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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

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

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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-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	$C_{16}H_{13}ClN_2O$	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-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	$C_{12}H_{13}N_3O_2$	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.

7.5 Sample vials, appropriate for the automated sample injector, e.g. crimp-top vial, nominal volume 1,5 ml with crimp cap and septum of rubber/PTFE.

NOTE Sample vials of polyethylene (PE) can be used in an additional run to minimize losses of macrolides.

7.6 HPLC column, preferably with precolumn, suitable for chromatography of the selected substances. See Annex C for examples.

7.7 High performance liquid chromatograph, coupled to mass spectrometer, consisting of the following.

7.7.1 Degasser unit, e.g. vacuum degasser.

7.7.2 Analytical pumping systems, low-pulsation, suitable for binary gradient elution.

7.7.3 Manual or automated sample injector.

7.7.4 Apparatus for thermostat control of the separation column, e.g. column thermostat.

7.7.5 Mass spectrometry detector (MS/MS, HRMS), preferably with electrospray ionization (ESI).

8 Sampling

Take the samples in accordance with the specifications given in ISO 5667-4, ISO 5667-5, ISO 5667-6, ISO 5667-10 and ISO 5667-11.

Use flat-bottomed bottles (7.1) for sampling and fill the bottles with the water to be examined.

When taking drinking water that may contain oxidants, also add approximately 50 mg of sodium thiosulfate pentahydrate (6.1.8) per litre.

Analyse the water sample as soon as possible after it is sampled.

Store the sample at temperatures of (3 ± 2) °C, protected from light, for a maximum of three weeks.

NOTE If longer storage times are necessary and/or in case of presumed or validated instability of individual substances, suitable measures can be implemented (e.g. preservation by freezing of samples).

9 Procedure

9.1 General

The implementation of the method depends on the type of calibration and the measures planned to recognize and, if necessary, correct for matrix effects.

9.2 Sample preparation

If the sample is not visibly absent of particles, filter the sample through a syringe filter (7.4).

During filtration of the sample, sorption may cause loss of analytes, particularly hydrophobic substances (e.g. macrolides). In this case, do not filter the sample before chromatography, but use an in-line filter as an alternative to protect the separation column from particles.

When calibrating with an external standard (see 10.2), obtain an aliquot of the sample to determine the recovery if necessary (see 11.2).

When calibrating with an internal standard (see 10.3), add the internal standards so that the mass concentrations of the internal standards in the sample are equal to those in the calibration samples (see 6.2.8).

Take into account the dilution of the sample caused by the addition of reagents when calculating the individual results (see 12.2) if it amounts to more than 1 %.

Monitor an early eluter, e.g. metformin (see Table E.1), in terms of retention time and peak shape. The level of organic solvents in a prepared sample shall not result in any additional peak broadening.

9.3 High performance liquid chromatography (HPLC)

Operate the HPLC instrumentation in accordance with the instructions provided by the manufacturer.

Use a suitable HPLC column (7.6) for chromatographic separation and optimize the separation of the analytes with gradient elution.

Select the chromatographic conditions to achieve optimal sensitivity for mass spectrometric detection (see Annex C for examples).

NOTE 1 The use of gradient programmes with acetonitrile/water/acetic acid is advantageous in terms of sensitivity for most substances.

NOTE 2 At the same linear flow rate of the eluent, columns with a lower internal diameter exhibit better sensitivities than those with wider diameter.

Complete separation of the substances is not necessary provided that interference of the quantitative determination does not occur during peak overlapping.

The shortest retention time shall correspond to a minimum of three times the HPLC column dead volume time.

Use chromatography to separate substances that cannot be completely separated from each other using spectrometry. In these cases, chromatographic resolution R should be a minimum of $R = 1{,}2$.

Choose an appropriate injection volume so that no interfering peak widening or interference of the quantitative determination occur.

NOTE 3 For larger injection volumes, e.g. 1 ml, column switching techniques with suitable enrichment columns is possible but is outside the scope of this method.

Check retention time standard deviation once during initial assessment. It shall not exceed 0,03 min for six consecutive chromatograms.

9.4 Detection

9.4.1 General

Operate the mass spectrometer in accordance with the manufacturer's instructions and select the correct settings for the device.

The ESI mode is typically preferred when ionizing substances. This usually produces quasi-molecular ions of the type $[M+H]^+$ or $[M-H]^-$. In isolated cases, adduct ions, e.g. $[M+NH_4]^+$ or $[M+Na]^+$, can also be formed under certain chromatographic conditions.

Most substances listed in Table 1 can be detected using the positive ESI mode. Substances that are to be detected in the negative ESI mode can be analysed in a single run by switching polarity or tested in a separate run (see Table D.1).

When performing simultaneous detection of negative and positive ions, choose switch times for changing polarity that are short enough to preserve a sufficient number of data points.

Each peak shall be registered with a minimum of eight data points.

Identify and set the method-specific settings for the source parameters and the MS parameters using less sensitive substances, e.g. ibuprofen.

NOTE The signal is usually smoothed prior to peak integration. Depending on the algorithm used this can result in a disproportionately high loss of signal intensity when the number of data points is too low. Reproducibility of peak integration can also be affected, especially for low peak values and peak areas, if there are too few data points.

Use chromatography to separate substances that cannot be completely separated from each other using mass spectrometry (see 9.3).

9.4.2 Tandem mass spectrometry (MS/MS)

Identify the optimal settings for ionization under the specified chromatographic conditions for each substance in positive or negative mode depending on its chemical properties.

Select the substance-specific settings so that two product ions can be preserved for each substance if possible. Optimize a second mass transfer relative to the isotopic mass of, for example, ^{37}Cl (see Table D.1) for substances that fragment into only one detectable product ion, if possible. In order to avoid interferences during quantification in these cases, only use internal standards for which the relative molar masses are at least four mass units higher than those of the target substances. Examples are given in Table D.3.

9.4.3 High-resolution mass spectrometry (HRMS)

When using a high-resolution mass spectrometer in full scan mode the complete mass spectrum is available at each point in time during chromatography.

Ensure and check that mass accuracy (see 12.1) and resolution are maintained over the mass area and the entire chromatogram.

Select the resolution to achieve sufficient differentiation of matrix signals.

Set the measuring method so that for each analyte at least one product ion is retained for confirmation (see 12.1), if possible.

When selecting isotope-marked standards, ensure that the mass difference to the non-marked analytes is a minimum of three mass units in order to avoid any interferences with the isotope signals of the analyte. Examples are given in Table D.3.

9.5 Blank value measurements

Perform blank value measurements for the complete method on a regular basis to check there is no interference from either the instrument or the reagents.

Inject, for example, water (6.1.1) to perform the blank value measurements.

If interfering blank values occur (over 50 % of the lowest reporting level), identify the cause using systematic examination and eliminate the sources of contamination.

10 Calibration

10.1 General

The calibration of the method of determination is to be performed under specified chromatographic conditions. The retention times of the individual analytes and possibly of the internal standard substances shall be determined beforehand. They can be determined by injecting multi-component mixtures, or solutions of individual substances using the mass of the product ions or the quasi-molecular ions under the specified chromatographic conditions.

Proceed as follows.

