

Application News

SSI-BioTech-001

Liquid Chromatography Mass Spectrometry

Fully Automated Sample Preparation and LCMS Analysis of Drugs in Oral Fluid

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Summary: The Clinical Laboratory Automation Module (CLAM-2000) is a fully automated sample preparation module that is integrated with a Shimadzu LC/MS analyzer. This system has been used for analysis of 122 drugs and deuterated internal standards in oral fluid matrix with detection limits of 2 ng/mL.

Background: The CLAM-2000 is the first sample preparation module that is fully integrated with LC separation and MS detection of small molecules. Additionally, the CLAM-2000 is capable of parallel processing up to four samples simultaneously, which enables it to keep up with current sub-five-minute LCMS methods. One key advantage of the CLAM-2000 is its great reproducibility as it commonly achieves %RSDs of 10%. This

system offers high sample throughput and increased safety for laboratory personnel.

Method: Oral fluid analysis was performed using the CLAM-2000 integrated with a Shimadzu Nexera LC system and a Shimadzu 8050 triple quadrupole mass spectrometer. A gradient of 10% to 60% methanol was implemented over seven minutes. All samples, calibrators and quality controls were made in Quantisol oral fluid diluent (Immunoanalysis, Pomona, CA).

Analytical Method Summary	
# Drug Compounds	122
Time for first result	12 mins
Sample to sample time	7 mins
LOQ	2 ng/mL
Average %RSD	7.5%
R ² Values	≥ 99

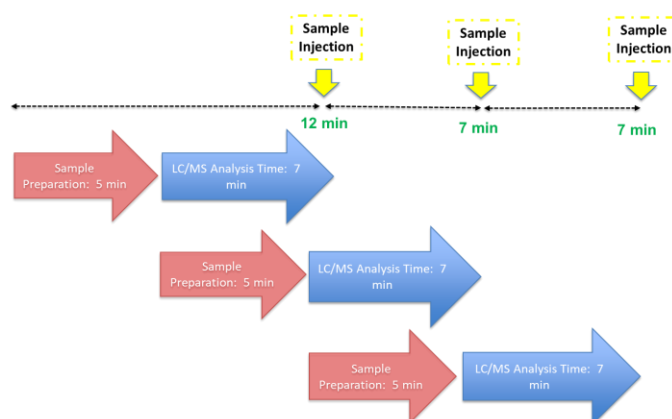
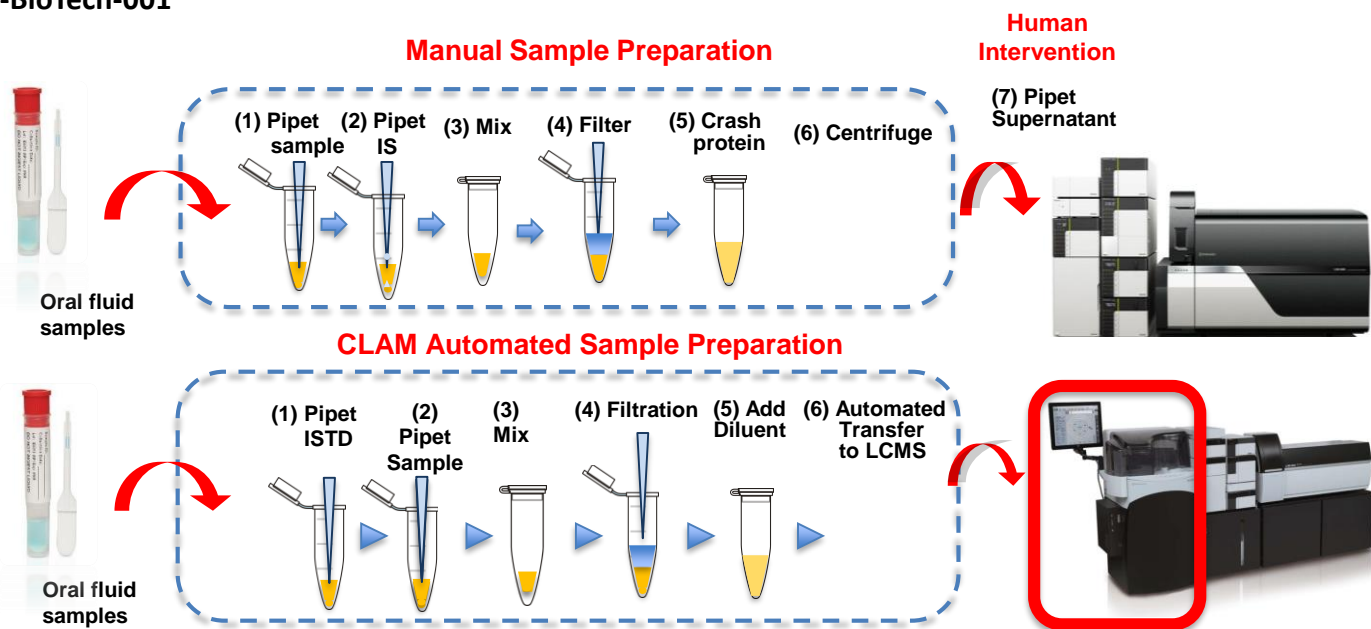


