Synthetic Cannabinoids: the Analytical Challenges

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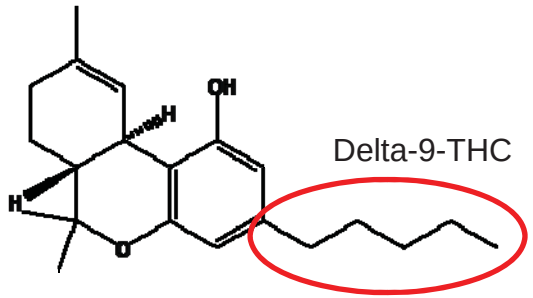
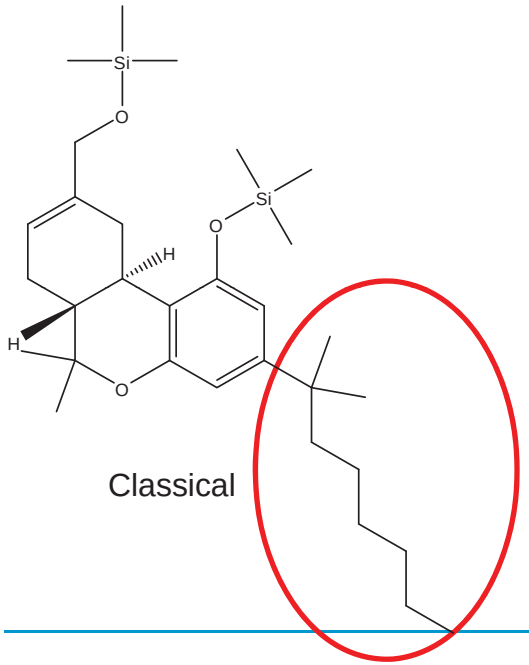
Historical Perspective

- Initially chemicals were synthesized for medicinal research.
- JWH prefaced compounds are named for John W. Huffman at Clemson University for research on the relationship between the structure of drugs and brain receptor activity.
- HU prefaced compounds are named for Hebrew University where these compounds were first synthesized and investigated.
- CP 47,497 was initially developed by Pfizer for its analgesic effects.

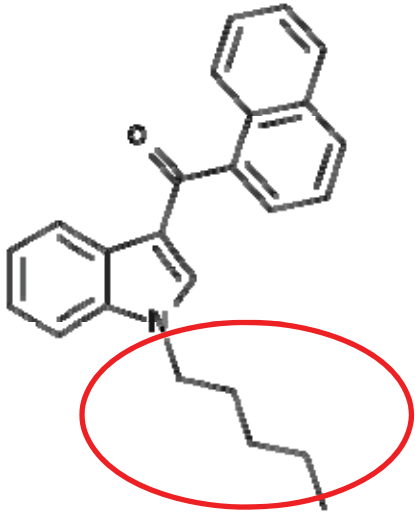
Molecular Structures



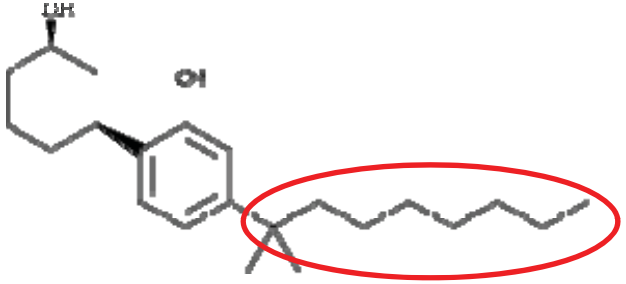
Delta-9-THC



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

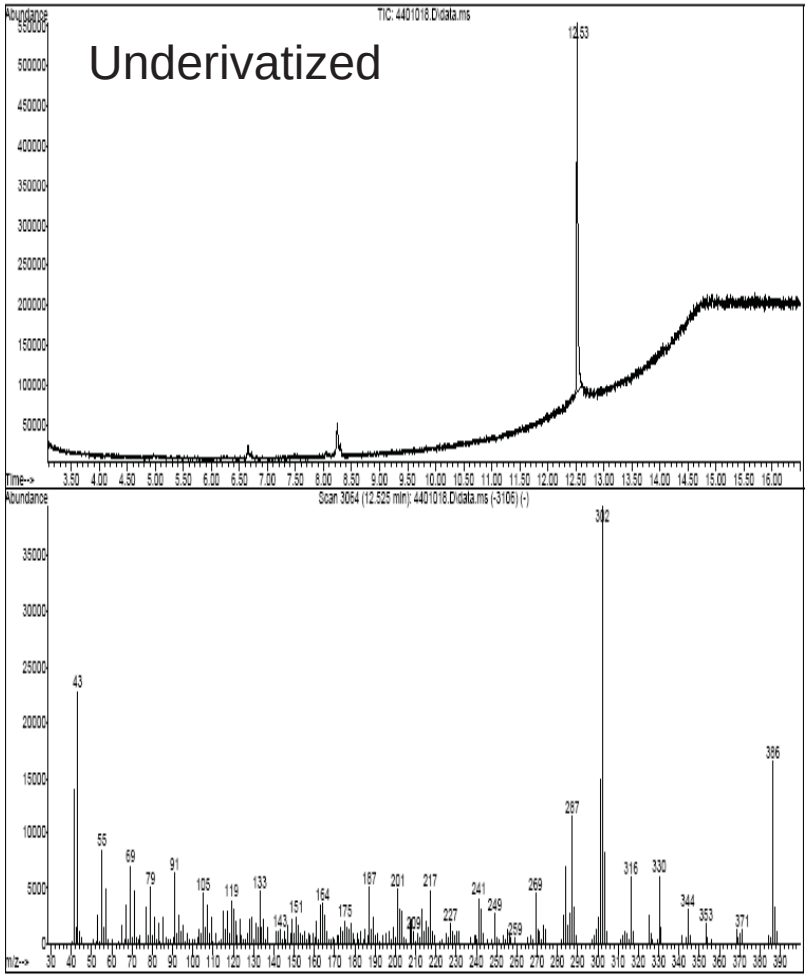
Sample Extraction

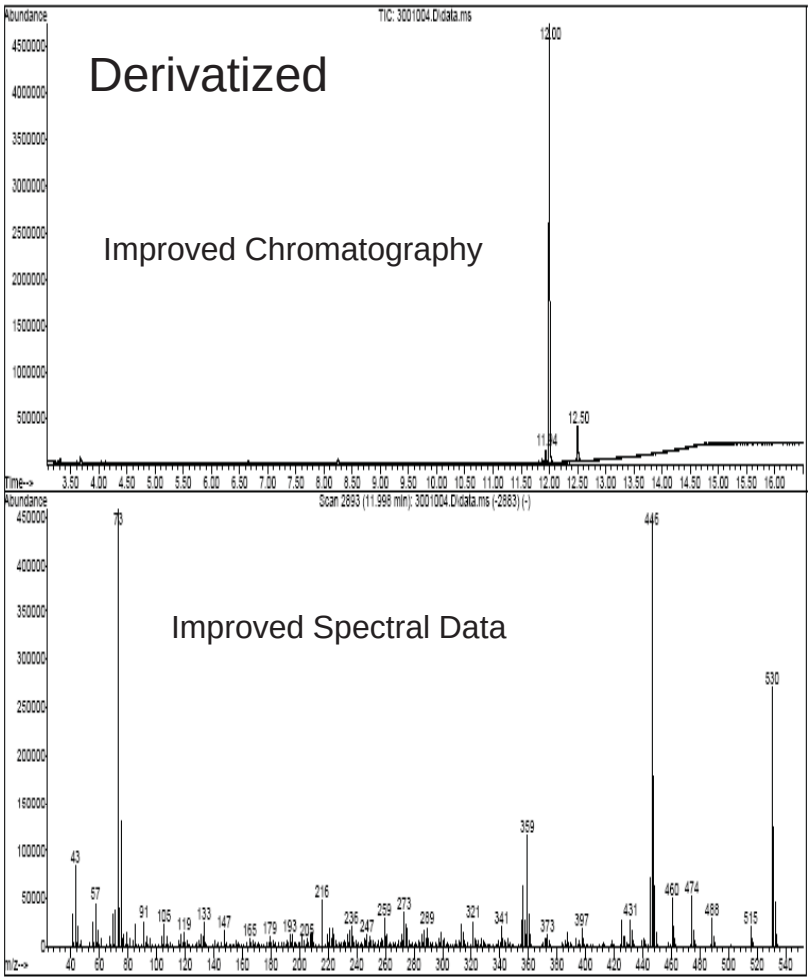
- Multiple function groups require a generalized approach. Compounds are phenols, alcohols, indoles, etc.
- Acid/base extraction
- Methanol digestion
- Matrix contains co-extracted materials

