FP 221

Multi-Target Screening and Quantitation of 24 Drugs in Blood, Serum and Urine Using Automated Sample Preparation Coupled Directly to LC-MS/MS

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1. Overview

Multi-target screening and quantitation for 24 drugs in human blood, serum and urine was established on a fully-automated platform using automated sample preparation module coupled directly to LC-MS/MS.

2. Introduction

In forensic toxicology, analysis of drugs in biological samples is conducted to determine drug-facilitated crimes, suspected drug abuses, or whether someone was driving under the influence (DUI(D)). Determining what analytes are present and the concentration of those compounds in a variety of matrices (e.g., blood, urine, or oral fluid) can be complex. Sample preparations in most forensic laboratories commonly use SPE or LLE, which are not only time-consuming, but also introduce human errors during the multi-step protocols. We describe here an automated alternative using a sample preparation module (CLAM-2030™) connected to a LC-MS/MS system (LCMS-8060) for multi-target screening and quantitation of 24 common abused drugs.

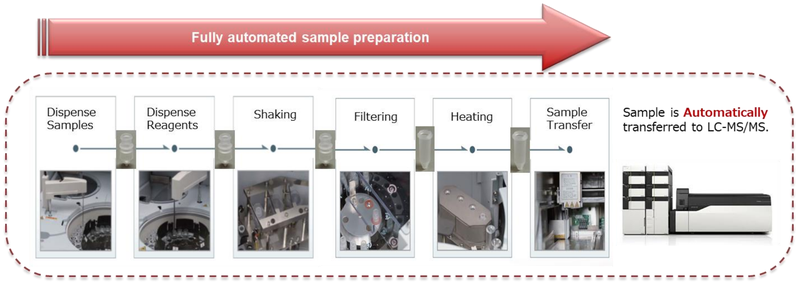

Figure 1. Workflow of CLAM-2030 for automated sample preparation coupled with LCMS-8060.

3. Method

A mixture of 24 drugs were spiked at varying concentrations using certified reference standards. The automated sample preparation module is fully integrated which conducts dilution, protein precipitation, stirring, and vacuum filtration all in one. Sample preparation was performed entirely inside the CLAM-2030 module involving the following pre-programmed steps: wetting PTFE filtering vials with water, reagent dispensing for internal standard (IS) addition, sample diluting 5 times with saline, sample dispensing, adding methanol and acetonitrile for protein precipitation, sample shaking inside the module for 30 seconds at 2000 rpm, vacuum filtration for 60 seconds into collection vial, and finally transferring the collection vial directly into the autosampler in the LCMS-8060 system. Compared to manual sample preparation, the time was reduced from over 2 hours to just 5 minutes[1].

A blank human serum sample was used as blank and the matrix for preparation of spiked samples. Postmortem human blood, serum, and urine samples were used for evaluation of the method performance. A ready-to-use method package Forensic Toxicology Database was used to set up the LCMS screening method for the analytes which minimized the efforts in LC and MRM method development. Following the method package LC conditions, the chromatographic separation of the drugs was achieved by a gradient elution in 17 minutes (Table 1). Additionally, screening results were compared to the forensic toxicology database library for identification and confirmation using similarity scores.

Table 1. LC-MS 8060 Analytical Conditions

Column:	Restek raptor biphenyl (2.1 mmI.D. x 100 mmL., 2.7 µm)	Nebulizing Gas:	3 L/min
Mobile phase:	A: 0.1% formic acid and 10 mM ammonium formate in water B: 0.1% formic acid and 10 mM ammonium formate in methanol	Ionization mode:	ESI positive and negative MRM
Flow Rate:	0.3 mL/min	Interface Temp.:	300 °C
Oven Temp.:	50°C	DL Temp.:	250 °C
Injection volume:	2.0 µL	Heat Block Temp.:	400 °C

4. Results and Discussion

The Forensic Toxicology Database, which includes methods, multiple reaction monitoring (MRM) transitions and libraries of over 2000 analytes, was used to set up the LC-MS/MS screening method for the analytes. Retention times of the compounds were updated using certified reference standards and MRM optimization of the ISs was carried out. A chromatogram of the 24 targets with 4 deuterated IS is shown in Figure 2.

