ACCURATE-MASS DATABASE FOR SCREENING OF PESTICIDE RESIDUES IN FRUITS AND VEGETABLES BY GAS CHROMATOGRAPHY TIME-OF-FLIGHT MASS SPECTROMETRY

European Commission

EURL-FV

MILAGROS MEZCUA

In practice, target analysis approach

(analysing 100-400 of GC and LC amenable compounds)

MEANS THAT THE MAJORITY OF THE LOW-FREQUENCY OR MISUSED COMPOUNDS ARE NOT LOOKED FOR

Target methods

Target methods

Very Expensive

Target methods

- Very Expensive

- Time Consuming

Screening methods

- ✓ Practically all applications have been focused on LC (Time of flight)
- ✓ LC-TOF-MS (libraries containing fragments of compounds and retention time).
- ✓ Focused in GC (single quadrupole), commercial libraries.

GC-TOF/MS (full scan)

(Ability to record an unlimited number of compounds)

- ✓ GC libraries (accurate masses of molecular ions and fragments and retention times)

Experimental conditions

Injection volume: 2 µL

Mode: Splitless MM Inlet

Inyector temperature: 280ºC

Agilent 7200 Q-TOF GC/MS

OVEN PROGRAM

Rate(ºC/min)	Value(ºC)	Hold Time(min)	Run Time(min)
	60	1	1
40	120	0	2.5
5	310	0	40.5

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OVEN PROGRAM

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5	310	0	40.5

Post Run Time: 2 min

Post Run temperature: 310ºC

BACKFLUSHING CAPABILITIES

Retention time locked with constant flow (Trifluralin)

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BACKFLUSHING CAPABILITIES

Retention time locked with constant flow (Trifluralin)

Electron Ionization
Acquisition in full scan at 2 GHz

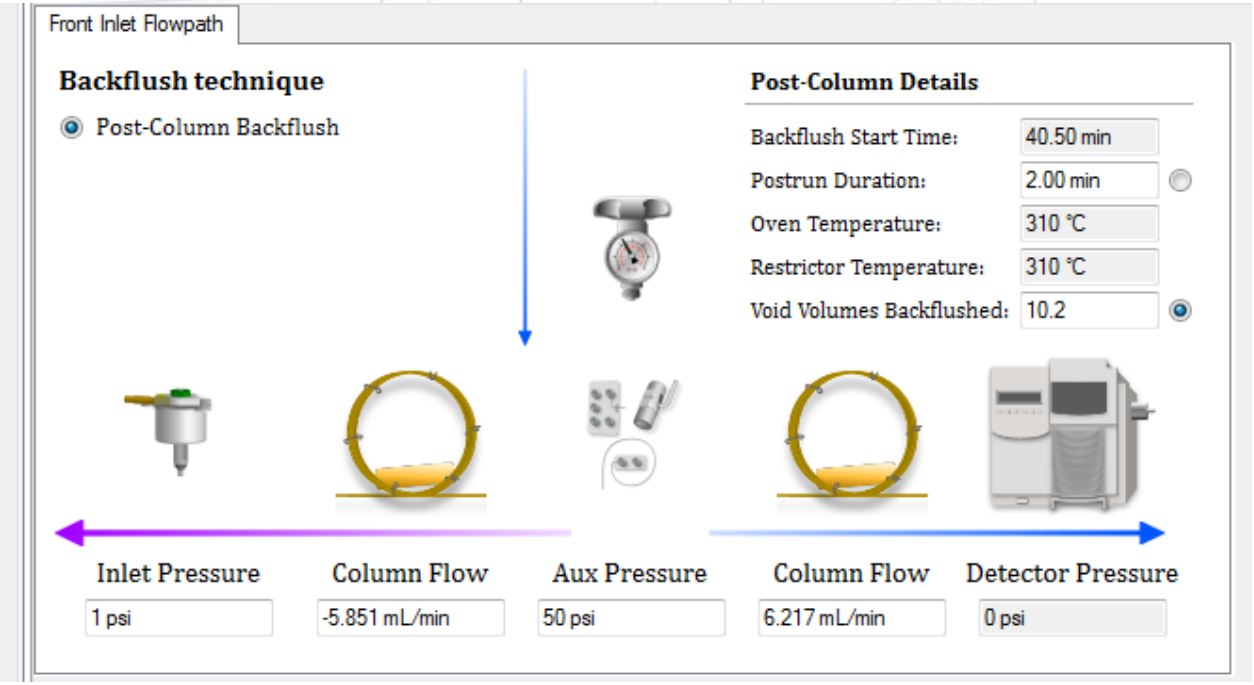
Ion source temperature: 280ºC

Transfer line temperature: 280ºC

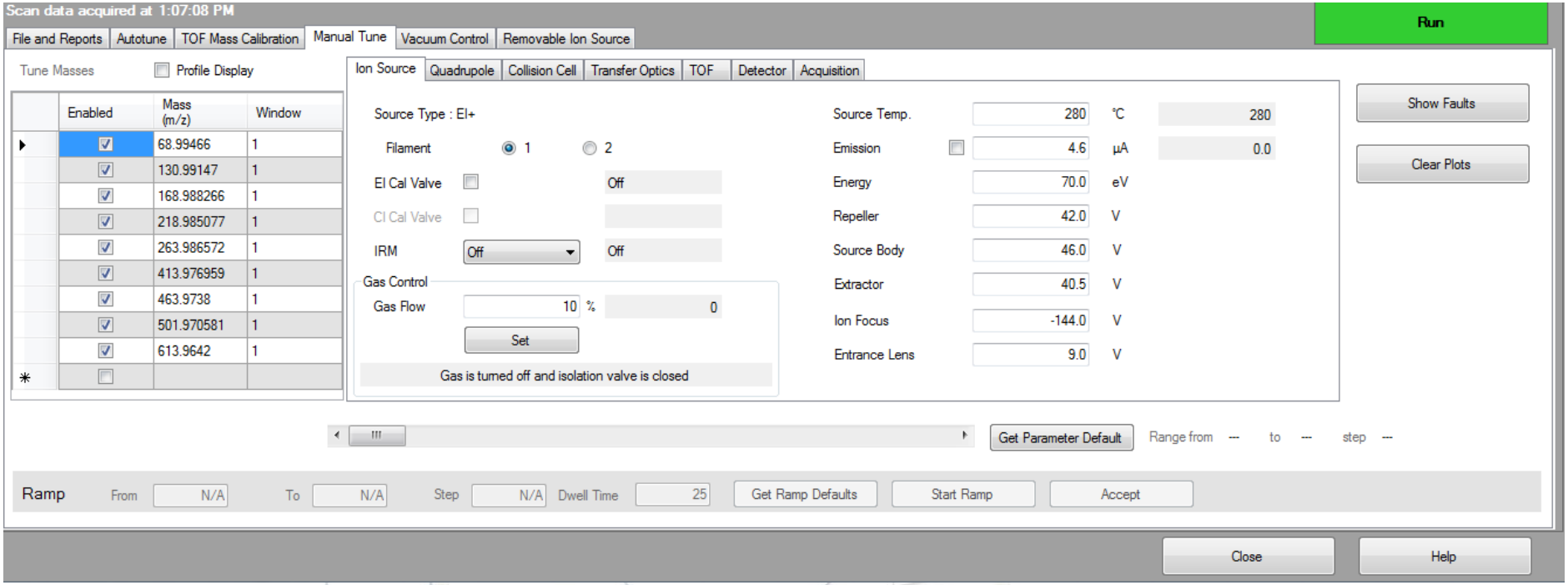
BACKFLUSH CONFIGURATION

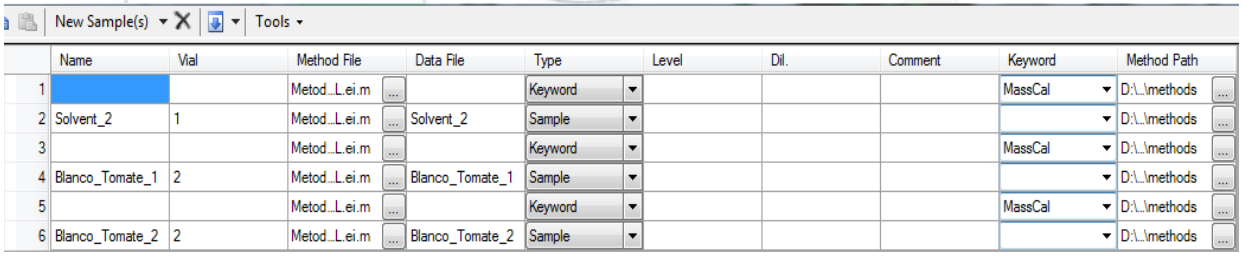
2 columns: HP5MSI (350ºC) 15mx250µmx0.25µm

Constant Flow
Column 1 = 1.225ml/min
Column 2= 1.425ml/min

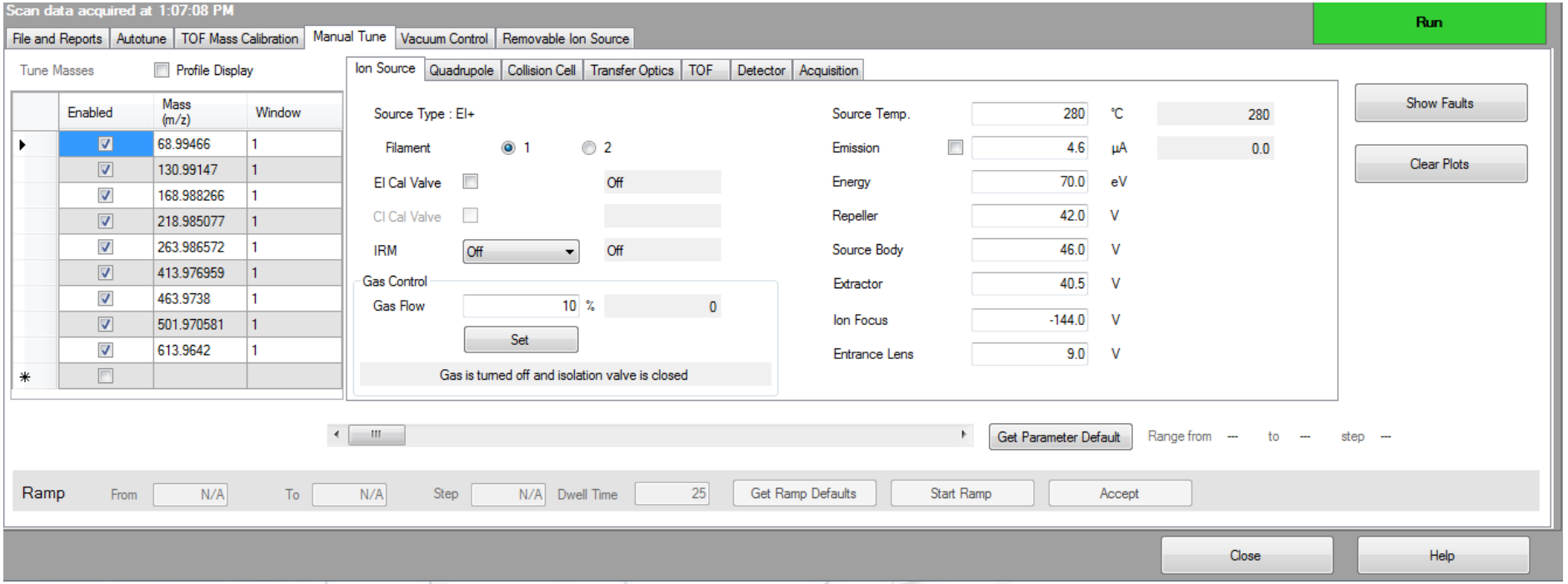


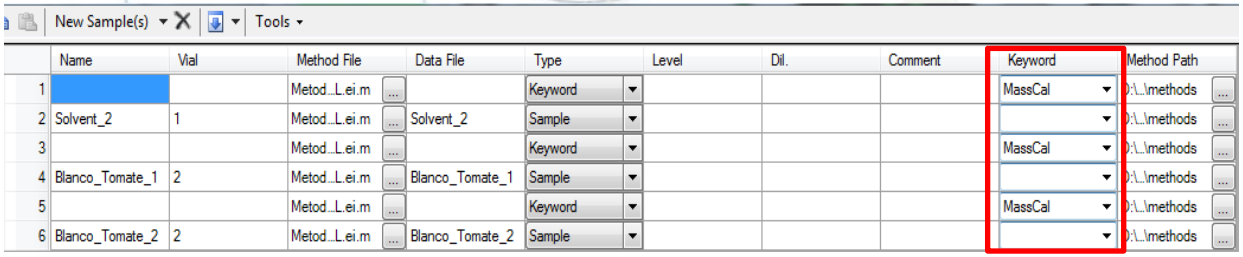
Internal Reference Mass





Internal Reference Mass

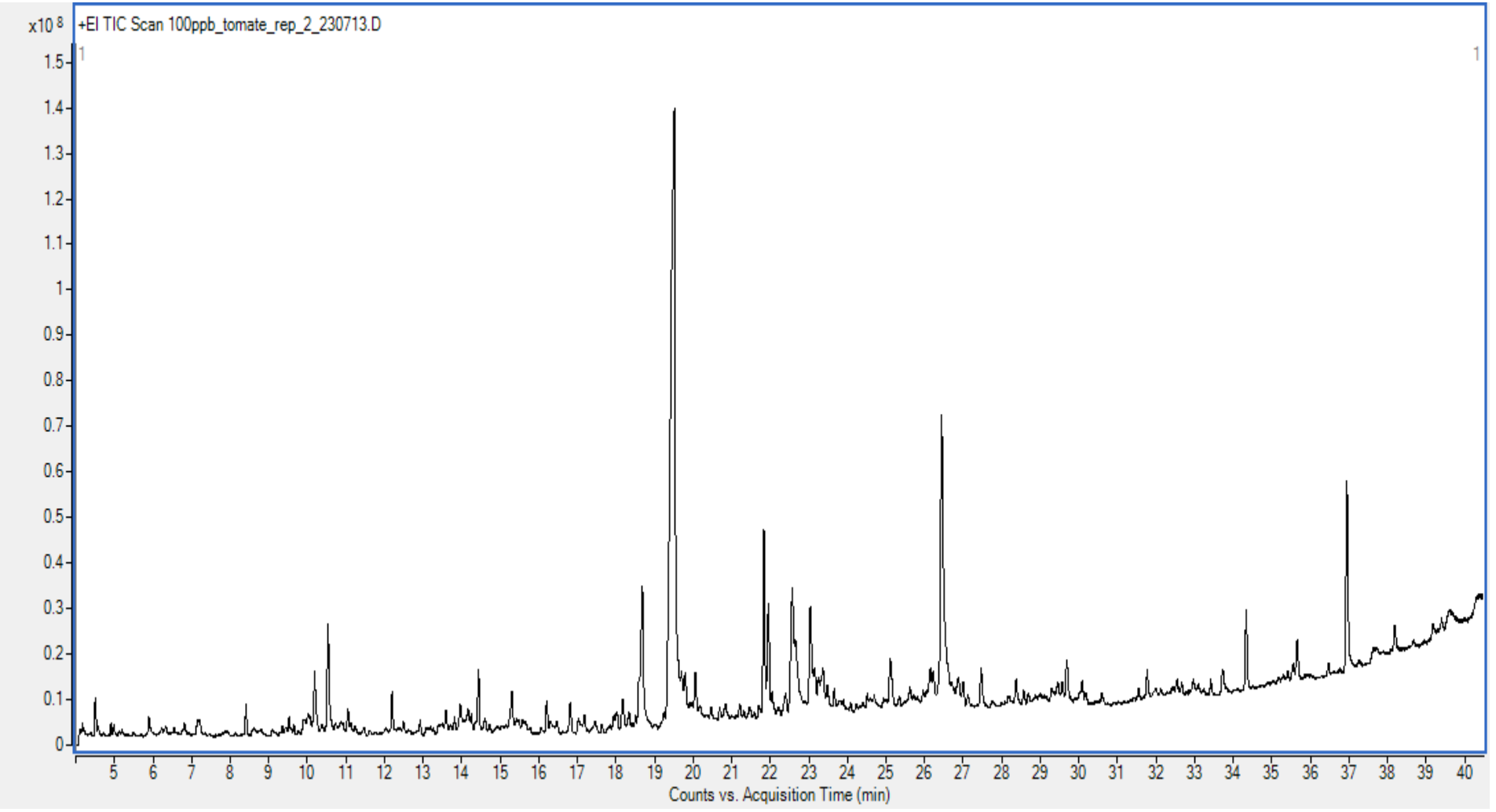




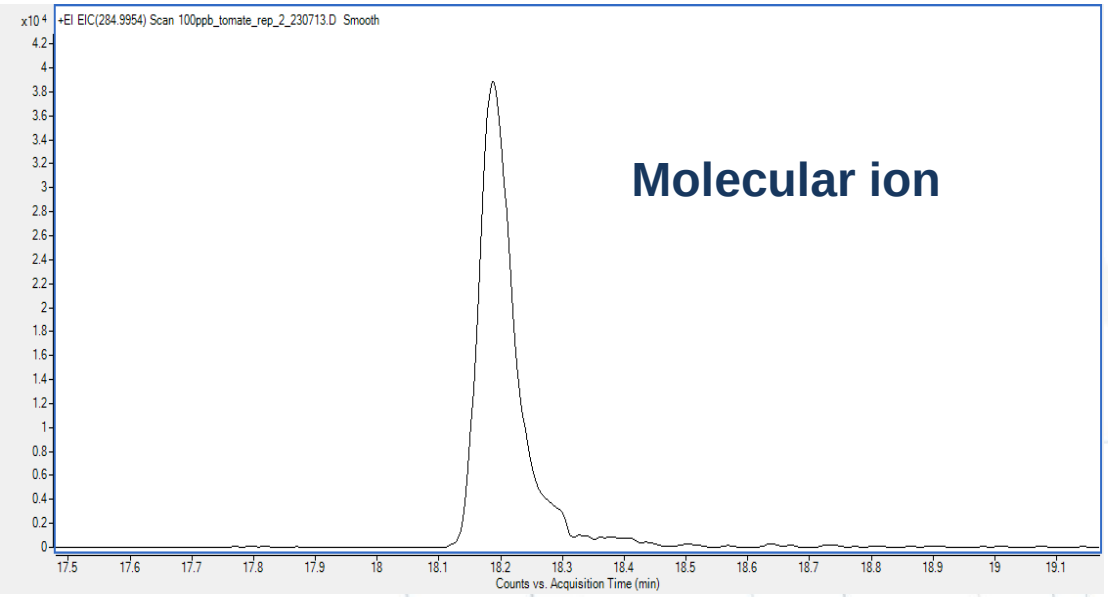
GC-Q-TOF DATABASE

- Many ions in the mass spectra.
- The molecular ion is not always present in the spectra.

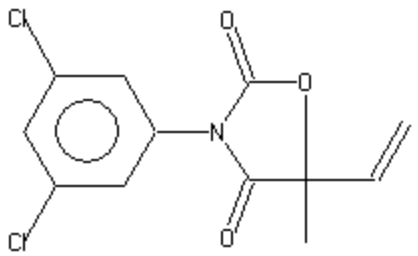
100ppb Tomate TIC



100ppb Tomate EIC (284.9954_Vinclozolin Parent Ion)



Molecular ion



Vinclozolin

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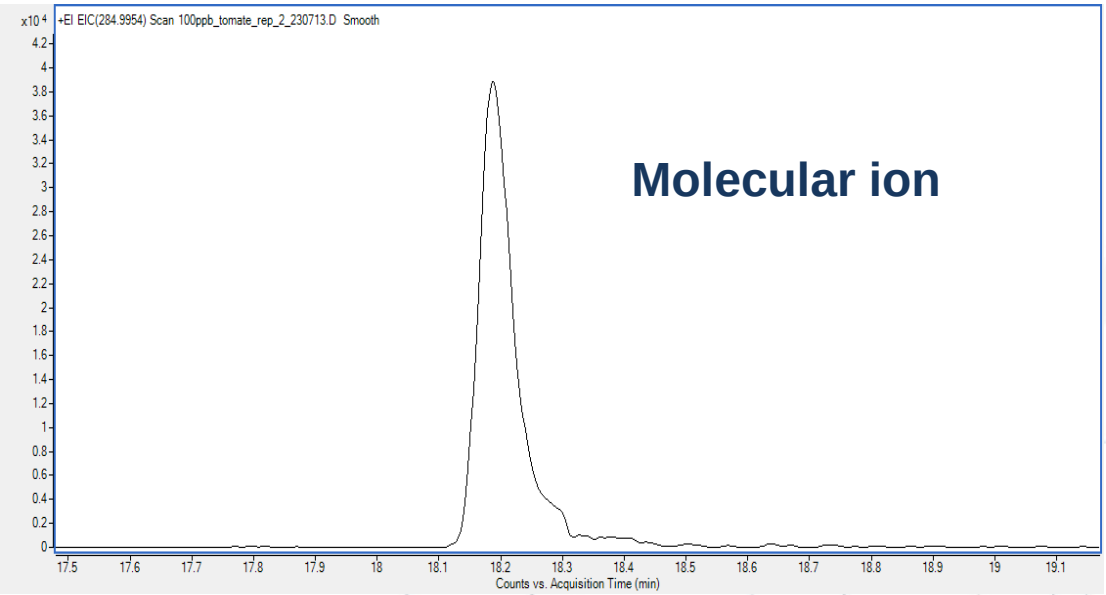
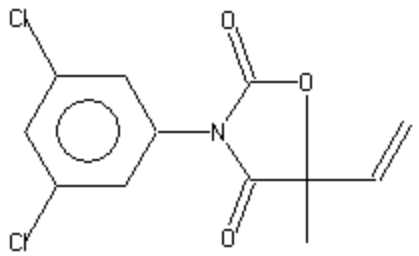
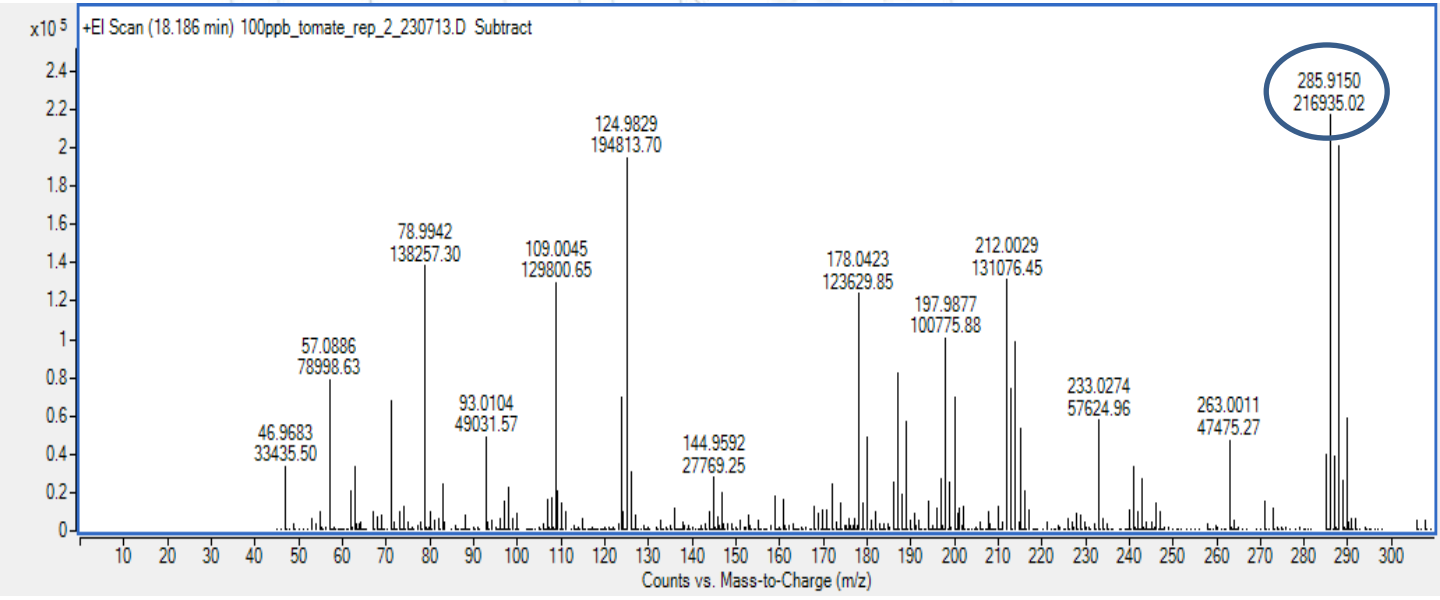


Molecular ion



Vinclozolin

100ppb Tomate Mass Spectrum (tr = 18.186 min)



