TECHNICAL SPECIFICATION

ISO/TS 11370

First edition
2000-11-15

Corrected and reprinted
2001-06-01

Water quality — Determination of selected organic plant-treatment agents — Automated multiple development (AMD) technique

Qualité de l'eau — Dosage de certains agents organiques de traitement des plantes — Méthode automatisée par développement multiple (ADM)

Reference number
ISO/TS 11370:2000(E)

ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.ch
Web www.iso.ch

Printed in Switzerland

Contents

Page

Annexes

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.

The main task of technical committees is to prepare International Standards. 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.

In other circumstances, particularly when there is an urgent market requirement for such documents, a technical committee may decide to publish other types of normative document:

— an ISO Publicly Available Specification (ISO/PAS) represents an agreement between technical experts in an ISO working group and is accepted for publication if it is approved by more than 50 % of the members of the parent committee casting a vote;

— an ISO Technical Specification (ISO/TS) represents an agreement between the members of a technical committee and is accepted for publication if it is approved by 2/3 of the members of the committee casting a vote.

An ISO/PAS or ISO/TS is reviewed every three years with a view to deciding whether it can be transformed into an International Standard.

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

ISO/TS 11370 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 2, *Physical, chemical, biochemical methods*.

Annexes A and B of this Technical Specification are for information only.

Water quality — Determination of selected organic plant-treatment agents — Automated multiple development (AMD) technique

1 Scope

The method described in this Technical Specification is applicable to the determination of selected plant-treatment agents and some of their main degradation products (metabolites) in drinking water, with a validated reporting limit of about > 0,05 µg/l (see examples in Table 1). The method may be extended to include additional substances and ground water, provided the method is validated for each individual case.

The selection of the plant-treatment agents and main degradation products in Table 1 and Table A.2 has been made according to the knowledge at the time of the interlaboratory trial (1992). Data for some other substances are given in annex A.

Table 1 — Plant-treatment agents determinable by this method

Name	Molecular formula	CAS No.[a]	Molar mass g/mol	Peak in Figure No.						
				1	2	3	4	5	6	7
Alachlor[b]	$C_{14}H_2OClNO_2$	015972-60-8	269,8	6				6		
Atrazine	$C_8H_{14}ClN_5$	001912-24-9	215,7	2				4		
Chlorfenvinphos[b]	$C_{12}H_{14}Cl_3O_4P$	000470-90-6	359,6	5				3		
Chlortoluron[b]	$C_{10}H_{13}ClN_2O$	015545-48-9	212,7	1						3
Cyanazine[b]	$C_9H_{13}ClN_6$	021725-46-2	240,7				1			4
2,4-D	$C_8H_6Cl_2O_3$	000094-75-7	221,0	4				1		
MCPA[b]	$C_9H_9ClO_3$	000094-74-6	200,6				2	2		
Metazachlor	$C_{14}H_{16}ClN_3O$	067129-08-2	277,8			3				5
Metobromuron	$C_9H_{11}BrN_2O_2$	003060-89-7	259,1			5				6
Metolachlor[b]	$C_{15}H_{22}ClNO_2$	051218-45-2	283,8			4				7
Metoxuron	$C_{10}H_{13}ClN_2O_2$	019937-59-8	228,7			1				1
Monuron[b]	$C_9H_{11}ClN_2O$	000150-68-5	198,7			2				2
Parathion[b]	$C_{10}H_{14}NO_5PS$	000056-38-2	291,3	7				7		
Pendimethalin	$C_{13}H_{19}N_3O_4$	040487-42-1	281,3		6				6	
Propazine[b]	$C_9H_{16}ClN_5$	000139-40-2	229,7	3				5		
Sebuthylazine[b]	$C_9H_{16}ClN_5$	007286-69-3	229,7		2				3	
Simazine	$C_7H_{12}ClN_5$	000122-34-9	201,7		1				2	
2,4,5-T[b]	$C_8H_5Cl_3O_3$	000093-76-5	255,5		4				1	
Terbutylazine[b]	$C_9H_{16}ClN_5$	005915-41-3	229,7		3				4	
Trifluralin[b]	$C_{13}H_{16}F_3N_3O_4$	001582-09-8	335,3	8				8		
Vinclozoline[b]	$C_{12}H_9Cl_2NO_3$	050471-44-8	286,1		5				5	

[a] CAS No.: Chemical abstracts system.

[b] Not included in the precision data (Table A.2).

2 Normative references

The following normative documents contain provisions which, through reference in this text, constitute provisions of this Technical Specification. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based on this Technical Specification 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 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*.

ISO 5667-3:1994, *Water quality — Sampling — Part 3: Guidance on the preservation and handling of samples*.

ISO 8466-1:1990, *Water quality — Calibration and evaluation of analytical methods and estimation of performance characteristics — Part 1: Statistical evaluation of the linear calibration function*.

ISO 8466-2:1993, *Water quality — Calibration and evaluation of analytical methods and estimation of performance characteristics — Part 2: Calibration strategy for non-linear second order calibration functions*.

ISO/TR 13530:1997, *Water quality — Guide to analytical quality control for water analysis*.

3 Interferences

3.1 Interferences with the extraction

The commercially available RP-C18 materials are often of varying quality. Considerable batch-to-batch differences regarding quality and selectivity of this material, even from one manufacturer, are possible. The recovery may vary with the concentration. Co-extractants eluated from the sorbent material can affect the blank and the recovery. Therefore the calibration and analysis shall be performed on exactly the same batch of sorbent. Also any UV-absorbing material occurring in the water which passes through the procedure and has a similar migration distance to that of the reference standard will interfere. Suspended matter in the water sample may clog the packing. In this case the water sample shall be filtered through a glass fibre filter prior to the extraction.

If the water sample has been acidified to pH 2, humic substances will also be extracted. They may interfere with the determination.

3.2 Interferences with the HPTLC measurement

A contaminated laboratory atmosphere may lead to interferences due to an uncontrolled contamination of the HPTLC-layer. Extremely concentrated solutions may crystallize during sample application, leading to incorrect quantification. Failure of the AMD vacuum will result in poor resolution.

Substances which absorb at the wavelengths of detection and have migration distances similar to those of the compounds to be investigated will interfere with the determination. This shall be taken into account especially when examining samples other than ground- and drinking water.

4 Principle

The substances in the water sample are extracted by solid-liquid extraction on RP-C18 material (RP = reversed phase), eluated with a solvent and then separated by high performance thin layer chromatography (HPTLC), using the Automated Multiple Development (AMD) technique. The detection and determination is performed by diffuse *in-situ* reflection measurement at different UV-wavelengths.

5 Reagents

Water, solvents and reagents shall be of sufficient purity (e.g. residue grade, HPLC grade or AMD grade) and as far as possible shall not contain any measurable UV-absorbing substances which could interfere with the compounds of interest.

