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Investigation on the rearrangement of triamcinolone

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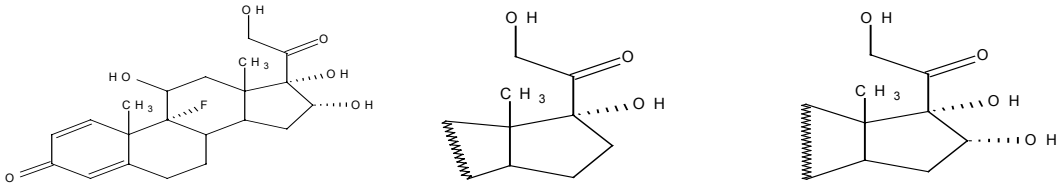
Introduction: Triamcinolone (9α-fluoro-11β-16α,17α,21-tetrahydroxypregna-1,4-diene-3,20-dione) is a synthetic corticosteroid (figure-1) can be used in certain sports to improve the performances of athletes. Administration of this substance per general route is prohibited by the WADA, while its use under local presentations is subjected to restrictions [1].



Triamcinolone 17-hydroxy-20-keto 16,17-dihydroxy-20-keto

Figure-1: Triamcinolone, 17-hydroxy-20-keto, 16,17-dihydroxy-20-keto steroids structures

Degradation studies of corticsteroids reported that D-ring acyloin rearrengement (D-Homo-annulation) of 17-hydroxy-20-keto steroids occured under both base and Lewis-acid catalysis (Figure-1). Moreever, Steroids possessing the 16,17-dihydroxy-20-keto moiety (Figure-1) are known to be more prone to rearrangement than 17-hydroxy-20-keto steroids. Triamcinolone, a representative of 16,17-dihydroxy-20-keto steroids, showed highly sensitivity to rearrangement catalyzed by traces of certain transition-metal and by dilute aqueous bases, leading to two products that were isolated and identificated by NMR spectroscopy [2,3]. However, none of these studies describe the rearrangement and/or transformation of triamcinolone in the organic solvents.

In the present study, we investigate the behavior of the triamcinolone in different organic solvents by HPLC–DAD, LC-MS/MS using a porous graphitic carbon column. This investigation shows that the pathway of transformation of triamcinolone is the same with the used organic solvents.

Working solution: Solutions were prepared in amber containers to protect triamcinolone from light degradation which were made by accurately weighing 1 mg of Triamcinolone standard and transferring the powder into 10 mL amber volumetric vial which was made up to the mark with methanol, ethanol, acetonitrile, acetone, propanol, acetic acid to give the final concentrations of 100 µg/mL and placed for one week at room temperature.

LC/MS/MS parameters: MS and MS/MS analysis were performed on a Quattromicro triple quadrupole mass spectrometer (Micromass, Manchester, UK), operating in the positive (ESI+) and negative (ESI−) electrospray ionization mode. Nitrogen was used at flow rates of 90 and 300 L. h^{-1}, respectively. Source and desolvation temperatures were set at 120 and 400 ◦C, respectively. Argon was used as the collision gas for collision-induced dissociation at a pressure of 5×10^{-4} mbar. The HPLC Agilent 1100 Series (Agilent Technologies, USA) was equipped with G1311A quaternary pump, G1379A degasser, G1313A autosampler with a G1316A column oven. Separations were obtained under isocratic conditions using a Hypercarb column (100 mm ×2.1 mm, 5µm) (Hypersil, Runcorn, England) with a guard column (XDB, 10 mm×4.6 mm, 5µm), (Agilent, USA). The mobile phase consisted of acetonitrile/water [1% acetic acid] (85/15) and acetonitrile/Ammonium Formate [5 mM, pH=3.5] (85/15). The flow rate was 300 µl. min^{-1} and the injected volume was 10 µL.

