



Multi-Active method

Jim Garvey on behalf of ESPAC



Summary



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MULTI-ACTIVE METHOD FOR THE ANALYSIS OF ACTIVE SUBSTANCES IN FORMULATED PRODUCTS

SCOPE

The method is suitable for determining a range of active substances in a range of formulated product types. The current GC scope is given in [Appendix 1](#) and the current HPLC scope is given in [Appendix 1](#).

1 Sampling: Ideally an intact product should be taken from the market as intended for use with an end user.

2 Identity tests:

2.1 DAD, UV, GC-MS, LC-MS or FTIR or any other suitable technique, depending on the product.

3.1 Active substances:

OUTLINE OF METHOD:

The sample preparation is different depending on the formulation type being [solid](#) or [liquid](#). Procedures are given for solid and liquid formulations. Once the samples are prepared the active substances are [analysed](#) by either gas chromatography with flame ionization detection (GC-FID) or high performance liquid chromatography with UV detector (HPLC-DAD) with internal or external standard calibration.

REAGENTS:

[Analytical standard](#) of known purity, stored in refrigeration.

[Ethyl Acetate](#): HPLC grade

[Acetonitrile](#): HPLC grade

[Water](#): HPLC grade

[Formic Acid](#): HPLC grade

[Dissolved potassium internal standard](#)

[Internal standard solution where applicable](#)

HPLC Mobile Phase: A = 98% Phosphoric acid
B = Acetonitrile

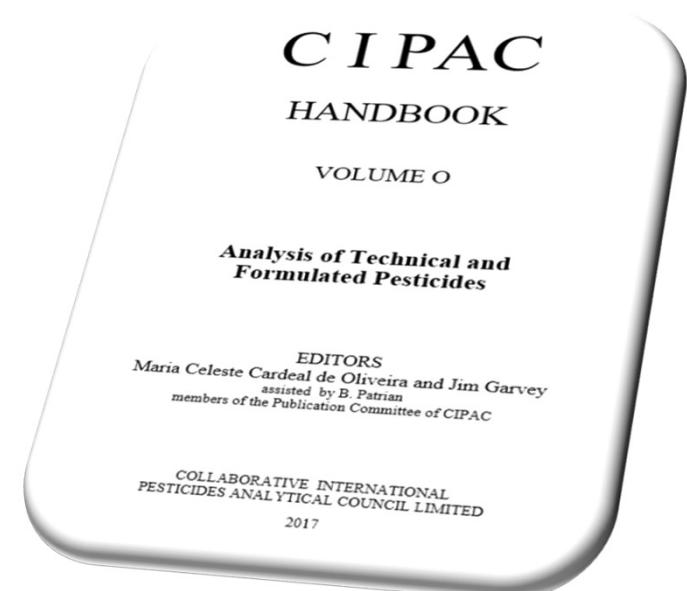
NOTE: [GC analysis with a wider temperature range](#)
[GC analysis with a wider temperature range](#)



ESPAC



- English Speaking Pesticide Advisory Council
- Local PAC within CIPAC
- Government and Industry representatives
- Members from Ireland, U.K., U.S.A,
Australia, South Africa, Switzerland,
Hungary, Czech Republic, Denmark, France,
Belgium and Netherlands





Introduction

- Currently Quality Control laboratories for Formulated products require access to:
 - GC and LC, usually FID and DAD detectors will suffice
 - Multiple GC and LC columns
 - Multiple sample preparation procedures
 - “Every sample’s a project” © DC
 - Deal with products from all companies

And they

- Waste a lot of time switching from one system to another





Introduction



- **Residues laboratories, on the other hand:**
 1. Have a small number of sample preparation procedures
 2. Use a limited number of GC and LC columns
 3. Analyse for a large number of compounds in a single method
 4. Maximise the efficiency of the analytical process by using multi residue methods.
- **Can Formulations Laboratories learn from Residues laboratories ?**





Introduction



- At CIPAC 2018 it was agreed that ESPAC would prepare a draft MAM to be presented at the next CIPAC meeting
- The first draft of the method was presented at the ESPAC meeting in October 2018

CIPAC

COLLABORATIVE INTERNATIONAL PESTICIDES ANALYTICAL COUNCIL

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The abbreviation CIPAC stands for Collaborative International Pesticides Analytical Council. We are an international, non-profit-oriented and non-governmental organization (see Contact) devoted to:

- promote the international agreement on methods for the analysis of pesticides and physico-chemical test methods for formulations;
- promote inter-laboratory programmes for the evaluation of test methods.

The methods are proposed by companies and are tested by laboratories all over the world. After evaluation of the results and adoption, the methods are published in the CIPAC Handbooks (see "CIPAC Methods" and "CIPAC Publication").

