SLOVENSKI STANDARD
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Kakovost vode - Določanje mikrocistinov - Metoda s tekočinsko kromatografijo s tandemsko masno spektrometrijo (LC-MS/MS)

Water quality - Determination of microcystins - Method using liquid chromatography and tandem mass spectrometry (LC-MS/MS)

Qualité de l'eau - Dosage des microcystines - Méthode par chromatographie en phase liquide couplée à la spectrométrie de masse en tandem (CL-SM/SM)

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Water quality — Determination of microcystins — Method using liquid chromatography and tandem mass spectrometry (LC-MS/MS)

Qualité de l'eau — Dosage des microcystines — Méthode par chromatographie en phase liquide couplée à la spectrométrie de masse en tandem (CL-SM/SM)

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Contents

Page

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 documents 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.

Water quality — Determination of microcystins — Method using liquid chromatography and tandem mass spectrometry (LC-MS/MS)

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 a method for the quantification of twelve microcystin variants (microcystin-LR, -LA, -YR, -RR, -LY, -WR, -HtyR, -HilR, -LW, -LF, [Dha^{7}]-microcystin-LR, and [Dha^{7}]-microcystin-RR) in drinking water and freshwater samples between 0,05 µg/l to 1,6 µg/l. The method can be used to determine further microcystins, provided that analytical conditions for chromatography and mass spectrometric detection has been tested and validated for each microcystin. Samples are analysed by LC-MS/MS using internal standard calibration.

This method is performance based. The laboratory is permitted to modify the method, e.g. increasing direct flow injection volume for low interference samples or diluting the samples to increase the upper working range limit, provided that all performance criteria in this method are met.

Detection of microcystins by high resolution mass spectrometry (HRMS) as an alternative for tandem mass spectrometry (MS/MS) is described in Annex A.

An alternative automated sample preparation method based on on-line solid phase extraction coupled to liquid chromatography is described in Annex B.

When instrumental sensitivity is not sufficient to reach the method detection limits by direct flow injection, a solid phase extraction clean-up and concentration step is described in Annex C.

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 3696, *Water for analytical laboratory use — Specification and test methods*

3 Terms and definitions

No terms and definitions are listed in this document.

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 http://www.electropedia.org/

4 Principle

This method is designed to identify and quantify total (free + intracellular) microcystins in water by direct flow injection liquid chromatography and tandem mass spectrometry (LC-MS/MS) with electrospray ionization[1],[2]. 12 microcystins (Table 1) are determined quantitatively by multi-point calibration using nodularin as internal standard.

Table 1 — Microcystin variants included in the method

Microcystin variant	CAS-RN[a]	Molecular formular
Microcystin-LR	101043-37-2	$C_{49}H_{74}N_{10}O_{12}$
Microcystin-RR	111755-374	$C_{49}H_{75}N_{13}O_{12}$
Microcystin-LA	96180-79-9	$C_{46}H_{67}N_7O_{12}$
Microcystin-YR	101064-48-6	$C_{52}H_{72}N_{10}O_{13}$
Microcystin-LY	123304-10-9	$C_{52}H_{71}N_7O_{13}$
Microcystin-WR	138234-58-9	$C_{54}H_{73}N_{11}O_{12}$
Microcystin-HtyR	913178-65-1	$C_{52}H_{72}N_{10}O_{13}$
Microcystin-HilR	N/A	$C_{50}H_{76}N_{10}O_{12}$
Microcystin-LW	157622-02-1	$C_{54}H_{72}N_8O_{12}$
Microcystin-LF	154037-70-4	$C_{52}H_{71}N_7O_{12}$
[Dha^7]-Microcystin-LR (dmLR)	120011-66-7	$C_{48}H_{72}N_{10}O_{12}$
[Dha^7]-Microcystin-RR (dmRR)	131022-02-1	$C_{48}H_{73}N_{13}O_{12}$

[a] CAS-RN: Chemical Abstracts System Registration Number.

Nodularin can be naturally occurring in brackish water samples. Blank levels should be checked before analysis for these samples. Alternatively, ^{15}N-labelled microcystin surrogates should be used if available.

