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SENSITIVE METHOD FOR THE ROUTINE DETERMINATION OF TRICYCLIC ANTIDEPRESSANTS IN PLASMA USING A SPECIFIC NITROGEN DETECTOR

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SUMMARY

The sensitivity of the alkali flame ionization detector to the tricyclic antidepressant drugs has been studied and limits of detection measured. A backflush system has been used to enable measurement in plasma extracts. A general method for the estimation of tricyclic plasma levels has been developed and exemplified by the measurement of imipramine at therapeutic levels.

INTRODUCTION

Tricyclic antidepressants are widely used in the treatment of mental disorders and recent evidence would suggest that routine monitoring of plasma levels should provide useful clinical information[1]. At present, however, the methods available for plasma estimations are only suitable for research purposes or overdose cases. Imipramine[2] and nortriptyline[3] have been estimated by gas chromatography–mass fragmentography and amitriptyline[4,5] by conventional gas chromatography.

Since the development of the alkali flame ionization detector (AFID), which was specific for organophosphorus[6], it has been used for the determination of S, As, Sb, Sn, Cl and N. The specific nitrogen detector has recently been used in the routine screening for drugs of abuse[7]. It is the intention of this paper to describe a sensitive method for the determination of the tricyclic antidepressants and is applied to the determination of imipramine as an example of its use.

EXPERIMENTAL

Materials

The tricyclic antidepressants were supplied by the following companies: imipramine, desimipramine, chlorimipramine and opiprimol by Geigy (Macclesfield, Great Britain); protriptyline by Merck, Sharp and Dohme (Hoddeson, Great Britain); amitriptyline by Roche (Welwyn Garden City, Great Britain); prothiaden by Boots (Nottingham, Great Britain); nortriptyline by Eli Lilly (Basingstoke, Great Britain); dibenzepin by Sandoz Products (Basle, Switzerland); trimipramine by May and Baker (Dagenham, Great Britain); and doxepin by Pfizer (Sandwich, Great Britain).

Apparatus

Analyses were performed using an isothermal chromatograph with heated nitrogen detector (Pye Unicam, Cambridge, Great Britain) and linear recorder. The back flush system recently reported by Warner *et al.*[8] was modified to suit the instrument used in this study. A conventional 9-ft. × $^1/_4$-in.-O.D. glass column was modified to include two auxiliary ports about 2 in. apart at approximately 1 ft. from the end of the column. The ports terminated in glass-to-metal seals which were connected by $^1/_{16}$-in.-O.D. stainless-steel tubing through the oven wall to the external backflush valve. A six-port micro-volume gas chromatography valve and two fine metering valves (Carle Instruments, Fullerton, Calif., U.S.A.) were used to control the direction and carrier gas flow-rate in the column. The flow system used is shown diagrammatically in Fig. 1. The column was silanized with a 5% solution of dimethyldichlorosilane in chloroform prior to packing with 3% OV-17 on Chromosorb W, 100–120 mesh. The packed column was conditioned at 300° for 24 h with carrier gas flowing and silylated with N,O-bis-(trimethylsilyl)-trifluoroacetamide prior to use.

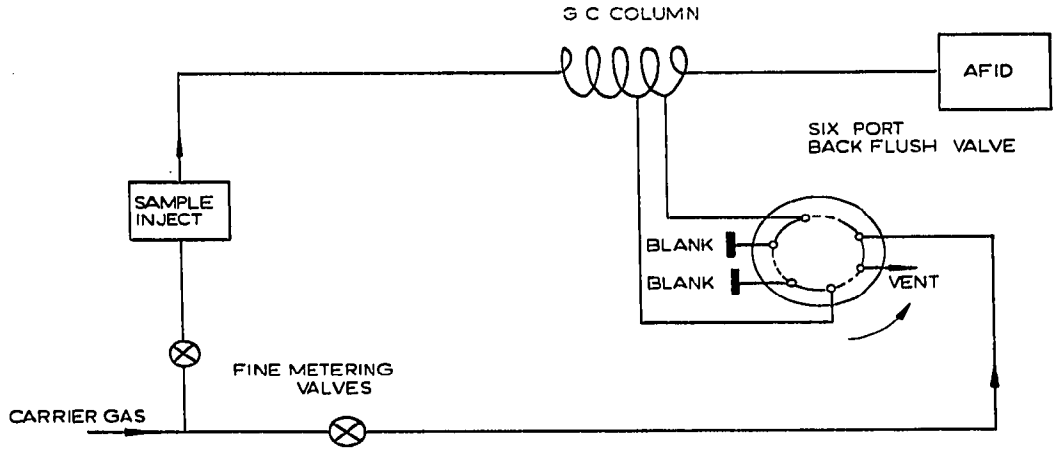

Fig. 1. Flow diagram incorporating six-port backflush valve. Rotation of the valve in the direction of the arrow changes the mode of operation from the conventional to the purge one.

