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RAPID, SENSITIVE GAS–LIQUID CHROMATOGRAPHIC METHOD FOR DETERMINATION OF AMITRIPTYLINE AND NORTRIPTYLINE IN PLASMA USING A NITROGEN-SENSITIVE DETECTOR

H. B. HUCKER and S. C. STAUFFER

Merck Institute for Therapeutic Research, West Point, Pa. 19486 (U.S.A.)

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SUMMARY

A simple gas chromatographic method for determination of amitriptyline and nortriptyline in plasma using a nitrogen-sensitive detector is described. Concentrations of both drugs as low as 10 ng per ml plasma were measured. The precision and accuracy of the method are within acceptable limits.

INTRODUCTION

Much progress has been made in recent years in development of methods for the determination of the tricyclic antidepressant drug amitriptyline, and its active metabolite nortriptyline in plasma. Steady-state levels of the two compounds were measured using gas–liquid chromatography (GLC) with hydrogen flame ionization detection[1–5].

Recently, an electron-capture detection was used to measure amitriptyline and nortriptyline after their oxidation to anthraquinone[6] but the method was unable to distinguish between the two individual compounds. This method has recently been modified[7] to enable separate determination of amitriptyline. The use of GLC with a nitrogen-sensitive detector has recently been reported for determination of amitriptyline and nortriptyline in plasma after therapeutic doses[8–12]. A mass fragmentographic procedure for their measurement in plasma has also been described[13].

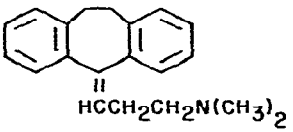
The present paper describes a rapid, sensitive procedure for measurement of the two drugs in human plasma, using GLC and a nitrogen-sensitive detector.

EXPERIMENTAL

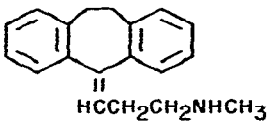
Reagents

Glass-distilled *n*-hexane was used as purchased from Burdick & Jackson (Muskegon, Mich., U.S.A.). Double-distilled water was used to prepare 0.5 *N* NaOH and 0.1 *N* HCl from analytical reagent-grade NaOH and HCl. Amitriptyline and nortriptyline were used as the hydrochloride salts. One of the following three internal standards was used: I, the diethyl analog of amitriptyline, 3-(10,11-dihydro-5H-

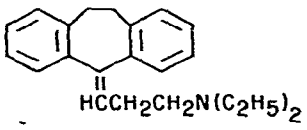
dibenzo[*a*,*d*]-cyclohepten-5-ylidene)-N,N-diethyl-1-propanamine·HCl; II, the diethyl analog of cyclobenzaprine, 3-(5H-dibenzo[*a*,*d*]cyclohepten-5-ylidene)-N,N-diethyl-1-propanamine·HCl; and III, the two-carbon side-chain analog of amitriptyline, 2-(10,11-dihydro-5H-dibenzo[*a*,*d*]-cyclohepten-5-ylidene)-N,N-dimethyl-1-ethanamine·HCl. Structures of the internal standards and of amitriptyline and nortriptyline are shown in Fig. 1. All concentrations are expressed in terms of the free bases. Solutions of the compounds were prepared in double-distilled water.



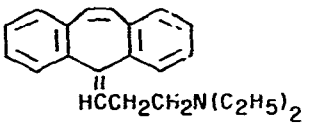
AMITRIPTYLINE



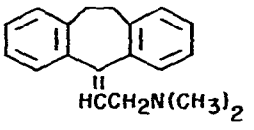
NORTRIPTYLINE



I



II



III

Fig. 1. Structures of amitriptyline, nortriptyline and internal standards I, II and III.

Equipment

Analyses were performed on a Perkin-Elmer (Norwalk, Conn., U.S.A.) gas chromatograph equipped with a nitrogen-sensitive detector. The glass column (1.82 m × 2 mm I.D.) was packed with 3% OV-17 on Gas-Chrom Q (100–120 mesh) and conditioned overnight at 275° with helium flow before use. The column, injector and interface temperatures were 240°, 260° and 275°, respectively. Hydrogen, helium and air flow-rates were 3.5, 500 and 100 ml/min, respectively. The electrometer sensitivity was $5 \cdot 10^{-12}$ A/mV. The rubidium glass bead detector was electrically heated, with a potentiometer setting of approximately 650. All glassware was washed with detergent and rinsed thoroughly with distilled water before use.

