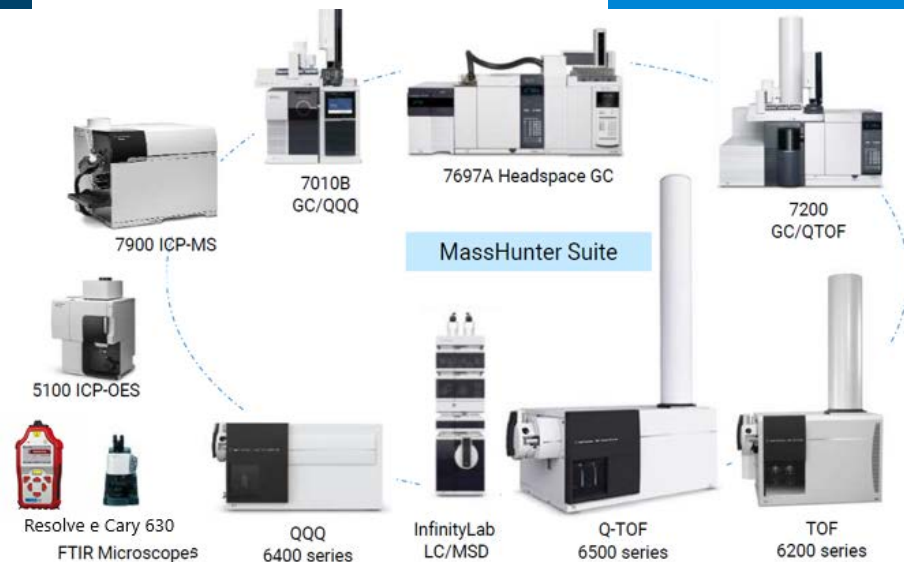


Técnicas Analíticas em Química Forense



Romão Beserra
Especialista de Produto GC e GCMS
romao_beserra-jr@agilent.com
18/10/19

Agenda

Introdução – A Cromatografia e a Espectrometria de Massas

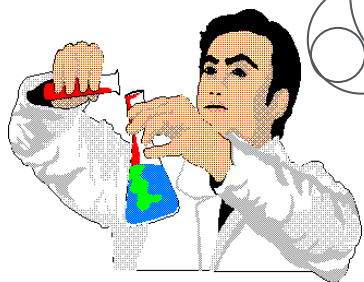
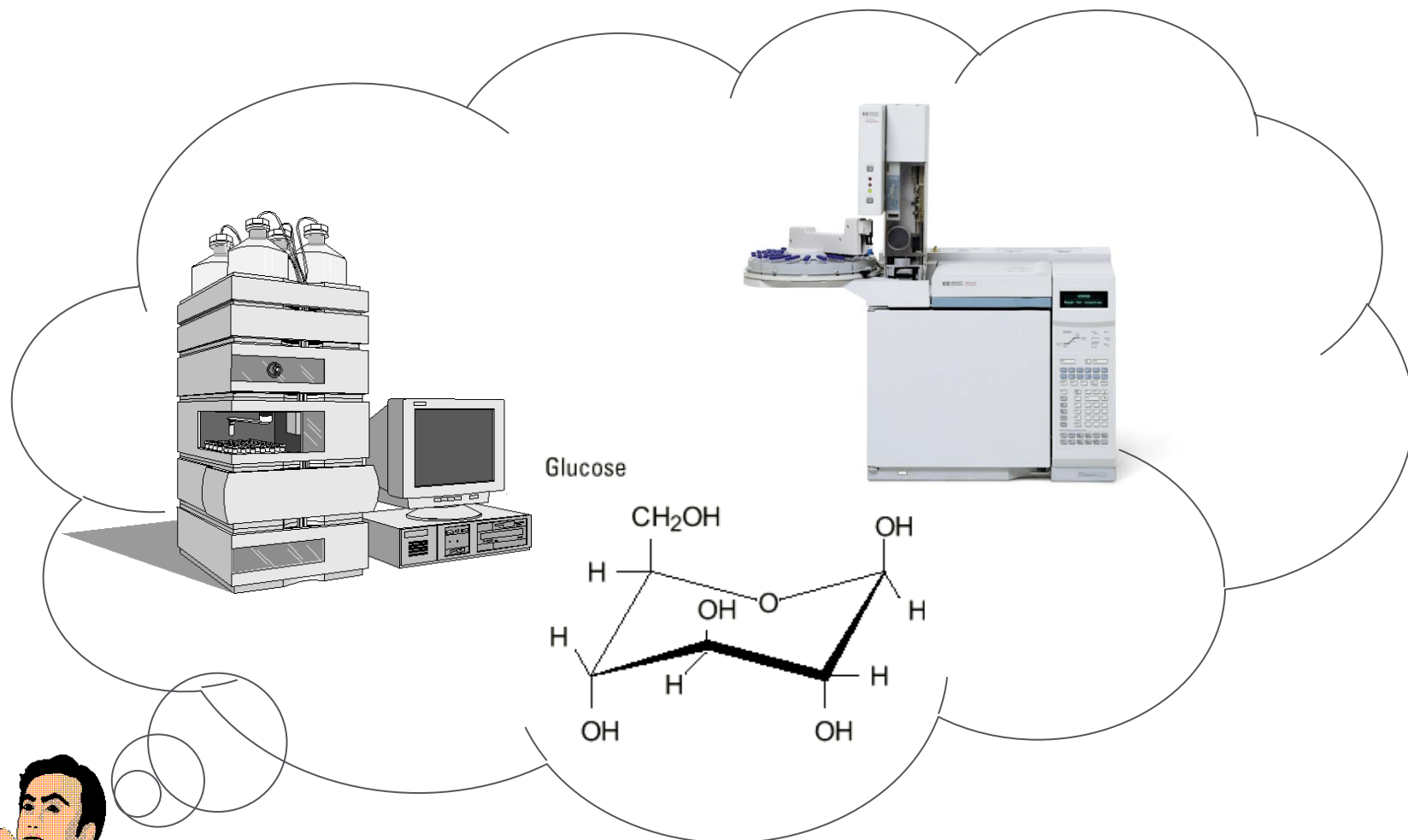
Aspectos do SQ

Aspectos do TQ

Aspectos do QTOF

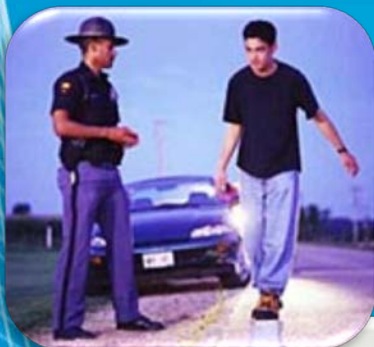
Outras Técnicas – LCMS e IR

Técnicas de separação



Eu tenho duas técnicas de separação no laboratório, LC e GC Qual eu devo usar para carboidratos?

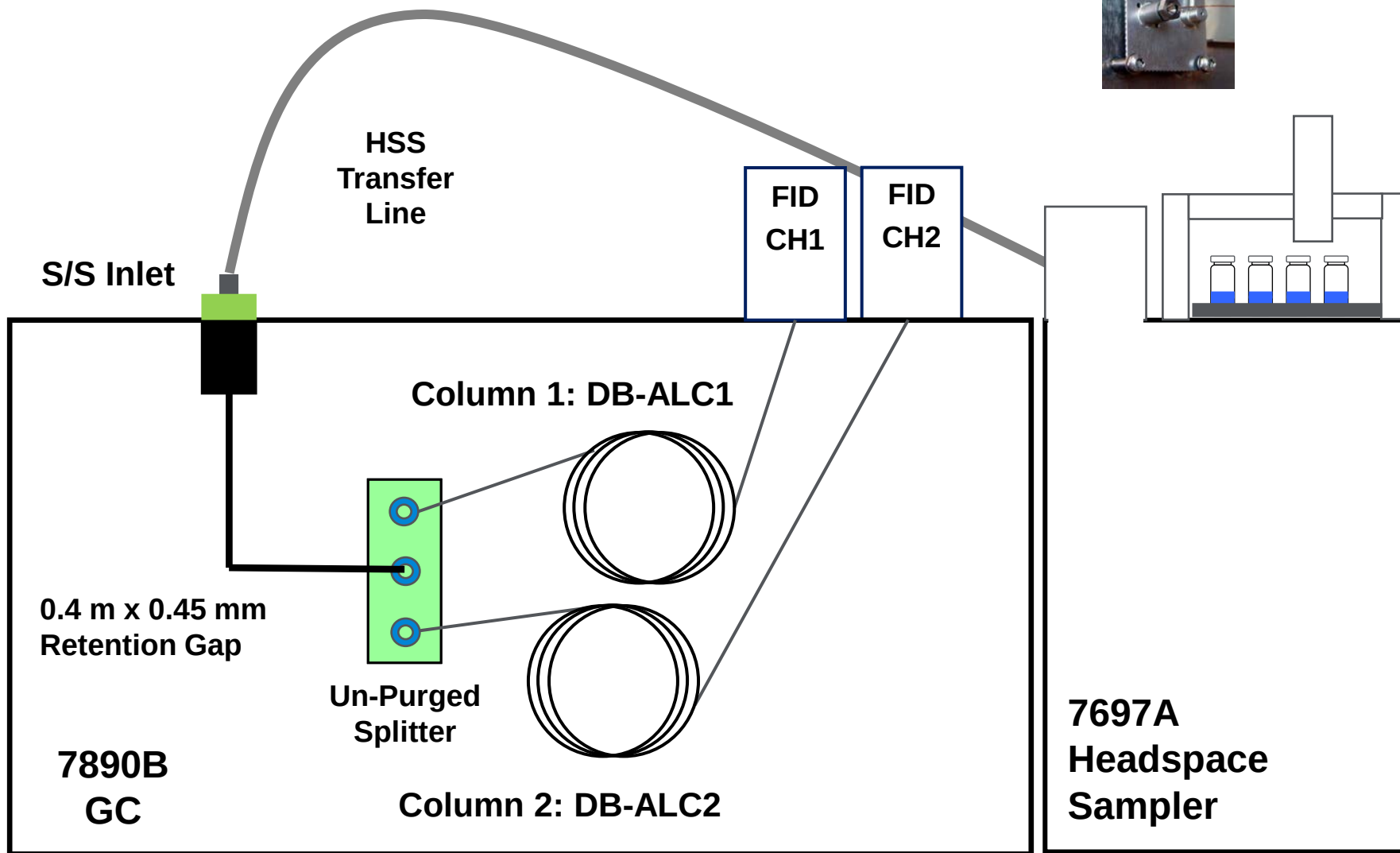
Blood Alcohol Analysis

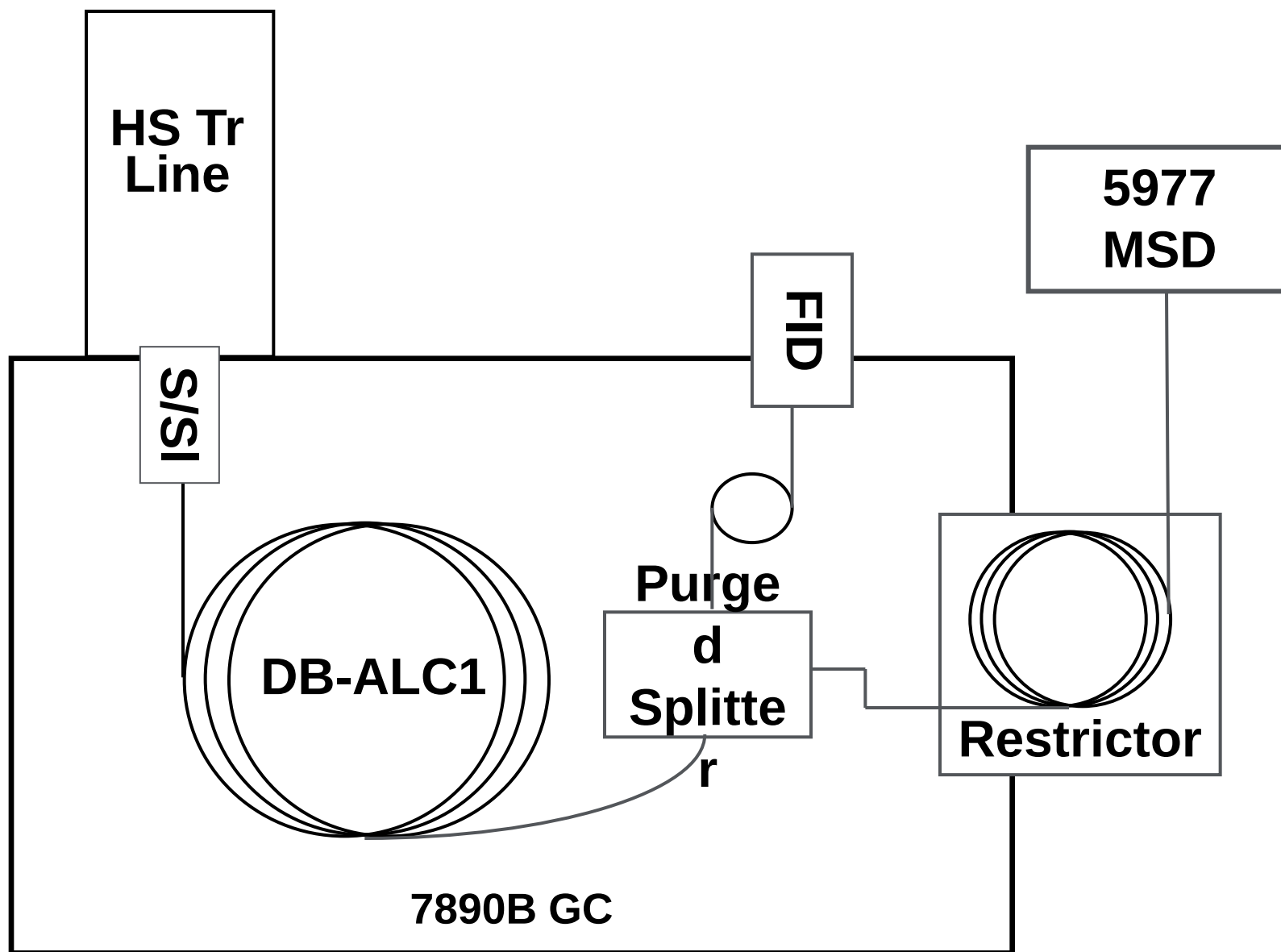


Agilent Technologies

Dual Column Blood Alcohol System

G3445B#683 Schematic





Blood Alcohol Analysis by GC/FID/FID

Dual Channel Blood Alcohol Analyzer (GC/FID/FID)



Determine Blood Alcohol with Dual Column/Dual FID for Precision and Reproducibility

Application Note

Forensics and Toxicology

Authors

Haleigh Boswell and Frank Dorman
The Penn State University
University Park, PA, USA

Ken Lynam
Agilent Technologies, Inc.

Abstract

This application note highlights the use of Agilent J&W DB-ALC1 and DB-ALC2 columns for the analysis of blood alcohol concentration by static headspace GC/FID, using a Dual Channel Blood Alcohol Analyzer. The combination of a dual-column/dual-FID configuration delivers precision and reproducibility of the determined alcohol concentration within a complex blood matrix.

Introduction

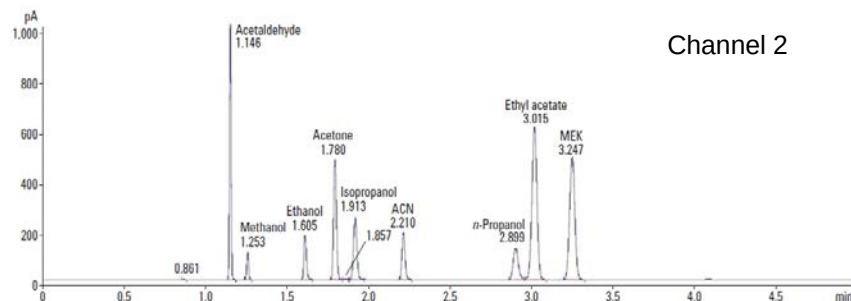
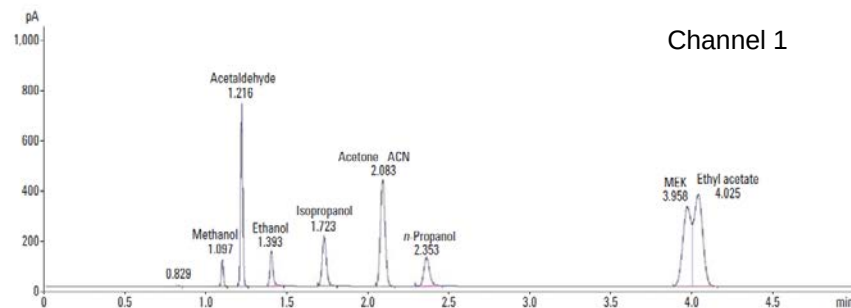
Determining the ethanol content of blood from those charged with driving while intoxicated is one of the most common and widely used applications of headspace-gas chromatography [1]. With a universal threshold value of 0.08 g/dL, a robust and optimized method must be used to ensure the reported values are accurate. Although forensic laboratories perform this analysis routinely, minute errors can occur, ultimately altering the reported value.

Using an internal standard method for quantitative analysis helps compensate for matrix differences [1]. Other alcohols with similar characteristics to ethanol, such as *n*-propanol and *t*-butanol, are typically chosen as internal standards. This compensation occurs due to the internal standards undergoing the same matrix effects as the ethanol within the blood, due to their equivalent chemical polarity. Calibration using the internal standard method characteristically results in lower percent error when compared to the external standard method.



