ANNEX B

Florasulam

B.4 Methods of analysis

B.4.1 Analytical methods for formulation analysis

B.4.1.1 Analytical methods for the determination of pure active substance in the active substance as manufactured (Annex IIA 4.1.1)

Analytical method EU-AM-97-001 : DE-570 Herbicidal chemical (Knowles, 1997a)
Calculation of the limits of detection of DE-570 impurities for validated methods EU-AM-97-002 and EU-AM-008: Supplemental information for European Union registration (Madsen, 1999)

GLP :

Yes

Principle of the method :

DE-570 technical (≈ 0.1 g) is dissolved in 10 mL internal standard solution (dimethylphthalate in acetonitrile), after which the solution is further diluted to 100 mL with acetonitrile.
DE-570 content is determined by HPLC (Magellen C-18; 5 µm; gradient elution) using UV detection at 260 nm. Quantification by internal standard method.

Findings :

Specificity - interferences : According to the representative chromatograms of solvent blank, DE-570 technical and analytical standard, the method is suitable to determine DE-570 in DE-570 technical; no interferences are observed.

Linearity : the response of the HPLC/UV system (= peak area) to DE-570 and IS was found to be linear

1) for DE-570 : $R^2 = 0.9976$; $y = 4.10^7 x + 121751$ (n = 7; range from 0.0432 to 0.260 g/100 mL)
2) for IS : $R^2 = 0.9977$; $y = 8.10^6 x + 98857$ (n = 7; range from 0.170 to 1.01 g/100 mL)

Accuracy : determined by analysis of a series of synthetic mixtures, covering a technical concentration range from 99.7 to 89.4%
mean recovery = 99.4% (n = 5)

Repeatability : determined by analysing the same sample 10 times over 2 days on each system
1) instrument 1 : RSD = 0.360%
2) instrument 2 : RSD = 0.324%
Instruments 1 and 2 have differences in the dead volume of the system

Conclusions :

The HPLC-method is suitable for the determination of DE-570 in DE-570 technical.
No CIPAC method exists for this active substance.

B.4.1.2 Analytical methods for the determination of significant and/or relevant impurities and additives in the active substance as manufactured (Annex IIA 4.1.2)

Analytical Method EU-AM-97-002 : The determination of a range of impurities in DE-570 technical (Knowles, 1997b)

GLP :

No GLP-compliance stated.

Principle of the method :

DE-570 technical is dissolved in acetonitrile, after which the impurities are determined by HPLC (Magellen C-18; 5 µm; gradient elution) with UV detection at 260 nm. Quantification by external standardization.

Findings :

Specificity - interferences : According to the representative chromatograms of solvent blank, DE-570 technical and impurity analytical standards mixture, the HPLC-method is able to separate impurities 1, 2, 3, 4, 5 and 8 from one another and from the a.s. No interferences were observed.

Linearity : The response of the HPLC-UV system (= peak area) to each of the impurities was found to be linear.

Accuracy : Determined by analysis of a series of 5 laboratory prepared mixtures

Repeatability : Determined by 5 analyses of the same batch of technical DE-570 on each system (instruments 1 and 2 have differences in the dead volume of the system).

Data : see table B.4.1.2-1

Table B.4.1.2-1 : Validation of method EU-AM-97-002 (Knowles, 1997b)

Impurity	Repeatability (% RSD) *instrument 1 instrument 2*	Mean Recovery (%)	Linearity (n=8)	LOD (%)
		Concentration range (mg/mL)	Concentration range (g/100 mL)	
1	1.18	102	$y = 9.10^7 x - 4909.8$ $R^2 = 0.9999$	0.0224
	0.65	0.000574 - 0.0230	0.000144 - 0.0115	
2	1.04	102	$y = 2.10^7 x + 293.95$ $R^2 = 1$	0.121
	0.80	0.00114 - 0.0456	0.000285 - 0.0228	
3	2.15	100	$y = 1.10^8 x - 140.82$ $R^2 = 1$	0.00966
	3.80	0.000536 - 0.0214	0.000134 - 0.0107	
4	1.61	99	$y = 1.10^8 x - 254.35$ $R^2 = 1$	0.00915
	1.75	0.000545 - 0.0218	0.000136 - 0.0109	
5	0.87	99	$y = 1.10^8 x - 238.64$ $R^2 = 1$	0.00750
	0.45	0.000545 - 0.0218	0.000136 - 0.0109	
8	2.49	102	$y = 9.10^7 x - 36.179$ $R^2 = 1$	0.00426
	9.10	0.000482 - 0.0193	0.000120 - 0.0096	

Conclusions :
The HPLC-method is suitable for determination of the structurally related impurities 1-5 and 8 in DE-570 technical.

