INTERNATIONAL STANDARD

ISO 15913

First edition
2000-04-01

Water quality — Determination of selected phenoxyalkanoic herbicides, including bentazones and hydroxybenzonitriles by gas chromatography and mass spectrometry after solid phase extraction and derivatization

Qualité de l'eau — Dosage de certains herbicides phénoxyalcanoïques, y compris bentazones et hydroxybenzonitriles, par chromatographie en phase gazeuse et spectrométrie de masse après extraction en phase solide et dérivatisation

Reference number
ISO 15913:2000(E)

ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 734 10 79
E-mail copyright@iso.ch
Web www.iso.ch

Printed in Switzerland

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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.

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 International Standard may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

International Standard ISO 15913 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 2, *Physical, chemical and biochemical methods*.

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

Water quality — Determination of selected phenoxyalkanoic herbicides, including bentazones and hydroxybenzonitriles by gas chromatography and mass spectrometry after solid phase extraction and derivatization

WARNING — Diazomethane is explosive, extremely toxic and severely irritating, causing pulmonary oedema when inhaled in high concentrations. Long-term, low-level exposure may lead to sensibilization, resulting in asthma-like symptoms. Also, diazomethane and several of its chemical precursors have been cited as carcinogens.

1 Scope

This International Standard specifies a method for the determination of phenoxyalkanoic acids in ground and drinking water in mass concentrations $\geqslant$ 50 ng/l (detailed information is given in Table A.1 of annex A). Examples of phenoxyalkanoic acids which can be determined by this method are given in Table 1.

This method may be applicable to compounds not mentioned in Table 1 or to other types of water. However, it is necessary to verify the applicability of this method for these special cases (see annex B).

Table 1 — Plant treatment agents determined by this method

Name	Molecular formula	Relative molecular mass	CAS registry No.
(2,4-Dichlorophenoxy) acetic acid	$C_8H_6Cl_2O_3$	221,0	94-75-7
Mecoprop	$C_{10}H_{11}ClO_3$	214,65	93-65-2
Dichlorprop	$C_9H_8Cl_2O_3$	235,06	120-36-5
MCPA	$C_9H_9ClO_3$	200,6	94-74-6
MCPB	$C_{11}H_{13}ClO_3$	228,67	94-81-5
(2,4,5-Trichlorophenoxy)acetic acid	$C_8H_5Cl_3O_3$	255,5	93-76-5
Bentazone	$C_{10}H_{12}N_2O_3S$	240,3	25057-89-0
Bromoxynil	$C_7H_3Br_2NO$	276,9	1689-84-5
4-(2,4-Dichlorophenoxy)-butanoic acid	$C_{10}H_{10}Cl_2O_3$	249,1	94-82-6
Fenoprop	$C_9H_7Cl_3O_3$	269,51	93-72-1

2 Normative references

The following normative documents contain provisions which, through reference in this text, constitute provisions of this International Standard. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based on this International Standard 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*.

3 Term, definition, abbreviations and subscripts

3.1 Term and definition

For the purposes of this International Standard, the following term and definition applies.

3.1.1
phenoxyalkanoic herbicides
herbicides which undergo derivatization with diazomethane and which may subsequently be determined by gas chromatography

EXAMPLE Typical phenoxyalkanoic herbicides include alkylhalogenated phenoxy acids, hydroxybenzonitriles and bentazone.

3.2 Abbreviations

2,4-D (2,4-dichlorophenoxy) acetic acid

2,4-DB 4-(2,4-dichlorophenoxy) butanoic acid

2,4-DP dichlorprop

MCPP mecoprop

2,4,5-T (2,4,5-trichlorophenoxy) acetic acid

2,4-TP fenoprop

3.3 Subscripts

c calibration step using an external standard

g overall procedure

i identity of the substance *i*

is internal standard

j consecutive figure *j* for pairs of values

sam sample

sol solvent

4 Principle

After acidification, substances are enriched on solid phase adsorbent material [for example RP[1)]-C18 material], eluted with solvent, methylated with diazomethane and then determined by gas chromatography using a mass spectrometric detector. In some cases, the substances may be present as their esters, for example octanoic esters. Hydrolysis of the water sample (see annex C) may lead to higher concentrations of the free acids.

5 Interferences

5.1 Occurrence

Interferences may occur especially when examining other types of water, for example surface water.

5.2 Sampling

To avoid interferences collect the sample as described in clause 8.

5.3 Enrichment

The commercially available adsorbent materials are often of varying quality. Considerable batch-to-batch differences in quality and selectivity of this material are possible. The recovery may vary with the concentration. Therefore, check recovery regularly at different concentrations. Perform calibration and analysis with material taken only from the same batch. Suspended matter in the water sample (such as iron hydroxide, calcium carbonate) occurring during sampling, storage and sample preparation, or an increase in the concentration of microorganisms may clog the packing. In this case, filter the water sample through a glass fibre filter prior to enrichment.