Agilent Technologies

FID Responses for DB-ALC1 and DB-ALC2 Channels

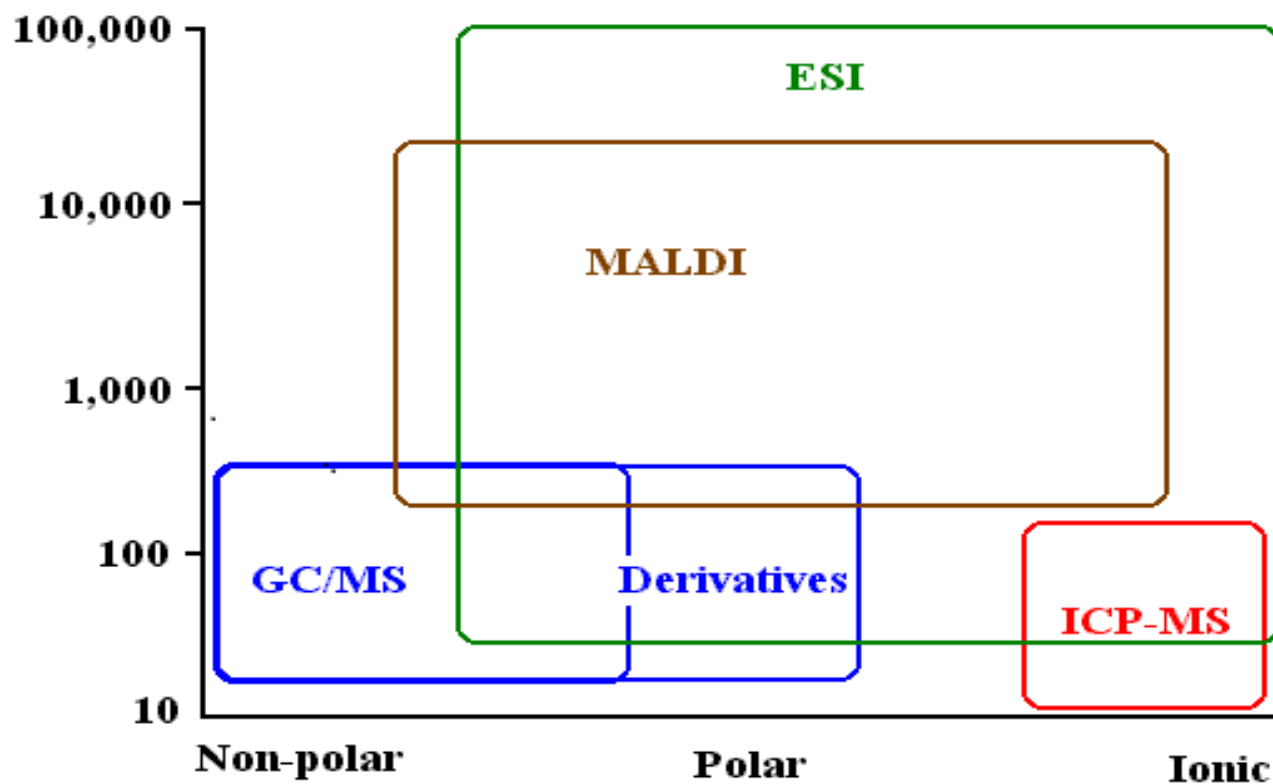


Order an Agilent M74xxxAA Series Analyzer with the following system option:

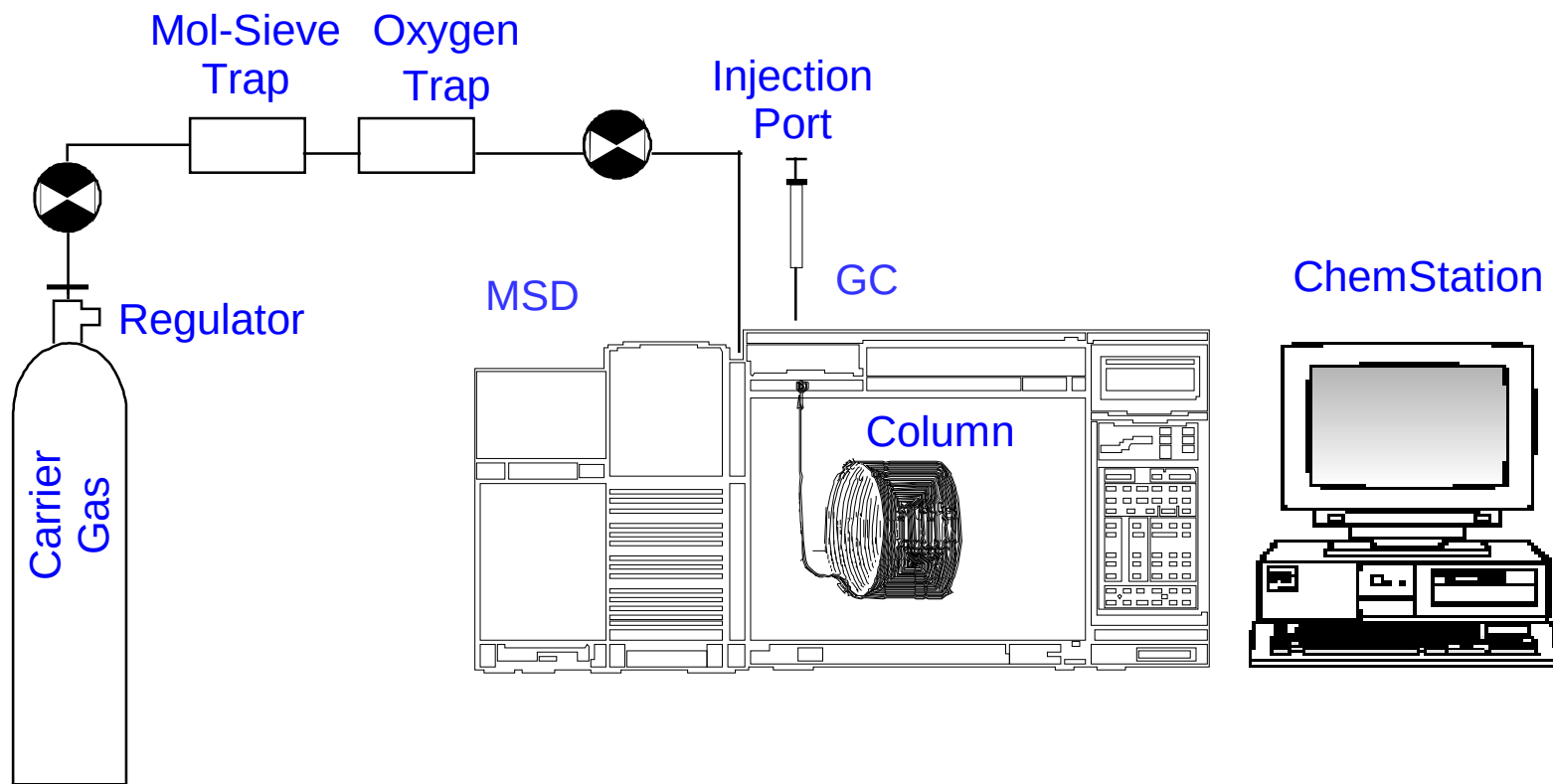
- **Option 683** Dual Channel Blood Alcohol Analyzer (GC/FID/FID)

<http://www.chem.agilent.com/Library/applications/5991-3671EN.pdf>

Espectrometria de massas

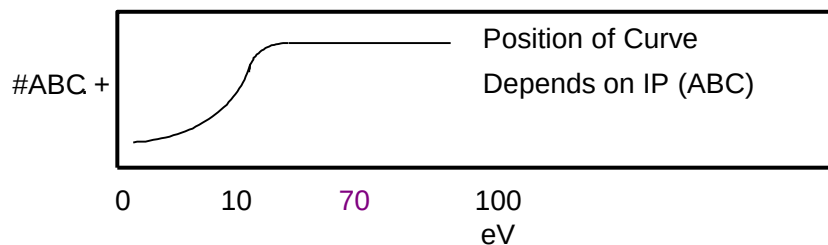
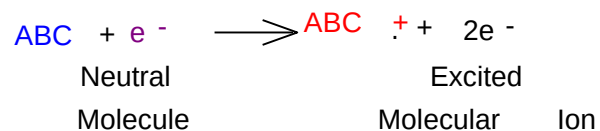


Typical Agilent GC-MSD System



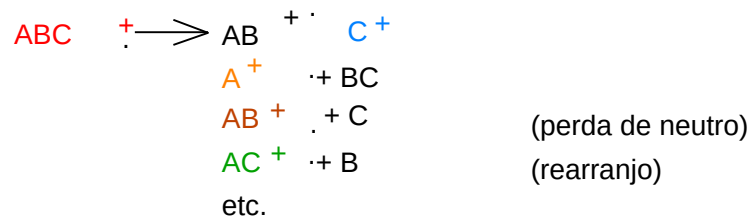
Ionização por impacto de elétrons (EI)

Ionização:

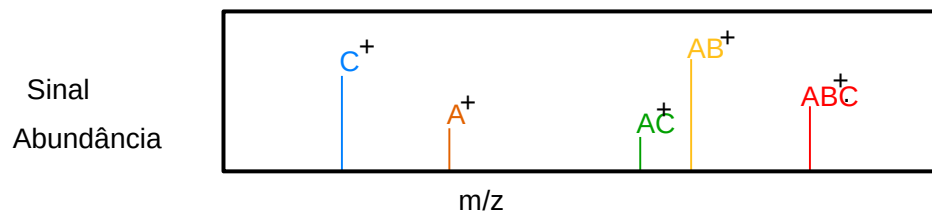


Energia do elétron

Fragmentação:



Resultando espectro massa:



GC/MS Positioning – Which MS?



5977 GC/MSD

The source of new possibilities – gives you the ability to improve your sample throughput, analytical performance, and business outcome



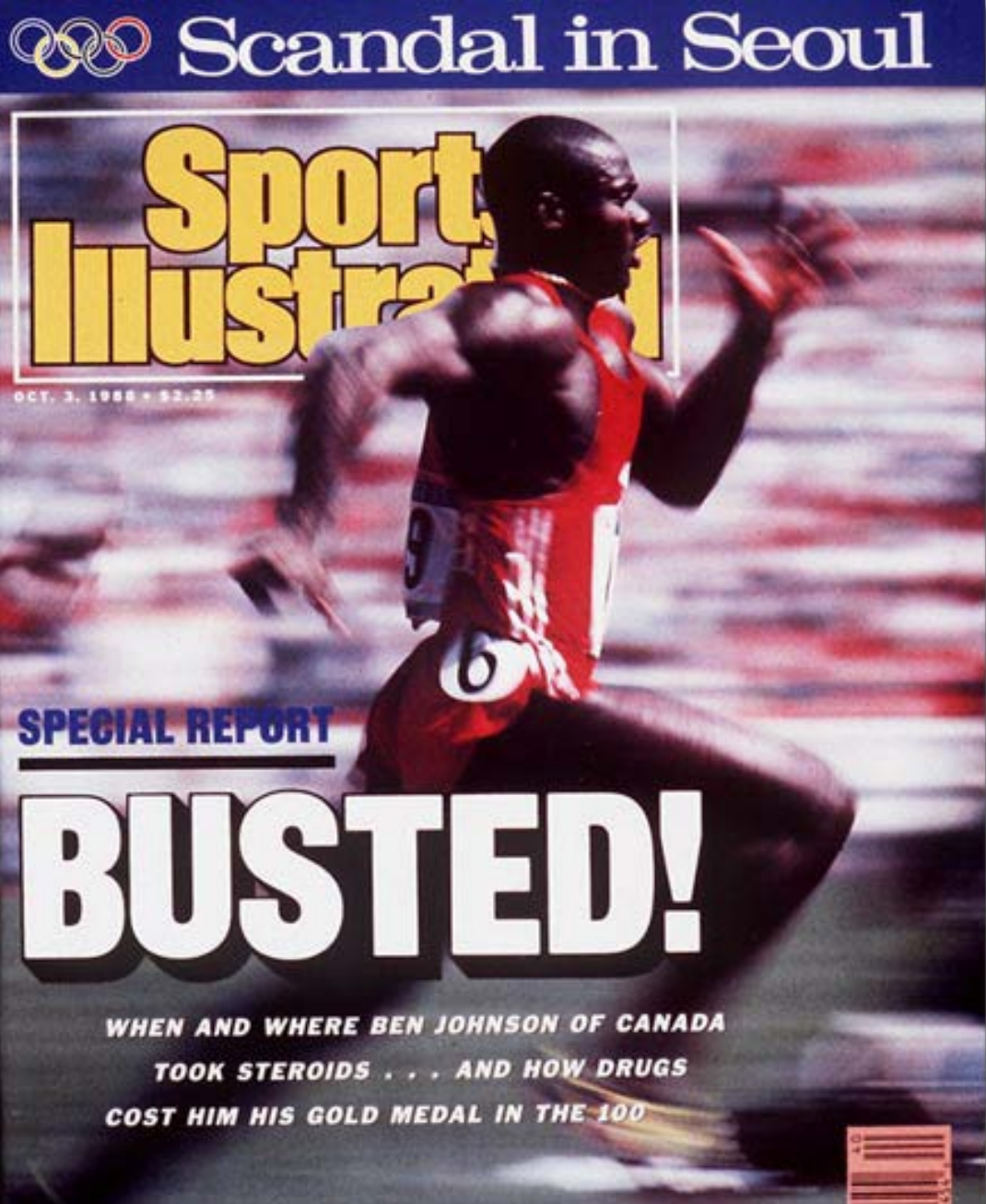
7000 Series GC/TQ

Expand your lab in multiple dimensions – take your lab to higher levels of efficiency and productivity for more confidence in your results at a lower cost of ownership



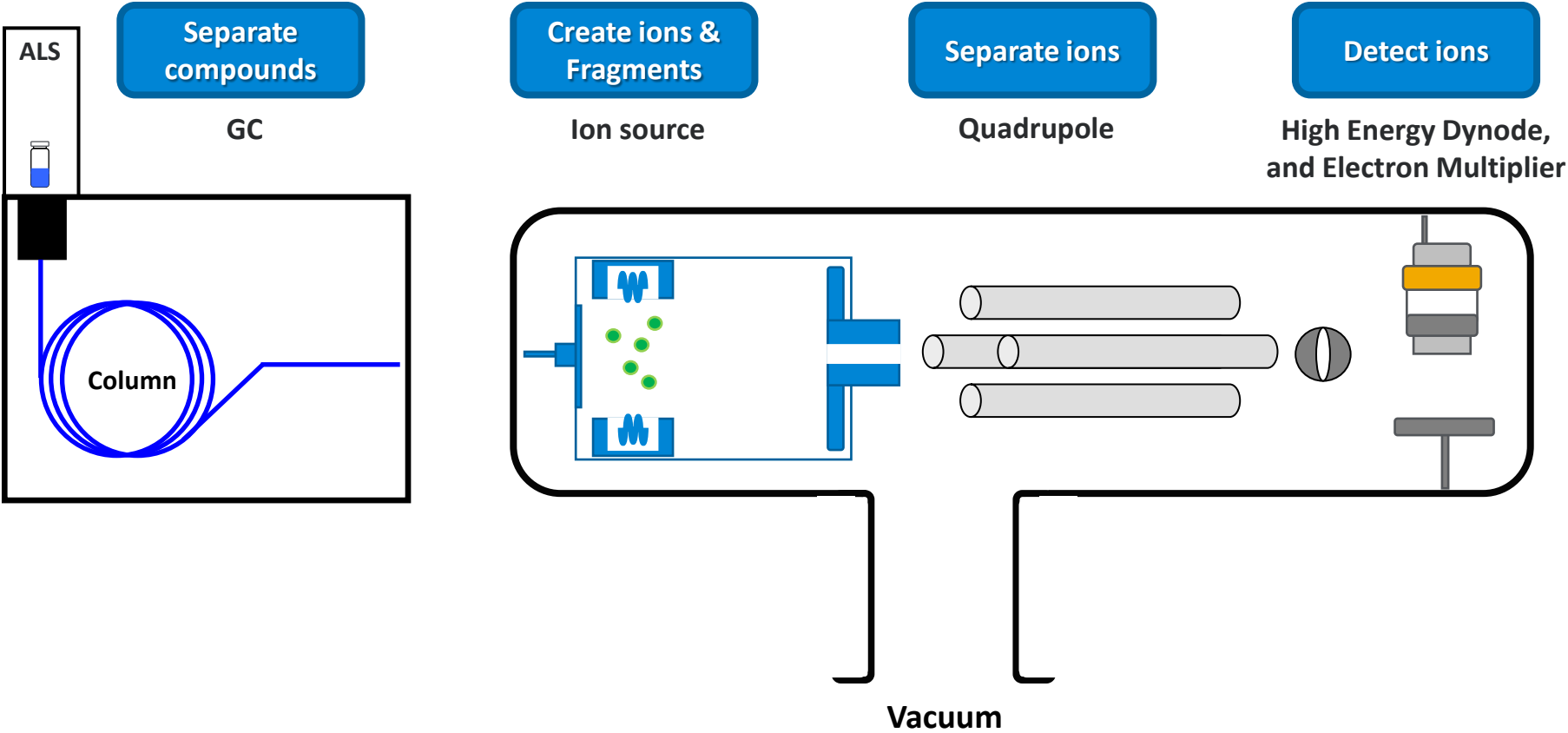
7250 GC/Q-TOF

See the whole picture – the premier instrument for all your GC/MS identification, quantification, and exploration challenges

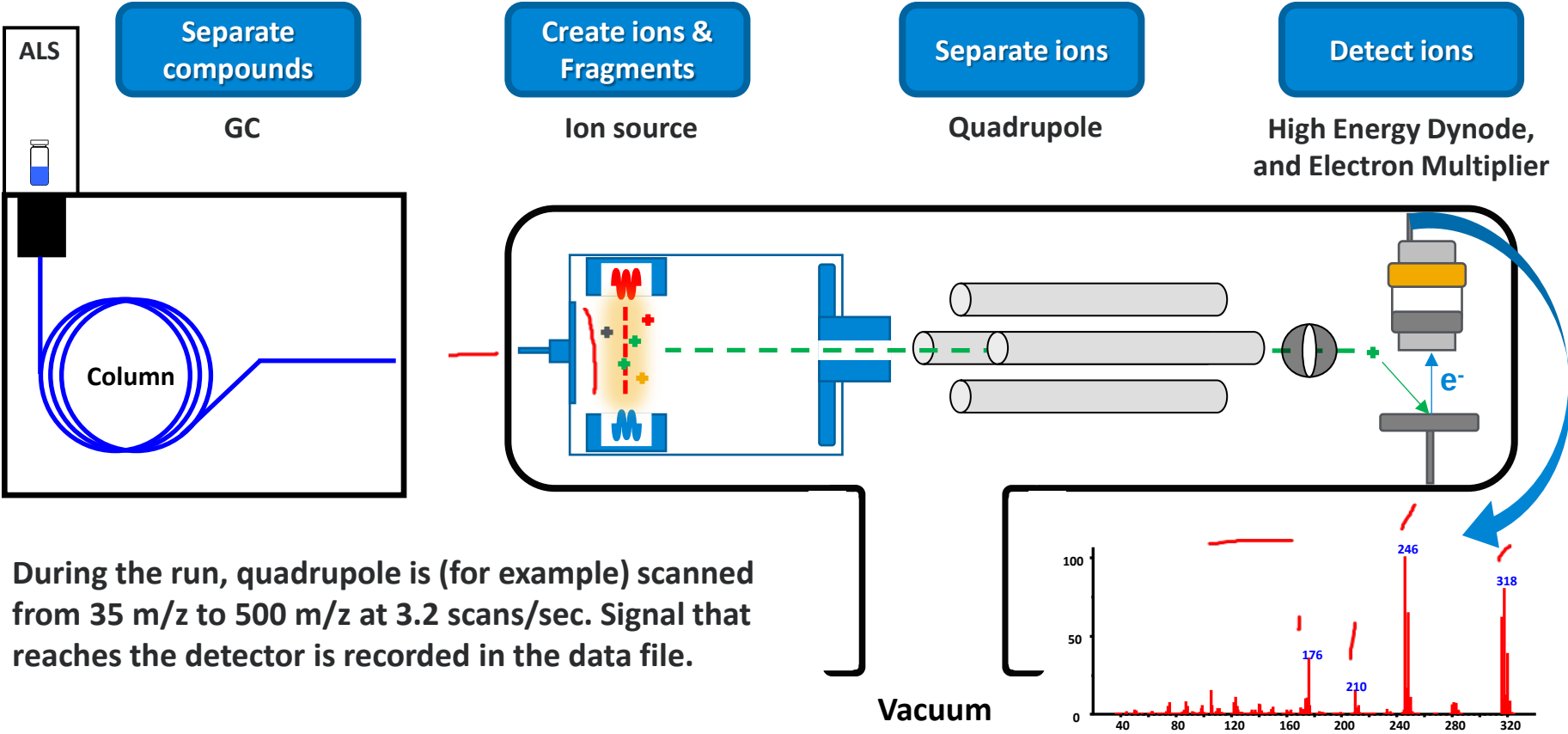


« GCMS da Hewlett-Packard foi o único capaz de parar o Ben Johnson ! »

