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Note

Determination of ryosidine in human plasma by gas—liquid chromatography

I.W. TAYLOR*, T. TAYLOR and L.F. CHASSEAUD

Department of Metabolism and Pharmacokinetics, Huntingdon Research Centre, Huntingdon, Cambridgeshire (U.K.)

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Ryosidine is a novel member of the 1,4-dihydropyridine-based class of calcium ion antagonists possessing potent vasodilation properties, which have recently been introduced for the treatment of ischaemic heart disease [1, 2] in particular, the variant form of angina pectoris or vasospastic angina [3, 4]. As part of the development and safety evaluation of this drug for eventual clinical use, initial pharmacokinetic and metabolism studies in rats and dogs [5] were extended to a study of the metabolic fate of ryosidine after oral administration of the ^{14}C-labelled drug to human subjects [5].

This paper describes a novel analytical method developed to measure concentrations of unchanged ryosidine in the plasma of these subjects.

EXPERIMENTAL

Materials

All reagents were of analytical grade (Fisons, Loughborough, U.K.), and all inorganic reagents were prepared in freshly glass-distilled water.

Toluene (Rathburn Chemicals, Walkerburn, U.K.) was re-distilled prior to use. *n*-Dodecane was puriss grade (Fluka, Derbyshire, U.K.). Standard solutions of ryosidine [dimethyl-4-(*o*-difluoromethoxyphenyl)-2,6,-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate] and internal standard [diethyl-4-(*o*-difluoromethoxyphenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate] were prepared in toluene at concentrations of 11.30 and 6.47 μg/ml, respectively, and stored at about 4°C. Samples of ryosidine and internal standard were supplied by Sanol Schwarz (Monheim, F.R.G.).

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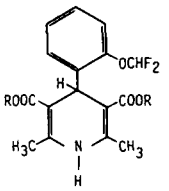

Fig. 1. Chemical structures of ryosidine (R = CH_3) and the internal standard (R = C_2H_5).

Extraction procedure

Plasma (1 ml) was transferred to a disposable screw-capped culture tube (Corning, through Fisons Scientific Apparatus, 10 ml) and internal standard (5 μl, containing 32.4 ng of internal standard) followed by saturated aqueous sodium chloride (2 ml) were added. The mixture was extracted with toluene (2 ml) by vortexing it for 30 sec on a whirlymixer (Fisons Scientific Apparatus). The extract was centrifuged at about 1000 *g* for 5 min (MSE Chilspin centrifuge; Fisons Scientific Apparatus) and the separated toluene phase carefully transferred to another culture tube and evaporated to dryness under nitrogen at 40°C. The residue was dissolved in *n*-dodecane (0.2 ml) and an aliquot (2 μl) injected into the chromatograph.

Calibration procedure

To samples of drug-free plasma (1 ml) were added ryosidine at concentrations of 2.3, 11.3, 22.6, 39.6 and 56.5 ng/ml and internal standard at a fixed concentration of 32.4 ng/ml. The samples were taken through the extraction procedure described above.

Instrumentation

The gas chromatograph was an HP 5880 chromatograph (Hewlett-Packard, Wokingham, U.K.), fitted with an automatic liquid injection system and an electron-capture detector (^{63}Ni).

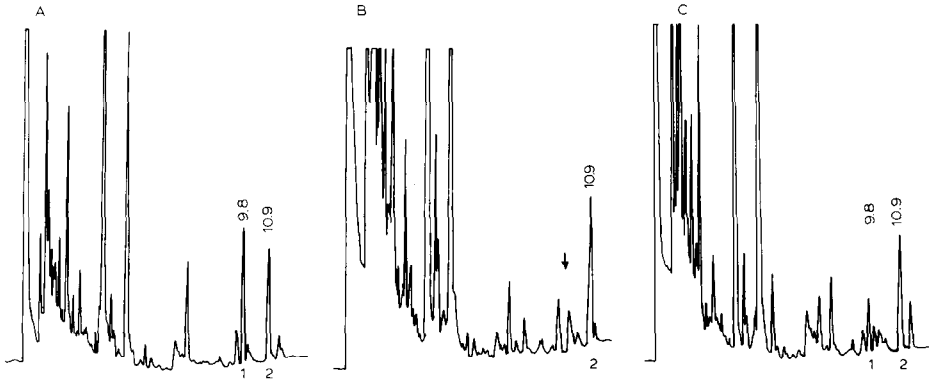

Fig. 2. Representative chromatograms of extracts of plasma: (A) containing 39.6 ng/ml ryosidine; (B) plasma blank; (C) containing 13.0 ng/ml ryosidine (samples of plasma from subject 2 at 3 h after dosing). Conditions: column, 25 m × 0.31 mm I.D. fused silica with 5% cross-linked phenylmethylsilicone gum (0.17 μm film thickness); carrier gas (nitrogen) flow-rate, 2 ml/min; temperature programme, 170°C for 3 min, raised at 7°C/min to 250°C for 10 min, raised at 30°C/min to 290°C for 3 min. Electron-capture detection at 300°C. Peaks: 1 = ryosidine; 2 = internal standard.

