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Water quality — Multi-compound class methods —

Part 2: Criteria for the quantitative determination of organic substances using a multi-compound class analytical method

Qualité de l'eau — Méthodes d'analyse de composés multi-classes —

Partie 2: Critères pour la détermination quantitative de composés organiques avec une méthode d'analyse de composés multi-classes

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Contents

Page

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

A list of all parts in the ISO 21253 series can be found on the ISO website.

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 — Multi-compound class methods —

Part 2: Criteria for the quantitative determination of organic substances using a multi-compound class analytical method

1 Scope

This document specifies the criteria for developing an in-house mass spectrometry-based method for quantitative analysis of multiple subgroups of organic substances in the scope of physical-chemical analysis of water.

This document supplements ISO/TS 13530 which provides guidance on the initial characterization of the measurement performances, by providing details to select the test matrix and internal standards and criteria for analyte and internal standard recoveries.

This document is not intended as a substitute for the currently applicable analytical standards dedicated to organic compounds but as a resource bringing additional characterization elements.

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, *Water quality — Calibration and evaluation of analytical methods and estimation of performance characteristics — Part 1: Statistical evaluation of the linear calibration function*

ISO 8466-2, *Water quality — Calibration and evaluation of analytical methods and estimation of performance characteristics — Part 2: Calibration strategy for non-linear second-order calibration functions*

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

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*

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 http://www.electropedia.org/

3.1
analyte
substance to be determined

[SOURCE: ISO/TS 28581:2012, 3.1]

3.2
blank
aliquot of reagent water (reagent blank) or of a matrix in which the *analyte* (3.1) 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.

3.3
calibration curve
expression of the relation between indication and corresponding measured quantity value

[SOURCE: ISO/IEC Guide 99:2007, 4.31]

3.4
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/TS 21231:2019, 3.2.5, modified — Note to entry has been excluded.]

3.5
analytical method
unambiguously written procedure describing all details required to carry out the analysis of the determinand or parameter, namely: scope and field of application, principle and/or reactions, definitions, reagents, apparatus, analytical procedures, calculations and presentation of results, performance data and test report

[SOURCE: ISO/TS 16489:2006, 3.3]

3.6
recovery
relative recovery
extent to which a known, added quantity of determinand 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]

3.7
relative retention time
ratio between the retention time of the target compound and the retention time of the calibration standard

[SOURCE: ISO 15680:2003, 3.5, modified — "retention-time standard" has been replaced by "calibration standard"]

3.8
injection standard
standard mixture added to a sample before injection into the GC-MS apparatus, to monitor variability of instrument response and to calculate internal standard recovery

Note 1 to entry: The same definition is applied for GC-MS/MS, LC-MS and LC-MS/MS.

[SOURCE: ISO 28540:2011, 3.4, modified — Note to entry has been replaced.]

3.9
subgroup of compounds
analytes (3.1) pre-classified into groups that share similar patterns of analytical behaviour in response to extraction mode and analysis

3.10
internal standard
compound added in a known amount to the sample from the beginning of the protocol and enabling analytical coverage throughout the procedure, and that is used to correct for losses during sample preparation and analysis by accounting for all-system matrix effects (recoveries, ionization effect, variability of the detector response of the instrument for example)

Note 1 to entry: Determining the ratio of characteristic molecule signal intensity to the internal standard makes it possible to calculate the quantitative ratio between molecule and internal standard and thereby to deduce molecule concentration in the sample. An identical amount is also added to the calibration standard solutions.

3.11
yield
absolute recovery
amount of *analyte* (3.1) 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.

4 Principle

This document specifies the critical points to be considered when developing a mass spectrometry-based method for the analysis of multiple subgroups of organic substances in water samples. The critical points addressed here involve:

— selection of the matrix;

— internal standards and internal standard recoveries;

— analyte recovery.

This document proposes a rating-scale method for analytical protocols dedicated to huge numbers of compounds, making it possible to give the method a reliability score for each compound. When a review of these critical points concludes that the method can reliably quantify the analytes, then the initial characterization of the measurement performances of the method can be started.

