An attempt to correlate the *in vivo* with the *in vitro* release of the indomethacin proved to be unsatisfactory because of the wide variance in the results from three *in vivo* treatments and three dissolution methods. However, the dialyzing tubing method gave the best correlation with the *in vivo* data in three suppository bases, whereas the USP dissolution method gave the best correlation only with the use of fatty suppository bases (esterified fatty acids (C_{10}–C_{18}) and theobroma oil). Attempts to use a sequential order correlation between the three dissolution methods and the *in vivo* results show that the best correlation was found during the first 45 min of the experiment.

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NOTES

High-Performance Liquid Chromatographic Determination of Proglumide in Plasma

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Received May 22, 1981, from the *Research Laboratory, Taiho Pharmaceutical Co., Ltd., Kawauchi-cho, Tokushima, 771-01, Japan.* Accepted for publication October 7, 1981.

Abstract □ A rapid and sensitive method for determining the anticholinergic agent, proglumide, in plasma by high-performance liquid chromatography is described. Samples were acidified with hydrochloric acid and extracted with chloroform. The dried extract was resolved in chloroform and chromatographed on an adsorption chromatographic column using a mobile phase of chloroform–methanol (24:1) on a high-performance liquid chromatograph equipped with a UV absorbance detector (240 nm). The detection limit for proglumide was 0.05 μg/ml.

Keyphrases □ Proglumide—high-performance liquid chromatographic determination in plasma □ High-performance liquid chromatography—analysis, proglumide in plasma □ Anticholinergic agent—proglumide, high-performance liquid chromatographic determination in plasma

Proglumide (xylamide, DL-4-benzamido-*N*,*N*-dipropylglutaramic acid) is a derivative of the amino acid that was developed as an anticholinergic agent (1–7). The GLC methods have been reported for the assay of proglumide in plasma (8–10). Proglumide has been assayed for its methyl derivative (8, 9), and its trimethylsilyl derivative determined (10).

The present report describes a rapid, precise, and sen-

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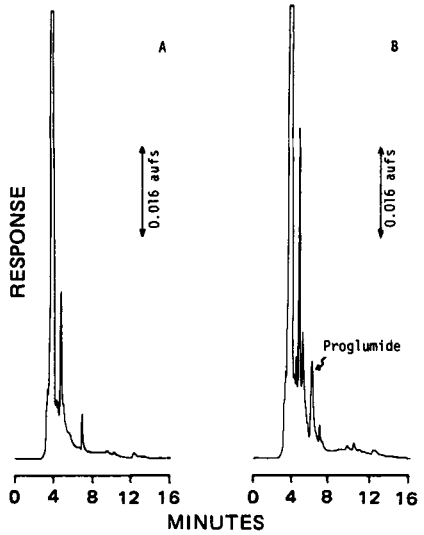

Figure 1—*High-performance liquid chromatograms of human control plasma (A) and human plasma extracts following addition of proglumide at 0.5 μg/ml (B). The conditions were: column (25-cm × 6.2-mm i.d.); mobile phase, chloroform–methanol (24:1); flow rate, 1.5 ml/min; and UV detector, 240 nm (room temperature).*

sitive high-performance liquid chromatographic (HPLC) method using an adsorption chromatographic column for determination of proglumide in plasma.

EXPERIMENTAL

Materials—Proglumide[1] was used as received. Chloroform and methanol were liquid chromatographic grade[2], and the other chemicals[2] were analytical reagent grade.

HPLC Conditions—The high-performance liquid chromatograph[3] was equipped with a high-pressure injection valve[4] and a variable-wavelength (240 nm) UV absorbance detector[5].

An adsorption chromatographic column[6] (25-cm × 6.2-mm i.d.) was used for the separation and maintained at room temperature. The mobile phase was chloroform–methanol (24:1) at a flow rate of 1.5 ml/min. A gradienter[7] was used for controlling the mobile phase concentration. A computer system[8] was employed for quantitative calculations.

Analytical Procedure—Blood samples were collected in heparinized containers and centrifuged to separate the plasma.

