Observations concerning…

☑ ***a compound*** ☐ ***a matrix*** ☑ ***a method*** ☐ ***other***

Analysis of dicofol via QuECHERS - use of isotope labeled dicofol to improve precision

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Description of problem/observation/solution:

Dicofol is well known to readily degrade to p,p´-dichlorobenzophenone (DCBP) both within crops and at various steps of analytical procedures (e.g. during milling, extraction, cleanup, extract storage and GC-injection). **DCBP is not part of the MRL residue definition of dicofol**. It is thus important to detect dicofol as such and to conduct analysis in a way that minimizes dicofol losses. **Analysis following complete conversion to DCBP is not reasonable** as there is no possibility afterwards to distinguish between the DCBP formed from dicofol during analysis and the DCBP already contained in the sample prior to analysis. Moreover, DCBP is not specific for dicofol as it is also a metabolite of chlorobenzylate. Nevertheless, DCBP can still serve as a valuable indicator especially in routine testing. In solution dicofol degradation is most pronounced at high pH and it is therefore important to avoid exposition to high pH during analysis (e.g. skip dSPE with PSA in QuEChERS). Degradation in standard solutions in acetonitrile has also been observed. Acidification of stock and working solutions in acetonitrile (e.g. with acetic acid) is thus necessary to protect dicofol. LC-MS/MS sensitivity for dicofol is very poor and the compound is thus typically analyzed via GC (e.g. using ECD, MSD or MS/MS). GC-analysis, however, is very challenging due to the poor reproducibility of dicofol decomposition within the hot GC-inlet and the risk of complete decomposition, e.g. when the GC-inlet surface is very active (contaminated with lots of non-volatiles) or in absence of matrix components. Dicofol decomposition in GC-inlets is practically unavoidable and the focus should be in achieving uniform decomposition rates in sample extracts and calibration solutions. Matrix-matching and the use of analyte protectants (APs)[1] can help to reduce GC-decomposition rates and to improve signal reproducibility, but experiments have shown that the robustness achieved is still not fully satisfactory. The most efficient way to eliminate all the above error-sources is the use of isotope-labeled dicofol (e.g. dicofol-D8) as internal standard (ISTD). The ISTD can match for many error-sources but the existence of a well measurable dicofol signal remains of paramount importance (laboratory reporting limits should also be met). This essentially means that measures should be taken to minimize degradation during sample preparation and GC-analysis. Skipping dSPE with PSA and the use of calibration standards in matrix or APs is thus recommended.

[1] http://www.eurl-pesticides.eu/library/docs/srm/EURL_Observation-APs.pdf

Compound profile:

Dicofol is a non-systemic acaricide used for foliar application on a wide variety of crops including fruit trees, fruiting vegetables, leafy vegetables and vines.

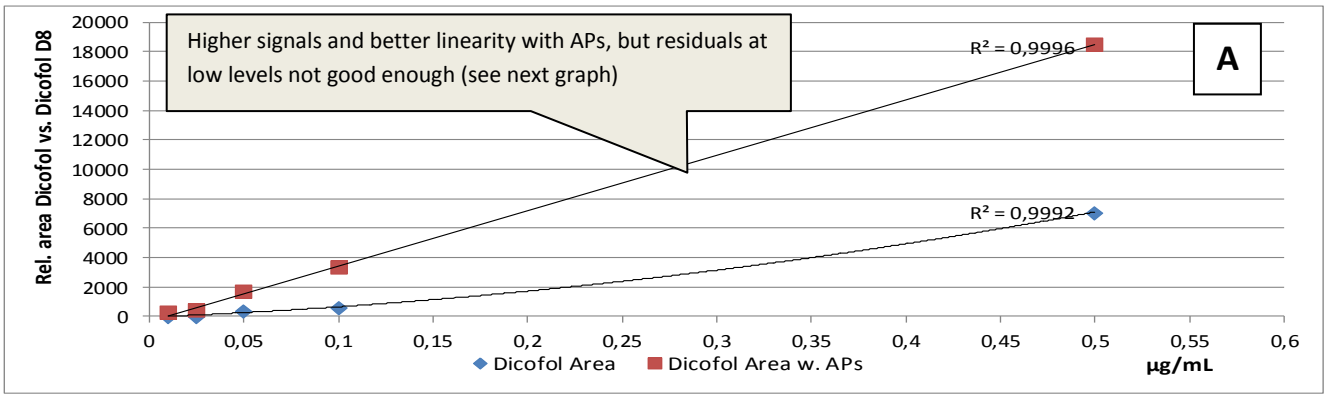
Dicofol		p,p'-Dicofol
Mode of action	Non-systemic acaricide with contact action	
Composition specifications	Min. 95% pure, composed of min. 84% p,p´-dicofol and 16% o,p´-dicofol[2] DDT should not exceed 0.1% (since 1987), previous limit was 2.5%	
LogP	4.3	
Water solubility	0.8 mg/L	
Stability	Stable in acidic media but unstable in alkaline media. Hydrolysis to DCBP and chloroform. Half lifes for p,p´-dicofol: 85 days at pH5, 64 hours at pH7, 26 minutes at pH 9. Photo-degradation also leads to DCBP. In general o,p´-dicofol dissipates more rapidly than p,p´-dicofol[3]	
Residue definition EU	Sum of p,p' and o,p' isomers (both for food of plant and animal origin)	
Registration Status	No authorization in place within the EU	
Main metabolites / degradation products	4,4'-dichlorobenzophenone (= p,p`-dichlorobenzophenone = DCBP) In animal products dechlorodicofol (DCD) is a relevant metabolite (not included in the EU residue definition).	p,p'-Dichlorbenzophenon