Chromatography

A 25 m × 0.31 mm I.D. fused-silica capillary column, coated with cross-linked 5% phenylmethylsilicone gum (0.17 μm film thickness) was used (Hewlett-Packard ultra performance column). The sample was introduced into a split—splitless injector maintained at 270°C and operated in the splitless mode for 40 sec. The initial column temperature of 170°C for 3 min was raised to 250°C for 10 min at a rate of 7°C/min, and thereafter to 290°C for 3 min at a rate of 30°C/min. The detector was operated at a temperature of 300°C. Column gas flow-rate (oxygen-free nitrogen) was 2 ml/min with a detector make-up gas flow-rate (oxygen-free nitrogen) of 20 ml/min. Under these conditions, ryosidine and internal standard eluted with retention times of 9.8 and 10.9 min, respectively (Fig. 2).

Plasma samples

The method of analysis was applied to plasma samples obtained from three male volunteer subjects after each had received an oral dose of 20 mg of ryosidine (^{14}C-labelled) contained in a gelatin capsule [5]. Blood samples, for measurement of unchanged ryosidine in plasma, were collected at pre-dose and at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10 and 12 h after dosing, and the separated plasma stored at about −20°C until analysis.

RESULTS AND DISCUSSION

A least-squares regression line of peak height ratio of drug to internal standard (y) against concentration of drug (x) was constructed over the concentration range 2.3—56.5 ng/ml, and was best described by the quadratic function $y = 0.064793 + 0.030923x - 0.000065x^2$. The coefficients of variation of the mean of five replicate analyses gave the precision of the method as 10% at 2.3 ng/ml, 9% at 22.6 ng/ml and 12% at 56.5 ng/ml (Table I). The accuracy of the method, defined by the standard error associated with fitting the least-squares regression line, i.e. taking the calibration line as an estimate of the concentration of ryosidine in plasma, was ± 4.0. The mean recovery of internal standard from plasma was 95 ± 7% S.D. (n = 5) at a concentration of 32.4 ng/ml. The mean recovery of ryosidine from plasma was 78 ± 7% S.D. (n = 5) determined at a concentration of 22.6 ng/ml, and was calculated by comparing peak height ratio measurements of non-extracted

TABLE I

PRECISION OF THE ANALYTICAL METHOD

Concentration of ryosidine added to plasma (ng/ml)	Mean peak height ratio	Coefficient of variation (%)
2.3	0.10	10
11.3	0.45	18
22.6	0.77	9
39.6	1.12	5
56.5	1.63	12

TABLE II

CONCENTRATIONS OF RYOSIDINE IN THE PLASMA OF HUMAN SUBJECTS AFTER SINGLE ORAL DOSES OF 20 mg OF RYOSIDINE

Time after administration (h)	Concentrations of ryosidine (ng/ml)			Mean ± S.D.
	Subject 1	Subject 2	Subject 3	
0.5	ND*	ND	ND	—
1	ND	ND	ND	—
1.5	ND	ND	3.8	—
2	6.2	3.1	4.8	4.7 ± 1.6
3	9.8	13.0	9.4	10.7 ± 2.0
4	10.5	11.5	8.9	10.3 ± 1.3
5	6.4	8.3	2.5	5.7 ± 3.0
6	2.5	3.7	ND	2.1 ± 1.9
8	ND	2.8	ND	—
10	ND	ND	ND	—
12	ND	ND	ND	—

*ND = Not detected (< 2.3 ng/ml). ND values entered as zero for calculation of the mean data.

standards to those of extracted standards corrected for the recovery of internal standard. The analytical procedure was selective for ryosidine, and the limit of detection was arbitrarily set at 2.3 ng/ml, being more than twice the background interference and the lowest datum point on the calibration line.

Concentrations of ryosidine in the plasma of three subjects after oral administration of 20 mg of ryosidine were measured using this procedure. Peak concentrations of ryosidine were 10.5, 13.0 and 9.4 ng/ml, occurring at 4, 3 and 3 h after dosing, respectively in subjects 1, 2 and 3, respectively (Table II). Concentrations of ryosidine in the plasma of these subjects declined from the peak concentrations in an apparent first-order manner with half-lives of about 1, 2 and 1 h, respectively. Concentrations of ryosidine had declined to levels below the limit of detection of the analytical procedure (2.3 ng/ml) at 8, 10 and 6 h after dosing in these subjects, respectively.

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