Improved methods for the determination of crop protection agents residues in tobaccos and tobacco products by LC-MS/MS using the QuEChERS extraction

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QuEChERS = Quick, Easy, Cheap, Effective, Rugged, Safe

Objective

- Improve LC methods used for the determination of crop protection agents (CPA) recommended in the CORESTA ACAC guideline N°1 *“Agrochemicals provided with Guidance Residue Levels“*
  - Previously used methods were time consuming (multi residues methods based on automated solvent extraction and solid phase extraction clean-up)
  - Examine QuEChERS methodology as an analytical tool

QuEChERS = Quick, Easy, Cheap, Effective, Rugged, Safe

Agenda

- Crop protection agents covered by the methods
- Background of QuEChERS methodology
- Need to have two methods
- Clean-up and analytical conditions
- Validation design
- Results
- Conclusion

Scope

- The CORESTA Guideline Nº1 * covers 118 CPA
- Around half of the CPA are analyzed by gas chromatography (GC) and the other half by liquid chromatography (LC)
- Some CPA are analyzed by wet chemistry
- In this presentation, we will cover only 51 CPA analyzed by LC

* http://www.coresta.org/Guides/Guide-No01-GRLs(2nd-Issue-June08-Addendum-June10).pdf

QuEChERS multi residue method

QuEChERS = Quick, Easy, Cheap, Effective, Rugged, Safe

- QuEChERS is a miniaturized, simple and rapid multi residue method developed by Anastiassades et al. (2003) * for the determination of pesticides from fresh commodities, such as fruits and vegetables

- QuEChERS proved to be a very powerful multi residue method that can cover a large variety of different pesticide compound classes with excellent recoveries

*** Anastassiades, M., S. J. Lehotay, D. Stajnbaher and F. J. Schenck (2003):** "Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce." Journal of AOAC International 86(2): 412-431

Principle of the QuEChERS method

Extraction

- extraction of a tobacco aqueous suspension with acetonitrile
- buffer salt induced phase separation

↓

Clean-up

- dispersive solid phase extraction with PSA (Primary Secondary Amine) in the acetonitrile phase

↓

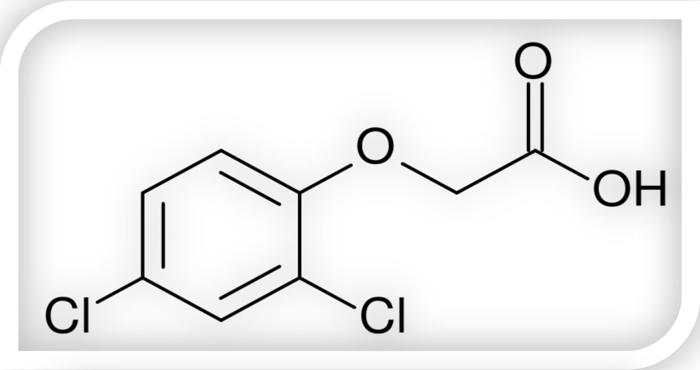
Detection

LC-MS/MS

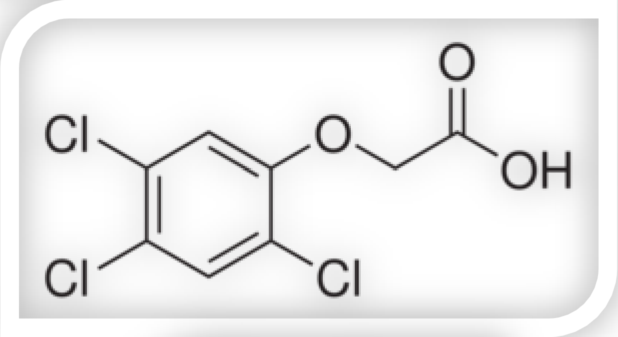
The issue of acidic herbicides

- For three CPA (2,4-Dichloro phenoxyacetic acid, Dicamba and 2,4,5-Trichloro phenoxyacetic acid), the QuEChERS methodology needed to be modified, due to their acidic properties. It resulted in the development of two methods
- Method 1
  - Analysis of 48 CPA residues in tobacco samples by LC-MS/MS using QuEChERS extraction method
- Method 2
  - Analysis of acidic CPA (2,4-Dichloro phenoxyacetic acid, Dicamba and 2,4,5-Trichloro phenoxyacetic acid) in tobacco samples by LC-MS/MS using *modified QuEChERS* extraction method

