

Pesticide Multiresidue Analysis in Cereal Grains using Modified QuEChERS Method

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in Wyndmoor, PA



in Minneapolis, MN

Objective

State-of-the-art approach for pesticide residue
analysis in cereal grains and other matrices



Traditional

Luke method

GC/ECD, FPD, NPD
Confirmation:
GC-ITD MS

HPLC-fluorescence
for carbamates

State-of-the-art

QuEChERS method

DSI-LVI-GC-TOF MS

UPLC-MS/MS

UPLC-MS/MS



Mobile phase:

A = 10 mM HCOONH₄ in water (pH 3)
B = 10 mM HCOONH₄ in methanol

UPLC:

Waters Acquity

MS/MS:

Waters Quattro Premier

Column:

BEH C18 2.1 x 50 mm, 1.7 μm

Temperature: 40°C

Flow: 450 μL/min

Injection volume: 2 μL

Gradient:

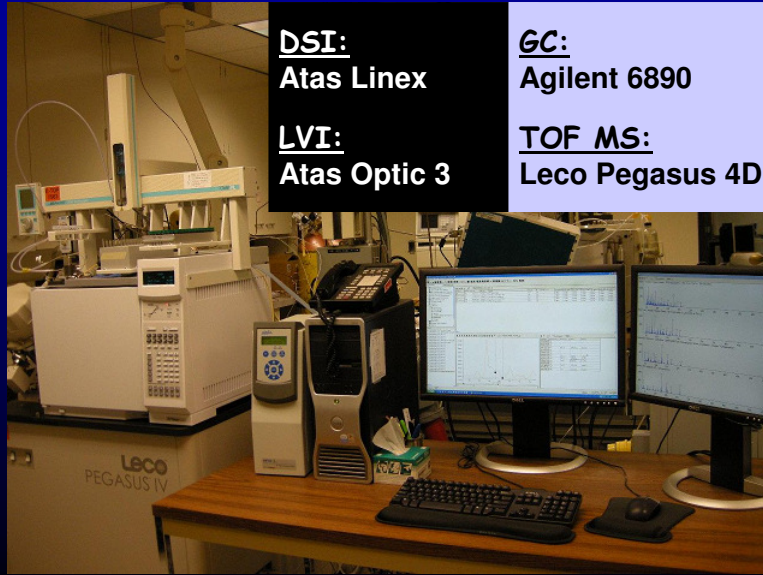
30% B (0 - 4 min)

→ 60% B (7.5 - 8.5 min)

→ 100% B (10.5 - 12.5 min)

→ 30% B (12.6 - 15 min)

DSI-LVI-GC-TOF MS



Direct Sample Introduction (DSI)

1) 10 μ L of the sample placed in a microvial in a DSI liner: = large volume injection (LVI)



2) the DSI liner is placed in the injection port



3) solvent evaporation (PTV)

