



ThermoFisher
S C I E N T I F I C

液质联用技术在真菌毒素检测中的应用

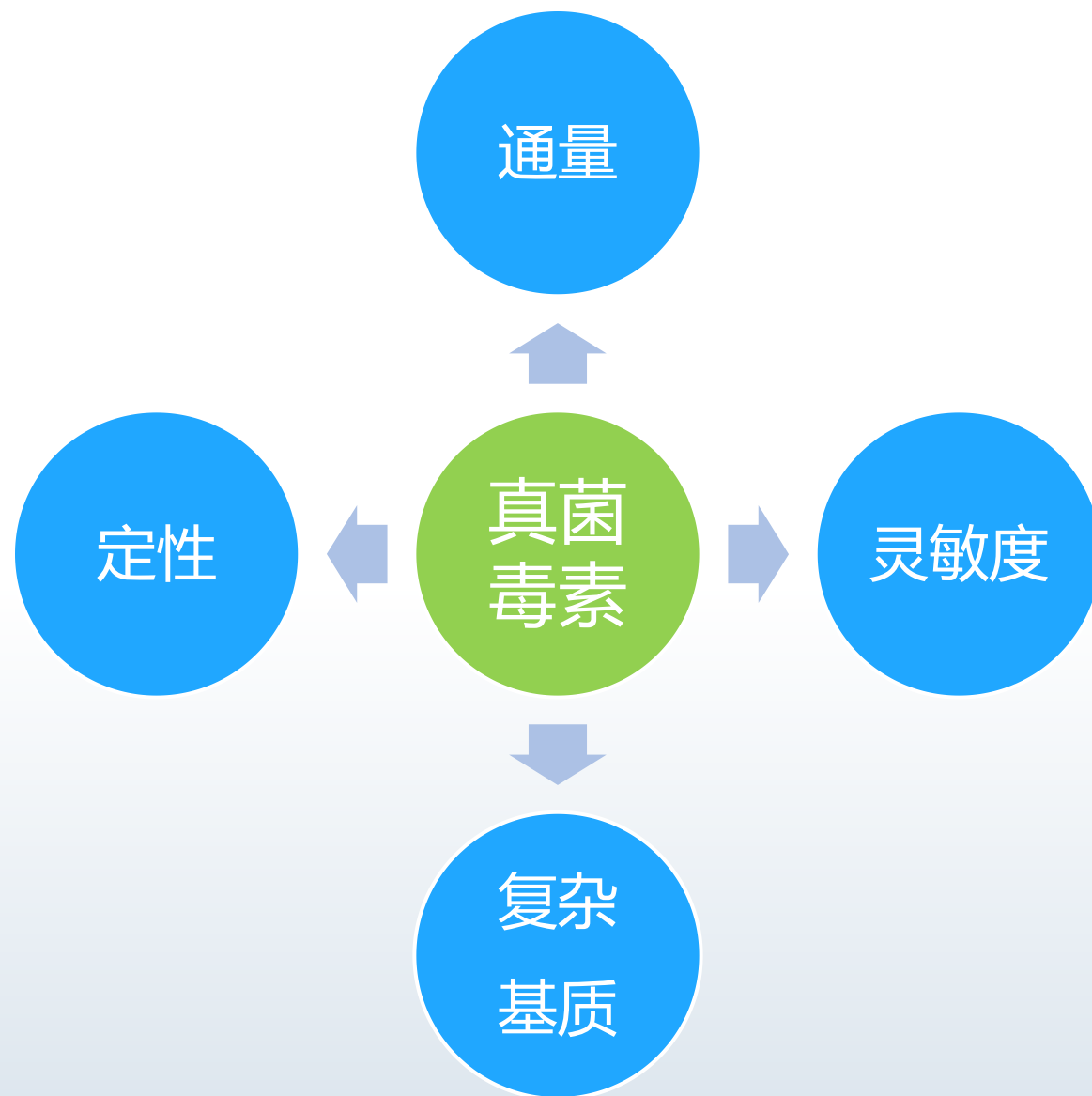
色谱质谱部 应用工程师
祝翔

xiang.zhu@thermofisher.com

2017-9-21

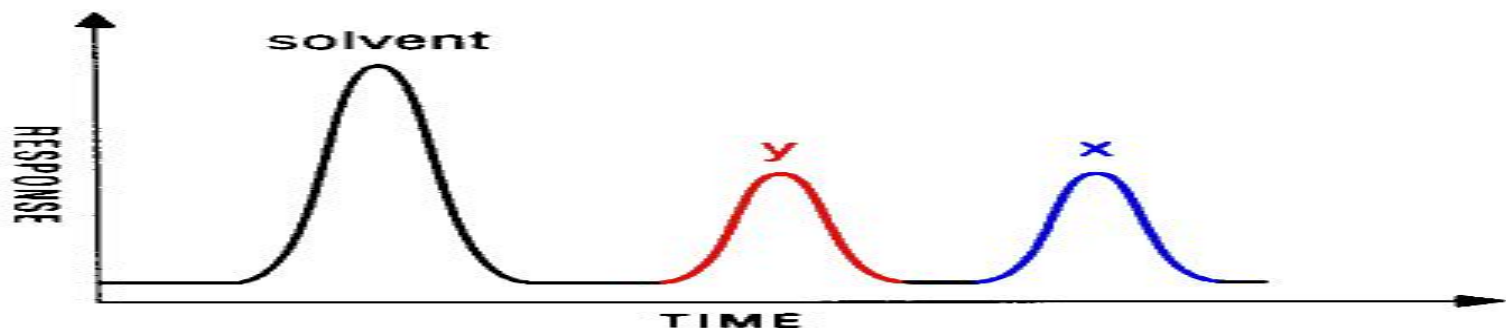
The world leader in serving science

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

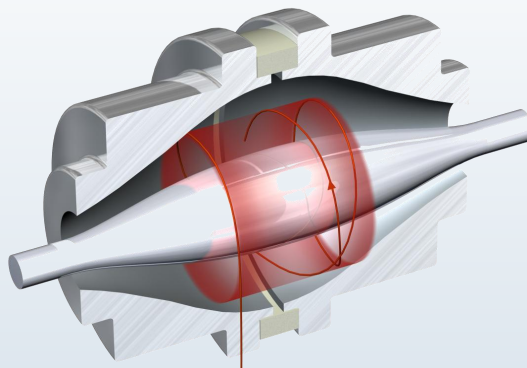
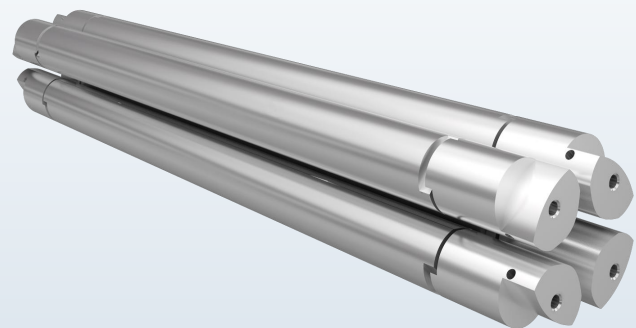
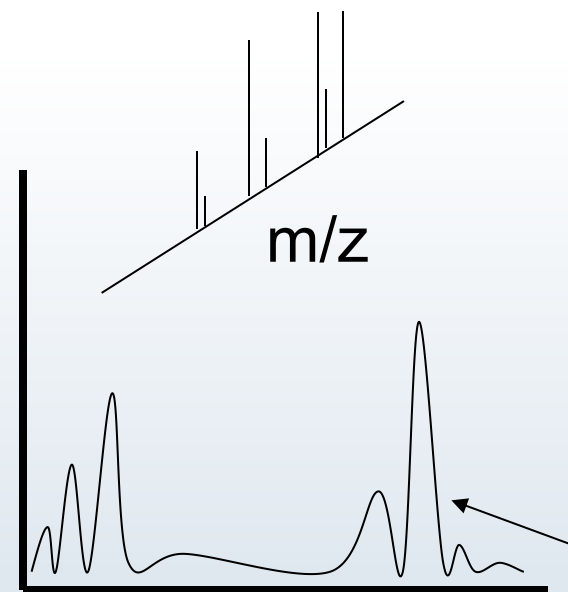


➤ 质谱检测器的选择性强

- 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



样品前处理

目标物分析

定量



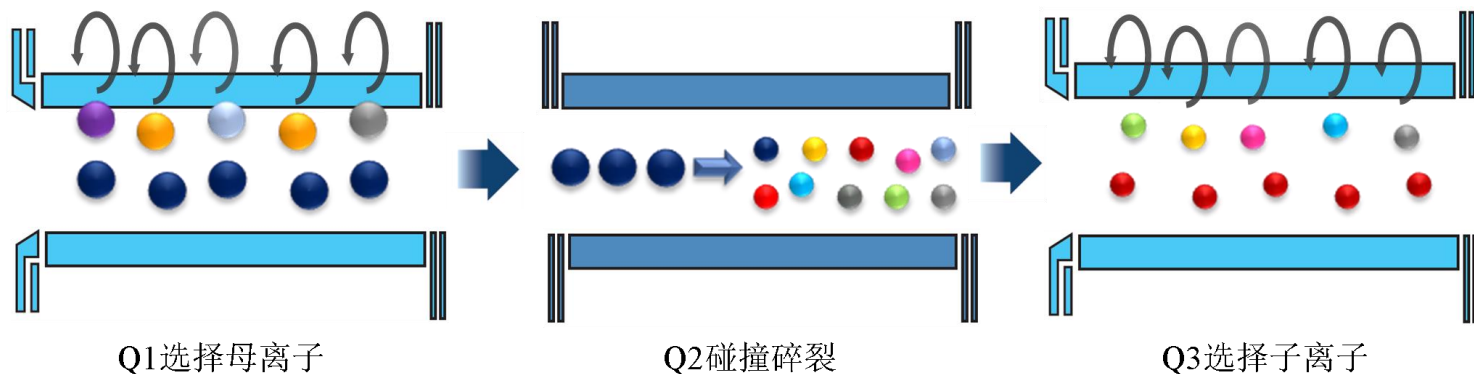
非目标物分析

定性

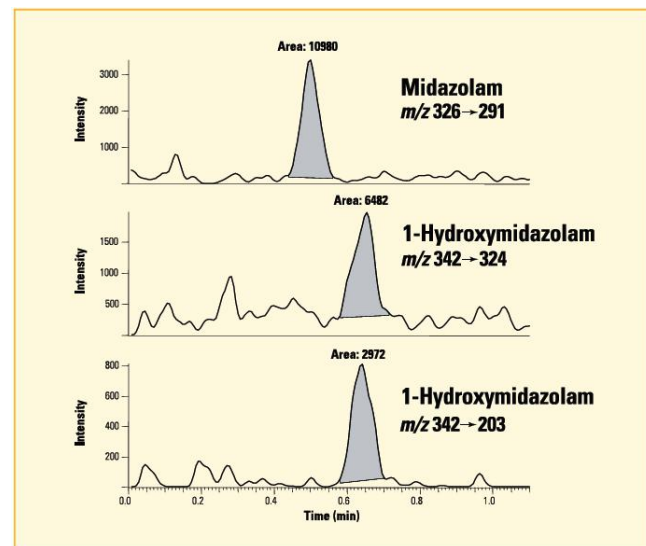


三重四级杆质谱定量原理

Selective Reaction Monitoring(SRM)



