INTERNATIONAL STANDARD

ISO 21676

First edition
2018-10

Water quality — Determination of the dissolved fraction of selected active pharmaceutical ingredients, transformation products and other organic substances in water and treated waste water — Method using high performance liquid chromatography and mass spectrometric detection (HPLC-MS/MS or -HRMS) after direct injection

Qualité de l'eau — Détermination de la fraction dissoute des ingrédients pharmaceutiques actifs sélectionnés, des produits de la transformation et d'autres substances organiques dans l'eau et dans l'eau résiduaire — Méthode par chromatographie en phase liquide à haute performance et détection par spectrométrie de masse (CLHP-MS/MS ou -HRSM) après l'injection directe

Reference number
ISO 21676:2018(E)

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Published in Switzerland

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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).

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. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

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.

Introduction

Pharmaceutical ingredients are essential for human and animal health. Through application or improper disposal, active pharmaceutical ingredients enter the water cycle unchanged or transformed. This can happen via municipal waste water, treated at treatment plants. There, some active pharmaceutical ingredients and transformation products cannot be removed completely from the waste water by conventional treatment techniques. Active pharmaceutical ingredients and their transformation products also travel through sludge to the soil and subsequently enter water bodies via leachate, depending on the nature of the ground and the active ingredients. Active pharmaceutical ingredients and their transformation products are therefore found in treated waste water, as well as in surface and ground water. This document specifies a liquid chromatography method with mass spectrometric detection for the determination of selected active pharmaceutical ingredients and their transformation products in the dissolved fraction.

Water quality — Determination of the dissolved fraction of selected active pharmaceutical ingredients, transformation products and other organic substances in water and treated waste water — Method using high performance liquid chromatography and mass spectrometric detection (HPLC-MS/MS or -HRMS) after direct injection

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 determination of the dissolved fraction of selected active pharmaceutical ingredients and transformation products, as well as other organic substances (see Table 1) in drinking water, ground water, surface water and treated waste water.

The lower application range of this method can vary depending on the sensitivity of the equipment used and the matrix of the sample. For most compounds to which this document applies, the range is ≥ 0,025 µg/l for drinking water, ground water and surface water, and ≥ 0,050 µg/l for treated waste water.

The method can be used to determine further organic substances or in other types of water (e.g. process water) provided that accuracy has been tested and verified for each case, and that storage conditions of both samples and reference solutions have been validated. Table 1 shows the substances for which a determination was tested in accordance with the method. Table E.1 provides examples of the determination of other organic substances.

Table 1 — Substances for which a determination was tested in accordance with this method

Common name Chemical name (IUPAC[a])	Molecular formula	Molar mass g/mol	CAS-RN[b]
4-Acetylaminoantipyrine N-(2,3-Dimethyl-5-oxo-1-phenyl-3-pyrazolin-4-yl)acetamide	$C_{13}H_{15}N_3O_2$	245,28	83-15-8
N4-Acetyl sulfamethoxazole N-{4-[(5-Methyl-1,2-oxazol-3-yl)sulfamoyl]phenyl}-acetamide	$C_{12}H_{13}N_3O_4S$	295,32	21312-10-7
Diatrizoic acid (amidotricoic acid) 3,5-Bis(acetamido)-2,4,6-triiodobenzoic acid	$C_{11}H_9I_3N_2O_4$	613,91	117-96-4
Atenolol (RS)-2-[4-[2-Hydroxy-3-(1-methylethylamino) propoxy]phenyl] ethanamide	$C_{14}H_{22}N_2O_3$	266,34	29122-68-7
[a] IUPAC: International Union of Pure and Applied Chemistry.			
[b] CAS-RN: Chemical Abstracts System Registration Number.			

