# Bruker Corporation

# 50 Years of Bruker History

| | |
|---|---|
| **1960** | Bruker was founded in 1960 with NMR products |
| **1974** | First Bruker FT-IR developed internally |
| **1980** | *Bruker acquires/develops mass spec business, later called Bruker Daltonics* |
| **1997** | Bruker acquires analytical X-ray business from Siemens, called Bruker AXS |
| **2000** | Bruker Daltonics IPO |
| **2001** | Bruker AXS IPO |
| **2003** | Merger of Bruker Daltonics and Bruker AXS into Bruker BioSciences (BRKR) |
| **2006** | Bruker BioSciences acquires Bruker Optics |
| **2007** | Bruker BioSciences (BRKR) and separate private Bruker BioSpin (NMR/MRI) |
| **2008** | Merger of Bruker BioSciences and Bruker BioSpin into Bruker Corporation (BRKR) |
| **2010** | Acquisition of GC, GC-QQQ and ICP-MS Technologies |

# Global Company with Diversified Markets

- Over 5,500 Employees in over 90 Countries around the world

# Bruker Corporation

- **Bruker AXS** –X-ray, optical emission spectroscopy, atomic force microscopy and stylus and optical metrology instrumentation
- **Bruker BioSpin** – Magnetic resonance spectroscopy and imaging (NMR, MRI, EPR)
- **Bruker Daltonics** - Mass spectrometry, gas chromatography and CBRN detection
- **Bruker Optics** – Optical Vibrational Spectroscopy (FT-IR , NIR & Raman)

**Bruker Energy & Supercon Technologies (BEST)**

- Super conducting magnets, high and low temperature wire development and manufacturing

BRUKER

Innovation with Integrity

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# Highlighting the new Impact HD QToF for Screening & Identification of Unknowns and Sample Data Review

Sandy Yates, Bruker

Impact HD:
High-Definition bench-top UHR-QTOF

# Outline

- Impact HD Q-ToF for Screening
- Identification of Unknowns
- Sample Data Review

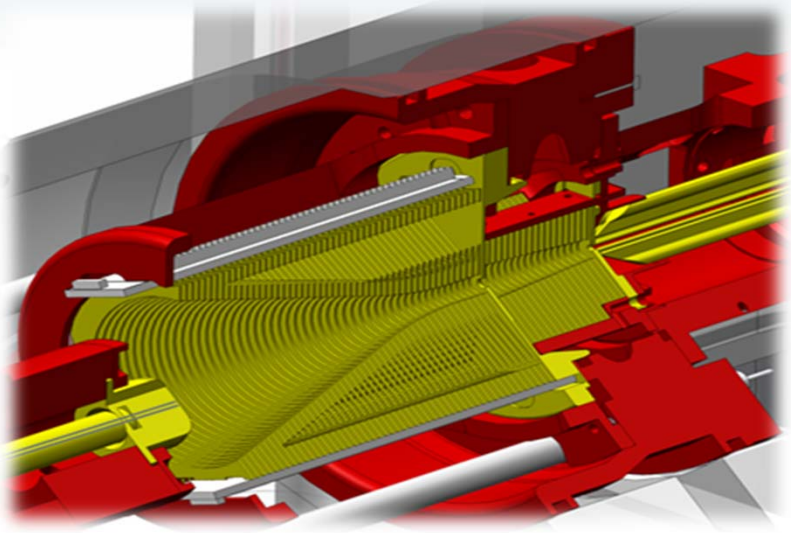
# Bruker's New impact HD Bench-top QTOF

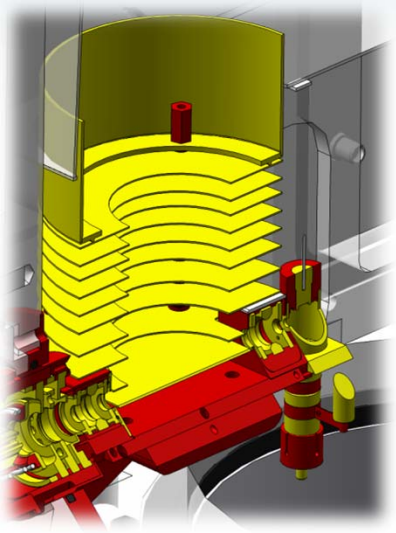
Introducing **High-Definition, Instant Expertise™** and in **Ultra-High performance LC-MS-MS** bench-top QTOF

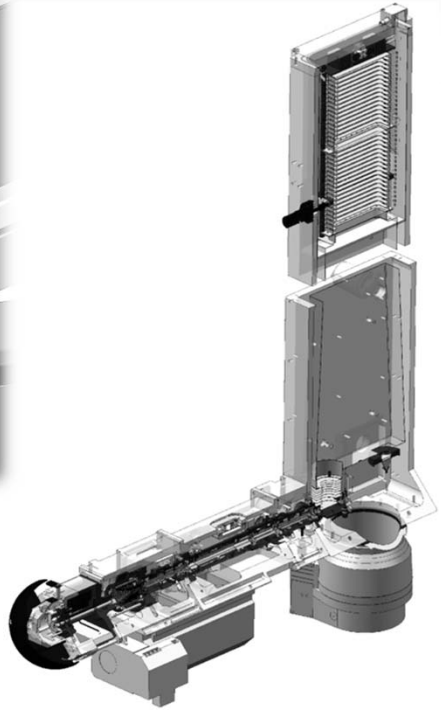
- **High Definition: 5 orders of magnitude dynamic range at UHPLC speed** from 10-Bit digitizer technology - definitive trace analysis from complex, high-background matrices
- Full mass resolution (40,000 @ m/z 1222) at full sensitivity
- **Instant Expertise: Intelligent dynamically optimizing** MS/MS for easier tandem MS work

## How does Impact HD achieve the performance?

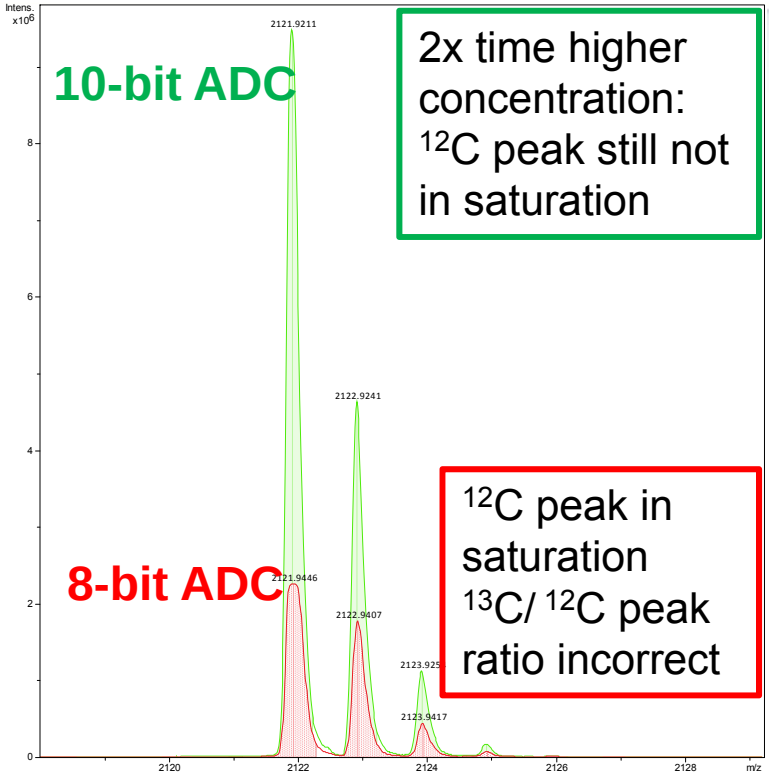
- Dual Ion Funnel Source ( Introduced 2005 )
- Increased sensitivity, reduced method tuning





