Atmospheric Pressure Chemical Ionisation as an alternative ion source for the analysis of problematic pesticides by GC-QQQ Mass Spectrometry.

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Summary

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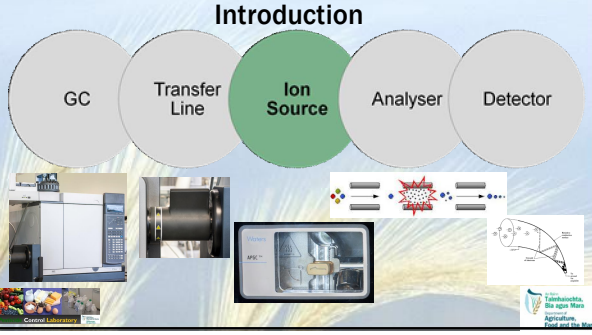
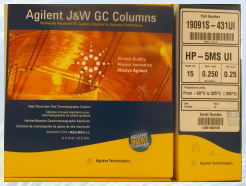
- Å2. GC Method
- Å3. Ionisation processes
- Å4. Difficult pesticides
- Å5. Method Development ï Fruit & Vegetables
- Å6. Method Validation
- Å7. APCI v EI - sample comparison
- Å8. Conclusion and future developments

Introduction



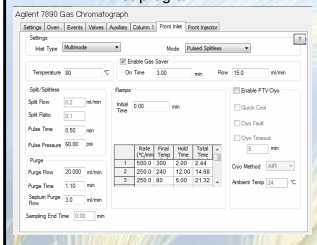
GC Method

- ÅAgilent J&W HP5 MS UI column
- Å15m with retention gap
- Å0.25mm (narrowbore) x 250mm
- ÅPulsed splitless injection
- Å2µl injection volume
- ÅConstant flow

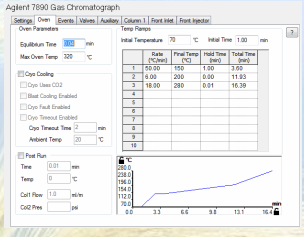


GC Method

Inlet program



GC Temperature program

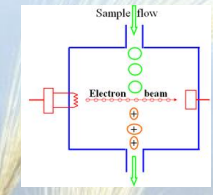


Introduction

Primary ion production

1. Electron impact (EI) ionisation
2. Chemical Ionisation ï Positive and Negative (PCI & NCI)
3. Atmospheric Pressure Chemical Ionisation (APCI)

Electron Impact Ionisation



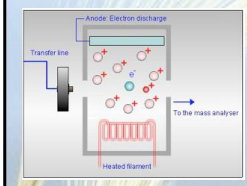
Â1. The sample is introduced into the ion source from the GC ï under vacuum

Â2. An electron beam (70 eV) passes through the sample causing ionisation / fragmentation

Â3. The ions are focussed into a beam and pass into the analyser.

Â4. In the case of QQQs secondary fragmentation occurs in the collision cell.

Chemical Ionisation



Â1. The sample is introduced into the ion source from the GC ï under vacuum

Â2. The sample is mixed with a reagent gas such as NH_4

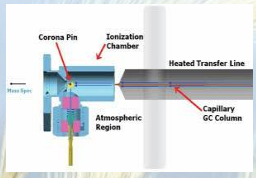
Â3. An electron beam (70 eV) passes through the reagent gas causing ionisation of the gas

Â4. Collisions between the ionised reagent gas and the sample causes ionisation of the sample

Â3. The ions are focussed into a beam and pass into the analyser.

Â4. In the case of QQQs secondary fragmentation occurs in the collision cell.

Atmospheric Pressure Chemical Ionisation



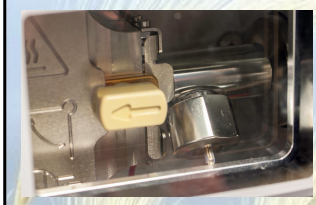
1. The effluent from the GC is mixed with a make-up gas ï high purity N_2

2. The gaseous mixture passes over a charged corona pin which induces ionisation in the make-up gas producing an N_2^+ plasma

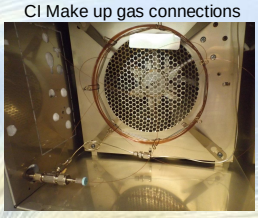
3.Collisions between the charged N_2^+ molecules and the sample induce ionisation in the sample