Table 1 *(continued)*

Common name Chemical name (IUPAC[a])	Molecular formula	Molar mass g/mol	CAS-RN[b]
Bezafibrate 2-{4-[2-(4-Chlorbenzamido)ethyl]phenoxyl}-2-methylpropanoic acid	$C_{19}H_{20}ClNO_4$	361,80	41859-67-0
Bisoprolol (RS)-1-[4-(2-Isopropoxyethoxymethyl)phenoxy]-3-isopropylamino-2-propanol	$C_{18}H_{31}NO_4$	325,45	66722-44-9
Carbamazepine 5H-Dibenzo[b,f]azepine-5-carbamide	$C_{15}H_{12}N_2O$	236,27	298-46-4
Clarithromycin (2R,3R,4S,5R,8R,9S,10S,11R,12R,14R)-11-[(2S,3R,4S,6R)-4-(dimethylamino)-3-hydroxy-6-methyloxan-2-yl]oxy-5-ethyl-3,4-dihydroxy-9-[(2R,4R,5S,6S)-5-hydroxy-4-methoxy-4,6-dimethyl-oxan-2-yl]oxy-12-methoxy-2,4,8,10,12,14-hexa-methyl-6-oxacyclotetradecane-1,7-dione	$C_{38}H_{69}NO_{13}$	747,95	81103-11-9
Clofibric acid 2-(4-Chlorophenoxy)-2-methylpropanoic acid	$C_{10}H_{11}ClO_3$	214,70	882-09-7
Dehydrato-Erythromycin (anhydro-erythromycin) (2R,3R,4S,5S,8R,9S,10S,11R,12R)-11-{[4-(dimethylamino)-3-hydroxy-6-methyloxan-2-yl]oxy}-5-ethyl-3-hydroxy-9-[(5-hydroxy-4-methoxy-4,6-dimethyloxan-2-yl)oxy]-2,4,8,10,12,14-hexame-thyl-6,15,16-trioxatricyclo[10.2.1.1{1,4}]hexadecane-7-one	$C_{37}H_{65}NO_{12}$	715,91	23893-13-2
Diazepam (RS)-7-Chlor-1-methyl-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepine-2-on	$C_{16}H_{13}ClN_2O$	284,74	439-14-5
Diclofenac 2-[2-[(2,6-Dichlorphenyl)amino]phenyl]acetic acid	$C_{14}H_{11}Cl_2NO_2$	296,15	15307-86-5
10,11-Dihydro-10,11-dihydroxy carbamazepine (5S,6S)-5,6-Dihydroxy-5,6-dihydrobenzo[b][1]benzazepie-11-carboxamide	$C_{15}H_{14}N_2O_3$	270,29	58955-93-4
Erythromycin 6-(4-Dimethylamino-3-hydroxy-6-methyl-oxan-2-yl)oxy-14-ethyl-7,12,13-trihydroxy-4-(5-hydroxy-4-methoxy-4,6-dimethyl-oxan-2-yl)-oxy-3,5,7,9,11,13-hexamethyl-1-oxacyclo-tetradecane-2,10-dione	$C_{37}H_{67}NO_{13}$	733,93	114-07-8
4-Formylaminoantipyrine N-(2,3-Dihydro-1,5-dimethyl-3-oxo-2-phenyl-1H-pyrazol-4-yl) formamide	$C_{12}H_{13}N_3O_2$	231,25	1672-58-8
Gemfibrozil 5-(2,5-Chlorophenoxy)-2,2-methylpropanoic acid	$C_{15}H_{22}O_3$	250,34	25812-30-0
Ibuprofen (RS)-2-[4-(2-Methylpropyl)phenyl]propanoic acid	$C_{13}H_{18}O_2$	206,28	15687-27-1

[a] IUPAC: International Union of Pure and Applied Chemistry.

