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FAO SPECIFICATIONS
FOR PLANT PROTECTION PRODUCTS

FENOPROP + MECOPROP

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Group on Pesticide Specifications

FAO Panel of Experts on Pesticide Specifications, Registration Requirements and Application Standards

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DISCLAIMER [1]

FAO specifications are developed with the basic objective of promoting, as far as practicable, the manufacture, distribution and use of pesticides that meet basic quality requirements.

Compliance with the specifications does not constitute an endorsement or warranty of the fitness of a particular pesticide for a particular purpose, including its suitability for the control of any given pest, or its suitability for use in a particular area. Owing to the complexity of the problems involved, the suitability of pesticides for a particular purpose and the content of the labelling instructions must be decided at the national or provincial level.

Furthermore, pesticides which are manufactured to comply with these specifications are not exempted from any safety regulation or other legal or administrative provision applicable to their manufacture, sale, transportation, storage, handling, preparation and/or use.

FAO disclaims any and all liability for any injury, death, loss, damage or other prejudice of any kind that may arise as a result of, or in connection with, the manufacture, sale, transportation, storage, handling, preparation and/or use of pesticides which are found, or are claimed, to have been manufactured to comply with these specifications.

Additionally, FAO wishes to alert users to the fact that improper storage, handling, preparation and/or use of pesticides can result in either a lowering or complete loss of safety and/or efficacy.

FAO is not responsible, and does not accept any liability, for the testing of pesticides for compliance with the specifications, nor for any methods recommended and/or used for testing compliance. As a result, FAO does not in any way warrant or represent that any pesticide claimed to comply with a FAO specification actually does so.

[1] This disclaimer applies to all specifications published by FAO.

INTRODUCTION TO FAO SPECIFICATIONS DEVELOPED UNDER THE OLD PROCEDURE

Between 1975 and 2000, FAO published booklets of specifications for technical materials and related formulations of plant protection products. Revisions of, and additions to, already published specifications will be issued when necessary. However, all changes and revisions of FAO specifications are now subject to the new procedure described in the *Manual on the development and use of FAO and WHO Specifications for Plant Protection Products*, FAO Plant Production and Protection Paper No. 173, Rome 2002 (*Revised First Edition* available only on the FAO home page of the Internet at: http://www.fao.org/pest-and-pesticide-management/en/)

FAO specifications developed under the old procedure are based on the requirements defined in the Fourth Edition of the *Manual on the development and use of FAO specifications for plant protection products*, Plant Production and Protection Paper No. 128, Rome 1995.

This manual contained detailed definitions and other essential background information on basic procedures and technical principles adopted by the group on Pesticide Specifications of the FAO Panel of Experts on Pesticide Specifications, Registration Requirements, Application Standards and Prior Informed Consent, such as:

1. Categories of Specifications (Section 3.1 of the Manual)

   FAO Tentative Specifications (Code 'S/T', formerly 'TS') are those which have been recommended by FAO as preliminary specifications and which are based on minimum requirements. The methods of analysis cited are normally supplied by the manufacturer or may already have been published or be the subject of collaborative work.

   FAO Provisional Specifications [Code 'S/P', formerly ('S')] are those for which more evidence of the necessary parameters is available and where some collaborative study of the methods of analysis has been carried out.

   FAO (full) Specifications (Code 'S/F', formerly 'S').
   Specifications that have all necessary requirements together with CIPAC (full) methods, or other collaboratively studied (proven) methods.[2, 3]

   Wherever possible, standards for apparatus and common names for pesticides are those approved by the International Organization for Standardization (ISO).

2. Expression of active ingredient content (Section 4.2.5 of the Manual)

   - for solids, liquid technical materials, volatile liquids (of maximum boiling point 50°C) and viscous liquids (with minimum kinematic viscosity of 1×10^3 m^2/s at 20°C) the FAO Specification shall be based on expression of the content as g/kg;

- for all other liquids the active ingredient content of the product shall be declared in terms of g/kg *or* g/l at 20°C. If the customer requires both g/kg *and* g/l at 20°C, then in case of dispute the analytical results shall be calculated as g/kg.

3. Tolerance on content (Section 4.2.7 of the Manual)

A declared content of active ingredient must be included in all specifications, and one of the problems immediately arising is the level of tolerance acceptable about the nominal figure. The tolerance is influenced by (a) the reproducibility of the method of analysis, (b) the sampling error and (c) the manufacturing variance.

Allowable variations in analytical results (i.e. tolerances in content of active ingredient) with respect to specific pesticide consignments are intended to cover reasonable variations in the contents of active ingredients. For examples of such tolerances, see the table in Section 4.2.7 of the Manual.

4. Containers/packaging

FAO guidelines are in preparation.

Containers shall comply with pertinent national and international transport and safety regulations.

Technical materials, dustable powders and granules

Containers shall be suitable, clean, dry and as specified, and shall not adversely affect, or be affected by, the contents, but shall adequately protect them against external conditions.

Wettable powders

The product shall be packed in suitable, clean, dry containers as specified in the order. The container shall provide all necessary protection against compaction, atmospheric moisture, loss by vaporization and/or contamination to ensure that the product suffers no deterioration under normal transit and storage conditions.

The product shall be protected by an adequate moisture barrier. This may be a suitable bag of polyethylene or alternative means of giving equal or better protection.

Solutions and emulsifiable concentrates

Containers shall be lined, where necessary, with a suitable material, or the interior surfaces shall be treated to prevent corrosion and/or deterioration of the contents.

Additional information should be given in all specifications where particular pesticides present problems in packaging.

5. Biological information

Phytotoxicity

No test can be specified to cover the possible phytotoxicity of a formulation to all crops. When a crop is not mentioned in the instructions for use, purchasers should check with the supplier that the material is suitable, always provided that such a use is not restricted or legally forbidden.

Wetting of crops

The dilute spray should satisfactorily wet the leaves of the specified crops when used in accordance with the instructions. Test method MT 53.2, CIPAC F, p.162, may be useful.

