
**Water quality — Determination of selected
plant treatment agents — Method using
high performance liquid chromatography
with UV detection after solid-liquid
extraction**

*Qualité de l'eau — Dosage de certains agents de traitement des plantes —
Méthode par chromatographie en phase liquide à haute performance (CLHP)
avec détection UV après extraction solide liquide*



Contents

Page

1	Scope	1
2	Interferences	2
3	Normatives references	2
4	Principle	2
5	Reagents	2
6	Apparatus	3
7	Sampling and samples	4
8	Procedure	5
9	Calibration	10
10	Evaluation	13
11	Expression of results	14
12	Test report	14
13	Precision data	14
Annex A (informative) Recovery rates		15
Annex B (informative) Results of interlaboratory trial		17

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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.

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.

International Standard ISO 11369 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 2, *Physical, chemical and biochemical methods*.

Annexes A and B of this International Standard are for information only.

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Water quality — Determination of selected plant treatment agents — Method using high performance liquid chromatography with UV detection after solid-liquid extraction

1 Scope

This International Standard describes a method for the determination of organic plant treatment agents in drinking and ground water using high performance liquid chromatography (HPLC) with UV detection after solid-liquid extraction.

The method described in this International Standard 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 0,1 µg/l. Limited additional data indicate that it can be extended to 0,05 µg/l (see table 1 for examples). 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 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 to which this International Standard applies

Name	Molecular formula	Molar mass	CAS No. ¹⁾	Substance family ²⁾
Atrazine	C ₈ H ₁₄ ClN ₅	215,7	001912-24-9	T
Chlorotoluron	C ₁₀ H ₁₃ ClN ₂ O	212,7	015545-48-9	H
Cyanazine**	C ₉ H ₁₃ ClN ₆	240,7	021725-46-2	T
Desethylatrazine *	C ₆ H ₉ ClN ₅	186,6	006190-65-4	T
Diuron	C ₉ H ₁₀ Cl ₂ N ₂ O	233,1	000330-54-1	H
Hexazinone**	C ₁₂ H ₂₀ N ₄ O ₂	252,3	051235-04-2	T
Isoproturon	C ₁₂ H ₁₈ N ₂ O	206,3	034123-59-6	H
Linuron	C ₉ H ₁₀ Cl ₂ N ₂ O ₂	249,1	000330-55-2	H
Metazachlor	C ₁₄ H ₁₆ ClN ₂ O ₃	277,8	067129-08-2	A
Methabenzthiazuron	C ₁₀ H ₁₁ N ₃ OS	221,3	018691-97-9	H
Metobromuron**	C ₉ H ₁₁ BrN ₂ O ₂	259,1	003060-89-7	H
Metolachlor	C ₁₅ H ₂₂ ClNO ₂	283,8	051218-45-2	A
Metoxuron**	C ₁₀ H ₁₃ ClN ₂ O ₂	228,7	19937-59-8	H
Monolinuron	C ₉ H ₁₁ ClN ₂ O ₂	214,6	1746-81-2	H
Sebutylazine**	C ₉ H ₁₅ ClN ₅	228,7	00728-69-3	T
Simazine	C ₇ H ₁₂ ClN ₅	201,7	000122-34-9	T
Terbutylazine	C ₉ H ₁₆ ClN ₅	229,7	005915-41-3	T

1) CAS No.: Chemical abstracts number
 2) Substance family: T: Triazine; H: Phenylurea herbicide; A: substituted anilide
 *: Main degradation product of atrazine
 **: Not included in the performance data

2 Interferences

2.1 Interferences with the enrichment

The commercially available RP (reversed phase)-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 eluted from the sorbent material can affect the blank and the recovery. Therefore the calibration and analysis are 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 retention time similar to the standard will interfere. Suspended matter in the water sample may clog the packing. In this case the water sample is filtered through a glass fibre filter prior to the enrichment.

2.2 Interferences with the HPLC measurement

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

3 Normative references

The following standards contain provisions which, through reference to this text, constitute provisions of this International Standard. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO 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/TR 13530:—¹⁾, *Water quality - General guidance to analytical quality control for water analysis*

4 Principle

The plant treatment substances in the water sample are extracted by solid-liquid extraction on RP-C18 material (RP = reversed phase), eluted with a solvent and then separated, identified and quantified by high performance liquid chromatography (HPLC) using UV detection.

5 Reagents

5.1 General requirements

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

5.2 Nitrogen, high purity, for drying solvents and, if need be, for concentration by evaporation of the eluates.

5.3 Helium, high purity, for degassing HPLC solvents (see also 6.13)

1) To be published.

