Determination of pesticides in filter papers in the framework of WHOPES IRS studies

Olivier Pigeon, Patricia De Vos & Nicolas Mabon
Walloon Agricultural Research Centre (CRA-W), D3/U10, Belgium

Rajpal Yadav
World Health Organization (WHO), NTD/VEM, Switzerland

CIPAC Symposium, Kiev, Ukraine, 11 June 2013

Centre wallon de Recherches agronomiques
Département Agriculture et Milieu naturel
Unité Physico-chimie et Résidus des Produits Phytopharmaceutiques et des biocides
www.cra.wallonie.be

cra-w
Centre wallon de Recherches agronomiques
Wallonie

Content

- **WHOPES IRS trials**
  - Objective
  - Methodology
  - Efficacy criteria
- **Chemical analysis in IRS studies**
  - Methodology
  - Method and method validation for :
    pirimiphos-methyl, deltamethrin, chlorfenapyr, bendiocarb & p,p'-DDT
- **Conclusion**

Centre wallon de Recherches agronomiques
Département Agriculture et Milieu naturel
Unité Physico-chimie et Résidus des Produits Phytopharmaceutiques et des biocides
www.cra.wallonie.be

WHOPES IRS trials

- **Pirimiphos-methyl CS 300 g AI/L**
  - Small-scale trials in India, South Africa & Vietnam
  - Phase III trials in India & The Gambia

- **Deltamethrin SC-PE 62.5g AI/L**
  - Small-scale trial in Vietnam
  - Phase III trials in India & Mexico

- **Chlorfenapyr SC 240 g AI/L**
  - Phase I (laboratory study) in Montpellier, France
  - Small-scale trials in Benin & Vietnam
  - Phase III trials in India & The Gambia

Centre wallon de Recherches agronomiques
Département Agriculture et Milieu naturel
Unité Physico-chimie et Résidus des Produits Phytopharmaceutiques et des biocides
www.cra.wallonie.be

Objective of WHOPES IRS trials

- **To determine the persistence of insecticidal activity**
  - Pirimiphos methyl CS: 0.5 and 1 g AI/m² doses
  - Deltamethrin SC: 20 and 25 mg AI/m² doses
  - Chlorfenapyr: 150 and 250 mg AI/m² doses

- **To evaluate impact on vectorial potential**
  (impact on mortality, blood feeding, survival rate, entry/exit rate, sporozoite rate / Entomological Inoculation Rate)

- **To record community acceptance of IRS**

Centre wallon de Recherches agronomiques
Département Agriculture et Milieu naturel
Unité Physico-chimie et Résidus des Produits Phytopharmaceutiques et des biocides
www.cra.wallonie.be

Methodology

- **WHOPES IRS guidelines followed**
- **Random allocation of villages to comparison arms**
- **IRS by trained spray people using compression sprayers**
- **Entomological studies**
  - Selection of villages and sentinel houses
  - Determine baseline susceptibility of local vectors
  - Mosquito collections: Floor sheet, Exit trap, Hand and Pyrethrum spray collection, UV Light trap collection, Human landing collections
  - Bioassays with susceptible colony mosquitoes

Efficacy criteria

Phase	Type of study	Parameters measured	Criteria
Phase I	1. Laboratory studies 2. Risk assessment	1. Intrinsic insecticidal activity 2. Diagnostic concentration 3. Irritant or excito-repellent effect 4. Cross-resistance to other insecticides 5. Efficacy and residual activity on relevant substrates	1. Establish dose-response line 2. Determine LC_{50} and LC_{90} 3. Establish a diagnostic concentration 4. Determine FT_{50} and FT_{90} 5. Efficacy & residual action on substrates 6. Cross-resistance determined
Phase II	Small-scale field trials	1. Efficacy & impact on mosquito behaviour in different ecological settings 2. Persistance on local surfaces 3. Optimum application dosage 4. Handling and application 5. Perceived adverse effects	1. Residual activity: cut off mortality > 80% 2. Deterrency (reduction of entry rate) 3. Exophily 4. Blood-feeding inhibition 5. Mortality- immediate & 24h post-exposure 6. Determine target dose
Phase III	Large-scale field trials	1. Efficacy - impact on vectorial potential 2. Residual activity 3. Operational & community acceptability	1. Duration of effective residual action (mortality > 80%); 2. Impact on vectorial potential 3. Worker safety 4. Community acceptability

Chemical analysis in IRS studies

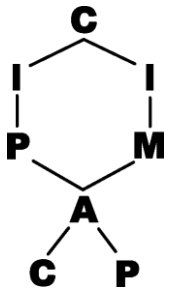
➢ Determination of pesticides in treated filter papers

Phase	Type of study	Parameters measured
Phase I	1. Laboratory studies 2. Risk assessment	**Efficacy and residual activity on relevant substrates**
Phase II	Small-scale field trials	**Persistance on local surfaces** **Optimum application dosage**
Phase III	Large-scale field trials	**Residual activity**

Centre wallon de Recherches agronomiques
Département Agriculture et Milieu naturel
Unité Physico-chimie et Résidus des Produits Phytopharmaceutiques et des biocides
www.cra.wallonie.be

Analysis of pesticides in filter papers
Methodology

- **Development of chromatographic analytical methods**
  - for pirimiphos-methyl, deltamethrin and chlorfenapyr
  - for bendiocarb and p,p'-DDT (reference products)
  based on CIPAC methods for ai content
  in TC and formulated products



- **Validation of the analytical methods**
  - Specificity and non-analyte interference
  - Linearity of chromatographic responses
  - Accuracy (recoveries and re-extractions)
  - Repeatability
  - Limit of quantification (LOQ)

- **Determination of pesticides in filter paper samples**

- **Performance verification during analysis of samples**

Centre wallon de Recherches agronomiques
Département Agriculture et Milieu naturel
Unité Physico-chimie et Résidus des Produits Phytopharmaceutiques et des biocides
www.cra.wallonie.be

Method for pirimiphos-methyl

➢ **Based on CIPAC method 4778/m (prepublished full method)**

Laboratory sampling
Measuring the surface (area) of the filter paper
Cutting into pieces of 1.5 cm x 1.5 cm
Introduction into a 100 mL screw cap glass bottle

↓

Extraction
Weighing (to the nearest 0.1 mg)
+ 2 mL internal standard solution (4,4'-dimethoxybenzophenone)
+ 23 mL acetone
Ultrasonic bath at ambient temperature for 15 minutes