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[1] *Should national pesticide specifications developed from these approved FAO specifications deviate from them, the National Authority responsible for making such changes is requested to inform the FAO Plant Protection Service of the nature of, and the reasons for, the modifications.*

[2] *Methods of analysis and miscellaneous techniques referred to in these specifications have been developed and adopted by CIPAC (Collaborative International Pesticides Analytical Council Ltd.). See CIPAC Handbooks 1 (1970), 1A (1980), 1B (1983), 1C (1985), D (1988), E (1993), F (1995), G (1995), CIPAC Proceedings 1980 and 1981, obtainable from Black Bear Press Limited, King's Hedges Road, Cambridge CB4 2PQ, England. The page numbers of specific methods are given in parentheses in the specifications. Copies of methods not yet published can be obtained from the FAO Plant Protection Service.*

[3] *Information on standard waters for laboratory evaluation of pesticidal formulations will be found in CIPAC Monograph 1*, Standard Waters and an FAO Survey on Naturally Occurring Waters *(1972), Black Bear Press Limited, King's Hedges Road, Cambridge CB4 2PQ, England.*

SUBMISSION OF DRAFT SPECIFICATIONS TO FAO

Any organization, commercial firm or interested individual is encouraged to submit relevant specifications, or proposals for revision of existing specifications, for pesticide products for consideration and possible adoption by FAO. Correspondence should be addressed to the Pesticide Management Group, Plant Production and Protection Division, FAO, Viale delle Terme di Caracalla, 00153 Rome, Italy.

General guidelines on preparing draft specifications are given in the *Manual on the development and use of FAO and WHO Specifications for Plant Protection Products*, FAO Plant Production and Protection Paper No. 173, Rome 2002 (Revised First Edition available only on the FAO home page of the Internet at: http://www.fao.org/pest-and-pesticide-management/en/).

Specifications which are considered suitable for further processing are assigned priorities and circulated to appropriate organizations and specialists to comment. Comments, together with other relevant information, are then reviewed in detail by the Group on Specifications of the FAO Panel of Experts on Pesticide Specifications, Registration Requirements, Application Standards and Prior Informed Consent. The drafts are converted into FAO Provisional Specifications, or full FAO Specifications.

INFORMATION

COMMON NAME:	FENOPROP
CIPAC CODE NO:	118
EMPIRICAL FORMULA:	$C_9H_7Cl_3O_3$
RMM:	269.5

CHEMICAL NAMES:

Fenoprop is the ISO common name for (±)-2-(2,4,5-trichlorophenoxy)propionic acid (IUPAC); (±)-2-(2,4,5-trichlorophenoxy) propionic acid (CA; Registry No. 93-72-1).

COMMON NAME:	MECOPROP
CIPAC CODE NO:	51
EMPIRICAL FORMULA:	$C_{10}H_{11}ClO_3$
RMM:	214.6

Mecoprop is the ISO common name for (±)-2-(4-chloro-o-tolyloxy)propionic acid (IUPAC); 2-(4-chloro-2-methylphenoxy) propanoic acid (CA; Registry No. 98-65-2).

FENOPROP + MECOPROP SALT AQUEOUS SOLUTIONS

FAO Tentative Specification October 1983
(118.1+51.1/SL/ts/-)

.1 DESCRIPTION

The product shall consist of fenoprop and mecoprop (complying with the respective FAO Provisional Specifications October 1983) as the active ingredients formulated as a fenoprop + mecoprop salt aqueous solution. It shall be free from visible suspended matter or sediment.

.2 ACTIVE INGREDIENT

.2.1 Salt(s)

The names of the fenoprop and mecoprop salt(s) present shall be stated. (Note 1).

.2.2 Identity tests*

Where the identity of the active ingredient is in doubt the extractable acids shall comply with any two of the following tests:

.2.2.1 GLC

The major components in the sample chromatogram shall have the same relative retention times as those from a standard fenoprop and mecoprop chromatographed under identical conditions.

.2.2.2 TLC

The major component in the sample chromatogram shall have the same Rf value as those from a standard fenoprop and mecoprop.

.2.2.3 HPLC

The major components in the sample chromatogram shall have the same retention times as those from a standard fenoprop and mecoprop chromatographed under identical conditions.

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* The analytical method for determination of the relevant impurity is available from the Pesticide Management Group of the FAO Plant Protection Service, or can be downloaded here

.2.3 Extractable acids*

The extractable acid content expressed as fenoprop shall be not more than l.lx + 1.25y where x is the content of fenoprop and y is the content of mecoprop found under .2.4 (Note 2).

.2.4 Fenoprop and mecoprop*

The nominal fenoprop and mecoprop contents (g/l at 20 C or g/kg; Note 3) shall be declared and, when determined, the content obtained shall differ from that declared by not more than +/- 5% of the declared content.

.3 IMPURITIES

.3.1 Free phenols*

Maximum: 1.5% (Note 4), expressed as 2,4,5-trichlorophenol (Note 5) of the total fenoprop and mecoprop contents found under .2.4 (Note 6).

.3.2 Water insolubles*

The product shall pass through a 250 µm test sieve and not more than 1 g/kg shall remain on a 150 µm test sieve.

.3.3 2,3,7,8- tetrachlorodibenzo-p-dioxin*

Maximum: 0.01 µg/g of the fenoprop content declared under .2.4.

.4 PHYSICAL PROPERTIES

.4.1 Stability on dilution (MT 41, CIPAC l,-p. 933)

The product, after dilution with CIPAC Standard Water C, shall give a clear or opalescent solution, i.e., free from more than a trace of sediment and/or visible solid particles.

.5 STORAGE STABILITY

.5.1 Stability at 0 C (MT 39.2, CIPAC 1, p. 932)

After storage at 0 C (Note 7) for 48 hours there shall be no separation of material from the product.