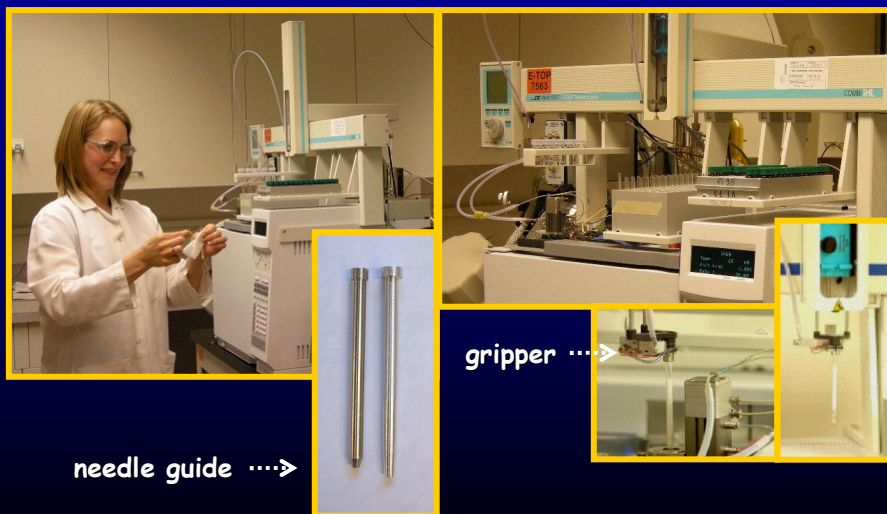


non-volatile matrix components removed from the system

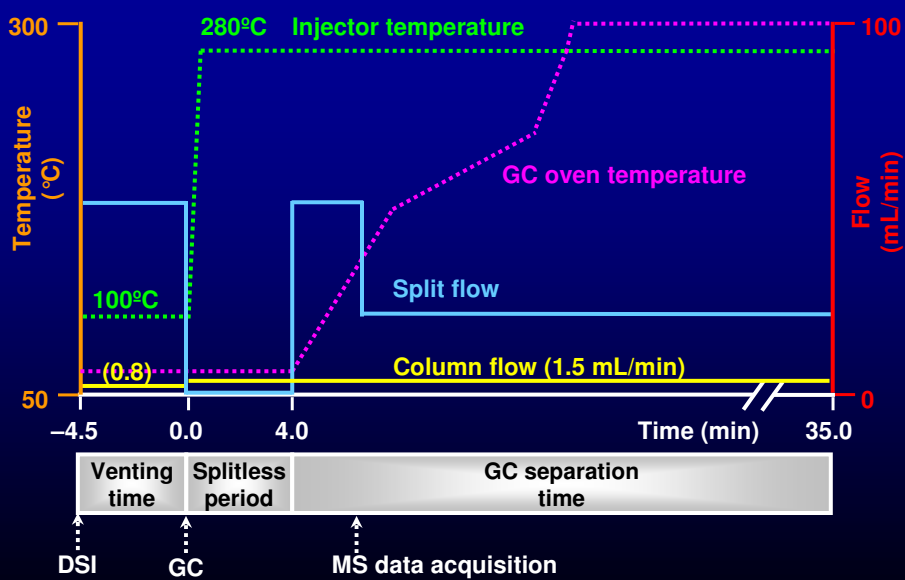


4) analytes transferred to the GC column

Liner Exchange (LINEX) - for automated DSI

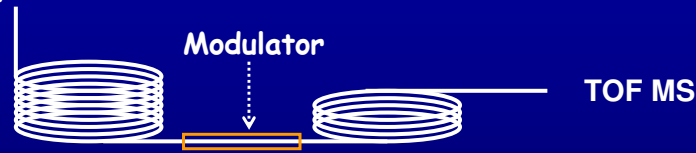


DSI-LVI-GC-TOF MS Optimization



DSI-LVI-GC-TOF MS Optimization

From injector



1st Dimension

RTX-5ms

20 m × 0.25 mm × 0.25 μm

2nd Dimension

RTX-CLpesticide2

1 m × 0.1 mm × 0.1 μm

1D separation: 60°C (4 min), 20°C/min to 180°C,
5°C/min to 230°C, 20°C/min to 280°C,
40°C to 300°C (12 min); † = 35 min



