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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

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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.

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

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).

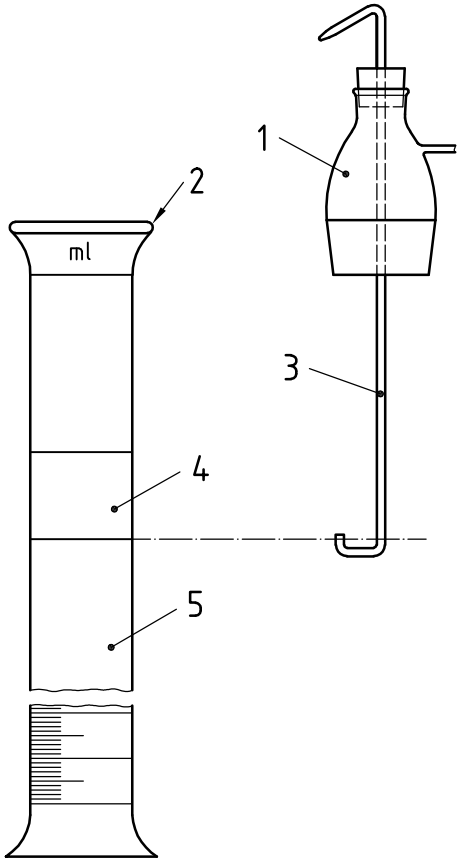
5 Apparatus

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.

Table 3 — Relative response, absorption maxima and relative retention of herbicides

No.	Compound	Relative response [a]	1st maximum nm	2nd maximum nm	Relative retention time [a]
1	Atrazine-desisopropyl	0,73	215		0,23
2	Metamitron	0,46	309		0,31
3	Atrazine-desethyl	1,04	215		0,36
4	Hexazinon	0,62	247		0,61
5	Metoxuron	0,77	209	245	0,62
6	Bromacil	0,25	212	278	0,64
7	Simazine	1,57	223		0,69
8	Monuron	1,11	248		0,71
9	Cyanazine	1,26	220		0,75
10	Methabenzthiazuron	1,42	225	269	0,86
11	Chlortoluron	1,39	211	245	0,91
12	Atrazine	1,96	223		0,92
13	Isoproturon	1,79	204	242	0,99
14	Diuron	1,00	212	251	1,00
15	Metobromuron	1,15	248		1,01
16	Metazachlor	0,25	200		1,05
17	Sebuthylazine	2,30	223		1,10
18	Propazine	2,08	223		1,11
19	Dichlobenil	1,03	211		1,15
20	Terbuthylazine	2,33	224		1,18
21	Chloroxuron	1,59	249		1,27
22	Propyzamide	0,87	207		1,28
23	Terbutryn	2,83	224		1,31
24	Ethofumesate	0,44	228	280	1,33
25	Metolachlor	0,28	200		1,33
26	Alachlor	0,14	200		1,35
27	Pendimethalin	3,39	240		1,71

NOTE If all the above substances are present, they are not completely resolved on the chromatogram, see Annex A.

[a] Relative to diuron, wavelength: 235 nm. See 6.5.4 for interferences. For additional compounds, see Annex C.

6.5.3 Calibration

6.5.3.1 First calibration

The retention times of the individual herbicides are established by measuring the working solutions I (4.13.3) and II (4.13.4).

The separating system is calibrated in the linear range of the UV-detector. At least 3 equidistantly distributed concentrations of individual-substance working solutions or the multi-component mixtures (4.13.6, 4.13.7 and 4.13.8) and related intermediate dilutions shall be prepared. For quantitative determination, signal heights (or signal areas) γ_1 are measured and compared to related concentration ρ_1. For every substance, specify the working range and establish a linear calibration curve.

6.5.3.2 Recalibration

Before any series of samples, the first calibration shall be verified by measuring two standards of different concentrations.

6.5.4 Measurement

The extract E2 (6.4.2) is chromatographed under the described conditions. Solvents and injection volumes shall be equally prepared for sample extracts and calibration solutions. Substances are qualitatively integrated with the retention times and quantitatively determined using signal heights (or signal areas) γ_{EX}. Compounds are considered to be detected as herbicide if the retention time is within a tolerance $\pm$ 0,1 min, compared to the herbicide present in the corresponding standard solutions.

NOTE Soils with a high content of organic material may give interfering peaks. If necessary, cleaning up using gel permeation chromatography (GPC) or absorbent chromatography (e.g. silica gel or florisil) can improve the results.

