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Methadone Extraction by Modified Quechers and Liquid-Liquid Extraction from Post-Mortem Urine by GC-MS

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Abstract

Background: Methadone abuse in drug addiction centers and increased methadone consumption among addicts is a new cause of mortality due to substance abuse in forensic medicine. The usual method of extracting urine from corpses in most legal centers is a long and costly method of liquid-liquid extraction, which in addition to the high consumption of solvents and environmental contamination causes severe damage to the column of laboratory analysis devices. As a result, QuEChERS extraction method was proposed as an easy, fast, cheap and green way.

Objective: In this paper, the results of methadone extracted from the urine after death using Liquid-Liquid extraction and modified QuEChERS method were compared.

Method: Liquid-liquid extraction (LLE) by acidifying the urine sample with Hcl and separating the organic phase at an alkaline pH, with a Chloroform and Isopropanol solvent. Extraction in the QuEChERS method is using $MgSO_4$ and NaCl salts and ethyl acetate solvent. Detect methadone in two methods with GC-MS.

Results: The recovery level of methadone of urine sample was obtained equal to 78% (N=15) in QuEChERS method and equal to 55% (N=15) in LLE method. According to the obtained results, there was significant difference between QuEChERS and LLE methods in the term of methadone test in post-mortem urine.

Conclusion: Cheap, fast, effective and green QuEChERS improved with better recovery could replace the LLE method in detecting methadone in post-mortem urine samples.

Keywords: LLE; QuEChERS; Methadone; Post-mortem urine; GC-MS

Introduction

QuEChERS stands for Quick, Easy, Cheap, Effective, Rugged and Safe. Alves et al. [1] first introduced this method to extract veterinary drugs from animal tissue. This method is widely used to determine pesticides [1]. In forensic medicine are usually use Liquid-Liquid extraction (LLE) and solid phase extraction (SPE) methods. The SPE method is expensive and the cleaning of the homogenizer probe creates a risk of contamination and cross-reactivity, and therefore the SPE method is somewhat tedious and time-consuming and cannot be performed in many centers, but the QuEChERS method is widely used as a simple, fast and reliable method is used [2]. Extraction is an important step in the analysis of toxicology in forensic medicine [3,4]. Clinical toxicology and forensic medicine are heavily dependent on the science of decomposition chemistry [5,6]. Liquid-liquid extraction is simple but reduces its value due to inadequate extraction of samples and contamination of tools and the environment. The SPE method with a high selective performance, but this method is relatively costly and time-consuming. The QuEChERS method was proposed for the analysis of legal toxicology [7]. The QuEChERS method is similar to LLE is simple and also similar to SPE have high selective performance [7,8]. The QuEChERS method is used to determine the residue of pesticides in food products [7,9]. QuEChERS is based on extraction with solvents such as acetonitrile or ethyl acetate and dehydration in the presence of salts such as magnesium sulfate and sodium chloride. Methadone is predominantly prescribed for treatment of heroin addiction and has a morphine-like function [10]. Some methadone is metabolized to the 2-Ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) by Cytochrome P450 (CYP3A4). The ratio EDDP to Methadone is used in the diagnosis of long-term

and short-term treatment [11]. However, EDDP is the product of the side effect of high temperature GC on methadone. Reducing the temperature of the GC inlet port from 260 to 180°C reduced the concentration of EDDP observed in a methadone sample at an initial concentration of 10,000 ng/mL from 201 ng/mL to 53 ng/mL. As a result, alternative methods such as HPLC or LC-MS should be considered [12].

In this paper, Methadone extraction by QuEChERS from post-mortem urine samples.

Materials and Method

The Methadone (ST) with concentration of 100 µg/mL (Sigma-al Rich), Methanol, Ammonia, Chloroform, Isopropanol, Acid hydrochloric acid, Ethyl acetate, Magnesium sulfate, Sodium hydrogen chloride **(Figure 1).**

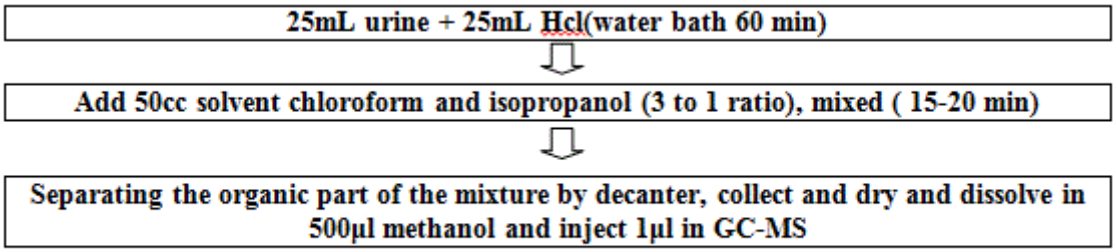

Figure 1 Liquid-liquid extraction method.

