Quantitative Analysis of Novel Synthetic Opioids, Morphine, and Buprenorphine in Oral Fluid by LC-MS/MS

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INTRODUCTION

The purpose of this research was to develop and validate a comprehensive analytical method for the detection and quantification of morphine, 6-acetylmorphine (6-AM), buprenorphine, U-47700, U-49900, U-50488, AH-7921, MT-45, W-18, and W-15 in oral fluid collected via Quantisal™ device. This was achieved by solid-phase extraction followed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). This method was applied to a small set of authentic samples acquired from a Texas detainee center.

MATERIALS AND METHODS

Instrumentation

An Agilent 1290 Infinity Liquid Chromatograph system equipped with an Agilent 6470 Triple Quadrupole Mass Spectrometer (Santa Clara, CA) was used for instrumental analysis. A Poroshell 120 EC-C18 column (100 mm x 3.0 mm x 2.7 µm) was utilized with a mobile phase consisting of 5 mM ammonium formate with 0.05% formic acid in water/ 0.1% formic acid in acetonitrile. Separation was achieved using gradient elution at a flow rate of 0.5 mL/min.

Analysis of Oral Fluid

- 1 mL • Oral Fluid/Buffer (1:3)
- 25 µL • Internal Standard and Standard
- 2 mL • Phosphate Buffer
- Add • Sample to Column (PolyChrom ClinII 3 cc)
- 1 mL • Deionized Water Wash
- 1 mL • 1 M Acetic Acid Wash
- Dry • Under Max Pressure for 5 Minutes
- 1 mL • Hexane Wash
- 1 mL • Elute Acidic Drugs with Ethyl Acetate
- 1 mL • Methanol Wash
- 1 mL • Elute Basic Drugs with DCM:IPA:Ammonium Hydroxide (80:20:5)
- Dry • Under Nitrogen at 50°C & Reconstitute in 95:5 Mobile Phase

Oral fluid specimens were collected via Quantisal™ devices from 18 anonymous detainees in a Texas adult detention center in accordance with a Sam Houston State University Institutional Review Board (IRB) approved protocol (# 2017-11-37550). All subjects gave written informed consent prior to collection. Specimens were refrigerated (4°C) and analyzed within 72 h. Oral fluid samples (1 mL) were extracted and analyzed using the validated method.

RESULTS AND DISCUSSION

Table 1. Validation according to SWGTOX parameters for morphine, 6-AM, buprenorphine, and Novel Synthetic Opioids in oral fluid

Analyte	LOD (ng/mL)	LLOQ (ng/mL)	ULOQ (ng/mL)	Bias (%, n=15)	Precision (%CV, n=15)	Matrix Effects (%, n=6)	Interferences (n=33) Qualitative	Interferences (n=33) Quantitative	Carryover (n=3)	Stability (%Difference, n=3) 24 h RT	Stability (%Difference, n=3) 72 h 4°C	Stability (%Difference, n=3) 60 h AS
Morphine	5	10	500	-5.6 to -4.3	4.5-9.1	<±3.7	None	None	None	<±1.6	<±6.2	<±2.5
6-acetylmorphine	5	10	500	-7.3 to -5.8	5.5-11.1	<±13.7	None	None	None	<±3.5	<±7.8	<±0.7
U-47700	5	10	500	-4.0 to -3.0	4.0-8.3	<±16.4	None	None	None	<±3.4	<±5.4	<±0.8
AH-7921	5	10	500	-8.8 to -6.0	4.2-8.1	<±10.7	None	None	None	<±7.6	<±9.7	<±2.7
Buprenorphine	5	10	500	-4.3 to -2.5	4.8-10.3	<±6.5	None	None	None	<±5.8	<±7.3	<±1.8
U-49900	5	10	500	-4.1 to -1.7	4.3-7.8	<±4.9	None	None	None	<±11.8	<±13.1	<±5.7
U-50488	5	10	500	-4.6 to -2.9	4.1-7.2	<±7.9	None	None	None	<±12.2	<±12.4	<±4.1
MT-45	5	10	500	-5.1 to -3.9	3.9-6.8	<±3.4	None	None	None	<±2.6	<±5.2	<±3.3
W-18	5	10	500	-6.1 to -3.8	4.4-8.0	<±3.7	None	None	None	<±6.6	<±4.5	<±0.7
W-15	5	10	500	-6.9 to -5.4	5.4-9.3	<±21.1	None	None	None	<±10.3	<±4.8	<±1.8

