Effects of triglycerides levels in human whole blood on the extraction of 19 commonly used drugs using liquid–liquid extraction and gas chromatography–mass spectrometry

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A B S T R A C T

Liquid–liquid extraction (LLE) is the most commonly sample preparation procedure used by forensic toxicologists in China for screening drugs in whole human blood. It extracts numerous substances from blood including targeted drugs and interfering substances, specifically triglycerides (TG). With increasing prevalence of hyperlipidemia, the influences of TG on LLE and on subsequent analysis with gas chromatography–mass spectrometry (GC–MS) may become a major issue for forensic laboratories. This study aims to elucidate the influences of TG on LLE and to provide possible solutions to this problem. Nineteen commonly encountered drugs in forensic cases were spiked to human whole blood with different TG concentrations. Diethyl ether, ethyl acetate/hexane mixed solutions, chlorobutane and several other frequently used solvents were tested for the extraction of drugs from spiked whole blood. The supernatant organic layer was evaporated to dryness and reconstituted with methanol. The resultant products were analyzed by GC–MS, and the extraction recovery was calculated. LLE with diethyl ether, ethyl acetate/hexane (9:1) and chlorobutane all possessed effective and reliable extraction recoveries for blood sample with low TG concentrations (0.63–6.85 mmol/L). At high TG concentrations, diethyl ether produced a highly turbid substance that could not be further analyzed using GC–MS. Extraction recoveries dramatically dropped for ethyl acetate/hexane (9:1) mixture at high TG concentrations, while chlorobutane experienced minimal drops in extraction recoveries. In conclusion, TG levels in whole blood noticeably influence drug recovery to variable extents depending on the LLE solvent. Chlorobutane showed minimal influences from TG content in whole blood and thus is the recommended LLE solvent for forensic drug extraction.

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1. Introduction

Human whole blood is a ubiquitous sample in the field of forensic toxicology. The most common procedure of choice for pretreating whole blood is initial pretreatment by liquid–liquid extraction (LLE). Because of its suitability for screening, ease of operation, low cost, and adaptability...
In LLE, an extraction solvent is used to extract and purify the analytes out of whole blood to be further analyzed. One main parameter for assessing LLE efficacy is the extraction recovery of targeted analytes. Other parameters that need to be considered include the extraction solvent’s specificity, volatility and toxicity [5]. Unfortunately, extraction solvents often can extract additional endogenous substances, such as triglyceride (TG) [14], which may interfere with subsequent analysis [1]. With increasing prevalence of hypertriglyceridemia in China (up to 11.3% for individuals over 18 years old) [11,12], the level of TG is now more likely to affect the detection of drugs in whole blood. It was believed that the saturated fatty acid chains of TG showed affinity to form tight bonds with certain drugs, depending on chemical structure and polarity of the drug. This could result in formation of complexes that cannot be extracted efficiently by LLE solvents [7,8]. This study hypothesizes that high TG levels can reduce the extraction recoveries of drugs when using LLE.

The primary purpose of this study was to determine the impact of TG content on LLE for human whole blood. This study also attempts to find an alternative extraction solvent that can counteract the negative impacts of TG.

2. Materials and methods

2.1. Chemicals and solutions

Amphetamine (AMP), methamphetamine (MAMP), 3,4-methylenedioxymethamphetamine (MDA), 3,4-methylenedioxyamphetamine (MDMA), ketamine, methadone, pethidine, secobarbital, lidocaine, clenbuterol, benzhexol, carbamazepine, diazepam, chlorpromazine, oланzapine, flurazepam, clozapine, alprazolam, triazolam and diphenoxylate were purchased from the National Institute for Control of Pharmaceutical and Biological Products (Beijing, PR China). HPLC grade chlorobutane was obtained from Sigma–Aldrich Co., Ltd. (St. Louis, USA). Analytical grade ethyl acetate, hexane, cyclohexane, heptane, isooctane, sodium hydroxide (NaOH) and HPLC grade methanol were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. Instrumentation and chromatographic conditions

The chromatographic system used was an Agilent 7890 GC. It was fitted with a 5975C mass detector (MSD) (Agilent Technologies, Palo Alto, CA, USA) and connected to HP Chemstation software for data recording.

