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QUANTITATIVE ANALYSIS OF TRICYCLIC ANTIDEPRESSANTS IN SERUM FROM PSYCHIATRIC PATIENTS

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SUMMARY

A method for the quantitative analysis of tricyclic antidepressants in the serum of psychiatric patients is described. The method can be used for determining amitriptyline, nortriptyline, imipramine, demethylimipramine, clomipramine, demethylclomipramine, trimipramine and protriptyline. The method consists in a series of extraction steps followed by gas chromatography with a flame-ionization detector. The drugs are determined in their native state. The internal standard method is used for the quantitation.

INTRODUCTION

In psychiatric clinics and hospitals there is an increasing demand for determinations of concentrations of tricyclic antidepressants in plasma or serum. Various methods for determining such concentrations are available but, with few exceptions [1,2], for only one or two drugs. As most psychiatric units use several antidepressants, a single method that is capable of determining as many antidepressants as possible as well as their main metabolites would be useful.

The method proposed here can be used for determining the concentrations of amitriptyline, nortriptyline, imipramine, demethylimipramine, clomipramine, demethylclomipramine, trimipramine and protriptyline in serum. The method consists in extraction from serum, followed by sepration and quantitation of the unchanged drug by gas chromatography employing a flameionization detector and an internal standard.

MATERIALS AND METHODS

Sampling routines

Venous blood samples were drawn from fasting patients in the morning before the first dose of medicine of the day, approximately 12 h after the last dose on the day before. A condition for determination of the serum level was that the patient had received the same daily dose of antidepressive drug for at least 1 week.

Reagents

All reagents were of analytical grade, except *n*-hexane, which was of pesticide grade (Fisher). All aqueous solutions were prepared with water re-distilled in an all-glass apparatus. Sodium hydroxide solution (2 *M*) was washed with diethyl ether and with *n*-hexane. The antidepressive drugs used as reference compounds were generously provided by the manufacturers.

Glassware

The centrifuge tubes used in the first two extraction steps were new and were used only once. Before use they were cleaned by rinsing with hot tap-water and then with distilled water in a dish-washing machine. The small glass-stoppered tubes used in the third extraction step were used only for these analyses and were rinsed with ethanol—*n*-heptane (1:10) and with formic acid—water (1:10) between each run. The small conical test-tubes in the final step were of disposable type made from new Pasteur pipettes that had first been rinsed with 99% ethanol.

Internal standard

Any of the tricyclic drugs could be used as an internal standard in the determination of another tricyclic drug. For each series of determinations a suitable internal standard was chosen from those tricyclic drugs which were not to be included in that series. Later it was found that cyproheptadine could serve as an internal standard for all of the drugs, and this substance was then generally used. A 0.1 m*M* solution of the internal standard in 99% ethanol was prepared and 2.00 nmole were added to each sample.

Extraction procedure

Mixing was performed either by slowly rotating the tubes at 20 rpm or by agitating them on a Whirlimixer. The phases were separated by centrifugation for 10 min at 1000 *g*. Transfers were made with the aid of disposable Pasteur pipettes.

Each sample of 4 ml of serum was pipetted into a polyethene stoppered centrifuge tube and the internal standard was added. The samples were then made alkaline by addition of 0.2 ml of 2 *M* NaOH and extracted with 7 ml of *n*-hexane—isoamyl alcohol (100:3) by rotation for 15 min. After separation of the phases, the hexane phase was transferred into another polyethene stoppered centrifuge tube and extracted with 2 ml of 0.1 *M* HCl, also by rotation for 15 min. The hydrochloric acid phase was transferred into a smaller glass-stoppered tube, made alkaline with 0.2 ml of 2 *M* NaOH and extracted with

0.5 ml of *n*-hexane—methyl isobutyl ketone (100:3) by agitating for 30 sec. The organic extract was transferred into a small conical test-tube and 20 μl of formic acid—methanol—water (1:5:5) were added. The tube was agitated for 60 sec and then allowed to stand for 5 min for the phases to separate, whereupon the organic phase was evaporated to dryness in a KOH-containing vacuum desiccator, which was evacuated with a water suction pump. The dry residue was dissolved in 7 μl of *n*-heptane—toluene—isoamyl alcohol—diethylamine (80:20:1.5:1) by sonication, and 2 μl were injected into the gas chromatograph.

Gas chromatography

The gas chromatograph was a Perkin-Elmer F 11 instrument equipped with a flame-ionization detector and 2 m × 2 mm I.D. silanized glass column packed with Carbowax 20M (1.4%) and KOH (1.4%) on Gas-Chrom Q, 60—80 mesh. The column temperature was 200° and the carrier gas was nitrogen at a pressure of 70 kPa, giving a flow-rate of 35 ml/min. The gas was purified by passing it through an oxygen filter.

