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Application News

SSI-LCMS-101

Liquid Chromatography Mass Spectrometry

Analysis of Postmortem Samples Using Automated Sample Preparation Coupled Directly to LC-MS/MS

■ Summary

A faster, highly accurate, and reproducible approach to analyzing postmortem samples using a fully integrated and automated sample preparation module with a LCMS system was developed.

■ Background

Currently, sample preparation in forensic laboratories involve using SPE or LLE which are not only time-consuming approaches, but can introduce human errors during the multi-step sample preparation process. An automated sample preparation approach offers a more reproducible solution which can help remove human error caused during manual preparations, and increase laboratory safety. The Clinical Laboratory Automation Module (CLAM) series was developed to meet these needs as well as a providing a solution to increase laboratory efficiency.

■ Method

A LC-MS/MS method was created for the Shimadzu 8060 triple quadrupole using 15 drug standards purchased from Restek Corporation (Bellefonte, PA) and Cerilliant Corporation (Round Rock, TX). Four deuterated internal standards (alprazolam-D5, morphine-D3, hydrocodone-D3, and delta-9-THC-D3) were purchased from Cerilliant Corporation.

All four internal standards were combined to create a working solution in methanol with a final concentration of 100 ng/mL for alprazolam-D5, morphine-D3, and hydrocodone-D3 and a final concentration of 10 ng/mL for delta-9-THC-D3. All 15 drug standards were combined in methanol making a 10 µg/mL stock solution. Spiked samples were prepared in blank human blood at concentrations of 10, 50, 100, 500, and 1000 ng/mL.

Postmortem human blood samples, human spleen tissue, human brain tissue, and spiked blood samples were used for this application. The tissue samples were homogenized at a 1:4 dilution prior to loading directly into the CLAM-2000 series for sample preparation. The CLAM-2000 series was programmed to add 20µL of distilled water followed by 10µL of each sample. Protein precipitation was then performed using 100µL 50/50 methanol/acetonitrile solution. This mixture was vortexed inside the CLAM-2000 series for 30 seconds at 1900 rpm. Next, 20µL of the internal standard solution was added followed by an additional 30 seconds of shaking. Following a 60 second filtration, the samples were collected and automatically transferred into the LCMS-8060 system.

A Raptor Biphenyl column from Restek (100 x 2.1mm x 2.7µm) was used with an EDX Direct connect holder and Raptor Biphenyl guard cartridges. A 12-minute linear binary gradient using 0.1% formic acid and 5mM ammonium formate in water with 0.1% formic acid and 5mM ammonium formate in methanol was used. The gradient is shown in **Table 1.** The column was set to 30° C and the injection volume was 2µL.

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

Table 1: LC Gradient conditions

Positive mode electrospray ionization with multiple reaction monitoring (MRM) transitions were used for analysis on the LCMS-8060. The MRM transitions for each analyte and internal standard are shown in **Table 2**. Conditions for the LCMS-8060 are in **Table 3**.

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 2: MRM transitions

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 3: MS Settings

■ Results and Discussion

A total of seven postmortem samples were analyzed in triplicate using the CLAM-LCMS-8060 system. Excellent reproducibility as well as good correlation was observed for all seven samples. The %RSD for each sample (N=3) were all <10% with the exception of the spleen sample which had a %RSD of 17% (**Table 4**).

The calibration curves for the 15 analytes are shown in **Figures 2** and **3**. Calibration samples were run in triplicate and all curves had a linear regression value of $r^2 \geq 0.997$. These postmortem samples were manually prepped and analyzed on a LCMS triple quadrupole prior to being run using the automated CLAM-LCMS-8060 analysis.

A comparison of results between using the CLAM-2000 and manual preparation is summarized in **Table 4.** The sample preparation time was reduced from over 2 hours down to 5 minutes, thereby accelerating sample throughput (see **Figure 4**). In addition, the CLAM-2000 series is capable of processing four samples simultaneously, which further increases sample throughput.

