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Soil quality — Determination of herbicides — Method using HPLC with UV-detection

Qualité du sol — Dosage des herbicides — Méthode par CLHP avec détection par UV

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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 2.

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.

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ISO 11264 was prepared by Technical Committee ISO/TC 190, *Soil quality*, Subcommittee SC 3, *Chemical methods and soil characteristics*.

Soil quality — Determination of herbicides — Method using HPLC with UV-detection

1 Scope

This International Standard specifies a high-performance liquid chromatography (HPLC) method for qualitative and quantitative determination of herbicides of various substance classes in soils. This method covers triazines including their related metabolites, phenyl urea compounds and other herbicides. Compounds are identified and quantified with UV-detection.

The limit of detection for triazines and phenyl urea compounds is ~ 0,01 mg/kg dry matter. It is dependent upon both the compound and the soil matrix.

2 Normative reference

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 10381-1, *Soil quality — Sampling —- Part 1: Guidance on the design of sampling programmes*

ISO 10381-2, *Soil quality — Sampling — Part 2: Guidance on sampling techniques*

ISO 11465, *Soil quality — Determination of dry matter and water content on a mass basis — Gravimetric method*

ISO 14507, *Soil quality — Pretreatment of samples for determination of organic contaminants*

3 Principle

The field-moist soil sample is extracted with a mixture of acetone and water (2:1). After addition of NaCl and dichloromethane or petroleum, the isolated organic phase is concentrated and transferred to a acetonitrile/water mixture and without further clean-up is analysed using RP-HPLC, gradient of acetonitrile and water, with UV-detection. Results can be confirmed using diode array UV spectra, GC-MS, GC-NPD or GC-AED (some may need derivatisation).

4 Reagents

All reagents shall be of known analytical grade. The purity of the reagents used shall be checked by running a blank determination as described in 6.5. If the blank value is unreasonably high, i.e. more than 10 % of the lowest value of interest, find the cause through a step-by-step examination of the whole procedure. For measurements at the limit of determination, even reagents suitable for residue analyses may not fulfil this criterion. In this case, sufficient blank determinations shall be incorporated in each series of samples.

4.1 **Water**, for residue analysis, normally tap water (drinking water) is suitable.

4.2 **Acetone**, for residue analysis.

4.3 Sodium chloride.

4.4 Dichloromethane, for residue analysis.

4.5 Petroleum ether, boiling range 40 °C to 60 °C, for residue analysis.

4.6 Sodium sulfate, anhydrous, for residue analysis.

4.7 Acetonitrile, HPLC grade.

4.8 Water, HPLC grade.

4.9 2-propanol, for chromatography.

4.10 Acetonitrile (4.7)/**water** (4.8) **mixture**, 1 + 1 (V + V) for dissolving the sample.

4.11 Methanol, HPLC grade.

4.12 Standard substances (including Chemical Abstracts Number).

Alachlor [015972-60-8], Atrazine [001912-24-9], Atrazine-desethyl [06190-65-4], Atrazine-desisopropyl [01007-28-9], Bromacil [000314-40-9], Chlortoluron [015545-48-9], Chloroxuron [001982-47-4], Cyanazine [021725-46-2], Dichlobenil [001194-65-6], Diuron [000330-54-1], Ethofumesate [026225-79-6], Hexazinon [051235-04-2], Isoproturon [034123-59-6], Metazachlor [061729-08-2], Metamitron [041394-05-2], Metabenzthiazuron [018691-97-9], Metobromuron [003060-89-7], Metolachlor [051218-45-2], Metoxuron [019937-59-8], Monuron [000150-68-5], Pendimethalin [040487-42-1], Propazine [000139-40-2], Propyzamide [023950-58-5], Sebuthylazine [00728-69-3], Simazine [000122-34-9], Terbutryn [000886-50-0], Terbutylazine [005915-41-3].

4.13 Standard solutions for HPLC determination

4.13.1 Stock solutions, ρ_{11} = 1 mg/ml

Weigh 10,0 mg of each standard substance (4.12) and place in individual 10 ml measuring flasks (5.13). Dissolve with 2-propanol (4.9) or, in the case of sebuthylazine and simazine, with small portions of acetone (4.2) or, in the case of metamitron, with methanol (4.11). Then make up to the mark with 2-propanol (4.9).

4.13.2 Intermediate dilutions, ρ_{12} = 100 µg/ml

Pipette 1,0 ml of each of the stock solutions (4.13.1) into an individual 10 ml measuring flask (5.13) and make up to the mark with 2-propanol (4.9).

