



SLOVENSKI STANDARD

SIST ISO 20179:2010

01-september-2010

Kakovost vode - Določevanje mikrocistinov - Metoda z ekstrakcijo na trdni fazi (SPE) in s tekočinsko kromatografijo visoke ločljivosti (HPLC) z ultravijolično (UV) detekcijo

Water quality - Determination of microcystins - Method using solid phase extraction (SPE) and high performance liquid chromatography (HPLC) with ultraviolet (UV) detection

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Qualité de l'eau - Dosage des microcystines - Méthode utilisant l'extraction en phase solide (SPE) et la chromatographie en phase liquide à haute performance (CLHP) avec détection dans l'ultraviolet (UV)

Ta slovenski standard je istoveten z: ISO 20179:2005

ICS:

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

ISO
20179

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**Water quality — Determination of
microcystins — Method using solid
phase extraction (SPE) and high
performance liquid chromatography
(HPLC) with ultraviolet (UV) detection**

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*Qualité de l'eau — Dosage des microcystines — Méthode utilisant
l'extraction en phase solide (SPE) et la chromatographie en phase
liquide à haute performance (CLHP) avec détection dans l'ultraviolet
(UV)*

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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.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

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

ISO 20179 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 2, *Physical, chemical and biochemical methods*.

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Introduction

The user should be aware that particular problems could require the specification of additional conditions.

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Water quality — Determination of microcystins — Method using solid phase extraction (SPE) and high performance liquid chromatography (HPLC) with ultraviolet (UV) detection

WARNING — The method requires use of microcystin-containing solutions. Microcystins are highly hepatotoxic to humans. Laboratory wastes of microcystins shall be collected separately and disposed as highly toxic chemical waste. Long-term decontamination with concentrated sodium hypochlorite (NaClO) solution is also possible.

Persons using this International Standard should be familiar with normal laboratory practice. This standard 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 and to ensure compliance with any national regulatory conditions.

IMPORTANT — It is absolutely essential that tests conducted according to this standard be carried out by suitably trained staff.

1 Scope

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This International Standard specifies a method for the determination and quantification of microcystins in raw water (containing biomass) and treated water, such as tap water. The method described is validated for MCYST-RR, MCYST-YR, and MCYST-LR. It is also applicable for the determination of several structure variants^[1] of these microcystins, but an unambiguous identification cannot be made due to the lack of commercially available standards and due to co-elution.

The threshold value of 1 µg/l of MCYST-LR in water, proposed by the World Health Organization, can be followed after microcystin enrichment using solid phase extraction (SPE).

2 Normative references

The following referenced documents are indispensable for the application 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 3696:1987, *Water for analytical laboratory use — Specification and test methods*

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*

3 Abbreviated terms

For the purposes of this document, the following abbreviated terms apply.

APCI	atmospheric pressure chemical ionization
MCYST	microcystin
MCYST-LR	microcystin containing leucine (L) and arginine (R)

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MCYST-RR	microcystin containing two arginine (R) units
MCYST-YR	microcystin containing tyrosine (Y) and arginine (R)
SIM	selected ion monitoring
SEC	size exclusion chromatography

4 Principle

Water samples containing cyanobacterial material (biomass) shall be filtered first. The biomass is extracted separately with a solvent (methanol/water). The extract is filtered, diluted and a solid phase extraction (SPE) is applied for sample clean-up. The filtrate is treated as a pure water sample (see below).

Pure water samples such as tap water are enriched using SPE. The microcystins are eluted from the SPE cartridges with methanol/water [90/10 by volume] containing 0,1 % by volume of trifluoroacetic acid (TFA).

Microcystins are quantified by reversed-phase high performance liquid chromatography (RP-HPLC) with ultraviolet/diode array detection at 238 nm.

5 Reagents

Use only reagents of recognized analytical grade and water complying with grade 3 as specified in ISO 3696:1987, unless otherwise specified.

