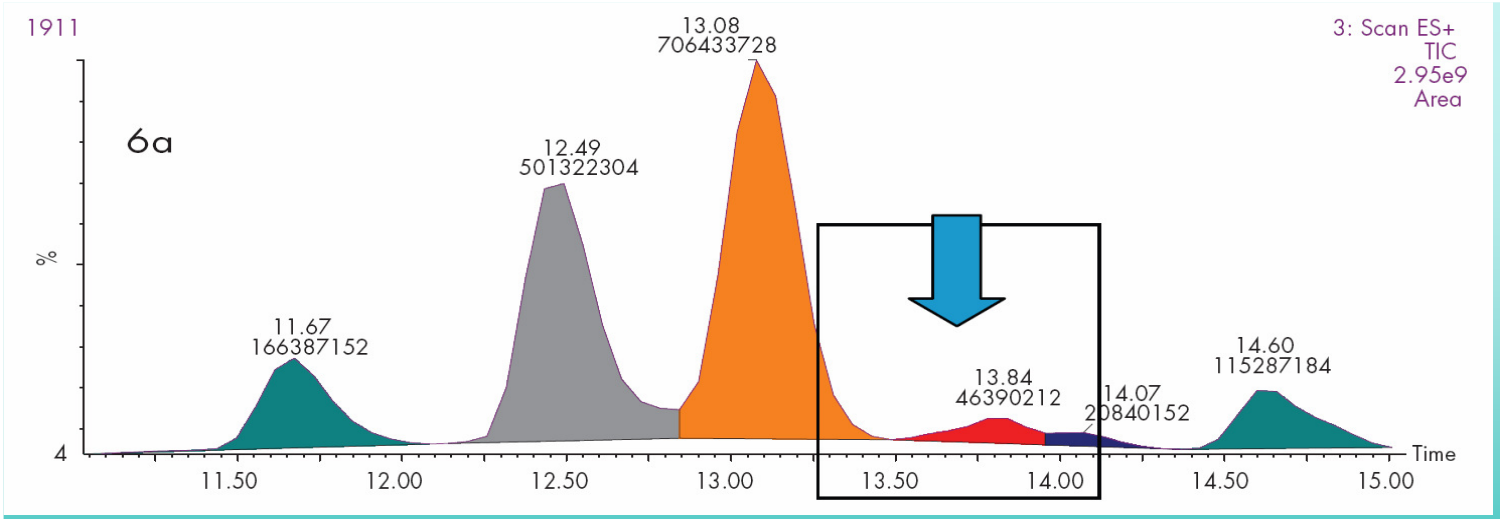


Mass spectra of Loxapine, recorded at ***6 different CV values*** using in-source CID.

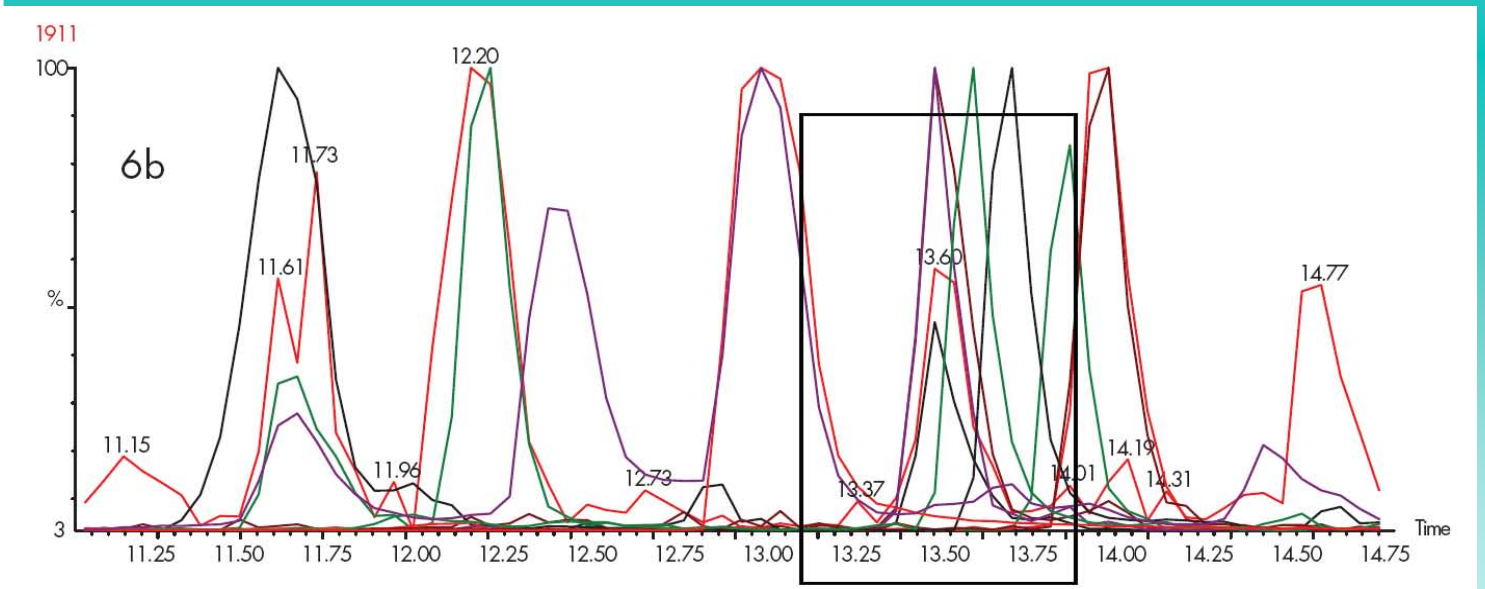
The ***degree of fragmentation*** increases with the cone voltage



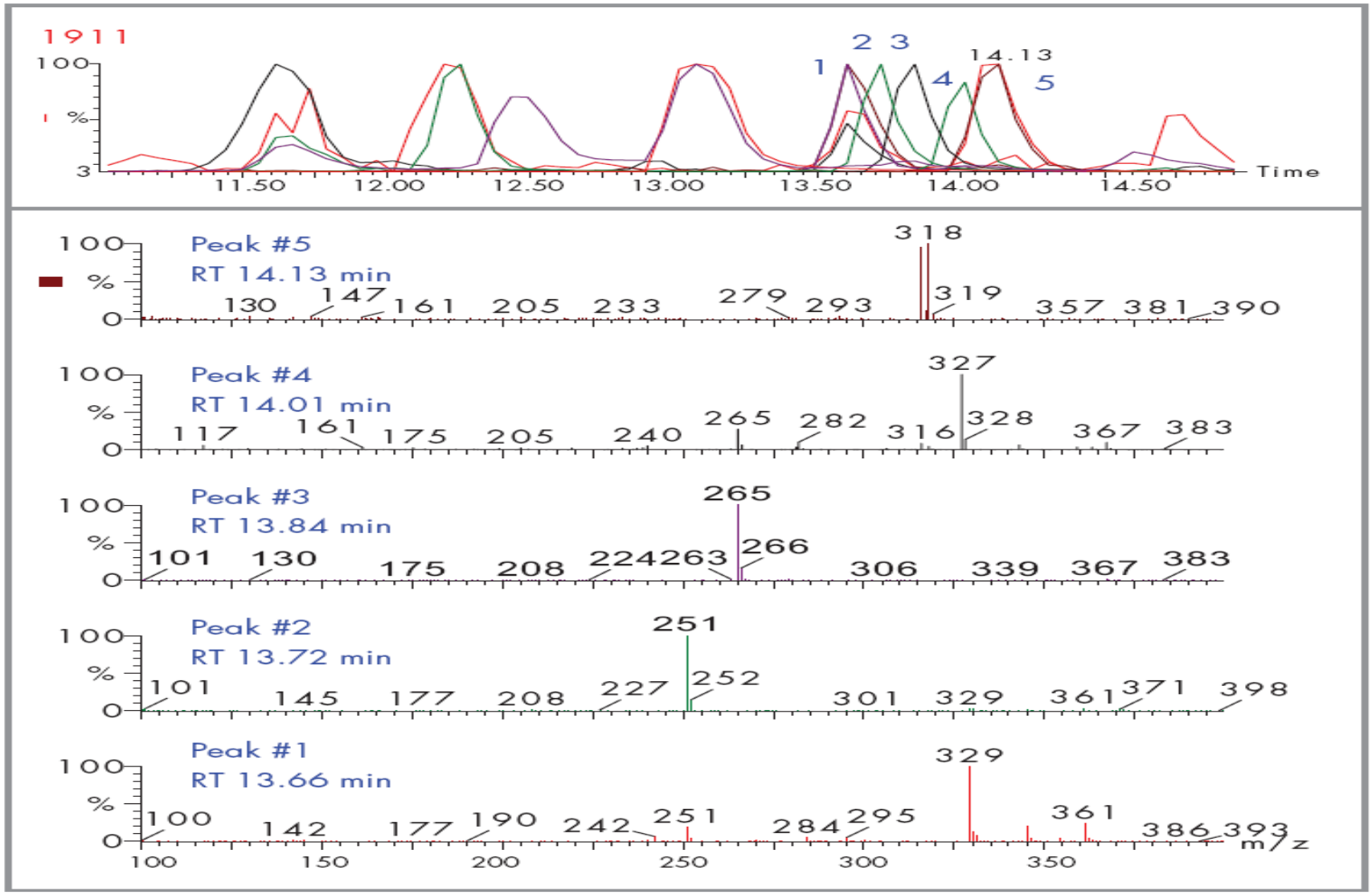
Structural information is stored for each component in the library, when mass spectra are ***stored at every significant cone voltage*** in both, positive and negative ion mode.



