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Excretion profile of inhaled formoterol: distinguishing between therapeutic use and abuse in sports

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Abstract

Formoterol is a potent and long acting beta-2 agonist which is frequently used to prevent exercise induced asthma and chronic bronchitis. The minimum required performance limit (MRPL) of formoterol is reduced from 100 to 30 ng/mL by WADA with its inclusion in the category of threshold substances which requires its quantitation to report adverse analytical finding (AAF). The aim of the present study is to develop a quantitation method for formoterol and to study excretion profile, to differentiate between the therapeutic use and abuse.
Excretion study of formoterol fumarate was performed by inhalation (24 µg) to two healthy male volunteers with budamate transcaps (formoterol 6 µg + budesonide 400 µg) and budamate inhaler (formoterol 6 µg + budesonide 400 µg) respectively. The maximum concentration of formoterol obtained after administration of therapeutic dose (24 µg daily) ranged between 9-13 ng/mL for transcaps and 6-9 ng/mL for aerosol inhaler. The excretion profile of budesonide was also studied in correlation with formoterol. The results show that threshold of 30 ng/mL is appropriate to facilitate therapeutic administration of formoterol for treatment of asthmatic athletes.

Introduction

Formoterol is a potent and long acting beta-2 agonist which is frequently used to prevent exercise induced asthma and chronic bronchitis [1-3]. The use of beta-2 agonists is banned in sports by World Anti Doping Agency (WADA) because of anabolic and stimulating effects. In the year 2012, the minimum required performance limit (MRPL) of formoterol is reduced from 100 to 30 ng/mL by WADA with its inclusion in the category of threshold substances which requires its quantitation to report adverse analytical finding (AAF) [4].
The aim of the present study was to develop a quantitation method for formoterol and to study dose dependant excretion profile to differentiate between the therapeutic use and abuse.

Experimental

Formoterol was added in the routine screening method on LC-MS/MS in January 2011 [5]. Certified reference standard of formoterol was obtained from Sigma Aldrich, USA and for D6-formoterol was obtained from Toronto Research Chemicals, Canada. The sample extraction procedure involves enzymatic hydrolysis and liquid-liquid extraction. To two/four mL of urine sample aliquots based on specific gravity, 30 ng/mL of internal standard (D6 formoterol) was added. Hydrolysis was performed using β-glucuronidase (E.coli) enzyme at 60 °C for an hour. The pH was adjusted to 9-10 with 7% K_2CO_3 and liquid-liquid extraction was performed using 5 mL tertiary butyl methyl ether (TBME). After mixing for 15 min and centrifugation for 10 min at 3000 rpm, the organic layer was separated and dried under nitrogen gas at 60°C. Finally, the residue was reconstituted in 100 µL of mobile phase (1% formic acid and acetonitrile) (50:50) *(v/v)* and transferred into conical autosampler vials for analysis.
All UPLC-MS/MS experiments were performed with a Waters Acquity UPLC and API 4000™ tandem mass spectrometer (electro spray ionization source). The internal standard and analytes were chromatographically resolved using a Acquity UPLC BEH C-18 column (1.7 µm, 100 mm x 2.1 mm). The mobile phase, delivered at a flow rate of 0.3 mL/min, consisted of solvent A (1% formic acid in water) and solvent B (acetonitrile). The mass spectral data was obtained in positive ionization mode.

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Formoterol fumarate was administered twice daily (24 µg by inhalation; two consecutive inhalations every 12 h) for two days in therapeutic dose to two healthy volunteers for each preparation of formoterol (budamate: transcaps and budamate: inhaler). Additionally, another volunteer (chronic asthmatic patient) who was on double of therapeutic dose of budamate transcaps was also included in the study (Table 1). The study was duly approved by Ethics Committee of NDTL, India. The excretion profile of budesonide and 16α-OH prednisolone was also studied in correlation of excretion of formoterol because budesonide is available in India in combination with formoterol.

Volunteer details	Drug preparation	Time of sample collection after administration from first dosage
Volunteer 1 & 2	• Budamate 400 Transcaps (Lupin, India) containing formoterol fumarate and budesonide powder for inhalation • Each capsule contained (formoterol 6 µg + budesonide 400 µg) • Two capsules administered twice for two days (2 capsules every 12 h)	0 h 30 min 11 h 22 h 35 h
Volunteer 3 & 4	• Budamate 400 Inharel (Lupin, India) containing formoterol fumarate and budesonide aerosol for inhalation • Each puff contained (formoterol 6 µg + budesonide 400 µg) • Two puffs administered twice for two days (2 puffs every 12 h)	0 h 30 min 11 h 22 h 35 h
Volunteer 5	• Budamate 400 Transcaps (Lupin, India) containing formoterol fumarate and budesonide powder for inhalation • Each capsule contained (formoterol 6 µg + budesonide 400 µg) • Four capsules administered twice (4 capsules every 12 h) for two days	0 h 30 min 5 h 8 h