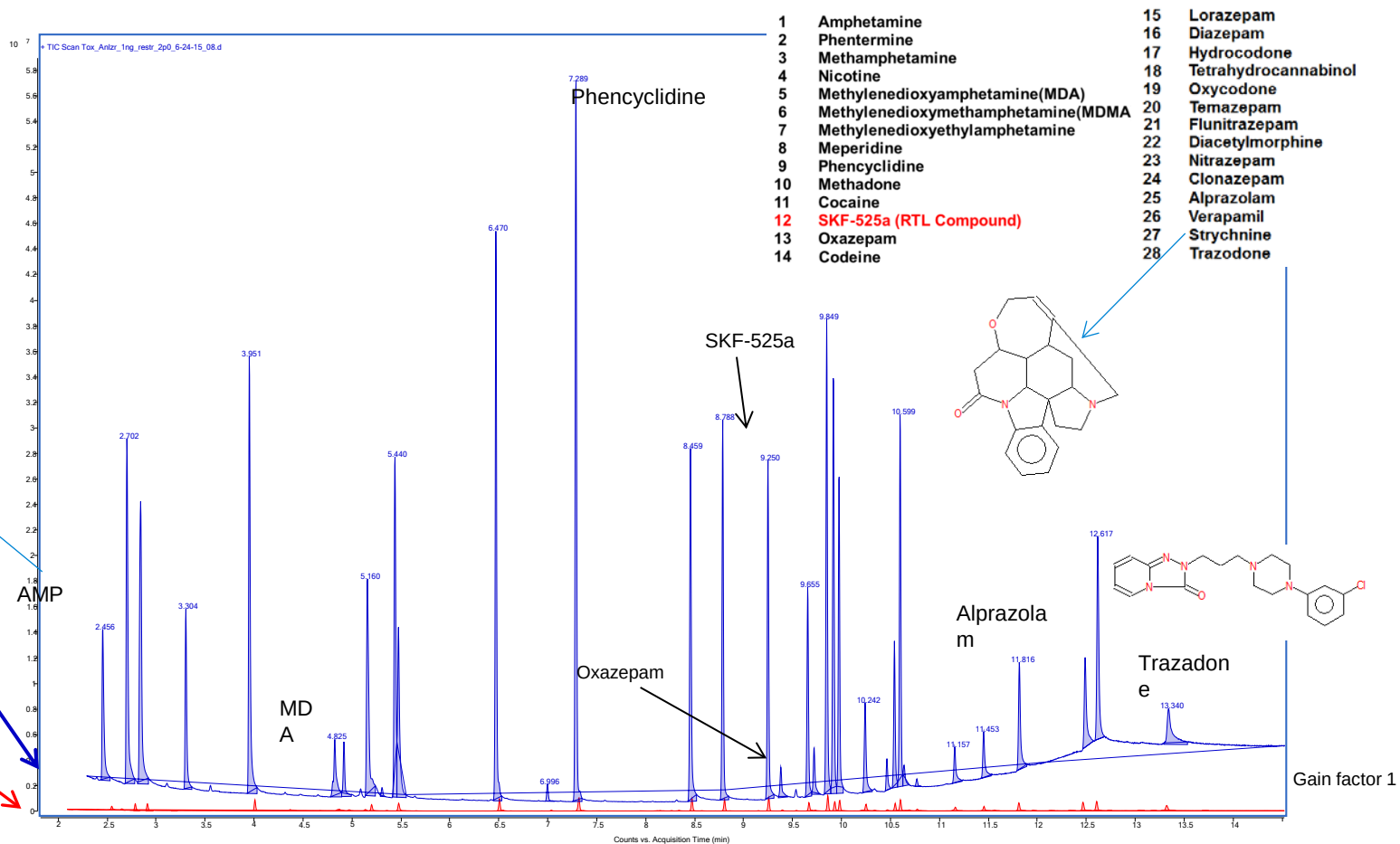
SQ GC/MSD



SQ GC/MSD



Toxicology Checkout Standard Mix – overlay Scan TIC



Detecção de Canabinóides em Fluido Oral usando GC-MS - App note 5989-5860EN

GC/MS Conditions

Instrument:	Agilent 6890 GC 5975 MSD; inert source; 220/240V oven
Detection mode:	Electron impact
Column:	DB-5 MS, 0.25 mm id, 0.25-µm film thickness, 15-m length
Injection temperature:	250 °C
Purge flow:	50 mL/min for 1 min
Carrier gas:	Helium
Injection mode:	Splitless
Injection volume:	2 µL
Mode of operation:	Constant flow at 1.5 mL/min
Transfer line:	280 °C
Quadrupole:	150 °C
Ion source:	230 °C
Dwell time:	50 ms
Oven program:	125 °C for 0.5 min; ramp at 40 °C/min to 250 °C; hold 1.3 min ramp at 70 °C/min to 300 °C
Retention times:	Deuterated THC: 4.27 min; THC 4.28 min; cannabidiol 3.88 min; cannabinol 4.61 min; 2-c-THC 5.66 min

Ions Monitored

Drug	Ions monitored
THC	Deuterated (d3) 374.3 , 389.3; Unlabeled THC 371.2 , 386.2, 303.1
CBN	367.3 , 382.2, 310.1
CBD	390.1 ; 301.2
2-carboxy-THC	487.3 , 488.2, 489.2

Quantitative ions in bold type

Resultados

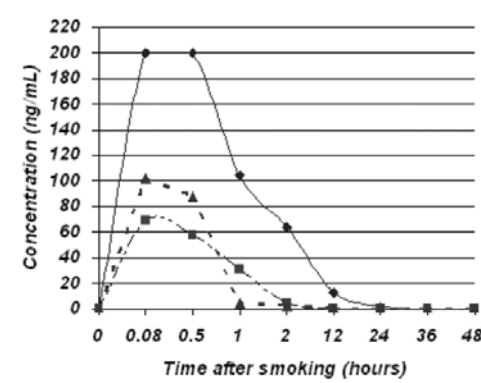


Figure 1. Cannabinoids in oral fluid following marijuana smoking.

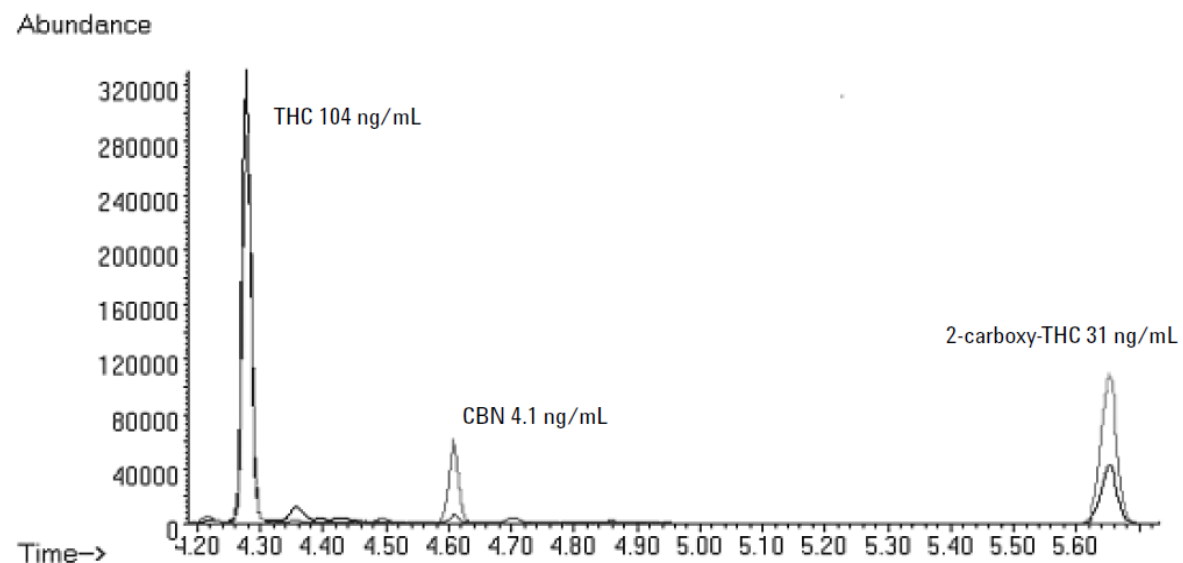


Figure 2. Oral fluid specimen collected 1 hour after marijuana smoking.

Analyte	LOQ (ng/mL)	Linear equation	Correlation r ²	Ion ratio range (%)
THC	0.5	y = 0.0266x + 0.00273	0.998	386/371:69.7–104.5 303/371:44.0–66.0
CBN	0.5	y = 0.138x + 0.0022	0.999	382/367:7.4–11.2 310/367:5.7–8.5
CBD	1	y = 0.0271x + 0.00178	0.998	301/390:17.1–25.7
2-carboxy-THC	1	y = 0.0571x + 0.0195	0.998	488/487:31.7–47.5 489/487:11.0–16.6

PROCESSO – SOFTWARE

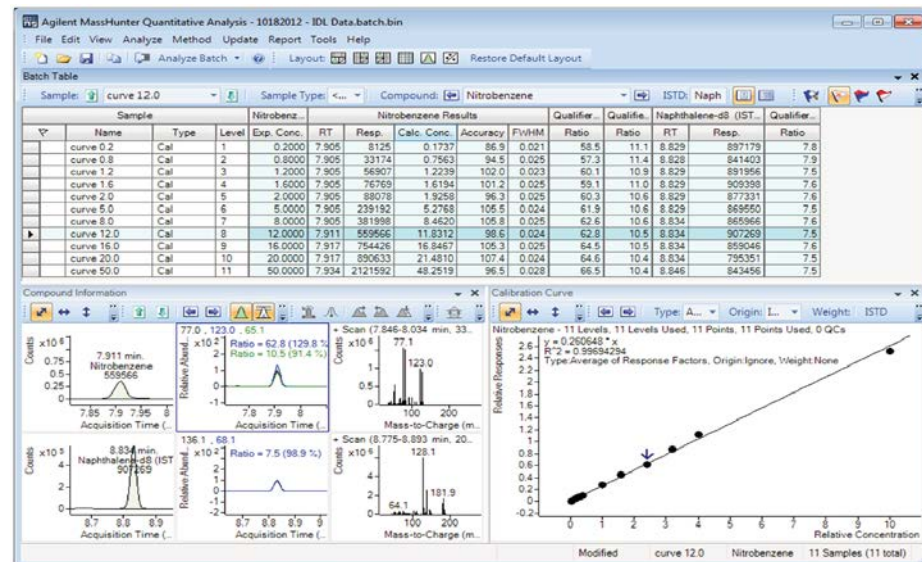
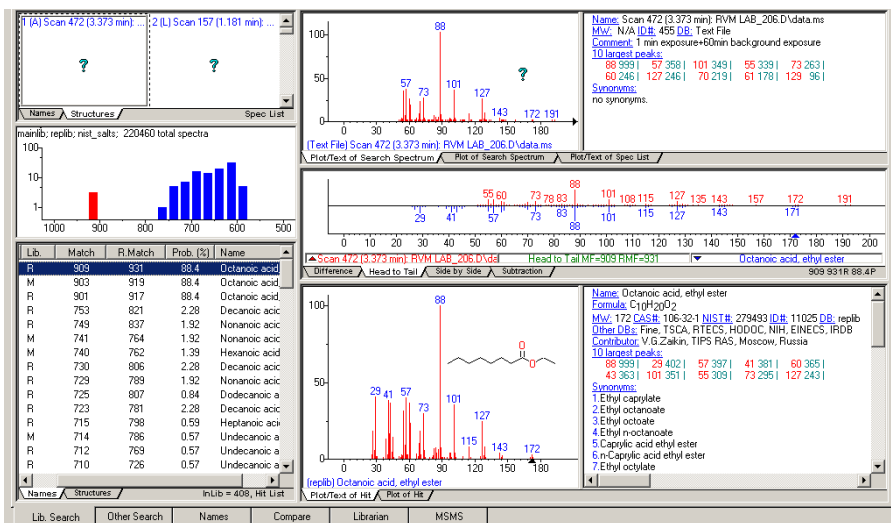
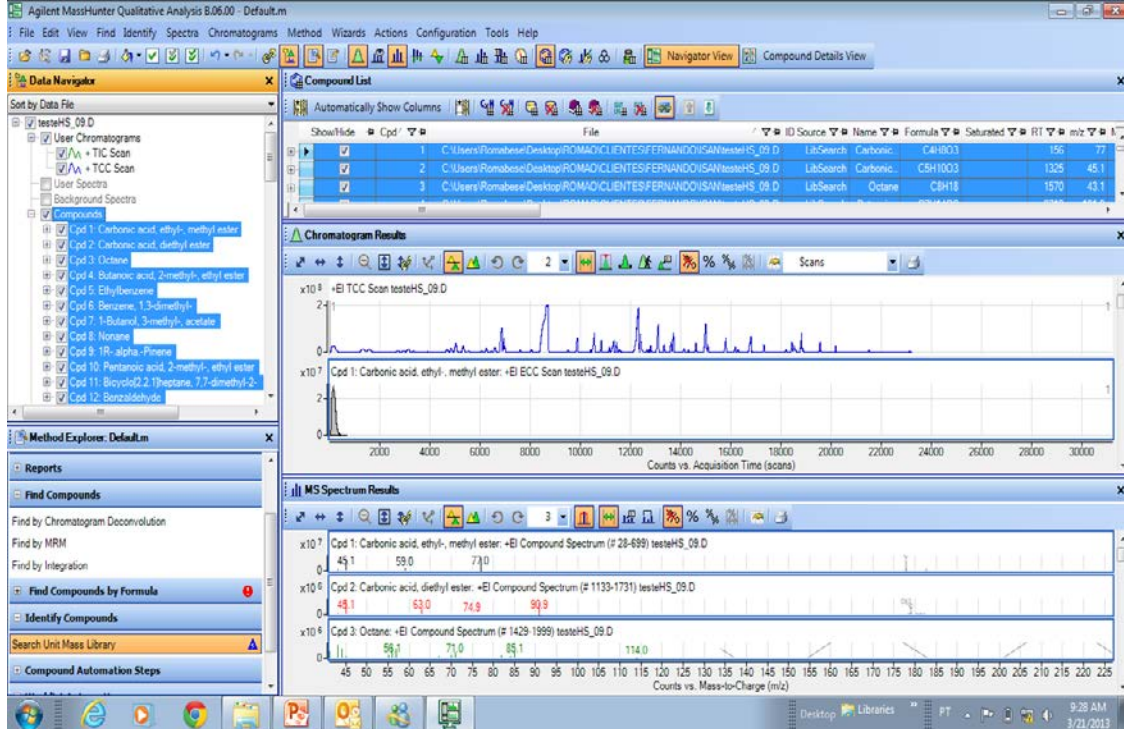
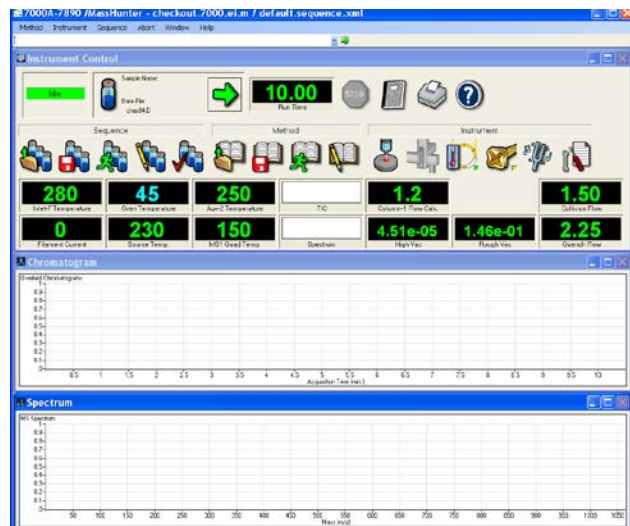


Figure 3. Evaluation of calibration using Agilent MassHunter Quantitative Analysis, in this case using the nitrobenzene component of the 8270 test mix.

- SIM/Scan simultâneos

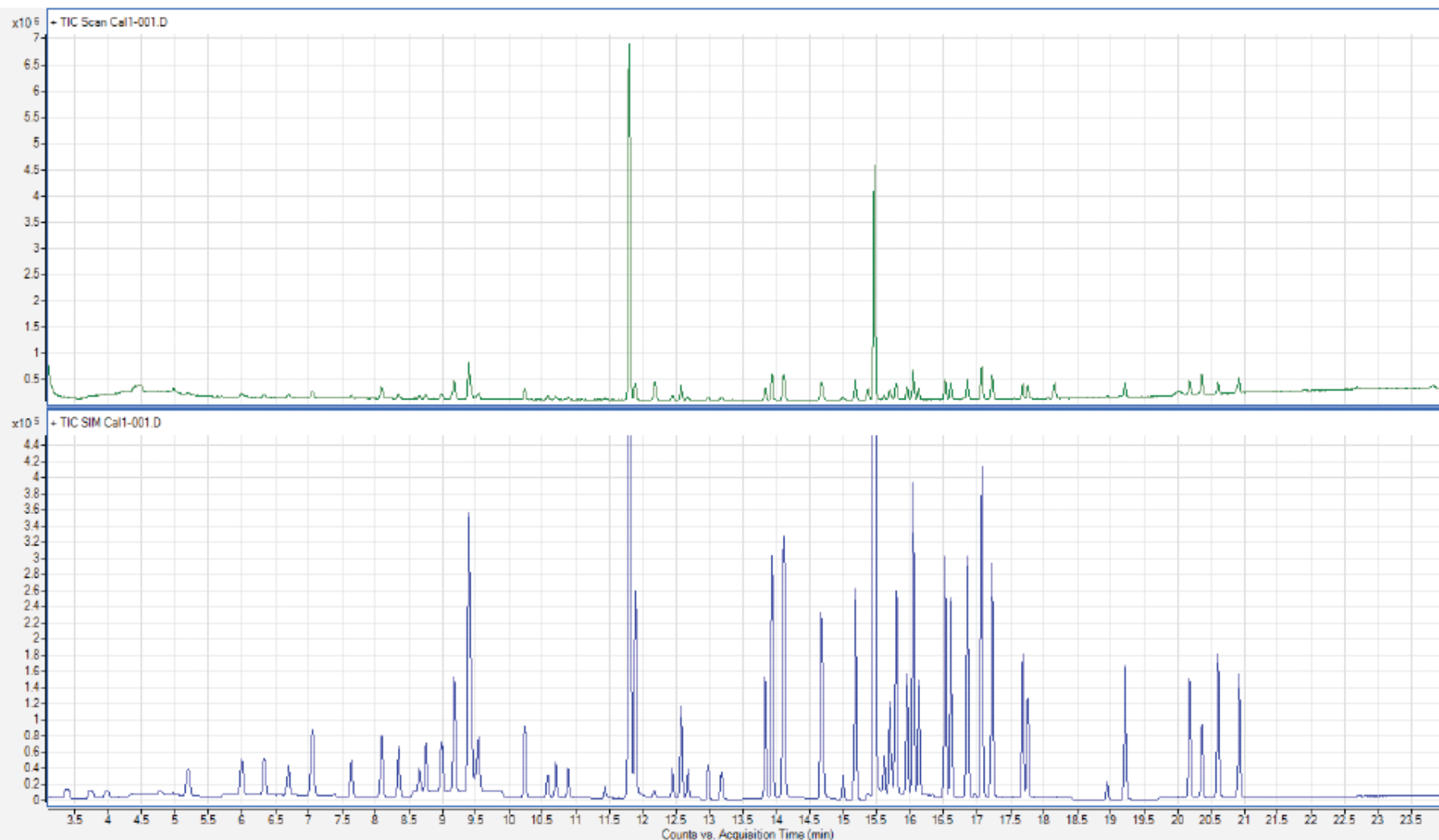
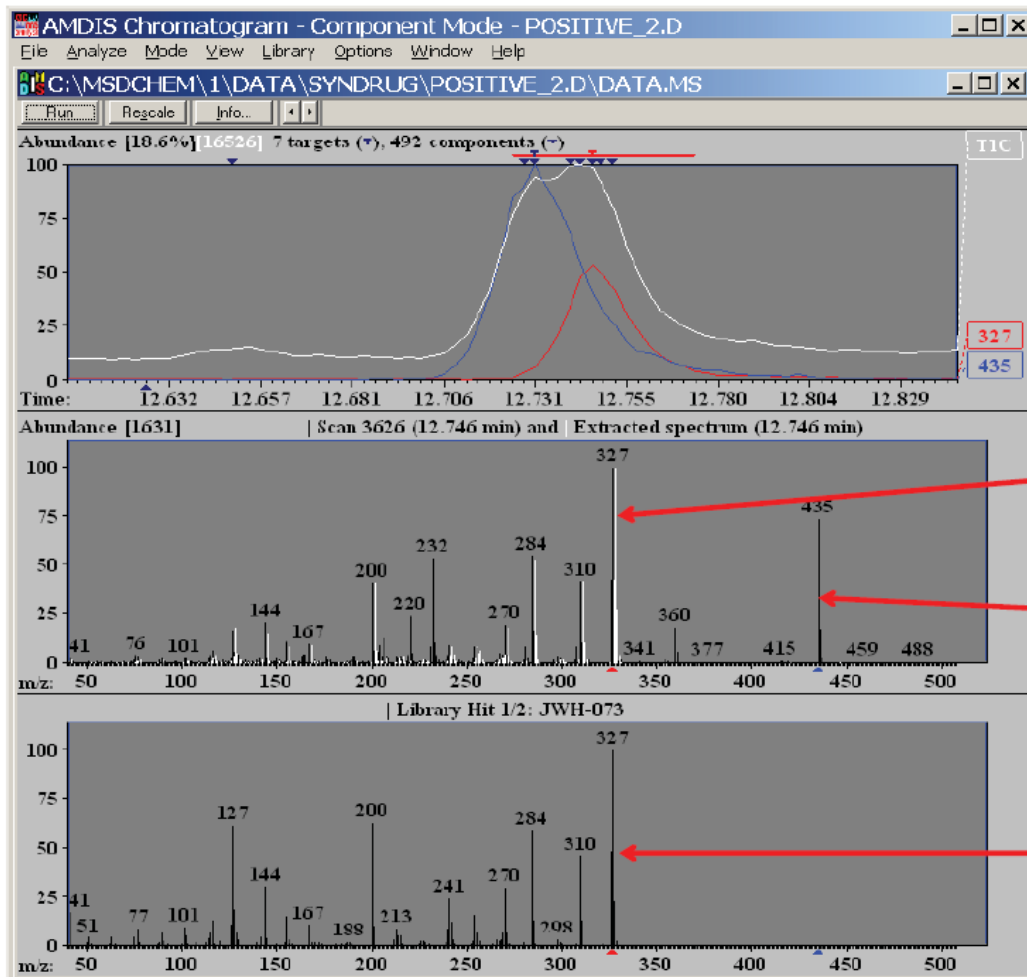


