

Detection and excretion study of fluticasone propionate by liquid chromatography–mass spectrometry

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Introduction

Fluticasone propionate (FP), a synthetic corticosteroid frequently used in the treatment of allergic rhinitis, asthma and dermatological disorders [1], is available for inhalation and as nasal drops. Unlike most other corticosteroids, the structure of FP is based on the androstane, rather than the pregnane, corticosteroid nucleus. The molecule is designed to maximise topical anti-inflammatory activity and minimise the unwanted systemic effects associated with other glucocorticoids (suppression of the hypothalamopituitary-adrenal axis) [2]. Its use in sports is allowed when medically necessary. Several studies have been reported for the detection of FP and its carboxylic acid metabolite in urinary samples [3-6]. However, no reports on the elimination profiles of this substance and its metabolites have been so far described. In the present investigation, a fast LC–MS method was developed for the identification of FP and its carboxylic acid metabolite in urine samples after inhalation of Flixotide® 200. The primary aim of this study was designed to answer the following questions: what is the elimination profiles of FP and its metabolites after inhalation and local application of drugs to two healthy volunteers; do high doses of inhaled FP suppress 48 h urinary cortisol, cortisone and tetrahydrocortisone levels.

2. Materials and Methods

2.1 Administration and purification

Following administration of a single 2000µg inhaled dose of FP, urine were collected at different time-points up to 48 h. Four milliliters of urine was spiked with beclomethasone (IS, 250 ng), the pH was adjusted to 6.5 using 1 mL of potassium phosphate buffer (0.2M). After an enzymatic hydrolysis by 20 µL β -glucuronidase at 55°C for 1 hour the samples were loaded into the extraction cartridges and eluted with 5 mL of MTBE. The organic phase was evaporated under a nitrogen stream. The dry residue was redissolved in 100 µ L of mobile phase.

2.2 Chemical and reagents

Fluticasone propionate (FP), beclomethasone (IS), cortisone, hydrocortisone, tetrahydrocortisol, acetic acid, ammonium acetate, water and methanol were purchased from Sigma Aldrich (Steinheim, Germany). Fluticasone carboxylic acid was prepared by alkaline hydrolysis of FP using the same condition as described by A. Gotzmann et al.[6].

2.3 LC-MS/MS parameters

Instrument: Agilent 1100 HPLC coupled to Quattro micro (Micromass)

Ionization mode/Data acquisition mode ESI (-)/MRM **419>329, 421>331, 425>335, 451>72 (FCA), 559>413 (FP), 467>377 (IS)**

High voltage electrodes: 3200V Source and Desolvation temperature: 120°C and 400 °C

Desolvation gas flow: 500L/h Nebulisation gas pressure: 7bar.

Collision gas: 2 10⁻³ mbar Column: Zorbax RX C-8 (2.1×150 mm, 5 µm)

Mobile phase: Ammonium acetate [5mM, pH = 3.5] -methanol at Flow rate of 0.3 mL. min⁻¹.

From 0 to 2min → 40% methanol; From 2 to 20min → 40 to 85% methanol; From 20 à 23min → 85% methanol.

3. Results and Discussion

3.1. Method validation

Using a least square fit, good linearity ($r^2 = 0.997$) was obtained for FP in the range 10 – 200 ng/mL. As shown in Table 1, RSD values for repeatability and reproducibility did not exceed the 12%. Deviation of the mean measured concentration from the theoretical concentration (accuracy) for FP was below the 11 % for the three concentration levels. Regarding the selectivity, interferences from other doping agents could not be found. In addition, analysis of 20 different blank urine samples did not result in the detection of interfering substances, proving the specificity of the method. The limit LOD and LOQ of the method were 5 ng/mL and 10 ng/mL, respectively. Recoveries varying from 80.6 to 89.8 % were obtained for PF. The intraday recovery indicated that the RSD of the PF was not more than 11%.

3.2. Excretion study

The elimination profile of FP and its metabolite was performed after the inhalation of a single inhaled dose of 10×200µg of Flixotide. The excretion study (Figures A and B) indicates that FP and its metabolite can be detected up to 48 hours after administration of 10×200µg to the two healthy volunteers. Figures A and B show also that the elimination of the FP and its carboxylic acid metabolite is important during the 8 hours after the inhalation of Flixotide® 200. In fact, the concentration of FP and its metabolite increase and reach a maximum levels 4 hours after inhalation. Furthermore, for the two tested subject carboxylic acid metabolite is

detectable at the concentration 10 times higher than the parent compound until 24 h after post administration (Inhalation), while FP concentration still below 5 ng/mL along the urinary excretion study. This investigation clearly indicates that the parent compound could not be detected up to 30 ng/mL in urine. So, it should not be a best target compound for its detection in urine. In addition, the urinary concentration of cortisone, cortisol and tetrahydrocortisol (Figures C and D) significantly decreased following inhaled administration, the minimum value of the urinary concentration of the endogenous was obtained when the concentration inhaled glucocorticoid and its main metabolite increase. This behaviour of endogenous corticosteroids is due to the negative retrocontrol of hypothalomo-hypophysary axis in agreement with the well known suppression of pituitary-adrenal function observed as a consequence glucocorticoid therapy.

