

Development of multi-pesticides / multi-matrix methods for determination of pesticides in LN and filter papers treated with IRS

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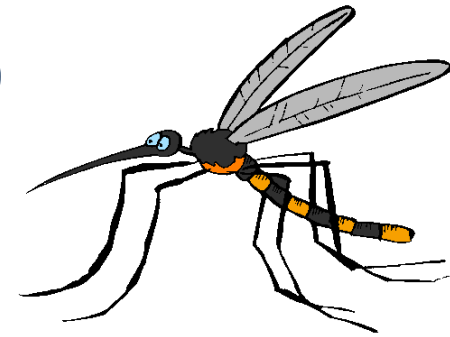
Outline

- Malaria
- Current analytical methods
- Objective
- Sample preparation
- Extraction conditions
- Analytical techniques
- Comparison multi-pesticides / CIPAC methods
- Conclusion

Malaria

Some informations about malaria disease

- Malaria, the most parasitic disease spread in the world
- Parasite: Plasmodium (falciparum)
- Transmission: mosquito (Genus Anopheles)
- 300-500 millions cases in the world
- 627 000 death (2012)
- \$2 billions financing (+/-)
- Objective: a 75 % decrease of the malaria in 2015



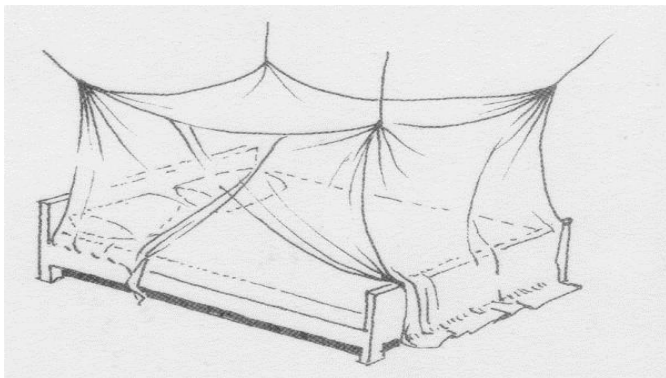
Malaria



2 ways to fight against the vector

Nets

- Long-lasting insecticidal nets (LNs)
- Polyester (« coated act. subst. »), polyethylene or polypropylene (« incorporated act. subst. »)
- Treated with pesticides (pyrethrinoides), synergistics



Indoor residual spraying (IRS)

- Spraying pesticides onto the house walls
- Pesticides: pyrethrinoids, organochlorinated, organophosphate and carbamates
- Verification of the target dose, analysing pieces of filter paper fixed to the wall during spraying



Malaria

WHO recommended long-lasting insecticidal nets

<i>Product name</i>	<i>Product type</i>	<i>Status of WHO recommendation</i>	<i>Status of publication of WHO specification</i>
<i>DawaPlus® 2.0</i>	Deltamethrin coated on polyester	Interim	Published
<i>Duranet®</i>	Alpha-cypermethrin incorporated into polyethylene	Full	Published
<i>Interceptor®</i>	Alpha-cypermethrin coated on polyester	Full	Published
<i>LifeNet®</i>	Deltamethrin incorporated into polypropylene	Interim	Published
<i>MAGNet™</i>	Alpha-cypermethrin incorporated into polyethylene	Full	Published
<i>Olyset Net®</i>	Permethrin incorporated into polyethylene	Full	Published
<i>Olyset® Plus</i>	Permethrin and PBO incorporated into polyethylene	Interim	Published
<i>PermaNet® 2.0</i>	Deltamethrin coated on polyester	Full	Published
<i>PermaNet® 3.0</i>	Combination of deltamethrin coated on polyester with strengthened border (side panels) and deltamethrin and PBO incorporated into polyethylene (roof)	Interim	Published
<i>Royal Sentry®</i>	Alpha-cypermethrin incorporated into polyethylene	Full	Published
<i>Yorkool® LN</i>	Deltamethrin coated on polyester	Full	Published

Malaria

WHO recommended insecticides for IRS

<i>Insecticide compounds and formulations¹</i>	<i>Class group²</i>	<i>Dosage (g a.i./m²)</i>	<i>Mode of action</i>	<i>Duration of effective action (months)</i>
<i>DDT WP</i>	OC	1-2	contact	>6
<i>Malathion WP</i>	OP	2	contact	2-3
<i>Fenitrothion WP</i>	OP	2	contact & airborne	3-6
<i>Pirimiphos-methyl WP & EC</i>	OP	1-2	contact & airborne	2-3
<i>Pirimiphos-methyl CS</i>	OP	1	contact & airborne	4-6
<i>Bendiocarb WP</i>	C	0.1-0.4	contact & airborne	2-6
<i>Propoxur WP</i>	C	1-2	contact & airborne	3-6
<i>Alpha-cypermethrin WP & SC</i>	PY	0.02-0.03	contact	4-6
<i>Bifenthrin WP</i>	PY	0.025-0.05	contact	3-6
<i>Cyfluthrin WP</i>	PY	0.02-0.05	contact	3-6
<i>Deltamethrin SC-PE</i>	PY	0.02-0.025	contact	6
<i>Deltamethrin WP, WG</i>	PY	0.02-0.025	contact	3-6
<i>Etofenprox WP</i>	PY	0.1-0.3	contact	3-6
<i>Lambda-cyhalothrin WP, CS</i>	PY	0.02-0.03	contact	3-6

