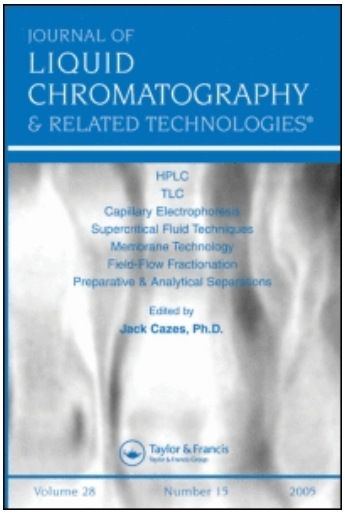

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A FAST, SIMPLE METHOD FOR ANALYSIS OF PHENOXY ACID SALT AND ESTER FORMULATIONS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

Salt formulations of 2,4-D (2,4-dichlorophenoxyacetic acid), 2,4,5-T (2,4,5-trichlorophenoxyacetic acid) and dichlorprop [2-(2,4-dichlorophenoxy)propanoic acid] have been analysed by reversed-phase HPLC using a C18-column with 50:50 (v/v) acetonitrile/2% acetic acid as eluant. Internal and external standard HPLC methods are compared.

Ester formulations of 2,4-D and 2,4,5-T are analysed, without hydrolysis, on the same column using 60:40 (v/v) acetonitrile/2% acetic acid as eluant. The method has been used in this laboratory to determine free phenoxy acid in ester formulations, and for the identification of esters in mixed ester formulations.

The methods are fast and accurate, and offer some advantages over previously-described methods.

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INTRODUCTION

High performance liquid chromatography (HPLC) methods of analysis of the formulated phenoxy acid herbicides have been described in detail by Lawrence (1; and references therein).

2,4-D (2,4-dichlorophenoxyacetic acid), 2,4,5-T (2,4,5-trichlorophenoxyacetic acid) and MCPA (2-methyl-4-chlorophenoxyacetic acid) salts are analysed by ion-exchange or reversed-phase chromatography, using salicylic acid or p-bromophenol as internal standards. Stevens (2) described a reversed-phase method for 2,4-D/picloram (4-amino-3,5,6-trichloropicolinic acid) formulations using automated 2 μL injections of undiluted formulation onto a 9 mm i.d. Partisil 10-25-ODS column. The large column required high solvent flow rates and high pressures, necessitating modifications to the injector so that reproducible results could be obtained. The method precision was then as good as ±0.6% relative without the use of an internal standard.

Alternatively, the phenoxy (and other) acids can be analysed by adsorption chromatography (1). The method requires extraction of the acids from an acidified aqueous solution with chloroform-diethyl ether.

Previously published methods of ester analysis require saponification of the esters with a KOH solution containing internal standard, and then analysing the acids as before (1). Using one of the above procedures, Skelly et al. (3) noted that the unhydrolysed ester had a retention time greater than two hours, and suggested the method could be used to determine free 2,4-D acid in ester products.

The methods described here are refinements of procedures which have been used in this laboratory for several years and have been found to be fast, accurate, and trouble-free.

MATERIALS AND METHODS

Equipment

LC : A Varian Vista series 5000 liquid chromatograph (LC) was fitted with a Varian 8055 autosampler with a 10 µL sample loop.

Column : Two columns have been used successfully:- a 30 cm x 4 mm i.d. stainless steel column containing octadecylsilane (C18)-bonded silica of 10 micron particle size (Varian MCH-10) or a Waters Radial-Pak C18 cartridge column. A guard column of pellicular C18 packing material was replaced as necessary.

Detector : Varian UV-50 variable wavelength detector set at 290 nm.

Integrator : Varian CDS401 Chromatography Data System.

HPLC Solvents

Waters HPLC grade methanol (MeOH) and acetonitrile (ACN) were used as received.

2% Acetic acid (HAc) (pH 2.62) was prepared from BDH AR grade acetic acid and glass-distilled water which had been passed through a 0.45 micron filter.

Column eluant was 50:50 or 60:40 ACN/2% HAc (v/v) for salt or ester formulations, respectively. Flow rate was 2 ml/minute.

Preparation of Pure Phenoxy Acids

Technical phenoxy acids were obtained from ICI Australia Operations Pty Ltd or Nufarm Chemicals Pty Ltd. Alternatively, technical acid can be extracted from commercial salt formulations by acidification with 10% H_2SO_4 and extraction of the precipitated acid with diethyl ether.