It is recommended to use an internal standard for recovery experiments in each series, or even in each sample. The substance can be one of the listed compounds not under investigation, or any other suitable organic compound that is unlikely to be present in the samples. The user has to check that there are no interfering peaks in the chromatogram.

6.5.5 Verification of positive findings

A false-positive result may occur due to co-elution of matrix substances. Verification of the HPLC determination can be limited by application of diode array or a scanning UV-detector, and measurement and comparison of the UV spectra of the samples and a standard.

NOTE Gas chromatography with a neutron/proton (NP)-selective or mass-spectrometry-selective detector may be used as an independent detection method for verification of results of some of the analytes. In the case of phenylurea compounds, degradation products are formed in the hot injector zone. Another possibility for determination of these compounds is derivatisation followed by gas chromatography. Liquid-column mass spectrometry (LC-MS) may also be used if available.

7 Calculation

7.1 Calculation of the mass concentration ρ_{EX} of the substance in extract solution E2 according to Equation (2)

$$\rho_{EX} = \frac{\gamma_{EX}}{\gamma_i} \times \rho_{in} \qquad (2)$$

where

ρ_{in} is the mass concentration of the substance i used for calibration, in µg/ml;

γ_i is the measured value (signal peak or signal area) of the substance i used for calibration;

γ_{EX} is the measured value (signal peak or signal area) of the substance x in the soil extract E.

7.2 Calculation of the content w of the substance in the soil extract solution E2 according to Equation (3)

$$w = \frac{\rho_{EX}}{\rho_{EN}} \times \frac{100 - w_{dm}}{100 - w_w} \quad (3)$$

where

w is the mass fraction of the substance, in mg/kg dry matter;

w_{dm} is the mass fraction of dry matter, in mg/kg;

ρ_{EX} is the mass concentration of the substance x in the soil extract E, in µg/ml;

ρ_{EN} is the equivalent mass concentration of the field-moist soil in the extract solution in g/ml; e.g. 20 g/2 ml (see 6.4.2);

w_w is the mass fraction of water of the soil, in %.

7.3 Expression of results

Express the result, as mg substance per kg dry matter, to two significant figures, for example:

Diuron: 1,1 mg/kg dry matter

Atrazine: 0,63 mg/kg dry matter

Atrazine-desethyl: 0,08 mg/kg dry matter

8 Test report

The test report shall contain at least the following information:

a) a complete identification of the sample;

b) a reference to this International Standard;

c) the contents of individual herbicides, in mg/kg on the basis of dry matter, rounded off in accordance with 7.3;

d) any details not specified in this International Standard or which are optional, as well as any factor which may have affected the results.

9 Accuracy

In Annex B, the results of validation studies are presented.

Recovery experiments were done by three independent laboratories at two levels in triplicate.

Interlaboratory trials were done with five different soils, three of them with herbicide residues and two of them with spiked herbicides. The number of participants were from eight to fourteen.

Annex A
(informative)