4.13.3 Working solutions I, ρ_{13} = 10 µg/ml

NOTE Working solutions are made to establish the retention time. Different concentrations are made because the analytes have different detector responses.

Pipette 1,0 ml of each of the intermediate dilutions (4.13.2) into an individual 10 ml measuring flask (5.13) and make up to the mark with acetonitrile/water mixture (4.10).

4.13.4 Working solutions II, ρ_{14} = 1 µg/ml

Transfer, with a microlitre syringe (5.14), 100 µl of each of the intermediate dilutions (4.13.2) into a 10 ml measuring flask (5.13) and make up to the mark with acetonitrile/water mixture (4.10).

4.13.5 Working solutions III, ρ_{15} = 0,1 µg/ml

Transfer, with a microlitre syringe (5.14), 100 µl of working solution I (4.13.3) into a 10 ml measuring flask (5.13) and make up to the mark with acetonitrile/water mixture (4.10).

4.13.6 Mixed solutions I, ρ_{16} = 5 µg/ml to 100 µg/ml

According to Table 1, transfer with a microlitre syringe (5.14), between 50 µl and 1 000 µl of the stock solutions (4.13.1) into a 10 ml measuring flask (5.13) and make up to the mark with 2-propanol (4.9).

4.13.7 Mixed solutions II, ρ_{17} = 0,5 µg/ml to 10 µg/ml

Pipette 1,0 ml of the mixed solution I (4.13.6) into a 10 ml measuring flask (5.13) and make up to the mark with acetonitrile/water mixture (4.10) (see Table 1).

4.13.8 Mixed solutions III, ρ_{18} = 0,05 µg/ml to 1 µg/ml

Pipette 1,0 ml of the mixed solution II (4.13.7) into a 10 ml measuring flask (5.13) and make up to the mark with acetonitrile/water mixture (4.10).

Table 1 — Concentration of compounds of mixed solutions I (4.13.6), II (4.13.7) and III (4.13.8)

No.	Compound [a]	Volume [b] µl	Mixed solution I µg/ml	Mixed solution II µg/ml	Mixed solution III µg/ml
1	Atrazine-desisopropyl	200	20	2	0,2
2	Metamitron	500	50	5	0,5
3	Atrazine-desethyl	200	20	2	0,2
4	Hexazinon	200	20	2	0,2
5	Metoxuron	200	20	2	0,2
6	Bromacil	400	40	4	0,4
7	Simazine	100	10	1	0,1
8	Monuron	100	10	1	0,1
9	Cyanazine	100	10	1	0,1
10	Methabenzthiazuron	100	10	1	0,1
11	Chlortoluron	100	10	1	0,1
12	Atrazine	100	10	1	0,1
13	Isoproturon	100	10	1	0,1
14	Diuron	200	20	2	0,2
15	Metobromuron	100	10	1	0,1
16	Metazachlor	500	50	5	0,5
17	Sebuthylazine	100	10	1	0,1
18	Propazine	100	10	1	0,1
19	Dichlobenil	200	20	2	0,2
20	Terbutylazine	100	10	1	0,1
21	Chloroxuron	100	10	1	0,1
22	Propyzamide	100	10	1	0,1
23	Terbutryn	50	5	0,5	0,05
24	Ethofumesate	500	50	5	0,5
25	Metolachlor	500	50	5	0,5
26	Alachlor	1 000	100	10	1
27	Pendimethalin	100	10	1	0,1

[a] Compounds are in elution order using the conditions stated in 6.5. For additional compounds, see Annex C.

[b] Volume to be taken from the stock solution (4.13.1) to prepare 10 ml of the mixed solution I (4.13.6).