Separations were conducted on a HP-5MS capillary column (30 m × 0.25 mm × 0.25 μm) (Agilent Technologies, Palo Alto, CA, USA). 1 μL of sample was injected in split mode (split ratio = 10:1) using an ionizing energy of 70 eV with temperatures of the inlet, MSD transfer line, quadrupole and ion source at 250 °C, 280 °C, 150 °C and 230 °C, respectively. Temperature of the column was set at 100 °C initially, maintained for 1 min and increased at a rate of 20 °C/min to 280 °C, which was kept constant for 23 min.

| Drug       | Retention time (RT) (min) | Significant ions (m/z) | Spiked concentration (μg/mL) |
|------------|---------------------------|------------------------|------------------------------|
| AMP        | 4.30                      | 44/91/65               | 5.0                          |
| MAMP       | 4.55                      | 58/91/149              | 5.0                          |
| MDA        | 6.20                      | 44/136/179             | 5.0                          |
| MDMA       | 6.45                      | 58/135/193             | 5.0                          |
| Pethidine  | 7.60                      | 71/247/172             | 5.0                          |
| Secobarbital | 7.80                  | 168/195/124            | 5.0                          |
| Ketamine   | 8.25                      | 180/209/152            | 5.0                          |
| Lidocaine  | 8.30                      | 86/58/234              | 5.0                          |
| Clenbuterol | 9.20                     | 86/57/127              | 5.0                          |
| Benzhexol  | 10.20                     | 98/218/118             | 5.0                          |
| Carbamazepine | 10.60                   | 193/236/165            | 5.0                          |
| Diazepam   | 11.20                     | 256/283                | 5.0                          |
| Chlorpromazine | 11.50                | 58/86/318              | 5.0                          |
| Olanzapine | 13.35                     | 242/229/312            | 5.0                          |
| Flurazepam | 13.65                     | 86/99/387              | 5.0                          |
| Clozapine  | 14.9                      | 243/256/192            | 5.0                          |
| Alprazolam | 16.0                      | 279/204/308            | 5.0                          |
| Triazolam  | 17.6                      | 313/238/342            | 5.0                          |
| Diphenoxylate | 27.8                    | 246/42/91              | 7.5                          |

AMP, amphetamine; MAMP, methamphetamine; MDA, 3,4-methylenedioxymethamphetamine; MDMA, 3,4-methylenedioxyamphetamine.

Helium was used as the carrier gas at a constant flow rate of 1 mL/min for a total GC runtime of 33 min. There was a 3 min solvent delay before the ion source was turned on. Selected ion monitoring (SIM) mode was utilized to collect chromatograms. Two or three fragment ions were used for each compound (Table 1).

2.3. Specimen

Whole blood samples for preliminary experiments were leftover blank blood from forensic cases. Hypertriglyceridemia whole blood samples were obtained from 96 volunteers, and they were divided into 5 groups according to TG concentrations, which were measured with a Hitachi 7600-120 Model Automatic Analyzer. Overall TG concentration ranged from 0.60 mmol/L to 33.35 mmol/L. All samples were stored at −20 °C for 2 months prior to LLE.

2.4. Sample preparation

2 mL of human whole blood was initially spiked with 19 drugs each reaching a concentration of 5 μg/mL except diphenoxylate which reached 7.5 μg/mL. 3 mL of extraction solvent was then added to the sample. The samples were mixed for 2 min followed by centrifugation at 4000 RPM for 5 min. The supernatant organic layer was collected. 200 μL of NaOH (10%) and 3 mL of the same extraction solvent used previously were added to the pellet remaining from centrifugation. The samples were again mixed for 2 min followed by centrifugation at 4000 RPM for 5 min. The supernatant organic layer was collected and combined with the previously collected supernatant. The combined supernatant was then evaporated to dryness under air at 50 °C. The residue was reconstituted in 100 μL of methanol, of which 1 μL was injected into the GC–MS system. The
Table 2
Recoveries of most common drugs for comparison between extraction solvents.