Figure 1: CLAM-2000 workflow with parallel sample processing



Graph 1: Comparison of manual sample preparation and LCMS analysis and CLAM-2000 fully automated sample preparation and LCMS analysis. The many steps of human intervention required for manual preparation not only introduce human error but costs more because employees are being paid to do routine tasks that are more easily accomplished by automation.

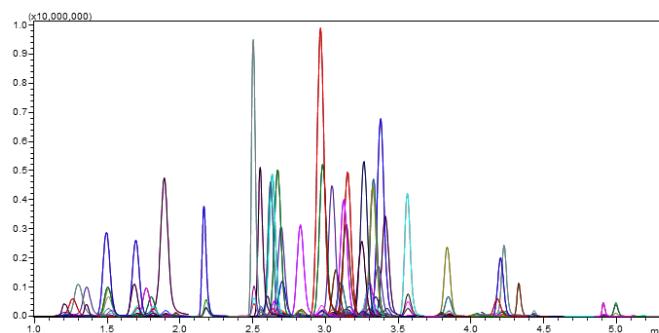
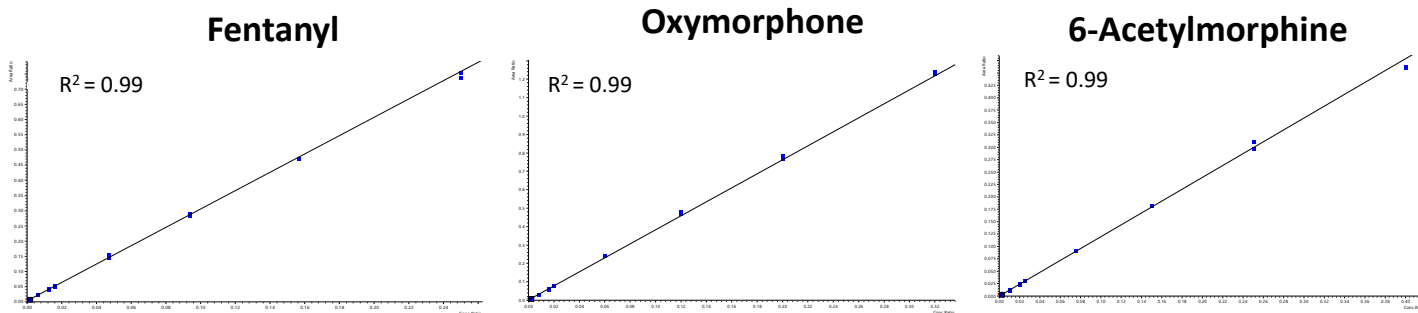
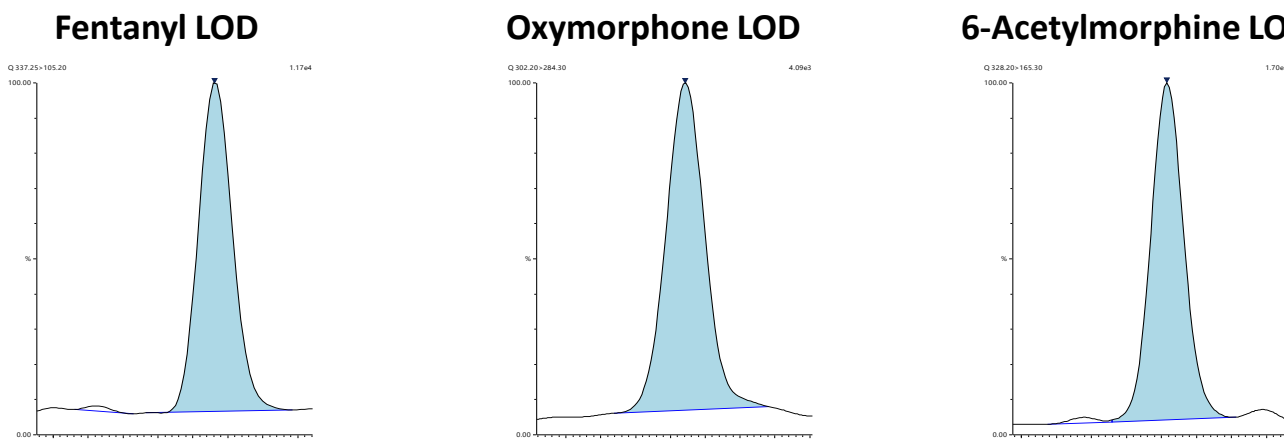


