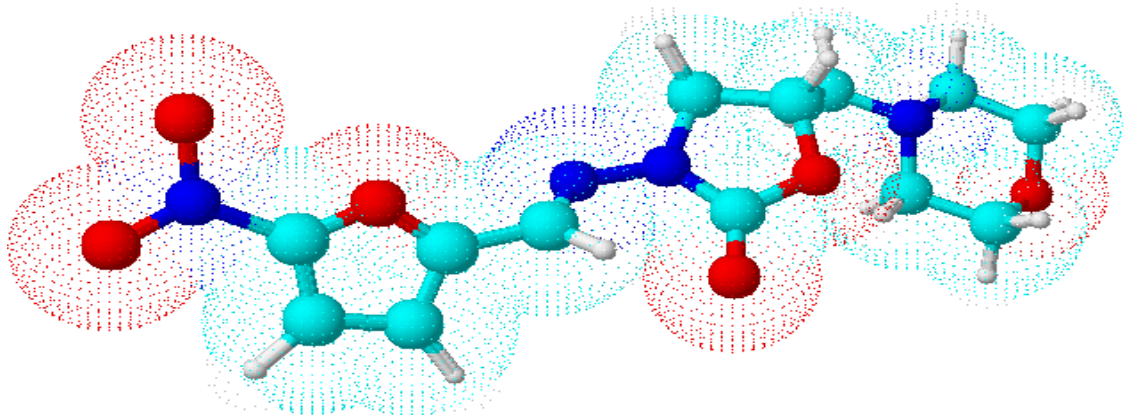
Agilent 6000系列液相色谱/质谱在食品分析中的应用

Agilent 6000系列液相色谱/质谱平台

- 包括:
  - 单四极
  - 串联四极质谱
  - 离子阱质谱
  - 飞行时间质谱（**TOF**）
  - 四极杆-飞行时间串联
- 食品分析解决方案
  - 最佳性能价格比的**Agilent 6410 Triple Quad LC/MS**
  - 高性能的 **Agilent 6210TOF LC/MS**

硝基呋喃代谢物的检测 - Agilent 6410 三重串联四极杆液质系统



硝基呋喃代谢物检测的背景

- 四种硝基呋喃作为抗生素药物(呋喃唑酮,呋喃妥因,呋喃 西林及呋喃它酮)被广泛地用于饲料添加剂以防止牛,鱼,家禽和猪等动物的病菌感染
- 欧盟和美国FDA分别于1995年和 2002年颁布法规禁止在动物饲养中使用硝基呋喃
- 欧盟设定的最低要求方法检测限 (MRPL)是:四个硝基呋喃代谢物均为 1 ug/kg

欧盟法规- 最低要求方法检测限（MRPL）

COMMISSION DECISION
of 12 August 2002
implementing Council Directive 96/23/EC concerning the performance of analytical methods and
the interpretation of results

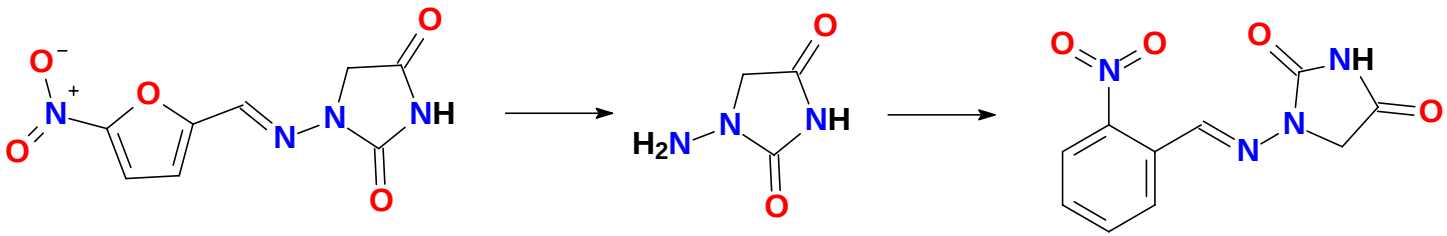
(2002/657/EC)

欧盟法规-硝基呋喃

COMMISSION DECISION
of 13 March 2003
amending Decision 2002/657/EC as regards the setting of minimum required performance limits (MRPLs) for certain residues in food of animal origin
(2003/181/EC)

硝基呋喃代谢物: —呋喃唑酮, —呋喃它酮 —呋喃妥因 —呋喃西林	家禽类肉 水产品	**1 ug/kg**

硝基呋喃及代谢物的化学结构

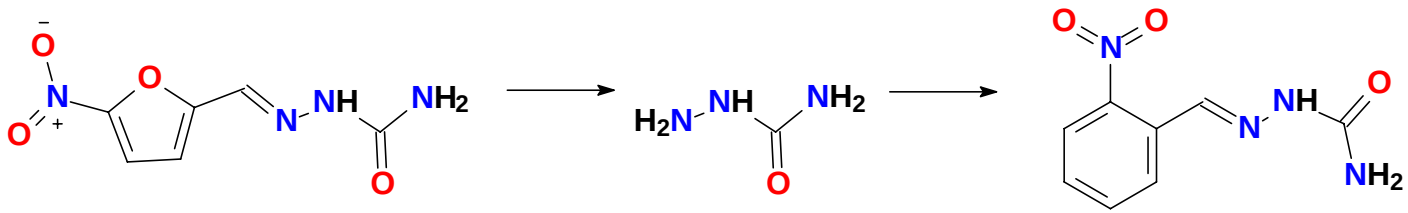


Nitrofurantoin

呋喃妥英

AHD

NPAHD



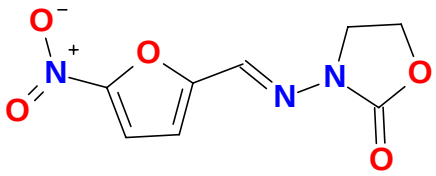
Nitrofuranzone

呋喃西林

SEM

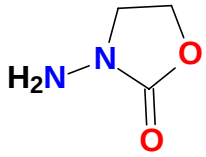
NPSEM

硝基呋喃及代谢物的化学结构

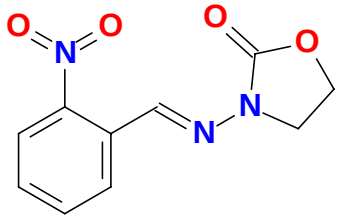


呋喃唑酮

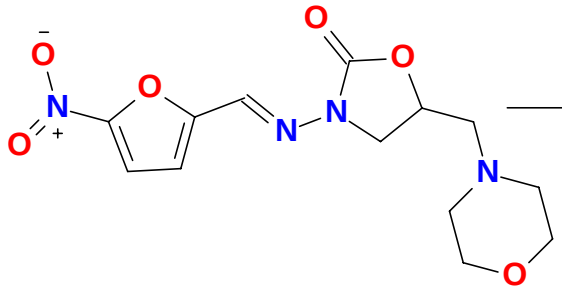
Furazolidone



AOZ

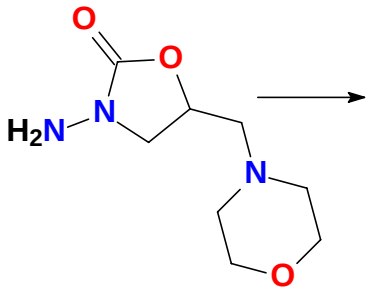


NPAOZ

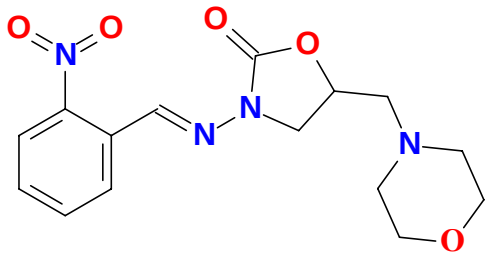


呋喃它酮

Furaltadone



AMOZ



NPAMOZ

液相色谱的分析条件

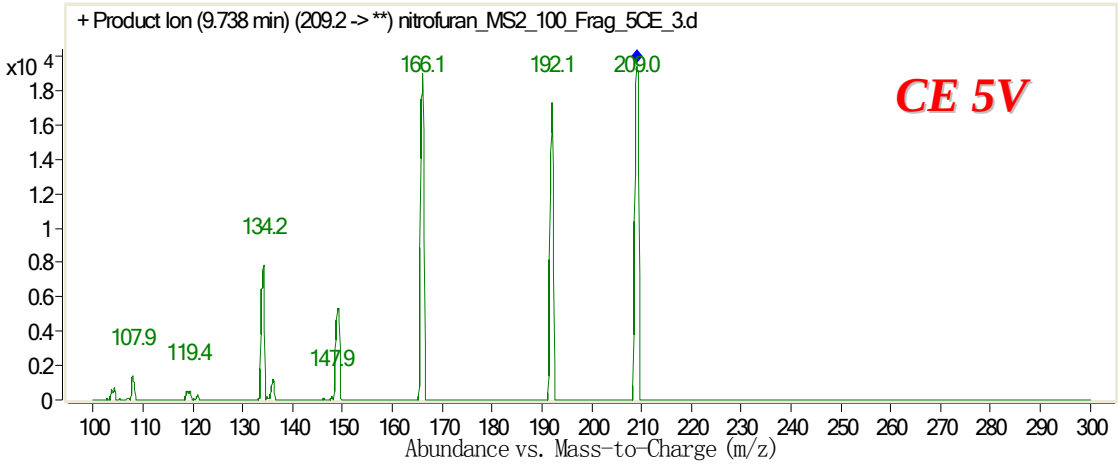
- **HPLC system : Agilent 1100 series**
- **Column : C_{18}, 2.1×150, 5 μm**
- **Injection Volumn : 50 μL**
- **Flow rate : 0.3 mL/min**
- **Temp : 40 ℃**
- **Mobile phase : A-0.1% Formic acid; B-Acetonitrile**
- **Gradient**

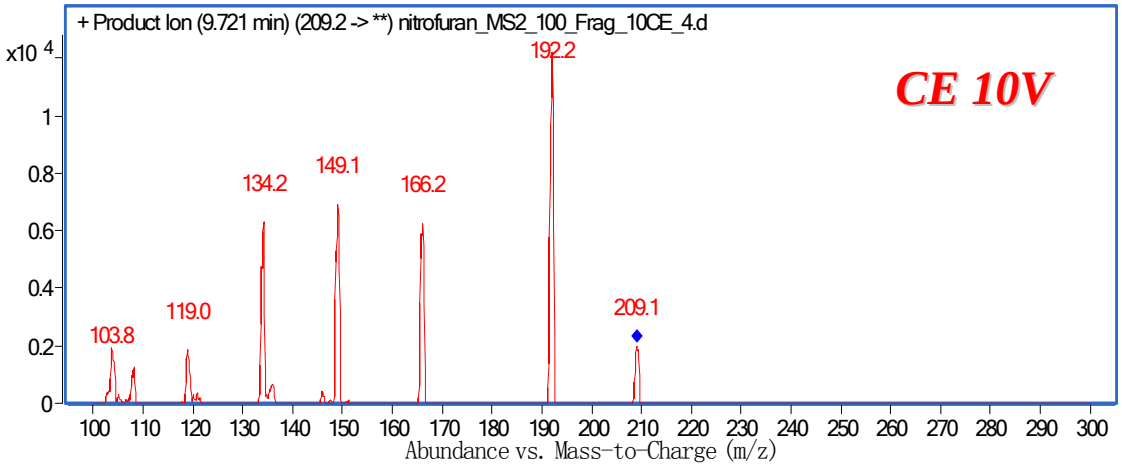
Time	A	B
0	90	10
10	50	50
12	40	60
12.01	5	95
17	5	95
17.01	90	10
22	90	10

质谱的分析条件

- MS system : Agilent 6410 LC/MS/MS
- Ion source : ESI
- Polarity : Positive
- Nebulizer gas : Nitrogen
- Ion spray voltage : 4000V
- Source temperature : 350℃
- Resolution : Q1 (unit) Q3 (unit)
- Scan mode : Multiple Reaction Monitoring (MRM)
- Conditions of MRM

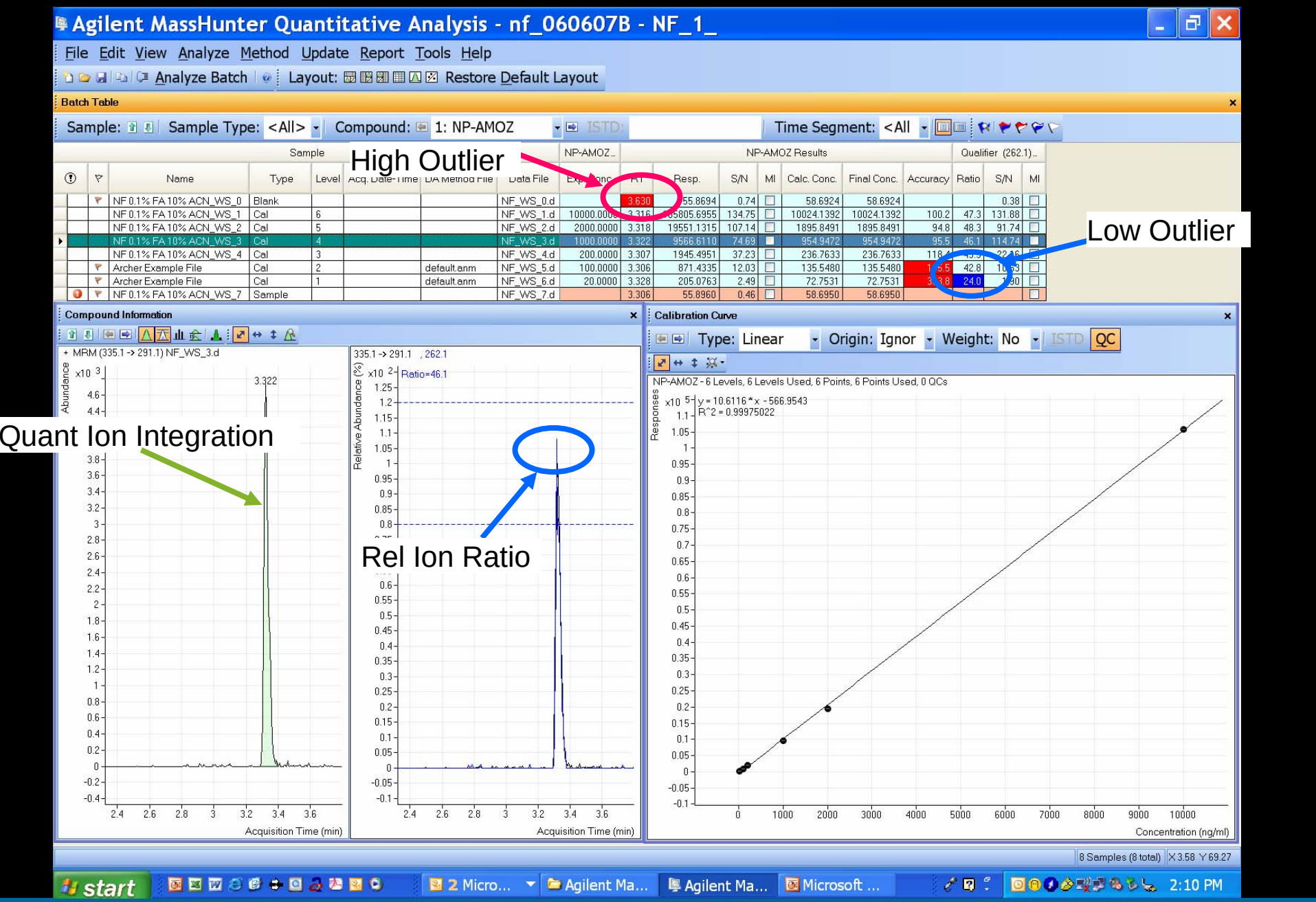
对于 SEM： 在不同碰撞能量下的二级质谱图





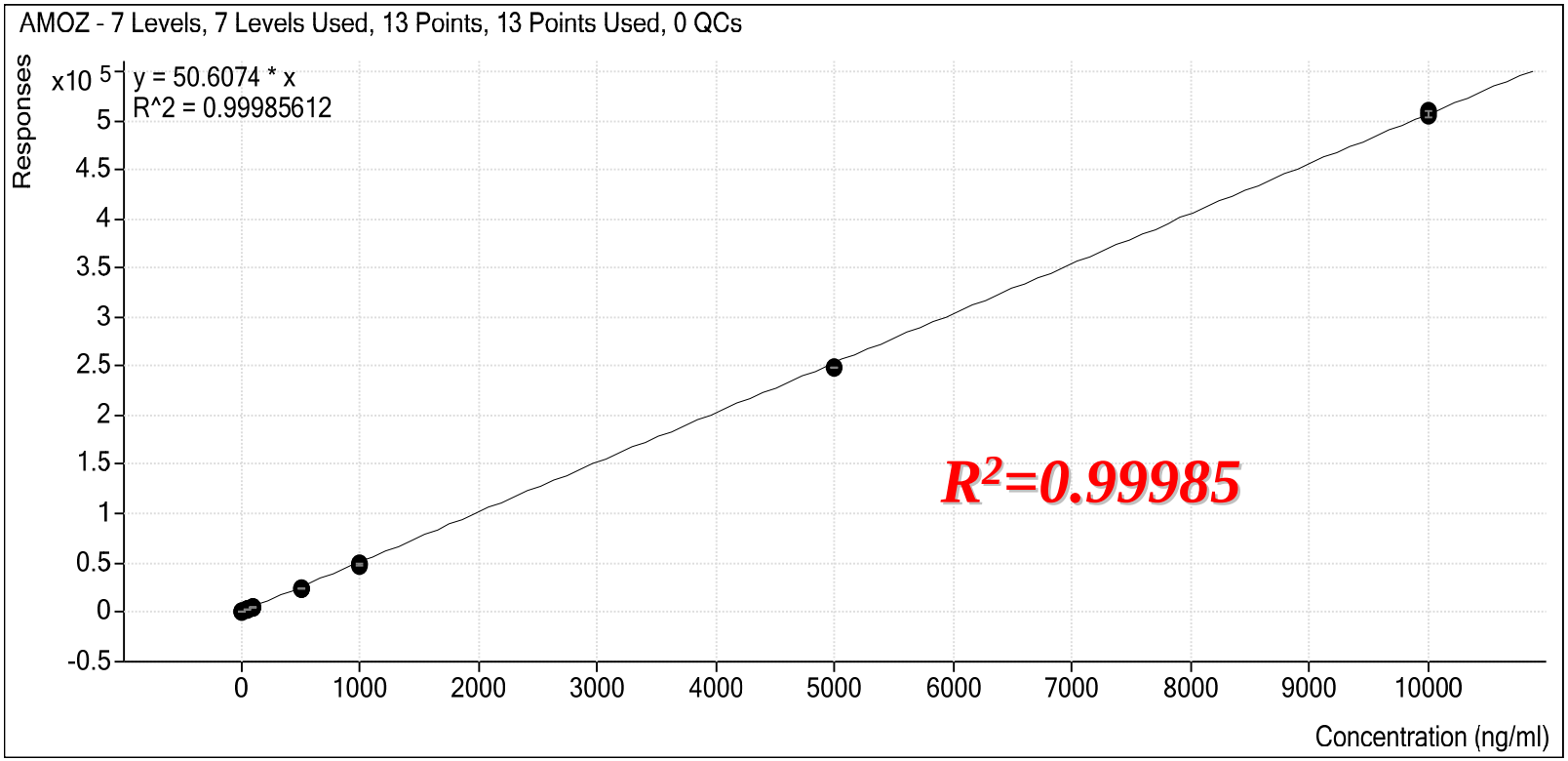
对于四个硝基呋喃代谢物的MRM分析条件

Time	Compound	Precursor	Product	Dwell (ms)	Fragmentor (V)	Collision Engergy (V)
0	AMOZ	335.1	291.4	150	92	5
		335.1	262.4	150	92	5
8	SEM	209.1	192.3	150	92	5
		209.1	166.3	150	92	5
9.95	AH	249.1	134.2	150	92	5
		249.1	104.2	150	92	5
10.8	AOZ	236.0	134.3	150	92	5
		236.0	104.3	150	92	5



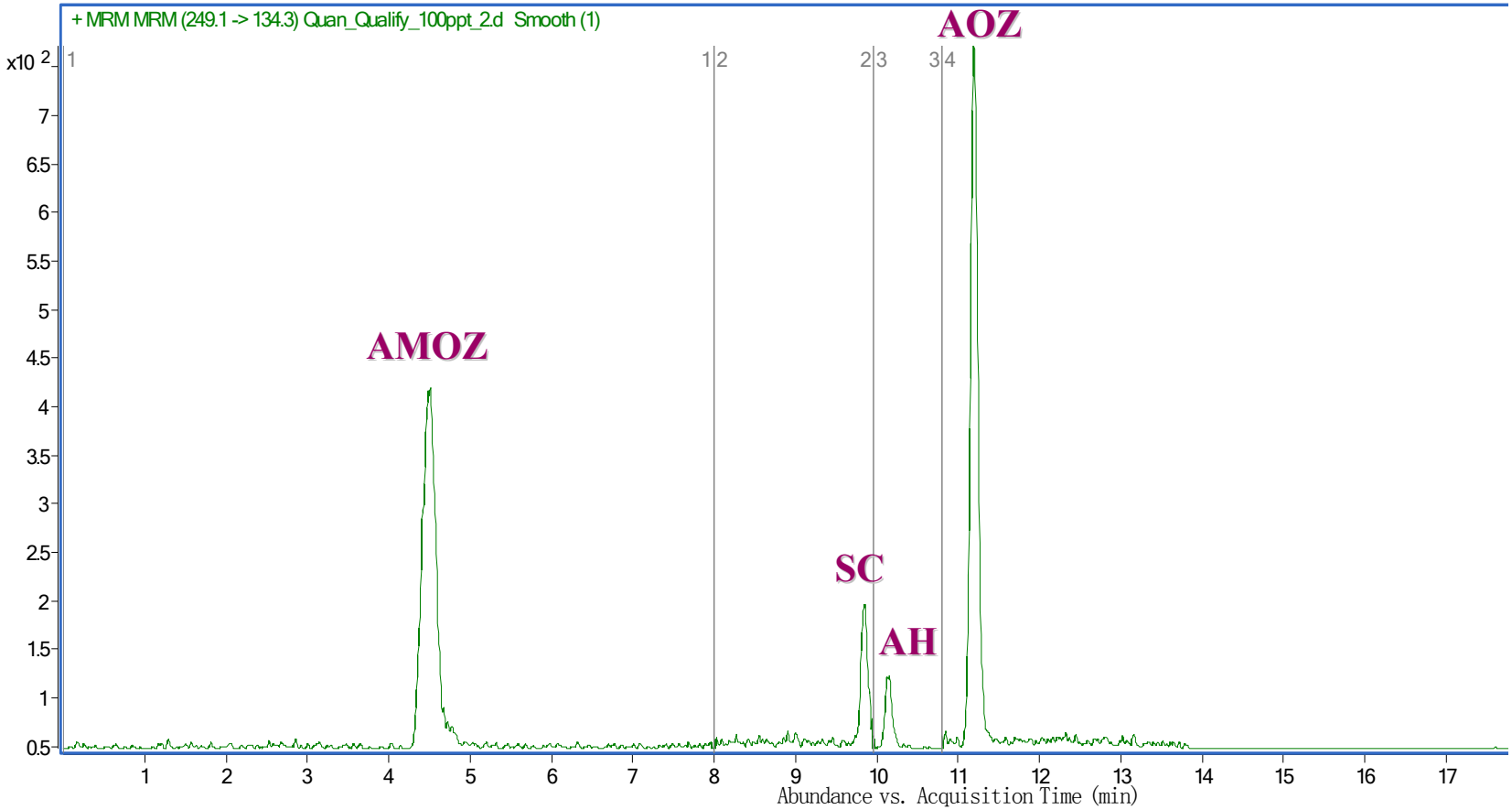
		Name	Type	Level	Acq. Date-Time	DA Method File	Data File	NP-AMOZ... Exp. Conc.	RT	Resp.	S/N	MI	Calc. Conc.	Final Conc.	Accuracy	Qualifier (262.1)... Ratio	S/N	MI
		NF 0.1% FA 10% ACN_WS_0	Blank				NF_WS_0.d		3.630	55.8694	0.74		58.6924	58.6924			0.38	
		NF 0.1% FA 10% ACN_WS_1	Cal	6			NF_WS_1.d	10000.0000	3.316	5805.6955	134.75		10024.1392	10024.1392	100.2	47.3	131.88	
		NF 0.1% FA 10% ACN_WS_2	Cal	5			NF_WS_2.d	2000.0000	3.318	19551.1315	107.14		1895.8491	1895.8491	94.8	48.3	91.74	
		NF 0.1% FA 10% ACN_WS_3	Cal	4			NF_WS_3.d	1000.0000	3.322	9566.6110	74.69		954.9472	954.9472	95.5	46.1	114.74	
		NF 0.1% FA 10% ACN_WS_4	Cal	3			NF_WS_4.d	200.0000	3.307	1945.4951	37.23		236.7633	236.7633	118.4			
		Archer Example File	Cal	2		default.anm	NF_WS_5.d	100.0000	3.306	871.4335	12.03		135.5480	135.5480		42.8		
		Archer Example File	Cal	1		default.anm	NF_WS_6.d	20.0000	3.328	205.0763	2.49		72.7531	72.7531		24.0		
		NF 0.1% FA 10% ACN_WS_7	Sample				NF_WS_7.d		3.306	55.8960	0.46		58.6950	58.6950				

AMOZ的校正曲线

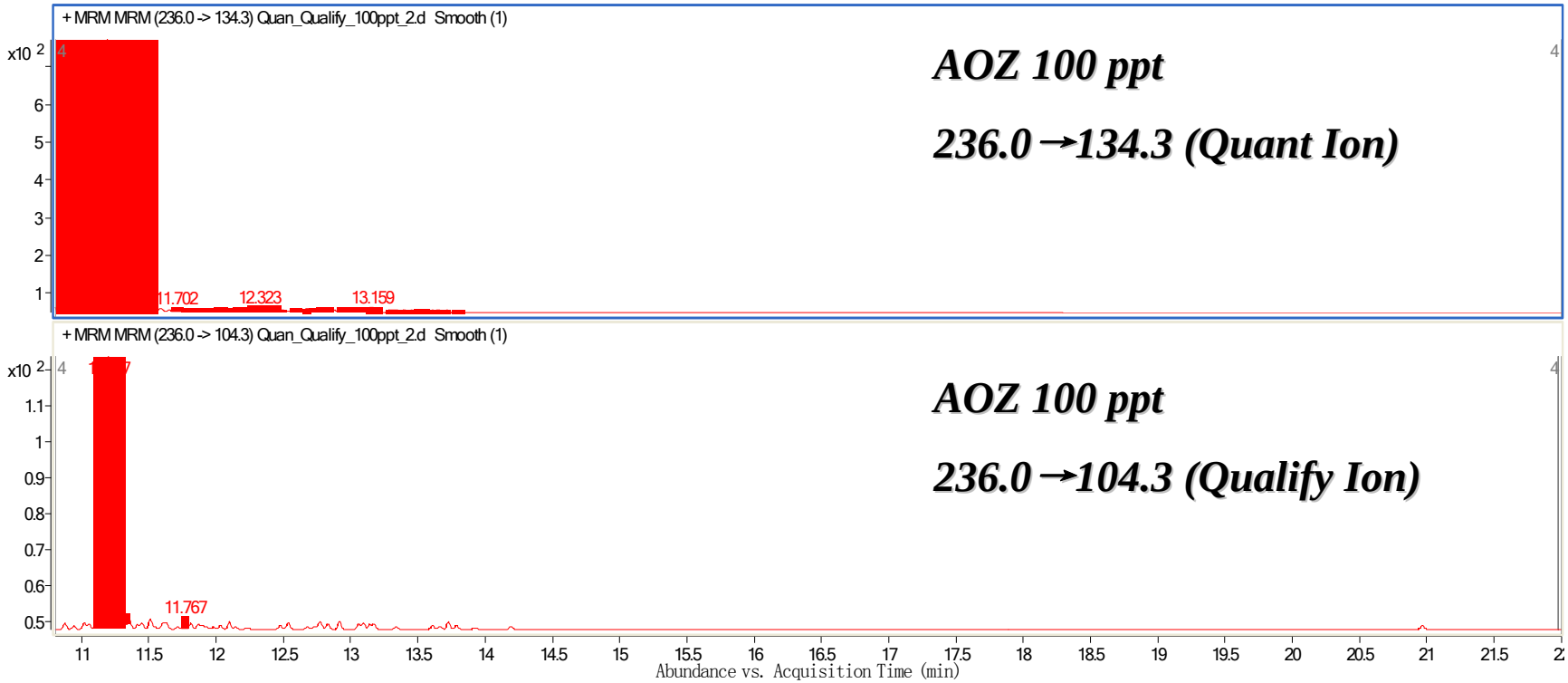


Linear range: 10 ppt ~ 10ppb

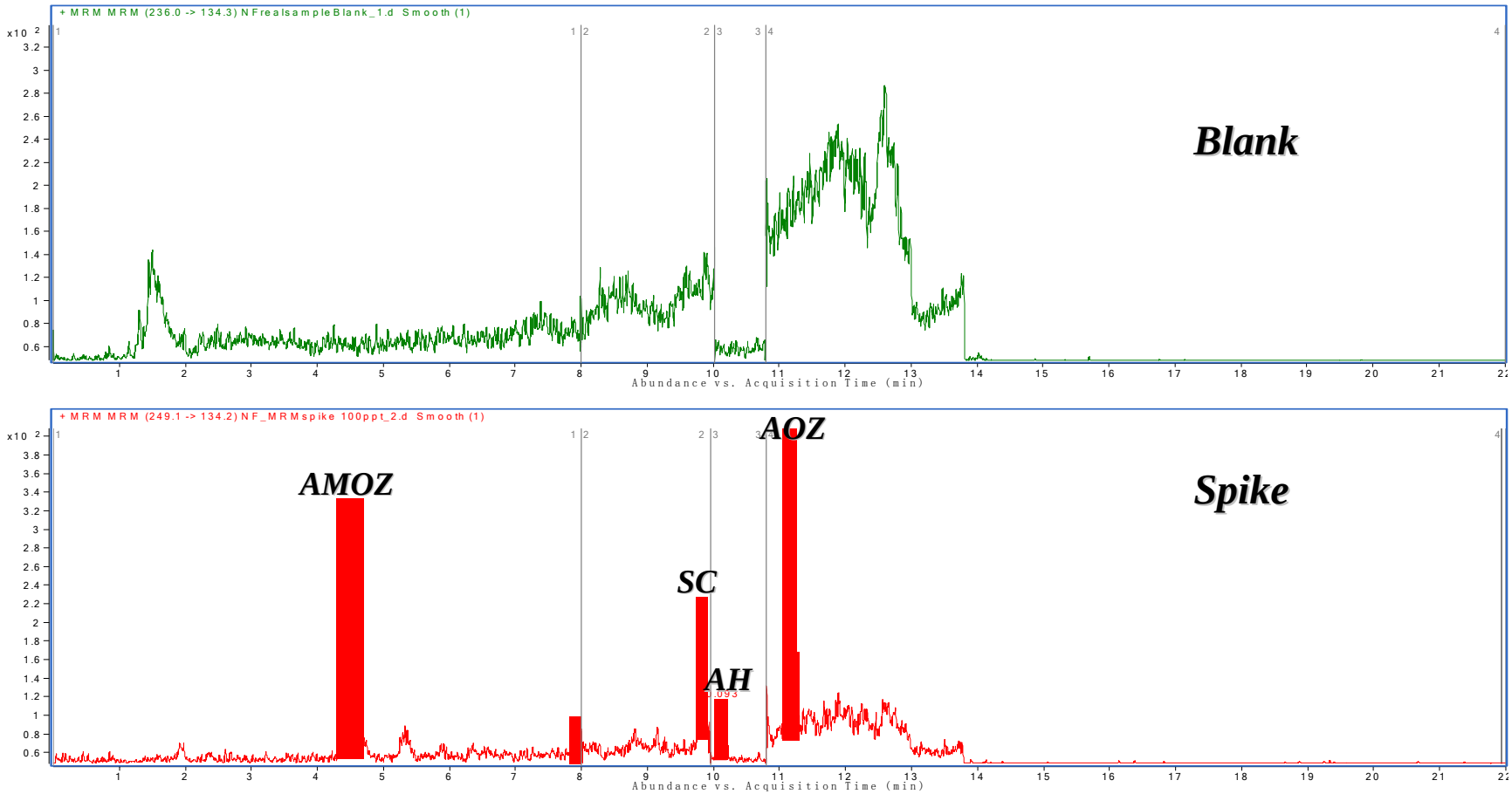
硝基呋喃代谢物(100 ppt)的MRM 分析结果



EIC Transitions at m/z 134 and m/z 104



鳗鱼添加样品的分析结果 (100 ppt)



硝基呋喃代谢物分析结果的精密度

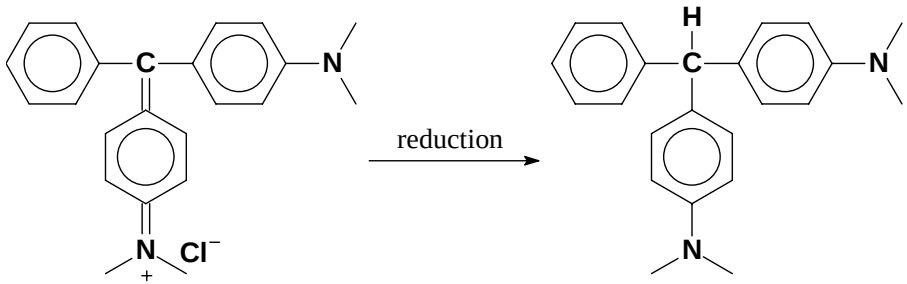
化合物	浓度水平 (ng/mL)	RSD (%)
AMOZ	0.1	1.05
SEM	0.1	2.01
AH	0.1	8.07
AOZ	0.1	1.58

[a] Each value was the average of 8 replicates (n=8).

