Analysis of Low-Level Acidic, Basic, & Neutral Drugs of Abuse in Oral Fluids via LC-MS/MS Detection

UCT Part Numbers

CSXCE103
Clean Screen® Xcel I
130 mg, 3 mL Column

SPHPHO6001-10
Select PH Buffer Pouches
100 mM Phosphate Buffer pH 6.0

SLPFPP100ID21-3UM
Selectra® PFPP HPLC Column
100 X 2.1 mm, 3 µm

SLPFPPGDC20-3UM
Selectra® PFPP Guard Column
10 X 2.0 mm, 3 µm

SLGRDHLDR
Guard Column Holder

VMFPPM13V2
Positive Pressure Manifold 2.0
With 13x100 Collection Rack

VMFSPEDRY-48
SPeDRY® Solvent Evaporator

VMFSPEDRYCR-4851
SPeDRY® Sample Tray, GC Vial

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Summary:

Oral fluid is a non-invasive approach to sample collection for drug screening. It is a quick procedure that is easy to collect in the field, difficult to adulterate and provides a more accurate indication of recent drug use. One of the biggest challenges in oral fluid testing is overcoming the diverse and typically unknown composition of the stabilization buffer, which, if not effectively cleaned up prior to injection can hinder accurate data analysis and/or result in instrument downtime.

This application note describes a simple and rapid solid-phase extraction (SPE) procedure for the analysis of a broad range of acidic, neutral, and basic drugs in oral fluids using UCT's Clean-Screen® XCEL I column. The functionality of the SPE sorbent effectively retains the analytes of interest while removing undesired matrix components resulting in cleaner extracts, greater method robustness and improved LC-MS/MS analysis from which both illicit and prescribed drugs can be accurately detected. HPLC separation was carried out using UCT's Selectra® PFPP column prior to detection by LC-MS/MS. The pentafluorophenylpropyl phase can undergo dipole–dipole, and pi–pi interactions, imparting unique selectivity and retention mechanisms to the column that distinguish it from a traditional biphenyl phase. Excellent recoveries (102-129%) were obtained in all cases, except for benzodiazepines which experience losses during the wash steps at the pH employed in this procedure. It is recommended to utilize an internal standard for accurate measurement of benzophenones in this application.

Sample Pretreatment:

1. To 1 mL sample (oral fluid/preservative buffer working solution), add appropriate amount of internal standard.

SPE Procedure:

1. APPLY SAMPLE DIRECTLY TO SPE Column: Load at 1 to 2 mL/minute

2. WASH:

   a) 1 x 2 mL pH 6.0 Phosphate Buffer.

   b) 1 x 3 mL DI H_2O.

   c) 1 x 3 mL 25% Acetonitrile.

   d) Dry column for 10 minutes at 80-100 psi.

3. ELUTE (Acidic/Neutral Drugs):

   a) 1 x 2mL of Hexane: Ethyl Acetate (80:20) directly into 12x75mm culture tubes. Place tubes aside for collection of basic drugs as directed in step 5.

4. WASH COLUMN (this step will mitigate matrix effects introduced by the oral fluid

   a) 1 x 2mL of Methanol: DI H_2O.

   b) Dry columns for 5-10 min. at 80-100 psi.

5. ELUTE (Basic Drugs):

   a) 1 x 2mL of Methanol: NH_4OH (98:2) directly into the same 12x75mm culture tubes used in collection of acidic/neutral drugs

6. DRY ELUATE:

   a) Evaporate the combined eluent in culture tubes to dryness.

   a) Add 100µL 1% HCl after 4 min. of drying.

7. RECONSTITUTE

   a) Reconstitute sample in 200 µL MeOH: H_2O (50:50)

   b) Inject 5µL

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LC-MS/MS Parameters:

System: Shimadzu LC30AD w/ MS-8050
Column: Selectra® PFPP HPLC Column 100 X 2.1 mm, 3 µm
Guard Column: Selectra® PFPP Guard Column 10 X 2.0 mm, 3 µm
Column Temperature: 40 °C
Column Flow Rate: 0.3 mL/min
Injection Volume: 5 µL
Auto-sampler temperature: 10 °C

Gradient Program:		
Time (min)	% Mobile Phase A 5 mM Ammonium Formate with 0.1% Formic Acid in H_2O	% Mobile Phase B 5 mM Ammonium Formate with 0.1% Formic Acid in CH_3OH
0	100	0
8.0	0	100
10.0	0	100
10.1	100	0
15.0	100	0

