Synthetic Cannabinoids: the Analytical Challenges

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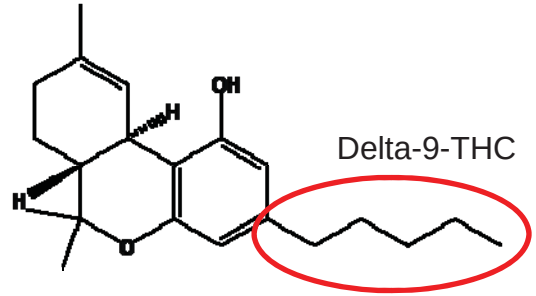
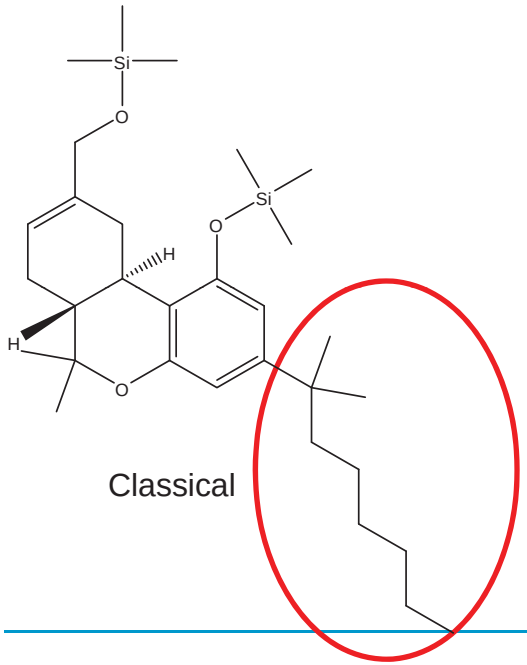
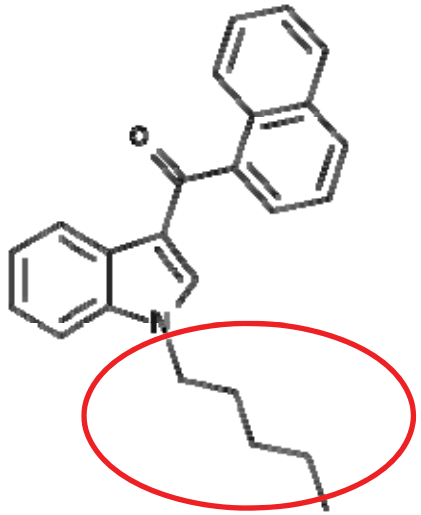
Molecular Structures



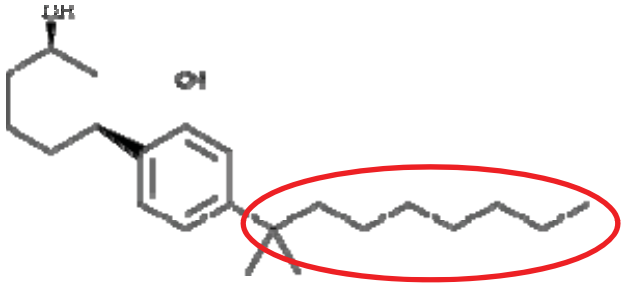
Delta-9-THC



Classical



Naphthoylindoles



Non-Classical

HU-210/HU-211
JWH-133

JWH-073
JWH-122
JWH-210
AM-2201
WIN55,212-2

JWH-081
JWH-200
JWH-398
AM-694

CP47,497(C7)
CP55,940
HU-308

Synthetic Cannabinoids

Current trends

- “Herbal Incense”
- Numerous compounds
- “legal” status
- Not picked up in standard urine drug test
- Many outlets
- Many distributors

Method Development

- Sample preparation
- Sample extraction
- Derivatization
- Acquisition
- Data analysis

Sample Preparation

- Lack of homogeneity requires some degree of mixing to ensure representative samples are tested.

- Matrix difficult to homogenize
  - Mortar and pestle
  - Hand-held herb grinder
  - Electrical devices
  - Sandpaper

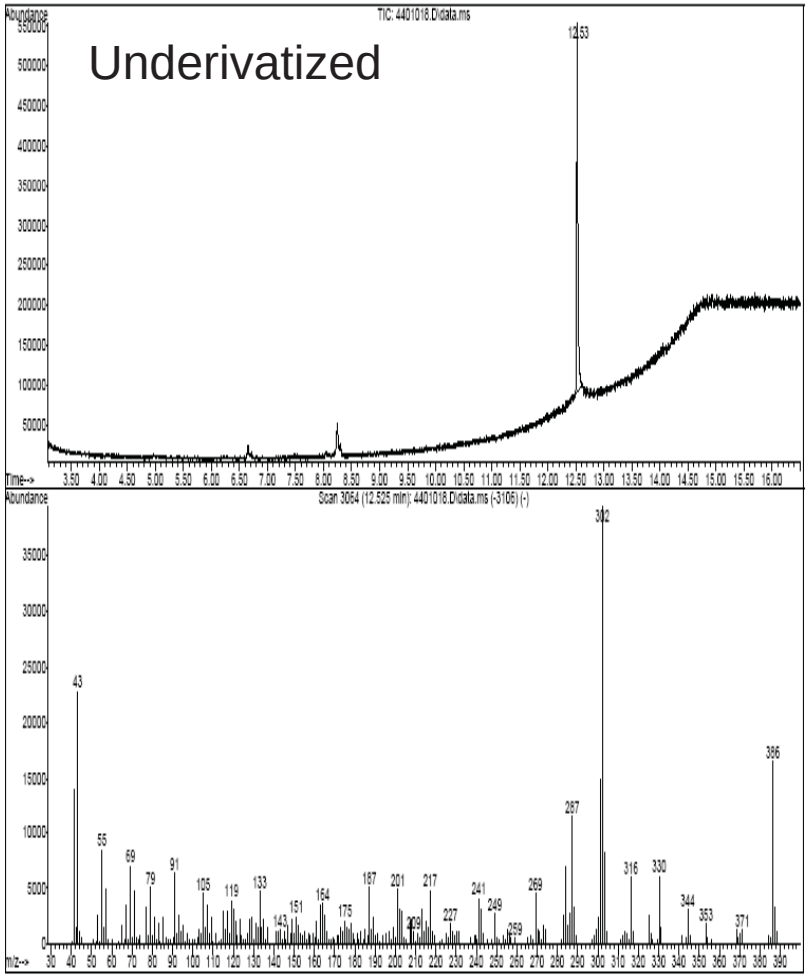
Sample Extraction

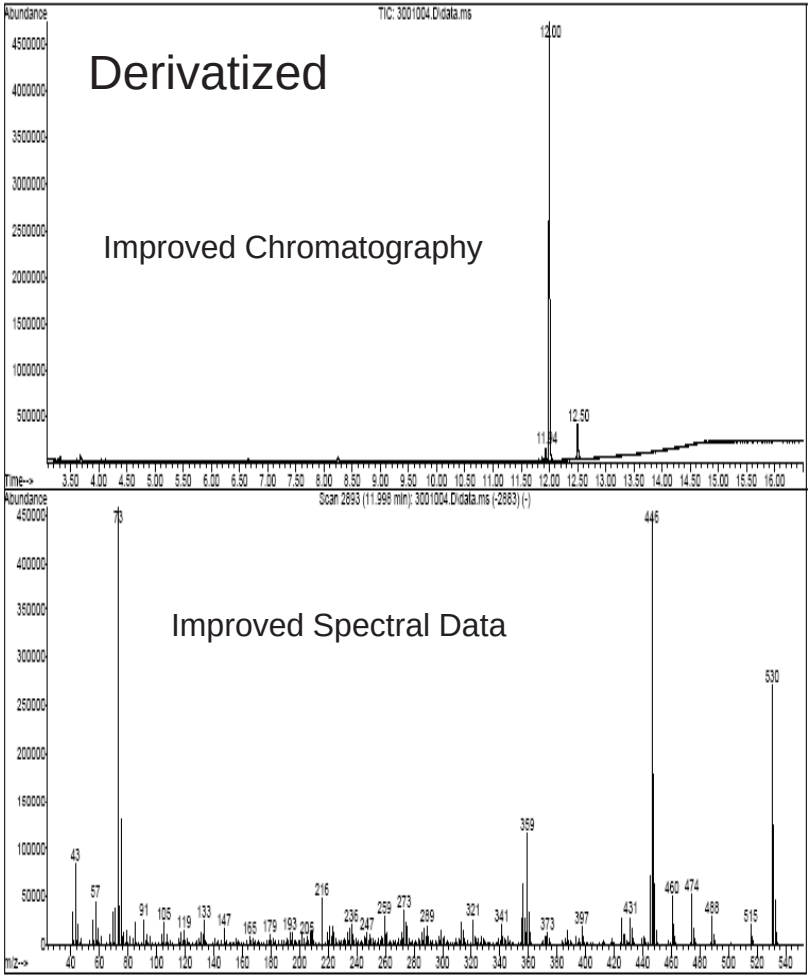
- Multiple function groups require a generalized approach. Compounds are phenols, alcohols, indoles, etc.
- Acid/base extraction
- Methanol digestion
- Matrix contains co-extracted materials

Derivatization

- Multiple polar function groups require derivatization for trace analysis
- Poor chromatography
- Poor response

HU-211 Standard



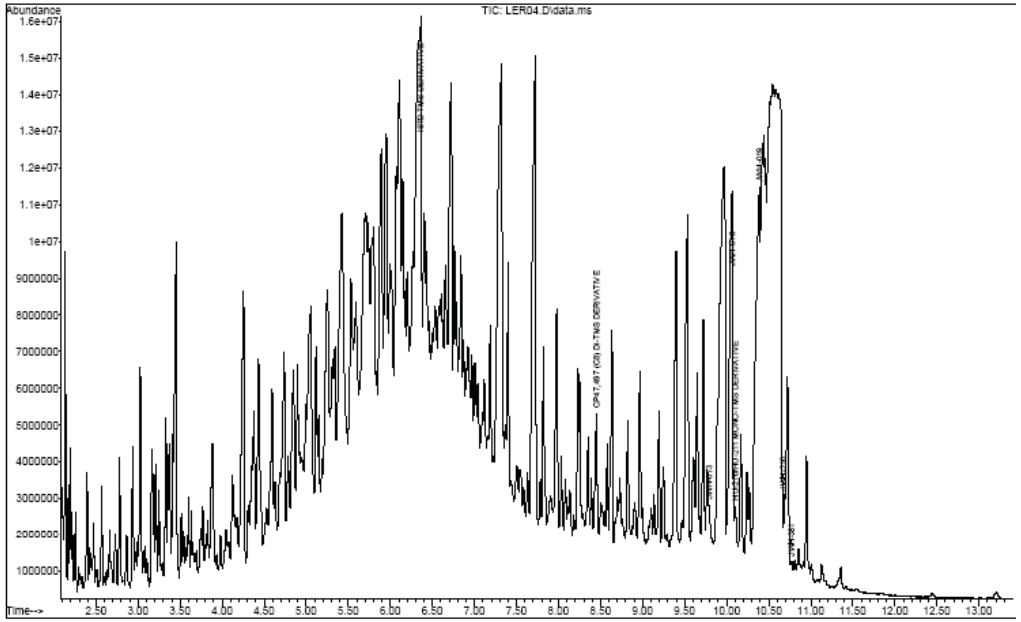


SQ Acquisition Parameters

- Agilent 5973N-turbo mass spectrometer
- Gas Chromatograph: 6890, autoinjector and tray
- DB-1 column (pt# 128-1012)
  - 12 meter
  - 0.2 mm i.d.
  - 0.33 um film thickness
- Helium Flow: 2.6 mL/min.
- Constant pressure
- Ramp: 50 - 340°C. @ 20°/min.
- Scan range: 40 – 620 amu

Data Analysis

- Numerous issues
- Trace level analysis
- Isomeric pairs
  - JWH-015 and JWH-073
  - JWH-019 and JWH-122
- Similar fragmentation
- Co-extractant interferences



Why Use Deconvolution Reporting Software?