CIPAC MEETING 2019

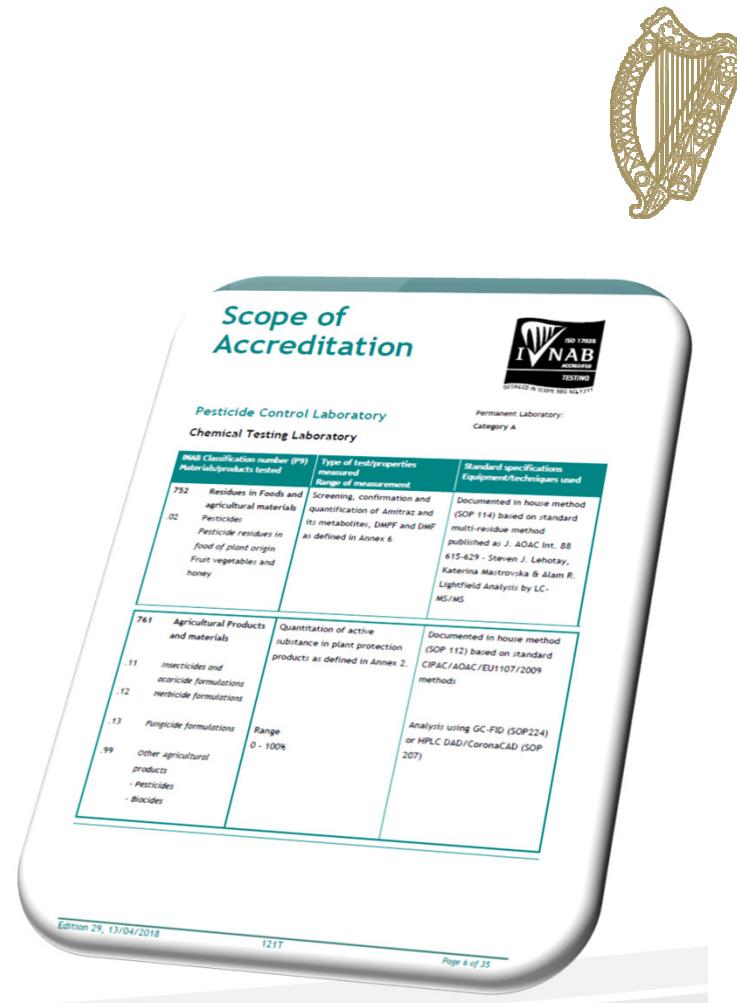
Next CIPAC meeting will be from 11th - 20th June 2019 Braunschweig, Germany





Scope

- The method used in the PCL has been used to cover a wide range of active substances and formulation types
- Each active and product is validated before being added to the scope
- The method is accredited by INAB for a flexible scope - <https://www.inab.ie/> Lab 121T
- The flexible scope must define the active substance, the analytical range and the determination method used.
- For a CIPAC method more information is required in the scope





Scope



Scope of Accreditation



Pesticide Control Laboratory
Chemical Testing Laboratory

Permanent Laboratory:
Category A

ANNEX 2 Quantitation of active substance in plant protection products. GC-FID & LC-DAD					
Pesticide	Range %	Pesticide	Range %	Pesticide	Range %
Fluazifop-butyl	0.1-100	Ioxynil	0.1-100	Metcloazole	0.1-100
Fludiconil	0.1-100	Iprodione	0.1-100	Methiocarb	0.1-100
Flufenacet	0.1-100	Ispururon	0.1-100	Metrabenonol	0.1-100
Flumioxazin	0.1-100	Ispyracazin	0.1-100	Metrabuzin	0.1-100
Fluopicolide	0.1-100	Isoxaben	0.1-100	Metsulfuron (-methyl)	0.1-100
Fluoxastrobe	0.1-100	Isoxafuthole	0.1-100	Myclobutanil	0.1-100
Flupyrsulfuron methyl	0.1-100	Kresoxim methyl	0.1-100	Nicosulfuron	0.1-100
Fluroxypyr	0.1-100	Lambda-cyhalothrin	0.1-100	Otanilactone	0.1-100
Flusilazole	0.1-100	Lenacil	0.1-100	Oxadiazon	0.1-100
Flutolomil	0.1-100	Limuron	0.1-100	Oxyfluorfen	0.1-100
Fluxapyroxad	0.1-100	Malathion	0.1-100	Paclobutrazol	0.1-100
Folpet	0.1-100	MCPA	0.1-100	Pencycuron	0.1-100
Fosetyl (aluminium)	0.1-100	Mecoprop	0.1-100	Pendimethalin	0.1-100
Fosfazate	0.1-100	Mecoprop-P	0.1-100	Penthiopyrad	0.1-100
Glufosinate (L- ammonium)	0.1-100	Mefenpyr Diethyl	0.1-100	Permethrin	0.1-100
Glyphosate	0.1-100	Mepiquat Chloride	0.1-100	Phenmedipham	0.1-100
Imazalil	0.1-100	Mercosulfuron - Methyl	0.1-100	Picolinafen	0.1-100
Imidacloprid	0.1-100	Merothion	0.1-100	Picosystrobin	0.1-100
Imazamox	0.1-100	Metalaxyl M	0.1-100	Pinoxaden	0.1-100
Imazaquin	0.1-100	Metaldehyde	0.1-100	Piperonyl butoxide	0.1-100
Indoxacarb	0.1-100	Metonantron	0.1-100	Pirimicarb	0.1-100
Iodosulfuron-methyl- iodine	0.1-100	Metazachlor	0.1-100	Pirimiphos-me	0.1-100

Flexible scope: Additional substances may be added and ranges extended in accordance with the laboratory's approved and documented procedures. For details refer to the laboratory's master list of flexible scope changes, available from the laboratory.

