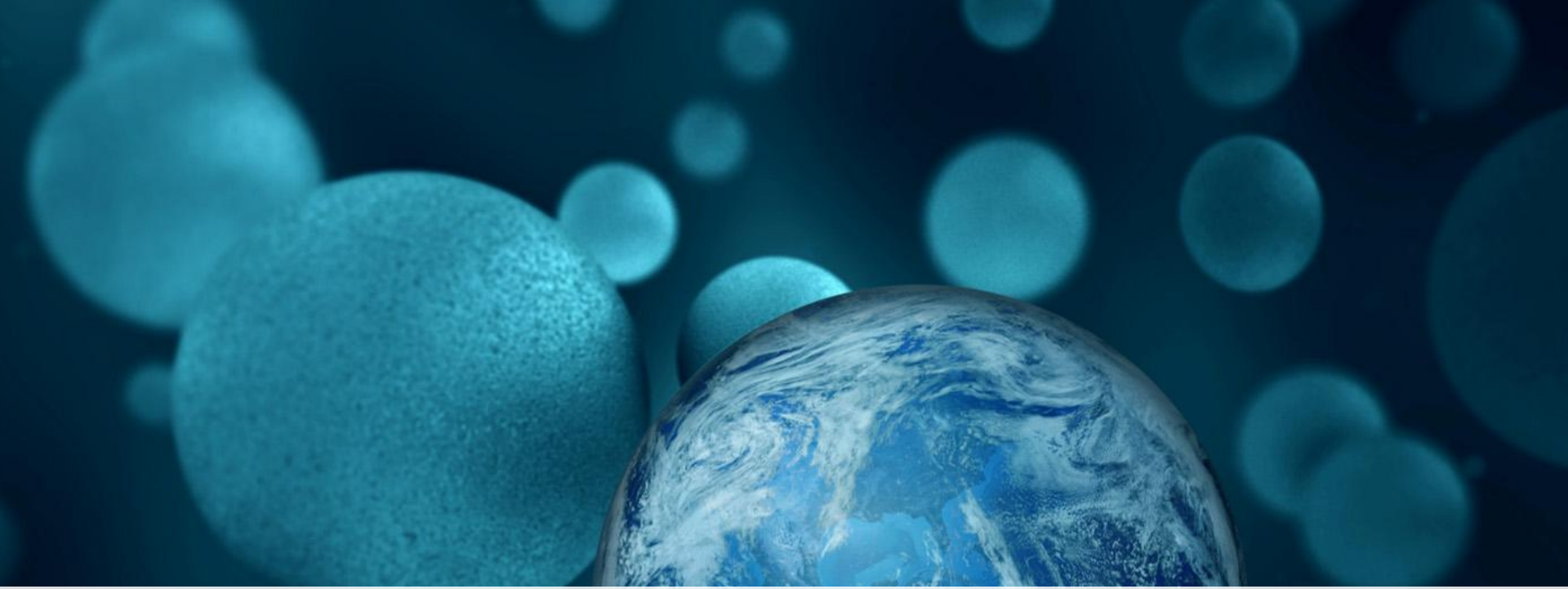
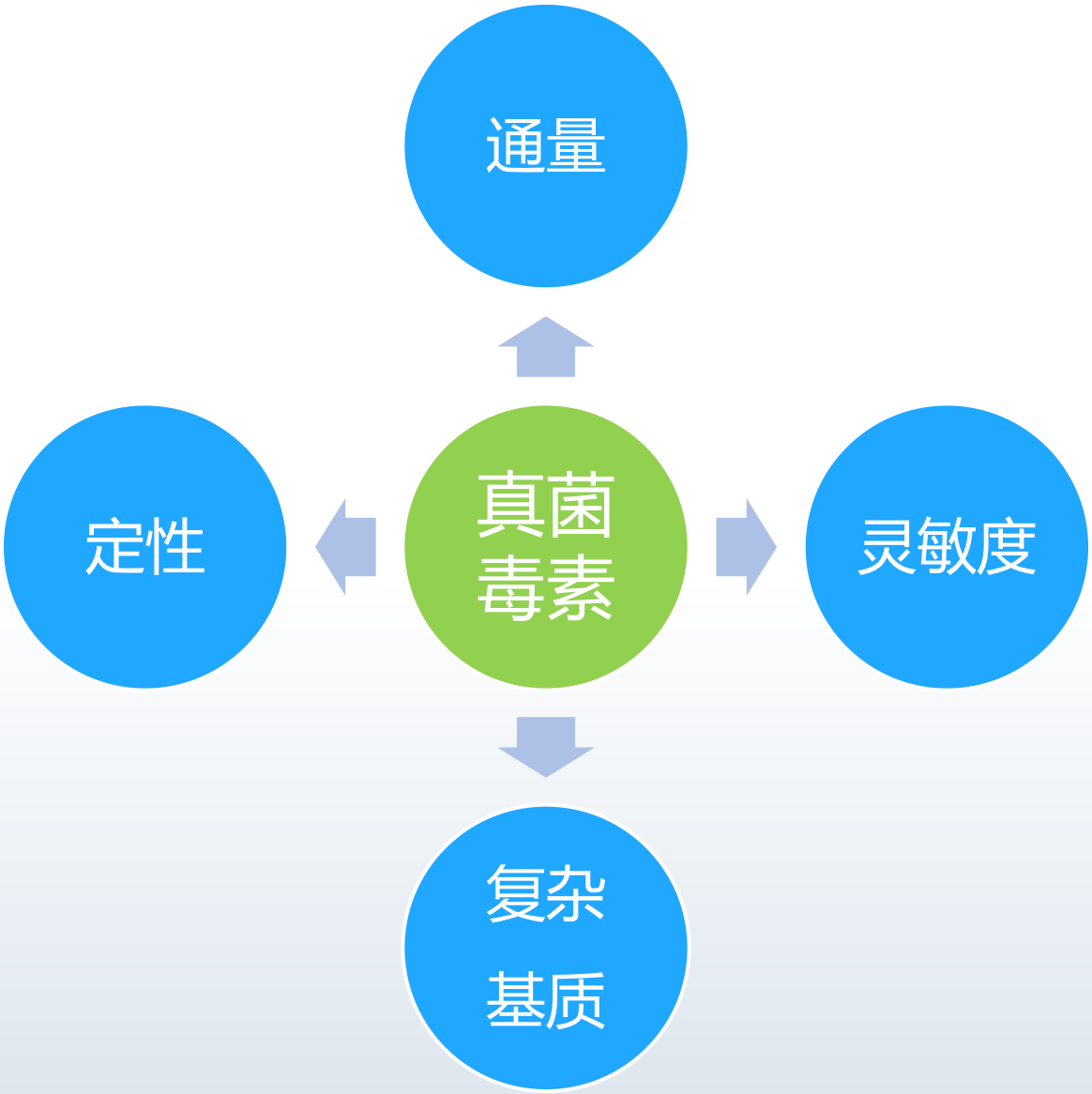


ThermoFisher SCIENTIFIC

液质联用技术在真菌毒素检测中的应用

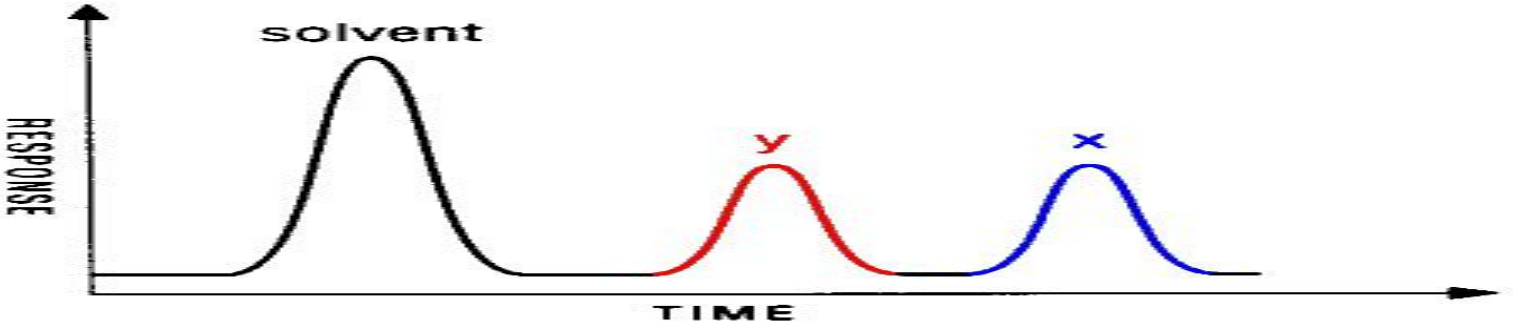
色谱质谱部 应用工程师
祝翔
xiang.zhu@thermofisher.com
2017-9-21

真菌毒素检测难点

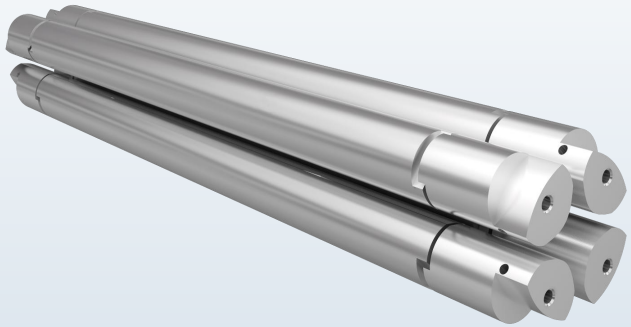


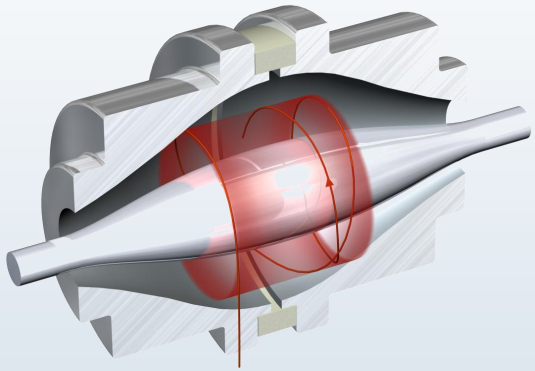
液质联用技术的优势

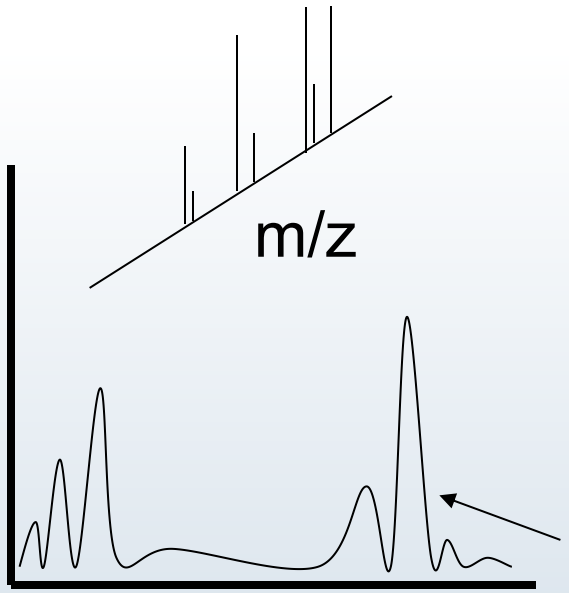
- 色谱分离和富集，能有效提高灵敏度



- 质谱检测器的选择性强
  - 无需基线分离
- 质谱是高灵敏度和高选择性的检测器
  - pg/ml级别,DAD的灵敏度一般是ug/ml
  - m/z即为化合物特有标签