5.1 Hydrochloric acid, c(HCl) = 1 mol/l (for example).

5.2 Sodium hydroxide solution, c(NaOH) = 1 mol/l (for example).

5.3 Ammonia solution, $w(NH_3) = 25$ %.

5.4 Gases, for drying, conditioning of the HPTLC plate in the AMD system and for evaporation, e.g. high-purity nitrogen.

5.5 Formic acid, w(HCOOH) = 98 % to 100 %.

5.6 Solvents, e.g. acetone, C_3H_6O; acetonitrile, CH_3CN; dichloromethane, CH_2Cl_2; 3,3-dimethyl-2-oxabutane, $C_6H_{12}O$; ethyl acetate, $C_4H_8O_2$; hexane, C_6H_{14}; methanol, CH_3OH; 2-propanol, C_3H_8O.

WARNING — These solvents are toxic agents. Caution shall be exercised when handling.

5.7 RP-C18 sorbent, for solid-phase extraction. For quality and selectivity of the material, see 3.1.

NOTE Other solid-phase adsorbents may be used, if the performance is comparable to this material and if it has been proved suitable according to 3.1.

5.8 Reference standards (see Table 1), of high purity or certified material.

5.9 Solutions of the individual reference standards.

Place, for example, 50 mg of the reference standards (5.8) in a 100 ml volumetric flask. Dissolve it in methanol or in another solvent (5.6) and make up to volume with the solvent.

NOTE Simazine is poorly soluble in acetonitrile.

Store the solutions at about 4 °C, protected from light. They are stable for at least one month depending on the compound of interest. For longer use, check regularly by comparison with an independent, preferably certified standard solution.

The solution of vinclozoline shall be prepared freshly every second day.

5.10 Stock solution.

As an example, pipette 1 ml each of the solution of the individual standards (5.9) into a 100 ml volumetric flask, and make up to volume with methanol or another solvent (5.6).

Store the solutions at about 4 °C, protected from light. They are stable for at least one month depending on the compound of interest.

5.11 Reference solution for the multipoint calibration.

Prepare the solution by an adequate dilution of the stock solution (5.10), e.g. $\rho_i = 10$ ng/µl.

Store the solution at about 4 °C, protected from light. It is stable for at least one week.

5.12 Reference solutions for the determination of the recovery.

Prepare the solution by an adequate dilution of the stock solution (5.10), e.g. $\rho_i = 20$ ng/ml to 200 ng/ml.

Store the solution at about 4 °C, protected from light. It is stable for at least one week.

5.13 Precoated HPTLC plates, with silica gel 60, 20 cm × 10 cm, preferably 100 m layer thickness, with fluorescence indicator.

The plate shall be prewashed before use (see 8.5.2).

6 Apparatus

Equipment or parts of it which may come into contact with the sample or its extract shall be free from residues that could cause unacceptable interference in blanks. It is recommended to use glass, stainless steel or polytetrafluoroethene (PTFE) and, for cartridges, also polypropylene.

6.1 Flat-bottom flasks or **bottles** for sampling, preferably brown glass, e.g. 1 000 ml and 2 000 ml, stoppered with ground glass stoppers or with polytetrafluoroethene-lined screw caps,

6.2 Graduated cylinders, e.g. 500 ml and 1 000 ml.

6.3 Volumetric flasks, e.g. 10 ml, 25 ml, 50 ml and 100 ml.

6.4 pH meter.

6.5 Vacuum- or overpressure assembly, for sample enrichment and extract concentration.

6.6 Cartridges, from polypropylene or glass, filled with RP-C18 (e.g. internal diameter 9 mm, length 8 cm) or commercially available prefilled cartridges.

6.7 Glass vessels with stopper, for the collection and evaporation of the eluates (e.g. 5 ml graduated flasks or sampling vessels with conical bottom).

6.8 Borosilicate glass fibre filter, diameter 0,75 µm to 1,5 µm, with inorganic binding material.

6.9 Equipment for the evaporation of the eluates, e.g. a rotary evaporator with vacuum stabilizer and temperature-controlled water bath, or equipment for the evaporation of solvent with nitrogen.

6.10 Microlitre syringes, e.g. 100 µl, 250 µl and 1 000 µl, for the preparation of the reference solution and for adding the solvent to redissolve the residue of the evaporated eluates.

6.11 TLC chamber, to wash the HPTLC plates.

6.12 Heating device, suitable to dry the HPTLC plates, e.g. temperature-controlled hot plate.

A drying oven with forced air recirculation is not suitable for this purpose.

6.13 Desiccator, to store the pre-washed and dried HPTLC plates.

6.14 Applicator, for the band-shaped application of the solution onto the HPTLC plates.

6.15 AMD system, with vacuum pump.

6.16 UV scanner, for measurement and evaluation of thin-layer chromatograms, preferably computer-controlled and interfaced to a multicolour plotter.

Measurements below $\lambda = 200$ nm require an instrument equipped with a photomultiplier, specifically suitable for measurements at $\lambda = 190$ nm. Flush the monochromator housing with nitrogen.

7 Sampling and samples

To avoid interferences collect samples as stated below and according to ISO 5667-1, ISO 5667-2 or ISO 5667-3.

Use thoroughly cleaned, preferably brown, flat-bottom flasks (6.1). Rinse the flasks with the water to be sampled; treat the ground glass stoppers or the lined caps in the same way.

Fill the bottles to the brim with the water to be examined.

Transport the cooled sample, protected from light.

Extract substances from the water samples as soon as possible after sample collection.

If storage is unavoidable, keep the water samples at about + 4 °C in the dark or freeze them in suitable containers to approximately −18 °C. Half-filled glass bottles, welded into polyethylene bags, have proved to be appropriate.

NOTE If the samples are stored at about + 4 °C for longer than 3 days, analyte losses may occur.

8 Procedure

8.1 General requirements

The same conditions (e.g. amount of adsorbent, type of cartridge, conditioning, sample volume and flow, eluting steps and volumes) shall be used for all samples within one batch, including the procedure recovery samples.

Low recovery rates can occur from an insufficient amount of RP-C18 sorbent or an insufficient volume of methanol for the conditioning or elution step. Before analysing, these conditions should be checked and optimized in each laboratory. For typical recoveries, see annex A.

8.2 Conditioning of the RP-C18 material

For a water sample of 1 000 ml, place 1 g to 2 g of RP-C18 material (5.7) into a cartridge or glass column or use an adequate commercial device.

NOTE For more polar substances, e.g. metabolites, bad recoveries arise when using 1 g of RP-C18 for a 1 000 ml sample.

Rinse the RP-C18 material in the cartridge or glass column with five times its bed volume of eluting solvent (see 5.6).

Rewash with water (see clause 5) (five times its volume) and use the moist carrier material for the extraction.

The sorbent shall remain moist.

