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DETERMINATION OF 2,4-DIAMINO-5-(3,4-DICHLOROPHENYL)-6-METHYLPYRIMIDINE (BW 197U) IN HUMAN PLASMA

J. S. CRIDLAND and B. C. WEATHERLEY

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

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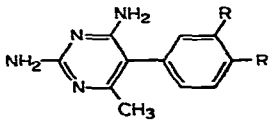
SUMMARY

A gas–liquid chromatographic method for the measurement of 2,4-diamino-5-(3,4-dichlorophenyl)-6-methylpyrimidine (BW 197U) in human plasma has been developed. After extraction from alkaline medium into ethyl acetate the compound is injected into a gas–liquid chromatograph and measured using a ^{63}Ni constant-current electron-capture detector. The range of concentrations measured was from 10 ng/ml to 10 μg/ml in plasma. An internal standard was employed and reproducibility between replicates was found to be good. The method has been semi-automated by the use of an auto-sampler controlled by a minicomputer which also processes the data.

INTRODUCTION

Compound BW 197U (Ia), also known as DDMP, methodichlorophen and metoprine, has been investigated for a number of years as a potential antineoplastic agent[1,2] and is now undergoing clinical trial in certain types of human cancer. Conduct of these trials is aided by knowledge of the concentration of the drug in the plasma of the patients involved.



Ia R = Cl
Ib R = Br

DeAngelis, Simmons and Nichol[3] have described a specific method for measuring BW 197U in plasma based on quantitative scanning of thin-layer chromatographic plates. However, the availability of a semi-automated gas–liquid chromatograph in these laboratories made analysis using this instrument seem attractive.

In this report a rapid and precise gas–liquid chromatographic (GLC) procedure for the analysis of BW 197U in human plasma is described.

EXPERIMENTAL

Reagents and materials

BW 197U is a weak base (p*K* 7.15), stable to prolonged heating in water and in 0.1 *M* aqueous sodium hydroxide, soluble in most organic solvents and in aqueous solutions of organic acids. Solutions in plasma are stable for at least 6 months at −20°. Its partition coefficient between octan-1-ol and water is 660 and the optimum pH range for extraction from plasma into ethyl acetate, butan-1-ol or nitromethane is 11–13.

The compound 2,4-diamino-5-(3,4-dibromophenyl)-6-methylpyrimidine (BW 214U; Ib) was chosen as internal standard because of its close similarity to BW 197U in physical properties and its appropriate retention time. Other chemicals used were ethyl acetate, lactic acid, trisodium orthophosphate (all AnalaR grade; BDH, Poole, Great Britain) and amyl acetate (mixture of isomers; laboratory reagent grade, BDH). The sodium phosphate was employed as a saturated solution in water (about 500 g/l).

Standard solutions of BW 197U were prepared in horse plasma for reasons of safety and because it proved, for the purposes of this assay, to be indistinguishable from human plasma. A stock solution was made by dissolving 14.76 mg of the drug in 100 ml water containing 20 μl lactic acid. Of this solution 1 ml was diluted to 50 ml in horse plasma to make the stock standard solution which, in turn, was further diluted to make the working standard solutions. The internal standard solution was prepared by dissolving BW 214U in a similar fashion in order to achieve a final concentration of about 7.5 μg/ml.

Glassware

Screw-capped 10-ml Sovirel tubes (V. A. Howe, London, Great Britain) were used in the extraction. Vials of 1.5 ml capacity were employed to hold solutions in the autosampler.

Gas–liquid chromatograph

A Perkin-Elmer F30 instrument, modified to allow sample injection from a Hewlett-Packard 7670A autosampler, was used with a ^{63}Ni electron-capture detector; this was operated in constant-current mode which was superior to constant-pulse mode in linearity of response and in dynamic range, especially under conditions of high column bleeding. Operation of the autosampler and data processing were carried out using a Hewlett-Packard 3352B minicomputer.

Extraction from plasma

Preliminary experiments having shown that extraction of BW 197U from plasma was most efficient at pH 11–13 into ethyl acetate, the following procedure was devised. Each 1-ml plasma sample (standard or unknown) was mixed with 0.2 ml internal standard solution and 0.2 ml sodium phosphate solution, giving a final pH of 11. Ethyl acetate (7 ml) was added and the phases mixed by tumbling end-over-end at 15 min^{-1} for 15–20 min. The phases were separated by centrifugation and the upper layer transferred as completely as possible to a clean tube. Solvent was removed by evaporation below 100° under a stream of nitrogen and the residue was dissolved in 1.5 ml amyl acetate; this solution was transferred to an autosampler vial for analysis.

Gas–liquid chromatography

Several stationary phases were investigated and it was concluded that 10% OV-17 on Chromosorb W HP (100–120 mesh) was the most efficient in terms of separating the drug and internal standard from endogenous plasma material, and of minimizing tailing. The column was maintained at 290°, the injection port at 300° and the detector at 350°; it was noticed that the sensitivity of detection of BW 197U and of BW 214U was to a minor extent a function of detector temperature. The carrier gas was argon, flowing through the column at 50 ml/min. Under these conditions the retention time of BW 197U was 3.0 min and that of BW 214U was 5.2 min.

RESULTS

Although peaks showed slight tailing (Fig. 1), as is often observed when basic compounds are analysed on the silicone phases necessary for operation at high temperatures, reproducibility between replicates was excellent (Table I). On a weight-for-weight basis, the sensitivity of detection of BW 214U was about twice that of BW 197U; the minimum detectable amount of the latter injected on to the column was less than 10 pg.