Edition 29, 13/04/2018

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Scope of Accreditation



Pesticide Control Laboratory
Chemical Testing Laboratory

Permanent Laboratory:
Category A

ANNEX 2 Quantitation of active substance in plant protection products. GC-FID & LC-DAD					
Pesticide	Range %	Pesticide	Range %	Pesticide	Range %
Prochloraz	0.1-100	Spiroxamine	0.1-100	Fluoroxypr-1-methyl heptyl ester	0.1-100
Prodamine	0.1-100	Spirotetramat	0.1-100	MCPB	0.1-100
Prohexadione Calcium	0.1-100	Sulfentrazone	0.1-100	Triisufuron-methyl	0.1-100
Propamocarb	0.1-100	Sulfosulfuron	0.1-100	Picloram	0.1-100
Propagrazofop	0.1-100	Tebucanoazole	0.1-100	Fluopyram	0.1-100
Propiconazole	0.1-100	Tepraloydim	0.1-100	Halausifen Methyl	0.1-100
Propyzamide	0.1-100	Terbutylazine	0.1-100	Maleic Hydrazide	0.1-100
Proquazid	0.1-100	Thiabendazole	0.1-100	Triclopyr	0.1-100
Prosulfofcarb	0.1-100	Thiaclorpid	0.1-100	Triclopyr butoxyethyl ester (BEE)	0.1-100
Prothioconazole	0.1-100	Thiamethoxan	0.1-100		
Pymetrozine	0.1-100	Thifensulfuron (-methyl)	0.1-100		
Pyraclostrobin	0.1-100	Thiophanate-methyl	0.1-100		
Pyrethrins	0.1-100	Tralkoxydim	0.1-100		
Pyridate	0.1-100	Tribenuron (-methyl)	0.1-100		
Pyrimethanil	0.1-100	Trifl oxystrobil	0.1-100		
Pyraflufen-Ethyl	0.1-100	Trifluralin	0.1-100		
Pyroxasulam	0.1-100	Trimecapac ethyl	0.1-100		
Quimerac	0.1-100	Triticinacole	0.1-100		
Quatzolop-P-ethyl	0.1-100	2,4-DB	0.1-100		
Rimsulfuron	0.1-100	2,4 D	0.1-100		
Silthiofam	0.1-100	2,4-D-ethyl heptyl ester	0.1-100		
Spinosad	0.1-100				

Flexible scope: Additional substances may be added and ranges extended in accordance with the laboratory's approved and documented procedures. For details refer to the laboratory's master list of flexible scope changes, available from the laboratory.

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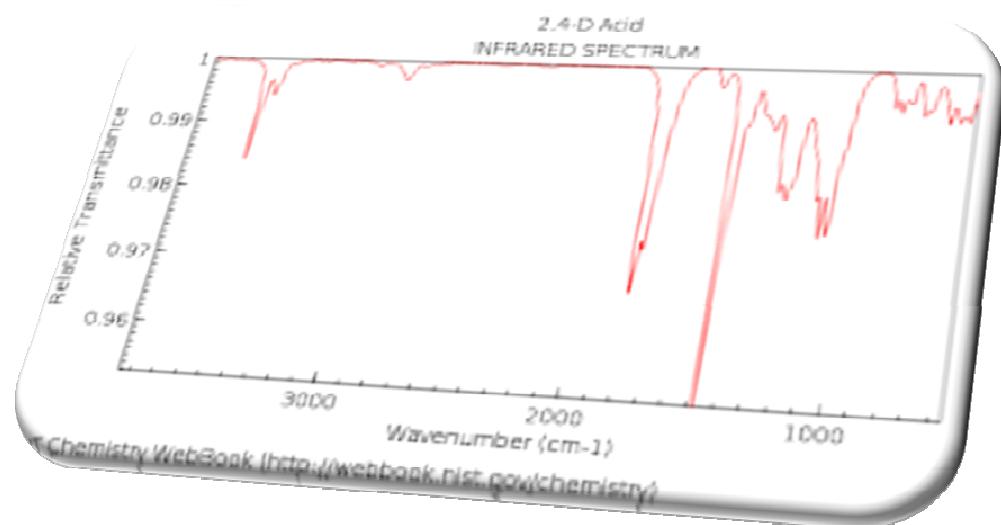
World Accreditation Day

9 June 2019



Outline of method

- Identity check
 - Any appropriate technique
 - MS, DAD, FTIR
- Specificity check
 - Mass spectrometry
 - DAD