A sample of 1.0 ml of plasma was diluted to 2.0 ml with distilled water, adjusted to pH 3.0 with 1 *N* HCl, and then shaken vigorously with 20 ml of chloroform at room temperature for 10 min. This extraction was repeated once. The combined chloroform layer was evaporated to a suitable volume under reduced pressure at water temperature, then transferred to a test tube (5-ml capacity) by washing with chloroform, and dried under nitrogen. The residue was dissolved in 100 μl of chloroform, and 30-μl portions of this solution were injected into the liquid chromatograph with a 100-μl syringe[9].

Calibration curve for the determination of proglumide by HPLC was prepared by plotting the peak area against the concentration of this compound. This calibration curve was linear.

RESULTS AND DISCUSSION

The HPLC separation of proglumide extracted from plasma was first examined using a reversed-phase column[10]. When a linear gradient system with methanol–5 m*M* KH_2PO_4 (0–100% methanol, 6%/min) was used as the mobile phase, proglumide was well separated from plasma constituents.

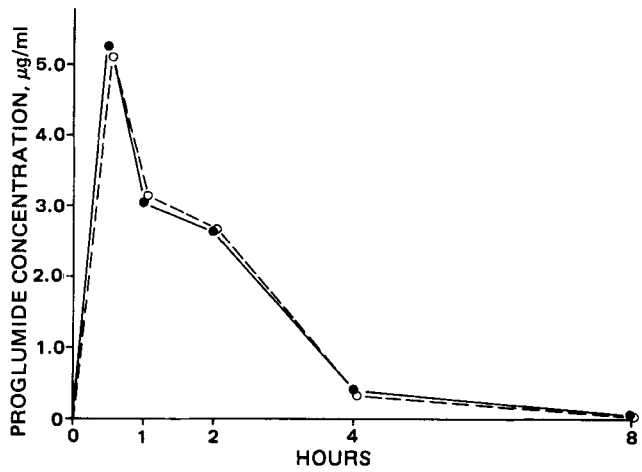

Figure 2—*Comparison of plasma levels of proglumide determined by HPLC (●) and GLC (○) methods after a single oral dose of 400 mg of proglumide to a healthy human.*

An adsorption chromatographic column[6] was used next for the separation of proglumide extracted from plasma. A mixture of a nonpolar solvent (*e.g.*, chloroform, methylene chloride, or ethylene chloride), containing 4–6% alcohol (*e.g.*, methanol or ethanol) as the mobile phase, gave a good separation of proglumide from plasma components. Of these columns and mobile phases tested, a column[6] and chloroform–methanol (24:1) as the mobile phase showed the most suitable chromatographic separation and analysis time and was chosen for the present experiments.

The HPLC separation pattern of proglumide extracted from human plasma following addition of 0.5 μg/ml of this compound and that of a control plasma extract are shown in Fig. 1. The retention time of proglumide was 6.0 min, and the time required for the assay was 15 min. The detection limit for proglumide under the present HPLC condition was 0.05 μg/ml of plasma. The present method had a precision of ±2.8% and good reproducibility.

Known amounts (0.1, 0.5, 1.0, 5.0, and 10.0 μg/ml) of proglumide were added to human plasma (1.0 ml) and the recovery of proglumide was examined five times at each concentration. The overall recovery of proglumide was 93.1 ± 3.2%.

A 400-mg dose of proglumide was administered orally to a healthy male, and the time course of change in concentration of proglumide in plasma was measured by the present HPLC method. The results were compared with those by the GLC method (8, 9). As shown in Fig. 2, the overall difference between the value obtained by the two methods was ± 3.7%.

The present HPLC method is simple and rapid since it does not require the preparation of derivatives and has high accuracy and sensitivity.

[1] Rotta Research Laboratorium S.p.A., Monza, Italy.
[2] Wako Pure Chemicals Co., Osaka, Japan.
[3] Model LC-3, Shimadzu, Kyoto, Japan.
[4] Model SIL-1A, Shimadzu.
[5] Model SPD-2, Shimadzu.
[6] Zorbax SIL (Du Pont), Shimadzu.
[7] Model GRE-2, Shimadzu.
[8] Chromatopac C-R1A, Shimadzu.
[9] Model 100A-RP-GP, Scientific Glass Engineering Ltd., North Melbourne, Australia.
[10] μ-Bondapak C_{18}, Waters Associates, Milford, Mass.

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