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Note

Reversed-phase high-performance liquid chromatography of phenylbutazone in body fluids

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Phenylbutazone (PBZ) is an effective anti-inflammatory agent used widely in horses to improve their racing performance. PBZ has been detected in body fluids by spectrophotometry [1—4], high-performance liquid chromatography (HPLC) [5] and gas—liquid chromatography [6]. However, difficulty has been encountered in measuring minute amounts of PBZ such as those remaining 24—48 h after drug administration. An HPLC method using a reversed-phase column, which allows the detection of 50—100 ng of PBZ per ml in 0.5—1 ml of plasma, urine, saliva and sweat of horses is described.

EXPERIMENTAL

Apparatus

The HPLC system consisted of a continuous-flow constant-volume delivery system (Model 6000A, Waters Assoc., Milford, Mass., U.S.A.), a U6K universal injector (Waters Assoc.) and a variable wavelength UV detector (GM 770, Schoeffel Instruments). A column (250 mm × 4.6 mm I.D.) packed with μBondapak C_{18} (Waters Assoc.) was included with the apparatus.

Reagents

The reagents were of analytical grade (Prolabo, Paris, France). Phenylbutazone was purchased from Vetoquinol (Lure, France).

Standards

A phenylbutazone standard stock solution was prepared containing 1 mg of PBZ per ml of methanol. The solution was diluted with mobile phase to obtain

1, 0.1, and 0.01 μg, respectively, in a constant injection volume of 10 μl. Standard solutions were stored at +4°.

Operating conditions

Analyses were performed with a mobile phase consisting of 2% glacial acetic acid in water–methanol (35:65, v/v). The flow-rate was set at 2 ml/min (inlet pressure of 2800 p.s.i.).

Before use the phase was degassed by applying a vacuum to the solvent reservoir for approximately 5 min. Detection of PBZ was achieved at 240 nm. The retention time of PBZ was 6 min and the system operated at an ambient temperature of 18–20°. A typical chromatogram for 10 ng of PBZ injected into the column with a detection sensitivity of 0.01 a.u.f.s. is shown in Fig. 1.

Extraction procedure

One millilitre of 1 *N* HCl and 10 ml of hexane were added to 0.5 ml of plasma, urine or sweat. The tubes were shaken for 30 sec and centrifuged for 10 min at 11,400 *g*. The organic phase was isolated by filtration on phase-separating paper (Whatman No. 1 Ps) and evaporated at 60° under a nitrogen gas stream to prevent oxidation. The residue was dissolved in 100 μl of elution solvent. Ten or 20 μl of this solution were injected into the column.

The recovery of the extraction procedure for both plasma and sweat was found to be 65 ± 2% with a concentration range of 100 ng to 10 μg.

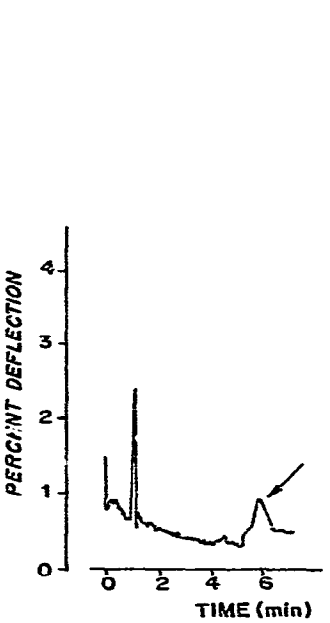

Fig. 1. Chromatogram corresponding to an injection of 10 ng of phenylbutazone.

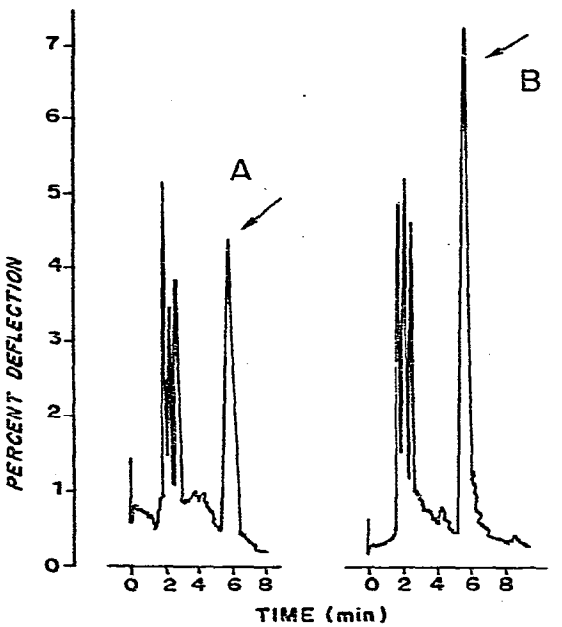

Fig. 2. Chromatogram of horse sweat containing phenylbutazone before (A) and after (B) addition of 50 ng of phenylbutazone.

RESULTS AND DISCUSSION

The method allows the determination of PBZ concentrations as low as 50 ng/ml in a variety of body fluids, especially sweat in racing horses. A typical chromatogram obtained by this method is shown in Fig. 2A from an extract of sweat collected immediately after a race from a horse of 500 kg body weight which, 18 h before, had received a tablet containing 2 g of PBZ.

Oxyphenylbutazone, the major metabolite of phenylbutazone, was also separated with a retention time of 3.2 min. However, the measured concentration in the sample remained low (probably due to its low solubility in hexane); polar organic solvents such as dichloromethane or diethyl ether could be used for a better extraction. Nevertheless, such a procedure would not be selective enough to detect low concentrations of PBZ without interference by other substances present in body fluids.

In summary, a specific and precise HPLC assay has been developed to permit estimation of minute quantities of PBZ in horses.

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REFERENCES

1 J.J. Burns, B.K. Rose, T. Chenkin, A. Golman, A. Schulert and B.B. Brodie, J. Pharmacol. Exp. Ther., 109 (1953) 346.
2 B. Hermann, Med. Exp., 1 (1960) 170.
3 E. Janchen and G. Levy, Clin. Chem., 18 (1972) 984.
4 R. Pulver, Schweiz. Med. Wochenschr., 80 (1957) 308.
5 N.J. Pound, I.J. McGilveray and R.W. Sears, J. Chromatogr., 89 (1974) 23.
6 R. Perego, E. Martinelli and P.C. Vanoni, J. Chromatogr., 54 (1971) 280.