Outline of method

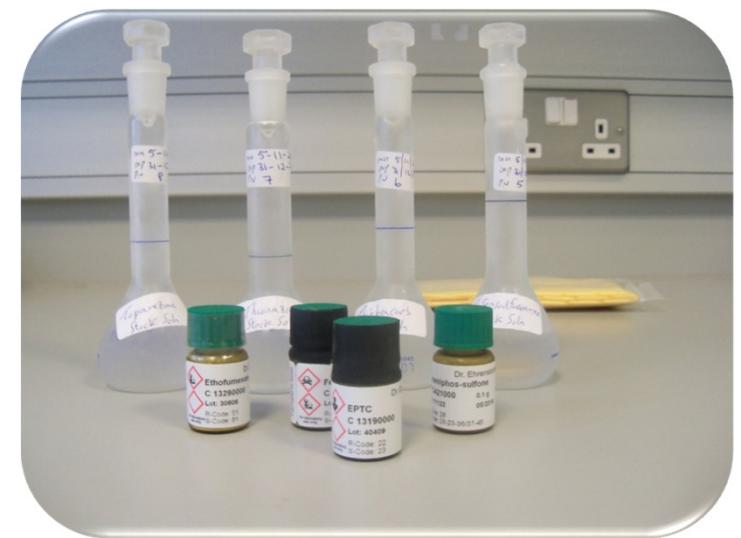
- The sample preparation is different depending on the formulation type being analysed.
- Procedures are given for solid and liquid formulations.
- Once the samples are prepared the active substances are analysed by either GC-FID or HPLC-DAD with external standard calibration.
- Internal standards can be used, if required.
- Reagents and apparatus are listed without attempting to be exhaustive





Preparation of calibration standards

- Weigh (to the nearest 0.1mg) about 10mg the required analytical standard (s mg) into a volumetric flask (100ml).
- Dissolve and fill to mark with solvent.
- Mix well. This gives a standard concentration of 100mg/l
- Two standards can be made for comparison purpose if necessary





Preparation of samples

- **Liquid or suspension formulations:** Prior to analysis the product container is shaken for 30min
- To achieve a homogeneous sample the laboratory should not have to do anything beyond what a discerning user would be required to do





Preparation of samples



- **Liquid or suspension formulations:** Thoroughly shake the sample container to homogenize the sample before use.
- Weigh in duplicate (to the nearest 0.1 mg) sufficient sample to contain 9 to 11 mg (w mg) of the active substance into a volumetric flask (100 ml). Sample concentration ~ 100mg/l
- Make up to volume with ethyl acetate or acetonitrile (for either GC or LC) and mix well (Solutions S_A and S_B).



Preparation of samples



- **Solid formulations:** Weigh ~15 -20g of the sample into a mortar and pestle and homogenise thoroughly.
- Subsample by weighing in duplicate (to the nearest 0.1 mg) sufficient sample to contain 9 to 11 mg (w mg) of the active substance into a volumetric flask (100 ml).
- Make up to volume with ethyl acetate or acetonitrile (for GC or LC) and mix well (Solutions S_A and S_B). Sample concentration ~ 100mg/l



GC Method

- (a) Chromatographic conditions
- Column HP5MS, 30 x 0.25mm x 0.25μm
- Injection system
- Injector Split injection
 Injection volume 0.2μl
 Split ratio 5:1
- Detector Flame ionisation detector
- Temperatures:
 Injection port 70°C
 Detector 310°C





GC Method



Temperature program

Temp °C	Rate °C/min	Time (min)
65	---	0.5
280	50	0
280	0	6

Total Run Time = 10.80 mins



HPLC Method

- **(a) Chromatographic conditions**
- **Column** HPLC column, Kinetex C₁₈, 100 mm x 4.6 mm (id) x 2.6 µm / 100Å or equivalent
- **Flow rate** 1.0 ml/min
- **Injection volume** 5 µL
- **Detector wavelength** 220 nm – 280 nm. The optimum wavelength can be established by analyzing the analytical standard on PDA detector prior to analysis
- **Run Time** 18 min





