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Axiron – a transdermal testosterone administration case study – steroid profiling & GC/C/IRMS

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Abstract

Traditional steroid profiling parameters have been shown to be limited in their ability to detect the administration of transdermal testosterone gels and creams.
Axiron is a recent transdermal pharmaceutical testosterone preparation for the treatment of testosterone deficiency in men. This case study looks at the steroid profiling and GC/C/IRMS results for a multiple dose administration of Axiron (60 mg per day) to a single volunteer over a four day period.
In this case study, the steroid profiling results were reviewed using the ABP steroid passport. This led to all samples from the 3rd day of administration onwards being identified for further investigation by GC/C/IRMS. The analysis of these samples by GC/C/IRMS would have resulted in an AAF according to positivity criteria v. from the now effective WADA technical document for GC/C/IRMS [8].

Introduction

Traditional steroid profiling parameters have been shown to be limited in their ability to detect the administration of transdermal testosterone gels and creams. This is particularly true in individuals whose T/E ratio is naturally low. With the implementation of the ABP steroid passport, it is anticipated that the administration of endogenous substances will be easier to detect [1-3].
Axiron is a recent pharmaceutical testosterone preparation for the treatment of testosterone deficiency in men. The preparation is in liquid form and is applied (via an applicator) to the underarms. A starting daily dose of 60 mg of testosterone per day is recommended. This is achieved by applying one 30 mg “pump” to each underarm. Steady state serum concentrations should be achieved within 14 days of daily dosing [4].

Experimental

This single subject case study looks at the steroid profiling, ABP steroid passport analysis and GC/C/IRMS results for a multiple dose administration of Axiron over a four day period.
A healthy male volunteer (age >60) was administered a daily dose of 60 mg of Axiron for three days. Four urine samples were collected prior to the administration, then all spontaneous urines (10) were collected during the administration period. All urine samples were stored frozen prior to steroid profiling and GC/C/IRMS analysis.
Steroid profiling was performed using the routine procedure applied to all doping control samples in the laboratory [5] (profile components – Testosterone (T), Epitestosterone (E), Androsterone (A), Etiocholanolone (Et), 5α-androstan-3α,17β-diol (5αdiol), 5β-androstan-3α,17β-diol (5βdiol), 5β-pregnan-3α,20α-diol (PD)). pH, sg and LH were also measured. After profiling, all samples were submitted for GC/C/IRMS analysis using a method adapted from Van Renterghem et al [6]. The UGT2B17 genotype of the volunteer was not determined.

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Steroid Profiling Extraction Procedure

- Hydrolyis (β-glucuronidase)
- SPE
- Dry
- Derivitisation (MSTFA/NH_4I/ethanethiol)
- GCMS analysis

GC/C/IRMS Extraction Procedure

- SPE
- Hydrolyis (β-glucuronidase)
- LLE (TBME)
- Derivitisation (Ac_2O/Pyr)
- HPLC
- F1, F2 & F3 collected
- GCMS analysis
- GC/C/IRMS analysis

Figure 1: Summary of the extraction procedures used for steroid profiling and GC/C/IRMS analysis.

Results and Discussion

GC/MS Steroid Profiling and ABP Analysis:

In this case study, none of the steroid profile parameters exceed traditional population based reference limits. This is not unexpected with low dosage transdermal T administrations. However, with the introduction of the ABP steroid passport, it may be possible to identify a number of samples for further investigation [7-8].

Four blank urine samples were collected prior to the administration. These four urines and the first post-administration sample (which was negative by GC/C/IRMS analysis) were added to the ABP software (version 2.4.2) as the first five points of a longitudinal study. The other post-administration samples were then treated individually as the sixth point of the study to determine whether the results would fall outside the reference limits. 5αdiol/E and A/E ratios were plotted in the same way, but without upper and lower reference limits.