Chromatograms

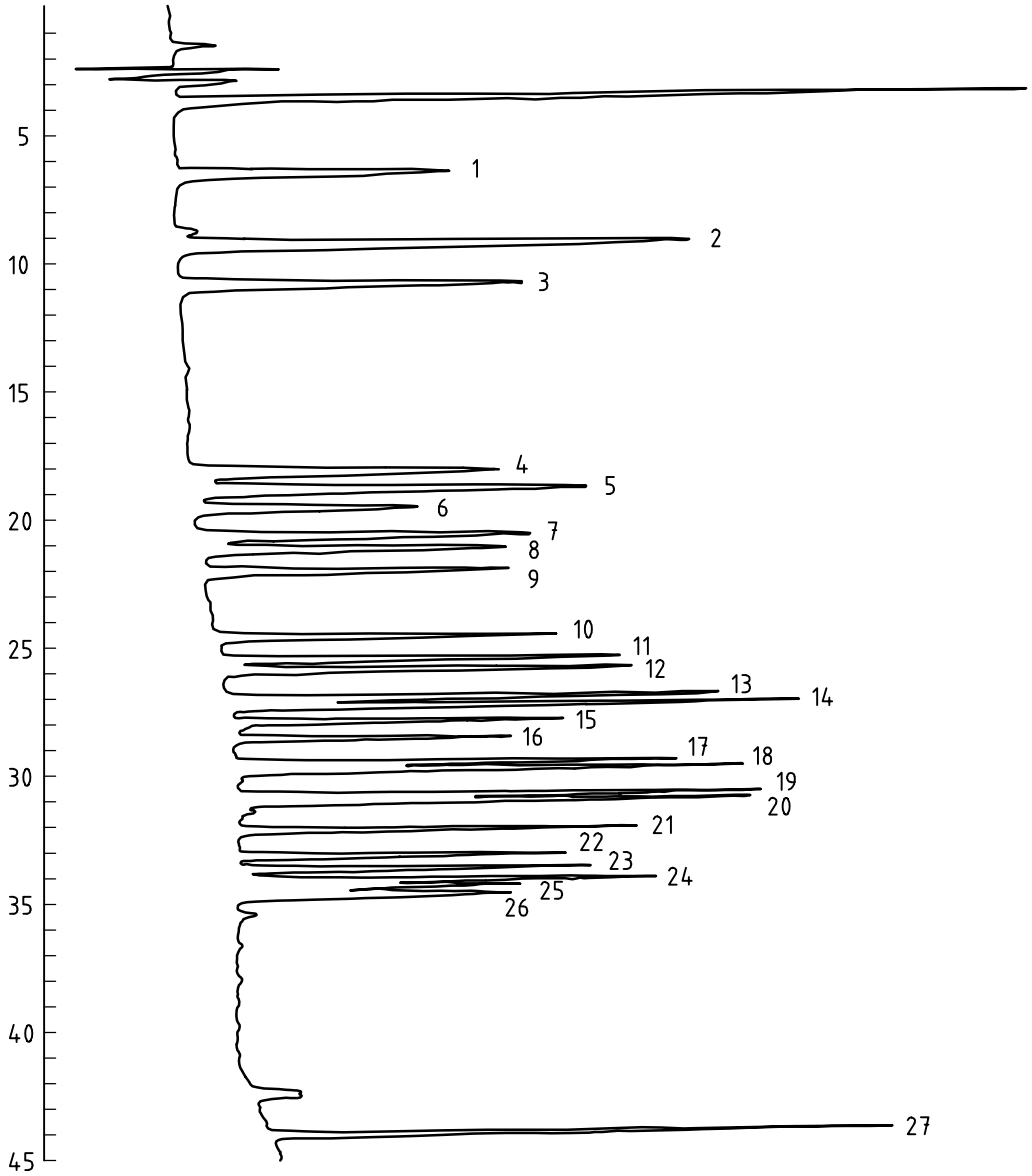


Figure A.1 — Chromatogram of herbicide mixed solution II (see 4.13.7) according to 6.4.2, wavelength 235 nm

The listing of the herbicides in Figure A.1 corresponds to Table 1.