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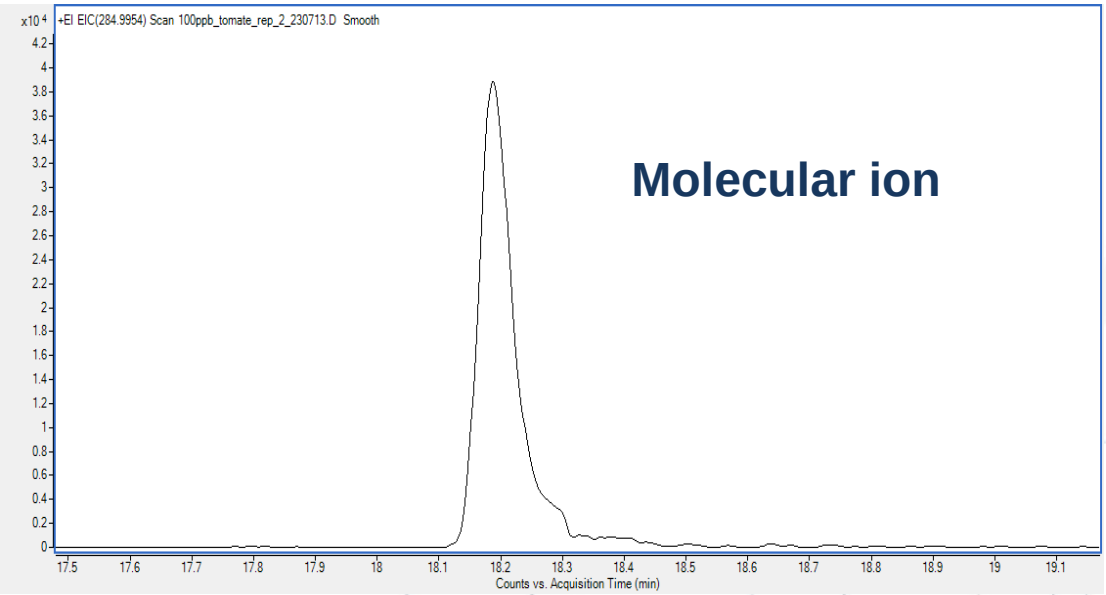
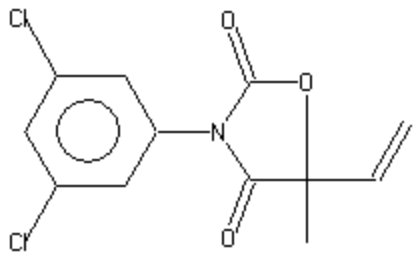


Molecular ion

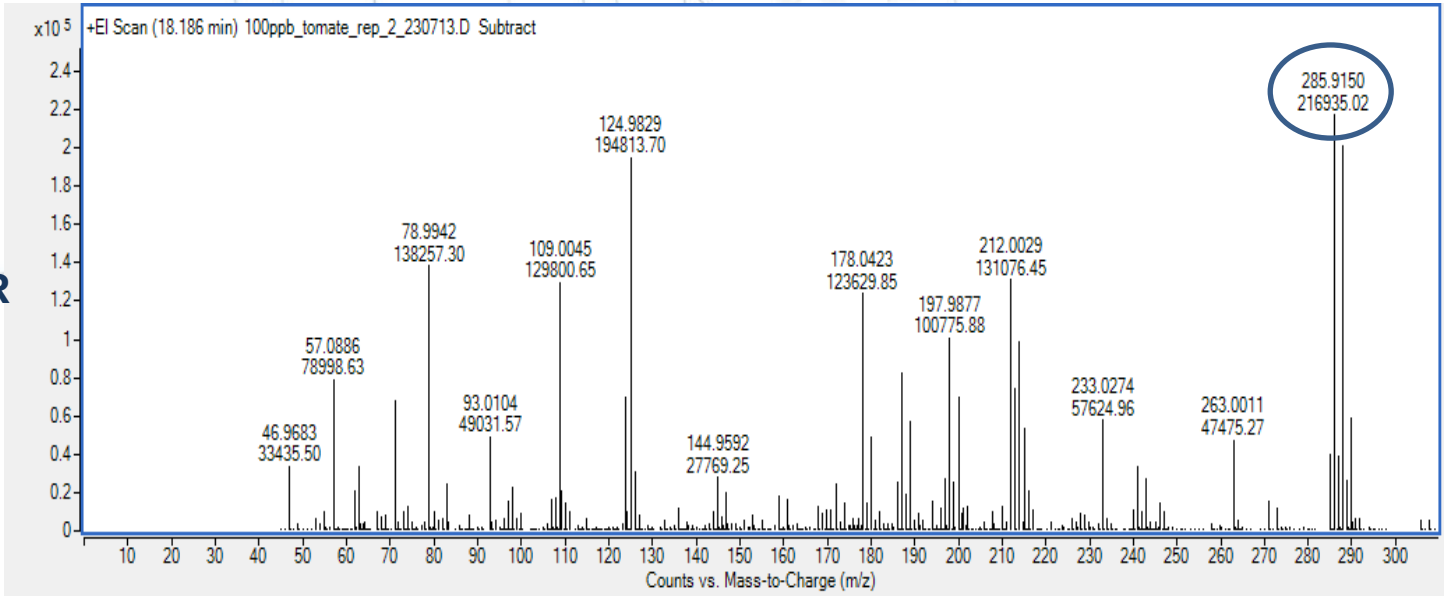


Vinclozolin

100ppb Tomate Mass Spectrum (tr = 18.186 min)

NIST

MASS INTERPRETER



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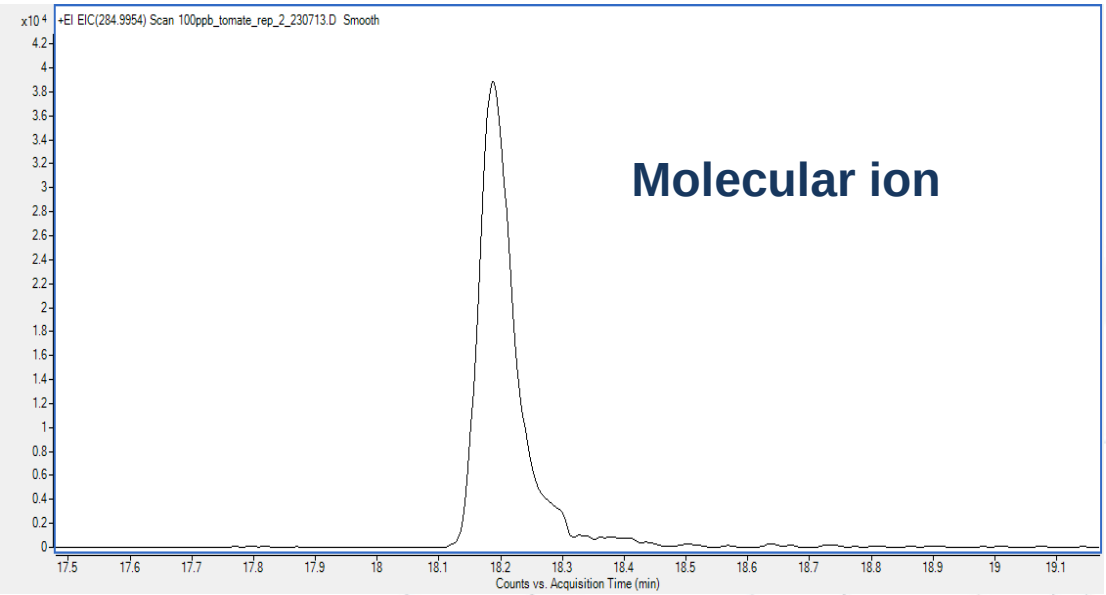
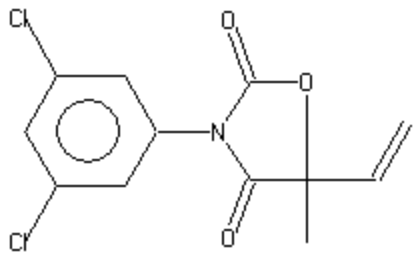


Molecular ion



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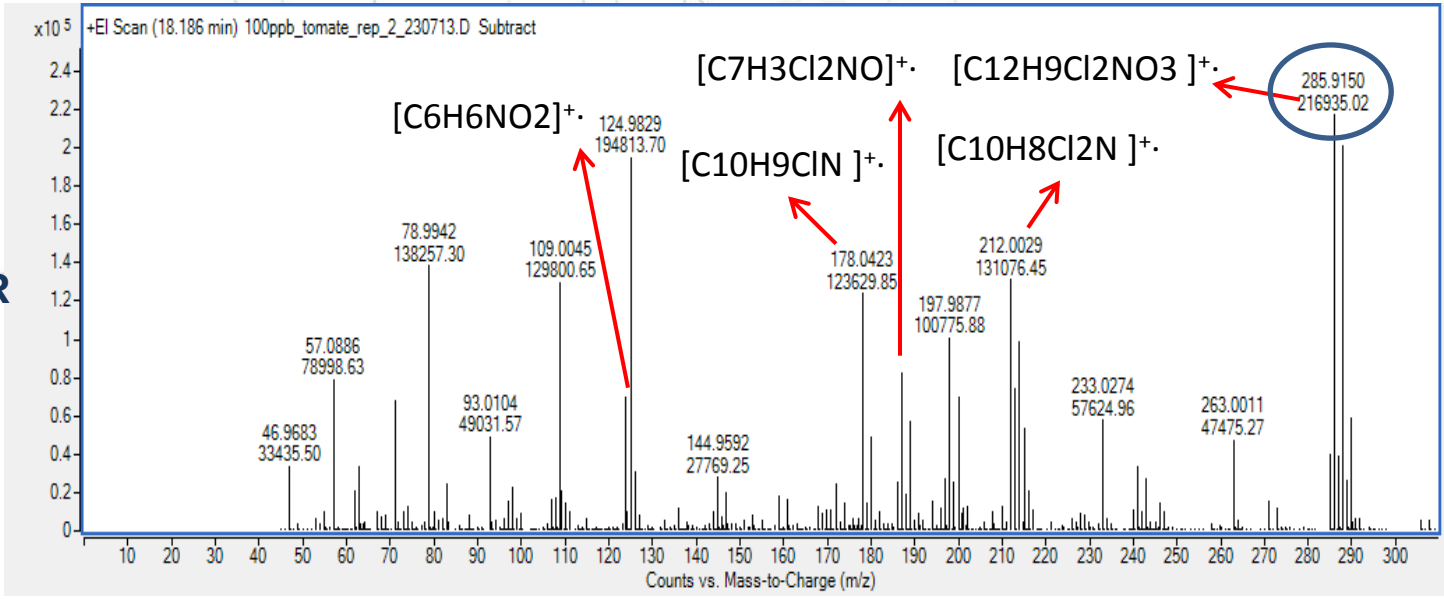
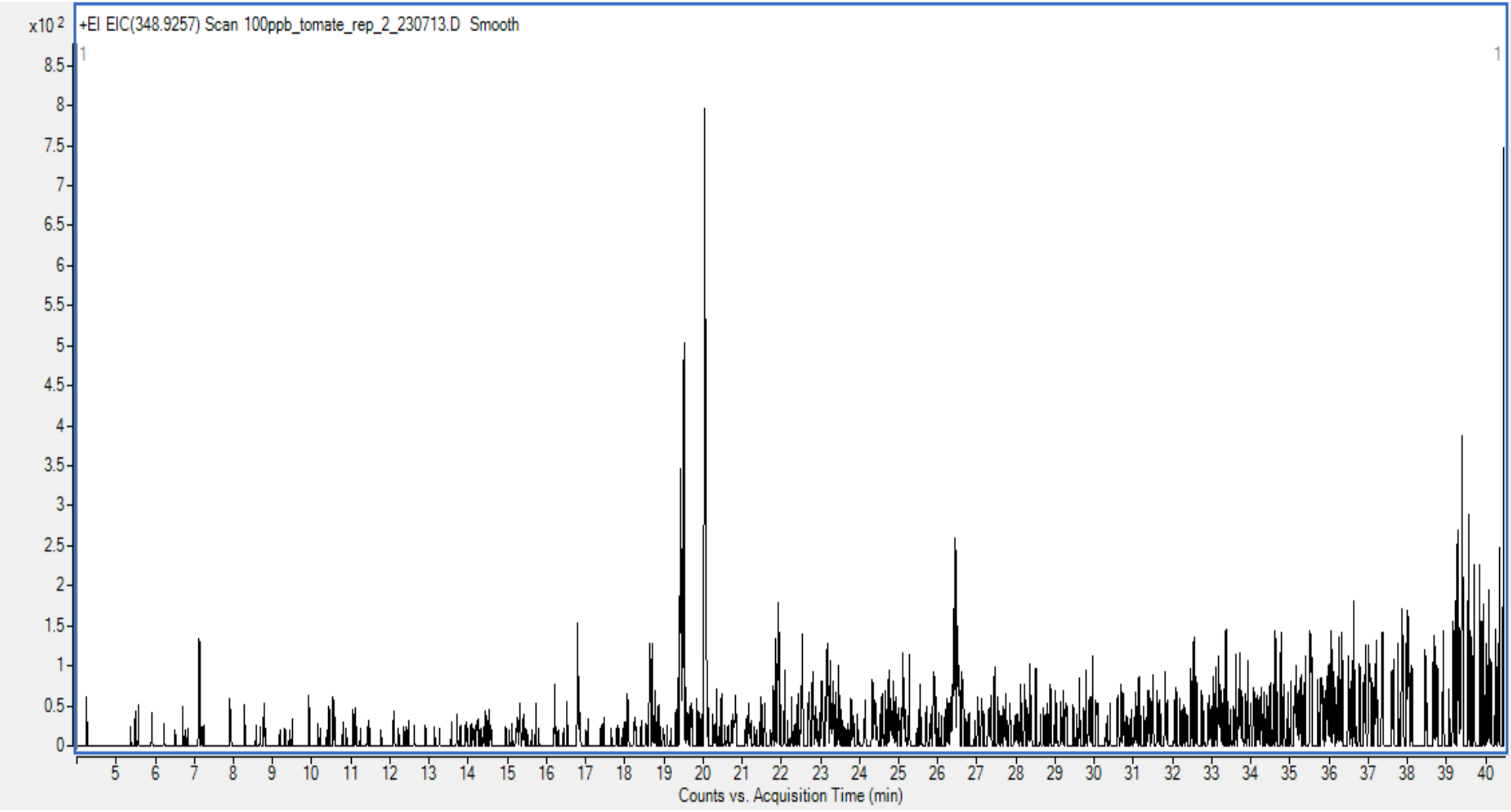
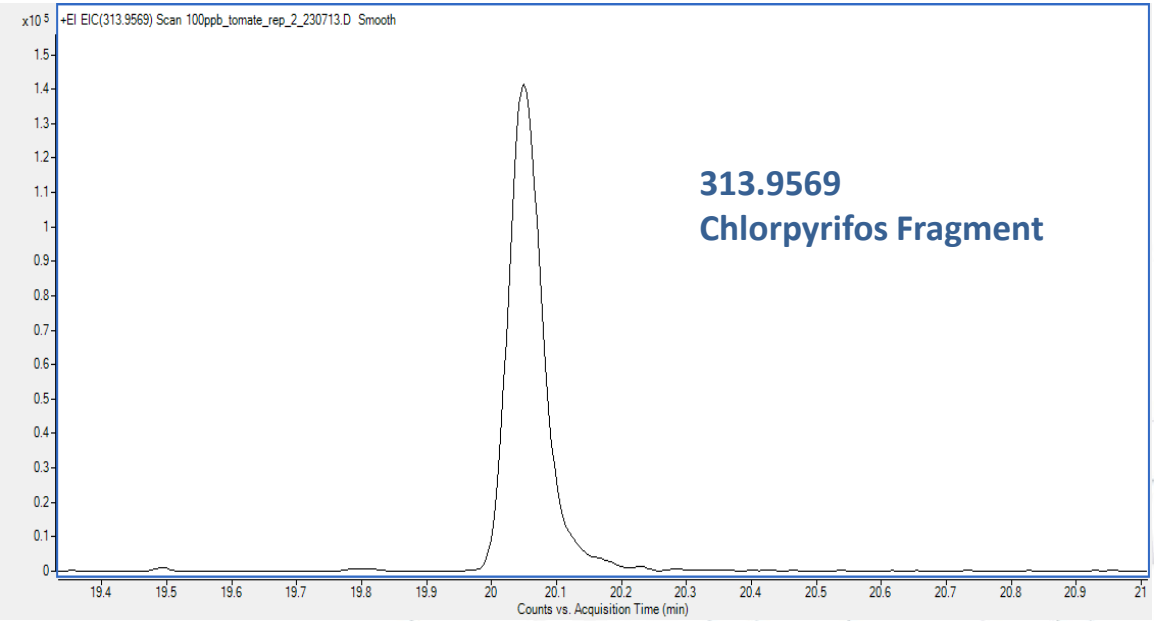


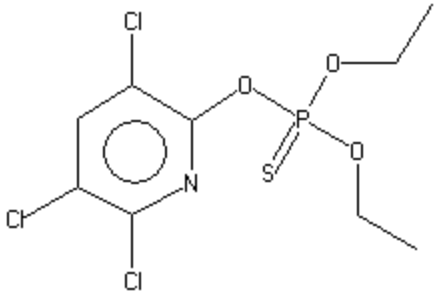
100ppb Tomate EIC (348.9257_Chlorpyrifos Parent Ion)



100ppb Tomate EIC (313.9569_Chlorpyrifos Fragment)

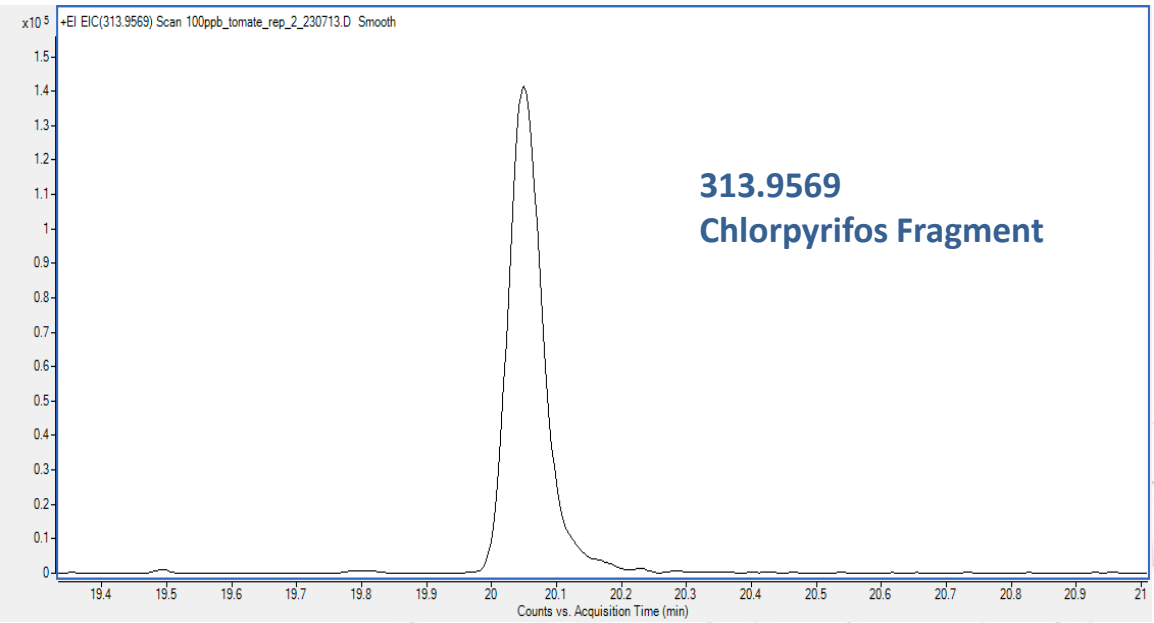


313.9569
Chlorpyrifos Fragment

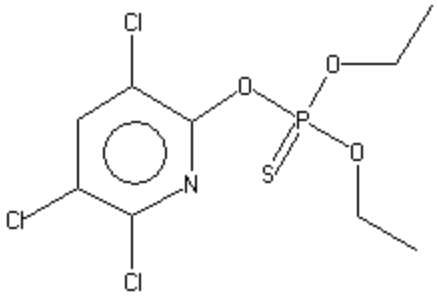


Chorpyrifos

100ppb Tomate EIC (313.9569_Chlorpyrifos Fragment)

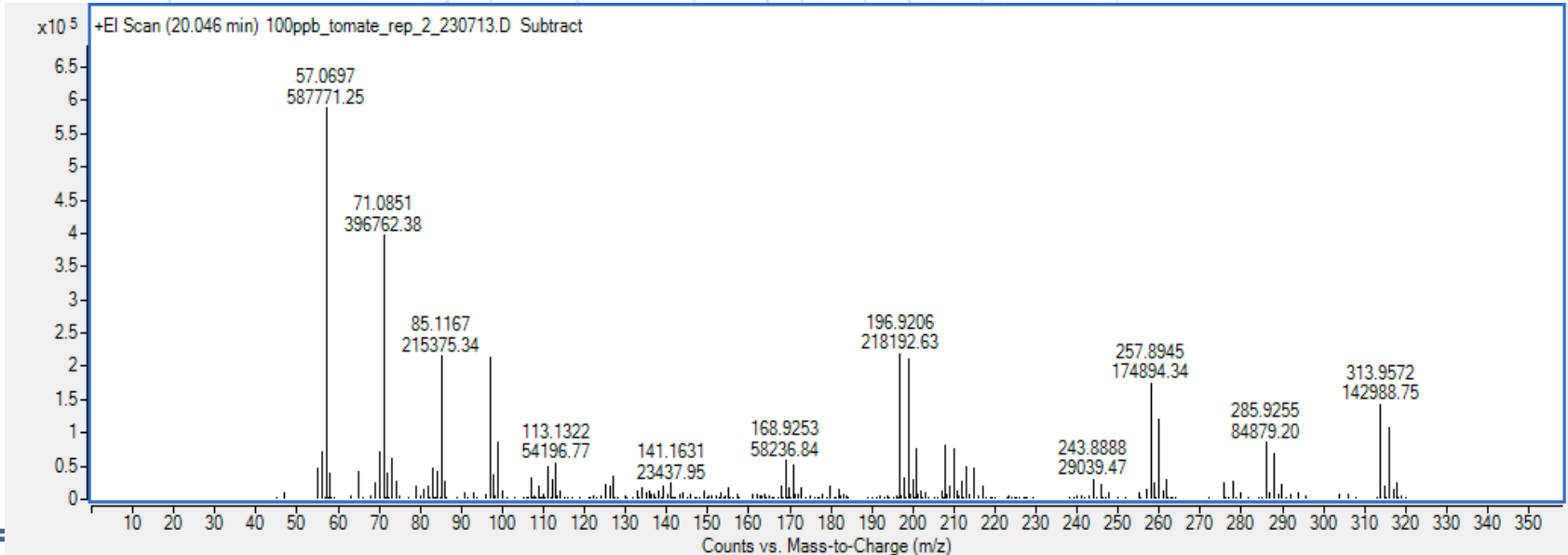


313.9569
Chlorpyrifos Fragment

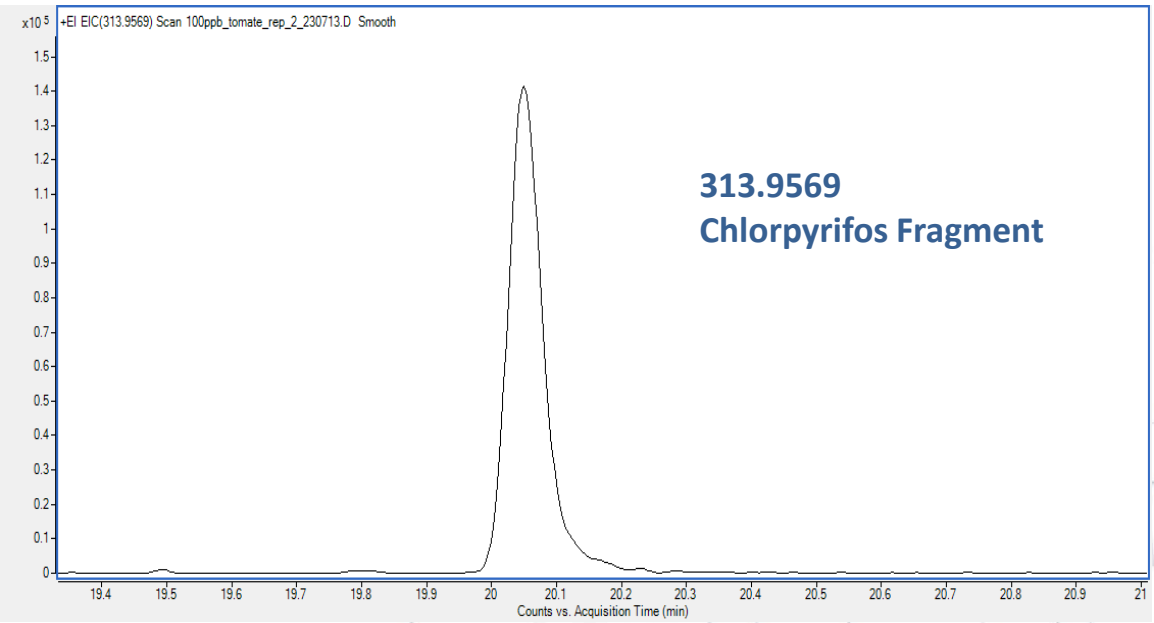


Chorpyrifos

100ppb Tomate Mass Spectrum (tr = 20.046 min)



100ppb Tomate EIC (313.9569_Chlorpyrifos Fragment)



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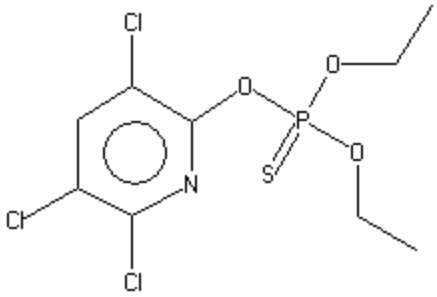
Chlorpyrifos Fragment