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* The analytical method for determination of the relevant impurity is available from the Pesticide Management Group of the FAO Plant Protection Service, or can be downloaded here

.5.2 Stability at 54°C*

After storage at 54 +/- 2 C for 14 days the product shall continue to comply with .2.4, .3.2, .4.1 and .5.1.

.6 CONTAINERS

They should be lined, where necessary, with a suitable material or the interior surfaces treated to prevent corrosion and/or deterioration of the contents.

They should comply with pertinent national and international transport and safety regulations.

NOTE 1 In the case of mixed salt formulations the approximate concentration of each shall be stated.

NOTE 2 On a fenoprop content of 200 g/l the maximum permitted extractable acid content would be 200 x 1.1 and 200 x 1.25 = 470 g/l.

NOTE 3 If the buyer requires both g/l at 20 C and g/kg then in cases of dispute the analytical results shall be calculated as g/kg. s

NOTE 4 Interim limit which will be reviewed when collaborative work is complete on determination of free phenols.

NOTE 5 The content of free phenols is limited to avoid possible taint of neighbouring crops and foodstuffs.

NOTE 6 On a content of 200 g/kg fenoprop and 200 g/kg mecoprop the maximum permitted free phenol content would be 6 g/kg of the product.

NOTE 7 A test temperature of 0 C may not be suitable for products intended for use in cold countries, and alternative test temperatures may be specified.

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* The analytical method for determination of the relevant impurity is available from the Pesticide Management Group of the FAO Plant Protection Service, or can be downloaded here

EDTA disodium salt solution ($C_{10}H_{14}O_8N_2Na_2 \cdot 2H_2O$) Dissolve EDTA disodium salt (37 g) and sodium hydroxide (9 g) in water and make up to 100 ml with water.

Butanone ($CH_3CH_2COCH_3$) ethyl methyl ketone

Butan-1-ol $\{CH_3[CH_2]_2CH_2OH\}$

Diethylamine $\{(CH_3CH_2)_2NH\}$

Eluant butanone + butan-1-ol + diethylamine + ammonia solution (6:2:1:1 by volume)

APPARATUS

TLC plate pre-coated with 0.25 mm silica gel

PROCEDURE

To 0.5 g of sample add methanol (10 ml) and EDTA solution (20 ml) and shake thoroughly. Make up with methanol + water (1:1) to 100 ml. Prepare solutions of the standards in the same way (solutions cannot be stored and must be freshly prepared).

Apply 10 µl of the test and standard solutions to the plate, place in a tank containing the eluant and allow to run for 15 min. Develop by spraying with copper(II) sulphate + ammonia solution. The dithiocarbamates are visualized as brown spots.

R_f values	anion of zineb	0.3
	anion of propineb	0.4
	anion of ziram	0.6

MT 155 ANALYTICAL HPLC METHOD FOR DETERMINATION OF PHENOLIC IMPURITIES IN PHENOXYALKANOIC HERBICIDES

155.1 Ultraviolet Detector Method

SCOPE

The method is used to determine phenolic impurities in technical and formulated products containing dichlorprop and mecoprop (acid or salt), but can be used for other phenoxyalkanoic herbicides.

OUTLINE OF METHOD

The phenolic impurities are separated on a reverse phase (µBondapak C18) column using methanol/acetate buffer solution 45 + 55 as mobile phase. Detection is performed by ultraviolet absorption at 280 nm. The determination is carried out

by an external standard technique. The identification of the chlorinated phenols is verified by separating samples and spiked samples with eluant(s) of varying pHs.

REAGENTS

Methanol (CH_3OH) HPLC grade

Acetic acid (CH_3COOH) glacial

Sodium acetate ($CH_3COONa{\cdot}3H_2O$)

Sodium hydroxide (NaOH) 1 mol/l (1N)

Water deionized and filtered through a 0.45 μm membrane

Dichlorprop standard of known purity, better than 99%

2-(2,4-dibromophenoxy)propionic acid reference standard (for relative retention times)

2-Chlorophenol standard of known purity

4-Chlorophenol standard of known purity

2,6-Dichlorophenol standard of known purity

2,4-Dichlorophenol standard of known purity

2,4,6-Trichlorophenol standard of known purity

o-Cresol (2-methylphenol) standard of known purity

4-Chloro-o-cresol (4-chloro-2-methylphenol) standard of known purity

6-Chloro-o-cresol (2-chloro-6-methylphenol) standard of known purity

4,6-Dichloro-o-cresol (2,4-dichloro-6-methylphenol) standard of known purity

Buffer solution Dissolve 1.36 g sodium acetate trihydrate in 2 l water and adjust the pH with glacial acetic acid to 2.75 for dichlorprop and 3.50 for mecoprop (Note 1).

Eluant Methanol/acetate buffer solution, 45+55 v/v. Mix 0.9 l methanol and 1.1 l buffer solution, cool, and degas by filtration through a 0.45 μm filter under moderate vacuum. The apparent pH of the eluant should be 3.40 for dichlorprop and 4.17 for mecoprop. (Note 1)

Mixed calibration solution of phenolic impurities (for dichlorprop and other chlorophenoxy herbicides) Weigh (to the nearest 0.1 mg) about 15 mg 2-chlorophenol standard, about 22 mg 4-chlorophenol standard, about 25 mg 2,6-dichlorophenol standard, about 40 mg 2,4-dichlorophenol standard and about 90 mg 2,4,6-trichlorophenol standard into a 50 ml volumetric flask and dissolve in 25 ml methanol. Add 1 mol/l sodium hydroxide, to apparent pH 11 and make up to volume with water and mix. Stock solutions should be prepared *in duplicate*. Dilute an aliquot of the stock solution 100 times with the eluant to give a working solution having acceptable peak heights at the highest usable sensitivity of the detector.