Atmospheric Pressure Chemical Ionisation

APCI Source



CI Make up gas connections



Atmospheric Pressure Chemical Ionisation

Advantages:

1.Softer ionisation

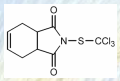
2.A higher probability of more unique ions

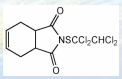
3.Can switch between GC and HPLC in minutes

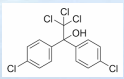
Comparison of ionisation mechanisms

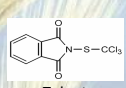
	EI	CI	APCI
Ionisation	Hard	Soft	Soft
Molecular ion formation	Poor	OK	Good
Charge	+	+ / -	+

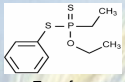
The Good, The Bad & The Ugly

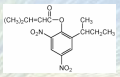

Captan


Captafol


Dicofol


Folpet


Fonofos


Binapacryl

The mix from Hell !!

Why are these pesticides difficult ?

Å1. Liner effects:
- ÅActive liners can adsorb some difficult pesticides

Å2. Fragmentation/degradation:
- ÅPrimary ionisation results in ions which are not necessarily unique

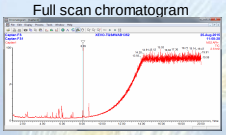
Å3. Matrix:
- ÅInterference

Method Development

Å1. MSScan method to determine the Retention Time & the parent ion
Å2. Daughter ion scan to determine possible product ions
Å3. Optimisation of cone voltage and collision energy
Å4. Build MS Method & check linearity
Å5. Validation

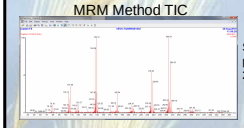
Captan

Full scan chromatogram



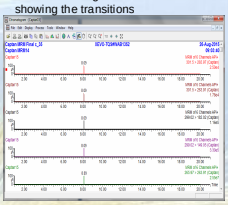
RT at 8 mins

MRM Method TIC

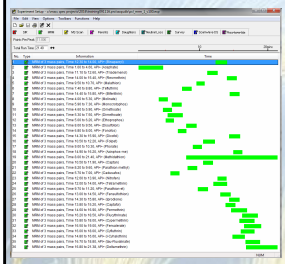


Select parent ion at 264

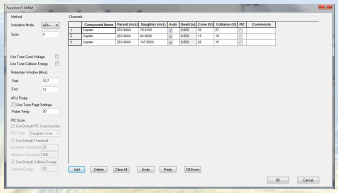
Results of Daughter ion scan showing the transitions



MS Method



Optimised transitions in compound dependent time windows



Comparison of Transitions

Pesticide	Mass	APCI	EI
Captan	300.6	263.9 → 79.01 263.9 → 94.9 263.9 → 147.95	79 → 77 79 → 51
Captafol	349.1	311.78 → 79.0 311.78 → 116.86 311.78 → 160.81	150.9 → 78.9 150.9 → 51.0 150.9 → 150.9
Dicofol	370.5	252.92 → 112.98 252.92 → 138.96 252.92 → 140.96	139 → 111 139 → 75
Folpet	296.6	259.81 → 94.91 259.81 → 102.01 259.81 → 129.99	147 → 103.1 147 → 76
Fonofos	246.3	246.342 → 80.92 246.344 → 125.99 246.346 → 108.97	246 →109.1 246 →81.1
Binapacryl	290.8	222.02 → 146.03 222.02 → 158.02 222.02 → 175.04	82.9 → 55.1 82.9 → 39

In general APCI transitions are ñmore uniqueò than those in EI

Validation plan

	Analyst 1				Analyst 2			
Sp Level ppb →	10	20	50	250	10	20	50	250
Apple	√	√	√	√	√	√	√	√
Orange	√	√	√	√	√	√	√	√
Sweet Potato	√	√	√	√	√	√	√	√

ÅRepeatability ï 6 spikes per set
ÅTotal = (3 x 8) = 24
ÅDifferent analysts
ÅDifferent days

ÅCalculations:
1. Repeatability mean, standard deviation and % RSD.
2. Reproducibility mean, standard deviation and % RSD
3. Linearity
4. Reporting Limits

Linearity

	Name	R^2
1	Molinate	0.99
2	Cadusafos	1.00
3	Dimethoate (1)	0.99
4	Tefluthrin	0.99
5	Parathion methyl	1.00
6	Malathion	0.99
7	Parathion ethyl	0.99
8	Captan	0.96
9	Folpet	0.99
10	Iprodione	0.94