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

Table 1 *(continued)*

Common name Chemical name (IUPAC[a])	Molecular formula	Molar mass g/mol	CAS-RN[b]
Iomeprol (±)-N,N′-Bis-(2,3-dihydroxypropyl)-5-[(2-hydroxy-acetyl) methylamino]-2,4,6-triiodo isophthalamide	$C_{17}H_{22}I_3N_3O_8$	777,09	78649-41-9
Iopamidol (S)-N,N′-Bis[2-hydroxy-1-(hydroxymethyl)ethyl]-5-[(2-hydroxypropanoyl)amino]-2,4,6-triiodobenzene-1,3-dicarbamide	$C_{17}H_{22}I_3N_3O_3$	777,08	60166-93-0
Iopromide (±)-N,N′-Bis(2,3-dihydroxypropyl)-2,4,6-triiodo-5-(2-methoxyacetamido)-N-methylisophthalamide	$C_{18}H_{24}I_3N_3O_8$	791,12	73334-07-3
Metoprolol (RS)-1-(Isopropylamino)-3-[4-(2-methoxyethyl) phenoxy] propan-2-ol	$C_{15}H_{25}NO_3$	267,36	37350-58-6
Naproxen (S)-2-(6-Methoxy-2-naphthyl)propanoic acid	$C_{14}H_{14}O_3$	230,26	22204-53-1
Oxazepam (RS)-7-Chloro-3-hydroxy-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-on	$C_{15}H_{11}ClN_2O_2$	286,71	604-75-1
Phenazone 1,5-Dimethyl-2-phenyl-2,3-dihydro-1H-pyrazol-3-on	$C_{11}H_{12}N_2O$	188,23	60-80-0
Primidone 5-Ethyl-5-phenylhexahydropyrimidin-4,6-dione	$C_{12}H_{14}N_2O_2$	218,25	125-33-7
Propyphenazone 1,5-Dimethyl-4-(1-methylethyl)-2-phenyl-1,2-dihydro-3H-pyrazol-3-one	$C_{14}H_{18}N_2O$	230,31	479-92-5
Roxithromycin (3R,4S,5S,6R,7R,9R,11S,12R,13S,14R)-6-{[(2S,3R,4S,6R)-4-(dimethylamino)-3-hydroxy-6-methyloxan-2-yl] oxy}-14-ethyl-7,12,13-trihydroxy-4-{[(2R,4R,5S,6S)-5-hydroxy-4-methoxy-4,6-dimethyloxan-2-yl]oxy}-3,5,7,9,11,13-hexamethyl-10-(2,4,7-trioxa-1-azaoctan-1-ylidene)-1-oxacyclotetradecane-2-one	$C_{41}H_{76}N_2O_{15}$	837,05	80214-83-1
Sotalol (RS)-4′-(1-Hydroxy-2-isopropylaminoethyl) methanesulfonanilide	$C_{12}H_{20}N_2O_3S$	272,36	3930-20-9
Sulfamethoxazole 4-Amino-N-(5-methyl-1,2-oxazol-3-yl)benzene-sulfonamide	$C_{10}H_{11}N_3O_3S$	253,28	723-46-6
Temazepam (RS)-7-Chloro-3-hydroxy-1-methyl-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one	$C_{16}H_{13}ClN_2O_2$	300,74	846-50-4
Trimethoprim 2,4-Diamino-5-(3,4,5-trimethoxybenzyl)pyrimidine	$C_{14}H_{18}N_4O_3$	290,32	738-70-5

[a] IUPAC: International Union of Pure and Applied Chemistry.

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

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 1042, *Laboratory glassware — One-mark volumetric flasks*

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

ISO 4796-2, *Laboratory glassware — Bottles — Part 2: Conical neck bottles*

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*

ISO 5667-6, *Water quality — Sampling — Part 6: Guidance on sampling of rivers and streams*

ISO 5667-10, *Water quality — Sampling — Part 10: Guidance on sampling of waste waters*

ISO 5667-11, *Water quality — Sampling — Part 11: Guidance on sampling of groundwaters*

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*

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

The water sample is injected directly into the analysis system. The identification and quantitative determination is performed using high performance liquid chromatography coupled with mass spectrometric detection (HPLC-MS/MS, HPLC-HRMS).