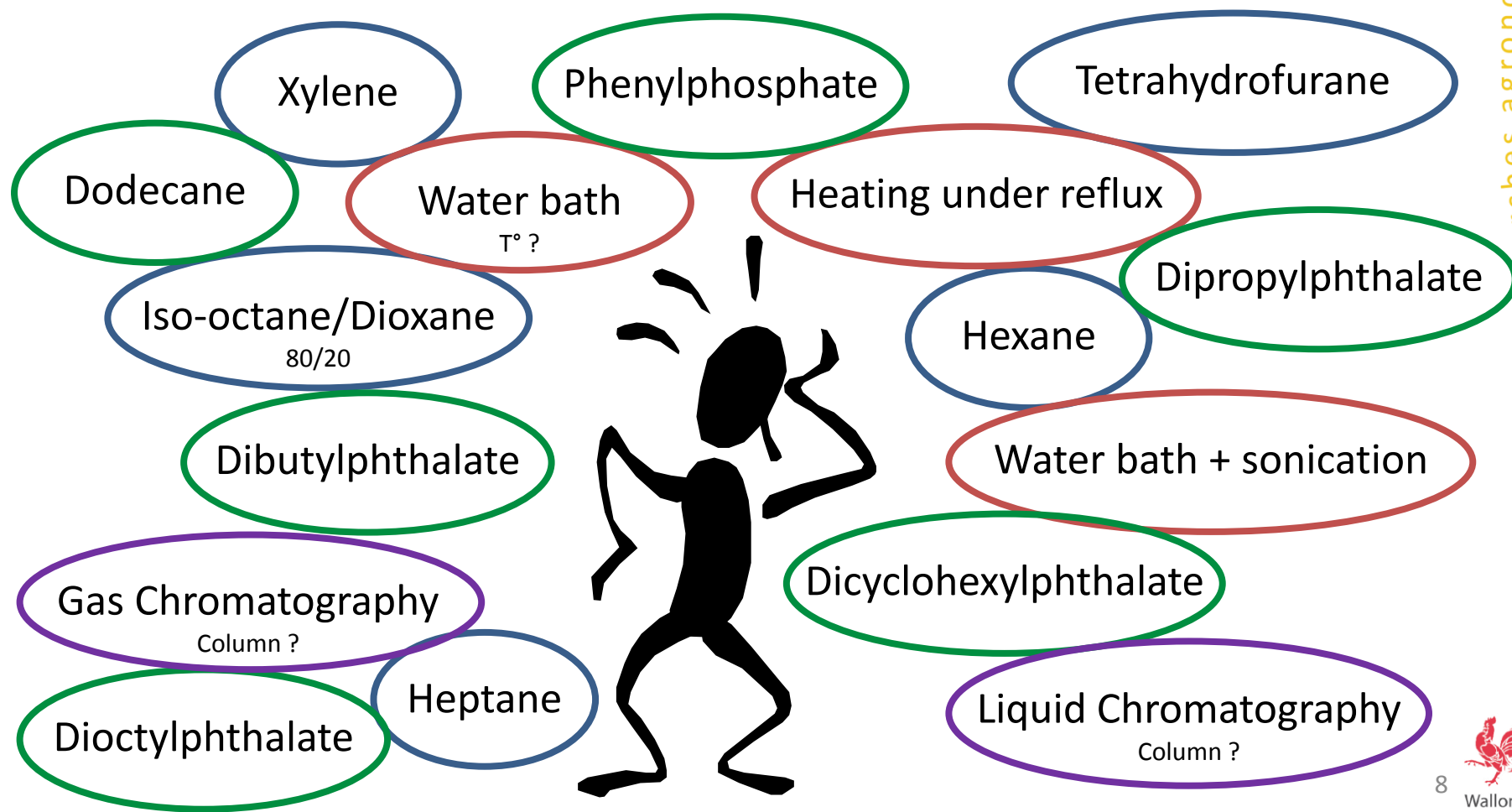
Current analytical methods

Determination: 1 act. substance => 1-2 method(s)

Active substance	Extraction Solvent	Extraction technique	Internal standard	Analytical parameters	Analytical technique
Deltamethrin (incorporated into PEt or PP) CIPAC method 333/LN/(M2)/3	Xylene	Heating under reflux (30 min)	Dibutylphthalate	isooctane/dioxane +0.15% water(94/6) Colum T°: 35°C	HPLC Column CN (5µm), 250 x 4.6 (i.d.) mm Diode array detector (DAD) 230nm
Deltamethrin (coated onto polyester) CIPAC method 333/LN/(M)/3	Isooctane/dioxane (80/20)	Water bath + sonication	Dipropylphthalate	Isooctane/dioxane + 0.15% water(94/6)	HPLC Column CN 5µm, 250 x 4.6 (i.d.) mm DAD (230nm)
Permethrin (incorporated into PEt) CIPAC method 331/LN/M/3	Heptane	Water bath 85-90°C for 45 min	Triphenylphosphate	Oven T°: 240°C Detector: 265°C	GC Dimethylpolysiloxane 5% 30m x 0.25 (i.d.) mm 0.25µm phase thickness Flame ionisation detect. (FID)

Current analytical methods

Managing the implementation of the methods



Development of multi-pesticides methods for QC

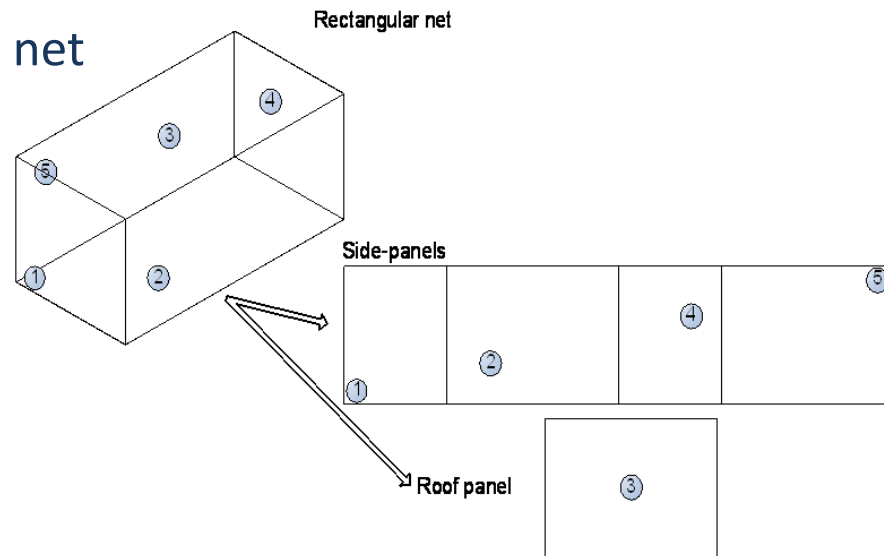
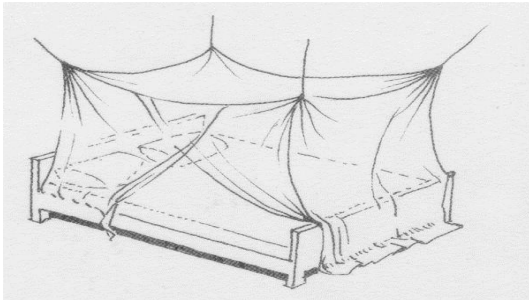
Determination of common conditions for analysis of the 18 actives substances recommended by WHO for LNs and IRS:

- Sample preparation
- Selection of an internal standard + colouring agent
- Determination of the extraction conditions
- Optimisation of the analytical methods (GC/HPLC)
- Validation of the methods
- Comparison of the developed methods with the CIPAC methods

