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Note

Determination of oxyphenbutazone in human plasma by high-performance liquid chromatography

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Oxyphenbutazone is not only a metabolite of phenylbutazone but it is also extensively used as a drug. Several methods have been described for its quantitative determination in biological fluids, including two laborious gas chromatographic methods [1, 2]. High-performance liquid chromatographic (HPLC) methods have been published recently. Most of them allow the simultaneous determination of phenylbutazone and its main metabolite oxyphenbutazone. Chromatography is performed either on an adsorption column [3, 4] with a sensitivity of 0.77—1.54 μmol/l or on a reversed-phase column [5—11], with the disadvantages of a poor limit of quantitation when the method is performed without extraction [5—7], a lack of accuracy when there is no internal standard [8—10], and a more laborious procedure when two extraction steps are required [11].

This paper describes a simple and rapid procedure which permits the determination of oxyphenbutazone down to 0.154 μmol/l in human plasma by HPLC with a one-step extraction and with phenylbutazone as internal standard.

EXPERIMENTAL

Chemicals and reagents

Oxyphenbutazone and phenylbutazone were supplied by Ciba-Geigy (Basle, Switzerland). Potassium dihydrogen phosphate, phosphoric acid 85% and pH 5 Titrisol buffer were purchased from Merck (Darmstadt, G.F.R.). Toluene (Pestipur, SDS, Peypin, France) and methanol were of analytical grade.

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Chromatography equipment and conditions

Chromatography is performed on a Hewlett-Packard 1081 B high-performance liquid chromatograph equipped with a fixed-wavelength (254 nm) UV detector. The peak areas are obtained with a Perkin-Elmer data system (Sigma 10) connected to the chromatograph. The chromatographic column (stainless-steel tube, 25 cm × 4.7 mm I.D.) is filled with LiChrosorb RP-18, 10 μm particle size (Merck 9334), using the slurry packing technique. Chromatography is performed at room temperature using the degassed mobile phase methanol—phosphate buffer pH 4 (63:37, v/v) at a constant flow-rate of 1.5 ml/min. The top pressure is about 90 bars.

Extraction

Five-hundred microliters (3.2 nmol) of a phenylbutazone methanolic solution are introduced into a glass tube and evaporated under nitrogen at 37°C. Then 1 ml of plasma, 1 ml of buffer pH 5 and 5 ml of toluene are added. The tube is stoppered and shaken mechanically for 15 min at 300 rpm (Infors shaker), then centrifuged for 10 min at 2500 *g*. A maximum volume of organic phase is transferred into another tube and taken to dryness under a nitrogen stream at 45°C. Two hundred and fifty microliters of the mobile phase are added to the dry residue, the tube is shaken on a Vortex mixer and 40 μl are injected onto the chromatographic column.

Calibration

The calibration samples are prepared by introducing 500 μl of the internal standard solution and 50 μl of suitable methanolic solutions containing oxyphenbutazone into glass tubes. Methanol is evaporated under nitrogen, the compounds are redissolved in 1 ml of plasma. The added amounts of oxyphenbutazone correspond to plasma concentrations ranging from 0.154 to 154 μmol/l. A calibration curve is generated on every analysis day.

RESULTS AND DISCUSSION

Recovery and precision

Various spiked plasma samples were prepared and analysed several times. Table I shows the within-day reproducibility over the concentration range 0.154—154 μmol/l. The six replicate samples of each concentration were analysed on the same day.

Table II shows the day-to-day reproducibility of standard curves. A least-squares ln—ln regression line was generated from the 29 data points of six standard curves and each concentration added to plasma was calculated from this regression line.

Reproducibility of calibration curves

Day-to-day reproducibility was tested by expressing each data point as a percentage of the value read off the ln—ln line for the corresponding concentration (Table II). The distribution of these normalized (concentration independent) data had an overall average (± C.V.) of 101.0 ± 12.9%. This result shows a variability especially for the lower concentrations, and it is necessary to establish a calibration curve daily.

TABLE I

PRECISION AND RECOVERY IN THE WITHIN-DAY DETERMINATION OF OXYPHENBUTAZONE APPLIED TO SPIKED HUMAN PLASMA SAMPLES

Concentration added (μmol/l)	Mean concentration found (n = 6) (μmol/l)	Coefficient of variation (%)	Mean recovery (%)
0.154	0.142	6.7	92
0.617	0.601	6.9	97
1.54	1.50	3.6	97
3.09	3.18	2.6	103
15.4	16.70	4.5	108.5
30.9	30.15	5.5	98
154	144	6.2	93

Mean recovery ± C.V. (%) 98.4 ± 7.1

TABLE II

DAY-TO-DAY REPRODUCIBILITY OF CALIBRATION CURVES USED TO DETERMINE OXYPHENBUTAZONE IN PLASMA

Concentration added to plasma (μmol/l)	Peak area ratios: Experimental (E) Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Calculated from ln—ln regression line (C)	$100 \times \frac{E}{C}$
0.154	0.017						0.0273	64
		0.033						120
			0.029					106
				0.031				113
					0.019			70
						0.032		117
0.617	0.111						0.114	97
		0.140						123
			0.115					101
				0.108				95
					0.105			92
						0.110		96
3.09	0.625						0.603	104
		0.651						108
			0.651					108
				0.693				115
					0.631			105
						0.701		116
30.9	—						6.49	—
		6.54						101
			6.24					96
				5.93				91
					7.13			110
						7.08		109
154	32.83						34.21	96
		33.19						97
			32.56					95
				31.22				91
					32.63			95
						33.85		99

