and as a result it is easy to separate the blue dyes from them except for Fast Green FCF.

Pharmacognosy Department, Welsh School of Pharmacy, University of Wales Institute of Science and Technology, Cathays Park, Cardiff, Glamorgan, U.K. T. D. TURNER

Elanco Division, Eli Lilly and Company Limited, Basingstoke, Hampshire, U.K. B. E. JONES

July 9, 1971

REFERENCES

BARRETT, J. F. & RYAN, A. J. (1963). *Nature, Lond.*, **199**, 372–373.
CHAPMAN, W. B. & OAKLAND, D. (1968). *Ass. publ. Analysts*, **6**, 124–128.
DAVIDEK, J. & DAVIDKOVA, E. (1967). *J. Chromat.*, **26**, 529–531.
GILL, J. P. (1962). *Chem. Prod.*, **25**, 56–62.
MOTTIER, M. & POTTERAT, M. (1955). *Analytica Chim. Acta*, **13**, 46–56.
NIEMAN, C. (1964). *Food colours recently authorised in 43 countries*, Supplement. Amsterdam: Consudel.
NEY, M., BERGER, K. G., SPERLICH, H. & MIETHKE, H. (1965). *Dt. LebensmittRsch.*, **61**, 148–150.
PARRISH, J. R. (1968). *J. Chromat.*, **33**, 542–543.
SAENZ LASCANO RUIZ, I. (1964). *Annls. Falsif. Expert. chim.*, **57**, 115–118.
WOLLENWEBER, P. (1962). *J. Chromat.*, **7**, 557–560.

Gas-liquid chromatographic estimation of paracetamol

Methods for the gas-liquid chromatographic estimation of paracetamol in plasma and urine have recently been described (Prescott, 1971). The drug could not be chromatographed directly in small amounts without significant absorption losses, and prior conversion to trimethylsilyl (TMS) derivatives was necessary. There are disadvantages however, in the use of silylating agents. The di-TMS derivatives of paracetamol formed with *N,O*-bis(trimethylsilyl)acetamide (BSA) is susceptible to hydrolysis, and silylation of other compounds in plasma extracts may give rise to unwanted peaks on the chromatograms. Although better results were obtained with *N*-trimethylsilylimidazole (TMSI), the sensitivity of the assay is limited by the broad solvent front of the slowly eluting TMSI. Furthermore, the flame ionization detector electrodes become contaminated by deposits of silica.

An improved method is now described in which both paracetamol and *N*-butyryl-*p*-aminophenol (the internal standard) are acetylated. The derivatives are stable, sensitivity is increased and the analysis can be completed in a much shorter time. Phenacetin does not interfere, and could be estimated in a sample at the same time as paracetamol.

Phosphate buffer (1·0 ml, M, pH 7·4) is added to plasma or urine (2·0 ml) containing up to 50 μg of paracetamol in a 15 ml glass-stoppered tube. Redistilled ethyl acetate (5·0 ml) containing *N*-butyryl-*p*-aminophenol (5 μg/ml) is then added and extraction effected by gentle mechanical shaking for 10 min. After centrifugation, the upper organic phase is transferred with Pasteur pipettes to 10 ml tapered stoppered centrifuge tubes and taken to dryness using a rotary vacuum evaporator. Pyridine (5 μl) and acetic anhydride (15 μl) are then added to the residue, the tubes stoppered and the contents mixed with a vortex mixer. The tubes are incubated on a water bath at 45° for 20 min and 1–3 μl aliquots are injected directly into the gas chromatograph. Samples containing paracetamol (50–500 μg/ml) are extracted with ethyl acetate containing *N*-butyryl-*p*-aminophenol (50 μg/ml), the residue is dissolved in pyridine (15 μl) and acetic anhydride (30 μl), and 1 μl aliquots are injected into the chromatograph. Appropriate dilutions are made of more concentrated samples, and total

Table 1. *Estimation of paracetamol added to plasma and urine—results of repeated analyses.*

Concentration (μg/ml)	No. of estimations	s.d. (%)
5–25	20	3·4
50–500	18	3·7
20	47*	3·4

* Each estimation was made on a different sample of plasma.

unchanged and conjugated paracetamol in plasma or urine can be determined by prior hydrolysis with glusulase as described by Prescott (1971).

A Hewlett-Packard Model 402 gas chromatograph with flame ionization detectors and a 2 ft long $\frac{1}{4}$ inch i.d. U-shaped glass tube column packed with 3% HI-EFF 8BP on 100/120 mesh Gaschrom Q (Applied Science) was used with the column temperature 220° and the nitrogen carrier gas flow rate 80 ml/min. The retention times of phenacetin, paracetamol and *N*-butyryl-*p*-aminophenol were 1·6, 3·4 and 4·5 min respectively. Satisfactory results were also obtained with 4 ft columns of 3% OV17 or 1% Carbowax 20 M on Gaschrom Q. *p*-Aminophenol will yield the same acetylated derivative as paracetamol, but this is of little consequence since, in man, *p*-aminophenol is not detectable in biological fluids following ingestion of paracetamol.

An appropriate aqueous standard of paracetamol is run with the samples and drug concentrations determined using the peak height ratios of drug to internal standard. The recovery of paracetamol added to plasma, urine or aqueous solutions is identical, and the precision of the assay is shown in Table 1.

Although interfering peaks were not normally observed with plasma extracts, a single large peak with a retention time approximately 4 times that of the internal standard was regularly encountered with both the above mentioned columns. This peak was not observed when the liquid phase was 3% XE60, but injection of acetic anhydride resulted in rapid deterioration of the column.

I am indebted to Dr. A. Robertson, Sterling Winthrop Research & Development, Fawdon, Newcastle upon Tyne, for a gift of *N*-butyryl-*p*-aminophenol, and I gratefully acknowledge technical assistance given by Mrs. M. E. Roberts and Miss N. Taylor. This work was supported by a grant from the Scottish Hospital Endowments Research Trust.

University Department of Therapeutics,
The Royal Infirmary,
Edinburgh, EH3 9YW, U.K.

L. F. PRESCOTT

July 2, 1971

REFERENCE

PRESCOTT, L. F. (1971). *J. Pharm. Pharmac.*, **23**, 111–115.