5.4 Mineral acid, e. g. phosphoric acid, $c(\text{H}_3\text{PO}_4) = 1 \text{ mol/l}$.

5.5 Sodium hydroxide solution $c(\text{NaOH}) = 1 \text{ mol/l}$.

5.6 RP-C18 sorbent, for the solid-phase extraction. For quality and selectivity of the material see 2.1.

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 2.1.

5.7 Solvents, e. g. methanol (CH_3OH), acetonitrile (CH_3CN), acetone ($\text{C}_3\text{H}_6\text{O}$).

WARNING These solvents, especially acetonitrile, are toxic agents. Caution shall be exercised when handling.

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

5.9 Solutions of the individual standards

Place 50 mg (for example) of the reference standards (see 5.8) into a 100 ml volumetric flask, dissolve it in methanol or another solvent (see 5.7) and make up to volume with the solvent.

NOTE: Simazine is only 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.

5.10 Stock solution

As an example, pipette 1 ml each of the solution of the individual substances (see 5.9) into a 100 ml volumetric flask, and make up to volume with methanol or another solvent (5.7).

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 solutions for multipoint calibration

Prepare the solutions by an adequate dilution of the stock solution (5.10) or several stock solutions to have at least 5 multicomponent reference solutions, e.g. $\rho_i = 20 \text{ to } 200 \text{ ng/ml}$. As solvent, use the initial HPLC eluant mixture.

Store the reference solutions at about 4 °C, protected from light. They are stable for at least one week.

5.12 Buffer solutions for gradient elution

As an example, aqueous solution of ammonium acetate ($\text{CH}_3\text{COONH}_4$), or sodium acetate CH_3COONa , concentration $\leq 20 \text{ mmol/l}$. (See also the figures).

Filter the solution through a membrane filter, pore size 0,45 μm , before use.

NOTE: Due to microbiological activity, the shelf-life of the buffer may be limited. Therefore it should be replaced every second day.

6 Apparatus

6.1 General requirements

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 polypropene.

6.2 Cartridges from polypropene or glass, filled with RP-C18, of e.g. internal diameter 9 mm, length 8 cm, or commercially available prefilled cartridges.

6.3 Flat-bottom flasks or bottles for sampling, preferably brown glass, 1000 ml and 2000 ml, stoppered with ground glass stoppers or with PTFE-lined screw caps.

6.4 Graduated cylinders, 10 ml and 1000 ml.

6.5 Glass vessels for the collection and evaporation of the eluates, such as centrifuge tubes, 12 ml, with ground glass stoppers.

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

6.7 Glass vials with inert stopper, such as PTFE-coated septum, for storage of extracts.

6.8 Volumetric flasks, 1 ml, 10 ml, and 100 ml.

6.9 Microlitre syringes, 25 μ l, 50 μ l, 100 μ l, 250 μ l and 1000 μ l, for manual injection into the HPLC system, for preparation of the reference solutions and for adding the solvent to redissolve the residue of evaporated eluates.

6.10 Borosilicate glass fibre filter diameter, 0,75 to 1,5 μ m, with inorganic binding material.

6.11 Membrane filter for clear filtration of the extracts, such as a polyamide or cellulose membrane, pore size 0,2 μ m to 0,45 μ m.

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

6.13 Degassing system for the HPLC instrument.

6.14 Analytical column

Typical analytical column, length up to 300 mm, internal diameter 2 mm to 4,6 mm, packed with RP-C18 material, particle size 3 μ m to 5 μ m. The column shall be capable of a baseline resolution of the compounds listed in table 1 (see also the figures).

6.15 High performance liquid chromatograph, consisting of

- a) solvent gradient elution system with manual or automatic sampling application, designed for the desired analytical working range;
- b) degassing assembly, if necessary;
- c) column thermostat, able to guarantee a constant temperature with less than ± 1 °C deviation;
- d) UV detector, preferably diode-array detector, for the on-line recording of absorption spectra in the range 200 nm to 350 nm, or alternatively, a detector capable of monitoring at least two different wavelengths.
- e) data processing or integration system.

7 Sampling and samples

Use for sampling carefully cleaned, preferably brown, flat-bottom glass flasks (see 6.3). 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.

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

To avoid interferences, collect samples as stated below and according to the appropriate part of ISO 5667.

If storage is unavoidable, keep the water sample at 4 °C in the dark.

NOTE: Water samples may be stored at 4 °C and for not longer than 1 week.

8 Procedure

8.1 General requirements

It is absolutely essential that tests conducted according to this International Standard are carried out by suitably qualified staff.

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.

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

NOTE: Low recovery rates can result from using an insufficient amount of 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 common recovery rates, see annex A.

8.2 Conditioning of the RP-C18 material

For a water volume of 1000 ml, place 1,0 g to 2,0 g of RP-C18 material (see 5.6) into a cartridge or a glass column, or use an adequate commercial device.

