SLOVENSKI STANDARD

SIST EN 15055:2006

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Nemastna ģivila - Doloļ evanje klormekvata in mepikvata - Metoda LC-MS/MS

Non fatty foods - Determination of chlormequat and mepiquat - LC-MS/MS method

Fettarme Lebensmittel - Bestimmung von Chlormequat und Mepiquat - LC-MS/MS-Verfahren

Aliments non gras - Détermination du chlorméquate et du mépiquate - Méthode CL-SM/SM

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Aliments non gras - Détermination de la teneur en chlormequate et mepiquate - Méthode LC-MS/MS

Fettarme Lebensmittel - Bestimmung von Chlormequat und Mepiquat - LC-MS/MS-Verfahren

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Foreword

This document (EN 15055:2006) has been prepared by Technical Committee CEN/TC 275 “Food analysis - Horizontal methods”, the secretariat of which is held by DIN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by November 2006, and conflicting national standards shall be withdrawn at the latest by November 2006.

As an alternative, there is also EN 15054 "Non fatty foods - Determination of chlormequat and mepiquat - LC-MS method" available.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland and United Kingdom.

1 Scope

This draft European Standard specifies a method using high performance liquid chromatography/tandem mass spectrometry (LC-MS/MS) for the determination of the growth regulators chlormequat and mepiquat in non-fatty foods as chlormequat and mepiquat cation, respectively.

The method is applicable to all kinds of fruits, vegetables and cereal products. It has been collaboratively studied on mushrooms, pears, wheat flour, fruit puree and, additionally, on infant formula, see [1].

2 Principle

The sample is mixed with deuterated internal standards, water and methanol and the homogenate is centrifuged. An aliquot portion of the supernatant is filtered. An aliquot portion of the filtrate is analysed by liquid chromatography with tandem mass spectrometric detection after positive electrospray ionisation. To achieve the required selectivity the mass spectrometer is operated in the multi reaction mode, see [2] to [7].

3 Reagents

3.1 General and safety aspects

Unless otherwise specified, use reagents of recognised analytical grade. Take every precaution to avoid possible contamination of water, solvents, inorganic salts, etc.

WARNING — The use of this standard may involve hazardous materials, operations and equipment. This standard does not purport to address all the safety problems associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and to determine the applicability of regulatory limitations prior to use.

3.2 Ammonium acetate

3.3 Ammonium formate

3.4 Filter aid, for example Celite® 545[1)]

3.5 Glacial acetic acid, mass fraction *w* at least 96 g/100 g

3.6 Acetonitrile, HPLC quality

3.7 Methanol, HPLC quality

3.8 Water, suitable for HPLC

3.9 Chlormequat chloride stock solution, mass concentration $\rho(C_5H_{13}NCl_2)$ = 4,30 µg/ml in methanol

This stock solution contains 3,33 µg/ml chlormequat cation.

1) Celite 545 is a product supplied by Celite Corp. (World Minerals Inc., Santa Barbara, CA, USA). This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN of the product named. Equivalent products may be used if they can be shown to lead to the same results.

3.10 Mepiquat chloride stock solution, $\rho(C_7H_{16}NCl)$ = 4,37 µg/ml in methanol

This stock solution contains 3,33 µg/ml mepiquat cation.

3.11 Internal standard solution 1 (d4-Chlormequat chloride), $\rho(C_5H_9D_4NCl_2)$ = 21,33 µg/ml in methanol

This stock solution contains 16,67 µg/ml d_4-chlormequat cation. For the availability of the standard, contact your national standardisation institute.

3.12 Internal standard solution 2 (d3-Mepiquat iodide), $\rho(C_7H_{13}D_3NI)$ = 34,70 µg/ml in methanol

This stock solution contains 16,67 µg/ml d_3-mepiquat cation. For the availability of the standard, contact your national standardisation institute.

3.13 Calibration solutions

Prepare six calibration solutions (no. 1 to no. 6) in 10 ml volumetric flasks as follows. Into the flasks, pipette 10 µl, 50 µl, 100 µl, 300 µl, 1 000 µl or 3 000 µl each of chlormequat chloride stock solution (3.9) and of mepiquat chloride stock solution (3.10). To all flasks add 10 µl of the internal standard solution 1 (3.11) and 10 µl of the internal standard solution 2 (3.12). Dilute the solutions no. 1 to no. 5 to the mark with a methanol/water mixture 1 + 1 (*V*+*V*) and solution no. 6 with water.