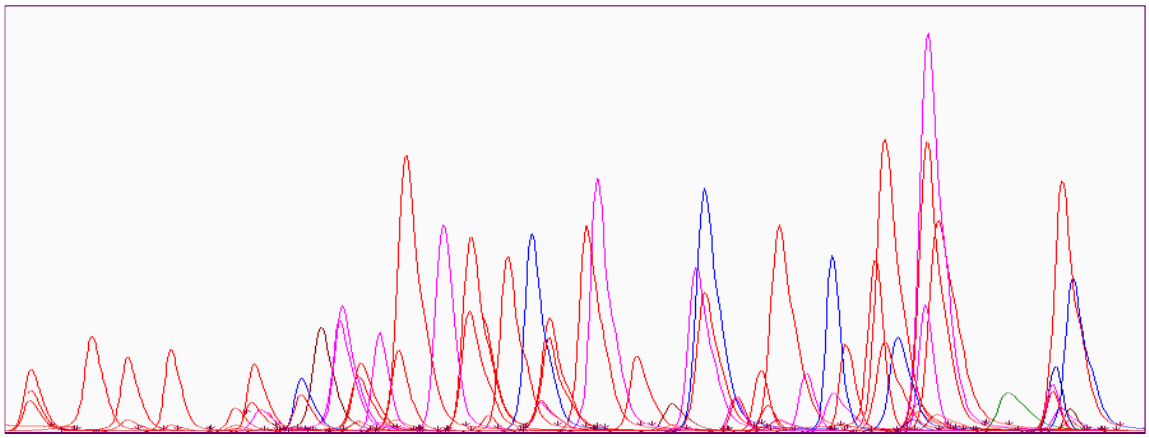

Figure 1: Chromatogram of Solvent Standard, 5 ng/mL

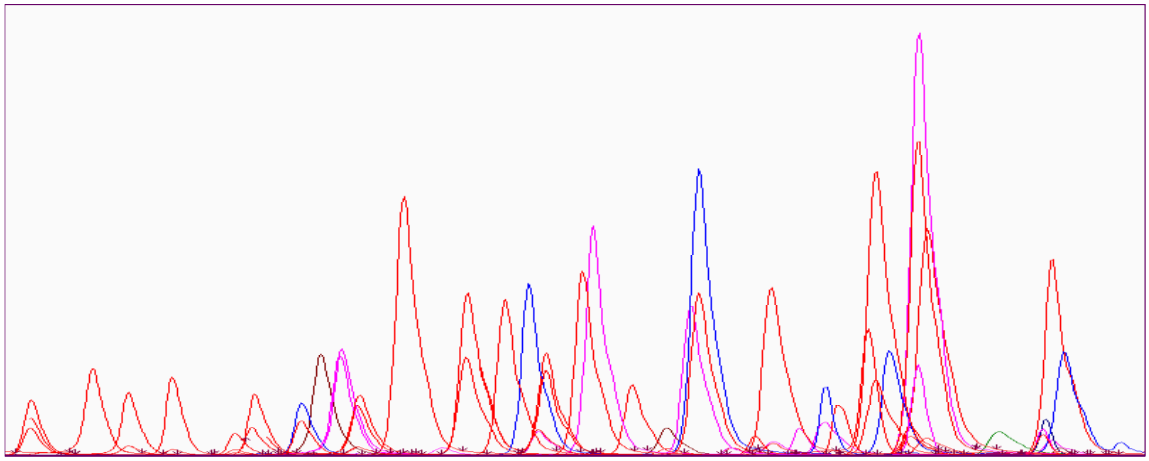

Figure 2: Chromatogram of Extracted Standard, 5 ng/mL

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Absolute Recovery - Oral Fluid				
Analyte	**1ng/mL (n=3)**	**Rel. Std Dev %**	**5ng/mL (n=3)**	**Rel. Std Dev %**
Alphahydroxy Alprazolam	35%	5%	42%	7%
Alprazolam	129%	5%	65%	1%
Amitriptyline	120%	1%	101%	7%
Amphetamine	118%	2%	100%	5%
Atenolol	117%	6%	99%	3%
Bupivicaine	117%	5%	92%	2%
Buprenorphine	119%	3%	98%	7%
CBD	103%	3%	84%	6%
CBN	117%	2%	90%	6%
Clonazepam	118%	5%	105%	7%
Cocaine	117%	5%	91%	5%
Codeine	120%	2%	99%	7%
COOH-THC	108%	9%	86%	4%
Dextorphan	117%	2%	91%	4%
Diazepam	107%	6%	101%	6%
EDDP	117%	3%	86%	4%
EME	114%	2%	96%	2%
Fentanyl	115%	2%	94%	6%
Hydrocodone	116%	3%	94%	7%
Hydromorphone	115%	2%	98%	8%
Imipramine	119%	1%	94%	4%
Ketamine	119%	3%	94%	3%
6 MAM	113%	3%	95%	3%
Lorazepam	36%	8%	40%	7%
MDPV	116%	3%	90%	6%
Meperidine	118%	1%	93%	6%
Methamphetamine	118%	2%	94%	4%
Methylphenidate	118%	6%	94%	3%
Midazolam	118%	5%	85%	6%
MDEA	120%	5%	95%	7%
MDMA	115%	4%	93%	6%
Morphine	113%	2%	96%	5%
Naltrexone	117%	6%	98%	6%
Norbuprenorphine	109%	3%	99%	7%
Norcodeine	115%	6%	105%	9%
Nordiazepam	102%	3%	101%	2%
Norketamine	122%	2%	100%	7%
Normeperidine	112%	4%	91%	5%
Nortriptyline	117%	1%	93%	4%
OH-THC	113%	7%	90%	5%
Oxazepam	51%	7%	55%	7%
Oxycodone	117%	4%	98%	5%
Oxymorphone	113%	3%	100%	4%
PCP	104%	8%	100%	6%
Phentermine	117%	2%	99%	5%
Temazepam	47%	5%	56%	8%
THC	114%	3%	92%	9%
Tramadol	117%	4%	93%	4%
Zolpidem	114%	1%	93%	2%