Compound Name	RT (min)	Compound Name	RT (min)
Acetylsalicylic acid	3.910	Naproxen	7.276
Amphetamine	3.683	Oxazepam	7.321
Atenolol	3.424	Phentermine	4.026
Benzoylecgonine	4.745	Sertraline	7.775
Bupropion (IS D9)	5.247	THC (IS D3)	10.122
Butalbital_neg	5.414	Fentanyl	6.566
Carbamazepine	6.896	Acetaminophen (IS D4)	3.071
Fluoxetine (IS D6)	6.498	Heroin	5.159
Lidocaine	4.313	MDMA	4.105
Meprobamate	4.870	MDEA	4.391
Methamphetamine	3.895	Methadone	7.726
Morphine	3.362	Phenylpropanolamine	3.157

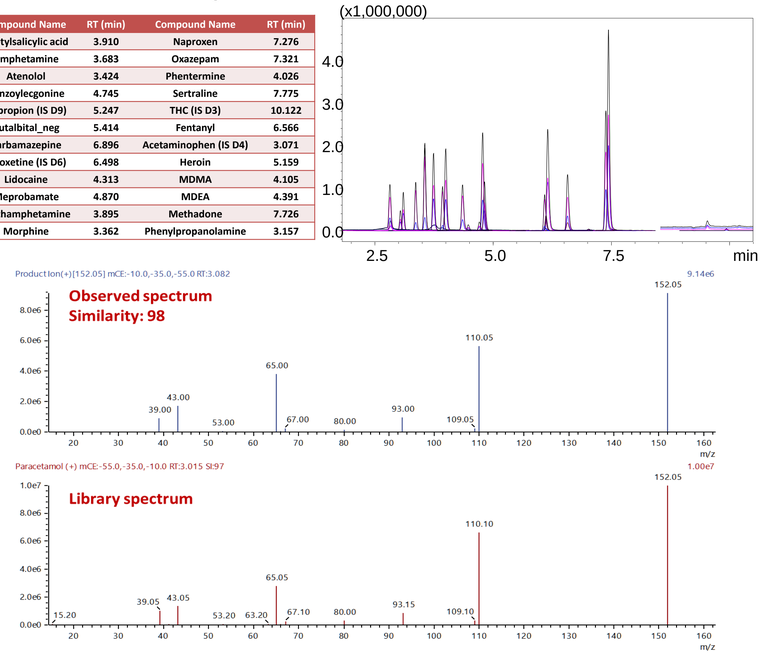

Figure 2. Upper: MS chromatogram of mixed standards of 24 targeted drugs with four ISs. Lower: MS/MS spectrum of paracetamol in blood sample matching the library with similarity score of 98 (100 is the highest).

Validation of quantitative method has been completed following ANSI/ASB Standard 036. Limit of detection (LOD) and limit of quantitation (LOQ) were as low as 10 ng/mL for all 24 analytes. Linear calibration curves in the range of 10-1000 ng/mL were run in triplicate and established for the 24 drugs with $R^2 \geq 0.990$ except for naproxen and heroin. Bias and precision were evaluated at three concentration levels (80 ppb, 400 ppb, 800 ppb) and did not exceed 20% acceptance range, except for naproxen, heroin and acetylsalicylic acid. No carryover was observed even after the highest calibrator. No interferences from matrix and internal standards were observed.

A total of seven postmortem samples were analyzed in triplicate using the CLAM-LCMS system. Excellent reproducibility as well as good correlation was observed for all seven samples. The %RSD for each sample were all <20%. The average differences between CLAM-LCMS results and target concentration were <20% for all the analytes in each sample.

The Synchronized Survey Scan function in LCMS-8060 automatically performs MS/MS when a precursor threshold is exceeded, thereby producing a combined MRM and MRM-dependent product ion scan in a single analysis. Library search results identify compounds using similarity scores (Figure 2). All the analytes in each sample were flagged without false negative or false positive detection using 80 as the similarity score cutoff.

Table 3. CLAM-LCMS library screening and quantitative results for biological samples

Sample ID	Matrix type	Drugs found above 80 score cutoff	CLAM-LCMS library search similarity score	Target concentration (ng/mL)	CLAM-LCMS quantitative results (ng/mL)	Average % difference between CLAM and target
1	Blood	Paracetamol	98	50,000	50,399	0.7
2	Blood	Benzoylecgonine	94	2000	1969.5	-1.5
		Fentanyl	85	15	12.89	-14.0
3	Serum	Carbamazepine	91	3000	2935.7	-2.1
4	Serum	Butalbital	97	2000	2171.3	8.6
		Meprobamate	94	20000	20495.1	2.5
5	Serum	Oxazepam	86	750	738.4	-1.5
6	Urine	Morphine	96	500	547.6	-0.4
7	Urine	Amphetamine	92	150	153.7	2.4
		Methamphetamine	98	1000	952.4	-4.8

5. Conclusions

A fully integrated and automated platform consisting of CLAM-2030 and LCMS-8060 was used in establishing a multi-target screening and quantitation for 24 drugs in biological matrices. The automated sample preparation approach offers a more reproducible solution which helps eliminate human errors during manual preparations and increase laboratory safety. By using the Forensic Toxicology Database, tedious method development work was avoided as only retention time adjustments and MRM optimization for some ISs was needed. This work demonstrates that the system, which couples automated sample preparation and LC-MS/MS, can be effective in high throughput screening of large numbers of targets in biological samples in forensic toxicological research and investigation.

Reference

1. Analysis of Postmortem Samples Using Automated Sample Preparation Coupled Directly to LC-MS/MS, Shimadzu Scientific Instruments, 2019.