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**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)*

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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.

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.

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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  $> 0,05 \mu\text{g/l}$  (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. <sup>a</sup>	Molar mass g/mol	Peak in Figure No.						
				1	2	3	4	5	6	7
Alachlor <sup>b</sup>	$\text{C}_{14}\text{H}_{20}\text{ClNO}_2$	015972-60-8	269,8	6				6		
Atrazine	$\text{C}_8\text{H}_{14}\text{ClN}_5$	001912-24-9	215,7	2				4		
Chlorfenvinphos <sup>b</sup>	$\text{C}_{12}\text{H}_{14}\text{Cl}_3\text{O}_4\text{P}$	000470-90-6	359,6	5				3		
Chlortoluron <sup>b</sup>	$\text{C}_{10}\text{H}_{13}\text{ClN}_2\text{O}$	015545-48-9	212,7	1						3
Cyanazine <sup>b</sup>	$\text{C}_9\text{H}_{13}\text{ClN}_6$	021725-46-2	240,7				1			4
2,4-D	$\text{C}_8\text{H}_6\text{Cl}_2\text{O}_3$	000094-75-7	221,0	4				1		
MCPA <sup>b</sup>	$\text{C}_9\text{H}_9\text{ClO}_3$	000094-74-6	200,6				2	2		
Metazachlor	$\text{C}_{14}\text{H}_{16}\text{ClN}_3\text{O}$	067129-08-2	277,8			3				5
Metobromuron	$\text{C}_9\text{H}_{11}\text{BrN}_2\text{O}_2$	003060-89-7	259,1			5				6
Metolachlor <sup>b</sup>	$\text{C}_{15}\text{H}_{22}\text{ClNO}_2$	051218-45-2	283,8			4				7
Metoxuron	$\text{C}_{10}\text{H}_{13}\text{ClN}_2\text{O}_2$	019937-59-8	228,7			1				1
Monuron <sup>b</sup>	$\text{C}_9\text{H}_{11}\text{ClN}_2\text{O}$	000150-68-5	198,7			2				2
Parathion <sup>b</sup>	$\text{C}_{10}\text{H}_{14}\text{NO}_5\text{PS}$	000056-38-2	291,3	7				7		
Pendimethalin	$\text{C}_{13}\text{H}_{19}\text{N}_3\text{O}_4$	040487-42-1	281,3		6					6
Propazine <sup>b</sup>	$\text{C}_9\text{H}_{16}\text{ClN}_5$	000139-40-2	229,7	3				5		
Sebuthylazine <sup>b</sup>	$\text{C}_9\text{H}_{16}\text{ClN}_5$	007286-69-3	229,7		2					3
Simazine	$\text{C}_7\text{H}_{12}\text{ClN}_5$	000122-34-9	201,7		1					2
2,4,5-T <sup>b</sup>	$\text{C}_8\text{H}_5\text{Cl}_3\text{O}_3$	000093-76-5	255,5		4					1
Terbutylazine <sup>b</sup>	$\text{C}_9\text{H}_{16}\text{ClN}_5$	005915-41-3	229,7		3					4
Trifluralin <sup>b</sup>	$\text{C}_{13}\text{H}_{16}\text{F}_3\text{N}_3\text{O}_4$	001582-09-8	335,3	8				8		
Vinclozoline <sup>b</sup>	$\text{C}_{12}\text{H}_9\text{Cl}_2\text{NO}_3$	050471-44-8	286,1		5					5

<sup>a</sup> CAS No.: Chemical abstracts system.

<sup>b</sup> 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

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### 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 eluted 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), eluted 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**,  $c(\text{HCl}) = 1 \text{ mol/l}$  (for example).

**5.2 Sodium hydroxide solution**,  $c(\text{NaOH}) = 1 \text{ mol/l}$  (for example).

**5.3 Ammonia solution**,  $w(\text{NH}_3) = 25 \%$ .

**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**,  $w(\text{HCOOH}) = 98 \%$  to  $100 \%$ .

**5.6 Solvents**, e.g. acetone,  $\text{C}_3\text{H}_6\text{O}$ ; acetonitrile,  $\text{CH}_3\text{CN}$ ; dichloromethane,  $\text{CH}_2\text{Cl}_2$ ; 3,3-dimethyl-2-oxabutane,  $\text{C}_6\text{H}_{12}\text{O}$ ; ethyl acetate,  $\text{C}_4\text{H}_8\text{O}_2$ ; hexane,  $\text{C}_6\text{H}_{14}$ ; methanol,  $\text{CH}_3\text{OH}$ ; 2-propanol,  $\text{C}_3\text{H}_8\text{O}$ .

**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  $4^\circ\text{C}$ , 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  $4^\circ\text{C}$ , 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.  $\rho_i = 10 \text{ ng}/\mu\text{l}$ .

Store the solution at about  $4^\circ\text{C}$ , 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.  $\rho_i = 20 \text{ ng/ml}$  to  $200 \text{ ng/ml}$ .

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 µm 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.

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### 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	—	—