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# SEPARATION OF THE ENANTIOMERS OF FLUAZIFOP AND OTHER 2-PHENOXYPROPIONIC ACIDS USING CHIRAL METAL CHELATE ADDITIVES IN REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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## SUMMARY

Most of the work on the use of chiral ligand-exchange systems in reversed-phase liquid chromatography has been focussed on the resolution of the enantiomers of amino acids. This paper describes the novel use of L-prolyl-*n*-octylamide-Ni(II) in the mobile phase for the resolution of the enantiomers of fluazifop and other phenoxypropionic acids.

## INTRODUCTION

Several approaches have been used for the separation of enantiomers by high-performance liquid chromatography (HPLC). These include chiral bonded phases on silica, chiral ligand-exchange bonded phases and the addition of chiral selective reagents to the mobile phase. The applicability of these methods for enantiomer resolution depends on the functional groups present on the compound of interest. The chiral bonded phases described by Pirkle are generally applicable to a broader range of compounds than the chiral ligand-exchange systems, where most of the work has been focussed on the resolution of the enantiomers of amino acids.

Using the Pirkle type 1A columns[1,2] we have separated the enantiomers of the selective herbicide fluazifop butyl (I) and its major metabolite fluazifop (II), as its methyl ester. The Pirkle column is an amino propyl derivatised silica modified to give chiral resolution by ionically bonding the chiral acid (D)-N-(3,5-dinitrobenzoyl) phenylglycine. This produces a stable stationary phase as long as relatively non-polar mobile phases are used. However when using this column for the analysis of residues of fluazifop butyl (I) and fluazifop (II) on crop samples, the column performance deteriorated with concomitant loss of chiral selectivity, after 4–5 injections.

The use of chiral selective additives to the mobile phase was considered as an alternative approach. The chiral modification of commercially available reversed-phase columns by the addition of chiral ligands to the mobile phase has been described by Davankov *et al.*[3] and Karger and co-workers[4,5]. The main advantages of

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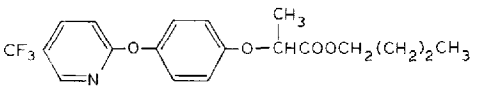
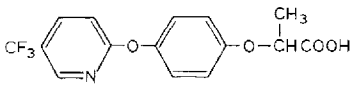


FLUAZIFOP BUTYL
(I)



FLUAZIFOP
(II)

this technique over the use of the Pirkle type 1A column is the higher stability of the chiral phase and its use with polar mobile phases. Karger has used both $C_3$–$C_8$ dien-Zn(II) and L-prolyl-*n*-octylamide-Ni(II) as the chiral metal chelate for the separation of amino acids and their dansyl derivatives. The alkyl chain ($C_8$) of the chiral ligand is adsorbed into the surface layer of the $C_8$ packing, with the ligand group on the surface available for chelation with metal ions and mobile ligands.

This paper describes the use of L-prolyl-*n*-octylamide-Ni(II) in the mobile phase for the resolution of the enantiomers of fluazifop and other phenoxy propionic acids.

## EXPERIMENTAL

### *Equipment*

High-performance liquid chromatography was performed using a Waters Assoc. 6000A pump, a U6-K manual injector and Model 440 absorbance detector. Chromatograms were recorded on a Phillips PM8251 single pen recorder.

An Ultrasphere ODS IP column was used (15 cm × 4.6 mm I.D.) supplied by Altex Scientific. The column temperature was controlled at 35°C using a Waters temperature control unit Model III.

The pH of the mobile phase was measured using an EIL 7050 laboratory pH meter.

### *Reagents*

L-Propyl-*n*-octylamide was synthesised following the method described by Tapuhi *et al.*[5]. HPLC grade acetonitrile and methanol were supplied by Rathburn Chemicals, Walkerburn, U.K. Analar grade acetic acid, ammonia solution and nickel sulphate were supplied by BDH, Poole, U.K. Purified water was obtained through a Milli-Q water purification system supplied by Millipore U.K., Harrow, U.K.

