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Urinary excretion profile of inhaled formoterol and budesonide in humans: correlation with revised threshold and permitted dose of formoterol

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Abstract

Formoterol is a potent long-acting β_2-adrenergic agonist and has a pronounced and very effective bronchodilating effect. It is banned in sports by the World Anti Doping Agency (WADA) because of its anabolic and stimulating effects. From year 2013, the urinary threshold value of formoterol was increased from 30 to 40 ng/mL and maximum permitted inhaled dosage (in 24 hrs) from 36 µg to 54 µg. Hence, the aim of the present work was to investigate the urinary excretion profile of inhaled formoterol and budesonide in correlation to the revised WADA threshold for formoterol.
The finding of the excretion profile showed that excreted concentration of formoterol was below the revised threshhold level of 40 ng/mL given by WADA and may not cause an adverse analytical finding (AAF) for formoterol but it may give an AAF for budesonide and its metabolite during competition period.

Introduction

Formoterol is a potent and long acting β_2-agonist which is frequently used to prevent exercise induced asthma, branchospam and chronic obstructive pulmonary disease (COPD) [1-3]. It is normally administered by inhalation either alone or in combination with glucocorticosteroids. It is banned by the World Anti Doping Agency (WADA) because of its anabolic and stimulating effects [4]. Budesonide (22(R,S)-16α,17α-butylidenedioxy-11β,21-dihydroxypregna-1,4-diene-3,20-dion) is a glucocorticosteroid widely used for the treatment of asthma and rhinitis [5]. In India, the most common medication for formoterol is available in combination with budesonide which is often found to be declared on the doping control forms. From 2013, the urinary threshold of formoterol has been increased from 30 ng/mL to 40 ng/mL and the maximum permitted inhaled delivered dosage of formoterol from 36 µg to 54 µg over 24 hours. Various studies have been published for evaluation of formoterol concentration in human blood and urine [6,7].
The aim of present work was to evaluate the threshold concentration of formoterol (40 ng/mL) by studying urinary concentrations in healthy volunteers after inhalation of different metered dosage to establish a difference between its therapeutic and non-therapeutic usage. The excretion profile of budesonide was also studied for correlation between its use during in-competition and out-of-competition period.

Experimental

Certified reference materials for formoterol fumarate dihydrate and budesonide were purchased from Sigma Aldrich (St. Louis, USA), and formoterol-d6 (internal standard) from Toronto Research Chemical (Canada). Budesonide metabolite (16α-OH-prednisolone) was a precious gift from Anti-Doping Laboratory, Rome. The sample Extraction procedure involves enzymatic hydrolysis and liquid-liquid extraction. The experiments were performed with Waters acquity UPLC and API 4000™ tandem mass spectrometer using electrospray ionization source. The ionization voltage and cone temperature was 5500 V and 500 °C respectively. The chromatographic column used was acquity UPLC BEH C-18 column (1.7µm, 100 mm x 2.1 mm) and the total run time was 5 minutes [8].
The analytical method for identification and quantitation of formoterol developed and validated as per the requirement of WADA ISL (version 7.0) [9].

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The five-level calibration curve was made by spiking defined volumes of ethanolic solution of the certified reference standards of formoterol in blank urine in triplicate at 15, 20, 30, 60 and 120 ng/mL. Precision was assessed as the percentage relative standard deviation (%RSD) of both repeatability (within-day) (n = 5) and reproducibility (between-day and different analysts) (n = 15). The inter-batch coefficient of variation had to be 15% for precision, and the mean value had to be within 15% of the actual value for accuracy. A linear regression was used with a weighting factor of 1/x. The coefficient of correlation has to achieve a degree of certainty of R = 0.99. Specificity was tested by analyzing ten blank urine samples as described above to evaluate the presence of interferences. Acceptable specificity was defined as area of possible interferences in blank urine samples. Repeatability and reproducibility was determined in multiple measurements of the samples under the same analytical conditions.

The excretion study of formoterol and budesonide in human volunteers was approved by the Ethics Committee of NDTL. The dosage of formoterol for the present study was decided on the basis of previous and revised permitted therapeutic dose recommended by WADA. Foracort (formoterol 6 µg + budesonide 200 µg) (Cipla India Ltd.) was administered through inhalation at three different dosages (36 µg, 54 µg and 108 µg) to volunteers (Table 1) and urine samples were collected for 48 hours.