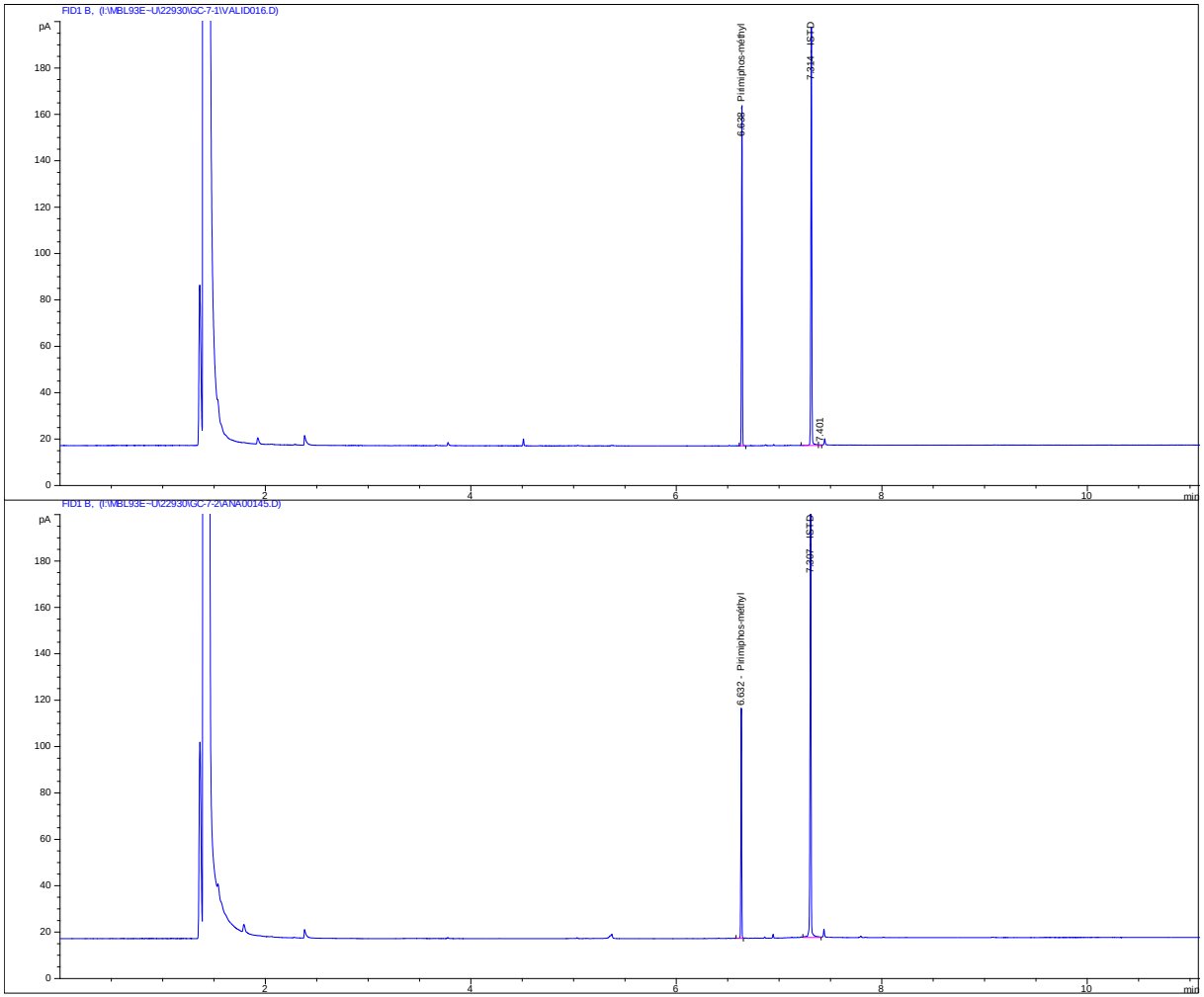
↓

GC-FID
Column : DB-1, 30 m x 0.25 mm i.d., 0.25 µm film thickness
Injection of 1 µL in split (20:1) – Temperature from 60°C to 280°C
5 points internal standard calibration curve (100 – 800 µg/mL)
Pirimiphos-methyl content as g/kg and mg/m²

Centre wallon de Recherches agronomiques
Département Agriculture et Milieu naturel
Unité Physico-chimie et Résidus des Produits Phytopharmaceutiques et des biocides
www.cra.wallonie.be

Method validation for pirimiphos-methyl

➢ Specificity and non-analyte interference



Calibration solution

Sample solution

Centre wallon de Recherches agronomiques

Centre wallon de Recherches agronomiques

Département Agriculture et Milieu naturel

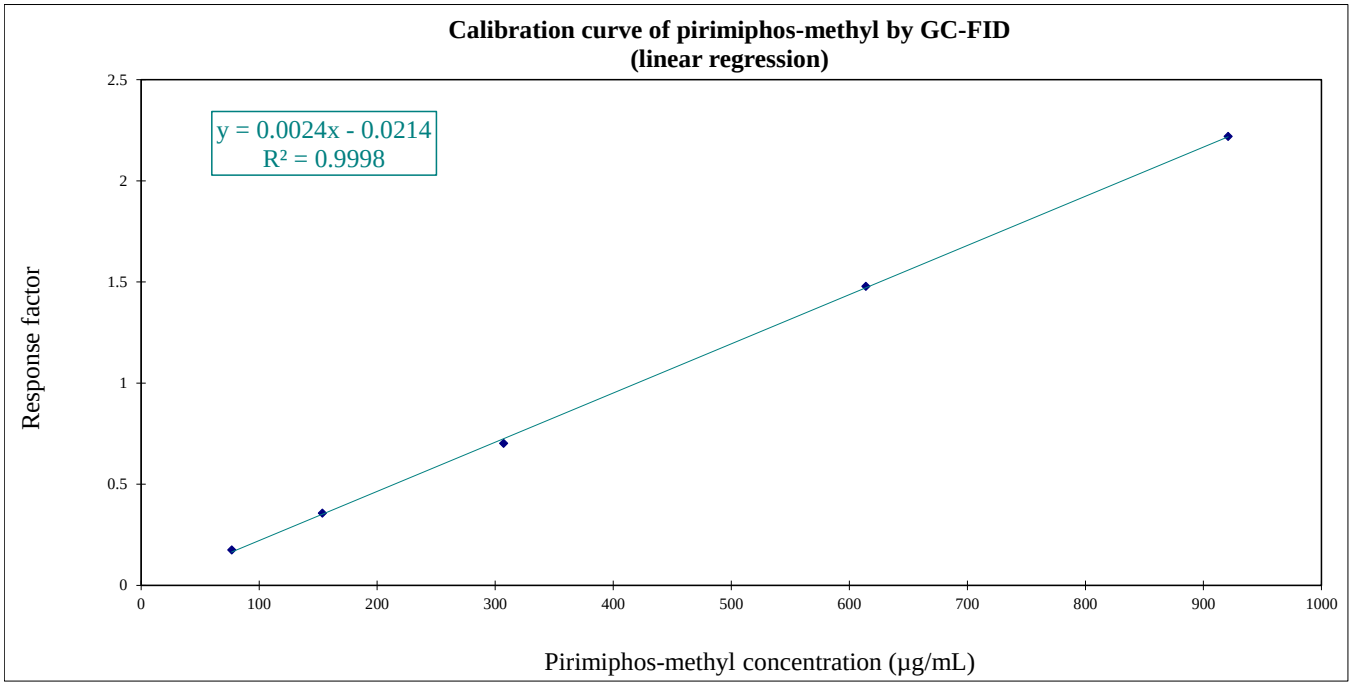
Unité Physico-chimie et Résidus des Produits Phytopharmaceutiques et des biocides

www.cra.wallonie.be

Wallonie

Method validation for pirimiphos-methyl

➢ Linearity of chromatographic response
(AI to ISTD peak area ratio versus AI concentration)



