Gas-chromatographic separation of chlorpromazine, diazepam and *N*-desmethyldiazepam

The g.l.c. assay of chlorpromazine, diazepam and their metabolites, on OV-17 as a stationary phase has proved difficult because of mutual interference (Curry, 1968, 1974; Christoph, Schmidt & others, 1972; Flint, Ferullo & others, 1971; Mackay, Healey & Baker, 1974, Spirtes, 1972; Rivera-Calimlin, Castaneda & Lasagna, 1973; Marcucci, Fanelli & Mussini, 1968; de Silva & Puglisi, 1970; van der Kleijn, Boelen & Frederick, 1971; Berharrel, Hailey & McLaurin, 1972; Zingales, 1973). The columns used for both groups of drugs have generally been of OV-17. We now find that improved separation can be achieved with columns of OV-225, and Fig. 1 gives a

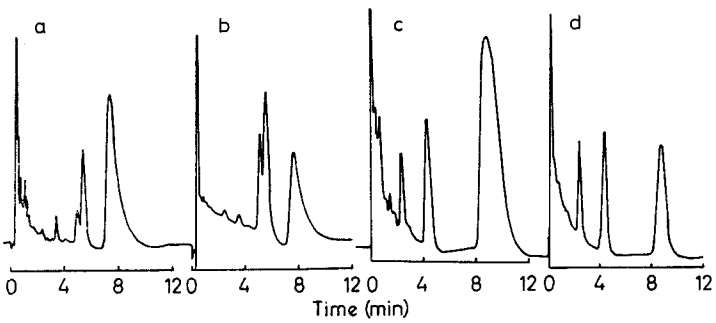

FIG. 1. G.l.c. traces of: (a) one μl of an extract prepared from plasma of a treated human subject, containing chlorpromazine, diazepam and *N*-desmethyldiazepam (OV-17); (b) 5 μl containing chlorpromazine (30 ng) and diazepam and *N*-desmethyldiazepam (2 ng of each) (standards) (OV-17); (c) 8 μl of the extract of (a) (OV-225); (d) 9 μl containing chlorpromazine (50 ng) and diazepam and *N*-desmethyldiazepam (4 ng of each) (standards) (OV-225).

comparison of OV-17 (9′, 3%, on Chromosorb WHP 80/100 mesh at 265°) and OV-225 (3′, 3%, on Chromosorb WHP 80/100 mesh at 245°) columns, in this respect. The retention times for the standards are as follows: OV-17, chlorpromazine 4·9 min, diazepam 5·4 min, *N*-desmethyldiazepam 7·3 min; OV-225, chlorpromazine 2·4 min, diazepam 4·4 min, *N*-desmethyldiazepam 8·8 min. The superiority of OV-225 for both standards and experimental solutions, particularly where both drugs have been administered, is obvious.

Department of Pharmacology and Therapeutics,
The London Hospital Medical College,
Turner Street, London, E1 2AD, U.K.

R. WHELPTON
S. H. CURRY

June 13, 1975

REFERENCES

BEHARREL, G. P., HAILEY, D. M. & MCLAURIN, M. K. (1972). *J. Chromat.*, **70**, 45.

CHRISTOPH, G. W., SCHMIDT, D., JANOWSKY, D. S. & DAVIES, J. M. (1972). *Clin. chim. Acta*, **38**, 265–268.

CURRY, S. H. (1968). *Analyt. Chem.*, **40**, 1251–1255.

CURRY, S. H. (1974). In: *Phenothiazines and Structurally Related Drugs.* pp. 335–346. Editors: Forrest, I. S., Carr, C. J. & Usdin, E., New York: Raven Press,

DE SILVA, J. A. F. & PUGLISI, C. V. (1970). *Analyt. Chem.*, **42**, 1725–1736.

FLINT, D. R., FERULLO, C. R., LEVANDOSKI, P. & HWANG, B. (1971). *Clin. Chem.*, **17**, 830–831.

MACKAY, A. V. P., HEALEY, A. F. & BAKER, J. (1974). *Br. J. Clin. Pharmac.*, **1**, 425–430.

MARCUCCI, F., FANELLI, R. & MUSSINI, E. (1968). *J. Chromat.*, **37**, 318–320.

RIVERA-CALIMLIN, L., CASTANEDA, L. & LASAGNA, L. (1973). *Clin. Pharmac. Ther.*, **14**, 978–984.
SPIRTES, M. A. (1972). *Clin. Chem.*, **18**, 151–152.
VAN DER KLEIJN, E., BOELEN, G. C. & FREDERICK, M. A. (1971). *Clin. chim. Acta*, **34**, 345–356.
ZINGALES, I. A. (1973). *J. Chromat.*, **75**, 55.

Identification of diastereomeric propranolol-*O*-glucuronides by gas chromatography-mass spectrometry

Propranolol, widely used for the treatment of hypertension, has been found by Drs. T. Walle and E. Conradi (unpublished findings) to be extensively metabolized in man by glucuronic acid conjugation. Evidence for the structure of the glucuronic acid conjugate is now presented.

The mass spectrometric analysis was carried out on an LKB 9000S instrument with an ionizing electron energy of 20 eV. The glass g.c. column (3′ × 1·5 mm, i.d.) was packed with 1% OV-1 on silanized 60/80 mesh Chromosorb W. The helium flow was 10 ml min^{-1} with a column temperature of 200°.

(±)-Propranolol was administered chronically to a patient (4 × 80 mg daily). The urine (5 ml) was adjusted to pH 11 (1M NaOH) and washed with 2 × 10 ml of diethyl ether (discarded). The remaining traces of ether were removed by evaporation at 60°. The pH was adjusted to 7 (1M HCl) and the sample was transferred to an Amberlite XAD-2 (20–50 mesh) column (25 × 1 cm) which had been washed with methanol, acetone and distilled water. The column was eluted with 150 ml of distilled water (discarded) and with methanol (10 ml fractions collected) (cf. Fujimoto & Haarstad, 1969). The major portion of the propranolol glucuronide was found in fraction 3 (determined by g.c. as trifluoroacetyl derivative after hydrolysis with Glusulase, Walle & Conradi, unpublished).

Fraction 3 was evaporated to dryness and the residue in methanol (200 μl) treated (15 min, 20°) with diazomethane in diethyl ether (prepared according to Stanley, 1966). Evaporation under nitrogen at 80° gave a residue which, mixed with ethyl acetate (400 μl) and trifluoroacetic anhydride (100 μl) was shaken for 15 min (Vortex mixer)

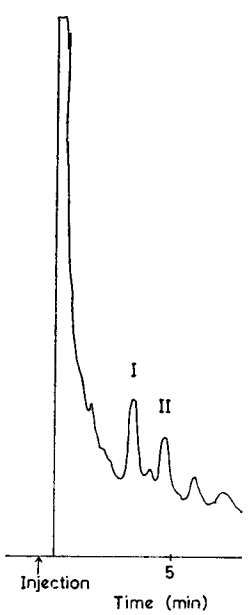

FIG. 1. Total ion current recording from human urine extract. Column: 1% OV-1. Column temperature: 200°. Helium flow: 10 ml min^{-1}.