	Name	R2
11	Bifenthrin	0.94
12	Azinphos methyl	0.90
13	Permethrin (1)	0.91
14	Cyfluthrin	0.99
15	Cypermethrin	0.95
16	Flucythrinate	0.96
17	Dicofol	0.94
18	Omethoate	0.99
19	Acephate	0.99
20	Fonofos	0.98

	Name	R^2
21	Fensulfothion	0.90
22	Ethoprophos	1.00
23	Triademenol	0.99
24	Nitrofen	0.91
25	tau-Fluvalinate	0.96
26	Fenvalerate	0.97
27	Deltamethrin	0.99
28	Tetramethrin	0.99
29	Binapacryl	0.97
30	l-Cyhalothrin	0.97
31	Captafol	0.90

Reproducibility -- Apple

#	Name	10 ppb WC	10 ppb ED	20 ppb WC	20 ppb ED	50 ppb WC	50 ppb ED	250 ppb WC	250 ppb ED	Mean	s	%RSD
1	Molinate	116.7	108.0	121.7	112.9	111.3	118.1	119.4	118.0	116.0	4.4	3.8
2	Cadusafos	104.9	92.0	100.1	94.8	100.4	103.1	108.0	109.8	101.6	6.1	6.0
3	Dimethoate (1)		98.8		112.1		110.4		110.3	107.9	6.1	5.7
4	Tefluthrin	95.9	94.4	98.4	93.2	103.4	107.3	111.5	103.3	100.9	6.5	6.5
5	Parathion methyl	123.8	100.0	105.3	100.0	109.6	110.5	105.0	107.9	107.8	7.6	7.0
6	Malathion	104.4	105.0	99.6	97.2	100.8	102.3	110.5	109.5	103.7	4.7	4.5
7	Parathion ethyl	109.2	92.3	111.7	99.2	104.1	103.4	115.3	103.5	104.8	7.3	6.9
8	Captan	113.7	#VALUE!	106.5	172.5	98.0	110.9	107.5	110.9	117.1	24.9	21.3
9	Folpet	99.3	89.2	100.9	90.0	110.8	110.1	114.1	107.0	102.7	9.5	9.2
10	Iprodione	89.3	106.8	89.3	92.2	107.8	115.6	101.7	97.1	100.0	9.6	9.6
11	Bifenthrin	96.4	86.3	108.0	83.0	123.4	125.5	121.5	119.6	108.0	17.3	16.0
12	Azinphos methyl	######	86.6	110.7	96.5	105.5	105.6	104.2	91.5	100.1	8.7	8.7
13	Permethrin (1)	98.5	91.5	156.7	80.1	89.2	121.1	115.5	105.3	107.2	24.2	22.5
14	Cyfluthrin	95.3	98.8	107.8	93.9	104.7	116.1	106.0	109.6	104.0	7.6	7.3
15	Cypermethrin	######	82.8	115.1	79.9	118.7	115.9	99.2	98.6	101.5	15.9	15.7
16	Flucythrinate	102.7	97.6	95.1	91.9	110.8	116.7	101.2	105.9	102.7	8.2	8.0
17	Dicofol	85.8	113.8	93.5	110.2	109.3	117.4	134.0	118.0	110.0	15.3	13.9
18	Omethoate	67.3	96.5	83.4	97.3	93.1	99.3	98.8	99.9	92.0	11.3	12.1
19	Acephate	120.3	102.8	111.1	94.7	102.2	111.2	102.3	101.1	105.7	8.0	7.6
20	Fonofos	177.0	145.5	132.0	135.8	135.3	155.4	142.6	148.9	146.6	14.6	9.9
21	Fensulfothion	132.2	114.3	134.3	117.5	125.3	128.1	118.3	128.8	123.6	8.1	6.5
22	Ethoprophos	99.7	103.6	95.3	103.0	104.1	108.8	119.1	106.6	105.0	7.0	6.7
23	Triademenol	93.5	93.0	88.0	89.1	97.4	101.9	102.4	100.6	95.9	5.5	5.7
24	Nitrofen	109.4	94.4	86.3	91.4	97.6	114.8	119.7	112.2	103.2	12.3	11.9
25	tau-Fluvalinate	103.2	116.2	100.1	84.0	107.8	122.4	105.2	113.0	106.5	11.7	11.0
26	Fenvalerate	82.2	125.6	95.2	102.3	123.5	127.7	125.5	120.6	112.8	17.2	15.3
27	Deltamethrin	97.1	103.7	93.6	83.6	109.6	102.9	105.7	96.8	99.1	8.2	8.3
28	Tetramethrin	89.2	117.8	89.2	107.9	106.3	113.3	112.8	103.6	105.0	10.7	10.2
29	Binapacryl	107.0	93.9	132.9	93.9	106.2	117.5	115.1	105.3	108.9	12.8	11.8
30	l-Cyhalothrin	126.4	124.7	106.9	101.1	102.7	109.5	104.1	95.0	108.8	11.2	10.3
31	Captafol	97.5	#VALUE!	95.7	119.9	66.2	113.9	115.6	131.3	105.7	21.4	20.2