Agilent 6410 三重串联四极杆质谱分析
孔雀石绿和隐性孔雀石绿

孔雀石绿 (MG)

- 在其它类兽药中，具有三苯基甲烷结构的染料，如孔雀石绿 (MG)是最常见的化合物， 它主要是被用于防止鱼类发生白斑点病，水霉病及纤毛虫
- MG 可以还原成隐性孔雀石绿 (LMG)，并且沉积在鱼的脂肪中



Malachite Green

(Green)

Leucomalachite Green

(Colorless)

欧盟对于孔雀石绿和其代谢物的要求限量

COMMISSION DECISION

of 22 December 2003

amending Decision 2002/657/EC as regards the setting of minimum required performance limits (MRPLs) for certain residues in food of animal origin

(notified under document number C(2003) 4961)

(Text with EEA relevance)

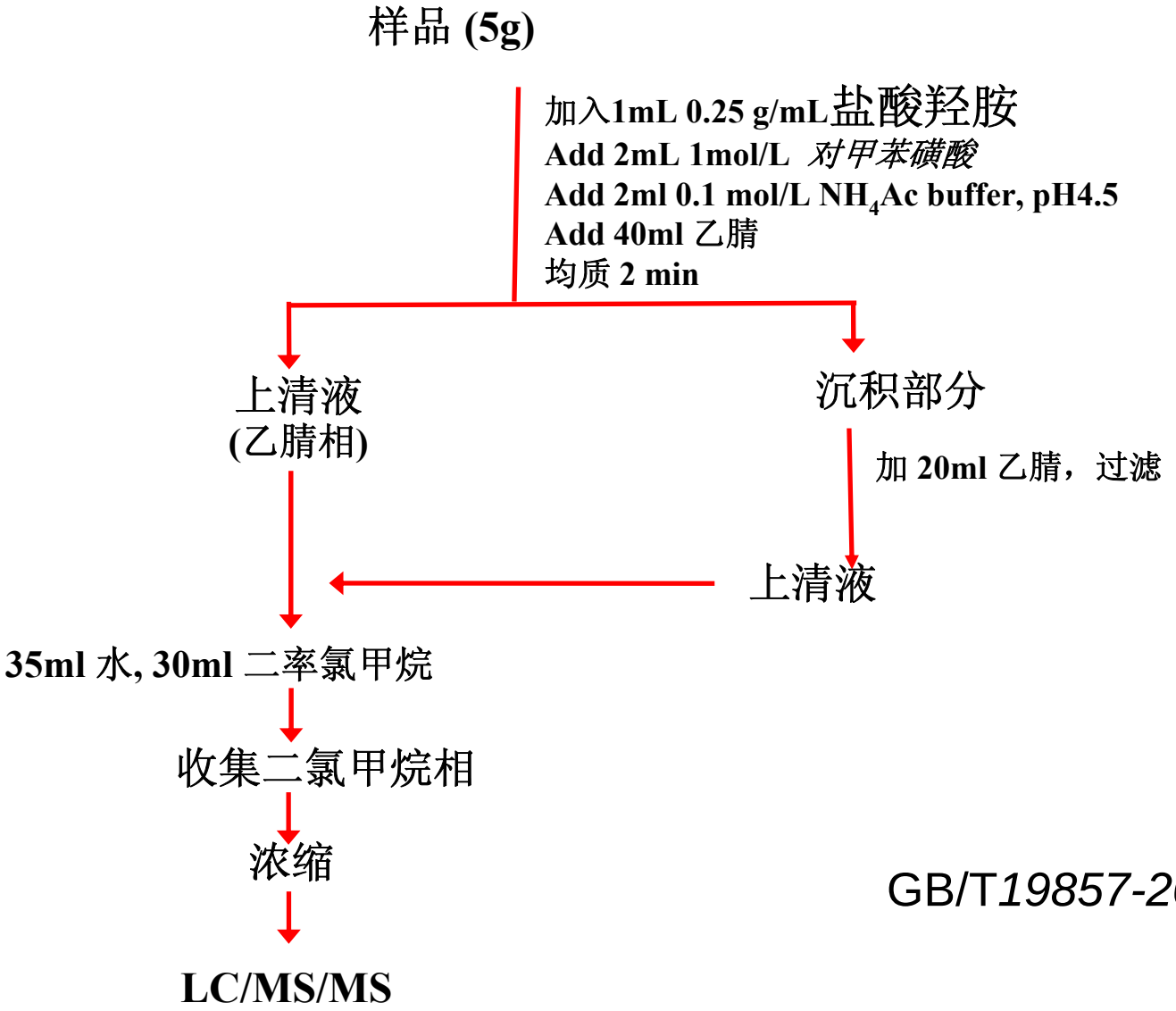
ANNEX

Commission Decision 2002/657/EC is amended as follows:

In Annex II, the following row is added:

'Substance and/or metabolite	Matrixes	MRPL
Sum of malachite green and leucomalachite green	Meat of aquaculture products	2 µg/kg'

孔雀石绿/隐性孔雀石绿的分析流程



GB/T19857-2005

液相色谱参数

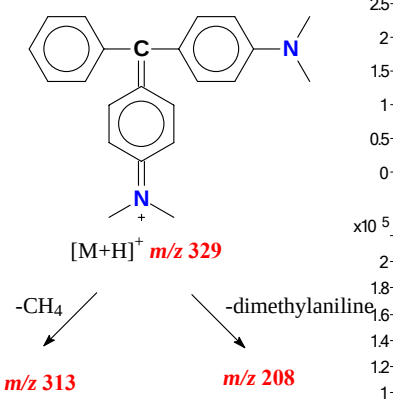
- **HPLC system : Agilent 1100 series**
- **Column : C_{18}, 2.1×150, 5 μm**
- **Injection Volumn : 10 μL**
- **Flow rate : 0.3 mL/min**
- **Mobile phase : A-10mmol NH_4Ac (pH=4.5); B-Acetonitrile**
- **Gradient**

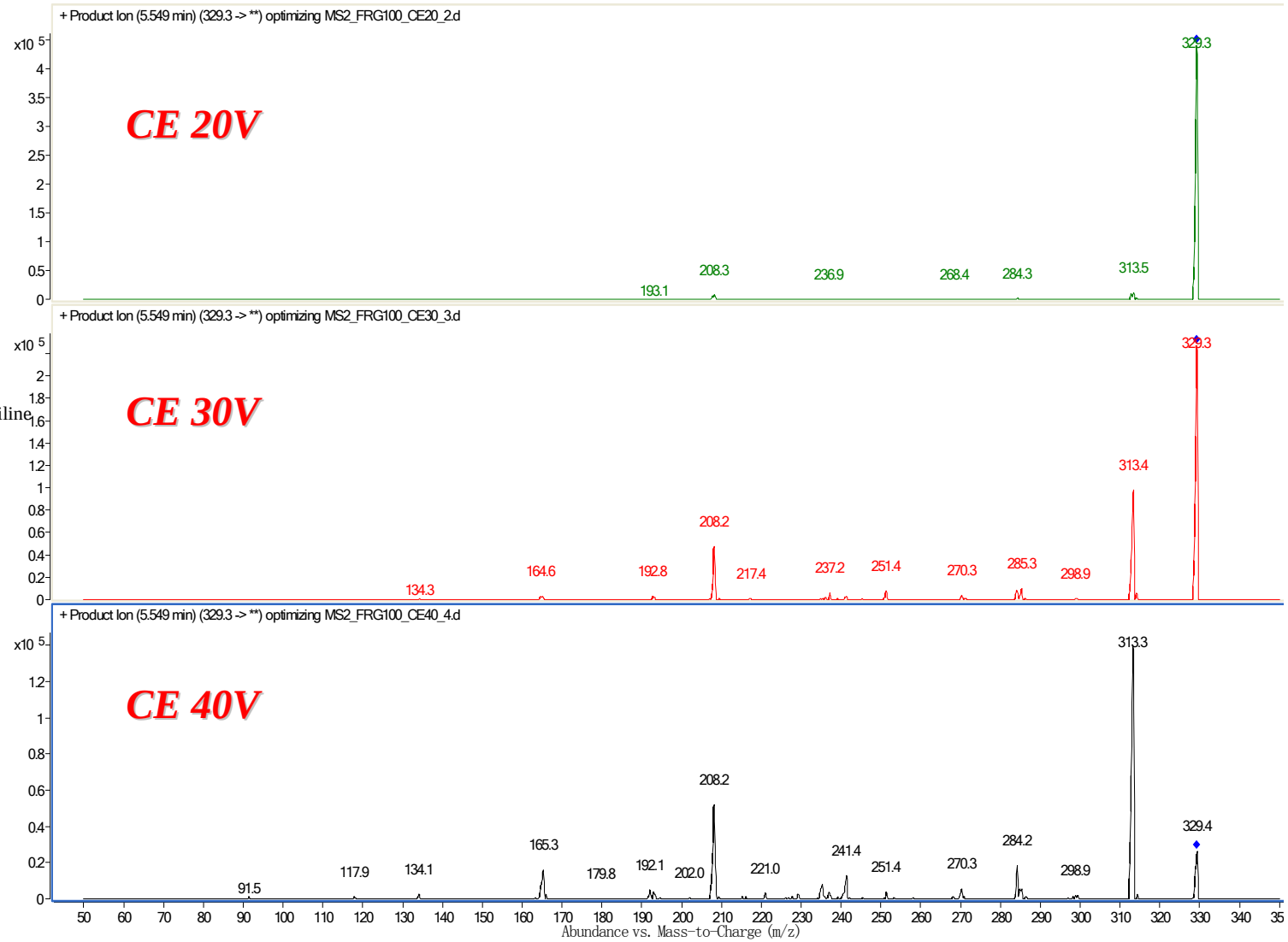
Time	A	B
0	70	30
1	50	50
2	5	95
8	5	95
8.01	70	30
13	70	30

质谱参数

- MS system : Agilent 6410 LC/MS/MS
- Ion source : ESI
- Polarity : Positive
- Nebulizer gas : Nitrogen
- Ion spray voltage : 4000V
- Source temperature : 350℃
- Resolution : Q1 (unit) Q3 (unit)
- Scan mode : Multiple Reaction Monitoring (MRM)
- Conditions of MRM

在不同碰撞电压下，孔雀石绿的二级质谱图

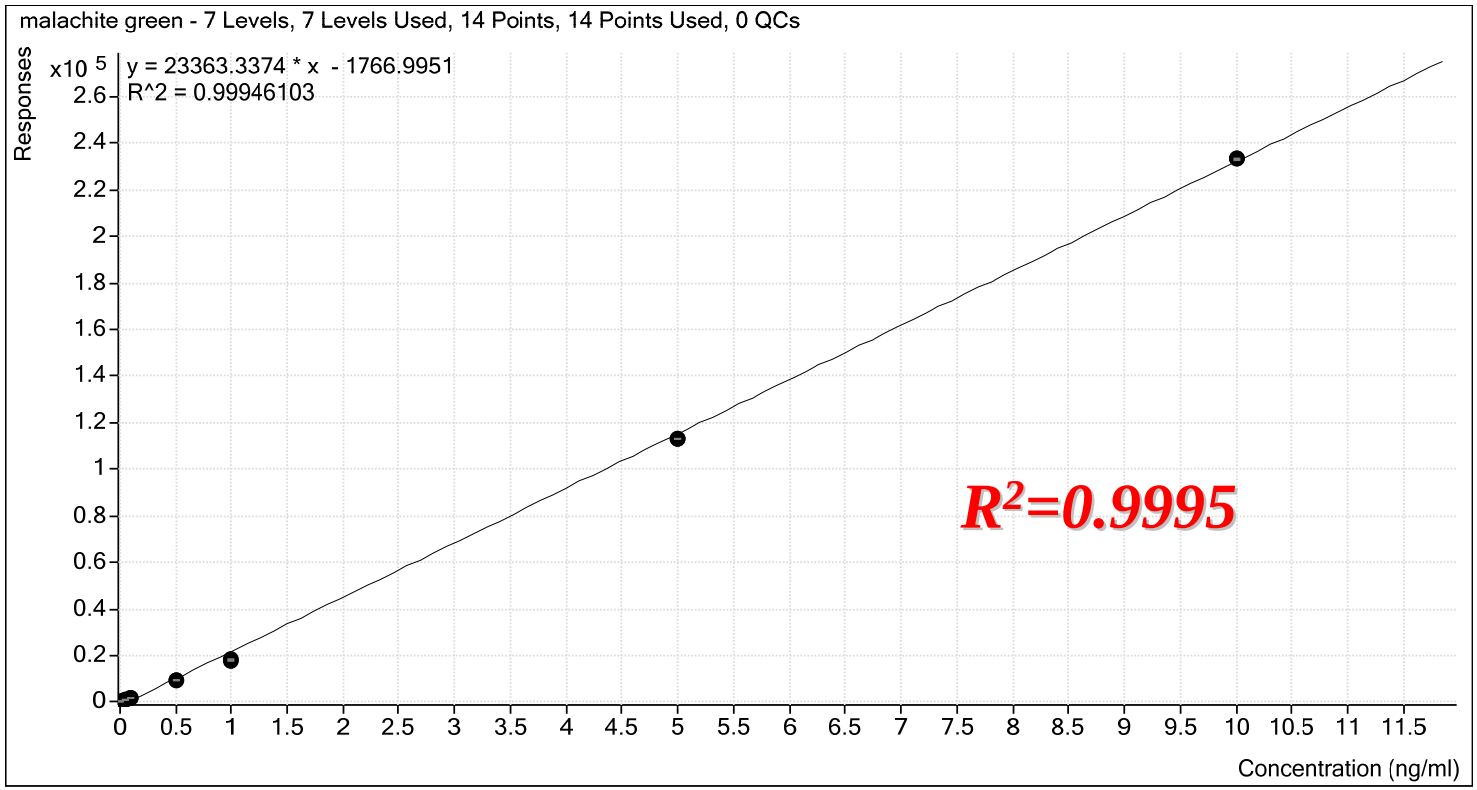




孔雀石绿/隐性孔雀石绿的MRM条件

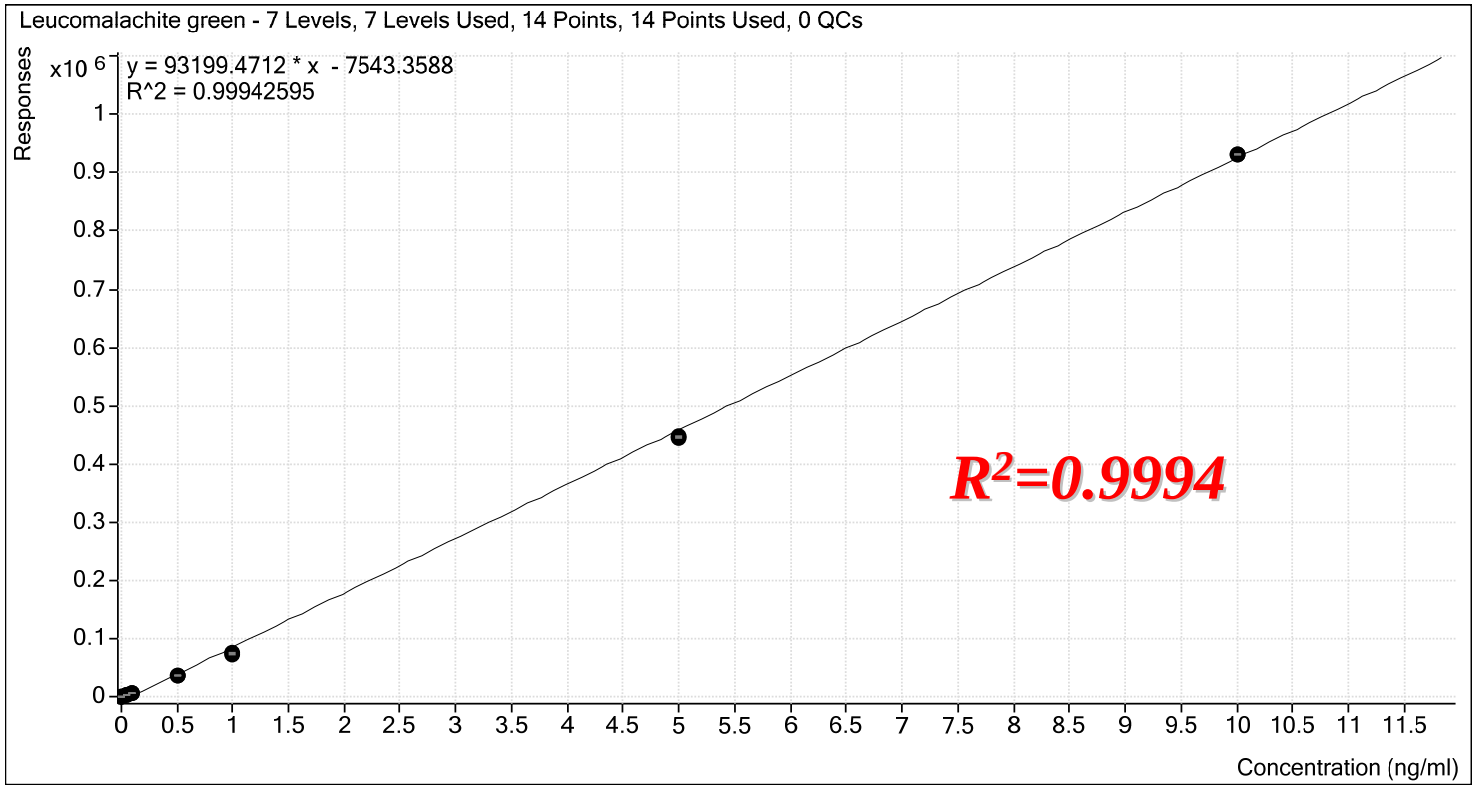
Time	Compound	Precursor	Product	Dwell (ms)	Fragmentor (V)	Collision Engergy (V)
0	Malachite green	329.3	313.3	40	100	40
		329.3	208.2	40	100	40
7	Leucomalachite green	331.3	316.3	40	100	30
		331.3	239.2	40	100	30

孔雀石绿的校正曲线



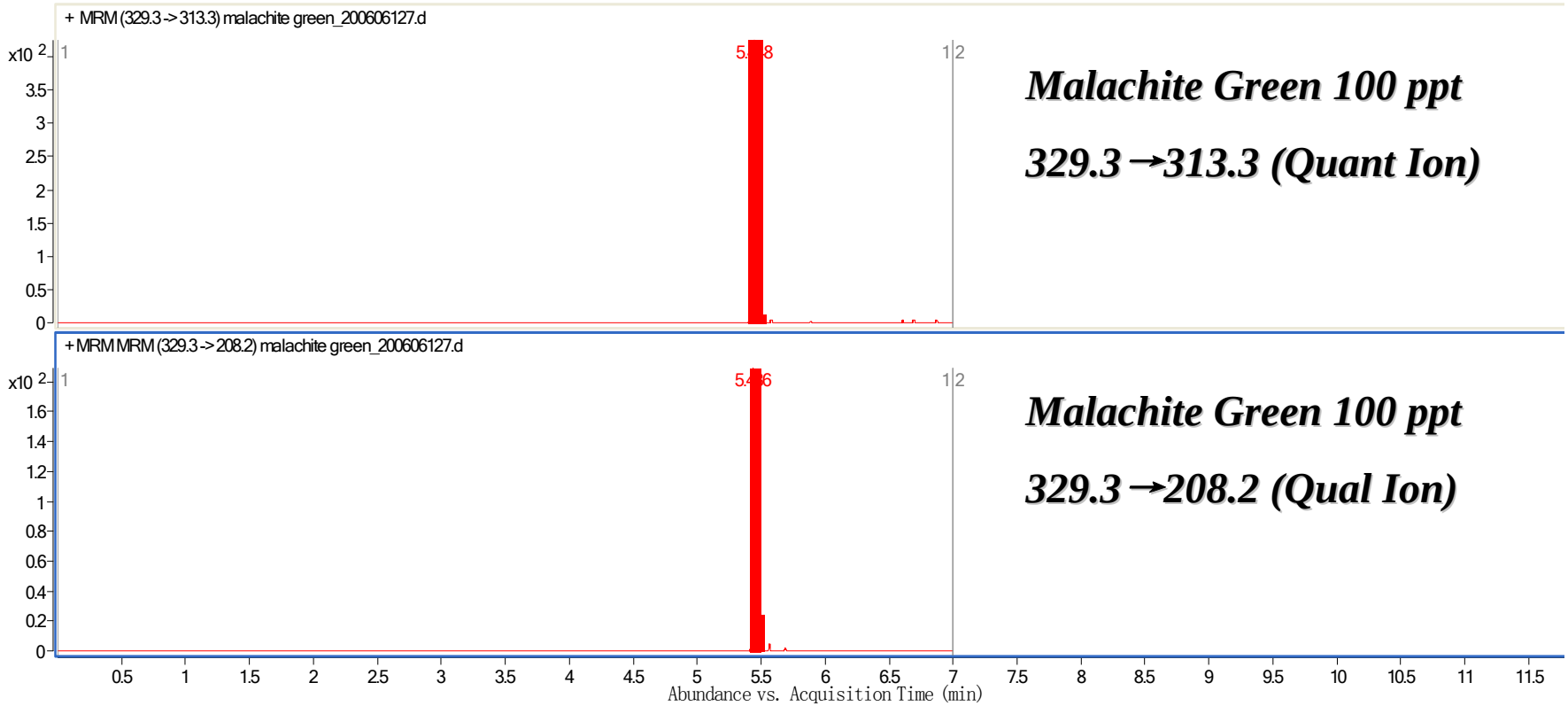
Linear range: 10 ppt ~ 10ppb

隐性孔雀石绿的校正曲线



Linear range: 10 ppt ~ 10ppb

孔绿MRM的提取离子流图
m/z 313 and m/z 208



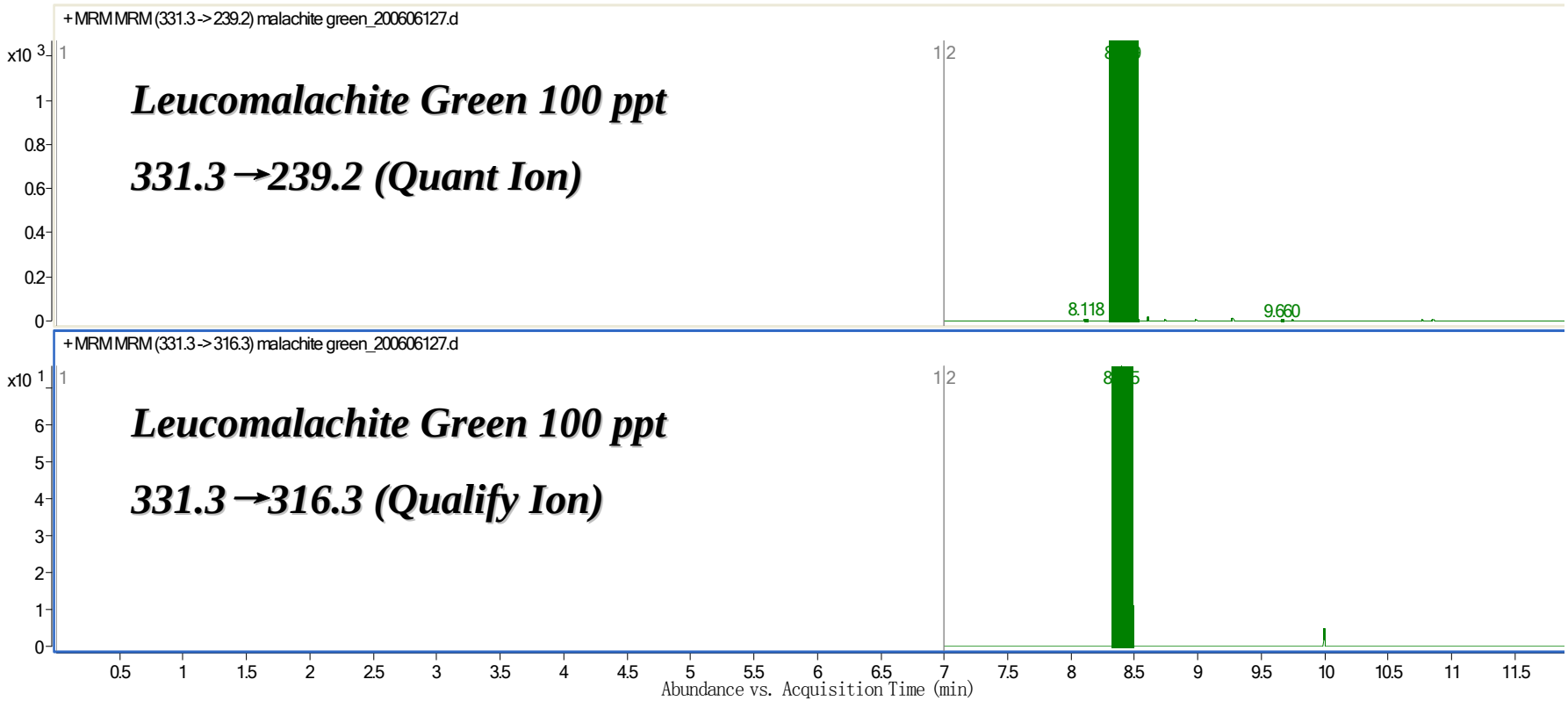
Malachite Green 100 ppt

329.3 →313.3 (Quant Ion)

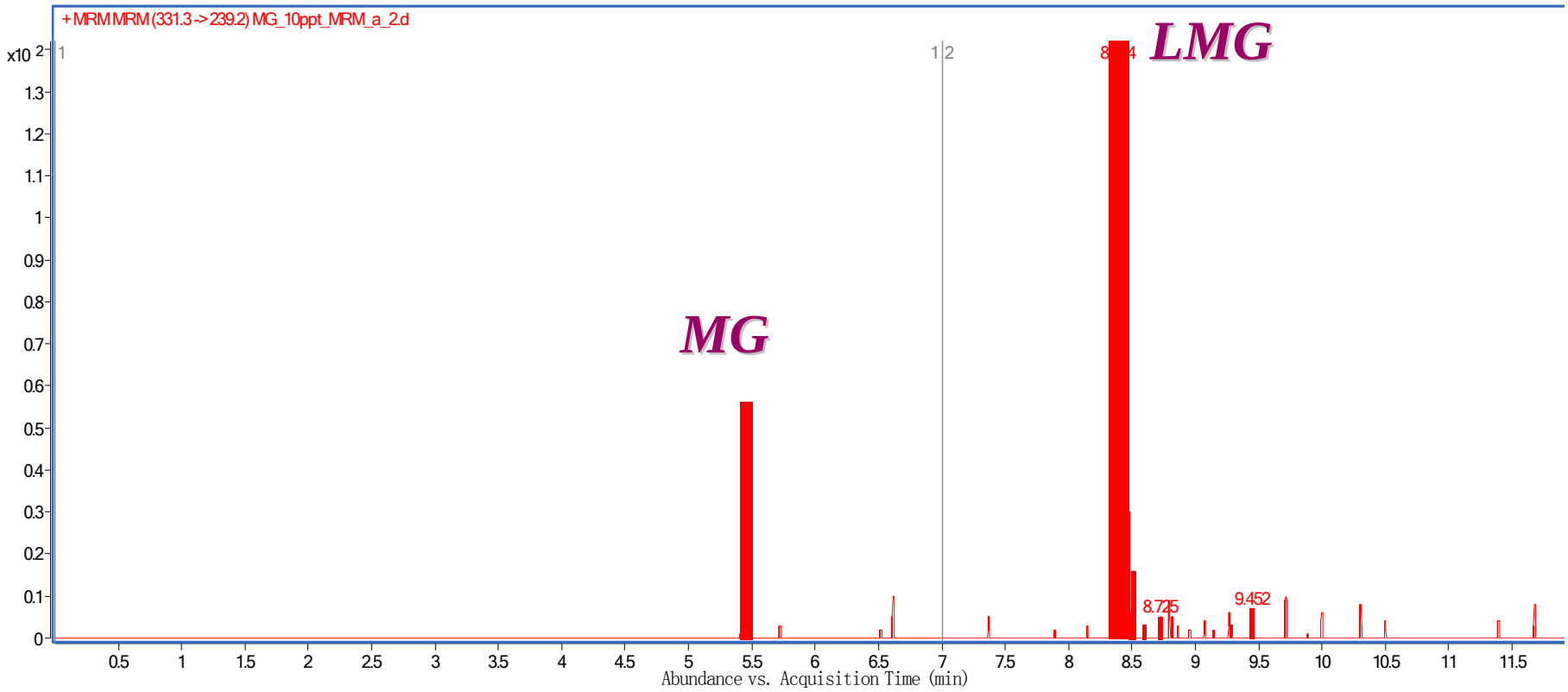
Malachite Green 100 ppt

329.3 →208.2 (Qual Ion)

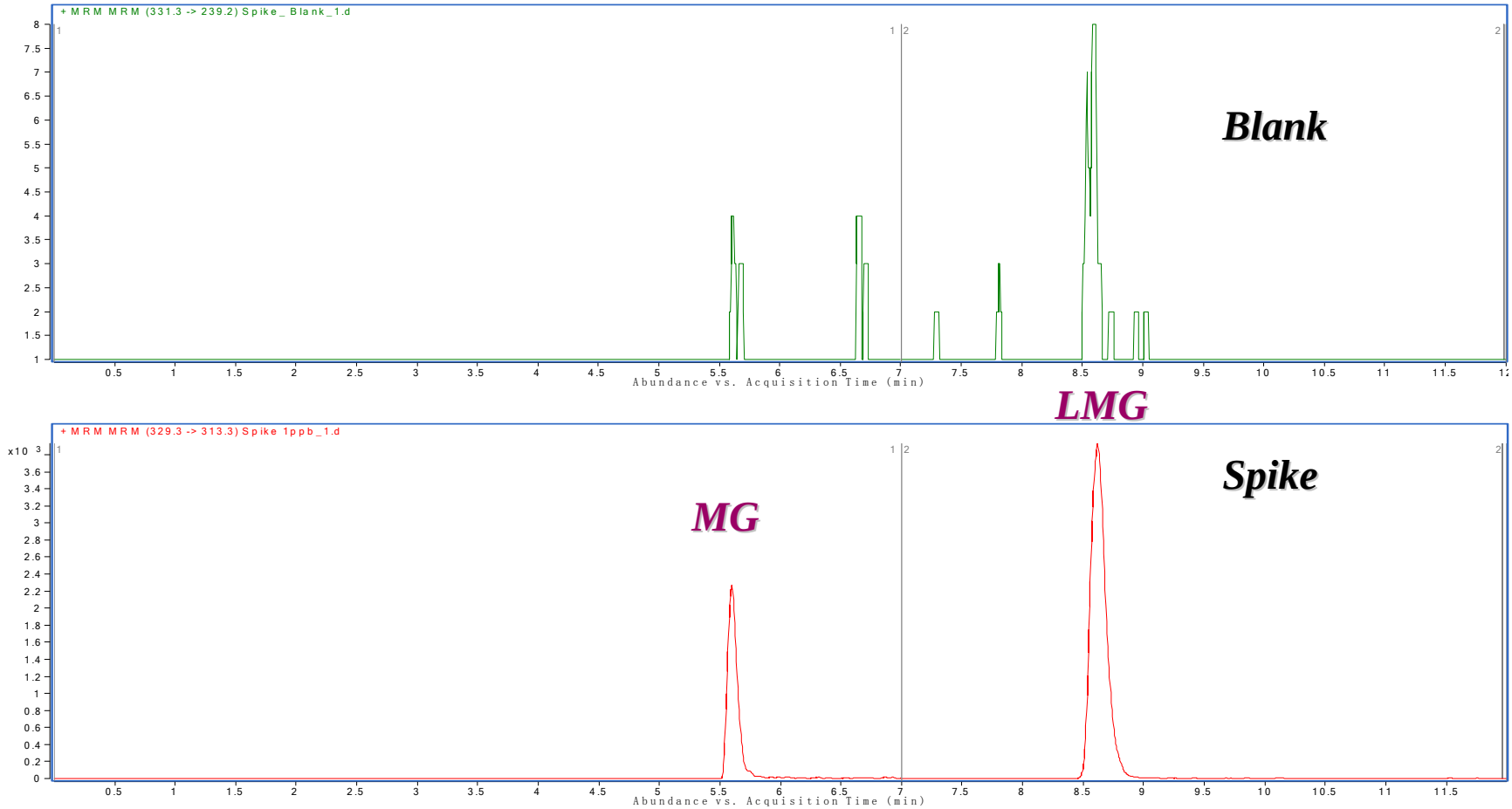
隐性孔绿MRM的提取离子流图
m/z 239 and m/z 316



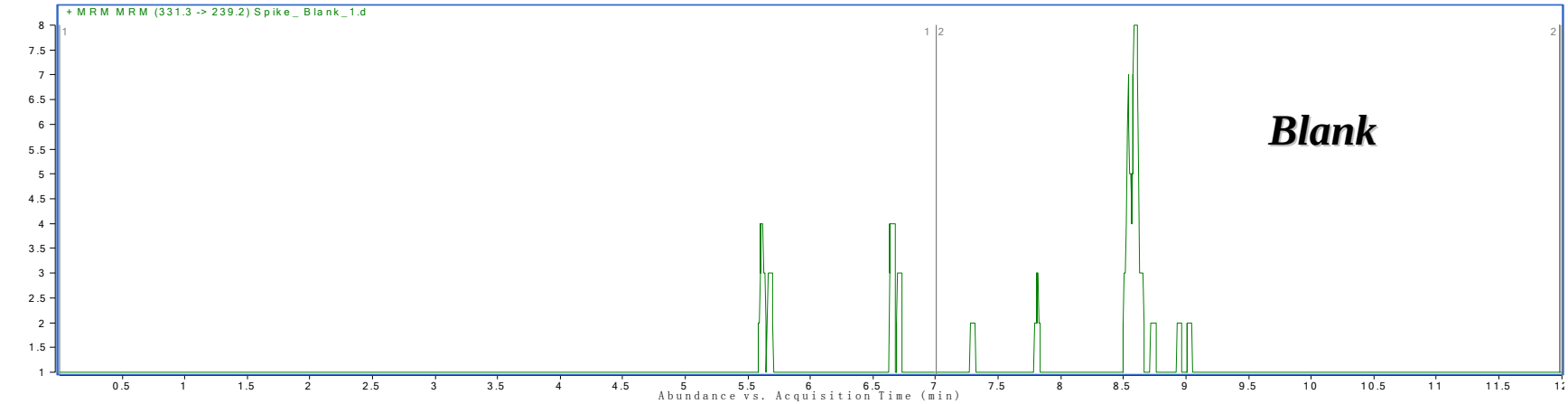
在10ppt的浓度下，孔绿和隐性孔绿的MRM图

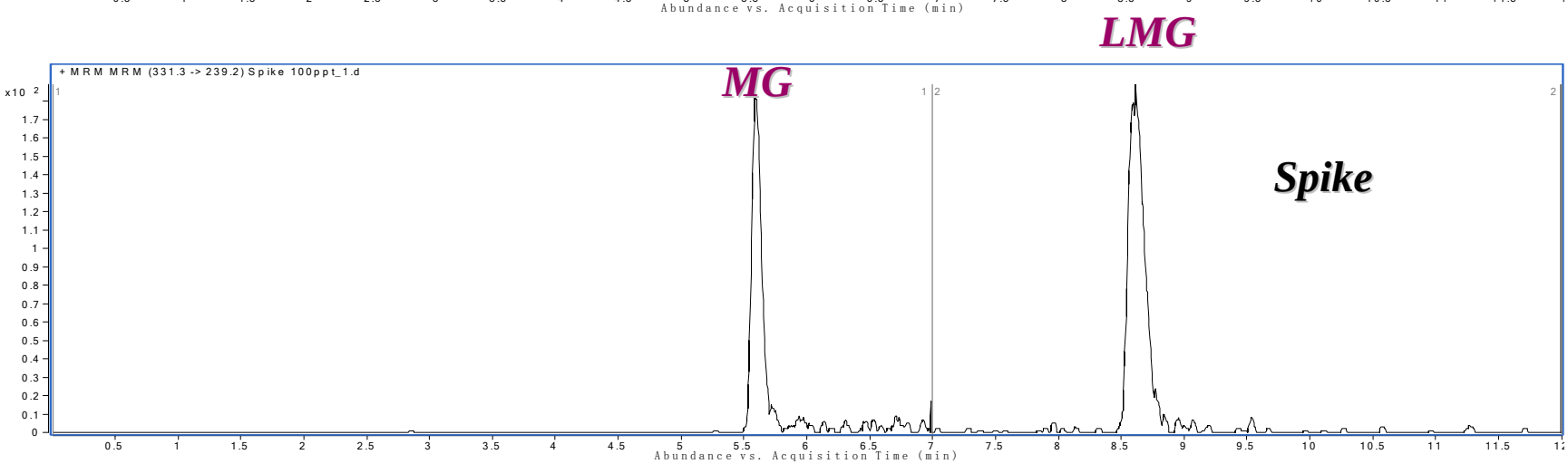


鳗鱼添加孔绿和隐性孔绿的分析结果 (1 ppb)



鳗鱼添加孔绿和隐性孔绿的分析结果 (100 ppt)





孔绿和隐性孔绿的分析结果的重现性

Compounds	Concentration (ng/mL)	RSD (%)
MG (m/z 329.3→313.3)	0.1	3.52
LMG (m/z 331.3→239.2)	0.1	2.25

[a] Each value was the average of 8 replicates (n=8).