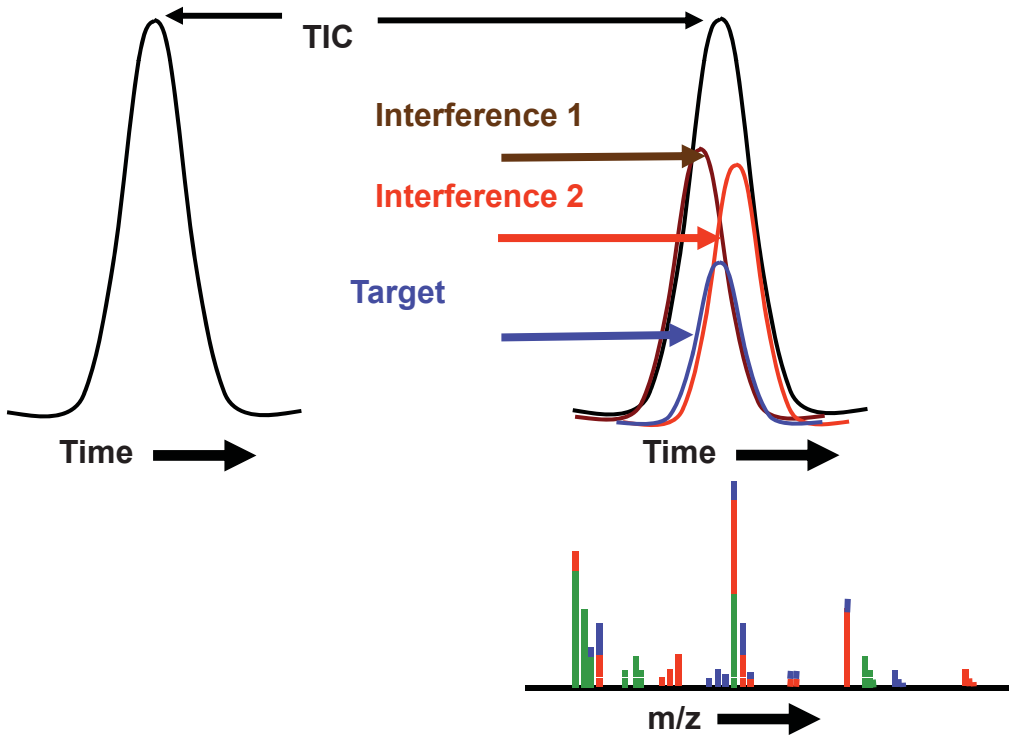
Deconvolution of mass spectra removes/reduces interferences from chromatographically overlapped peaks:

- Better identification and confirmation of analytes in high matrix samples
- Reduces data review time, especially for large forensic screening methods
- Reduces errors in dirty samples
- Identification based on matching entire spectrum cleaned of interferences against library. Much more reliable than target/qualifier ratio method.

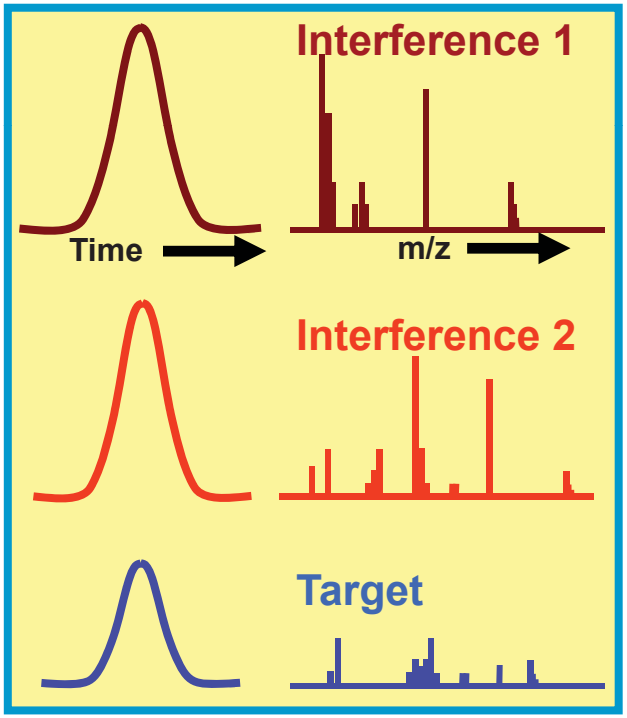
How Does Deconvolution Work?

Ions with the same abundance vs time profile are grouped together to create spectra "cleaned" of interferences from overlapped peaks

Three Overlapped Chromatographic Peaks



Deconvolution



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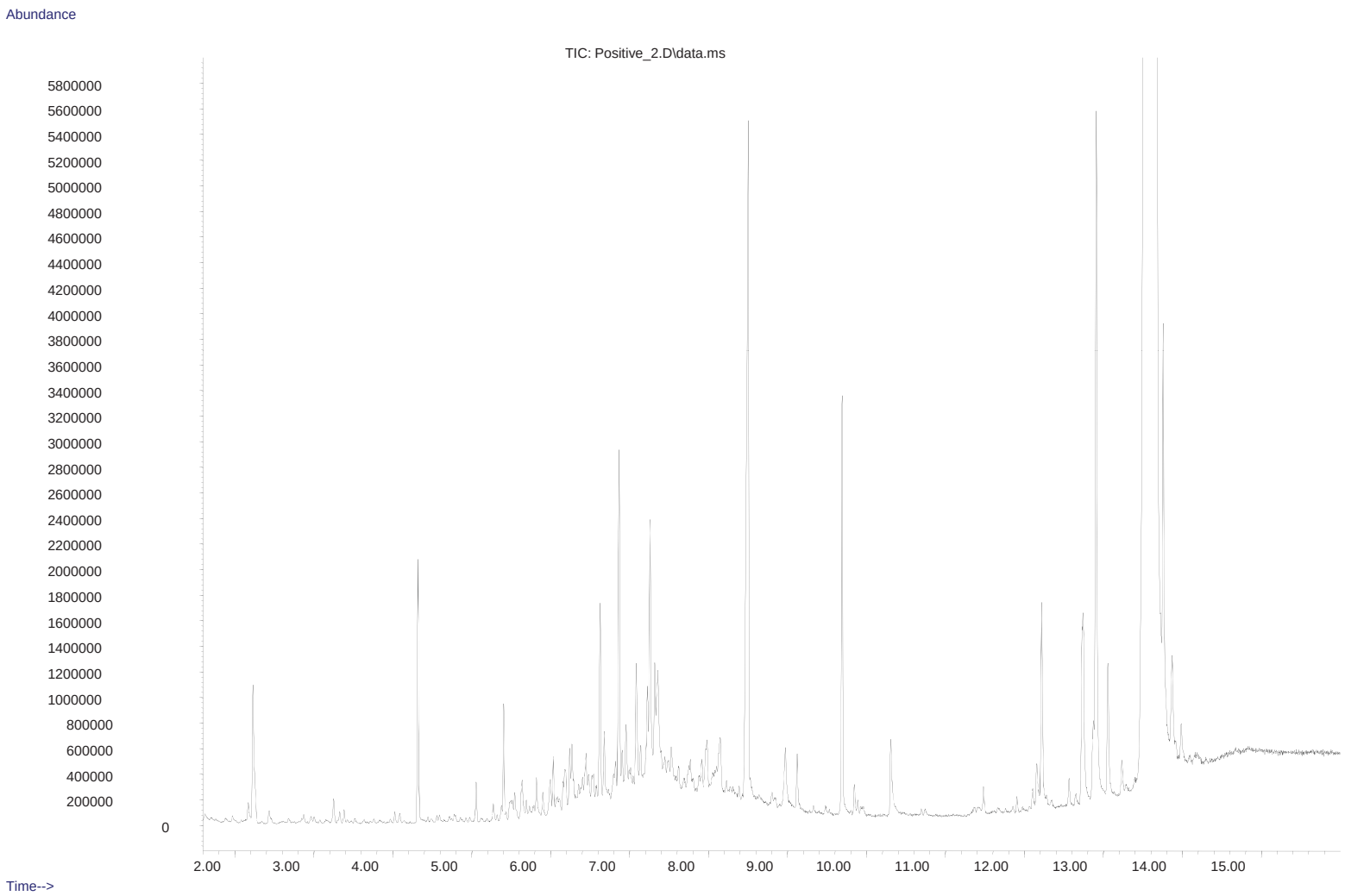
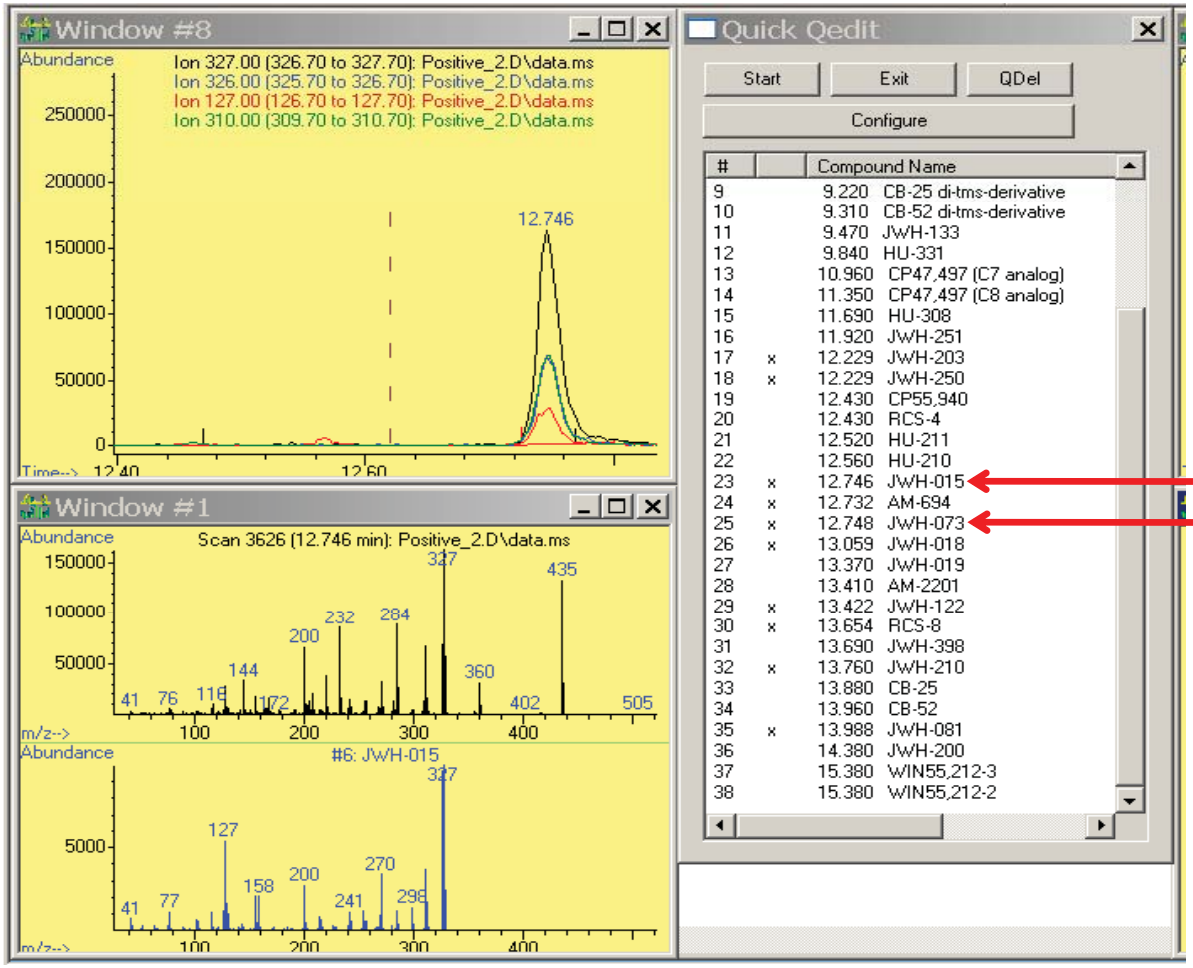
Example: Positive “Herbal Incense” Sample



