Note

Liquid chromatography of amitriptyline and related tricyclic compounds

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Tricyclic antidepressants are widely prescribed. However, the large variability in the elimination rates for these drugs between individuals hampers their use. The differences in these rates can be as high as 30–40 fold; thus, patients on the same dose can have steady-state levels varying from 25 ng/ml to 1000 ng/ml[1,2]. While the former is therapeutically ineffectual, the latter can be seriously toxic. Thus, measurement of drug concentration in a readily available compartment such as plasma permits rational dosage adjustment.

Mellström and co-workers[3,4] have reported on the use of ion-pair partition chromatography to measure these drugs. Although their system appears to be adequate, modification presented in this report resulted in an improved system with several advantages. First, the solvent contains only two components simplifying solvent preparation and enhancing daily reproducibility. Second, the diisopropyl ether is not used, thus making the solvent less hazardous for routine use. Moreover, trials of their system showed that several different brands of diisopropyl ether contained substances which interfered at 254 nm and therefore appreciably reduced the assay's sensitivity. Finally, this assay achieves good separation in less than 15 min at lower pump pressure thus extending longevity of the system. This assay has been used to quantitate amitriptyline and its metabolites as well as the other commercially available tricyclic antidepressants.

EXPERIMENTAL

Apparatus

The chromatograph from Waters Assoc. (Milford, MA, U.S.A.) consisted of a U6K injector, a Model 6000A solvent delivery system, and a Model 440 absorbance detector operated at 254 nm. The stainless steel separation column (300 × 3.9 mm I.D.) was packed with μBondapak C_{18} (Waters Assoc.).

Chromatographic system

The mobile phase consisted of acetonitrile and a perchlorate solution at pH 2.5 (44:56). The perchlorate solution was prepared by mixing 0.005 M perchloric acid with 0.045 M sodium perchlorate (9:1). The solvent was degassed by filtering through organic:aqueous filter paper (Millipore FH UP 04700) primed with methanol. The flow-rate was 1.0 ml/min.

Chemicals and materials

The acetonitrile used was Baker HPLC grade. The water used in making the perchlorate solution was HPLC grade. All other chemicals were analytical-reagent grade. The internal standard, 2-(dibenz[b^5]azepin-5-yl)-N-methylethylamine, obtained from Ciba-Geigy (Basel, Switzerland), was used as a 1 μg/ml solution. Standard solutions of amitriptyline, nortriptyline, and the internal standard were prepared in 0.005 M sulfuric acid.

Analytical method

Plasma was separated from the heparinized whole blood by centrifugation at 2000 rpm for 20 min. A 3.0-ml sample was spiked with 100 μl of the internal standard solution. A double extraction was then performed. First, the drugs were extracted into 4 ml of hexane–isoamyl alcohol (99:1) by alkalinization using 200 μl of 1.5 M sodium hydroxide and gentle shaking for 60 min. The organic phase was separated from the plasma by centrifugation. The drugs were then back-extracted and concentrated by addition of 200 μl of 0.1 M perchloric acid and shaking for 20 min. The organic phase was discarded. A variable quantity of the aqueous phase, usually 50 μl, was injected onto the column. Larger volumes can be delivered for dilute samples without altering peak resolution.

Standard curves using five different concentrations of the drugs to be measured were determined daily immediately preceding experimental sample analysis. These standards were prepared by the addition of known amounts of the tricyclics to drug-free plasma. These samples were analyzed using the method described, and the ratio of peak height of drug to peak height of internal standard was plotted *versus* the drug concentration in the spiked samples. The resulting standard curves were then used for quantitation of the clinical samples. For clinical assays, the concentrations used were: 50, 100, 150, 200 and 250 ng/ml. The therapeutic range for amitriptyline is 150–250 ng/ml.

RESULTS AND DISCUSSION

The theory underlying ion-pair chromatography is available in the literature[4,5]. However, this procedure, which utilizes perchlorate as the counter ion, has several advantages over earlier methods. The solvent contains only acetonitrile and the perchlorate solution, making it less complex and less hazardous than previous solvents requiring diisopropyl ether[4]. Furthermore, several brands of diisopropyl ether contained substances which absorbed light at 254 nm thus reducing assay sensitivity. Unlike the method described by Kraak and Bijster[6], the mobile phase is acidic thus extending column longevity. The same column, used only for this assay, has been in service for over six months without loss of resolving capacity. The peaks of even the most retained tricyclic compounds remain symmetrical and return to baseline.

