Journal of Chromatography, 588 (1991) 348–351
Elsevier Science Publishers B.V., Amsterdam

CHROM. 23 772

Short Communication

High-performance liquid chromatographic analysis of diastereomers and enantiomers of pyrroloisoquinoline antidepressants

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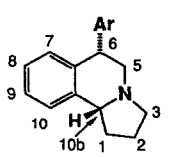
(First received September 10th, 1991; revised manuscript received September 26th, 1991)

ABSTRACT

To determine the diastereomeric and enantiomeric purity of different sets of isomers of pyrroloisoquinoline compounds in a reaction mixture, two general direct high-performance liquid chromatographic (HPLC) methods were developed: a reversed-phase HPLC method for diastereomers and a normal-phase HPLC method for enantiomers. These methods allowed us to monitor the course of reactions; changes in reaction variables were made until optimized conditions were identified to provide enantiomerically pure products.

INTRODUCTION

Hexahydropyrrolo[2,1-a]isoquinoline compounds (**1**) were developed as potential antidepressant agents [1–5]. Biological activity in the series resides with the *trans*-[6a,10b] diastereomers [3]. In general, the active enantiomer has the *R* configuration at the 10b position [6,7]. Herein we present methods to determine diastereomeric and enantiomeric purity in the series.



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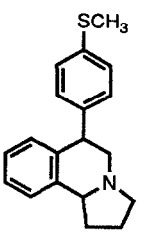
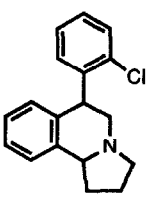
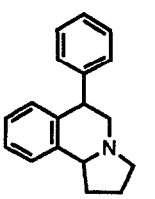
For this study, three sets of pyrroloisoquinoline compounds (**1A**,**1B** and **1C**) were prepared [7–9]. A reversed-phase high-performance liquid chromatography (HPLC) method, using a 25 cm × 4.6 mm Supelcosil-LC-18-DB (5 μm) column, was developed to determine the diastereomeric purity of each set. A normal-phase system, using a 25 cm × 4.6 mm Chiralcel OD (10 μm) column, was developed to determine the enantiomeric purity.



1A



1B



1C

These methods do not require derivatization of a compound before and/or after analysis. Both methods are linear in the tested concentration range

0021-9673/91/$03.50

(0.01–0.50 mg/ml for diastereomeric purity and 0.01–0.15 mg/ml for enantiomeric purity) with a correlation coefficient of 0.99. The lower detection limit of both methods is 0.006 mg.

EXPERIMENTAL

Materials

Pure standards of sets **1A** [McN-5652 (RWJ 35652) and McN-5655 (RWJ 35655)], sets **1B** [McN-4612 (RWJ 34612) and McN-4111 (RWJ 34111)], and sets **1C** [McN-5707 (RWJ 35707) and McN-5706 (RWJ 35706)] were prepared in our laboratory [7–9]. HPLC-grade acetonitrile, hexane, isopropanol and reagent-grade triethylamine were purchased from Fisher Scientific. Water was purified with a Millipore Milli-Q system.

Instrumentation

The chromatographic system consisted of a Waters (Milford, MA, USA) Model 600 pump, Model 490 variable-wavelength detector, U6K injector and Model 740 data system. The 25 cm × 4.6 mm Supelcosil-LC-18-DB (5 μm) column was purchased from Supelco (Bellefonte, PA, USA) and 25 cm × 4.6 mm Chiralcel OD (10 μm) column was purchased from J. T. Baker (Phillipsburg, NJ, USA).

Chromatographic conditions

The HPLC conditions are listed below.

(1) Diastereomeric purity method: mobile phase, 0.01 *M* triethylamine buffer (pH 6.2)–acetonitrile (25:75), flow-rate, 2.0 ml/min; injection volume, 10 μl; detection wavelength, 220 nm; detector sensitivity, 0.5 a.u.f.s.; column temperature, 35°C.

(2) Enantiomeric purity method: mobile phase, hexane–isopropanol–triethylamine (80:20:0.1); flow-rate, 1.0 ml/min; injection volume, 10 μl; detection wavelength, 220 nm; detector sensitivity, 0.5 a.u.f.s.; column temperature, ambient.

Calibration graphs

(1) Diastereomeric method: stock solutions of three sets of diastereomers, **1A**, **1B** and **1C** (as defined above), were prepared in acetonitrile at 2.0 mg/ml each. A series of dilutions were made to obtain the concentration range of 0.01–0.40 mg/ml. Four standards were analyzed and the calibration graphs of concentration *versus* area were plotted to check the linearity of the method. Using a least-squares analysis it was determined that the method is linear with a correlation coefficient of 0.99 in the tested concentration range. The calibration graphs were straight lines passing through the origin.

(2) Enantiomeric method: stock solution of racemic McN-5652 (RWJ 35652), McN-5707 (RWJ 35707) and McN-4612 (RWJ 34612) were prepared at 1.0 mg/ml concentration in hexane–isopropanol (80:20) solution. Four standards of each in the concentration range of 0.01–0.15 mg/ml were prepared by serial dilution and analyzed. The graph of concentration *versus* area was plotted to check the linearity of the method. Using a least-squares analysis it was determined that the method is linear with a correlation coefficient of 0.99 in the tested concentration range. The calibration graphs were straight lines which passed through the origin.

RESULTS AND DISCUSSION

Figs. 1–3 represent typical chromatograms of each set of diastereomers illustrating the separation achieved in the diastereomeric method. In each set of diastereomers, the *trans*-[6a,10b] diastereomer eluted as the second peak.