NOTE: For more polar substances, e.g. metabolites, poor recoveries result when using 1 g/l for a one-litre sample.

Rinse the RP-C18 material in the cartridge or glass columns with five times its bed volume of eluting solvent (see 5.7).

Rewash with water (see 5.1) (using five times its volume) and use the moist carrier material for the enrichment. The sorbent shall remain moist.

8.3 Enrichment

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 to 6 to 8 with either mineral acid (see 5.4) or sodium hydroxide solution (see 5.5).

Pass the water sample through 1 g of adsorbent at a flowrate of between 3 ml/min to 15 ml/min. If 2 g of adsorbent are used, the flowrate should not exceed 25 ml/min.

Regulate the flowrate by adjusting the vacuum or the overpressure, respectively.

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

8.4 Elution

Elute in the following way with at least 1 ml of solvent (see 5.7) per 500 mg of RP-C18 material (see 5.6).

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

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

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

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

Dissolve the residue and make up to a defined volume, e.g. 1 ml, using the initial HPLC eluant as solvent. Ultrasonic treatment will help to redissolve the substances.

Filter the extract through a membrane filter, if necessary.

Use an aliquot of this solution for the HPLC determination.

8.5 High performance liquid chromatography (HPLC)

8.5.1 General requirements

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

8.5.2 Chromatographic separation

Use analytical columns packed with reversed phase material (see 6.14) capable of separating the compounds listed in table 1.

Achieve optimization of the separation by adjusting either the initial solvent composition or the solvent gradient or the final solvent composition (see figures 1 and 2).

NOTES

1 Because of its higher optical transparency and lower viscosity, use of acetonitrile solvent should be given preference to methanol. Particular attention is drawn to the toxicity of acetonitrile.

2 The maximum injectable sample volume which can be used without perceptible band broadening depends on various parameters, including the internal diameter of the analytical column. As an example, the injection volume should not exceed 100 µl for columns of 4 mm internal diameter.

8.5.2.1 Chromatographic conditions for the separation in figure 1 a) to c)

Injection volume: 25 µl of plant treatment standard solution ($\rho_i = 100$ ng/ml each)

Solvent: 2 mmol of potassium acetate buffer (pH = 6,5)

Column: ODS Hypersil 3 µm (250 x 4 mm)

Eluant gradient: A 2 mmol of potassium acetate buffer (pH = 6,5) /acetonitrile/8:2