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

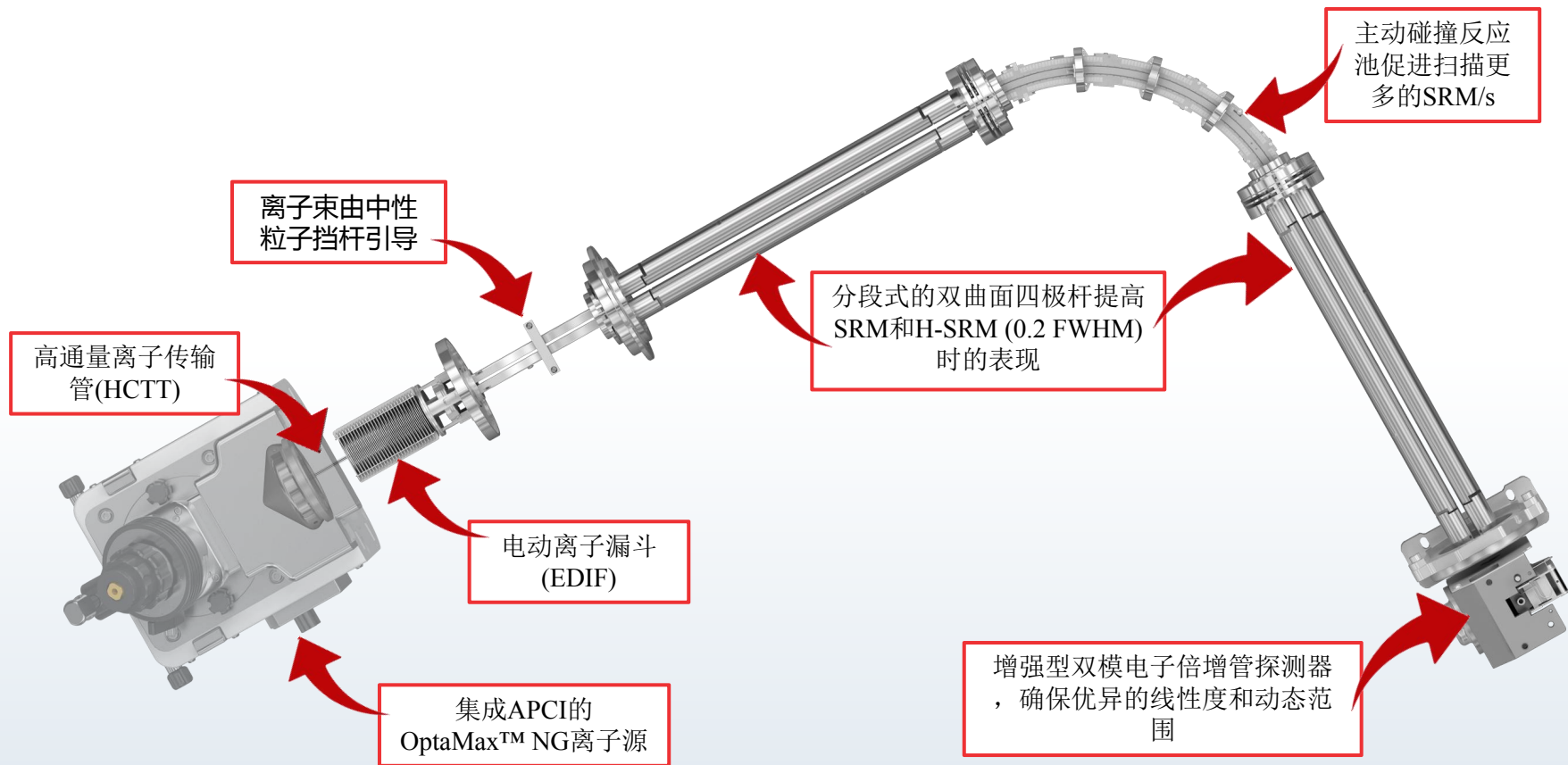


- 灵敏度高，选择性好，通量高
- 目标化合物定量金标准
- 准确性、耐用性、灵敏性

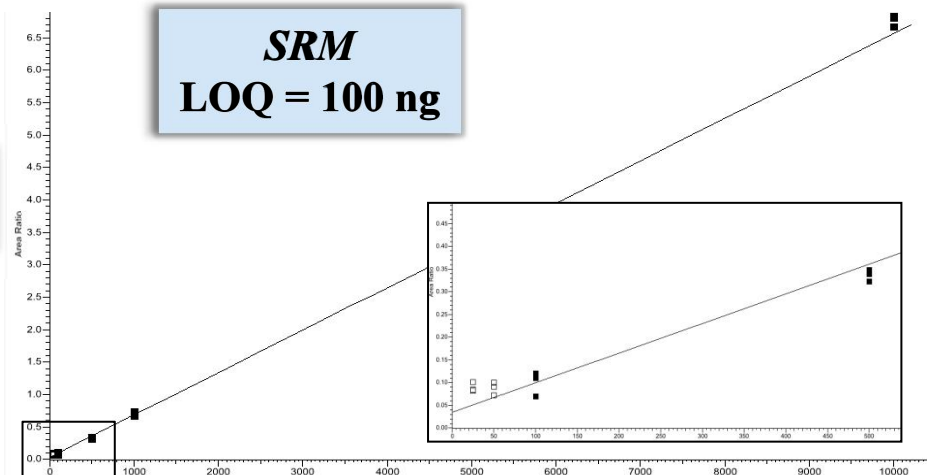
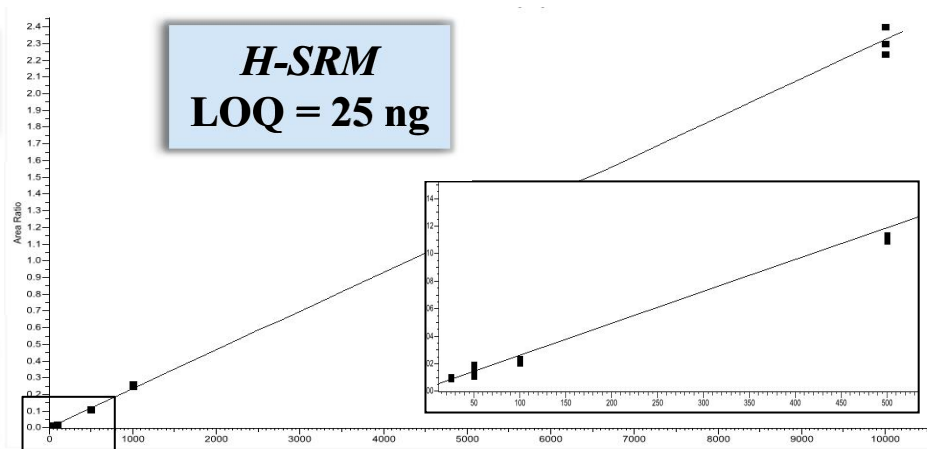
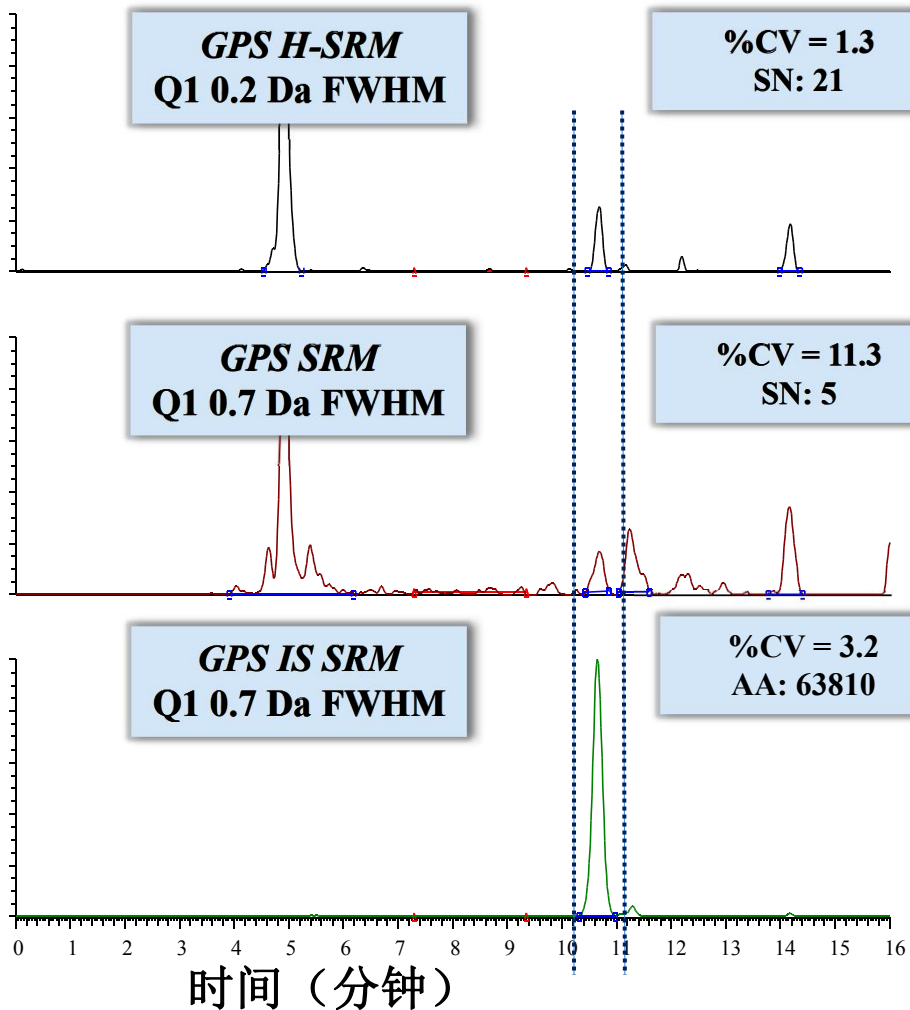
TSQ Altis三重四极杆质谱仪：AIM+技术带来的关键优势