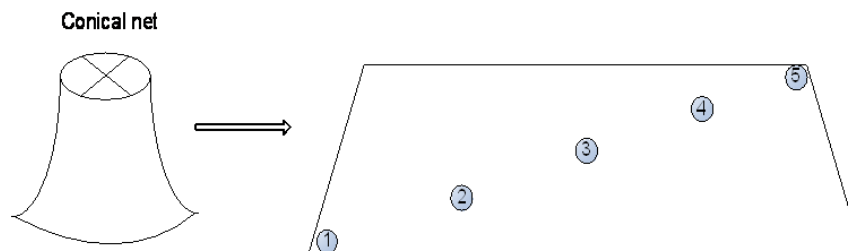
Sample preparation

Sampling method, 5 pieces of 25cm*25cm
according to FAO/WHO Specification Manual

Rectangular net

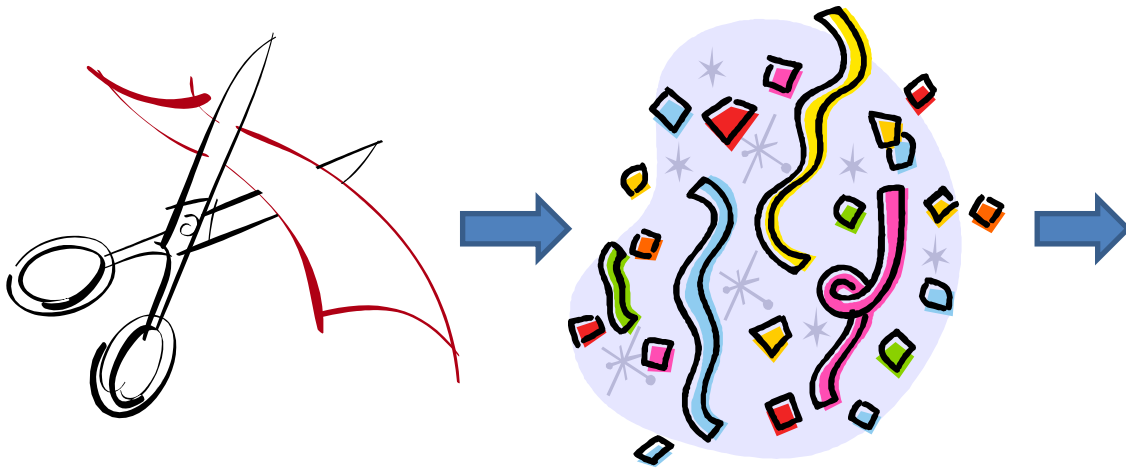


Conical net



Sample preparation

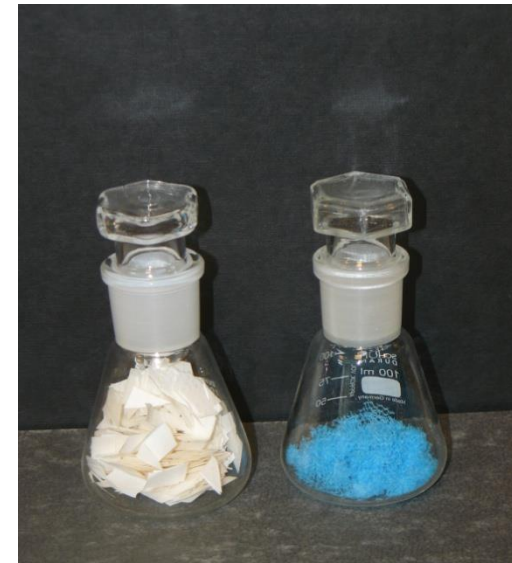
Cutting and homogenisation of samples



Cut the pieces of

- Long lasting Net (LN)
- Filter paper

in small pieces of 5-10 mm square, mix efficiently.



Filter paper

Net

Extraction conditions

Selection of extraction solvents

Suitable characteristics

- Able to solubilise the active substances of interest
- Efficient in fully impregnation of net fibers
- Compatible with GC and LC analysis
- With the lower toxicity
- As cheap as possible

2 Selected solvents

- **Heptane** for **LN**s (GC / LC normal phase)
- **Acetonitrile** for **filter papers** (LC reversed phase)

Extraction conditions

Selected technique: internal calibration

Advantages of internal calibration

- Easy manipulations and very simple glassware
- Few steps of procedure (no depletion of samples)
- High repeatability and accuracy

Suitable characteristics of internal standard

- Compatible with extraction solvents and analytical methods (GC/LC)
- Similar polarity as the substances of interest
- No interference with the focused substances
- Detector response close to the one of active substances
- Not too expensive

Selected internal standard: **Dicyclohexylphthalate**

Selection of a colouring agent

Aim of using a colouring agent

- To show the dispense of internal standard
- Eliminate operator error

Suitable characteristics

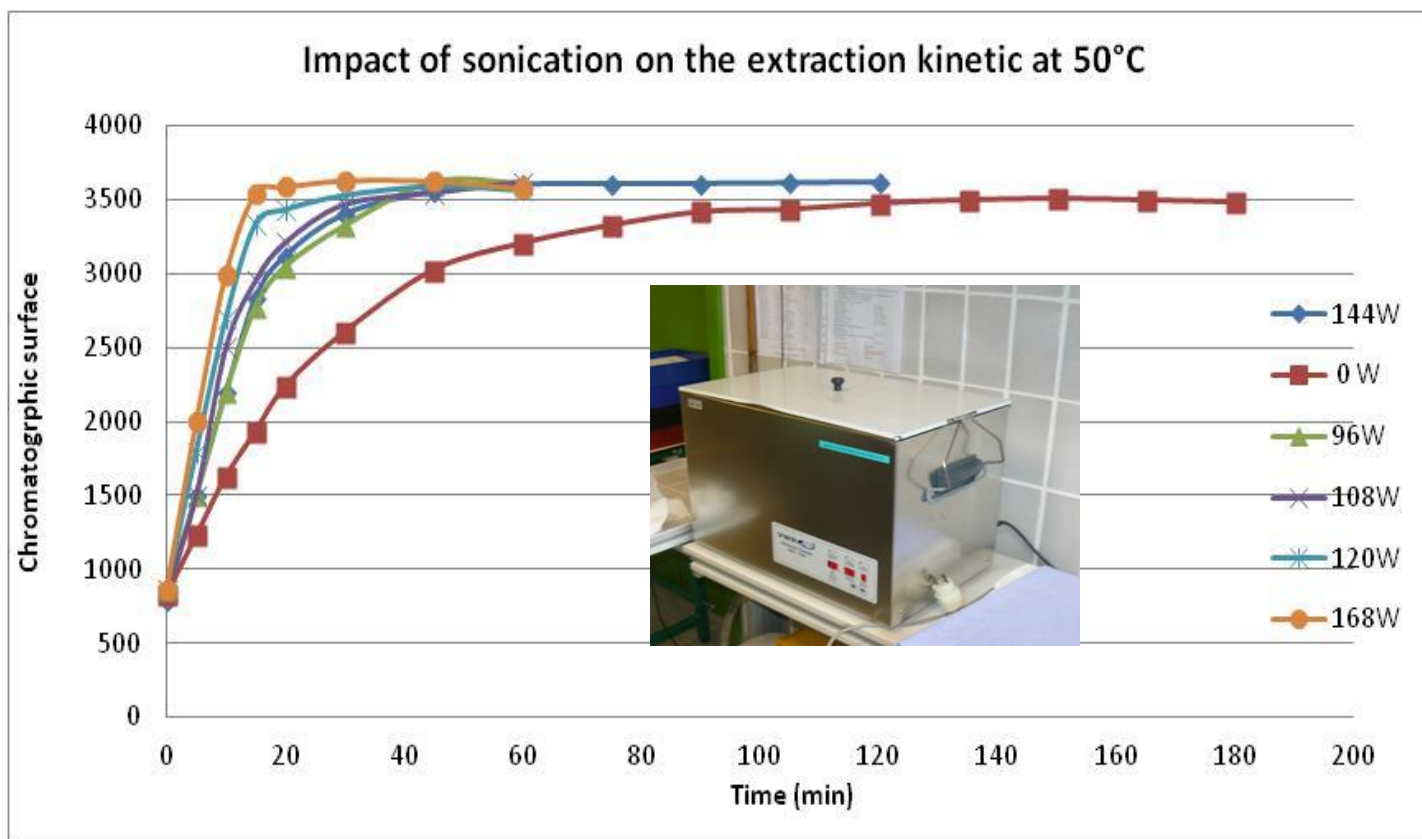
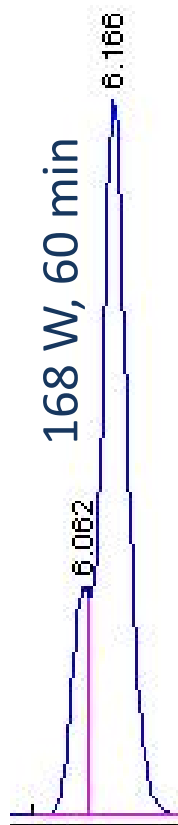
- Soluble in the selected solvents
- No interference with the focused substances
- Detectable to the naked eye

→ **Unisol Blue** (Mixed with the internal standard)



Extraction conditions

Determination of optimal conditions (T° ...)



