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Note

Simultaneous high-performance liquid chromatographic assay for 5-(2-bromo-*E*-ethenyl)-2′-deoxyuridine and its metabolite, 5-(2-bromo-*E*-ethenyl)uracil, in plasma

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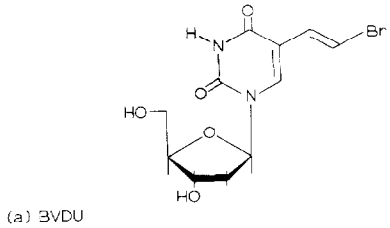
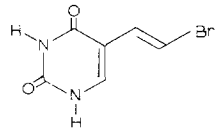
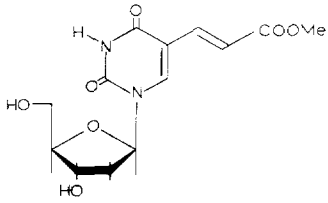
Many modified nucleosides have been synthesised and tested for activity against the *Herpes* viruses. Recently, a derivative of 2′-deoxyuridine, 5-(2-bromo-*E*-ethenyl)-2′-deoxyuridine (Fig. 1, BVDU, SC 38394), has been shown to be a potent anti-herpetic compound [1, 2]. It has shown activity in vitro [1] and in vivo [2] against *Herpes labialis* and *Varicella zoster*, and has proved effective in the treatment of herpetic keratitis and "shingles" in man [3].



(a) BVDU



(b) BVU



(c) Internal standard

Fig. 1. Structures of (a) BVDU, (b) BVU and (c) the internal standard.

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During studies of the disposition of BVDU, 5-(2-bromo-*E*-ethenyl)uracil (Fig. 1, BVU) was identified as a prominent plasma metabolite in animals and man [4]. Further investigation of BVDU required a sensitive assay for the drug and its metabolite, BVU, in the plasma of animals and man. The present report describes the development of the assay and some preliminary results of its application to studies of the pharmacokinetics of BVDU.

EXPERIMENTAL

Materials

BVDU, its metabolite BVU and the internal standard 5-(2-carbomethoxy-*E*-ethenyl)-2′-deoxyuridine (Fig. 1) were supplied by Chemical Development, G.D. Searle (High Wycombe, U.K.). All other reagents and HPLC-grade solvents were purchased from BDH (Poole, U.K.), unless otherwise stated.

Aqueous solutions of potassium dihydrogen orthophosphate (0.2 *M*, 19.5 ml) and disodium hydrogen orthophosphate (0.2 *M*, 30.5 ml) were mixed and diluted to 1500 ml with glass-distilled water. Suitable volumes of this solution were mixed with acetonitrile to give mobile phases containing different proportions of acetonitrile and 0.007 *M* phosphate buffer (pH ~7). The mobile phases were helium-degassed and vacuum-filtered before use.

Stock solutions (0.1 mg/ml) of BVDU, BVU and internal standard were prepared by dissolving each compound (5 mg) in methanol (30 ml) and diluting with glass-distilled water to 50 ml in a volumetric flask. Working standard solutions of these compounds were prepared by suitable dilutions of the stock solutions with glass-distilled water or plasma as required.

Apparatus

Chromatography was carried out with a Series 3B pump, ISS-100 sample processor and LC85 UV detector set at 254 nm (Perkin-Elmer, Beaconsfield, U.K.). The analysis was carried out on a μBondapak C_{18} column (10 μm; 300 mm × 3.9 mm I.D.) fitted with a Bondapak C_{18}/Corasil guard column (37 μm; 20 mm × 3.9 mm I.D.) (Waters Assoc., Hartford, U.K.). The output from the UV detector was linked to a Perkin-Elmer RO56 dual-pen potentiometric recorder and an HP3390A integrator (Hewlett-Packard, Wokingham, U.K.).

Selection of HPLC conditions

The separation of BVDU, BVU and the internal standard from ethyl acetate extractable plasma components was optimised using different proportions of acetonitrile and phosphate buffer. The optimal solvent composition was acetonitrile—0.007 *M* phosphate buffer (pH 7; 12.5:87.5, v/v).

Assay procedure

The internal standard working solution (0.5 ml; 3 μg/ml) was added to plasma (0.5 ml) in an amber glass test tube and mixed. The plasma was extracted with ethyl acetate (2 × 2.5 ml) on a partitioning extractor, centrifuged at 2500 *g* for 10 min, and the combined organic phases were evaporated to dryness under a nitrogen stream at 45°C (SC-3 evaporator, Techne, Cambridge, U.K.).