- Design the complete method of determination to produce a linear relationship between the measured signal and the concentration for each of the substances to be determined.
- For this, determine the linear working range of the instrument by injecting at least five calibration samples (see 6.2.8) of various concentrations for each of the substances to be determined (see ISO 8466-1).
- Select a linear calibration range that covers the actual concentrations, e.g. a calibration range of 0,025 µg/l to 1 µg/l for the examination of drinking, ground and surface water.

The lowest concentration level within the calibration shall be higher or equal to the limit of quantification. The identification of the detection limit and the limit of quantification are performed in accordance with documented methods, e.g. in accordance with ISO 8466-1.

- For routine operation, it is sufficient to perform multiple-point calibration using at least three concentration levels.
- For multiple-point calibration, distribute the concentration levels evenly over the calibration range and perform the calibration in accordance with ISO 8466-1.
- Keep the injection volume constant for calibration and sample measurement.

The calibration function determined for a particular substance is valid only for the applicable concentration range. It is also dependent on the operating status of the measurement system and shall be tested in each measurement series.

Two procedures are described for setting up the calibration functions:

a) calibration with external standard;

b) calibration with internal standard.

Internal standard calibration is preferred and highly recommended where labelled standards are available.

When examining drinking, ground and surface water, calibration with an external standard (see 10.2) leads to results that will not deviate by more than 25 % from their true value for most of the substances in Table 1 without corrections made by the recovery. There can be exceptions, especially with polar substances that exhibit low retention, e.g. some X-ray contrast agents, for which higher systematic deviations can occur due to matrix effects. In these instances, it can be necessary to use internal standards (see 10.3) or corrections with sample-specific recovery (see 11.2).

Matrix effects can occur, especially in treated waste water samples, and exert an interfering influence throughout the further course of chromatography on the quantitative determination as a result of ion suppression or enhancement. The matrix effects can be lessened by diluting the sample.

NOTE 1 Performing correction using sample-specific recovery (see 11.2) and applying internal standardization (see 10.3) both increase the amount of deviation contributing to measurement uncertainty. The correction can also result in artificially high values. To confirm the quantitative results, the method of standard addition can be applied with several spiking steps.

Matrix effects can depend on the working conditions or the type and condition of the equipment, and shall be identified, e.g. by determination of the recovery, when applying the method for the sample types of interest. If possible, the composition of the calibration samples (see 6.2.8) should be similar to that of the samples to be examined.

NOTE 2 Matrix effects can also be detected through post-column infusion of analytes or internal standards over the complete time used for chromatography of the real samples based on a reduction in intensity.

Table 2 gives an explanation of the subscripts used in the following formulae and text.

Table 2 — Definition of subscripts

Index	Meaning
i	identity of substance
j	consecutive figure for pairs of values
e	measuring variables for the calibration
a	measuring variables for addition
I	internal standard
M	measurement solution
P	sample
A	addition

10.2 Calibration with external standard

Inject the calibration samples (see 6.2.8) to perform calibration.

Chart the calibration function graphically. For this, plot the measurement values y_{ie} for each substance i on the y-axis and the corresponding mass concentration ρ_{ie} on the x-axis.

Determine the reference function from the pairs of values y_{iej} and ρ_{iej} by using linear regression given in Formula (1):

$$y_{\mathrm{ie}} = b_i \cdot \rho_{\mathrm{ie}} + a_i \tag{1}$$

where

y_{ie} is the measurement value (dependent variable) of the substance i during calibration as a function of ρ_{ie}, e.g. area unit;

b_i is the slope of the linear regression for the substance i (corresponding to the substance-specific response factor), e.g. area unit × litres per microgram (l/µg);

ρ_{ie} is the mass concentration (independent variable) of the substance i in the calibration sample, in micrograms per litre (µg/l);

a_i is the axis intercept of the linear regression for the substance i on the ordinate, e.g. area unit.

10.3 Calibration with internal standard

The use of internal standards for quantitative analysis can compensate for the interferences that can occur during mass spectrometric measurement (see Clause 5).

The internal standard shall not be contained in the water sample to be examined. It should exhibit chemical similarities to the substance to be determined and should act like the substance to be determined during filtration, chromatography and mass spectrometric measurement.

Use compounds as internal standards that are equal to substances under investigation, which are structured with different isotopes, e.g. deuterated or ^{13}C-marked compounds (see Table D.3).

Analytes for which no isotope-marked compounds are available may be evaluated using other internal standards, if it has been ensured and documented that the analyte recovery calculated from addition in the examined sample types is within the same range as the recovery of the selected internal standard.

To perform the calibration, inject calibration samples (see 6.2.8) that contain all of the substances to be determined as well as the internal standards (6.1.11).

To graphically display the calibration function for each substance *i*, plot the ratios of the measured values y_{ie}/y_{Iie} on the y-axis and the corresponding mass concentration ratios ρ_{ie}/ρ_{Iie} on the x-axis.

Determine the reference function from the pairs of values y_{iej}/y_{Iiej} and ρ_{iej}/ρ_{Iiej} by using linear regression given in Formula (2):

$$\frac{y_{ie}}{y_{Iie}} = b_{Ii} \cdot \frac{\rho_{ie}}{\rho_{Iie}} + a_{Ii} \qquad (2)$$

where

y_{ie} see Formula (1);

y_{Iie} is the measured value for the internal substance I of the substance *i* during calibration, e.g. area unit;

ρ_{ie} see Formula (1);

ρ_{Iie} is the mass concentration for the internal standard I of the substance *i* in the calibration sample, in micrograms per litre (µg/l);

b_{Ii} is the slope of the linear regression y_{ie}/y_{Iie} dependent on the ratio ρ_{ie}/ρ_{Iie} of the substance *i*, dimensionless;

a_{Ii} is the axis intercept of the linear regression on the ordinate of the substance *i*, e.g. area unit, dimensionless.

11 Calculation of recovery

11.1 General

Analyte recovery may provide indications of matrix influences. For example, these can be identified by performing addition on the sample as given in 11.2.

NOTE 1 Correction performed using the sample-specific recovery can result in higher measurement uncertainty.

When performing calibration and evaluation with an internal standard (see 10.3 and 12.3), the recoveries of the internal standards are a measure to assess the validity of the instrumental analysis. They shall be determined in accordance with 11.3.

The sample-specific recovery of the analyte or the recovery of an internal standard for the analyte shall fall within the range of 50 % to 150 %.

NOTE 2 A low recovery results in a higher limit of quantification and higher measurement uncertainty.

11.2 Calculation of analyte recovery using samples

To determine the recovery, perform addition with the analyte on the sample, e.g. by adding 50 µl of the intermediate dilution B (see 6.2.4) on 5 ml of the sample and then analysing the sample both with and without addition over the course of the entire process.

The amount of the addition should be in the middle working range, e.g. 0,5 µg/l. The mass concentrations of the analytes used in samples undergoing addition shall not exceed the calibration range. The sample shall be diluted prior to addition if necessary.