Mixed calibration solution of phenolic impurities (for mecoprop and other chloromethylphenoxy herbicides) Weigh (to the nearest 0.1 mg) about 20 mg *o*-cresol standard, about 35 mg 6-chloro-*o*-cresol standard, about

40 mg 4-chloro-*o*-cresol standard, and about 85 mg 4,6-dichloro-*o*-cresol standard into a 50 ml volumetric flask and dissolve in 25 ml methanol. Add 1 mol/l sodium hydroxide, to give apparent pH 11 and make up to volume with water and mix. Stock solutions should be prepared *in duplicate*. Dilute with eluant about a 100 times to give acceptable peak heights at the highest usable sensitivity of the detector.

Check linearity in the concentration range used.

Before use, check that the phenoxyalkanoic herbicide standards, the internal standard and the chlorinated phenols do not contain impurities that will interfere with the chromatographic separation given in this method.

APPARATUS

Liquid chromatograph. Waters Associates pump model 6000A, injector model 710A WISP and UV detector model 440 (280 nm) or equivalent. 10 mV recorder or electronic integrator. A column jacket giving temperature control at 22°C is recommended.

Liquid chromatographic column. Stainless steel 300 mm × 3.9 mm (i.d.) containing 10 μm particles of silica gel with chemically-bonded octadecyl groups. (C18). This material should have a minimal number of theoretical plates of 3000. (Waters Associates, μBondapak C18 or equivalent). A guard column containing the same C18 silica gel packing, or a coarser grade is recommended.

Solvent clarification and degassing. An all-glass filter apparatus with suitable PTFE disc filters of 0.45 μm pore size.

pH-meter and suitable electrodes

PROCEDURE

(a) *HPLC Operating conditions.*
Eluant flow rate 2 ml/min
Detector wavelength 280 nm
Detector sensitivity 0.005 absorbance unit full scale
Temperature 22°C
Injection volume 25 μl

(b) *Preparation of sample.*

Weigh to the nearest 0.1 mg sufficient sample (*w* mg) to contain about 0.33 g dichlorprop into a 50 ml volumetric flask and dissolve in 10 ml methanol. Make up to volume with eluant and mix to give a *sample stock solution*. Sample stock solutions are prepared *in duplicate*. Prepare sample stock solutions spiked with

phenolic impurities e.g. 5 ml sample stock solution + 25 µl mixed calibration solution of phenolic impurities.

(c) *System suitability test.*

To ensure proper resolution of peaks, a test solution containing dichlorprop, probable phenolic and phenoxy acid impurities and the internal standard is injected and the relative retention times compared with those given in Table 00. As a guideline, procedure and concentration corresponding to the calibration solution in the HPLC method for determination of dichlorprop, CIPAC 1C, p. 2088, and of mecoprop, p. 2158, could be used for preparing a suitable test solution. All components of the test solution should be identified in the chromatogram (Note 2).

As the elution order of phenols relative to phenoxy acids depends on the pH of the eluant and possibly on the column material used, it is important to inject the mixed standard solution of phenolic impurities alternatively with the test solution and then compare the retention times.

(d) *Determination.*
Inject 25 µl of the mixed calibration solution of phenolic impurities, the sample stock solution, and the sample stock solutions spiked with phenolic impurities, according to the following sequence:

- - -, X_1, C_1, S_1, S_2, C_2 - - -

where:

X_1	=	S_1 spiked with phenolic impurities
C_1	=	standard solution of phenolic impurities, preparation 1
C_2	=	standard solution of phenolic impurities, preparation 2
S_1	=	sample solution from 1st sample stock solution
S_2	=	sample solution from 2nd sample stock solution

The solution should be clear before injection. If not, separate undissolved matter by centrifugation or filtration. Identify the peaks of phenolic impurities by comparing the chromatograms of the spiked and the unspiked sample solutions, and check the relative retention times.

If necessary, repeat the injection with smaller or larger injection volumes of sample or inject dilutions of sample solutions in the eluant to give acceptable peak heights of the phenolic impurities at the highest usable sensitivity of the detector.
If the sample contains impurities other than those used in the calibration solution, e.g. some of the phenoxy acids listed in Table 00, their peaks may interfere with

some peaks of interest. Proper resolution is likely to be achieved by changing the pH of the eluant. Generally, raising the pH gives relatively longer retention times for phenols with respect to phenoxy acids - and vice versa, see Table I.

Verify the identification of peaks of phenolic impurities must be verified by running the X_1 and S_1 solutions with eluting solvents of alternative pH value. Suitable alternative eluants could be selected from Table I.

The time of analysis for the 'X_1' injections should be about four times the retention time for dichlorprop, in order to detect any late-eluting impurities and to determine the time of analysis for the actual sample.

(e) *Calculation*

Measure the peak heights (to 0.1 mm).

For each determination, make the calculation on the basis of the standard injections preceding and following the one or two actual sample injections.

$$\text{Content of impurity} = \frac{H_s \cdot v \cdot p}{v_1 \cdot w \cdot F_{ave}} \quad \text{g/kg}$$

where:

H_s = peak height of the impurity for sample
v_1 = dilution volume for mixed standards (ml)
v = dilution volume for sample (ml)
w = mass of sample taken (mg)
p = purity of impurity standard (g/kg)
F_{ave} = average response factor

where:

F = the individual response factor = $\frac{H}{m}$
H = peak height of the impurity for mixed standard solution
m = phenolic impurity standard weighed in mixed calibration solution of phenolic impurities (mg)

Note 1 For other phenoxyalkanoic herbicides use Table I as guideline for the selection of suitable pH values.

Note 2 2,6-Dichlorophenol is probably poorly separated from 2-(2-chloro-phenoxy)propionic acid under the prescribed conditions. If necessary, it is possible to separate 2,6-dichlorophenol completely from other components in the test solution by using an eluant of pH 4.06, 4.64 or 6.14 (Table I).

