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17ß-19-nortestosterone metabolite profiling abuse or natural occurrence?

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Introduction

17β-19-nortestosterone (17β-NT) is a powerful steroid that may be used for fattening purposes in cattle [1]. Even though the presence/absence criteria of 17β-NT in urine may seem adequate, its occurrence in boar urine can be of endogenous origin [2]. Also in female pigs, 17β-NT is detected in low concentrations [3]. The origin of these findings can be either abuse or endogenous production. Intersex or hermaphrodite animals are thought to produce 17β-NT. Therefore, non-ambiguous criteria, possibly based on different metabolites, are required for confirmation of 17β-NT administration in pigs.

A GC-MS/MS method for the analysis of 17β-NT and 16 known metabolites was developed and validated. With this method urine samples of an animal trial where sows were treated with Nandrosol® (17ß-NT phenylpropionate) were analysed and a profiling model was made from these results. Additionally a survey on Dutch boar and sow urine samples was carried out to gain insight in the possible abuse of 17ß-NT.

Results method development

Table 1. RT (minutes) and MRM transitions for 17ß-NT and metabolites

Compound	Abrevation	RT(min)	MRM-1	MRM-2
Nortestosterone-17β	NT-17β	16.99	418>182	418>194
Nortestosterone-17α	NT-17α	16.42	418>182	418>194
Norandrosterone		14.21	405>225	405>315
Noretiocholanolone		14.81	405>225	405>315
Norepiandrosterone		15.01	405>225	405>315
Norandrostenediol		15.45	330>225	330>240
Norandrostanedione		15.83	403>171	403>223
Norandrostenedione		16.95	416>220	416>234
5β-estrane-17β-ol-3-one		14.55	420>240	420>330
5α-estrane-17β-ol-3-one		15.85	420>240	420>330
5α-estrane-3α,17α-diol	AAA	13.65	407>199	407>241
5β-estrane-3α,17α-diol	BAA	13.97	407>199	407>241
5β-estrane-3β,17α-diol	BBA	14.11	407>199	407>241
5α-estrane-3α,17β-diol	AAB	14.22	407>199	407>241
5α-estrane-3β,17α-diol	ABA	14.55	407>199	407>241
5β-estrane-3β,17β –diol	BBB	14.68	407>199	407>241
5β-estrane-3α,17β –diol	BAB	14.87	407>199	407>241
5α-estrane-3β,17β –diol	ABB	15.03	407>199	407>241

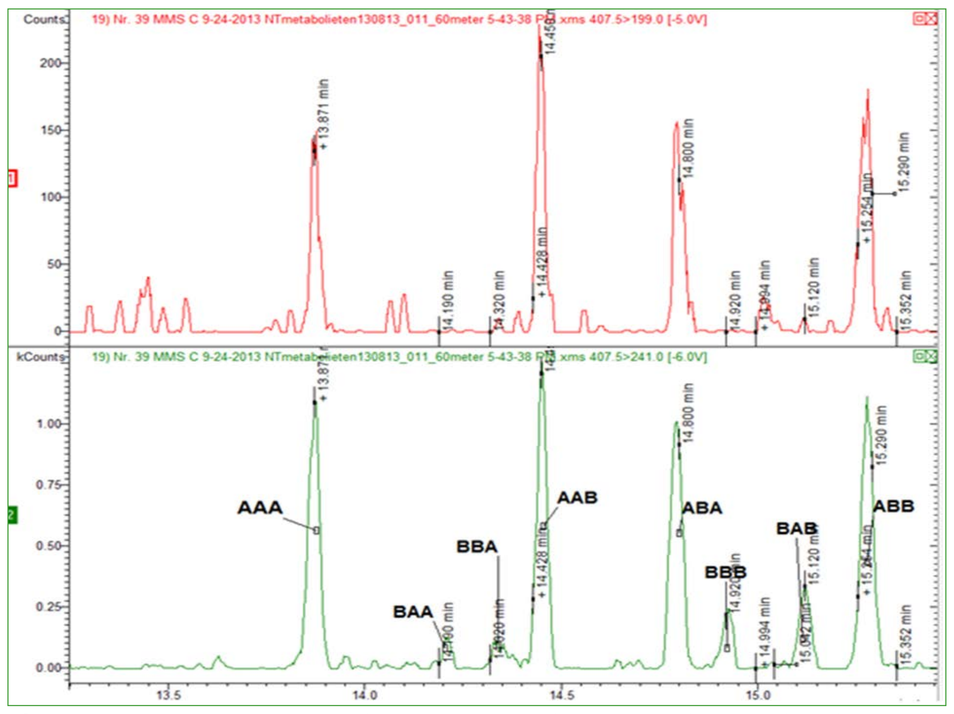

Figure 1. Examples of MRM chromatograms (MRM-1 and MRM-2) of isomers from 17ß-NT of a matrix matched standard pig urine spiked at 1.0 µg/L level.

Results survey and profiling

From the results of the animal trial (Nandrosol® administration) a profiling model was created in which treated sows can be separated from non-treated sows (figure 2). This is done by calculating the ratio of the concentrations of norepiandrosterone and norandrostenedione. If the ratio falls within the 95% confidence interval of the model the sample can be considered as suspect. The results of the survey samples were inserted in the model (figure 4). One of the samples (sow) falls within the 95% confidence interval.

