Modified QuEChERS-Method for the Analysis of Chlorothalonil in Fruits and Vegetables

- Brief Description -

Introduction:

Chlorothalonil is a widely employed non-systemic fungicide. Due to its tendency to experience losses during sample preparation, storage of sample extracts and standard solutions as well as during GC-analysis, analysis of chlorothalonil via multiresidue methods (e.g. QuEChERS) is highly challenging. The susceptibility of chlorothalonil to losses largely depends on the pH value as well as on the commodity type, with allium and brassica crops, containing components that reportedly undergo reactions with chlorothalonil having a particularly negative impact on its stability. During QuEChERS procedure dispersive SPE using PSA sorbent is the most critical step for chlorothalonil losses as it causes a considerable pH increment.

In the following, a modification of the QuEChERS[1] method for the analysis of chlorothalonil is briefly described involving acidification of the analytical sample with sulphuric acid to pH~1 at the beginning of the procedure. **No buffer salts** are used in the initial extraction/partitioning step and **no dispersive-SPE cleanup** is performed. Determinative analysis is performed via GC-MSD or LC-MS/MS in the APCI neg. mode. In the LC-MS/MS ion source (APCI-neg. -mode) chlorothalonil undergoes hydrolytic dechlorination to 4-hydroxy-chlorothalonil that forms the parent ion. 4-hydroxy-chlorothalonil can also be determined with the same method giving the same mass transitions but a peak at a different retention time. Virtually quantitative chlorothalonil recoveries, at low variations, were achieved on various representative commodities. Reliable chlorothalonil-determinations at 0,01 mg/kg levels are possible both by GC-MSD and LC-MS/MS in the APCI neg. mode. The stability of extracts was studied over 9 days and was shown to be highly satisfactory.

Overview of Procedure

- Weigh 10 g of the homogeneous, frozen sample into a 50 ml centrifugation tube
- Acidify with 100 µL H_2SO_4 (conc.) to pH of ~1
- Add 10 ml acetonitrile and 100 µl ISTD-solution[2]; close tube and shake vigorously for 1 min.
- Add 4 g magnesium sulphate anhydrous, 1 g sodium chloride, and shake for 1 min; centrifuge for 5 min at 3000 rpm
- Fill an aliquot of the raw extract into a vial and employ for GC-MS or LC-MS/MS analysis using calibration standards prepared in blank extracts (not necessarily of the same kind as the samples) derived as described above but without addition of ISTD.

[1] See also CEN method EN 15662 (Determination of pesticide residues using GC-MS and/or LC-MS (/MS) following acetonitrile extraction/partitioning and cleanup by dispersive SPE - QuEChERS method) as well as www.quechers.de.

[2] Internal standard solution containing 1 µg/mL of PCB138 (for GC-MS) and/or 1 µg/mL Nicarbazin (for LC-MS/MS) dissolved in acetonitrile

Determinative Analysis:

a) Via LC-MS/MS *(Please consider the LC-MS/MS data as exemplary)*

Instrument: API 4000, AB SCIEX
Mode: APCI negative
Column: Waters Acquity BEH C18 1,7 um 2.1x100
Eluents: **A:** 95 % water + 5 % ACN + 0.01 % acetic acid; **B:** ACN + 0.01 % acetic acid
Gradient: Start with 30% B and go to 90% B within 3 min, stay for 3 min. The re-equilibrate column at 30% B for 5 minutes.
Flow: 0.5 mL/min
Injection volume: 2 µL
Retention time: chlorothalonil: ≈2.3 min; 4-OH-chlorothalonil: 1,1 min
Mass transitions: (same for chlorothalonil and 4-OH-chlorothalonil):

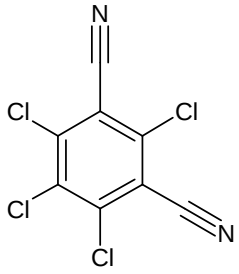
	First Mass (m/z)	Second Mass (m/z)		DP	CE	CXP
1	245	245		-75	-10	-11
2	247	247		-75	-10	-11
3	245	182		-75	-40	-9
4	247	184		-75	-42	-9
5	247	175		-75	-36	-7