Extraction procedure

To 4 ml of plasma, containing the drug to be estimated, was added 1.5 ml of aqueous internal standard containing 200 ng/ml of internal standard. In the analysis of imipramine the internal standard was butriptyline. The plasma was then diluted with 4 ml distilled water and made alkaline by the addition of 1 ml 10 *M* NaOH. The drugs were extracted into 10 ml *n*-heptane by gently shaking on a horizontal shaker for 15 min. The organic phase was then separated by centrifuging for 10 min and 7 ml of the upper organic phase aspirated. A 1-ml aliquot of *n*-heptane containing 10% glacial acetic acid and 20% amyl alcohol was added to the aspirated heptane fraction in order to reduce volatilisation of the tricyclic amines during the concentration procedure. The organic extract was evaporated to a final volume of 50 μl in a constant-temperature water-bath at 60° with the aid of a gentle stream of air. 5-μl Aliquots of the final extract were injected into the chromatograph for quantitation.

RESULTS AND DISCUSSION

Column operation

The operating conditions were fixed with the backflush valve set in the conventional mode. The carrier gas flow-rate through the column was adjusted to 30 ml/min, the hydrogen flow-rate was adjusted to 30 ml/min and the air flow-rate to 250 ml/min. The column oven temperature was maintained at 275° and the injector and detector temperatures were both set at 335°. The height of the rubidium chloride tip above the flame was set to give maximum sensitivity from an injection of 2 μl of a 30 μg/ml solution of imipramine in methanol. The position of the crystal was noted by insertion of feeler gauges and for a new rubidium chloride tip was of the order 0.025–0.030 in. Under these operating conditions the standing current was found to be approximately 5×10^{-11} A.

The backflush valve was then operated in the purge mode and the fine needle valve controlling the flow of purge gas was adjusted until there was no change in baseline. Samples were injected with the valve in the purge mode. After the solvent front and interfering compounds had eluted from the column the position of the valve was changed to the conventional mode of operation and the drugs recorded as normal (Fig. 2). The timing of the change over from the purge mode to the conventional mode was determined from inspection of a complete chromatographic trace of a mixture obtained by operating the instrument in the conventional manner. By using the backflush valve fractions of any sample could thus be vented before they reached the detector.

Retention parameters

The retention index was taken as the ratio of the drug elution time to the elution of the leading edge of solvent from the point of sample injection. The retention indices for the tricyclic antidepressants studied are given in Table I. In any analysis of a mixture it is desirable that the individual components should have retention times sufficiently different from one another to allow for adequate resolution. It can be seen that the retention indices of some of these drugs are similar, however, this is advantageous in this instance since it allows a choice of internal standard having a retention index similar to that of the drug being estimated. A greater level of precision can thus be achieved by minimising changes in response to drug and internal standard as a result of changing detector sensitivity during elution. A further consideration which influences the choice of internal standard is the presence of metabolic products which have retention indices similar to that of the parent drug. This can be seen for imipramine and its metabolite desimipramine with retention indices of 3.2 and 3.5, respectively. A suitable internal standard for the quantitative estimation of imipramine would, therefore, be butriptyline with a retention index of 2.9.

Sensitivity

The sensitivity of the AFID to the tricyclic antidepressants was calculated from the minimum detectable amount at twice the noise level under normal operating conditions. Minimum detectable limits expressed in nanogram of drug on injection are given in Table I. AFID response to the 5-H-dibenzo[*a*,*d*]cycloheptenes and to the 5-H-dibenz[*b*,*f*]azepines is shown in Figs. 3 and 4. The detection limits fall in the

