EURL-SRM – Analytical Method Report

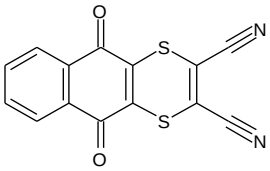
Analysis of Dithianon in Food of Plant Origin using acidified QuEChERS and LC-MS/MS

Version 2 (last update: 29.04.16)

Short description

A method is presented for the analysis of Dithianon in fruits and vegetables. The pesticide is extracted using the QuEChERS method under acidic conditions and directly analysed by LC-MS/MS in the ESI (neg.) mode without dSPE cleanup involving PSA-sorbent.

Compound details

DITHIANON (CAS: 3347-22-6), IUPAC: 5,10-dihydro-5,10-dioxonaphtho[2,3-b]-1,4-dithiine-2,3-dicarbonitrile			
Parameter	**Value**	**Notes**	
Molecular Mass	296.3 g/mol		
Pka	No dissociation in water [4]		
LogPow	3,2	(20°C; pH 2), pH independent	
Water solubility	0.31 mg/L	(20°C; pH 4) [4]	
Stability	Decomposed by alkaline media, concentrated acids, and prolonged heating [1]		
Hydrolysis rates in water (DT50) According to EFSA Peer Review report[1]	12 days	20°C; pH 5	**Hydrolysis products reported …** Phthalic acid, Phthaldialdehyde, 1,2-benzenedimethanol
	0.6 days	20°C; pH 7	
	8 min.	20°C; pH 9	
Hydrolysis rates in water (DT50) According to BASF report[2]	11.6 days	50°C; pH 4	
	6.3 days	30°C; pH 7	
	1.5 hours	40°C; pH 7	
	0.12 hours	25°C; pH 9	
	stable	pH 1.2	
Residue definition EU	Dithianon [2]		
Approved in…	AT, BE, BG, CY, CZ, DE, DK, EE, EL, ES, FI, FR, HR, HU, IE, IT, LT, LU, LV, NL, PL, PT, RO, SE, SI, SK, UK		
ADI / ARfD	0.01 mg/kg bw/day / 0.12 mg/kg bw [4]		

Dithianon is a foliar fungicide with protective and curative action used to control a variety of foliar diseases on a wide range of crops including table and wine grapes, pome fruit, stone fruit, berries, spinach, lettuce, brassica crops, solanaceous crops and rice. In connection with the current MRLs, which were established in 2008[3], acute and chronic intake concerns were identified by EFSA[4,5]. An acute

[1] EFSA Peer Review report: **http://www.efsa.europa.eu/en/scdocs/doc/1904.pdf**
[2] **http://www.bvl.bund.de/SharedDocs/Downloads/04_Pflanzenschutzmittel/01_zulassungsberichte/006459-00-00.pdf?__blob=publicationFile&v=2**
[3] Reg. (EC) No 839/2008 Applicable from: 01/09/2008

intake concern was identified for grapes (MRL= 3.0 mg/kg), whereas the theoretical exceedance of the chronic toxicological thresholds (TMDI) are mainly driven by the MRLs for pears, apples, table grapes and oranges.

Materials[6]:

- Dithianon (purity 99.9%) from Riedel-de-Haen,
- Dithianon D4 (99.1 atom %) was purchased from HPC Standards GmbH / Germany;
- Nicarbazin - containing N,N'-bis(4-Nitrophenyl)urea (BNPU) - was purchased from Sigma-Aldrich
- Sulfuric acid (>95%) was purchased from Fluka
- Formic acid (>96%) was purchased from Sigma-Aldrich
- Stock solutions of native dithianon and ILIS at 1 mg/ml as well as any working solutions were prepared in acetonitrile containing 0.4 % acetic acid (v/v)
- All other materials and chemicals used as listed in EN 15662

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 Procedures:
General remark: *It is important homogenizing samples in frozen condition, keeping homogenates frozen until analysis and conducting analysis quickly under acidic conditions. No dispersive SPE step with PSA should be conducted.*

a) **QuEChERS + 1 % FA:** Weigh 10 g of frozen fruit or vegetable homogenate; add 10 mL acetonitrile containing 1 % (v/v) formic acid (conc.) and internal standard (e.g. 100µL of an appropriately concentrated solution of BNPU or dithianon-D4). Shake 15 min using a mechanical shaker. Add a mixture of 4g $MgSO_4$ and 1g NaCl (no citrate buffer salts), shake 1 min and centrifuge. Measure by LC-MS/MS in the ESI-negative mode. 1mL final extract will represent approximately 1 g matrix.
Note: This procedure provides good recoveries of dithianon from commodities of high acid content. For other commodities it is highly recommended using dithianon D4 as IS to compensate for recovery losses. In absence of dithianon-D4 the use of method b) below is recommended.