Section of chromatogram area acquired in positive ESI @ 30 V.
Here the total ion chromatogram (TIC) indicates that only one component elutes at 13.8 min



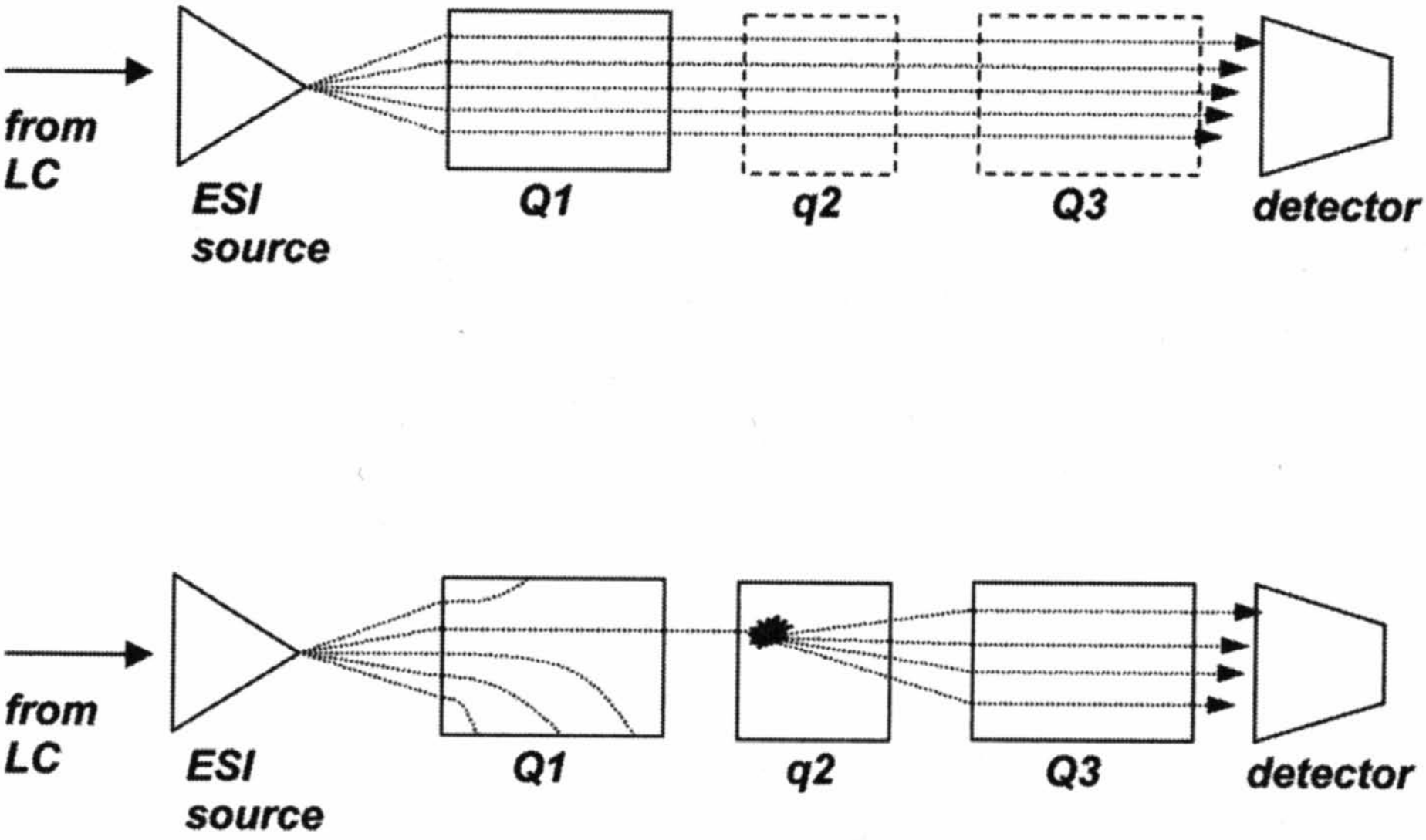
Section of chromatogram area for function 3 acquired in positive ESI @ 30 V. Using extracted ion chromatograms shows that at least three components elute between 13.5 and 13.8 minutes.

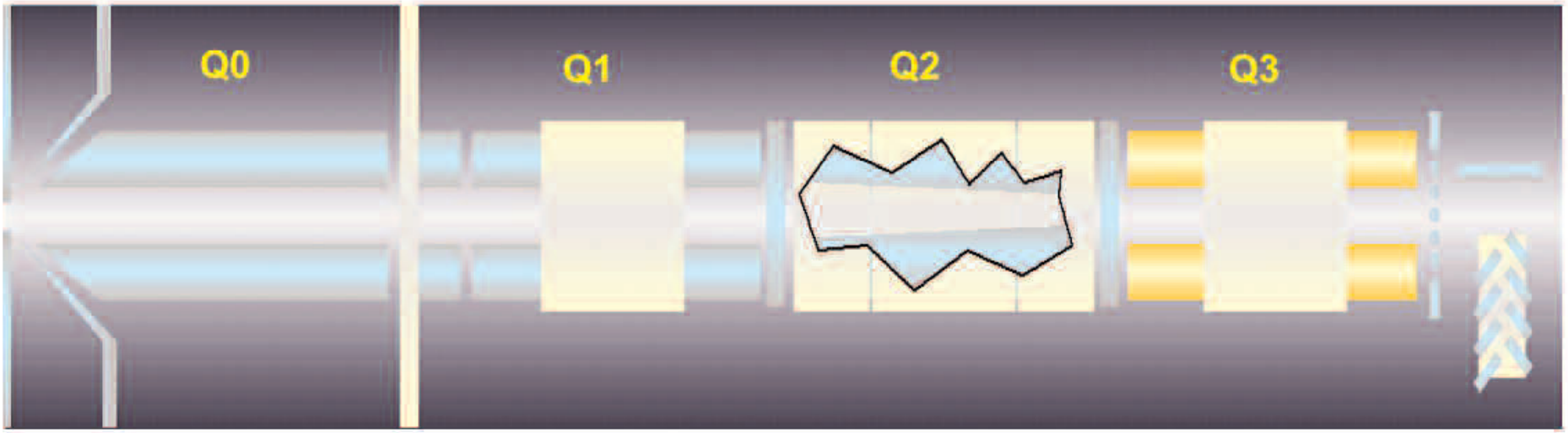


Chromatogram acquired in positive ESI @ 30 V and corresponding mass spectra of 5 components. Automated spectral deconvolution allows extraction of clean mass spectra that can be used for library searching.

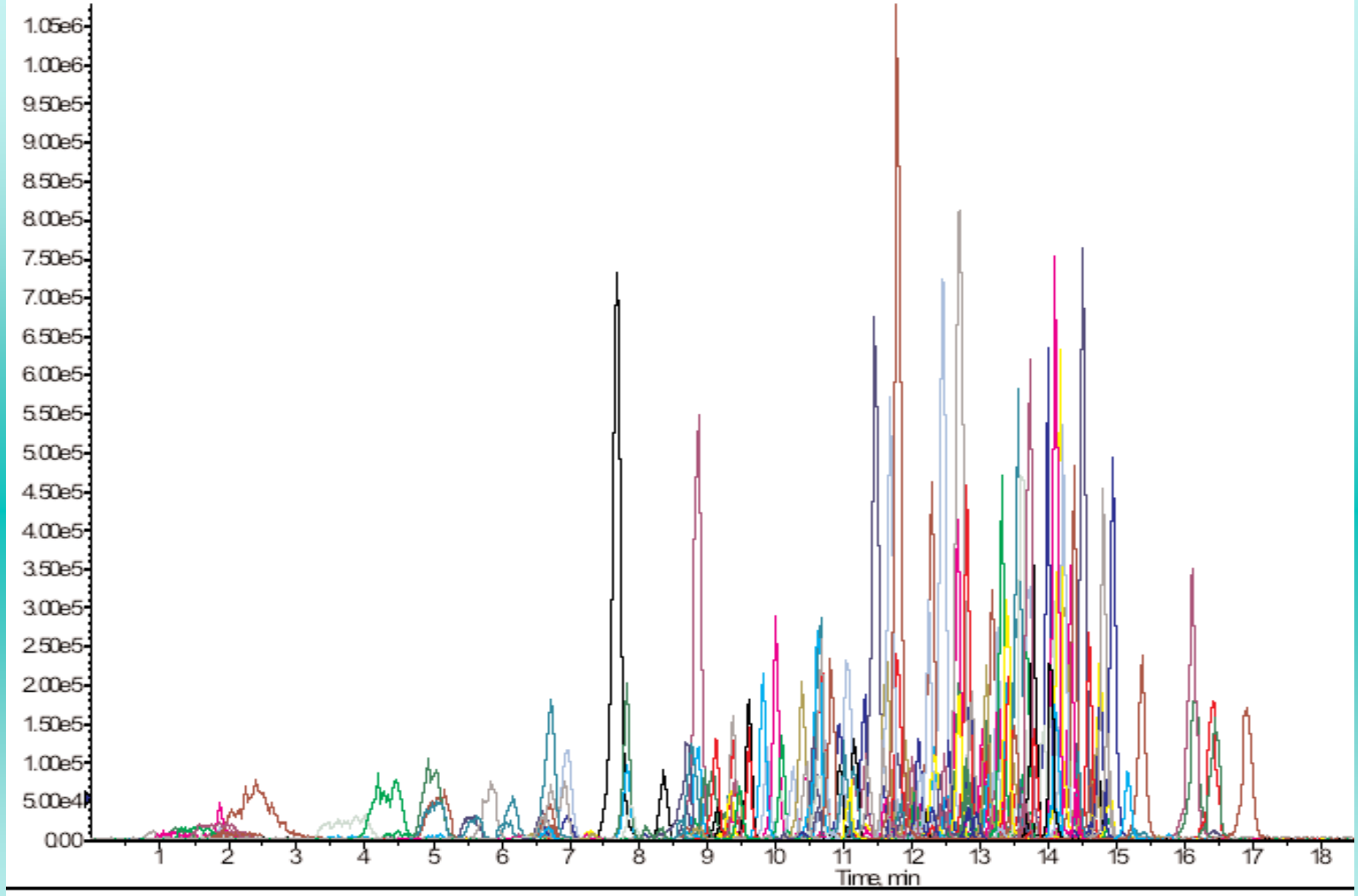
				Candidate Average Fit (%)
#	Analyte Name	Status	Origin	6 Functions
1	Nicotine	Unexpected molecule	Smoker / Contamination	56.1
2	Trimetazidine	Expected molecule	Medication	63.3
3	Acetaminophen	Expected molecule	Medication	62.3
4	Caffeine	Expected molecule	Medication	74.0
5	Quinine	Expected molecule	Medication	70.3
6	Zolpidem	Expected molecule	Medication	94.7
7	Meprobamate	Unexpected molecule	Unknown	55.3
8	Mianserin	Expected molecule	Medication	67.1
9	Acepromazine	Unexpected molecule	Unknown	57.6
10	Bromazepam	Unexpected molecule	Unknown	53.1
11	Hydroxyzine	Expected molecule	Medication	88.2
12	Propoxyphene	Expected molecule	Medication	62.1
13	Tramadol	Expected molecule	Medication	Not found

