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Note

Ligand-exchange chromatography

I. Resolution of L- and D-proline on a copper(II)–proline complex bound to microparticulate silica gel

K. SUGDEN, C. HUNTER and G. LLOYD-JONES

Reckitt and Colman Ltd., Pharmaceutical Division, Dansom Lane, Kingston-upon-Hull HU8 7DS (Great Britain)

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Of the two principal methods for achieving chromatographic resolution of amino acid enantiomers the conventional one involves reaction with a chiral compound to form diastereoisomers with subsequent separation by gas (GC)[1,2] or ion-exchange[3] chromatography. This method has the disadvantage in quantitative analysis in that derivatisation procedures can lead to the formation of interfering artefacts or result in racemisation of the enantiomers. The alternative method, involving on-column formation of diastereoisomers by interaction with optically active stationary phases is less likely to lead to racemisation and has been used extensively to resolve racemic acid esters by GC[4–9]. Some literature reports indicate that D- and L-amino acids can also be directly resolved by liquid chromatography using either a chiral eluent[10] or a ligand-exchange technique involving a copper(II)–proline complex bound to either a styrene–divinylstyrene copolymer[11] or an acrylamide porous gel[12]. This paper describes a rapid and efficient separation achieved by high-performance liquid chromatography using a copper(II)–proline complex bound to microparticulate silica gel.

EXPERIMENTAL

Preparation of the chiral stationary phase

LiChrosorb SI 60 (5 μm silica gel) (BDH, Poole, Great Britain) was refluxed with 2 *M* hydrochloric acid for 4 h, filtered, washed with water and acetone and dried in an oven at 110° for 16 h. The acid-washed material (10 g) was suspended in dry dioxane (200 ml) and 3-chloropropyltrichlorosilane (4 ml) was added. The mixture was refluxed for 4 h, filtered, washed with dioxane, water and acetone and dried on a water pump. The silanized silica gel was suspended in chloroform–methanol (85:15) (200 ml) containing L-proline (3 g), potassium iodide (1 g) and diisopropylethylamine (2.8 ml). The mixture was refluxed for 17 h, filtered, washed with methanol and acetone and dried on a water pump.

Column

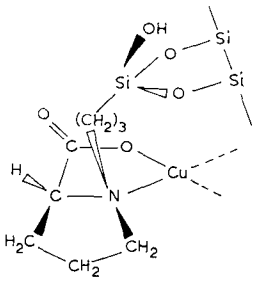
The chiral phase was packed, as a methanol slurry, into a 25 × 0.46 cm I.D. stainless-steel tube using a Magnus P5000 Slurry Packing Unit (Magnus Scientific, Sandbach, Great Britain). The copper(II)–proline complex was formed *in situ* by eluting the column with a mobile phase of 1 m*M* copper(II) acetate.

Apparatus

A Waters Model 6000A constant flow pump (Waters Assoc., Northwich, Great Britain) was used to provide mobile phase flow and an ACS ultraviolet detector model 750/11 (Applied Chromatography System, Luton, Great Britain), fitted with a 240 nm filter, was employed to monitor column eluent.

RESULTS AND DISCUSSION

The surface of the silica gel was chemically modified and then activated by the elution of Cu^{2+} to give a structure of the type:



The amount of L-proline bound to the silica gel was determined by elemental analyses (Table I) which, from the percentage carbon figures, indicate a 67.5% conversion of the silanized intermediate. Atomic absorption analysis of the complexed phase indicate that it contained 0.17% (w/w) copper.

TABLE I

ELEMENTAL ANALYSIS OF THE CHEMICALLY MODIFIED SILICA GEL

Sample	*C (%, w/w)*	*H (%, w/w)*	*N (%, w/w)*
Silanized intermediate	2.10	0.75	−0.02
3-Prolylpropyl–silica gel	3.62	0.89	0.27

The chiral phase was initially evaluated in the absence of any bound Cu^{2+} using water only as the mobile phase and was found to give capacity factors, $k' > 15$ with both L- and D-proline. On the introduction of 1 m*M* ammonium acetate into the mobile phase elution of the amino acid was rapid ($k' = 1.4$) but resolution of the two isomers was not observed. With 1 m*M* copper (II) acetate as the mobile phase, however, ligand exchange occurred and the L- and D- isomers were resolved

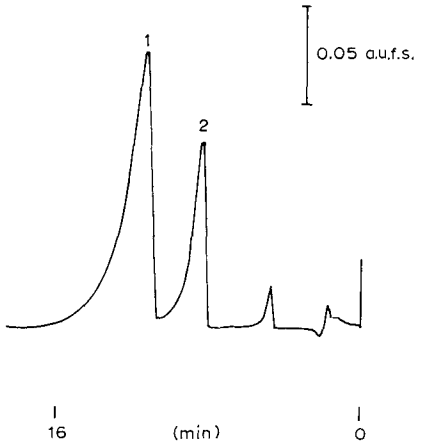


Fig. 1. Separation of D-proline (1) and L-proline (2). Column, copper (II)–proline complex bound to LiChrosorb 5 μm silica gel, 25 × 0.46 cm I.D. Mobile phase, 1 mM copper(II) acetate (pH 4.6) delivered at 3 ml min^{-1}. Detection, UV at 240 nm.

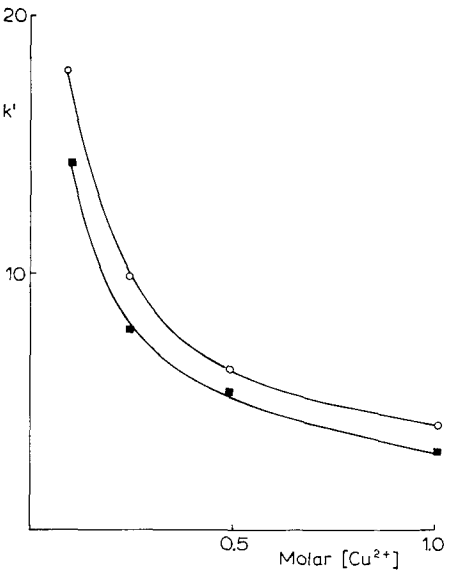


Fig. 2. Effect of Cu^{2+} ionic strength of the mobile phase on the k' values of D-(○) and L-proline (■).

(Fig. 1). Some peak tailing was observed and was thought to be due to the slow kinetics of ligand exchange in the bound metal coordination sphere.

The absolute retention of the proline isomers is inversely dependent upon the Cu^{2+} ionic strength of the mobile phase (Fig. 2) but, over the range studied, resolution is largely unaffected. The presence of Cu^{2+} in the mobile phase has the advantage that it allows the amino acids to be eluted as their copper(II) complexes, which exhibit a wavelength of maximum absorption of 240 nm, and thus avoids the need

to use end-absorption to achieve good sensitivity. The on-column limit of detection was found to be 400 ng L-proline at this wavelength.

The mobile phase pH range over which this chiral stationary phase can be successfully employed is narrow; at pH <3.9, the copper(II)–proline complex is unstable whereas at pH >5.6 column efficiency is observed to deteriorate.

Some work has been carried out using mobile phases containing other metal ions, such as Zn^{2+}, but complexation with the stationary phase has been found to be minimal with no observable resolution of the enantiomers.

L- and D-3,3-dimethylprolines have been completely resolved using the copper(II)-complexed stationary phase and partial resolution has been achieved with L- and D-phenylalanines. Attempts to resolve the isomers of histidine, alanine and glutamic acid have as yet proved unsuccessful but with further development the procedure may well prove to be useful as a method for the rapid evaluation of the stereochemical purity of α-amino acids.

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