Figure 2: 1 µg/L VOC Standard Scan and SIM Traces.

Deconvolução



AMDIS sorts out confusion:

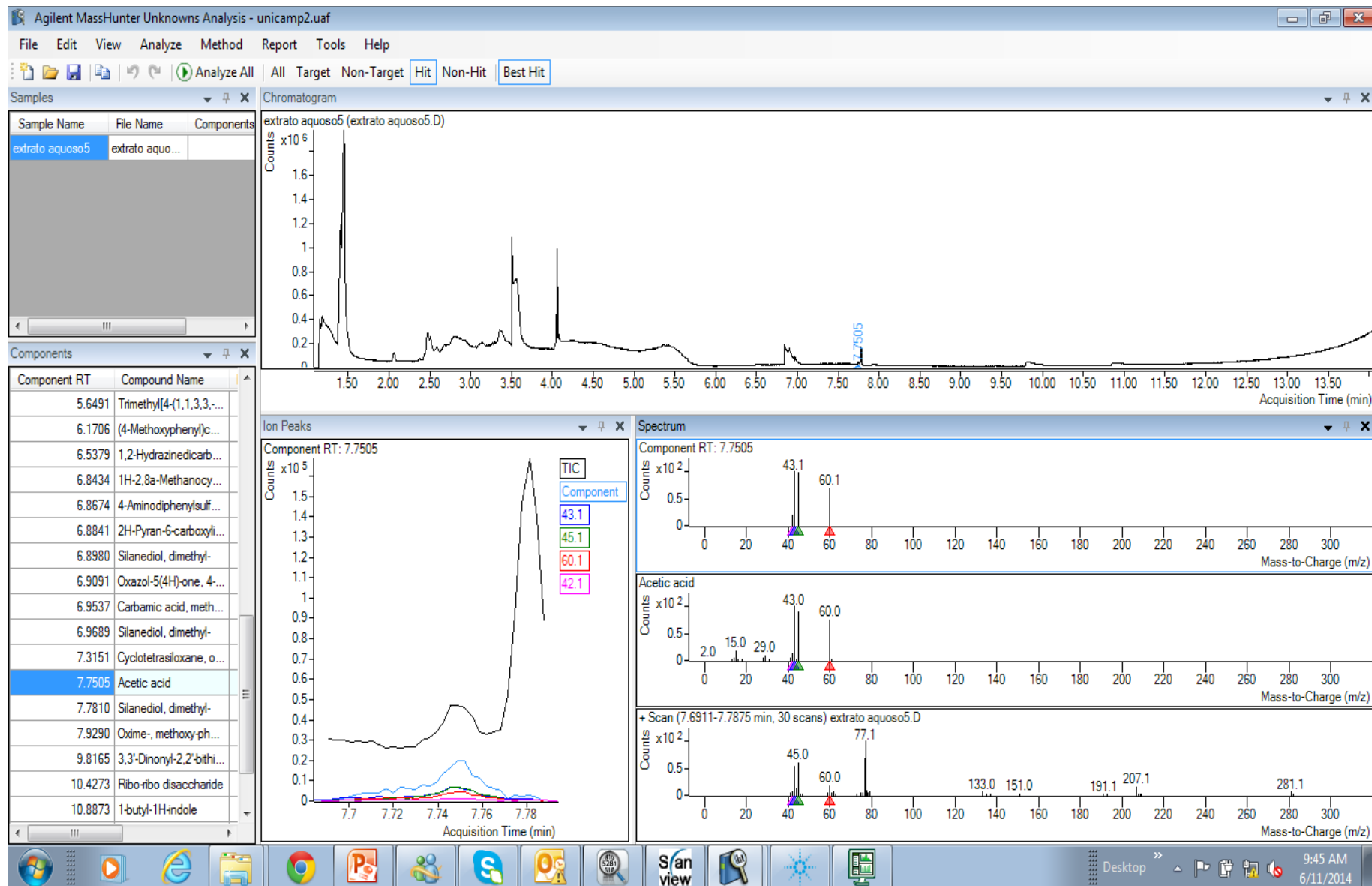
AM-694 (blue 435 trace) is overlapped with JWH-073 (red trace).

White is deconvoluted

Black is raw spectrum

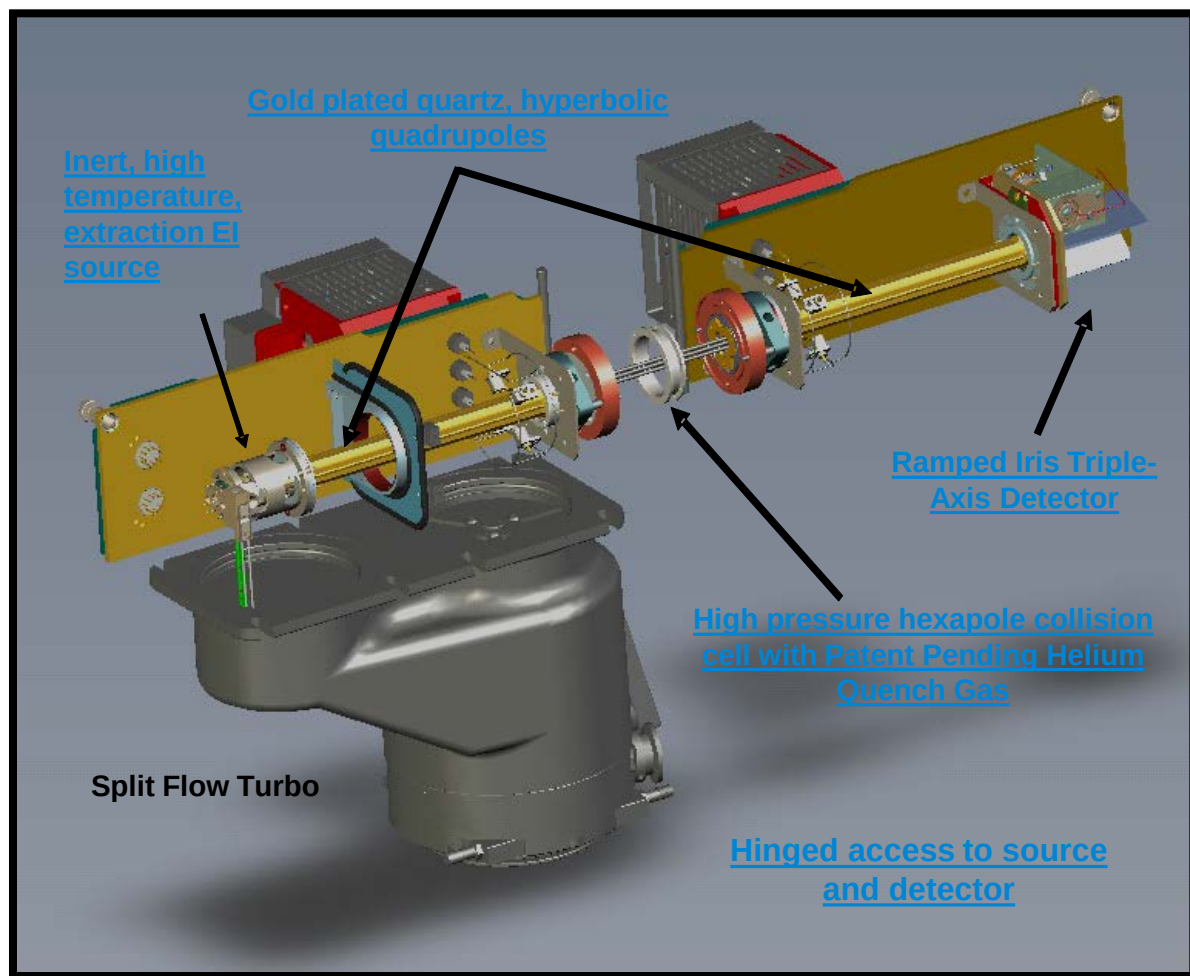
JWH-073 Library spectrum

Unknown Analysis



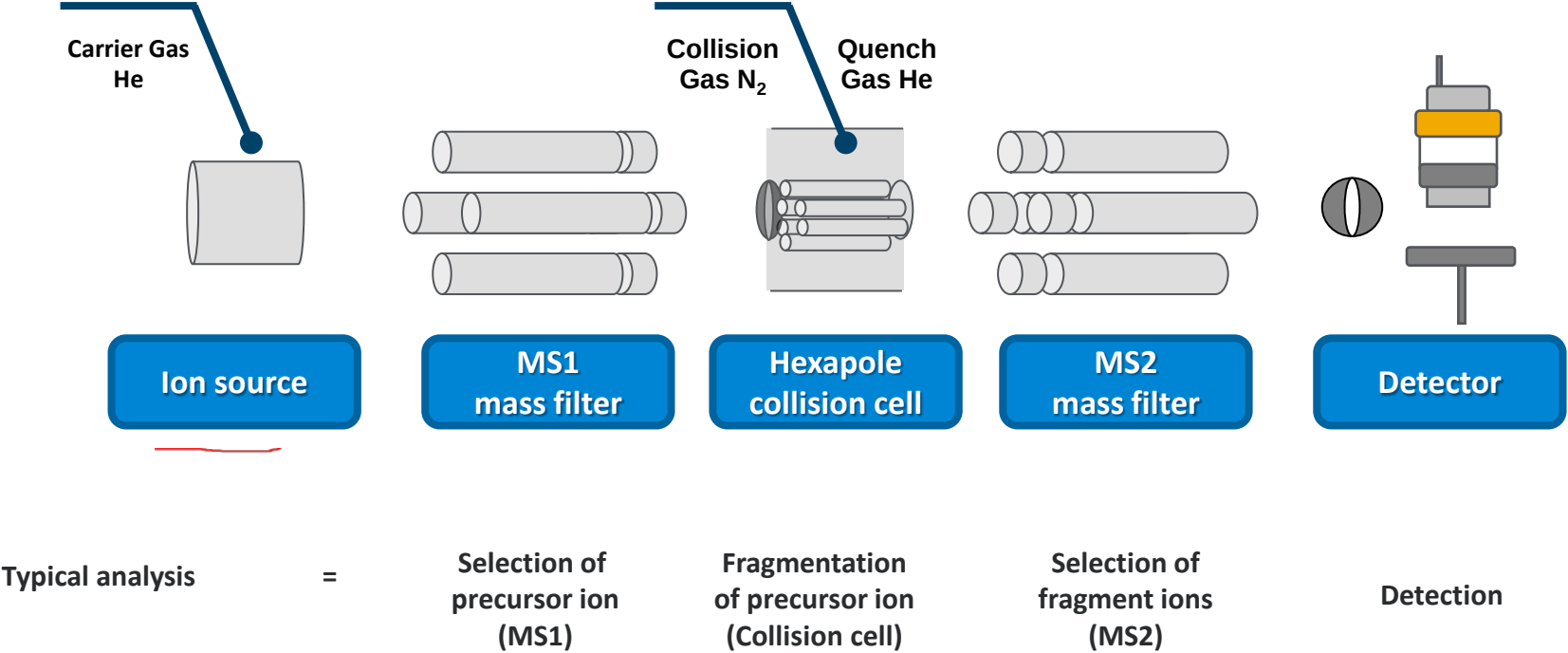
GC-TQ 7000D/7010B : Built on Proven Technologies

- Reliable, inert, high performance ion source
- Heated monolithic gold plated quartz quadrupole
- High Signal/Low Noise Triple-Axis Detector
- PCI/NCI ion source
- Linear acceleration enhanced hexapole collision cell
- MassHunter software

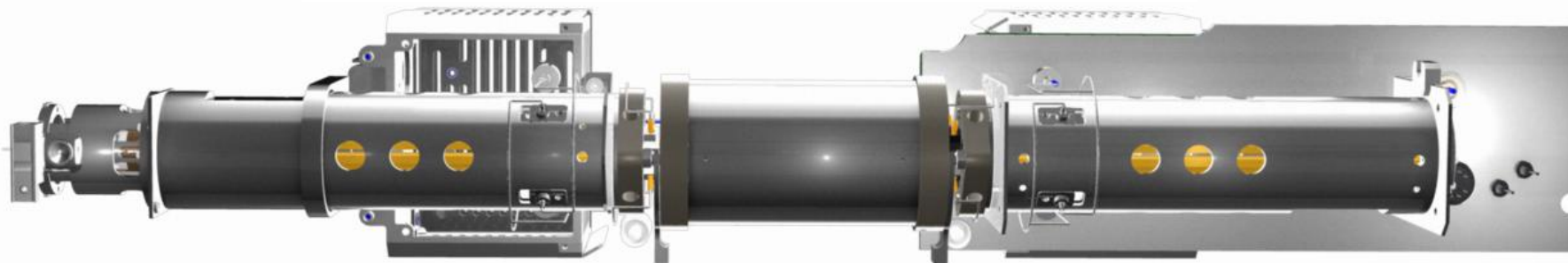


TQ GC/MS: What is MS/MS analysis?

MS/MS requires multiple mass filters in series.



7000 Series Quadrupole MS/MS *Optimized for Gas Chromatography*

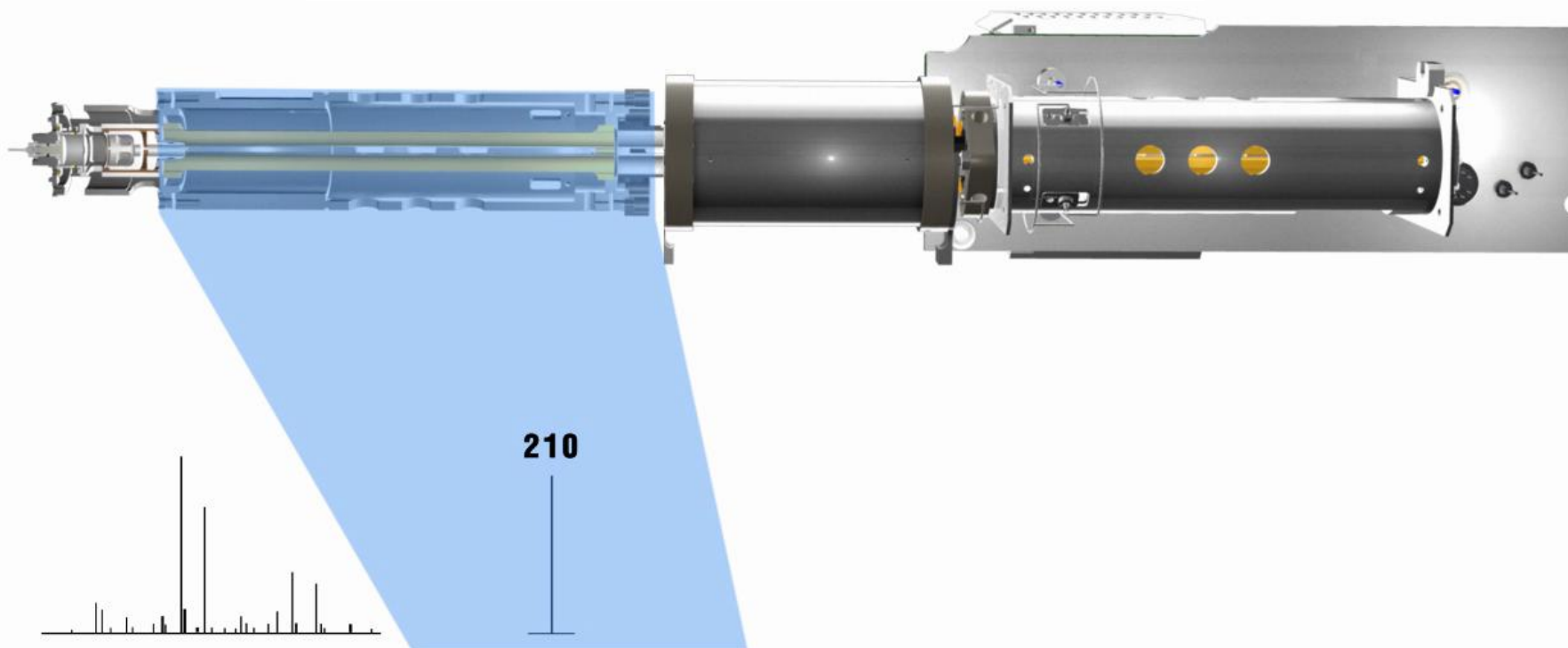


7000 Series Quadrupole MS/MS *Optimized for Gas Chromatography*



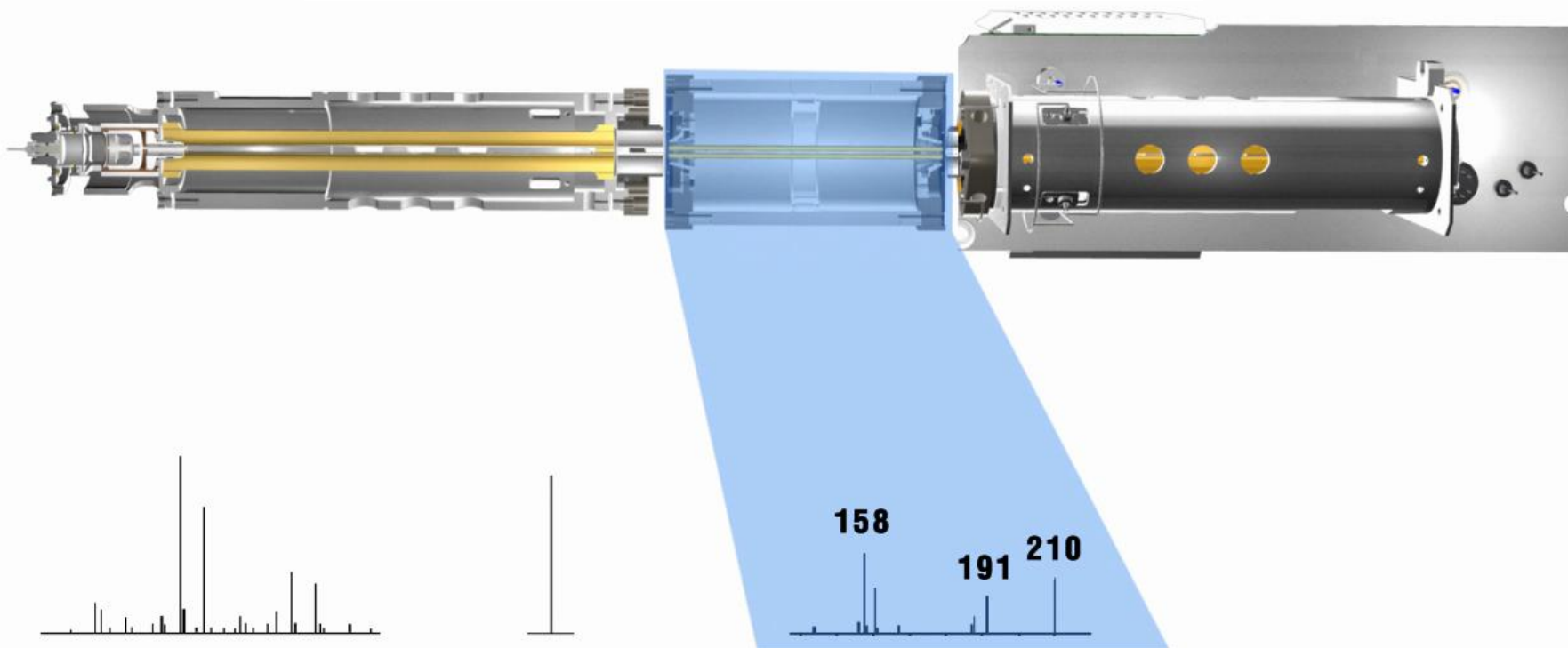
EI Spectrum with
all analyte and
background ions

