SHIMADZU

Novel Platform for Online Sample Preparation and LC-MS/MS Analysis of Drugs in Biological Matrices

Sarah Olive (1), Aria McCall (2), Ruth Gordillo (3), Rachel Lieberman (1), Brian Feild (1), Joshua Emory (1), Robert English (1), Benjamin Figard (1) (1) Shimadzu Scientific Instruments, Columbia, MD (2) Tarrant County Medical Examiner's Office, Fort Worth, TX (3) University of Texas Medical Center, Dallas, TX

1. Overview

- Fast, reproducible sample preparation module for postmortem samples which transfers the extract directly into LCMS
- Reduces operator error
- Increase sample throughput by processing 4 sample simultaneously



Increases laboratory safety

2. Introduction

- Currently, sample preparation in forensic laboratories involves using time-consuming SPE or LLE. The multi-step sample preparation can lead to more human error, irreproducible results, and endanger the analyst by exposing them to biohazardous materials
- The Clinical Laboratory Automation Module (CLAM) was developed to meet these needs and offer a safer, hands-free approach to postmortem sample analysis.

3. Methods

CLAM 2000-series module in front of LCMS-8060 (Figure 1)



Figure 1. CLAM-2000 with LCMS 8060

• Postmortem human blood samples, homogenized human spleen tissue, and homogenized human brain tissue were loaded directly into the CLAM module.

• The CLAM was programmed to perform the following steps:

Add water Add	Add Int	Vortex Add 1:1 m	Nix of Vortex	Filter Transfer	
	Siu				

• 12 minute LCMS method for the separation of 15 compounds using Restek Biphenyl column (100 x 2.1mm x 2.7 µm). See Tables 1, 2, and 3 for LCMS parameters. Mobile phases were 0.1% formic acid and 5mM ammonium formate in water and acetonitrile.

Time (min)	%B				
0	5				
9	100				
10	100				
10.01	5				
12	Stop				

LCMS-8060 Mass Spect	rometer conditions
Nebulizing Gas Flow	2 L/min
Drying Gas Pressure	10 L/min
Heating Gas Flow	10 L/min
DL Temperature	250°C
Heat Block Temperature	400°C
Interface Temperature	300°C
Ionization	Heated ESI (+ mode)

Table 1. LC gradient

Table 2. MS parameters

Analyte	Precursor Ion	Product Ion (Quant)	Product Ion (Qual)		
Morphine-d3	289.2	152.1			
Morphine	286.2	152.1	165.0		
Hydromorphone	286.2	185.0	128.0		
Codeine	300.2	165.1	152.0		
6-AM	328.2	165.0	211.0		
Hydrocodone-d3	303.1	199.0			
Hydrocodone	300.1	199.0	128.0		
7-aminoclonazepam	286.1	121.2	195.0		
Fentanyl	337.3	188.0	105.1		
Buprenorphine	468.3	55.1	396.0		
Lorazepam	321.1	275.0	229.0		
Clonazepam	316.1	270.0	214.1		
Nordiazepam	271.0	140.0	165.0		
Alprazolam-d5	314.1	286.0			
Alprazolam	309.1	281.0	205.0		
THC-COOH	345.0	299.2	193.2		
THC-d3	318.0	196.0			
delta-9-THC	315.2	193.0	259.0		
THC-OH	331.0	193.0	201.0		

4. Results

Calibration curves (10-1000 ng/mL) run in triplicate had r² all >0.997.

• 7 postmortem samples (human blood from various sites, human spleen, and human brain) were run in triplicate.

%RSD for each sample (N=3) were all <10% with exception of spleen which had 17% RSD.

Excellent correlation between samples extracted using SPE sample preparation and CLAM sample preparation (Table 4).

Sample ID	Postmortem Specimen	Drugs found above 10 ng/mL cutoff	Results from manual preparation (ng/mL)	CLA LCMS	M-2000 S-8060 r (ng/mL)	with esults		Average % difference between manual prep and CLAM	
				Rep 1	Rep 2	Rep 3	%RSD		
		Morphine	400	446.1	445.4	442.1	0.476	11%	
A	Heart blood	Codeine	19	22.3	20.9	21.2	3.478	13%	
		Alprazolam	593	557.9	558.1	557.7	0.043	-6%	
	Chasterity	Morphine	363	373.0	365.1	369.7	1.071	2%	
В	blood	Codeine	31	31.9	32.3	33.2	1.952	5%	
	biood	Nordiazepam	135	154.8	149.5	156.2	2.317	14%	
C	Femoral	Hydrocodone	177	175.9	177.9	174.2	1.048	-1%	
	blood	Hydromorphone	30	30.3	29.9	30.8	1.438	1%	
D	Heart blood	-7 aminoclonazepam	72	48.5	47.7	46.4	2.147	-34%	
Е	Heart blood	THC-COOH	Detected*	42.2	42.3	49.9	9.937	n/a	
F	Spleen** (ng/g) homogenate dil. factor = 5	Morphine	493	493.7	457.1	347.7	17.551	-12%	
G	Brain** (ng/g) homogenate dil. factor = 5	Morphine	147	169.7	168.9	176.6	2.460	17%	

*Lab does not quantitate THC in any postmortem sample.

