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Note

Determination of therapeutic levels of amitriptyline in serum by gas–liquid chromatography

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Amitriptyline is a tricyclic antidepressant drug and the determination of therapeutic levels in serum is required. The literature on the determination of low levels of amitriptyline is very scanty. Braithwaite and Widdop[1] applied gas–liquid chromatography (GLC) to the determination of plasma levels. Riedmann[2] also used gas chromatography, with the aid of a nitrogen flame ionization detector. Most published work concerns toxicological analysis.

Amitriptyline is mainly metabolized to nortriptyline by N-demethylation. Norheim[3] described a GLC method for the simultaneous determination of amitriptyline and nortriptyline. He determined amitriptyline in blood from individuals who had succumbed to overdoses.

In this paper, a GLC method is described for the determination of amitriptyline alone, using the method of Norheim[3]. This method has been modified so as to achieve the determination of amitriptyline at lower levels in serum.

EXPERIMENTAL

Chemicals

All of the chemicals used in the extraction procedure were of pro analysi quality (E. Merck, Darmstadt, G.F.R.). Amitriptyline hydrochloride was kindly supplied by Merck, Sharp & Dohme, Haarlem, The Netherlands, and promazine hydrochloride by Wyeth, Amsterdam, The Netherlands.

Extraction procedure

Samples of 5–8 ml of serum, prepared from fresh blood, were subjected to the extraction procedure of Norheim[3], which involves extraction with diethyl ether followed by extraction with 0.1 *N* sulphuric acid and finally with chloroform. The chloroform extract was evaporated to a small volume (about 1 or 2 ml) in a Büchi rotavapor and then evaporated to dryness under reduced air pressure (*in vacuo*). A 50-μl volume of methanol containing 25 μg of promazine hydrochloride as internal standard was added and the solution was evaporated to dryness *in vacuo*. Finally, the residue was dissolved in 50 μl of hexamethyldisilazane (HMDS) and, after heating at 75° for 2½ h in a closed vial, 3 μl of the reaction mixture were injected into the gas chromatograph.

Gas–liquid chromatography

The analysis was carried out on a Hewlett-Packard Model 5750 G research chromatograph equipped wit a flame ionization detector, with the following conditions: column, 6 ft. × 2 mm I.D.; liquid phase, 3% Carbowax 20M; support, Chromosorb W AW DMCS, 80–100 mesh; column temperature, 230°; detector temperature, 300°; injection port temperature, 300°; attenuation, $4 \cdot 10^2$; supporting gas, helium at the flow-rate of 16 ml/min; internal standard, 50 mg of promazine hydrochloride in 100 ml of methanol.

RESULTS

The injection of HMDS alone gives peak H (Fig. 1), while the injection of HMDS containing promazine hydrochloride gives peaks H and P. The promazine peak, P, is not silylated by HMDS. Injection of HMDS containing promazine hydrochloride and amitriptyline gives peaks A, H and P. The amitriptyline, peak A, is a silylated derivative.

The injection of standard solutions of amitriptyline, such as 333 ng of amitriptyline per 50 μl of HMDS, 500 ng per 50 μl of HMDS, etc., gives peak ratios that show a good correlation with amounts of amitriptyline of 20 ng, 30 ng, etc. The response is linear.

Standard samples of extracted nortriptyline, the major metabolite of amitriptyline, give no peaks and this method is therefore suitable for the determination of amitriptyline alone in the presence of nortriptyline. Blank serum samples give no interfering peaks.

In silylation of the reaction mixture with HMDS at room temperature, standing overnight is recommended. However, the silylation is also complete after warming the mixture at 75° for 2½ h. The use of HMDS with pyridine as catalyst does not give better results, even after heating the solution at 75° for several hours. The pyridine peak tends to overlap that of amitriptyline.

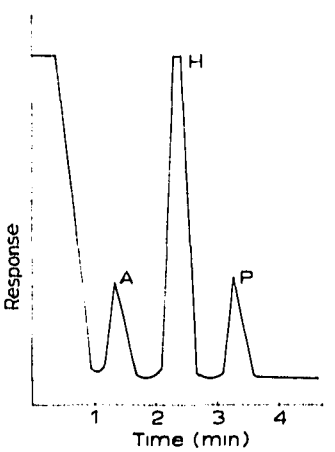

Fig. 1. Typical chromatogram from an extract of a serum sample. A = silyl derivative of amitriptyline; H = unknown HMDS product; P = promazine.

It is known that ammonium salts can be very effective in silylation with HMDS. Ammonium sulphate may be present in the residue after evaporating the chloroform layer and the presence of ammonium sulphate in the residue must therefore be prevented.

A sample of 8 ml of serum from a depressive patient who had been treated with three daily 35-mg doses of amitriptyline hydrochloride for 2 weeks gave a peak ratio that corresponded to 40 ng of amitriptyline, which means that the serum level was about 80 ng/ml. Braithwaite *et al.*[4], who measured the plasma concentrations of amitriptyline in depressive patients, found a mean concentration of 70 ng/ml in plasma. The minimum amount of amitriptyline in serum that can be determined by our method is 40 ng/ml, calculated from an original sample of 8 ml of serum. The minimum sensitivity of the method is 20 ng per 3 μl of amitriptyline standard injected into the gas chromatograph. The method is, of course, also suitable for toxicological analysis.

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