Analytical Method EU-AM-97-008 : Determination of process solvents in technical grade DE-570 (Knowles, 1997c)
Calculation of the limits of detection of DE-570 impurities for validated methods EU-AM-97-002 and EU-AM-008: Supplemental information for European Union registration (Madsen, 1999)

GLP :
Yes

Principle of the method :
DE-570 technical is dissolved in ethyl acetate, after which the process solvents are determined by GC (HP Plot Q; 30m x 0.32mm i.d.; 0.20µm film divinyl benzene with HP deactivated retention gap; 1m x 0.53mm i.d.) with FID. Quantification by external standardization.

Findings :
Specificity - interferences : According to the representative chromatograms of solvent blank, DE-570 technical, spiked DE-570 technical and process solvent analytical standards, the GC-method is able to separate both solvents (= impurity 6 and 9) from one another. No interferences were observed.
Linearity : The response of the GC-FID system (= peak area) to each solvent was found to be linear.
Accuracy : Determined by analysis of a series of 3 laboratory prepared mixtures (impurity levels ranging between 0.04 and 0.5% w/w)
Repeatability : Determined by 5 analyses of the same sample on the same day

Data : see table B.4.1.2-2

Conclusions :

The GC-method is suitable for determination of the process solvents in DE-570 technical.

Table B.4.1.2-2 : Validation of method EU-AM-97-008 (Knowles, 1997c)

Impurity	Repeatability (% RSD)	Mean Recovery (%)	Linearity (n=8)	LOD (%)
		Concentration range (g/100 mL)	Concentration range (g/100 mL)	
6	0.69	97	$y = 511309\ x + 102.66$ $R^2 = 0.9939$	0.0116
		0.0394 - 0.494	0.00165 - 0.00990	
9	0.83	101	$y = 1.10^6\ x + 222.16$ $R^2 = 0.9944$	0.0292
		0.0370 - 0.465	0.00175 - 0.0106	

B.4.1.3 Analytical methods for the determination of pure active substance in plant protection products (Annex IIIA 5.1.1)

- Analytical Method EU-AM-96-005 : XDE-570 SC Herbicide (Boothroyd, 1997)

GLP :

No GLP-compliance stated.

Principle of the method :

The formulation sample (ca. 0.5 g) is dispersed by swirling with 10 mL water, after which 5 mL internal standard solution (dipropylphthalate in acetonitrile) and 85 mL acetonitrile is added. The mixture is sonicated to dissolve the active substance and subsequently centrifuged to obtain a clear supernatant for analysis.

DE-570 is determined by HPLC (Zorbax SB Phenyl; 5 µm; isocratic elution) with UV detection at 260 nm; quantification by internal standard method.

Findings :

Specificity - interferences : According to representative chromatograms of DE-570 standard solution and SC formulation, the method is suitable to determine DE-570. Relative retention times of a.s. and IS show adequate separation; no interferences were observed.
A formulation blank was however not examined, so that it cannot be unequivocally established that the co-formulants exhibit no interferences at the retention time of DE-570.

Linearity : the response of the HPLC/UV system (= peak area) to DE-570 and IS was found to be linear
1) for DE-570 : $R^2 = 0.9999$; range from 0.010 to 0.060 g/100 mL
2) for IS : $R^2 = 0.9998$; range from 0.062 to 0.375 g/100 mL

Accuracy : determined by analysis of a series of 7 synthetic mixtures (a.s. content ranging between 4.13 and 5.58% w/w)
mean recovery = 98.8% (RSD = 0.6%)

Repeatability : determined by analyzing 2 samples five times on 2 different chromatographic systems
1) instrument 1 : RSD = 0.24% - 0.98%
2) instrument 2 : RSD = 0.11% - 0.27%

Conclusions :

The HPLC-method appears suitable for determination of the DE-570 content in SC-formulations (50 g/L), but specificity/interferences could have been demonstrated in a more conclusive manner.