5.4 Gas chromatography and mass spectrometry

Use the operational conditions set in accordance with manufacturer's instructions. Check these settings at regular intervals.

General interferences, caused by the injection system or insufficient separation can be eliminated with the help of special laboratory experience and the instrument's manuals.

6 Reagents

6.1 General

Use, as far as available, "for residual analysis" reagents. Use only reagents and water with negligibly low impurities, i.e. resulting in clean blanks.

6.2 Operating gases for the gas chromatography/mass spectrometry, of high purity and in accordance with manufacturer's specifications.

6.3 Nitrogen, of high purity, i.e. minimum 99,996 % by volume, for drying and eventually for concentration by evaporation.

6.4 Hydrochloric acid, c(HCl) = 2 mol/l.

6.5 Diethyl ether, $C_4H_{10}O$, stabilized.

1) RP = reversed phase

6.6 Ethanol, C_2H_5OH.

6.7 Acetic acid, CH_3COOH, 10 % by volume, aqueous solution (used to destroy diazomethane).

6.8 Sodium hydroxide solution, c(NaOH) = 6 mol/l.

6.9 Solvents for the elution, for example acetone C_3H_6O, or methanol, CH_3OH.

6.10 Methanol, CH_3OH, as conditioning agent.

6.11 Potassium hydroxide solution, KOH, volumic mass of 60 %.

6.12 Diazald (*N*-methyl-*N*-nitroso-4-toluenesulfonamide), $C_8H_{10}N_2O_3S$.

6.13 Solid phase adsorbent material, most commonly RP-C18-material, in the form of commercially available cartridges or adequately glass columns filled according to 7.4 with a minimum packing of 1,0 g.

For selectivity of the material see 5.3.

6.14 Internal standard, preferably deuterated or ^{13}C-labelled compounds.

The standards are often commercially available at a concentration of 100 µg/ml. Dilute this standard with acetone. The final concentration in the water sample shall be for example about 100 ng/l.

6.15 Diazomethane solution.

WARNING — Diazald is an irritant and all skin contact should be avoided.

Prepare diazomethane in a distillation apparatus, such as the one shown in Figure 1.

For security reasons, install two wash bottles; keep the first one empty for the purpose of protecting the solution from backflush and fill the second with acetic acid (6.7).

Insert 8 ml of the KOH solution (6.11) and 10 ml of ethanol (6.6) in a 250 ml reaction flask.

Suspend 5,0 g of diazald (6.12) in 45 ml of diethyl ether (6.5) in a pressure-equalizing funnel.

Cautiously warm the reaction flask to about 60 °C (water bath) and, within 20 min, dropwise add the diazald suspension from the filter funnel.

Collect the diazomethane being formed during this process and the diethyl ether in the trap (cooled with ice/NaCl).

After this reaction, add an additional 10 ml of diethyl ether through the filter funnel and distil the remaining diazomethane.

Stopper the trap and store it at about −18 °C. Check the stability of the diazomethane which should have an intensive yellow colour.

NOTE Excess diazomethane may be destroyed by adding a solution of acetic acid (6.7).

Prior to cleaning, rinse all diazomethane glassware with acetic acid (6.7).

