SLOVENSKI STANDARD
SIST EN ISO 21570:2006

01-marec-2006

Živila – Analitske metode za odkrivanje gensko spremenjenih organizmov in njihovih produktov – Kvantitativne metode na osnovi nukleinske kisline (ISO 21570:2005)

Foodstuffs - Methods of analysis for the detection of genetically modified organisms and derived products - Quantitative nucleic acid based methods (ISO 21570:2005)

Lebensmittel - Verfahren zum Nachweis von gentechnisch modifizierten Organismen und ihren Produkten - Quantitative auf Nukleinsäuren basierende Verfahren (ISO 21570:2005)

Produits alimentaires - Méthodes d'analyse pour la détection des organismes génétiquement modifiés et des produits dérivés - Méthodes quantitatives basées sur l'utilisation des acides nucléiques (ISO 21570:2005)

Ta slovenski standard je istoveten z: **EN ISO 21570:2005**

ICS:

67.050	Splošne preskusne in analizne metode za živilske proizvode	General methods of tests and analysis for food products

SIST EN ISO 21570:2006 **en**

EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

EN ISO 21570

November 2005

ICS 67.050

English Version

Foodstuffs - Methods of analysis for the detection of genetically modified organisms and derived products - Quantitative nucleic acid based methods (ISO 21570:2005)

Produits alimentaires - Méthodes d'analyse pour la détection des organismes génétiquement modifiés et des produits dérivés - Méthodes quantitatives basées sur l'utilisation des acides nucléiques (ISO 21570:2005)

Lebensmittel - Verfahren zum Nachweis von gentechnisch modifizierten Organismen und ihren Produkten - Quantitative auf Nukleinsäuren basierende Verfahren (ISO 21570:2005)

This European Standard was approved by CEN on 26 October 2005.

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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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COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

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Foreword

This document (EN ISO 21570:2005) has been prepared by Technical Committee CEN/TC 275 "Food analysis - Horizontal methods", the secretariat of which is held by DIN, in collaboration with Technical Committee ISO/TC 34 "Agricultural food products".

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 May 2006, and conflicting national standards shall be withdrawn at the latest by May 2006.

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, Slovakia, Slovenia, Spain, Sweden, Switzerland and United Kingdom.

INTERNATIONAL STANDARD

ISO 21570

First edition
2005-11-01

Foodstuffs — Methods of analysis for the detection of genetically modified organisms and derived products — Quantitative nucleic acid based methods

Produits alimentaires — Méthodes d'analyse pour la détection des organismes génétiquement modifiés et des produits dérivés — Méthodes quantitatives basées sur l'utilisation des acides nucléiques

Reference number
ISO 21570:2005(E)

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Published in Switzerland

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 21570 was prepared by the European Committee for Standardization (CEN) Technical Committee CEN/TC 275, *Food Analysis — Horizontal methods*, in collaboration with Technical Committee ISO/TC 34, *Food products*, in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

Introduction

The search for ingredients of genetically modified origin is performed by means of the following successive (or simultaneous) steps. After sample collection, nucleic acids are extracted from the test portion. Extracted nucleic acids can be further purified, simultaneously or after the extraction process. Afterwards, they are quantified (if necessary), diluted (if necessary) and subjected to analytical procedures (such as PCR). These steps are detailed in the present and in the following International Standards:

ISO 21569, *Foodstuffs — Methods of analysis for the detection of genetically modified organisms and derived products — Qualitative nucleic acid based methods*

ISO 21570, *Foodstuffs — Methods of analysis for the detection of genetically modified organisms and derived products — Quantitative nucleic acid based methods*

Further information about definitions and general items involving the steps cited above are collected in:

ISO 24276, *Foodstuffs — Methods of analysis for the detection of genetically modified organisms and derived products — General requirements and definitions*.

The International Organization for Standardization (ISO) draws attention to the fact that it is claimed that compliance with this document may involve the use of a patent concerning the PCR technology.

ISO takes no position concerning the evidence, validity and scope of these patent rights.

ISO has been informed that Applied Biosystems, Roche Molecular Systems, Inc. and Hoffman-La Roche hold patent rights concerning PCR technology. The companies have assured the ISO that they are willing to negotiate licences under reasonable and non-discriminatory terms and conditions with applicants throughout the world. In this respect, the statements of the holders of these patent rights are registered with ISO. Information may be obtained from:

Licensing Department
Applied Biosystems
850 Lincoln Centre Drive
Foster City, CA 94404,
USA

and

Roche Molecular Systems, Inc.
Licensing Department
1145 Atlantic Avenue
Alameda, CA 94501,
USA

Attention is drawn to the possibility that some of the elements of this document may be subject of patent rights other than those identified above. ISO shall not be held responsible for identifying any or all such patent rights.

