INTERNATIONAL STANDARD

ISO 16588

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Water quality — Determination of six complexing agents — Gas-chromatographic method

Qualité de l'eau — Dosage de six agents complexants — Méthode par chromatographie en phase gazeuse

Reference number
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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.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 3.

Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this International Standard may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

International Standard ISO 16588 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 2, *Physical, chemical and biochemical methods*.

Annex A of this International Standard is for information only.

Introduction

It is essential that the test described in this International Standard be carried out by suitably qualified staff.

It should be investigated whether and to what extent particular problems will require the specification of additional conditions.

Water quality — Determination of six complexing agents — Gas-chromatographic method

WARNING — Persons using this International Standard should be familiar with normal laboratory practice. This standard 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 and to ensure compliance with any national regulatory conditions.

1 Scope

This International Standard specifies a method for the determination of the water-soluble organic complexing agents listed in Table 1 in the concentration range from 0,5 μg/l to 200 μg/l, if a sample volume between 50 ml and 100 ml is used. The concentration range may change if diluted solutions are analysed. The method is applicable to drinking, ground, surface and waste water.

Table 1 — Complexing agents determinable by this method

No.	Name	Composition	Molecular mass	CAS number[a]
1	EDTA — ethylenedinitrilotetraacetic acid	$C_{10}H_{16}O_8N_2$	292,25	60-00-4
2	NTA — nitrilotriacetic acid	$C_6H_9O_6N$	191,14	139-13-9
3	DTPA — diethylenetrinitrilopentaacetic acid	$C_{14}H_{23}O_{10}N_3$	393,35	67-43-6
4	MGDA — methylglycinediacetic acid	$C_7H_{11}O_6N$	205,17	29578-05-0
5	β-ADA — β-alaninediacetic acid	$C_7H_{11}O_6N$	205,17	6245-75-6
6	1,3-PDTA — 1,3-propylenedinitrilotetraacetic acid	$C_{11}H_{18}O_8N_2$	306,27	1939-36-2

[a] CAS: Chemical Abstracts System

In waste water analysis, it is recommended that a smaller sample volume, e.g. 5 ml or 10 ml, be used in order to reduce matrix effects.

The adsorption of the six complexing agents on solid materials is negligibly low.

Other complexing agents of similar composition may also be determined using this method, provided they behave similarly during sample pretreatment, derivatization and gas chromatography. This shall be checked in each individual case.

2 Normative references

The following normative documents contain provisions which, through reference in this text, constitute provisions of this International Standard. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the normative documents indicated below. For undated references, the latest edition of the normative document referred to applies. Members of ISO and IEC maintain registers of currently valid International Standards.

ISO 3696:1987, *Water for analytical laboratory use — Specification and test methods*

ISO 5667-1:1980, *Water quality — Sampling — Part 1: Guidance on the design of sampling programmes*

ISO 5667-2:1991, *Water quality — Sampling — Part 2: Guidance on sampling techniques*

3 Principle

A test sample is stabilized with formaldehyde and evaporated to dryness. Hydrochloric or formic acid is added and the sample again evaporated to dryness. The complexing agents are then esterified to the *n*-propyl, iso-propyl or *n*-butyl esters. Water is added and the esters are extracted with *n*-hexane, separated and identified by gas chromatography, and determined quantitatively with a nitrogen-sensitive detector or by mass spectrometry.

For the determination of EDTA, DTPA, and 1,3-PDTA, 1,2-propylenedinitrotetraacetic acid (1,2-PDTA) is used as an internal standard through the whole procedure. When a nitrogen-sensitive detector is used, heptadecane- and/or octadecanenitrile is used as a control standard in the gas-chromatographic step. 1-Chorotetradecane may be used as the control standard if detection by mass spectrometry is chosen.

Alternatively, ^{13}C-labelled standards may be used.

4 Interferences

In spite of their stability, complexes of these complexing agents with heavy metals are broken up and the complexing agents determined, except in the case of bismuth. With samples containing bismuth in concentrations $> 100\ \mu g/l$, losses can be expected.

In the case of high salt concentrations (> 2 g/l of NaCl, corresponding to an electrical conductivity of about 400 mS/m), complete evaporation to dryness may be difficult. The complete removal of water is necessary, however, for the subsequent esterification. Therefore, samples have to be diluted or smaller sample volumes have to be taken. In the presence of calcium ions in concentrations exceeding 200 mg/l of Ca^{2+}, losses of EDTA will occur.