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液质联用工作流程

样品前处理

目标物分析

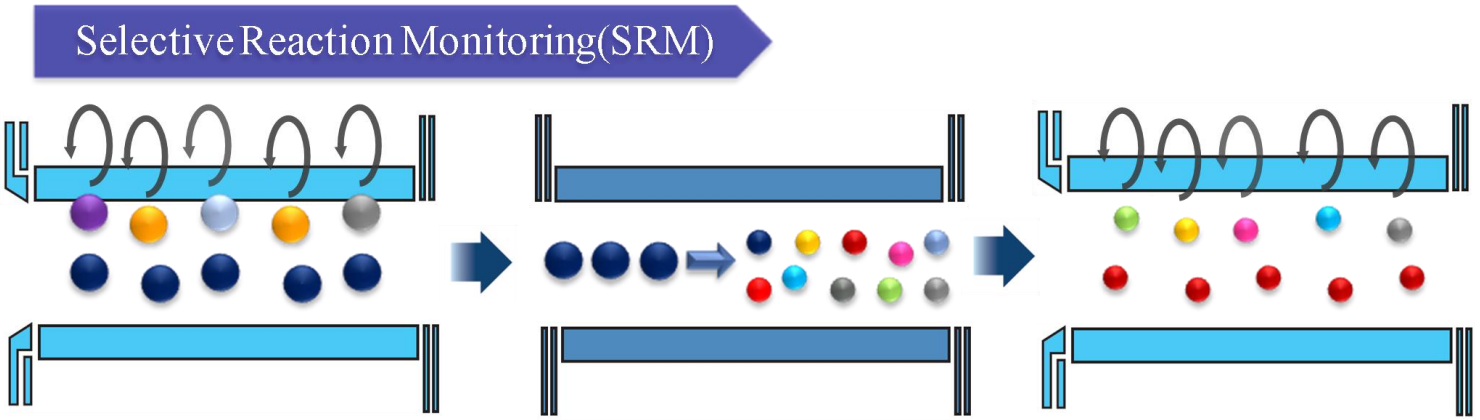
定量

非目标物分析

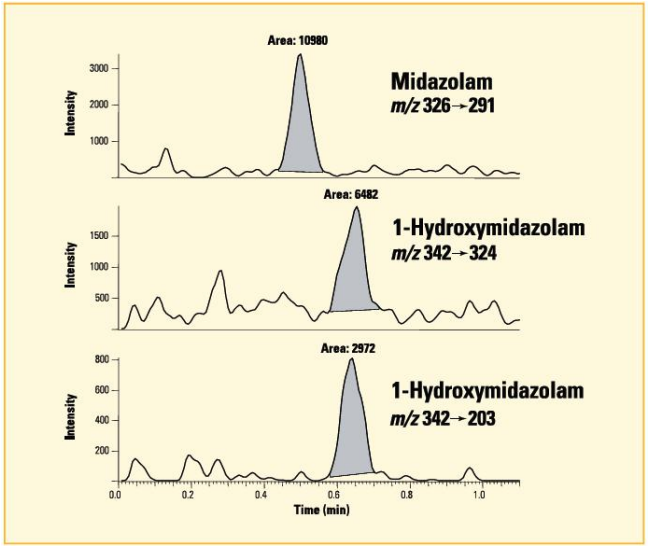
定性

ThermoFisher SCIENTIFIC

三重四级杆质谱定量原理



Compound	H-SRM Transition	Collision Energy (ev)	Scan Time (s)
Midazolam	326.06 → 291.08	26	0.1
1-Hydroxymidazolam	342.06 → 324.03	20	0.1
1-Hydroxymidazolam	342.06 → 203.00	26	0.1



- 灵敏度高，选择性好，通量高
- 目标化合物定量金标准
- 准确性、耐用性、灵敏性

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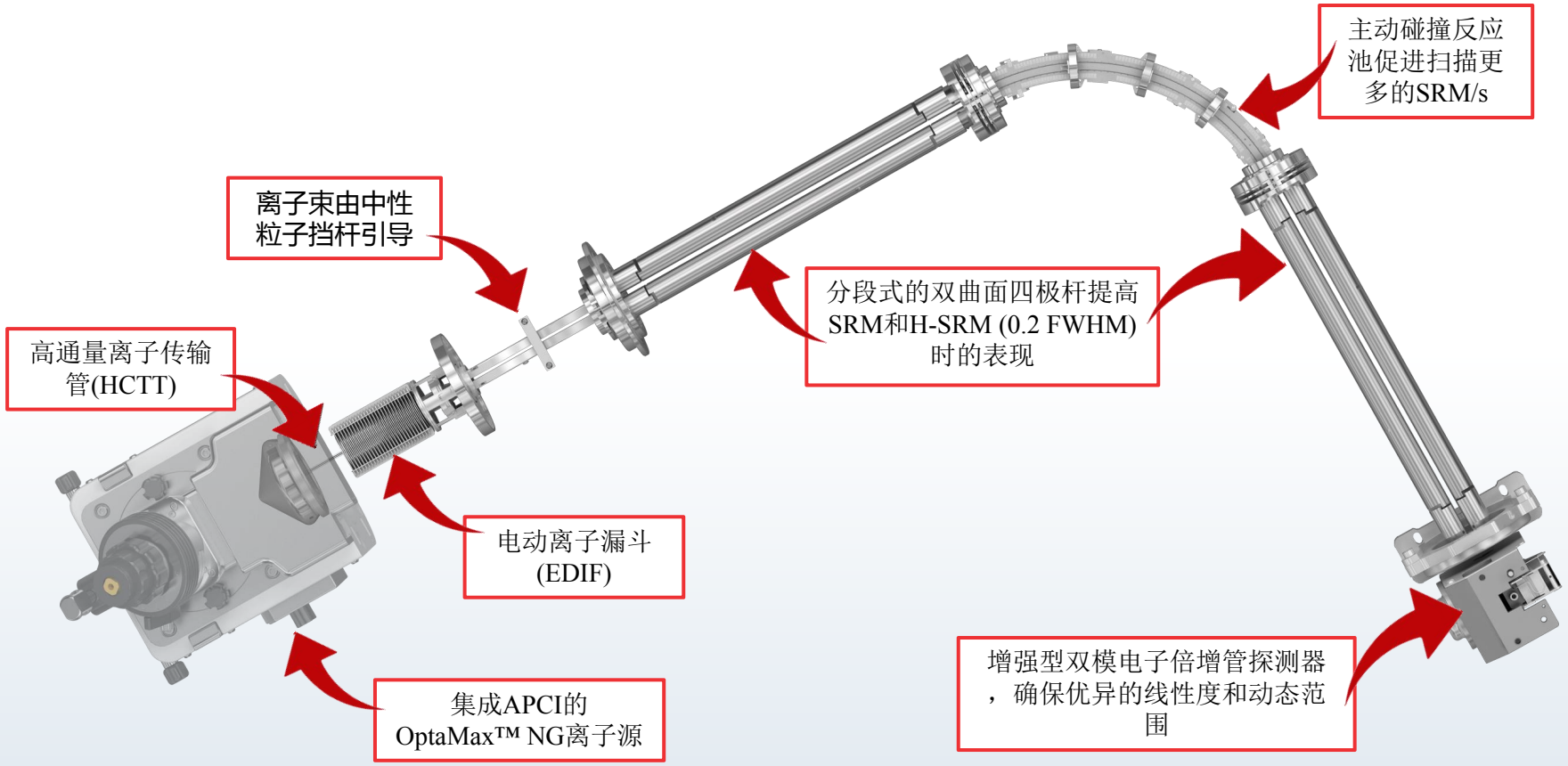
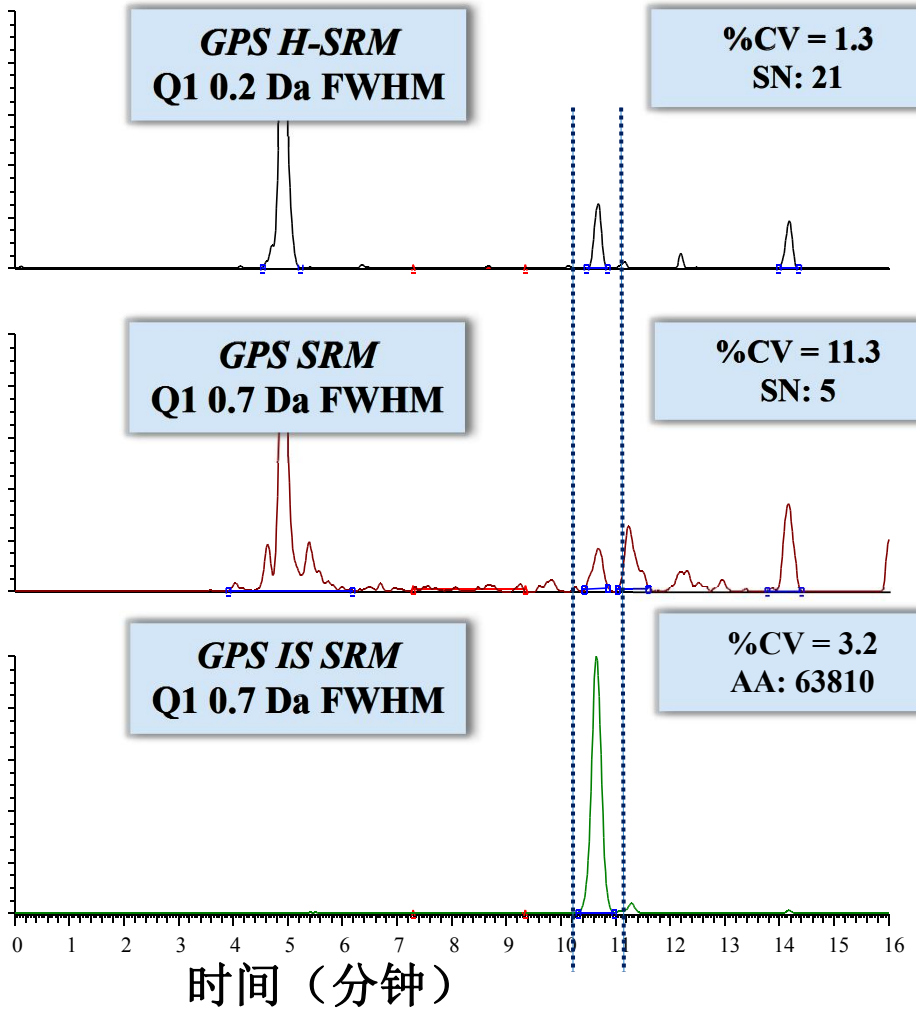
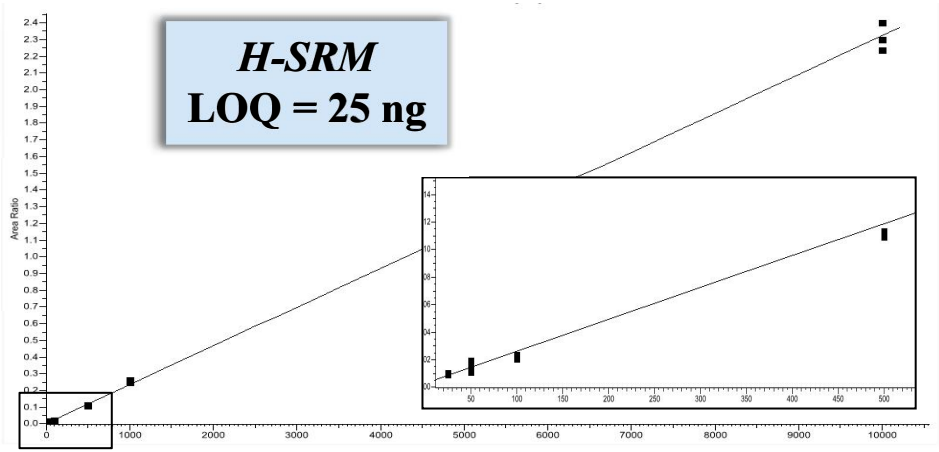
TSQ Altis三重四极杆质谱仪：AIM+技术带来的关键优势

