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A New Ultraperformance–Tandem Mass Spectrometry Oral Fluid Assay for 29 Illicit Drugs and Medications

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Oral fluid (saliva) testing—including the numbers of tests, the analytical procedures used, and laboratories offering the service—is increasing rapidly throughout Europe, North America, and Australia (1, 2). Although the monitoring of therapeutic drugs in oral fluid offers a noninvasive means of estimating the free concentrations of drugs in plasma or serum, this technique is used infrequently (3). There currently is great interest in the use of this alternative matrix for documenting driving under the influence of drugs (DUID)[2] and for workplace and drug-treatment testing. Oral fluid can be collected under direct observation without requiring same-sex collectors and specialized collection facilities, and its use reduces the opportunities for sample adulteration. Weak bases are ion-trapped in oral fluid because of its lower pH, yielding higher concentrations and easier detection than in blood. The risk to analysts of infectious-disease exposure is lower than for blood, and the presence of the parent drug in oral fluid may provide a better correlation with ongoing pharmacodynamic effects than with urine testing (4). Oral fluid also offers improved identification of heroin use because of the frequent presence of 6-acetylmorphine and even heroin itself, whereas this biomarker has a short window of detection in urine (5).

Oral fluid testing also has disadvantages (6), as for any biological matrix. The volume of oral fluid is limited and may be reduced by drug consumption. Drug concentrations in oral fluid also are lower than in urine (e.g., benzodiazepines and cannabinoids), and their measurement therefore requires highly sensitive assays. Inhalation, smoking, oral, or insufflation administration may contaminate the oral mucosa, increasing concentrations and disrupting correlations with blood results. Another disadvantage is that excretion and concentrations vary with the pH of oral fluid given that the pH increases with the stimulation of oral fluid flow. Yet another limitation is the variation in the amounts of oral fluid collected within and between collection devices, which makes measurement of drug concentrations difficult. Such devices include buffers and surfactants to reduce drug adsorption to the container and collection pad that help improve drug recovery, but these additives dilute drug concentrations and can produce matrix interferences if oral fluid or diluted sample is injected directly into LC-MS instruments. Many investigators initially attempted direct injection or simple dilution procedures, but matrix effects frequently affected drug quantification. This consideration is important because additional preparation

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[2]Nonstandard abbreviations: DUID, driving under the influence of drugs; LC-MS/MS, liquid chromatography–tandem mass spectrometry; SPE, solid-phase extraction; THC, Δ^9-tetrahydrocannabinol.

of oral fluid samples is usually necessary, increasing assay turnaround times, costs, and labor.

The small volume of oral fluid available for a chromatographic analysis of a large number of analytes is a major limiting factor. Liquid chromatography–tandem mass spectrometry (LC-MS/MS) has proved especially effective for broadening the number of analytes at low concentrations that can be assayed simultaneously. In the previous issue of *Clinical Chemistry*, Badawi et al. reported a new analytical method for the simultaneous quantification of 29 medications and illicit drugs in oral fluid (7). This assay includes solid-phase extraction (SPE) of only 200 mg of oral fluid collected with the Saliva-Sampler™ (StatSure Diagnostic Systems) followed by ultraperformance–tandem mass spectrometry. Many of the challenges of oral fluid testing were successfully addressed with this new assay.

Each collection device was weighed before analysis to obtain the amount of oral fluid collected and to estimate drug concentration (assuming the equivalence of 1 μg/kg to 1 μg/L of oral fluid). The mean weight of 10 devices before collection was subtracted from the weight of each vial after oral fluid collection. This approach provides a means of correcting drug amounts for individual samples, because it is difficult to be certain of the amount of oral fluid collected and the ratio of the oral fluid volume to the volume of the buffer included in the device. Another approach is to determine the amount of oral fluid collected by measuring the volume of the oral fluid/buffer mixture in the device and subtracting the mean buffer volume of unused devices. The Greiner Bio-One device uses a novel tactic that determines the amount of oral fluid by measuring the dilution of a dye in the extraction solution (8). For this device, the sample donor rinses the mouth with the extraction solution, which is then expectorated with the oral fluid into a collection beaker. The absorbance of the resulting solution is read in a spectrophotometer, and the volume of oral fluid is calculated. Obtaining an objective estimate of the amount of oral fluid collected improves the ability to interpret oral fluid results. Although regulatory agencies generally state oralfluid cutoffs in terms of concentration rather than amount, the authors state that the same approach could be applied with measurements of volume rather than weight.