Calculate the recovery $A_{i\mathrm{P}}$ for the substance *i* in the sample using Formula (3):

$$A_{i\mathrm{P}} = \frac{\rho_{ia\mathrm{P}} - \rho_i}{\rho_{i\mathrm{A}}} \cdot f \tag{3}$$

where

$A_{i\mathrm{P}}$ is the recovery for the substance *i* in the sample, in per cent (%);

$\rho_{ia\mathrm{P}}$ is the determined mass concentration for the substance *i* in the sample with addition of analyte, calculated using Formula (1), in micrograms per litre (µg/l);

ρ_i is the determined mass concentration for the substance *i* in the sample without addition of analyte, calculated using Formula (1), in micrograms per litre (µg/l);

$\rho_{i\mathrm{A}}$ is the added mass concentration for the substance *i* in the sample with addition of analyte in micrograms per litre (µg/l);

f is the conversion factor, here: $f = 100$, in per cent (%).

11.3 Recovery of internal standards

Calculate the recovery for internal standards using Formula (4):

$$A_{\mathrm{I}i\mathrm{P}} = \frac{\rho_{\mathrm{I}i\mathrm{M}}}{\rho_{\mathrm{I}i\mathrm{e}}} \cdot f \tag{4}$$

where

$\mathrm{A}_{\mathrm{I}i\mathrm{P}}$ the recovery for the internal standard I of the substance *i*, in per cent (%);

$\rho_{\mathrm{I}i\mathrm{M}}$ is the determined mass concentration for the internal standard I of the substance *i* in the measurement solution, in micrograms per litre (µg/l);

$\rho_{\mathrm{I}i\mathrm{e}}$ see Formula (2);

f see Formula (3).

12 Evaluation

12.1 Verification of individual substances

When using an HPLC MS/MS method, a substance in a sample is considered to be verified if

a) in the MS/MS chromatogram of the mass trace produced by a product ion of this substance, a signal is registered for which the retention time corresponds within a tolerance of ±0,15 min with the retention time produced by the corresponding reference substance under standard conditions, and

b) a second product ion from the same precursor ion or from another precursor ion of this substance is detected on a further mass trace in the same retention time, and

c) the intensities of the product ions are in a relationship to one another that corresponds to the ratio for these ions determined for the reference substance under the standard conditions within a tolerance of ±30 %. This tolerance may be as large as 50 % at the lower application limit and especially at the limit of quantification for this analysis method.

When using an HPLC-HRMS method, a mass difference of 5 ppm[1] between the measured (accurate) mass and the theoretical (exact) mass of the substance or the ion of the substance shall not be exceeded. Under these conditions, a substance in a sample is considered to be verified if

- in the HRMS chromatogram of the mass trace produced by a quasi-molecular ion (or adduct ion) of the substance, a signal is registered for which the retention time corresponds within a tolerance of ±0,15 min with the retention time produced by the corresponding reference substance under the same conditions, and
- a signal from at least one product ion from the substance is detected during the same retention time.

NOTE A product ion spectrum with exact mass is typically attained when applying HRMS/MS.

The substance is also considered to be verified if, instead of a product ion from the substance being detected in the HRMS chromatogram, the mass trace of an isotope of the quasi-molecular ion, e.g. ^{37}Cl, ^{81}Br, is detected in the same retention time and its intensity ratio is within a tolerance of ±30 %. This tolerance may be as large as 50 % at the lower application limit and especially at the limit of quantification for this analysis method.

For analytes with the same retention time and identical elemental composition, verification shall be performed using a product ion. If these masses are also identical, chromatographic separation of the analytes is necessary.

If the criteria for verification are met only partially, e.g. with just one product ion of sufficient intensity in a HPLC-MS/MS method, a positive finding, if judged by a specialist to be necessary, can be supported by:

- applying another ionization technique that would result in different ions, e.g. ESI negative or APCI;
- the retention time with a HPLC column of a different selectivity.

12.2 Calculation of the individual results using calibration with an external standard

Calculate the mass concentration $\rho_{i\mathrm{P}}$ of the substance i in the sample using Formula (5):

$$\rho_{i\mathrm{P}} = \frac{\left(y_{i\mathrm{M}} - a_i\right)}{b_i \cdot A_{i\mathrm{P}}} \cdot f \qquad (5)$$

where

$\rho_{i\mathrm{P}}$ is the mass concentration of the substance i in the sample, in micrograms per litre (µg/l);

$y_{i\mathrm{M}}$ is the measured value for the substance i in the measured solution, e.g. area unit;

f see Formula (3) (only if using recovery for calculation);

a_i, b_i see Formula (1);

$A_{i\mathrm{P}}$ see Formula (3) and 10.1 (only if using recovery for calculation).

12.3 Calculation of the individual results using calibration with an internal standard

Calculate the mass concentration $\rho_{i\mathrm{P}}$ of the substance i in the sample using Formula (6):

$$\rho_{i\mathrm{P}} = \frac{\dfrac{y_{i\mathrm{M}}}{y_{\mathrm{I}i\mathrm{M}}} - a_{\mathrm{I}i}}{b_{\mathrm{I}i}} \cdot \rho_{\mathrm{I}i\mathrm{P}} \qquad (6)$$

where

---

[1] Parts per million (ppm) is a deprecated unit, i.e. not accepted by the International System of Units, SI.

$\rho_{i\mathrm{P}}$	see Formula (5);
$y_{i\mathrm{M}}$	see Formula (5);
$y_{\mathrm{I}i\mathrm{M}}$	is the measured value for the internal substance I of the substance *i* in the measured solution, e.g. area unit;
$\rho_{\mathrm{I}i\mathrm{P}}$	is the predefined mass concentration for the internal standard I of the substance *i* in the sample, in micrograms per litre (µg/l);
$a_{\mathrm{I}i}$, $b_{\mathrm{I}i}$	see Formula (2).

13 Expression of results

The analysis results obtained when applying this document are subject to a measurement uncertainty, see ISO 11352[1], that is to be considered in the interpretation of the results (see Annex A).

The mass concentrations of the substances in accordance with Table 1 are indicated in micrograms per litre at two significant figures.

EXAMPLES atenolol: 0,091 µg/l

0,15 µg/l

1,5 µg/l

14 Test report

The test report shall contain at least the following information:

a) the test method used, together with a reference to this document, i.e. ISO 21676:2018;

b) identity of the sample;

c) expression of the results in accordance with Clause 13;

d) any deviation from this method;

e) report of all circumstances that can have affected the results.

Annex A
(informative)

Performance data

The performance data given in Tables A.2 to A.5 were determined in an interlaboratory trial for validation carried out in Germany from 13 January 2014 to 31 January 2014 including four samples (see Table A.1), which were spiked with analytes within a concentration range of 0,035 µg/l to 0,90 µg/l. Some of the substances to be quantified were already present in the original samples. The assigned values are based on the spiking of analyte and the initial level of pollution of the original samples determined in the interlaboratory study. Of the 18 participating laboratories, 16 used tandem mass spectrometry (MS/MS) and 2 used high-resolution mass spectrometry (HRMS), each without a previous sample filtration step. The evaluation was made in accordance with ISO 5725-2[2].

Table A.1 — Characterization of the unfiltered interlaboratory study samples

Parameter	Unit	Drinking water (two samples)	Surface water	Waste water treatment plant effluent
pH value	—	8,2	8,0	8,0
TOC	mg/l	< 0,5	1,85	5,5
Conductivity	mS/m	67	37	94
Na	mg/l	35,8	24,1	106
Ca	mg/l	85,8	39,8	69,1

Tables A.2 to A.5 contain the performance data.

