

1 *Supplementary*

2 **Development of a derivatization method for**
3 **investigating testosterone and**
4 **dehydroepiandrosterone using tandem mass**
5 **spectrometry in saliva samples from young**
6 **professional soccer players pre- and post-training**

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18 **Supplemental material content:**

19 **Supplementary Figure 1.** Structures of derivatisation 2-Hydrazino-1-methylpyridine derivative
20 (HMP) with testosterone, dehydroepiandrosterone and epitestosterone.

21 **Supplementary Figure 2.** Optimisation of reaction conditions in derivatisation steroids with
22 HMP.

23 **Supplementary Figure 3.** Solid phase extraction cartridge selection.

24 **Supplementary Figure 4.** Matrix Effect.

25 **Supplementary Figure 5.** Linearity and calibration of standards spiked into saliva.

26 **Supplementary Figure 6.** Correlation between the concentrations of Sal-DHEA and Sal-T (n=20)
27 were (A) pre-training and (B) post-training.

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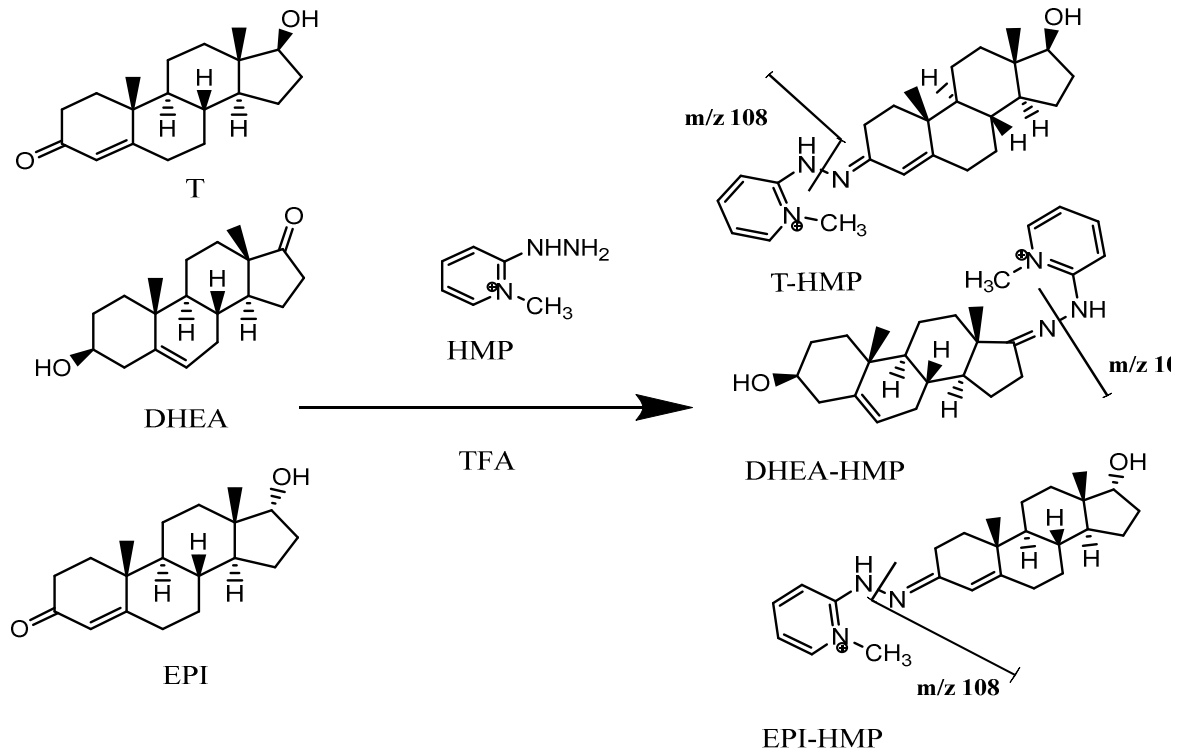
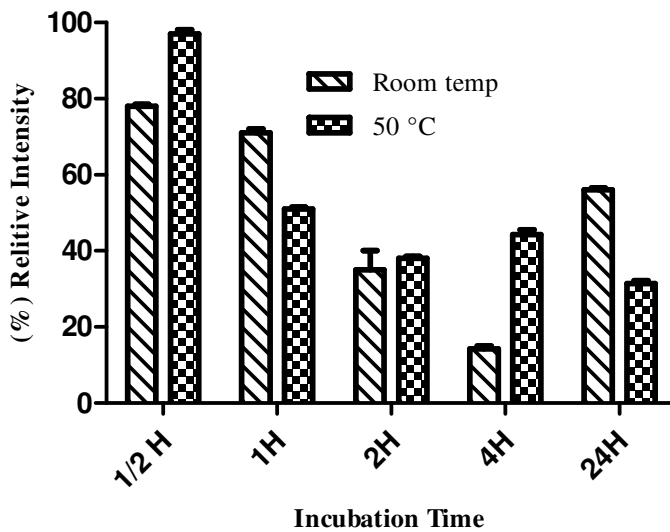