| Drug          | Chlorobutane | Diethyl ether | Ethyl acetate | Heptane | Hexane | Isooctane |
|---------------|--------------|---------------|---------------|---------|--------|-----------|
| Diazepam      | 56.3%        | 46.9%         | 65.1%         | 38.8%   | 22.1%  | 14.3%     |
| Chlorpromazine| 47.9%        | 42.6%         | 36.7%         | 23.2%   | 3.2%   | 8.4%      |
| Flurazepam    | 59.2%        | 47.5%         | 52.6%         | 38.4%   | 4.4%   | 15.8%     |
| Clozapine     | 50.1%        | 45.5%         | 60.5%         | 24.5%   | 34.8%  | 1.3%      |
| Alprazolam    | 33.9%        | 33.0%         | 54.6%         | 0.10%   | 56.4%  | 0.12%     |
| Glenbuterol   | 42.5%        | 59.0%         | 69.8%         | 44.9%   | 12.3%  | 2.7%      |
| Carbamazepine | 34.5%        | 40.6%         | 78.8%         | 0.6%    | 0.10%  | 0.08%     |

The extraction recovery values were calculated by comparing the peak areas of the analytes extracted from whole blood to the areas obtained by injecting the standard solutions at the same concentrations.

2.5. Statistical analysis

Statistical analyses in this study were conducted using Statistical Product and Service Solutions (SPSS) version 15.0.

3. Results and discussion

3.1. Preliminary selection of organic solvents

The organic solvents used for the extraction of drugs in human whole blood were selected based on frequency of usage in research and blood detection in forensic practice [2–4,13,15,16,18–21,23], and on their corresponding toxicities. Thus, solvents of high toxicity such as benzene, chloroform and dichloromethane were not included in our list despite favorable extraction efficiency. Our preliminary list contained pure solvents (diethyl ether, chlorobutane, hexane, ethyl acetate, heptane, and isooctane), and mixed solutions (chlorobutane/isopropanol solution and ethyl acetate/hexane solution). These extraction solvents were evaluated based on recoveries using the same spiked whole blood.

3.1.1. Evaluation of pure solvent

Six pure solvents were studied, and it was found that, the extraction solvents that possessed the highest and most consistent recoveries were chlorobutane, diethyl ether and ethyl acetate (Table 2). Hexane possessed low recovery specifically for chlorpromazine, flurazepam, and carbamazepine, while heptane and isooctane possessed low recovery for alprazolam and chlorpromazine. Although heptane, hexane and isooctane are commonly used as the extraction solvents in other researches, we believe their weak polarities constitute to their low recoveries, making them unsuitable for LLE. Thus, chlorobutane, ethyl acetate and diethyl ether were selected as the extraction solvents for further testing.

3.1.2. Evaluation of ethyl acetate/hexane mixed solutions

For ethyl acetate/hexane solution, the ratio of ethyl acetate to hexane varied greatly in research [15,21] and required a selection of an optimal mixture. The recoveries for pure ethyl acetate were compared to those of ethyl acetate/hexane mixture at ratios of 2:1, 6:1 and 9:1. The results showed little difference between pure ethyl acetate and three ethyl acetate/hexane mixtures, showing a relative standard deviation (RSD) lower than 11.1%. Using MDMA as an example, the recovery was 51.5% when using ethyl acetate alone, and 51.7%, 49.2% and 51.3% at ratios of 2:1, 6:1, 9:1, respectively (RSD = 2.2%, n = 4). The only notable improvement was the 70% increase in extraction recovery for chlorpromazine by ethyl acetate/hexane mixture at 9:1.

Furthermore, from the gas chromatogram, we discovered that the ethyl acetate/hexane mixtures consistently produced significantly lower background noise than pure ethyl acetate from impurities (Fig. 1). This phenomenon
was most recognizable for column bleeding at retention time 5.2 min. We believed that the addition of hexane to ethyl acetate helped protect the gas chromatographic column but further investigation would be required for confirmation.