Derivatization

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

HU-211 Standard



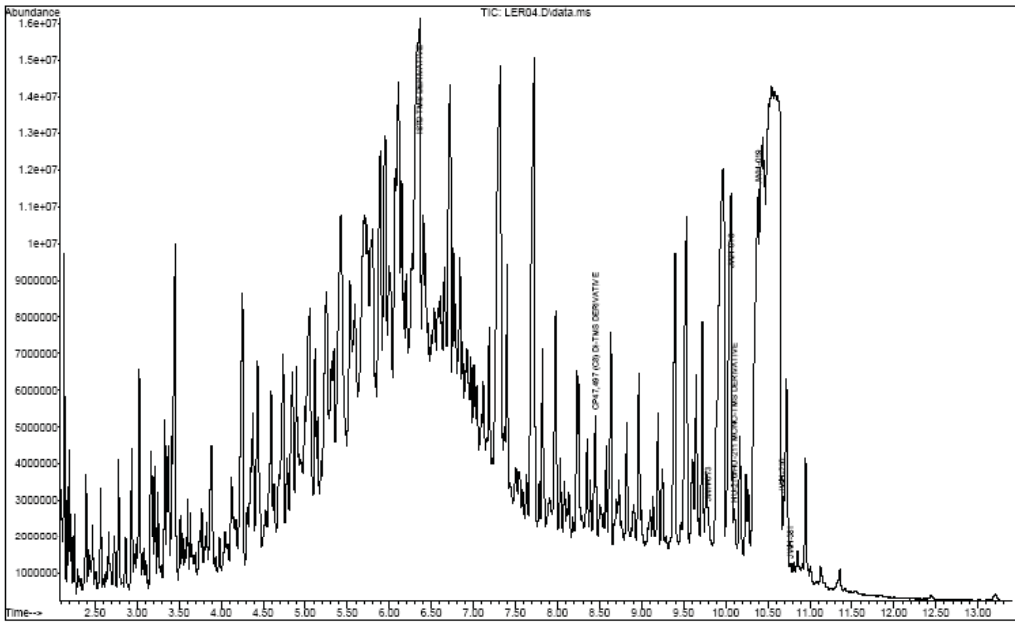


SQ Acquisition Parameters

- 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



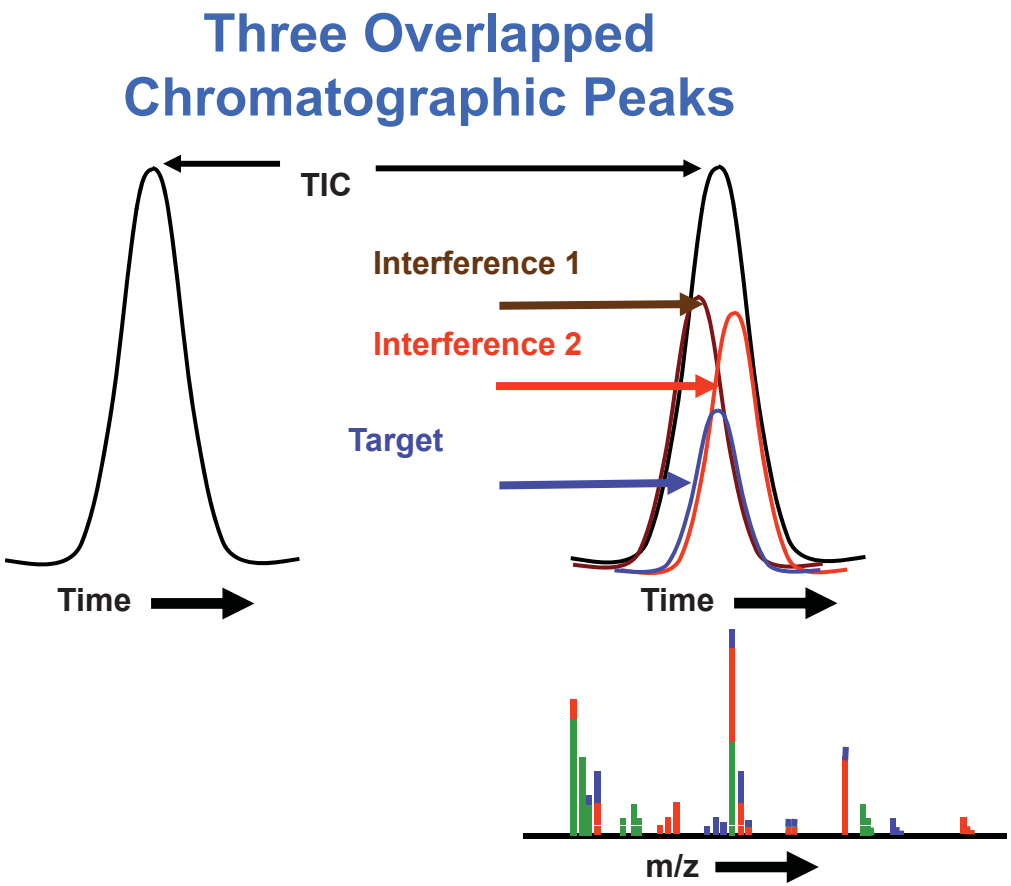
Why Use Deconvolution Reporting Software?

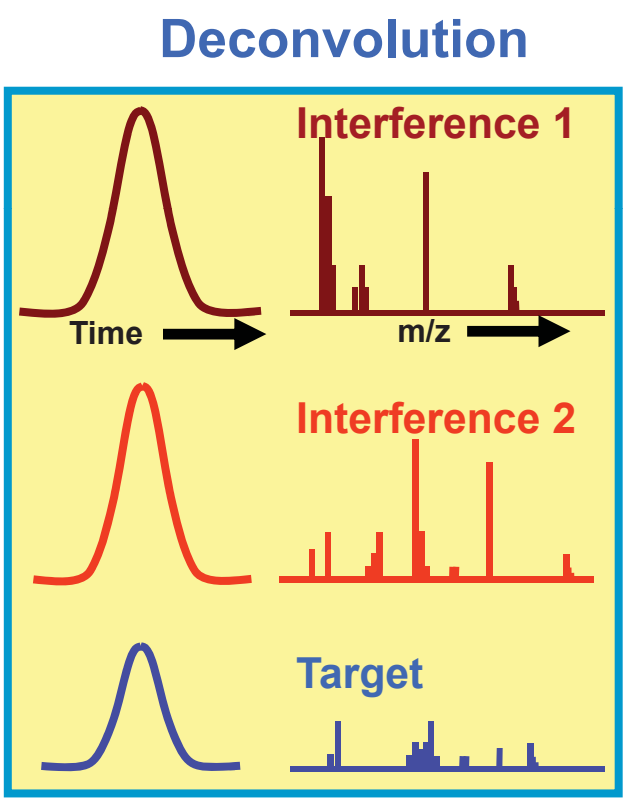
Deconvolution of mass spectra removes/reduces interferences from chromatographically overlapped peaks:

- Better identification and confirmation of analytes in high matrix samples
- Reduces data review time, especially for large screening methods
- Reduces false positives and false negatives 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





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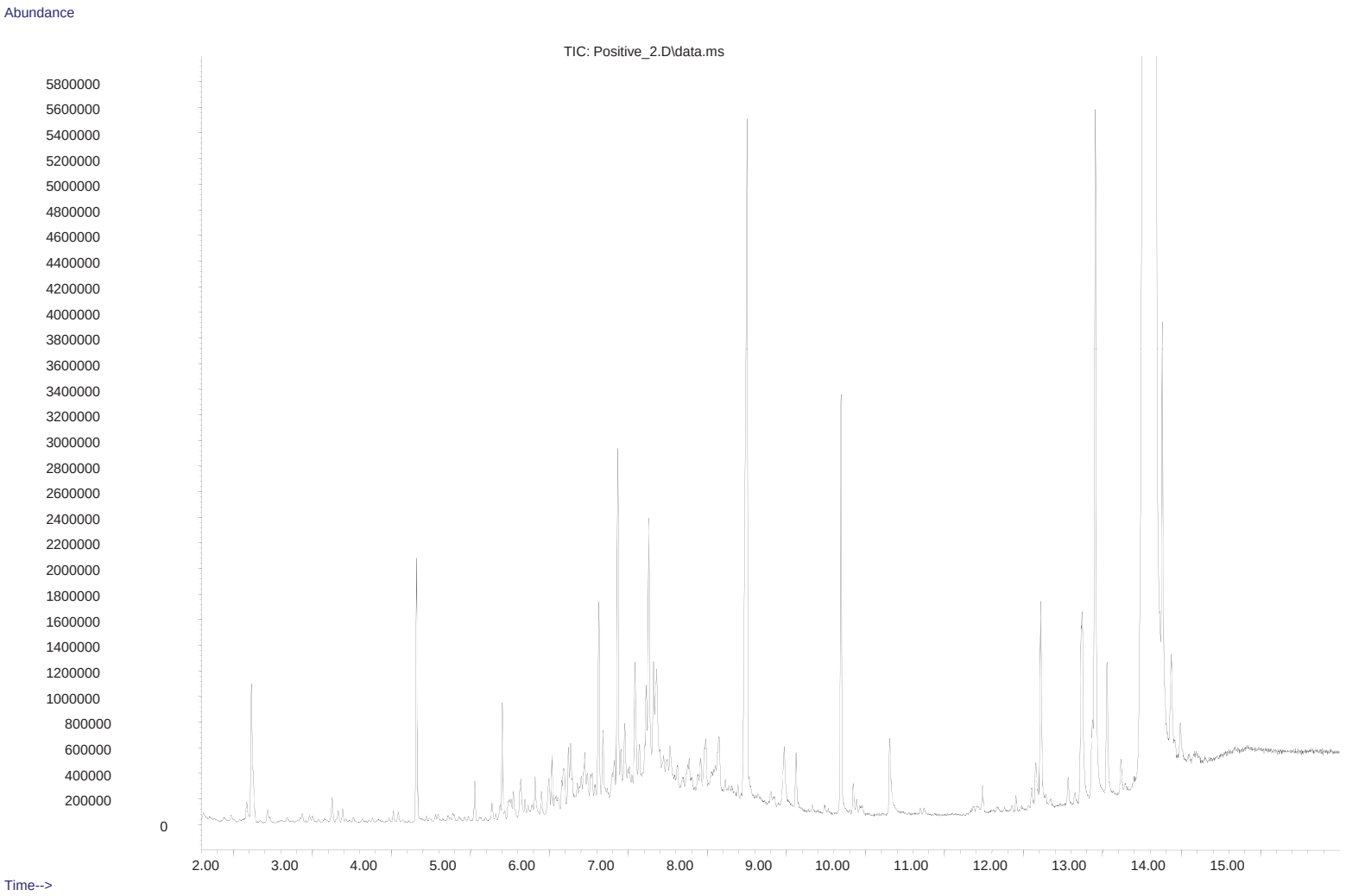
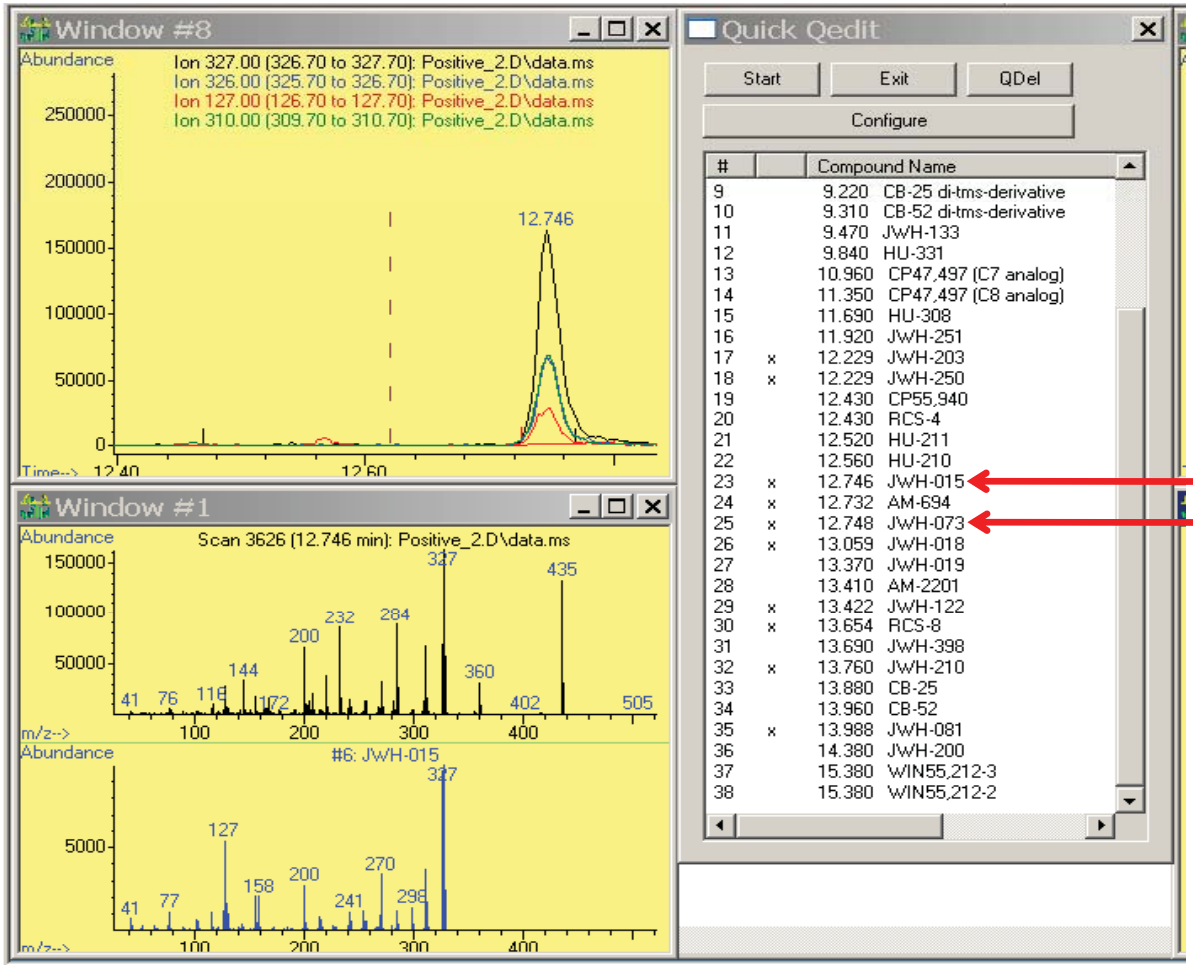
Example: Positive “Herbal Incense” Sample