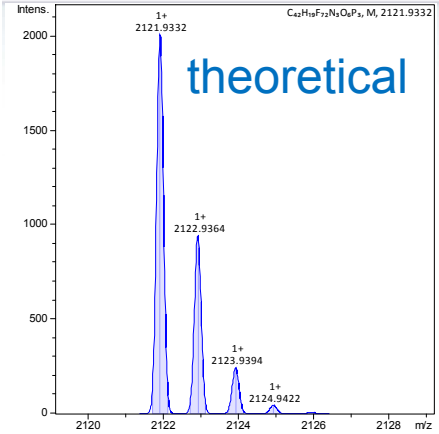


# Impact HD:
## More Dynamic Range = Accurate Quantitation




2x time higher concentration: $^{12}C$ peak still not in saturation

$^{12}C$ peak in saturation $^{13}C/^{12}C$ peak ratio incorrect



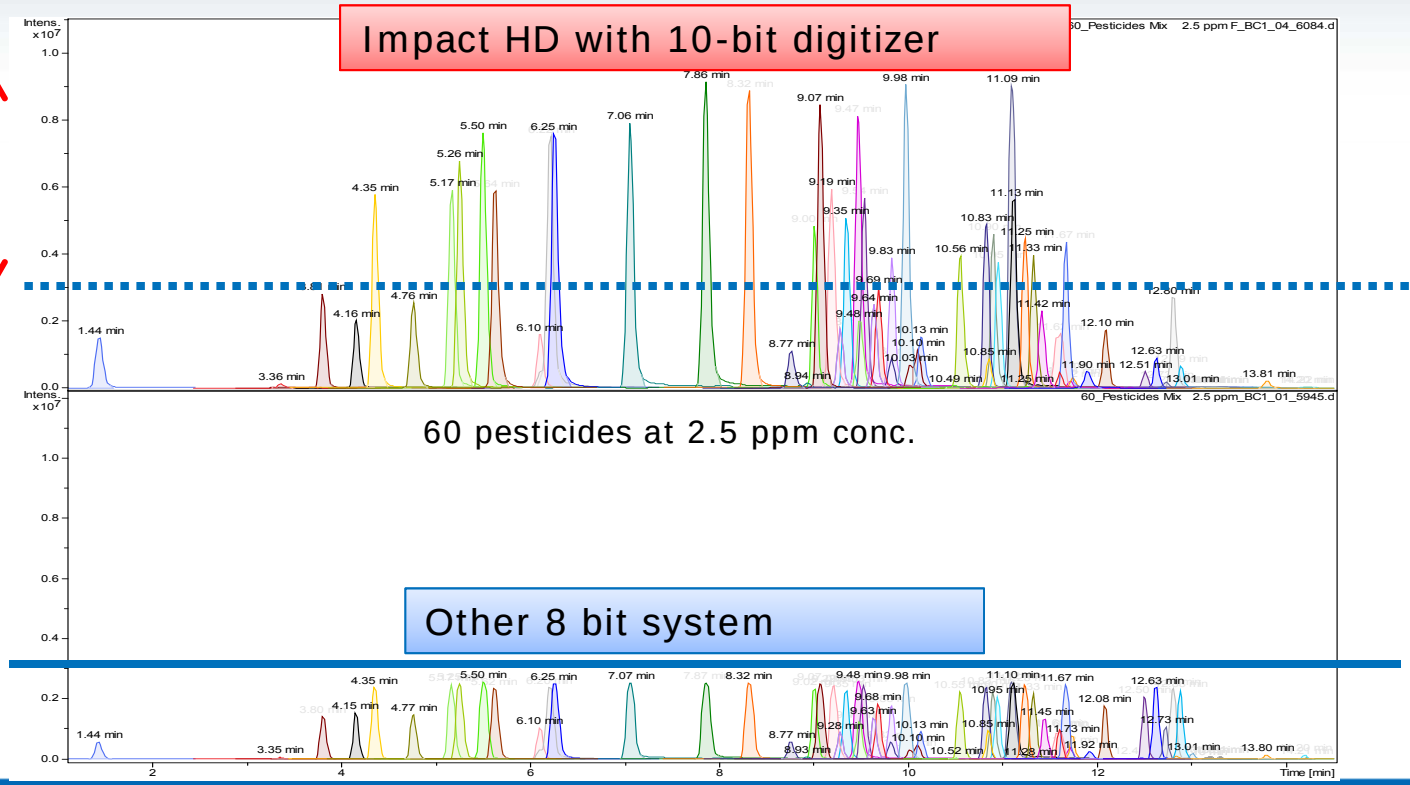

**Direct benefit for quantitation and identification**

- 3-4 x higher dynamic range from 10-bit ADC technology
- Provides highest sampling speeds and maintains high resolution

# Screening:
## more dynamic range - more confidence

Enhanced sensitivity through broad mass transfer

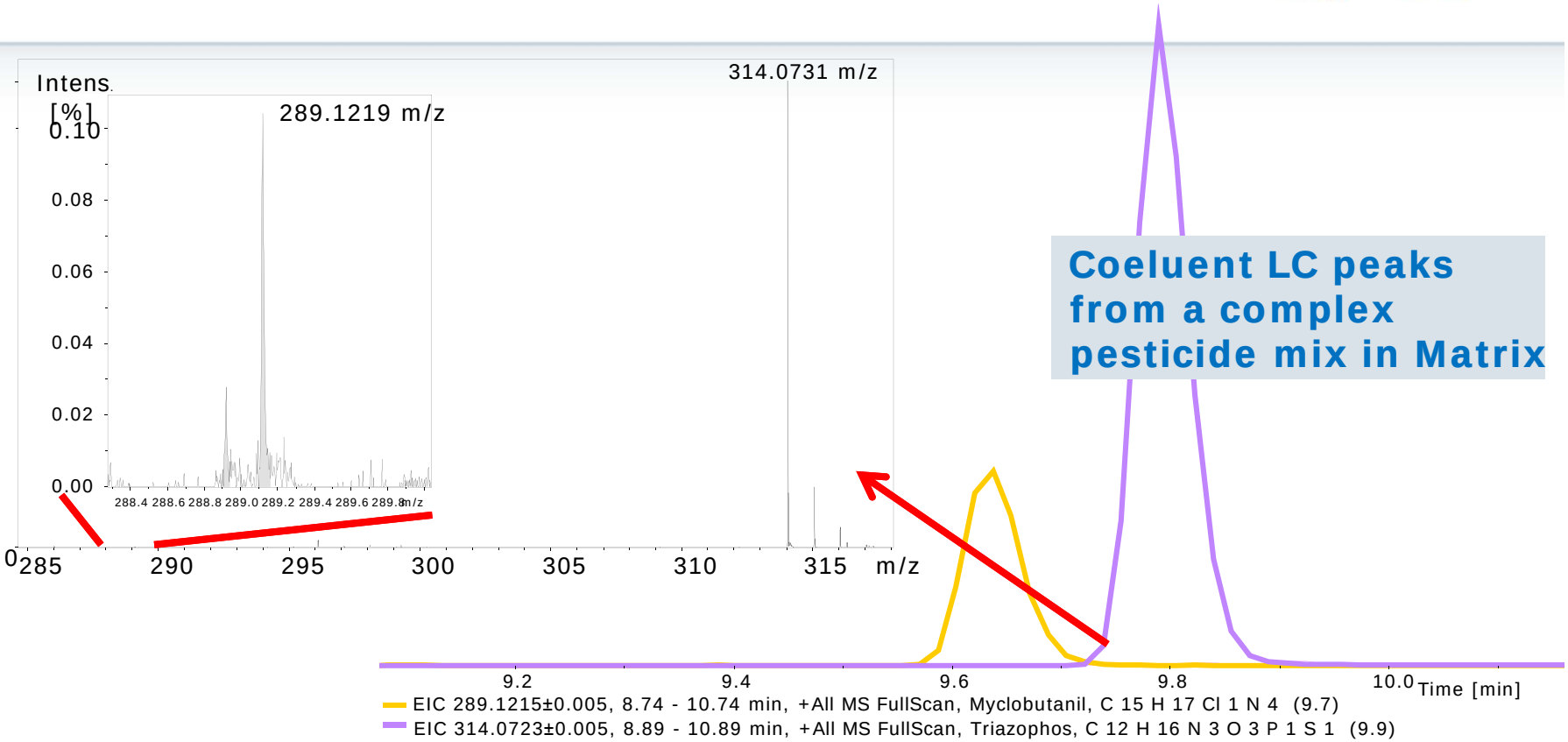
Gain in dynamic range




60 pesticides at 2.5 ppm conc.