Quantitation

Standard serum samples containing known amounts of the substances to be determined were included in each set of determinations. The concentrations in the test samples were then calculated from the peak heights. Duplicate analyses were run as a routine.

RESULTS

This method has been used for routine clinical determinations of amitriptyline and nortriptyline, of imipramine and demethylimipramine, and of clomipramine and demethylclomipramine. It can also be used for measuring trimipramine and protriptyline. The relative retention times of the drugs are listed in Table I, and Fig. 1 shows gas chromatograms of serum extracts from patients.

There were linear relationships between the serum concentration of each drug and the peak-height ratio between the drug and the internal standard within the concentration ranges found in patients. This was tested by adding known amounts of the drugs to blank serum samples and then analyzing the samples.

To test the yield of the extraction procedure, known amounts of the drugs were added to blank serum samples and the extraction procedure was performed without the internal standard, which was not added until just before the injection into the gas chromatograph (Table II).

The precision of the method was calculated from duplicate values obtained from routine analyses of amitriptyline, nortriptyline, imipramine, demethylimipramine, clomipramine and demethylclomipramine (Table III). The precision of the determination of trimipramine and protriptyline was calculated by repeatedly analyzing pooled serum samples containing these drugs (Table IV).

Most other psychoactive drugs did not interfere in the determinations. Non-

TABLE I

RELATIVE RETENTION TIMES OF TRICYCLIC ANTIDEPRESSANTS

The retention times were calculated relative to cyproheptatidine, which had an absolute retention time of 5 min.

Drug	Relative retention time
Trimipramine	0.44
Amitriptyline	0.46
Imipramine	0.56
Nortriptyline	0.68
Demethylimipramine	0.84
Protriptyline	0.90
Clomipramine	1.10
Demethylclomipramine	1.74

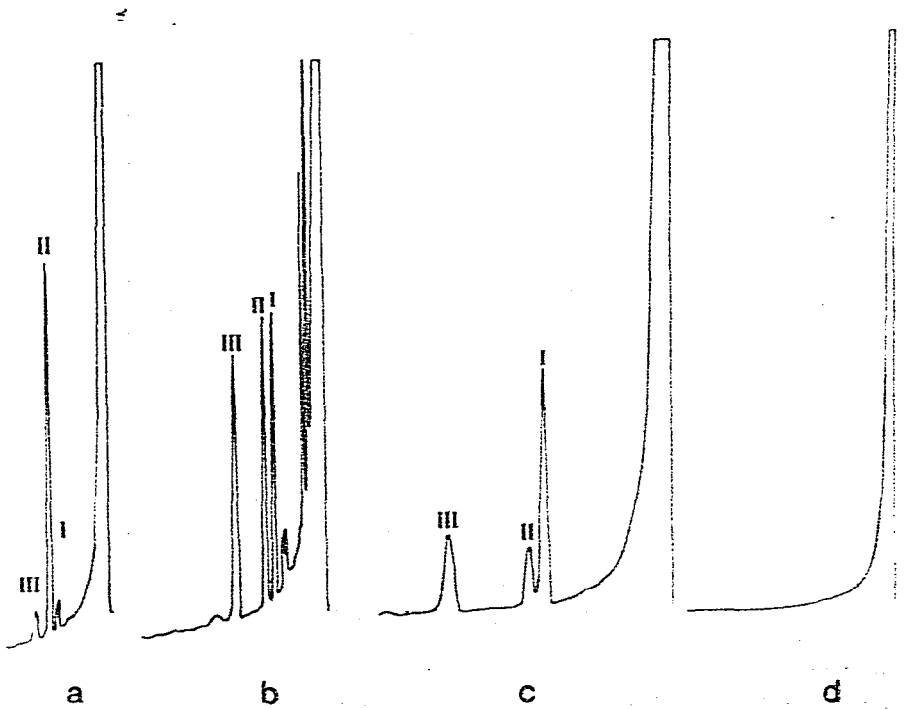

Fig. 1. Gas chromatograms of serum extracts from patients. (a) Patient treated with 30 mg of amitriptyline per day. I = Amitriptyline (0.04 μM); II = imipramine (internal standard); III = nortriptyline (0.08 μM). (b) Patient treated with 150 mg of imipramine per day. I = Amitriptyline (internal standard); II = imipramine (0.34 μM); III = demethylimipramine (0.55 μM). (c) Patient treated with 75 mg of clomipramine per day. I = Cyproheptadine (internal standard); II = clomipramine (0.16 μM); III = demethylclomipramine (0.48 μM). (d) Patient not treated with any tricyclic antidepressant.