Reproducibility -- Orange

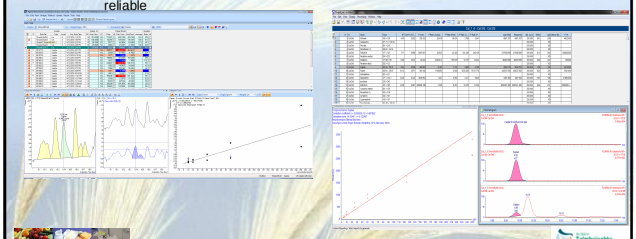
#	Name	10 ppb WC	10 ppb ED	20 ppb WC	20 ppb ED	50 ppb WC	50 ppb ED	250 ppb WC	250 ppb ED	Mean	s	%RSD
1	Molinate	74.8	66.9	85.9	82.8	99.1	109.6	121.4	115.7	94.7	19.9	21.0
2	Cadusafos	41.3	52.2	75.5	73.2	81.9	98.3	98.1	130.0	81.3	28.0	34.4
3	Dimethoate (1)	67.8	59.7	77.0	76.5	88.6	102.4	104.9	109.2	85.8	18.4	21.4
4	Tefluthrin	60.8	70.3	85.5	83.8	92.7	107.1	114.1	119.9	91.8	20.9	22.8
5	Parathion methyl	58.8	60.9	81.8	79.8	91.0	112.1	113.6	119.2	89.6	23.5	26.3
6	Malathion	92.1	67.9	95.5	87.2	118.1	118.9	112.3	121.4	101.7	19.1	18.8
7	Parathion ethyl	66.4	47.6	85.0	87.5	86.8	115.4	109.5	111.4	88.7	23.5	26.5
8	Captan	26.6	67.8	50.2	53.4	58.6	84.9	94.2	123.1	76.0	26.4	34.7
9	Folpet	58.5	102.6	84.0	98.6	90.4	113.5	119.6	127.5	105.2	15.8	15.0
10	Iprodione	76.1	111.3	79.7	101.5	101.9	124.8	97.7	109.3	100.3	16.1	16.1
11	Bifenthrin	69.8	80.0	73.9	74.1	94.2	98.5	93.8	90.9	84.4	11.2	13.2
12	Azinphos methyl	102.3	#VALUE!	62.3	460.7	87.4	101.5	93.5	96.3	143.4	152.7	106.5
13	Permethrin (1)	64.4	234.5	91.7	90.9	103.7	107.1	102.8	105.3	112.5	51.2	45.5
14	Cyfluthrin	98.2	64.9	86.5	117.7	102.0	93.2	90.2	93.7	93.3	14.9	16.0
15	Cypermethrin	85.5	6666.7	78.4	###### #	25.7	#VALUE!	79.9	83.0	#VALUE!	#VALUE!	#VALUE!
16	Flucythrinate	120.3	166.7	84.5	125.4	83.2	94.2	81.8	87.0	105.4	30.1	28.5
17	Dicofol	98.8	155.3	93.3	146.7	94.9	134.5	112.3	132.5	121.0	24.4	20.2
18	Omethoate	59.1	53.7	67.4	61.6	76.9	90.5	87.7	92.2	73.6	15.2	20.7
19	Acephate	590.8	131.3	82.3	121.4	###### ##	96.6	#VALU E!	119.5	#VALU UE!	#VALU UE!	#VALUE!
20	Fonofos	110.4	104.6	123.1	118.4	145.2	145.8	164.6	153.3	133.2	21.9	16.4
21	Fensulfothion	103.8	126.5	93.0	104.6	126.0	116.9	107.0	104.4	110.3	11.8	10.7
22	Ethoprophos	50.9	64.1	73.2	76.9	82.2	101.6	114.9	114.6	84.8	23.5	27.7
23	Triademenol	67.7	56.4	72.7	89.9	89.2	99.3	102.8	102.5	85.1	17.5	20.5
24	Nitrofen	54.2	69.5	78.1	80.3	104.2	106.4	106.0	114.1	89.1	21.5	24.1
25	tau-Fluvalinate	60.9	98.7	61.7	102.6	78.2	105.5	83.4	103.0	84.0	20.6	24.5
26	Fenvalerate	64.6	56.9	52.3	74.1	103.0 ######	110.3 #VALU	87.6 #VALU	91.0 #VALU	80.0 #VALU	21.4 #VALU	26.7 #VALUE!
27	Deltamethrin	74.7	#VALUE!	79.3	2190.9	##	109.5 E!	100.2 E!	UE!	#VALUE!		
28	Tetramethrin	56.6	114.8	93.9	68.7	105.4	104.5	100.9	121.9	95.8	22.4	23.4
29	Binapacryl	33.6	126.6	53.3	94.0	78.0	100.0	101.6	103.8	93.9	23.0	24.5
30	l-Cyhalothrin	71.0	52.3	74.5	44.8	92.7	98.4	91.6	99.8	78.1	21.1	27.0
31	Captafol	92.4	#VALUE!	92.8	125.8	91.1	113.4	109.0	132.7	108.2	16.9	15.6

