LC/MS Analysis of Comprehensive Drugs in Oral Fluid Using SEC Tips

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HIGHLIGHTS: Sensitive, reduced solvent volumes

SEC

INTRODUCTION

Many LC/MS methods require comprehensive analysis, which is contrary to conventional sample preparation. Instead of separating compounds based on chemical differences, it is possible to remove interferences such as phospholipids, surfactants, and proteins by size exclusion chromatography (SEC). In this application, we demonstrate the utility of 300 µL SEC Tips to isolate drugs from oral fluid buffer solutions. The SEC Tips are dry initially, and are readily swollen into a gel by simple capillary action - a patent-pending method known as INTip™ Swelling. The pores of the SEC resin are too small for the larger compounds such as surfactants and phospholipids, so these interferences pass through the resin quickly and are readily separated from common drugs and metabolites. We present limits of detection that show the usefulness of this method for meeting the suggested guidelines for oral fluid drug testing.

MATERIALS AND METHODS

SEC Tips (300 µL size, manual format from DPX Technologies, Columbia, SC) were placed in a vial of PBS buffer and allowed to swell (less than 1 min). After adding 50 µL PBS to gravity flow to equilibrate the gel (app. 2 min), spiked negative Quantisal™ oral fluid buffer (from 1 µL to 500 µL) (Abbott, Pomona, CA) was added and allowed to flow to waste. Then 70 µL of solvent (20% or 50% methanol) was added as void volume to remove large compounds. Subsequently, 200 µL of solvent (20% or 50% methanol) was added and collected for direct injection into the LC/MS system. Analysis was performed using a Restek biphenyl LC column, Shimadzu LC-40DXR system and SCIEX 6500+ triple quadrupole instrument.

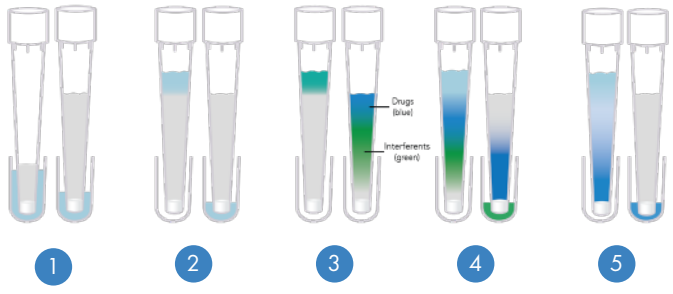

Figure 1. *Sample preparation using 300 µL SEC Tips, following the method outlined in* ***Table 1****.*

Table 1. *Method using 300 µL SEC Tips for sample preparation for LC/MS analysis of comprehensive drugs in oral fluid.*

1	INTip Swelling	Allow to swell via capillary action in PBS.
2	Equilibrate	Add 50 µL PBS. Allow to gravity flow.
3	Load Sample	Load desired sample volume. Allow to gravity flow to waste.
4	Remove Interferents	Add 70 µL solvent. Allow to gravity flow to waste.
5	Collect	Add 100-200 µL solvent. Collect for direct injection into LC/MS.

RESULTS

Drugs of interest for oral fluid drug testing by Substance Abuse and Mental Health Services Administration (SAMHSA) guidelines include delta-9-tetrahydrocannabinol (THC), cocaine/benzoylecgonine, codeine/morphine, hydrocodone/hydromorphone, oxycodone/oxymorphone, 6-acetylmorphine, phencyclidine (PCP), amphetamine/methamphetamine, and methylenedioxymethamphetamine/methylenedioxyamphetamine (MDMA/MDA). All of these compounds showed similar rates of passing through the SEC Tips, while THC (as well as benzodiazepines) was much slower. Plots of area counts for some representative drugs are shown (**Figure 2**) vs. volume of collection solvent added.

