Chlorophenoxycarboxylic acids (4-Chloro-2-methylphenoxy-acetic acid; 2,4-Dichlorophenoxyacetic acid; 4-Chloro-2-methylphenoxy-propionic acid; 2,4-Dichlorophenoxypropionic acid)

Application	Determination in urine
Analytical principle	Capillary gas chromatography/mass selective detection (MSD)
Completed in	January 1995

Summary

The capillary gas chromatographic method described here for the determination of chlorophenoxycarboxylic acids in urine is suitable for investigating the exposure of people who come into contact with chlorophenoxycarboxylic acids at their workplace.

Hydrochloric acid and the internal standard 4-chloro-2-methylbutyric acid are added to the urine. The mixture is drawn into a previously conditioned C18 cartridge in which the chloro-phenoxycarboxylic acids are enriched. After elution they are converted to their derivatives with sulphuric acid/methanol. Calibration is carried out using aqueous standard solutions which are processed in the same way as the urine samples and are determined by gas chromatography and subsequent mass selective detection. The peak areas of the chlorophenoxycarboxylic acids obtained are divided by the peak areas of the internal standard. The resulting quotients are plotted as a function of the concentration of the chlorophenoxycarboxylic acids to obtain a calibration curve.

4-Chloro-2-methylphenoxyacetic acid (MCPA)

Within-series imprecision:	Standard deviation (rel.)	s_w = 2.6 %
	Prognostic range	u = 6 %
	At a concentration of 31.7 µg 4-chloro-2-methylphenoxyacetic acid per litre urine and where n = 8 determinations	
Between-day imprecision:	Standard deviation (rel.)	s = 3.6–6.9 %
	Prognostic range	u = 8.3–15.9 %
	At concentrations of 4-chloro-2-methylphenoxyacetic acid between 9.9 and 35.5 µg per litre urine and where n = 8 days	
Inaccuracy:	Recovery rate	r = 95 %
Detection limit:	10 µg 4-chloro-2-methylphenoxyacetic acid per litre urine	

2,4-Dichlorophenoxyacetic acid (2,4-D)

Within-series imprecision:	Standard deviation (rel.)	s_w = 2.4 %
	Prognostic range	u = 5.5 %
	At a concentration of 39.1 µg 2,4-dichlorophenoxyacetic acid per litre urine and where n = determinations	
Between-day imprecision:	Standard deviation (rel.)	s = 4.8–11.3 %
	Prognostic range	u = 11–26.2 %
	At concentrations of 2,4-dichlorophenoxyacetic acid between 5.4 and 20.7 µg per litre urine and where n = 8 days	
Inaccuracy:	Recovery rate	r = 93 %
Detection limit:	10 µg 2,4-dichlorophenoxyacetic acid per litre urine	

4-Chloro-2-methylphenoxypropionic acid

Within-series imprecision:	Standard deviation (rel.)	s_w = 3.4 %
	Prognostic range	u = 7.8 %
	At a concentration of 40.6 µg 4-chloro-2-methylphenoxypropionic acid per litre urine and where n = 8 determinations	

Between-day imprecision:	Standard deviation (rel.)	s = 4.5–9.9 %
	Prognostic range	u = 10.4–22.8 %
	At concentrations of 4-chloro-2-methylphenoxypropionic acid between 10 and 35.7 µg per litre urine and where n = 8 days	
Inaccuracy:	Recovery rate	r = 91 %
Detection limit:	10 µg 4-chloro-2-methylphenoxypropionic acid per litre urine	

2,4-Dichlorophenoxypropionic acid

Within-series imprecision:	Standard deviation (rel.)	s_w = 4.2 %
	Prognostic range	u = 9.7 %
	At a concentration of 33.5 µg 2,4-dichlorophenoxypropionic acid per litre urine and where n = 8 determinations	
Between-day imprecision:	Standard deviation (rel.)	s = 7.3–9.9 %
	Prognostic range	u = 16.8–22.8 %
	At concentrations of 2,4-dichlorophenoxypropionic acid between 17.4 and 63.3 µg per litre urine and where n = 8 days	
Inaccuracy:	Recovery rate	r = 90 %
Detection limit:	10 µg 2,4-dichlorophenoxypropionic acid per litre urine	

Chlorophenoxycarboxylic acids

Chlorophenoxycarboxylic acids are widely used as selective herbicides in the cultivation of cereals. Moreover, chlorophenoxycarboxylic acids are frequently used as total herbicides in combination with amitrole and triazines on paths and local areas.

