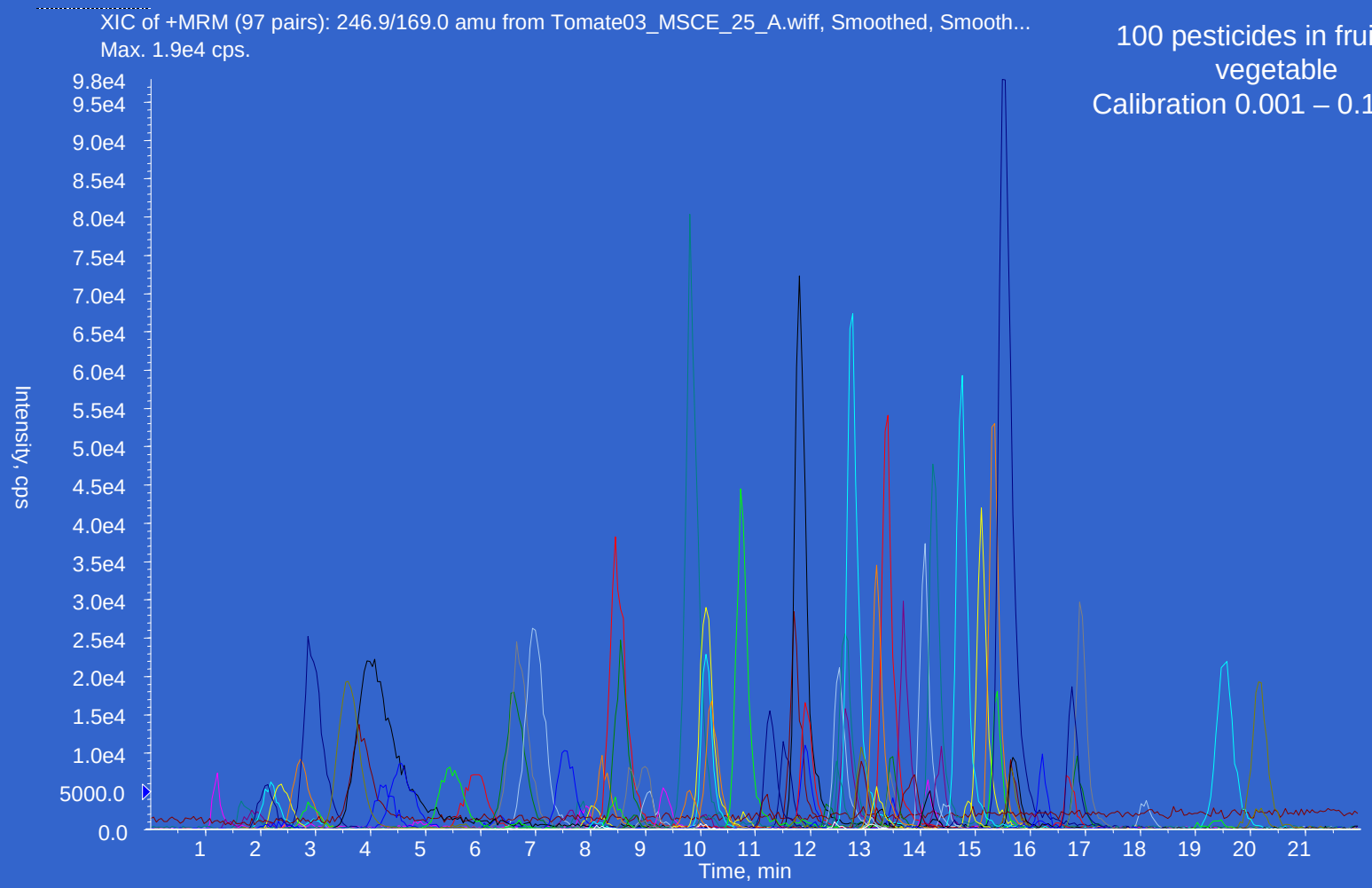
1st CRL/NRL PESTICIDE RESIDUE TRAINING WORKSHOP

6th – 7th December, 2006 – Stuttgart
GERMANY

LARGE SCALE PESTICIDE MULTIRRESIDUE METHODS BY LC-TOF/MS FOLLOWED BY LC-QqQ/MS

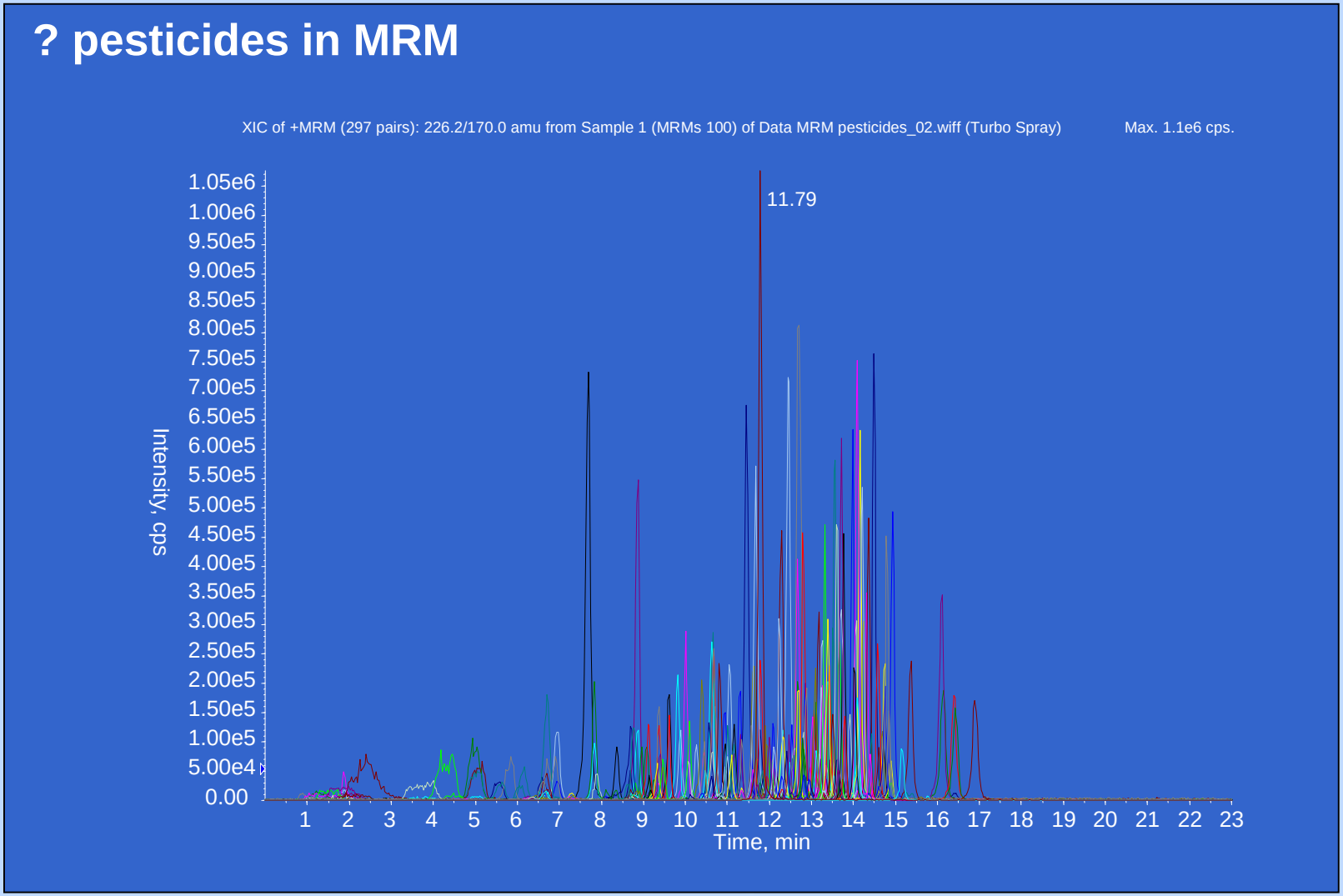
Amadeo R. Fernández-Alba
CRL Pesticides for Fruits and Vegetables
University of Almería
SPAIN

Starting point

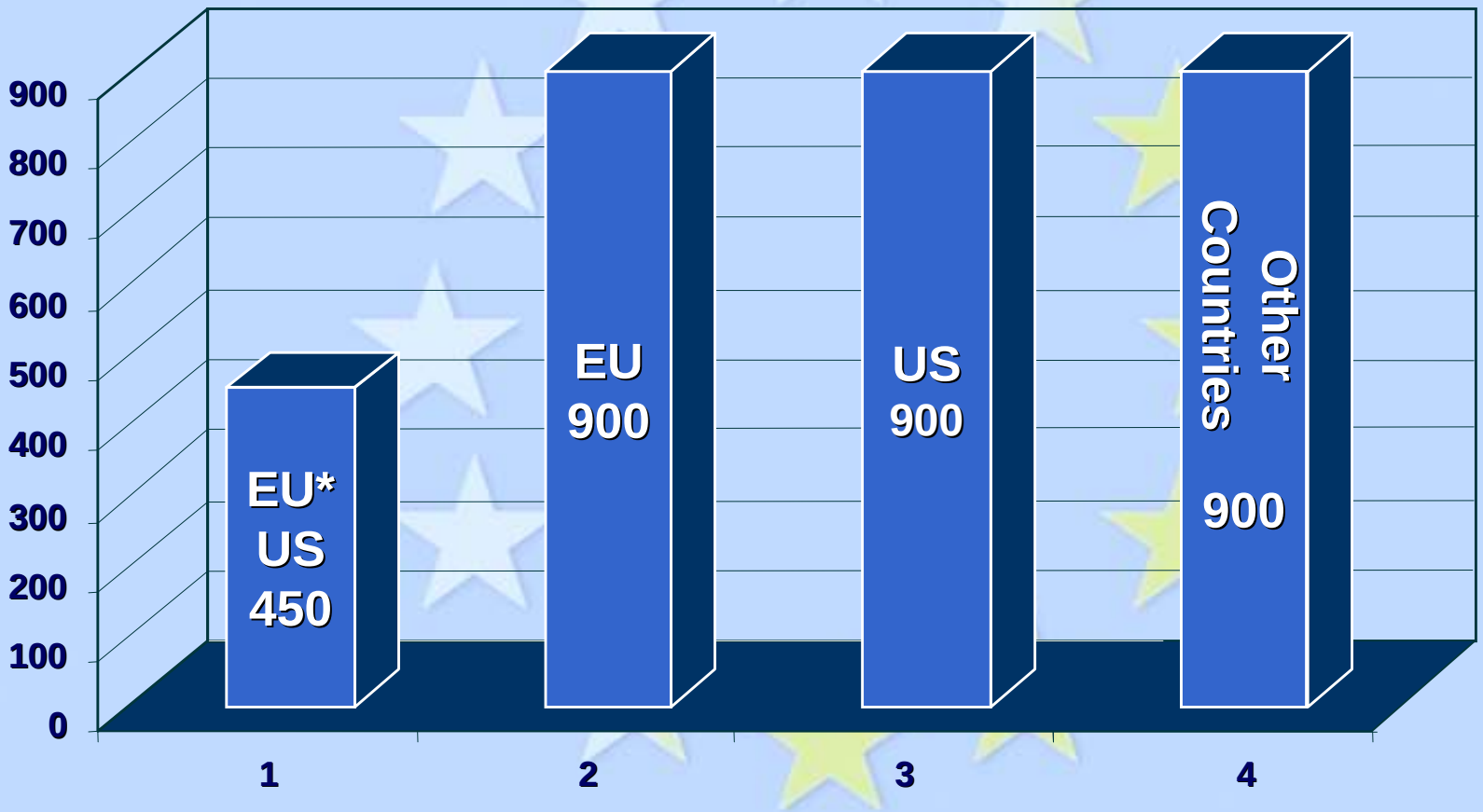


100 pesticides in fruit and vegetable
Calibration 0.001 – 0.1 mg/kg

? pesticides in MRM



Number of Registered Pesticides



0 9 0 0

EUPT 6

PESTICIDES	Nº of reported results	Nº of NA reported	False negatives	% of results from the total 127
Acrinathrin	69	52	4	58
Azoxystrobin	91	27	7	77
Bromopropylate	117	7	1	93
Chlorothalonil	113	9	3	91
Diazinon	123	2	0	97
Dimethoate	113	4	8	95
Endosulfan	118	5	2	95
Imazalil	87	23	15	80
Imidacloprid	51	69	5	44
CS2	77	45	3	63
Oxydemeton-methyl	42	72	11	42
Procymidone	119	6	0	94
Thiabendazole	87	31	7	74

EUPT 7

PESTICIDES	Nº of reported results	Nº of NA (not sought)	False negatives	% of results from the total 125
Acetamiprid	56	67	2	44.8
Carbaryl	101	24	0	80.8
Cyprodinil	99	24	2	79.2
Diazinon	123	2	0	98.4
Dimethoate	119	2	4	95.2
Fenhexamid	89	36	0	71.2
Fludioxonil	85	36	4	68.0
Imidacloprid	64	60	1	51.2
Iprodione	113	8	4	90.4
Kresoxim-methyl	104	20	1	83.2
Methomyl	71	45	9	56.8
Monocrotophos	89	30	5	71.2
Procymidone	121	4	0	96.8
Pyrimethanil	98	25	2	78.4
Tetraconazole	70	49	6	56.0
Thiabendazole	104	17	3	83.2

EUPT 8

PESTICIDES	Nº of reported results	Nº of NA (not sought)	False negatives	% of results from the total 128
Acetamiprid	78	47	3	61
Azoxystrobin	113	15	0	88
Bifenthrin	119	9	0	93
Bromopropylate	125	3	0	98
Carbaryl	108	17	3	84
Carbendazim	94	34	0	74
Chlorpyrifos	127	1	0	99
Cyprodinil	114	13	1	89
Diazinon	127	1	0	99
Dichlofluanid	113	5	10	88
Fludioxonil	92	33	3	72
Imazalil	107	14	7	84
Lambda-cyhalothrin	121	5	2	95
Myclobutanil	114	14	0	89
Parathion	114	10	4	89
Pirimicarb	114	14	0	89

Confirmation

1) Compare RT with standard

2) Compare spectrum with standard

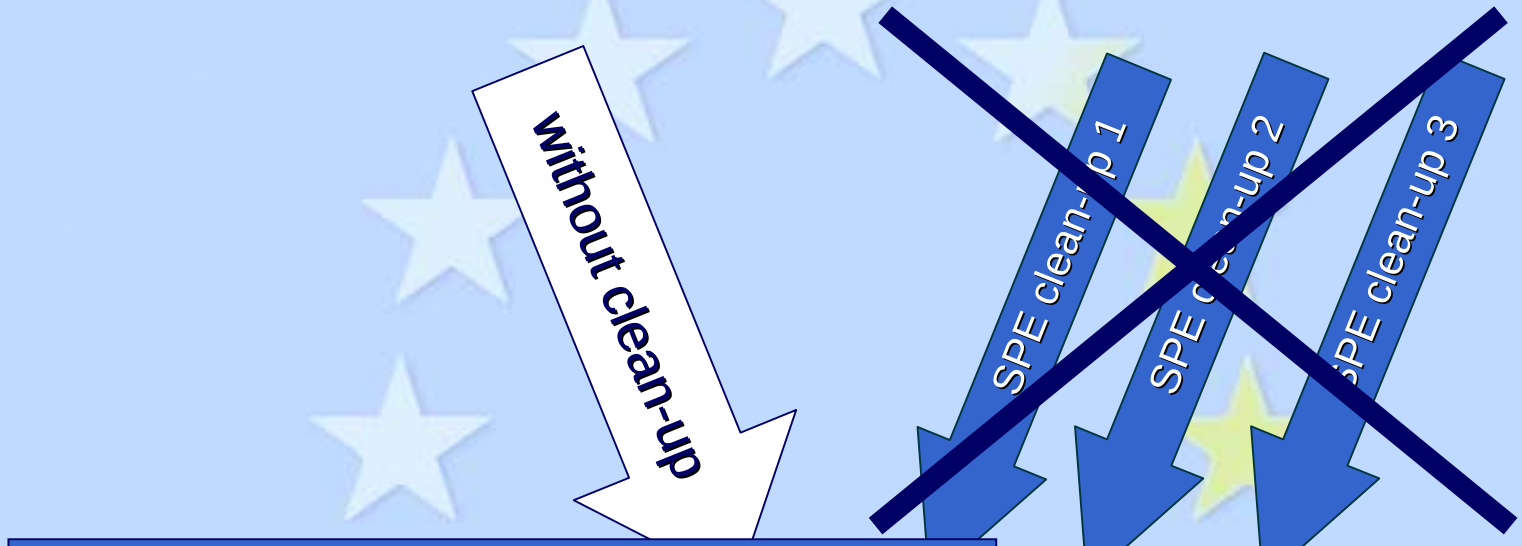
(match 1, 2, 3, 4,... ions)

Confirmation

Confirmation

Types of analyte encountered in GC-LC/MS relative to the associated identification processes and respective techniques

SOLVENT EXTRACTION METHOD

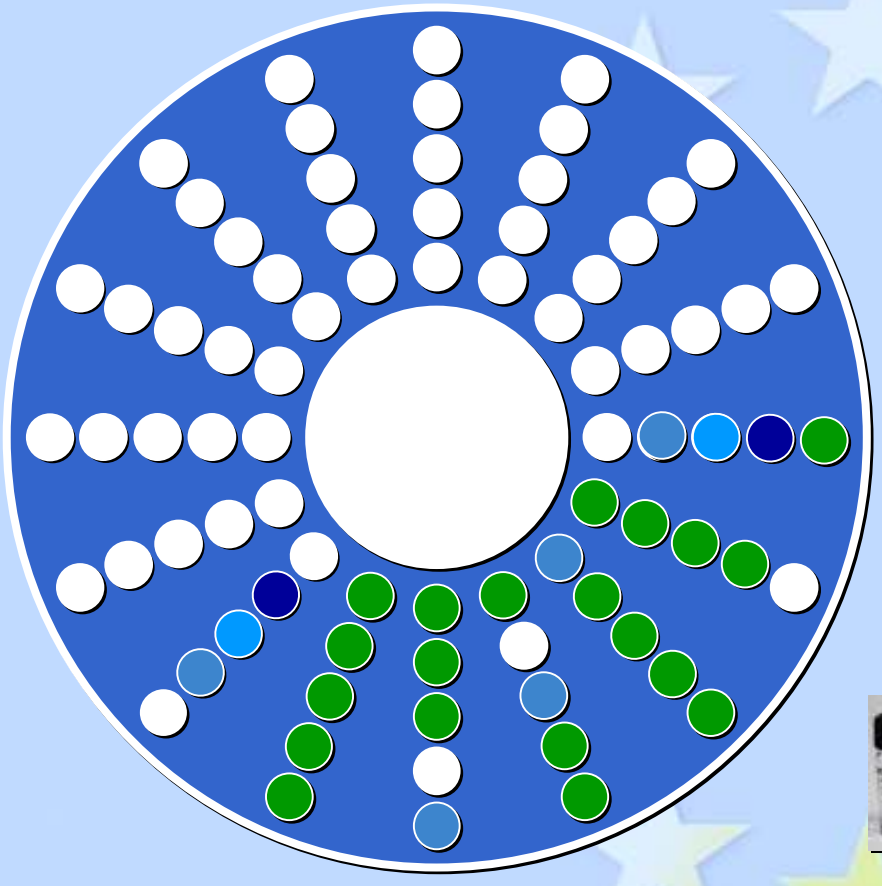


Commodities with high water content
(e.g. lettuce, tomatoes, strawberries)
Fruits with high acid content
(e.g. lemons, oranges, grapefruits)

PI - MS

High Sensitivity (LOQ)
Multiclass compounds
High identification criteria

Practical design of a batch of samples



QC PROCEDURES FOR PEST. RES. ANAL. Guidelines for Residues Monitoring Control in the European Union

Document 7826/VI/97

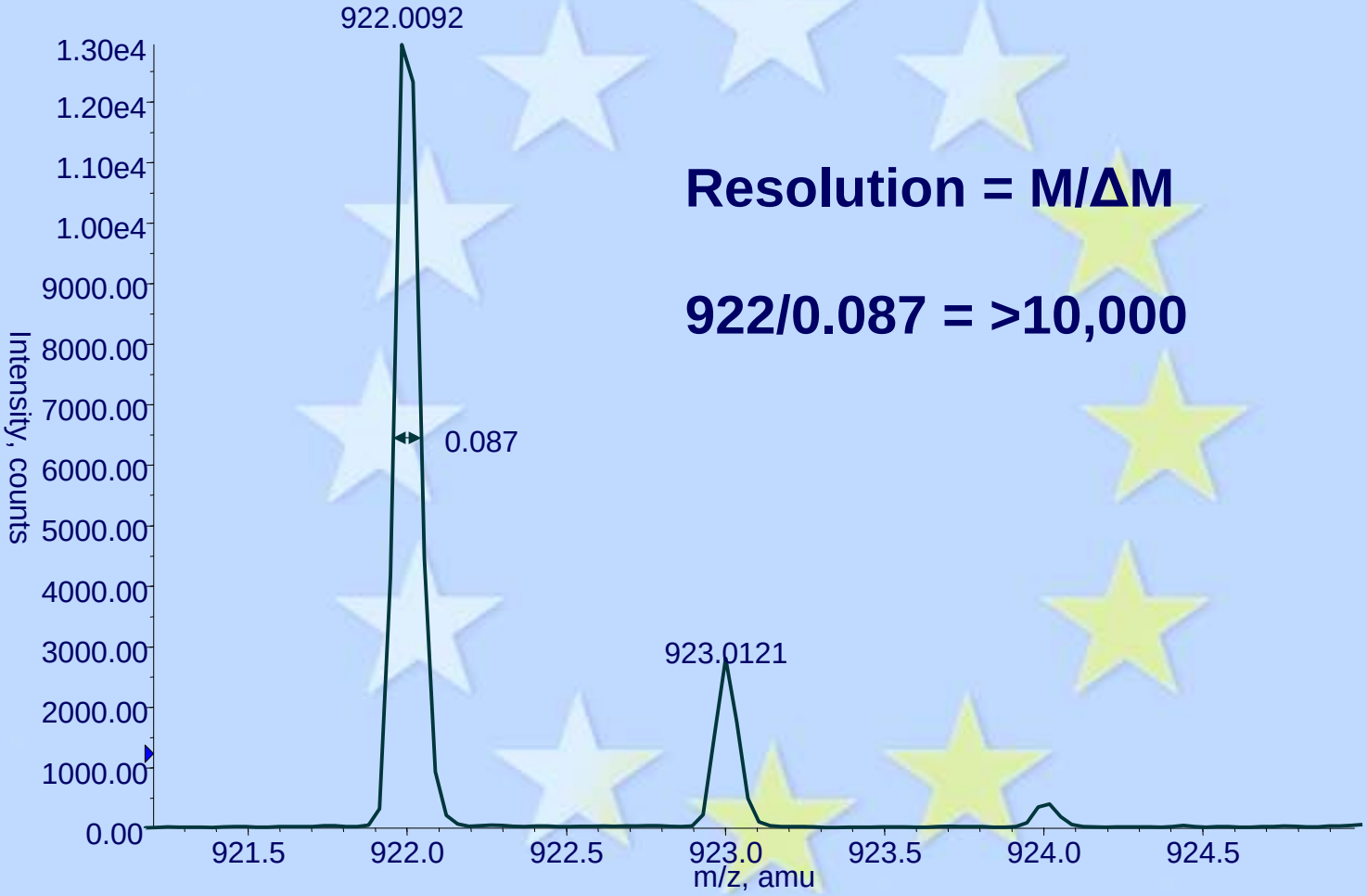
- **20 min (analysis time) + 10 min (equilibration) = 30 min/analysis**

around 50 analysis/day

samples???

LC-TOF/MS

LC-TOF-MS – Mass Resolution

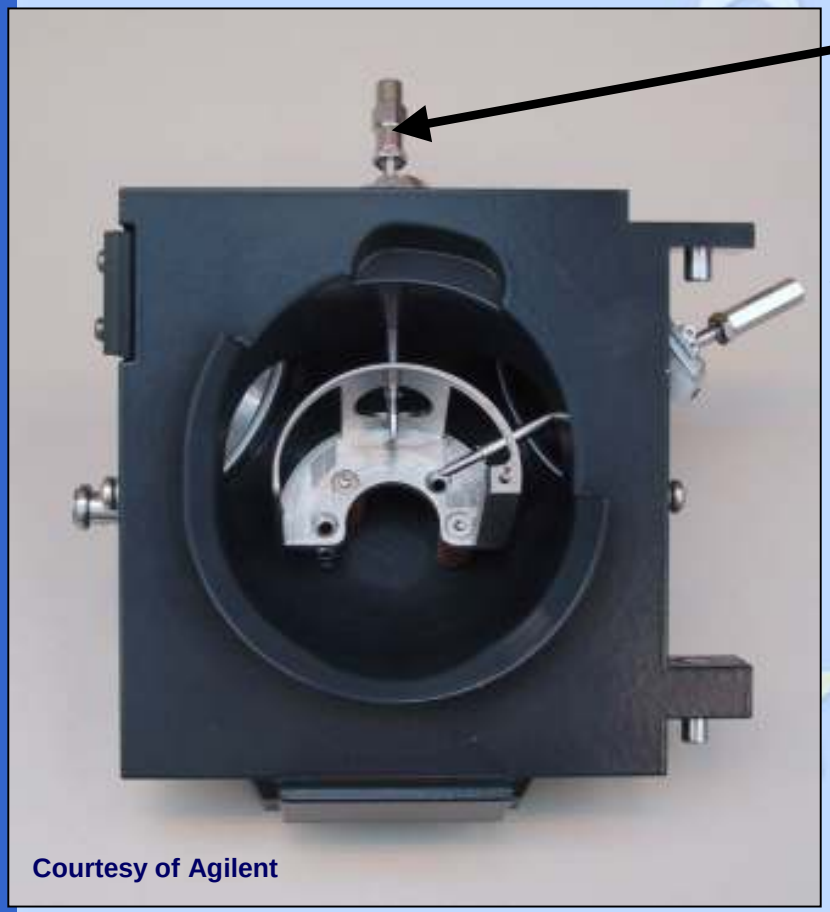


Resolution = M/ΔM

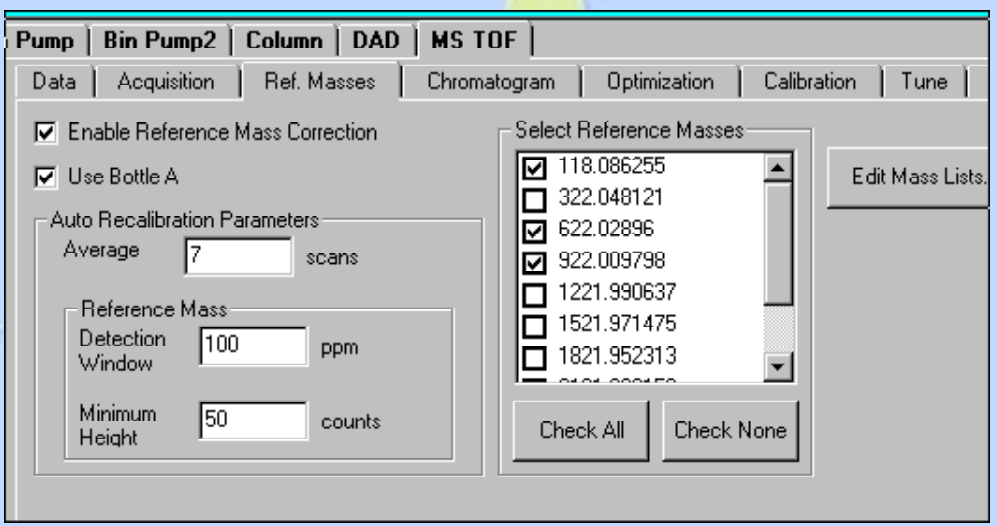
922/0.087 = >10,000

Inlets – Electrospray Ion Source

Dual sprayer design for Sample and lock mass compound

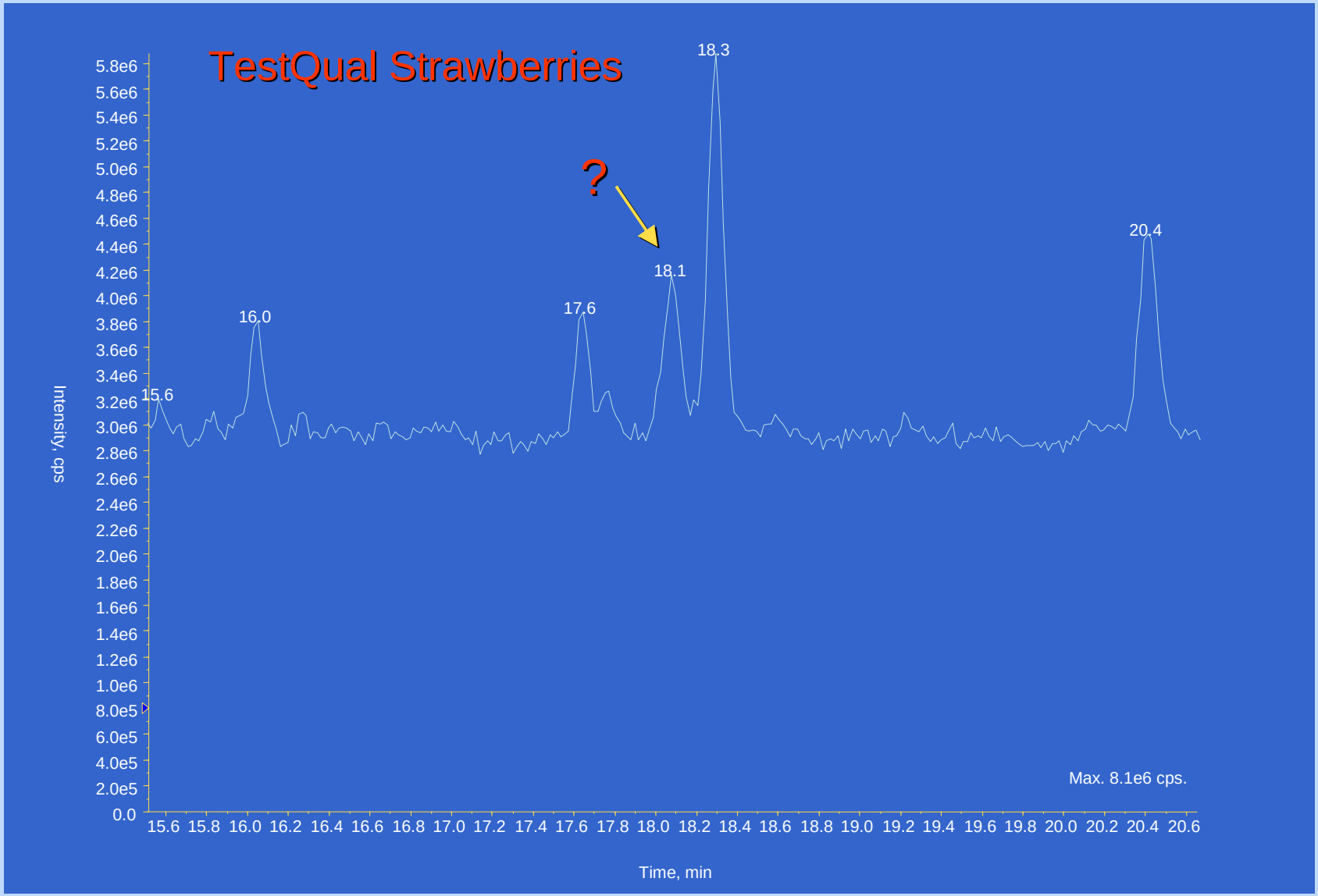


Courtesy of Agilent

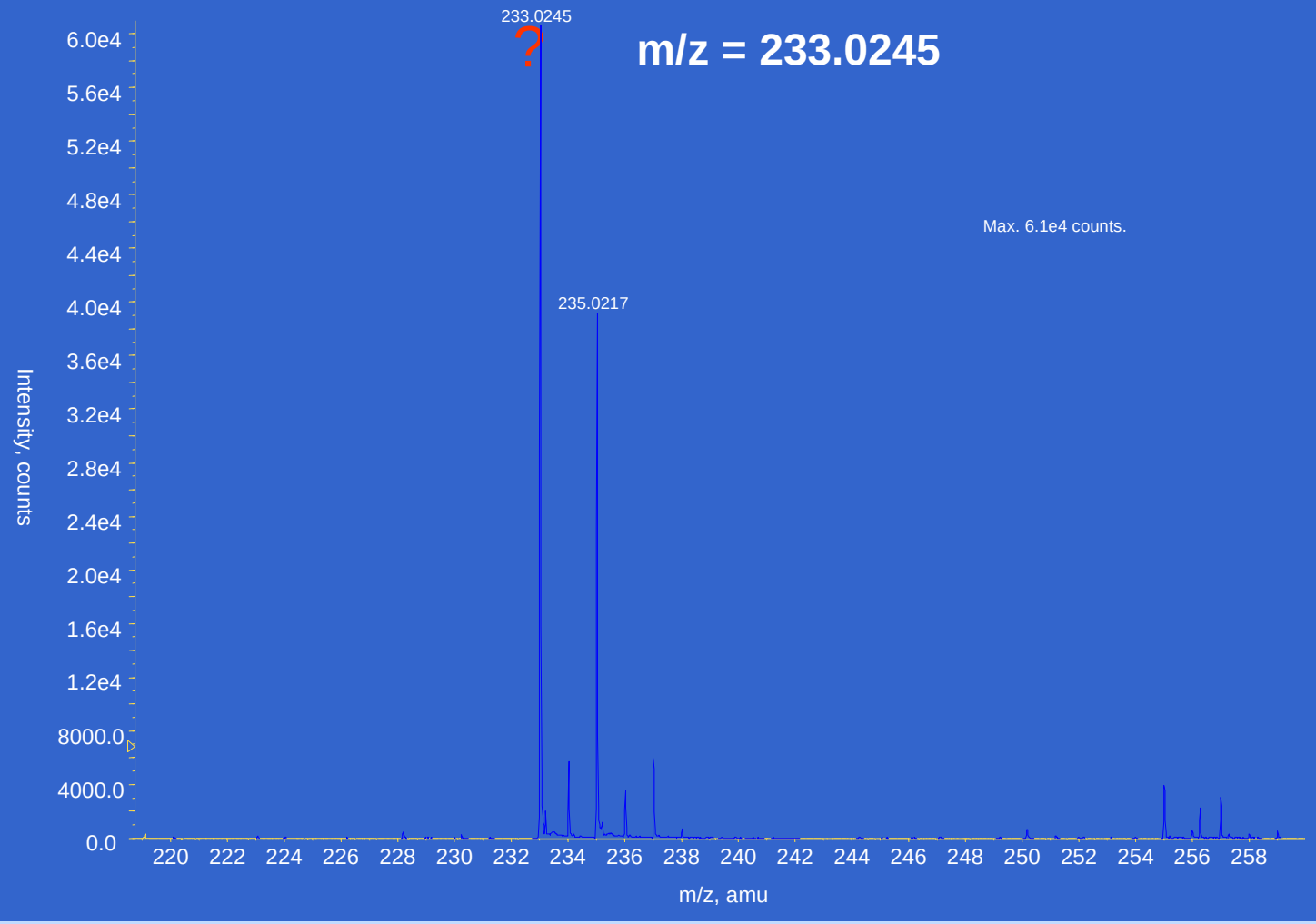


Accurate Mass in Small Molecules

- Single quad reports mass to +/- 0.1 = 165 ppm
- Number of possible formulas (approx.) using only C, H, O & N:
  - 165 ppm (quad) 209
  - 10 ppm 13
  - 5 ppm 7
  - 3 ppm 4
  - 2 ppm 2
- Accurate mass reduces the number of possible molecules.


TestQual Strawberries
?
15.6
16.0
17.6
18.1
18.3
20.4
Intensity, cps
Time, min
Max. 8.1e6 cps.
5.8e6
5.6e6
5.4e6
5.2e6
5.0e6
4.8e6
4.6e6
4.4e6
4.2e6
4.0e6
3.8e6
3.6e6
3.4e6
3.2e6
3.0e6
2.8e6
2.6e6
2.4e6
2.2e6
2.0e6
1.8e6
1.6e6
1.4e6
1.2e6
1.0e6
8.0e5
6.0e5
4.0e5
2.0e5
0.0
15.6 15.8 16.0 16.2 16.4 16.6 16.8 17.0 17.2 17.4 17.6 17.8 18.0 18.2 18.4 18.6 18.8 19.0 19.2 19.4 19.6 19.8 20.0 20.2 20.4 20.6

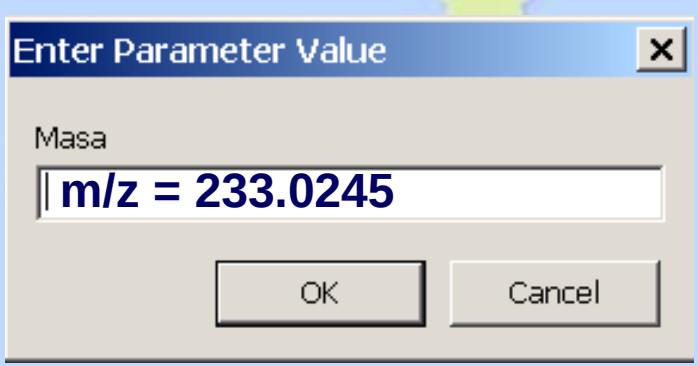
Database-assisted LC/TOF/MS accurate mass identification of non-targeted pesticide residues



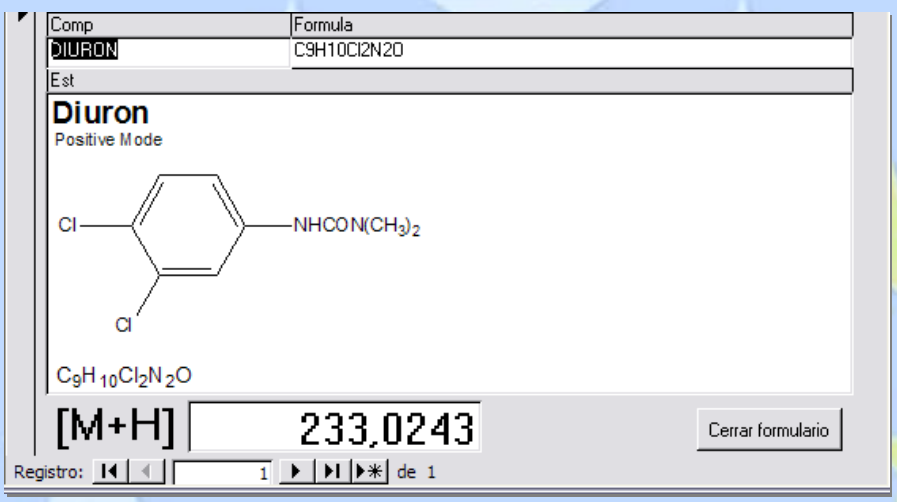
Database-assisted LC/TOF/MS accurate mass identification of non-targeted pesticide residues

Database

Search criterion: accurate mass



Database-assisted LC/TOF/MS accurate mass identification of non-targeted pesticide residues



Does it match with the elemental composition provided by the instrument?

