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Study of methylprednisolone elimination profile in human urine using LC-ESI-MS/MS

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Introduction

Methylprednisolone (MP) is a synthetic corticosteroid used in particularly for their antiinflammatory and immunosuppressive actions [1]. Due to its significant side effects, the systemic use of methylprednisolone during competitions has been prohibited by the WADA [2]. Several analytical assays have been developed for the determination of MP and its metabolites in biological samples using HPLC, LC-MS and GC-MS [3-9]. In drug metabolism and pharmacokinetic studies, reversed-phase was adopted for the separation of corticosteroids and their metabolites. However, reversed-phase is not suitable for drug metabolism studies since compounds and their metabolites with very similar polarities will not be resolved. In present study, an attempt was made to increase the stereochemical differences between theses metabolites using a porous graphitic carbon column and ESI (+/-) tandem mass spectrometry. The developed method was used to identify the MP metabolites and to investigate urinary profiles of parent drug and its metabolites, after IM injection and 32mg oral dose of MP.

Materials and Methods

Excretion study and purification

The study was approved by the local Ethics Committee of the Charles Nicholle Hospital (Tunisia). Urinary samples were obtained from three healthy volunteers (two men: 22 and 25 years old and one woman 26 years old). After clinical examination the volunteers received orally two tablets of 16 mg MP (Medrol® 16 mg tablets, Pfizer Holding). The urine samples were collected at different time-points up to 60 h post-dose. After 14 days of wash out, the volunteers received an IM injection of Solu-Medrol (Solu-Medrol® 40 mg, Pfizer

Pharmaceuticals) and urines were collected at different time-points up to 60 h post-dose. Samples were then extracted by SampliQ (3 mL/500 mg) cartridges (Agilent Technologies, USA).

LC-ESI-MS parameters

Agilent HPLC coupled to Quattro micro (Micromass).

Ionization mode	ES-/ES+
MRM transition	419.3>373.3 (MP), 435.2>359.2 (M1), 435.2>389.3 (M2/M3), 437.2>391.2 (M4/M5), 419.3>343.3 (M6/M7), 415.2>339.2 (M9), 417.2>341.3 (M10), 421.3>375.3 (M12), 437.2>361.3 (M11/M13), 433.2>357.3 (M8/M14), 303>109 (IS, ESI+)
High voltage electrodes	3.2 kV
Source temperature	120ºC
Desolvation temperature	400 ºC
Nebulisation gas pressure	7 bars
Desolvation gas flow	650L/h
Collision gas	$2\ 10^{-3}$ mbar
Column	Hypercarb (2.1×150 mm, 5 μm)
Mobile phase	Ammonium formate-methanol [5mM, pH = 3.5] (10:90) at 0.3 mL/min

Results and Discussion

The total ion chromatograms of excretion urine after ingesting of 32 mg of and IM injection of MP were compared with blank urine one to detect suspicious peaks. Identification of MP 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. Using this methodology, 14 MP metabolites were detected in the excretion study, as shown in Figure 1. The investigation indicates the conversion of the prodrug MP 21-hemisuccinate (IM) to the parent drug MP leading finally to 14 metabolites including 6β-hydroxymethylprednisolone (M1), 20α and 20β- dihydro-11-oxo-6β-hydroxymethylprednisolone (M2/M3), 20α and 20β-dihydro-6β-hydroxymethyl-prednisolone (M4/M5), 20α- and 20β dihydro-11-oxo-methylprednisolone (M6/M7), 11-oxo-21-hydroxymethylprednisolone (M8), 11-oxo-6-7-dehydromethylprednisolone (M9), 11-oxo-methylprednisolone (M10), 1,2-dihydro-6β-hydroxymethylprednisolone (M11), 20α- and 20β- dihydromethyl-prednisolone (M12), 1, 2, 3α, 3β-tetrahydro-11-oxo-6β-hydroxymethyl-prednisolone (M13), 11-oxo-6β-hydroxymethylprednisolone (M14). Maximum urinary concentrations of all metabolites were obtained at 0–4h and 2-8h after IM and oral

administration on MP, respectively. Figures 2A and 2B show that the concentration of MP exceeded 30 ng/mL for up to 48 h post administration (IM and Oral). This study shows also that the urinary concentration of metabolites **M2**, **M3**, **M8**, **M10** and **M12** are 10 to 400 times higher than conentration of MP after 10h post administration (Figures 2C and 2D). In addition this investigation suggest that metabolites **M2**, **M3**, **M8**, **M10** and **M12** provide a prolonged detection period and are a powerful tool for long-term detection of MP abuse.