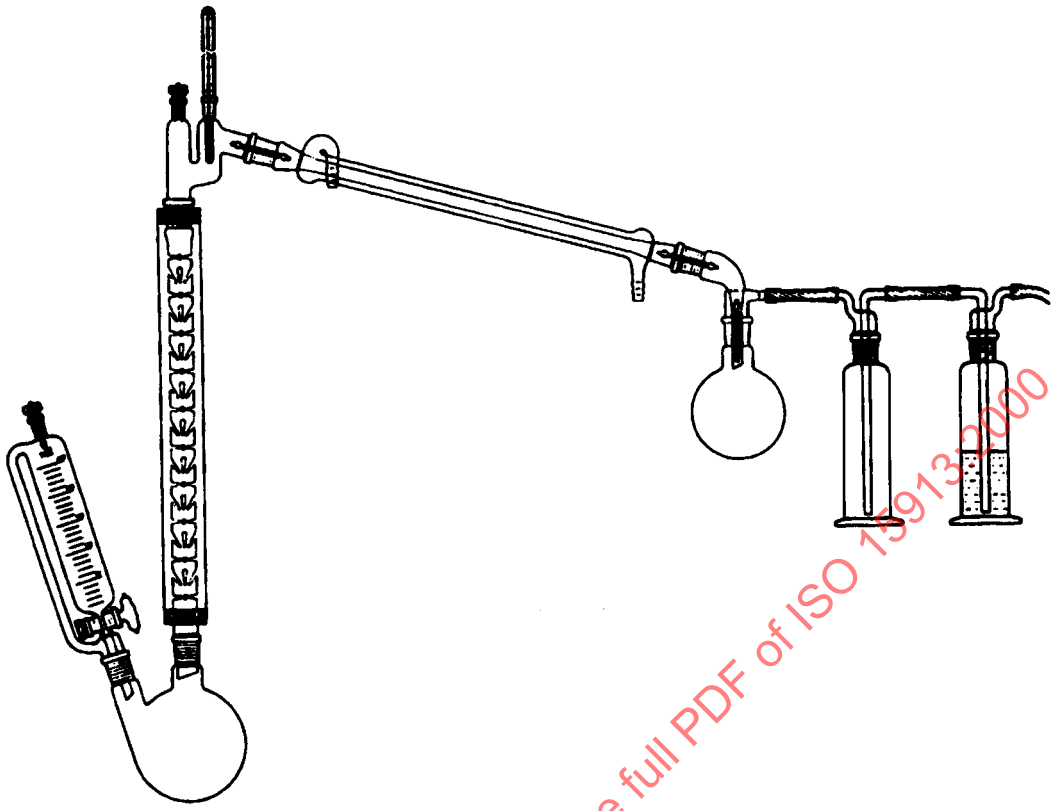

Figure 1 — Example of a distillation apparatus

6.16 Reference substances:

6.16.1 Methyl ester reference substances (methyl esters of the acids listed in Table 1) of defined concentration suitable for the preparation of reference solutions for gas chromatography.

6.16.1.1 Solutions of individual methyl esters.

As an example, place 50,0 mg each of a reference substance into a 100 ml volumetric flask, dissolve with acetone (6.9) and dilute to volume.

Store the solution at −18 °C, protected from light.

Check the concentration regularly.

6.16.1.2 Methyl ester stock solutions.

As an example, pipette 1 ml of each of the solution of the individual substance (6.16.1.1) into a 100 ml volumetric flask and dilute to volume with acetone (see 6.9).

Store the solutions at −18 °C, protected from light.

Check the concentration of the stock solutions regularly.

6.16.1.3 Methyl ester reference solutions (working standard solution).

Prepare the reference solutions by an adequate dilution of the stock solution (6.16.1.2).

Store the reference solution in the refrigerator. Reference solutions are stable for about 6 months.

6.16.2 Free-acid reference substances:

6.16.2.1 Solutions of the individual free acid.

As an example, place 50,0 mg of each of the reference substance into a 100 ml volumetric flask, dissolve with acetone (6.9) and dilute to volume.

Store the solutions at −18 °C, protected from light.

Check the concentration of the stock solutions regularly.

6.16.2.2 **Free-acid stock solution** (intermediate standard solutions).

As an example, pipette 1 ml of each of the solutions of the individual free acid (6.16.2.1) into a 100 ml volumetric flask and dilute to volume.

Store the solutions at −18 °C, protected from light.

Check the concentration of the free-acid stock solutions regularly.

6.16.2.3 **Free-acid reference solutions** (working standard solutions).

Prepare the solutions by adequate dilution of the stock solution (6.16.2.2).

Store the free-acid reference solutions in a refrigerator. Their shelf-life is limited.

7 Apparatus

7.1 General requirements

Equipment or parts of it which are likely to come into contact with the water sample or its extract shall be free from residues causing interferences. It is recommended to use vessels made of glass or stainless steel.

7.2 Flat-bottomed flasks, preferably brown glass, 1 000 ml and 2 000 ml, with glass stoppers.

7.3 Graduated cylinders, 1 000 ml.

7.4 Cartridges, made of polypropene or glass, filled with solid-phase material, for example RP-C18 material, (6.13).

NOTE The cartridges are commercially available.

7.5 Vacuum pump or **pressure assembly**.

7.6 Vials, suitable for automatic or manual injection.

7.7 Volumetric flasks, 10 ml or 100 ml.

7.8 Capillary gas chromatograph, equipped with a non-discriminating injection system and a mass-spectrometric detector.

7.9 Capillary columns, for gas chromatography; for examples see annex D, Figures D.5 and D.6.

7.10 Glass-fibre filters, made of borosilicate glass, of fibre diameter for example from 0,75 µm to 1,5 µm, with inorganic binding material.

7.11 pH meter.

