EURL-SRM - Analytical Observations Report

concerning the following…

- **Compound(s)**: Dithianon, Dithianon D4
- **Commodities**: Fruit and vegetables, cereals
- **Extraction Method(s)**: QuEChERS, QuEChERS (variations)
- **Instrumental analysis**: LC-MS/MS, ESI (neg.)

Analysis of Dithianon by the QuEChERS Method - Impact of pH on recovery rates

Version 2.1 (last update: 09.05.2016)

Background information / Initial Observations:

Using QuEChERS (EN 15662), dithianon often shows low or variable recovery rates from various commodities. Especially from commodities exhibiting high natural pH, recoveries are often very low. In acidic commodities recoveries are typically acceptable (see examples in Table 1). However, cleanup with PSA also leads to low recoveries.

Table 1: Exemplary Recovery rates of dithianon extracted from the EURL-DataPool:

Extraction	Level (mg/kg)	dSPE cleanup	Mean Recovery %	RSD %	n
Cucumber	0.1	None	68	12,5	5
Lemon	0.1	None	100	13.9	5
Apple	0.1	with PSA	5	43%	6

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[1], acute and chronic intake concerns were identified by EFSA[2,3]. An acute 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.

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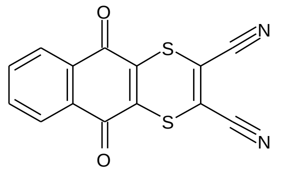
[1] Reg. (EC) No 839/2008 Applicable from: 01/09/2008

[2] 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

[3] 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)

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[4]	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[5]	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]		

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
- Acetic acid (>99%) was purchased from Sigma-Aldrich
- Stock solutions of native dithianon and ILIS at 1 mg/ml was prepared by dissolving 15mg of the compound in 1 mL acetone and filling it up to 15 mL with acetonitrile containing 0.4 % acetic acid (v/v)
- Working solutions were prepared by appropriately diluting stock solutions with acetonitrile containing 0.4% acetic acid (v/v)
- All other materials and chemicals used as listed in EN 15662

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[4] EFSA Peer Review report: **http://www.efsa.europa.eu/en/scdocs/doc/1904.pdf**

[5] **http://www.bvl.bund.de/SharedDocs/Downloads/04_Pflanzenschutzmittel/01_zulassungsberichte/006459-00-00.pdf?__blob=publicationFile&v=2**

[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

Measurement conditions

Measurement was conducted by LC-MS/MS instrument (ESI-negative mode). Details are given in Table 2 and Table 3

Table 2: Instrumentation details

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

Tab. 3: 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

Experiments conducted and observations:

In order to explore the impact of sample pH on dithianon recovery rates we have conducted recovery experiments on lettuce and blueberries using the QuEChERS procedure in 4 different versions as follows:

1) **QuEChERS**: EN-15662[7];

2) **QuEChERS + 4 % HAc**: EN-15662 modified by using 10 mL ACN containing 4 % (v/v) acetic acid for the 1st extraction step and 4g $MgSO_4$ / 1g NaCl for partitioning;

3) **QuEChERS + 1 % FA:** EN-15662 modified by using 10 mL ACN containing 1 % (v/v) formic acid for the 1st extraction step and 4g $MgSO_4$ / 1g NaCl for partitioning;

4) **QuEChERS + 1 % SA:** EN-15662 modified by using 10 mL ACN containing 1 % (v/v) H_2SO_4 conc. for the 1st extraction step and 4g $MgSO_4$ / 1g NaCl for partitioning;

In none of the four versions d-SPE cleanup was performed. Two different internal standards were tested: BNPU[8] was used in the earlier experiments, and dithianon D4 (ILIS) was used in parallel later on.

Matrix-matched calibration standards were prepared using blank extracts prepared with the respective procedure, however without addition of an IS. The results of these experiments are shown in Table 4.

Tab. 4: Recovery data for Dithianon from various commodities:

Matrix Type	Matrix	Spiking Level	IS	QuEChERS			QuEChERS + 4 % HAc			QuEChERS + 1 % FA			QuEChERS + 1 % SA		
				n	Mean Rec.%	RSD %	n	Mean Rec.%	RSD %	n	Mean Rec.%	RSD %	n	Mean Rec.%	RSD %
High water	**Lettuce**	0.1	BNPU	3	**22**	13.7	3	**46**	12.7	5	**76**	3.2	5	**90**	4.1
	Cucumber	0.1	BNPU	-	-	-	-	-	-	3	**66**	7.4	5	**84**	6.9
			ILIS								**110**	6.7		**103**	7.4
	Spinach	0.1	BNPU	5	**4**	23.5	-	-	-	5	**70**	8.0	5	**93**	6.0
			ILIS								**96**	5.1		**104**	5.1
		0.1 (waiting time ca. 10 min)	BNPU							5	**14**	17.3			
High water + low pH	**Blueberry 1**	0.1	BNPU	3	**61**	4.9	3	**63**	6.6	5	**82**	10.3	5	**79**	9.4
	Blueberry 2	0.01	BNPU	-	-	-	-	-	-	5	**94**	3.1	-	-	-
Dry	**Rice**	0.1	BNPU	5	No peak	n.d.	-	-	-	-	-	-	5	**85**	8.9
		0.1 (waiting time ca. 10 min)	BNPU							5	**17**	15.0	5	**39**	3.6