Although the chlorophenoxycarboxylic acids are in common use and individual derivatives, such as MCPA, dichlorprop, mecoprop are increasingly employed, the most frequently used derivative of the chlorophenoxycarboxylic acids is still 2,4-dichlorophenoxyacetic acid (2,4-D).

Figure 1 shows the most important chemicals in this group.

According to United Nations reports 2,4-D was produced by 48 companies in 15 countries and 231 commercially available products contain it. As a rule 2,4-D is synthesized from 2,4-dichlorophenol with monochloroacetic acid in a strongly alkaline medium. On account of its ready solubility in water 2,4-D is rarely used in its pure state.

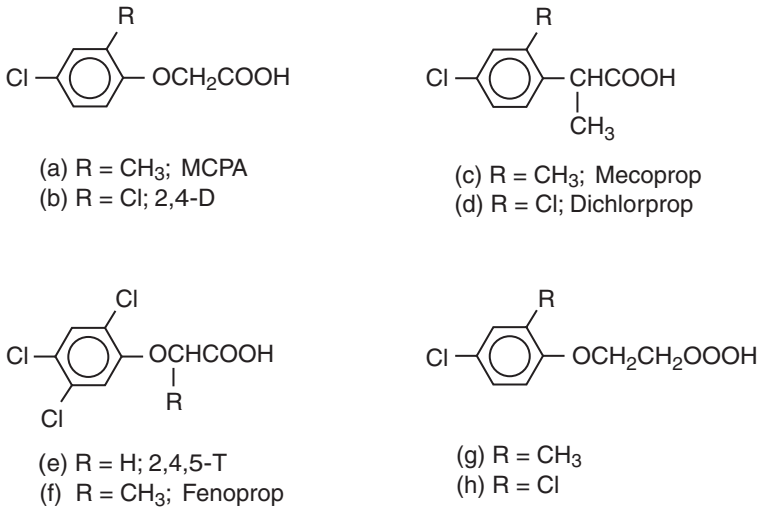

Figure 1. Chlorophenoxycarboxylic acid herbicides ((a) 4-chloro-2-methylphenoxyacetic acid; (b) 2,4-dichlorophenoxyacetic acid; (c) 2'(4-chloro-2-methylphenoxy)propionic acid; (d) 2'(2,4-dichlorophenoxy)propionic acid; (e) 2,4,5-trichlorophenoxyacetic acid; (f) 2'(2,4,5-trichlorophenoxy)propionic acid; 4-chloro-2-methylphenoxypropionic acid; 2,4-dichlorophenoxypropionic acid)

It is frequently used as an alkaline salt, ammonium salt or ester [1]. These herbicides are contaminated by mono and tetrachlorobiphenyls as well as di-, tri- and tetrachlorodibenzo-p-dioxins, but not 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) [2, 3]. In older samples of the dimethylammonium salt up to 2.8 ppm of N-nitrosodimethylamine could be detected in some cases [4, 5].

Several review articles give information about the toxicity of 2,4-D and its compounds [6, 7].

Doses of 2,4-D exceeding 5 mg per kg body weight administered to rats caused histological changes to their kidneys. At doses exceeding 15 mg per kg body weight temporary changes in the blood parameters, such as a diminished erythrocyte count and lowered haemoglobin and haematocrit were observed [6].

Intake of 2,4-D can occur by inhalation [8], as a result of oral administration [9–12] and by absorption of the compound through the intact skin [10, 13–15]. In humans 2,4-D is mainly excreted unchanged in urine via the kidneys. A small proportion of conjugates could be detected (12.8 %) in only a few cases. Five people, who each volunteered to take an oral dose of 5 mg per kg body weight, excreted up to 100 % of the 2,4-D amount they had taken within 87 days [12]. In contrast, after dermal intake of 2,4-D excretion was determined over a longer period of time. This can be explained by the fact that the skin serves as a storage medium until final absorption of 2,4-D.