Average ± C.V. (%): 101.0 ± 12.9

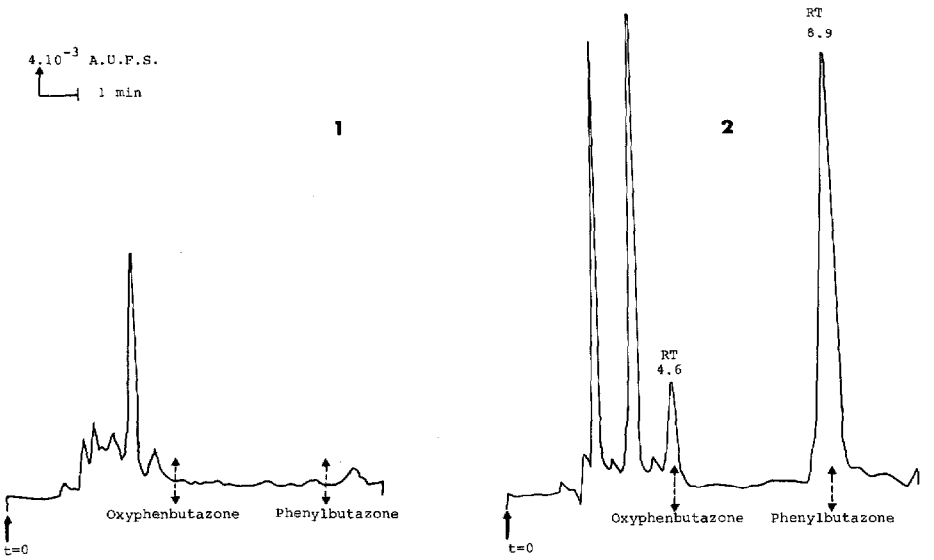

Fig. 1. Examples of chromatograms: (1) human plasma blank (1 ml of plasma); (2) human plasma containing 0.617 μmol/l oxyphenbutazone and 3.2 nmol of phenylbutazone (internal standard). RT = retention time (min).

Plasma interference and selectivity

Fig. 1 shows the chromatograms corresponding to extracts of 1 ml of human plasma and of the same plasma spiked with 617 nmol/l oxyphenbutazone and 3.2 nmol of internal standard. These chromatograms demonstrate that there is no interference from the normal plasma components.

Oxyphenbutazone in urine

The HPLC method described above was not applied to determine oxyphenbutazone in human urine. A very low percentage of the dose (2.3%) has been found as the unchanged drug in human urine [12]. Moreover, the metabolism of oxyphenbutazone is not well known: it was only reported that about 25% of the dose is found in urine as the oxyphenbutazone O-glucuronide [13, 14].

Stability

Oxyphenbutazone was found to be stable in plasma for at least three months when kept at −20°C.

Application

This method was applied to study the oxyphenbutazone plasma concentrations in six subjects after a single administration of one 250-mg oxyphenbutazone suppository (Fig. 2).

CONCLUSIONS

With the described method, plasma concentrations down to 154 nmol/l (50 ng/ml) of oxyphenbutazone can be determined using a 1-ml sample. This limit of quantitation is convenient for pharmacokinetic and bioavailability

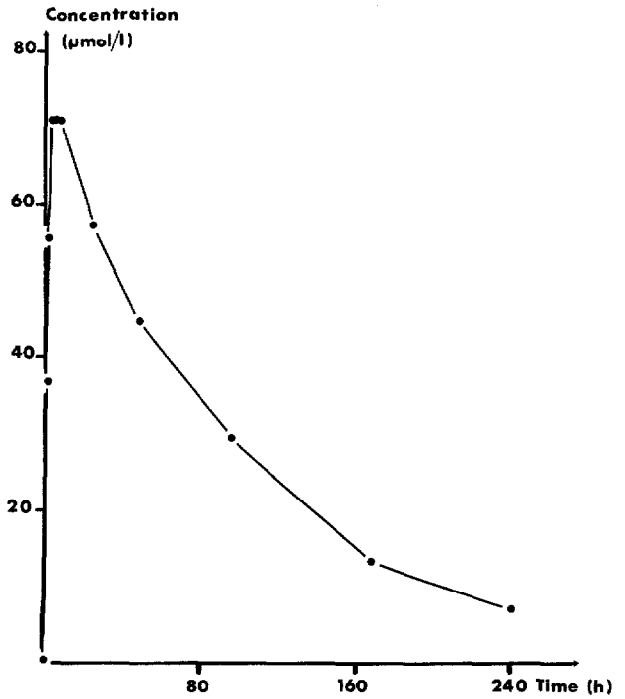

Fig. 2. Oxyphenbutazone mean concentrations in plasma after administration of one 250-mg oxyphenbutazone suppository to six subjects.

studies. It also permits oxyphenbutazone determinations in small volumes of plasma (100 μl), which could be useful when the drug is administered to children.

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