Funktion eines Triple-Quad Mass Spectrometer

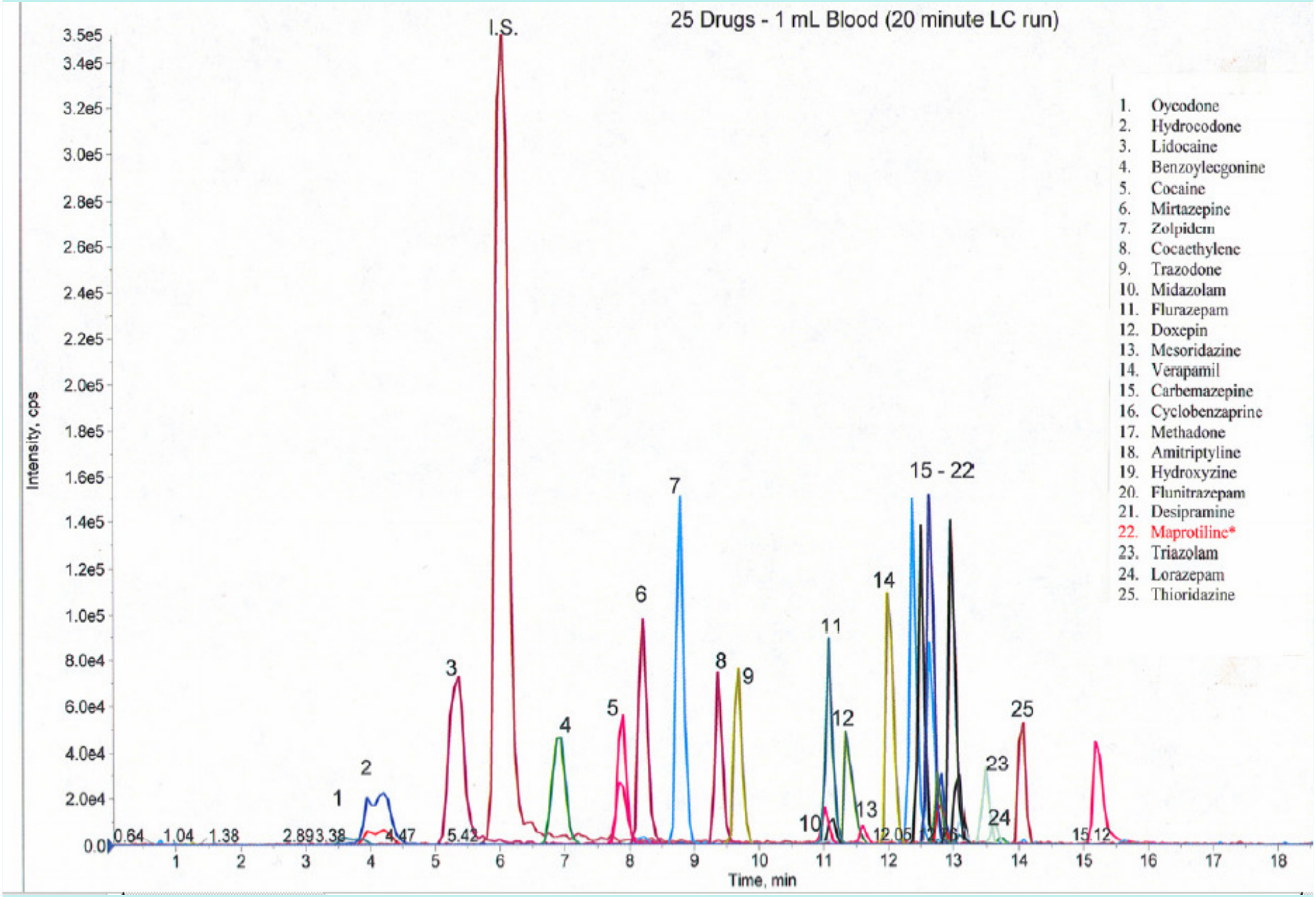


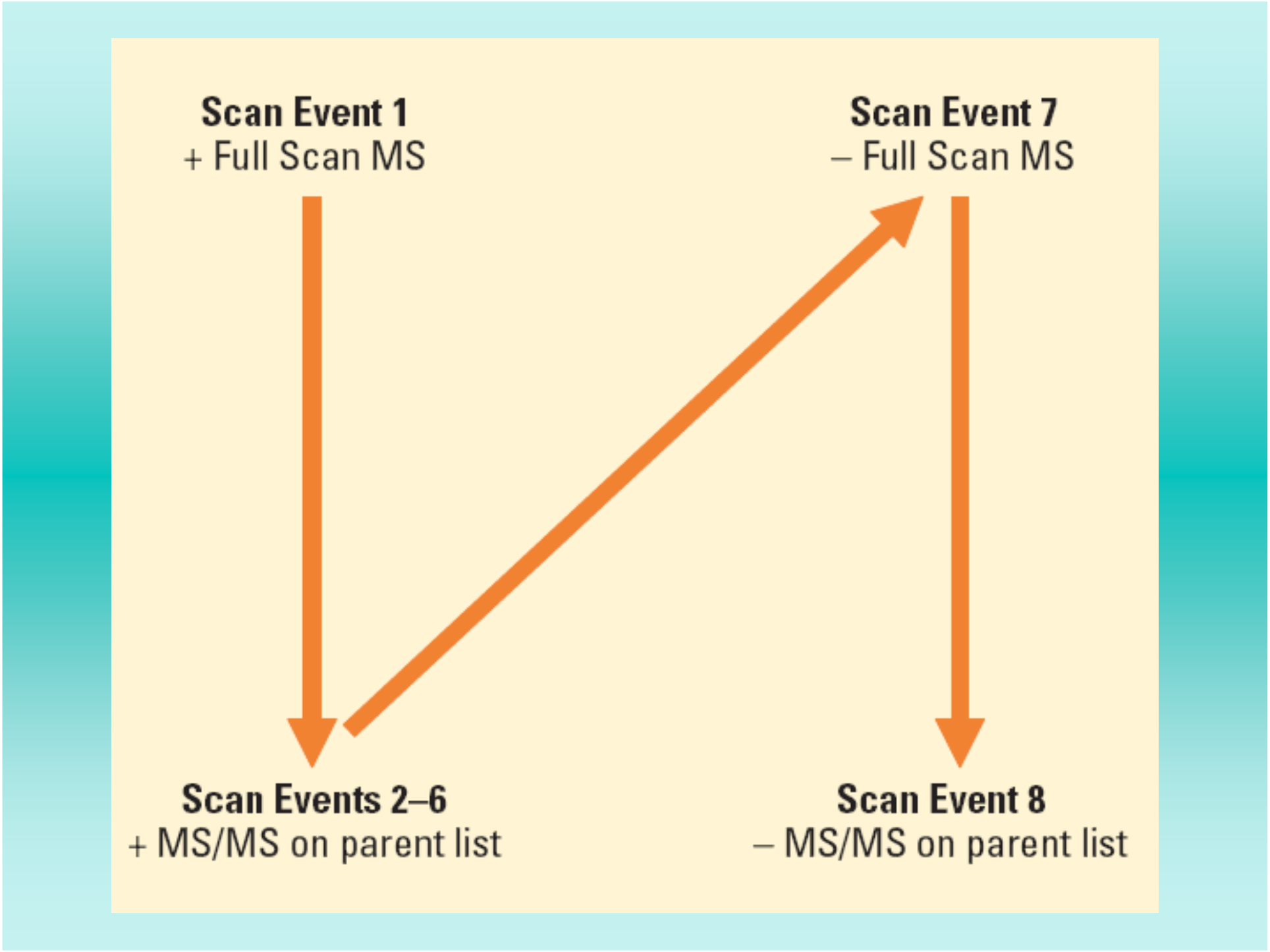


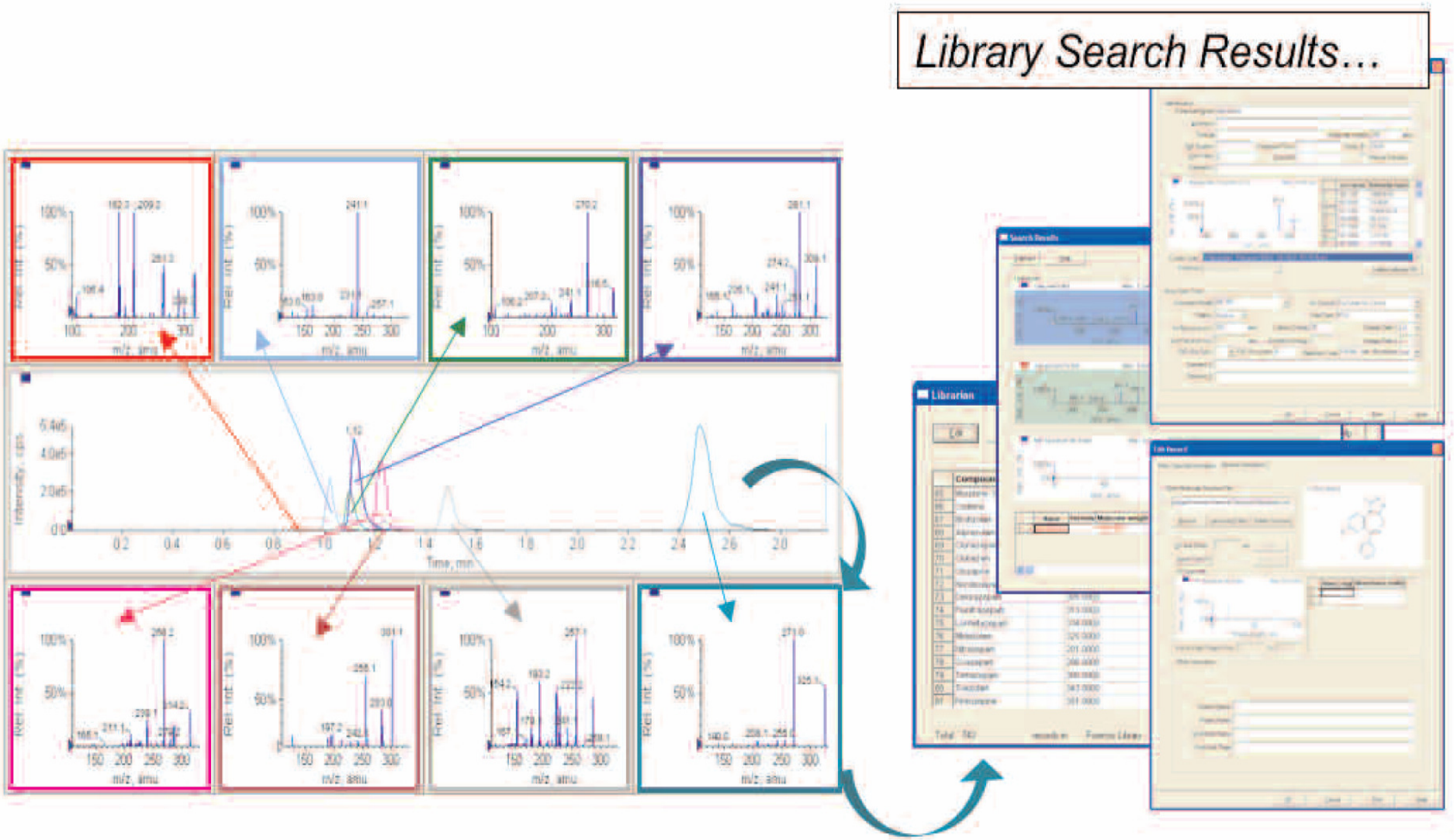
Schematic diagram of the ion path of a hybrid triple quadrupole/ linear ion trap (QQQ/LIT) instrument.
The ion path is virtually identical to a standard triple quadrupole, except Q3 has been modified to operate not only as a quadrupole, but also as an ion trap.



Analysis and detection of 300 compounds in a single assay. All compounds were analyzed in under 20 minutes.


25 Drugs - 1 mL Blood (20 minute LC run)
I.S.
Intensity, cps
Time, min
1. Oxycodone
2. Hydrocodone
3. Lidocaine
4. Benzoylecgonine
5. Cocaine
6. Mirtazepine
7. Zolpidem
8. Cocaethylene
9. Trazodone
10. Midazolam
11. Flurazepam
12. Doxepin
13. Mesoridazine
14. Verapamil
15. Carbemazepine
16. Cyclobenzaprine