AIM+
TECHNOLOGY

Active Ion Management Plus (AIM+) - 精密设计从Thermo Scientific OptaMax™ 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



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

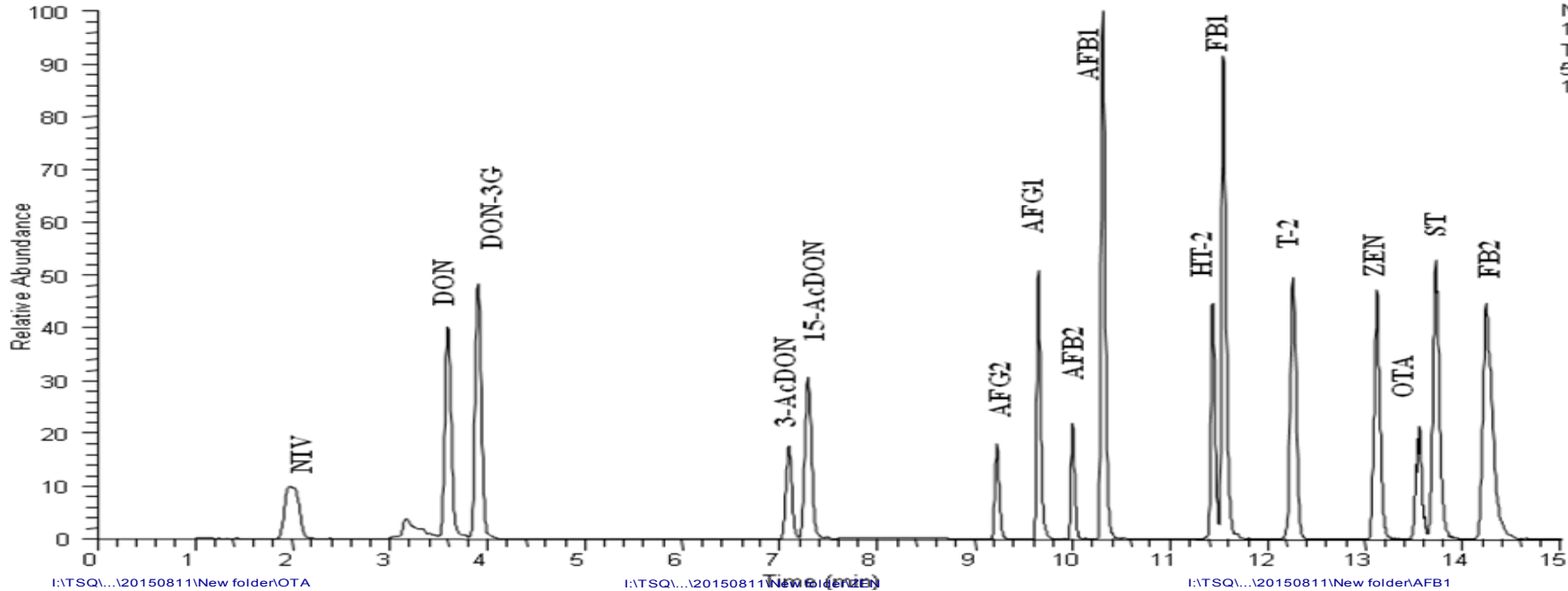
A: 甲醇

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

RT: 0.00 - 15.00 SM: 15G



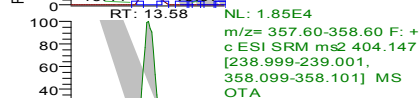
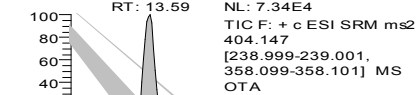
NL:
1.37E5
TIC MS
5P3-PURE-
1

I:\TSQL...\20150811\New folder\OTA

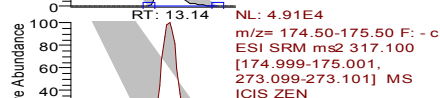
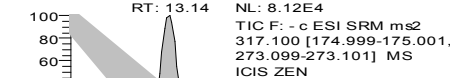
I:\TSQL...\20150811\New folder\ZEN

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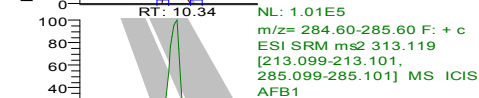
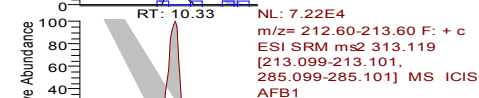
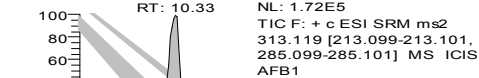
RT: 12.89 - 14.24 SM: 7B



RT: 12.56 - 13.52 SM: 7B



RT: 9.54 - 10.99 SM: 7B



Eurofins: Confirmation

Positive Sample:

=> Mebendazole-2-amine (氨基甲苯哒唑)

Same Sample by FS-ddMS (HR-MS)

=> negative

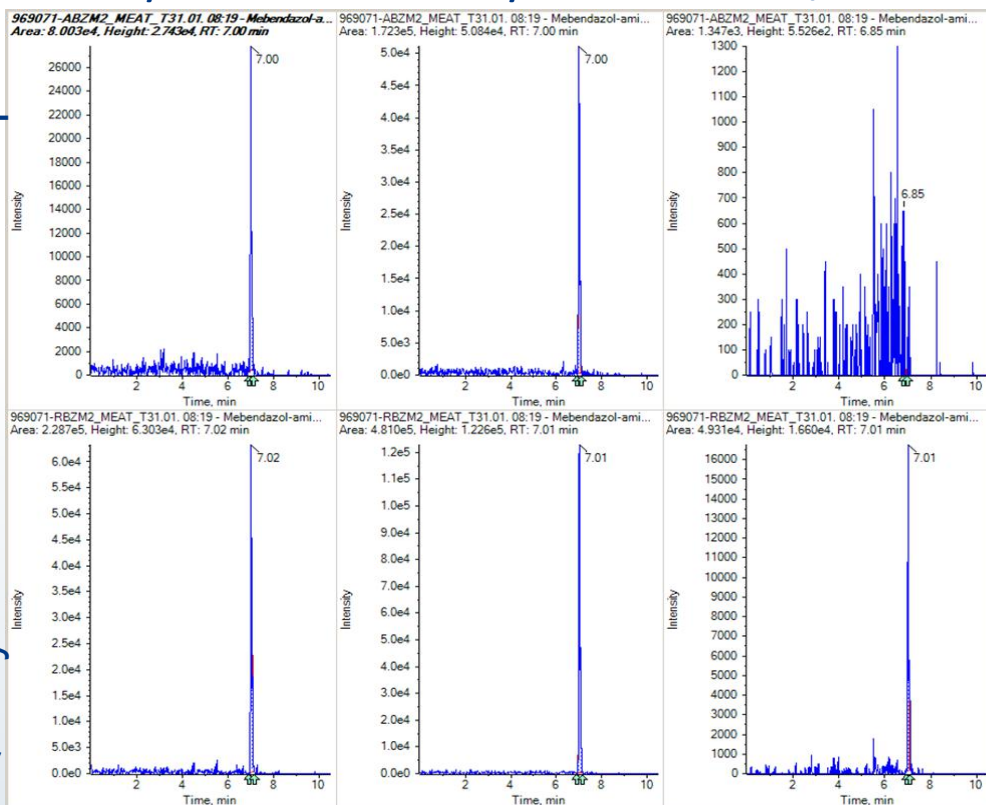
HR-MS resolution 70 000

Unknown sample
Quality control

238/76

238/105

238/133



Compounds				
Target Screening Results				
	Flag	Compound Name	Match Result Name	Formula
	Aa	Marbofloxacin	Marbofloxacin@RT 4.15	C17H19FN4O4
	Aa	Mebendazol	Mebendazol@RT 6.18	C16H13N3O3
	Aa	Mebendazol-2-amin	Mebendazol-2-amin@RT 4.5	C14H11N3O
	Aa	Methylenblau	Methylenblau@RT 4.9	C16H17N3S

Commission Decision 2002/657/EC: fulfilled criteria

Eurofins: Confirmation

Screening: Full scan (resolution 70 000)

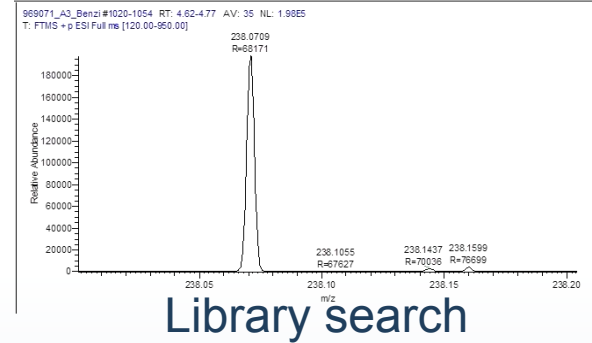
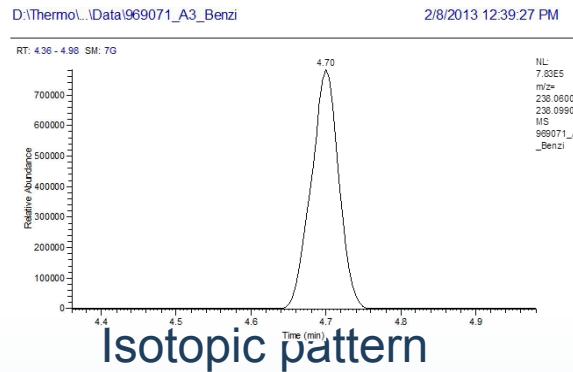
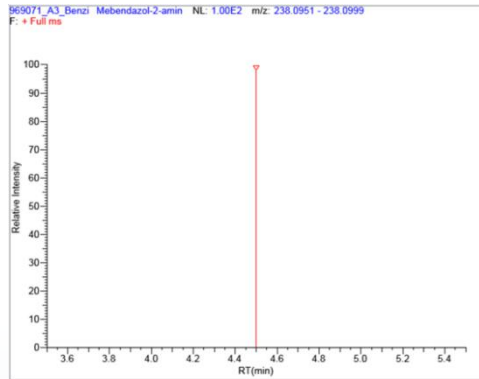
Data-dependent Fragmentation (resolution 17 500)

