

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

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## 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:



- 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

Table 1. LC gradient

LCMS-8060 Mass Spectrometer 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 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

Table 3. MS transitions

## 4. Results

- Calibration curves (10-1000 ng/mL) run in triplicate had  $r^2$  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)	CLAM-2000 with LCMS-8060 results (ng/mL)			%RSD	Average % difference between manual prep and CLAM
				Rep 1	Rep 2	Rep 3		
A	Heart blood	Morphine	400	446.1	445.4	442.1	0.476	11%
		Codeine	19	22.3	20.9	21.2	3.478	13%
		Alprazolam	593	557.9	558.1	557.7	0.043	-6%
B	Chest cavity blood	Morphine	363	373.0	365.1	369.7	1.071	2%
		Codeine	31	31.9	32.3	33.2	1.952	5%
		Nordiazepam	135	154.8	149.5	156.2	2.317	14%
C	Femoral blood	Hydrocodone	177	175.9	177.9	174.2	1.048	-1%
		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%
E	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.

Table 4. Comparison of manual preparation versus CLAM-2000 results

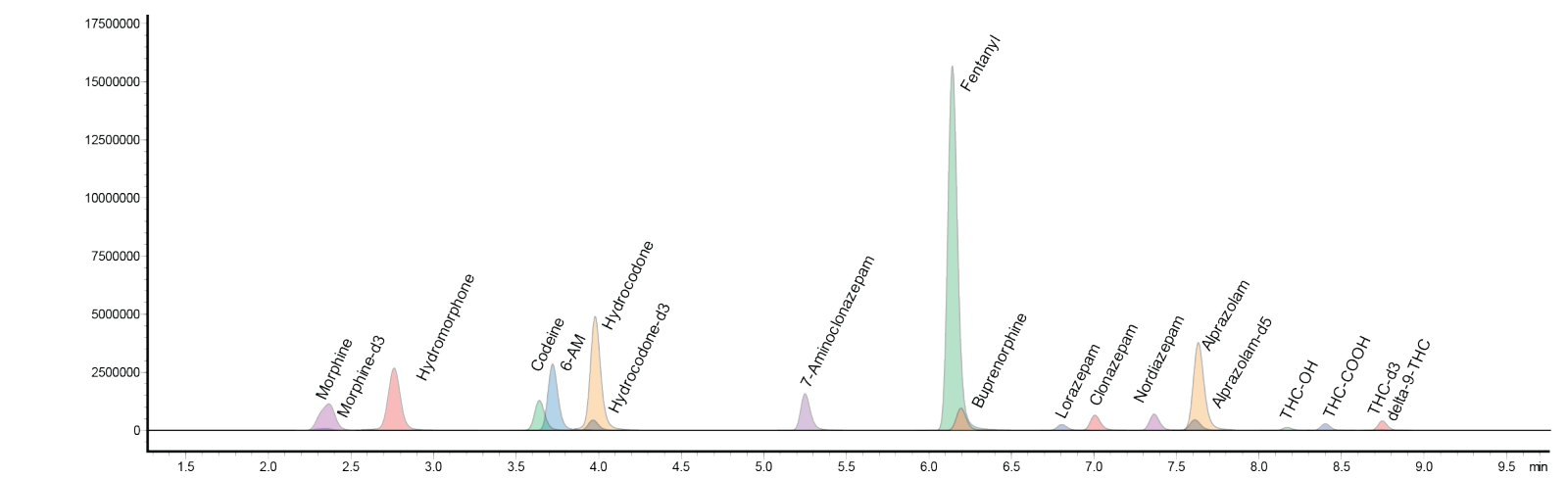


Figure 2. Sample chromatogram of 1000 ng/mL spiked blood standard

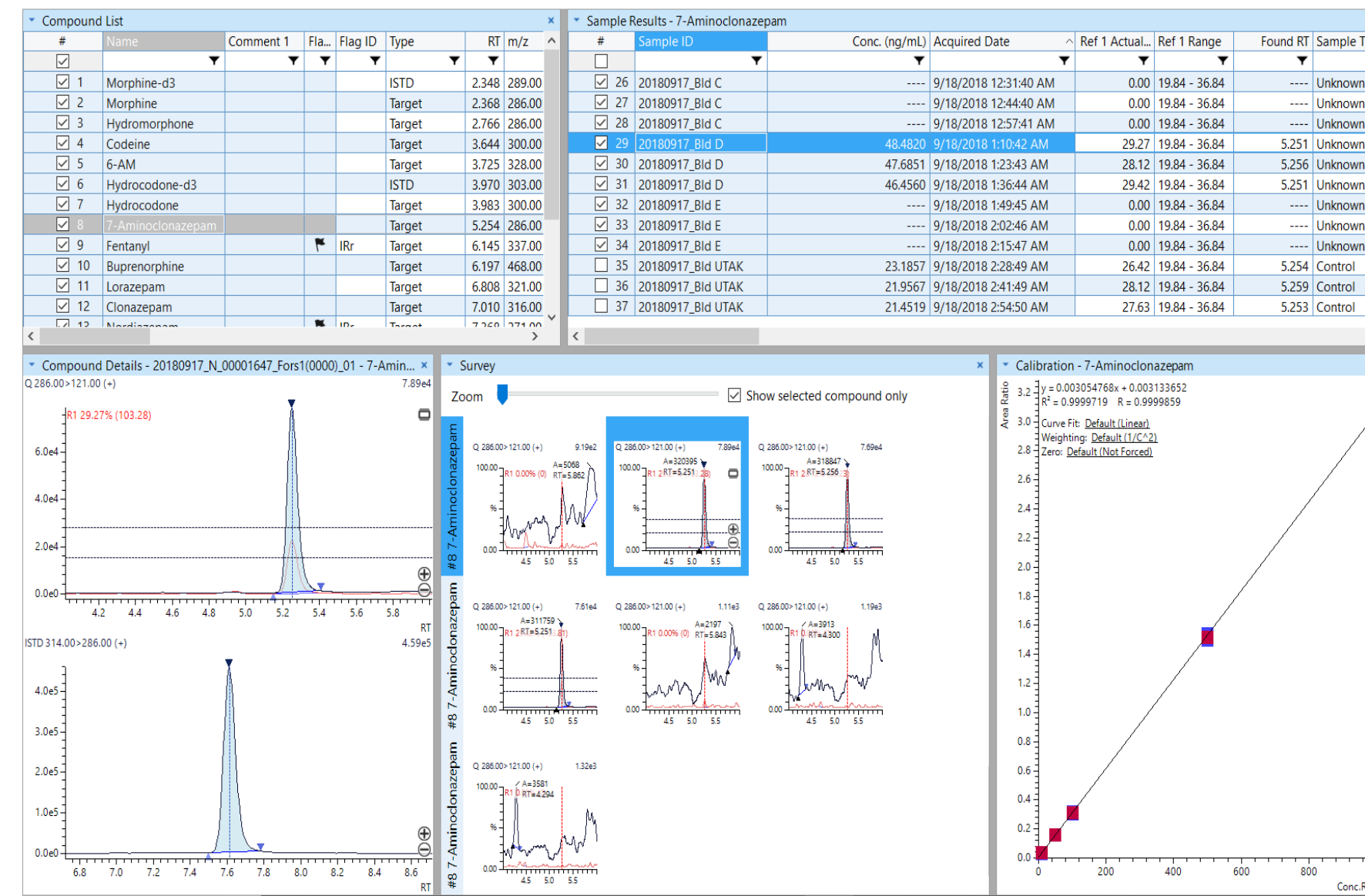


Figure 3. Data analysis in Insight software

## 5. Conclusion

- 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.