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# Analytical and metabolic study of clenbuterol in human and rats' urine

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## Introduction

Clenbuterol (4-Ammino-3,5-dichloro-α-[[(1,1-dimethylethyl) amino]methyl]benzenemethanol) is an extremely potent ß-2 agonist that is used to treat asthma. It is also an anabolic reagent. It promotes muscle growth and alters body composition in the direction of increased muscle mass and decreased fat. So, this drug was banned by the medical commission of the IOC. Because the active dose is very low, the resulting urine concentration of clenbuterol following oral administration of the drug is very small.

Some GC/MS methods were found to be satisfactory for the determination[1,2,3], but they were time-cosuming and troublesome. This assay has been successfully adapted to the detection of clenbuterol in human urine. The methods were also used to analyse rats' urine and one metabolite was detected.

## Experimental

### Chemicals

Clenbuterol hydrochloride standard was kindly supplied by INRS-Sante Labotories(Canada).The other standards and derivatization reagents were purchased from Sigma(USA), The other solvents and reagents were of analytical-reagent grade, and the solvents were re-distilled.

### Urine extration

To 5ml of urine sample were successively added 2g of solid carbonate buffer($K_2CO_3$:$NaHCO_3$=2:3) and 3ml of diethylether-isopropanol(9:1) mixture containing 1μg of nadolol(internal

standard). After urine sample was extracted on a shaker for 10min. The organic layer was evaporated to dryness under nitrogen at 50 °C.

**Derivatization procedure**

To the residue were added 100µl of MSTFA/NH4I (1000:2,v/w), the solution was heated at 80°C for 20min.

**Chromatography**

All GC/MS measurements were performed on a HP5890-HP5970 gas chromatograph-mass spectrometer in the EI mode. A fused-silica capillary column(HP-5; 25m x 0.25mm ID;0.33µm film thickness;USA) was used with helium as carrier gas(0.98ml/min). The mass spectrometer was operated in the repetitive SIM mode. The oven temperature was programmed as follows: initial temperature 180°C, temperature programming rate 10°C/min to 220°C, then 5°C/min to 260°C.

**Drug administraton(human)**

Clenbuterol hydrochloride(80µg) was administered orally to a healthy man, and urine samples collected after 0,2,5,7,9,13, 22,27,32,48 and 72h. Theses were stored frozen until analysed.

**Drug administration(rats)**

Clenbuterol hydrochloride (3mg) in deioned water(3ml) was administered orally to 8 rats and samples were stored frozen until analysed.

**Results and discussion**

Extration methods

Enzymic hydrolysis was used by many authors to analyse this drug before liquid-liquid extration, but it has been found that enzymic or acidic hydrolysis can destroy drug. The direct

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liquid-liquid extraction procedure without hydrolysis was found more sensitive than that of two kinds of hydrolysis methods. Maybe the drug existed in human urine in free form.

Derivatization methods

n-butylboric acid was usually utilized as a derivatization reagent to react with bifunctional drug molecules, but it had very low sensitivity for clenbuterol. MSTFA or other silylated reagents can derivatize the drug, but the reaction rates were too slow. NH4I or TMSI was used to catalyze the reaction. The reaction can complete in 20min at 80°C. The characteristic ions 86,335,337 were chosen to minitor the exist of clenbuterol in urine.

Urine excretion curve of human

The collected urine samples were analysed by th above methods. The Ion 86 abundance versus time curve was shown in Fig.1.

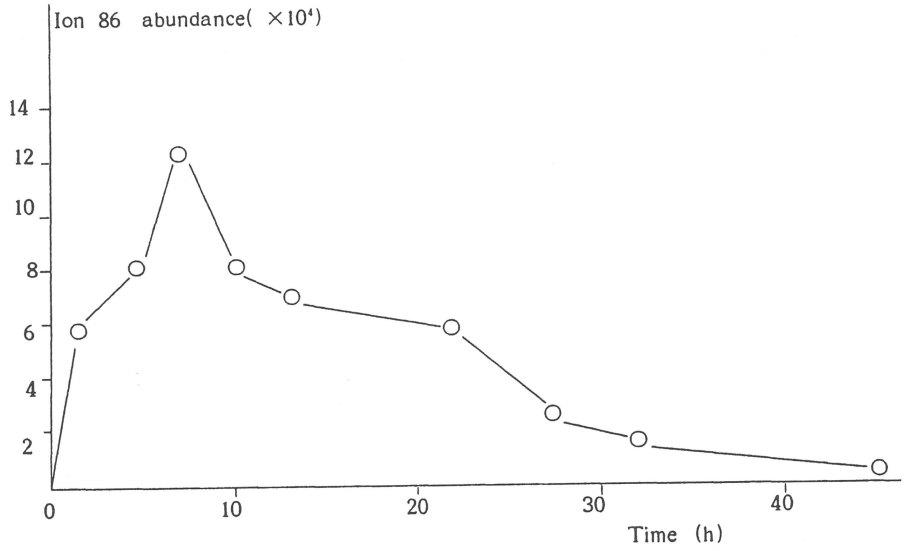


Fig.1 Urine excretion curve of clenbuterol after administration of 80μg clenbuterol hydrochloride orally to a male volunteer.

