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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

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

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Contents

International Organization for Standardization
Case postale 56 • CH-1211 Genève 20 • Switzerland
Internet: central@iso.ch
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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	$C_8H_{14}ClN_5$	215,7	001912-24-9	T
Chlorotoluron	$C_{10}H_{13}ClN_2O$	212,7	015545-48-9	H
Cyanazine**	$C_9H_{13}ClN_6$	240,7	021725-46-2	T
Desethylatrazine *	$C_6H_9ClN_5$	186,6	006190-65-4	T
Diuron	$C_9H_{10}Cl_2N_2O$	233,1	000330-54-1	H
Hexazinone**	$C_{12}H_{20}N_4O_2$	252,3	051235-04-2	T
Isoproturon	$C_{12}H_{18}N_2O$	206,3	034123-59-6	H
Linuron	$C_9H_{10}Cl_2N_2O_2$	249,1	000330-55-2	H
Metazachlor	$C_{14}H_{16}ClN_2O_3$	277,8	067129-08-2	A
Methabenzthiazuron	$C_{10}H_{11}N_3OS$	221,3	018691-97-9	H
Metobromuron**	$C_9H_{11}BrN_2O_2$	259,1	003060-89-7	H
Metolachlor	$C_{15}H_{22}ClNO_2$	283,8	051218-45-2	A
Metoxuron**	$C_{10}H_{13}ClN_2O_2$	228,7	19937-59-8	H
Monolinuron	$C_9H_{11}ClN_2O_2$	214,6	1746-81-2	H
Sebutylazine**	$C_9H_{15}ClN_5$	228,7	00728-69-3	T
Simazine	$C_7H_{12}ClN_5$	201,7	000122-34-9	T
Terbutylazine	$C_9H_{16}ClN_5$	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 possiblity 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, $c(H_3PO_4)$ = 1 mol/l.

5.5 Sodium hydroxide solution c(NaOH) = 1 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 (C_3H_6O).

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. ρ_i = 20 to 200 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 (CH_3COONH_4), or sodium acetate CH_3COONa, concentration ≤ 20 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 µl, 50 µl, 100 µl, 250 µl and 1000 µl, 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 µm, 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 µm to 0,45 µm.

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 µm to 5 µm. 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 approriate 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

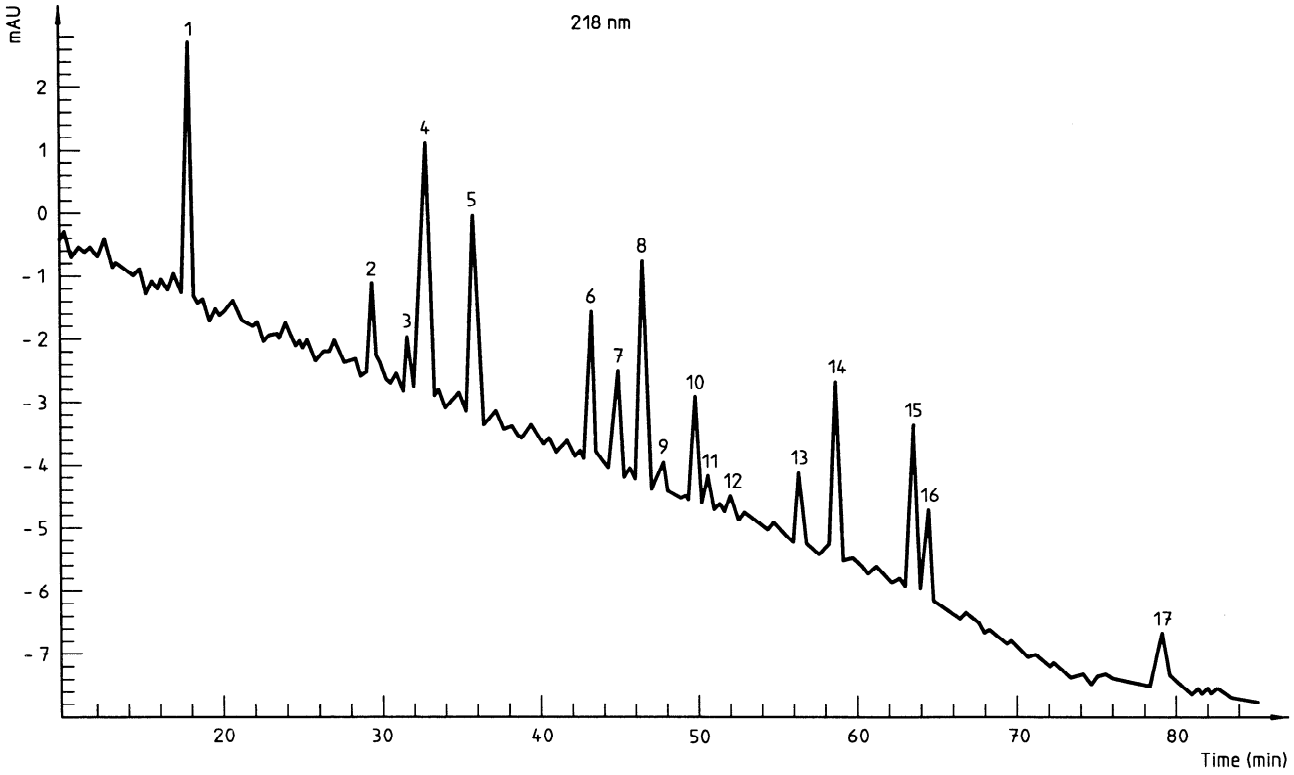
1 Because of its higher optical transparency and lower viscosity, use of acetonitrile solvent should be given preference to methanol. Particular attention is drawn to the toxicity of acetonitrile.