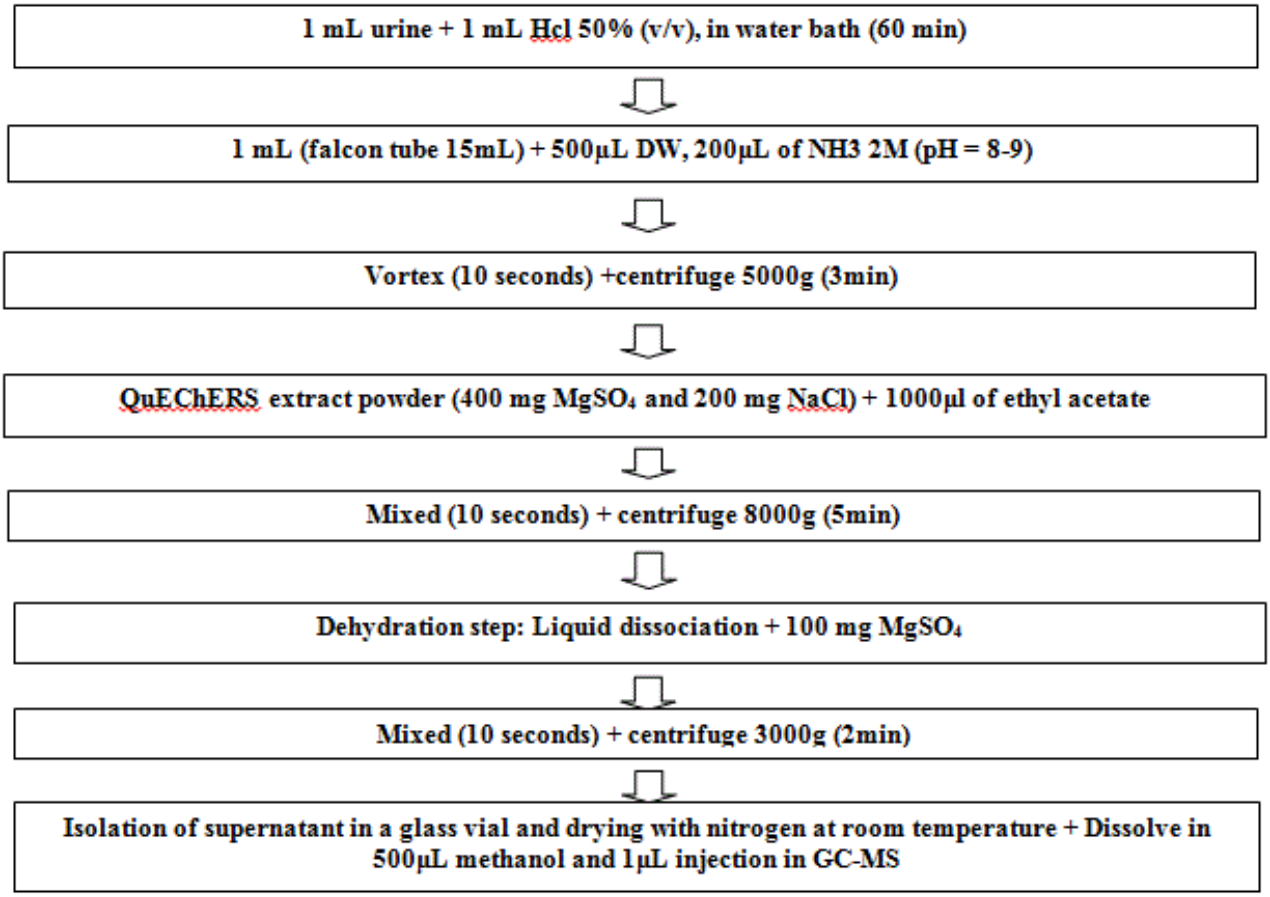

Figure 2 QuEChERS method.

The oven temperature increased from 60°C to 280°C at a temperature of 10°C and was kept constant at 280°C for 10 min. Helium gas was used with a purity of 999/99 at a flow rate of 1 mL/min and a mass energy of 70 ev. Split less injection mode

and run time is 30 min. The methadone identification method was based on the SIM index ion **(Table 1)**.

Table 1 Index ions in GC-MS.

Analyte	Quantifier (m/z)	Qualifier(m/z)	RT
Methadone	72	294223165	11.7
EDDP	276	262220165	11.1

For analysis of the Gas chromatography apparatus model 7890A connected to Agilent mass spectrometer Model 5975C equipped with a column (30 m*0.25 mm*0.25 µm thin film thickness), which used as a fixed phase with 5% phenyl methyl **(Figure 2)**.

The results were analyzed with the Wiley7n.1 library software GC-MS according to the index (m/z) **(Figure 3)**. Methadone validation and its results in **Table 2**.

Table 2 methadone validation and its results.