TABLE I *Relative retention times for dichlorprop, mecoprop, probable phenolic and phenoxy acid impurities, six other phenoxy acid herbicides and the reference standard (2-(2,4-dibromophenoxy)propionic acid).*

pH of the eluant	3.40	4.06	4.17	4.64	4.93	5.40	6.14
pH of the buffer solution	2.75	3.35	3.50	3.90	4.10	4.70	5.40
Relative retention times							
Reference standard	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Approximate retention time (min)	23	20	18	14	13	12	9
Dichlorprop	0.70	0.68	0.68	0.69	0.70	0.69	0.71
Mecoprop	0.81	0.84	0.83	0.83	0.75	0.73	0.71
2-Chlorophenol	0.20	0.24	0.28	0.38	0.41	0.47	0.63
4-Chlorophenol	0.24	0.31	0.35	0.44	0.51	0.60	0.81
2,6-Dichlorophenol	0.31	0.40	0.44	0.56	0.67	0.78	1.00
2,4-Dichlorophenol	0.48	0.64	0.70	0.90	1.06	1.27	1.64
2,4,6-Trichlorophenol	0.90	1.22	1.32	1.71	2.00	2.42	2.76
o-Cresol	0.19	0.24	0.25	0.34	0.38	0.45	0.60
6-Chloro-*o*-cresol	0.35	0.45	0.48	0.63	0.69	0.86	1.11
4-Chloro-*o*-cresol	0.44	0.58	0.62	0.84	0.91	1.14	1.48
4,6-Dichloro-*o*-cresol	0.90	1.21	1.33	1.75	1.86	2.43	3.01
2-(2-Chlorophenoxy)propionic acid	0.31	0.30	0.30	0.31	0.32	0.31	0.35
2-(4-Chlorophenoxy)propionic acid	0.36	0.35	0.35	0.35	0.35	0.35	0.39
2-*o*-Tolyloxypropionic acid	0.37	0.38	0.39	0.41	0.38	0.35	0.37
2-(2,6-Dichlorophenoxy)propionic acid	0.43	0.47	0.48	0.45	0.43	0.37	0.39
2-(6-Chloro-*o*-tolyloxy)propionic acid	0.50	0.55	0.57	0.57	0.50	0.41	0.42
2-(2,4,6-Trichlorophenoxy)propionic acid	1.13	1.18	1.17	1.04	0.98	0.85	0.86
2-(4,6-Dichloro-*o*-tolyloxy)propionic acid	1.30	1.43	1.42	1.36	1.15	1.03	0.94
2,4-D	0.40	0.39	0.38	0.42	0.44	0.46	0.49
MCPA	0.47	0.47	0.46	0.49	0.49	0.47	0.49
2,4,5-T	0.72	0.70	0.70	0.74	0.77	0.82	0.85
Fenoprop	1.33	1.29	1.30	1.28	1.30	1.34	1.33
2,4-DB	1.28	1.73	2.00	2.47	2.63	2.59	1.78
MCPB	1.35	1.81	2.12	2.62	2.81	2.77	1.91

The eluants are all mixtures of methanol and acetate buffer solution 45 + 45 v/v.

In the table are given the pH values to which the sodium acetate solutions have to be adjusted with glacial acetic acid to give the apparent pH values of eluant listed in the table.

155.2 **Electrochemical Detector Method**

SCOPE

The method is used only for determining phenolic impurities in technical and formulated products containing the ionic salts of 2,4-D, MCPA, dichlorprop and mecoprop, but can also be used for other herbicides.

The active ingredient(s) can also be determined by using a UV-detector.

OUTLINE OF METHOD

The phenolic impurities (and the active ingredients) are separated on a reverse phase column using methanol/acetonitrile/buffer as mobile phase. Phenolic impurities are detected with an electrochemical detector at +0.9 V. Determination is carried out by using 4-bromo-2-chlorophenol as internal standard. Identification of the impurities is confirmed by spiking, and/or analyzing samples and spiked samples with eluant(s) of alternative pH value(s) (See CIPAC 1C pp. 2091 and 2160). Determination of active ingredients can also be done by UV-detection at 280 nm using 2-(2,4-dibromophenoxy)propionic acid as internal standard.

REAGENTS

Methanol (CH_3OH) HPLC grade
Acetonitrile (CH_3CN) HPLC grade
Sodium hydroxide (NaOH) 1 mol/l (1N)
- 0.1 mol/l (0.1N)
Citric acid, monohydrate ($C_6H_9O_7 \cdot H_2O$)
Disodium hydrogenphosphate dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$)
Water de-ionized, filtered through a 0.45 µm membrane
o-Cresol standard of known purity
2-Chlorophenol standard of known purity
4-Chlorophenol standard of known purity
2,6-Dichlorophenol standard of known purity
6-Chloro-o-cresol standard of known purity
4-Chloro-o-cresol standard of known purity
2,4-Dichlorophenol standard of known purity
4,6-*Dichloro-o-cresol* standard of known purity
2,4,6-Trichlorophenol standard of known purity
4-Bromo-2-chlorophenol (internal standard for phenolic impurities)

2,4-D standard of known purity
MCPA standard of known purity
Dichlorprop standard of known purity
Mecoprop standard of known purity
2-(2,4-Dibromophenoxy)propionic acid (internal standard for active ingredients)

Buffer solution. Dissolve 21.01 g citric acid monohydrate in 1000 ml water (giving a 0.1 mol/l citric acid solution) and in a separate bottle 17.9 g of disodium hydrogenphosphate dodecahydrate in 250 ml water (giving a 0.2 mol/l solution). Mix 1000 ml citric acid solution with 140 ml disodium hydrogenphosphate solution, to give a buffer solution of pH 2.70.

Eluant. Methanol/acetonitrile/buffer solution, 20+20+60 v/v. Mix 900 ml buffer with 300 ml methanol and 300 ml acetonitrile, cool and de-gas by filtration through a 0.45 µm membrane under moderate vacuum. The apparent pH of the eluant should be 3.40.

Phenolic impurities internal standard solution. Weigh (to the nearest 0.1 mg) 50.0 mg 4-bromo-2-chlorophenol and transfer to a 1000 ml volumetric flask. Dissolve in 1 mol/l sodium hydroxide (40 ml), make up to volume with water and mix.

