

# Problematic Compounds: Dithianon

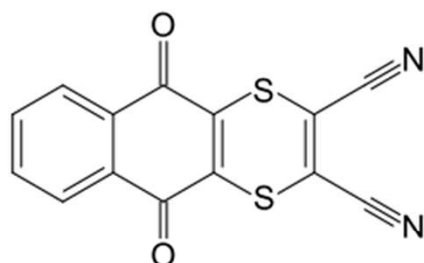
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Austrian Agency for Health and Food Safety

## Structure and Reactivity



$C_{14}H_4N_2O_2S_2$  (296.33 g/mol)  
mp: 216 °C (decomposition)  
 $\lg K_{o/w} = 3.2$   
 $p_{vap} = 2.71 \times 10^{-11}$  mbar  
pH = 4.4 – 4.8 (1 wt% aq.)  
MRL: 3 (grapes) to 100  
(hops) mg/kg

- Quinone Fungicide (prevents spore forming)
- **Hydrolyzation under basic conditions**
- Photo-degradation by first order ( $t_{1/2} \approx 1$  week)

[1] US EPA: Pesticide Fact Sheet Dithianon

[2] UN FAO, Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Expert Group on Pesticide Residues, Rome September 21st – 30th, 1992, Evaluations (Pt. I), p. 428

[3] Codex Alimentarius: MRLs for Pesticide Residues in Food, 2008

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Dithianon is a quinone type fungicide, which is legally used up to comparably high MRL. However, due to its instability this analyte may be lost to a certain extent during analysis. Hydrolysis as well as photo-degradation of the compound are reported in literature. This presentation is not intended to present entirely new data, but to draw attention to problems known but easily overlooked associated with dithianon. Due to its polarity, dithianon should be extracted by the QuEChERS method in a facile way, analyte losses caused by evaporation are not to be expected.

## EU Control Program



- Matrices and approximate pH values:

- 2014: Beans with pod (6), carrots (6), cucumbers (5.5), oranges or mandarins (4), pears (4), potatoes (5.8), rice, spinach (6), wheat flour (6)
- 2015: Aubergines, bananas (5), cauliflower (5.6) or broccoli (6.6), table grapes (3), orange juice (3.7), peas without pod (6), peppers (sweet) (5.5), wheat (6), virgin olive oil

[1] Commission Implementing Regulation EU 788/2012, 31.08.2012

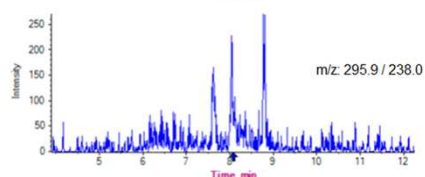
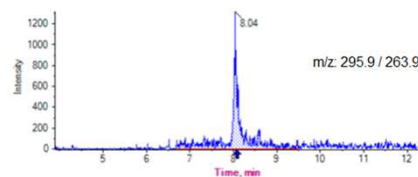
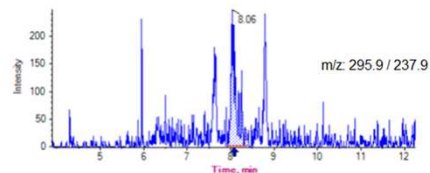
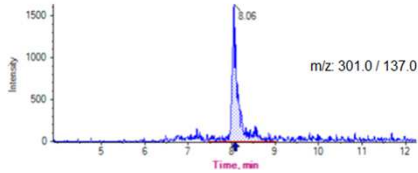
[2] pH Values of Common Foods and Ingredients ([www.foodsafety.wisc.edu/business\\_food/files/Approximate\\_pH.pdf](http://www.foodsafety.wisc.edu/business_food/files/Approximate_pH.pdf))

The European Union Control Program makes dithianon analysis in the food commodities listed mandatory. As hydrolysis of dithianon is reported to be effective under basic conditions, one should not expect significant analyte losses: approximate pH values are found smaller than pH = 7, thus (mild) acidic.

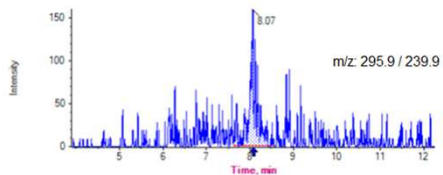
## Example



- Wheat flour: bio quality?