5 Interferences

5.1 During sample preparation

Loss of analytes can occur during filtration of the sample as a result of sorption.

5.2 During high performance liquid chromatography and mass spectrometry

Peak tailing, peak fronting and/or wide peaks are indications of a malfunctioning of HPLC and/or interferences occurring during chromatography. However, some compounds tend to show more signal tailing than others depending on the chromatographic conditions.

Interferences from accompanying substances (matrix) can occur in both positive and negative ionization modes depending on the measured compound (e.g. diclofenac in negative ESI mode).

Accompanying substances (matrix) can affect the ionization of the target substances (e.g. ion suppression or signal enhancement). This can result in underestimation or overestimation of concentration during

quantification. These interferences can be detected and corrected for as needed using analyte recovery (11.2 and Annex B) and/or internal standardization (10.3 and Table D.3).

6 Reagents

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 interference shall be negligible. This shall be checked regularly (see 9.5).

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

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

6.1.1 Water, complying with the requirements of ISO 3696, grade 1 or equivalent without any interfering blank values.

6.1.2 Methanol, CH_3OH.

6.1.3 Acetonitrile, CH_3CN.

6.1.4 Acetic acid, $w(CH_3COOH) = 100$ % mass fraction.

6.1.5 Formic acid, $w(HCOOH)$ not less than 98 % mass fraction.

6.1.6 Ammonium acetate, $w(CH_3COONH_4)$ not less than 99 % mass fraction.

6.1.7 Ammonium formate, $w(HCOONH_4)$ not less than 99 % mass fraction.

6.1.8 Sodium thiosulfate pentahydrate, $Na_2S_2O_3 \cdot 5H_2O$.

6.1.9 Operating gases for the mass spectrometer, in accordance with the specifications of the instrument manufacturer.

6.1.10 Reference substances, as listed in Table 1, with known mass fraction.

6.1.11 Internal standard substances, preferably isotope-labelled compounds of reference substances (see Table D.3).

The internal standards shall not lead to analyte interferences (see 9.5).

6.2 Preparation of solutions

6.2.1 General

Solutions of internal standard substances are needed only once calibration and evaluation have been performed in accordance with 10.3 and 12.3.

Test the accuracy of the reference substance solutions against a control standard (see 6.2.9), e.g. during calibration (see 10.1).

NOTE Reference substance solutions and internal standard substances are available commercially.

6.2.2 Stock solutions (reference substances/internal standard substances)

Prepare solutions with a mass concentration of, for example, 0,1 mg/ml of each substance.

For this, use, for example, a 5 mg amount of a substance (6.1.10) in separate 50 ml volumetric flasks (7.2), dissolve them in acetonitrile (6.1.3) or methanol (6.1.2), and then add solvent to solution until it reaches the mark.

NOTE Alternatively, commercially available (or custom made) stock solutions of individual reference substances (or internal standard substances) in organic solvent can be used for preparing further dilutions.

Store the solutions at temperatures below −15 °C and protected from light and evaporation. Under these conditions they are stable for one year.

6.2.3 Intermediate dilution A (reference substances)

Prepare an intermediate solution with substance mass concentrations of, for example, 1 µg/ml each.

This involves transferring, for example, 0,5 ml of each reference substance stock solution (see 6.2.2) to a 50 ml volumetric flask (7.2) and then making the solution up to the mark with acetonitrile (6.1.3) to the mark.

Store the solution at temperatures below −15 °C and protected from light and evaporation. Under these conditions it is stable for one year.

6.2.4 Intermediate dilution B (reference substances)

Prepare an intermediate solution with substance mass concentrations of, for example, 50 ng/ml each.

This involves transferring, for example, 0,5 ml of the intermediate dilution A (see 6.2.3) to a 10 ml volumetric flask (7.2) and then making the solution up to the mark with water (6.1.1) to the mark.