HPLC Method



Mobile Phase

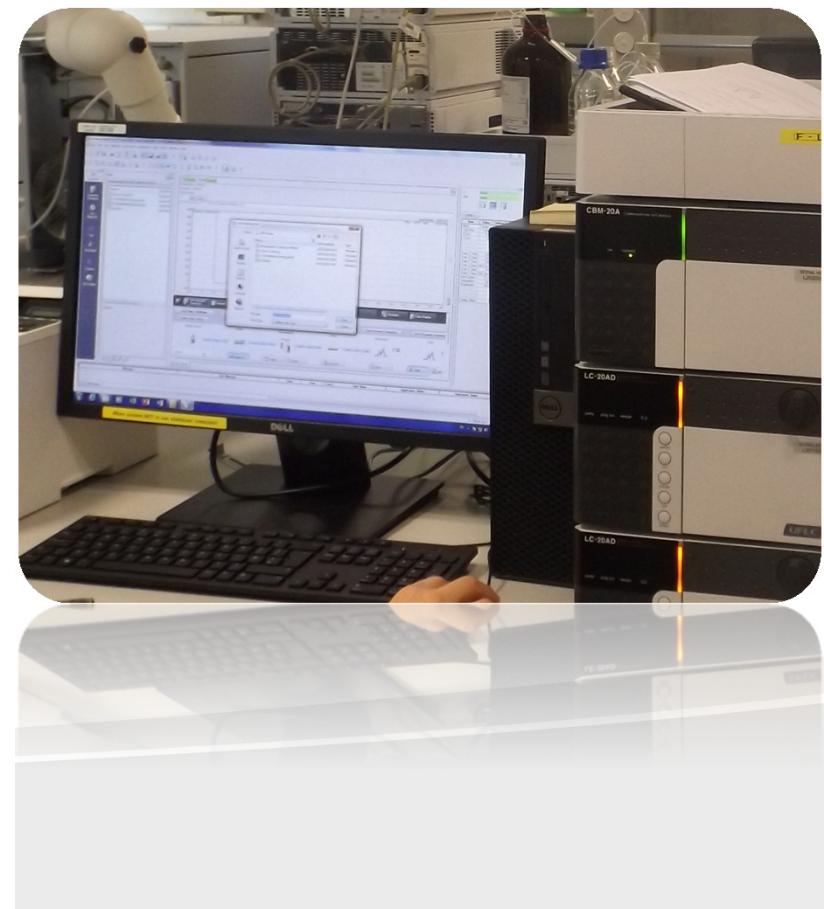
A = 65% - 0.1% Aqueous Formic acid (or o-Phosphoric acid)

B = 35% Acetonitrile

Pump Gradient:

Time (min)	% A	%B
0.01	65	35
10.0	15	85
16.0	15	85
16.4	65	35
18.0	65	35

Column temperature 25 °C





Typical sequence

- **Determination:**
- Inject in duplicate required portions of each sample solution
- Bracketing them by injections of the calibration solutions as follows;
- $C_A, S_{1A}, S_{1B}, C_B, S_{2A}, S_{2B}, C_A$, and so on.
- Measure the relevant peak areas.





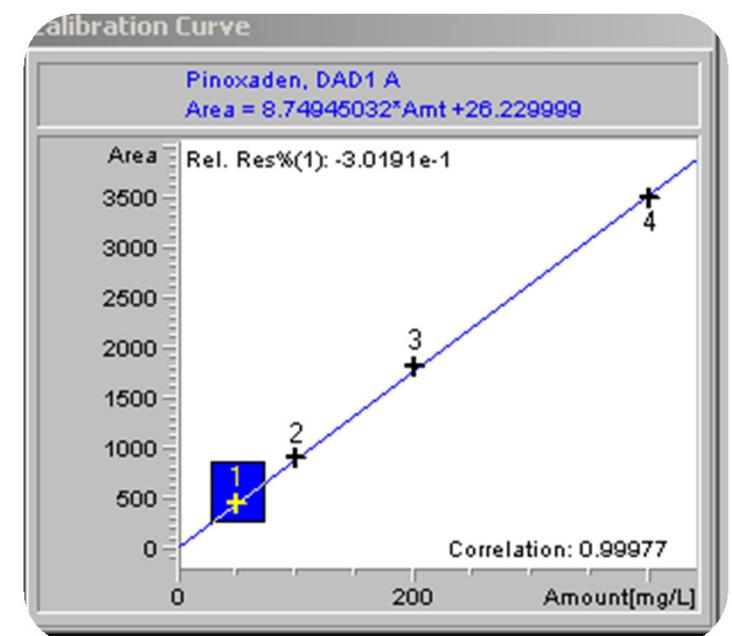
Calculations

- (d) **Calculation.** Calculate the mean value of each pair of response factors bracketing the two injections of a sample and use this value for calculating the active substance contents of the bracketed sample injections.
- $f_i = \frac{s \times P}{H_s}$
- Content of active substance = $\frac{f \times H_w}{w}$ g/kg
- where:
- f_i = individual response factor
- f = mean response factor
- H_s = peak area of active substance in the calibration solution
- H_w = peak area of the active substance in the sample solution
- s = mass of the active substance working standard in the calibration solution (mg)
- w = mass of sample taken (mg)
- P = purity of the active substance working standard (g/l)



Extending the scope

- To add a new active substance to the scope of this method the following validation data must be provided:
- An unequivocal identity test
- Specificity check
- Injection repeatability data – should be better than 1%
- Linearity should be demonstrated between at least 50 and 150ppm
- PT or collaborative trial data would also be beneficial