0.11 mg/kg dithianon



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In a practical example, wheat flour –claimed to be bio grade- was monitored using LC-MS/MS and a standard QuEChERS clean-up procedure. Measurement was conducted 16 hours after sample preparation. Solvent used for sample preparation was 1% formic acid. The sample contained (was spiked with) 0.11 mg/kg dithianon. However, responses are very poor.

## Critical Steps in Clean-Up



(Sample homogenization)

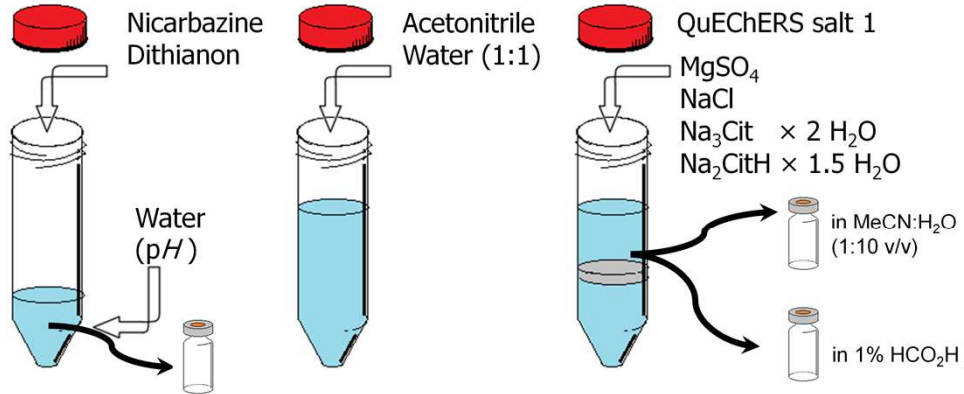
- Extraction (acetonitrile – water)
- Addition of QuEChERS salt mixture
- Centrifugation
- Dispersive SPE

(Sample concentration)

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Loss of analyte may occur during the clean-up procedure at the step of extraction by reaction with the solvents, upon addition of the QuEChERS salt mixture by reaction with mixture components, during centrifugation hydrolysis can proceed further, and – if applied – during dSPE by reaction with the absorbent.

## Experiment



Aqueous solution of dithionon is „cleaned up“ like a proper sample, pH and dithionon concentration are monitored.

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In order to determine the analyte loss experimentally, a very simple set-up neglecting reactions with matrix constituents was chosen. An aqueous solution of dithionon was cleaned up using QuEChERS method. Aqueous acetonitrile was applied for extraction followed by addition of QuEChERS salt mixture and further extraction. Three samples at different pH were investigated, nicarbazine was used as internal standard in order to realte LC-MS/MS measurement results. Samples for LC-MS/MS were taken as indicated, these samples were either dilluted with acetonitrile and water (1:10, v/v) or formic acid (1% in water).

## Results



„sample“:

<i>pH</i>	2.6	6.0	11.0
$c_0$ (mg/kg)	0.036	0.036	0.036

QuEChERS extract:

<i>pH</i>	4.0	4.0	4.0
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Time from preparation to measurement: 46 hrs

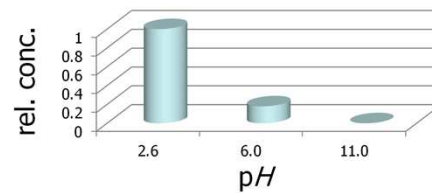
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Starting pH values of the samples were 2.6 (hydrochloric acid added), 6.0 and 11.0 (sodium hydroxide added). Due to the citrate salts of the QuEChERS salt mixture, the solutions were buffered after salt addition to pH of 4. Dithianon was monitored prior to work-up and from the QuEChERS extract. LC-MS/MS measurements were conducted 46 hours after sample preparation.