Active ingredients internal standard solution (Note 1). Weigh (to the nearest mg) 2.4 g of 2-(2,4-dibromophenoxy)propionic acid and transfer to a 1000 ml volumetric flask. Dissolve in 1 mol/l sodium hydroxide (40 ml), make up to volume with water and mix.

Stock standard solution. The stock standard solutions are prepared by weighing into separate 50 ml volumetric flasks the amount for each impurity as given in Table II. Dissolve and make up to volume with 0.1 mol/l sodium hydroxide.

TABLE II

Impurity	mass (mg)
o-cresol	49
2-chlorophenol	114
4-chlorophenol	198
2,6-dichlorophenol	109
6-chloro-*o*-cresol	75
4-chloro-*o*-cresol	98
2,4-dichlorophenol	164
4,6-dichloro-*o*-cresol	103
2,4,6-trichlorophenol	111

Stock mixed standard solutions. As 4,6-dichloro-*o*-cresol and 2,4,6-trichlorophenol will elute at the same time by this procedure, it is necessary to make two separate stock mixed standard solutions (I and II) and have the response factor for each. Prepare the two stock mixed standard solutions by transferring by pipette the volumes given in Table III from the stock standard solutions into two 100 ml volumetric flasks, make each up to volume with water and shake.

TABLE III

	Volume of Mixed Standard I per 100 ml	Volume of Mixed Standard II per 100 ml
o-cresol	1.0	1.0
2-chlorophenol	1.0	1.0
4-chlorophenol	1.0	1.0
2,6-dichlorophenol	1.0	1.0
6-chloro-*o*-cresol	1.0	1.0
4-chloro-*o*-cresol	1.0	1.0
2,4-dichlorophenol	1.0	1.0
4,6-dichloro-*o*-cresol	-	1.0
2,4,6-trichlorophenol	1.0	-

Calibration solutions. Transfer by pipette 1.00 ml of stock mixed standard solution I into a 100 ml volumetric flask, add 1.00 ml of phenolic impurities internal standard solution (4-bromo-2-chlorophenol) and dilute to the mark with eluant. This gives calibration solution I. Dilute stock mixed standard solution II in the same manner to give calibration solution II. The calibration solutions should be made in duplicate to give solutions IA, IB and IIA, IIB. Solution I is used for chlorophenoxy herbicides samples (e.g. dichlorprop), and solution II for methylchlorophenoxy herbicides samples (e.g. mecoprop). For mixed samples the mean response factor for 2,4,6-trichlorophenol and 4,6-dichloro-*o*-cresol should be used.

Calibration solution with active ingredients. (Note 1) Weigh (to the nearest 0.1 mg) into a 50 ml volumetric flask the amounts of standard of the relevant active ingredients as given in Table IV, dissolve and make up to the volume with 0.1 mol/l sodium hydroxide.

The calibration solution of active ingredient is prepared by transferring with a pipette the volume of each active ingredient listed in Table IV into a 100 ml volumetric flask together with 20 ml of the active ingredients internal standard solution, mixing, and making up to volume with eluant.

TABLE IV

Active ingredient	Mass (mg) taken for 50 ml of solution	Volume of solution (ml) to be diluted with eluant
2,4-D	144	6
MCPA	165	6
dichlorprop	140	8
mecoprop	150	10
Internal standard 2-(2,4-dibromophenoxy)propionic acid		20

APPARATUS

Liquid chromatograph. Waters Associates pump model 6000A, injector model 710A WISP, and UV detector model 440 (280 nm) BAS EC-detector model LC4B (+0.9V) or equivalent. Waters datamodule model 730 or an equivalent 2 pen recorder, or a 2-channel integrator e.g. Hitachi model D-2000. A column jacket giving temperature control at 25°C is recommended. The UV-detector and the EC-detector are connected in series, with the UV-detector first. (Note 2)

Liquid chromatographic column. (Note 3) Stainless steel 150 mm × 3.9 mm i.d., 4 µm diameter particles of chemically bonded octadecyl groups on silica gel (C18), (minimum number of 3000 plates). (Waters Associates, Novapak C18 or equivalent). A guard column containing the same C18 silica gel packing, or a coarser grade, is recommended.

Solvent clarification and degassing.

An all glass filter apparatus with suitable PTFE disc filters of 0.45 µm pore size.

pH-meter and suitable electrodes

PROCEDURE

(a) *HPLC operating conditions.*

Eluant flow rate	1 ml/min	
Injection volume	25 µl	
Temperature	25°C	
Detector sensitivity.	UV:	0.1 absorbance unit full scale (Note 2)
	EC:	50 nA full scale for 1 V output signal or 5 nA full scale for 10 mV output signal +0.9 V
Detector wavelength	280 nm (Note 2)	

(b) *System suitability test.*

To ensure proper resolution of peaks, the calibration standards of impurities (and, if wanted, the active ingredients) are injected and compared with the relative retention times given in Table V. The relative retention times for the phenolic components are based on 4-bromo-2-chlorophenol as internal standard. The relative retention times for active ingredients are based on 2-(2,4-dibromophenoxy)propionic acid. Compare the chromatogram with the attached chromatogram of the calibration standard (Fig. 46).

TABLE V *Relative Retention Times*

Component	EC-detector	UV-detector
o-cresol	0.33	
2-chlorophenol	0.35	
4-chlorophenol	0.41	
2,6-dichlorophenol	0.56	
6-chloro-*o*-cresol	0.62	
4-chloro-*o*-cresol	0.74	
2,4-dichlorophenol	0.83	
4-bromo-2-chlorophenol (int.std.)	1.00 (14 min)	
4,6-dichloro-*o*-cresol	1.59	
2,4,6-trichlorophenol	1.59	
2,4-D	0.65	0.39
MCPA	0.72	0.43
dichlorprop	1.19	0.68
mecoprop	1.27	0.72
2-(2,4-dibromophenoxy)propionic acid (int.std.)		1.00 (24.6 min)

(c) *Preparation of sample.*

Weigh (to the nearest 0.1 mg) sufficient sample (*w* mg) to contain about 0.75 g active ingredient into a 100 ml volumetric flask (giving peak height/areas, after dilution 5-10 times, similar to those of the calibration solution of the active ingredients; Note 1). Dissolve in 40 ml methanol and make up to volume with eluant and mix. Samples are prepared in duplicate.
Make at least two dilutions of each sample.

