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Technical Report No. 15

Methods of analysis for herbicide residues in use at the Weed Research Organization

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Contents

Introduction

The analytical methods in this report are in routine use by the Chemistry Section of the Weed Research Organization. The report has been written as a reference manual for use by our staff. It is the authors' intention to amend and update the methods when necessary and to add new methods.

Reagents

The reagents used in these methods are general purpose grade unless 'AR' grade is specified. All solvents are distilled in glass before use.

Gas Chromatography Detectors

Two tritium and one Nickel 63 electron capture detectors are used in the methods described. A flame ionisation detector is available but has insufficient sensitivity and selectivity for residue analysis. A description and the uses of the three detectors is summarised in the Table.

Detector make	Construction	Source	Mode	Temp. limit	Methods
Varian Aerograph	glass/kovar	250 mc tritium	D.C.	200°C	tri-allate dichlobenil chlorthiamid
Laboratory built (McKone & Hance 1968)	brass/PTFE	250 mc tritium	D.C.	200°C	substituted ureas tri-allate dichlobenil chlorthiamid
Pye-Unicam	stainless steel	10 mc Nickel 63	pulsed D.C. 150 and 500 µsec interval	350°C	all methods

Because of the temperature limitation on the tritium detectors, contamination of the foil and gradual loss of standing current occurs during normal use. Extracts that are not adequately cleaned up cause rapid contamination demonstrated by low sensitivity and below baseline excursions of the recorder pen after each peak. When this occurs and measurement becomes difficult, the foil should be removed and cleaned. It should be handled with forceps at all times taking care to avoid scratching the surface. Appropriate regulations for the handling of radioactive materials should be followed at all times. The foil may be cleaned by placing it in an ultrasonic bath containing 5% potassium hydroxide in ethanol. After half an hour the foil is removed, rinsed with alcohol and hexane, and polished with cotton wool. A more satisfactory method of cleaning (Tayler 1962) is to polish the foil with Solvol Autosol, a proprietary chrome cleaner. After polishing, the foil is rinsed with hexane and polished with cotton wool.

The Nickel 63 detector may be operated at temperatures up to 350° and is less subject to contamination and loss of standing current. It is used in the pulse mode and has given excellent results for all the methods described. After one year's service the detector was cleaned. It was removed from the oven and filled with a 5% aqueous solution of hot basic detergent. After 15 minutes it was rinsed by drawing distilled water through it followed by methanol and hexane. The detector was dried in an oven at 110° for 15 minutes and refitted. A stable baseline and normal performance was obtained after 2 to 3 hours.

McKone, C.E. & Hance, R.J. (1968) J. Chromatog., 36, 234

Taylor, M.P. (1962) J. Chromatog., 9 , 28.

Preparation of columns for gas chromatography

Apparatus and reagents:

250 ml beaker
12 cm evaporating basin
water bath
vacuum pump
oven (250° min)
liquid phase
support material
glass twist (solvent washed)
oxygen-free nitrogen.

Column cleaning:

One end of the column is connected to a water vacuum pump and 100-ml portions of the following solvents are drawn through; aqueous detergent, distilled water, acetone or methanol and finally chloroform. The column is dried before packing by passing air through it.

Batch size:

A 5g to 10g batch of packing should be prepared for a 5 ft x $\frac{1}{8}$ in. column and a 20g batch for a 5 ft x $\frac{1}{4}$ in. column. The appropriate column size is described in each method. Column packings are prepared on a weight/weight basis so a 5% column packing requires 1g of liquid phase and 19g of support material.

Coating:

The appropriate amount of liquid phase is weighed into a 250-ml beaker and dissolved in 50 ml of solvent. The dried support (2 hours at 110°) is weighed into an evaporating basin and the solution of liquid phase is added. The basin is placed on a water bath and the solvent is evaporated gently stirring from time to time with a spatula to prevent bumping. Stirring is kept to a minimum to avoid disintegration of the support. When dry the coated support is thoroughly mixed and sieved.

Sieving:

All support materials are supplied with a nominal mesh size e.g. 60/80 and 80/100. The coated material is placed on the larger of the two appropriate sieves, e.g. with a 60 mesh on top of an 80 mesh. The support material is gently brushed and the sieves are tapped and rotated. The material passing the 60 mesh and retained by the 80 mesh is collected and used for packing the column.

Packing:

A 1 cm plug of solvent washed glass twist is placed in the detector end of the column and a funnel is connected to the injection end. The column is clamped with the funnel uppermost and a gentle vacuum is applied to the detector end. The column is filled by adding small amounts of packing material with a spatula while tapping the column gently to ensure uniform consolidation. The column is filled to within 1 cm of the end and a plug of glass twist is inserted.

Conditioning:

It is essential to condition columns before use in order to remove volatile impurities.

The packed column is placed in an oven preferably at a temperature some 10°C higher than that at which the column is to be operated and oxygen-free nitrogen is passed through it at 100 ml/min for a minimum of 24 hours. The column may then be used immediately or capped and stored.

Notes:

1. Suitable solvents for liquid phases and maximum temperature limits are quoted in suppliers' catalogues.
2. Violent agitation while filling the column is to be avoided as this may fracture the particles of support material exposing adsorptive sites and also cause back pressure in the column with consequent loss of resolution.

Soil sampling

Methods for soil sampling at W.R.O. for chemical residue analysis use 1 inch diameter core samples. Twelve to twenty 0-6 inch cores are randomly taken from field plots that vary in size from 20 to 90 square yards. Occasionally samples may be taken at 6 inch intervals down to 30 inches.

The core samples are mixed and passed through a 3 mm mesh sieve to remove stones. The sieved material is thoroughly mixed by shaking in a plastic bag or jar. The soil is subsampled by quartering and approximately 1 kilogram is retained for analysis. Soil samples containing volatile herbicides are stored moist in polyethylene bags in a deep freeze at - 15°C. Moisture content is determined at the time of analysis. Samples containing relatively non volatile herbicides are spread out in a thin layer on polyethylene sheet and allowed to air dry at 20°. This procedure is only adopted after checks have been made to ensure that losses do not occur during drying. The dried samples are stored in polyethylene bags in the deep freeze.

Fortification of substrates

In this laboratory recovery determinations are made for each new method and for each application of an existing method to a new substrate or soil.

Soils normally are fortified in the wet state and are allowed to stand for 3 days before the analytical procedure is begun. Crop and water samples are usually fortified immediately before analysis. Fortification levels are chosen to cover the expected range of residues. Commonly the rates used correspond to 1, 0.5, 0.1 and 0.05 ppm.

Such experiments should not be considered to give a very accurate estimate of rates of recovery, but they do give a useful indication and are the only means of obtaining this quickly and simply for routine purposes. In addition, they provide a good guide to the efficacy of the analytical technique after the extraction stage.

The limit of detection of a method is determined on the basis of the blank value obtained with unfortified substrate. The rule of thumb used in this laboratory is to set the limit of detection at twice the signal obtained from an appropriate blank. Lower values are reported as "less than" the detection limit .

Evaluation of data

Two methods are in use for the quantitation of chromatograms; peak height measurement and automatic integration with an Infotronics CRS 108 electronic integrator. Whenever peak heights are to be measured, chromatographic conditions are adjusted as far as possible to give narrow symmetrical peaks. A line is drawn across the base of the peak where it emerges from the background. The height of the peak is measured in centimetres to the nearest 0.5 millimetre along a line perpendicular to the base or to a point on the baseline which is reproducible from chromatogram to chromatogram. Standard curves are obtained by plotting log peak height against log ng of herbicides. The amplifier attenuation, standards injected and detector pulse intervals are adjusted so that the calibration graph is strictly linear.