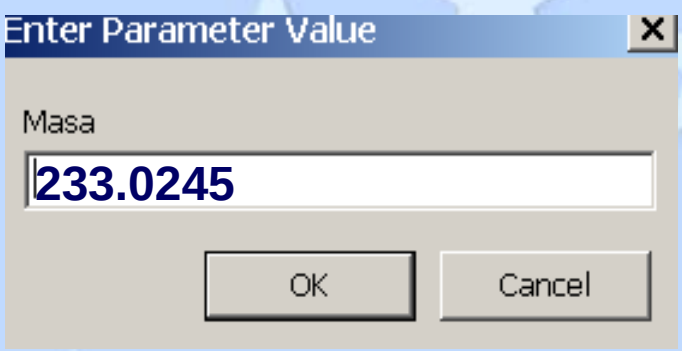
YES

	Formula ...	Calculated m/z (amu)	mDa Error	ppm Error	DBE
1	C9 H11 N2 O Cl2	233.02429	0.205	0.8798	4.5

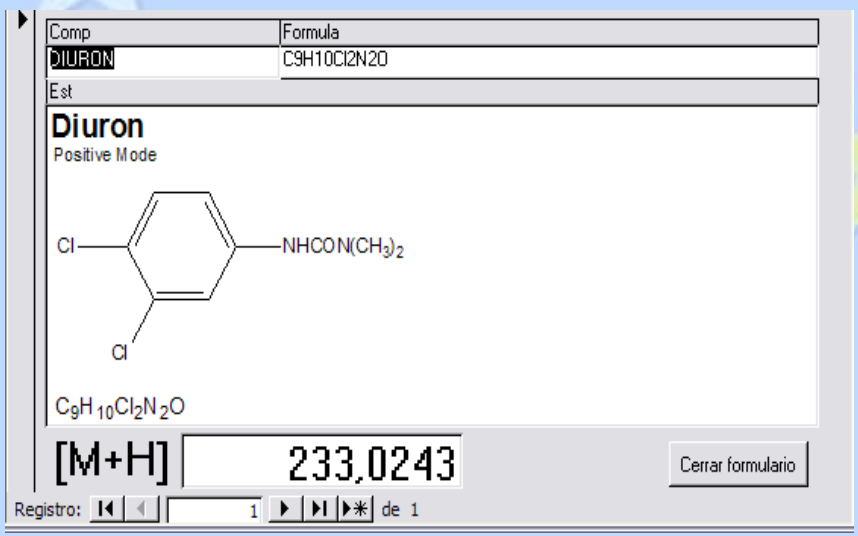
$-H^+$ → $C_9H_{10}N_2OCl_2$

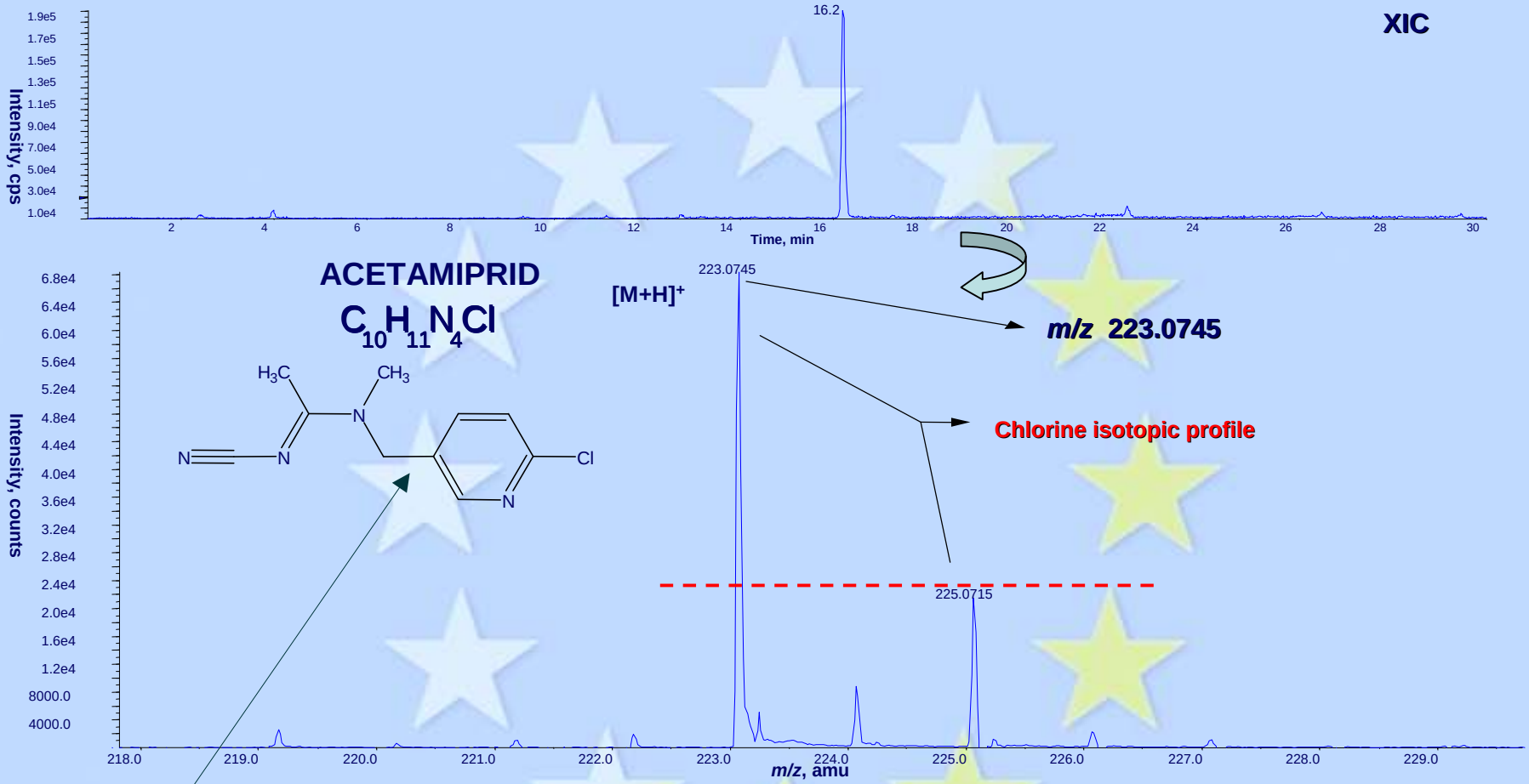
0.9 ppm

Database-assisted LC/TOF/MS accurate mass identification of non-targeted pesticide residues



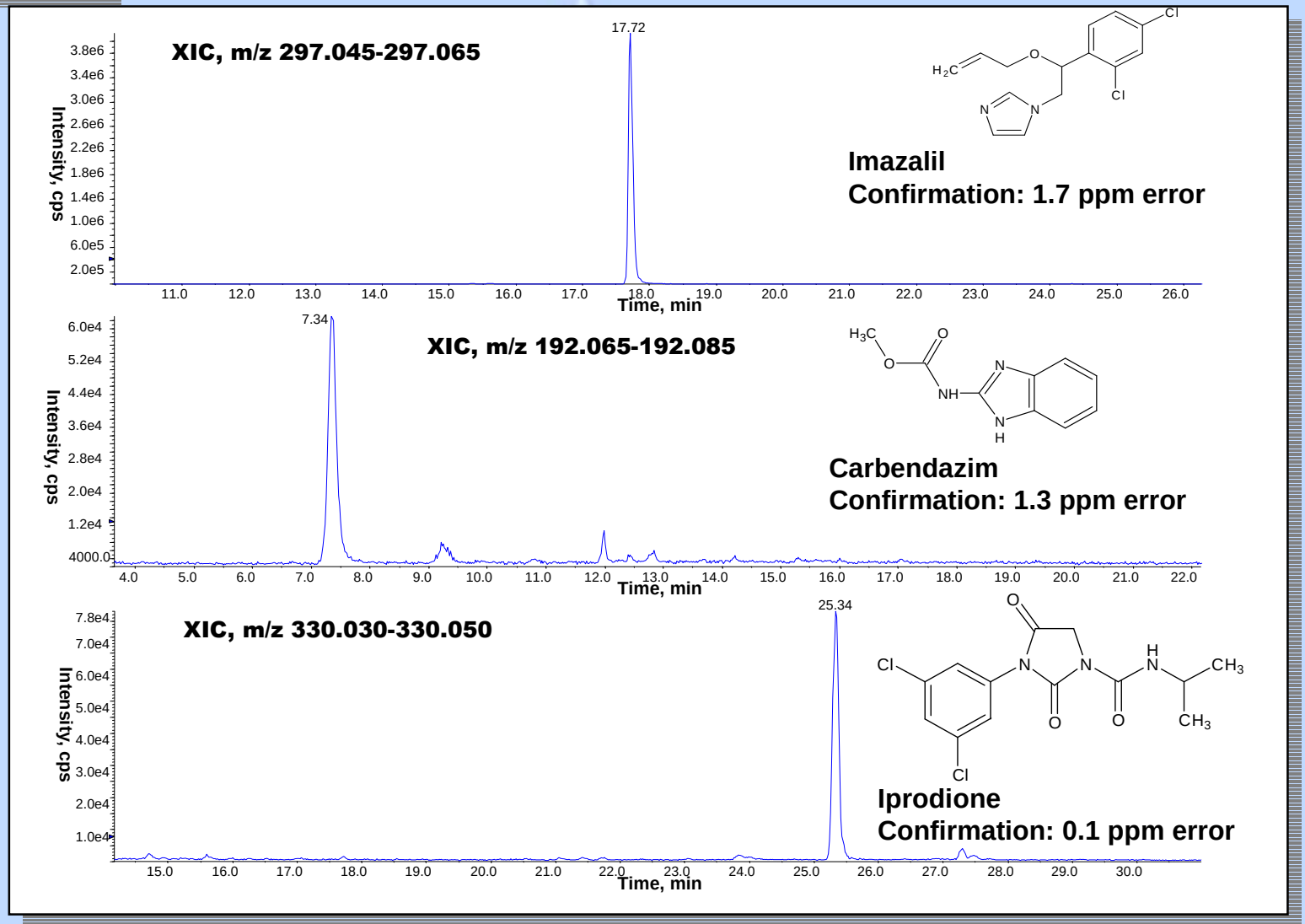
Database results____1 match



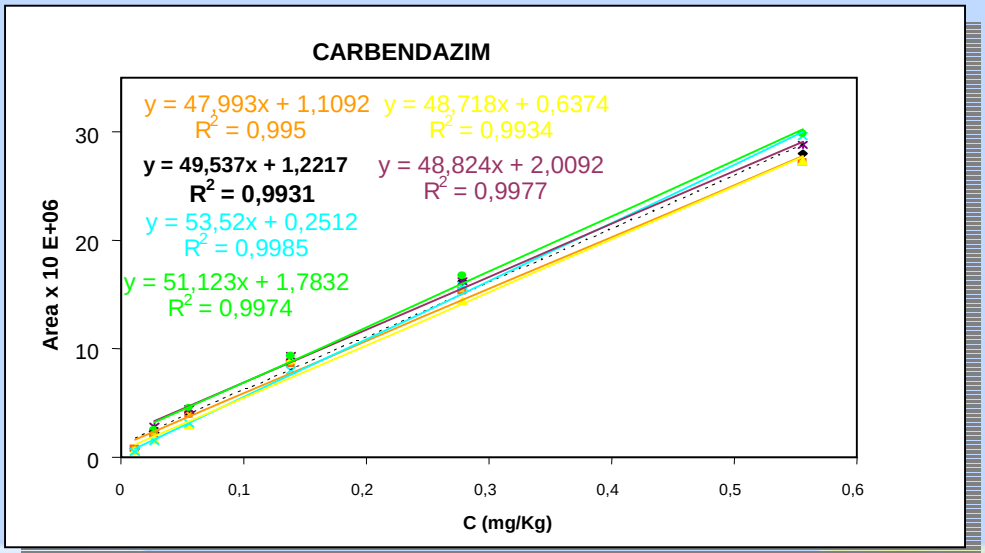


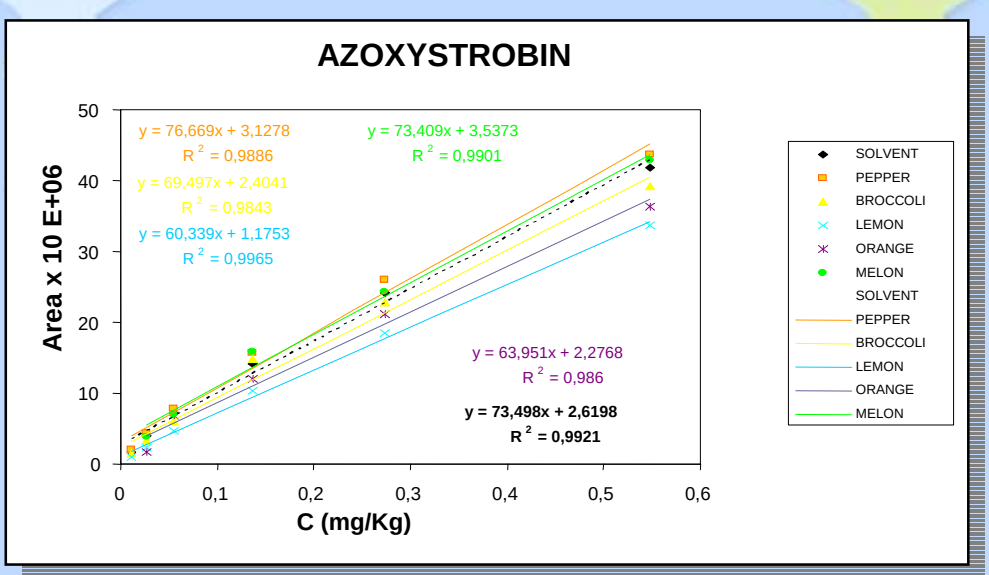
Formula ...	Calculated m/z (amu)	mDa Error	ppm Error
C10 H12 N4 Cl	223.0745	-0.0006	-0.0029
H10 N10 F2 Cl	223.0741	0.3988	1.788
C7 H13 N4 O F Cl	223.07564	-1.1435	-5.1264
C9 H16 O4 Cl	223.07316	1.3366	5.9919

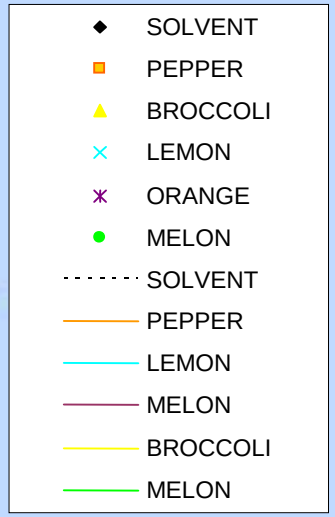
Apple # 22080

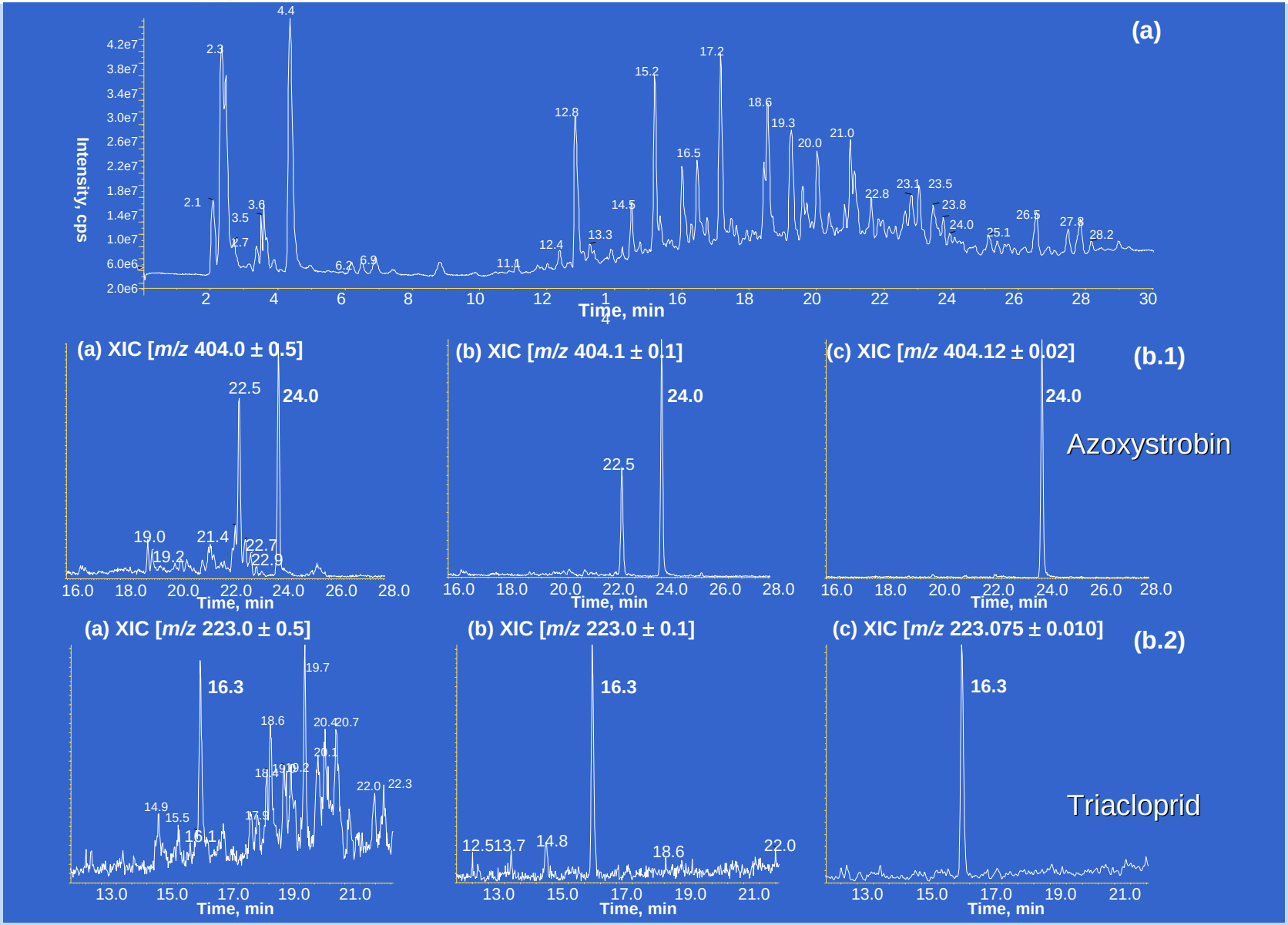

XIC, m/z 297.045-297.065
17.72
Intensity, cps
Time, min
Imazalil
Confirmation: 1.7 ppm error
XIC, m/z 192.065-192.085
7.34
Carbendazim
Confirmation: 1.3 ppm error
XIC, m/z 330.030-330.050
25.34
Iprodione
Confirmation: 0.1 ppm error

Linearity and matrix effects LC-TOF/MS





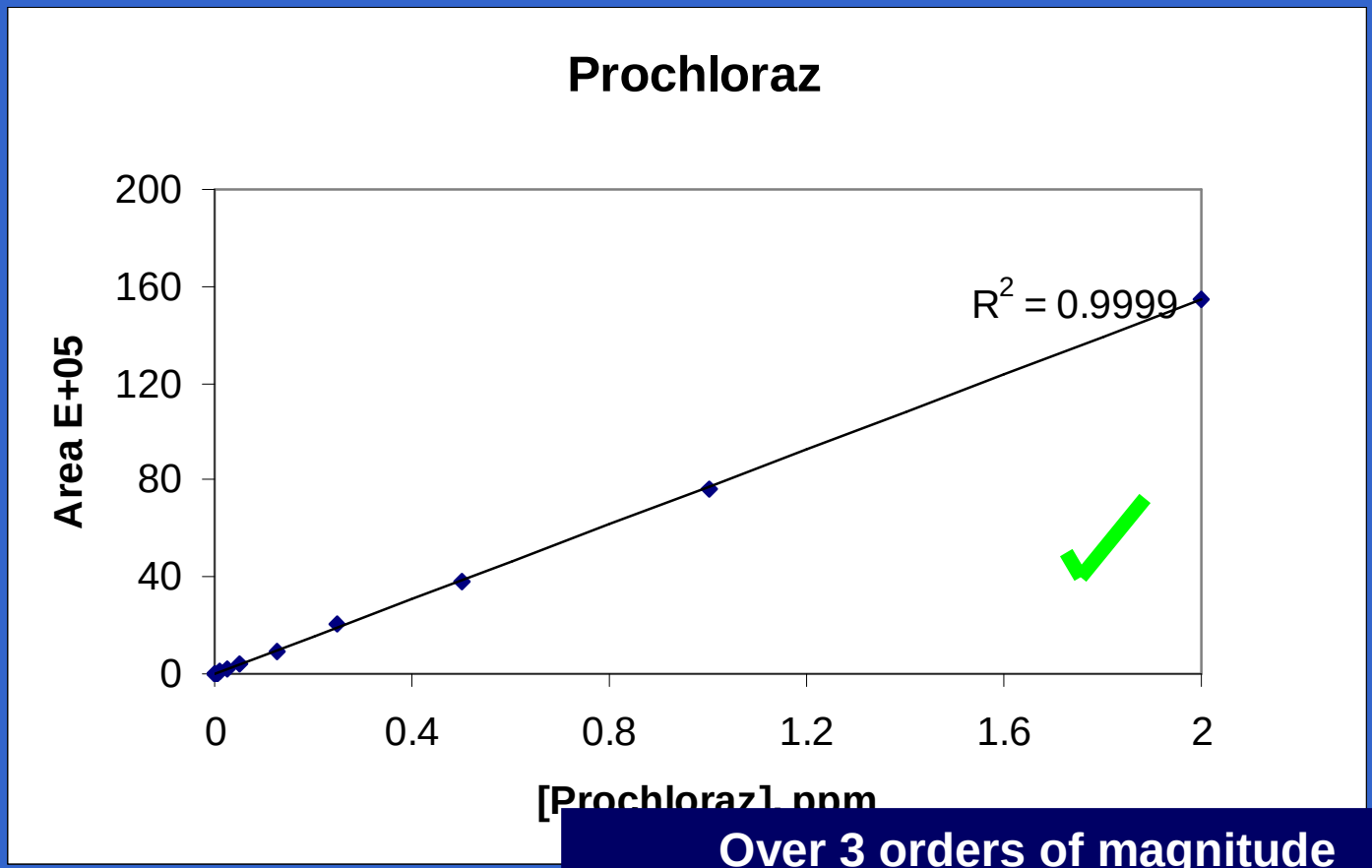



(a)
Intensity, cps
Time, min
(a) XIC [m/z 404.0 ± 0.5]
(b) XIC [m/z 404.1 ± 0.1]
(c) XIC [m/z 404.12 ± 0.02]
(b.1)
Azoxystrobin
(a) XIC [m/z 223.0 ± 0.5]
(b) XIC [m/z 223.0 ± 0.1]
(c) XIC [m/z 223.075 ± 0.010]
(b.2)
Triacloprid

PESTICIDE RESIDUE ANALYSIS by MASS SPECTROMETRIC TECHNIQUES

TARGET	SUSPECTED	UNKNOWN
Parent compounds with "a priori" method information (retention time, MRM transitions)	Parent compounds no method information (retention time, fragmentation)	Degradation products impurities from commercial formulations
STANDARDS AVAILABLE	STANDARDS AVAILABLE	STANDARDS NON AVAILABLE

Linearity by LC/TOFMS
(3 orders of magnitude)

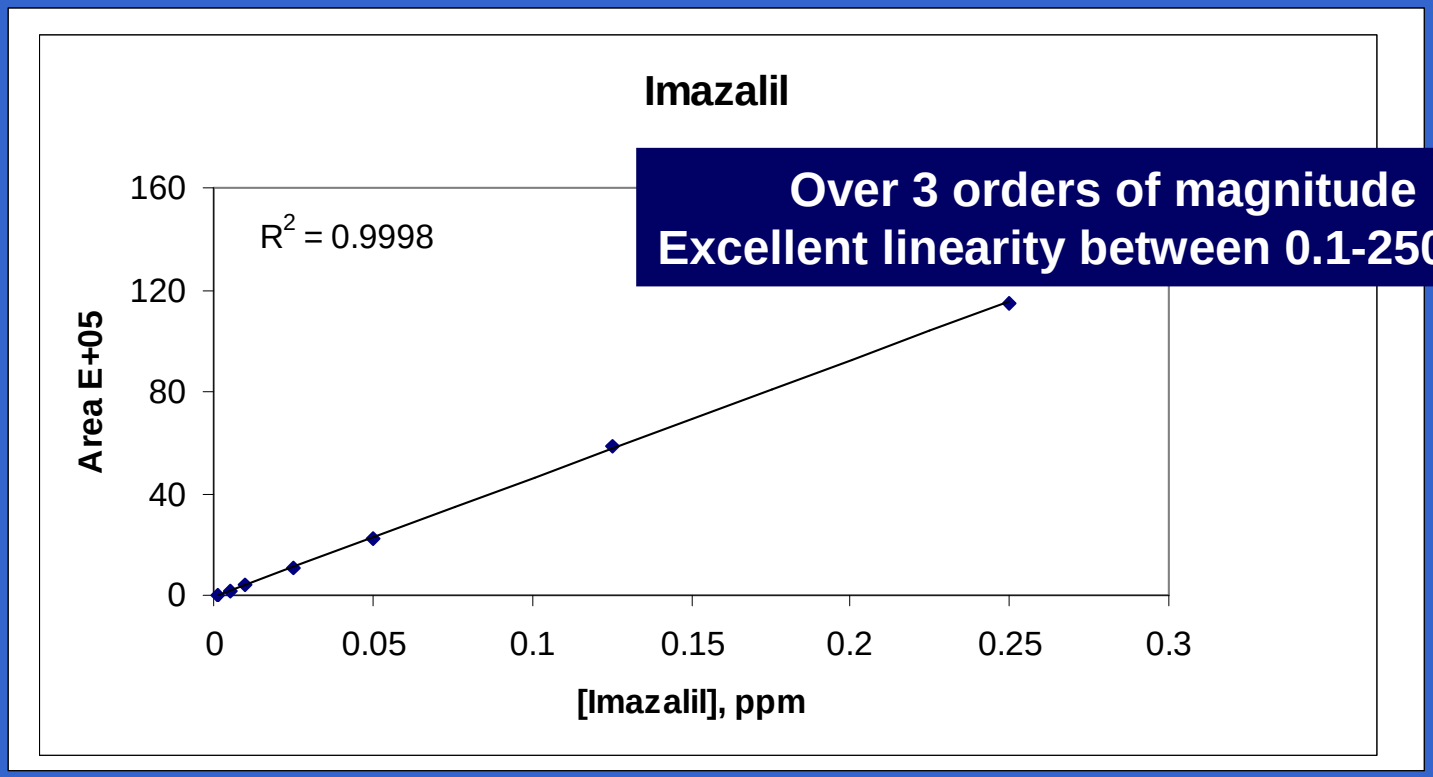


Over 3 orders of magnitude
Excellent linearity between 0.001-2 ppm

Drawbacks of LC/TOFMS

Linearity over 3 orders of magnitude

Quantitation performance might depend upon each individual compound, its sensitivity and MRLs



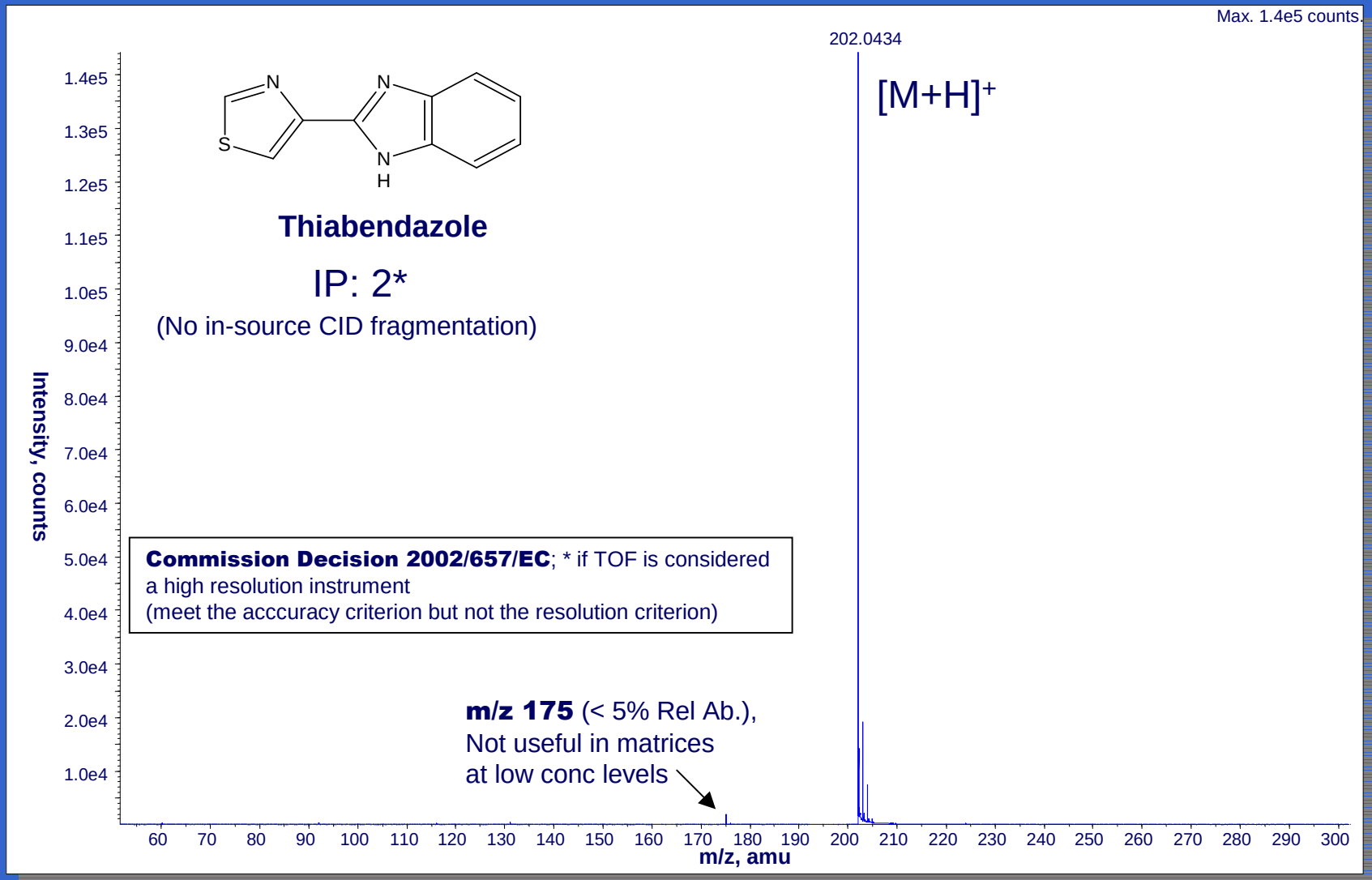
Over 3 orders of magnitude
Excellent linearity between 0.1-250 ppb

Imazalil MRL in citrus 5-10 mg/kg

Drawbacks of LC/TOFMS

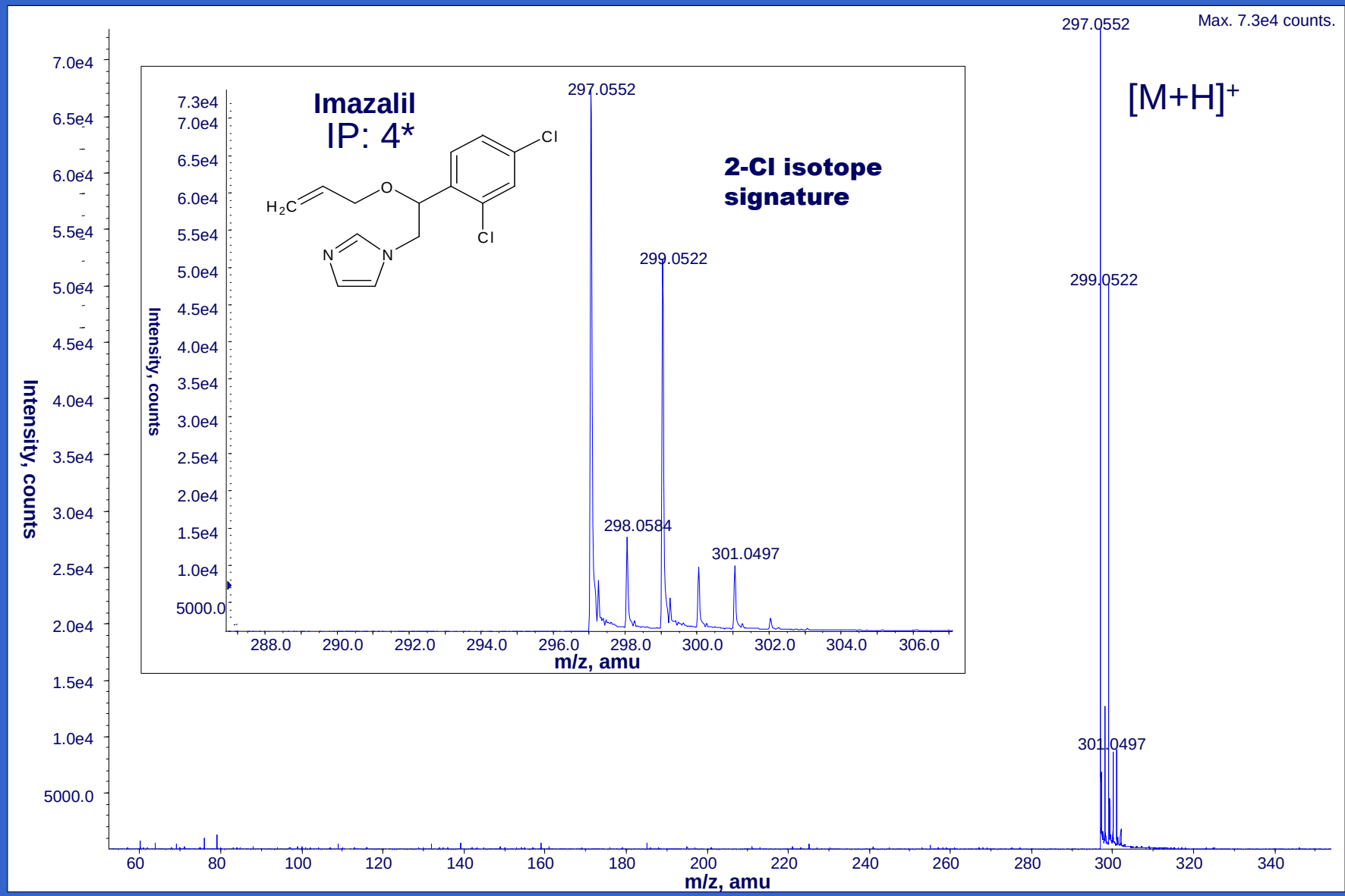
Confirmation criteria: Identification Points (IPs)

Selected pesticides often yield poor in-source CID fragmentation and does not meet 3(4) IP Confirmation criterion



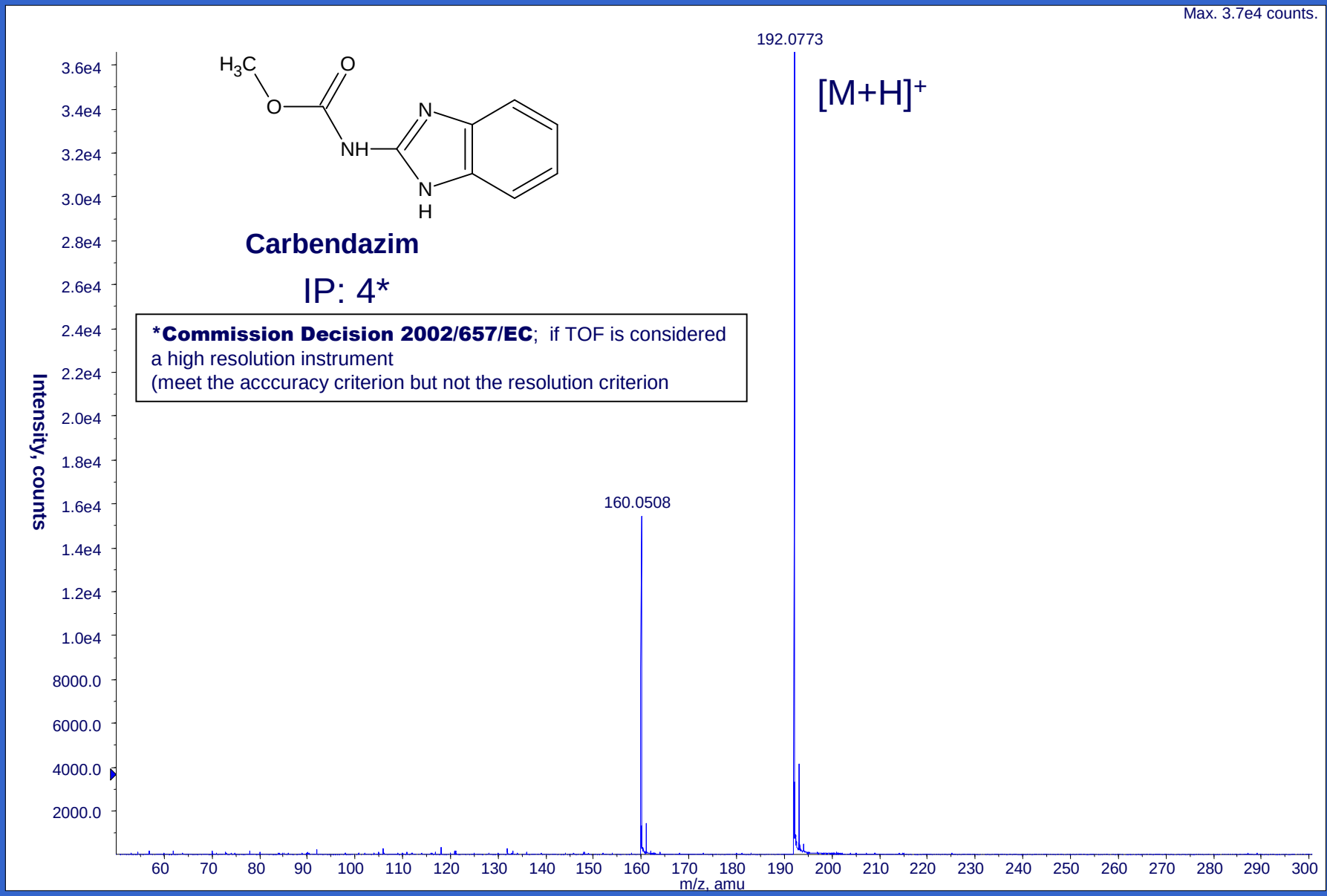
Drawbacks of LC/TOFMS

Confirmation criteria: Identification Points (IPs)



Drawbacks of LC/TOFMS

Confirmation criteria: Identification Points (IPs)



