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Short Communication

Rapid determination of propyphenazone in plasma by high-performance liquid chromatography

M. C. Rouan*, J. Campestrini, J. B. Lecaillon and J. Godbillon

Laboratoires CIBA-GEIGY, Biopharmaceutical Research Center, B.P. 308, 92506 Rueil-Malmaison Cedex (France)

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ABSTRACT

A rapid and simple high-performance liquid chromatographic assay for the determination of propyphenazone in plasma is described. Phenylbutazone was used as the internal standard. Plasma proteins were precipitated with acetonitrile before injection onto a 3-μm Supelcosil LC-18 column. The mobile phase, ethanol containing 0.2% (v/v) heptylamine–0.005 *M* potassium dihydrogenphosphate (30:70, v/v), was used at a flow-rate of 1.3 ml/min. The quantitation was performed by ultraviolet detection at a wavelength of 270 nm. The chromatographic time was 7 min. The within- and between-day coefficients of variation were less than 6% and the recoveries close to 100% for concentrations between 0.4 and 22 μmol/l. The limit of quantitation was 0.4 μmol/l (*ca.* 100 ng/ml).

INTRODUCTION

Propyphenazone is an antipyretic, analgesic and anti-inflammatory agent. A few methods have been reported for the determination of this compound in plasma. Sioufi and Marfil [1] determined propyphenazone by gas chromatography (GC) down to 125 ng/ml of plasma. Kosmeas and Clerc [2] reported a high-performance thin-layer chromatographic (HPTLC) procedure for the simultaneous determination of paracetamol, phenobarbital, caffeine and propyphenazone in plasma down to 250–500 ng/ml. Both methods involved liquid–liquid extraction prior to chromatography.

This paper describes an assay for the determination of propyphenazone in plasma by high-performance liquid chromatography (HPLC). The sample preparation is more simple and rapid than in previously published methods. It involves only protein precipitation.

EXPERIMENTAL

Materials

Propyphenazone and the internal standard, phenylbutazone, were provided by Ciba-Geigy (Basle, Switzerland). Their molecular structures are shown in Fig. 1.

Reagents

Potassium dihydrogenphosphate was purchased from E. Merck (Darmstadt, Germany) and heptylamine from Aldrich (Strasbourg, France). Acetonitrile (Carlo Erba France, Puteaux, France) and ethanol (Prolabo, Paris, France) were of HPLC grade. Water was deionized and purified using a Milli-Q reagent-grade water system (Millipore, Bedford, MA, USA).

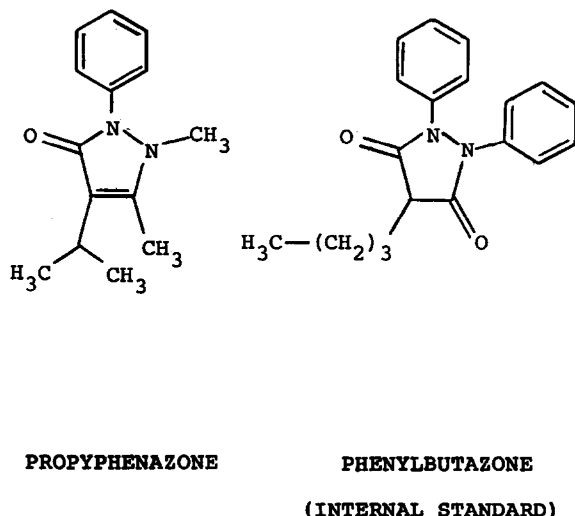

Fig. 1. Structures of propyphenazone and the internal standard, phenylbutazone.

Apparatus

The chromatographic system consisted of a Model 410-LC pump (Perkin-Elmer, Norwalk, CT, USA), a Model 231 automatic injector (Gilson, Villiers-le-Bel, France) and a Model SM 4000 variable-wavelength UV detector (LDC, Riviera Beach, FL, USA) set at 270 nm with a response time of 1 s. A Model C-R3A integrator recorder (Shimadzu, Kyoto, Japan) was used for data capture.

Columns and filter

The stainless-steel analytical column (3.3 cm × 4.6 mm I.D.) was prepacked with Supelcosil LC-18, 3 μm particle size (Supelco France, St.-Germain-en-Laye, France). A filter (No. FL 01, Société Française Chromato Colonne, Neuilly-Plaisance, France) with a replaceable 2-μm-pore frit was inserted between the injector and the analytical column.

A small stainless-steel column (3.3 cm × 4.7 mm I.D.) was placed between the pump and the injector to protect the analytical column from mobile phase particles. It was tap-filled in our laboratory with pellicular ODS material, 37–53 μm particle size (Whatman, Clifton, NJ, USA).

Calibration solutions

An internal standard solution was prepared by dissolving 5 mg of phenylbutazone in 100 ml of ethanol. The master reference solution was then prepared by dissolving 1 mg of propyphenazone in 10 ml of the internal standard solution. The reference solutions for calibration were obtained by dilution with the internal standard solution. They were stable for at least one month at 4°C.

Sample preparation

A 200-μl volume of plasma, 20 μl of internal standard or reference solution and 200 μl of acetonitrile were introduced in a polypropylene tube and shaken on a vortex mixer for a few seconds. The tube was then centrifuged for 5 min at 2500 *g*, 100 μl of the supernatant were diluted with 300 μl of water and 50 μl were injected onto the analytical column.

Chromatography

The chromatography was carried out at room temperature. The mobile phase was ethanol containing 0.2% (v/v) heptylamine–0.005 *M* potassium dihydrogenphosphate (30:70, v/v). The flow-rate of the mobile phase was 1.3 ml/min, and the total pressure drop across the guard column and the analytical column was *ca.* 90 bar.

