Journal of Chromatography, 224 (1981) 492—495
Biomedical Applications
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROMBIO. 879

Note

Measurement of pyrimethamine in human plasma by gas—liquid chromatography

C.R. JONES*, P.R. RYLE and B.C. WEATHERLEY

Department of Drug Metabolism, Wellcome Research Laboratories, Langley Court, Beckenham, Kent BR3 3BS (Great Britain)

(First received November 7th, 1980; revised manuscript received February 25th, 1981)

Pyrimethamine is an antimalarial drug which is sometimes used alone but often used in combination with dapsone as a chemoprophylactic. A method based upon high-performance liquid chromatography (HPLC) for determining pyrimethamine and dapsone simultaneously in human plasma was recently reported from these laboratories [1]. This method, using a UV detector, is conveniently applicable to both compounds but the lower limit of detection is about 5 ng injected for pyrimethamine and 2.5 ng for dapsone. Dapsone however, can be measured at much greater sensitivity by the use of a fluorescence detector [2], a technique not applicable for pyrimethamine which is only poorly fluorescent. The recommended dose of pyrimethamine when used on its own for malaria prophylaxis is 25 mg weekly but no data have been published for the plasma concentrations at this dose level. It was proposed to determine by direct measurement the plasma concentrations in volunteers so dosed and to correlate these with in vitro antimalarial activity as determined by means of a Plasmodium falciparum culture in human erythrocytes.

For this, a method for measuring pyrimethamine at greater sensitivity than hitherto was therefore developed using gas—liquid chromatography (GLC). A GLC assay method for a closely related compound, metoprine [2,4-diamino-5-(3,4-dichlorophenyl)-6-methylpyrimidine] (BW197U) has been published from these laboratories [3] and this served as a useful basis for developing the method. The results from the volunteer study will be reported separately.

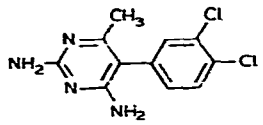
MATERIALS AND METHODS

Reagents and materials

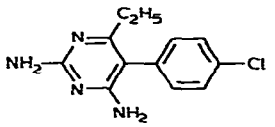
Pyrimethamine and BW197U were used as reference compounds as stock

0378-4347/81/0000—0000/$02.50

solutions at 1 mg/ml in methanol and these were diluted 1:100 with water before use.



BW 197 U



Pyrimethamine

Toluene AR grade was distilled before use. Amyl acetate (mixture of isomers; laboratory reagent grade, BDH, Poole, Great Britain) was distilled before use.

Human plasma was used for the validation experiments and was spiked with the diluted stock solutions of pyrimethamine and BW197U before extraction.

Glassware

Screw-capped 10-ml glass tubes were used in the extraction. Capped 200-μl vials were used for the autosampler.

Extraction procedure

Replicate 1-ml plasma samples or control plasma samples spiked with a range of standard quantities of pyrimethamine and with 20 μl BW197U solution as internal standard were mixed thoroughly and left at 4°C for at least 4 h to ensure equilibration. These samples were diluted with 1 ml water, 0.5 ml 1 *M* sodium hydroxide was added and roller-mixed for 20 min (1100 turns) with two 5-ml portions of toluene. After centrifugation at 1600 *g* for 10 min the pooled organic layers were transferred to clean tubes and dried under a stream of nitrogen gas at room temperature. The residue was redissolved in 100 μl amyl acetate and transferred to the GLC microvial which was then capped ready for chromatographic analysis.

Gas—liquid chromatography

Previous experience with the analysis of metoprine [3] indicated that 10% OV-17 on Chromosorb W HP (100—120 mesh) on a 2 m × 2 mm I.D. glass column would be suitable for the analysis of pyrimethamine and the conditions were optimised to separate the drug and internal standard from endogenous material extracted from plasma. The injection port of the gas chromatograph was maintained at 300°C, the detector at 350°C and the column at 235°C. After the BW197U had eluted, the column temperature was programmed to rise at 16°C/min to 280°C for 4 min, in order to elute late plasma peaks and reduce the overall analysis time for each sample to 26 min. Carrier gas was nitrogen at a flow-rate of 35 ml/min, and under these conditions pyrimethamine and BW197U had retention times of 7.3 and 10.8 min, respectively (Fig. 1).