$R^2 \geq 0.99$

→ Meet the CIPAC and EU SANCO requirements

Centre wallon de Recherches agronomiques
Département Agriculture et Milieu naturel
Unité Physico-chimie et Résidus des Produits Phytopharmaceutiques et des biocides
www.cra.wallonie.be

Method validation for pirimiphos-methyl

- **Accuracy and repeatability (recoveries)**

Spiking level	N	Individual recovery values (%)	Mean Recovery	RSD
3.3 g/kg	5	112, 112, 113, 112, 106	**111 %**	**2.4 %**
13.3 g/kg	5	94, 96, 94, 96, 95	**95 %**	**0.7 %**

Mean R% in the range 80-120% for spiking levels < 1 % and RSD < 5 %
Mean R% in the range 90-110% for spiking levels ≥ 1 % and RSD < 3 %
→ Meet the CIPAC and EU SANCO requirements

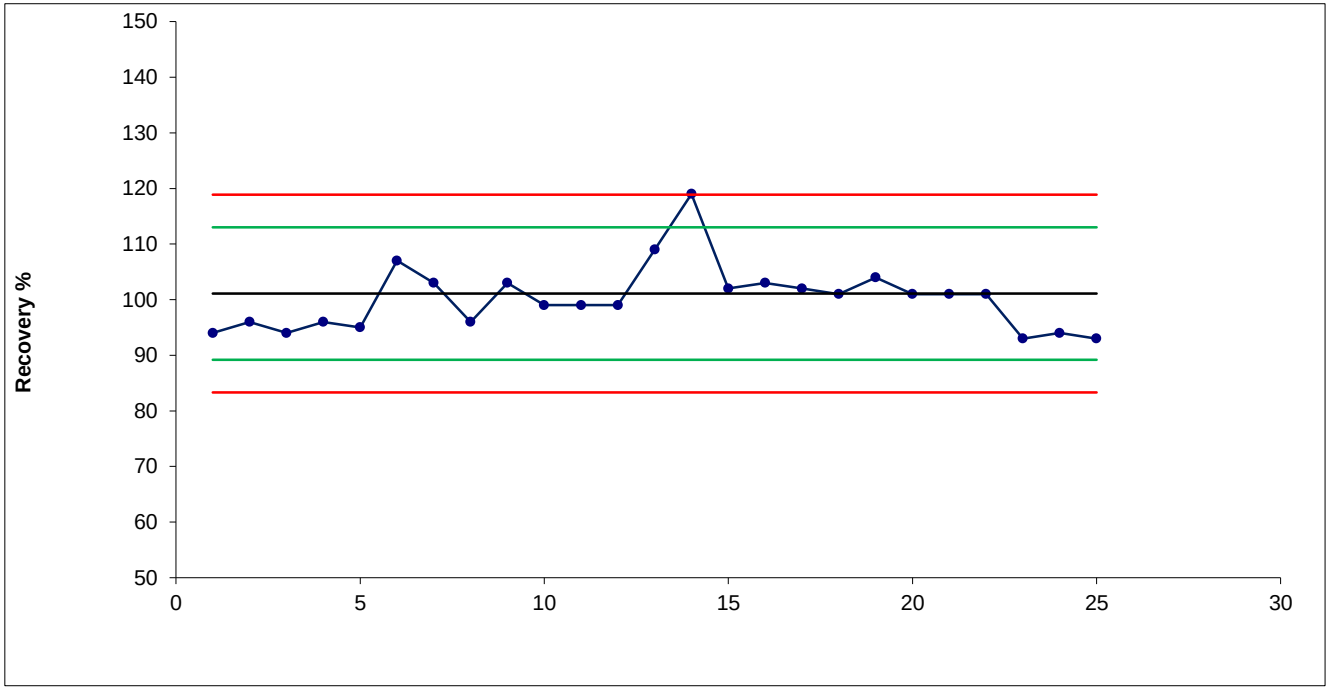
- **Accuracy (re-extractions)**
  AI content in re-extracted samples < 0.05 g/kg (n = 5)

- **LOQ = 0.05 g/kg or 4 mg/m²**

Centre wallon de Recherches agronomiques
Département Agriculture et Milieu naturel
Unité Physico-chimie et Résidus des Produits Phytopharmaceutiques et des biocides
www.cra.wallonie.be

QC during analysis of samples for pirimiphos-methyl

Accuracy and reproducibility
(recoveries from 01/2012 up to 03/2013)



24/25 recoveries within the warning limits

25/25 recoveries within the action limits

Centre wallon de Recherches agronomiques
Département Agriculture et Milieu naturel
Unité Physico-chimie et Résidus des Produits Phytopharmaceutiques et des biocides
www.cra.wallonie.be

Method for deltamethrin

➢ **Based on CIPAC method 333/LN/(M)/3 (CIPAC Handbook M)**

Laboratory sampling
Measuring the surface (area) of the filter paper
Cutting into pieces of 1.5 cm x 1.5 cm
Introduction into a 100 mL screw cap glass bottle

↓

Extraction
Weighing (to the nearest 0.1 mg)
+ 1 mL internal standard solution (dipropyl phthalate)
+ 24 mL isooctane / dioxane (80/20 , v/v)
Ultrasonic bath at 70°C for 15 minutes
Horizontal shaker (150-200 beats / min) for 30 minutes