7.12 Injection syringes, nominal capacity 5 µl and higher.

7.13 Apparatus for preparing diazomethane, (see example in Figure 1), comprising the following:

— double-necked, round-bottomed flask, 250 ml capacity;

— pressure-equalizing funnel, 100 ml capacity;

— distillation column, for example Vigreux column;

— distillation head;

— condenser, for example Liebig condenser;

— flask for absorption of diazomethane;

— security flask;

or a commercial distillation apparatus.

8 Sampling

Collect samples in accordance with ISO 5667-1, ISO 5667-2, and ISO 5667-3.

Use thoroughly-cleaned, preferably brown, flat-bottomed glass flasks (see 7.2) for sampling.

Fill the bottles completely with the water to be examined.

Treat and analyse the samples as soon as possible after the sample collection.

If storage is unavoidable, store the sample at 4 °C in the dark, but not for longer than 3 days.

9 Procedure

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

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

9.1 Solid phase adsorption and derivatization of test samples

9.1.1 Conditioning of the RP-C18 adsorbent material

Wash the RP-C18 material in the cartridge or glass column (7.4) with a volume of methanol (6.10) five times that of the column volume.

Rewash the column with a volume of water (6.1), five times that of the column volume and use the conditioned material for enrichment.

Do not let the cartridge/column run dry.

9.1.2 Enrichment and derivatization

Measure the volume of the sample (see clause 8) (for example 1 000 ml) in a graduated cylinder or by weighing.

Adjust the pH with hydrochloric acid (6.4) to (2 ± 0,2).

If calibration is carried out using an internal standard, add 10 µl of the commercially available internal standard (6.14), or 1 ml of the dilute internal standard solution (6.14).

Run the water sample at a regulated flow rate of < 1 000 ml/h over the adsorbent material previously conditioned according to 9.1.1. (Regulate the flow rate by altering the vacuum or the overpressure, respectively).

After enrichment, dry the adsorbent with an inert gas, for instance in a nitrogen stream (6.3) (30 min, at a flow rate of approximately 100 ml/min at room temperature).

Elute in small portions with at least 4 ml of eluent (6.9) per 1 g of RP-C18 material.

Add half of the solvent (6.9) to the column (cartridge) and allow to equilibrate (for instance for 10 min).

Add the remaining solvent and collect the eluent in a small volumetric flask.

Transfer any remaining eluent from the adsorbent by means of vacuum or pressure into the volumetric flask.

Carefully evaporate the solvent under a stream of nitrogen, nearly to dryness, and carry out the derivatization step immediately.

9.1.3 Derivatization with diazomethane

Add 0,5 ml of diazomethane solution (6.15) to the evaporated extract (9.1.2) or to the standard solution, respectively, to react in the dark for 1 h. The residual solution shall remain yellow, otherwise add more diazomethane. Note the solvent volume, V_{sol}, used for dissolution. Evaporate the solvent almost to dryness using a stream of nitrogen and dissolve the residue acetone (6.9).

9.2 Blank monitoring

The proper condition of instruments and reagents shall be checked by blank monitoring at regular intervals, at least each time after any change has been made.

For the blank measurements prepare and analyse 1 000 ml of water (6.1) in the same way as the sample.

If the blanks are higher than the limit of determination, systematic investigations shall be carried out to detect and consequently eliminate the source of contamination.

9.3 Determination

9.3.1 Operating conditions — Gas chromatography

Optimize the instrumental parameters as described in the operator's manual.

Use a capillary column (7.9) for the separation (see annex D, key to Figures D.5 and D.6).

Ensure that the compounds of interest are separated sufficiently to allow proper identification.

NOTE Identification by retention times and spectra data may be supported by the available methyl ester reference substances (6.16.1).

9.3.2 Identification of individual compounds

For the identification of a substance the following conditions shall apply:

Ionization method:	Electron impact, electron energy at least 45 eV
Mass range of the system:	The spectrum may be started at 46 amu (atomic mass units) (due to interferences by, for example, CO_2). At least 10 amu above the highest atomic mass of the substance shall be determined.
Cycle time:	Less than 2 s and such that at least five spectra may be recorded per substance peak.

If only single masses are registered in order to increase the sensitivity, register the base peak and at least 2 more ions (if within the spectrum), with the same cycle duration as above.

Consider individual compounds in the sample to be identified if:

— the retention times (t_R) of the respective peaks in the total ion-current chromatograms or in the individual mass chromatograms lie within a tolerance of $t_R = \pm 0{,}08$ min (5 s), compared with the retention times of the peaks of the substances in the total ion current chromatograms or individual mass chromatogram of a reference solution, measured under identical conditions; and

— if complete, background-corrected mass spectra of the reference compounds agree within specified tolerances with the background corrected mass spectra obtained at the respective retention time in the total ion-current chromatogram of the sample; or

— if at least the characteristic molecular ions or fragment ions of the reference compounds (see Table 2) agree, within specified tolerances, with those of the compounds to be identified as to their relative peak intensities.