Figs. 4–6 represent typical chromatograms of McN-5652 (RWJ 35652), McN-5707 (RWJ 35707)

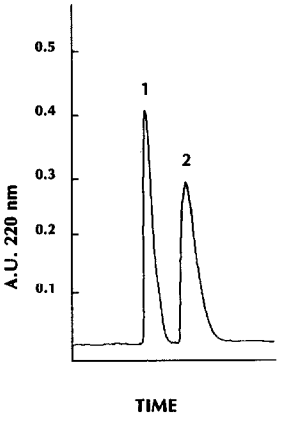


Fig. 1. Separation of McN-5652 (RWJ 35652) and McN-5655 (RWJ 35655) diastereomers. Chromatographic conditions: 25 cm × 4.6 mm I.D. Supelcosil-LC-18-DB (5 μm) column; 0.01 *M* triethylamine buffer (pH 6.2)–acetonitrile (25:75); flow-rate 2.0 ml/min; column temperature 35°C; detector wavelength 220 nm. Peaks: 1 = McN-5655 (retention time 3.25 min); 2 = McN-5652 (retention time 4.13 min).

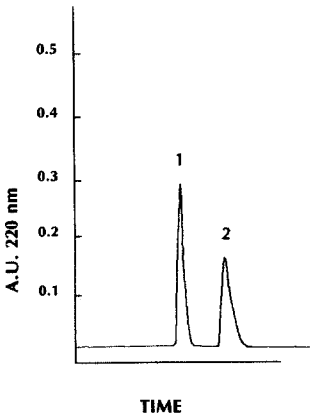


Fig. 2. Separation of McN-5707 (RWJ 35707) and McN-5706 (RWJ 35706) diastereomers. Chromatographic conditions as in Fig. 1. Peaks: 1 = McN-5706 (retention time 5.47 min); 2 = McN-5707 (retention time 7.46 min).

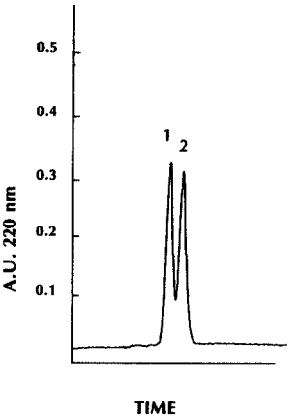


Fig. 4. Separation of McN-5652 (RWJ 35652) enantiomers. Chromatographic conditions: 25 cm × 4.6 mm I.D. Chiralcel OD (10 μm) column; hexane–isopropyl alcohol–triethylamine (80:20:1); flow-rate 1.0 ml/min; column temperature ambient; detector wavelength 220 nm. Peaks: 1 = 4.09 min; 2 = 4.40 min.

and McN-4612 (RWJ 34612) enantiomers demonstrating the separation achieved in the enantiomeric method. In each set of enantiomers the active enantiomer (*R*-configuration at the 10b position) eluted as the second peak.

Using calibration graphs of each with a linear regression equation we determined that both methods are linear throughout the concentration range studied with the correlation coefficient of 0.99.

These methods allowed us to monitor the course of reactions; changes in experimental conditions were made until optimized conditions were identified to provide enantiomerically pure products.

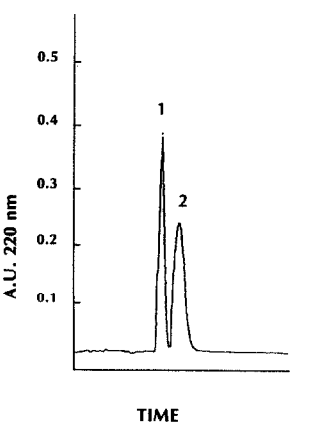


Fig. 3. Separation of McN-4612 (RWJ 34612) and McN-4111 (RWJ 34111) diastereomers. Chromatographic conditions as in Fig. 1. Peaks: 1 = McN-4111 (retention time 4.21 min); 2 = McN-4612 (retention time 5.34 min).

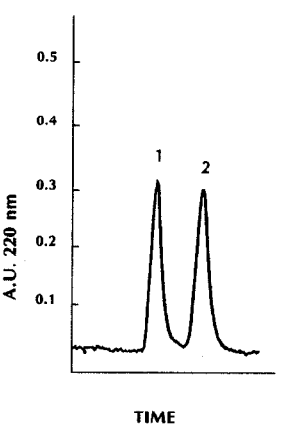


Fig. 5. Separation of McN-5707 (RWJ 35707) enantiomers. Chromatographic conditions as in Fig. 4. Peaks: 1 = 3.75 min; 2 = 4.37 min.

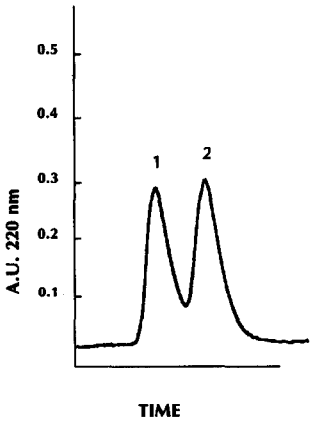


Fig. 6. Separation of McN-4612 (RWJ 34612) enantiomers. Chromatographic conditions as in Fig. 4. Peaks: 1 = 4.12 min; 2 = 4.47 min.

ACKNOWLEDGEMENTS

We thank B. E. Maryanoff, K. L. Sorgi and D. F. McComsey for supplying samples for analysis.

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