Table 1: Details of drug preparations and dosage schedule

Results and Discussion

The runtime of the method was 5 min and formoterol and formoterol D6 (ISTD) eluted at 2.3 min in positive ionization (Figure 1). The MRM ion transition used for quantitation were 345.2-327 (formoterol) and 351.2-155.3 (formoterol d6). The concentration of formoterol after administration of therapeutic dose (24 µg daily) ranged between 1.2-13 ng/mL for transcaps and 1-6.9 ng/mL for aerosol inhaler (Figure 2a). The concentration of formoterol after administration of double of therapeutic dose of transcaps was found between 4.84-19 ng/mL (Figure 2b).
Formoterol is available in combination with budesonide in India. Budesonide and its metabolite 16α-OH prednisolone were monitored using MRM transitions 431-341 and 377-359, respectively. The excretion profile of budesonide studied in correlation with formoterol showed concentration of budesonide parent between 5-42 ng/mL for transcaps and 3-35 ng/mL for aerosol inhaler (Figure 3a) while that of budesonide metabolite (16α-OH-prednisolone) ranged between 09-994 ng/mL for transcaps and 2-410 ng/mL for aerosol inhaler (Figure 3b).

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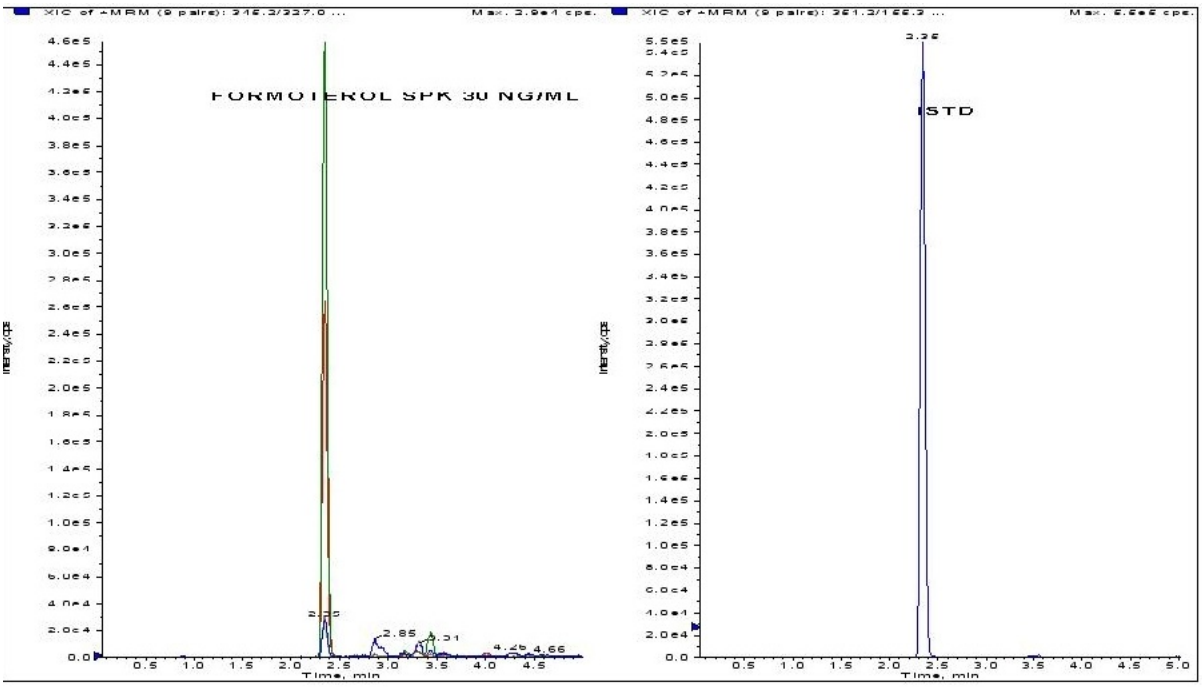