NOTE 1 Generally, after background correction, no ion with greater mass should be present in the mass spectrum than the greatest possible mass for a compound being identified.

NOTE 2 The identification via the molecular ion or via a main fragment ion is often not sufficient for an identification, therefore, at least a further typical fragment mass should be used for confirmation.

NOTE 3 Low limits of detection can be achieved by identification via single mass detection with individual mass recording (SIM technique) or with multiple mass recording (MID technique). However, less information is obtained from these techniques. Therefore SIM and MID should be used only with a certain knowledge of the sample matrix. With inadequate background information of the sample to be investigated at least two further characteristic masses should be examined to get an additional confirmation.

9.4 Calibration

9.4.1 General requirements

For practical reasons, use reference standards.

Use the same injection volume for calibration as for the measurement of the sample solutions.

For each compound a separate calibration function and graph, consisting of at least five points from five different concentrations, shall be established.

Table 2 — Identification and quantification of methylated phenoxyalkanoic herbicides, hydroxynitriles and bentazone

Compound	Masses used for quantification	Further mass fragments used for identification
MCPP	169, 228	230, 142
MCPA	155, 214	141, 216
Dichlorprop	162, 248	164, 250
2,4-D	99, 234	201, 236
Fenoprop	196, 198	282, 284
2,4,5-T	233, 268	235, 270
2,4-DB	162, 164, 231	101
Bentazone	212, 254	175
Bromoxynil	291, 276	289, 299
MCPB	242, 107	101
2,4-Dichlorophenylacetic acid	159, 183	
2,4-D (ring D_3) (for example as internal standard)	202, 237, 178	

9.4.2 Calibration strategy

9.4.2.1 Step 1 — Initial calibration

It is necessary to determine the recovery in the following way:

a) directly inject the methyl ester reference solutions (6.16.1.3) (see 9.4.3.1);

b) inject the methylated extracts of spiked aqueous standard solutions (see 9.4.3.2).

The data obtained from a) are compared with those from b) in order to calculate the recovery of each substance determined (see 9.4.3.3).

9.4.2.2 Step 2 — Recalibration

Recalibration is carried out using two reference solutions (about 10 % and 80 % of the working range) with each batch of samples to analyse (see 9.4.3.4).

NOTE It is permitted to use the derivatized (methylated) extracts of the spiked aqueous standard solutions to recalibrate if these extracts are stable.

9.4.2.3 Step 3 — Evaluation of data

The data shall be evaluated as follows:

a) if batch recalibration is carried out using methyl ester reference solutions (6.16.1), the results obtained shall be corrected by mean recovery, using equation (7);

b) if batch recalibration is carried out using methylated extracts of spiked aqueous standard solutions, the results obtained include direct correction for recovery (see 10.1.1);

c) if a complete calibration of the overall procedure (see 9.4.3.2) is carried out for each batch of samples to analyse, the results obtained include direct correction for recovery (see 10.1.2).

9.4.3 Use of external standards

9.4.3.1 Reference solutions (not using the overall procedure)

Inject volumes in the range of 1 µl to 10 µl of the methyl ester reference solutions (6.16.1.3) into the gas chromatograph.

Measure the gas chromatographic signals for each substance (peak heights or area integration units respectively).

For a graphical presentation of the calibration curve, plot the respective measured values $y_{i,c}$ on the ordinate against the respective mass concentrations $\rho_{i,c}$ of the substance i on the abscissa.

The series of measured values thus obtained shall be used to establish the linear regression function as follows:

$$y_{i,c} = a_i \cdot \rho_{i,c} + b_i \quad (1)$$

where

$y_{i,c}$ is the (dependent variable) measured response of substance i, depending on $\rho_{i,c}$; the unit depends on the evaluation; for example expressed as an area value;

$\rho_{i,c}$ is the (independent variable) mass concentration, in micrograms per litre, of substance i (external standard), in the methyl ester reference solution;

a_i is the slope of the calibration function of substance i, the unit depends on the evaluation, for example area value × litres per micrograms;

b_i is the ordinate intercept of the calibration curve; the unit depends on the evaluation, for example area value.

NOTE As a rule, the intercept b is very small.

The working range is defined as the linear part of the curve.

9.4.3.2 Overall procedure (spiked water)

To calibrate the entire procedure, prepare aqueous solutions of the compounds to be determined in an individual concentration range within the linear dynamic range of the detector, as follows.

9.4.3.2.1 Preparation of the spiked aqueous standard solutions

Prepare at least five aqueous standard solutions covering the range 0,02 µg/l to 0,5 µg/l by adding different volumes of the free-acid stock solution (6.16.2.2) to water (6.1).