TOF MS: 45-550 amu
10 spectra/s

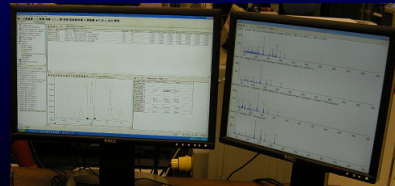
GC-TOF MS Advantages

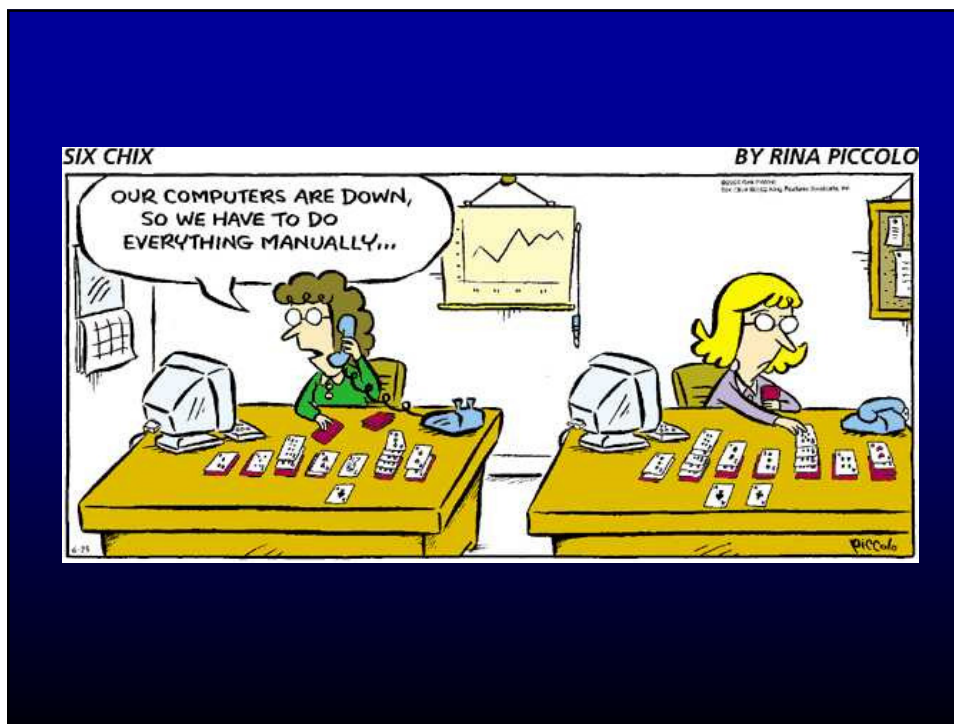
- ☐ Fast acquisition of full mass spectra (up to 500 spectra/s)
- ☐ High mass analyzer efficiency
- ☐ Mass spectra deconvolution

Non-targeted analysis at levels suitable for pesticide residue analysis



Routine data processing:
a list of target pesticides!





QuEChERS for Fruits and Vegetables

extraction

10 g sample + 10 mL MeCN

add internal standard



add 4 g MgSO_4 + 1 g NaCl
shake vigorously for 1 min

centrifuge for 1 min at 3450 rcf

dispersive SPE
clean-up

1 mL of the upper layer
+ 50 mg PSA + 150 mg MgSO_4
mix for 30 s

centrifuge for 1 min at 3450 rcf



Buffered QuEChERS (AOAC 2007.01)

extraction

15 g sample + 15 mL 1% HOAc in MeCN

add internal standard



add 6 g $MgSO_4$ + 1.5 g NaOAc
shake vigorously for 1 min

centrifuge for 1 min at 3450 rcf

dispersive SPE
clean-up

1 mL of the upper layer
+ 50 mg PSA + 150 mg $MgSO_4$
mix for 30 s
centrifuge for 1 min at 3450 rcf