Therefore, ethyl acetate/hexane mixed solution (9:1) replaced pure ethyl acetate in further testing.

3.1.3. Evaluation of chlorobutane/isopropanol (4:1) mixed solution

We discovered that the addition of chlorobutane to blood followed by mixing often resulted in emulsification of the sample. This phenomenon formed a thick, turbid layer that effectively trapped and prevented the drugs from separating into the supernatant organic layer during centrifugation. As a result, the drugs remained in the pellet and recoveries drop drastically. To deal with this problem, chlorobutane/isopropanol (4:1) mixture was used instead of pure chlorobutane [20]. Isopropanol acted as a de-emulsifier and subsequent mixing effectively counteracted the emulsification. However, this mixed solution produced varying results for drug recoveries. The mixed solution reduced recoveries for MDMA, clenbuterol, carbamazepine, clozapine and alprazolam while only increasing recoveries for MDA and diphendil. Furthermore, the mixed solution produced more background noise on chromatogram than pure chlorobutane. Therefore, pure chlorobutane was preferable for further testing. In regards to emulsification, saturated salt water equal to the amount of chlorobutane was added to the emulsified substance. Subsequent mixing and centrifugation counteracted emulsification while also preserving recoveries.

3.2. Effect of TG concentration on the extraction recovery when using diethyl ether, ethyl acetate/hexane mixture and chlorobutane as the extraction solvents

19 drugs spiked to whole blood samples with varying TG concentrations were extracted by the solvents obtained from preliminary selection, and the recoveries were compared.

3.2.1. Diethyl ether as the extraction solvent

Under low TG concentrations (1.67, 3.79, and 6.87 mmol/L), diethyl ether produced recoveries ranging from 11.7% to 81.1%, and 16 out of 19 drugs had consistent recoveries above 50%. The recovery data was highly precise. The RSDs of the recoveries for MDA, MDMA, carbamazepine, chlorpromazine, clozapine and diphenoxylate were 7.6%, 11.3%, 11.4%, 14.1%, 16.2% and 16.2% respectively for the three trials. However, diethyl ether produced low recoveries for olanzapine, AMP, and MAMP at 32.7% (RSD = 16.8%, n = 3), 21.3% (RSD = 35.0%, n = 3) and 11.7% (RSD = 47.9%, n = 3) respectively. Regarding the poor recovery associated with amphetamines, we, as well as other researchers, have observed this phenomenon where amphetamines are extremely volatile by nature and can easily evaporate along with the supernatant during evaporation [9,10,22].

In this study, diethyl ether consistently produced serious procedural problems when pretreating whole blood under high TG concentrations. This problem occurred for all trials with TG concentrations of 11.43 mmol/L, 15.06 mmol/L, 19.76 mmol/L and 26.78 mmol/L. Evaporation of the supernatant organic layer to dryness after LLE with diethyl ether resulted in a brownish yellow, turbid and oily substance that could not reconstitute in methanol. This resulted in a turbid mixture which could not be injected for further instrumental analysis. In fact, this phenomenon has been frequently encountered in routine practice during forensic blood analysis in our lab. In certain cases such as hemorrhagic shock and cardiac rupture, the collected blood sample is extremely scarce, sometimes less than 2 mL. According to the standard protocol, a 2 mL of sample can supply enough blood for only one analytical procedure. Thus, if the extracted residue becomes turbid after LLE with diethyl ether, the blood analysis would become inconclusive as there may be inadequate blood left for additional analysis. Therefore, diethyl ether can only be used reliably for whole blood sample with low TG concentrations.

3.2.2. Ethyl acetate/n-hexane mixture (9:1) as the extraction solvent

At low TG concentrations ranging from 1.12 to 6.85 mmol/L, ethyl acetate/hexane (9:1) mixed solution consistently produced high recoveries, especially for MAMP, clozapine, lidocaine, ketamine, carbamazepine and chlorpromazine, which averaged to 90.5 ± 4.7%, 91.5 ± 3.6%, 91.7 ± 2.7%, 94.5 ± 3.0%, 92.7 ± 5.7% and 91.5 ± 4.6%, respectively. However, a rise in TG concentration resulted in dramatic drops in recoveries (Fig. 2). This was especially evident in the highest TG concentration of 23.35 mmol/L where recoveries dropped for all 19 drugs. This showed that high TG concentrations could dramatically reduce the recoveries when using ethyl acetate/hexane (9:1) mixed solution.