Fig. S1. The scheme shows the derivatisation of testosterone (T), dehydroepiandrosterone (DHEA) and epitestosterone (EPI) with 2-hydrazino-1-methylpyridine (HMP) in the presence of Trifluoroacetic acid (TFA).

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Fig S2. The yield of the HMP derivative of testosterone depending on reaction temperature and time.

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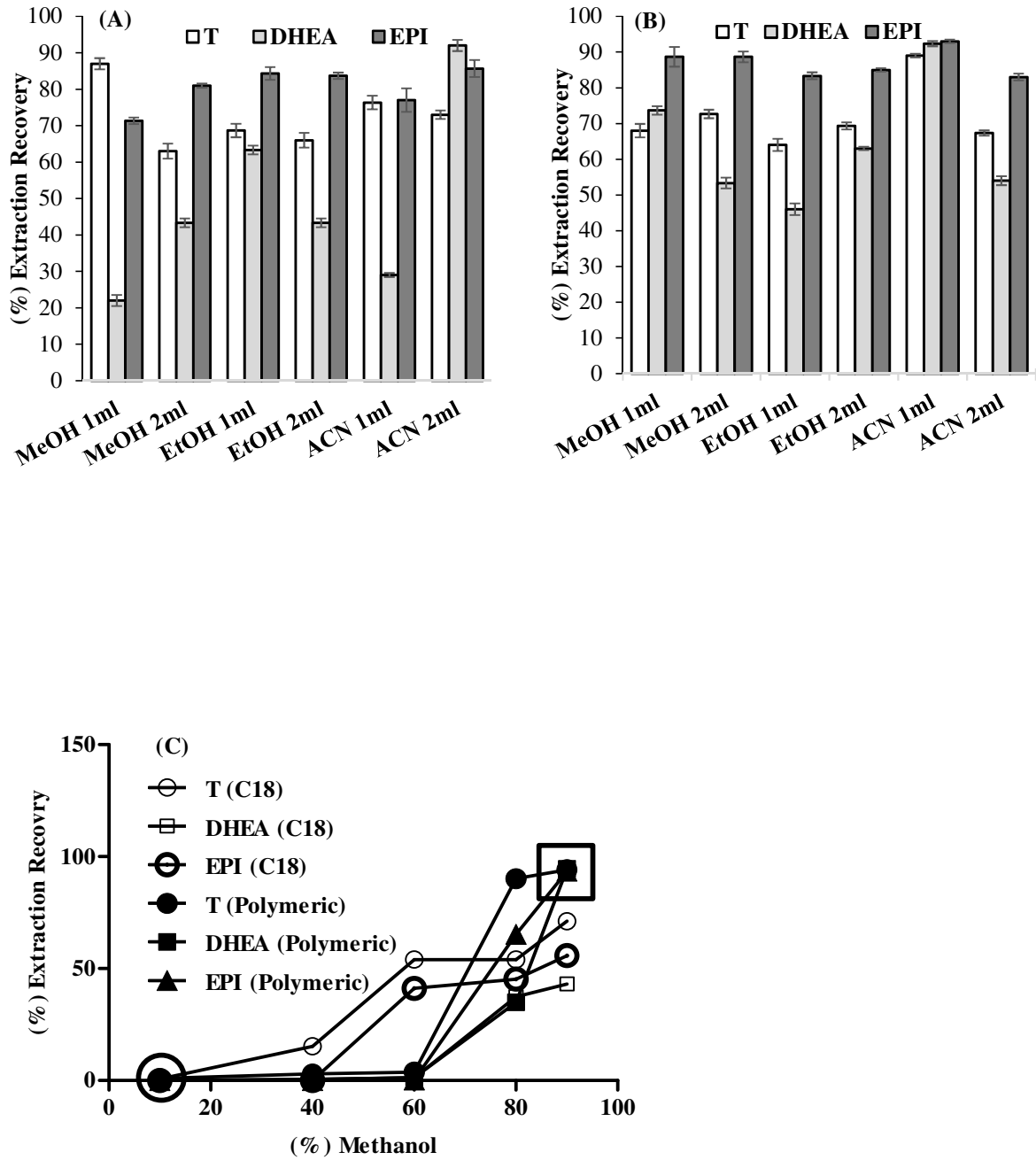
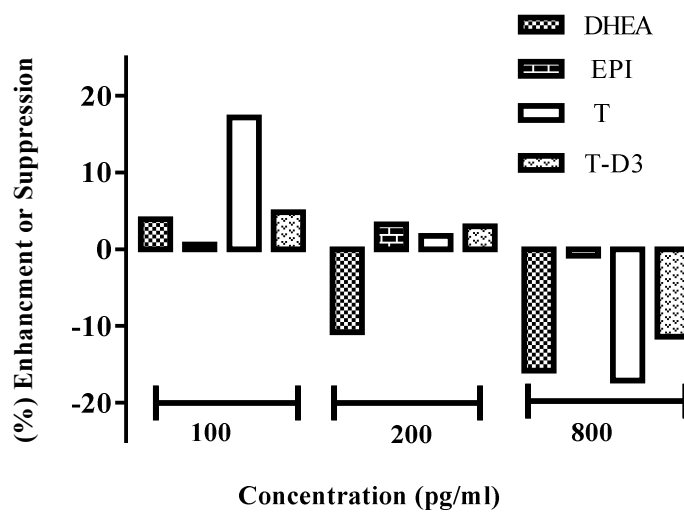