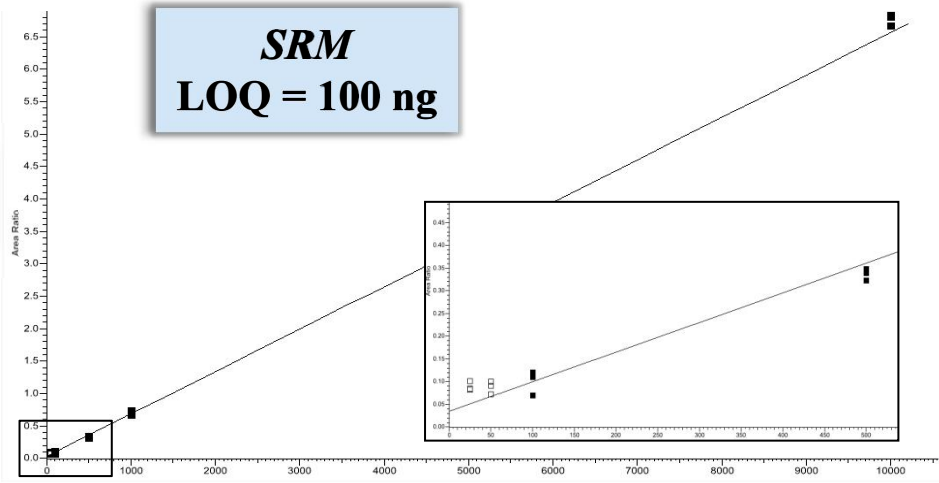
Active Ion Management Plus (AIM+) - 精密设计从Thermo Scientific OptaMaxTM 离子源到增强型电子倍增器的离子管控、结合分段式双曲面四极杆以及增强型RF电子元件，以进一步优化离子管控精度、可靠性、速度和重现性。



H-SRM (0.2 Da FWHM)的优异灵敏度







ThermoFisher SCIENTIFIC

粮食中16中真菌毒素的测定

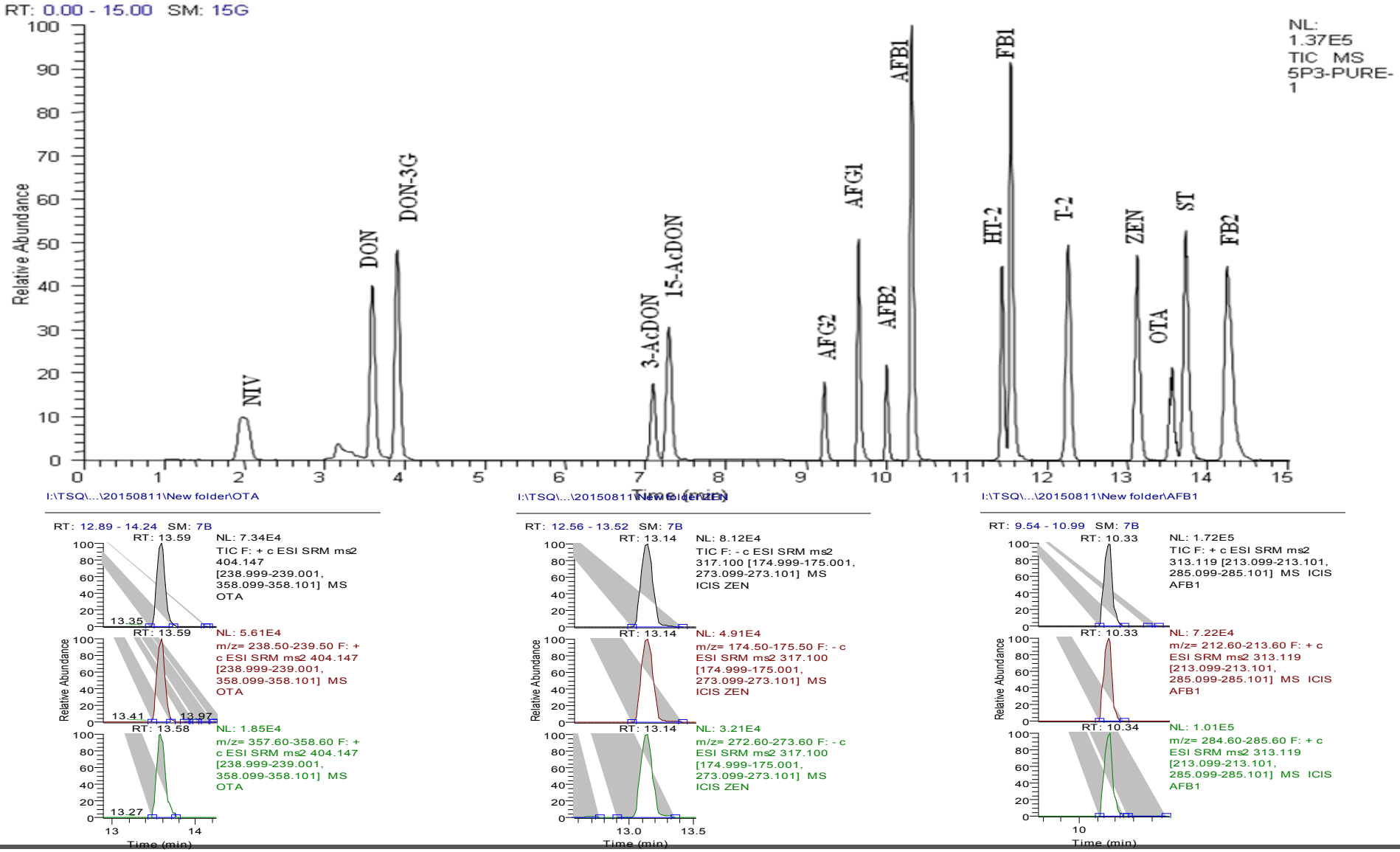
色谱柱：C18，100*2.1 mm，粒径1.6~1.8 μm
柱温：40°C
进样量：2 μL
流速：0.3 mL/min

时间	A(4.17)	B(4.17)
Initial	10.0	90.0
2.0	10.0	90.0
3.0	20.0	80.0
4.0	21.0	79.0
5.0	26.0	74.0
7.0	26.0	74.0
10.5	60.0	40.0
13.5	60.0	40.0
14.5	95.0	5.0
17.0	95.0	5.0
18.0	10.0	90.0
20.0	10.0	90.0

A: 甲醇
B：含0.1%甲酸(体积比)和1 mMol/L乙酸铵的水溶液

名称	电喷雾模式	母离子(m/z)	子离子(m/z)	碰撞能量(V)	透镜电压(V)
NIV	-	357.1	281.2*/311.1	16/13	42
[^{13}C]-NIV	-	372.1	295.1	16	42
DON	+	297.2	249.1*/203.1	11/13	99
[^{13}C]-DON	+	312.2	263.1	11	99
DON-3G	+	476.2	297.0*/459.2	11/5	94
3–AcDON	+	339.2	231.1*/213.1	12/19	116
[^{13}C]-3-AcDON	+	356.2	245.1	12	116
15–AcDON	+	339.2	137.0*/261.1	16/16	98
AFG_2	+	331.1	313.1*/245.0	24/30	109
[^{13}C]-AFG_2	+	348.1	330.1	24	109
AFG_1	+	329.1	243.1*/311.1	29/21	106
[^{13}C]-AFG_1	+	346.1	257.1	29	106
AFB_2	+	315.1	287.1*/259.0	25/31	133
[^{13}C]-AFB_2	+	332.1	303.1	25	133
AFB_1	+	313.1	285.1*/213.1	22/45	133
[^{13}C]-AFB_1	+	330.1	301.1	22	133
HT–2	+	442.3	263.0*/215.1	12/13	123
[^{13}C]-HT-2	+	464.3	278.1	12	123
FB_1	+	722.5	334.2*/352.3	42/39	155
[^{13}C]-FB_1	+	756.5	356.4	42	155
T–2	+	484.3	305.1*/185.0	13/20	113
[^{13}C]-T-2	+	508.3	322.1	13	113
FB_2	+	706.5	336.3*/318.2	40/41	155
[^{13}C]-FB_2	+	740.4	358.4	40	155
ZEN	–	317.1	175.0*/273.1	26/19	-140
[^{13}C]-ZEN	–	335.1	185	26	-140
OTA	+	404.1	239.0*/358.1	26/13	125
[^{13}C]-OTA	+	424.1	250.0	26	125
ST	+	325.1	281.0*/310.1	35/23	99
[^{13}C]-ST	+	343.1	327.1	23	99

粮食中16中真菌毒素的测定



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Eurofins: Confirmation

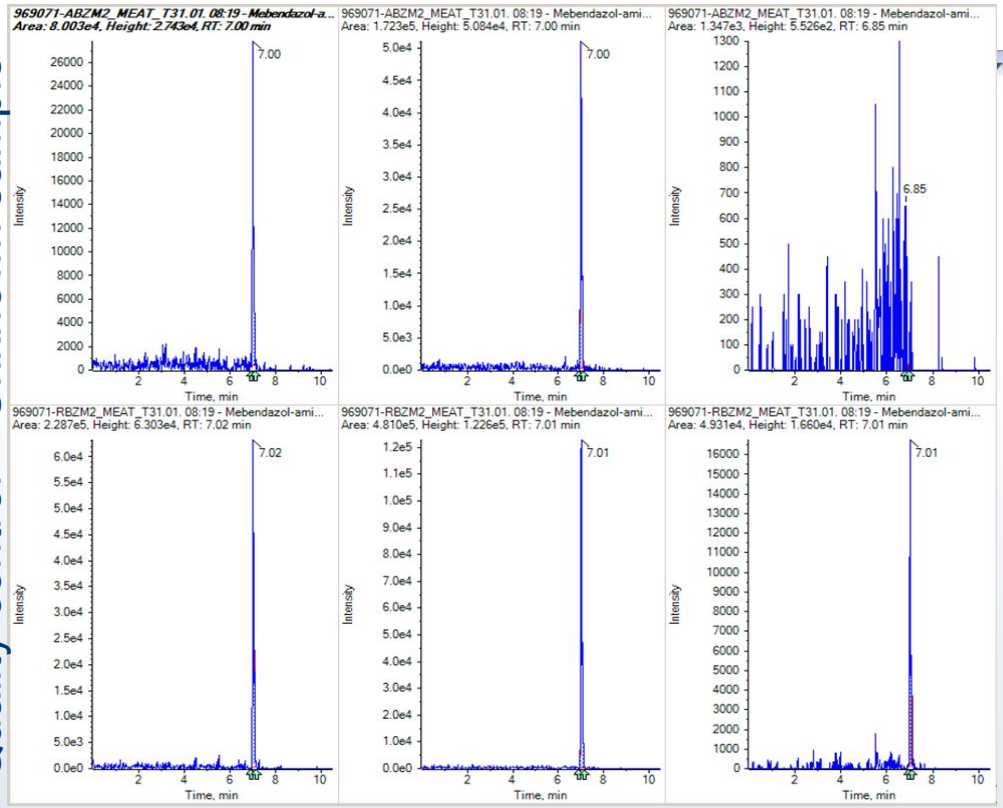
Positive Sample:
=> Mebendazole-2-amine （氨基甲苯哒唑）

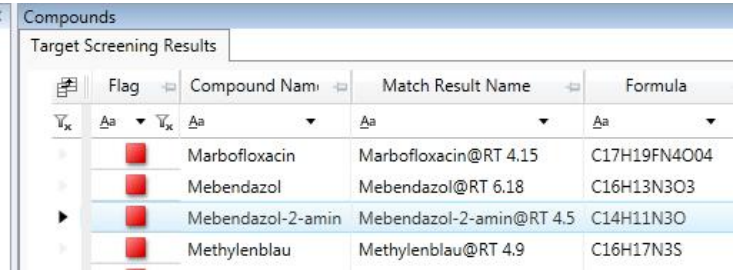
Same Sample by FS-ddMS (HR-MS)
=> negative
HR-MS resolution 70 000