### *General procedures*

Mobile phases were prepared by dissolving L-prolyl-*n*-octylamide in the organic component of the mobile phase and the metal sulphate in the water component. The two components were then combined and the required volume of acetic acid added. Finally the pH was adjusted to the desired value using ammonia solution (sg. 0.880).

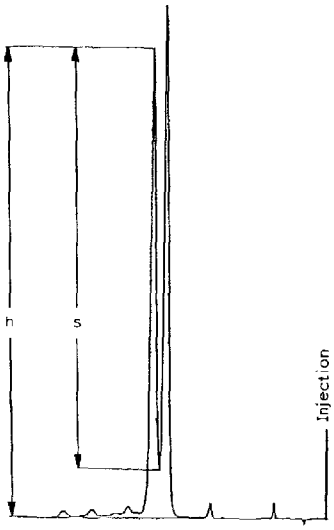



Fig. 1. Calculation of the separation factor. Separation factor = $(s/h) \cdot 100$.

The effects of the following factors were investigated on the separation of the enantiomers of fluazifop (II) as the model compound: pH, temperature, buffer concentration, solvent composition and the concentration of L-prolyl-*n*-octylamide-Ni(II) complex. The effects of other metal ions were also investigated using zinc acetate and copper sulphate as alternative salts to nickel sulphate.

The separation factor was used as a means of displaying the degree of enantiomer separation. A value of 100 indicates that complete separation has been achieved. Although the α-value [the ratio of $k'$(D) to $k'$(L)] were more sensitive to the conditions used, they do not indicate whether peak separation to the base-line has been obtained.

$$\text{Separation factor} = \frac{s}{h} \cdot 100$$

where $s$ and $h$ are measured as shown in Fig. 1.

## RESULTS AND DISCUSSION

Lindner *et al.*[4] found that the pH of the mobile phase was the major factor affecting complex formation and therefore chiral selectivity with the dansyl derivatives of amino acids. Using $C_3$–$C_8$ dien-Zn(II) complex as the chiral additive to the mobile phase, they achieved optimum chiral resolution at pH 9. The same pH was used in work with L-prolyl-*n*-octylamide-Ni(II). Our investigations have also shown that the pH of the mobile phase is the key factor in the separation of the enantiomers of fluazifop using L-prolyl-*n*-octylamide-Ni(II) complex in the mobile phase. However with fluazifop the greatest chiral recognition occurred at pH 7.0–7.5 as shown in Fig. 2. Retention ($k'$) of fluazifop also increased to a maximum at pH 7.0–7.5

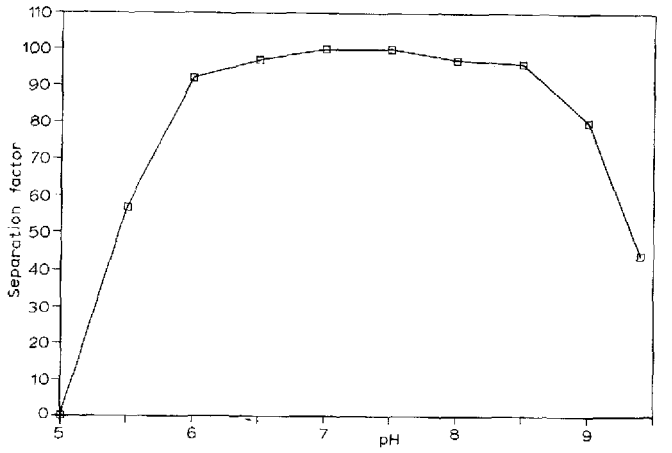



Fig. 2. Change in separation factor as a function of pH. Mobile phase: acetonitrile–methanol–water (35:15:50, v/v/v); L-prolyl-*n*-octylamide-Ni(II), 4 m*M*; ammonium acetate, 88 m*M*.

