Excretion Study of Methylprednisolone using HPLC-MS Ion Trap

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Synthetic corticosteroid methylprednisolone (MP; 11ß, 17α,21-trihydroxy-6α-methylpregna-1,4-diene-3,20-dione) is a widely used anti-inflammatory drug. Corticosteroids are prohibited by the World Anti Doping Agency in competition, and their HPLC-MS detection is a must for accredited laboratory. Nowadays HPLC-MS technique is a method of choice. In former times, before HPLC-MS era, the metabolites of MP in humans have been investigated in Moscow Antidoping Centre [1] using capillary gas chromatography – EI mass spectrometry (GC-MS) and methoxyamine-trimethylsilyl (MO-TMS) derivatization. After 19 years excretion study have been repeated to confirm the previous findings.

Sample work-up protocol included enzymatic hydrolysis followed by liquid-liquid extraction with mixture of diethyl ether/toluene 50/50. HPLC Separation was performed using Restek Ultra C18 column, 100 × 2.1 mm I.D., 5 µm, connected to a guard column 4 × 12,5 mm The mobile phase was 0.05% formic acid (A) – acetonitrile (B) at a flow rate of 0.2 ml/min. The gradient was as following: 0 min – 15% B; 10 min – 60% B; 15 min – 75% B; 25 min – 85% B.

Eight mg dose of MP was administrated orally. The urine steroids profiles before and after administration of MP were studied using Agilent 1100 HPLC-ESI(-)/MS Ion Trap SL (Superior Line) System. Following 3 hours after administration the amount of natural corticosteroids decreased significantly, and maximal concentration of MP was observed. Parent MP is excreted from human organism during 36 hours. Major metabolites were 6,7-dehydro-MP and 20-hydroxy-MP as was previously reported [1]. For screening purposes and confirmation major metabolites and unchanged MP are monitored.

REFERENCES:

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