238/76 238/105 238/133

Unknown sample

Quality control





Commission Decision 2002/657/EC: fulfilled criteria

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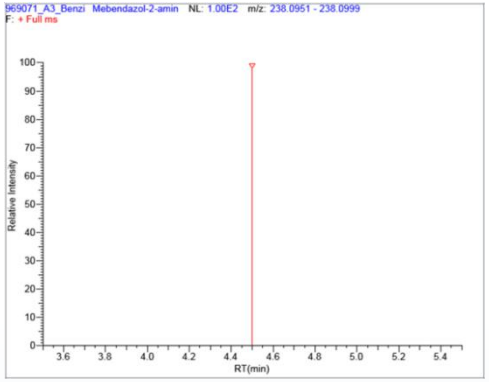
10

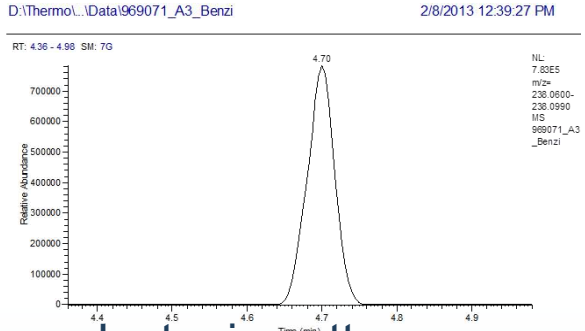
Eurofins: Confirmation

Screening: Full scan (resolution 70 000)
Data-dependent Fragmentation (resolution17 500)
Mebendazole-2-amine: 238.0975 Da
（氨基甲苯哒唑）

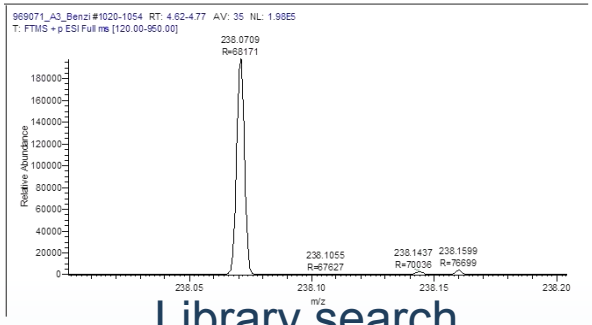
Xcalibur: mass ranges 238.06-238.0999

Unkown Sample



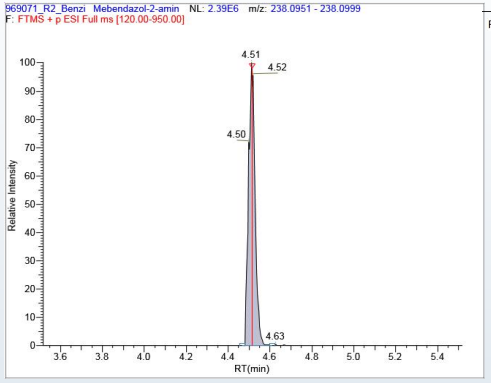


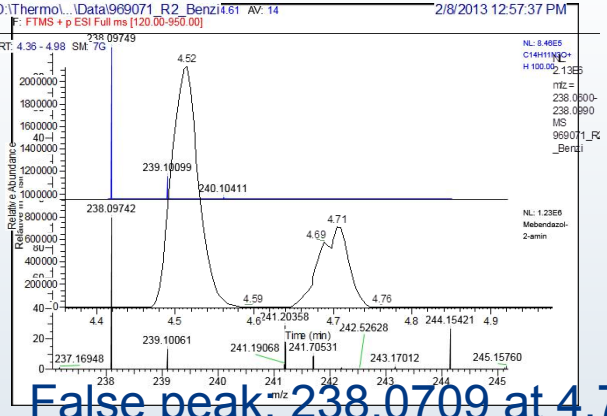
Isotopic pattern

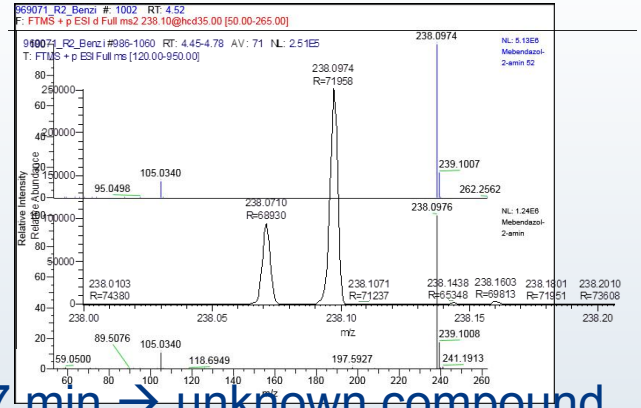


Library search

Quality coltrol

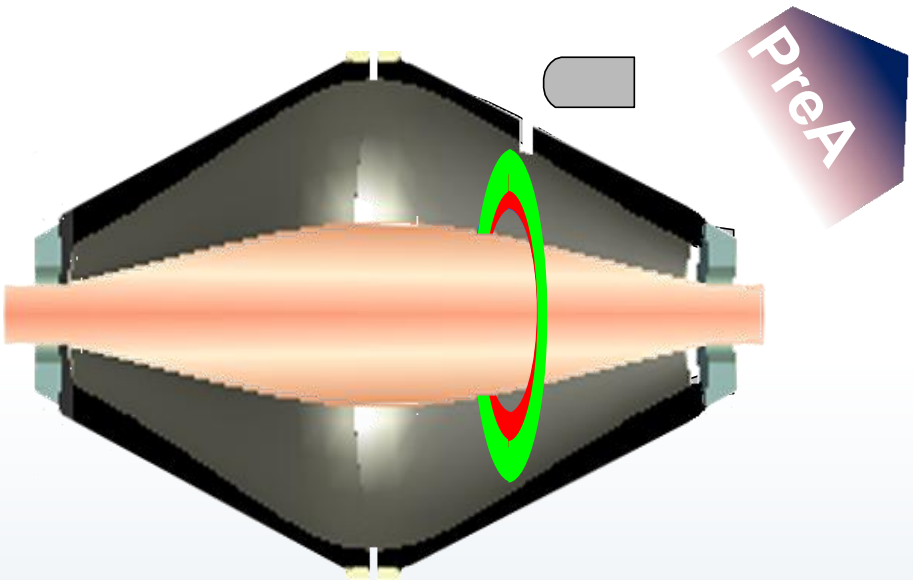


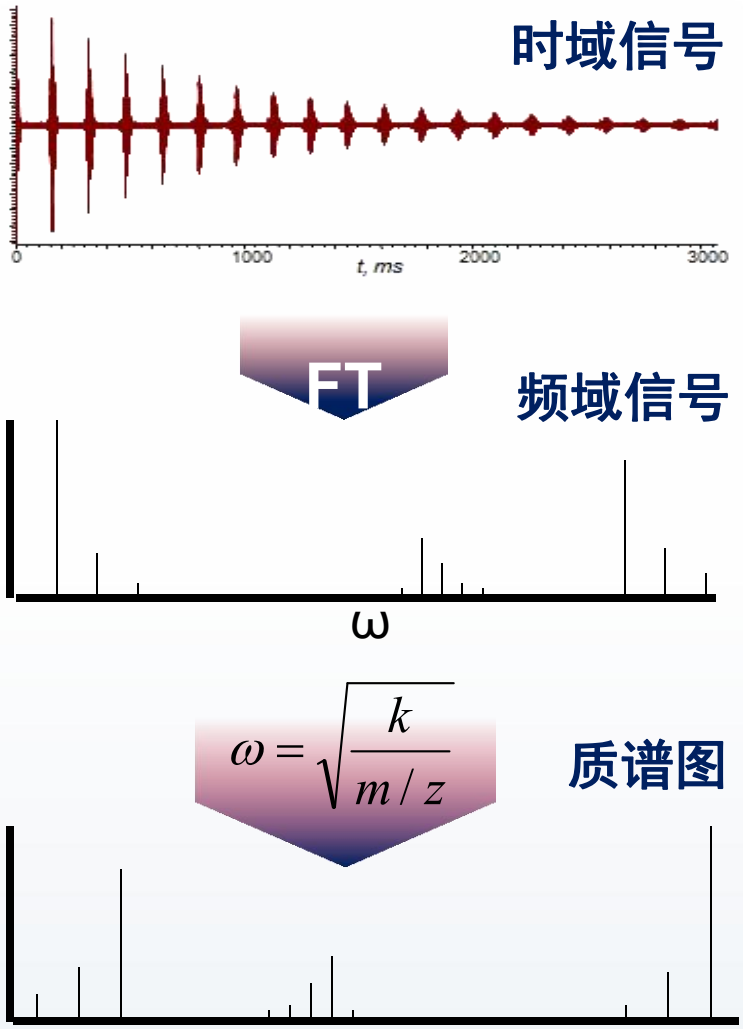




False peak: 238.0709 at 4.7 min → unknown compound

Orbitrap 静电场轨道阱高分辨质谱原理





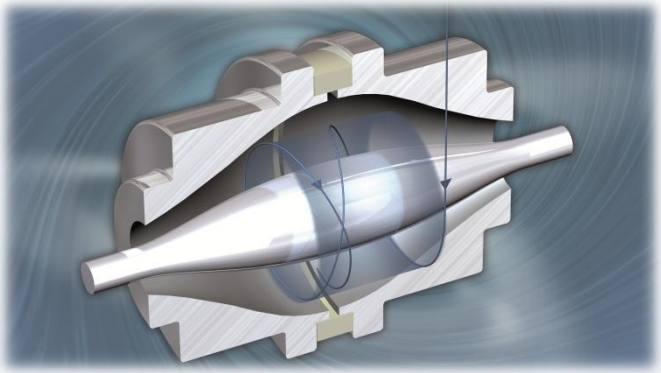
30年来唯一基于全新理论的质量分析器，真空度比传统质量分析器高3个数量级以上，稳定性极强。