(Fig. 3). The different mobile phase pHs required for optimum chiral recognition by fluazifop and the dansyl derivatives of amino acids reflect the nature of the interactions when complexation takes place. Lidner *et al.*[4] has postulated that the dansyl derivatives of amino acids act as dianionic species in the basic mobile phase required for chiral recognition with $C_3$–$C_8$ dien-Zn(II) chelates. With fluazifop, the attraction between the anion of the carboxylic acid and the nickel cation in the L-prolyl-*n*-octylamide-Ni(II) will be the predominant effect and further coordination is probably between the ether linkage of the 2-phenoxypropionoic acid residue in fluazifop and the nickel in the L-prolyl-*n*-octylamide-Ni(II). As this involves the lone pair of electrons of the oxygens, the association will not be influenced by pH, so that the pH requirements will be governed solely by the needs for optimising the attraction be-

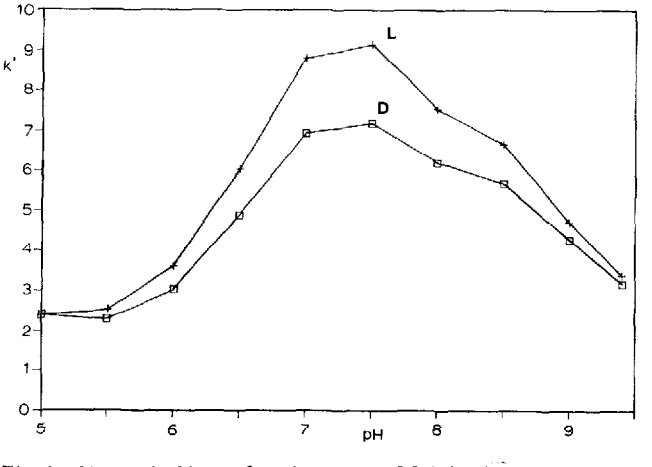
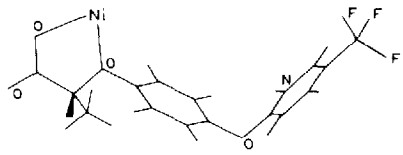
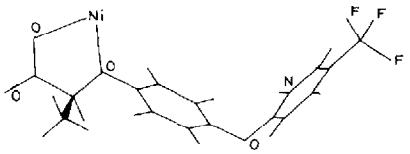



Fig. 3. Change in $k'$ as a function of pH. Mobile phase as in Fig. 2.




D-Fluazifop




L-Fluazifop

Fig. 4. Structures of D- and L-fluazifop showing the location of the nickel atom in the L-prolyl-*n*-octylamide-Ni(II) complex.

tween the carboxylic acid group and the nickel in the L-prolyl-*n*-octylamide complex.

Coordination between the ether linkage of the 2-phenoxypropionic acid residue and the nickel of the L-propyl-*n*-octylamide complex allows the formation of a five membered ring (Fig. 4). Lidner *et al.*[4] found that the dansyl derivatives of amino acids that formed five membered ring complexes with $C_3$–$C_8$ dien-Zn(II) gave high $k'$ values and greater selectivity due to the higher stability of the complex than those which formed six- or seven-membered ring complexes.

Apart from the pH of the mobile phase, other factors such as temperature, complex and metal ion concentration and organic modifier concentration had less effect on the chiral resolution.

We found that temperature had only a marginal effect on the retention of the enantiomers of fluazifop in contrast to the findings of Lidner *et al.*[4] with $C_3$–$C_8$ dien-Zn(II) complex. Over the temperature range 15–45°C $k'$(D) was reduced by 2% compared to 6% for the L-enantiomer of fluazifop. At 45–70°C the effect was slightly greater with $k'$(D) reduced by 10% and $k'$(L) by 11%. Chiral recognition was reduced, the value of $\alpha - 1$ falling by 19% from 15°C to 70°C. However the effect on the separation factor was very slight, the values being 100% at 15°C and 98% at 70°C. We selected 35°C as the optimal temperature for studying the effect of other factors.

