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Excretion study of budesonide metabolites after inhalation and oral administration using LC-ESI-MS/MS

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Introduction

Budesonide (BUD) is a corticosteroid, clinically used in the treatment of respiratory diseases, such as asthma and rhinitis [1]. Several studies have been reported for the determination of BUD and its metabolites in biological samples [2–10]. Despite this, there are a few data on the detection of BUD and its metabolites associated to doping analysis [11]. In addition, no reports on the urinary excretion profiles of this corticosteroid and its metabolites have been so far described. The present work describes a sensitive and specific LC-ESI-MS/MS method for the identification of BUD metabolites in urine after inhalation and oral administration. Five BUD metabolites were therefore separated and identified. Analysis of urine samples taken after inhalation of Budiar® (5×400 µg) and ingestion of three capsules of Entocort® (3×3 mg) revealed that the five metabolites could be detected up to 50 hours after administration.

2. Materials and Methods

2.1. Excretion studies and purification

The study was approved by the local Ethics Committee of the Charles Nicholle Hospital (Tunisia). Urinary samples were obtained from three healthy women volunteers (volunteers 1, 2 and 3 at age 22, 26 and 53 years old respectively) who was signed a written consent form. After clinical examination and biochemical tests the volunteer received 2000 µg (maximum therapeutic dose) of BUD in successive inhalated puffs (Budiar® 400 µg/dose, Chiesi, Italy) and urine was collected at different time-points up to 60 h post-dose. After 14 days of wash out, the volunteer received orally 9 mg of budesonide (Entocort® 3 mg, AstraZeneca, France) and urine was collected at different time-points up to 60 h post-dose. Samples were then extracted by AccuBond (3 mL/200 mg) cartridges (Agilent Technologies, USA)

2.2. LC-ESI-MS parameters

Agilent HPLC coupled to Quattro micro (Micromass, UK).

Ionization mode	ESI-
MRM transition	489.2>357.2 (BUD), 375>327.2 (Met 1/Met 3), 373.1>325.2 (Met 2), 505.2>373.3 (Met 4), 505.2>357.3 (Met 5), 511.4>431.5 (IS, Fluocinolone acetonide)
High voltage electrodes	3.2 kV
Source and Desolvation temperature	120ºC and 400 ºC
Nebulisation gas pressure/Desolvation gas flow	7 bars/650L/h
Collision gas	2 10^{-3} mbar
Column	Zorbax C-8 (2.1×150 mm, 5 µm)
Mobile phase	Ammonium Acetate (A) -Acetonitrile (B)[5mM, pH = 4.5] (10:90) at 0.3 mL/min. 20 % of B from 0 to 2 min, followed by a 14 min gradient to 80% B; then 80% B for 2 min.

3. Results and discussion

The total ion chromatograms of excretion urine after ingesting of three capsules (maximum therapeutic dose) of Entocort® (3×3 mg) and Inhalation of 2000 µg of BUD were compared with blank urine one to detect suspicious peaks. Identification of BUD metabolites were performed by comparing their retention-times, differences between molecular masses (Δm) and MS/MS (ESI+ and ESI-) spectra with those of the parent drug. This investigation showed that BUD is converted to five metabolites namely 16α-hydroxyprednisolone (Met 1), 16α-hydroxyprednisone (Met 2), 3α, 3β–dihydro-16α-hydroxyprednisone (Met 3), 6β-hydroxybudesonide (Met 4) and 23-hydroxy-budesonide (Met 5). BUD was only detected in urine at a few time points in most subjects and therefore the elimination profiles of BUD could not be reliably characterized**.** The urinary excretion profiles for 16α-hydroxy-prednisolone are shown in Figures 1A and 1B. The maximum urinary concentration of 16α-hydroxyprednisolone was reached 2 hours after inhalation for the three volunteers while that relative to oral administration was reached after 5.5 h. For inhalation and oral administration, the maximum concentrations ranged between 670 and 740 $ng.mL^{-1}$ and varied between 1500 and 3000 $ng.mL^{-1}$, respectively. This study indicates also that for all tested subjects 16α-hydroxyprednisolone concentration (Figure 1B) is still higher than 30 $ng.mL^{-1}$ until 12 h for inhaled and 36 h for oral administration. The excretion profiles for the five metabolites are presented in Figures 2-3. Metabolites were substantially excreted during the first 12 h after intake and the concentration remains significant until the 24 h post

