ApplicationNOTE

Waters

SOLID-PHASE EXTRACTION AND TANDEM LC/MS DETERMINATION OF DRUGS OF ABUSE IN PRESERVED SALIVA

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INTRODUCTION

Saliva is a less invasive alternative to urine or blood testing in forensics analysis for drugs of abuse. In terms of job site testing, employers seek alternatives for testing employees that are easy to use and that are convenient for random testing. Oral testing devices are now becoming a popular alternative that fits the employers' criteria and allows the employee to be tested in a more dignified manner.

Oral fluid testing devices are designed for detection of recent drug usage, typically within 1 to 3 days of administration. Drug residue levels found in saliva are typically lower than the corresponding levels in other biological fluids such as blood or urine. Therefore, quantification limits for saliva samples must be considerably lower than those for urine and blood. For our study the trace analyte levels were tested in the low ng/mL range.

GC/MS is most commonly used to detect and quantify these types of analytes even though LC/MS is a simpler analytical approach that requires no derivatization. However, the saliva collection fluid contains ingredients (stabilizers and preservatives) that present some interference problems for LC/MS. This application uses an effective mixed-mode (cation-exchange) SPE procedure to provide effective sample cleanup for tandem LC/MS determination of basic drugs of abuse in preserved saliva.

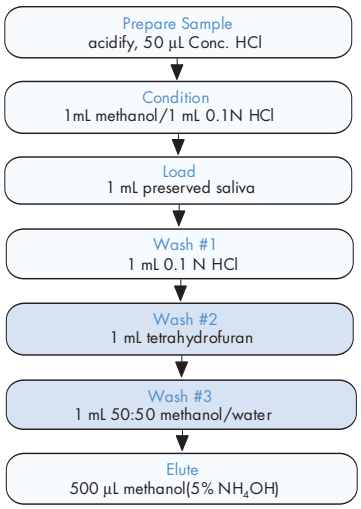
METHODS

Sample Preparation:

A 1 mL preserved saliva sample was acidified using 50 µL of concentrated HCl. The sample was then loaded onto a preconditioned 30 mg 1 cc SPE cartridge (Oasis® MCX) at 2 mL/min. Clean-up was accomplished with successive 1 mL washes of 0.1 N HCl, THF, and 50:50 methanol:water. The cartridge was dried by passing air through the sorbent bed for 5 minutes before elution with 0.5 mL of 5% ammonia in methanol. The extracted sample was diluted with 1 mL of water before injection onto the LC.

LC/MS Conditions:

The analytes were separated using a gradient mobile phase consisting of 10 mM ammonium bicarbonate and methanol in less than 15 minutes (flow: 0.3 mL/min; temperature: 30 °C; column dimensions 2.1 x 150 mm). Two parent-to-daughter transitions were monitored to confirm the identity of the analytes.



The Oral Collection Device Fluid contains stabilizing salts, non-ionic surfactants for surface wetting and antibacterial agents.

Wash #1 locks the analytes and matrix onto the sorbent using ion-exchange mechanism. Any salts and weakly retained species are removed.

Wash #2 removes acids and neutrals (i.e. THC-COOH) that are retained by reversed-phase interaction. This fraction may be collected and analyzed for those compounds, if desired.

Wash #3 removes any remaining surfactants.

The eluent was diluted 2:1 with water prior to LC/MS analysis

The elution solution was chosen to elute the analytes while retaining any strong organic bases (antibacterial) in the preservative solution.