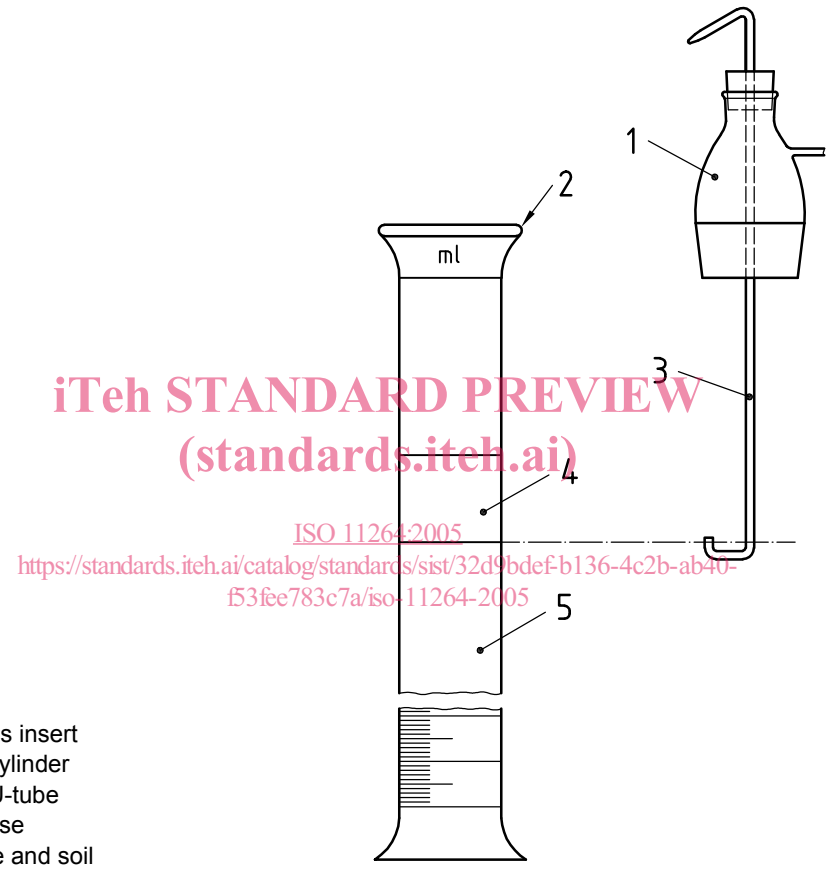
5 Apparatus

5.1 Glass bottles, with screw cap and sealing ring of polytetrafluor ethylene, nominal capacity 250 ml and 1 000 ml.

5.2 Extraction apparatus (see Figure 1), consisting of the following.

5.2.1 Measuring cylinder, of 1 000 ml nominal volume with ground neck and ground-glass stopper.

5.2.2 Ground-glass insert (wash-bottle principle) with adjustable U-tube, the bent end of which can be placed at the interface between the two phases.



Key

1 Ground-glass insert
2 Measuring cylinder
3 Adjustable U-tube
4 Organic phase
5 Water phase and soil

Figure 1 — Extraction apparatus

5.3 Shaking apparatus, head-over-head or horizontal. Complete mixing of the soil and extractant should be obtained.

5.4 Vacuum rotating evaporator, with water bath and vacuum control.

5.5 Pear-shaped flask, of 10 ml nominal volume, with graduations at 0,5 ml and 1,0 ml and a ground-glass stopper.

5.6 Membrane filter, porosity at least 0,45 µm, solvent-resistant.

5.7 Test tube, of 2 ml nominal volume, sealable, with polytetrafluor ethylene disc.

5.8 HPLC apparatus, consisting of high-pressure pumps with gradient programmer, injection valve of 20 µl to 50 µl, column thermostat, solvent de-gassing device.

5.9 UV-detector, with variable wavelengths or diode array detector (DAD).

5.10 Electronic recording device (integrator or laboratory data system).

5.11 Separation column, length 250 mm, inner diameter 4 mm, and pre-column holders (see 6.5.1).

5.12 Pre-column, length 4 mm, inner diameter 4 mm (see 6.5.1)

5.13 Measuring flasks, 10 ml nominal volume, with glass stopper.

5.14 Microlitre syringe, 50 µl to 1 000 µl.

5.15 Ultrasonic bath or **vortex mixer**.

6 Procedure

6.1 Sample preparation

Obtain representative field-moist soil samples in accordance with ISO 10381-1, using sampling apparatus in accordance with ISO 10381-2.

6.2 Sample preservation and pretreatment

Field-moist samples shall be pretreated in accordance with ISO 14507 as soon as possible (no chemical drying).

Store the samples in a dark place at a temperature below − 20 °C. Herbicides may be subject to microbial conversion under certain conditions. It is recommended that samples be frozen, if they are stored for more than 2 days.

Determine the water and dry matter contents of a subpart of the sample in accordance with ISO 11465.

6.3 Addition of water to the sample

Add to the soil mass a specific mass of water calculated according to Equation (1) (each prepared sample will have a water mass of 100 g).

$$m_{\mathrm{w}} = 100 - \frac{m_{\mathrm{s}} \cdot w_{\mathrm{w}}}{100} \qquad (1)$$

where

m_{w} is the mass of added water, in g;

m_{s} is the mass of sample, in g;

w_{w} is the mass fraction of water, in %.

NOTE Addition of water is important to have a fixed ratio (water acetone dichloromethane of 1:2:1,5). Under these conditions, the organic phase will be the upper layer.

