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Note

Rapid gas chromatographic method for the determination of clonazepam in serum and cerebrospinal fluid

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Clonazepam is becoming widely used as an anticonvulsant in status epilepticus[1] and in epilepsy prophylaxis[2–4]. Thus there is a need for a rapid and sensitive assay of this drug for therapeutic monitoring and for further pharmacological evaluation. The published assay procedures are too time-consuming for routine use[5,6], lack sufficient sensitivity for detailed pharmacokinetic studies[7] or coextract interfering contaminants[8]. We have developed a rapid, sensitive and reproducible method which is now in routine use for both therapeutic monitoring and pharmacokinetic studies.

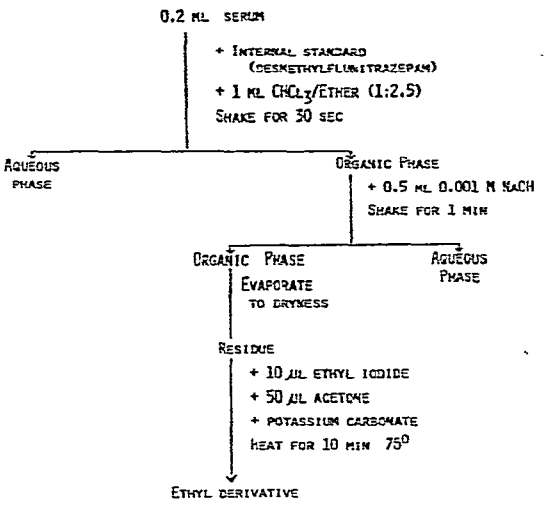
EXPERIMENTAL

Materials

Clonazepam and the internal standard, desmethylflunitrazepam were supplied by Roche (Sydney, Australia). All reagents and solvents were analytical grade and were redistilled shortly before use. Aqueous sodium hydroxide solution (0.001 *M*, pH 9–10) was extracted with an equal volume of chloroform. Extraction and derivative formation was carried out in test tubes with PTFE-lined screw caps. The tubes were silanised with dimethyldichlorosilane and rinsed with the extracting solvent mixture. 0.4 μg ml solutions of clonazepam and desmethylflunitrazepam were prepared in acetone.

Extraction (Fig. 1)

Internal standard (0.02 ml) was added to 0.2 ml of serum and extracted by shaking vigorously for 30 sec with 1 ml of a mixture of chloroform–diethyl ether (1:2.5). The tubes were centrifuged briefly to separate the phases. The organic phase was decanted to another tube and washed with 0.5 ml of 0.001 *M* sodium hydroxide by shaking for 1 min. After centrifuging, the organic phase was transferred to another tube and evaporated to dryness for derivative formation. Known standards were assayed with each set of samples.



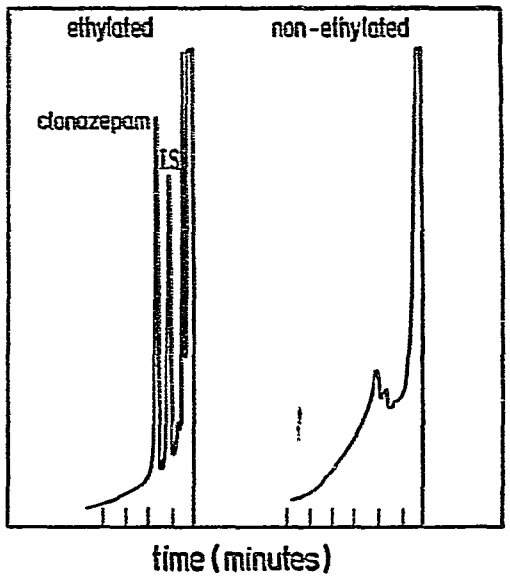


Fig. 1. Clonazepam extraction and derivative formation.

Fig. 2. Chromatogram obtained from assaying 0.2 ml of serum containing 4 ng of internal standard and 8 ng of clonazepam.

Derivative formation

To the residue was added 50 µl acetone, 10 µl ethyl iodide and a small amount of potassium carbonate. The tubes were capped and heated for 10 min at 75° to form the ethyl derivative. After evaporation the residue was dissolved in 0.1–0.2 ml hexane and 2–3 µl were injected into the gas chromatograph.

