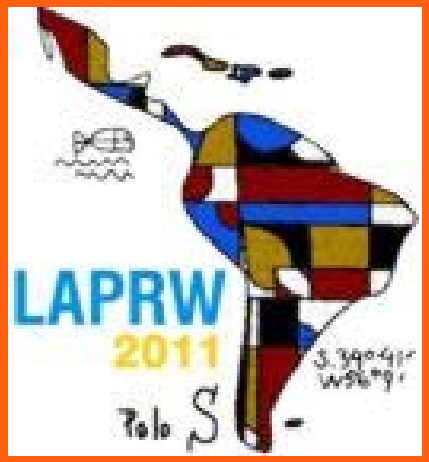
WETENSCHAPPELIJK INSTITUUT
VOLKSGEZONDHEID
INSTITUT SCIENTIFIQUE
DE SANTÉ PUBLIQUE

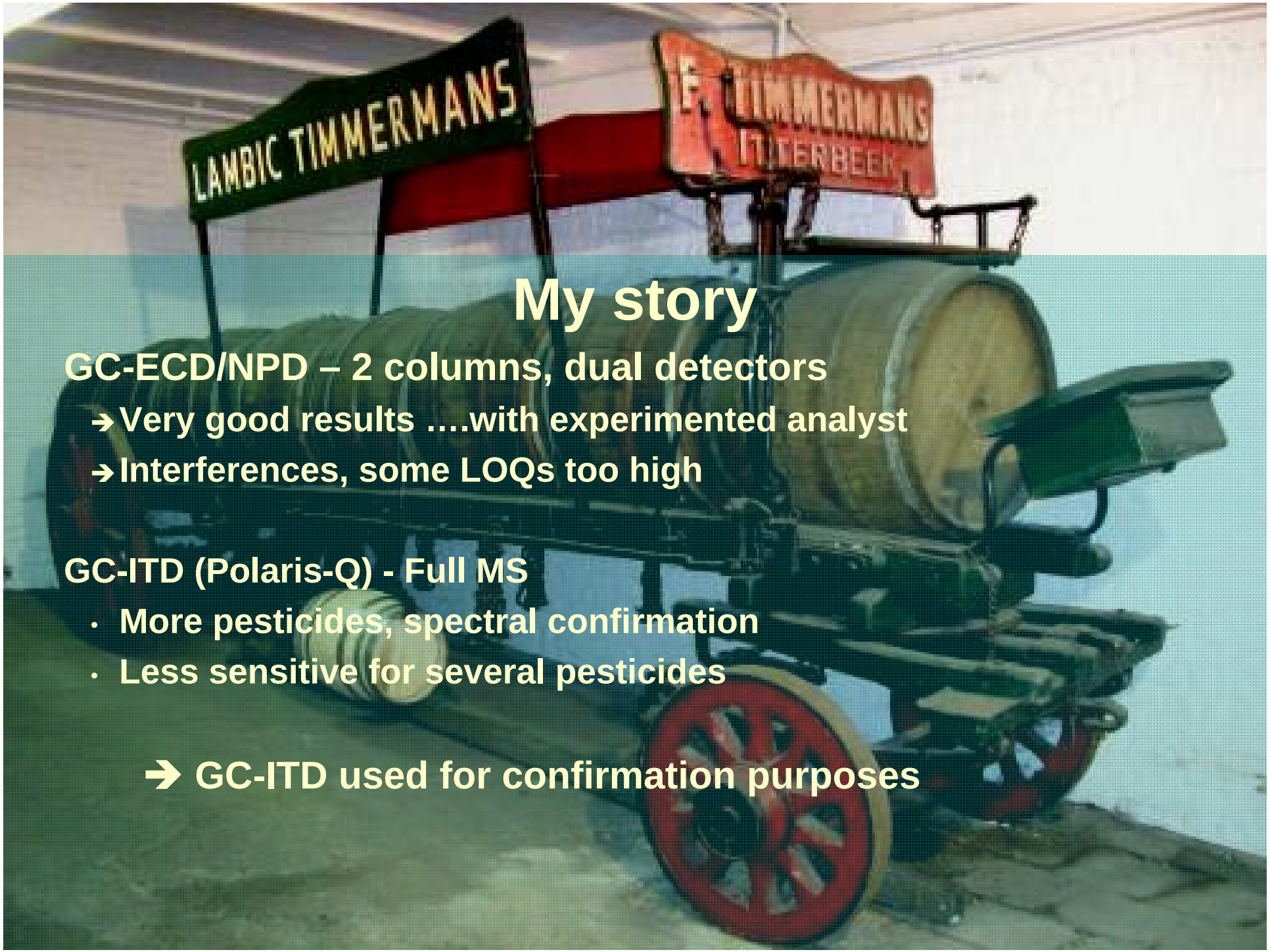
Comparison of Multi-residue methods to determine pesticide residues in Fruit, Vegetables and Cereals by GC – Tandem Mass Spectrometry

