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Note

# Reversed-phase high-performance liquid chromatographic determination of salbutamol in rabbit plasma

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Although salbutamol, $\alpha^1$-[(*tert.*-butylamino)methyl]-4-hydroxy-*m*-xylene-$\alpha,\alpha'$-diol, is widely used as a bronchodilator drug, the pharmacokinetic studies of this drug are limited because of the difficulty of determining the blood levels. Evans et al. [1] reported the disposition of salbutamol in various animals using radioactive salbutamol. Martin *et al.* [2] and Leferink *et al.* [3] developed a gas chromatographic—mass spectrometric method to determine salbutamol concentrations in human plasma. However, these methods are very complicated and technically limited. Recently, a high-performance liquid chromatographic (HPLC) method using two columns (ion-exchange column and reversed-phase column) connected to an electrochemical detector was reported by Oosterhuis and Van Boxtel [4]. In spite of the high sensitivity and selectivity, the method seems to have some drawbacks of short lives of columns and detector.

In this report we describe a new HPLC method to study the relations between administration routes and bioavailability in rabbits. This method includes the clean-up procedure by Sep-Pak cartridge and fluorometric determination after separation on a reversed-phase column. Salbutamol concentrations in rabbit plasma could be determined without any interference after oral, rectal and intravenous administration.

## EXPERIMENTAL

### *Materials and reagents*

Salbutamol sulphate was obtained from Chemische Fabrik Schweizerhall (Basel, Switzerland) and its free base, which was used as a standard substance,

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was prepared from salbutamol sulphate by the method of Plavšić [5]. Ethenzamide as an internal standard was purchased from Shizuoka Caffeine (Shizuoka, Japan). Sodium-1-heptane sulphonate (specially prepared for ion-pair reagent) was purchased from Nakarai Chemical (Kyoto, Japan). Methanol (HPLC grade, Cica-Merck) was purchased from Kanto Chemical (Tokyo, Japan). Diethyl ether (extra pure reagent, Kanto Chemical) was used after further purification by distillation. The water used was double-distilled in an all-glass still after passage through an ion-exchange column. The other reagents employed were of analytical grade. The Sep-Pak $C_{18}$ cartridge was purchased from Water Assoc. (Milford, MA, U.S.A.).

*Apparatus and conditions*

A Shimadzu (Kyoto, Japan) Model LC-3A high-performance liquid chromatograph equipped with a fluorescence spectromonitor (Shimadzu Model RF-530) was employed. The analytical column and the guard column were Zorbax $C_8$ (25 cm × 4.6 mm, DuPont Instruments) and LiChrosorb RP-8 (particle size 10 μm, RP-GU, Brown Labs.), respectively, and were warmed at 60°C. The mobile phase was a mixture methanol—5.8 m*M* phosphate buffer (pH 6.0) (31:69), containing 5 m*M* sodium heptane sulphonate as an ion-pair reagent. The flow-rate was 1.0 ml/min, and detection wavelengths were 273 nm for excitation and 310 nm for emission.

*Experiment in rabbits*

White rabbits (3.5—4.0 kg) with stomach-emptying rate controlled by the method of Maeda et al. [6] received oral, rectal or intravenous administration of salbutamol (as salbutamol sulphate) in the form of an aqueous solution (4 mg per 0.4 ml per body volume). Heparinized blood samples were taken from the marginal vein over a period of 9 h, then the separated plasma samples were stored at −20°C until analysis.

*Sample preparation*

The Sep-Pak cartridge, connected to a 5-ml syringe as eluent reservoir, was previously washed with 20 ml of methanol—diethyl ether (1:1) and 20 ml of water. Two millilitres of 2.5 m*M* sodium heptanesulphonate, 0.5 ml of plasma sample and 200 μl of ethenzamide aqueous solution (2 μg/ml) were poured into the cartridge, and were passed through with 2 ml of water twice. After centrifuging at 2300 *g* for 10 min to exclude the aqueous solution, the cartridge was twice eluted with 7 ml of methanol—diethyl ether (1:1) and the eluate was evaporated under vacuum at 20°C. The residue was redissolved in 200 μl of the mobile phase, then a 60-μl aliquot of the solution was injected into the chromatograph.

## RESULTS AND DISCUSSION

The separation of salbutamol from biological components using solvent extraction is relatively difficult because of its hydrophilic character. In our preliminary experiments, salbutamol was poorly extracted with diethyl ether and some other organic solvents. Salbutamol extraction using the Sep-Pak cartridge [4] was more effective; however, the extraction ratio from the aque-