This document concludes by proposing quality assurance criteria that should be made routine to ensure that performance levels do not degrade over time.

If there is a need to expand the initial list of reliably quantifiable analytes in a method, then verify the analogue transitions, retention times, LOQ performances and accuracy. Modification of method performances of previously characterized analytes to new performance levels requires an update of the method characterization in the way described before.

For each substance, the method can only be claimed as reliable for those matrices that were effectively tested during the characterization process.

If one or more purification steps need to be carried out, and if substances are present that are liable to create interferences in the chromatogram or contaminate the analytical system, then the measured yield for each substance shall account for the purification step(s).

5 Selection of the matrix

The method shall be characterized on a matrix that is representative of the claimed scope. The water matrix is characterized by its physicochemical properties (SPM, TOC, conductivity, pH, major ions, etc.).

Additional information on the source of the sample (marine water, waste water, groundwater, drinking water, etc.) can inform the final user on the likelihood of certain physicochemical parameters. These parameters shall be identified at the start of of the method development and recorded.

The representative matrix can be natural or synthetic. Laboratory-grade water (distilled water, deionized water) is not representative of any natural water.

In the absence of a suitable analyte-free real matrix blank (especially for the characterization of the LOQ), then the natural matrix can be diluted (with drinking water or laboratory-grade water, for example) in order to lower the concentration of organic compounds, taking care to appropriately adjust the modified properties (SPM, TOC, conductivity, pH, etc.) as specified in ISO/TS 21231:2019, 7.1.

6 Sample preservation prior to analysis

In the absence of normative requirements expressly covering the analytes, storage time and sample preservation mode shall be defined and verified from collection of the sample through completion of all analysis-related operations. The storage time defined commences at the date of sample collection.

Sample preservation integrity tests shall be conducted on the representative matrix (see Clause 5) and, where appropriate, in presence of the preservative. They shall be conducted at least one level at or near 10 × LOQ or at the regulatory value, with field/laboratory duplicates; ISO 5667-3:2018, Annex C provides a more complete protocol, for example, where appropriate, these tests shall also extend to preservation time for sample extracts.

Special attention shall be paid to any breakdown products of analyte molecules (parent molecules and metabolites) if they are included in the scope of the method.

7 Internal standards and injection standards

7.1 General

One or more internal standards shall be used and introduced into the sample right at the start of the protocol. This is the most efficient way to screen for and correct any potential matrix-effect interferences at extraction and analysis of each sample.

The use of stable isotopically labelled internal standards is recommended to account for specific losses of individual analytes and for matrix effects.

The injection standards are added just before the analysis step. They provide additional information on the instrumental analysis, detector response for example, and are used to calculate internal standard recoveries.

Both these types of standards can be used for the quantification step, although the use of internal standards is preferred.

7.2 Selection of standards

The internal standard and the injection standard shall possess properties making them representative of the analytes. Suitable criteria are available in ISO/TS 13530:2009, 4.4.3.1. The near-identical retention time may also be a useful criterion.

For methods featuring a large number of compounds and in those cases where it is impossible to get an internal standard for each analyte, it is recommended to assign one internal standard or injection standard per subgroup of compounds, giving a suitable justification for the rationale involved.

For complex matrices liable to create strong interferences, for example signal enhancement, signal suppression, or altered retention time, it is recommended to have a labelled analogue for each substance subject to such interferences.

Every effort shall be made to ensure the internal or injection standards are well distributed along the full duration of the chromatogram, and if necessary, in each ionization mode.

Cumulating a series of internal standards and injection standards may help to overcome the analytical difficulties involved when implementing multi-compound class methods and to make the quantification reliable (see 11.2).

For the mass spectrometric detection, it is recommended to use a stable isotopically labelled analogue as an internal standard; ^{13}C or ^{15}N-labelled compounds are preferred rather than deuterated compounds. There is generally an observable drift in retention time compared to the natural compound when working with isotopically labelled standards (isotope effect).