Fig S3. Development of an SPE method for androgens using two sorbents of SPE cartridges: (A) effect of elution different solvents and volumes using C18 cartridge; (B) effect of elution with different solvent mixtures and volumes using the polymeric cartridge; (C) effect of different % of methanol in the washing step (n=3).



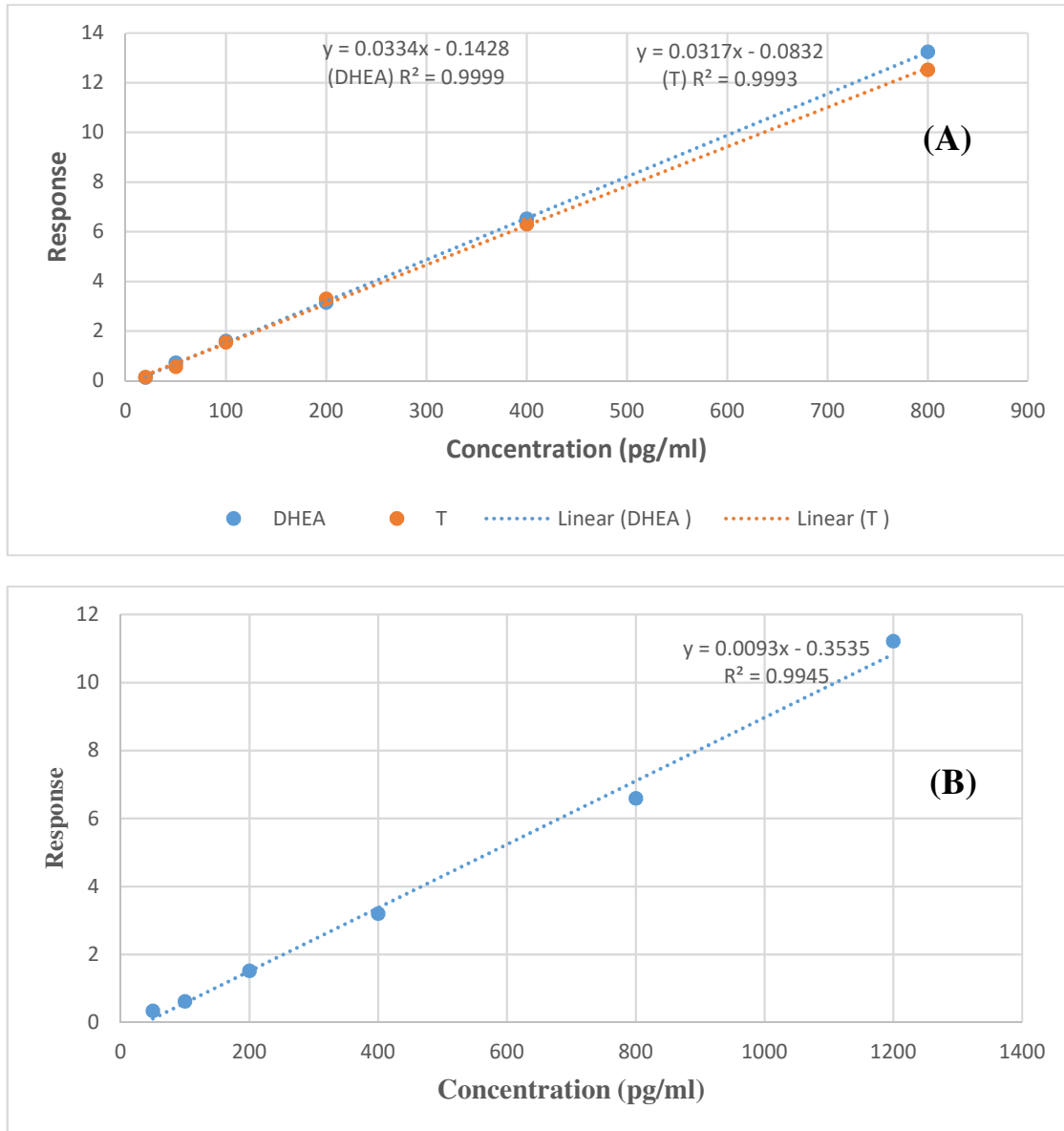
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Figure S4. Mean matrix effect (n = 3).

