Massenspektrometrie und Methodenentwicklung

Dr. Volker Gnau
Produktspezialist LC/MS
Hamburg 02. Oktober 2012

Agenda

- **Ionization Sources**
- **6100 Single Quad Series**
- **6500 QTOF Series**
- **6400 QQQ Series**

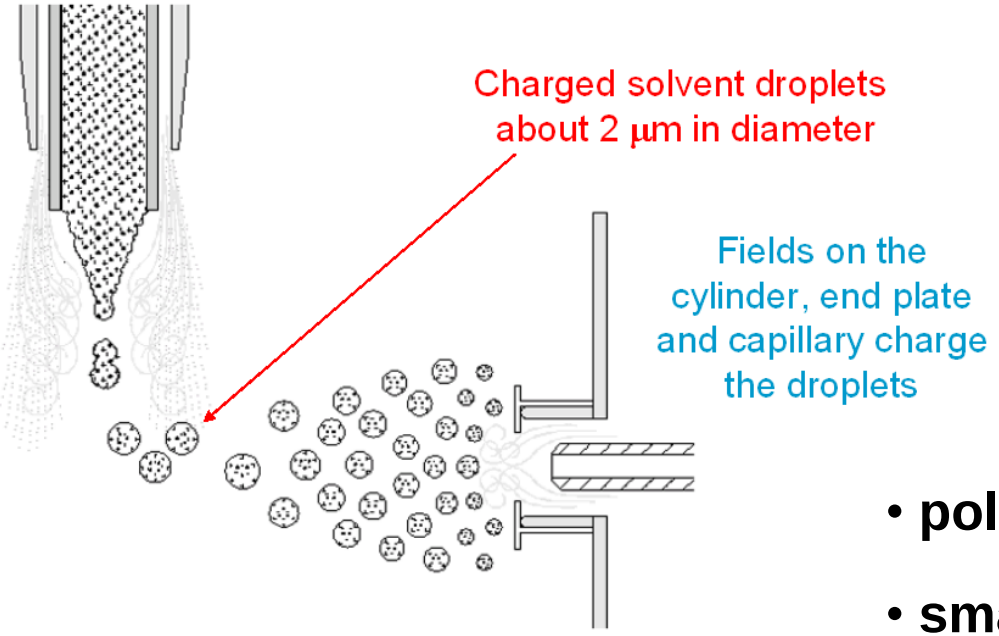
Ionization Techniques

ESI

APCI

APPI

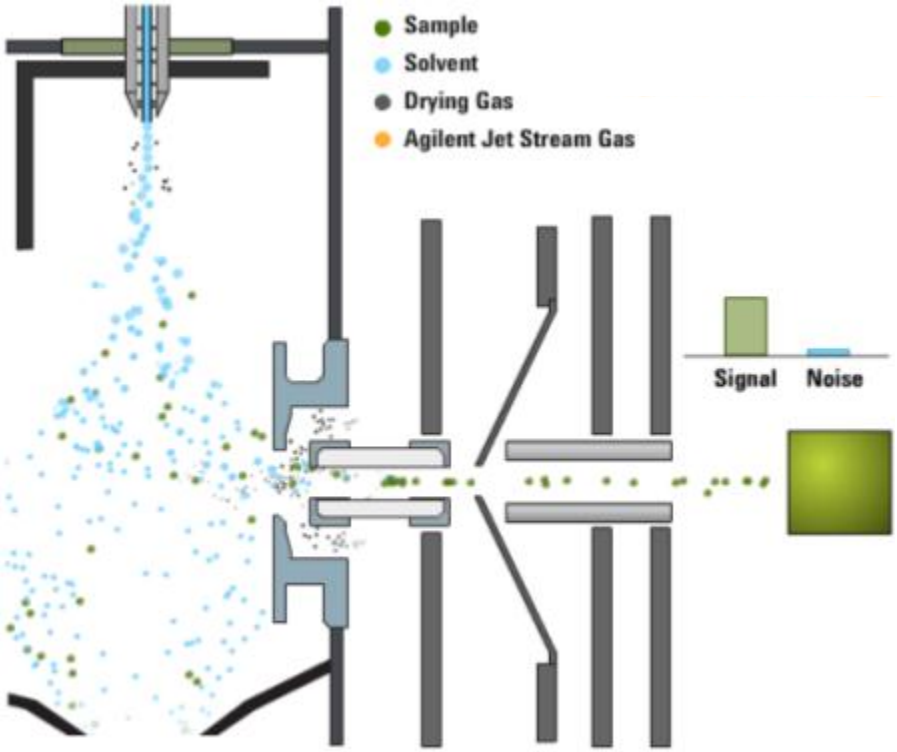
Electrospray Ionization - ESI



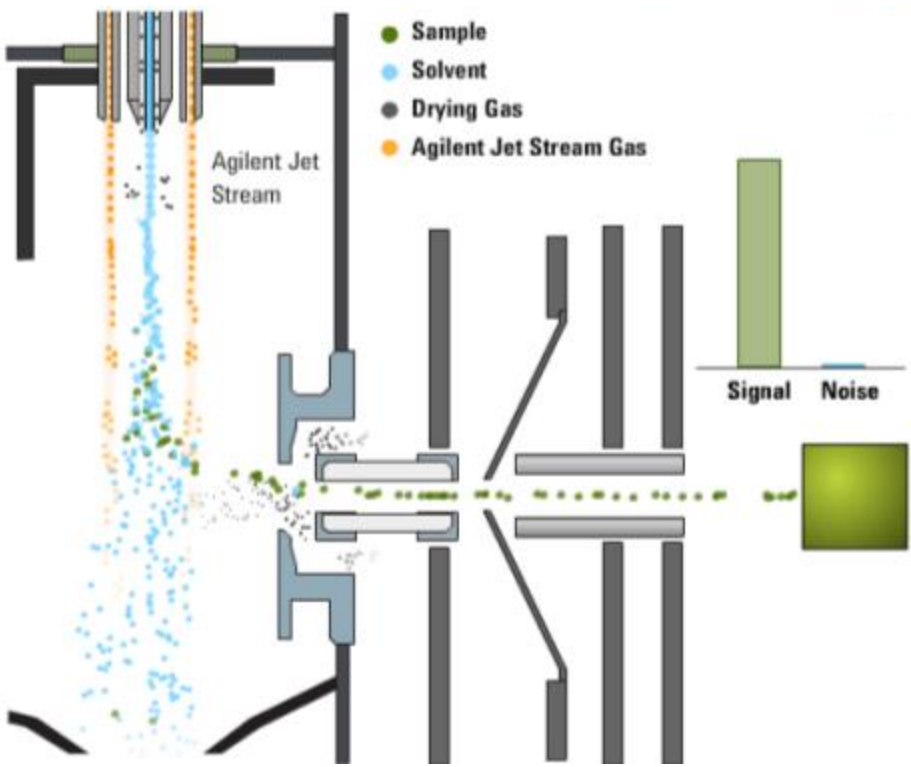
- **polar compounds**
- **small and large molecules**
- **(multiple) charged compounds**
- **thermal labile compounds**
- **Samples with small amounts**

Agilent Jet Stream

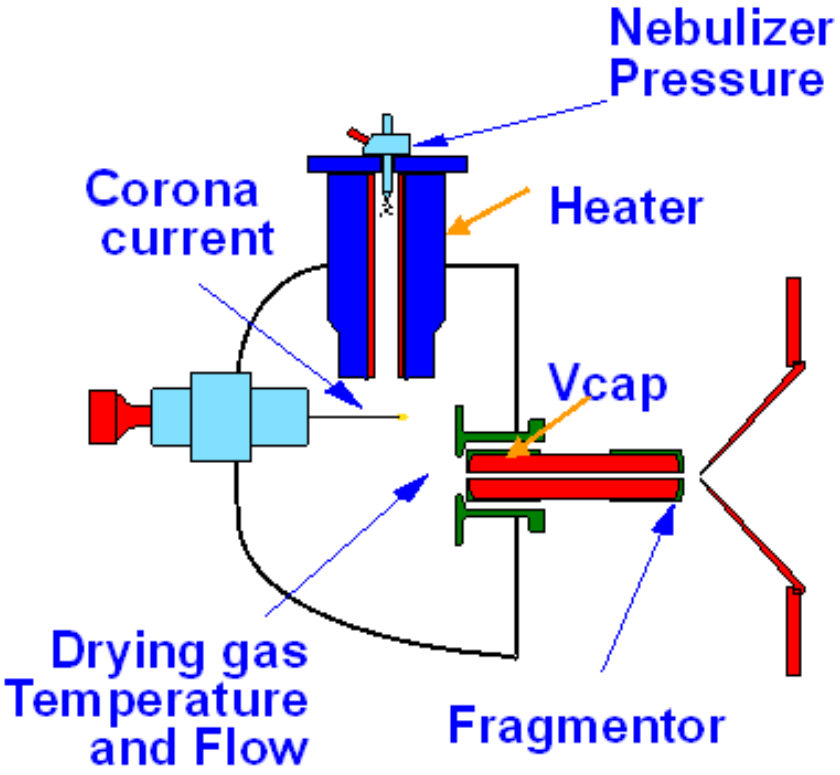
Off



On

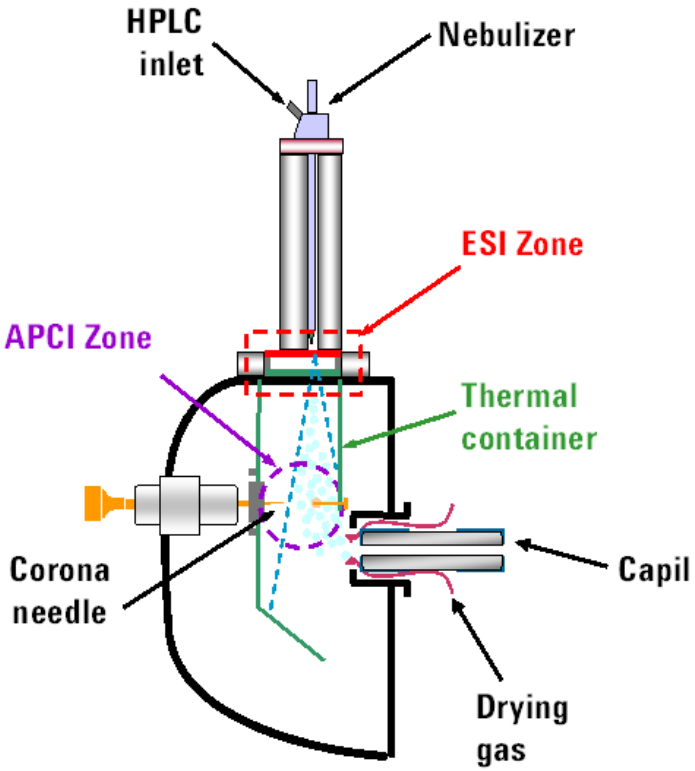


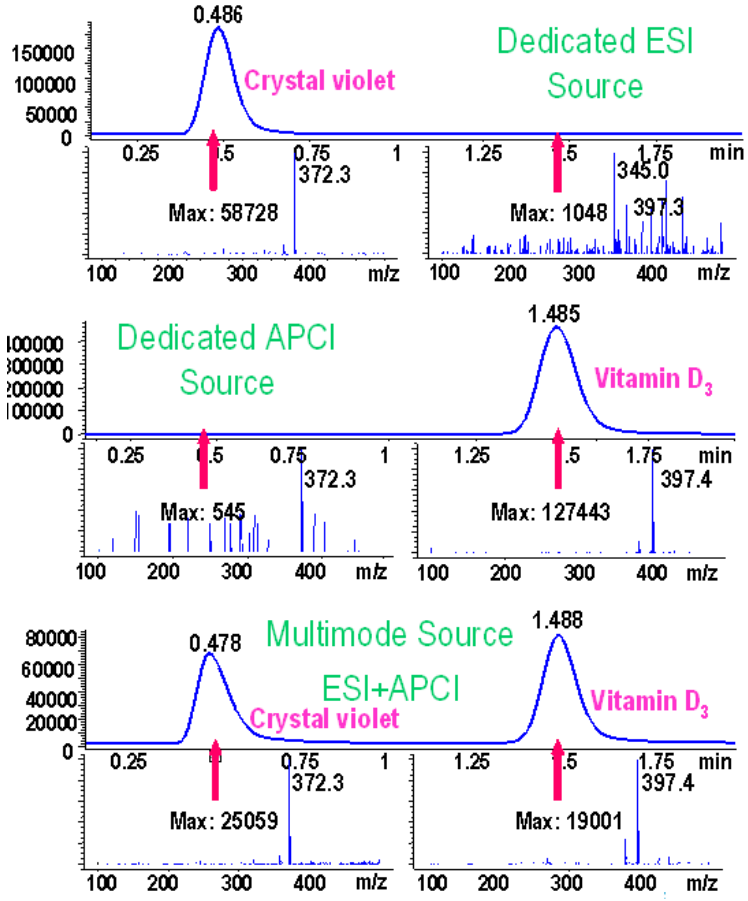
Atmospheric Pressure Chemical Ionization (APCI)