Other 8 bit system

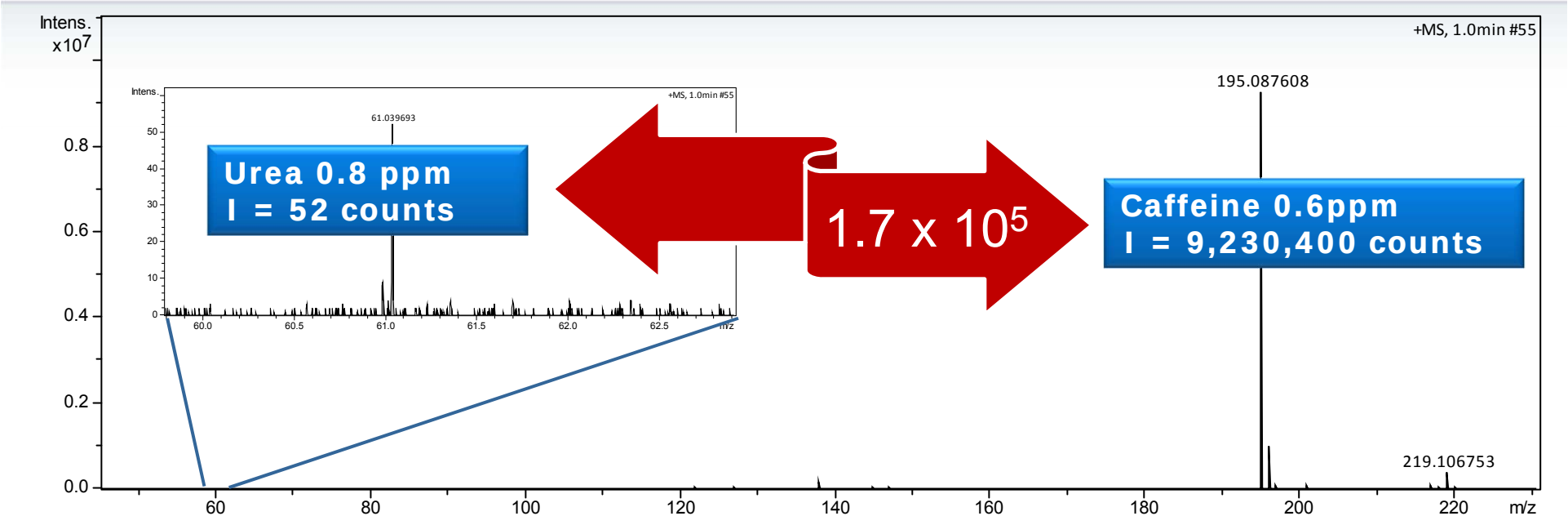
# Intra-Scan Dynamic Range




**Coeluent LC peaks from a complex pesticide mix in Matrix**

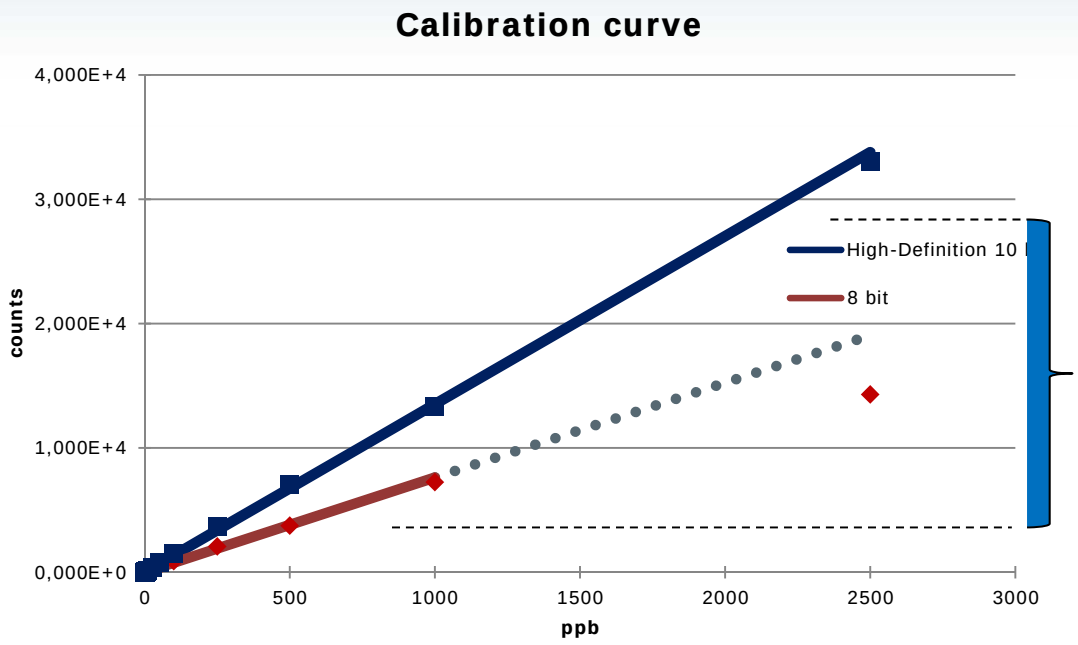
# 10-bit = Dynamic range at real LC speed

Much higher dynamic range in concentration reachable




**>5 orders of magnitude dynamic range @ 1 Hz speed LC timescale**

# High-Definition Quantitation with hrEIC over a large dynamic range



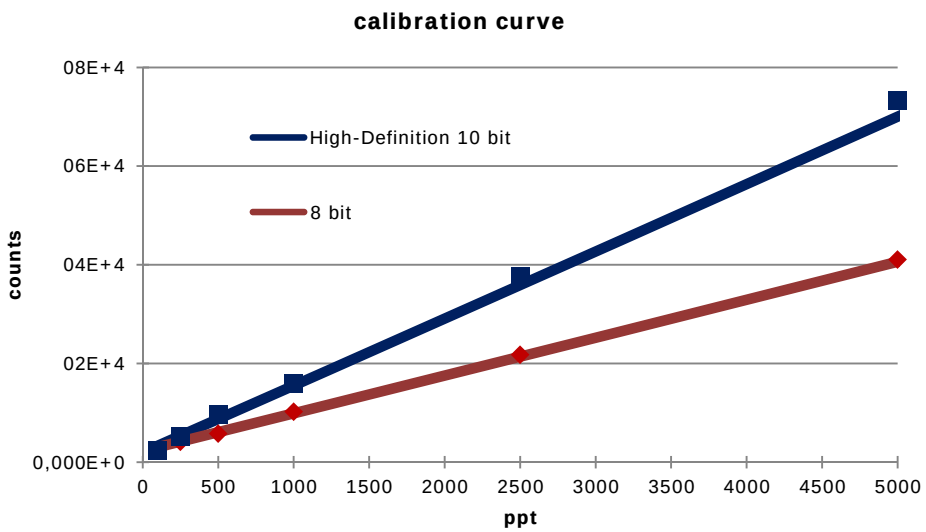

$y = 1995 + x*13,513$

$R^2 = 0.9996$

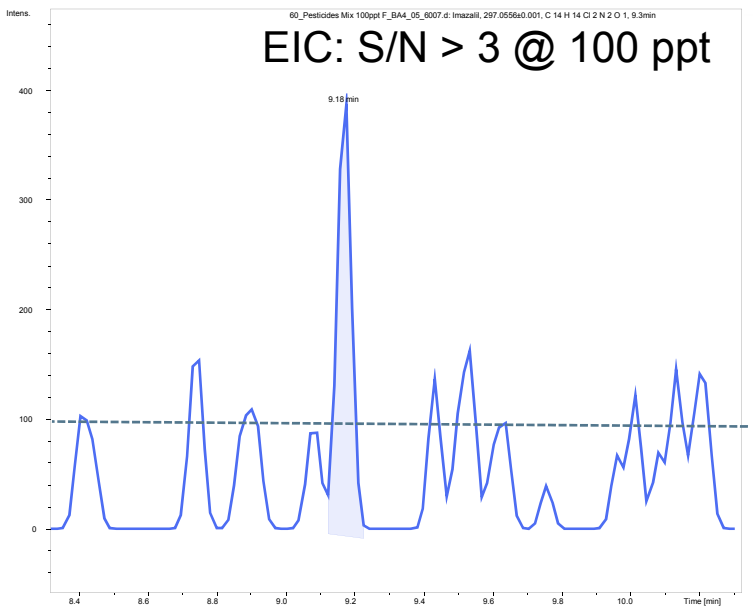
Gain in dynamic range with High-Definition 10-bit Digitizer

More than 4 orders of magnitude: 100 ppt up to 2.5 ppm

# High-Definition Quantitation with hrEIC




$R^2 = 0.9996$




Imazalil at 100ppt measured with Impact HD hrEIC @ 1 mDa width

# Data Dependant Tandem MS (AutoMS)

*Now made easy with Instant Expertise™*

# Instant Expertise:
# Optimal quant and ID results in One acquisition

Challenge: Classical Auto MS/MS use basically the same pre-set conditions for fragmentation, independent of the peptide amount and chromatographic resolution - dynamic range and MS/MS quality is compromised

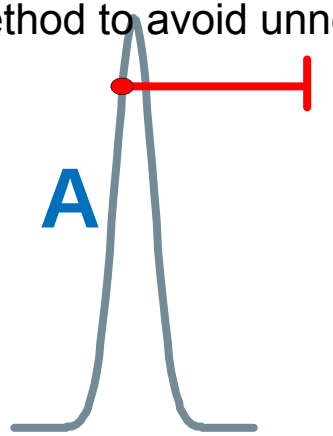
Solution: Instant Expertise Software with new autoMSMS strategies

- IDAS™ (Intensity Dependent Acquisition Speed)
- RT²™ (Real-Time Re-Think)

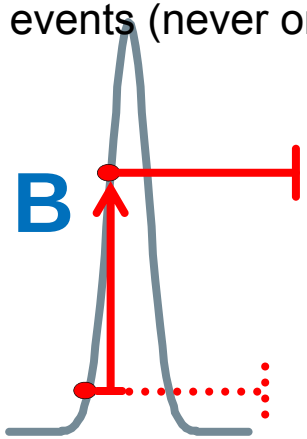
- ✓ **One acquisition method for all concentration levels**
- ✓ **Higher yield of excellent MS/MS spectra => higher ID rates**
- ✓ **Accuracy for quantitation**

# Instant Expertise™
# RT² : Real Time Re-Think

**R**eal**T**ime **R**e-**T**hink managements of the Dynamic exclusion. The basic exclusion time is fixed to slightly longer than the base width of the chromatographic peak. **Robust** method to avoid unnecessary AutoMSMS events (never on falling peak flank)




A: The MS/MS is triggered near the peak maximum
=> the precursor is not fragmented again