The electronic integrator prints out a 3 or 4 figure number proportional to the area of the peak. The log of these numbers are plotted against log nanograms of herbicide, and instrument conditions and amounts of herbicide are adjusted to produce linear calibration graphs. The automatic integrator is the major method used as it integrates broad peaks which would not be accurately measured by peak height. It is not necessary to handle the chromatogram and the calibration may be computed from the printed areas without drawing calibration graphs manually.

Methylation

Acid compounds must be esterified in order to increase their volatility before they can be successfully gas chromatographed. Diazomethane and BF_3/methanol have been investigated as methylating reagents and it has been found that the former is superior as it produces a much lower level of background interference. The following procedure is used routinely: the herbicide residue is dissolved in 10 ml diethyl ether in a 100 ml conical flask fitted with a ground glass neck. A solution is prepared containing 400 mg N-methyl N-nitroso p-toluene sulphonamide in 4.6 ml diethylether and 6 ml methanol. One ml of 60% KOH is added and the diazomethane generated is bubbled into the ethereal residue solution. The process is continued until the solution turns yellow. The flask is then stoppered and allowed to stand for 15 minutes. The ether and excess diazomethane are then removed with a gentle stream of air in a fume cabinet and the residue dissolved in an appropriate solvent.

ATRAZINE

(2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine)

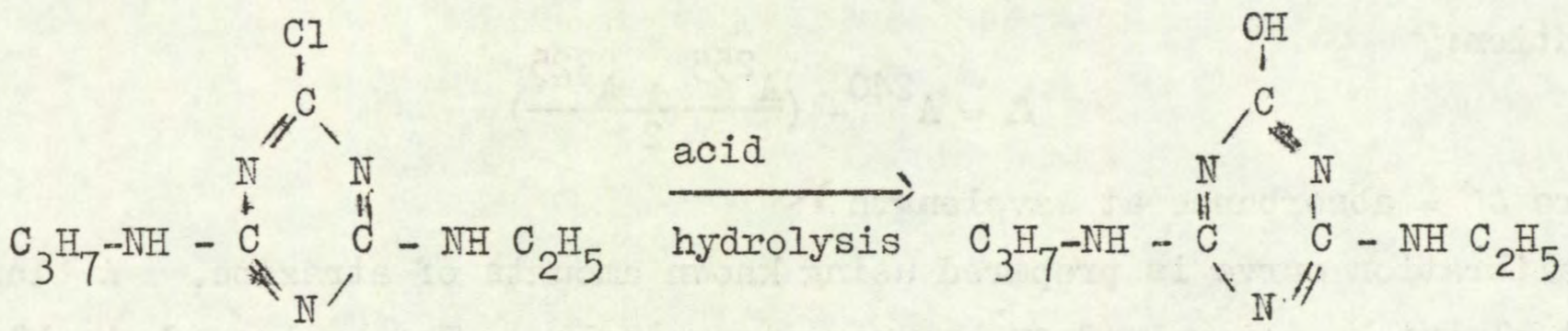
Substrate: Soil

Sample preparation and storage:

The soil is mixed and passed through a 3 mm sieve, stones being discarded. The soil may be stored either wet or dry at -15°.

Method

The method is based on that of Delley as described by Gysin and Knüsli (1960) and Knüsli *et al* (1964). The residue is taken up in chloroform and the solution washed with aqueous solvents. The atrazine is simultaneously hydrolysed and extracted from the organic phase by shaking with 50% sulphuric acid. The hydroxy triazine produced shows a maximum absorption in acid solution at 240 nm.



Apparatus and reagents:

Soxhlet extraction apparatus
Ultra-violet spectrophotometer
Sulphuric acid, A.R. 50%
Chloroform
Diethylether
Methanol (for wet soils)
250-ml round bottom flasks
Leibig condensers to fit Soxhlet extractors
28 x 80 mm single extraction thimbles
Test tubes (25 x 150 mm) with ground glass stoppers
1 ml and 10 ml pipettes
10 ml graduated pipette

Procedure:

Air dry soils: 10-50g soil is extracted in a Soxhlet extractor with 150 ml chloroform for 2 hours at a rate of 1 cycle per 5 min. The extract is concentrated to 10-25 ml and transferred quantitatively to a 35 ml stcppered test tube. The chloroform solution is shaken successively with 10 ml 0.5N NaOH, 10 ml 0.5N H Cl and 10 ml H_2O. The aqueous layers are discarded in each case. The chloroform solution is then filtered through a small plug of non-absorbent cotton wool into a second test tube to remove suspended water droplets and the filter washed with a little chloroform. One ml 50% H_2SO_4 is added and the tube shaken for 30 sec. then allowed to stand for 30 min. This process is repeated a further 3 times to give a total hydrolysis period of at least 2 hours. A 9 ml aliquot of water is then added and the tube shaken. About 7 ml of the aqueous layer is transferred to another tube by pipette and it is then shaken with 10 ml diethyl ether, to remove chloroform from the aqueous solution. The ether is removed by suction at a water pump. The absorbance of the aqueous layer is read at 225, 240 and 255 nm on a U-V spectrophotometer.

Wet soils:

10-50g soil is extracted on a Soxhlet apparatus for 2 hours with 150 ml methanol at a rate of 1 cycle per 5 min. The extract is evaporated to 1-2 ml by heating and the residue finally dried in an air stream at room temperature. The residue is dissolved in 10 ml chloroform and transferred to a 35 ml stoppered test tube. The flask is washed with 2 x 5 ml chloroform and the washings added to the tube. The procedure then follows that described for air dry soils. The water content of the soil is determined on a further subsample.

Calculation:

Spectrophotometer readings are taken at three wavelengths to provide a correction for the background. The readings give the difference Δ from the following equation:

$$\Delta = A^{240} - \left(\frac{A^{255} + A^{225}}{2}\right)$$

where A^{λ} = absorbance at wavelength λ

A calibration curve is prepared using known amounts of atrazine. A blank value must always be determined on an untreated sample. The Δ value of the blank is frequently negative so that subtraction of the value from that obtained from the treated sample will involve the addition of the appropriate figure. The amount of atrazine present is then determined from the calibration curve.

Interferences:

All the currently used herbicidal triazines will interfere and so probably will derivatives in which the amino-alkyl substituent is different. The hydroxy-triazines do not interfere.

With some soils it is not necessary to wash the chloroform extract prior to hydrolysis. This depends on the organic matter content of the soil and the weight of soil taken.

Reports in the literature, see Sheets and Kearney (1964) and McGlamery et al. (1967) indicate that chloroform will not extract chlorotriazines efficiently from all soils. In this case the wet soil procedure must be used.

Recovery and limits of detection

Recoveries of 70 to 95+% may be expected depending on the soil. This method will detect about 2 µg of atrazine but the limit of detection will depend upon the level of background interference. For 50g soil this is usually about 0.05 ppm.

Other substrates:

Water: A 100 ml aliquot of water is successively shaken with 25, 10 and 10 ml chloroform. The chloroform extracts are bulked and evaporated to about 10 ml. The procedure is then as for air dry soils beginning "the chloroform solution is filtered ....".

For water the limit of detection approaches 0.02 ppm.

References:

Gysin, H. & Knüsli, E. (1960) Adv. Pest Control Res., 3, 289.