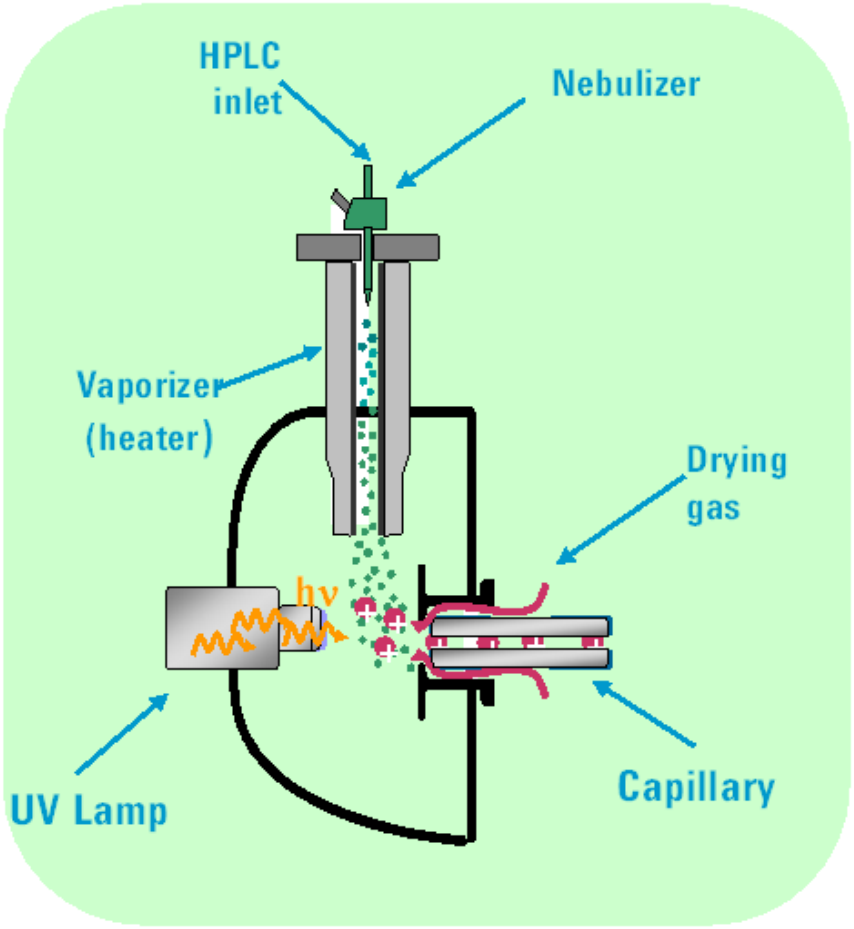
- apolar compounds
- small molecules
- single charged compounds
- thermal stable compounds

Atmospheric Pressure with Multimode Source ESI and APCI Ionization





Atmospheric Pressure Photo Ionization (APPI)



- compounds with inadequate ESI and APCI
- small molecules
- mainly single charged compounds
- thermal stable compounds

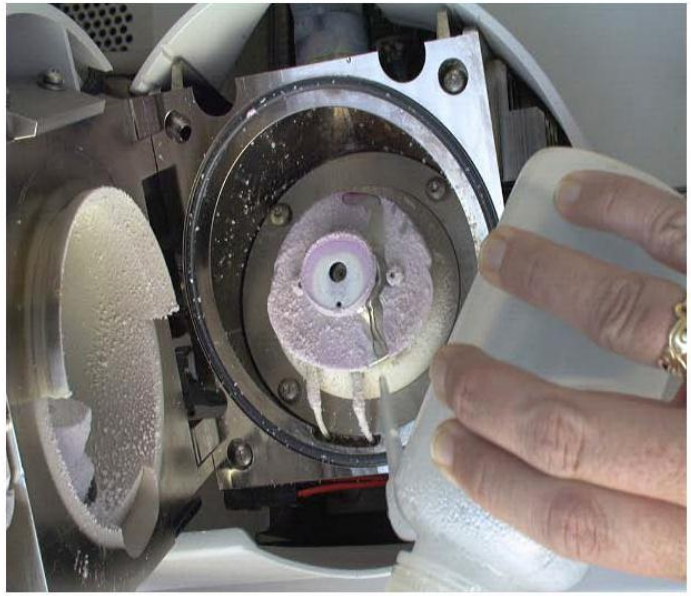
Atmospheric Pressure Ionization
Solvents and Buffers

- Acetonitrile, Methanol, THF, Aceton
- Acetate acid and salts, pH 3-4
- Formate acid and salts, pH 2-3
- TFA, pH 1-2
- Ammonia, pH >7
- TEA, pH > 7

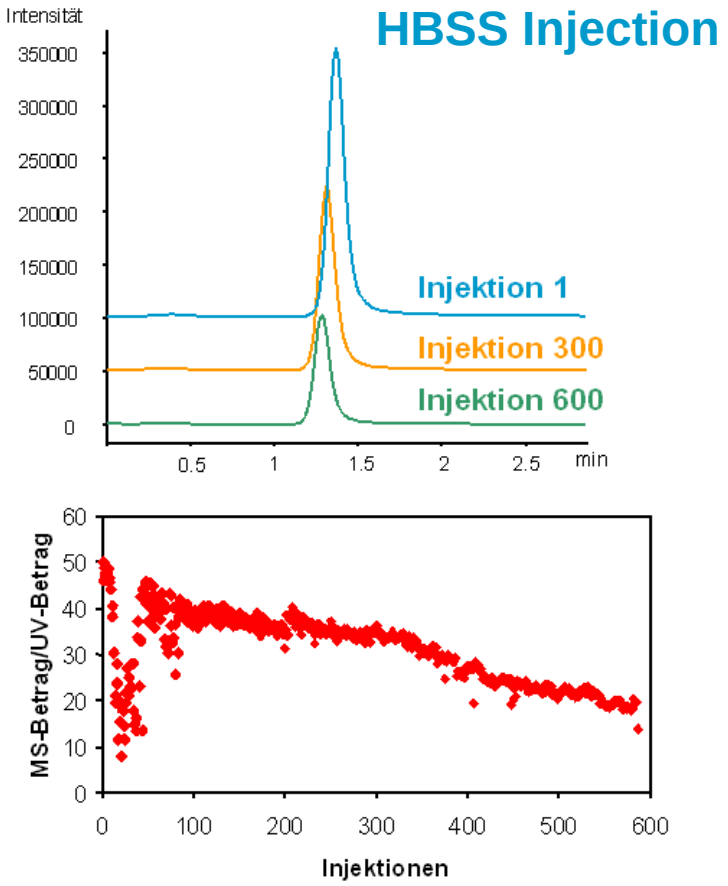
Use only volatile solvents and buffers

TFA (neg, m/z 113), TEA (pos, m/z 102) cause high background

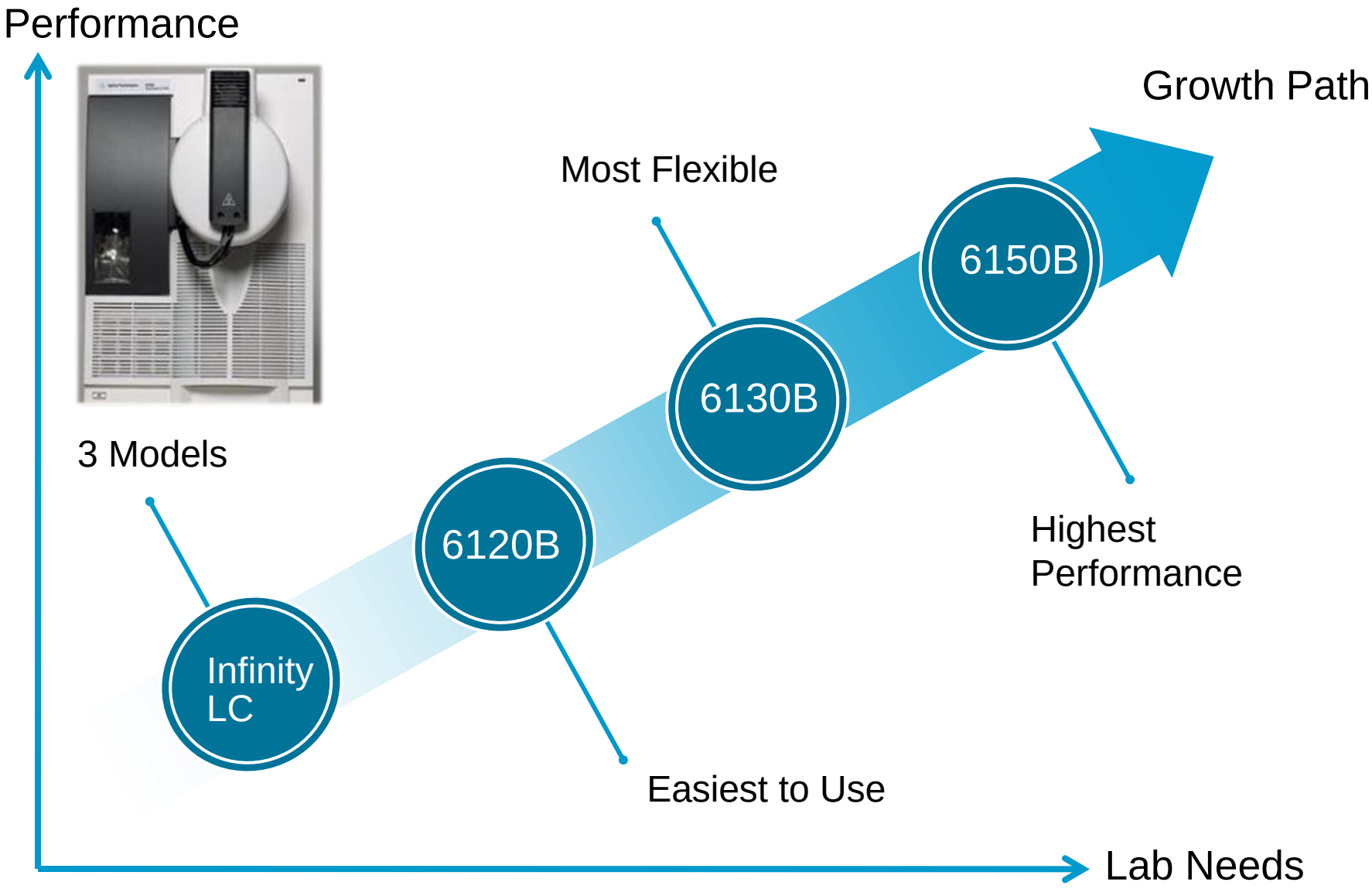
Influence of non-volatile buffers and samples on API Signal



APCI-SprayChamber after 6hrs with 25mmol phosphate buffer (0.7mL/min)



Singlequadrupol - Aufrüstbarkeit



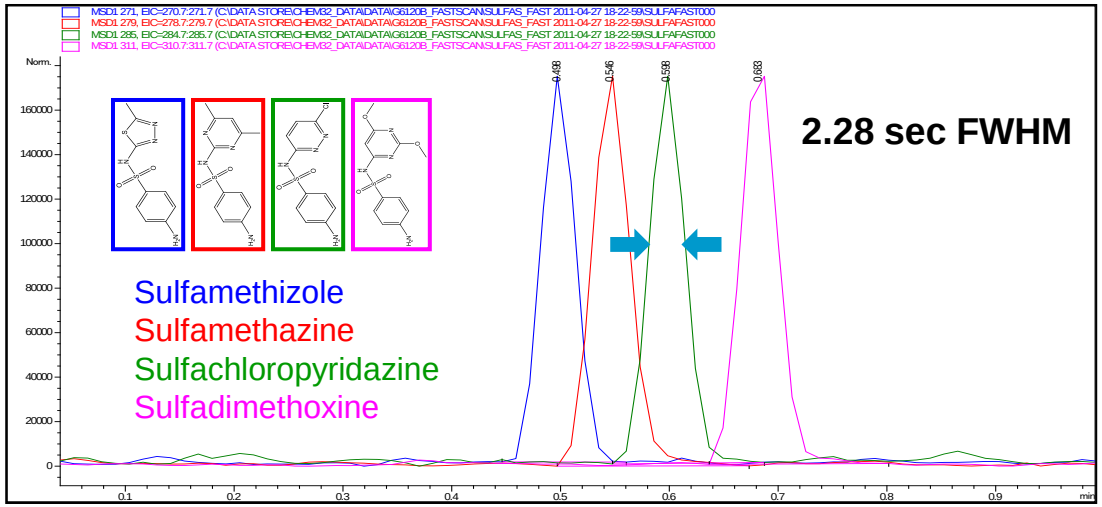
Enhanced 6100B Installation Specifications