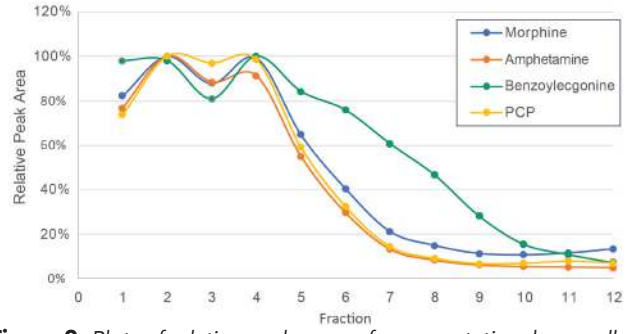

Figure 2. *Plots of relative peak areas of representative drugs collected in fractions of 10 µL aliquots after passing the void volume of 70 µL.*

It was determined that THC could not be efficiently collected in 20% methanol, but 50% methanol worked well. The void volume of the SEC Tips used was pre-determined to be 65 µL (from a study of dye-stained proteins), so 70 µL was used as the void volume to ensure efficient removal of surfactants (**Figure 3**). With most of the drugs having abundance collected between 90 and 120 µL, the acceptable loading volume for recovery purposes

would be about 30 to 40 µL. However, much higher volumes of sample can be loaded to increase the recovery of THC. Extracted ion chromatograms for THC with various volumes of sample loaded is shown (**Figure 4**), indicating that the drug is concentrating on the gel during loading.

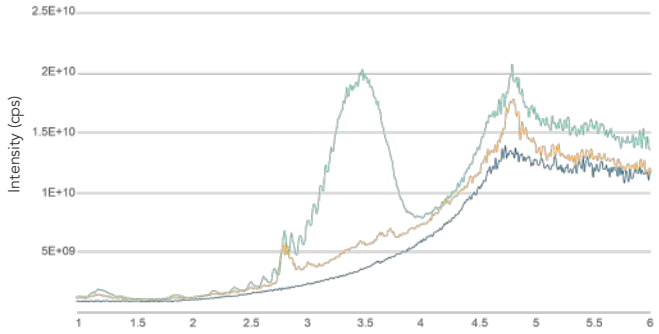

Figure 3. *Total ion chromatograms of: a) 20 µL oral fluid diluted to 200 µL with 50% methanol (green); b) 100 µL 50% methanol fraction (diluted to 200 µL) collected post SEC cleanup of 400 µL negative oral fluid buffer (yellow); c) 50% methanol blank solvent (blue).*

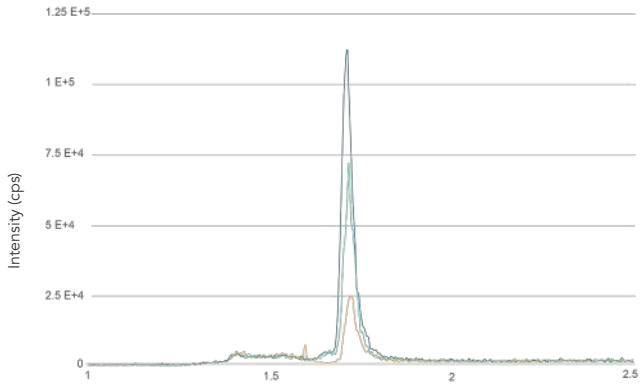

Figure 4. *Extracted ion chromatograms of THC from collected fractions of: a) 250 µL oral fluid sample (yellow); b) 500 µL oral fluid sample (green); and c) 1 mL oral fluid sample (blue).*

For a single chromatographic analysis of all of the drugs of interest, we have found loading 400 µL of the oral fluid buffer, passing 70 µL 50% methanol for the void volume, and collecting 200 µL of 50% methanol allows for sensitive detection of all the analytes of interest. Limits of detection are provided (**Table 2**) for each drug with this method.

Table 2. *Limits of detection and SAMHSA cutoffs for drugs of abuse in oral fluid.*

Analyte	Screen Cutoff (ng/mL)	Confirmation Cutoff (ng/mL)	LOD (ng/mL)
Delta-9-tetrahydrocannabinol (THC)	4.0	2.0	0.5
Cocaine/benzoylecgonine	15.0	8.0	0.5
Codeine/morphine	30.0	15.0	1.0
Hydrocodone/hydromorphone	30.0	15.0	2.0
Oxycodone/oxymorphone	30.0	15.0	2.0
6-monoacetylmorphine	4.0	2.0	0.5
Phencyclidine (PCP)	10.0	10.0	0.5
Amphetamine/methamphetamine	50.0	25.0	1.0
Methylenedioxymethamphetamine (MDMA)/Methylenedioxyamphetamine (MDA)	50.0	25.0	0.5

DISCUSSION

SEC Tips can be used to analyze drugs in oral fluid samples by removing matrix interferences including surfactants. Sensitive analysis of drugs like THC can be achieved without solvent evaporation or the use of large volumes of organic solvent.