Several Compounds Have Similar Times And Spectra



This peak might be JWH-015 or JWH-073. Spectrum is dirty, so it is not definitive

JWH-015

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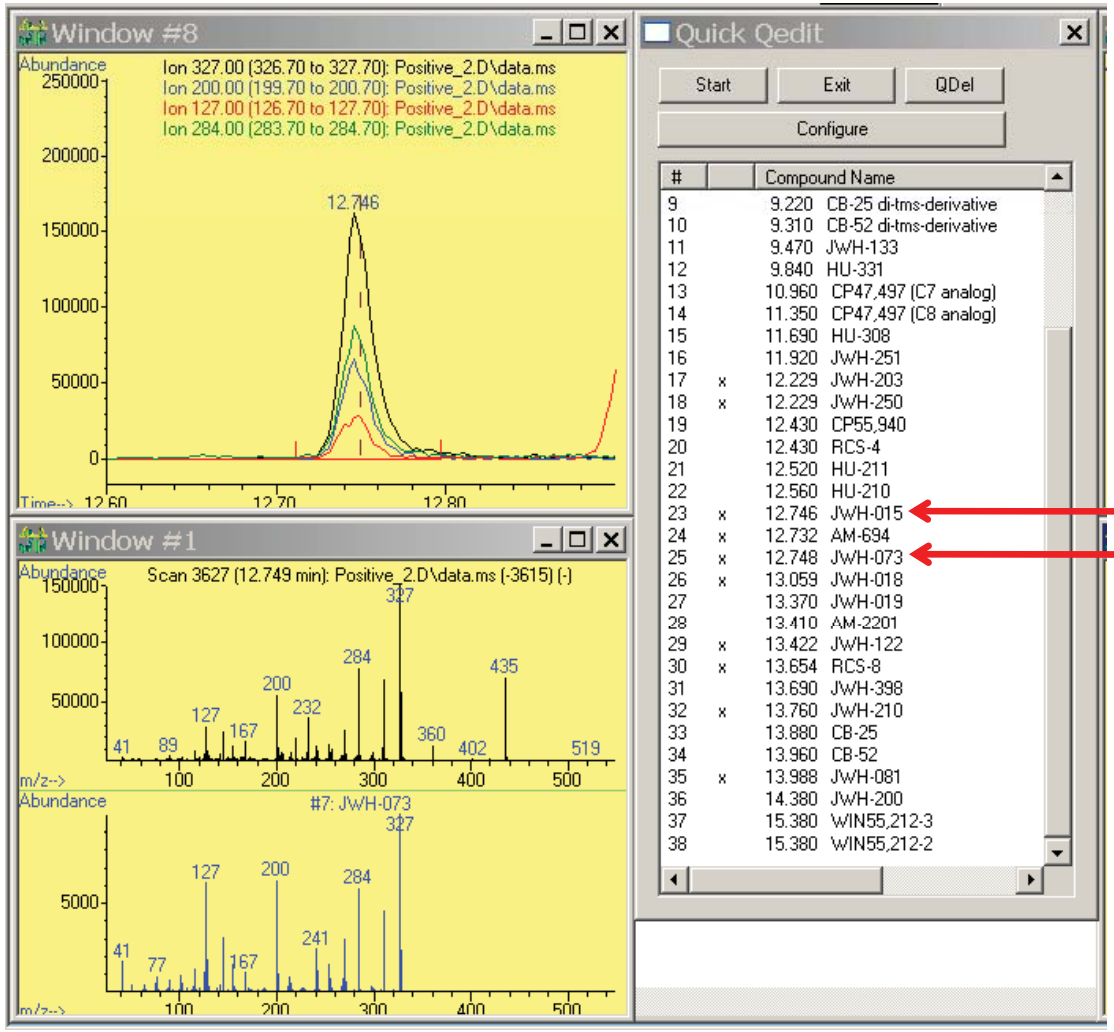
JWH-073 Spectrum Looks Better But Is Still Dirty



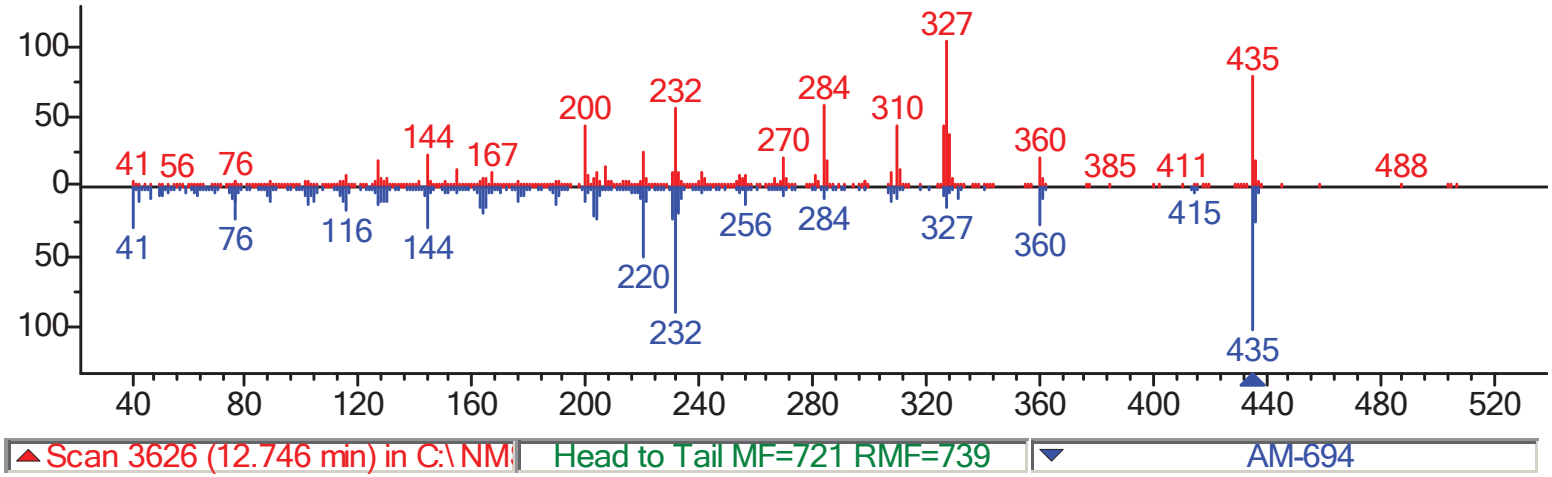
JWH-073 looks more likely.

Spectrum is dirty, so it is not definitive

JWH-073

Search Dirty Spectrum Against Library: Not Definitive

First hit in NIST Search is AM-694, with 72 match factor
Second hit is JWH-073 with 65 match factor
Third hit is JWH-015 with 59 match factor



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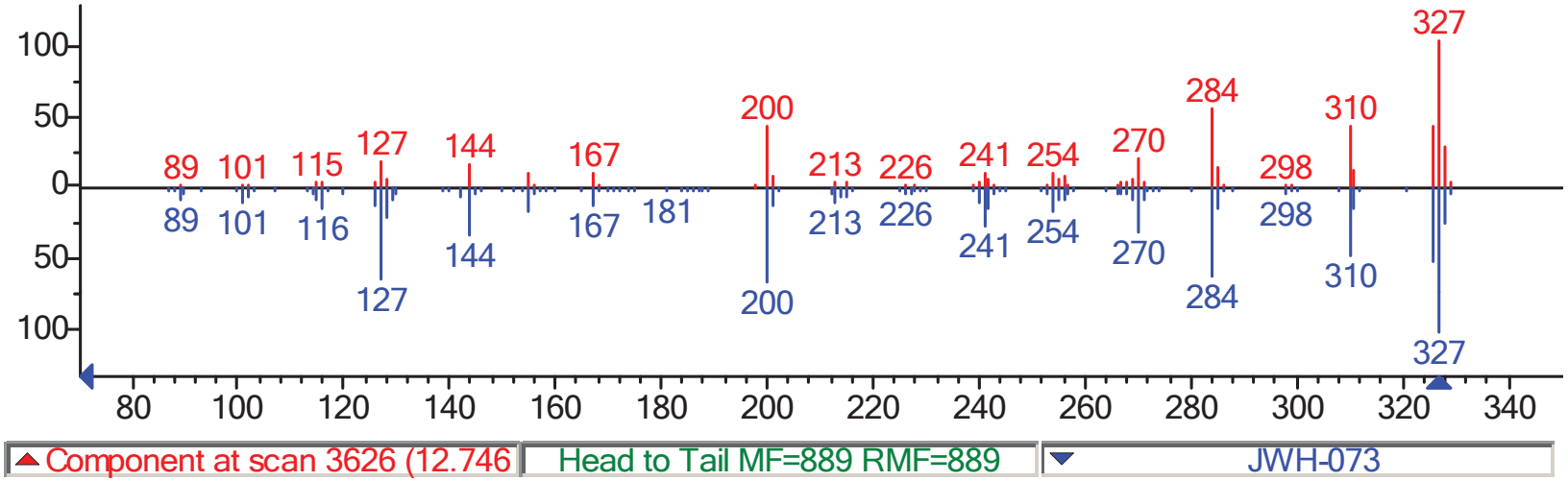
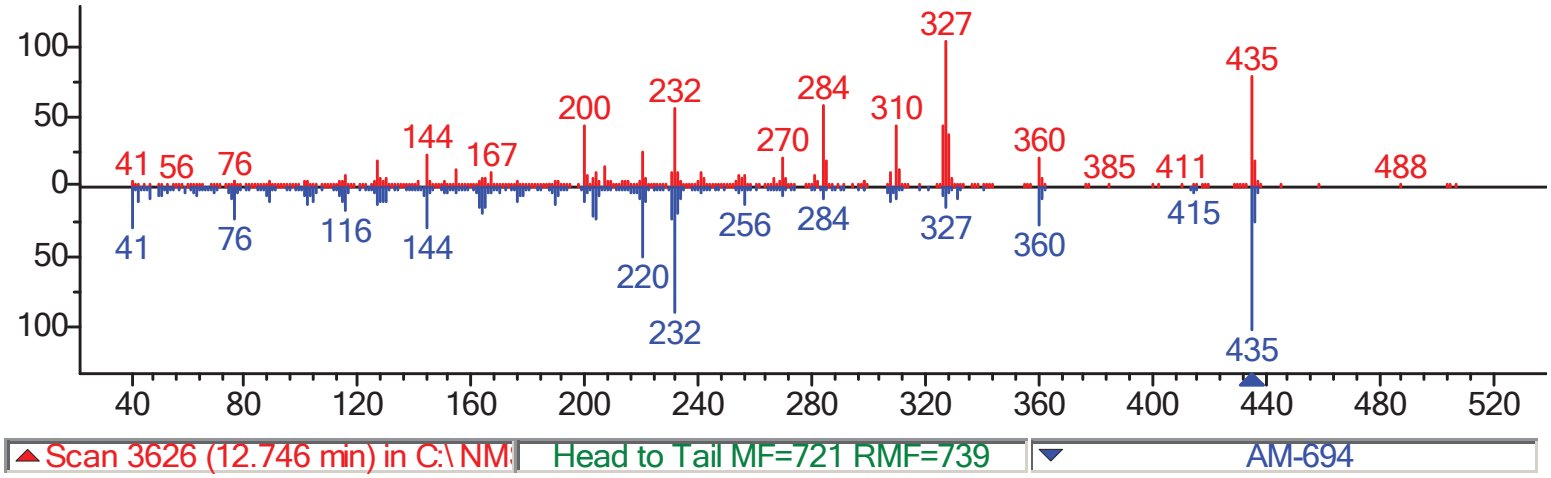
Now Use Deconvolution With AMDIS