Changing the metal ion to copper or zinc had very little effect. The separation of D- and L-fluazifop given by the Ni(II) and Zn(II) complexes of L-prolyl-*n*-octylamide is given in Fig. 5. The Zn(II) complex was less effective than the Ni(II) complex. Lidner *et al.*[4] had found that the elution order of the enantiomers of the dansylated amino acids was reversed on changing the metal from nickel to zinc. In our hands, the elution order for the fluazifop enantiomers was unchanged. The copper

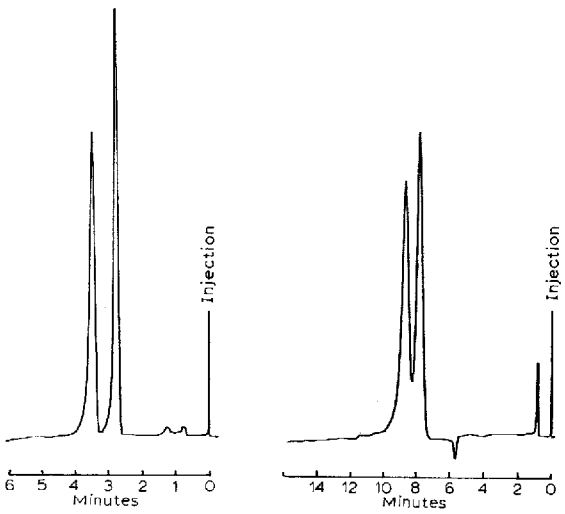



Fig. 5. Separation of the enantiomers of fluazifop (left) with mobile phase containing 4 m*M* L-prolyl-*n*-octylamide-Ni(II) and (right) with mobile phase containing 4 m*M* L-prolyl-*n*-octylamide-Zn(II).

complex gave a high background UV absorbtion, which made its use inappropriate.

Changing the mobile phase composition progressively from methanol–water (50:50, v/v) to acetonitrile–water (50:50, v/v) reduced the retention ($k'$) of the enantiomers and increased the separation factor as shown in Figs. 6 and 7. Davenkov *et al.*[3] noted a similar effect when using N-decyl-L-histidine-Cu(II) for the resolution of α-amino acids.

In view of the improved performance given by acetonitrile–water (50:50, v/v) this mobile phase composition was used in most of the remaining investigations.

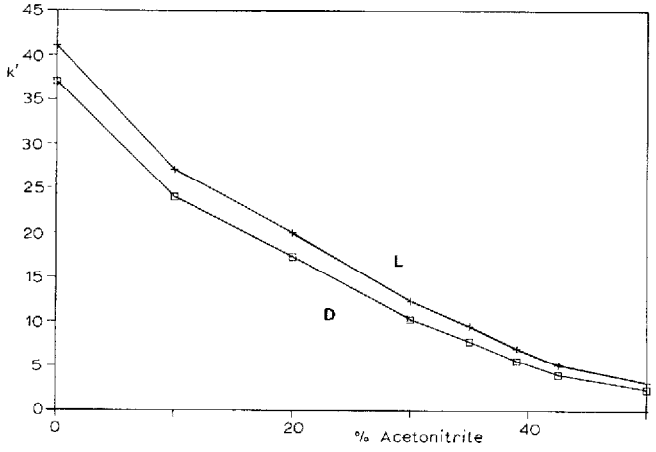



Fig. 6. Change in $k'$ as a function of solvent composition. Mobile phase: water, 50% (v/v); L-prolyl-*n*-octylamide-Ni(II), 4 m*M*; ammonium acetate, 88 m*M*; pH 7.5.