Specification	G6120B	G6130B	G6150B
Mass Range	10-2000 m/z	2-3000 m/z AJS ready	10-1350 m/z
Scan Speed	5000u/s	2500 u/s, 5000 u/s	10000 u/s, 2500 u/s
POS/NEG Switching	30 msec	30 msec	20 msec
SIM Sensitivity ESI POS reserpine	----------10 pg 50:1 p-p	1 pg 20:1 p-p ----------10 pg 200:1 p-p	1 pg 100:1 p-p ----------10 pg 1000:1 p-p
Scan Sensitivity reserpine	None	50pg 20:1 p-p	50pg 100:1 p-p
Mass Accuracy Mass Axis Stab.	+-0.13u *+-0.1u or 100ppm 12hrs*	+-0.13u *+-0.1u or 100ppm 12hrs*	+-0.13u *+-0.1u or 100ppm 12hrs*

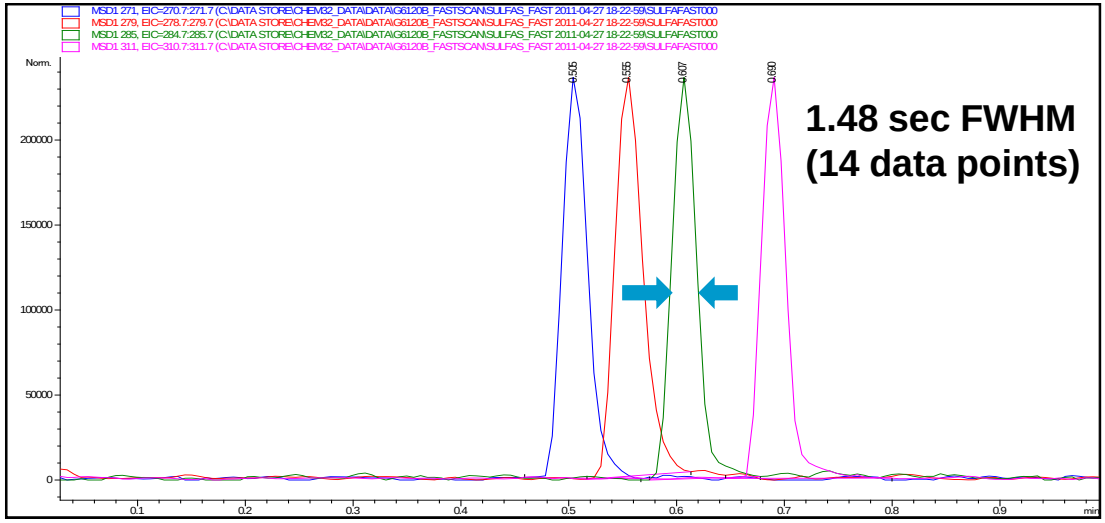
Improved Peak Widths with Faster Scanning

Improved chromatographic resolution and compound identification

2500 u/sec



5250 u/sec



35% Improvement

6500 QTOF Product Line

Excellent Value and Performance -

6530 QTOF LC/MS

- Agilent JetStream for greatest sensitivity
- Robust, easy to use
- Wide dynamic range for qual/quan applications

6540 UHD QTOF LC/MS

- Sub 1-ppm mass accuracy for highest confidence compound identification
- Versatile system with highest resolution
- Fast scan speed to match UHPLC separations

6550 iFunnel QTOF LC/MS

- Agilent iFunnel for highest sensitivity
- Perfect for most demanding applications
- Wide dynamic range for qual/quan applications
- Fastest scan speed to match UHPLC separations

Informationsgehalt

Analyse unbekannter Verbindungen mit hochauflösender MS/MS

Beispiel für Strukturvielfalt: M = 149

Massengenauigkeit von 2 ppm = ± 0,0003

Zugelassene Elemente:	Anzahl an Strukturformeln *
C, H, N, O, S, P, F, Cl, Br:	120
C, H, N, O, S, P:	38
C, H, N, O:	27

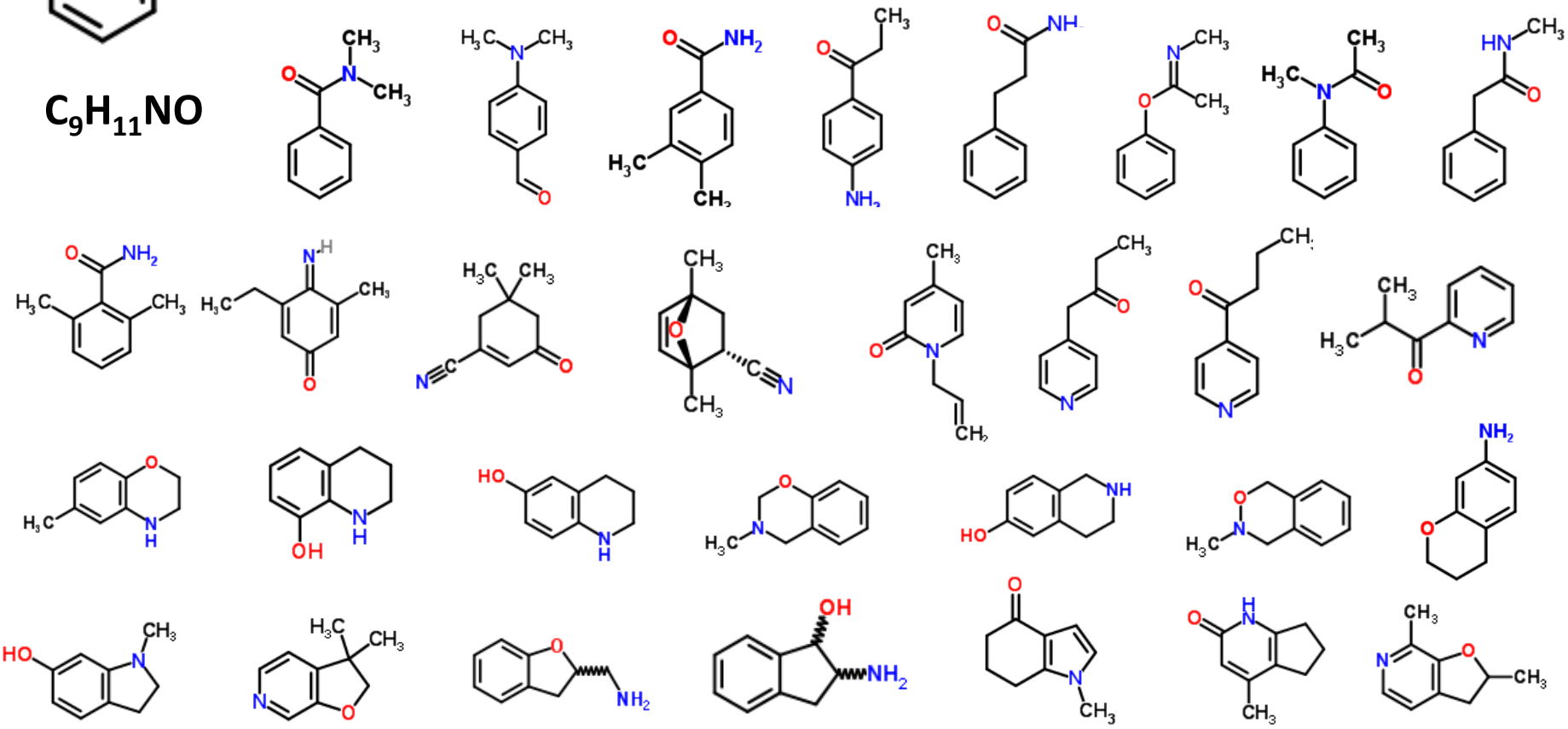
*Number of isomers according to MOLGEN
(Molecular structure generation; http://molgen.de)

Beispiel für Strukturisomere von Cathinon

$C_9H_{11}NO$

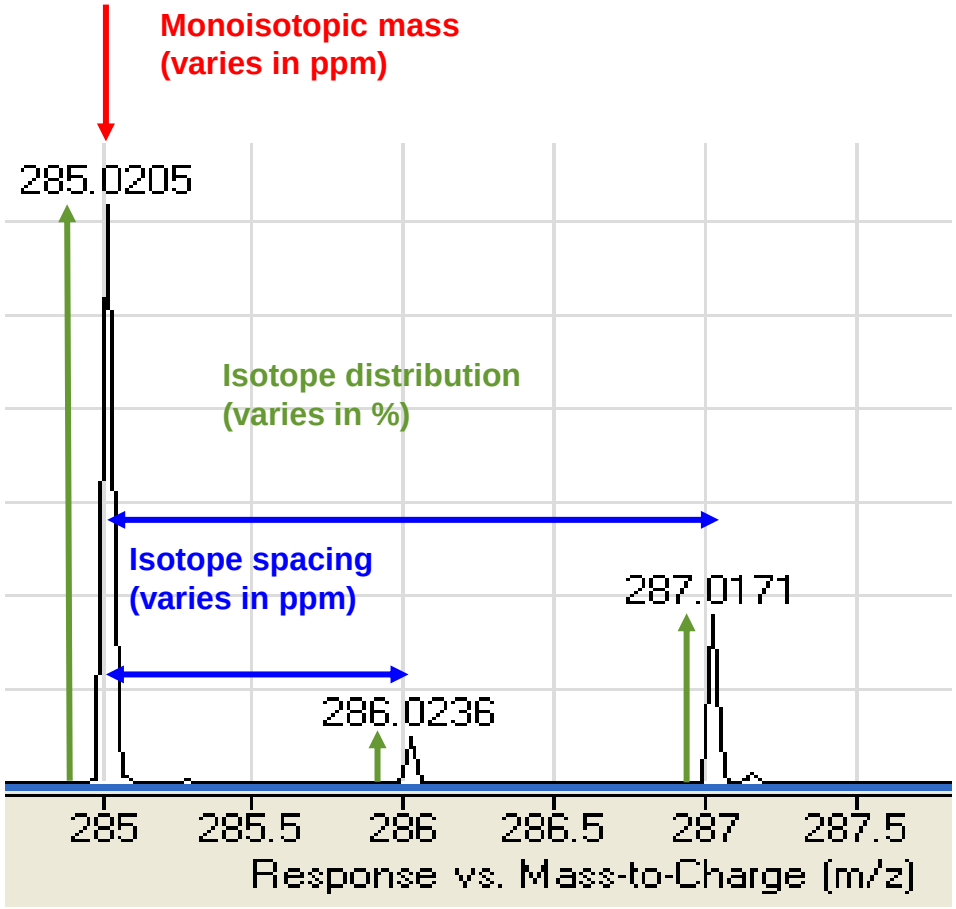
(www.chemspider.com, 829 Treffer)

Insgesammt sind 25 895 621 Strukturisomere möglich
(Stereoisomere kommen noch dazu!!!)