Best Match is JWH-073 with 89 match factor. Since RT and spectral match are better than for JWH-015, this peak is JWH-073





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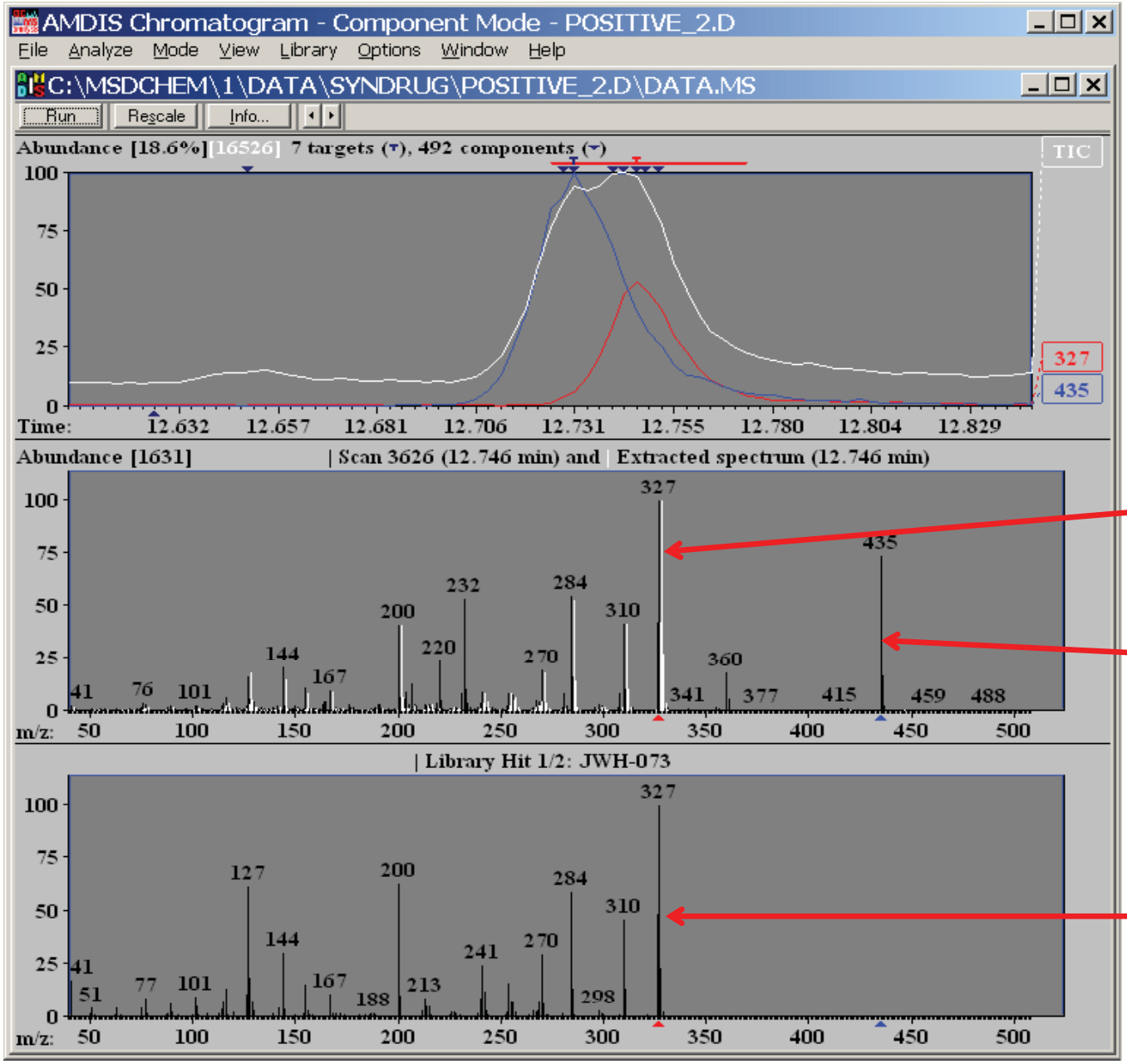

AMDIS sorts out confusion:

AM-694 (blue 435 trace) is overlapped with JWH-073 (red trace).

White is deconvoluted

Black is raw spectrum

JWH-073 Library spectrum

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Agilent Synthetic Cannabinoid Compendium

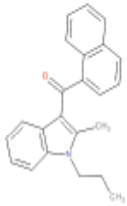
Identification of Synthetic Cannabinoids in Herbal Incense Blends by GC/MS

Application Compendium

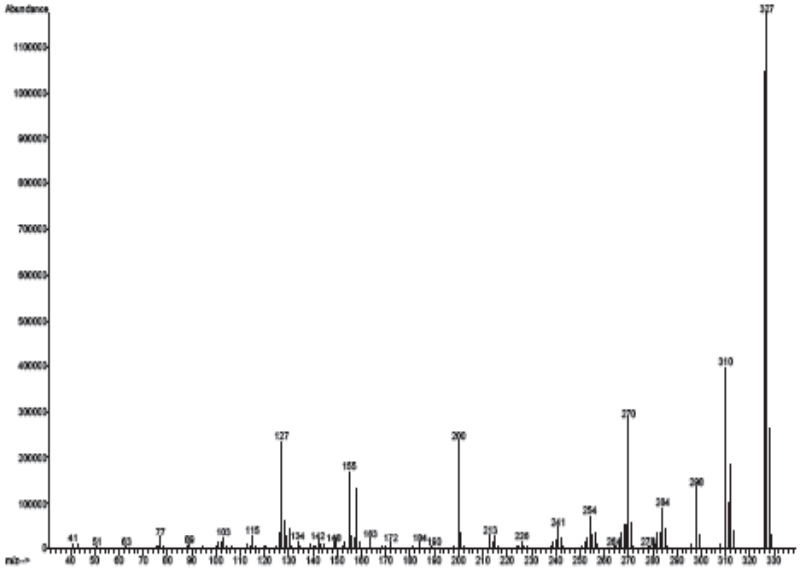
JWH-015

Chemical name	(2-Methyl-1-propyl-1H-indol-3-yl)-1-naphthalenylmethanone	
Molecular formula	$C_{23}H_{21}NO$	
Molecular mass	327.16	
Major GC/MS ions	327.3, 326.3, 310.3, 270.2, 200.2, 127.1	
Ions used for analysis	Target	327.3
	Qualifier-1	310.3
	Qualifier-2	270.2
Retention time	12.62 minutes	
LOD	Not yet established	
Additional comments	JWH-015 does not derivatize. JWH-015 and JWH-073 are formula isomers. They have similar fragmentation patterns with slight differences in retention time.	

Molecular Structure:



Mass Spectrum:



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Synthetic Cannabinoid Applications CD (5990-7857EN)

- Synthetic Cannabinoid pdf Compendium (5990-7967EN)
- Overview presentation on Synthetic Cannabinoids
- Agilent ChemStation library files
- DRS Library files
- Read me files (instructions for use)

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Synthetic Cannabinoids: the Analytical Challenges

GC/QQQ (TANDEM MASS SPECTROMETRY)

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GC/QQQ: An Alternative to DRS

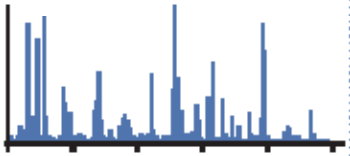
DRS uses a mathematical algorithm to deconvolute complex spectra into its component parts to facilitate interpretation

GC/QQQ uses dual stage mass spectrometry to physically isolate the target compounds of interest from potential interferences

The Agilent 7000A Triple Quadrupole GC/MS: How it works

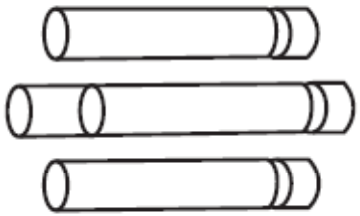
*Analytes are ionized in the **inert ion source** after chromatographic separation by the GC.*

Inert Ion Source

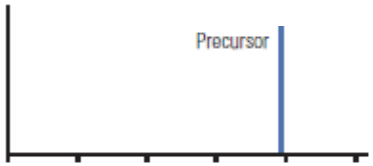


Target and matrix Ions created from ionization

*The target precursor ion is isolated from the matrix in the first **quartz quadrupole** mass analyzer.*

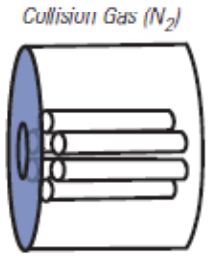


Quartz Quadrupole



Target precursor ion isolated from non-target ions

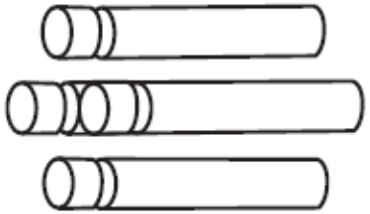
*Collisional processes in the **hexapole collision cell** dissociate the precursor ion into unique product ions.*



Hexapole Collision Cell

Unique product ions transmitted rapidly and efficiently, avoiding ion ghosting and cross-talk.

*Product ions are further separated from interferences in the second **quartz quadrupole** mass analyzer.*



Quartz Quadrupole

Product ions measured against reduced chemical noise

*Ions exit the second quadrupole and are guided through a **triple-axis detector** to a high-energy dynode.*

Triple-Axis Detector

The unique ion path in the triple-axis detector significantly reduces neutral noise from secondary ions resulting in femtogram level detection of target analytes.