B acetonitrile

Gradient: 10 % B to 45 % in 75 min linear

Flush time: 10 min with eluant B/10 % A

Equilibration: 10 min under starting conditions

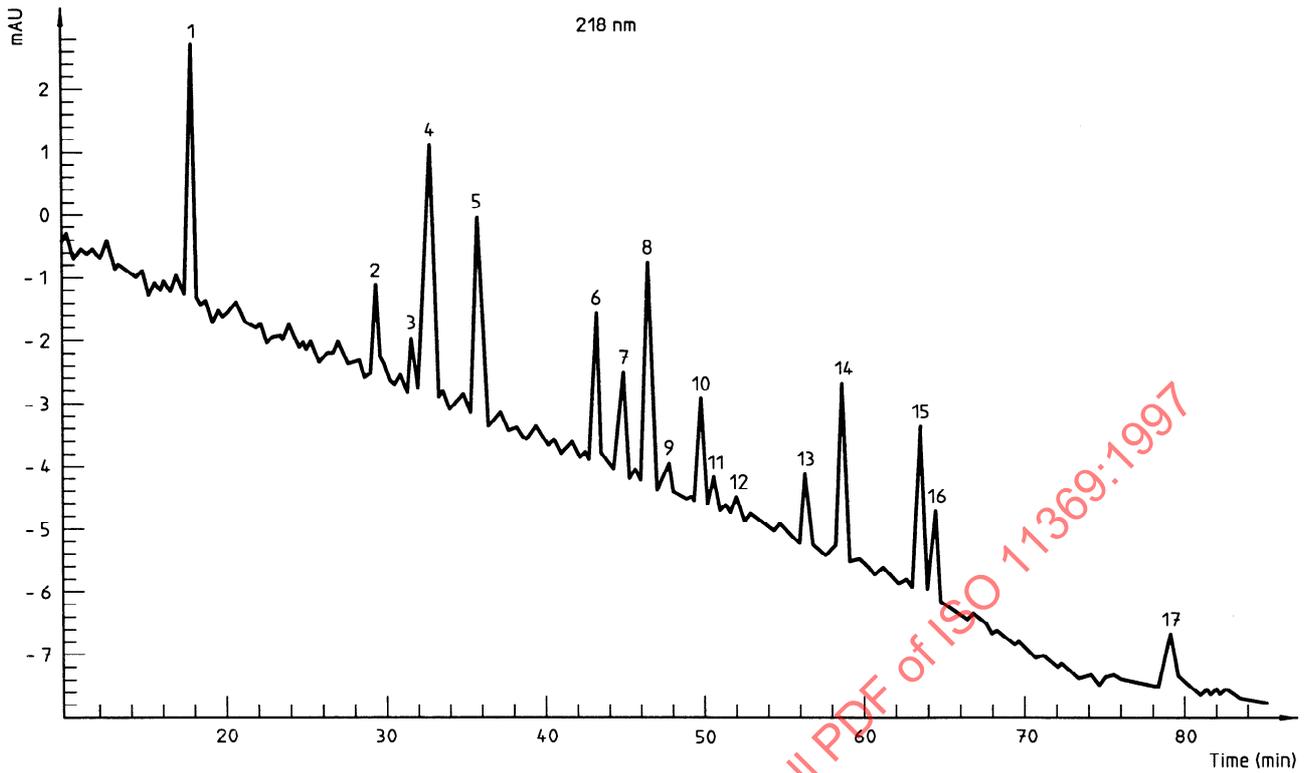
Flowrate: 0,35 ml/min

Temperature: 40 °C

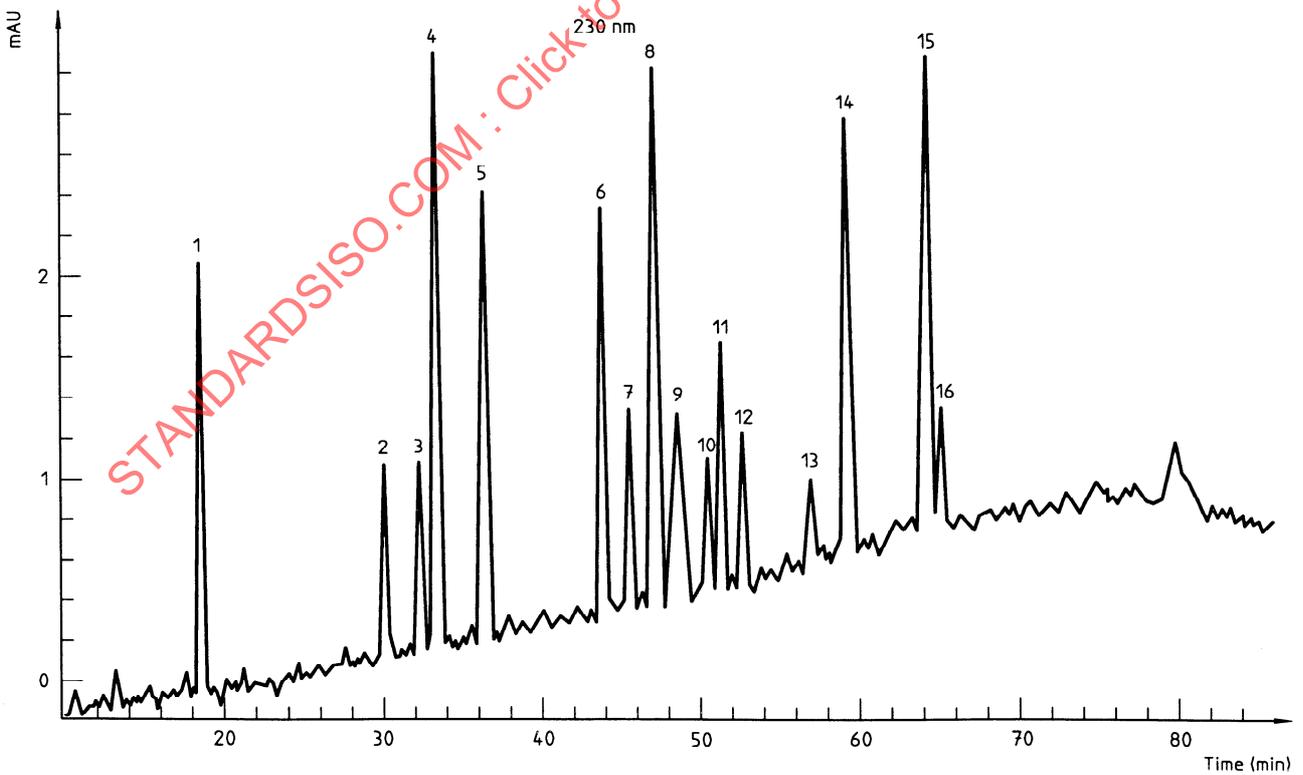
Diode array detector; flow cell $d = 10$ nm, time constant 640 ms

Wavelengths 218 nm, 230 nm, 245 nm, bandwidth 4 nm.