8.3 Extraction

If necessary remove suspended matter by filtration through a glass fibre filter and record this in the final report.

If filtration is carried out, use spiked samples in order to verify that the recovery is not influenced by this additional step.

Measure the water sample to be examined, e.g. 1 000 ml, adjust the pH from 6 to 8 with either hydrochloric acid (5.1) or sodium hydroxide solution (5.2).

If acidic compounds are to be determined, adjust the pH to between 1,5 and 2 with hydrochloric acid (5.1) immediately before the enrichment step.

NOTE Some plant-treatment agents may be destroyed by pH adjustment (e.g. vinclozoline, parathion).

Pass the water samples through 1 g of adsorbent at a flow rate of between 3 ml/min to 15 ml/min. If 2 g of adsorbent are used, the flow rate should not exceed 25 ml/min. Regulate the flow rate by adjusting the vacuum or the overpressure respectively.

Dry the sorbent, for example in a stream of nitrogen or air (at least 45 min at a flow rate of 200 ml/min of nitrogen or air at room temperature).

The degree of dryness can strongly affect the recovery rate. Therefore the analyst shall optimize this drying step.

8.4 Elution and enrichment

Elute with at least 2 ml of solvent per gram of sorbent. For example, the following solvents may be used: methanol, acetonitrile, ethyl acetate, *n*-hexane. Elute in the following way.

Place half of the respective quantity of the eluant onto the column or cartridge and elute into a glass vessel with conical bottom.

Add, after about 15 min, the remainder of the eluant and collect the eluate in the same glass vessel as before.

Transfer the residual solvent remaining on the sorbent by means of vacuum or overpressure into the receiving vessel.

Carefully concentrate the eluate, for example by evaporation in a nitrogen stream at about 35 °C, or with a rotary evaporator under reduced pressure at about 30 °C. As an alternative, evaporate just to dryness.

Dissolve the residue and make up to a defined volume, e.g. with 200 µl of methanol or another solvent. Ultrasonic treatment will help to redissolve the substances.

If trifluralin is to be determined, the dried cartridge shall first be eluted with *n*-hexane as described. Subsequently a second elution is made, e.g. with methanol. The *n*-hexane and methanol eluates are collected in two different glass vessels, separately evaporated to a small volume and analysed. Because *n*-hexane has a lower evaporating temperature, losses of trifluralin can thus be minimized.

8.5 High-performance thin-layer chromatography (HPTLC-AMD)

8.5.1 General requirements

Set up the instrument in accordance with the manufacturer's instructions before starting the analysis. Ensure that the background and baseline drift are sufficiently low.

8.5.2 Purification of the plates

Thoroughly wash the HPTLC plates (5.13) before use, for example by completely immersing them in 2-propanol for at least 1 h.

Dry at approximately 100 °C for at least 30 min, and keep the plates in a closed chamber until use, covered with a clean glass plate (desiccator).

8.5.3 Application of the eluate

Apply 20 µl to 150 µl of the concentrated eluate (see 8.4) onto the HPTLC plate, preferably as bands with a length of 7 mm. The distance from the lower plate edge shall be 8 mm. The distance between the tracks shall be 3 mm. The distance from the left and the right plate edge should be at least 20 mm.

Carefully dry the application zones, e.g. in a stream of ambient air.

It is advantageous to apply more diluted solutions. To avoid overloading effects, the eluates may be sprayed in portions of, for example, three bands, parallel to each other with a distance apart of 2 mm.

8.5.4 Chromatography

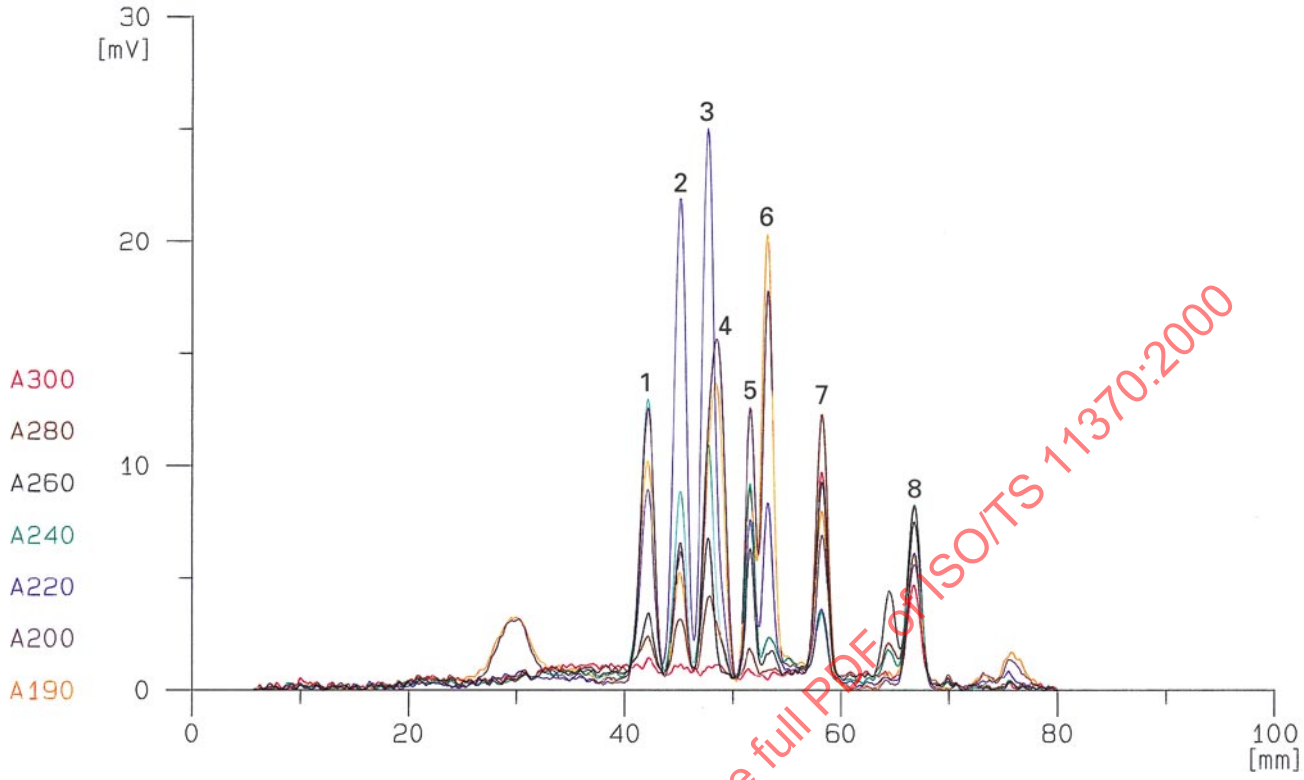
Place the HPTLC plate in the separation chamber and start the run programme, e.g. focus ten times for 0,8 min, then perform 23 runs, empty the mixer after run 15. The development time of the subsequent runs is: 1,1; 1,4; 1,9; 2,4; 3,0; 3,6; 4,1; 5,1; 5,8; 6,6; 7,4; 8,3; 9,3; 10,3; 11,2; 12,2; 13,3; 14,4; 15,4; 16,6; 17,6; 18,5; 22,2. As drying time between the individual runs, choose 1 min to 4 min, depending on the layer thickness of the HPTLC plate and the quality of the vacuum. A longer drying time may be necessary for the last run, e.g. 10 min for 3,3-dimethyl-2-oxabutane, to avoid zone spreading. Before each run the layer is conditioned with e.g. nitrogen.

