SLOVENSKI STANDARD
oSIST prEN 17644:2021

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Živila - Odkrivanje prisotnosti alergenov v živilih z metodo tekočinske kromatografije z masno spektrometrijsko detekcijo (LC-MS) - Splošne ugotovitve

Foodstuffs - Detection of food allergens by liquid chromatography - mass spectrometry (LC-MS) methods - General considerations

Lebensmittel - Nachweis von Lebensmittelallergenen mit flüssigkeitschromatographisch-massenspektrometrischen (LC-MS) Verfahren - Allgemeine Betrachtungen

Produits alimentaires - Détection des allergènes alimentaires par des méthodes de chromatographie en phase liquide couplée à la spectrométrie de masse (CL-SM) - Considérations générales

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

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Foodstuffs - Detection of food allergens by liquid chromatography - mass spectrometry (LC-MS) methods - General considerations

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COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

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European foreword

This document (prEN 17644:2021) has been prepared by Technical Committee CEN/TC 275 “Food analysis - Horizontal methods”, the secretariat of which is held by DIN.

This document is currently submitted to CEN Enquiry.

Introduction

Specific peptides or groups of peptides deriving from specific proteins can serve as markers for the presence of food or food ingredients provoking allergic reactions. This document describes the procedure for the development of qualitative and/or quantitative mass spectrometry-based methods for the determination of protein-derived peptides as markers for potentially allergenic food ingredients or constituents by analysing the protein/s extracted from a sample. Appropriate procedures for the extraction of the targeted proteins are an essential part of each method. This document describes general considerations for the application of liquid chromatography mass spectrometry-based methods in qualitative or quantitative targeted analysis of specific peptides (derived from specific proteins) that are representative for a food allergen. The document includes recommendations for method validation and for the conversion of the analytical results to units of mg protein/kg food.

1 Scope

This document establishes an overall framework covering qualitative and quantitative methods for the determination of food allergens and allergenic ingredients using mass spectrometry-based methods for the determination of specific peptides/proteins. This document provides general guidelines and performance criteria applicable to this methodology. Guidelines, minimum requirements and performance criteria laid down in this document are intended to ensure that comparable and reproducible results are obtained by different analysts, instrumentation and laboratories.

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.

EN 15842, *Foodstuffs - Detection of food allergens - General considerations and validation of methods*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in EN 15842 and the following apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— IEC Electropedia: available at http://www.electropedia.org/

— ISO Online browsing platform: available at https://www.iso.org/obp

3.1
high-performance liquid chromatography–mass spectrometry
HPLC-MS
analytical chemistry technique that combines the separation capabilities of high-performance liquid chromatography with the detection capabilities of mass spectrometry (MS)

Note 1 to entry: The abbreviation LC-MS is also used.

3.2
tandem mass spectrometry
MS/MS
MS^2
sequential combination of two mass analyses

Note 1 to entry: Different mass spectrometer instrument types exist, combining different principles of mass detection, e.g. quadrupole, time-of-flight, ion trap, Fourier-Transform mass spectrometer.

3.3
multi-stage mass spectrometry
MS^n
sequential combination of more than two mass analyses

3.4
targeted mass spectrometry
mass spectrometry application analysing only specific ions (m/z) at specific times (RT)

Note 1 to entry: The targets are defined in an inclusion list.

Note 2 to entry: The opposite is untargeted MS measuring any ion present.

Note 3 to entry: In general targeted MS increases method sensitivity.

3.5
peptide
amide that consists of two or more amino acids

Note 1 to entry: Peptides are formed by amide bonding of the amino group of one amino acid (AA) with the carboxyl group of another AA.

Note 2 to entry: Peptides are usually obtained by enzymatic hydrolysis of proteins during sample preparation for mass spectrometry-based methods.

3.6
marker peptide
peptide that is specific/unique for a definite protein and used as analyte in mass spectrometry-based methods

Note 1 to entry: Portion of a protein used for its identification, recovery and purification.

[SOURCE: ISO 20418-1]

3.7
analyte
substance or chemical constituent that is subjected to measurement

[SOURCE: CEN/TS 15968:2010, 3.1]

3.8
measurand
quantity intended to be measured

Note 1 to entry: The specification of a measurand requires knowledge of the kind of quantity, description of the state of the phenomenon, body, or substance carrying the quantity, including any relevant component, and the chemical entities involved.

Note 2 to entry: In the second edition of the VIM and in IEC 60050-300:2001, the measurand is defined as the “particular quantity subject to measurement”.

Note 3 to entry: The measurement, including the measuring system and the conditions under which the measurement is carried out, might change the phenomenon, body, or substance such that the quantity being measured may differ from the measurand as defined. In this case, adequate correction is necessary.

[SOURCE: ISO/IEC Guide 99:2007, 2.3, modified – removed Examples and note 4 to entry]

3.9
incurred samples
material produced by adding a specific amount of allergenic ingredient to a relevant matrix before it is processed by food manufacturing techniques

3.10
mass-to-charge ratio
m/z
dimensionless quantity formed by dividing the ratio of the mass (m) of an ion to the unified atomic mass unit by its charge number (z)

[SOURCE: IUPAC Recommendations 2013: Definitions of terms relating to mass spectrometry, Pure Appl. Chem., Vol. 85, No. 7, pp. 1515–1609]

3.11
retention time
RT
time taken for an analyte to pass through a chromatography column from injection to detection

3.12
selected reaction monitoring
SRM
determination of a targeted analyte by measuring the precursor-ion to product-ion transition in MS/MS fragmentation

Note 1 to entry: The mass filters in the mass spectrometer are adjusted to the ion masses of, respectively, the precursor ion and the product ion ("mass windows"), increasing the selectivity and sensitivity of the measurement.