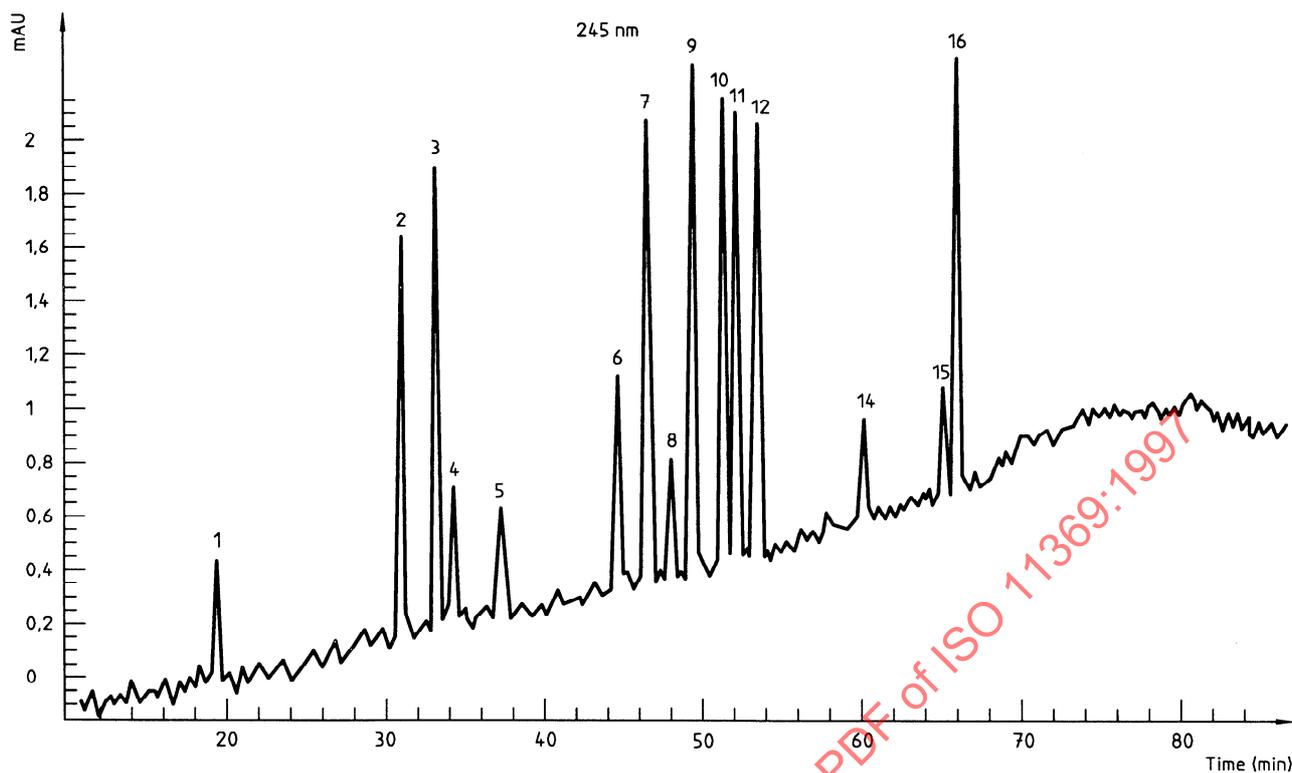
Reference wavelengths: 460 nm, bandwidth 80 nm.



a) Chromatogram measured at 218 nm



b) Chromatogram measured at 230 nm



c) Chromatogram measured at 245 nm

Figure 1 — Chromatograms of a low-concentration standard of plant treatment agents, separated with acetonitrile buffer gradient