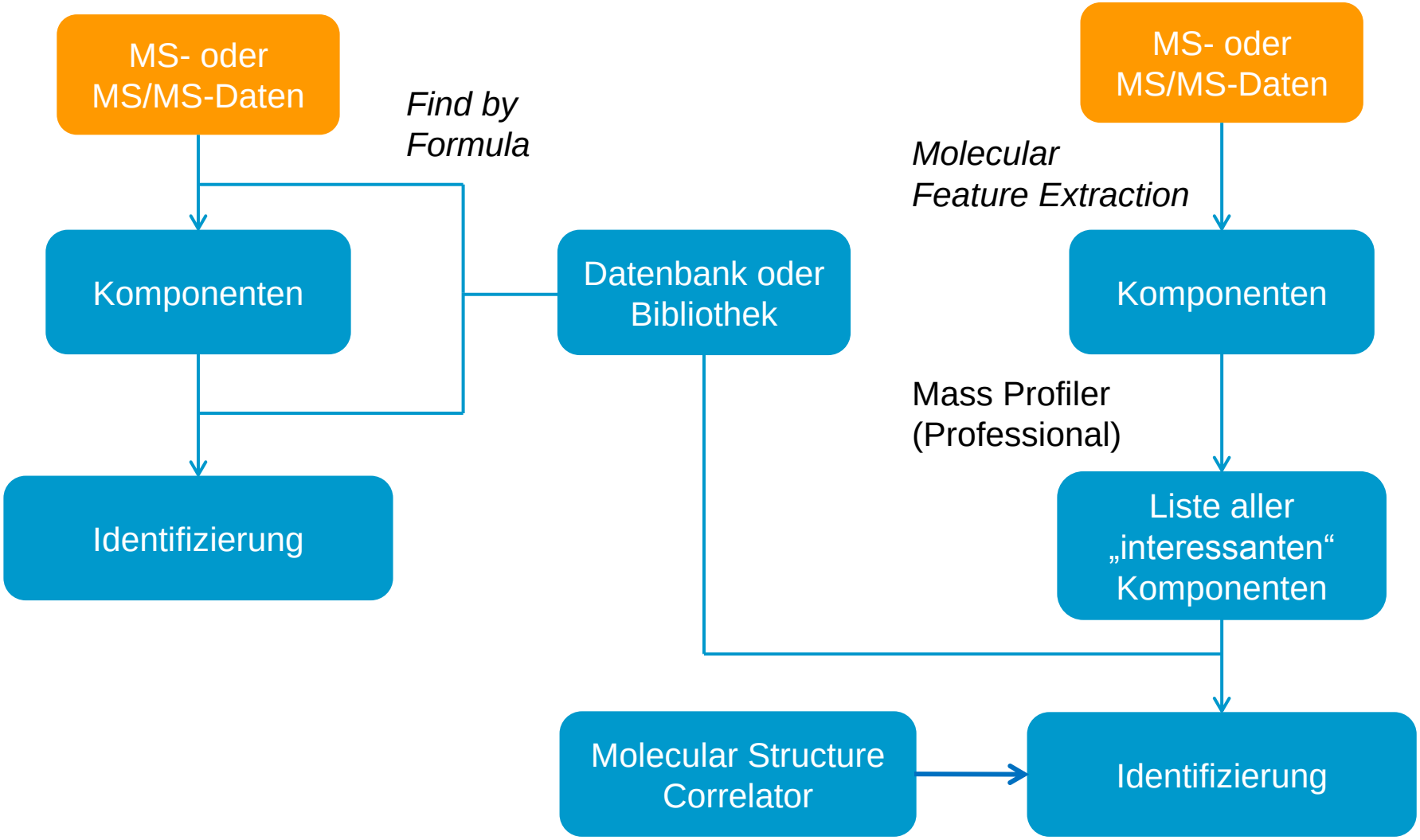


Q-TOF Software Tools

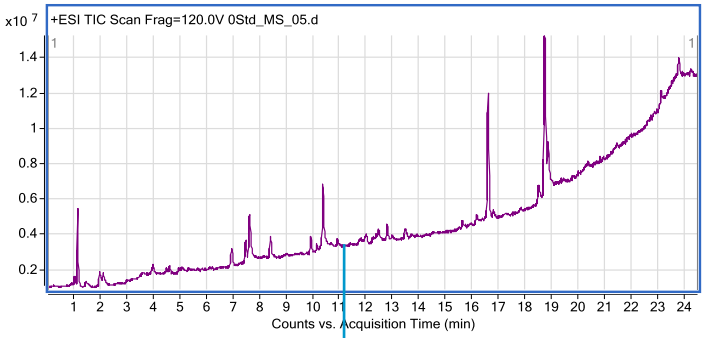
Calculation of chemical formular
Scoring based on:

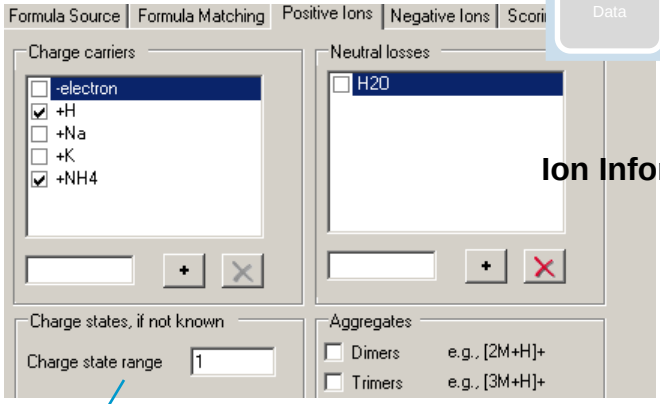


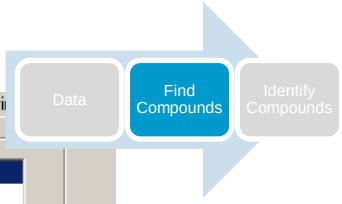
(Q)TOF Software: *Screening* und *Profiling*



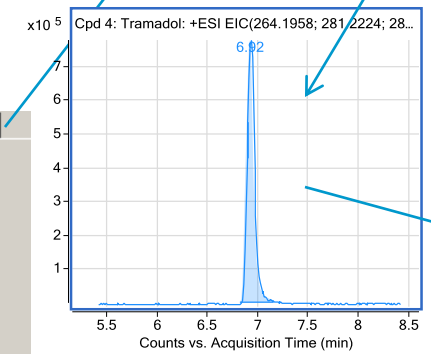
Find by Formula





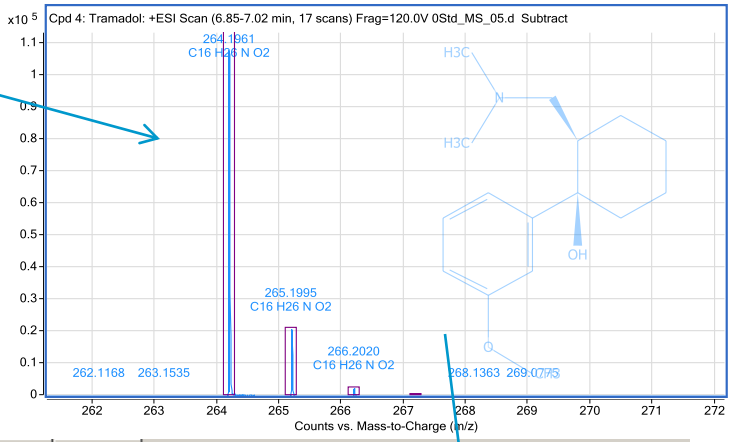


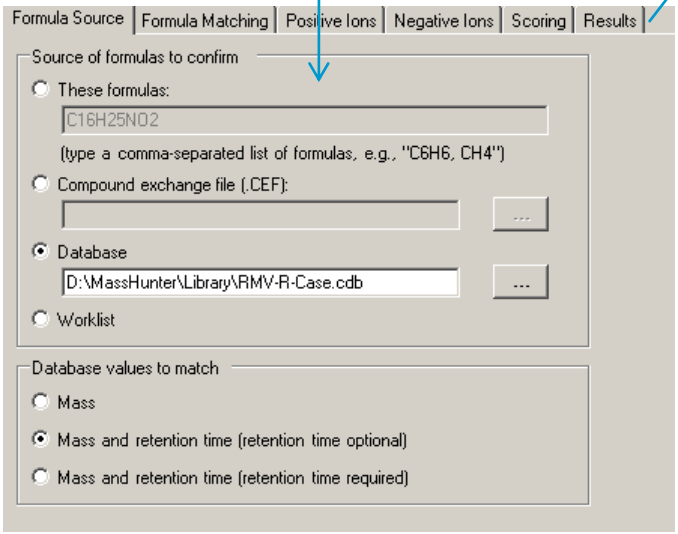
Ion Information



Ion chromatogram drawn from information

Spectra extracted from integrated peak





Compound information from formula or Database

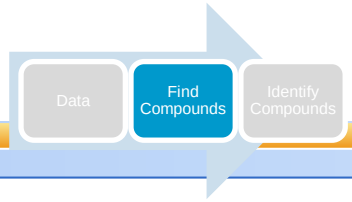
m/z	Ion	Formula	Abundance
264.1961	(M+H)+	C16 H26 N O2	117959.3

Best	Formula (M)	Ion Formula	Score	Cross Score	Calc m/z	Diff (ppm)	Mass Match	Abund Match	Spacing Match
☑	C16 H25 N O2	C16 H26 N O2	99.64		264.1958	-1.02	99.41	99.87	99.82

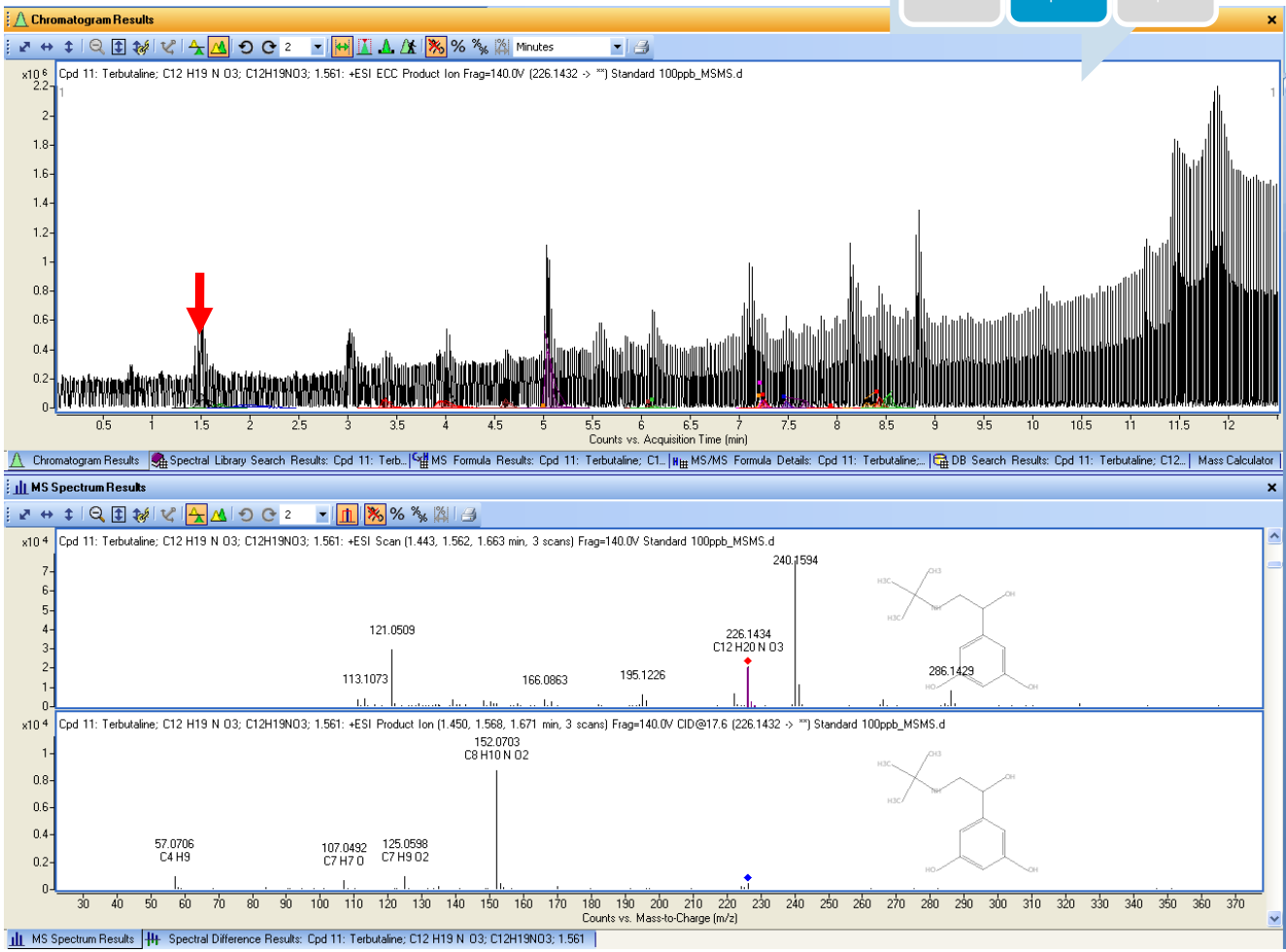
Isotope	Calc Abund Sum%	Abund Sum%	m/z	Calc m/z	Diff (ppm)
1	83.23	83.49	264.1961	264.1958	-1.01
2	15.02	14.75	265.1995	265.1991	-1.61
3	1.62	1.64	266.202	266.2019	-0.37
4	0.13	0.12	267.2029	267.2045	6.04

Spectral performance calculated

Find by Auto MS/MS or Targeted MS/MS

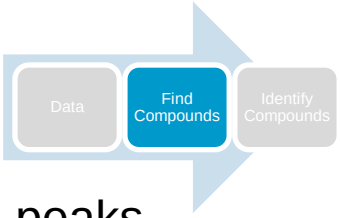


Find by Auto MS/MS or Targeted MS/MS automatically extract the MS and MS/MS information, aligns the spectra sorts and display the according spectra as a single compound. The data can be used to calculate empirical formulas for MS and MS/MS. In addition the information can be used to search the database with the MS data and library search the MS/MS data using the accurate mass MS/MS spectral library.



