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Using liquid chromatography-mass spectrometry to determine testosterone concentrations in dolphin saliva and blow

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Historically internal physiology of great whales was assessed during the whaling days. Since whaling ceased in the mid-twentieth century, there has been no effective way in which to assess reproductive hormones in whales. As some whale populations are showing poor signs of recovery a greater understanding of their reproductive cycles is required. This knowledge can lead to better population management and information into potential causes of reproductive dysfunction. Saliva has been used in both humans and some animal species to determine reproductive hormones. This study assesses the feasibility of using blow exudate from whales to determine reproductive status by determining reproductive hormone concentrations in saliva and blow of captive bottlenose dolphins using liquid chromatography-mass spectrometry (LC-MS). A rapid, accurate and reproducible assay utilising LC-MS has been developed and validated for determining testosterone concentrations in saliva and blow of dolphins. Sample preparation used solid phase extraction with specific preconditioning of cartridges. Analytes were eluted with 100% acetonitrile, dried under nitrogen and stored at -80°C. Samples were reconstituted in 60% acetonitrile for LC-MS analysis. Chromatographic separation was achieved with an Alltech Macrosphere C8 stainless steel analytical column (2.1 I.D. x 150 mm, 5µm particle size, 300 Å pore size) using a Shimadzu dual pump LC with 55% mobile phase B isocratic method (mobile phase A = 0.5% acetic acid; mobile phase B = 0.5% acetic acid, 90% acetonitrile). Samples were analysed on a Shimadzu LCMS-2010 in SIM at m/z 289.20 (testosterone mw 288.40) and a positive ion ESI. The limit of quantification was 0.5 ng/ml with a limit of detection of 0.2 ng/ml. The concentration curve was linear from 0.5 - 50 ng/ml ($r^2 = 0.959$). The RSDs of intra- and inter-batch precision were less than 15% for saliva and 11% blow. Recovery of the assay for saliva was $93.0 \pm 7.9\%$ (50ng/ml) and $91.5 \pm 3.72\%$ (1ng/ml), and for blow was $83.3 \pm 6.8 \%$ (50 ng/ml) and $85.8 \pm 4.6 \%$ (1 ng/ml). The described assay was used to determine the presence of endogenous testosterone in saliva (9.73 - 23 ng/ml, n = 10) and blow (14.71 - 86.20 ng/ml, n = 11) samples of captive bottlenose dolphins.