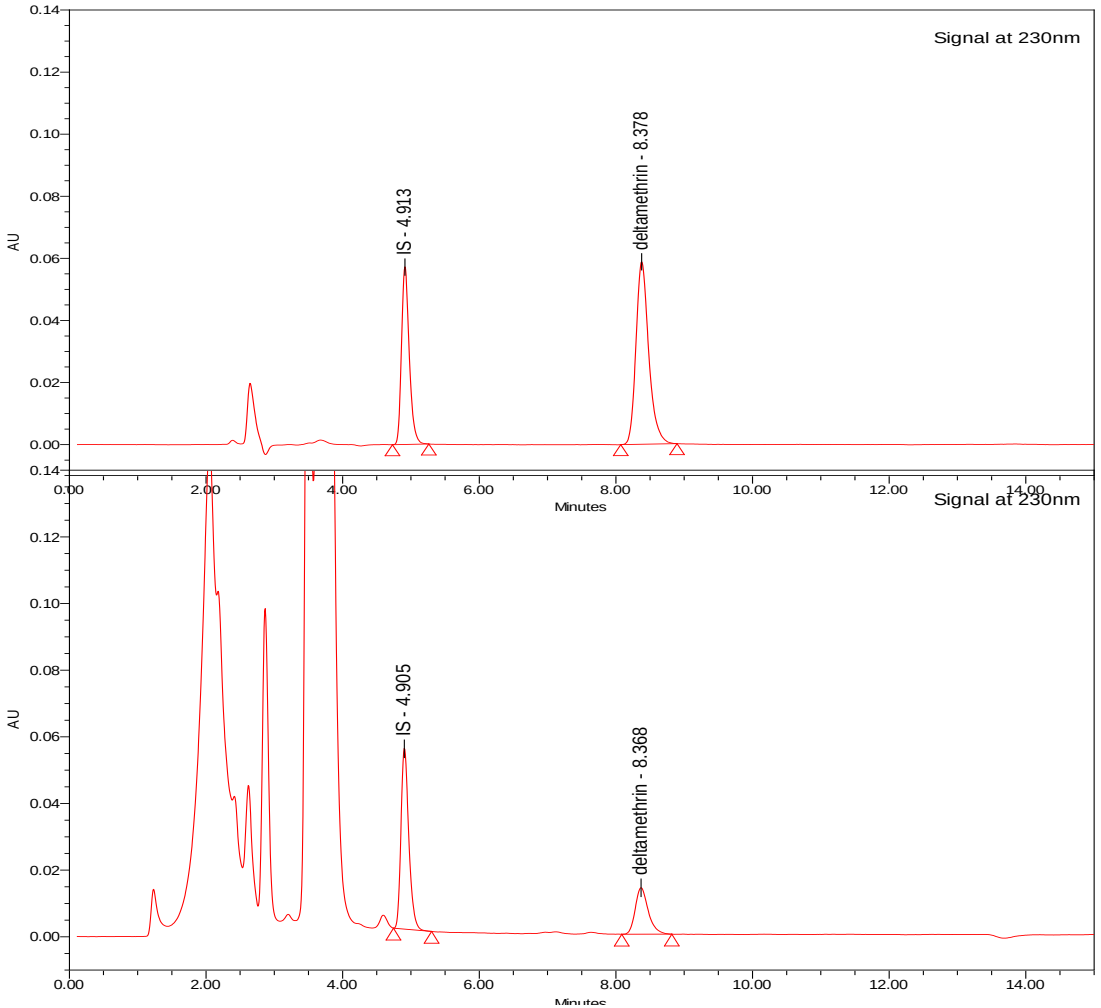
↓

HPLC-UV (DAD)
Column : Phenomenex Luna CN, 5 µm, 250 x 4.6 mm
Mobile phase : isooctane / dioxane (+ 0.15 % water) (94/6, v/v) - 1.5 mL / min
Injection of 20 µL – Detection at 230 nm
5 points internal standard calibration curve (5 – 50 µg/mL)
Deltamethrin content as g/kg and mg/m²

Centre wallon de Recherches agronomiques
Département Agriculture et Milieu naturel
Unité Physico-chimie et Résidus des Produits Phytopharmaceutiques et des biocides
www.cra.wallonie.be

Method validation for deltamethrin

➢ Specificity and non-analyte interference



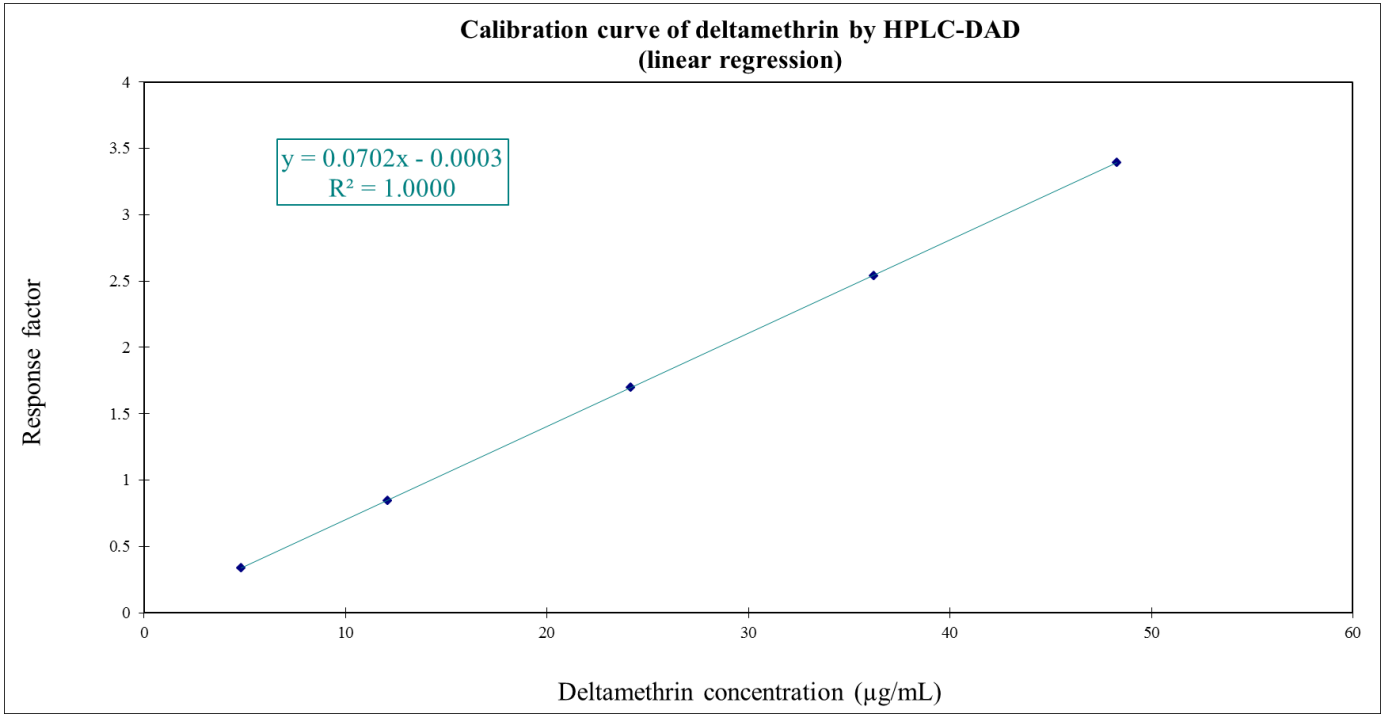
Calibration solution

Sample solution

Centre wallon de Recherches agronomiques
Département Agriculture et Milieu naturel
Unité Physico-chimie et Résidus des Produits Phytopharmaceutiques et des biocides
www.cra.wallonie.be

Method validation for deltamethrin

➢ Linearity of chromatographic response
(AI to ISTD peak area ratio versus AI concentration)



$R^2 \geq 0.99$

→ Meet the CIPAC and EU SANCO requirements

Centre wallon de Recherches agronomiques
Département Agriculture et Milieu naturel
Unité Physico-chimie et Résidus des Produits Phytopharmaceutiques et des biocides
www.cra.wallonie.be

Method validation for deltamethrin

- **Accuracy and repeatability (recoveries)**

Spiking level	N	Individual recovery values (%)	Mean Recovery	RSD
0.2 g/kg	5	101, 101, 100, 100, 96	**100 %**	**1.9 %**
1 g/kg	5	99, 100, 100, 100, 98	**99 %**	**0.9 %**