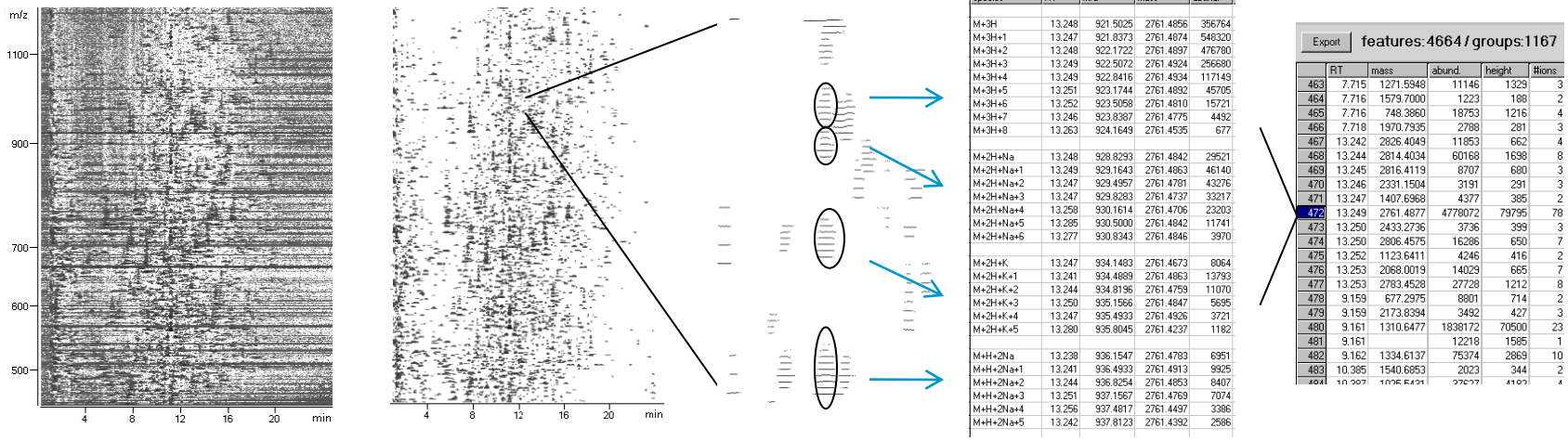
Qual B.04 extracts MFE from Auto MSMS and aligns possible product ion spectra to found features. This allows for a database search of accurate MFE compounds and an MSMS library search of the attached product ion spectra.

Molecular Feature Extractor (MFE)



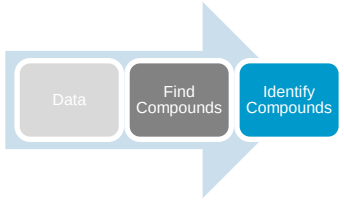
- MFE is extremely fast
  - Does not use exhaustive EIC extraction
  - Uses total ion count abundance information, not just base peak
  - Can yield more false negatives than exhaustive approach

- Find chromatographic peaks
  - Subtract background
  - Locate the groups of co-variant ions in each chromatogram;
    - Adducts, dimers, isotopes ($[M+H]^+$, $[M+H+1]^+$,…
  - Sum all ion signals into one value



Raw data → Background noise removed → Individual m/z peaks grouped into isotope clusters → Isotopes grouped into features

Databases and Libraries



Database

Consists of

- Name
- Empirical formula
- Accurate monoisotopic mass

Optional

- Structure
- CAS_Number
- Chemspider Number
- Retention time

Library

Same as database plus structure

In addition it contains

- Spectra from
- Different collision energies
- Different ion sources
- Different polarities

Q-TOF- Software Tools

MassHunter B.05. (Windows 64 bit)

- MassHunter Aquisition
  - Auto MS/MS
- MassHunter Qual
  - MFE (Molecular Feature Extractor)
  - Find by formular
  - MSC (Molecular Structure Correlator)
- PCDL Manager
- MassHunter Quant

PCDLs (Datenbanken und Bibliotheken)

Met ID

MPP (Mass Profiler Professional)

Qualitative und Quantitative Analyse

Bekannt, Unbekannt und Unerwartet

„unknown screening“

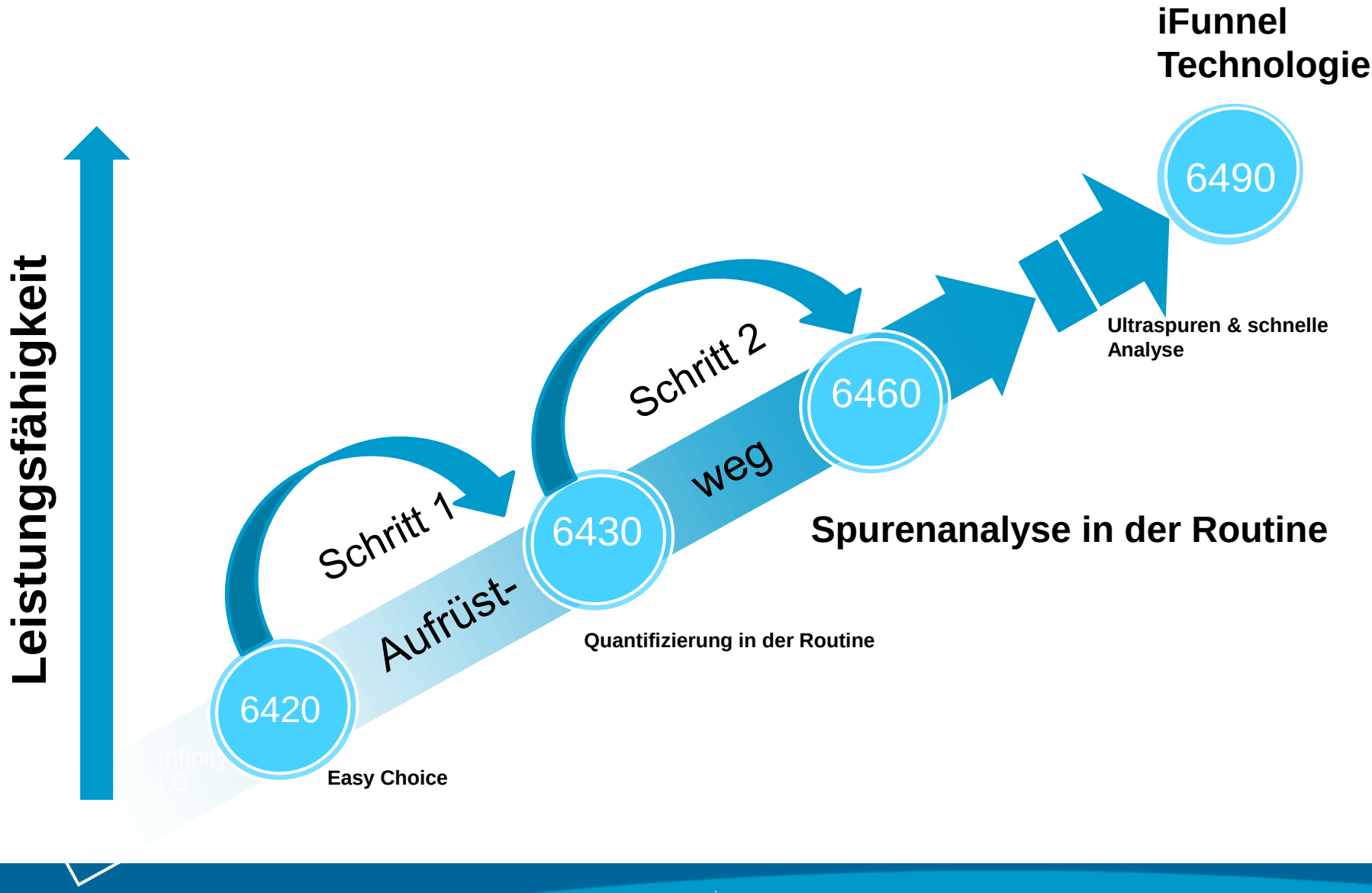
“Target Screening “

Quantifizierung

nicht-selektiv

selektiv

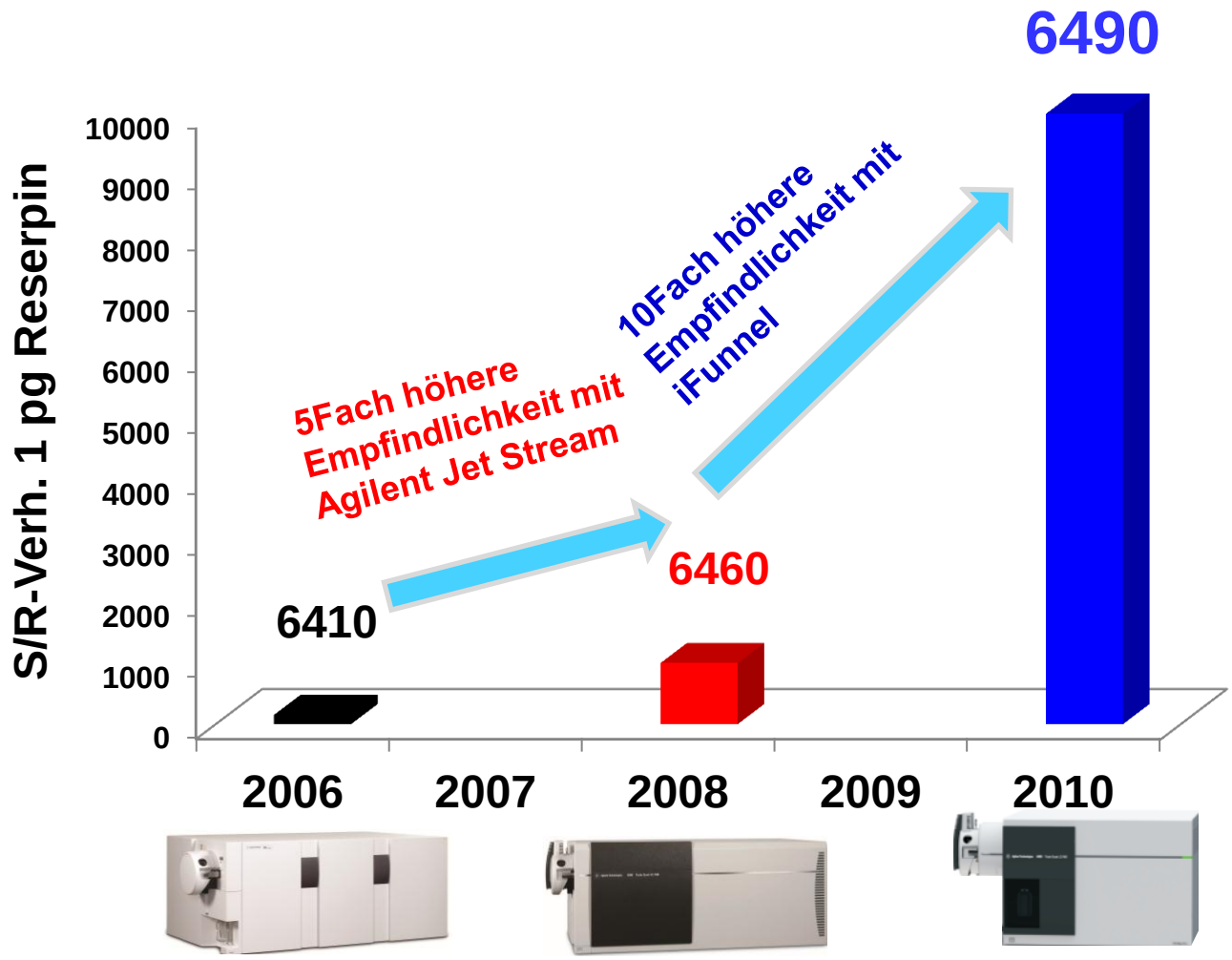
Agilent QQQ-Systeme - Aufrüstbarkeit…



6460 und 6490 Spezifikationen

Specification	6460	6490
S/N 1 pg reserpine	1,000:1	10,000:1
Mass Range (m/z)	3,000	1,400
Mass Resolution	0.7 Da	0.4 Da
Scan Speed, Da/s	5,000	10,000
Polarity Switching	30 msec	30 msec
MRMs/Sec no cross-talk	200	300
Data Dependent	YES	YES
Triggered MRM	YES	YES