Chorpyrifos

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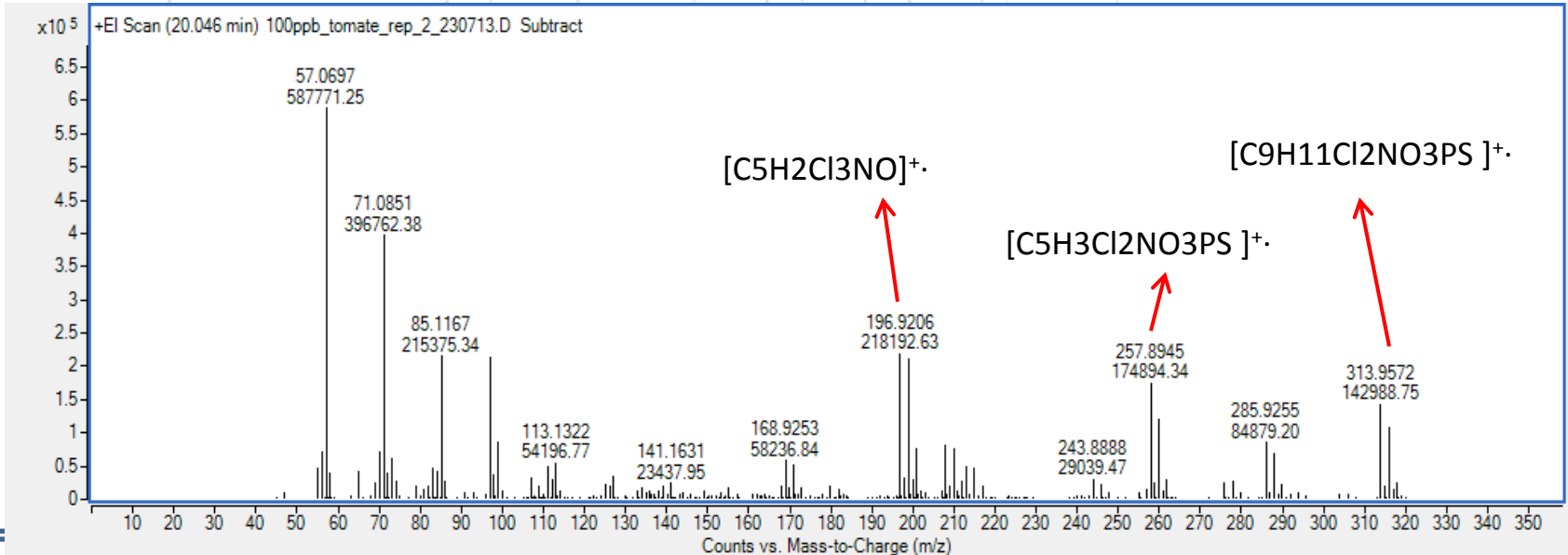

ACCURATE MASS DATABASE

### Formula	Retention Time	Mass	Compound name
# Formula	RT	Mass	Cpd
C17H16Br2O3	28,12	425,9466	Bromopropylate
C13H9Br2O	28,12	338,9020	Bromopropylate F1
C7H6BrO	28,12	184,9602	Bromopropylate F2
C6H4Br	28,12	154,9496	Bromopropylate F3
C13H12BrCl2N3O	27,92	374,9541	Bromuconazole
C10H8BrCl2O	27,92	292,9136	Bromuconazole F1
C13H12Cl2N3O	27,92	296,0357	Bromuconazole F2
C10H9Cl2O	27,92	215,0030	Bromuconazole F3
C7H3Cl2O	27,92	172,9561	Bromuconazole F4
C8H7Cl2	27,92	172,9925	Bromuconazole F5
C13H24N4O3S	24,01	316,1569	Bupirimate
C11H18N3O3S	24,01	272,1069	Bupirimate F1
C11H18N3O	24,01	208,1450	Bupirimate F2
C5H4N2O	24,01	108,0324	Bupirimate F3
C16H23N3OS	23,85	305,1562	Buprofezin
C9H9N2S	23,85	177,0486	Buprofezin F1
C7H5NO	23,85	119,0371	Buprofezin F2
C7H8N	23,85	106,0657	Buprofezin F3
C7H11N2OS	23,85	171,0592	Buprofezin F4
C8H16N2S	23,85	172,1034	Buprofezin F5
Continue	….	….	….

ACCURATE MASS DATABASE

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C5H4N2O	24,01	108,0324	Bupirimate F3
C16H23N3OS	23,85	305,1562	Buprofezin
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C7H5NO	23,85	119,0371	Buprofezin F2
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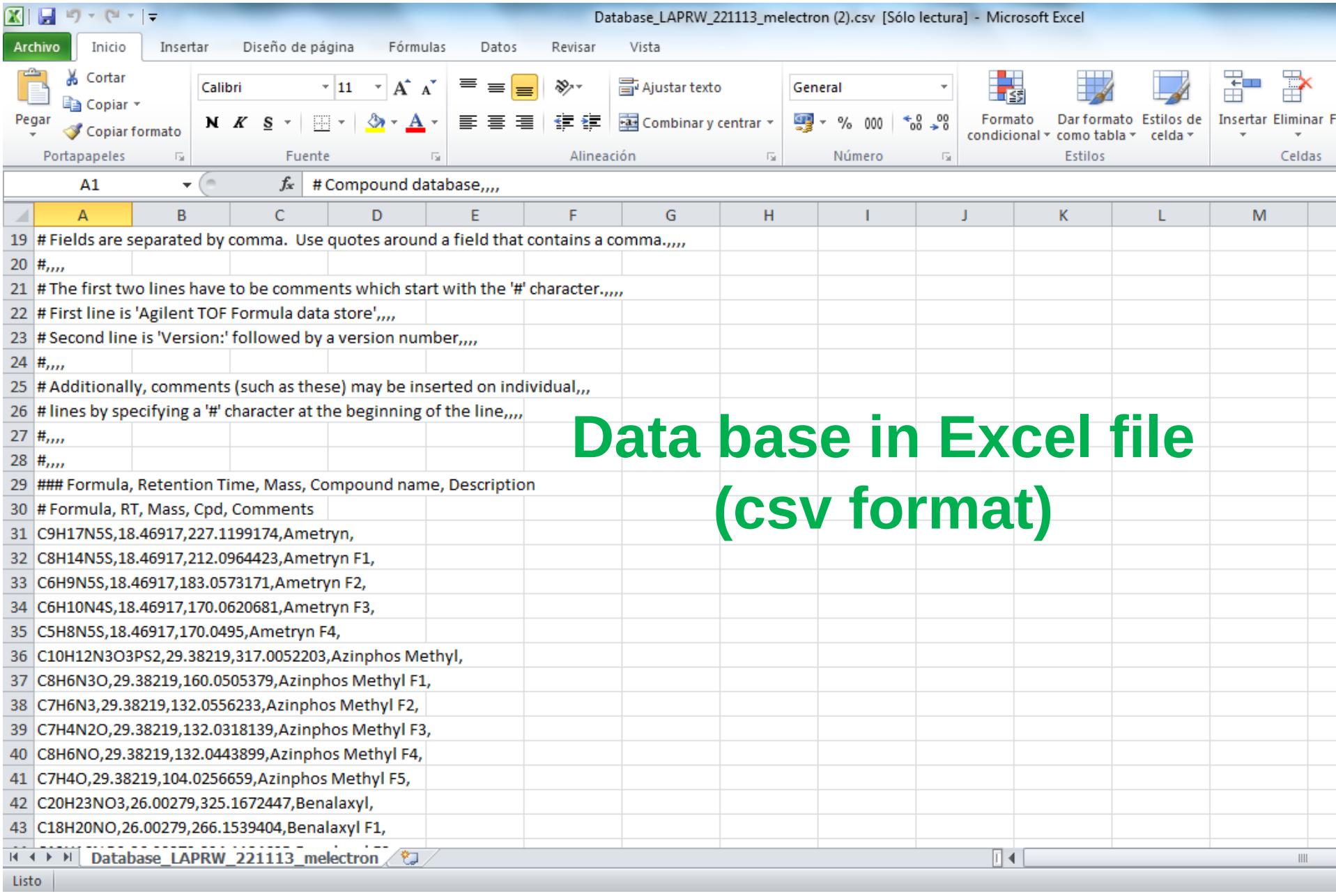
C7H11N2OS	23,85	171,0592	Buprofezin F4
C8H16N2S	23,85	172,1034	Buprofezin F5
Continue	....	....	....

87 PESTICIDES

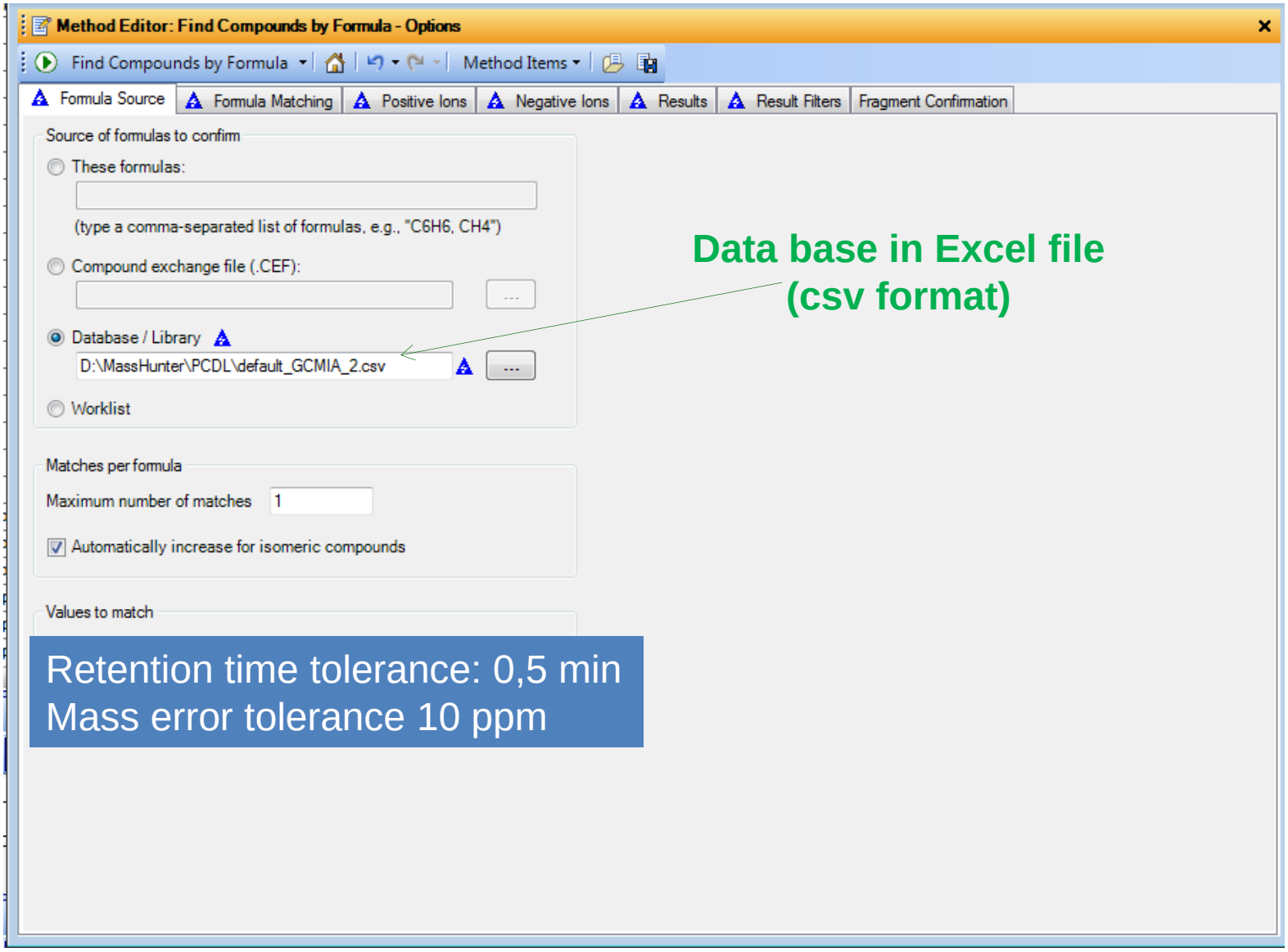
2 FRAGMENTS AT LEAST FOR EACH PESTICIDE

RETENTION TIMES OF EACH PESTICIDE



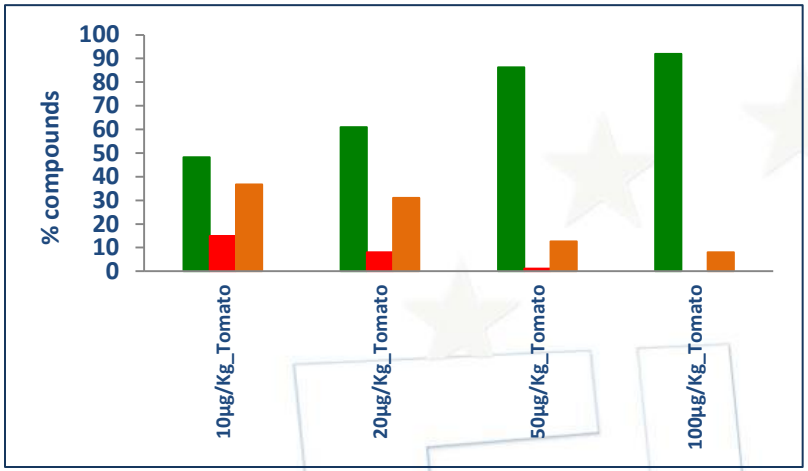
	A
19	# Fields are separated by comma. Use quotes around a field that contains a comma.,,,,
20	#,,,,
21	# The first two lines have to be comments which start with the '#' character.,,,,
22	# First line is 'Agilent TOF Formula data store',,,,
23	# Second line is 'Version:' followed by a version number,,,,
24	#,,,,
25	# Additionally, comments (such as these) may be inserted on individual,,,
26	# lines by specifying a '#' character at the beginning of the line,,,,
27	#,,,,
28	#,,,,
29	### Formula, Retention Time, Mass, Compound name, Description
30	# Formula, RT, Mass, Cpd, Comments
31	C9H17N5S,18.46917,227.1199174,Ametryn,
32	C8H14N5S,18.46917,212.0964423,Ametryn F1,
33	C6H9N5S,18.46917,183.0573171,Ametryn F2,
34	C6H10N4S,18.46917,170.0620681,Ametryn F3,
35	C5H8N5S,18.46917,170.0495,Ametryn F4,
36	C10H12N3O3PS2,29.38219,317.0052203,Azinphos Methyl,
37	C8H6N3O,29.38219,160.0505379,Azinphos Methyl F1,
38	C7H6N3,29.38219,132.0556233,Azinphos Methyl F2,
39	C7H4N2O,29.38219,132.0318139,Azinphos Methyl F3,
40	C8H6NO,29.38219,132.0443899,Azinphos Methyl F4,
41	C7H4O,29.38219,104.0256659,Azinphos Methyl F5,
42	C20H23NO3,26.00279,325.1672447,Benalaxyl,
43	C18H20NO,26.00279,266.1539404,Benalaxyl F1,

Data base in Excel file (csv format)

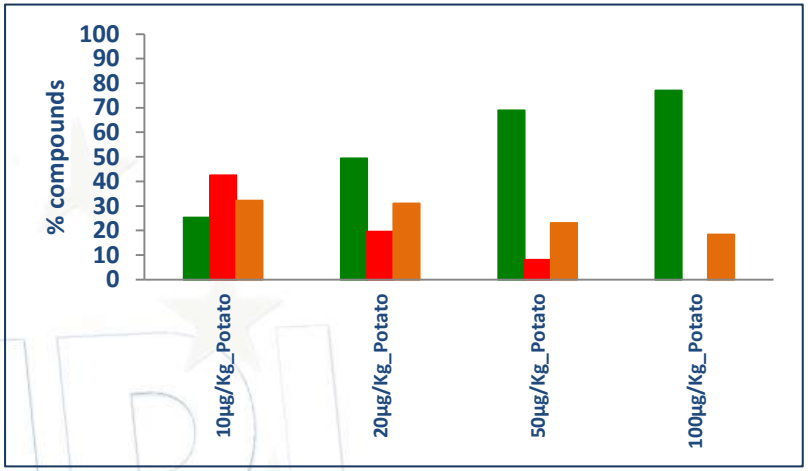

Method Editor: Find Compounds by Formula - Options
Find Compounds by Formula
Method Items
Formula Source
Formula Matching
Positive Ions
Negative Ions
Results
Result Filters
Fragment Confirmation
Source of formulas to confirm
These formulas:
(type a comma-separated list of formulas, e.g., "C6H6, CH4")
Compound exchange file (.CEF):
Database / Library
D:\MassHunter\PCDL\default_GCMIA_2.csv
Worklist
Matches per formula
Maximum number of matches
1
Automatically increase for isomeric compounds
Values to match
Retention time tolerance: 0,5 min
Mass error tolerance 10 ppm
Data base in Excel file
(csv format)

AUTOMATIC IDENTIFICATION

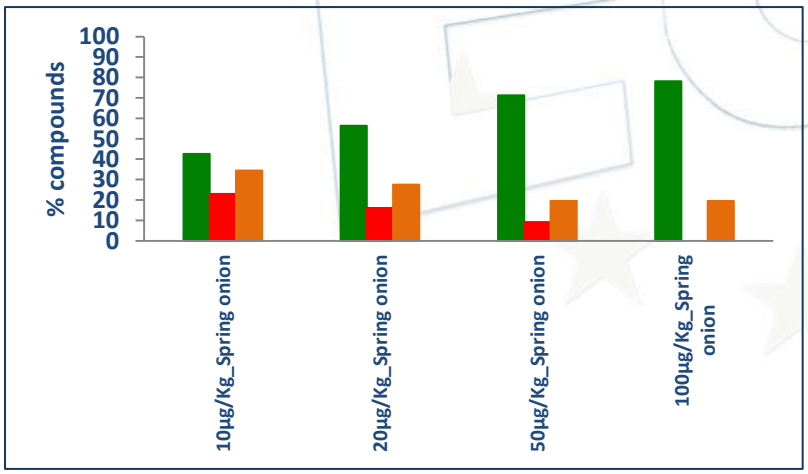
TOMATO



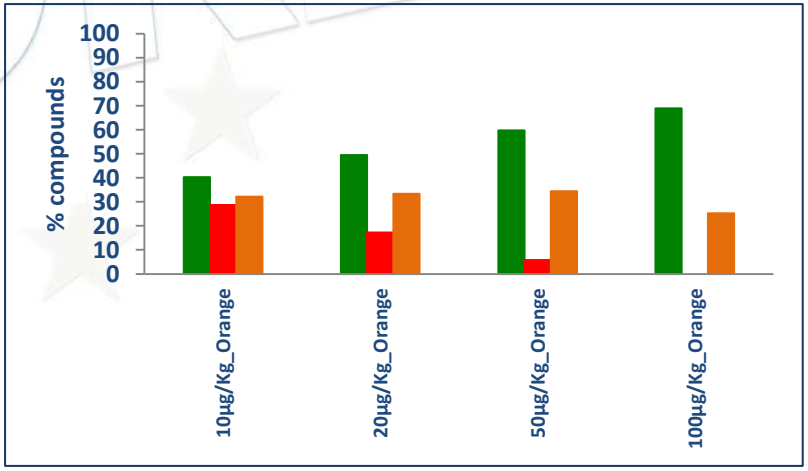
POTATO



SPRING ONION



ORANGE

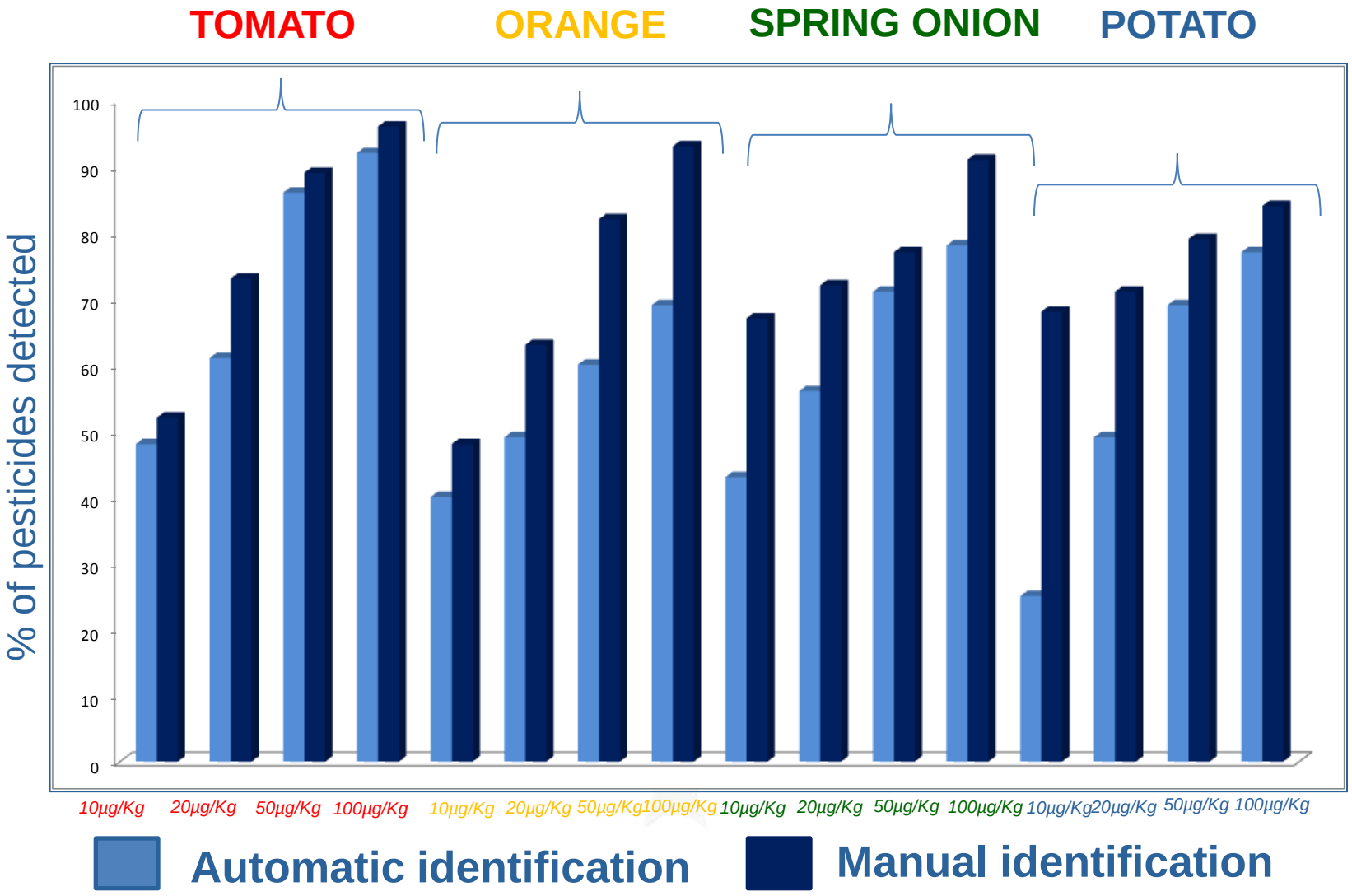


% DETECTED

% NOT DETECTED

% ONE FRAGMENT DETECTED

AUTOMATIC IDENTIFICATION VS MANUAL IDENTIFICATION

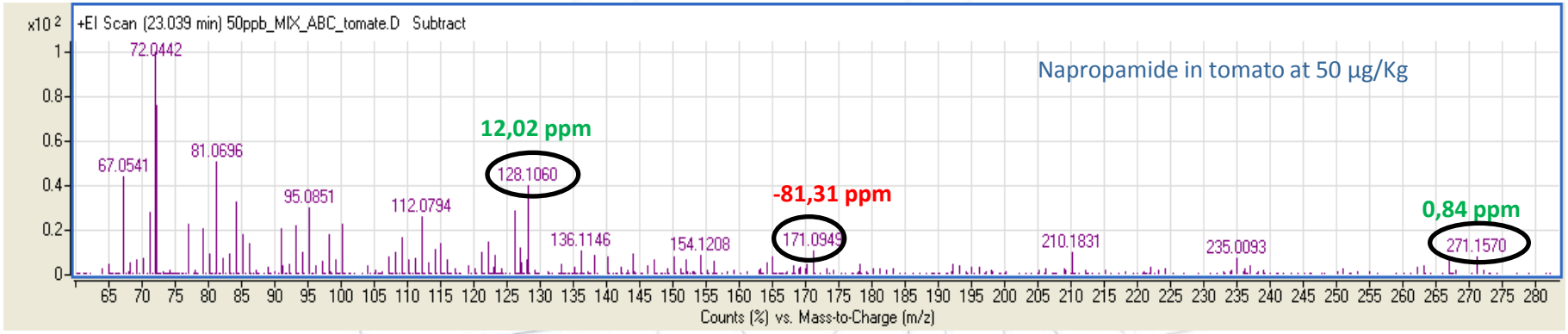


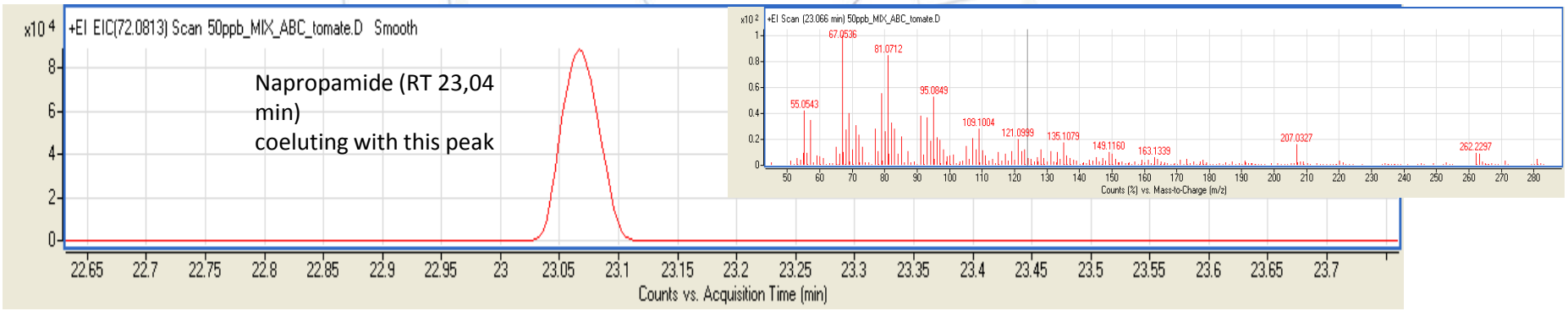
NOT AUTOMATICALLY IDENTIFIED

One ion is automatically detected

Cpd	Label	Name	Formula	Score	Mass	Mass (DB)	Diff (Tgt, ppm)	Diff (Tgt, mDa)
106	Cpd 106: Napropamide	Napropamide	C17 H21 N O2	81,36	271,1563	271,1572	-3,6	-0,98

