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Pesticides — Determination of total cypermethrin content and diastero isomer ratio

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Contents

Page

Foreword ivv

1 Scope 68

2 Normative references 68

3 Terms and definitions 68

4 Outline of method 68

5 Reagents 68

6 Apparatus 79

7 Sampling 810

8 Procedure 810
8.1 Preparation of the sample solutions 810
8.2 HPLC operating conditions 810
8.3 Equilibration of the system 911
8.4 Analysis of sample 911
8.5 Calculation of results 911
8.5.1 Total cypermethrin content 911
8.5.2 Individual diasteroisomer content 1

9 Test report 2

Annex A (normative) Annex title 4
A.1 General 4

Foreword

Rwanda Standards are prepared by Technical Committees and approved by Rwanda Standards Board (RSB) Board of Directors in accordance with the procedures of RSB, in compliance with Annex 3 of the WTO/TBT agreement on the preparation, adoption and application of standards.

The main task of technical committees is to prepare national standards. Final Draft Rwanda Standards adopted by Technical committees are ratified by members of RSB Board of Directors for publication and gazettment as Rwanda Standards.

DRS 591 was prepared by Technical Committee RSB/TC 64, *Pesticides.*

In the preparation of this standard, reference was made to the following standard:

ES 72: Pesticides — Determination of total cypermethrin content and diastero isomer ratio

The assistance derived from the above source is hereby acknowledged with thanks.

Committee membership

The following organizations were represented on the Technical Committee on *Pesticides* (RSB/TC 64) in the preparation of this standard.

Rwanda Food and Drugs Authority

Rwanda Forensic Institute

University of Rwanda/College of Sciences and Technology

Standards of Sustainability

CYIRA Ltd

P-TECHNIKS Ltd

Rwanda Inspectorate, Competition and Consumer Protection Authority

Rwanda Investigation Bureau

RAIDO

Rwanda Standards Board (RSB) – Secretariat

Pesticides — Determination of total cypermethrin content and diastero isomer ratio

1 Scope

This Draft Rwanda Standard gives the method for the determination of total cypermethrin content and diastero isomer ratio in technical and technical concentrates of cypermethrin by the high performance liquid chromatographic (HPLC) method.

NOTE The test method given in DRS 590 for the determination of total cypermethrin gives results of greater precision.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

RS 405, *Pesticides — Sampling*

RS 406, *Pesticides — Terminology*

DRS 590, *Pesticides — Determination of total cypermethrin content*

3 Terms and definitions

For the purposes of this standard, the terms and definitions given in RS 406 apply.

4 Outline of method

The sample is dissolved in ethyl acetate/iso-octane containing methyl benzoate as internal standard. Separation is achieved using high performance liquid chromatography with ultra-violet detection. The peak areas of internal standard and each cypermethrin diasteroisomer (A, B, C, D) are measured by a data handling system. These areas are used to calculate the total cypermethrin content of the sample and individual cypermethrin diasteroisomer content based on peak area normalization.

5 Reagents

Unless otherwise specified, use the following reagents of recognized analytical grade.

5.1 2,2,4-Trimethylpentane (iso-octane) HPLC grade.

5.2 Ethyl acetate HPLC grade

5.3 Stock solution

Transfer 20 ml ethyl acetate into a 100 ml flask and make up to volume with iso-octane

5.4 Mobile phase

0.50% v/v ethyl acetate in iso-octane. Pipette 10 ml ethyl acetate into a 2 liters flask and make up to volume with iso-octane. Filter before use and allow to stabilize at room temperature.

5.5 Methyl benzoate

99% pure, internal standard

5.6 Methyl benzoate solution

Weigh accurately approximately 5 g of methyl benzoate into a 50 ml beaker. Transfer with 200 ml ethyl acetate into a 1 liter graduate flask. Make up to volume with is-octane. (Solution I).

5.7 Cypermethrin working standard

Known cypermethrin content (minimum 900 g/kg), with a ratio of cis to trans - isomer content similar to that of the sample being analyzed. Store the standard in a cool dry place, preferably in a desiccator. The isomers may crystallize out of the mixture at ambient temperature and the analytical standard must be homogenous before use.

