



Jun 17, 2024

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

 [PLOS One](#)

DOI

<https://dx.doi.org/10.17504/protocols.io.e6nvw1b3dlmk/v1>

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Protocol Citation: Ruolan S. Wu, Jordan E. Hamden, Melody Salehzadeh, Michael X. Li, Asmita Poudel, Kim L. Schmidt, Michael S. Kobor, Kiran K. Soma 2024. Teeth Steroid Extraction for "Steroid profiling in human primary teeth via liquid chromatography-tandem mass spectrometry for long-term retrospective steroid measurement". **protocols.io**

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Protocol status: Working

We use this protocol and it's working

Created: June 15, 2024

Last Modified: June 17, 2024

Protocol Integer ID: 101886

Keywords: steroid profiling in primary teeth, steroid levels in primary teeth, steroids in human primary teeth, steroids in primary teeth, teeth steroid extraction, steroid hormone, steroid level, steroid profiling, steroid levels over the long term, measurements of steroid, human primary teeth, ms in human primary teeth, primary teeth, levels of dehydroepiandrosterone, levels of androgen, multiple steroid, steroid, tandem mass spectrometry, levels of glucocorticoid, dehydroepiandrosterone, androgen, developmental endocrine pathology, liquid chromatography, using liquid chromatography, progesterone, baby teeth, endocrine pathology, tandem spectrometry, glucocorticoid, corticosterone, first assay, dehydrocorticosterone, testosterone

Funders Acknowledgements:

Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Grant

Grant ID: RGPIN-2019-04837

Discovery Accelerator Supplement

Grant ID: RGDAS-2019- 00033

UBC Grants for Catalysing Research Clusters

Grant ID: F18-05858

ew Frontiers in Research Fund Gran

Grant ID: F21-04739

Michael Smith Health Research BC and CLEAR Foundation Postdoctoral Fellowshi

NSERC CGS-M and CGS-D

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 polypropylene microcentrifuge tubes to correspond with bead ruptor tubes
- 4 Label LC-MS/MS vials to correspond 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 previously made 5X IS.
- 9 Make enough 1X I.S. to use for this assay
- 10 Preheat oven to 60C
- 11 Rinse all 12×75mm 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 12×75mm 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