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ION-PAIR LIQUID CHROMATOGRAPHY OF AMITRIPTYLINE AND METABOLITES IN PLASMA

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SUMMARY

A method is described for the determination of amitriptyline and nortriptyline in the plasma of patients during treatment. The method is based on liquid chromatography of the amines as ion-pairs with perchlorate. The separation column is packed with silica gel, and a mixture of diisopropyl ether, dichloromethane, methanol and water containing the counter ion is used as eluent. High efficacy and stability of the system is achieved. The separation of metabolites is demonstrated. The *cis*- and *trans*-10-hydroxy isomers of amitriptyline and nortriptyline are easily resolved with this chromatographic system.

INTRODUCTION

Amitriptyline and its N-demethylated metabolite (nortriptyline) are two important tricyclic antidepressant drugs in widespread clinical use. Studies have shown that the therapeutic as well as the adverse effects of these drugs are related to the plasma concentrations achieved during treatment[1–8] and several liquid chromatographic methods for the determination of tricyclic antidepressant drugs have been described[9–12].

Ion-pair partition chromatography is a highly suitable technique for the measurement of these drugs[13–15] and the present study is a modification of this technique that offers advantages with respect to stability and reproducibility compared with an earlier system[15]. By using this method, therapeutic plasma concentrations of amitriptyline and nortriptyline have been measured; the separation of the major metabolites of the drugs is also described.

EXPERIMENTAL

Apparatus

The liquid chromatograph incorporated an LDC 711-26 solvent delivering

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system. The detector unit was an Altex Model 153 analytical UV detector operated at 254 nm. The injector was a Rheodyne Model 70-10 sample injection valve (Altex Scientific, Berkeley, Calif., U.S.A.). The volume of the injection loop was 70 μl.

Chromatographic system

The separation column (100 or 125 mm × 4.6 mm I.D.) was of stainless steel and was packed with Partisil 5 (H. Reeve Angel, Clifton, N.J., U.S.A.) by the balanced-density slurry technique[16]. Equal volumes of tetrabromoethane, carbon tetrachloride and dioxan were used as suspending medium for the particles. The mobile phase consisted of perchloric acid, sodium perchlorate, methanol, dichloromethane and diisopropyl ether and was degassed in an ultrasonic bath for 45 min before use.

Reagents and chemicals

Diisopropyl ether was of synthesis grade (E. Merck, Darmstadt, G.F.R.; stabilized with 2,6-di-*tert.*-butyl-4-methylphenol); all other chemicals and solvents were of analytical grade. The internal standard, 2-(dibenz[*b*,^{5}]azepin-5-yl)-N-methyl ethylamine (Fig. 1), was obtained from Ciba Geigy (Basel, Switzerland).

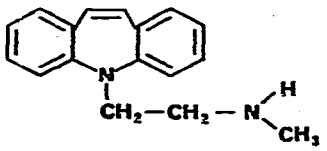

Fig. 1. Structure of internal standard.

Analytical procedure

Plasma samples (1 ml) were extracted by methods previously described[15,17]. Briefly, 50 μl of the internal standard solution (2.74 μM) and 1 ml of 2 M carbonate buffer (pH 10.5) were added to the samples and extraction of the drugs into *n*-hexane (5 ml) was achieved by gentle shaking to avoid the formation of an emulsion. The compounds were back-extracted into 1 ml of 0.25 N sulphuric acid and, after this solution had been made alkaline with 0.2 ml of 2.5 M sodium hydroxide, they were finally extracted into 0.1 ml of diisopropyl ether; this extract was used for the liquid chromatography.

RESULTS AND DISCUSSION

Chromatography

In previous ion-pair partitition chromatographic systems for tricyclic anti-depressant drugs[13–15], the stationary aqueous phase was pre-coated on the support. In the present system, a mobile phase that is under-saturated with the aqueous phase containing the counter ion is used. This technique gave highly stable and reproducible columns[18], without the need for thermostatic control of the chromatographic system that is essential with pre-coated columns.