**there might be no transition, but the masses are still very characteristic and useful for identification*

Transformation of chlorothalonil to 4-OH-chlorothalonil in the APCI ion source in negative mode:

Monoisotopic Mass = 263.881559 Da

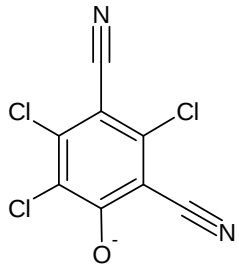
Molecular Formula = $C_8Cl_4N_2$



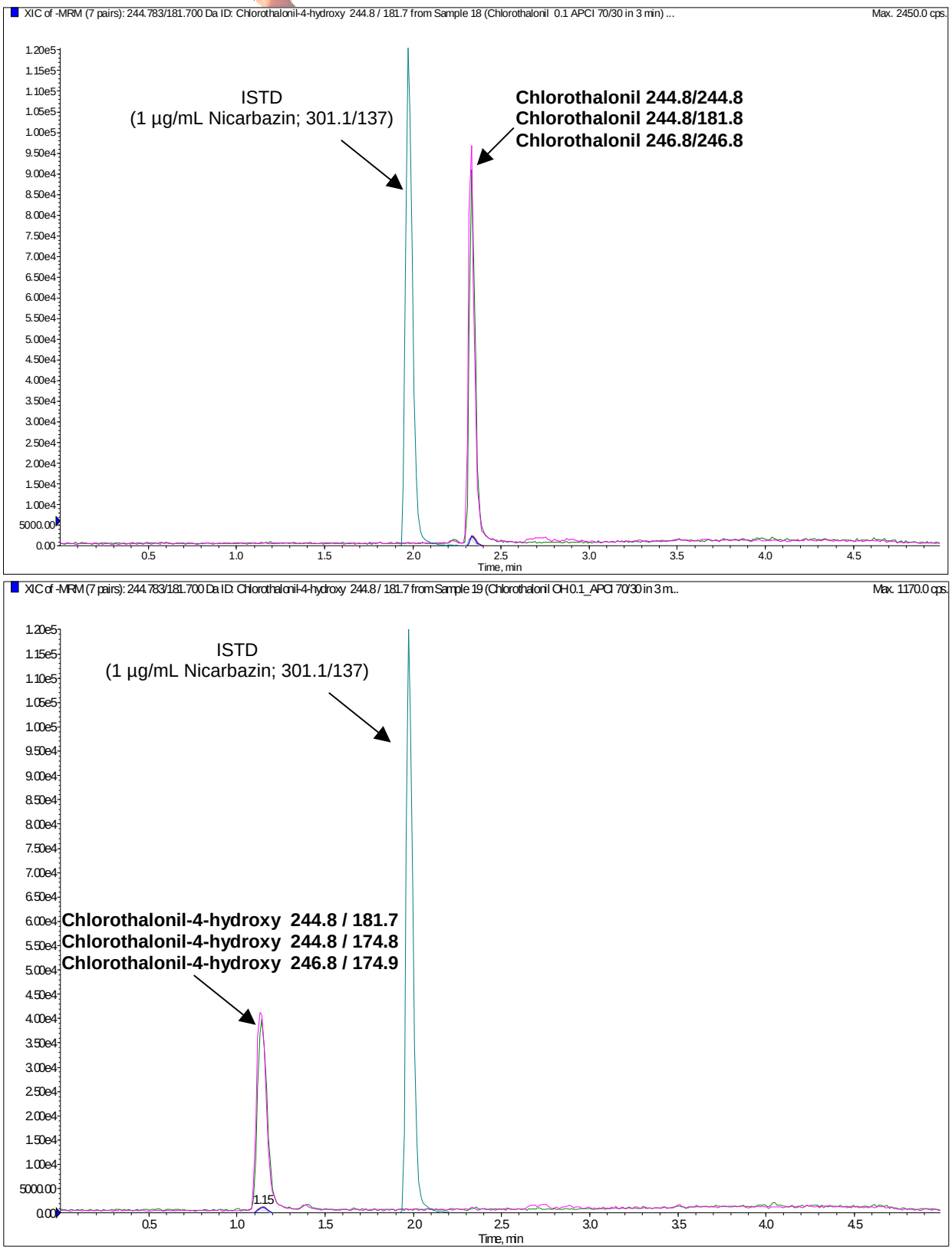
Chlorothalonil

APCI negative mode

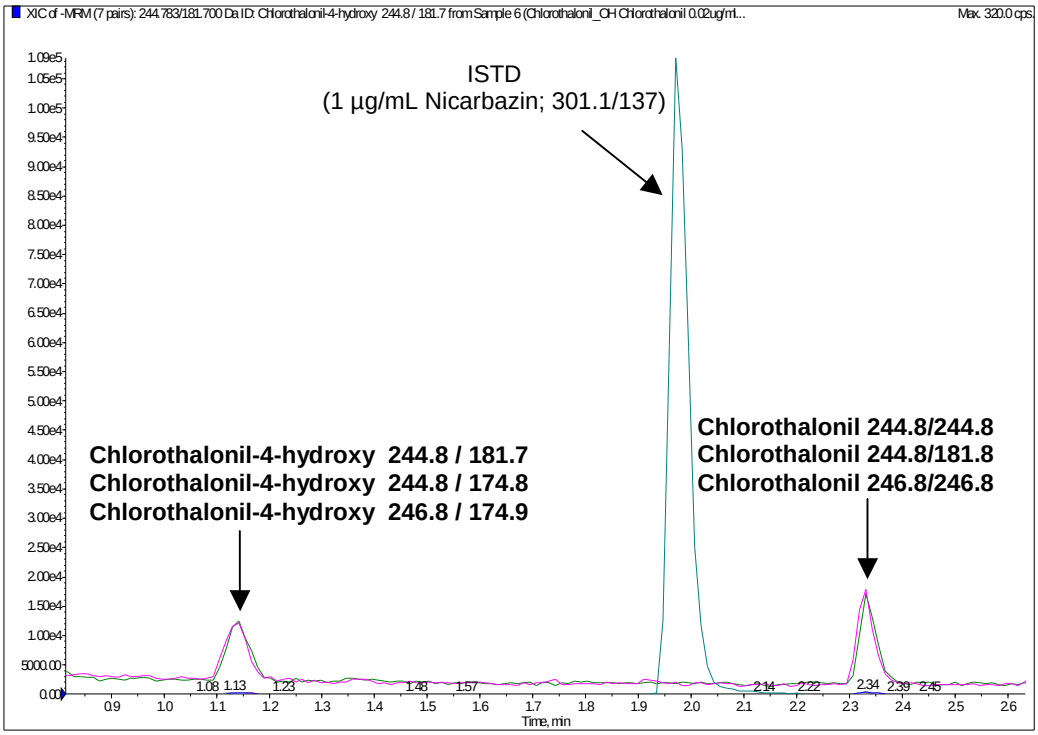
Monoisotopic Mass = 244.908169 Da



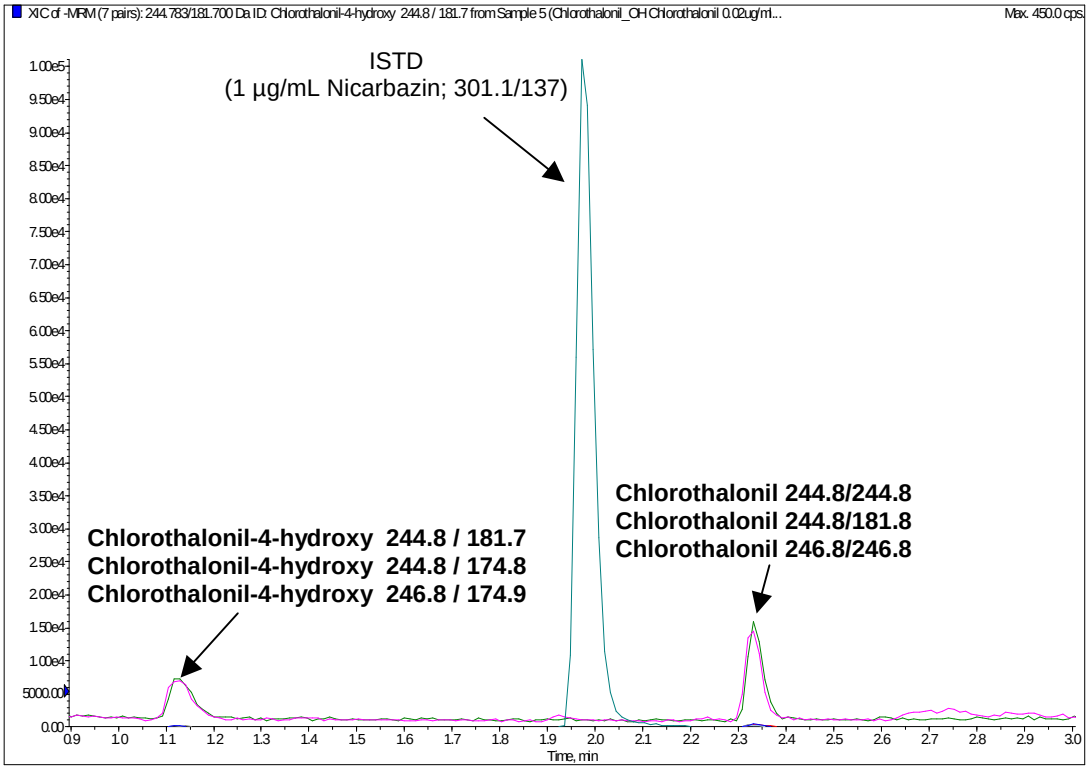
4-OH-Chlorothalonil = 4-hydroxy-trichloroisophthalonitrile



LC-MS/MS (APCI-neg.) chromatogram of chlorothalonil at 0.1 µg/ISTD in acidified acetonitril (by 0.4 Vol.% glacial acetic acid) and 4-OH-chlorothalonil at 0.1 µg/ISTD in acetonitril.



Exemplary LC-MS/MS (APCI-neg.) chromatogram of chlorothalonil und 4-OH-chlorothalonil at 0.02 µg/mL in tomato extract.