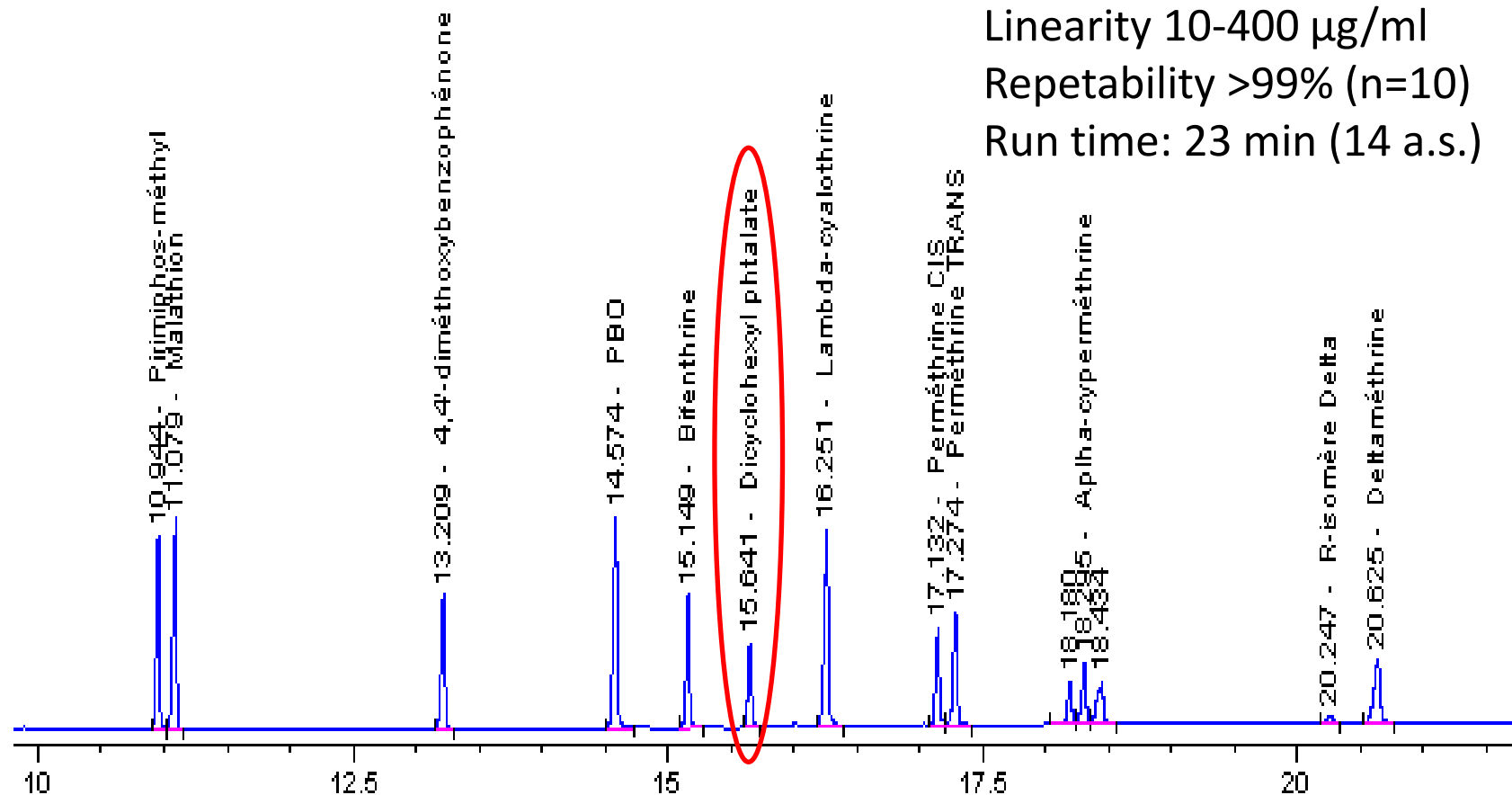
→ 50°C + sonication 108 W, for 60 minutes

Analytical techniques

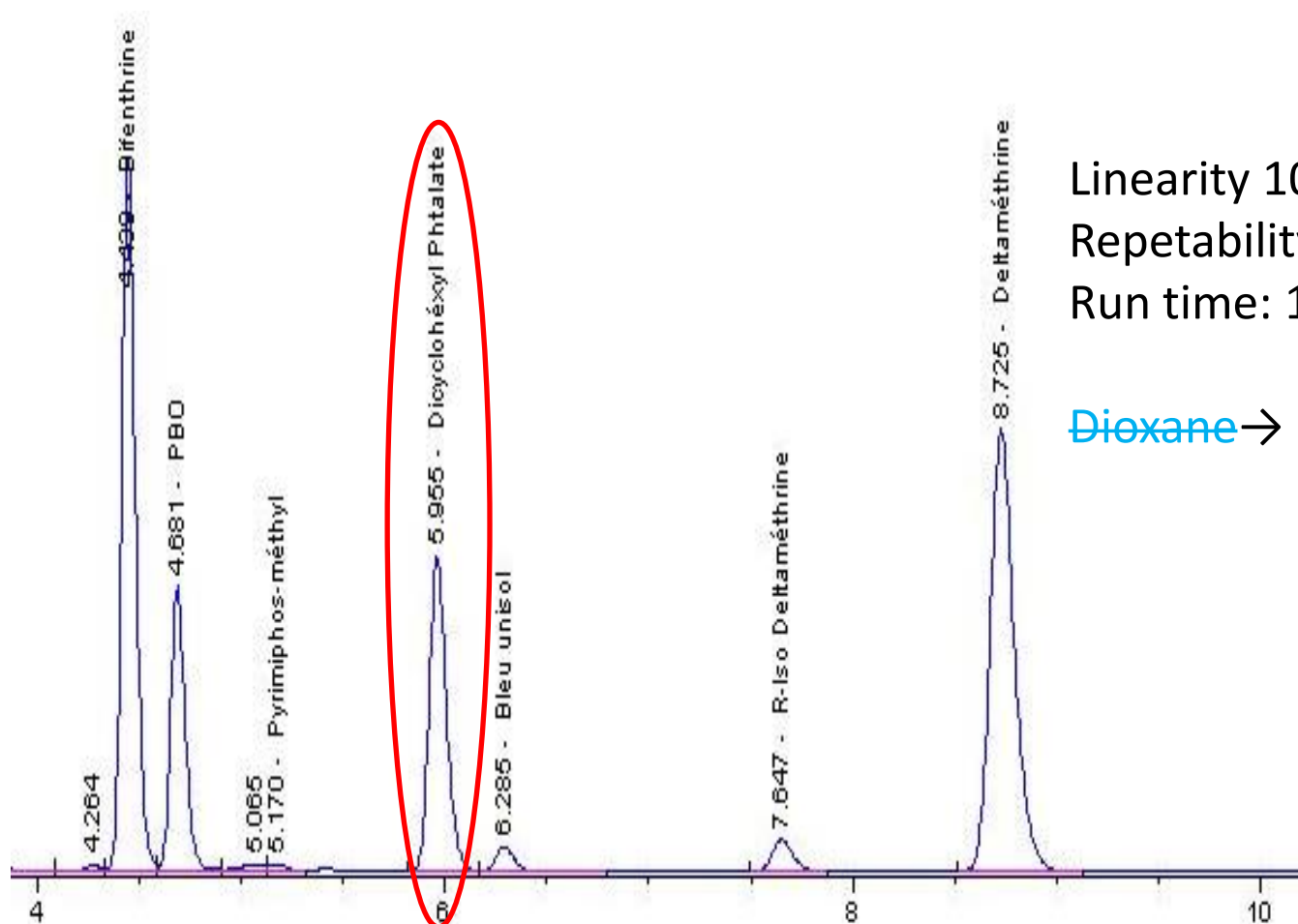
- Long-lasting nets: **heptane** → GC
→ HPLC Normal phase
- Filter papers for IRS: **acetonitrile** → HPLC Reversed phase