All six calibration solutions contain 16,7 ng/ml of d_4-chlormequat cation and 16,7 ng/ml of d_3-mepiquat cation. Solutions no. 1 to no. 6 contain 3,3 ng/ml, 16,7 ng/ml, 33,3 ng/ml, 100 ng/ml, 333 ng/ml and 1 000 ng/ml each of chlormequat cation and of mepiquat cation respectively. On the basis of a 20 g test portion, this corresponds to 0,01 mg/kg, 0,05 mg/kg, 0,10 mg/kg, 0,30 mg/kg, 1,00 mg/kg or 3,00 mg/kg each of chlormequat cation and mepiquat cation.

4 Apparatus

Usual laboratory apparatus and, in particular, the following:

4.1 Homogenizer or high speed blender, fitted with jar

4.2 Centrifuge, capable of producing a centrifugal acceleration of at least 3 000 *g* at the bases of the centrifuge tubes

4.3 Syringe, capacity of at least 2 ml

4.4 Syringe filter, pore size 0,45 µm (polyamide or polytetrafluoroethylene)

4.5 Glass vials, 1,8 ml volume; applicable for automatic injection into an autosampler

4.6 LC-MS/MS system, equipped with electrospray interface

5 Procedure

5.1 Preparation of the samples

Where possible, carry out the analysis of samples immediately upon their arrival in the laboratory. Do not analyse a laboratory sample which is wholly or extensively spoiled.

For analysis take only the portion of the laboratory sample to which the maximum residue level applies. No further plant-parts may be removed. A record of the plant parts which have been removed shall be kept. The sample thus prepared is the test sample.

If the test sample cannot be analysed immediately, store it at 0 °C to 5 °C for no longer than 3 days before analysis.

The reduction of the test sample shall be carried out in such a way that representative portions are obtained (e. g. by division into four and selection of opposite quadrants). When the samples are in small units (e. g. small fruits, legumes, cereals), the test sample shall be thoroughly mixed before weighing out the test portion. When the samples are in larger units, take wedge-shaped sections (e. g. large fruits and vegetables) or cross sections (e. g. cucumbers) which include the outer surface from each unit.

From each test sample, remove those parts which would interfere with the analytical procedure. In the case of stone fruits, the stones should be removed. Care shall be taken that as little as possible of the remainder such as juice or flesh is lost. The basis for the calculation of the residue mass fraction is the mass of the original test sample (with stones).

Chop the test sample and weigh out test portions of masses of 20 g to an accuracy of ± 1 %.

If samples have to be stored for more than 3 days, they should be deep-frozen at – 20 °C. To ensure that, even after thawing, representative samples can be taken, prepare portions of the product which are each sufficient for one analysis.

5.2 Extraction

Transfer a representative test portion of m_A = 20 g into the blender cup (4.1). For dry sample materials like cereal products, weigh a homogenised portion of 10 g (m_A) into the cup. Add 60 µl of internal standard solution 1 (3.11) and 60 µl of internal standard solution 2 (3.12). Add sufficient water, that a total volume (added and natural) of 20 ml water is obtained. In the case of dry sample materials wait 10 min after addition of water. Add 40 ml of methanol (3.7) and blend for 2 min. The total volume of liquid extract (taking into account the natural water content of the sample) is 60 ml. Centrifuge an aliquot at approximately 3 000 g (4.2), transfer approximately 2 ml of the supernatant into the syringe (4.3) and filter the solution through the syringe filter (4.4) into a 1,8 ml glass vial (4.5). If the filter is likely to be blocked by suspended matter, place a 1 cm layer of filter aid (3.4) onto the bottom of the syringe before filling it with the extract.

5.3 Determination

Inject equal volumes of the sample test solution derived from 5.2 and of the calibration solutions (3.13) into the LC-MS/MS system. The LC-MS/MS system shall be operated in the multi reaction mode with transitions selective for chlormequat, mepiquat and the internal standards (see Annex A).

Make sure that the liquid chromatographic conditions (column length, stationary phase type, injection volume, column temperature, electrospray interface parameters, etc.) are such that the separation of chlormequat and mepiquat from possible interfering peaks originating from the samples is as complete as possible.

Typical LC-MS/MS conditions are given in Annex A.

5.4 Test for interference and recovery

Prepare reagent blanks and carry out spiked recovery tests at levels appropriate to the maximum residue level. The chromatogram of the reagent blank should not show any significant peak at the retention time of chlormequat or mepiquat.