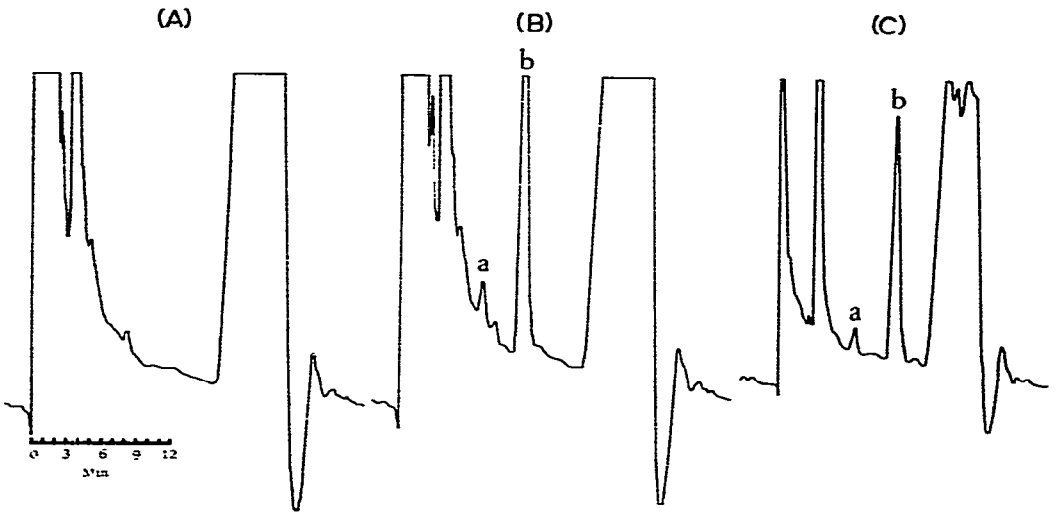


Fig. 1. GLC traces of plasma extracts assayed for pyrimethamine. (A) Unspiked plasma extract. Attenuation x 32. (B) Plasma extract spiked with pyrimethamine at 5 ng/ml (a) and internal standard at 200 ng/ml (b). Attenuation x 32. (C) Volunteer plasma extract containing pyrimethamine at 12 ng/ml (a) and spiked with internal standard at 200 ng/ml (b).

Instrumentation

The gas chromatograph was a Hewlett-Packard 5735A equipped with a constant-current electron-capture detector, with a 7671A automatic injector (5-μl injection used throughout) and linked to a Hewlett-Packard 3352B data system for controlling sample injection and processing chromatograms.

RESULTS AND DISCUSSION

The Hewlett-Packard 3352B data system identified by retention time, measured peak areas of pyrimethamine and BW197U, and calculated peak area ratios. Analysis of quadruplicate samples spiked with pyrimethamine over the range 5—400 ng/ml gave a coefficient of variation of 5.5%, being essentially constant over the whole concentration range. Recoveries of pyrimethamine and BW197U, which were also independent of concentration, were 75.6 ± 0.9% S.D. (n = 6) and 74.7 ± 1.4% S.D., respectively.

Regression of the logarithm of peak area ratios versus logarithm of pyrimethamine concentrations gave a correlation coefficient of 0.9994 (n = 23). The minimum detectable quantity of pure compound (signal-to-noise ratio = 2) was 50 pg injected on column and this allowed plasma concentrations ranging from 5—400 ng/ml to be comfortably assayed from volunteers receiving this drug. These results will be reported separately. The only other determination for pyrimethamine using GLC which appears to have been published [4] had a lower limit of sensitivity of 100 ng per g tissue. Using quantitative thin-layer chromatography, DeAngelis et al. [5] achieved a limit of 10 ng/ml as did Jones and Ovenell [1] using HPLC. In order to measure the lowest concentrations by the HPLC method it was necessary to inject over half of the extract. Using this

GLC method the errors are smaller and because only one twentieth of the total extract is injected at one time, replicate injections are possible. By this means, plasma levels can be monitored in volunteers receiving a single prophylactic dose (25 mg) of pyrimethamine for several weeks after dosing.

REFERENCES

1 C.R. Jones and S.M. Ovenell, J. Chromatogr., 163 (1979) 179.
2 C.A. Mannan, G.J. Krol and B.T. Kho, J. Pharm. Sci., 66 (1977) 1618.
3 J.S. Cridland and B.C. Weatherley, J. Chromatogr., 137 (1977) 449.
4 P.C. Cala, N.R. Trenner, R.P. Buhs, G.V. Downing, Jr., J.L. Smith and W.J.A. VandenHeuvel, J. Agr. Food Chem., 20 (1972) 337.
5 R.L. DeAngelis, W.S. Simmons and C.A. Nichol, J. Chromatogr., 106 (1975) 41.