C17H21NO2	22,97	271,1572	Napropamide
C12H11O	22,97	171,081	Napropamide F1
C7H14NO	22,97	128,1075	Napropamide F3



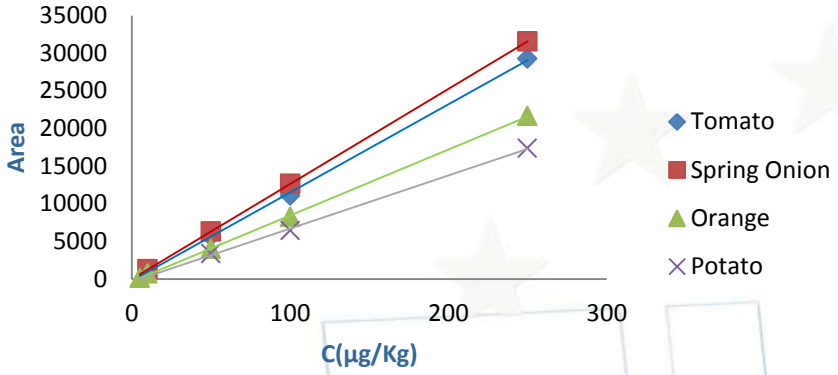


QUANTIFICATION

MATRIX EFFECT

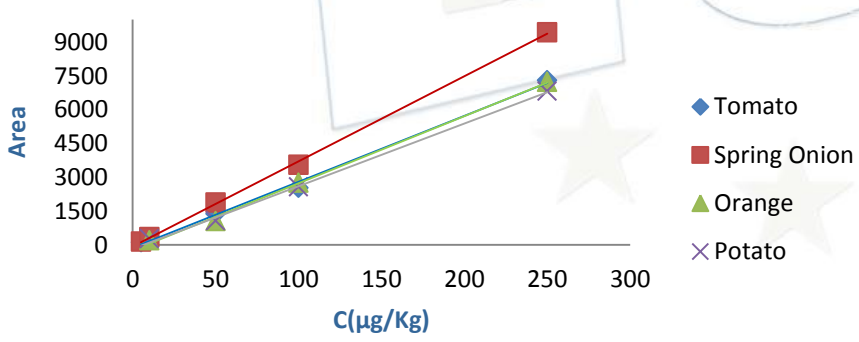
GC-QqQ/MS

Chlozolinate



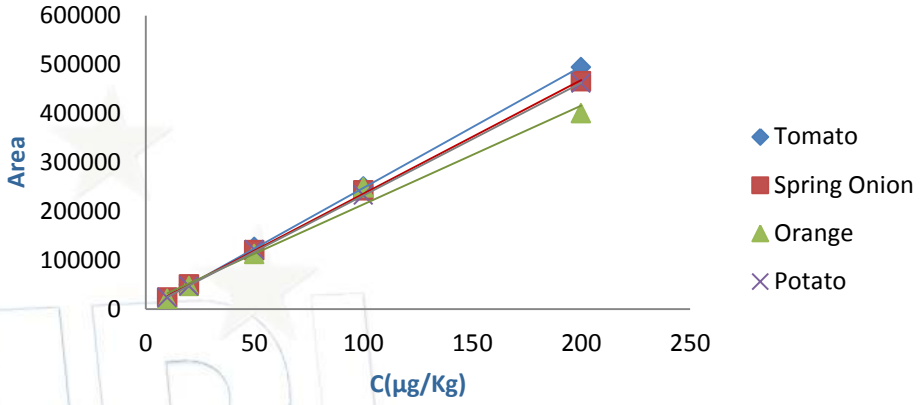
5-10-50-100-250µg/Kg

Endosulphan sulfate



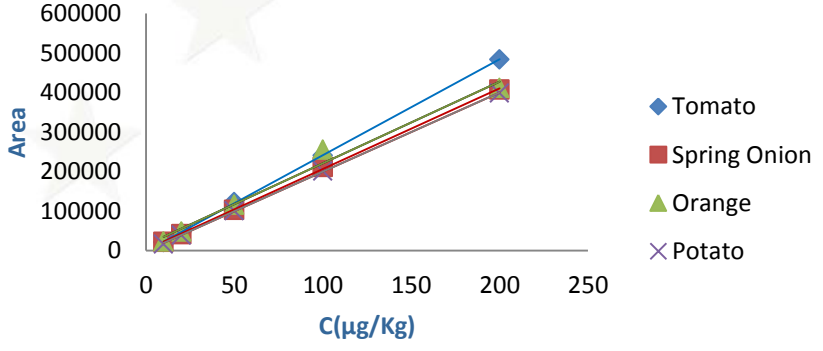
GC-QTOF/MS

Chlozolinate



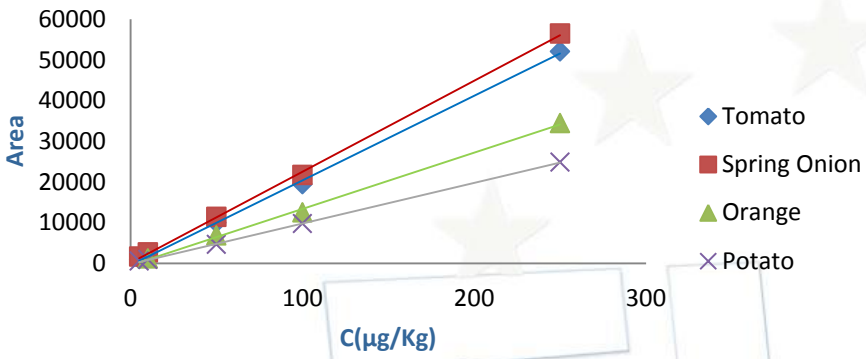
10-20-50-100-200µg/Kg

Endosulphan sulfate



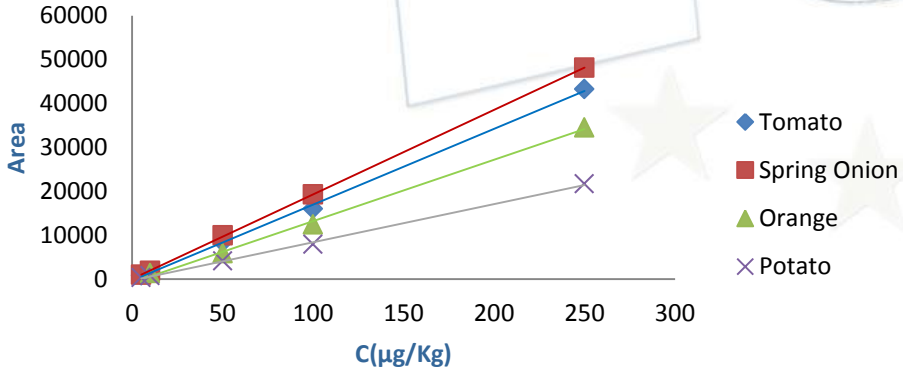
GC-QqQ/MS

Metalaxyl



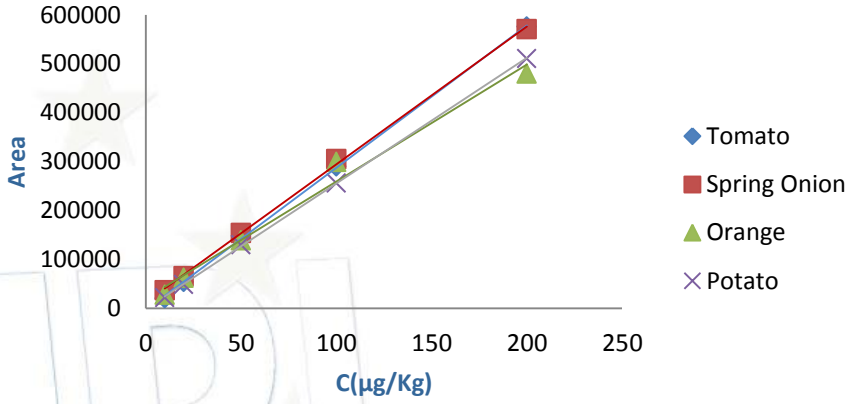
5-10-50-100-250µg/Kg

Vinclozolin



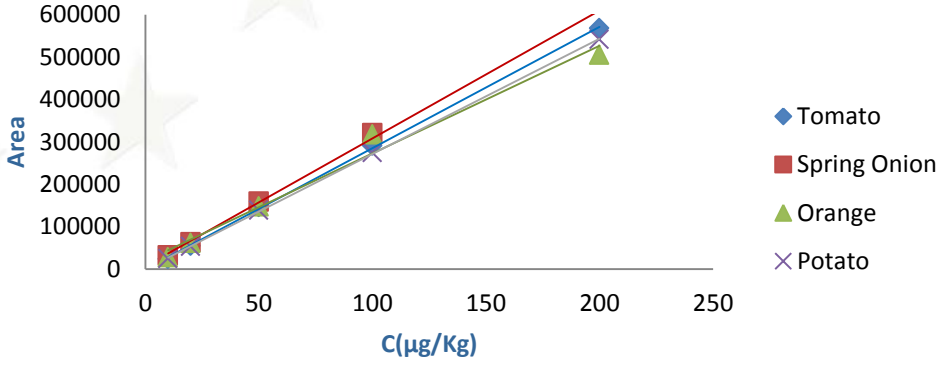
GC-QTOF/MS

Metalaxyl



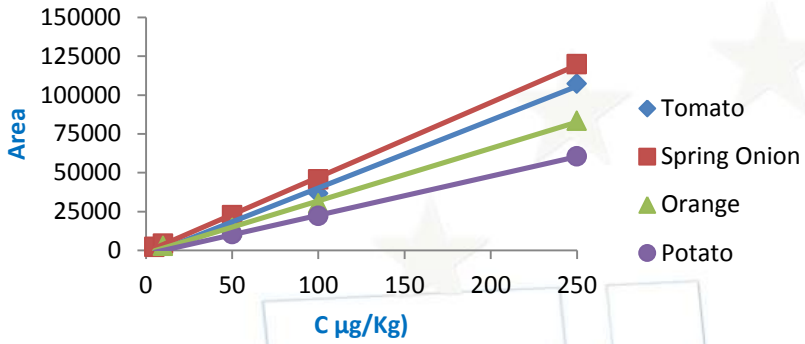
10-20-50-100-200µg/Kg

Vinclozolin



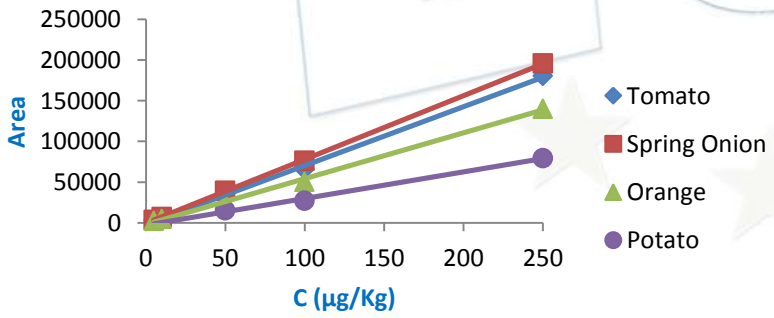
GC-QqQ/MS

Ametryn



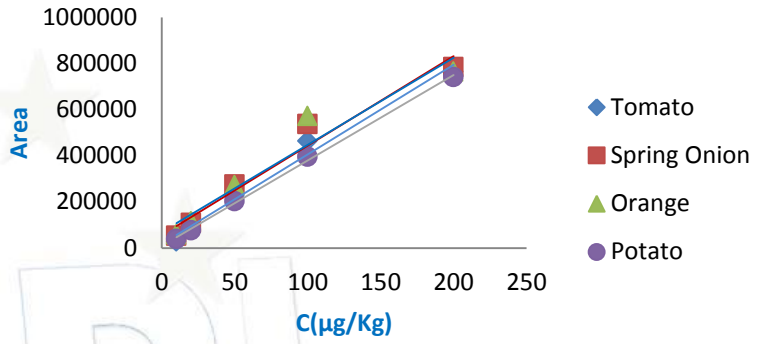
5-10-50-100-250µg/Kg

Cadusafos



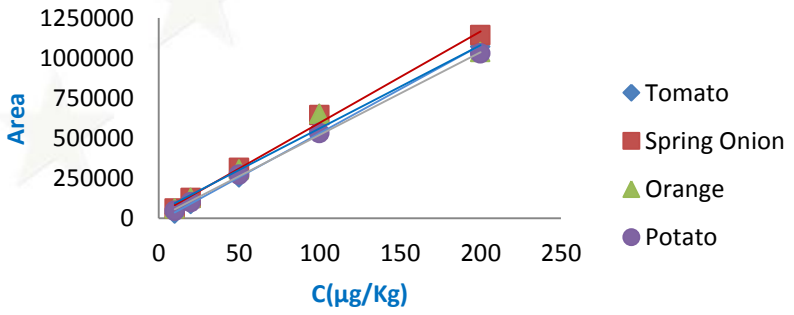
GC-QTOF/MS

Ametryn



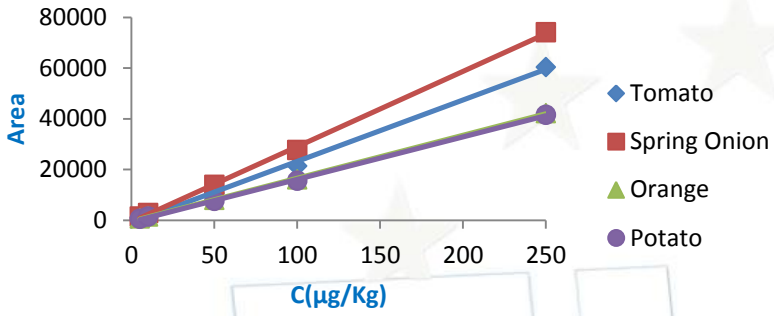
10-20-50-100-200µg/Kg

Cadusafos



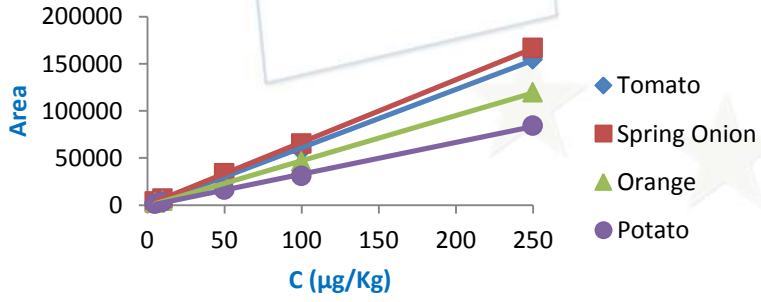
GC-QqQ/MS

Chlorpyrifos-Methyl



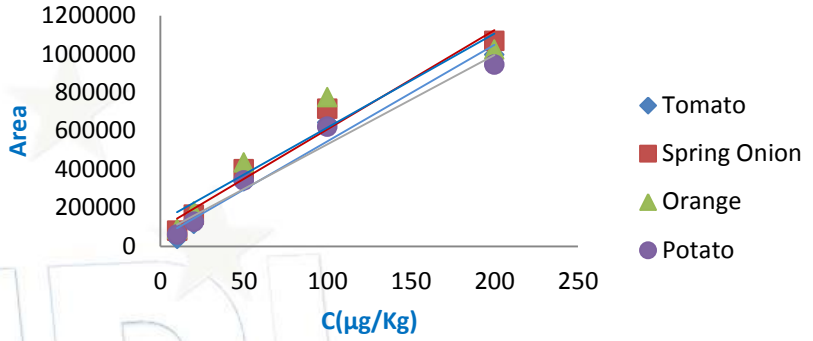
5-10-50-100-250µg/Kg

Ethofumesate



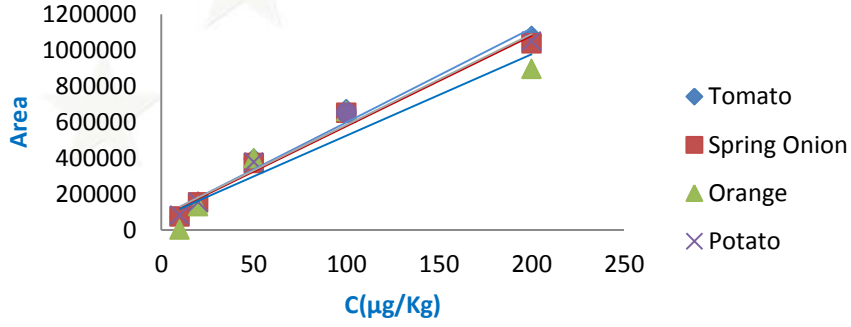
GC-QTOF/MS

Chlorpyrifos_Methyl



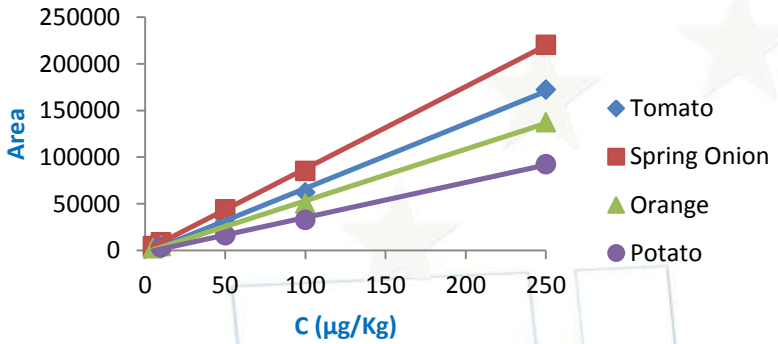
10-20-50-100-200µg/Kg

Ethofumesate



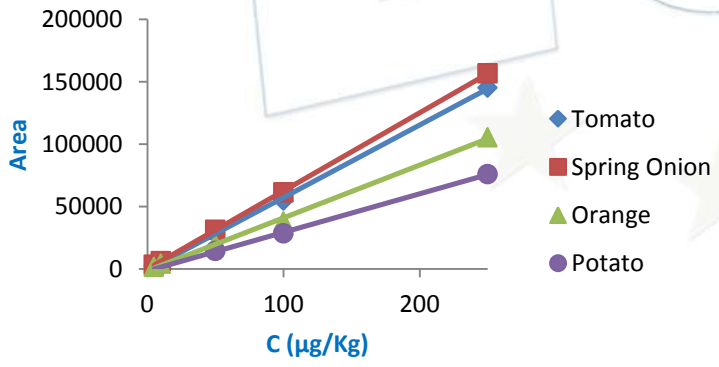
GC-QqQ/MS

Malathion



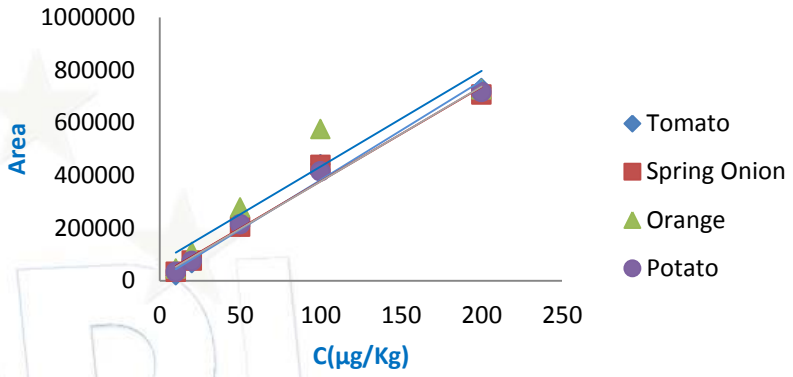
5-10-50-100-250µg/Kg

Nuarimol



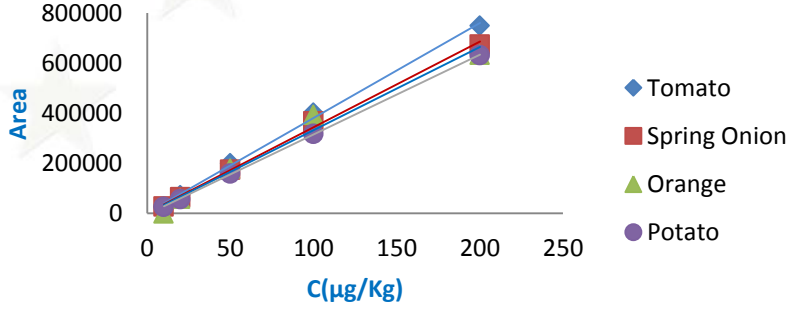
GC-QTOF/MS

Malathion



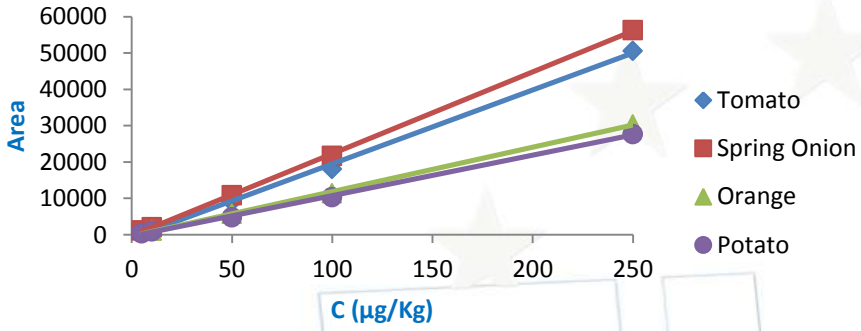
10-20-50-100-200µg/Kg

Nuarimol



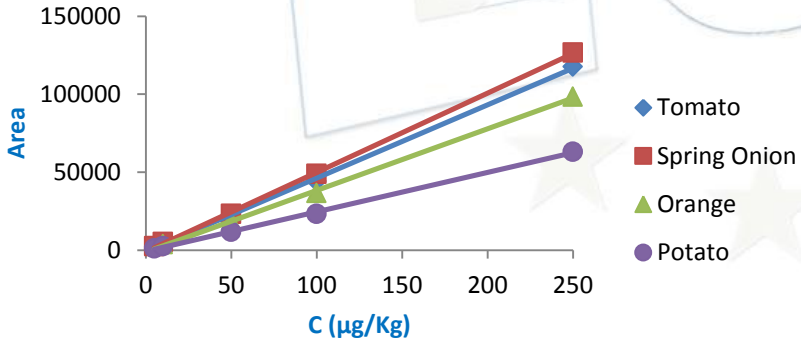
GC-QqQ/MS

Pirimiphos-Methyl