Drug formulation	Dosage	Schedule	Volunteers
Foracort 200 (formoterol 6 µg + budesonide 200 µg)	36 µg	3 puffs, two times every 12 hours	Volunteer 1 & 2
Foracort 200 (formoterol 6 µg + budesonide 200 µg)	54 µg	3 puffs, three times every 6 hours	Volunteer 3, 4 and 5
Foracort 200 formoterol 6 µg + budesonide 200 µg)	108 µg	6 puffs, three times every 6 hours	Volunteer 6

Table 1: Dosage schedule and volunteer detail

Results and Discussion

Formoterol and its deuterated analogue (d6-formoterol) were efficiently ionized using positive electrospray ionization (ESI) because of the presence of the basic nitrogen which is easily protonable in the positive ionization mode (Figure 1a). The formoterol and d6-formoterol (ISTD) were eluted at 2.3 minutes (Figure 1b) and run time of the method was 5 minutes. The concentration of formoterol in urine samples ranged from 1.7-22.1 ng/mL (Fig 2a) after administration of 36 µg in 24 hours, whereas for 54 µg it ranged between 2-29.6 ng/mL (Figure 2b). The concentration of formoterol after administration of higher dose of 108 µg in 24 hours ranged between 5.71 - 34.5 ng/mL (Figure 2c). The urinary concentration of formoterol did not cross the revised threshold level of 40 ng/mL in any volunteer, which may be due to the fact that drug was administered only for 24 hours.

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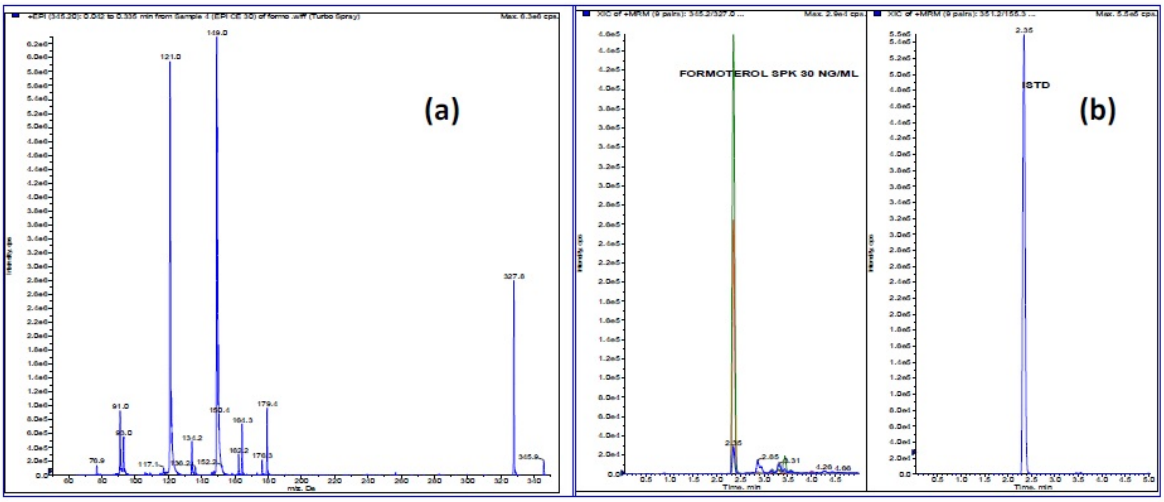

Figure 1: (a) Enhanced product ion trap spectrum of m/z 345.2 for formoterol, (b) total ion chromatogram of formoterol and d6-formoterol (ISTD)