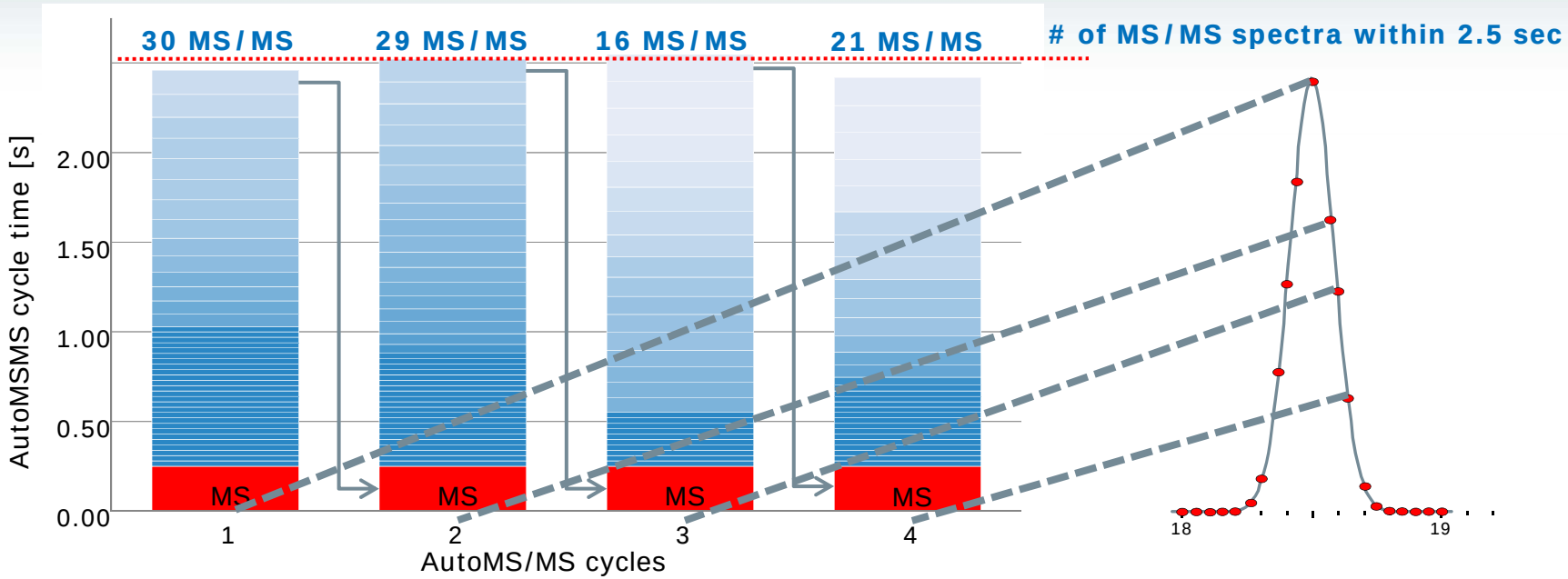



B: The MS/MS is triggered a first time near the peak base
=> the precursor is reconsidered for fragmentation

- **Higher yield of good MS/MS => higher ID rates**

# IDAS (Intensity Dependent Acquisition Speed)

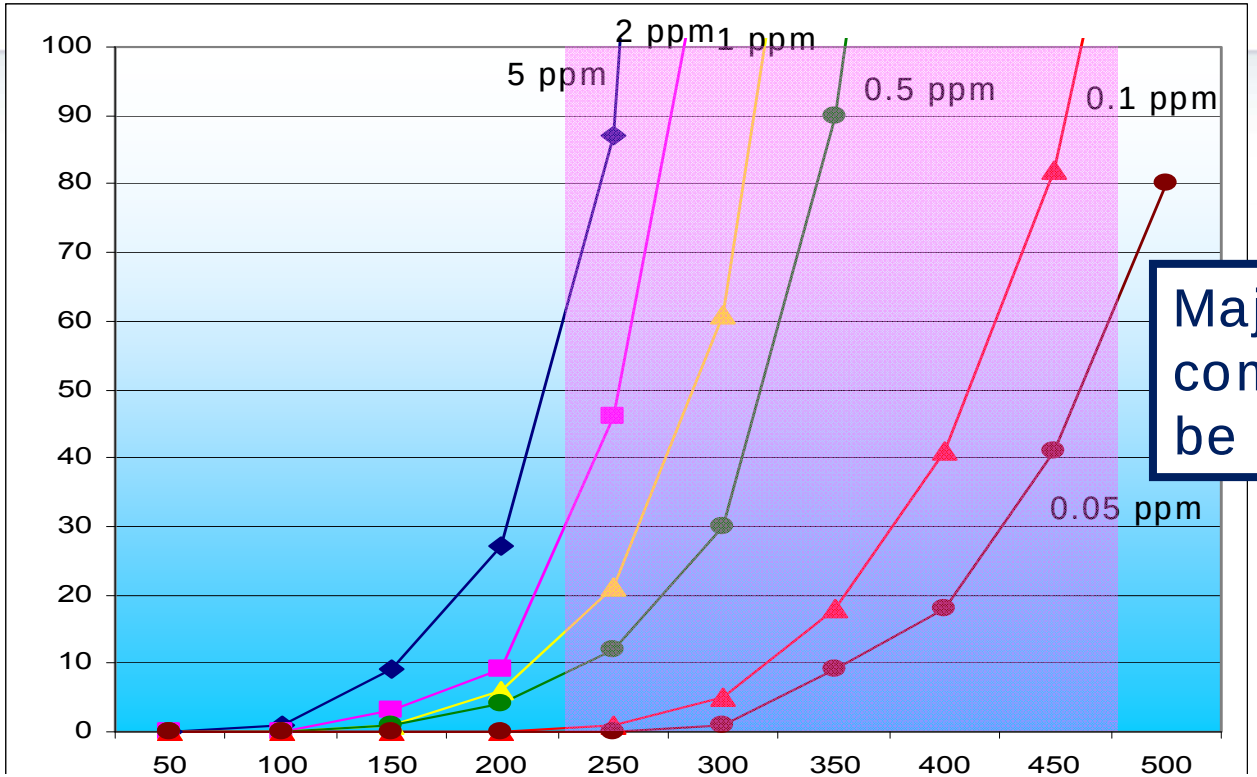
Number of MS/MS precursor ions automatically adjusted to precursor signal intensity




Always optimal results independent from sample amount or complexity

# Identifcations of unknowns

# Mass accuracy alone is insufficient for a unique compound **identification** or **confirmation!**




Majority of compounds to be analyzed

maximum of $C_{200}H_{400}N_{50}O_{50}S_5P_5F_5Cl_5Br_5I_5$, even electron configuration, nitrogen rule

BMC Bioinformatics

BioMed Central

Research article

Open Access

# Metabolomic database annotations *via* query of elemental compositions: Mass accuracy is insufficient even at less than 1 ppm

Tobias Kind* and Oliver Fiehn

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Email: Tobias Kind* - tkind@ucdavis.edu; Oliver Fiehn - ofiehn@ucdavis.edu

* Corresponding author

Published: 28 April 2006

*BMC Bioinformatics* 2006, **7**:234 doi:10.1186/1471-2105-7-234

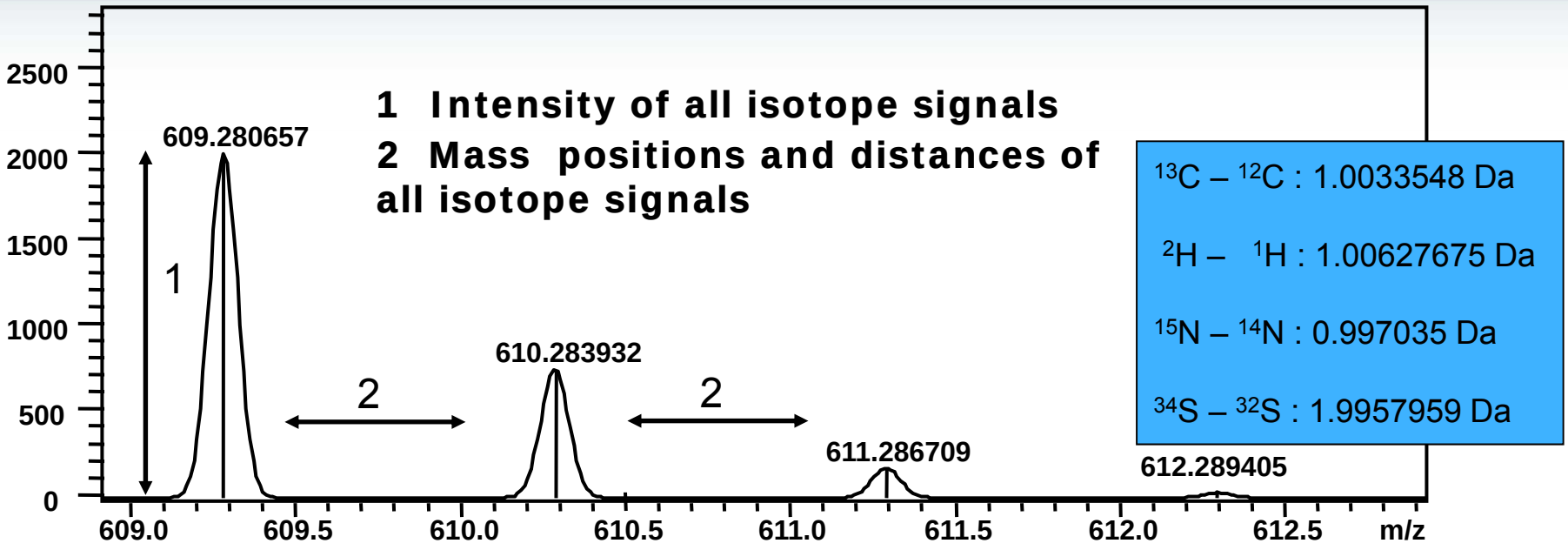
Received: 22 December 2005

Accepted: 28 April 2006

*… However, even with very high mass accuracy (<1 ppm) many chemically possible formulae are obtained in higher mass regions. In automatic routines an additional orthogonal filter therefore needs to be applied in order to reduce the number of potential elemental compositions. …*