Figure 1: Extracted ion chromatogram of formoterol and D6 formoterol

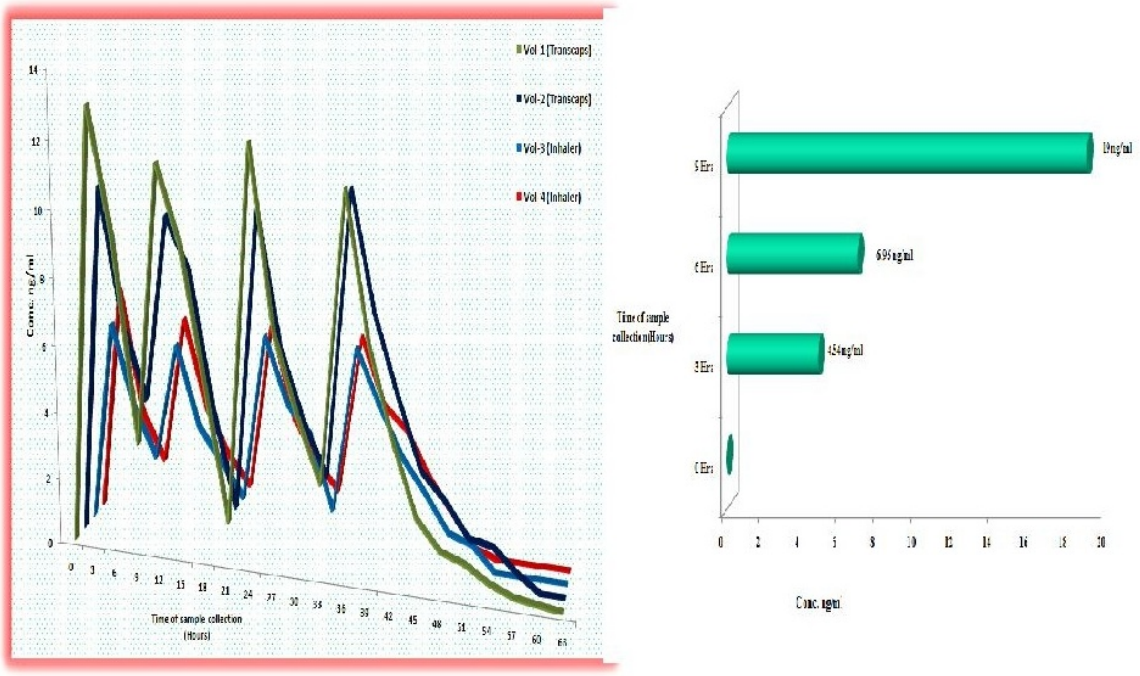

Figure 2: (a) Excretion profile of formoterol (transcaps & inhaler) at therapeutic dose, (b) excretion profile of formoterol (inhaler) at double of therapeutic dose

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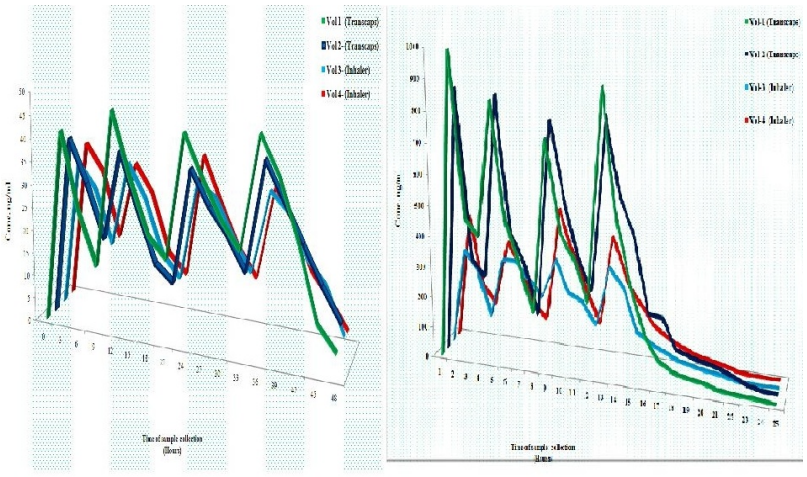

Figure 3: (a) Excretion profile of budesonide (transcaps & inhaler) at therapeutic dose, (b) Excretion profile of budesonide metabolite (transcaps & inhaler) at therapeutic dose

Two samples reported as adverse analytical finding (AAF) for budesonide in 2011 at NDTL, India were re-analyzed and quantitated for formoterol showed presence of 1.5 and 5 ng/mL of formoterol respectively which is much below the threshold level of 30 ng/mL.

Conclusions

It is inferred that the therapeutic administration of formoterol for treating asthmatic athletes does not cross the threshold of 30 ng/mL, hence is appropriate to facilitate treatment of asthmatic athletes. The combination of budesonide with formoterol taken for therapeutic purpose during training period will not cause an AAF for formoterol. Further work is in progress to perform the uncertainty measurement of the method and to study the detectability of formoterol in various Indian drug preparations.

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