Several studies describe the effect of exposure on workers who were engaged in the production and packing of 2,4-D herbicides. The concentrations measured in air fluctuated from below the detection limit to 93 mg/m^3 [8]. Within the framework of a recent investigation air concentrations of 2,4-D up to 0.5 mg/m^3 were determined, whereby the high exposure correlated well with the excretion of 2,4-D in urine [16].

Further studies reported on the effect of exposure to this substance on workers as a result of agricultural application (cf. Table 1).

Table 1. Inner stress of workers as a result of the application of 2,4-D and its derivatives

Product used	No. of Persons	Type of use	Concentration of 2,4-D in urine [mg/L]	References
2,4-D and dicamba (3,6-dichloro-2-methoxybenzoic acid) in aqueous solution	2	spraying once	1–4	[17]
	2	spraying several times	3–20	
2,4-D/2,4,5-T-butoxyethyl ester as 2 % emulsion in water	4	spraying	1–14	[18, 19]
2,4-D/2,4-DP and 2,4-D/pichloram	23	working at the roadside	< 0.01–8	[20]

In a series of further studies the relationship between the exposure of farm workers to 2,4-D and other plant protection products and the incidence of non-Hodgkin's lymphomas was investigated. Due to difficulties in determining the composition of the exposure mixture, especially the contaminants in the herbicides (dioxins), these studies give only an indication of a possible connection between exposure to 2,4-D herbicides and the incidence of non-Hodgkin's lymphomas [21–25].

A further study, in which 578 people suffering from leukaemia and 1245 control persons participated, indicated a slightly, but not significantly, increased risk of contracting leukaemia for 98 persons who had been exposed to 2,4-D in agriculture [26].

In 1994 the Deutsche Forschungsgemeinschafts Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area stipulated a MAK value of 1 mg/m^3 for 2,4-D including its salts and esters [6]. An "H" in the relevant column of the list of MAK and BAT values indicates the risk of absorption through the skin.

Moreover, the Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area has assigned 2,4-D to pregnancy risk "C" in the list of MAK values. This means that there is no reason to fear a risk of damage to the developing embryo or foetus when the MAK value is adhered to [6].

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Chlorophenoxycarboxylic acids (4-Chloro-2-methylphenoxy-acetic acid; 2,4-Dichlorophenoxyacetic acid; 4-Chloro-2-methylphenoxy-propionic acid; 2,4-Dichlorophenoxypropionic acid)

Application	Determination in urine
Analytical principle	Capillary gas chromatography/mass selective detection (MSD)
Completed in	January 1995

Contents

1 General principles

Hydrochloric acid and the internal standard 4-chloro-2-methylbutyric acid are added to the urine. The mixture is drawn into a previously conditioned C18 cartridge in which the chlorophenoxycarboxylic acids are enriched. After elution they are converted to their derivatives with sulphuric acid/methanol. Calibration is carried out using aqueous standard solutions which are processed in the same way as the urine samples and are determined by gas chromatography and subsequent mass selective detection. The peak areas of the chlorophenoxycarboxylic acids obtained are divided by the peak areas of the internal standard. The resulting quotients are plotted as a function of the concentration of the chlorophenoxycarboxylic acids to obtain a calibration curve.

2 Equipment, chemicals and solutions

2.1 Equipment

Gas chromatograph with split/splitless injector, mass selective detector (MSD) and compensation recorder or integrator

Gas chromatographic column:
Length 30 m, inner diameter 0.25 mm; stationary phase DB-WAX, film thickness 0.25 µm (e.g. from J & W Scientific)

5 µl syringe for gas chromatography, preferably an autosampler

3 mL C18 cartridges (e.g. Bakerbond spc, from Baker)

Sampler vials (approx. 1.5 mL) with crimp caps and crimping tongs

10 mL volumetric flask with a ground glass stopper

1, 2, 5 and 10 mL pipettes

Microlitre pipettes, adjustable between 100 and 1000 µL (e.g. from Eppendorf)

20 mL crimp-top vials with PTFE-coated crimp caps and crimping tongs

100 mL volumetric flasks

Apparatus for evaporation in a flow of nitrogen

Work station for vacuum extraction (e.g. from Baker)

2.2 Chemicals

96 % Sulphuric acid (Suprapur, e.g. from Merck)

37 % Hydrochloric acid p.a. (e.g. from Merck)

n-Hexane p.a. (e.g. from Merck)