Der neue 6490: Verbesserung der Empfindlichkeit um Faktor 50 in 4 Jahren

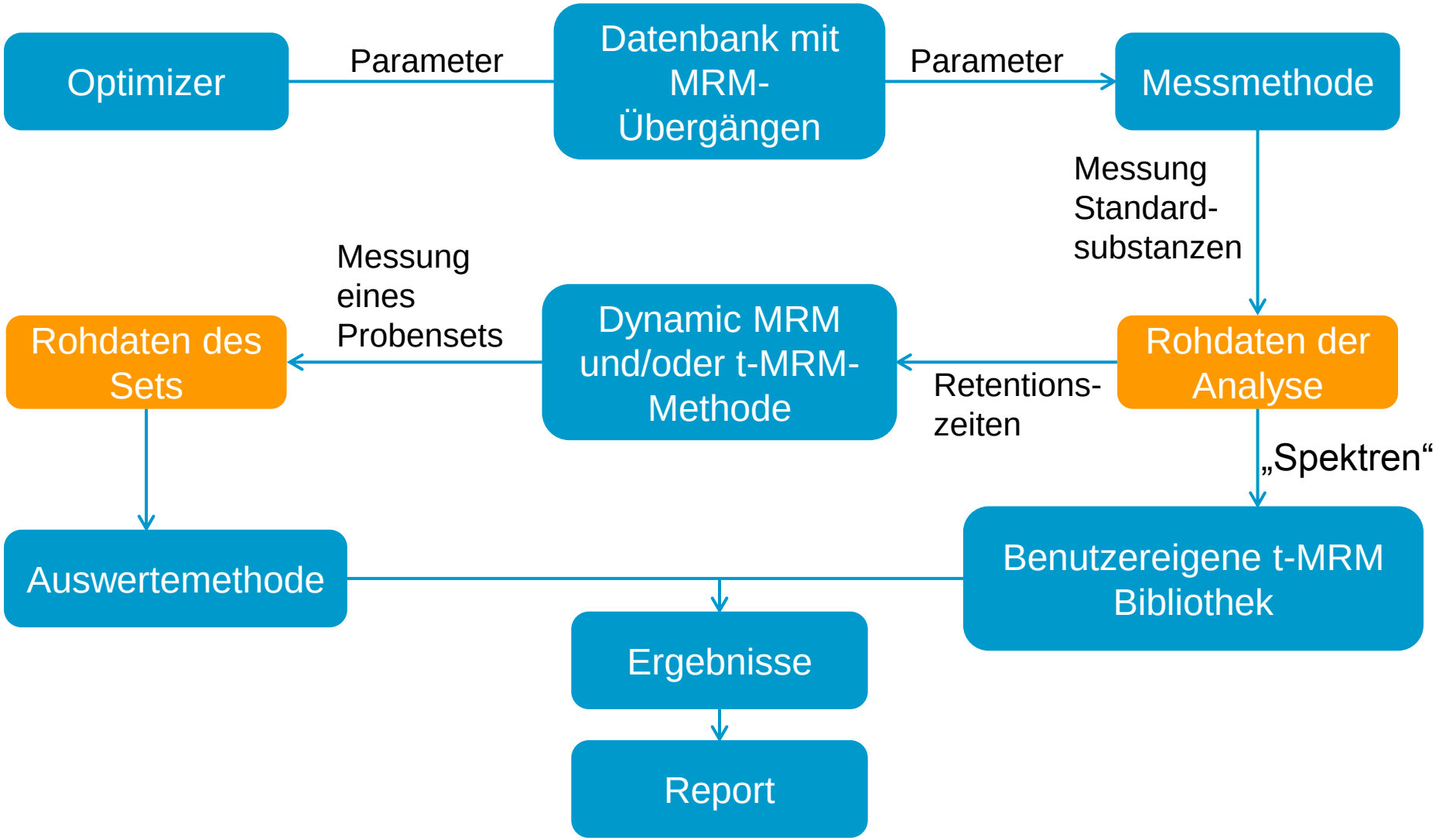


QQQ- Softwaretools

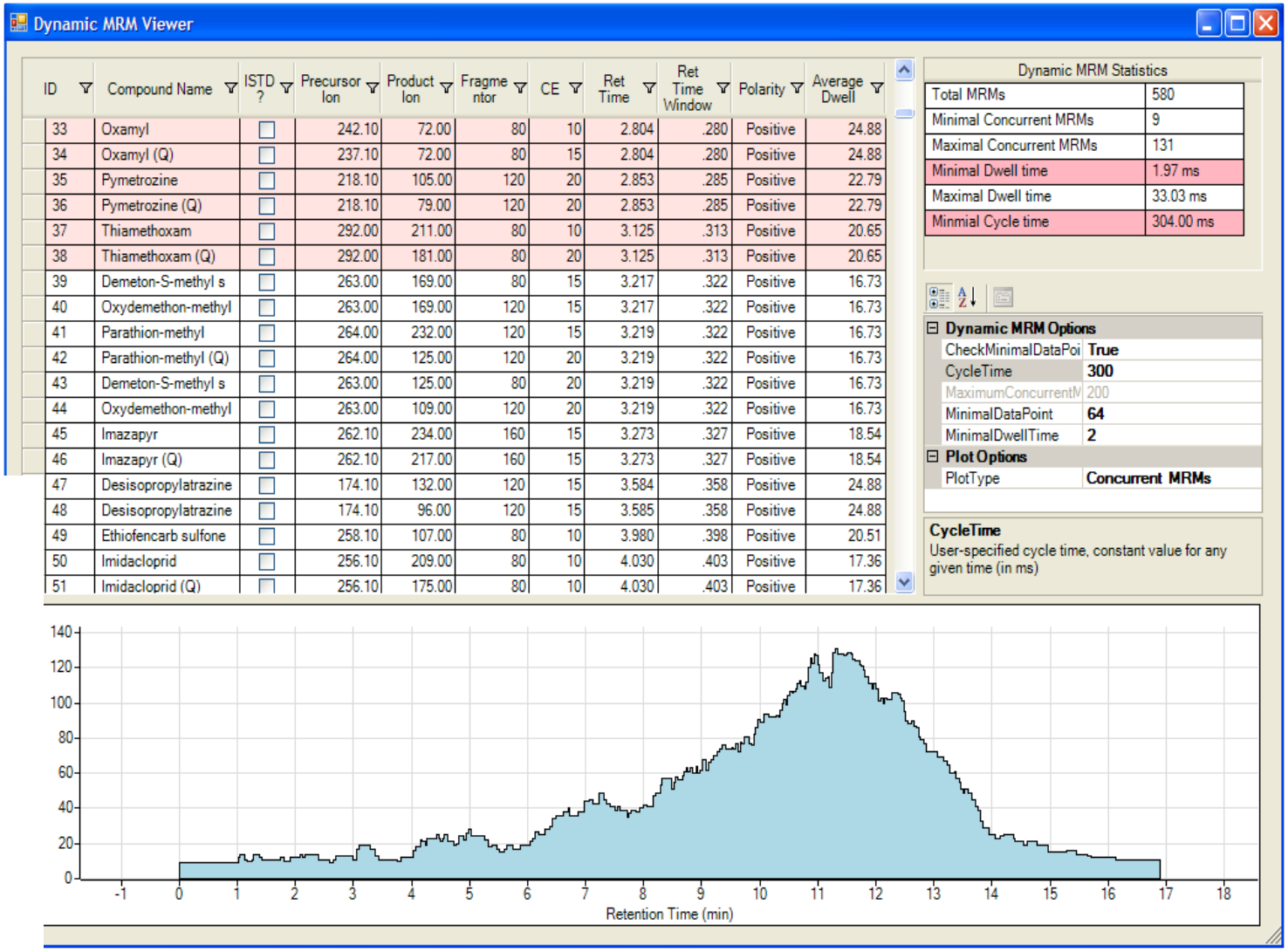
MassHunter

- Optimizer
  - d-MRM Datenbanken
- MassHunter Aquisition
  - dynamic MRM
  - Triggered MRM
- MassHunter Quant

