SLOVENSKI STANDARD
oSIST prEN ISO 13646:2024

01-februar-2024

Kakovost vode - Določanje izbranih estrogenov v celotnem vzorcu vode - Metoda tekočinske kromatografije (LC) ali plinske kromatografije (GC) z masno selektivnim detektorjem po ekstrakciji na trdni fazi (SPE) (ISO/DIS 13646:2023)

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 (ISO/DIS 13646:2023)

Wasserbeschaffenheit - Bestimmung ausgewählter Estrogene in Gesamtwasserproben - Verfahren mittels Festphasenextraktion (SPE) und anschließender Flüssigkeitschromatographie (LC) oder Gaschromatographie (GC) gekoppelt mit massenspektrometrischer Detektion (MS) (ISO/DIS 13646:2024)

Qualité des eaux - Dosage destrogènes sélectionnés dans des échantillons d'eau totale - Méthode par extraction en phase solide (SPE), avec analyse par couplage chromatographie-spectrométrie de masse (SM) (ISO/DIS 13646:2023)

Ta slovenski standard je istoveten z: **prEN ISO 13646**

ICS:

13.060.50	Preiskava vode na kemične snovi	Examination of water for chemical substances

oSIST prEN ISO 13646:2024 **en,fr,de**

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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

ICS: 13.060.50

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Foreword

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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*.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

Natural and synthetic oestrogens 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 may 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). Beside feminised fish and other endocrine disruptive effects in water ecosystems also they may be a factor in biodiversity loss.[1] Therefore, appropriate measurement methods are necessary which allow estrogen levels below their ecotoxicological level (e.g. or predicted no effect concentration (PNEC) or environmental quality standard (EQS) to be monitored and to demonstrate if a water body is at risk.

This International Standard specifies validated methods for analysing whole water samples satisfying future requirements in support of the European Water Framework Directive WFD and any others regulation worldwide aiming at qualifying the quality of the water environment with respects to the selected estrogens.

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.

ISO takes no position concerning the evidence, validity and scope of this patent right.

The holder of this patent right has assured ISO that he/she is willing to negotiate licences under reasonable and non-discriminatory terms and conditions with applicants throughout the world. In this respect, the statement of the holder of this patent right is registered with ISO. Information may be obtained from the patent database available at www.iso.org/patents.

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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. The methods are based on solid-phase extraction (SPE disk and/or cartridge) followed by liquid or gas chromatography-mass spectrometry detection (tandem mass spectrometry and/or High Resolution Mass Spectrometry). Depending on the sample preparation chosen, it is applicable to the analysis of selected estrogens in drinking water, groundwater and surface water containing suspended particulate matter (SPM) up to 500 mg/l, 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 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 ng/l.

For application that targets the measurements of very low level of concentrations (between the lowest LOQ and 0,1 ng/l) every single step of the methods become critical and imply to fix some additional requirements.

The method can be used to determine further estrogens or hormones in other types of water e.g. 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.

Table 1 — The table summarizes names, abbreviations, structures, CAS numbers, formulas, molecular weights, log Kow of the 5 selected estrogens

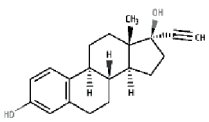
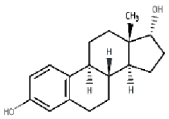
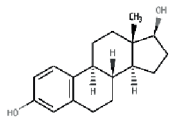
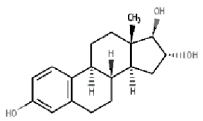
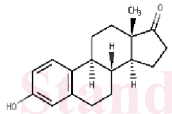
Names	Structure	CAS-RN [a]	Formul [a]	Molecular weight (g/mol)	Log K_{ow}
17alpha- ethinylestradiol (17αEE2) IUPAC name: (13R,17S)-17-ethynyl-13-methyl-7,8,9,11,12,14,15,16-octahydro-6H-cyclopenta[a]phenanthrene-3,17-diol		57-63-6	$C_{20}H_{24}O_2$	296,40	4,52
[a] CAS-RN: Chemical Abstracts System Registration Number					

Table 1 *(continued)*

Names	Structure	CAS-RN [a]	Formul [a]	Molecular weight (g/mol)	Log K_{ow}
17alpha-estradiol (17αE2) IUPAC name: (8R,9S,13S,14S,17R)-13-methyl-6,7,8,9,11,12,14,15,16,17-decahydrocyclopenta[a]phenanthrene-3,17-diol		57-91-0	$C_{18}H_{24}O_2$	272,38	4,13
17beta-estradiol (17βE2) IUPAC name: (8R,9S,13S,14S,17S)-13-methyl-6,7,8,9,11,12,14,15,16,17-decahydrocyclopenta[a]phenanthrene-3,17-diol		50-28-2	$C_{18}H_{24}O_2$	272,38	4,13
Estriol (E3) IUPAC name: (8R,9S,13S,14S,16R,17R)-13-methyl-6,7,8,9,11,12,14,15,16,17-decahydrocyclopenta[a]phenanthrene-3,16,17-triol		50-27-1	$C_{18}H_{24}O_3$	288,38	2,94
Estrone (E1) IUPAC name: (8R,9S,13S,14S)-3-hydroxy-13-methyl-7,8,9,11,12,14,15,16-octahydro-6H-cyclopenta[a]phenanthren-17-one		53-16-7	$C_{18}H_{22}O_2$	270,37	3,69
[a] CAS-RN: Chemical Abstracts System Registration Number					