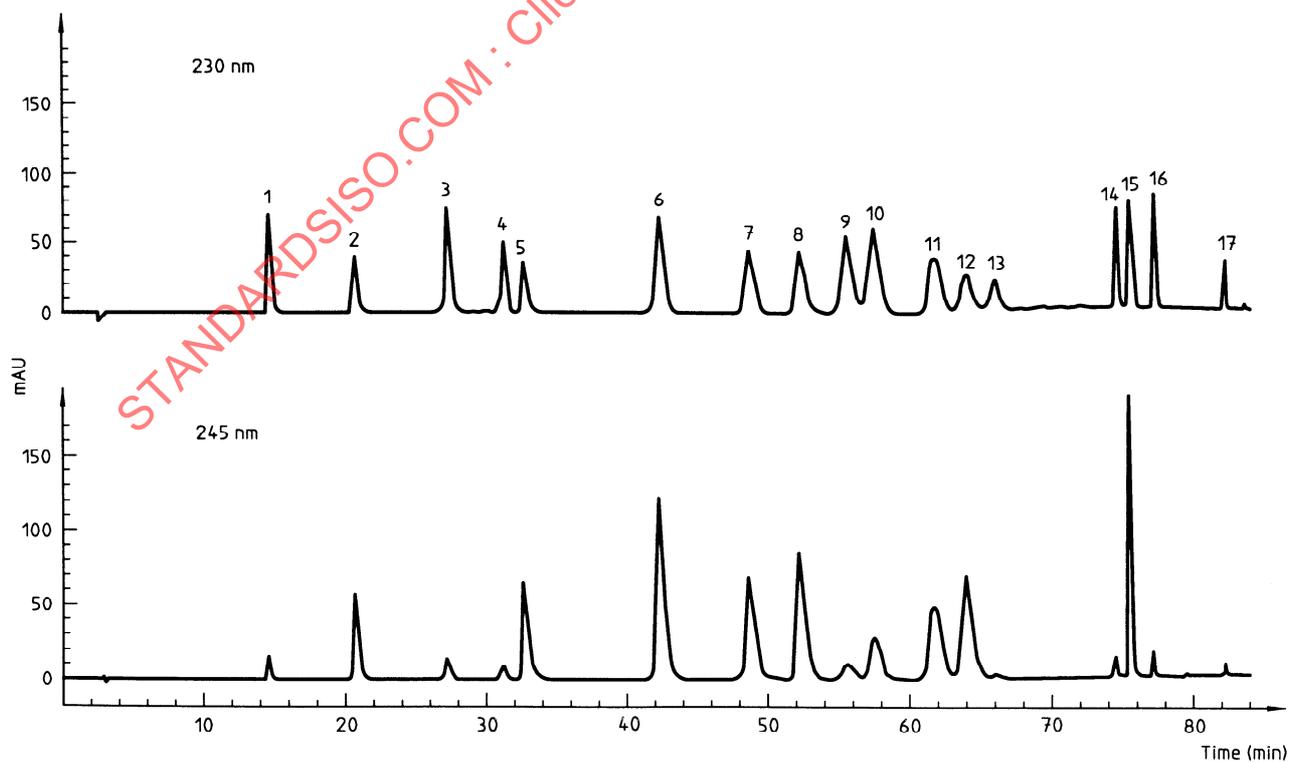


Figure 2 — Chromatograms of a high-concentration standard of plant treatment agents, separated with methanol/water gradient

Peak allocation

1 Desethylatrazine	10 Diuron
2 Metoxuron	11 Isoproturon
3 Hexazinone	12 Metobromuron
4 Simazine	13 Metazachlor
5 Cyanazine	14 Sebutylazine
6 Methabenzthiazuron	15 Terbutylazine
7 Chlorotoluron	16 Linuron
8 Atrazine	17 Metolachlor
9 Monolinuron	

8.5.2.2 Chromatographic conditions for the separation in figure 2 for an elution with methanol/water

Injection volume: 50 µl of plant treatment standard solution ($\rho_i = 5 - 20 \text{ ng}/\mu\text{l}$ each)

Solvent: eluant at starting conditions

Column: Nucleosil 120-3; 3 µm (250 x 4 mm)

Eluant: A: 20 mmol of ammonium acetate, aqueous solution/methanol; 75/25, B: methanol

Gradient: 0 % B to 9,5 % B in 30 min linear up to 60 min isocratic, linear gradient B to 87 % in 30 min

Flush time: 5 min with methanol

Equilibration: 15 min with eluant at starting conditions

Flowrate: 1 ml/min

Temperature: 40 °C

Diode array detector; flow cell 4,5 µl, $d = 6 \text{ mm}$,

Wavelengths 218 nm, 230 nm, 245 nm, bandwidth 4 nm.

Reference wavelength: 550 nm, bandwidth 100 nm.

Peak allocation

1 Desethylatrazine	10 Metabenzthiazuron
2 Metoxuron	11 Isoproturon
3 Cyanazine	12 Diuron
4 Simazine	13 Metazachlor
5 Hexazinone	14 Sebutylazine
6 Monolinuron	15 Linuron
7 Chlorotolurone	16 Terbutylazine
8 Metobromuron	17 Metolachlor
9 Atrazine	

8.5.3 Detection

Monitor substances at several wavelengths (for example at 218 nm, 230 nm and 245 nm depending on the solvent system) and each time choose the most favorable one for the quantification of single substances.

With the diode array detector, absorption spectra may be recorded over a range from 200 nm to 350 nm.

8.6 Blank monitoring

For quality control of the analytical procedure, determine the contribution of the reagents and equipment to the HPLC chromatograms by analysing the same volume of water (see 5.1) 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 1 Substances which absorb at the wavelengths of detection and have a retention time 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 water 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 standard are also an indication of interferences. Information on identity and chromatographic purity of a compound allocated by its retention time can be obtained from the

absorption spectra of the signals (see 8.5.3). In borderline cases, an independent method should additionally be used.

