

Section A4.2/01

Analytical Methods for Detection and Identification

Annex Point IIA, IV.4.2

a) Residues in Soil

Official
use only

- 1 REFERENCE**
- 1.1 Reference** Hellstern J, 2007, Development and Validation of a Residual Analytical Method for the Determination of 2-(n-Octyl)-isothiazol-3(2H)-one (OIT) in Soil, RCC Ltd, Itingen, Switzerland [REDACTED]
- 1.2 Data protection** Yes
- 1.2.1 Data owner Thor GmbH, Speyer, Germany
- 1.2.2 Companies with letter of access None
- 1.2.3 Criteria for data protection Data submitted on existing A.S. for the purpose of its entry into Annex I.

- 2 GUIDELINES AND QUALITY ASSURANCE**
- 2.1 Guideline study** European Commission, Guidance Document on Residue Analytical Methods, SANCO/825/00 rev. 7, March 17, 2004
- 2.2 GLP** Yes
- 2.3 Deviations** None

- 3 MATERIALS AND METHODS**
- 3.1 Preliminary treatment**
- 3.1.1 Extraction Soil samples are extracted three times using 30 mL ethyl acetate. The organic phases are concentrated under reduced pressure in a rotary evaporator at 30°C and the resulting sample re-dissolved in a volume of about 5 mL water/acetonitrile (1:1, v/v). X
- 3.1.2 Cleanup No further cleanup necessary
- 3.2 Detection** LC/MS
- 3.2.1 Separation method Pump: TSP P4000 (Thermo Separation Products)
Autosampler: TSP AS3000 (Thermo Separation Products)
Pre-column: 4 mm x 2 mm, Security guard C18
Column: 150 mm x 4.6 mm, 5 µm Phenomenex Luna C8
Mobile Phase: Solvent A: 5 mM ammonium acetate in water
Solvent B: acetonitrile

Time, min.	0	9	16	16.1	20
Solvent A, %	50	5	5	50	50
Solvent B, %	50	95	95	50	50

Flow: 0.5 mL/min

Sample: A volume of 100 µL was injected

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3.2.2	Detector	Instrument: Mass Spectrometer TSQ , Finnigan MAT Ionisation Mode: Atmospheric Pressure Chemical Ionisation (APCI) Detection Mode: Positive ion detection Scan Mode: SIM: m/z 214 (M+H+), width 0.6 Da	X																
3.2.3	Standard(s)	OIT determined by external standardisation with reference substance.																	
3.2.4	Interfering substance(s)	None																	
3.3	Linearity																		
3.3.1	Calibration range	0.05 – 1.28 µg/mL	X																
3.3.2	Number of measurements	7 concentration levels, mostly duplicate determination																	
3.3.3	Linearity	$r^2 = 0.9975$																	
3.4	Specificity: interfering substances	No interference observed at the retention time of OIT higher than 6% of the LOQ of 0.01 mg/kg.																	
3.5	Recovery rates at different levels	Samples of Speyer 2.2 topsoil (0-20 cm) were fortified with OIT. Due to degradation of OIT under non-sterile conditions, the soil was sterilised by autoclaving at 120°C for 30 min.																	
		<table border="1"> <thead> <tr> <th>Fortification level, mg/kg</th> <th>No. of analyses</th> <th>Mean recovery, %</th> <th>Relative standard deviation, %</th> </tr> </thead> <tbody> <tr> <td>0.01</td> <td>5</td> <td>97.9</td> <td>2.4</td> </tr> <tr> <td>0.10</td> <td>5</td> <td>84.3</td> <td>3.8</td> </tr> <tr> <td>Overall</td> <td>10</td> <td>91.1</td> <td>3.1</td> </tr> </tbody> </table>	Fortification level, mg/kg	No. of analyses	Mean recovery, %	Relative standard deviation, %	0.01	5	97.9	2.4	0.10	5	84.3	3.8	Overall	10	91.1	3.1	
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0.01	5	97.9	2.4																
0.10	5	84.3	3.8																
Overall	10	91.1	3.1																
3.5.1	Relative standard deviation	see table above																	
3.6	Limit of determination	0.01 mg/kg for OIT in sterilised soil, based on the lowest fortification level that yielded acceptable recovery and precision.																	
3.7	Precision																		
3.7.1	Repeatability	Relative standard deviations see point 3.5																	
3.7.2	Independent laboratory validation	Not required																	

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	4	APPLICANT'S SUMMARY AND CONCLUSION
4.1	Materials and methods	<p>A method was validated for the determination of OIT in soil. OIT is extracted from soil using ethyl acetate, and extracts are re-constituted in water/acetonitrile. Separation is carried out on HPLC, with mass-spectrometric detection. OIT is quantified by external standardisation.</p> <p>Recovery experiments in non-sterile soil were impossible due to the rapid degradation of OIT. Therefore, recovery was analysed in sterilised soil.</p>
4.2	Conclusion	<p>A fully validated LC/MS method is available for the determination of OIT in soil. Acceptable recovery, precision and the absence of interferences were demonstrated. The LOQ is 0.01 mg/kg in sterilised soil.</p>
4.2.1	Reliability	1 (reliable without restrictions)
4.2.2	Deficiencies	None

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Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	02/11/2009
Materials and methods	<p>Applicant's version acceptable with the following comments;</p> <p>Section 3.1.1 – Fortified soil samples were extracted</p> <p>Section 3.2.2 – only one ion has been used for monitoring and hence method is not regarded as highly specific.</p> <p>Section 3.3.1 – Calibration range, concentration equivalent in soil is 0.005 – 0.128 mg/kg.</p>
Conclusion	Adopt applicant's version. A confirmatory method will be required.
Reliability	2
Acceptability	acceptable
Remarks	<p>The active substance undergoes rapid degradation on soil so it was questioned whether the analysis should look at the degradation products. The degradation study however found no relevant degradation products present with >6% of the applied radioactivity. The fate evaluation has concluded that OIT is the relevant residue for monitoring in soil.</p> <p>There is no NOEC for soil, as chronic studies were not submitted. The EC50 (based on seedling growth) is 45.0 mg OIT/kg soil dry weight, resulting in a PNEC of 45 µg OIT/kg soil (assessment factor of 1000). Therefore, the LOQ demonstrated is acceptable.</p> <p>The method used LC/MS with only one ion monitored. Therefore, the method cannot be regarded as highly specific. A confirmatory method is required. This can be addressed post approval of the active but prior to the date of entry into force.</p>
	COMMENTS FROM ...
Date	<i>Give date of comments submitted</i>
Results and discussion	<i>Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion. Discuss if deviating from view of rapporteur member state</i>
Conclusion	<i>Discuss if deviating from view of rapporteur member state</i>
Reliability	<i>Discuss if deviating from view of rapporteur member state</i>
Acceptability	<i>Discuss if deviating from view of rapporteur member state</i>
Remarks	