Mean R% in the range 80-120% for spiking levels < 1 % and RSD < 5 %
Mean R% in the range 90-110% for spiking levels ≥ 1 % and RSD < 3 %
→ Meet the CIPAC and EU SANCO requirements

- **Accuracy (re-extractions)**
  AI content in re-extracted samples < 0.01 g/kg (n = 5)

- **LOQ = 0.01 g/kg or 1 mg/m²**

Centre wallon de Recherches agronomiques
Département Agriculture et Milieu naturel
Unité Physico-chimie et Résidus des Produits Phytopharmaceutiques et des biocides
www.cra.wallonie.be

Method for chlorfenapyr

➢ **Based on CIPAC method 4825/m (prepublished provisional method)**

Laboratory sampling
Measuring the surface (area) of the filter paper
Cutting into pieces of 1.5 cm x 1.5 cm
Introduction into a 100 mL screw cap glass bottle

↓

Extraction
Weighing (to the nearest 0.1 mg)
+ 50 mL acetonitrile
Ultrasonic bath at ambient temperature for 20 minutes

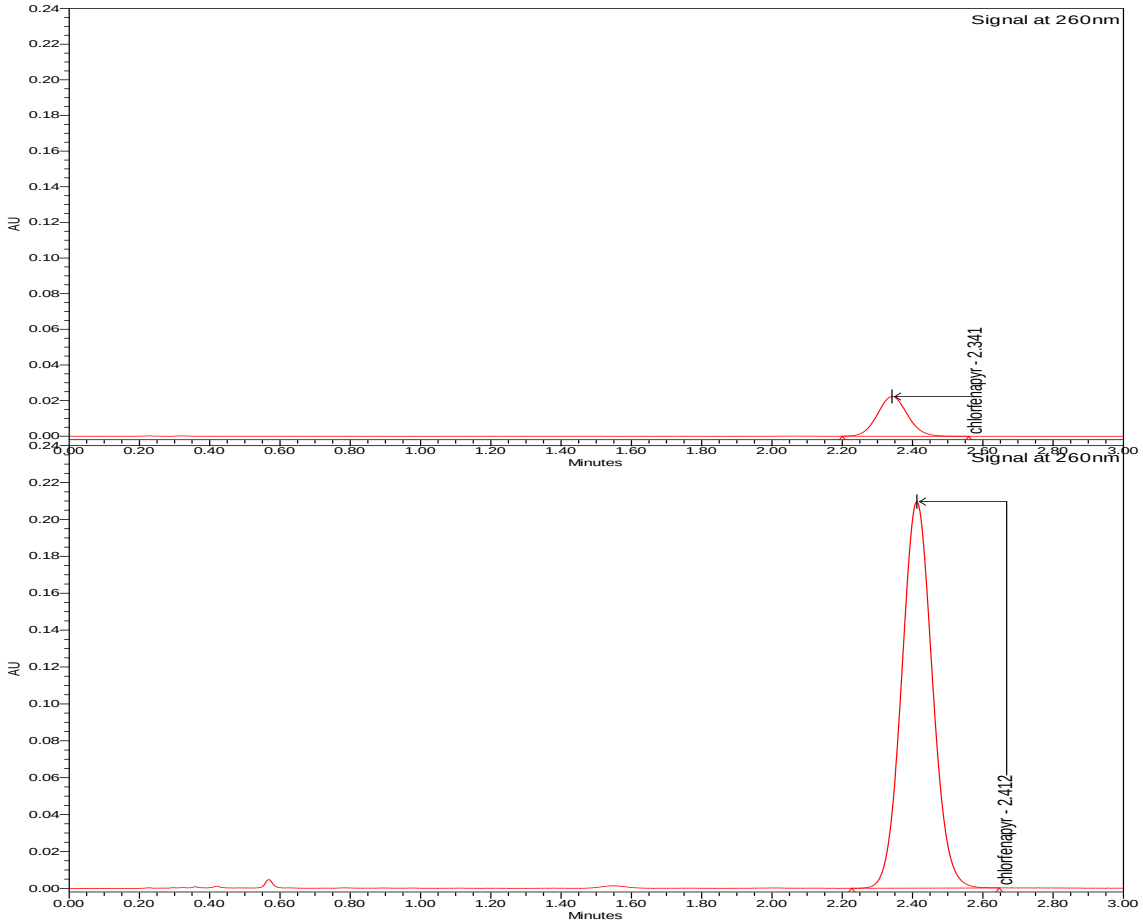
↓

HPLC-UV (DAD)
Column : Synergi Fusion RP80A, 4 µm, 50 x 4.6 mm
Mobile phase : acetonitrile / water (65/35, v/v) (+ 0.05% acetic acid) – 2.0 mL / min
Injection of 5 µL – Detection at 260 nm
5 points external standard calibration curve (25 – 500 µg/mL)
Chlorfenapyr content as g/kg and mg/m²

Centre wallon de Recherches agronomiques
Département Agriculture et Milieu naturel
Unité Physico-chimie et Résidus des Produits Phytopharmaceutiques et des biocides
www.cra.wallonie.be

Method validation for chlorfenapyr

➢ Specificity and non-analyte interference



Calibration solution

Sample solution

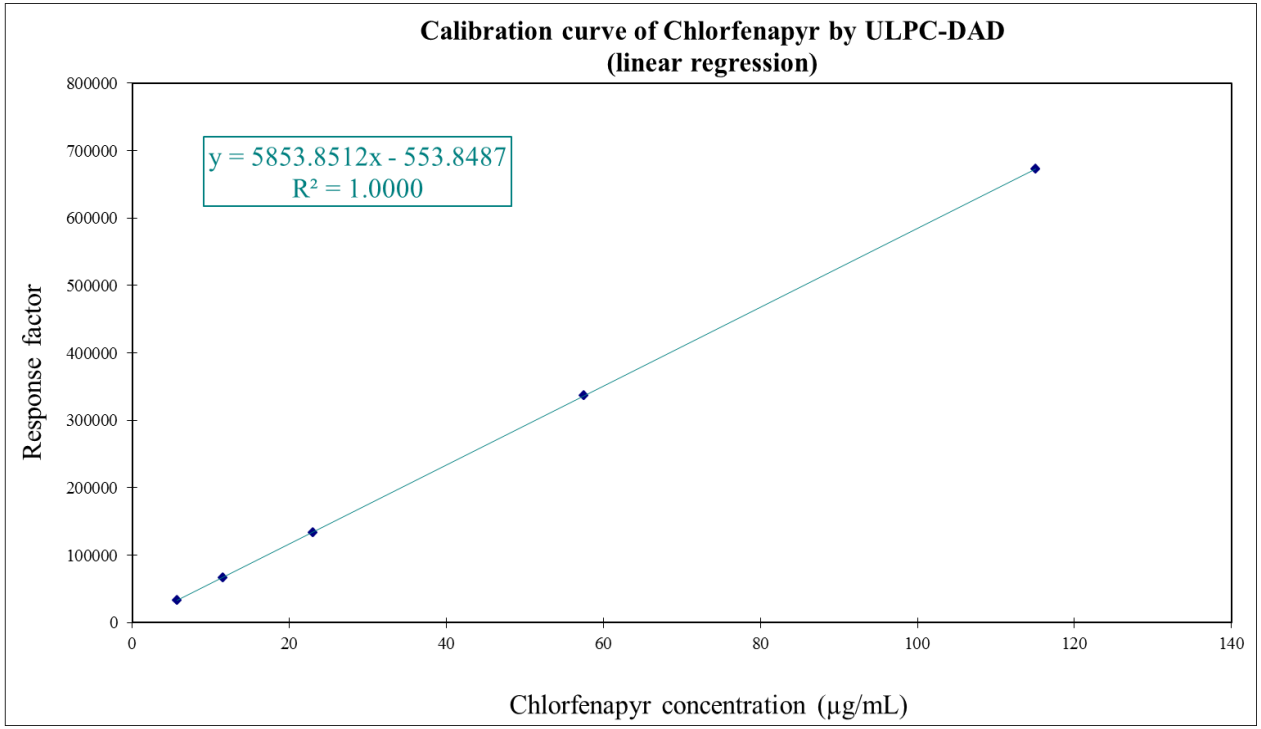
Centre wallon de Recherches agronomiques
Département Agriculture et Milieu naturel
Unité Physico-chimie et Résidus des Produits Phytopharmaceutiques et des biocides
www.cra.wallonie.be