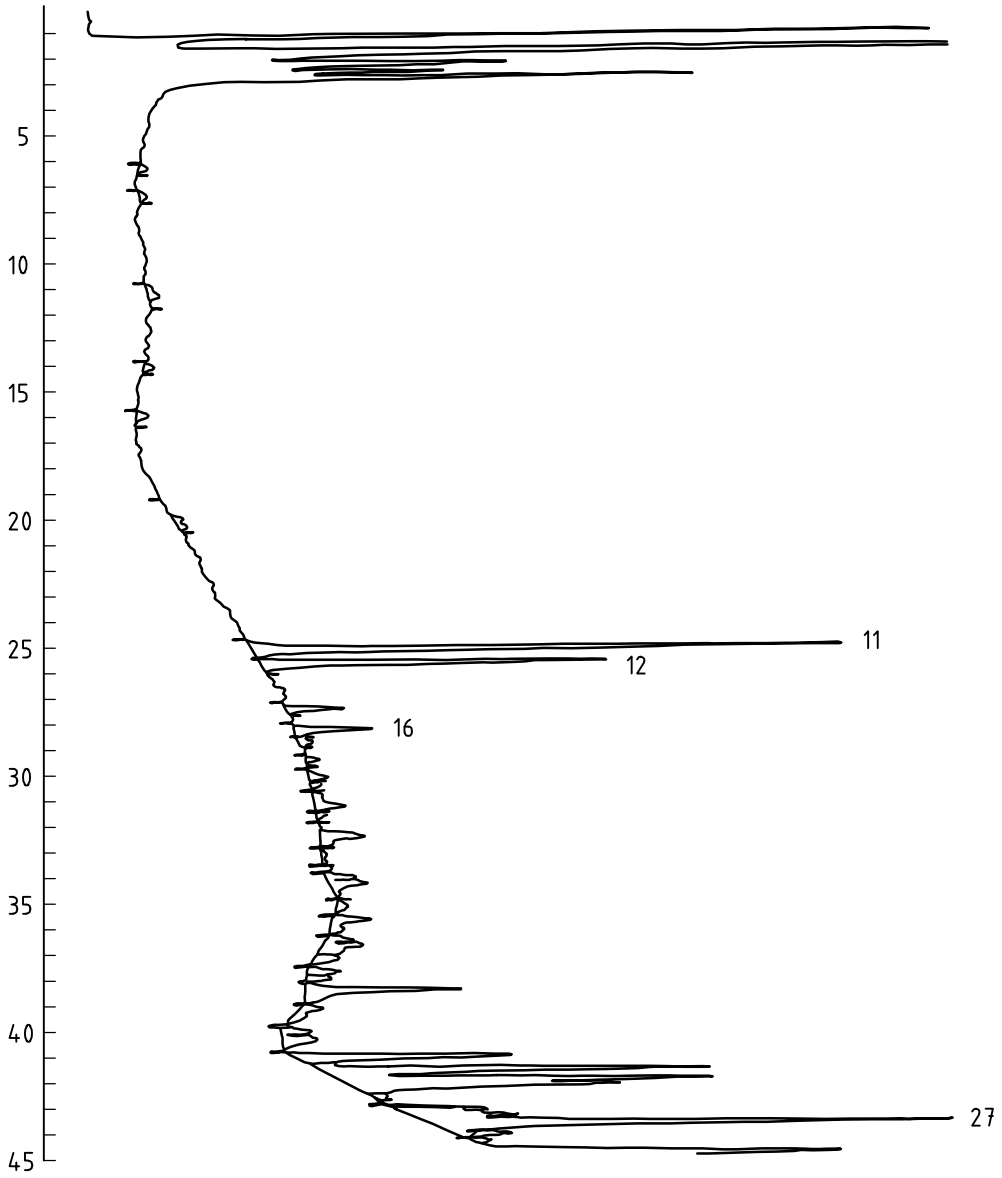


Figure A.2 — Chromatogram of a soil sample, taken from extract E2 (ρ_{E2} = 10 g/ml according to 6.4.2)

Atrazine (12): 0,28 mg/kg m_{md}; metazachlor (16): 0,48 mg/kg m_T and pendimethalin (27): 0,17 mg/kg m_T.

The listing of the herbicides in Figure A.2 corresponds to Table 1.

Annex B
(normative)

Validation

B.1 Determination of recoveries

It is not possible to obtain a natural soil with 27 herbicides as grown residues. It was the aim of the interlaboratory trial to check a list of 27 compounds and to identify the residues first in a qualitative manner. This case is more difficult than analysing the given 27 substances.

The method has been validated by three independent laboratories, by spiking an uncontaminated soil sample with two concentration levels in threefold. Level 1 compared to 0,04 mg/kg m_{dm}, level 2 compared to 0,4 mg/kg m_r, each level referring to atrazine. The remaining herbicides have been added according to 4.13.6 and 4.13.7. Recoveries in most cases have been between 70 % and 120 %. The average recovery had been 108 % (see Table B.1). The recoveries are based on spiked samples.

Table B.1 — Recovery rates

No.		Level [a] 0,04 mg/kg m_{dm}		Level [a] 0,4 mg/kg m_{dm}		Average value	
		w_f 1 %	S_R 1	w_f 2 %	S_R 2	w_f %	S_R
1	Atrazine-desisopropyl	(129)	19	108	9	119	14
2	Metamitron	110	9	107	9	109	9
3	Atrazine-desethyl	115	7	110	9	113	8
4	Hexazinon	111	9	108	10	109	9
5	Metoxuron	117	10	110	8	113	9
6	Bromacil	115	7	109	9	120	8
7	Simazine	115	5	110	8	113	7
8	Monuron	115	7	111	8	113	8
9	Cyanazine	102	15	106	11	104	13
10	Methabenzthiazuron	117	27	108	14	113	20
11	Chlortoluron	106	11	100	12	103	12
12	Atrazine	108	11	104	8	106	10
13	Isoproturon	(269) [b]	15	119	9		
14	Diuron	113	11	107	10	110	11
15	Metobromuron	97	35	85	59		
16	Metazachlor	107	10	103	8	105	9
17	Sebuthylazine	96	14	96	16	96	15
18	Propazine	87	18	71	13	79	15
19	Dichlobenil	115	14	99	28	107	21
20	Terbuthylazine	118	15	114	11	116	13
21	Chloroxuron	106	14	106	14	106	14
22	Propyzamide	93	27	101	14	99	20
23	Terbutryn	(127)	8	(153)	60		
24	Ethofumesate [c]						
25	Metolachlor	112	8	104	10	108	
26	Alachlor	112	10	104	13	108	11
27	Pendimenthalin	(173)	56	106	17		
	All compounds	110	12	105	12	108	12