Results and discussion: The ion chromatogram obtained using positive electrospray in scan mode at cone voltage 15V, for a fresh preparation of triamcinolone in methanolic solution (Figure-2), shows one peak at t_R =5.8min.The corresponding DAD spectrum shows a maxima at 238 nm. Furthermore, the mass spectrum gives five fragement at m/z=393, 375, 357, 339 and 321 corresponding to $[M+H]^+$, $[M+H-HF]^+$, $[M+H -HF-H_2O]^+$, $[M+H -HF-2H_2O]^+$ and $[M+H-HF -3H_2O]^+$ respectively. The TIC obtained for the methanolic solution stored for one week at room temperature (figure -3) shows two resolved peaks which appears at t_R = 3.95 min and at t_R = 5.8 min. The diode array and mass spectra of the peak apearing at t_R = 3.95 min were qualitatively similar to those of triamcinolone. The difference lies only in the intensity of absorbance and in the abundance. In addition, the daughter ion chromatogram of the methanolic solution of triamcinolone (stored for one week at ambiant temperature) in positive and negative electrospray were carried out. The selected daughter ion m/z= 395 in positive mode and m/z =453= $[M+Acetate]^-$, m/z =393= $[M-H]^-$, m/z =363= $[M-H- CH_2O]^-$ in negative mode were illustrated in figure-5a, 5b and 5c. The obtained daughter ion spectra of the unknown substance eluted at 3.95 min were comparably to those of triamcinolone. This finding argues that triamcinolone undergoes rearrangement in methanolic solution giving a new product with the same spectrum and fragmentation ions. The same observation was

made whenever the triamcinolone was dessolved in protic solvent such as ethanol, propanol, isopropanol and acetic acid (Figure-6). The rate of transformation of triamcinolone depends on the carbon chain length of the alcohol. In fact, the rate of rearrangement of triamcinolone increase when the polarity of alcohol increase. Howerer, this transformation was not observed in aprotic solvant, such as acetone, acetronitrile and dimethyformamide (Figure-7).

Conclusion: This investigation clearly shows that triamcinolone undergoes rearrangement and points out the role of solvent in its stability in solution. It appears that the use of acetone, acetonitrile and dimethylformamide is recomended for better stability of triamcinolone.

Finnally the identification of the rearrangement product will be useful for the elucidation of the mechanism of this transformation.

References:

[1]: The WADA Antidoping Code: The 2005 prohibited List international Standard, Available on line at hptt://www.wada-ama.org/rtecontent/document/list_2005.pdf.

[2]: L. S. Leland, E. L. Ezell, Steroids., 53, 3-5, 1989.

[3]: E. J. Delaney, R. G. Sherrill, V. Palaniswamy, T. C. Sedergran, Steroids., 59, 3, 1994.

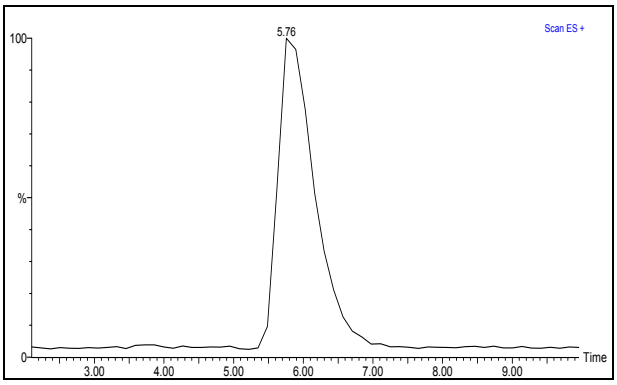


Figure -2: Ion scan chromatogram in ES+ of fresh preparation triamcinolone in methanolic solution with Acetonitrile-Ammonium formate [5mM, pH=3.5] as mobile phase at flow rate of 0.3 mL/min.

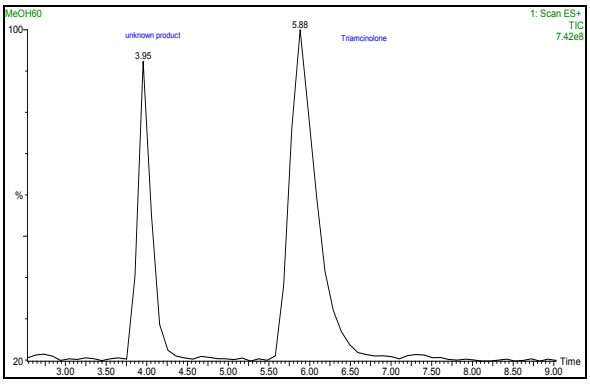


Figure -3: Ion scan chromatogram in ES+ of triamcinolone in methanolic solution stored for one week at ambiant temperature with acetonitrile-ammonium formate [5mM, pH=3.5] as mobile phase at flow rate of 0.3 mL/min.