The high recovery for diatrizoic acid in both drinking water samples (Tables A.2 and A.3) can be traced back to the initial level of pollution in the drinking water at a level of approximately 0,06 µg/l.

The high recovery and $C_{V,R}$ for the substances erythromycin, dehydrato-erythromycin, clarithromycin and roxithromycin can be traced back to the results from laboratories that have used calibration solutions prepared with ultrapure water.

Table A.2 — Performance data for drinking water in the lower application range

Substance	l	n	o	X	$\bar{\bar{x}}$	η	s_R	$C_{V,R}$	s_r	$C_{V,r}$
			%	µg/l	µg/l	%	µg/l	%	µg/l	%
4-Acetylaminoantipyrine	13	49	7,5	0,035 0	0,030 8	87,9	0,004 6	15,0	0,001 3	4,1
N4-Acetyl sulfamethoxazole	16	61	0,0	0,035 0	0,034 5	98,6	0,005 3	15,4	0,002 2	6,4
Diatrizoic acid	13	52	7,1	0,035 0	0,085 5	244,3	0,024 5	28,7	0,005 6	6,5
Atenolol	16	60	0,0	0,035 0	0,038 9	111,1	0,006 7	17,2	0,002 2	5,6
Bezafibrate	12	48	7,7	0,035 0	0,033 6	96,0	0,003 5	10,4	0,001 3	3,9
Bisoprolol	13	49	19,7	0,035 0	0,040 2	114,7	0,004 7	11,8	0,001 0	2,5
Carbamazepine	15	57	0,0	0,035 0	0,035 4	101,1	0,005 5	15,4	0,001 5	4,3
Clarithromycin	13	49	14,0	0,035 0	0,036 4	104,1	0,009 0	24,6	0,001 3	3,6
Clofibric acid	14	53	7,0	0,035 0	0,033 1	94,6	0,004 2	12,8	0,000 9	2,8
Dehydrato-Erythromycin	11	44	8,3	0,035 0	0,044 9	128,2	0,015 1	33,6	0,002 8	6,2
Diazepam	16	61	0,0	0,035 0	0,034 9	99,7	0,003 9	11,2	0,001 7	4,8
Diclofenac	14	53	7,0	0,035 0	0,036 2	103,4	0,004 3	11,8	0,001 7	4,7

Table A.2 *(continued)*

Substance	l	n	o %	X µg/l	$\overline{\overline{x}}$ µg/l	η %	s_R µg/l	$C_{V,R}$ %	s_r µg/l	$C_{V,r}$ %
10,11-Dihydro-10,11-dihydroxy carbamazepine	13	49	14,0	0,035 0	0,033 3	95,1	0,003 9	11,7	0,001 9	5,6
Erythromycin	15	57	6,6	0,035 0	0,038 3	109,3	0,010 3	26,8	0,002 4	6,3
4-Formylaminoantipyrine	16	60	0,0	0,035 0	0,035 3	100,9	0,006 9	19,5	0,001 6	4,6
Gemfibrozil	14	53	7,0	0,035 0	0,032 1	91,8	0,003 4	10,5	0,001 6	5,0
Ibuprofen	11	41	0,0	0,035 0	0,038 0	108,4	0,008 5	22,5	0,003 0	7,8
Iomeprol	13	51	0,0	0,035 0	0,037 2	106,3	0,008 4	22,6	0,003 9	10,4
Iopamidol	12	48	7,7	0,035 0	0,037 4	107,0	0,006 4	17,2	0,004 3	11,4
Iopromide	14	56	6,7	0,035 0	0,037 6	107,3	0,008 8	23,5	0,002 8	7,5
Metoprolol	16	61	0,0	0,035 0	0,040 1	114,4	0,004 6	11,6	0,001 7	4,2
Naproxen	11	41	16,3	0,035 0	0,034 9	99,8	0,006 1	17,3	0,001 3	3,6
Oxazepam	14	53	13,1	0,035 0	0,033 9	96,7	0,003 2	9,6	0,001 5	4,3
Phenazone	16	61	0,0	0,035 0	0,035 6	101,7	0,003 5	9,9	0,001 7	4,9
Primidone	15	57	6,6	0,035 0	0,034 7	99,1	0,003 4	9,7	0,002 1	6,2
Propyphenazone	15	57	6,6	0,035 0	0,036 0	102,9	0,003 3	9,2	0,001 3	3,5
Roxithromycin	8	29	12,1	0,035 0	0,052 6	150,2	0,022 0	41,8	0,004 8	9,2
Sotalol	15	57	6,6	0,035 0	0,036 8	105,2	0,005 1	13,8	0,001 6	4,3
Sulfamethoxazole	12	48	14,3	0,035 0	0,033 5	95,6	0,003 2	9,6	0,001 4	4,3
Temazepam	12	45	21,1	0,035 0	0,033 8	96,5	0,003 5	10,5	0,001 0	2,8
Trimethoprim	16	61	0,0	0,035 0	0,037 8	107,9	0,004 5	11,9	0,001 8	4,7

l	number of laboratories after outlier rejection
n	number of individual test results after outlier rejection
o	percentage of outliers
X	conventionally correct (assigned) value of the test sample
$\overline{\overline{x}}$	overall mean of results (without outliers)
η	recovery
s_R	reproducibility standard deviation
$C_{V,R}$	coefficient of variation of reproducibility
s_r	repeatability standard deviation
$C_{V,r}$	coefficient of variation of repeatability

Table A.3 — Performance data for drinking water

Substance	l	n	o %	X µg/l	$\overline{\overline{x}}$ µg/l	η %	s_R µg/l	$C_{V,R}$ %	s_r µg/l	$C_{V,r}$ %
4-Acetylaminoantipyrine	16	60	1,6	0,085 0	0,071 5	84,2	0,010 9	15,3	0,002 9	4,0
N4-Acetyl sulfamethoxazole	16	61	0,0	0,085 0	0,082 6	97,2	0,008 1	9,8	0,003 9	4,7
Diatrizoic acid	15	57	0,0	0,085 0	0,154 1	181,2	0,064 6	41,9	0,008 4	5,5
Atenolol	15	57	6,6	0,085 0	0,092 7	109,0	0,015 7	16,9	0,002 9	3,2
Bezafibrate	16	61	0,0	0,085 0	0,079 3	93,3	0,010 2	12,9	0,003 5	4,4

NOTE For an explanation of symbols, see Table A.2.

