INTRODUCTION
Measurement of hormone levels such as 17-Hydroxyprogesterone, Cortisol, Estradiol, Estrone, Estriol, and Testosterone can be used to help researchers identify potential markers of disease states in men, women, and children. Steroids are present in oral fluids at low concentrations so methods are needed that provide rapid and reliable analysis with high sensitivity. Traditional methods of analysis, such as immunoassays, can suffer from poor accuracy and reproducibility. A sensitive, selective and robust method was developed to enable the quantification of endogenous steroids in oral fluids.

MATERIALS AND METHODS
Sample Preparation:
1 mL of human oral fluid sample was spiked with deuterated internal standards corresponding to each of the 7 steroid analytes, and the sample was extracted with Biotage Evolute ABN SPE. The organic extracts were dried under nitrogen gas and derivatized with 100 µL of dansyl chloride solution. 2 µL of the derivatized sample was directly injected for LC-MS/MS analysis.

LC Conditions:
Separation was performed on the Eksigent ekspert™ microLC 200 system with a Halo C18 50x0.5mm, 2.7µm column. Mobile phase consisted of water and methanol with 0.1% formic acid. The flow rate was 25µL/min with injection volume of 2µL. A 25µm ID PEEKSil® capillary ion spray electrode with stainless steel tip was used in order to reduce band broadening due to the low flow rate of 30 µL/minute.

RESULTS
The method is sensitive for quantification of endogenous steroids in oral fluids. With and without DMS, lower limits of quantification (LOQ) were established at 1 pg/mL for Testosterone, 5 pg/mL for 17-Hydroxyprogesterone, Cortisol, and Progesterone, and at 0.5 pg/mL for derivatives of Estradiol, Estrone, and Estriol.

The use of SelexION™ ion mobility technology, in combination with MRM analysis, increased data quality by removing endogenous background, and improving peak shapes, which is especially important for low concentrations. When the SelexION device was employed, linearity was also improved: 0.9993 (17-Hydroxyprogesterone), 0.9995 (Cortisol), 0.9994 (Estradiol), 0.9998 (Estrone), 0.9993 (Estriol), 0.9962 (Progesterone), and 0.9975 (Testosterone).

CONCLUSIONS
The method proved to be accurate, precise and easy to perform for the routine measurement of endogenous steroids in oral fluids. Use of the SelexION™ ion mobility technology provided a significant improvement to data quality, by removing endogenous interferences.

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