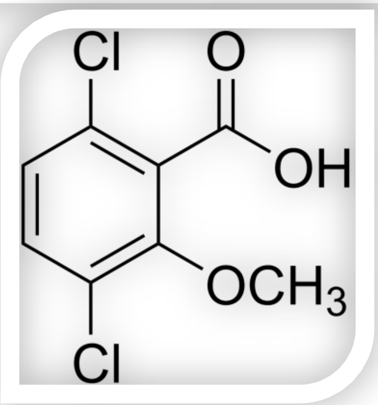
Chemical structure of acid herbicides



2,4-Dichlorophenoxyacetic acid
(2,4-D)



2,4,5-Trichlorophenoxyacetic acid (2,4,5-T)



3,6-dichloro-2-methoxybenzoic acid
(Dicamba)

List of analyzed CPAs

Method 1

1	Acetamiprid
2	Acibenzolar-S-methyl
3	Alachlor
4	Aldicarb (Sum)
	Aldicarb-sulfone
	Aldicarb-sulfoxide
5	Benalaxyl
6	Benomyl
7	Butralin
8	Carbaryl
9	Carbendazim (Sum)
	Benomyl
	Thiophanate-methyl
10	Carbofuran (sum)
	3-hydroxycarbofuran
11	Clomazone
12	Cymoxanil
13	Demeton-S-methyl (sum)
	Oxydemeton-methyl
	Demethon-s-methyl-sulfone
14	Dichlorvos (sum)
	Naled
	Trichlorfon
15	Diflubenzuron
16	Dimethoate (sum)
	Omethoate
17	Dimethomorph (sum)
18	Diphenamid
19	Disulfoton (Sum)
	Disulfoton-sulfone
	Disulfoton-sulfoxide
20	Ethoprophos
21	Fenamiphos (sum)
	Fenamiphos sulfoxide
	Fenamiphos sulfone
22	Fluazifop-P-Butyl (sum isomers)
23	Imidacloprid
24	Iprodione (sum)
	N-3,5-dichlorophenyl-3-isopropyl-2,4-dio.
25	Metalaxyl (sum)
	Metalaxyl-M
	Mefenoxam
26	Methamidophos
27	Methiocarb (sum)
	Methiocarb sulfoxide
	Methiocarb sulfone
28	Methomyl (Sum)
	Methomyl oxim
	Thiodicarb
29	Methoprene
30	Monocrotophos
31	Naled
32	Omethoate
33	Oxadixyl
34	Oxamyl
35	Pebulate
36	Penconazole
37	Phoxim
38	Piperonylbutoxide
39	Pirimicarb
40	Propoxur
41	Pymetrozine
42	Pyrethrins (sum)
	Pyrethrins 1
	Pyrethrins 2
	Cinerins 1
	Cinerins 2
	Jasmolin 1
	Jasmolin 1
43	Thiametoxam
44	Thiodicarb
45	Thionazin
46	Thiophanatne-methyl
47	Trichlorfon
48	Vamidothion (sum)
	Vamidothion sulfone
	Vamidothion sulfoxide

Method 2

49 Dicamba
50 2,4-Dichlorophenoxy acetic acid
51 2,4,5-Trichlorophenoxy acetic acid

- **51 Active substances and some of their metabolites**
- **Method 1 QuEChERS**
- **Method 2 Modified QuEChERS**

