Short title: UPC^2-MS/MS analysis of Steroids in Mammalian Whiskers

What's in a whisker? High-throughput analysis of twenty-eight C_{19} and C_{21} steroids in mammalian whiskers by ultra-performance convergence chromatography-tandem mass spectrometry.

Nico Lübcker[1,*],

Liezl M. Bloem[2],

Therina du Toit[2],

Pieter Swart[2],

P.J. Nico de Bruyn[1],

Amanda C. Swart[2],

Robert P. Millar[3]

[1]Mammal Research Institute, Department of Zoology and Entomology, University of Pretoria, Private Bag X20, Hatfield 0028, South Africa.

[2]Department of Biochemistry, Stellenbosch University, Stellenbosch 7600, South Africa.

[3]Centre for Neuroendocrinology, Department of Immunology, Faculty of Health Sciences, University of Pretoria, Pretoria 0001, South Africa.

*corresponding author: nlubcker@zoology.up.ac.za, +27720370130.

A. Supplementary Materials

A.1. List of Supplementary Figures.

Figure 1S: Chromatographs of steroids detected during the sample pre-treatment steps (panel B, C). Panel A shows the retention time and peak intensity of the standards of progesterone (PROG) and androstenedione (A4) measured at a concentration of 0.2 ng/mL for reference. PROG and A4 were the only steroids detected above the LOD during the 3-minute water washing step (panel B), as well as during the first isopropanol washing step (panel C), albeit below the LOQ. No other steroids were detected during the any of the wash steps and no PROG and A4 were detected during the second (final) 3-minute isopropanol wash step (data not shown). This suggests that our washing protocol successfully removed external contaminants without extracting quantifiable amounts of intrinsic steroids (contained in the shaft of the whiskers). The peak intensity of A4 measured in the 0.2 ng/mL standard is $x10^3$ larger than measured during the wash steps, for example (Panel B, C).

Figure 2S: Whisker matrix ion suppression or enhancement on the peak area of four internal standards, assessed at four whisker matrix concentrations (0.1 mg/µL; 0.13 mg/µL, 0.2 mg/µL, and 0.4 mg/µL), spiked with internal standards at the same concentration as the whisker samples (0.1 ng/µL). The concentration of the whisker matrix is determined by the initial whisker sample mass extracted and the resuspension volume. Limited linear increases or decreases was observed in the peak area of D9PROG ($y = 157.5x + 960856$), D2T ($y = 52.8x + 151333$), and D711OHA4 ($y = 695.4x$). More D7A4 ion suppression occurred at the higher matrix concentrations ($y = 973.6 + 960856$).

Figure 3S: Principal component analysis (PCA) expressing the multivariate relationships between the steroids measured above the LOQ of ≥30% of the whisker samples of (a) three seal

species, and (b) between the tip, middle, base of the whisker segments analyzed. SES = southern elephant seals (*Mirounga leonina*); *A. tropicalis* = *Arctocephalus tropicalis*.