Knüsli, E., Burchfield, H.P., & Storrs, E.E. (1964) Analytical methods for pesticides, plant growth regulators and food additives. Vol. 4. Herbicides (Zweig, G., ed.). Academic Press, p. 213.

Sheets, T.J. & Kearney, P.C. (1964) Abstr. 1964 Meeting Weed Soc. Amer, 10.

McGlamery, M.D., Slife, F.W. & Butler, H. (1967) Weeds, 15, 35.

CHLORTHIAMID

(2,6-dichlorothiobenzamide)

Substrate: Soil

Sample preparation and storage:

Moist soil samples from the field are crushed, mixed and passed through a 3 mm sieve to remove stones. Soil samples awaiting analysis are stored at -15°C in polyethylene bags.

Method

Moist soil is extracted with 20% acetone in hexane after which the extract is shaken with aqueous alkaline permanganate to convert the chlorthiamid to 2,6-dichlorobenzonitrile which moves into the hexane layer. Aliquots of the hexane solution are injected into a gas chromatograph and the 2,6-dichlorobenzonitrile is measured with an electron capture detector. The results are expressed as total nitriles (Beynon et al. 1966).

Apparatus and reagents:

Wrist-action flask shaker
gas chromatograph fitted with an electron capture detector
10 inch pestle and mortar
250-ml stoppered conical flasks
100-ml separating funnels
stoppered test tubes (25 x 150 mm)
2, 5, 10 and 50-ml pipettes
10 & 25 µl microsyringes
100-ml measuring cylinder
acetone
hexane
Na_2SO_4 anhydrous
$Na_2S_2O_5$
$KMnO_4$

Procedure:

Samples are thawed immediately prior to analysis and the moisture content is determined on a sub-sample. A 25g sample is ground with an equal weight of anhydrous Na_2SO_4 and transferred to a 250-ml stoppered conical flask. After 1 hour 50 ml of 20% acetone in hexane is added and the flask shaken for two hours on a wrist-action shaker. After shaking the soil slurry is allowed to settle and a 10-ml aliquot of the supernatant liquid is transferred by pipette to a 100-ml separating funnel. Two millitres of saturated $KMnO_4$ solution in 0.1M NaOH is added and the funnel shaken for 1 minute. Water (60 ml) is added and sufficient $Na_2S_2O_5$ to clarify the solution. The aqueous layer is removed and the hexane layer is washed with 5 ml of water, transferred to a stoppered test tube and dried with

anhydrous Na_2SO_4. Aliquots (5 µl) of this solution are taken for gas chromatography.

Gas chromatography:

Operating conditions using a Pye 104 with ^{63}Ni detector. Column:

1.5m x 4 mm i.d. stainless steel packed with 4% E.301 on 80/100 mesh Gas Chrom Q.

Injector temperature: 260° Carrier gas flow: 100 ml/min oxygen-free nitrogen

Column temperature: 180°

Detector temperature: 300° Detector voltage: pulse mode 150 µsec.

Attenuation: 5 or 10 x 10^2

Operating conditions using a Varian Aerograph with ^{3}H detector. Column:

1.5m x 2 mm i.d. stainless steel packed with 5% E. 301 on 60/80 mesh Gas Chrom Q.

The conditions for injector, column and carrier gas are as above.

Detector temperature: 200° Detector voltage: 90 D.C.

Attenuation: 8 or 16 Sensitivity: x1

Calibration:

A solution of 2,6-dichlorobenzonitrile containing 1 mg/ml is prepared in acetone. A 25 µl aliquot of this solution is transferred with a microsyringe to a 50-ml graduated flask and diluted to volume with hexane. This solution containing 2.5 ng of 2,6-dichlorobenzonitrile in 5 µl is diluted with hexane to give a range of standards containing 0.1ng to 1.0 ng herbicide in 5 µl. The hexane solutions obtained from extracts of the soil are diluted where necessary so that the standard injection volume of 5 µl contains a 2,6-dichlorobenzonitrile concentration within the linear portion of the calibration curve. Application of chlorthiamid to the soil results in a mixture of 2,6-dichlorothiobenzamide and 2,6-dichlorobenzonitrile being present. The residue found is normally reported in the form of 'total nitriles' based on a 2,6-dichlorobenzonitrile measurement.

Calculation:

$$\frac{\text{ng 2,6-dichlorobenzonitrile in 5 µl}}{1000} \times \frac{\text{Vol. of hexane phase (µl)}}{5} \times \frac{1}{\text{air dry wt. of soil}} \times 1.2 = \text{ppm chlorthiamid}$$

To express result in terms of 'total nitrile' the 1.2 conversion factor is omitted.

Interferences:

Clean-up has not proved necessary for sandy loam soils (organic carbon 1.9%) but if the chromatogram of an untreated soil contains interfering peaks, then a clean-up procedure as described in the original publication should be used.

Recoveries and limits of detection:

The mean recovery of chlorthiamid from a sandy loam soil (organic carbon 1.9%) was 70%. The practical limit for the detection of chlorthiamid in the sandy

loam soil analysed was 0.05 ppm.

Other substrates:

Gooseberries:

Samples are stored in a deep freeze at-15°C.

Method

Additional apparatus

Macerator (e.g. Waring Blendor)

250 ml Volumetric flasks

50 or 100 ml separating funnels

Filter funnels

Procedure:

The sample is thawed and 100g are macerated for 2 min with 200 ml acetone + 50g anhydrous Na_2SO_4. The slurry is then filtered through Whatman No. 1 paper into a 250 ml volumetric flask. The filter is washed with acetone and the extract made to volume. An aliquot of 10 ml of the acetone solution (≡4g crop) is shaken for 1 minute with 8 ml hexane & 2 ml saturated $KMnO_4$ in 0.1 N NaOH in a separating funnel. Water (60 ml) is then added plus sufficient $Na_2S_2O_5$ to clarify the solution. The aqueous layer is run off and discarded and the hexane washed with 5 ml H_2O which is also discarded. The hexane layer is then dried with anhydrous Na_2SO_4. Aliquots (5 ul) are chromatographed as before.

This procedure again converts chlorthiamid to dichlobenil so results are reported as 'total nitriles'.

Interferences

No additional clean-up steps have proved necessary.

Recoveries and limits of detection

Recoveries of better than 70% may be expected and the detection limit is about .005 ppm.

Blackcurrants

The same method is applicable.

References

Beynon, K.K., Davies, L., Elgar, K. & Wright, A.N. (1966) J. Sci Fd Agric 17, 4.

DICHLOBENIL

(2,6-dichlorobenzonitrile)

Substrate: Soil

Sample preparation and storage: Moist soil samples from the field are crushed, mixed and passed through a 3 mm sieve to remove stones. Soil samples awaiting analysis are stored in polyethylene bags at -15°C.

Method

The method of Beynon et al. 1966 is used.

Moist soil is extracted with 20% acetone in hexane, after which the extract is shaken with water. Dichlobenil remains in the hexane layer and this solution is analysed by electron capture gas chromatography.

Apparatus and reagents:

Wrist action flask shaker
Gas chromatograph fitted with a ^{63}Ni or ^{3}H electron capture detector
10-inch pestle and mortar
250-ml stoppered conical flasks
100-ml separating funnels
Stoppered test tubes (25 x 150 mm)
2, 5, 10 and 50-ml pipettes
10 and 25 µl microsyringes
acetone
hexane
anhydrous Na_2SO_4 A.R.