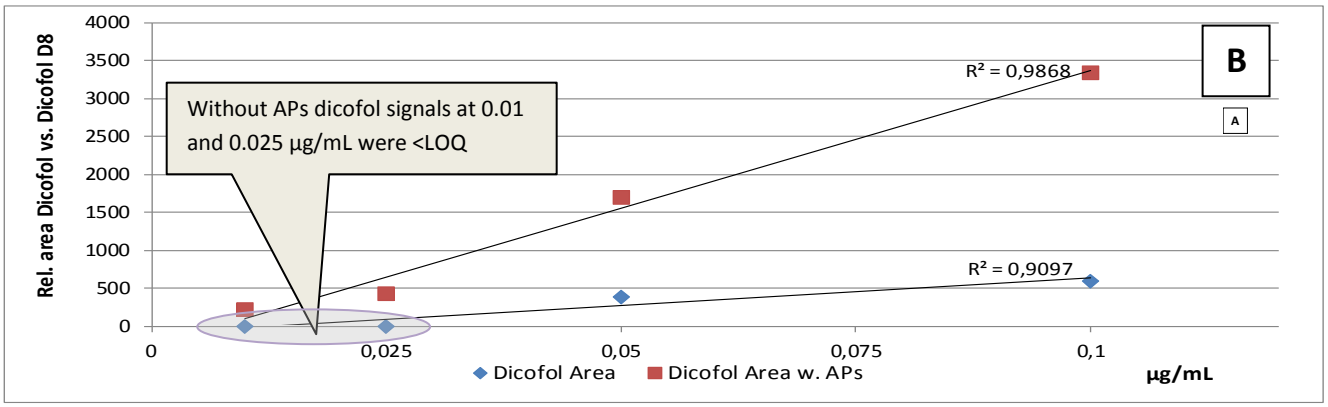
[2] www.fao.org/ag/AGP/agpp/Pesticid/Specs/docs/word/DICO.DOC
[3] www.epa.gov/oppsrrd1/REDs/0021red.pdf

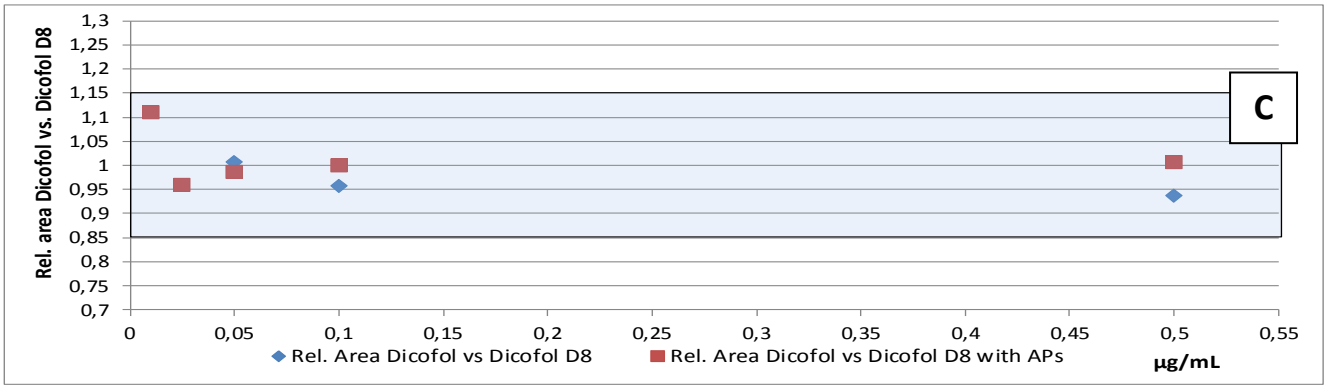
Experiments conducted and observations:

2 series of blank cucumber extracts each spiked at 0.01, 0.025, 0.05, 0.1 and 0.5 µg/mL with dicofol and dicofol-D8 were prepared. One series was spiked with APs and both were analyzed by GC-MS/MS. In absence of APs the response curve was quadratic indicating that the percent decomposition rate increases at low concentrations. The signals for dicofol and dicofol-D8 even fell below the LOQ at 0.01 and 0.025 µg/mL. Better linearity and higher signals were obtained in presence of APs (figure 1A). The curve quality was, however, still not fully satisfactory in the presence of APs (figure 1B). The dicofol/dicofol-D8 ratio remained practically constant irrespective of the presence/absence of APs (see figure 1C).

Figure 1: Response curves of dicofol in cucumber extract in presence and absence of APs



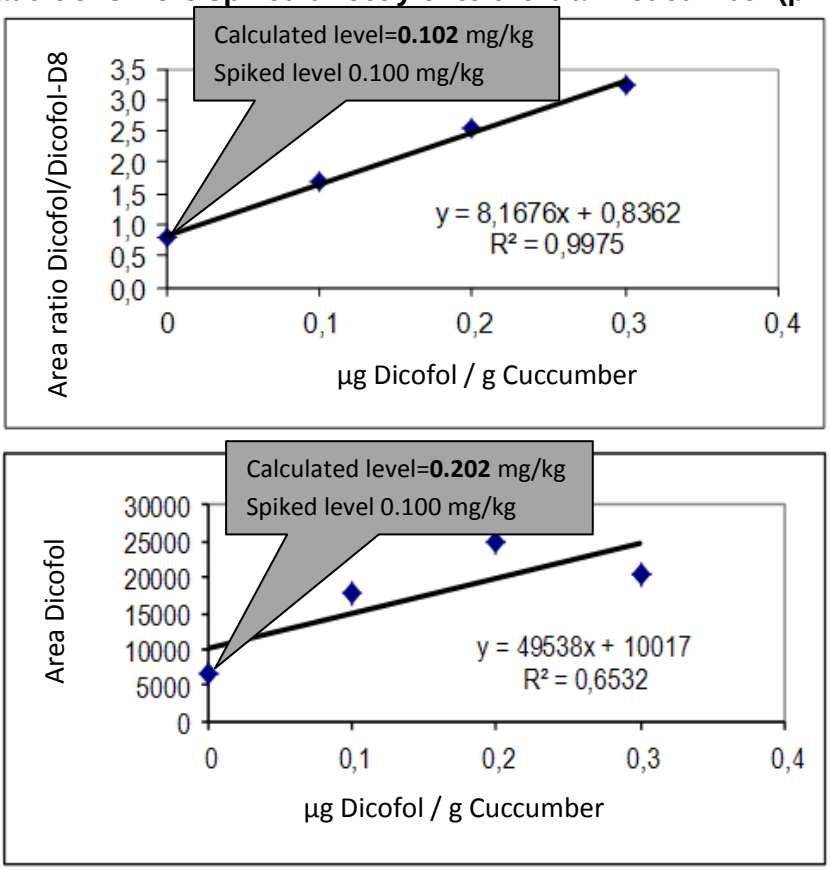




To assess dicofol losses during dSPE-cleanup (with 25 mg PSA/mL) and to test the ability of dicofol-D8 (ISTD) to match for these losses the following experiment was run. Blank cucumber extract was spiked with dicofol and dicofol-D8 and the dSPE cleanup was performed. Following cleanup the extract was acidified with formic acid immediately in one case and after 1 hour in the second. Recoveries calculated via the area were 50 and 13% respectively. Corrected via the ISTD the recoveries achieved were 106 and 103% respectively clearly showing that the ISTD can effectively and accurately correct for recovery even in cases where the real recoveries are very low.