Long-lasting insecticidal nets: Gas Chromatography (GC)



Long-lasting insecticidal nets: Liquid Chromatography (LC)

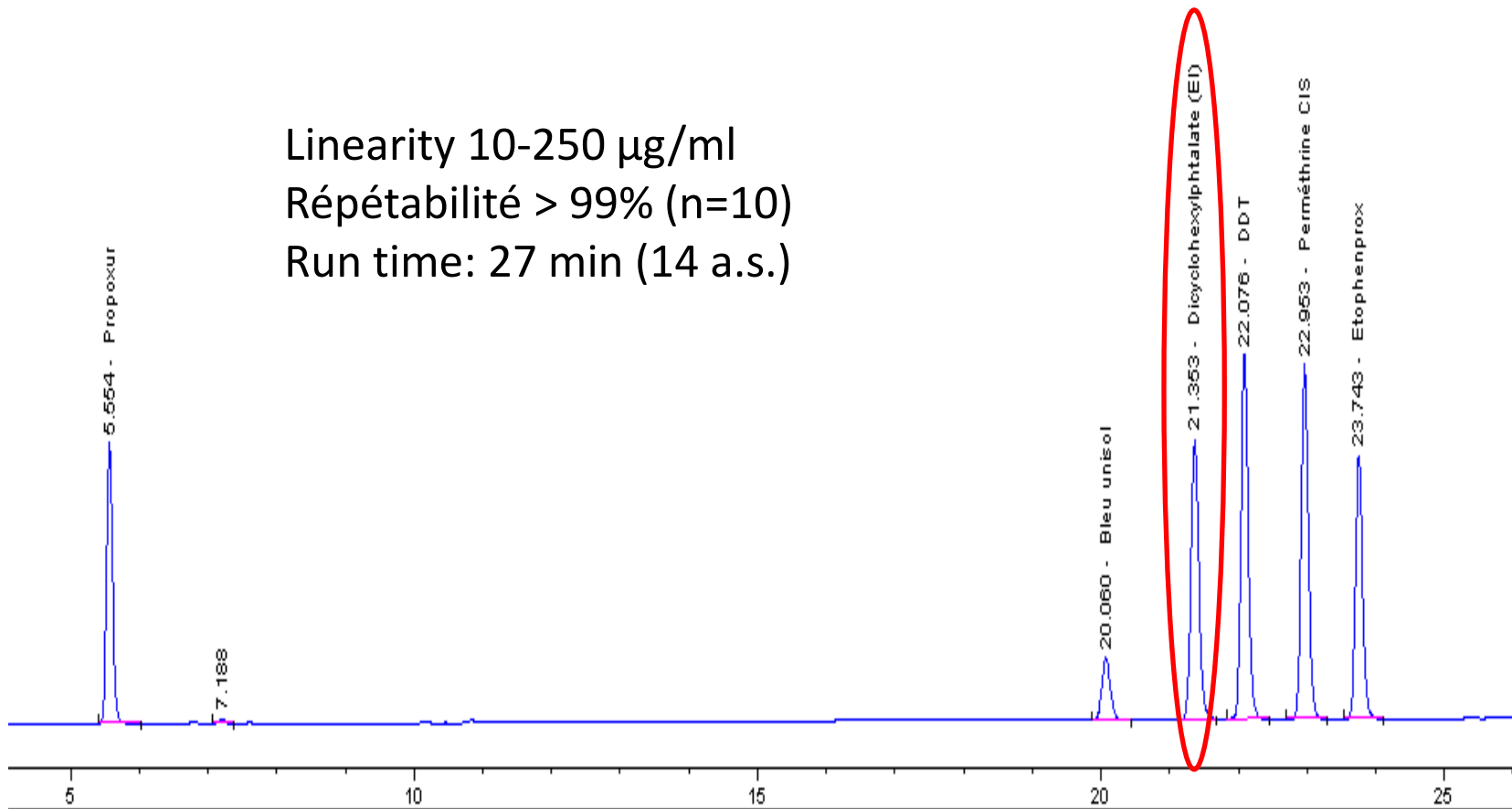


Linearity 10-250 $\mu\text{g/ml}$
Repetability > 99% (n=10)
Run time: 13 min (12 a.s.)

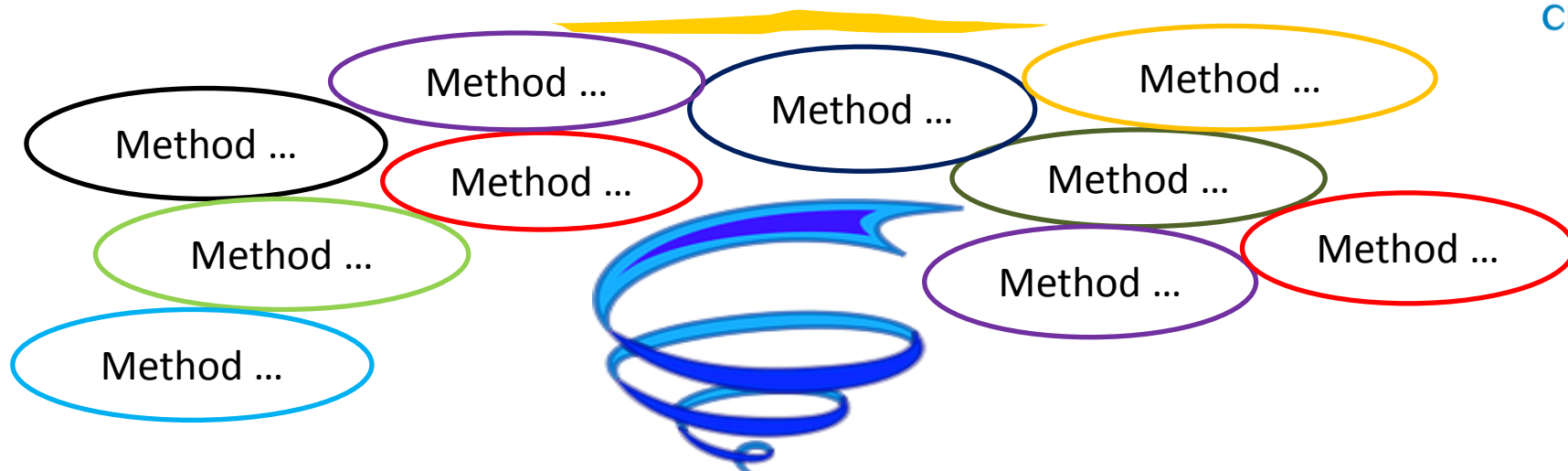
Dioxane \rightarrow Isopropanol

IRS: Reversed phase Liquid Chromatography (RP-LC)

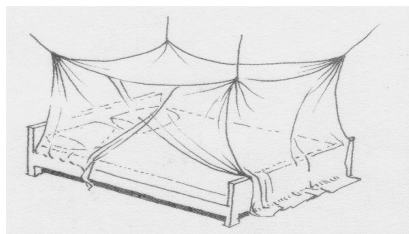
Linearity 10-250 µg/ml
Répétabilité > 99% (n=10)
Run time: 27 min (14 a.s.)