Vincent HANOT & Martine Van Houche



Rue Juliette Wytsmanstraat 14 | 1050 Brussels | Belgium
T +32 2 642 51 89 | F +32 2 642 56 91 | email: vincent.hanot@wiv-isp.be | www.wiv-isp.be

.be



My story

GC-ECD/NPD – 2 columns, dual detectors

- ➔ **Very good results ….with experimented analyst**
- ➔ **Interferences, some LOQs too high**

GC-ITD (Polaris-Q) - Full MS

- **More pesticides, spectral confirmation**
- **Less sensitive for several pesticides**

➔ **GC-ITD used for confirmation purposes**

My story

GC-MS (Single Quad)

- Many systems working, very good reputation

- Discussion with some colleagues…

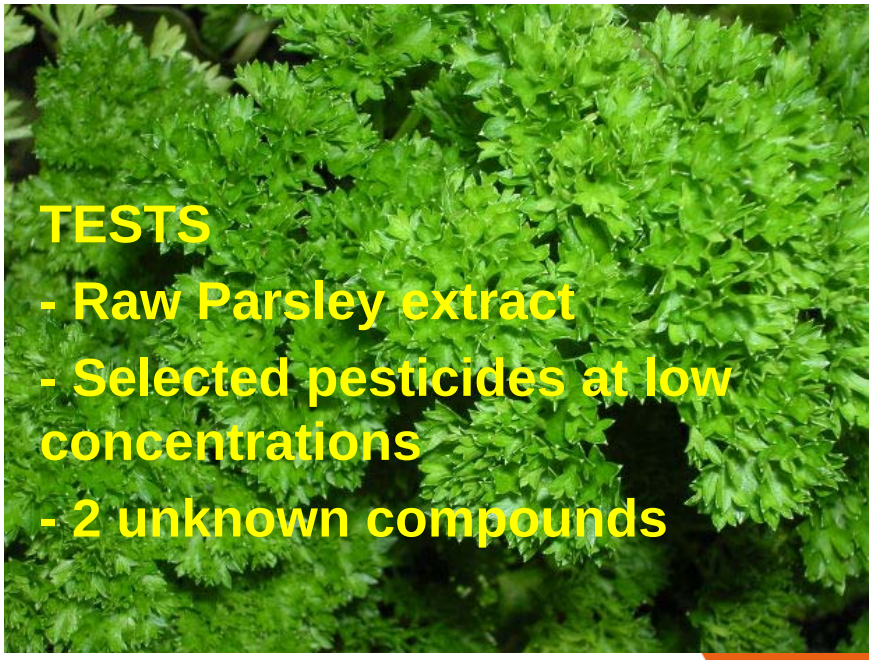
  ..after a few Strong Belgian Beers… in fact troubles for many pesticide/matrix combination

➔ Other systems?

Interbrew

« New » Alliance

- ➔ GC - TOF HRMS
- ➔ GC - TOF LRMS
- ➔ GCxGC - TOF LRMS
- ➔ GC - MS/MS



Performance

System	Unknowns	Quantitative	Remarks
GC - TOF HRMS	Not found	Good	LOQ not OK for all Screening possible
GC - TOF LRMS	Not found	Not OK for all pesticides	ELIMINATED
GC x GC – TOF LRMS	Not found	Very Good	Some bad peak shapes LOQ not OK for all Screening possible
GC - MS/MS	/	Very Good	

From GC-ECD/NPD to GC-MS/MS

- New FULL validation
- GOOD OPPORTUNITY to use another MRM

➔ Need to compare

The best Method

My best selected method is…..

