Mouth-Watering Prospects: Generic Extraction of Drugs of Abuse from Oral Fluid Samples

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Abstract

Saliva collection is a robust, high integrity and reliable method for on-site drug sampling, where sample manipulation or adulteration is difficult. Most drugs of abuse extraction methods for saliva have historically focussed on liquid-liquid extraction and simple SPE sorbents which require extensive method development. Saliva testing has some limitations due to sample volume and drug concentration often being low. Additives present in the collection device can also cause sensitivity issues in LC MS/MS. We describe an innovative polymeric SPE sorbent, which has excellent extraction properties and uses generic methods with excellent reproducibility. It also has special surface chemistry that allows for exclusion of saliva collection device preservatives. Examples of extractions on LC MS/MS and GC MS/MS are shown for several drug classes.

Introducing Plexa DAS: Hydrophilic Sorbent for Oral Fluid SPE

Plexa DAS is a highly engineered hydrophilic polymer that contains cation exchange sites and other oligomeric chemistries which allow for excellent phase transfer of analytes out of saliva matrices. Specially engineered pores exclude endogenous material such as salivary proteins, and excellent elution profiles allow isolation of analytes in small elution volumes.

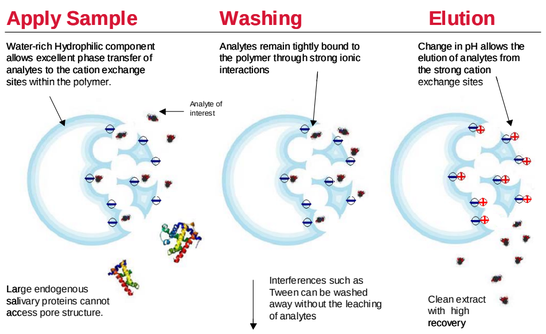


Figure 1 The three stages of extraction and key functional properties when using Plexa DAS.

Removal of Oral Collection Device Additives

The integrity of saliva sample is a vital component of drug analysis. All of the major saliva sampling devices contain a preservative solution which stabilizes and buffers testing samples prior to testing. One of the major components in the storage liquor, Tween 20, is known to cause issues with ion suppression and column build-up.

In the study below, a series of well known oral fluid SPE devices were tested against Plexa DAS for Tween removal properties. The top chromatogram control was fluid from one of the most common testing devices in the industry. It is very clear that Plexa DAS shows no Tween contamination in the final elution extract, whereas other materials still retain and elute high levels of the preservative.

Column: Pursuit XRs $^{2.8}$ C18 30 x 2.0 mm
Mobile Phase : A: 0.1% Formic Acid B: Methanol
Isocratic: t = 0-3 min, 20% A : 80% B

All samples dried and reconstituted in 100 µL of 1:1 0.1% aq. formic acid: MeOH.

MS conditions:

Compound	Q1	Q3	CE
Tween 20	227.1	95.0	15.0V

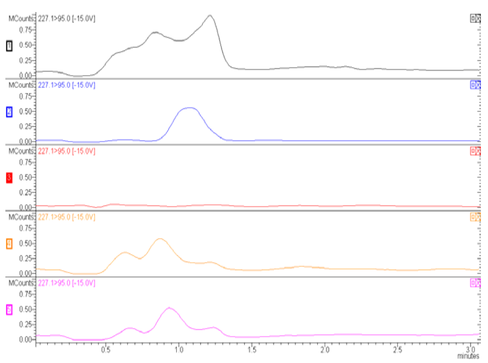


Figure 2 LC/MS responses for Tween 20 in elution fractions of several extraction devices including Plexa DAS.

Analysis of Opiates in Oral Fluid using Plexa DAS and LC/MSMS

The opiates are a commonly screened class of molecules in most drugs of abuse laboratories. We have developed a very simple and robust extraction protocol that covers all commonly observed opiates in drug screening. Extraction efficiency and reproducibility are excellent across the board. The very important 6-monoacetylmorphine (6-MAM) metabolite was extracted at 1 ng/mL levels while the other members of the group (morphine, codeine, hydrocodone and hydromorphone) were extracted at the 10 ng/mL level.