Comparison multi-pesticides / CIPAC methods



- 1) Sampling/Cuting/ Weighting
- 2) 2 solvents (Heptane / Acetonitrile)
- 3) Internal standard + colouring agent
- 4) Extraction Conditions (60min, 50°C + sonication)



Nets

GC (23 min) – Lc_{NP} (13 min)

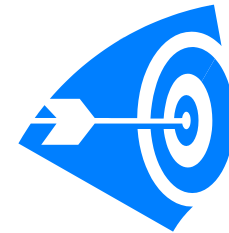
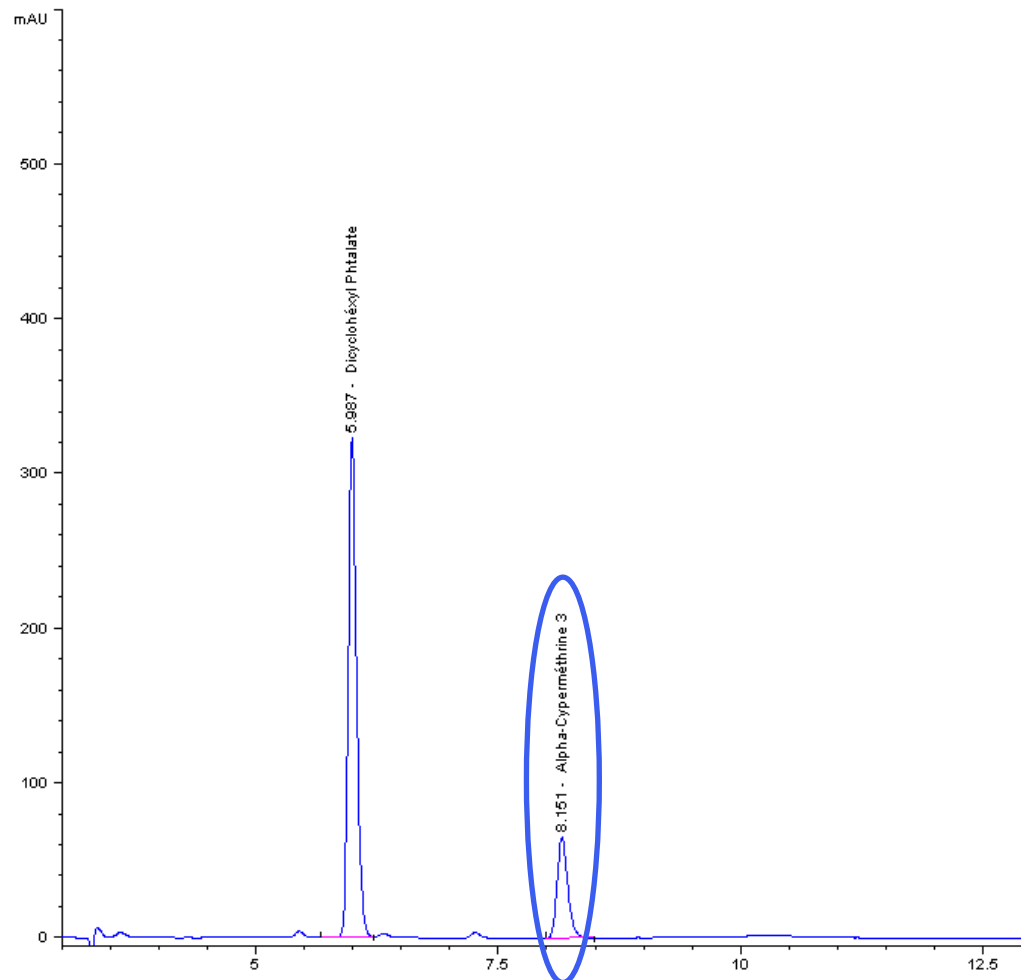


Filter papers

LC_{RP} (27 min)

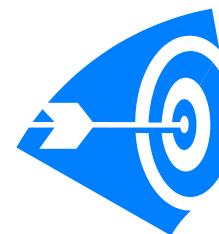
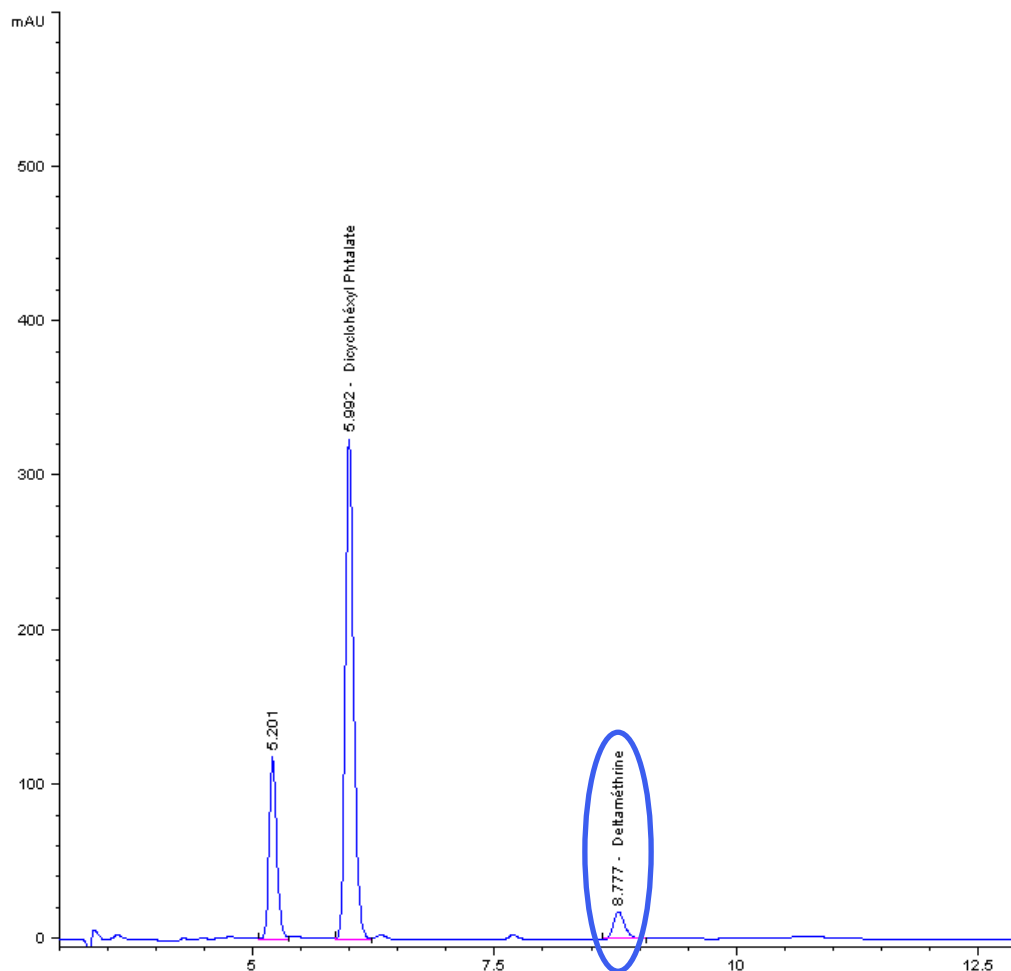
Comparison multi-pesticides / CIPAC methods

Analysis of MAGNet®: Alpha-cypermethrin into PEt (5.8 g/kg)



