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Note

Determination of xylazine in plasma using high-performance liquid chromatography

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Xylazine hydrochloride (Rompun Bay, 1470) is a sedative analgesic drug which was first evaluated extensively in Germany. Xylazine [2-(2,6-dimethylphenylamino)-4H-5,6-dihydro-1,3-thiazine] was first synthesized in 1962 and since then has been used in many animal species including man. Its sedative properties have been used to good advantage in horses, cattle, sheep, goats, dogs, cats, and many different wild and zoo animals. In the United States, xylazine has been cleared by the FDA for use in horses, dogs and cats, but it has not been cleared for use in food-producing animals.

Assay methods reported for the determination of xylazine [1] in biological fluids include spectrophotometric studies and thin-layer chromatography. But these techniques are slow and not sufficiently sensitive. Moreover, the ionic character and low volatility of xylazine does not permit direct quantitation by gas chromatography.

In the present study, a rapid, sensitive and specific high-performance liquid chromatographic (HPLC) technique was developed for the quantitation of xylazine in plasma and for pharmacokinetic studies.

EXPERIMENTAL

Reagents

Xylazine hydrochloride was purchased from Bayer (Puteaux, France); the reagents were of analytical grade (Prolabo, Paris, France).

Apparatus

The HPLC system consisted of a continuous-flow constant-volume delivery system (Model 6000A; Waters Assoc., Milford, MA, U.S.A.), a U6K universal

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injector (Waters) and a variable-wavelength UV detector (GM 770; Schoeffel Instruments). A stainless-steel column (30 cm × 4 mm I.D.) packed with a stable reversed-phase stationary phase consisting of porous silica beads (mean diameter 10 μm) coated with a chemically bonded monolayer of octadecylsilane (μBondapak C_{18}; Waters) was included with the apparatus.

Standards

A xylazine standard stock solution was prepared containing 1 mg/ml in methanol. The solution was diluted with mobile phase in order to obtain 1, 0.1 and 0.01 μg in a constant injection volume (10 μl). Standard solutions were stored at 4°C.

Drug extraction from plasma samples

Aliquots of plasma (0.5 ml) were pipetted into 21 × 150 mm tubes (Corex; Corning, Corning, NY, U.S.A.). After the addition of 1 μg of internal standard (10 μl of a solution containing 10 mg of doxapram in 100 ml of methanol), 1 ml of buffer solution (0.05 *M* borax, pH 11) was added. The sample was vortexed for 5 sec and then shaken with 10 ml of chloroform for 10 min. After centrifugation at 11,400 *g* for 10 min, the organic phase was isolated by filtration on phase-separating paper (No. 1 PS; Whatman,Clifton, NJ, U.S.A.) and evaporated at 70°C under a nitrogen gas stream. The evaporated extract was redissolved in 100 μl of mobile phase and the entire sample injected into the liquid chromatograph.

Operating conditions

Analysis was performed with a mobile phase permitting ion-pair chromatography by adjusting the pH so that the sample was present in its ionic form (pH 3.5). A strongly ionic counter-ion was chosen with a very lipophilic group attached (heptanesulfonic acid, Pic B7, Waters Assoc.).

The composition of the mobile phase was 2% glacial acetic acid in water—methanol—heptanesulfonic acid (55:45:0.2, v/v). Before use, the mobile phase was degassed by applying vacuum to the solvent reservoir for approximately 5 min. The system was operated at ambient temperature at a flow-rate of 2 ml/min. The UV detector was operated at a wavelength of 225 nm.

The retention times were xylazine 4 min, and doxapram 5.5 min.

Calibration and reproducibility

Known quantities of xylazine chloride in the concentration range 0.1—1 μg were added to blank plasma samples. Calibration curves were constructed by plotting the peak height ratios between xylazine and the internal standard doxapram, versus the amount of xylazine added. The reproducibility of the analytical procedure was checked by determining the calibration curve on four different days. The data were analyzed by analysis of variance and were not found to be significantly different.

RESULTS AND DISCUSSION

The system reported here provides a reliable method for the extraction and quantification of xylazine in plasma. The use of doxapram as internal standard was supported by the fact that its dosage required similar conditions (unpublished observation). Fig. 1 shows chromatograms typical of those obtained for the separation of xylazine and the internal standard, doxapram, following injection of plasma extracts. The linear relationship calculated between the peak height ratio (R) and the concentration (x) of xylazine in plasma up to 1 μg/ml was $R = 4.79x + 0.23$ ($r = 0.998$).

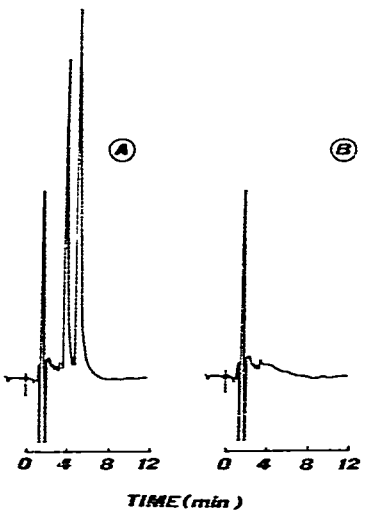

Fig. 1. Chromatograms of sheep plasma extracts (A) containing xylazine (0.4 μg) and doxapram (1 μg) and of blank plasma (B) (0.1 absorbance units full scale).

When standard curves were constructed on four different days over a xylazine concentration range of 0.1—1 μg/ml, an excellent linear relationship was obtained each time. The slopes of the calibration curves were quite reproducible with a coefficient of variation of 4.5%; the coefficients of variation of the different experimental data are between 2.08 and 8.1%.

Recovery of xylazine from plasma was found to be 76.4 ± 3.4% within the concentration range 0.1—2 μg. The proposed assay procedure can be used to estimate levels below 20 ng/ml by using a plasma sample larger than 0.5 ml. The method is sufficiently sensitive to measure pharmacokinetic parameters of xylazine in domestic animals (unpublished data). Moreover, it can be used for the determination of doxapram, for which an HPLC method has not previously been described.

REFERENCE

1 J. Putter and G. Sagner, Vet. Med. Rev., 73 (1973) 145.