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Determination of formoterol in rat plasma by liquid chromatography-electrospray ionisation mass spectrometry

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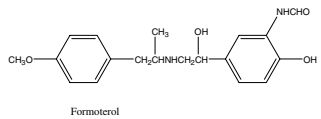
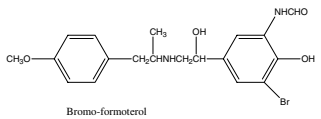
A sensitive method for the determination of formoterol in rat plasma is described, using high performance liquid chromatographic separation with tandem mass spectrometry. Samples were purified using liquid-liquid extraction and separated on CAPCELL PAK C18 UG120 (2.0 × 150 mm) with a mobile phase consisting of a mixture of methanol- 50 mM ammonium hydrogen carbonate (1 : 1 v/v). Detection was performed with a TSQ 7000 mass spectrometer using positive ion electrospray ionisation, monitoring the shift from precursor ions for formoterol at m/z 344.9 to product ions of m/z 121.0. The limit of quantitation of the method was found to be 0.1 ng/ml, when using 0.1 ml plasma. Plasma concentrations of formoterol could be quantified from 0.15 to 7.01 ng/ml, allowing the analysis of samples up to 32 h after a single oral dose of formoterol fumarate (0.25 mg) to rats.

1. Introduction

Formoterol is a long-acting selective β_2-adrenocepteor agonist therapeutically used as a bronchodilator in patients with obstructive airway disease, mainly by the inhalation route, but also orally. A significant response occurs within a few minutes after inhalation of a therapeutic dose of formoterol and maximum bronchodilation is achieved within 2 h, the effect then persisting for 12 h (Bartow and Brogden 1998; Faulds et al. 1991).



Formoterol



Bromo-formoterol

Plasma levels of formoterol can be analyzed using high performance liquid chromatography with electrochemical detection (van den Berg et al. 1994), this approach having high sensitivity with a limit of detection of 20 pg/ml using 2 ml plasma samples, not attainable with other previously available methods (Kamimura et al. 1982; Yokoi et al. 1983). However, the method is not sufficiently sensitive to allow determination of plasma concentrations after sustained release and percutaneous application in animal studies. We have therefore developed of a more sensitive method for the determination of formoterol in rat plasma by liquid chromatography-mass spectrometry.

2. Investigations, results and discussion

Good linear relationships were noted over the concentration range 0.1–50 ng/ml of formoterol: $y = 1.221x \times 0.004137$. The calibration graph was derived by plotting the peak area ratio of formoterol to the internal standard versus the plasma concentration of formoterol; a linear regression with 1/y weighting was used. During the validation, the correlation coefficient obtained was 0.9998. The residual error of the calibration graph, as determined by the percentage error of the back-calculated sample, was $-4.48 \sim 8.08\%$.

The data determined by analyzing five replicates at 0.3, 8.0 and 40.0 ng/ml are summarized in the Table. The accuracy of the method was determined by calculating the percentage relative error, and the precision was determined by calculating the percentage relative standard deviation. With rat plasma, the precision ranged from 0.015 to 1.263 and the accuracy ranged from −2.20 to 0.08, for the three concentrations evaluated.

The sensitivity of a method is very important for trace analysis and mainly depends upon instrument sensitivity. The limit of quantification (LOQ) was obtained by deter-

Table: Summary of intra- day quality control results for formoterol (n = 5)

Parameter	Validation sample level (ng/ml)		
	0.3	8.0	40.0
Average (ng/ml)	0.2934	8.006	39.94
S.D. (n-1)	0.01468	0.3285	1.263
C.V. (%)	5.00	4.10	3.16
R.E. (%)	−2.20	0.08	−0.15

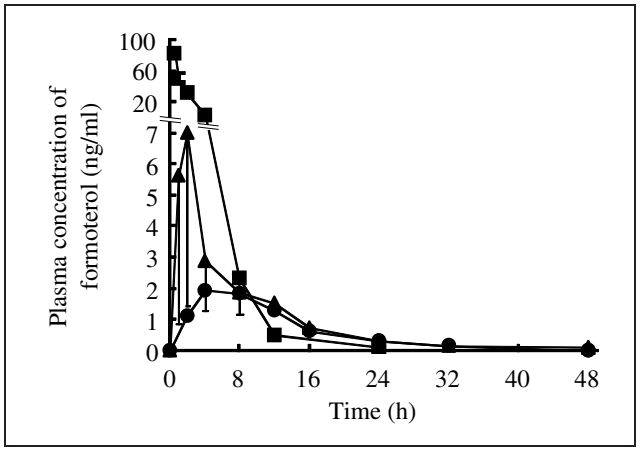


Fig.: Plasma concentrations of FF after percutaneous application and intravenous administration to rats. ●, percutaneous; ▲, oral; ■, intravenous. Each point represents the mean ± S.E. for five rats

mining the lowest amount of 0.1 ng/ml. The acceptable variability of the measured amount was set at ±20%. The precision and the mean accuracy were 17.45 and −14.60%, respectively.