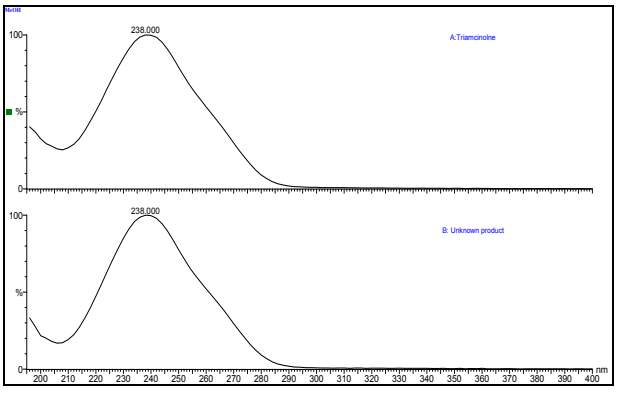


Figure-4a: DAD spectra of: (A) Triamcinolone [t_R= 5.8min], (B) Unknown product [t_R= 3.95 min].

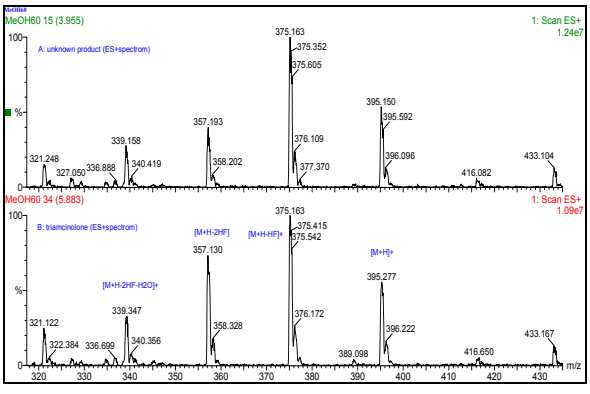


Figure-4b: (MS, ES+) spectrum of: (A) Triamcinolone [t_R= 5.8min], (B) unknown product [t_R = 3.95 min].

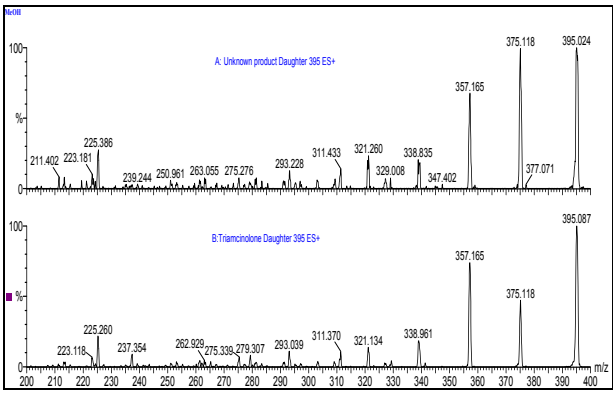


Figure-5a: Daughters ions spectrums {m/z= 395=$[M+H]^+$} in ES+ [Cone voltage =18 V and Collision energy=15eV] of : (A) unknown product [t_R =3.95 min], (B) Triamcinolone [t_R=6.8min] with Acetonitrile- ammonium Formate[5mM, pH=3,5] (85:15)as mobile phase at flow rate 0.3mL/min.

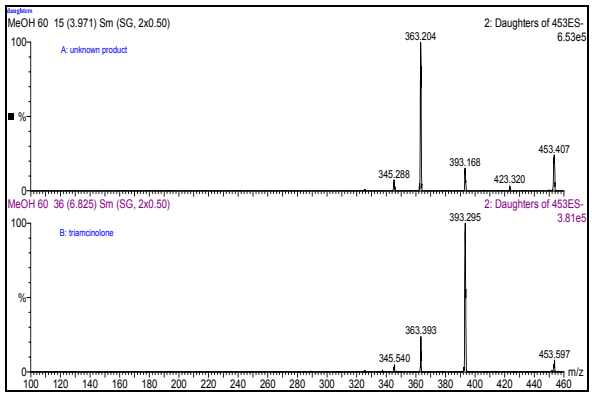


Figure-5b: Daughters ions spectrums {m/z=453=[M+Acetate]$^-$} in ES- [Cone voltage=15 V and Collision energy= 12 eV] of: (A) unknown product [t_R= 3.95min], (B) Triamcinolone [t_R= 6.8min] with Acetonitrile- Acetic acid 1% (85:15) as mobile phase at flow rate of 0.3mL/min.

