

## Original article

# Modified QuEChERS in blood sample preparation for drug abuse determination by LC-MS/MS

Bundhita Boonchaleaw<sup>a</sup>, Nat Tansrisawad<sup>b</sup>, Udomsak Hoonwijit<sup>b,\*</sup>

<sup>a</sup>Program in Medical Sciences, Department of Forensic Medicine, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand

<sup>b</sup>Department of Forensic Medicine, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand

**Background:** Liquid chromatography-tandem mass spectrometry (LC-MS/MS) becomes a common technique in the routine forensic toxicological analysis because of its accurate result. Solid-phase extraction (SPE) and liquid-liquid extraction (LLE) are the sample preparation methods having disadvantages from time-consuming and using a high quantity of harmful substances. Therefore, modified QuEChERS has been developed for routine toxicological laboratory for simpler sample preparation that could save time and decrease the use of toxic solvent for routine laboratory.

**Objective:** To validate the modified QuEChERS technique for the blood sample preparation and to determine drug abuse in the blood sample using LC-MS/MS.

**Methods:** Amphetamine, methadone, midazolam, morphine and triazolam were added to the blood. Modified QuEChERS was used for blood sample preparation and the analysis by LC-MS/MS was performed. Validation of the technique was tested and the application to the routine cases was done.

**Results:** The chromatographic data of the interested abused drugs were obtained within 14 minutes. Modified QuEChERS extracted products could recover the 5 drugs in which the coefficient of determination of the calibration curves was higher than 0.99 and no influence of other drugs was found in the selectivity test.

**Conclusion:** Modified QuEChERS is suitable for sample preparation before analysis with LC-MS/MS in routine forensic toxicology laboratory.

**Keywords:** Modified QuEChERS, drug abuse, LC-MS/MS.

Amphetamine, methadone, midazolam, morphine and triazolam have been worldwide drug abuse problems for years.<sup>(1,2)</sup> In Thailand, there are still a lot of people die from drug abuses.<sup>(3)</sup> The deceased with the suspect of drug intoxication must be sent to the institute of forensic medicine to perform an autopsy and collect the samples to confirm the cause and manner of death. Blood and urine are the most common samples from the autopsy sent to the forensic toxicology laboratory for analysis.<sup>(4,5)</sup>

Recently, the standard methods of forensic toxicology analysis are gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-tandem mass spectrometry (LC-MS/MS). Liquid

Chromatography-tandem mass spectrometry<sup>(6)</sup> becomes a common method used in the past few years because it can be performed quickly and has high accuracy and sensitivity.<sup>(7)</sup>

LC-MS/MS analysis requires sample preparation. There are two common sample preparation methods which are solid-phase extraction (SPE) and liquid-liquid extraction (LLE).<sup>(8)</sup> However, due to their time consuming, high quantity of harmful substances and expensive, in 2003 Anastasiades M, *et al.*<sup>(9)</sup> dedicated to the determination of pesticide residues in fruits and vegetables by using the sample preparation called QuEChERS that stands for Quick, Easy, Cheap, Effective, Rugged and Safe. QuEChERS consists of two steps: the first step is the extraction-partitioning step in which the sample is added by acetonitrile (ACN) before adding salts and secondly, the dispersive SPE is introduced for cleaning up the sample. Later, a modified QuEChERS technique has been developed and adapted to the work in forensic toxicology for decreasing the steps, saving time and the cost to

\*Correspondence to: Udomsak Hoonwijit, Department of Forensic Medicine, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand.

E-mail: udomsakhoon@hotmail.com

Received: September 16, 2020

Revised: October 7, 2020

Accepted: October 14, 2020

prepare samples. Therefore, the purposes of this study were to validate the modified QuEChERS technique for blood sample preparation and to determine drug abuse in the blood sample using LC-MS/MS.

### Materials and methods

This study has been approved by the Institutional Review Board (IRB), the Faculty of Medicine, Chulalongkorn University (IRB no. 465/62).