Accuracy (%, n=3)	Precision (%, n=3)	Quality control Concentration (ng/mL)	LOQ (ng/mL)	LOD (ng/mL)	N=3	
89	13.5	62.5			Day=1	
95	8	250	97.3	29.1	Day=2	Methadone
101	11	2000			Day=3	

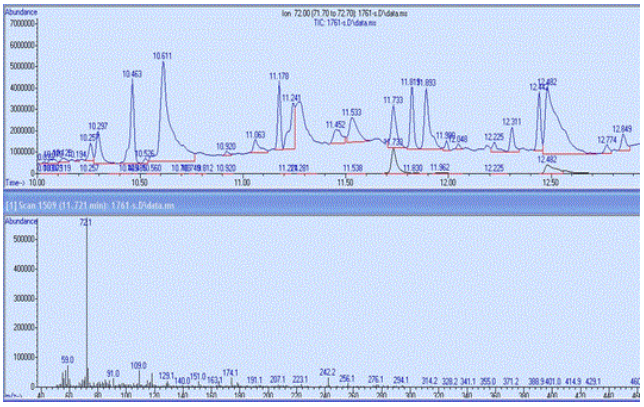

Figure 3 Methadone analysis based on index ion in GC-MS.

Results

Methadone recovery rates in different samples in QuEChERS method were 65-78% **(Figure 4)**. Generally, the rate of recovery in studies is between 80% and 120% **(Figure 5)**. Jones et al. showed that for QuEChERS, 60-70% improvement for medium and non-polar compounds is acceptable [13].

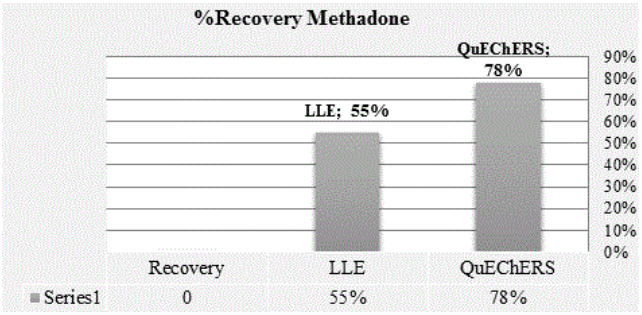

Figure 4 Compare recovery with two extraction methods.

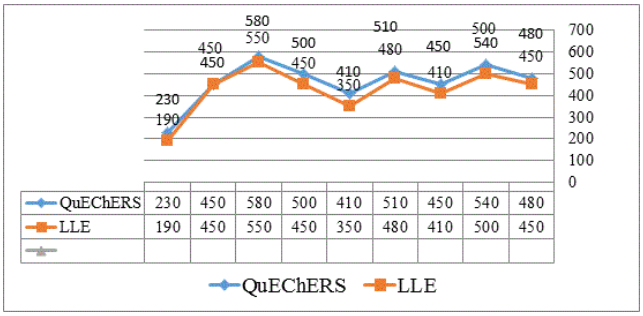

Figure 5 Comparison of methadone levels in Post-mortem urine by two methods of extracting ng/ml.

Discussion

Extraction method in most of the forensic centers is LLE. Large volume of samples and solvents, inadequate extraction and contamination of tools and the environment are main disadvantages of LLE method [1]. Compounds of carcinogen and mutagen such as Chloroform and Ammonia, threat to the health of staff and experts in forensic centers. In addition, utilizing large

amounts of solvent and chemicals seriously threatens the enviroment and human community. As a result, finding an efficient method with fewer risks force us to use new techniques such as SPE and QuEChERS.

QuEChERS was approved as a sensitive, renewable and relatively simple method for qualitative and quantitative analysis of drugs and medicine. Since this method does not require specific equipment and requires less time, this method has a great potential for analyzing clinical and legal samples.A review of the results from previous studies in QuEChERS extraction has proved its ability to detect drugs and drugs in biological samples. In a study by Emanuele Amorin Alves and colleagues in 2016 aimed at refining and developing the QuEChERS method, he was able to detect 13 opioid , methadone, and cocaine and their metabolites in total blood with the GC-MS device, Showed that this method has a high operational capability in various forensic cases [2]. It's easy to detect analyte with varying amounts of detection with a reagent and calculate the highest recovery, but the implementation of these methods is based on actual samples that are metabolized in the body or in samples of corpses that are corrupted after Death is different. There are numerous interventions in these samples, such as binding and conjugation of analyte to vital molecules, Postmortem Redistribution, metabolism, and the formation of secondary metabolites and post-mortem corruption makes it difficult to work. The results were much better in comparison with the LLE method in terms of ease, fastness and low cost.

Conclusion

The results of this study did not show significant difference betweenthe results of costly and time-consuming LLE method with QuEChERS method. Recovery of methadone in a much smaller volume of urine samples inQuEChERS, Showed that this method could replace LLE in detecting methadone in urine after death.

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