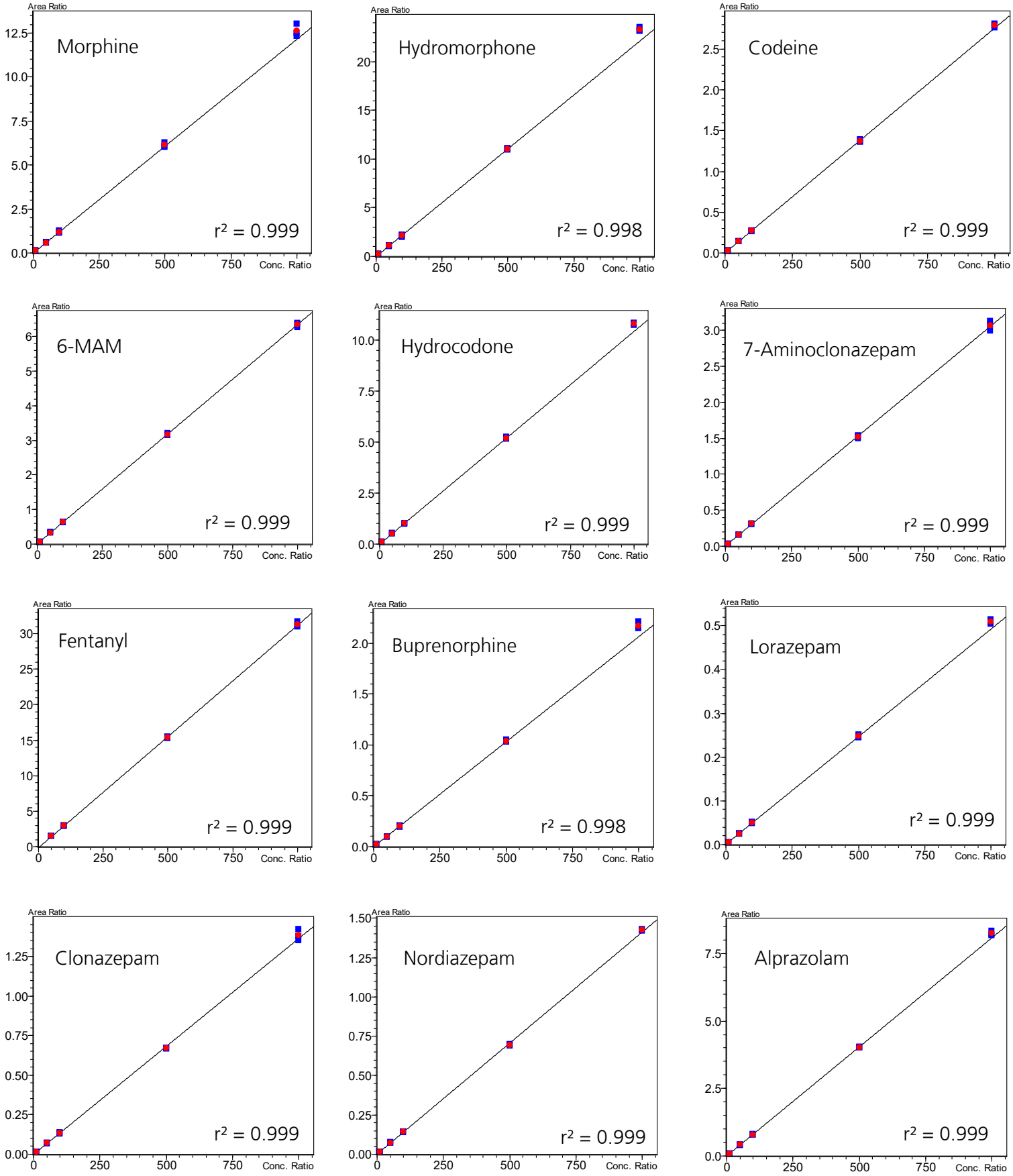

Figure 2: Calibration curves for the twelve of the 15 analytes in the assay.