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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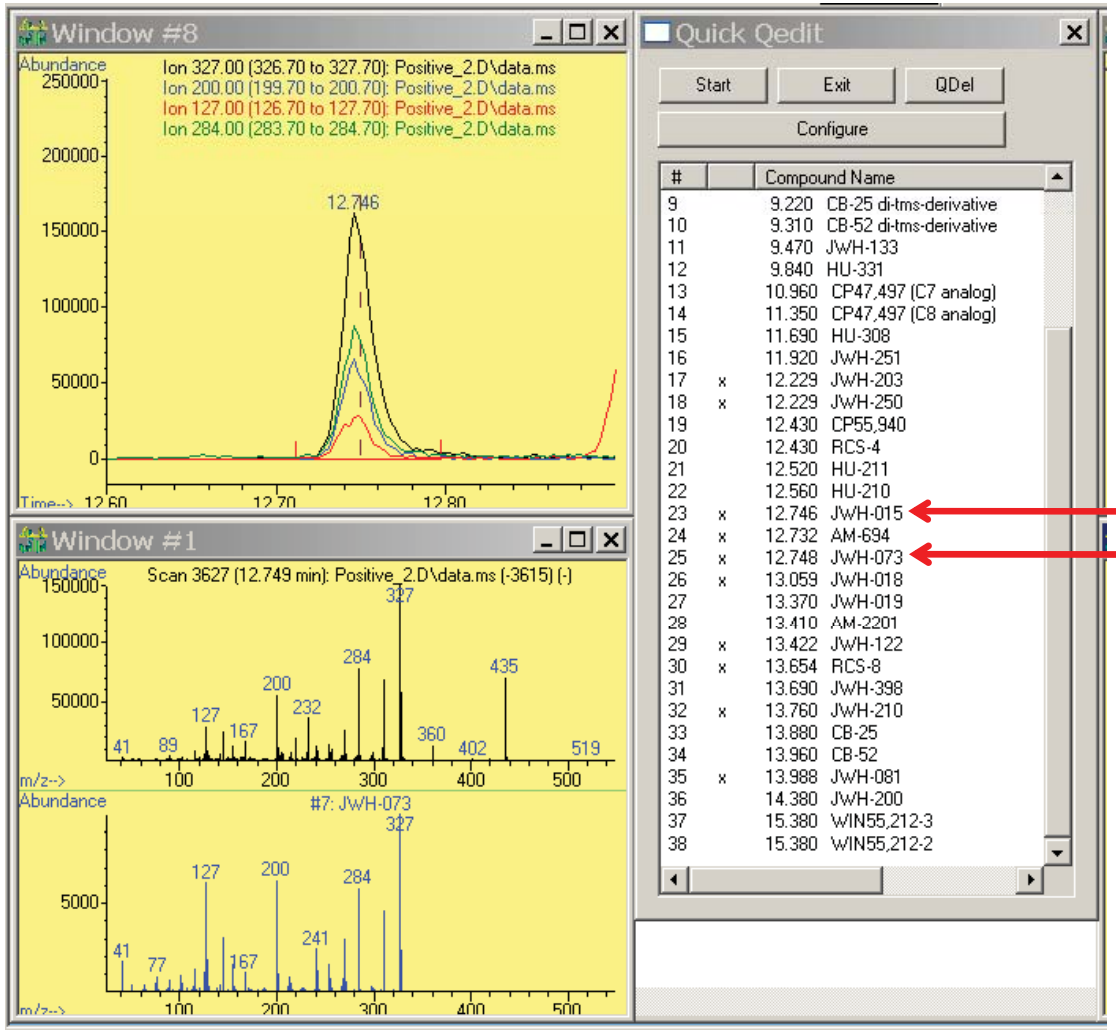
JWH-073 Spectrum Looks Better But Is Still Dirty



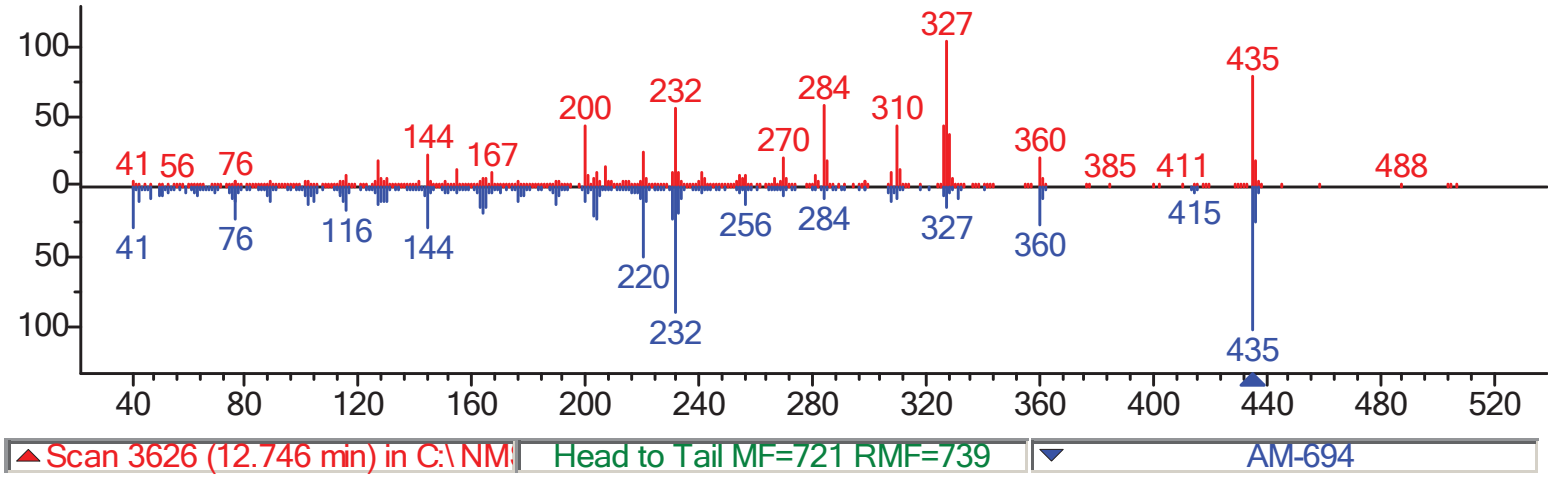
JWH-073 looks more likely.

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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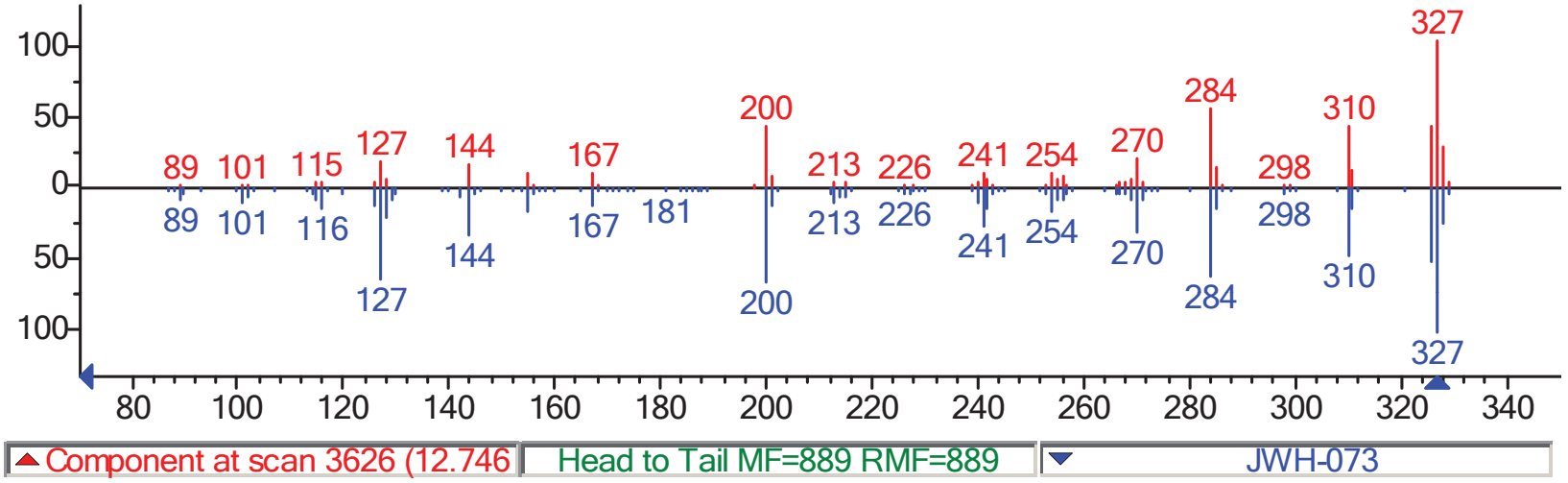
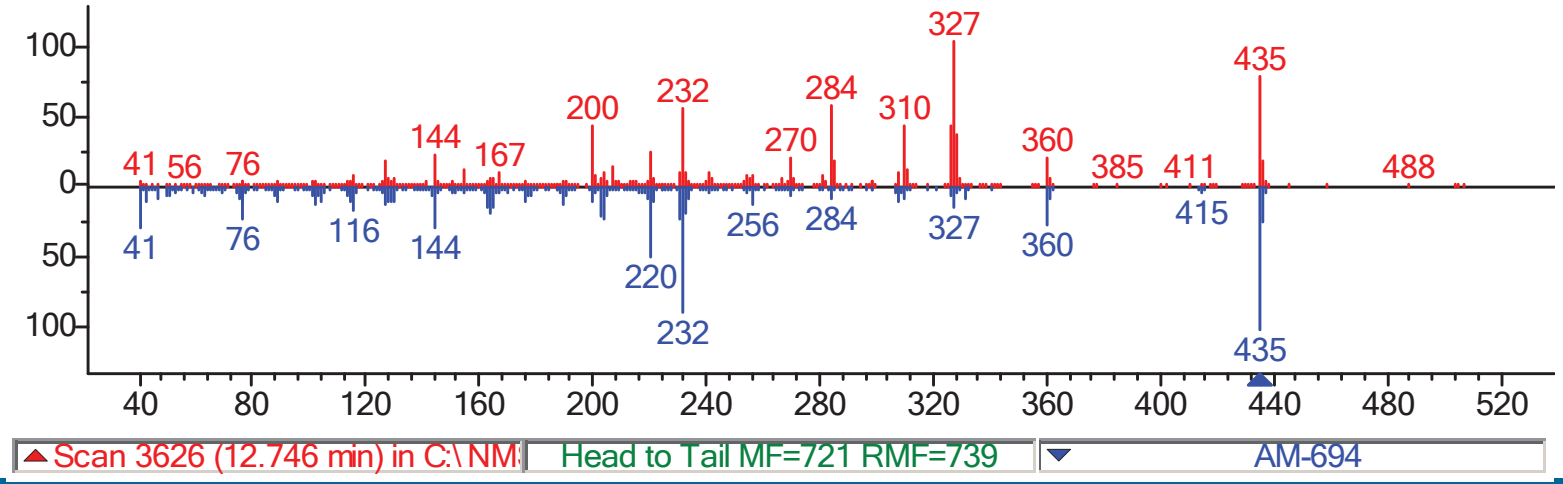
Now Use Deconvolution With AMDIS

