
**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*



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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.

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.

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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.

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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 $\geq 0,025 \mu\text{g/l}$ for drinking water, ground water and surface water, and $\geq 0,050 \mu\text{g/l}$ 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 ₃ O 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 ₃ O ₄ S	295,32	21312-10-7
Diatrizoic acid (amidotricic acid) 3,5-Bis(acetamido)-2,4,6-triiodobenzoic acid	C ₁₁ H ₉ I ₃ N ₂ O ₄	613,91	117-96-4
Atenolol (RS)-2-[4-[2-Hydroxy-3-(1-methylethylamino) propoxy]phenyl] ethanamide	C ₁₄ H ₂₂ N ₂ O 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]phenoxy}-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 ₂ O	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-hexamethyl-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-hexamethyl-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 ₂ O	284,74	439-14-5
Diclofenac 2-[2-[(2,6-Dichlorophenyl)amino]phenyl]acetic acid	C ₁₄ H ₁₁ Cl ₂ NO 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 ₂ O 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-oxacyclotetradecane-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 ₃ O 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 ₃ N ₃ O ₈	777,09	78649-41-9
Iopamidol (S)-N,N'-Bis[2-hydroxy-1-(hydroxymethyl)ethyl]-5-[(2-hy- droxypropanoyl)amino]-2,4,6-triiodobenzene-1,3-dicarbamide	C ₁₇ H ₂₂ I ₃ N ₃ O 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 ₃ N ₃ O 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 ₂ O ₂	286,71	604-75-1
Phenazone 1,5-Dimethyl-2-phenyl-2,3-dihydro-1H-pyrazol-3-on	C ₁₁ H ₁₂ N ₂ O	188,23	60-80-0
Primidone 5-Ethyl-5-phenylhexahydropyrimidin-4,6-dione	C ₁₂ H ₁₄ N ₂ O 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 ₂ O	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-hy- droxy-4-methoxy-4,6-dimethyloxan-2-yl]oxy]-3,5,7,9,11,13- hexamethyl-10-(2,4,7-trioxa-1-azaocan-1-ylidene)-1- oxacyclotetradecane-2-one	C ₄₁ H ₇₆ N ₂ O ₁₅	837,05	80214-83-1
Sotalol (RS)-4'-(1-Hydroxy-2-isopropylaminoethyl) methanesulfonanilide	C ₁₂ H ₂₀ N ₂ O ₃ S	272,36	3930-20-9
Sulfamethoxazole 4-Amino-N-(5-methyl-1,2-oxazol-3-yl)benzene-sulfonamide	C ₁₀ H ₁₁ N ₃ O ₃ S	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 ₂ O ₂	300,74	846-50-4
Trimethoprim 2,4-Diamino-5-(3,4,5-trimethoxybenzyl)pyrimidine	C ₁₄ H ₁₈ N ₄ O 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, $w(\text{CH}_3\text{COOH}) = 100\%$ mass fraction.

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

6.1.6 Ammonium acetate, $w(\text{CH}_3\text{COONH}_4)$ not less than 99 % mass fraction.

6.1.7 Ammonium formate, $w(\text{HCOONH}_4)$ not less than 99 % mass fraction.

6.1.8 Sodium thiosulfate pentahydrate, $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$.

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.