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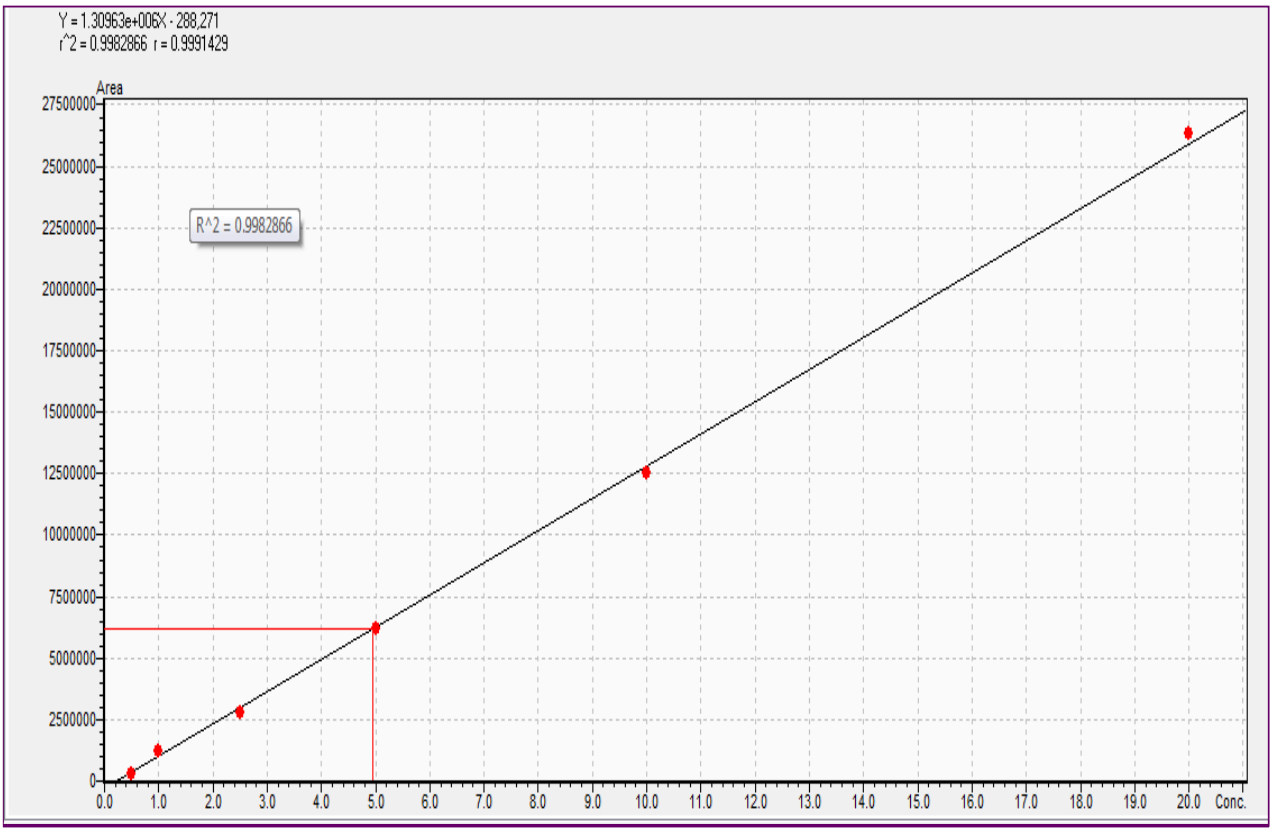
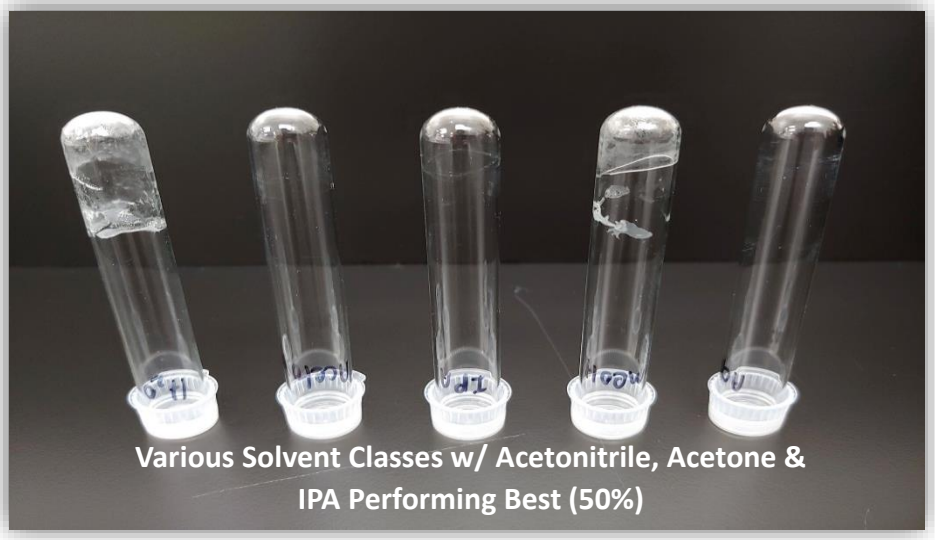