The resolving capacity of this method permitted quantification of amitriptyline and its metabolites as well as other commercially available tricyclic antidepressants (Figs. 1 and 2). The assay time was less than 15 min for all of these drugs. No interfering substances were detected in blank human or laboraroty rat plasma samples. Chromatograms of amitriptyline and its metabolites from a patient within the therapeutic range and from an overdosed patient are presented in Fig. 3.
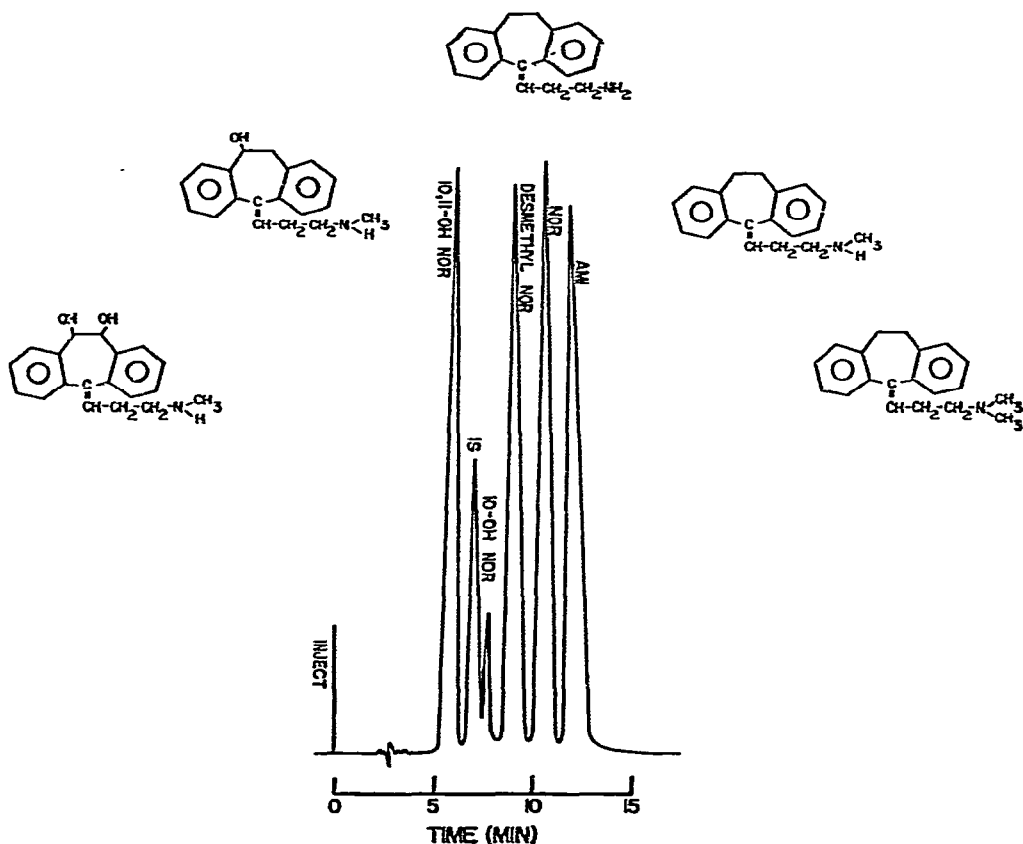
Fig. 1. Separation of amitriptyline and its metabolites. Mobile phase: 0.01 M perchlorate solution (pH 2.5)–acetonitrile (56:44); flow-rate 1.0 ml/min. From left to right, the chemical structure and corresponding peak (structure of internal standard, not illustrated). NOR = nortriptyline, AMI = amitriptyline. 10-hydroxyamitriptyline was not available for testing.

The reproducibility of the method for clinical purposes was assessed by correlating results from the measurement of 35 duplicate human plasma samples spiked with known amounts of drug to obtain concentrations from 5 to 400 ng/ml ($r = 0.9887$ for nortriptyline; $r = 0.9984$ for amitriptyline). Drug recovery exceeded 80% over this concentration range. The within-run variation for five determinations of the same plasma sample spiked with 100 ng/ml of drug was 100 ± 1.3 S.E. for nortriptyline and 100 ± 2.6 S.E. for amitriptyline. Nine consecutive daily assays of five plasma samples (range: 50–250 ng/ml) had a coefficient of variation of 6.4% for nortriptyline and 7.7% for amitriptyline. The correlation coefficient for these standard curves were 0.9969 for nortriptyline and 0.9967 for amitriptyline.

