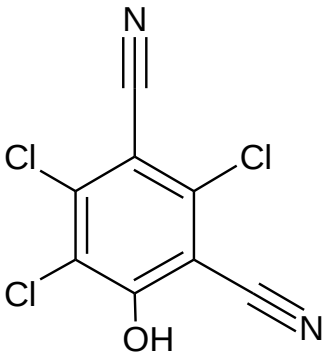
Analysis of 4-Hydroxy-Chlorothalonil (SDS-3701) in Milk using QuEChERS and LC-MS/MS

Version 2.1 (last update: 06.05.14)

Short description

A method is presented for the analysis of 4-Hydroxy-Chlorothalonil in milk. The pesticide is extracted using the QuEChERS method and analysed by LC-MS/MS in the ESI (neg.) mode following cleanup by freeze-out.



Compound details

4-Hydroxy-Chlorothalonil or SDS-3701, IUPAC name: 2,5,6-trichloro-4-hydroxyphtalonitrile is the main metabolite of chlorthalonil. Chlorthalonil is a non-systemic foliar fungicide with protective action. It is used against many fungal diseases in a wide range of crops, including pome fruit, stone fruit, citrus fruit, cranberries, strawberries, bananas, mangoes, coconut palms, oil palms, rubber, pepper, vines, hops, vegetables, cucurbits, tobacco, coffee, tea, rice, soya beans, peanuts, potatoes, sugar beet, cotton, maize, and mushrooms [3].

In a recent reasoned opinion by EFSA, significant chlorothalonil intakes were calculated for dairy ruminants, meat ruminants, poultry and pigs. Metabolism in lactating ruminants was sufficiently investigated. In all commodities of ruminant origin, including milk, the relevant residue definition for enforcement was proposed as 2,5,6-trichloro-4-hydroxyphtalonitrile (SDS-3701) [2].

Current Residue definitions:

The residue definitions for Chlorothalonil in food of animal origin are as follows [1]:

Matrix-code 1011000 (Swine products):	Chlorothalonil only
Matrix-code 1012000 (Bovine products including milk):	SDS-3701
Matrix-code 1013000 (Sheep products including milk):	SDS-3701
Matrix-code 1014000 (Goat products including milk):	SDS-3701
Matrix-code 1015000 (Horses, asses, mules or hinnies):	Chlorothalonil only
Matrix-code 1016000 (Poultry products):	Chlorothalonil only
Matrix-code 1017000 (Other farm animals (Rabbit, Kangaroo):	Chlorothalonil only

SDS-3701 follows a different toxicological mechanism than the parent compound. For plant commodities EFSA proposed two separate residue definitions for enforcement, of chlorothalonil alone and of SDS-3701.

Sources of Supply (exemplary):
Toronto Research Chemicals (TRC), Order Nr.: H825060, 100mg
Dr. Ehrenstorfer, Order Nr.: LA11510400AL, 1mL (100ng/µL)

Apparatus and Consumables:
Use materials described in the QuEChERS standard procedure (EN15662). As a mechanical shaker you can use a horizontally or vertically reciprocating shaker or a rotatory shaker (e.g. HS260 by IKA or GenoGrinder by Spex or SSL1 Labscale Orbital Shaker by Stuart). To filter the extract use e.g. polyester disposable syringe filters of 0.45 µm pore size.

Extraction and Cleanup Procedure:
Extraction: Weigh 10 g of milk, add 10 mL acetonitrile and internal standard (e.g. 100µL of an appropriately concentrated solution) and shake 15 min using a mechanical shaker. Add QuEChERS citrate-buffer mix, shake 1 min and centrifuge --> Raw Extract.
Cleanup: Transfer e.g. 6-8 mL of the raw extract into a vial and place it in a freezer for at least 2 h. Take the vial out of the freezer and either a) quickly filter 1mL through a syringe filter into an LC-MS/MS vial, paying attention to avoid heating up the mixture as heating-up would progressively re-dissolve the lipids; OR b) quickly decant 1 mL into an LC-MS/MS vial paying attention to leave precipitates behind and to avoid heating up the mixture; OR c) quickly filter some milliliters through a cotton-wool-filled funnel into a separate vessel and transfer 1 mL from there into an LC-MS/MS vial.

Preparation of calibration standards:
Matrix matched calibration standards are prepared using an extract of blank milk (not containing any of the pesticides of interest). The blank extract is produced as described above however without addition of an IS. 1mL final extract will represent approximately 1 g matrix.

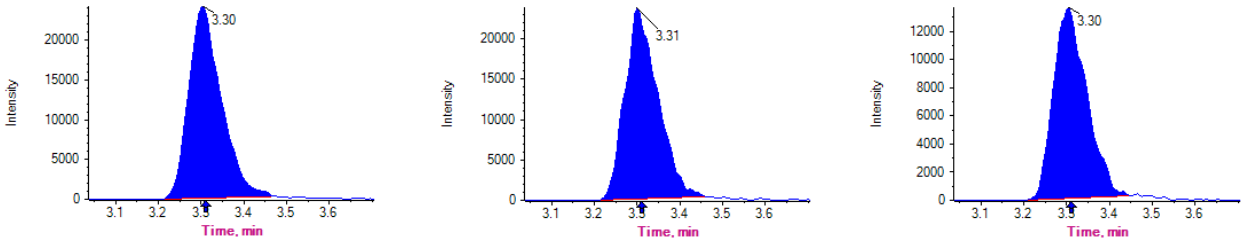
Measurement:
Inject the extracts into LC-MS/MS instrument (ESI-negative mode).