Classes of compounds

- Organochlorines
- Carbamates
- Triazoles
- Neonicotinoids
- Ureas
- Amides
- Organophosphorous
- …

Method 1, Step by step (1/2)

1. Weigh 2 g of fine ground tobacco sample into a 50 mL Teflon centrifuge tube with screw cap

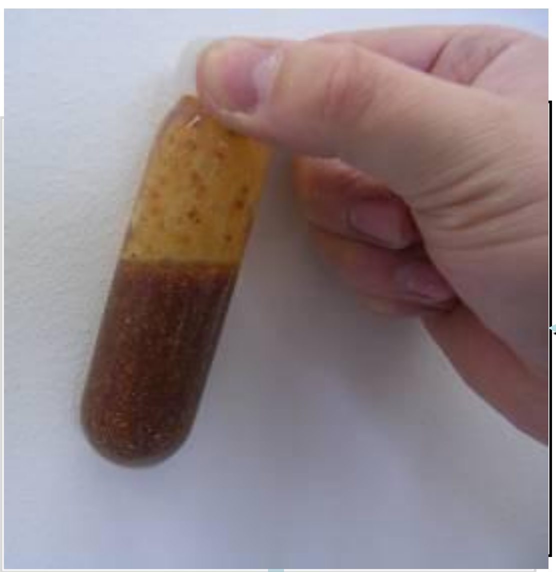
2. Add 10 mL water and let 15 min swell

3. Add 10 mL acetonitrile

4. Add Internal standard solution

Shake

5. Add 6.5 g buffer salt mixture [1)]



1) ... 4 g magnesium sulfate
+ 1 g sodium chloride
+ 1 g trisodium citrate dihydrate
+ 0,5 g disodium hydrogencitrate sesquihydrate

Method 1 step by step (2/2)

7. Centrifuge for 2 min at 4.000 rpm

8. Prepare a small cap-lock tube with 225 mg $MgSO_4$ and 40 mg PSA sorbent

9. Transfer an aliquote of 1.5 mL acetonitrile extract

10. Shake for 30 sec by hand or vortexer

11. Centrifuge for 2 min at 6.000 rpm

12. Ready-to-dilute-and-shoot acetonitrile extract

13. Analyze!

Method 2, step by step

- Same process as for method 1, with following modifications
  - pH adjustment, before the addition of acetonitrile
  - No clean-up step with PSA, $MgSO_4$
  - No dilution of final solution

Chromatographic conditions

- HPLC 1100 system (Agilent) coupled to an API 4000 tandem mass spectrometer (Applied Biosystems)
- Phenomenex Luna C18(2) column, 150 x 2mm, 5 µm with pre-column

	Method 1	Method 2
Flow Rate	0.4 mL/min	0.4 mL/min
Autosample Dilution	1:10 (2 µL sample extract + 18 µL ACN/0,05% acetic acid (200+1600))	-
HPLC Solvents	A=Water+0.05% acetic acid B=Methanol+0.05% acetic acid Gradient from 10 to 90 % B	A=Water+0.1% formic acid B=Methanol+0.1% formic acid Gradient from 5 to 35% B
Ionisation Mode	Positive ionization	Negative ionization