5.1 Methanol, CH₃OH, HPLC grade. (standards.iteh.ai)

5.2 Acetonitrile, CH₃CN, HPLC grade. [SIST ISO 20179:2010](https://standards.iteh.ai/catalog/standards/sist/d3a7b88f-a8bb-4173-aeb5-2fdcf73e767/sist-iso-20179-2010)
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5.3 Trifluoroacetic acid, TFA, CF₃COOH. [SIST ISO 20179:2010](https://standards.iteh.ai/catalog/standards/sist/d3a7b88f-a8bb-4173-aeb5-2fdcf73e767/sist-iso-20179-2010)
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5.4 Standard dilution solution, SPE rinsing solvent, and re-dissolving solvent.

Methanol/water [20/80 by volume].

5.5 Extraction solution

Methanol/water [75/25 by volume].

5.6 SPE elution solution

Methanol/water [90/10 by volume] containing 0,1 % by volume TFA.

5.7 Sodium thiosulfate, solution.

Dissolve 1 g of sodium thiosulfate Na₂S₂O₃ (anhydrous or with 5 H₂O) in 100 ml of water. The final concentration is $\rho = 10$ g/l (63 mmolar in case of anhydrous Na₂SO₃).

5.8 Ammonium hydroxide solution

Commercially available ~ 1 mol/l of ammonium hydroxide solution, NH₄OH.

5.9 Solid phase extraction cartridges (SPE) for microcystin enrichment

The column shall have a minimum capacity (amount of analyte to be retained by the column) of not less than 100 µg of each microcystin and shall give a recovery of not less than 80 % for MCYST-LR and not less than 70 % for MCYST-RR and MCYST-YR when applied as a standard solution in water containing 0,05 µg of each microcystin.

NOTE The recovery strongly depends on the SPE cartridge material/brand, material specifications such as carbon load, particle size etc. The recovery data are based on C-18 cartridges determined by a single measurement. The material should have the following material specifications: carbon load (16,9 %), particle diameter (54 µm), surface coverage (333 µg/m² based on % C) cartridge volume (3 ml), material per cartridge (500 mg). If the above required recovery values can not be reached, changing the brand of the SPE cartridge is recommended.

Disk-type SPE cartridges may also be used for the microcystin enrichment from water samples [2].

5.10 HPLC mobile phase solution (A)

To a 1 000 ml volumetric flask, add 800 ml of acetonitrile (5.2) and 500 µl of TFA (5.3) and bring to volume with acetonitrile. Transfer this solution in a HPLC-eluent bottle. Degas the solution before use.

This solution is stable at room temperature for about 3 weeks.

5.11 HPLC mobile phase solution (B)

To a 1 000 ml volumetric flask, add 800 ml of water and 500 µl of TFA (5.3) and bring to volume with water. Transfer this solution in a HPLC-eluent bottle. Degas this solution before use.

This solution is stable at room temperature for about 2 weeks.

5.12 HPLC mobile phase gradient (an example)

Table 1 — HPLC mobile phase gradient

Time min	HPLC mobile phase solution (A) Acetonitrile with 0,05 % TFA (5.10) %	HPLC mobile phase solution (B); water with 0,05 % TFA (5.11) %	Total volume flow rate, depending on the column ml/min
0	30	70	0,3 to 1,0
10	35	65	0,3 to 1,0
40	70	30	0,3 to 1,0
42	100	0	0,3 to 1,0
44	100	0	0,3 to 1,0
46	30	70	0,3 to 1,0
55	30	70	0,3 to 1,0

5.13 Microcystins, commercially available film in ampoules.

NOTE The quality of commercially available microcystins is very variable. Thus, it is important to follow the procedure given in 5.14.

5.14 Microcystin stock solutions

To determine the exact concentration of microcystins, dissolve in each stock solution the individual microcystin delivered from the supplier in pure methanol (5.1). Record the absorption curve between 220 nm and 250 nm in 1 cm quartz glass cells in a spectrophotometer with methanol (5.1) in the reference cell.