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Application of Liquid chromatography tandem mass spectrometry for the detection of ten long-term metabolites of Prednisolone

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Introduction:

The use of synthetic corticosteroids for treating diseases is prevalent since 1940s. The misuse of glucocorticosteroids in sports has indeed been recognized since 1975 and their use is banned in sports since 1986. Prednisolone (pregna-1, 4-diene-11- β , 17, 21-triol-3, 20-diol) is a potent synthetic glucocorticosteroid mainly used for its anti-inflammatory and immunosuppressive actions. It is readily metabolized in the first pass liver metabolism. There is no adequate method for screening the large numbers of metabolic products implicated. Prednisolone is metabolized to a large extent in to various hydroxylated metabolites. However, detection of prednisolone after various routes of administration is reported earlier. (1-3)

The purpose of the present work was to explore the possibility of identification of possible metabolites of Prednisolone in excretion study samples after oral administration of drug in different dosage to healthy volunteers. The work explicates the HPLC-MS/MS method for the qualitative identification of long term metabolites of Prednisolone in human urine. Structural assignments of metabolites were based on changes in molecular masses and retention times.

Experimental:

Reference Standards: Reference Standards of Prednisolone and Prednisone were purchased from Sigma-Aldrich (Germany) and 20- β -OH-prednisolone was a gift from Rome-Italy lab.

Instrumentation: Agilent 1100 series, HPLC coupled with an API 3200TM tandem mass spectrometer.

Column: Inertsil[®] C-18 ODS-3 (3.0 μ m, 50 mm x 4.6 mm), Ionization mode: ESI positive, IS Voltage: 5500 V, IS Temperature: 550 °C.

Drug administration and excretion study: Three healthy male volunteers (25 \pm 2 years, 70 \pm 5 kg) gave their informed consent to participate in the study. The study protocol was reviewed

and approved by the ethical committee of NDTL, India. Prednisolone was administered orally in three different dosage viz. 10mg, 20mg and 40mg and urine samples collected up to 72 hours and immediately frozen at -20°C .

Method validation: The Analytical method was validated as per the requirement of WADA ISL (version 6.0) keeping in view sensitivity, recovery, accuracy, precision, linearity, specificity, reproducibility, and repeatability. ⁽⁴⁾

Result and Discussion:

In this study, it was possible to identify ten urinary metabolites of prednisolone namely prednisone (11-oxo metabolite) [M-1], 6- β -OH-Prednisolone [M-2], 20- β -OH-Prednisolone [M-3], 20- α -OH-Prednisolone [M-4], 20- α -OH-Prednisone [M-5], 20- β -OH-Prednisone [M-6], 2 epimers of 20- β -OH-prednisolone (5- α -20- β -tetrahydroprednisolone & 5- β -20- β -tetrahydroprednisolone) [M-7], 2 epimers of 20- α -OH-prednisolone (5- α -20- α -tetrahydroprednisolone & 5- β -20- α -tetrahydroprednisolone) [M-8], 2 epimers of 20- β -OH-prednisone (5- α - 20- β -tetrahydroprednisone & 5- β - 20- β -tetrahydroprednisone) [M-9], 2 epimers of 20- α -OH-prednisone (5- α -20- α -tetrahydroxyprednisone & 5- β -20- α -tetrahydroprednisone) [M-10]. The corresponding retention times, MS and MS/MS spectra were used to obtain structural information.

The analysis has shown that there is no formation of adduct ions with formic acid from mobile phase and the compounds are characterized by $(\text{M}+\text{H}^+)$ ion. M-1 formed due to the C-11 hydroxyl group oxidation of parent gives the parent ion 359. M-2 is formed due to the hydroxylation of parent drug at C-6 position, giving 377 as the parent ion. The metabolites M-3 and M-4, formed due to reduction of C=O bond at C-20 position in the parent drug are detected as epimers and give the parent ion 363. Similarly, the metabolites M-5 and M-6 are the epimers of M-1 with reduced C=O bond at C-20 position giving 360 as the parent ion. M-7 and M-8 are the metabolite formed due to the hydrogenation of C-5 atom of M-3 and M-4 respectively. Both M-7 and M-8 at gives the parent ion 365. Similarly, M-9 and M-10 are the metabolite formed due to the hydrogenation of C-5 atom of M-6 and M-5 respectively giving 363 as their parent ion. The last four metabolites M-7, M-8, M-9, and M-10 are the epimeric pairs which could not be resolved in our chromatographic system.