实验条件

建立在三重串联四极质谱上分析100种农药方法

优化离子驻留时间得到最好的检测灵敏度

在溶剂和样品基质下，找出在一组中可以设置的最多的MRM离子数

同样的样品，将分析结果与在飞行时间质谱的结果比较

三重串联四极质谱上分析结果

建立分析100种农药的质谱条件

最佳碰撞电压

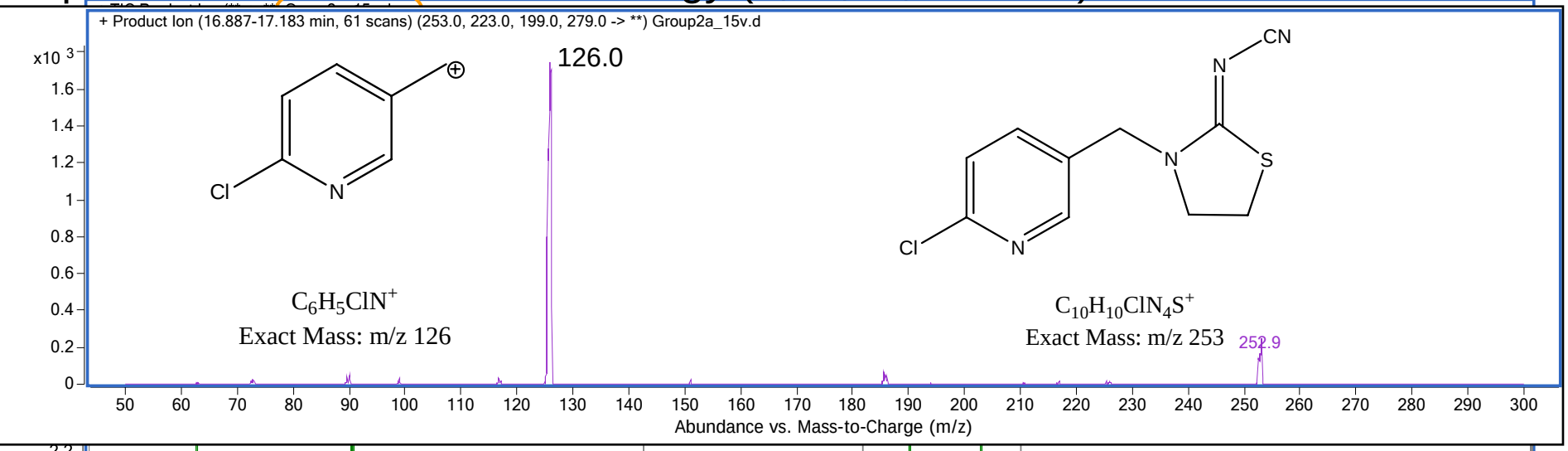
建立离子列表

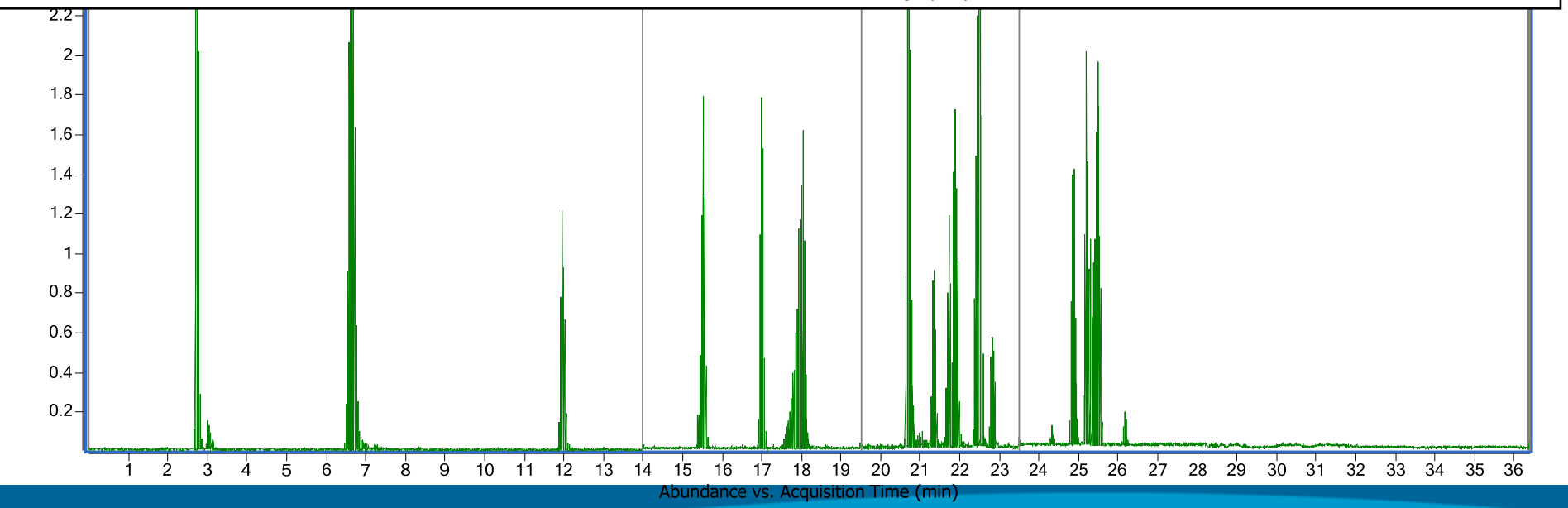
所有化合物的保留时间表

分析标样和实际样品

全扫描：获得MS/MS碎片离子...

Optimization of the Collision Energy (5,10,15, 20 eV...)





100种农药分析的MRM离子列表

Name.	Rt.	Nominal Mass	Frag. Ion 1	Collision energy
Cyromazine	2.7	167	125	20
Thiosultap	2.7	312	232	10
Cartap	3	150	105	15
Thiocyclam	4.5	182	137	10
Aldicarb Sulfoxide	6.4	207	89	5
Carbendazim	6.6	192	160	15
Thiabendazole	7.9	202	175	25
Aldicarb Sulfone	10.8	223	148	5
Nitenpyram	11	271	225	10
Hydroxyatrazine	11.2	198	156	15
Methomyl	11.5	163	88	5
Deisopropylatrazine	11.9	174	132	15
Imazapyr	12.5	262	234	15
Metamitron	13.9	203	175	15
Fenuron	14.5	165	72	15
Deethylatrazine	14.8	188	146	15
Imidacloprid	14.8	256	209	10
Dimethoate	15.4	230	199	5
Acetamiprid	15.5	223	126	15
Prometon	15.7	226	184	20
Irgarol metabolite	16	214	158	15
Methiocarb sulfone	16.4	258	122	5
Nicosulfuron	16.9	411	182	15
Thiacloprid	17	253	126	15
Imazalil	17.2	297	159	15
mebendazole	17.2	296	264	20
Aldicarb	17.5	213	89	10

Group 1

...MRM transitions

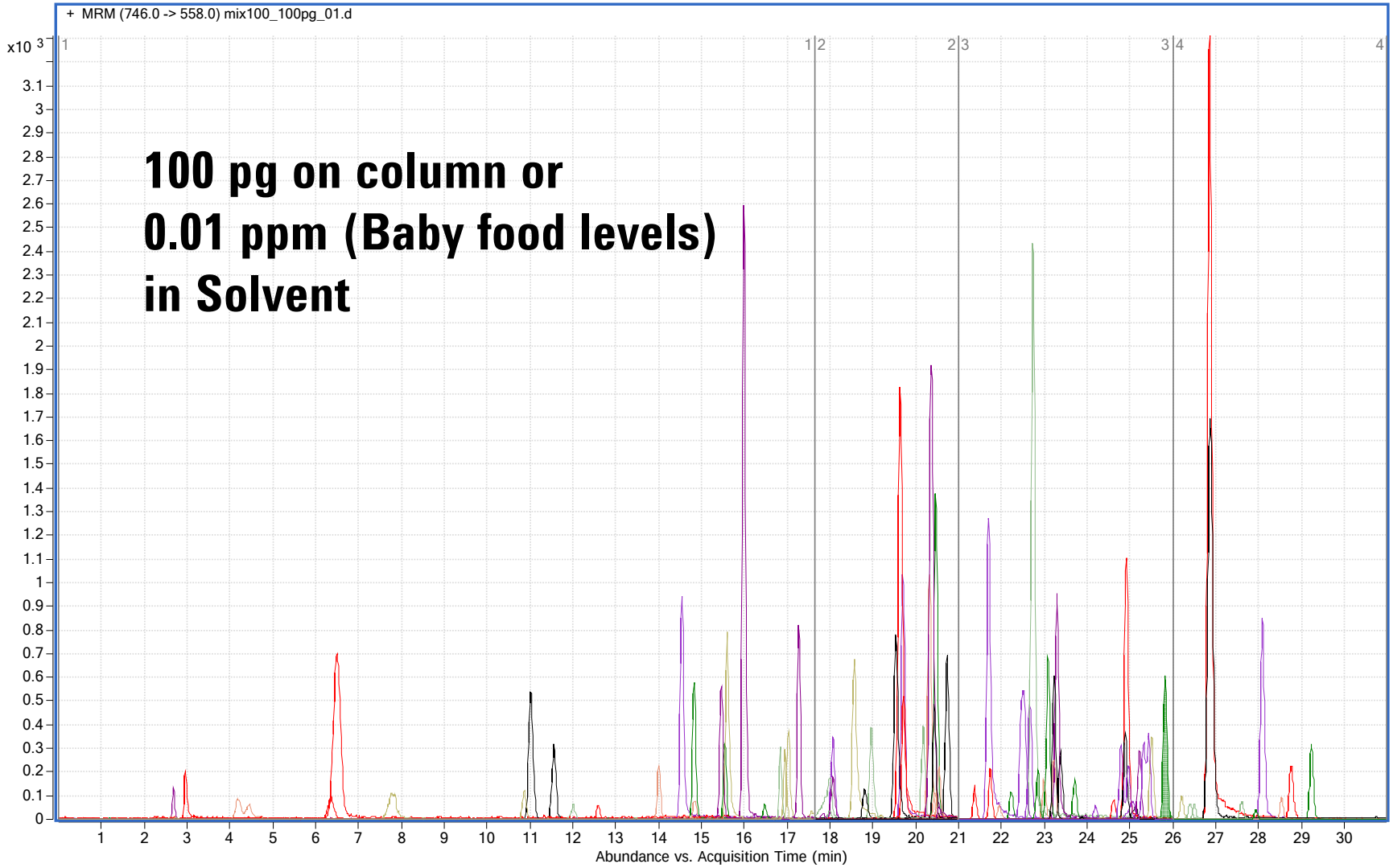
Name.	Rt.	Nominal Mass	Frag. Ion 1	Collision energy	
Imazaquin	17.8	312	284	20	
Oxadixyl	17.9	279	219	10	
Fluroxypyr	17.9	255	209	10	
Simazine	18	202	132	15	
Monuron	18	199	72	10	
Lenacil	18.4	235	153	10	
Cyanazine	18.5	241	214	10	
Metolcarb	18.5	166	109	5	
Dichlorvos	18.7	221	109	15	
Metribuzin	18.9	215	187	15	**Group 1**
Prometryn	19.5	242	200	20	
Terbutryn	19.5	242	186	15	
Carbofuran	19.6	222	165	10	
Bendiocarb	19.7	224	167	5	
Spinosad A	20	732	142	5	
Carbaryl	20.1	202	145	5	
Irgarol 1051	20.3	254	198	15	
Atrazine	20.3	216	174	15	
Metalaxyl	20.4	280	248	10	
Difenoxuron	20.4	287	123	15	
Isoproturon	20.4	207	72	15	**Group 2**
Bensultap	20.5	432	290	15	
Diuron	20.5	233	72	15	
Spinosa D	20.7	746	558	5	
Ethiofencarb	20.7	226	107	5	

...MRM transitions...

Name.	Rt.	Nominal Mass	Frag. Ion 1	Collision energy
Dimethomorph	21.3	388	301	20
Propachlor	21.6	212	170	10
Prochloraz	21.9	376	308	10
Propanil	22.2	218	162	15
Cyproconazole	22.5	292	70	10
Methiocarb	22.6	226	169	5
Terbutyylazine	22.7	230	174	15
Bromuconazole	22.8	376	159	20
Fenamiphos	23	304	217	15
Mehtidathion	23	303	145	5
Azoxystrobin	23.2	404	372	10
Phosmet	23.2	318	160	5
Dimethenamide	23.3	276	244	10
Promecarb	23.3	208	151	10
Molinate	23.7	188	126	10
Diflubenzuron	24.1	311	158	10
Iprodione	24.6	330	245	10
Propiconazole	24.7	342	159	20
Malathion	24.8	331	127	5
Metolachlor	24.9	284	252	10
Alachlor	25	270	238	10
Acetochlor	25.1	270	224	10
Flufenacet	25.2	364	194	5
Difeconazole	25.3	406	251	20
Chlorfenvinphos	25.5	359	155	10
Benalaxyl	25.8	326	294	5

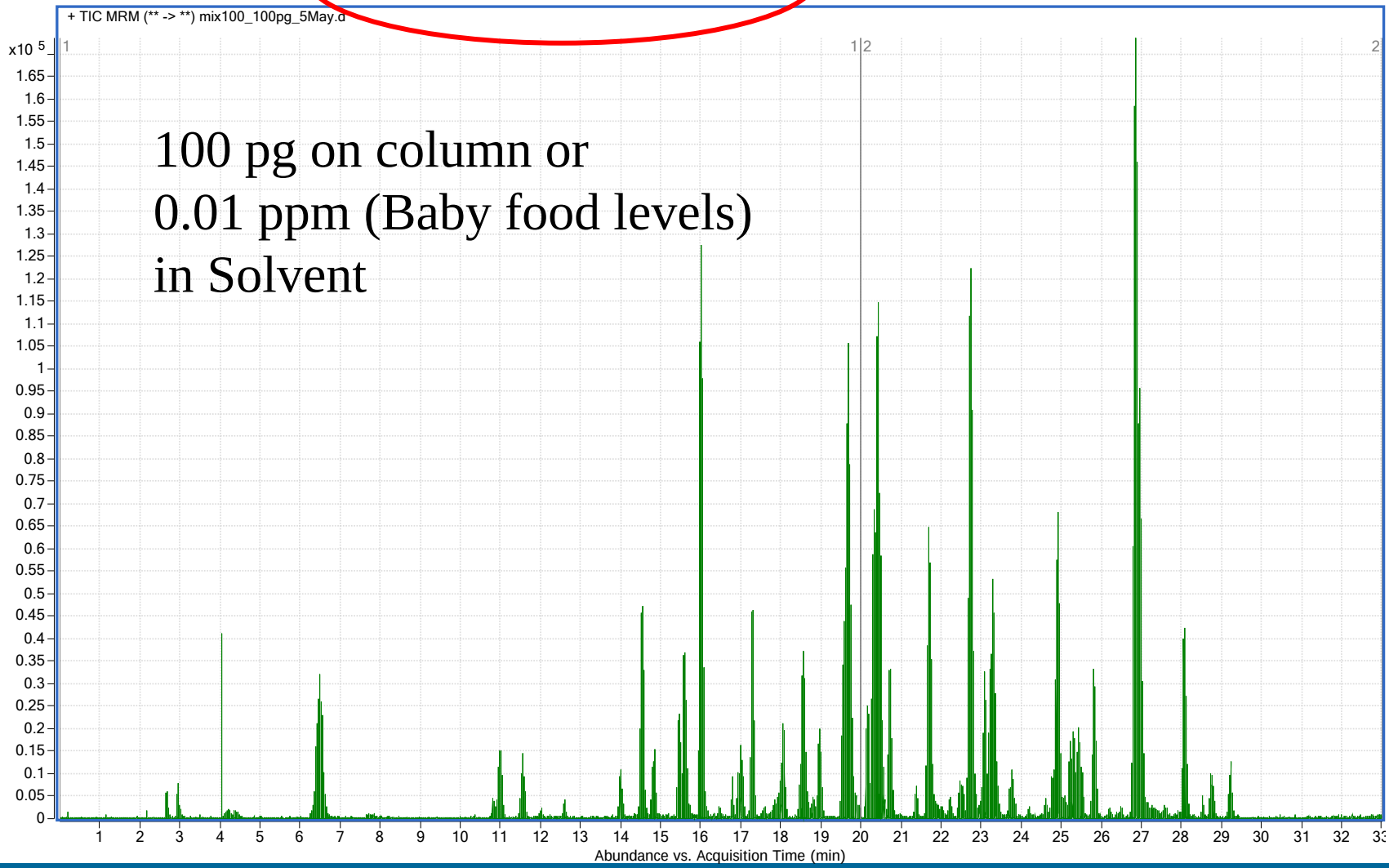
Group 2

100种农药化合物的提取离子流图

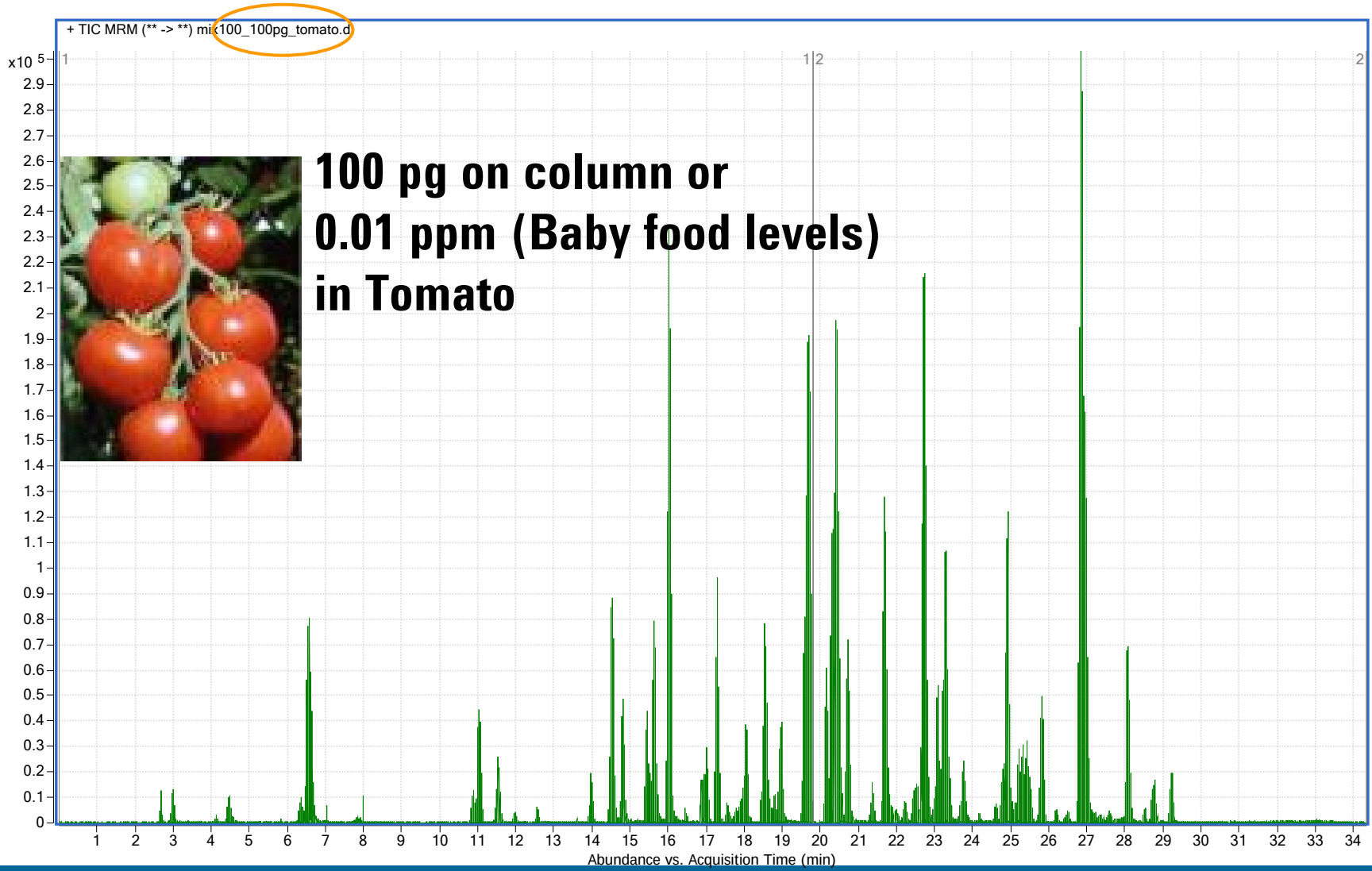


100种农药化合物的MRM提取离子流图

Two time segments: 15 msec. dwell time

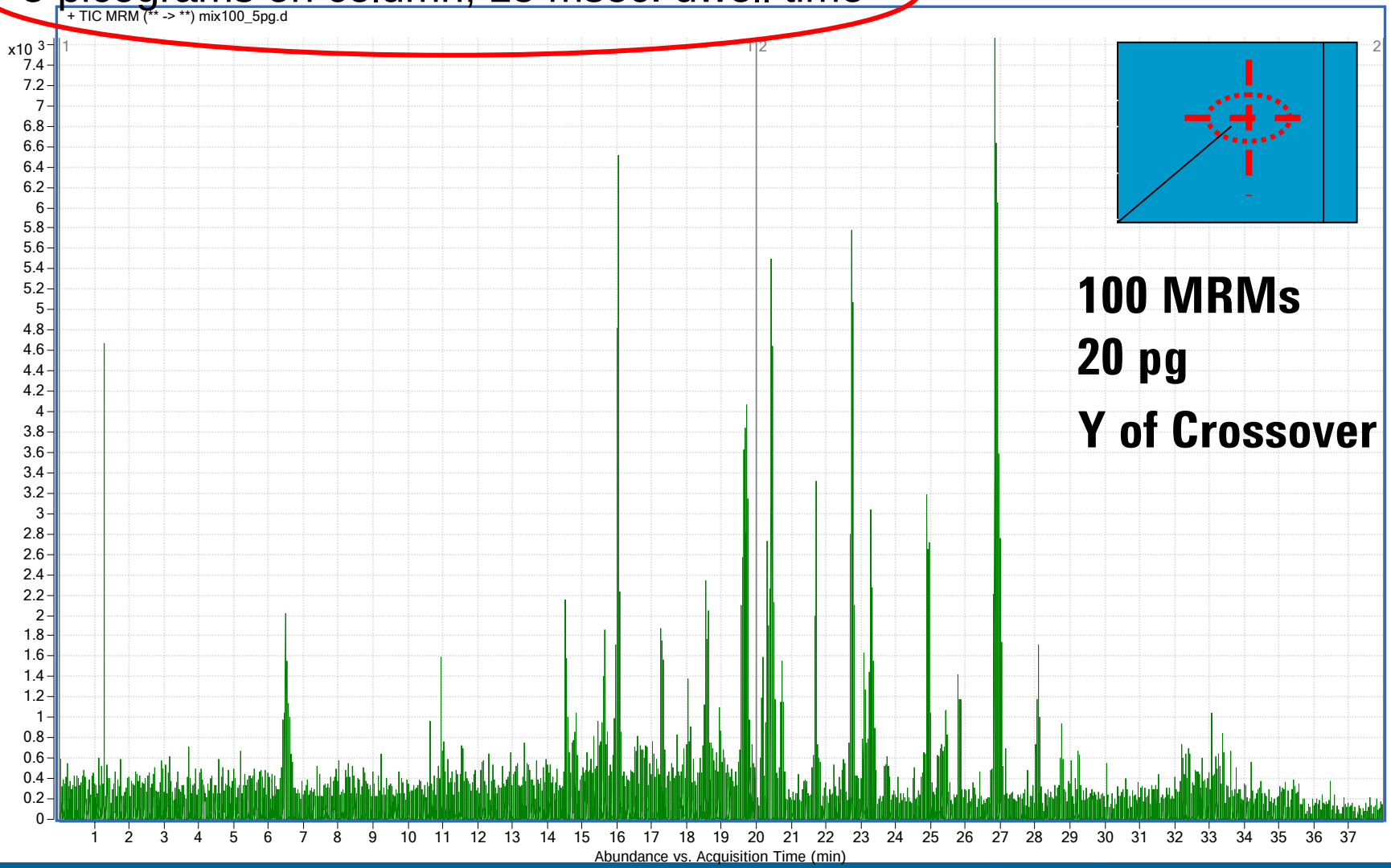


番茄样品添加：几乎没有基质影响



...检测限

5 picograms on column, 15 msec. dwell time



日本肯定列表中的81种农药残留化合物的LC/MS/MS分析方法

LC	**: 1200LC**
Column	**: Zorbax Extend C18(100mm,2.1mm,1.8 μ m)**
Mobile phase	**: A: ACN, B: 0.1%HCOOH+10mM$HCOONH_4$**
	10%A/90%B---(30min)---100%A
Column temp	**: 40℃**
Sample volume	**: 5uL**
Flow rate	**: 0.2mL/min**
MS	**: Agilent 6410 LC-MS**
Ionization	**: ESI (Positive)**
Collision energy	**: see next table(N2 gas)**
Scan range	**: m/z 100-450**
Drying gas	**: 10L/min at 350C**
Nebulizer gas	**: 345kPa**
Fragmentor	**: 100V**

研究的农药化合物列表

No	Pesticides	No	Pesticides	No	Pesticides
1	Azafenidin	30	Spinosyn D	59	Propaquizafop
2	Acibenzolar-S-methyl	31	Isoxaflutole	60	Hexaflumuron
3	Azoxystorvin	32	Sethoxydim	61	Hexythiazox
4	Aramite	33	Diallate	62	Pebulate
5	Aldicarb	34	Tetrachlorvinphos E	63	Pencycuron
6	Aldoxycarb	35	Tetrachlorvinphos Z	64	Benzyladenine
7	Iprodione	36	Tebuthiuron	65	Bendiocarb
8	Imazalil	37	Tebufenozide	66	Benfuracarb
9	Ethoxyquin	38	Tepraloxidim	67	Benfluralin
10	Ethofenprox	39	Teflubenzuron	68	Boscalid
11	Epoxiconazole	40	Triflumizole	69	Thiodicarb
12	Oxamyl	41	Triflumuron	70	Methomyl
13	Carbaryl	42	Novaluron	71	Methabenthiazuron
14	Carbofuran	43	Hydramethylnon	72	Methiocarb
15	Quizalofop-ethyl	44	Pinoxaden	73	Mepanipyrim
16	Clethodim	45	Bifenazate	74	Monolinuron
17	Clofentezine	46	Pyraclostrobin	75	Linuron
18	Chlorfluazuron	47	Pyridate	76	Lufenuron
19	Chloroxuron	48	Pirimicarb	77	Warfarin
20	Diuron	49	Pindone	78	Inabenfide
21	Cycloate	50	Fenobucarb	79	Indanofan
22	Cycloprothrin	51	Fenamidone	80	Ethiofencarb
23	Dinotefuran	52	Fenpyroximate E	81	Etobenzanide
24	Diflubenzuron	53	Fenpyroximate Z	82	Oxaziclomefone
25	Cyprodinil	54	Butroxydim	83	Carpropamide
26	Dimethomorph E	55	Flufenacet	84	Cumyluron
27	Dimethomorph Z	56	Flufenoxuron	85	Daimuron
28	Silafluofen	57	Fluridone	86	Furametpyr
29	Spinosyn A	58	Brodifacoum	87	Pentoxazone

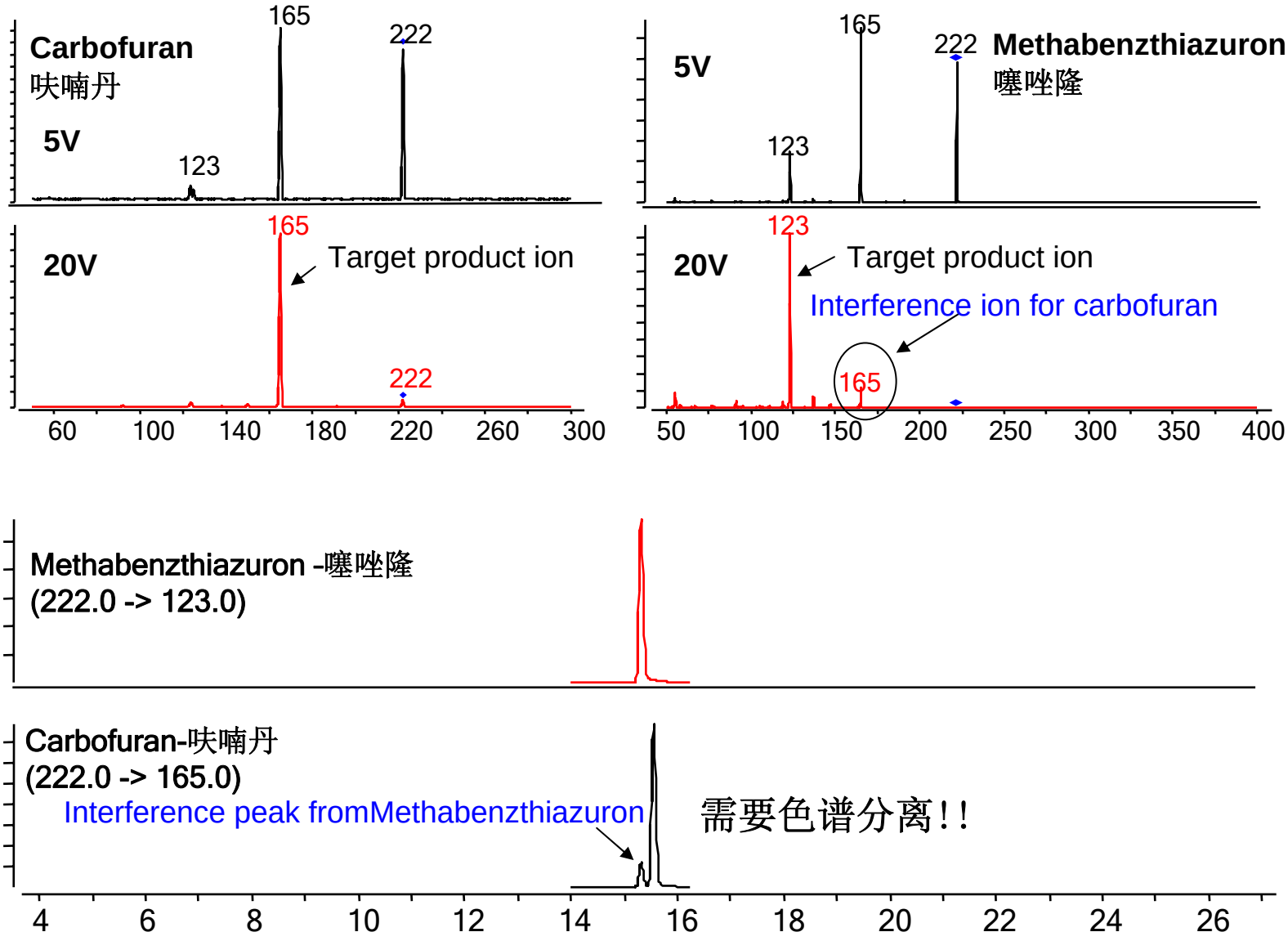
MRM 分析方法(正离子模式)

No	Pesticides	Q1	Q3	CV	R.T	TS
1	Dinotefuran	203	129	15	2.2	1
2	Methomyl	163	88	12	3.8	1
3	Aldoxycarb	240	223	4	3.8	1
4	Oxamyl	237	72	8	4.4	1
5	Benzyladenine	226	91	25	10.3	2
6	Aldicarb	116	89	10	12.7	3
7	Tebuthiuron	229	172	20	12.9	3
8	Pirimicarb	239	72	20	13.1	3
9	Imazalil	297	159	20	15.2	4
10	Thiodicarb	355	88	5	15.2	4
11	Bendiocarb	224	109	15	15.2	4
12	Carbofuran	222	123	20	15.3	4
13	Methabenthiazuron	222	165	15	15.5	4
14	Carbaryl	202	145	18	16.0	4
15	Ethiofencarb	226	107	15	16.5	5
16	Monolinuron	215	126	10	16.5	5
17	Diuron	233	72	20	16.7	5
18	Furametpyr	334	290	15	16.7	5
19	Inabenfide	339	321	20	18.0	6
20	Dimethomorph E	388	301	25	18.5	6
21	Dimethomorph Z	388	301	25	18.9	6
22	Warfarin	309	163	10	19.0	6
23	Methiocarb	226	121	15	19.1	6
24	Fluridone	330	309	35	19.2	6
25	Fenobucarb	208	95	15	19.2	6
26	Linuron	249	160	17	19.5	6
27	Acibenzolar-S-	211	91	25	19.6	6
28	Chloroxuron	291	72	18	19.9	6,7
29	Cumyluron	303	119	20	20.3	7
30	Azoxystorvin	404	372	10	20.3	
31	Epoxiconazole	330	121	15	20.3	7
32	Spinosyn A	733.4	143	25	20.4	7
33	Fenamidone	312	236	10	20.5	
34	Daimuron	269	151	15	20.6	7
35	Boscalid	342.9	307.9	20	20.7	7
36	Tepraloxidim	342	250	10	20.7	7,8
37	Mepanipyrim	224	106	28	21.1	7,8
38	Bifenazate	301	198	5	21.4	8
39	Diflubenzuron	311	158	17	21.4	8
40	Spinosyn D	747.4	143	25	21.5	8
41	Ethoxyquin	218	148	25	21.8	8
42	Iprodione	329.9	245	15	21.8	8
43	Tetrachlorvinphos E	366.8	127	10	21.9	8
44	Pinoxaden	401	317	10	22.1	8
45	Cyprodinil	226	226	15	22.1	8
46	Flufenacet	364	152	20	22.5	8,9
47	Tebufenozide	297	133	15	22.9	9
48	Indanofan	341	175	20	23.0	9
49	Triflumuron	359	156	15	23.4	9
50	Hydramethylhon	495	495	15	23.6	9
51	Carpropamide	333.9	138.9	20	23.8	9
52	Triflumizole	346	278	10	24.0	9
53	Etobenzanide	339.9	179	20	24.3	9
54	Pyraclostrobin	388	194	15	24.5	9
55	Clofentezine	302.9	138	10	24.7	9
56	Hexaflumuron	460.8	158	20	24.8	9
57	Pencycuron	329	125	20	24.9	9
58	Teflubenzuron	380.8	158	15	25.1	9
59	Pebulate	204	128	10	25.3	9
60	Cycloate	216	83	20	25.3	9
61	Novaluron	492.8	158	12	25.6	9,10
62	Quizalofop-ethyl	373	299	20	26.1	10
63	Propaquizafop	443.9	100	20	26.5	10
64	Clethodim	360	164	15	26.5	10
65	Diallate	270	86	15	26.5	10
66	Lufenuron	510.8	158	10	26.7	10
67	Fenpyroximate E	422	366	15	26.8	10
68	Pentoxazone	371	286	15	26.9	10
69	Benfuracarb	411	195	20	27.0	10
70	Oxaziclomefone	375.9	190	15	27.2	10
71	Sethoxydim	328	282	10	27.4	10,11
72	Flufenoxuron	488.8	158	20	27.7	10,11
73	Hexythiazox	353	228	20	28.1	11
74	Fenpyroximate Z	422	366	15	28.3	11
75	Benfluralin	336	236	10	28.5	11
76	Chlorfluazuron	539.8	382.9	25	28.5	11
77	Brodifacoum-1	522.9	335	20	29.2	11
78	Cycloprothrin	499	228	10	29.7	11
79	Brodifacoum-2	522.9	335	20	29.8	11
80	Ethofenprox	394	358.9	15	32.1	12
81	Pyridate	379	207	10	32.1	12