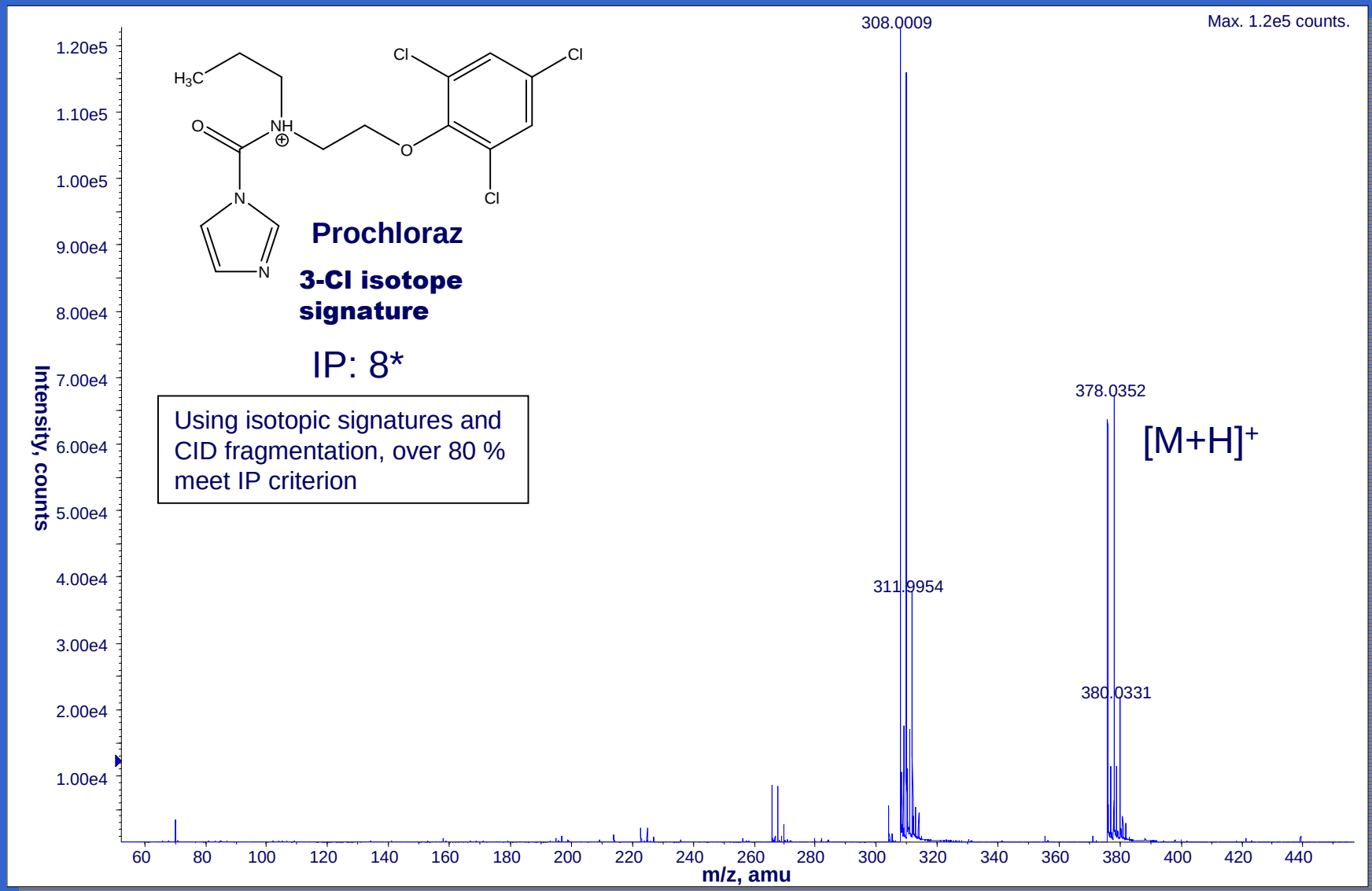
Carbendazim

IP: 4*

***Commission Decision 2002/657/EC**; if TOF is considered a high resolution instrument
(meet the acccuracy criterion but not the resolution criterion

Drawbacks of LC/TOFMS

Confirmation criteria: Identification Points (IPs)



Prochloraz

3-Cl isotope signature

IP: 8*

Using isotopic signatures and CID fragmentation, over 80 % meet IP criterion

Comparative evaluation of LC-TOF/MS and LC/MS/MS for pesticide residue analysis

	TARGET	NON-TARGET	UNKNOWNS
TQuad - QTRAP (SRM)	OK [Average LOD < 1 ng/g] ✓	KO	KO
TOF	OK [Average LOD 1-10 ng/g]	OK ✓	OK ✓

TOF offers complementary features to MS/MS (SRM) instruments for comprehensive investigations of pesticides on FOOD

PROPOSED WORKFLOW FOR THE SCREENING, CONFIRMATION AND QUANTITATION OF "TARGET" PESTICIDE RESIDUES IN VEGETABLE SAMPLES

Screening
LC-TOF/MS
(presence, time retention, *m/z*)

→

Confirmation (1)
LC-TOF/MS
(accurate mass measurements & isotopic pattern matching)

→

Confirmation (2) + Quantitation
LC-QqQ-MS/MS

Development of an LC-TOF/MS automated procedure to identify target pesticides at low concentration levels using retention time / m/z data

↓

Binary response
YES/NO
(presence/absence)
(PEAK AREA THRESHOLD)

← NO

→ YES

↓

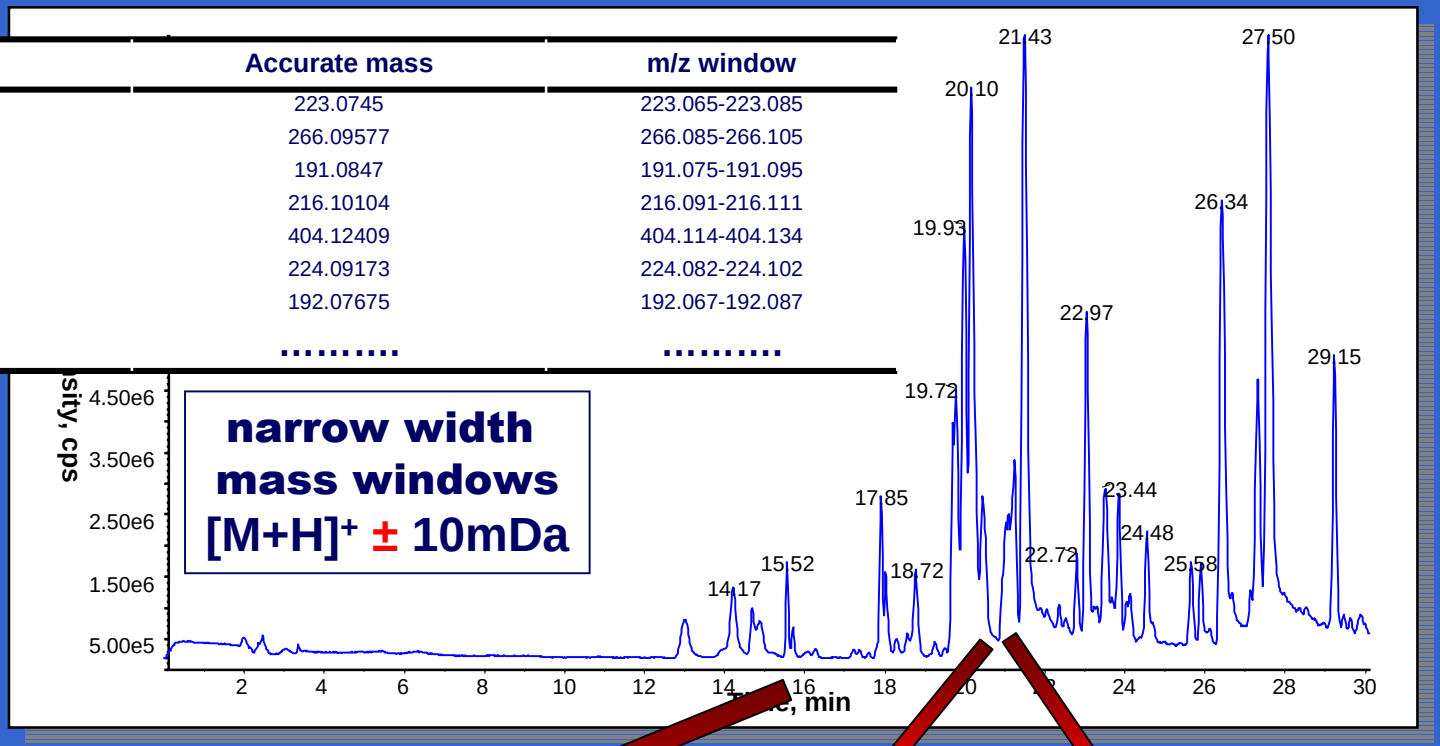
Confirmation by LC-TOF/MS accurate mass and isotopic pattern matching (Mass error < 5 ppm)

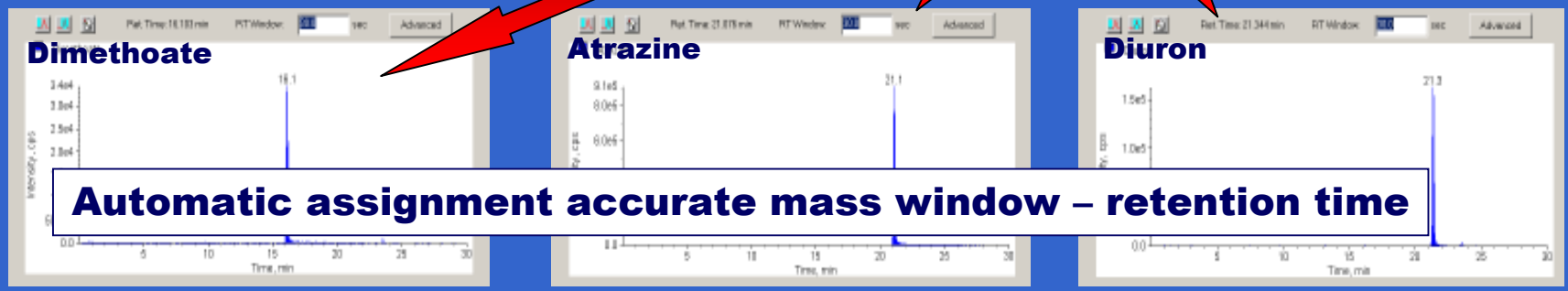
↓

Confirmation (2 MRM transitions) and quantitation by LC-MS/MS

Development of an LC-TOF/MS automated procedure to identify target pesticides at low concentration levels using retention time / m/z data

Pesticide	Accurate mass	m/z window
Acetamiprid	223.0745	223.065-223.085
Albendazole	266.09577	266.085-266.105
Aldicarb	191.0847	191.075-191.095
Atrazine	216.10104	216.091-216.111
Azoxystrobin	404.12409	404.114-404.134
Bendiocarb	224.09173	224.082-224.102
Carbendazim	192.07675	192.067-192.087
.....	.....	.....



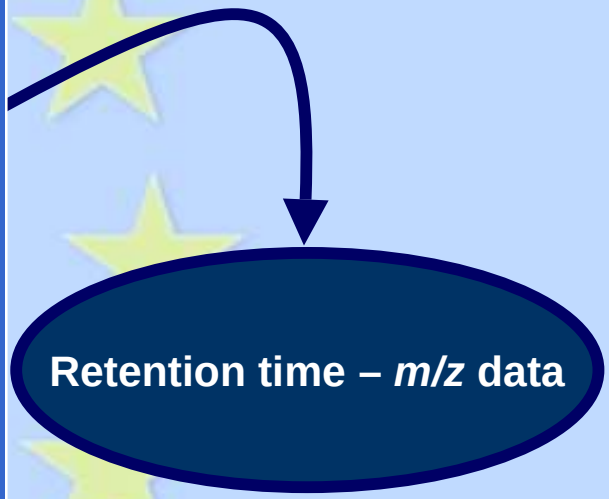


As example: 104 MULTICLASS PESTICIDES IN FRUITS, VEGETABLES,…

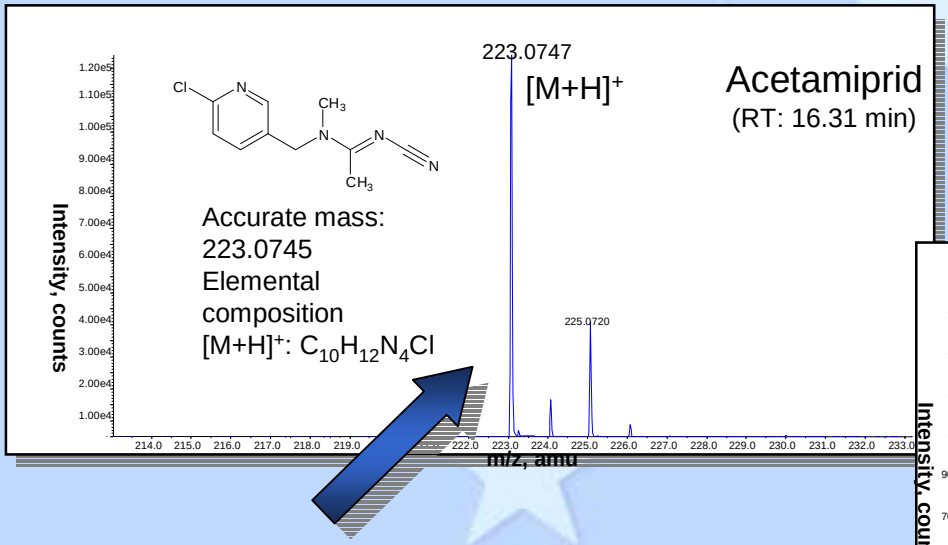
Acetamiprid	Chloroxuron	Fluometuron	Methidathion	Simazine
Albendazole	Cymoxanil	Fluroxypir	Methomyl	Spinosyn A
Aldicarb	Cyproconazole	Hexaflumuron	Methyl-thiophanate	Spinosyn D
Aldicarb sulfoxide	Cyromazine	Hexythiazox	Metobromuron	Spiroxamine
Aldicarb sulphone	Diazinon	Imazalil	Miconazole	Tebuconazole
Atrazine	Diazoxon	Imazalil metabolite	Monolinuron	Teflubenzuron
Azoxystrobin	Dichlofluanid	Imidacloprid	Monuron	Tetraconazole
Bendiocarb	Difenoxuron	Ioxynil	Neburon	Terbuthylazine
Bromacil	Diflubenzuron	Iprodione	Nitempyram	Thiabendazole
Bromoxynil	Dimethoate	Isoproturon	Oxadixyl	Thiacloprid
Bromuconazole	Dimethomorph	Lenacil	Oxamyl	Thiamethoxam
Buprofezin	Diuron	Linuron	Oxfendazole	Thiobencarb
Butocarboxin	Ethiofencarb	Lufenuron	Prochloraz	Thiofanox
Butoxicarboxin	Ethiofencarb sulphone	Malathion	Prochloraz-metabolite	Thiofanox sulfone
Cambendazole	Ethiofencarb sulfoxide	Metalaxyl	Procymidone	Thiofanox sulfoxide
Carbendazim	Ethoxyquin	Mebendazole	Prometryn	Thiram
Carbofuran	Fenamiphos	Mecarbam	Propazine	Triadimenol
Chloridazon	Fenbendazole	Metamytron	Promecarb	Triadimefon
Chlorotoluron	Flazasulfuron	Methiocarb	Propoxur	Triflumuron
Chlorobromuron	Flubendazole	Methiocarb sulphone	Pyrimethanil	Vinclozolin
Chlorfluazuron	Flufenoxuron	Methiocarb sulfoxide	Pyriproxifen	**∑ = 104 pests**

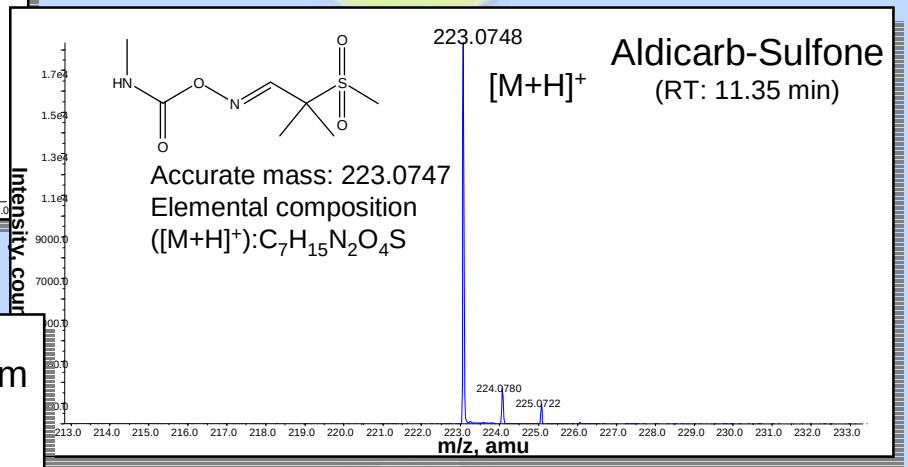
Automatic assignment of retention time/*m/z* data for the targeted pesticides

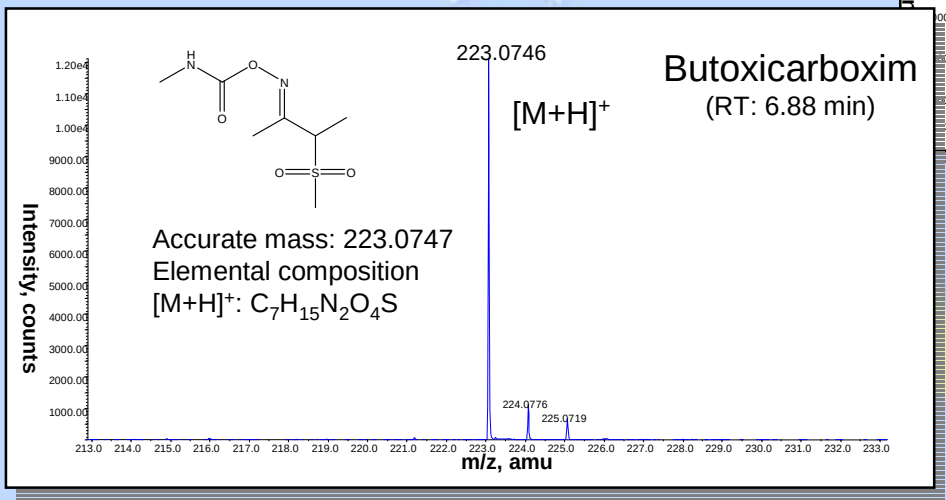
COMPOUND	ACCURATE MASS	M/Z WINDOW
Acetamiprid	223.0745	223.02-223.12
Albendazole	266.09577	266.04-266.14
Aldicarb	191.0847	191.03-191.13
Atrazine	216.10104	216.05-216.15
Azoxystrobin	404.12409	404.07-404.17
Bendiocarb	224.09173	224.04-224.14
Bromacil	261.02331	260.97-261.07
Bromoxynil	275.86541	275.81-275.91
Bromuconazole	375.96135	375.91-376.01
Buprofezin	306.1639	306.11-306.21
Butocarboxin	213.06682	213.01-213.11
Cambendazole	303.09102	303.04-303.14
Carbendazim	192.07675	192.03-192.13
Carbofuran	222.11246	222.06-222.16
Chloridazon	222.04286	222.0-222.1
Chlorotoluron	213.07891	213.03-213.13
Chlorobromuron	292.96869	292.92-293.02
…….	…….	…….



RETENTION TIME INFORMATION LC-TOF/MS Automated screening method (104 pest screened)

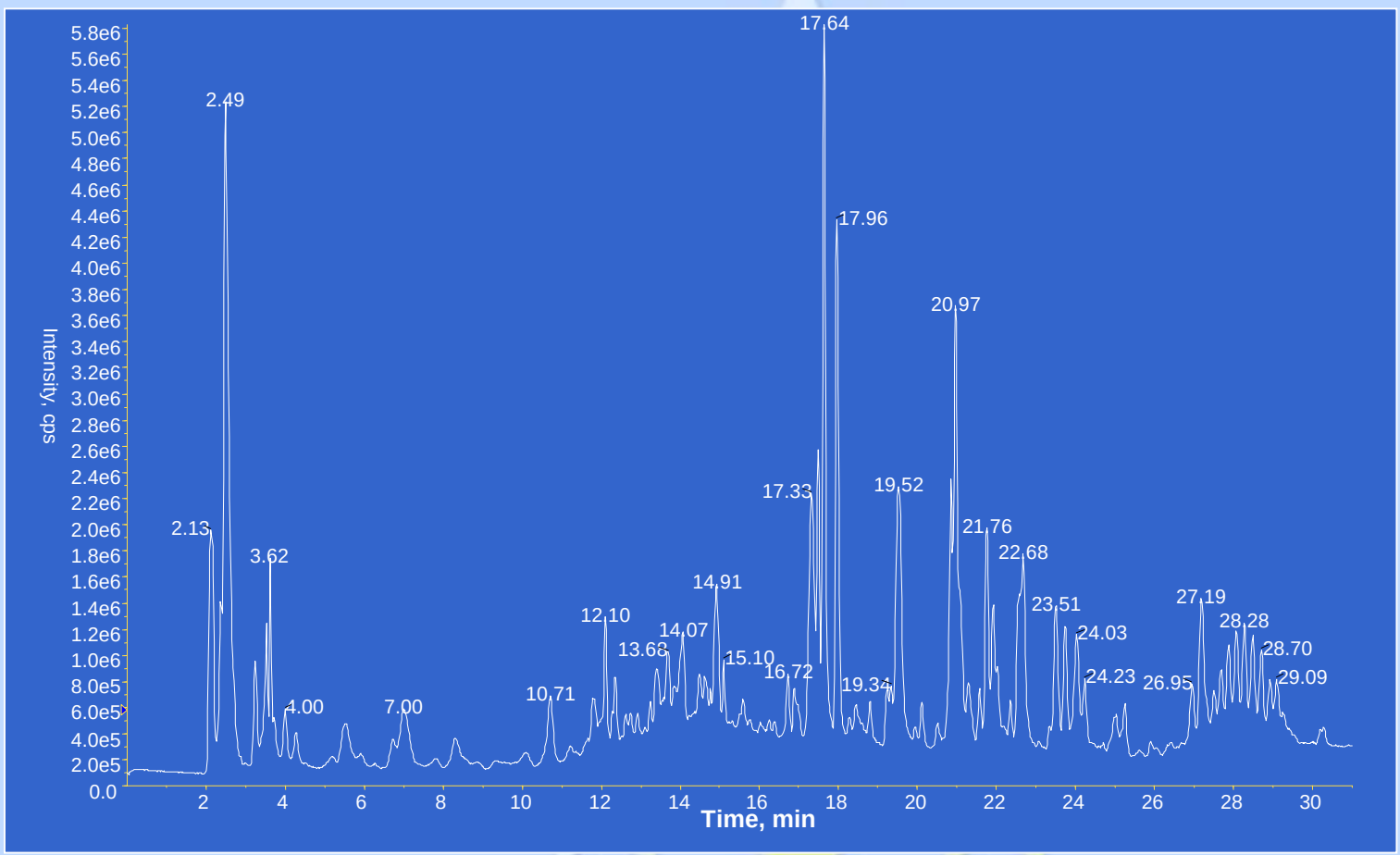






RESULTS

Lemon extract spiked with 0.05 mg/Kg of 25 targeted pesticides



The Sample matrices can be complex and the target compounds do not necesarily appear as distinguisable peaks

Lemon extract spiked with 0.05 mg/Kg of 25 targeted pesticides

Acetamiprid	Chloroxuron	Fluometuron	Methidathion	**Simazine**
Albendazole	Cymoxanil	Fluroxypir	Methomyl	**Spinosyn A**
Aldicarb	Cyproconazole	**Hexaflumuron**	**Methyl-thiophanate**	**Spinosyn D**
Aldicarb sulfoxide	**Cyromazine**	Hexythiazox	Metobromuron	Spiroxamine
Aldicarb sulphone	Diazinon	**Imazalil**	Miconazole	Tebuconazole
Atrazine	Diazoxon	Imazalil metabolite	Monolinuron	**Teflubenzuron**
Azoxystrobin	Dichlofluanid	**Imidacloprid**	Monuron	Tetraconazole
Bendiocarb	Difenoxuron	Ioxynil	Neburon	**Terbuthylazine**
Bromacil	Diflubenzuron	Iprodione	Nitempyram	**Thiabendazole**
Bromoxynil	**Dimethoate**	**Isoproturon**	Oxadixyl	Thiacloprid
Bromuconazole	**Dimethomorph**	Lenacil	Oxamyl	Thiamethoxam
Buprofezin	**Diuron**	Linuron	Oxfendazole	Thiobencarb
Butocarboxin	Ethiofencarb	**Lufenuron**	**Prochloraz**	Thiofanox
Butoxicarboxin	Ethiofencarb sulphone	Malathion	Prochloraz-metabolite	Thiofanox sulfone
Cambendazole	Ethiofencarb sulfoxide	Metalaxyl	Procymidone	Thiofanox sulfoxide
Carbendazim	Ethoxyquin	Mebendazole	Prometryn	Thiram
Carbofuran	Fenamiphos	Mecarbam	Propazine	Triadimenol
Chloridazon	Fenbendazole	Metamytron	Promecarb	Triadimefon
Chlorotoluron	Flazasulfuron	Methiocarb	Propoxur	**Triflumuron**
Chlorobromuron	Flubendazole	Methiocarb sulphone	Pyrimethanil	Vinclozolin
Chlorfluazuron	**Flufenoxuron**	Methiocarb sulfoxide	Pyriproxifen	

Using the automatic screening procedure…….. 28 out of 104 pesticides found

Acetamiprid	Chloroxuron	**Fluometuron**	Methidathion	**Simazine**
Albendazole	Cymoxanil	Fluroxypir	Methomyl	**Spinosyn A**
Aldicarb	Cyproconazole	**Hexaflumuron**	**Methyl-thiophanate**	**Spinosyn D**
Aldicarb sulfoxide	**Cyromazine**	Hexythiazox	Metobromuron	Spiroxamine
Aldicarb sulphone	Diazinon	**Imazalil**	Miconazole	Tebuconazole
Atrazine	Diazoxon	Imazalil metabolite	Monolinuron	**Teflubenzuron**
Azoxystrobin	Dichlofluanid	**Imidacloprid**	Monuron	Tetraconazole
Bendiocarb	**Difenoxuron**	Ioxynil	Neburon	**Terbuthylazine**
Bromacil	Diflubenzuron	Iprodione	Nitempyram	**Thiabendazole**
Bromoxynil	**Dimethoate**	**Isoproturon**	Oxadixyl	Thiacloprid
Bromuconazole	**Dimethomorph**	Lenacil	Oxamyl	Thiamethoxam
Buprofezin	**Diuron**	Linuron	Oxfendazole	Thiobencarb
Butocarboxin	Ethiofencarb	**Lufenuron**		ox
Butoxicarboxin	Ethiofencarb sulphone	Malath		e
Cambendazole	Ethiofencarb sulfoxide	Metal		ide
Carbendazim	Ethoxyquin	Mebendaz		
Carbofuran	Fenamiphos	Mecarbam		Triadimenol
Chloridazon	Fenbendazole	Metamytron	Promecarb	Triadimefon
Chlorotoluron	Flazasulfuron	Methiocarb	Propoxur	**Triflumuron**
Chlorobromuron	Flubendazole	Methiocarb sulphone	Pyrimethanil	Vinclozolin
Chlorfluazuron	**Flufenoxuron**	Methiocarb sulfoxide	Pyriproxifen	

Results:
Identification of 25 spiked
3 "false positives"

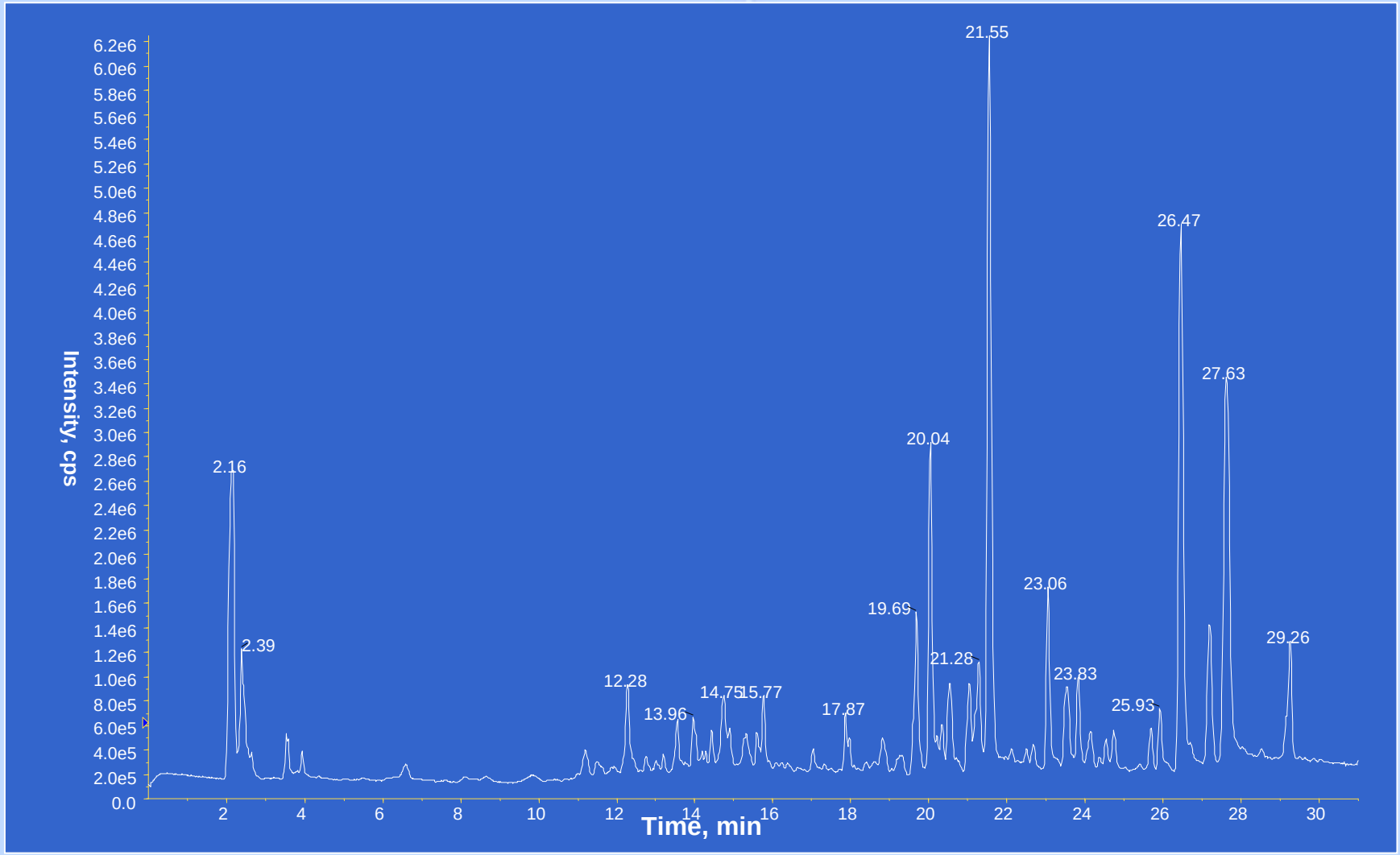
Final confirmation by LC/TOF/MS accurate mass measurements

Pesticide	Accurate mass (experimental)	Theoretical value	Error (ppm)	Confirmation
Acetamiprid	223.0744	223.0745	0.5	YES
Atrazine	216.1006	216.1014	2.0	YES
Azoxystrobin	404.1243	404.12409	0.5	YES
Buprofezin	306.1635	306.16346	0.1	YES
Carbendazim	192.0764	192.07675	1.8	YES
Dimethoate	230.0070	230.0069	0.4	YES
Dimethomorph	388.1313	388.13101	0.7	YES
Diuron	233.0240	233.02429	1.3	YES
Flufenoxuron	489.0414	489.04351	4.3	YES
Imazalil	297.0557	297.05559	0.4	YES
Imidacloprid	256.0593	256.05957	1.1	YES
Isoproturon	207.1487	207.14918	2.4	YES
Lufenuron	510.9861	510.98570	0.8	YES
Methyl-thiophanate	343.0524	343.05292	1.5	YES
Piriproxifen	322.1442	322.14377	1.3	YES
Prochloraz	376.0376	376.03808	1.3	YES
.....	.....	.....	.....	.....

Final confirmation by LC/TOF/MS accurate mass measurements (CONT….)

Pesticide	Accurate mass (experimental)	Theoretical value	Error (ppm)	Confirmation
Simazine	202.0850	202.08539	2.0	YES
Spinosyn A	732.4682	732.46812	0.1	YES
Spinosyn D	746.4844	746.48377	0.8	YES
Teflubenzuron	380.9828	380.98152	3.3	YES
Terbuthylazine	230.1168	230.11669	0.4	YES
Thiabendazole	202.0433	202.04334	0.2	YES
Triflumizol	346.0932	346.09285	1.0	YES
Cambendazole	303.0861	303.09102	15 ppm (5 mDa)	NO
Difenoxuron	287.0906	287.13901	>100 ppm (>50mDa)	NO
Fluometuron	233.0428	233.08962	>100 ppm (>40mDa)	NO
……	……	……	……	……

Apple extract spiked with 0.2 mg/Kg of 15 targeted pesticides



Apple extract spiked with 0.2 mg/Kg of 15 targeted pesticides

Acetamiprid	Chloroxuron	Fluometuron	**Methidathion**	Simazine
Albendazole	Cymoxanil	**Fluroxypir**	Methomyl	Spinosyn A
Aldicarb	Cyproconazole	Hexaflumuron	Methyl-thiophanate	Spinosyn D
Aldicarb sulfoxide	Cyromazine	Hexythiazox	Metobromuron	Spiroxamine
Aldicarb sulphone	**Diazinon**	**Imazalil**	Miconazole	Tebuconazole
Atrazine	Diazoxon	Imazalil metabolite	Monolinuron	Teflubenzuron
Azoxystrobin	**Dichlofluanid**	Imidacloprid	Monuron	Tetraconazole
Bendiocarb	Difenoxuron	**Ioxynil**	Neburon	Terbuthylazine
Bromacil	Diflubenzuron	Iprodione	Nitempyram	Thiabendazole
Bromoxynil	Dimethoate	Isoproturon	Oxadixyl	Thiacloprid
Bromuconazole	Dimethomorph	**Lenacil**	Oxamyl	Thiamethoxam
Buprofezin	Diuron	**Linuron**	Oxfendazole	Thiobencarb
Butocarboxin	Ethiofencarb	Lufenuron	Prochloraz	Thiofanox
Butoxicarboxin	Ethiofencarb sulphone	**Malathion**	Prochloraz-metabolite	Thiofanox sulfone
Cambendazole	Ethiofencarb sulfoxide	Metalaxyl	Procymidone	Thiofanox sulfoxide
Carbendazim	Ethoxyquin	Mebendazole	**Prometryn**	Thiram
Carbofuran	Fenamiphos	Mecarbam	**Propazine**	Triadimenol
Chloridazon	Fenbendazole	**Metamytron**	Promecarb	Triadimefon
Chlorotoluron	Flazasulfuron	Methiocarb	Propoxur	Triflumuron
Chlorobromuron	Flubendazole	Methiocarb sulphone	Pyrimethanil	Vinclozolin
Chlorfluazuron	Flufenoxuron	Methiocarb sulfoxide	Pyriproxifen	

Using the automatic screening procedure…….. 20 out of 104 pesticides found

Acetamiprid	Chloroxuron	**Fluometuron**	**Methidathion**	Simazine
Albendazole	Cymoxanil	**Fluroxypir**	Methomyl	Spinosyn A
Aldicarb	Cyproconazole	Hexaflumuron	Methyl-thiophanate	Spinosyn D
Aldicarb sulfoxide	**Cyromazine**	Hexythiazox	Metobromuron	Spiroxamine
Aldicarb sulphone	**Diazinon**	**Imazalil**	Miconazole	Tebuconazole
Atrazine	Diazoxon	Imazalil metabolite	**Monolinuron**	Teflubenzuron
Azoxystrobin	**Dichlofluanid**	Imidacloprid	Monuron	Tetraconazole
Bendiocarb	Difenoxuron	**Ioxynil**	Neburon	Terbuthylazine
Bromacil	Diflubenzuron	Iprodione	Nitempyram	Thiabendazole
Bromoxynil	Dimethoate	Isoproturon	Oxadixyl	Thiacloprid
Bromuconazole	**Dimethomorph**	**Lenacil**	Oxamyl	Thiamethoxam
Buprofezin	Diuron	**Linuron**	Oxfendazole	Thiobencarb
Butocarboxin	Ethiofencarb	Lufenuron		fenox
Butoxicarboxin	Ethiofencarb sulphone	**Malathi**		ne
Cambendazole	Ethiofencarb sulfoxide	Metala		e
Carbendazim	Ethoxyquin	Mebendaz		
Carbofuran	Fenamiphos	Mecarbam		Triadimenol
Chloridazon	Fenbendazole	**Metamytron**	Promecarb	Triadimefon
Chlorotoluron	Flazasulfuron	Methiocarb	Propoxur	Triflumuron
Chlorobromuron	Flubendazole	Methiocarb sulphone	Pyrimethanil	Vinclozolin
Chlorfluazuron	Flufenoxuron	Methiocarb sulfoxide	Pyriproxifen	

Results:
Identification of 15 spiked
5 "false positives"

Final confirmation by LC/TOF/MS accurate mass measurements

Pesticide	Accurate mass (experimental)	theoretical value	Error (ppm)	Confirmation
Butocarboxin	213.0667	213.06682	0.6	YES
Chloridazon	222.0426	222.04286	1.2	YES
Chlorotoluron	213.0794	213.07891	2.3	YES
Diazinon	305.1585	305.10832	0.6	YES
Dichlofluanid	332.9704	332.96958	2.4	YES
Fluroxypir	254.9735	254.9734	0.4	YES
Imazalil	297.0565	297.05557	3.0	YES
Ioxynil	371.8376	371.83769	0.3	YES
Lenacil	235.1440	235.1441	0.4	YES
Linuron	249.0192	249.0192	<0.1	YES
Malathion	331.0437	331.04334	1.1	YES
Metamytron	203.0928	203.09273	0.3	YES
Methidathion	324.9509	324.95108	0.6	YES
Prometryn	242.1433	242.14339	0.4	YES
.....	.....	.....	.....	.....

Final confirmation by LC/TOF/MS accurate mass measurements (CONT….)