It indicated that the drug concentration had a maximum value at about 7 hours after administration and the drug could be detected in the urine samples from 2 to 48 hours.No metabolites were detected in the samples.

The metabolite in rats' urine

By using the same methods, The rats' urine samples were analysed. One metabolite was detected in the urine samples 1 to 6 days after oral administration. The variation of the metabolite and its parent drug concentration in urine were determined (Fig.2). The mass spectrum of the metabolite was shown in Fig.3 and the possible structure of the metabolite was therefore obtained by mass fragment mechanism.(Fig.4)

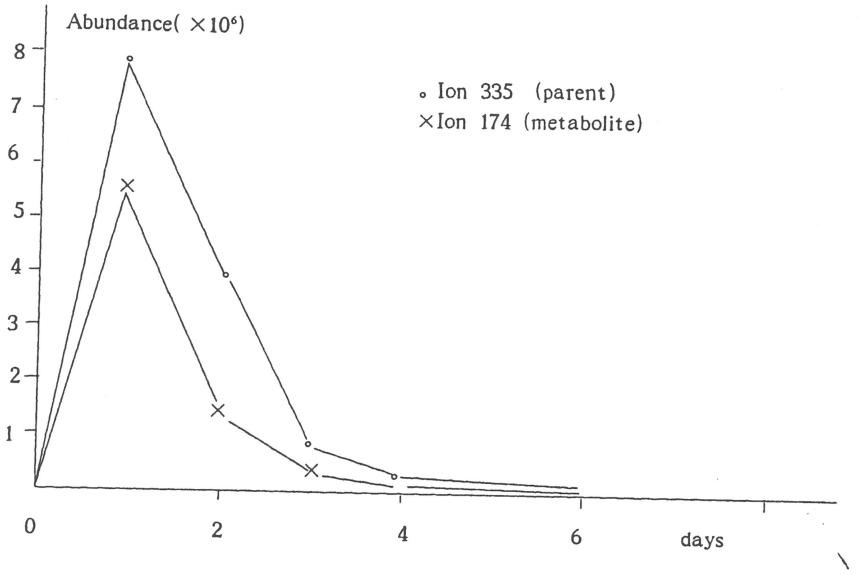


Fig.2 urine excretion curves of clenbuterol after administration of 3mg clenbuterol hydrochloride orally to 8 rats.

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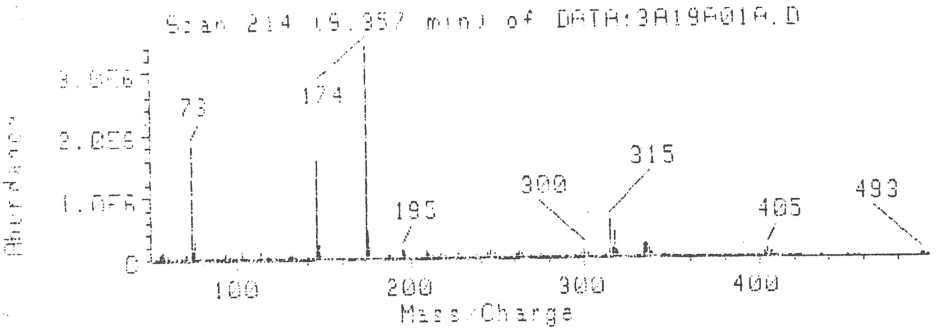
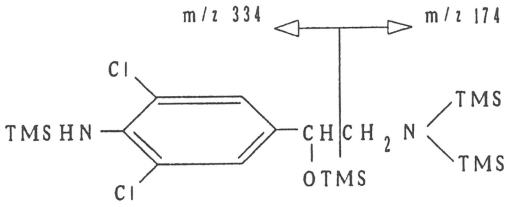



Fig.3 Mass spectrum of the metabolite TMS derivatives




$M^+ - CH_3$ =493
$M^+ - NHTMS - CH_3$ =405
$174 - CH_2 - CH_3$ =145

Fig.4 The fragment mechanism of the metabolite TMS derivatives.

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