**Not provided blank tissues for cal curve prep. These quants were based off the blank blood curve. The brain and spleen were from the same case file.









5. Conclusion



Figure 2. Sample chromatogram of 1000 ng/mL spiked blood standard

	 Sample Results - 7-Aminoclonazepam 										×				
Туре		RT	m/z	^	#		Sample ID	Conc. (ng/mL	Acquired D	ate ^	Ref 1 Actual	Ref 1 Range	Found RT	Sample Typ 🔨	
	Ŧ	Ŧ]	· · · · · ·	۲ T	·	Ŧ	Ŧ	Ŧ	Y		
ISTD		2.348	289.00			26	20180917_Bld C		9/18/2018	12:31:40 AM	0.00	19.84 - 36.84		Unknown	
Target		2.368	286.00			27	20180917_Bld C		9/18/2018	12:44:40 AM	0.00	19.84 - 36.84		Unknown	
Target		2.766	286.00		✓	28	20180917_Bld C		9/18/2018	12:57:41 AM	0.00	19.84 - 36.84		Unknown	
Target		3.644	300.00			29	20180917_Bld D	48.4820	9/18/2018	1:10:42 AM	29.27	19.84 - 36.84	5.251	Unknown	
Target		3.725	328.00		✓	30	20180917_Bld D	47.6851	9/18/2018	1:23:43 AM	28.12	19.84 - 36.84	5.256	Unknown	
ISTD		3.970	303.00			31	20180917_Bld D	46.4560	9/18/2018	1:36:44 AM	29.42	19.84 - 36.84	5.251	Unknown	
Target		3.983	300.00			32	20180917_BId E		9/18/2018	1:49:45 AM	0.00	19.84 - 36.84		Unknown	
Target		5.254	286.00			33	20180917_Bld E		9/18/2018	2:02:46 AM	0.00	19.84 - 36.84		Unknown	
Target		6.145	337.00			34	20180917_Bld E		9/18/2018	2:15:47 AM	0.00	19.84 - 36.84		Unknown	
Target		6.197	468.00			35	20180917_BId UTAK	23.1857	9/18/2018	2:28:49 AM	26.42	19.84 - 36.84	5.254	Control	
Target		6.808	321.00			36	20180917_BId UTAK	21.9567	9/18/2018	2:41:49 AM	28.12	19.84 - 36.84	5.259	Control	
Target		7.010	316.00			37	20180917_BId UTAK	21.4519	9/18/2018	2:54:50 AM	27.63	19.84 - 36.84	5.253	Control	
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min ×	• S	urvey							×	 Calibration 	- 7-Aminoclor	azepam		×	
7.89e4	70						Chow selected compound only			을 3.2 크y = 0.00	3054768x + 0.003	133652			
	200									$\frac{2}{3}$ $\frac{3}{3}$ $R^2 = 0.9999719$ R = 0.9999859					
	Ε									₹ 3.0 Curve Fit: <u>Default (Linear)</u>					
	ebe	Q 286.00>121.00 (+) 9.19e2 Q 286.00>121.0				Q 28	06.00>121.00 (+) 7.89e4	121.00 (+) 7.89e4 Q 286.00>121.00 (+) 7.69e4			2.8 - Zero: <u>Default (Not Forced)</u>				
	naz	100.00	100.00 R1 0.00% (0) RT=5.862			100	A=30395 100.00 R1 2 RT = 5251 280			2.6					
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RT	A=311759 100.00 R1 2RI=5.2513.81))	100.	.00 R1 0.00% (0) RT⊨5.843	100.00 A=3913 R1 0.00T=4300		1.6					
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Figure 3. Data analysis in Insight software

Automated sample preparation capabilities of the CLAM-2000 series coupled with the Shimadzu LCMS offers a new approach for drug analysis in biological matrices.

This automated approach increases throughput of sample analysis by overlapping sample prep with analytical runs and allowing an analyst to perform additional tasks.

The automated sample approach demonstrated analysis of drugs within 10% RSD of standard manual procedures.