Mebendazole-2-amine: 238.0975 Da

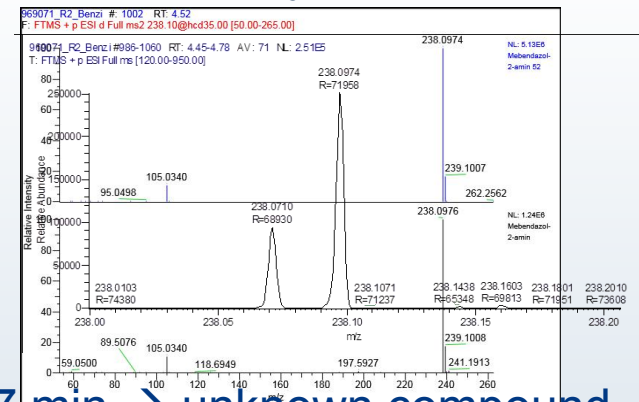
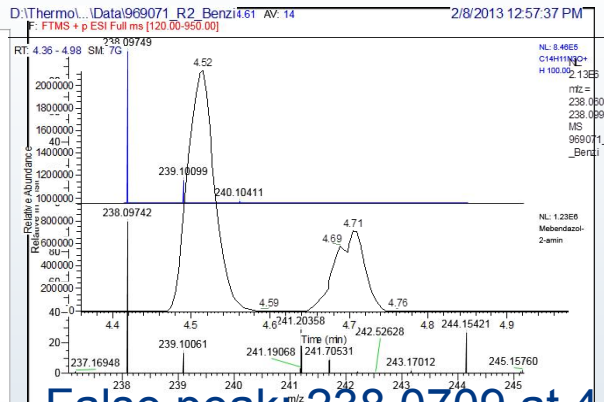
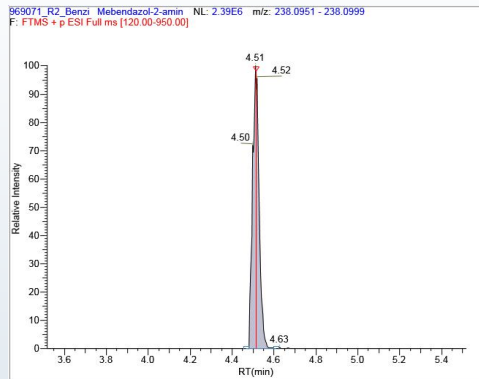
(氨基甲苯哒唑)