Pesticide	Accurate mass (experimental)	Theoretical value	Error (ppm)	Confirmation
Cambendazole	303.0499	303.09102	>100 ppm (>40 mDa)	NO
Cyromazine	167.0843	167.10397	>100 ppm (20 mDa)	NO
Dimethomorph	388.1823	388.13101	>100 ppm (50 mDa)	NO
Fluometuron	233.1542	233.08962	>100 ppm (60 mDa)	NO
Monolinuron	215.0681	215.0580	50 ppm (10 mDa)	NO
……	……	……	……	……

REAL SAMPLES (FROM ANDALUSIAN FOOD SAFETY AUTHORITY (SAS))

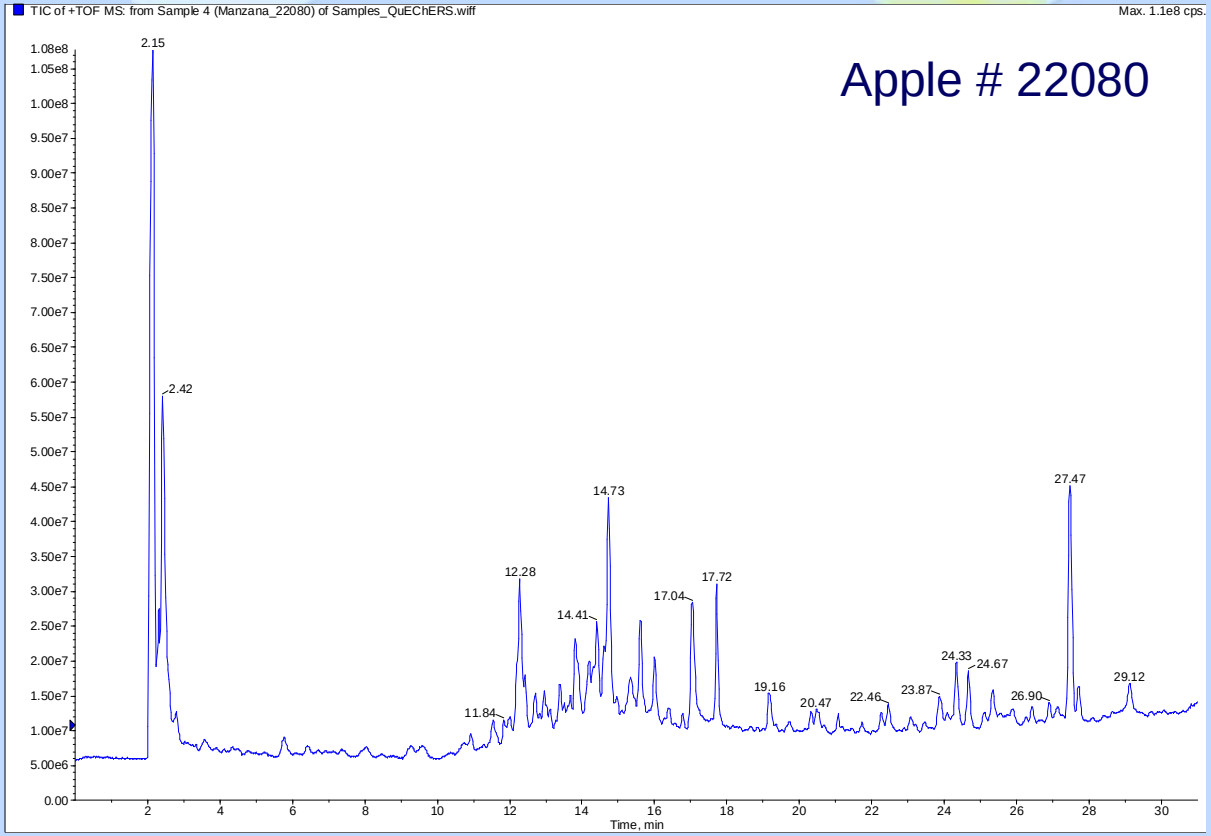
Screening
LC-TOF/MS
(presence, time retention, *m/z*)

→

Confirmation (1)
LC-TOF/MS
(accurate mass measurements & isotopic pattern matching)

→

Confirmation (2) + Quantitation
LC-MS/MS



REAL SAMPLES (FROM ANDALUSIAN FOOD SAFETY AUTHORITY (SAS))

Apple # 22080

Screening
LC-TOF/MS
(presence, time retention, *m/z*)

→

Confirmation (1)
LC-TOF/MS
(accurate mass measurements & isotopic pattern matching)

Compound detected	RT (min) Expected	RT (min) Experimental	Peak Area (counts x10$^{4)}$	*m/z* calculated	m/z experimental	Error, ppm	Confirmation
Carbendazim	7.34	7.30	47.5	192.07675	192.0765	1.3	YES
Ethoxyquin	20.01	19.98	63.8	218.15394	218.1541	0.7	YES
Imazalil	17.72	17.78	1350	297.05557	297.0561	1.7	YES
Imazalil-met	14.49	14.42	8.82	257.02429	257.0236	2.7	YES
Malathion	25.33	25.32	1.84	353.0256	353.0239	4.8	YES
Lenacil	19.20	19.22	1.48	235.1441	235.1026	> 10ppm	NO
Thiacloprid	17.78	17.83	2.42	253.03092	253.0305	1.6	YES
Thiofanox sulfone	16.38	16.42	1.49	251.10600	251.1645	> 10ppm	NO
Thiabendazole	9.00	9.04	2.33	202.04334	202.0439	2.7	YES

REAL SAMPLES (FROM ANDALUSIAN FOOD SAFETY AUTHORITY (SAS))

Apple # 22080

Confirmation (2) + Quantitation
LC-MS/MS

Sample	Pesticides	MRM1	MRM2	MRM ratio standard	MRM ratio Sample	Concentration ($\mu g.Kg^{-1}$)
22080	Carbendazim	192→160	192→132	5.5	5.1	49.7
	Imazalil	297→159	297→109	3.5	5.6	79.2
	Thiacloprid	253→126	253→99	4.4	2.4	0.3
	Thiabendazole	202→131	202→175	3.3	2.2	1.27
	Malathion	331 →127	331 →285	2.1	2.5	10.2
	Ethoxyquin	218→146	218→160	2.8	3.2	6.7
	Imazalil-Me					

REAL SAMPLES (FROM ANDALUSIAN FOOD SAFETY AUTHORITY (SAS))

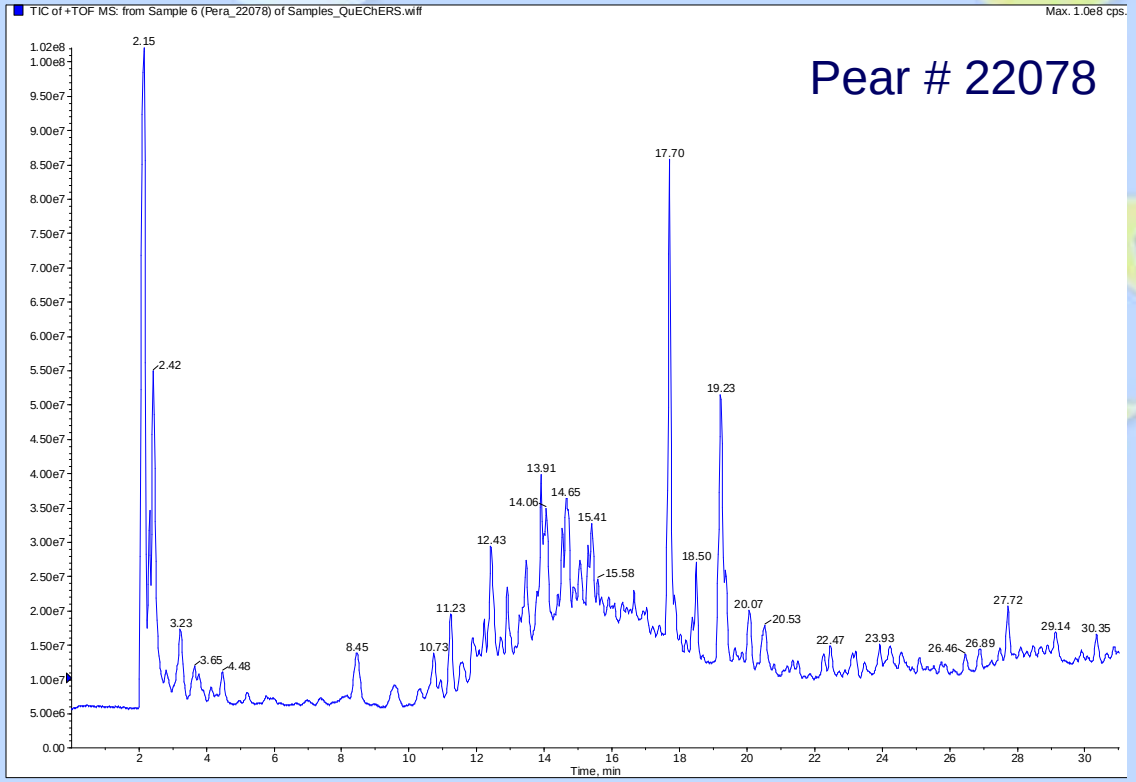
Screening
LC-TOF/MS
(presence, time retention, *m/z*)

→

Confirmation (1)
LC-TOF/MS
(accurate mass measurements & isotopic pattern matching)

→

Confirmation (2) + Quantitation
LC-MS/MS



REAL SAMPLES (FROM ANDALUSIAN FOOD SAFETY AUTHORITY (SAS))

Pear # 22078

Screening
LC-TOF/MS
(presence, time retention, *m/z*)

→

Confirmation (1)
LC-TOF/MS
(accurate mass measurements & isotopic pattern matching)

Compound detected	RT (min) Expected	RT (min) Experimental	Peak Area (counts x10^{4})	*m/z* calculated	m/z experimental	Error ppm	Confirmation
Albendazole	17.86	17.71	10.2	266.09577	266.1430	> 10 ppm	NO
Aldicarb sulfone	11.35	10.75	3.37	223.0747	223.0561	> 10 ppm	NO
Carbendazim	7.33	7.30	12.7	192.07675	192.0771	1.8	YES
Ethoxyquin	20.01	20.07	1850	218.15394	218.1539	0.2	YES
Imazalil	17.72	17.71	4820	297.05557	297.0553	0.9	YES
Imazalil-met	14.49	14.42	8.82	257.02429	257.0236	2.7	YES
Tebuconazole	24.50	24.54	269	308.15241	308.1525	0.3	YES
Thiabendazole	9.00	8.98	71.3	202.04334	202.0434	0.3	YES

REAL SAMPLES (FROM ANDALUSIAN FOOD SAFETY AUTHORITY (SAS))

Pear # 22078

Confirmation (2) + Quantitation
LC-MS/MS

Sample	Pesticides	MRM1	MRM2	MRM ratio standard	MRM ratio Sample	Concentration ($\mu g.Kg^{-1}$)
22078	Imazalil	297→159	297→109	3.5	3.6	297.3
	Thiabendazole	202→131	202→175	3.3	5.8	9.1
	Tebuconalzole	308→70	308→125	5.6	4.2	12.8
	Ethoxyquin	218→160	218→146	1.6	1.2	10.0
	Imazalil-Me					
	Carbendazim	192→160	192→132	5.5	1.3	1.1

REAL SAMPLES (FROM ANDALUSIAN FOOD SAFETY AUTHORITY (SAS))

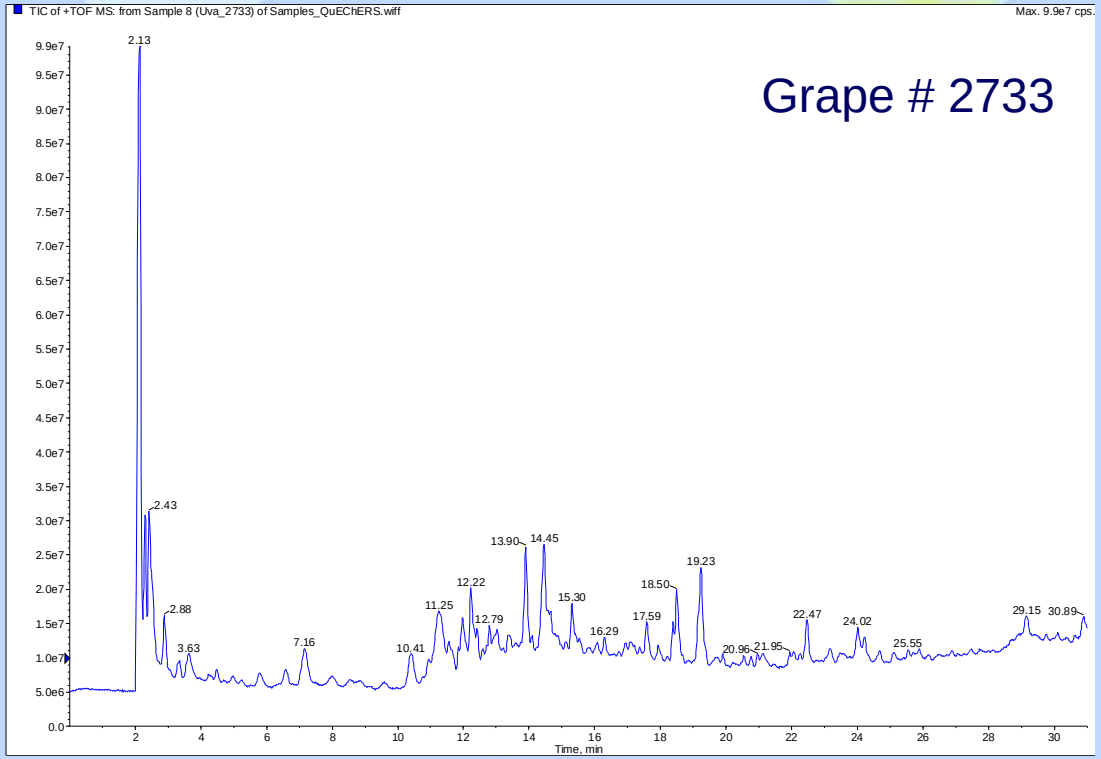
Screening
LC-TOF/MS
(presence, time retention, *m/z*)

→

Confirmation (1)
LC-TOF/MS
(accurate mass measurements & isotopic pattern matching)

→

Confirmation (2) + Quantitation
LC-MS/MS



REAL SAMPLES (FROM ANDALUSIAN FOOD SAFETY AUTHORITY (SAS))

Grape # 2733

Screening
LC-TOF/MS
(presence, time retention, *m/z*)

→

Confirmation (1)
LC-TOF/MS
(accurate mass measurements & isotopic pattern matching)

Compound detected	RT (min) Expected	RT (min) Experimental	Peak Area (counts x10^{4})	*m/z* calculated	m/z experimental	Error ppm	Confirmation
Azoxystrobin	24.07	24.02	236	404.12409	404.1237	1.0	YES
Cambendazole	14.88	14.77	21.2	303.09102	303.0511	> 10 ppm	NO
Carbendazim	7.34	7.31	57.3	192.07675	192.0772	2.3	YES
Oxfendazole	15.54	15.58	1.04	316.07503	316.0642	> 10 ppm	NO
Thiabendazole	9.00	9.01	12.9	202.04334	202.0432	0.7	YES

REAL SAMPLES (FROM ANDALUSIAN FOOD SAFETY AUTHORITY (SAS))

Grape # 2733

Confirmation (2) + Quantitation
LC-MS/MS

Sample	Pesticides	MRM1	MRM2	MRM ratio standard	MRM ratio Sample	Concentration ($\mu g.Kg^{-1}$)
2733	Azoxystrobin	404→372	404→344	2.9	3.8	19.4
	Carbendazim	192→160	192→132	5.5	1.3	1.5
	Thiabendazole	202→131	202→175	3.3	2.2	5.2

RESULTS (SAS)

< 0.01 mg/kg	0.01-0.05 mg/kg	0.05-0.1 mg/kg	0.1-0.5 mg/kg	>0.5 mg/kg
17	41	13	20	

Total samples:..... 304

Total positive findings:..... 91 (30%)

Total positive findings (0.01-0.1mg/kg) :..... 54 (18%)

RESULTS (Coexphal)

< 0.01 mg/kg	0.01-0.05 mg/kg	0.05-0.1 mg/kg	0.1-0.5 mg/kg	>0.5 mg/kg
7	37	14	34	41

Total samples:..... 318

Total positive findings:.....133 (42%)

Total positive findings (0.01-0.1mg/kg) :..... 58 (19%)

1st CRL/NRL Pesticide Residue Training Workshop
6th – 7th December, 2006 - Stuttgart, Germany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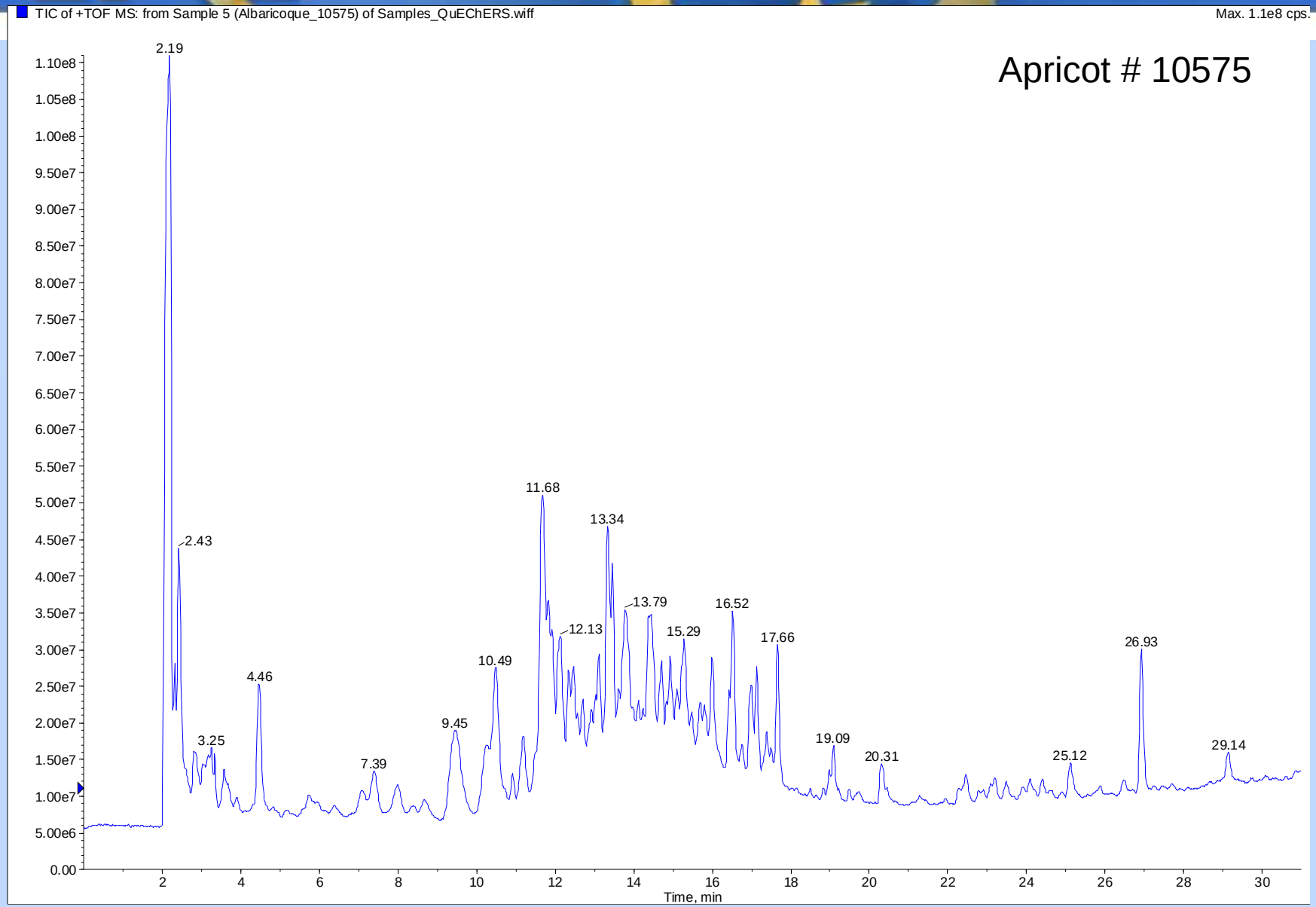
ACKNOWLDGEMENTS

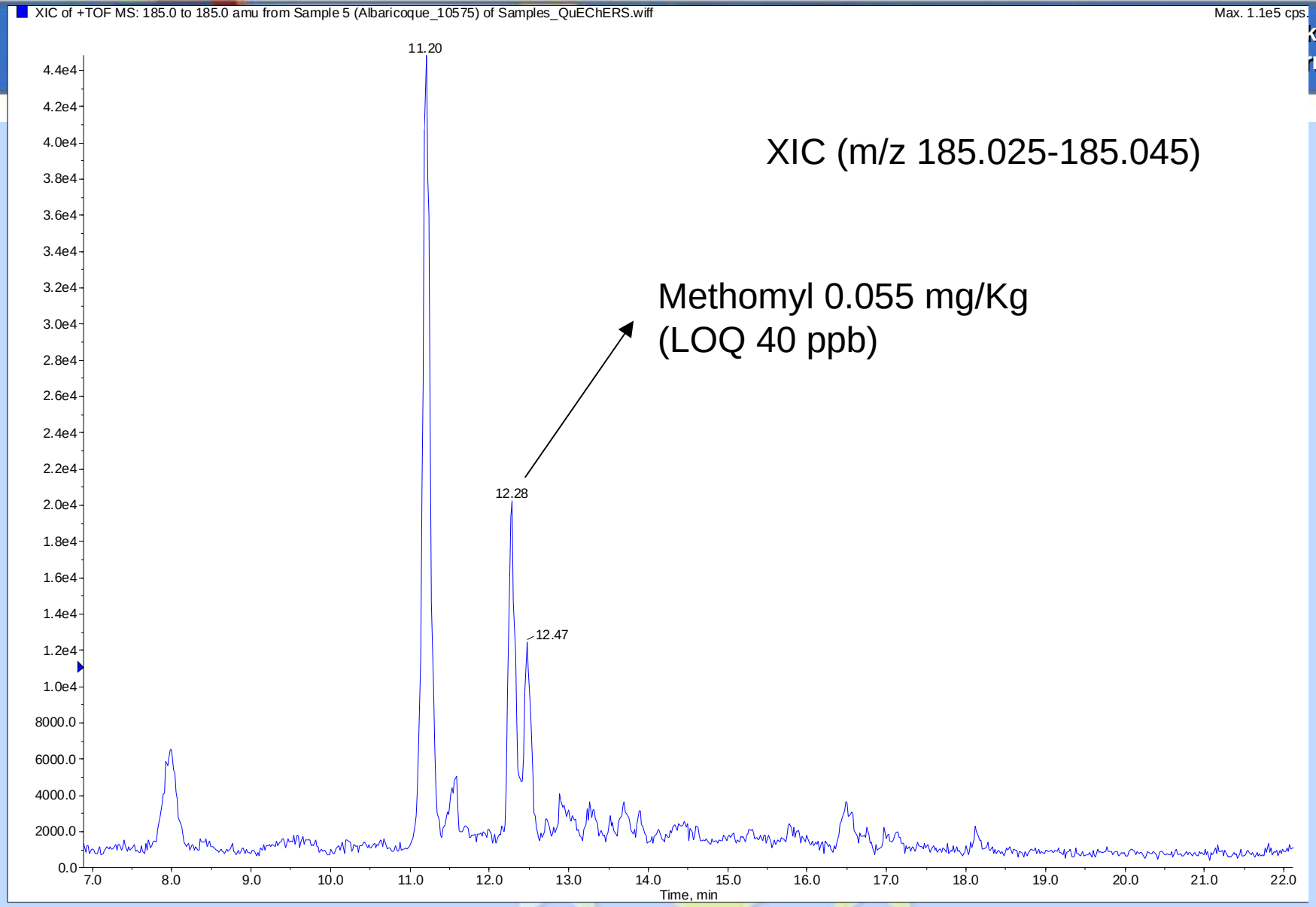
DG SANCO European Commission
for financial support

for instrumentacion facilities

Drawbacks of LC/TOFMS

Limited sensitivity for some compounds LOQ > 20 ppb

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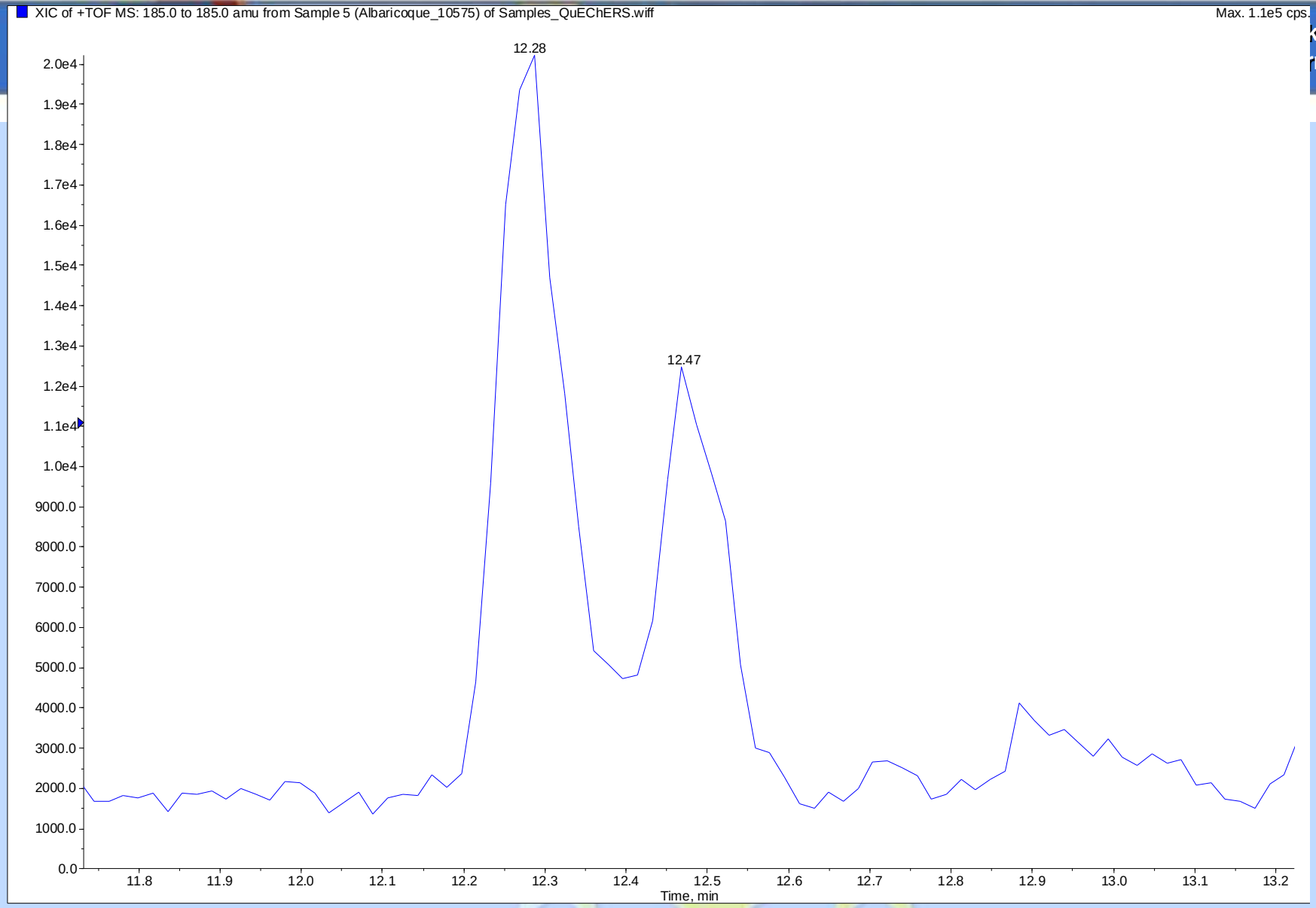
12.47
XIC (m/z 185.025-185.045)
Methomyl 0.055 mg/Kg
(LOQ 40 ppb)
Time, min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Max. 1.1e5 cps.
12.28
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2.0e4
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1.8e4
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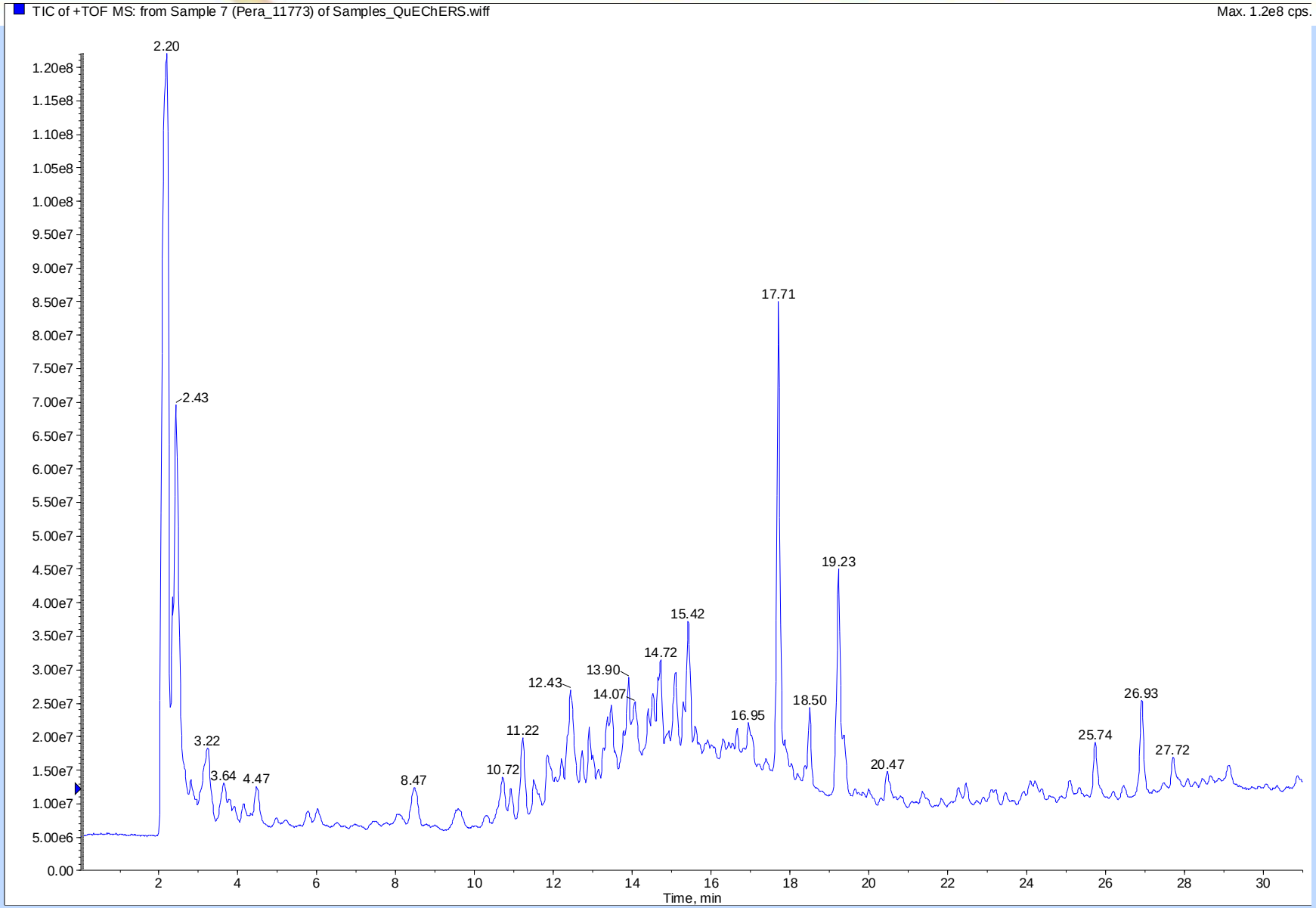
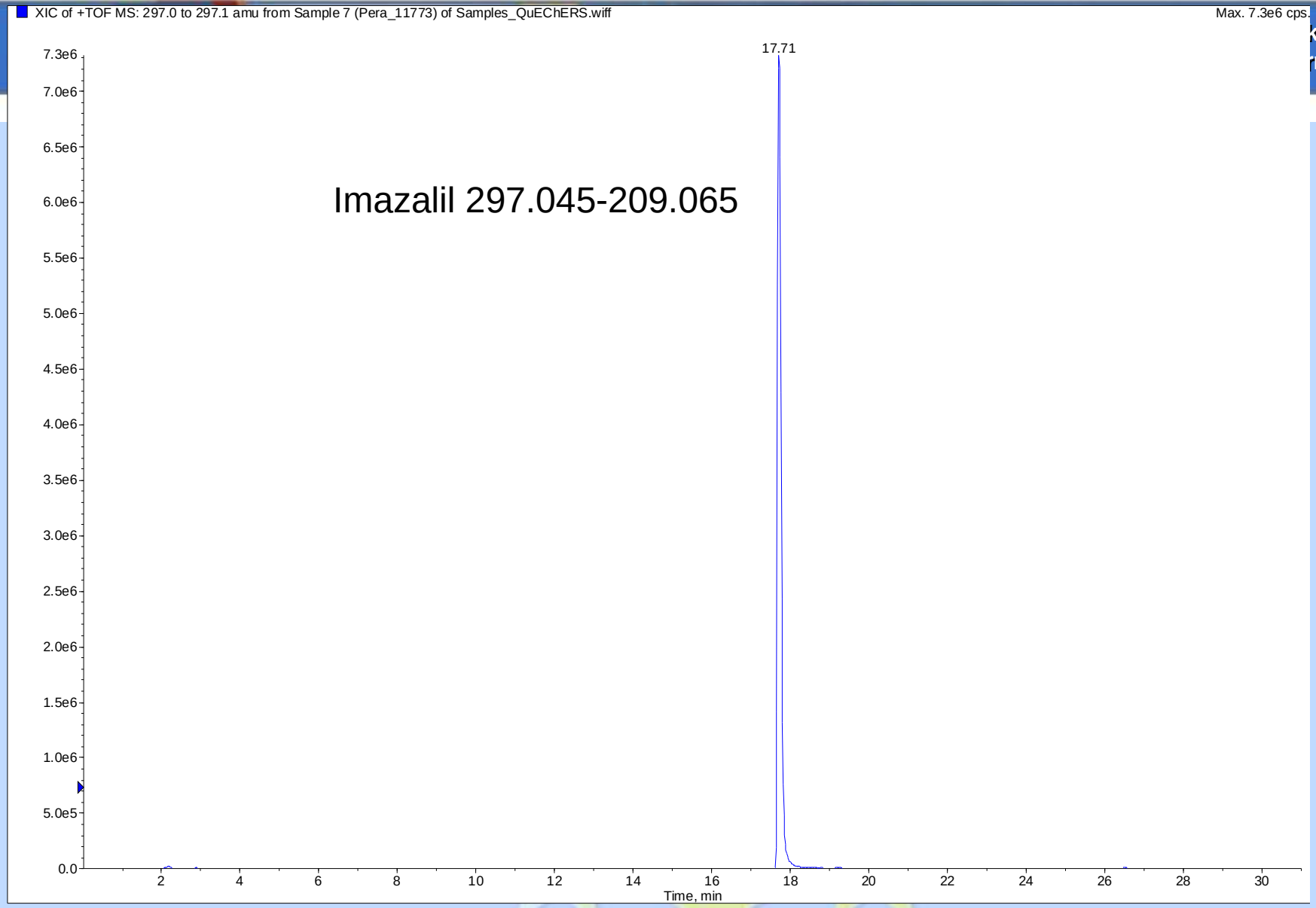
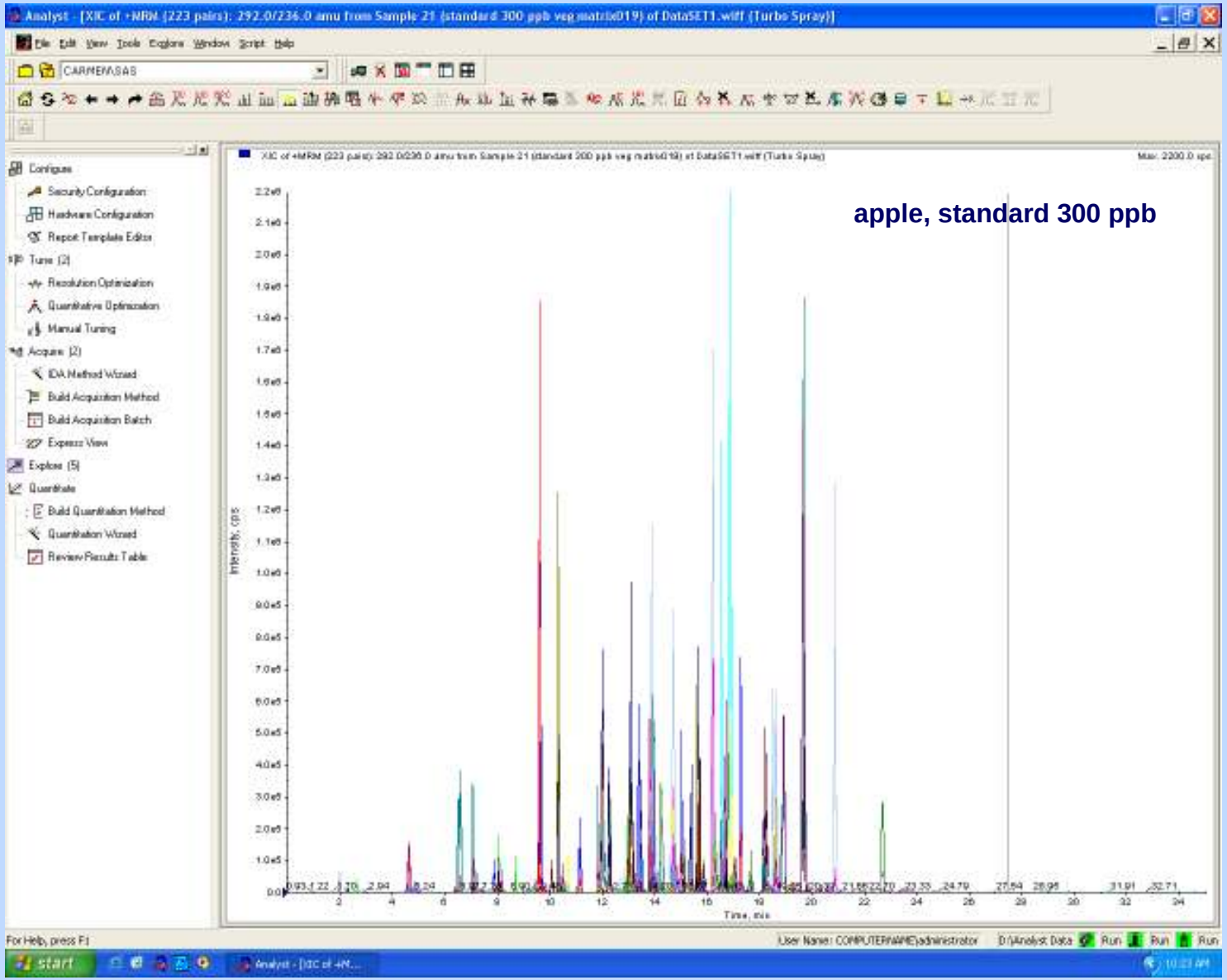
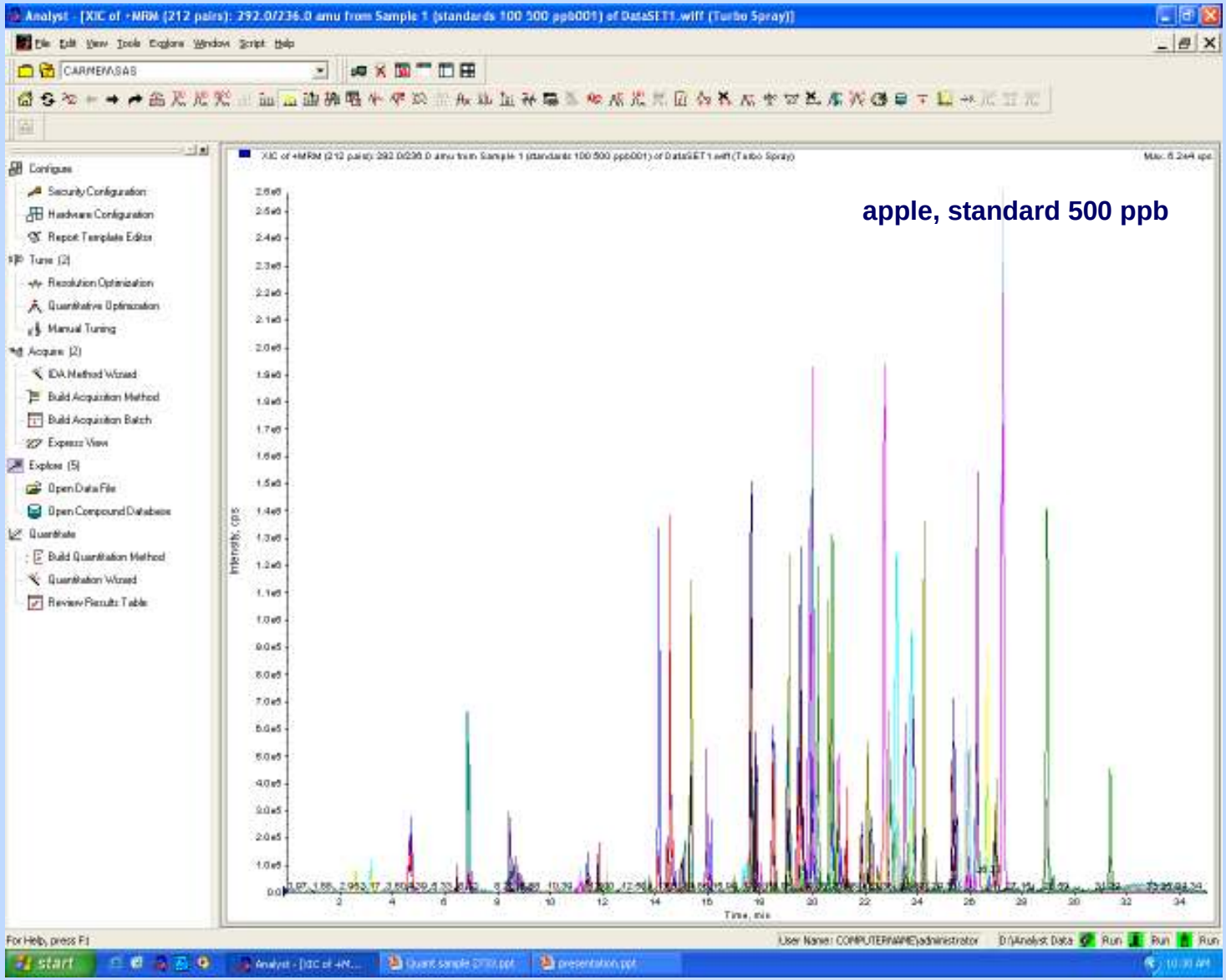
13.1
13.2
Time, min