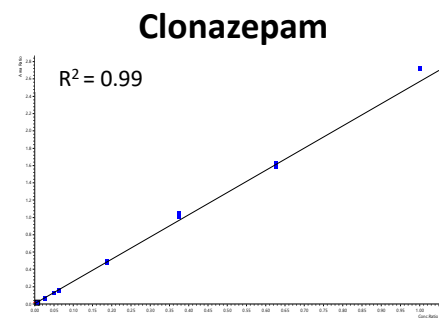
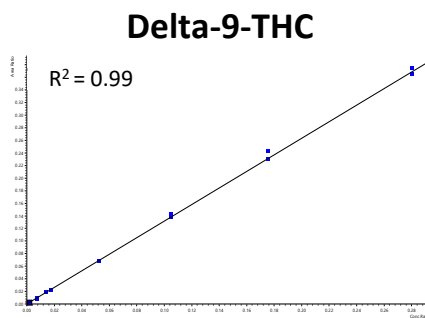
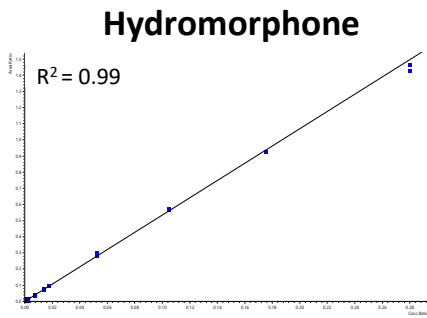
Figure 2: Simultaneous acquisition of 61 compounds in five minutes



Figures 3-5: Calibration curves for Fentanyl, Oxymorphone, 6-Acetylmorphine. All have R² values of greater than 0.99

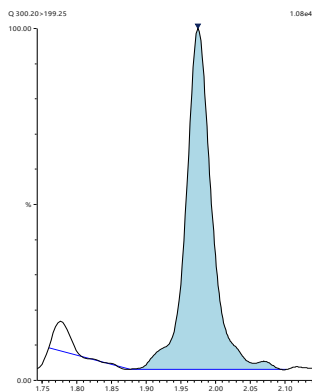


Figures 6-8: Extracted ion chromatograms at limits of quantification for Fentanyl, Oxymorphone, 6-Acetylmorphine.

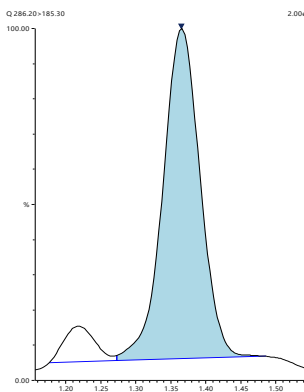


Figures 9-11: Calibration curves for Hydromorphone, Delta-9-THC, and Clonazepam. All have R^2 values ≥ 0.99

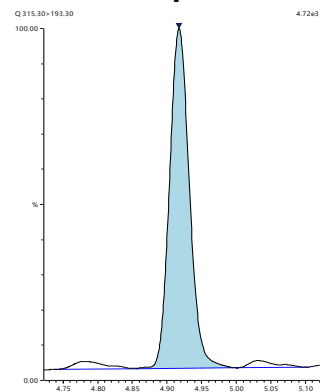
Hydromorphone LOD



Delta-9-THC LOD

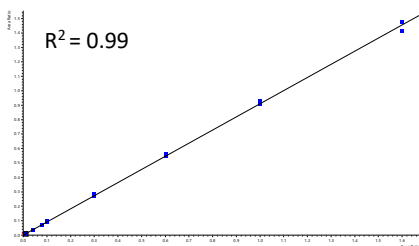


Clonazepam LOD

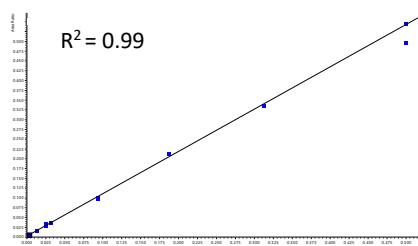


Figures 12-14: Extracted ion chromatograms at limits of quantification for Hydromorphone, Delta-9-THC, and Clonazepam.