NOTE Some microcystins (e.g. demethylated RR variants) have the same exact mass and a similar chromatographic behaviour. While some can be distinguished by their fragmentation (e.g. [Asp^3, Mdha^7] MC-RR and [MeAsp^3, Dha^7)] MC-RR), others even show the same fragmentation (e.g. Asp^3, Mdha^7] MC-RR and [Asp^3, Dhb^7] MC-RR).

Water samples are homogenized to disperse cell aggregates. A 5 ml aliquot is transferred to a 15 ml centrifuge tube, internal standard is added, and cells are lysed by three cycles of freeze/thaw. Solid particles and cell debris are centrifuged and syringe filtered directly into an autosampler vial. Quantification of microcystins is done by an internal standard method using LC-MS/MS or HRMS (Annex A).

Alternatively, lysed and filtered samples can be injected using an on-line SPE instrumental configuration (Annex B) or manual SPE (Annex C) for an increased sensitivity.

5 Interferences

This analysis was developed using liquid chromatography (LC) tandem mass spectrometry (MS/MS) with electrospray ionization (ESI), on a triple quadrupole mass spectrometer. Acquisition mode was based on multiple reaction monitoring (MRM). Isobaric interferences that are not resolved by chromatography or the unit mass resolution of the tandem quadrupoles may be present in some samples. These samples may require additional selectivity via additional sample clean-up and/or high-resolution mass spectrometry (HRMS). Some microcystins with the same exact mass might not be able to be distinguished by HRMS, but by their different fragmentation patterns, a few congeners cannot be distinguished by either of these approaches.

Variable instrument response and/or inconsistent retention times may be observed in the first gradient runs of the day. The column requires conditioning by running at least one gradient program prior to the first sample injection of the day.

5.1 Biases

All labware that contacts microcystins should have relatively inert surfaces; otherwise, compound losses may occur by adsorption onto the glass. Unscratched borosilicate glassware or polyethylene is recommended. To further minimize this effect, sample preparation should be carried out in a timely manner and quantification by matrix matched calibration standards is preferred.

Analytical results (method precision and accuracy) are calculated by internal standard quantitation methods and may be affected by differences in the recovery of the internal standard relative to that of the target compounds. When available, ^{15}N-labelled microcystins should be used for this purpose.

The concentration of on-site samples will vary greatly depending on the density of algae at each sampling point, and the concentration difference will also be large for each microcystins. Given this, the multi-point calibration curves for the microcystins, using a fixed amount of internal standard, are non-linear. Quantification is done by a second order (quadratic) curve-fitting procedure.

5.2 Limitations

The sample preparation method is restricted to water samples. Applicability of the method to samples with very high organic content, such as water containing high concentrations of humic materials, is unknown.

The working range of this method is 0,05 µg/l to 1,6 µg/l. If samples with a higher microcystin concentration than 1,5 µg/l are found or predicted, a smaller aliquot of sample should be taken, and a dilution factor applied to the final result. Surface waters containing thick cyanobacterial blooms may interfere with the instrumental analysis. In these cases, a smaller amount of sample can be diluted, and volume should be recorded for the final calculation of microcystins concentration.

Standards of specific microcystin variants are not always available on a continuous basis. Foreign suppliers are sometimes restricted by law and are not always able to export algal toxin standards to different countries. Before being used, newly prepared standards shall be compared to standards in current use. Purity of the different lots of standards should be checked against reference materials when available. Alternatively, purity can also be confirmed using universal detector like HPLC-UV (ISO 20179).

6 Reagents and standards

6.1 General

If available, reagents of purity grade “for analysis” or “for residue analysis” are used. The amount of impurities contributing to the blank value or causing signal interferences shall be negligible. This shall be checked regularly (see section for blank value measurements).

Solvent, water and reagents intended for use as elution agents shall be compatible with HPLC and mass spectrometry.

Microcystins are potent hepatotoxins. Laboratory safety measures should be strictly followed throughout the sample preparation (including lab gloves, labcoat, safety glasses) to prevent human exposure to these toxins.

NOTE 1 High purity grades of solvent applicable for use are available commercially.

NOTE 2 Reagents listed as “prepare as required” have an expiry date of one year from the moment they were prepared.

NOTE 3 Prepared standard solutions are stored at (5 ± 3) °C, with an expiry date of one year from the moment they were prepared.

Stock and intermediate standard solutions should be used as a reference, other stock and intermediate concentrations are acceptable to prepare the final working solutions.