5 Reagents

All reagents shall be free from impurities which could interfere with the reactions. Throughout the procedure, use only deionized water (5.1).

5.1 Deionized water, grade 1 as specified in ISO 3696.

5.2 Gases for gas chromatography and mass spectrometry, as specified by the instrument manufacturers.

5.3 Nitrogen, of purity $\geqslant$ 99,996 %.

5.4 *n*-Propanol or **iso-propanol** or ***n*-butanol** for the preparation of esterification reagent (5.6).

5.5 Acetyl chloride.

5.6 Esterification reagent.

Cautiously mix by swirling in a 400 ml beaker 90 ml of *n*-propanol or iso-propanol or *n*-butanol (5.4) with 10 ml of acetyl chloride (5.5).

CAUTION — Considerable heat is evolved.

The mixture is stable for at least 1 month if stored at room temperature.

5.7 Reference compounds.

5.7.1 Nitrilotriacetic acid (NTA), $C_6H_9O_6N$, of purity $\geqslant$ 99 %.

5.7.2 Ethylenedinitrilotetraacetic acid (EDTA), $C_{10}H_{16}O_8N_2$, of purity $\geqslant$ 99 %.

5.7.3 Diethylenetrinitrilopentaacetic acid (DTPA), $C_{14}H_{23}O_{10}N_3$, of purity $\geqslant$ 99 %.

5.7.4 Methylglycinediacetic acid (MGDA), $C_7H_{11}O_6N$.

5.7.5 β-Alaninediacetic acid (β-ADA), $C_7H_{11}O_6N$.

5.7.6 1,3-Propylenedinitrilotetraacetic acid (1,3-PDTA), $C_{11}H_{18}O_8N_2$, of purity $\geqslant$ 99 %.

5.7.7 ^{13}C-labelled reference compounds as listed in 5.7.1 to 5.7.6 (optional).

5.8 Control standards and internal standard.

5.8.1 Octadecanenitrile, $C_{18}H_{35}N$, of purity $>$ 98 %.

5.8.2 Heptadecanenitrile, $C_{17}H_{33}N$, of purity $>$ 98 %.

5.8.3 1-Chlorotetradecane, $C_{14}H_{29}Cl$, of purity $>$ 98 %.

5.8.4 1,2-Propylenedinitrilotetraacetic acid (1,2-PDTA), $C_{11}H_{18}O_8N_2$, of purity $>$ 98 %.

5.9 Formaldehyde, 37 % by volume aqueous solution.

5.10 *n*-Hexane.

5.11 Sodium sulfate, anhydrous.

5.12 Sodium hydroxide solution, $c(\mathrm{NaOH}) = 1$ mol/l.

5.13 Hydrochloric acid, $c(\mathrm{HCl}) = 5$ mol/l.

5.14 Hydrochloric acid, $c(\mathrm{HCl}) = 1$ mol/l.

5.15 Formic acid, 50 % by volume aqueous solution.

5.16 Stock solutions for calibration purposes, 1 g/l.

Prepare 1 g/l stock solutions as follows. Weigh 100 mg of each of the complexing agents 5.7.1 to 5.7.6 and 5.8.4 into 100 ml volumetric flasks, dissolve in 2 ml of sodium hydroxide solution (5.12) and make up to the mark with water (5.1).

Stored in a refrigerator in brown glass bottles, the stock solutions are stable for at least 3 months.

5.17 Intermediate stock solutions, 1 mg/l and 10 mg/l.

Prior to each series of analyses, prepare intermediate stock solutions of 10 mg/l and 1 mg/l by diluting the 1 g/l stock solutions (5.16) with water (5.1).

5.18 Nitrile control standard, 0,5 mg/l solution in hexane.

Dissolve 100 mg of octadecanenitrile (5.8.1) and/or heptadecanenitrile (5.8.2) in 100 ml of *n*-hexane (5.10).

Store the solution in a refrigerator at 4 °C.

The solution is stable for at least 3 months.

Before use, dilute this solution to 0,5 mg/l.

5.19 1-Chlorotetradecane control standard, 0,5 mg/l solution in hexane.

Dissolve 100 mg of 1-chlorotetradecane (5.8.3) in 100 ml of *n*-hexane (5.10).

Store the solution in a refrigerator at 4 °C.