…not yours

Ask to a Belgian, which beer is the best one?

Not only ONE answer

The best one is function of

- GC system (configuration,…)
- Apparatus in the lab
- Scope of pesticides, matrices
- Number of samples (+ number/batch)
- Time response needed for the client
- …$$...

MRM Methods

The most known and used

- Mini-LUKE & others acetone based extraction (CEN)
- Quechers (CEN)
- Swedish (CEN) & other ethyl acetate extraction

Others…., Modifications..

Selection criteria

- GC determination
- Include a Clean-up step
- CEN (or ISO)
  - Internationally recognized → better confidence (Client, Accreditation bodies,...)
  - Fully validated (incl inter-laboratory)
  - Good experimental description with even different conditions (Systems, injectors, …)

Selected MRM methods

	Mini-LUKE / EN12393 - Modified	QuEChERS EN15662	RIKILT* (Ethyl Acetate - DSP)
Extraction	Acetone	ACN	Ethyl acetate
Clean-up	L/L	phase separation + PSA (+ C_{gr})	PSA (+ C_{gr})

*H Mol et al -Anal Bioanal Chem (2007) 389:1715-1754

Method Modifications in our lab

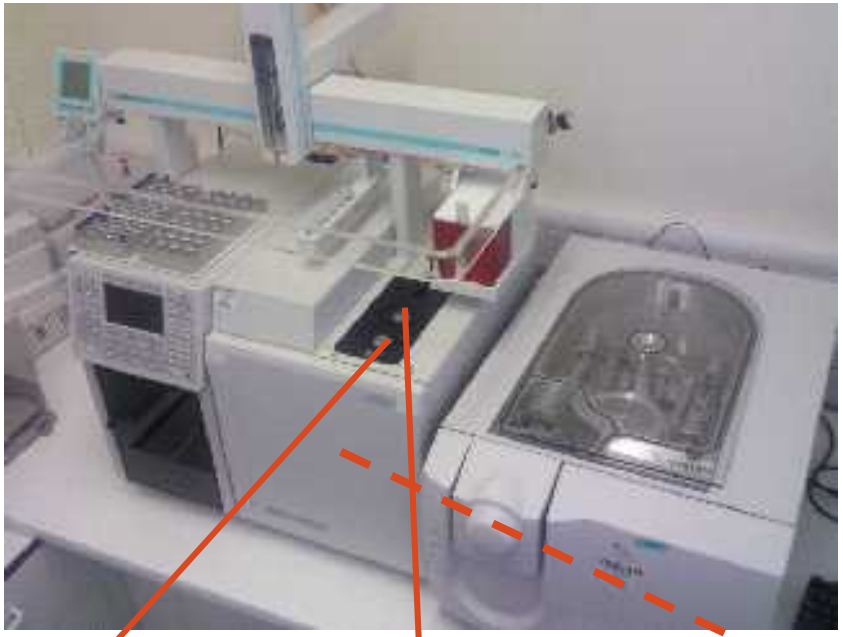
- Addition of a mixture H_3PO_4/NH_4Cl on the acetone extract

Raw extract  After addition

- L/L clean-up : Dichloromethane / Petroleum Ether replaced by di-isopropylether

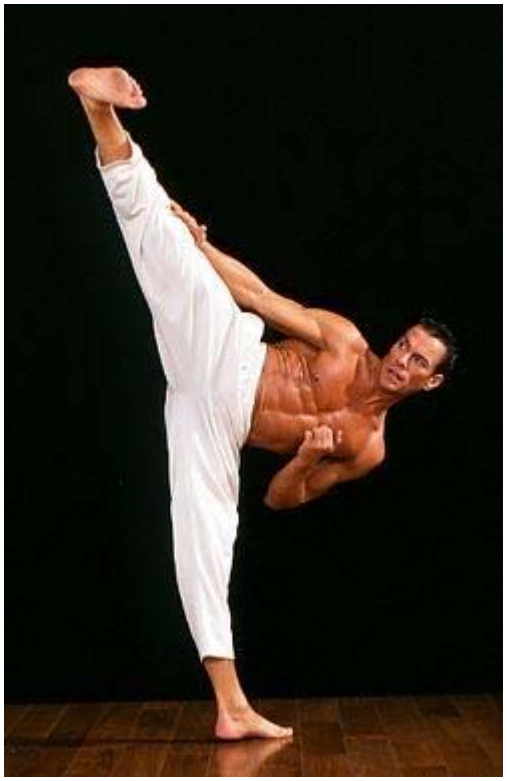
Our GC-MS/MS

Combi PAL - VARIAN 3800 – 320 MS



PTV Split/splitless Switching Valve

How to compare methods ?