Similar reproducibility results were obtained for samples from laboratory animals. This method has been used to quantitate amitriptyline in brain, heart, and liver tissue samples from laboratory animals following intraperitoneal drug administration[7]. The sensitivity of the method (< 5 ng/ml) has permitted repeat determination in laboratory mice on plasma samples as small as 100 μl.
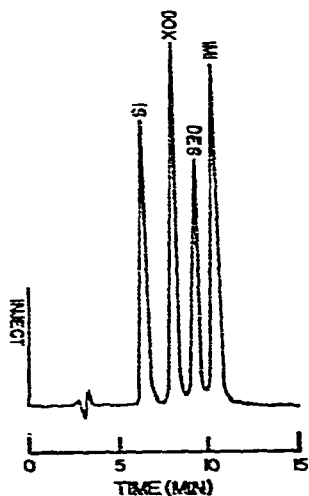
Fig. 2. Separation of other tricyclic antidepressants. Same conditions as in Fig. 1. IMI = imipramine, DOX = doxepin, DES = desipramine, IS = internal standard.
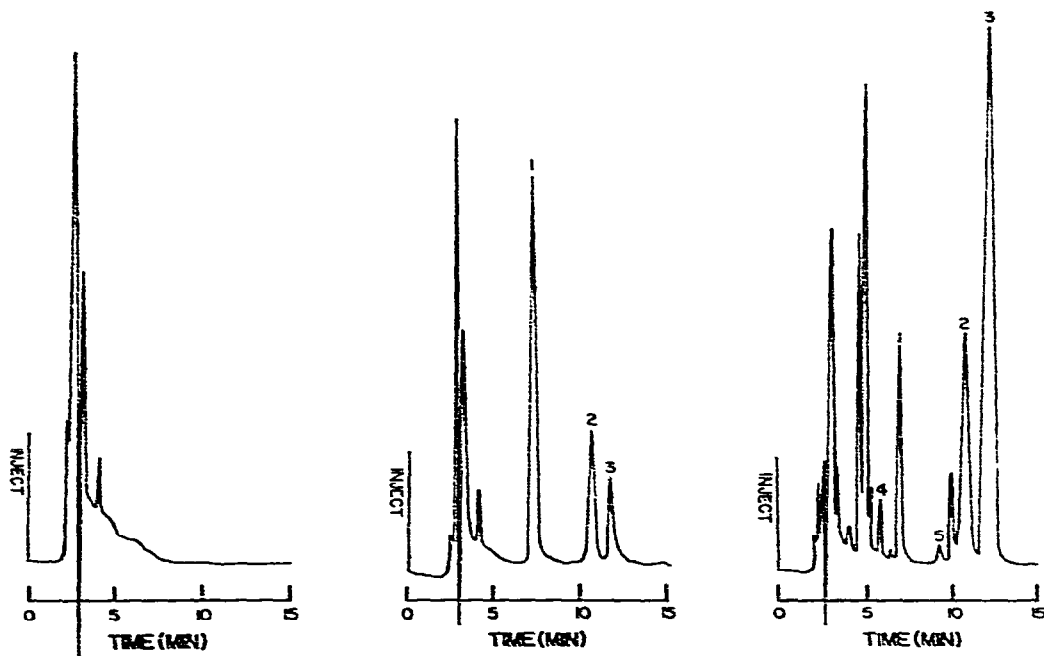

Fig. 3. Chromatograms from human plasma samples. Same conditions as in Fig. 1. From left to right, a blank sample, a sample in the therapeutic range, and a sample in the toxic range. I = internal standard, 2 = nortriptyline, 3 = amitriptyline, 4 = desmethylnortriptyline and 5 = 10,11-dihydroxynortriptyline.

Although desipramine and protriptyline elute simultaneously (Fig. 2), such interference should not represent a limitation for either clinical or experimental assays, since these drugs are rarely used concomitantly. Doxepin can be substituted for the internal standard used in this study, if the latter is unavailable. Of a variety of commonly used medications, only diazepam elutes near the tricyclic antidepressants (Table I).

TABLE I

RELATIVE RETENTION (K') FOR TRICYCLIC AND OTHER DRUGS ASSAYED BY THIS METHOD

$K' = (V_1 - V_0)/V_0$, V_1 = retention time of compound, V_0 = void volume retention time.

Drug	K'	Drug	K'
Tricyclic antidepressants		Cardiac drugs	
Amitriptyline	2.93	Propranolol	1.00
Nortriptyline	2.53	Quinidine	0.33
Doxepin	1.73	Procainamide	0.20
Protriptyline	2.07	Anticonvulsants	
Desipramine	2.07	Phenobarbital	0.67
Imipramine	2.47	Carbamizapine	1.07
Neuroleptics		Analgesics and others	
Chlorpromazine	3.87	Aspirin	0.60
Mesoridazine	1.20	Acetaminophen	0.07
Fluphenazine	2.3⁷	Codeine	0.20
Benzodiazepines		Theophylline	1.20
Diazepam	2.40		
Flurazepam	1.47		
Oxazepam	1.20		

REFERENCES

1 F. Sjöqvist, Int. Pharmacopsych., 6 (1971) 147.
2 S. Preskorn and J. Biggs, N. Engl. J. Med., 298 (1978) 166.
3 B. Mellström and S. Eksborg, J. Chromatogr., 116 (1976) 475.
4 B. Mellström and R. Braithwaite, J. Chromatogr., 157 (1978) 379.
5 G. Schill, in J. A. Marinsky and Y. Marcus (Editors), Ion Exchange and Solvent Extraction, Vol. 6, Marcel Dekker, New York, 1975, Ch. 1.
6 J. C. Kraak and P. Bijster, J. Chromatogr., 14 (1977) 499.
7 S. Preskorn, B. Hartman, M. Raichle and H. Clark, J. Pharm. Exp. Therap., 213 (1980) 313.