Preparation of standards

Standard solutions of 1 mg/ml amitriptyline and nortriptyline and internal standard were prepared in double-distilled deionized water for each series of analyses. The amitriptyline and nortriptyline standards were diluted to final concentrations of 0.2–4 μg/ml. The internal standard solutions were diluted to a final concentration of 0.5 μg/ml.

Assay procedure

Plasma (2.0 ml), 50 ng of internal standard (0.1 ml) and 0.5 ml of 0.5 *N* NaOH are pipetted into a 13-ml glass-stoppered centrifuge tube. *n*-Hexane (5 ml) is added and the tube is shaken for 20 min. After centrifugation, the organic phase is carefully

transferred, with a Pasteur pipet, to a 5-ml glass-stoppered centrifuge containing 0.2 ml of 0.1 *N* HCl. The tube is shaken for 10 min and centrifuged. The organic phase is discarded by aspiration. Fresh hexane (5 ml) is added and the tube shaken for 5 min, centrifuged and the organic phase aspirated.

The residue is made alkaline with 0.1 ml of 0.5 *N* NaOH. *n*-Hexane (25–50 μl) is added, the contents are vigorously mixed (Vortex mixer) for 1 min and centrifuged. A 5-μl volume of the organic phase is injected into the column.

Under these conditions, the retention times, t_R, of amitriptyline and nortriptyline were 3.2 and 3.7 min, respectively. The retention times of the internal standards I, II and III were 4.4, 5.2 and 2.5 min, respectively.

Plasma standards containing known concentrations (10, 25, 50, 100, 150 and 200 ng/ml) of amitriptyline and nortriptyline were analyzed concurrently with unknowns.

A standard curve is prepared for each series of analyses by plotting the ratio of peak height of amitriptyline or nortriptyline standards to the peak height of internal standard *vs.* the concentration of amitriptyline or nortriptyline. Concentrations of the two compounds in unknown samples are obtained by reference of the particular peak height ratio obtained to the standard curve.

RESULTS AND DISCUSSION

A GLC method using a nitrogen-sensitive detector for amitriptyline and nortriptyline in human plasma is described. The procedure involves extraction of the drugs from plasma with hexane, followed by back-extraction into dilute acid. The drugs are then re-extracted into a very small volume (50 μl) of hexane for analysis, thereby avoiding any necessity for evaporation of solvent, which can lead to loss of drug and concentration of solvent impurities. Actual recoveries for amitriptyline and nortriptyline were approximately 86 and 93%, respectively.

Replicate analyses of plasma samples, to which known amounts of amitriptyline and nortriptyline were added, demonstrated that the method was of acceptable accuracy and precision (Tables I and II). Concentrations as low as 10 ng/ml were determined in this study. The actual limit of sensitivity is approximately 5 ng/ml. Analysis of control human plasma showed that no interfering peaks were present.

TABLE I

PRECISION AND ACCURACY OF ANALYSIS OF AMITRIPTYLINE IN PLASMA

The mean ± standard deviation in ng/ml for 6 determinations with each internal standard is given followed by the relative deviation (in parentheses).

Conc. added (ng/ml)	*Conc. found using internal standard*		
	I	*II*	*III*
200	204 ± 10 (4.9%)	201 ± 12 (6.0%)	198 ± 12 (6.1%)
150	154 ± 10 (6.5%)	147 ± 6 (4.1%)	153 ± 1 (0.6%)
100	99 ± 8 (8.1%)	102 ± 5 (4.9%)	104 ± 4 (3.8%)
50	49 ± 2 (4.1%)	49 ± 5 (10.2%)	49 ± 2 (4.1%)
25	24 ± 3 (12.5%)	25 ± 2 (8.0%)	26 ± 1 (3.8%)
10	10.2 ± 1.5 (14.7%)	10.2 ± 0.8 (7.8%)	10.7 ± 0.5 (4.7%)

TABLE II

PRECISION AND ACCURACY OF ANALYSIS OF NORTRIPTYLINE IN PLASMA

The mean ± standard deviation in ng/ml for 6 determinations with each internal standard is given followed by the relative standard deviation (in parentheses).