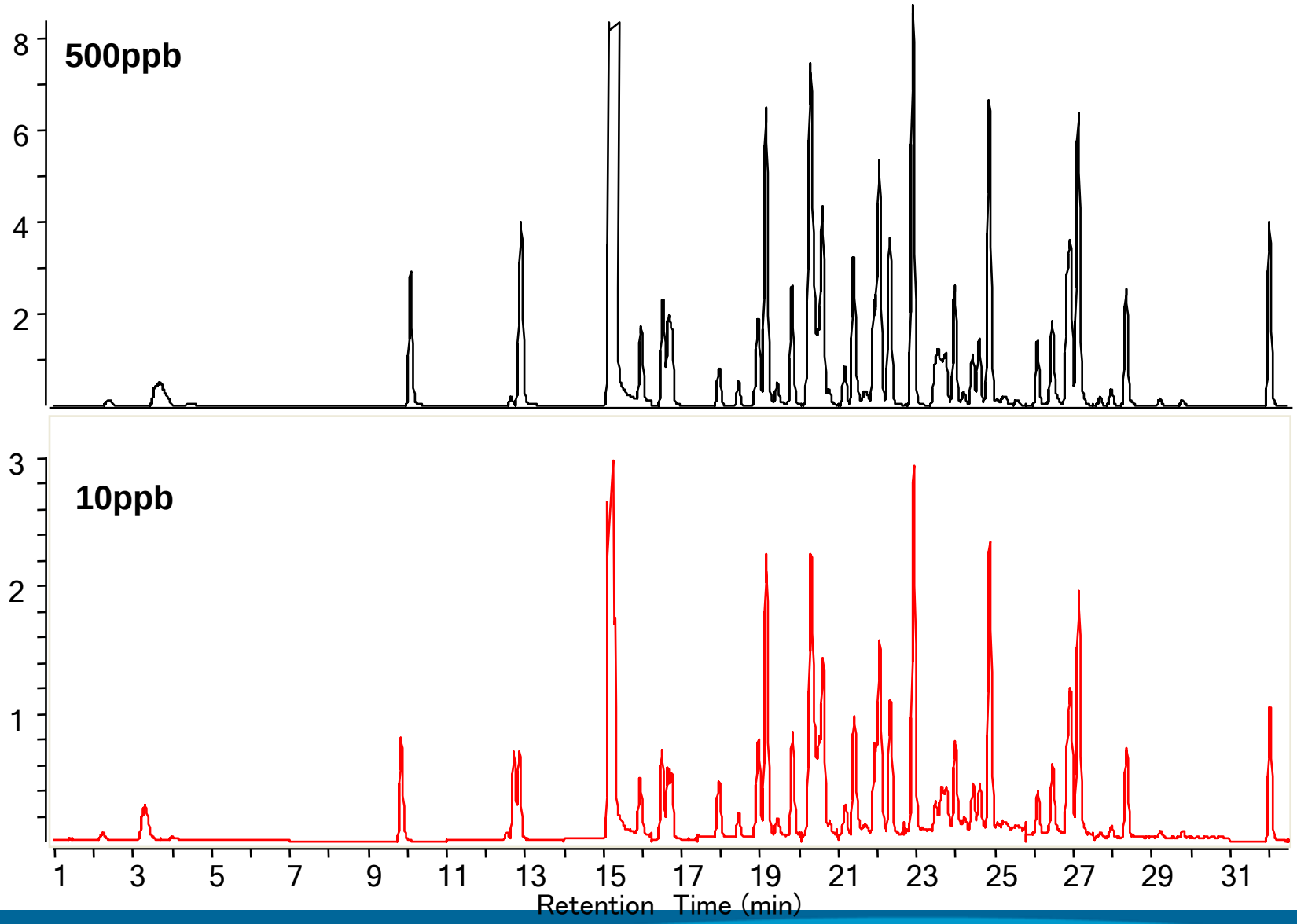
MRM 分析方法(正离子)

No	name	Precerson	Product	Collision	R.time
1	Benzyladenine	224	133	20	9.9
2	Diuron	231	186	23	16.7
3	Inabenfide	337	122	20	18.0
4	Warfarin	307	161	20	19.0
5	Daimuron	313	106	5	20.6
5	Tepraloxidim	340	248	15	20.8
6	Diflubenzuron	309	289	7	21.4
7	Tebufenozide	351	149	15	22.9
9	Triflumuron	357	154	10	23.5
10	Carpropamide	380	334	3	23.7
11	Hexaflumuron	459	439	8	24.8
12	Teflubenzuron	379	339	15	25.1
13	Novaluron	491	471	15	25.6
14	Clethodim	358	266	12	26.5
15	Lufenuron	509	326	15	26.7
16	Flufenoxuron	487	156	10	27.7
17	Chlorfluazuron	540	519	10	28.6
18	Brodifacoum-1	523	523	20	29.2
18	Brodifacoum-2	523	523	20	29.8

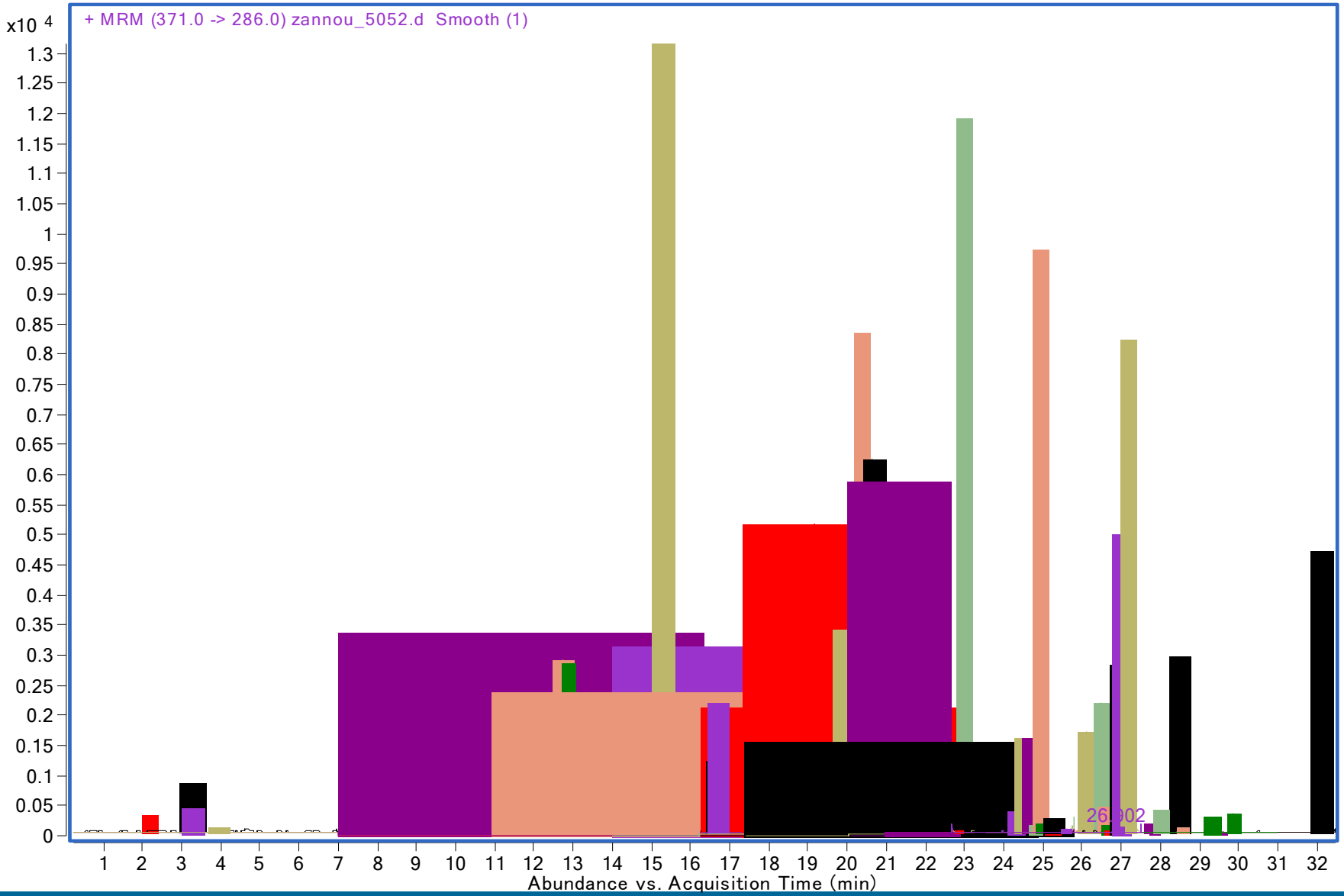
MRM分析条件的优化


Carbofuran
呋喃丹
5V
165
222
123
Methabenzthiazuron
噻唑隆
5V
165
222
123
20V
165
Target product ion
222
20V
123
Target product ion
Interference ion for carbofuran
165
60 100 140 180 220 260 300
50 100 150 200 250 300 350 400
Methabenzthiazuron -噻唑隆
(222.0 -> 123.0)
Carbofuran-呋喃丹
(222.0 -> 165.0)
Interference peak fromMethabenzthiazuron
需要色谱分离!!
4 6 8 10 12 14 16 18 20 22 24 26

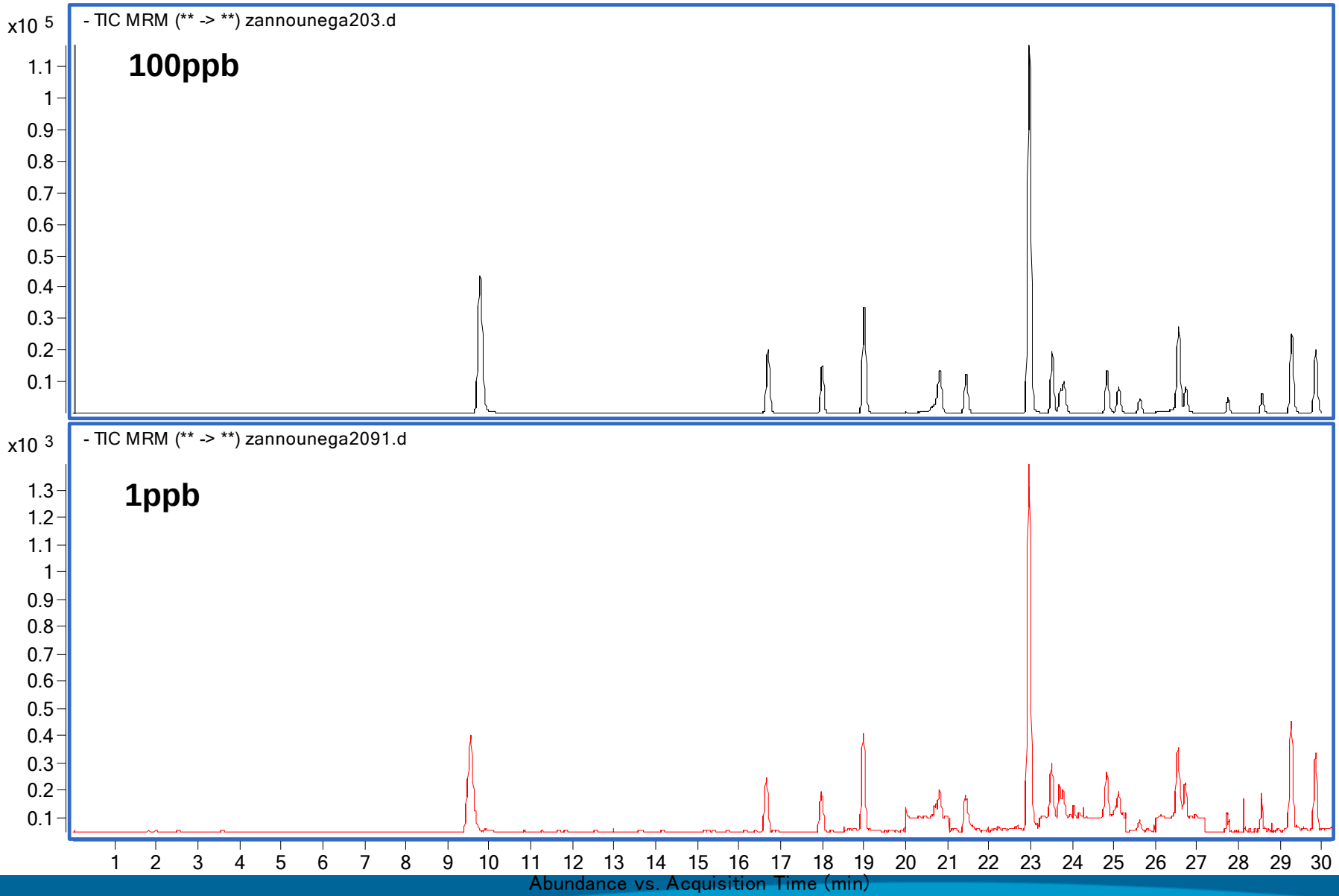
农药化合物的MRM的提取离子流图(正离子模式)



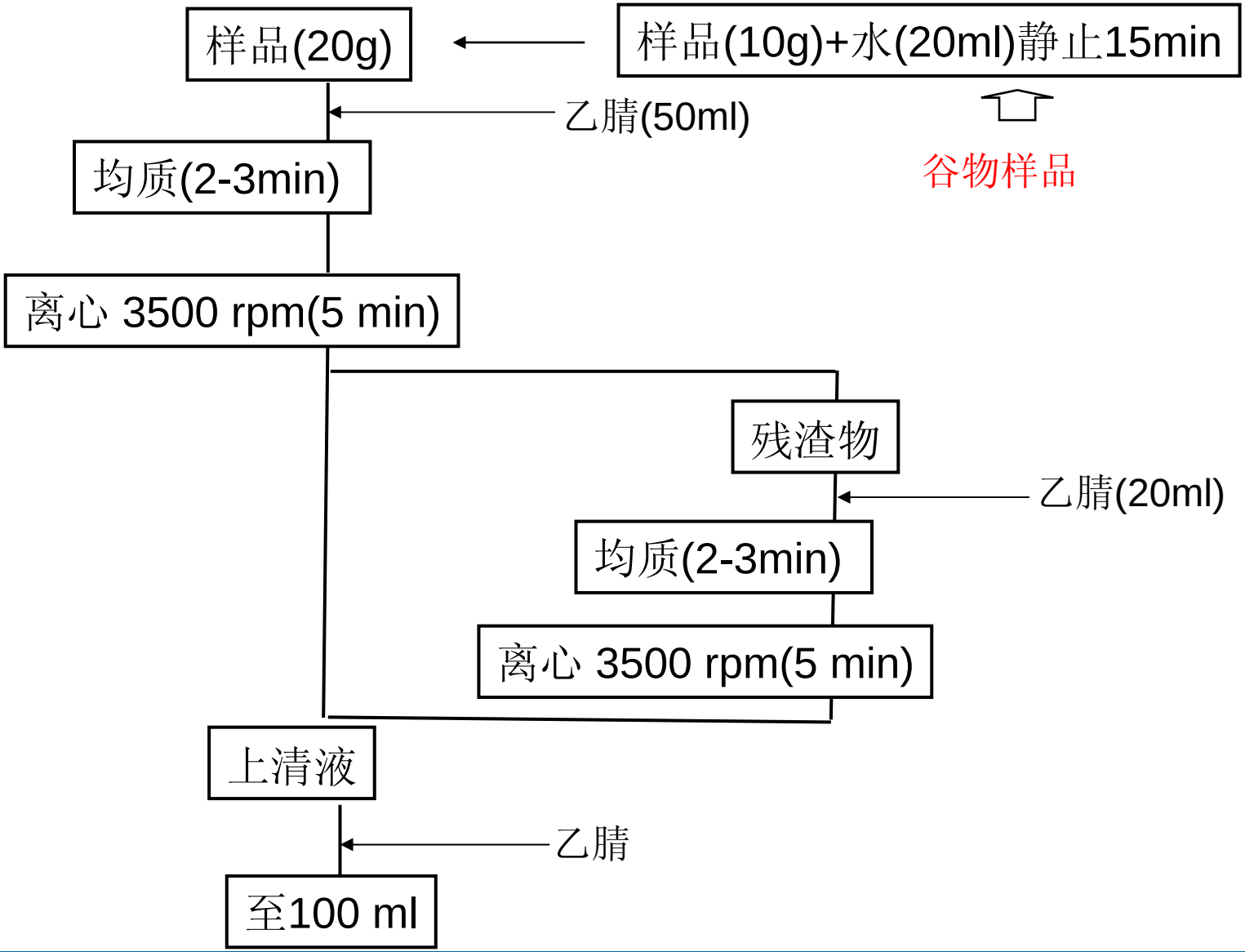
农药化合物的MRM的提取离子流图(正离子模式，1ppb)



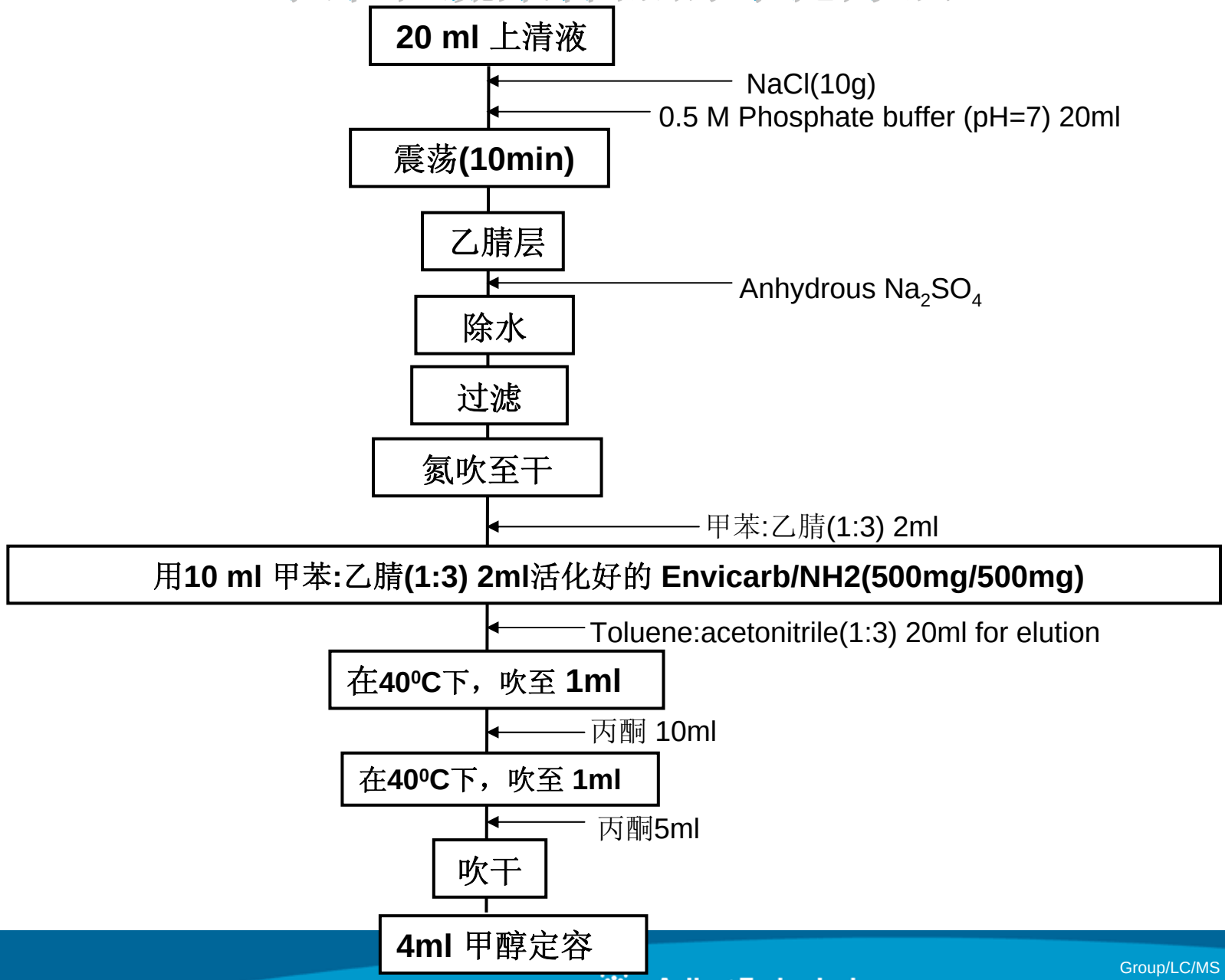
农药化合物的MRM的提取离子流图(负离子模式)



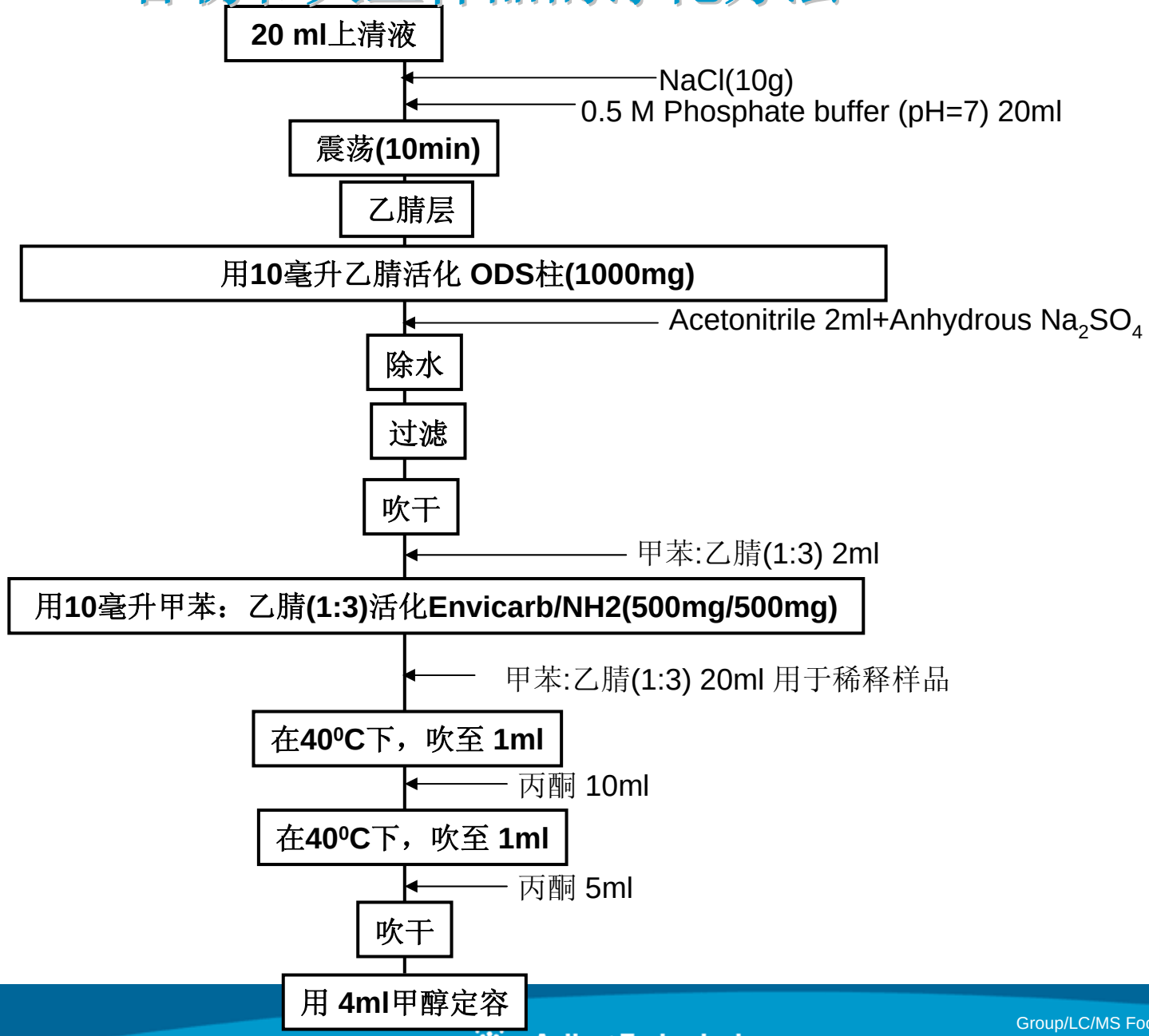
食品样品的提取方法


样品(10g)+水(20ml)静止15min
谷物样品
样品(20g)
乙腈(50ml)
均质(2-3min)
离心 3500 rpm(5 min)
残渣物
乙腈(20ml)
均质(2-3min)
离心 3500 rpm(5 min)
上清液
乙腈
至100 ml

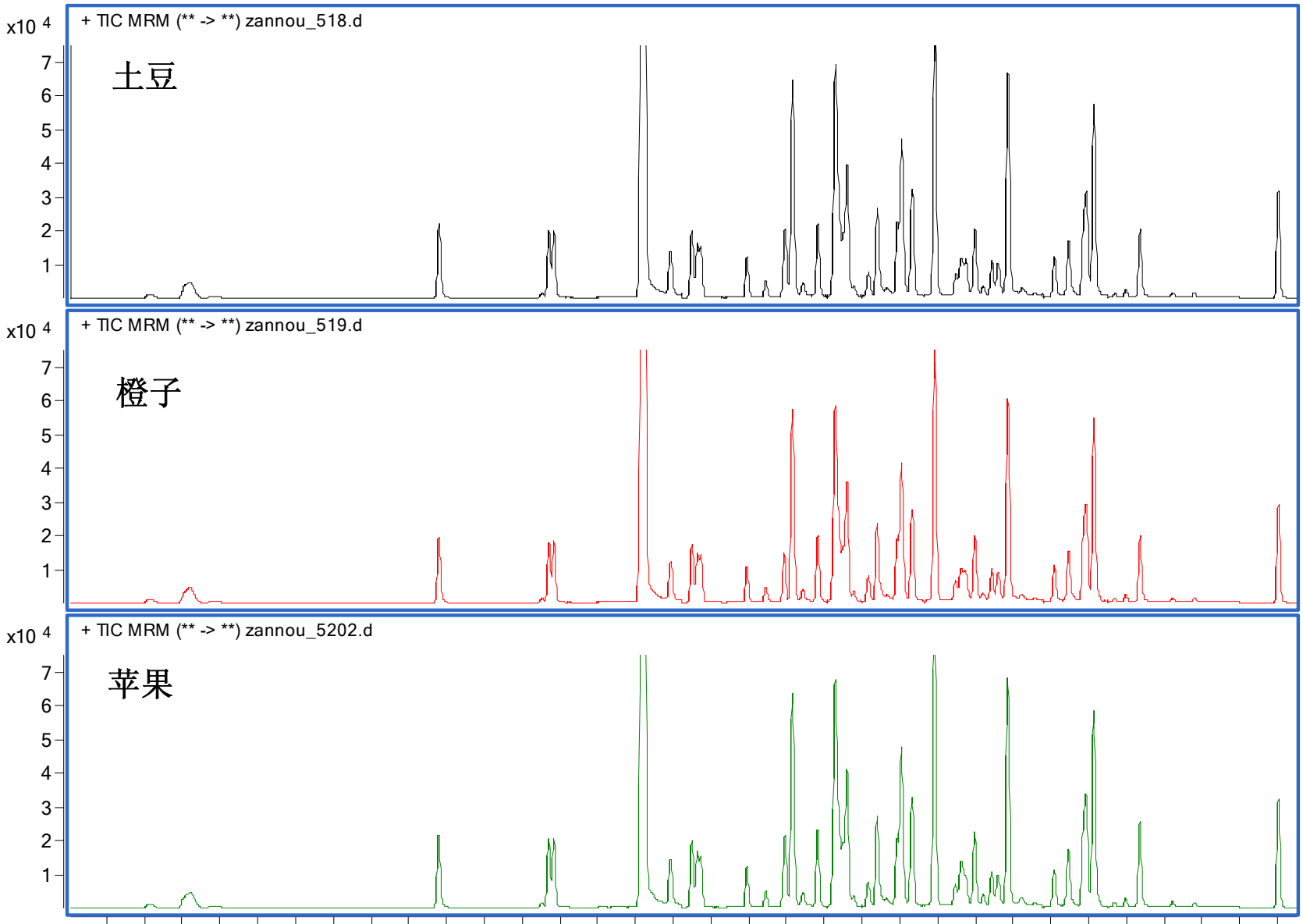
水果和蔬菜样品的净化方法



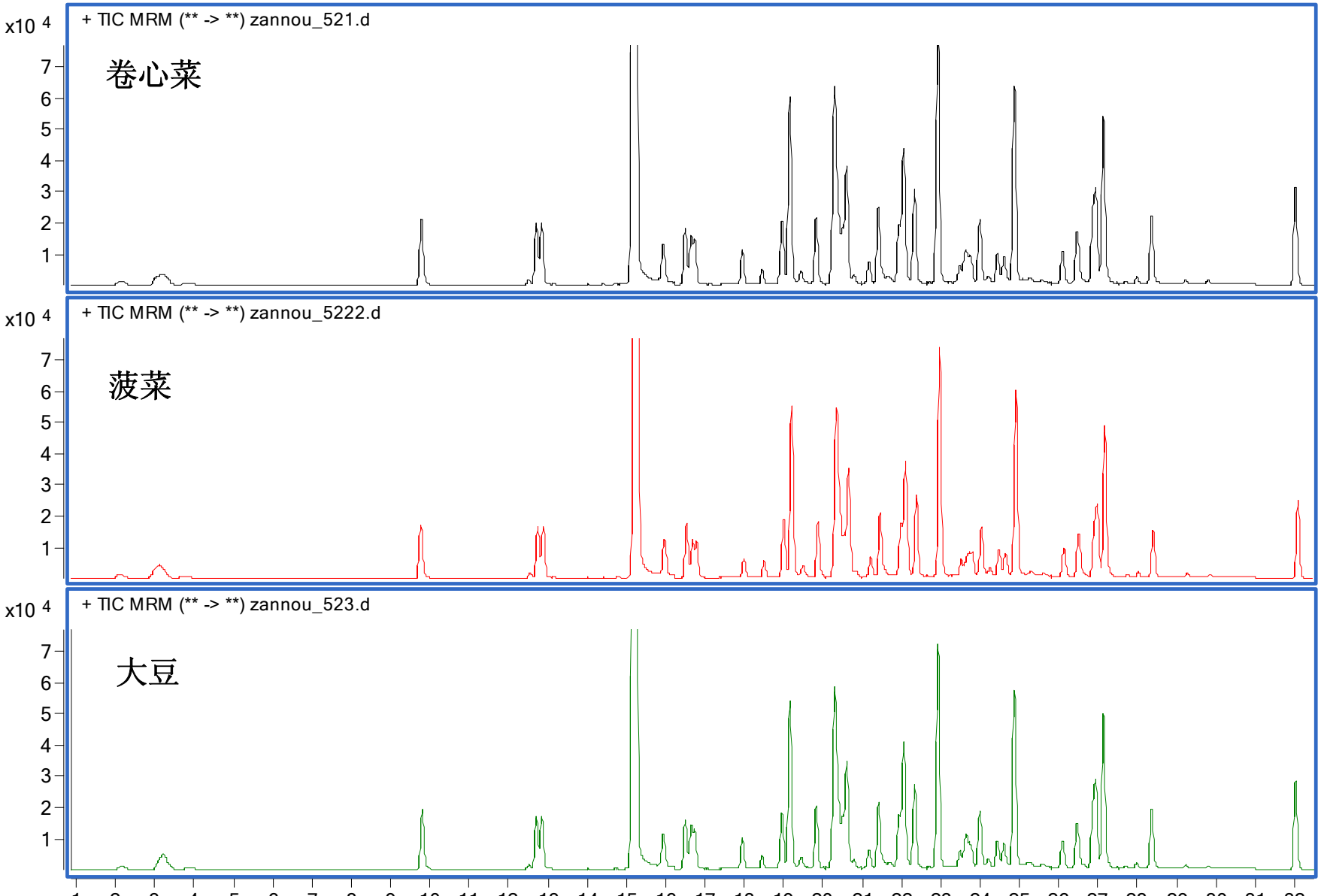
谷物和大豆样品的净化方法



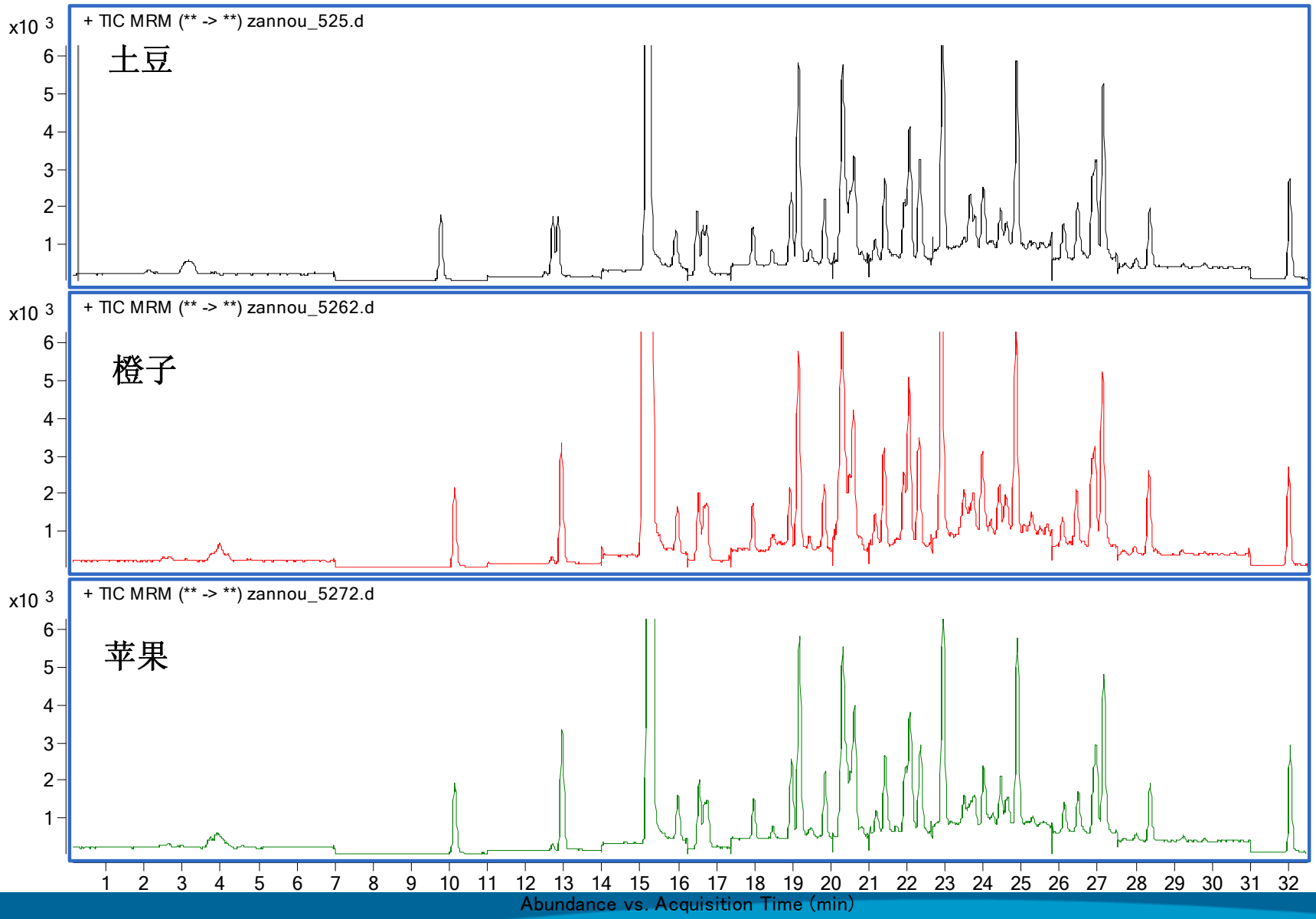
食品中农药化合物的MRM 提取离子流 (10ppb，正离子)