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[7] Detailed instructions on the QuEChERS method are given in the CEN method EN 15662 (citrate buffered), see also brief description under www.quechers.de .

[8] BNPU (1,3-bis(4-nitrophenyl)urea) is one of the two components of Nicarbazin

These experiments show that strong acidic conditions are needed for achieving good recoveries. Even formic acid alone does give sufficient protection. If no ILIS is used to correct for recovery losses acidification with sulfuric acid is preferred.
By using ILIS recovery correction is possible.
It was further observed that in commodities with high pH the degradation is very fast so that extraction at acidic conditions should also start immediately. Even with short waiting times of 5-10 min between spiking the blank sample portions and the start of the analysis a strong decline in the recoveries can be seen.
Given this strong recovery decline it is obvious that if ILIS is employed to correct for recoveries it should spiked to the sample portions immediately. To study these effects an experiment was conducted where the addition of the dithianon-ILIS as well as the start of the extraction was delayed by 10 min after spiking with the native dithianon standard. The results of this experiment are shown in Table 5.

Tab. 5: Recovery data for Dithianon depending on the delay time between spiking and extraction under acidic conditions:

Extraction Method	IS	**Delay** between spiking native dithianon and **spiking of ILIS**	**Delay** between spiking native dithianon and **extraction**	Recovery rates [%]						RSD (%)
				1	2	3	4	5	Avg	
QuEChERS + 1 % FA	ILIS	**No delay**	**No delay**	118	109	103			**110**	6,7
	BNPU			67	70	60			**66**	7,4
QuEChERS + 1 % SA	ILIS	**No delay**	**No delay**	114	97	108	98	97	**103**	7,4
	BNPU			93	79	85	83	79	**84**	6,9
	ILIS	**No delay**	**ca. 10 min**	115	100	94	-	-	**103**	10,4
	BNPU			73	67	62	-	-	**68**	8,1
	ILIS	**ca. 10 min**	**ca. 10 min (shortly after ILIS-addition)**	57	57	54	-	-	**56**	3,5
	BNPU			51	49	50	-	-	**50**	2,3

The experiments shown in Table 5 confirm that extraction with formic acid does not always provide acceptable recoveries. They also confirm the effectiveness of the ILIS to compensate matrix effects. As can be seen a delay of just 10 minutes causes severe recovery drop that can be still effectively corrected through the use of ILIS provided that ILIS was added at the same time as dithianon D4. At a delayed addition of ILIS any losses occurred prior to the addition of the ILIS cannot be compensated. Thus if ILIS is used to match for the losses the addition of ILIS should also be done as early in the procedure as possible.

Impact of cleanup:
No significant losses were observed during the dSPE cleanup step with GCB. The test was conducted on cucumber extracts obtained using two different methods a) **QuEChERS + 1 % SA** and b) **QuEChERS + 1 % FA**. 2.5 mg GCB and 150 mg $MgSO_4$ per mL cucumber extract were used in each case.

Discussion and conclusions:

Using the original citrate buffered QuEChERS method very low recoveries of dithianon are obtained from commodities with high pH. Also low recoveries are obtained when dSPE cleanup with PSA is conducted, whereas dSPE cleanup with GCB does not seem to induce losses. Acidification during the QuEChERS extraction step helps to improve the recovery rates. Sulfuric acid offers better protection than formic acid or acetic acid. If no ILIS is used to compensate recovery losses extraction with 1% sulfuric acid is indicated, especially for commodities having high natural pH. Using dithianon-D4 as ILIS recovery losses can be effectively compensated provided that it is added at a very early step in the procedure. As losses of dithianon are very rapid in high-pH commodities it is of paramount importance to conduct analysis as fast as possible and keep sample temperature low. Acidification during sample comminution helps to reduce losses further.

History

Action	When	Document Version
Initial Experiments	May 2013 – March 2014, November 2014	
Further Experiments	November 2014,	
Observation document placed on-line	22.11.2014	V1
Further Experiment	March 2015 January 2016, March/April 2016	
Major revision of document	April 2016	V2
Addition of a detail regarding preparation of the stock solution	09.May 2016	V2.1