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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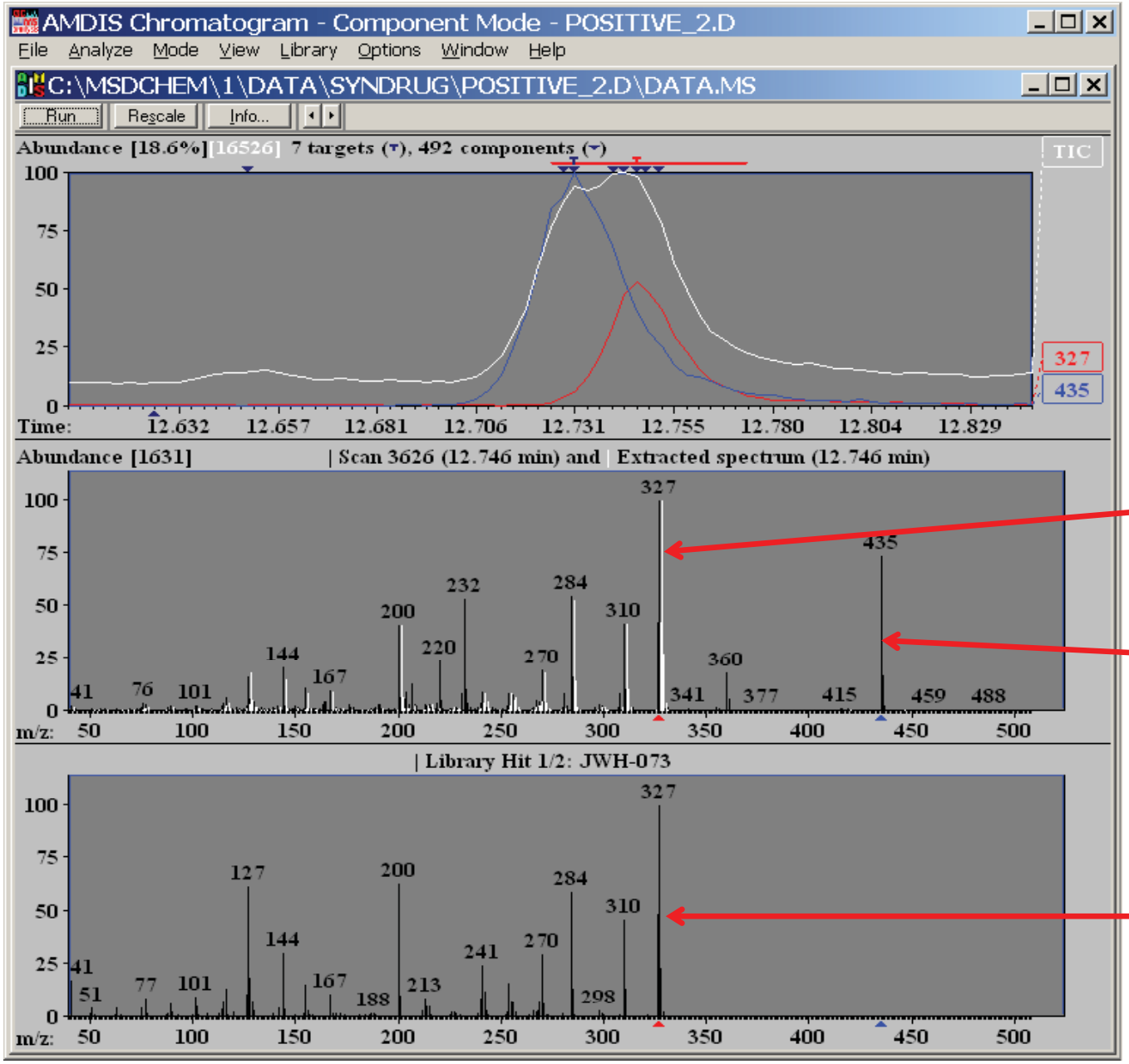

AMDIS sorts out confusion:

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

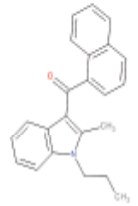
Identification of Synthetic Cannabinoids in Herbal Incense Blends by GC/MS

Application Compendium

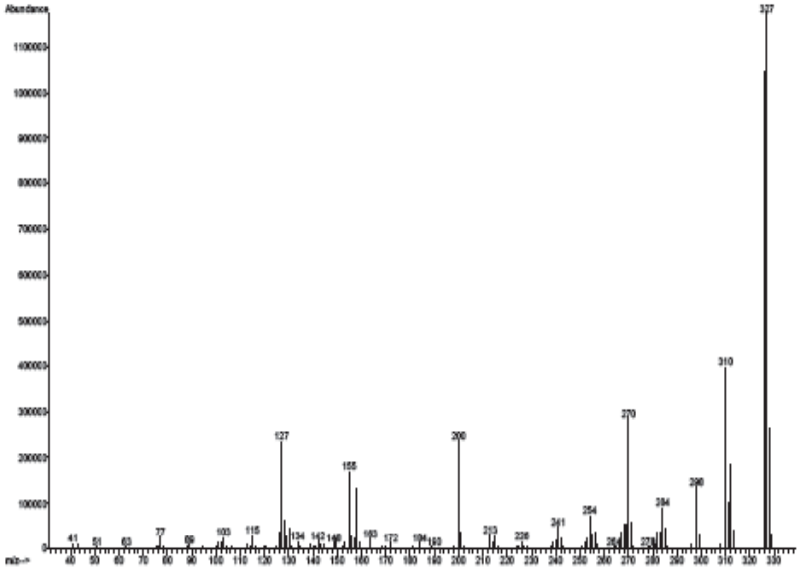
JWH-015

Chemical name	(2-Methyl-1-propyl-1H-indol-3-yl)-1-naphthalenylmethanone	
Molecular formula	$C_{25}H_{21}NO$	
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:



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)

www.agilent.com/chem/cannabinoidcd

Synthetic Cannabinoids: the Analytical Challenges

GC/QQQ (TANDEM MASS SPECTROMETRY)

GC/QQQ: An Alternative to DRS

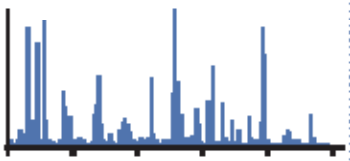
DRS uses a mathematical algorithm to deconvolute complex spectra into its component parts to facilitate interpretation

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

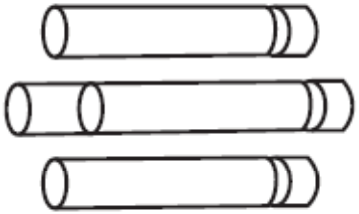
*Analytes are ionized in the **inert ion source** after chromatographic separation by the GC.*

Inert Ion Source

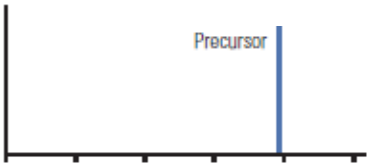


Target and matrix Ions created from ionization

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

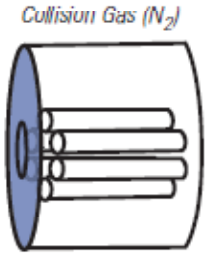


Quartz Quadrupole



Target precursor ion isolated from non-target ions

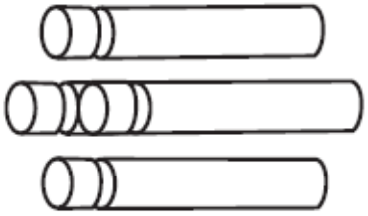
*Collisional processes in the **hexapole collision cell** dissociate the precursor ion into unique product ions.*



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.