Xcalibur: mass ranges 238.06-238.0999

Unkown Sample

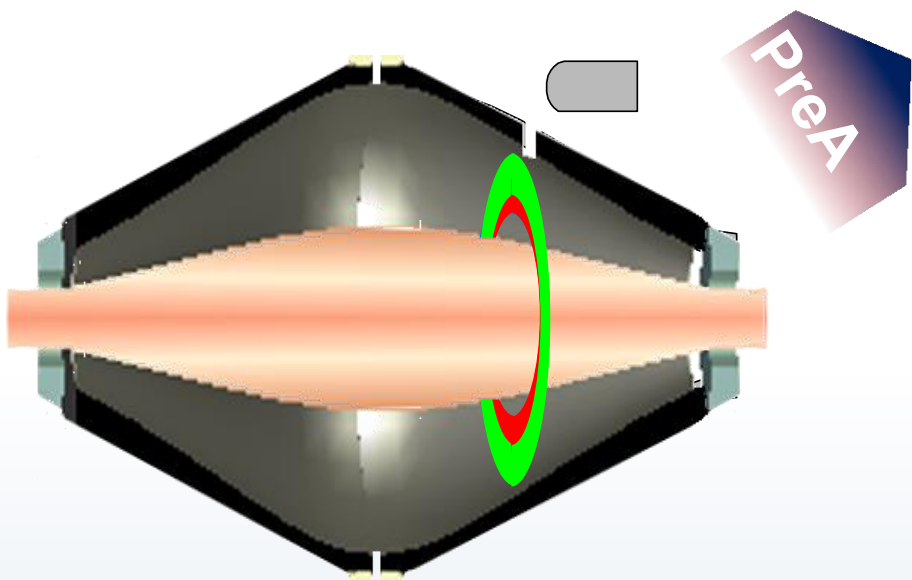


Quality control

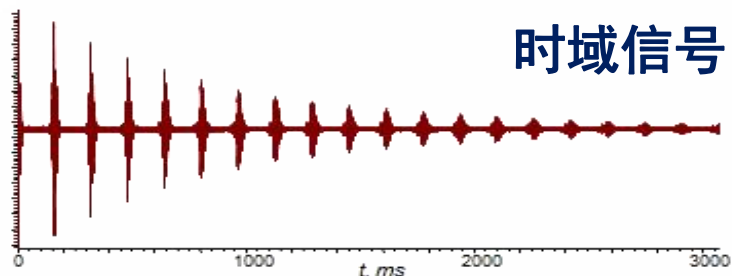


False peak: 238.0709 at 4.7 min → unknown compound

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。



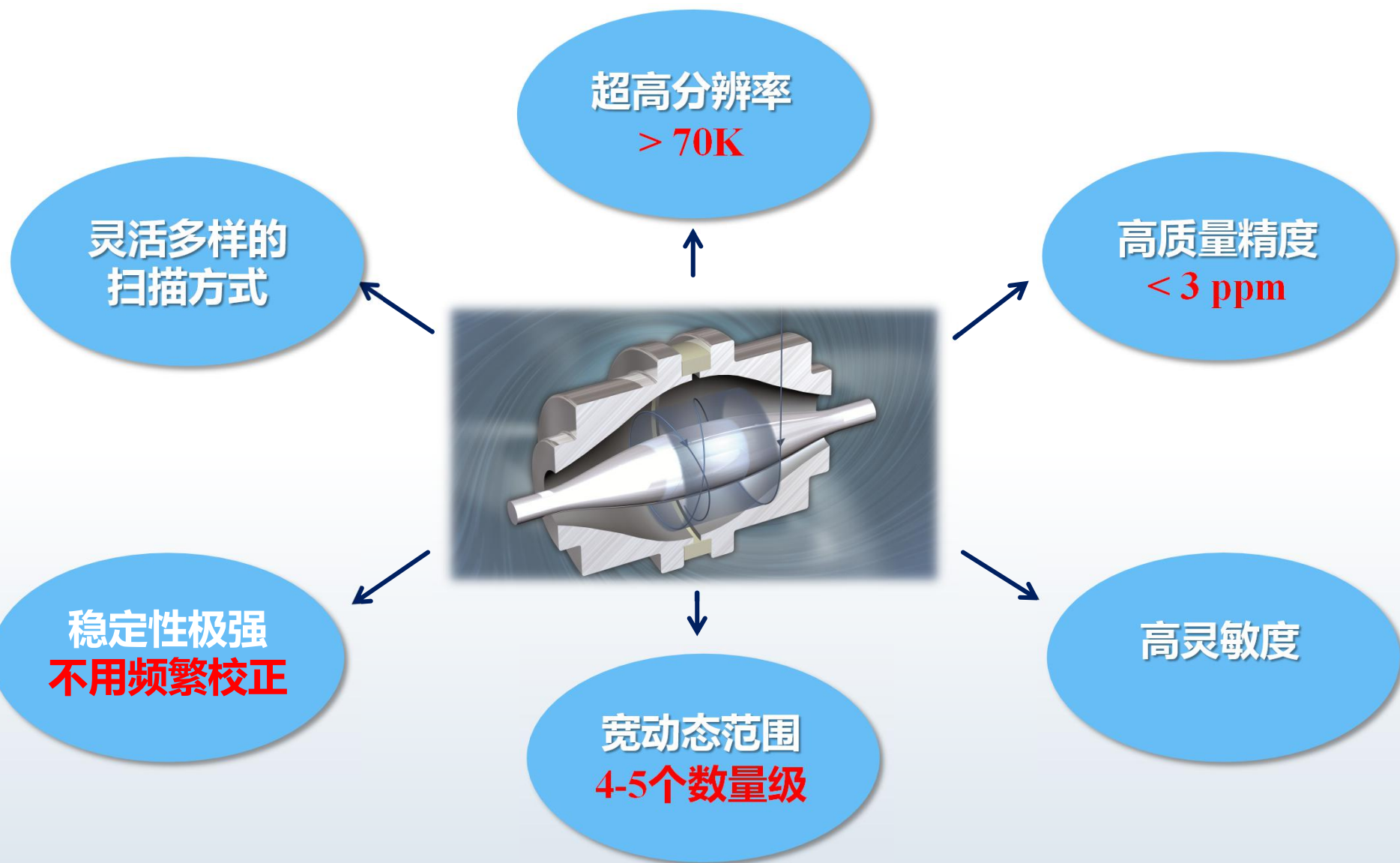
FT



$$\omega = \sqrt{\frac{k}{m/z}}$$



高分辨质谱性能特点

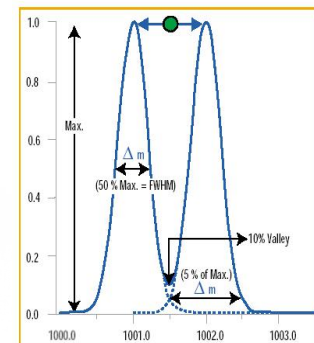


为什么需要高分辨

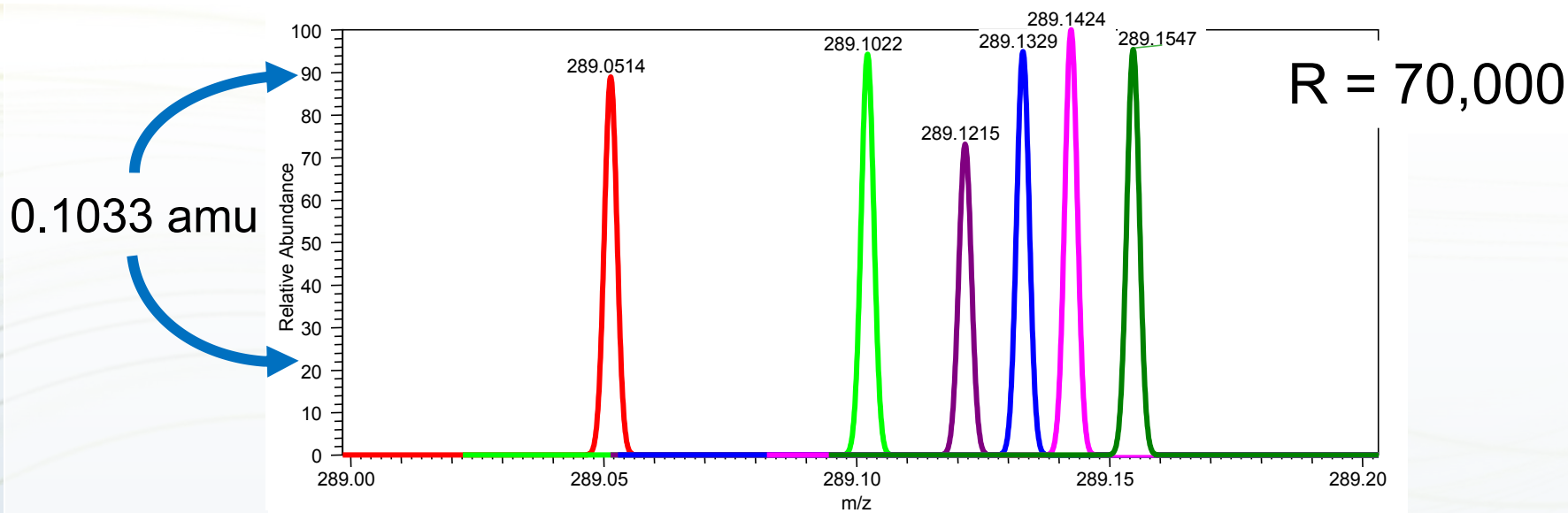
为什么需要
高分辨率？

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

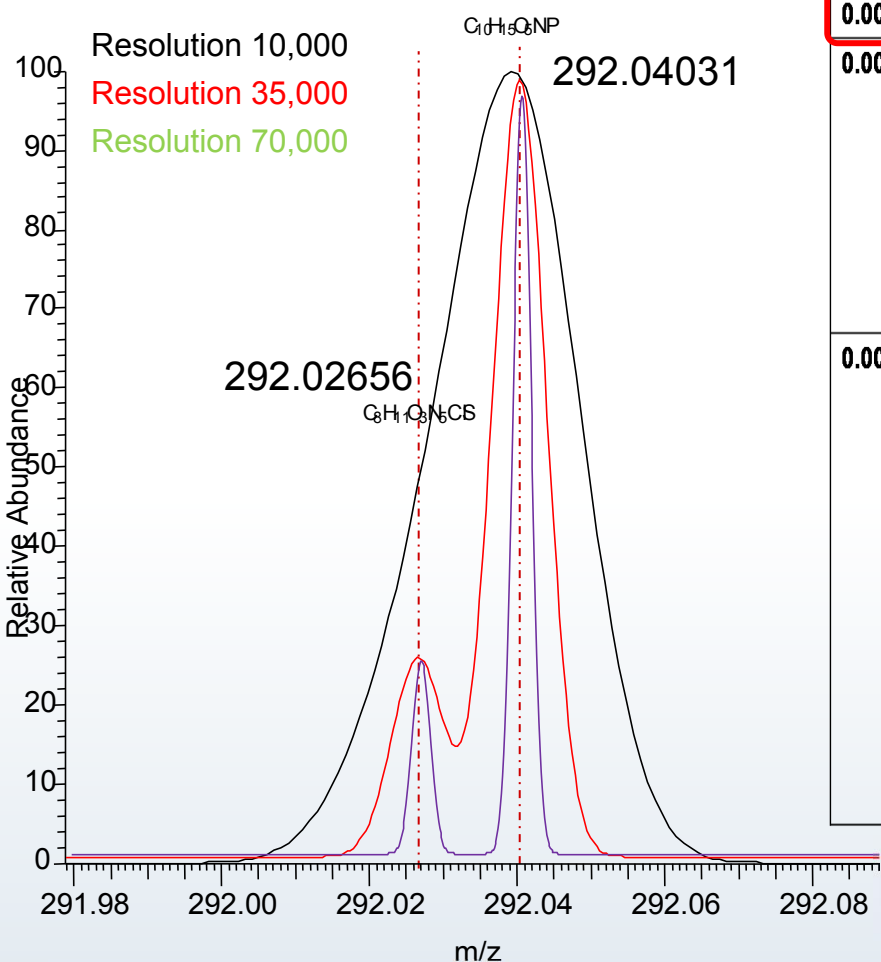


Yes, at high resolution !

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 mass	Delta (ppm)	Delta (mmu)	RDB	Composition
279.0913					
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

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

esc-2 #2165-2217 RT: 11.85-12.12
T: FTMS + p ESI Full ms [80.00-800.00]

NL: 1.98E9

m/z=

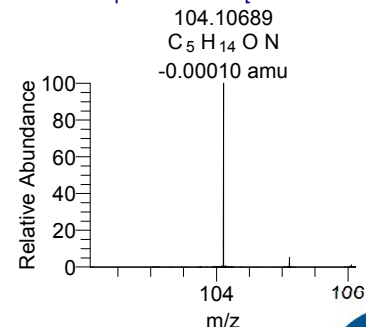
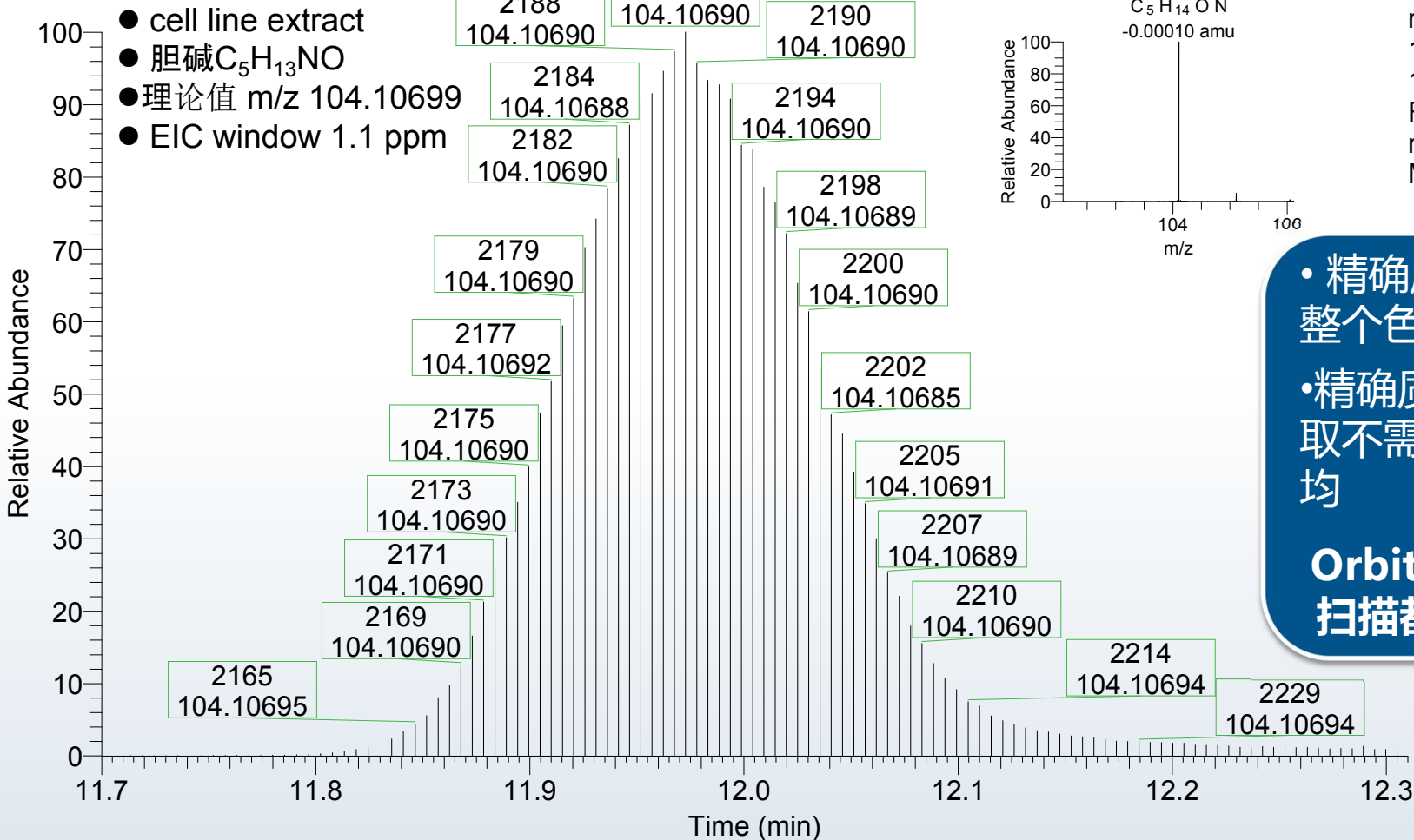
104.10675-

104.10697 F:

FTMS + p ESI Full

ms [80.00-800.00]

