Analysis of Meptyldinocap by QuEChERS, Followed by Alkaline Hydrolysis and LC-MS/MS Measurement

E. Scherbaum, E. Eichhorn, S. Goerlich, A. Barth and M. Anastassiades
E-Mail: eurl-srm@cvuas.bwl.de

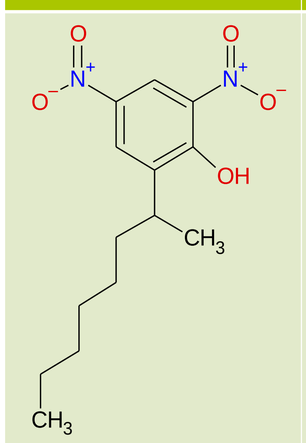
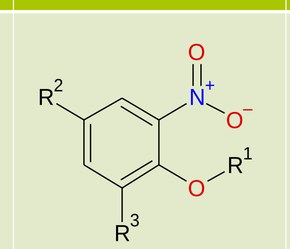
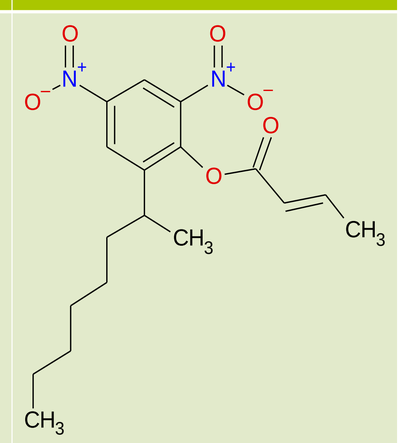
Introduction

Meptyldinocap is a contact fungicide with protective and curative properties. It is primarily used against powdery mildews in a variety of crops, such as cucurbits and other fruiting vegetables, various tree fruits, as well as berries.

Legal Aspects

Meptyldinocap is the major and most active compound of dinocap (6 pairs of stereoisomers), which is no longer approved in the EU. Meptyldinocap (one pair of stereoisomers) is approved under Reg. 1107/2009/EC and is authorized in fourteen member states. Dinocap is still in use elsewhere in the world, however.

The residue is defined as meptyldinocap and its corresponding phenol (2,4-DNOP); the same definition is used for dinocap.

Meptyldinocap phenol	General Formula	Meptyldinocap
		

Standard Stability

Analysis of meptyldinocap (and dinocap) requires taking measures to ensure stability of meptyldinocap in standard solutions. This includes acidification (when acetonitrile is used) and keeping the standard solutions in a cool and dark place to minimize hydrolysis and photolysis. Still, small amounts of 2,4-DNOP are typically observed as impurity in standard solutions.

Analytical Methods

Citrate buffered QuEChERS (EN 15662) is applied for the extraction. Separation of all dinocap esters and corresponding phenols is possible in GC-MS CI negative mode (CH_4), which is rarely used in labs. In GC (meptyl)dinocap has proven to be very sensitive to temperature and liner contamination.

Using LC-MS/MS, meptyldinocap shows in-source fragmentation to the corresponding phenol, but the latter gives a much more intensive signal in the ESI (neg.) mode. Therefore, despite being greatly underrepresented in the meptyldinocap standard solution (in this case meptyl-dinocap:2,4-DNOP ratio ~ 80:1), the phenol impurity shows a more sensitive signal than meptyldinocap when injected (see Fig. 1).

Transforming meptyldinocap to its phenol (2,4-DNOP) via alkaline hydrolysis in the QuEChERS extracts is a simple way of improving sensitivity and reducing the number of analytes to be covered in analysis.

Sample preparation: The samples are extracted according to the QuEChERS (citrate-buffered; EN-15662) method. High oil content commodities are extracted according to the QuOil method (CEN/TS 17062:2019). For the derivatization step, 1000 µL is transferred into a vial, 25 µL of 25% ammonia solution (75 µL in case of dry commodities or commodities of animal origin) is added, and the vial is left standing for at least 12 h at RT (e.g. overnight) or left to react for 2 h at 60° C. The hydrolysate is "neutralized" with 25 µL of concentrated acetic acid (75 µL in the case of dry commodities and commodities of animal origin) and is then directly employed for analysis.

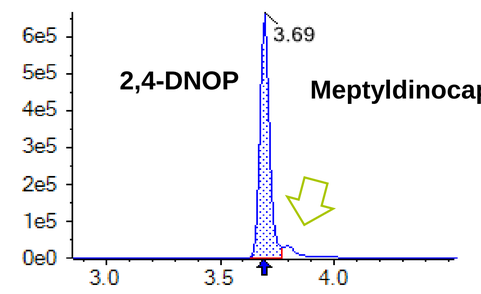


Figure 1: 2,4-DNOP at 0.005 and meptyldinocap at 0.02 µg/mL were injected ESI neg. mode (m/z 295/194)