## SigmaFit: used in SmartFormula(3D)

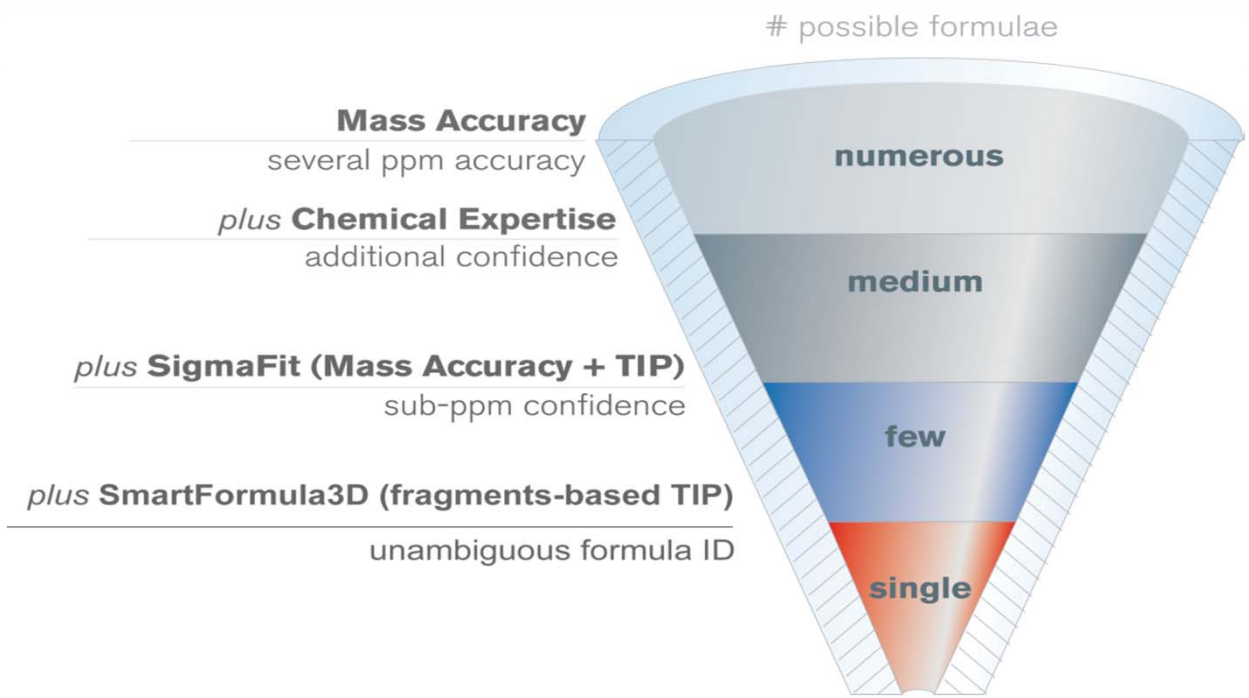
– Compares Isotope Pattern: measured versus theoretical




simulated spectrum of Reserpine $[M+H]^+$ $C_{33}H_{41}N_2O_9$

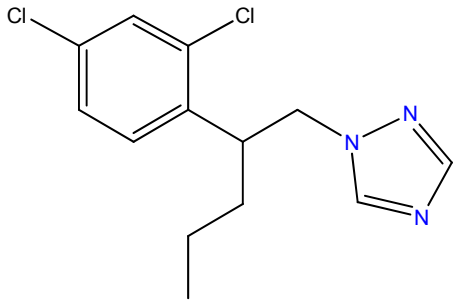
## Molecular Confidence Solution
- Identification of unknowns
- Verification of knowns (accurate mass & Isotopes )

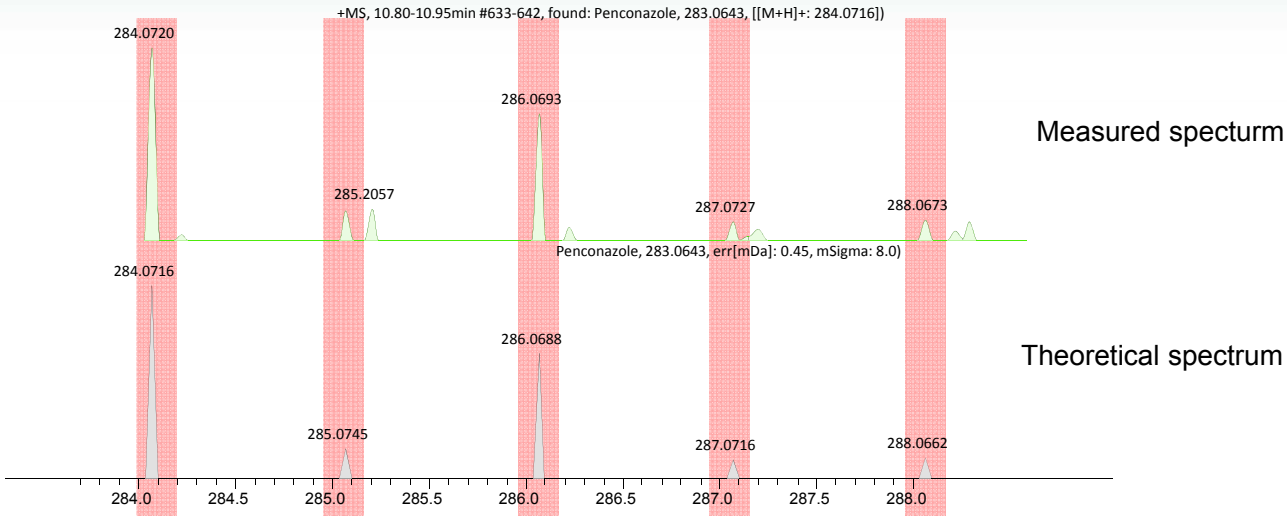
### Unambiguous Molecular Formula Determination with the new **SmartFormula3D**



# Confirmation of sum formulae with instant accurate mass and isotopic fidelity

Penconazole ($C_{13}H_{15}Cl_2N_3$)

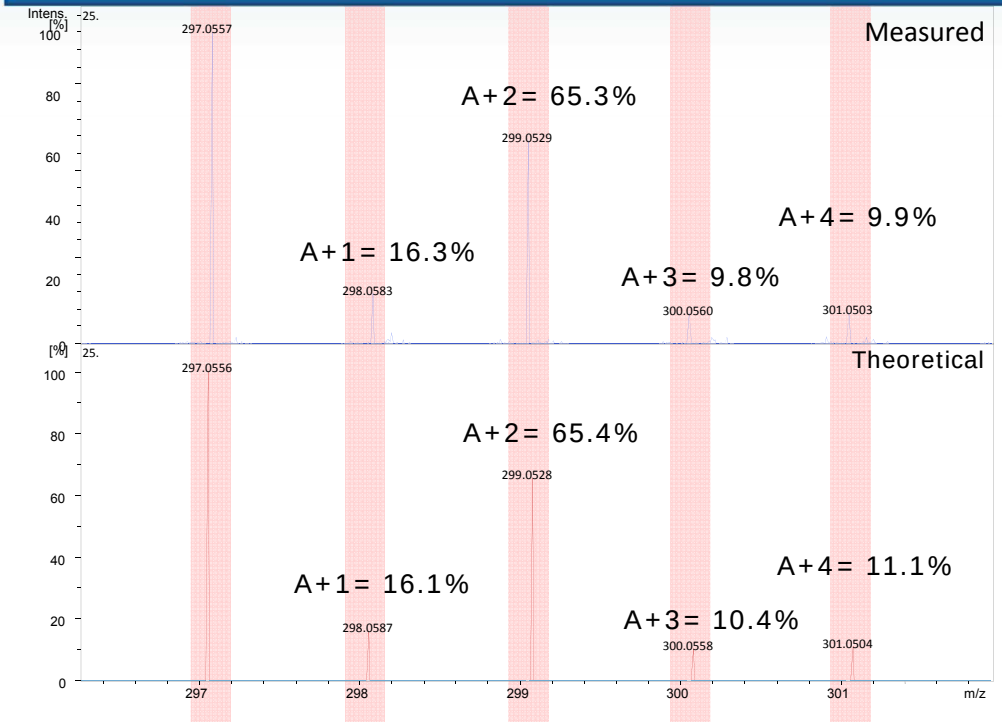



- ✓ **Perfect fit of isotopic ratios and spacing**
- ✓ **SmartFormula™ does confirm the identification in spite of matrix background signals.**

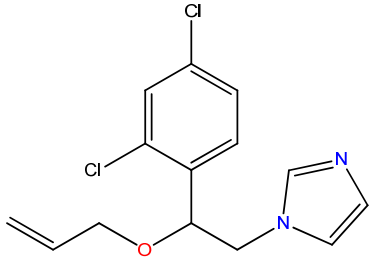
# Highest Confidence in Identification and Confirmation of Sum Formula due to Spectral Fidelity and SmartFormula™

## 10ppb Imazalil - 0.5 ppm mass accuracy - true isotopic pattern




- All 5 isotopes match
- Unambiguous identification of $C_{14}H_{15}Cl_2N_2O$, Imazalil with SmartFormula™

| Meas. m/z | # | Ion Formula | m/z | err [ppm] | err [mDa] | mSigma | # Sigma | rdb | N-Rule | e⁻ Conf |
|---|---|---|---|---|---|---|---|---|---|---|
| 297.0557 | 1 | C14H15Cl2N2O | 297.0556 | -0.5 | -0.1 | 6.2 | n.a. | 7.5 | ok | even |
| | 2 | C12H13Cl2N5 | 297.0543 | -5.0 | -1.5 | 7.4 | n.a. | 8.0 | ok | odd |
| | 3 | C17H12ClNO2 | 297.0551 | -2.1 | -0.6 | 180.0 | n.a. | 12.0 | ok | odd |
| | 4 | C15H10ClN4O | 297.0538 | -6.6 | -2.0 | 181.9 | n.a. | 12.5 | ok | even |
| | 5 | C20H9O3 | 297.0546 | -3.8 | -1.1 | 361.1 | n.a. | 16.5 | ok | even |
| | 6 | C23H7N | 297.0573 | 5.3 | 1.6 | 362.3 | n.a. | 21.0 | ok | odd |