Foodstuffs — Methods of analysis for the detection of genetically modified organisms and derived products — Quantitative nucleic acid based methods

1 Scope

This International Standard provides the overall framework of quantitative methods for the detection of genetically modified organisms (GMOs) in foodstuffs, using the polymerase chain reaction (PCR).

It defines general requirements for the specific amplification of DNA target sequences, in order to quantify the relative GMO-derived DNA content and to confirm the identity of the amplified DNA sequence.

Guidelines, minimum requirements and performance criteria laid down in this International Standard are intended to ensure that comparable, accurate and reproducible results are obtained in different laboratories.

This International Standard has been established for food matrices, but is also applicable to other matrices, e.g. feed and plant samples from the environment.

Specific examples of methods are provided in Annexes A to D.

2 Normative references

The following referenced documents are indispensable for the application 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.

ISO 21569:2005, *Foodstuffs — Methods of analysis for the detection of genetically modified organisms and derived products — Qualitative nucleic acid based methods*

ISO 21571, *Foodstuffs — Methods of analysis for the detection of genetically modified organisms and derived products — Nucleic acid extraction*

ISO 24276:—[1)], *Foodstuffs — Methods of analysis for the detection of genetically modified organisms and derived products — General requirements and definitions*

ISO Guide 32, *Calibration in analytical chemistry and use of certified reference materials*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 24276 apply.

1) To be published.

4 Principle

4.1 General

Quantitative analysis consists of the quantitation of target DNA sequences in the test samples. Each method specifies the target sequences(s).

Quantitation may be performed using competitive [1],[2] or real-time PCR [3],[4].

A quantitative analysis should clearly express the quantity of the target genetic element, relative to the quantity of a specific reference, appropriate calibrants and controls, and be within the dynamic range of the analytical method used and the test portion analysed.

The analysis generally consists of

— amplification of one or more specific target sequences,

— detection and confirmation of the specificity of the PCR product(s), and

— quantitation of the amplified fragments relative to calibrants.

NOTE In the case of real-time PCR analysis, amplification, detection and confirmation occur simultaneously.

4.2 Amplification, detection and confirmation of PCR products

See ISO 21569 for the principles of amplification, detection and confirmation of the DNA sequences.

4.3 Quantitation of PCR products

The principle of quantitation is usually to determine the ratio (expressed as a percent) of two DNA target sequences; i.e. a sequence representing the genetically modified organism of interest and an (endogenous) target taxon-specific sequence. However, in some cases, quantitation can also be carried out relative to a specified amount of food matrix (e.g. when detecting GM microorganisms in foods).

Calibrants (calibration materials) used for quantitation should be traceable to certified reference materials (CRMs), if available. If not available, other suitable reference material should be used. Example guidance is provided in Reference [5]. Information on validation studies and measurement uncertainty has been gathered from international studies [6],[7],[8],[9].

5 Reagents

All reagents and materials used in the analysis should be identical, or equivalent, to those specified in the method. Otherwise, all reagents and materials should be of molecular biology grade. These reagents shall be stored and used as recommended by the supplier or according to the laboratory quality assurance specifications. For a list of reagents, see the specific annex.

6 Apparatus and equipment

See Annexes A to D and ISO 24276.

7 Guidelines concerning the procedure

7.1 General

General considerations relevant to PCR amplification for the detection of GMOs are described in ISO 21569.

Annexes A to D specify PCR detection methods together with details of their scope of application. The demonstrated performance characteristics for each method are detailed.

The concentration of the DNA sequence of interest should be within the dynamic range of the method.

NOTE A target taxon specific monitor run can be undertaken to determine whether the template DNA is of sufficient quality (length and structural integrity), purity and quantity to allow the detection and quantitation of a GMO belonging to the target taxon. This may be of particular relevance when DNA is extracted from composite or highly processed matrices.

The DNA extracted from each test portion should be analysed at least in duplicate.

Appropriate controls shall be included (see ISO 24276:—, Table 1).

7.2 Target sequence stability

The allelic and copy number stability of the target sequence should be considered for cultivars of different geographic and phylogenic origins.

7.3 Calibration of the analysis

An appropriate number of calibration points and replicates covering the range of quantitation shall be applied [e.g. four calibration points with two replicates (altogether 4×2 values) or six calibration points with one measurement at each point (altogether 6 values)]. The quality of the calibration influences the measurement uncertainty [9].

As an alternative to genomic DNA calibration reference materials, for example, a dilution series of a plasmid or synthetic dsDNA containing the target sequence may be used, provided that it is demonstrated to perform in an equivalent way to the genomic DNA reference material and the genomic DNA extracted from the sample.

7.4 Quantitation considerations

PCR methods should be appropriately designed to minimize the variability.

NOTE Depending on the method used and/or the material analysed, the presence of stacked genes can lead to overestimation of the true GMO content.