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

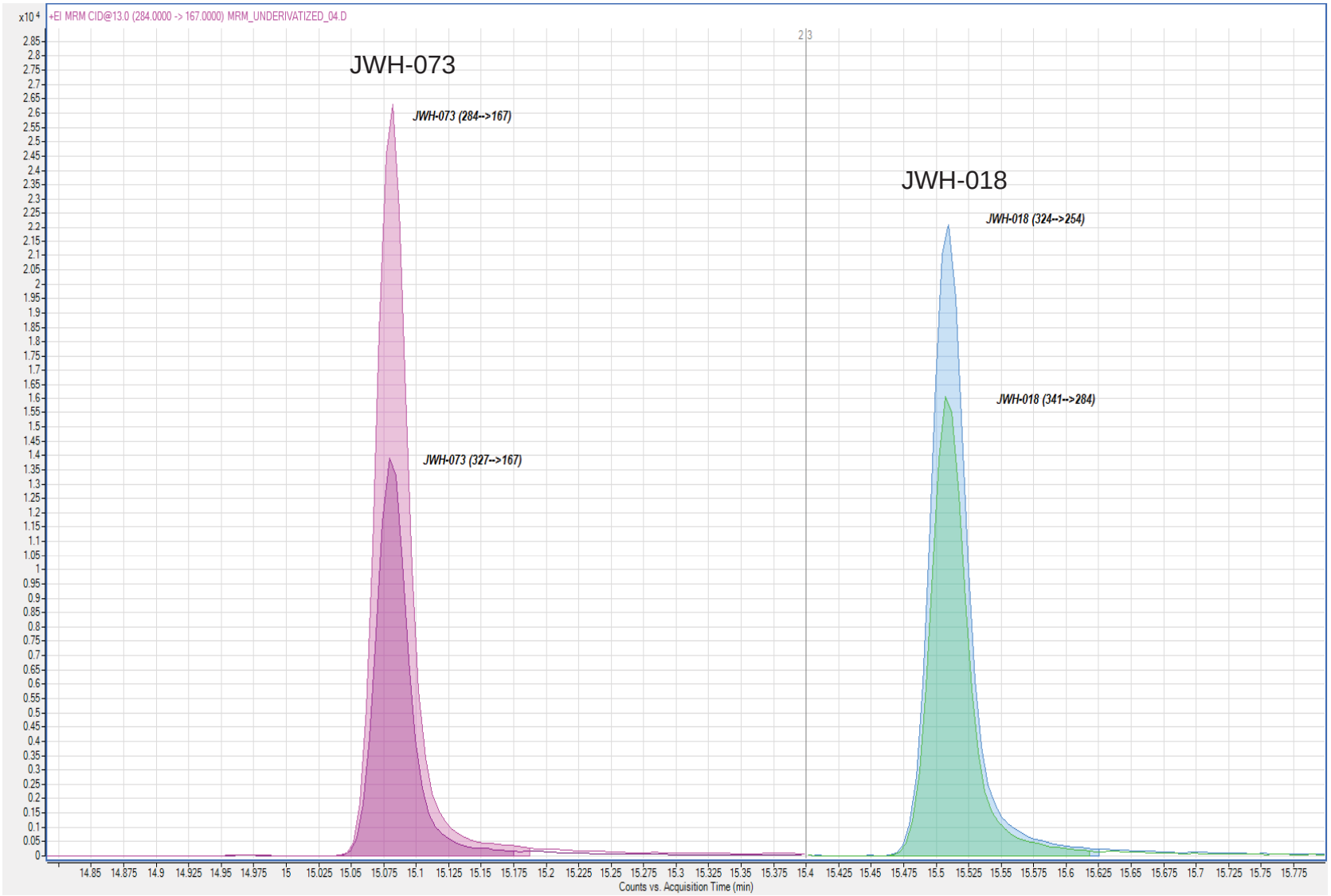
He Quench Gas 2.25 mL/min

N2 Collision Gas 1.5 mL/min

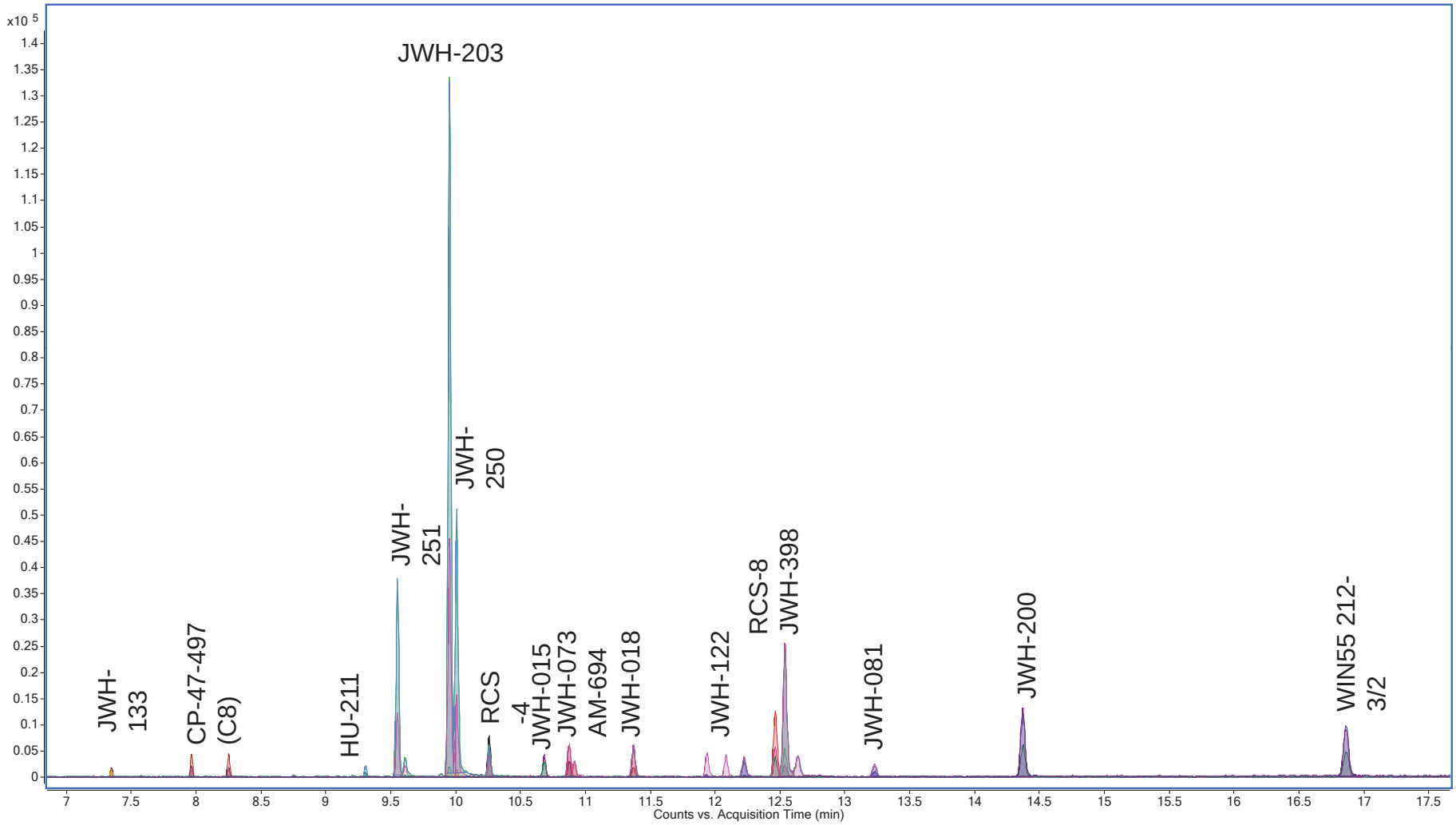
SRM Transitions

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

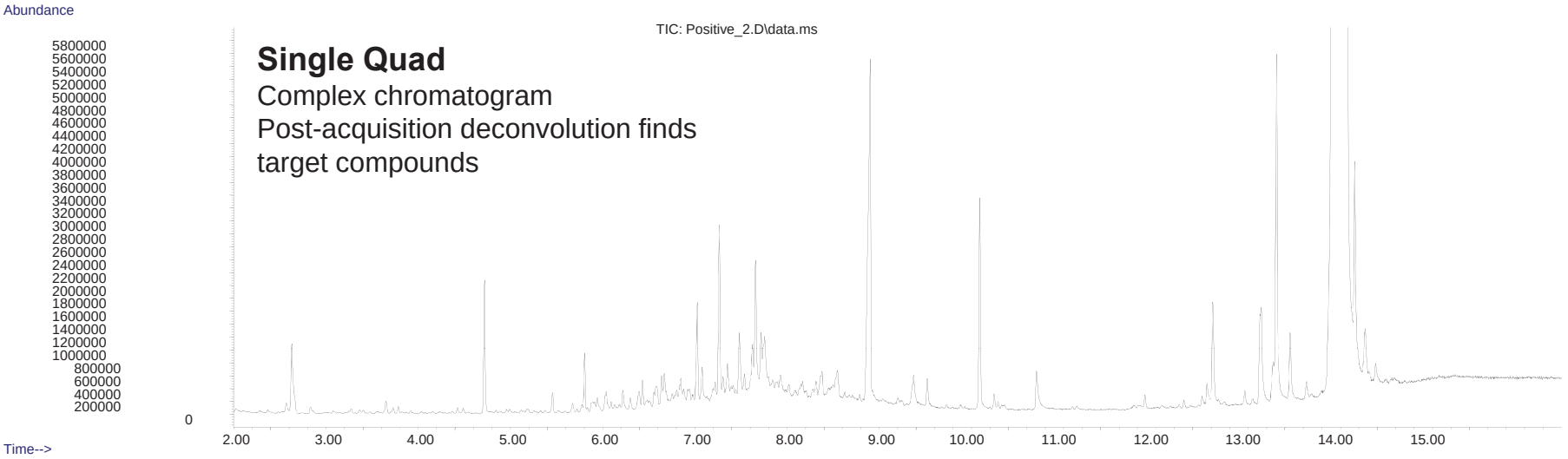
Compound Name	Precursor Ion	Product Ion	Collision Energy
JWH-200	384	100	23
JWH-200	100	56	17
JWH-203	339	214	3
JWH-203	214	144	17
JWH-250	335	214	3
JWH-250	214	144	17
JWH-251	214	144	17
JWH-251	144	116	12
JWH-398	375	201	23
JWH-398	318	189	23
RCS-4	321	264	19
RCS-4	264	135	17
RCS-8	254	158	13
RCS-8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