MS esc-2



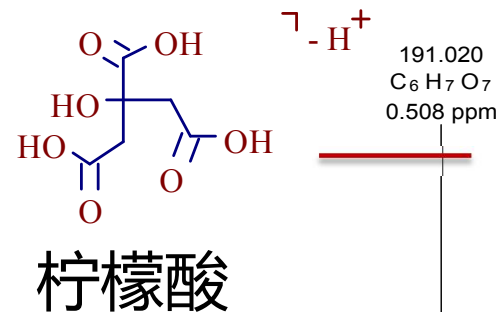
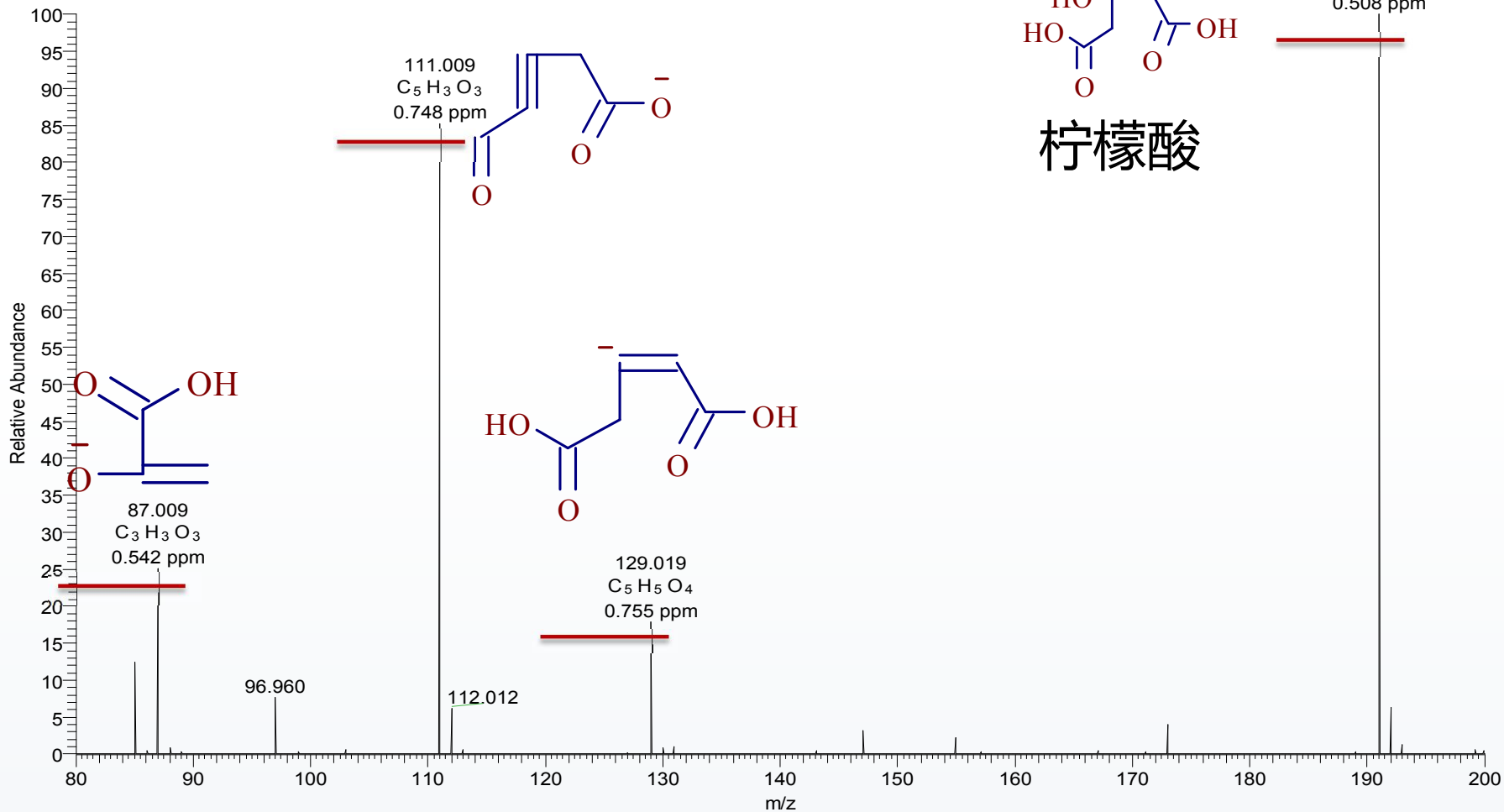
• 精确质量数贯穿整个色谱峰

• 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

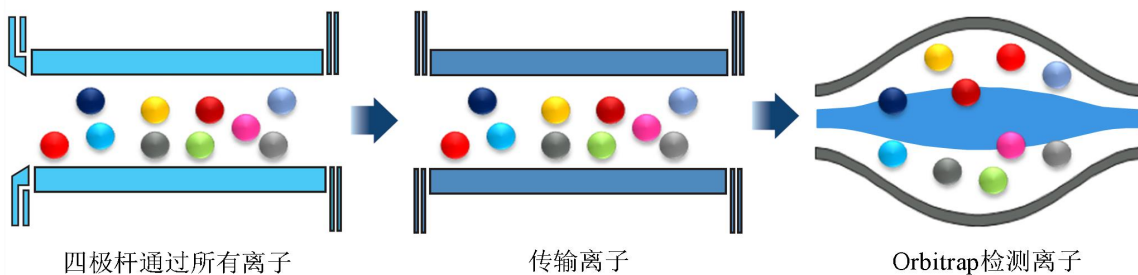
二级质谱质量精度

after CUSTOMER negcalibration_accuracy test #1191-2312 RT: 10.62-20.62 AV: 1122 NL: 6.09E6
T: FTMS - p ESI sid=20.00 Full ms [80.00-600.00]



灵活多样的高分辨定量模式

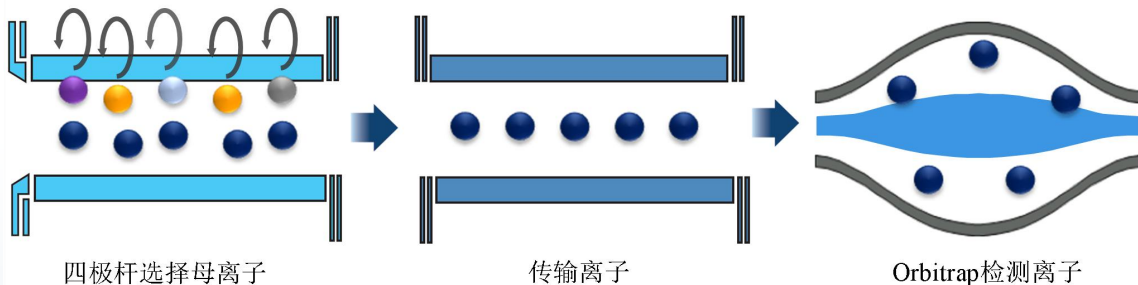
Full scan(FS)



全扫描 (FullScan)定量

- ◆通用性
- ◆数据回溯分析

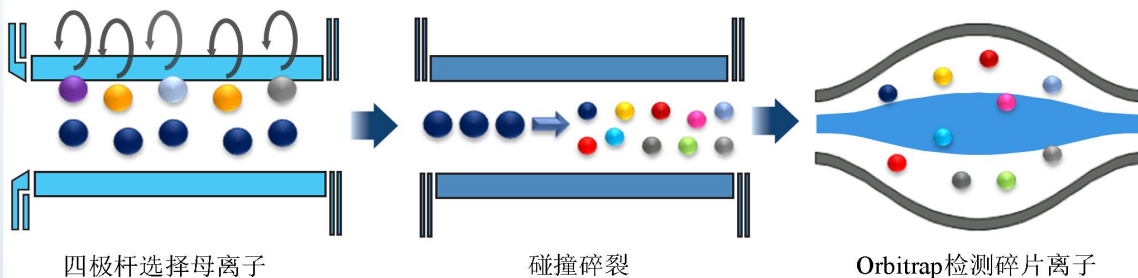
Selected-ion monitoring(SIM)



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

Parallel-reaction monitoring(PRM)



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	
<input checked="" type="checkbox"/>	China-Toxicology-China-20150327-step2	open
<input type="checkbox"/>	11	open
<input type="checkbox"/>	111	open
<input type="checkbox"/>	12	open
<input type="checkbox"/>	4500	open
<input type="checkbox"/>	4500_endogenous_metabolites	open
<input type="checkbox"/>	Benzodiazepines Example Database	open
<input type="checkbox"/>	China-Toxicology-China-20150327-step3	open
<input type="checkbox"/>	China-Toxicology-China-20150327	open
<input type="checkbox"/>	China-Toxicology	open
<input type="checkbox"/>	ChinaEFS_Verterinary Drugs@TSQ Endura	open
<input type="checkbox"/>	DefaultGC	open
<input type="checkbox"/>	DefaultLC	open
<input type="checkbox"/>	EFS_Database	open
<input type="checkbox"/>	EFS_HRAM_Compound_Database	open
<input type="checkbox"/>	EFS_TOX_China	open
<input type="checkbox"/>	GB-Pesticide_tSRM@TSQ Endura-RT	open
<input type="checkbox"/>	GB-Pesticide_tSRM@TSQ Endura	open
<input type="checkbox"/>	jinyong-neg	open
<input type="checkbox"/>	jinyong-pos	open
<input type="checkbox"/>	Quantiva for EFS pesticide China	open
<input type="checkbox"/>	Quantiva for EFS Veterinary drug-negative	open
<input type="checkbox"/>	Quantiva for EFS Veterinary drug-positive	open