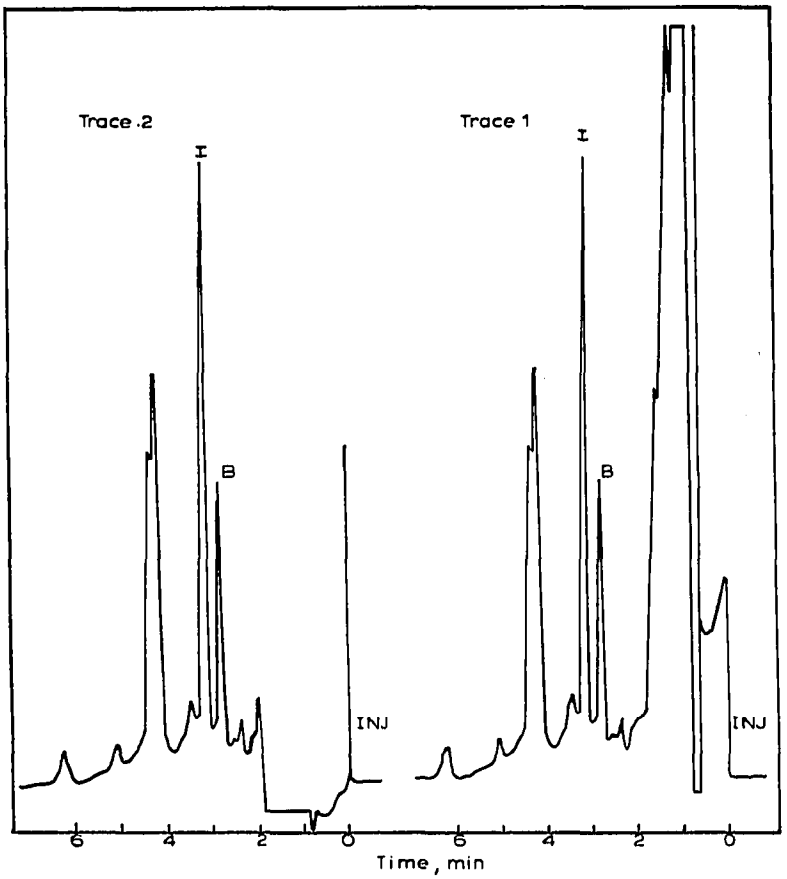

Fig. 2. Trace 1 shows the conventional trace from a plasma extract of 100 ng/ml imipramine (I) with butriptyline standard (B). Attenuation, 1×10^{-10} A f.s.d. Trace 2 shows the same sample after operation of the backflush valve.

TABLE I

RELATIVE RETENTION INDICES AND ABSOLUTE DETECTION LIMITS FOR TRICYCLIC ANTIDEPRESSANTS

Compound	*Relative retention index*	*Detection limit (ng)*
5-H-Dibenz[b,f]azepines		
Imipramine	3.2	1.0
Desimipramine	3.5	3.0
Trimipramine	3.0	2.0
Chlorimipramine	4.6	5.0
Opiprimol	2.6	30.0
5-H-Dibenzo[a,d]cycloheptenes		
Amitriptyline	3.0	1.0
Nortriptyline	3.3	5.0
Butriptyline	2.9	1.0
Protriptyline	3.6	2.0
Doxepin	3.3	3.0
Dothiepin	4.7	4.0

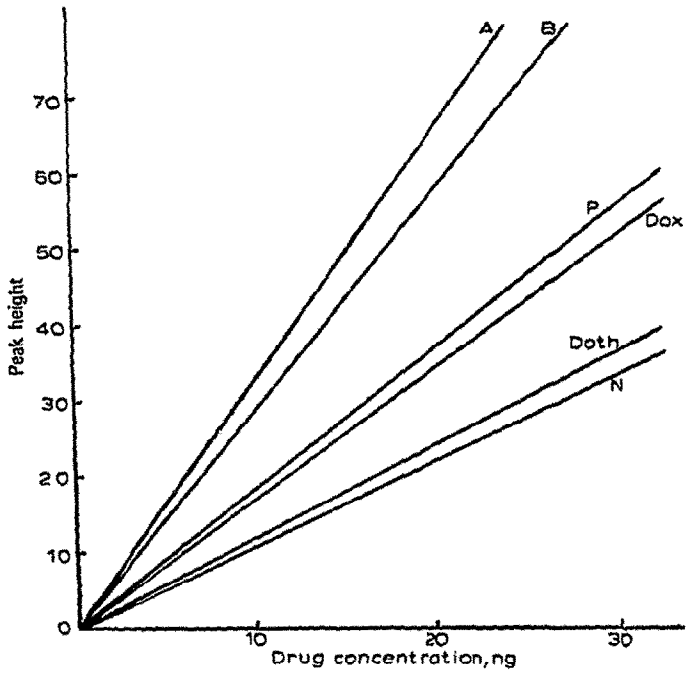

Fig. 3. AFID response to 5-H-dibenzo[*a*,*d*]cycloheptenes. Attenuation, 10^{-10} A f.s.d. A = Amitriptyline; B = butriptyline; P = protriptyline; Dox = doxepin; Doth = dothiepin; N = nortriptyline.