7000 Series Quadrupole MS/MS *Optimized for Gas Chromatography*



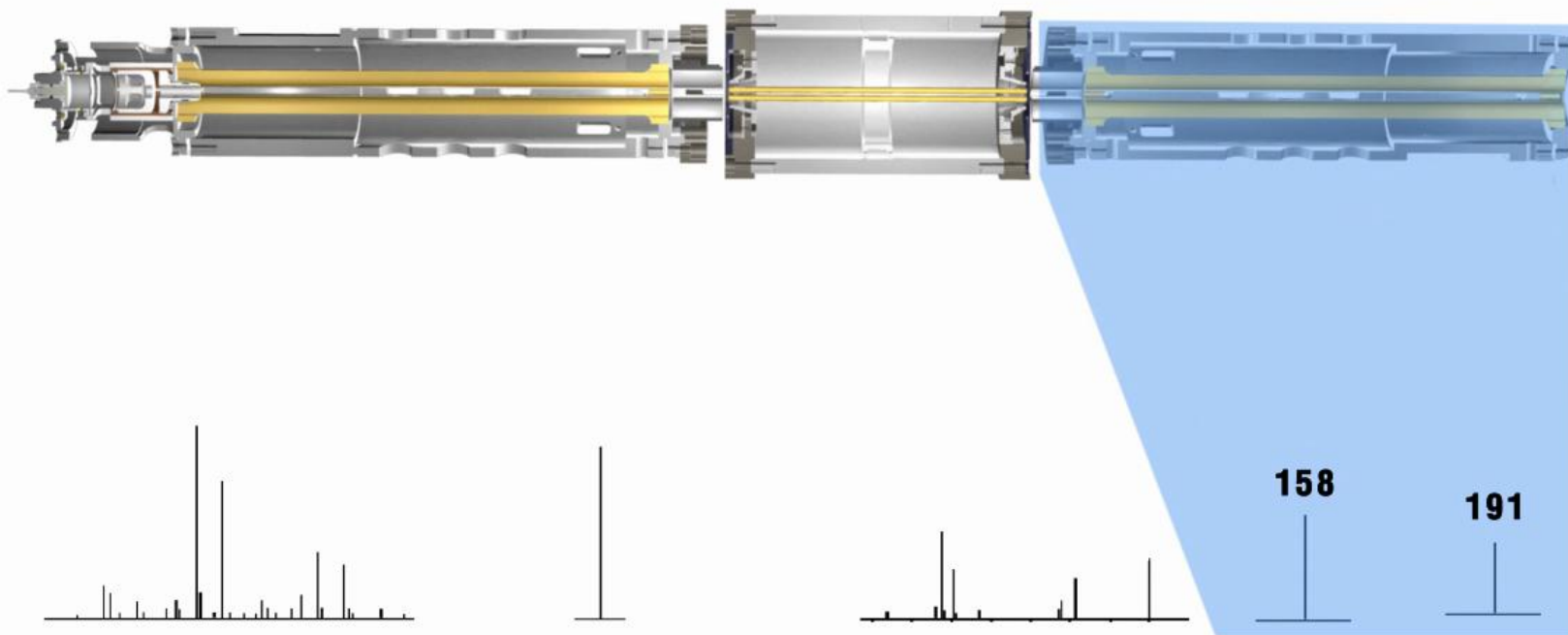
Q1 mass filters all ions other than the precursor; **only** target ion 210 pass through

7000 Series Quadrupole MS/MS *Optimized for Gas Chromatography*



Collision cell
dissociates m/z 210
into product ions

7000 Series Quadrupole MS/MS *Optimized for Gas Chromatography*

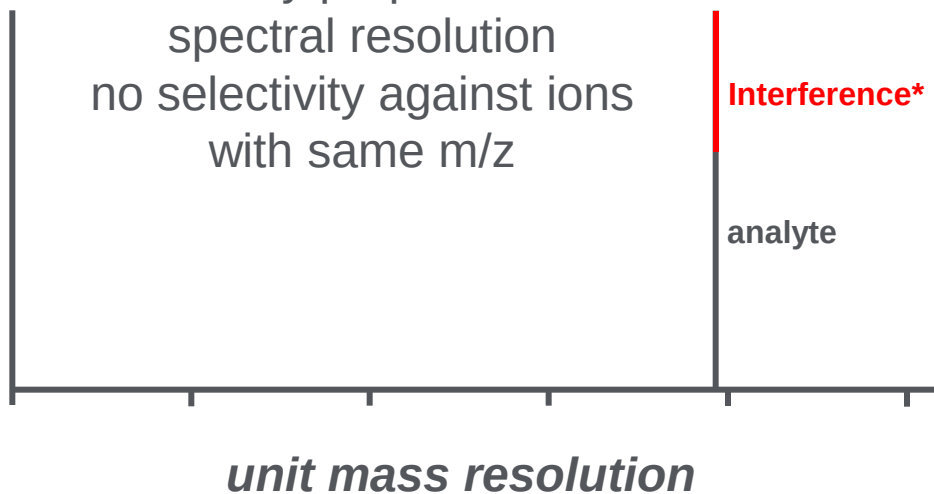


Q2 monitors **only**
characteristic fragments
158 and 191 from m/z
210 for quant and qual

MS/MS Eliminates Scan and SIM Interferences

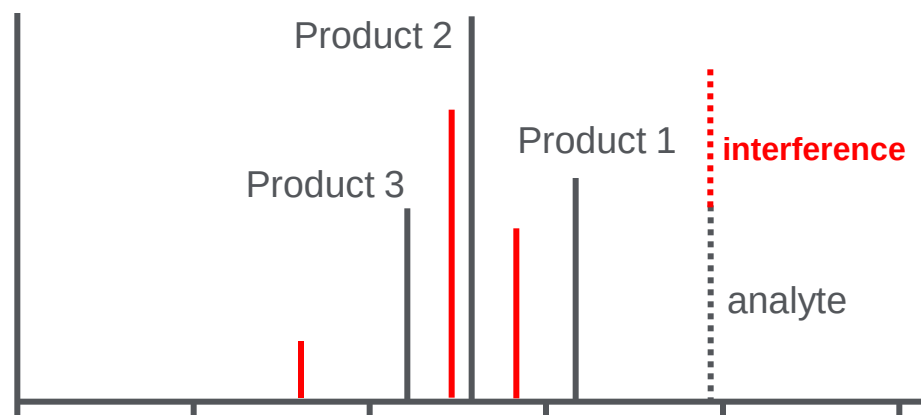
Single Quad MS

selectivity proportional to
spectral resolution
no selectivity against ions
with same m/z



Triple Quad MS

Precursor selectivity same as MS but
high probability that one or more of the
product ions will be a unique dissociation
product of the precursor only
AND NOT the interference



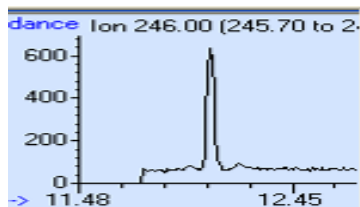
***Because the concentrate of the matrix may be much greater than the analyte, even matrix isotope ions (A+1, A+2, etc) may be a significant interference**

Consistent, High Quality Results, p,p'DDE 10 ppb

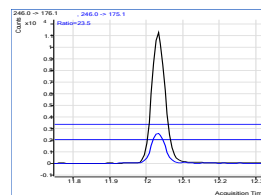
SIM versus MS/MS Comparison: Food Safety

SIM - EIC (246)

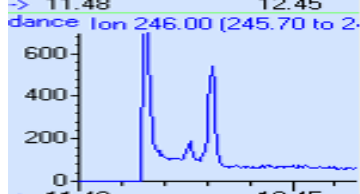
MRM (2 transitions)



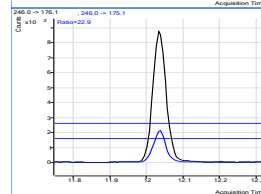
Apple



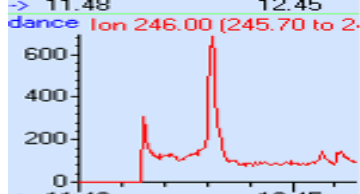
S/N = 448



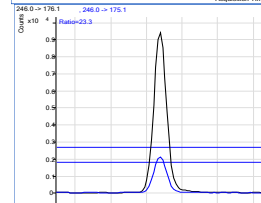
Cabbage



S/N = 241



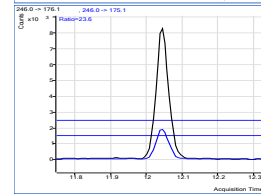
Ginseng



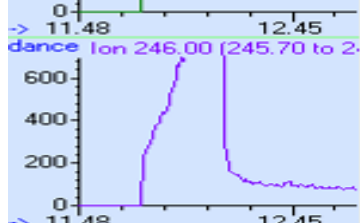
S/N = 446



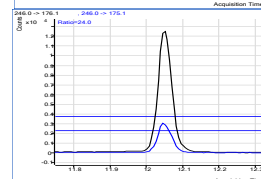
Orange



S/N = 456



Spinach



S/N = 260

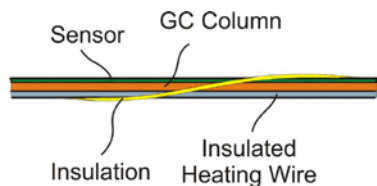
MRM (246→175)

THC em cabelo 5990-7535EN

Unique LTM Column Modules enable rapid temperature ramping and cooling



Figure 2. Low thermal mass (LTM) column modules interfaced with the Agilent 7890A GC.



A unique system for rapid and robust detection of THCA in hair

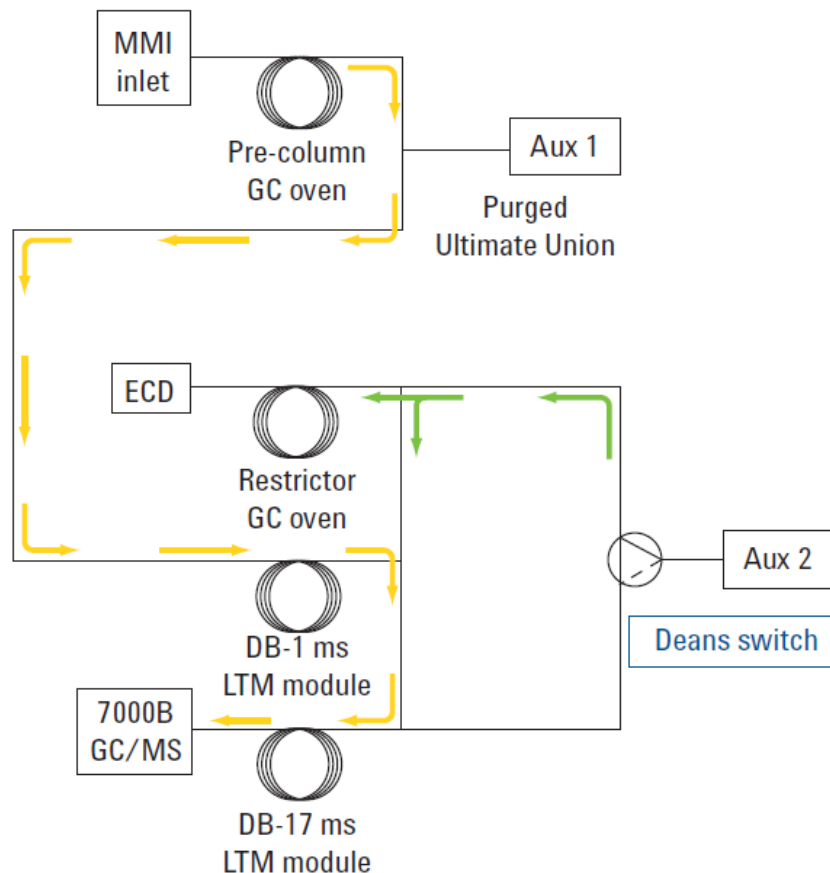


Figure 1. Schematic representation of the system used to develop the THCA method.

THC em cabelo 5990-7535EN



A unique system for rapid and robust detection of THCA in hair

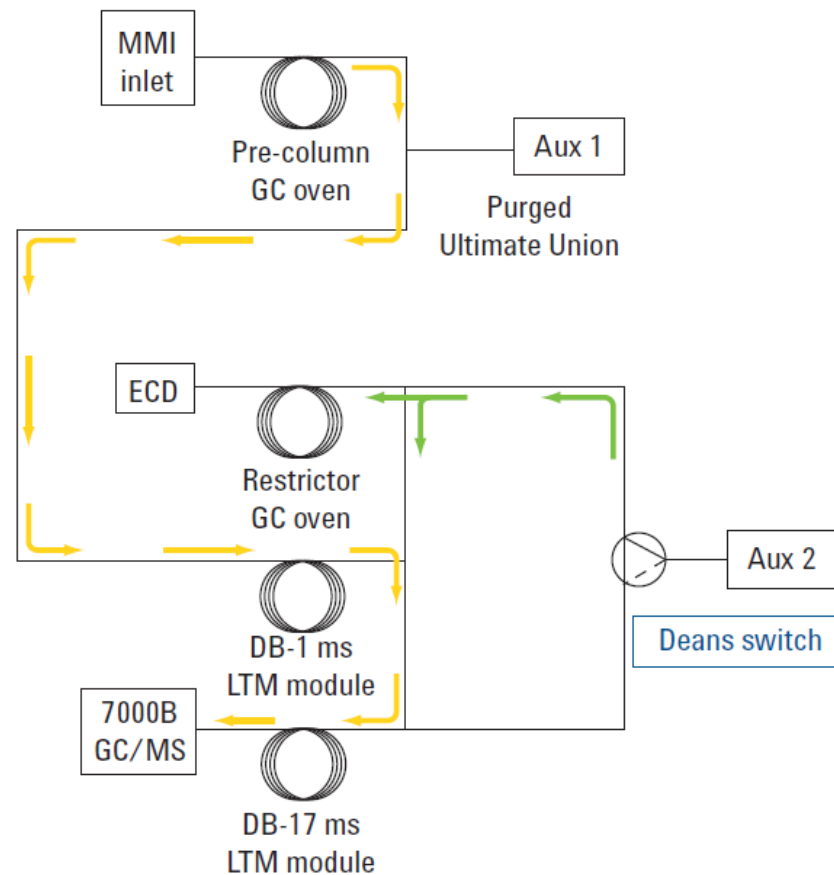


Figure 1. Schematic representation of the system used to develop the THCA method.