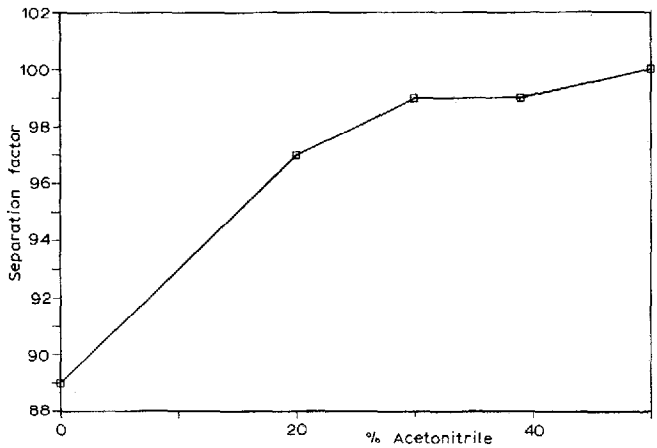



Fig. 7. Change in separation factor as a function of solvent composition. Mobile phase as in Fig. 6.

Increasing the ammonium acetate buffer concentration reduced the retention of the enantiomers of fluazifop as shown in Fig. 8. It is to be expected that the stability of the complex between fluazifop and L-prolyl-*n*-octylamide-Ni(II) would be reduced with increasing concentration of acetate ion.

$$[\text{L-propyl-Ni(II)}\ (OCOCH_3)_2] + [RCOO^-] \rightleftharpoons$$

$$[\text{L-prolyl-Ni(II)}\ RCOO\ CH_3COO] + [CH_3COO^-]$$

Over the range 5–40 m*M* ammonium acetate with the concentration of L-prolyl-*n*-octylamide-Ni(II) and 4 m*M*, the separation factor remained at 100%.

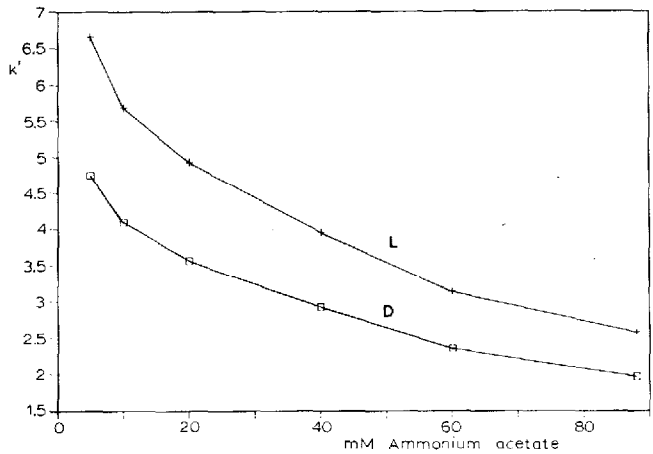



Fig. 8. Change in *k'* as a function of ammonium acetate concentration. Mobile phase: acetonitrile–water (50:50, v/v); L-prolyl-*n*-octylamide-Ni(II), 4 m*M*; pH 7.0.

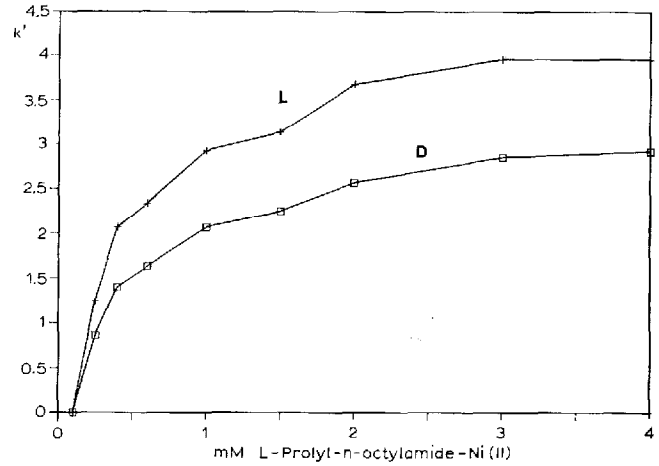



Fig. 9. Change in $k'$ as a function of the concentration of L-prolyl-*n*-octylamide-Ni(II). Mobile phase: acetonitrile–water (50:50, v/v), ammonium acetate at 10 times the concentration of nickel complex, pH 7.0.