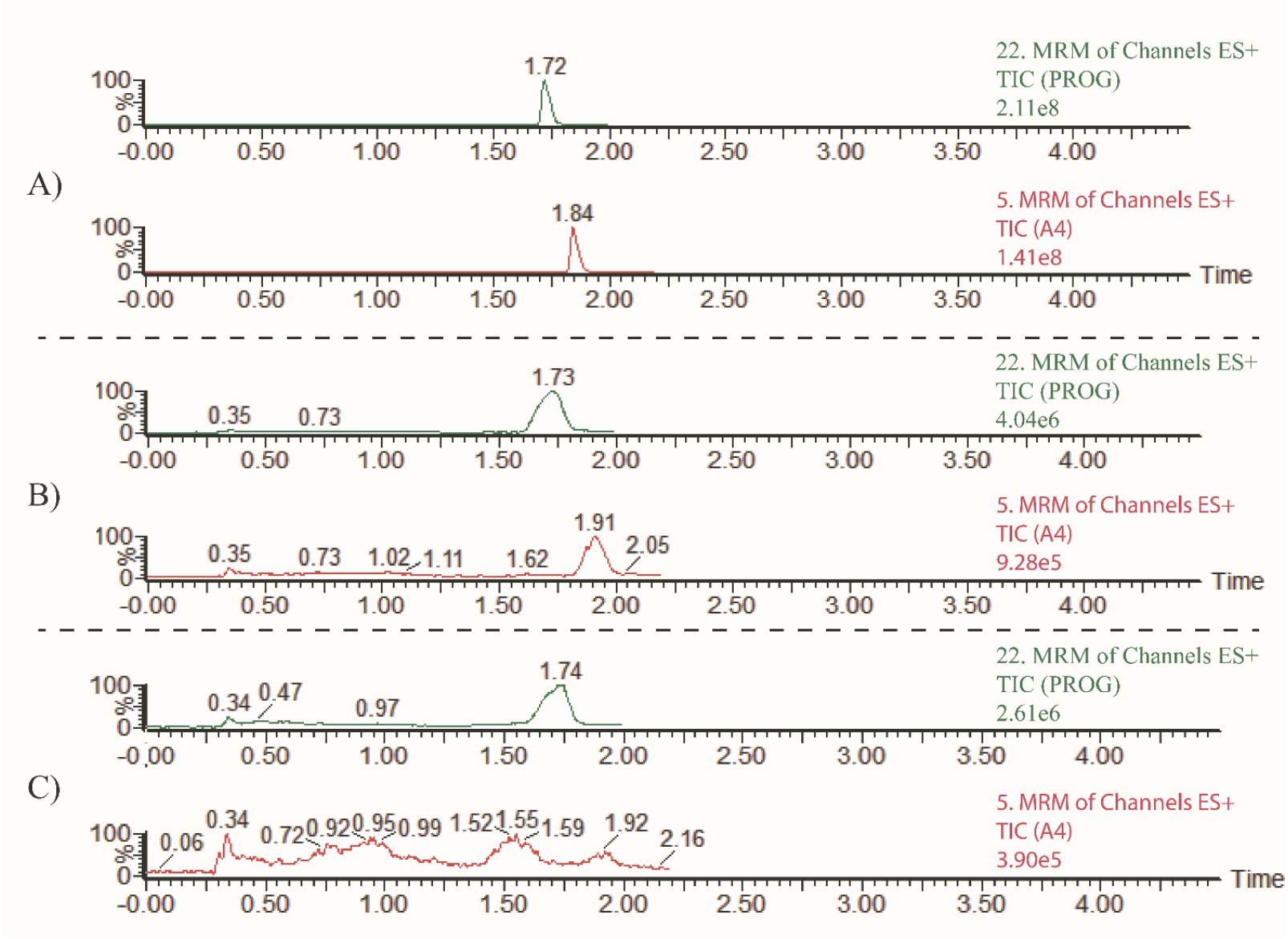
A)
22. MRM of Channels ES+
TIC (PROG)
2.11e8
1.72
5. MRM of Channels ES+
TIC (A4)
1.41e8
1.84
Time
B)
22. MRM of Channels ES+
TIC (PROG)
4.04e6
1.73
0.35
0.73
5. MRM of Channels ES+
TIC (A4)
9.28e5
1.91
2.05
0.35
0.73
1.02
1.11
1.62
Time
C)
22. MRM of Channels ES+
TIC (PROG)
2.61e6
1.74
0.34
0.47
0.97
5. MRM of Channels ES+
TIC (A4)
3.90e5
0.06
0.34
0.72
0.92
0.95
0.99
1.52
1.55
1.59
1.92
2.16
Time
100
%
0
-0.00
0.50
1.00
1.50
2.00
2.50
3.00
3.50
4.00

Figure 1S.