Centre wallon de Recherches agronomiques

Wallonie

Method validation for chlorfenapyr

- **Linearity of chromatographic response**
  (AI peak area versus AI concentration)



$R^2 \geq 0.99$

→ Meet the CIPAC and EU SANCO requirements

Centre wallon de Recherches agronomiques
Département Agriculture et Milieu naturel
Unité Physico-chimie et Résidus des Produits Phytopharmaceutiques et des biocides
www.cra.wallonie.be

Method validation for chlorfenapyr

- **Accuracy and repeatability (recoveries)**

Spiking level	N	Individual recovery values (%)	Mean Recovery	RSD
0.2 g/kg	5	99, 101, 100, 100, 99	**100 %**	**0.8 %**
0.7 g/kg	5	97, 96, 95, 96, 968	**96 %**	**0.7 %**

Mean R% in the range 80-120% for spiking levels < 1 % and RSD < 5 %
→ Meet the CIPAC and EU SANCO requirements

- **Accuracy (re-extractions)**
  AI content in re-extracted samples < 0.02 g/kg (n = 5)

- **LOQ = 0.02 g/kg or 2 mg/m²**

Centre wallon de Recherches agronomiques
Département Agriculture et Milieu naturel
Unité Physico-chimie et Résidus des Produits Phytopharmaceutiques et des biocides
www.cra.wallonie.be

Method for bendiocarb

➢ **Based on CIPAC method 232/WP/(M)/3 (CIPAC Handbook D)**

Laboratory sampling
Measuring the surface (area) of the filter paper
Cutting into pieces of 1.5 cm x 1.5 cm
Introduction into a 100 mL screw cap glass bottle

↓

Extraction
Weighing (to the nearest 0.1 mg)
+ 5 mL internal standard solution (propiophenone)
+ 20 mL acetonitrile
Ultrasonic bath at ambient temperature for 20 minutes

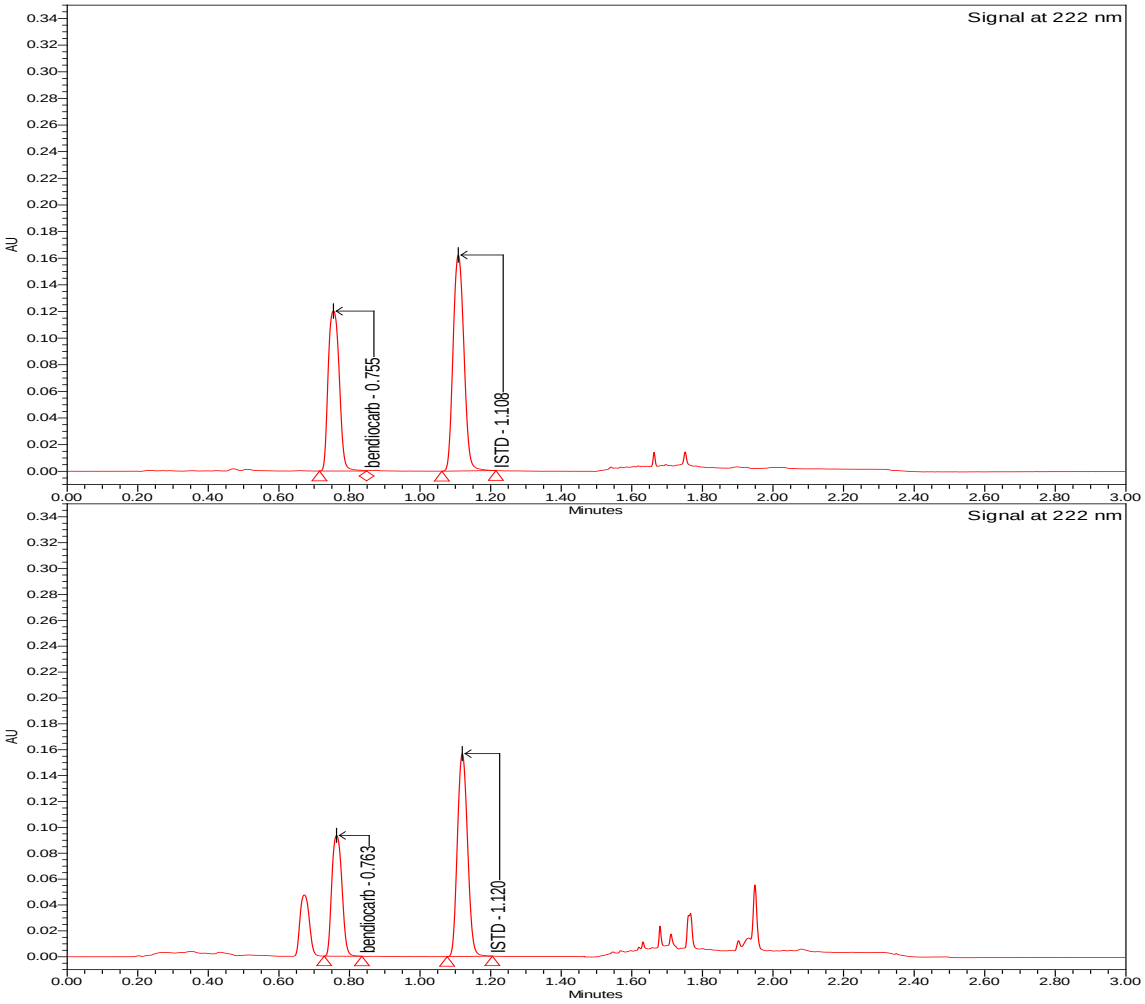
↓

UHPLC-UV (DAD)
Column : Acquity UPLC HSS, 1.8 µm, 100 x 2.1 mm
Mobile phase : water / acetonitrile (60/40, v/v) – 0.9 mL / min
Injection of 3 µL – Detection at 222 nm
5 points internal standard calibration curve (2 – 20 µg/mL)
Bendiocarb content as g/kg and mg/m²