The urine samples of volunteers after different dosage of drugs showed that all the above stated metabolites could be detected up to 24 hrs. However, of all the ten detectable metabolites M-1, M-3, M-4, M-5 and M-6 are the metabolites which show maximum abundance in 24 hrs. (Figure-1)

Conclusion:

The study made possible, to identify ten urinary metabolites of prednisolone after oral administration of drug at different dosages. The study explores newer metabolite of the drug which can further be used for the confirmatory purposes.

References:

1. Deventer, K, Delbeke, FT. (2004) Excretion studies with Corticosteroids. In: Schänzer W, Geyer H, Gotzmann A, Mareck U. (eds.) *Recent Advances in Doping Analysis* (12), Köln, pp 37-44.
2. Mazzarino, M, Bragano, M.C, Garribba, F, Rossi, F, Botre, F. (2005) The idea of a threshold value for synthetic glucocorticoids: urinary excretion studies following different routes of administration. In: Schänzer W, Geyer H, Gotzmann A, Mareck U. (eds.) *Recent Advances in Doping Analysis* (13), Köln, pp 465-468.
3. Kraiem, H, Oueslati, F, Amor SSB, Fenina. N, Loueslati. H. (2008) Structural elucidation of seven prednisolone metabolites by LC-ESI-MS/MS using a PGC column. In: Schänzer W, Geyer H, Gotzmann A, Mareck U. (eds.) *Recent Advances in Doping Analysis* (16), Köln, pp 379-382.
4. The World Anti Doping Code. WADA International Standard for Laboratories Version 6.0 (2009) http://www.wada-ama.org/rtecontent/document/International_Standard_for_Laboratories_v6_0_January_2009.pdf (access date: 15.01.09)

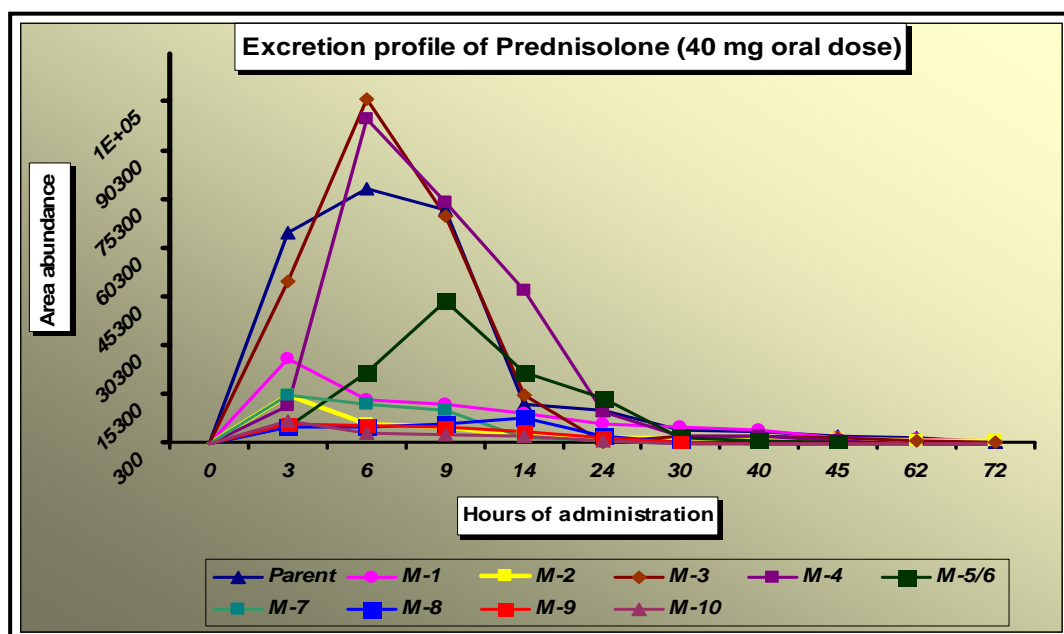


Figure 1: Excretion profile of prednisolone showing parent and 10 metabolites in 72 hours