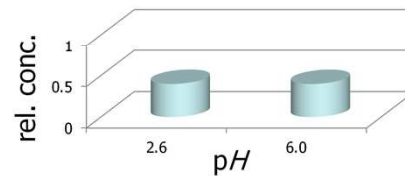
## Results



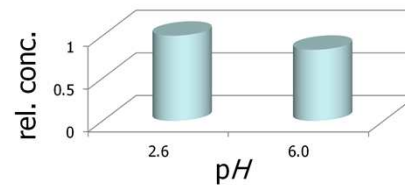
„sample“ (no work-up)



QuEChERS extract (buffered)



QuEChERS extract (1% HCO<sub>2</sub>H)



(Relative concentration of dithianon in acidic sample equals sample w/o work-up!)

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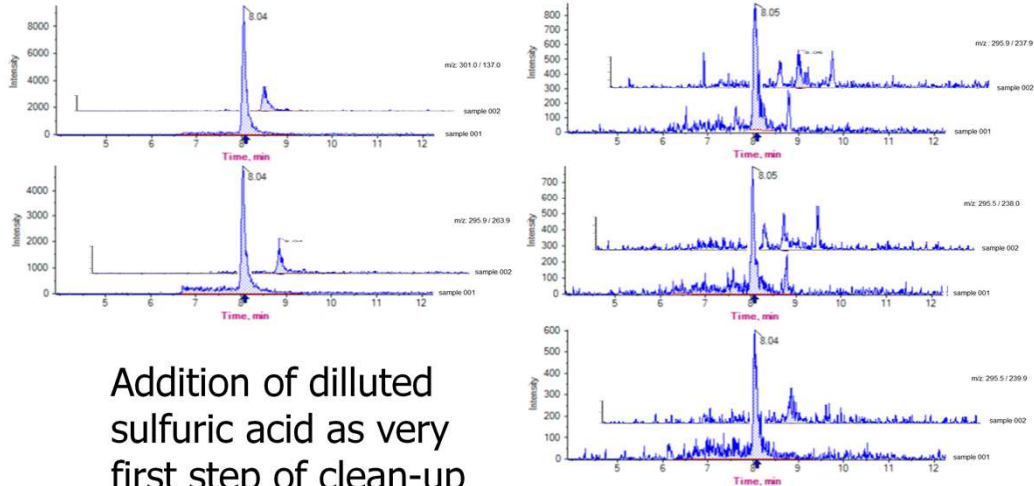
During the 46 hours, dithianon concentration sunk to 18 % of its original value at pH 6, remained almost unchanged at pH 2.6 and completely vanished in the basic sample. Unsurprisingly, no dithianon was discovered in the QuEChERS extracts of the basic samples.

In the buffered QuEChERS extracts, dithianon levels sunk to 40 %. However, as the QuEChERS extract aliquots were dilluted with 1 % formic acid, dithianon losses were diminished. The acidic sample did not show any losses of dithianon, in the pH = 6 sample, dithianon concentration sunk to 85 % of its original value. Clearly, 15 % of dithianon in the „neutral“ sample were lost during clean-up, alone.

Apparently, acidification of the sample prior to work-up or – at least – acidification of the dilluted analytical sample for LC measurements can hinder dithianon hydrolysis effectively.



## Re-Check



Addition of diluted sulfuric acid as very first step of clean-up  
(Up to 4.6-fold area increase)

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The experiment with the „bio grade“ wheat sample was repeated. As very first step of the clean-up procedure, the flour was suspended in diluted sulfuric acid with a pH-value of 0.8 instead of water and then processed using QuEChERS procedure. The sample for LC-MS/MS measurement was dissolved in 1% formic acid. As you can see, the addition of diluted acid as very first work-up step, resulted in an up to 4.6-fold increase in peak areas and thus lowered the LOD significantly.

## Consequences and Conclusion



- Problems with dithianon in neutral to weakly basic moieties can be countered
- Samples with „high“ pH should be acidified as soon as possible
- QuEChERS extracts should be diluted with acidic solvents to obtain and preserve samples suitable for LC-based measurements

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Concluding, it has been shown that dithianon hydrolysis can be effectively prevented or at least significantly reduced.

Acidification of the samples to be analyzed is the first important step and - even more important – analytical samples for LC-MS/MS investigation should be acidified in order to prevent dithianon hydrolyzation.

## Acknowledgements



Ms. Jasmin Aldrian and Mr. Roman Fischer are cordially thanked for fruitful discussions and conducting the LC-MS/MS measurements.