Centre wallon de Recherches agronomiques
Département Agriculture et Milieu naturel
Unité Physico-chimie et Résidus des Produits Phytopharmaceutiques et des biocides
www.cra.wallonie.be

Method validation for bendiocarb

➢ Specificity and non-analyte interference



Calibration solution

Sample solution

Centre wallon de Recherches agronomiques

Département Agriculture et Milieu naturel

Unité Physico-chimie et Résidus des Produits Phytopharmaceutiques et des biocides

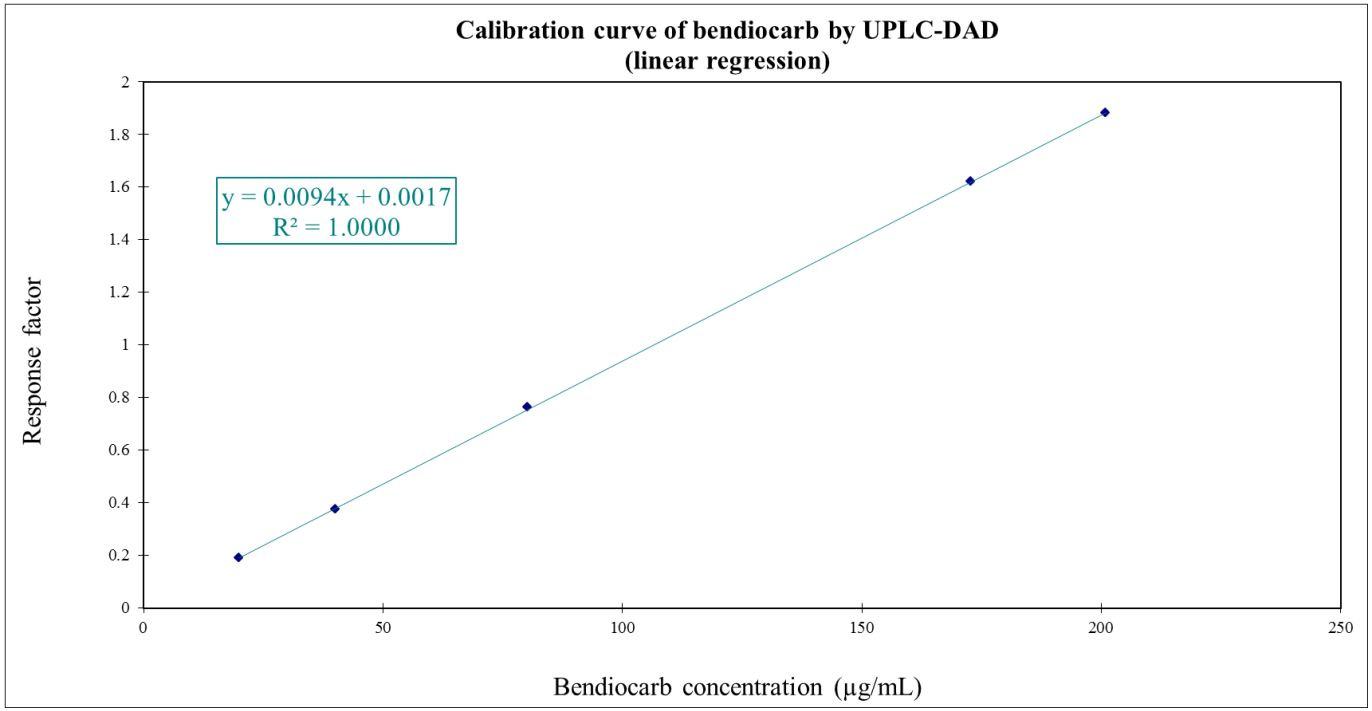
www.cra.wallonie.be

Centre wallon de Recherches agronomiques

Wallonie

Method validation for bendiocarb

➢ Linearity of chromatographic response
(AI to ISTD peak area ratio versus AI concentration)



$R^2 \geq 0.99$

→ Meet the CIPAC and EU SANCO requirements

Centre wallon de Recherches agronomiques
Département Agriculture et Milieu naturel
Unité Physico-chimie et Résidus des Produits Phytopharmaceutiques et des biocides
www.cra.wallonie.be

Method validation for bendiocarb

- **Accuracy and repeatability (recoveries)**

Spiking level	N	Individual recovery values (%)	Mean Recovery	RSD
0.8 g/kg	5	101, 100, 101, 101, 100	**101 %**	**0.6 %**
3.2 g/kg	5	101, 100, 100, 110, 101	**101 %**	**0.5 %**

Mean R% in the range 80-120% for spiking levels < 1 % and RSD < 5 %
→ Meet the CIPAC and EU SANCO requirements

- **Accuracy (re-extractions)**
  AI content in re-extracted samples < 0.1 g/kg (n = 5)

- **LOQ = 0.1 g/kg or 10 mg/m²**

Centre wallon de Recherches agronomiques
Département Agriculture et Milieu naturel
Unité Physico-chimie et Résidus des Produits Phytopharmaceutiques et des biocides
www.cra.wallonie.be

Method for p,p'-DDT

➢ **Based on CIPAC method 3/WP/M/3 (CIPAC Handbook E)**

Laboratory sampling
Measuring the surface (area) of the filter paper
Cutting into pieces of 1.5 cm x 1.5 cm
Introduction into a 100 mL screw cap glass bottle

↓

Extraction
Weighing (to the nearest 0.1 mg)
+ 50 mL acetone
Ultrasonic bath at ambient temperature for 15 minutes

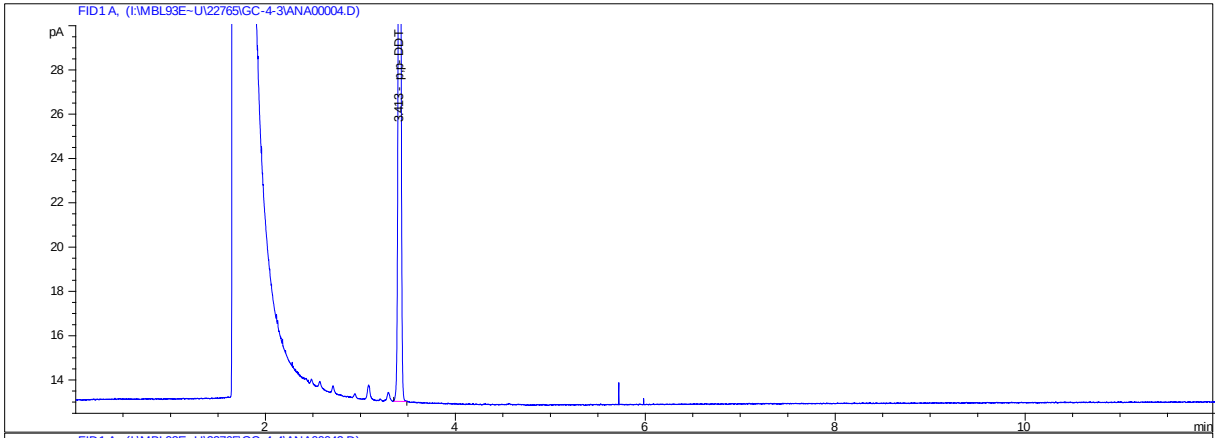
↓

GC-FID
Column : DB-210, 30 m x 0.32 mm i.d., 0.25 µm film thickness
Injection of 1 µL in split (10:1) – Temperature of 240°C
5 points external standard calibration curve (2 – 50 µg/mL)
p,p'-DDT content content as g/kg and mg/m²