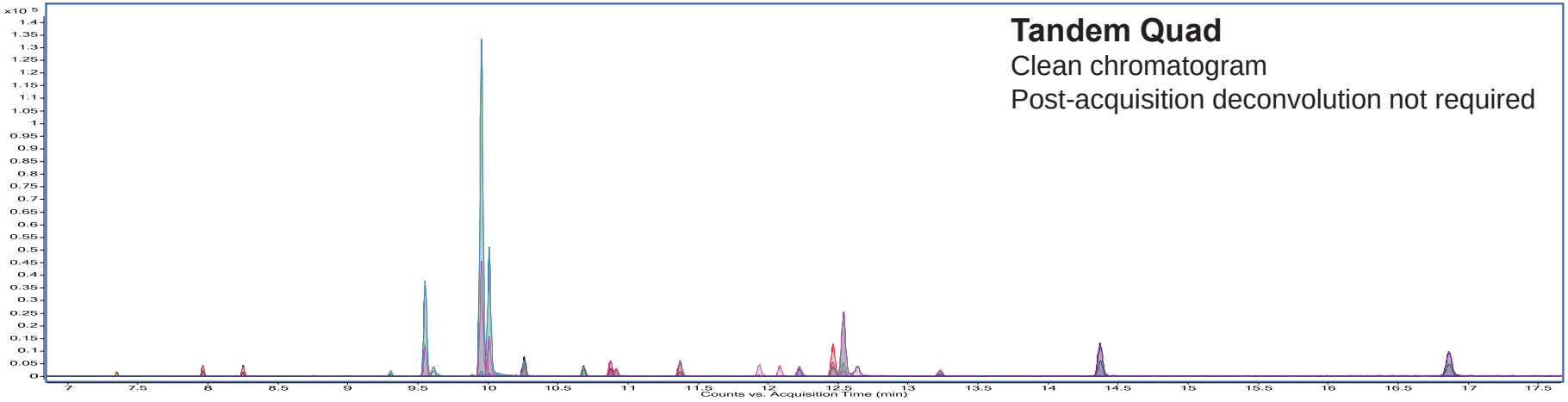


SRM TIC Chromatogram of SPICE Compounds

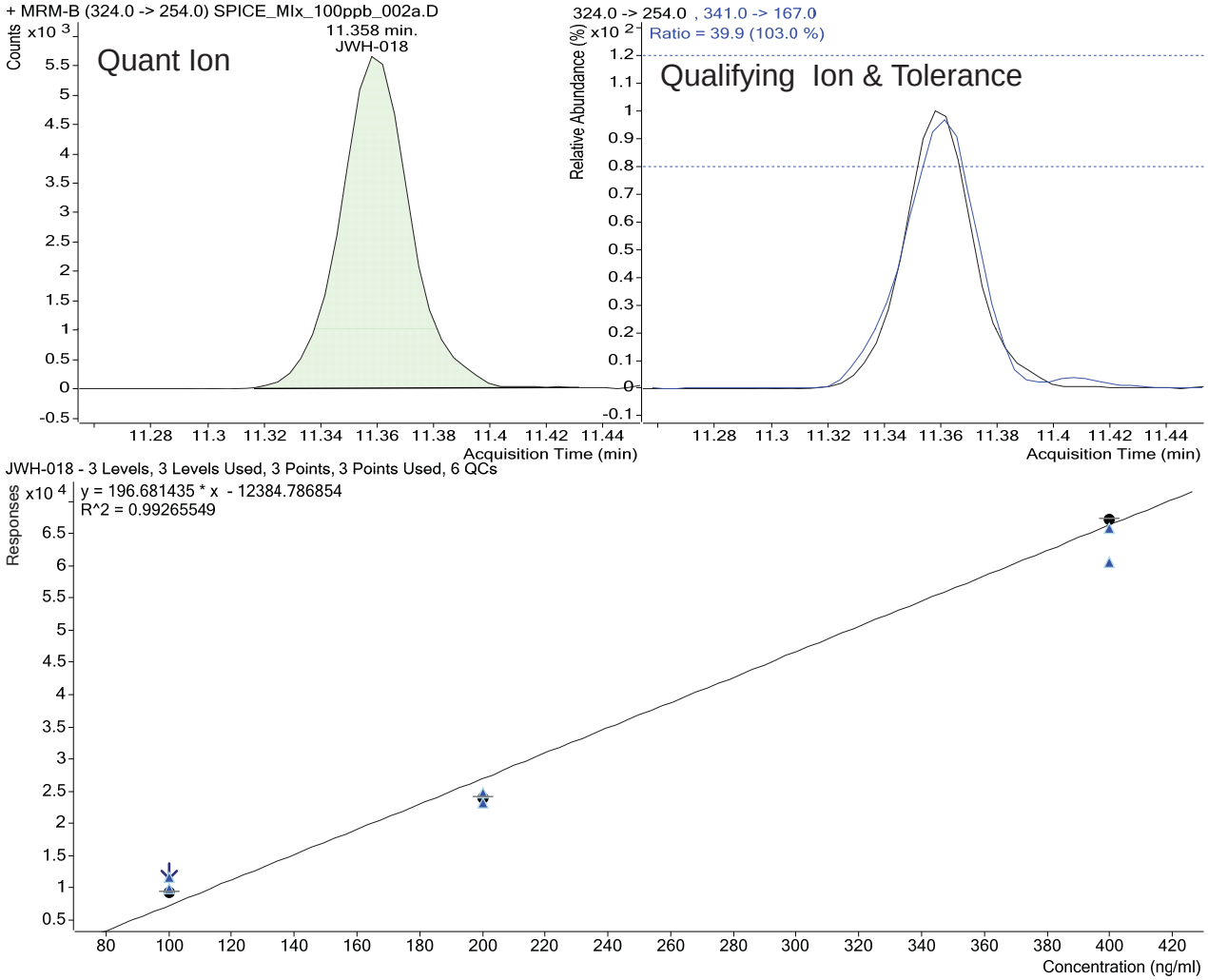


SQ vs. TQ “TICS”


Abundance
TIC: Positive_2.D\data.ms
Single Quad
Complex chromatogram
Post-acquisition deconvolution finds target compounds
Time-->


Tandem Quad
Clean chromatogram
Post-acquisition deconvolution not required
Counts vs. Acquisition Time (min)

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



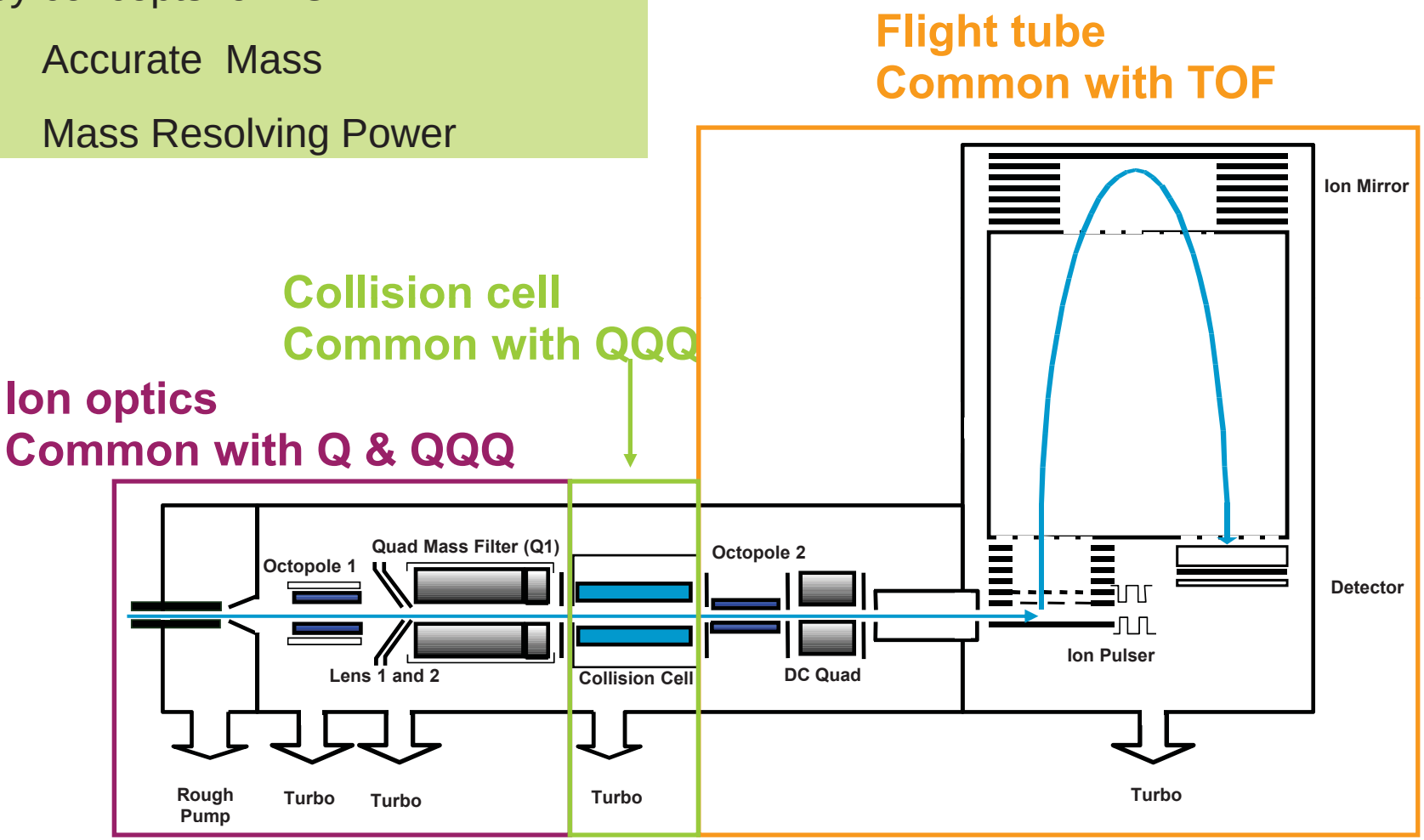
Synthetic Cannabinoids: the Analytical Challenges

GC/Q-TOF

Q-TOF Fundamentals

Key concepts for TOF:

1. Accurate Mass
2. Mass Resolving Power



Mass Analysis for TOF

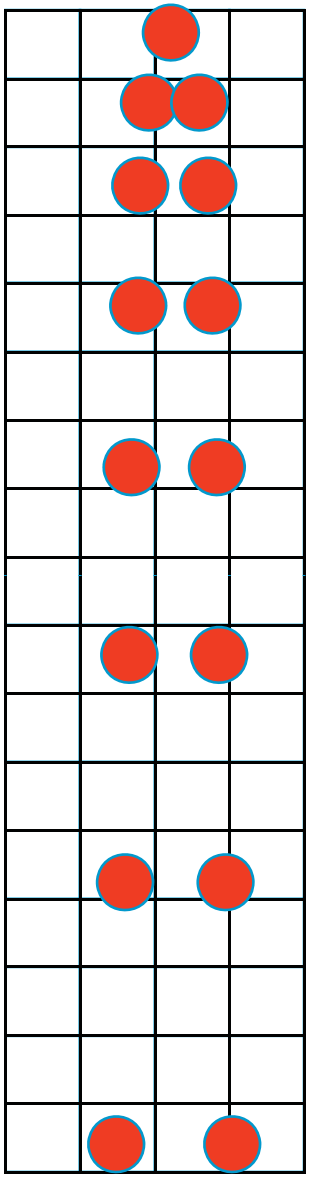
$v = d/t$

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

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

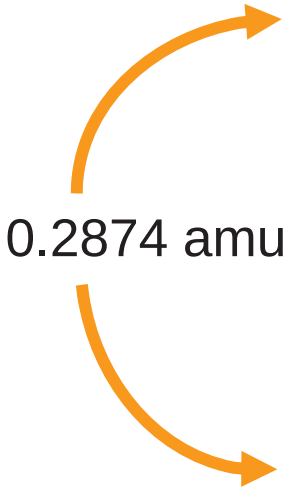
Energy (E) and Distance (d) are fixed

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



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



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

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

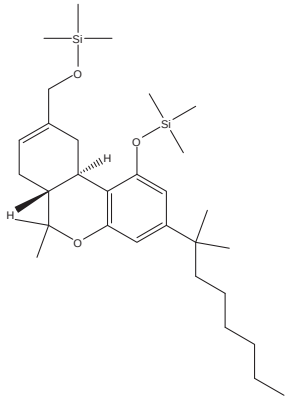
Pulsed, split-less injection

100 C-> 340 C @ 25 C/min

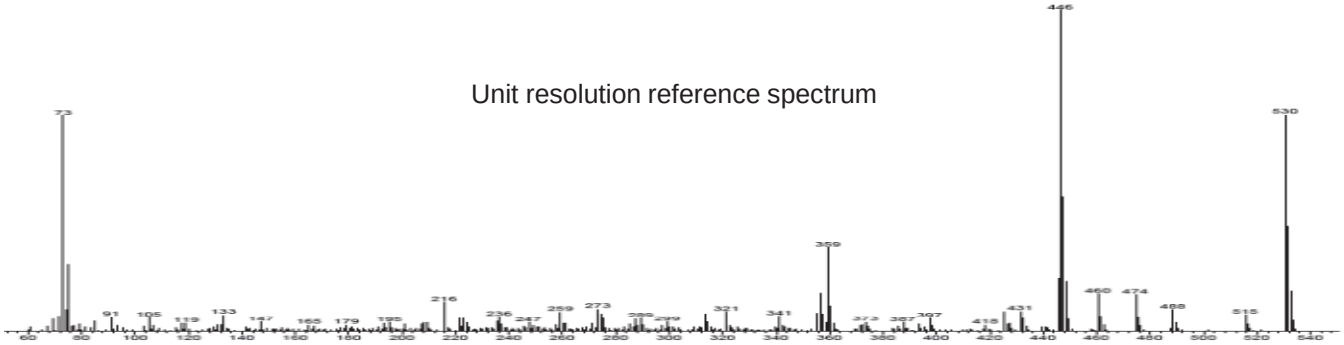
EI

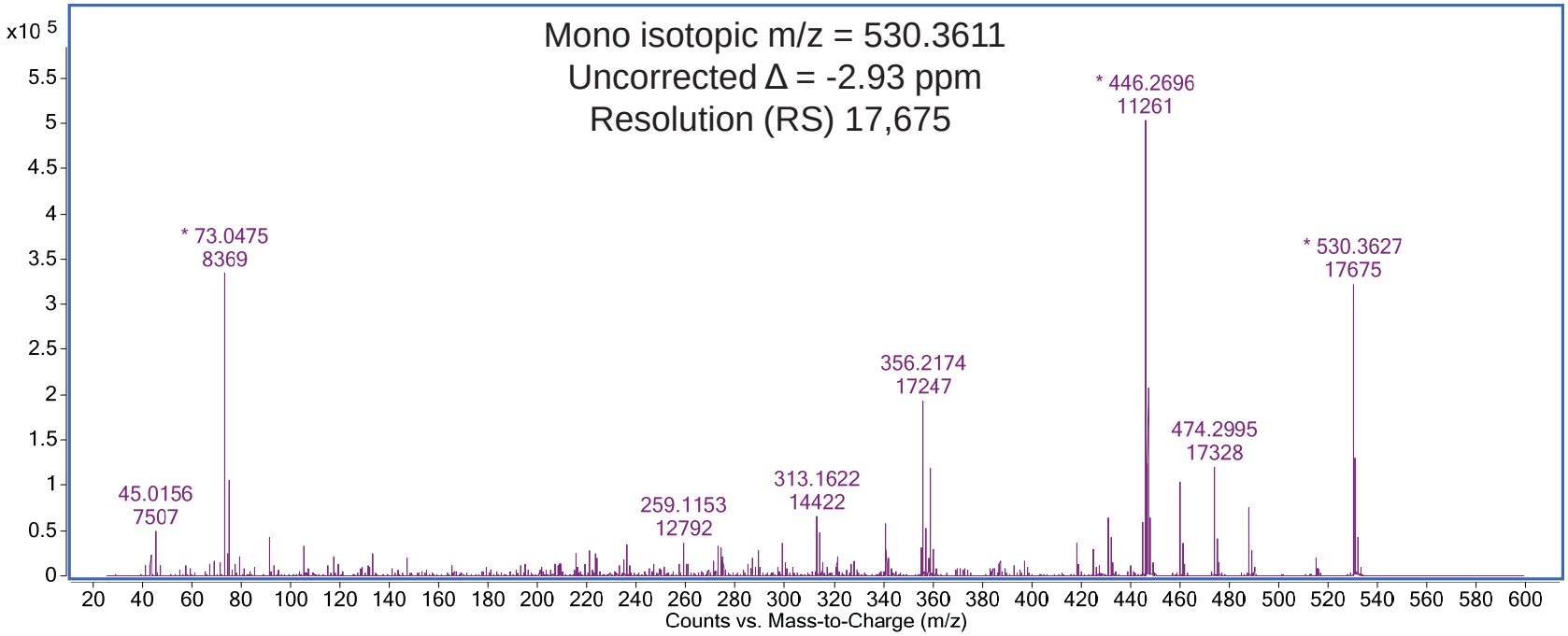
- High resolution mode (HRM)

20 ppb HU-210: HRM

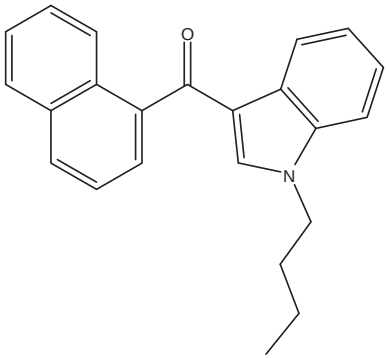


Unit resolution reference spectrum

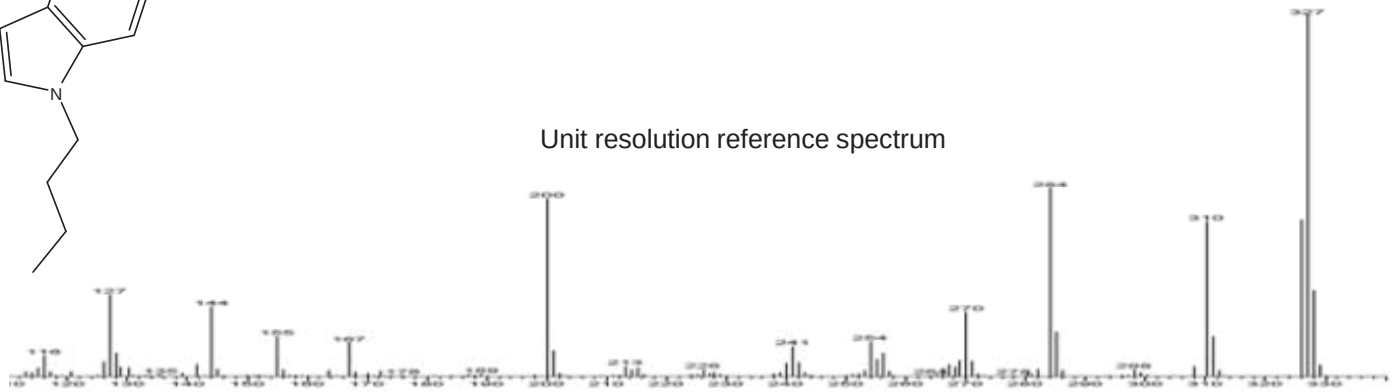




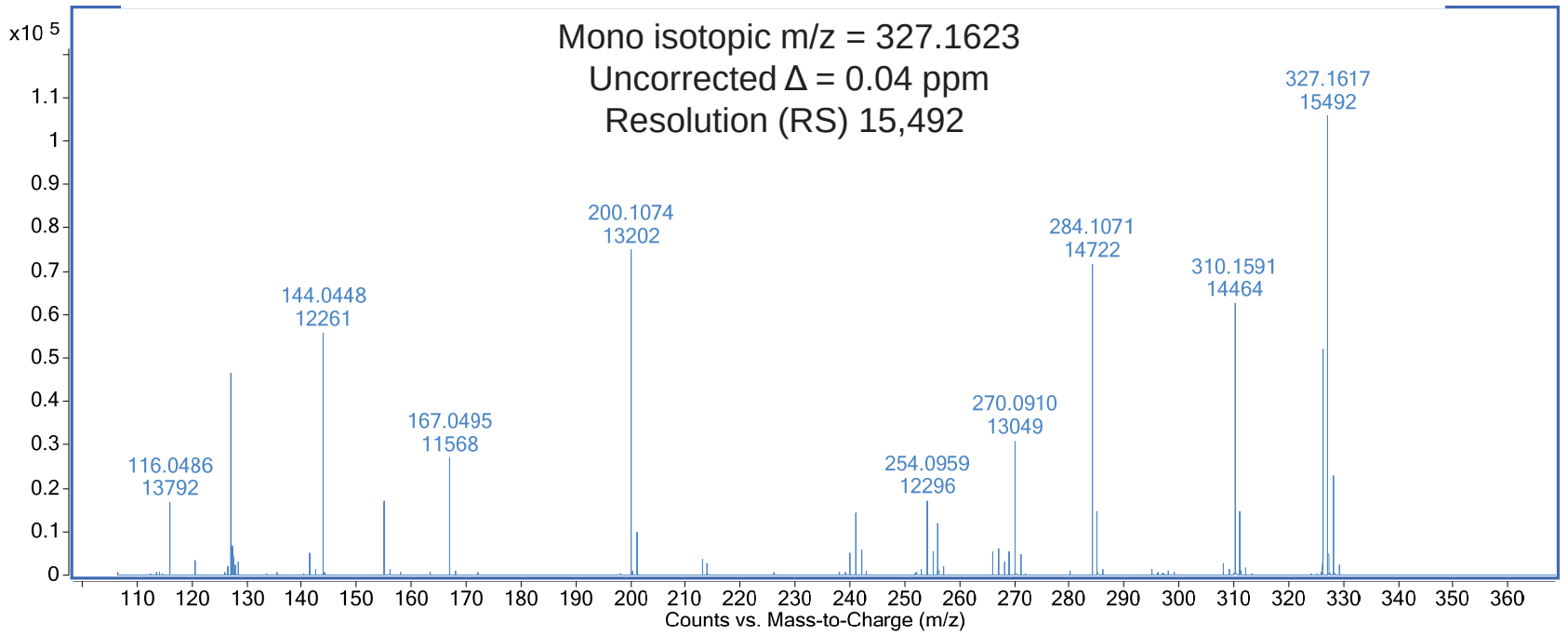
20 ppb JWH-073: HRM



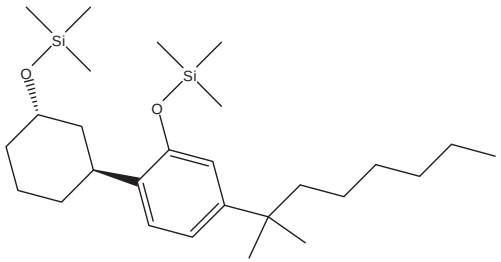
Unit resolution reference spectrum



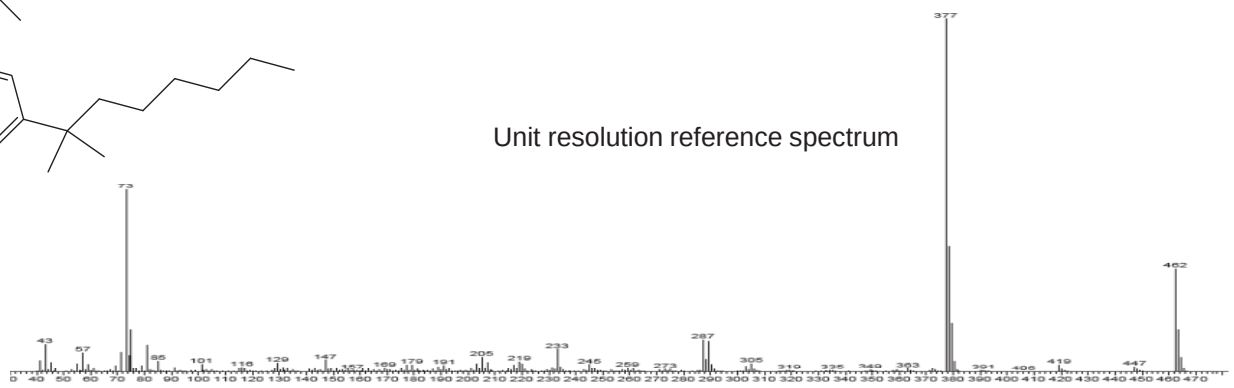
Mono isotopic m/z = 327.1623
Uncorrected Δ = 0.04 ppm
Resolution (RS) 15,492