Chromatography

The instrument used was a Pye series 104 chromatograph equipped with a ^{63}Ni electron capture detector. The 2 m × 2 mm I.D. glass column was packed with 80–100 mesh Chromosorb W, AW DMCS, coated with 4% OV-101. Operating conditions were: column temperature, 280°; detector temperature, 300°; carrier gas was oxygen-free nitrogen at 25 ml/min with an added detector purge of 20 ml/min; pulse interval, 500 µsec.

RESULTS AND DISCUSSION

A typical chromatogram using this procedure is shown in Fig. 2. The retention times of the internal standard and clonazepam were 65 and 90 sec, respectively. The recovery was 95–100% of clonazepam added to serum at concentrations of 10–100 ng/ml. A calibration curve is shown in Fig. 3. 0.1–0.15 ng of clonazepam gives a full scale recorder deflection at an attenuation of 5×10^2. Reproducible results can be obtained from assaying concentrations as low as 0.1 ng/ml in cerebrospinal fluid (CSF) and ultrafiltrate or 0.5 ng/ml in serum. It was necessary to silanise the glassware to minimise adsorption losses and to rinse glassware with the extracting solvent to reduce contamination which might interfere with chromatographic analysis. The wash with 0.001 *M* sodium hydroxide removes a considerable amount of coextractive which

elutes before the internal standard and permits more rapid chromatographic analysis. The derivative formation procedure is based on that described by Dünges *et al.*[9]. The reaction is complete in 3–5 min at 75° and uses only small amounts of reagents which are easily purified. The formation of an ethyl derivative considerably enhanced the detector response under the conditions employed (Fig. 2). An ethyl, rather than methyl derivative improved chromatographic separation.

This method is equally applicable to the assay of serum ultrafiltrate and CSF using slightly larger volumes. A single sample can be assayed in less than 30 min or 15 samples and 4 standards, all in duplicate, can be assayed in half a day.

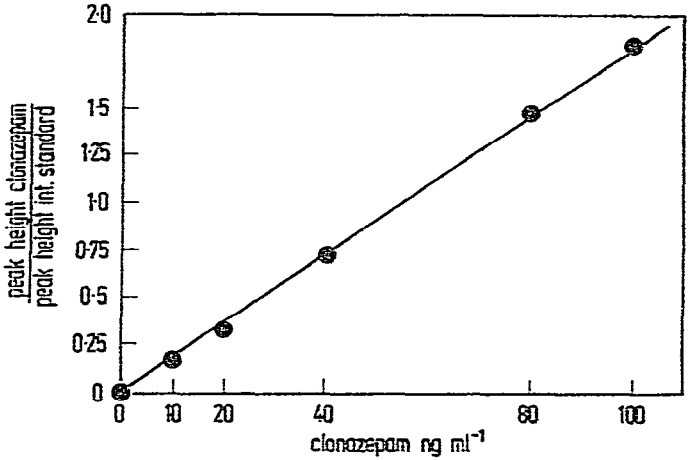


Fig. 3. Calibration curve obtained from adding clonazepam to serum in concentrations of 10–100 ng/ml. Internal standard concentration is 40 ng/ml.

The method described has been applied to an investigation of the pharmacokinetics of clonazepam in the sheep which involves clonazepam assay in serum, ultrafiltrate and CSF[10], and for the therapeutic monitoring of clonazepam in epileptic patients.

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REFERENCES

1 H. Gastaut, J. Courjon, R. Poiré and M. Weber, *Epilepsia*, 12 (1971) 197.
2 C. Y. Huang, J. G. McLeod, D. Sampson and W. J. Hensley, *Med. J. Aust.*, 2 (1974) 5.
3 F. E. Dreifus, J. K. Penry, S. W. Rose, H. J. Kupferberg, P. Dyken and S. Sato, *Neurology*, 25 (1975) 255.
4 J. A. Aarli, *Acta Neurol. Scand., Suppl.*, 53 (1973) 11.
5 J. A. F. de Silva, C. V. Puglisi and N. Munno, *J. Pharm. Sci.*, 63 (1974) 520.
6 J. Naestoft and N.-E. Larsen, *J. Chromatogr.*, 93 (1974) 113.
7 M. Gerna and P. L. Morselli, *J. Chromatogr.*, 116 (1976) 445.
8 J. A. F. de Silva and I. Bekersky, *J. Chromatogr.*, 99 (1974) 447.
9 W. Dünges, H. Heinemann and K. J. Netter, in S. G. Perry (Editor), *Gas Chromatography*, Applied Science Publishers, Barking, 1972, p. 99.
10 G. J. G. Parry, in preparation.