高分辨质谱性能特点

超高分辨率
> 70K

灵活多样的
扫描方式

高质量精度
< 3 ppm



稳定性极强
不用频繁校正

宽动态范围
4-5个数量级

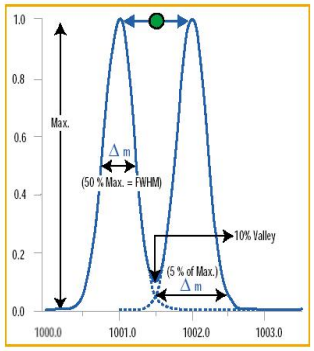
高灵敏度

为什么需要高分辨

为什么需要高分辨率？

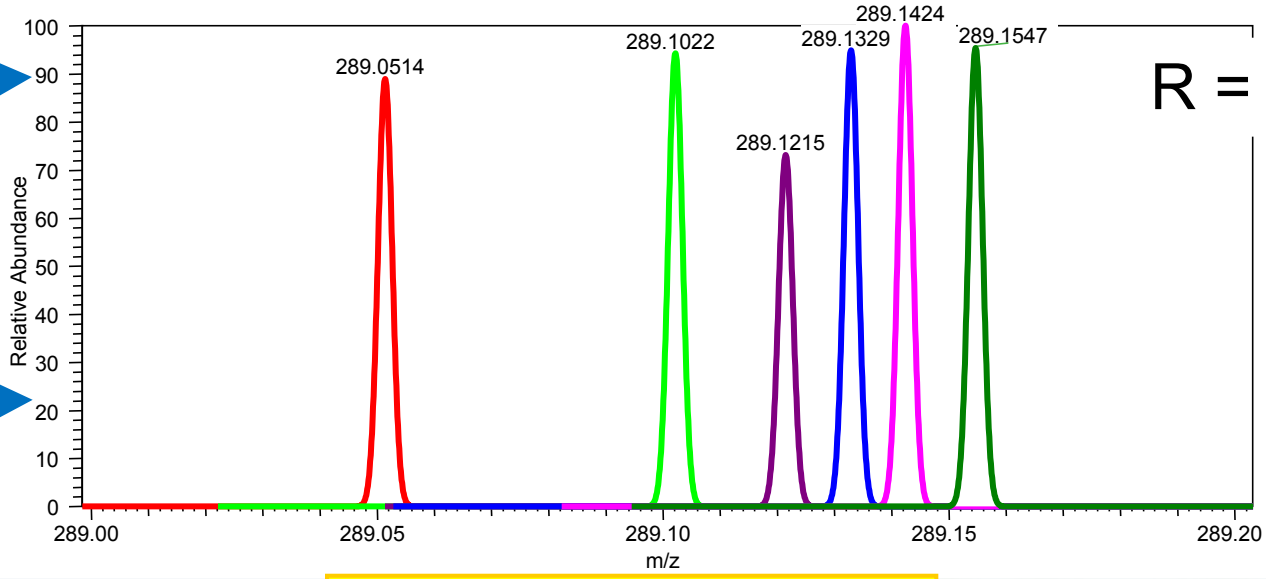
Element	Exact Mass
H	1.007825
C	12.000000
N	14.003074
O	15.994915

$$R = \frac{m}{\Delta m}$$



超高分辨率以区分基质中的同质异位素干扰物

0.1033 amu



R = 70,000

Yes, at high resolution !

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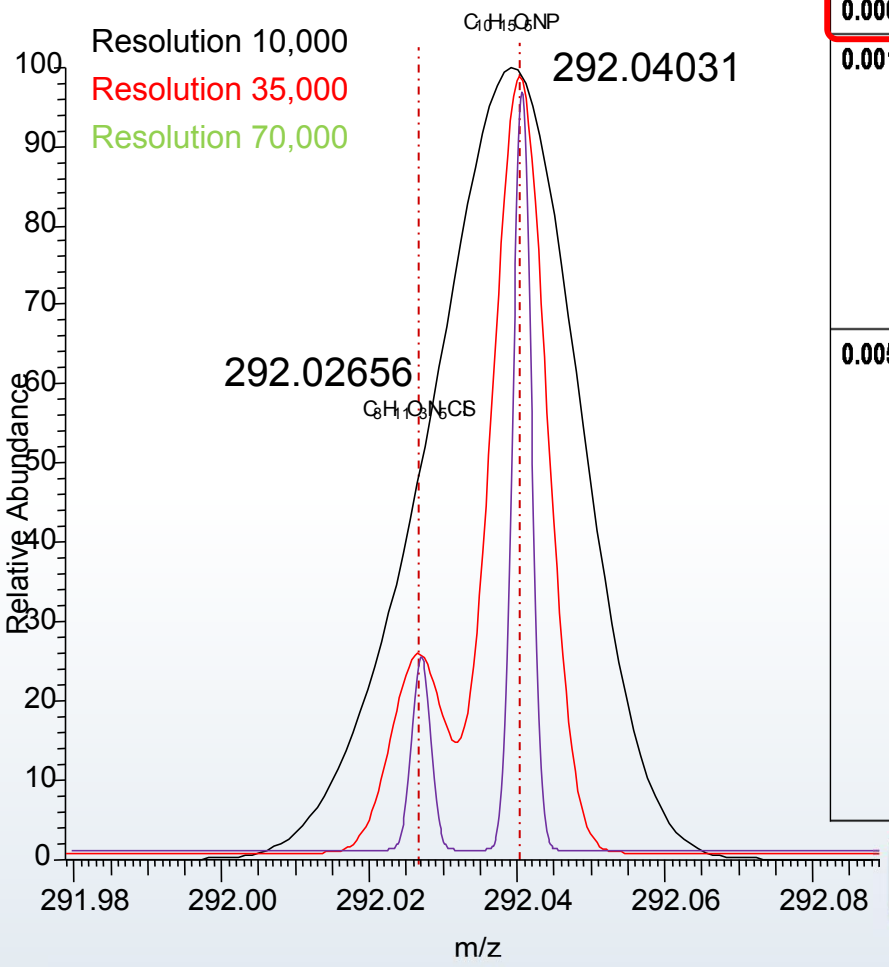
分辨率和质量精度的实际工作意义

噻虫嗪 $C_8H_{11}CLN_5O_3S$ $[M+H]^+$ = (292.02656)
对硫磷 $C_{10}H_{15}NO_5PS$ $[M+H]^+$ = (292.04031)



m/z	Theoretical	Delta	Delta	RDB	Composition
279.0913	mass	(ppm)	(mmu)		
0.0003(1)	279.0910	-1.1	-0.3	7.5	C12H15O2N4S1
0.001(6)	279.0910	-1.1	-0.3	7.5	C12H15O2N4S1
	279.0917	1.4	0.4	16.5	C20H11N2
	279.0917	1.4	0.4	3.5	C5H15N10S2
	279.0908	-1.8	-0.5	-1	C5H17O10N3
	279.0908	-1.8	-0.5	4.5	C4H11O5N10
	279.0906	-2.5	-0.7	1.5	C12H23O1S3
0.005(10)	279.0910	-1.1	-0.3	7.5	C12H15O2N4S1
	279.0917	1.4	0.4	16.5	C20H11N2
	279.0917	1.4	0.4	3.5	C5H15N10S2
	279.0908	-1.8	-0.5	-1	C5H17O10N3
	279.0908	-1.8	-0.5	4.5	C4H11O5N10
	279.0906	-2.5	-0.7	1.5	C12H23O1S3
	279.0922	3.2	0.9	4	C6H13O6N7
	279.0924	3.9	1.1	7	C14H17O3N1S1
	279.0897	-5.7	-1.6	2.5	C11H19O6S1
	279.0890	-8.2	-2.3	12	C17H13O3N1

高质量精度的质谱数据能够帮助准确定性和定量

高分辨率的实际工作意义在于复杂样本的质荷比相近的组分的准确分析

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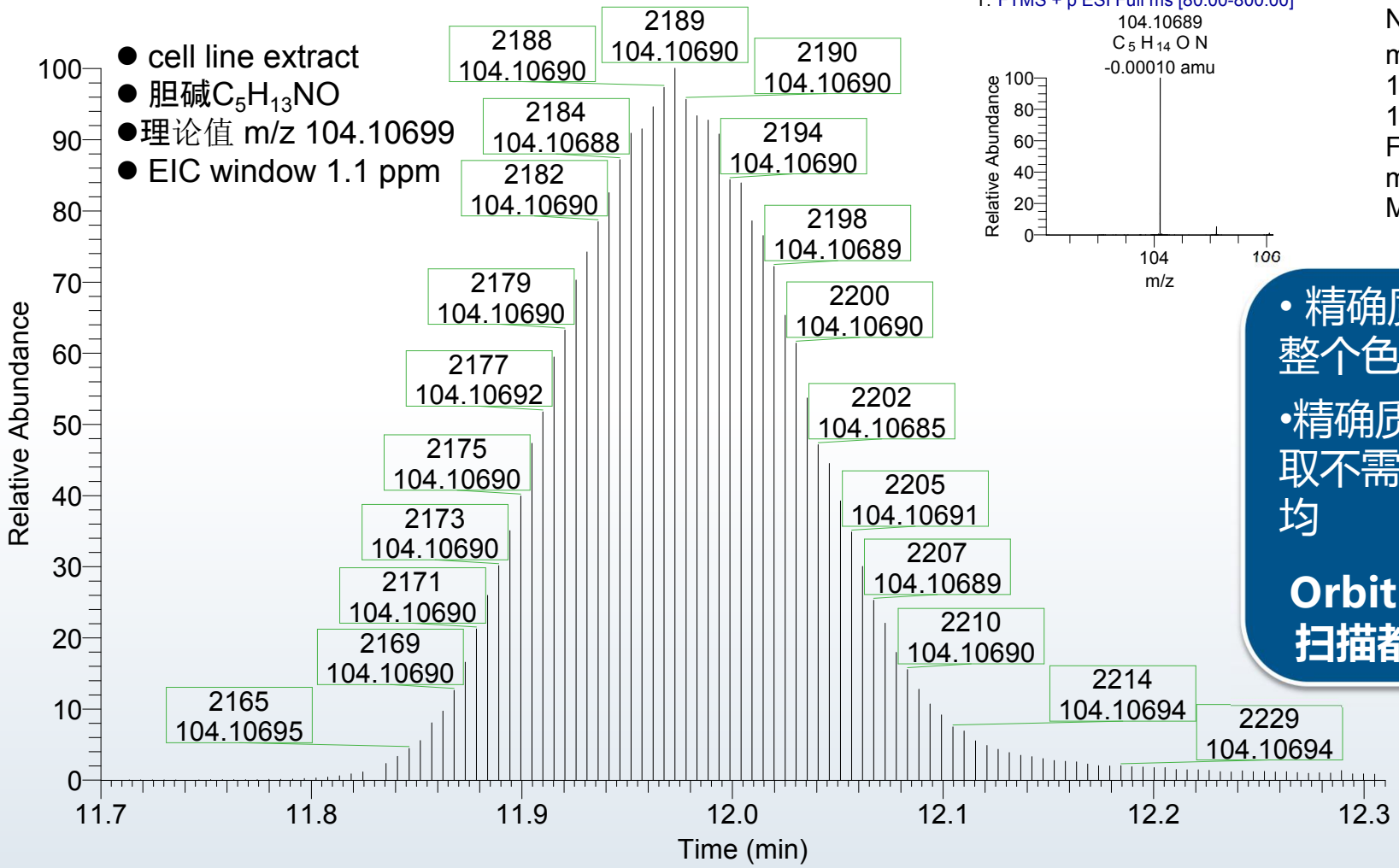
贯穿整个色谱峰的质量精度