- Recoveries
- Safe
- Speed
- Clean-up efficiency
- Cost

OK but how to really compare?

Full validation for all different matrices?

Which level ?

Recoveries - Level

Fixed level : 100 ng/ml

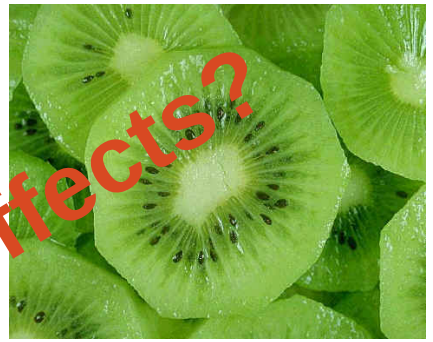
Why not LOQ ?

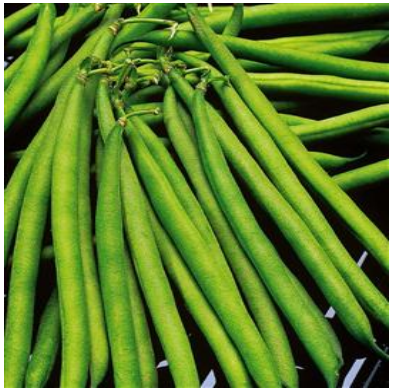
- Could be different with methods
- Higher CVs (small S/N,…..)


WHICH MATRIX?

Mix of matrixes

Mix of effects?





Recoveries – Pesticides list

aclonifen	clomazone	dimoxystrobin	flusilazol	oxadixyl	pyrazophos
acrinathrin	coumaphos	diniconazole	flutolanil	parathion-ethyl	quinoxyfen
benalaxyl	cyflufenamid	diphenylamine	fluvalinate	parathion-methyl	spiromesifen
bifenthrin	cyfluthrin	disulfoton	folpet	PCNB	sulfotep
binapacryl	cyhalothrin-l	endosulfan-a	fonofos	penconazole	tecnazene
bioallethrin	cypermethrin	endosulfan-b	HCH-b	pendimethalin	tefluthrin
bitertanol	DDD-p,p	endosulfan-sulfate	HCH-g	pentachloraniline	tetrachlorvinphos
bromophos-ethyl	DDE-p,p	epoxiconazole	hexaconazole	permethrin	tetradifon
bromophos-methyl	DDT-o,p	esfenvalerate	iprodione	phorate	thiometon
bromopropylate	DDT-p,p	ethion	kresoxim-methyl	phosalone	tolclofos-methyl
bupirimate	deltamethrin	etofenprox	malathion	phosmet	tolylfluanid
buprofezin	diazinon	etrimfos	mecarbam	picostrobin	triadimefon
captafol	Dichlobenil	fenamidone	mepronil	pirimiphos-ethyl	triadimenol
captan	dichlofluanid	fenarimol	Methacrifos	pirimiphos-methyl	triapenthenol
carbophenothion	Dichloroaniline 3,5-	fenchlorphos	methidathion	procymidon	triazophos
chlorfenvinphos-cis	diclobutrazol	fenitrothion	myclobutanil	profenofos	trifloxystrobin
chlorfenvinphos-trans	dicloran	fenpropathrin	nitrothal-isopropyl	propetamphos	triflumizole
chlorothalonil	dicofol	fenthion	nuarimol	propiconazole	trifluralin
chlorpyriphos-ethyl	difenoconazole	fenvalerate	OPP	propoxur	vinclozolin
chlorpyriphos-methyl	dimethenamid-p	fluquinconazole	orbencarb	propyzamide	