One of the gradients given in Table 2 may be chosen. If confirmation of the results is necessary, each gradient can confirm the other one.

NOTE By means of an alkaline development during the first runs, the interfering matrix, e.g. humic acids, is fixed at the start. The acidic development during the later runs permits chromatography of the phenoxyalkane carboxylic acids without elution of the humic acids.

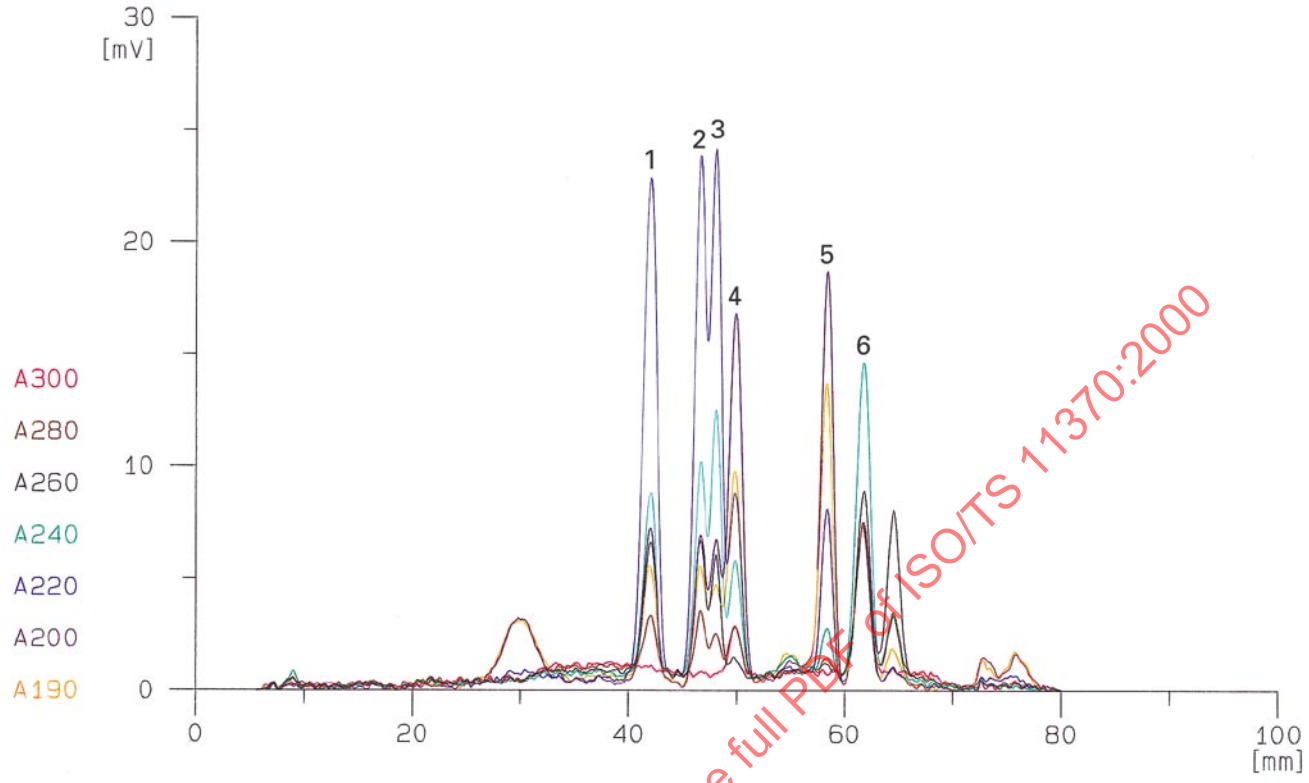
Table 2 — Example of gradient programmes

Gradient I (see Figures 1 to 4)						
Bottle No.	1	2	3	4	5	6
Run	1 to 10	11 to 15	16 to 20	21 to 25	26 to 30	31 to 33
Drying time, min	3	3	3	3	3	3
Empty the mixer after run 15						
Solvent components (volume portions)						
Acetonitrile	30	–	–	–	–	–
Dichloromethane	70	100	100	100	–	–
Hexane	–	–	–	–	100	100
Ammonia solution	0,1	–	–	–	–	–
Formic acid	–	–	0,1	0,1	–	–
Gradient II (see Figures 5 to 7)						
Bottle No.	1	2	3	4	5	6
Run	1 to 10	11 to 14	15	16 to 20	21 to 30	31 to 33
Drying time, min	3	3	3	3	3	3
Empty the mixer after run 14						
Solvent components (volume portions)						
3,3-Dimethyl-2-oxabutane + acetonitrile (90+10 by volume)	100	50	50	25	–	–
Hexane	–	50	50	75	100	100
Ammonia solution	0,2	–	–	–	–	–
Formic acid	–	–	0,5	0,5	–	–



