Section A4.2/01 Analytical Methods for Detection and Identification

Annex Point IIA, IV.4.2 *c) Residues in Water*

		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 Surface Water, 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	Water samples are partitioned three times with 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 water/acetonitrile.	
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	0.002 – 0.046 µg/mL	X
3.3.2	Number of measurements	10 concentration levels, mostly duplicate determination	
3.3.3	Linearity	$r^2 = 0.9995$	
3.4	**Specifity: interfering substances**	No interference observed at the retention time of OIT higher than 5% of the LOQ.	
3.5	**Recovery rates at different levels**	Surface water from a natural river in Switzerland was fortified with OIT	

Fortification level, µg/L	No. of analyses	Mean recovery, %	Relative standard deviation, %
0.1	5	89.5	8.3
1.0	5	100.4	4.6
Overall	10	94.9	6.4

3.5.1	Relative standard deviation	see table above	
3.6	**Limit of determination**	0.1 µg/L for OIT in surface water, 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	

4 APPLICANT'S SUMMARY AND CONCLUSION

4.1 Materials and methods	A method was validated for the determination of OIT in surface water. OIT is extracted from water samples with ethyl acetate, and reconstituted in water/acetonitrile. Separation is achieved with HPLC and mass-spectrometric detection. OIT is quantified by external standardisation.
4.2 Conclusion	A fully validated LC/MS method is available for the determination of OIT in surface water. Acceptable recovery, precision and the absence of interferences were demonstrated. The LOQ is 0.1 µg/L in surface water.
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	Accept applicant's version, except that: 3.2.2 Detector: Single ion was monitored and hence method is not regarded as highly specific. 3.3.1: Calibration range 100 ml samples of water were taken for analysis. following extraction the sample was reduced to dryness and then re-dissolved in water/acetonitrile ranging from 1 – 4 ml, the volume used being appropriate to ensure the concentration was within the linear range demonstrated: • Re-dissolving in 1 ml covers a linear range of 0.02 µg/L – 0.46 µg/L • Re-dissolving in 4 ml covers a linear range of 0.08 µg/L – 1.84 µg/L
Conclusion	*Adopt applicant´s version*
Reliability	*2*
Acceptability	acceptable
Remarks	The Fate evaluation has proposed that OIT only is the relevant residue for monitoring in water. The LOQ of 0.1 µg/L complies with the EU drinking water directive and also supports the NOEC for surface water which is 0.38 µg/L. The validation data generated for surface water covers drinking and ground water. Only a single ion was monitored. Therefore, the method cannot be regarded as highly specific. A confirmatory method is required with an LOQ of 0.005 µg/l (based on the agreed $\text{PNEC}_{\text{freshwater}}$ of 0.0071 µg/l).
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Results and discussion	*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*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	