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 4796-2, *Laboratory glassware — Bottles — Part 2: Conical neck bottles*

ISO 5667:3, *Water quality — Sampling — Part 3: Preservation and handling of water samples*

ISO 5667-4, *Water quality — Sampling — Part 4: Guidance on sampling from lakes, natural and man-made*

ISO 5667-5, *Water quality — Sampling — Part 5: Guidance on sampling of drinking water from treatment works and piped distribution systems*

ISO 5667-6, *Water quality — Sampling — Part 6: Guidance on sampling of rivers and streams*

ISO 5667-11, *Water quality — Sampling — Part 11: Guidance on sampling of groundwaters*

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

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

ISO 21253-2, *Water quality — Multi-compound class methods — Part 2: Criteria for the quantitative determination of organic substances using a multi-compound class analytical method*

ISO/TS 13530:2009, *Water quality — Guidance on analytical quality control for chemical and physicochemical water analysis*

ISO 11352:2012, *Water quality — Estimation of measurement uncertainty based on validation and quality control data*

3 Terms and definitions

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

ISO and IEC maintain terminological 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
accuracy
closeness of agreement between a measured quantity value and a true quantity value of a measurand

Note 1 to entry: The concept "measurement accuracy" is not a quantity and is not given a numerical quantity value. A measurement is said to be more accurate when it offers a smaller measurement error.

Note 2 to entry: The term "measurement accuracy" should not be used for measurement trueness and the term measurement precision should not be used for 'measurement accuracy', which, however, is related to both these concepts.

Note 3 to entry: "Measurement accuracy" is sometimes understood as closeness of agreement between measured quantity values that are being attributed to the measurand.

[SOURCE: ISO/IEC Guide 99:2007, 2.13]

3.2
analyte
substance to be analyzed

[SOURCE: ISO 21253-2:2019, definition 3.1]

3.3
blank
aliquot of reagent water (reagent blank) or of a matrix in which the *analyte* (3.2) 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 21253-1: 2019, definition 3.2]

3.4
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, definition 2.39]

3.5
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, definition 5.14]

3.6
integrity
property that the parameter(s) of interest, information or content of the sample container has not been altered or lost in an unauthorized manner or subject to loss of representativeness

[SOURCE: ISO 5667-3, definition 3.1]

3.7
isotope-dilution quantification
process where isotopically labelled standards (eg.deuterium- or carbon 13-labeled) chemically similar isotopic analogs of the target analytes, are added to all environmental and quality-control and quality-assurance samples before extraction and follow all the analytical procedure. It improve quantitative accuracy by accounting for sample-specific procedural losses in the determined analyte concentration

3.8
limit of quantification
LOQ
lowest value of a determinand that can be determined with an acceptable level of accuracy, which could be estimated by different means and shall be verified in the intended matrix

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

3.9
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]

3.10
traceability
property of a measurement result whereby the result can be related to a reference through a documented unbroken chain of calibrations, each contributing to the measurement uncertainty

[SOURCE: ISO/IEC Guide 99:2007, definition 2.41]

3.11
yield
absolute recovery
amount of *analyte* (3.2) 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, definition 3.11]

4 Principle

The water sample is spiked with an appropriate amount of isotopic labelled standard analogous of each targeted estrogens, before the sample to be 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 implementing isotope dilution calibration.

5 Interferences

5.1 General

Solvents, reagents, glassware, and other sample processing hardware may yield artefacts, elevated baselines, and/or lock-mass suppression causing misinterpretation of chromatograms.

Proper cleaning of glassware is extremely important, because glassware may not only contaminate the samples but may also remove the analytes of interest by adsorption on the glass surface.

Glassware should be rinsed with solvent and washed with a detergent solution as soon after use as is practical. Sonication of glassware containing a detergent solution for approximately 30 s may aid in cleaning. After detergent washing, glassware should be rinsed first with solvent (e.g. methanol) and then with ultrapure water (6.1). Baking of glassware in an oven, programmable and capable of heating to at least 450 °C for 2 h may be warranted.

Sample contamination is a concern because estrogenic substances are biogenic and can be present on human skin or might be used as pharmaceuticals or personal care products. It is important that field and laboratory personnel exercise care to avoid contamination of the samples by avoiding consumption or contact with such materials immediately before and during sample collection and processing procedures. Exercising care is important for both the acquisition and subsequent handling of samples and sample extracts to avoid contamination.