For blank measurements, add to one bottle of water (6.1) the same quantity of solvent used for the preparation of the aqueous standard solutions.

Prepare the spiked aqueous standard solutions on the day of use.

9.4.3.2.2 Calibration curve

Extract these aqueous solutions, concentrate and derivatize as given in 9.1.2.

Inject volumes in the range of 1 µl to 10 µl of the methylated extracts of the blank and of at least five spiked aqueous standard solutions with concentrations $\rho_{i,cg}$ in ascending order, into the gas chromatograph.

Measure the peak values $y_{i,cg}$ of these solutions.

Calculate a regression function for each substance using the pairs of values $y_{i,cg}$ and $\rho_{i,cg}$ as follows:

$$y_{i,cg} = a_{i,g} \cdot \rho_{i,cg} + b_{i,g} \quad (2)$$

where

$y_{i,cg}$ is the (dependent variable) measured response of substance i during calibration, depending on $\rho_{i,cg}$, the unit depends on evaluation; for example area value;

$\rho_{i,cg}$ is the (independent variable) mass concentration, in micrograms per litre, of substance i in the spiked aqueous standard solution;

$a_{i,g}$ is the slope of the calibration curve of substance i, often referred to as f_i the unit depends on the evaluation, for example area value × litres per micrograms;

$b_{i,g}$ is the ordinate intercept of the calibration curve; the unit depends on the evaluation, for example area values.

Plot the reference functions in a diagram with the ordinate, as the substance specific measured signals $y_{i,cg}$, and the abscissa as the mass concentration $\rho_{i,cg}$ of the substance i in the spiked aqueous standard solution.

The working range is defined as the linear part of this curve.

9.4.3.3 Determination of the recovery

Determine by means of the calibration procedure according to 9.4.3.2 and 9.4.3.3 the substance specific mean recovery A_i for the substance i [see equation (3)].

$$A_i = \frac{\frac{a_{i,g}}{a_i}}{F_V} = \frac{a_{i,g} \cdot V_{sam}}{a_i \cdot V_{sol}} \quad (3)$$

where

A_i is the specific mean recovery for the substance i, dimensionless;

a_i is defined in equation (1);

$a_{i,g}$ is defined in equation (2);

F_V is the ratio of the volume of extraction solvent and sample. This factor is calculated taking into account the sample volume, the extraction solvent volume, and the dilution factors (if applicable). The following equation applies:

$$F_V = \frac{V_{sol}}{V_{sam}} \quad (4)$$

where

V_{sol} is the solvent volume, in millilitres, used for dissolution (see 9.1.3);

V_{sam} is the sample volume, in millilitres.

NOTE Equation (3) is valid if b_i and $b_{i,g}$ are relatively small and if calibration according to equations (1) and (2) refers to the same range of concentration (in the extract and in the solvent standard solution) for example, comparable values for $y_{i,c}$ and $y_{i,cg}$.

A constant recovery is an essential prerequisite for good precision and accuracy of the analytical result. Variations of these values will indicate problems in some stages of the analysis.

The recovery depends on the distribution coefficient and derivatization efficiency and is characteristic for each substance and the working conditions. For the purposes of this International Standard, consider a recovery of greater than 60 % as "a good recovery".

9.4.3.4 Recalibration with each batch

For routine recalibration of the method, it is essential to work within the previously established linear range (see 9.4.3.1 or 9.4.3.2). This shall be updated regularly especially when contaminated samples are analysed, as these may affect the detector and hence the linear range.

The minimum requirement for batch recalibration shall be injections of two methyl ester reference solutions (6.16.1.3) or two derivatized extracts of spiked aqueous standard solutions (9.4.3.2.1). The concentration of the first solution shall be about 20 % of the selected linear working range, the concentration of the second solution about 80 %.

Calculate a regression function.

Compare this line to the previous established calibration curve (see 9.4.3.1 or 9.4.3.2). If the values are within the range of the confidence limits of the previously established calibration curve (9.4.3.1 or 9.4.3.2), use this new line as the calibration line for evaluation.

If not, check the system and establish a completely new calibration curve.

9.4.4 Use of internal standards

The procedure of the internal standard is a very powerful technique, if the compound used as an internal standard is well chosen. Ensure the chemical structure and behaviour are equivalent to the analysed compounds and the retention time is in an area of the chromatogram free from interfering peaks.

Follow a similar procedure to that used for the calibration using external standards (see 9.4.3), except that each spiked working sample and reference solution are spiked with the same amount of internal standard.

Keep the same solvent composition and internal standard concentration for the reference solutions and the extracts.