Instrumentation details for 4-Hydroxy-Chlorothalonil:

Instrument	Waters Acquity, ABSciex API 4000 QTrap
Ionisation mode	ESI neg
Column	Acquity UPLC BEH Shield RP 18, 1.7 µm; 2.1 x 100 mm
Pre-column	Van Guard BEH Shield RP 18, 1.7 µm
Eluent A	0.01 % acetic acid in water (with 5% acetonitrile)
Eluent B	0.01 % acetic acid in acetonitrile
Gradient	time / flow [µL/min] / A% / B% 0 / 400 / 80 / 20 4 / 400 / 70 / 30 7 / 400 / 10 / 90 8.5 / 400 / 10 / 90 8.6 / 400 / 80 / 20 13.5 / 400 / 80 / 20
Internal Standard	Nicarbazin

Tab. 2: MRM Details for Chlorothalonil-4-hydroxy (ESI-neg. mode using ABSciex API 4000 QTrap):

Name of Transition	Rel. Sensitivity	Parent mass $[M-H]^-$	Daughter mass	DP	CE	CXP	Mode
4-OH Chlorothalonil 245/175	1	245	175	-60	-38	-2	ESI neg.
4-OH Chlorothalonil 245/182	2	245	182	-60	-40	-2	ESI neg.
4-OH Chlorothalonil 245/210	3	245	210	-60	-34	-2	ESI neg.
Internal Standard (option)							
Nicarbazin		301	137	-45	-16	-7	ESI neg.



Chlorothalonil -4-hydroxy 245/175 T | **Chlorothalonil -4-hydroxy 245/182** | **Chlorothalonil -4-hydroxy 245/210**

Fig 1: Exemplary chromatograms of Chlorothalonil-4-hydroxy 0.1 µg/mL in Acetonitrile.

Validation data:

Tab. 3: Recovery experiments for 4-Hydroxy-Chlorothalonil from whole milk at 0.1 mg/kg (n=5)

Matrix	Spiking Level	Mean recovery rates (RSDs)
Whole Milk	0.1 mg/kg	93.6% (8.2%)
Whole Milk	0.01 mg/kg	97.2% (15.8%)

Validation data with modified methods:

Tab. 4: Recovery experiment for 4-Hydroxy-Chlorothalonil from Whole Milk at 0.05 mg/kg (n=3)
Cleanup: freeze-out -> filtration through cotton->dilution with water (1:1) -> filtration via syringe filter

Matrix	Spiking Level	Mean recovery rate (RSDs)
Whole Milk	0.05 mg/kg	111% (3.5%)

Tab. 5: Recovery experiments for 4-Hydroxy-Chlorothalonil from Whole Milk (n=5),
Cleanup: dilution with water (1:1) + ODS 25mg/mL raw extract

Matrix	Spiking Level	Mean recovery rate (RSDs)
Whole Milk	0.025 mg/kg	104% (4.2%)

Recoveries can also be found at **www.eurl-pesticides-datapool.eu**

Tab. 6: Impact of cleanup on dry residue of extracts

Cleanup approach	Dry residue %	Notes
None (Raw milk extract)	100%	3,3 mg dry residue/mL (=0,033%), compared to ca. 12% dry residue in whole milk
dSPE w. C18 (25 mg/mL)	64%	w. additional PSA/$MgSO_4$ (25/150 mg/mL) 53%
Freeze-out and decanting though cotton	44%	w. additional PSA/$MgSO_4$ (25/150 mg/mL) 35%
1:1 dilution w. water -> filtration	35%	Calculated on basis of non-diluted extract (x2)
1:1 dilution w. water + simultaneous dSPE w. C18 (25 mg/mL) -> filtration	23%	Calculated on basis of non-diluted extract (x2). **Substantial recovery losses of QACs observed!!**
Freeze-out -> filtration ->dilution with water (1:1) -> filtration	10%	Calculated on basis of non-diluted extract (x2)

References

[1] The ePesticide Manual, ISBN 1-901396-31-2, The British Crop Protection Council
[2] **http://www.efsa.europa.eu/de/efsajournal/doc/2940.pdf** (Reasoned opinion on the review of the existing maximum residue levels (MRLs) for chlorothalonil according to Article 12 of Regulation (EC) No 396/2005)
[3] EN L 135/22 Official Journal of the European Union 25.5.2012

History

Action	When	Version
Placing method on-line	08.04.2014	V1
Extensive revision and expansion	05.05.2014	V2
Corrections of MRMs in Table 2 and Figure 1 and update of MS-settings in Table 2	06.05.2014	V2.1