[a] Referred to atrazine.

[b] Signal overlap: w_f recovery rate (%)

[c] Ethofumesate was not involved in this trial

S_R Comparison variations coefficient (relative %)

B.2 Interlaboratory trial

The method had been tested in three interlaboratory trials with five soil samples. In the trials designated Bn Bo 2, Bn Bo 3 and 37/94 Q, soils with original residues were used. Trials B 2.2 and B 2.3 used soils with spiked substances and subsequent homogenisation. Evaluation was performed according to ISO 5725-2 (see Table B.2).

Table B.2 — Results of interlaboratory trials

Sample	Trial	$\overline{X}$ mg/kg m_{dm}	V_r %	V_R %	Number of participants	Outliers
Atrazine	Bn Bo 1	1,4	11	27	10	0
Atrazine	Bn Bo 3	0,017	6	34	9	2
Atrazine	B 2.2	0,33	6	24	11	1
Atrazine	B 2.3	0,34	9	21	11	0
Atrazine	37/94 Q	0,017	30	52	9	0
Atrazine-desethyl	Bn Bo 2	0,043	3	22	8	2
Atrazine-desethyl	B 2.2	0,14	13	27	11	1
Simazine	Bn Bo 2	0,025	14	53	8	1
Simazine	37/94 Q	0,14	12	43	14	0
Pendimethalin	Bn Bo 2	0,58	15	24	7	0
Pendimethalin	B 2.2	0,10	15	30	10	0
Pendimethalin	B 2.2	0,19	7	34	11	2
Chlortoluron	B 2.2	0,79	5	41	9	1
Chlortoluron	B 2.3	0,29	8	34	10	0
Isoproturon	B 2.2	0,64	11	21	11	0
Chlorofuron	Bn Bo 2	2,8	5	24	8	1
Diuron	37/94 Q	2,2	10	17	12	1

$\overline{X}$ Average value

V_r Coefficient of repeatability

V_R Coefficient of reproducibility

Annex C
(informative)

Additional compounds which were tested with this method
(see 6.4 and 6.5)

C.1 Herbicides

The listing is in the elution order.

Chloriazon, Carbetamide, Metribuzin, Terbuthylazine-desethyl, Lenacil, 1-(3,4-Dichlorophenyl)urea (Diuron metabolite 1), 1-(3,4-Dichlorphenyl)-3-methylurea (Diuron metabolite 2), Linuron, Phenmedipham, Chloroxuron, Propyzamide, Chlorpropham, EPTC, Fluorochloridone, Diflufenican, Prosulfocarb, Triallate, Trifluralin.

C.2 Additional insecticides and fungicides which were tested with this method
(see 6.4 and 6.5)

Pymetrozine, Pirimicarb, Pyrimethanil, Myclobutanil, Fluquinconazole, Aclonifen, Parathion, Quinoxyfen, Chlorpyrifos, Fenpropathrin.

The limit of detection for these compounds is between 0,27 mg/kg m_{dm} and 0,82 mg/kg m_{dm}.

C.3 Further insecticides and fungicides which were extracted with this method
(see 6.4)

Determination was done by gas chromatography and PN detector or electron-capture detector (ECD).

Aclonifen, Chlorpyrifos, Chlorthalonil, Cyproconazole, Dimethoat, Endosulfan, Epoxiconazole, Fenpropathrin, Fenpropimorph, Fluquinconazole, Flusilazole, Lindane, Myclobutanil, Parathion, Parathion-methyl, Pirimicarb, Propiconazole, Pyrimethalin, Quinoxyfen, Terbuthylazine-desethyl.

The limit of detection for these compounds is between 0,01 mg/kg m_{dm} and 0,1 mg/kg m_{dm}.

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