Reproducibility – Sweet Potato

#	Name	10 ppb WC	10 ppb ED	20 ppb WC	20 ppb ED	50 ppb WC	50 ppb ED	250 ppb WC	250 ppb ED	Mean	s	%RSD
1	Molinate	108.1	92.0	119.0	107.1	125.2	117.8	134.4	133	117.1	13.4	11.4
2	Cadusafos	49.4	51.7	68.6	75.1	96.9	87.6	97.0	109.9	79.5	20.7	26.0
3	Dimethoate (1)	60.4	72.9	84.5	80.1	100.6	95.7	103.4	105.8	87.9	15.2	17.2
4	Tefluthrin	56.6	50.5	72.7	69.6	93.1	88.4	102.2	103.6	79.6	18.9	23.8
5	Parathion methyl	66.5	48.5	85.4	69.6	96.0	91.4	95.9	107.5	82.6	18.2	22.0
6	Malathion	44.3	70.8	66.6	72.5	92.2	96.5	87.6	109.6	80.0	19.2	24.0
7	Parathion ethyl	62.9	67.1	74.8	60.3	97.8	99.5	100.7	110.4	84.2	18.7	22.2
8	Captan	126.5	69.7	101.9	56.8	76.6	71.8	92.9	103.5	87.5	21.3	24.4
9	Folpet	57.2	58.7	75.7	65.8	97.4	92.9	98.0	99.3	80.6	17.2	21.3
10	Iprodione	70.5	70.1	73.6	51.6	93.1	121.5	91.4	98.1	83.8	20.2	24.2
11	Bifenthrin	58.3	57.6	72.5	70.0	100.1	108.2	92.7	98.1	82.2	18.6	22.7
12	Azinphos methyl	92.0	96.0	102.5	104.1	92.4	153.1	105.2	100.2	105.7	18.5	17.5
13	Permethrin (1)	85.3	156.5	57.4	110.3	112.0	131.7	97.8	106.6	107.2	27.6	25.8
14	Cyfluthrin	65.4	81.9	76.2	94.6	90.2	94.1	76.3	97.0	84.5	10.6	12.5
15	Cypermethrin	115.7	ND	73.7	ND	92.7	108.5	93.1	90.7	95.7	13.5	14.1
16	Flucythrinate	92.9	64.7	105.7	79.3	99.5	98.3	82.0	94.1	89.6	12.5	13.9
17	Dicofol	49.8	61.1	103.8	62.5	97.1	107.0	101.6	109.4	86.5	22.8	26.3
18	Omethoate	72.9	40.9	65.8	62.1	85.5	84.4	89.4	104.7	75.7	18.4	24.3
19	Acephate	99.9	95.1	80.1	100.5	102.2	116.5	99.7	106.9	100.1	9.6	9.6
20	Fonofos	153.4	138.6	119.0	141.3	146.7	166.7	143.9	168.2	147.2	14.9	10.1
21	Fensulfothion	55.5	128.2	127.7	116.8	86.1	139.0	103.1	135.7	111.5	26.8	24.1
22	Ethoprophos	55.8	42.2	77.8	74.0	91.8	86.2	91.7	107.5	78.6	19.9	25.3
23	Triademenol	61.4	82.3	77.4	73.6	92.4	104.4	89.1	117.4	87.3	16.6	19.0
24	Nitrofen	53.3	68.7	65.5	80.6	102.4	101.4	99.6	103.4	84.4	18.7	22.1
25	tau-Fluvalinate	119.4	72.4	101.5	77.1	105.6	102.7	92.6	98.3	96.2	14.3	14.9
26	Fenvalerate	64.1	75.9	72.9	75.9	110.6	108.4	91.1	79.0	84.7	15.9	18.8
27	Deltamethrin	106.2	101.4	90.5	93.9	115.5	98.3	89.6	100.9	99.5	8.0	8.1
28	Tetramethrin	74.8	101.4	94.0	94.1	120.5	98.1	88.1	100.6	96.5	12.1	12.6
29	Binapacryl	54.9	78.3	70.2	85.4	125.4	90.7	86.6	103.7	86.9	19.9	22.9
30	l-Cyhalothrin	88.5	78.5	94.1	89.4	130.4	102.7	85.1	99.1	96.0	14.9	15.5
31	Captafol	38.7	ND	77.2	101.9	135.4	111.5	83.6	102.6	93.0	28.3	30.4