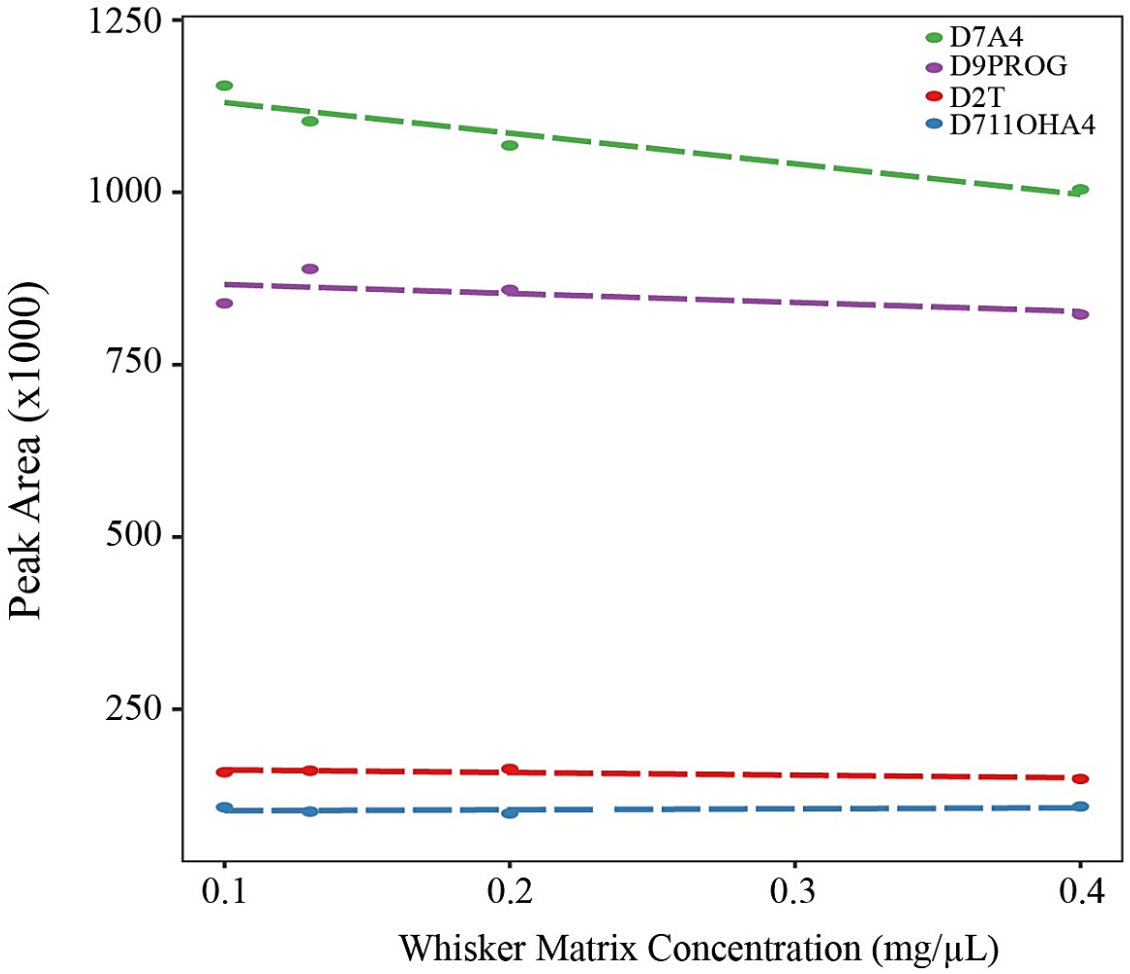
1250
1000
750
500
250
Peak Area (x1000)
D7A4
D9PROG
D2T
D711OHA4
0.1
0.2
0.3
0.4
Whisker Matrix Concentration (mg/µL)

Figure 2S.

