SLOVENSKI STANDARD
SIST-TS CEN/TS 18267:2026

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Nanotehnologije - Smernice za pripravo vzorcev, odkrivanje, identifikacijo in karakterizacijo nanoobjektov v anorganskih dodatkih, vključenih v živilske matrice, s spICP-MS in EM-EDX

Nanotechnologies - Guidelines for sample preparation, detection, identification and characterization by spICP-MS and EM-EDX of nano-objects in inorganic additives incorporated in food matrices

Nanotechnologien - Richtlinien zur Charakterisierung von nanoobjekthaltigen Zusatzstoffen in Lebensmitteln

Nanotechnologies - Lignes directrices pour la préparation, la détection, l'identification et la caractérisation d'échantillons par spICP-MS et EM-EDX de nano-objets dans des additifs inorganiques incorporés dans des matrices alimentaires

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Nanotechnologies - Guidelines for sample preparation, detection, identification and characterization by spICP-MS and EM-EDX of nano-objects in inorganic additives incorporated in food matrices

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This Technical Specification (CEN/TS) was approved by CEN on 20 March 2026 for provisional application.

The period of validity of this CEN/TS is limited initially to three years. After two years the members of CEN will be requested to submit their comments, particularly on the question whether the CEN/TS can be converted into a European Standard.

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European foreword

This document (CEN/TS 18267:2026) has been prepared by Technical Committee CEN/TC 352 “Nanotechnologies”, the secretariat of which is held by AFNOR.

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

This document has been prepared under a standardization request addressed to CEN by the European Commission. The Standing Committee of the EFTA States subsequently approves these requests for its Member States.

The purpose of this document is to assist in the use of the following standards in the context of extraction, detection identification and quantification of nano-objects-in pure additives and in additives contained in food matrices:

— EN ISO 3696:1995, *Water for analytical laboratory use — Specification and test methods (ISO 3696:1987)*

— CEN/TS 17273:2018, *Nanotechnologies — Guidance on detection and identification of nano-objects in complex matrices*

— CEN ISO/TS 19590:2024, *Nanotechnologies — Characterization of nano-objects using single particle inductively coupled plasma mass spectrometry (ISO/TS 19590:2024)*

— EN ISO 19749:2023, *Nanotechnologies — Measurements of particle size and shape distributions by scanning electron microscopy (ISO 19749:2021)*

— EN ISO 21363:2022, *Nanotechnologies — Measurements of particle size and shape distributions by transmission electron microscopy (ISO 21363:2020)*

— CEN ISO/TS 23302:2022, *Nanotechnologies — Requirements and recommendations for the identification of measurands that characterise nano-objects and materials that contain them (ISO/TS 23302:2021)*

Any feedback and questions on this document should be directed to the users’ national standards body. A complete listing of these bodies can be found on the CEN website.

According to the CEN-CENELEC Internal Regulations, the national standards organizations of the following countries are bound to announce this Technical Specification: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Republic of North Macedonia, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Türkiye and the United Kingdom.

Introduction

Nano-objects can be present in food/feed matrices and have various origins, such as certain additives and processing aids. These substances containing nano-objects can be categorized as:

— ‘engineered nanomaterial’ as defined in Regulation (EU) 2015/2283 on novel foods and in Regulation (EU) No 1169/2011 of the European Parliament and of the Council on the provision of food information to consumers;

— ‘nanomaterial’ as defined by the Commission Recommendation (2022/C 229/01) on the definition of nanomaterial;

— materials not meeting a regulatory definition of a nanomaterial but which contain a fraction of nano-objects or small particles, as for example defined by the EFSA [1] [2].

However, it should be noted that nano-objects can also arise e.g. as by-products of the production process, or during handling and processing of foods, or they can be of natural origin. This document focuses on nano-objects in food matrices regardless of their origin, as the current analytical methodologies do not allow a reliable determination of the origin of nano-objects. The presented approaches, useful for analysis of nano-objects in food, are equally applicable to analysis of small particles defined in the EFSA Guidance on Particle – Technical Requirements [1].

