Collection of oral fluid for drug testing is non-invasive, easy, and can be done under surveillance or other difficult conditions in clinics and workplace. This study analyzed four opiates and metabolite, five amphetamines, flunitrazepam and its two metabolites, and five cocaine and metabolites in the oral fluid with ultra-performance liquid chromatography/tandem mass spectrometry (UPLC-MS/MS) at selected-reaction monitoring (SRM) with isotope-dilution techniques. The most intense product ions from the most abundant precursor ions were used for quantification, and the next abundant product ions were used for confirmation. A BEH-HILIC column could not retain the 17 analytes well than that in HSS-T3 column.

This study simplified the sample preparation of oral fluid. A 100-μL sample of oral fluid was diluted with two times of deionized and distilled water (DDW) then was spiked with isotope-labelled internal standards. The sample was centrifuged for 20 min at 14,800 rpm (16,162 ×*g*), and the supernatant was collected for analysis. The recovery of sample preparation ranged from 81% to108%. For positive electrospray ionization (ESI+), the ion suppression of most analytes ranged from 28% to 78%; a post-column flow split (1:5) did not reduce the matrix effect. For positive atmospheric pressure chemical ionization (APCI+) and atmospheric pressure photoionization (APPI+), the ion suppression of most analytes ranged from 45% to 89% and from 74% to 96%, respectively.

Limits of quantification on the 17 drugs in oral fluid on ESI, APCI and APPI were ranged from 0.11−0.87 ng/mL, 0.02−0.74 ng/mL and 0.02−0.43 ng/mL, respectively. The methods were more sensitive on APCI and APPI than on ESI.

Methods were validated using spiked oral fluid at three levels on the three different ionization probes. The error percentage (accuracy) and relative standard deviations (precision) of intra-day and inter-day quantifications were most smaller than 15%.

This study extensively investigated the matrix effects of oral fluid on three ionization probes. The sample preparation was much simplified and the chromatographic time was only 7.5 min per run, with a sensitivity reached ppt levels.

Keywords: UPLC/MS/MS, isotope-dilution techniques, electrospray ionization, atmospheric pressure chemical ionization, atmospheric pressure photoionization, matrix effect, illicit drugs