5.8 Calibration solution

Homogenize the standard by warming the sealed bottle of cypermethrin standard (purity pg/kg) at between (40- 50)ºC until no crystals remain, and then shake the bottle. Weigh in duplicate (to the nearest 0.1 mg) approximately 0.2g of standard (MA and MB), in to suitable screw-capped vials. Add by pipette 10.0 ml of methylbenzoate internal standard solution and 5 ml stock solution (solutions CA and CB). Prepare a solution without internal standard by dissolving 0.2 g of cypermethrin standard in 3 ml of ethylacetate and diluting to 15.0 ml with iso-octane-shake for 20 minutes to effect solution (solution Co).

6 Apparatus

6.1 Liquid chramatograph

Fitted with an ultra-violet spectophotometric detector capable of operating at 278 nm, and pulse-free pump. The injection system should be capable of operating against a pressure of about 6MPa and an injection valve is recommended, e.g. Rheodyne 7125.

6.2 HPLC column.

An unused, 0.15 m X 4.6 mm i.d. stainless steel column packed with Spherisorb S5W. Alternatively, an unused, laboratory packed column may be used. Details of a suitable packing procedure, which involves the use of specialized equipment, are described in J. Chromatog. (1976) 122, 243-258.

6.3 Thermostatically controlled water bath

Maintained at 500C (± 2ºC) and with internal bath dimensions suitable for use with a 15 cm column. It is recommended that the water bath be fitted with a fail-safe device e.g. water level sensor cur-out or a suitable HPLC column oven.

6.4 Electronic integrator

Compatible with the liquid chromatograph.

6.5 Screw-capped vials.

6.6 Laboratory shaker

7 Sampling

Homogenize the bulk material by heating to about (40-50)0C before taking the sample. Take at least 25 g in accordance with ES 694 and rehomogenize before taking a sub-sample for analysis.

8 Procedure

8.1 Preparation of the sample solutions

8.1.1 Homogenize the material by the method given for the standard. Prepare a solution without internal standard by dissolving sufficient sample to contain approximately 0.2 g of cypermethrin in 3 ml of ethyl acetate and diluting to 15.0 ml with iso-octane (solution S0). Shake for 20 min to effect solution.

8.1.2 Weigh, in duplicate (to the nearest 0.1 mg), into suitable screw-capped vials sufficient sample (WA and WB, g) to contain approximately 0.2 g of cypermethrin. Add by pipette 10.0 ml of internal standard solution plus 5 ml of stock solution to bring the final volume to 15 ml. Shake for 20 min to effect solution (Solution SA and SB). If the solution is not clear after this time, transfer the solution to a suitable centrifuge tube and centrifuge at 3000 rpm until the solution is clear.

8.2 HPLC operating conditions

8.2.1 Column

Material	Stainless steel
Length X i.d.	0.15 m X 4.6 mm
Packing	Spherisorb S5W
Typical No of theoretical plates	3000 calculated using diastereoisomer peak 'D'

8.2.2 Detector system

Type	UV detector operated at 278 nm
Sensitivity	No special requirements
O.D. range	0.1 AUFS

8.2.3 Temperature

Column	$50^0C \pm 2^0C$
Mobile phase	

	Injection port	Ambient ± 2^{0}C up to 30^{0}C	
	Detector		
8.2.4	**Mobile phase**	0.5% v/v ethyl acetate in iso-octane, degassed before use. Pipette 10 ml ethyl acetate into a 2 litre flask and make up to volume with iso-octane. Flow rate 2.0 ml min-1. Constant flow is essential for repeatable results	
8.2.5	**Calibration**	Internal. Response factor peak area measurement	
8.2.6	**Retention times (typical**	Methyl benzoate	3.9 min
		(R)-∝, (1R)-cis + (S)-∝, (1R)-cis	8.5 min
		(S)-∝, (1R)-cis + (R)-∝, (1R)-cis	9.5 min
		(R)-∝, (1R)-trans + (S)-∝, (1R)-trans	11.6 min
		(S)-∝, (1R)-trans + (R)-∝, (1R)-trans	13.0 min (see Figure 1)

NOTE The column should be conditioned by recycling the mobile phase, from a 2 litre reservoir, overnight.