For the determination of the limit of quantitation (LOQ), see ISO 24276.

Calculation of the GMO content based on copy numbers of target sequences per haploid genome is influenced by the homo- and heterozygosity of the species under investigation. For details, see Annexes A to D.

Use of the $\Delta\Delta C_t$ (cycle of threshold) method is only valid if the amplification efficiencies of the target taxon-specific assay and the GMO-specific assay are very similar.

7.5 Quality assurance requirements

Consistency between measurements is desirable to obtain reliable estimates of target sequence quantities. However, knowledge of the relative standard deviation of repeatability of the method is required to establish whether the measurements are consistent (see the ISO 5725 series for details). To calculate the relative standard deviation of repeatability, the number of separate measurements per laboratory sample may exceed what is feasible in practice in terms of acceptable costs. Consequently, if a specified GMO-derived DNA is to be reported (in percent), a feasible solution should require the following as a minimum:

a) within test portion consistency:

   — through rejection of measurements <LOQ, and

   — through maximum deviation observed between dilutions and individual measurements equals the value expected from the corresponding dilution factor ± 33 %;

b) between test portion consistency:

   — estimated relative GMO-derived DNA concentrations obtained under a) for each test portion should not differ by values greater than –50 % to +100 % of the estimated quantity value (equal to a ΔC_t of 1 in real-time PCR) (i.e. for two test portions, measurements of 1,0 % and 2,0 % are acceptable, measurements of 0,9 % and 2,1 % are not).

In order to guarantee accuracy of the measurements, a reference material (RM), preferably certified (CRM), for the quantity of the event concerned, with an appropriate level of metrological reliability and with reasonable similarity of matrix shall be selected and analysed. In the absence of a CRM, in-house RM may be prepared by a procedure demonstrating stability, homogeneity and traceability, and ensuring the absence of bias. The quantified uncertainty shall fulfil the required uncertainty for the calibration (see ISO Guide 32).

8 Interpretation

The PCR result will be either

a) fit for quantitation of the target sequence provided

   — the result is positive according to ISO 21569:2005, 8.1,

   — the observable inhibition of the reaction is negligible,

   — the analysis produces an unambiguous measurement value,

   — the target sequence content is within the dynamic range of the method, and

   — the analysis is calibrated in an acceptable way (see 7.3), or

b) not fit for quantitation of the target sequence if any of the conditions listed above are not fulfilled.

The measurement uncertainty shall be sufficiently small to enable the laboratory to draw the relevant conclusions.

Annexes A to D describe the measurement of the target DNA quantities. These quantities can be used to calculate the GMO content. These calculations usually take into consideration relevant biological factors, such as the homo- or heterozygosity of the target sequences.

If the GM target sequence content or the taxon-specific target sequence content is below the limit of quantitation, the result shall only be expressed qualitatively.

NOTE Stating that the GMO-derived DNA content is below the practical LOQ accompanied by a specification of that LOQ is considered to be a qualitative expression of the result.

9 Expression of results

The results shall clearly state the quantity of the GM target sequence relative to the target taxon-specific sequence. The results should also provide values for the measurement uncertainty, such as the standard

deviation or relative standard deviation. Furthermore, the LOD and LOQ of the method and the practical LOD and LOQ should be reported.

The target sequences may or may not be detected, or the quantity of at least one of them may be below the limit of quantitation. Table 1 describes the four alternative cases and the corresponding expression of the result to be included into the test report.

Table 1 — Expression of results

Result	Expression of the result
Target taxon-specific sequence is not detected.	See ISO 21569. "For species x, DNA was not detected."
Target taxon-specific sequence is detected but GM target sequence is not detected.	According to ISO 21569. "For species x, GMO-derived DNA was not detected." In addition, if applicable, add: "The practical limit of detection is X %." (Specify unit used.)
The target taxon-specific sequence and the GM target sequence are both detected but the quantity is below the LOQ of at least one of the target sequences.	For each GMO, state: "GMO (specify the GMO) derived DNA as determined by detection of (specify target sequence) derived from (specify species) was detected." In addition, if applicable: "The practical limit of quantitation is X %." (Specify unit used.)
The target taxon-specific sequence and the GM target sequence are both detected and the quantity is above the LOQ for both target sequences.	For each GMO, state: "The content of GMO (specify the GMO) derived DNA as determined by detection of (specify target sequence) derived from (specify species) is X ± uncertainty %." (Specify unit used.)

The GMO-derived DNA content may also be reported as being above or below a specific value, taking into account the measurement uncertainty.

10 Test report

The test report shall be written in accordance with ISO 24276 and ISO 21569 and shall contain at least the following additional information:

a) LOQ of the method and the matrix used to establish it;

b) the practical LOQ;

c) a reference to the method which has been used for the extraction of DNA;

d) the reference material used;

e) the results expressed according to Clause 9.