QQQ Software: Entwicklung neuer Methoden



MRM viewer makes optimizing pesticides easy



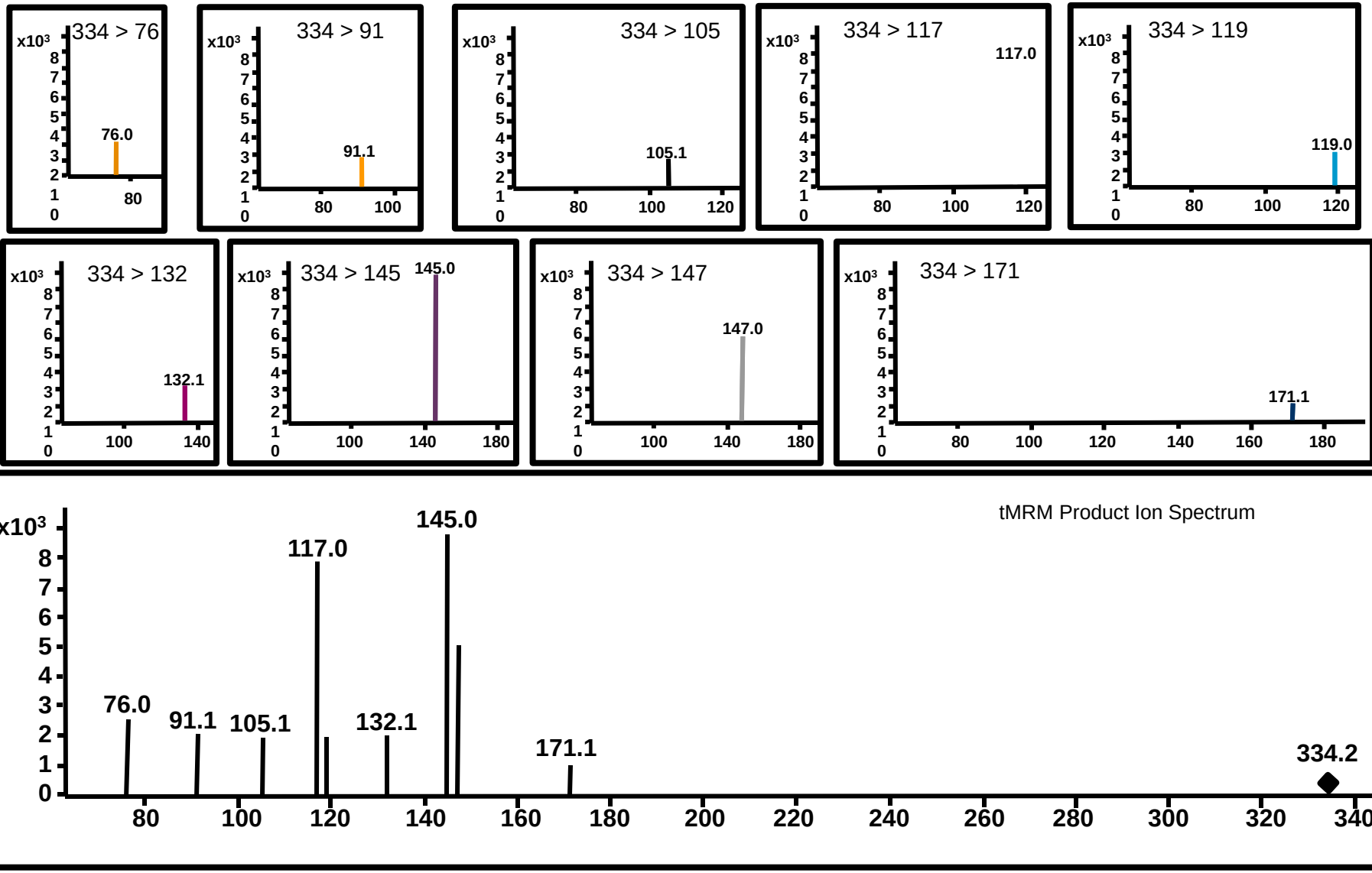
Min Dwell Time/method 2 msec

Max Dwell Time/method 33 msec

Max concurrent MRMs = 131

Min concurrent MRMs - 9

tMRM Product Ion Spectrum



tMRM Library Searching

Tebufenpyrad Standard 50 ppb

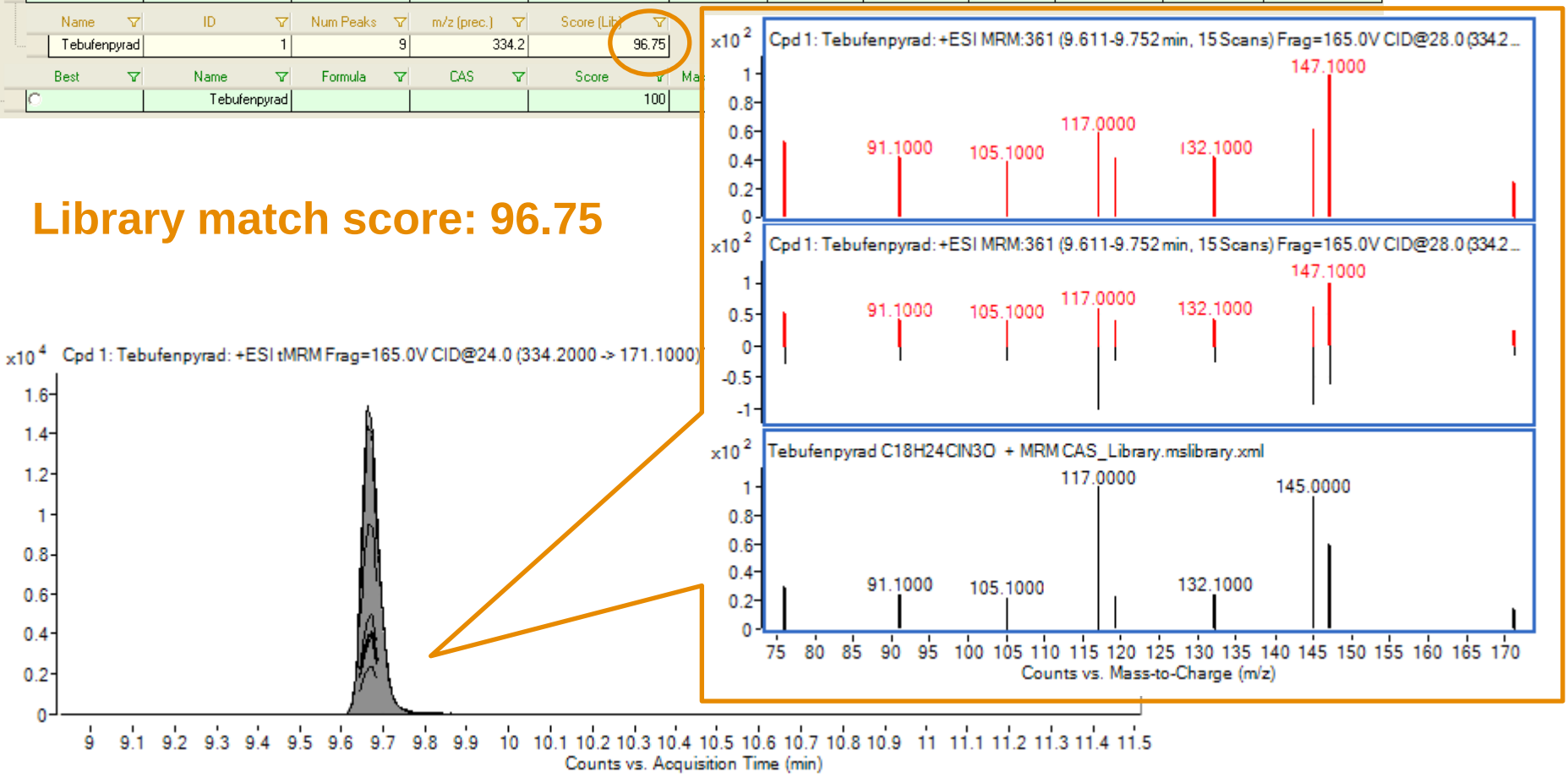
Show/Hide	Label	Polarity	Cpd	Name	Score	Start	RT	End	Width	m/z	Mass (DB)
☑	Cpd 1: Tebufenpyrad	Positive	1	Tebufenpyrad	96.75	9.602	9.664	9.761	0.046	334.2	333.8557

Best	Name	Formula	CAS	Score	Mass (DB)	RT	Score (Lib)	Precursor	Find by MRM	Score (Acq)	ID Source
◉	Tebufenpyrad	C18H24ClN3O	119168-77-3	96.75	333.8557	9.664	96.75				LibSearch

Name	ID	Num Peaks	m/z (prec.)	Score (Lib)
Tebufenpyrad	1	9	334.2	96.75

Best	Name	Formula	CAS	Score
○	Tebufenpyrad			100

Library match score: 96.75



Agilent Technologies

Summary

- **Ionization Sources**
- **6100 Single Quad Series**
- **6500 QTOF Series**
- **6400 QQQ Series**

7200 Series Q-TOF for GC/MS

Das GC-QTOF zeigt neue Wege in der GC-MS

5975E SQ

5975C SQ

5975T LTM SQ

ASMS 2011

More Choices – Better Solutions

Agilent GC/MS & GC/MS/MS

220 IT

240 IT

7000 TQ

7200 Q-TOF

Was kann dieses System leisten?

TOF mode

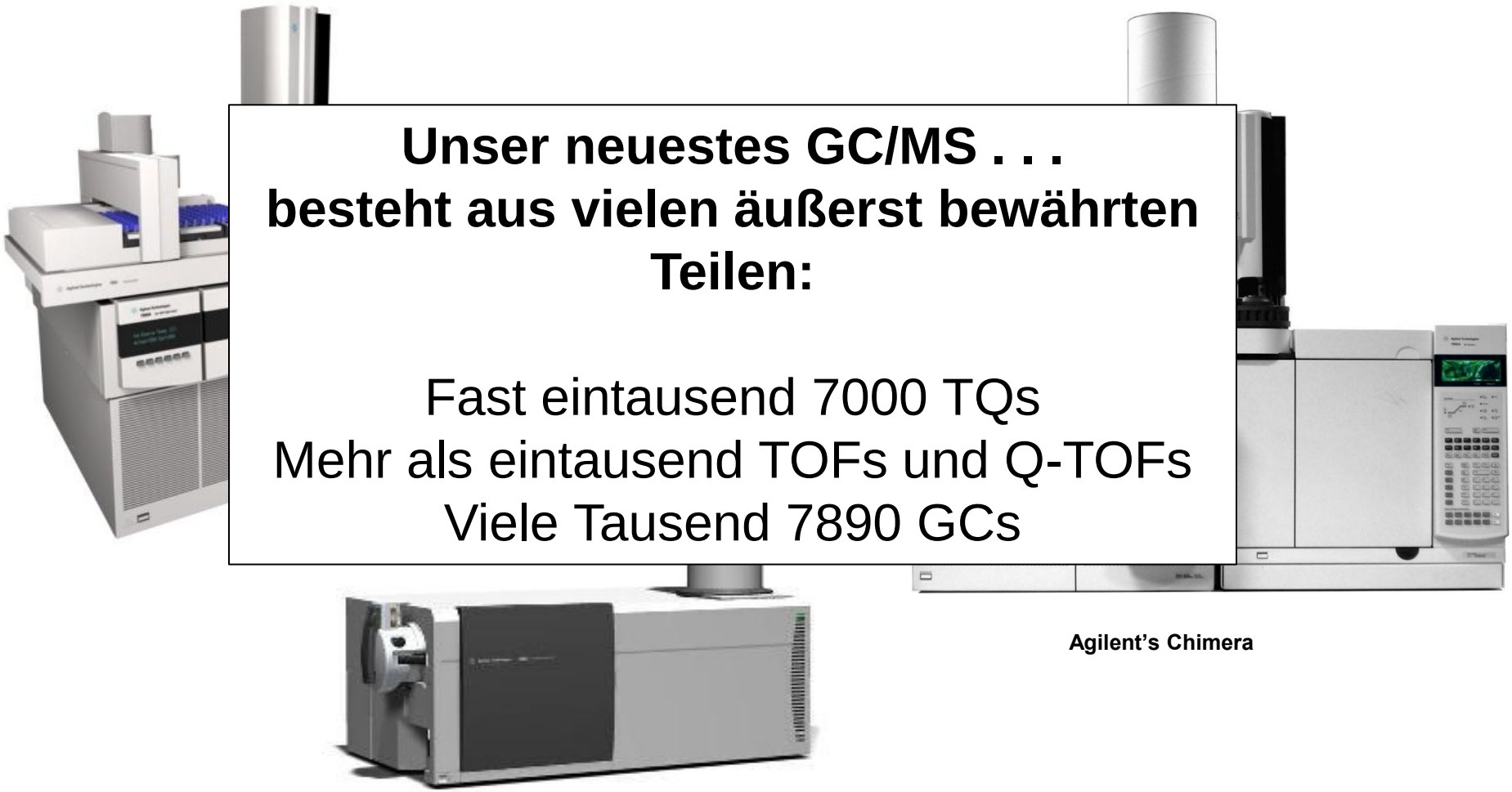
- Hoch Auflösende Full Scan Spektren
- Genaue Massenbestimmung
- Schnelles Scannen von Spektren im FullScan

MS/MS mode

- Triple Quadrupol MS/MS Product Ionen Spektren
- Full Scan Product Ionen Spektren – Hochauflösung genaue Massen

Ideales Werkzeug zur Lösung komplexer analytischer Probleme

7890 + 7000 + 6500 = 7200 GC/Q-TOF



Unser neuestes GC/MS . . .
besteht aus vielen äußerst bewährten Teilen:

Fast eintausend 7000 TQs
Mehr als eintausend TOFs und Q-TOFs
Viele Tausend 7890 GCs

Agilent's Chimera

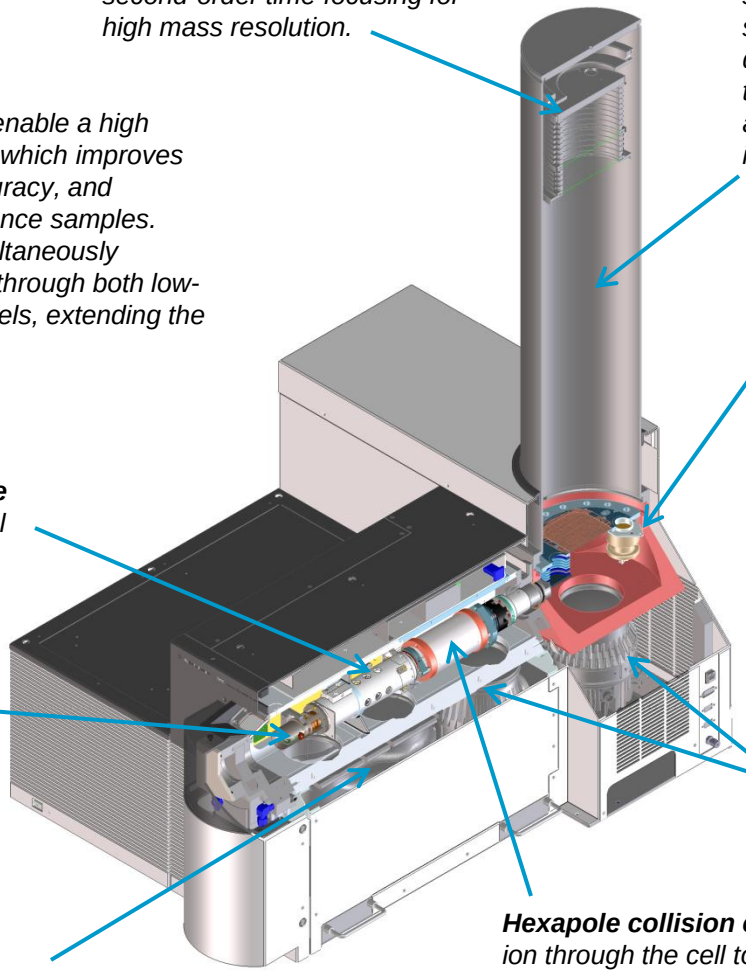
Neu . . . und dennoch bewährt

From 3 pg of brochure

Dual-stage ion mirror *improves second-order time focusing for high mass resolution.*