The solution is stable for at least 3 months.

Before use, dilute this solution to 0,5 mg/l.

6 Apparatus

6.1 Glassware, to be used only for the determination of complexing agents.

The use of detergents may lead to contamination. If contamination occurs, rinse the glassware with sodium hydroxide (5.12).

6.2 Ultrasonic bath.

6.3 Heating device, preferably a drying oven, suitable for evaporating water samples to dryness.

6.4 Equipment for passing an adjustable flow of nitrogen over the surface of the water samples during evaporation in the heating device (6.3).

6.5 Rotary evaporator.

6.6 Heating block, suitable for esterifying samples in sample vials (6.8) at (90 ± 3) °C.

During esterification, insert the sample vials into the heating block to about half of their volume (use metal rings to hold them in place).

6.7 Pipettes, 0,1 ml to 10 ml, or dispensers.

6.8 Disposable vials with PTFE-lined septa (PTFE = polytetrafluoroethylene), 3 ml and 12 ml.

6.9 pH meter, accuracy ± 0,1.

6.10 Beakers, 400 ml.

6.11 Gas chromatograph with mass-spectrometric detector.

6.12 Gas chromatograph with nitrogen-sensitive (NPD) detector.

6.13 Capillary column for gas chromatography, made of fused silica, length e.g. 20 m to 30 m, inner diameter e.g. 0,25 mm to 0,33 mm, stationary phase e.g. 100 % dimethyl polysiloxane or 95 % dimethyl polysiloxane plus 5 % diphenyl polysiloxane, film thickness 0,1 μm to 0,3 μm (see annex A).

6.14 Microlitre syringes, of suitable sizes.

6.15 Volumetric flasks, 50 ml and 100 ml.

6.16 Measuring cylinders, 50 ml and 100 ml.

6.17 Conductivity-measuring device.

6.18 Microseparator, e.g. as described in reference [7] (see the Bibliography).

7 Sampling and sample stabilization

Take samples in accordance with ISO 5667-1 and ISO 5667-2.

Place samples in glass or plastics bottles. For cleaning of the bottles, see 6.1. In order to avoid losses of some of the complexing agents by biological degradation, add, immediately after sampling, formaldehyde solution (5.9) in the ratio 1:100.

Store the stabilized samples at 4 °C in the dark. Do not keep for longer than one month.

8 Procedure

8.1 Sample pretreatment

Withdraw test samples directly from the settled sample. Alternatively, the sample may be centrifuged.

Measure the dissolved organic carbon (DOC) and conductivity of the sample.

The adsorption of the six complexing agents on settled solids may be neglected. However, if the method is applied to other complexing agents, this shall be checked for each individual compound.

If the DOC is $<$ 20 mg/l, add 1,2-PDTA (or ^{13}C-labelled compounds) as the internal standard (see 9.2) in about the same concentration as the complexing agents to be determined.

Evaporate 50 ml to 100 ml of the sample to dryness, preferably in a drying oven (6.3). Dissolve the residue in 10 ml of hydrochloric acid (5.14) or formic acid (5.15) and transfer quantitatively to a 12 ml vial (6.8). Evaporate the acidified sample to dryness in a heating block (6.6) or rotary evaporator (6.5) at (90 ± 3) °C under a continuous stream of nitrogen.

In the case of samples with a DOC concentration $>$ 20 mg/l, use a smaller sample volume, so that the DOC does not exceed 2 mg (absolute). In addition, if the salt concentration is high, modify the procedure accordingly (see clause 4). Smaller sample volumes may be transferred directly to the 12 ml sample vial and treated with hydrochloric acid.

Strongly alkaline waste waters may possibly require the addition of more hydrochloric acid.

8.2 Esterification of the sample

Add 2 ml of the esterification reagent (5.6) to the dry residue from 8.1. Close the vial and transfer it to the heating block (6.6). Insert it to half of its volume and leave it in the heating block at (90 ± 3) °C for at least 30 min for butyl esters or at least 3 h for propyl esters.

Butyl esters are more easily extracted. Especially in the case of the determination of DTPA, however, they may lead to problems during gas chromatography due to their higher boiling points.