Column: Pursuit UPS $^{2.4}$ 50 x 2 mm
Mobile Phase: A: 0.1% aqueous formic acid B: MeOH
Pump Program: Flow rate 200 µL/ min.

Time 0 - A: 95%, B: 5%
Time 3:15 - A: 75%, B: 25%
Time 4:00 - A: 20%, B: 80%
Time 4:30 - A: 20%, B: 80%
Time 4:31 - A: 95%, B: 5%
Time 5:30 - A: 95%, B: 5% Run time = 5:30 min

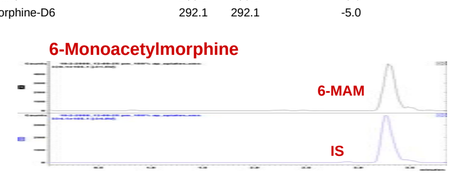
Samples were evaporated to dryness and reconstituted in 100 µL 0.1% formic acid.

Inj Vol: 10 µL
Instrument: Varian 320-MS LC/MS

MS Conditions

Compound	Q1 ion	Product ion	CE (eV)
6-Monoacetylmorphine	328.1	165.1	-31.5
IS: 6-Monoacetylmorphine-D6	334.1	165.1	-34.0
Codeine	300.1	300.1	-5.0
IS: Codeine-D6	306.1	306.1	-5.0
Hydrocodone	300.1	199.1	-26.5
IS: Hydrocodone-D6	306.1	202.1	-27.5
Hydromorphone	286.1	185.1	-25.5
IS: Hydromorphone-D6	292.1	185.1	-27.5
Morphine	286.1	286.1	-5.0
IS: Morphine-D6	292.1	292.1	-5.0

	Plexa DAS (30 mg)
Sample	**0.5 mL oral fluid**
Pretreatment	**Dilute 1 mL 2% formic acid**
Conditioning	**1. 1 mL MeOH** **2. 1 mL H_2O**
Washes	**1. 1 mL 2% formic acid** **2. 1 mL MeOH** **3. 1 mL EtAc** **Dry 5 min**
Elution	**1 mL 98:2 EtAc:NH_3**



Amt ng/mL	% Rec
1.04	104%
1.01	101%
1.00	100%
0.94	94%
1.06	106%
1.11	111%
Average Rec	**105%**
RSD	**6%**

Amt ng/mL	% Rec
10.0	100%
10.8	108%
10.1	101%
9.7	97%
9.5	95%
9.6	96%
Average Rec	**99%**
RSD	**5%**

Amt ng/mL	% Rec
9.5	95%
10.6	106%
9.3	9.3%
10.1	101%
10.1	101%
9.1	91%
Average Rec	**98%**
RSD	**6%**

Amt ng/mL	% Rec
10.0	100%
9.2	92%
10.5	105%
9.6	96%
10.3	103%
9.7	97%
Average Rec	**97%**
RSD	**5%**

Amt ng/mL	% Rec
10.1	101%
10.2	102%
9.7	97%
9.3	93%
100	100%
9.3	93%
Average Rec	**97%**
RSD	**5%**

Figure 3 Data bundle showing extraction, LC and MS detection method for a suite of opiates extracted from saliva using a Plexa DAS cartridge.

Analysis of THC in Oral Fluid using Plexa DAS and LC MS/MS

Tetrahydrocannabinol, also known as THC, is the main psychoactive substance found in the cannabis plant. THC has relatively quick clearance from saliva, therefore more sensitive analysis and improved extraction methods are attractive in drug screening laboratories. A simple Plexa DAS method using three generic wash steps affords excellent recoveries of THC with excellent RSDs at concentrations of 0.5 ng/mL.