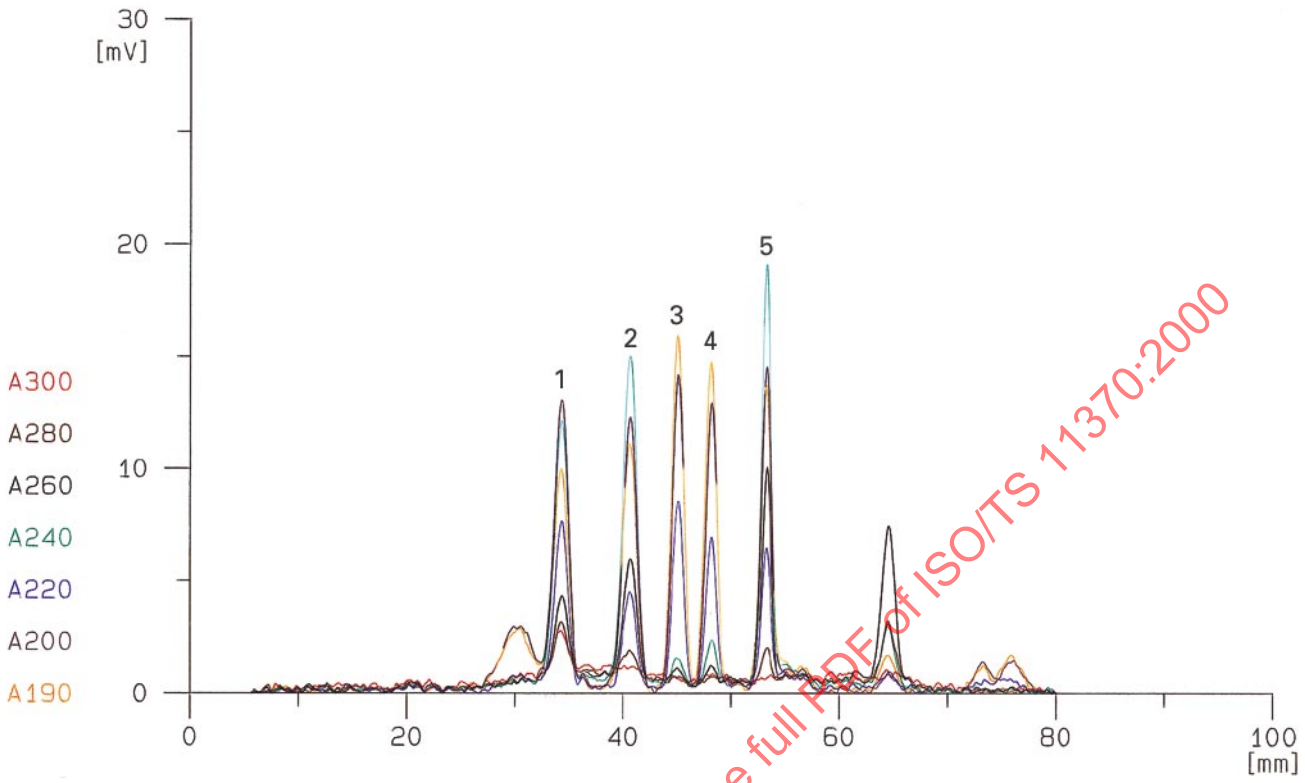
NOTE For peak allocation, see Table 1.

Figure 1 — Thin-layer chromatogram, silica gel 60 WRF254 s, extra thin layer
Example 1: Gradient I: standard solution 1; 100 ng/component



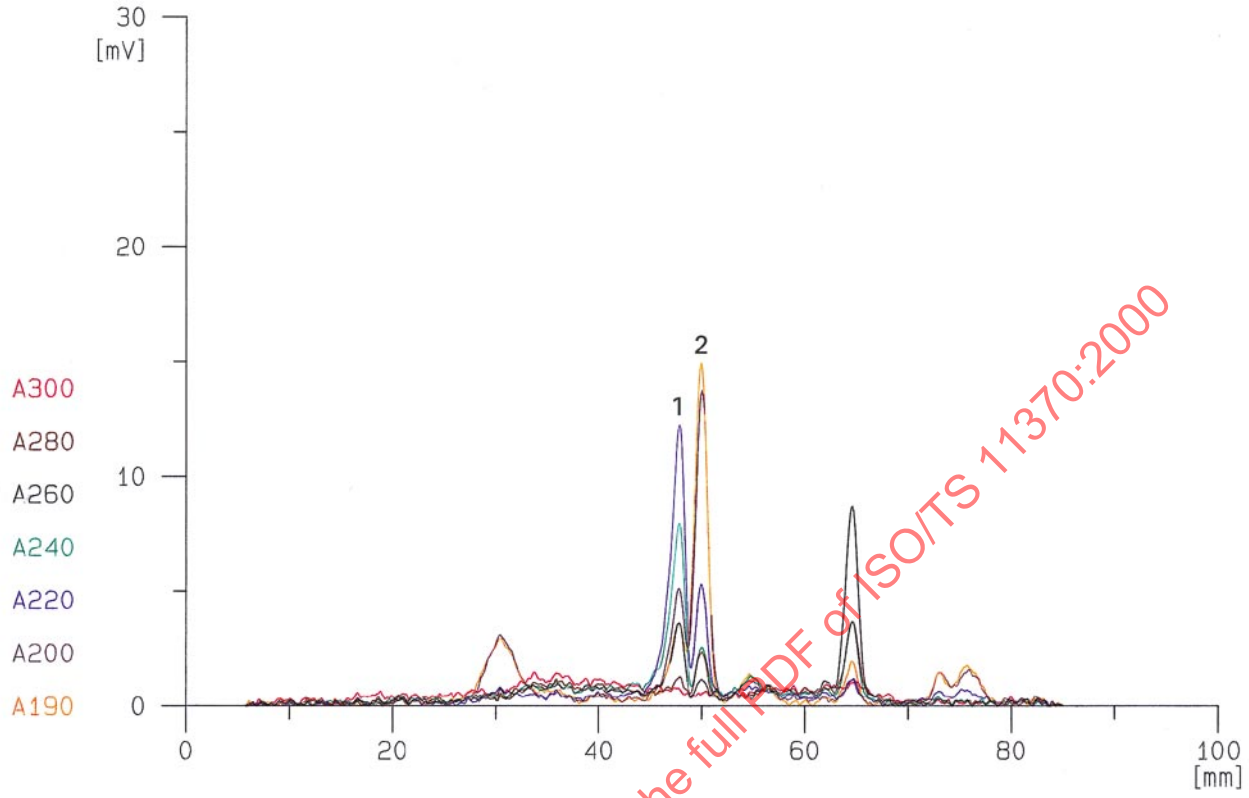
NOTE For peak allocation, see Table 1.

Figure 2 — Thin-layer chromatogram, silica gel 60 WRF254 s, extra thin layer
Example 2: Gradient I: standard solution 2; 100 ng/component