The effect of the concentration of L-prolyl-*n*-octylamide-Ni(II) on the retention ($k'$) is given in Fig. 9 and on the separation factor in Fig. 10. Both $k'$ and the separation factor increase as the concentration of the nickel complex is increased, the increase in the separation factor was very rapid over the concentration range 0.1 m*M* (zero) to 0.4 m*M* (97), after which there was a gradual increase to a value of 100 at 2.0 m*M*. When working at low concentrations of the complex (0.25–0.4 m*M*) it was necessary to pump at least 45 column volumes before the system reached equilibrium. The process was more rapid at higher concentrations of the complex.

Residues of D-fluazifop in crops have been determined satisfactorily on a

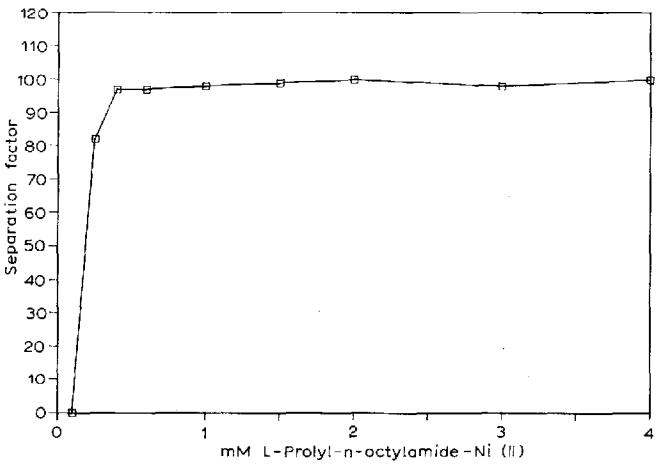



Fig. 10. Change in the separation factor as a function of the concentration of L-prolyl-*n*-octylamide-Ni(II). Conditions as in Fig. 9.

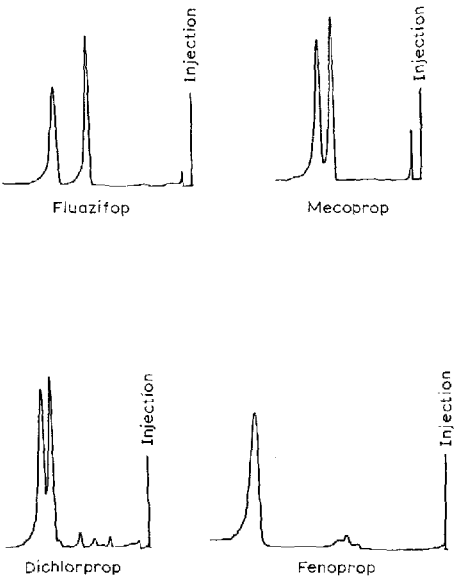



Fig. 11. Enantiomer separation for four 2-phenoxypropionic acid herbicides. Mobile phase: acetonitrile–water (40:60, v/v); L-prolyl-*n*-octylamide-Ni(II), 3.2 m*M*; ammonium acetate, 8 m*M*; pH 7.0.

routine basis by reversed-phase HPLC using L-prolyl-*n*-octylamide-Ni(II) in the mobile phase.

With suitable adjustments to the solvent ratio of the mobile phase, we were able to separate the enantiomers of 2-(4-hydroxyphenoxy)propionic acid, 2-(2-methyl-4-chlorophenoxy)propionic acid (Mecoprop), 2-(2,4-dichlorophenoxy)propionic acid (Dichlorprop), and 2-[4-(4-trifluoromethylphenoxy)phenoxy]propionic acid. We were, however, unable to separate the enantiomers of 2-(2,4,5-trichlorophenoxy)propionic acid (Fenoprop). The separation of the enantiomers of fluazifop, mecoprop and dichlorprop, together with the trace for fenoprop is shown in Fig. 11. The progressive loss of chiral recognition with increasing chlorine substitution of the phenoxy ring is believed to be due to the inductive effect of the chlorine atoms on the ion pair of electrons on the ether oxygen atoms.

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