Originally, a mixture of methanol and dichloromethane was used as the organic solvent for the mobile phase; however, the presence of diisopropyl ether was found to be necessary to retain the compounds and for good column performance.

It is our hypothesis that the polar silica gel support partly adsorbs the more polar components of the mobile phase. Support for this theory was obtained by measurements of the interstitial volume (V_m) of the column with tetrachloroethylene as the marker during equilibration of a column. The results are illustrated in Fig. 2. The intercept value, 0.74, was obtained with dry dichloromethane as mobile phase and probably represents the total porosity (ε_t) of the column[18]. The support availability for the mobile phase, represented by ε_m which is equal to V_m/V_0, where V_0 is the volume of the empty column), decreases to an equilibrium value of 0.68 or 0.62 after the passage of about 12 or 90 columns volumes, respectively, indicating that adsorption of a stationary phase occurs. It can be calculated from $\varepsilon_t = \varepsilon_m + \varepsilon_s$ (where ε_s is given by V_s/V_0 is the volume of the stationary phase) that this corresponds to the presence of 0.12 or 0.21 ml of stationary phase, which is about one-fifth and one-third, respectively, of the volume for a pre-coated ion-pair partition system. The adsorbed phase probably consists of a mixture of the aqueous phase and methanol.

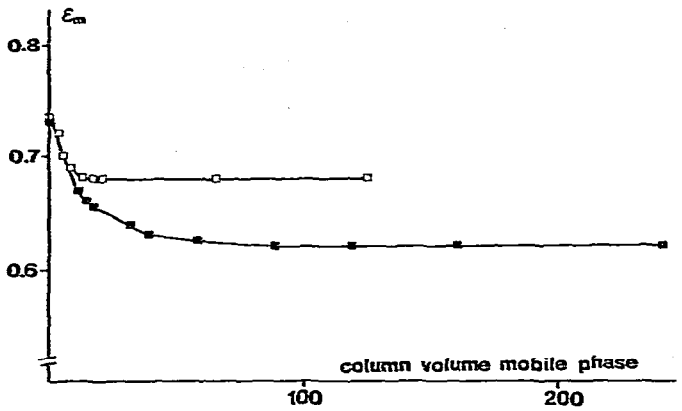

Fig. 2. Curves of porosity *vs.* number of column volumes of mobile phase. Column, 125 × 4.6 mm I.D. Mobile phase, 0.01 *M* perchloric acid + 0.09 *M* sodium perchlorate–methanol–dichloromethane–diisopropyl ether; □, 0.9:10:12:30; ■, 0.9:5.2:12:30. Flow-rate, 1.0 ml/min.

In straight-phase ion-pair chromatography the capacity factor (k') is given by

$$k' = \frac{V_s}{V_m \cdot E^*_{HAX} \cdot (X)} \qquad (1)$$

where E^*_{HAX} is the conditional ion-pair extraction constant and (X) is the concentration of counter-ion in the stationary phase. The capacity factors decrease with increasing concentration of perchlorate ions in accordance with eqn. 1 as illustrated in Fig. 3. However, the relationship is not rectilinear, partly because of the varying ionic strength and also because of the influence of other retention mechanisms. The possibility of regulating the capacity factors by adding methanol to the mobile phase is demonstrated in Fig. 4. The main features are that the capacity factors decrease as the methanol concentration increases above 11.8% (for the mobile phase studied). Some selectivity changes, especially between amitriptyline and the 10-hydroxy