Pure phenoxy acids were obtained by recrystallization of the technical acid from commercial grade toluene or benzene followed by a second recrystallization from LR grade MeOH/distilled H_2O of

indefinite proportions (50/50 to 80/20, v/v). The resulting acids were clean, white and chromatographically pure, with the exception of 2,4,5-T and fenoprop [2-(2,4,5-trichlorophenoxy)-propanoic acid] which always contained a small (3 to 5%) proportion of 2,4-D and dichlorprop [2-(2,4-dichlorophenoxy)-propanoic acid], respectively.

Melting points of the acids so prepared were determined using a Mettler FP61 melting point apparatus. The measured values were in excellent agreement with published literature values (4,5).

Preparation of 2,4-D and 2,4,5-T Esters

Esters were prepared from technical grade 2,4-D and 2,4,5-T after recrystallization from laboratory grade toluene.

100 g 2,4-D (≃ 0.45 mole) or 2,4,5-T (≃ 0.39 mole) was heated with constant stirring under reflux with an equivalent molar volume of the required alcohol plus 40 ml toluene, with 1 ml 98% H_2SO_4 as catalyst. Toluene/water azeotrope was collected using a modified Dean & Starke apparatus. Esterification was judged complete (40 to 60 mins) when temperature in the reaction vessel began to rise rapidly (above 110°C). Heating was stopped immediately as excessive or prolonged heat led to the breakdown of the esters and the production of phenols.

The ester was taken up in 50 to 100 ml diethyl ether, washed with 5% $NaHCO_3$ solution (250 ml) plus 1 or 2 ml triethanolamine, then with 3 x 200 ml distilled water. The aqueous layers were discarded, and the ether layer filtered through a Whatman 1PS phase separating paper into a column (6 x 2.5 cm) of anhydrous Na_2SO_4. The ether was distilled off with mild heat and constant stirring, and the ester analysed by HPLC to determine free acid content and by a titrimetric procedure to determine the % w/w 2,4-D or 2,4,5-T.

Preparation of Phenoxy Acid Standards

(a) Internal standard method

(i) Internal standard 3 mg/ml salicylic acid. Weigh accurately 3 g salicylic acid (AR grade) into a one litre volumetric flask and dissolve in 50:50 ACN/2% HAc.

(ii) Phenoxy acid standards 6 mg/ml. Weigh accurately 150 mg phenoxy acid standard into a 25 ml volumetric flask and make up to volume with internal standard solution.

(iii) Samples - Weigh the formulated acids as their salts into a 25 ml volumetric flask, to give an acid concentration (150 mg) approximately equal to the standard. Make the formulation just acid to phenolphthalein indicator by the addition of one or two drops of concentrated acetic acid, and add 25.0 ml internal standard solution. (A drop of phenolphthalein indicator does not interfere with the HPLC analysis).

(b) External standard method

(i) Phenoxy acid standards 6 mg/ml. Dissolve 150 mg phenoxy acid standard in 50:50 ACN/2% HAc in a 25 ml volumetric flask.

(ii) Samples - Prepare samples as before by weighing sufficient of the formulated acid to give 150 mg acid/25 ml solvent. Make the formulation just acid to phenolphthalein indicator with concentrated acetic acid, and make up to the mark with 50:50 ACN/2% HAc.

2,4-D and 2,4,5-T Esters

(a) Internal standard of 3 mg/ml salicylic acid was prepared as before in 60:40 ACN/2% HAc.

Pure (95 to 99%) 2,4-D and 2,4,5-T esters prepared as above were dissolved in internal standard solution to give standard solutions containing 6 mg/ml acid equivalent 2,4-D or 2,4,5-T.

(b) Samples

Sufficient commercial formulated ester (200 to 400 mg) was weighed into a 25 ml flask to give a final concentration about 6 mg/ml ae 2,4-D or 2,4,5-T. Twenty-five ml internal standard solution were added, and the solution filtered through a 0.2 micron teflon filter, using solvent resistant syringes and filter-holders.

Linearity of the Detector

The useful linear range of the detector was tested using a number of 2,4-D standards ranging from 60 to 300 mg/25 ml (2.4 to 12 mg/ml).

Quantitative Volumetric Method

The same samples as were used in the HPLC procedures were analysed by a quantitative volumetric procedure (4). The method does not distinguish the acids, so that, for mixed 2,4-D/2,4,5-T esters, the results are calculated in gram equivalents of 2,4-D.

UV-Spectra

The spectra of selected acids and esters from 240 to 320 nm were recorded on a Varian DMS100 spectrophotometer using 1 cm silica cells. Most of the spectra were then checked on a Jasco Uvidec 650. The recorded peak maxima thus obtained were within ± 1 nm, while the calcualted values of ε agreed within 3%.

Concentrations of the acids and esters were all approximately 170 µg/ml (6 to 8 x 10^{-3} moles/L) in ethanol.