No CIPAC method is available for the a.s. in the preparation.

B.4.1.4 Analytical methods for the determination of relevant impurities, additives and formulants in plant protection products (Annex IIIA 5.1.2)

No methods required since none of the impurities and formulants are considered to be of toxicological, ecotoxicological or environmental concern**.**

B.4.2 Analytical methods (residue) for food and feed (Annex IIA 4.2.1; Annex IIIA 5.2.1)

B.4.2.1 Analytical methods (residue) for target crops

A HPLC-UV method involving derivatisation of DE-570 was submitted.

- ERC 95.6 : Determination of XDE-570 residues in wheat and barley (Butcher et al., 1996a)
- Independent laboratory confirmation of an analytical method for the determination of residues of XDE-570 in cereal whole plant, grain and straw (Rawle, 1996)

in : Independent validation of DOWELANCO analytical method ERC 95.6 for the determination of XDE-570 residues in wheat and barley (Butcher, 1996)

GLP :

GLP-compliance stated for the independent laboratory validation study (Rawle, 1996).

Principle of the method :

Crop samples are extracted with aqueous acetic acid/acetone (20:1:80 v/v). After addition of water, 1M sulphuric acid and sodium chloride to an aliquot of the extract, XDE-570 is partitioned into methyl-tertiary-butyl ether (MTBE). The ether is partitioned with aqueous sodium bicarbonate solution, which is then acidified and XDE-570 partitioned back into MTBE. The ether is evaporated to dryness.

For *grain samples*, the residuum is reconstituted in MTBE. The extract is purified using an aminopropyl solid phase extraction cartridge eluting XDE-570 with 97:3 (v/v) MTBE/formic acid, after which the eluate is evaporated to dryness. The residuum is purified by extractive methylation, which utilises the formation of an ion pair with tetrabutyl ammonium hydroxide followed by methylation with methyl iodide and extraction into toluene. The toluene phase is then further purified using a silica solid phase extraction cartridge, eluting XDE-570 methyl with 97:3 (v/v) toluene/acetone.

For *straw and whole plant*, the residuum is reconstituted in ethyl acetate (EtOAc). The extract is purified using a silica solid phase extraction cartridge eluting XDE-570 with 50:50 (v/v) EtOAc/hexane. The eluate is evaporated to dryness and extractive methylation as above is carried out. The toluene phase is then further purified using a silica solid phase extraction cartridge, eluting XDE-570 methyl with 5:95 (v/v) EtOAc/toluene.

For all three substrates, the eluate is evaporated to dryness and the residuum reconstituted in 50:50 (v/v) acetonitrile/water. The XDE-570 methyl derivative is quantified by HPLC (Kromasil 100 C18; 5µm) using UV detection at 260 nm; quantification by external standardization.

Findings :

Specificity - interferences : According to the representative chromatograms of standard solutions, controls and spiked samples, the method is able to determine parent XDE-570 as its methyl derivative.
Untreated control samples exhibit no significant interfering peaks at the retention time of XDE-570 methyl (control values : 0.0000 mg/kg).

Linearity : The response of the HPLC-UV system (= peak height) to XDE-570 methyl was stated to be linear over a concentration range from 0.025 to 1.00 mg/L (XDE-570 equivalents).

Recovery - precision : see Table B.4.2.1-1

Validation by an independent laboratory : the method was independently validated in the laboratories of CEMAS, without any significant changes to the procedure.

Limit of determination (LOQ) : 0.01 mg/kg for grain
0.05 mg/kg for straw and whole plant

Conclusions :

The method is suitable for XDE-570 residue analysis in wheat and barley fractions with a LOQ of 0.05 mg/kg for straw and whole plant and a LOQ of 0.01 mg/kg for grain.