## Summary: Impact HD Bench-top QTOF

- **High Definition: 5 orders of magnitude dynamic range at UHPLC speed** from 10-Bit digitizer technology - definitive trace analysis from complex, high-background matrices
- mSigma (Isotope Pattern Comparision), ID unknowns, conformation of known compounds
- Full mass resolution (40,000 @ m/z 1222) at full sensitivity
- **Instant Expertise: Intelligent dynamically optimizing** MS/MS for easier tandem MS work

# Targeted Screening

# Triple Quad and/or QToF:

## Applied Markets Product Positioning Continuum

Targeted Quantitative Analysis

Increasing throughput in routine quant

Increasing confidence in ID

Unknown (Qualitative) Screening

***LC-Triple Quad***

***LC-QTOF***

**Food Testing:**
- High sensitivity, routine quant of pesticides, veterinary drugs or toxin residues

**Environmental Testing:**
- High sensitivity, routine quant of pesticides, veterinary drugs or toxin residues

**Clinical Research:**
- Targeted quant of drugs or metabolites

**Food Testing:**
- Rapid screening of pesticides, veterinary drugs or toxin residues

**Toxicology** (forensic and clinical):
- Detailed screening and confirmation of drugs of abuse or poisons

**Environmental Testing:**
- Detailed discovery and screening of pesticides, pharmaceuticals and personal care products (PPCP's)

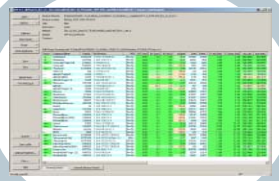
# Bruker‘s Target Screening Solution

**Sample**

**Pesticides**
food, feed, water
enivronmental

**LC-QTOF-MS:**

**UHPLC + impact**
High sensitivity
high resolution
accurate mass data
true isotope pattern



**Processing**:

**TargetAnalysis**

**Identification**
**Confirmation**
**Report**

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# High Resolution Full Scan Analysis For Rapid Screening

**Generic Extraction**

↓

**UHPLC-TOF/MS** analysis (sample 1 ... sample *n*)

↓

**Find** all components:
Get hrXIC of every compound, detect peaks and integrate

↓

**Screen**: Check compounds against database, potential residues

↓

**Confirm** compound:
Match and score retention time, mass, isotopes, fragments, etc. Exceeds MRL?

↓

**Screening Report**

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# Screener High Quality Database

## Multiple dimensions of confirmation, required for larger databases due to false positives

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 27 | 542.1396684 | 9.16 | C26H21F6NO5 | Acrinathrin | 101007-06-1 | 181.085921 | 213.112135 | 230.138685 |
| 28 | 564.1216131 | 9.16 | C26H21F6NO5 Na^1+ | Acrinathrin (Na) | 101007-06-1 | 181.085921 | 213.112135 | 230.138685 |
| 29 | 559.1662175 | 9.16 | C26H21F6NO5 NH3 | Acrinathrin (NH4) | 101007-06-1 | 181.085921 | 213.112135 | 230.138685 |
| 30 | 181.085921 | 9.17 | C 10 H 12 O 3 | Acrinathrin Fragm. 181 | 101007-06-1 | 181.085921 | 213.112135 | 230.138685 |
| 31 | 213.112135 | 9.17 | C 11 H 16 O 4 | Acrinathrin Fragm. 213 | 101007-06-2 | 181.085921 | 213.112135 | 230.138685 |
| 32 | 230.138685 | 9.19 | C 11 H 19 N O 4 | Acrinathrin Fragm. 230 | 101007-06-3 | 181.085921 | 213.112135 | 230.138685 |
| 33 | 270.1255331 | 10.27 | C14H20ClNO2 | Alachlor | 15972-60-8 | | | |
| 34 | 238.099318 | 10.27 | C13 H16 Cl N O | Alachlor Fragm 238 | 15972-60-8 | | | |
| 35 | 400.1359241 | 10.76 | C17H25N3O4S2 | Alanycarb | 83130-01-2 | 238.089626 | | |
| 36 | 238.089626 | 10.76 | C12H15NO2S | Alanycarb Fragm 238 | 83130-01-2 | | | |
| 37 | 191.0848748 | 6.12 | C7H14N2O2S | Aldicarb | 116-06-3 | 116.0528464 | | |
| 38 | 208.1114239 | 6.12 | C7H14N2O2S NH3 | Aldicarb (NH4) | 116-06-3 | 116.052846 | | |
| 39 | 116.0528464 | 6.12 | C5H9NS | Aldicarb Fragm 116 | 116-06-3 | 116.0528464 | | |
| 40 | 89.041947 | 6.12 | C4H8S | Aldicarb Fragm 89 | 116-06-3 | 116.0528464 | | |
| 41 | 223.0747041 | 3.7 | C7H14N2O4S | Aldicarb-sulfone (aldoxycarb) | 1646-88-4 | | | |
| 42 | 240.1012532 | 3.7 | C7H14N2O4S NH3 | Aldicarb-sulfone (aldoxycarb) (NH4) | 1646-88-4 | | | |
| 43 | 207.0797895 | 3.44 | C7H14N2O3S | Aldicarb-sulfoxide | 1646-87-3 | 132.047761 | | |
| 44 | 224.1063386 | 3.44 | C7H14N2O3S NH3 | Aldicarb-sulfoxide (NH4) | 1646-87-3 | 132.047761 | | |
| 45 | 132.0477611 | 3.43 | C5H9NOS | Aldicarb-sulfoxide Fragm 132 | 1646-87-3 | 89.041947 | | |
| 46 | 89.041947 | 3.43 | C4H8S | Aldicarb-sulfoxide Fragm 89 | 1646-87-3 | | | |
| 47 | 303.1954712 | 12.14 | C19H26O3 | Allethrin | 584-79-2 | | | |
| 48 | 174.0680182 | 6.32 | C8H12ClNO | Allidochlor | 93-71-0 | | | |
| 49 | 416.0814754 | | C22H19Cl2NO3 | alpha-Cypermethrin | 67375-30-8 | | | |
| 50 | 228.1277427 | 9.11 | C9H17N5S | Ametryn | 834-12-8 | 186.080793 | | |
| 51 | 209.1284542 | 4.68 | C11H16N2O2 | Aminocarb (Metacil) | 2032-59-9 | 152.1072 | 137.0836 | |
| 61 | 216.1010496 | 8.13 | C8H14ClN5 | Atrazine | 1912-24-9 | 174.054099 | | |
| 62 | 188.0697495 | 5.67 | C6H10ClN5 | Atrazine-Desethyl | 6190-65-4 | | | |
| 63 | 174.0540994 | 4.68 | C5H8ClN5 | [illegible] | 1007-28-9 | | | |
| 64 | 873.4994835 | 13.63 | C48H72O14 | AvermectinB1a (Abamectin) | 71751-41-2 | | | |
| 65 | 895.4814281 | 13.63 | C48H72O14 Na^1+ | AvermectinB1a (Abamectin) (Na) | 71751-41-2 | | | |
| 66 | 890.5260326 | 13.63 | C48H72O14 NH3 | AvermectinB1a (Abamectin) (NH4) | 71751-41-2 | | | |
| 67 | 859.4838334 | 13.36 | C47H70O14 | AvermectinB1b (Abamectin) | 71751-41-2 | | | |
| 68 | 881.465778 | 13.36 | C47H70O14 Na^1+ | AvermectinB1b (Abamectin) (Na) | 71751-41-2 | | | |
| 69 | 876.5103825 | 13.36 | C47H70O14 NH3 | AvermectinB1b (Abamectin) (NH4) | 71751-41-2 | | | |
| 70 | 300.0301085 | 7.82 | C12H11Cl2N3O2 | Azaconazole Peak 1 | 60207-31-0 | | | |
| 71 | 300.0301085 | 8.51 | C12H11Cl2N3O2 | Azaconazole Peak 2 | 60207-31-0 | | | |

- Not just a "list of accurate mass compounds"
  - Retention times for the matched UHPLC method
  - In-source Fragment ions
  - Isotopic pattern
  - Adduct information
  - Isomer information
- MS/MS Qualifier Ions for extra confirmation (broadband CID)

**Enabling fast and reliable identification of selected compounds at ppb levels in a complex sample matrix, with very low false positive rates.**

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# Automated Data Processing – Target Analysis

