APPLICATION NOTE

Tip-on-Tip SPE for the Extraction of Hormones from Saliva

BACKGROUND

Hormone analysis in blood and serum has been used to diagnose various diseases and even indicate early signs of a health decline. Saliva can be collected in relatively large amounts (mL) without any invasive measures taken, making it the perfect candidate for clinically relevant testing. Nine hormones were evaluated in pooled neat saliva with a Tip-on-Tip SPE technique (ToT SPE Patent No.: US 11,567,067) with Supel™ Swift HLB (MilliporeSigma).Levels were calculated at part-per-trillion levels with most average extraction recoveries greater than 85%.

SAMPLE PREPARATION

A MICROLAB® NIMBUS96 liquid handler was utilized for sample preparation. Internal standard (10 µL) and 30% MeOH (500 µL) were added to saliva samples. Sample/sorbent solution was then added to well plate containing 10mg HLB.

METHOD

Agilent 1260 LC system coupled with a SCIEX 6500+ tandem mass spectrometer. Agilent InfinityLab Poroshell 120 EC-C18, 3.0 x 100 mm, 2.7 µm, solvent saver LC with a 10 µL injection volume. The column was heated to 45 °C. Mobile Phases: [A] 0.1% formic acid in water and [B] 0.1% formic acid in MeOH. The LC method began with 50% [A] and 50% [B] for 0.5 minutes. The system ramped to 55% [B] at 3.7 minutes, ramped again to 80% [B] at 3.8 minutes, and finally ramped to 90% [B] at 6.0 minutes. A flush is done by ramping to 100% [B] at 6.5 minutes and holding for 4 minutes. Finally, the system ramped back to 50% [A] and 50% [B] at 10.6 minutes and held for 1.4 minutes. MS Source: positive mode, curtain gas of 35 psi, collision gas of 8 psi, IonSpray voltage of 4500, source temperature was 600 C, and ion source gas was 60. Matching internal standards were used for each analyte.

WORKFLOW

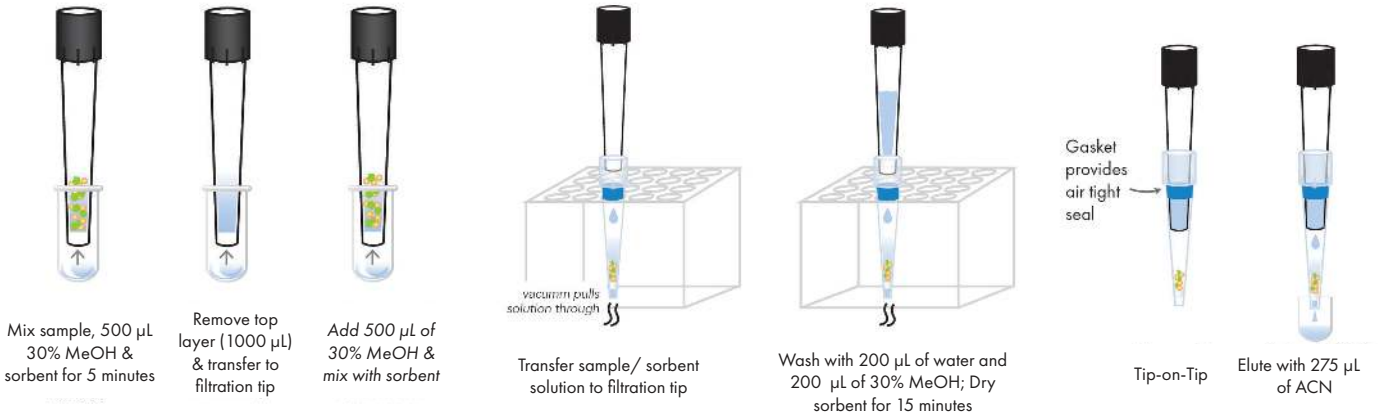

Figure 1. Schematic represents workflow for ToT Filtration method for extraction of hormones from saliva.

RESULTS

All analytes had an average Coefficient of determination (R^2)value of 0.999, except for OH-Progesterone, which had 0.997. Matrix effects ranged from 6% ion suppression to 18% for 5 ng/mL samples and ranged from 15% to 28% ion suppression for 50 ng/mL. All analytes at all QC levels had intra-day precision values between 2.36%-9.64% and inter-day precision between 0.23%-12.32%.

CONCLUSION

Using Tip-on-Tip SPE on viscous saliva samples has been shown to be reliable and reproducible. Recoveries for all analytes ranged from 85%-100% with minimal matrix effects of 5%-18% ion suppression at 5 ng/mL concentration. This method of extracting and analyzing steroids is reproducible, high throughput, and provides rapid turn around time for patient samples.

Table 1. *The results from a 3-day SWGTOX guidelines study evaluating the limit of detection (LOD) and quantification (LOQ) based on signal-to-noise (S/N) above 10 (LOQ) or 3 (LOD).*

Analyte	LOQ (ng/mL)	LOD (ng/mL)	Linear Dynamic Range (ng/mL)
Cortisone	0.030	0.010	0.009 - 19.7
Cortisol	0.026	0.009	0.009 - 19.7
11-Deoxycortisol	0.004	0.001	0.003 - 19.7
Androstenedione	0.013	0.004	0.001 - 2.19
Testosterone	0.002	0.001	0.003 - 19.7
DHT	0.026	0.009	0.009 - 19.7
DHEA	0.071	0.023	0.045 - 98.4
OH-Progesterone	0.032	0.010	0.027 - 19.7
Progesterone	0.020	0.007	0.009 - 19.7

Table 2. *The results from a 3-day SWGTOX guidelines study evaluating the inter-day and intra-day precision and accuracy.*

Analyte	QC1: 1 ng/mL Accuracy	QC 2: 0.1 ng/mL Accuracy	QC 3: 0.01 ng/mL Accuracy
Cortisone	107	91.0	99.4
Cortisol	109	93.7	96.2
11-Deoxycortisol	106	91.1	96.7
Androstenedione	106	90.9	104
Testosterone	108	90.6	91.4
DHT	105	86.8	89.2
DHEA	106	96.3	95.8
OH-Progesterone	105	89.5	N/A
Progesterone	94.2	87.3	83.6