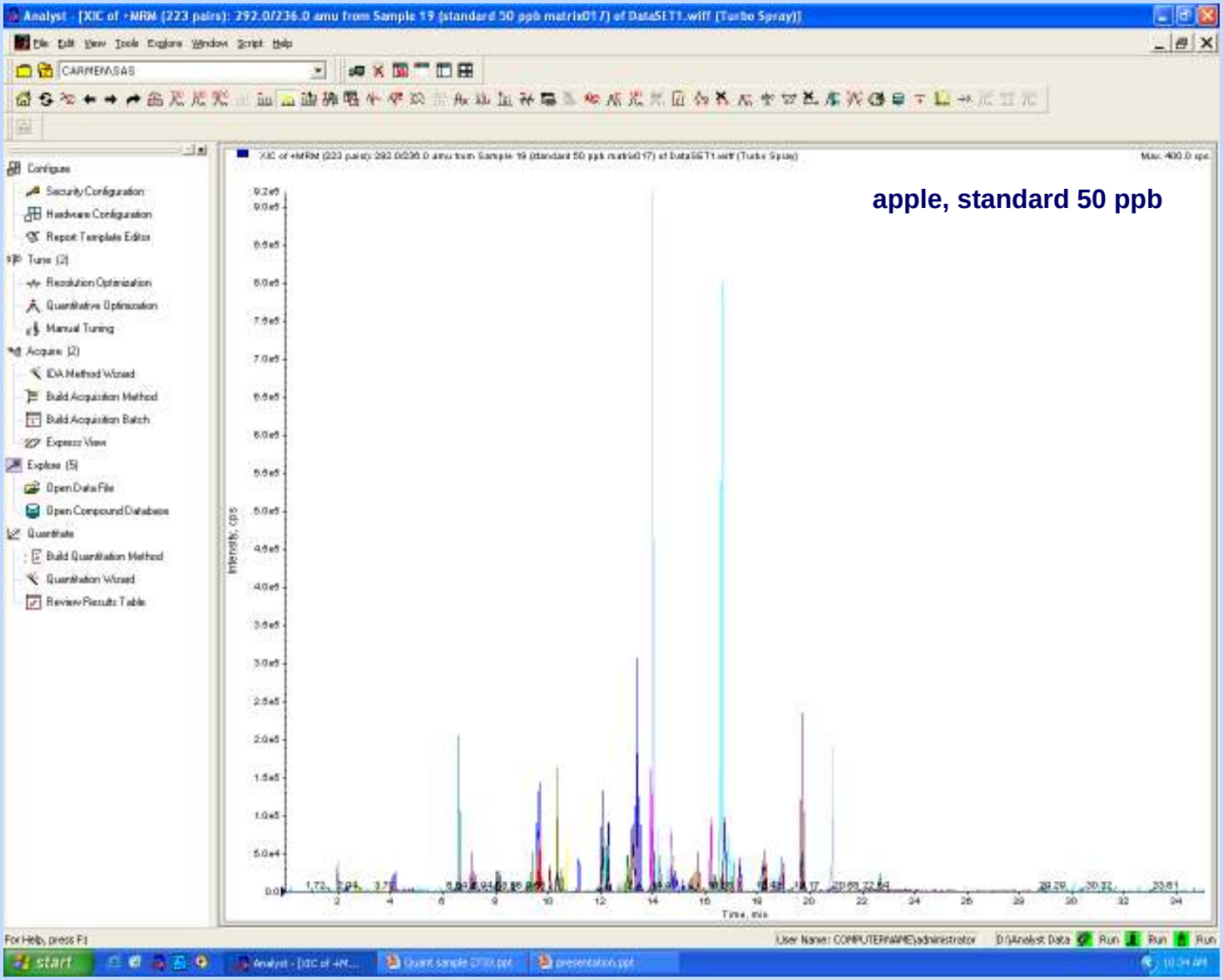
POSIBILIDAD DE FALSOS POSITIVOS

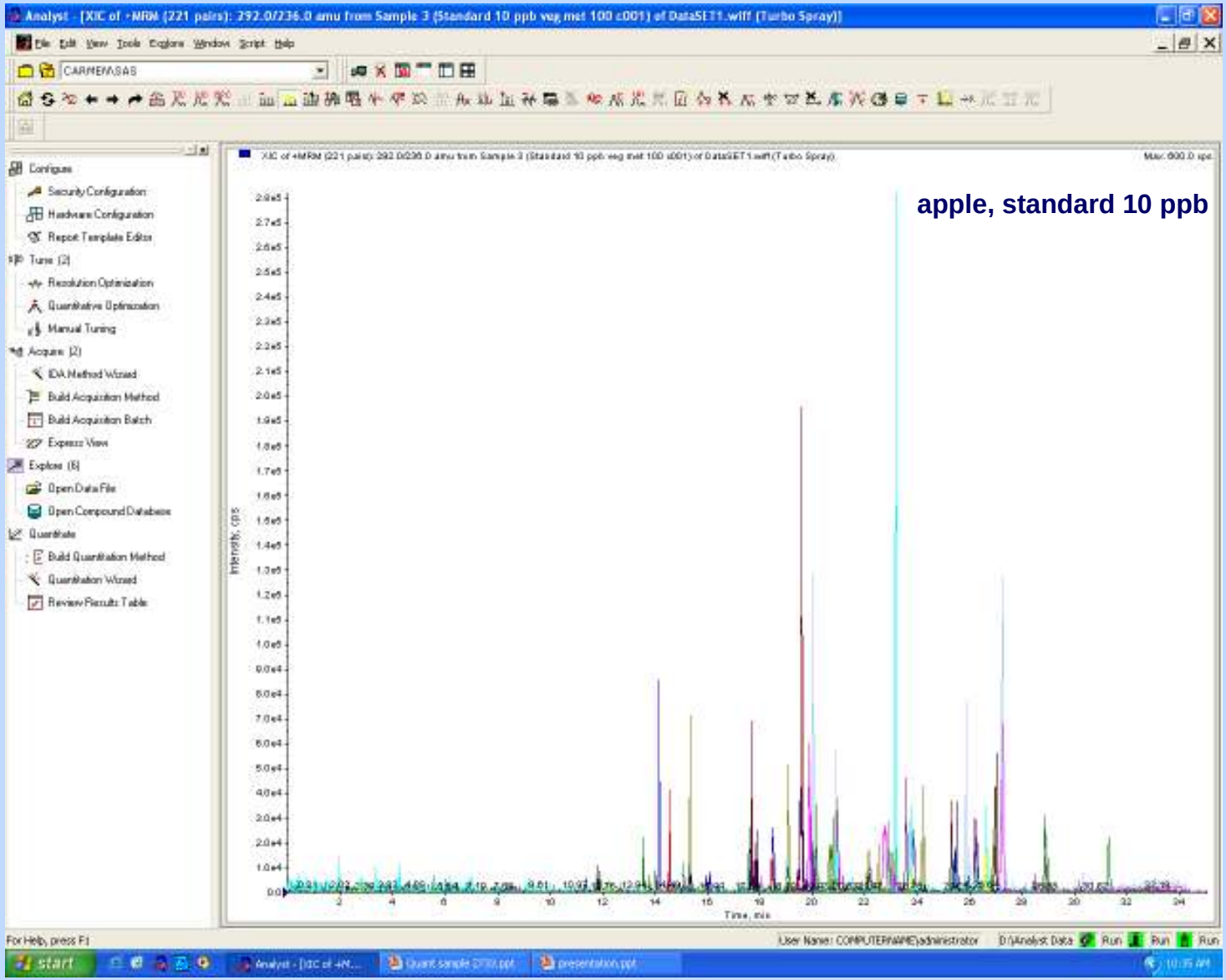


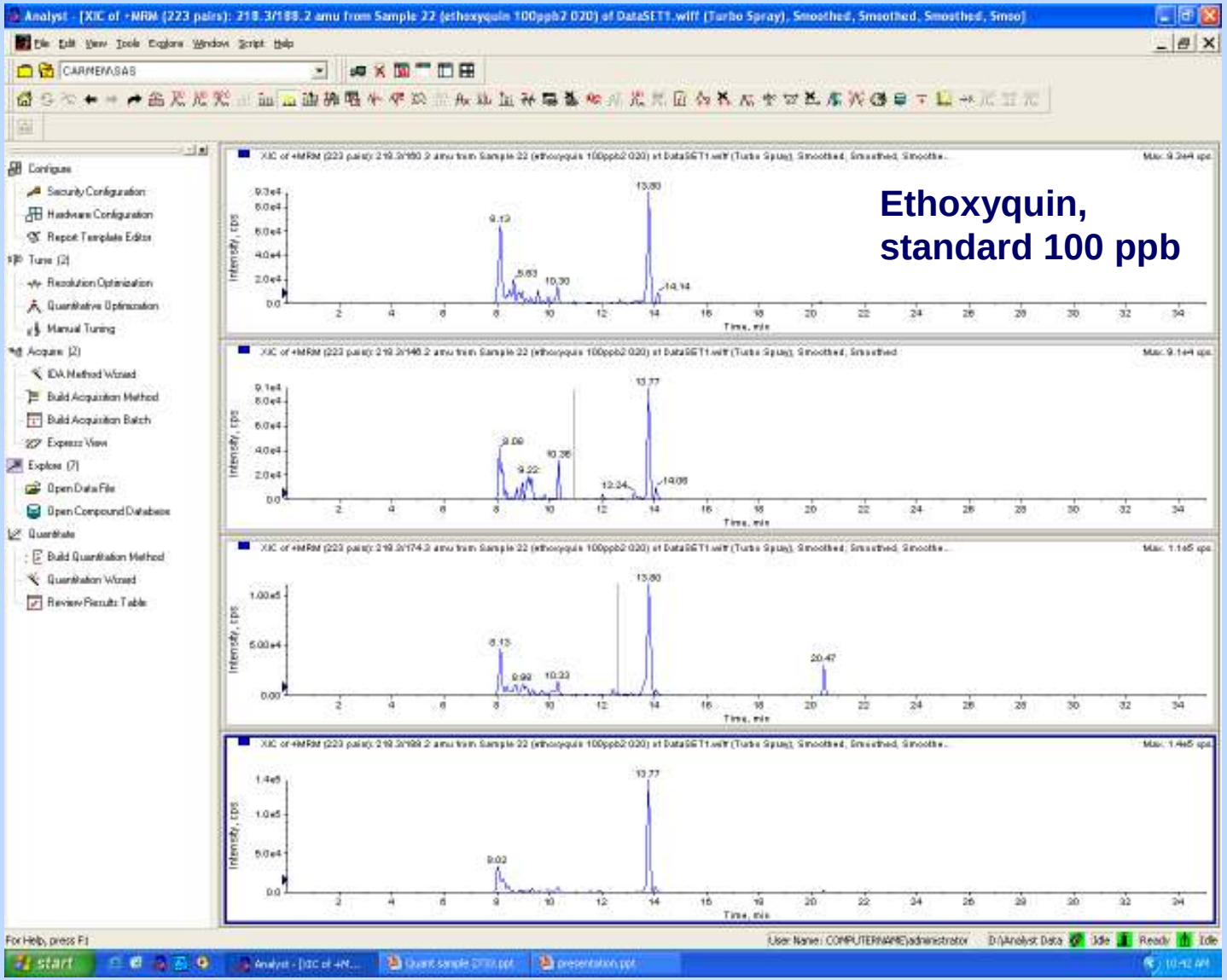

XIC of +TOF MS: 297.0 to 297.1 amu from Sample 7 (Pera_11773) of Samples_QuEChERS.wiff
Max. 7.3e6 cps.
17.71
Imazalil 297.045-209.065
7.3e6
7.0e6
6.5e6
6.0e6
5.5e6
5.0e6
4.5e6
4.0e6
3.5e6
3.0e6
2.5e6
2.0e6
1.5e6
1.0e6
5.0e5
0.0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
Time, min


apple, standard 300 ppb

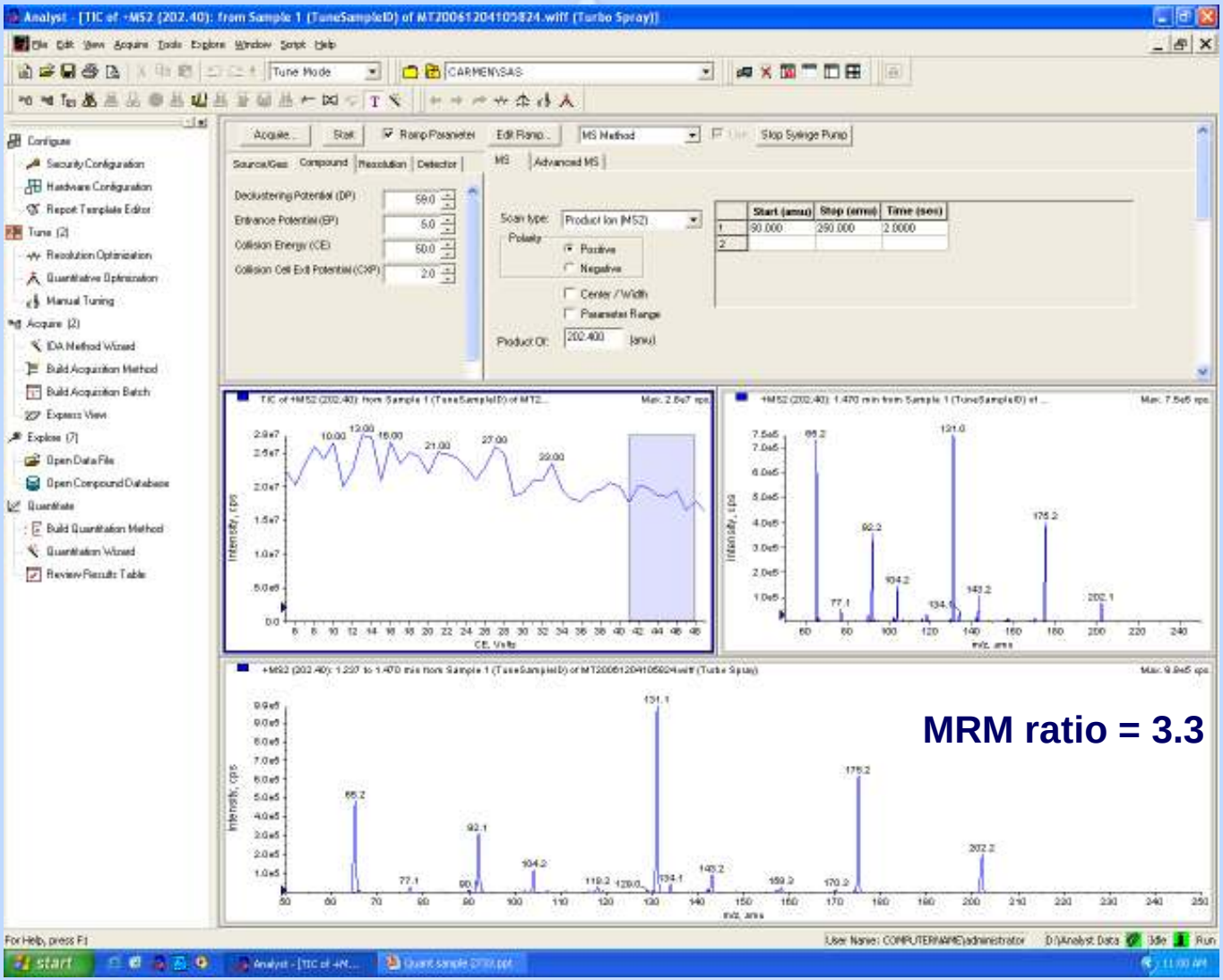

apple, standard 500 ppb


apple, standard 50 ppb

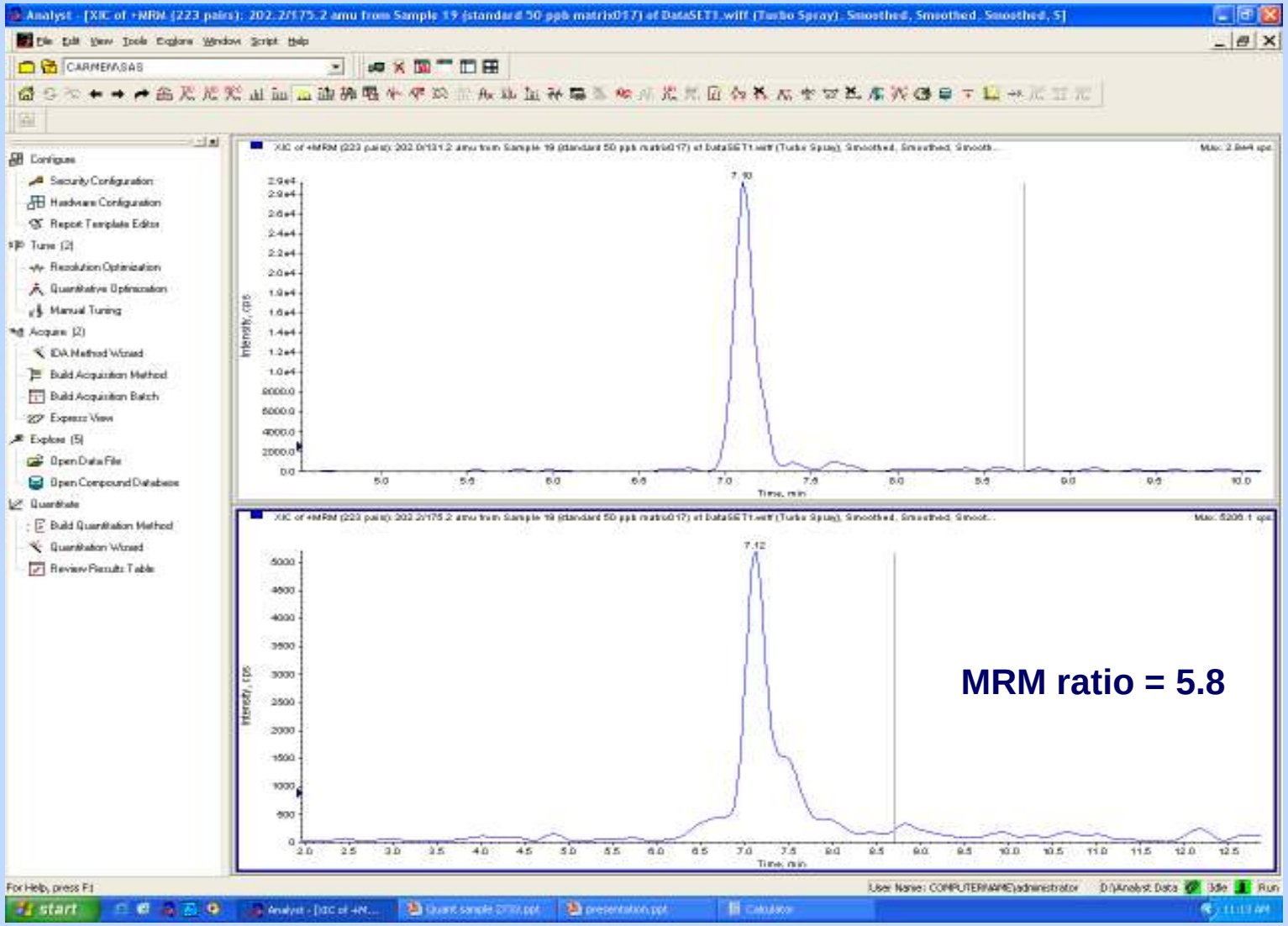

apple, standard 10 ppb


Analyst - [XIC of +MRM (223 pairs): 218.3/188.2 amu from Sample 22 (ethoxyquin 100ppb2 020) of DataSET1.wiff (Turbo Spray), Smoothed, Smoothed, Smoothed, Smoo]
Ethoxyquin,
standard 100 ppb
Intensity, cps
Time, min