Our study was designed to study the bioavailability and *in vivo* pharmacokinetics of formoterol following topical application to rats. The Fig. shows plasma concentrations after oral, intravenous or transdermal administration of 0.25 mg of formoterol fumarate to rats. The C_{max} values were 1.93 ng/ml 4 h after percutaneous application and 7.01 ng/ml 1 h after oral administration. The AUC_{0-24} values after transdermal, intravenous and oral administration were 25.6, 168.7 and 43.9 ng · h/ml, respectively. The bioavailability after percutaneous and oral exposure was equivalent to 15.2 and 26.0%, respectively, of the AUC_{0-24} after intravenous administration.

A highly sensitive and selective method for the quantification of formoterol in rat plasma was developed and validated. The limit of quantitation of the method was shown to be 0.1 ng/ml, when using 0.1 ml plasma. Plasma concentrations of formoterol could be quantified from 0.15 to 7.01 ng/ml, allowing an analysis of samples up to 32 h after a single oral dose of 0.25 mg formoterol fumarate to rats. This LC/MS/MS method is far more sensitive and precise than any other approach hitherto described.

3. Experimental

3.1. Materials

Formoterol fumarate and bromo-formoterol were supplied by Yamanouchi Pharmaceutical Co., Ltd. (Tokyo, Japan). HPLC grade methanol was obtained from Kokusan Chemical (Tokyo, Japan) and *tert.*-butylmethyl ether and ammonium hydrogen carbonate were purchased from Kanto Chemical (Tokyo, Japan). Water was purified with a Milli-Q reagent water system from Millipore Corp. All other chemicals employed were of the highest quality and used without further purification.

3.2. Equipment and analytical conditions

The HPLC system featured of a 2690 alliance (Waters) with a CAPCELL PAK C18 UG120 column (2.0 × 150 mm, Shiseido). The mobile phase consisted of a mixture of methanol- 50 mM ammonium hydrogen carbonate (1 : 1 v/v), the flow rate in the mobile phase being 0.2 ml/min. The column oven temperature was maintained at 40 °C and the injection volume was 20 µl.

Mass spectrometric detection was performed on a TSQ 7000 (Thermo electron) operating in the positive ion electrospray ionisation mode. Interface variable were: spray voltage 4.5 kV; sheath gas (N_2) 70 psi; auxiliary gas (N_2) 20 units; heated capillary temperature 250 °C; collision induced dissociation (CID) voltage for formoterol at −37 V and bromo-formoterol at −42 V; CID gas (Ar) 2.5 mtorr. The precursor ions for formoterol were detected at m/z 344.9 and bromo-formoterol at m/z 422.8, and the product ions at m/z 121.0 and 120.9, respectively.

3.3. Standard solutions

A standard stock solution of formoterol was prepared by dissolving 12.77 mg of formoterol fumarate in methanol so that the final concentration was 1 mg/ml. This solution was diluted with water to give a series of working solutions with concentrations of 500, 100, 50, 10, 5 and 1 ng/ml. A standard stock solution of bromo-formoterol (internal standard) was also prepared by dissolving 10.00 mg in methanol so that the final concentration was 1 mg/ml. This solution was diluted with water to give a working standard solution of 10 ng/ml. The stock solutions were stored at approximately −80 °C prior to use.

3.4. Sample preparation

After centrifugation of 0.3 ml blood samples, aliquots of plasma (100 µl) were mixed with 50 µl of an aqueous solution containing 0.5 ng of internal standard (bromo-formoterol) and 1.0 ml of 0.8 mol phosphate buffer (pH 9.5) and then extracted with 3.0 ml of *tert.*-butylmethylether. After vigorous shaking for 15 min, the organic layer was separated by centrifugation at 3000 rpm for 10 min, transferred to a centrifuge tube, evaporated in a nitrogen stream at 45 °C and redissolved in 200 µl of methanol-50 mM ammonium hydrogen carbonate (1 : 1 v/v). The concentration of formoterol was finally determined using the LC/MS/MS method.

3.5. Animal study

Male Wistar rats weighing 180–200 g were used, five rats being assigned to each experimental group. On the day before application, the abdominal hair was removed with an electric animal clipper. Matrix patches (2 × 1.5 cm, 0.25 mg/rat) were then applied to skin with porous stretching bandages to prevent licking or removal. For oral or intravenous application, formoterol fumarate was dissolved in saline solution and administered by gastric incubation (0.5 mg/ml) and bolus injection into the tail vein (0.5 ml/rat), respectively. The treated rats were anesthetized with ethyl ether and blood samples were taken via the jugular vein.

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