Peaks m/z

一级精确质量数

Threshold Override 5,000

S/N Ratio Threshold 5.0

Mass Tolerance: 5.00 ppm

Retention Time Identify Confirm

保留时间

Ignore if Not Defined

Window Override (sec) 30

Fragment Ions Identify Confirm

碎片离子匹配

Ignore if Not Defined

Min. # of Fragments 1

Intensity Threshold 10,000

Mass tolerance 5.00 ppm

Isotopic Pattern Identify Confirm

同位素

Fit Threshold (%) 90

Allowed Mass Deviation (ppm) 5

Allowed Intensity Deviation (%) 10

Use Internal Mass Calibration

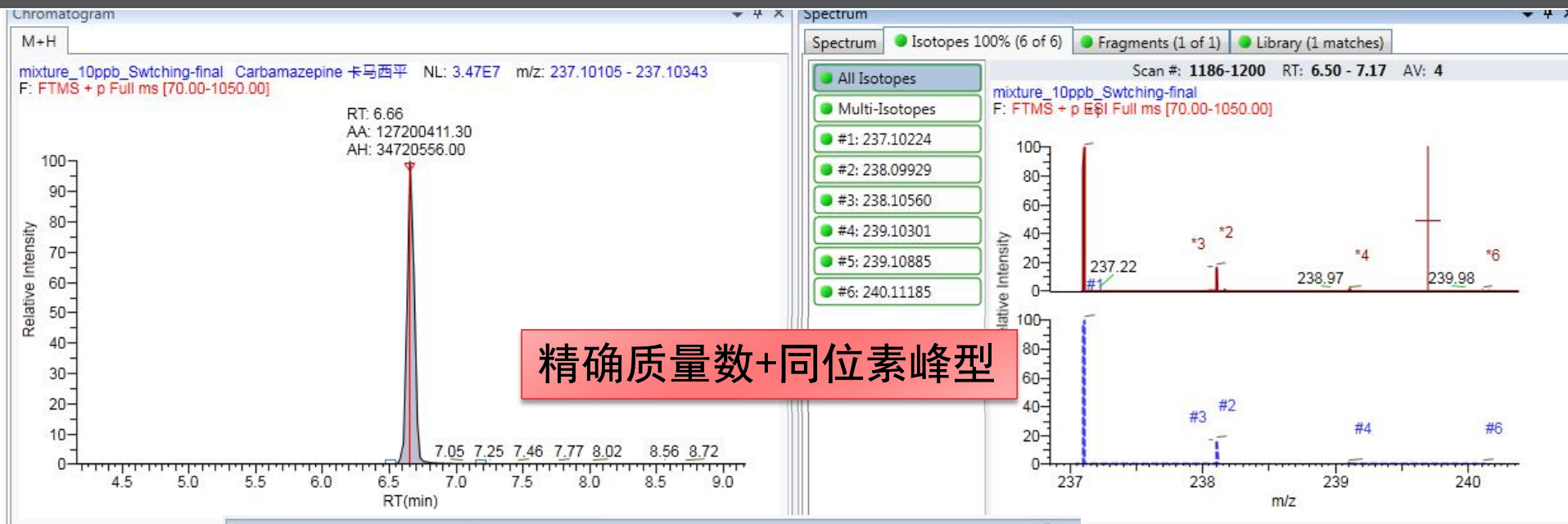
Library Search Identify Confirm

二级谱图匹配

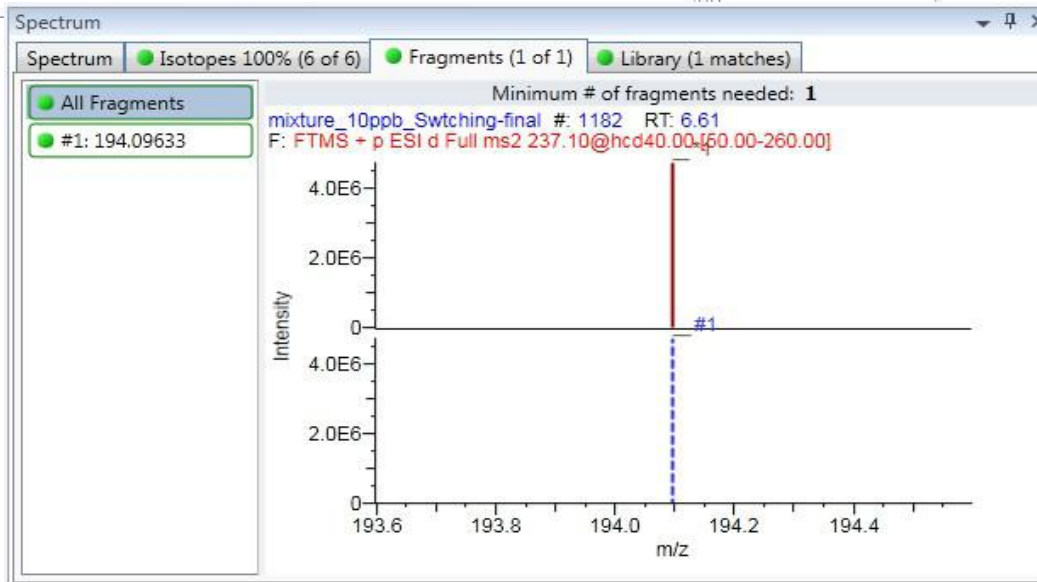
Library Search Type: Library Manager

Score Threshold (%) 80

高分辨筛查



精确质量数+同位素峰型



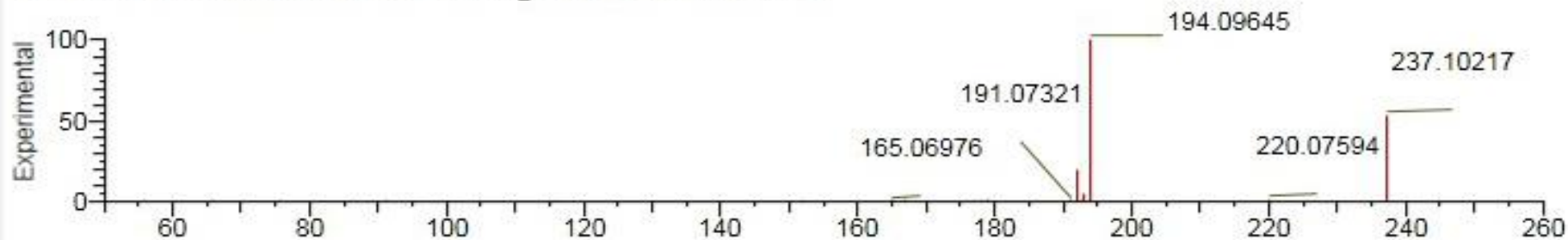
碎片离子

高分辨筛查

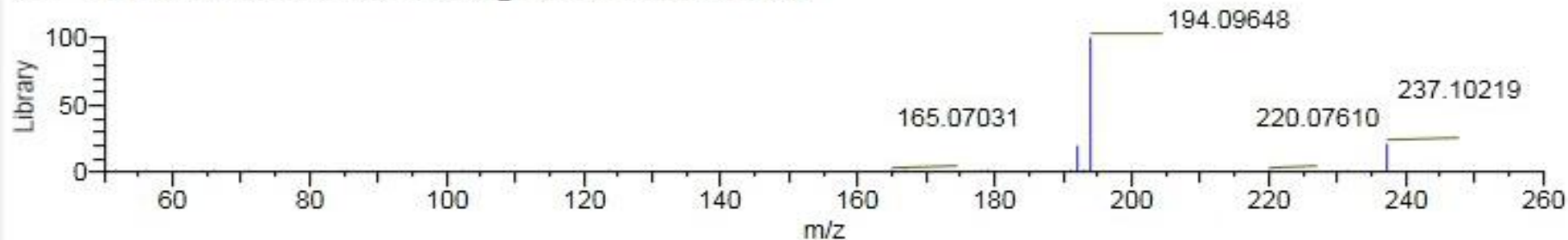
100% (6 of 6) ● Fragments (1 of 1) ● Library (1 matches)

M+H #1: **Carbamazepine ??????????????** **C₁₅H₁₂N₂O** Score: 68 Rank: 1 of 1 Id: 15

#1182 F:FTMS + p ESI d Full ms2 237.10@hcd40.00 [50.00-260.00]



#15 F:FTMS + p ESI d Full ms2 237.10@hcd40.00 [50.00-260.00]



高分辨筛查

AFB1 得到确证

精确质量数+同位素峰型；
保留时间；
碎片离子+二级谱图

Analysis

- Batch View
- Samples
- Data Review**
- Target Screening
- Report View
- Local Method
- Acquisition
- Target Screening
- Processing
- Peak Detection
- Reports
- Acquisition
- Analysis**
- Method Development

Data Review - AFB1-screening [Screening]

Selected	MZ	RT	IP	FI	LS	Flag	Compound Name	Match Result Name	Formula	Addu
<input checked="" type="checkbox"/>							AFB1	AFB1@RT 15.71	C17H12O6	M+H

Chromatogram

M+H

3-1 AFB1 NL: 1.90E8 m/z: 313.06753 - 313.07379
F: FTMS + p ESI Full ms [50.00-750.00]

RT: 15.71
AA: 1454248572.07
AH: 187879337.37

Relative Intensity

RT(min)

Spectrum

Spectrum Isotopes 100% (5 of 5) Fragments (4 of 4) Library (1 matches)

All Isotopes

- Multi-Isotopes
- #1: 313.07066
- #2: 314.07404
- #3: 315.07641
- #4: 316.07883
- #5: 317.08145

Scan #: 6526-6619 RT: 15.58 - 15.93 AV: 32 Score: 100

3-1
F: FTMS + p ESI Full ms [50.00-750.00]

100
50
0

313.27 *1 *2 *3 *4 *5

316.42

100
50
0

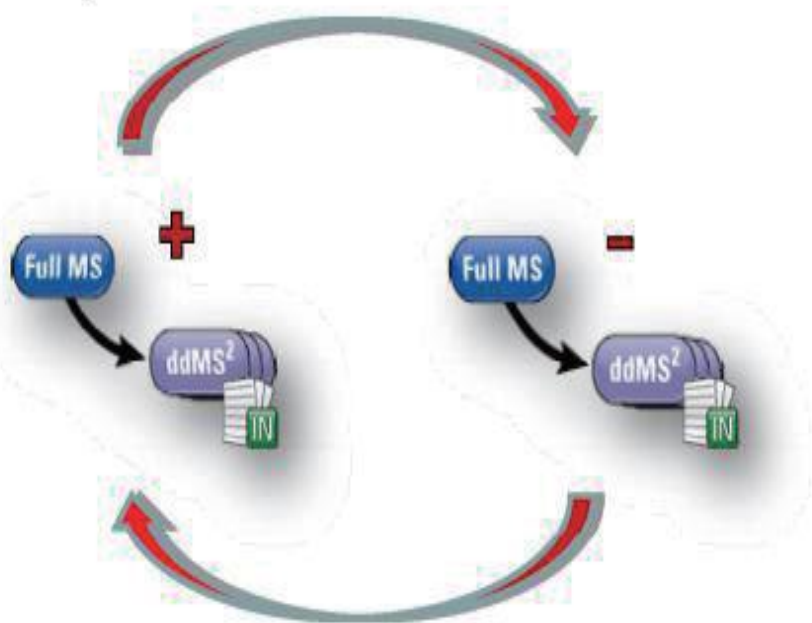
#1 #2 #3 #4 #5

313 314 315 316 317

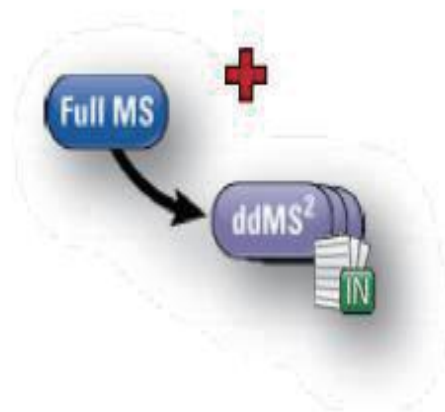
m/z

同时定性定量分析

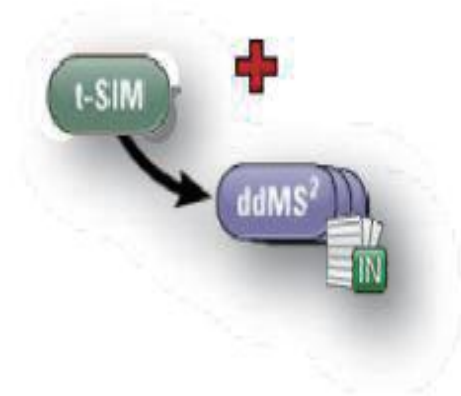
a)



b)



c)