Table B.4.2.1-1 : Validation of method ERC 95.6

Matrix	Analyte	Fortification level (mg/kg commodity)	Recovery			
			Number of samples	Range (%)	Mean (%)	RSD (%)
Validation by DOW (Butcher et al., 1996a)						
grain	XDE-570 (as methyl derivative)	0.01 0.02 0.05 0.10	8 4 4 4	87 - 112 74 - 99 89 - 105 89 - 97	98 91 97 93	8.5 12.6 8.4 4.1
		0.01 - 0.10	20	74 - 112	95	8.6
straw		0.05 0.20 0.50 1.00	8 4 4 4	92 - 104 92 - 98 94 - 99 91 - 96	95 95 96 94	4.5 2.9 2.3 2.2
		0.05 - 1.00	20	91 - 104	95	3.3
whole plant		0.05 0.20 0.50 1.00	8 4 4 4	85 - 93 82 - 90 87 - 96 84 - 91	89 87 91 88	3.0 4.1 4.1 4.0
		0.05 - 1.00	20	82 - 96	89	3.7
Validation by CEMAS (Rawle, 1996 in : Butcher, 1996)						
grain	XDE-570 (as methyl derivative)	0.01 0.02 0.05 0.10	8 4 4 4	69 - 86 75 - 86 82 - 86 80 - 94	79 82 84 87	6.7 6.4 2.2 6.7
		0.01 - 0.10	20	69 - 94	82	6.7
straw		0.05 0.20 0.50 1.00	8 4 4 4	76 - 91 82 - 90 90 - 93 89 - 93	82 87 92 90	7.4 4.1 1.6 2.1
		0.05 - 1.00	20	76 - 93	87	6.8
whole plant		0.05 0.20 0.50 1.00	8 4 4 4	76 - 91 81 - 90 91 - 98 85 - 94	83 86 94 89	5.2 4.7 3.3 5.3
		0.05 - 1.00	20	76 - 98	87	6.7

Examination of the applicability of DFG Method S19 for the determination of XDE-570 : analytical preliminaries (Hastings and Schmidt, 1997)

GLP :

GLP-compliance stated

Materials and methods :

As DFG Method S19 uses GC to quantify any pesticide residues found, it was first of all investigated whether standard XDE-570 solutions could be quantified using GC with either Electron Capture Detection (ECD), Nitrogen Phosphorous Detection (NPD) or Mass Selective Detection (MSD, scan mode).

Findings :

1) MSD, capillary column HP-5 MS :
   no peak originating from XDE-570 was found for a solution of 4 µg/mL
2) NPD, capillary column DB-1 :
   several peaks were found for a solution of 4 µg/mL; the 0.4µg/mL solution showed no congruence
3) ECD, capillary column DB-1 :
   several peaks were found; solutions of 4 µg/mL and 0.4 µg/mL showed congruence; relative peak heights depending on injector temperature
4) ECD, capillary column DB-1701 :
   only one peak was found; solutions of 4 µg/mL and 0.4 µg/mL showed congruence

The results of the experiments indicate that XDE-570 behaves unpredictably during GC-analysis and is therefore unsuitable for routine analysis by GC.

Further investigations into other aspects of DFG S19 applicability (extractability, liquid-liquid partitioning and gel permeation chromatography clean-up) were consequently not carried out.

Conclusions :

Multi residue DFG Method S19 is not suitable for routine analysis of XDE-570 residues.

B.4.2.2 Analytical methods (residue) for food of animal origin

No methods were submitted for foodstuffs of animal origin

Residue levels in edible tissues are predicted to be very low and not present at levels which would be appropriate for monitoring.

B.4.3 Analytical methods (residue) in soil, water, air (Annex IIA 4.2.2 to 4.2.4; Annex IIIA 5.2.2 to 5.2.4)

B.4.3.1 Analytical methods for soil (Annex IIA 4.2.2; Annex IIIA 5.2.2)

Several methods were presented :

· **for determination of extractable residue (organic solvent extraction) :**
- 2 ESP LC-MS/MS methods, determining DE-570 and 5-OH DE-570 : ERC 95.1 and ERC 96.21 (the latter being a revision of ERC 95.1, incorporating additional clean-up steps)
- 1 ESP LC-MS method, determining DE-570 and 5-OH DE-570 : ERC 96.23
- 1 GC-MSD method involving derivatisation, determining only DE-570 : ERC 97.07

· **for determination of bioavailable residue (aqueous extraction) :**
- 1 ESP LC-MS/MS method, determining DE-570 and 5-OH DE-570 : ERC 95.2
- 1 immunoassay method, determining DE-570 : ERC 97.04

Extractable residue

- ERC 95.1 : Determination of residues of XDE-570 and 5-hydroxy XDE-570 in soil using organic extraction (Ghosh et al., 1996)

GLP :
No GLP-compliance stated.