3.2.3. Chlorobutane as the extraction solvent

Chlorobutane produced the most consistent and reliable drug recoveries at all levels of TG concentration. Most drugs produced recoveries ranging from 30.3% to 85.8%, with the exception of four amphetamines (AMP, MAMP, MDA and MDMA) at any TG concentrations and of olanzapine at high TG concentrations (Table 3). These reduced recoveries for the amphetamines were also found in a study by Demme where chlorobutane was used to extract over 200 drugs from water [6]. Demme discovered that chlorobutane was an extremely potent extraction solvent for most drugs except amphetamines. As previously mentioned for diethyl ether, this was likely due to the loss of amphetamines during evaporation. The recoveries for most drugs only dropped minimally with increasing TG concentration. Most notable among varying TG concentrations were for dolantin, ketamine, lidocaine, clenbuterol, diazepam, flurazepam and triazolam whose recovery RSDs were only 13.9%, 15.4%, 13.6%, 13.2%, 15.6%, 15.6% and 16.5%, respectively. However, the extraction recoveries of olanzapine dropped to 1.0% and 3.6% when TG concentration reached 15.06 and 33.35 mmol/L. The non-polar piperazine ring of olanzapine may strengthen the affinity of the drug to bind to the saturated fatty acid chains of TG. This resulted in the formation of drug-TG complexes that
could not be extracted by LLE. Thus, olanzapine showed drastically reduced recoveries under high TG concentrations. Meanwhile, secobarbital, clenbuterol, diphenoxylate also possessed relatively low recoveries, which averaged 45.6% (RSD = 23.8%, n = 8), 55.0% (RSD = 13.2%, n = 8), and 30.3% (RSD = 30.3%, n = 8), respectively. The 2-pentyl group and the 3-propenyl group of secobarbital, the tert-butyl group of clenbuterol and the tetrahydropyridine structure of diphenoxylate increased each of their overall lipophilicity. Thus, we believe these drugs were also more likely to bind to TG to form complexes that could not be effectively extracted, resulting in decreased recoveries. These results suggested the extraction efficacy of chlorobutane was thwarted by TG levels only toward specific drugs such as olanzapine but remained consistent for most other drugs.

### 3.2.4. Cross comparison of diethyl ether, ethyl acetate/n-hexane and chlorobutane

For a particular blood sample, the recoveries of all 19 drugs were horizontally compared for diethyl ether, chlorobutane and ethyl acetate/hexane (9:1) mixed solution by extracting chemically stable drugs, particularly diazepam and benzhexol (Table 4). The recoveries of the

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### Table 3

Recovery of 19 drugs at varying TG concentrations using chlorobutane.