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QQQ GC/MS/MS Conditions

Multi Mode Inlet, Pulsed Splitless Mode @ 300 °C: Injection Volume 1 µL

Column: Agilent HP-5MS UI (19091S-433UI) 30 m x 250 µm x 0.25 µl, Constant Flow (He) 1.2 mL/min

Oven Program

80 °C for 0.17 min

then 30 °C/min to 300 °C for 0.5 min

then 5 °C/min to 340 °C for 5 min

Run Time 21.003 min

Transfer Line 325 °C

EI Mode

Source 300 °C

Collision Cell

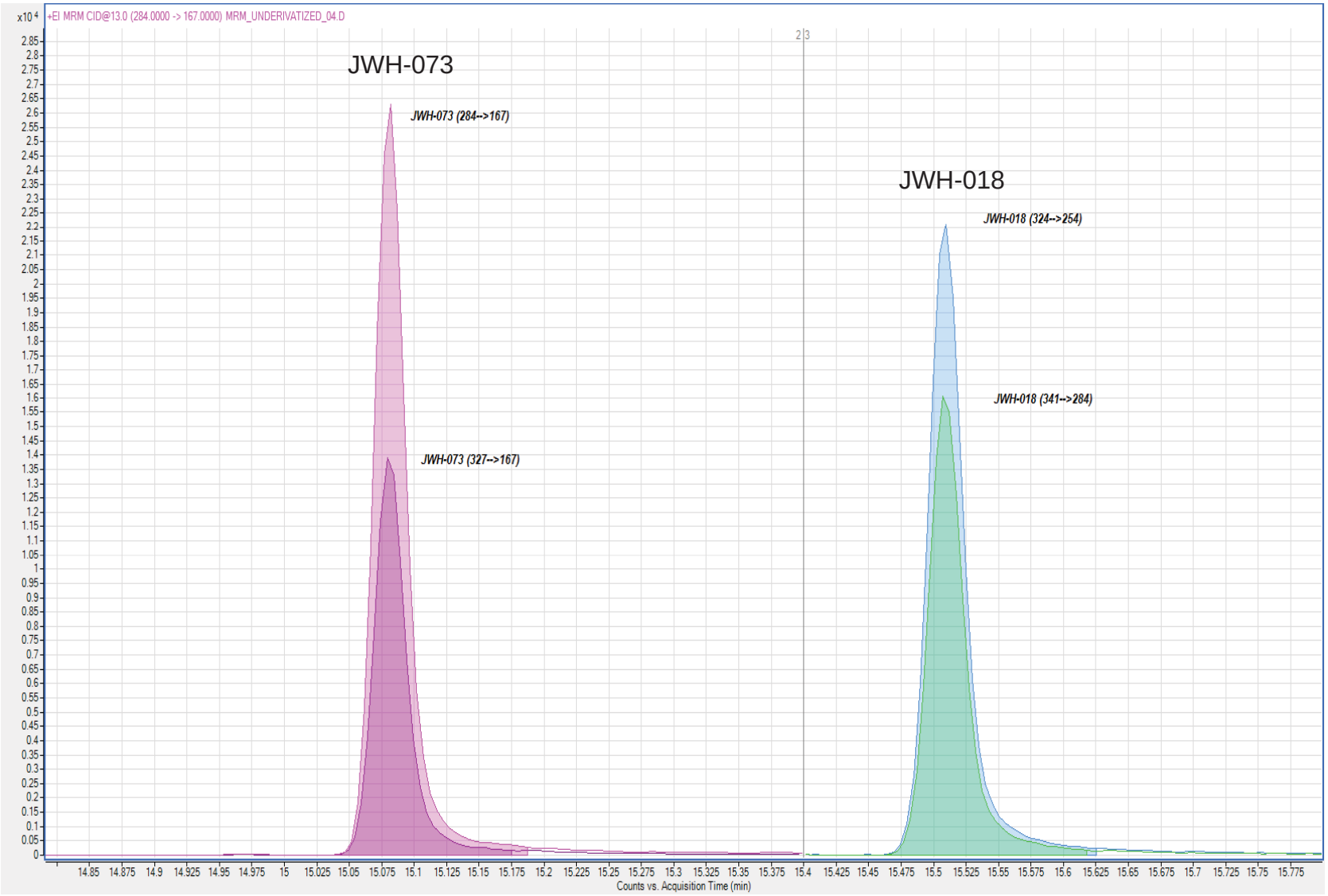
He Quench Gas 2.25 mL/min

N2 Collision Gas 1.5 mL/min

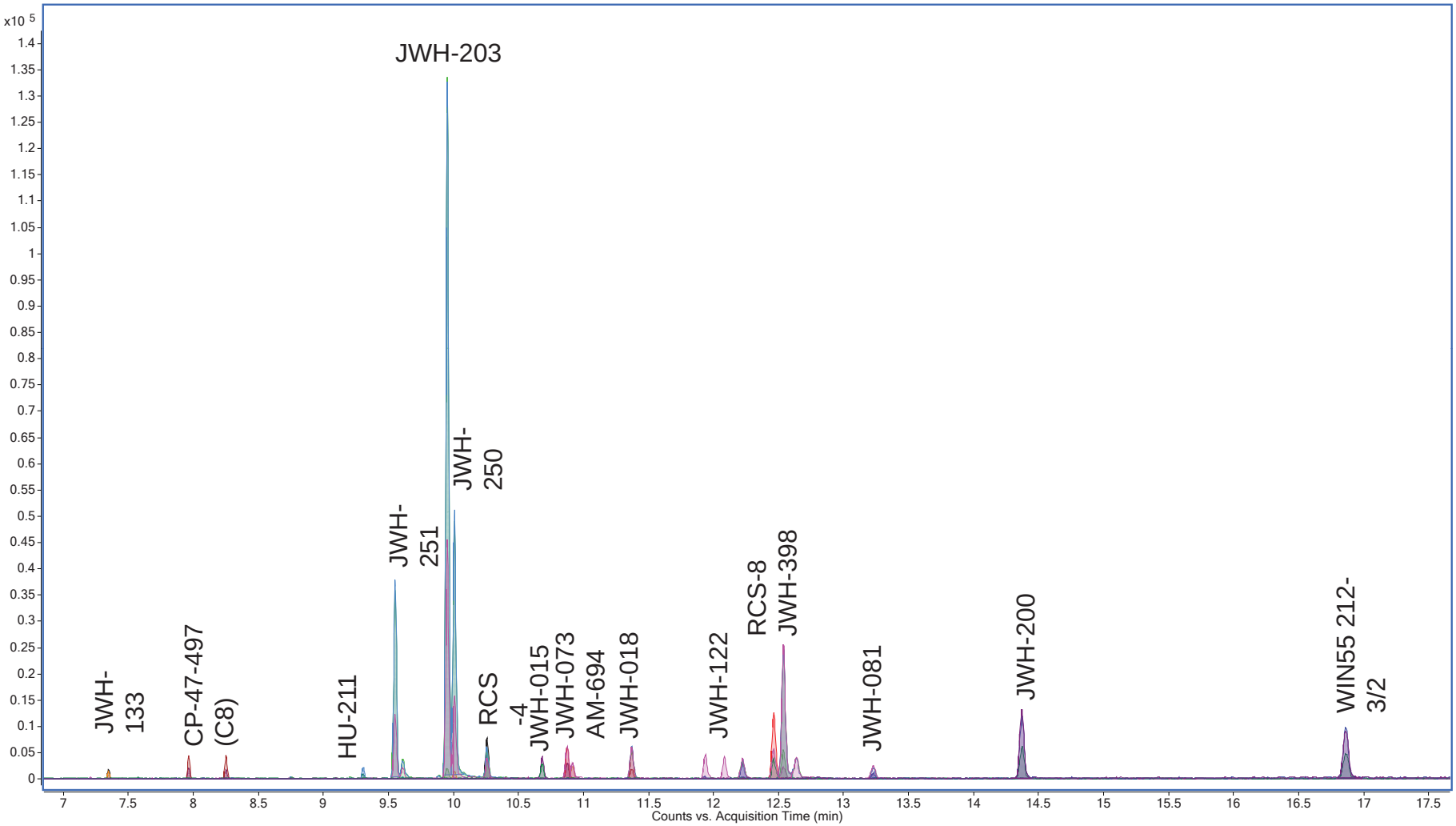
SRM Transitions

Compound Name	Precursor Ion	Product Ion	Collision Energy
AM-694	435	232	27
AM-694	435	220	13
CP-47-497-C8	377	191	29
CP-47-497-C8	377	167	33
HU-211	530	446	13
HU-211	446	299	21
JWH-015	327	310	10
JWH-015	310	268	23
JWH-018	341	167	23
JWH-018	324	254	23
JWH-073	327	167	23
JWH-073	310	254	23
JWH-081	371	197	23
JWH-081	354	269	31
JWH-122	338	268	23
JWH-122	298	181	12
JWH-133	312	269	12
JWH-133	269	93	23

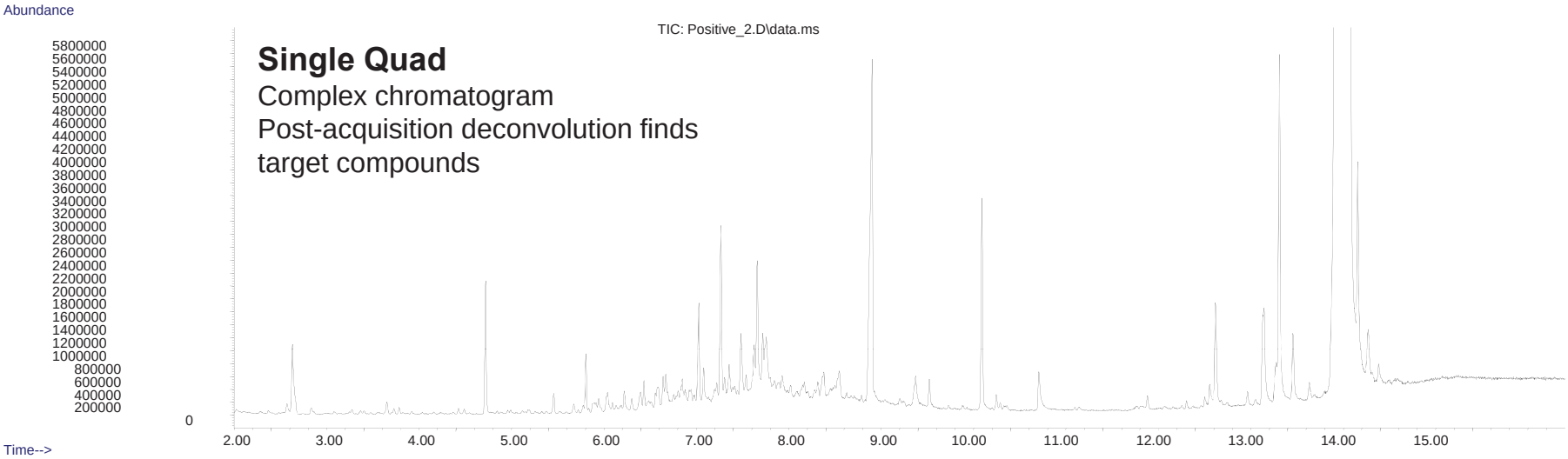
Compound Name	Precursor Ion	Product Ion	Collision Energy
JWH-200	384	100	23
JWH-200	100	56	17
JWH-203	339	214	3
JWH-203	214	144	17
JWH-250	335	214	3
JWH-250	214	144	17
JWH-251	214	144	17
JWH-251	144	116	12
JWH-398	375	201	23
JWH-398	318	189	23
RCS-4	321	264	19
RCS-4	264	135	17
RCS-8	254	158	13
RCS-8	254	144	19
WIN55 212-3/2	100	70	13
WIN55 212-3/2	100	56	15

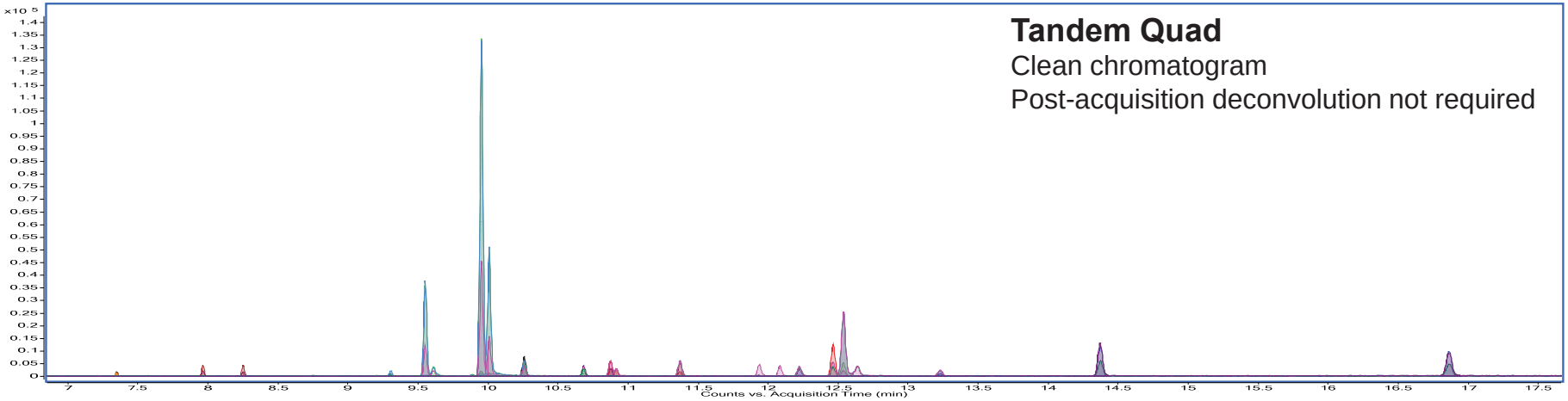

+EI MRM CID@13.0 (284.0000 -> 167.0000) MRM_UNDERIVATIZED_04.D
x10 4
JWH-073
JWH-073 (284-->167)
JWH-073 (327-->167)
JWH-018
JWH-018 (324-->254)
JWH-018 (341-->284)
Counts vs. Acquisition Time (min)