17. Methadone
18. Amitriptyline
19. Hydroxyzine
20. Flunitrazepam
21. Desipramine
22. Maprotiline*
23. Triazolam
24. Lorazepam
25. Thioridazine
15 - 22

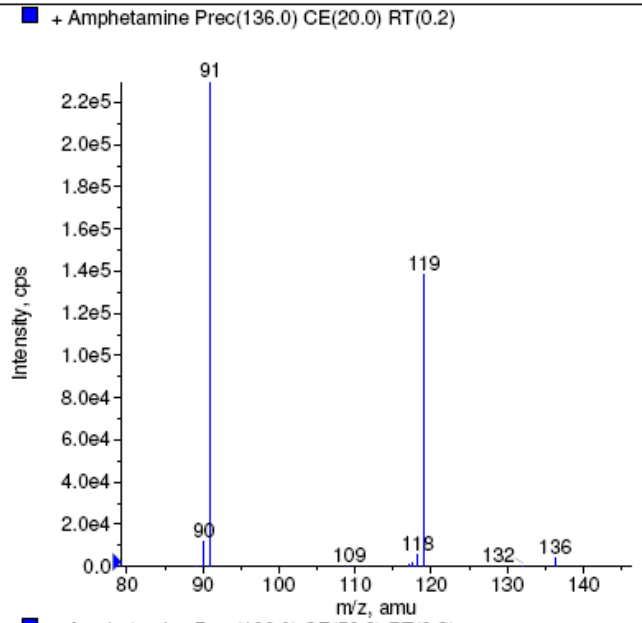

Scan Event 1
+ Full Scan MS
Scan Event 7
– Full Scan MS
Scan Events 2–6
+ MS/MS on parent list
Scan Event 8
– MS/MS on parent list

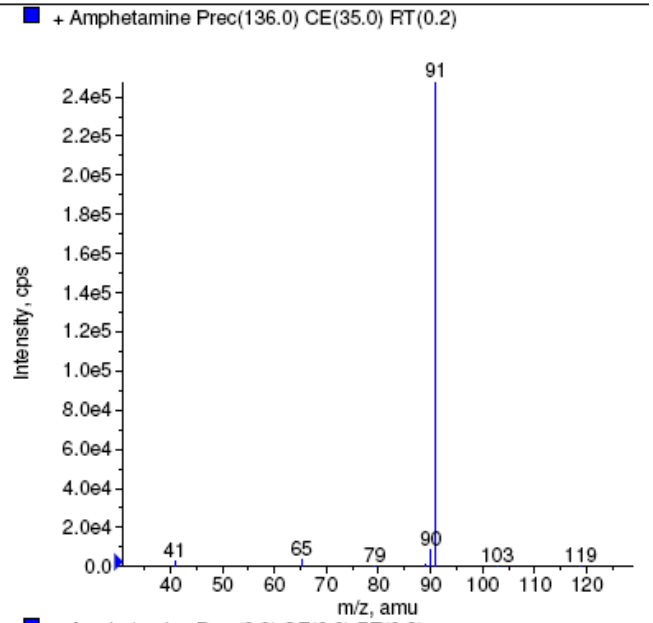


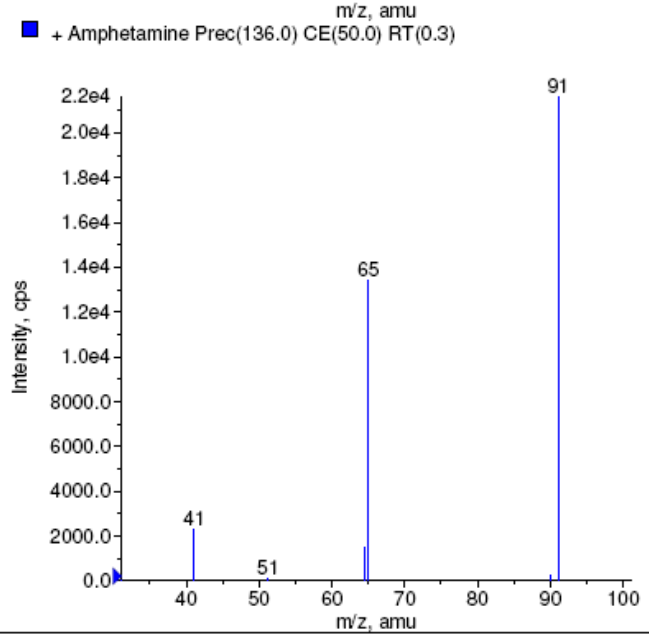
Analysis and detection on a QQQ/LIT instrument using MRM-IDA.
The chromatograms for each MRM transition is shown, along with the LIT MS/MS spectra that are acquired from each signal. Quality spectra were obtained, even from compounds obtained at low levels. Also, unique spectrawere acquired even in cases where two compounds co-eluted. The MS/MS spectra were used to search against a spectral library for analyte confirmation.