b) **QuEChERS + 1 % SA:** Weigh 10 g of frozen fruit or vegetable homogenate; add 10 mL acetonitrile containing 1 % (v/v) sulfuric acid (conc.) and internal standard (e.g. 100µL of an appropriately concentrated solution of BNPU or dithianon-D4). Shake 15 min using a mechanical shaker. Add a mixture of 4g $MgSO_4$ and 1g NaCl (no citrate buffer salts), shake 1 min and

[4] EFSA (European Food Safety Authority), 2010. Conclusion on the peer review of the pesticide risk assessment of the active substance dithianon. EFSA Journal 2010;8(11):1904, 121 pp. doi:10.2903 /j.efsa.2010.1904
[5] Peer review of the pesticide risk assessment for the active substance dithianon in light of confirmatory data submitted (**http://www.efsa.europa.eu/sites/default/files/scientific_otput/files/main_documents/4278.pdf**), November 2015)
[6] **Disclaimer**: Names of companies are given for the convenience of the reader and do not indicate any preference by the EURL-SRM towards these companies and their products

centrifuge. Measure by LC-MS/MS in the ESI-negative mode. 1mL final extract will represent approximately 1 g matrix.

Preparation of calibration standards:
For these tests matrix-matched calibration standards were prepared using an extract of a blank commodity produced as described above, however without addition of an IS. In routine analysis, use matrix-matched calibration standards prepared, if available, using a commodity of the same type as the samples to be analyzed.

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

Table 1: Instrumentation details

LC	Waters Acquity			
MS/MS	ABSCIEX 5500, run in ESI negative mode			
MRMs	296 / 264 (target); 296 / 164 (qualifier), 296 / 238 (qualifier)			
Column	Acquity UPLC BEH Shield RP 18, 2.1x100 mm, 1.7 µm			
Pre-column	Van Guard BEH Shield RP 18; 1.7µm			
Mobile Phase	A: 0.01% acetic acid in water + 5 % ACN (use brown bottles to avoid formation of algae) B: 0.01% acetic acid in ACN			
Gradient	**Time** (min)	**Mobile Phase A** (%)	**Mobile Phase B** (%)	**Flow** (mL/min)
	0	80	20	0.4
	4	70	30	0.4
	7	10	90	0.4
	8.5	10	90	0.4
	8.6	80	20	0.4
	13.5	80	20	0.4
Column temperature	40 °C			
Injection volume	2 µL			
Internal Standard	BNPU, Dithianon D4			

Tab. 2: MRM Details for Dithianon (ESI-neg. mode using ABSciex API 5500 QTrap):

Name of Transition	Rel. Sensitivity	Parent mass	Daughter mass	DP	CE	CXP	Mode
Dithianon 296/264	1	296*	264	-80	-30	-8	ESI neg.
Dithianon 296/164	2	296*	164	-80	-38	-0	ESI neg.
Dithianon 296/238	3	296*	238	-80	-24	-2	ESI neg.
Internal Standard (option)							
BNPU		301**	137	-25	-16	-7	ESI neg.
Dithianon D4		300*	268	-80	-30	-1	ESI neg.

* $[M\bullet]^-$ in the case of Dithianon and Dithianon D4 (due to the formation of a stabilized radical)
** $[M-H]^-$ in the case of BNPU

Validation data:

Tab. 3: Recovery experiments for Dithianon

Commodity	Method	n	Spiking Level [mg/kg]	Mean Recovery %	RSD %	IS
Lettuce	"QuEChERS + 1 % FA"	5	0.1	76	3.2	BNPU
	"QuEChERS + 1 % SA"	5	0.1	90	4.1	BNPU
Cucumber	"QuEChERS + 1 % FA"	5	0.1	66	7.4	BNPU
				110	6.7	ILIS
	"QuEChERS + 1 % SA"	5	0.1	84	6.9	BNPU
				103	7.4	ILIS
Spinach	"QuEChERS + 1 % FA"	5	0.1	70	8.0	BNPU
				96	5.1	ILIS
	"QuEChERS + 1 % SA"	5	0.1	93	6.0	BNPU
				104	5.1	ILIS
Blueberries	QuEChERS + 1 % FA"	5	0.1	82	10.3	BNPU
Rice	"QuEChERS + 1 % SA"	5	0.1	85	8.9	BNPU

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	Document Version
Initial Experiments	May 2013 – March 2014, November 2014	
Further Experiments	November 2014,	
Method document placed on-line	22.11.2014	V1
Further Experiment	March 2015, January 2016, March/April 2016	
Major revision of document	April 2016	V2