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Stability study of 3'-hydroxystanozolol glucuronide

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Abstract

One representative target compound for the detection of stanozol misuse is 3´-hydroxystanozolol glucuronide. An accelerated stability study along 62 days at 37°C was performed. Three different storage conditions were evaluated: two aqueous environments (pH 0.5 and 5.2) and one organic solvent (methanol). This study reveals that this commercially available compound is in general terms stable in solution. A graphical representation of the logarithmic areas as a function of time and the determination of the half-life for every storage conditions allows us to understand the behavior of 3´-hydroxystanozolol glucuronide in methanol, acetate buffer (pH 5.2) and hydrochloric acid 0.1M (pH 0.5).

Introduction

Stanozolol is still one of the most frequently detected anabolic steroids in doping control samples [1]. Stanozolol is intensively metabolized [2,3] and, in humans, the main metabolic products excreted in urine as glucuronidated conjugates are 3´-hydroxystanozolol, 4ß-hydroxystonozolol, 16ß-hydroxystanozolol and 4,16-dihydroxystanozolol. Among all these metabolites, 3´-hydroxystanozolol glucuronide (3STANG) has been described as a good target compound for the long-term detection of stanozolol [4] and is commercially available.

Experimental

Storage Conditions
Three different storage conditions were evaluated: 2 aqueous (acetate buffer pH 5.2 and hydrochloric acid 0.1M pH 0.5) and the organic solvent methanol. The starting-volume of every solution was 5 mL. 3STANG was spiked at 1 µg/mL and the solutions were incubated at 37°C.

Sample Preparation
From every solution, 33 samples (aliquots of 100 µL for every solution) were taken during 62 days. Samples were stored at -20°C until the experiment was finished. Then, the samples were diluted with 100 µL of methanol (for aqueous solutions) or water (for the methanolic solution) and 20 ng of the internal standard (d3-epitestosterone glucuronide, EpiTGd3) was added.

Instrumentation
The liquid chromatographic system consist of a Finnigan Surveyor HPLC system which is interfaced to a TSQ Quantum Discovery MAX triple quadrupole mass spectrometer (Thermo, San Jose, USA) using the electrospray ionization (ESI) interface. Separation was performed on a Sunfire C_{18} column (2.1 mm x 50 mm, 3.5µm, Waters, Milford, MA, USA) at a flow rate of 250 µl/min. The temperature of the autosampler and column oven were 15°C and 35°C respectively. The aqueous and methanolic mobile phases consisted both of 1mM ammonium acetate and 0.1% acetic acid. The percentage of organic solvent on the gradient program was modified as follows: 0 min, 40%; 0.5 min, 40%; 2 min, 55%; 8.9 min 70%; 9.0 min, 100%; 10 min, 100%; 10.10 min, 40%; 14 min, 40%. Nitrogen was used as sheath gas and auxiliary gas at flow rates of 50 and 20 arbitrary units, respectively. Sample ionization was carried out in positive mode ESI using spray voltages of 4500 V. The capillary temperature was set at 350°C. The collision gas was argon (Air Liquide, Desteldonk, Belgium) with a collision gas pressure of 1.5 mTorr. In Table 1, the mass spectrometric settings are displayed.

ISBN 978-3-86884-039-1

Compound	Ionization Mode	Ion Transition	Collision Energy (eV)	Tube Lens (V)	Retention Time (min)
3STANG	ESI+	521.4/345.3	40	107	8.82
EpiTGd3	ESI+	468.0/97.0	30	110	8.51

Table 1: Mass spectrometric settings

Results and Discussion

Knowledge on the stability of doping compounds is of utmost importance to ensure appropriate storage of the samples and thus, identification and quantification of the analytes. For this reason stability of several categories of doping agents has been investigated [5-7]. However only a little information on stability of glucuronidated steroids is available.The variation in relative signal intensity with time provides a detailed description of how fast is the degradation of 3STANG in different solvent environments. The obtained results for 3STANG are presented in Figure 1. Linear curves were fitted through the logarithmic areas according to $\log(A_t) = \log(A_{t0}) - 0{,}43k*t$, where $\log(A_t)$ denotes the area at time "t" among subjects with initial area (A_{t0}), with k = degradation speed; t_0= start time of the degradation experiment. The half-life ($t_{1/2}$) provides a measure of the degradation reaction rate. The calculation of the half-life ($t_{1/2}$) from the curves showed that in methanol, which resembles storage conditions of the standard, 153 days at 37°C are required to reduce the initial concentration by 50%. In acetate buffer, resembling physiological pH value of urine, more than double the time, 376 days, are required. As expected, the fastest degradation was observed at pH 0.5. However, even in this extreme condition the half-life was 15 days.

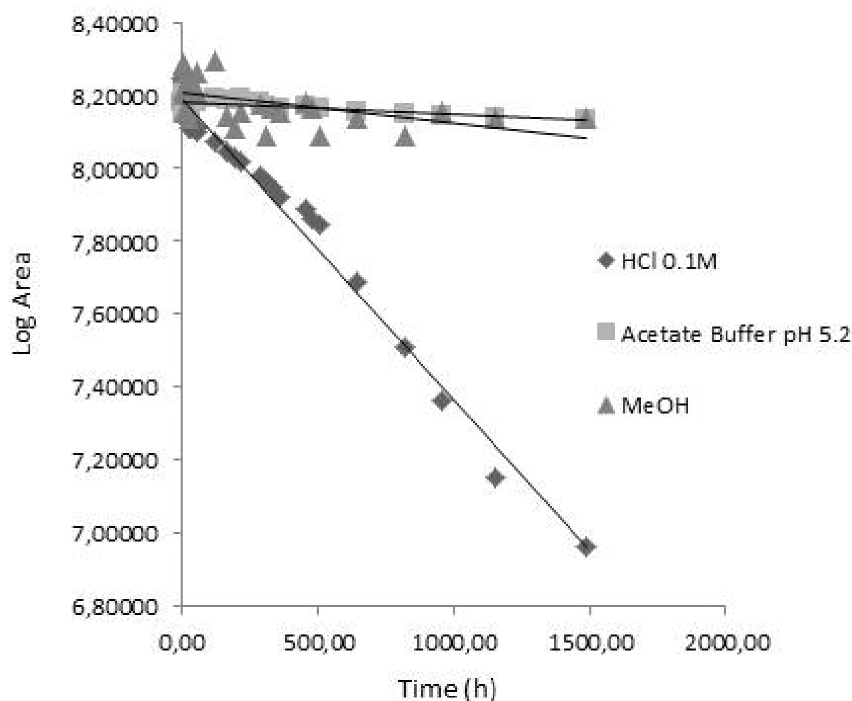

Figure 1. Degradation curves for 3STANG in HCl 0.1M, acetate buffer pH 5.2 and methanol

 ISBN 978-3-86884-039-1

Conclusions

It can be concluded that 3STANG is a stable compound. Even in the strong acidic solution, 15 days are needed to decrease the concentration to half of the original level. Taking into account that the stability study was performed at elevated temperature (37°C), long term storage of standards/samples refrigerated or frozen should guarantee the stability of 3STANG.

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Acknowledgements

Financial support of WADA (grant 11A17PV).

 ISBN 978-3-86884-039-1