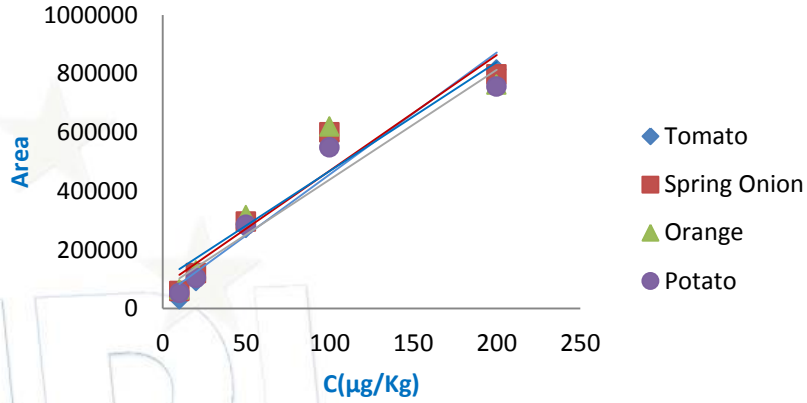
5-10-50-100-250µg/Kg

Triadimefon



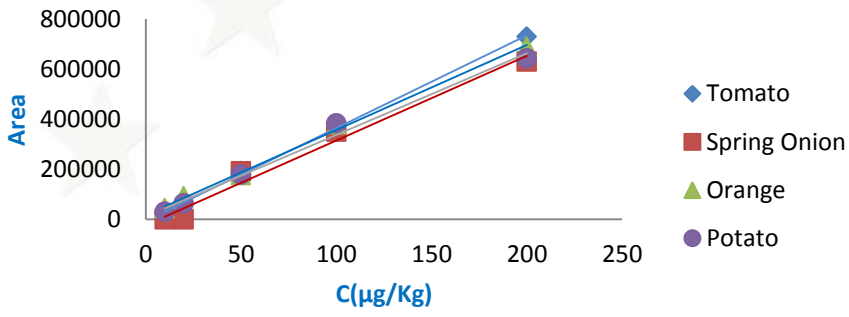
GC-QTOF/MS

Pirimiphos-Methyl



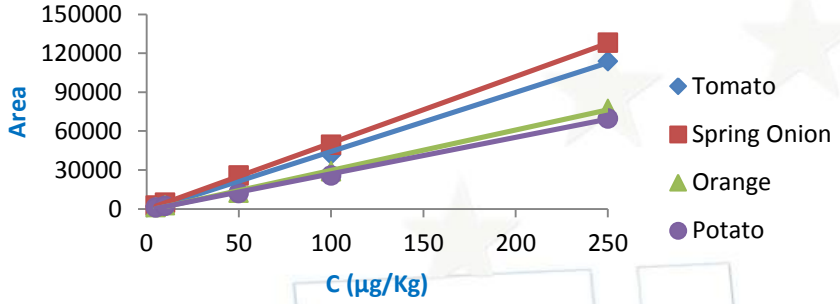
10-20-50-100-200µg/Kg

Triadimefon



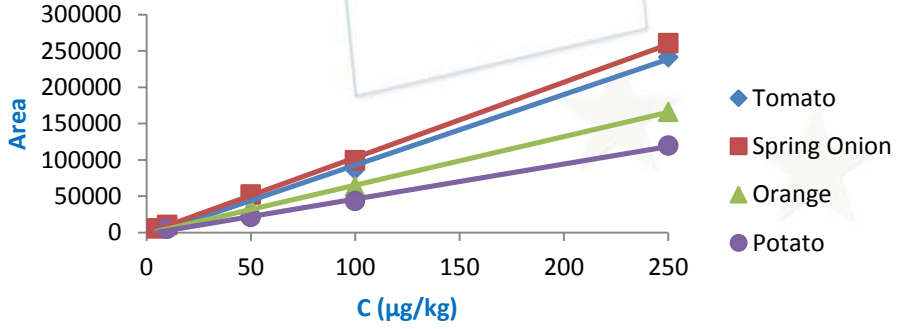
GC-QqQ/MS

Prothiophos



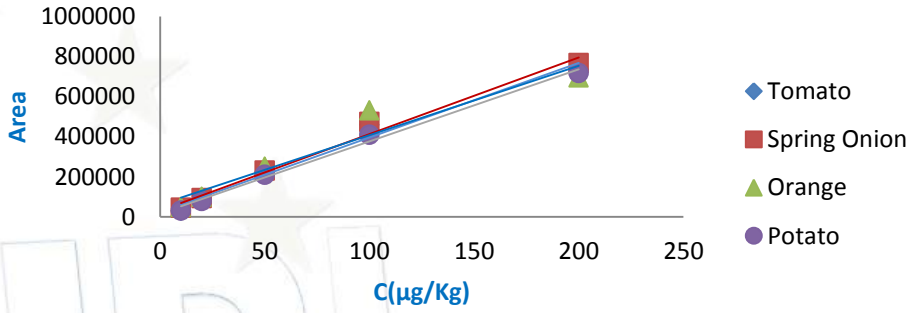
5-10-50-100-250µg/Kg

Terbumeton



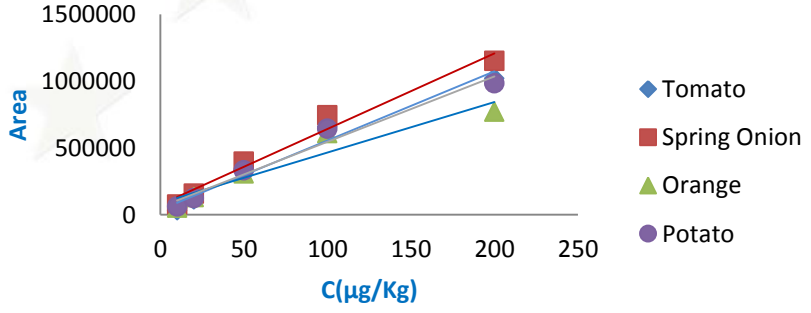
GC-QTOF/MS

Prothiofos



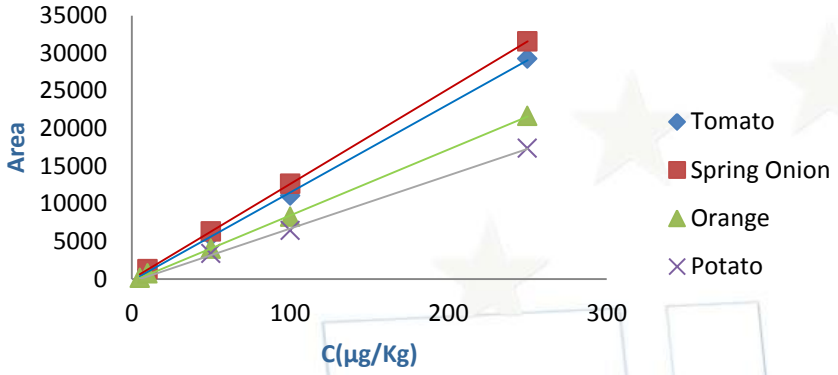
10-20-50-100-200µg/Kg

Terbumeton



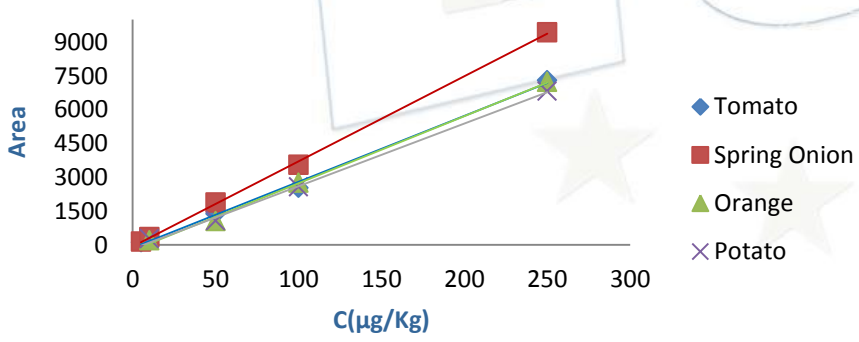
GC-QqQ/MS

Chlozolinate



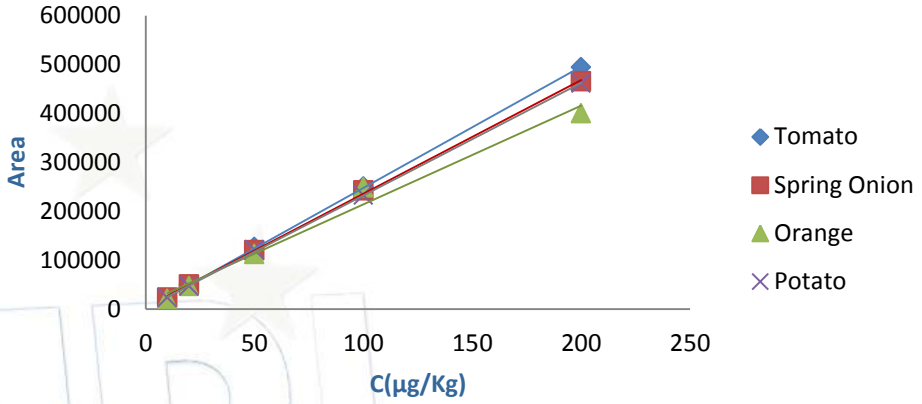
5-10-50-100-250µg/Kg

Endosulphan sulfate



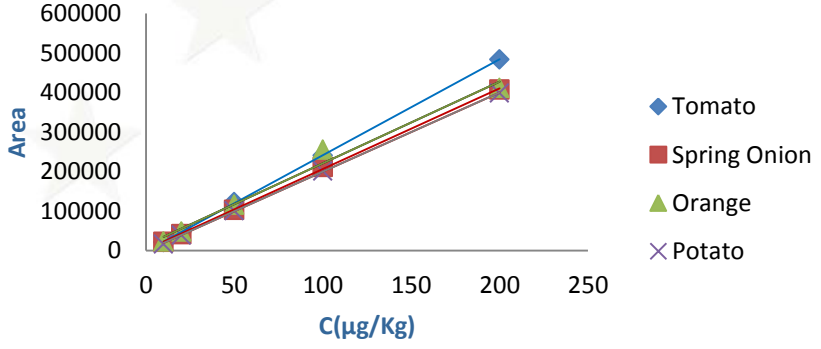
GC-QTOF/MS

Chlozolinate

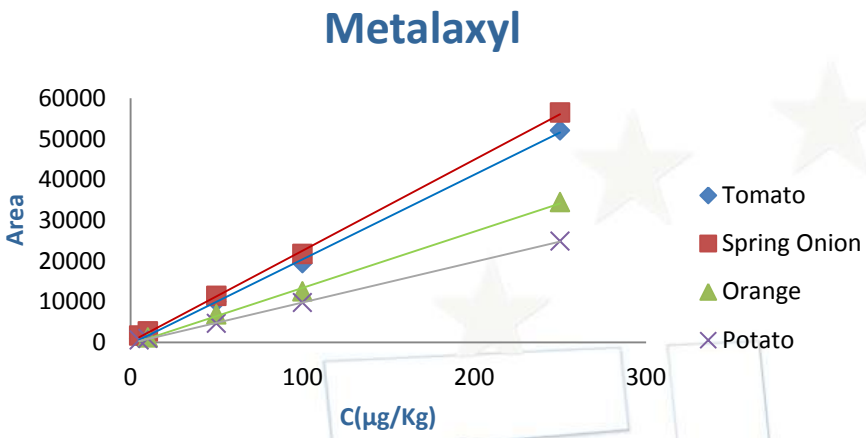


10-20-50-100-200µg/Kg

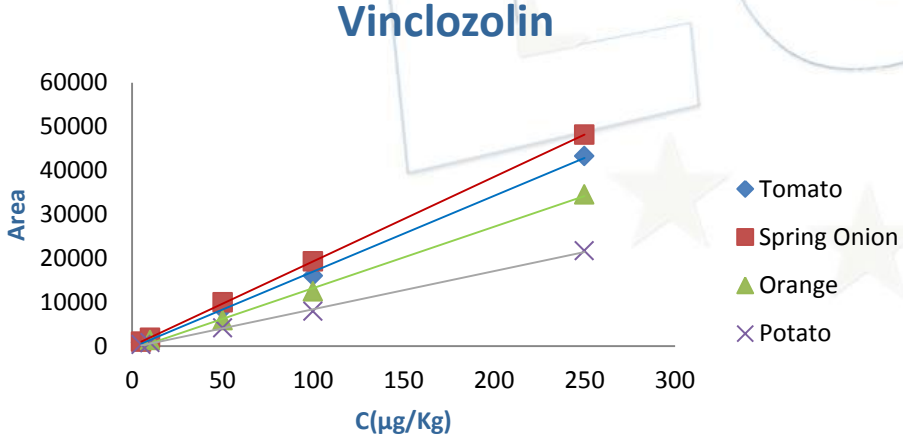
Endosulphan sulfate



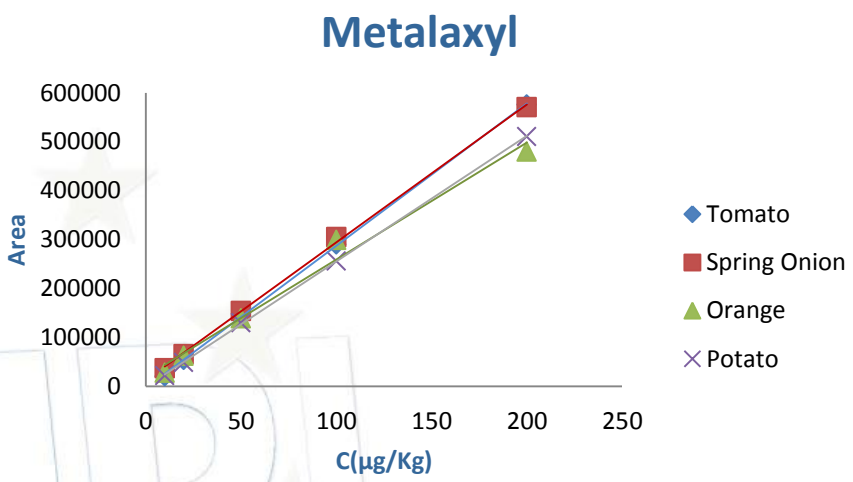
GC-QqQ/MS



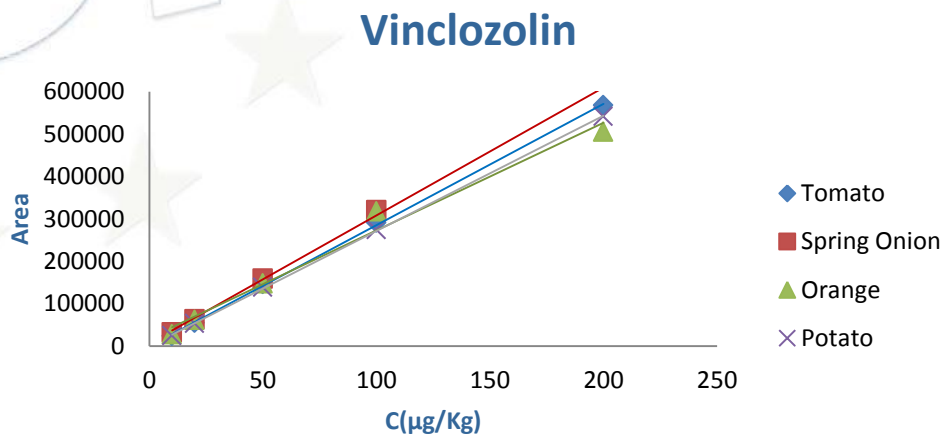
5-10-50-100-250µg/Kg



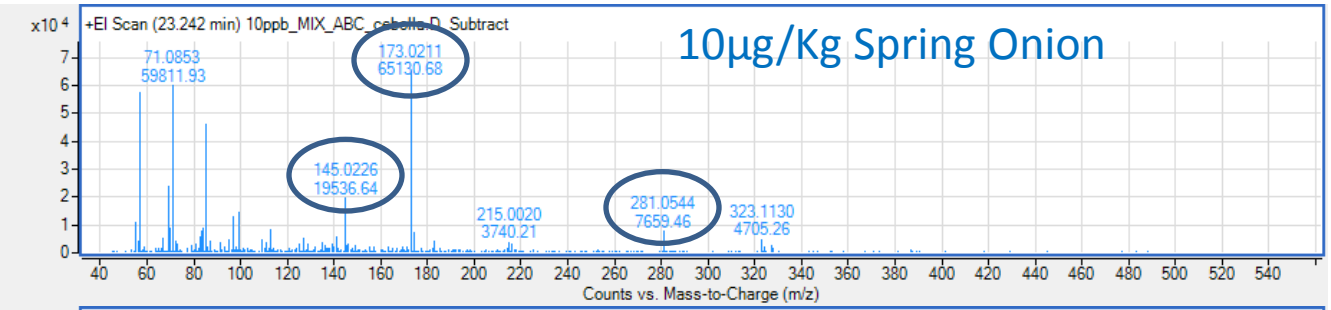
GC-QTOF/MS

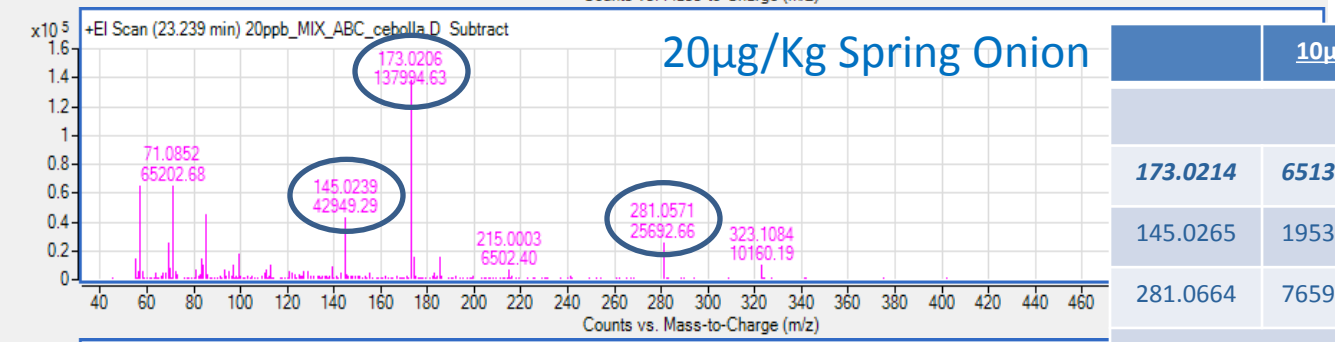


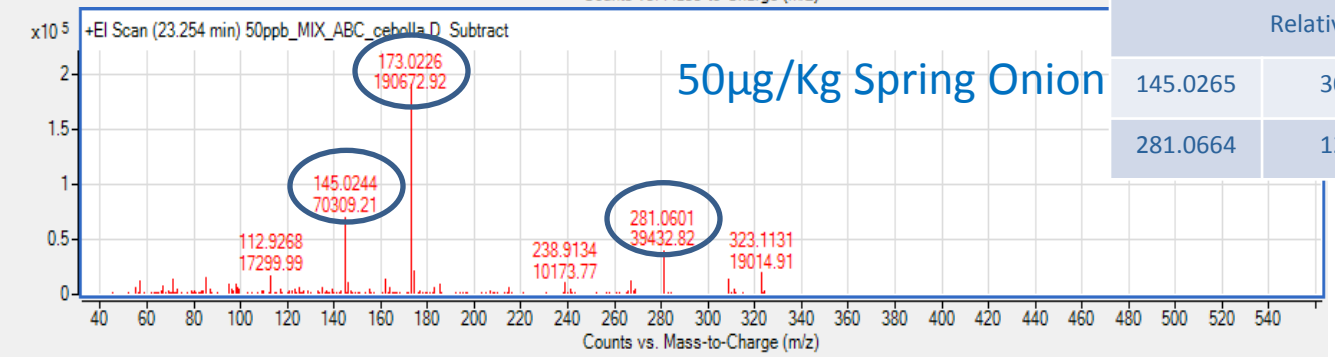
10-20-50-100-200µg/Kg

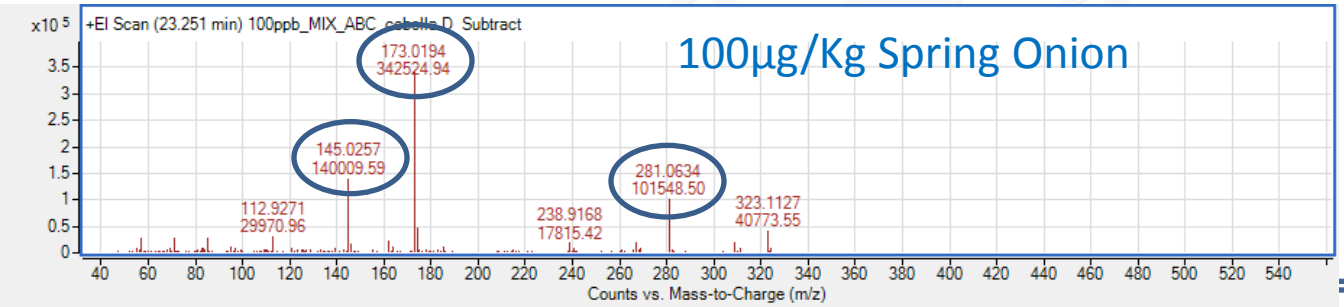


RELATIVE ABUNDANCES AT DIFFERENT CONCENTRATIONS



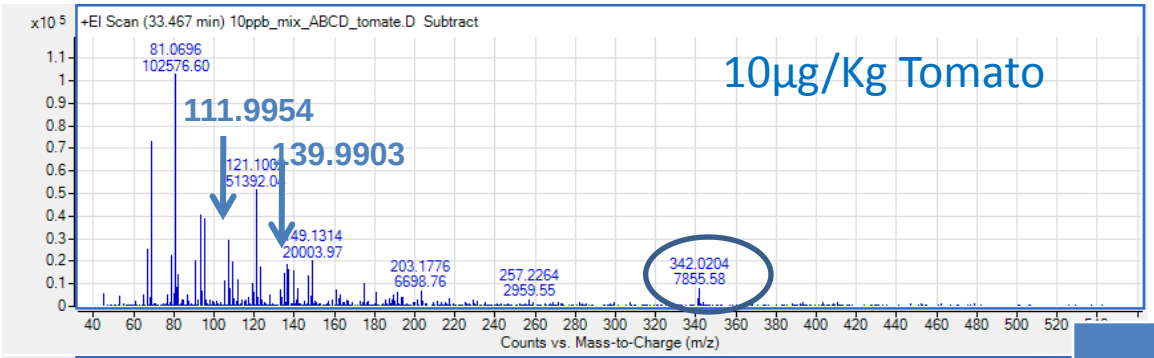


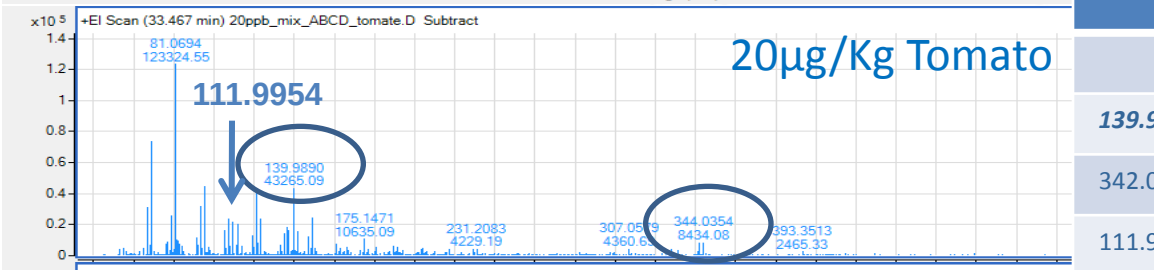


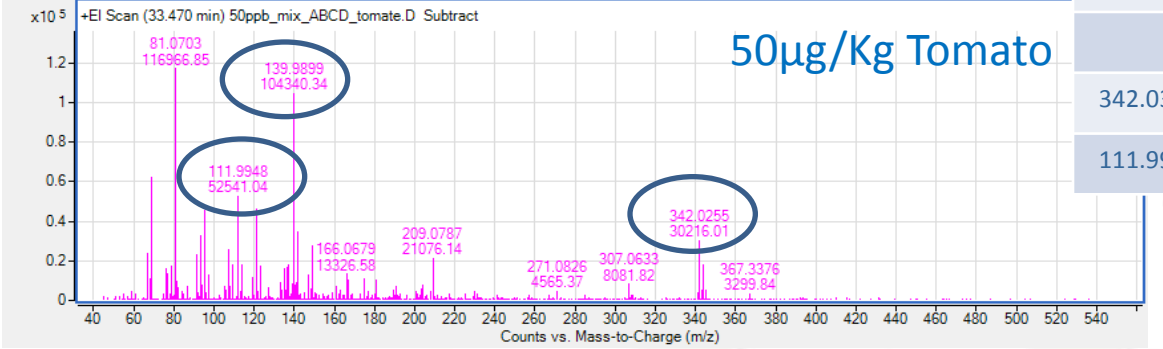


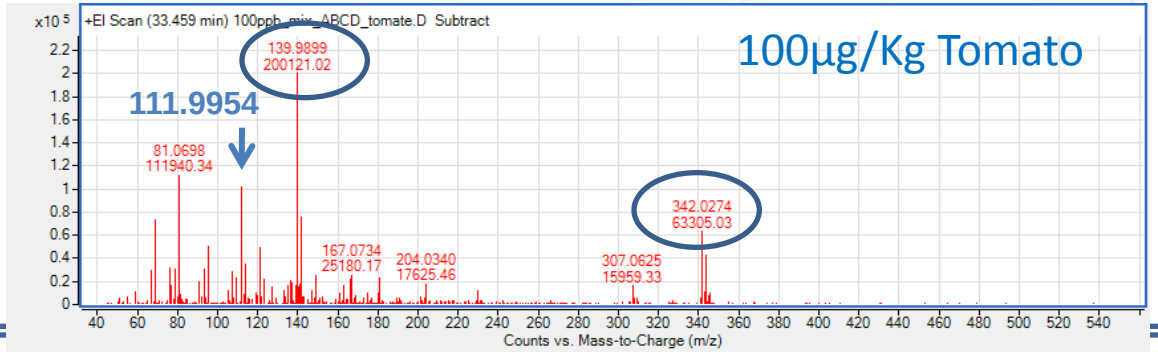
FLUTOLANIL

	10µg/Kg	20µg/Kg	50µg/Kg	100µg/Kg
Absolute Abundances				
173.0214	***65130.68***	***137994.63***	***190672.92***	***342524.94***
145.0265	19536.64	42949.29	70309.21	140009.59
281.0664	7659.46	25692.66	39432.82	101548.50
Relative Abundances to base peak 173.0214				
145.0265	30%	31%	37%	41%
281.0664	12%	19%	21%	30%