Level of confidence

| Found | Compound Name | Reg.No. | Mol.Formula | PMI | d RT [... | Err [ppm] | Err [m... | mSigma | Area | Intens. | RT,ex... | RT,me... | m/z,calc. | m/z,m... | Algorithm |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| +++ | Aclonifen | 7407005 | C 12 H 10 Cl 1 N 2 O 3 | [M+H]+ | 0.00 | 0.7 | 0.2 | 4.9 | 10368 | 2485 | 10.83 | 10.83 | 265.0374 | 265.0373 | Chrom... |
| ++ | Aldicarb Fragm 116 | 11603 | C 5 H 10 N 1 S 1 | [M+H]+ | -0.01 | 1.2 | 0.1 | 2.6 | 54389 | 11368 | 6.09 | 6.10 | 116.0528 | 116.0527 | Chrom... |
| ++ | Aldicarb Fragm 89 | 11603 | C 4 H 9 S 1 | [M+H]+ | 0.00 | 1.1 | 0.1 | 4.8 | 7452 | 1566 | 6.09 | 6.09 | 89.0419 | 89.0419 | Chrom... |
| +++ | Carbaryl | 6302 | C 12 H 12 N 1 O 2 | [M+H]+ | 0.00 | 0.3 | 0.1 | 2.9 | 6169 | 1203 | 7.39 | 7.39 | 202.0863 | 202.0862 | Chrom... |
| ++ | Carbaryl Fragm 145 | 6302 | C 10 H 9 O 1 | [M+H]+ | -0.00 | 1.0 | 0.1 | 7.6 | 16038 | 3079 | 7.39 | 7.39 | 145.0648 | 145.0646 | Chrom... |
| + | Cypermethrin | 5231508 | C 22 H 20 Cl 2 N 1 O 3 | [M+H]+ | -13.02 | 0.3 | 0.1 | 41.9 | 3223 | 1068 | 0.00 | 13.02 | 416.0815 | 416.0814 | Chrom... |
| + | Cypermethrin | 5231508 | C 22 H 20 Cl 2 N 1 O 3 | [M+H]+ | -13.10 | 0.2 | 0.1 | 23.1 | 12302 | 2770 | 0.00 | 13.10 | 416.0815 | 416.0814 | Chrom... |
| + | Cypermethrin | 5231508 | C 22 H 20 Cl 2 N 1 O 3 | [M+H]+ | -13.21 | 0.9 | 0.4 | 47.8 | 5778 | 1630 | 0.00 | 13.21 | 416.0815 | 416.0811 | Chrom... |
| ++ | Fenpropimorph | 6756404 | C 20 H 34 N 1 O 1 | [M+H]+ | 0.00 | 1.2 | 0.4 | 27.7 | 7725 | 1037 | 9.31 | 9.31 | 304.2635 | 304.2631 | Chrom... |
| +++ | Methamidophos | 1026506 | C 2 H 9 N 1 O 2 P 1 S 1 | [M+H]+ | -0.04 | 0.5 | 0.1 | 12.3 | 7505 | 1589 | 2.61 | 2.65 | 142.0086 | 142.0085 | Chrom... |
| ++ | Parathion | 5602 | C 10 H 15 N 1 O 5 P 1 S 1 | [M+H]+ | -0.00 | 0.8 | 0.2 | 31.0 | 2590 | 524 | 10.67 | 10.67 | 292.0403 | 292.0401 | Chrom... |
| --- | Pirimicarb | 2310302 | C 11 H 19 N 4 O 2 | [M+H]+ | 0.10 | 6.0 | 1.4 | 19.0 | 3176 | 7541 | 7.41 | 7.31 | 239.1503 | 239.1488 | Chrom... |
| --- | Simazine | 12209 | C 7 H 13 Cl 1 N 5 | [M+H]+ | 0.01 | 1.7 | 0.3 | 106.7 | 4587 | 940 | 7.00 | 6.99 | 202.0854 | 202.0851 | Chrom... |

- Visual review of the residues screened & scored against the database information
  - Retention Times, Accurate Mass, Isotopic Pattern (mSigma)
  - Broadband MS/MS qualifier ions (and their intensity ratios) for additional confirmation
- Enables fast and reliable identification of residues at ppb levels, with very low false positive rates even in complex matrices

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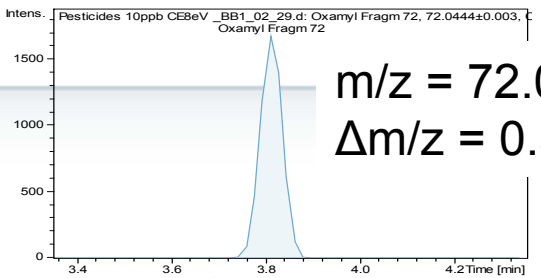
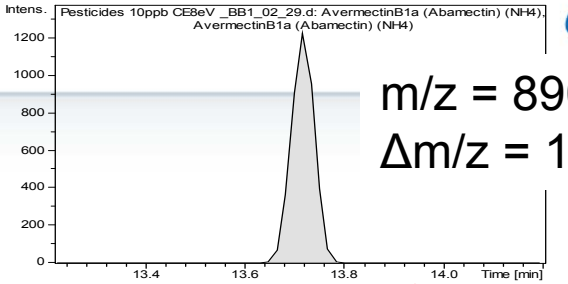
# Multi-Target Screening - 60 pesticides at 10ppb

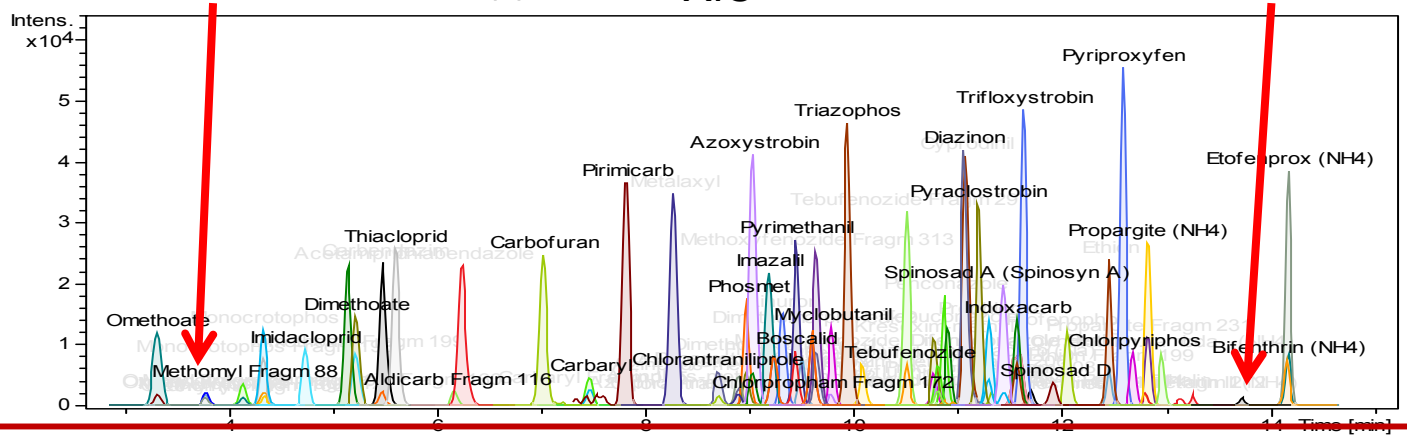



m/z = 72.0444
Δm/z = 0.5 ppm




m/z = 890.5267
Δm/z = 1.6 ppm

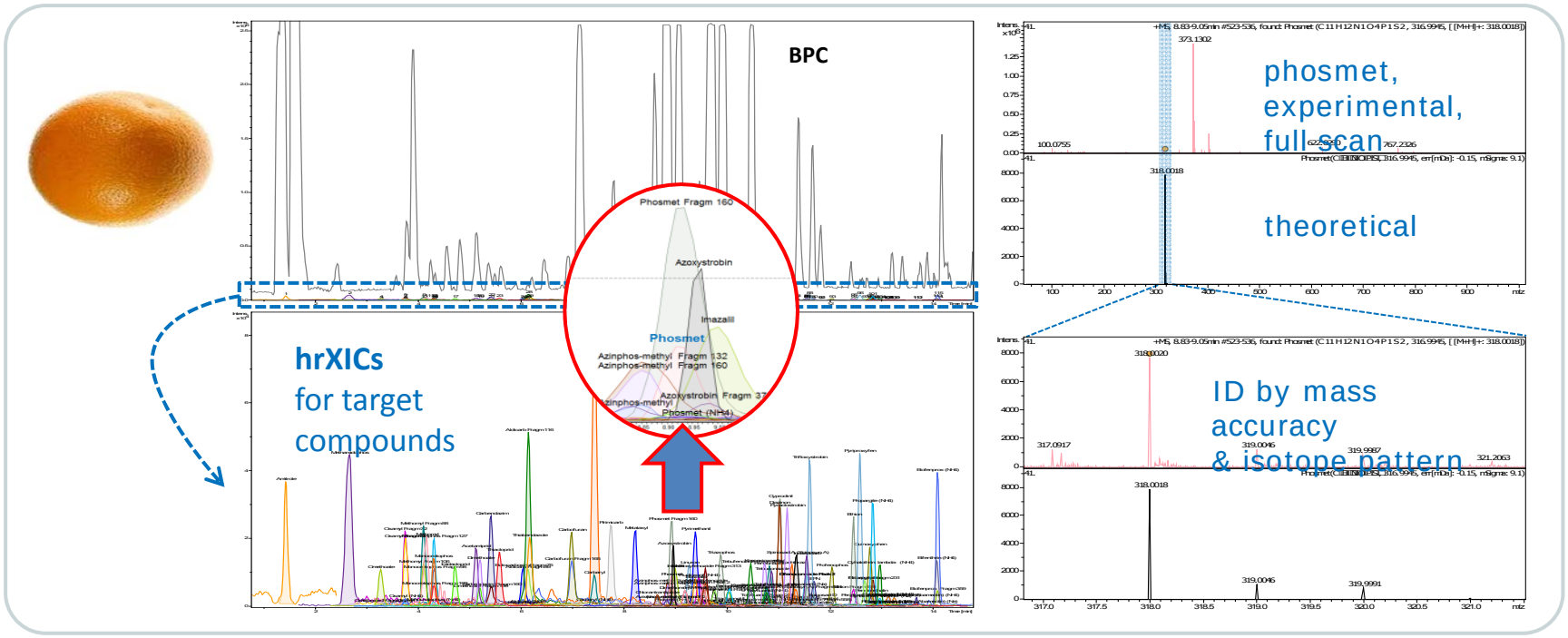
XIC




- Single MS method for all analytes
- Uncompromised mass range and sensitivity for small molecule screening, e.g. 50-1000