RESULTS

Linearity and Accuracy Tests

The detector was found to be linear over the range 0 (blank run, solvent only) to 300 mg 2,4-D/25 ml. Concentrations of

TABLE 1

Phenoxy Acid Content of Formulated Herbicides

Sample	Nominal Value (g/L)	Found		
		HPLC Values*		Total extractable acid* (CIPAC Method)
		Int. std.	Ext. std.	
2,4-D amine salt	500	504	495	511
2,4-D amine salt	500	509	497	511
dichlorprop potassium salt	600	584	581	615
dichlorprop potassium salt	600	584	581	614
dichlorprop potassium salt	600	584	582	615
2,4,5-T amine salt	200	197	190	211

* Means of three replicates

TABLE 2

Unadjusted Retention Times of Phenoxy Acid Herbicides on a Waters Radial-Pak C18 Cartridge Column Using 50:50 ACN/2% HAc as Eluant at a Flow Rate of 2 ml/minute

Herbicide	Retention time (minutes)
salicylic acid (internal standard)	2.55
2,4-D	3.81
2,4,5-T	5.12
dichlorprop	4.98
fenoprop	6.87
MCPA	3.98
2,4-DB	6.73

TABLE 3

2,4-D and 2,4,5-T Content (g/L Acid Equivalents) of Some Commercial Ester Formulations

Sample	Nominal Value (g/L)	Found	
		HPLC Procedure	Titrimetric* Procedure
2,4-D ethyl ester	800	713	684
2,4,5-T butyl ester	400	393	400
2,4,5-T butyl ester	400	393	402
2,4,5-T butyl ester	800	799	818
2,4-D ethyl/2,4,5-T butyl mixed esters	400	383	380**
2,4-D ethyl/2,4,5-T butyl mixed esters	400	420	391**

* Means of three replicates
** as 2,4-D (see Materials and Methods)

2,4-D greater than 12 mg/ml "saturated" the detector/integrator system.

Sample results

Analysis of Variance of the above data revealed that the results of the internal and external standard methods did not differ ($F_{1,11} = 1.01$) but that the two HPLC methods differed significantly from the titrimetric procedure ($F_{2,17} = 16.4$).

DISCUSSION

The HPLC methods described here are largely the same as the AOAC methods (1). However, the changes, though small, offer the chromatographer some advantages over the AOAC methods.

TABLE 4

Unadjusted Retention Times of Phenols, Free 2,4-D and 2,4,5-T Acids, and 2,4-D and 2,4,5-T Esters on a Waters Radial-Pak C18 Cartridge Column, Using 60:40 (v/v) ACN/2% HAc at 2 ml/minute

Compound	Retention Time (minutes)
2,4-D	2.24
2,4,5-T	2.64
2,4-dichlorophenol	2.67
2,4-DB	3.07
2,4,5-trichlorophenol	3.37
2,4,6-trichlorophenol	3.51
2,4-D methyl ester	3.71
2,4-D methoxyethyl ester	4.04
2,4-D ethyl ester	4.49
2,4-D iso-propyl ester	5.40
2,4-D n-propyl ester	5.53
2,4,5-T ethyl ester	5.80
2,4-D iso-butyl ester	6.70
2,4-D n-butyl ester	7.02
2,4-D amyl* ester	8.68
2,4-D n-pentyl ester	9.05
2,4,5-T butyl ester	9.30
2,4,5-T amyl* ester	11.67
2,4-D n-hexyl ester	12.10
2,4-D n-heptyl ester	16.71
2,4-D iso-octyl** ester	18.26)
	19.80)
2,4-D n-octyl ester	22.59
2,4,5-T iso-octyl** ester	24.90)
	26.84)
2,4-D n-decyl ester	48.10

* 3-methylbutyl ester

** prepared from commercial iso-octyl alcohol mixture

TABLE 5

Peak Maxima (λ max) and Calculated Molar Extinction Coefficients (ε) of Some Herbicides Recorded on a Varian DMS100 Spectrophotometer

Herbicide	λ max (nm)	ε	Herbicide	λ max (nm)	ε
2,4-D	284	2400	MCPA	279	2057
	292	2086		286	1792
2,4,5-T	289	2871	2,4-DB	285	2573
	297	2620		293	2243
dichlorprop	284	2230	2,4-D methyl ester	283	2390
	292	1889		291	2048
fenoprop	290	3006	2,4-D amyl ester	283	2886
	298	2690		291	2490

The ACN/2% HAc solvent (6) is more easily prepared than the other solvents given by Lawrence (1), and offers practical advantages over buffer solvents for the chromatographer who must change solvents and columns constantly for different procedures. A forgetful change to an immiscible solvent can precipitate buffer salts in a pump and/or column, and ruin both. The concentration of the acetic acid is not crucial, and a change from 2% HAc (pH 2.62) to 3% HAc (pH 2.60) does not affect the results obtained.

