Collection of oral fluid is easy and non-invasive, and can be done under surveillance to reduce the chances of adulteration or exchange of the specimens. Concentrations of drugs in the oral fluid can usually be related to those in plasma, which represent the body burden at sampling.

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is suitable for analyzing polar, non-volatile compounds and no needs to derivatize drugs comparing with gas chromatography-mass spectrometry (GC-MS). Besides, ultra-high performance liquid chromatography (UHPLC) provides better separation and much higher sample throughput than traditional HPLC. This study developed and validated a method using isotope-dilution UHPLC-MS/MS with selective reaction monitoring to analyze seven opiates and metabolites, twelve amphetamines-type stimulants (ATS), flunitrazepam/ketamine and their three metabolites, five cocaine and metabolites and two serotonin-based hallucinogens in the oral fluid. The sensitivity and matrix effects on positive electrospray ionization (ESI+) and atmospheric pressure photoionization (APPI+) were compared.

One hundred and twenty micro-liter of oral fluid was diluted with two times of Milli-Q water then the mixture was spiked with isotope-labeled internal standards. The sample was centrifuged for 20 min at 14,800 rpm (16,162 ×g), and the supernatant was filtered before instrumental analysis. The ion suppression of most analytes at ESI+ and APPI+ ranged from -28% to 78% and 40% to 100%, respectively, and the recoveries of sample preparation most ranged from 83% to 108%. Limits of detection (LODs) and limits of quantification (LOQs) of the analytes at ESI+ in oral fluid were about 0.003–0.39 ng/mL and 0.011–1.29 ng/mL, respectively. The accuracy and the precision of the method were tested on ESI+ at three spiked levels,

which the tested concentration ranged from 0.6 ng/mL to 540 ng/mL, and the bias (accuracy) and the relative standard deviation (precision, RSDs) were lower than 15% and 14.7%, respectively. The intra- and inter-day RSDs of analysis tested at ESI+ were found to be about 0.6% to 13.9% and 0.6% to 14.0%, respectively.

This study also evaluated the performance of micro-elution plates of Oasis MCX on sample preparation, an adsorbent for solid-phase extraction (SPE) with ultra-low elution volumes. The ion suppression of oral fluid using the SPE was most below 10% at ESI+ and was smaller than 20% at APPI+ for most analytes. The recovery of this procedure ranged from 59% to 86% for most analytes. The LODs of the analytes using the SPE were all below 0.91 ng/mL at ESI+ (LOQs were smaller than 3.02 ng/mL) and ranged from 0.009 ng/mL to 1.29 ng/mL for most analytes at APPI+ (LOQs ranged from 0.032 ng/mL to 4.31 ng/mL). The bias percent and the RSDs were below 25% and 16% for most analytes at ESI+, respectively.

This study investigated the separation of the 31 illicit drugs on the Ascentis Express RP-amide column. The RP-amide column is packed with fused-core particles coating with porous-shell stationary phase, which provides fast mass transfer and good separation efficiency that is comparable to a sub-2 μm UHPLC column. In addition, the backpressure of the RP-amide column was 4,000 psi (only half of that of a sub-2 μm column) at the same chromatographic conditions; therefore, it could be applied on conventional HPLC systems.

This study investigated the difference of the matrix effects and the recoveries between OraFlx Negative (an artificial oral fluid) and oral fluid was investigated in this study to evaluate if OraFlx Negative is a suitable alternative to oral fluid for method development. The result showed that the viscosity of OraFlx Negative was

much lower than that of oral fluid; besides, analytes spiked in OraFlx Negative were more susceptible to ion suppression than that in oral fluid either at ESI or APPI; therefore, OraFlx Negative may not be a suitable substitute to oral fluid for method development.

This method simplified the sample pretreatment to dilution and centrifugation; besides, SPE by micro-elution plates was also time- and labor-saving compared with traditional SPE cartridges (by eliminating the steps of post-extraction evaporation and reconstitution) and could significantly reduce the matrix effects. The isotope-dilution technique decreased the quantitative inaccuracy resulting from the analytical variations during sample preparation and from matrix effects. The chromatographic time was shortened to only 9 min per run using UHPLC (including column re-equilibration), and the sensitivity was improved for two to five times for most analytes comparing with that using a HPLC column because of the narrower peak widths of analytes, which significantly improved the signal-to-noise ratio. Therefore, this method could handle a large number of oral fluid samples containing trace amount of illicit drugs.

keyword：UHPLC/MS/MS, isotope-dilution techniques, electrospray ionization, matrix effect, atmospheric pressure photoionization, drug of abuse