Data evaluation

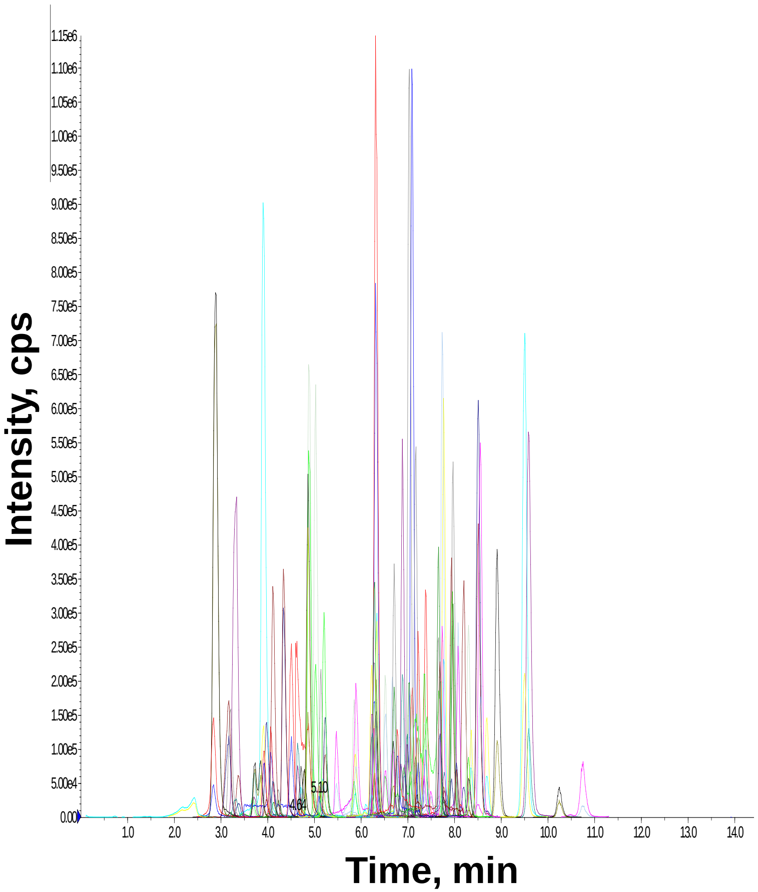
- Automatic integration, each peak is manually checked for correct peak finding and integration
- Calibration samples are prepared in the tobacco matrix (matrix-matched standards)
- Quantitation is done against linear calibration curves (7 concentration levels) with origin through zero
- 2 specific mass transitions per analyte with dwell time each 18 msec
- Qualitative confirmation criteria: (i) Peak area ratio of quantifier and qualifier mass transition (MRM ratio), and (ii) the retention time

Validation design

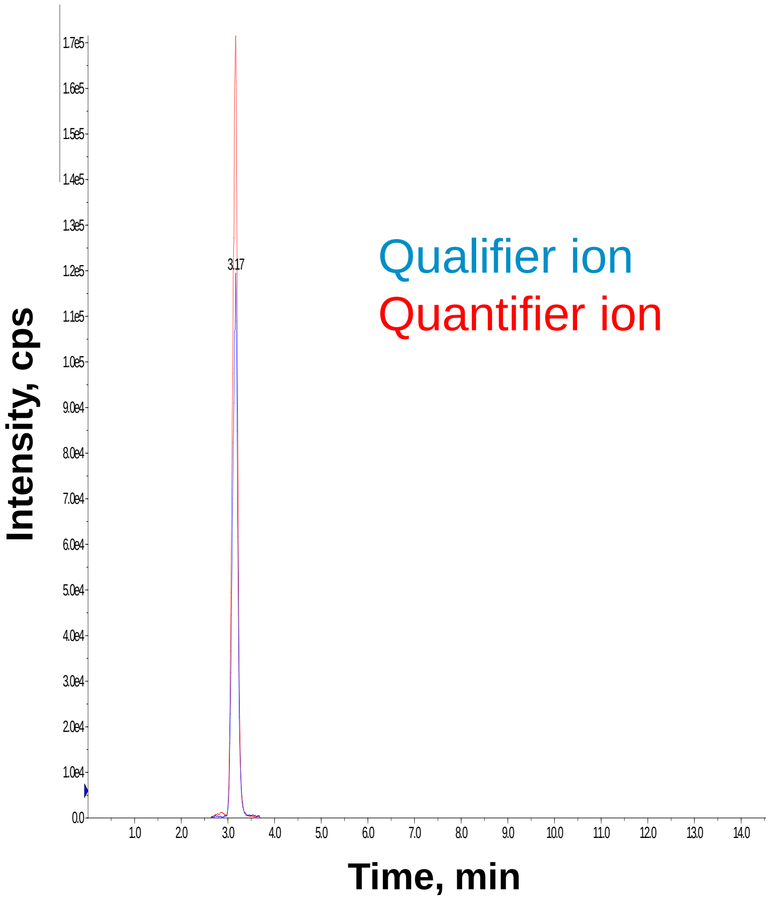
- The validation experiments were performed with a mixture of blank FC and BU (50-50, w/w)
- 3 spiking levels at 0.05, 0.5 and 1.25 mg/kg each analyte to the mixed tobaccos
- 3 replicates at each spiking level -> n=9
- 1 control blank sample
- Recovery rates were determined by quantifying against linear calibration curve of matrix-matched standards at 8 concentration levels
- Acceptance criteria: recovery 70-120%, relative standard deviation <20% (According to CORESTA Guide No 5, Technical guideline for pesticide residues analysis on tobacco and tobacco products)