Procedure:

Samples are thawed immediately prior to analysis and the moisture content is determined on a sub-sample. The soil (25g) is ground with an equal weight of anhydrous Na_2SO_4 and transferred to a stoppered 250-ml conical flask. After 1 hour the soil is shaken with 50 ml of 20% acetone in hexane for two hours on a wrist action shaker. After shaking the soil slurry is allowed to settle and a 10-ml aliquot of the supernatant liquid is transferred by a pipette to a 100-ml separating funnel. Distilled water (10 ml) is added and the funnel is shaken for 1 minute. The aqueous layer is discarded and the hexane is transferred to a stoppered test tube containing anhydrous Na_2SO_4. Aliquots of this solution are taken for gas chromatography.

Gas chromatography:

see chlorthiamid

Calibration:

see chlorthiamid

Calculation:

$$\frac{\text{ng dichlobenil in 5 µl}}{1000} \times \frac{\text{Total volume of hexane phase (µl)}}{5} \times \frac{1}{\text{wt soil sample}} \times \frac{100}{\% \text{ dry soil}} \times \text{ppm dichlobenil in dry soil}$$

Interferences:

see chlorthiamid

Recoverios and limits of detection:

The mean recovery of dichlobenil from a sandy loam scil (organic carbon 1.9%) was 71%. The practical limit for detection of dichlobenil in the sandy loam soil analysed was 0.05 ppm.

Other substrates:

Water. If storage is necessary, samples are kept in glass bottles in the dark at 5°.

Additional apparatus

100-ml measuring cylinders, 100 and 250-ml separating funnels.

Procedure

The water is not filtered prior to analysis but any large pieces of flora or fauna are removed. A 100 ml aliquot of water is measured into a 250-ml separating funnel and shaken for 1 minute each with 25 + 10 + 10 ml portions of hexane. The combined hexane extracts are washed with 5 ml of distilled water in a 100-ml separating funnel. The hexane is transferred to a 50-ml graduated flask through a funnel containing a small plug of hexane washed cotton wool and a layer of anhydrous Na_2SO_4. The funnel and cotton wool are rinsed with hexane. Aliquots of this solution are taken for gas chromatography.

Gas chromatography:

as for soil

Interferences:

It is important for all the glassware to be washed with re-distilled hexane immediately prior to use. The alkaline permanganate clean-up used for chlorthiamid residues in soil may be used if necessary. The analysis of a variety of natural waters in this laboratory has not shown the need for a clean-up stage.

Recoveries and limits of detection

Recovery of dichlobenil from a naturally occurring surface water was 95% with a practical limit of detection of 0.005 ppm.

Gooseberries:

see chlorthiamid

Blackcurrants:

see chlorthiamid

References

Beynon, K.I., Davies, L., Elgar, K., and Wright, A.N. (1966) J. Sci. Fd. Agric., 17,4.

DIQUAT

(9,10-dihydro-8a,10a-diazoniaphenanthrene-2A)

Substrate: Soil, Water

Methods:

The methods are the same as those described for paraquat. Extinctions should be read at 375, 379, 383 and 385 nm.

The correction formulae used are:

$$E\ 379 = 3.79\ E\ 379 - 2.28\ E\ 375 - 1.52\ E\ 385$$

$$E\ 379 = 2.49\ (2\ E\ 379 - E\ 375 - E\ 383)$$

Polarographic peaks in $0.1N H_2SO_4$ occur at about 0.55V and 0.99V. The latter peak is larger.

References

Calderbank, A. & Yuen, S.H. (1966) Analyst, 91, 625.

DIURON

(N'-(3,4-dichlorophenyl)-N,N-dimethylurea)

Substrate: soil

Sample preparation and storage:

see linuron

Method

see linuron

Gas chromatography

A column temperature of 155° is used. All other operating conditions are the same as for linuron.

Calibration:

Method as for linuron

Interferences:

see linuron

Recoveries and limits of detection:

Recoveries of diuron from a silty clay loam soil containing 3.7% organic carbon were 80 and 86% at the 0.1 and 1.0 ppm level respectively. The practical limit for the above soil was 0.05 ppm. In low organic matter soils a lower limit may be possible.

Other substrates: Water

If storage is necessary, samples are kept in glass bottles in the dark at 5°.

Method

Samples are shaken with dichloromethane to extract diuron. The solvent is evaporated and the residue dissolved in 2,2,4-trimethylpentane. Aliquots of this solution are analysed by electron capture gas chromatography (McKone & Hance, 1969).

Additional apparatus and reagents:

100-ml measuring cylinders 100-ml and 250-ml separating funnels.

Procedure:

The water is not filtered prior to analysis but any large pieces of flora or fauna are removed. One hundred ml of water is measured into a 250-ml separating funnel and shaken for 1 minute each with two 25-ml portions of dichloromethane. The lower organic layers together with any emulsion are combined in a 100-ml separating funnel. If any emulsion is present this may usually be cleared by shaking with 5 ml glass-distilled water. The dichloromethane is run into a 100-ml stoppered conical flask through a funnel containing a small plug of dichloromethane-washed cotton wool. The funnel and cotton wool are rinsed with dichloromethane. A glass still head is then fitted to the flask and the solution evaporated to about 0.5 ml under reduced pressure on a water bath at 35°, then the remaining solvent is removed with a gentle stream of air at room temperature. The residue is dissolved in 5 ml of 2,2,4-trimethylpentane and the flask is shaken

vigorously for 1 minute. Aliquots of this solution are injected into the gas chromatograph

Gas chromatography:

as for diuron in soil

Interferences:

It is important for the glassware to be rinsed with redistilled dichloromethane immediately prior to use. If possible the water should not be filtered. The washing of the combined dichloromethane layers with water is omitted if emulsions are absent. Diuron, linuron, metoxymarc, neburon and benzomarc all have the same retention time and therefore are indistinguishable. An unknown urea herbicide can be identified using gas chromatography in conjunction with thin-layer chromatography (Hance 1969). The metabolite N-(3,4-dichlorophenyl)-N'methylurea would also be estimated as diuron in this procedure, but may be separated using the thin-layer procedure of Katz & Nieh (1967) using the solvent system chloroform 100:methanol 5: pyridine 1.

Recoveries and limit of detection:

Recoveries of diuron from 3 naturally occurring surface waters were typically 95% at 0.001 ppm and 88% at 1.0 ppm. The mean of 11 determinations on untreated control samples of three waters was 0.0005 ppm. For practical purposes a limit for the detection of diuron in these waters was 0.001 ppm.

References

McKone, C.E. and Hance, R.J. (1969) Bull. environ. Contamin. Toxicol. 4, 31.

Hance, R.J. (1969) J. Chromatog. 44, 419

Katz, S.E. and Nieh, M.T. (1967) Bull. environ. Contam. Toxic. 2, 75.

