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Water quality — Determination of selected estrogens in whole water samples — Method using solid phase extraction (SPE) followed by liquid chromatography (LC) or gas chromatography (GC) coupled to mass spectrometry (MS) detection

Qualité de l'eau — Dosage d'œstrogènes sélectionnés dans des échantillons d'eau totale — Méthode par extraction en phase solide (SPE) suivie d'une détection par chromatographie en phase liquide (CL) ou en phase gazeuse (CG) couplée à la spectrométrie de masse (SM)

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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.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO document should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 2, *Physical, chemical and biochemical methods*,in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 230, *Water analysis,* in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

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Introduction

Natural and synthetic estrogens are widely used worldwide, e.g. for contraception. Through application or improper disposal, these estrogens can enter the water cycle unchanged or transformed. They can therefore be detected in surface and groundwater, as well as in treated wastewater. It is known that estrogens can end up in surface waters via wastewater, and due to their physicochemical properties, they can partition in the different compartments [water and suspended particulate matter (SPM)] of water systems. They are of rising concern, due to their high estrogenic activity even at the measured ultra-trace levels (far below ng/l). Besides feminised fish and other endocrine disruptive effects in water ecosystems, they can also be a factor in biodiversity loss.[16] Therefore, appropriate measurement methods are required to monitor estrogen levels below their ecotoxicological level [e.g. predicted no effect concentration (PNEC) or environmental quality standard (EQS)] and accordingly demonstrate if a water body is at risk.

This document specifies validated methods for analysing water samples in monitoring programs aiming at qualifying the quality of the water environment with respects to the selected estrogens.

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Water quality — Determination of selected estrogens in whole water samples — Method using solid phase extraction (SPE) followed by liquid chromatography (LC) or gas chromatography (GC) coupled to mass spectrometry (MS) detection

WARNING — Persons using this document should be familiar with normal laboratory practice. This document does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices.

IMPORTANT — It is absolutely essential that tests conducted in accordance with this document be carried out by suitably qualified staff.

1 Scope

This document specifies methods for the determination of five selected estrogens in whole water samples listed in Table 1 (see Clause 4). The methods are based on solid-phase extraction (SPE; disk or cartridge) followed by liquid or gas chromatography-mass spectrometry detection (tandem mass spectrometry or high resolution mass spectrometry). Depending on the sample preparation chosen, the sample preparation can be applicable to the analysis of selected estrogens in drinking water, groundwater and surface water containing suspended particulate matter (SPM) up to 500 mg/l, dissolved organic carbon (DOC) content up to 14 mg/l (whole water samples).

The lower application range defined as verified limit of quantification can vary depending on the methods, the sensitivity of the equipment used and the matrix of the sample. The range is 0,006 ng/l to 1 ng/l for 17alpha-ethinylestradiol (EE2) and 0,038 ng/l to 1 ng/l for the other estrogens in drinking water, ground water and surface water. The upper limit of the working range is approximately tens of nanograms per litre.

For application that targets the measurements of very low level concentrations (between the lowest LOQ and 0,1 ng/l), every single step of the procedure becomes critical.

The methods can be used to determine further estrogens or hormones in other types of water, for example treated wastewater, if accuracy has been tested and verified for each case as well as storage conditions of both samples and reference solutions have been validated.

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.

ISO 8466-1:2021, *Water quality — Calibration and evaluation of analytical methods — Part 1: Linear calibration function*

ISO 21253-1:2019, *Water quality — Multi-compound class methods — Part 1: Criteria for the identification of target compounds by gas and liquid chromatography and mass spectrometry*

ISO 11352:2025, *Water quality — Estimation of measurement uncertainty based on validation and quality control data*

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3 Terms, definitions and subscripts

3.1 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/

3.1.1
accuracy
closeness of agreement between a measured quantity value and a true quantity value of a measurand

[SOURCE: ISO/IEC Guide 99:2007, 2.13, modified — the terms “measurement accuracy” and “accuracy of measurement” and Notes 1, 2 and 3 to entry have been deleted.]

3.1.2
analyte
substance to be analyzed

[SOURCE: ISO 6107:2021, 3.31, modified — the domain and Notes 1 and 2 to entry have been deleted.]