SRM TIC Chromatogram of SPICE Compounds



SQ vs. TQ “TICS”





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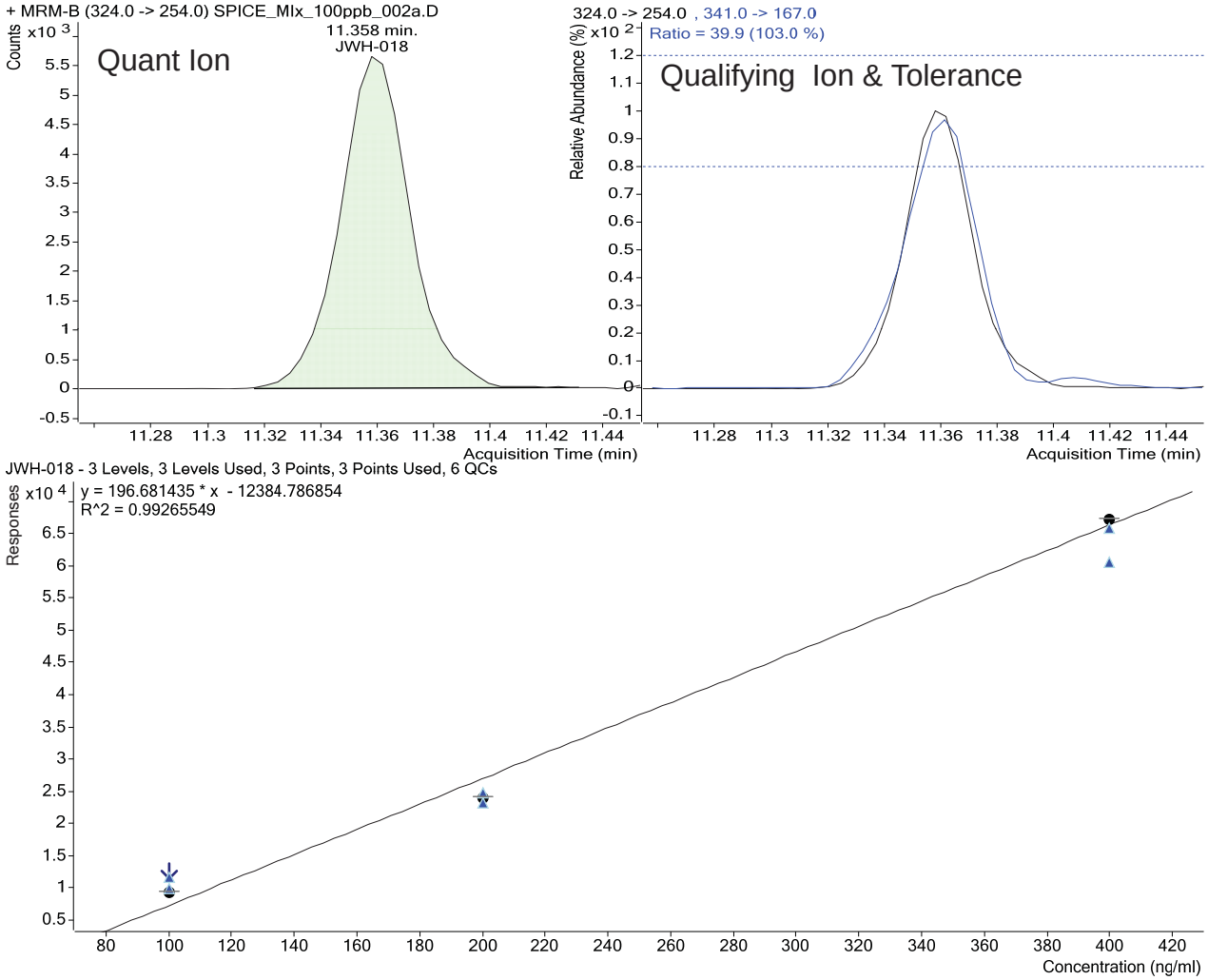
Example Compound
JWH-018, 100 ng/ml – 400 ng/ml



Synthetic Cannabinoids: the Analytical Challenges

GC/Q-TOF

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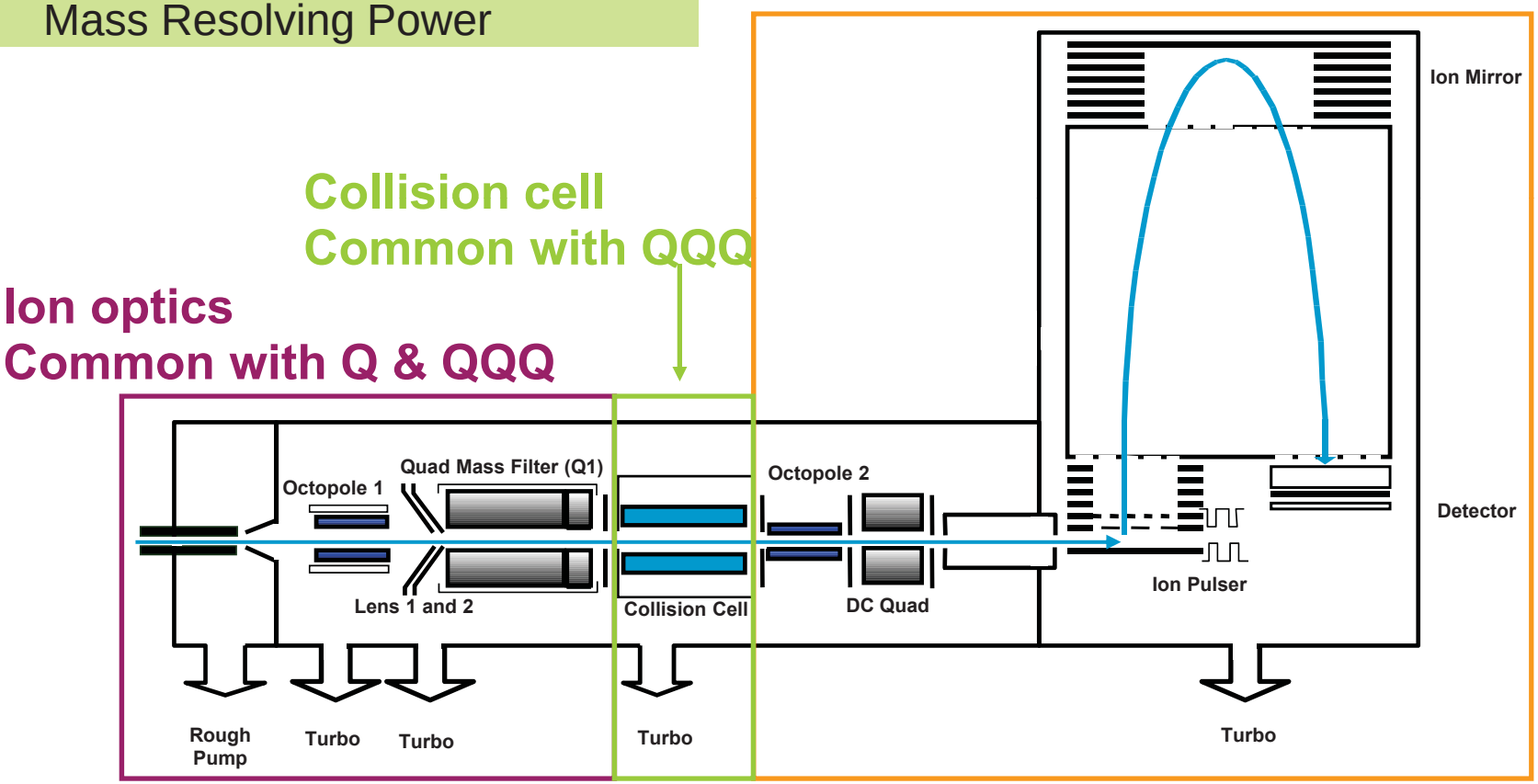
Q-TOF Fundamentals

Key concepts for TOF:

1. Accurate Mass
2. Mass Resolving Power



Mass Analysis for TOF

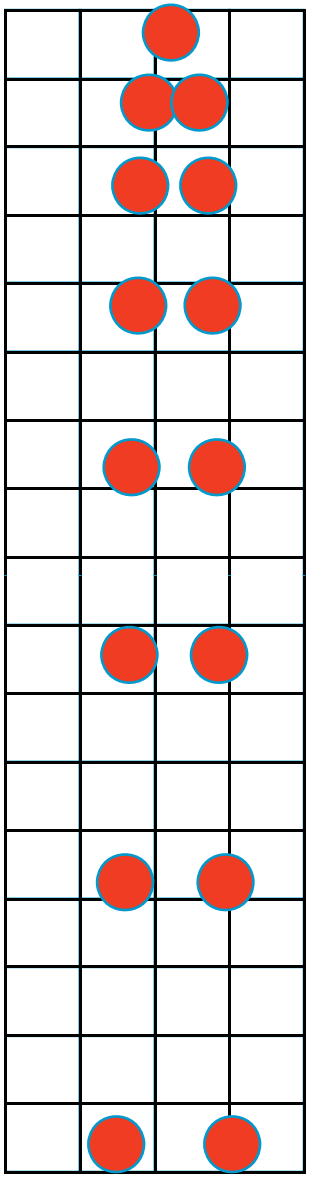
$v = d/t$

$E = \frac{1}{2} m v^2 = \frac{1}{2} m (d/t)^2$

$m = (2E/d^2)\, t^2$

Energy (E) and Distance (d) are fixed

The measured mass is proportional to the flight time (time-of-flight).



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What does “Exact Mass” mean?

Element	Atomic Number	Exact Mass
H	1	1.007825
C	6	12.000000
N	7	14.003074
O	8	15.994915

0.2874 amu

C6H6Cl6	287.8600665	Lindane
C10H12N2O6S	288.0416000	Carbasulam
C9H21O2PS3	288.0441285	Terbufos
C13H21O3PS	288.0949000	Iprobenfos
C15H17N4Cl	288.1141743	Myclobutanil
C12H21N2O4P	288.1238937	Diazoxon
C11H20N4O3PS	288.1256000	Epronaz
C11H21N4O3P	288.1351000	Pirimetaphos
C16H20N2O3	288.1473925	Imazamethabenz

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Why / When TOF/Q-TOF

SQ and TQ

- Target analysis, enforcement
  - However, what else is present?