Evaluate the gas chromatographic signals for each substance against concentration.

Plot the response value ratio $y_{i,cj}/y_{is,cj}$ (peak areas, peaks heights or integration units) for each substance i on the ordinate and the associated value of mass concentration ratio $\rho_{i,cj}/\rho_{is,cg}$ on the abscissa.

Establish the linear regression function using the pairs of values $y_{i,cj}/y_{is,cj}$ and $\rho_{i,cj}/\rho_{is,cg}$ of the measured series in the following equation:

$$\frac{y_{i,c}}{y_{is,c}} = a_{i,is}\frac{\rho_{i,c}}{\rho_{is,c}} + b_{i,is} \qquad (5)$$

where

$y_{i,c}$ is the (dependent variable) measured response of the substance i in the calibration, depending on $\rho_{i,c}$; the unit depends on the evaluation, for example, area value;

$y_{is,c}$ is the measured response of the internal standard "is" in the calibration, depending on $\rho_{is,c}$; the unit depends on the evaluation, for example expressed as an area value;

$\rho_{i,c}$ is the (independent variable) mass concentration, in micrograms per litre, of the substance i in the calibration solution;

$\rho_{is,c}$ is the (independent variable) mass concentration, in micrograms per litre, of the internal standard "is";

$a_{i,is}$ is the slope of the calibration curve from response ratio, $y_{i,c}/y_{is,c}$, as a function of the mass concentration ratio, $\rho_{i,c}/\rho_{is,c}$, often called the response factor;

$b_{i,is}$ is the axis intercept of the calibration curve on the ordinate.

For recalibration see 9.4.3.4.

10 Calculation

10.1 Mass concentration

10.1.1 Determination using a (re)calibration of the reference solution

Calculate the mass concentration $\rho_{i,c}$ of the substance i using equation (6) after solving equation (1) for the mass concentration $\rho_{i,c}$:

$$\rho_{i,c} = \frac{y_i - b_i}{a_i \cdot A_i} \quad (6)$$

where

$\rho_{i,c}$ is the mass concentration, in nanograms per litre, of the substance i in the water sample (**corrected for mean recovery**);

y_i is the measured value of the substance i in the extract of the water sample (on the condition of the same procedure being applied as with the calibration and the sample measurement); the unit depends on the evaluation, for example expressed as an area value;

a_i is the slope of the calibration curve (9.4.3.1 or 9.4.3.4) of the substance i, the unit depends on the evaluation, for example expressed as the area value × (litres per nanograms);

b_i is the axis intercept of the reference line on the ordinate; the unit depends on the evaluation, for example, expressed as an area value;

A_i is the specific mean recovery for the substance i.

10.1.2 Determination using a calibration of the overall procedure

Calculate the mass concentration $\rho_{i,g}$ of the substance i in the water sample using equation (7) after solving equation (2) for the mass concentration $\rho_{i,g}$;

$$\rho_{i,g} = \frac{y_{i,g} - b_{i,g}}{a_{i,g}} \quad (7)$$

where

$\rho_{i,g}$ is the mass concentration, in micrograms per litre, of the substance i in the water sample **(corrected for recovery)**;

$y_{i,g}$ is the measured value of the substance i in the extract of the water sample (on the condition the same procedure being applied as with the calibration and the sample measurement); the unit depends on the evaluation, for example expressed as an area value;

$a_{i,g}$ is the slope of the calibration curve (see 9.4.3.2 or 9.4.3.4) of the substance i; the unit depends on the evaluation, for example expressed as the area value × (litres per micrograms);

$b_{i,g}$ is the axis intercept of the reference line on the ordinate; the unit depends on the evaluation, for example expressed as area value.

10.1.3 Determination using an internal standard

Calculate the mass concentration ρ_i of the substance using equation (8) after solving equations (3), (4) and (5).

$$\rho_i = \frac{\left(\frac{y_i}{y_{is}} \cdot b_{i,is}\right)\rho_{is}}{a_{i,is}} \cdot \frac{F_V}{A_i} \qquad (8)$$

where

y_i is the measured value of the substance i in the water sample; the unit depends on the evaluation, for example expressed as an area value;

y_{is} is the measured value of the internal standard "is" in the water sample; the unit depends on the evaluation, for example expressed as an area value;

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

ρ_{is} is the mass concentration, in micrograms per litre, of the internal standard "is";

$b_{i,is}$ is defined in equation (5);

$a_{i,is}$ is defined in equation (5);

A_i is the specific mean recovery for the substance i;

F_V is the ratio of the final extract volume to sample volume.

10.2 Expression of results

Report the mass concentration of the individual plant treatment agents in terms of µg/l to two significant figures.