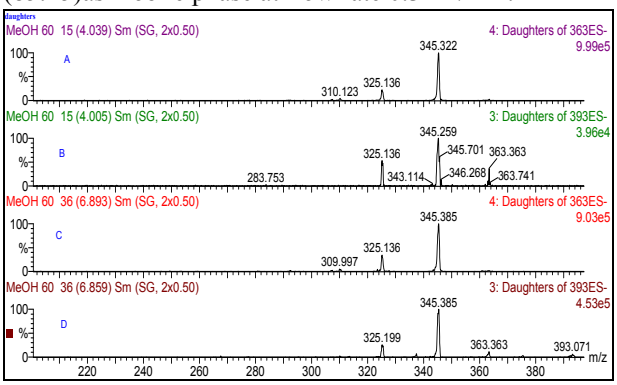


Figure -5c: Daughters ions spectrums {m/z= 393= $[M-H]^-$, m/z= 363= $[M-H-CH_2O]^-$} in ES- [Cone voltage= 20 V and Collision energy=20 eV] of: (A) and (B) unknown product [t_R = 3.95 min], (C) and (D) Triamcinolone [t_R = 5.8min] with Acetonitrile- Acetic acid 1% (85:15) as mobile phase at flow rate of 0.3mL/min.

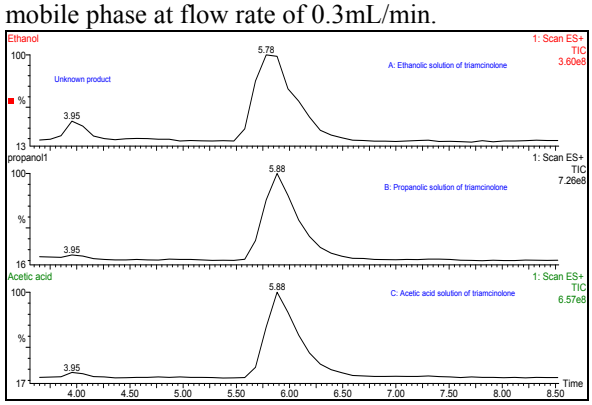


Figure -6: Ion scan chromatogram in ES+ of triamcinolone in (A) Ethanol, (B) Propanol and (C) Acetic Acid solution stored for one week at ambiante temperature with Acetonitrile- Ammonium formate [5mM, pH=3.5] as mobile phase at flow rate of 0.3 mL/min.

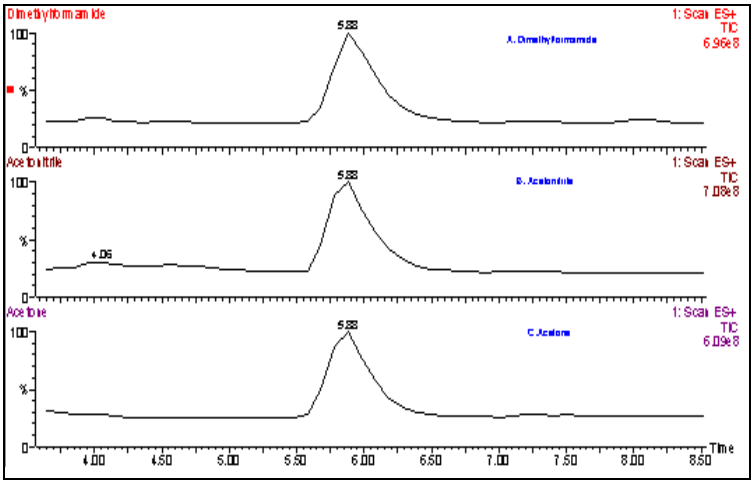


Figure-7: Ion scan chromatogram in ES+ of triamcinolone in (A) Dimethylformamide, (B) Acetonitrile and (C) Acetone solution stored for one week at ambiente temperature with acetonitrile-ammonium formate [5mM, pH=3.5] as mobile phase at flow rate of 0.3 mL/min.