Automated SPE of Drugs and their Metabolites from Oral Fluid Samples.

OVERVIEW
- Analysis of oral fluid samples in toxicology and clinical laboratories is replacing the analysis of blood and urine samples due to its non-invasive ease of collection.
- Oral fluid collection devices typically contain a buffer and/or surfactants to solubilize and stabilize the sample. In some devices (Quantisaal, Immunoassay Corp.), the buffer includes a dye that may co-extract with analytes of interest.
- For this work, solid phase extraction was performed using Corex OFXQ, a sorbent specifically created to extract OF samples. The process may be automated using a Tecan SP A200 sample processor in combination with a Tecan robotic liquid handler.
- The use of Narrow Bore Extraction columns permits the use of low elution volumes and selective elution solvents.

INTRODUCTION
Drug and drug metabolite concentrations in OF are substantially lower than those found in blood. Sample preparation via solid phase extraction is useful to provide both sample cleanup (desalting, removal of mucoid content, etc.) and sample concentration. The proprietary OFXQ sorbent in Narrow Bore Extraction columns was created specifically for the extraction of oral fluid specimens. A specific goal during product development was to produce extracts of samples collected using the Quantisaal device that do not contain the blue dye found in the product. This feature holds true for many elution solvents commonly used in this type of SPE sample preparation.

The columns feature high-capacity, high-efficiency, low bed mass sorbent that permits the use of low elution volumes. This allows the use of selective elution solvents, resulting in cleaner extracts than would be possible using high solvent strength (low specifically) solvents. Analysis of the extracts was performed on a SCIEX 5500 mass spectrometer interfaced to a Shimadzu Nexera XR UHPLC. The acquisition program was built using scheduled MRM (two product ions for each internal standard).

EXPERIMENTAL
Sample matrices
OF materials were obtained from Immunoassay Corp. (Pomona, CA), Quantisaal Synthetic Negative Saliva Pre-diluted in Extraction Buffer), Oasis (Burlington, MA), Negative Calibrator Oral Fluid, and Thermo Scientific (Waltham, MA, Oral-Eze Oral Fluid Preservative Buffer).

Spiking solutions
All spiking solutions were prepared in water to a single concentration and then appropriately diluted for use with the low bed mass sorbent that permits the use of low bed mass sorbent that permits the use of low elution volumes.

SPE method
Condition columns with methanol, then water.

Dry sorbent for 10 minutes 45-50 psi

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Transfer the SPE plate to collection
Elute with 300 μL 80:18:2 dichloromethane:IPA:conc. NH4OH.

Dry the elution solvent at room temperature.

Residue in 100 μL 90:10 0.1% aqueous formic acid:methanol (vortex well).

SUMMARY
OFXQ NBE SPE columns provide a convenient and simple means of preparing oral fluid samples for analysis by LC-MS. The extraction procedure uses few reagents, and automating the process reduces hands-on-time dramatically. Sub-nanogram/mL LLOQs are easily achieved. The data clearly demonstrate that not all collection device buffers behave identically for a given extraction method. Protocols must be individually optimized for the collection device to be used in order to achieve best performance.

RESULTS AND CONCLUSIONS
As shown in Figure 1, the use of OFXQ extraction columns for the extraction of oral fluid samples provides clear, colorless extracts over a wide range of elution solvent polarities. Extracts of samples collected using Quantisaal collection devices, produced using conventional polymeric SCX sorbents, may exhibit the blue color depending on the choice of elution solvent.

Example chromatograms and calibration curves are shown in Figure 2. All chromatograms were smoothed using the single-point Gaussian algorithm in the SCIEX software. The curve fit algorithms were quadratic, 1/x2 weighted, not forced through the origin.

Assessments of curve accuracy (requiring ≥20% from nominal value at the low calibrator, >15% at all other points), s/n ratios of both quantitation and qualifier ions, and qualifier ion area ratios, were used to estimate LLOQs and are shown below (Table 1).

A few differences were noted in the analytical results between the three matrices. Most notable were the results for oxazepam extracted from Oral-Eze. In that instance, the LLOQ was limited by interference in the qualifier ion (287 > 289) chromatogram, not by qualifier ion abundance and s/n ratio. This situation could have been resolved by the selection of an alternative qualifier ion.

Oxazepam and tramadol saturated the detector at the high calibration (250 ng/mL, data not shown), producing a decidedly curved regression plot. Other compounds, e.g. lorazepam (see curves in Figure 2), approached this limit as well but produced acceptable calibration curves.

The method shown is scalable to less sensitive mass spectrometers by adjustments to reconstitution and injection volumes. Suitable changes to the calibration curve parameters are suggested (depending on the chosen reporting cutoff) would be appropriate.