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Abstract

Clenbuterol is a β2-agonist and a stimulant of the central nervous system; it is used as an antiasthmatic and tocolytic agent. However, it has been reported that Clenbuterol was being used as growth promoter in the cattle industry, bringing up the fact that it could also be an anabolic agent. Specialized Bodybuilding literature began to describe the use of Clenbuterol a few years ago and it appeared that the administration of this substance could be widespread among athletes seeking for muscle mass enhancement and strength.

The review of analytical methods available for the identification of Clenbuterol showed that due to the low dose administered (20 to 80 μg/day for the anti-asthma therapy), low limits of detection were needed to detect it (high picogram level/mL of urine).

Since the TMS-derivatization was already used for the screening of adrogenic anabolic steroids, we established at first the limits of detection in the routine procedure IV. The mass spectrum of the di-TMS derivative (molecular ion at m/z 420) showed very characteristic ions at m/z 405 (-Me+), 335 (ArOH+), 300 (ArOH-Cl+) and 86 (base peak, CH2=NHC(Me)3+). Through normal procedure of derivatization (MSTFA:TMSI, dithioerythritol), Clenbuterol could be detected up to 2.5 ng/mL. These results were also confirmed by spiking urine samples with Clenbuterol hydrochloride. For confirmation purposes, the free fraction isolated from Sep-Pack C18 cartridges and diethyl ether extraction at pH 9 was used: the aliquot was concentrated four times by extracting twice the amount used in the screening procedure and then derivatized in a final volume of 50 μL instead of 100 μL: the extraction recovery estimated analytically with reference to the injection of the standard was around 75%.

Finally, an excretion study was performed with a single dose of 100 μg of Clenbuterol hydrochloride. Clenbuterol was identified in the urine samples up to the last collection time ie: 24 hours.

Since November 1991, Clenbuterol has been identified in nine urine samples (mainly Body Builder's).
PROCEDURE IV

3 ml urine
↓
add
5 μg cholic acids
500 ng 17α-methyl-androstan-3β,17β-diol
↓
apply on Sep-Pack- C18 cartridge
wash with 5 ml of water
wash with 3 ml of n-hexane
elute with 5 ml of methanol
evaporate methanol to dryness
↓
add
1 ml phosphate buffer 0.2M pH: 6.9
β-glucuronidase from E. Coli (type IX-A, 3500 units)
↓
37°C for 16 hours or 55°C for 3 hours
↓
add
100 mg solid buffer 1:10 W/W
5 ml diethyl ether
↓
shake mechanically 10 min. and centrifuge 5 min.
evaporate organic phase to dryness
↓
dissolve residue in 250 μl of methanol
transfer to vial containing 500 ng of 5α-androstan-17-one
↓
evaporate to dryness
↓
add
dithioerythritol
98 μl MSTFA
2 μl TMSI
↓
70°C for 30 min.
↓
Inject into GC/MS and GC/FID
Mass Spectrum of Clenbuterol di-TMS (procedure IV)
SIM analyses of clenbuterol (standard)-diTMS (procedure IV)

Screening procedure: limit of detection: 2.5 ng/mL
SIM analysis of spiked urine samples (screening procedure IV)

Limit of detection: 2.5 ng/mL
SIM analysis of spiked urine samples (confirmation procedure)

Free fraction: 10 mL/50 uL
SIM analysis of clenbuterol excretion study (100 ug per os)

Screening procedure IV: 3 or 6 mL/100 uL
SIM analysis of clenbuterol excretion study (continued)
SIM analysis of clenbuterol excretion study (confirmation procedure)

Free fraction (procedure IV): 6 and 16 mL/50 uL
SIM analysis of clenbuterol excretion study (confirmation procedure) (continued)
Mass spectrum obtained from excretion study

Confirmation procedure (free fraction): 23:30 h after 100 ug
A: SIM analysis of a urine sample positive to clenbuterol (screening procedure IV)

B: Mass spectrum of clenbuterol di-TMS obtained in confirmation procedure