EPTC

(S-ethyl N N-dipropyl (thiocarbamate))

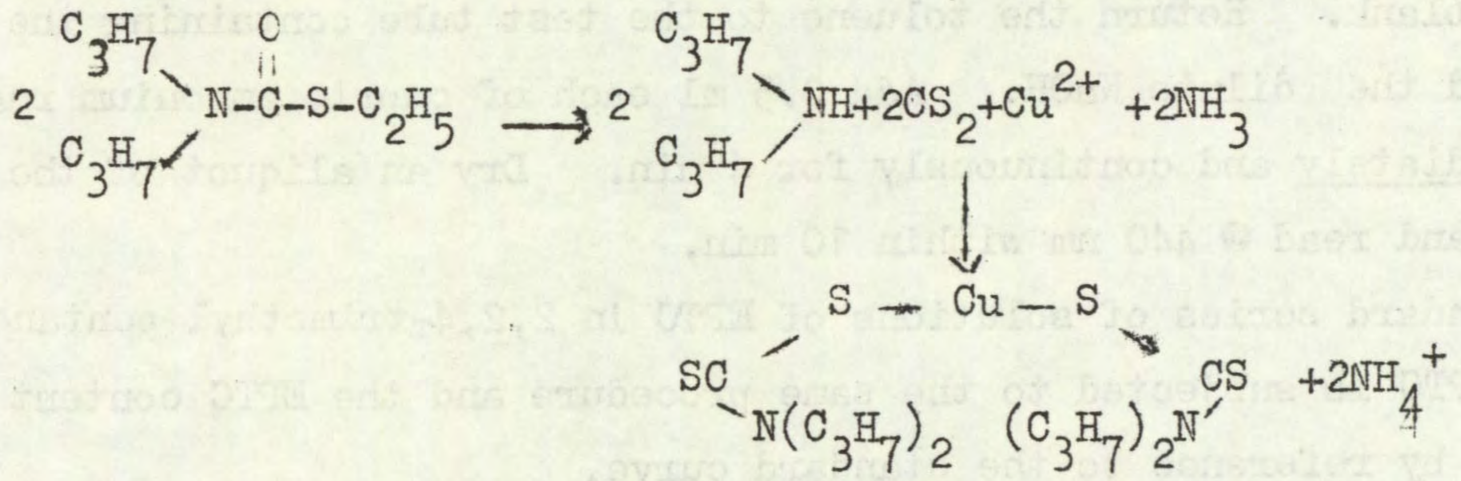
Substrate: Soil

Sample preparation and storage:

The soil is mixed and passed through a 3 mm sieve, stones being discarded. The soil is stored wet in the deep freeze.

Method

The method used is that of Batchelder & Patchett (1960). EPTC is steam distilled from the soil, hydrolysed in sulphuric acid to produce di-n-propylamine which is converted to the yellow cupric dithiocarbamate for colorimetric estimation.



Apparatus and reagents

Spectrophotometer

1 litre conical flask with still head and condenser set up for steam distillation

100 ml separating funnel

35 ml stoppered test tube (25 x 150 mm)

2,2,4-trimethylpentane, reagent grade

Conc. HCl, AR

Conc. H_2SO_4, AR

50% NaOH, AR

1% phenolphthalein in methanol

Toluene

Copper-ammonium reagent: dissolve 1g $CuSO_4.5H_2O$ in 5 ml H_2O and dilute to 250 ml with .880 ammonia

NA_2SO_4

CS_2

Procedure:

The soil (50g) is weighed into a 1 litre conical flask and 200 ml water and 5 ml glacial acetic acid are added. The steam distillation apparatus is assembled and the EPTC steam distilled into a 100 ml separating funnel. At least 50 ml distillate is collected. If foaming occurs in the distillation flask a little silicone anti-foam is added. After distillation 5 drops conc. HCl are added to the distillate and the EPTC is extracted from the aqueous phase with two 10 ml portions of 2,2,4-trimethylpentane. The extracts are combined in a 35 ml stoppered tube, 1 ml conc. H_2SO_4 is added and the tube shaken vigorously. The trimethyl

pentane layer is then discarded. The EPTC is hydrolysed by placing the tube in a water bath @ 85 ± 1°C for 20 minutes. The tube is rolled twice during this time to wash the sides with hot acid. The tube is then cooled in an ice bath and the acid diluted by the cautious addition of 10 ml water.

One drop 1% phenolphthalein is added and the acid is neutralised with 50% NaOH while swirling the tube in the ice-bath. Three additional drops of NaOH are added and the contents extracted by shaking with 10 ml toluene. Transfer about 4 ml of the toluene layer to a test tube containing 1g anhydrous Na_2SO_4. Shake until clear and then read the background absorbance @ 440 nm in a 1 cm cell against a toluene blank. Return the toluene to the test tube containing the rest of the toluene and the dilute NaOH. Add 0.5 ml each of cupric ammonium reagent and CS_2. Shake <u>immediately</u> and continuously for 4 min. Dry an aliquot of the toluene phase as before and read @ 440 nm within 10 min.

A standard series of solutions of EPTC in 2,2,4-trimethyl pentane containing 4-400 µg EPTC is subjected to the same procedure and the EPTC content of the soil determined by reference to the standard curve.

The water content of the soil is determined in a further sub-sample.

<u>Interferences:</u>

Normally these are low. If significant they can be reduced by discarding the aqueous phase obtained after shaking the hydrolysate with toluene. The toluene is then extracted with 10 ml H_2O made acid to phenolphthalein by conc. HCl and then adding a further 3 drops HCl. The toluene layer is then discarded. An additional 10 ml toluene is then added and the aqueous phase made alkaline as before and the procedure continued.

The method does not distinguish EPTC from PEBC.

<u>Recovery and limits of detection</u>

Recoveries of better than 80% should be achieved. The method will detect 4 µg EPTC, equivalent to 0.08 ppm on a 50g soil sample.

<u>Notes</u>

The colour formation step should be carried out at 20-25°. The reaction may be incomplete at lower temperatures and side reactions occur at higher temperatures. Side reactions may also occur if there is any delay before starting the 4 minute shaking period.

<u>Reference</u>

Batchelder, G.H. & Patchett, G.G. (1960) J. agric. Fd. Chem., 8, 214.

LINURON

(N'-(3,4-dichlorophenyl)N-methoxy-N-methylurea)

Substrate: Soil

Sample preparation and storage:

Soil samples from the field are allowed to air dry at 20°. The dry soil is crushed, mixed and passed through a 3 mm sieve to remove stones. Soil samples awaiting analysis are stored dry at -15° in polythene bags.

Method

Principle:

Samples are extracted with methanol. An aliquot of the methanol extract is evaporated and the residue dissolved in 2,2,4-trimethylpentane. Aliquots of this solution are analysed by electron capture gas chromatography (McKone 1969).

Apparatus and reagents:

Wrist-action flask shaker

Gas chromatograph fitted with the tritium electron capture detector described by McKone and Hance (1968[2]) or a ^{63}Ni detector

Water bath at 50°, 100 and 250-ml stoppered conical flasks

Glass stoppered test tubes (25 x 150 mm)

5 and 50-ml pipettes

7 cm glass filter funnels

Whatman No. 42, 12.5 cm filter papers

10 and 25 µl microsyringes

Anhydrous Na_2SO_4 'AR' grade

methanol

2,2,4-trimethylpentane

Procedure:

Air dried soil (25g) is placed in a stoppered 250-ml conical flask with 50 ml of methanol and shaken on a wrist-action shaker for 1 hour. The soil slurry is allowed to settle and the supernatant liquid is filtered through a fluted No. 42 filter paper into a stoppered tube. As soon as 10 to 15 ml of filtrate has been collected, the filter funnel is removed and the tube is stoppered. A 5-ml aliquot is transferred by pipette to a 100-ml stoppered conical flask. A glass still head is fitted and the solution is concentrated to about 0.5 ml under reduced pressure in a water bath at 50°. The remaining methanol is then removed with a gentle stream of dry air at room temperature. The residue is dissolved in 5 ml of 2,2,4-trimethylpentane; 0.5g of anhydrous Na_2SO_4 is added and the flask is stoppered and shaken vigorously for 1 minute. Aliquots (5 µl) of this solution are taken for gas chromatography.