THC em cabelo 5990-7535EN

Table 1. Agilent 7890N/7000B Gas Chromatograph and Triple Quadrupole Mass Spectrometer Conditions

GC Run Conditions

Pre-column	1 m × 0.15 mm × 1.2 µm DB-1 (p/n 12A-1015)
Analytical columns	
Column 1	15 m × 0.25 mm × 0.25 µm DB-1ms LTM Column Module (p/n 122-0112LTM)
Column 2	15 m × 0.25 mm × 0.25 µm DB-17ms LTM Column Module (p/n 122-4712LTM)
Injection volume	2 µL
Inlet temperature	Isothermal at 250 °C
Injection mode	0.75 minute pulsed splitless at 35 psi

Oven temperatures

GC oven	7 minute hold at 250 °C (isothermal)
1st LTM module	50 sec hold at 100 °C 100 °C to 210 °C at 200 °C/min 210 °C to 267 °C at 10 °C/min Hold at 267 °C for 2 min
2nd LTM module	324 sec hold at 100 °C 100 °C to 230 °C at 200 °C/min 230 °C to 240 °C at 10 °C/min Hold at 240 °C for 2 min
Carrier gas	Helium in constant pressure mode. Pre-column: 1 psi; Column 1: 26.6 psi; Column 2: 19.6 psi
Transfer line temp	300 °C

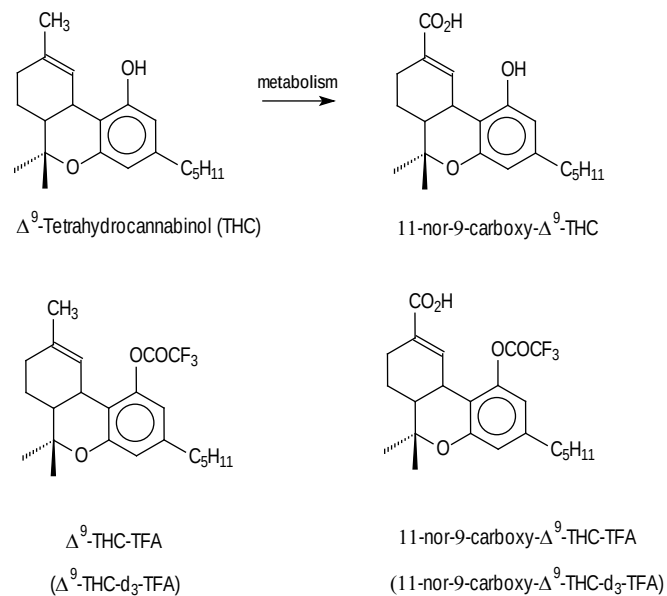
MS conditions

Tune	Autotune
EMV Delta	1200 V
Acquisition parameters	NCI mode; multiple reaction monitoring (MRM)
Reagent gas	Ammonia, 35% flow
Collision gas	Argon, constant flow, 0.9 mL/min
Quench gas	Helium, constant flow, 0.5 mL/min
Solvent delay	6.2 min
MS temperatures	Source 150 °C; Quadrupole 150 °C

Table 2. Agilent 7000B Triple Quadrupole GC/MS System Analysis Parameters

Compound	RT (min)	MRM	Dwell time (ms)	Collision energy (EV)
THCA*	6.714	620→492	50	5
		620→383	50	5
THCA-d3	6.710	623→495	20	5
		623→386	20	5

*11-nor- Δ^9 -Tetrahydrocannabinol-9-Carboxylic Acid



THC em cabelo 5990-7535EN

LOD of 0.002 pg/mg

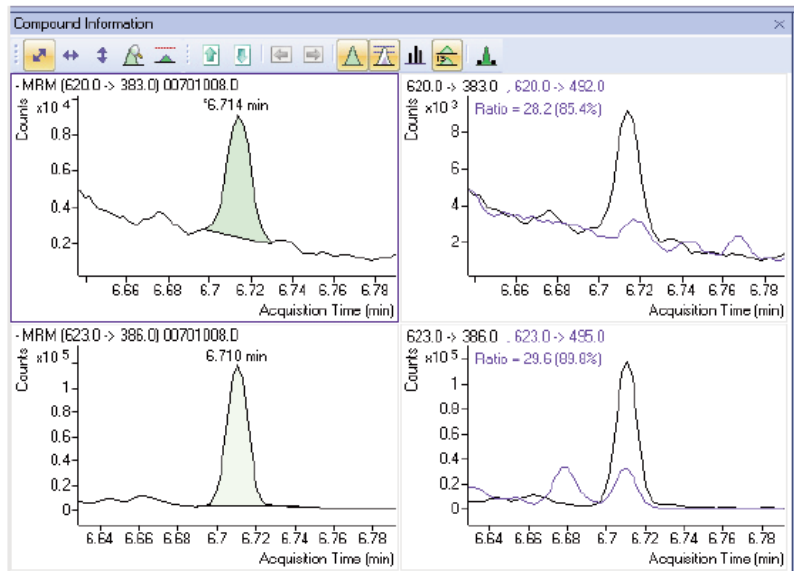


Figure 3. MRM traces for the quantifying transition (left) and both the quantifying and qualifying transitions (right) for the 0.002 pg/mg LOD of THCA (upper panel) and the deuterated standard (lower panel) spiked into a hair sample.

Reliable calibration

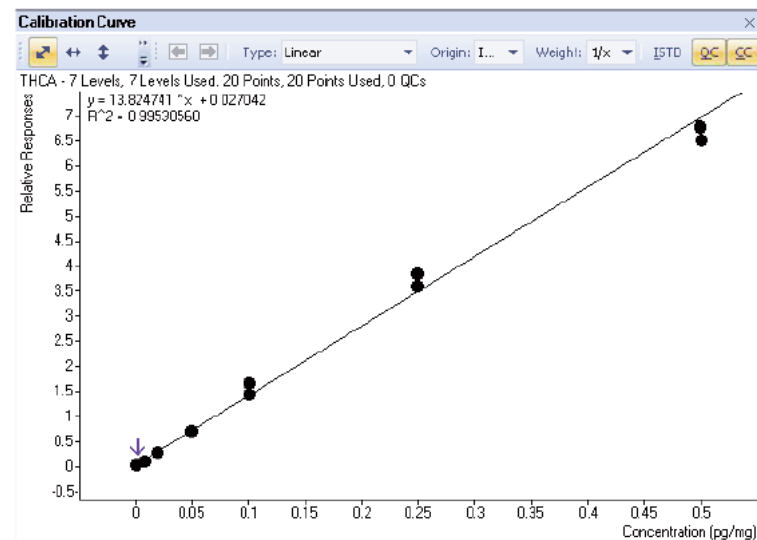
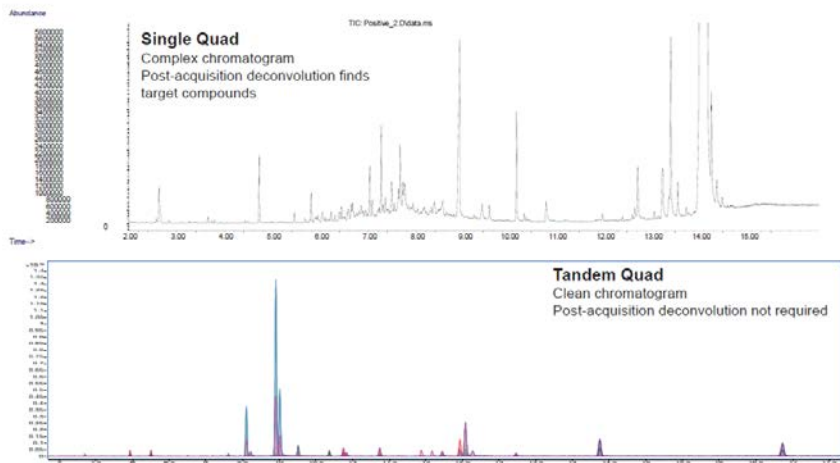


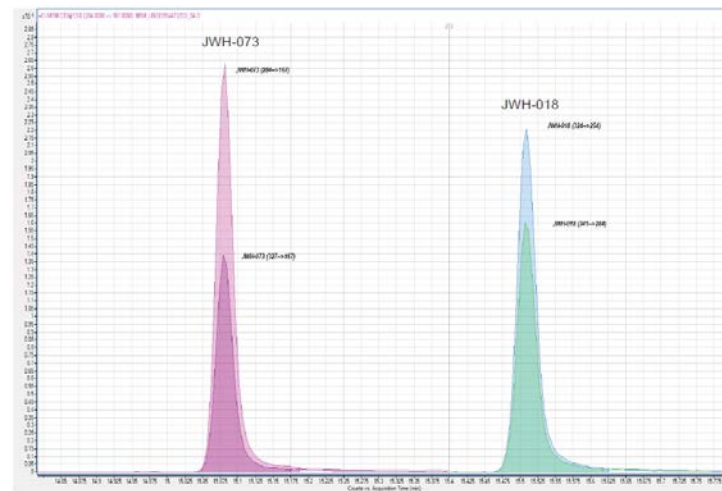
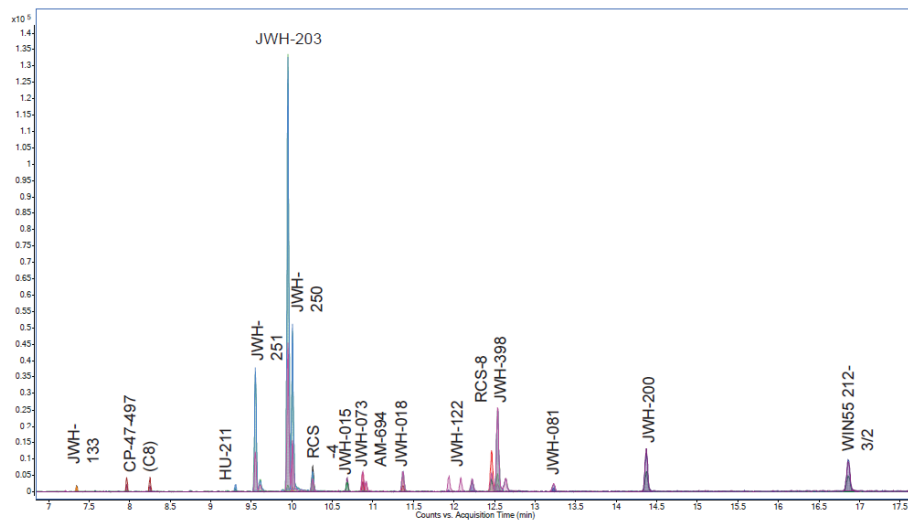
Figure 4. Calibration curve for THCA spiked into hair samples at 0.002, 0.01, 0.02, 0.05, 0.1, and 0.5 pg/mg of hair.

Comparação de Análise de Sintéticos GCQ x GCTQ

SQ vs. TQ "TICS"



Compound Name	Precursor Ion	Product Ion	Collision Energy	Compound Name	Precursor Ion	Product Ion	Collision Energy
AM-694	435	232	27	JWH-200	384	100	23
AM-694	435	220	13	JWH-200	100	56	17
CP-47-497-C8	377	191	29	JWH-203	339	214	3
CP-47-497-C8	377	167	33	JWH-203	214	144	17
HU-211	530	446	13	JWH-250	335	214	3
HU-211	446	299	21	JWH-250	214	144	17
JWH-015	327	310	10	JWH-251	214	144	17
JWH-015	310	268	23	JWH-251	144	116	12
JWH-018	341	167	23	JWH-398	375	201	23
JWH-018	324	254	23	JWH-398	318	189	23
JWH-073	327	167	23	RCS-4	321	264	19
JWH-073	310	254	23	RCS-4	264	135	17
JWH-081	371	197	23	RCS-8	254	158	13
JWH-081	354	269	31	RCS-8	254	144	19
JWH-122	338	268	23	WIN55 212-3/2	100	70	13
JWH-122	298	181	12	WIN55 212-3/2	100	56	15
JWH-133	312	269	12				
JWH-133	269	93	23				

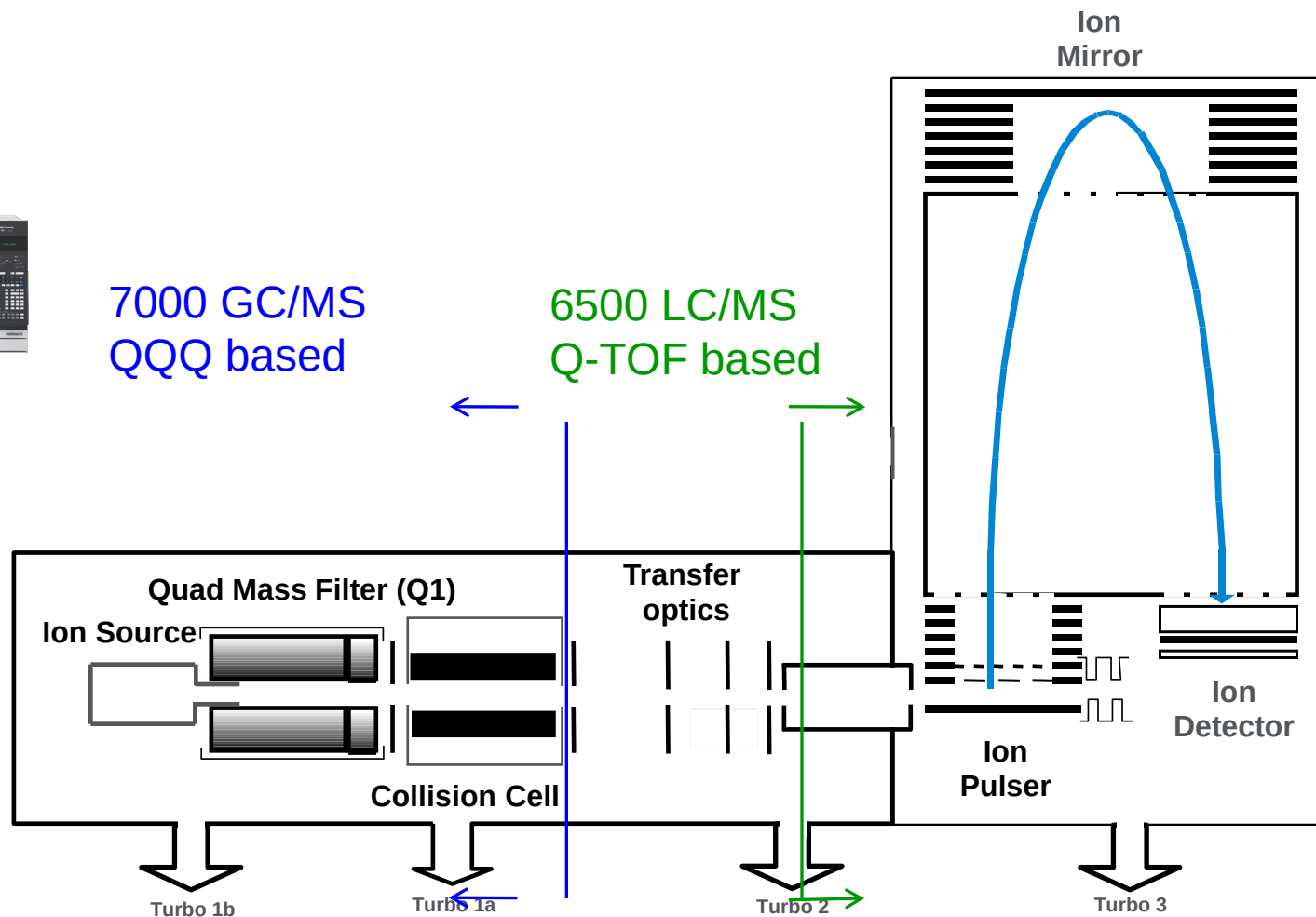


The merging of two platforms



7000 GC/MS
QQQ based

6500 LC/MS
Q-TOF based



TOF MS Theory: Idealized

Mass Analysis for TOF

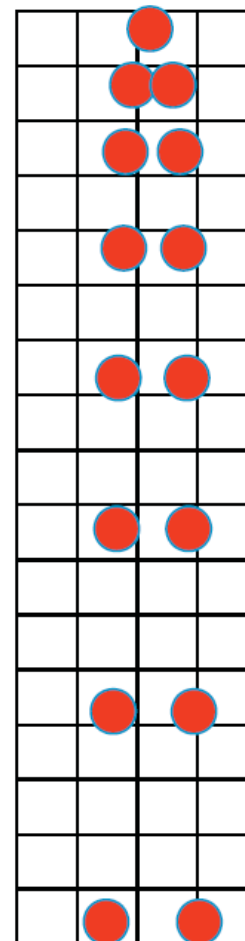
$$v = d/t$$

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

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

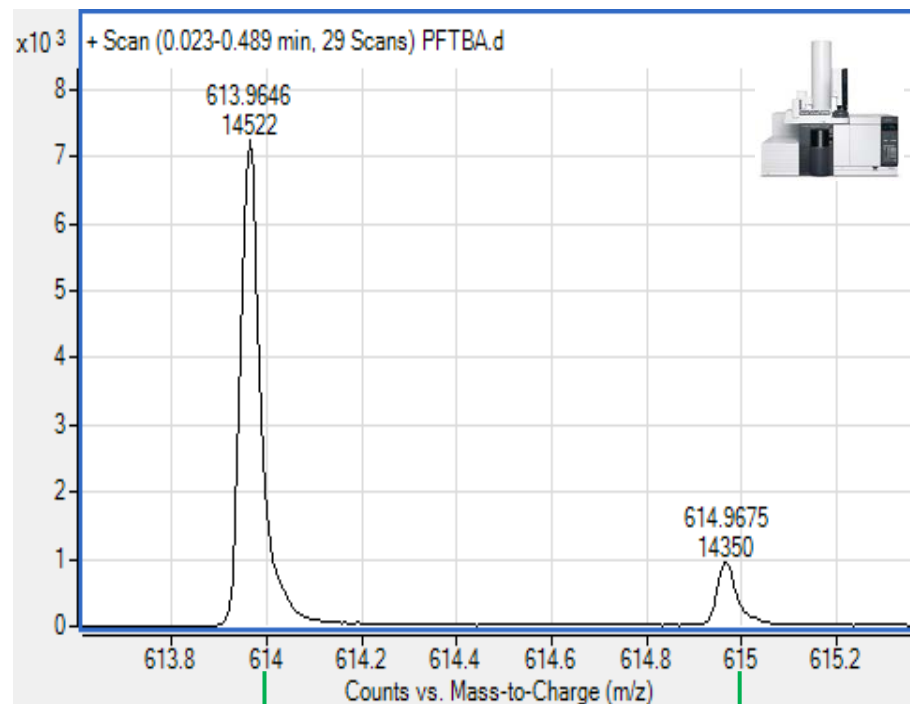
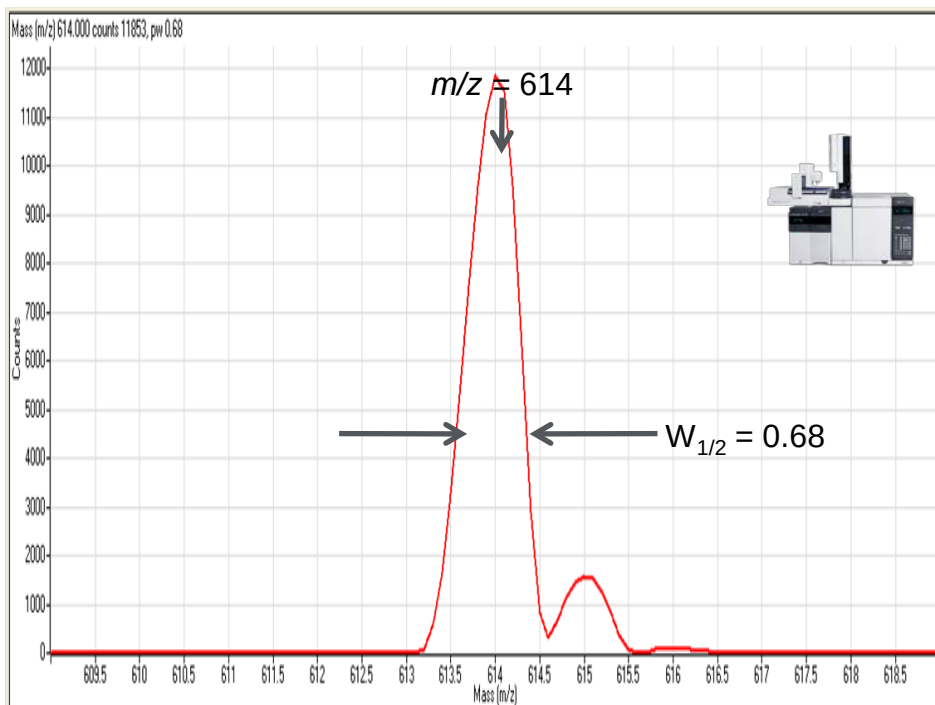
Energy (E) and Distance (d) are fixed