Methanol p.a. (e.g. from Merck)

Ethyl acetate for trace analysis (e.g. from Baker)

Sodium hydroxide pellets p.a. (e.g. from Merck)

4-Chloro-2-methylphenoxybutyric acid, 99 % (e.g. Pestanal from Riedel-de Haën)

4-Chloro-2-methylphenoxybutyric acid methyl ester, 99 % (e.g. Pestanal from Riedel-de Haën)

4-Chloro-2-methylphenoxyacetic acid methyl ester, 99 % (e.g. Pestanal from Riedel-de Haën)

4-Chloro-2-mefhylphenoxyacetic acid, 99 % (e.g. Pestanal from Riedel-de Haën)

2,4-Dichlorophenoxyacetic acid, 99 % (e.g. Pestanal from Riedel-de Haën)

4-Chloro-2-methylphenoxypropionic acid, 99 % (e.g. Pestanal from Riedel-de Haën)

4-Chloro-2-methylphenoxypropionic acid methyl ester, 99 % (e.g. Pestanal from Riedel-de Haën)

2,4-Dichlorophenoxypropionic acid, 99 % (e.g. Pestanal from Riedel-de Haën)

2,4-Dichlorophenoxypropionic acid methyl ester, 99 % (e.g. Pestanal from Riedel-de Haën)

Ultrapure water (equivalent to ASTM type 1) or double-distilled water

Helium for GC

Purified nitrogen

2.3 Solutions

Solution of the internal standard:
About 10 mg of 4-chloro-2-methylphenoxybutyric acid are weighed exactly in a 100 mL volumetric flask which is then filled to the mark with ultrapure water while being swirled carefully (content: 100 mg/L). 10 mL of this solution are pipetted into a 100 mL volumetric flask. It is then filled to the mark with ultrapure water (content: 10 mg/L).
This solution can be stored in the refrigerator at 4 °C for about four weeks.

2.4 Calibration standards

Starting solution:
About 50 mg of 4-chloro-2-methylphenoxyacetic acid, 2,4-dichlorophenoxyacetic acid, 4-chloro-2-methylphenoxypropionic acid and 2,4-dichlorophenoxypropionic acid are exactly weighed in a 100 mL volumetric flask. The flask is subsequently filled to the mark with methanol (0.5 g/L).

This solution can be stored in the refrigerator at 4 °C for about four weeks.

Stock solution:
1 mL of the starting solution is pipetted into a 100 mL volumetric flask. The flask is subsequently filled to the mark with ultrapure water and carefully swirled around several times (content: 5 mg/L).

This solution can be stored in the refrigerator at 4 °C for about four weeks.

The calibration standards are prepared from this stock solution by diluting it with ultrapure water. They contain between 10 and 100 µg of each chlorophenoxycarboxylic acid per litre (cf. Table 2).

Table 2. Pipetting scheme for the preparation of the calibration standards

Volume of the stock solution [mL]	Final volume of the calibration standards [mL]	Concentration of the calibration standards [µg/L]
0.2	100	10
0.4	100	20
0.5	100	25
1	100	50
2	100	100

Thus prepared, the calibration standard solutions can be stored in the refrigerator at 4 °C for four weeks.

2.5 Preparation of the C 18 cartridge

An octadecyl cartridge is conditioned with 3 mL ethyl acetate, 3 mL n-hexane, 6 mL methanol and 9 mL ultrapure water in that order. The prepared cartridge must still be moist when the urine is introduced into it.

3 Specimen collection and sample preparation

Specimens are collected over a period of 24 hours in sealable plastic bottles and the total volume is determined.

The pH of a 25 mL urine sample is adjusted to 0–3 using 37 % HCl (1 mL 37 % HCl per 100 mL urine) and 1 mL of the internal standard is added. Thus prepared, the urine is slowly drawn through the still moist C 18 cartridge previously prepared as described in Section 2.5. For sample preparation by means of liquid-solid extraction, it is convenient to use equipment which can deal with several cartridges under vacuum at the same time.

After enrichment of the chlorophenoxycarboxylic acids, the cartridge is rinsed with 6 mL ultrapure water and subsequently sucked dry.