| Drugs     | TG concentration (mmol/L) | Mean  | RSD (%) |
|-----------|---------------------------|-------|---------|
| AMP       | 0.63                      | 1.47  | 1.99    |
|           | 2.24                      | 3.81  | 7.04    | 15.06 | 26.78 |
| MAMP      | 3.22                      | 18.4  | 35.4    | 35.8  | 33.4  | 31.9  | 6.5  | 29.4  | 26.4  | 38.8  |
| MDA       | 3.6                       | 8.6   | 18.7    | 43.7  | 29.8  | 46.0  | 0.2  | 49.9  | 23.0  | 87.2  |
| MDMA      | 12.4                      | 46.0  | 67.5    | 57.0  | 69.4  | 38.7  | 32.9 | 75.7  | 48.9  | 44.0  |
| Pethidine | 63.4                      | 71.1  | 82.6    | 76.0  | 86.4  | 95.6  | 79.5 | 65.7  | 78.0  | 13.9  |
| Secobarbital | 40.6                  | 44.5  | 49.8    | 42.6  | 48.7  | 62.2  | 44.1 | 23.4  | 45.6  | 23.8  |
| Ketamine  | 60.8                      | 75.6  | 88.5    | 72.7  | 86.2  | 97.2  | 80.3 | 65.8  | 78.6  | 15.4  |
| Lidocaine | 69.6                      | 85.7  | 95.5    | 80.9  | 92.7  | 102.3 | 83.6 | 70.0  | 85.8  | 13.6  |
| Clenbuterol| 46.6                      | 57.0  | 65.2    | 53.5  | 62.1  | 53.2  | 44.0 | 58.8  | 55.0  | 13.2  |
| Benzhexol | 71.1                      | 80.0  | 83.4    | 72.2  | 85.6  | 102.9 | 77.7 | 53.2  | 77.5  | 18.3  |
| Carbamazepine | 55.6                   | 74.4  | 77.8    | 61.2  | 66.5  | 89.8  | 60.1 | 49.6  | 68.2  | 19.2  |
| Diazepam  | 73.2                      | 87.6  | 96.8    | 78.9  | 89.3  | 99.8  | 73.6 | 62.2  | 82.6  | 15.6  |
| Chlorpromazine | 67.8                  | 70.8  | 74.2    | 67.2  | 71.9  | 83.1  | 49.3 | 40.9  | 65.0  | 21.2  |
| Olanzapine | 38.7                      | 63.3  | 79.4    | 44.3  | 23.4  | 80.7  | 1.0  | 3.6   | 45.0  | 69.6  |
| Flurazepam | 71.3                      | 83.9  | 92.6    | 78.2  | 85.8  | 100.5 | 70.0 | 62.1  | 81.4  | 15.6  |
| Clozapine | 69.5                      | 92.8  | 108.4   | 75.3  | 82.2  | 117.2 | 54.9 | 57.4  | 83.8  | 27.2  |
| Alprazolam| 66.7                      | 86.6  | 95.6    | 75.5  | 83.4  | 105.8 | 75.5 | 59.4  | 82.5  | 18.3  |
| Triazolam | 65.6                      | 78.9  | 86.5    | 68.4  | 76.5  | 91.3  | 68.1 | 52.6  | 75.0  | 16.5  |
| Diphenoxylate | 25.9                    | 30.3  | 39.7    | 28.6  | 31.7  | 74.4  | 36.2 | 15.2  | 30.3  | 24.7  |

TG, triglycerides; RSD, relative standard deviation; AMP, amphetamine; MAMP, methamphetamine; MDA, 3,4-methylenedioxyamphetamine; MDMA, 3,4-methylenedioxymethamphetamine.
drugs extracted by the three extraction solvents differed negligibly in samples with TG concentrations ranging from 3.79 mmol/L to 6.87 mmol/L. Diethyl ether showed lower recoveries for diazepam and benzhexol than the other two solvents in samples with TG concentration at 1.67 mmol/L and 7.75 mmol/L (Table 4). When TG concentration was above 6.87 mmol/L, recoveries obtained using diethyl ether as the extraction solvent declined drastically, which suggested that diethyl ether was greatly affected by TG concentration. Chlorobutane, on the other hand, produced the most consistent recoveries for all drugs even at high TG concentrations (Table 3), thus making it the extraction solvent of choice when facing unknown or high concentrations of TG in human whole blood.