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



Resolution and Accurate Mass

PFTBA ($C_{12}F_{24}N$) = 613.9642



$$\text{Resolution} = (m/z)/w_{1/2}$$

1 Da

1 Da

$$\text{Mass accuracy} = \left(\frac{\text{Theoretical} - \text{Experimental}}{\text{Theoretical}} \right) \times 10^6$$

$$R = 614/0.68 = \mathbf{903}$$

$$= ((613.9642 - 614)/613.9642) \times 10^6$$

$$= \mathbf{-58.3 \text{ ppm}}$$

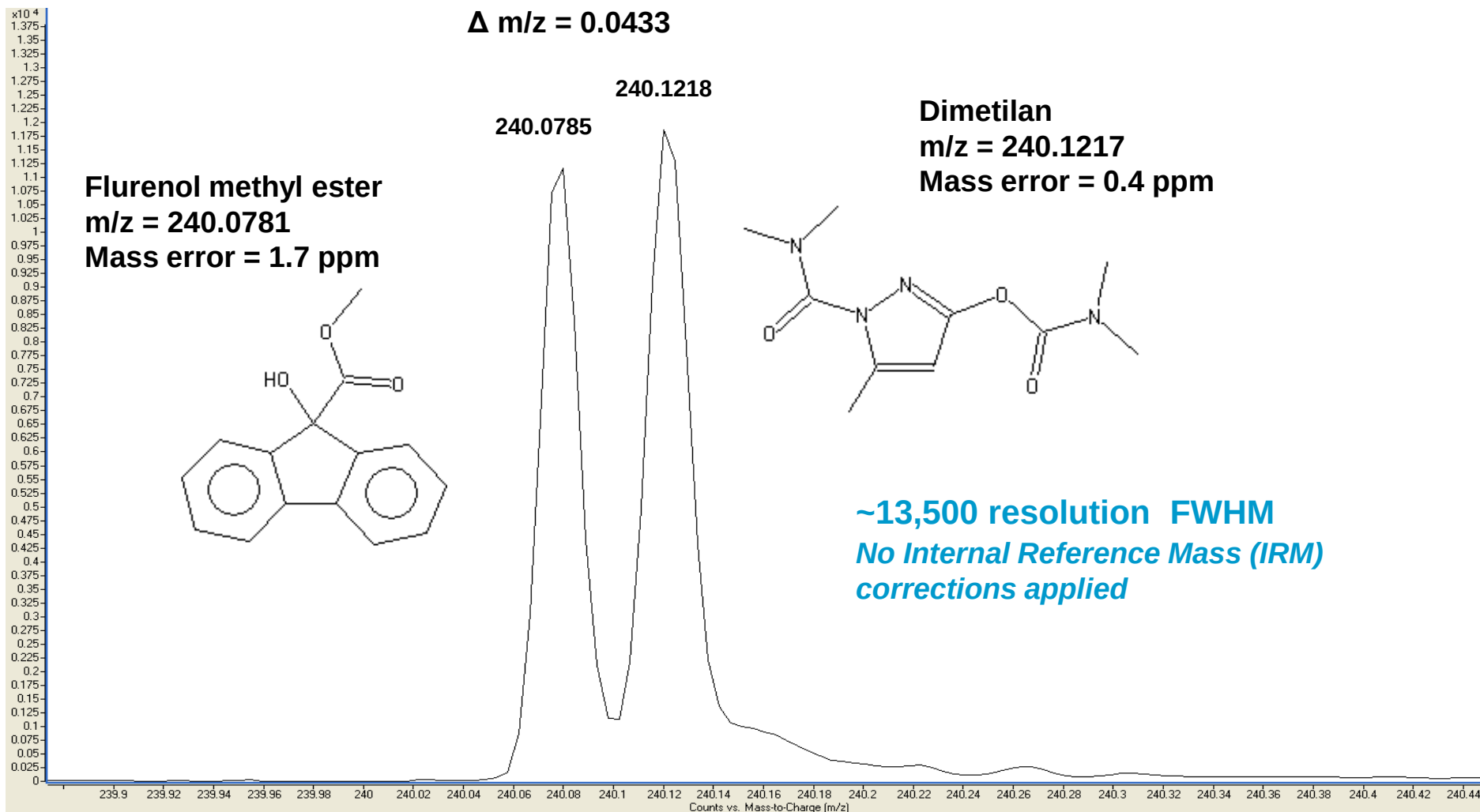
$$R = 614/0.0423 = \mathbf{14,522}$$

$$= ((613.9642 - 613.9646)/613.9642) \times 10^6$$

$$= \mathbf{-0.65 \text{ ppm}}$$

High Res can Eliminate Isobaric Interferences

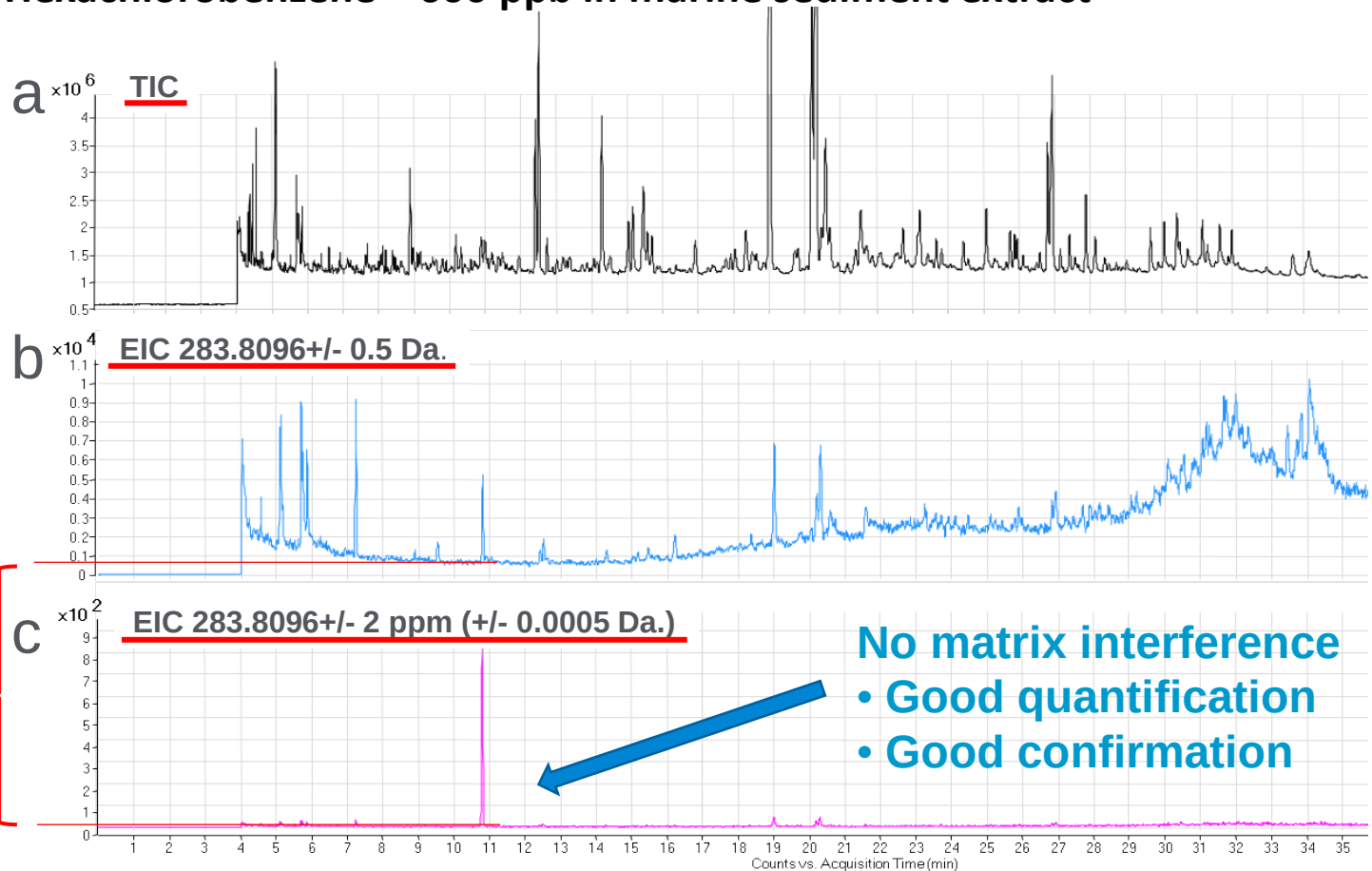
Appears as single m/z 240 peak for any unit mass resolution MS



Porque alta resolução e massa exata?

Quantitativa de compostos alvo e confirmação

Hexachlorobenzene – 600 ppb in marine sediment extract



Canabinóides sintéticos

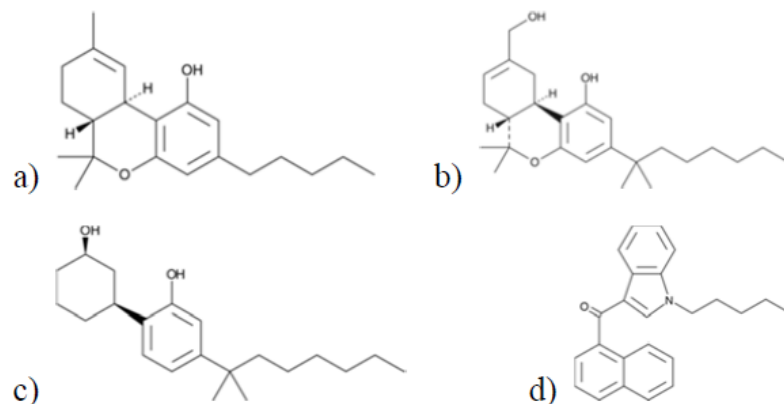
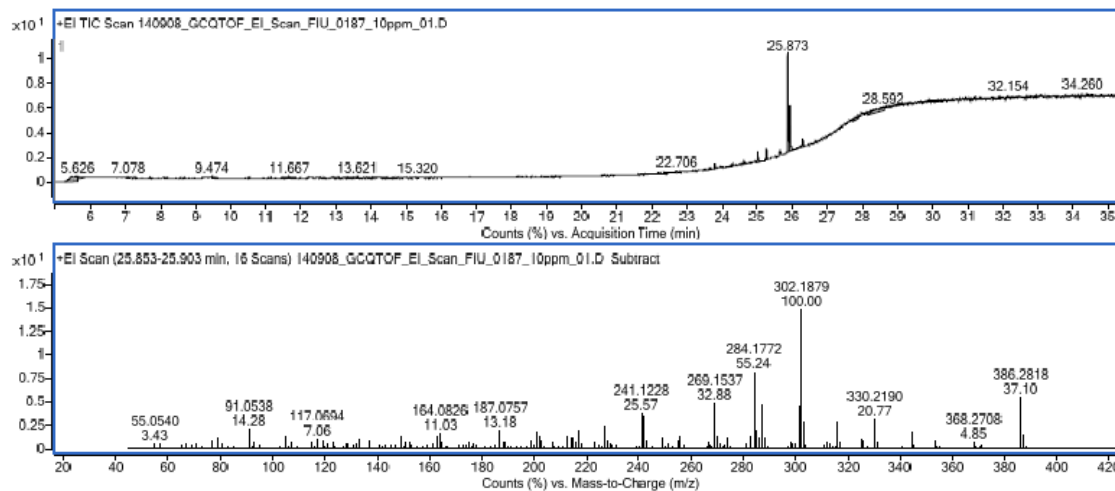
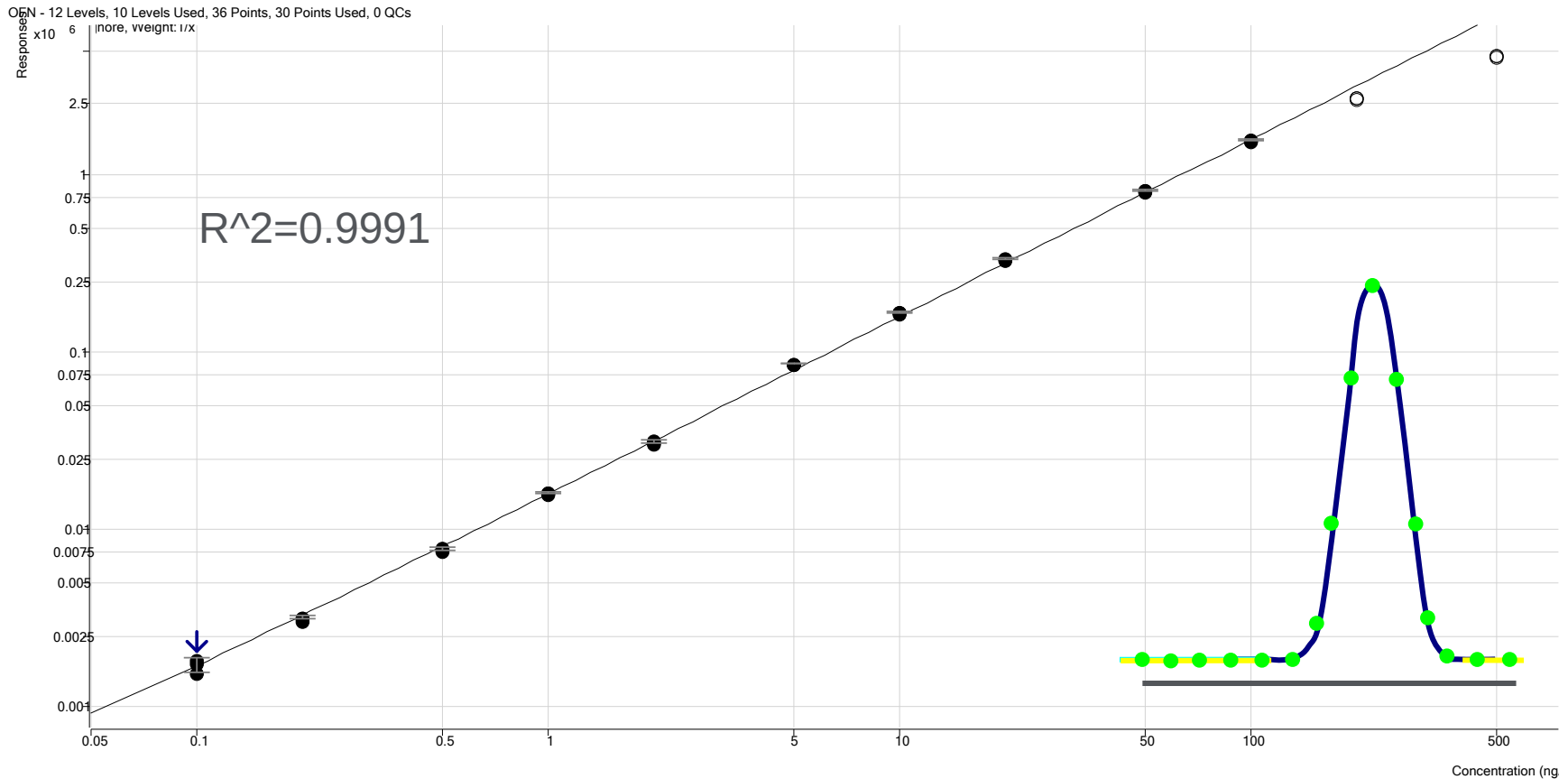


Figure 2. Chemical structures of THC and 3 main synthetic cannabinoid classifications
a) THC b) HU-210 c) CP 47,497 d) JWH 018.



Response linearity and mass accuracy

ADC advantages



5 Hz acquisition, RIS

LOD about 0.01 pg

Outros Acessórios para complementar as técnicas de Análise

Acessórios / portfólio



Headspace Estático



HS , SPME , Itex



Dessorvedor Térmico



Probe de inserção

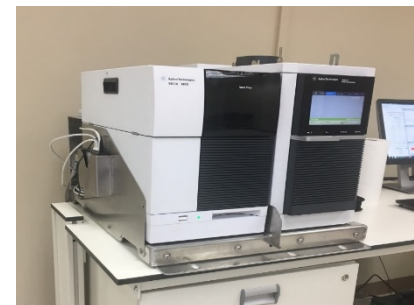


Headspace Dinâmico – P&T



Jet Clean

Unidades Móveis



LC/MS

Table I provides some points for comparison of GC–MS, for an electron ionization (EI) source and a single-quadrupole mass analyzer, with LC–MS, using electrospray ionization (ESI) and tandem mass spectrometry (MS/MS) on a triple-quadrupole instrument.

Table I. Comparison of GC-MS and LC-MS for trace analysis of drugs in biological fluids		
Aspect	GC–EI-MS	LC–ESI-MS/MS
General application	Volatile, thermally stable molecules (low- to mid-polarity); low-volatility compounds need to be derivatized	Nonvolatile, polar and ionic molecules (mid- to high-polarity)
Cost	~\$100k	~\$200k
Available? Established?	Yes, yes	Yes, yes
Sample preparation for biological fluids	Liquid–liquid extraction; solid-phase extraction; derivatization (to make polar compounds more volatile); finish in volatile solvent	Dilute-and-inject (urine); Protein precipitation; liquid–liquid extraction; solid-phase extraction; finish in polar solvent (water ideal)
Limits of detection and quantification	Nanogram (10^{-9} g) to <u>picogram</u> (10^{-12} g)	Picogram to femtogram (10^{-15} g)
Specificity?	Yes, via EI mass spectrum (library matching; abundant diagnostic fragment ions from EI); lack of molecular ion is a problem	Yes, via MS/MS; monitor specific fragments of desired molecular ion using triple quadrupole; no universal library matching
Matrix effects?	Minimal	Yes, require stable isotopically labeled internal standards for each analyte

Benefícios da Espectrometria de Massas

- ✓ **Universal**: medidas de massa-carga
- ✓ Maior **seletividade** e **sensibilidade** para análises quantitativas
- ✓ Mais **informações ortogonais** (t_R , m/z , MS/MS) para análises qualitativas
- ✓ **Menor preparo de amostra**
- ✓ **Alta velocidade**
- ✓ **Sem limitação de m/z**