Elution of the enriched substances is carried out using 3mL methanol. The eluate is collected in a crimp-top vial in which 1 mL 98 % sulphuric acid has already been placed. After waiting about 5 minutes, 2 mL n-hexane are pipetted into the crimp-top vial. When the vial has been sealed with a Teflon-coated butyl rubber stopper the solution is shaken mechanically for 10 minutes. The organic phase is subsequently transferred to an approximately 1.5 mL glass sampler vial and reduced to a volume of about 300 µL in a flow of nitrogen.

4 Operational parameters for gas chromatography

Capillary column:	Material:	Quartz (fused silica)
	Stationary phase:	DB-WAX
	Length:	30 m
	Inner diameter:	0.25 mm
	Film thickness:	0.25 µm
Detector:	Mass selective detector (MSD)	
Temperatures:	Column:	2 minutes at 35 °C; then increase 50 °C per minute to 150 °C; then increase 10 °C per minute to 220 °C; 9 minutes at the final temperature

	Injector:	210 °C
Carrier gas:	Helium	at a precolumn pressure of 1300 hPa
Splitless time :	1 minute	
Sample volume:	1 µL	

5 Analytical determination

For the gas chromatographic analysis 1 µL of each prepared sample is injected into the gas chromatograph (cf. Figures 2 and 3).

The analytical determination is carried out using a mass selective detector (MSD) in the SIM mode (selected ion monitoring). For the determination of the chlorophenoxycarboxylic acids the following conditions were selected on the MSD:

Table 3. Selected conditions for the determination of the methyl esters of the chlorophenoxycarboxylic acid using MSD

Compound	Retention time [min]	Mass [g/mol]	Percentage area [%]
4-Chloro-2-methylphenoxyacetic acid methyl ester	11.47	214.05	100
		215.95	33
		155.00	58
2,4-Dichlorophenoxyacetic acid methyl ester	13.11	199.00	100
		233.95	62
		235.95	40
4-Chloro-2-methylphenoxypropionic acid methyl ester	10.26	228.05	100
		169.00	97
		230.05	34
2,4-Dichlorophenoxypropionic acid methyl ester	11.41	161.90	100
		247.95	65
		249.95	44
4-Chloro-2-methylphenoxybutyric acid methyl ester (internal standard)	13.62	242.05	100
		211.05	91
		244.05	33

If the results do not lie within the linear range of the calibration curve the samples are diluted with ultrapure water and processed anew.

6 Calibration

The aqueous calibration standards (Section 2.4) are processed in the same way as the urine samples (Section 3) and analysed as described in Sections 4 and 5 by means of gas chromatography/mass spectrometry. A calibration curve is obtained by plotting the quotients of the peak areas of the chlorophenoxycarboxylic acids with the internal standard as a function of the concentrations used (cf. Figure 4). It is not necessary to plot a complete calibration curve for every analytical series. It is sufficient to analyse one aqueous calibration standard with every new analytical series. The ratio of the result obtained for this standard and the result for the equivalent standard in the calibration curve is calculated. Using this quotient the results read off the calibration curve can be adjusted for each series. A new calibration curve should be plotted if systematic deviation of the quality control results is observed.

The calibration curve is linear between the detection limit and 120 µg of the chlorophenoxycarboxylic acids per litre urine.

7 Calculation of the analytical result

The recorded peak areas of the chlorophenoxycarboxylic acids are divided by the peak area of the internal standard. The quotients thus obtained are used to read off the appropriate concentrations of the chlorophenoxycarboxylic acids in µg per litre urine from the relevant calibration curve. The results are adjusted as described in Section 6. If necessary, a reagent blank value must be taken into account.

8 Standardization and quality control

Quality control of the analytical results is carried out as stipulated in TRgA 410 (Regulation 410 of the German Code on Hazardous Working Materials) [27] and in the Special Preliminary Remarks in this series. In order to determine the precision of the method, a sample containing a constant concentration of the individual chlorophenoxycarboxylic acids is analysed. As material for quality control is not commercially available, it must be prepared in the laboratory. For this purpose a defined amount of the individual chlorophenoxycarboxylic acids is added to urine. Aliquots of this solution can be stored in the deep-freezer for up to one year and used for quality control. The mean expected value and the tolerance range of this quality material is obtained in a pre-analytical period (one determinatin of the control material on 20 different days) [28].