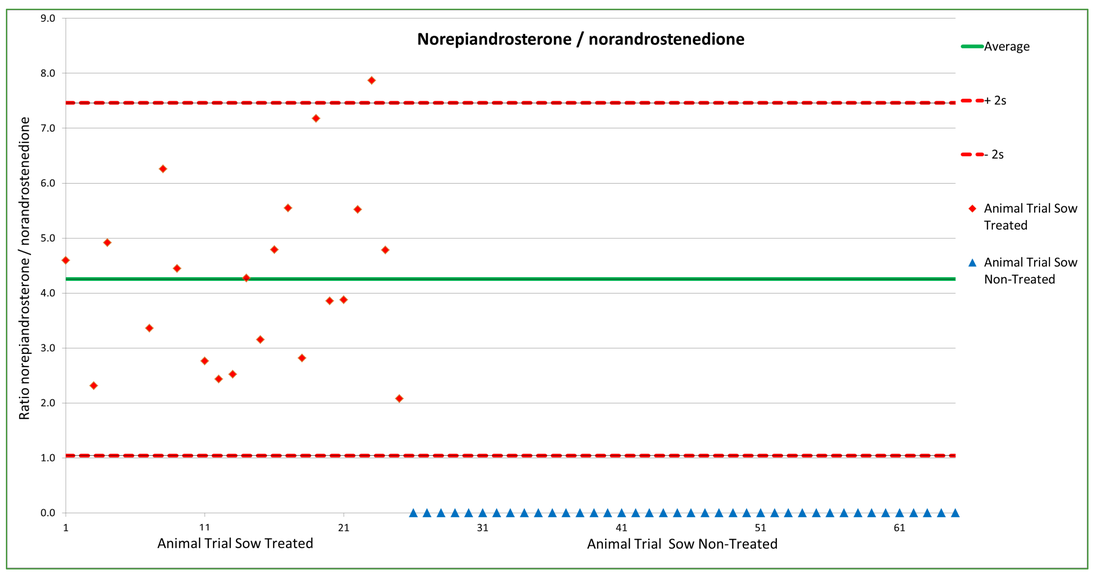

Figure 2. Ratio norepiandrosterone / norandrostenedione of urine of 17ß-NT treated sows and non-treated sows with the 95% confidence interval for suspicion of 17ß-NT.

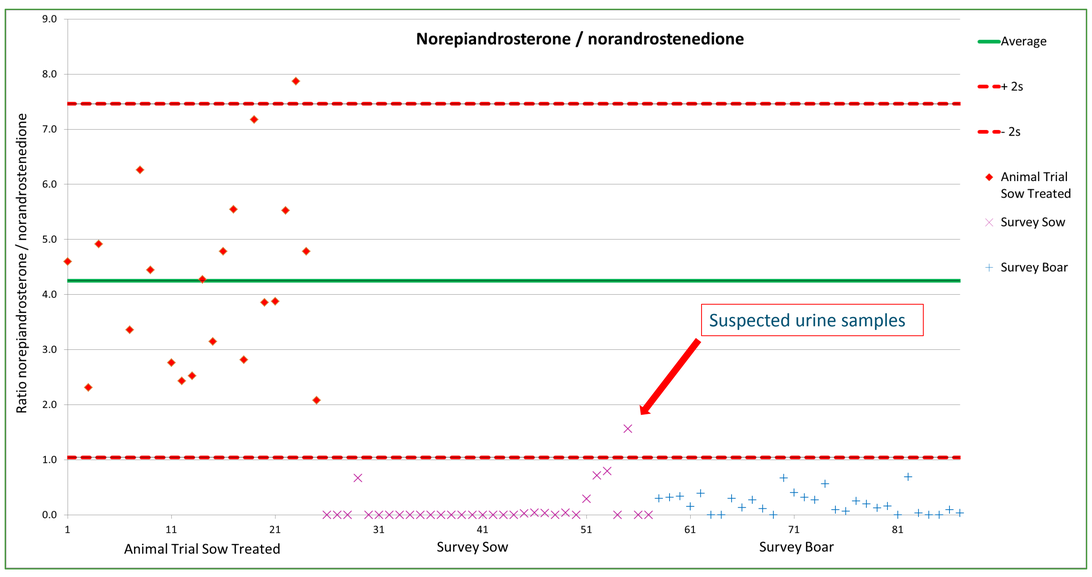

Figure 3. Ratio norepiandrosterone / norandrostenedione of urine of 17ß-NT treated sows and survey samples (sow and boar) from Dutch farms with the 95% confidence interval for suspicion of 17ß-NT.

Conclusions

- A method was developed and validated which is capable of detecting 17ß-NT and 16 known metabolites.
- A profiling model which uses the ratio of the concentration of norepiandrosterone and norandrostenedione was developed. The model can make a distinction between urine of treated sows and urine of non-treated sows as well as urine of non-treated boars and barrows.
- 1 sow urine out of 62 urine samples (sow and boar) from a survey of Dutch farms was classified by this model as a suspected sample. To confirm the animal was treated with 17ß-NT or if the animal could be an intersex animal or hermaphrodite, the sample must be analysed with GC-c-IRMS [4].

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