

## **Analytical and Bioanalytical Chemistry**

### **Electronic Supplementary Material**

#### **Rapid and reliable steroid hormone profiling in *Tursiops truncatus* blubber using liquid chromatography tandem mass spectrometry (LC-MS/MS)**

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**Table S1** Calibration standard masses in ng per vial and isotopically labeled internal standard masses (ng) used for the quantitation of steroid hormones in dolphin blubber validation experiments

| Steroid  | CAL-A | CAL-B | CAL-C | CAL-D | CAL-E | CAL-F | IS   |
|--|-------|-------|-------|-------|-------|-------|------|
| Cortisol   | 8.89  | 3.66  | 1.49  | 0.84  | 0.4   | 0.237 |      |
| Cortisone  | 9.84  | 5.79  | 3.11  | 2.78  | 1.36  | 0.821 |      |
| 11-Deoxycortisol                                 | 9.01  | 4.42  | 1.8   | 0.851 | 0.483 | 0.24  |      |
| Corticosterone                                   | 9.45  | 4.47  | 1.82  | 0.892 | 0.488 | 0.252 |      |
| 17-OH-Progesterone                               | 8.88  | 4.18  | 1.7   | 0.839 | 0.457 | 0.237 |      |
| Testosterone                                     | 8.87  | 4.38  | 1.78  | 0.838 | 0.478 | 0.236 |      |
| Androstenedione                                  | 8.92  | 4.25  | 1.73  | 0.843 | 0.464 | 0.238 |      |
| Progesterone                                     | 9.26  | 3.65  | 1.48  | 0.875 | 0.398 | 0.247 |      |
| Androstenedione- <sup>13</sup> C <sub>3</sub>    |       |       |       |       |       |       | 5.05 |
| Testosterone- <sup>13</sup> C <sub>3</sub>       |       |       |       |       |       |       | 5.51 |
| 17-OH-Progesterone- <sup>13</sup> C <sub>3</sub> |       |       |       |       |       |       | 4.48 |
| Progesterone- <sup>13</sup> C <sub>3</sub>       |       |       |       |       |       |       | 5.26 |
| Cortisol- <i>d</i> <sub>4</sub>                  |       |       |       |       |       |       | 3.79 |

**Table S2** Calibration curves and linear regression information for detected steroids in bottlenose dolphin blubber validation experiments

| Analyte            | Associated Standard                              | R <sup>2</sup> | Slope (m) | Intercept (b) | Calibrant Range (ng/g) |
|--------------------|--|----------------|-----------|---------------|------------------------|
| Cortisol           | Cortisol- <i>d</i> <sub>4</sub>                  | 1.00           | 0.994     | 0.096         | 0.84 - 8.89            |
| Cortisone          | Cortisol- <i>d</i> <sub>4</sub>                  | 1.00           | 3.57      | -0.073        | 0.821 - 5.79           |
| 11-Deoxycortisol   | Cortisol- <i>d</i> <sub>4</sub>                  | 1.00           | 4.28      | -0.055        | 0.24 - 1.8             |
| Corticosterone     | Cortisol- <i>d</i> <sub>4</sub>                  | 1.00           | 0.452     | -0.008        | 0.252 - 1.82           |
| 17-OH-Progesterone | 17-OH-Progesterone- <sup>13</sup> C <sub>3</sub> | 1.00           | 0.928     | 0.004         | 0.237 - 1.7            |
| Testosterone       | Testosterone- <sup>13</sup> C <sub>3</sub>       | 1.00           | 1.30      | -0.001        | 0.236 - 1.78           |
| Androstenedione    | Androstenedione- <sup>13</sup> C <sub>3</sub>    | 1.00           | 0.976     | 0.035         | 0.464 - 1.73           |
| Progesterone       | Progesterone- <sup>13</sup> C <sub>3</sub>       | 1.00           | 0.808     | -0.006        | 0.247 - 1.48           |

**Table S3** Calibration standard masses in ng per vial and spike mass (ng) used for the quantitation of accuracy experiments for steroid hormones in dolphin blubber

| Steroid (ng)           | Cal A | Cal B | Cal C | Cal D | Cal E  | Cal F   | Spike |
|------------------------|-------|-------|-------|-------|--------|---------|-------|
| 11-Deoxycorticosterone | 100   | 5.71  | 5.00  | 2.07  | 0.118  | 0.104   | 2.95  |
| 11-Deoxycortisol       | 75.2  | 8.42  | 3.75  | 1.55  | 0.174  | 0.0780  | 4.34  |
| 17-OH-Progesterone     | 110   | 26.0  | 5.50  | 2.28  | 0.537  | 0.115   | 13.4  |
| Androstenedione        | 9.42  | 2.65  | 0.469 | 0.194 | 0.0549 | 0.00977 | 1.37  |
| Corticosterone         | 78.0  | 21.8  | 3.89  | 1.61  | 0.450  | 0.0809  | 11.2  |
| Cortisol               | 87.3  | 41.6  | 4.35  | 1.80  | 0.860  | 0.0905  | 21.4  |
| Cortisone              | 80.8  | 9.74  | 4.03  | 1.67  | 0.201  | 0.0838  | 5.03  |
| Progesterone           | 113   | 22.4  | 5.62  | 2.33  | 0.462  | 0.117   | 11.5  |
| Testosterone           | 251   | 28.3  | 12.5  | 5.18  | 0.585  | 0.260   | 14.6  |