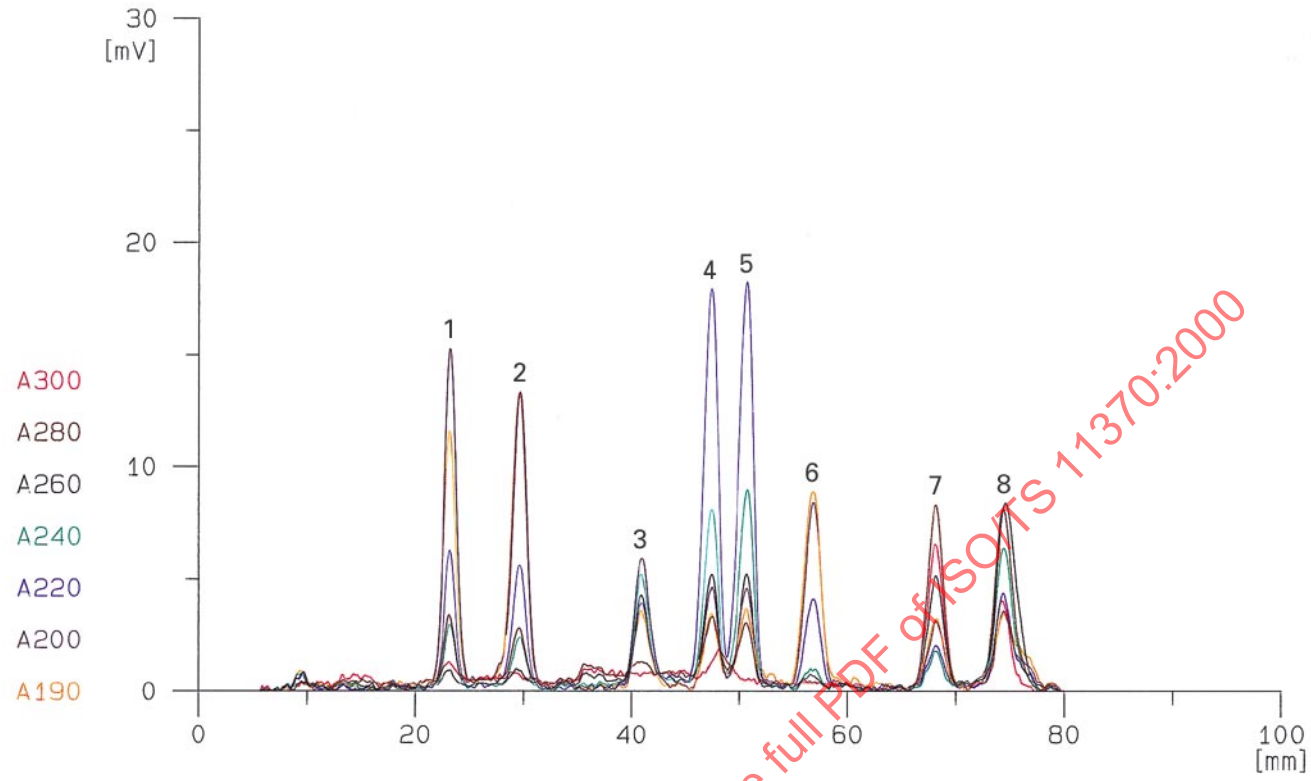
NOTE For peak allocation, see Table 1.

Figure 3 — Thin-layer chromatogram, silica gel 60 WRF254 s, extra thin layer
Example 3: Gradient I: standard solution 3; 100 ng/component



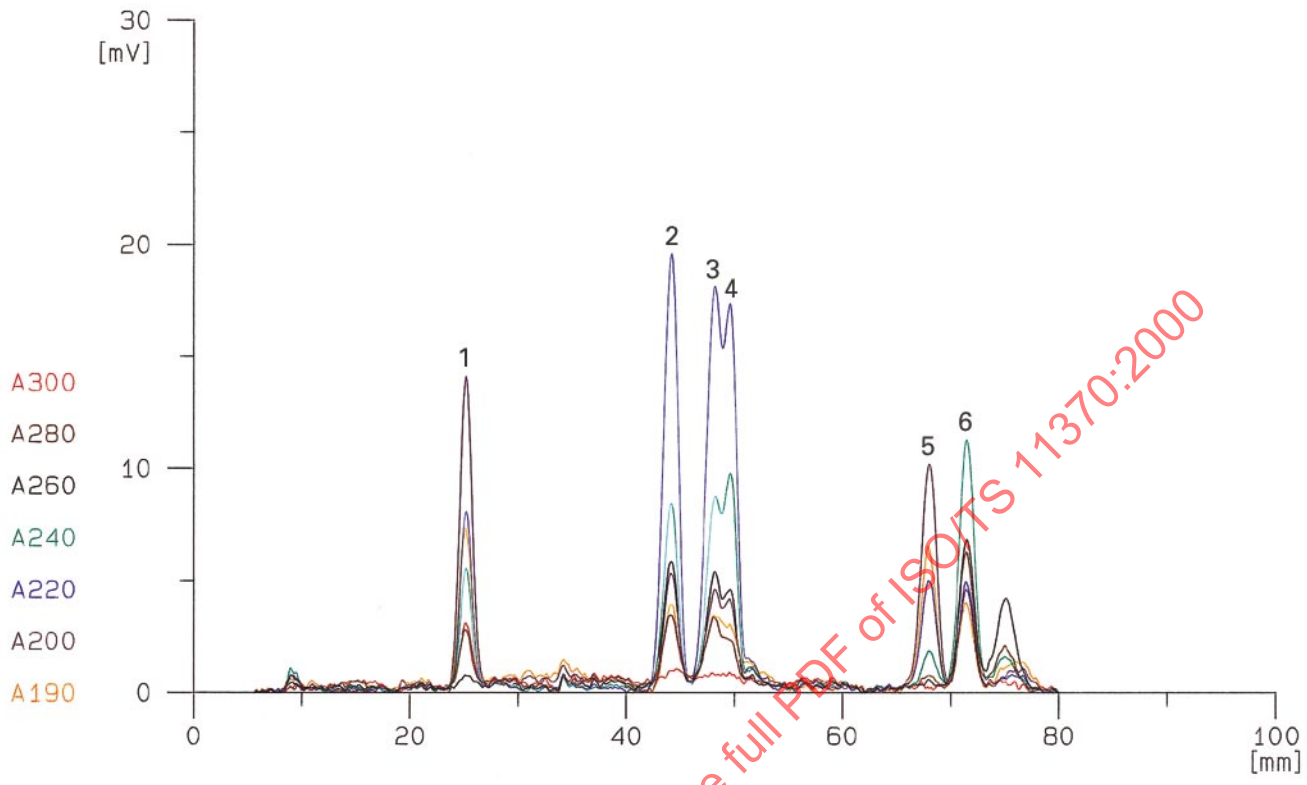
NOTE For peak allocation, see Table 1.