Centre wallon de Recherches agronomiques
Département Agriculture et Milieu naturel
Unité Physico-chimie et Résidus des Produits Phytopharmaceutiques et des biocides
www.cra.wallonie.be

Method validation for p,p'-DDT

➢ Specificity and non-analyte interference



Calibration solution

Sample solution

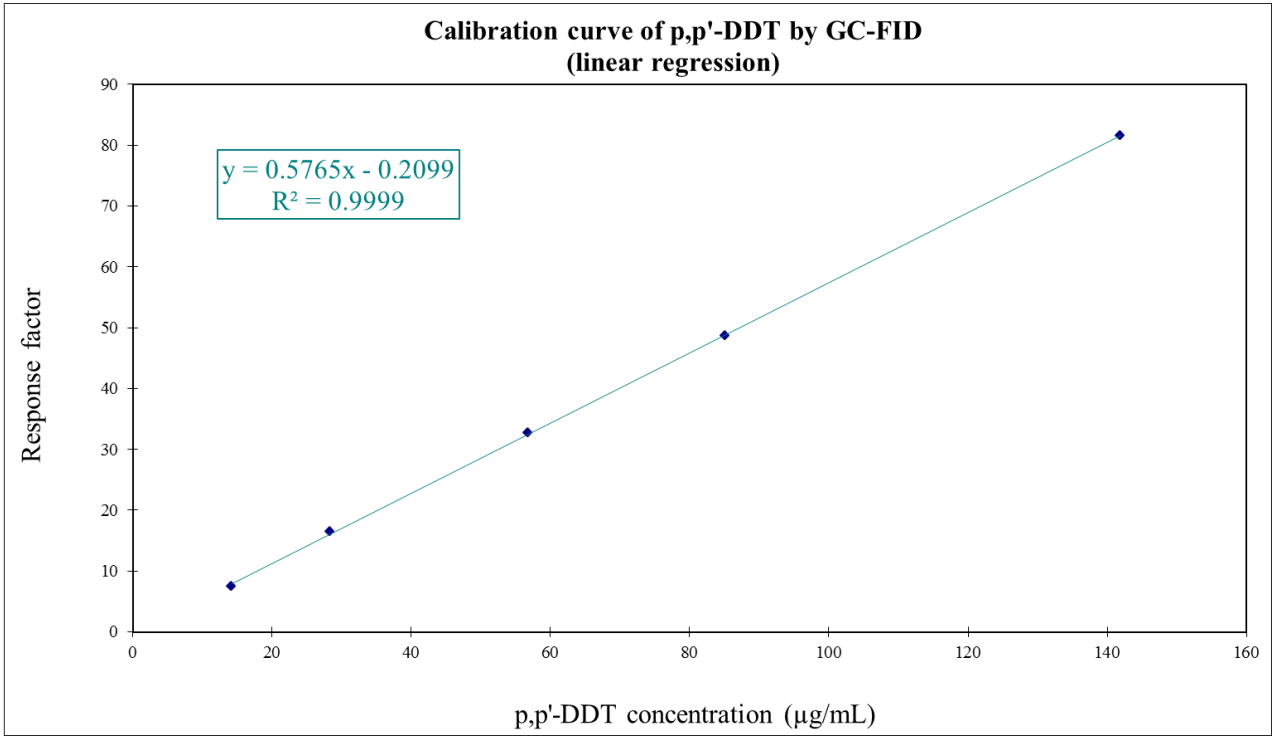
Centre wallon de Recherches agronomiques

Centre wallon de Recherches agronomiques
Département Agriculture et Milieu naturel
Unité Physico-chimie et Résidus des Produits Phytopharmaceutiques et des biocides
www.cra.wallonie.be

Wallonie

Method validation for p,p'-DDT

➢ Linearity of chromatographic response
(AI peak area ratio versus AI concentration)



$R^2 \geq 0.99$

→ Meet the CIPAC and EU SANCO requirements

Centre wallon de Recherches agronomiques
Département Agriculture et Milieu naturel
Unité Physico-chimie et Résidus des Produits Phytopharmaceutiques et des biocides
www.cra.wallonie.be

Method validation for p,p'-DDT

- **Accuracy and repeatability (recoveries)**

Spiking level	N	Individual recovery values (%)	Mean Recovery	RSD
1.1 g/kg	5	102, 100, 101, 94, 90	**97 %**	**5.1 %**
4.2 g/kg	5	97, 97, 99, 98, 96	**97 %**	**1.2 %**

Mean R% in the range 80-120% for spiking levels < 1 % and RSD < 5 %
→ Meet the CIPAC and EU SANCO requirements

- **Accuracy (re-extractions)**
  AI content in re-extracted samples < 0.2 g/kg (n = 5)

- **LOQ = 0.2 g/kg or 20 mg/m²**

Centre wallon de Recherches agronomiques
Département Agriculture et Milieu naturel
Unité Physico-chimie et Résidus des Produits Phytopharmaceutiques et des biocides
www.cra.wallonie.be

Conclusion

- **Analytical methods successfully validated for** pirimiphos-methyl (GC-FID), deltamethrin (HPLC-DAD), chlorphenapyr (HPLC-DAD), bendiocarb (HPLC-DAD) & p,p'-DDT (GC-FID) **in filter papers**

- **More than 1200 filter paper samples have been analysed since 2011**
  Performance verification (blanks, recoveries, re-extractions) during analysis of samples permitted to validate the analytical results

- **Chemical analysis of pesticides in filter papers**
  → an essential quality control measure for the WHOPES testing and evaluation of IRS

cra-w

Centre wallon de Recherches agronomiques

Centre wallon de Recherches agronomiques
Département Agriculture et Milieu naturel
Unité Physico-chimie et Résidus des Produits Phytopharmaceutiques et des biocides
www.cra.wallonie.be

Wallonie

Thank you for your attention

Thanks to Aurore Bayot, Nadia Boinon, Aurore Dineur, Natacha Garcia, Isabelle Istace Jean-Paul Vandenberghe & Aurore Wellin

Centre wallon de Recherches agronomiques
Département Agriculture et Milieu naturel
Unité Physico-chimie et Résidus des Produits Phytopharmaceutiques et des Biocides
Bât. Carson, Rue du Bordia, 11 - B-5030 GEMBLOUX - Tél : ++ 32 (0)81 62 52 62 - Fax : ++ 32 (0)81 62 52 72
pesticides@cra.wallonie.be - http://www.cra.wallonie.be