Let the vial cool down to room temperature, open it and add 1 ml of a 0,5 mg/l solution of nitrile control standard (5.18) (when using a nitrogen-sensitive detector) or 1-chlorotetradecane control standard (5.19) (when using a mass-spectrometric detector) in hexane and shake vigorously. Transfer the contents to a 50 ml volumetric flask and add 1 ml of sodium hydroxide solution (5.12). Rinse the vial several times with water, add the water to the flask and make up to the mark with water. Shake vigorously for 1 min. Immediately after phase separation, withdraw as much of the organic layer as possible using a pipette or a microseparator (6.18) and transfer to a 3 ml vial (6.8). Add 0,5 g of sodium sulfate (5.11), shake for 5 min to 10 min and transfer the dried extract to another 3 ml vial (6.8). The extract may be stored in a refrigerator for a maximum of 2 weeks. In the case of low concentrations (1 µg/l to 10 µg/l), reduce the volume to about one-tenth using nitrogen.

8.3 Gas-chromatographic determination

In order to avoid discrimination effects during sample injection, it is preferable to use a cold-vapour system or cold on-column injection.

Use capillary columns. Use only immobilized stationary phases because of their high thermal stability (see annex A). Prior to the analysis, optimize the conditions and allow the GC system to stabilize.

8.4 Identification of the complexing agents

8.4.1 General

Identify individual complexing agents in the sample by comparing the retention times of the peaks in the chromatogram with the retention times of peaks produced by calibration solutions.

If the chromatogram contains no peak at the substance-specific retention time, record the compound as not detected.

If a peak is identified at a distinct, substance-specific, retention time, the presence of the compound is possible. Make additional investigations to verify the identity of the compound.

8.4.2 Identification of complexing agents using a nitrogen-sensitive detector

For identification and quantification by GC/NPD, use at least two columns of different polarity. A compound is regarded as identified if the retention time differs by no more than 0,03 min compared to that of a reference compound.

In the case of samples of low matrix concentration or if information on the origin of the sample and the matrix is available, identifications made using only one column may be regarded as highly probable.

8.4.3 Identification of complexing agents using a mass-spectrometric detector

Individual complexing agents in the sample are regarded as identified if:

— the retention time of the respective peak in the total-ion-current or selective-ion-current chromatogram is the same as the retention time of the respective peak produced by a calibration solution; and

— the complete background-corrected mass spectrum of the reference compound matches the background-corrected mass spectrum of the calibration solution; or

— the relative peak intensity of at least two sufficiently characterized molecule or fragment ions of the reference compound (see Table 2) matches that of the complexing agent to be identified.

Table 2 — Mass-spectral fragments of reference compounds

Reference compound	Main fragments m/z[a]		
	n-propyl ester	iso-propyl ester	*n*-butyl ester
EDTA	(460), 373, 230[b], 146	(460), 373, 230[b]	(516), 415, 258[b], 158
NTA	(317), 230[b], 146	(317), 230[b], 146	(359), 258[b], 158
DTPA	(673), 373[b], 244	(673), 516, 373[b], 244	(743), 415[b], 272, 158
MGDA	(331), 244[b], 160	(331), 244[b], 160	(373), 272[b], 216
ß-ADA	(331), 244[b], 230, 160	(331), 244[b], 160	(373), 272[b], 258
1,3-PDTA	(474), 230, 144[b]	(474), 230, 144[b]	(530), 184, 158[b]
1,2-PDTA (internal standard)	(474), 387, 244[b], 160	(474), 244[b], 216, 160	(530), 429, 272[b]

[a] m/z = mass to charge ratio. The first value (in brackets) corresponds to the molecular mass of the ester.

[b] Most abundant fragments.

Table 3 — Mass-spectral fragments of ^{13}C-labelled reference compounds

Reference compound	Main fragments m/z		
	n-propyl ester	iso-propyl ester	*n*-butyl ester
^{13}C-EDTA	(464), 377, 232	(464), 377, 232	(520), 419, 260
^{13}C-NTA	(320), 233, 147	(320), 233, 149	(362), 261, 161
^{13}C-DTPA	(678), 521, 376, 246	(678), 521, 376, 246	(748), 577, 418, 274
^{13}C-MGDA	(333), 246, 158	(333), 246, 162	(375), 274, 218, 172
^{13}C-ß-ADA	(333), 246, 160	(333), 246, 162	(375), 274, 260, 174
^{13}C-1,3-PDTA	(478), 391, 232	(478), 391, 232	(534), 433, 260, 186, 160

8.5 Blank determination

Check for possible contamination of glassware (see 6.1) and reagents by regular blank measurements.