Proprietary INVAR flight tube *sealed in a vacuum-insulated shell eliminates thermal mass drift due to temperature changes to maintain excellent mass accuracy, 24/7. Added length improves mass resolution.*

4GHz ADC electronics *enable a high sampling rate (32 Gbit/s) which improves the resolution, mass accuracy, and sensitivity for low-abundance samples. Dual gain amplifiers simultaneously process detector signals through both low-gain and high gain channels, extending the dynamic range to 105.*



Analog-to-digital (ADC) Detector*: Unlike time-to-digital (TDC) detectors which record single ion events,* ADC detection *records multiple ion events, allowing very accurate mass assignments over a wide mass range and dynamic range of concentrations.*

Hot, quartz monolithic quadrupole analyzer and collision cell *identical to the 7000 Quadrupole MS/MS*

New ***Internal Reference Mass*** *can be delivered to the source at a low and high concentration*

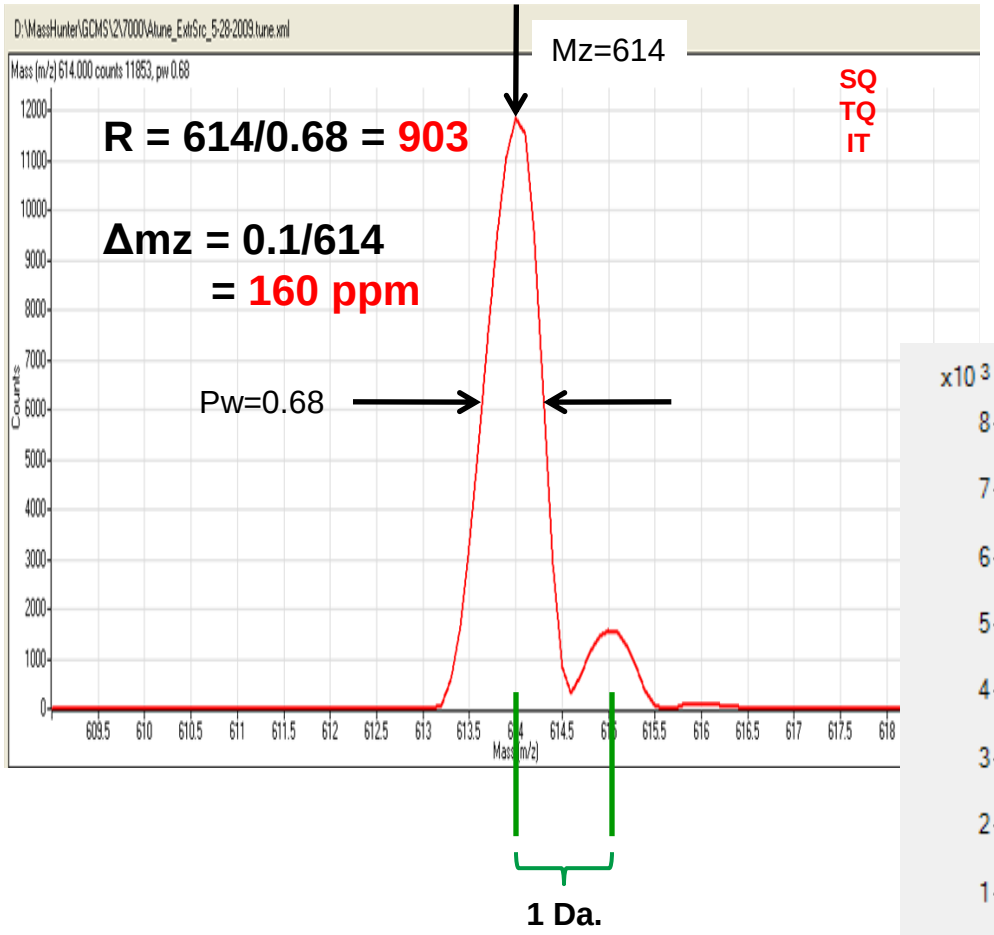
New ***Removable Ion Source*** *includes repeller, ion volume, extraction lens and dual filaments*

Two 300L/s t urbos *pump the focusing optics and flight tube*

Split-flow turbo *differentially pumps the ion source and quadrupole analyzer compartments*

Hexapole collision cell *accelerates ion through the cell to enable faster generation of high-quality MS/MS spectra without cross-talk*

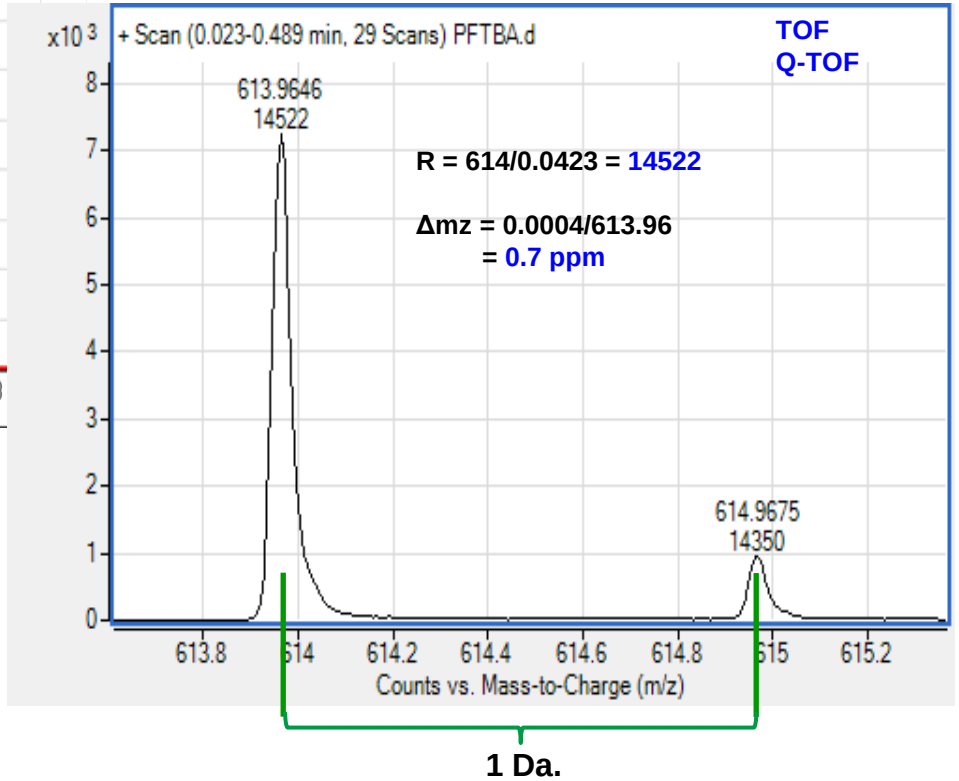
Auflösung & Massengenauigkeit



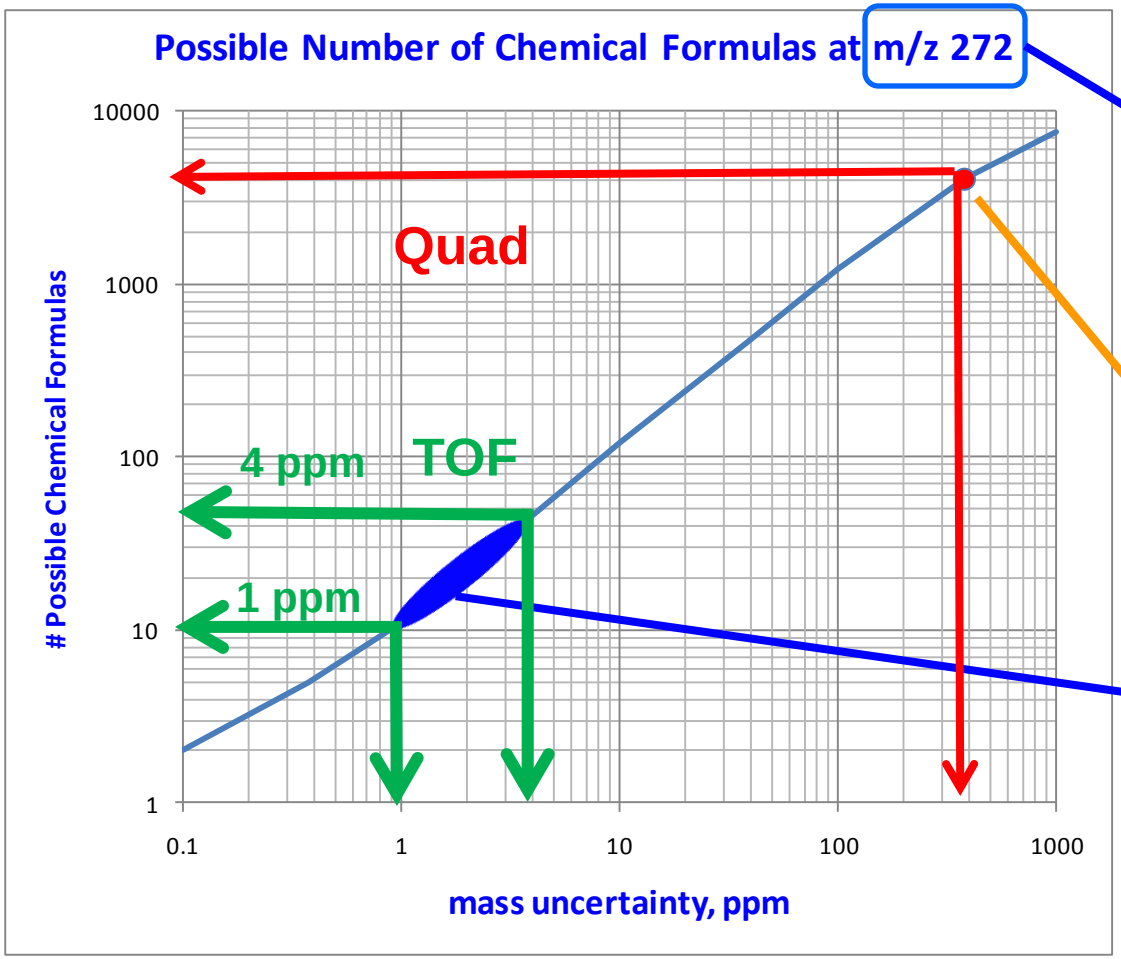
Auflösung “Resolving Power:”
R=mz/FWHM
(FWMH = full width at half maximum)

Massengenauigkeit:
$\Delta mz = dm/mz * 10^6$, parts per million (ppm)

PFTBA mass 614
C12F24N=613.964203



Viele mögliche Formeln mit MSD oder IT
Aber nur einige wenige mit TOF



Octafluoronaphthalene (CAS 313-72-4)

$C_{10}F_8 = 271.98667$

mass uncertainty		# of Possible Formulas
ppm	amu	
1000	0.3	7657
368	0.1	4050
100	0.03	1223
37	0.01	466
10	0.003	120
4	0.001	43
1	0.0003	11
0.4	0.0001	5
0.1	0.00003	2

Formulas made of: C,H,N,O,F, & Cl

Genaue Massen verringern das Risiko in das falsche Molekül Arbeit zu investieren

7200 Instrument Performance Spezifkationen…

Auflösung: >12.5 K at m/z 272, typisch 13500

Massen Genauigkeit: <5 ppm at m/z 272

MS Empfindlichkeit ist zwischen SIM und Scan eines Single Quad Instrument

MS/MS Empfindlichkeit ist zwischen SRM und Produkt Ionen Scan eines Tandem Quad Instrument

Dynamischer Bereich: 3-5

Quad Massen Bereich: 20-1050 Da (0.7-4.0 Da FWHM)

TOF Massen Bereich : 20-1700 Da

Spektrale Rate: 1-50 Spektren/sec

Zusammenfassung – was Sie beachten sollten

- Die Welt um uns ändert sich dauernd und neue Probleme können jederzeit auftreten
- Neue Probleme erfordern oft neue Instrumente um eine Lösung zu finden
- Das GC Q-TOF bietet Leistungsmerkmale um neue Probleme auf neue Weise zu lösen
- Höhere Auflösung (HR), bessere Massen Genauigkeit schnellere Scan Geschwindigkeit verbessern immer die analytischen Ergebnisse
- Erweitere MS/MS Produkt-Ionen durch HR und MA und Strukturaufklärung wird möglich

- **Agilent bietet die größte Palette leistungs-fähiger GC/MS Geräte – SQ, IT, TQ, & Q-TOF**

Vielen Dank !!!

Fragen ???

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