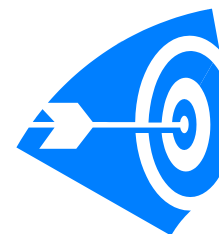
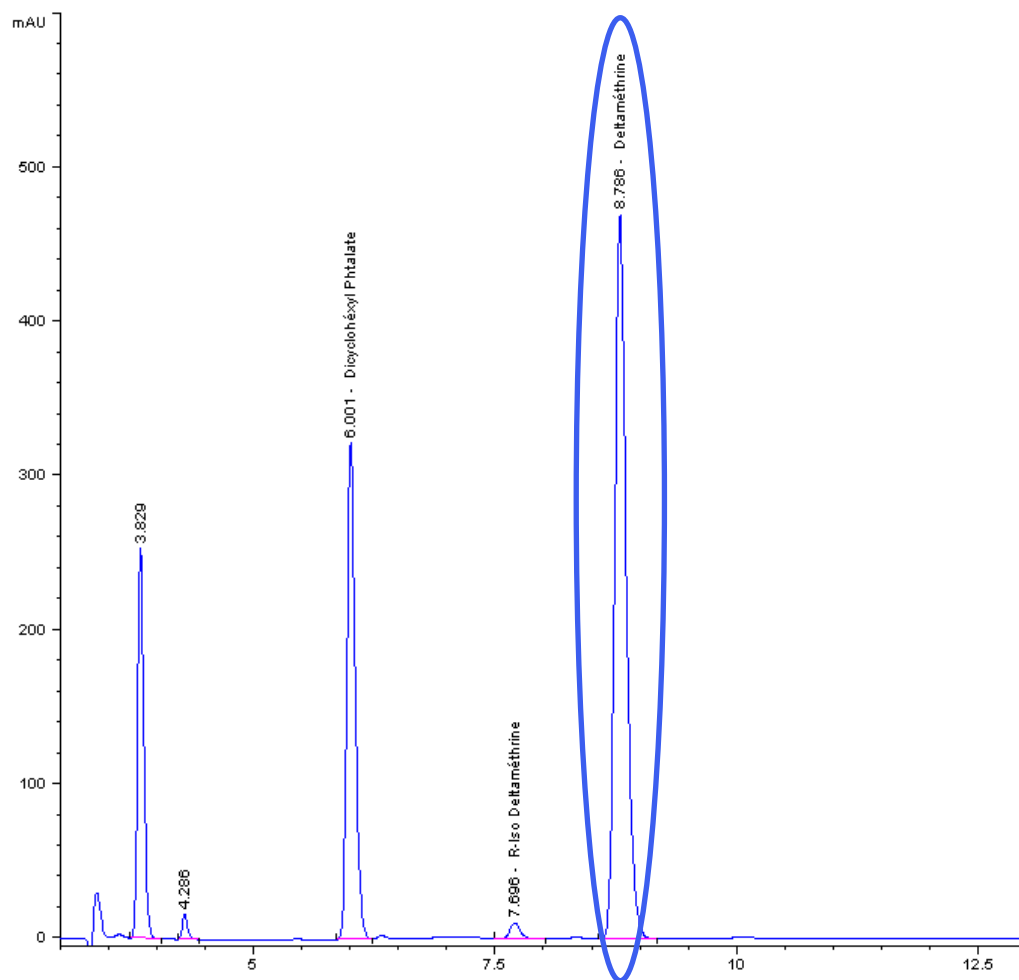
Comparison multi-pesticides / CIPAC methods

Analysis of Netprotect®: Deltamethrin into PEt (1.8 g/kg)



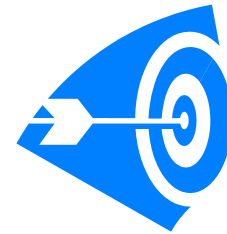
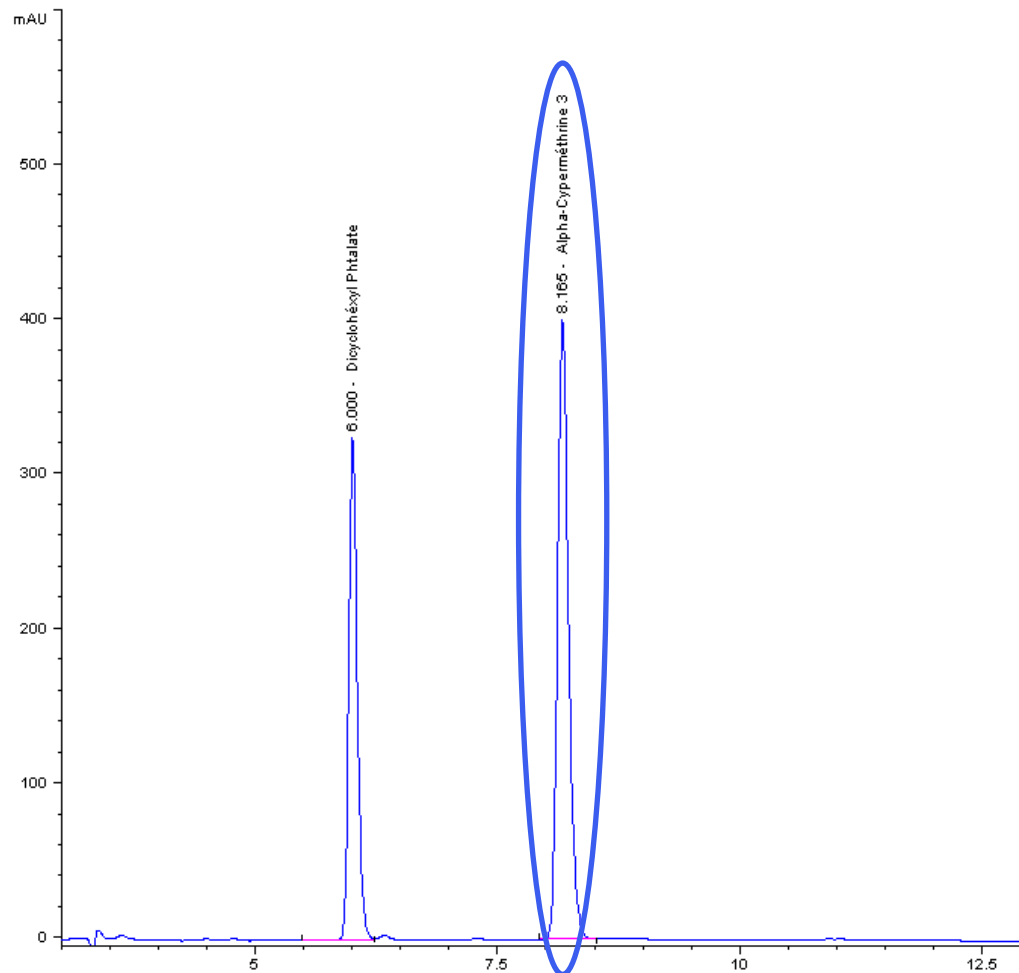
Comparison multi-pesticides / CIPAC methods

Analysis of LifeNet®: Deltamethrin into PP (8.5 g/kg)