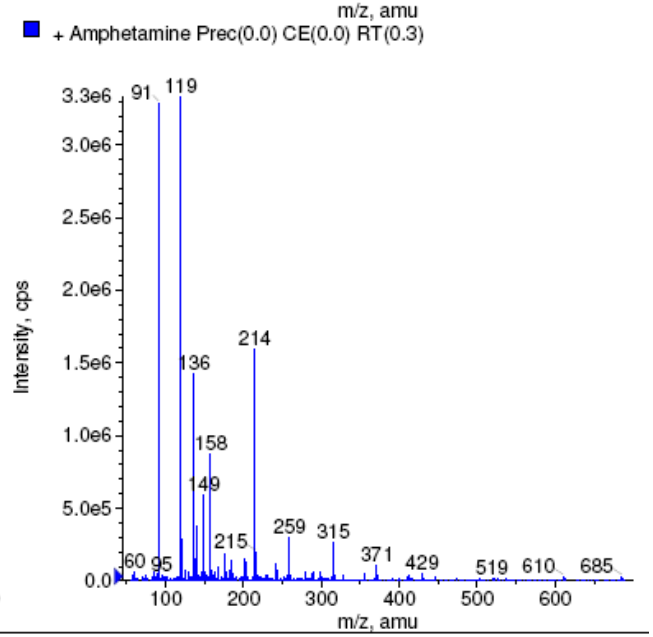
Compound Name:	Amphetamine
Synonyms:	
Formula:	C9H13N
CAS Number:	300-62-9
Molecular weight(amu):	135.0000
Compound Class:	illegal drug
Compound ID:	A022
User Value:	0.0000
Keyword:	
Internal Standard:	No
Comment1:	

Instrument Model:	API 365
Ion Source:	Turbo Spray
Polarity:	Positive
Scan Type:	MS2
1st Precursor m/z:	136.0000
Collision Energy1(V):	20.0000
Charge State1:	1
CAD Gas Type:	N2
CAD Gas Value:	4.0000
Retention Time(min):	0.20
Resolution:	unit
Comment2:	
Comment3:	









Weinmann, W., PDF-Files of ESI-MS/MS-Product Ion Spectra (800 compounds)
Instrumentation: AB/Sciex API 365
http://www.chemicalsoft.de/msmslib2005/MS2-2005-index.html

Efficiency of the different toxicological test measures…

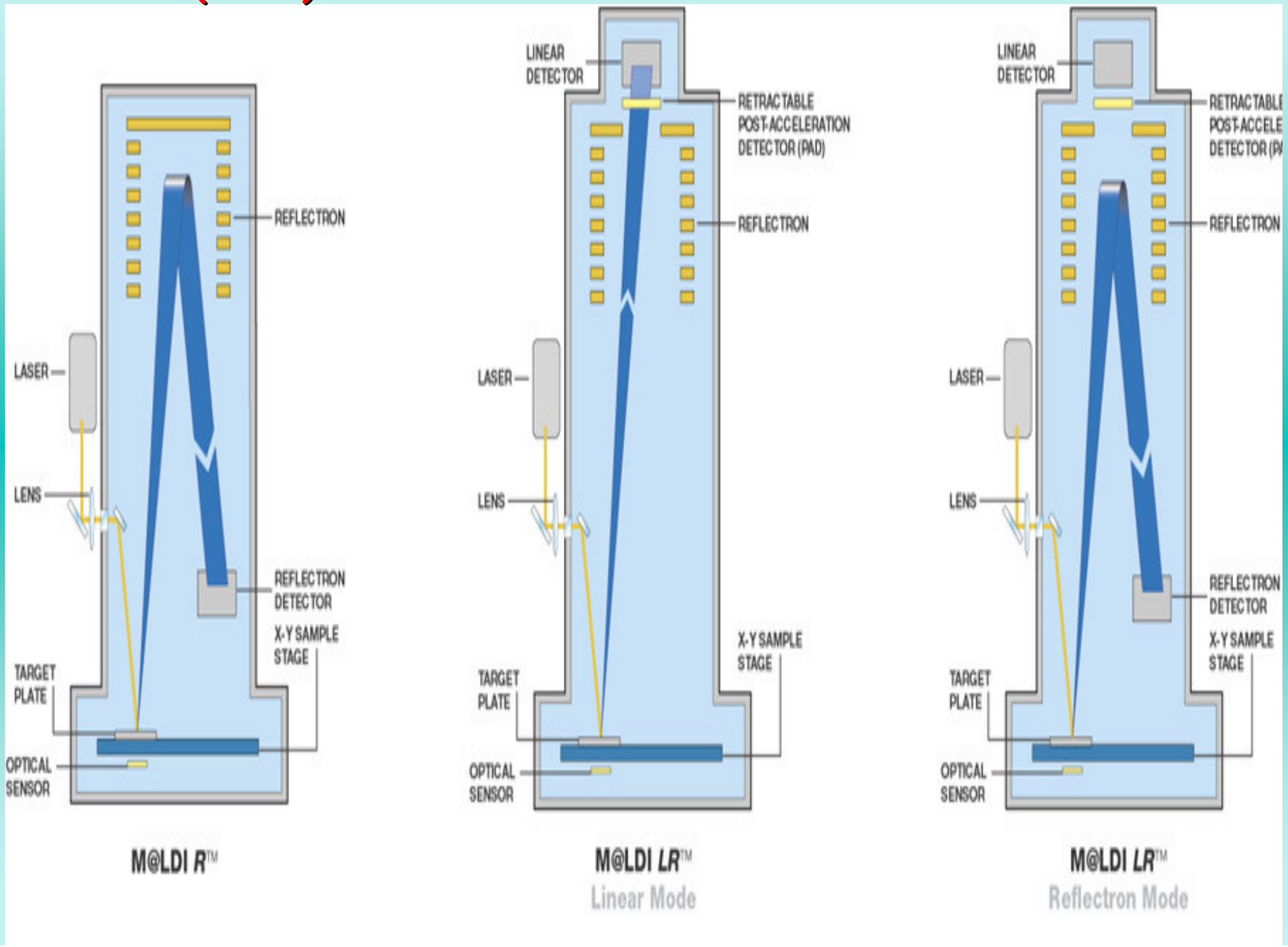
LXQ 30 Minute	LXQ 13 Minute	LC-UV	Immunoassay
Nortriptyline	Nortriptyline	Nortriptyline	Barbiturates
Amitriptyline	Amitriptyline	Amitriptyline	Benzodiazepines
Benzoylecgonine	Benzoylecgonine	Benzoylecgonine	Cocaine
Cocaine	Cocaine	Cocaine	Opiates
Cocaethylene	—	Cocaethylene	THC
Cyclobenzaprine	—	—	—
Norbenzoylecgonine	Norbenzoylecgonine	—	—
Morphine	Morphine	—	—
Norcocaine	Norcocaine	—	—
Codeine	—	—	—
Norcocaethylene	Norcocaethylene	—	—
Methadone	—	—	—
Quinidine/Quinine	Quinidine/Quinine	—	—
Hydroxyzine	Hydroxyzine	—	—
Noskapine	Noskapine	—	—
Diltiazem	Diltiazem	—	—
Morphine-3-beta-Glucuronide	Morphine-3-beta-Glucuronide	—	—

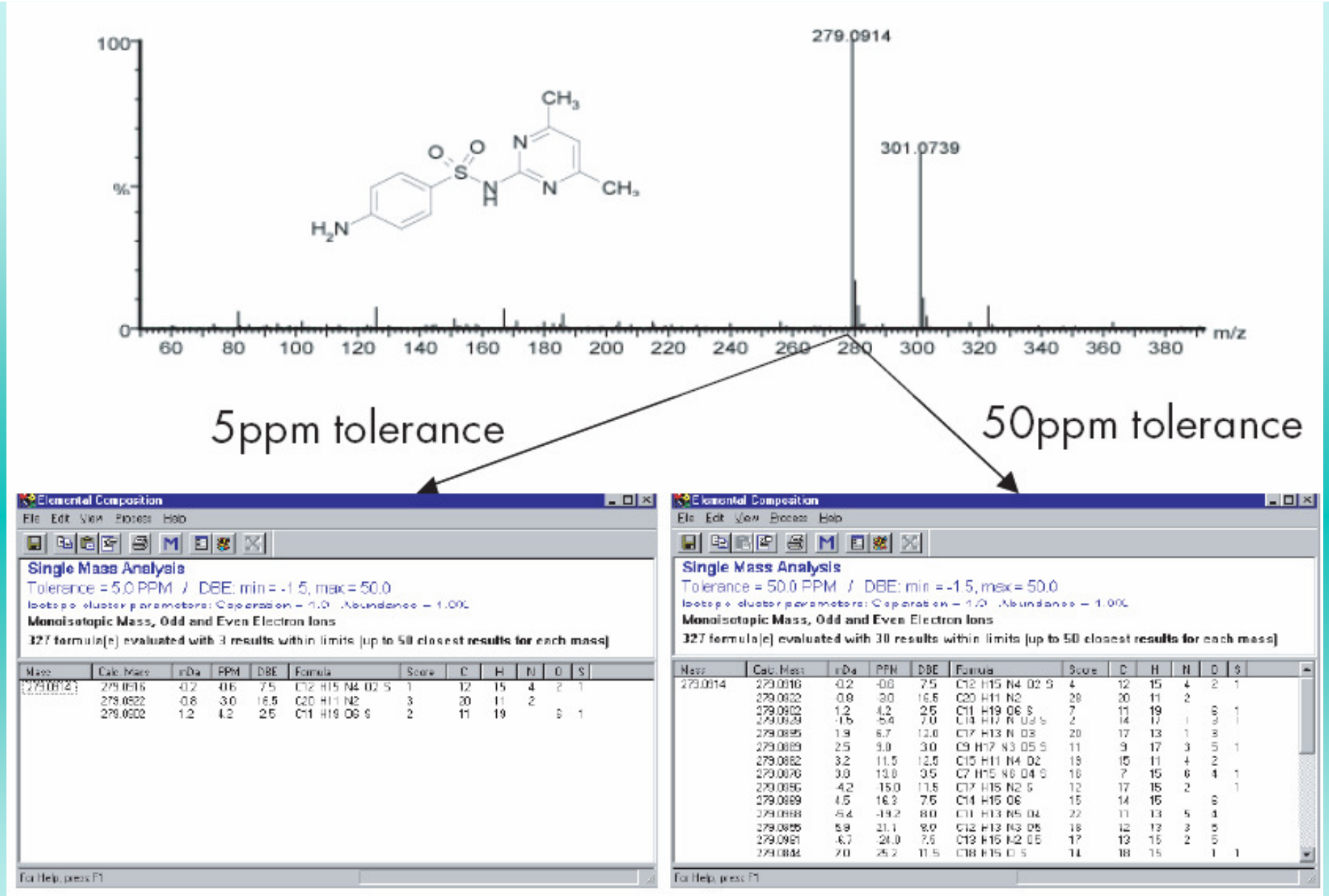
Exact Mass Determination…

Effect of *Mass Accuracy* and *Mass Tolerance* on (Peptide) Mass Fingerprinting Search Results