GC Scope



	Active substance	Form.	Type	unit	Spec	Tol (±)
1	Azoxystrobin		EC	g/L	62.5	6.3
2	b-Cyfluthrin		WP	w/w	10	1
3	Chlorothalonil		SC	g/L	500	25
4	Chlorpyrifos		EC	g/L	480	24
5	Chlorpyrifos methyl		EC	g/L	225	13.5
6	Clodinafop propargil ester		EC	g/L	100	10
7	Cypermethrin		EC	g/L	100	10
8	Cypermethrin		EW	g/L	100	10
9	Cyprodinil		EC	g/L	187.5	11.3
10	Deltamethrin		DP	w/w	0.05	0.013
11	Difenoconazole		SC	g/L	250	15
12	Dimethoate		EC	g/L	400	20
13	Epoxiconazole		SC	g/L	125	7.5
14	Esfenvalerate		EC	g/L	25	3.8
15	Fenoxaprop p ethyl		EW	g/L	69	6.9
16	Fenpropidin		EC	g/L	750	25
17	Fenpropimorph		SC	g/L	250	15
18	Fluazifop-P-Butyl		EC	g/L	125	7.5
19	Fluroxypyr 1 MHE		EC	g/L	144	8.6
20	Folpet		SC	g/L	500	25
21	Ioxynil Octanoate		EC	g/L	268.1	13.4
22	Isopyrazam		EC	g/L	62.5	6.3



GC Scope - cont



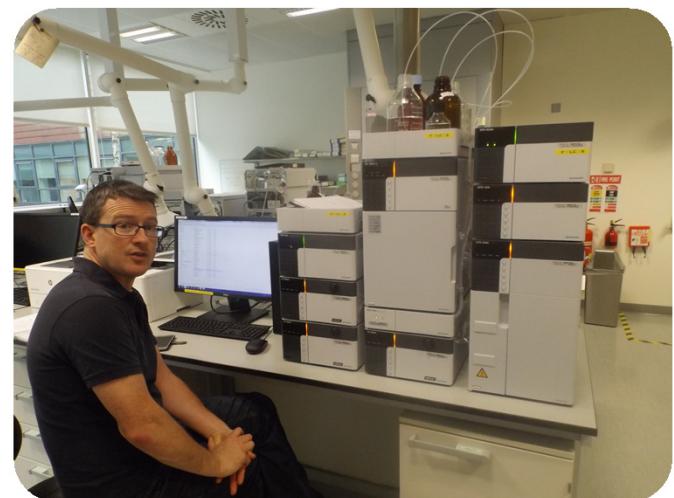
	Active substance	Form.	Type	unit	Spec	Tol (±)
23	λ - Cyhalothrin		CS	g/L	100	10
24	Metconazole		EC	g/L	25	3.8
25	Metribuzin		WG	%w/w	70	2.5
26	Myclobutanil		EW	g/L	200	12
27	Paclobutrazol		SC	g/L	125	7.5
28	Permethrin		DP	w/w	0.488	0.12
29	Picoxystrobin		SC	%w/w	17.9	1.07
30	Piperonyl butoxide		XX	w/w	1.0	0.15
31	Pirimicarb		WG	w/w	50	2.5
32	Pirimiphos-me		EC	g/L	50	5
33	Procloraz		EC	g/L	300	15
34	Prodiamine		XX	w/w	2.73	0.27
35	Prometron		SC	w/w	14.3	0.86
36	Propiconazole		EC	g/L	104	6.2
37	Propyzamide		WG	w/w	4.0	1.0
38	Prosulfocarb		EC	g/L	800	25
39	Pyrethrins		XX	w/w	0.1	0.02
40	Spiroxamine		EC	g/L	250	15
41	Tebuconazole		SC	g/L	250	15
42	Terbutylazine		SC	g/L	330	16.5
43	Triclopyr butoxy ethyl ester		EO	g/L	334	16.7
44	Trifluralin		WG	w/w	1.47	0.37



HPLC Scope



Active substance	Form. Type	l (nm)	unit	Spec	Tol (±)
1 Abamectin	EC	240	g/L	18	2.7
2 Acetamiprid	ME	260	w/w	0.05	0.008
3 Acetamiprid	ME	260	g/kg	0.05	0.008
4 Amidosulfuron	WG	240	w/w	75	2.5
5 Amisulbrom	WG	260	g/kg	50	5
6 Asulam	SL	260	g/L	400	20
7 Atrazine	SL	280	g/L	4	0.6
8 Benzovindiflupyr	EC	260	g/L	75	7.5
9 Bifenthrin	ME	260	g/L	0.02	0.003
10 Bixafen	EC	260	g/L	60	6
11 Boscalid	WG	260	g/kg	500	25
12 Carfentrazone-et	WG	260	w/w	33.3	1.67
13 Chlоридазон	WP	260	w/w	650	25
14 Chlороантринилипроле	FS	280	w/w	50	25
15 Chlороантринилипроле	WG	280	g/kg	350	17.5
16 Chlороантринилипроле	SC	280	g/kg	184	11.04
17 Chlороантринилипроле	Tech	280	g/kg	1000	25
18 Члороталонил*	SC	260	g/L	500	25
19 Члоротулурон	SC	240	g/L	250	15
20 Cloquintocet-mexyl	EC	340	g/L	25	2.5
21 Cyantraniliprole	OD	280	g/L	100	10
22 Cymoxanil	WG	260	w/w	500	25
23 Daminozide	WG	220	w/w	85	8.5
24 Dazomet	GR	280	g/kg	970	25
25 Difenacoum	RB	260	w/w	0.005	0.0013
26 Diflufenican	SC	280	w/w	200	12
27 Dimethomorph	WP	260	w/w	500	25
28 Epoxiconazole	SC	260	g/L	50	5

