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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

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*.

Introduction

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

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

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)

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, CH_3OH, HPLC grade.

5.2 Acetonitrile, CH_3CN, HPLC grade.

5.3 Trifluoroacetic acid, TFA, CF_3COOH.

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 $Na_2S_2O_3$ (anhydrous or with 5 H_2O) in 100 ml of water. The final concentration is ρ = 10 g/l (63 mmolar in case of anhydrous Na_2SO_3).

5.8 Ammonium hydroxide solution

Commercially available ~ 1 mol/l of ammonium hydroxide solution, NH_4OH.

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/m^2 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	**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**
min	%	%	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.

Calculate the mass concentration of each microcystin, ρ_i, in micrograms per millilitre, µg/ml, using Equation (1):

$$\rho_i = \frac{A_{max} \cdot M_i \cdot 1000}{\varepsilon_i \cdot d} \quad (1)$$

where

A_{max} is the absorbance determined at the maximum of the absorption curve;

M_i is the molar mass of each microcystin, in grams per mol; g/mol;

ε_i is the molar absorptivity of each microcystin in methanol (5.1), in litres per (mole × centimetre), l/(mol × cm);

d is the optical path length of the cell, in centimetres, cm;

1 000 is a calculation factor to achieve the final unit micrograms per millilitre, µg/ml.

M_i and ε_i are tabulated in Table 2.

Table 2 — Molar mass and molar absorptivity of MCYST-LR, -YR, and -RR (in methanol, at 238 nm)

Microcystin	M_i g mol^{-1}	ε_i l mol^{-1} cm^{-1}
-LR	994	39 800
-YR	1 044	39 800
-RR	1 037	39 800
NOTE Data taken from Reference [1]. For further details refer to this reference.		

For further HPLC analysis, the solvent methanol/water ratio for the MCYST-LR, -YR, and -RR standards can be adjusted to 20/80 by volume (i.e. to the standard dilution solution described in 5.4), by adding water and allowing a concentration of 10 µg/ml for each microcystin.

5.15 Mixed microcystin stock solutions

Prepare a standard solution containing 2,5 µg/ml each of MCYST-LR, -YR, and -RR in the standard dilution solution (5.4). Store it below −16 °C. To avoid incorporation of water by condensation, do not open the vial until its contents have reached room temperature.

If the solution is to be stored for a long period, use a hermetic vial. In case of doubt, weigh the vial and record any changes in mass during storage.

5.16 Mixed microcystin standard solutions

Pipette the volumes of microcystin stock solutions (5.14) given in Table 3 into 1 ml vials.

To each vial, add the volume of the standard dilution solution (5.4) given in Table 3 to achieve a final volume of 1 000 µl, and shake well.

Table 3 — Pipetting scheme for the mixed microcystin standard solutions

Standard solution	Withdrawal volume from each microcystin stock solution [MCYST-LR, -YR, -RR (5.14)] µl	Volume to add from the standard dilution solution (5.4) to achieve a final volume of 1 000 µl µl	Mass concentration of standard solution µg/ml		
			MCYST-LR	MCYST-YR	MCYST-RR
1	20	940	0,2	0,2	0,2
2	40	880	0,4	0,4	0,4
3	100	700	1,0	1,0	1,0
4	200	400	2,0	2,0	2,0
5	300	100	3,0	3,0	3,0

5.17 Spiking solution for method control

Prepare a spiking solution by pipetting 200 µl of the mixed microcystin stock solution (prepared according to 5.15) into a 500 ml volumetric flask. Dilute it to the mark with water (tap-water or blank water from a natural lake), and shake well.

The concentration of this spiking solution is 1 µg/l for MCYST-LR, -YR, and -RR.

6 Apparatus

6.1 General

The laboratory glassware and equipment to be used is not specified in this International Standard, as the choice of apparatus will depend on the specific applications and circumstances.

Avoid the use of plastics whenever possible. This is necessary because the use of plastics (e.g. plastic pipettes, plastic tubing or plastic cartridges) may cause losses of microcystins through absorption on the surface walls.

6.2 Adjustable horizontal shaker, needed only for the analysis of samples containing phytoplankton.

6.3 Glass microfibre filter paper, retention size 1 µm to 2 µm.

The maximum diameter of the filter should be 47 mm.

Filtration is needed only for the analysis of samples containing phytoplankton.

6.4 SPE reservoir, 500 ml with connector for cartridges.

6.5 Vacuum pump for SPE

6.6 Laboratory centrifuge, $\geqslant$ 4 000 min^{-1}, relative centrifugal force (RCF) $\geqslant$ 10 000 g.

The use of an explosion-proof centrifuge is strongly advised due to the use of inflammable extraction solvents.

6.7 Ultrasonic probe, with characteristics of ~ 60 W, ~ 20 kHz.

6.8 Ultrasonic bath