Store the solution at between 2 °C and 8 °C and protected from light and evaporation. Under these conditions it is stable for one month.

Use the solution to spike analytes to the samples to determine the recovery (see 11.2).

6.2.5 Intermediate dilution C (reference substances)

Prepare an intermediate solution with substance mass concentrations of, for example, 5 ng/ml each.

This involves transferring, for example, 0,25 ml of the intermediate dilution A (see 6.2.3) to a 50 ml volumetric flask (7.2) and then making the solution up to the mark with water (6.1.1) to the mark.

Store the solution at between 2 °C and 8 °C and protected from light and evaporation. Under these conditions it is stable for one month.

6.2.6 Intermediate dilution D (internal standards)

Prepare an intermediate solution with substance mass concentrations of, for example, 1 µg/ml each.

This involves transferring, for example, 0,5 ml of each internal standard substance stock solution (see 6.2.2) to a 50 ml volumetric flask (7.2) and then making the solution up to the mark with acetonitrile (6.1.3) to the mark.

Store the solution at temperatures below −15 °C and protected from light and evaporation. Under these conditions it is stable for one year.

6.2.7 Intermediate dilution E (internal standards)

Prepare an intermediate solution with substance mass concentrations of, for example, 50 ng/ml each.

This involves transferring, for example, 0,5 ml of the intermediate dilution D (see 6.2.6) to a 10 ml volumetric flask (7.2) and then making the solution up to the mark with water (6.1.1) to the mark.

Store the solution at between 2 °C and 8 °C and protected from light and evaporation. Under these conditions it is stable for one month.

Use the solution for generating calibration samples and for spiked samples.

6.2.8 Calibration samples

Prepare calibration samples from the corresponding dilutions of the intermediate dilution C (see 6.2.5). For calibration with an internal standard (see 10.3), apply the same amount of internal standards to each calibration sample.

Prepare calibration samples, e.g. solutions in which the mass concentrations of the substances to be determined correspond to 0,025 µg/l and those of the internal standard substances correspond to 0,250 µg/l (see 10.1).

This involves transferring, for example, 50 µl of the intermediate dilution C (see 6.2.5) to a 10 ml volumetric flask, mixing 50 µl of the intermediate dilution E (see 6.2.7) and then making the solution up to the mark with, for example, water (6.1.1).

If possible, the composition of the calibration samples should be similar to that of the samples to be examined and shall not result in interfering peak broadening. When using calibration samples in drinking, ground or surface water, ensure that the substances to be determined are not present.

NOTE When calibration samples are prepared in ultrapure water, this can lead to lower findings of macrolides. In these cases, the use of ultrapure water is not preferred, but matrix matched calibration instead.

Prepare new calibration samples for each new measurement sequence if their stability cannot be verified.

6.2.9 Control standard

The control standard is a reference substance solution produced independently of the stock solutions, e.g. a solution from an alternative batch or manufacturer. The solution should contain all of the substances to be determined.

7 Apparatus

Equipment or parts of equipment that come into contact with the water sample shall have no blank values for the compounds measured within this method. All equipment used should preferably be made of glass, stainless steel or polytetrafluoroethylene (PTFE).

7.1 Narrow-neck flat-bottomed bottles, preferably of brown glass conical joint with glass stoppers, e.g. laboratory bottles, volume of 250 ml, as per ISO 4796-2 — NS 250.

7.2 Volumetric flasks, nominal volume 10 ml, 25 ml, 50 ml, e.g. volumetric flasks, as per ISO 1042 — A50-C.

7.3 Microsyringes.

7.4 Syringe filters, with low dead volume, e.g. diameter of 13 mm with a regenerated cellulose membrane.

Filtration should not lead to significant losses of individual substances, and the type of filter used shall be selected by testing this. Verify no contamination or significant loss from filtering by passing blanks and reference substance solutions through the same filters.