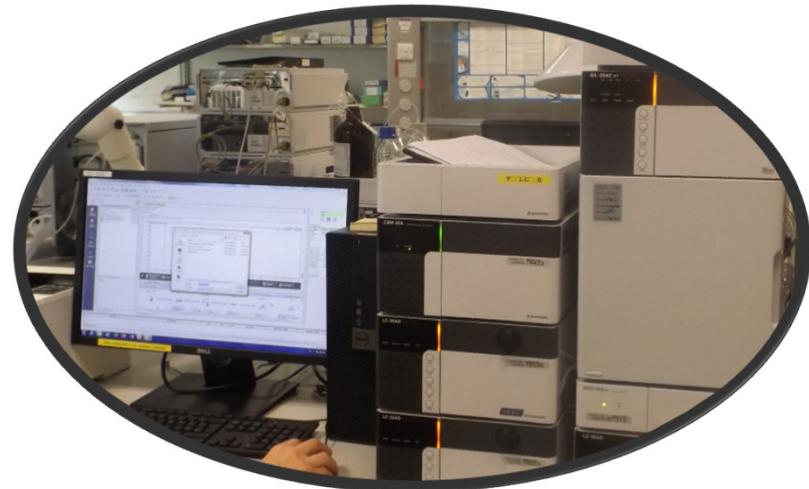




HPLC Scope cont



Active substance	Form. Type	l (nm)	unit	Spec	Tol (±)
30 Fipronil	XX	260	g/kg	0.14	0.035
31 Flazasulfuron	WG	260	w/w	250	12.5
32 Flonicamid	WG	260	w/w	500	25
33 Florasulam	SC	260	g/L	50	5
34 Fluazinam	EC	260	g/L	400	20
35 Flufenacet	SC	245	g/L	400	20
36 Flumioxazin	SC	280	g/L	300	15
37 Fluopicolide	SC	260	w/w	5-53	0.55
38 Fluopyram	EC	260	g/L	65	6.5
39 Fluoxastrobin	EC	260	g/L	75	7.5
40 Flurprimidol	XX	260	g/kg	130	0.78
41 Flurtamone	SC	280	g/L	120	7.2
42 Fluxapyroxad	EC	260	g/L	62.5	6.25
43 Halauxifen-methyl	XX	260	g/L	6.3	0.9
44 Imidacloprid	SL	260	g/L	0.125	0.0188
45 Iodosulfuron-Me-Na	SC	240	g/L	0.3	0.05
46 Iprodione	SC	240	g/L	256	12.8
47 Isoproturon	SC	260	g/L	500	25
48 Isoxaben	SC	260	g/L	500	25
49 Isoxaflutole	WG	260	w/w	100	10
50 Linuron	SC	260	g/L	450	22.5
51 Mesosulfuron-Me	WG	240	w/w	0.9	0.14
52 Mesotrione	SC	260	g/L	70	7
53 Metamitron	SC	260	g/L	700	70
54 Metazachlor	SC	260	g/L	375	18.8
55 Metconazole*	EC	260	g/L	60	6
56 Methomyl	SC	240	w/w	20	1.2
57 Metsulfuron-methyl	WG	260	w/w	68	10
58 Myclobutanil*	ME	260	g/L	0.075	0.0113
59 Nicosulfuron	WG	260	w/w	750	25





HPLC Scope cont



Active substance	Form. Type	λ (nm)	unit	Spec	Tol (±)
60 Oxadiazon	SC	260	g/L	4.8	0.72
61 Oxamyl	GR	240	w/w	5	0.5
62 Pendimethalin	SC	260	g/L	455	22.8
63 Penoxsulam	WG	280	w/w	0.04	0.01
64 Penthiopyrad	SC	260	g/L	100	10
65 Picloram	SL	260	g/L	67	6.7
66 Pinoxaden	EC	260	g/L	100	10
67 Prohexadione calcium	WG	260	g/kg	50	12.5
68 Propaquizafop	EC	340	g/L	100	10
69 Proquinazid	EC	260	g/L	200	12
70 Prothioconazole	EC	260	g/L	200	12
71 Pyraclostrobin	SC	260	g/L	133	8
72 Pyraflufen-ethyl	SL	260	g/L	0.33	0.05
73 Pyriate	WP	260	w/w	45	2.25
74 Pyrimethanil	SC	260	g/L	400	20
75 Pyroxsulam	WG	310	w/w	7.1	0.71
76 Quinoclamine	WP	240	g/kg	250	12.5
77 Quizalofop-p-tefuryl	EC	240	g/L	40	4
78 Sulfoxaflor	WG	260	g/kg	500	25
79 Tebuthiuron	WG	260	w/w	20	1.2
80 Tepraloxydim	EC	260	g/L	50	5
81 Thiacloprid	SC	240	g/L	40.4	2.02
82 Thifensulfuron-methyl	WG	260	w/w	682	25
83 Tribenuron-methyl	SG	260	w/w	40	2
84 Trifloxystrobin	SC	260	g/L	16	1.6
85 Triflusulfuron-methyl	WG	260	w/w	500	25
86 Trinexapac-ethyl	EC	260	g/L	250	12.5
87 Triticonazole	ME	260	g/L	7.5	1.1