Characterize an individual compound in the chromatogram by comparing its retention time with the retention time of the reference standard. For a correct assignment, the retention time shall be within 1 %, or not more than 10 s, of the retention time of the standard.

NOTE 2 To check the stability of retention, it is helpful to add an appropriate UV-absorbing substance as a reference peak to the final extract. For this purpose the peak of *N*-benzoylaniline (benzanilide) may be used.

If there is no peak at the characteristic retention time and the chromatogram is normal in all other respects, the substance is regarded not to be present.

If a peak corresponds to a standard, the presence of the substance is possible and 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 substance (both measurements at similar concentration) agree within < 10 % deviation (relative to the smaller value) the qualitative identification is probable and, in the case of drinking water, 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 its retention time may be considered as identified if the absorption spectrum is in agreement with that of the reference substance (explanations and instructions of spectrometer manufacturers shall be observed).

NOTE 3 An additional or alternative confirmation may be obtained by the application of another procedure [e.g. gas chromatography (GC), GC-mass spectrometry, or high performance thin layer chromatography (HPTLC)].

9 Calibration

9.1 General requirements

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

- a) Calibration of the HPLC step by direct injection of the reference solutions (see 9.2)

This procedure delivers information on the linear working range of the detector, retention times and relative responses of determinands.

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

Both methods need to produce a linear relationship between the measured signal value and the standard concentration based on at least five concentration levels. Establish the linear regression function (calibration function) for each substance.

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

The recoveries can be obtained by comparison of the slopes m_1 and m_2 of these two methods or according to 9.4.

The calibration function established (see 9.3) for a substance is valid for the concentration range covered; it additionally depends on the operational conditions of the HPLC instrument, and shall be checked regularly. For routine work an adjustment of the calibration function performed as a two-point calibration is adequate (see 10.1).

The subscripts used in the following context are defined in table 2:

Table 2 — 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 HPLC step (Calibration with external standard, not covering the overall procedure)

Establish a calibration function for each determinand using at least 5 different concentrations. It is appropriate to examine all compounds mentioned in table 1 in one working cycle.

The knowledge of the retention time of the individual substance under investigation is a prerequisite. These data are determined using the solutions of the individual substances (see 5.9).

Establish the calibration function after injection of the reference solutions (see 5.11). The injection volume for the calibration shall be identical with that of the sample measurement.

Display the calibration function graphically.

Plot the measured values y_{ie} (peak areas, peak heights or integration units, respectively) for each substance i on the ordinate and the associated mass concentration ρ_{ie} on the abscissa.

Determine the linear calibration function using the pairs of values y_{ie} and ρ_{ie} of the measurement series

$$y_{ie} = m_i \rho_{ie} + b_i \quad (1)$$

where

y_{ie} is the measured value of substance i obtained from the calibration, depending on ρ_{ie} , unit depends on the evaluation; e.g. area value;

ρ_{ie} is the mass concentration of substance i (external standard in the reference solution), in micrograms per litre;

m_i is the slope of the calibration function of substance i , (corresponds to the substance-specific response factor), e.g. area value in litres per micrograms;

b_i is the ordinate intercept of the calibration line.

9.3 Calibration with external standard (covering the overall procedure)

Calibrate the overall procedure by fortification of control water samples (see 5.1) (e.g. 1000 ml) with 1 ml of a range (minimum 5) of reference solutions (see 5.11)

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

Display the calibration function graphically using the pairs of values y_{ieg} and ρ_{ieg} as described in 9.2.

Determine the linear calibration function by regression, using the pairs of values y_{ieg} and ρ_{ieg} of the measurement series

$$y_{ieg} = m_{ig} \rho_{ieg} + b_{ig} \quad (2)$$

where

y_{ig} is the measured value of substance i obtained from the calibration as a function of ρ_e , unit depends on evaluation, e.g. area value

ρ_{eg} is the mass concentration of substance i in the reference solution, in micrograms per litre

m_{ig} is the slope of the calibration function of substance i

b_{ig} is the ordinate intercept of the calibration line of substance i .

9.4 Determination of procedural recovery values

Reliable recovery data are obtained from analysis of water and fortified control water samples (see 5.1) at different concentration levels, equidistantly spread over the working range. From these individual results a mean specific recovery \bar{A}_i is calculated.

Add to e.g. 1000 ml of water (see 5.1) 1 ml each of the respective reference solution (see 5.11) and proceed in the same way as described for a natural sample.