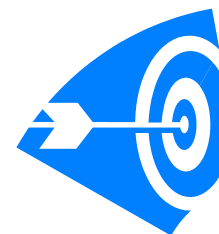
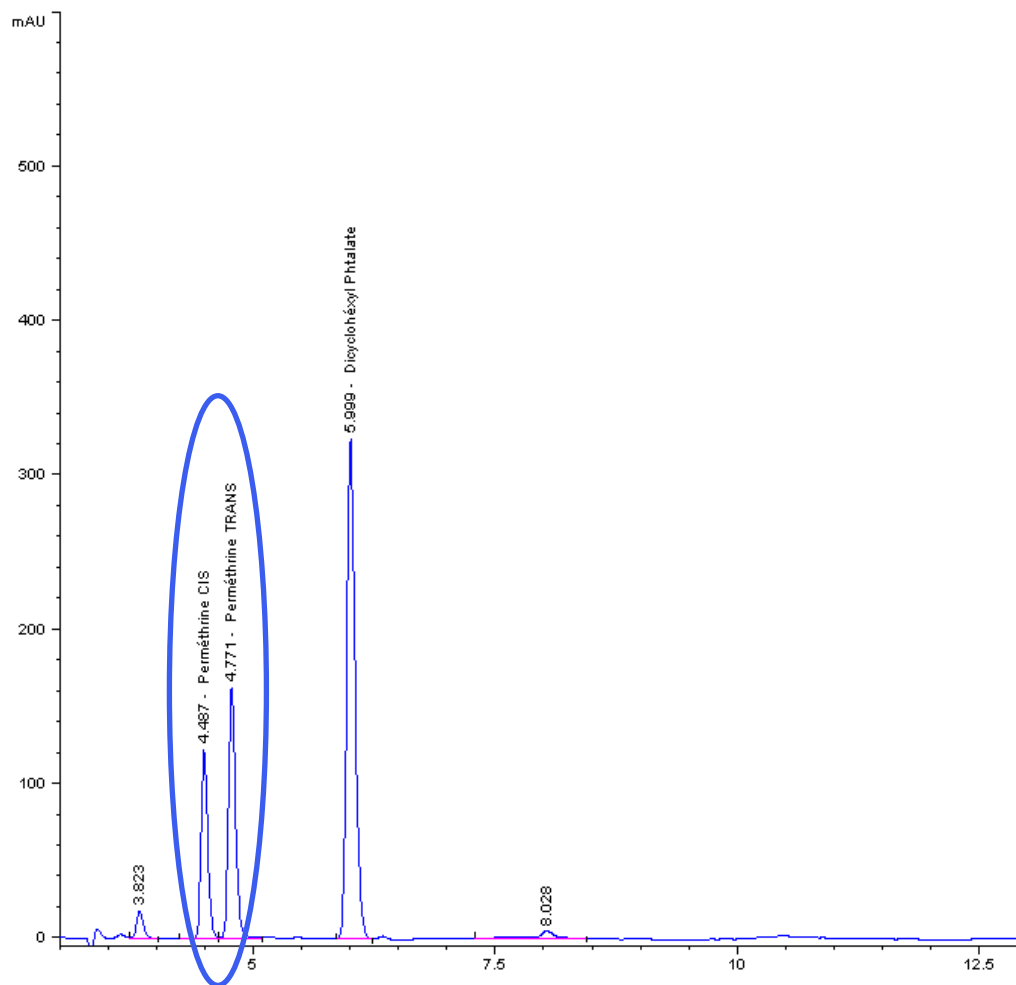
Comparison multi-pesticides / CIPAC methods

Analysis of Interceptor®: Alpha-cypermethrin on PEst (5 or 6.7 g/kg)



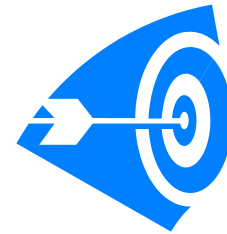
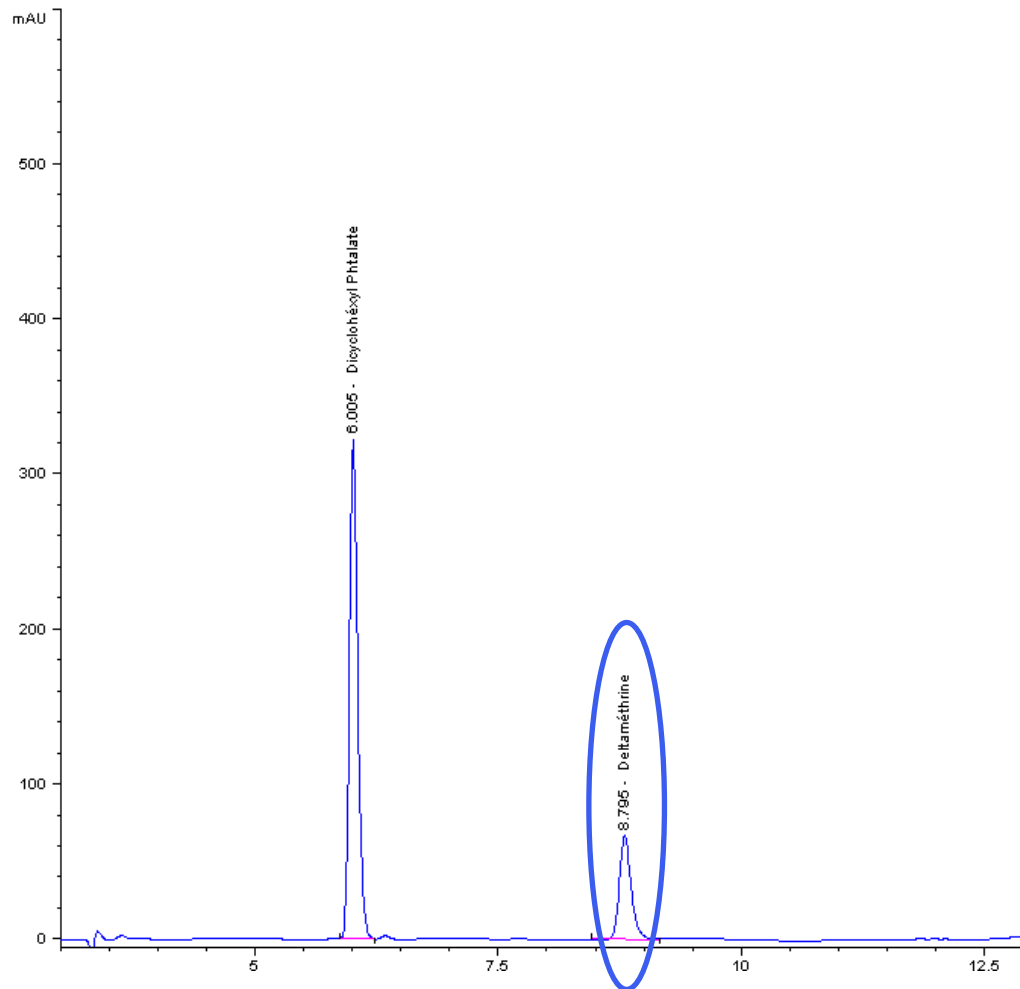
Comparison multi-pesticides / CIPAC methods

Analysis of Olyset®: Permethrin into PEt (20 g/kg)



Comparison multi-pesticides / CIPAC methods

Permanet 2.0[®]: Deltamethrin on PEst (1.4 or 1.8 g/kg)





Conclusion

The objective of a “common” method has been reached

- Time saving method: few steps of procedure
 - Easy manipulations and very simple glassware
 - One way preparation of samples (for both Nets and filter papers)
 - Common Internal standard + colouring agent (for Nets and filter papers)
 - No more than 2 extraction solvents (with moderate toxicity)
 - Limited consumption of extraction solvents (+/- 20ml per sample)
 - Common extraction conditions
 - Optimised analytical methods (GC and LC)
 - Perfect Linearity of detectors response in wide range of concentration
 - High accuracy and repeatability
 - Applicable for the analysis of the different matrices, 18 active substances
- Well adapted for Quality control

Thank you for your attention



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