Figure 3: Hydromorphone Calibration Curve (0.5, 1, 2.5, 5, 10, 20ng/mL) R^2= 0.9982

Conclusion:

Use of UCT's protocol for the screening of oral fluids is efficient in the extraction of Acidic/Neutral/Basic Drugs while minimizing matrix effects introduced by oral fluid buffer components. The use of the highly selective Clean Screen® Xcel I SPE column allows for increased analyte sensitivity, enhanced specificity for selected functional groups, low organic solvent consumption, and optimizied chromatographic resolution.

In this procedure, a wash step using 3mL DI H_2O followed by 3mL 25% Acetonitrile was used prior to elution of analytes if interest. The choice of 25% acetonitrile was optimal for removal of dyes and buffer salts in the storage mechanism while ensuring optimal recoveries (see pictures below for concentrated results using different wash solvent systems).



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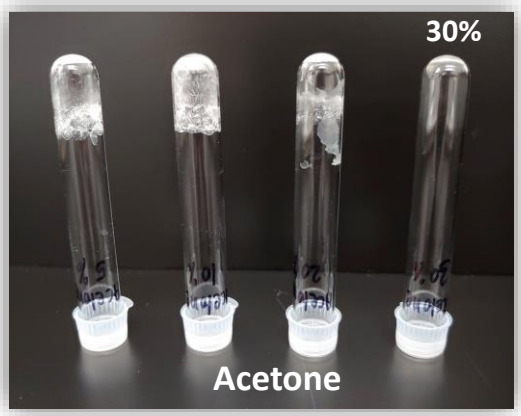

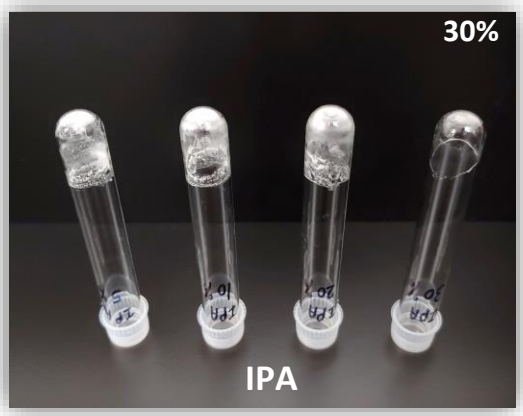

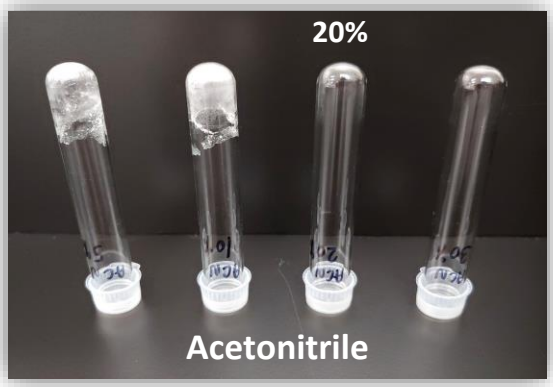

Also critical to this application was the use of a two-step elution procedure which allowed the wash of oral fluid buffer constituents from the column between elution of acidic/neutral, and basic drug eluents.

Excellent results were obtained for all drug classes, except for benzodiazepines which experienced loss during the wash steps at the pH employed in this procedure. Utilization of an internal standard will aid in accurate measurement of the benzodiazepine compounds studied in this application.

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