The use of 50:50 ACN/2% HAc at 2 ml/minute allows an analysis of the phenoxy acids in as little as five minutes, but does not separate all peaks of interest. However, the phenoxy acids which are not well separated are not commonly found in mixtures (in Australia), so that few problems should arise. For others, increasing the amount of aqueous phase (2% HAc) and reducing the flow rate of solvent improves resolution and allows separation of difficult pairs, including phenols, but increases the analysis time.

Acetonitrile was preferred to methanol as solvent because it gives better resolution at lower column back-pressures, but ACN is more expensive. System changes which involve the use of methanol rather than acetonitrile and the use of other C18 columns should not materially affect the results.

Accuracy of the external standard method, determined by running a series of 2,4-D standards against each other, was 3 to 4%, but this improved to ± 1 to 2%, when the standard and unknown were within 50 mg/25 ml of each other, i.e. in the range 100-200 mg/25 ml for a 150 mg/25 ml standard. Triplicate analyses usually gave a coefficient of variation (CV) of ± 0.5-1.0%, with only a few analyses giving a CV of ± 1.5%. This compares favourably with the ± 0.6% reported by Stevens (2), and is generally better than the results reported for the AOAC method (3,7). Both accuracy and reproducibility were improved by the inclusion of an internal standard, triplicate analyses then being accurate to within ± 1% with a CV of ± 0.5%.

Reducing the eluant flow rate from 2.0 to 1.5 or 1.0 ml/minute does not affect either the accuracy or the reproducibility of the results, but does allow for analysis of picloram and dicamba (2-methoxy-3,6-dichlorobenzoic acid) formulations.

The CIPAC titrimetric procedure (4) gave results which were statistically significantly greater than the two HPLC procedures. One source of error is that the CIPAK method measures impurities which may include other phenoxy acids, whereas only the specific phenoxy acid identified on the label need be measured by the HPLC procedure. However, even when such impurities are included in the HPLC analysis, the CIPAK procedure still overestimates the amount of phenoxy acid present.

The ester method reported here is again simple and fast, allowing triplicate analysis of many mixed ester formulations in less than 45 minutes.

A single injection allows simultaneous determination of the phenols and free phenoxy acids present in the formulation, as

well as both quantitative and qualitative analysis of the individual esters. In this the method is superior to the CIPAK procedure (4), where hydrolysis of the esters can lead to overestimation of the amount of free acid present in a formulation (8), and the other HPLC procedures (1) in which hydrolysis of the esters precludes estimation of the free acid or identification of the esters. Precision of the method is better than ± 1%, which compares favourably with other HPLC procedures (1,3,7).

A Consideration of the Use of Spectral Data in the Analysis

Peak maxima (λ max) and molar extinction coefficients (ε) of some phenoxy herbicides are listed in Table 5. The data are in good agreement with previously published values (9), except for the odd case where previously published values are erroneous (e.g. 2,4,5-T data in 10). The 2,4-dichloro- and 2,4,5-trichlorophenoxy herbicides exhibit a λ max at 290 ± 3 nm, so that a wavelength setting of 290 nm on a UV-detector allows sensitive and reproducible results to be obtained.

Spectra-structure correlations are discussed by Aly et al. (11) and will not be discussed here, except to say that the effect of -Cl substitution can be seen in the shift of λ max, e.g. in the series MCPA → 2,4-D → 2,4,5-T, while substitution of the acid group acetic → propionic → butyric produces little or no change. According to Van Peteghem and Heyndrickx (12), the absorption patterns and λ max are determined solely by the substitution of the aromatic nucleus; there are almost no differences among the spectra of (the methyl esters of) compounds with identical aromatic moieties. The constancy of ε with ester substitution thus allows HPLC calibration factors for all esters of the phenoxy acids to be calculated from a few selected esters of those acids.

One issue which has not been otherwise addressed in this paper is the consideration of whether this assay is isomer-

specific (3,7). Small changes in solvent composition and/or flow rate should allow the separation of the herbicidal 2,4-dichloro- and 2,4,5-trichloro- etc. phenoxy acids from their 2,5-, 2,6-, and 3,4- etc. analogues. If doubt remains or further confirmation is necessary, the use of UV-detectors which gather spectral data or determining absorbance ratios from chromatograms monitored at more than one wavelength should allow absolute confirmation of peak identity.

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