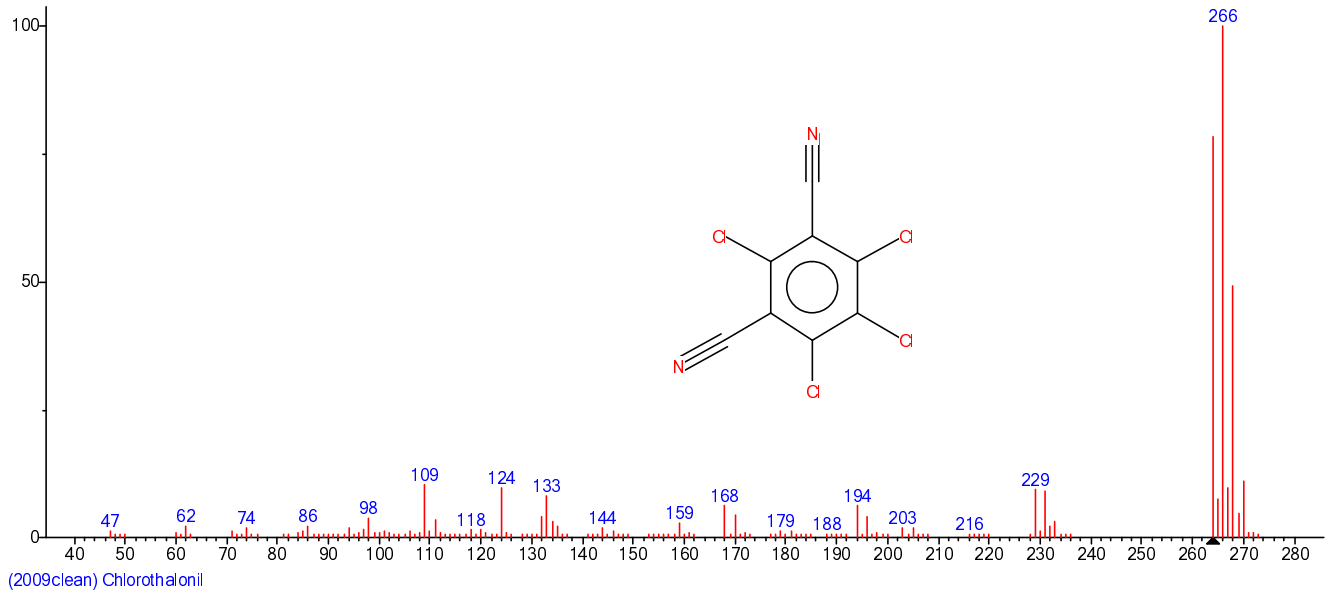


Exemplary LC-MS/MS (APCI-neg.) chromatogram of chlorothalonil und 4-OH-chlorothalonil at 0.02 µg/mL in kiwi extract.

a) <u>Via GC-MSD</u> (Please consider the GC-MSD data as exemplary)

Instrument:	Agilent 6890 and Agilent 5973 with PTV injection system
Column:	DB 5 MS crosslinked, 30 m x 0.25 mm x 0.25 µm, 5 %Ph Me Silicon
Carrier gas:	Helium, constant flow 2 mL/min
GC temperature program	2 min at 40 °C then with 30 °C /min to 220 °C then with 5 °C /min to 260 °C then with 20 °C/min to 280 °C (15 min)
Transfer-line:	280 °C
Injection volume :	3 µL (PTV, solvent vent mode),
PTV temperature program	0,8 min at 50 °C then with 720 °C / min to 300 °C, hold 5 min then with 720°C /min to 280 °C hold 10 min
PTV gas flow	Vent flow 20 mL/min until 0,5 min Purge flow 47.2 mL/min starting at 2 min Gas saver 20 mL/min starting at 6 min

SIM-masses for chlorothalonil: 266; 264; 268, 229, 231



Recovery studies for Chlorothalonil:

Recovery studies were performed for 4 different commodities at the 0.1 mg/kg-level. Results are shown in the following table.

Commodity	Recoveries [%]			
	Via the present QuEChERS modification		via trad. QuEChERS with d-SPE*	via trad. QuEChERS w/o d-SPE
	LC-MS/MS	GC-MSD	LC-MS/MS	LC-MS/MS
Cucumber	104	112	52	97
	99	109	49	97
	96	115		
	95	117		
	94	116		
Mean / (RSD) [%]	**97 / 3.5**	**114 / 2.6**	**51**	**97**
Kiwi	92	113	59	98
	92	116	59	98
	89	116		
	93	117		
	88	115		
Mean / (RSD) [%]	**91 / 2.1**	**116 / 1.3**	**59**	**98**
Spring Onions	100	87	5	-
	99	84	5	-
	99	81		
	100	79		
	101	75		
Mean / (RSD) [%]	**100 /1.0**	**81 /4.9**	**5**	**-**
Cauliflower	89	93	0	62
	98	93	0	64
	83	94		
	93	92		
	79	90		
Mean / (RSD) [%]	**88 / 7.7**	**93 / 1.3**	**0**	**63**

* involving d-SPE with PSA/MgSO4

Extract Stability:

Extract stability in the vial was checked after 3 and 9 days of storage at room temperature against freshly prepared calibration solutions. Virtually no degradation was noticed.

Stability of Chlorothalonil in extracts derived using the above method

	Recovery	Recovery Same vial 3 days later	Recovery Same vial 9 days later
Cucumber	**104%**	96%	88%
	99%	91%	89%
Kiwi	**92%**	85%	98%
	92%	93%	99%
Spring Onions	**100%**	96%	102%
	99%	94%	100%
Cauliflower	**89%**	91%	90%
	98%	85%	92%

The stability of chlorothalonil in extracts derived with the traditional QuEChERS method (CEN 15662), involving d-SPE with PSA and re-acidification with formic acid was not satisfactory, especially in spring onion and cauliflower extracts.