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Short Communication

Isotachophoretic analysis of some antidepressants

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ABSTRACT

Optimal electrolyte systems for the analysis of the substances with tricyclic structure, clomipramine and imipramine, and tetracyclic, maprotiline, were determined. The analysis conditions were applied to the determination of these compounds in antidepressant drugs.

INTRODUCTION

Depressive illnesses are very often treated with antidepressant drugs. These drugs can be divided into three groups. The first-generation drugs were thymoleptics with a tricyclic structure. The second generation of tetracyclic antidepressants share a number of the basic therapeutic properties of tricyclics in various kinds of depression (endogenous, somatogenic, etc.). The third and newest generation of drugs enhance serotoninergic neurotransmission.

To control the amount of antidepressants in drugs and human blood, plasma or serum chromatographic analytical techniques are used. Reviews of GC, HPLC and HPTLC for tricyclic antidepressants are presented in refs. 1 and 2. Other methods of preconcentration and derivatization of antidepressants were published later [3–6].

Electrophoretic methods have not often been used. Seven tricyclic antidepressants were analysed by capillary electrophoresis [7], and some of them were determined by capillary isotachophoresis [8,9].

Isotachophoresis (ITP) with coupled columns can provide both qualitative and quantitative analysis on ionic solutes without sample pretreatment in a relatively short analysis time. Most antidepressants are in ionic form. If they are not, changing the pH of their water solutions makes it possible to analyse them in electrolyte systems for cations. ITP analysis of compounds of our

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interest—imipramine and clomipramine tricyclic antidepressants and maprotiline, a second-generation antidepressant—has not yet been published. These compounds are the basic substances in the drugs very often prescribed by psychiatrists. The optimal conditions for ITP analysis of these compounds were applied for the ITP analysis of antidepressant drugs.

EXPERIMENTAL

Instrumentation

For the isotachophoretic separations a coupled column system in a ZKI 02 instrument (Spišská Nová Ves, Slovak Republic) and a conductivity detector were used. The preseparation column was 160 mm × 0.8 mm I.D. The zone of antidepressants was trapped in the analytical column which was 160 mm × 0.3 mm I.D. The columns were made from a copolymer of fluorinated ethylene and propylene. The voltages varied between 1 and 15 kV.

A PC AT with twelve-bit A/D–D/A converter with 100-Hz sample frequency and connected on-line to the ITP instrument was used, and the results were processed with the ITP-PC version 2 program by KasComp (Bratislava, Slovak Republic).

Chemicals

All chemicals were of analytical grade or additionally purified by the usual methods. Imipramine, clomipramine and maprotiline (all as hydrochlorides) were obtained from Ciba-Geigy (Basle, Switzerland). Analysed drugs Anafranil and Ludiomil were produced by Ciba-Geigy and Melipramine by Egis Pharmaceutical (Hungary).

RESULTS AND DISCUSSION

Two tertiary amines (imipramine and clomipramine) were chosen because there is only small difference in their chemical structure (R = H or Cl). Their pK_a in water reaches 9.8. Maprotiline is a tetracyclic compound which differs chemically from the others by virtue of a bridge in the central ring. Its pK_a in water is 10.5. The structures of imipramine, clomipramine and maprotiline are shown in Fig. 1.

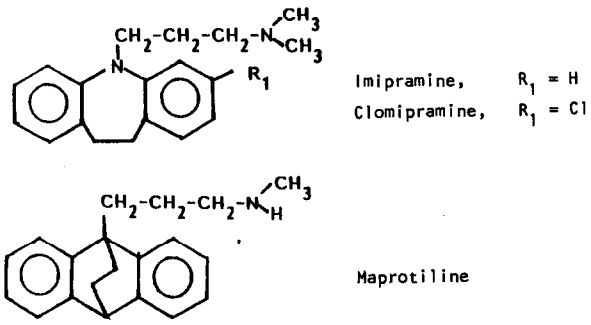

Fig. 1. Structures of analysed antidepressants.

Tested electrolyte systems which are the same in preseparation and separation columns are listed in Table I.

The best leading systems contained sodium acetate and sodium glutamate. The driving current in the preseparation column was 250 μA and in the analytical column 40 μA. The terminating components β-alanine, ε-caproic acid, acetic acid and glutamic acid were tested. Acetic acid as a terminator was too quick for the very large antidepressant molecules. The values of the principal qualitative parameter, $r_{s,h}$, for analysed standards are given in Table II.