Safe <> extraction technique

	Mini-LUKE modified	RIKILT	QuEChERS
Solvent Toxicity	Acetone	Ethyl acetate	ACN
Extraction technique	Omni-mixer®	Ultra-turrax®	Vortex®[1] or Ultra-turrax®
"Safe"	*** (Omni : closed vessel)	** (strong odor)	** (ACN - closed vessel if vortex)

[1]if cryo-milled

Extraction techniques*

Matrix : Lemon with incured systemic pesticides

Not cryo-milled

Efficiency:

Ultra-Turrax ~ Omnimixer > Vortex (-10%) > Ultrasonication (-25%)

* Poster V. Hanot et al, EPRW 2006

Extraction techniques – New results

Solvent: **Ethyl Acetate** (Swedish Method)	**SYSTEMIC PESTICIDES IN CELERY** **Mean Concentration (µg/kg), RSD in brackets**			
	Not "cryo"		**Cryo-homogenization**	
	Ultra-Turrax®	**Vortex + Ultrasonication**	**Vortex**	**Vortex + Ultrasonication**
Pirimicarb	**165.4 (2.2)**	**139.7 (3.9)**	**157.2 (0.7)**	**160.0 (3.7)**

- 15 %

Extraction techniques – New results

Solvent: **Ethyl Acetate** (Swedish Method)	**SYSTEMIC PESTICIDES IN CELERY** **Mean Concentration (µg/kg), RSD in brackets**			
	Not "cryo"		**Cryo-homogenization**	
	Ultra-Turrax®	**Vortex + Ultrasonication**	**Vortex**	**Vortex + Ultrasonication**
Difenconazole	275.9 (3.9)	163.6 (12.5)	212.1 (2.4)	215.9 (3.7)

- 40 %

- 22 %

Recovery in Mix 4F&4V

	Mini-LUKE Derived	RIKILT	QuEChERS
Sample/ml	0.5g/ml	0.5g/ml	1g/ml
Level in final extract	100 ng/ml	100 ng/ml	100 ng/ml
Average (RSD)	90.4 (13.6)	101 (5.5)	95.7 (16.1)
Pest in 70-120	93.9 %	97.7%	92.4 %
Pest in 70-110	93.9 %	93.2 %	87.8 %
Tendency	Several lower	/	Several Higher
Rate for recoveries	**	***	**

Recovery - Some specials case

Mini-LUKE Derived		QuEChERS	
Dichloroaniline 3,5-	Rec << 70	Captafol	Rec < 20
Oxadixyl	Rec < 20	Captan	Rec << 70
		Chlorothalonil	Rec << 70 RSD >> 20
		Dicofol	Rec << 70 RSD >> 20
		Fenchlorphos	Rec >> 120

Practical…ask the analyst in the lab!!!

	Mini-LUKE Derived	RIKILT	QuEChERS
Time & Practical	All several steps		
	Filtration[1] (one by one)	Centrifugation[1] 2X	Centrifugation[1]
	Waiting L/L	Weighting time	Use of pre-weighted tubes (⚠ different composition & proportions)
	Washing	Waste	Waste
Global	**	**	**

[1]A few samples < > many samples in one batch

Selection of QuEChERS KIT from Agilent

	Kit	Quantity & Size/Pack	AOAC 2007.01 METHOD Contents and Part No.	EUROPEAN METHOD – EN 15662 Contents and Part No.
	**GENERAL FRUITS AND VEGETABLES:** Removes polar organic acids, some sugars and lipids	100 – 2 mL tubes	50 mg PSA 150 mg $MgSO_4$ **5982-5022** **5982-5022CH**	25 mg PSA 150 mg $MgSO_4$ **5982-5021** **5982-5021CH**
		50 – 15 mL tubes	400 mg PSA 1200 mg $MgSO_4$ **5982-5058** **5982-5058CH**	150 mg PSA 900 mg $MgSO_4$ **5982-5056** **5982-5056CH**
	**PIGMENTED FRUITS AND VEGETABLES:** Removes polar organic acids, some sugars and lipids, and carotinoides and chlorophyll; not for use with planar pesticides.	100 – 2 mL tubes	50 mg PSA 50 mg GCB 150 mg $MgSO_4$ **5982-5222** **5982-5222CH**	25 mg PSA 2.5 mg GCB 150 mg $MgSO_4$ **5982-5221** **5982-5221CH**
		50 – 15 mL tubes	400 mg PSA 400 mg GCB 1200 mg $MgSO_4$ **5982-5258**	150 mg PSA 15 mg GCB 900 mg $MgSO_4$ **5982-5256** **5982-5256CH**
	**HIGHLY PIGMENTED FRUITS AND VEGETABLES:** Removes polar organic acids, some sugars and lipids, plus high levels of carotenoides and chlorophyll; not for use with planar pesticides.	100 – 2 mL tubes		25 mg PSA 7.5 mg GCB 150 mg $MgSO_4$ **5982-5321** **5982-5321CH**
		50 – 15 mL tubes		150 mg PSA 45 mg GCB 900 mg $MgSO_4$ **5982-5356** **5982-5356CH**