9 Reliability of the method

9.1 Precision

In order to determine the precision in the series, urine of a person who had not been occupationally exposed to the chlorophenoxycarboxylic acids was spiked with different defined amounts of the chlorophenoxycarboxylic acids. Solutions containing the chlorophenoxycarboxylic acids in concentrations between 31.7 and 40.6 µg per litre urine were prepared. When these urine samples were analysed eight times, a relative standard deviation of between 2.4 % and 4.2 % was found and the corresponding prognostic range was between 5.5 % and 9.7 % (see Table 4).

Table 4. Precision in the series for the gas chromatographic determination of chlorophenoxycarboxylic acids (n = 8)

Substance	Expected value [µg/L]	Standard deviation (rel.) [%]	Prognostic range [%]
4-Chloro-2-methylphenoxyacetic acid	31.7	2.6	6.0
2,4-Dichlorophenoxyacetic acid	39.1	2.4	5.5
4-Chloro-2-methylphenoxypropionic acid	40.6	3.4	7.8
2,4-Dichlorophenoxypropionic acid	33.5	4.2	9.7

Moreover, the precision from day to day was checked using urine from a person who had not been exposed to chlorophenoxycarboxylic acids. The urine was spiked with specific amounts of the chlorophenoxycarboxylic acids to give three different concentration ranges, and was processed and analysed on eight different days. This resulted in relative standard deviations of between 3.6 % and 11.3 % and the corresponding prognostic ranges were between 8.3 % and 26.2% (cf. Table 5).

Table 5. Precision from day to day for the gas chromatographic determination of chlorophenoxycarboxylic acids (n = 8 days)

Substance	Expected value [µg/L]	Standard deviation (rel.) [%]	Prognostic range [%]
4-Chloro-2-methylphenoxyacetic acid	9.9	6.9	15.9
	23.3	5.5	12.7
	35.5	3.6	8.3
2,4-Dichlorophenoxyacetic acid	5.4	11.3	26.2
	12.8	7.8	18.0
	20.7	4.8	11.0
4-Chloro-2-methylphenoxypropionic acid	9.9	9.9	22.8
	22.9	8.0	18.4
	35.7	4.5	10.4
2,4-Dichloro-2-phenoxypropionic acid	17.4	9.9	22.8
	38.7	8.6	19.8
	63.3	7.3	16.8

9.2 Accuracy

In order to check for losses which occur during the sample preparation, a solution of the appropriate chlorophenoxycarboxylic acid esters in n-hexane was prepared and analysed by gas chromatography without further processing. The results obtained were compared with those obtained for the urine samples which contained the same concentrations of chlorophenoxycarboxylic acids and were subjected to the processing described above. The losses due to sample processing were between 5 % and 9 % (cf. Table 6).

Table 6. Losses due to sample processing

Substance	Recovery [%]	Losses due to processing [%]
4-Chloro-2-methylphenoxyacetic acid	95	5
2,4-Dichlorophenoxyacetic acid	93	7
4-Chloro-2-methylphenoxypropionic acid	91	9

As the methyl ester of 2,4-dichlorophenoxypropionic acid was not available, the urine of a person who had not been exposed to 2,4-dichlorophenoxypropionic acid was spiked with a specific quantity of 2,4-dichlorophenoxypropionic acid, processed as described in Section 3 and analysed. The recovery rate was 90 %.

9.3 Detection limit

Under the given conditions for sample preparation and gas chromatographic determination the detection limit was 10 µg of the individual chlorophenoxycarboxylic acids per litre urine. As no reagent blank value occurred, the detection limit was calculated as three times the signal-noise ratio.

The determination limit of the method, calculated as ten times the standard deviation of the background noise, was 30 µg of the individual chlorophenoxycarboxylic acids per litre urine.

9.4 Sources of error

Interference from other substances has not been observed i.e. in the investigations carried out so far no gas chromatographic peak has occurred at or near the characteristic retention times of the individual chlorophenoxycarboxylic acids for urine samples from persons who were not occupationally exposed to chlorophenoxycarboxylic acids. Moreover, it has been established that chlorophenoxycarboxylic acids cannot be detected in the urine of people who are not exposed to them at the workplace.