EXAMPLES

2,4-D 0,81 µg/l;

2,4,5-T 1,5 µg/l.

11 Precision

Results of precision from an interlaboratory trial carried out in Germany are presented for information in annex A.

12 Test report

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

a) the identity of the sample;

b) the expression of the results, in accordance with clause 10.2;

c) any deviations from this procedure and all circumstances which may have affected the results;

d) the measuring conditions: report whether the substances were identified by the full spectra mode or only by identification of some masses.

Annex A
(informative)

Results of an interlaboratory trial

An interlaboratory trial, carried out in Germany in 1997, resulted in the values given in Table A.1.

NOTE In the interlaboratory trial, the choice of the solvent was left to the analyst's discretion.

Table A.1 — Precision data

Sample	Substance	l	n	o %	x_{corr} µg/l	x µg/l	RR %	s_R µg/l	CV_R %	s_r µg/l	CV_r %
Drinking water	Bentazone	15	60	6,3	0,143	0,156	109,4	0,024	15,6	0,011	7,2
	2,4-D	14	56	12,5	0,209	0,224	107,1	0,037	16,6	0,018	8,0
	Fenoprop	13	52	13,3	0,175	0,197	112,3	0,035	17,7	0,015	7,6
	MCPB	14	56	6,7	0,149	0,169	113,3	0,035	20,7	0,019	11,4
	Mecoprop	16	64	0	0,121	0,136	112,1	0,031	23,1	0,014	10,1
Ground water	Bentazone	14	53	7,0	0,048	0,055	113,8	0,080	14,0	0,004	8,1
	Bromoxynil	13	49	7,5	0,117	0,127	108,4	0,030	18,1	0,012	9,0
	2,4-DB	15	57	0	0,149	0,159	107,0	0,034	21,4	0,021	12,8
	Dichlorprop	16	61	0	0,102	0,115	112,7	0,023	19,9	0,009	7,9
	MCPA	16	61	0	0,193	0,205	106,4	0,040	19,6	0,014	6,8
	2,4,5-T	14	52	1,9	0,079	0,093	117,8	0,021	22,0	0,006	6,8
Standard	Bentazone	15	60	4,8	0,239	0,243	101,7	0,044	18,2	0,016	6,5
	Bromoxynil	14	54	1,8	0,390	0,382	98,1	0,065	17,0	0,020	5,3
	Mecoprop	16	63	0	0,503	0,483	96,0	0,109	22,6	0,028	5,8
	2,4,5-T	15	59	0	0,517	0,517	87,0	0,101	19,5	0,034	6,5

l is the number of laboratory sets;

n is the number of outlier-free individual analytical values;

o is the relative portion of outliers;

x_{corr} is the correct value, by convention;

x is the total mean;

RR is the recovery rate;

s_R is the reproducibility standard deviation;

CV_R is the reproducibility coefficient of variation

s_r is the repeatability standard deviation;

CV_r is the repeatability coefficient of variation

Annex B
(informative)

Further substances which may be analysed by this procedure

Table B.1 gives a list of other substances which can be analysed using this International Standard.

Figure B.1 — Further substances which can be analysed using this International Standard

Name	Formula	CAS registry No.
Ioxynil	$C_7H_3I_2NO$	1689-83-4
Triclopyr	$C_7H_4Cl_3NO_3$	55335-06-3
Fluroxypyr	$C_7H_5Cl_2FN_2O_3$	69377-81-7
Picloram	$C_6H_3Cl_3N_2O_2$	1918-02-1
Pentachlorophenol	C_6HCl_5O	87-86-5
2,3,6-TBA	$C_7H_3Cl_3O_2$	50-31-7
Benazolin	$C_9H_6ClNO_3S$	3813-05-6
Chlorthal	$C_8H_2Cl_4O_4$	2136-79-0
Clopyralid	$C_6H_3Cl_2NO_2$	1702-17-6
Dicamba	$C_8H_6Cl_2O_3$	1918-00-9
Diclofop	$C_{15}H_{12}Cl_2O_4$	40843-25-2
DNOC	$C_7H_6N_2O_5$	534-52-1
Fenoxaprop	$C_{16}H_{12}ClNO_5$	95617-09-7
Fluazifop	$C_{15}H_{12}F_3NO_4$	69335-91-7
Imazamethabenz	$C_{15}H_{18}N_2O_3$	100728-84-5
Imazapyr	$C_{13}H_{15}N_3O_3$	81334-34-1

Annex C
(informative)

Example of hydrolysis of phenoxyalkanoic carbonic esters

Add 17 ml of sodium hydroxide solution (6.8) to about 1 000 ml of sample in the sample bottle. Mix well and store the sample overnight at room temperature.