Table A.3 *(continued)*

Substance	l	n	o %	X µg/l	$\bar{\bar{x}}$ µg/l	η %	s_R µg/l	$C_{V,R}$ %	s_r µg/l	$C_{V,r}$ %
Bisoprolol	14	53	13,1	0,085 0	0,095 5	112,4	0,016 6	17,4	0,002 4	2,6
Carbamazepine	14	53	13,1	0,085 0	0,087 2	102,6	0,011 6	13,3	0,002 0	2,3
Clarithromycin	16	61	0,0	0,085 0	0,094 0	110,6	0,031 5	33,6	0,005 5	5,8
Clofibric acid	14	53	13,1	0,085 0	0,083 2	97,8	0,009 6	11,6	0,001 8	2,2
Dehydrato-Erythromycin	13	52	7,1	0,085 0	0,093 1	109,5	0,027 4	29,4	0,007 2	7,7
Diazepam	16	61	0,0	0,085 0	0,084 6	99,6	0,010 6	12,6	0,003 9	4,6
Diclofenac	15	57	6,6	0,085 0	0,088 7	104,3	0,011 1	12,5	0,002 7	3,1
10,11-Dihydro-10,11-dihydroxy carbamazepine	15	57	0,0	0,085 0	0,081 0	95,3	0,010 2	12,6	0,004 3	5,3
Erythromycin	15	57	6,6	0,085 0	0,088 3	103,9	0,024 6	27,9	0,003 7	4,2
4-Formylaminoantipyrine	13	49	19,7	0,085 0	0,086 0	101,1	0,013 1	15,3	0,002 2	2,6
Gemfibrozil	15	57	0,0	0,085 0	0,078 6	92,5	0,008 8	11,2	0,003 0	3,8
Ibuprofen	12	45	8,2	0,085 0	0,086 6	101,9	0,011 2	13,0	0,005 0	5,8
Iomeprol	15	57	0,0	0,085 0	0,088 3	103,9	0,016 0	18,1	0,006 9	7,9
Iopamidol	12	45	21,1	0,085 0	0,077 7	91,4	0,011 0	14,1	0,004 0	5,2
Iopromide	14	53	13,1	0,085 0	0,084 3	99,1	0,012 1	14,3	0,006 3	7,4
Metoprolol	15	56	8,2	0,085 0	0,093 6	110,1	0,009 8	10,4	0,002 9	3,1
Naproxen	13	49	14,0	0,085 0	0,085 5	100,6	0,012 7	14,9	0,002 9	3,4
Oxazepam	13	49	19,7	0,085 0	0,082 1	96,6	0,008 4	10,2	0,002 0	2,5
Phenazone	16	61	0,0	0,085 0	0,085 8	100,9	0,006 9	8,0	0,003 3	3,9
Primidone	15	57	6,6	0,085 0	0,083 4	98,2	0,009 7	11,7	0,004 5	5,4
Propyphenazone	15	57	6,6	0,085 0	0,086 5	101,7	0,005 6	6,5	0,003 1	3,6
Roxithromycin	16	61	0,0	0,085 0	0,080 7	94,9	0,042 8	53,1	0,006 8	8,5
Sotalol	13	49	19,7	0,085 0	0,088 5	104,2	0,008 9	10,0	0,002 3	2,6
Sulfamethoxazole	15	57	6,6	0,085 0	0,080 1	94,3	0,010 6	13,3	0,003 8	4,8
Temazepam	15	57	0,0	0,085 0	0,080 4	94,6	0,007 9	9,8	0,004 3	5,4
Trimethoprim	13	49	19,7	0,085 0	0,088 9	104,6	0,008 5	9,6	0,002 7	3,0

NOTE For an explanation of symbols, see Table A.2.

Table A.4 — Performance data for surface water

Substance	l	n	o %	X µg/l	$\bar{\bar{x}}$ µg/l	η %	s_R µg/l	$C_{V,R}$ %	s_r µg/l	$C_{V,r}$ %
4-Acetylaminoantipyrine	14	53	13,1	0,317	0,300	94,6	0,039 4	13,1	0,006 3	2,1
N4-Acetyl sulfamethoxazole	16	61	0,0	0,125	0,128	102,4	0,014 1	11,0	0,004 6	3,6
Diatrizoic acid	15	57	0,0	0,253	0,260	102,8	0,063 6	24,5	0,014 0	5,4
Atenolol	15	57	6,6	0,125	0,144	115,2	0,022 2	15,4	0,006 1	4,2
Bezafibrate	16	61	0,0	0,157	0,147	93,6	0,016 3	11,1	0,003 2	2,1
Bisoprolol	16	61	0,0	0,155	0,164	105,8	0,034 0	20,7	0,004 6	2,8
Carbamazepine	12	45	26,2	0,179	0,180	100,6	0,018 8	10,5	0,002 9	1,6
Clarithromycin	15	57	6,6	0,125	0,163	130,4	0,060 2	36,9	0,006 2	3,8

NOTE For an explanation of symbols, see Table A.2.

Table A.4 *(continued)*

Substance	l	n	o	X	$\bar{\bar{x}}$	η	s_R	$C_{V,R}$	s_r	$C_{V,r}$
			%	µg/l	µg/l	%	µg/l	%	µg/l	%
Clofibric acid	12	45	26,2	0,125	0,120	96,0	0,008 6	7,2	0,001 8	1,5
Dehydrato-Erythromycin	14	56	0,0	0,125	0,166	132,8	0,061 9	37,3	0,011 6	7,0
Diazepam	15	57	6,6	0,125	0,121	96,8	0,012 8	10,6	0,002 5	2,1
Diclofenac	16	61	0,0	0,234	0,228	97,4	0,023 3	10,2	0,005 4	2,4
10,11-Dihydro-10,11-dihydroxy carbamazepine	15	57	0,0	0,262	0,283	108,0	0,098 8	34,9	0,007 1	2,5
Erythromycin	14	53	13,1	0,125	0,146	116,8	0,043 3	29,7	0,005 6	3,8
4-Formylaminoantipyrine	16	61	0,0	0,376	0,382	101,6	0,061 1	16,0	0,010 7	2,8
Gemfibrozil	15	57	0,0	0,125	0,117	93,6	0,012 6	10,8	0,003 4	2,9
Ibuprofen	11	41	16,3	0,125	0,128	102,4	0,020 9	16,4	0,004 8	3,7
Iomeprol	15	57	0,0	0,360	0,391	108,6	0,077 3	19,8	0,023 3	6,0
Iopamidol	15	57	0,0	0,297	0,296	99,7	0,056 6	19,1	0,014 9	5,1
Iopromide	16	61	0,0	0,224	0,235	104,9	0,033 5	14,2	0,012 3	5,2
Metoprolol	15	57	6,6	0,288	0,292	101,4	0,031 7	10,9	0,007 5	2,6
Naproxen	15	57	0,0	0,125	0,139	111,2	0,011 5	8,3	0,006 0	4,3
Oxazepam	15	56	8,2	0,125	0,138	110,4	0,019 1	13,9	0,003 9	2,8
Phenazone	15	57	6,6	0,125	0,125	100,0	0,009 9	7,9	0,002 6	2,1
Primidone	13	49	19,7	0,125	0,136	108,8	0,014 8	10,9	0,004 5	3,3
Propyphenazone	16	61	0,0	0,125	0,123	98,4	0,010 3	8,3	0,002 7	2,2
Roxithromycin	16	61	0,0	0,125	0,207	165,6	0,137 0	66,2	0,012 4	6,0
Sotalol	15	57	6,6	0,157	0,150	95,5	0,014 5	9,6	0,003 5	2,3
Sulfamethoxazole	14	53	13,1	0,155	0,137	88,4	0,022 0	16,1	0,002 7	2,0
Temazepam	14	53	7,0	0,125	0,116	92,8	0,014 0	12,1	0,004 2	3,6
Trimethoprim	16	61	0,0	0,125	0,134	107,2	0,015 5	11,6	0,003 9	2,9

NOTE For an explanation of symbols, see Table A.2.