It is essential to ensure that the solvent used for the extraction is of the highest purity, as this varies greatly from one manufacturer to another.

At present analyte loss cannot be excluded when urine is transported in plastic sample bottles. It is possible that adsorption of the analyte may take place on the vessel walls. In order to remobilize absorbed substances, it may be advisable to adjust the pH to between 12 and 14 just before sample processing e.g. using sodium hydroxide pellets.

10 Discussion of the method

The method described here permits a simple, reliable and accurate determination of chlorophenoxycarboxylic acids in urine. It requires notably little effort and shows a low susceptibility to interference. Thus, the examiner of the method was able to reproduce the data and the quality control results achieved by the authors on his first attempt.

As a result of the unproblematic type of derivatization of the chlorophenoxy-carboxylic acids using sulphuric acid/methanol, it is unnecessary to use carcinogenic derivatizing reagents (such as diazomethane, methyl iodide, etc). As the results of the examiner of the method show, replacing n-hexane by n-heptane presents no problem.

The use of a mass selective detector enables achievement of a detection limit which is more than adequate for the purpose of occupational medicine. Furthermore, a high specificity is achieved. In principle, an electron capture detector (ECD) can also be used. However, increased interference from the other substances occurring in urine must be expected.

Detection of ecological exposure to the chlorophenoxycarboxylic acids is not possible using this method. The detection limit can, however, be lowered by further concentrating the sample solution so that the ecological range may be reached.

Instruments used:

Gas chromatograph HP 5890 with mass selective detector HP 5970B with Unix work station and Autosampler HP 7673A from Hewlett-Packard

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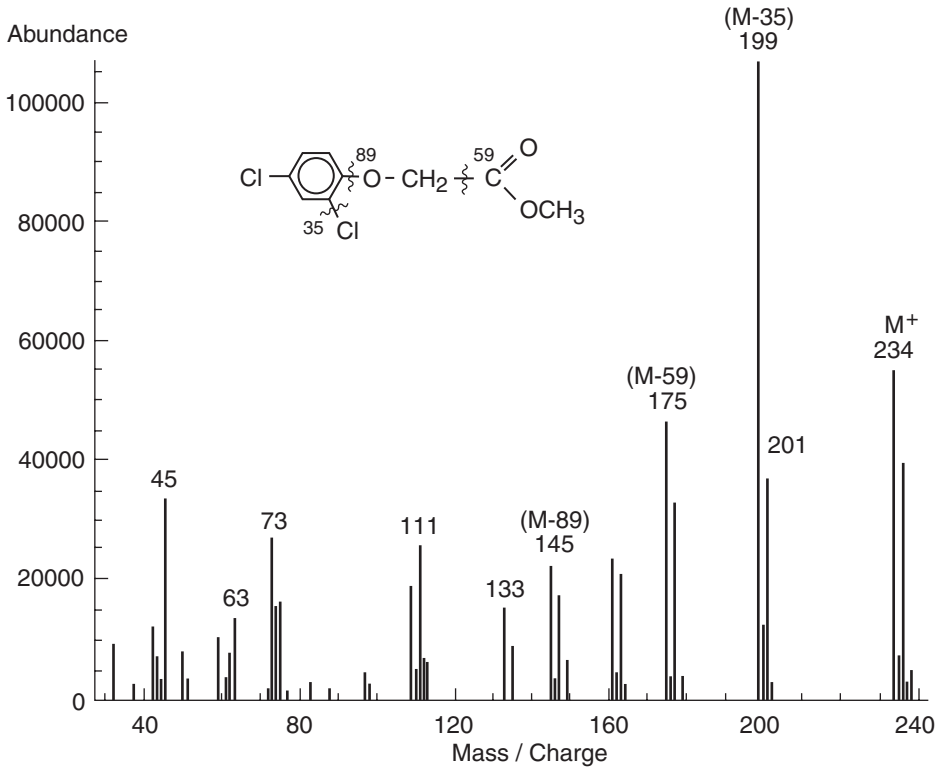

Figure 2. Mass spectrum of the methyl ester of 2,4-dichlorophenoxyacetic acid (The wavy lines in the molecular structure symbolize fragments of the molecule)