Gas chromatography:

Operating Conditions using a Varian Aerograph with a laboratory-built ^{3}H detector

Column: 1.5 m x 2 mm i.d. stainless steel packed with 5% E 301 on 60/80 mesh Gas Chrom Q.

Injector temperature: 265°
Column temperature: 150°
Detector temperature: 200°
Detector voltage: 90 D.C.
Retention time: 0.95 min

Carrier gas flow: 50 ml/min oxygen-free nitrogen
Attenuation: 4 and 8
Sensitivity x1
Chart speed 30 inches/hour

Operating conditions: using a Pye 104 with a ^{63}Ni detector

Column: 1.5 m x 4 mm i.d. stainless steel packed with 4% E 301 on 60/80 mesh Gas Chrom Q.

The conditions for injector and carrier gas are as above.

Column temperature: 180°
Detector temperature: 300°
Detector voltage: pulse mode 150 µsec

Attenuation: 5 or 10 x 10^{2}

Calibration:

A solution of linuron containing 1 mg/ml is prepared in methanol. Using a 25 µl microsyringe, 25 µl of the solution is transferred to a 50-ml graduated flask and diluted to volume with 2,2,4-trimethylpentane. Aliquots of this solution which contains 2.5 ng linuron in 5 µl are diluted with 2,2,4-trimethylpentane to give a range of standards containing 0.1-1.0 ng linuron in 5 µl. The 2,2,4-trimethylpentane solutions obtained from extracts of the soil are diluted where necessary so that the standard injection volume of 5 µl contains a linuron concentration within the linear portion of the calibration curve.

Calculation:

To determine the ppm linuron in the sample use the following calculation:

$$\frac{\text{ng linuron in final 5 µl}}{1000} \times \frac{\text{volume of final dilution in µl}}{5\ \mu l} \times \text{dilution factor} \times \frac{1}{\text{wt. of sample}} = \text{ppm linuron (air dry basis}$$

Interferences:

Linuron has been successfully determined in soils containing up to 12% organic carbon without clean up techniques. Any peaks occurring in untreated control soil at the same retention time as linuron should be treated as 'apparent' linuron and their value subtracted as a blank from treated samples.

Diuron, linuron, metoxymarc, neburon and benzomarc all have the same retention time and are therefore indistinguishable. An unknown urea herbicide may be identified using gas chromatography in conjunction with thin layer chromatography (Hance 1969). The metabolites N-(3,4-dichlorophenyl)-N'-methylurea and N-(3,4-dichlorophenyl)-N'-methoxyurea

are also estimated as linuron by the procedure but may be separated using the thin layer method of Katz & Nieh (1967).

Recoveries and limits of detection:

The mean recovery of linuron from three soils ranging in organic carbon from 1.9 to 12% was 88%. The practical limit of detection was 0.05 ppm.

References

McKone, C.E. (1969) J. Chromatog., 44, 60

McKone, C.E. & Hance, R.J. (1968) J. Chromatog., 36, 234

Hance, R.J. (1969) J. Chromatog., 44, 419

Katz, S.E. & Nieh, M.T. (1967) Bull. environ. Contam. Toxic., 2, 75

PARAQUAT

(1,1'-dimethyl-4,4'-bipyridilium-2A)

Substrate: soil

Sample preparation and storage:

The soil is mixed and passed through a 3 mm sieve, stones being discarded. It may be stored air dry at room temperature or wet in the deep freeze.

Method

Source:

The method has been developed by I.C.I. and is described in various publications e.g. Tucker *et al.* (1967).

Principle:

The soil is digested with strong H_2SO_4. After dilution the extract is passed through an ion exchange resin which retains the paraquat. Paraquat is eluted from the resin with NH_4Cl and is then reduced with dithionite to produce a blue free radical which is estimated spectrophotometrically.

Apparatus and reagents

Spectrophotometer

500 ml round bottomed flasks with ground glass necks attached to reflux condensers

16 cm Buchner funnel

Chromatographic tubes

50 ml volumetric flasks

1 litre separating funnels

2N HCl

18N H_2SO_4

1% sodium dithionite in N NaOH. Use within 3 hours of preparation

Saturated NH_4Cl

Zeocarb 225, 52 to 100 mesh 8% DVB Na form

Celite 545

Whatman No. 5 filter paper

Procedure:

About 100g soil is ground to pass a 3 mm sieve and 25g is weighed into a 500 ml boiling flask. 100 ml 18N H_2SO_4 is added and the contents of the flask refluxed for 5 hours. For some soils 12N or even 5N acid may be adequate but this can only be determined by experiment.

A Whatman's No. 5 paper is placed in a 16 cm Buchner funnel attached to a 2l. filter flask and moistened. Gentle suction is applied and 150 ml of an aqueous suspension of about 10g Celite 545 is poured in the funnel. The filter is sucked dry and the washings discarded.

The soil digest is cooled, cautiously diluted to about 500 ml and filtered through the Celite pad which is then washed with 2 100 ml portions of water.

The filtrate is transferred to a 1-litre separating funnel and diluted to about 1 l. with water.

An ion exchange column is prepared by weighing 3.5g Zeocarb 225 into a 25 ml burette containing a glass wool pad above the stopcock. The column is eluted successively with 20 ml 2N HCl and 50 ml H_2O at a flow rate of 5 ml/min.

The 1-litre separating funnel is now connected to the resin column and the solution is allowed to percolate at 5-10 ml/min. The column is then washed at 3-4 ml/min with 25 ml H_2O, 100 ml 2N HCl, 25 ml H_2O, 50 ml 2.5% NH_4CL and 25 ml H_2O. These eluates are discarded. The paraquat is then eluted at 1 ml/min with 50 ml saturated NH_4Cl, the effluent being collected in a 50 ml volumetric flask.

A 10 ml aliquot of effluent is pipetted with a 15 ml glass-stoppered test tube and 2 ml dithionite reagent are added. Within 5 minutes the absorbence of the solution is measured at 392, 396, 400 and 401 nm in 4 cm cells. Standards in the range 0.25-10 ppm are treated similarly.

Calculation

The extinction at 396 nm (E 396) is corrected for background absorption using the following equations:

1) E 396 (corr) = 2.91 E 396 - 1.61 E 392 - 1.28 E 401

2) E 396 (corr) = 1.68 (2 E 296 - E 392 - E 400)

The mean of the two E 396 (corr) values is used for calculation of paraquat content from the calibration curve.

Interferences

The clean-up procedure appears to be adequate for all soils

Recovery and limits of detection

Recoveries of the order of 70-95% should be possible. Using a 25g sample the limit of detection is about 0.1 ppm but lower levels can be detected with larger samples.

Note

It is important to use the exact grade of Zeocarb 225 specified. Inconsistent results may be obtained if resins of other divinyl benzene content are used. Columns should not be re-used.

Other substrates

Water

Storage - if necessary the sample is stored in the dark at 4°C.

Procedure: A suitable aliquot of the water sample is passed through a treated ion exchange column and the procedure is then carried through as for soil.

Alternative procedure

Paraquat is polarographically active. Using a Davis-Southern Analytical Cathode Ray Polarograph Type A 1670 in the derivative mode the limit of detection for paraquat in 0.1 N H_2SO_4 is about 0.04 ppm. If the water sample contains paraquat above this level and provided the background from untreated water is sufficiently low, then paraquat can be estimated directly by this means after acidification of the sample. Paraquat gives two peaks under these conditions at 0.68 and 1.12 V versus a Hg pool electrode. The peak at 1.12 V should be used.