Only a stable part of the molecule shall be labelled, and degree of labelling shall not change over the course of analysis. Exchanges may take place, via tautomerization processes for example, and so this type of mechanism shall be experimentally verified in the event that the abundance of internal standard is shown to vary.

Spectral overlapping between labelled analogue and native substances shall be avoided. Consequently, for the majority of small-molecule applications, the labelled analogue should preferably have a monoisotopic mass greater than at least 3 Da.

The isotopic purity of the internal standards shall be checked. It is not a critical factor provided that its impurities, including unlabelled analogue, have preferably zero contribution on target compounds of the analytical method. In case it is not possible, the contribution of the labelled analogue to the unlabelled analogue should be negligible at LOQ level.

To confirm the accuracy of the method, it is necessary to confirm the right balance between labelled analogue and native substances. It may be necessary to run pre-investigative studies to check that:

— the internal standard has been fully extracted without degradation, or

— that internal standard is in equilibrium with the sample and bound to suspended particulate matter (SPM) in the same way as the native substance.

If a filtration is intended in the laboratory, the internal standards can be added before filtration, allowing delay for the equilibrium to take place, and the applicability of the internal standards for this step shall be verified (see 9.3).

8 Calibration

In order to analyse and quantify a substance of interest, the analyst shall acquire the compound as a calibration standard of the highest purity possible. This analytical standard may be sourced either pure or in solution. Firstly, it will guarantee that instrumental analysis is both feasible and under control. It will also confirm that the substance is present in the sample and will serve to establish the calibration curve needed to quantify, and further to establish the performance of the extraction step.

Important information (whether for in-house prepared solutions or commercially-sourced solutions) is:

— the identity of the substances: special attention is needed when dealing with mixtures of substances (nonylphenols for example) and with separately distributed isomers or isomer mixtures (endosulfan for example);

— their purity;

— the uncertainty on their assessed concentrations;

— the behaviour patterns of the internal standards and injection standards, which is acquired by individually injecting them;

— the behaviour pattern of the compounds in the course of analysis, whether injected individually or in mixture of a small number of substances on one hand, and mixed with all the other intended substances,

internal standards, injection standards in calibration solvent solutions on the other hand, so as to check for any synergistic or antagonistic substance-to-substance effects in the course of analysis. If there are differences in the responses, it means the instrumental analysis method is not optimized. If this method nevertheless remains the technique employed by the laboratory, then the selection of internal standards should include the stable isotopically labelled analogue of each compound whose response presents the above-stated behaviour, or to apply the standard addition method.

Identity of the standard and trueness of the calibration shall be double-checked by using a cross-check solution prepared with the same substance but obtained through a different reference frame: for example, different supplier, different batch number, different preparation —exclusively if made up from the pure compound, or a certified reference (CRM) if available.

Special attention should be given to those substances that show similar chemical structures (same family, parent substances and metabolites) and the same retention time in order to preclude a potential loss of specificity. Loss of specificity can be detected by injecting individual standard solutions and scrutinizing all of the transitions specified for the method.

Some instruments feature an automatic ionization optimization mode for injecting mixtures of substances which, in some cases, can lead to loss of specificity. The optimization parameters proposed shall therefore be verified with the individual compounds.

For calibration protocols, see the ISO 8466 series and ISO/TS 13530. Internal standard calibration shall be preferred to external calibration.

9 Recovery determination

9.1 General

The analytical method cannot be developed further without knowing the matrix-matched recovery of each substance. Matrix effect on the analysis and compound extraction recovery can be distinguished using Annex A. This information helps guiding further development of the method, particularly in terms of selecting a calibration system.

If a filtration is intended in the method, the effect of a filtration step on the recovery shall be determined.

9.2 Quantification by external calibration

In the absence of regulatory or normative required performance criteria, the following criteria for the recovery apply.

— For each level of analyte concentration, obtain a mean recovery at intermediate precision (n = at least 10, 5 × 2 replicates, for example) in the range 70 % to 120 % with a coefficient of variation ≤ 20 %.

— In cases where recovery is less than 70 %, the coefficient of variation in recovery shall not vary more than 20 % around this recovery value. Otherwise, the analyst shall use an internal standard.