TOF/Q-TOF

- Targeted and non-targeted analysis
- Profiling: Origin, manufacturer, adulterants
- Structure elucidation / confirmation (Q-TOF)

GC/Q-TOF Attributes

Resolving Power

- More detection selectivity

Mass Accuracy

- More qualitative information about the compound
- Better library search results if database has accurate mass information

Higher Sensitivity compared to SQ SIM

Extended Dynamic Range

MS/MS with Accurate Mass and High Resolving Power

- High selectivity and information for structural elucidation

Method

DB-1 (12m x 0.2 mm x 0.33um)

1.5 ml/min constant Flow

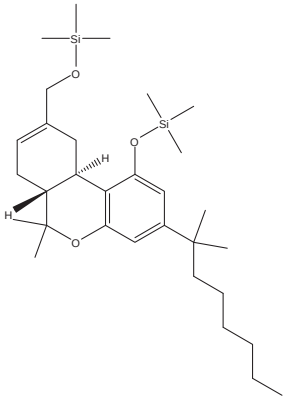
Pulsed, split-less injection

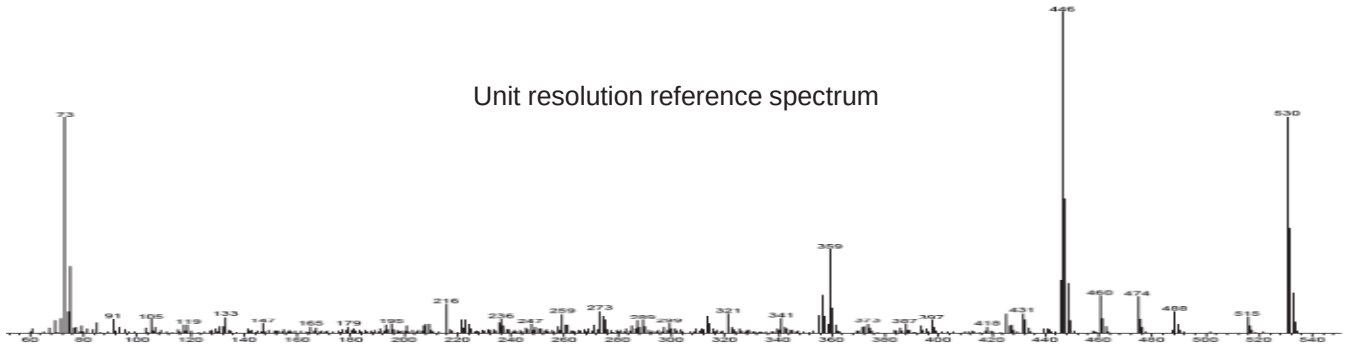
100 C-> 340 C @ 25 C/min

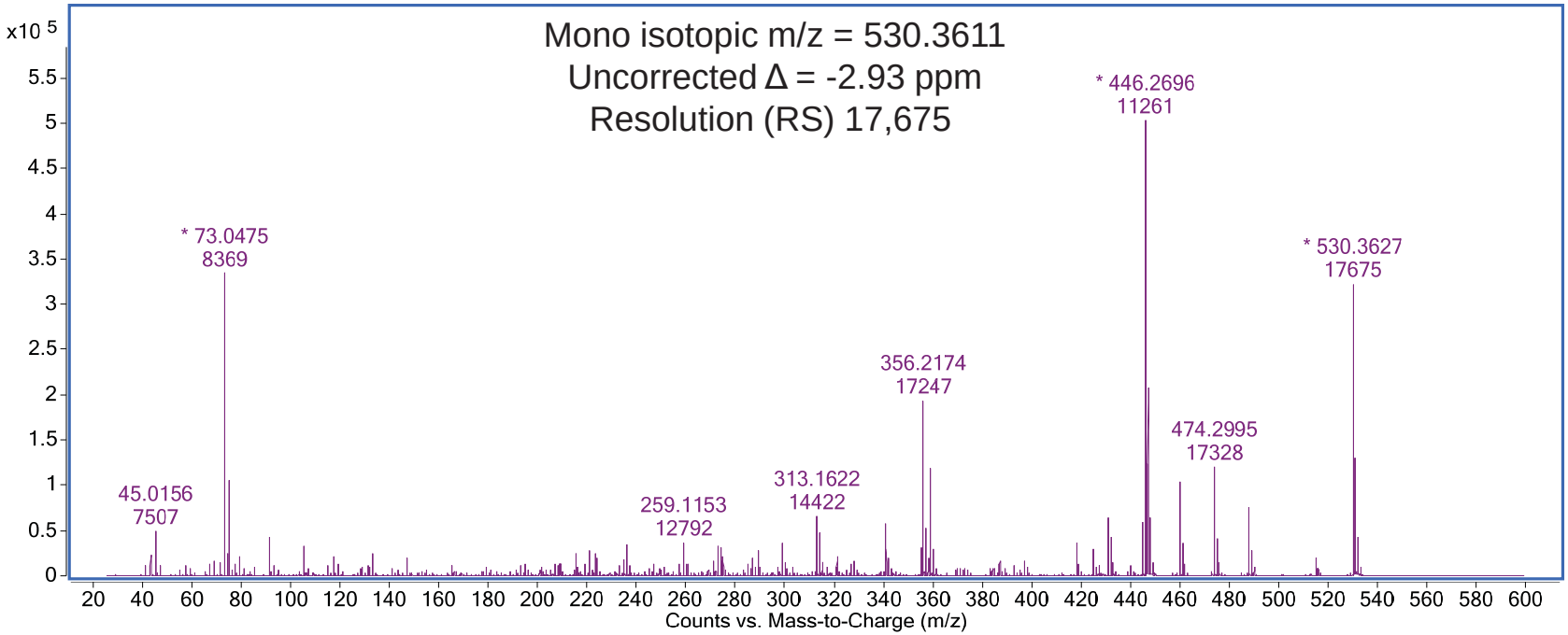
EI

- High resolution mode (HRM)