Principle of the method :
Soil samples are extracted twice by shaking with acetone/1% aqueous acetic acid (9:1 v/v), after which the combined extracts are evaporated to a small volume. Following the addition of aqueous sodium hydrogen carbonate, the analytes are purified from the co-extractive material using a strong anion exchange (SAX) Bond Elut solid phase extraction column, followed by partitioning into ethyl acetate. Aqueous acetic acid is added and the organic phase is evaporated, after which the remaining aqueous solution is made to volume in further aqueous acetic acid.
Residues of XDE-570 and 5-hydroxy XDE-570 are quantified by ESP LC-MS/MS (Spherisorb ODS B; 5 µm) with multiple reaction monitoring (m/z 360>129 for XDE-570 and m/z 346>129 for 5-hydroxy XDE-570). Quantification by external standardization.

Findings :

Specificity - interferences : According to the representative chromatograms of standard solutions, controls and fortified samples, the method is suitable to determine XDE-570 and its metabolite 5-hydroxy XDE-570.
Untreated control samples exhibit no interfering peaks at the retention time of XDE-570 or 5-hydroxy XDE-570 (control values : 0.0000 µg/kg).

Linearity : The MS response (= peak area) to XDE-570 and 5-hydroxy XDE-570 was demonstrated to be linear for each compound over a concentration range from 0.50 to 30 ng/mL.

Recovery - precision : see Table B.4.3.1-1

Limit of determination (LOQ): 0.05 µg/kg for both compounds

Conclusions :
The method is suitable for determination of XDE-570 and metabolite 5-hydroxy XDE-570 residues in soil with a LOQ of 0.05 µg/kg.
However, the method involves LC/MS-MS, which is currently not considered to be commonly available.

Table B.4.3.1-1 : Validation of method ERC 95.1 (Ghosh et al., 1996)

Matrix	Analyte	Fortification level (µg/kg commodity)	Recovery			
			Number of samples	Range (%)	Mean (%)	RSD (%)
soil	XDE-570	0.05	8	80 - 102	87	7.8
		0.2	2	70 - 95	83	-
		0.5	2	99 - 101	100	-
		1.0	2	70 - 72	71	-
		5.0	2	79 - 88	84	-
		20.0	2	71 - 86	79	-
		50.0	2	71 - 73	72	-
		0.05 - 50.0	20	70 - 102	84	12.4
	5-hydroxy XDE-570	0.05	8	70 - 102	81	15.8
		0.2	2	86 - 92	89	-
		0.5	2	99 - 101	100	-
		1.0	2	84 - 92	88	-
		5.0	2	102 - 103	103	-
		20.0	2	101 - 119	110	-
		50.0	2	82 - 102	92	-
		0.05 - 50.0	20	70 - 119	91	14.9

- ERC 96.21 : Determination of residues of XDE-570 and 5-hydroxy XDE-570 in soil using organic extraction (Butcher et al., 1997)

GLP :

No GLP-compliance stated.

Principle of the method :

Soil samples are extracted twice by shaking with acetone/1% aqueous acetic acid (9:1 v/v), after which the combined extracts are evaporated to a small volume. Following the addition of water, the analytes are first purified from the co-extractive material by a non-polar (C18) solid phase extraction column. Further purification is achieved using a strong anion exchange (SAX) Bond Elut solid phase extraction column, followed by partitioning into ethyl acetate. Aqueous acetic acid is added and the organic phase is evaporated, after which the remaining aqueous solution is made to volume in further aqueous acetic acid.

Residues of XDE-570 and 5-hydroxy XDE-570 are quantified by ESP LC-MS/MS as in method ERC 95.1

Findings :

Specificity - interferences : According to the representative chromatograms of standard solutions, controls and fortified samples, the method is suitable to determine XDE-570 and its metabolite 5-hydroxy XDE-570.

Untreated control samples exhibit no interfering peaks at the retention time of XDE-570 or 5-hydroxy XDE-570 (control values : 0.0000 µg/kg).

Linearity : The MS response (= peak area) to XDE-570 and 5-hydroxy XDE-570 was demonstrated to be linear for each compound over a concentration range from 0.30 to 30 ng/mL.