To improve information on the presence of nano-objects in food originating from additives, industrial players along the value chain and the EU Member State (MS) control laboratories need to be able to evaluate their presence and to characterize the number-based particle size distribution of the food additive after it has been incorporated in food matrices. This will enable a more efficient implementation of the regulatory requirements applicable to food ingredients that fall within the scope of the ‘engineered nanomaterial’ definition. Additionally, it will also be of general value in assessing particles at the nanoscale.

Existing guidance and standards may be general e.g. CEN/TS 17273:2018, CEN ISO/TS 19590:2024, EN ISO 19749, EN ISO 21363:2022, which strongly focus on the provision of general measurement principles for several techniques. Details on e.g. sample preparation are not included, although this step has a critical impact on the (nano)particles size distribution measurements.

This document focuses on describing method-specific sample preparation approaches required to extract nano-objects from food matrices and providing a library of specific protocols for ex situ characterization of nanoparticles. It addresses approaches that destruct and remove various types of food matrices, purify and concentrate the nanoparticles of interest, and disperse them so that individual particles can be measured, without altering their physicochemical properties. This information can be complementary to the guidance on detection and identification of nano-objects in complex matrices provided in CEN/TS 17273:2018. Please note that sample preparation approaches for organic food additives are not covered, as the state-of-the-art analytical methods do not reliably allow the separation and identification of such substances.

Once extracted, the “constituent particles” composing the material can be identified and counted. It should be noted that the terminologies between ISO, CEN and European Commission regulatory framework are different as detailed in subclause 6.3 of FprCEN/TS 18269, “Nanotechnologies - Guidance on the determination of the aggregation and agglomeration state of nano-objects”. The counting of constituent particles in various legislation varies in different countries and application domains [3]. Electron microscopy (EM) is important as it can measure the size of constituent particles in agglomerates and aggregates, in contrast to most other techniques (e.g. ensemble techniques, single particle inductively coupled plasma mass spectrometry (spICP-MS)) [4]. spICP-MS and EM coupled to Energy-dispersive X-ray spectroscopy -(EDX) are key techniques to determine the number size distribution of a given particulate substance in complex samples where several substances may be present, since they can target a given chemical composition. spICP-MS cannot distinguish constituent particles in agglomerates/aggregates.

In this document, spICP-MS and EM-EDX methods are therefore selected as they provide complementary information on the key chemical and physical attributes required to support the implementation of the food legislation. The measurement performance of the methods presented in this document has been assessed through inter-laboratory comparison on selected inorganic food additives contained in food matrices present on the market, used as case studies.

The procedures reported in this document can also be applied to the characterization of nano-objects in pristine additives. This document can also be considered as a starting point to develop methodologies for measuring other substances containing nano-objects that are either i) added to food, but that are not classified as food additives (e.g. minerals, vitamins, enzymes, flavourings), or ii) inorganic additives contained in medicinal products, feed additives and cosmetics.

1 Scope

This document provides guidance to the food industry, service providers and control laboratories on methodologies to be used for sample preparation, detection, identification and measurement of nano-objects in inorganic food additives incorporated in food matrices.

Electron microscopy combined with energy dispersive X-ray spectroscopy (EM-EDX) and inductively coupled plasma mass spectrometry (ICP-MS) operated in single particle mode (spICP-MS) are the selected measurement methodologies to provide information on (i) the chemical composition and (ii) number-based particle size distribution of the nano-objects.

Special attention is given to the sample preparation, including matrix digestion, sample extraction and dilution steps to be used according to the combination of (i) the chemical nature of the food additive, (ii) the type of food matrix and (iii) the analytical technique of choice (EM-EDX or spICP-MS).

2 Normative references

There are no normative references in this document.