Conc. added (ng/ml)	*Conc. found using internal standard*		
	I	*II*	*III*
200	201 ± 18 (9.0%)	193 ± 16 (8.3%)	213 ± 23 (10.8%)
150	158 ± 14 (8.9%)	152 ± 11 (7.2%)	146 ± 15 (10.3%)
100	103 ± 6 (5.8%)	100 ± 5 (5.0%)	101 ± 11 (10.9%)
50	54 ± 10 (18.5%)	48 ± 5 (10.4%)	48 ± 4 (8.3%)
25	24 ± 4 (16.6%)	24 ± 2 (8.3%)	26 ± 1 (3.8%)
10	10.1 ± 1.5 (14.8%)	9.5 ± 1.2 (12.6%)	9.7 ± 1.0 (10.3%)

Typical calibration curves obtained are shown in Fig. 2. The correlation coefficients, r, were 0.99 for amitriptyline and 0.94 for nortriptyline over the range of concentrations studied.

Occasionally analysis of a pre-test control plasma sample from a patient may indicate that a contaminant is present in the extract which has the same retention time as the internal standard. For this reason, three different internal standards were evaluated for the present method and found to be equally suitable. Thus, if analysis of a pre-test sample showed the presence of a significant peak at 4.4 or 5.2 min, which would interfere with internal standard I or II, then internal standard III ($t_R = 2.5$ min) was used in the assay.

The method has been in use in this laboratory for almost one year in connection

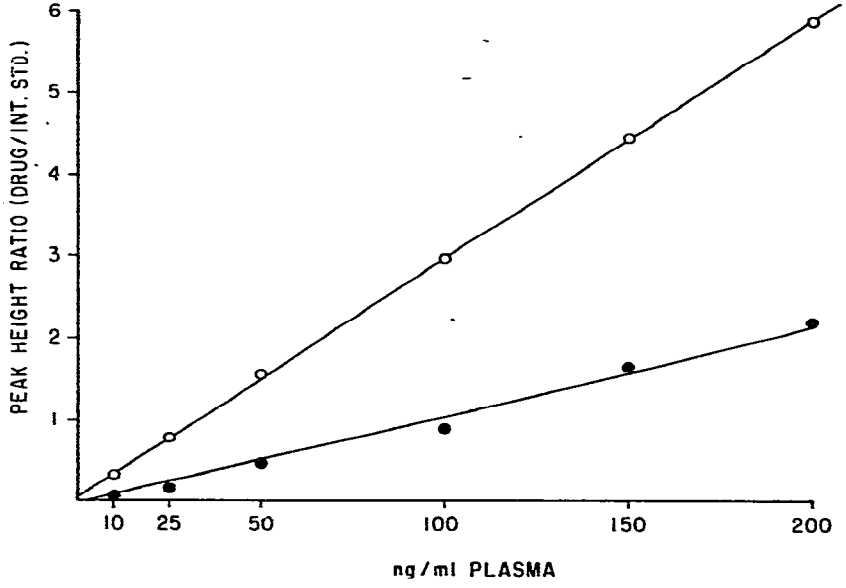


Fig. 2. Typical standard curve obtained by analysis of plasma samples ($n = 6$ for each point) containing known concentrations of amitriptyline (○) and nortriptyline (●) and internal standard III.