Table A.5 — Performance data for treated waste water

Substance	l	n	o	X	$\bar{\bar{x}}$	η	s_R	$C_{V,R}$	s_r	$C_{V,r}$
			%	µg/l	µg/l	%	µg/l	%	µg/l	%
4-Acetylaminoantipyrine	14	53	13,1	1,306	1,135	86,9	0,187 3	16,5	0,023 4	2,1
N4-Acetyl sulfamethoxazole	16	61	0,0	0,900	0,827	91,9	0,132 9	16,1	0,031 9	3,9
Diatrizoic acid	15	57	0,0	2,616	2,587	98,9	0,703 1	27,2	0,107 6	4,2
Atenolol	15	60	0,0	1,019	0,935	91,8	0,237 1	25,3	0,026 9	2,9
Bezafibrate	15	57	6,6	0,942	0,912	96,8	0,118 4	13,0	0,031 7	3,5
Bisoprolol	14	53	13,1	1,034	1,039	100,4	0,131 3	12,6	0,022 7	2,2
Carbamazepine	16	61	0,0	1,603	1,592	99,3	0,174 6	11,0	0,035 3	2,2
Clarithromycin	16	61	0,0	1,053	1,413	134,2	0,631 0	44,7	0,068 3	4,8
Clofibric acid	14	53	13,1	0,900	0,849	94,3	0,097 3	11,5	0,023 1	2,7
Dehydrato-Erythromycin	14	56	0,0	0,984	1,366	138,8	0,603 6	44,2	0,073 0	5,3
Diazepam	15	57	6,6	0,900	0,815	90,5	0,097 3	11,9	0,024 5	3,0
Diclofenac	14	53	13,1	2,221	2,228	100,3	0,193 0	8,7	0,037 9	1,7

NOTE For an explanation of symbols, see Table A.2.

Table A.5 *(continued)*

Substance	l	n	o %	X µg/l	$\bar{\bar{x}}$ µg/l	η %	s_R µg/l	$C_{V,R}$ %	s_r µg/l	$C_{V,r}$ %
10,11-Dihydro-10,11-dihydroxy carbamazepine	14	53	7,0	2,090	1,968	94,2	0,325 2	16,5	0,065 3	3,3
Erythromycin	15	57	6,6	0,950	1,141	120,1	0,415 0	36,4	0,038 5	3,4
4-Formylaminoantipyrine	14	53	13,1	1,445	1,439	99,6	0,198 3	13,8	0,028 5	2,0
Gemfibrozil	15	57	0,0	0,900	0,805	89,4	0,169 0	21,0	0,029 0	3,6
Ibuprofen	12	43	8,5	0,900	0,817	90,8	0,177 6	21,7	0,025 9	3,2
Iomeprol	15	57	0,0	2,407	2,444	101,5	0,368 7	15,1	0,107 5	4,4
Iopamidol	13	48	14,3	1,028	1,006	97,8	0,201 1	20,0	0,042 0	4,2
Iopromide	15	56	0,0	3,759	3,739	99,5	0,575 3	15,4	0,120 9	3,2
Metoprolol	15	56	8,2	1,744	1,839	105,4	0,233 0	12,7	0,044 0	2,4
Naproxen	13	49	12,5	0,957	0,860	89,8	0,077 2	9,0	0,028 3	3,3
Oxazepam	15	56	8,2	0,995	0,918	92,3	0,133 2	14,5	0,029 3	3,2
Phenazone	16	59	0,0	0,900	0,836	92,9	0,106 1	12,7	0,022 9	2,7
Primidone	16	61	0,0	1,162	1,106	95,2	0,169 7	15,3	0,029 3	2,7
Propyphenazone	15	57	6,6	0,900	0,830	92,2	0,079 6	9,6	0,017 7	2,1
Roxithromycin	15	57	6,6	1,062	1,866	175,7	1,003 4	53,8	0,114 3	6,1
Sotalol	16	61	0,0	1,141	1,055	92,4	0,271 3	25,7	0,035 2	3,3
Sulfamethoxazole	14	52	8,8	1,104	0,969	87,8	0,198 6	20,5	0,025 3	2,6
Temazepam	14	53	7,0	0,900	0,786	87,3	0,131 5	16,7	0,020 4	2,6
Trimethoprim	16	61	0,0	0,946	0,873	92,3	0,168 0	19,3	0,019 5	2,2
NOTE For an explanation of symbols, see Table A.2.										

Annex B
(informative)

Examples of recovery

Table B.1 — Recovery during filtration of calibration samples[c]

Substance	A_1	A_2	A_3	A_4	A_{iF}	s
4-Acetamidoantipyrine	100	101	98	99	99,5	1,3
N-Acetyl sulfamethoxazole	100	93	99	102	98,5	3,9
Atenolol	99	101	100	102	100,5	1,3
Atorvastatin[a]	84	83	87	83	84,3	2,2
Bezafibrate	98	94	100	100	98,0	2,9
Bisoprolol	91	92	94	90	91,8	1,9
Candesartan[a]	99	98	103	100	100,0	2,2
Carbamazepine	103	104	108	102	104,3	2,5
Clarithromycin	71	74	80	73	74,5	5,2
Clenbuterol[a]	98	95	100	95	97,0	2,5
Clofibric acid	97	94	97	95	95,8	1,6
Codeine[a]	96	101	100	99	99,0	2,2
Dehydrato-erythromycin	64	66	70	62	65,5	5,2
Desvenlafaxine[a]	97	94	96	92	94,8	2,3
Diazepam	96	95	97	92	95,0	2,3
Diclofenac	108	101	101	100	102,5	3,6
Dihydrocodeine[a]	99	97	95	94	96,3	2,3
Dihydro-dihydroxy-carbamazepine	95	97	97	94	95,8	1,6
Erythromycin	80	81	83	80	81,0	1,7
4-Formylaminoantipyrine	100	97	95	93	96,3	3,1
Fenofibric acid[a]	93	91	90	90	91,0	1,6
Furosemide[a]	92	95	92	91	92,5	1,9
Gabapentin[a]	99	96	101	98	98,5	2,1
Gemfibrozil	90	91	91	91	90,8	0,6
Ibuprofen	99	97	99	97	98,0	1,2
Indometacin[a]	95	97	93	91	94,0	2,7
Losartan[a]	100	105	102	103	102,5	2,0
Metformin[a]	91	92	89	89	90,3	1,7
Metolprolol	95	101	98	96	97,5	2,7
Metronidazole[a]	103	105	104	104	104,0	0,8

A recovery (repeat measurements)[b].

A_{iF} recovery for substance i during filtration, in per cent (%).

s standard deviation, in per cent (%).

[a] Other substances in accordance with Table E.1.

[b] Data processed in accordance with Clause 10.

[c] Syringe filters, with low dead volume, diameter of 13 mm with a regenerated cellulose membrane.