5.2 Interferences with sampling, extraction and concentration

Use sampling containers of materials (7.1) that do not affect the analyte content during the contact time, preferably glass. Avoid plastics and organic materials during sampling, sample storage at (5 ± 3) °C or extraction especially if very low level of concentrations are targeted. High-density polyethylene (HDPE) or polytetrafluoroethylene (PTFE) may be used but are not recommended if very low level of concentrations (< 0,1 ng/l) is to be measured.

If automatic samplers are used, avoid the use of silicone or rubber material for the tubes. If these materials are present, ensure that the contact time is minimized. Rinse the sampling line with the water to be sampled before taking the test sample. ISO 5667-1 and ISO 5667-3 provide guidance.

Storage temperature is at (5 ± 3) °C. For sampling and sample preservation see Clause 8.

During storage of the test samples, losses of components may occur due to adsorption on the walls of the containers. The extent of the losses may depend on the storage time.

Commercially available solid-phase extraction (SPE cartridge or disk) may differ in quality. Variations in the selectivity of the materials also frequently occur from batch to batch, thus possibly causing significant deviations in extraction yield. This does not basically impair their suitability, apart from a resulting higher quantification limit for individual substances.

Avoid major fluctuations in the extraction times and elution procedures within one sample sequence when analyzing the samples.

Water samples containing high content of SPM or DOC can lead to clogging in case of SPE cartridge (see subclause 9.2.2) that could be prejudicial to extraction recovery. To overpass, reduction of sample

volume or switching to SPE disk (see subclause 9.2.3) extraction may be implemented. Another solution is the application of glass wool or sand to the cartridge (filling height 1 cm to 2 cm) to achieve higher sample volumes.

Repeated uses and cleaning procedures of glassware may cause active sites on the glass surface that may irreversibly adsorb the selected estrogens and also be responsible of cross contamination.

To prevent cross contamination between series, SPE apparatus, tubing and pieces in contact with samples shall be dismantled and cleaned with successively e.g. (hot) water with detergents, solvents (e.g. methanol, acetone, ethanol) then rinsed with water and dried.

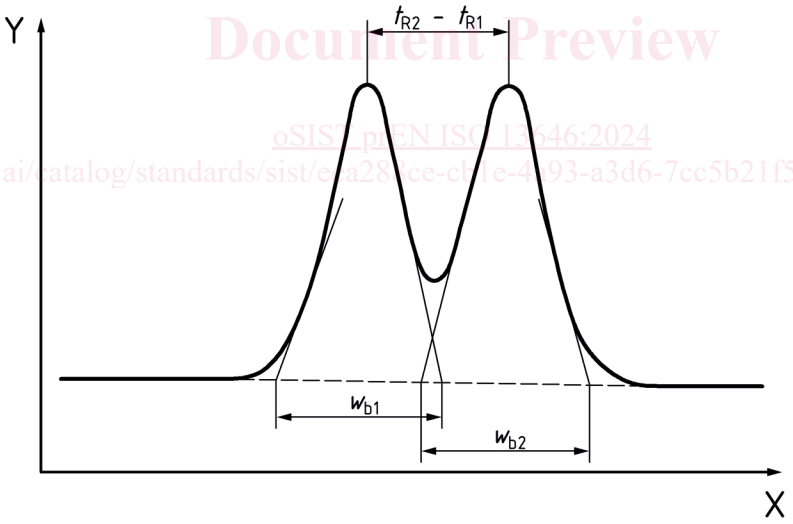
Interferences co-extracted from samples will vary considerably from source to source, depending on the diversity of the site being sampled. Interfering substances may be present at concentrations several orders of magnitude higher than the selected estrogens. The most frequently encountered interferences are humic and other acids. Because very low levels of estrogens shall be measured by this method, elimination of interferences is essential (implementation of clean-up (see subclause 9.3).

5.3 Interferences during high performance liquid chromatography and mass spectrometry

Substances with similar retention times and masses as the target estrogens may lead to interferences and overlapping or incompletely resolved peaks in the chromatogram. Depending on their intensity those co-eluents can affect the trueness of the analysis.

17αE2 and 17βE2 are epi-isomers and as consequence have the same transitions in MS. It is critical to separate both substances. A minimal chromatographic resolution (R) ≥ 1,2 is suitable. If the criterion cannot be reached, a suitable column shall be chosen to meet the required resolution [see Annex E and Annex F for examples].

The chromatographic resolution is calculated according to Figure 1 and Formula (1).



Key

X	time
Y	intensity
t_{R1}, t_{R2}	retention time of eluting substances 1 and 2 in seconds (s)
w_{b1}, w_{b2}	peak width at the base of each peak in seconds (s)

Figure 1 — Resolution of chromatographic peaks