Single Quad



QQQ



TOF

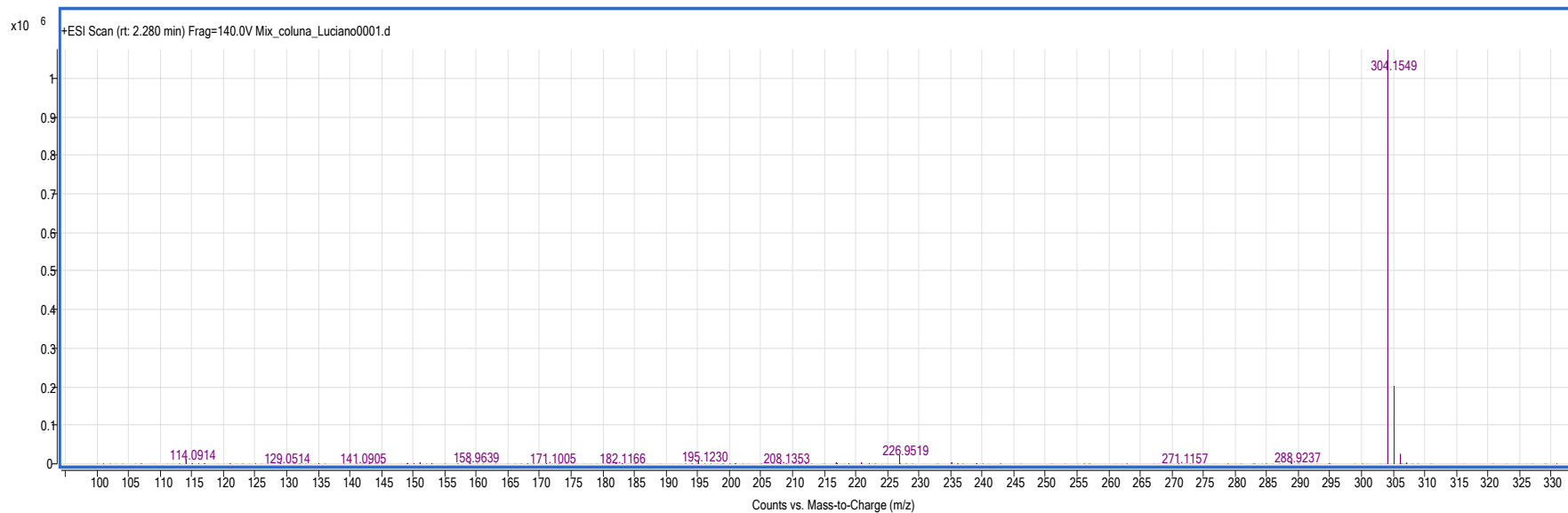


QTOF

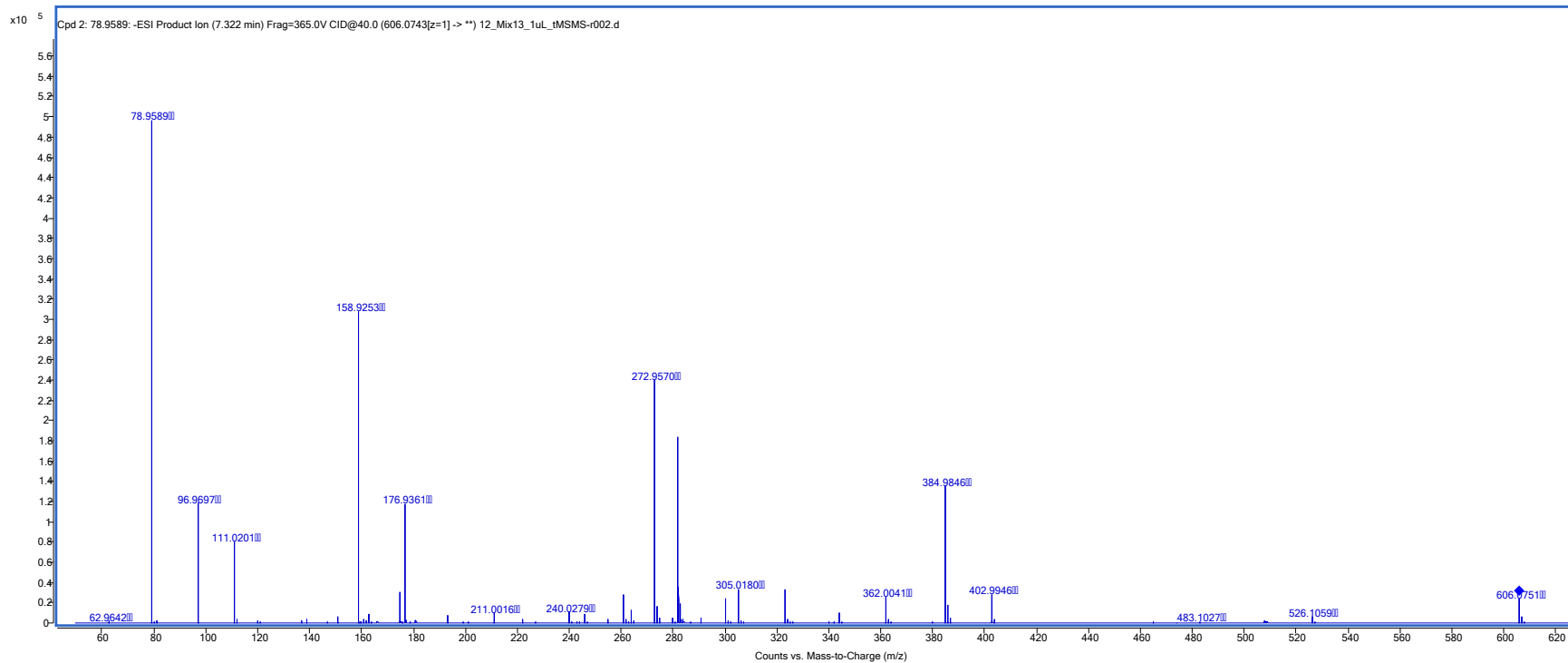
Sistema LCMS



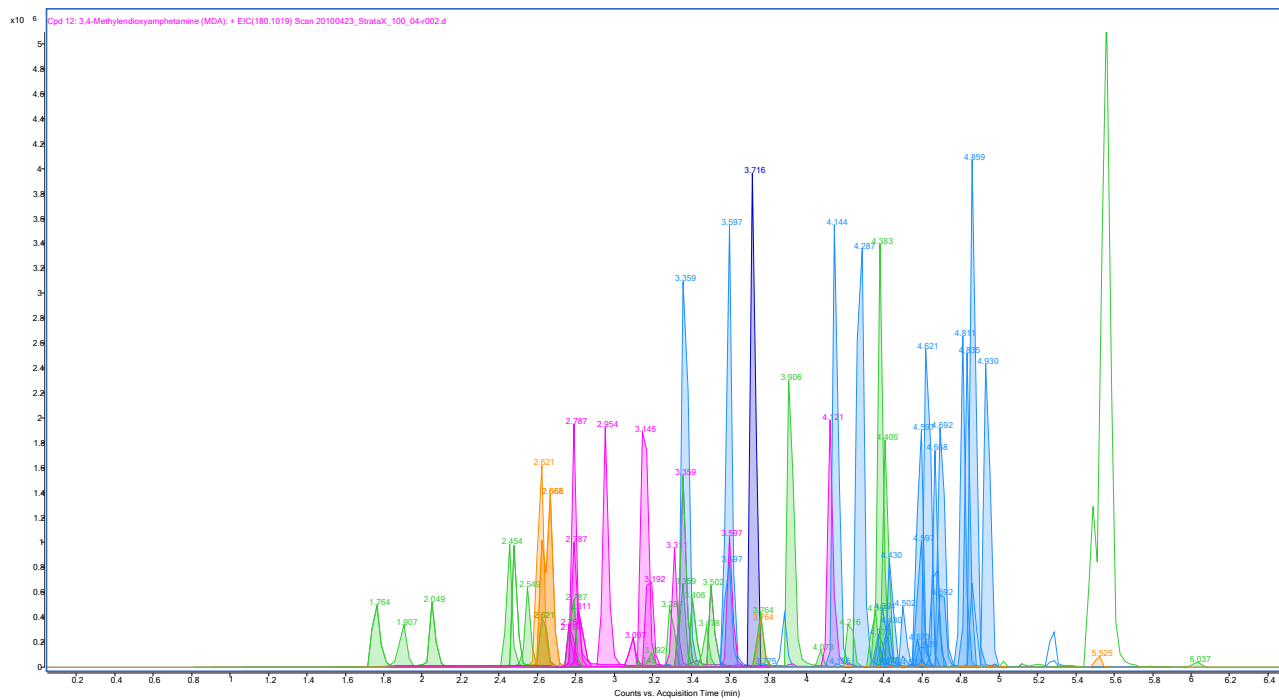
Espectro de MS



Espectro de MSMS

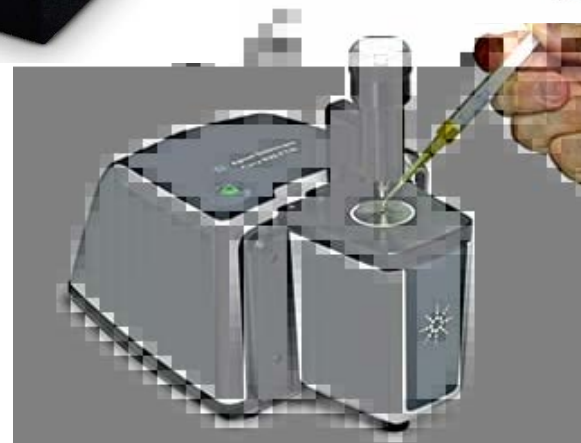


Cromatograma



POLICE LINE DO NOT CROSS

Soluções FTIR e Raman Agilent para o segmento Forense

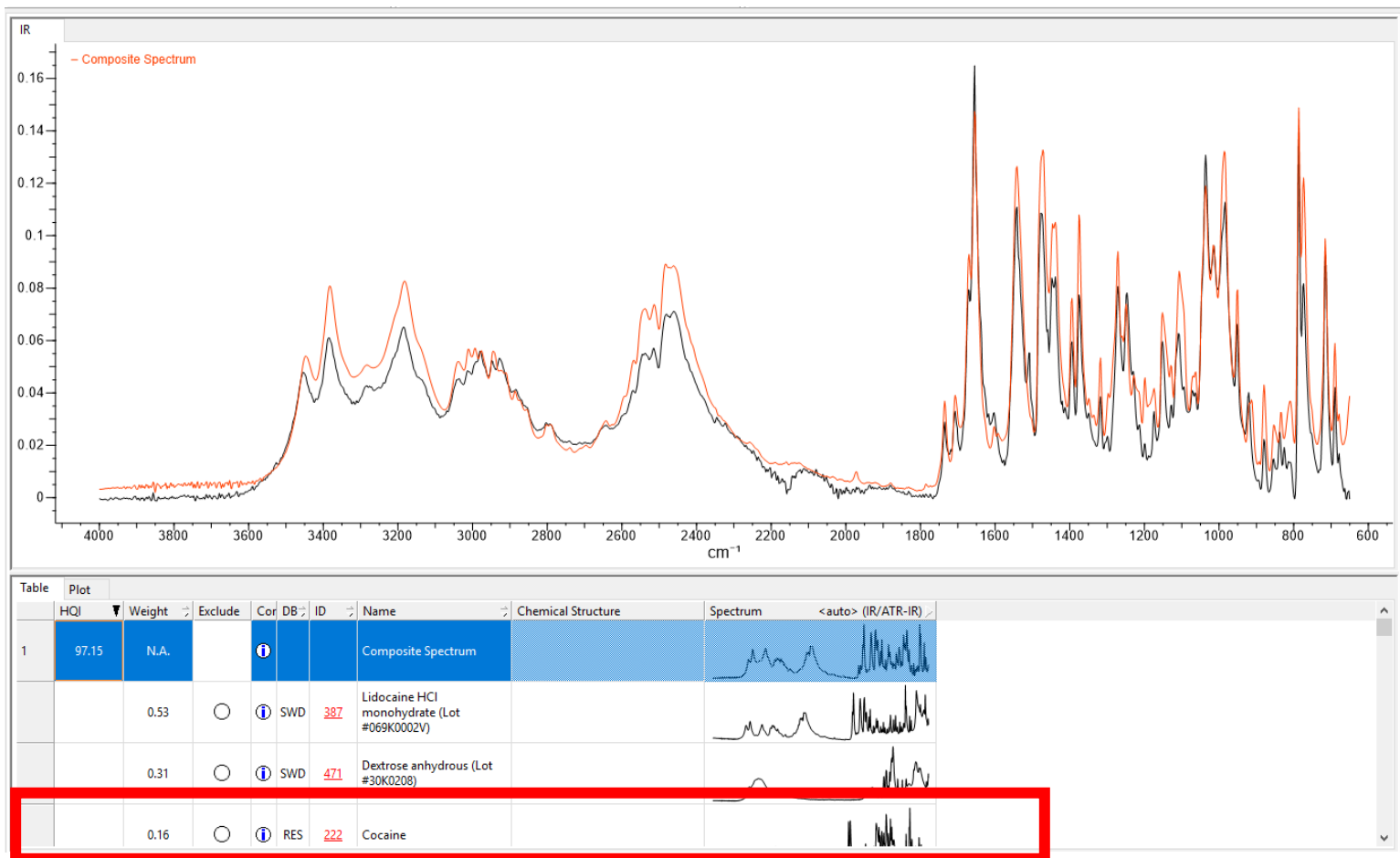


FTIR Cary 630 – ATR de diamante



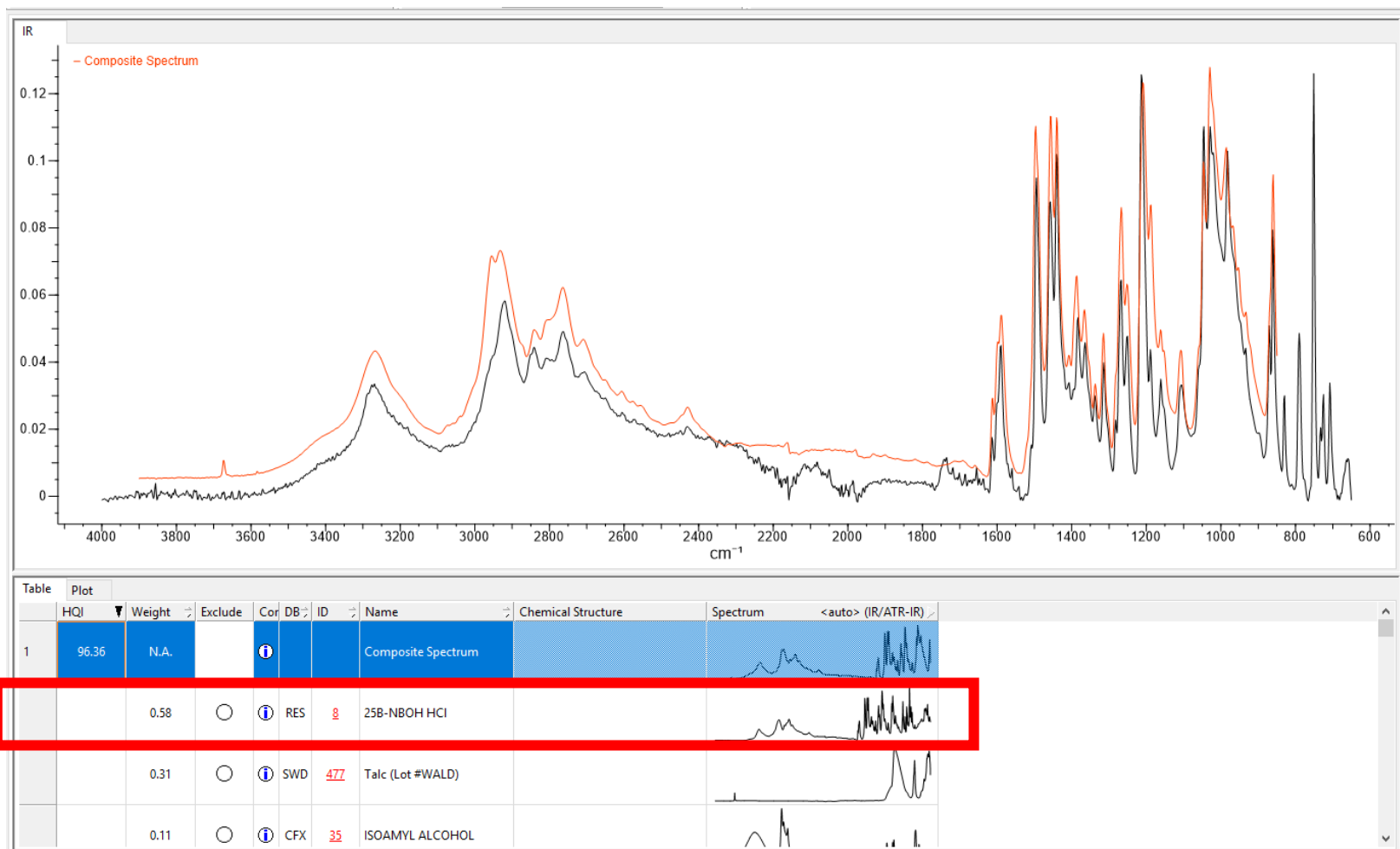
- Sistema FTIR mais **robusto** do mercado com **óptica permanentemente alinhada** que confere maior **estabilidade analítica** e **portabilidade**;
- **Todos os materiais ópticos em ZnSe**; Não trabalhamos com ópticas mistas com materiais menos nobres e sem necessidade de purga para operação;
- Alta **performance** e **velocidade**;
- Hardware e software **completos** e de **fácil** utilização;
- **Garantia** 10 anos interferômetro e laser e 5 anos para fonte;

Exemplo 1: pó branco



Identificação com sucesso de cocaína em uma mistura através de busca avançada de multicomponentes

Exemplo 2: extrato selo



Identificação com sucesso de 25B-NBOH após extração através de busca avançada de multicomponentes

Raman Resolve

Identifica compostos através da embalagem com tecnologia SORS!!!



Diferente dos Ramans convencionais que tentam ajuste de foco manual e o ID através da embalagem é uma questão da habilidade ou sorte do usuário!

Raman Resolve

Aumentado o poder da identificação Raman portátil

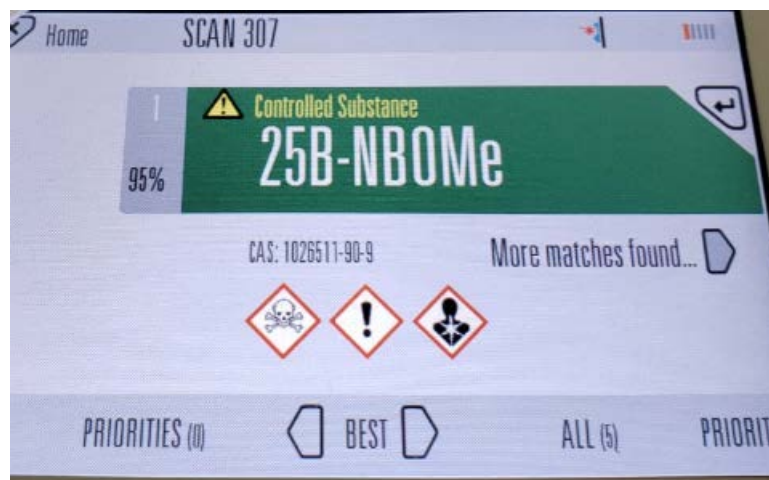


■ Geralmente rotineiro

■ Geralmente mais difícil*

* O sucesso da identificação depende da combinação de recipiente e conteúdo.

Raman Resolve



Raman Resolve

análise de combustíveis

OBRIGADO PELA ATENÇÃO !!!
DÚVIDAS ???

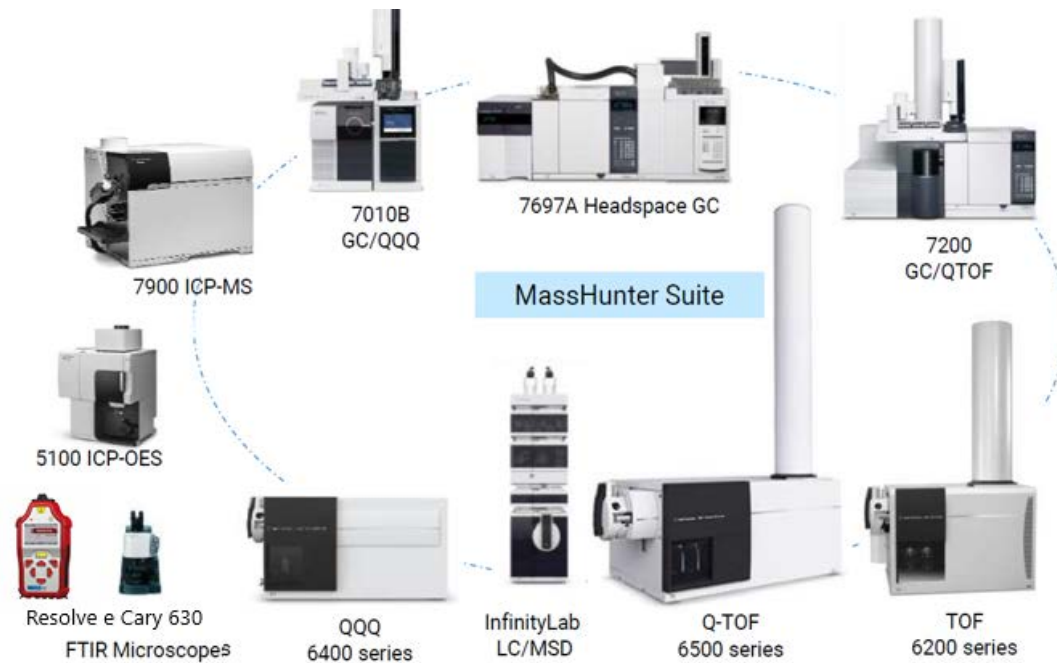
Romão Beserra

11-96423-0132 cel

11-4197-3500 geral

0800-728-1405 Suporte

romao_beserra-jr@agilent.com



Agilent

Trusted Answers