Table B.1 *(continued)*

Substance	A_1	A_2	A_3	A_4	A_{iF}	s
Moxifloxacin[a]	84	85	83	82	83,5	1,5
Nadolol[a]	96	97	99	100	98,0	1,9
Naproxen	94	94	92	92	93,0	1,2
Oxazepam	95	94	91	91	92,8	2,2
Phenazone	93	96	99	93	95,3	3,0
Primidone	102	103	105	96	101,5	3,8
Propranolol[a]	107	106	105	104	105,5	1,2
Propyphenazone	98	97	97	97	97,3	0,5
Ritalinic acid[a]	96	102	100	100	99,5	2,5
Roxithromycin	55	54	54	46	52,3	8,0
Sotalol	101	103	104	97	101,3	3,1
Sulfadiazine[a]	93	94	96	100	95,8	3,2
Sulfadimethoxine[a]	105	104	106	103	104,5	1,2
Sulfadoxine[a]	90	88	86	87	87,8	1,9
Sulfamerazine[a]	96	95	95	92	94,5	1,8
Sulfamethazine[a]	96	95	94	93	94,5	1,4
Sulfamethoxazole	98	95	97	96	96,5	1,3
Sulfathiazole[a]	104	101	101	99	101,3	2,0
Temazepam	93	96	95	94	94,5	1,4
Tramadol[a]	98	100	100	99	99,3	1,0
Trimethoprim	101	97	103	104	101,3	3,1
Valsartan[a]	106	104	107	102	104,8	2,1
Venlafaxine[a]	96	95	96	94	95,3	1,0

A recovery (repeat measurements)[b].

A_{iF} recovery for substance i during filtration, in per cent (%).

s standard deviation, in per cent (%).

[a] Other substances in accordance with Table E.1.

[b] Data processed in accordance with Clause 10.

[c] Syringe filters, with low dead volume, diameter of 13 mm with a regenerated cellulose membrane.

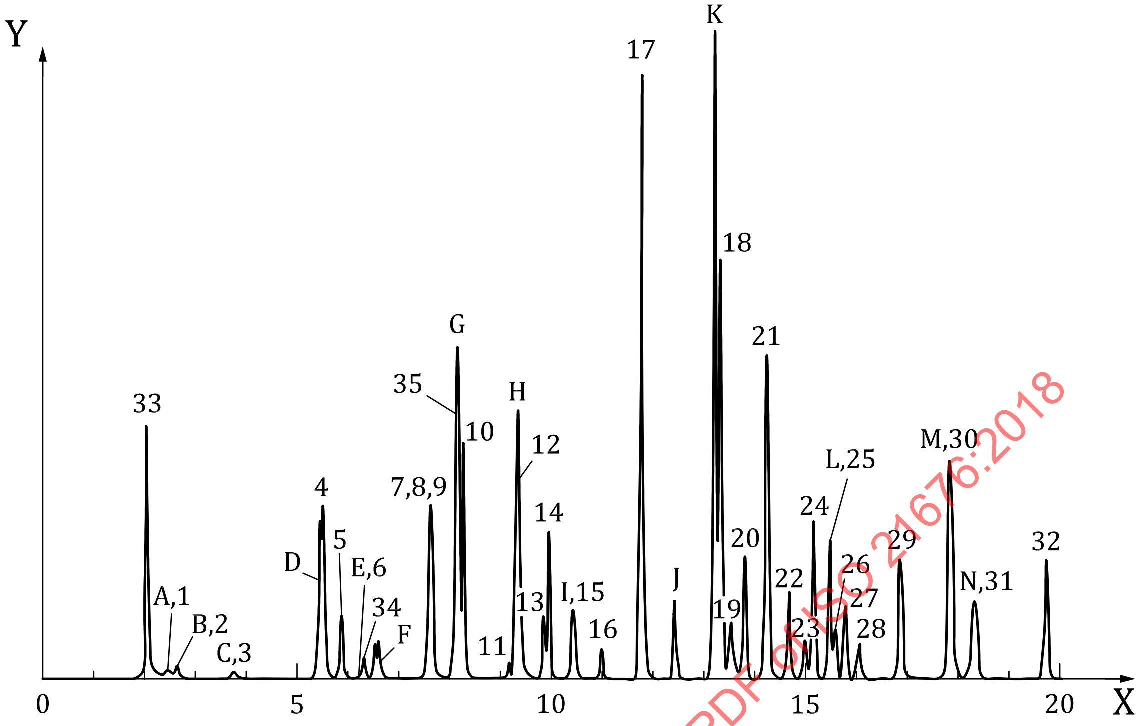
Annex C
(informative)

Examples of HPLC columns and chromatograms

C.1 Chromatographic conditions for the chromatogram in Figure C.1

Precolumn/ separation column:	Ultra Cartridges C-18 ID 2,1 mm, Synergi Hydro-RP[a] 2,5 µm, 100 mm × 2 mm
Injection:	100 µl standard solution in drinking water, ρ = 0,1 µg/l
Mobile phase:	A: 0,1 % acetic acid with 1 mmol ammonium acetate in water B: 0,1 % acetic acid in acetonitrile
Gradient:	0 min to 2 min; 4 % B, isocratic; 2 min to 20 min: 4 % B to 75 % B, linear; 20 min to 25 min: 95 % B, isocratic; 25 min to 35 min: 4 % B, isocratic
Flow:	0,25 ml/min
Column temperature:	40 °C
Pressure:	16 MPa at initial conditions

[a] Ultra Cartridges C-18, Synergi Hydro-RP are examples of suitable products available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of these products.



Key

X	time, min
Y	signal intensity (ESI positive/negative)