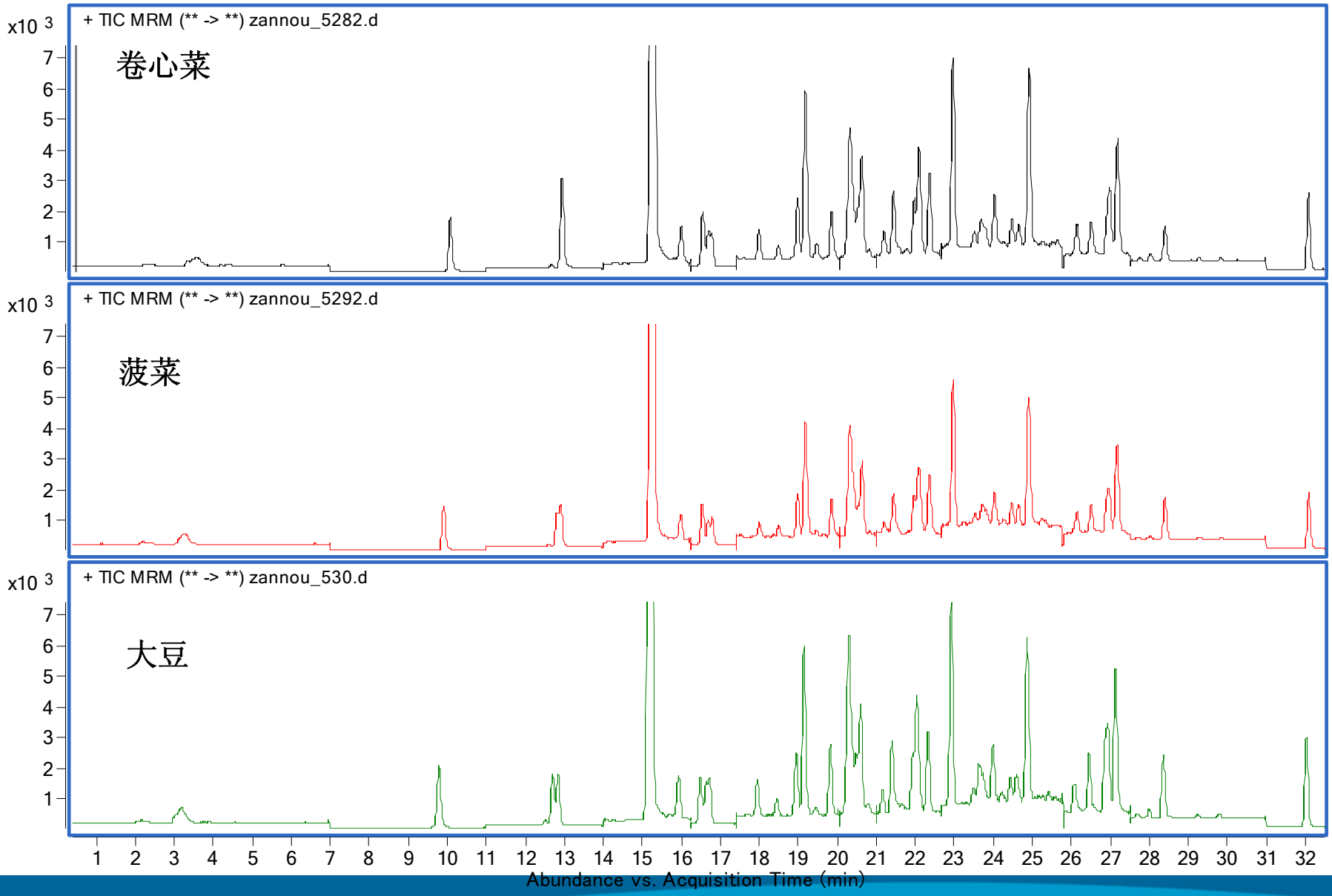
食品中农药化合物的MRM 提取离子流 (10ppb，正离子)



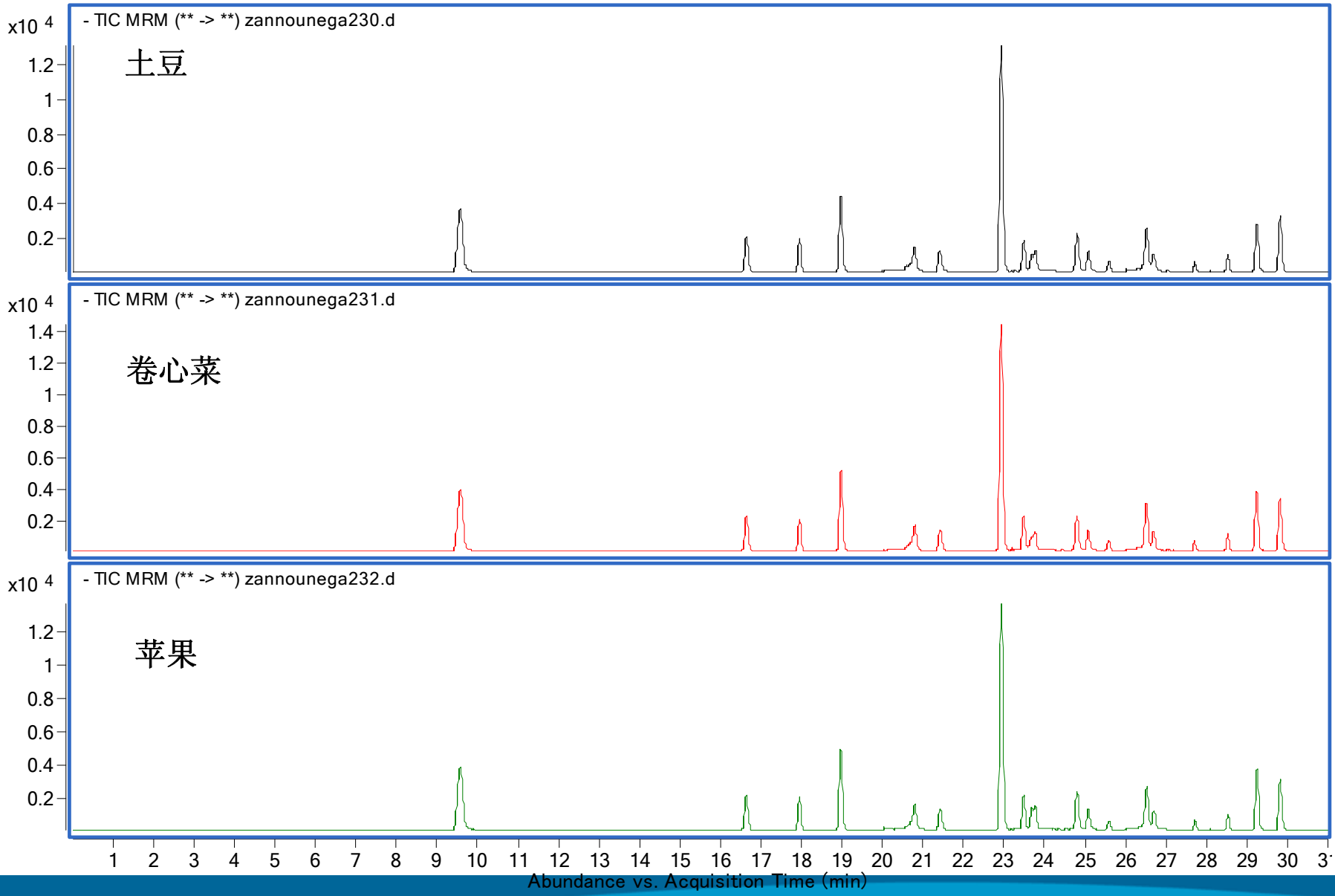
食品中农药化合物的MRM 提取离子流 (1ppb，正离子)



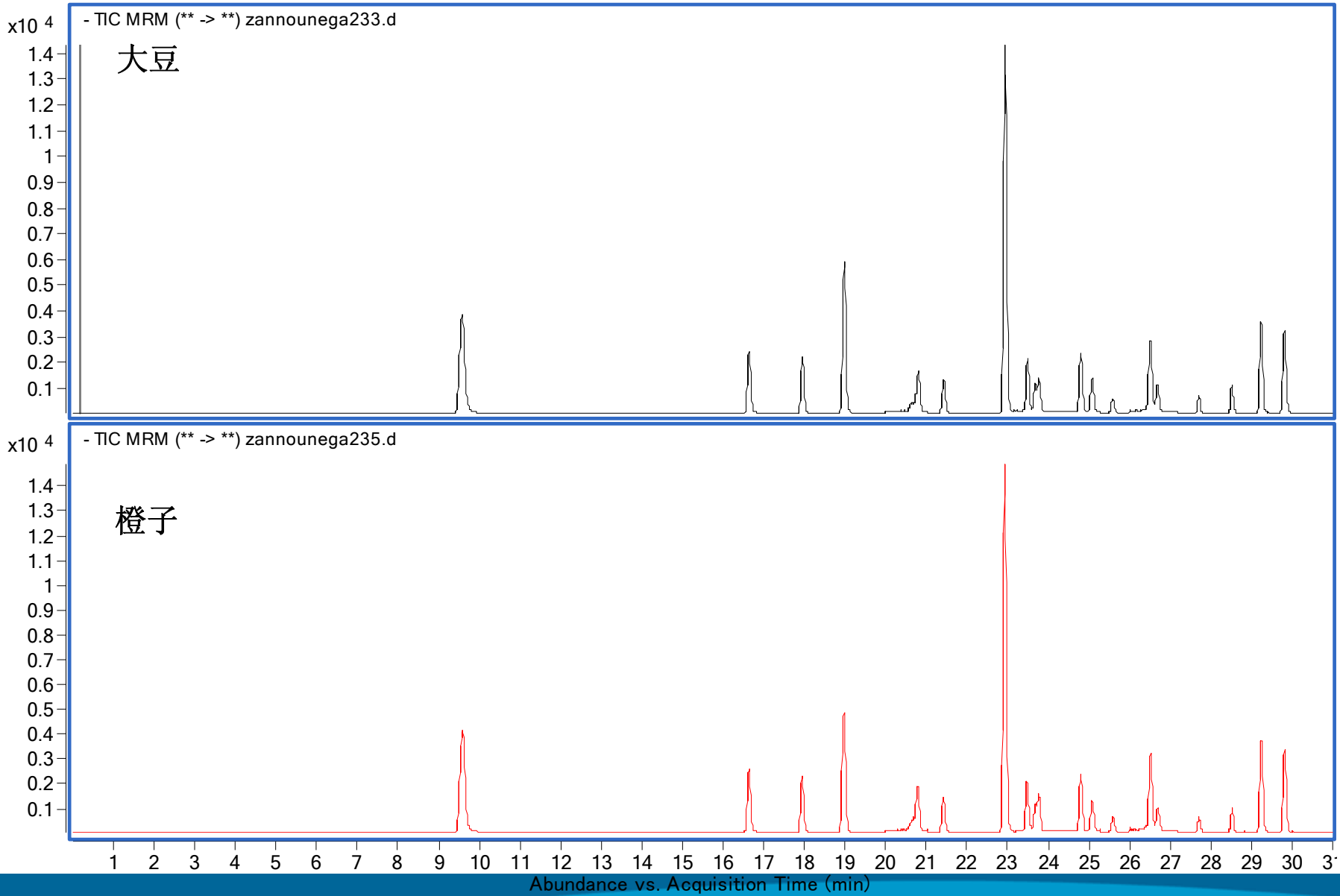
食品中农药化合物的MRM 提取离子流 (1ppb，正离子)



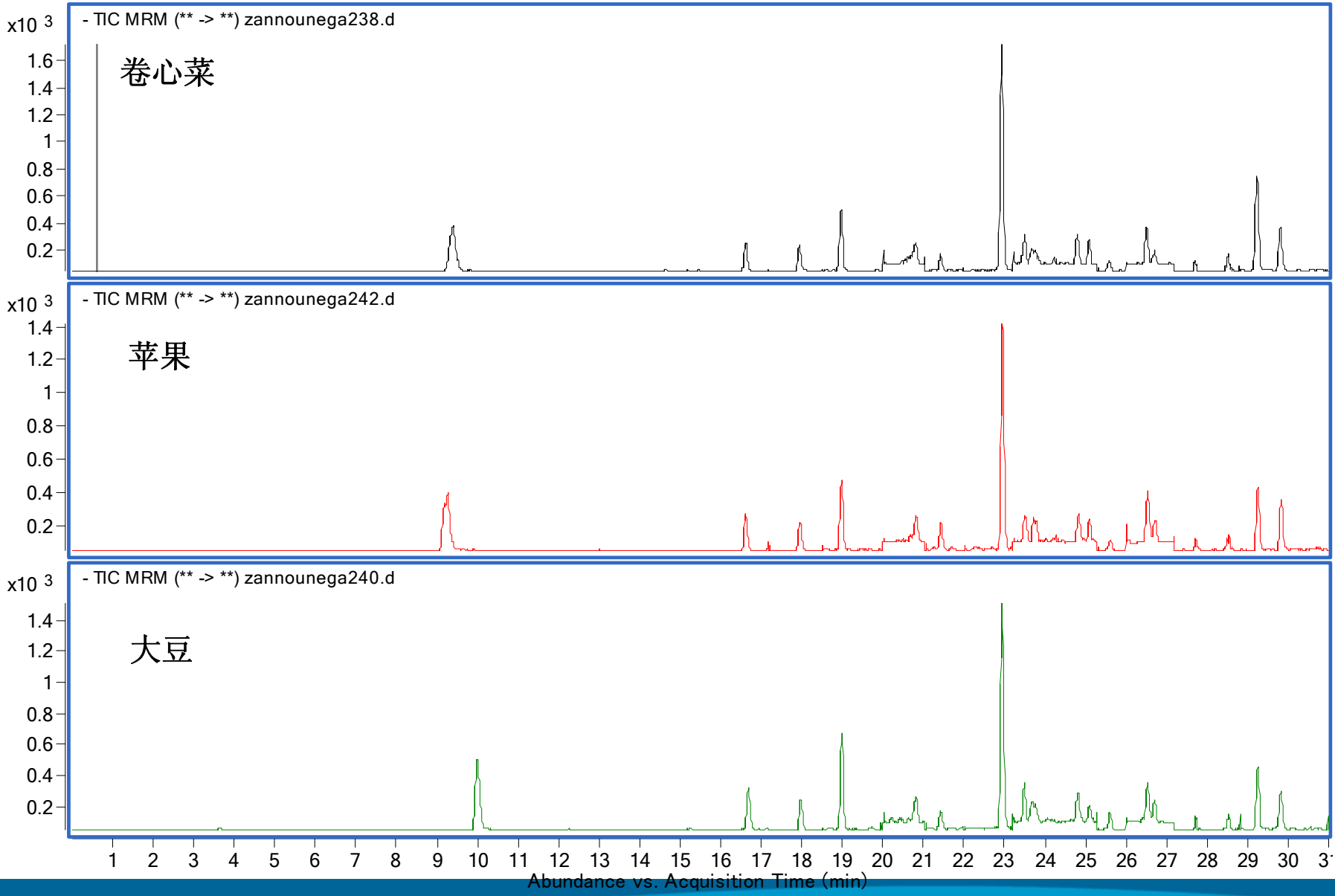
食品中农药化合物的MRM 提取离子流 (10ppb，负离子)



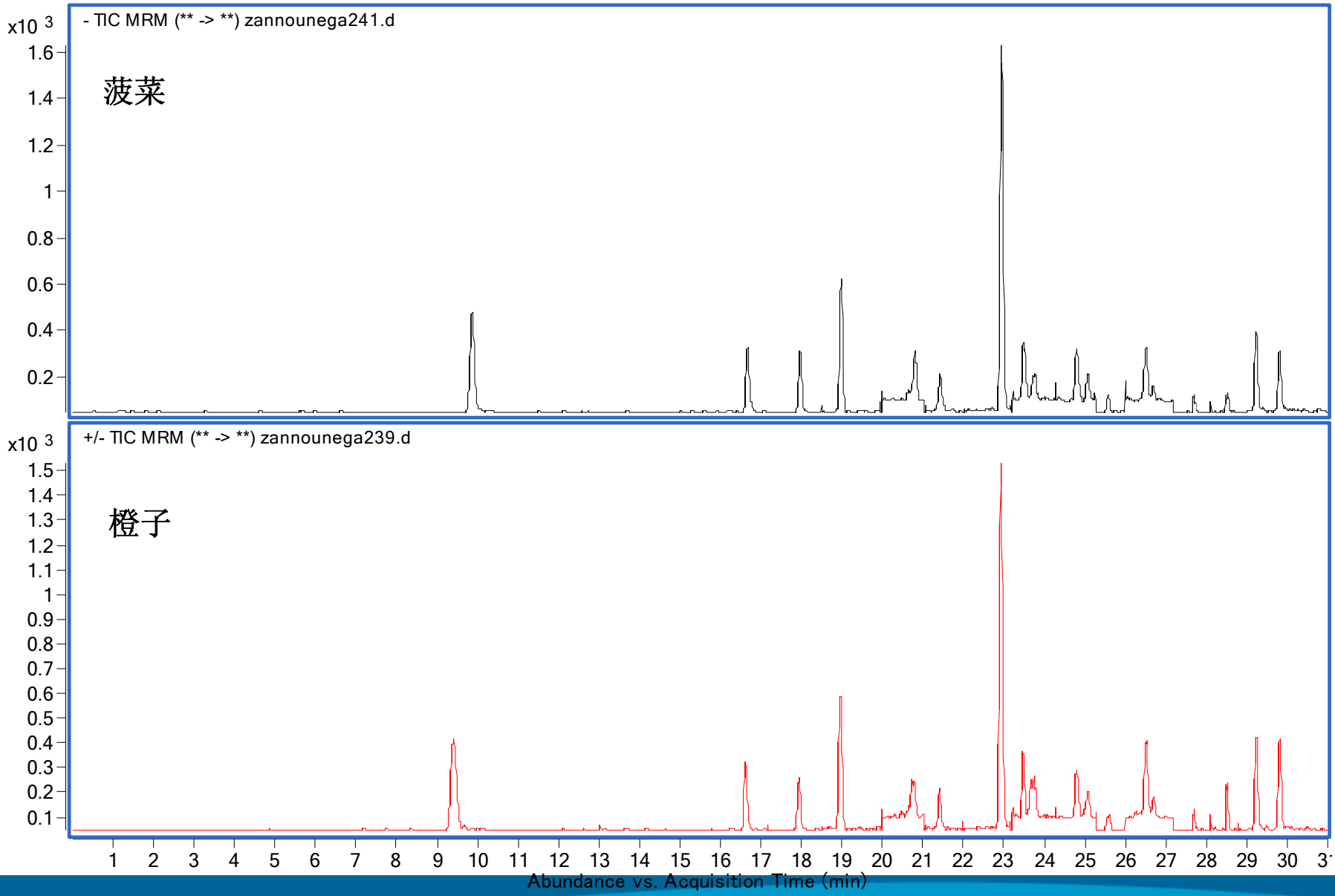
食品中农药化合物的MRM 提取离子流 (10ppb，负离子)



食品中农药化合物的MRM 提取离子流 (1ppb，负离子)



食品中农药化合物的MRM 提取离子流 (1ppb，负离子)



LC/MS/MS用于微量有害化合物的检测

1: 土壤中氯代酸类除草剂的检测

2: LC/MS/MS用于多种残留化合物的检测

3. 食品中真菌毒素的检测

- Aflatoxins（黄曲霉素）
- Fumonisin（伏马菌素）

氯代酸类除草剂的用途及可能的污染

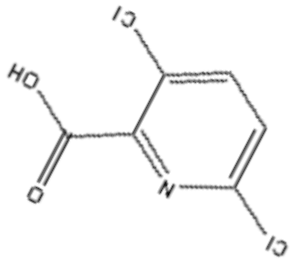
常用除草剂，用于清除草坪和农田里的阔叶类杂草

潜在的地下水污染物

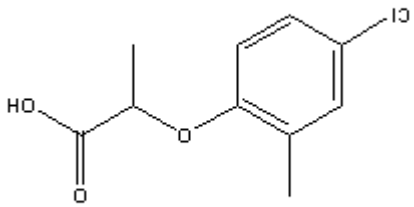
经常被滥用

需要对微量的残留物进行定量分析

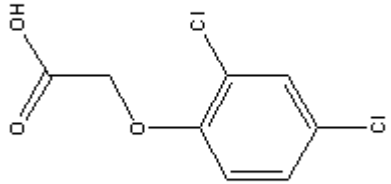
氯代酸类除草剂的化学结构



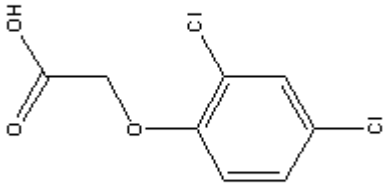
二氯皮考啉酸
Clopyralid



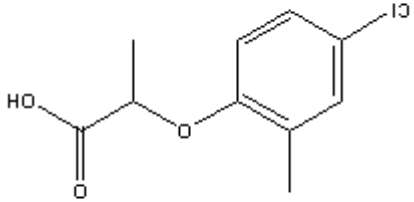
毒莠定
Picloram



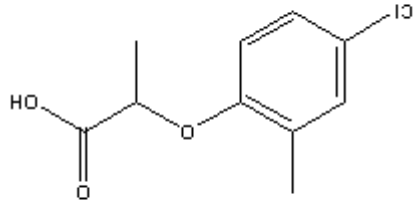
杀草畏
Dicamba



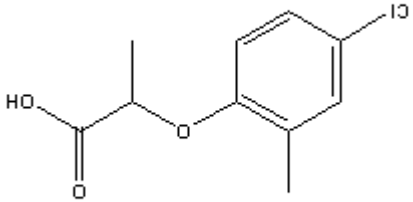
2,4-D



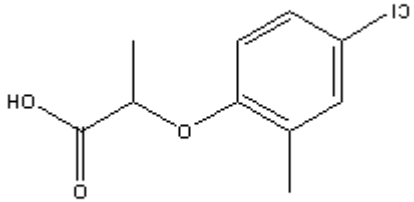
MCPA



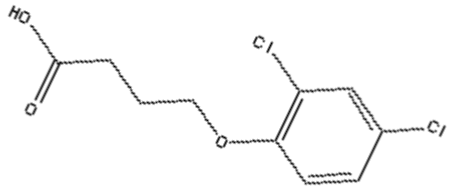
绿草定
Triclopyr



2,4-滴丙酸
2,4-DP



MCPP



2,4-滴丁酯
2,4-DB

常用的检测方法

液-液萃取

重氮甲烷衍生化

气相色谱/电子捕获检测器和电解检测器

对于阳性样品，需要进一步确认

问题:

溶剂使用量大

问题数据的解析

甲烷化试剂的安全性

为什么要使用 HPLC/MS/MS?

无需化学衍生化

确认和定量一次完成

复杂基体类样品中的检测限低

提高实验室的分析效率 (使用固相萃取)

实验结果更准确

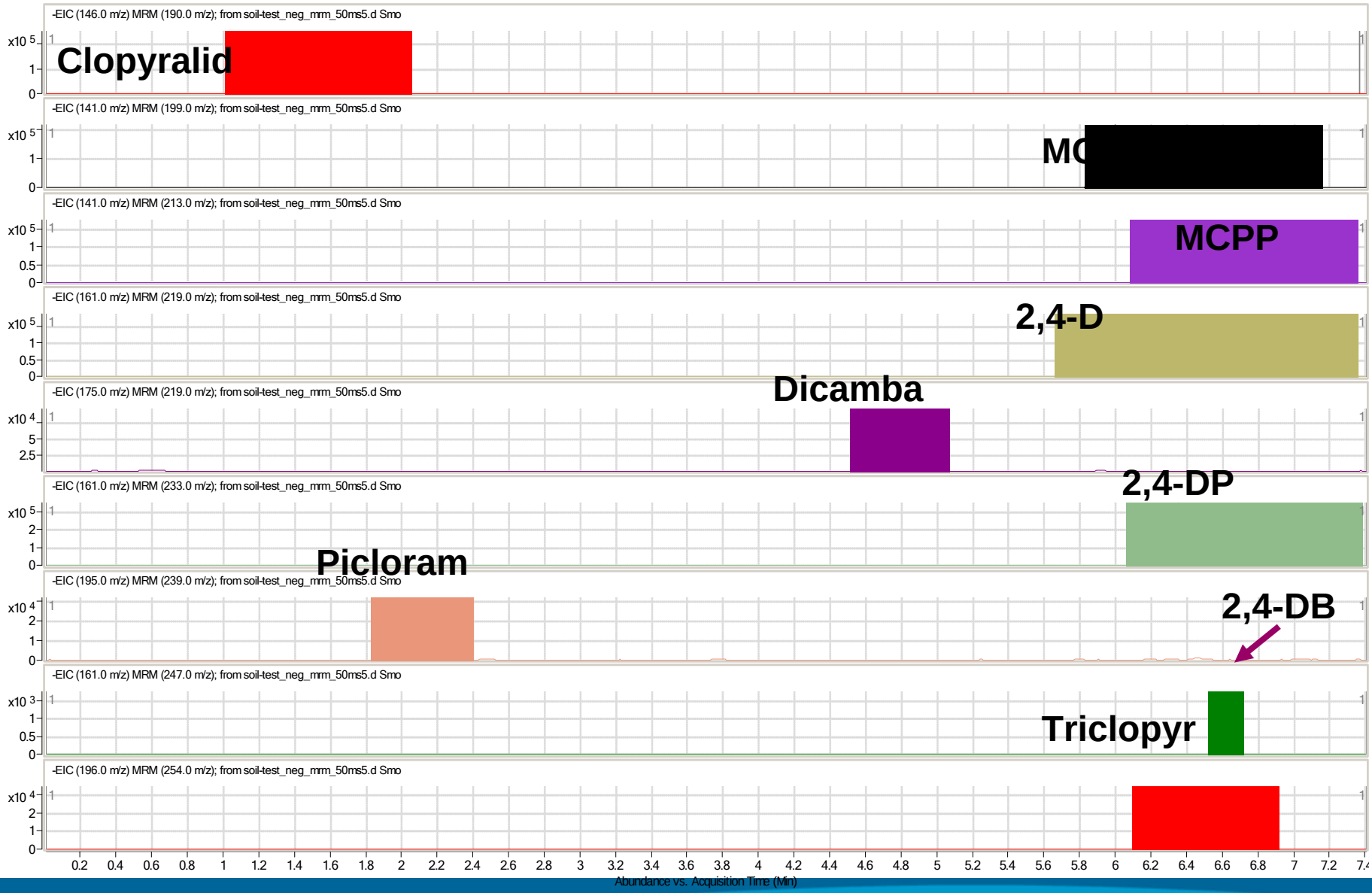
氯代酸类除草剂的分析条件

LC	: 1100LC
Column	:Zorbax Exlipse XDB C18(50mm,2.1mm,3.5μm)
Mobile phase	: A: MeOH, B:0.04%CH_3COOH
	10%A/B---(3min)---70%A---(3min)---95%A/1min
Column temp	: 40℃
Sample volume	: 1ul
Flow rate	: 0.4ml/min
MS	: G 6410 QQQ
Ionization	: ESI(Positive)
Dwell time	: 50msec
Collision energy	: 25V(N2 gas)
Scan range	: m/z 100-450
Drying gas	: 10l/min at 350C
Nebulizer gas	: 45psi
Fragmentor	: 80V

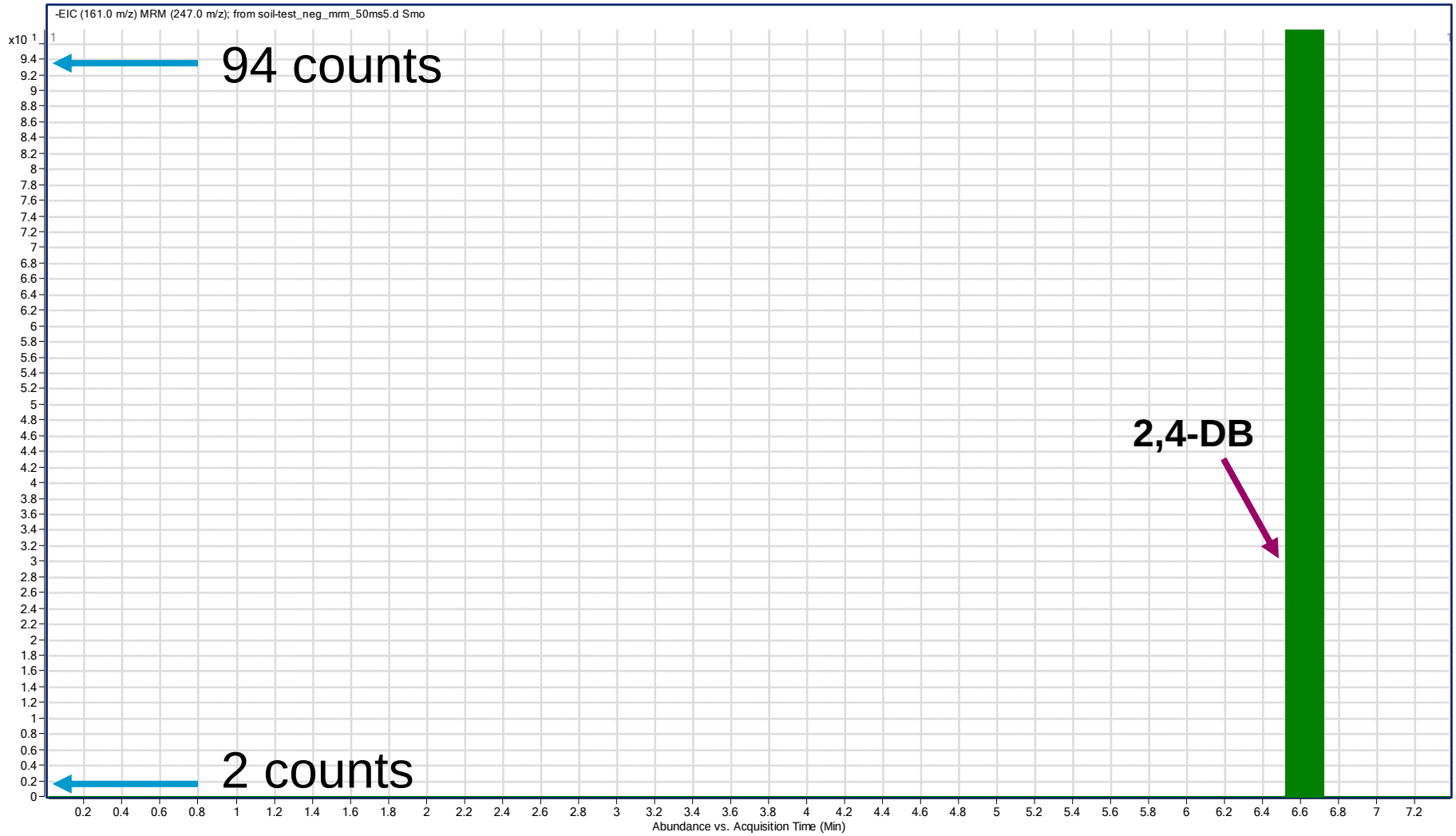
MRM ions (M1>M2)

Clopyralid	190>146	2,4-DP	233>161
Picloram	239>195	Triclopyr	254>196
Dicamba	219>175	MCPP	213>141
2,4-D	219>161	2,4-DB	247>161
MCPA	199>141		