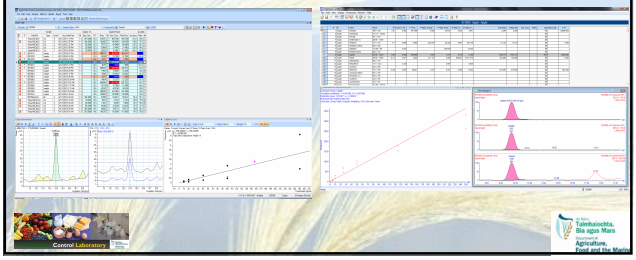
Captan – Standard Comparison

EI data very poor and unreliable ï APCI data much cleaner and more reliable



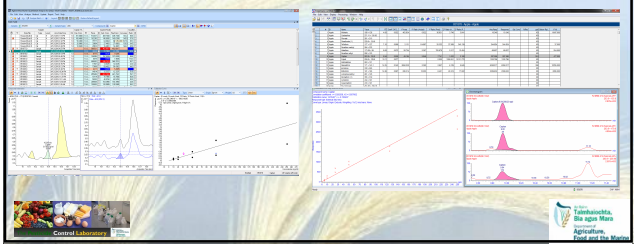
Captan - Apple comparison

Reasonable EI data confirmed by APCI



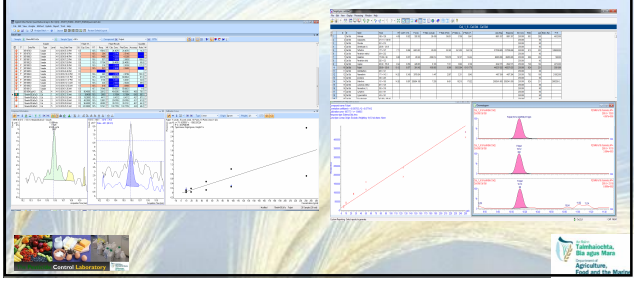
Captan - Apple comparison 2

Very poor EI data but APCI data excellent ï possible false negative avoided !!

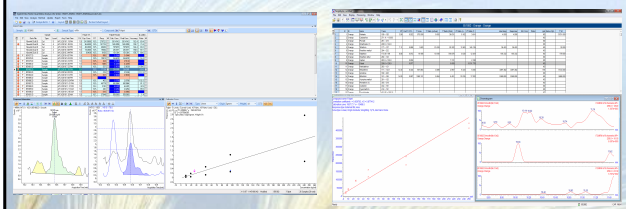


Folpet – Standard Comparison

Poor EI data v excellent APCI data



Folpet – Orange Comparison



EI data easily misinterpreted v APCI data with clear interpretation ï false positive avoided !!

Future work

- ÅExpand the method to:
  - ÅCereals
  - ÅMilk and eggs
  - ÅHoney
  - ÅInfant formula
  - ÅFats
- ÅExpand scope of pesticides and metabolites
- ÅAccreditation

Conclusion

- ÅHave we tamed the mix from Hell ?
  - Probably not but they are much calmer and the data is much more reliable with this method
- ÅThis work is in itõ infancy ï work in progress
- ÅThe number of false positive and false negatives is reduced greatly
- ÅThis technique has potential
- ÅCould also work for difficult LC compounds


Thank you for your attention
Any Questions