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Note

Resolution of the neuroexcitatory N-methylaspartic acid (2-methylaminosuccinic acid) enantiomers by ligand-exchange thin-layer chromatography

MELVIN R. EUERBY[a]

Department of Pharmaceutical Chemistry, School of Pharmacy, University of London, 29–39 Brunswick Square, London WC1N 1AX (U.K.)

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Excitatory amino acid receptors which are thought to be activated physiologically by L-aspartic acid and/or L-glutamic acid[1–3] in the central nervous system of vertebrates have been classified into different types[3–6]. The N-methyl-D-aspartate receptor is probably the best characterised of all the receptor subtypes[7]. It is activated by N-methyl-D-(−)-aspartic acid (NMDA) and blocked by 2-amino-ω-phosphonoalkanoic acids, such as D-2-aminophosphonoheptanoic acid and D-2-aminophosphonovaleric acid[8]. Activation of the NMDA receptor is thought to play a role in learning, memory and central control of muscle tone. Alzheimer's and Huntington's diseases may also be linked to overactivation of the NMDA receptor, leading to progressive neuronal pathology and death[3].

The L-isomer of N-methylaspartic acid (NMA) is less effective in activating the NMDA receptor than its corresponding D-enantiomer, which is 10–1000 times more potent than L-glutamic acid depending on the type of test preparation[9,10]. Hence it is essential to establish the enantiomeric purity of the NMA enantiomers from commercial sources prior to pharmacological or biochemical studies. To date, the optical purity of these compounds has been ensured by optical rotation measurements. However, this method is insensitive to contamination with small quantities of minor enantiomers arising from racemisation on synthesis or storage.

Recently a ligand-exchange high-performance liquid chromatographic (HPLC) separation has been reported of the NMA enantiomers using a silica-bonded L-proline and a copper(II) acetate mobile phase system[11]. Whereas the separation of the enantiomers was excellent, the method was time-consuming requiring costly equipment. There has also been interest in the enantioselective separation of amino acids and derivatives by thin-layer chromatography (TLC) using a Chiral plate coated with a reversed-phase silica gel and impregnated with a chiral selector (a proline derivative) and copper(II) ions[12–14]. The separation of enantiomers is based on li-

[a] Address for correspondence: Analytical Development Section, Research and Development Laboratories, Fisons Pharmaceuticals PLC, Bakewll Road, Loughborough, Leicestershire LE11 0RH, U.K.

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gand-exchange chromatography as described by Davankov[15]. Therefore it was decided to evaluate the use of ligand-exchange TLC using the commercially available Chiral plates in the development of a convenient and enantioselective assay of NMA enantiomers.

EXPERIMENTAL

Reagents and chemicals

All chemicals and solvents were of analytical or HPLC grade. Ultra-pure water was obtained by means of a Milli-Q system (Millipore). NMDA was purchased from the following suppliers: Sigma, Aldrich and Cambridge Biochemical Research. L- and DL-NMA were purchased from Aldrich. Pre-coated TLC plates (Chiral plates®, catalogue No. 811055/056, size 10 cm × 20 cm, layer thickness 0.25 mm) were purchased from Machery–Nagel.

Chromatographic conditions

The Chiral plates were used immediately after activation, which involved heating the plates at 110°C for 15 min and cooling, as recommended by the manufacturers. The mobile phases were made up as described in Table I. The TLC chambers were equilibrated for 1 h prior to the chromatographic development.

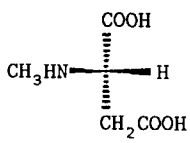
After development (15–18 cm, 30–90 min), the plates were left in the air to dry and subsequently sprayed with a 0.2% (w/v) solution of ninhydrin in acetone. The enantiomers were revealed as red-coloured derivatives on a white background after heating at 100°C for 10 min.

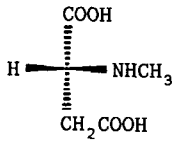
Preparation of the standard NMA solutions

Stock solutions of the individual enantiomers were prepared, freshly each day,

TABLE I

EFFECT OF ELUENT COMPOSITION ON THE R_F VALUES OF THE N-METHYLASPARTIC ACID ENANTIOMERS





Acetonitrile–methanol–water ratio	*R_F NMLA*	*R_F NMDA*
5.0:1.0:1.0	0.42	0.34
4.0:1.0:1.0	0.45	0.39
3.0:1.0:1.0	0.49	0.45
2.0:1.0:1.0	0.52	0.52
1.0:1.0:1.0	0.60	0.66
0.6:1.0:1.0	0.58	0.67
0.0:1.0:1.0	0.60	0.68

in water at a concentration of 10 mg/ml. A 2-μl volume of each solution was applied to the plate and dried with the aid of a cold-blowing hair drier before development.

RESULTS AND DISCUSSION

Ligand-exchange TLC utilising either home-prepared or commercially available Chiral plates which are coated with the chiral selector (2*S*,4*R*,2′*RS*)-4-hydroxy-1-(2′-hydroxydodecyl)proline have been most commonly developed with acetonitrile–methanol–water mixtures, the most popular ratios being 4:1:1 and 0.6:1:1 (v/v/v) mixtures as mobile phases. With the latter system, excellent separation and reproducible R_F values could be obtained for the enantiomers of NMA. The level of detection of the L- in the D-enantiomer was less then 0.5% (w/w) using ninhydrin spray to visualise the enantiomers. The optical purity of the L- and D-enantiomers of NMA from differing commercial sources was shown to be better than 99.5% (no detectable levels of the opposite enantiomer were observed). Use of the 4:1:1 (v/v/v) mobile phase failed to give such tight separation of the enantiomers but surprisingly the elution order of the enantiomers had been reversed, *i.e.* the elution order was L- before D-enantiomer (4:1:1) where, as previously, it had been the D- before the L-enantiomer (0.6:1:1).

To investigate this phenomenon further, acetonitrile–methanol–water mobile phases with ratios varying from 0:1:1 to 5:1:1 (v/v/v) were employed. The results shown in Table I show that at ratios of 3:1:1 to 5:1:1 the L-enantiomer elutes before the D-enantiomer, whereas at ratios of 1:1:1 to 0:1:1 the D-enantiomer elutes before the L-enantiomer. At the latter ratios the R_F values for both enantiomers reached a plateau. A ratio of 2:1:1 resulted in co-elution of the enantiomers.

Brinkman and Kamminga[12] have previously examined the effect of varying the acetonitrile content in the mobile phase on the R_F values for the enantiomers of glutamine, phenylalanine, isoleucine, norleucine and norvaline but failed to observe a reversal in enantioselectivity on changing the acetonitrile content of the mobile phase. The reversal of enantioselectivity observed in this paper may be attributed to acidic amino acids only.

The involvement of hydrophobic interactions in the separation mechanism is thought to be small as the R_F values did not increase on increasing the acetonitrile content of the mobile phase. The change in selectivity of the system on varying the acetonitrile to methanol and water concentration of the eluent is probably a direct consequence of the change in polarity of the resultant eluent. The TLC method described is sensitive, fast and very convenient, the plates being commercial available; it has been reported that the method is amenable to quantification by the use of a densitometer[13].

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