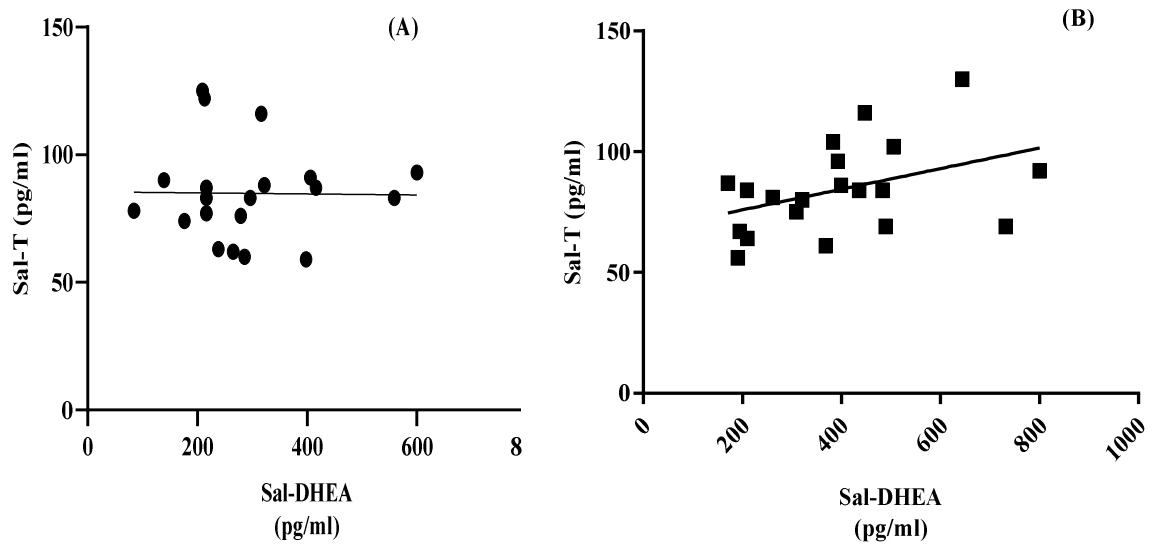
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Fig S5. Calibration curve for (A): (T) testosterone (orange colour), (DHEA) Dehydroepiandrosterone (blue colour) in the range of 20-800 pg/ml each, and (B): (EPI) Epitestosterone in the range of 50-1200 pg/ml.



41 **Fig S6.** Correlation between the concentrations of Sal-DHEA and Sal-T (n=20) were (A) pre-training
42 and (B) post-training.

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