Search *m/z*	Mass tolerance (Da)	# Hits
1529	1	478
1529.7	0.1	164
1529.73	0.01	25
1529.734	0.001	4
1529.7348	0.0001	2

MALDI - (TOF) instruments

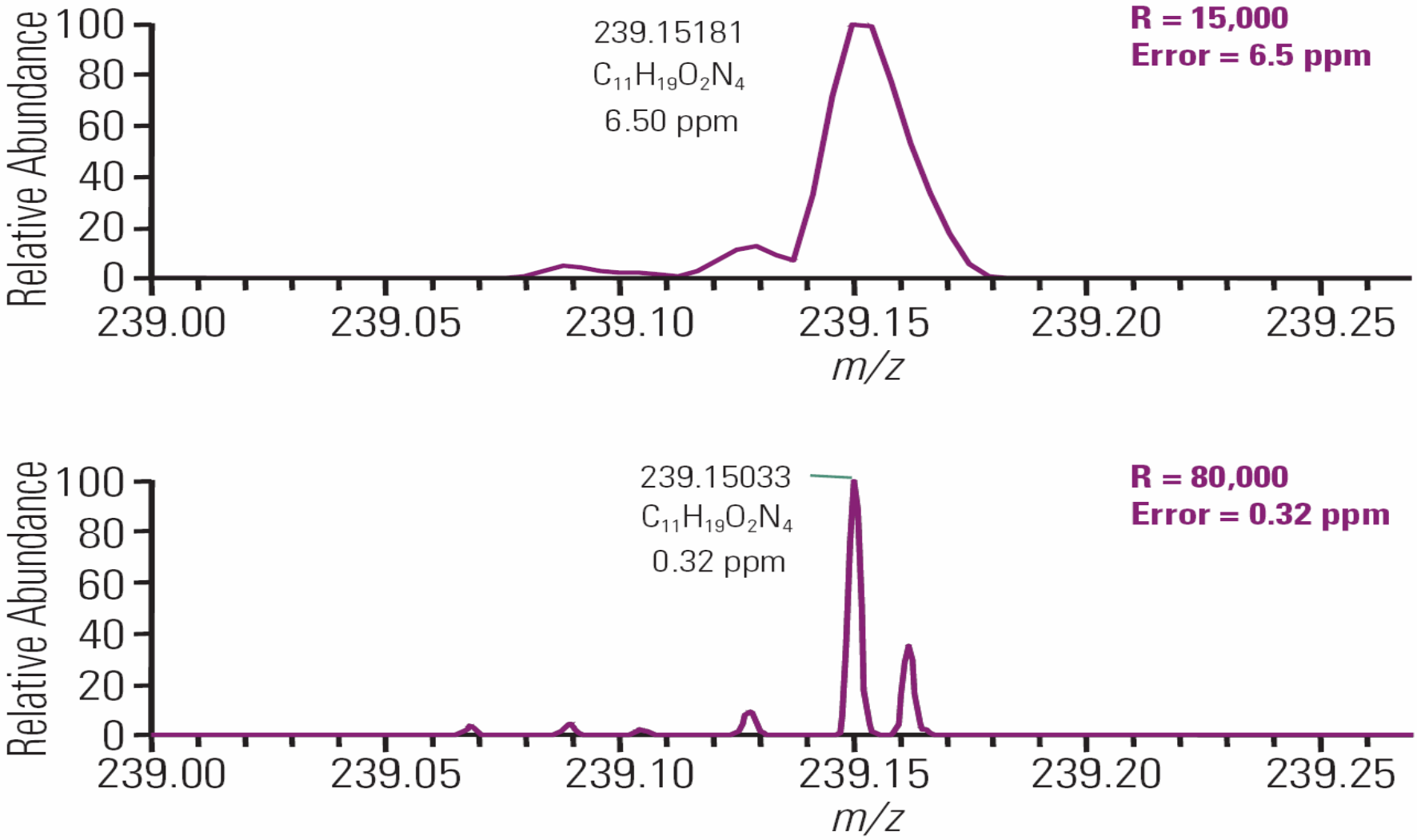




Influence of exact mass resolution on identification power

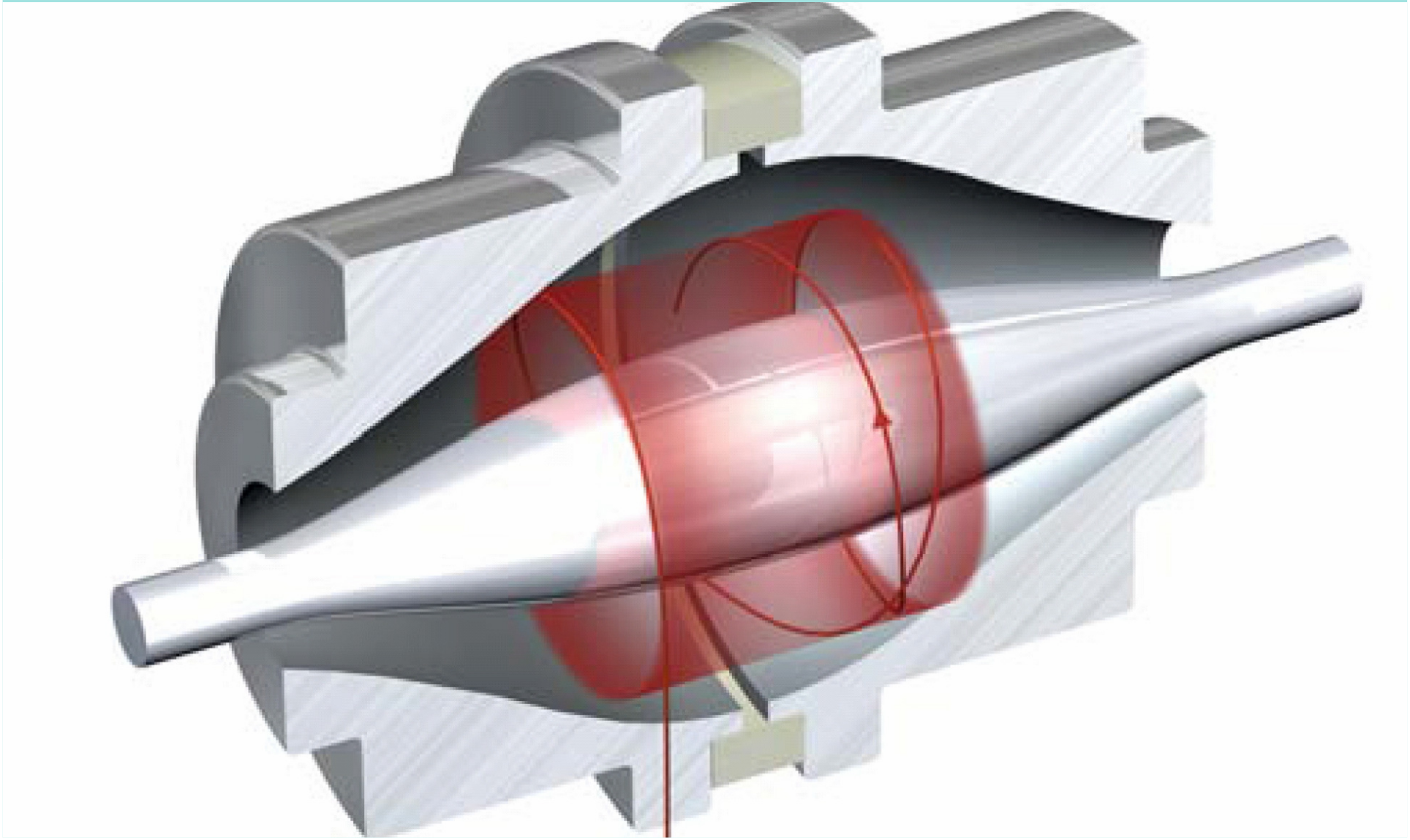
Compound	Actual Mass	Measured Mass	mDa Error	ppm Error
4-acetamidophenol	152.0711	152.0707	-0.4	-2.6
sulfaguanidine	215.0602	215.0597	-0.5	-2.3
Sulfadimethoxine	311.0814	311.081	-0.4	-1.3
Val-Tyr-Val	380.2185	380.219	0.5	1.3
Terfenadine	472.3215	472.321	-0.5	-1.1
Leucine enkephalin	556.2771	556.2775	0.4	0.7
Reserpine	609.2812	609.2828	1.6	2.6
Erythromycin	734.469	734.4695	0.5	0.7
			RMS ppm error	1.8

