LETTERS TO THE EDITOR

Estimation of phenacetin and paracetamol in plasma and urine by gas-liquid chromatography

Prescott, Steel & Ferrier (1970) described methods for estimating phenacetin and paracetamol in plasma and urine by gas-liquid chromatography. We experienced difficulties with the methods arising from incomplete silylation of the drugs and from interference in the g.l.c. analysis by extraneous compounds in the ethyl acetate extracts of plasma. The conditions for silylation were not as ideal as those described by Prescott (1971). We have therefore modified the methods by substituting a more powerful silylating reagent (Regisil) and a more selective extractant (diethyl ether).

Estimation of total paracetamol in urine. Urine (1·0 ml), diluted if necessary, 0·2M sodium acetate buffer pH 5·0 (1·0 ml) and Glusulase (0·05 ml) was incubated overnight at 37°. Incubate (1·0 ml) and 1·9 ml of 0·2M phosphate buffer pH 9·9 was saturated with sodium chloride and extracted with diethyl ether (5·0 ml) containing *p*-bromoacetanilide (3 μg/ml). The ether layer was removed, dried with a current of dry nitrogen, and the residue dissolved in 40 μl of Regisil [bis(trimethylsilyl)trifluoroacetamide], (Regis Chemical Co., Chicago, Illinois 60610) and kept at 50° for 30 min. After cooling, 1–2 μl of the sample was injected into a Varian 2100 gas chromatograph. The conditions were: 3% OV-1 on Gas Chrom Q in a 6 ft ¼ inch i.d. glass column. Nitrogen was used as the carrier gas nitrogen flow rate 50 ml/min; hydrogen and air flow rates were 30 and 300 ml/min respectively. Column temperature 160°.

Estimation of phenacetin and free paracetamol in plasma and urine. Urine (diluted if necessary) or plasma (2·0 ml) and 1M phosphate buffer pH 7·4 (1·0 ml) was saturated with sodium chloride and extracted with diethyl ether (5·0 ml) containing *p*-bromoacetanilide (3 μg/ml). From this point the ether extract was treated as already described.

We found that the column packing should be renewed after about 500 injections. The recovery of both phenacetin and paracetamol in the ether extracts was almost complete and reproducibility in plasma was good down to concentrations of 1 μg/ml.

The ether extract is cleaner than that obtained with ethyl acetate giving fewer extraneous peaks on the gas chromatograph. Regisil is a more powerful silylating reagent than TMSI (*N*-trimethylsilylimidazole) or BSA [*N,O*-bis(trimethylsilyl)acetamide] and thus permits the use of less carefully controlled conditions. Regisil is also more volatile and causes less detector contamination than TMSI or BSA. Over 300 urines and 100 plasma samples have been successfully analysed.

Our thanks are due to Messrs. G. Solomonraj and W. Zeitz for technical assistance.

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December 8, 1971

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