Cereal Grains

Corn
Oat
Rice
Wheat

- ☐ Dry matrices
- ☐ High content of fatty acids

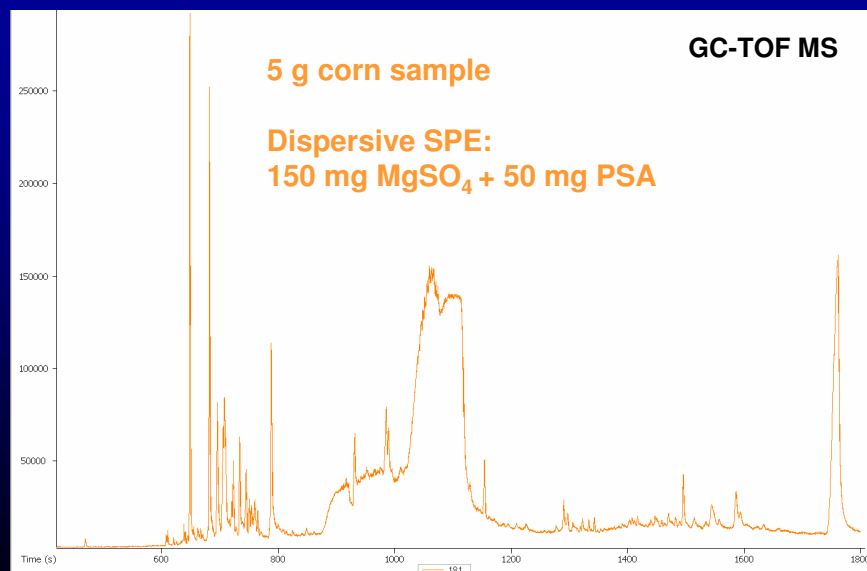


QuEChERS method optimization:

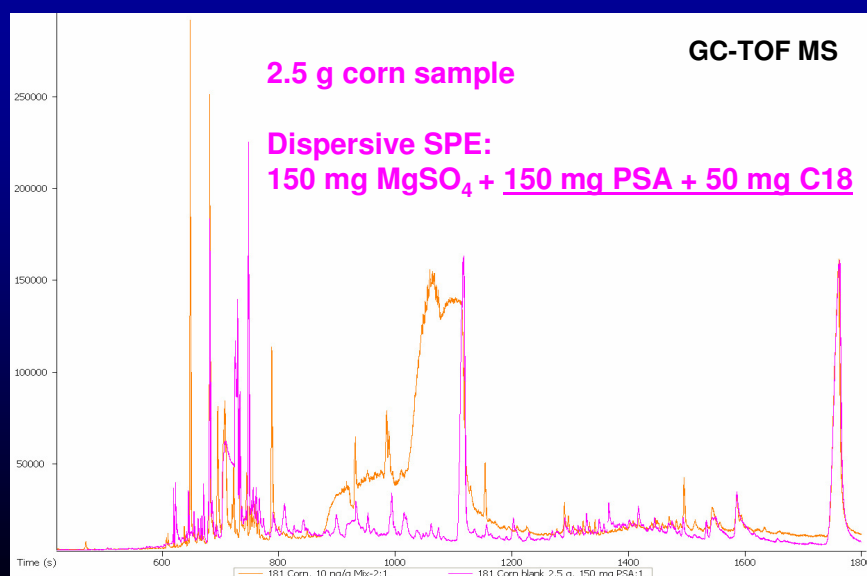
- Addition of water for swelling
- Sample to solvent ratio
- Extraction time
- Dispersive SPE clean-up



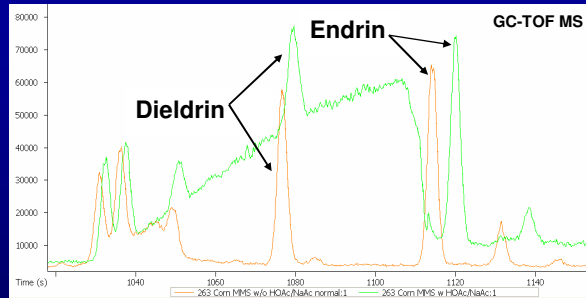
Method Optimization for Corn



Method Optimization for Corn



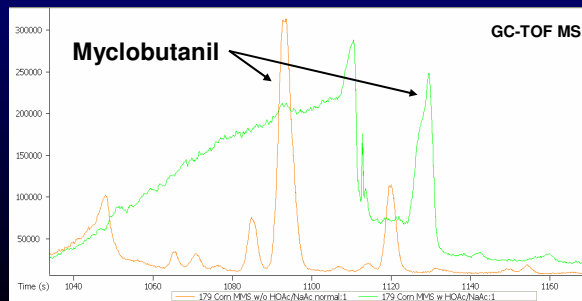
Buffered vs. non-buffered approach



Extraction:

Buffered:
1% HOAc in MeCN
MgSO₄ + NaOAc

Original:
MeCN
MgSO₄ + NaCl



Sample to Solvent Ratio

For 10 mL of acetonitrile (10 mL of extract):

Matrix	Sample size	Water volume
Wheat	5.0 g	10 mL
Rice	5.0 g	15 mL
Oat	3.5 g	10 mL
Corn	2.5 g	10 mL

Swelling/extraction with both water and MeCN
for 1 hour improved analyte recoveries!



extraction

dispersive SPE
clean-up

QuEChERS for Grains

2.5 - 5 g sample + 10 mL water*
+ 10 mL MeCN + internal standards

shake for 1 h

add 4 g MgSO₄ + 1 g NaCl
shake vigorously for 1 min

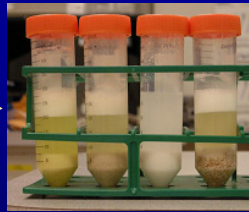
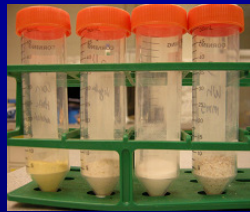
centrifuge for 1 min

*15 mL water for 5 g of rice

1 mL of the upper layer
+ 150 mg PSA + 50 mg C18 + 150 mg MgSO₄
mix for 30 s
centrifuge for 1 min



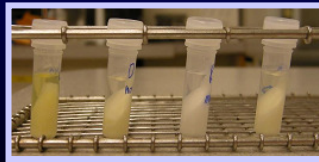
QuEChERS for Grains



Corn
Oat
Rice
Wheat



dispersive SPE
clean-up



Syringeless filters

Mini-UniPrep™ (Whatman)



1) Place unfiltered sample (max. 0.5 mL) in chamber.

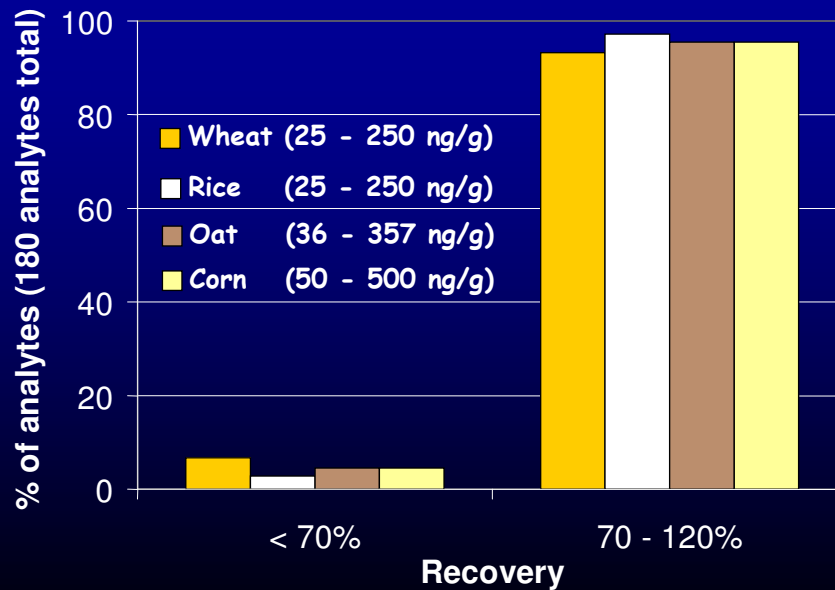
2) Compress filter plunger into sample chamber. Clean filtrate fills reservoir bottom up.

3) Place the Mini-UniPrep™ vial in an autosampler.