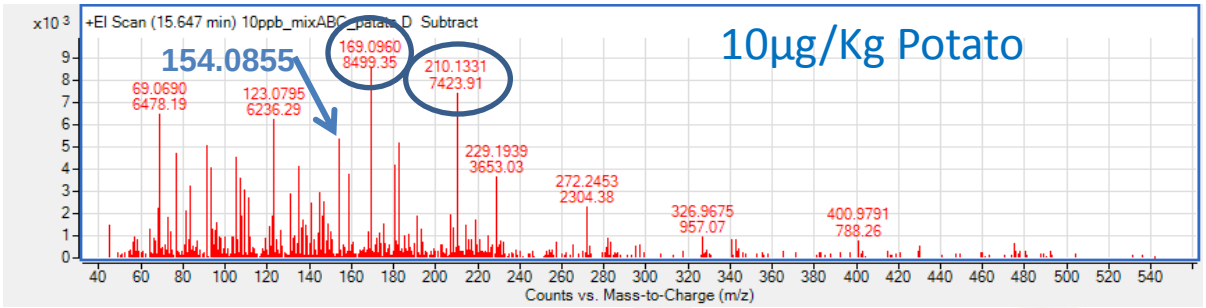


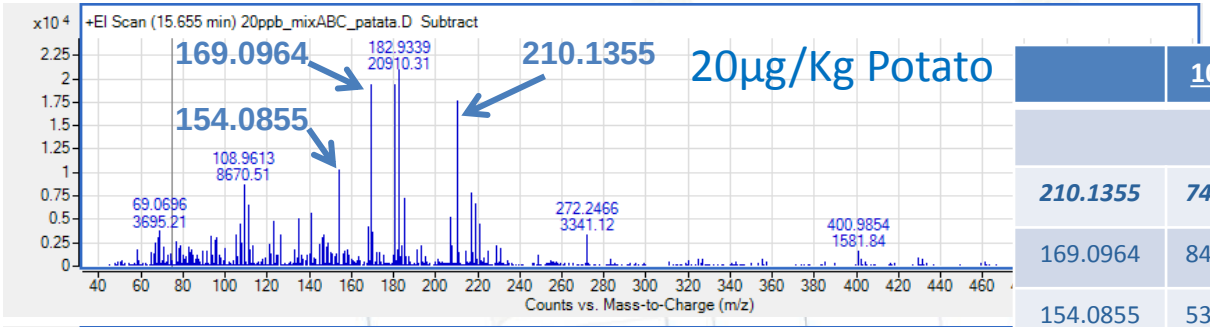


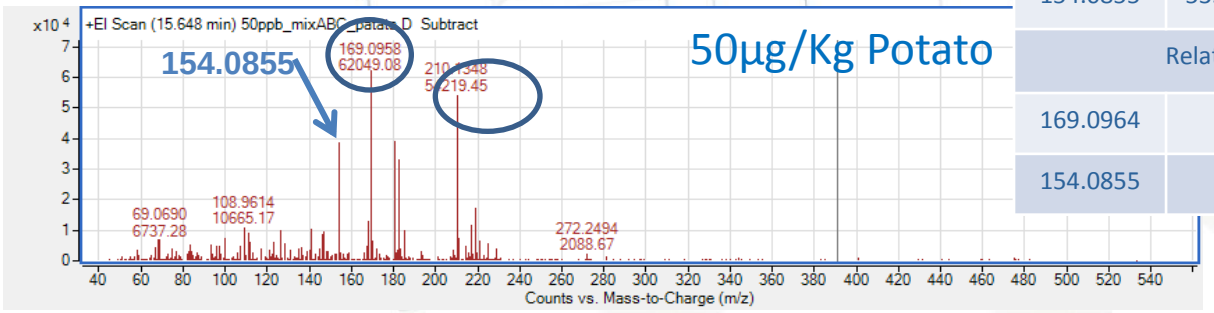


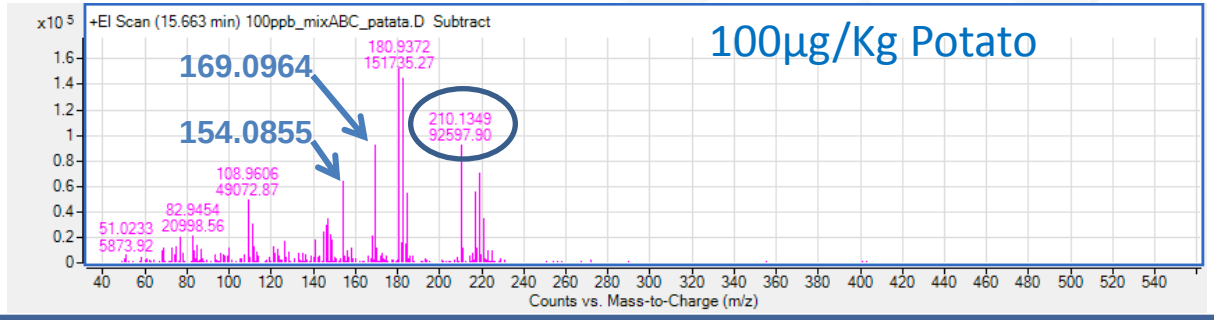
BOSCALID

	10µg/Kg	20µg/Kg	50µg/Kg	100µg/Kg
Absolute Abundances				
139.9903	***15757.57***	***43265.09***	***104340***	***200121***
342.0327	7855.58	8177.92	30216	63305.03
111.9954	11613.48	20109.98	52541	102291
Relative Abundances to base peak 139.9903				
342.0327	44%	19%	30%	32%
111.9954	**74%**	46%	50%	51%





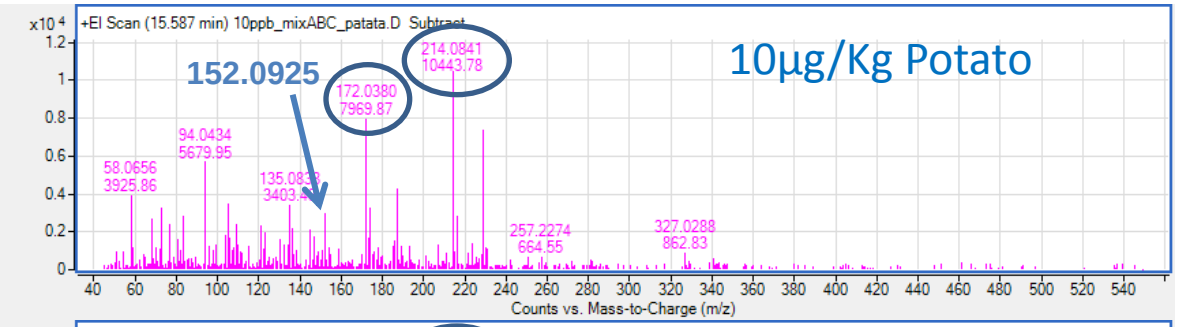




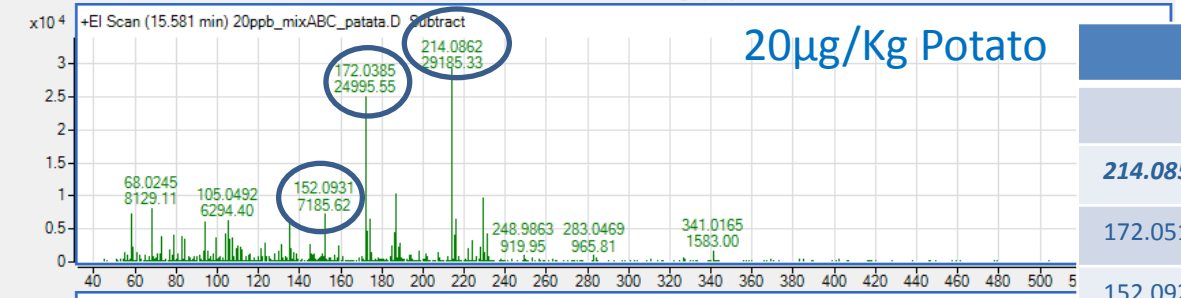
TERBUMETON

	10µg/Kg	20µg/Kg	50µg/Kg	100µg/Kg
Absolute Abundances				
210.1355	***7423.91***	***17674.94***	***54219.45***	***92597.90***
169.0964	8499.35	19325.86	62049.08	91847.98
154.0855	5352.16	10216.07	38487.03	63805.87
Relative Abundances to base peak 210.1355				
169.0964	114%	109%	114%	99%
154.0855	72%	58%	71%	69%

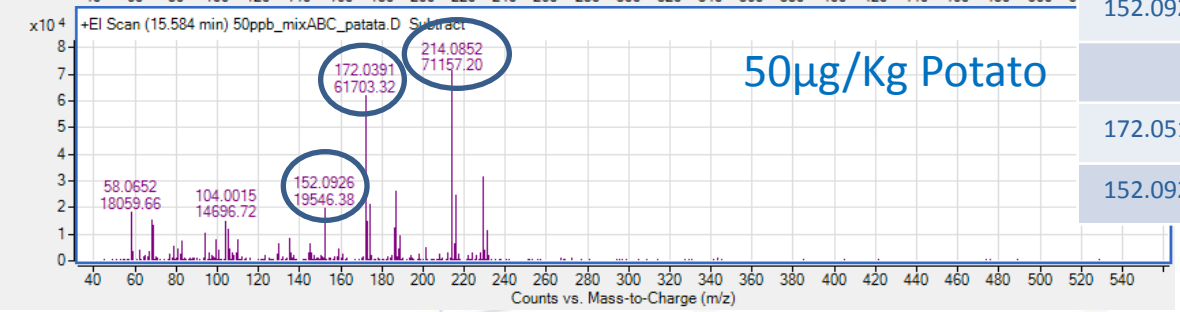
RELATIVE ABUNDANCES IN DIFFERENT MATRICES



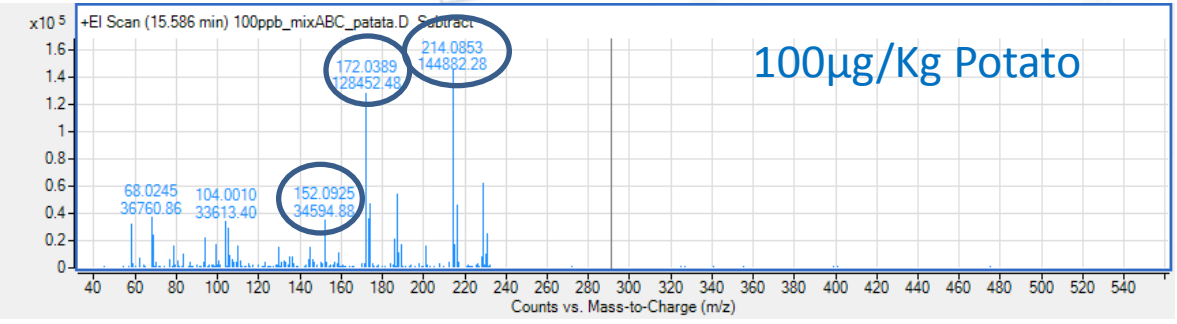
10µg/Kg Potato



20µg/Kg Potato



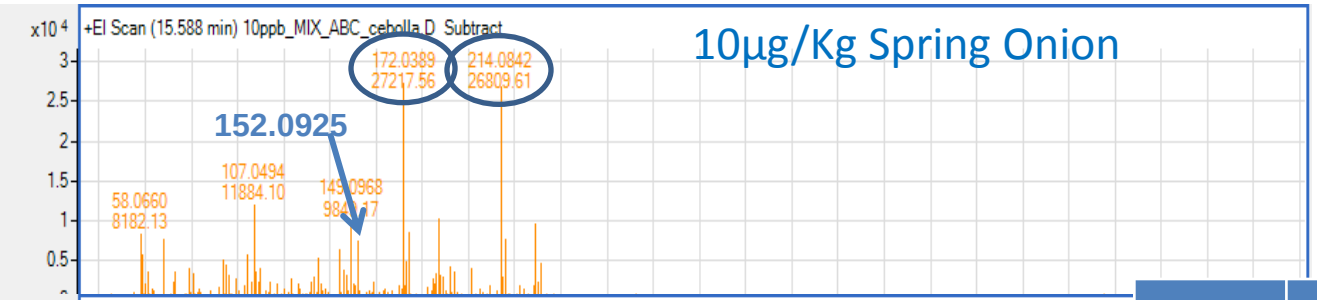
50µg/Kg Potato

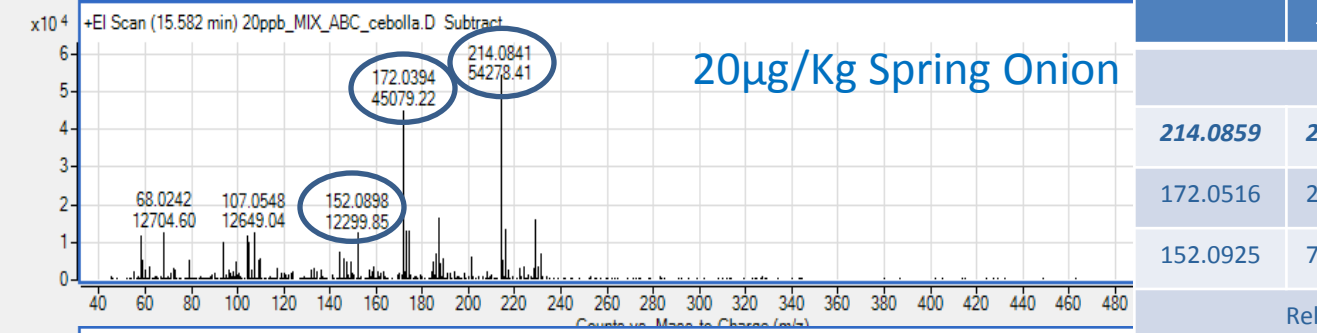


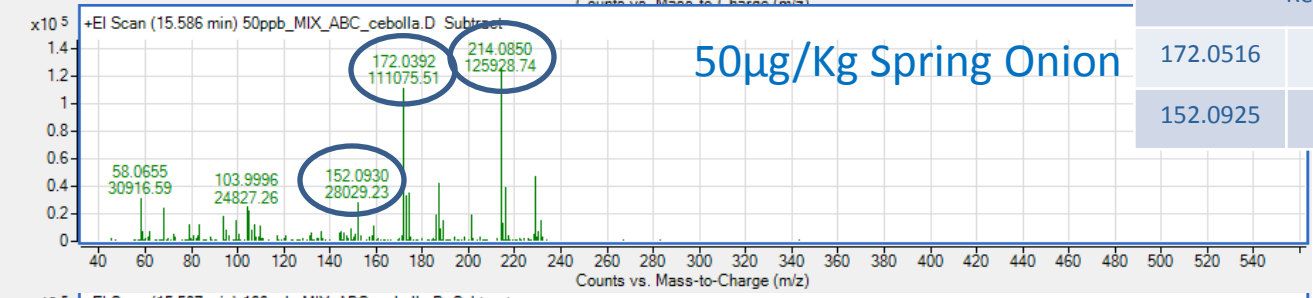
100µg/Kg Potato

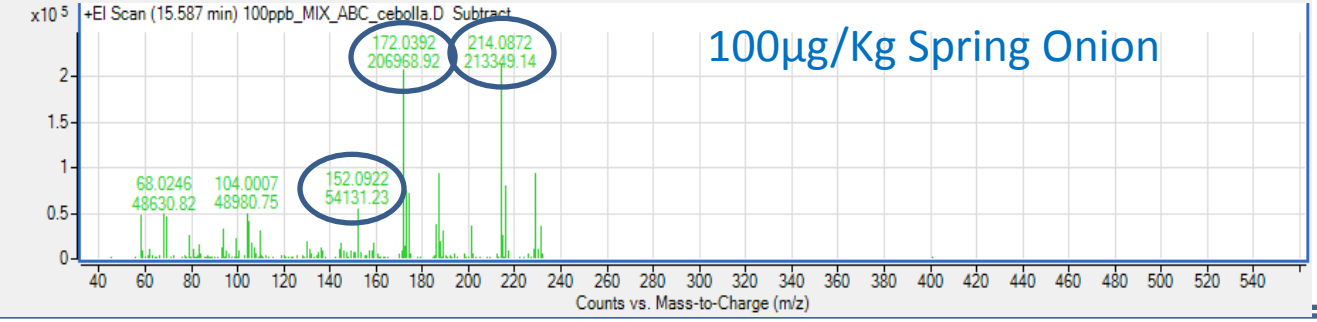
PROPAZINE

	10µg/Kg	20µg/Kg	50µg/Kg	100µg/Kg
Absolute Abundances				
214.0859	***10443.78***	***29185.33***	***71157.20***	***144882.28***
172.0516	7969.87	24995.55	61703.32	128452.48
152.0925	2947.45	7185.62	19546.38	34594.88
Relative Abundances to base peak 214.0859				
172.0516	76%	86%	87%	89%
152.0925	28%	25%	27%	24%



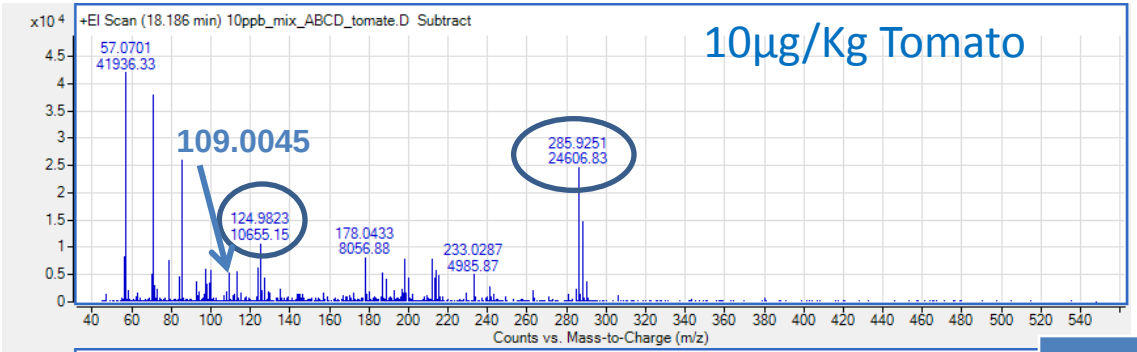


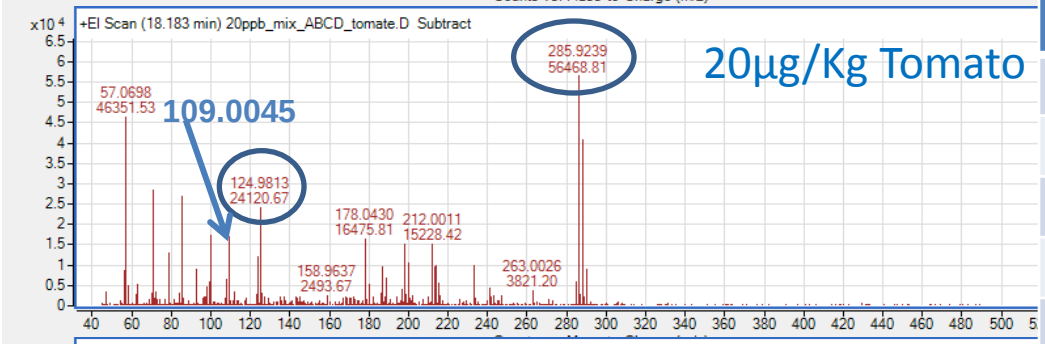


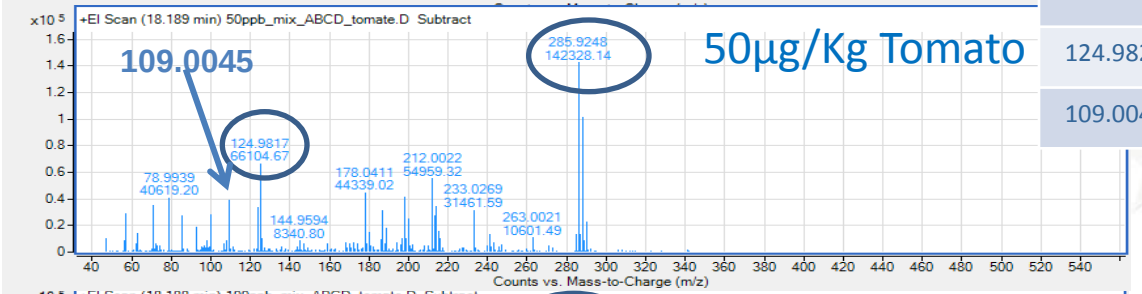


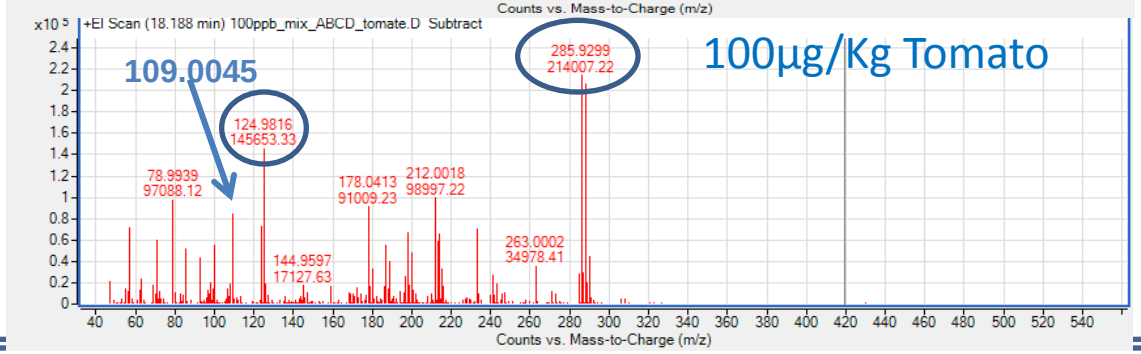
PROPAZINE

	10µg/Kg	20µg/Kg	50µg/Kg	100µg/Kg
Absolute Abundances				
214.0859	***26809.61***	***54278.41***	***125928.74***	***213349.14***
172.0516	27217.56	45079.22	111075.51	206968.92
152.0925	7313.01	12299.85	28029.23	54131.23
Relative Abundances to base peak 214.0859				
172.0516	102%	83%	88%	97%
152.0925	27%	23%	22%	25%