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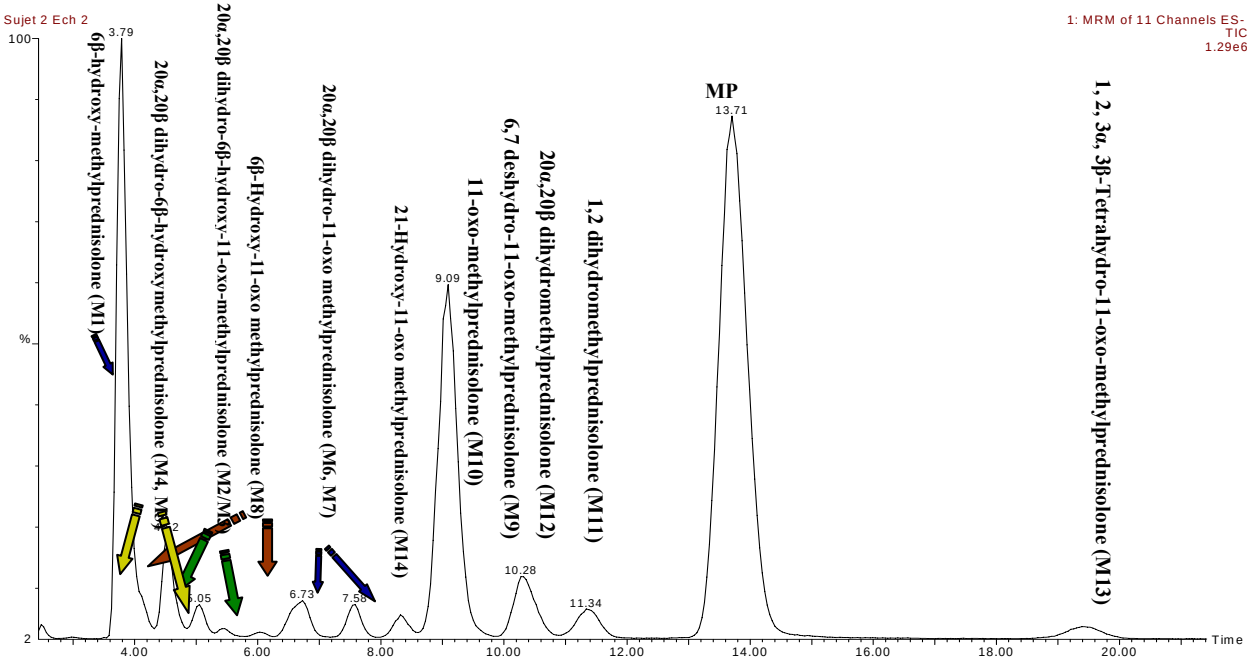

Figure 1: MRM chromatogram of sample 2h after oral administration of MP

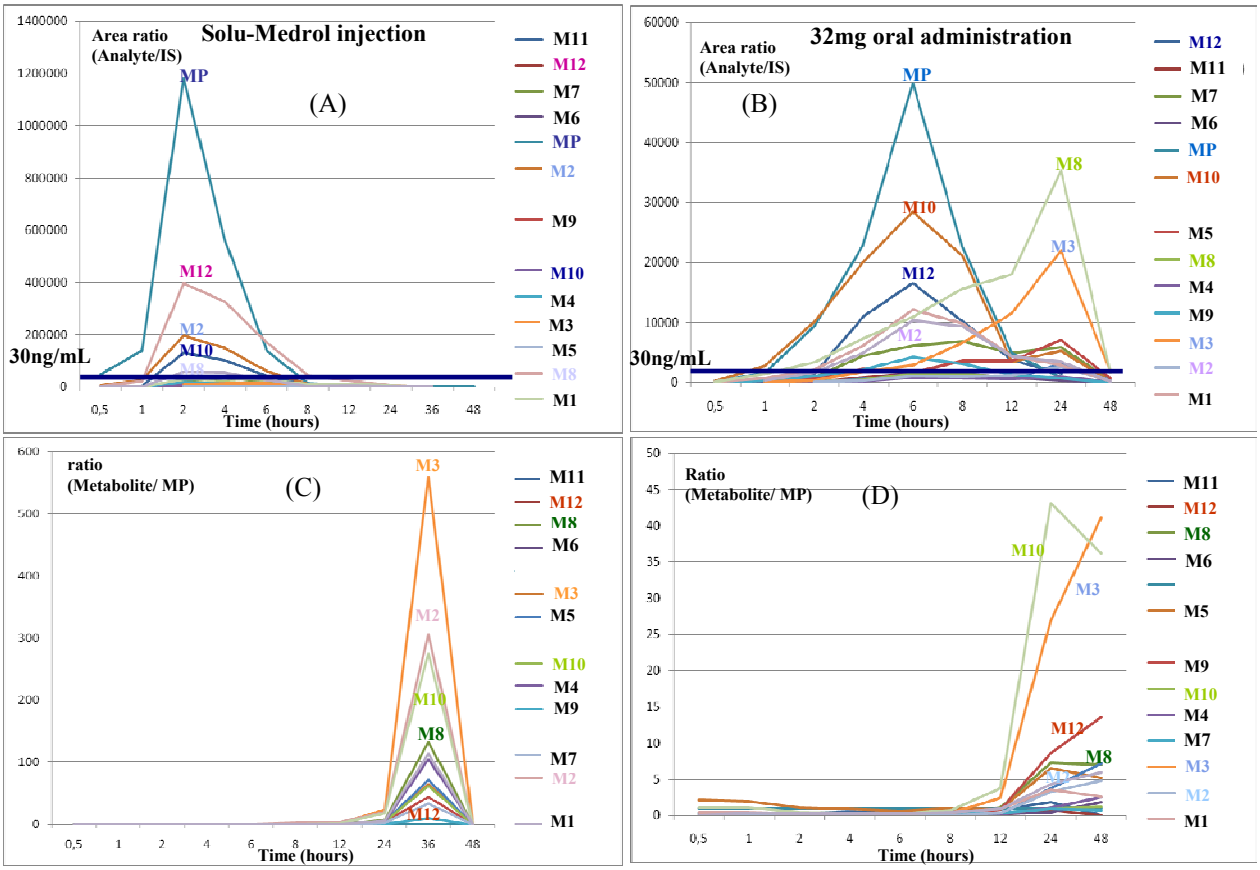

Figure 2: Excretion of profiles of MP metabolites after IM (A) and oral (B) administration and (Metabolites/ MP) ratios after IM (C) and oral (D) administration.