External calibration data@70K resolution setting, **70 dots@20s base width**

RT: 11.70 - 12.31



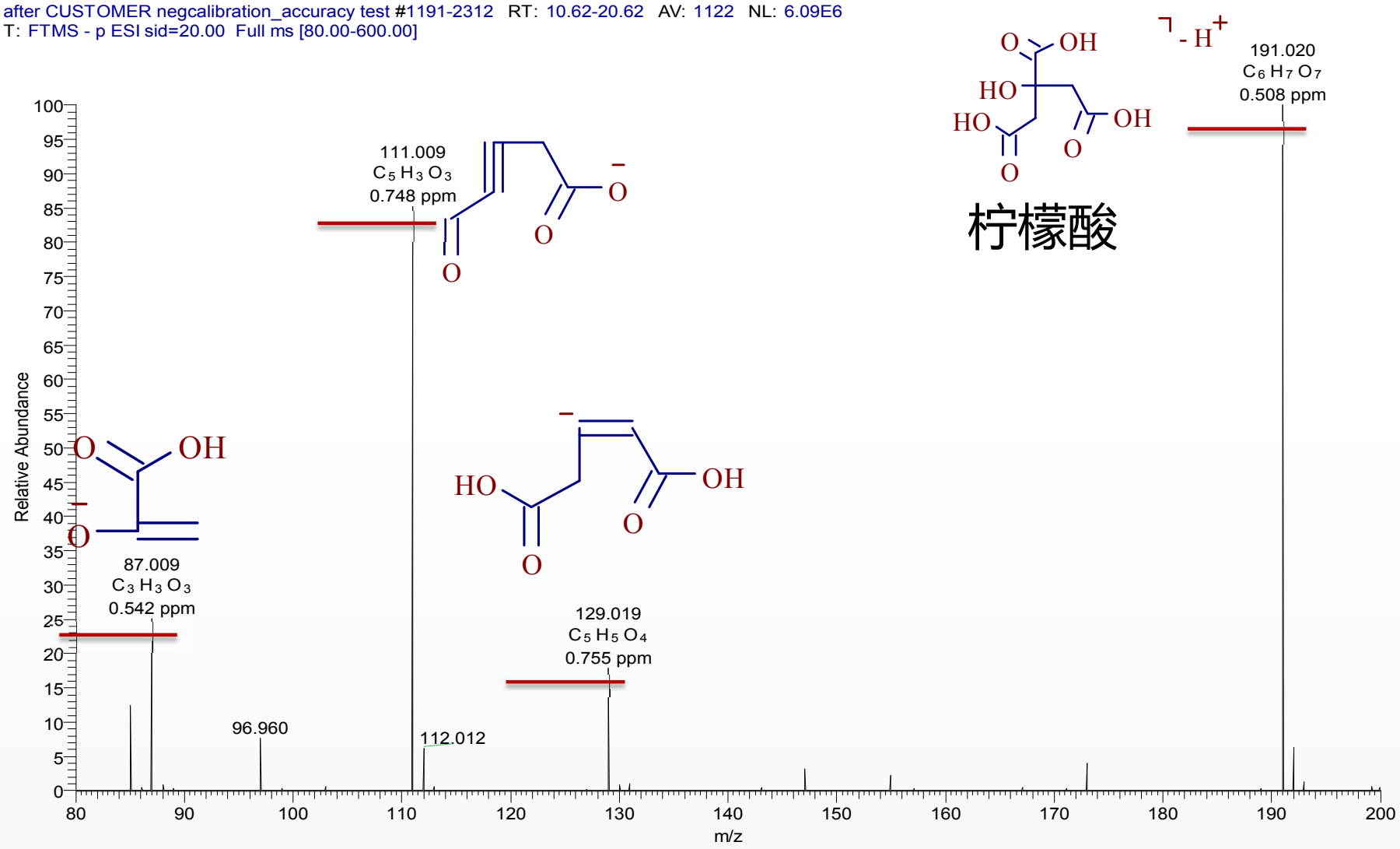
NL: 1.98E9
m/z= 104.10675-104.10697 F: FTMS + p ESI Full ms [80.00-800.00] MS esc-2

- 精确质量数贯穿整个色谱峰
- 精确质量数的获取不需要谱图平均

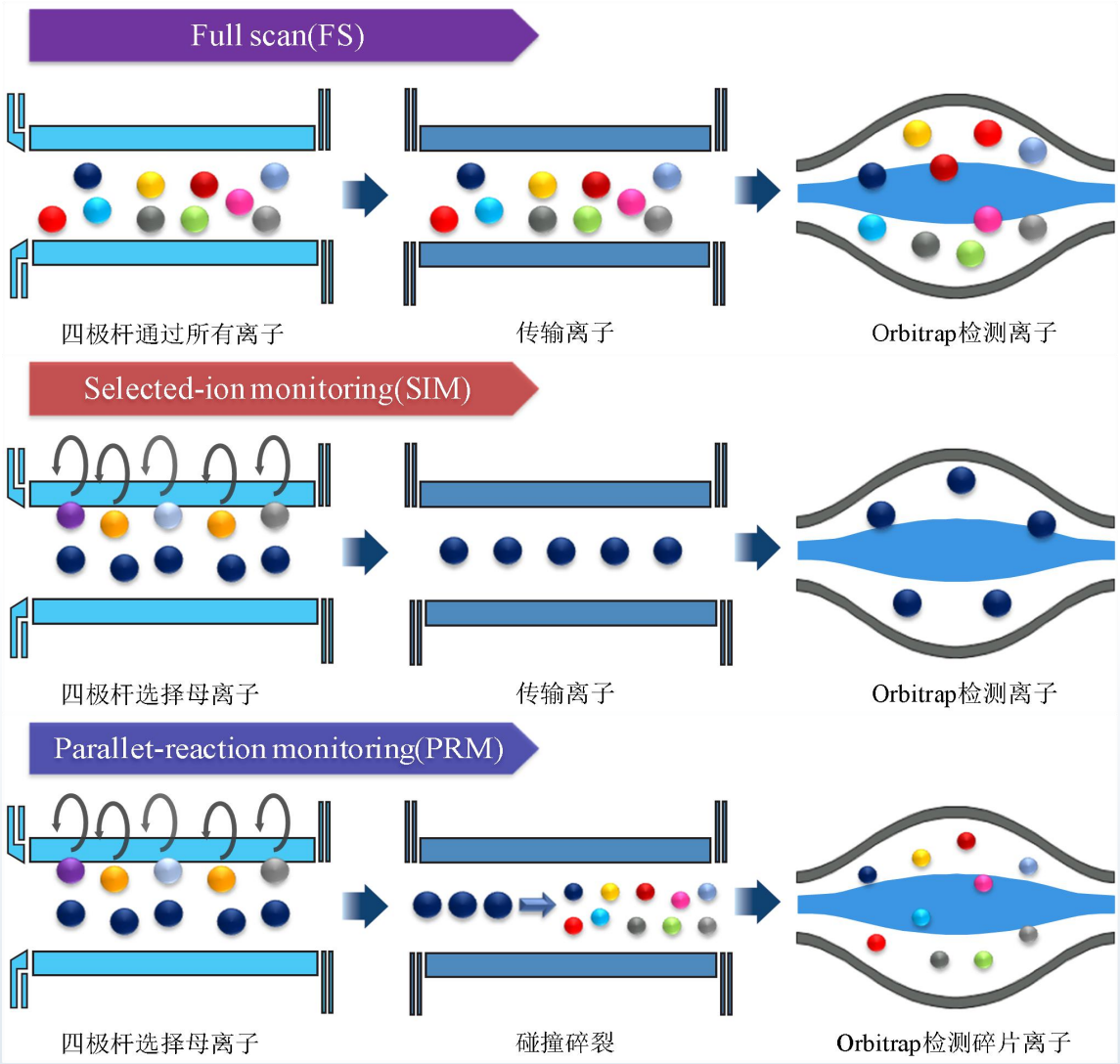
Orbitrap的每次扫描都值得信赖

ThermoFisher SCIENTIFIC

二级质谱质量精度



灵活多样的高分辨定量模式



全扫描 (FullScan)定量

◆通用性

◆数据回溯分析

选择离子检测(SIM)定量

◆ SIM-SIM (Todd Zhou)

◆四极杆过滤干扰离子

◆Ctrap富集目标离子，更高的灵敏度

平行反应检测（PRM）

◆高分辨子离子定量

◆更高的专属性

高分辨筛查

Calibration file last used:

	Enabled	Database Name	
1	☑	China-Toxicology-China-20150327-step2	open
2	☐	11	open
3	☐	111	open
4	☐	12	open
5	☐	4500	open
6	☐	4500_endogenous_metabolites	open
7	☐	Benzodiazepines Example Database	open
8	☐	China-Toxicology-China-20150327-step3	open
9	☐	China-Toxicology-China-20150327	open
10	☐	China-Toxicology	open
11	☐	ChinaEFS_Verterinary Drugs@TSQ Endura	open
12	☐	DefaultGC	open
13	☐	DefaultLC	open
14	☐	EFS_Database	open
15	☐	EFS_HRAM_Compound_Database	open
16	☐	EFS_TOX_China	open
17	☐	GB-Pesticide_tSRM@TSQ Endura-RT	open
18	☐	GB-Pesticide_tSRM@TSQ Endura	open
19	☐	jinyong-neg	open
20	☐	jinyong-pos	open
21	☐	Quantiva for EFS pesticide China	open
22	☐	Quantiva for EFS Veterinary drug-negativ	open
23	☐	Quantiva for EFS Veterinary drug-positive	open