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

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

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

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

3.1
agglomerate
collection of weakly or medium strongly bound *particles* where the resulting external surface area is similar to the sum of the surface areas of the individual components

Note 1 to entry: The forces holding an agglomerate together are weak forces, for example van der Waals forces or simple physical entanglement.

Note 2 to entry: Agglomerates are also termed secondary particles and the original source particles are termed primary particles.

[SOURCE: EN ISO 80004-1:2023, 3.2.4]

3.2
aggregate
particle comprising strongly bonded or fused particles where the resulting external surface area is significantly smaller than the sum of surface areas of the individual components

Note 1 to entry: The forces holding an aggregate together are strong forces, for example, covalent or ionic bonds, or those resulting from sintering or complex physical entanglement.

Note 2 to entry: Aggregates are also termed secondary particles and the original source particles are termed primary particles.

[SOURCE: EN ISO 80004-1:2023, 3.2.5]

3.3
constituent particle
identifiable, integral component of a larger *particle*

Note 1 to entry: The constituent particle structures can be *primary particles* or *aggregates*.

Note 2 to entry: According to the EC Recommendation 2022/C 229/01, constituent particles can be present, either as particles that are present on their own or as identifiable particles in aggregates and agglomerates.

Note 3 to entry: The EC defines the constituent particle as 'the (morphologically) identifiable particles, including those inside an *aggregate* or *agglomerate*. In agglomerates the constituent particles are only weakly bound. In *aggregates* the constituent particles are strongly bound. Ensemble-based techniques cannot be used to measure the size of constituent particles in *aggregates* and *agglomerates* [4, 5].

[SOURCE: EN ISO 80004-1:2023, 3.2.3, modified – added Notes 2 and 3 to entry.]

3.4
minimum feret diameter
minimum length of an object whatever its orientation

Note 1 to entry: The Feret diameter or Feret's diameter is a measure of an object size along a specified direction; it is applied to projections of a three-dimensional object on a two-dimensional plane. It is also called the caliper diameter.

Note 2 to entry: The maximum Feret diameter (x_{Fmax}) is the "length" of the particle. The minimum Feret diameter (x_{Fmin}) is the "breadth" of the particle.

Note 3 to entry: The Feret diameter depends on the orientation of the particle with respect to tangents, so a single measurement cannot always be representative. If all possible orientations are considered, for a convex particle with the particle perimeter P: $P = \pi\, x_{\mathrm{Fmean}}$ (Cauchy theorem). There is no such relation between P and x_{Fmean} for a concave object.

[SOURCE: EN ISO 19749:2023]

3.5
food additive
substance that is not normally consumed as a food by itself and that is not normally used as a typical ingredient of food, but are intentionally added to food to exert a technological purpose in the manufacture, processing, preparation, treatment, packing, packaging, transport or holding of such food

Note 1 to entry: In the European Union all food additives are identified by an E number. Food additives are always included in the ingredient lists of foods in which they are used. Product labels must identify both the function of the additive in the finished food (e.g. colour, preservative) and the specific substance used either by referring to the appropriate E number or its name (e.g. E 415 or Xanthan gum). The most common additives to appear on food labels are antioxidants (to prevent deterioration caused by oxidation), colours, emulsifiers, stabilisers, gelling agents and thickeners, preservatives and sweeteners.

Note 2 to entry: See Annex A (informative).

[SOURCE: Codex Alimentarius — General Standard for the Labelling of Prepackaged Food (CODEX STAN 1-1985)]

3.6
nano-object
discrete piece of material with one, two or three external dimensions in the *nanoscale*

[SOURCE: EN ISO 80004-1:2023, 3.1.5]

3.7
nanoscale
length range approximately from 1 nm to 100 nm

[SOURCE: EN ISO 80004-1:2023, 3.1.1]

3.8
particle
minute piece of matter with defined physical boundaries

Note 1 to entry: A physical boundary can also be described as an interface.

