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Teeth Steroid Extraction for "Steroid profiling in human primary teeth via liquid chromatography-tandem mass spectrometry for long-term retrospective steroid measurement"

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We use this protocol and it's working

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Abstract

Steroid hormones are important modulators of many physiological processes, and measurements of steroids in blood, saliva, and urine matrices are widely used to assess endocrine pathologies and stress. However, these matrices cannot be used to retrospectively assess early-life stress and developmental endocrine pathologies, because they do not integrate steroid levels over the long term. A novel biological matrix in which to measure steroids is primary teeth (or “baby teeth”). Primary teeth develop early in life and accumulate various endogenous molecules during their gradual formation. Here, we developed and validated the first assay to measure steroids in human primary teeth using liquid chromatography-tandem spectrometry (LC-MS/MS). Our assay is highly sensitive, specific, accurate, and precise. It allows for the simultaneous quantification of 17 steroids in primary teeth (16 of which have not been examined previously in primary teeth). Overall, steroid levels in primary teeth were relatively low, and 8 steroids were quantifiable. Levels of dehydroepiandrosterone, cortisol, and progesterone were the highest of the 17 steroids examined. Next, we used this assay to perform steroid profiling in primary teeth from males and females. The same 8 steroids were quantifiable, and no sex differences were found. Levels of androgens (androstenedione and testosterone) were positively correlated, and levels of glucocorticoids (cortisol, cortisone, corticosterone, 11-dehydrocorticosterone) were also positively correlated. These data demonstrate that multiple steroids can be quantified by LC-MS/MS in human primary teeth, and this method potentially provides a powerful new way to retrospectively assess early-life stress and developmental endocrine pathologies.

First Day

1 Put 5 1.4mm beads in a 2mL Bead ruptor tube

2 Label bead ruptor tubes

3 Label 0.6mL polypropelene microcentrifuge tubes to correspond with bead ruptor tubes

4 Label LC-MS/MS vials to corresond with bead ruptor tubes and put glass inserts in vials

5 Make 50% MeOH

6 Make 25% MeOH

7 Use previously diluted standard curve.

8 Use previoulsy made 5X IS.

9 Make enough 1X I.S. to use for this asay

10 Preheat oven to 60C

11 Rinse all 12x75mm Fisher culture tubes needed for experiment with 1mL MeOH, vortex for 2sec, dump MeOH waste into waste beaker, add another 1mL MeOH to each tube, vortex for 2 sec, dump MeOH into waste and place inverted tubes in tube rack in drying oven at 60C

12 Label rinsed and dried culture tubes

13 Cover all rinsed, dried and labelled tubes (right-side up) with aluminum foil ensuring complete covering of tubes

14 Wash all teeth to remove saliva and blood

2nd Day

15 Preheat speed vac to 60C

16 Remove all reagents from 4C or -20C and allow to come to room temp

17 Retrieve the cleaned pieces of three teeth from the drawer and record the weight

18 Crush each piece into powder with the tooth crusher

19 Collect and weigh the powder

20 Put all tooth powder into corresponding bead ruptor tube

21 Add 10ul STD to appropriate bead ruptor tubes

22 Add 1mL room temp HPLC ACN to all tubes

23 Add 50ul I.S. to all tubes excpet BLKBLK

24 Add 50uL of 50% MeOH to BLKBLK

25 Vortex 2sec

26 Homogenize 4m/s for 30sec

27 Centrifuge at 16,100g for 5min

28 Remove 1000ul supernatant (94%) and put in 12x75mm culture tube

29 Add 500ul Hexane to all samples

30 Vortex 5sec

31 Centrifuge at 3200g for 2min

32 Remove hexane and put in waste

33 Dry ACN at 60C for 45min

34 Resuspend in 55ul 25% MeOH

35 Vortex 5 sec

36 Centrifuge 3200g for 1min

37 Transfer all supernatant to 0.6mL centrifuge tube

38 Centrifuge 16100g for 2min

39 Transfer 50ul supernatant to LC insert using gel loading tips

40 Store in -20C until injection