

INTRODUCTION

Pesticides containing carboxylic groups are applied in crop production either as free acids or as esters linked to a variety of alcohol groups. Most esters are reportedly quickly hydrolysed in soil or plants to the free acids.

RESIDUE DEFINITIONS

Within the EU, the residues of acidic pesticides are not uniformly regulated with residue definitions (RDs) entailing sometimes only the free acids, sometimes acids and esters and sometimes additionally conjugates. Conjugated residues are formed secondarily in crops when acids covalently bind to various matrix components via ester-, glycoside- or other bonds.

New Approach: Enzymatic Hydrolysis (EH)

To achieve full hydrolysis of sterically hindered esters a new approach was introduced by the EURL-SRM that employs esterase enzymes. Similar to AH enzymatic hydrolysis (EH) is conveniently performed directly prior to QuEChERS.

Various esterase types were tested and esterase from porcine liver was proven the most appropriate. 2 mg esterase (100 µL suspension) are added to the samples following pH-adjustment at levels between 6-9. The mixture is left to react for 3 h or over night (16h) at room temperature before continuing with the QuEChERS procedure. Optionally EH can be combined with AH as described above depending on the application.

Matrix: Method:	Cucumber QuEChERS					Residue Definition	Experiments performed in advance to QuEChERS							
	Ester	Acid	Esters	Conjug.	Residue Definition		I	II	III	IV	V	VI	VII	VIII
							AH RT 30 min	AH 80°C, 30 min	AH RT 16 h	EH (1 mg) RT 3h	EH (2mg) RT 3h	EH (5mg) RT 3h	EH (2mg) RT 16 h	VII + II
2,4,5-T	2-butyl				2,4,5-T (F)	92	88	91	91	91	91	91	91	91
	butoxyethyl					0	0	0	0	0	0	0	0	0
	ethylhexyl					103	47	26	7	4	2	0	0	0
	isooctyl	x				104	48	26	3	5	3	0	0	0
	isopropyl					11	0	0	0	0	0	0	0	0
	methyl					11	0	1	2	2	2	1	0	0
	octyl					38	10	3	3	2	1	1	0	0
2,4-D	methyl	x	x		2,4-D (sum of 2,4-D and its esters expr. as 2,4-D)	0	0	0	0	0	0	0	0	
2,4-DP	methyl	x			Dichlorprop, incl. Dichlorprop-p	0	0	0	0	0	0	0	0	
Carfentrazone	ethyl	x	x		Carfentrazone-ethyl (determined as carfentrazone and expr. as carfentrazone-ethyl)	0	0	0	0	0	0	0	0	
Chlorthal	dimethyl		x		Chlorthal-dimethyl	100	61	76	83	89	78	57	24	
Cinidon	ethyl		x		Cinidon-ethyl (sum of cinidon ethyl and its E-isomer)	10	0	0	2	1	0	0	0	
Clodinafop	propargyl	x			Clodinafop and its S-isomers, expr. as clodinafop (F)	2	0	0	0	0	0	0	0	
Cyhalofop	butyl	x	x		Cyhalofop-butyl (sum of cyhalofop butyl and its free acids)	78	2	0	0	0	0	0	0	
Dichlorprop	2-ethylhexyl	x			Dichlorprop, incl. Dichlorprop-p	70	58	41	8	3	1	1	0	
Diclofop	methyl	x	x		Diclofop (sum diclofop-methyl and diclofop acid expr. as diclofop-methyl)	60	1	0	1	1	1	0	0	
Diethatyl	ethyl					8	0	0	12	2	0	0	0	
Dinoseb	acetate	x			Dinoseb	8	0	0	0	0	0	0	0	
Fenoxaprop	P-ethyl					37	0	0	1	0	0	0	0	
Flamprop	isopropyl					103	33	20	97	92	76	53	15	
Fluazifop	butyl					77	42	1	1	0	0	0	0	
Flumiclorac	pentyl					13	0	0	0	0	0	0	0	
Haloxifop	ethoxyethyl	x	x	x	Haloxifop incl. haloxifop-R (Haloxifop-R methyl ester, haloxifop-R and conjugates of haloxifop-R expr. as haloxifop-R) (F) (R)	3	0	0	0	0	0	0	0	
	methyl					6	0	0	1	1	1	0	0	
Ioxynil	octanoat	(x)	x		Ioxynil, incl. its esters expr. as Ioxynil (F)	70	16	0	3	2	1	0	0	
MCPA	thioethyl	x	x	x	MCPA and MCPB (MCPA, MCPB incl. their salts, esters and conjugates expr. as MCPA) (F) (R)	3	0	0	0	0	0	0	0	
Mecoprop	methyl				Mecoprop (sum of mecoprop-p and mecoprop expressed as mecoprop)	0	0	0	0	0	0	0	0	
	1-octyl	x				103	85	54	8	5	3	2	1	
	2,4,4-trimethylpentyl					104	83	44	7	4	3	0	0	
Mefenpyr	diethyl				2	0	0	0	0	0	0	0		
Nitrothal	di-isopropyl				19	0	0	2	1	0	0	0		
Trinexapac	ethyl	x			Trinexapac	6	0	0	82	84	29	10	0	

AH = alkaline hydrolysis, pH 12, re-neutralized with H₂SO₄

EH = incubation of buffered sample with esterase from porcine liver (buffering at pH 6-9 with phosphate buffer)

remaining esters < 5%
 remaining esters 5-20%
 remaining esters >20%

ANALYSIS

Current Approach: Alkaline Hydrolysis (AH)

Where RDs include esters and/or conjugates analysis typically involves alkaline hydrolysis (AH) to release acids.

A few years ago, the EURL-SRM distributed a method where AH is directly performed at room temperature prior to QuEChERS in a simple procedure. Following neutralization, sample preparation continues exactly as described in QuEChERS with dispersive SPE, using PSA, being skipped to avoid losses. AH releases conjugated residues but does not quantitatively hydrolyse esters composed of sterically hindered alcohol and/or acid groups even at extended hydrolysis times and elevated temperatures. This raises the need to still analyze remaining un-hydrolysed esters even after AH.

RESULTS

Esterase hydrolysis (EH) was shown to effectively hydrolyse even sterically highly hindered esters that are insufficiently hydrolysed by AH. Only in one case (trinexapac ethyl) AH was superior to EH. EH and AH seem not to release the same types of conjugated residues (e.g. observed in cereals with incurred residues) suggesting the need for a combined procedure depending on the RD. EH was shown to work best at pH between 6-10. At pH 5 or less enzyme activity was compromised.

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