administration. It's worth to note that the urinary concentration of **Met 1** and **Met 2** are 5 to 10 times higher than **Met 3**, **Met 4** and **Met 5** 24 h after intake of the drug. Although urinary concentration of BUD is still below the MRPL of 30 $ng.mL^{-1}$, this study indicates that **Met 1** and **Met 2** could be used as primary target compounds for the detection of BUD abuse in doping analysis.

4. Conclusion

Analysis of samples taken after inhalation of Budiar® (5×400 μg) and ingestion of three capsules of Entocort® (3×3 mg) showed that BUD is converted to 16α-hydroxyprednisolone, 16α-hydroxyprednisone, 6β-hydroxybudesonide, 3α, 3β–dihydro-16α-hydroxy-prednisone and 23-hydroxybudesonide. The maximum concentration of BUD metabolites was reached in 2 and 5.5 h after inhalation and oral administration, respectively. Furthermore, the excretion study showed that the urinary concentration of metabolites **Met 1** and **Met 2** was 5 to 10 times higher than the other metabolites until 24 h after intake of the drug. It was shown that 16α-hydroxyprednisolone and 16α-hydroxyprednisone could be used as primary target compounds for the detection of BUD abuse in doping analysis.

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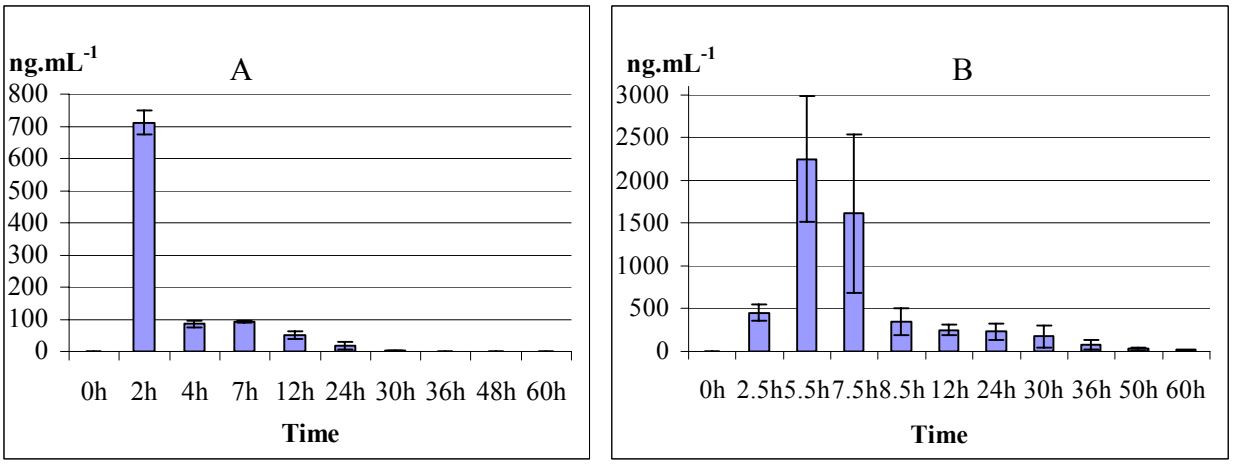

Figure 1: 16α-hydroxyprednisolone excretion profiles after (A) inhalation and (B) oral administration of the drug for three volunteers.