6.4 Preparation of soil extract

6.4.1 Extraction and liquid/liquid distribution

Weigh 50 g of soil prepared according to 6.1 into a glass bottle (5.1) or glass cylinder (5.2.1), and add m_W grams of water (4.1) and 200 ml of acetone (4.2). Close and shake for at least 6 h. Appropriate mixing of the two phases shall be observed during shaking. Then add 30 g of sodium chloride (4.3) and 150 ml of dichloromethane (4.4) or petroleum ether (4.5). Close and shake for 5 min.

Other extraction techniques, such as ultrasonic extraction, microwave or pressurised extraction, may be suitable. However, if other extraction techniques are used, the comparability to the method described in this International Standard has to be proven.

The use of dichloromethane should be restricted to an unavoidable minimum, for health and environmental reasons. The substitution of dichloromethane by petroleum ether (boiling range 40 °C to 60 °C) for some soils and compounds leads to reductions in the concentrations of these compounds of up to 10 %. With ethylacetate and diethylether instead of dichloromethane, poor recoveries were sometimes found, depending on the sample matrix.

In the case of some compounds (e.g. metamitron), recovery rates less than 70 % were found. For a quantitative evaluation, it is recommended to apply an extraction technique suited to the individual compound.

Place approximately 50 g of anhydrous sodium sulfate (4.6) in a conical flask, of nominal volume 500 ml. Transfer the organic phase into the conical flask, either by decanting or using the ground-glass insert (5.2.2) and the use of nitrogen. Close the flask and mix (see 5.3) for at least 2 h. Measure an aliquot of 140 ml, 40 % of the total aliquot (referring to an extract of 20 g soil), and place it in a round flask (5.5). Concentrate to about 1 ml using a vacuum rotating evaporator at reduced pressure and at a water-bath temperature of max. 40 °C. Concentration to complete dryness shall be avoided. This residue concentration is designated as extract 1 “E1”.

If dichloromethane is applied, remove the water layer and filter the organic layer into the conical flask to remove the soil particles.

6.4.2 Concentration and dissolution

Transfer the extract E1 (6.4.1) with a Pasteur pipette into a graduated pear-shaped flask (5.5). Rinse the conical flask with approximately 2 ml of acetonitrile (4.7). For complete removal of acetone concentrate to less than 1 ml, subsequently make up with acetonitrile (4.7) to 1,0 ml. Add, using a pipette, 1,0 ml of HPLC-water (4.8) and mix with a vortex mixer or ultrasonic bath (5.15). If necessary, filter (see 5.6) and transfer the solution toin a sealable test tube (5.7). This residue is designated as extract “E2”. It consists of the extract of 10 g soil/ml, ρ_{E2} = 10 g/ml.

NOTE 1 Using this extraction procedure (6.4.1) also allows the determination of other plant protection agents (e.g. insecticides and fungicides) and environmentally important compounds (e.g. polychlorinated biphenyls and polycyclic aromatic hydrocarbons).

NOTE 2 This method can also be carried out as a micro-scale method with limited (proportional) sample and reagent masses (e.g. 5 g soil, 10 ml water, 20 ml acetone, 15 ml dichloromethane and 3 g sodium chloride). In this case, poor homogeneity of some samples may cause problems.

6.5 HPLC-determination

6.5.1 General

The following conditions should be regarded as guide values. The user has to optimise these, depending on the column used, the other devices and the separation problem.

6.5.2 Measuring and operating conditions

The HPLC can be operated under the following conditions (example).

Stationary phase: C18 Column[1)]

Mobile phase: The relevant ratio of water (4.8) and acetonitrile (4.7) is determined by the gradient programme (see Table 2).

Injection volume: 50 µl

Oven temperature: 30 °C

Flow rate: 1,0 ml/min

Detection wavelength: 235 nm

By variation of the wavelength between 220 nm and 309 nm, signal intensities of different substance classes or individual compounds can be optimised (see Table 3). For example, 220 nm for triazine compounds, 245 nm for phenyl urea compounds.

Eluent A: Water (4.8)

Eluent B: Acetonitrile (4.7)

NOTE The given eluents A and B, as mixtures, are preferred to 100 % water and 100 % acetonitrile

The maximum injection volume is 100 µl with the mentioned column. Volumes above this should not be used.

By regularly changing the pre-column (5.12), interferences caused by pressure rise due to deposition of sample particles at the column can be avoided for a considerable time.

Table 2 — Gradient programme

Time min	Eluent A %	Eluent B %
0	80	20
5	81	19
10	81	19
30	38	62
35	38	62
40	10	90
50	10	90
55	80	20
65	80	20

1) Lichrospher®60RP-select B (see 5.11 and 5.12) or Nucleosil-AB are examples of suitable products found available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of these products.