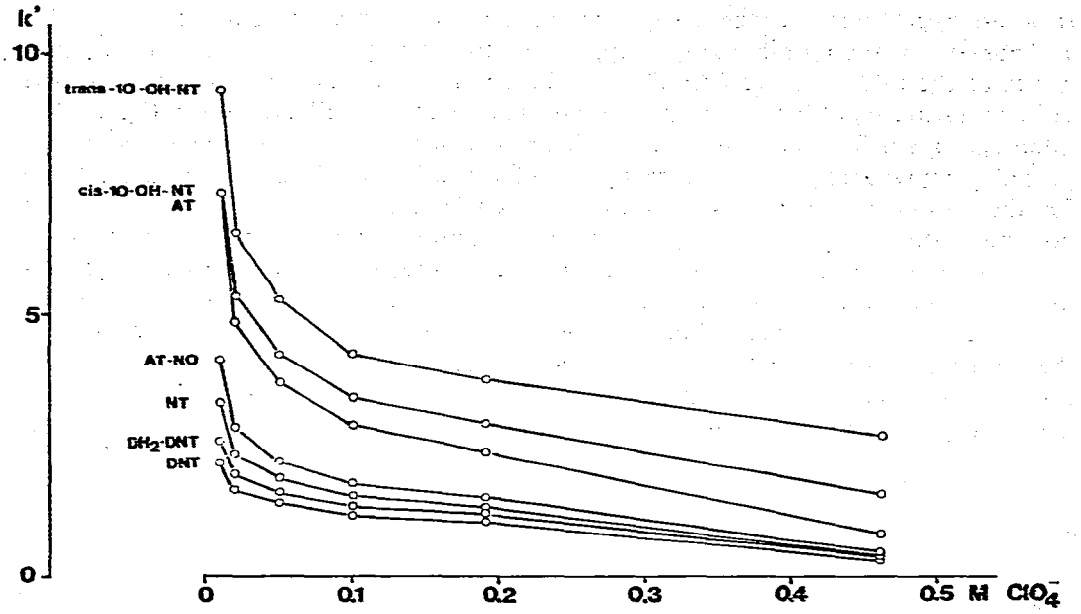

Fig. 3. Influence of counter-ion concentration on capacity factor (k'). Column, as in Fig. 1. Mobile phase, perchloric acid + sodium perchlorate–methanol–dichloromethane–diisopropyl ether (0.9:5.2:12:30). Flow-rate, 1.1 ml/min. For abbreviations see Fig. 7.