# Detection & Identification

## - Full Scan MS, Orange, Spiked 10 ppb, 60 compounds




"**hrXIC**"= High-resolution extracted ion chromatogram of the mass +/- **2** mDa → **selectivity** for detection

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## Summary: Impact HD Bench-top QTOF

- **High Definition: 5 orders of magnitude dynamic range at UHPLC speed** from 10-Bit digitizer technology - definitive trace analysis from complex, high-background matrices
- mSigma (Isotope Pattern Comparision), ID unknowns, conformation of known compounds
- Targeted Screening, Quantification
- Full mass resolution (40,000 @ m/z 1222) at full sensitivity
- **Instant Expertise: Intelligent dynamically optimizing** MS/MS for easier tandem MS work

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# Sample Analysis:
# Impact HD Bench-Top QTOF

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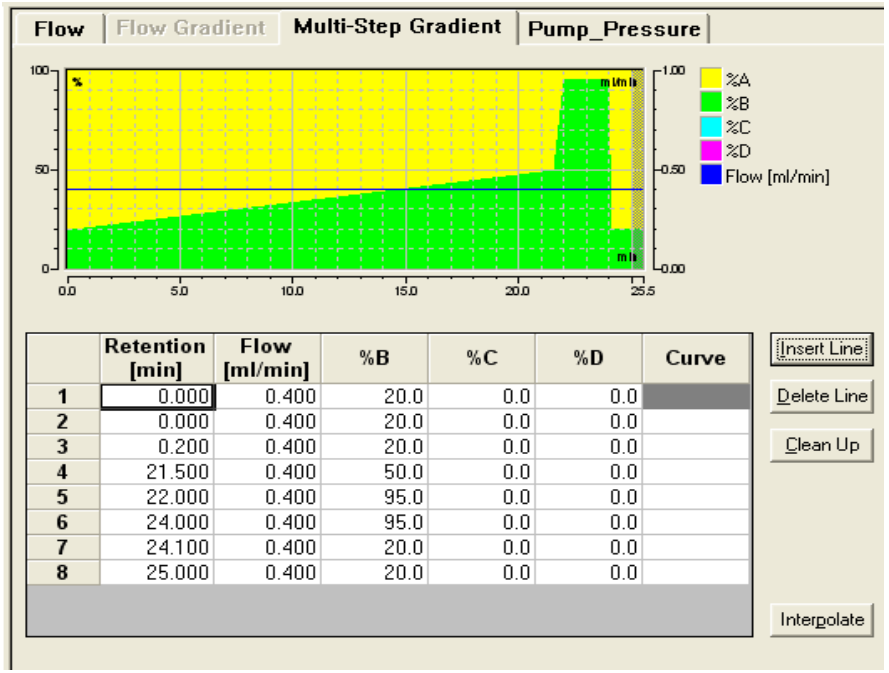
# LC Conditions

LC Method, (for reference only)

- HPLC system: Dionex RSLC
- Column: Phenomenex Gemini 150x2 mm, 3 µm
- Column Temp: 30C
- Mobile Phase:
  - MP-A : 0.1% AcOH in H2O
  - MP-B : 0.1% AcOH in ACN
- Injection Volume: 5 µL
- Gradient:




| | Retention [min] | Flow [ml/min] | %B | %C | %D | Curve |
|---|---|---|---|---|---|---|
| 1 | 0.000 | 0.400 | 20.0 | 0.0 | 0.0 | |
| 2 | 0.000 | 0.400 | 20.0 | 0.0 | 0.0 | |
| 3 | 0.200 | 0.400 | 20.0 | 0.0 | 0.0 | |
| 4 | 21.500 | 0.400 | 50.0 | 0.0 | 0.0 | |
| 5 | 22.000 | 0.400 | 95.0 | 0.0 | 0.0 | |
| 6 | 24.000 | 0.400 | 95.0 | 0.0 | 0.0 | |
| 7 | 24.100 | 0.400 | 20.0 | 0.0 | 0.0 | |
| 8 | 25.000 | 0.400 | 20.0 | 0.0 | 0.0 | |

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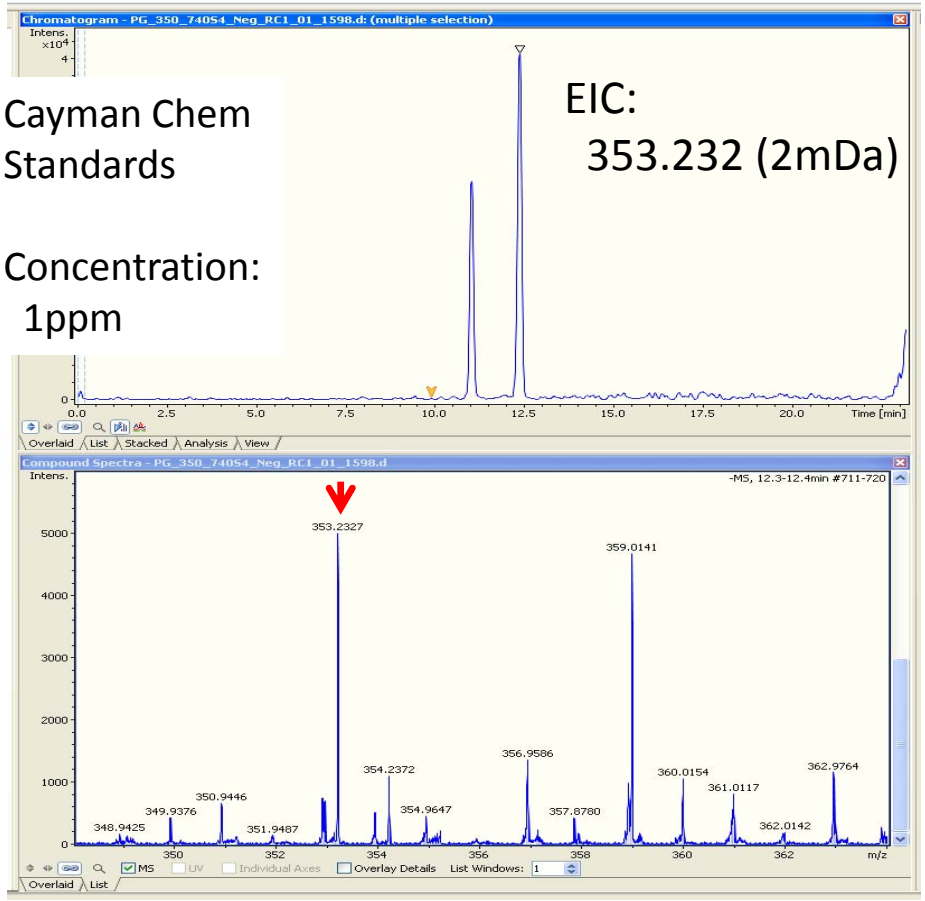
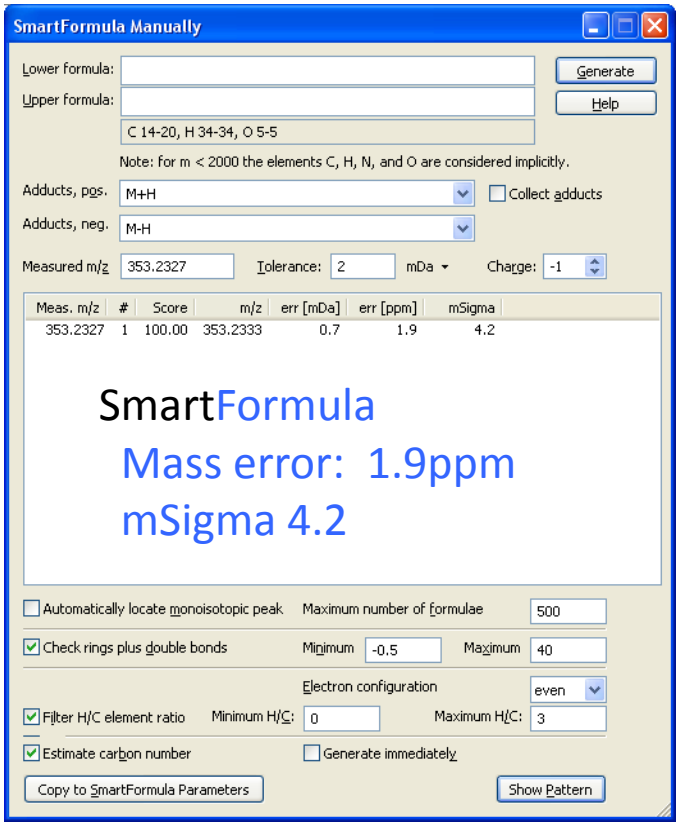
# Initial Results: Cayman Chem: Standards

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