RESULTS AND DISCUSSION

Propyphenazone and the internal standard, phenylbutazone, were separated from plasma components within 7 min (Fig. 2). The addition of heptylamine to the eluent improved the peak shape of propyphenazone. A simple protein precipitation was applied to plasma samples. Acetonitrile was chosen as the deproteinization agent rather than methanol, because more interfering substances were removed when using acetonitrile. The supernatant was diluted with water before injection to prevent band-broadening. When a guard column (Supelguard LC-18-DB, 2 cm × 4.6 mm I.D., 5 μm particle size) was placed between the injector and the analytical column to protect the column, a loss in peak height of *ca.* 30% due to band-broadening occurred. There-

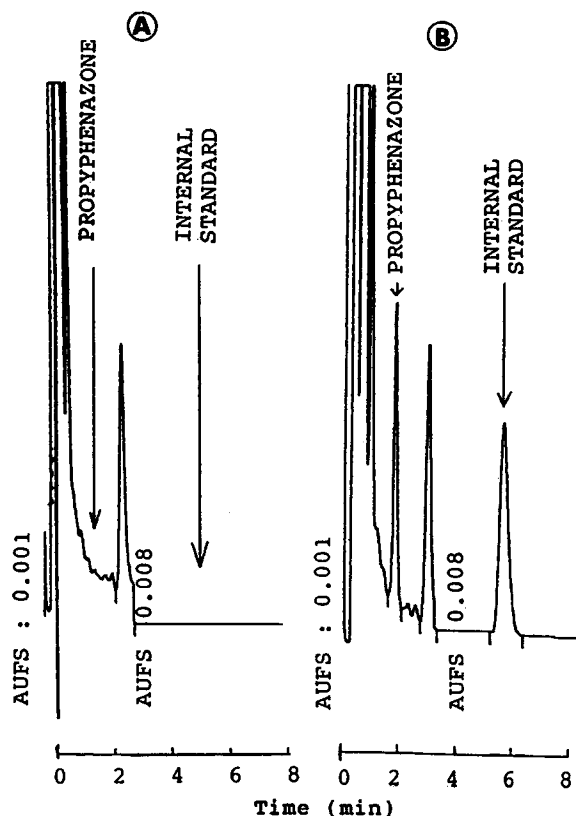

Fig. 2. Chromatograms of (A) drug-free human plasma and (B) human plasma containing 2.2 μmol/l propyphenazone and 16 μmol/l internal standard.

fore, deproteinized plasma samples were injected directly via only a filter to protect the analytical column. The filter frit was changed once a week. An average of 250 plasma samples have been injected per column without degradation of the separation.

Deproteinized plasma samples spiked with propyphenazone and the internal standard were stable for at least 8 h at room temperature on the rack of the injector.

Linearity, accuracy and precision

Calibration graphs were obtained by plotting the peak-area ratio (propyphenazone/internal standard) *versus* the concentration of propyphenazone in the sample. The equation was calculated by the least-squares method using weighted linear regression with a weighting factor of 1/(concentration)^2 [3]. The coefficient of correlation was higher than 0.9990. The within-day accuracy and precision of the method were assessed by using series of six plasma samples spiked with different concentrations of propyphenazone. These samples were prepared and analysed on the same day. The between-day accuracy and precision were assessed by analysing on different days plasma samples spiked with different concentrations. The coefficients of variation (C.V.) were less than 6%, and the recoveries (amount found × 100)/(amount introduced) were close to 100% (Table I). The limit of quantitation was 0.4 μmol/l (*ca.* 100 ng/ml).

TABLE I

WITHIN- AND BETWEEN-DAY PRECISION AND ACCURACY OF THE ASSAY

Nominal concentration (μmol/l)[a]	Within-day (n = 6)		Between-day (n = 8)	
	Mean recovery (%)	C.V. (%)	Mean recovery (%)	C.V. (%)
0.4	97	3	101	6[b]
1	99	2	101	6
2	102	3	103	4
4	101	0.5	102	1
11	101	1	102	3
22	102	0.5	100	2

[a] To convert into μg/ml, multiply the values by 0.2303.
[b] n = 7.

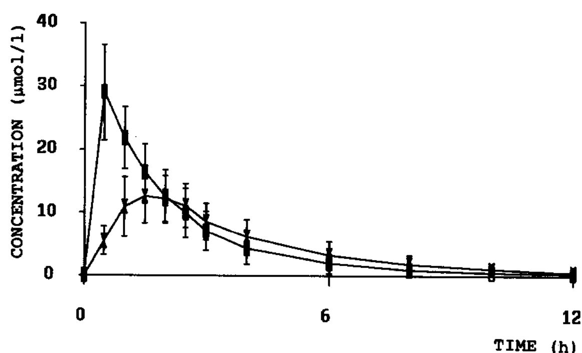

Fig. 3. Mean (± S.D.) plasma profiles of propyphenazone obtained for twelve healthy volunteers given (×) a Spasmo-Cibalgin compositum suppository (500 mg of propyphenazone, 40 mg of codeine phosphate and 50 mg of drofenine hydrochloride) and (□) an oral suspension of the active ingredients.

Application

The method was used in a bioavailability study to determine the plasma concentrations of propyphenazone in twelve healthy volunteers given a Spasmo-Cibalgin compositum suppository (500 mg of propyphenazone, 40 mg of codeine phosphate and 50 mg of drofenine hydrochloride) and an oral suspension of the active ingredients. Codeine, drofenine and their metabolites did not interfere with the determination of propyphenazone. The mean plasma profiles obtained for propyphenazone are shown in Fig. 3.

CONCLUSION

The described procedure is simple and rapid. It permits the determination of propyphenazone with a sensitivity suitable for pharmacokinetic investigations.

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