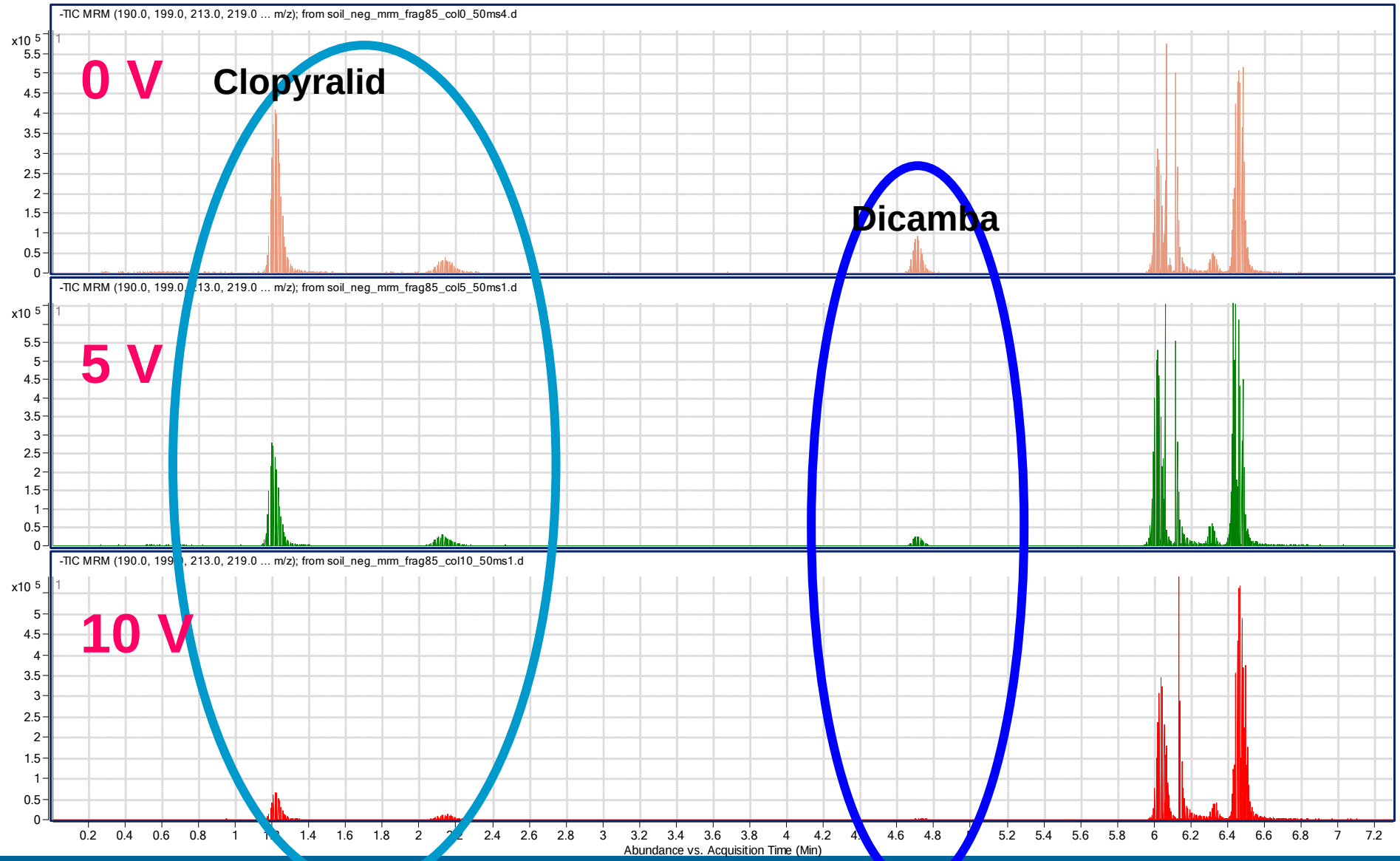
氯代酸类除草剂的MRM图



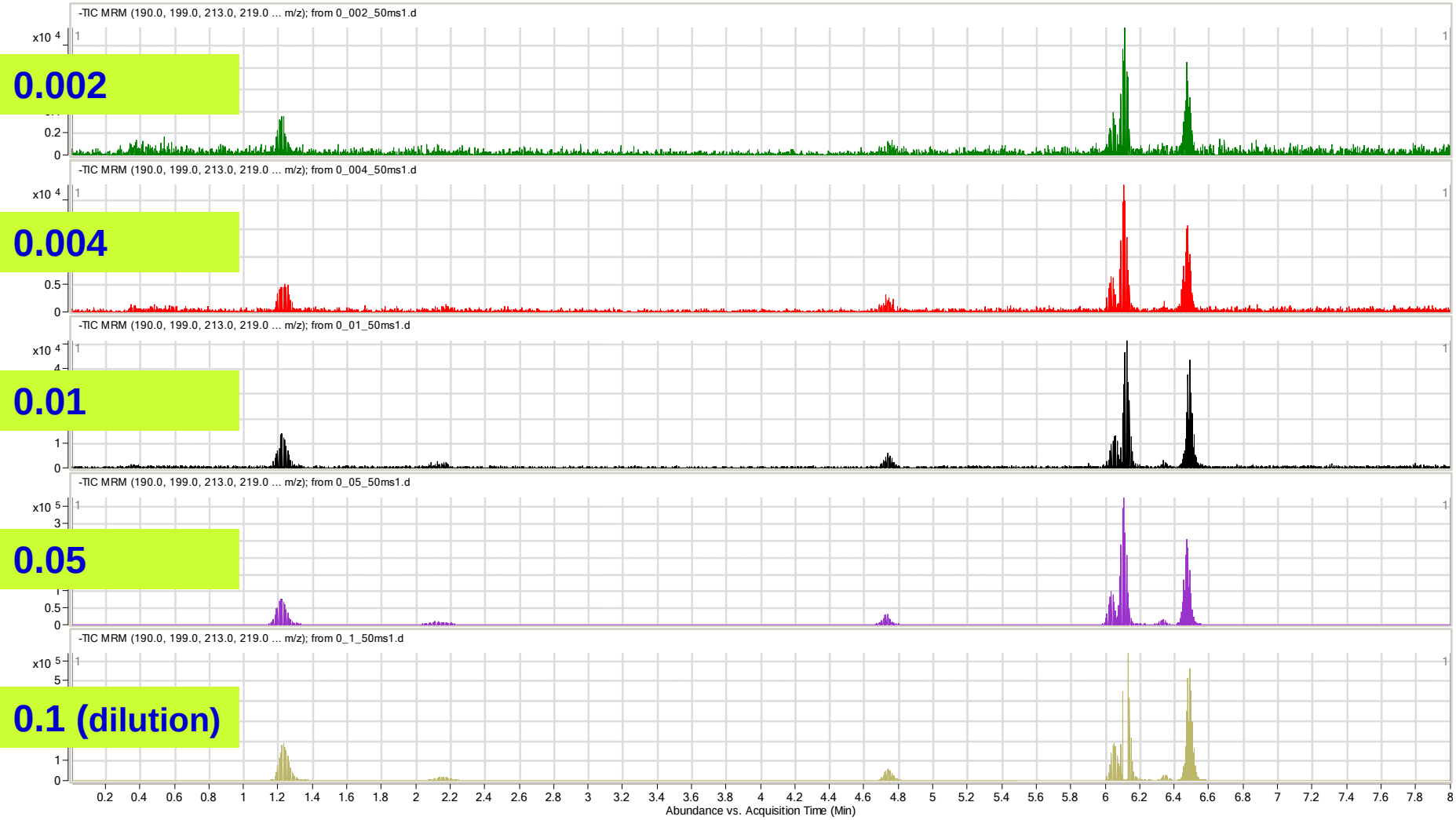
在MRM模式下的离子流图中几乎没有背景



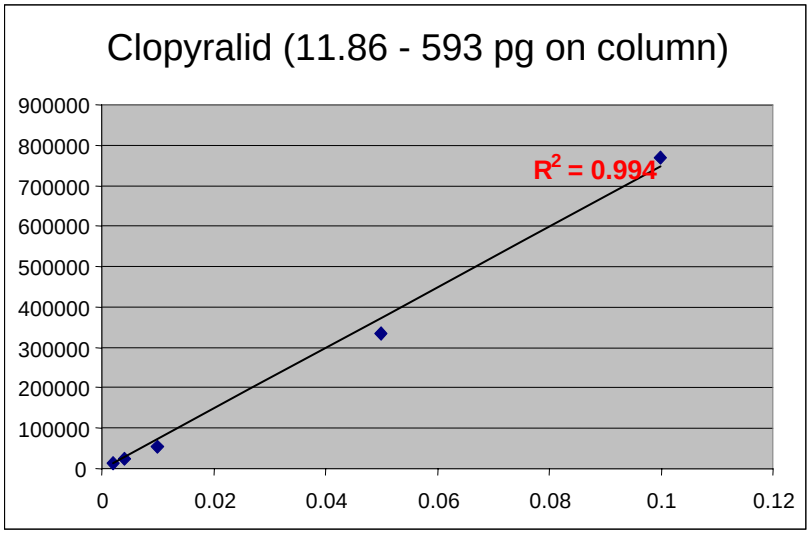
碰撞能量的优化

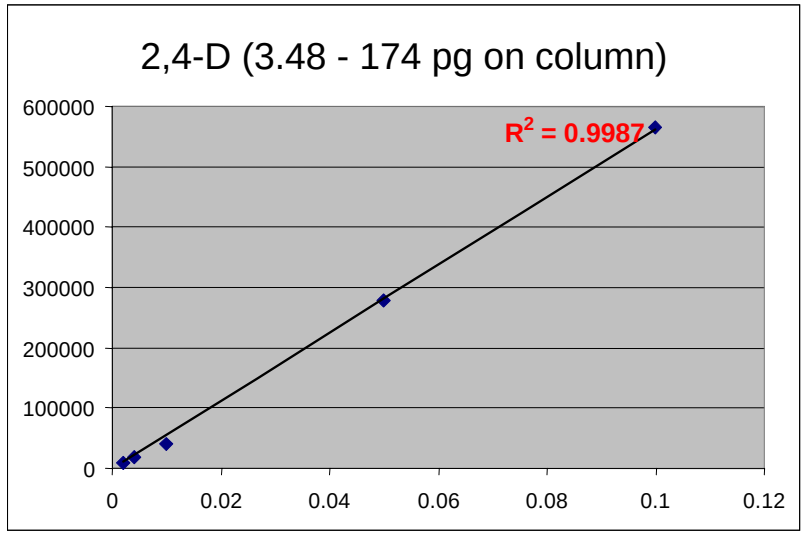

-TIC MRM (190.0, 199.0, 213.0, 219.0 ... m/z); from soil_neg_mrm_frag85_col0_50ms4.d
0 V
Clopyralid
Dicamba
-TIC MRM (190.0, 199.0, 213.0, 219.0 ... m/z); from soil_neg_mrm_frag85_col5_50ms1.d
5 V
-TIC MRM (190.0, 199.0, 213.0, 219.0 ... m/z); from soil_neg_mrm_frag85_col10_50ms1.d
10 V
x10 5
Abundance vs. Acquisition Time (Min)

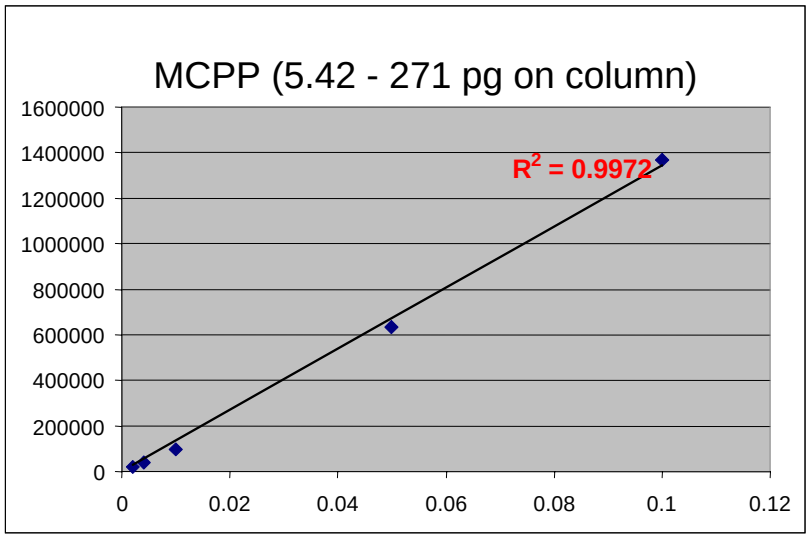
MRM chromatograms by dilution

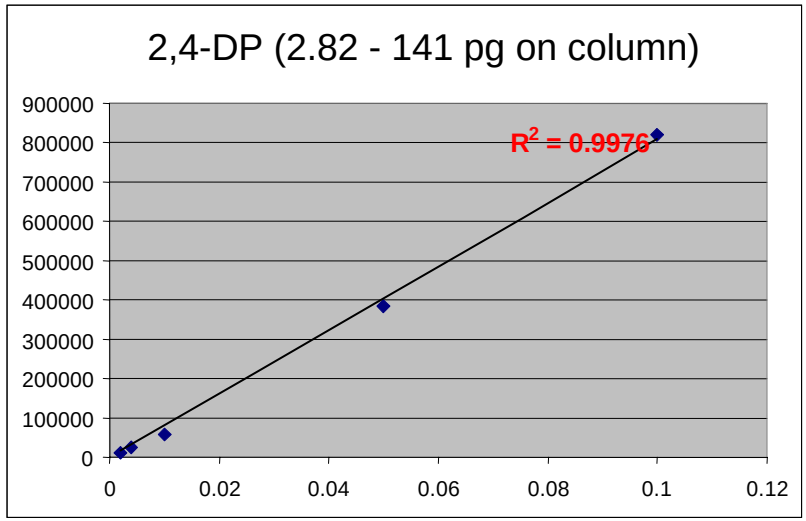


氯代酸类除草剂的定量校正曲线







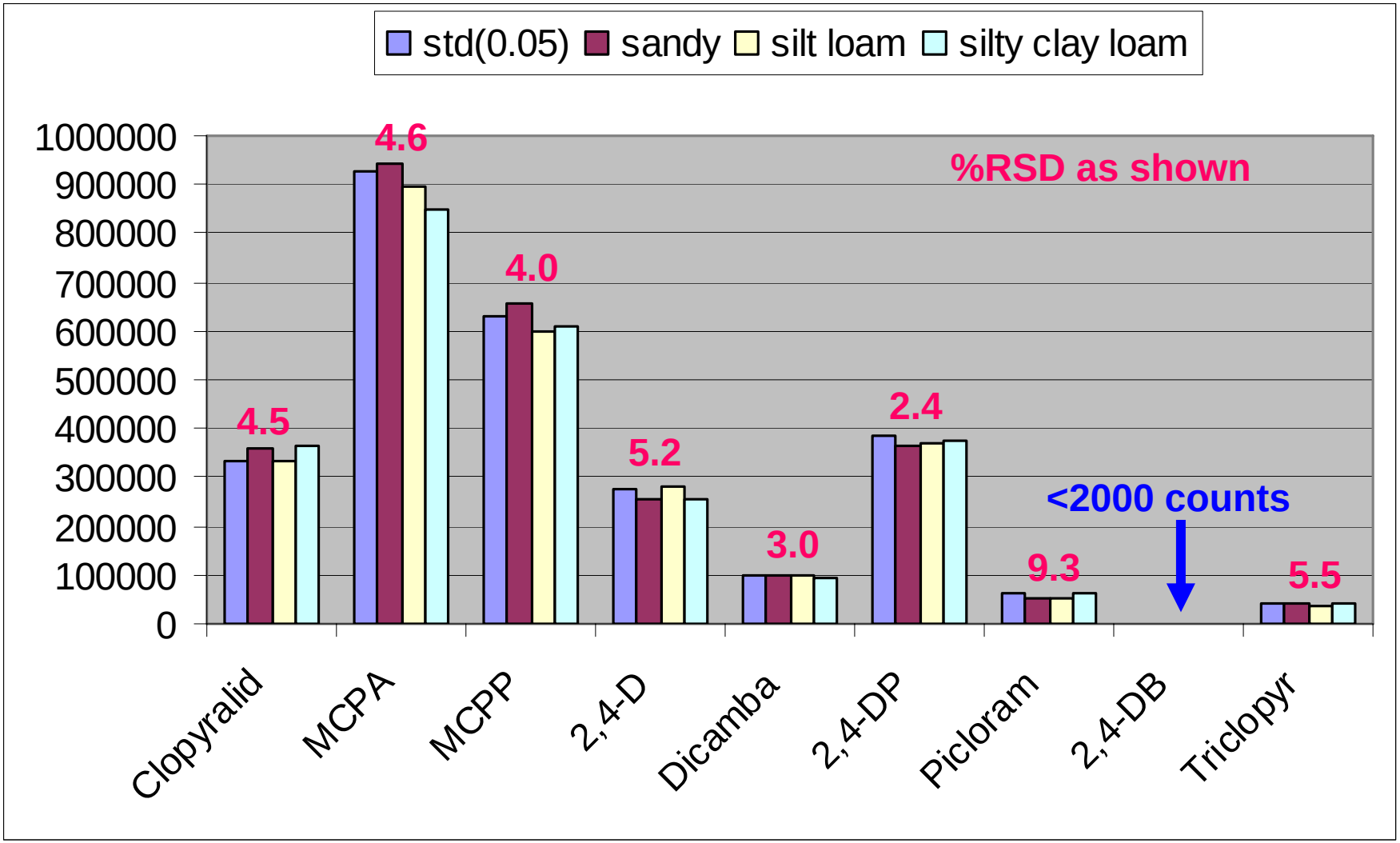


稀释倍数及柱上绝对进样量(pg)

Dilution →	mixed std.	0.1	0.05	0.01	0.004	0.002	Soil (LODs) (pg/µL)
Clopyralid	5930	593	297	59.3	23.7	11.86	40
Picloram	1800	180	90	18.0	7.2	3.60	10
Dicamba	8200	820	410	82.0	32.8	16.40	50
2,4-D	1740	174	87	17.4	7.0	3.48	10
MCPA	5480	548	274	54.8	21.9	10.96	40
Triclopyr	1240	124	62	12.4	5.0	-	10
2,4-DP	1410	141	71	14.1	5.6	2.82	10
MCPP	2710	271	136	27.1	10.8	5.42	20
2,4-DB	6900	690	345	-	-	-	50

All detected except shaded spaces.

不同基质的应影响(soil)



Spike 20 µL of mixed std. into 380 µL of each soil extract

结论

LC/MS/MS是一种快速，简单和灵敏的用于检测土壤中氯代酸除草剂的方法

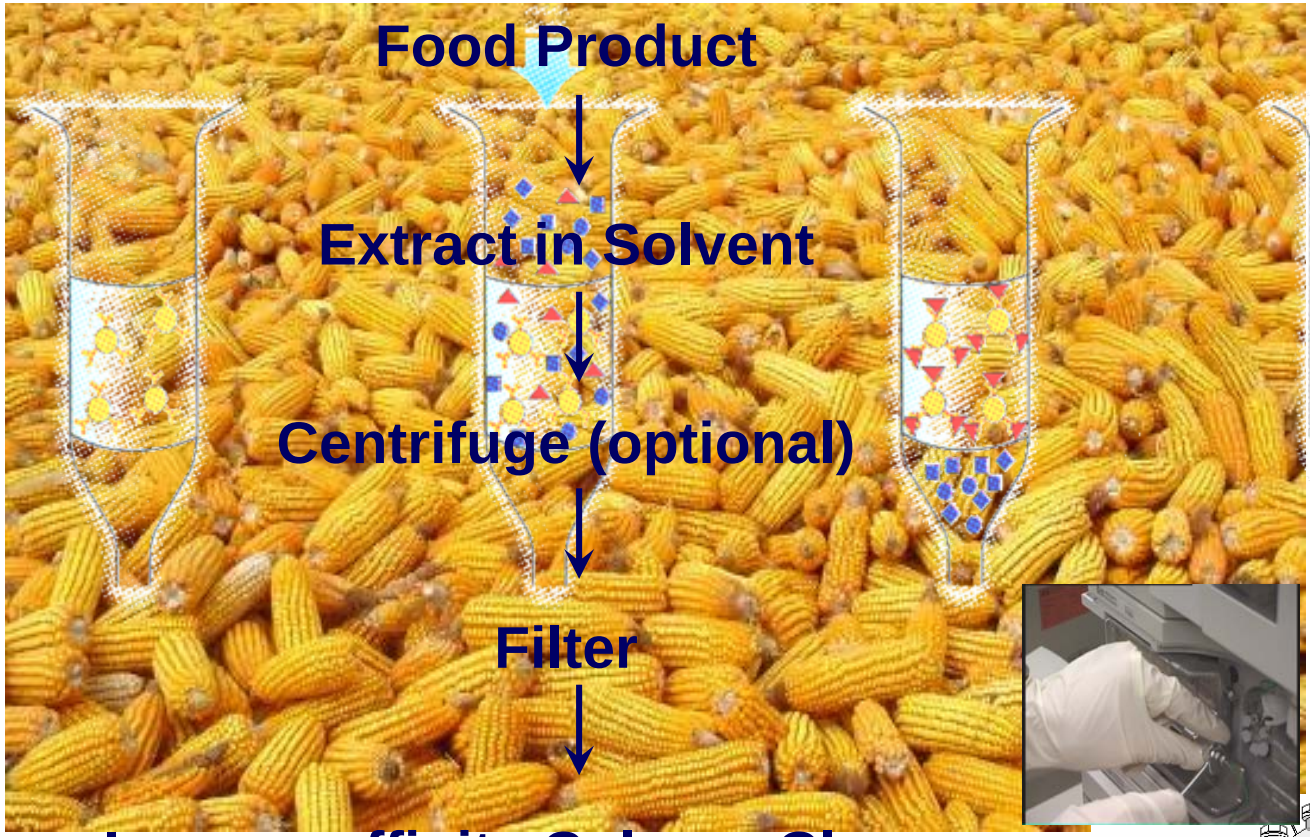
不用甲基化衍生化反应，大大提高了实验室效率，同时提高了实验室的安全性。

多反应检测(MRM)用于化合物的定性定量分析

极佳的定量线性

未发现基质效应

Analysis of Mycotoxins

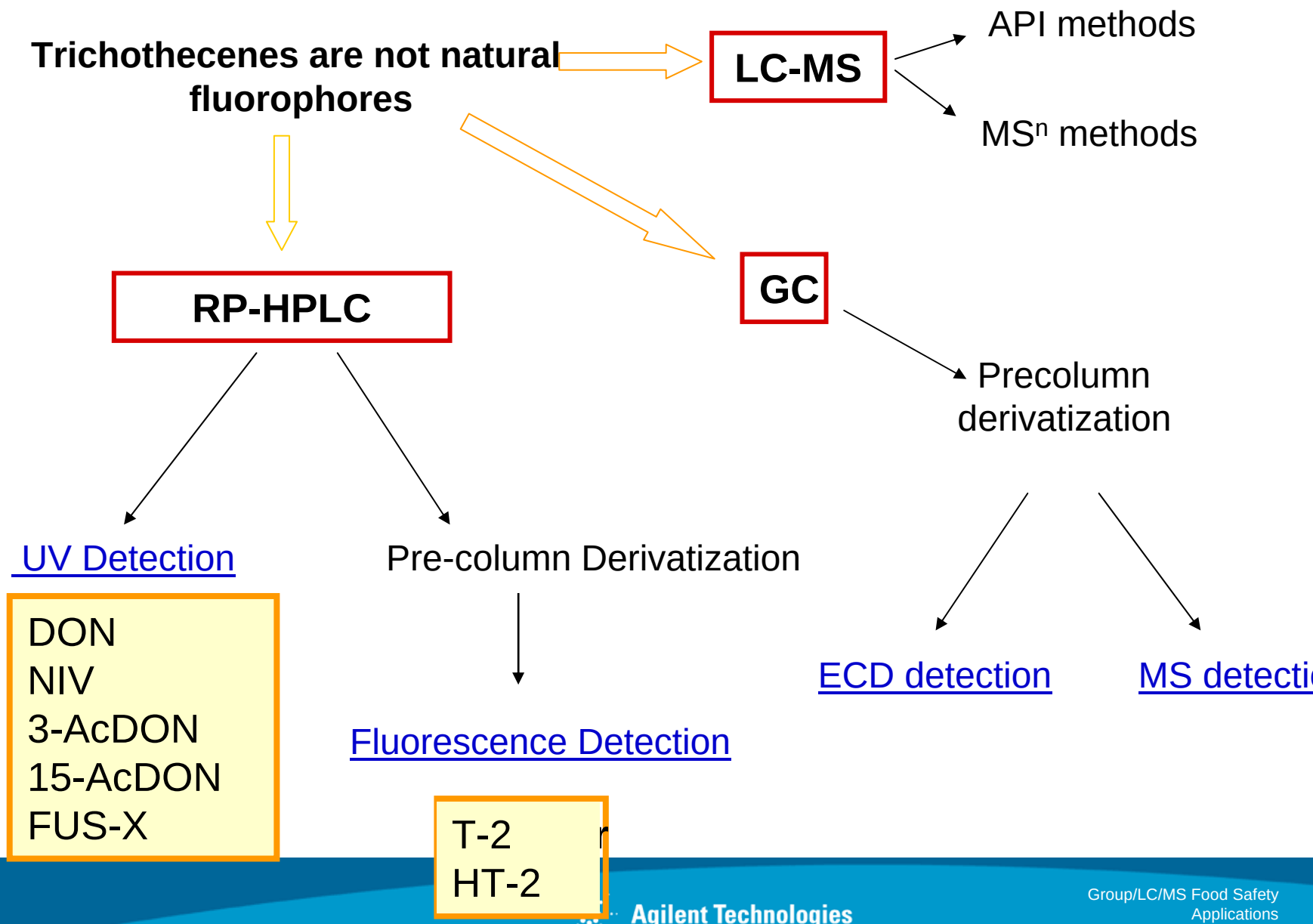


Immunoaffinity Column Clean-up

Evaporate and Redissolve

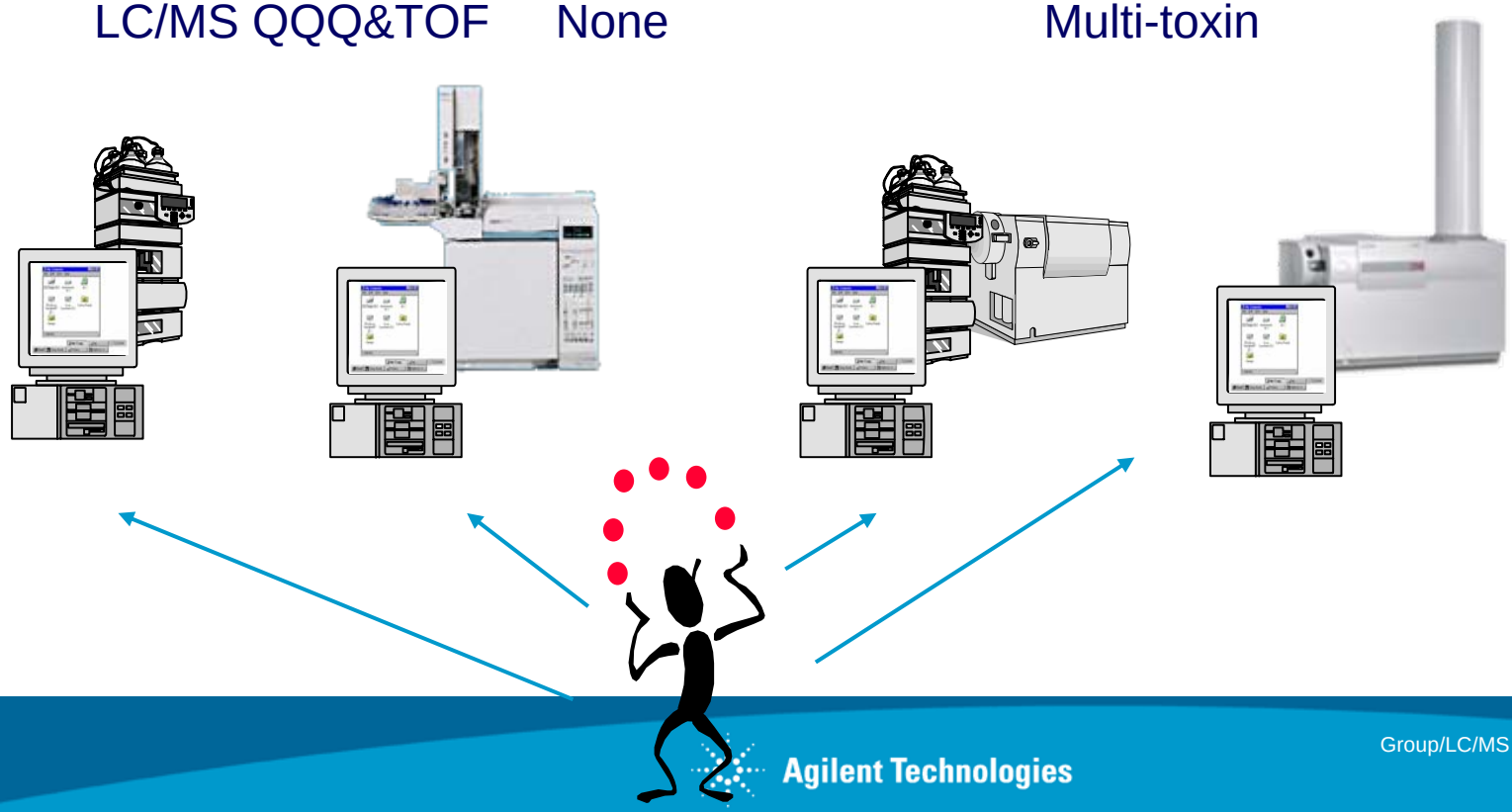
Detection

Selection of Appropriate Instrumental methods for Mycotoxin Analysis



Selection of Appropriate Instrumental methods for Mycotoxin Analysis

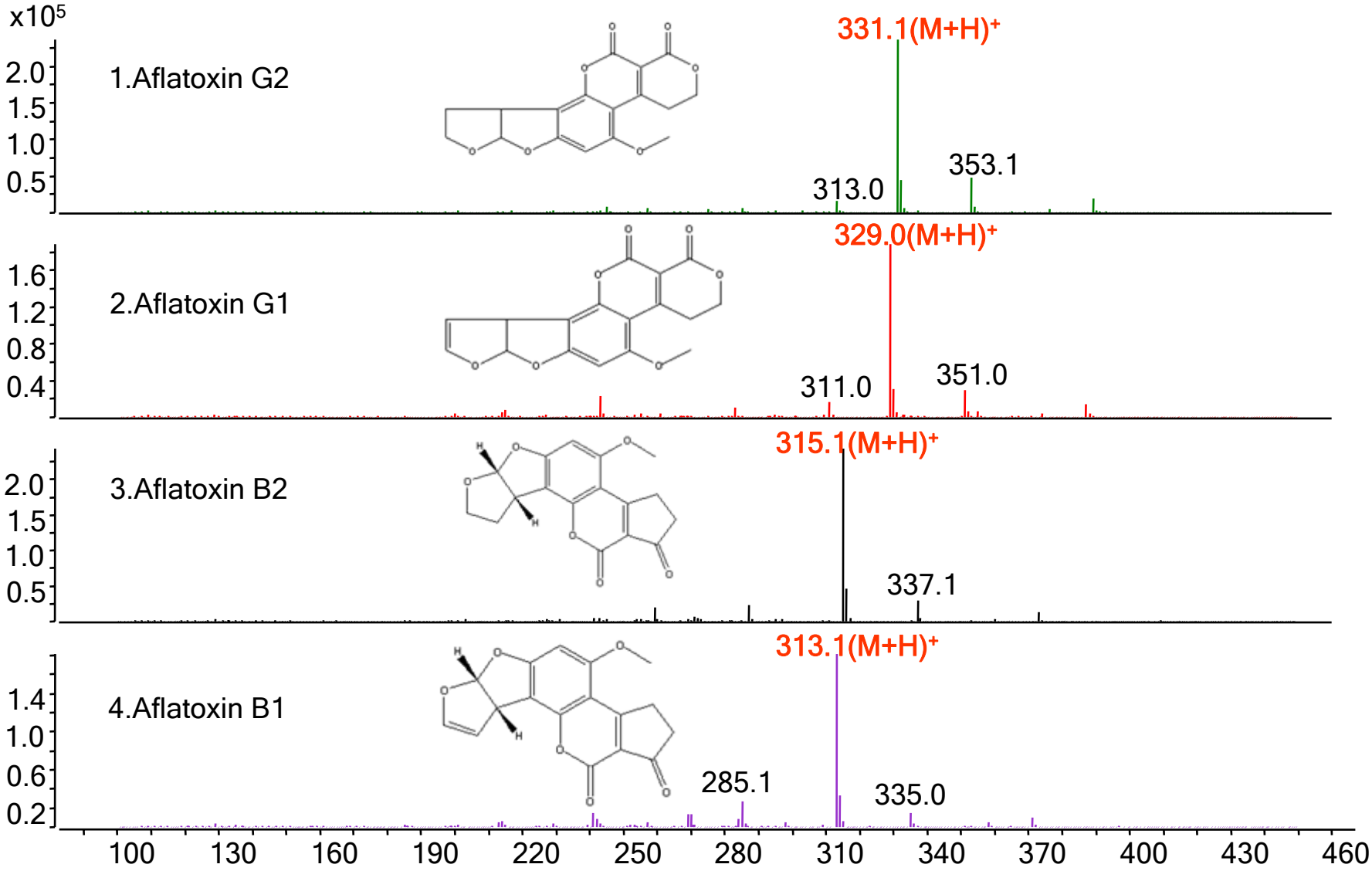
Technique	Derivatization	Mycotoxin
HPLC (FLD)	OPA	Fumonisins
HPLC (FLD)	None	ZON
HPLC (UV)	None	DON
GC&GC/MS	TFA	DON, NIV, T2
LC/MS QQQ&TOF	None	Multi-toxin



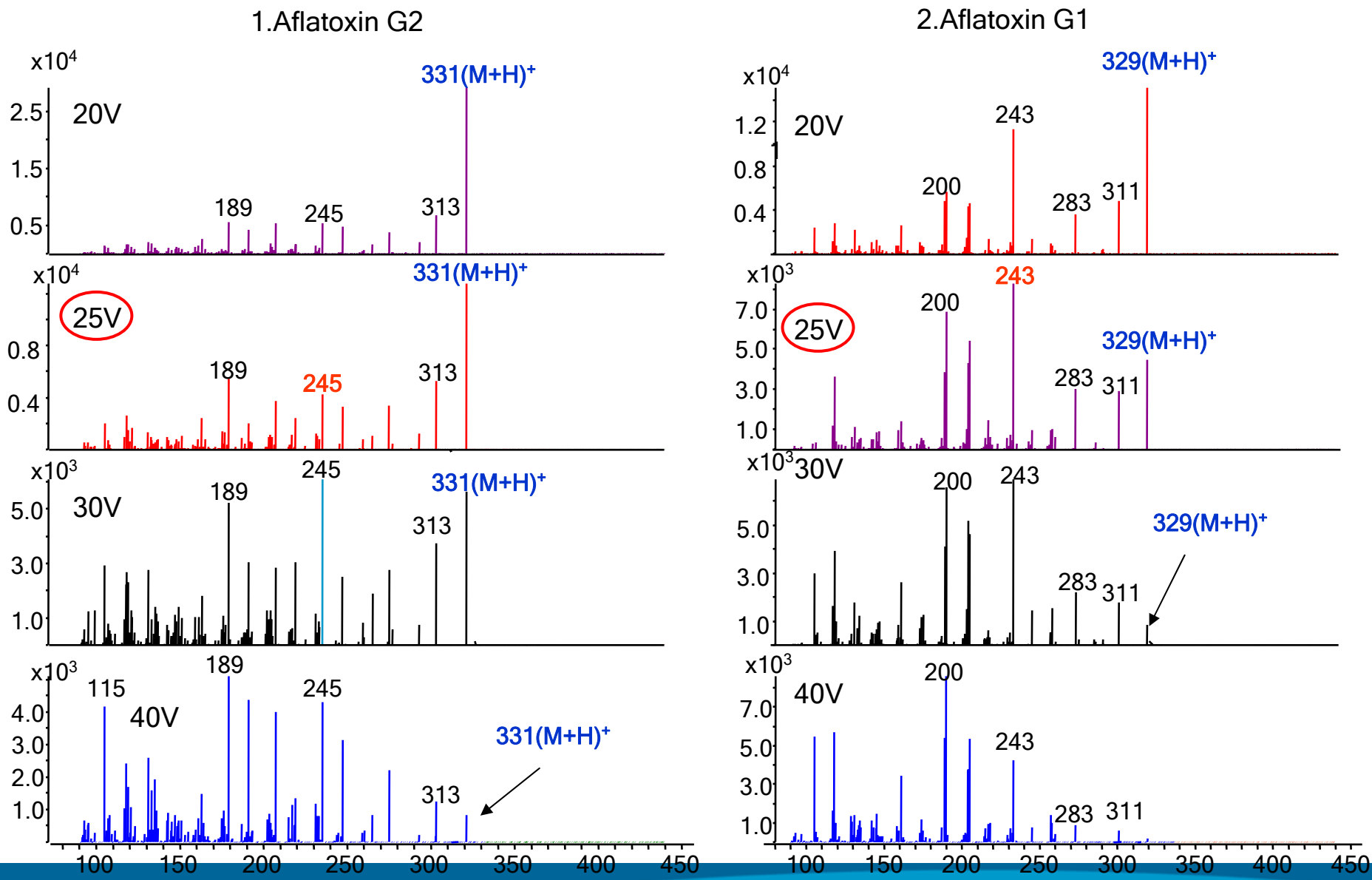
黄曲霉素的分析条件

LC	**: 1100LC**
Column	**:Zorbax Exlipse XDB C18(150mm,2.1mm,3.5μm)**
Mobile phase	**: A: MeOH, B:10mMCH_3COONH_4**
	40%A/B
Column temp	**: 40℃**
Sample volume	**: 5ul**
Flow rate	**: 0.25ml/min**
MS	**: G 6410 QQQ**
Ionization	**: ESI(Positive)**
MS1	**: m/z=331(AFG2),329(AFG1),315(AFB2),313(AFB1)**
MS2	**: m/z=245(AFG2),243(AFG1),287(AFB2),241(AFB1)**
Dwell time	**: 50msec**
Collision energy	**: 25V(N2 gas)**
Scan range	**: m/z 100-450**
Drying gas	**: 10l/min at 350C**
Nebulizer gas	**: 50psi**
Fragmentor	**: 100V**