### Chemical and reagents

The following compounds were purchased from Cerilliant<sup>®</sup>: analytical reference standard; amphetamine, amphetamine-D5, methadone, methadone-D3, morphine, morphine-D3, midazolam, and triazolam. Acetonitrile and formic acid (HPLC grade) were purchased from Merck millipore<sup>®</sup>. 10 mM ammonium formate (Crystalline/Certified ACS) was purchased from Fisher chemical<sup>®</sup>. Ready-to-use QuEChERS salts were purchased from UCT<sup>®</sup> (Mg<sub>2</sub>SO<sub>4</sub> 4000 mg/NaCl 1,000 mg).

### Sample preparation

The sample preparation method was modified from Dularent S, *et al.*<sup>(10)</sup> Blood from the blood bank was stored at -20°C until the extraction study. 20 µL internal standard (IS) prepared at a concentration of 200 ng/mL in ACN was added to 100 µL of blood samples. Then the standard of the compounds in this study was added into the blood samples to get the interested concentrations of this study ACN stored at -20°C was added and the final volume was 320 µL. The mixture was vortexed for 30 sec. After 10 minutes left in the room temperature; 40 mg of QuEChERS salts was added, then shaken by shaker 5 min and centrifuged at 15,000 g for 10 min. Fifty microliters of supernatant were transferred to an injection vial with 150 µL of diluted mobile phase (A: B, 1: 3). The final 1 µL was injected into the LC-MS/MS system.

Five points of calibration standards were prepared in blood samples (5, 50, 100, 200, and 500 ng/mL) by adding the volume of working standard solution to the blood samples.

### LC-MS/MS conditions

A Shimadzu 8,060 triple quadrupole mass spectrometer was used with the positive electrospray ionization mode (ESI). The setting parameters were

as follows (after mass calibration): nebulizing gas flow 3 L/min, heating gas flow 10 L/min, interface temperature 300°C, desolvation Line (DL) temperature 250°C, heat block temperature 400°C and drying gas flow 10 L/min and multiple reaction monitoring (MRM) mode. Mobile phase: (A) 10 mM ammonium formate in water + 0.1% formic acid and (B) 10 mM ammonium formate in methanol + 0.1% formic acid. Using gradient mode: 0.3 mL/min; 0.00 - 2.00 min, 15.0% (B); 2.00 - 10.00 min, 15.0 - 50.0% (B); 10.00 - 12.00 min, 50 - 95% (B); 12.00 - 20.00 min, 95 - 5.0% (B); 21.00 - 26.00 min, column equilibration with 5.0% (B) at 40°C of oven temperature.

### Validation for blood sample preparation and analysis by LC-MS/MS

The set of acceptance criteria were as follows:

**Precision and accuracy:** The intra-assay precision (%RSD; relative standard deviation) and accuracy were assessed at 5, 50, and 500 ng/mL for all compounds. Acceptance criteria were intra-assay precision (%RSD) and accuracy less than 20.0%.

**Linearity:** Calibration graphs of the compounds of interest-to-internal standard peak area ratios of the quantification versus the expected concentrations used the linear regression. A value greater than 0.99 was expected for the coefficient of determination ( $r^2$ ).

**Recovery:** The analyte/internal standard peak area ratios obtained after the extraction was compared to the ratio of the standards/internal standard. %RSD in the extraction recovery had to be less than 20.0%.

**Selectivity:** To test impurities from degradant and matrix, daily common compounds in blood samples such as caffeine, nicotine, acetaminophen, and estazepam were added to test the selectivity.

**Limit of detection (LOD):** This study used signal/noise (S/N) ratio > 3 in the chromatogram for the determination of the lowest concentration of the compound to be LOD.