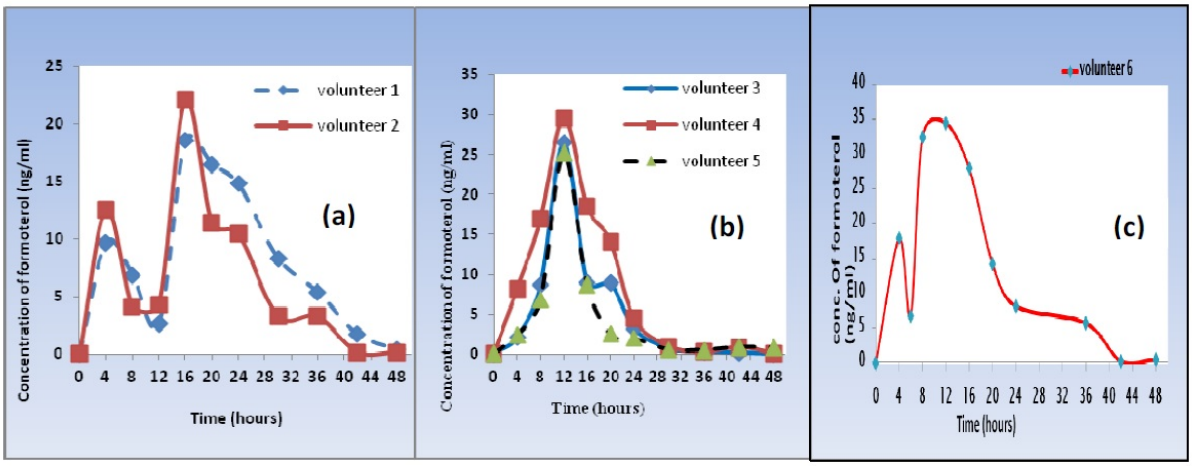

Figure 2: Urinary excretion profile of formoterol after administration of formoterol at 36 µg/24 hrs (a), 54 µg/24 hrs (b) & 108 µg/24 hrs (c)

The excretion profile of budesonide studied along with formoterol at different dosage of 1.2, 1.8 and 3.6 mg administered for 24 hours showed maximum concentration of budesonide parent at 19.7, 49.5 and 65.4 ng/mL respectively (Figure 3 a). The maximum concentration of budesonide metabolite (16α-OH-prednisolone) was found to be 275, 373 and 446 ng/mL after the dosage of 1.2, 1.8 and 3.6 mg respectively (Figure 3b).

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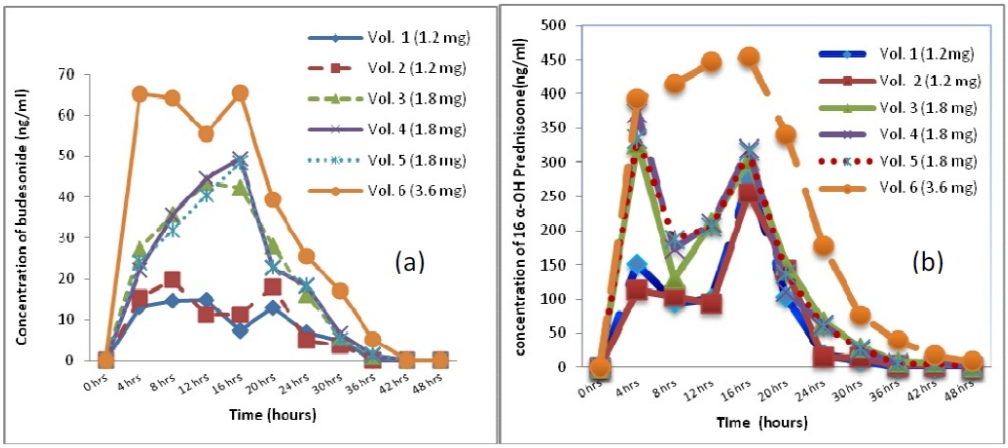

Figure 3: Urinary excretion profile of budesonide (a) & 16α-OH prednisolone (b).

Conclusions

Excretion profiles of formoterol in healthy volunteers revealed that the threshold level of 40 ng/mL was not crossed with the administered dose of 36 µg, 54 µg and 108 µg in 24 hours. A combined preparation of formoterol and budesonide provides relevant information that if taken for therapeutic purpose, may not cause an Adverse Analytical Finding (AAF) for formoterol but it may give an AAF for budesonide and its metabolite during the competition period. The athlete should declare the therapeutic use of formoterol & budesonide as prolonged use at high dosage may lead to an adverse analytical finding.

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