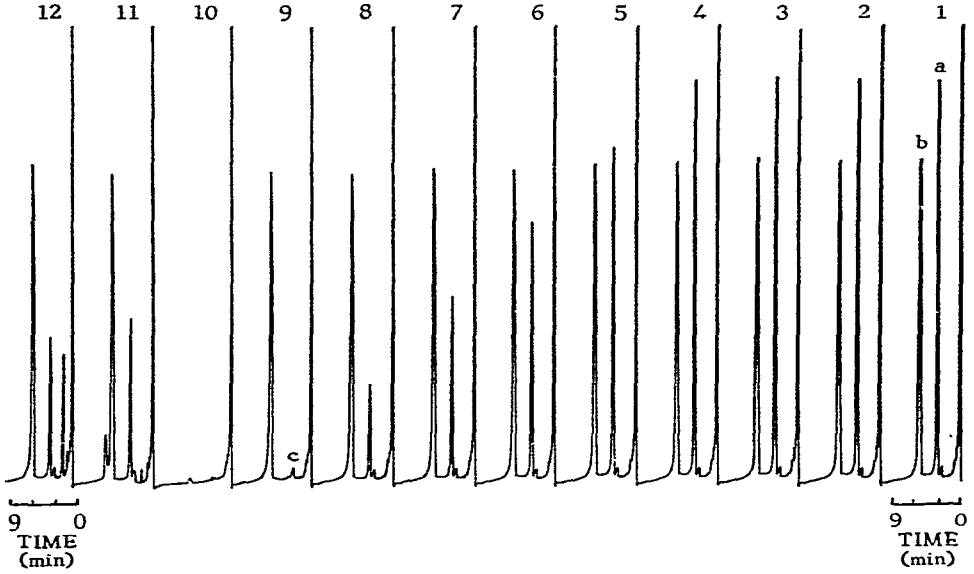


Fig. 1. Traces from GLC of plasma samples extracted and analysed for BW 197U. a = BW 197U; b = BW 214U; c = trace impurity in b. 1–4 are replicate extractions of the standard solution containing 2.96 μg/ml BW 197U; 4–9 are the series of standard solutions containing 1.5 μg/ml BW 214U and 2.96, 2.36, 1.77, 1.18, 0.59 and 0 μg/ml BW 197U; 10 is blank human plasma, and 11 and 12 are plasma samples from patients.

Computation of results

Data from the chromatograph were processed by the computer to give, for each sample, the ratio of the areas of the BW 197U and the BW 214U peak. Using

TABLE I

REPRODUCIBILITY OF THE ASSAY ON REPEATED ANALYSIS OF THE STANDARD SOLUTIONS

S.D. is the standard deviation; ratio is the area of the BW 197U peak divided by that of the BW 214U peak. Each standard solution was analysed in octuplicate.

Concentrations (μg/ml)		*Mean ratios (S.D.)*
Added	*Found (S.D.)*	
0.59	0.58 (0.02)	0.149 (0.002)
1.18	1.19 (0.03)	0.308 (0.008)
1.77	1.77 (0.04)	0.456 (0.010)
2.36	2.34 (0.03)	0.596 (0.007)
2.95	2.95 (0.05)	0.745 (0.012)

results obtained from analyses of the standard solutions a calibration curve could be constructed of the form:

$$R_i = \frac{A}{B + C_i} - \frac{A}{B} + \frac{C}{(B + C_i)^2} - \frac{C}{B^2} \tag{1}$$

where R_i is the ratio obtained by analysing the standard solution of concentration C_i and A, B and C were constants determined by minimizing the sum-of-squares function:

$$\sum_i \frac{(R_i - R_{i_{obs.}})^2}{C_i^2} \tag{2}$$

where $R_{i_{obs.}}$ was the observed ratio. This procedure was used since calibration curves calculated from data derived by the use of electron-capture detectors are often sigmoidal in shape.

The computer was programmed to use the values of the constants A, B and C in order to calculate concentrations of BW 197U in the plasma of patients on treatment with the drug from the corresponding ratios obtained by analysis.

Validation

Concentrations in the range 20 ng/ml to 10 μg/ml BW 197U in plasma have been measured using the method described and were found in all cases to fit the calibration curve of the form given in eqn. 1.

Each standard solution used for work described in this paper was analysed in octuplicate. Ratios and concentrations found, calculated using the described calibration curve (eqns. 1 and 2), are shown in Table I.

The present method has been compared with the thin-layer chromatographic scanning method of DeAngelis, Simmons and Nichol[3] but using a Farrand scanner. Samples of plasma from 17 patients treated with BW 197U and from one untreated patient were assayed by both techniques anda good agreement was observed (Table II).

A comparison of results was also made with those obtained by a spectrophotometric enzymic assay[4] for the drug using 51 plasma samples from 7 patients (Table II). Good agreement was also found in this comparison.

TABLE II

CORRELATIONS BETWEEN PRESENT AND OTHER METHODS

Methods: 1, quantitative scanning of thin-layer chromatographic plates[3] and 2, enzyme inhibition by BW 197U measured by spectrophotometry[4].

Parameter	*Method*	
	1	*2*
Number of data	18	51
Correlation coefficient	0.977	0.936
Student t statistic	18.5	18.6

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