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.

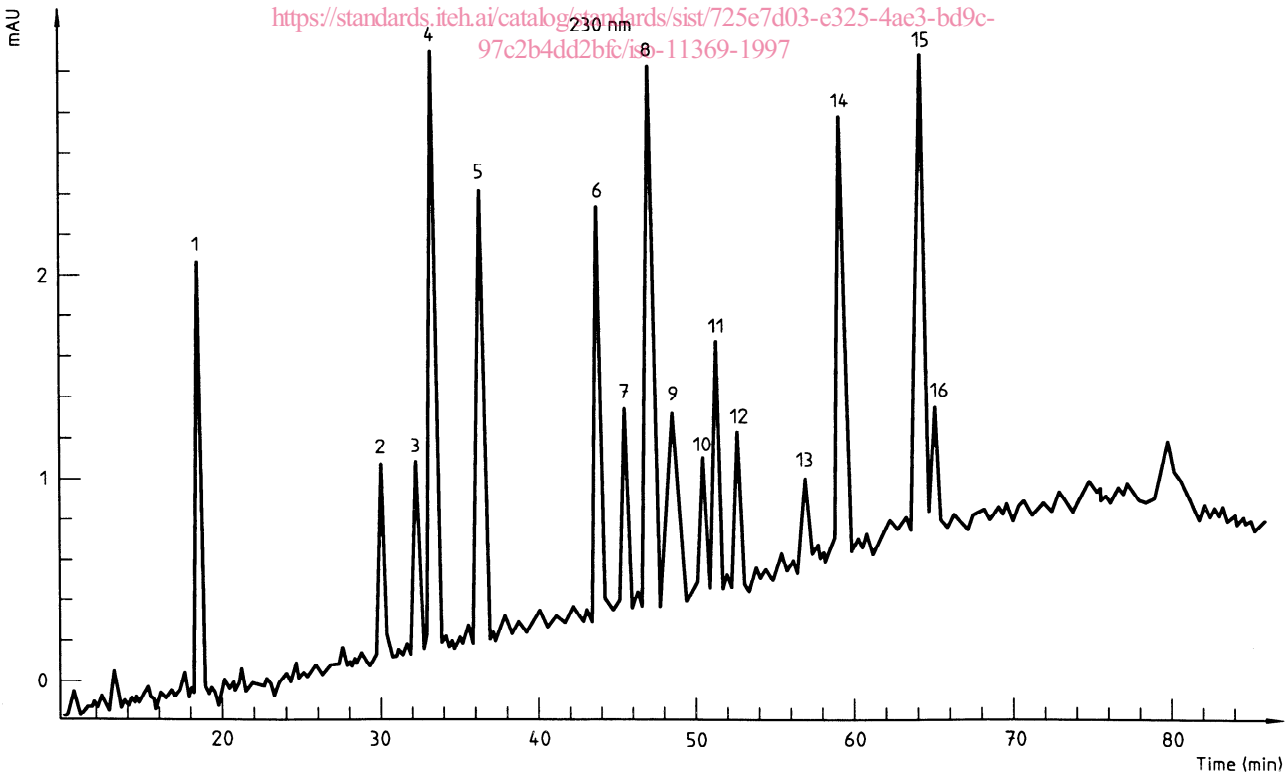
8.5.2.1 Chromatographic conditions for the separation in figure 1 a) to c)

Injection volume: 25 µl of plant treatment standard solution (ρ_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