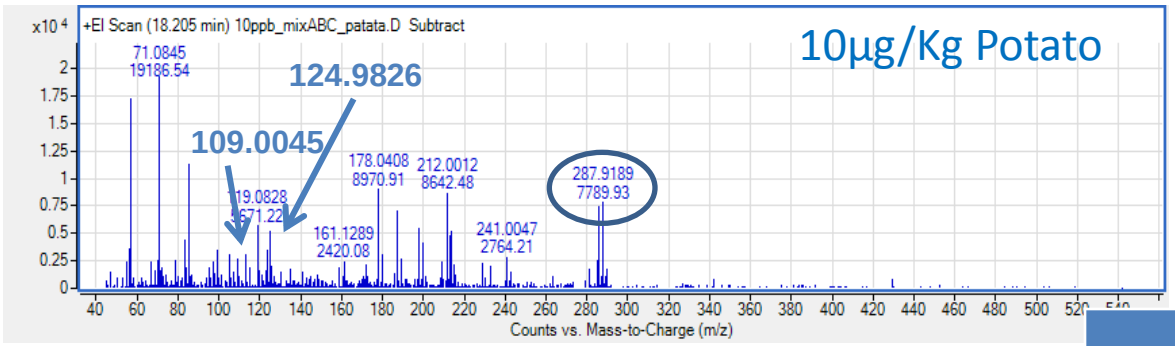


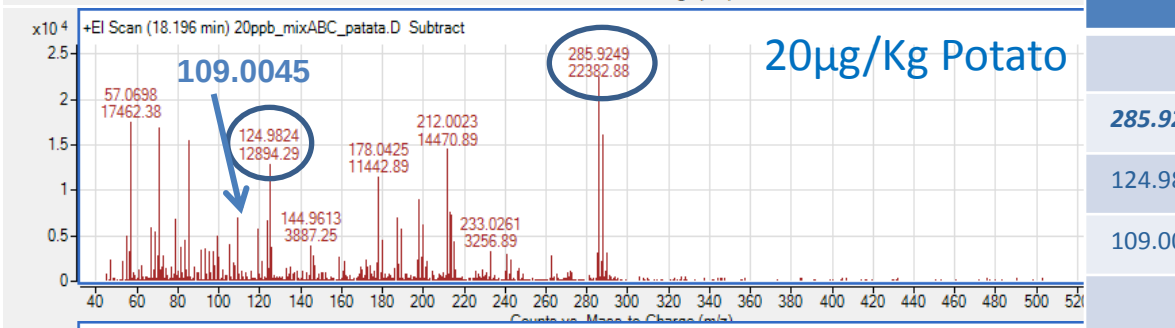


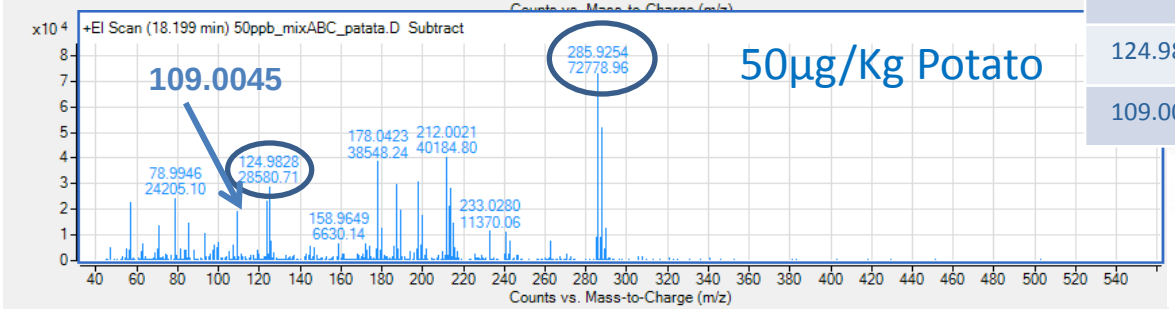


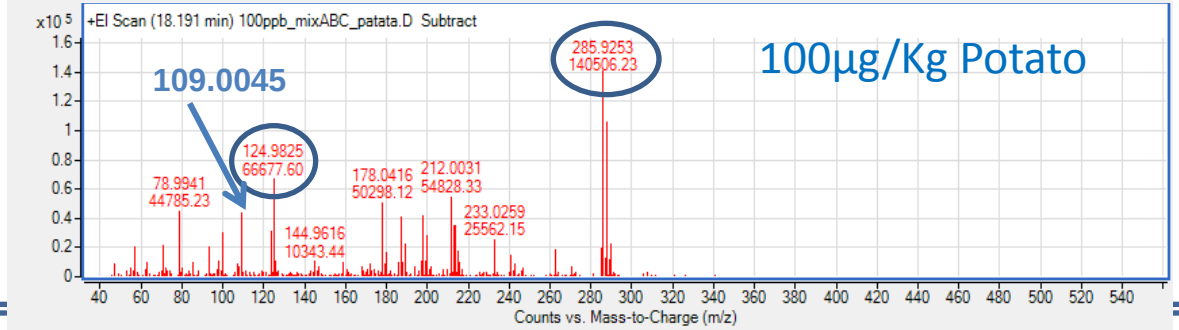
CHLORPYRIFOS METHYL

	10µg/Kg	20µg/Kg	50µg/Kg	100µg/Kg
Absolute Abundances				
285.9261	***24606.83***	***56468.81***	***142328***	***214007.22***
124.9826	10655.15	24120.67	66104.67	145653.33
109.0045	5369.61	16857.85	39130.32	84063.63
Relative Abundances to base peak 285.9261				
124.9826	43%	43%	46%	**68%**
109.0045	22%	30%	27%	39%



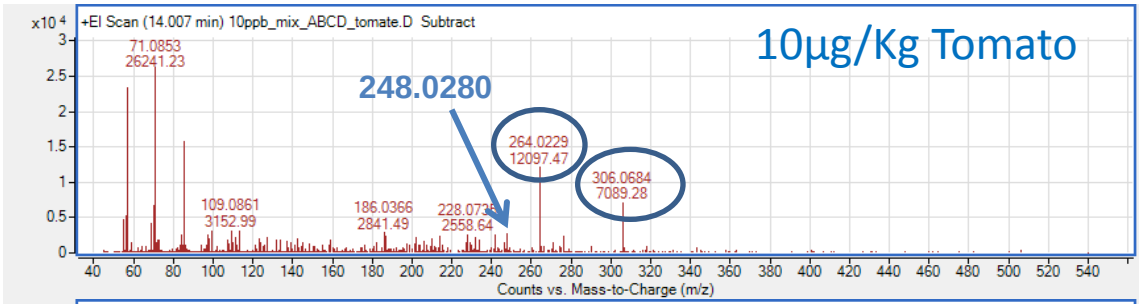




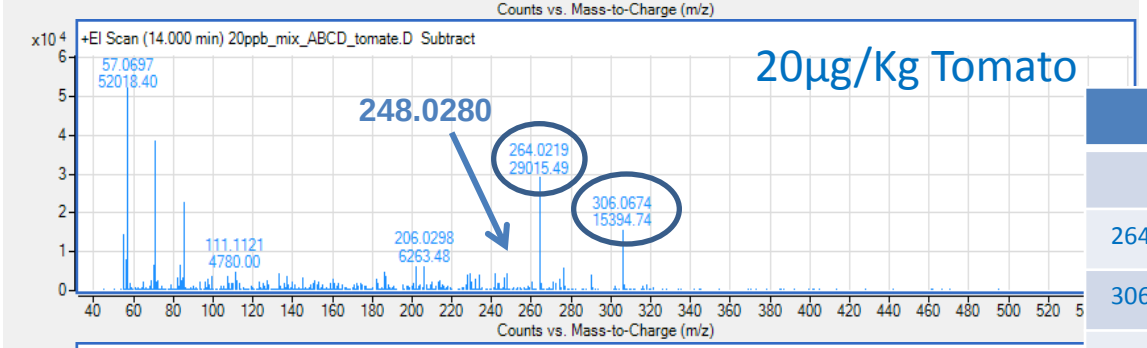


CHLORPYRIFOS METHYL

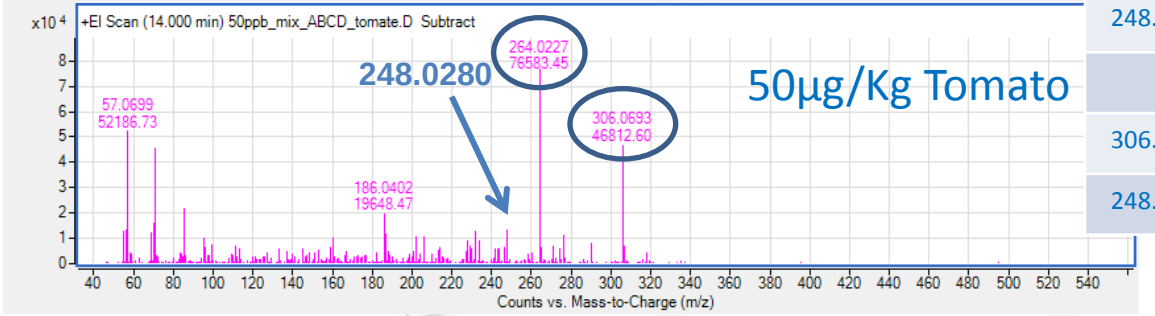
	10µg/Kg	20µg/Kg	50µg/Kg	100µg/Kg
Absolute Abundances				
285.9261	***7434.42***	***22382.88***	***72778.96***	***140506.23***
124.9826	5132.83	12894.29	28580.71	66677.60
109.0045	2606.15	6930.35	19063.65	44127.69
Relative Abundances to base peak 285.9261				
124.9826	69%	58%	39%	47%
109.0045	35%	31%	26%	31%



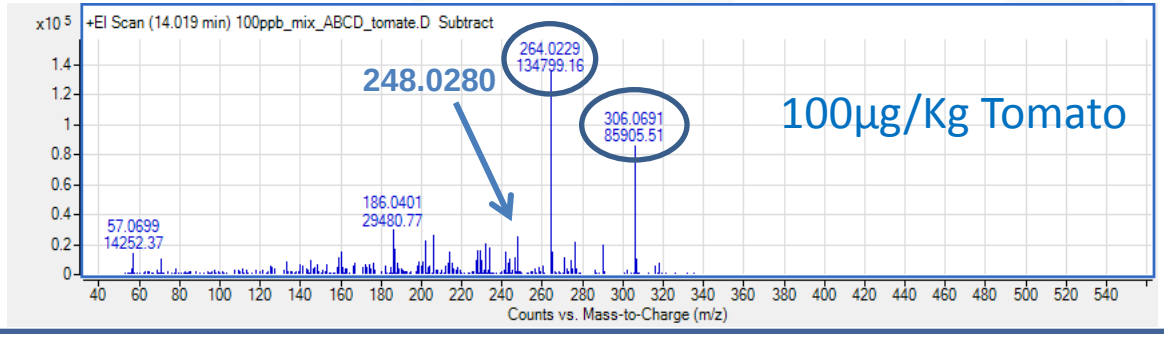
10µg/Kg Tomato



20µg/Kg Tomato



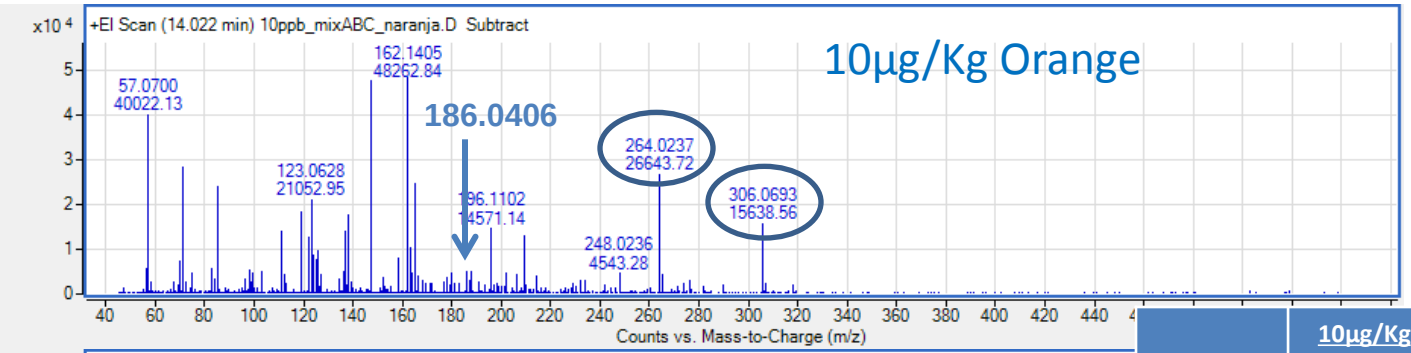
50µg/Kg Tomato

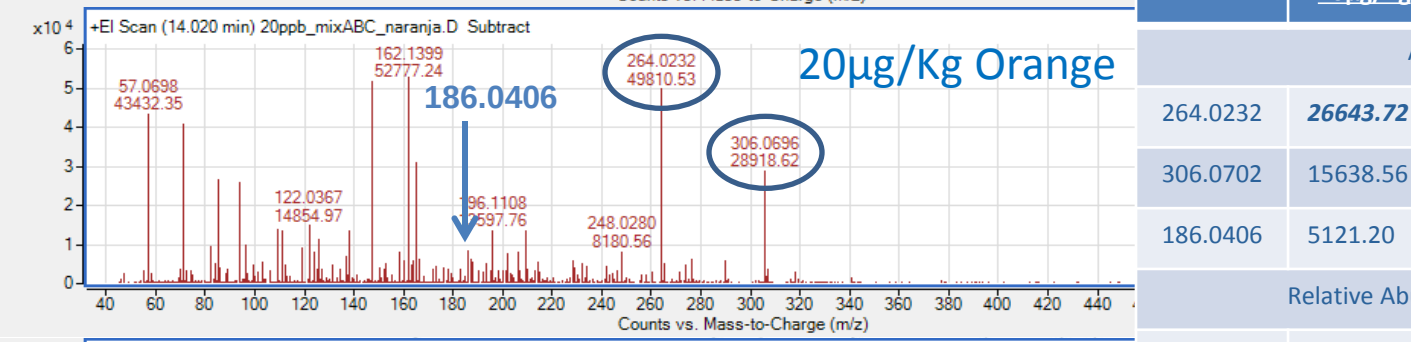


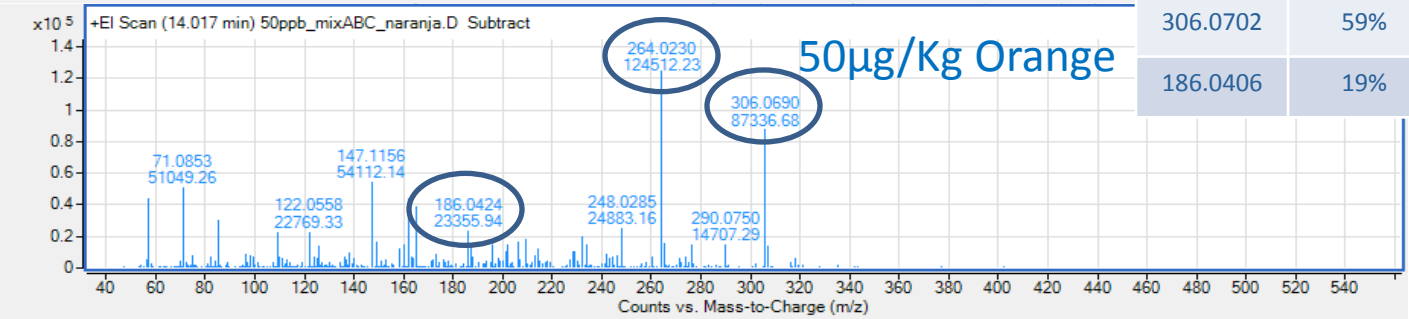
100µg/Kg Tomato

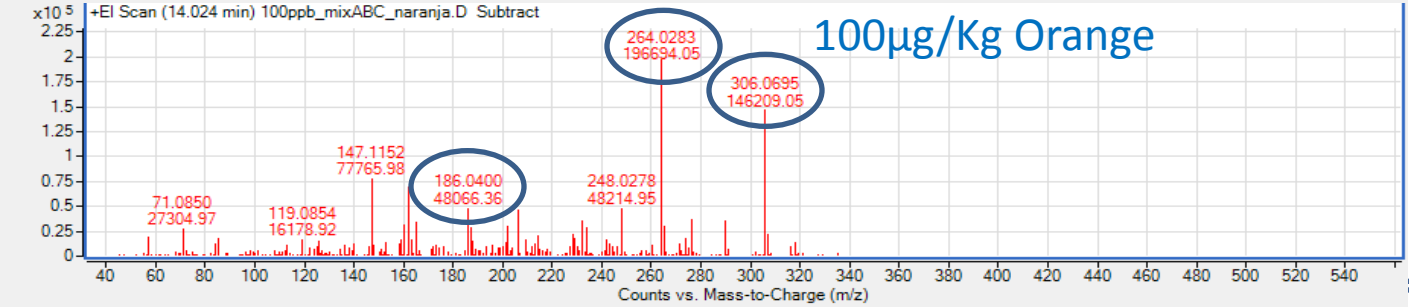
TRIFLURALIN

	10µg/Kg	20µg/Kg	50µg/Kg	100µg/Kg
Absolute Abundances				
264.0232	***12097.47***	***29015.49***	***76583.45***	***134799.16***
306.0702	7089.28	15394.74	46812.60	85905.51
248.0280	2802.65	4210.54	13306	24920.55
Relative Abundances to base peak 264.0232				
306.0702	59%	53%	61%	64%
248.0280	23%	15%	17%	18%





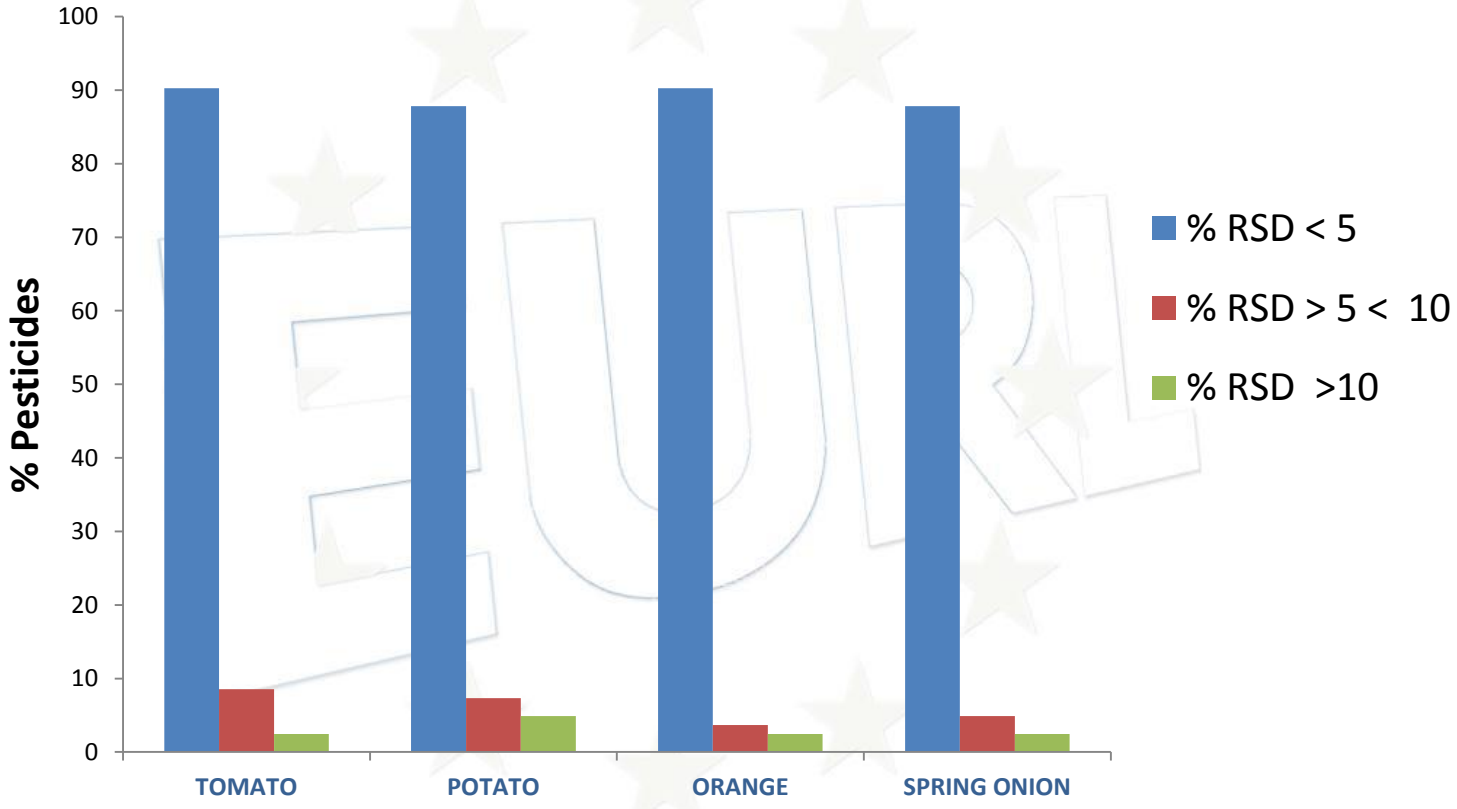




TRIFLURALIN

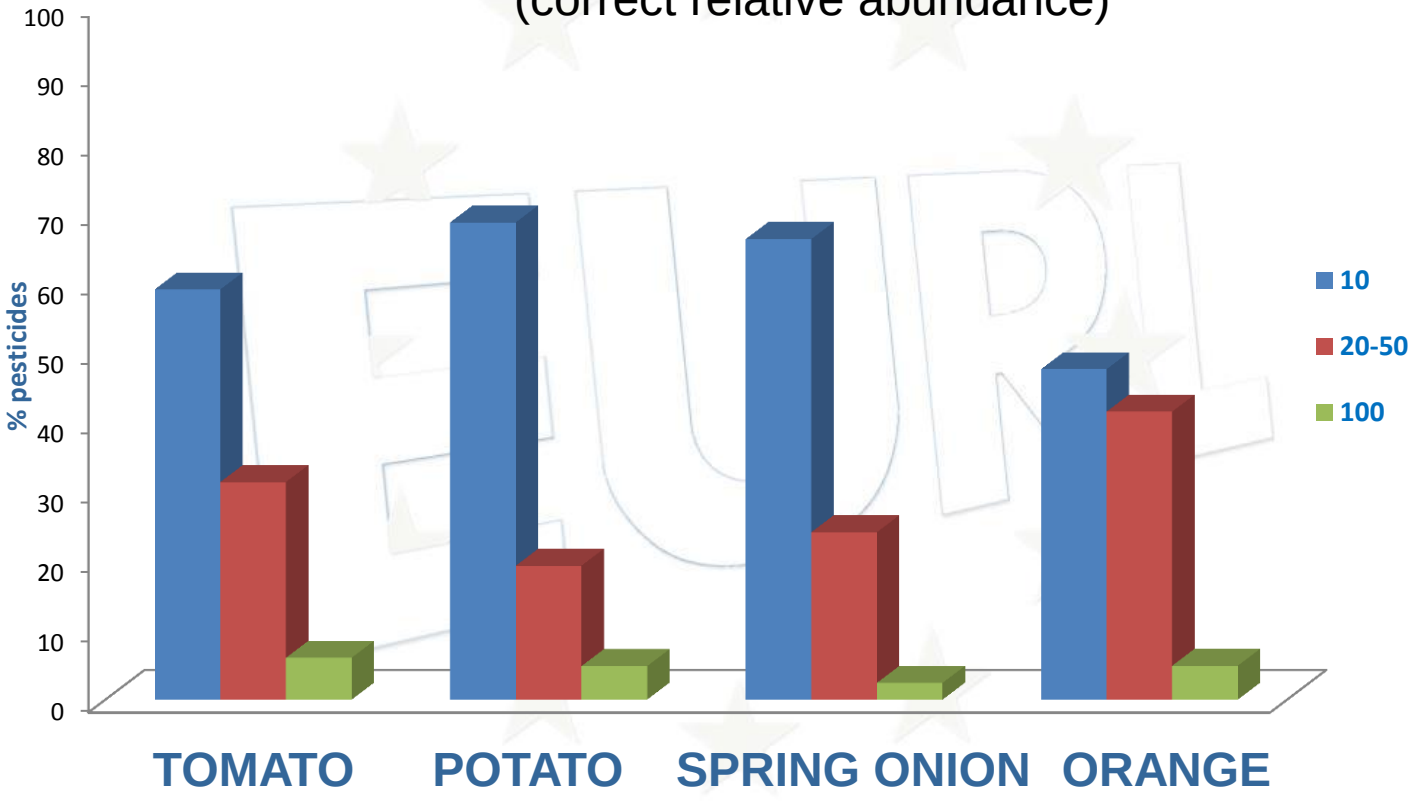
	10µg/Kg	20µg/Kg	50µg/Kg	100µg/Kg
Absolute Abundances				
264.0232	***26643.72***	***49810.53***	***124512.23***	***196694.05***
306.0702	15638.56	28918.62	87336.68	146209.05
186.0406	5121.20	8316.86	23355.94	48066.36
Relative Abundances to base peak 264.0232				
306.0702	59%	58%	70%	74%
186.0406	19%	17%	19%	24%

ACCURACY (%RSD, n =10)



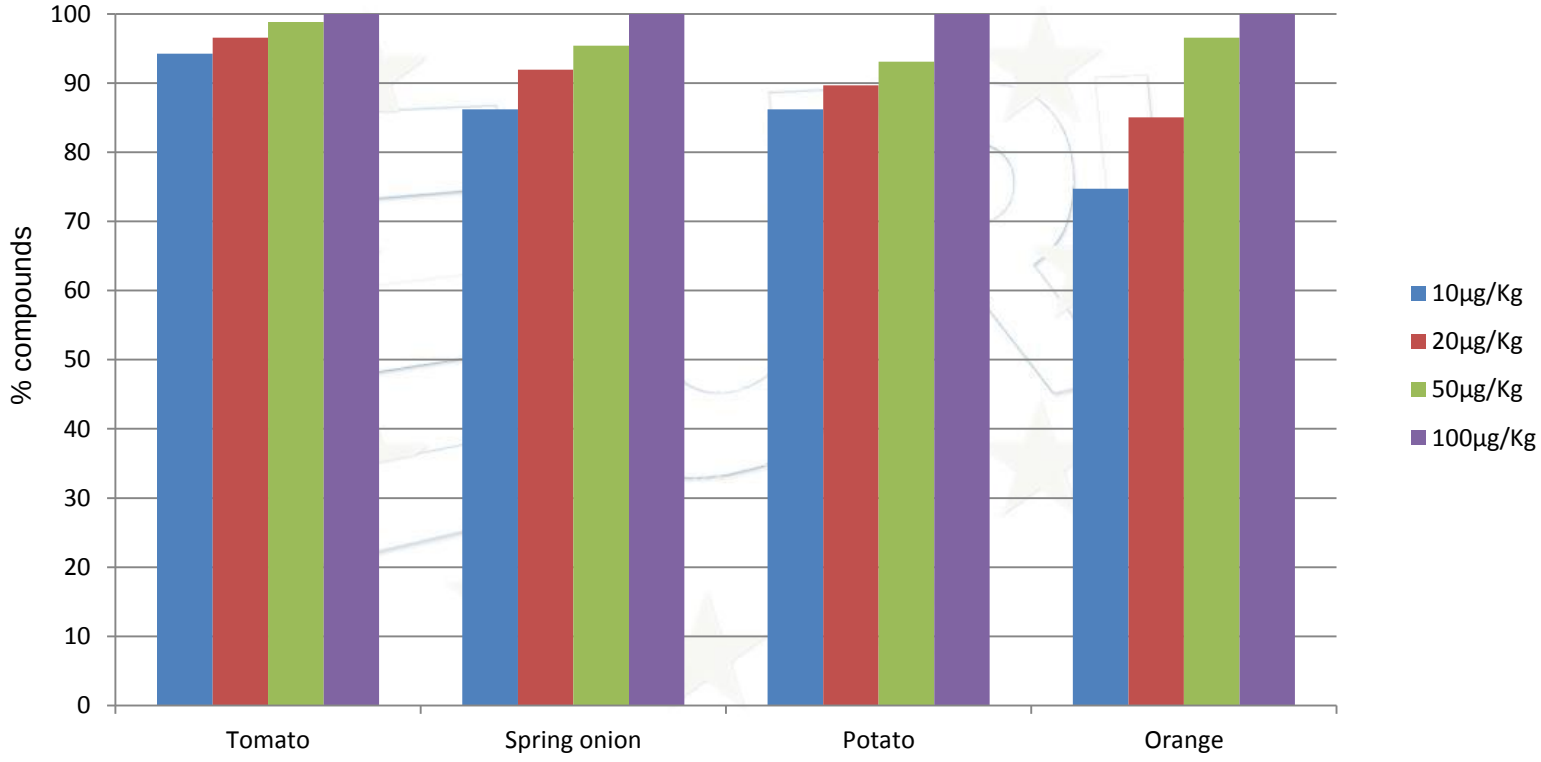
LIMITS OF DETECTION

Retention time and at least two fragments with error lower than 5 ppm (correct relative abundance)



