Analysis of Dinocap and Meptyldinocap by modified QuEChERS

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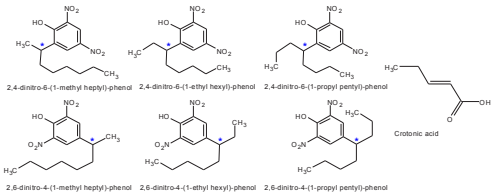
Introduction

Dinocap is a contact fungicide primarily used against powdery mildews in pome fruits, stone fruits, citrus, vines, berries and other. It is additionally effective as a non-systemic acaricide.

Commercially available dinocap is a mixture of six different dinitrophenyl crotonate isomers, one of which is meptyldinocap (2,4-dinitro-6-(methyl-heptyl)-phenyl crotonate, (2,4-DNOPC)).



Legal Aspects

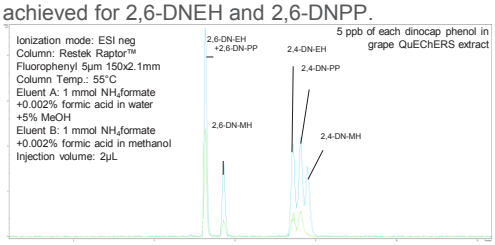
The single isomer meptyldinocap is approved under Reg. 1107/2009/EC and authorized at national level in fourteen Member States, whereas the use of dinocap (the isomers mixture) is no longer authorized within the EU. Dinocap is however still in use elsewhere in the world.

To judge dinocap and meptyldinocap residues, two different residue definitions as per Reg. 396/2005/EC need to be taken into account. The first one refers to the sum of all dinocap isomers, and their corresponding phenols, and the second to the sum of the single isomer meptyldinocap and its corresponding phenol (2,4-DNOP). However, pursuant to Reg. 1127/2014/EU, the residue definition for dinocap is not applicable if only meptyldinocap and/or it corresponding phenol is detected. This is relevant for legal evaluation as MRLs of dinocap and meptyldinocap can be different. With MRL*s being as low as 0.02 mg/kg in fruits and vegetables, a sensitive and specific analytical method is needed in order to correctly identify, quantitate and evaluate residues of dinocap and its phenolic metabolites.

Development of Method

Analysis of dinocap has proven challenging for various reasons including the following: a) standards of individual dinocap isomers or their phenols, except meptyldinocap, are commercially unavailable*; b) the isomers (phenols and esters) are structurally very similar and thus difficult to separate chromatographically and mass-spectrometrically; c) formation of the phenols either thermally in GC or via in-source fragmentation in LC-MS/MS; d) crotonate esters readily undergo hydrolysis and photolysis to their corresponding phenols in solvent, unless kept in acidic medium in a dark and a cool environment.

First, identity and stability of the available standards (six individual esters, two phenols and two phenol mixtures) was tested using LC-MS/MS, high resolution time-of-flight (TOF) mass spectrometry and nuclear magnetic resonance (NMR), as well as GC-MSD in CI-neg.-mode. Separation of all dinocap esters and corresponding phenols was possible in GC, but dinocap proved very sensitive to injector temperature and liner condition. To reduce the number of components to be analyzed, further experiments focused on transformation of esters to phenols and chroma-tographic separation of the latter. This proved very challenging and could finally not be achieved for 2,6-DNEH and 2,6-DNPP.



The conversion of esters to their corresp. phenols, was accomplished in QuEChERS extracts by addition of 10µL 25% aq. NH_3 to 0.5 mL extract. Quantitative hydrolysis of dinocap esters was achieved at room temperature within 2 hours (2,6-DNOPCs) and 12 hours (2,4-DNOPCs). Increasing temperature (40°C) and/or pH resulted in significantly shortened reaction times. Phenols and internal standard BNPH remained stable.

Applied Method

Extraction: Apply citrate buffered QuEChERS (EN 15662).

Hydrolysis: Transfer 0.5 mL of the QuEChERS raw extract into a vial with an integrated PTFE filter (0.45 µm pore size), add 10 µL 25% aqueous NH_3 solution and place for 12 h at room temperature and then filter the hydrolysate.

LC-MS/MS analysis: In ESI negative mode, dinocap phenols can easily be detected at levels below 1 ppb for screening purposes in QuEChERS raw extracts. In case of positive findings, hydrolysis of raw extract and LC-MS/MS analysis of the hydrolysate should be performed. Complete separation of phenols is optional as distinction between phenols can still be accomplished using isomer / group-specific mass transitions.

Note: for meptyldinocap the 2,4-DNOP is commercially available and can be used to quantitate residues. For dinocap, the commercially available mixture has to be hydrolyzed and used for calibration (for details see upcoming publication of analytical observation) .

Results

The method was validated at the 0.005 mg/kg level for all phenols in grapes, strawberries and cucumber, with good recovery and reproducibility rates (data not shown). Real samples with positive findings of dinocap were hydrolysed and phenol levels were found to have increased up to 6-fold

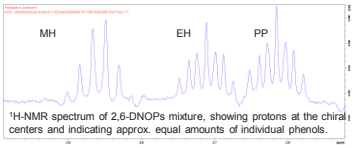
Summary

We developed a quick, easy and reliable QuEChERS-based method for the extraction and quantification of all dinocap isomers as their corresponding phenols, using a single additional step featuring alkaline hydrolysis on an aliquot of the final extract, combined with sensitive and selective measurement by LC-MS/MS.

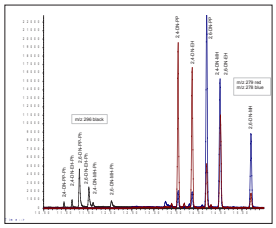
References

1. Review report for the active substance Dinocap, SANCO/4345/2000 – final, 5 January 2007
2. Final Review report for the active substance Meptyldinocap, SANCO/10742/2014 rev2
3. Observation: in preparation (see EURL website)

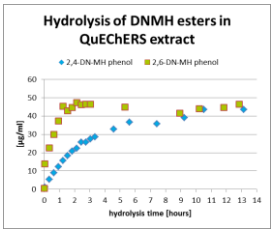
*samples of dinocap isomers were kindly provided by Dow AgroSciences.

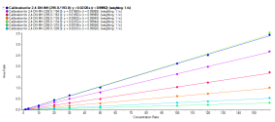


^{1}H-NMR spectrum of 2,6-DNOPs mixture, showing protons at the chiral centers and indicating approx. equal amounts of individual phenols.

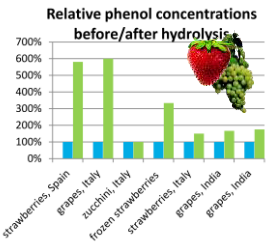


GC-CI neg. chromatogram of dinocap esters (100 ppb each). Note that small amounts of their corresp. phenols are also present.





Linearity from 1 ppb to 160 ppb for relevant mass transitions of dinocap phenols



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