



**SLOVENSKI STANDARD**  
**SIST EN 15054:2006**  
**01-september-2006**

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

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

Aliments non gras - Détermination du chlorméquat et du mépiquat - Méthode CL-SM

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**Ta slovenski standard je istoveten z: EN 15054:2006**

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ICS 67.060; 67.080.01

English Version

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

Aliments non gras - Détermination du chlorméquat et du  
mépiquat - Méthode CL-SM

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

This European Standard was approved by CEN on 20 April 2006.

CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration. Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the Central Secretariat or to any CEN member.

This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the Central Secretariat has the same status as the official versions.

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EUROPEAN COMMITTEE FOR STANDARDIZATION  
COMITÉ EUROPÉEN DE NORMALISATION  
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## Foreword

This document (EN 15054:2006) 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of an identical text or by endorsement, at the latest by November 2006, and conflicting national standards shall be withdrawn at the latest by November 2006.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 15055 "Non fatty foods - Determination of chlormequat and mepiquat - LC-MS/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.

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## 1 Scope

This draft European Standard specifies a method using high performance liquid chromatography/mass spectrometry (LC-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 and fruit puree, 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 analyzed by high performance liquid chromatography (HPLC) with mass spectrometric detection after positive electrospray ionisation, see also [2] to [7].

## 3 Reagents

### 3.1 General and safety aspects

Unless otherwise specified, use reagents of recognized 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 Filter aid, for example Celite® 545<sup>1)</sup>

### 3.4 Glacial acetic acid, mass fraction w at least 96 g/100 g

### 3.5 Acetonitrile, HPLC quality

### 3.6 Methanol, HPLC quality

### 3.7 Water, suitable for HPLC

### 3.8 Chlormequat chloride stock solution, mass concentration $\rho(\text{C}_5\text{H}_{13}\text{NCl}_2) = 4,30 \mu\text{g/ml}$ in methanol

This stock solution contains 3,33  $\mu\text{g/ml}$  chlormequat cation.

### 3.9 Mepiquat chloride stock solution, $\rho(\text{C}_7\text{H}_{16}\text{NCl}) = 4,37 \mu\text{g/ml}$ in methanol

This stock solution contains 3,33  $\mu\text{g/ml}$  mepiquat cation.

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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 Internal standard solution 1 (d<sub>4</sub>-Chlormequat chloride), $\rho(\text{C}_5\text{H}_9\text{D}_4\text{NCl}_2) = 21,33 \mu\text{g/ml}$ in methanol

This stock solution contains 16,67  $\mu\text{g/ml}$  d<sub>4</sub>-chlormequat cation. For the availability of the standard, contact your national standardisation institute.

### 3.11 Internal standard solution 2 (d<sub>3</sub>-Mepiquat iodide), $\rho(\text{C}_7\text{H}_{13}\text{D}_3\text{NI}) = 34,70 \mu\text{g/ml}$ in methanol

This stock solution contains 16,67  $\mu\text{g/ml}$  d<sub>3</sub>-mepiquat cation. For the availability of the standard, contact your national standardisation institute.

### 3.12 Calibration solutions

Prepare six calibration solutions (no. 1 to no. 6) in 10 ml volumetric flasks as follows. Into the flasks, pipette 10  $\mu\text{l}$ , 50  $\mu\text{l}$ , 100  $\mu\text{l}$ , 300  $\mu\text{l}$ , 1 000  $\mu\text{l}$  or 3 000  $\mu\text{l}$  each of chlormequat chloride stock solution (3.8) and of mepiquat chloride stock solution (3.9). To all flasks add 60  $\mu\text{l}$  of the internal standard solution 1 (3.10) and 60  $\mu\text{l}$  of the internal standard solution 2 (3.11). 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 100 ng/ml of d<sub>4</sub>-chlormequat cation and 100 ng/ml of d<sub>3</sub>-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.

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## 4 Apparatus

Usual laboratory apparatus and, in particular, the following:

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### 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  $\mu\text{m}$  (polyamide or polytetrafluoroethylene)

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

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

## 5 Procedure

### 5.1 Preparation of the samples

Where possible, carry out the analysis of samples immediately on 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 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).

If samples have to be stored for more than 3 days, they shall 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.