Column: Pursuit™ Ultra 2.8, 50 x 2 mm
Mobile Phase: A: 0.1% aqueous formic acid. B: 0.1 % formic acid in ACN
Pump Program: Flow rate 200 µL/ min

0 min 80% A, 20% B
5 min 10% A, 90% B, hold 1 min
6 min 80% A, 20% B, hold 1 min
Run time = 7.0 min

Samples were evaporated to dryness and reconstituted in 100 µL methanol.
Inj Vol: 10 µL
Instrument: Varian 320 LC MS/MS

MS Conditions:

Compound	Q1 ion	Product ion
THC	315.1	293.1
IS: THC-IS	318.1	296.1

	Plexa DAS (30 mg) LC (LC/MS compatible conditions)
Sample	**0.5 mL oral fluid**
Pretreatment	**Dilute 1 mL** **2% 0.5 mL KH_2PO_4, 0.8 mL MeOH**
Conditioning	**1 mL MeOH**
Washes	**1. 1 mL H_2O** **2. 1 mL 40% MeOH** **Dry 5 min** **3. 0.2 mL hexane**
Elution	**1 mL 80:20 hexane:EtAc**

Amt ng/mL	% Rec
0.523	105%
0.511	102%
0.468	94%
0.502	100%
0.518	104%
0.523	105%
Average Rec	**102%**
RSD	**4.1%**

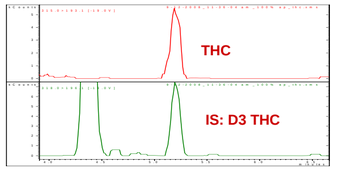


Figure 4 Data bundle showing extraction, LC and MS detection methods for saliva samples containing 0.5 ng/ mL of THC.

Analysis of PCP in Oral Fluid using Plexa DAS and GC MS/MS

Phencyclidine (PCP) is a dissociative drug formerly used as an anaesthetic agent, exhibiting hallucinogenic and neurotoxic effects. PCP is registered as a Class A substance in the United Kingdom. Extraction and detection of PCP can be difficult when present in low levels. In the GC MS/MS method described below, Plexa DAS extracts PCP with excellent recovery and RSD at a concentration of 1 ng/mL. The same extraction method can also be used in LC MS/MS derivatisation.

Column: Factor Four VF-5ms 0.15 mm x 20 m x 0.15 µm
Oven Temp: 150 °C hold for 1 min
150 – 325 °C at 25 °C/min
Run time = 8 min
Samples were evaporated to dryness and reconstituted in 25 µL EtAc
Inj Vol: 2 µL
Instrument: Varian 320-MS GC/MS
Inj Temp: 280 °C, transfer line: 280 °C
Split at 3 mL/min. 80 psi pressure pulse for 1 min

MS Conditions

Compound	Quant ion	Qualifiers
PCP	200.2	242.6,186.0
IS: PCP-D5	205.2	246.3

	Plexa DAS (30 mg) LC (LC/MS compatible conditions)
Sample	**0.5 mL oral fluid**
Pretreatment	**Dilute 1 mL** **2% formic acid**
Conditioning	**1. 1 mL MeOH** **2. 1 mL H_2O**
Washes	**1. 1 mL H_2O** **2. 1 mL MeOH** **3. 1 mL EtAc** **Dry 5 min**
Elution	**1 mL 98:2 EtAc:NH_3**

Amt ng/mL	% Rec
1.044	104%
0.968	97%
0.975	98%
1.021	102%
0.93	93%
0.908	91%
Average Rec	**97%**
RSD	**5.3%**

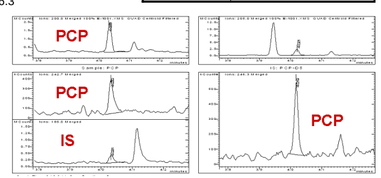


Figure 5 Data bundle showing extraction, GC and MS detection methods for a saliva sample containing 1 ng/ mL PCP.

Conclusion

Plexa DAS is a highly versatile, robust and reproducible extraction device for saliva drugs of abuse analysis. The specially designed sorbent allows for excellent phase transfer and extraction of analytes while other functionalities help exclude interferences such as salivary proteins and oligomeric preservatives such as Tween 20, which may cause ion suppression in LC MS/MS applications.

Method ruggedness allows similar extraction protocols to be used in LC and GC applications. A broad range of drug suites can be extracted using this sorbent with minimal method development.