Dilution A Transfer by pipette 2.00 ml of the sample and 1.0 ml of the phenolic impurities internal standard solution (4-bromo-2-chlorophenol) into a 100 ml volumetric flask, mix and make up to the volume with the eluant. This dilution should give measurable peak heights of the *major phenolic impurities.*

Dilution B Transfer by pipette 20.00 ml of the sample, 1.0 ml of phenolic impurities standard solution (4-bromo-2-chlorophenol) and 20.00 ml of the active ingredients internal standard solution (2-(2,4-dibromophenoxy)propionic acid), into a 100 ml volumetric flask, mix and make up to volume with the eluant. This dilution should give measurable peak heights for the minor phenolic impurities and for the active ingredients (Note 1). If not, continue to make dilutions until all the main phenolic impurities give measurable peak heights.

For each dilution it is necessary to confirm the identity of the phenolic compounds by spiking the solution. This is done by mixing 1.00 ml sample, 0.5 ml stock mixed phenolic standard solution and 0.5 ml 4-bromo-2-chlorophenol in a 50 ml volumetric flask, and then diluting to volume with eluant.
Identify the peaks of phenolic impurities by comparing the chromatograms of the spiked and unspiked sample solutions and by checking the relative retention times. (Table V). If the sample contains impurities other than those expected, their peaks in the chromatogram may interfere with peaks of interest. Table VI shows the relative retention times of other possible impurities.

(d) *Determination*

Inject alternately 25 µl of the two calibration solutions until the response factors (*F*) for two successive injections agree within ±1.0% of their mean.
Without changing the conditions, inject 25 µl of the calibration solution, the diluted sample solutions, and of the diluted sample solutions spiked with phenolic impurities according to the following sequence:

--- X, IA, $S_{1(1)}$, IB, $S_{1(2)}$, S_1S, IA, $S_{2(1)}$, IB, $S_{2(2)}$ ---

X = sample without internal standards
IA = first calibration solution I.
$S_{1(1)}$ = sample with internal standard for major phenolic impurities, preparation 1
$S_{1(2)}$ = as S_1, preparation 2
IB = second calibration solution I.
S_1S = S_1 spiked with phenolic standard.
$S_{2(1)}$ = sample with both internal standards and diluted for minor phenolic impurities and active ingredients, preparation 1. (Note 1)
$S_{2(2)}$ = as $S_{2(1)}$, preparation 2.

(e) *Calculation*

Measure the peak heights for the phenolic impurities. Measure the peak areas or heights for the active ingredient(s).

For each determination, calculate the phenolic impurities on the basis of the standard injections, preceding and following sample injections, as follows:

$$\text{Content of phenolic impurity} = \frac{R \cdot P \cdot v}{F_{ave} \cdot w} \text{ g/kg}$$

where:

R = peak height ratio of impurity and internal standard for the sample solution.
w = mass of sample (mg)
v = dilution volume for the sample (ml)
F_{ave} = average response factor
P = purity of standard (g/kg)

where the individual response factor :

$$F = \frac{R_1}{s}$$

R_1 = peak height ratio of impurity and internal standard for the calibration solution.
s = concentration of the impurity in the calibration solution (mg/ml)

Note 1 Preparation and addition of the active ingredients internal standard solution is only necessary if the determination of active ingredient is wanted.
Note 2 The UV-detector is only needed for active ingredient determination.
Note 3 The Novapak packing is recommended, because the operating conditions are optimized with respect to this column material and even slightly different materials may provide poorer separation of some of the peaks.

TABLE VI *Relative retention times of possible impurities*

Component	RRT [1] EC	RRT [2] UV	RRT [3] UV
3,6-dichloropyridine-2-carboxylic acid		0.14	0.08
2,3,6-TBA		0.21	0.12
phenol	0.23		
dicamba		0.29	0.17
m-cresol	0.31		
p-cresol	0.31		
(2-chlorophenoxy)acetic acid		0.33	0.19
cyanazin		0.33	0.19
o-cresol	0.33		
2-chlorophenol	0.35		
o-tolyloxyacetic acid		0.36	0.21
(4-chlorophenoxy)acetic acid		0.38	0.22
(2,6-dichlorophenoxy)acetic acid		0.41	0.24
2-bromophenol	0.40		
4-chlorophenol	0.41		
3,4-xylenol	0.45		
3-chlorophenol	0.43		
6-chloro-*o*-tolyloxyacetic acid	0.46	0.27	
2,3-xylenol	0.48		
3,5-xylenol	0.46		
2,6-xylenol	0.49		
2,4-xylenol	0.50		
2,5-xylenol	0.52		
2-(2-chlorophenoxy)propionic acid		0.50	0.29
6-chloro-*m*-cresol	0.56		
2-(4-chlorophenoxy)propionic acid		0.55	0.32
2,6-dichlorophenol	0.56		
2-(3-chlorophenoxy)propionic acid		0.57	0.33
2-*o*-tolyloxypropionic acid		0.57	0.33