6 Evaluation of results

To identify residues of chlormequat or mepiquat compare the retention times obtained from the sample test solution with those obtained from the calibration solutions. Positive findings are confirmed by comparing the peak intensity ratios of the first and second compound specific transition (e. g. transition 122 → 58 and 124 → 58 for chlormequat) with the expected peak intensity ratios from the standards. If the peak ratio of a residue peak differs more than 30 % from the expected response ratio, additional measures are necessary, e.g. the use of other LC column, another eluent or an additional transition.

Use the calibration solutions to check linearity and to determine the calibration graphs based on the response ratio of chlormequat/d_4-chlormequat and mepiquat/d_3-mepiquat. As units on the x-axis, use the corresponding mass fraction of chlormequat cation or mepiquat cation respectively in the test portion (see 3.13).

NOTE 1 Typically, a response ratio of about 1 should be obtained with the calibration solution no. 2 (corresponding to a mass fraction of 0,05 mg/kg for the 20 g test portion). The resulting slope of the calibration graphs should be approximately 20 kg/mg.

NOTE 2 Since the calibration range shall be appropriate to the residue concentrations found, it may be necessary to construct more than one calibration graph from the results of the calibration measurements.

Measure the peak height (or peak area) obtained for chlormequat and mepiquat cation in the sample test solution and divide it by the peak height (or peak area) obtained for the peak of the corresponding internal standard (d_4-chlormequat or d_3-mepiquat, respectively). Based on the resulting response ratios and the two calibration graphs calculate the mass fraction w_R of chlormequat cation and mepiquat cation, in milligrams per kilogram of sample, using equation (1):

$$w_R = \frac{(A_A / A_{Istd}) - a}{b} \times \frac{20\ \text{g}}{m_A} \qquad (1)$$

where:

A_A is the peak response of chlormequat (or mepiquat) cation;

A_{Istd} is the peak response of internal standard (d_4-chlormequat cation or d_3-mepiquat cation, respectively);

a is the intercept of the calibration graph for chlormequat (or mepiquat) without dimensions;

b is the slope of the calibration graph for chlormequat (or mepiquat) in (kilogram per milligram);

m_A mass of the test portion (in gram).

If the results indicate that the amount of residue approaches or exceeds the maximum residue level, at least one further test portion shall be analysed.

7 Confirmatory tests

To confirm positive findings, an HPLC column with different analyte retention properties may be used (for examples see Annex A).

8 Precision

8.1 General

Details of the inter-laboratory test of the precision of the method according to ISO 5725-1 and ISO 5725-2 are summarised in Annex B. The values derived from the inter-laboratory test may not be applicable to analyte concentration ranges and matrices other than those given in Annex B.

8.2 Repeatability

The absolute difference between two single test results found on identical test material by one operator using the same apparatus within the shortest feasible time interval will exceed the repeatability limit *r* in not more than 5 % of the cases. The values are given in Table 1.

Table 1 — Repeatability

	Mushroom 1	Mushroom 2	Pear	Wheat flour	Fruit puree	Infant formula
Chlormequat						
mean value $\bar{x}$ (mg/kg)	1,03	0,10	0,24	0,055	0,007	0,010
repeatability limit *r* (mg/kg)	0,237	0,013	0,033	0,023	0,002	0,004
Mepiquat						
mean value $\bar{x}$ (mg/kg)	0,39	0,043	0,72	0,078	0,008	0,009
repeatability limit *r* (mg/kg)	0,059	0,017	0,152	0,022	0,002	0,004

8.3 Reproducibility

The absolute difference between two single test results on identical test material reported by two laboratories will exceed the reproducibility limit *R* in not more than 5 % of the cases. The values are given in Table 2.

Table 2 — Reproducibility

	Mushroom 1	Mushroom 2	Pear	Wheat flour	Fruit puree	Infant formula
Chlormequat						
mean value $\bar{x}$ (mg/kg)	1,03	0,10	0,24	0,055	0,007	0,010
reproducibility limit *R* (mg/kg)	0,353	0,034	0,035	0,031	0,009	0,006
Mepiquat						
mean value $\bar{x}$ (mg/kg)	0,39	0,043	0,72	0,078	0,008	0,009
reproducibility limit *R* (mg/kg)	0,104	0,023	0,206	0,038	0,014	0,018

9 Test report

The test report shall contain at least the following:

— all information necessary for the identification of the sample;

— reference to this European Standard;

— date and type of sampling procedure (if possible);

— date of receipt of sample in the laboratory;

— date of test;

— results and the units in which the results have been expressed;

— any particular points observed in the course of the test;

— any operations not specified in the method or regarded as optional which might have affected the results.