References

Tucker, B.V., Pack, D.E. & Ospenson, J.N. (1967) J. agric. Fd. Chem., 15,1005.

Calderbank, A. & Yuen, S.H. (1965) Analyst, 90, 99.

Hance, R.J. (1970) Pestic. Sci., 1, 112.

PEBULATE

(S-propyl N-butyl-N-ethylthiocarbamate)

The procedure for EPTC is used.

PICLORAM

(4-amino-3,5,6-trichloropicolinic acid)

Substrate: Soil

Sample preparation and storage:

Soil samples from the field are allowed to air dry at 20°. The dry soil is crushed, mixed and passed through a 3 mm sieve to remove stones. Soil samples awaiting analysis are stored dry at -15° in polyethylene bags.

Method

Source:

The method was developed by Leahy and Taylor (1967). A shortened version of this method is used with slight modifications.

Principle:

Samples are extracted by shaking with 0.05N potassium hydroxide in 10% potassium chloride. After filtration, aliquots are acidified and extracted with diethyl ether. The ether extracts are methylated with diazomethane and the resultant methyl ester is measured by electron capture gas chromatography.

Apparatus and reagents:

Wrist-action flask shaker

Isothermal gas chromotograph fitted with a ^{63}Ni electron capture detector

Water bath at 35°

100 and 250-ml stoppered conical flasks

100-ml separating funnels

Glass stoppered test tubes (25 x 150 mm)

1, 5, 10, 25 and 50 ml pipettes

7 cm glass filter funnels

10 and 25 µl microsyringes

Whatman No. 42, 12.5 cm filter papers

Anhydrous Na_3SO_4 ('AR' grade)

KoH " "

K Cl " "

H_2SO_4

N-Methyl N-nitroso-p-toluene sulphonamide

Diethyl ether, purified and distilled (Vogel 1956)

Methanol

Hexane

Procedure:

Air dried soil (25 g) is placed in a stoppered 250-ml conical flask with 50 ml of 0.05N potassium hydroxide containing 10% potassium chloride and shaken on a wrist-action shaker for 1 hour. After shaking the soil slurry is allowed to settle and the supernatant liquid is filtered through a fluted No. 42 filter

paper into a test tube. A 25-ml aliquot of the filtrate is transferred by pipette into a 100-ml stoppered conical flask and shaken vigorously for 1 minute with 25 ml of diethyl ether. The contents of the flask are poured into a 100-ml separat . funnel. The lower aqueous layer is run back into the 100-ml conical flask and the ether layer is discarded. The aqueous extract is adjusted to pH2 with N sulphuric acid (about 10-15 ml) and shaken vigorously for 1 minute with a further 25 ml of diethyl ether. The contents of the flask are returned to the separating funnel and the aqueous layer is run back into the 100-ml conical flask. The aqueous layer is extracted with two further 10-ml portions of diethyl ether in a similar manner. The combined ether extracts are washed with 5 ml of distilled water. After separation the upper ether layer is transferred to a clean 100-ml conical flask through a funnel containing a small plug of cotton wool and a layer of anhydrous sodium sulphate. The funnel and cotton wool are rinsed with 5 ml of diethyl ether. A glass still head is added and the solution is evaporated to about 0.5 ml under reduced pressure on a water bath at 35°. The remaining solvent is removed with a gentle stream of air. The residue is dissolved in 10 ml of diethyl ether (dried with Na_2SO_4) and the flask is stoppered to await methylation. After methylation with diazomethane (see "Methylation") the residue is dissolved in hexane, dried with anhydrous sodium sulphate and aliquots of this solution are injected into the gas chromatograph.

Gas chromatography:

Operating conditions: using a Pye 104 with a ^{63}Ni detector.
Column 1.5 m x 4 mm i.d. stainless steel packed with 4% E 301 (methyl silicone) on 80/100 mesh Gas Chrom Q.

Injector temperature: 260°
Column temperature: 200°
Detector temperature: 300°
Attenuation: 5 or 10 x 10^2
Carrier gas flow: 100 ml/min oxygen-free nitrogen
Detector voltage: pulse mode 150 µsec

Calibration:

A solution of picloram methyl ester containing 1 mg/ml is prepared in methanol. Using a 25 µl microsyringe, 25 µl of this solution is transferred to a 50-ml graduated flask and diluted to volume with hexane. This solution containing 2.5 ng methyl ester in 5 µl is diluted with hexane to give a range of standards containing 0.1 to 1.0 ng methyl ester in 5 ul. The hexane solutions obtained from extracts of the soil are diluted where necessary so that the standard injection volume of 5 µl contains a methyl ester concentration within the linear portion of the calibration curve. Using the calibration, the amount of picloram methyl ester in the 5 µl of soil extract is determined and by calculation the amount of original picloram acid. A daily calibration is prepared and occasional injections of standards are made throughout a series of analyses to observe and

correct for any change in detector response.

Calculation:

$$\text{ng methyl ester in final 5 µl} \times \frac{\text{volume of final dilution in µl}}{\text{5 ul}} \times \frac{\text{dilution factor}}{1000} \times \frac{0.94}{\text{wt of sample (25g)}} \times \text{ppm picloram acid (air dry basis)}$$

Interferences:

Picloram has been successfully determined in soils containing up to 3.45% organic carbon using this method. Methods of clean-up are described in the original publication if required.

Recoveries and limits of detection:

The mean recovery of picloram for two soils of 3.45% and 3.09% organic carbon was 93%. Recovery determinations should be made with all unknown soils (see Fortification of soil). The practical limit for the detection of picloram in the two soils analysed was 0.05 ppm.

References

Leahy, J.S., and Taylor T. (1967) Analyst, 92, 371.

Vogel, A.T. (1956) Practical Organic Chemistry 3rd Edition, Longmans Green and Co. Ltd.

TRI-ALLATE

(S-2,3,3-trichloroallyl N,N-diisopropyl thiolcarbamate)

Substrate: Soil

Sample preparation and storage:

Moist soil samples from the field are crushed, mixed and passed through a 3 mm sieve to remove stones. If necessary the soil is allowed to air dry at 20°C until a suitable physical condition for the manipulations is attained. Complete drying is avoided as this causes loss of the herbicide. Soil samples awaiting analysis are deep frozen at -15° in polythene bags.

Method

Principle:

Samples are extracted with a mixture of 2,2,4-trimethylpentane and isopropyl alcohol, after which the extract is shaken with water and the aqueous phase discarded. Aliquots of the 2,2,4-trimethylpentane are injected into a gas chromatograph fitted with an electron capture detector, McKone & Hance (1967).

Apparatus and reagents:

Wrist-action flask shaker

Isothermal gas chromatograph fitted with a ^{63}Ni or tritium electron capture detector

250-ml stoppered conical flasks

7 cm glass filter funnels

stoppered test tubes (25 x 150 mm)

100-ml separating funnels

25 and 50-ml graduated flasks

2, 5, 10 and 50-ml pipettes

10 and 25 µl microsyringes

2,2,4-trimethylpentane

isopropyl alcohol

12.5 cm Whatman no. 42 filter paper

Na Cl 'AR' grade

anhydrous $Na_2 SO_4$ 'AR' grade

Procedure:

Samples are thawed out immediately prior to analysis and the moisture content is determined on a portion of the sample. A 25 g sample is weighed into a 250-ml stoppered conical flask. Fifty ml of mixed solvent (2,2,4-trimethylpentane and isopropyl alcohol, 2 to 1) is added, and the slurry is shaken for 30 minutes on a wrist-action shaker. After settling, the mixture is decanted and the supernatant liquid filtered through a fluted Whatman no. 42 paper into a test tube. A 25-ml aliquot of the filtrate is transferred by pipette into a 100-ml separating funnel and extracted with two 25-ml portions of distilled water, the lower aqueous phase being discarded each time. Approximately 0.5g sodium chloride is added at each

extraction to inhibit the formation of emulsions and to aid separation. After the second extraction, the remaining 2,2,4-trimethylpentane is run into a stoppered tube and shaken with anhydrous sodium sulphate. Aliquots of this solution are taken for gas chromatography.

