



International
Standard

ISO 13646

**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**

**First edition
2025-10**

*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

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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).

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.

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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*