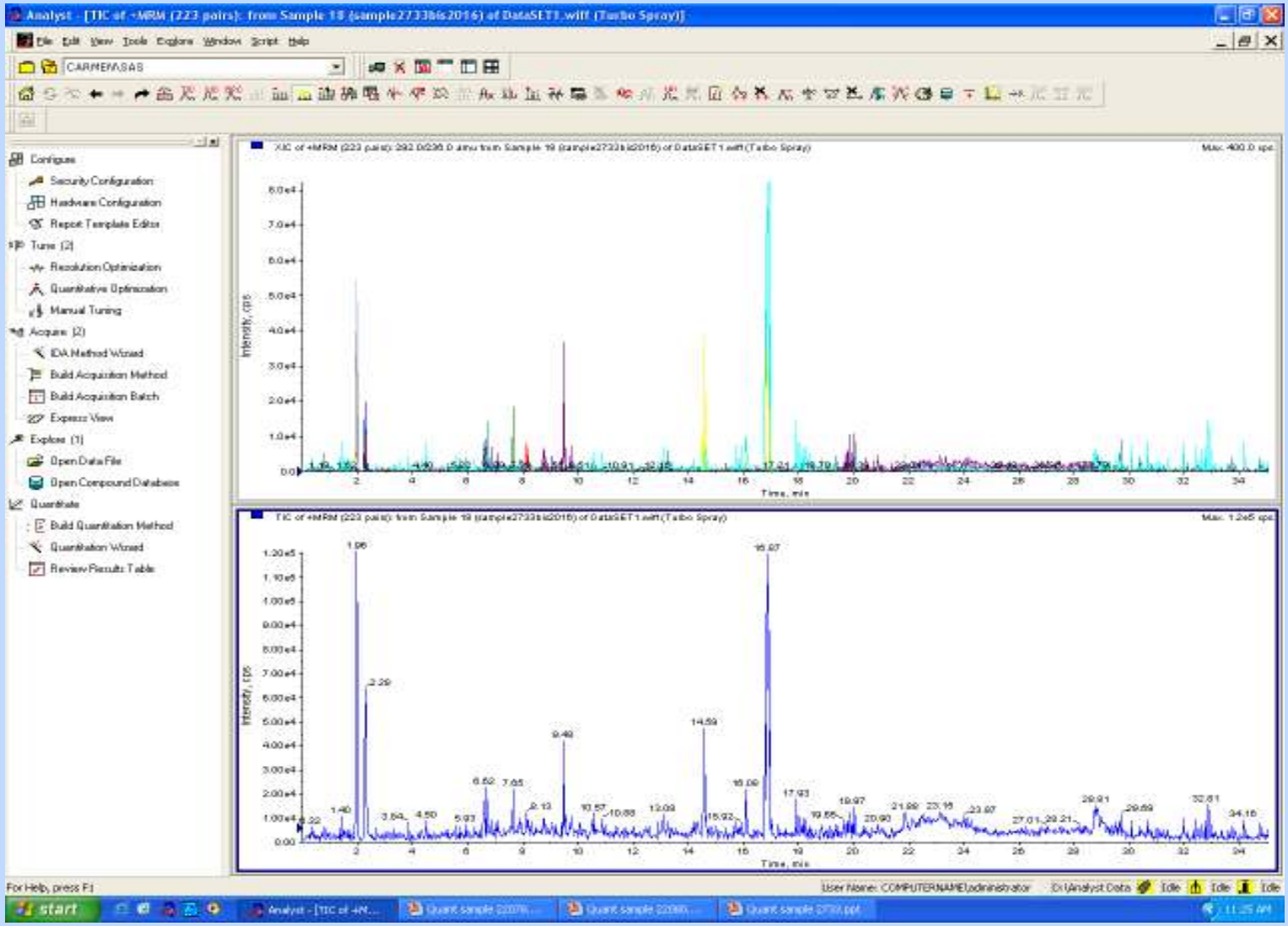
Thiabendazole, MRM optimization



Thiabendazole, matrix



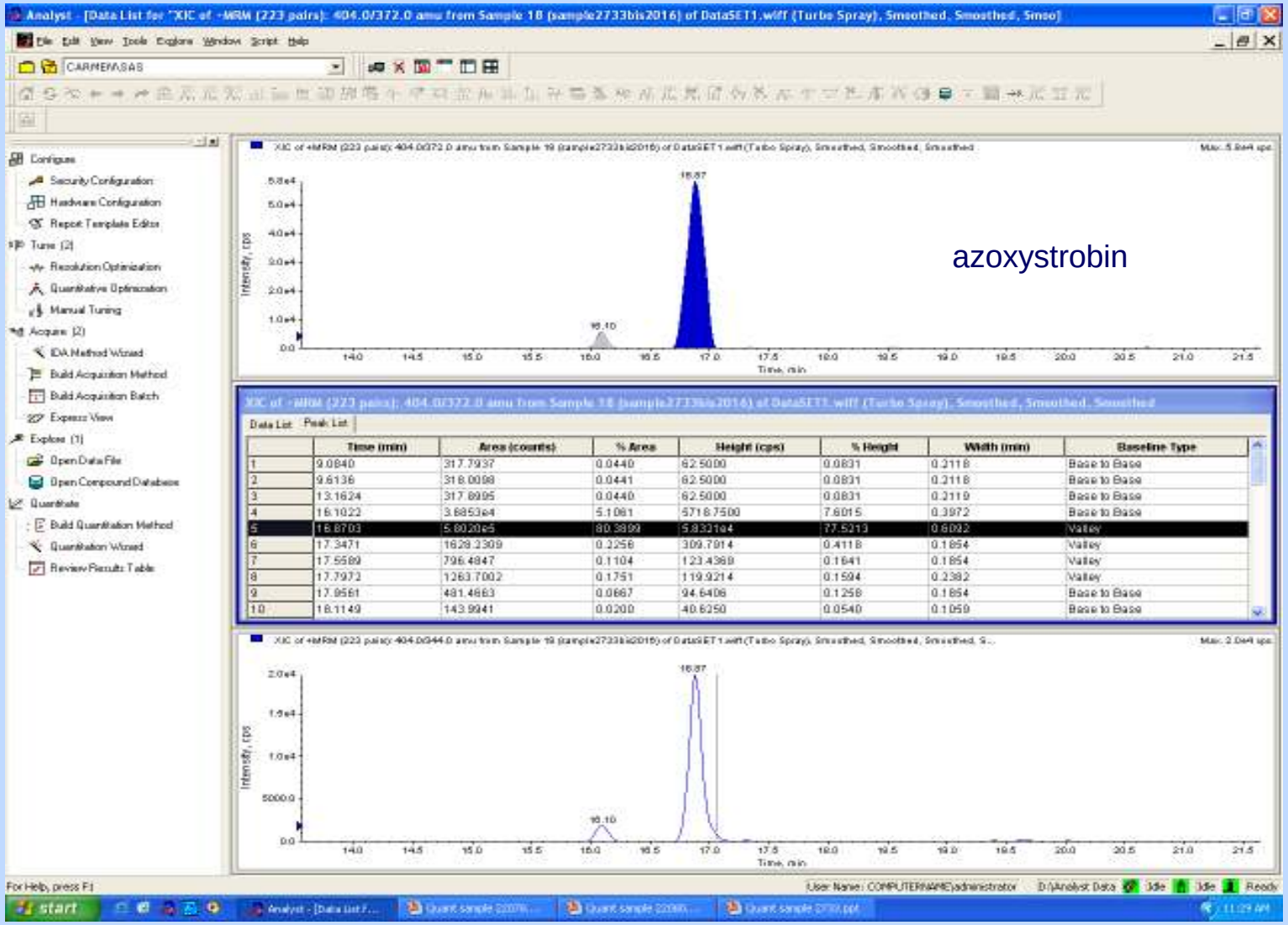
Sample #2733, grapes



Sample #2733, grapes

positive

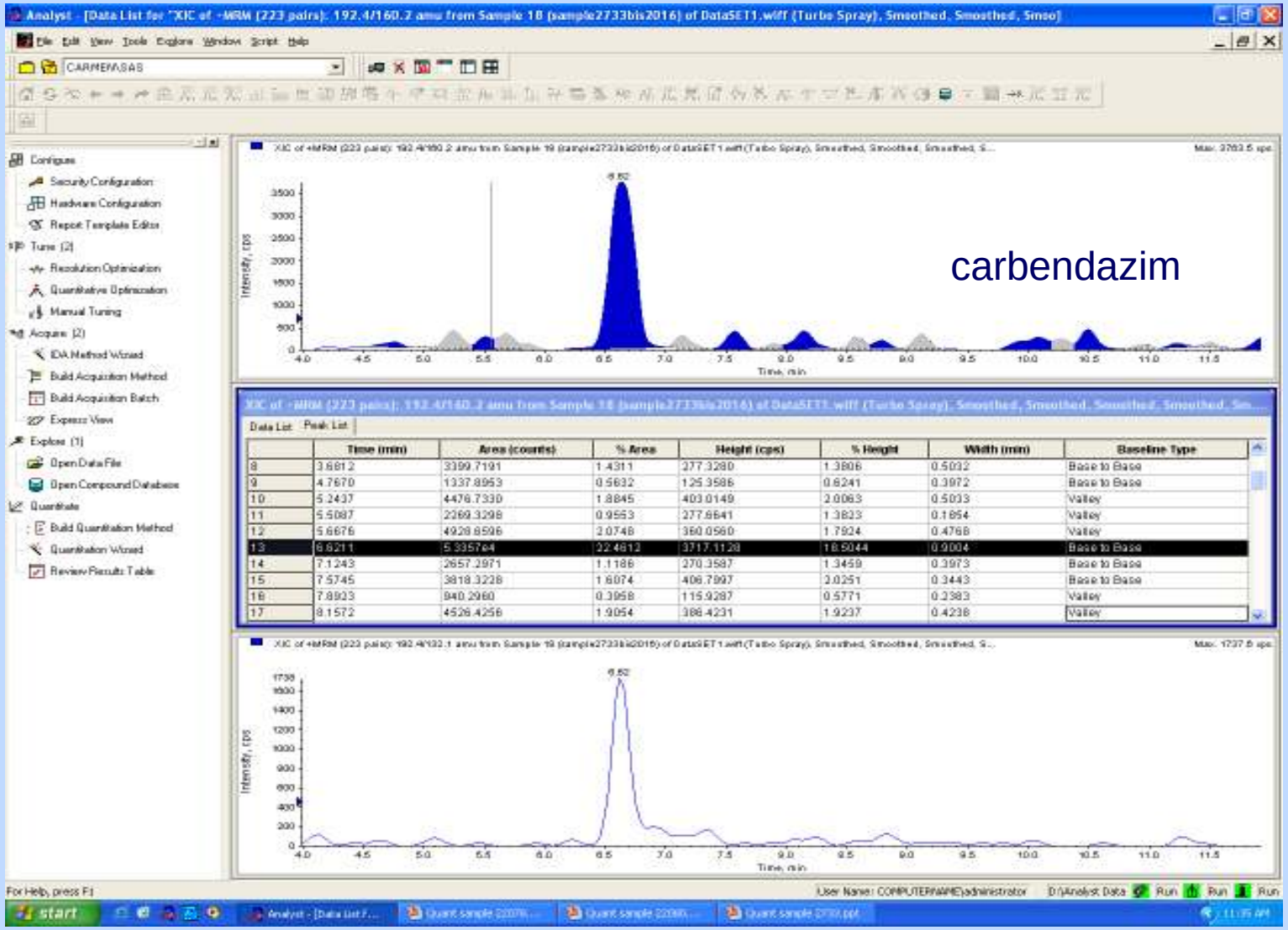
19.4 ppb



Sample #2733, grapes

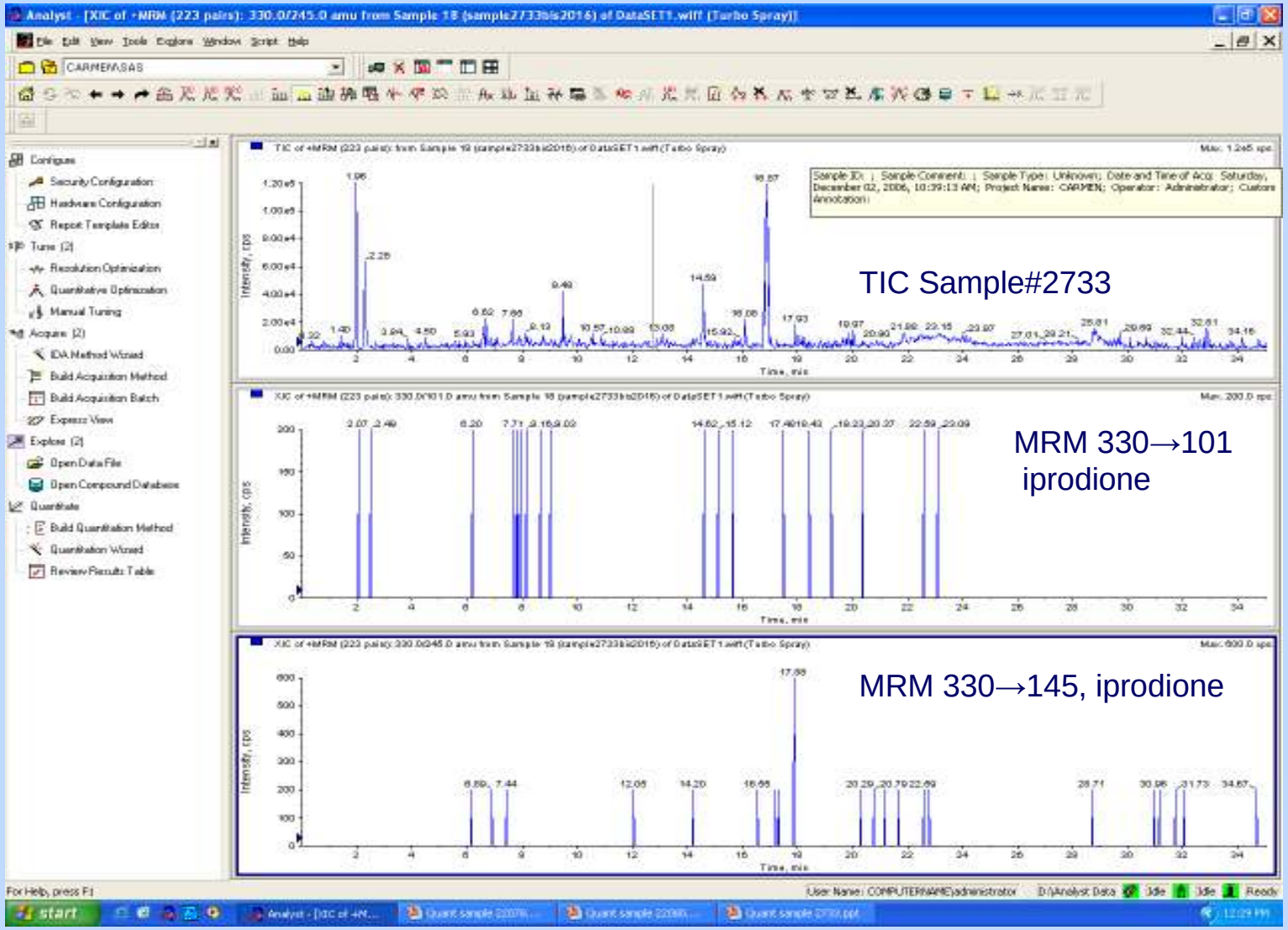
positive

1.5 ppb



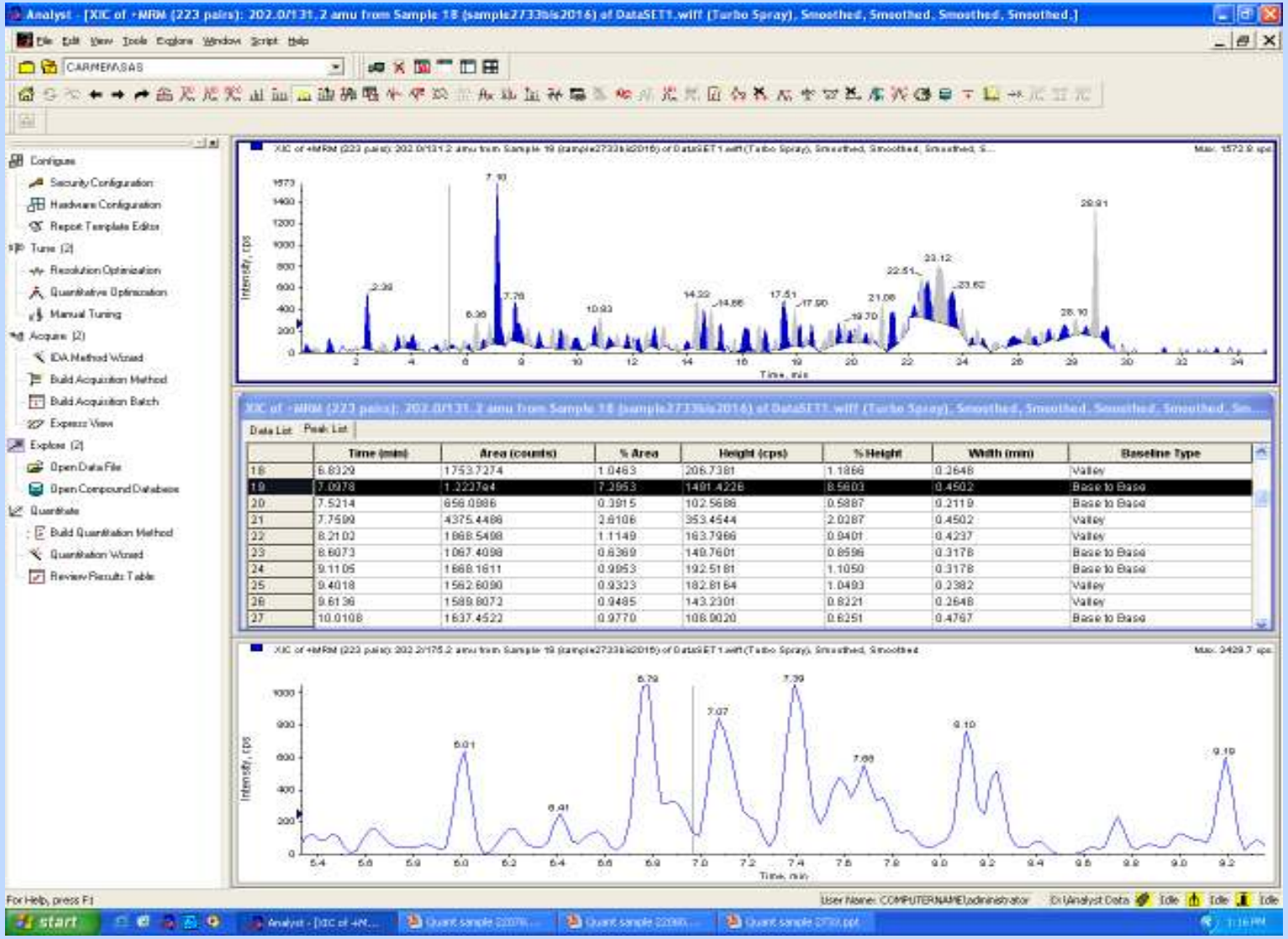
Sample #2733, grapes

negative

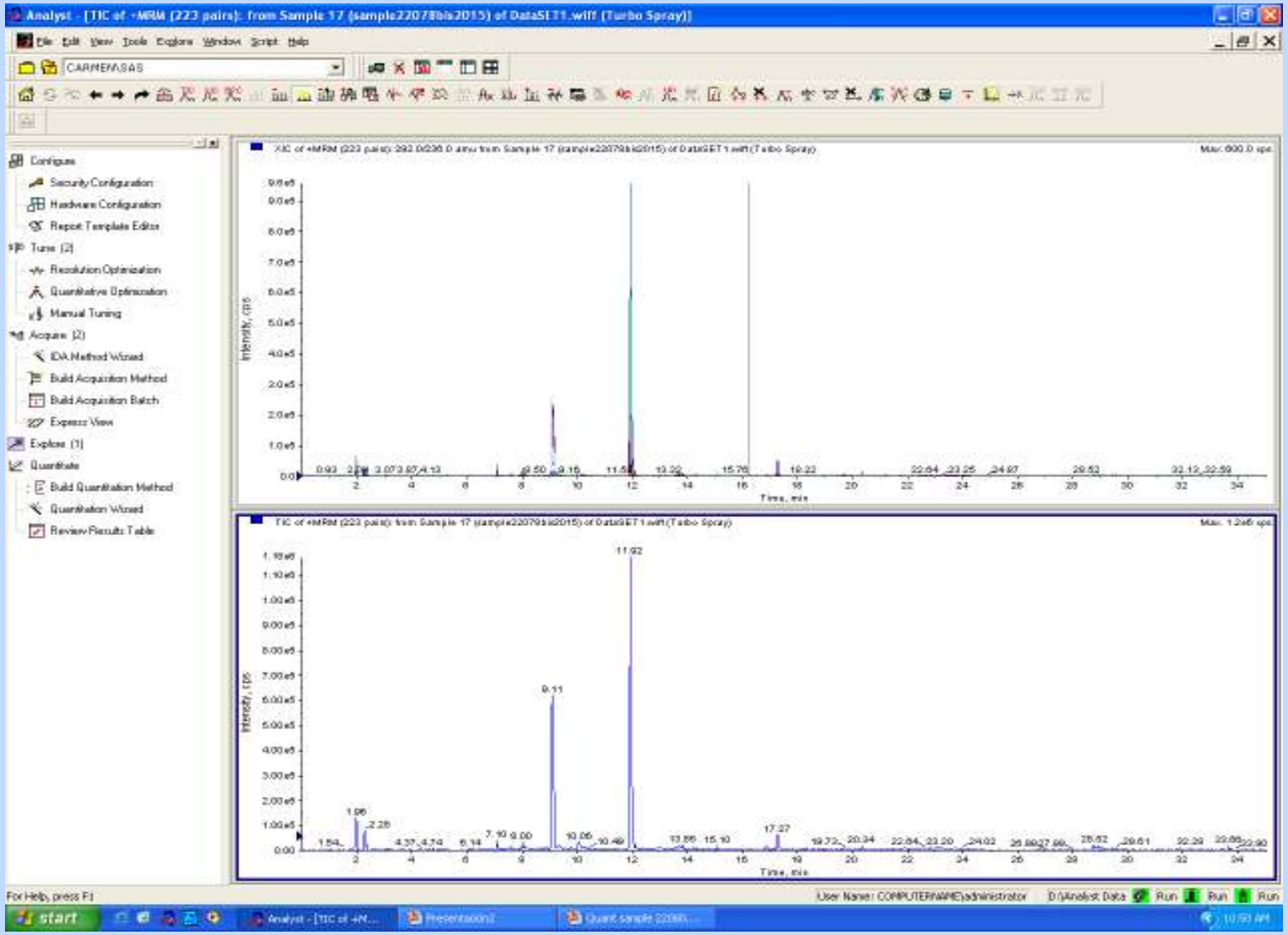


Sample #2733, grapes

Negative?

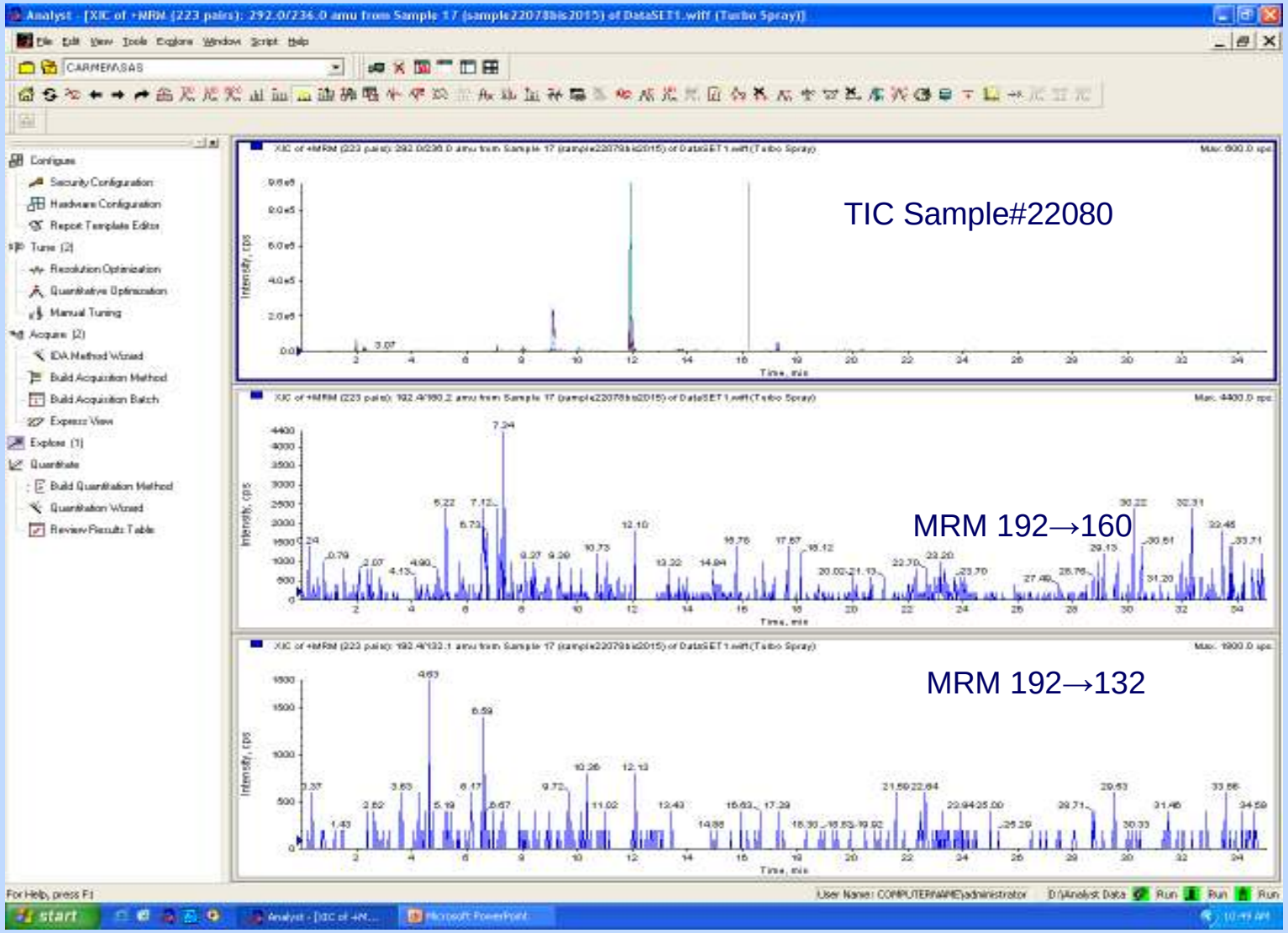


Sample #22078, pear



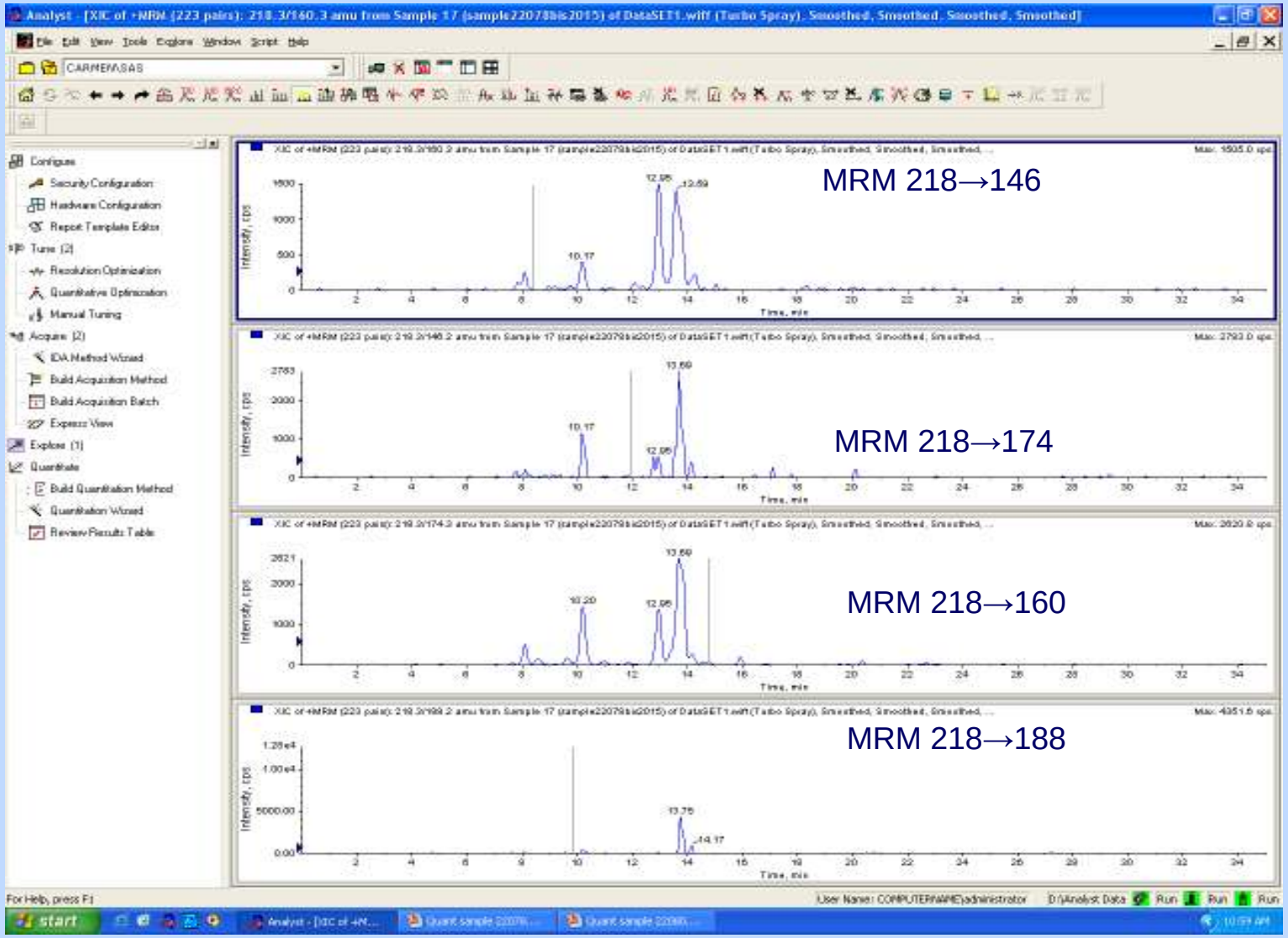
Sample #22078, pear

Carbendazim Negative



Sample #22078, pear

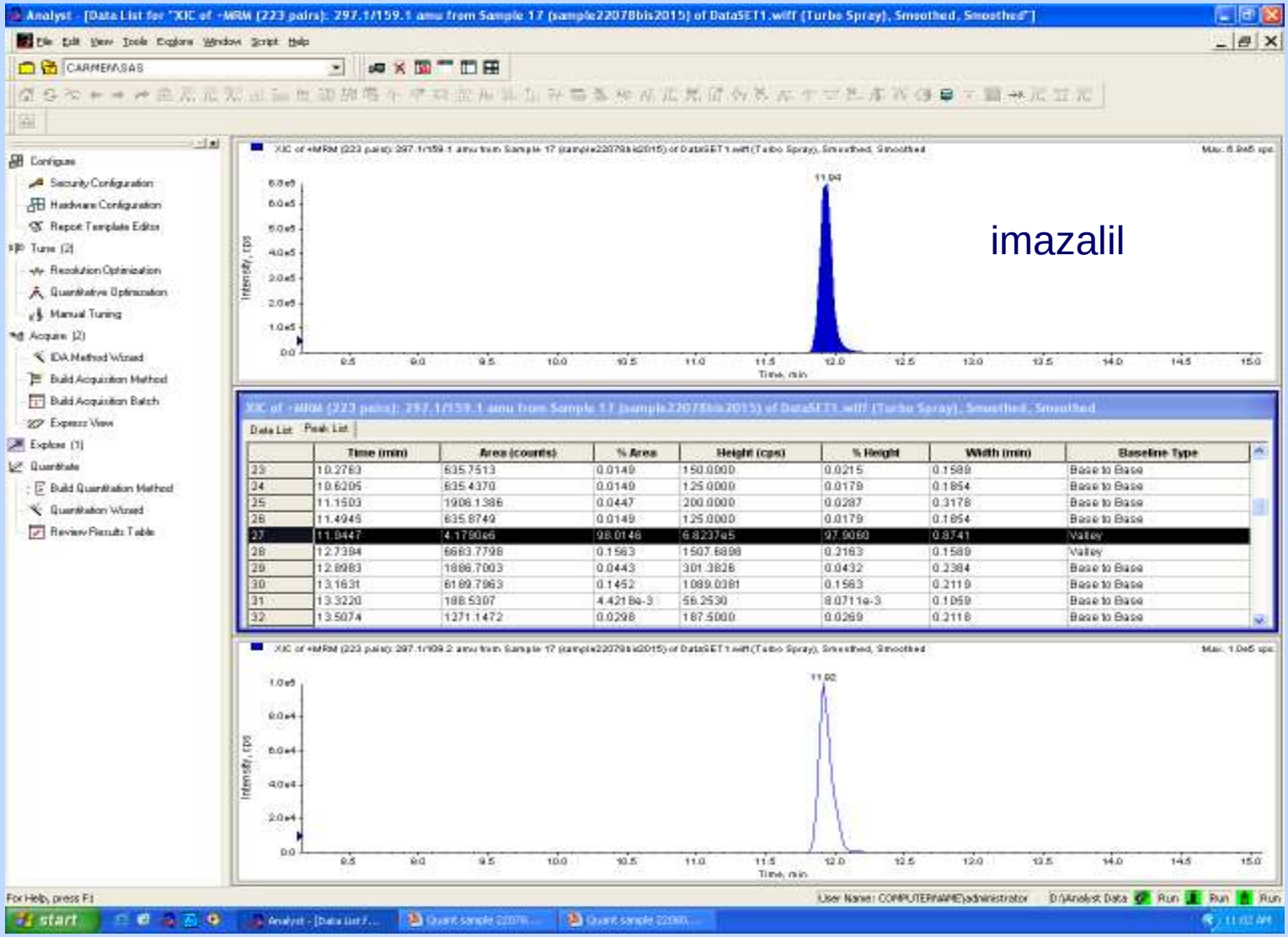
Ethoxyquin Positive?



Sample #22078, pear

Positive

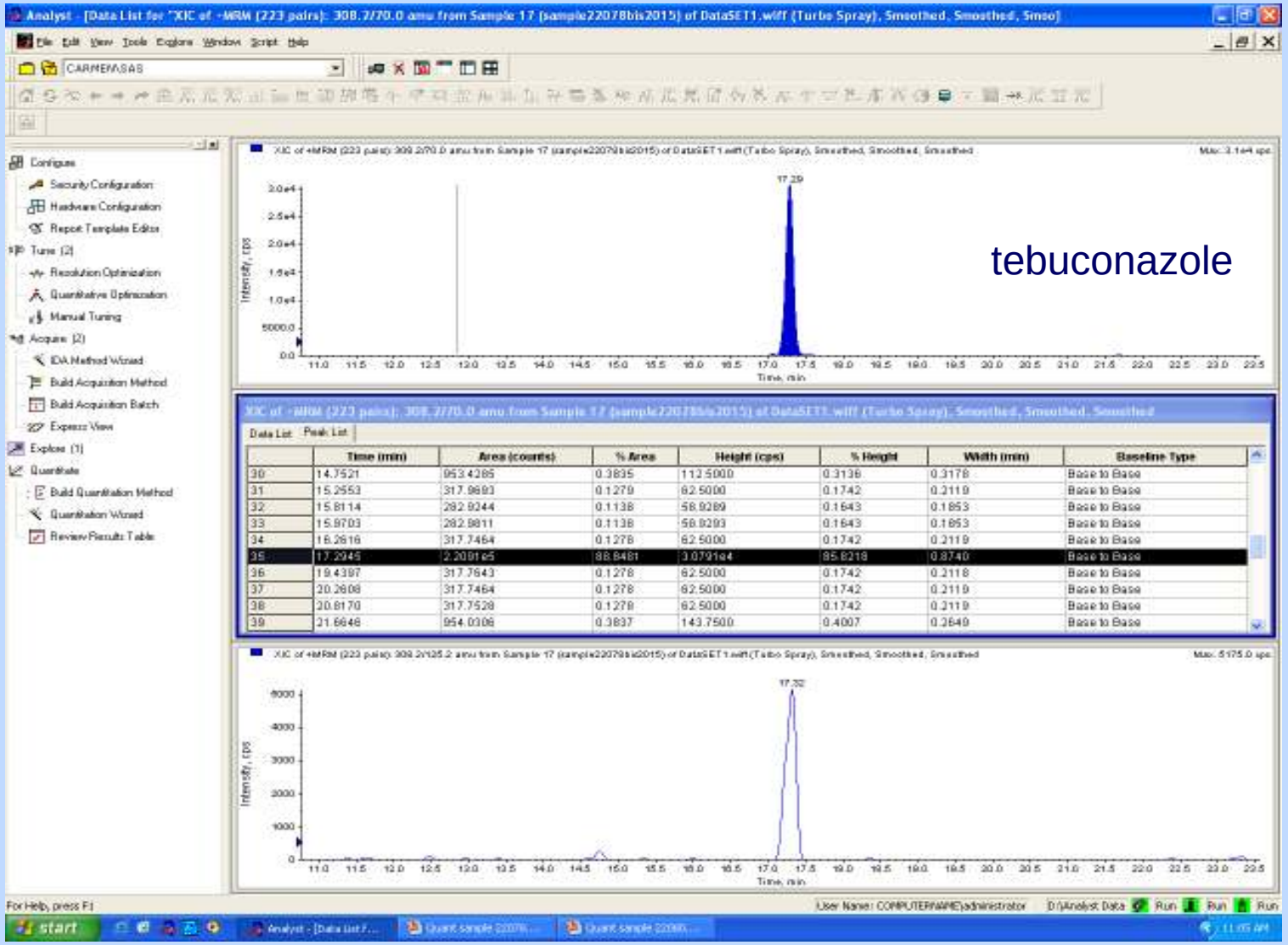
297.3 ppb



Sample #22078, pear

Positive

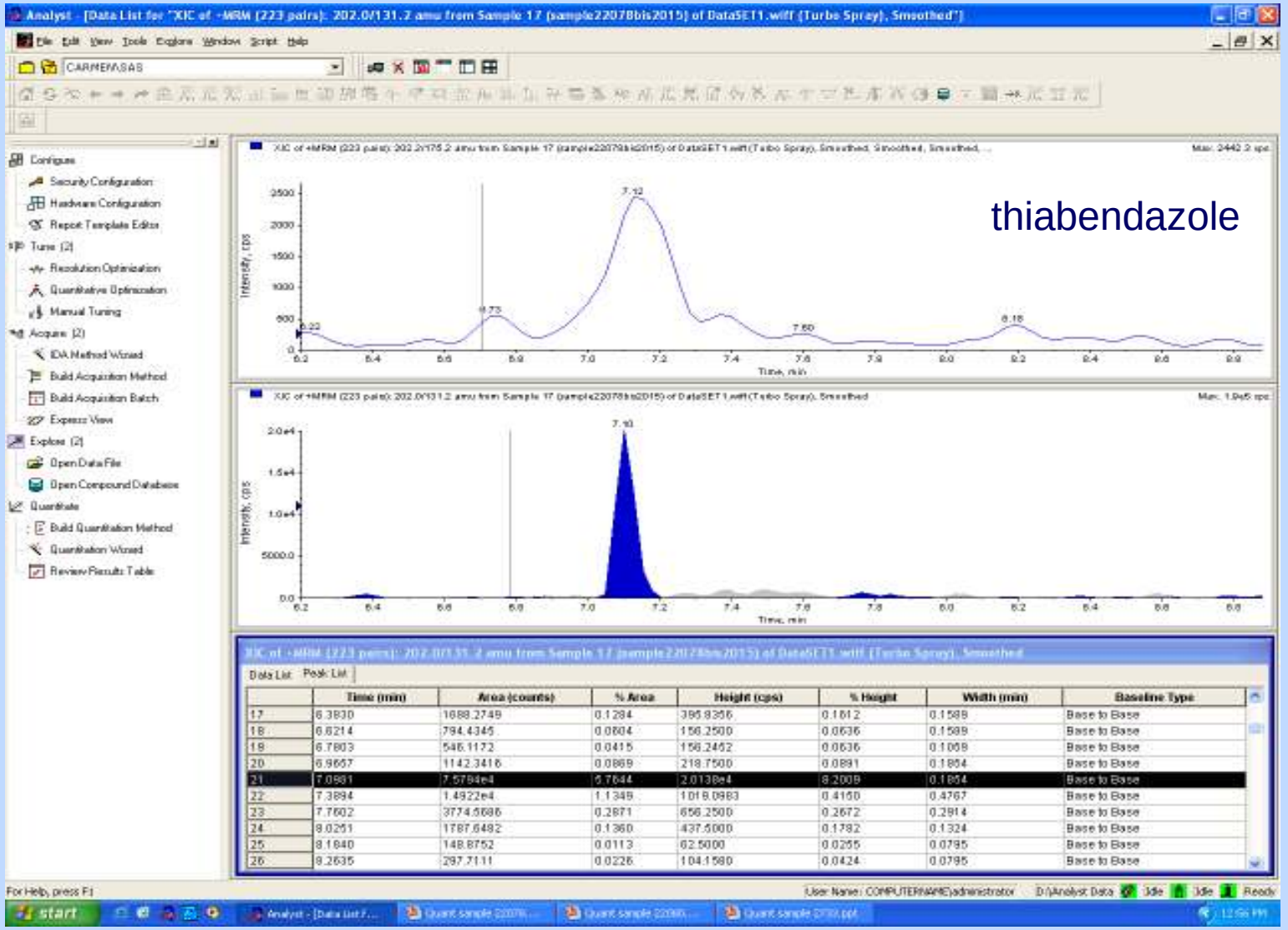
12.8 ppb



Sample #22078, pear

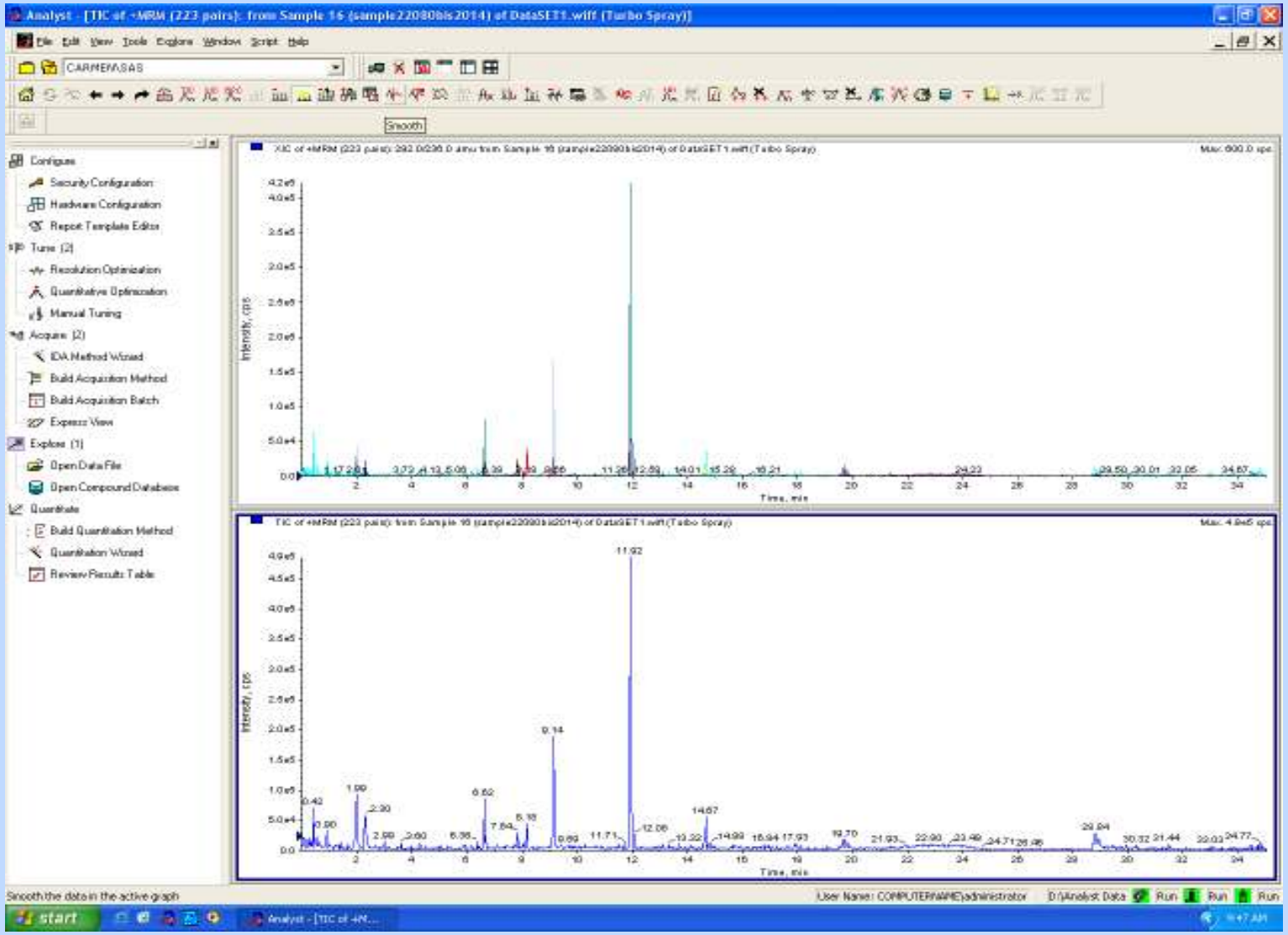
Positive

9.1 ppb



	Time (min)	Area (counts)	% Area	Height (cps)	% Height	Width (min)	Baseline Type
17	6.3830	1688.2749	0.1284	395.8356	0.1612	0.1589	Base to Base
18	6.6214	784.4345	0.0804	156.2500	0.0636	0.1589	Base to Base
19	6.7803	546.1172	0.0415	156.2452	0.0636	0.1059	Base to Base
20	6.9667	1142.3416	0.0869	218.7500	0.0891	0.1854	Base to Base
21	7.0881	7.5794e4	5.7644	2.0138e4	8.2009	0.1854	Base to Base
22	7.3894	1.4922e4	1.1349	1018.0983	0.4150	0.4767	Base to Base
23	7.7602	3774.5686	0.2871	656.2500	0.2672	0.2914	Base to Base
24	8.0251	1787.6482	0.1360	437.5000	0.1782	0.1324	Base to Base
25	8.1840	148.8752	0.0113	62.5000	0.0255	0.0795	Base to Base
26	8.2635	297.7111	0.0226	104.1580	0.0424	0.0795	Base to Base

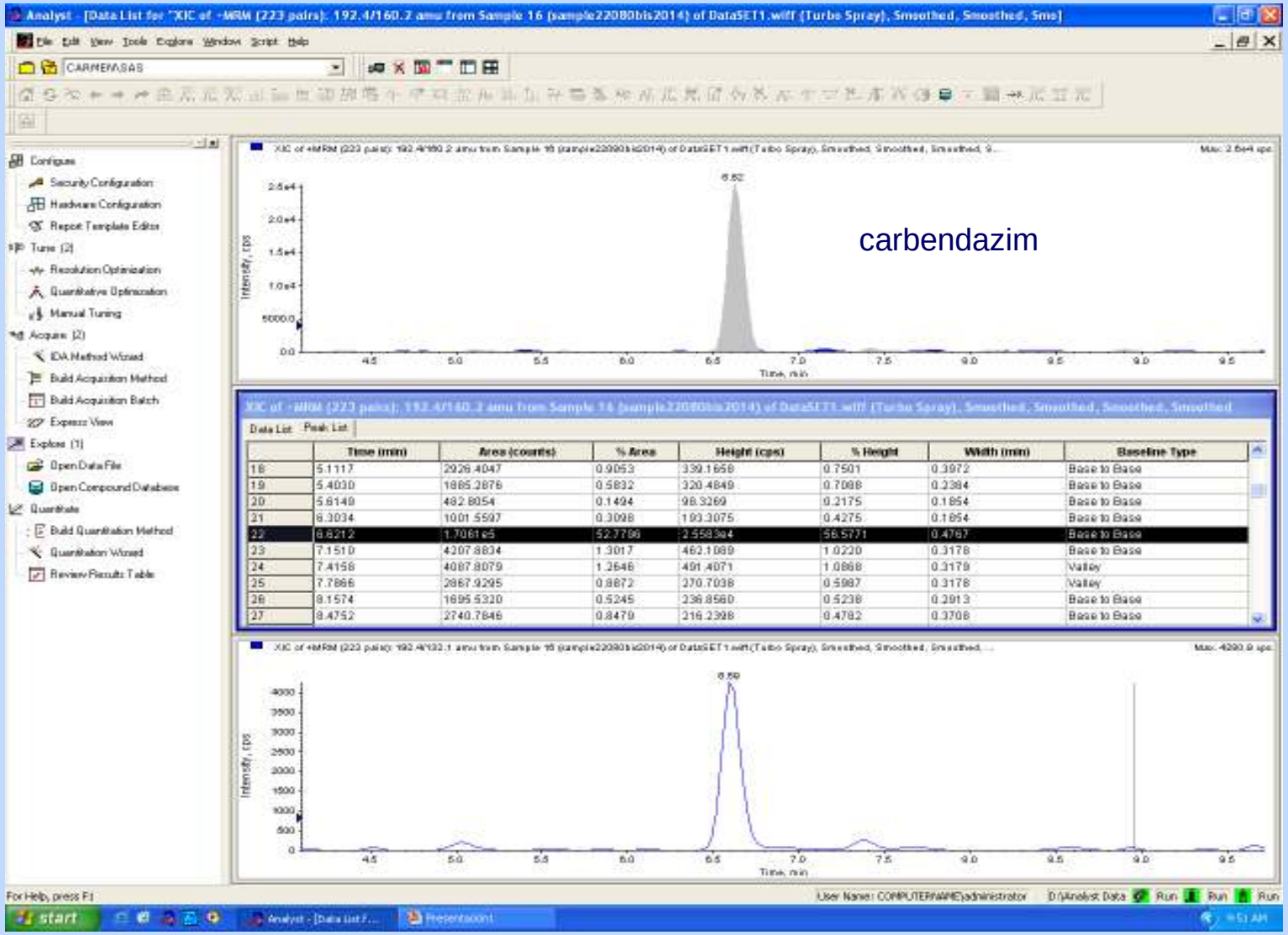
Sample #22080, apple



Sample #22080, apple

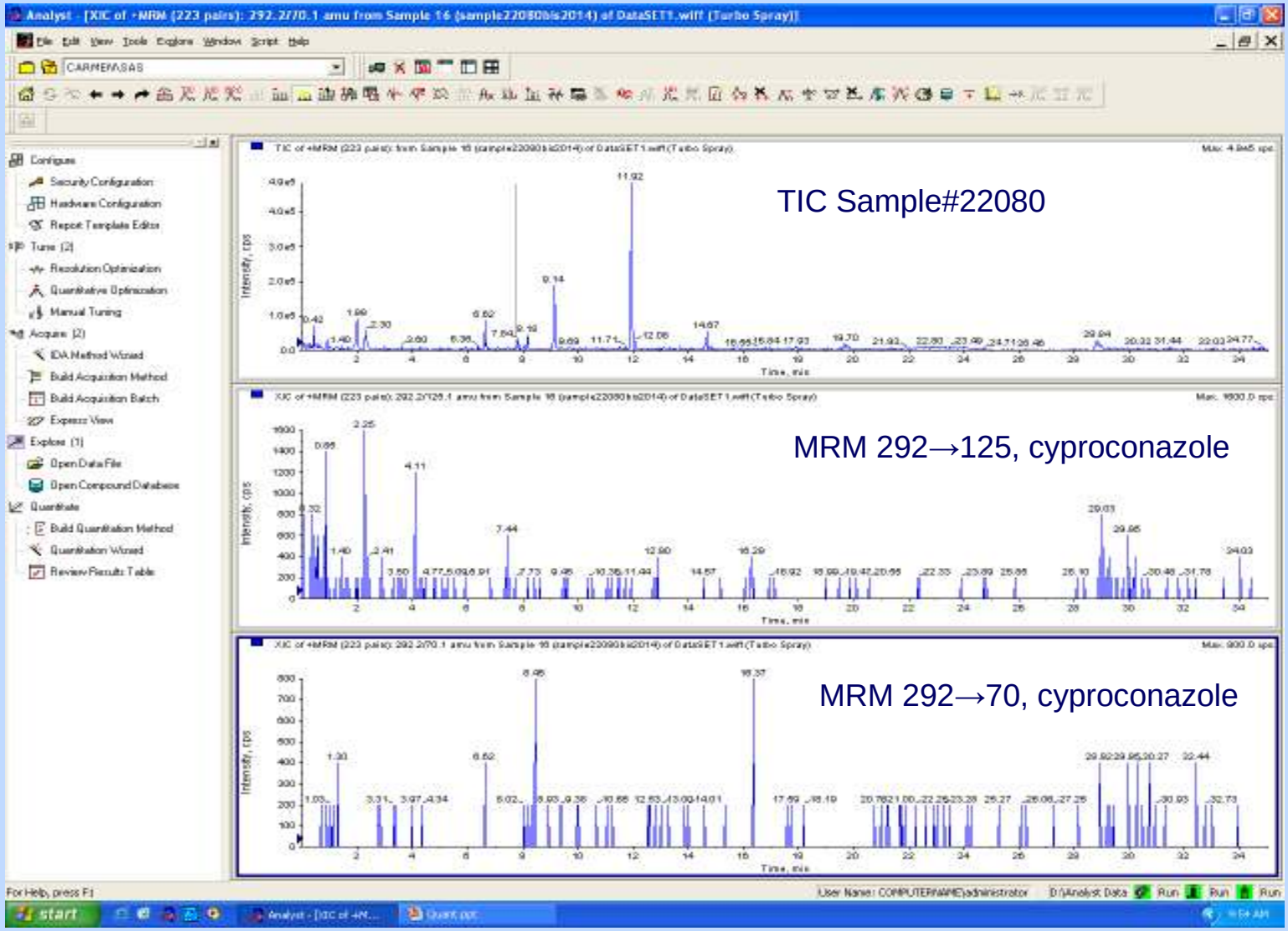
positive

49.7 ppb



Sample #22080, apple

negative



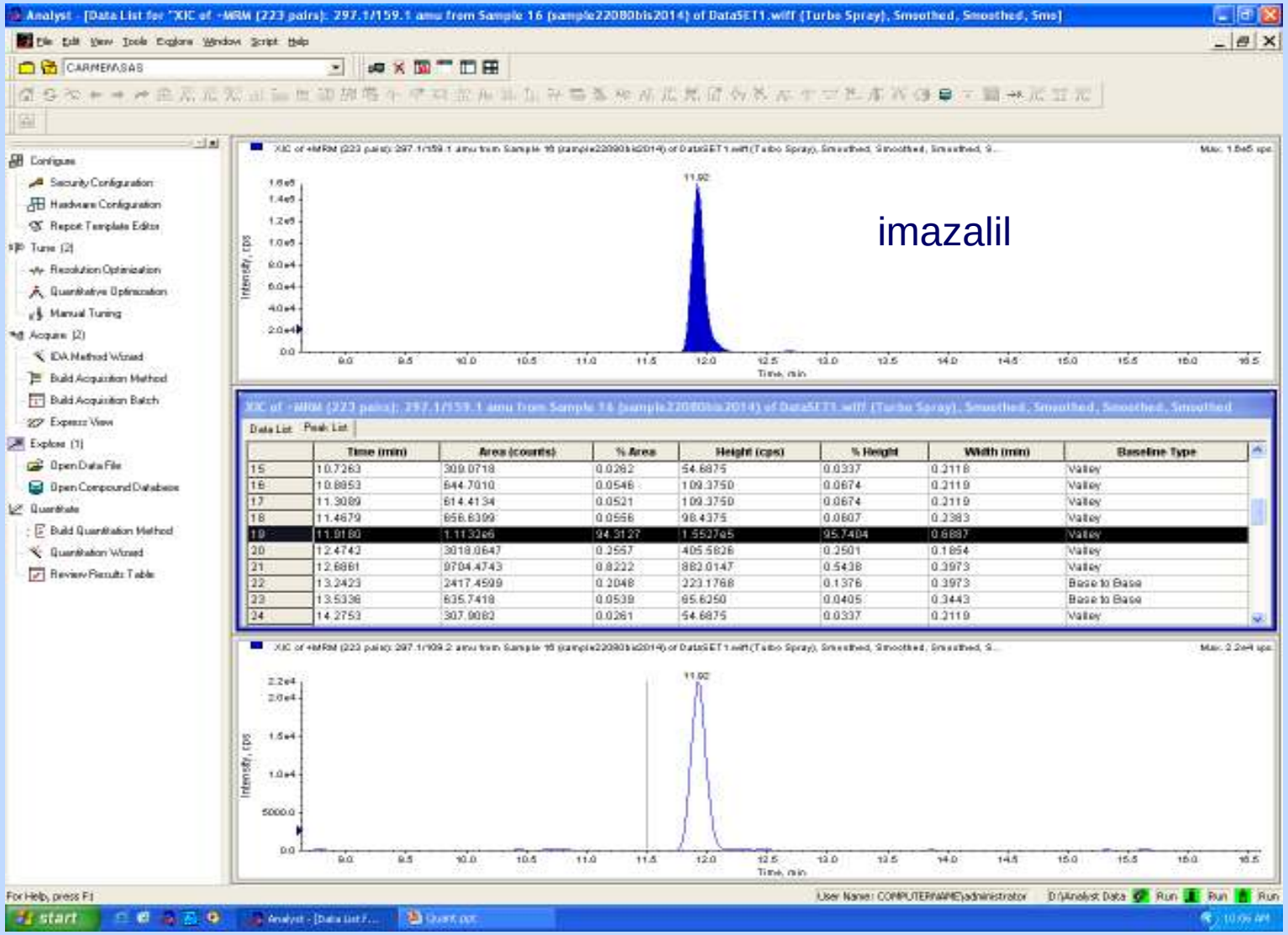
Sample #22080, apple

Ethoxyquin Positive?