Calculate with the aid of the calibration procedure according to 10.1 and 10.2 the substance-specific mean recovery \bar{A}_i for the substance i with equations (3) and (4):

$$\bar{A}_i = \frac{\sum_{N=1}^N A_{i,n}}{N} \quad (3)$$

where

N is the number of individual measurement values A_i

$$A_{i,n} = \frac{\rho_{i,nfd}}{\rho_{i,nnom}} \quad (4)$$

where:

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

$\rho_{i,nfd}$ is the mass concentration of the substance i found at the concentration level n , calculated with equation (1) in 9.3, in micrograms per litre;

$\rho_{i,nnom}$ is the given (nominal) mass concentration of the substance i at the concentration level n , in micrograms per litre. With the ratios of phase material and sample volume given in clause 8, high recoveries will normally be achieved. Low and diverging recoveries indicate matrix effects and/or problems with the extraction.

\bar{A}_i has to be calculated for a typical water.

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:

$$\rho_i = \frac{(y_i - b_i) V_0}{m_i V_i \bar{A}_i} \quad (5)$$

where:

ρ_i is the mass concentration of the determinand i in the water sample, in micrograms per litre;

y_i is the measured value for determinand i in the water sample, e.g. area value;

m_i, b_i see equation (1);

\bar{A}_i is the mean recovery of the determinand;

V_0 is the volume of the final extract from which the injection was made, in millilitres;

V_i is the volume of the extracted water sample, in millilitres.

When applying this method, the reliability of the analytical procedure shall be checked for each batch of analysis by two recovery tests, one with a concentration of about 20 % of the working range, the other at about 80 %.

If the actual A_i are in the same range as \bar{A}_i , the method is proved to be stable and \bar{A}_i may be used. Otherwise \bar{A}_i shall be determined again.

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

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

$$\rho_{ig} = \frac{(y_{ig} - b_{ig})}{m_{ig}} \quad (6)$$

where:

ρ_{ig} is the mass concentration of the determinand i in the water sample, in micrograms per litre;

y_{ig} is the measured value for determinand i in the water sample, e.g. area value;

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

11 Expression of results

The mass concentrations of the individual plant treatment agents are reported in terms of $\mu\text{g/l}$ with not more than two significant figures.

EXAMPLES:

Atrazine 0,05 $\mu\text{g/l}$

Chlorotoluron 1,2 $\mu\text{g/l}$

12 Test report

The report shall refer to this International Standard and contain the following detailed information:

- a) identity of the water sample;
- b) expression of the results, according to clause 11;
- c) additional treatment, e.g. filtration;
- d) grade of confirmation;
- e) time between sampling and extraction;
- f) any deviation from this procedure and all circumstances that may have affected the results.

13 Precision data

An interlaboratory trial, carried out in autumn 1992, provided the results given in annex B, tables B.1 to B.4.

Annex A (informative)

Recovery rates

Recovery rates for plant treatment agents at the concentration level of about 0,05 µg/l and under different enrichment conditions. Data collected in 1993.

Substance	Laboratory 1				Laboratory 2		
	Spiked concentration (ng/l)	Recovery (%)				Spiked concentration (ng/l)	Recovery (%)
		1	2	3	4		
Atrazine	50	98	76	86	90	50	99,9
Desethylatrazine	50	94	74	86	90	50	83,3
Desisopropylatrazine	25	36	64	36	40	–	–
Metamitron	50	24	72	70	28	–	–
Chloridazon	25	72	64	72	72	–	–
Metoxuron	25	92	76	88	88	50	102,4
Carbetamid	50	94	80	88	88	–	–
Bromacil	100	95	76	81	86	–	–
Simazine	50	94	76	84	86	50	92,4
Cyanazine	50	94	76	84	80	50	90,6
Methabenzthiazuron	25	92	76	84	84	50	86,8
Chlorotoluron	25	100	84	92	92	50	94,2
Monolinuron	50	66	54	58	64	50	64,7
Diuron	25	100	88	92	96	50	93,3
Isoproturon	25	112	96	100	104	50	95,6
Metobromuron	25	88	80	80	80	50	80,3
Metazachlor	50	94	80	80	86	50	88,9
Sebutylazine	25	96	80	84	92	50	89,2
Propazine	25	96	84	88	92	–	–
Terbutylazine	50	94	80	86	86	50	79,5
Linuron	25	96	80	88	92	50	67,4
Terbutryn	50	82	74	74	82	–	–
Metolachlor	100	91	75	82	83	50	63,7
Hexazinone	100	91	90	–	–	50	87,4

Enrichment conditions

Laboratory 1:

- 1 l tap water with 0,5 g NaCl, spiked with 1 ml of standard solution (methanol/water 1 : 5) commercially available polypropene cartridge with 1 g RP-C18 (40 µm)
- Conditioning: 2×1 ml of hexane, 2×1 ml of dichloromethane, 2×1 ml of methanol, 2×5 ml of ultrapure water
- Flowrate: about 5,5 ml/min; dried 1 h with N₂ at about 60 °C ;
- Elution: 3×1 ml of methanol