4. Conclusions

Initial findings showed that ethyl ether, ethyl acetate and chlorobutane produced higher recoveries than hexane, heptane and isooctane. Furthermore, the addition of hexane to ethyl acetate drastically reduced background noise in gas chromatogram while maintaining recoveries. Therefore, diethyl ether, ethyl acetate/n-hexane (9:1) and chlorobutane were compared with respect to their ability to extract drugs from human whole blood under different TG concentrations. Extraction by diethyl ether performed well for blood samples with low TG concentrations, but often formed turbid residues when TG concentrations were high, which prevented further instrumental analysis. Ethyl acetate/hexane (9:1) mixed solution also effectively extracted all 19 drugs from blood samples with low TG concentrations but recoveries drastically fell with increases in TG concentration. Finally, chlorobutane effectively and reliably extracted most drugs, except the amphetamines, from blood samples with TG content in the range of 0.63–26.78 mmol/L. The amphetamines typically possessed low recovery and therefore traditional LLE might not be suitable for the extraction of amphetamines from whole blood samples. Chlorobutane sporadically formed an emulsified substance that drastically decreased recovery when mixed with blood. However, the addition of saturated salt water effectively eliminated the emulsification and restored recovery. Despite this drawback of chlorobutane, it still produced reliable and consistent recovery for drugs spiked to whole blood at varying TG concentrations. Therefore, we recommended chlorobutane as the primary extraction solvent for LLE pretreatment of human whole blood for forensic purposes.

Conflict of interest

We declare that we have no conflict of interest.

**Table 4**

Recoveries of diazepam and benzhexol at four TG concentrations obtained using diethyl ether, ethyl acetate/hexane mixed solution (9:1) and chlorobutane as the extraction solvents.

| TG concentration (mmol/L) | 1.67 | 3.79 | 6.87 | 7.75 | Benzhexol | 1.67 | 3.79 | 6.87 | 7.75 |
|---------------------------|------|------|------|------|-----------|------|------|------|------|
| Diethyl ether             | 57.7%| 69.6%| 82.0%| 58.4%| 58.5%     | 64.1%| 82.0%| 58.4%| 82.0%|
| Ethyl acetate/hexane mixture (9:1) | 76.0%| 73.1%| 76.6%| 87.6%| 80.1%     | 75.6%| 79.2%| 90.9%| 84.0%|
| Chlorobutane              | 81.3%| 76.4%| 71.4%| 98.6%| 82.4%     | 73.3%| 73.2%| 84.0%|      |

**Transparency document**

The Transparency document associated with this article can be found in the online version.

**References**

[1] M.Y. Ali, R.B. Cole, SFE-plus-C(18) lipid cleanup and selective extraction method for GC/MS quantitation of polycyclic aromatic hydrocarbons in smoked meat, J. Agric. Food Chem. 49 (2001) 4192–4198.

[2] A. Alves, M. Gonçalves, M. Bernardo, B. Mendes, Dispersive liquid–liquid microextraction of organophosphorous pesticides using nonhalogenated solvents, J. Sep. Sci. 35 (2012) 2653–2658.

[3] Chen, J. Hass, P. Albro, A screening method for polychlorinated dibenzo-p-dioxins (PCDDs), dibenzo furans (PCDFs), and nonortho polychlorinated biphenyls (PCBs) in biological matrices, Environ. Sci. Technol. 34 (2000) 5172–5176.

[4] Z. Chloubkova, C. Swiegoda, P. Kocsieliak, W. Piekoszewski, Identification and determination of flunitrazepam and its metabolites in blood by gas chromatography, Chem. Anal. (Wars). 49 (2004) 71–77.

[5] L. Coughman, P. Morgan, LC-MS in analytical toxicology: some practical considerations, Biomed. Chromatogr. 25 (2011) 100–123.

[6] U.B.J. Demme, H. Bussemans, T. Daldrup, F. Erdmann, M. Ermens, P.K. Iten, H. Kaferstein, K.J. Lusthof, H.J. Mageri, A. Meyer Le Reiter, G. Rocholz, A. Schmoldt, E. Schneider, H.W. Schultz, T. Stimpfl, F. Tarbaj, J. Teske, W. Vycudilik, J.P. Wellmer, W. Weinmann, Systematic evaluation of 1-chlorobutane for liquid–liquid extraction of drugs, 2005, http://www.ggfr.org/ChlorobutaneExtraction.pdf, http://www.ggfr.org/chlorobutaneextraction.pdf

[7] J.A. Donald, BURGER’S Medicinal Chemistry & Drug Discovery, John Wiley & Sons Inc., 2003.

[8] W.O. Foye, Principles of Medicinal Chemistry, Lea Fegiber, Philadelphia, 2002.