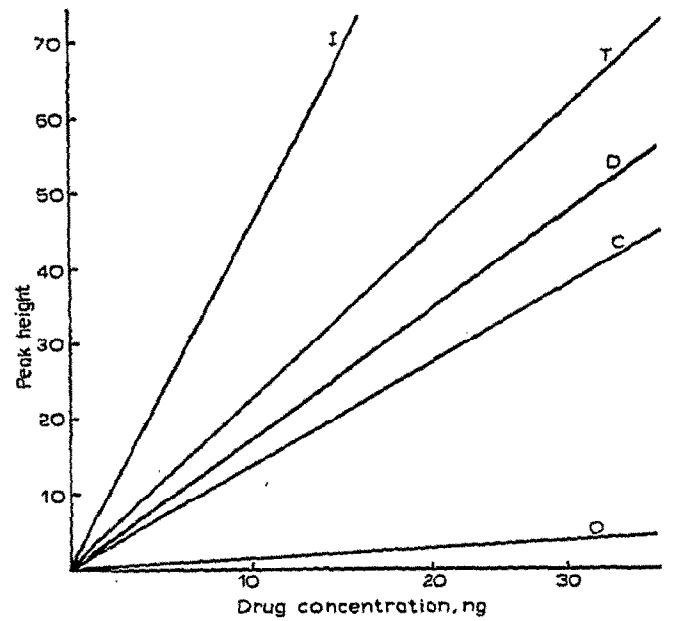

Fig. 4. AFID response to 5-H-dibenz[*b*,*f*]azepines. Attenuation, 10^{-10} A f.s.d. I = Imipramine; T = trimipramine; D = desimipramine; C = chlorimipramine; O = opiprimol.

range of 1–10 ng for these drugs with the exception of opiprimol and between 5–50 pg calculated as nitrogen content.

Reproducibility

The inherent instability of the AFID detector as a result of its extreme sensitivity to small changes in gas flow has been well documented[9–12]. As mentioned previously, the incorporation of an internal standard with a retention index similar to the drug being estimated overcomes this problem. In a study of the on-column reproducibility of the system a test solution containing 50 ng/μl of amitriptyline and desimipramine was analysed ten times on each of three successive days. Each day the instrument was turned off after use and set up again the following day. Using this method a coefficient of variation of less than 3% was found for the ratio of the two drugs.

The reproducibility of the whole procedure was tested on three concentrations of imipramine in plasma over the therapeutic range. Standard curves were prepared each day using blood bank plasma containing 0–125 ng/ml imipramine hydrochloride. A solution of butriptyline hydrochloride 200 ng/ml was used as internal standard. The test solutions and calibration standards were extracted simultaneously thus eliminating variation due to extraction conditions. The ratio of imipramine to butriptyline was calculated and compared with the values obtained from the standard solutions. The results from six determinations of each of the three concentrations studied are shown in Table II. Coefficient of variation for concentrations in the range 25–100 ng/ml were found to be 3–10% and recoveries were in the range 98–103%.

TABLE II

REPRODUCIBILITY OF IMIPRAMINE ESTIMATION FROM PLASMA AT THERAPEUTIC CONCENTRATIONS

Imipramine concentration (ng/ml)	*Amount found (ng/ml)*	*Recovery (%)*	*Standard deviation (%)**
25	25.8	103	10.5
50	49.0	98	2.4
100	103.3	103	2.0

* Six determinations.

CONCLUSIONS

The AFID, selective to nitrogen, has been shown to be extremely sensitive to the tricyclic antidepressants. By use of the backflush system described, interferences from extraneous extraction products were considerably reduced and reproducibility was improved by eliminating detector saturation. The improved detector sensitivity over the conventional flame ionization detector systems allows a simple rapid extraction procedure to be used without the necessity of lengthy derivatisation procedures. The system is equally sensitive to secondary and tertiary amines, thus enabling routine analyses of imipramine and amitriptyline at therapeutic concentrations which, until now, have been difficult.

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