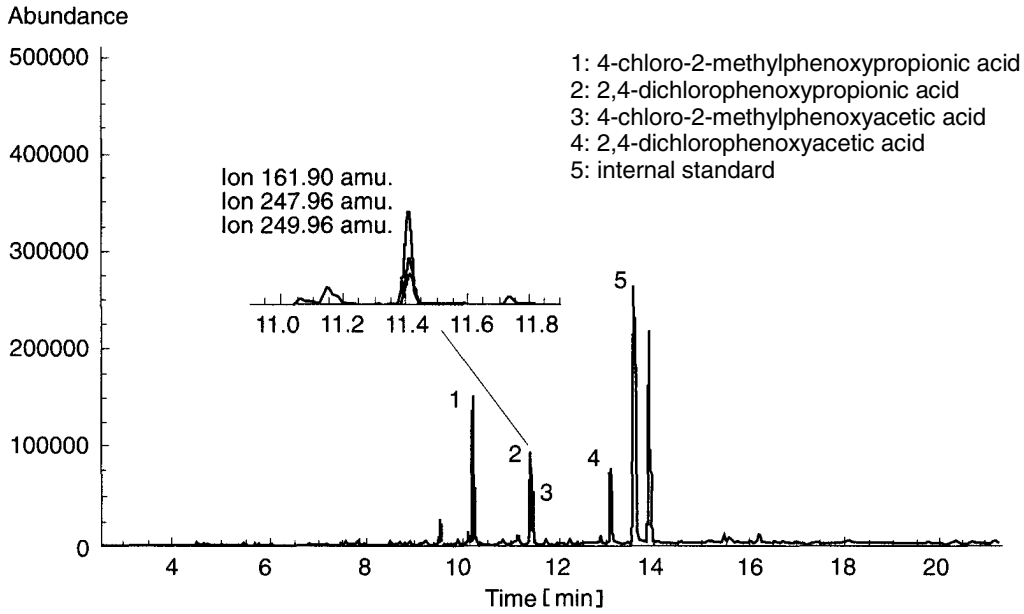

Figure 3. Gas chromatogram of a urine sample spiked with 30–40 g/L of each chlorophenoxycarboxylic acid

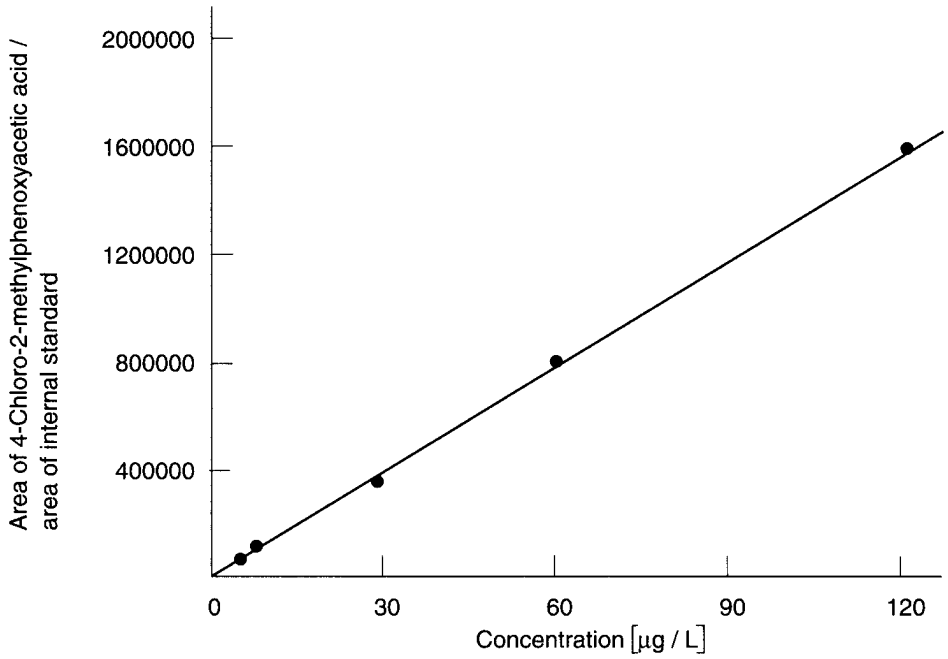

Figure 4. Example of a calibration curve for the determination of 4-chloro-2-methylphenoxy acetic acid