Sample #22080, apple

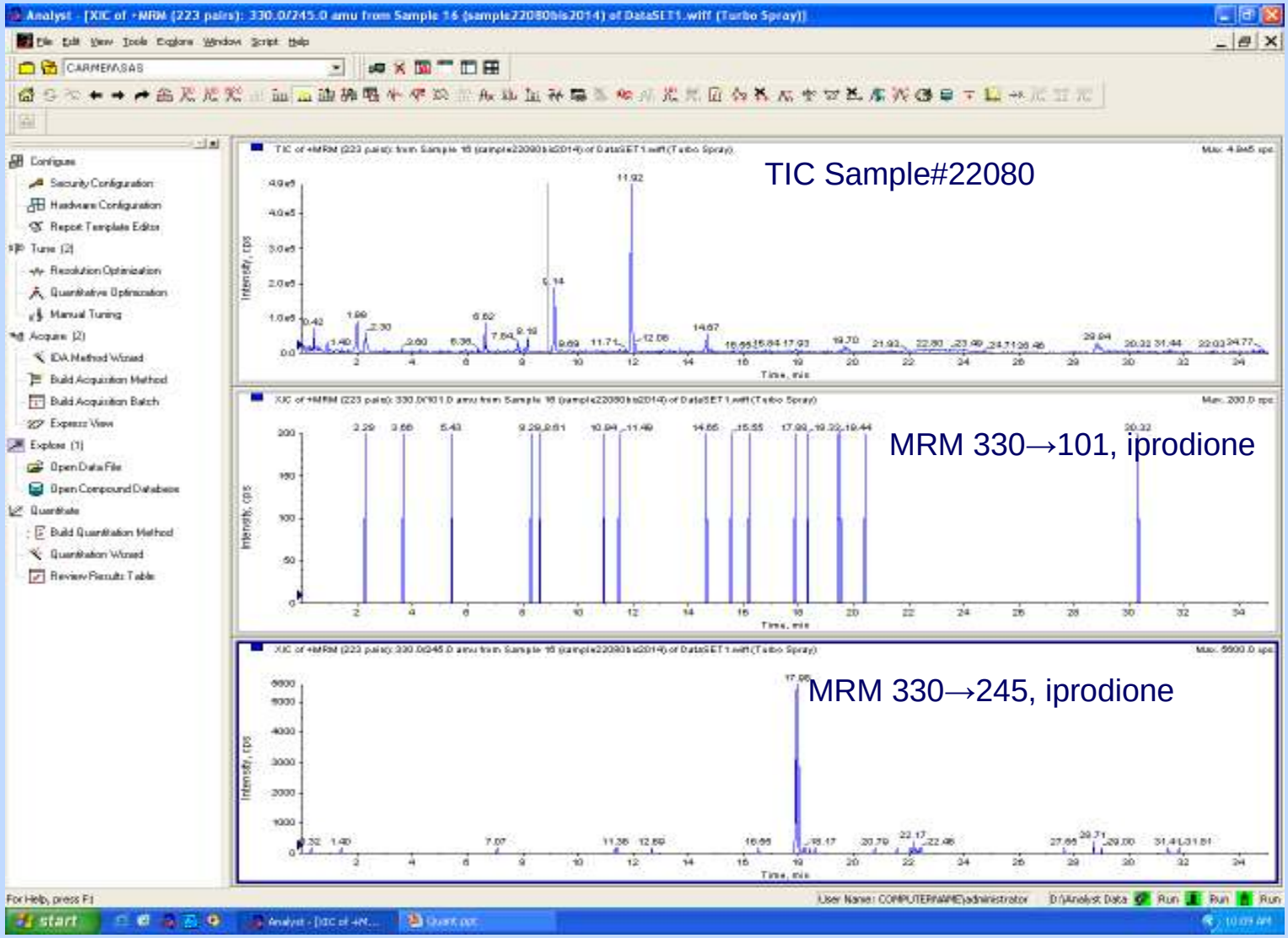
positive

79.2 ppb

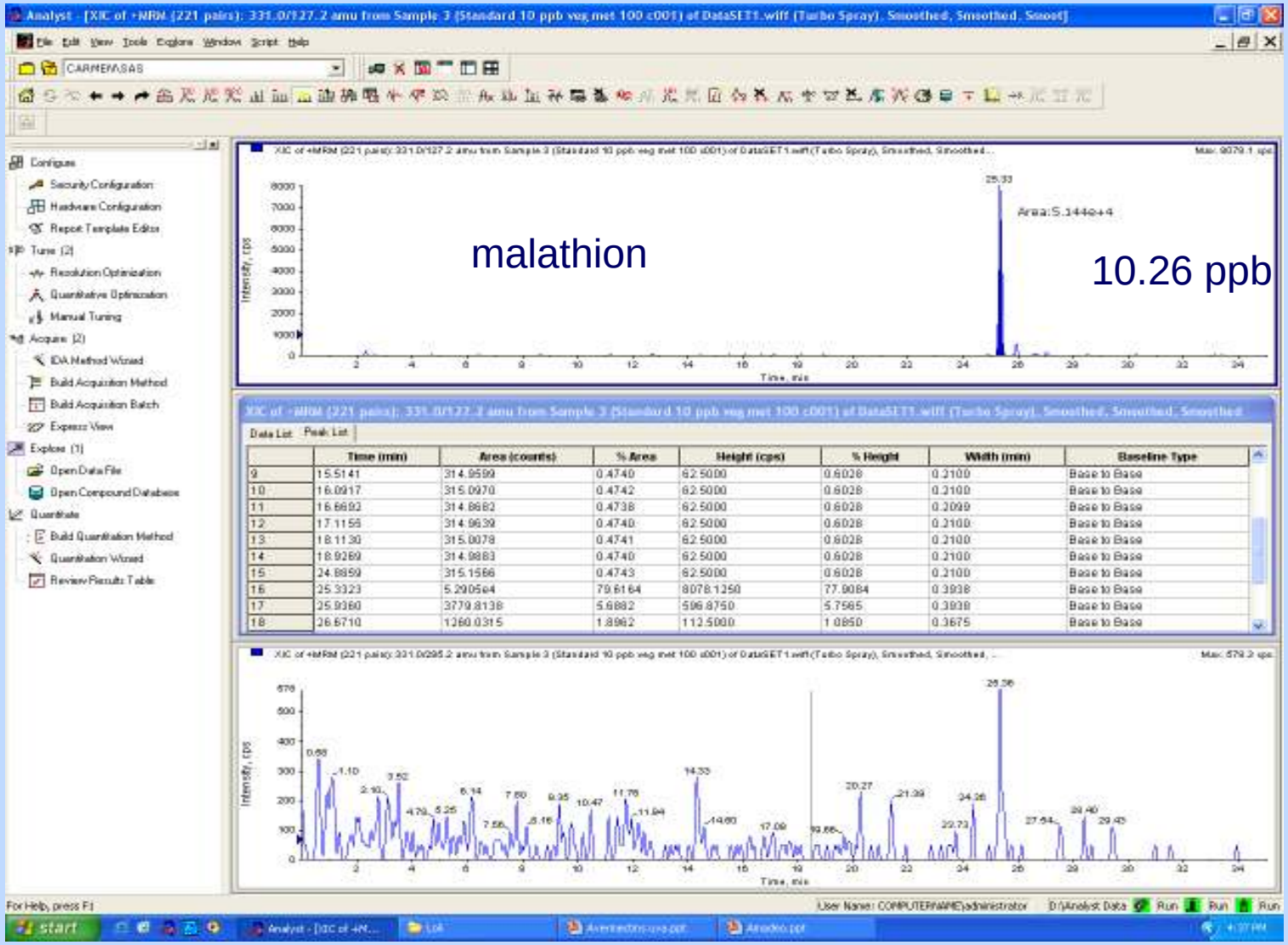


Sample #22080, apple

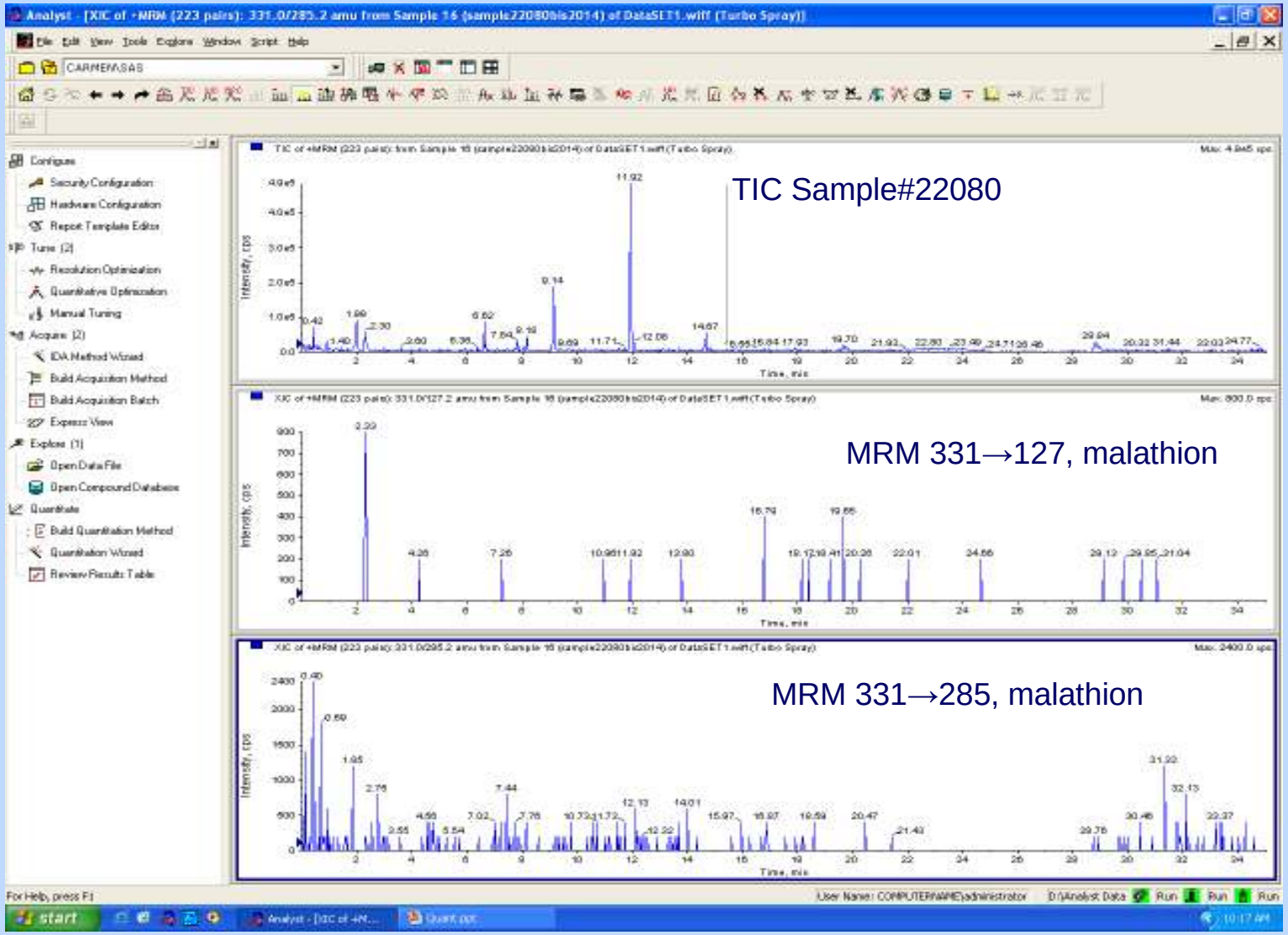
negative



Sample #22080 apple Analysis 1 positive

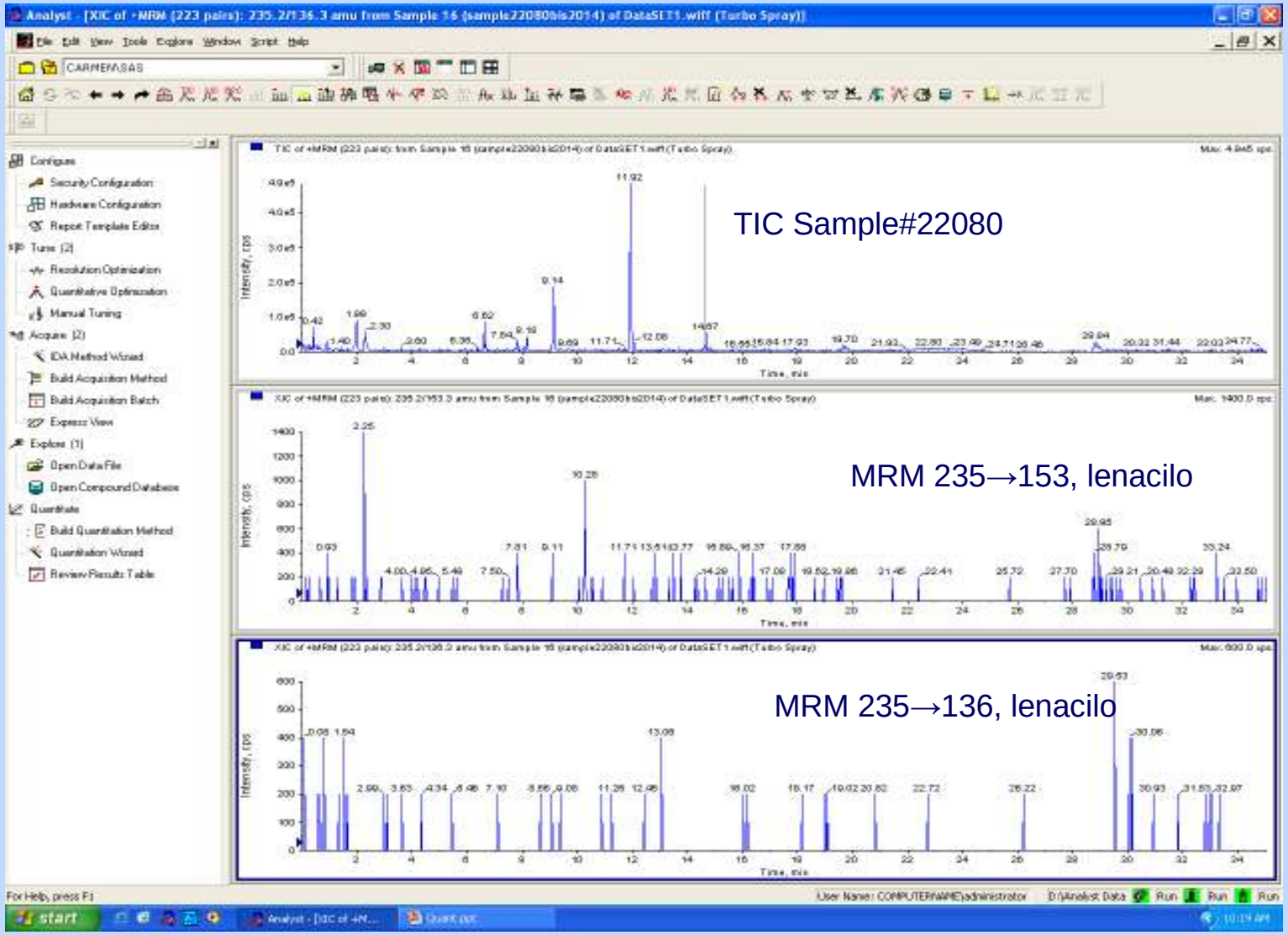


Sample #22080, apple Analysis 2 negative



Sample #22080, apple

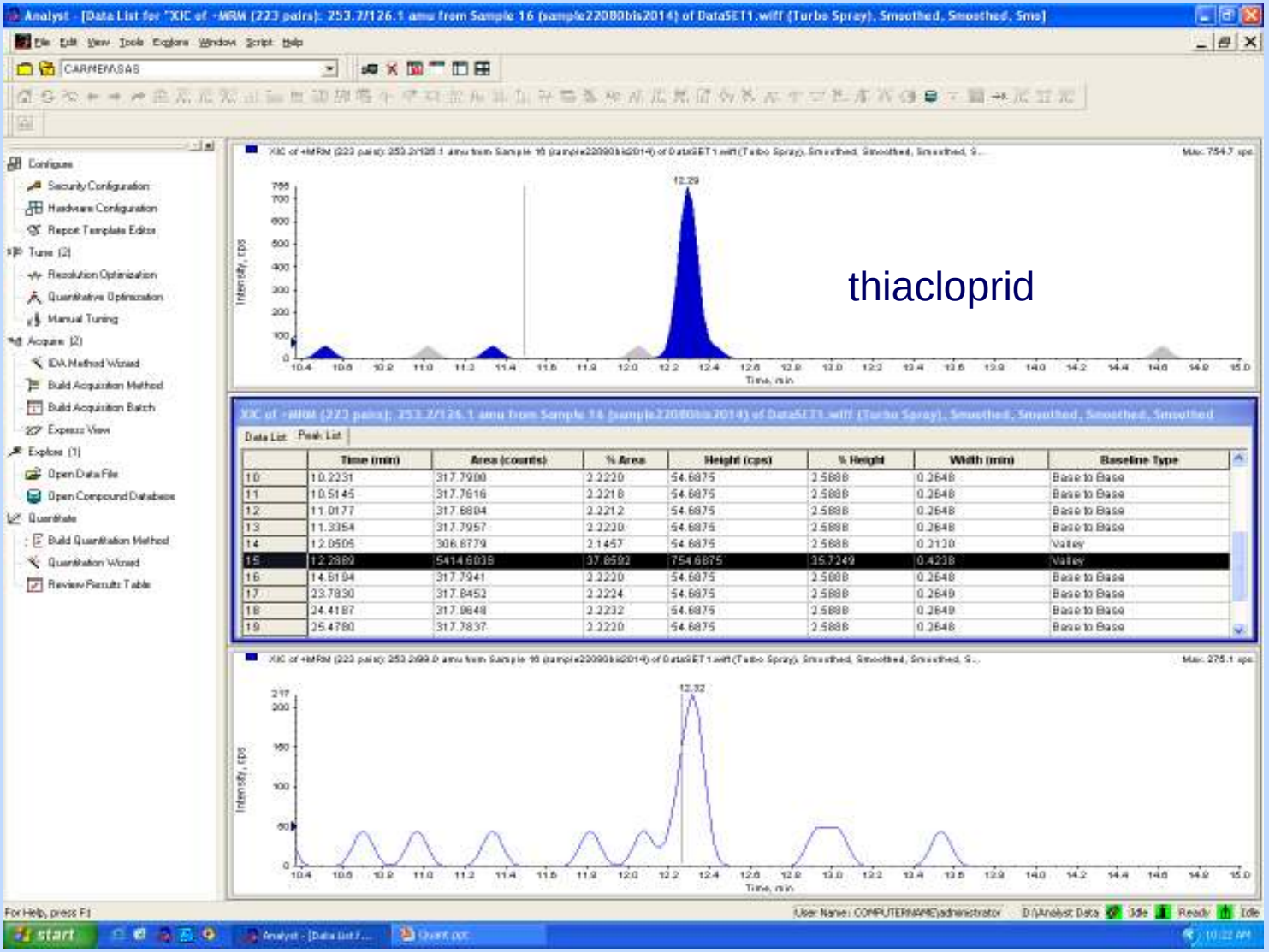
negative



Sample #22080, apple

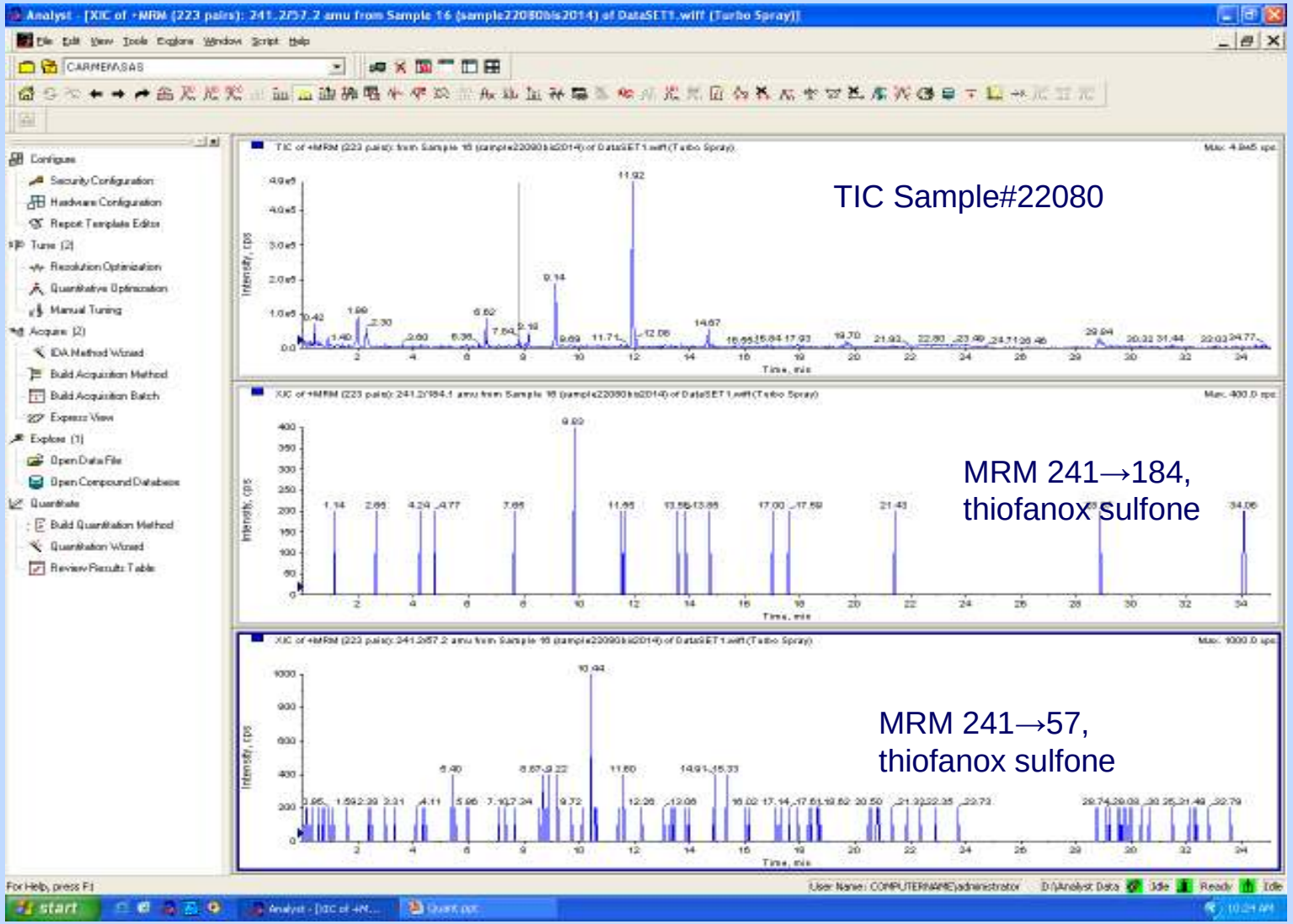
positive

0.3 ppb



Sample #22080, apple

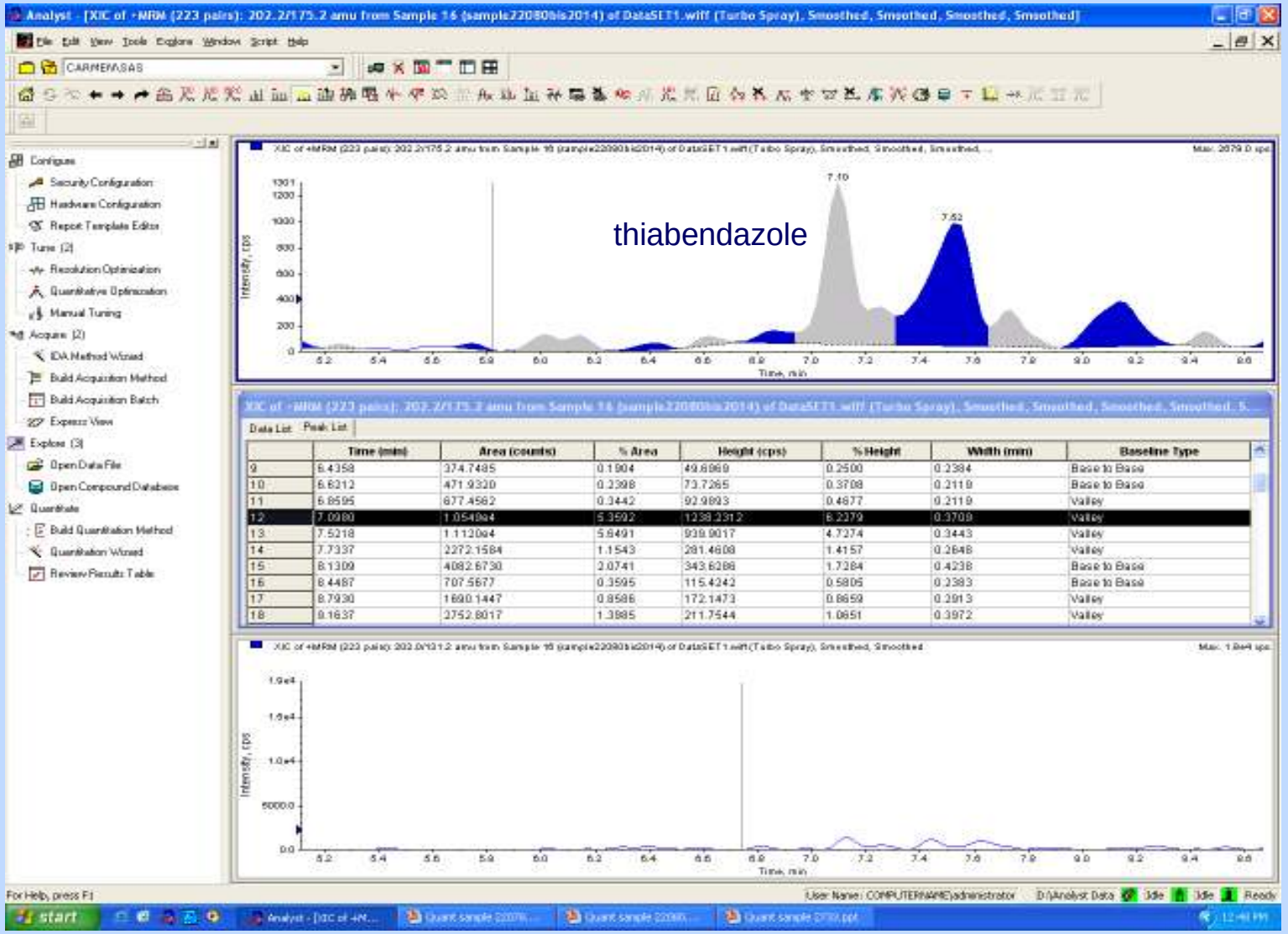
negative

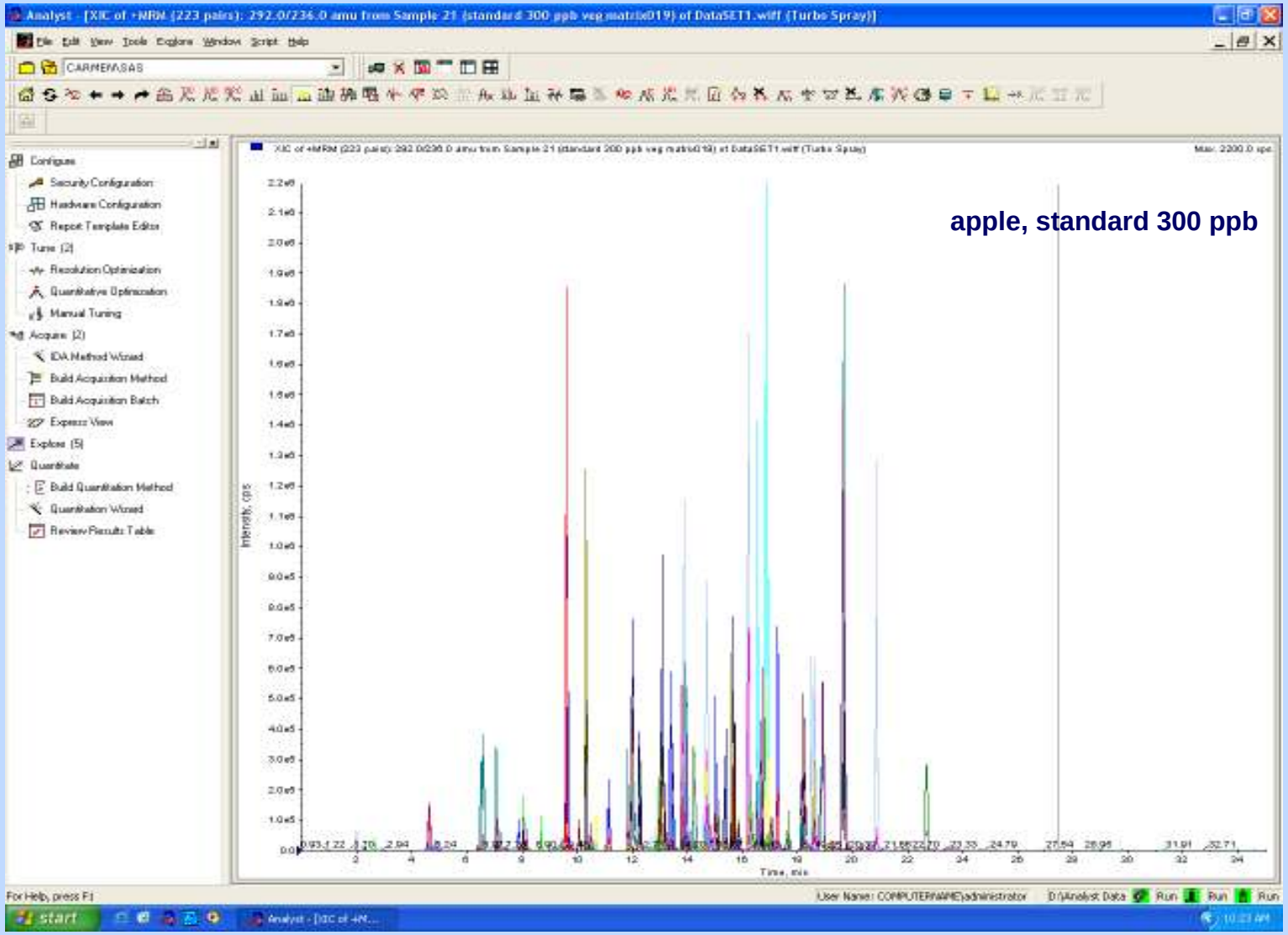


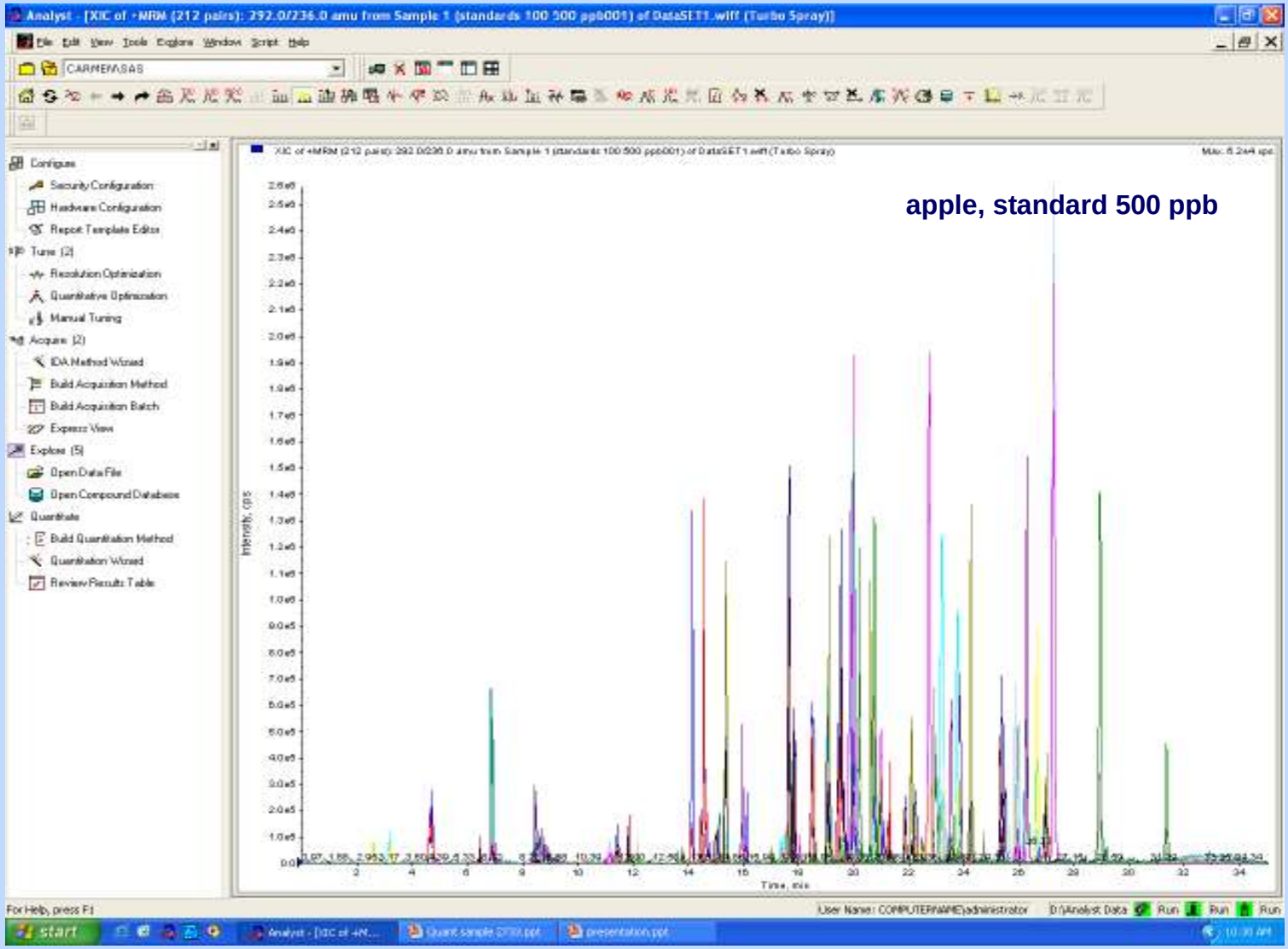
Sample #22080, apple

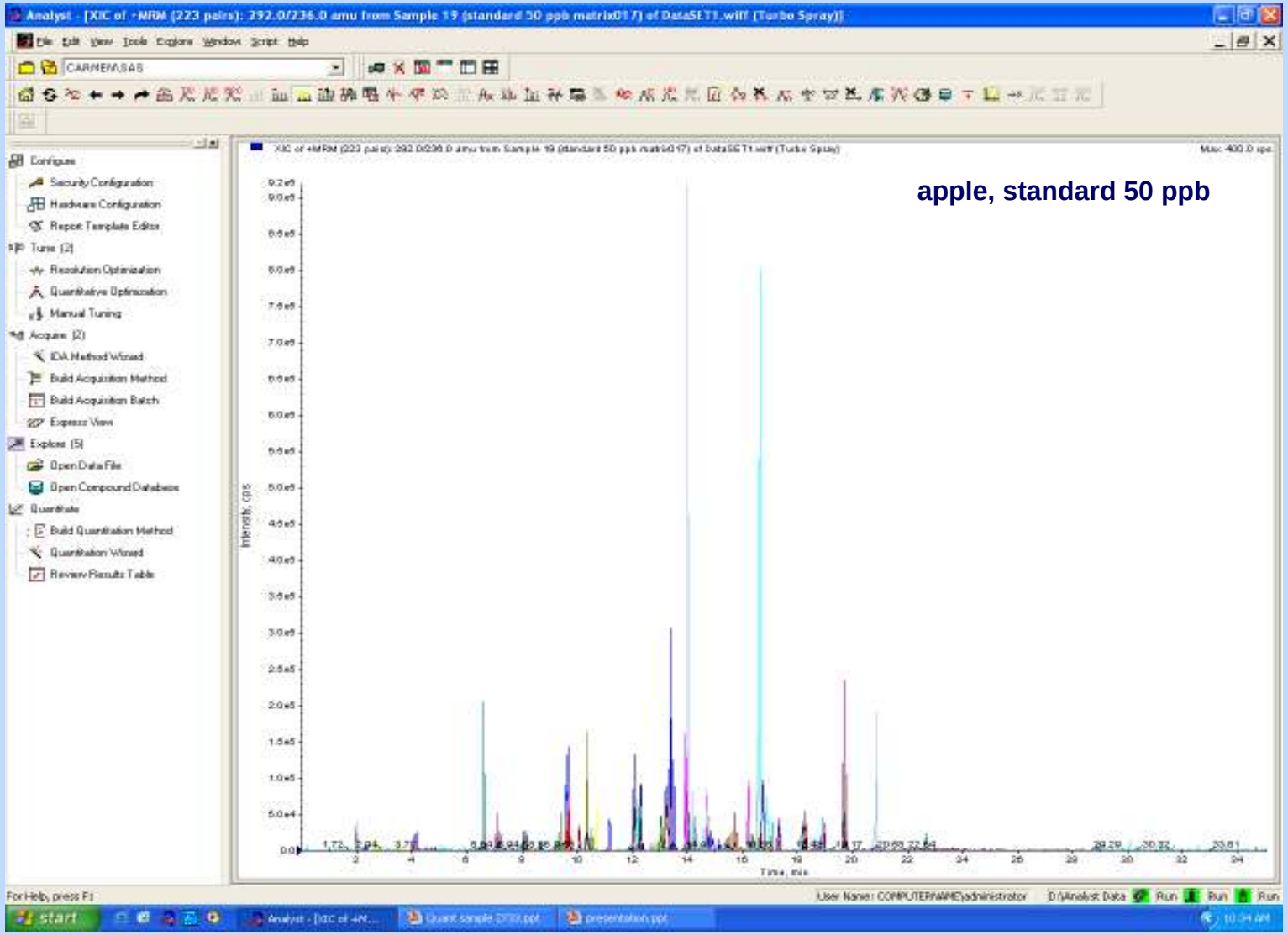
positive

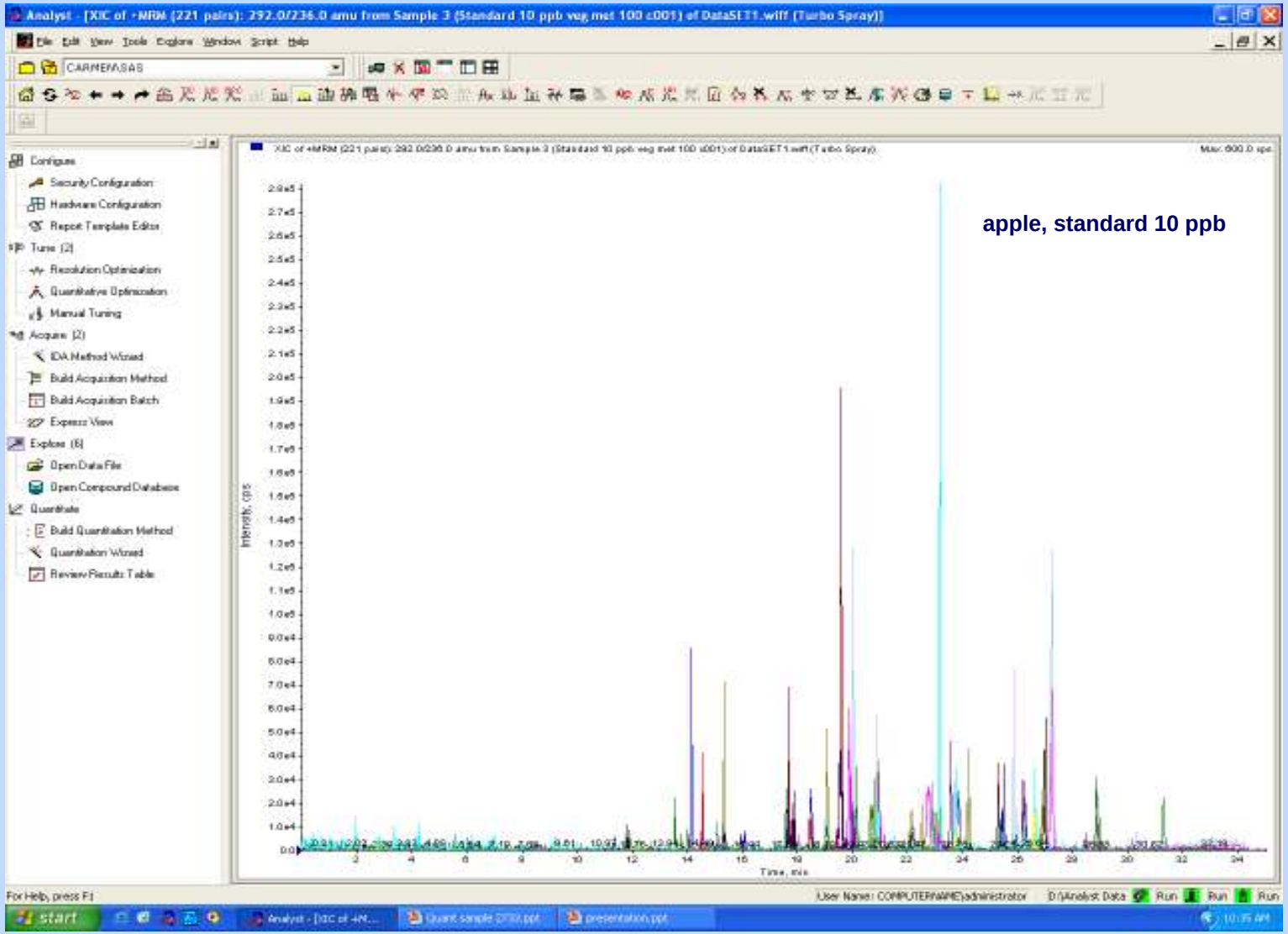
1.27 ppb

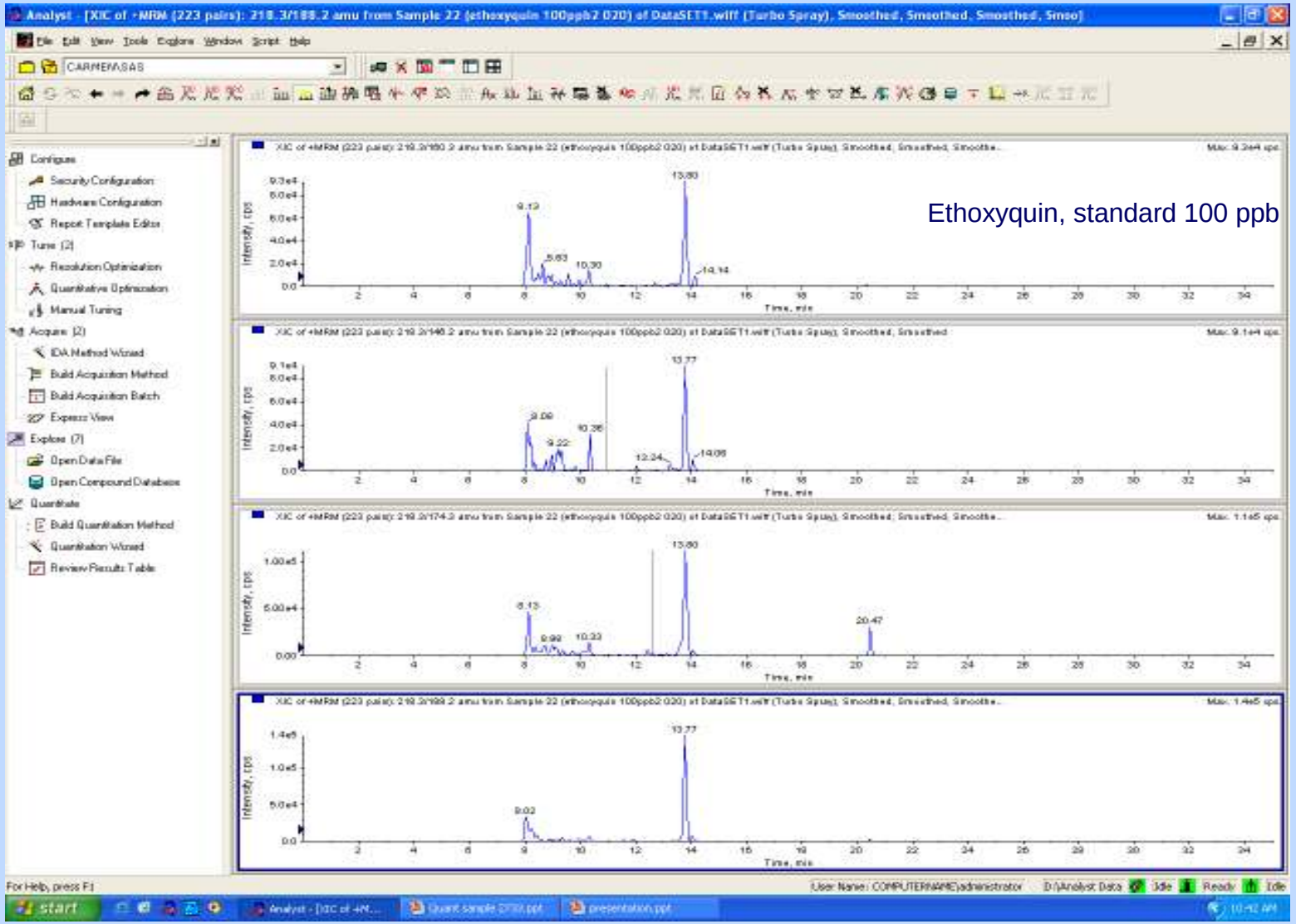


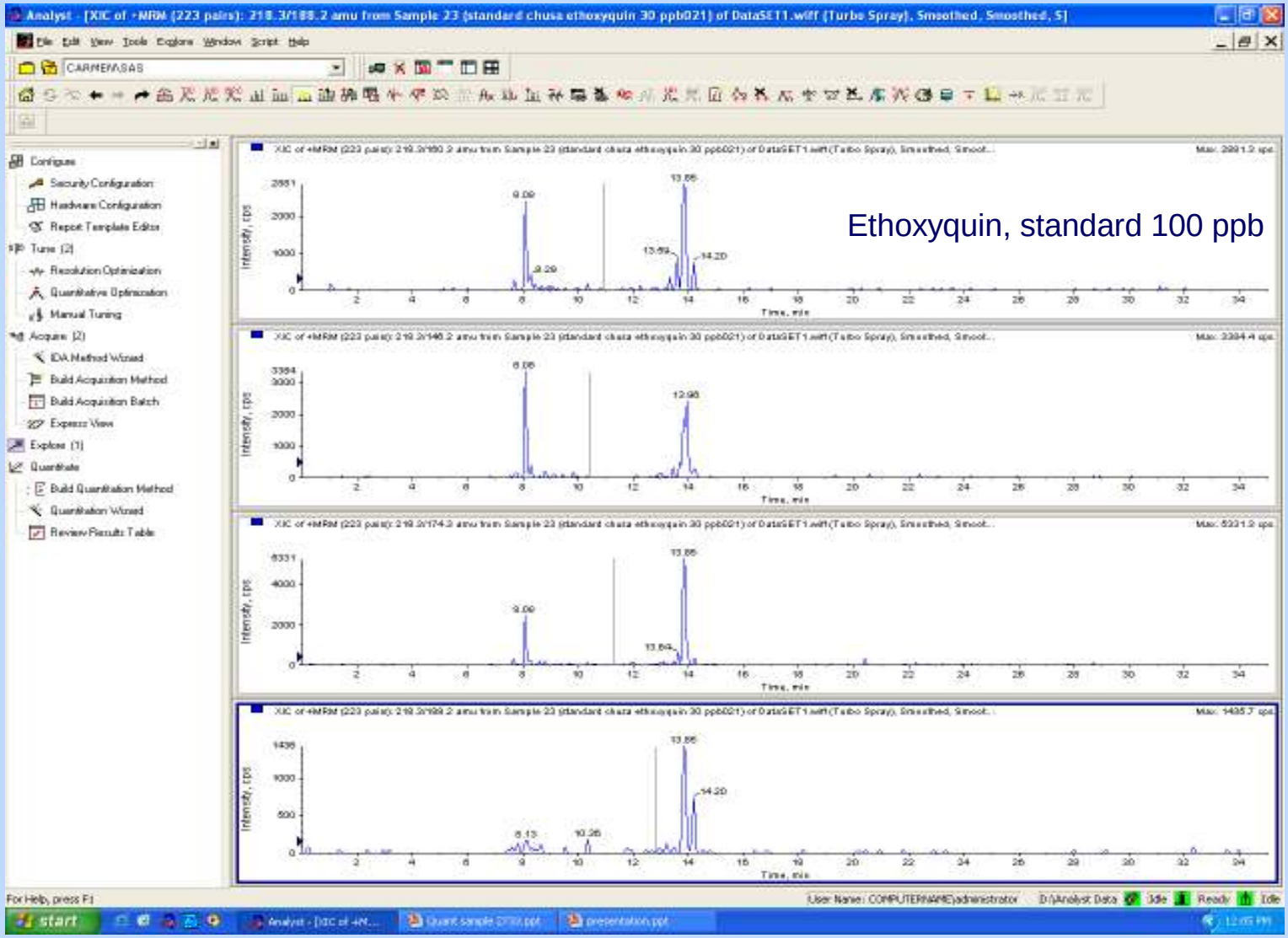

apple, standard 300 ppb


Analyst - [XIC of +MRM (212 pairs): 292.0/236.0 amu from Sample 1 (standards 100 500 ppb001) of DataSET1.wiff (Turbo Spray)]
apple, standard 500 ppb
Intensity, cps
Time, min

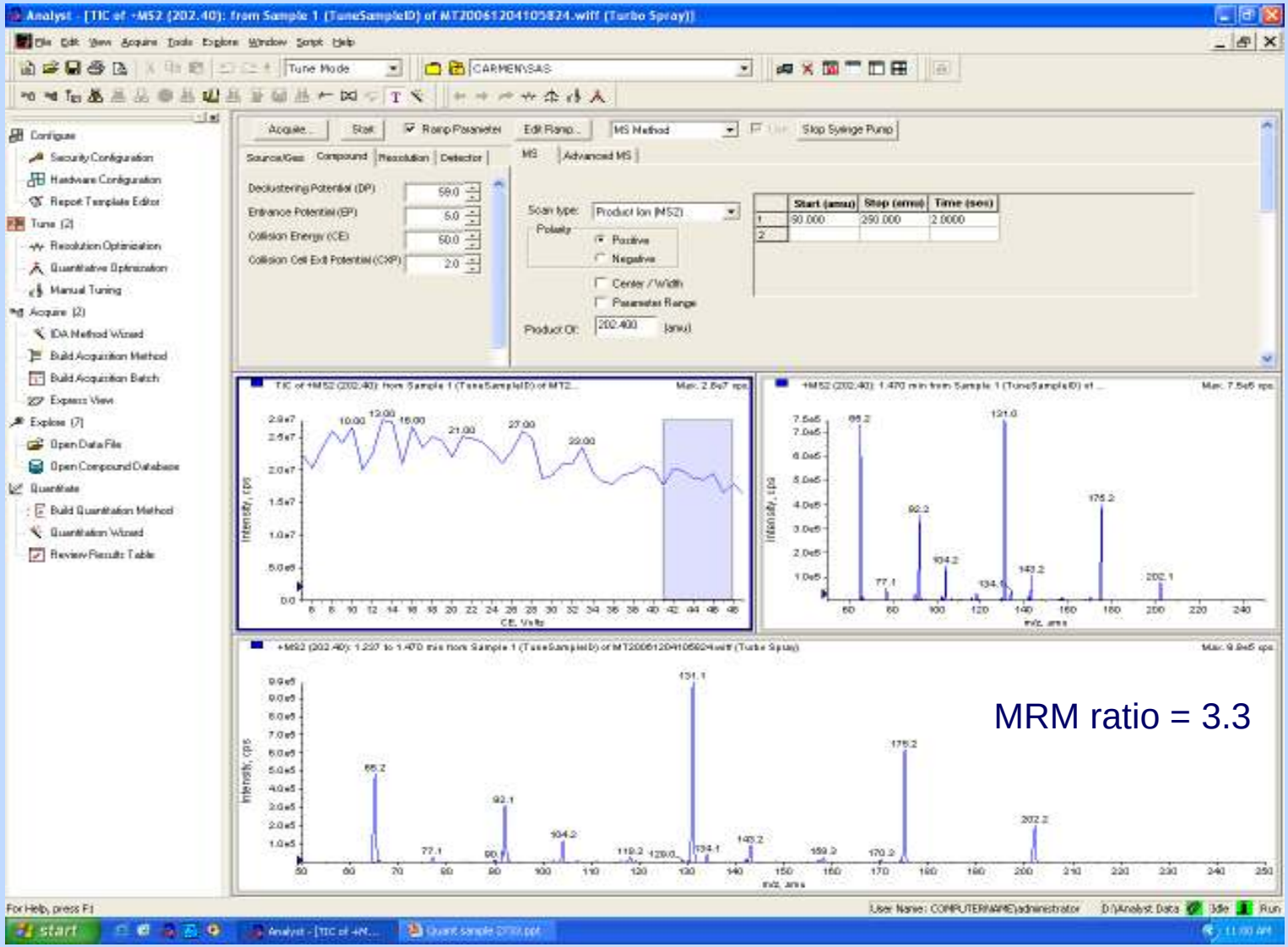

apple, standard 50 ppb


Analyst - [XIC of +MRM (221 pairs): 292.0/236.0 amu from Sample 3 (Standard 10 ppb veg met 100 c001) of DataSET1.wiff (Turbo Spray)]
Intensity, cps
Time, min
apple, standard 10 ppb

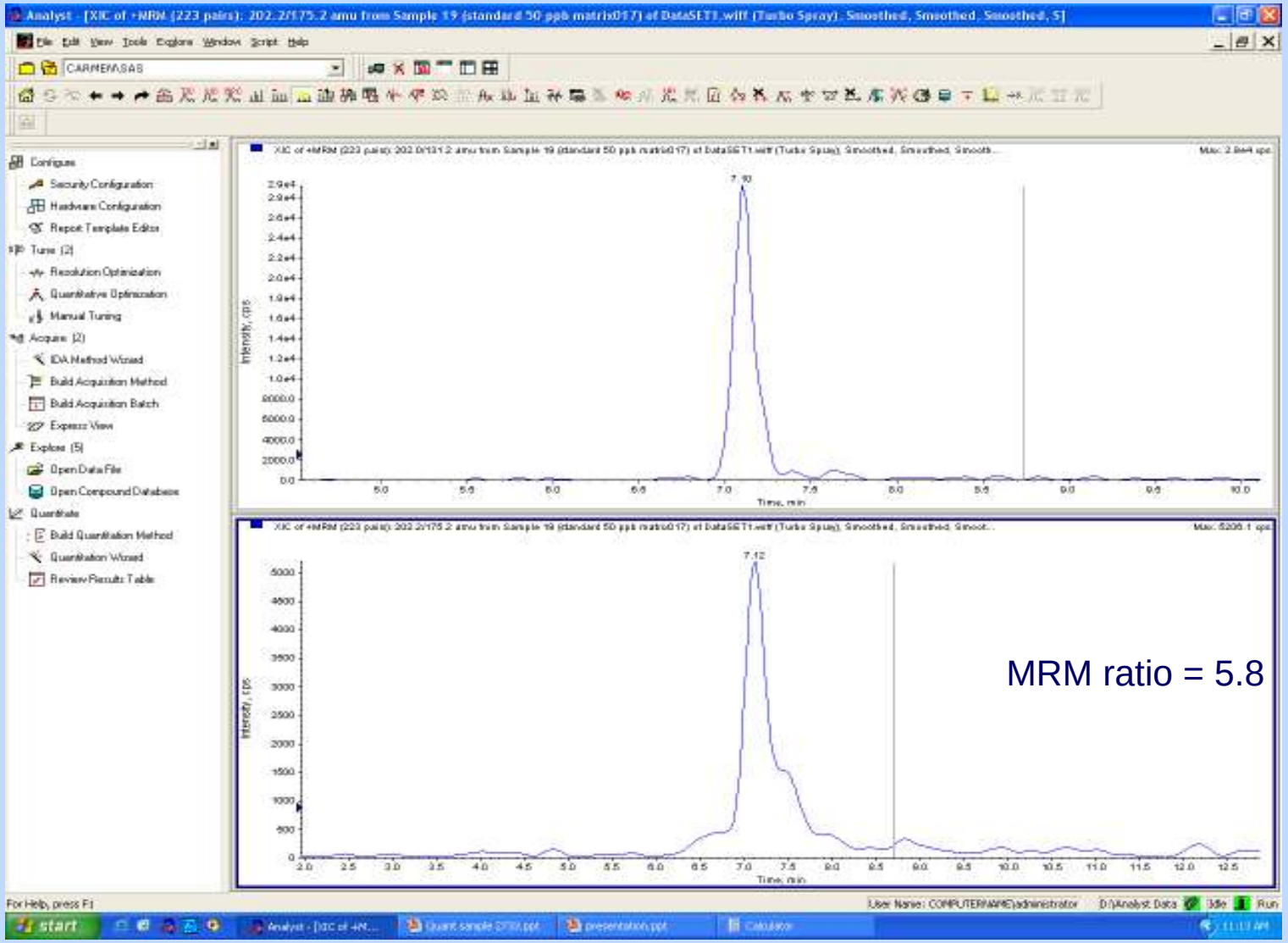

Analyst - [XIC of +MRM (223 pairs): 218.3/188.2 amu from Sample 22 (ethoxyquin 100ppb2 020) of DataSET1.wiff (Turbo Spray), Smoothed, Smoothed, Smoothed, Smoo]
Intensity, cps
Time, min
Ethoxyquin, standard 100 ppb


Analyst - [XIC of +MRM (223 pairs): 218.3/188.2 amu from Sample 23 (standard chusa ethoxyquin 30 ppb021) of DataSET1.wiff (Turbo Spray), Smoothed, Smoothed, 5]
Ethoxyquin, standard 100 ppb

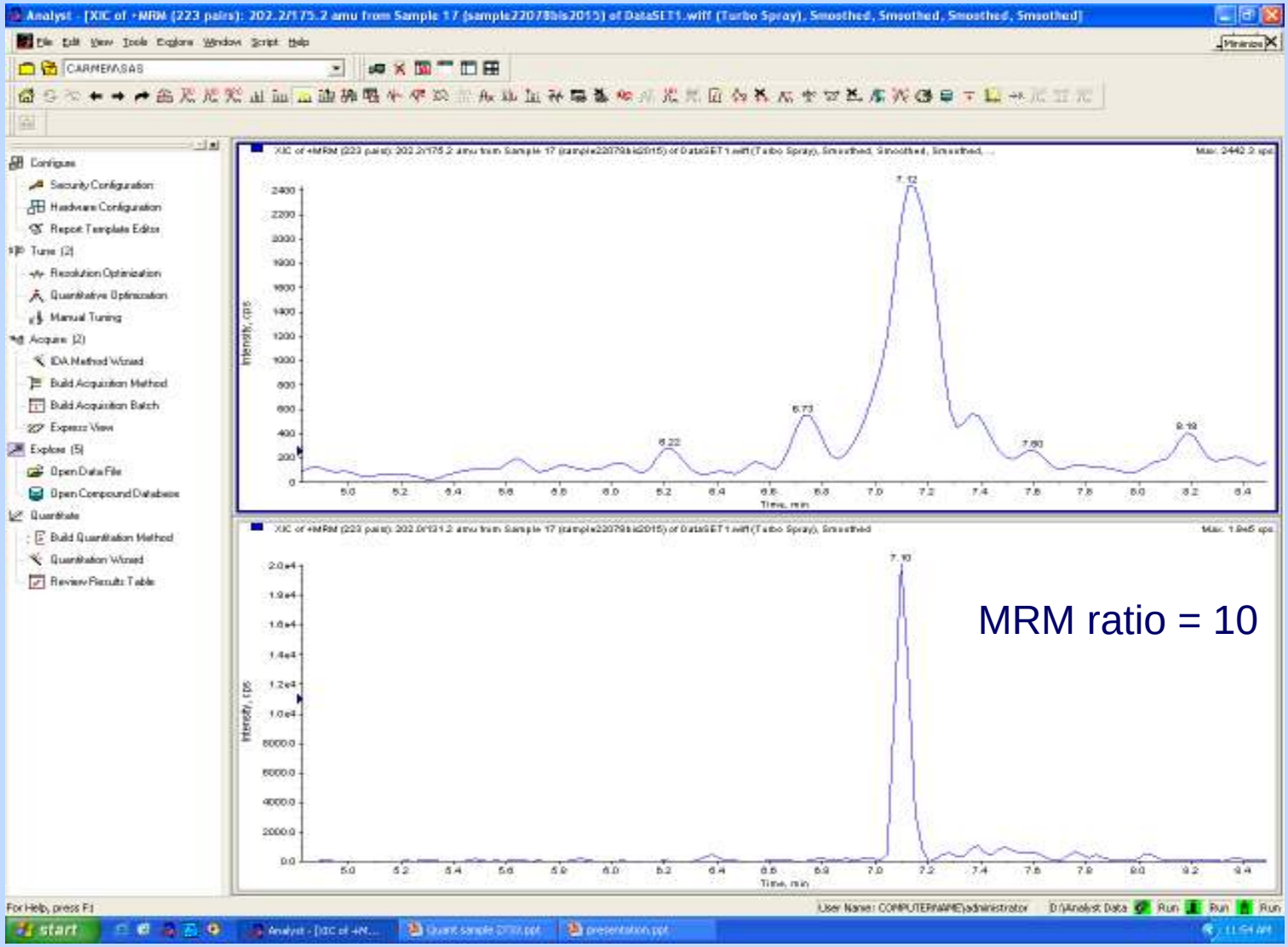
Thiabendazole, MRM optimization



Thiabendazole, matrix



Thiabendazole, pear Sample

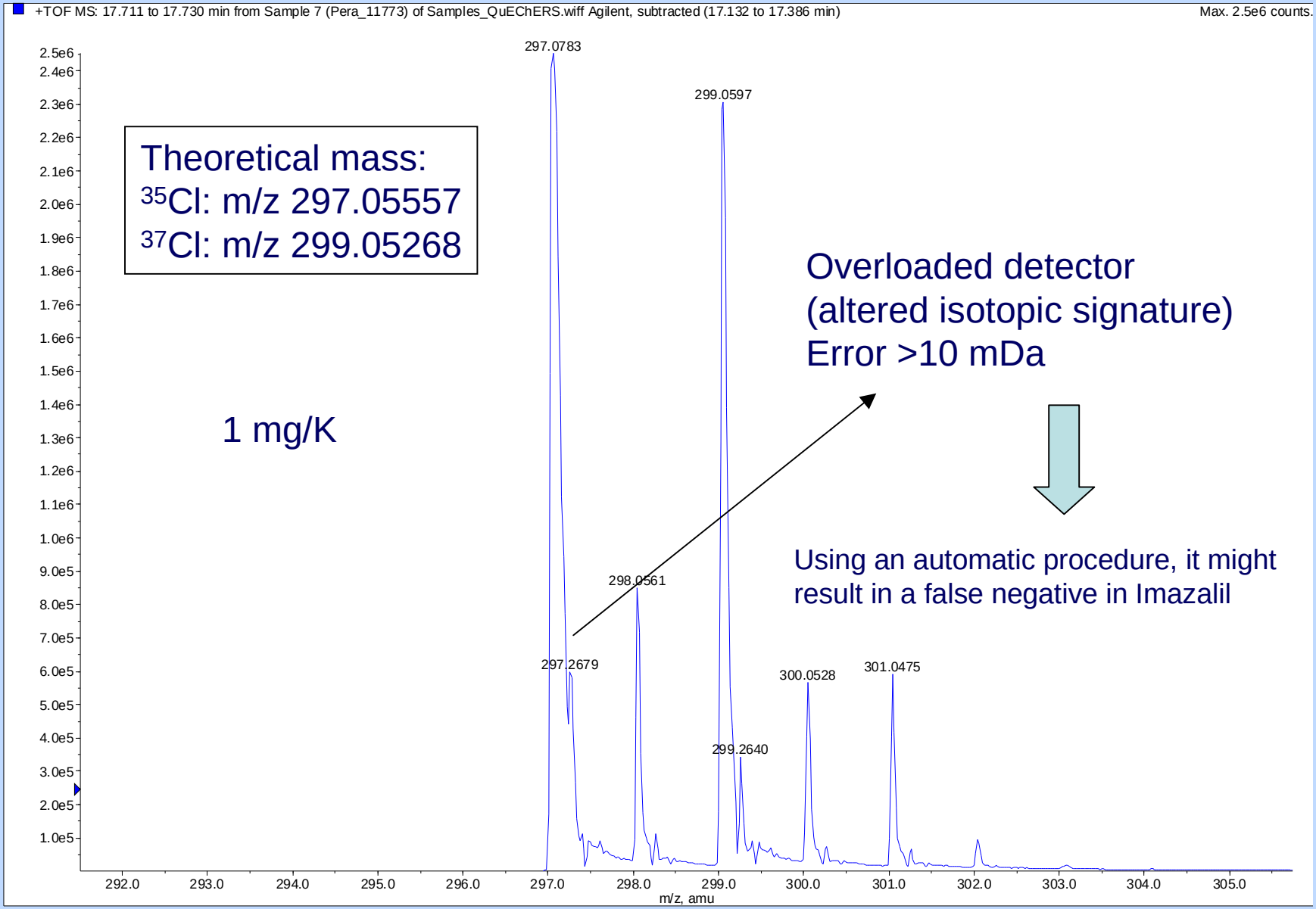


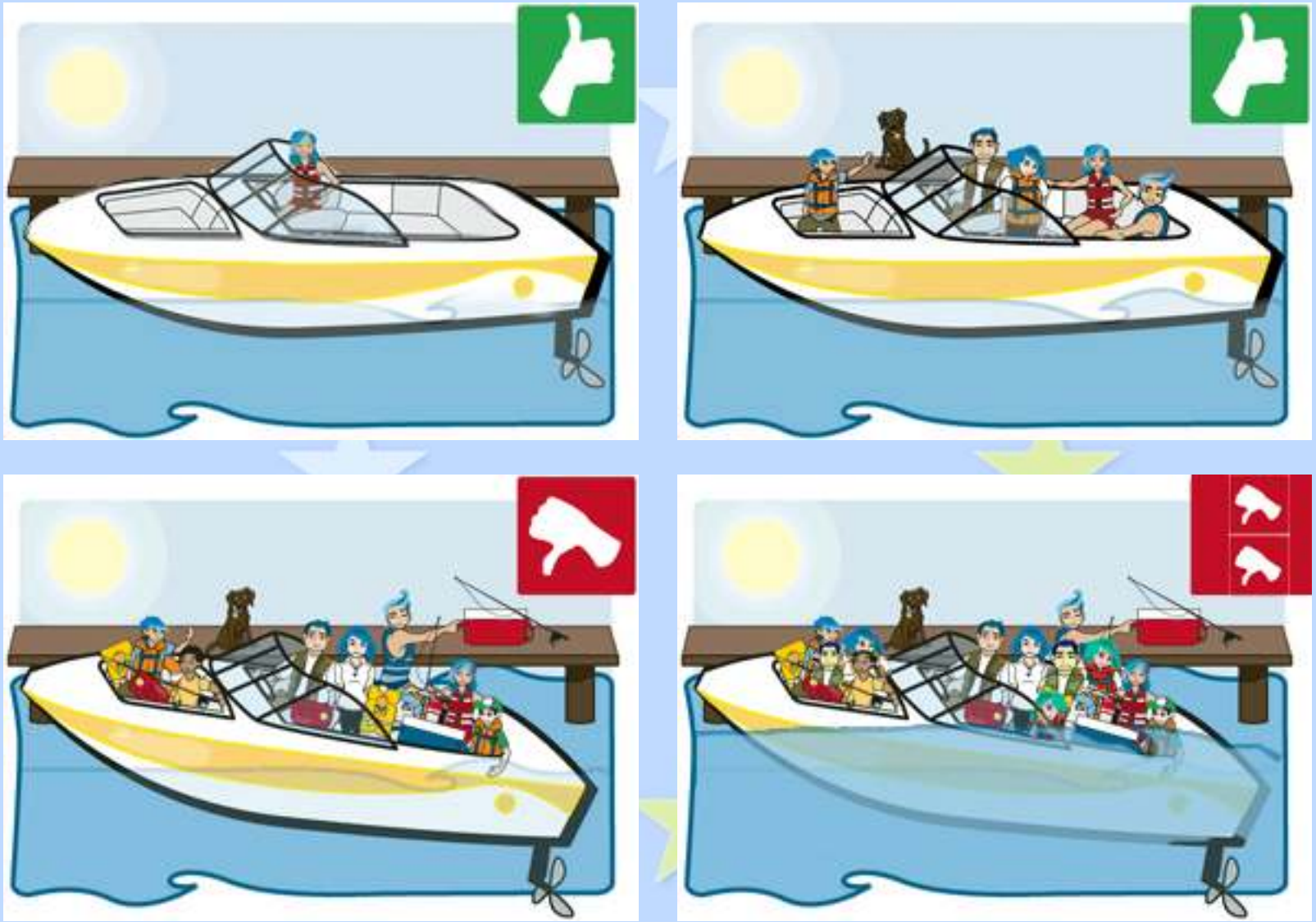
99 pesticides, Rt and MRM transitions

Pesticides	Rt	MRM1	MRM2	Pesticides	Rt	MRM1	MRM2
Prometrym	19.8	242→200	242→158	Diuron	20.7	233→72	233→160
Parathion	26.7	292→236	292→123	Imazalil	17.7	297→159	297→109
Spiromesifen	31.3	371→273	371→255	Imidacloprid	15.3	256→209	256→175
Promecarb	23.8	208→151	208→109	Methomyl	211.8	163→88	163→106
Metolcarb	19.0	166→109	166→94	Dimetomorph	20.7	388→301	388→165
Monuron	18.6	199→72	199→126	Buprofezin	27.0	306→201	306→116
Fenuron	19.0	166→109	166→72	Acetamiprid	16.1	223→126	223→56
Chlorfenvinphos	25.9	361→155	361→127	Thiacloprid	17.6	253→126	253→99
Thiocyclam	4.7	182→137	182→73	Difenoxuron	21.0	287→72	287→123
Methidation	23.5	302.9→145	302.9→84.9	Benalaxyl	26.3	326→208	326→148
Difenoconazole	25.8	406→251	406→337	Terbutilazina	23.1	230→174	230→146
Thiabendazole	8.4	202→131	202→175	Alachlor	15.5	270→162	270→147
Azoxystrobin	23.7	404→372	404→344	Metolachlor	25.3	284→176	284→252
Metiocarb	23.1	226→169	226→121	Isoproturon	20.9	207→72	207→165