TABLE II

PERCENTAGE YIELD OF THE EXTRACTION PROCEDURE

Drug	Concentration (μM)		
	1	0.2	0.1
Amitriptyline	84		86
Nortriptyline	79	79	
Imipramine	92		90
Demethylimipramine	81	71	
Clomipramine	71		71
Demethylclomipramine	65	59	
Trimipramine	75		75
Protriptyline	85	94	

basic drugs were separated by the extraction procedure, while basic drugs, such as opiates, benzodiazepines, butyrophenones and carbamazepine, as well as most phenothiazines, had longer retention times in the gas chromatographic system. Interfering drugs were alimemazin and promethazine, which were not completely separated from demethylimipramine, and promazine, which was not separated completely from clomipramine.

The serum concentrations of all of the drugs found in patients were of the same magnitude and ranged between 0.05 and 1.5 μM. The daily dose of antidepressant varied between 30 and 225 mg. As has been shown repeatedly before, there was a considerable inter-individual variation of the serum concentration in patients treated with an identical dose. Thus, in a group of 20 pa-

TABLE III

PRECISION OF THE METHOD CALCULATED FROM DUPLICATE VALUES

The coefficient of variation was calculated from the following equations:

$$S = \sqrt{\frac{\Sigma(x_{i1} \times x_{i2})^2}{2n}} \; ; \qquad V = \frac{S \times 100}{x}$$

where V = coefficient of variation as percentage of the mean, S = standard deviation, x_{i1} and x_{i2} = duplicate analysis of the same sample, x = mean of all analysis values and n = number of samples.

Drug	Coefficient of variation (% of the mean)	n
Amitriptyline	6.6	74
Nortriptyline	9.4	83
Imipramine	10.7	23
Demethylimipramine	9.1	21
Clomipramine	7.8	31
Demethylclomipramine	13.2	31

TABLE IV

PRECISION OF THE METHOD CALCULATED FROM REPEATED ANALYSES OF THE SAME SAMPLE

Drug	Concentration (μM)	Coefficient of variation (%)	n
Trimipramine	0.1	10.5	9
	1.0	4.8	9
Protriptyline	0.1	11.8	9

tients given 150 mg of amitriptyline a day in three equal doses, the serum concentration of amitriptyline varied between 0.06 and 0.60 μM and the nortriptyline concentration between 0.1 and 1.25 μM. The ratio between the amount of demethylated metabolite and that of the parent substance also showed a considerable inter-individual variation between 0.44 and 7.5, with a mean of 2.37.

DISCUSSION

The proposed method can be used for the quantitative analysis of most tricyclic antidepressive drugs in serum (all but one of those listed in the Swedish Pharmacopeia) and some demethylated metabolites. The method is simple enough for routine clinical use. The substances are analysed in their unchanged state and no preparation of derivatives was found to be necessary. Also, the type of gas chromatograph used is fairly simple and inexpensive, with an ordinary flame-ionization detector. Its sensitivity is sufficient for the determination of serum levels in patients and even levels far below those which can be regarded as therapeutically optimal. The method can also be used for kinetic studies. With the aid of mass fragmentography [1], it is also possible to determine all tricyclic antidepressants, but such a sophisticated and expensive instrument is hardly suitable for routine clinical analyses.

A general gas chromatographic method employing a nitrogen detector for the determination of plasma levels of these drugs has recently been described [2], but its sensitivity is essentially the same as that of our method. The former method was exemplified with plasma determinations of imipramine only.

One difficulty in this type of analysis is that the sample extracts may contain substances that are eluted from the gas chromatograph together with the drugs to be determined. Such interfering substances may be present in serum, but the samples may also have been contaminated during the extraction procedure. Owing to the high sensitivity of the method, even trace amounts of impurities, which may also be present in chemicals of good quality, may interfere in the analyses. Another source of contamination is the rinsing procedures, in which glassware in poor condition may become contaminated. To mitigate the risk of contamination, the extraction procedure had to be made more elaborate than is usual for this type of analysis. In addition, it is essential to adhere to very strict standards in the preparation of solvents and handling of glassware. The

routines described here have given satisfactory results, but the procedures may, of course, be modified according to the conditions in different laboratories and give equally good results.

Patients treated with an antidepressant very often also receive other psychoactive drugs, mostly sedatives and tranquillizers. As most psychoactive drugs are fairly closely related chemically, there is always a considerable risk that one drug may interfere in the determination of another. In the proposed method, this risk is small as most other psychoactive drugs are either separated by the extraction or have clearly different retention times in the gas chromatographic system.

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