In a separate experiment blank cucumbers were spiked with dicofol at 0.1, 0.2, 0.3, and 0.4 mg/kg and with dicofol-D8 at 0.1 mg/kg. The samples were extracted by the QuEhERS method and dicofol as well as dicofol-D8 were measured by LC-MS/MS. This experiment was conducted in duplicate. The spiking levels were plotted both against the dicofol area and against the area ratio (versus the ISTD). Very good linearity was achieved in both cases. In an experiment simulating standard additions approach the first spiking level (0.1 mg/kg) was assumed to be unknown and was calculated via linear extrapolation using the other three levels (see figure 2). The result obtained via the ISTD was excellent whereas the result calculated directly using the area deviated by a factor of 2 due to the poor linearity.

Figure 2: Simulated standard additions approach of dicofol on cucumber. The standard additions were spiked directly onto the blank cucumber (prior to extraction).



Recovery experiments for dicofol in potato lentil and oranges by QuEChERS (EN-15662) without dSPE and using Dicofol-D8 as ISTD (added at the beginning of the procedure), resulted in recoveries between 95 and 104%. Calculation directly through dicofol peak areas also resulted in acceptable average recoveries but a much higher variation was noticed.

Materials:

Dicofol (purity 99%), purchased from Dr. Ehrenstorfer (Cat #: C12570000)
Dicofol D8 (purity 100%) purchased from Dr. Ehrenstorfer (Cat #: XA12570100CY)
p,p'-Dichlorobenzophenone (purity 99.5%), purchased from Dr. Ehrenstorfer (Cat #: C12410000)
p,p'-Dichlorobenzophenone D8 (purity 99%), purchased from Dr. Ehrenstorfer (Cat #: XA12410100AC)

Instrumentation details:

GC	GC Agilent Typ:6890N, Injector Gerstel CIS4
MS/MS	Waters Quatro micro GC, run in ESI positive mode
Column	Fused-silica capillary column Restek Rxi®-5Sil MS (30 m length, 0.25 mm ID, film thickness 0.25 µm; integrated pre column without film, 10 m length, 0.25 mm ID)
Injection volume	3 µL (MeCN), solvent vent mode
Injection	Injection at 50 °C, split valve open, split flow 50 mL/min Keep temperature at 50 °C for 0.5 min, split valve open, split flow 20 mL/min (vent flow), close split valve at 0.5 min, at 0.8 min increase split flow to 50 mL/min, start heating with 12°C/s to 280 °C and hold for 15 min. Reopen split valve at 2 min. Split flow at 50 mL/min, at 6 min activate gas saver mode (20 mL/min split flow).
Oven program	2 min at 40 °C, increase with 30 °C/min to 220 °C, with 5 °C/min to 260 °C, with 20 °C/min to 280 °C, 15 min isotherm at 280 °C
Flow	Helium, 2 ml/min, constant flow
Transfer line	260 °C
Detector	EI-MSMS, 70 eV, Source temperature 200 °C
Resolution	Q1 and Q3 0.7 amu
Collision gas	1.5 mTorr

Compound name	Retention time (min)	Transition	Used for	Dwell time (s)	Collision energy (eV)
p,p'-Dicofol	14.9	251>139	quantification	0.050	10
		139>111	confirmation	0.050	10
p,p'-Dicofol D8	14.9	259>143	quantification	0.050	10
		143>115	confirmation	0.050	10
p,p'-Dichlorobenzophenone	10.4	250>139	quantification	0.050	10
		250>215	confirmation	0.050	5
p,p'-Dichlorobenzophenone D8	10.4	258>143	quantification	0.050	10
		258>223	confirmation	0.050	5

Discussion and conclusions:

As dicofol is extensively (often completely) degraded during analysis. This results in poor recoveries and poor analytical precision. The use of isotope-labeled dicofol (e.g. dicofol-D8) as internal standard (ISTD) is the most efficient and convenient way to eliminate most sources of errors. If added to the final extract the ISTD can match for any decomposition and signal fluctuations in GC. If added at the beginning of the procedure it will also match for any losses during extraction. Of high importance is, however, that a well measurable signal is obtained for both dicofol and dicofol-D8. Thus measures should be taken to minimize dicofol degradation during entire procedure. Skipping dSPE with PSA and the use of calibration standards on matrix or APs is thus recommended.