Figure 4 — Thin-layer chromatogram, silica gel 60 WRF254 s, extra thin layer
Example 4: Gradient I: standard solution 4; 100 ng/component



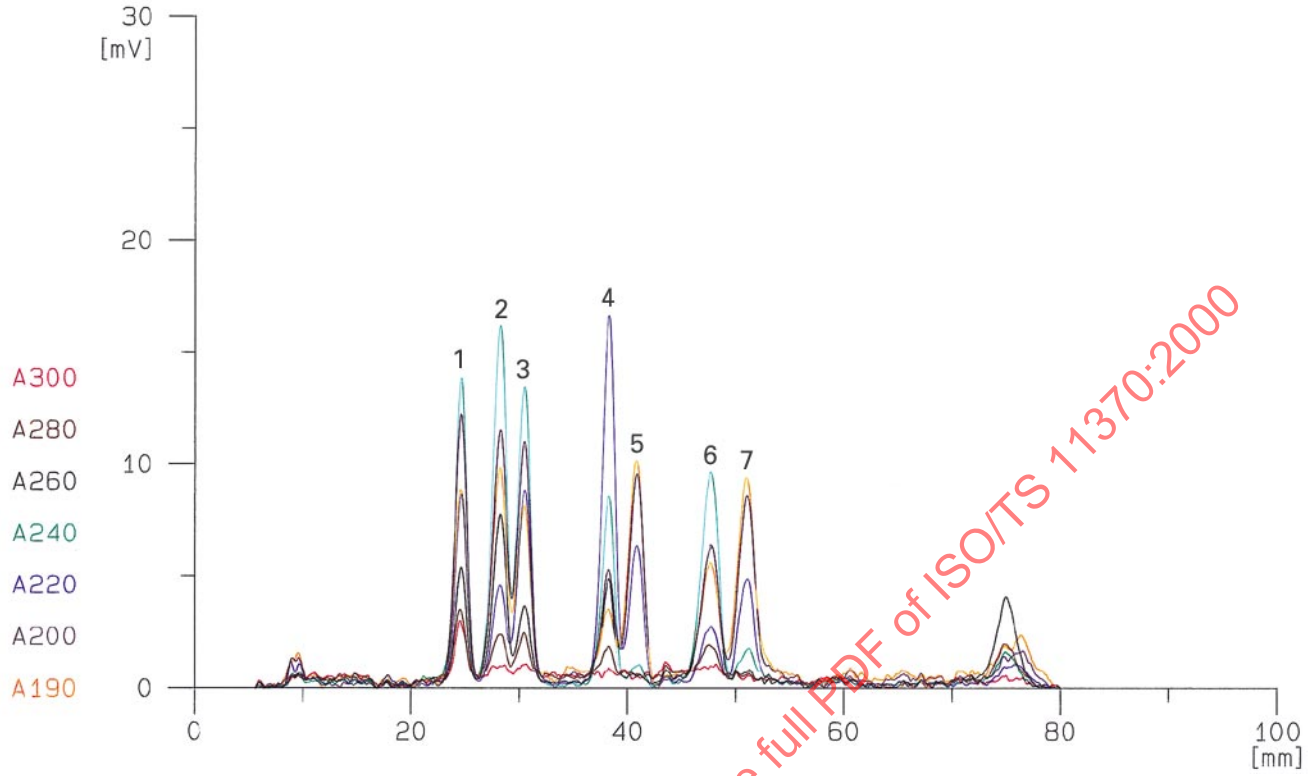
NOTE For peak allocation, see Table 1.

Figure 5 — Thin-layer chromatogram, silica gel 60 WRF254 s, extra thin layer
Example 5: Gradient II: standard solution 1; 100 ng/component



NOTE For peak allocation, see Table 1.

Figure 6 — Thin-layer chromatogram, silica gel 60 WRF254 s, extra thin layer
Example 6: Gradient II: standard solution 2; 100 ng/component



NOTE For peak allocation, see Table 1.

Figure 7 — Thin-layer chromatogram, silica gel 60 WRF254 s, extra thin layer
Example 7: Gradient II: standard solution 3; 100 ng/component

8.5.5 Detection

Record the chromatograms with a UV scanner (6.16), for example at the following wavelengths: $\lambda = 190$ nm, 220 nm, 240 nm, 280 nm and 300 nm.

It is recommended to superimpose the individual scans at the various wavelengths on a multicolour plotter for visual evaluation.

If necessary, measure the UV spectra in the range of 190 nm to 400 nm for identification or purity tests.

8.6 Blank monitoring

For quality control of the analytical procedure, determine the contribution of the reagents and equipment to the HPTLC chromatograms by analysing the same volume of water (see clause 5) corresponding to the sample volume and following the analytical procedure in all steps (8.1 to 8.5). If interfering peaks are detected in the blank (typically greater than 10 % of the lowest measured value), carry out systematic investigations to detect and eliminate the source of contamination.

8.7 Confirmation and identification of substances

NOTE Substances which absorb at the wavelengths of detection and have a migration distance similar to those of the compounds to be investigated will interfere with the determination. This is more likely to occur when examining samples other than ground- and drinking water. Depending on the quality of resolution, these interferences may lead to unresolved peaks, and these will affect the accuracy of the results. Additionally unsymmetrical peaks and peaks broader than those obtained with the reference standard are also an indication of interferences. Information on the identity and chromatographic purity of a compound allocated by its migration distance may be obtained from the absorption spectra of the signals (see 8.5.5). In borderline cases, an independent method should additionally be used.

Characterize an individual compound in the chromatogram by comparing its migration distance with the migration distance of the reference standard. For a correct assignment, the difference between the compared migration distances should be less than 5 %.

NOTE To check the stability of the migration distances it is helpful to add an appropriate UV-absorbing substance as a reference peak to the final extract. For this purpose *N*-benzoylaniline (benzanilide) may be used.

If there is no peak at the characteristic migration distance and the chromatogram is normal in all other respects, the substance is regarded as not being present.

If a peak corresponds to a reference standard, the presence of the substance is possible; the identity of the substance shall be confirmed by further analysis.

If the response of the measuring signals at different wavelengths for the sample and the reference standard (both measurements at similar concentration) agree to within $<$ 10 % deviation (related to the smaller value) the qualitative result is probable, in the case of drinking water it is very probable. Alternatively, the results, calculated independently (see clause 9 to 10.2) at two (normally the most intense) different wavelengths, shall not differ by more than 10 %.

A substance being assigned by the migration distance may be considered as identified if the absorption spectrum is in agreement with that of the reference standard (explanations and instructions of spectrometer manufacturers shall be observed).

NOTE An additional confirmation is obtained if the quantitative results of two different gradients (Table 2) deviate by less than 10 %. Another possibility is the confirmation by post-chromatographic derivatization reactions; furthermore the limit of detection may be lowered in this way. An alternative confirmation is obtained by the application of another procedure (e.g. gas chromatography GC, GC-mass spectrometry, or high-performance liquid chromatography HPLC).

9 Calibration

9.1 General requirements

Initially, it is necessary to determine the recovery rate. This recovery rate is obtained by the two following calibration steps:

a) calibration of the HPTLC step by direct application of the reference solutions (9.2);

b) calibration of the overall procedure (9.3) using water samples, spiked and extracted.

Both methods deliver information on the type of calibration function, migration distances and relative responses of determinands.

The data obtained from the chromatograms (9.3) are compared with the previous (9.2) in order to calculate the recovery rate (9.4) of each determinand.

For routine calibration, the procedure according to 9.2 should preferably be applied, with calibration carried out on each individual HPTLC plate. The calibration function achieved for one substance is valid only for the calibration range applied.

Adapt the working range to the real requirements (see clause 1). At least five different calibrating points, uniformly distributed over the entire working range, are necessary.

The subscripts used in the following context are defined in Table 3.