Treat blank samples in the same way as described in 8.1 for the sample (following the entire procedure) and following the instructions in 8.3.

When using 50 ml of sample, the blank value shall not exceed the value corresponding to 0,5 µg/l of an individual complexing agent.

9 Calibration

9.1 Preparation of calibration solutions

Prepare calibration solutions by diluting the stock solutions of the complexing agents (5.16). The calibration shall cover the expected working range evenly.

Prepare solutions freshly prior to use. For a calibration curve from 5 µg/l to 100 µg/l, use the concentrations given in Table 4, or for a calibration curve from 0,5 µg/l to 10 µg/l use the concentrations given in Table 5 and add 2 ml of 5 mol/l hydrochloric acid (5.13) or formic acid (5.15) to each solution.

Table 4 — Examples of calibration solutions (5 µg/l to 100 µg/l)

Aliquot of 10 mg/l **stock solution** µl	**Mass of reference compound in aliquot** µg	**Corresponding to a concentration (in** 50 ml **of calibration solution) of** µg/l
25	0,25	5
50	0,5	10
100	1,0	20
200	2,0	40
300	3,0	60
400	4,0	80
500	5,0	100

9.2 Calibration with internal standard or control standard

A prerequisite for the use of an internal standard is that its chemical and gas-chromatographic behaviour is very close, or at least similar, to that of the determinand, and it is absent from the original sample. In the determination of EDTA, DTPA and 1,3-PDTA, use 1,2-PDTA as the internal standard over the entire procedure. In the determination of NTA, ß-ADA and MGDA, use a control standard.

Table 5 — Examples of calibration solutions (0,5 μg/l to 10 μg/l)

Aliquot of 1 mg/l **stock solution** μl	**Mass of reference compound in aliquot** μg	**Corresponding to a concentration (in** 50 ml **of calibration solution) of** μg/l
25	0,025	0,5
50	0,05	1
100	0,1	2
200	0,2	4
300	0,3	6
400	0,4	8
500	0,5	10

NOTE The results of interlaboratory trials have shown that there is no useful internal standard for NTA, β-ADA and MGDA.

Add the same volume of 1,2-PDTA solution or control standard solution to the test sample and to each calibration solution. For example, add 200 μl of the appropriate 10 mg/l calibration solution (5.17), corresponding to 2 μg. Use the same volume of test sample and calibration solution.

Treat the test sample and calibration solutions as described in 8.1 and carry out the esterification and the extraction as descibed in 8.2.

Establish the calibration curves from the chromatograms as follows:

— Measure the peak heights or areas for the determinands and the internal standard or control standard.

— Calculate the ratios of the values measured for the determinands and the standards.

— From these ratios and the respective concentrations, plot, or calculate using regression techniques, the best-fit straight-line calibration curves:

$$y_i = m_i \times \rho_i + b_i \quad (1)$$

where

y_i is the ratio calculated for the propyl or butyl ester of complexing agent i;

ρ_i is the concentration, in micrograms per litre, of complexing agent i;

m_i is the slope of the calibration curve, in litres per microgram, for complexing agent i (will depend on the response factor for the complexing agent concerned);

b_i is the intercept on the ordinate axis of the calibration curve for complexing agent i.

^{13}C-labelled complexing agents are especially suitable as internal standards.

9.3 Calculation

Only calculate the concentration ρ of a complexing agent in the water sample if the value measured is within the calibration range.

Only calculate results for samples for which recovery of the internal standard is at least 60 %. This criterion is met if the ratio of the signal intensities of 1,2-PDTA and the control standards in the gas-chromatographic step in the test sample and the ratio of the signal intensities of 1,2-PDTA and the control standards in the calibration solutions are $>$ 0,6.

Rearranging equation (1) gives:

$$\rho_i = \frac{y_i - b_i}{m_i} \quad (2)$$

from which ρ may be calculated for each complexing agent.

If the values of the ratio y are derived from chromatograms obtained with test samples prepared as described in 9.2, the concentrations of the complexing agents in the samples are given directly for a sample volume of 50 ml. If different sample volumes are used for the calibration and the determination, this shall be taken into account in the calculation.

10 Expression of results

Express the concentrations of the complexing agents, in µg/l, to not more than two significant figures, and rounded to the nearest 0,1 µg/l where applicable.