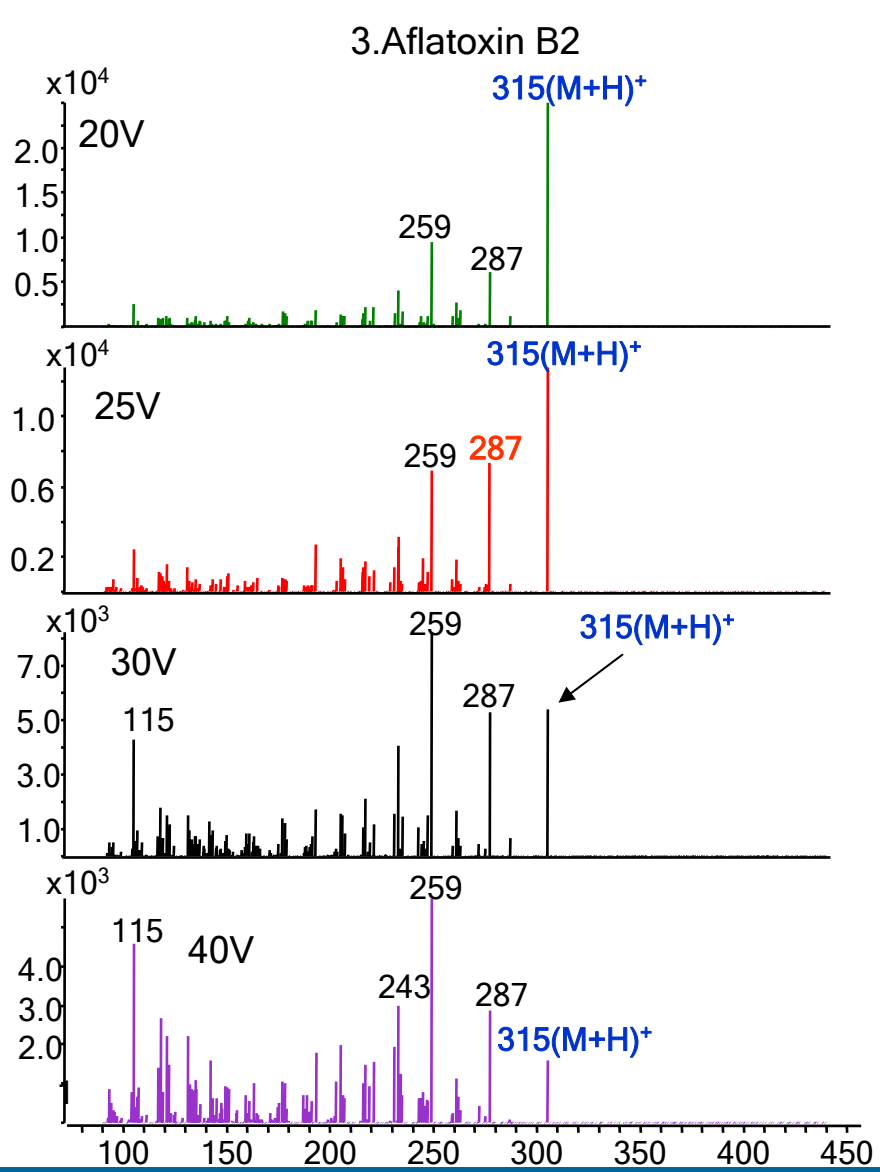
黄曲霉素的质谱图

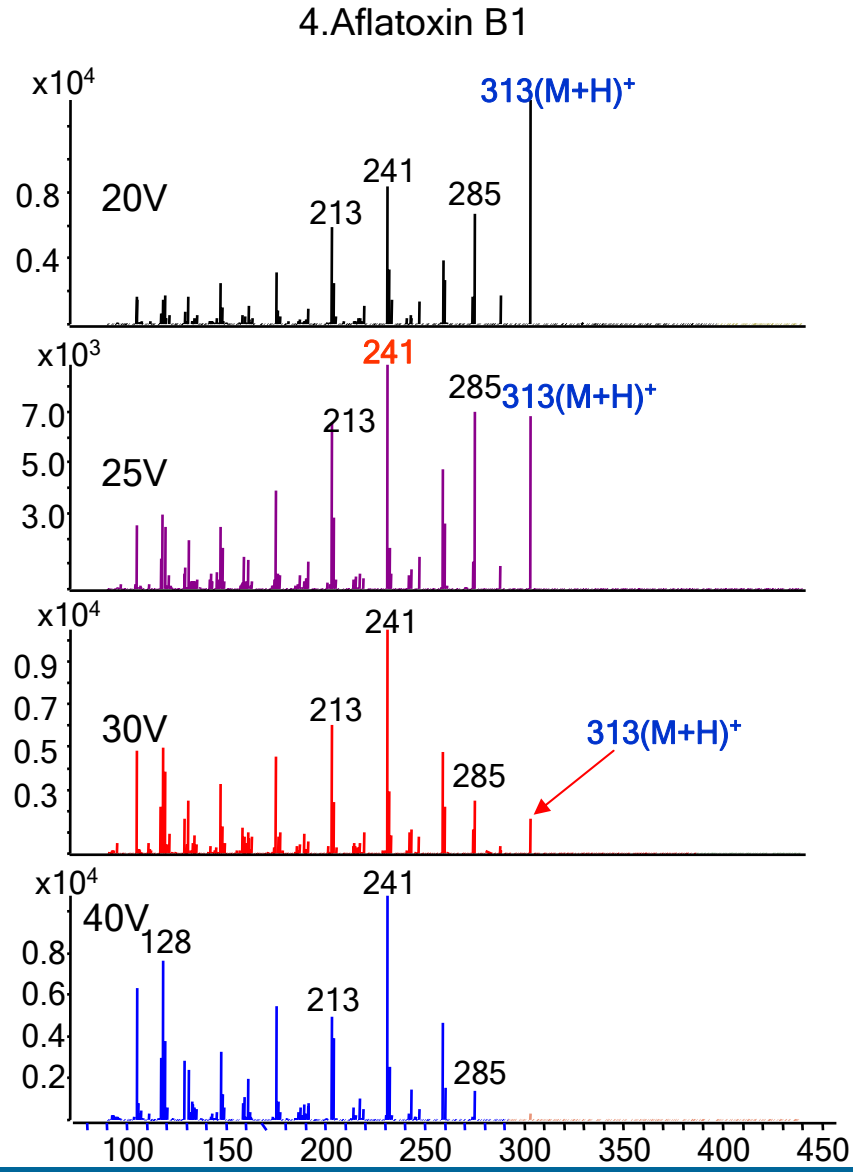

x10^5
1.Aflatoxin G2
331.1(M+H)+
313.0
353.1
2.Aflatoxin G1
329.0(M+H)+
311.0
351.0
3.Aflatoxin B2
315.1(M+H)+
337.1
4.Aflatoxin B1
313.1(M+H)+
285.1
335.0
100 130 160 190 220 250 280 310 340 370 400 430 460

黄曲霉素的MS/MS图

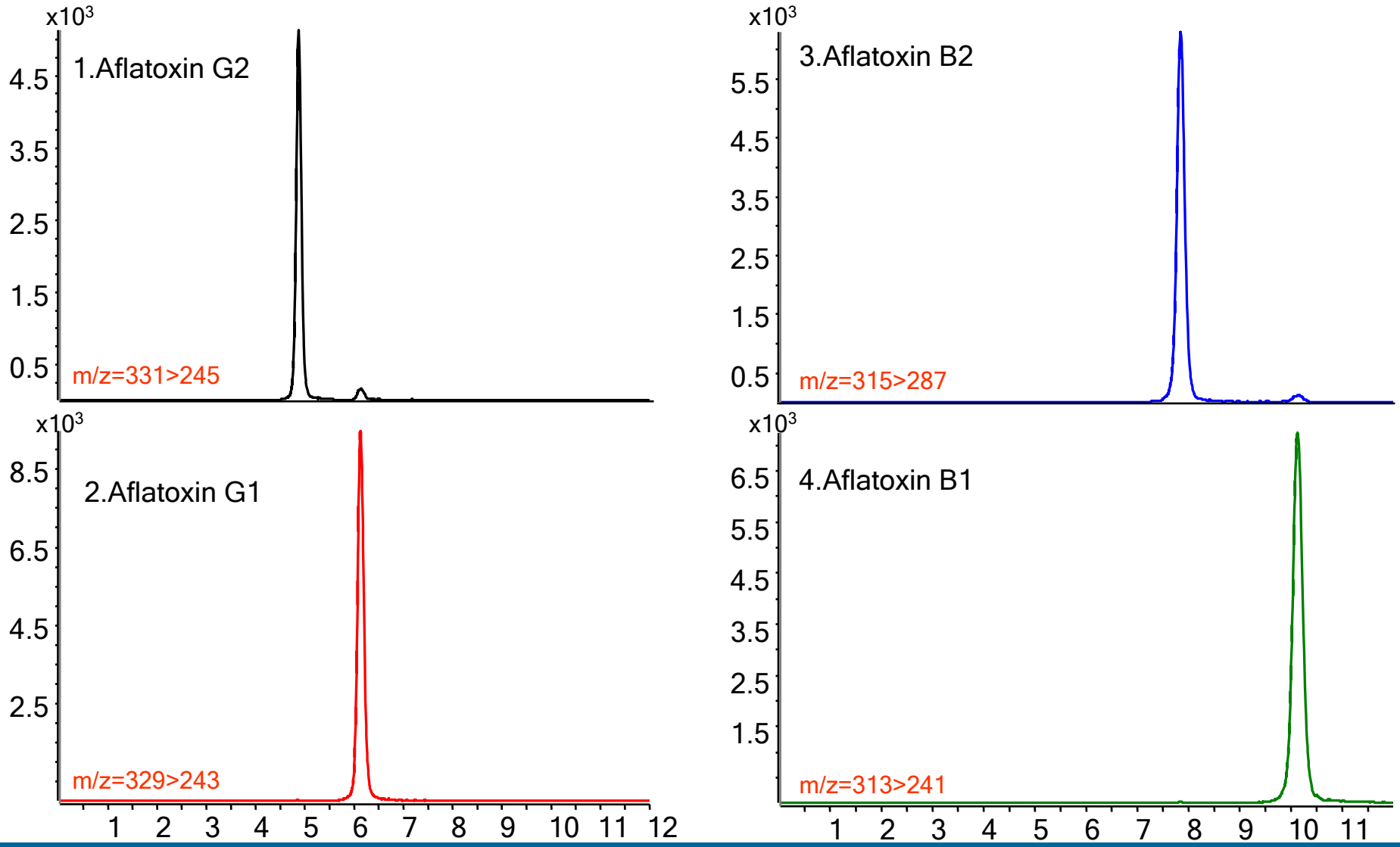

1.Aflatoxin G2
2.Aflatoxin G1
x10^4
20V
331(M+H)+
189
245
313
25V
30V
40V
115
x10^3
329(M+H)+
243
200
283
311
100
150
200
250
300
350
400
450

黄曲霉素的MS/MS图

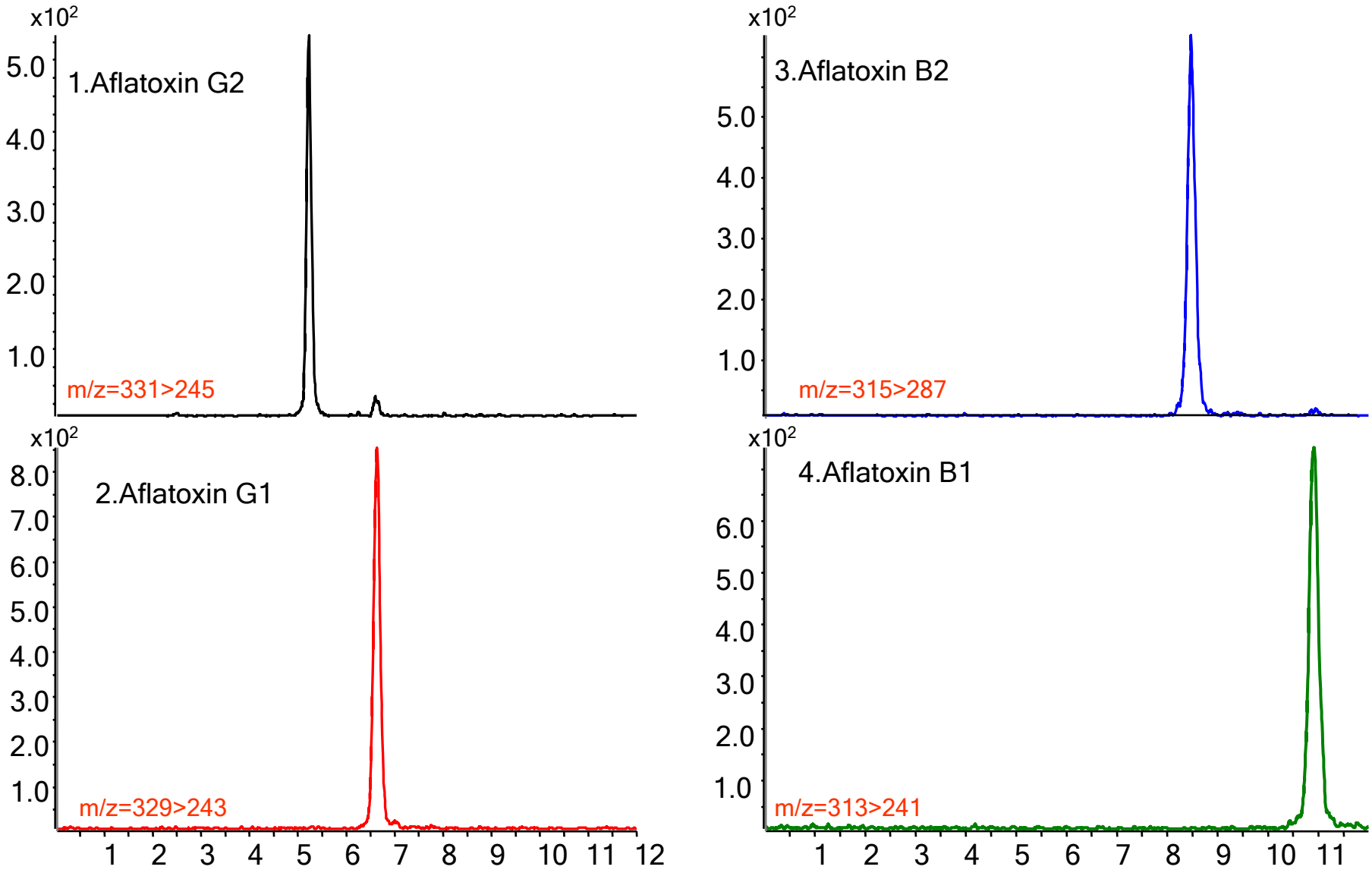

3.Aflatoxin B2
x10^4
20V
315(M+H)+
259
287
x10^4
25V
315(M+H)+
259
287
x10^3
30V
259
315(M+H)+
115
287
x10^3
115
40V
259
243
287
315(M+H)+
100
150
200
250
300
350
400
450


4.Aflatoxin B1
x10^4
20V
313(M+H)+
241
213
285
x10^3
241
25V
285
313(M+H)+
213
x10^4
241
30V
213
313(M+H)+
285
x10^4
241
40V
128
213
285
100
150
200
250
300
350
400
450

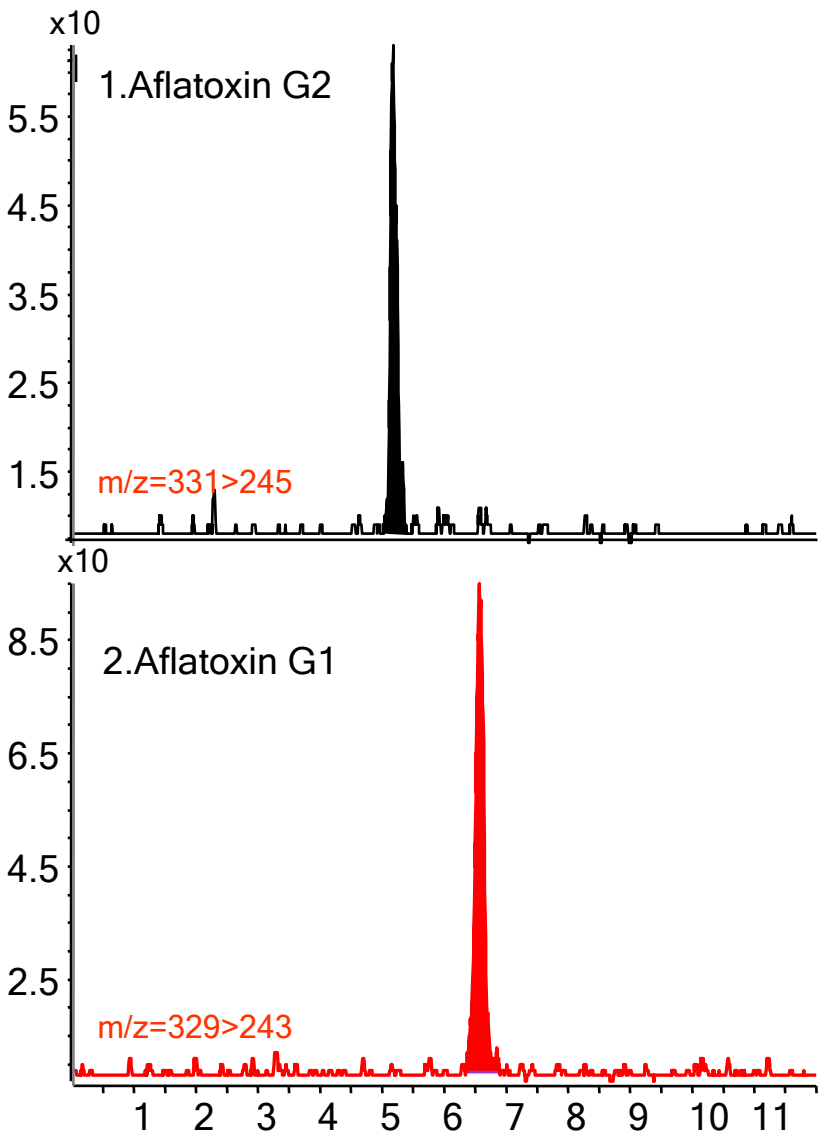
黄曲霉素的MRM总离子流图(100ppb)

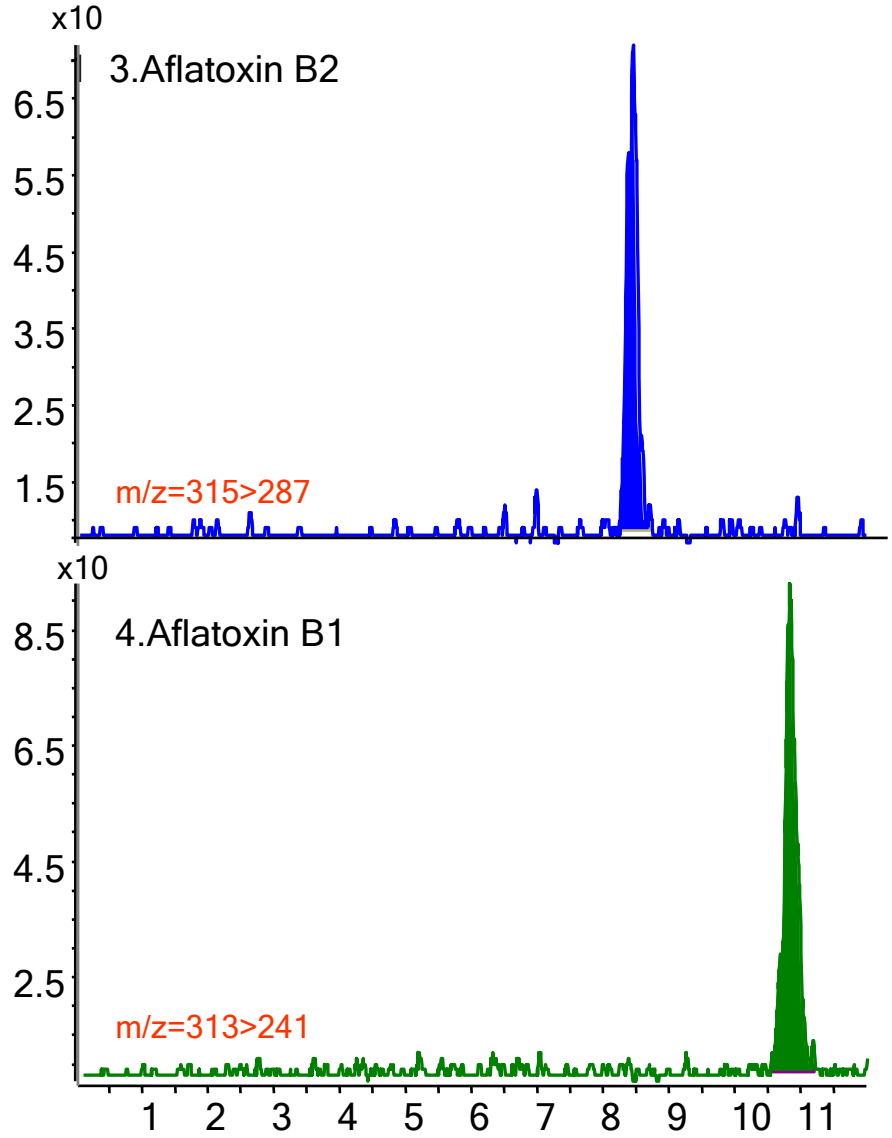


黄曲霉素的MRM总离子流图(10ppb)

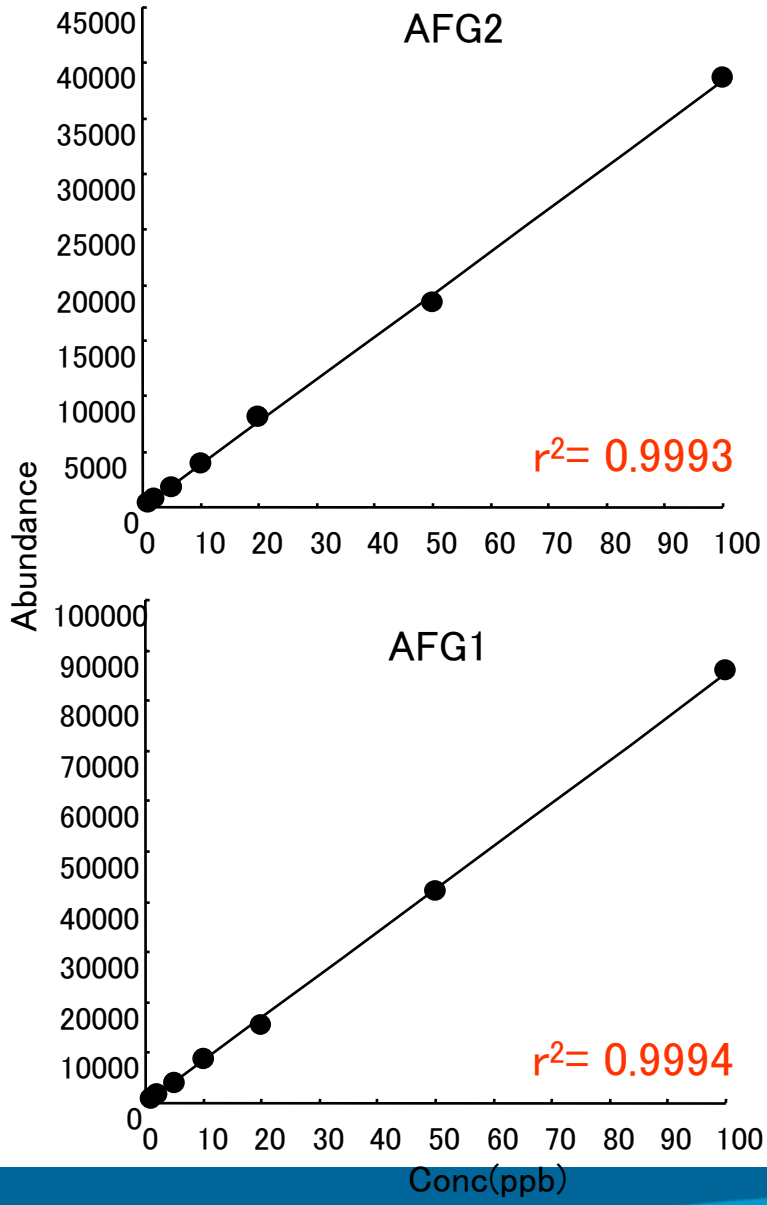


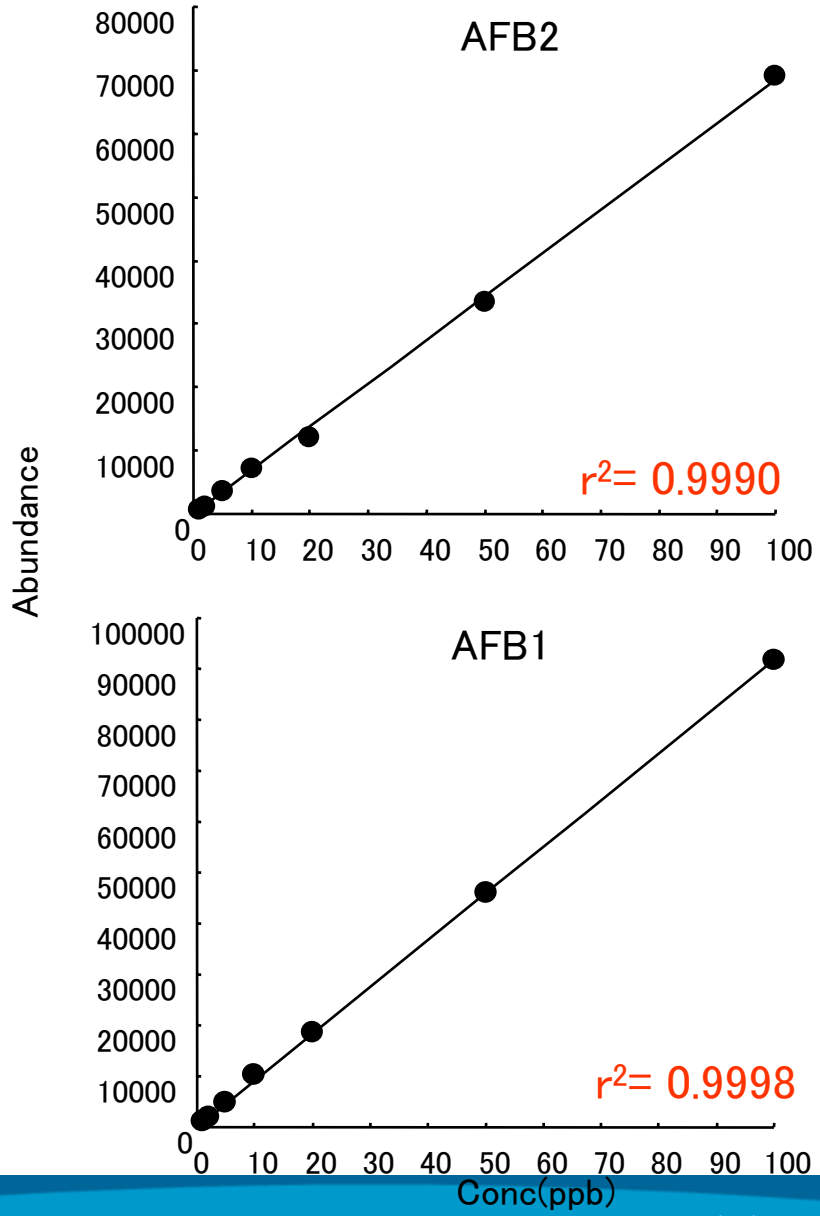
黄曲霉素的MRM总离子流图(1ppb)





黄曲霉素的定量校正曲线(100-1ppb)





黄曲霉素的样品处理方法

提取

20g的经过磨细的样品加入40毫升乙腈/水(9/1)的溶剂振荡30分钟

混合物在1650 *g* 的转速下，离心5分钟，取出上清液

净化

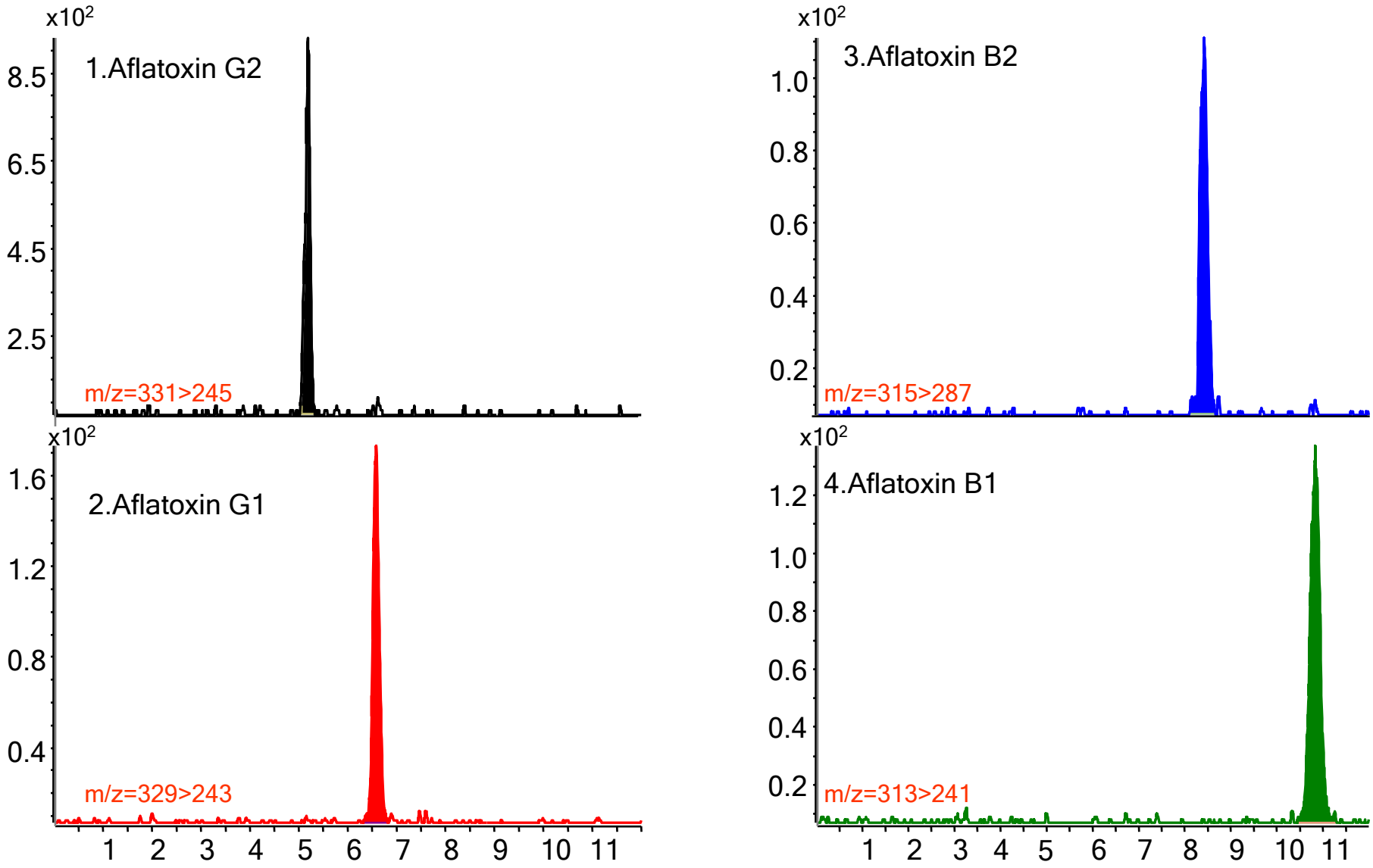
取5 mL 的提取液用Multi Sep #228净化柱净化.

以1mLmin的流速通过净化柱，收集前2毫升的流出液

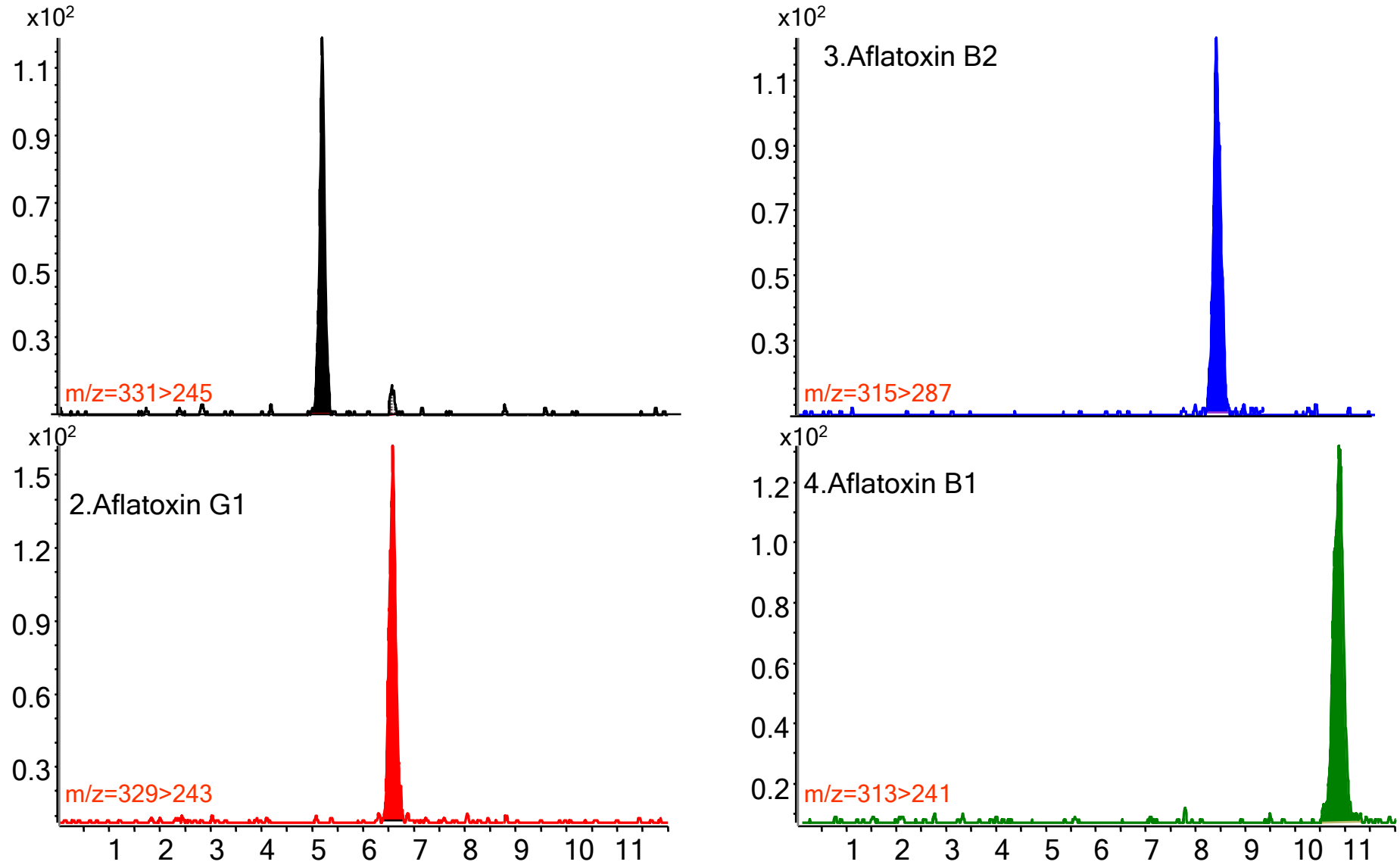
收集的流出液在40^{0}C下，氮气吹干

残留物用1mL 的甲醇/水(4/6)再溶。

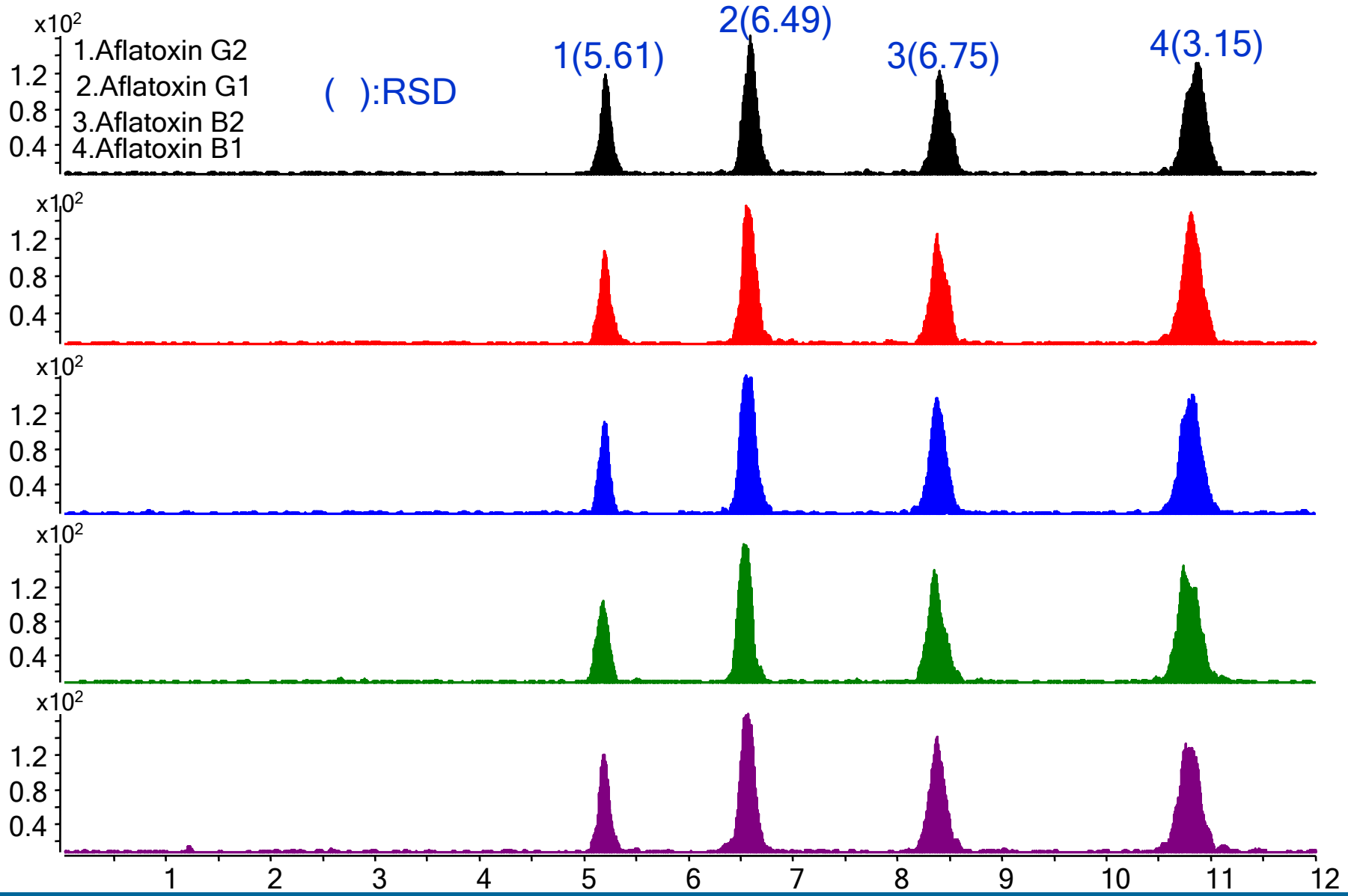
谷物中黄曲霉素的MRM图(2ppb)



