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Metabolite identification and excretion profile of deflazacort on ultra performance liquid chromatography-tandem mass spectrometry

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Introduction

The use of glucocorticosteroids in sports is banned World Anti Doping Agency (WADA) by due to their anti inflammatory effect. Deflazacort (11 β, 16 β)-21-(acetoxyl)-11-hydroxy-2'-methyl-5'H-pregna-1, 4-dieno [17, 16-d] oxazole-3, 20-dione) is recently included in the WADA banned list of glucocorticosteroids in 2010. [1] Deflazacort, is the oxazoline derivative of synthetic corticosteroid prednisolone-21-acetate. It is an inactive prodrug which rapidly converts to its active metabolite 21-des acetyl deflazacort. [2] The objective of the present work was to develop a fast, sensitive and specific UPLC-MS/MS method for the detection of maximum possible metabolites of deflazacort and to study their excretion profile so as to identify the marker metabolite/s.

Materials and methods:

The samples were prepared using enzymatic hydrolysis and liquid- liquid extraction [3] and analysis was performed on Waters Acquity TM Ultra performance LC and an API 4000 TM tandem mass spectrometer (Table-1). The Analytical method was developed and validated as per the requirement of WADA ISL (version 6.0) keeping in view sensitivity, recovery, accuracy, precision, linearity, specificity, reproducibility, and repeatability. (4) The excretion study was performed by administering drug to three healthy male volunteers (25±2 years, 70±5 kg) with their informed consent to participate in the study. The study protocol was reviewed and approved by the ethical committee of NDTL, India. The drug (Deflazacort , Defcort, Macleods Pharma, Ltd) was given in a dose of 12 mg orally and urine samples were collected up to 72 hours and immediately frozen at -20°C.

Result and Discussion:

Method Development

Based on the molecular weight, full scan positive ion mass spectra were obtained for the possible metabolites. The unchanged parent drug deflazacort could not be detected as it deaceylates rapidly into its active compound desacetyl deflazacort [M-1] yielding precursor ion *m/z* 400 in positive ionization mode. Desacetyl 6-β hydroxy deflazacort [M-2] and 3-OH-21 desacetyl deflazacort [M-3] were also detected giving the precursor ion *m/z* 416 and 402 respectively. (Figure-1)

Based on the results, the mass spectrometer was operated in product ion mode. The fragmentation patterns of all three analytes contained typical fragments of steroids with androst-1, 4-diene-3-one structure. [(2)] The mass spectrometer was operated in the Multiple Reaction Monitoring Mode (MRM) for identification of all the three metabolites. (Table-2) All the three metabolites were eluted within 5 minutes of runtime. No significant interference from matrices at the retention times of the targeted ion transitions was observed. The calibration curve for desacetyl deflazacort was found to be linear in range from 15-120 ng/ml and the coefficient of regression (r^2) was 0.9996. The percentage recovery at 30 ng/ml ranged from 82.9 % to 87.8 %. The method showed good precision and accuracy.

Excretion Study

The excretion study profile of all the metabolites in four healthy volunteers shows that M-1 was detectable up to 36 hours, M-2 was detectable up to 30 hours whereas, M-3 was detectable up to 48 hours. (Figure-2) All the three metabolites could be identified in excretion study sample received from World Association of Anti Doping Scientists (WAADS) (Figure-3). Our study shows detection of M-3 as an additional metabolite iIn comparison to earlier work of Bredehoft *et al* [2], which can be used as a long-term marker for deflazacort abuse.

Hence, the present study shows a fast and sensitive method for the identification of three metabolites of deflazacort, out of which 3-OH-21 desacetyl deflazacort [M-3] is the additional metabolite not reported earlier which can be used as a long-term marker for deflazacort abuse.