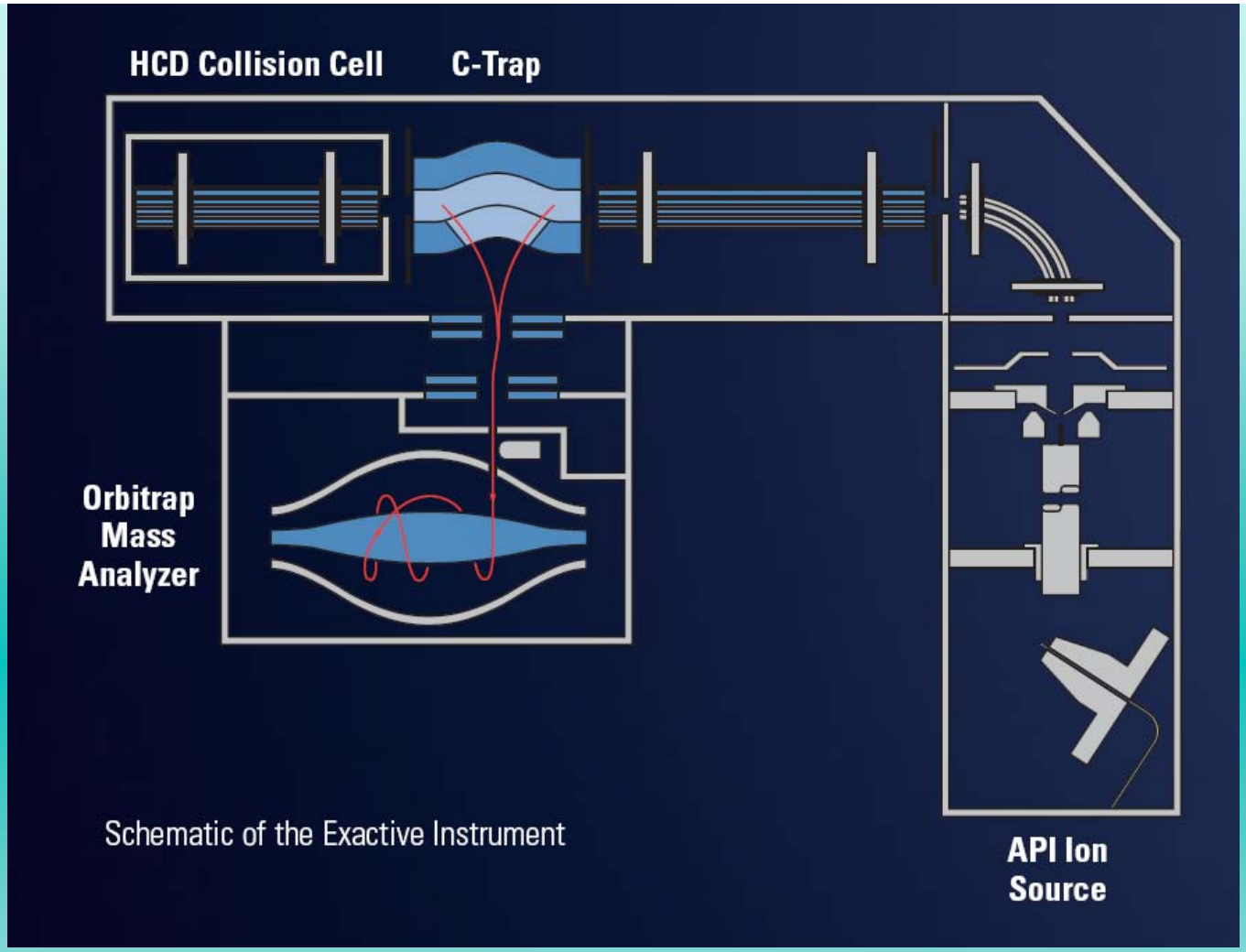
Identification of drugs by exact mass measurements



The unsually high error of 6.5 ppm (top) indicates the need for higher resolution. Re-analysis performed at 80,000 (bottom) showing baseline separation of the targeted pesticide from the matrix interference

The Orbitrap - Technology



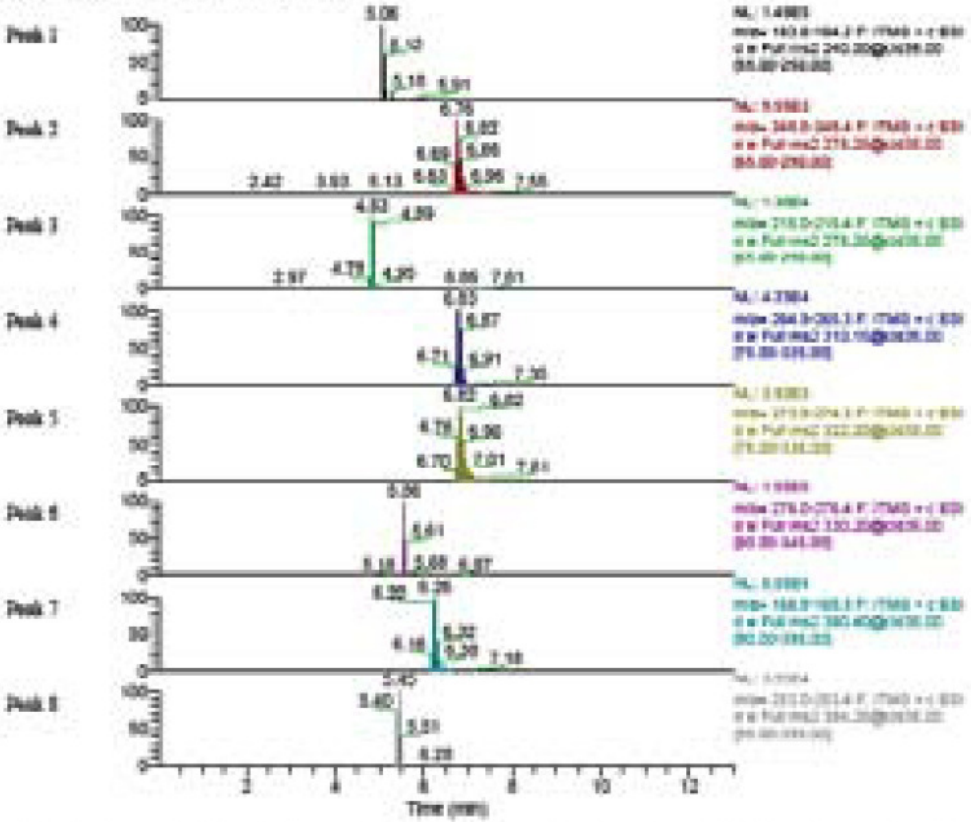


Schematic of the Exactive Instrument

But there are softwaretools needed...

ToxID Summary Report

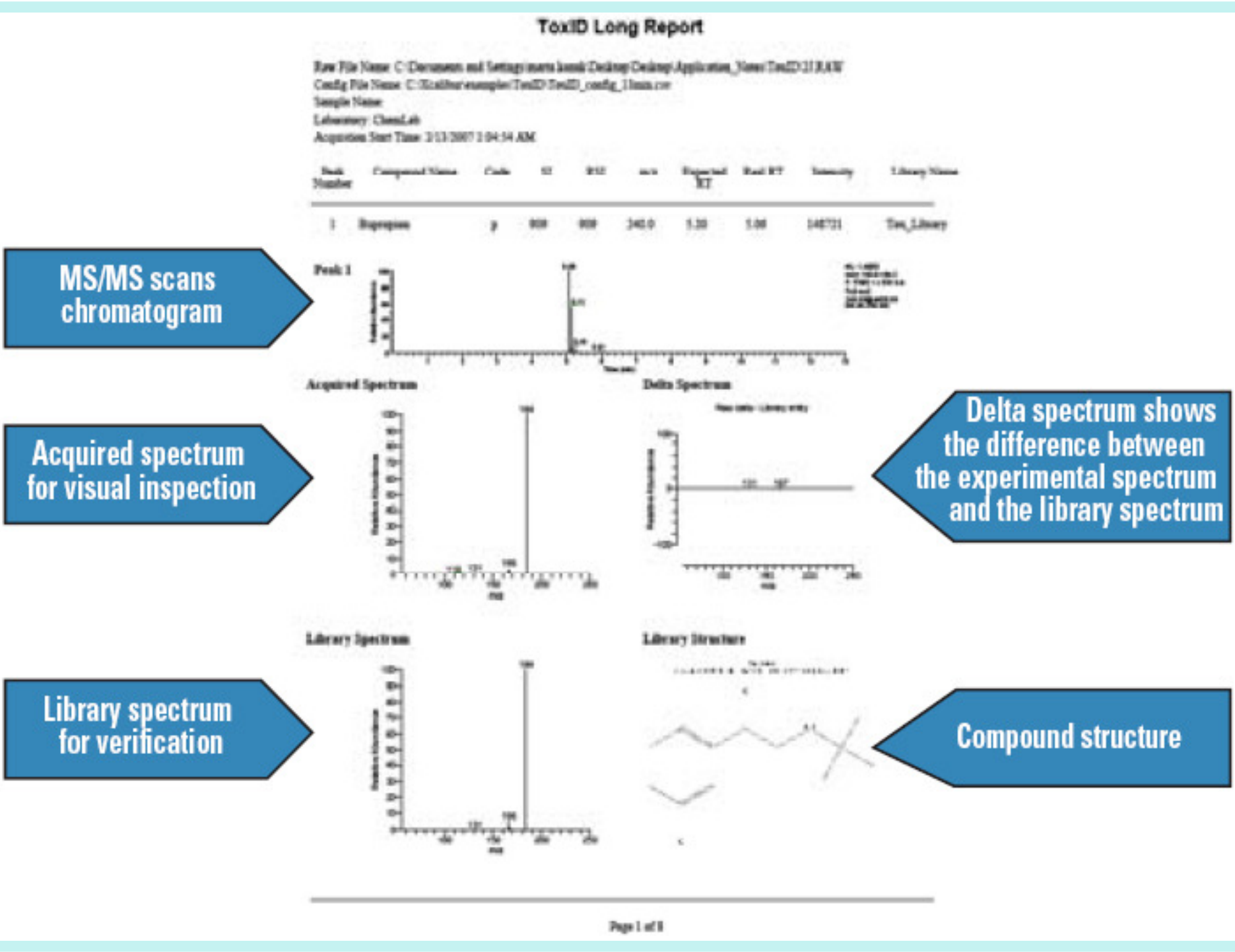
Raw File Name: C:\Documents and Settings\asura kazak\Desktop\Desktop\Applications_Notes\ToxID\10.RAW
Config File Name: C:\Xcalibur\examples\ToxID\ToxID_config_10min.csv
Sample Name:
Laboratory: ChemLab
Acquisition Start Time: 3/13/2007 1:04:54 AM