玉米中黄曲霉素的MRM图(2ppb)



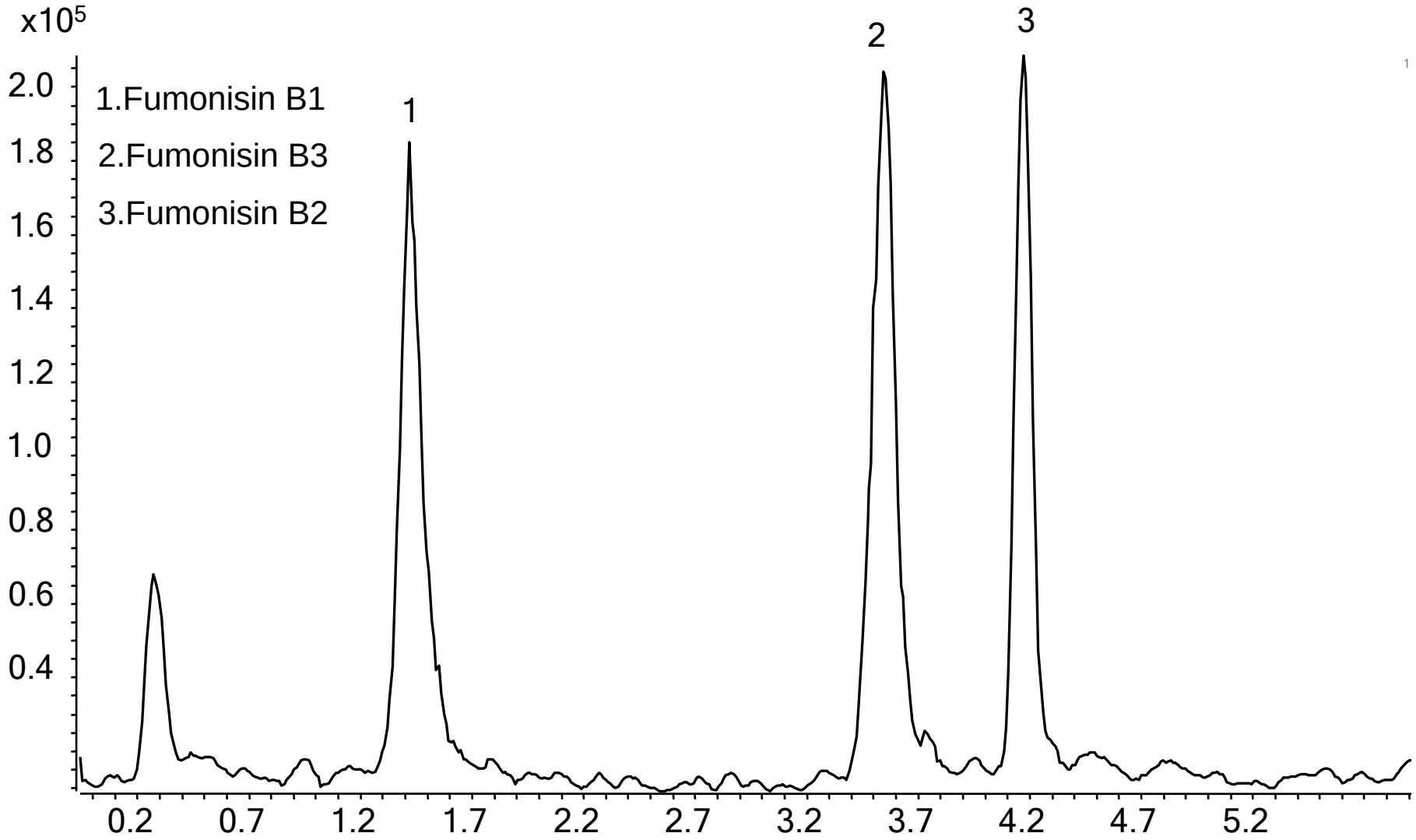
玉米中黄曲霉素分析结果的精密度(2ppb)



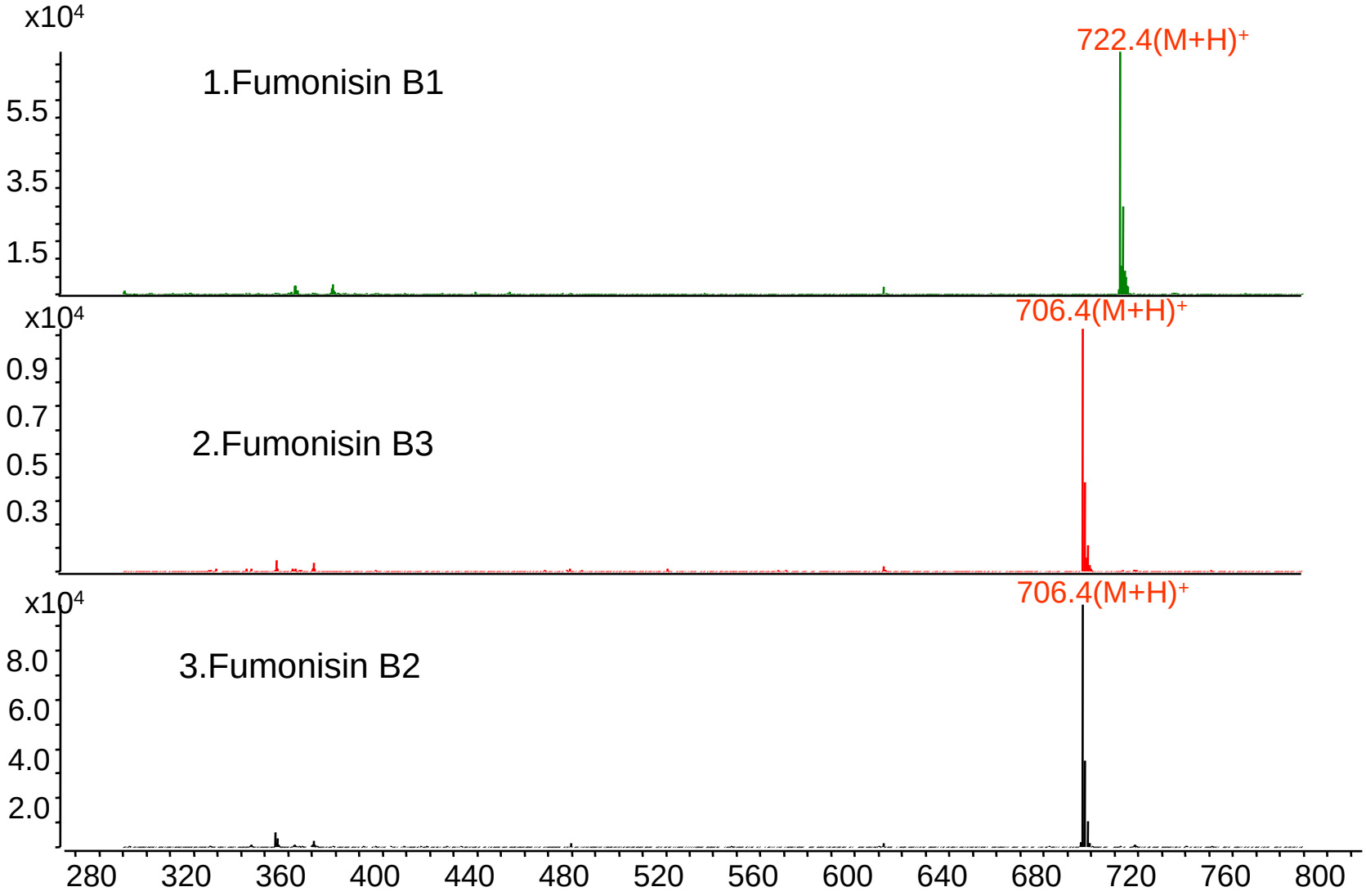
伏马菌素的分析条件

LC	**: 1100LC**
Column	**:Zorbax Exlipse XDB C18(50mm,2.1mm,3.5μm)**
Mobile phase	**: A: ACN, B: 0.1%HCOOH**
	30%A/70%B---(5min)---80%A
Column temp	**: 40℃**
Sample volume	**: 1ul**
Flow rate	**: 0.35ml/min**
MS	**: G 6410 QQQ**
Ionization	**: ESI(Positive)**
MS1	**: m/z=722(Fumonisin B1),706(Fumonisin B3,B2)**
MS2	**: m/z=334(Fumonisin B1),336(Fumonisin B3,B2)**
Collision energy	**: 40V(N2 gas)**
Scan range	**: m/z 100-450**
Drying gas	**: 10l/min at 350C**
Nebulizer gas	**: 50psi**
Fragmentor	**: 100V**

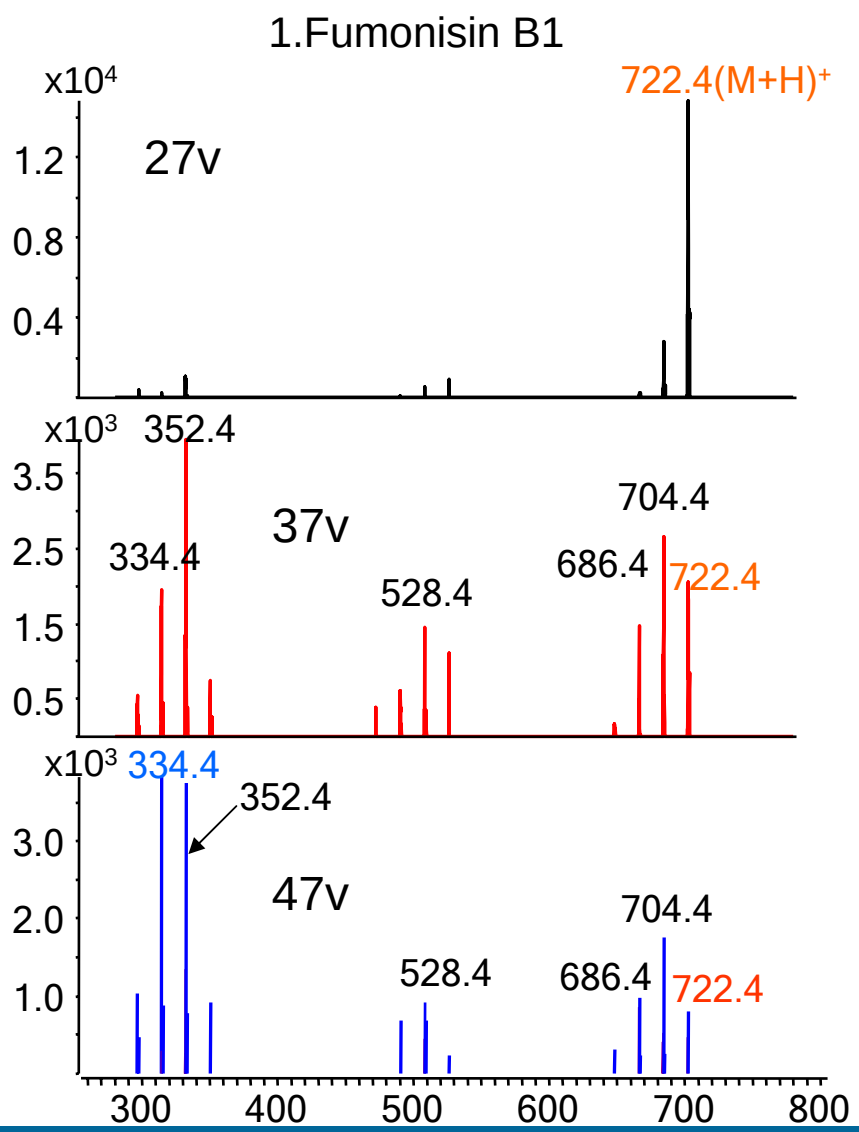
伏马菌素的总离子流图(2ppm)

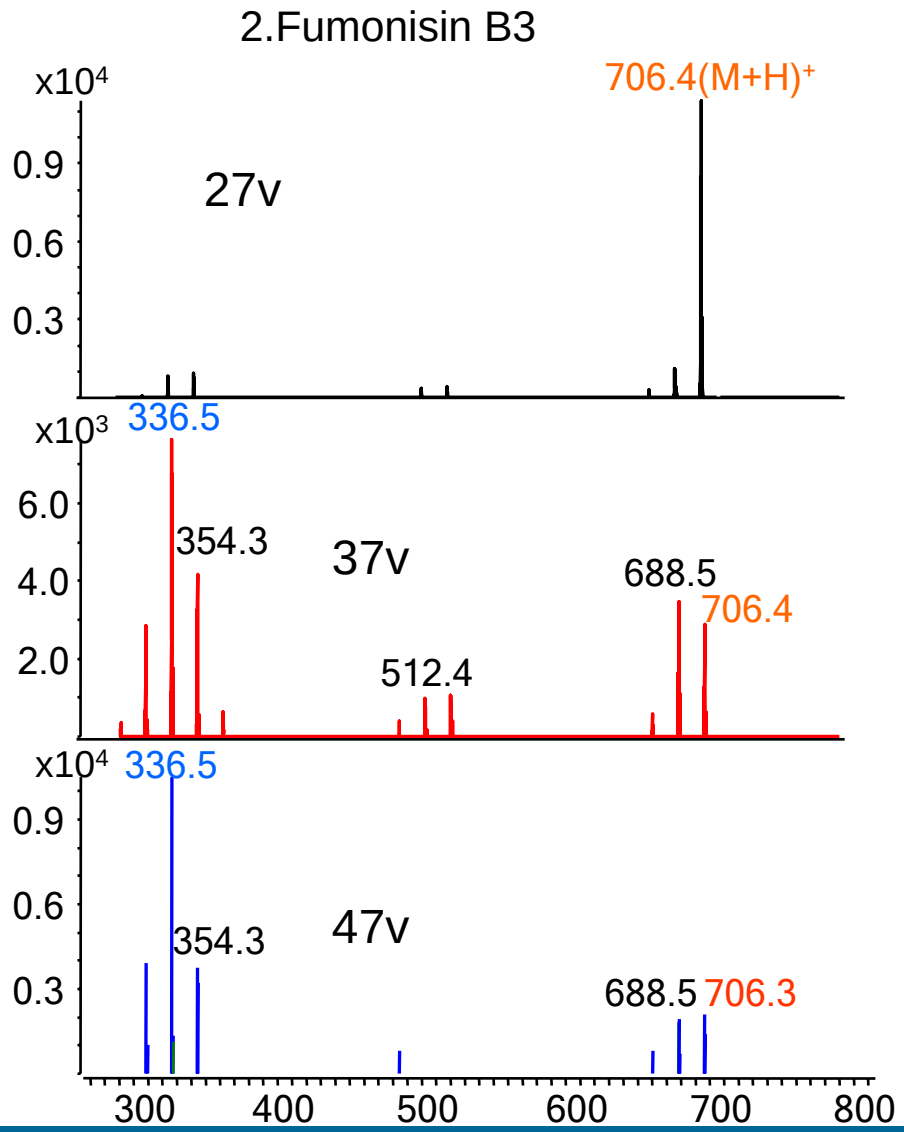


伏马菌素的质谱图(2ppm)

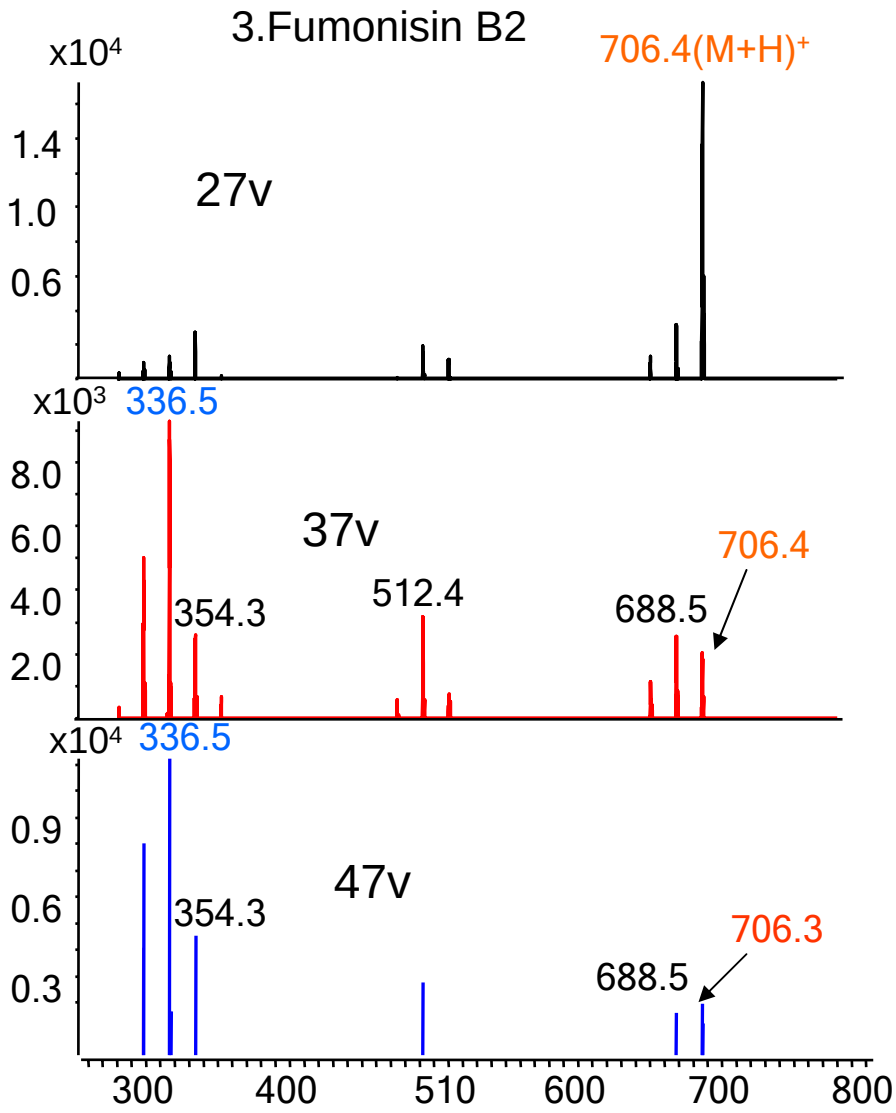


伏马菌素的MS/MS图(2ppm)

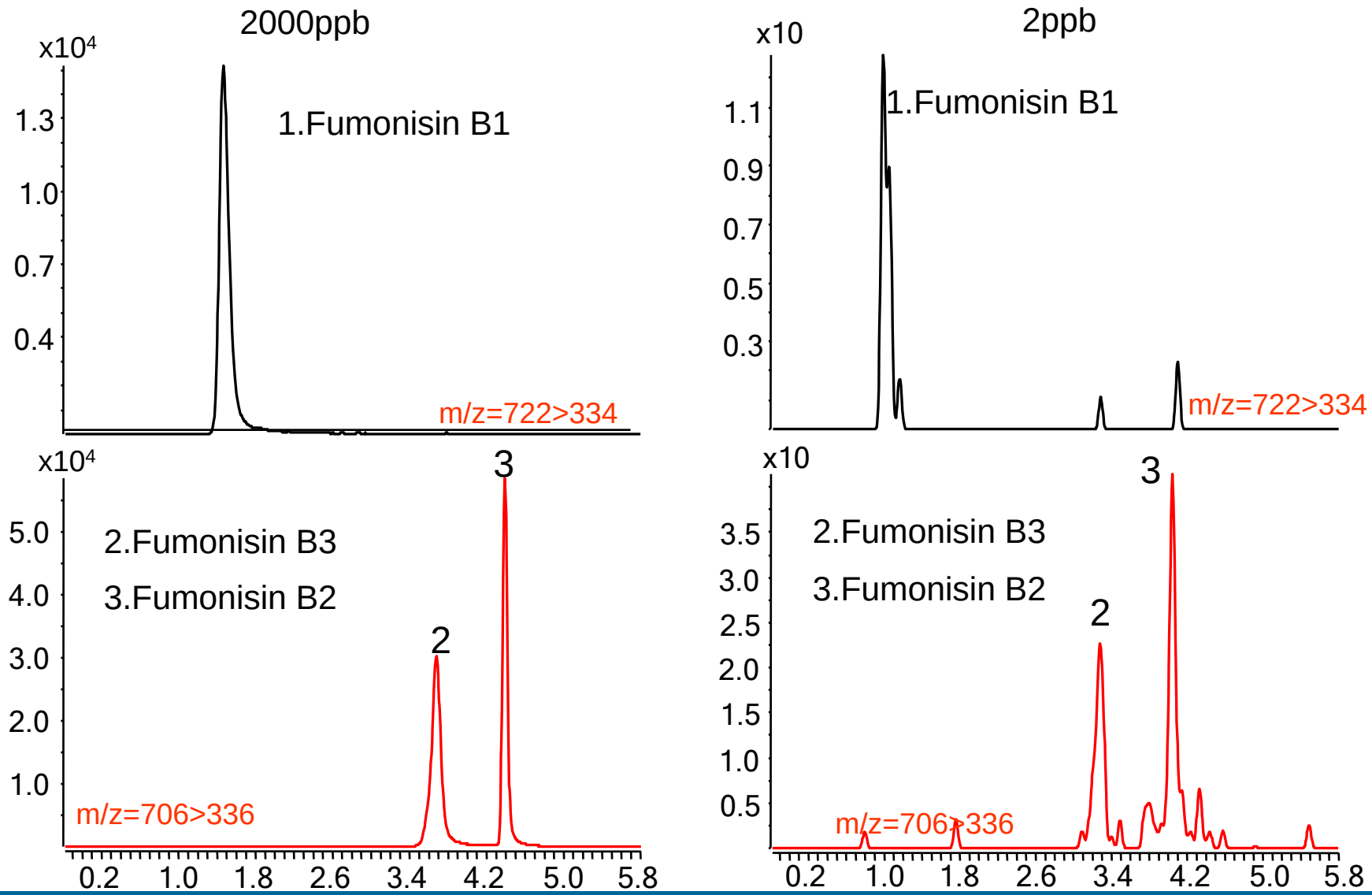




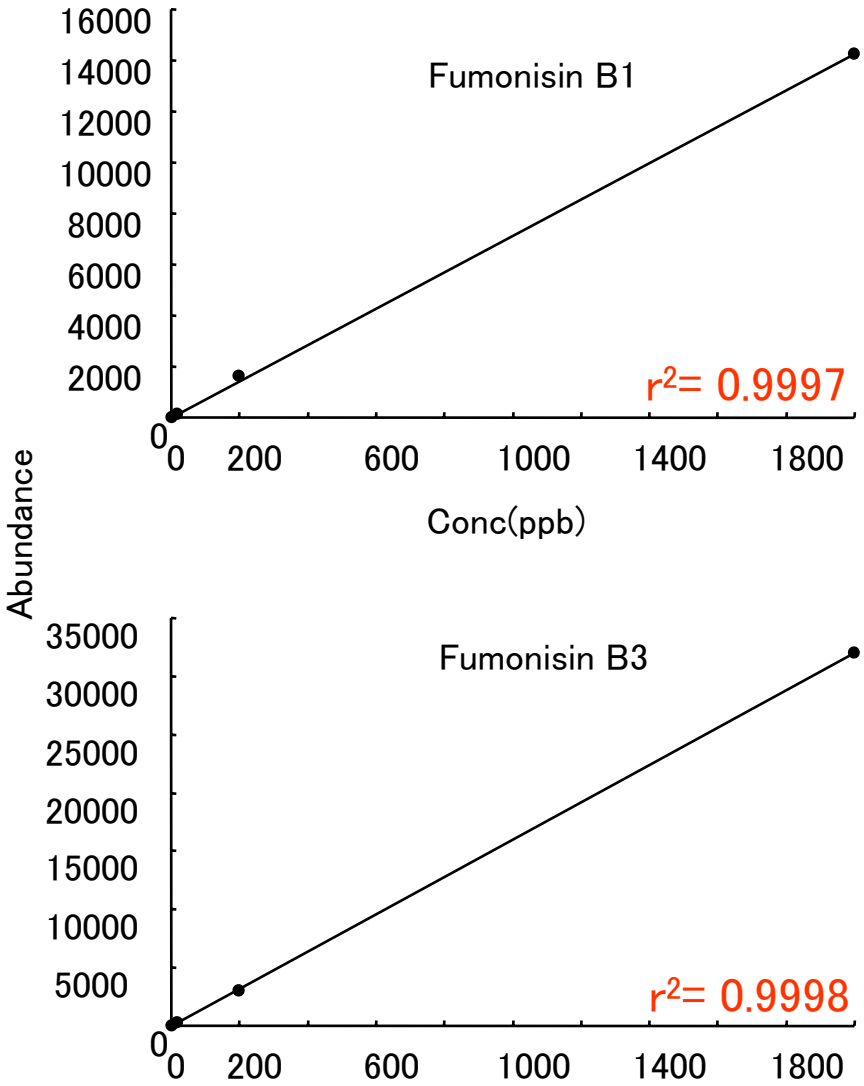
伏马菌素的MS/MS图(2ppm)

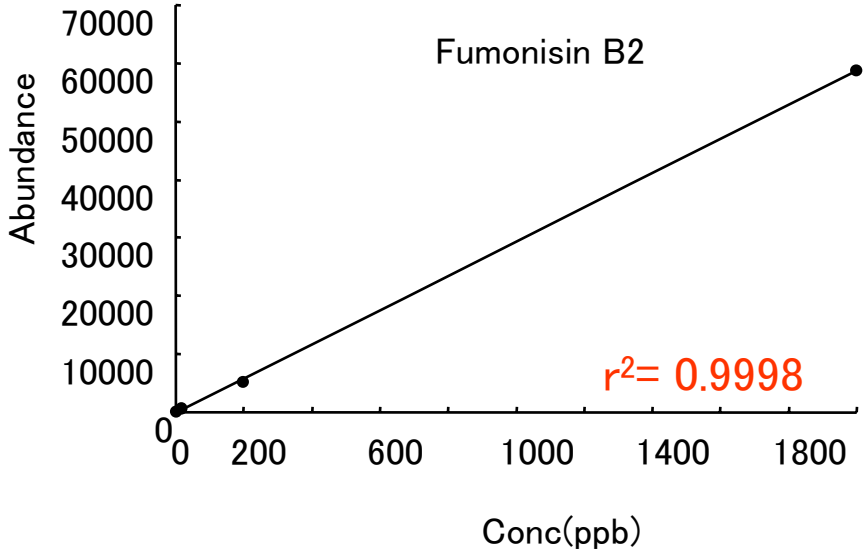


伏马菌素的MRM图(2ppm)



伏马菌素的定量校正曲线(2000-2ppb)





对于伏马菌素的样品前处理方法

萃取

20g样品加100 mL 甲醇+水(3+1)，振荡15分钟

混合物在 3500 rpm离心10min，取出上清液。

净化

SAX净化柱，分别用 8 mL甲醇 和 8 mL甲醇/水(3/1)活化

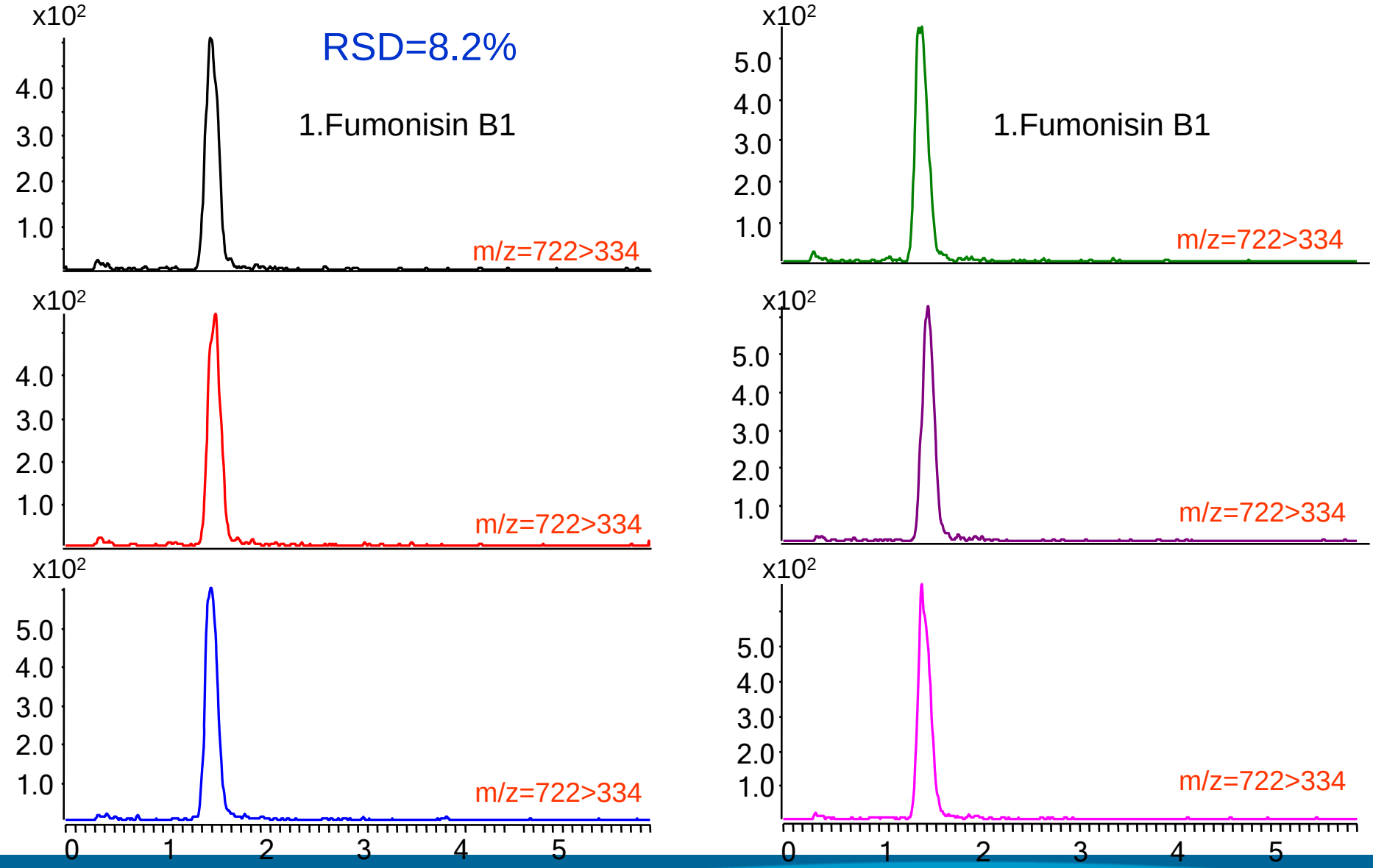
10 mL上清液，倒入 SAX 净化柱

SAX 净化柱用8 mL 甲醇/水(3/1)和8 mL甲醇淋洗

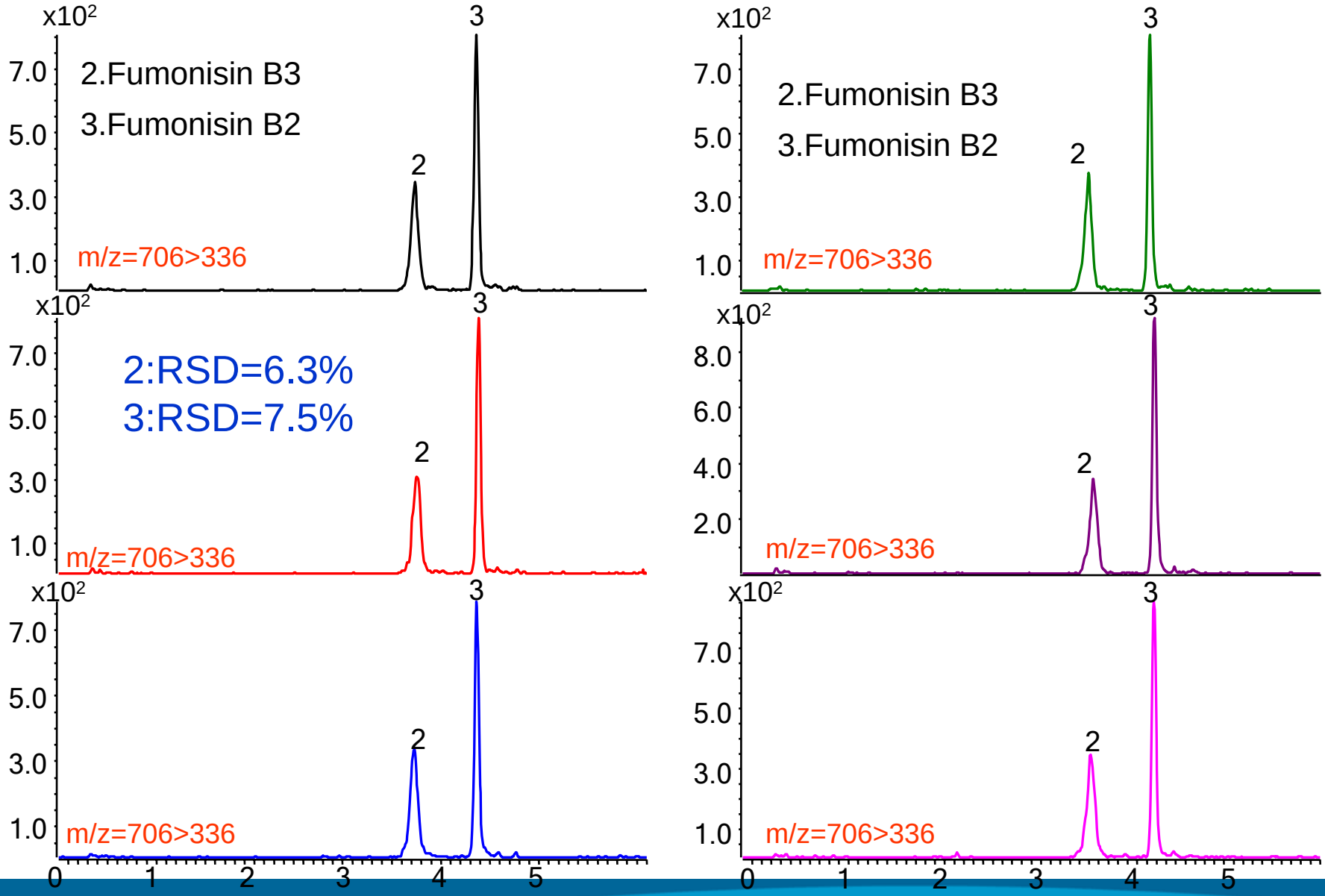
用14 mL 1% 乙酸/甲醇的溶液将赭曲霉素洗脱出来

洗脱液用真空旋转蒸发器蒸干，最后溶解于1ml 甲醇/水(1/1)

爆米花中伏马菌素的MRM图



爆米花中伏马菌素的MRM图



结论

LC/MS/MS具有很高的选择性。适合于检测食品中霉菌毒素类化合物 (黄曲霉素和伏马菌素) 最低检测限（LOD）<1ppb

在样品基质的条件下，MRM的选择性远远高于SIM

对于黄曲霉素，其S/N提高了10倍

谷物类样品中霉菌毒素的分析结果的精密度<10% RSDs

LC/MS/MS 的线性： r^2>0.999