### Results

The chromatographic separation of the 5 compounds was obtained within 14 minutes, with the first retention time of morphine 2.57 min to 13.09 min of triazolam (Table 1). Table 2 shows acceptance criteria obtaining for intra-assay precision and accuracy. The extraction recovery %RSD data were less than 20.0% (Table 3). The limit of detection of the 5 substances is presented in Table 3. The

calibration curve used linear regression and the coefficients of determination of the calibration curve were higher than 0.99. No interference in the selectivity test was found (Figure 1). The application of the method was applied to the previous cases in

which extracted by SPE and the results were similar.

The study revealed two points out of expected outcomes which were the precisions of morphine at 5 ng/mL and amphetamine at 5 ng/mL (Table 2).

**Table 1.** MRM transitions and retention time of the 5 compounds.

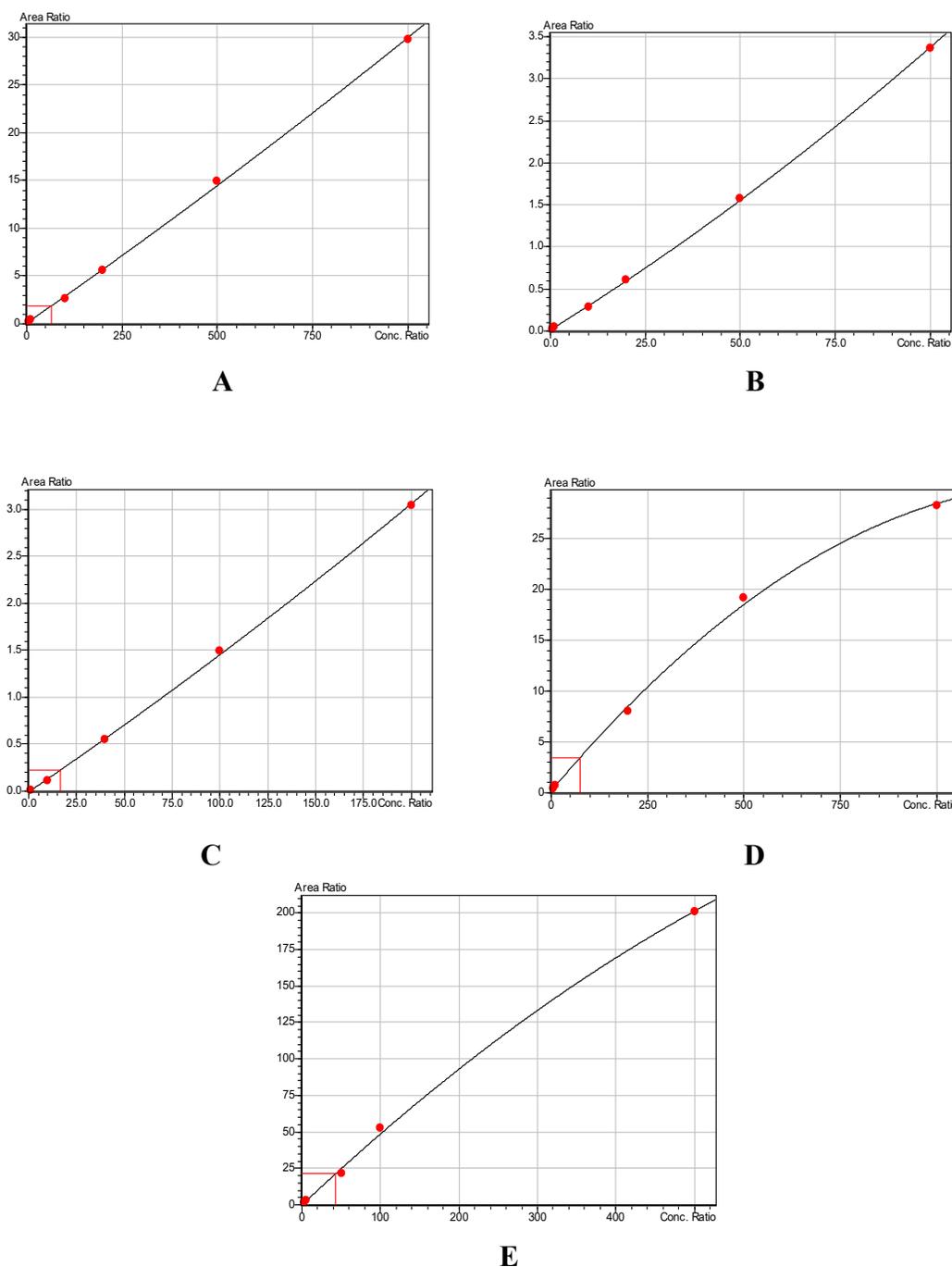
Standard	Retention time (min)	Precursor ion (m/z)	Product ion (m/z)
Amphetamine	5.52	136.10	119.15, 91.10
Methadone	12.96	310.20	265.15, 105.05
Midazolam	12.54	326.10	291.00, 223.10
Morphine	2.57	286.15	201.10, 152.10
Triazolam	13.09	343.05	308.20, 315.00

