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# Monitoring serum clobazam by isothermal gas–liquid chromatography with nitrogen detector*

MARIA ISABEL ARRANZ PEÑA†§ and ENRIQUE SAENZ LOPE‡

† *Biochemistry Service, "Ramón y Cajal" Hospital, C Colmenar Km/9.100, 28034 Madrid, Spain*
‡ *Neurology Service, Centro Nacional de Rehabilitación, Madrid, Spain*

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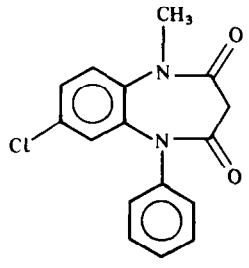
## Introduction

Clobazam (I) is a 1,5-benzodiazepine derivative which is widely used as an anxiolytic antiepileptic agent [1–4].

The major metabolic pathway of clobazam in humans appears to involve hepatic *N*-demethylation to yield desmethylclobazam (II) [5], a metabolite that also has pharmacological activity [6].

The present paper describes a fast and simple gas–liquid chromatographic method, involving the use of a nitrogen selective detector, for the simultaneous determination of clobazam and desmethylclobazam in serum.

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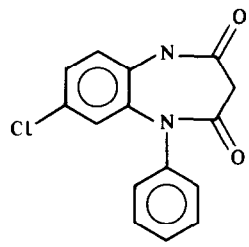


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§To whom correspondence should be addressed.

## Experimental

*Apparatus and chromatographic conditions*

The analytical instrument was a gas chromatograph (Perkin–Elmer model 3920 B) equipped with a nitrogen detector and an electronic integrator. The column, constructed from Pyrex glass (200 cm × 2 mm i.d.) was packed with 3% SP 2250 on 100–120 mesh Supelcoport (Supelco Inc., Bellefonte, PA). Nitrogen was used as carrier gas at a flow rate of 30 ml $min^{-1}$. The operating temperatures were: injection port, 350°C; column 280°C, and detector 350°C. At the beginning of each working day, three 5 μl volumes of egg lecithin in benzene (10 mg $ml^{-1}$) were injected in order to prime the column.

*Preparation of stock solutions*

Clobazam, desmethylclobazam (Hoescht Aktiengesellschaft, 6230 Frankfurt, Main 80) and the internal standard flunitrazepam (Hoffman-La Roche, Nutley, NJ 07110) were dissolved in ethyl acetate to produce solutions containing 100 mg $l^{-1}$ of each compound. The stock solutions were stored in amber bottles at −4°C.

Working standards were prepared containing up to 750 μg $l^{-1}$ for clobazam, up to 2250 μg $l^{-1}$ for *N*-desmethylclobazam and 200 μg $l^{-1}$ flunitrazepam were prepared by appropriate dilution with ethyl acetate.

*Preparation of samples*

A constant amount of serum (500 μl) was added to a glass stoppered centrifuge tube, then evaporated carefully to dryness in a stream of nitrogen at 40–50°C. Calibration standards were prepared by addition of known amounts of clobazam and desmethylclobazam and the solvents again evaporated to dryness. Serum (500 μl) of drug-free control was added to each calibration tube and 250–1000 μl of test sample was added to each tube that contained only the internal standard. The calibration standards were extracted and analysed daily together with each batch of unknowns.

*Extraction*

To each tube was added 5 ml of extraction solvent (toluene–ethyl acetate 75:25, v/v). The tube contents were vortex-mixed for 15 min and then centrifuged for 5 min. An accurate volume of the organic layer was transferred to another tube and evaporated carefully to dryness with nitrogen. The residue was dissolved in 50 μl of ethyl acetate and 2.5–5 μl was injected into the chromatograph.

## Results

*Evaluation of the method*

Under the described chromatographic conditions, the relative retention times (internal standard = 1.0) were found to be 0.88 for clobazam and 1.24 for desmethylclobazam (Fig. 1).