Gas Chromatography

Operating conditions using a Pye 104 with ^{63}Ni detector

Column: 1.5 m x 4 mm i.d. stainless steel packed with 4% E 301 on 60/80 mesh Gas Chrom Q

Injector temperature: 200° Carrier gas flow 75 ml/min)

Column temperature: 185° Purge gas flow 25 ml/min) oxygen-free nitrogen

Detector temperature: 300° Detector voltage: pulse mode 150 usec

Attenuation: 5 or 10 x 10^2

Operating conditions using a Varian Aerograph with ^{3}H detector

Column: 1.5 m x 2 mm i.d. stainless steel packed with 5% E 301 on 60/80 mesh Gas Chrom Q

The conditions for injector and column are as above

Detector temperature: 200° Carrier gas flow: 100 ml/min oxygen-free nitrogen

Attenuation: 8 or 16 Sensitivity: x1

Detector voltage: 90 D.C.

Calibration:

A solution of tri-allate containing 1 mg/ml is prepared in 2,2,4-trimethylpentane. Using a 25 µl syringe, 25 µl of the solution is transferred to a 50-ml graduated flask and diluted to volume with 2,2,4-trimethylpentane. This solution containing 2.5 ng tri-allate in 5 ul is diluted with solvent to give a range of standards containing 0.1-1.0 ng tri-allate in 5 µl. The 2,2,4-trimethylpentane solutions obtained from extracts of the soil are diluted where necessary so that the standard injection volume of 5 µl contains a tri-allate concentration within the linear portion of the calibration curve. Using the calibration, the amount of tri-allate in the 5 µl of soil extract is determined. A daily calibration is prepared and occasional injections of standards are made throughout a series of analyses to observe and correct for any change in detector response.

Calculation:

To determine the ppm tri-allate in the sample use the following calculation:

$$\text{ng tri-allate in final 5 µl} \times \frac{\text{volume of final dilution in µl}}{5\ \mu l} \times \frac{\text{dilution factor}}{1000} \times \frac{1}{\text{wt of sample}} = \text{ppm tri-allate (wet basis)}$$

All results are normally reported in ppm on 100% dry soil basis.

Interferences:

Clean-up has not proved necessary for sandy loam soils (organic carbon 1.9%) but if the chromatogram of an untreated high organic matter soil shows evidence of interfering peaks, then a simple clean up procedure using Nuchar attaclay may be used (see other substrates).

Recoveries and limits of detection:

Experiments to determine the recovery of tri-allate from a fortified sandy loam soil (organic carbon 1.9%) showed that recovery was complete (see Fortification of soil). The limit of 0.025 ppm was arbitrarily chosen for routine use when this sandy loam soil is analysed.

Other substrates: straw and grain

Additional apparatus and reagents;

M.S.E. top drive macerator
3" diam. sintered glass funnel
vacuum filter flask
Hyflo Supercel (Hopkin & Williams Ltd)
Nuchar Attaclay (Varian Aerograph)
100-ml measuring cylinder

Procedure:

Chopped straw and grain samples are milled to pass a 2 mm screen. Teh grams of straw or 20 grams of grain is weighed into a macerator jar. Mixed solvent (50 ml) is added and the mixture blended for 3 minutes. The extract is filtered under vacuum through a $\frac{1}{2}$-inch layer of Hyflo Supercel prepared in a 3-inch diameter sintered glass funnel. The residue is washed with 40 ml of solvent and residual solvent pressed out with a small beaker. The filtrate is transferred to a 100-ml graduated cylinder and adjusted to 100 ml with solvent. A 25-ml aliquot is transferred by pipette into a 100-ml separating funnel and washed with two 25-ml portions of distilled water as in the soil method. The 2,2,4-trimethylpentane layer is run into a stoppered tube, 0.5g Nuchar attaclay is added and the mixture is shaken vigorously for 1 minute and then filtered immediately through a fluted Whatman no. 42 paper into a stoppered tube. Aliquots of this solution are taken for gas chromatography.

Gas chromatography: see method for soil

Recoveries and limits of detection:

Recovery experiments using fortified control samples showed 85% tri-allate recovered from straw and 95% from grain with practical limits of detection 0.05 and 0.025 ppm respectively

References

McKone, C.E. and Hance, R.J. (1967) J. agric. Fd. Chem., 15, 935

SIMAZINE

(2-chloro-4,6-bisethylamino-1,3,5-triazine)

The procedures described for atrazine are used.

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1. Susceptibility of ornamental plants to simazine and other chemicals. Trees and shrubs. November, 1964. G.W. Ivens. Out of print.

2. 3,5-Di-iodo-4-hydroxybenzonitrile. A progress report on experimental work by the A.R.C. Weed Research Organization. May-October, 1963. K. Holly and J. Holroyd. Out of print.

3. Chemical control of bracken. 1964. G.L. Hodgson. Out of print.

4. Susceptibility of ornamental plants to simazine and other chemicals. Annuals, biennials and herbaceous perennials. April, 1965. G.W. Ivens. Out of print.

5. A survey of the problem of aquatic weed control in England and Wales. October, 1967. T.O. Robson. Price - £0.25.

6. The botany, ecology, agronomy and control of Poa trivialis L. rough-stalked meadow-grass. November, 1966. G.P. Allen. Price - £0.25.

7. Flame cultivation experiments 1965. October, 1966. G.W. Ivens. Price - £0.25.

8. The development of selective herbicides for kale in the United Kingdom. 2. The methylthiotriazines. Price - £0.25.

9. The post-emergence selectivity of some newly developed herbicides (NC 6627, NC 4780, NC 4762, BH 584, BH 1455). December, 1967. K. Holly and Mrs. A.K. Wilson. Price - U.K. and overseas surface mail - £0.25; overseas airmail - £0.50.

10. The Liverwort, Marchantia polymorpha, L. as a weed problem in Horticulture; its extent and control. July, 1968. I.E. Henson Price - £0.25.

11. Raising plants for herbicide evaluation; a comparison of compost types. July, 1968. I.E. Henson. Price - £0.25.

12. Studies on the regeneration of perennial weeds in the glasshouse: I. Temperate species. May, 1969. I.E. Henson. Price - £0.25.

13. Changes in the germination capacity of three Polygonum species following low temperature moist storage. June, 1969. I.E. Henson. Price - £0.25.

14. Studies on the regeneration of perennial weeds in the glasshouse. II. Tropical species. May, 1970. I.E. Henson. Price - U.K. and overseas surface mail - £0.25; overseas airmail £0.50.

15. Methods of analysis for herbicide residues in use at the Weed Research Organization. December, 1970. R.J. Hance & C.E. McKone. Price - U.K. and overseas surface mail - £0.25; overseas airmail - £0.50.