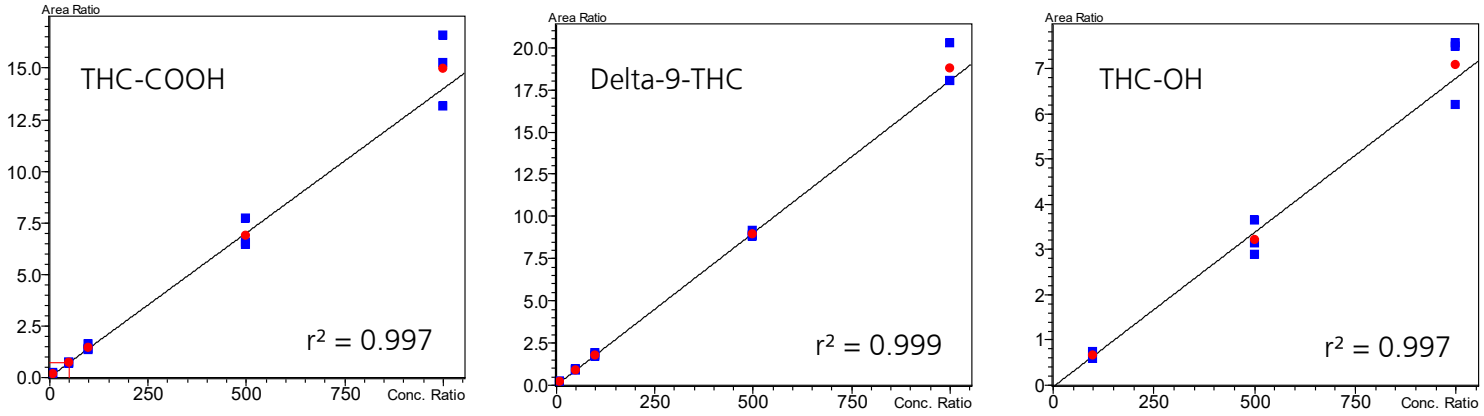

Figure 3: Calibration curves for the remaining three THC analytes.

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)				Average % difference between manual prep and CLAM
				Rep 1	Rep 2	Rep 3	%RSD	
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%

Table 4: Comparison of manual prep and CLAM-2000 results

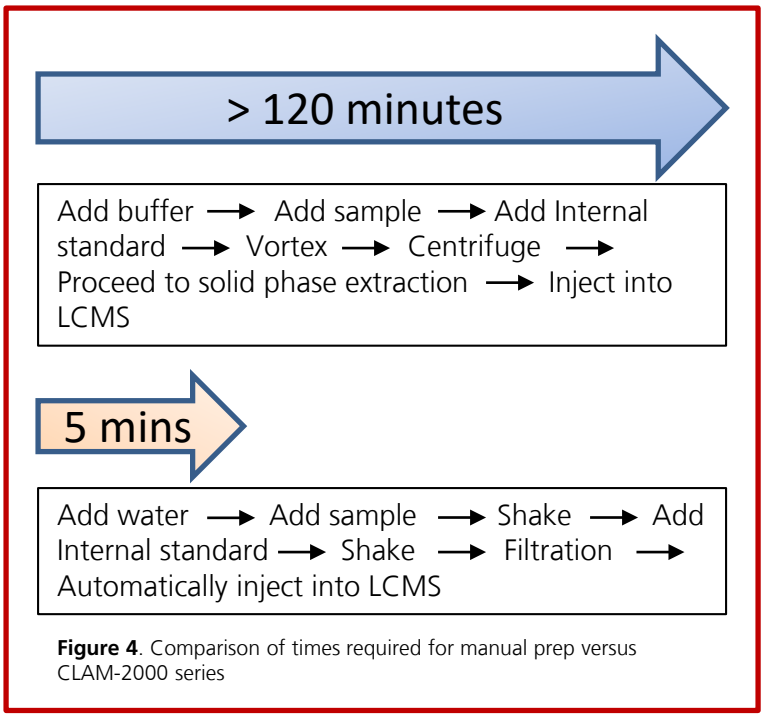

Figure 4. Comparison of times required for manual prep versus CLAM-2000 series

■ Conclusion

The automated sample preparation capabilities of the CLAM-2000 series coupled with the Shimadzu LCMS offers a new, hands-free approach for drug analysis in biological matrices. This automated approach increases the throughput of sample analysis by overlapping sample prep with analytical runs and allowing an analyst to perform additional tasks. The automated sample preparation approach demonstrated analysis of drugs within 10% RSD of standard manual procedures.

■ Acknowledgements

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ULTRA FAST MASS SPECTROMETRY


LCMS-8040


LCMS-8045


LCMS-8050


LCMS-8060


LCMS-2020


Q-TOF LCMS-9030

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