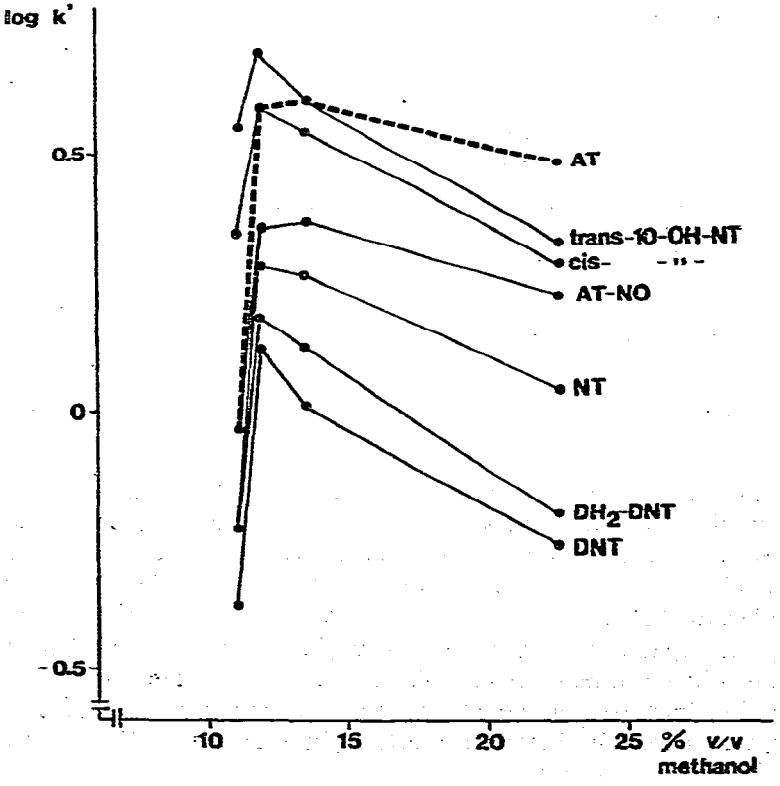

Fig. 4. Influence of methanol concentration on capacity factor (k'). Column, as in Fig. 1. Mobile phase, 0.01 M perchloric acid + 0.09 M sodium perchlorate–dichloromethane–diisopropyl ether (0.9:8:30)–methanol. Flow-rate, 1.1 ml/min. For abbreviations see Fig. 7.

metabolites of nortriptyline, can be seen. With decreasing methanol concentration below 11.8%, the mobile phase will approach saturation with respect to water and counter ion. The column efficiency with the system, calculated as the height of a theoretical plate, is between 0.02 and 0.04 mm.

Bioanalysis

The system has been successfully applied to the measurement of plasma concentrations of amitriptyline and nortriptyline in patients receiving therapeutic doses of these drugs. Typical traces obtained from the analysis of 1-ml plasma samples (a) containing no drug and (b) obtained from a patient receiving amitriptyline are shown in Fig. 5. Chlorimipramine and demethylchlorimpiramine have also been measured by using this chromatographic system and the extraction procedure described earlier[15].

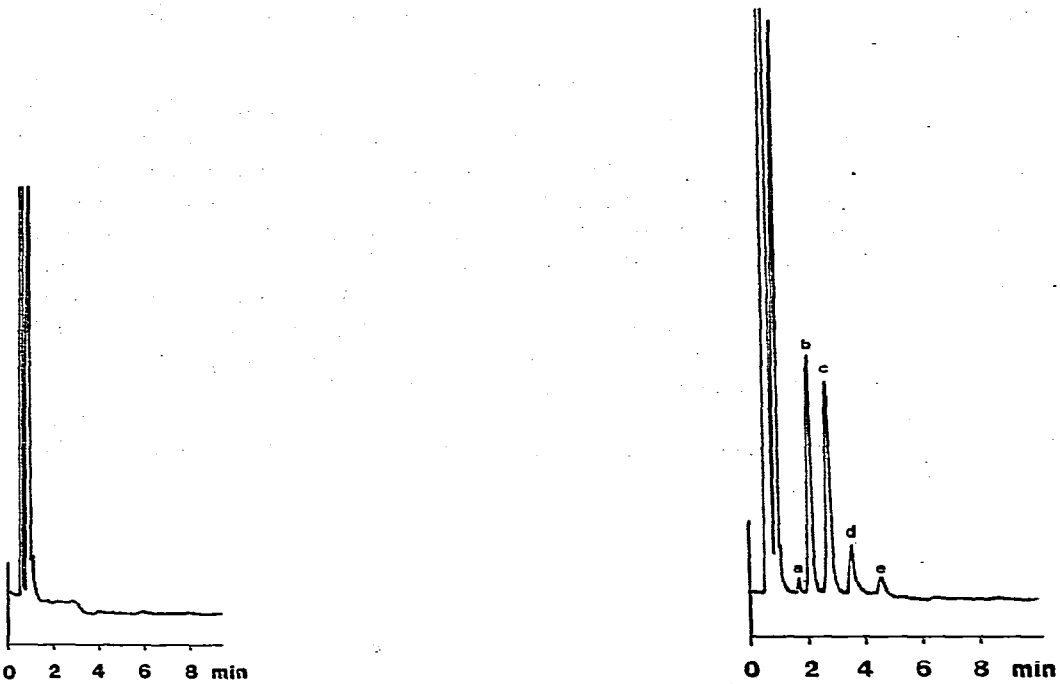

Fig. 5. Chromatograms from plasma samples. Column, as in Fig. 1. Mobile phase, 0.1 *M* perchloric acid–methanol–dichloromethane–diisopropyl ether (0.9:5.2:10:30). Flow-rate, 1.9 ml/min. (a) Blank human plasma, (b) plasma of a patient receiving amitriptyline. Peaks: a = demethylnortriptyline, b = nortriptyline (462 n*M*), c = internal standard (135 n*M*), d = amitriptyline (226 n*M*), e = *trans*-10-hydroxynortriptyline.