TABLE VI, *continued*

Component	RRT [1] EC	RRT [2] UV	RRT [3] UV
6-chloro-*o*-cresol	0.62		
4-chloro-*m*-tolyloxyacetic acid		0.64	0.37
2-(2,6-dichlorophenoxy)propionic acid		0.65	0.38
4-chloro-*m*-cresol	0.66		
2,4-D		0.67	0.39
4-chloro-*o*-cresol	0.74		
MCPA		0.74	0.43
2-(6-chloro-*o*-tolyloxy)propionic acid		0.74	0.43
5-chloro-*o*-cresol	0.75		
4-(2-chlorophenoxy)butyric acid		0.76	0.44
3-chloro-*o*-cresol	0.80		
2,4-dichlorophenol	0.83		
6-chloro-2,4-xylyloxyacetic acid		0.88	0.51
4-*o*-tolyloxybutyric acid		0.88	0.51
2-(4-chloro-*m*-tolyloxy)propionic acid		0.96	0.56
(2,4,6-trichlorophenoxy)acetic acid		0.98	0.57
4-chloro-2,6-xylyloxyacetic acid		1.00	0.58
4-bromo-2-chlorophenol (int. std.)	1.00		
4-(4-chlorophenoxy)butyric acid		1.02	0.59
4-chloro-3,5-xylenol	1.11		
4,6-dichloro-*o*-tolyloxyacetic acid		1.12	0.65
4-chloro-2,6-xylenol	1.19		
dichlorprop		1.17	0.68
4-(6-chloro-*o*-tolyloxy)butyric acid		1.17	0.68
2,4-dibromophenol	1.23		
4-(2,6-dichlorophenoxy)butyric acid		1.22	0.71
2,4,5-T		1.22	0.71
2,3,6-trichlorophenol	1.24		
3-(2,4-dichlorophenoxy)propionic acid		1.22	0.71

TABLE VI, *continued*

Component	RRT [1] EC	RRT [2] UV	RRT [3] UV
mecoprop		1.24	0.72
2-(6-chloro-2,4-xylyloxy)propionic acid		1.46	0.85
3,5-dichlorophenol	1.58		
4,6-dichloro-*o*-cresol	1.59		
2,4,6-trichlorophenol	1.59		
2,3,4-trichlorophenol	1.60		
2-(4-chloro-2,6-xylyloxy)propionic acid		1.65	0.96
2-(2,4-dibromophenoxy)propionic acid (int. std.)		1.72	1.00
2-(2,4,6-trichlorophenoxy)propionic acid		1.72	1.00
2-(4,6-dichloro-*o*-tolyloxy)propionic acid		2.01	1.17
2,4-DB		2.03	1.18
2,3,5-trichlorophenol	1.77		
2,4,5-trichlorophenol	1.88		
MCPB		2.12	1.23
fenoprop		2.20	1.28
3,4,5-trichlorophenol	2.18		
2,3,4,6-tetrachlorophenol	3.27		
2,3,5,6-tetrachlorophenol	3.50		
4-(4,6-dichloro-*o*-tolyloxy)butyric acid		3.39	1.97
2,3,4,5-tetrachlorophenol	3.46		
pentachlorophenol	7.57		

1): Approximate relative retention times of the phenolic impurities to 4-bromo-2-chlorophenol at the EC-detector. The average retention time of 4-bromo-2-chlorophenol is 12.59 min.

2)+3): Approximate relative retention times of the phenoxy acid herbicides and other herbicides 2) to 4-bromo-2-chlorophenol and 3) to 2-(2,4-dibromophenoxy)propionic acid, determined by the UV-detector. The average retention time of 2-(2,4-dibromophenoxy)propionic acid is 21.67 min.

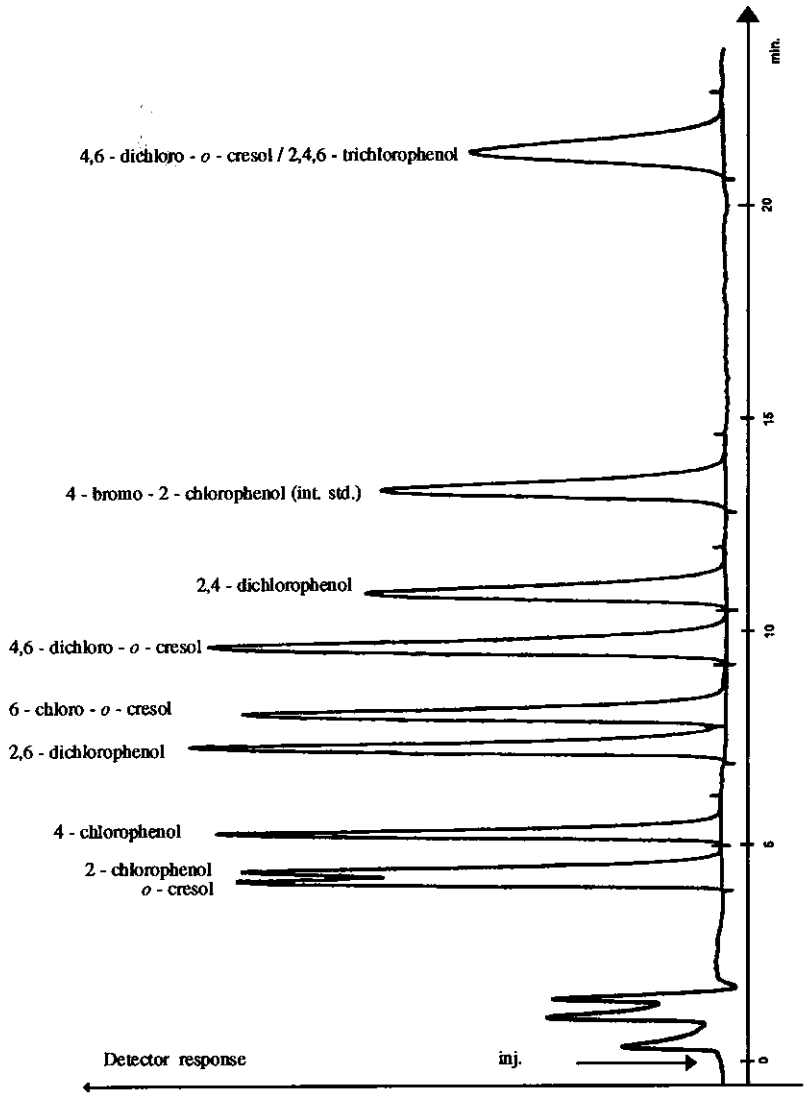

Figure 45 Chromatogram of calibration standard.