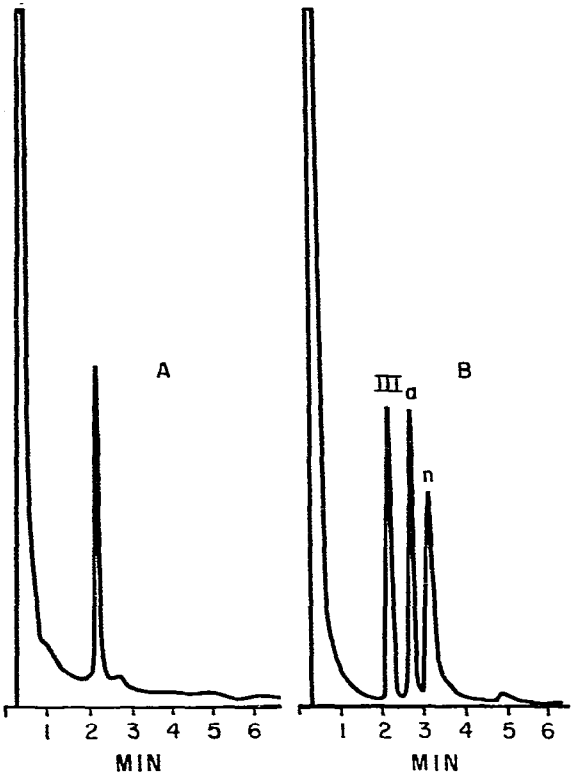


Fig. 3. Gas chromatograms obtained on analysis of (A) control human plasma to which 25 ng/ml of internal standard III was added, and (B) plasma from a patient containing 32 ng/ml of amitriptyline (a), 70 ng/ml of nortriptyline (n) and 25 ng/ml of III.

with a clinical pharmacology study. Fig. 3 shows a typical gas chromatogram from one of the patients being evaluated. This patient had received 150 mg of amitriptyline daily for 6 weeks before the sample was taken.

Although no attempt has as yet been made to measure drug levels in human subjects after a single dose of amitriptyline with the present method, it would appear to have the required sensitivity based on reported values[8] of approximately 10–50 ng/ml for two volunteers given a single oral dose of 50 mg of drug.

The present method appears to offer advantages over other published procedures for the following reasons: (1) an evaporation step, required in other methods[8–10,12], which is time-consuming and which may lead to loss of drug and concentration of solvent impurities, is avoided; (2) redissolving drug residues in very small volumes (25 μl) of solvent, required in other methods[8–10,12], with possible loss of drug and/or internal standard in unequal amounts with consequent error, is avoided; (3) a single, rather than a multiple extraction used by others[8,9] is sufficient, resulting in shortening the method; (4) nortriptyline is determined directly, avoiding the additional derivatization step used by others[8,9] which saves additional time; (5) special cleaning of glassware or reagent purification, employed by others[8,10], is unnecessary with the present method; the instrument used requires no daily cali-

bration method of hydrogen and air flow-rates or adjustment of head position stipulated by Reite *et al.*[9]; (6) the internal standards employed in the present method are more similar to amitriptyline than protriptyline, used by others[8,10–12], which will increase the certainty that both internal standard and amitriptyline are extracted to the same extent in all samples, with consequent increase in accuracy and precision of the method and a choice of three suitable internal standards gives greater flexibility to the present method; (7) daily priming of the column, used by others[11] is unnecessary with the present method.

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REFERENCES

1 R. A. Braithwaite and B. Widdop, *Clin. Chim. Acta*, 35 (1971) 461.
2 R. A. Braithwaite, R. Goulding, G. Theano, J. Bailey and A. Coppen, *Lancet*, (1972) 1297.
3 R. A. Braithwaite, B. R. S. Nakra and R. Gaind, *Psychol. Med.*, 4 (1974) 338.
4 H. B. Hucker and S. C. Stauffer, *J. Pharm. Sci.*, 63 (1974) 296.
5 H. B. Hucker, S. C. Stauffer, F. G. Clayton, B. R. S. Nakra and R. Gaind, *J. Clin. Pharmacol.*, 15 (1975) 168.
6 J. E. Wallace, H. E. Hamilton, L. K. Goggin and K. Blum, *Anal. Chem.*, 47 (1975) 1516.
7 P. Hartvig, S. Strandberg and B. Näslund, *J. Chromatogr.*, 118 (1976) 65.
8 A. Jorgensen, *Acta Pharmacol. Toxicol.*, 36 (1975) 79.
9 S. Reite, B. Salvesen and O. Skang, *Medd. Nor. Farm. Selsk.*, 37 (1975) 55.
10 D. N. Bailey and P. I. Jatlow, *Clin. Chem.*, 22 (1976) 777.
11 T. B. Cooper, D. Allen and G. M. Simpson, *Psychopharm. Commun.*, 2 (1976) 105.
12 J. Vasiliades and K. C. Bush, *Anal. Chem.*, 48 (1976) 1708.
13 J. T. Biggs, W. H. Holland, S. Chang, P. T. Hipps and W. R. Sherman, *J. Pharm. Sci.*, 65 (1976) 261.