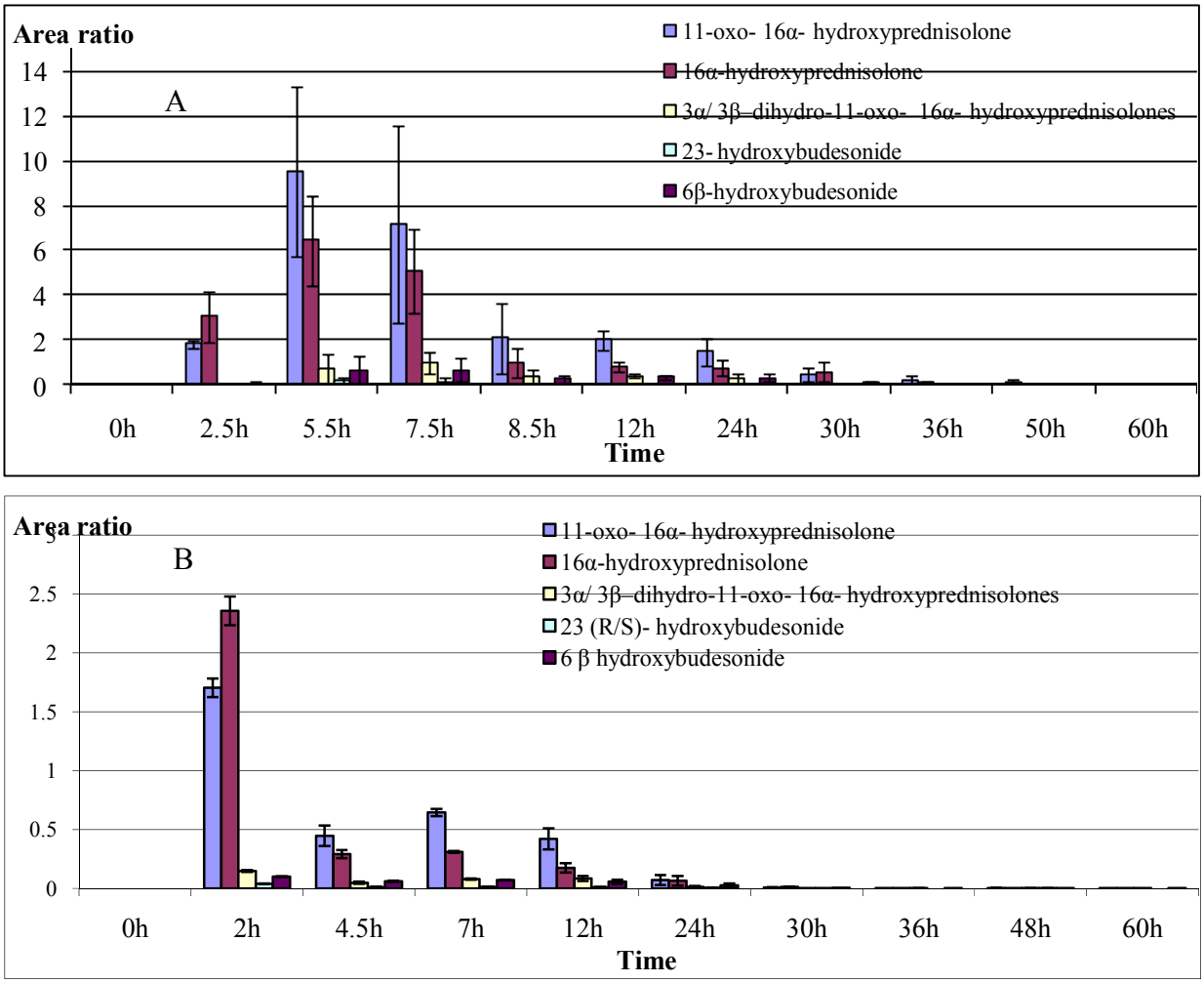

Figure 2: Elimination profiles of budesonide metabolites after inhalation (A) oral administration (B) for three volunteers.