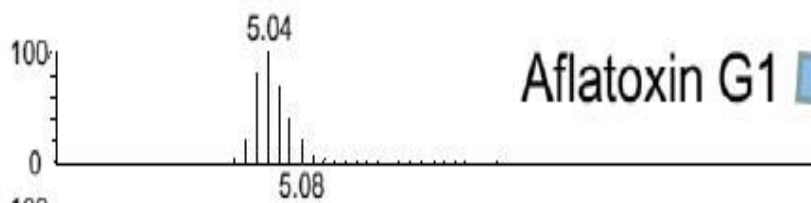


- a) 快速正负切换的筛查方法
- b) Full MS 定量方法
- c) t-SIM 定量方法

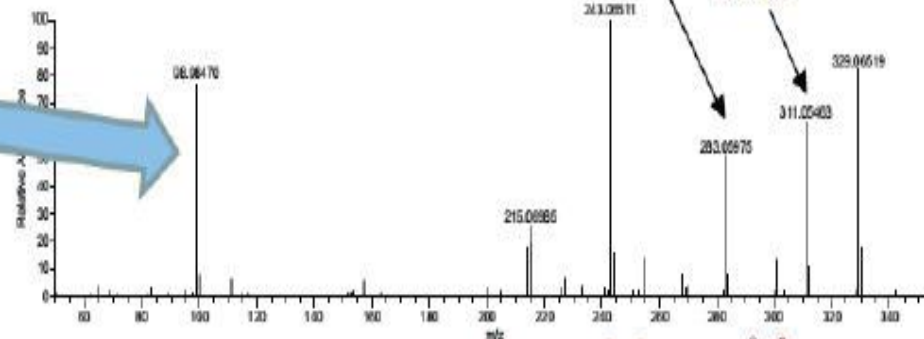
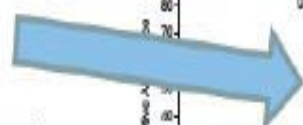
同时定性定量分析

Compound Name	Elemental Composition	Polarity	Exact Mass [M+H] ⁺ / [M-H] ⁻
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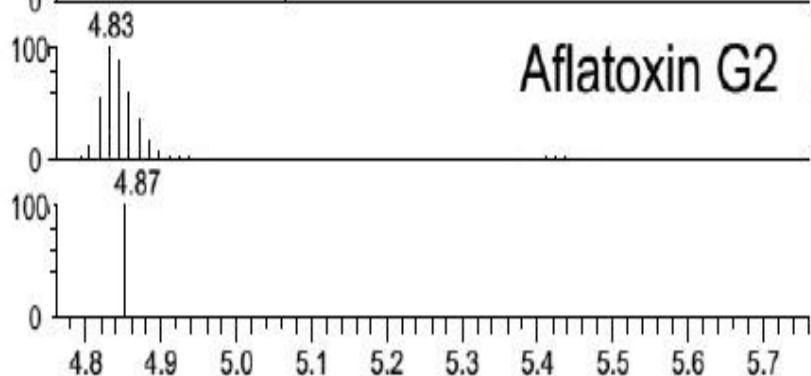
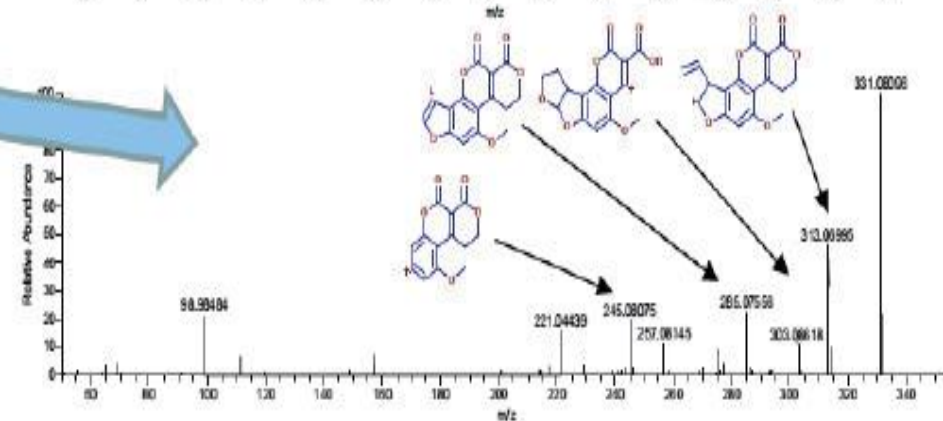
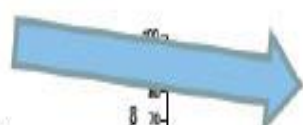
RT: 4.76 - 5.77



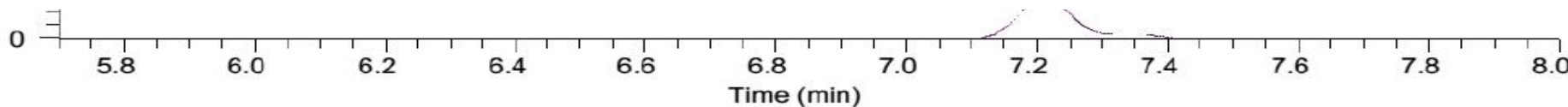
Aflatoxin G1



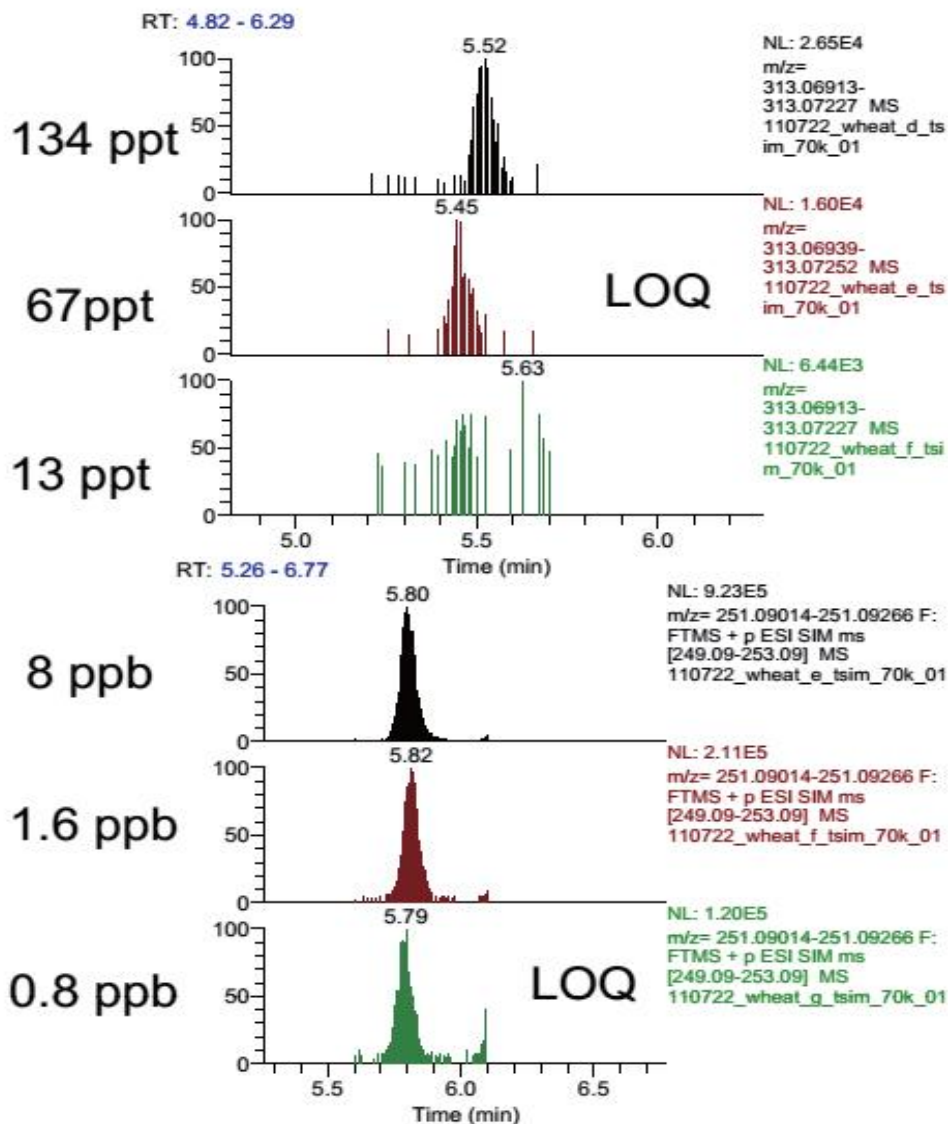
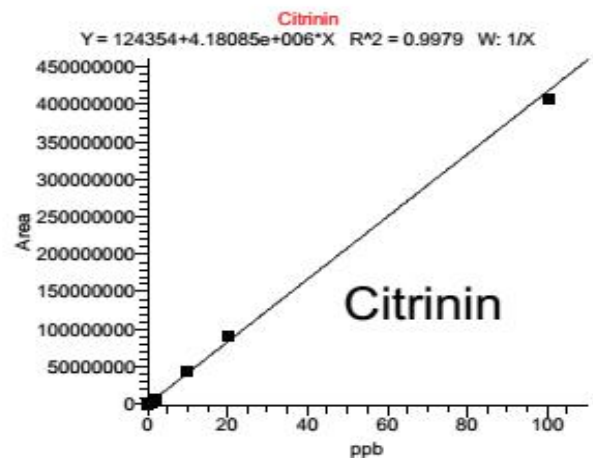
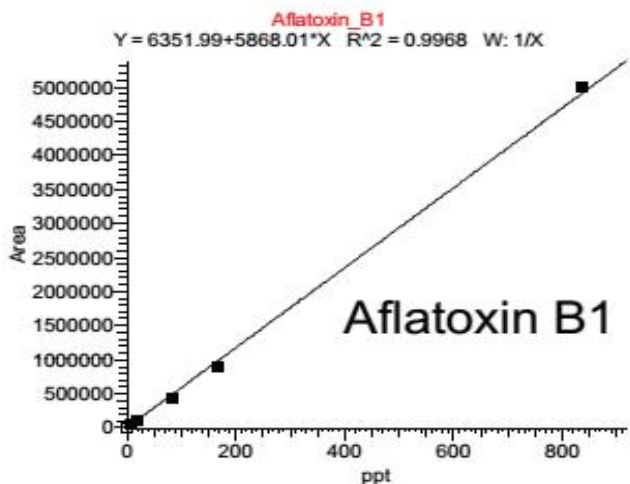
Aflatoxin G2



Time (min)



同时定性定量分析





Thermo
S C I E N T I F I C

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