4. Conclusion

The urinary concentration of PF and its carboxylic acid metabolite increase 60 min after administration of Flixotide until to reach a maximum at 240 min for the two volunteers. Furthermore, this study shows that the major part of the inhaled drug is metabolized in 17 β -carboxylic acid of fluticasone. This study indicated also that the urinary concentration of cortisone, hydrocortisone and tetrahydrocortisol decrease when the concentration of the FP and its métabolite reach a maximum values.

References

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Table: Accuracy, repeatability, reproductibility and tolerance limits of method at three FP concentrations obtained from the analysis of spiked urine using beclomethasone as an IS.

Concentration (ng.mL ⁻¹)	Accuracy (%), n=18	Repeatability (%), n=6	Reproductibility (%), n=18	Recovery ±RSD (%), n=18
10	10.2	9.9	10.4	89.8 ± 10.2
50	8.6	10.4	8.9	82.3 ± 8.6
200	4.1	6.3	3.8	80.6 ± 6.5

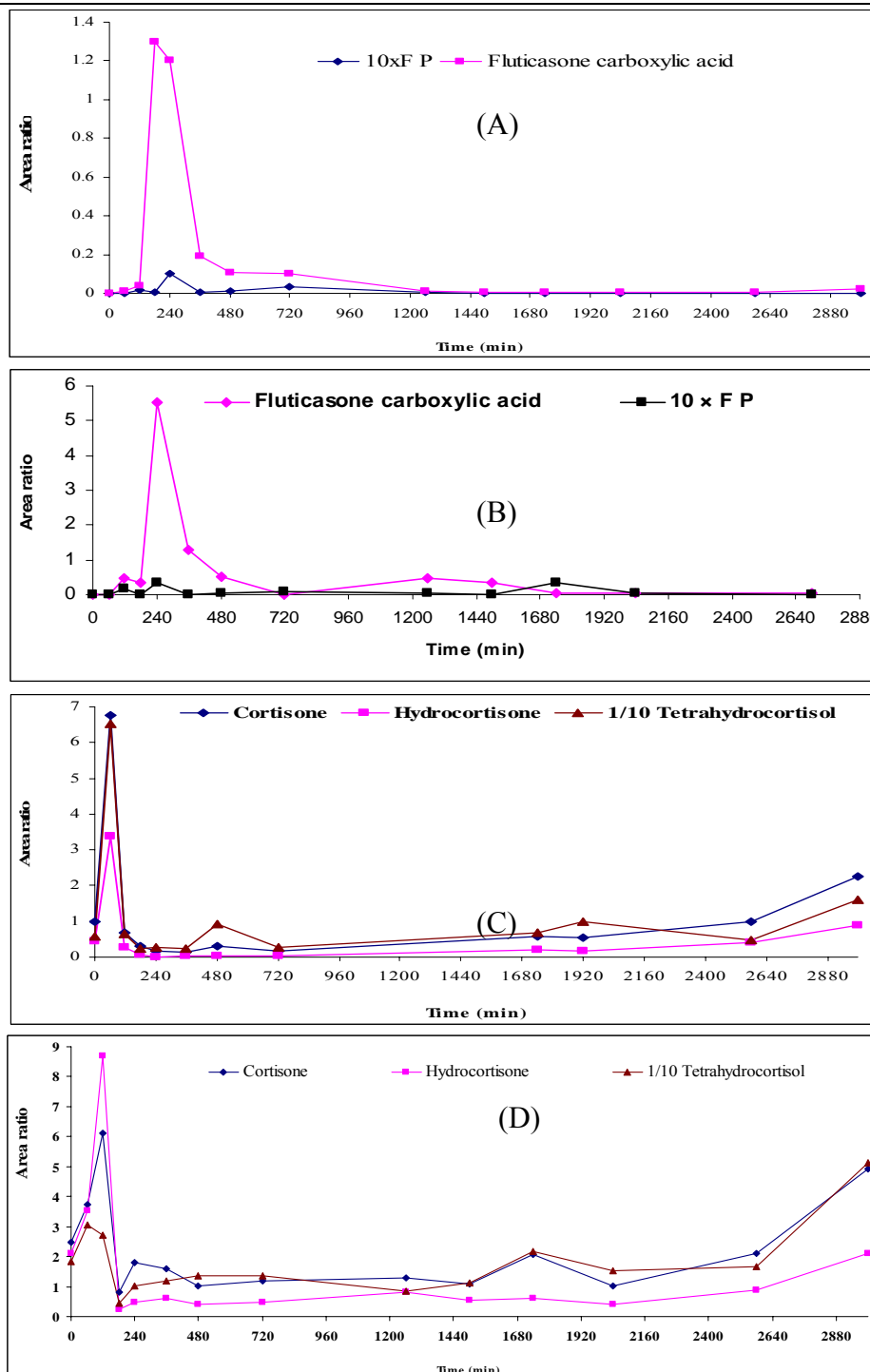


Figure : (A) and (B) Excretion profiles of PF and Fluticasoone carboxylic acid of after inhalation of Flixotide. (C) and (D) elimination profiles of Cortisol, Cortisone and Tetrahydrocortisol after inhalation of Fluoxetine