Note 2 to entry: This general particle definition applies to *nano-objects*.

[SOURCE: EN ISO 80004-1:2023, 3.2.1]

3.9
processing aid
substance which is added during food processing to confer particular characteristics; for example, yeast added to bread

[SOURCE: EFSA glossary [6]]

4 General considerations

Over the past decade an increasing number of food additives and processing aids has been shown to contain a fraction of nano-objects, in particular due to the progress of analytical techniques.

Nano-objects in food can be a part of engineered/manufactured ingredients, such as certain food additives, or they can be of natural origin, as for example the nanostructured amorphous silica found in rice [8], or in plant fibres [9].

The European Food Safety Authority (EFSA) released a *Guidance on technical requirements for regulated food and feed product applications to establish the presence of small particles including nanoparticles* [1] which sets out mandatory information requirements in accordance with Regulation (EC) No 1925/2006. It indicates when the EFSA *Guidance on risk assessment of nanomaterials in the food and feed chain* [2] needs to be applied. Information regarding the fraction of nanoparticles, such as their number-based size distribution, is required to help assess possible risks that can be related to the particulate character of a food additive, and to evaluate compliance with its specifications.

However, standardized methods for sample preparation, detection, identification and measurement of particles in inorganic additives incorporated in food are missing today. These are required for monitoring exposure levels to nano-objects and small particles in regulated food and feed applications and verifying associated regulatory requirements. Such methods require that the particles of interest are identified among the food matrix material which often also consists of other particles, and that their relevant properties, including their size, can be accurately measured.

CEN/TS 17273:2018 provides guidance on detection and identification of nano-objects in complex matrices. It addresses specifically their detection in complex food matrices which can contain an elevated level of inorganic salts, organic contaminants and larger organic and inorganic particles. It stipulates that for each type of particle two measurands shall be considered: the size is needed for classification of particles as nano-objects, and the elemental composition is needed to discriminate the target particles with an *a priori* known elemental composition from the matrix and background particles.

CEN/TS 17273:2018 proposes as applicable characterization methods: i) the use of EM equipped with energy dispersive X-ray spectroscopy (EDX) to determine the elemental composition of the particles, additionally to their geometrical measures and ii) single particle inductively coupled plasma – mass spectrometry (spICP-MS) as an elemental specific detection system that gives as well size related information. EM-EDX and spICP-MS are most suitable for the analysis of inorganic nano-objects. The techniques cannot determine the origin of the detected inorganic particles, i.e. whether the particles are of natural origin or engineered/manufactured particles. Field Flow Fractionation (FFF) based techniques, proposed in CEN/TS 17273:2018, are not considered within the scope of this document because they require extensive optimization for each particle/food matrix combination.

The analysis requires *a priori* information regarding the elemental composition of the particles of interest. This can be obtained from the ingredients list, which is mandatory and includes food additives, or, in a control setting, from chemical analysis measuring total element content (e.g. ICP-MS). The sample preparation is key in all cases as the particles should be extracted from the food matrices before their characterization.

The approach for sample preparation prior to the analysis of the nano-objects incorporated in food matrices should be considered as a step-by-step process as described in the flow chart presented in Figure 1.

It includes the following main steps:

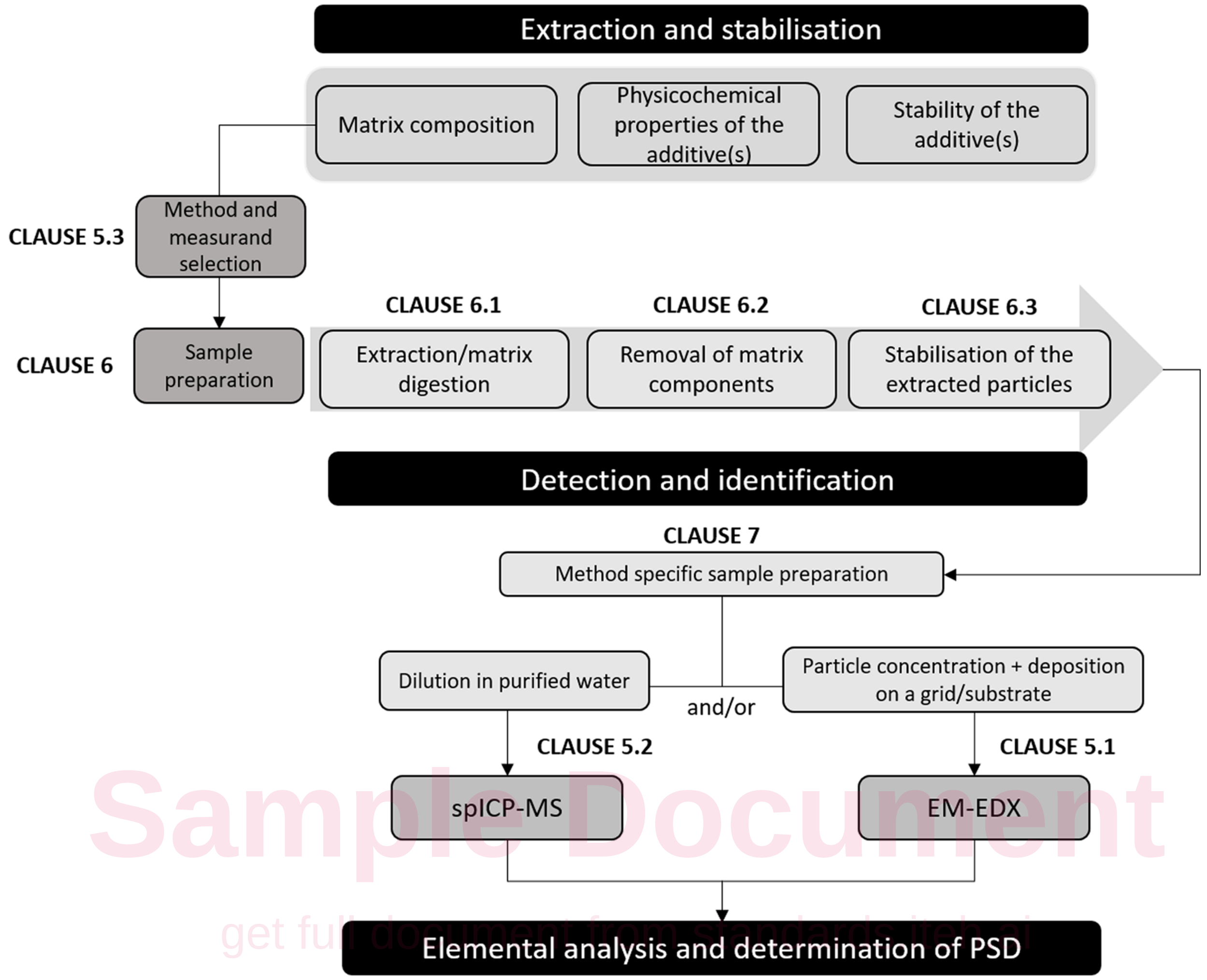
1. matrix dissolution or destruction and extraction of the particles from the food matrix;

2. removal of matrix components and residues;

3. particles deagglomeration;

4. specific sample preparation steps, associated to the measurement technique(s) and measurands selected.

Those steps should be optimized depending on the physicochemical properties of the additives to be analysed and its concentration in the food matrix, the composition of the food matrices and the measurement technique selected (see Clause 7).



Purified water refers to the following specifications: resistivity of 18,2 MΩ*cm at 25 °C and additionally passed through a filter with cut-off of 0,2 µm or lower.