In systems with sodium glutamate as the leading electrolyte and glutamic acid or β-alanine as the terminating electrolyte, clomipramine, imipramine and maprotiline can be distinguished in a mixture. In other systems the separation of these three antidepressants was not explicit. However, their separation is not important because these three compounds are not found together in drugs, nor are they used together in multiple therapy.

TABLE I

LEADING ELECTROLYTE SYSTEMS FOR ITP ANALYSIS OF ANTIDEPRESSANT CATIONS

Leading electrolyte	Concentration (*M*)	Counter-constituent	pH
Sodium acetate	0.01	Acetic acid	4.6
Potassium acetate	0.02	Acetic acid	4.6
Calcium hydroxide	0.01	Acetic acid	4.5
Sodium glutamate	0.01	Glutamic acid	5.3

TABLE II

PARAMETER $r_{s,h}$ OF ANALYSED COMPOUNDS

Leading electrolyte	Terminating *electrolyte*	$r_{s,h}$ [a]		
		Clomipramine	Imipramine	Maprotiline
Sodium acetate	β-Alanine	0.482	0.431	0.444
Calcium acetate	ε-Caproic acid	0.737	0.753	0.769
Sodium glutamate	Glutamic acid	0.959	0.803	0.839
Sodium glutamate	β-Alanine	0.734	0.647	0.870

[a] $r_{s,h}$ = Relative step height = $(h_x - h_L)/(h_T - h_L)$.

The determination of clomipramine, maprotiline and imipramine as basic substances in the drugs Anafranil, Ludiomil and Melipramine was achieved by the calibration and standard addition methods and statistically evaluated by linear regression. Seven calibration points repeated five times were measured with standard solutions. Samples of drugs as tablets and injections containing various amounts of the basic compounds were tested. Ten tablets and five injections for each type of drug were analysed, and every analysis was repeated five times (Fig. 2). Changes in analysed solutions from day to day were not observed. The relative standard deviation for a drug concentration 0.1 mg/ml was less than 0.5%, for the concentration range 0.01–0.1 mg/ml less than 3% and for concentrations less than 0.01 mg/ml reached 8%. The limits of detection of clomipramine, imipramine and maprotiline were around 200 ng/ml, and correlation coefficients ranged from 0.9996 to 0.9999. Table III shows the overall determinations of the basic substances in several drugs.

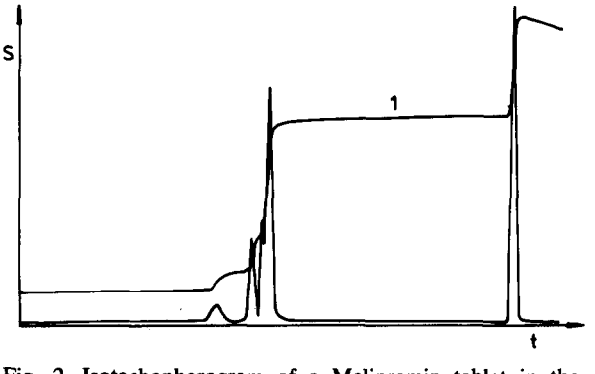

Fig. 2. Isotachopherogram of a Melipramin tablet in the following electrolyte system: leading electrolyte, sodium acetate + acetic acid pH 4.6; terminating electrolyte, β-alanine. 1 = Imipramine.

TABLE III

AMOUNTS OF THE BASIC COMPOUNDS FOUND IN TABLETS AND INJECTIONS

The amounts declared by the manufacturers are: 25 or 10 mg in tablets and 25 mg per 2-ml injection of solution; $n = 2$

Drug	Amount of basic compound (mg per tablet or injection)
Anafranil tablet	24.72 ± 0.111
Anafranil tablet	9.41 ± 0.050
Anafranil injection	24.55 ± 0.160
Melipramin tablet	24.92 ± 0.115
Melipramin tablet	9.94 ± 0.045
Melipramin injection	25.11 ± 0.133
Ludiomil tablet	26.73 ± 0.150
Ludiomil tablet	9.98 ± 0.050
Ludiomil injection	26.81 ± 0.174

CONCLUSIONS

It is shown that the ITP technique is well suited to the analysis of Anafranil, Tofranil and Ludiomil, out of this important group of drugs. The limit of detection of these drugs (around 200 ng/ml) was obtained with the injection of 50 μl of drug solution and zone width of 0.1 mm. The multiple injections of these volumes (50 μl) of plasma could be a problem in ITP detection systems.

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