The extract residue was redissolved in acetonitrile—0.007 *M* phosphate buffer (70 μl; pH ~7; 12.5:87.5, v/v), the mobile phase, and a 40-μl aliquot was analysed on a μBondapak C_{18} column (300 × 3.9 mm I.D.) fitted with a pre-column (20 × 3.9 mm I.D.) containing Bondapak C_{18} Corasil, at a mobile phase flow-rate of 2 ml/min.

The peak height ratios for BVDU and BVU versus internal standard were measured by the integrator. The concentrations of BVDU and BVU in plasma were determined by the integrator using a response factor obtained by the analysis of calibration standards with each batch of samples. The accuracy and precision of the assay were assessed by the analysis of plasma samples containing added BVDU and BVU at concentrations which were unknown by the analyst.

RESULTS AND DISCUSSION

The assay method efficiently resolved BVDU (retention time, t_R 8.5 min), BVU (t_R 7.5 min) and the internal standard (t_R 6 min) from the co-extracted components in the plasma of rat, dog and man (Fig. 2).

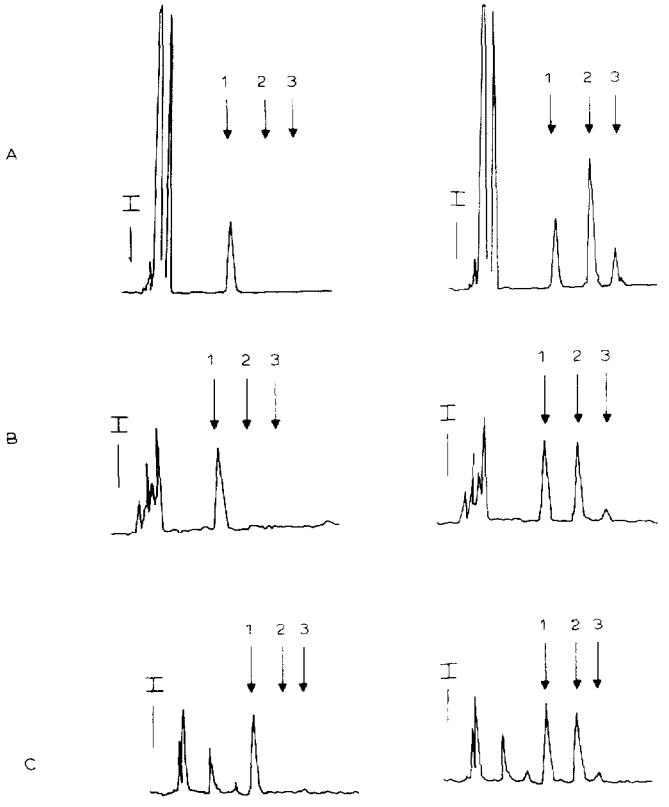

Fig. 2. HPLC profiles of plasma extracts from (A) rat, (B) dog and (C) man before and after oral doses of BVDU. Column: μBondapak C_{18}. Solvent: acetonitrile—0.007 *M* phosphate buffer (12.5:87.5, v/v). I = injection. Peaks: 1 = internal standard; 2 = metabolite (BVU); 3 = BVDU.

There was a linear correlation between peak height ratio and concentration for BVDU and BVU in the plasma of each species over the concentration ranges 0—5 μg/ml and 0—15 μg/ml, respectively. The 95% confidence intervals for the repeated analysis of plasma containing 0.025 μg/ml BVDU and BVU showed that the sensitivity of the assay was < 0.025 μg/ml.

Analysis of quality control plasma samples showed an acceptable level of accuracy and precision of the assay for both compounds (Tables I and II). These results indicated that accurate quantitation was possible down to 0.05 μg/ml for both compounds in rat and dog plasma and 0.125 μg/ml in human plasma, based on achieving a between-assay coefficient of variation ⩽ 15%.

Plasma samples obtained from rat and man following the oral administration of BVDU were analysed by the assay procedure described. The results of these analyses are shown in Fig. 3. Orally administered BVDU appears to be rapidly absorbed and metabolised to BVU in both species, with plasma BVDU levels some three- to ten-fold lower than those of BVU.