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LC Conditions

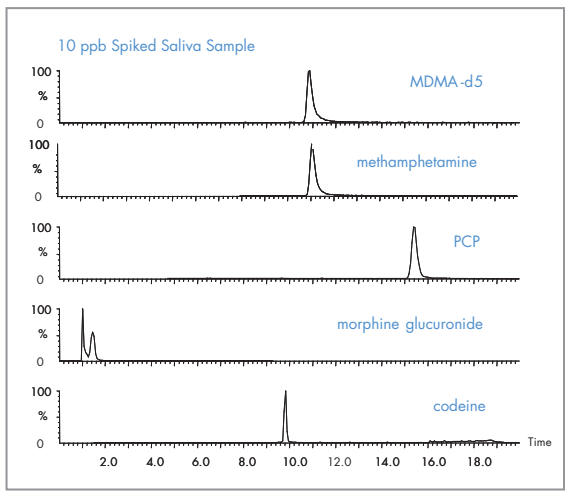
Waters Alliance™ 2695 Separations Module

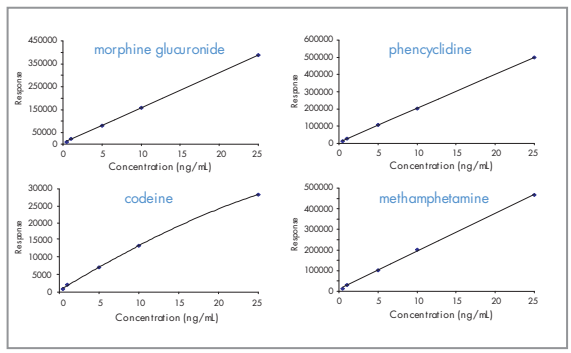
Column:	2.1 x 150 mm, XTerrra® MSC_{18}, 5 µm
Mobile Phase:	A: 10 mM ammonium bicarbonate (pH 10)
	B: Methanol
Gradient:	30% B for 3 min, to 80% B in 7 minutes, hold for 3 min, then step to 100% B for 3 min
Flow:	0.3 mL/min
Temperature:	30 °C
Injection:	40 _L

Tandem Mass-Spectrometry

Waters/Micromass Quattro Micro™
ESI+, Multiple Reaction Monitoring (MRM)

Compound	MRM	Cone(V)	Collision(eV)
MDMA	194.0>162.9	25	12
amphetamine	135.9> 90.9	17	15
methamphetamine	49.9> 90.0	20	15
benzoylecgonine	289.9>168.0	40	20
phencyclidine	243.9>159.0	18	15
morphine	285.9>165.0	50	38
morphine glucuronide	461.9>286.0	40	40
codeine	300.0>165.0	50	40





		10 ng/mL			50 ng/mL		
MRM	Compound	Response	RSD	% Recovery	Response	RSD	% Recovery
461.9>286	Morph-Gluc	1.50	6.93	91.7	6.55	6.93	93.5
243.9>159	Phencyclidine	1.86	6.12	59.3	7.46	4.23	81.4
285.9>165	Morphine	0.108	5.13	85.7	0.486	5.18	97.1
300>165	Codeine	0.142	2.24	87.0	0.612	4.41	94.9
289.9>168	Benzoyl EG	0.475	9.76	78.6	1.03	12.3	81.2
149.9>90.0	Methamphetamine	1.95	4.29	65.9	8.13	4.78	85.7
135.9>90.9	Amphetamine	1.14	5.67	69.5	4.79	5.37	86.6
194>162.9	MDMA	2.28	3.07	56.7	9.62	4.18	78.2

Discussion and Conclusions

This protocol demonstrates the utility of LC/MS to determine trace analyte levels in preserved saliva samples using Orasure Intercept® drug screening kits. The interference problems encountered for the LC/MS analysis were minimal from the saliva itself, but fairly significant interferences resulted from the solution used for collection and preservation. A mixed-mode SPE procedure overcame these interferences. The drugs studied were cocaine, codeine, morphine, amphetamine, methamphetamine, ecstasy (MDMA) and phencyclidine (PCP). MDMA-d5 was used as an internal standard. Calibration was accomplished in the range from 5 to 250 ng/mL. For some analytes (e.g. codeine) the calibration curve was non-linear. For these analytes, the data were fit to a quadratic function. Correlation coefficients (r2) for all constituents were better than 0.998. Reproducibility for all constituents was better than 10 % (RSD) for 6 replicate samples fortified at the 10 ng/mL and 50 ng/mL levels. Sample recoveries were calculated against post extract matrix standards. The calculated recoveries were greater than 85% for all constituents.

References

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