— In cases where the coefficient of variation in recovery (in the range 70 % to 120 %) is >20 %, if this parameter cannot be optimized (another extraction method, another analytical method, etc.), then an internal standard shall be used.

— If recovery is >120 %, it is strongly recommended to identify an alternative (another extraction method, another analytical method, etc.).

If it proves impossible to meet the characterization criteria stipulated in this article, it is recommended to use the standard additions method in 9.2.

In all cases, attention shall be drawn to the impact of the recovery and its coefficient of variation on the uncertainty.

9.3 Quantification by internal calibration

When quantification is done by internal calibration, the only criterion to consider is the criterion applied to yield. Mean yield shall be in the range 70 % to 120 %, with a coefficient of variation ≤20 %.

The recommended minimum recovery for an internal standard is 30 %.

In any cases and mainly in case of low mean recovery, attention shall be drawn to the impact on the uncertainty.

10 Limit of quantification (LOQ)

LOQ shall be established by matrix-matched measurement and for each analyte claimed as in the scope of the method. The determination of LOQ covers the method's entire protocol.

In the absence of any other requirement or national regulation, the LOQ can be obtained as explained in ISO/TS 13530:2009, 3.1.7 and CEN/TS 16800:2016, 6.4.5[11].

In the case of mass spectrometry analyses for quantification and qualification where molecules are identified via transitions or ions, the LOQ shall be verified for the MS quantification transition or ion, provided than qualification transitions or ions are present (see ISO 21253-1).

When studying the LOQ, at least one matrix blank shall be processed in exactly the same way as the samples (including all pre-processing steps). Concentration of substances in the matrix blank shall not go above the LOQ. Otherwise, an investigation should be led to find and remove the cause. If the blank remains above the LOQ, then it shall not be subtracted from the values obtained for the sample's quantification, but the LOQ shall be re-assessed instead.

11 Results

11.1 Identification of the compounds

According to ISO 21253-1.

11.2 Quantification

Quantification requires an adequate number of appropriately distributed calibration points. Calibration accuracy shall meet a set of minimum requirements in accordance with ISO 8466-1 and ISO 8466-2.

The laboratory shall specify any yield correction factors applied. If this correction is not applied, then this uncorrected bias shall be accounted for in the uncertainty calculations.

11.3 Measurement uncertainty

Measurement uncertainty estimations shall be calculated for each analyte and each matrix at the characterization stage using ISO 11352.

12 Quality controls

12.1 General

It is important to regularly challenge the analytical sequencies by introducing control samples undergoing the whole analytical process (including filtration step, if relevant). These control samples are obtained by directly adding standard solutions in a sampling bottle containing exactly the same amount of representative matrix as the test portion volume sampled. Regular participation in proficiency testing (interlaboratory trials) is also recommended.

It should be checked that the initial uncertainty values obtained at the characterization stage remain consistent with uncertainty values obtained over the method's lifetime, via approaches based on internal quality assurance or interlaboratory testing.

12.2 Quality control checks on the blank

In each analytical sequence, at least one blank shall be processed in exactly the same way as the samples, including all pre-treatment steps. It is recommended to perform at least one blank determination before analysing real samples so as to ensure that the full procedure is contamination-free.

If the concentration in the blank is greater than the LOQ, then it shall not be subtracted from the concentration in the samples, either the analytical batch shall be invalidated or the LOQ raised.

12.3 Quality control checks on the internal standards

Response of standard solutions and internal standards shall be subjected to acceptability criteria and regularly monitored, using a control chart for example.

12.4 Quality control checks on the limit of quantification

The LOQ shall be verified at each measurement series containing samples below LOQ, in a matrix that is representative of the samples under test.

Any adaptations made to the LOQ due to:

— accounting for a blank value;

— or diluting the sample or extract

shall be clearly stated and appropriately documented.

If interference is suspected, it may be necessary to perform additional measurements to confirm the identity of the analyte, such as using different chromatographic separation conditions or evaluating additional m/z ratios or SRM transitions.