NOTE A second-order polynominal regression is described in the following. Within a restricted measuring range, a first-order function may be used for the evaluation.

Table 3 — Definition of subscripts

Subscript	Meaning
i	Identity of the substance
e	Calibration step
g	Overall procedure
j	Consecutive figure for pairs of values

9.2 Calibration of the HPTLC step (Calibration with external standard, not covering the overall procedure)

Establish a calibration function for each determinand i using at least five different concentrations or absolute quantities, respectively. It is appropriate to examine all compounds mentioned in Table 1, with as few multicomponent solutions as possible.

Knowledge of the migration distance of the individual substance under investigation is prerequisite. These data are determined with the solutions of the individual standards (5.9).

Establish the calibration function by application of different volumes of the reference solution (5.11).

The application volume should not be less than 20 µl.

Display the calibration function graphically.

Plot the measuring values $y_{i\text{ej}}$ (peak areas or peak heights, respectively) for each substance i on the ordinate and the associated mass concentration $\rho_{i\text{ej}}$ on the abscissa.

Determine the second order function using the pairs of values $y_{i\text{ej}}$ and $\rho_{i\text{ej}}$ of the measuring series.

$$y_{i\text{e}} = m_i \cdot \rho_{i\text{e}}^2 + a_i \cdot \rho_{i\text{e}} + b_i \quad (1)$$

where

$y_{i\text{e}}$ is the measuring value of substance i obtained from the calibration, depending on $\rho_{i\text{e}}$; the unit depends on the evaluation; e.g. peak height value;

m_i is the coefficient of the quadratic term of the calibration function of substance i (e.g. in peak height value)/(ng/band)^2;

$\rho_{i\text{e}}$ is the mass concentration of substance i (external standard in the reference solution), in ng/band;

a_i is the coefficient of the linear term in the calibration function of substance i (e.g. in peak height value)/(ng/band);

b_i is the coefficient of the linear term in the calibration function of substance i (e.g. in peak height value)/(ng/band).

9.3 Calibration with external standard, covering the overall procedure

Calibrate the overall procedure by fortification of control water samples (see clause 5) (e.g. 1 000 ml) with 1 ml of a range (minimum 5) of reference solutions (5.12).

Extract, enrich and analyse these solutions following the analytical procedure.

Display the calibration function graphically, using the pairs of values $y_{i\text{egj}}$ and $\rho_{i\text{egj}}$ as described in 9.2.

Determine the second-order function using the pairs of values $y_{i\text{egj}}$ and $\rho_{i\text{egj}}$ of the measuring series:

$$y_{i\text{eg}} = m_{i\text{g}} \cdot \rho_{i\text{eg}}^2 + a_{i\text{g}} \cdot \rho_{i\text{eg}} + b_{i\text{g}} \quad (2)$$

where

$y_{i\text{eg}}$ is the measuring value of substance i, obtained from the calibration as a function of $\rho_{i\text{eg}}$; the unit depends on the evaluation, e.g. peak height value;

$\rho_{i\text{eg}}$ is the mass concentration of substance i in the spiked control water sample, in ng/l;

$m_{i\text{g}}$ is the coefficient of the quadratic term in the calibration function of substance i (e.g. in peak height value)/(ng/l)^2;

$a_{i\text{g}}$ is the coefficient of the linear term in the calibration function of substance i (e.g. in peak height value)/(ng/l);

$b_{i\text{g}}$ is the ordinate intercept of the calibration line of substance i (e.g. in peak height values).

9.4 Determination of the procedural recovery values

Reliable recovery data are obtained from analysis of water and fortified control water samples (see clause 5) at different concentration levels, equidistantly spread over the working range. From these individual results a mean specific recovery $\overline{A}_i$ is calculated.

Add to, for example, 1 000 ml of water (see clause 5) 1 ml each of the respective reference solutions (5.12) and proceed in the same way as described for a natural sample.

Calculate with the aid of the calibration procedure according to 9.2 the substance-specific mean recovery $\overline{A}_i$ for the substance i with equations (3) and (4). $\overline{A}_i$ has to be calculated for a typical water.

$$\overline{A}_i = \sum_{N=1}^{N} A_{in}/N \quad (3)$$

where N is the number of individual measuring values A_i.

$$A_{in} = \frac{\rho_{in\text{fd}}}{\rho_{in,\,\text{nom}}} \cdot f \quad (4)$$

where

A_{in} is the recovery of substance i at the concentration level n;

$\rho_{in\text{fd}}$ is the found mass concentration of the substance i at the concentration level n, calculated with equation (2) in 9.3, in ng/l;

$\rho_{in,\,\text{nom}}$ is the given (nominal) mass concentration of the substance i at the concentration level n, in ng/l;

f is a factor, here 100.

NOTE With the extraction procedure described in clause 8, high recoveries (see Table A.1 in annex A) will normally be achieved. Low and diverging recoveries indicate matrix effects and/or problems with the extraction (e.g. insufficient drying).

10 Evaluation

10.1 Calculation of the individual result using external standard calibration

Calculate, with equation (5), the mass concentration ρ_i of substance i in the water sample:

$$\rho_i = \left(-\frac{a_i}{2m_i} - \sqrt{\frac{a_i^2}{4m_i^2} - \frac{b_i - y_i}{m_i}} \right) \cdot \frac{V_0}{V_\mathrm{p} \cdot V_\mathrm{A} \cdot \overline{A}_i \cdot f} \qquad (5)$$

where

ρ_i is the mass concentration of the determinand i in the water sample, in ng/l;

y_i is the measuring value of the determinand i in the measuring solution, e.g. peak height value;

a_i, b_i, m_i see equation (1);

V_0 is the volume of the measuring solution from which the applied sample is taken, in ml;

V_p is the volume of the extracted water sample, in ml;

V_A is the volume applied of the measuring solution, in ml;

f is the conversion factor, here $f = 105$;

$\overline{A}_i$ see equation (3).

10.2 Calculation of the individual results for the calibration with external standard, covering the overall procedure

Calculate the mass concentration ρ_{ig} of the substance i in the water sample with equation (6):

$$\rho_{ig} = -\frac{a_{ig}}{2m_{ig}} - \sqrt{\frac{a_{ig}{}^2}{4m_{ig}{}^2} - \frac{b_{ig} - y_{ig}}{m_{ig}}} \qquad (6)$$

where

ρ_{ig} is the mass concentration of the determinand i in the water sample, in ng/l;

y_{ig} is is the measuring value of the determinand i in the water sample, e.g. peak height value;

a_{ig}, b_{ig}, m_{ig} see equation (2).

11 Expression of results

The mass concentration of the individual plant-treatment agents are reported in micrograms per litre to not more than two significant figures.

EXAMPLES

Chlorotoluron 0,08 µg/l

Atrazine 0,12 µg/l