Figure 1 — Guidance chart for the sample preparation, detection, identification and measurement by spICP-MS and EM-EDX

5 Overview of the applicability and limitations of EM and spICP-MS

5.1 General

EM and spICP-MS described in this document are amongst the most established analytical approaches, able to detect and identify inorganic nano-objects extracted from food matrices.

5.2 EM-EDX

5.2.1 General

Electron microscopy (EM) is a versatile technique to analyse the morphology, chemical composition and crystallographic structure of a wide range of particles.

It can be considered highly suitable for the characterization of nano-objects for several reasons [5]. First, EM analysis is one of the few methods that can reliably provide a spatial resolution covering the complete nanoscale size range from 1 nm to 100 nm. The combination of EM imaging with image analysis allows determining the physical properties (size, shape and surface morphology) of constituent particles quantitatively, based on the characteristics of their 2D projections.

Multiple properties can be measured simultaneously for each constituent particle, from which descriptive statistics and corresponding number-based distributions can be determined, as requested in legislation and guidelines [5, 10]. EM allows assessing in a qualitative way whether a material contains aggregates/agglomerates, e.g. in relation to the efficacy of the dispersion protocol. Spectroscopy methods such as EDX and electron energy loss spectroscopy (EELS) can be incorporated in the electron microscope for elemental analysis of nano-objects allowing characterization of subpopulations of nano-objects in mixtures, and nano-objects in the context of a complex matrix.

Despite the wealth of information that can be obtained, the applicability of EM to characterize particles is currently limited by the following factors:

a) The particles transferred to the grid or substrate should be representative for the population of particles present in the original food/feed product. This is influenced by the sample preparation, including removal of matrix components and residues and concentration steps, and by the particle deposition on the grid or substrate used for EM observations [11, 12].

b) EM methods typically measure the two dimensions of particles perpendicular to the electron beam, but do not assess the dimension parallel to the beam. As a result, measurements of particles that have a preferential orientation on the EM-grid, such as platelets, can lead to biased results. For such cases, more advanced EM based methods, such as tomography, or alternative sample preparation methods can be considered [13].

This document focuses on approaches for EM characterization of nano-objects using SEM, TEM and STEM equipped with EDX. More advanced EM methodologies are excluded from this document.

5.2.2 Scanning electron microscope (SEM)

Scanning electron microscopy (SEM) produces images by scanning the surface of a sample with a focused electron beam. The electrons interact with atoms in the sample, producing various signals that contain information about the surface topography and composition of the sample. The energy exchange between the electron beam and the sample results in the reflection of high-energy electrons by elastic scattering, emission of secondary electrons by inelastic scattering and the emission of electromagnetic radiation, each of which can be detected independently by specialized detectors. The dimensional parameters can be derived from SEM images by the observation and counting of isolated and constituent particles. More detailed information on the measurement principle and the related formulae can be found in EN ISO 19749:2023.

Some SEM instruments can be equipped with a transmission detector placed underneath the specimen to detect transmitted primary electrons [14]. For some materials this method, which is called TSEM, can provide an economical and efficient alternative for obtaining micrographs with a higher resolution than achieved with SEM in normal scanning mode.

5.2.3 Transmission electron microscopy (TEM)

A transmission electron microscope (TEM) uses an electron source to generate a primary electron beam, which is accelerated by an electric potential and projected onto a thin specimen through a set of lenses and apertures. Part of the electron beam can pass through the specimen without interacting with it. Other electrons will be scattered by the specimen. The information contained in the electron waves that exit from the specimen is used to create an image. At low magnifications, TEM image contrast originates from the absorption and scattering of electrons in the specimen, due to the thickness and composition of the material (i.e. mass-thickness contrast), and from the crystal orientation (i.e. diffraction contrast). By analysing the image determined by the mass-thickness contrast of the nano-objects, information on the number-based size and shape distributions can be obtained. Electron diffraction can be applied to determine their crystal structure. More detailed information on the measurement principle and the related formulae can be found in EN ISO 19749:2023.