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## Note

# Determination of practolol in plasma by high-performance liquid chromatography

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Practolol, 4-(2-hydroxy-3-isopropylaminopropoxy)acetanilide, is a selective antagonist of cardiac beta-receptors ($\beta_1$), and was the prototypic drug in this category. It has been used for the treatment of cardiac arrhythmias; the oral dosage ranges from 1.5 to 12 mg/kg. Few methods have been reported for the determination of practolol in biological specimens. Bodem and Chidsey [1] described a fluorometric method. Gas chromatographic methods have also been reported [2—4] in which the drug is derivatized before analysis. The present report describes a simple and rapid method for the determination of practolol in plasma, utilizing high-performance liquid chromatography (HPLC) with ultraviolet detection.

## EXPERIMENTAL

Glassware was washed with concentrated nitric acid and silanized with 5% v/v dichlorodimethylsilane in pyridine.

### *Apparatus*

The liquid chromatograph consisted of a Waters Assoc. Model 6000 pump and U6K universal injector coupled to a Schoeffel Instrument Spectroflow SF 770 multiwavelength monitor. The chromatographic conditions were as follows: column, $\mu$Bondapak $C_{18}$ (30 cm × 4 mm I.D.), particle size 10 $\mu$m (Waters Assoc., Milford, Mass., U.S.A.); mobile phase, 0.1% phosphoric acid in ethanol—water (1:9), flow-rate, 1.0 ml/min; column temperature, ambient; detection at 254 nm.

### *Chemicals and reagents*

All solvents and reagents were analytical grade and were used without further purification.

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*Extraction procedure*

Blood samples obtained from pregnant sheep receiving 10 mg/kg of practolol were centrifuged at 4° for 10 min and the plasma was removed. Sodium hydroxide (5 *N*, 0.1 ml) and ethyl acetate (10 ml) were added to 1 ml plasma. This was agitated on a Vortex mixer for 1 min and centrifuged for 10 min. An 8-ml aliquot of the organic layer was evaporated to dryness in vacuo at 50°, and redissolved in 0.1 ml of the chromatographic mobile phase.

*High-performance liquid chromatography*

For samples containing high drug levels, 5-μl injection volumes were used at an attenuation of 0.1 a.u.f.s. Plasma specimens containing low concentrations of practolol were injected in volumes up to 60 μl at an attenuation of 0.02 a.u.f.s. Peak heights were used for quantitation.

## RESULTS AND DISCUSSION

The chromatograms of representative sheep plasma samples are illustrated in Fig. 1. As can be seen, the control sample was free from any interfering peaks. The lower limit of detection was 50 ng/ml plasma, using 1-ml volumes and the recovery of practolol added to serum over the range of 0.05—8 μg/ml was 96.9 ± 12.7% (mean ± S.D., $n$ = 16). The standard curves were linear in this concentration range and passed through the origin. The same procedure can also be used for human plasma and blank samples showed no interfering peaks.

This procedure has several advantages over previously reported techniques. The simple extraction and rapid elution (retention time of practolol is 6 min) enable the analysis of numerous samples a day. Moreover, the method is sensitive enough to study drug disposition in clinical situations where only small sample volumes are required. Though the previously reported gas chromatographic procedures [3, 4] can detect lower drug levels, the sample workup time is longer.

The method reported here is currently being used to study the disposition of practolol in the ovine maternal-placental-fetal model and the results will be published elsewhere.

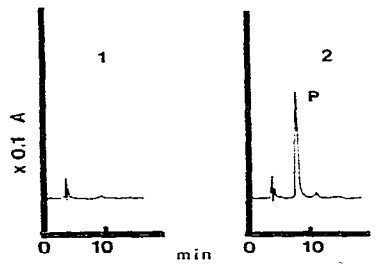


Fig. 1. HPLC of sheep plasma samples. 1, Control plasma; 2, plasma containing 7.2 μg/ml practolol (P). Conditions: column, 30 cm × 4 mm I.D. μBondapak $C_{18}$; eluent, 0.1% phosphoric acid in ethanol—water (1:9), flow-rate, 1.0 ml/min; column temperature, ambient; detection, 254 nm.

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