The relation between peak height ratio and serum concentration was found to be linear for clobazam and desmethylclobazam for serum concentrations up to 750 μg $l^{-1}$ for clobazam and 2250 μg $l^{-1}$ for desmethylclobazam, respectively. Results obtained by using linear regression analysis were for clobazam: $y = 0.009x + 0.012$ and for *N*-desmethylclobazam: $y = 0.003x + 0.000$. The precision of the method was determined by assaying serum samples which contained known quantities of the drugs. As shown in

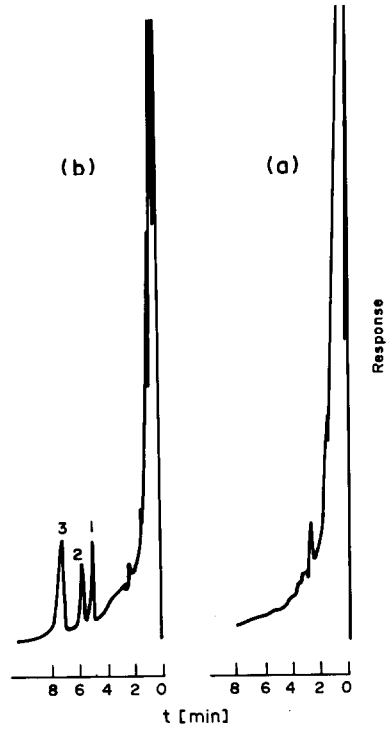


**Figure 1**
Chromatogram of a drug free control serum extract (a) and chromatogram of a patient receiving clobazam (b). The peaks shown are: (1) clobazam (122 $\mu g\ l^{-1}$); (2) flunitrazepam (internal standard); (3) desmethylclobazam (290 $\mu g\ l^{-1}$).

**Table 1**
Precision for simultaneous determination of drugs in serum

| Drug | Clobazam Mean $\mu g\ l^{-1}$ | RSD % | Bias | *N*-desmethylclobazam Mean $\mu g\ l^{-1}$ | RSD % | Bias |
|---|---|---|---|---|---|---|
| Within-day | 13 | 4.4 | 0.48 | 57 | 6.8 | 0.60 |
| $N = 14$ | 32 | 4.2 | 0.85 | 112 | 4.9 | 0.59 |
| | 114 | 2.9 | 0.28 | 302 | 3.9 | 0.38 |
| | 183 | 1.4 | 0.17 | 459 | 4.4 | 0.48 |
| Between-day | 240 | 7.7 | 0.53 | 750 | 4.1 | 0.43 |
| $N = 31$ | 95 | 6.6 | 0.58 | 249 | 6.6 | 0.52 |
| Run to run | 99 | 3.8 | 0.13 | 381 | 4.3 | 0.87 |
| $N = 20$ | | | | | | |

Table 1 the within-day precision ranged from 1.4 to 6.8, between-day precision ranged from 5.7 to 7.1 and run-to-run precision between 3.8 and 4.3.

The approximate detection limits, were 2–5 $\mu g\ l^{-1}$ of the original sample when 1 ml serum was extracted. The analytical recovery was evaluated by spiking drug free serum with working standard solutions and comparing the results with those obtained by direct injection of some amount of working standard solutions. The percentage recoveries were about 95%.

## Discussion

The sensitivity and specificity of the nitrogen detector has facilitated the development of quantitative methods for a number of drugs in human serum following therapeutic dosage.

The present report describes the application of this approach to the simultaneous determination of clobazam and its major pharmacologically active metabolite, desmethylclobazam.

When columns containing the phase SP 2250 are primed with lecithin, improved chromatographic performance is observed for desmethylclobazam so that complete separation of clobazam from its desmethyl metabolite, the internal standard and from endogenous serum contaminants is observed.

The proposed method is simple and fast since it analyses the drugs in free form and under isothermal conditions with only one neutral extraction step.

## References

[1] A. G. Chapman, R. W. Horton and B. S. Meldrum, *Epilepsia* **19**, 293–295 (1978).
[2] B. S. Meldrum, A. G. Chapman and R. W. Horton, *Br. J. Clin. Pharmacol.* **7**, 55S–60S (1979).
[3] H. Gastaud, *Nouv. Press Med.* **7**, 2400 (1979).
[4] H. Gastaud and M. D. Low, *Epilepsia* **20**, 437–446 (1979).
[5] M. Volz, O. Christ, H. M. Kellner, H. W. Fehlhaber, D. Gantz, P. Hadju and F. Cavagna, *Br. J. Clin. Pharmacol.* **7**, 41S–50S (1979).
[6] S. Fielding and I. Hoffman, *ibid.* **7**, 7S (1979).

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