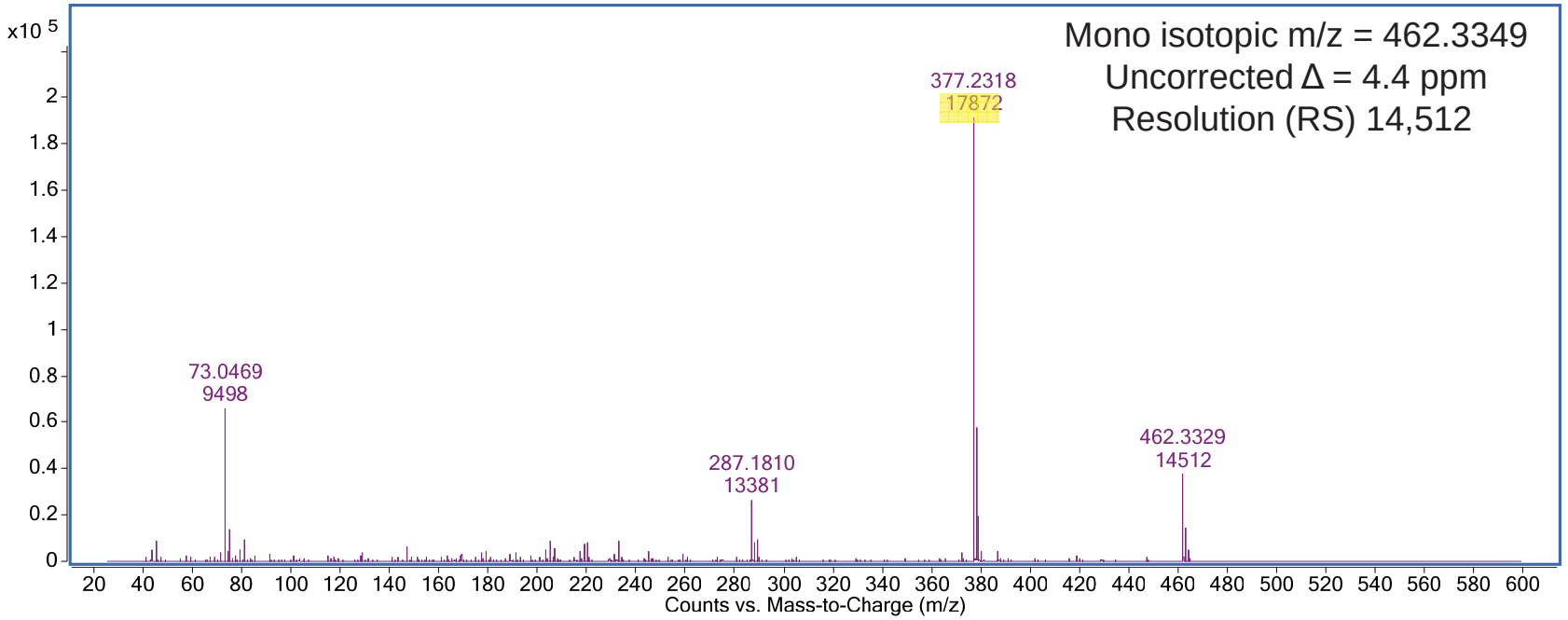


20 ppb CP-47,497 (C7 analogue): HRM



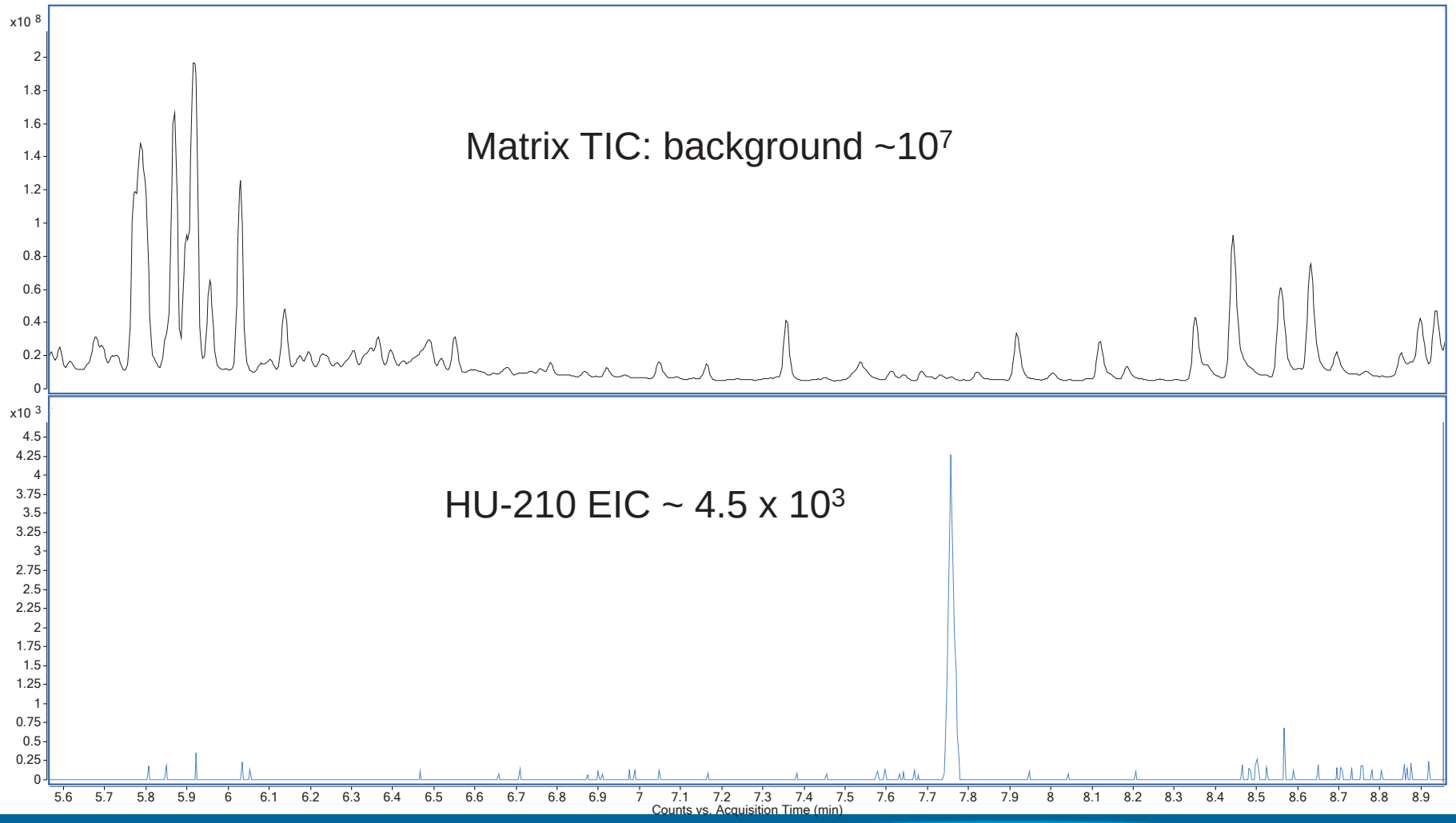
Unit resolution reference spectrum



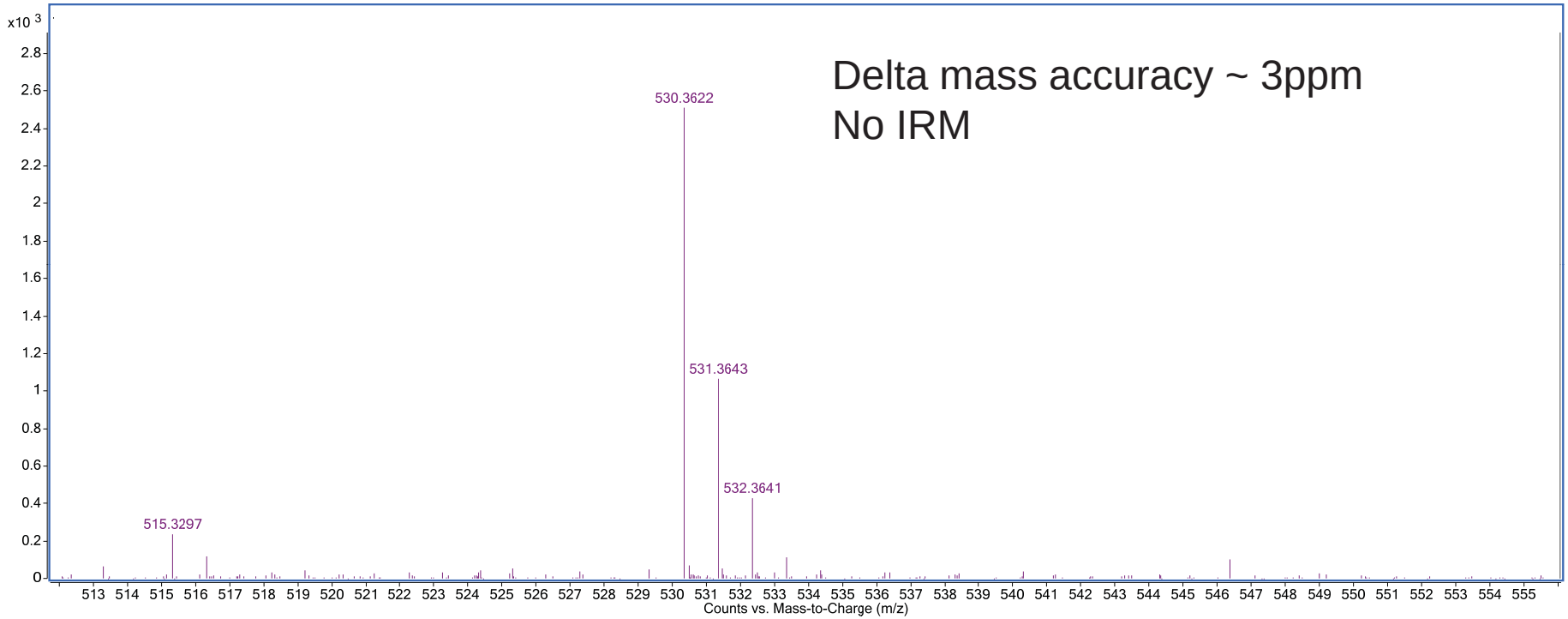


Mono isotopic m/z = 462.3349
Uncorrected Δ = 4.4 ppm
Resolution (RS) 14,512

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



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



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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