

**Simultaneous Detection of Androgen and Estrogen Abuse in Breeding Animals by Gas  
Chromatography-Mass Spectrometry/Combustion/Isotope Ratio Mass Spectrometry  
(GC-MS/C/IRMS) Evaluated against Alternative Methods**

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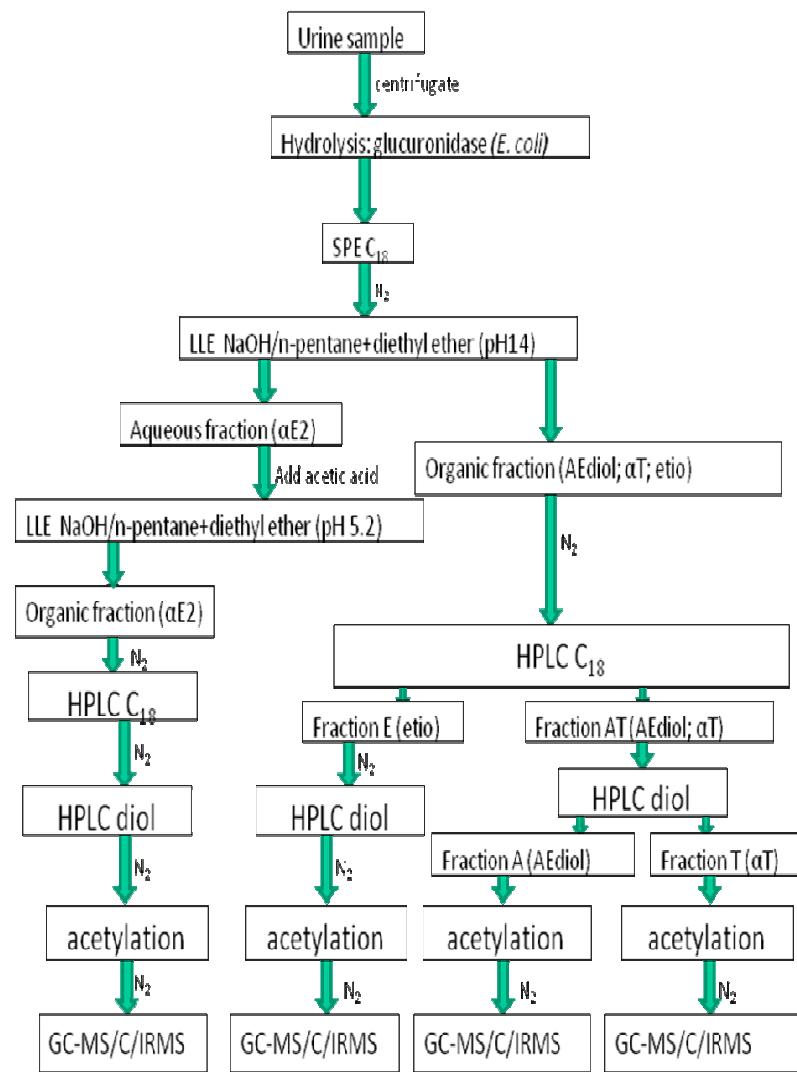
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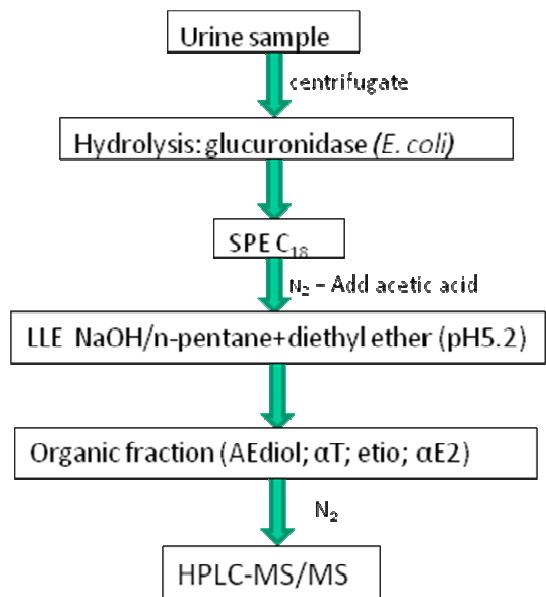
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## Supporting information

**Figure S1.** Analytical protocol for the extraction and purification of 17 $\alpha$ -estradiol ( $\alpha$ E2), 17 $\alpha$ -testosterone ( $\alpha$ T), etiocholanolone (Etio) and the endogenous reference compound 5-androstene-3 $\beta$ ,17 $\alpha$ -diol (AEdiol), prior to GC-MS/C/IRMS analysis. LLE stands for liquid-liquid extraction.



**Figure S2.** Analytical protocol for the sample preparation prior to HPLC-MS/MS analysis. LLE stands for liquid-liquid extraction.



**Figure S3.**  $\Delta^{13}\text{C}_{\text{VPDB}}$  values (expressed in ‰) of 17 $\alpha$ -estradiol ( $\alpha\text{E}2$ ), 17 $\alpha$ -testosterone ( $\alpha\text{T}$ ) and etiocholanolone (Etio), with 5-androstene-3 $\beta$ ,17 $\alpha$ -diol as endogenous reference compound, in the urine samples from a bull (above) and a heifer (below) after treatment with 17 $\beta$ -testosterone propionate and 17 $\beta$ -estradiol benzoate.