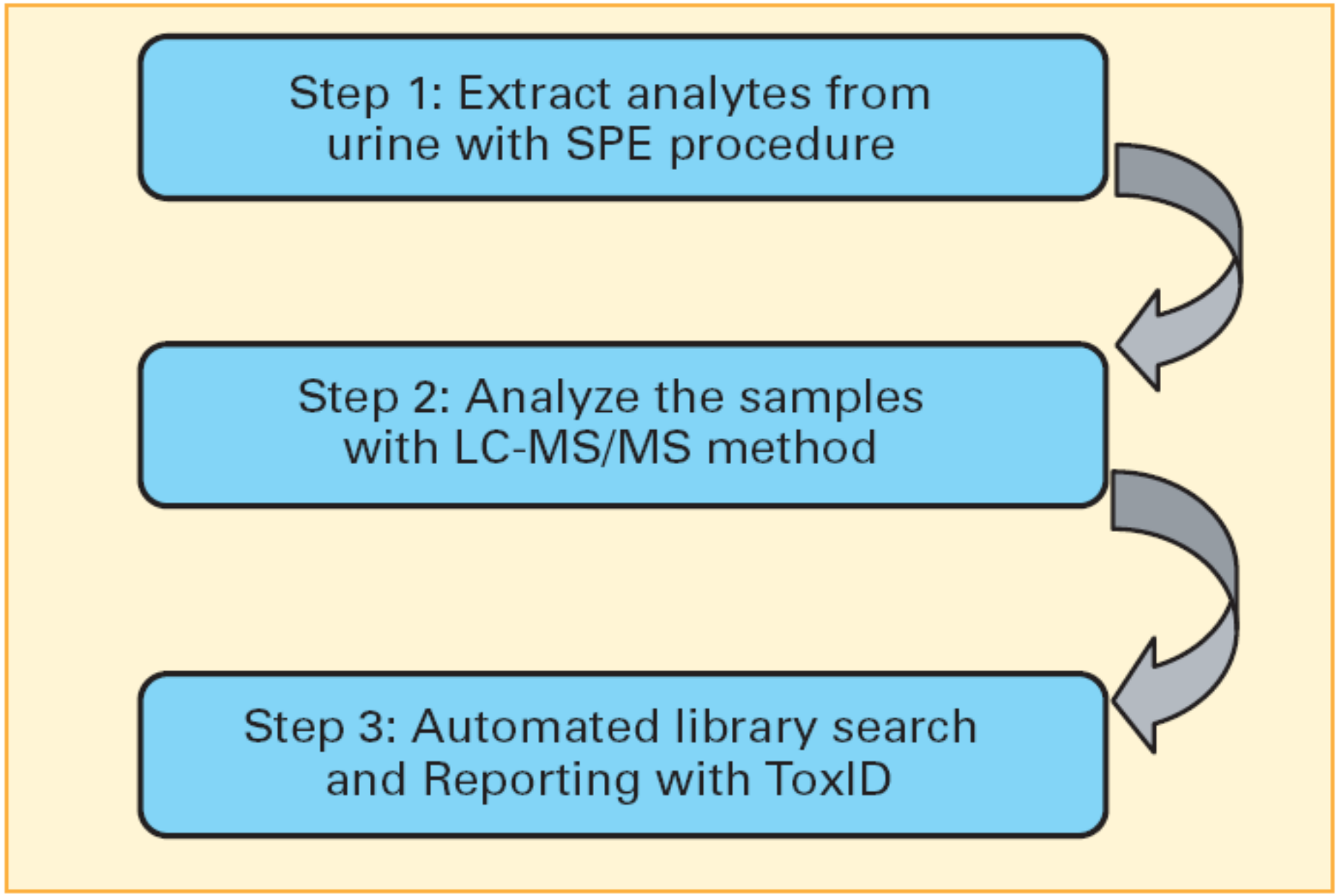
Identified compounds MS/MS scans related chromatograms

Peak Number	Compound Name	Code	SI	RSI	m/z	Expected RT	Real RT	Intensity	Library Name
1	Bupropion	p	909	909	240.0	5.50	5.66	148751	Tox_Library
2	EDDP	p	857	873	278.2	6.60	6.76	9549	Tox_Library
3	Venlafaxine	p	836	837	278.2	4.90	4.83	12984	Tox_Library
4	Methadone	p	932	932	310.2	6.70	6.83	42262	Tox_Library
5	Chlorpromazine-D3	i	859	859	321.2	6.80	6.82	3924	Tox_Library
6	Prazepam-D5	i	969	974	330.2	5.60	5.56	154827	Tox_Library
7	Haloperidol-D4	i	830	837	380.4	6.20	6.26	85589	Tox_Library
8	Quetiapine	p	870	871	384.2	5.40	5.43	39512	Tox_Library

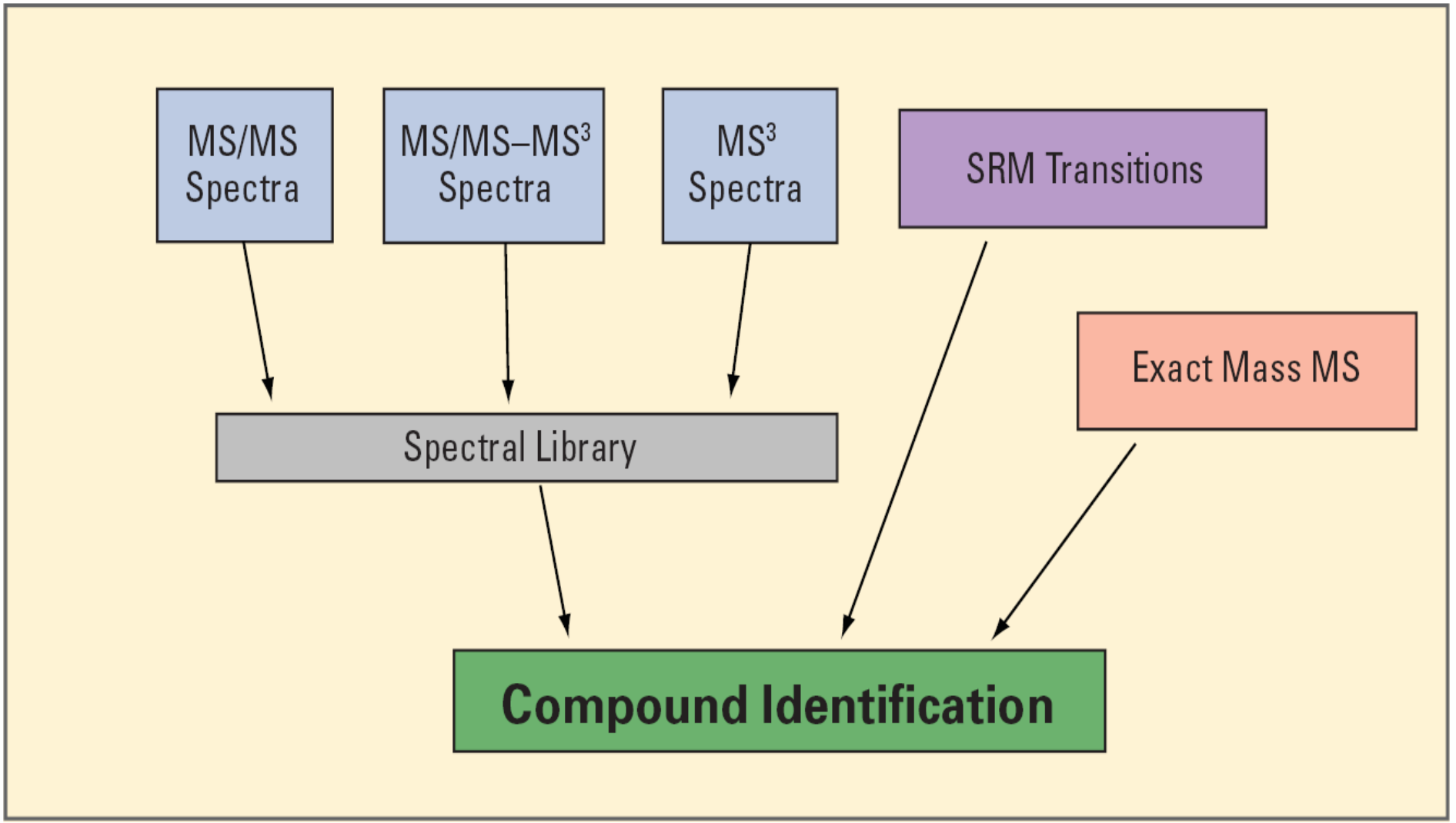
Identified compounds with search indexes


ToxID Long Report
Peak 1
Acquired Spectrum
Delta Spectrum
Library Spectrum
Library Structure
MS/MS scans chromatogram
Acquired spectrum for visual inspection
Delta spectrum shows the difference between the experimental spectrum and the library spectrum
Library spectrum for verification
Compound structure

Practical strategies in toxicological analysis….



Practical strategies in toxicological analysis…. (2)



Edited by W. R. Külpmann

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Clinical Toxicological Analysis

Procedures, Results, Interpretation

Volume 1

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Aufmerksamkeit!