Note 2 to entry: SRM is monitoring only a single fixed mass window.

Note 3 to entry: SRM is also called single reaction monitoring.

Note 4 to entry: Parent ion is another expression for precursor ion.

Note 5 to entry: Fragment ion is another expression for product ion.

3.13
multiple reaction monitoring
MRM
application of SRM to multiple product ions from one or more precursor ions

Note 1 to entry: MRM scans rapidly over multiple (narrow) mass windows and thus captures traces of multiple product/fragment ion masses in parallel.

3.14
basic local alignment search tool
BLAST[1]
bioinformatic algorithm for the comparison of primary sequence information such as amino acid sequences of peptides or/and proteins or nucleotide sequences of DNA and/or RNA molecules

1 National Center for Biotechnology Information (NCBI), U.S. National Library of Medicine, Bethesda MD, USA

3.15
internal standard
IS
ISTD
substance, which is similar in the chemical behaviour (chemical structure, polarity) and analytical response to a certain target analyte

Note 1 to entry: Stable-isotope labelled ISTDs are preferred in mass spectrometry-based methods, which are then sometimes called absolute quantitation (AQUA)-methods.

[SOURCE: IEC 62697-1:2012, 3.12]

3.16
standard addition
procedure in which a known amount of an analyte is added to a test sample

Note 1 to entry: To perform standard addition procedure, the test sample is divided in two (or more) test portions. One test portion is analysed as such, whereas known amounts of an analyte are added to the second test portion before analysis.

3.17
conversion factor
factor for the conversion of measurement results to a reporting unit

Note 1 to entry: The measurement results are converted into e.g. mg protein/kg food.

4 General laboratory requirements

4.1 Principle

Samples are extracted for proteins with a high-yielding, reproducible and matrix-specific procedure including enzymatic digestion for the generation of peptides. A processing step for the reduction and alkylation may be included. Specific marker peptides are measured by HPLC-MS/MS using targeted MRM for qualitative or quantitative analysis. The signal (usually the peak area) of a marker peptide measured at a specific chromatographic retention time is used for quantitation.

4.2 Apparatus and equipment

The laboratory shall use properly maintained equipment suitable for the method employed, e.g. according to the requirements outlined by EN ISO/IEC 17025. In addition to standard laboratory equipment, additional apparatus are described in the specific methods.

Apparatus and equipment should be maintained according to manufacturer's instructions. Calibration systems shall be available and calibration shall be routinely performed for measuring equipment, according to laboratory quality assurance programmes.

In a tandem mass spectrometer, ions are formed in the ion source and separated by mass-to-charge ratio in the first stage of mass spectrometry (MS1). In targeted mass spectrometry, ions of a particular mass-to-charge ratio (precursor ions) are selected and product ions are created by collision-induced dissociation, ion-molecule reaction, photodissociation, or other processes. The resulting ions are then separated and detected in a second stage of mass spectrometry (MS2).

In MRM mode, the mass spectrometer is set to scan a very small mass range in MS1, typically one mass unit, at the expected masses of the targeted precursor ions. After fragmentation, the product ions are detected in MS2 by successively scanning small mass ranges at their expected masses. Alternatively,

some MS instruments (e.g. high-resolution instruments (HRMS) with fast switching MS to MS/MS capacity) allow the simultaneous detection of all product ions (Parallel Reaction Monitoring (PRM)).

In MS-based ion fragmentation analysis, the precursor ion selected for analysis shall be clearly defined, e.g. as the molecular ion, a characteristic ion adduct of the molecular ion, a characteristic fragment ion or a typical isotope ion. The selected product ions should not exclusively originate from the same part in the molecule of the marker peptide that is to be analysed. The signal-to-noise ratio for each product ion should be ≥ 3:1.

4.3 Material and reagents

Analytes shall be clearly described, including the type of standard (e.g. synthetic peptides, purified protein, protein extracts), and information on purity, protein profiles …), storage conditions and shelf-life.

Only reagents of MS quality grade and only de-ionized or distilled water or water that has been purified should be applied for HPLC-MS analysis, unless otherwise stated in specific method descriptions. Other reagents, such as enzymes, reducing and alkylating agents, should be of MS-grade. Buffer components, organic solvents standards, analyte, reference material, controls, and samples are method-specific. Storage conditions and shelf-life of reagents and samples should be determined in method validation (method robustness) and clearly specified in the method protocol.

5 Method development

5.1 General

For the use of this document, general requirements of quality assurance for laboratories shall be observed (e.g. concerning calibration of apparatus, extraction of samples and measurement of replicates, blanks, use of reference materials, preparation of calibration curves, etc.). The scope of the method, including applicability to certain food matrices needs to be clearly defined.

Before conducting food allergen analysis, special considerations should be made regarding:

a) the laboratory lay-out (e.g. ideally, extraction working area should be spatially separated from detection working area);

b) the current workflow (e.g. other activities in the laboratories that can increase the potential for cross-contamination should be separated);

c) sample types handled (because cross-contamination issues can undermine the capability to perform analysis reliably);

d) equipment in the laboratories (e.g. the risk of cross-contamination should be considered if equipment is shared; use dedicated equipment when appropriate);

e) containers (e.g. disposable consumables are preferable, those that exhibit low protein binding - not polystyrene);

f) general house-keeping tasks (e.g. effective cleaning of items and surfaces is important and should be considered). Specific cleaning routines for rooms housing mass spectrometry instruments might be required because cleaning chemicals might disturb the analysis, depending on the MS instrument type.