EXAMPLE 1 NTA 7,1 µg/l

EXAMPLE 2 EDTA 61 µg/l

11 Test report

The test report shall contain the following information:

a) a reference to this International Standard (i.e. ISO 16588);

b) the identity of the water sample;

c) details of sample pretreatment and the type of detector used;

d) the reference compounds used;

e) details of the calibration procedure used;

f) the results as specified in clause 10;

g) any deviations from the method which may have influenced the result.

Annex A
(informative)

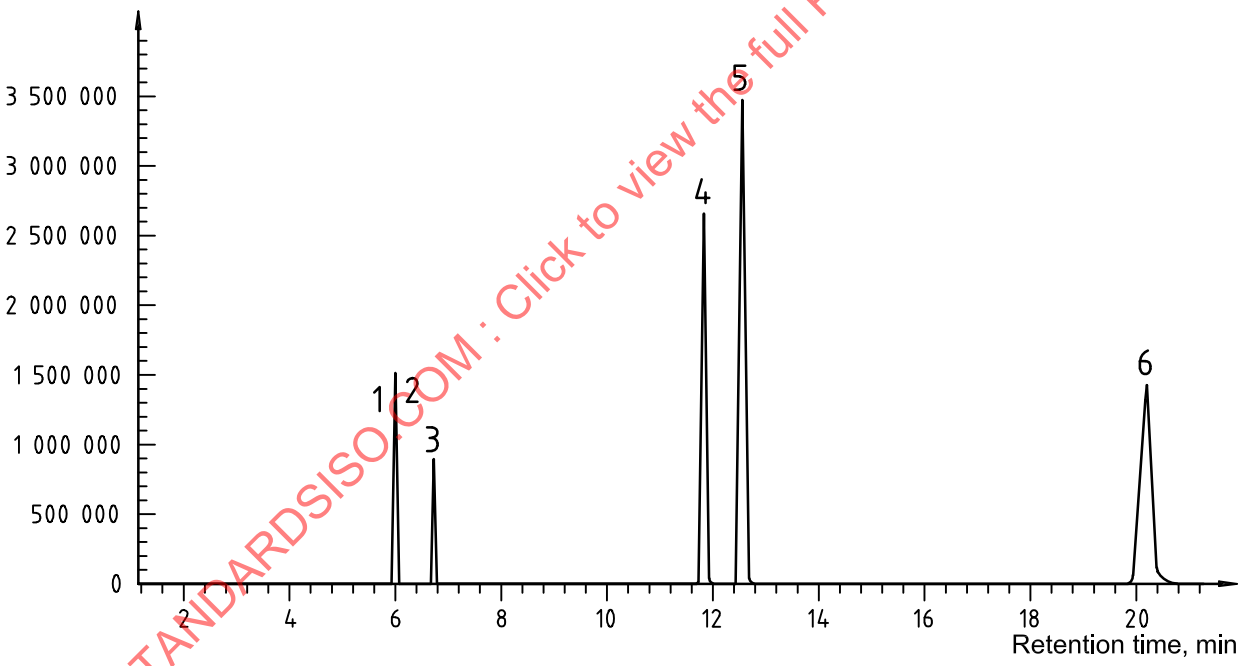
Examples of columns, chromatograms and mass spectra

A.1 Examples of columns

Table A.1 — GC capillary columns (examples)

Column material	Stationary phase	Trade name
Fused silica	100 % Dimethyl polysiloxane	Durabond-1, HP-1
Fused silica	95 % Dimethyl polysiloxane + 5 % Diphenyl polysiloxane	Durabond-5, SE-54
Fused silica	50 % Dimethyl polysiloxane + 50 % Diphenyl polysiloxane	OV-17, DB-17, HP-17

A.2 Example of a chromatogram



Key

1 NTA
2 MGDA
3 β-ADA
4 EDTA
5 1,3-PDTA
6 1,2-PDTA

Gas-chromatographic conditions: cold-injection system, 60 °C, 6 °C/s to 290 °C (5 min)
Column: DB1, 12 m × 0,20 mm × 0,33 µm
Temperature programme: 150 °C (0,4 min), 30 °C/min to 180 °C, 10 °C/min to 290 °C (8,6 min)
Detector: mass spectrometer (TIC)

Figure A.1 — Example of a gas chromatogram of *n*-butyl esters of standards (each 100 µg/l)