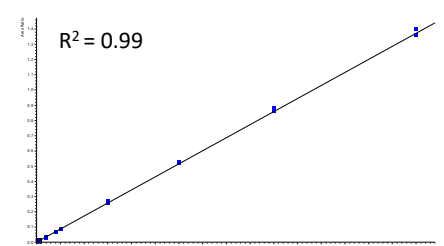
Oxazepam



MDA

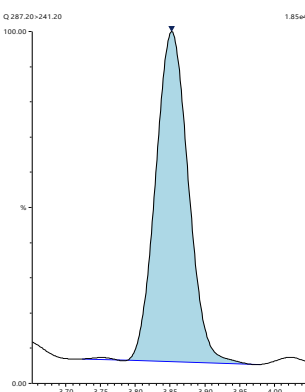


Benzoylcegonine

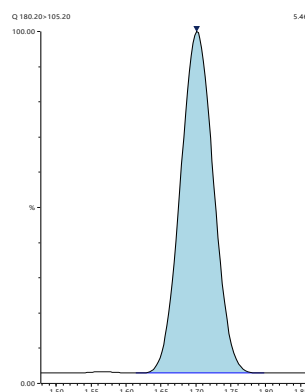


Figures 15-17: Calibration curves for Oxazepam, MDA, and Benzoylcegonine. All have R^2 values of greater than 0.99

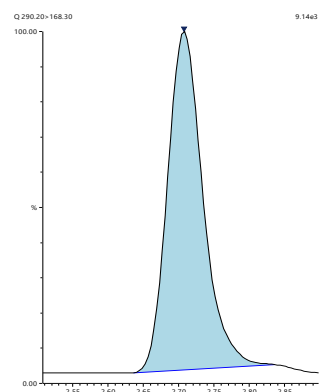
Oxazepam LOD



MDA LOD



Benzoylcegonine LOD



Figures 18-20: Extracted ion chromatograms at limits of quantification for Oxazepam, MDA, and Benzoylcegonine

Oral Fluid Laboratory Cutoff Concentrations		
	SAMSHA	Laboratory
THC	2	2
Cocaine	8	1.25
Benzoyllecgonine	8	1.25
Codeine	15	2
Morphine	15	2
Hydrocodone	15	1.25
Hydromorphone	15	1.25
Oxycodone	15	2
Oxymorphone	15	2
6-Acetylmorphine	2	1
Phencyclidine	2	1.25
Amphetamine	15	7.5
Methamphetamine	15	1.25
MDMA	15	1.25
MDA	15	1.25
MDEA	15	1.25

Table 1: Laboratory SAMSHA cutoff concentrations for oral fluid analysis of drugs of abuse and cutoff concentrations from laboratory validated oral fluid analysis method

Table 2: Standard deviation and coefficient of variation for three QC levels from Fentanyl, Oxymorphone and 6-Acetylmorphine. Both Interday and Intraday results show CV's ≤ 10

Precision Results: n = 7			
Interday			
	Conc (ng/mL)	SD	CV
Fentanyl	0.125	0.008	6.296
	1.25	0.101	8.16
	12.5	0.458	3.623
	Conc (ng/mL)	SD	CV
Oxymorphone	2	0.112	5.826
	20	0.815	4.197
	200	3.025	1.526
	Conc (ng/mL)	SD	CV
6-Acetylmorphine	1	0.082	8.296
	10	0.283	2.842
	100	3.263	3.255
Intraday			
	Conc (ng/mL)	SD	CV
Fentanyl	0.125	0.011	8.963
	1.25	0.059	5.221
	12.5	0.628	5.347
	Conc (ng/mL)	SD	CV
Oxymorphone	2	0.142	6.982
	20	1.076	5.424
	200	6.097	3.066
	Conc (ng/mL)	SD	CV
6-Acetylmorphine	1	0.064	6.553
	10	0.309	3.198
	100	4.325	4.496

Results and Discussion: Fully automated sample preparation, LC separation and MS analysis of 122 drugs and deuterated internal standards were performed using the CLAM-2000 LC/MS system. No sample preparation was performed by lab personnel aside from loading the oral fluid collection devices into the instrument carousel.

After the first sample preparation and LCMS analysis, which takes eleven minutes, the sample to sample analysis time is equal to the LCMS method time (seven minutes). Calibration curves for the drugs of abuse exhibited R^2 values ≥ 0.99 and limits of quantification ranged from 2 ng/mL for most compounds to 7.5 ng/mL for Amphetamine. This novel sample preparation method achieved percent relative standard deviations of $\sim 10\%$ or less for all compounds

Conclusion: The fully automated sample preparation and analysis of over one hundred drugs and internal standards was performed with the CLAM-2000 LCMS system. A high degree of reproducibility from sample to sample was exhibited using the CLAM-2000. Additionally, this fully automated sample preparation and LC/MS analysis system reduces sample preparation time by humans, increases laboratory efficiency, improves safety while providing high accuracy and reproducibility. A complete sample preparation and analysis solution for drug analysis is possible when the CLAM-2000 is coupled with an LCMS system and Insight software which allows importation of data files into modern LIMS systems for an efficient and productive laboratory workflow.

UPLC-MS

ULTRA FAST MASS SPECTROMETRY



LCMS-8030



LCMS-8040



LCMS-8050



LCMS-8060



LCMS-2020



LCMS-IT-TOF

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