Pessimist: Half Empty

Complexity to manage these Kits

Cost

Risk of errors

Optimist: Half Full

Adapted for all kind of matrices and always the best conditions

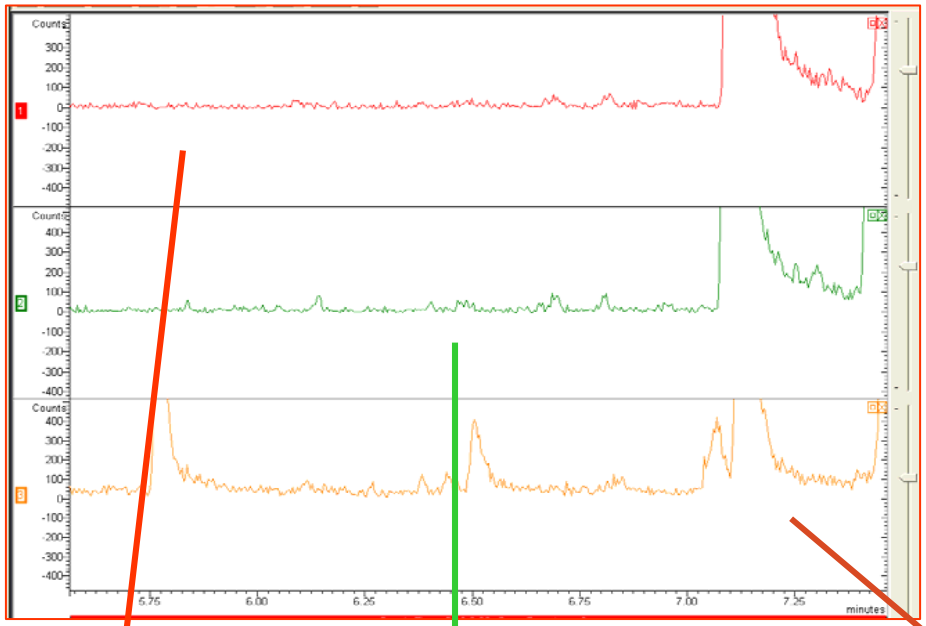
Nice chemistry

Cost

	Mini-LUKE Derived	RIKILT	QuEChERS
Cheap	*** (PTV Liner)	** PSA, PTV Liner	* ACN: 185 $ / Liter PSA, tubes, PTV Liner

Clean-up efficiency

GC-MS/MS



	Mini-LUKE Derived	RIKILT	QuEChERS
Clean-up	***	**	**

Overall Results – F&V

	Mini-LUKE -Derived	RIKILT	Quechers
“Safe”	***	**	**
Time	**	**	**
Cheap	***	**	*
Clean-up	***	**	**
Recoveries	**	***	**
Global	**13 ***	**11 ***	**9 ***


For cereals…
…..let’s do it again


For two methods
Mini-LUKE modified and Rikilt (C18)