Method 1 typical chromatogram

Matrix-matched standards, 10 µg/mL

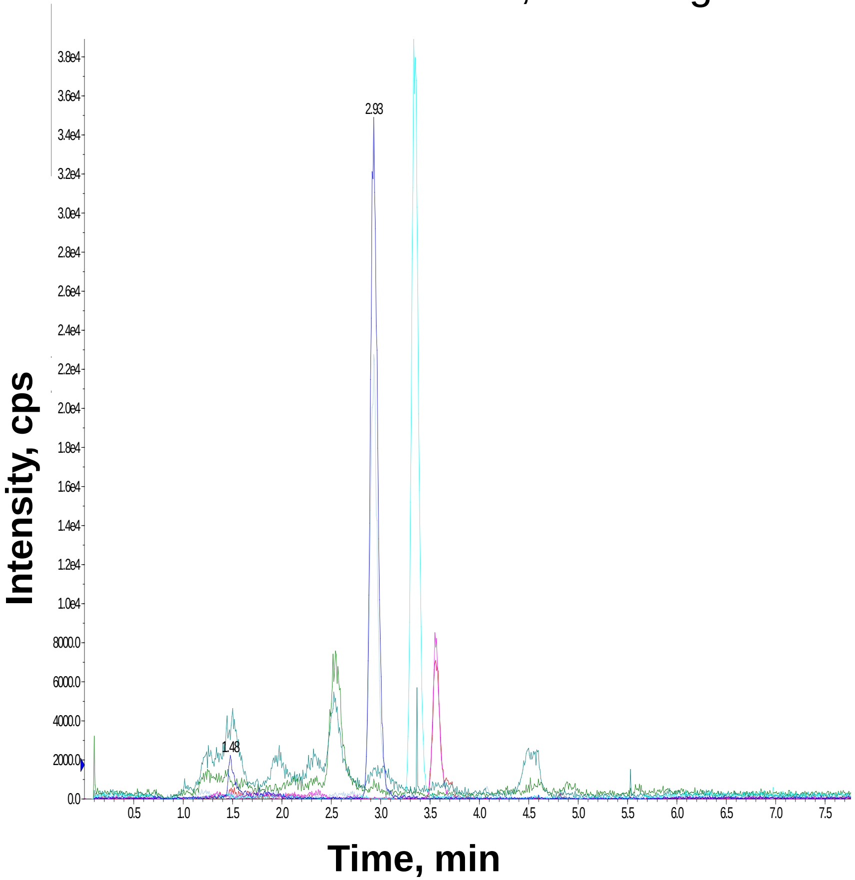


e.g. Omethoate, m/z 214->183 amu

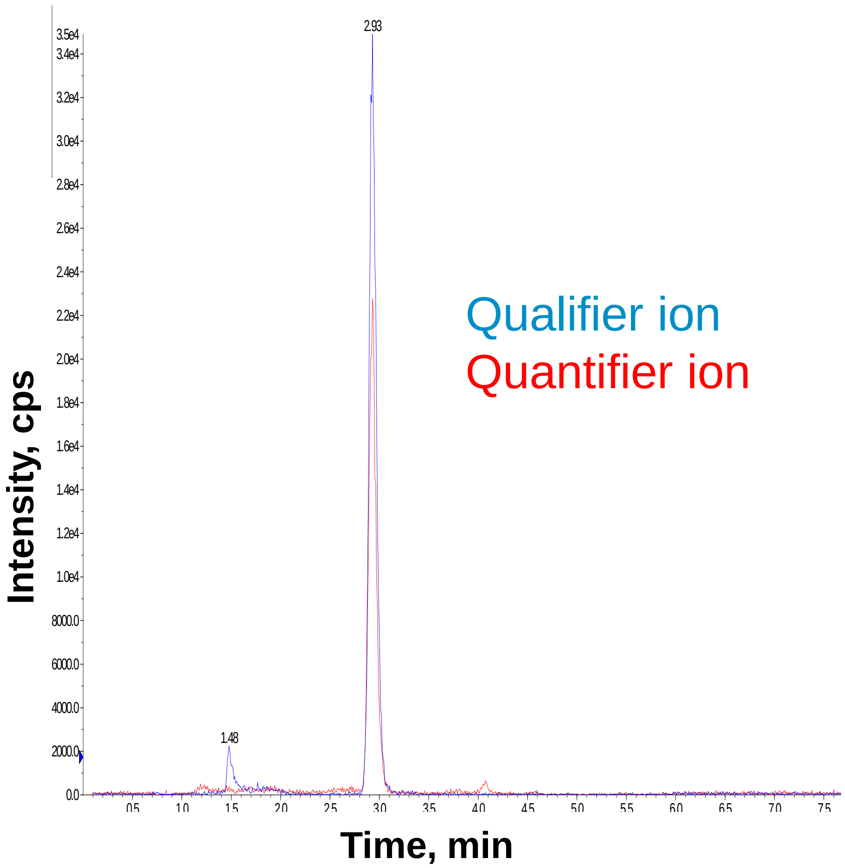


Method 2 typical chromatogram

Matrix-matched standards, 15-60 ng/mL



e.g. 2,4-Dichloro phenoxyacetic acid, m/z 219->161 amu



Results for method 1

Calibration (R^2)	0.999 – 0.9931
Limit of quantification	Accordance with the CORESTA requirement
Repeatability of extraction (coefficient variation)	<10%
Instrument repeatability (coefficient variation)	<10%
Recovery	Accordance with the CORESTA requirement*

*****_Except 62% Recovery for Pymetrozin because of a lack of extraction efficiency. Nevertheless, the low recoveries had been proven to be very stable in the same range._
The method can be used for Pymetrozin.

Results for method 2 (acid herbicides)

Calibration (R^2)	0.997 – 0.988
Limit of quantification	Accordance with the CORESTA requirement
Repeatability of extraction (coefficient variation)	<12%
Instrument repeatability (coefficient variation)	<12%
Recovery	93% - 97%

Conclusion

- Multi residue method with reduced analysis time
  - Previous multi residue method — 28 samples / 6 days
  - QuEChERS — 28 samples /1.5 days
- Lower consumption of solvents
  - Previous multi residue method — ca. 60 mL / sample
  - QuEChERS — ca. 10 mL / sample
- Wide range of extractable CPA with very good recovery rates

Results in collaborative test

- We participated to the last FAPAS* collaborative test
- Our results were all in the acceptable range (z score <2) for the active substances (8) analyzed with the QUECHERS LC MS/MS method

* Food Analysis Performance Assessment Scheme

Aknowledgment

- Françoise Mischler (Practical work)
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