The matrix effect is one of the major concerns with LC-MS/MS analyses and must be fully investigated during method validation. When multiple analytes and matrix components coelute, the ionization of specific drugs may be enhanced (or, more commonly, suppressed), thereby affecting quantification. In the featured method of Badawi et al. (7), SPE of only 200 mg of oral fluid and buffer/surfactant mixture and the inclusion of deuterated internal standards for 25 of 29 analytes helped compensate for matrix effects and achieved good quantification results. It is critical to determine whether analyte recovery and assay imprecision are equivalent for authentic samples and synthetic matrix. Badawi et al. documented an increased matrix effect for synthetic oral fluid (7), a finding that could represent problems for laboratories that use calibrators and controls prepared in synthetic oral fluid for quantifying authentic oral fluid, and for proficiency surveys prepared in this matrix. Another challenge to the multianalyte approach is the longer run times that may be necessary to provide adequate dwell times for each ion to obtain accurate integration.

Other recently published LC-MS/MS assays also target similar illicit drugs and medications for either the Roadside Testing Assessment (ROSITA) or Driving Under the Influence of Drugs (DRUID) programs in the European Union (9-11). It quickly became apparent that extraction and removal of the matrix was necessary to achieve the high sensitivity and specificity required for analysis of 23–32 drugs and 9–13 deuterated internal standards in <1 mL of oral fluid. LC-MS/MS methods that use liquid–liquid extraction with the Varian Toxi-Tube A® devices (11), SPE (10) of oral fluid collected with the StatSure Saliva-Sampler collection device, and SPE of oral fluid collected with the Intercept collection

device (OraSure Technologies) (9) are available. All of the mentioned methods include quantification of Δ^9-tetrahydrocannabinol (THC), an analyte that has been difficult to incorporate into multianalyte assays yet is essential to include because it has the highest prevalence in workplace and DUID testing.

Oral-fluid testing by LC-MS/MS is becoming the standard for DUID testing and is increasingly the choice for workplace and treatment drug testing. Drug quantification of oral fluid by LC-MS/MS is reliable and accurate when properly validated, but protocols of data interpretation are still in development. Preanalytical factors that affect the interpretation of results include variable individual and pH-dependent salivary excretion, effects from ingested drugs, the lack of controlled drug-administration studies, variation in drug recovery from the collection device, and inconsistent oral fluid collection volumes.

The correlation of drug concentrations measured in oral fluid and blood/serum/plasma with impairment is an important interpretation issue. For 6 h after smoking of 18- and 36-mg THC cigarettes, the mean (SD) oral fluid/serum ratios for samples simultaneously collected from 10 participants were 46 (27) and 36 (20), respectively (12). The authors concluded that the highly variable ratios did not provide a reliable basis for correlating THC concentrations in oral fluid and serum. Wille et al. recently compared the oral fluid and blood concentrations of multiple drugs of abuse in DUID cases (13). Oral fluid/blood ratios for basic drugs such as amphetamines, cocaine, and opiates were >1, the ratios for benzodiazepines were low (0.02– 0.1) because of high protein binding and weak acid polarity, and those for THC were approximately 15. The current consensus in the field is that the wide variation in oral fluid/blood ratios in drivers thought to be under the influence of drugs does not allow reliable calculation of blood concentrations from the concentrations in oral fluid.

Additional research is needed to identify new oral-fluid biomarkers, to determine drug-detection windows, to characterize oral fluid adulteration techniques, and to evaluate analyte stability in oral fluid, but there is no doubt that oral fluid offers multiple advantages as an alternative matrix for monitoring licit and illicit drug use. Nonetheless, this new analytical method (7) should aid clinical chemists and toxicologists who are interested in implementing oral fluid analysis for DUID, workplace, or drug-treatment testing.

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