In conclusion, the assay method was adequately selective, sensitive, accurate and precise to measure BVDU and BVU in the plasma of rat, dog and man

TABLE I

ACCURACY AND PRECISION FOR THE MEASUREMENT OF 5-(2-BROMO-*E*-ETHENYL)-2′-DEOXYURIDINE IN THE PLASMA OF RAT, DOG AND MAN BY HPLC

Nominal (μg/ml)	Recovery (%)	Coefficient of variation* (%)	
		Within-assay	Between-assay**
Rat plasma			
0.025	90.0	41.6 (5)	41.6 (4)
0.049	82.7	16.4 (5)	16.6 (4)
0.350	108.0	5.2 (5)	7.4 (4)
0.875	113.5	10.3 (5)	11.3 (4)
2.1	105.0	4.0 (5)	4.0 (4)
3.5	104.7	4.3 (5)	4.6 (4)
Dog plasma			
0.05	89.0	14.9 (7)	15.6 (2)
0.10	93.2	7.5 (6)	10.1 (1)
0.25	86.8	1.7 (2)	8.6 (1)
1.0	99.5	9.7 (3)	9.7 (2)
2.5	94.3	5.5 (4)	6.7 (2)
4.5	95.5	3.4 (4)	5.7 (2)
Human plasma			
0.049	107.6	30.8 (5)	50.0 (4)
0.125	102.5	3.5 (4)	4.7 (3)
0.350	103.3	1.6 (5)	7.5 (4)
0.875	95.9	9.8 (5)	12.1 (4)
2.1	98.7	8.3 (5)	8.9 (4)
3.5	98.1	3.1 (5)	4.1 (4)

*Figures in brackets are degrees of freedom.
**Expected precision for singleton analysis.

TABLE II

ACCURACY AND PRECISION FOR THE MEASUREMENT OF 5-(2-BROMO-*E*-ETHENYL)URACIL IN THE PLASMA OF RAT, DOG AND MAN BY HPLC

Nominal (μg/ml)	Recovery (%)	Coefficient of variation* (%)	
		Within-assay	Between-assay**
Rat plasma			
0.046	115.7	6.9 (5)	7.5 (4)
0.288	107.3	8.5 (5)	8.5 (4)
0.575	106.7	5.1 (5)	10.4 (4)
2.875	110.7	9.7 (5)	11.2 (4)
4.60	94.7	4.1 (5)	7.0 (4)
11.5	106.6	3.6 (5)	7.3 (4)
Dog plasma			
0.04	85.0	8.6 (2)	8.6 (1)
0.08	86.3	4.3 (2)	6.9 (1)
0.15	84.0	11.3 (3)	11.3 (2)
0.30	83.5	4.0 (2)	13.3 (1)
0.75	82.5	5.1 (2)	8.2 (1)
3.0	98.7	1.4 (3)	5.3 (2)
7.5	91.1	4.2 (4)	13.1 (2)
15.0	89.8	4.1 (4)	9.6 (2)
Human plasma			
0.046	130.9	37.1 (5)	39.5 (4)
0.125	120.9	1.7 (5)	12.9 (4)
0.288	107.2	8.7 (5)	11.2 (4)
0.575	112.6	4.1 (5)	7.5 (4)
2.875	102.2	8.5 (5)	9.5 (4)
4.6	95.9	8.4 (5)	9.1 (4)
11.5	106.3	4.1 (5)	4.7 (4)

*Figures in brackets are degrees of freedom.
**Expected precision for singleton analysis.

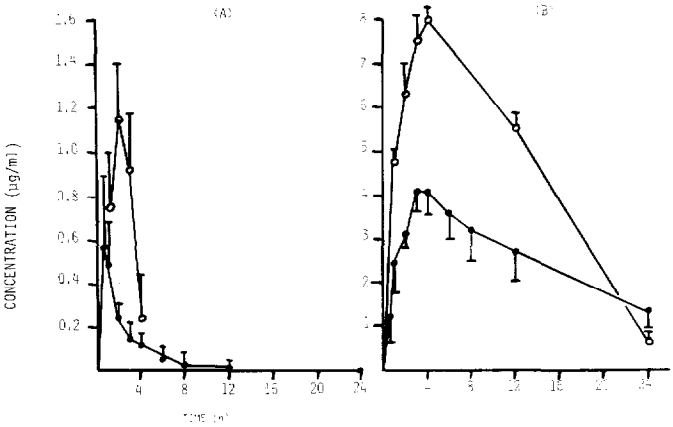

Fig. 3. Plasma levels of (A) BVDU and (B) BVU in the rat (○) after a 30 mg/kg oral dose and in man (●) after a 250-mg oral dose of BVDU.

following oral administration of BVDU. Recent work in these laboratories indicates that a similar assay procedure can be used for the analysis of these compounds in human urine and blood cells.

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