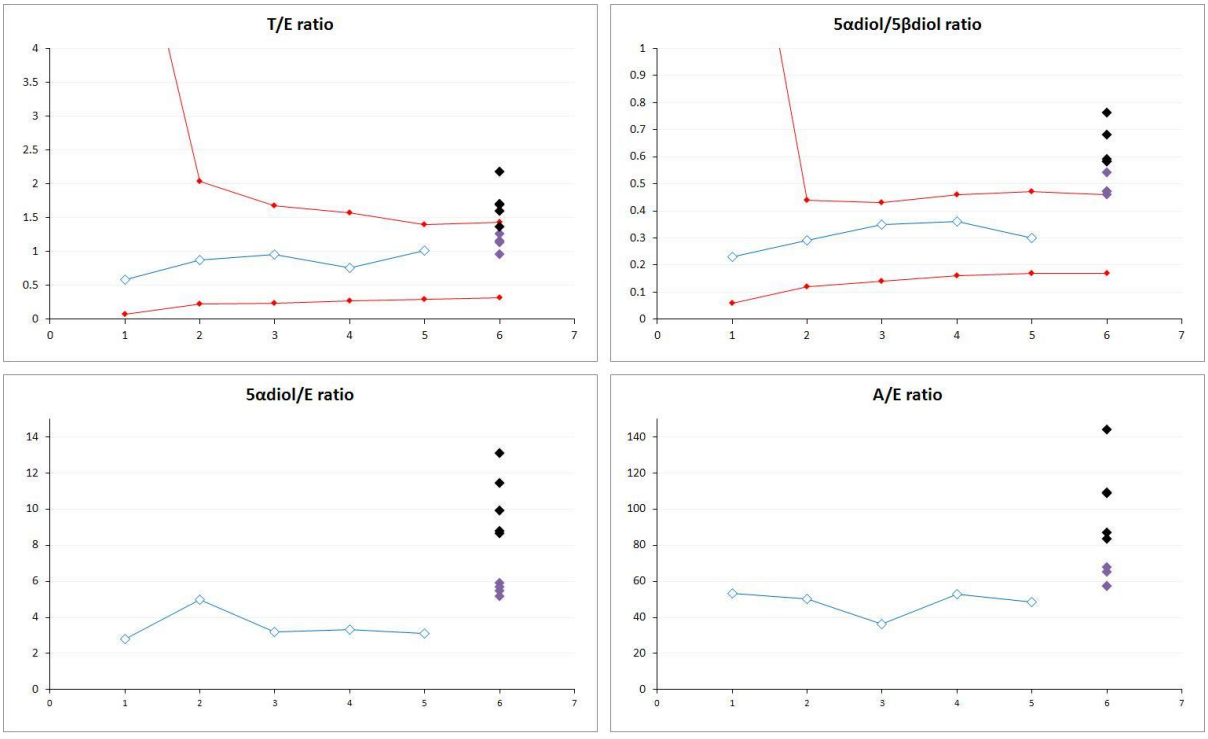

Figure 2: Steroid profiling ratios. Pre-admin (white), post-admin day 1 & 2 (grey), post-admin day 3 & 4 (black), reference limits (red).

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ABP steroid passport results for the day 1 and day 2 samples were not found to differ significantly from the blank urine samples. These samples would not have been identified for further analysis.
Day 3 and day 4 samples were found to have T/E, 5αdiol/5βdiol, 5αdiol/E and A/E ratios that were above the upper reference limits. These samples may have been identified for further analysis. They also demonstrate the usefulness of the 5αdiol/E and A/E ratios for the detection of transdermal T administration as previously highlighted by Geyer et al [2].

GC/C/IRMS Analysis:
$\delta^{13}C$ values for Et, A, 5αdiol, 5βdiol & PD were obtained after acetate correction [6]. Post- administration samples were easily distinguished (with the exception of day 1-1) from the pre-administration samples, with all samples meeting positivity criteria v. from the now effective WADA technical document for GC/C/IRMS [8]. It is expected that positivity criteria i. would have also been met if the subjects urinary T concentration was high enough to measure (Axiron's T δ value was measured at -28.5 ‰ [9]). Δ values appear to be typical of transdermal T administration in that the greatest change in δ value is to the 5α metabolites, particularly the 5αdiol.

Sample	GC-C-IRMS δ values (Ac-corrected)					Δ values				+ve
Description	Et	A	5βdiol	5αdiol	PD	PD-Et	PD-A	PD-5βdiol	PD-5αdiol	Criteria
Blank 1	-24.2	-24.1	-23.8	-24.8	-23.0	1.26	1.09	0.84	1.79	
Blank 2	-24.4	-24.5	-23.6	-23.9	-23.0	1.33	1.41	0.53	0.86	
Blank 3	-24.9	-23.6	-24.3	-24.6	-23.0	1.89	0.63	1.30	1.63	
Blank 4	-24.6	-23.4	-24.8	-24.4	-23.2	1.49	0.26	1.66	1.22	
Day 1 - 1	-24.2	-24.4	-24.3	-25.7	-23.0	1.16	1.44	1.29	2.71	
Day 1 - 2	-25.1	-26.7	-24.5	**-28.8**	-22.9	2.17	**3.80**	1.57	**5.88**	v, vi
Day 2 - 1	-25.7	-26.7	-25.9	**-27.7**	-23.4	2.34	**3.27**	2.48	**4.33**	v, vi
Day 2 - 2	-25.2	-26.7	-24.5	**-28.2**	-23.0	2.14	**3.66**	1.52	**5.20**	v, vi
Day 2 - 3	-26.0	-27.0	-24.2	**-28.3**	-22.6	**3.39**	**4.39**	1.60	**5.67**	v, vi
Day 3 - 1	-26.2	-26.7	-24.3	**-27.5**	-22.7	**3.55**	**4.06**	1.58	**4.82**	v, vi
Day 3 - 2	-26.6	-26.2	-24.8	**-27.9**	-22.8	**3.77**	**3.41**	1.98	**5.03**	v, vi
Day 3 - 3	-25.3	-27.2	-25.1	**-28.0**	-23.7	1.57	**3.50**	1.38	**4.27**	v, vi
Day 3 - 4	-26.0	-26.3	-25.5	-27.0	-23.3	2.73	**3.04**	2.18	**3.69**	v
Day 4 - 1	-25.6	-25.6	-25.4	**-27.4**	-22.7	2.90	**2.91**	2.74	**4.71**	v, vi

Table 1: GC/C/IRMS results for all samples, δ values and Δ values.

Sample description	Number of samples		
	Total	Identified by ABP	GC/C/IRMS AAF
Blanks	4	0	0
Day 1 & 2	5	0	4
Day 3 & 4	5	5	5

Table 2: Summary of sample numbers identified by ABP steroid passport analysis and resulting in a GC/C/IRMS AAF.

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Conclusions

• Axiron GC/C/IRMS results are similar to results published for other transdermal testosterone administrations.

• The ABP steroid passport is important for the detection of the administration of low doses of endogenous substances. In this case study, the use of testosterone could be detected with ABP steroid passport analysis (followed by GC/C/IRMS) from the 3rd day of administration onwards, given that pre-administration samples were tested.

• It is important to include both 5αdiol and T in routine GC/C/IRMS analysis when concentrations are sufficient for analysis. This is a requirement of the now effective WADA technical document for GC/C/IRMS [8].

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