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

## 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 test portion of 10 g ( $m_A$ ) into the cup. Add 360 µl of internal standard solution 1 (3.10) and 360 µl of internal standard solution 2 (3.11). Add so much 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 the addition of water. Add 40 ml of methanol (3.6) 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 portion 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 (3.3) 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 (3.12) into the LC-MS system. The selected ions shall be appropriate for the sensitive and selective determination of 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 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 ions (e. g. ions  $m/z$  122 and  $m/z$  124 for chlormequat, see A.8) 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.

Use the calibration solutions to check linearity and to determine the calibration graphs based on the response ratio of chlormequat/d<sub>4</sub>-chlormequat and mepiquat/d<sub>3</sub>-mepiquat, respectively. As units on the x-axis use the corresponding mass fraction of chlormequat cation or mepiquat cation respectively in 20 g of the test portion (see 3.12).

NOTE 1 Typically, a response ratio of about 1 should be obtained with the calibration solution no. 4 (corresponding to a mass fraction of 0,3 mg/kg for the 20 g test portion). The resulting slope of the calibration graphs should be approximately 3,3 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 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<sub>4</sub>-chlormequat or d<sub>3</sub>-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):

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

where:

$A_A$  is the peak response of chlormequat cation or mepiquat cation;

$A_{Istd}$  is the peak response of internal standard (d<sub>4</sub>-chlormequat cation or d<sub>3</sub>-mepiquat cation, respectively);

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

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

$m_A$  is the mass of the test portion (in grams).

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 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	Pear 1	Pear 2	Wheat flour	Fruit puree
Chlormequat					
mean value $\bar{x}$ (mg/kg)	1,19	0,16	0,063	0,11	0,04
repeatability limit $r$ (mg/kg)	0,127	0,028	0,008	0,035	0,016
Mepiquat					
mean value $\bar{x}$ (mg/kg)	0,31	0,24	0,11	0,39	0,034
repeatability limit $r$ (mg/kg)	0,027	0,094	0,039	0,096	0,013

## 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	Pear 1	Pear 2	Wheat flour	Fruit puree
Chlormequat					
mean value $\bar{x}$ (mg/kg)	1,19	0,16	0,063	0,11	0,04
reproducibility limit $R$ (mg/kg)	0,490	0,080	0,046	0,075	0,029
Mepiquat					
mean value $\bar{x}$ (mg/kg)	0,31	0,24	0,11	0,39	0,034
reproducibility limit $R$ (mg/kg)	0,155	0,110	0,064	0,188	0,066

## 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;
- results and the units in which the results have been expressed;
- date and type of sampling procedure (if possible);
- date of receipt of sample in the laboratory;
- date of test;
- 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.

## Annex A (informative)

### Examples of experimental conditions

#### A.1 General

The following LC-MS operating conditions have been proven to be satisfactory.

#### A.2 LC-System 1

HPLC Pump	G1312A Binary Pump from system HP1100
Autosampler	G1313A from system HP1100
Injection volume	20 µl
Column	Shodex RSpak DE 413, 4 µm, 150 mm x 4,6 mm, guard column Shodex RSpak DE-G
Mobile Phase	Acetonitrile/methanol/acetic acid/water 17,5 + 7,5 + 3,75 + 71,25 (V+V+V+V) containing 50 mmol/l ammonium acetate (3.2)
Flow rate	0,3 ml/min (standards.iteh.ai)
Column temperature	ambient
Retention time for	<a href="https://standards.iteh.ai/catalog/standards/sist/78b5cc65-9b02-4669-9ea2-946654195985/sist-en-15054-2006">https://standards.iteh.ai/catalog/standards/sist/78b5cc65-9b02-4669-9ea2-946654195985/sist-en-15054-2006</a>
chlormequat	5,9 min
mepiquat	6,0 min

#### A.3 LC-System 2

HPLC pump	G1312A Binary Pump from system HP1100
Autosampler	G1313A from system HP1100
Injection volume	20 µl
Column	Shodex RSpak DE 613, 6 µm, 150 mm × 6 mm
Mobile phase	Methanol/water 50 + 50 (V+V) containing 25 mmol/l ammonium acetate (3.2)
Flow rate	0,3 ml/min
Column temperature	40 °C
Retention time for	
chlormequat	11,6 min
mepiquat	10,6 min