For peak numbers 1 to 32, see Table C.1

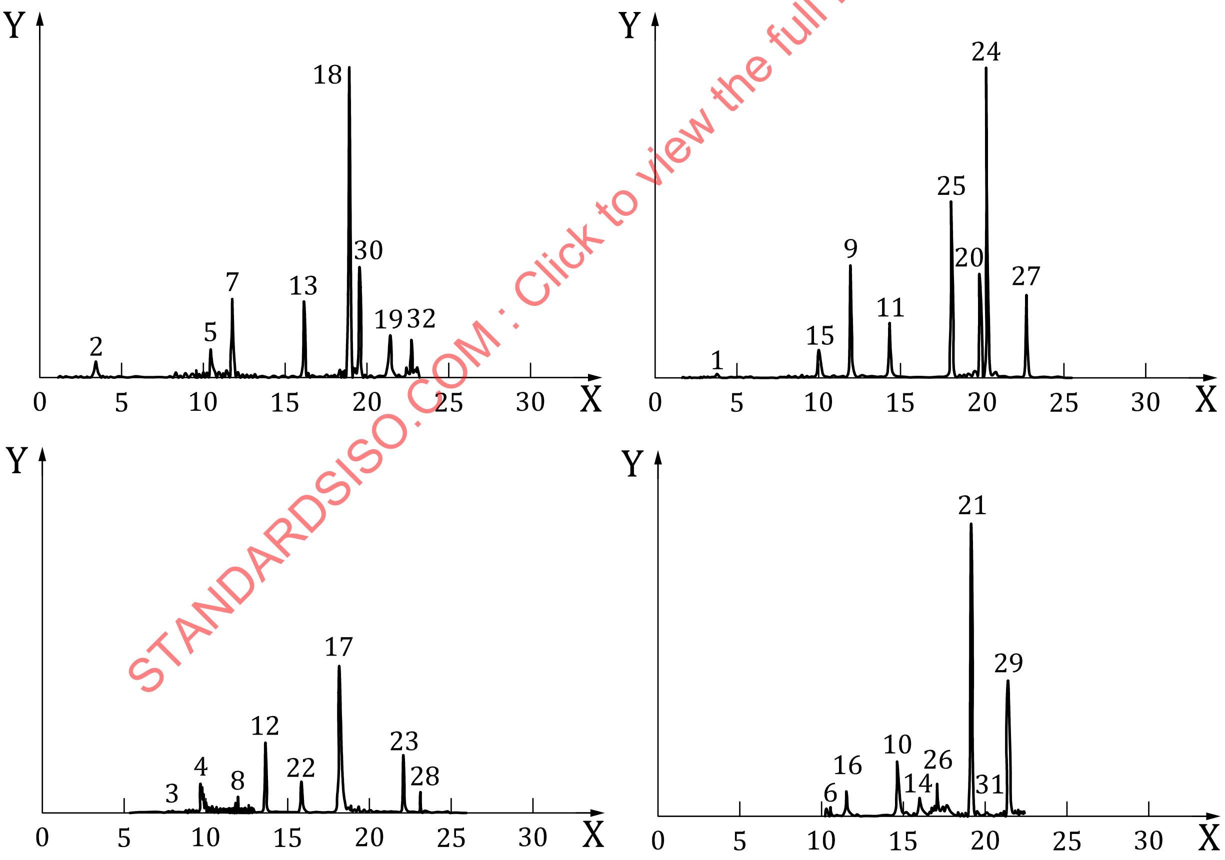
33	metformin
34	gabapentin
35	ritalinic acid
A	iopamidol-D3
B	diatrizoic acid-D6
C	iomeprol-D3
D	sotalol-D6
E	iopromide-D3
F	sulfadiazine-D4
G	trimethoprim-D9
H	phenazone-D3
I	sulfamethoxazole-D4
J	propranolol-D7
K	carbamazepine-D10
L	bezafibrate-D4
M	diclofenac-D4
N	ibuprofen-D3

Figure C.1 — Chromatographic separation, example 1, TIC chromatogram

C.2 Chromatographic conditions for the chromatogram in Figure C.2

Separation column:	Kinetex EVO C18[a] 100A (150 × 2,1 mm; 5 µm)
Injection:	40 µl
Mobile phase:	A: water with 1 mmol/l ammonium acetate B: methanol with 1 mmol/l ammonium acetate
Gradient:	5 min 98 % A, 20 min 98 % A to 2 % A (linear), 9 min 2 % A, 1 min 2 % A to 98 % A, 7 min 98 % A
Flow:	0,2 ml/min
Column temperature:	40 °C
Pressure:	15 MPa at initial conditions

[a] Kinetex EVO C18 is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.



Key

X	time, min
Y	relative signal intensity (ESI positive/negative)

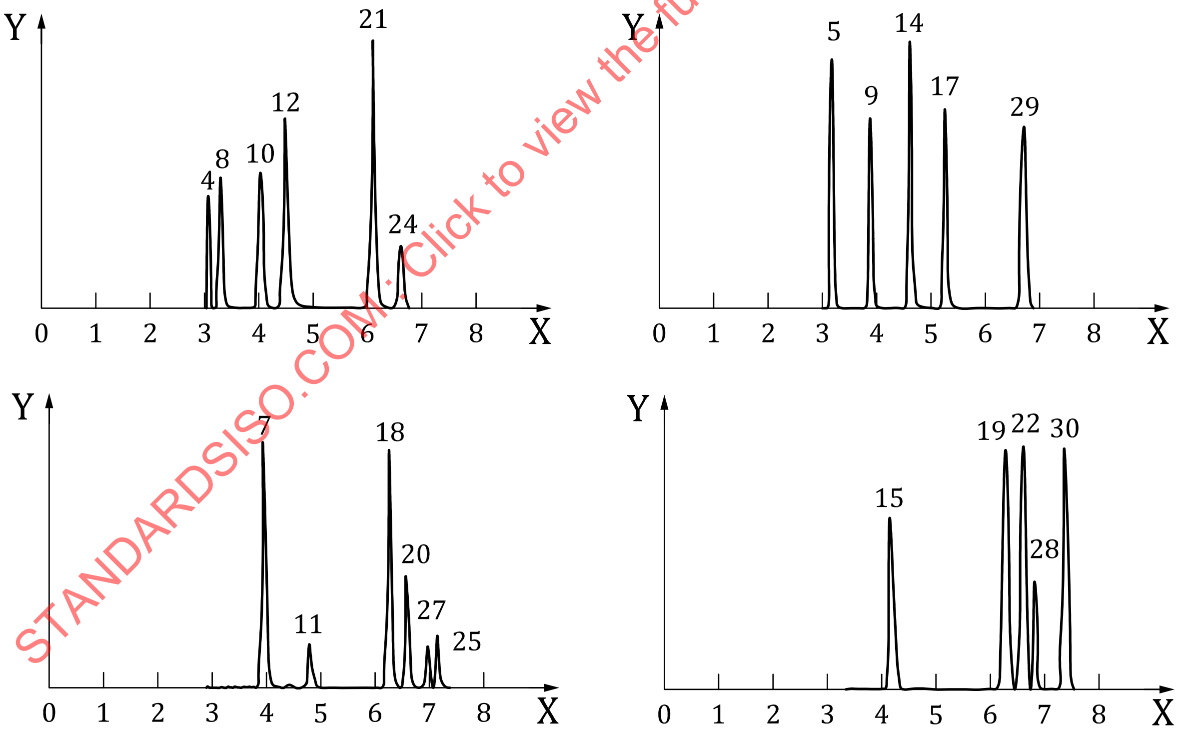
For peak numbers 1 to 32, see Table C.1

Figure C.2 — Chromatographic separation and signal intensity for the quantification transitions at 25 ng/l, example 2, MS/MS chromatograms

C.3 Chromatographic conditions for the chromatogram in Figure C.3

Separation column:	ACQUITY UPLC HSS T3[a] 50 × 2,1 mm, 1,8 µm (modified C18 material; Waters)
Enrichment column:	Hypersil Gold C18[a], 20 × 2,1 mm, 12 µm
Injection:	1 ml to the enrichment column (1 ml/min, 1 %MeOH addition)
Mobile phase:	A: water with 1 % MeOH, 0,1 % formic acid B: methanol with 0,1 % formic acid
Gradient:	1,3 min 99 % A, to 8 min to 5 % A to 12 min linear from 12 min to 15 min 99 % A
Flow:	0,6 ml/min
Column temperature:	30 °C
Pressure:	30 MPa at initial conditions

[a] ACQUITY UPLC HSS T3, Hypersil Gold C18 are examples of suitable products available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of these products.



Key

X time, min

Y relative signal intensity (ESI positive/negative)

For peak numbers 1 to 32, see Table C.1

Figure C.3 — Chromatographic separation and signal intensity (±5 ppm mass accuracy) for the quantification transitions at 125 ng/l, example 3, HRMS chromatograms