The reproducibility of the method determined from measurements on 38 duplicate plasma samples and calculated as the coefficient of variation was 10.1% for amitriptyline and 5.2% for nortriptyline; the concentration range was 90–900 n*M*.

A comparison between the proposed technique and a mass fragmentographic method[19] was carried out for the analyses of plasma nortriptyline in a group of 10

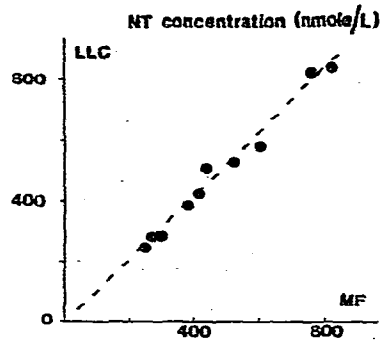

Fig. 6. Comparison of results obtained by mass fragmentography (abscissa) and by liquid chromatography (ordinate) for nortriptyline in plasma. Line of best fit: $y = 1.04x - 8.60$ ($n = 10$, $r = 0.99$).

patients; agreement between the results obtained by the two methods was close ($r = 0.99$; Fig. 6).

The high resolving capacity of the system is demonstrated in the separation of amitriptyline and nortriptyline from their respective metabolites (Fig. 7). It was possible to resolve the stereoisomeric forms of 10-hydroxyamitriptyline and 10-hydroxynortriptyline. The stereoisomers of 10-hydroxynortriptyline saved from an earlier study in this laboratory[20] gave retention times of 4.5 and 6.0 min, respectively. The absolute configuration of *trans*-10-hydroxynortriptyline has now been established[21], and it has the same retention time as isomer I, showing that the isomers I and II reported by Bertilsson and Alexanderson[20] represent the *trans*- and *cis*-forms, respectively. For the stereoisomers of 10-hydroxyamitriptyline (Fig. 7), it is possible to argue that isomers I and II represent the *trans*- and the *cis*-forms, respectively, by analogy with the order obtained with 10-hydroxynortriptyline.

Using this chromatographic system, quantitation of the metabolites of amitriptyline is possible. The clinical application of this study will be reported in a later publication.

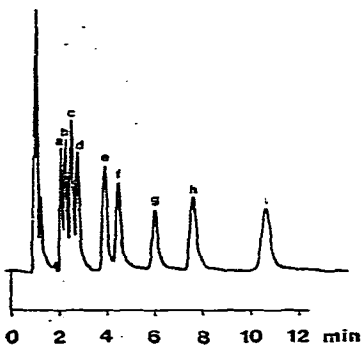

Fig. 7. Separation of amitriptyline and its metabolites. Column, as in Fig. 1. Mobile phase, 0.1 *M* perchloric acid + 0.9 *M* sodium perchlorate–methanol–dichloromethane–diisopropyl ether (0.85:5.1:5:30). Flow-rate, 1.1 ml/min. Peaks: a = demethylnortriptyline (DNT), b = 10,11-dehydrodemethylnortriptyline (DH_2-DNT), c = nortriptyline (NT), d = amitriptyline-N-oxide (AT-NO), e = amitriptyline (AT), f = *cis*-10-hydroxynortriptyline (*cis*-10-OH-NT), g = *trans*-10-hydroxynortriptyline (*trans*-10-OH-NT), h = 10-hydroxyamitriptyline II and i = 10-hydroxyamitriptyline I.

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