Screening Method for Barbiturates, Benzodiazepines and THC in Oral Fluid by LC-MS/MS using Styre Screen® HLD SPE Column

UCT Part Numbers

SSHLD063
Styre Screen® HLD
60 mg, 3 mL

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

SLPFPPGDC20-3UM
Selectra® PFPP Guard Column
10 X 2.1 mm, 3 µm

SLGRDHLDR-HPOPT
Guard Column Holder

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. As the cut-off levels for oral fluid are usually very low, sensitive screening and confirmatory assays are required for analysis. This also often necessitates the need to include a concentration step in the method to enhance sensitivity. The typically unknown buffer composition used to preserve oral fluid makes it a considerably challenging matrix to deal with and therefore it becomes imperative that the method of choice is robust and able to yield the cleanest extract possible.

This featured application note exploits the highly retentive but universal nature of UCT's polymeric Styre Screen® HLD sorbent for the analysis of a wide range of neutral drugs in oral fluid. By targeting the hydrophobic properties of the neutral drugs, multiple drug panels can be simultaneously extracted. The procedure is extremely simple to perform and does not require any complex recipes of chemical solutions. HPLC separation of the neutral drugs was carried out using UCT's Selectra® PFPP column prior to detection by LC-MS/MS. The Pentafluorophenylpropyl (PFPP) 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.

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SPE Procedure:

1. Sample Preparation

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

2. Condition the Styre Screen® HLD column

a) 1 x 1 mL CH_3OH
b) 1 x 1 mL H_2O

3. Apply Sample

a) Load at 1 to 2 mL/minute

4. Wash Column

a) 1 x 2 mL H_2O
b) 1 x 2 mL 40% CH_3OH in water

5. Dry Columns

a) Dry for 5 mins at 80-100 psi

6. Elute

a) 1 x 2 mL Ethyl acetate

7. Evaporate

a) Evaporate the eluent to complete dryness

8. Reconstitute

a) Reconstitute sample in 200 µL 50:50 CH_3OH: H_2O

Note: Add CH_3OH first, vortex and then add H_2O

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LC-MS/MS Parameters	
LC-MS/MS System	Shimadzu LCMS-8050
HPLC Column	Selectra® PFPP (100 X 2.1 mm, 3 µm) (PN: **SLPFPP100ID21-3UM**)
Guard Column	Selectra® PFPP (10 X 2.1 mm, 3 µm) (PN: **SLPFPPGDC21-3UM**)
Column Temperature	40 °C
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 Acetate in Water	**% Mobile Phase B** Methanol
0	100	0
8.0	0	100
10.0	0	100
10.1	100	0
12.0	100	0

Chromatogram:

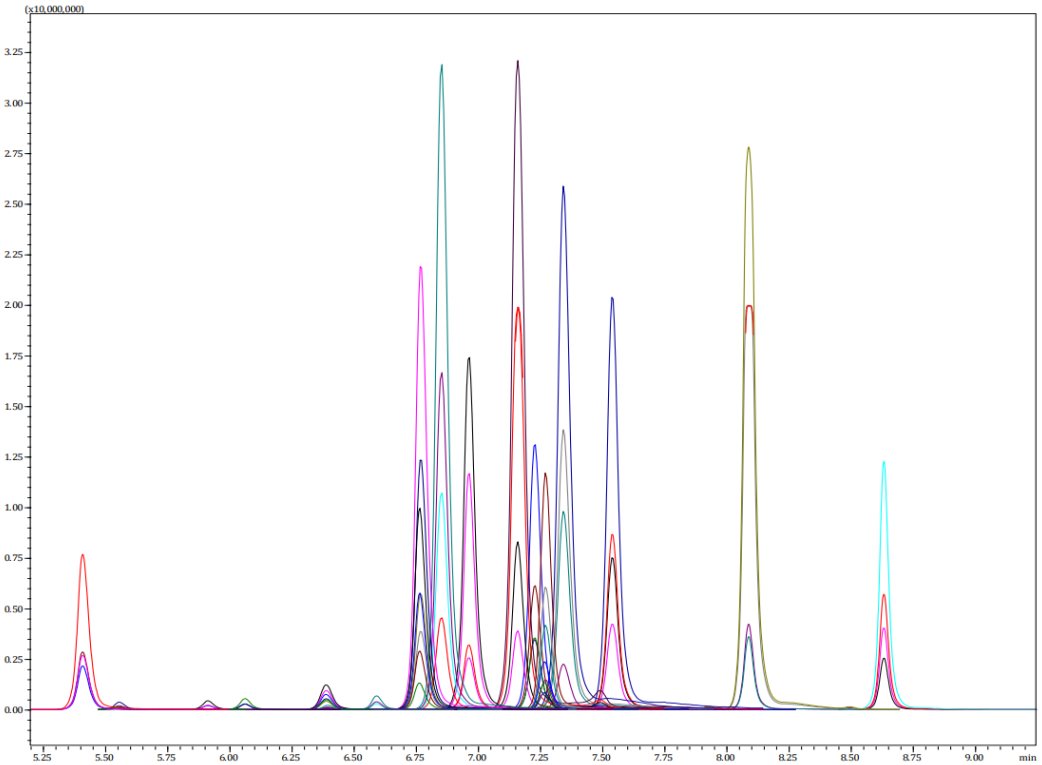

Figure 1. Chromatogram of 100 ng/mL extracted sample

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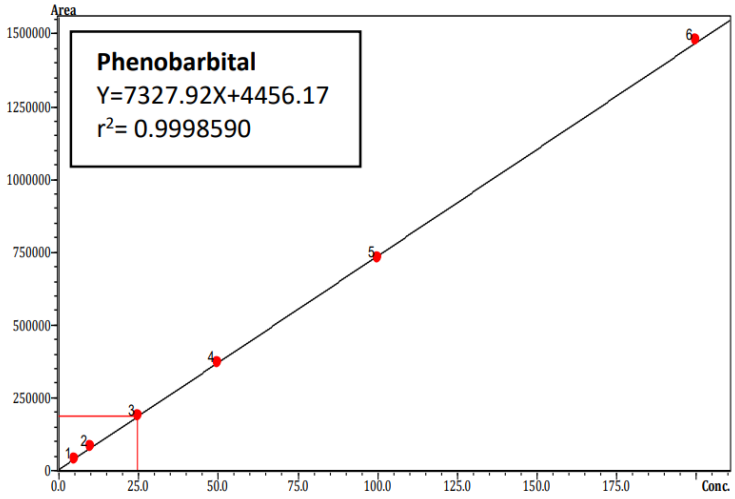
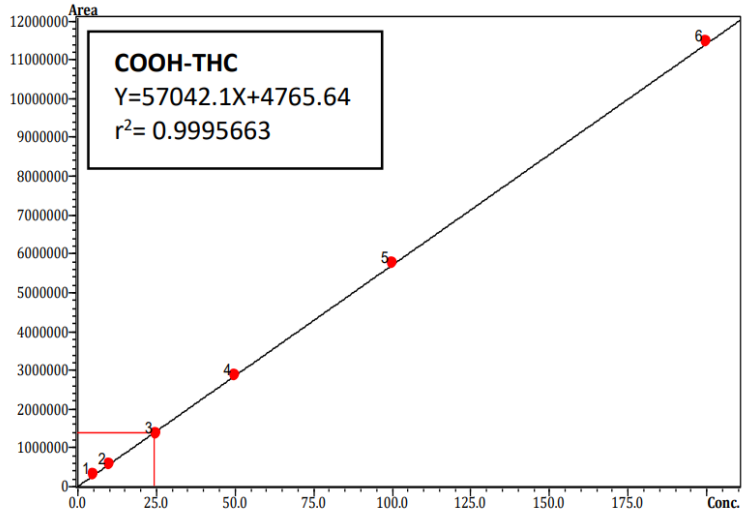
Matrix Matched Calibration Curve:





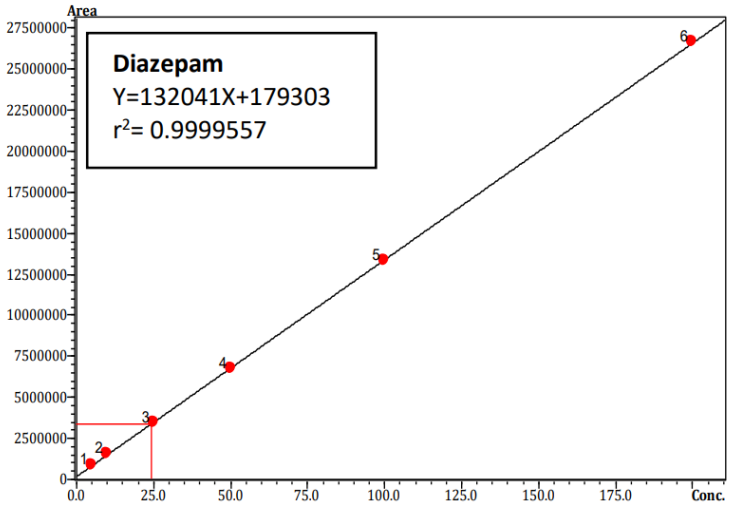


Figure 2. Calibration Curve Examples (5, 10, 25, 50, 100 & 200 ng/mL)

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Results:

Recovery - Barbiturates				
Analyte	**25 ng/mL (n=3)**	**Rel. Std Dev (%)**	**100 ng/mL (n=3)**	**Rel. Std Dev (%)**
Amobarbital	93%	4.1	89%	0.6
Butabarbital	94%	5.6	87%	1.6
Butalbital	93%	3.2	88%	0.7
Pentobarbital	94%	4.1	89%	0.8
Phenobarbital	99%	3.1	93%	0.5
Secobarbital	93%	3.3	85%	0.5

Recovery - Benzodiazepines				
Analyte	**25 ng/mL (n=3)**	**Rel. Std Dev (%)**	**100 ng/mL (n=3)**	**Rel. Std Dev (%)**
Clonazepam	77%	2.7	79%	1.0
Diazepam	78%	1.5	82%	0.7
Lorazepam	77%	2.4	80%	1.0
Nordiazepam	65%	2.9	70%	2.2
Oxazepam	72%	2.3	72%	1.0
Temazepam	71%	2.8	74%	2.1

Recovery - THCs				
Analyte	**25 ng/mL (n=3)**	**Rel. Std Dev (%)**	**100 ng/mL (n=3)**	**Rel. Std Dev (%)**
COOH-THC	63%	7.8	67%	3.0
OH-THC	75%	3.4	80%	6.4
THC	80%	3.3	76%	7.9

Note: Recovery was calculated using a neat solvent calibration curve and without the use of internal standards. The use of isotopically labelled internal standards and/or matrix-matched standard would further improve the results obtained with this method.

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Conclusion:

Good recoveries (absolute) and low relative standard deviation (RSD) values demonstrate that this method is efficient in extracting neutral drugs from oral fluid samples. The use of highlycrosslinked polymeric Styre Screen® HLD sorbent allows for increased analyte sensitivity and enhanced specificity for selective functional groups. The resulting cleaner extracts helps to significantly reduce matrix effects, clogging of the HPLC column, and overall instrument downtime. The easy clean-up procedure not only extracts barbiturates, benzodiazepines and THCs, but also allows the user to add other neutral drugs into the mix.

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