References:

1. The World Anti Doping Code. The 2010 prohibited list international standard. Available online at http://www.wada-ama.org/rtecontent/document/list_2010.pdf
2. Bredehoft M, Thevis M, Schanzer W. (2009) "Deflazacort and its main metabolites-preparation and analysis using LC-ESI-MS/MS" " Recent advances In Doping Analysis, Sport und Buch Strauß, Koln, 2009, 17
3. Reddy M.I., Beotra A., Jain S., Ahi S. (2009) "A simple and rapid ESI-LC-MS/MS method for simultaneous screening of doping agents in urine samples" Ind. J. Pharm., 41, 80-86
4. The World Anti Doping Code. WADA International Standard for Laboratories Version 6.0. Available online at http://www.wada-ama.org/rtecontent/document/International_Standard_for_Laboratories_v6_0_January_2009.pdf

Table-1Analytical parameters

UPLC	**Waters Acquity TM Ultra performance LC**
Column	Acquity UPLC BEH C-18 (1.7 µm, 100 mm x 2.1 mm)
Solvent A	1% aqueous solution of formic acid
Solvent B	Acetonitrile
Flow	300 µl/minute
Run Time	5 Minutes
Injection volume	5 µl
Mass Spectrometer	**Sciex API 4000, triple quadruple Mass Spectrometer QTrap**
Ionization	ESI Positive
Collision Gas	Nitrogen
Source Temperature	550°C

Table-2 Mass Spectrometric Details of the metabolites

Name	**Molecular Weight**	**Parent Ion (M+H+)**	**Product Ion**
21 desacetyldeflazacort [M-1]	399	400	124, 121, 147,
6-β hydroxy deflazacort [M-2]	415	416	121, 124, 171
3-OH-21 desacetyl deflazacort [M-3]	401	402	121, 304, 282

Figure-1 Molecular Structure of Deflazacort metabolite

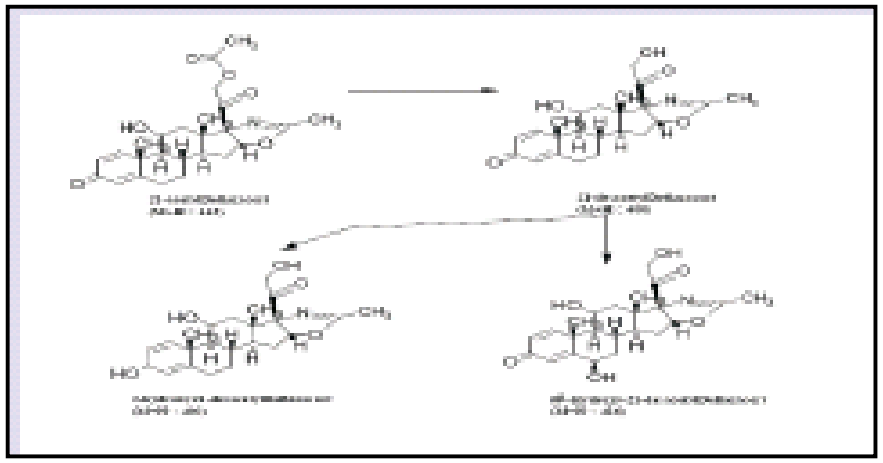

Figure 2- Excretion study profile of deflazacort metabolites

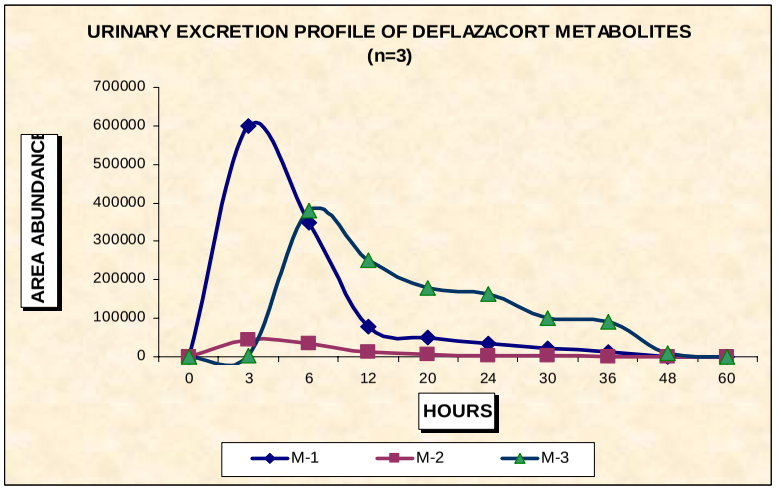

Figure-3 Identification of three metabolites of Deflazacort in WAADS Sample