8.3 Equilibration of the system

Check for interfering components by injecting solutions I, C0, and S0, under the conditions described in clause 8.2. If any interfering peaks are present, use external calibration as the method of quantitation. Inject calibration solutions CA and CB to set the integrator parameters. The total cypermethrin methyl benzoate peak area ratio in solutions CA and CB should not differ by more than 2% of their weight adjusted mean (area ratio obtained divided by standard weight).

8.4 Analysis of sample

Carry out injection of 5 □l of calibration solution CA and CB and samples solutions SA and SB in the following sequence and record the integrated areas of the peaks.

Injection sequence shall be CA1, SA1 SA2, CB1, CA2, SB1, SB2, CB2 (Giving total cypermethrin to methyl benzoate peak area ratios of [RCA1, RSA1, RSA2, RCB1, RCA2, RSB1, RSB2, RCB2]

8.5 Calculation of results

8.5.1 Total cypermethrin content

For each sample injection calculate the cypermethrin content:

$$Cypermethrin\ content = \frac{RMP}{R1w}\ g/kg$$

Where;

R is total cypermethrin (A + B+ C + D) to methyl benzoate peak area ratio of the sample injection;

R^1 is mean total cypermethrin (A + B + C + D) to methyl benzoate peak area ration of the calibration solution injections which bracket the sample injection;

M is mass of cypermethrin standard in calibration solution (g);

w is mass of sample (g); and

P is purity of the cypermethrin standard (g/kg).

The mass of internal standard is common to both calibration and sample solutions and so does not enter into the calculation. Report the values obtained for total cypermethrin content for each injection of the sample solutions.

8.5.2 Individual diasteroisomer content

8.5.2.1 The relative ratio of diasteroisomers present in each sample can be calculated by peak area normalization, since the detector response to each diasteroisomer may be considered to be the same and the% relative ratio of diasteroisomer shall be calculated as follows:

$$e.g.\ 'A'in\ the\ sample = \frac{K}{K+L+M+N} X100$$

Where;

K is area of peak 'A' in sample solution

L is area of peak 'B' in sample solution

M is area of peak 'C' in sample solution; and

N is area of peak 'D' in sample solution.

8.5.2.2 Report the ratio of diastereoisomers for each sample injection. Peaks B and D contain the isomers predominantly responsible for the insecticidal activity.

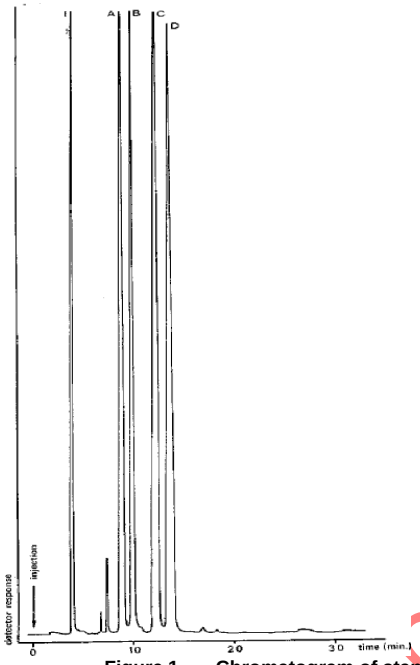

Figure 1 – Chromatogram of standard solution cypermethrin + internal standard (I)

(A) αR,1R-cis + αS, 1S- cis.

(B) αS, 1R - cis + αR, 1S - cis.

(C) αR, 1R - trans + αS, 1S - trans.

(D) αS, 1R - trans + αR, 1S - trans.

NOTE For the repeatability and reproducibility of the method, see clause 8.2.6.2 of CD xxx

9 Test report

The test report shall include the following information:

a) the result and method of expression used;

b) all information necessary for complete identification of sample;

c) a reference to this Ethiopian Standard;

d) any unusual feature noted during the determination;

e) any operation included in this Ethiopian Standard or in the Ethiopian standard to which reference is made or regarded as optional; and

f) the date of test

Annex A
(normative)

Annex title

A.1 General

Bibliography

[1] ISO/IEC Directives, Part 2, *Rules for the structure and drafting of International Standards*, 2016

DRS 591: 2024

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