Proficiency test results - AAPCO

			Reported	Median	s	z
2016						
2016 - 1	Pale amber liquid	Propiconazole	14.74	14.3	0.519	0.57
2016 - 2	White dust	Deltamethrin	0.035	0.038	0.0036	-0.56
3026 - 3	Opaque yellow liquid	Sulfentrazone	1.325	1.33	0.059	-0.06
2026 - 4	Opaque yellow liquid	Prodiamine	2.64	2.676	0.115	-0.21
2017			Reported	Median	s	z
2017 - 01	White pressed liquid	Prometon	4.82	4.995	0.479	-0.23
2017 - 02	White opaque liquid	Atrazine	4.11	4.01	0.432	0.675
2017 - 03	Transparent amber liquid	Acetamiprid	8.63	8.779	0.468	-0.762
2017 - 04	Brown granules	Penoxsulam	0.0401	0.04	0.012	-0.067
2018			Reported	Median	s	z
2018 - 01	Grey pressed pellets	Tebuthiuron	22.4	21.94	1.181	0.667
2018 - 02	White emulsified liquid	Iprodione	24	23.51	0.974	0.868
2018 - 03	Grey wettable powder	Cyfluthrin	9.855	10.2	0.709	-0.675



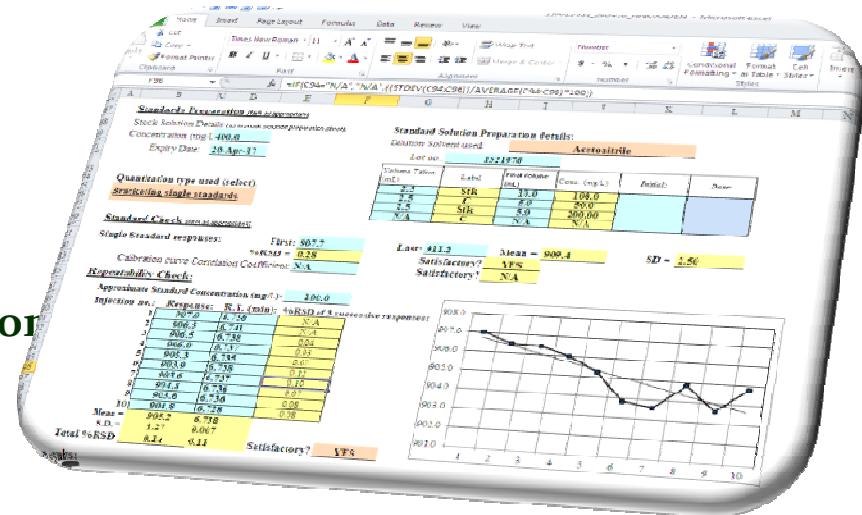
Proficiency test results

2018			Reported	Median	s	z
	Grey pellets	Fipronil	0.01545	0.0131	0.0015	1.9
	Transparent green liquid	Flurprimidol	13.55	13.41	0.508	0.285
		Trinexapac-ethyl	3.59	3.626	0.363	-0.326
ITPT 2018			Reported %	Average %	s	z
2018_01	Wettable powder	Cymoxanil	20	20	0.78	0
2018_02	Soluble liquid	Methomyl	19.3	19.4	1.05	-0.92
2018_03	Granuales	Oxamyl	5.23	5.27	0.16	-0.11
ITPT 2019			Reported %	Average %	s	z
2019_01	Water dispersible granules	Amisulbron	4.75	4.96	0.25	-0.84
2019_02	Wettable powder	Dimethomorph	6.05	6.07	0.14	-0.14
2019_03	Liquid	Pirimiphos-methyl	5.1	5.3	0.5	-0.40
2019_04	Emulsifiable concentrate	Propiconazole	26.05	25.8	3.41	0.07



Conclusion

- The reality is that Quality Control Laboratories are using MAM's – could become methods of choice within the EU
- These methods are cost efficient and minimise the downtime changing from one batch of samples to another
- These methods are already recognised by accreditation bodies
- They can potentially give companies a starting point when trying to develop new analytical methods
- They will not cover all active substance – product types – if they can cover over 90% then that is a major simplification





Conclusion

- Acknowledgement of these methods would be helpful for laboratories preparing for accreditation
- CIPAC methods extended to cover the full range of laboratory activities
- Maybe need to create a new method “type” or accept as MT methods
- Acceptance of reality