**Table 2.** Intra-assay and accuracy of the 5 compounds.

Standard	Intra-assay Precision (% (%RSD))			Accuracy (%RSD) average
	5 ng/mL	50 ng/mL	500 ng/mL	
Amphetamine	-4.44 (20.73)	2.92 (1.48)	4.174 (4.68)	15.02
Methadone	22.04 (5.33)	13.01 (5.79)	14.65 (5.44)	13.73
Midazolam	14.26 (5.22)	10.94 (3.15)	10.42 (4.60)	9.14
Morphine	-3.57 (out)	15.00 (14.16)	10.39 (4.96)	4.08
Triazolam	25.30 (16.50)	8.94 (3.81)	11.89 (3.78)	12.45

**Table 3.** Recovery and limit of detection of the 5 compounds.

Standard	Recovery (% (%RSD))		LOD (ng/mL)
	5 ng/mL	500 ng/mL	
Amphetamine	95.33 (3.80)	90.47 (4.24)	0.50
Methadone	90.31 (3.49)	75.89 (6.98)	0.05
Midazolam	96.95 (17.82)	70.49 (3.46)	0.20
Morphine	80.73 (8.27)	82.65 (13.29)	0.05
Triazolam	82.42 (12.31)	78.91 (2.21)	0.25



**Figure 1.** Calibration curve of (A) amphetamine, (B) methadone, (C) midazolam, (D) morphine and (E) triazolam.

### Discussion

This study demonstrated blood sample preparation by QuEChERS for determining amphetamine, methadone, midazolam, morphine and triazolam which all validated data presented in acceptance criteria. But precision values in the low concentrations of amphetamine and morphine showed that the method might not be suitable for these two compounds. So other sample preparations should have been

developed and validated for better analysis of these drugs in the low concentration. For routine sample preparations such as solid-phase extraction (SPE) and liquid-liquid extraction (LLE), the extracts might be clean but they were time-consuming, using a lot of solvents and required multiple-steps for sample preparation. Recently, Usui, *et al.*<sup>(11)</sup> reported QuEChERS extraction in whole blood. The proposed method needs only 2 extraction steps which are still

time-consuming. In 2014, they reported modified QuEChERS to determine drugs from liver samples,<sup>(12)</sup> also they still required two extraction steps. In 2017, Asl, *et al.*<sup>(13)</sup> compared modified QuEChERS and LLE for drug analysis in urine and they showed that modified QuEChERS provided good results but they also required to reconstitute step in sample preparation. In 2018, Dybowski, *et al.*<sup>(14)</sup> reported the quantification of drugs in whole blood by QuEChERS extraction with good results but they have multiple, time-consuming step. Therefore, this study showed modified QuEChERS with one step sample preparation to determine drugs of abuse in routine forensic toxicology laboratory which less time and less hazard solvent was used in the preparation. The validation results were acceptable so this technique could be applied for routine use in a forensic toxicology laboratory.