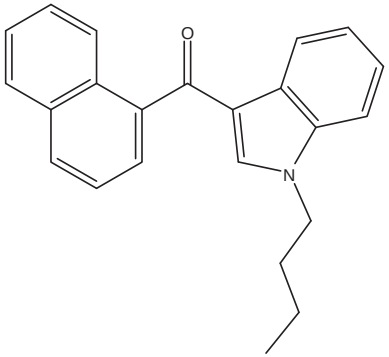
20 ppb HU-210: HRM

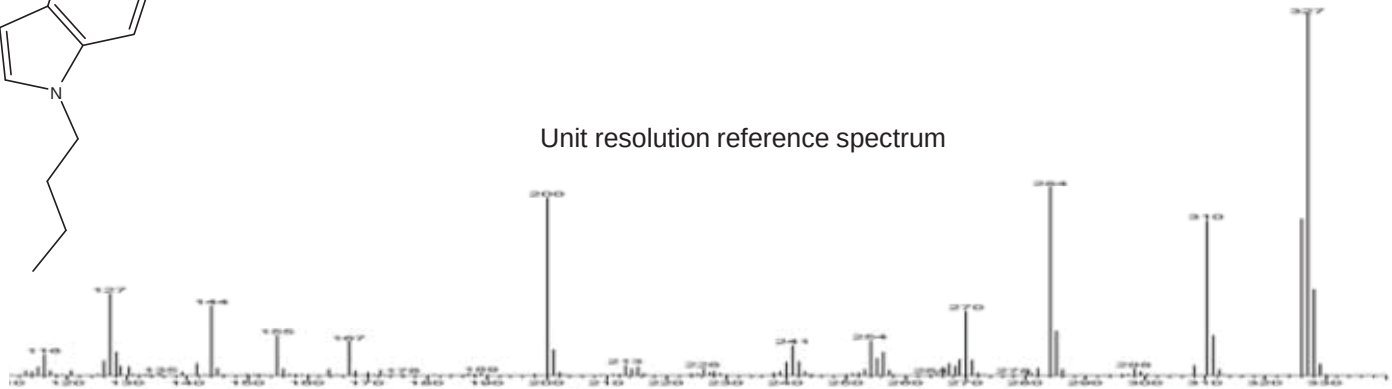


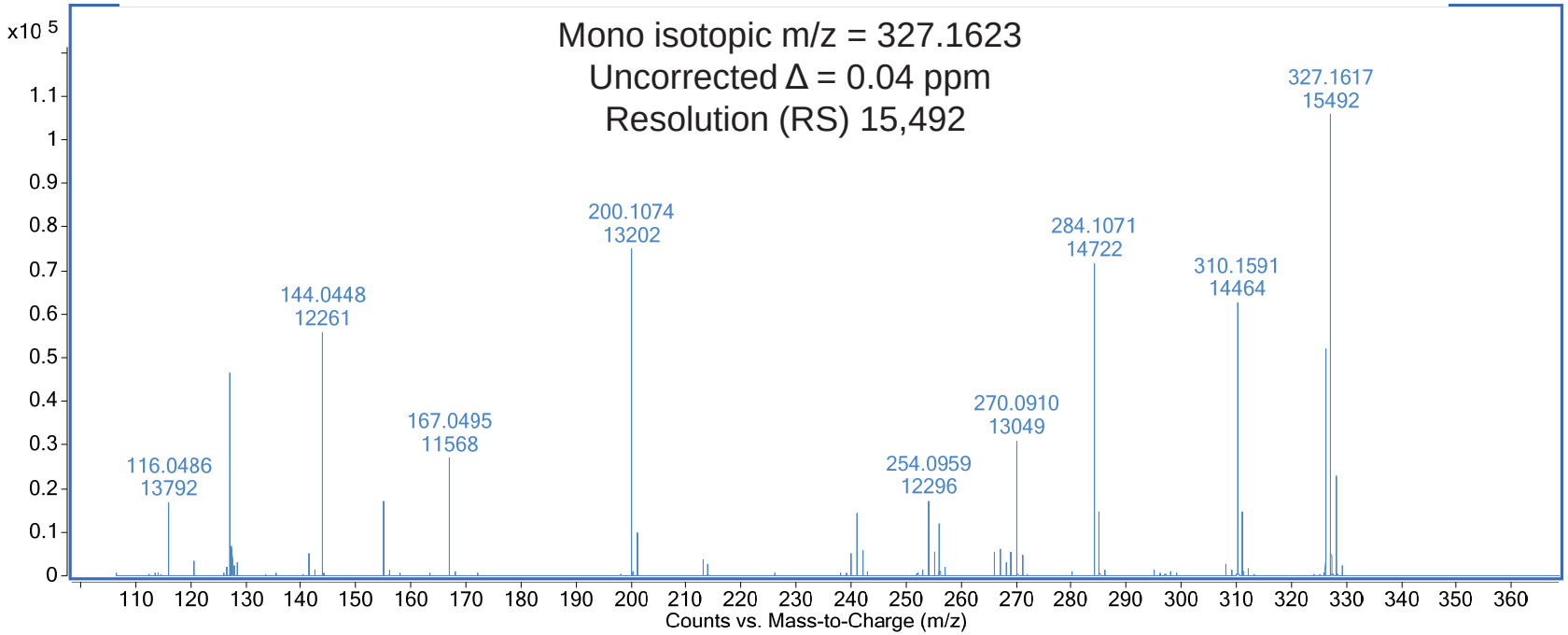




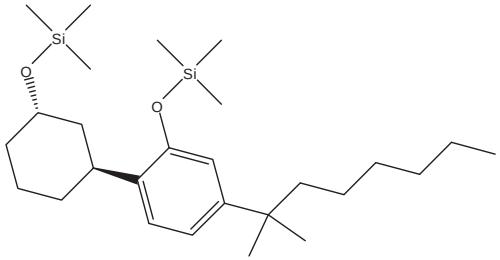
20 ppb JWH-073: HRM

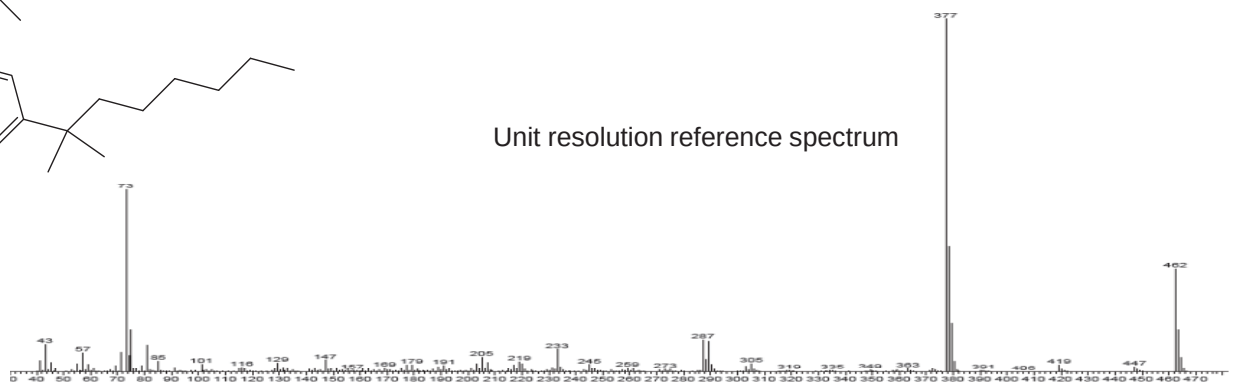


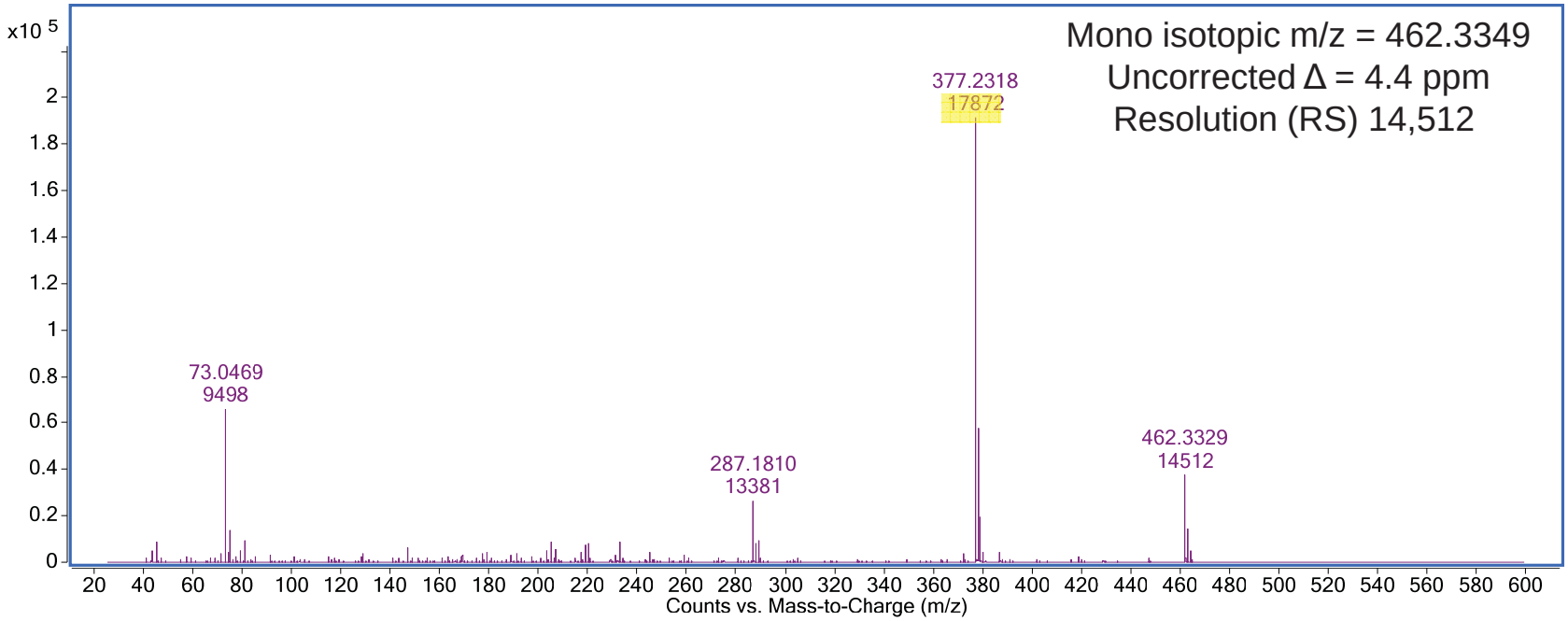




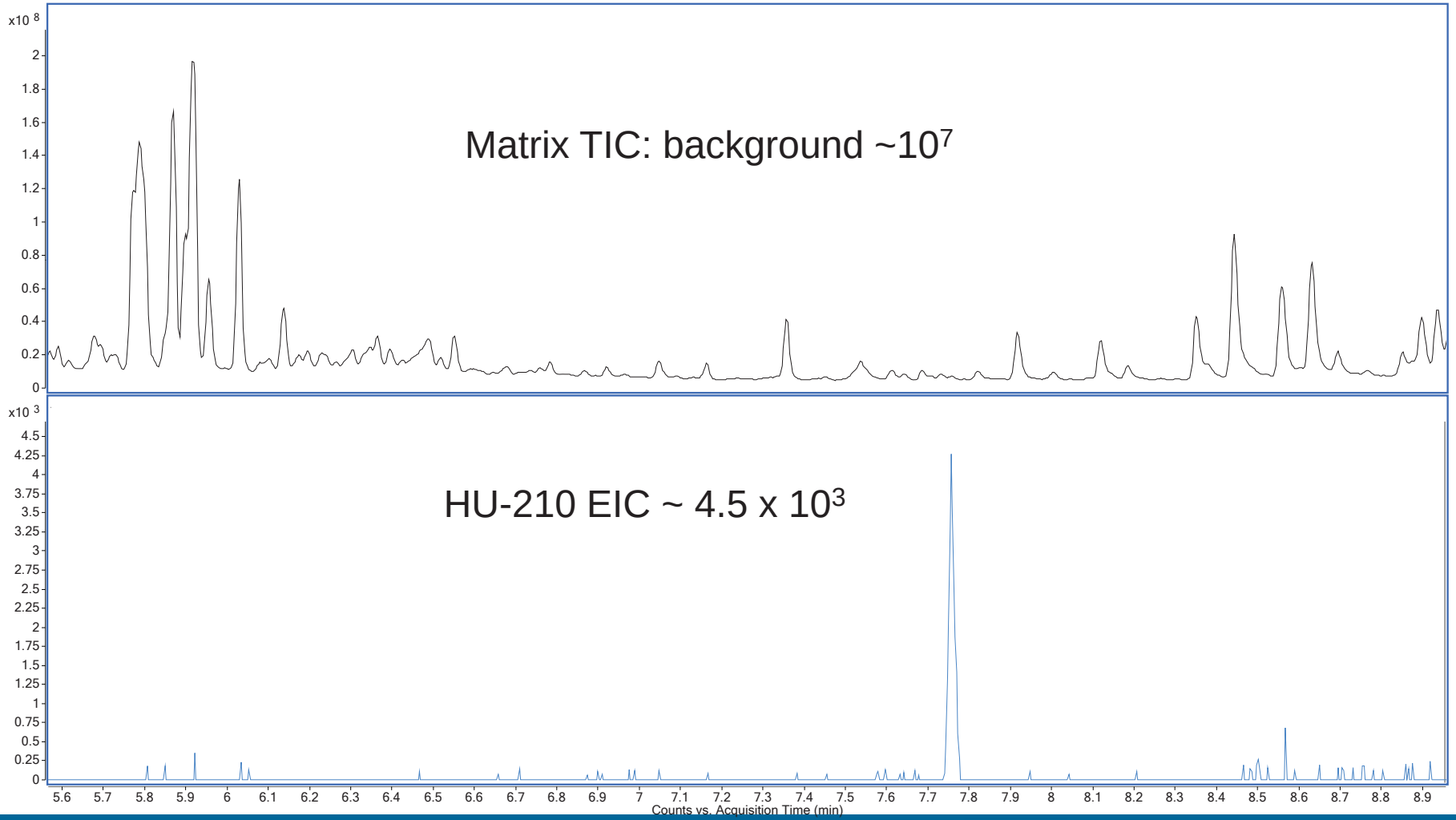
20 ppb CP-47,497 (C7 analogue): HRM







HU-210: Extraction of analyte from heavy matrix
Mass Resolving Power = 10,000



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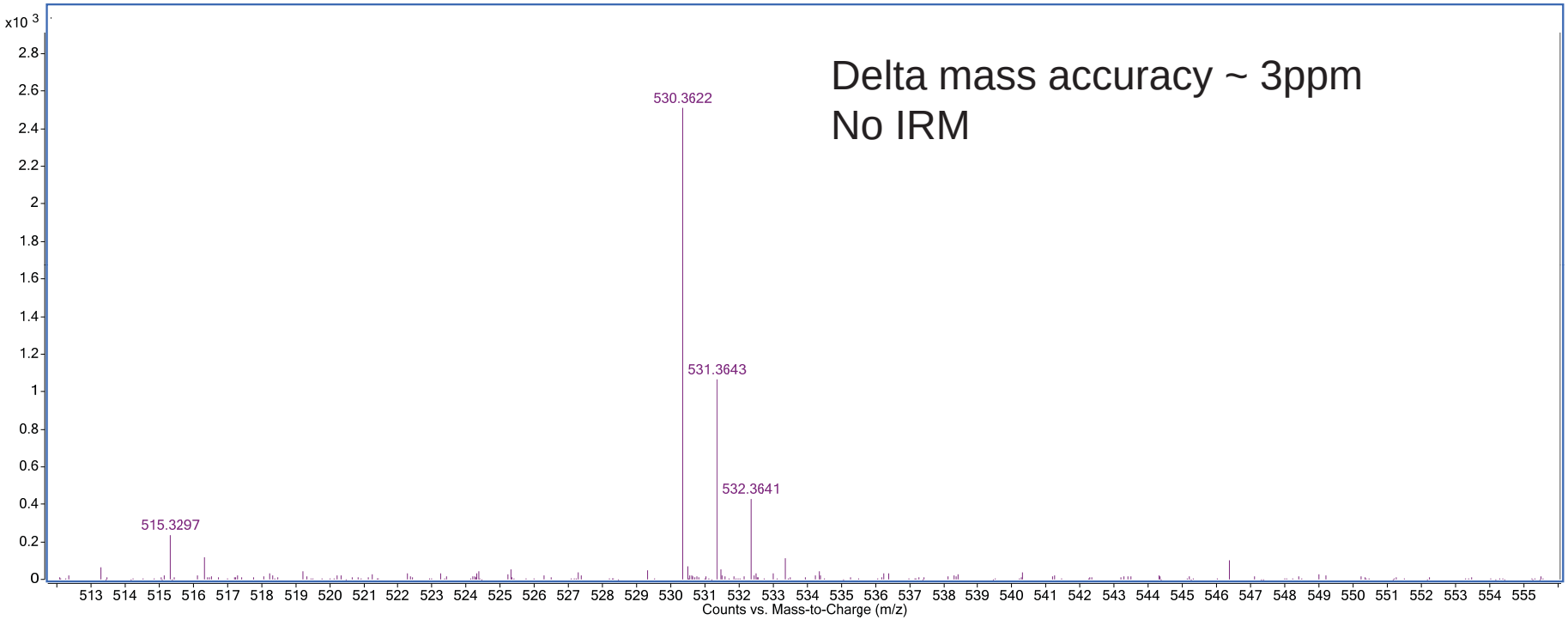
For Forensic Use.

Mass Accuracy: HU-210
True Mass = 530.361, Measured Mass = 530.3622



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Conclusions

The described SQ-DRS method and associated library is an effective means of confirming the presence of synthetic cannabinoids by GC/MS

- DRS greatly facilitates data interpretation particularly when addressing complex matrices such as herbal blends

GC/QQQ provides an alternative means of facilitating data interpretation in complex matrices while improving the ratio of signal to chemical noise

GC/Q-TOF offers the investigator the ability to perform targeted and non-targeted analysis, structure elucidation and confirmation

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