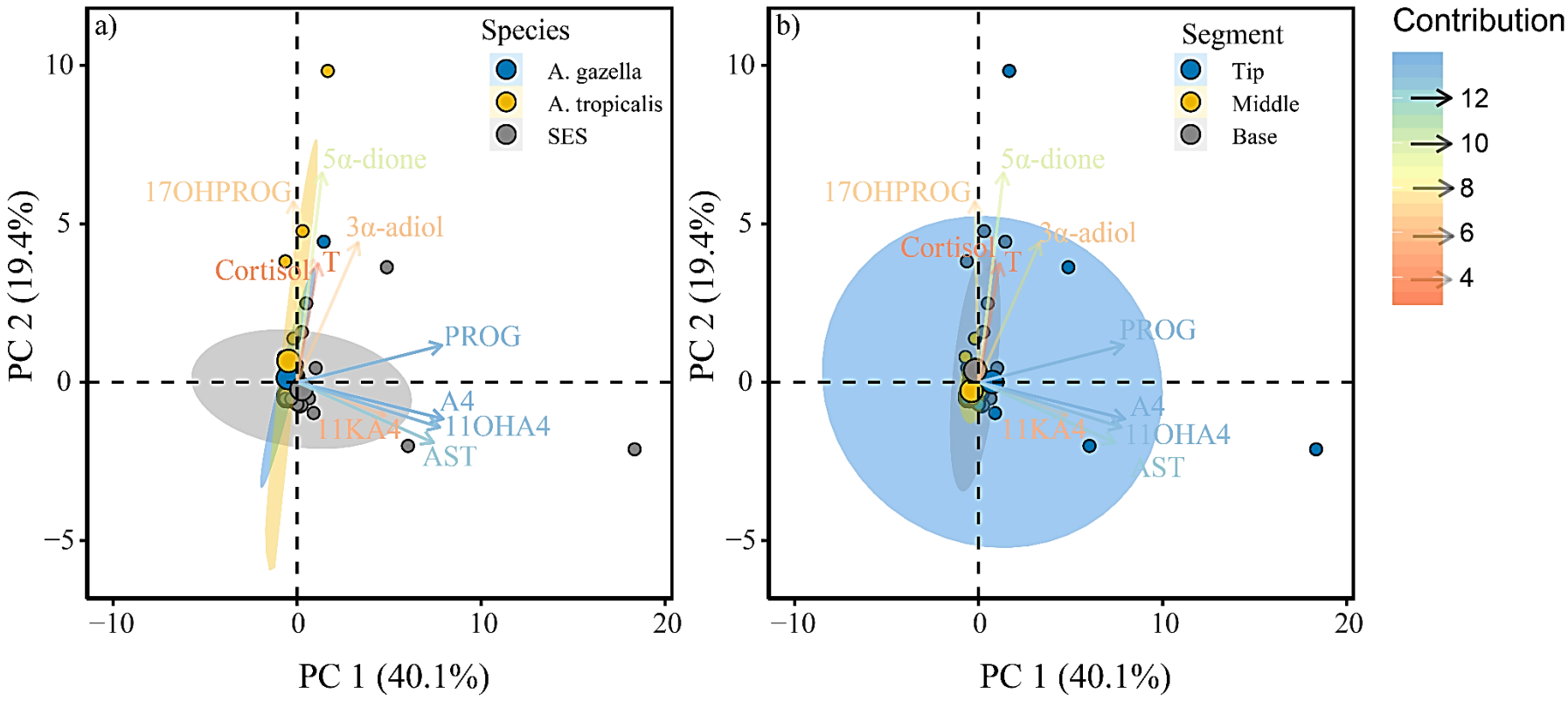
a)
Species
A. gazella
A. tropicalis
SES
5α-dione
17OHPROG
3α-adiol
Cortisol
T
PROG
A4
11KA4
11OHA4
AST
PC 2 (19.4%)
PC 1 (40.1%)
b)
Segment
Tip
Middle
Base
Contribution
12
10
8
6
4

Figure 3S.