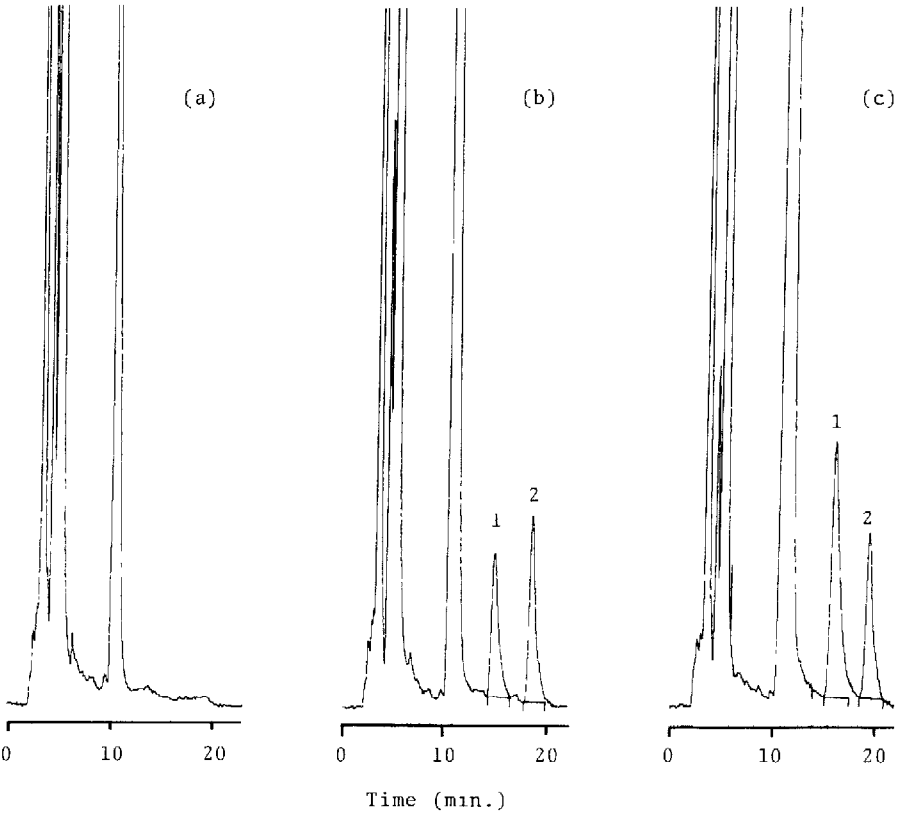


**Fig. 1. Chromatograms of (a) rabbit plasma blank, (b) plasma spiked with salbutamol (50 ng) and internal standard, and (c) plasma obtained 1 h after intravenous administration of salbutamol. 1 = salbutamol, 2 = ethenzamide (internal standard).**

ous solution was not sufficient: 50.5% ± 16.23 [mean ± C.V. (%), $n$ = 3]. To modify the extraction ratio, we used an ion-pair reagent for the Sep-Pak extraction. In this method, the average extraction ratio was sufficiently high and had less variation: 89.9% ± 2.79 [mean ± C.V. (%), $n$ = 3].

Typical chromatograms of rabbit plasma samples are shown in Fig. 1. The retention times of salbutamol and ethenzamide were approximately 15 and 19 min, respectively. There was no chromatographic interference by plasma constituents.

The calibration curve obtained from the peak height ratios and the amounts of salbutamol extracted from the aqueous standard solution was linear over the range 10—300 ng/ml and almost passed through the origin ($Y$ = 0.0083$X$ + 0.0174; $r$ = 0.999).

The recovery of the drug from the rabbit plasma was satisfactory as shown in Table I. The minimum detection limit estimated from the signal-to-noise ratio was about 4 ng/ml plasma.

Fig. 2 shows the plasma concentration curves of salbutamol after oral, rectal and intravenous administration of the drug to rabbits. The maximum plasma concentrations after oral and rectal administration were about 30 ng/ml. As these levels were very low compared with that after intravenous administration,

TABLE I

RECOVERY OF SALBUTAMOL FROM RABBIT PLASMA

| Amount added (ng/ml) | Recovery (%) | |
|---|---|---|
| | Mean ± S.D.* | C.V. (%) |
| 10 | 107.6 ± 8.60 | 7.99 |
| 100 | 100.1 ± 2.76 | 2.77 |
| 300 | 104.9 ± 2.75 | 2.62 |

*$n = 5$.

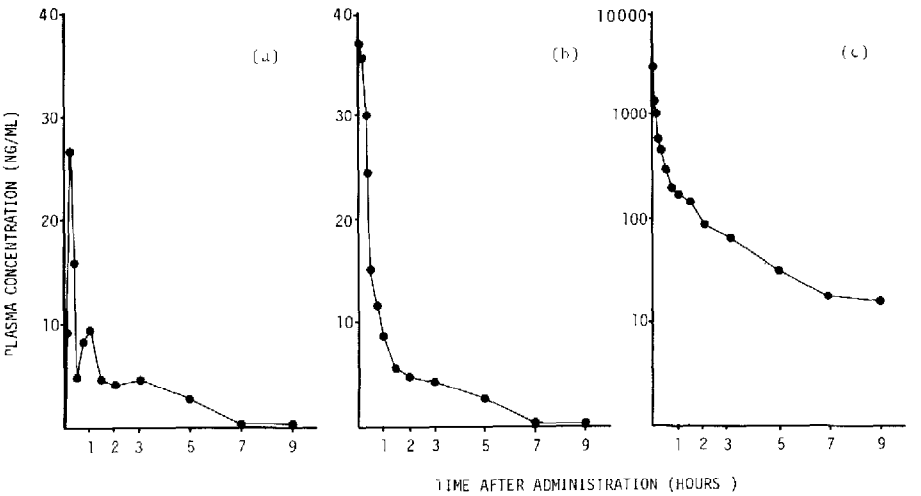


Fig. 2. Plasma concentrations of salbutamol after (a) oral, (b) rectal and (c) intravenous administration of 4 mg of salbutamol to rabbit.

it was suggested that the high metabolic first-pass effect might be in the oral and rectal routes.

In conclusion, the relatively simple method for plasma salbutamol determination described here has enough sensitivity and reproducibility for a salbutamol bioavailability study in rabbits, and could be extended to the determination of the salbutamol conjugates [7] in plasma.

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