There are some Limitations in this study. This study validated the proposed method but had not compared to the analytical results to the gold standard technique. The sample matrix effect is another limitation since the matrix of blood from blood bank is different from the matrix of blood in forensic cases.

### Conclusion

This study validated the modified QuEChERS method for blood sample preparation for the determination of 5 drugs of abuse by LC-MS/MS. The method is suitable for routine sample preparation in the forensic toxicology laboratory. It might be further studied in the extraction of an alternative specimen such as vitreous fluid.

### Acknowledgements

This study was supported by the Ratchadapiseksompotch Fund, Faculty of Medicine, Chulalongkorn University. The authors would like to thank all the staff members of the forensic toxicology laboratory, Faculty of Medicine, Chulalongkorn University for assistance and support of this project.

### Conflict of interest

The authors, hereby, declare no conflict of interest.

### References

1. Tobias JD, Leder M. Procedural sedation: A review of sedative agents, monitoring, and management of complications. *Saudi J Anaesth* 2011;5:395-410.
2. Wu LT. Substance abuse and rehabilitation: responding to the global burden of diseases attributable to substance abuse. *Subst Abuse Rehabil* 2010;1:5-11.
3. Angkurawaranon C, Jiraporncharoen W, Likhitsathian S, Thaikla K, Kanato M, Perngparn U, et al. Trends in the use of illicit substances in Thailand: Results from national household surveys. *Drug Alcohol Rev* 2018;37:658-63.
4. Flanagan RJ, Braithwaite RA, Brown SS, Widdop B, de Wolff FA. *Basic analytical toxicology*. Geneva: WHO; 1995.
5. Forrest AR. ACP Broadsheet no 137: April 1993. Obtaining samples at post mortem examination for toxicological and biochemical analyses. *J Clin Pathol* 1993;46:292-6.
6. Mbughuni MM, Jannetto PJ, Langman LJ. Mass spectrometry applications for toxicology. *Ejifcc* 2016; 27:272-87.
7. Mogollon NGS, Quiroz-Moreno CD, Prata PS, de Almeida JR, Cevallos AS, Torres-Guierrez R, et al. New advances in toxicological forensic analysis using mass spectrometry techniques. *J Anal Methods Chem* 2018;2018:1-17.
8. Shintani H. Liquid-liquid extraction vs solid phase extraction in biological fluids and drugs. *Int J Clin Pharmacol Toxicol* 2013;2:1.
9. Anastassiades M, Lehota SJ, Stajnbaher D, Schenck FJ. Fast and easy multiresidue method employing acetonitrile extraction/partitioning and “dispersive solid-phase extraction” for the determination of pesticide residues in produce. *J AOAC Int* 2003;86: 412-31.
10. Dulaurent S, El Balkhi S, Poncelet L, Gaulier JM, Marquet P, Saint-Marcoux F. QuEChERS sample preparation prior to LC-MS/MS determination of opiates, amphetamines, and cocaine metabolites in whole blood. *Anal Bioanal Chem* 2016;408:1467-74.
11. Usui K, Hayashizaki Y, Hashiyada M, Funayama M. Rapid drug extraction from human whole blood using a modified QuEChERS extraction method. *Leg Med (Tokyo)* 2012;14:286-96.
12. Usui K, Hashiyada M, Hayashizaki Y, Hosoya T, Igari Y, Sakai J, et al. Application of modified QuEChERS method to liver samples for forensic toxicological analysis. *Forensic Toxicol* 2014;32:139-47.
13. Asl S, Khodayar M, Mousavi Z, Akhgari M. Methadone extraction by modified QuEChERS and liquid-liquid extraction from post-mortem urine by GC-MS. *J Med Toxicol Clin Forensic Med* 2017;3:1-4.
14. Dybowski MP, Dawidowicz AL. Application of the QuEChERS procedure for analysis of delta (9)-tetrahydrocannabinol and its metabolites in authentic whole blood samples by GC-MS/MS. *Forensic Toxicol* 2018;36:415-23.