Peaks ☑ *m/z*

Threshold Override ☐ 5,000

S/N Ratio Threshold 5.0

Mass Tolerance: 5.00 ppm

一级精确质量数

Retention Time ☐ Identify ☑ Confirm

Ignore if Not Defined ☑

Window Override (sec) ☑ 30

保留时间

Fragment Ions ☐ Identify ☑ Confirm

Ignore if Not Defined ☑

Min. # of Fragments 1

Intensity Threshold 10,000

Mass tolerance 5.00 ppm

碎片离子匹配

Isotopic Pattern ☐ Identify ☑ Confirm

Fit Threshold (%) 90

Allowed Mass Deviation (ppm) 5

Allowed Intensity Deviation (%) 10

Use Internal Mass Calibration ☐

同位素

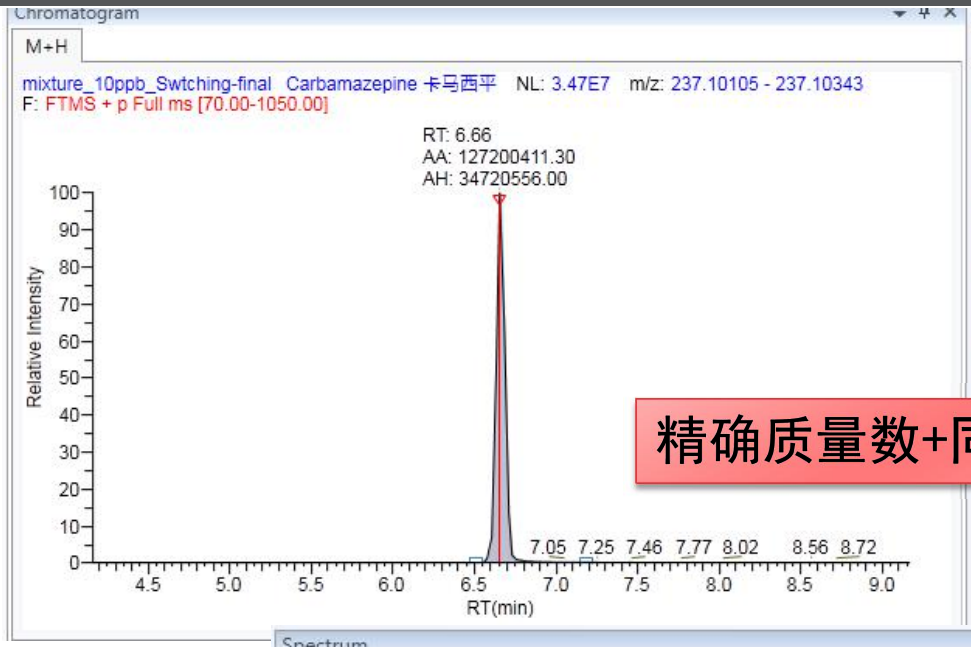
Library Search ☐ Identify ☑ Confirm

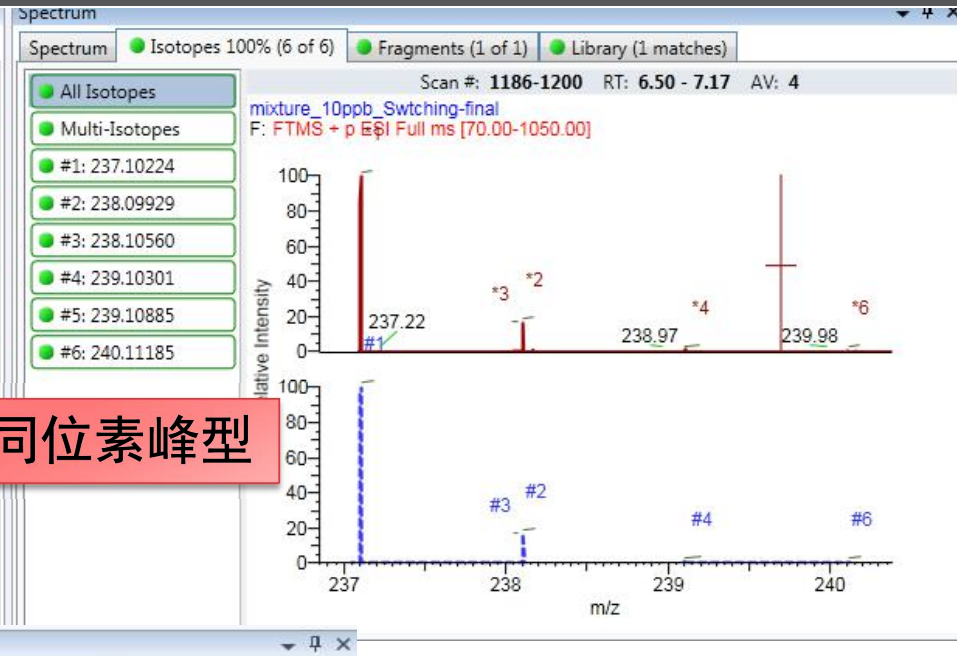
Library Search Type: Library Manager

Score Threshold (%) 80

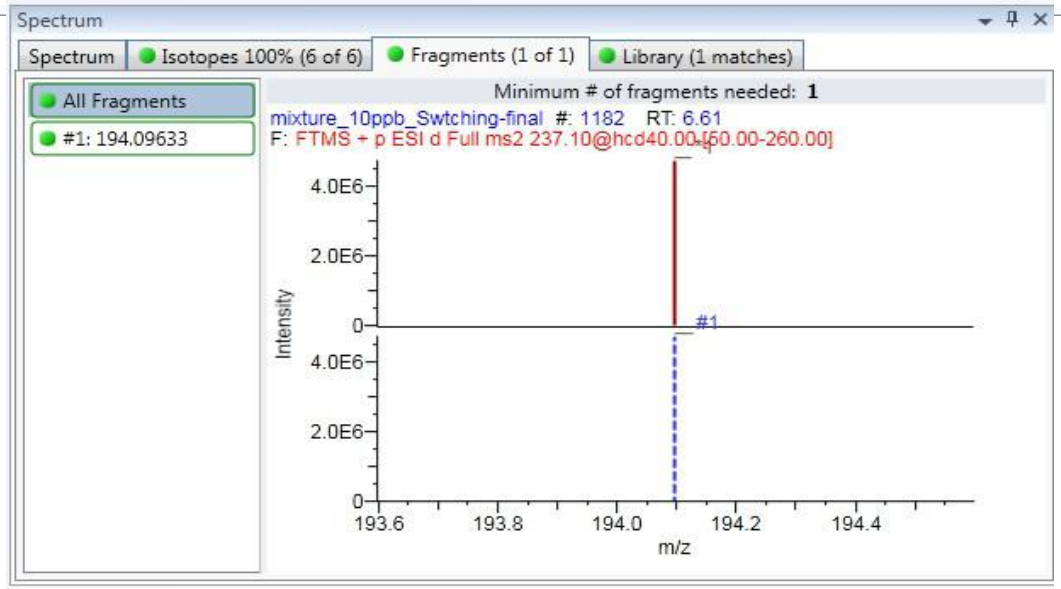
二级谱图匹配

高分辨筛查





精确质量数+同位素峰型



碎片离子

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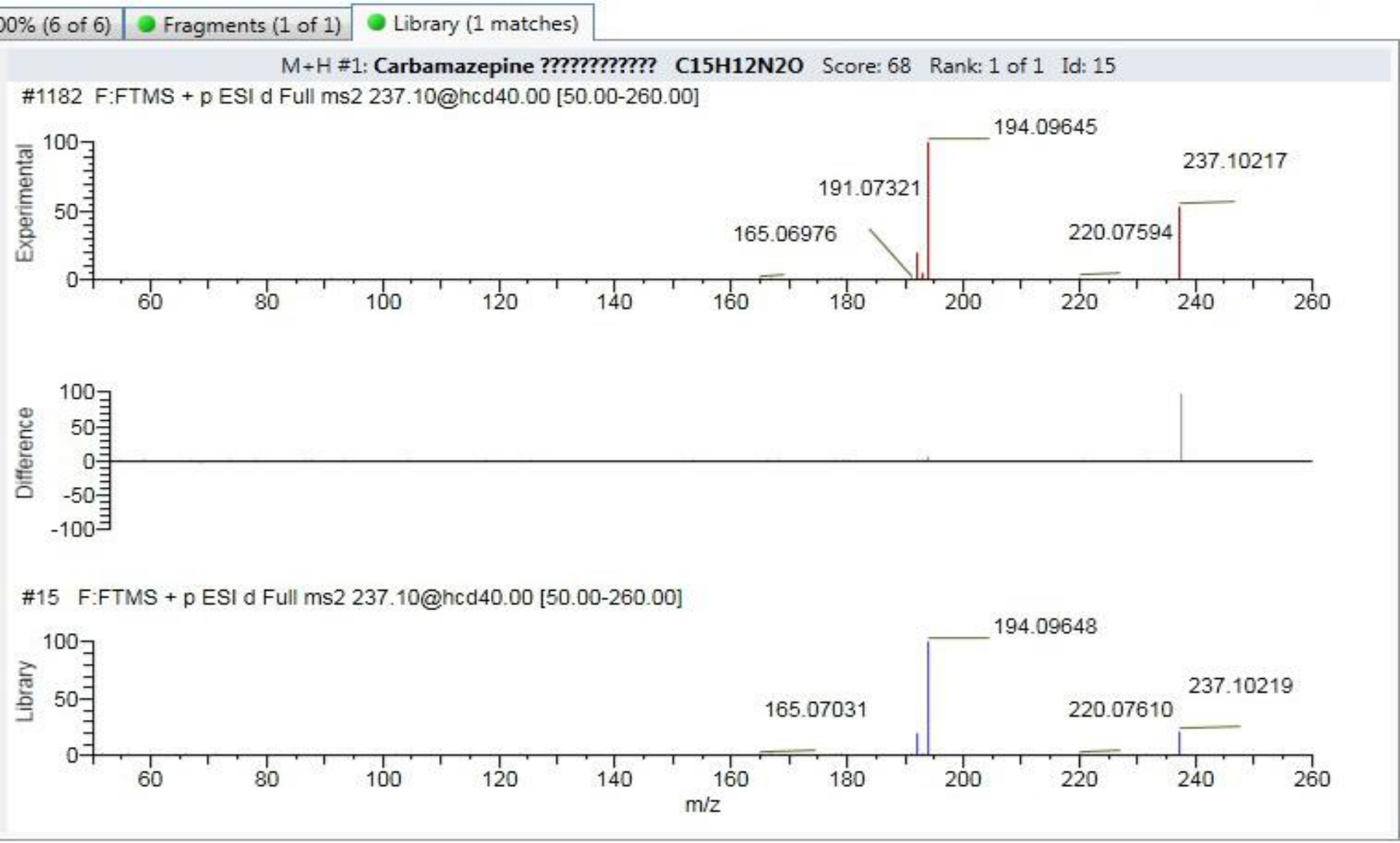
高分辨筛查



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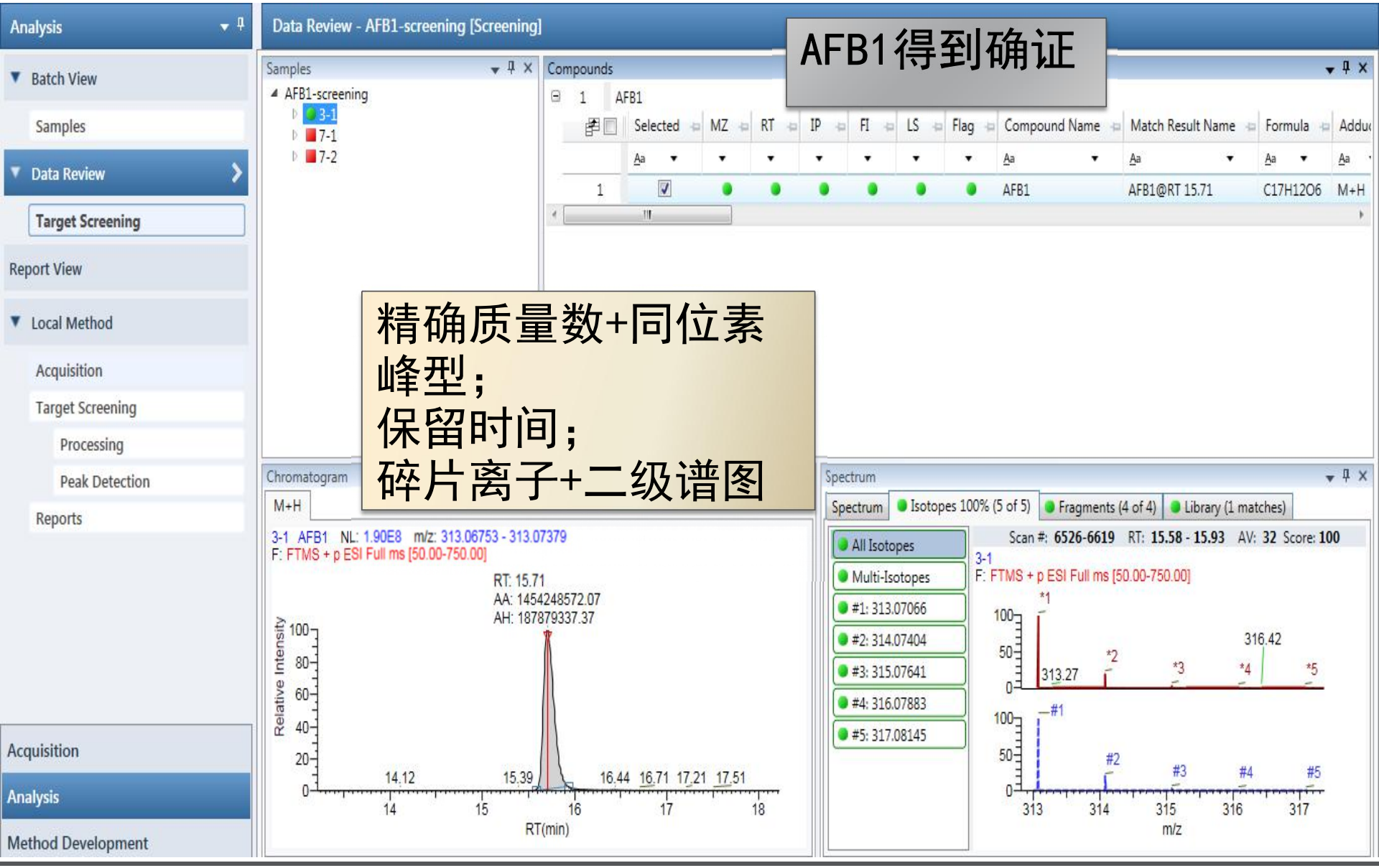
高分辨筛查