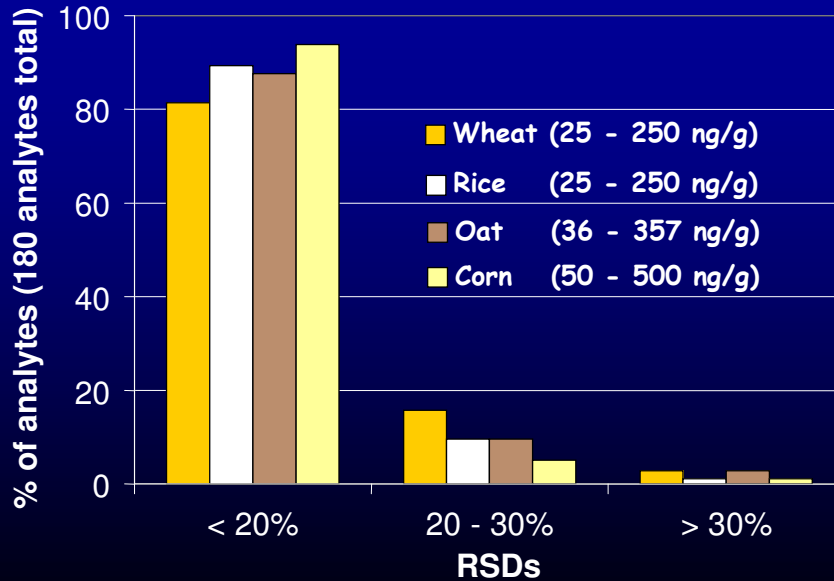


PVDF (polyvinylidene fluoride) filter

Method Validation



Method Validation



Problematic Analytes

☐ Degradation issues:

- Degradation products monitored for:
captan, captafol, folpet, dicofol, tolylfluanid
chlorothalonil (62-69%), amitraz (51-72%)

☐ Highly lipophilic analytes:

HCB (51-67%), mirex (52-70%), DDE (62-81%), aldrin (64-81%)

☐ Affinity to PSA/polarity issues:

piperalin (32-43%), methamidophos (64-69%),
acephate (67-73%), spinosad (68-81%)

Approximate Cost Comparison

Method	Approx. cost per sample	
	Luke-based approach	QuEChERS approach
Disposable materials for sample preparation (solvents, sorbents, vials ...)	\$12.50	\$6.80
Analysis operational costs (instrument operation, service contracts, maintenance, data processing)	\$180*	\$114*

*based on 1100 samples/year

Conclusions QuEChERS vs. Traditional Approach

☐ Reduced cost per sample

About 40-50% reduction in sample prep and operational costs

☐ Reduced and less hazardous waste

1:100 reduction; no chlorinated solvents

☐ Increased sample throughput

About 3-fold increase



☐ Increased scope of pesticides

- Currently successfully validated for about 180 pesticides
- Inclusion of more LC-amenable pesticides in the screen (currently 70 pesticides analyzed by the UPLC-MS/MS)
- Potential for non-targeted GC-TOF MS screening

Acknowledgments

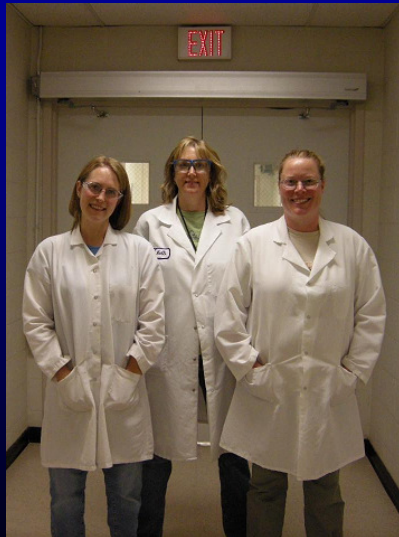
Medallion Laboratories, General Mills:

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Thank you for your attention!

Your questions?



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