LIMITS OF DETECTION

Retention time and at least one fragment with error lower than 5 ppm (other fragments with error higher than 5 ppm but with the correct relative abundance)

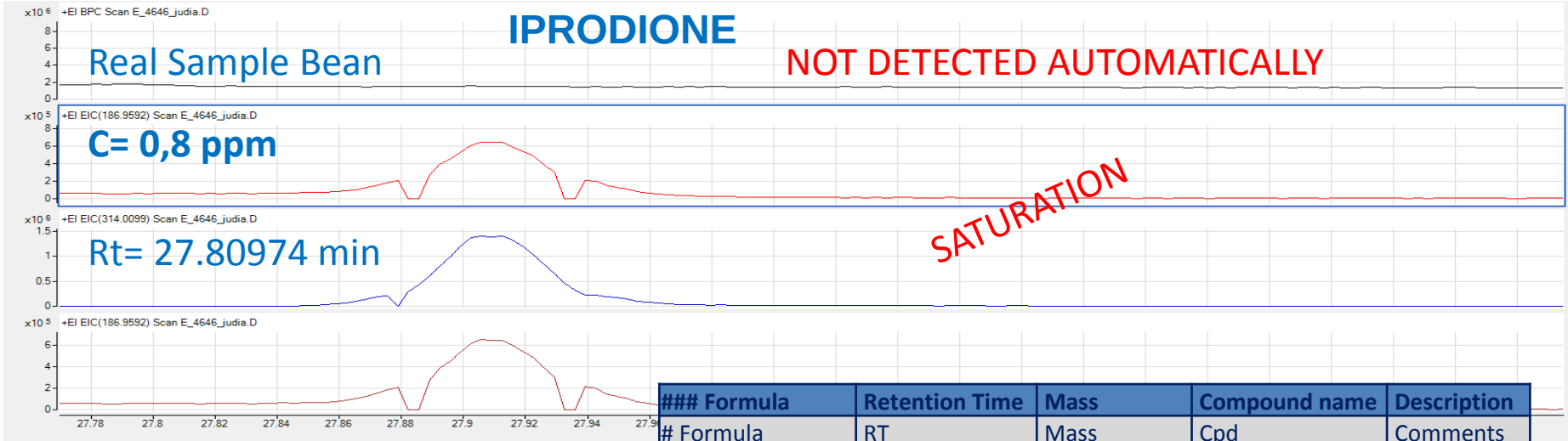


REAL SAMPLES

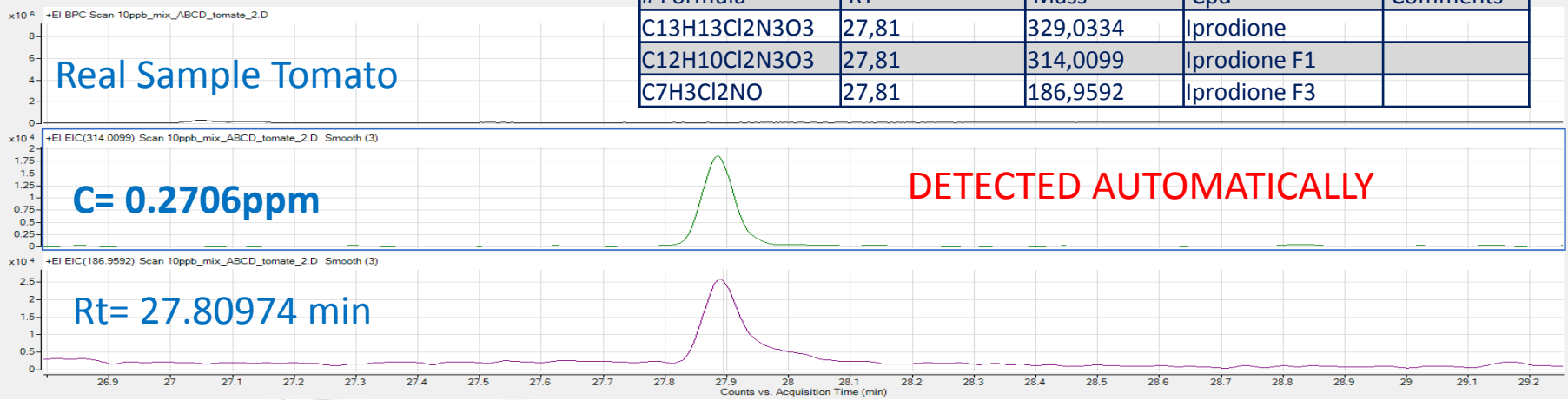
Muestra	GC-TOF automatico	GCQQQMS	GC-TOF manual
Pepper	Boscalid (0.25mg/Kg)	Boscalid(0.23mg/Kg)	
Tomato_1	Iprodione (0.20mg/Kg)	Iprodione(0.27mg/Kg)	
	Pyriproxifen (0.054mg/Kg)	Pyriproxifen (0.062mg/Kg)	
Tomato_2	**Bifenthrin 1F(0,12**mg/Kg)	**Bifenthrin (0.13mg/Kg)**	**Ion 166.099 error higher than10 ppm**
Melon_1	Bupirimate (0.15mg/Kg)	Bupirimate(0.13mg/Kg)	
Tomato_3	Buprofezin(0.49mg/Kg)	Buprofezin(0.16mg/Kg)	
	Metalaxyl(0.060mg/Kg)	Metalaxyl(0.068mg/Kg)	
Zucchini	Pirimicarb(0.33mg/Kg)	Pirimicarb(0.24mg/Kg)	
	Bupirimate(0.11mg/Kg)	Bupirimate(0.048mg/Kg)	
	Penconazole(0.091mg/Kg)	Penconazole(0.074mg/Kg)	
Brussels sprouts	Boscalid(0.21mg/Kg)	Boscalid(0.12mg/Kg)	
Tomato_4	**Bifenthrin (0.10mg/Kg)**	**Bifenthrin (0.125mg/Kg)**	**Ion 166.099 error higher than10 ppm**
Bean_1*	**Iprodione 1F(0.830mg/Kg)**	**Iprodione(1.02mg/Kg)**	**Saturation***
Bean_2	Azoxystrobin ND	Azoxystrobin(0.337mg/Kg)	**Coelution**
	Iprodione(0.36mg/Kg)	Iprodione(0.25mg/Kg)	
Bean_3	Azoxystrobin ND	Azoxystrobin(0.11mg/Kg)	**Coelution**
	Iprodione(0.19mg/Kg)	Iprodione(0.078mg/Kg)	
Melon_2	Azoxystrobin ND	Azoxystrobin(0.16mg/Kg)	**Coelution**
	Bupirimate(0.11mg/Kg)	Bupirimate(0.08mg/Kg)	
Bean_4*	Iprodione ND(1.82mg/Kg)	**Iprodione(1.70mg/Kg)**	**Saturation***
Bean_5*	Iprodione ND(1.27mg/Kg)	**Iprodione(1.70mg/Kg)**	**Saturation***
Grape	Iprodione(0.15mg/Kg)	Iprodione(0.11mg/Kg)	
	Myclobutanil 0.041mg/Kg	Myclobutanil(0.041mg/Kg)	

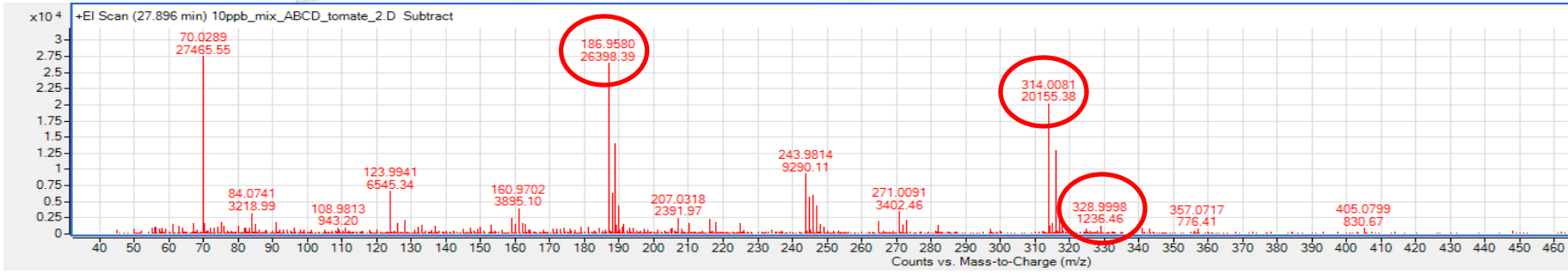
***These samples were injecting diluted 1:2 to obtain a correct identification and quantification and the mass spetrum show at least 2 fragments ions.**

IPRODIONE



### Formula	Retention Time	Mass	Compound name	Description
# Formula	RT	Mass	Cpd	Comments
C13H13Cl2N3O3	27,81	329,0334	Iprodione	
C12H10Cl2N3O3	27,81	314,0099	Iprodione F1	
C7H3Cl2NO	27,81	186,9592	Iprodione F3	



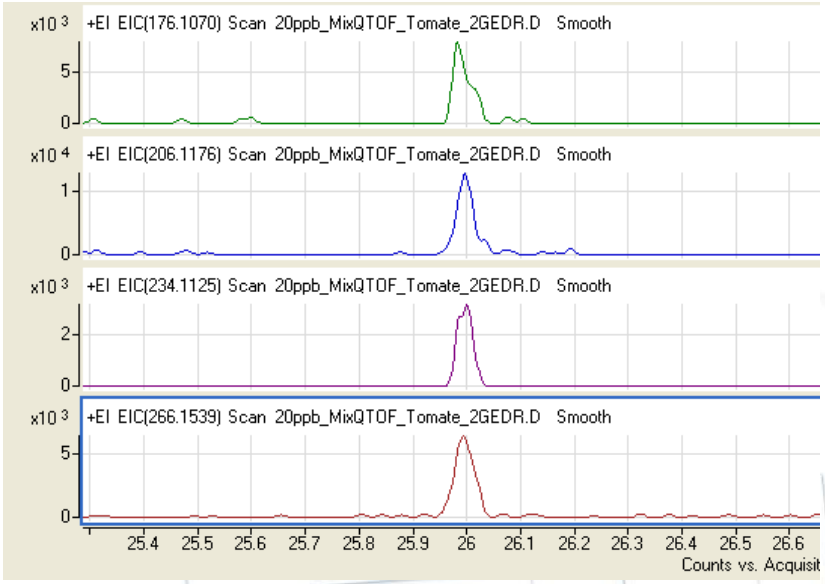


The system has 2 modes of operation :

- **2GHz Extended Dynamic Range (EDR) mode**
- **4Ghz High Resolution (HR) mode**

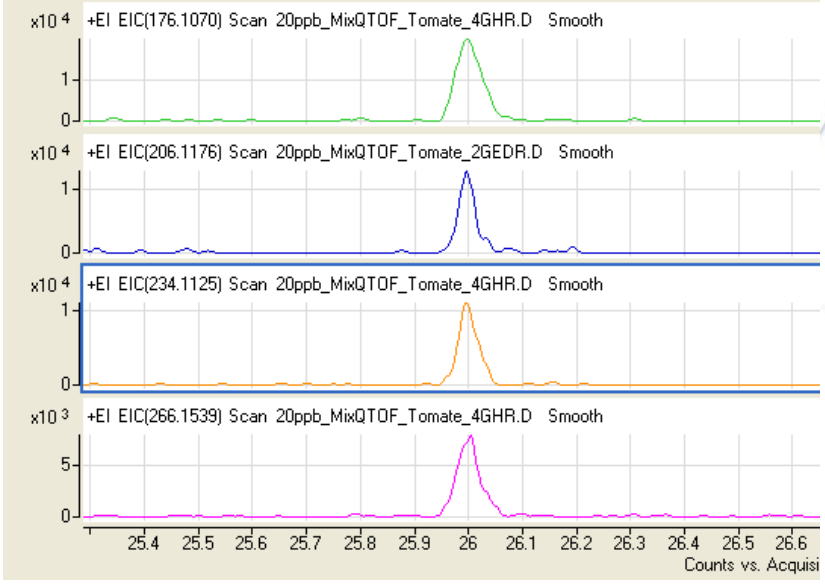
Benalaxyl

2GHz



Fragment	Molecular formula	Experimental mass	Theorethical mass	Error (ppm)
Benalaxyl F1	C18 H20 N O	266,1542	266,1539	0,83

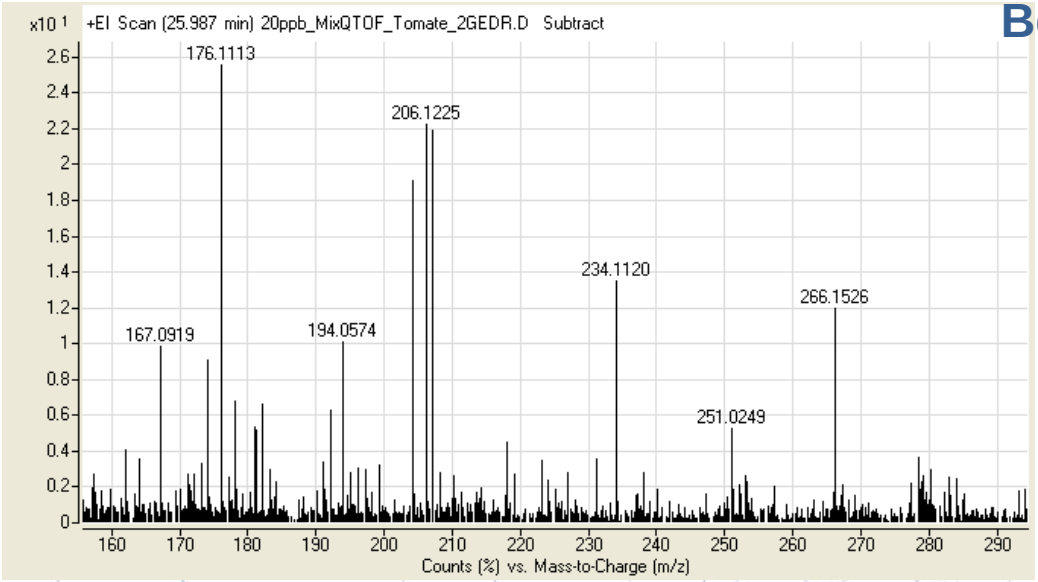
4GHz



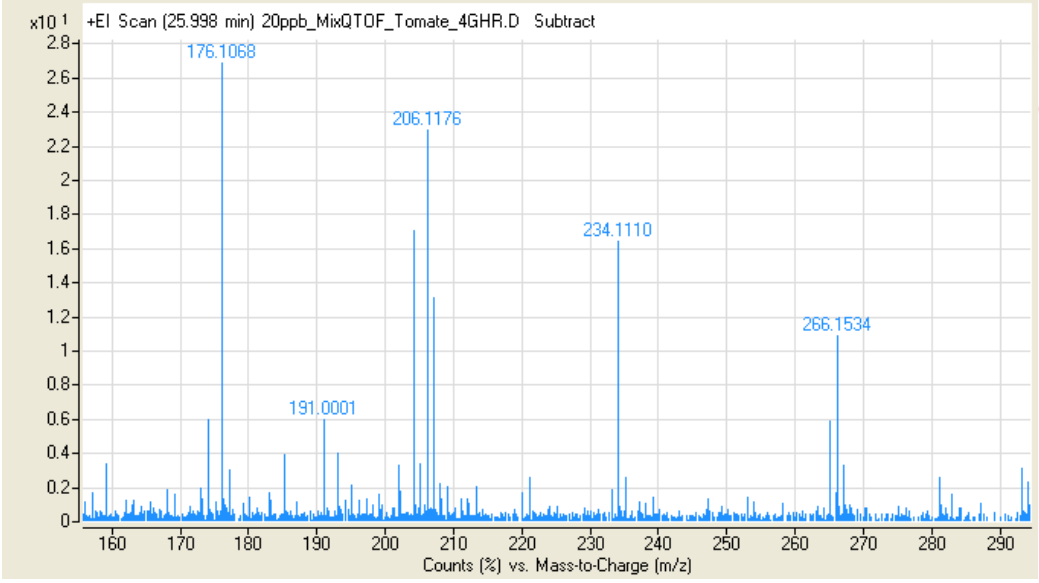
Fragment	Molecular formula	Experimental mass	Theorethical mass	Error (ppm)
Benalaxyl F1	C18 H20 N O	266,152	266,1539	0,83
Benalaxyl F2	C13 H16 N O3	234,112	234,1125	-2,19
Benalaxyl F4	C12 H16 N O2	206,1174	206,1176	-0,81
Benalaxyl F5	C11 H14 N O	176,1073	176,107	1,67

Benalaxyl

2GHz

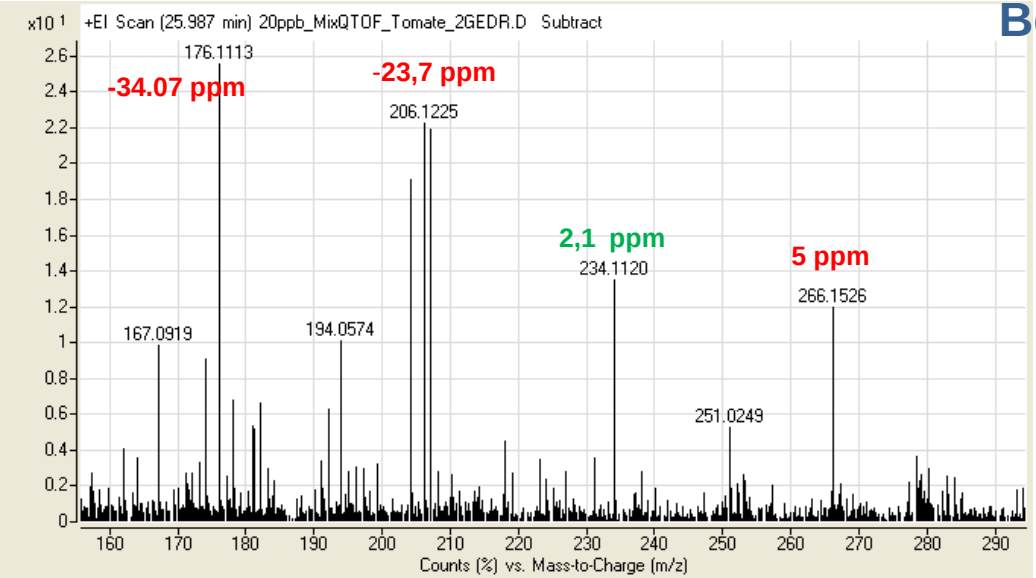


4GHz

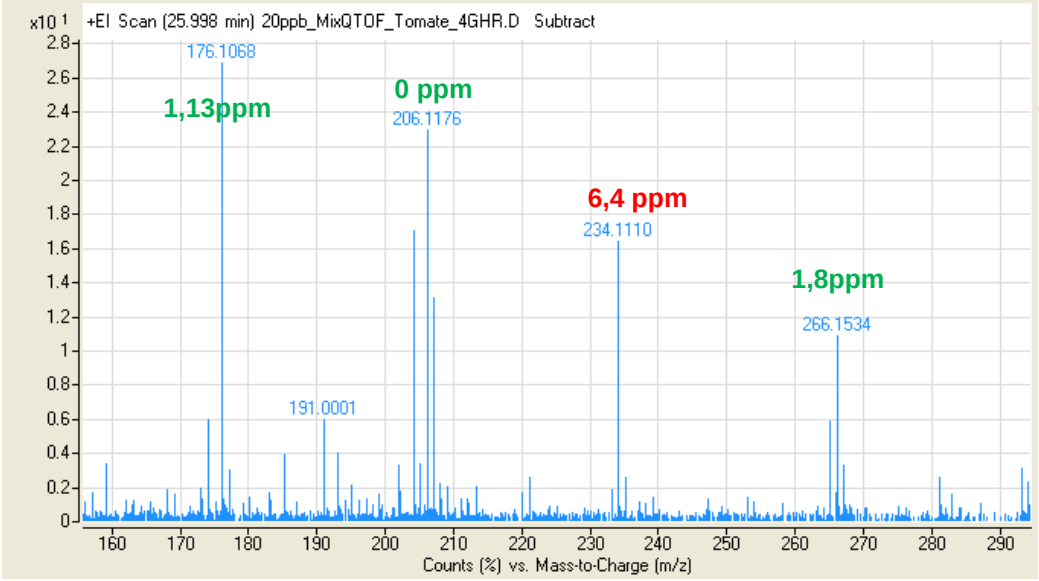


Benalaxyl

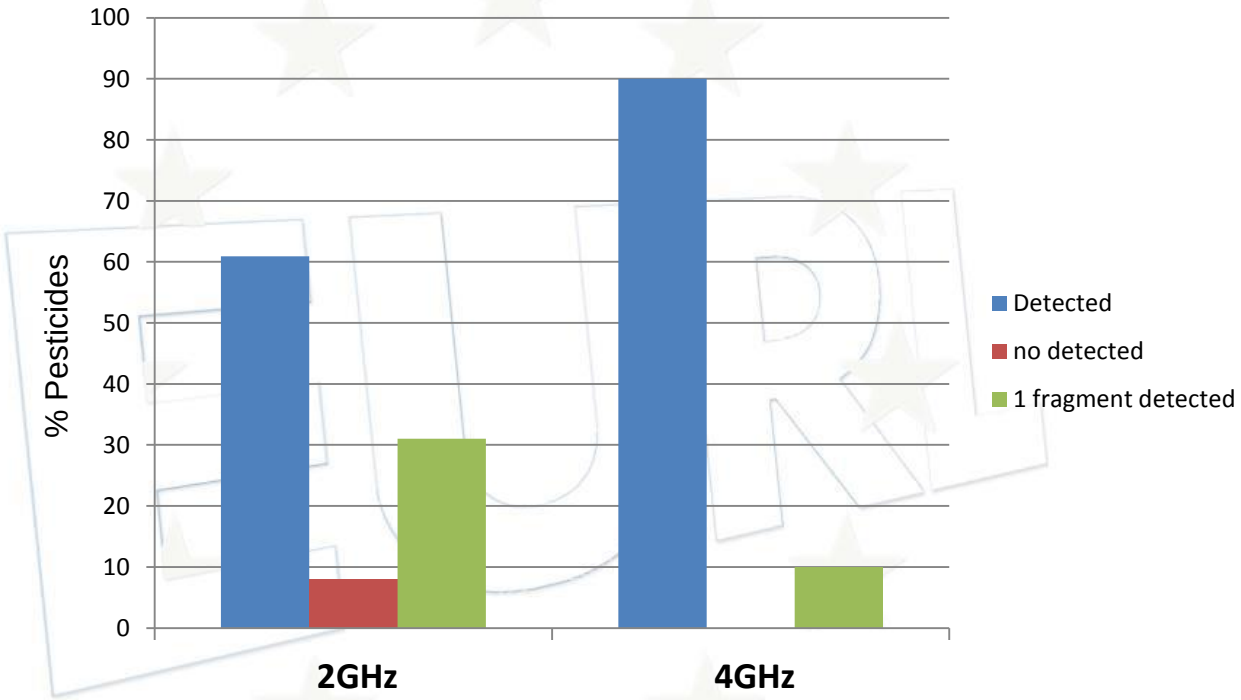
2GHz



4GHz



20 ppb in Tomato
Automatic identification



CONCLUSIONS

- 15 real samples were processed with the developed method, results were compared with those obtained by GC-QQQ-MS, from a total of 22 positives 14 were correctly identified automatically, and three pesticides were not identified correctly by GCQTOF because the high concentration saturate the detector, five pesticide were not correctly identified because the some of the ions showed errors higher than 10 ppm.

- This work is a first approach to the accurate mass database for automatic identification and quantification in GC-TOF-MS. It can be considered as a starting point for a large database and for an improved methodology for automatic identification.

- High Resolution (HR) mode can be considered as a alternative to improve the effectiveness of the automatic identification.