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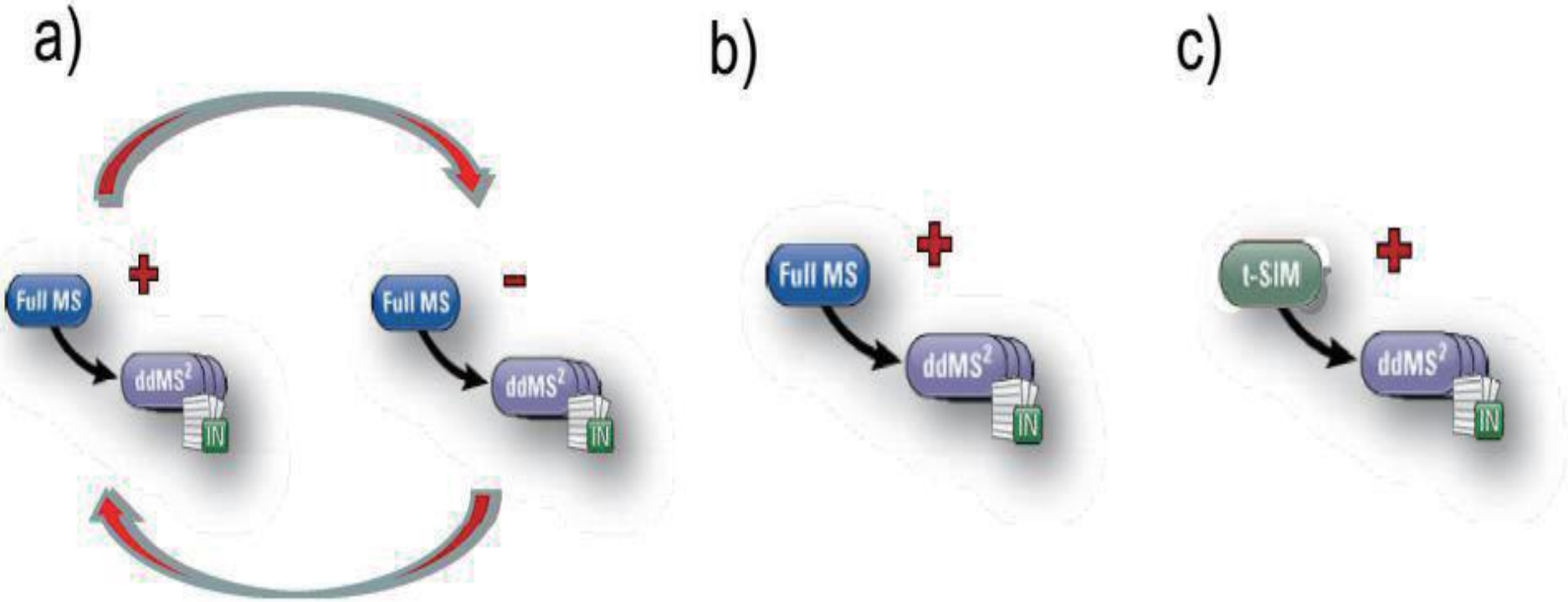
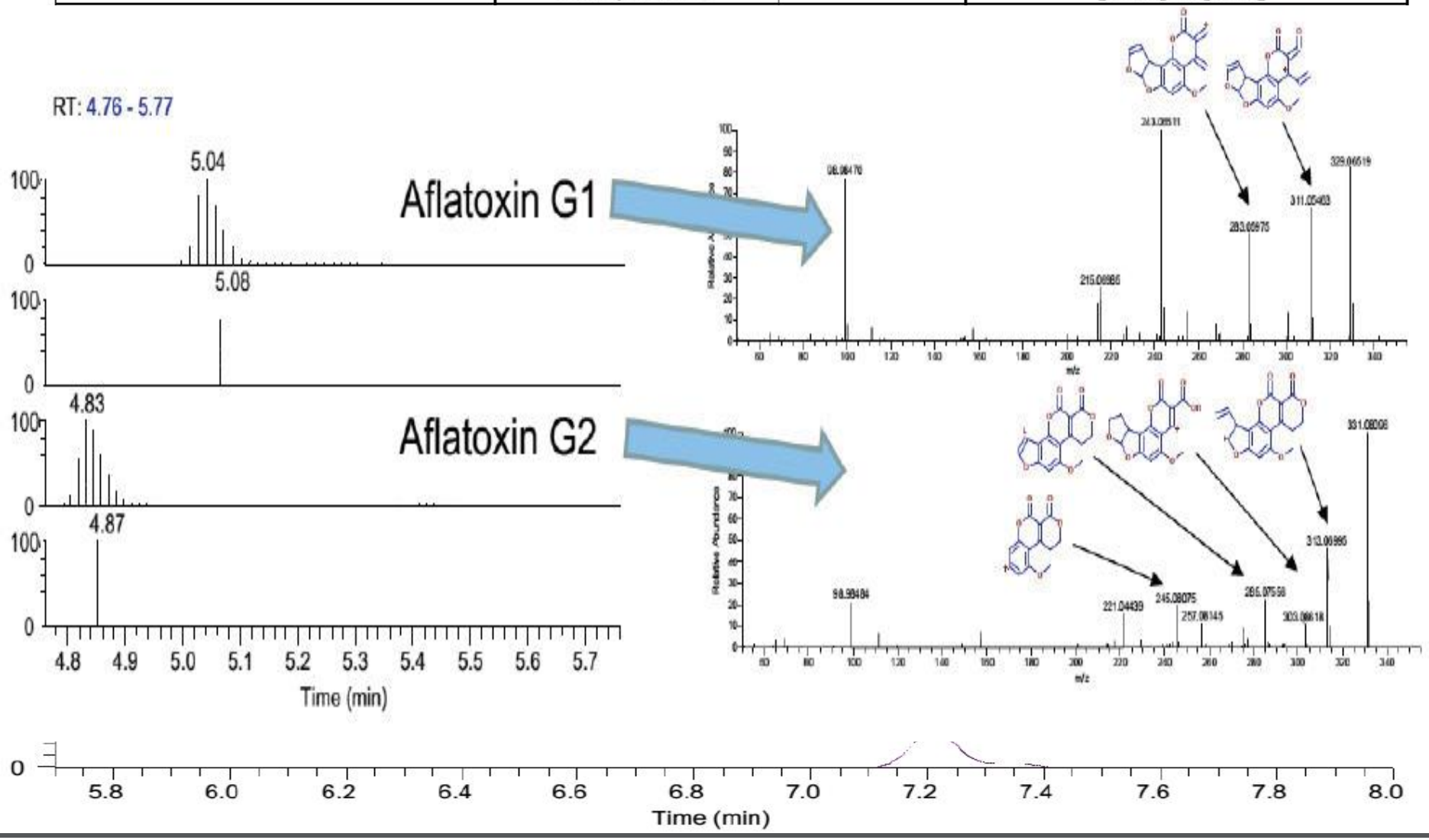
同时定性定量分析



a) 快速正负切换的筛查方法
b) Full MS 定量方法
c) t-SIM 定量方法

同时定性定量分析

Compound Name	Elemental Composition	Polarity	Exact Mass [M+H]+ / [M-H]-



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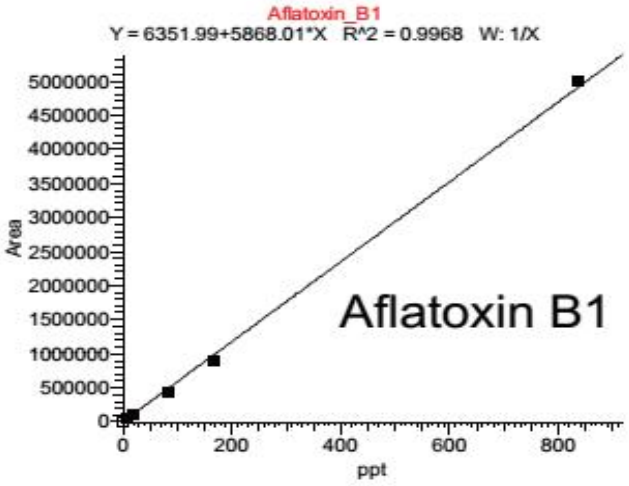
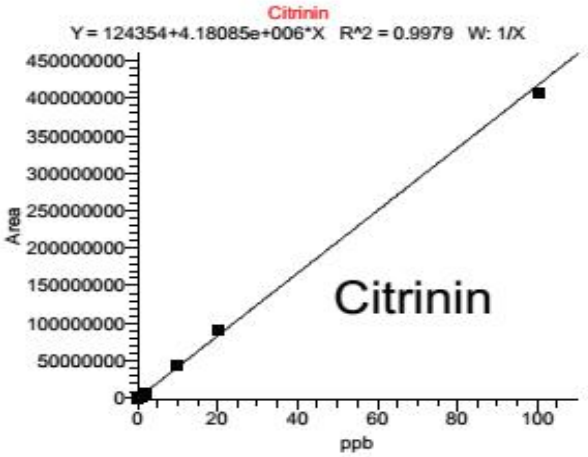
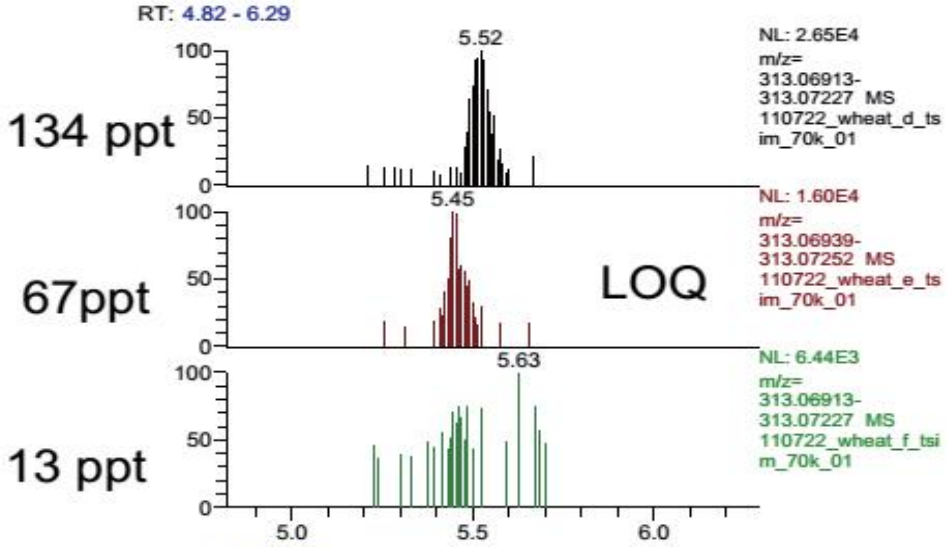
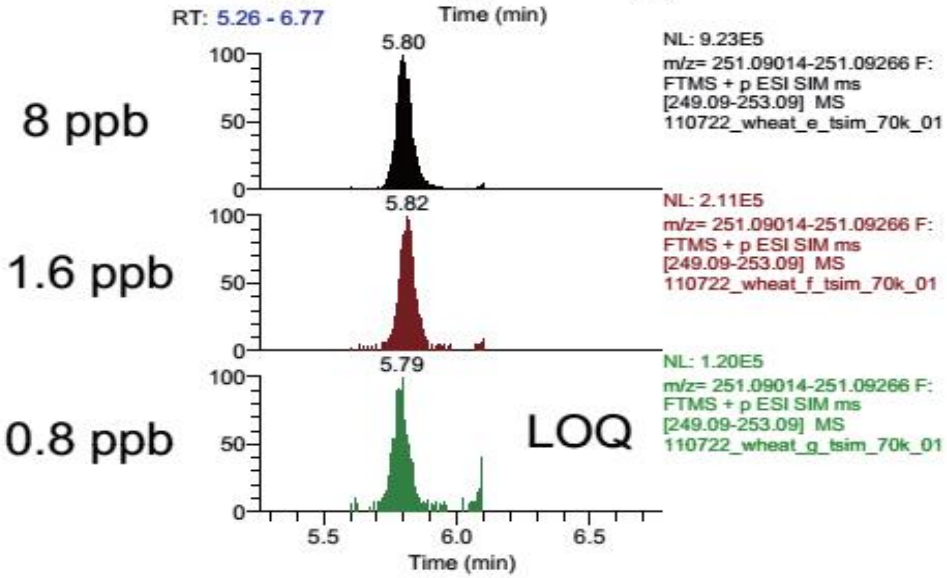
同时定性定量分析









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