Validation data

Matrix	Spiking level (mg/kg)	Mass trace	Calculation using matrix-matched calibration						Amount of aqueous ammonia solution (25%) added
			w/ ISTD Propyzamide-D_3		w/ ISTD BNPU		w/o ISTD		
			Mean Rec.	RSD	Mean Rec.	RSD	Mean Rec.	RSD	
Cucumber	0.005	295/194	83 %	10 %	80 %	17 %	82 %	8 %	+ 25 µL (per mL extract)
		295/193	87 %	9 %	85 %	15 %	85 %	6 %	
		295/134	95 %	15 %	95 %	25 %	94 %	14 %	
Grapes	0.005	295/194	83 %	10 %	77 %	8 %	80 %	7 %	
		295/193	89 %	11 %	83 %	9 %	86 %	8 %	
		295/134	75 %	8 %	70 %	7 %	72 %	4 %	
Bovine liver	0.005	295/194	83 %	2 %	91 %	10 %	87 %	12 %	
		295/193	83 %	3 %	91 %	10 %	86 %	12 %	
		295/134	83 %	2 %	91 %	10 %	86 %	13 %	
Whole wheat flour	0.005	295/194	85 %	2 %	89 %	4 %	89 %	3 %	+ 75 µL (per mL extract)
		295/193	89 %	2 %	92 %	3 %	92 %	2 %	
		295/134	87 %	6 %	89 %	4 %	89 %	6 %	
Peanut butter	0.005	295/194	83 %	6 %	86 %	6 %	85 %	7 %	
		295/193	80 %	4 %	82 %	3 %	81 %	4 %	
		295/134	86 %	4 %	87 %	3 %	87 %	2 %	

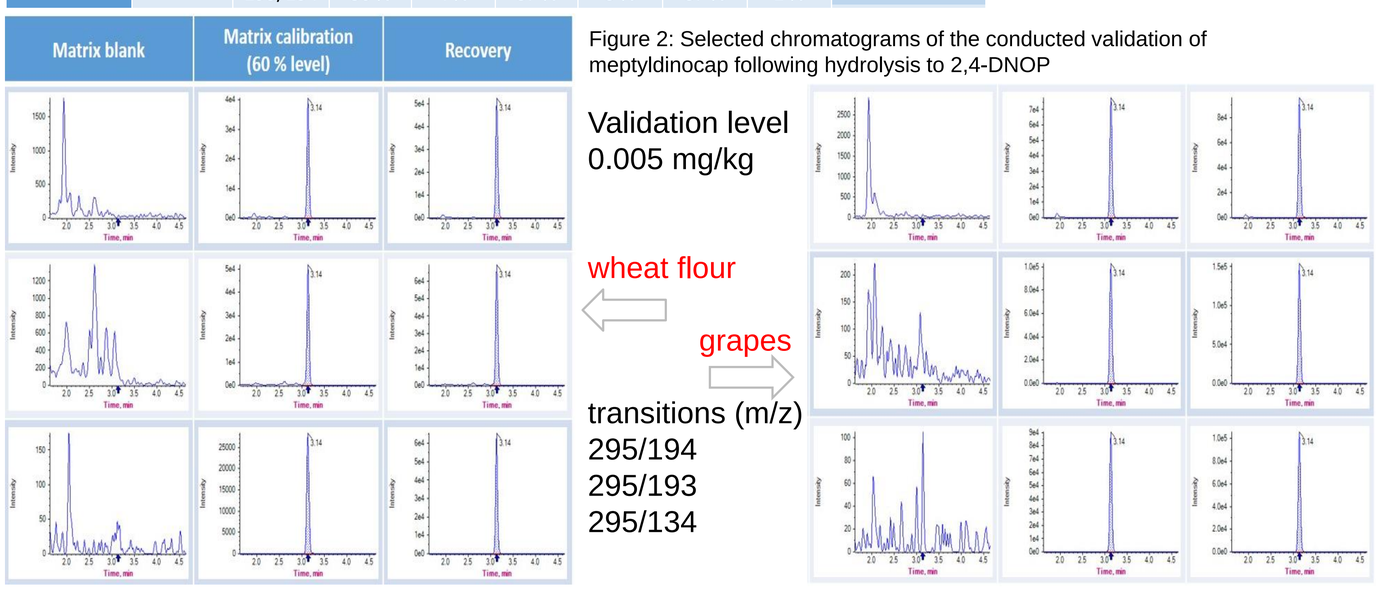


Figure 2: Selected chromatograms of the conducted validation of meptyldinocap following hydrolysis to 2,4-DNOP

Typically, dinocap is well distinguishable from meptyldinocap, as it shows a more complex LC-MS/MS peak pattern.

Further work will focus on improving the chromatographic separation of all dinocap components, to enable proper quantitative analysis of dinocap (sum). The availability of analytical standards for the six parents and the six phenols is, however, a prerequisite for this.

Summary

We developed a QuEChERS-based method for the extraction and quantification of meptyldinocap and its corresponding phenol, by introducing a single additional step featuring alkaline hydrolysis on an aliquot of the final extract, combined with sensitive and selective measurement by LC-MS/MS in ESI neg. mode.

Literature

[1] Observation on Meptyldinocap; see EURL-SRM

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