[9] L. Guo, Z.B. Lin, Z.B. Huang, H. Liang, Y. Jiang, Y.H. Ye, Z.P. Wu, R.S. Zhang, Y.R. Zhang, Y.L. Rao, Simple and rapid analysis of four amphetamines in human whole blood and urine by liquid–liquid extraction without evaporation/derivationization and by gas chromatography–mass spectrometry, Forensic Toxicol. 33 (2015) 104–111.

[10] J.M. Holler, S.P. Vorce, T.S. Bosy, A. Jacobs, Quantitative and isomeric determination of amphetamine and methamphetamine from urine using a nonpuriﬁc elution solvent and R(-)-alpha-methoxy-alpha-trifluoromethylphenylacetic acid chloride derivatization, J. Anal. Toxicol. 29 (2005) 652–657.

[11] J.H. Li, S.Q. Mi, Y.C. Li, M. Zhang, Y.F. Bi, Y. Jiang, L.M. Wang, G. Ning, W.H. Zhao, The levels and distribution of the serum lipids in Chinese adults, 2010, Zhonghua Yu Fang Yi Xue Za Zhi 46 (2012) 607–612.

[12] J.H. Li, L.M. Wang, Y.C. Li, Y.F. Bi, Y. Jiang, S.Q. Mi, M. Dai, Y. Xu, G. Ning, W.H. Zhao, Epidemiologic characteristics of dyslipidemia in Chinese adults 2010, Zhonghua Yu Fang Yi Xue Za Zhi 46 (2012) 414–418.

[13] I. Malakova, H. Brozmanova, V. Vorisek, V. Prochazkova, V. Palicka, A capillary GC method using nitrogen phosphate detector for determination of topiramate in patients with epilepsy, Chromatographia 66 (2007) 363–367.

[14] C. Megremis, Medium-chain triglycerides – a nonconventional fat, Food Technol. 45 (1991) 108–110.

[15] J. Owens, D. Holstege, A. Clifford, High-throughput method for the quantitation of total folate in whole blood using LC-MS/MS, J. Agric. Food Chem. 55 (2007) 3292–3297.

[16] I. Papoutsis, P. Nikolou, C. Spiliopoulos, C. Pistos, M. Stefanidou, S. Athanasiadis, A simple and sensitive GC/MS method for the determination of atropine during therapy of anticholinesterase poisoning in serum samples, Drug Test. Anal. 4 (2012) 229–234.

[17] E. Saar, D. Gerostamouli, O. Drummer, J. Beyer, Comparison of extraction efficiencies and LC–MS–MS matrix effects using LLE and
SPE methods for 19 antipsychotics in human blood, Anal. Bioanal. Chem. 393 (2009) 727–734.

[18] F. Sporkert, C. Brunel, M. Augsburger, P. Mangin, Fatal tolperisone poisoning: autopsy and toxicology findings in three suicide cases, Forensic Sci. Int. 215 (2012) 101–104.

[19] D. Tennakoon, W. Karunarathna, U. Udugampala, Carbofuran concentrations in blood, bile and tissues in fatal cases of homicide and suicide, Forensic Sci. Int. 227 (2013) 106–110.

[20] F. Versace, F. Sporkert, P. Mangin, C. Staub, Rapid sample pretreatment prior to GC–MS and GC–MS/MS urinary toxicological screening, Talanta 101 (2012) 299–306.

[21] B. Watzer, H. Seyberth, H. Schweer, Determination of misoprostol free acid in human breast milk and serum by gas chromatography/negative ion chemical ionization tandem mass spectrometry, J. Mass Spectrom. 37 (2002) 927–933.

[22] A. Wohlfarth, W. Weinmann, S. Dresen, LC-MS/MS screening method for designer amphetamines, tryptamines, and piperazines in serum, Anal. Bioanal. Chem. 396 (2010) 2403–2414.

[23] Y. Zhang, H. Lee, Application of ultrasound-assisted emulsification microextraction based on applying low-density organic solvent for the determination of organochlorine pesticides in water samples, J. Chromatogr. A 1252 (2012) 67–73.