3.1.3
blank
aliquot of reagent water (reagent blank) or of a matrix in which the *analyte* (3.1.2) is absent (matrix blank) that is treated exactly as a sample through the complete analytical procedure including extraction, clean-up, identification and quantification including all the relevant reagents and materials

Note 1 to entry: It is crucial that the laboratory specifies which blank is considered.

[SOURCE: ISO 21253-2:2019, 3.2]

3.1.4
calibration
operation that, under specified conditions, in a first step, establishes a relation between the quantity values with measurement uncertainties provided by measurement standards and corresponding indications with associated measurement uncertainties and, in a second step, uses this information to establish a relation for obtaining a measurement result from an indication

Note 1 to entry: A calibration may be expressed by a statement, calibration function, calibration diagram, calibration curve, or calibration table. In some cases, it may consist of an additive or multiplicative correction of the indication with associated measurement uncertainty.

Note 2 to entry: Calibration should not be confused with adjustment of a measuring system, often mistakenly called “self-calibration”, nor with verification of calibration.

[SOURCE: ISO/IEC Guide 99:2007, 2.39, modified — Note 1 to entry has been deleted.]

3.1.5
certified reference material
CRM
reference material, accompanied by documentation issued by an authoritative body and providing one or more specified property values with associated uncertainties and traceabilities, using valid procedures

[SOURCE: ISO/IEC Guide 99:2007, 5.14, modified — Notes 1, 2, 3, 4 and 5 to entry have been deleted.]

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3.1.6
integrity
property of the parameter(s) of interest, information or content of a sample stored in a container that has not been altered or lost in an unauthorized manner or that has been subject to loss of representativeness

[SOURCE: ISO 5667-3:2024, 3.2]

3.1.7
isotope dilution quantification
process where isotopically labelled standards (e.g. deuterium- or carbon 13-labeled), which are chemically similar isotopic analogs of the target *analytes* (3.1.2), are added to all environmental and quality control and quality assurance samples before extraction and that follow the analytical procedure

Note 1 to entry: Isotope dilution quantification improves quantitative *accuracy* (3.1.1) by accounting for sample-specific procedural losses in the determined analyte concentration

3.1.8
limit of quantification
LOQ
lowest value of a determinand that can be determined with an acceptable level of *accuracy* (3.1.1), which could be estimated by different means and verified in the intended matrix

[SOURCE: ISO 21253-2:2019, 3.4]

3.1.9
recovery
relative recovery
extent to which a known, added quantity of determinant in a sample can be measured by an analytical system

Note 1 to entry: Recovery is calculated from the difference between results obtained from a spiked and an unspiked aliquot of sample and is usually expressed as a percentage.

[SOURCE: ISO 5667-14:2014, 3.8]

3.1.10
traceability
property of a measurement result whereby the result can be related to a reference through a documented unbroken chain of *calibrations* (3.1.4), each contributing to the measurement uncertainty

[SOURCE: ISO/IEC Guide 99:2007, 2.41, modified — Notes 1, 2, 3, 4, 5, 6, 7 and 8 to entry have been deleted.]

3.1.11
yield
absolute recovery
amount of *analyte* (3.1.2) added in the test sample corrected by the relative recovery of the internal standard (analyte-to-internal standard ratio)

Note 1 to entry: Yield is a value that accounts for both sample matrix effect and compound recovery.

[SOURCE: ISO 21253-2:2019, 3.11]

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3.2 Subscripts

a measuring variables for addition

A addition

e measuring variables for the calibration

i identity of the substance

I internal standard

j consecutive number for value pairs

g found value

v default value

M measuring solution

P sample

R reference

4 Principle

A water sample is spiked with an appropriate amount of isotopic labelled standard analogous of each targeted estrogen, before the sample is extracted by solid phase extraction (SPE) cartridge or disk and then cleaned up by SPE. The separation of the substances is achieved by liquid chromatography (LC) or gas chromatography (GC) with an identification and quantification based on mass spectrometry [tandem mass spectrometry (MS/MS) or high-resolution mass spectrometry (HRMS)]. The result is calculated by implementing isotope dilution calibration.

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