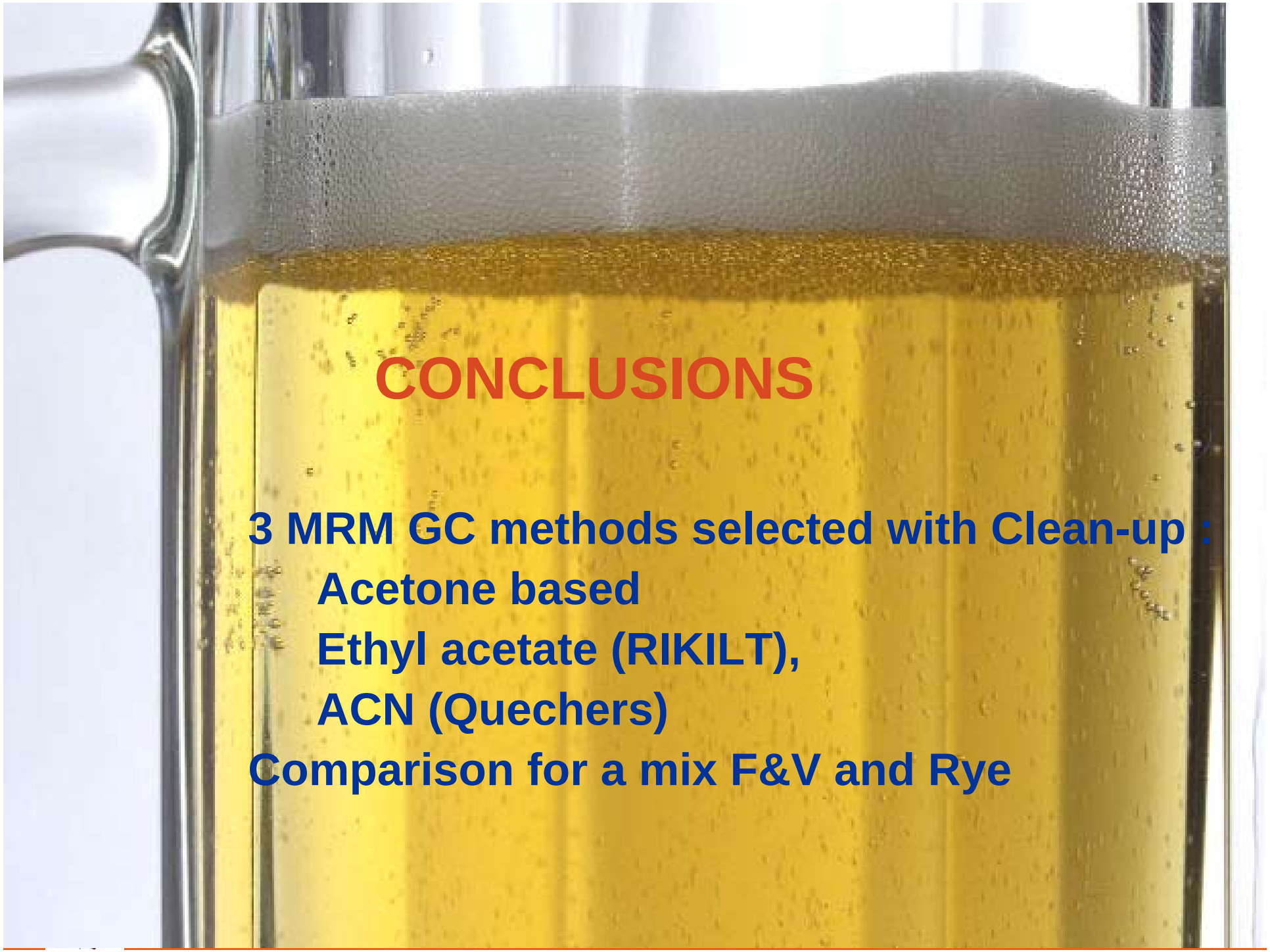
Recovery in Cereals (Rye)

Addition of water in both methods

- Same general observations
- More pronounced trends

  i.e lower recovery for many pyrethroïds (for acetone extraction)

  NB: paradox of water addition for acetone



CONCLUSIONS

3 MRM GC methods selected with Clean-up :

- **Acetone based**
- **Ethyl acetate (RIKILT),**
- **ACN (Quechers)**

Comparison for a mix F&V and Rye

Conclusions of the method comparison

- **Good recoveries for all 3 methods**

  Ethyl Acetate > Acetone > ACN

- **Considering all aspects (Safe, Time, Cost, Clean-up, Practical…)**

  Modified Mini Luke (acetone) for F&V

  Rikilt (Ethyl Acetate) for Cereals

…draft your own conclusions considering your specificity


Let’s take a break
Leffe