Recovery - precision : see Table B.4.3.1-2

Limit of determination (LOQ): 0.05 µg/kg for both compounds

Conclusions :

The method is suitable for determination of XDE-570 and metabolite 5-hydroxy XDE-570 residues in soil with a LOQ of 0.05 µg/kg.

However, the method involves LC/MS-MS, which is currently not considered to be commonly available.

Table B.4.3.1-2 : Validation of method ERC 96.21 (Butcher et al., 1997)

Matrix	Analyte	Fortification level (µg/kg commodity)	Recovery			
			Number of samples	Range (%)	Mean (%)	RSD (%)
soil	XDE-570	0.05 0.5 5.0 50.0	8 4 4 4	83 - 104 79 - 96 89 - 97 87 - 97	92 89 93 94	7.8 8.9 3.6 4.8
		0.05 - 50.0	20	79 - 104	92	6.5
	5-hydroxy XDE-570	0.05 0.5 5.0 50.0	8 4 4 4	72 - 110 78 - 91 89 - 94 92 - 96	92 85 93 95	15.5 6.4 2.6 2.0
		0.05 - 50.0	20	72 - 110	91	10.6

- ERC 96.23 : Determination of the residues of XDE-570 and its 5-hydroxy metabolite in soil (Butcher et al., 1996b)

GLP :

No GLP-compliance stated.

Principle of the method :

Soil samples are extracted twice by shaking with acetone/1% aqueous acetic acid (9:1 v/v), after which the combined extracts are evaporated to a 10-15 mL volume. Following the addition of 1M sulphuric acid and sodium chloride, the analytes are partitioned into MTBE/acetonitrile (9:1 v/v). The organic phase is partitioned with aqueous sodium bicarbonate solution, which is then acidified and the analytes partitioned back into MTBE/acetonitrile (9:1 v/v). This organic phase is then further purified using an aminopropyl solid phase extraction cartridge eluting with MTBE/acetonitrile/formic acid (72:25:3 v/v/v). The eluate is evaporated to dryness and the residuum reconstituted in toluene/1% formic acid in acetonitrile (10:1 v/v). This solution is applied to a silica solid phase extraction cartridge and the eluate which contains XDE-570 is collected. 5-hydroxy XDE-570 is retained on the cartridge and eluted separately using toluene/2% formic acid in acetonitrile (70:30 v/v). The elution fractions are evaporated to dryness and each reconstituted in 1% aqueous acetic acid/acetonitrile (80:20 v/v).

Residues of XDE-570 and 5-hydroxy XDE-570 are quantified by ESP LC-MS (Kromasil 100 C8; 5 µm; gradient elution), monitoring $[M-H]^-$ ions at m/z 358 (XDE-570) and 344 (5-hydroxy XDE-570). Quantification by external standardization.

Findings :

Specificity - interferences : According to the representative chromatograms of standard solutions, controls and fortified samples, the method is suitable to determine XDE-570 and its metabolite 5-hydroxy XDE-570.

Untreated control samples exhibit no significant interfering peaks at the retention time of XDE-570 or 5-hydroxy XDE-570 (mean control values : 0.0045 µg/kg and 0.0039 µg/kg resp. (<30% of LOQ)).

Linearity : The MS response (= peak area) to XDE-570 and 5-hydroxy XDE-570 was demonstrated to be linear for each compound over a concentration range from 0.001 to 0.05 µg/mL.

Recovery - precision : see Table B.4.3.1-3

Limit of determination (LOQ): 0.05 µg/kg for both compounds

Conclusions :

The method is suitable for determination of XDE-570 and metabolite 5-hydroxy XDE-570 residues in soil with a LOQ of 0.05 µg/kg.

Table B.4.3.1-3 : Validation of method ERC 96.23 (Butcher et al., 1996b)

Matrix	Analyte	Fortification level (µg/kg commodity)	Recovery			
			Number of samples	Range (%)	Mean (%)	RSD (%)
soil	XDE-570	0.05 0.20 1.00 5.00	8 3 4 4	73 - 94 76 - 80 80 - 89 88 – 94	86 78 86 92	7.7 2.7 4.5 2.9
		0.05 - 5.00	19	73 – 94	86	7.3
	5-hydroxy XDE-570	0.05 0.20 1.00 5.00	8 4 4 4	71 - 100 75 - 92 71 - 77 73 - 76	82 82 75 75	10.7 9.0 3.5 1.9
		0.05 - 5.00	20	71 - 100	79	8.9

- ERC 97.07 : Determination of residues of DE-570 in soil (Gambie and McLaughlin, 1997)

GLP :

No GLP-compliance stated.