Triflumizole	25.3	346→278	346→73	Triclocarban	26.9	315→127	315→162
Spynosyn A	20.6	732→142	732→189	Diphenylsulfone	22.2	219→141	219→77
Spynosyn D	21.2	746→189	746→142	Fenbendazole	19.9	300→268	300→268
Diflubenzuron	24.7	311→158	331→141	Malathion	25.3	331→127	331→285
Aldicarb	18.0	213→89	213→116	Iprodione	27.0	330→101	330→245
Flufenoxuron	29.0	489→158	489→141	Chloroxuron	23.2	291→72	291→218
Hexaflumuron	26.9	461→158	461→141	Aldicarb sulfoxide	6.4	207→89	207→132
Lufenuron	28.3	511→158	511→141	Lenacil	18.9	235→153	235→136
Prochloraz	22.5	376→308	376→308	Flumeturon	20.7	233→72	233→213
Ethiophencarb	21.2	226→107	226→164	Chlorbromuron	23.8	293→204	293→182
Carbofuran	20.2	222→165	222→123	Bromuconazole	23.0	376→159	376→70
Vinclozolin	25.3	286→214	286→179	Tebuconazole	24.2	308→70	308→125

99 pesticides, Rt and MRM transitions

Pesticides	Rt	MRM1	MRM2	Pesticides	Rt	MRM1	MRM2
Chloridazon	15.1	222→70	222→77	Chlortoluron	20.2	213→72	213→140
Bromacil	18.3	261→205	261→187	Spiroxamine	19.5	298→144	298→100
Dichlofluanid	26.4	333→224	333→123	Flazasulfuron	22.0	408→182	408→226
Albendazol	17.6	266→234	266→191	Teflubenzuron	27.3	381→158	381→141
Oxfendazol	15.3	316→159	316→191	Thiametoxam	13.5	292→211	292→132
Butoxicarboxim	10.3	223→106	223→63	Triflumuron	26.2	359→156	359→139
Metobromuron	21.7	259→170	259→148	Carbendazim	6.8	192→160	192→132
Propazine	22.7	230→146	230→188	Oxamilo	10.8	220→90	220→72
Fluroxypyr	18.6	255→237	255→209	Mebendazole	17.8	296→264	296→105
Butocarboxin	17.3	213→75	213→156	Monolinuron	21.3	215→126	215→148
Neburon	25.6	275→88	275→57	Terbuthrin	19.8	242→186	242→72
Metamitron	14.4	203→104	203→175	Carbaryl	20.6	202→145	202→127
Methiocarb sulfone	17.0	258→122	258→201	Ethoxyquin	13.6	218→160	218→146
Methiocarb sulfoxide	14.1	242→185	242→170	Monocrotophos	11.8	224→127	224→98
Cyproconazole	22.9	292→125	292→70	Desethylterbuthylazin	19.1	202→146	202→104
Miconazole	20.8	415→159	415→227	Simazina	18.5	202→132	202→124
Fenamiphos	23.5	304→276	304→217	Atrazina	20.7	216→174	216→104
Metalaxyl	20.8	280→220	280→192	Diazinon	27.2	305→169	305→153
Oxadixyl	18.5	279→219	279→102	Dimethoate	15.9	230→199	230→125
Nytempyram	11.4	271→225	271→99	Cymoxanil	17.1	199→128	199→111
Linuron	23.4	249→160	249→182	Bendiocarb	12.0	224→167	224→109
Cambendazol	14.5	303→217	303→261	Pyrimethamyl	11.3	200→77	200→67
Aldicarb sulfone	11.1	223→86	223→148	Cyromacine	3.1	167→85	167→108
Pyriproxyphen	28.9	322→96	322→227				


+TOF MS: 17.711 to 17.730 min from Sample 7 (Pera_11773) of Samples_QuEChERS.wiff Agilent, subtracted (17.132 to 17.386 min)
Max. 2.5e6 counts.
297.0783
299.0597
298.0561
297.2679
300.0528
301.0475
299.2640
m/z, amu
Theoretical mass:
35Cl: m/z 297.05557
37Cl: m/z 299.05268
1 mg/K
Overloaded detector
(altered isotopic signature)
Error >10 mDa
Using an automatic procedure, it might result in a false negative in Imazalil

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