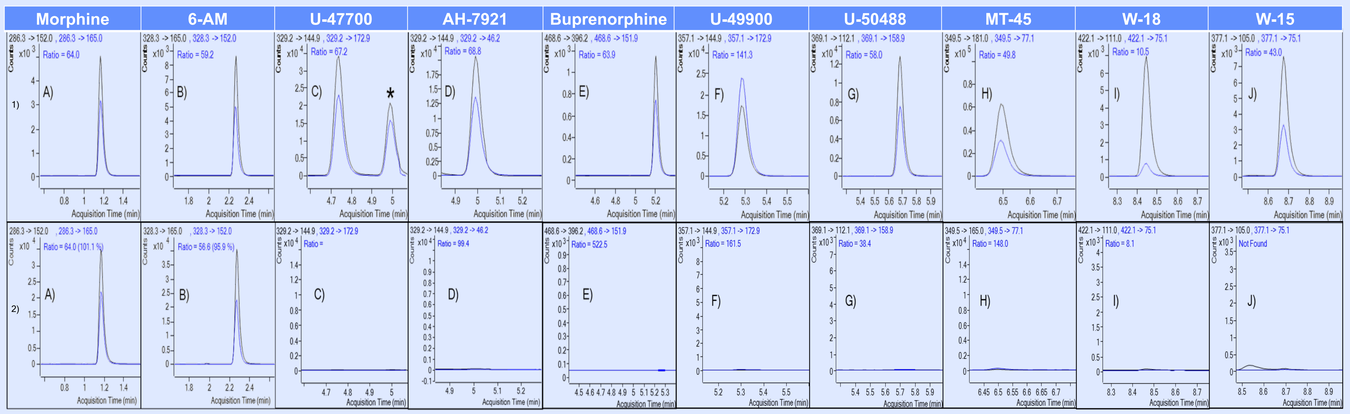
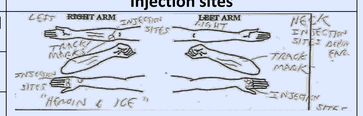
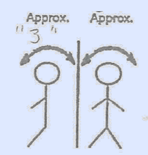
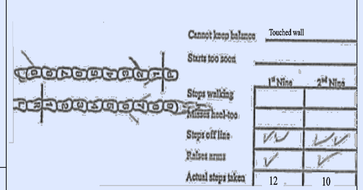


Figure 1 . Extracted Ion Chromatograms for morphine, 6-AM, U-47700, AH-7921, buprenorphine, U-49900, U-50488, MT-45, W-18, W-15 in 1) blank oral fluid fortified at analyte limits of quantification (LOQ), and in 2) authentic oral fluid

Figure 2. Track marks of admitted heroin and methamphetamine user (SHSU-0005)

Table 2. Drug Recognition Expert evaluation for one detainee (SHSU-0001) from San Antonio

Are you sick or injured?		**Are you taking any medications or drugs?**		**What drugs or medications have you been taking? When? How much?**	
Yes, Hep C, HIV, Heart/Liver Failure		Yes, Unknown		Meth, Heroin, K2	3 to 4 hrs ago / Blunt
Speech:	Slurred	**Eyelids:**	Droopy	**Injection sites**	
Breath odor:	Normal	**Face:**	On the nod		
Nasal area:	Redness	**Pupil size:**	Normal		
Coordination:	Unsteady	**HGN:**	None		
Pulse and Time		**Modified Romberg Balance**		**Walk and Turn Test**	
46	2:52 PM				
44	3:30 PM				
44	3:50 PM				
Urine presumptive results					
Benzos, Cocaine, Amp, Meth, Opiate, THC					
Opinion of Evaluator		72 seconds estimated as 30 seconds			
Narcotic Analgesic		Leaned forward			

Oral fluid was collected from 18 adults: 9 males (24 - 42 years) and 9 females (23 - 47 years) in the San Antonio area. These samples were collected while officers were completing field training for the Drug Recognition Expert (DRE) program. Each oral fluid sample was accompanied by a DRE evaluation. An example of information collected in these evaluations can be seen in **Table 2.** Injection sites were noted and sometimes photographed (**Figure 2**). From the authentic samples analyzed, morphine was detected in 4 cases and 6-AM was detected in 3 of those 4 subjects as seen in **Table 3.**

Table 3. Concentrations from authentic oral fluid samples

Sample	Age	Sex	Race	Location	Concentration (ng/mL) Morphine	Concentration (ng/mL) 6-AM	Evaluator Opinion
SHSU-0001	37	M	Hisp	TX	145	109	Narcotic analgesic
SHSU-0002	32	F	Hisp	TX	32.2	15.2	Narcotic analgesic
SHSU-0004	36	F	Cauc	TX	103	6.82	Narcotic analgesic
SHSU-0005	23	F	Hisp	TX	<LOQ	ND	CNS stim/dep

CONCLUSION

This method is currently being used to determine drug trends in several groups. Analysis of oral fluid specimens from sensitive populations (i.e. DUID, prisoners, arrestees, parolees) that may be seeking alternative or substitute opioid use will allow us to examine prevalence of novel synthetic drugs in the recreational drug market.

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