Principle of the method :

Soil samples are extracted twice by shaking with acetone/1% aqueous acetic acid (9:1 v/v), after which the combined extracts are evaporated to a 10-15 mL volume. Following the addition of water, the analyte is first purified from the co-extractive material by a non-polar (C18 PolarPlus) solid phase extraction column. Further purification is achieved using a strong anion exchange (SAX) Bond Elut solid phase extraction column, followed by partitioning into ethyl acetate. After evaporation of the organic phase, DE-570 is derivatised in 1 mL of acetone by adding triethylamine and triethyloxonium tetrafluoroborate (1 M in dichloromethane). The sample is evaporated and then partitioned twice into toluene from 5% sodium chloride solution in acid. The combined organic extracts are further purified using a Silica Isolute solid phase extraction column, after which the final eluate is evaporated and the residuum reconstituted in 250 µL of toluene solution containing 0.05 µg/mL of N-methyl DE-570 internal standard.

Residues are quantified by GC (HP-5MS (crosslinked 5% phenyl methyl siloxane); 0.25 µm) with mass selective detection (MSD), monitoring ions at 156 (N-ethyl DE-570) and 142 (N-methyl DE-570) amu. Quantification by internal standardization.

Findings :

Specificity - interferences : According to the representative chromatograms of standard solutions, controls and fortified samples, the method is suitable to determine DE-570 as its N-ethyl derivative. Untreated control samples exhibit no interfering peaks at the retention time of N-ethyl DE-570 (mean control value : 0.0008 µg/kg (< 30% of LOQ)).

Linearity : The MSD response to N-ethyl DE-570 (= peak area ratio 156 ion/142 ion) was demonstrated to be linear over a concentration range from 0.001 to 0.10 µg/mL (DE-570 equivalents).

Recovery - precision : see Table B.4.3.1-4

Limit of determination (LOQ): 0.05 µg/kg

Conclusions :

The method is suitable for determination of DE-570 residues in soil with a LOQ of 0.05 µg/kg.

Table B.4.3.1-4 : Validation of method ERC 97.07 (Gambie and McLaughlin, 1997)

Matrix	Analyte	Fortification level (µg/kg commodity)	Recovery			
			Number of samples	Range (%)	Mean (%)	RSD (%)
soil	DE-570 (as N-ethyl derivative)	0.05	8	75 - 107	87	11.5
		0.20	4	79 - 83	82	2.1
		1.00	4	75 - 79	77	2.4
		5.00	6	69 - 93	82	11.4
		0.05 - 5.00	22	69 - 107	83	10.0

Bioavailable residue

- ERC 95.2 : Determination of residues of XDE-570 and 5-hydroxy XDE-570 in soil using aqueous extraction (Ghosh et al., 1997**)**

GLP :

No GLP-compliance stated.

Principle of the method :

Soil samples are extracted twice by shaking with distilled water. The combined extracts are then acidified and the analytes are purified from the co-extractive material by partitioning into ethyl acetate twice. Aqueous acetic acid is added and the organic phase evaporated, after which the remaining aqueous solution is made to volume in further aqueous acetic acid.

Residues of XDE-570 and 5-hydroxy XDE-570 are quantified by ESP LC-MS/MS as in method ERC 95.1.

Findings :

Specificity - interferences : According to the representative chromatograms of standard solutions, controls and fortified samples, the method is suitable to determine XDE-570 and its metabolite 5-hydroxy XDE-570.

Untreated control samples exhibit no interfering peaks at the retention time of XDE-570 or 5-hydroxy XDE-570 (control values : 0.0000 µg/kg).

Linearity : The MS response (= peak area) to XDE-570 and 5-hydroxy XDE-570 was demonstrated to be linear for each compound over a concentration range from 0.30 to 30 ng/mL.

Recovery - precision : see Table B.4.3.1-5

Recovery tests were carried out by fortification of the combined initial aqueous extracts, instead of by fortification of the weighed soil samples.

Limit of determination (LOQ): 0.05 µg/kg for both compounds

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