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# 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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ABSTRACT

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 drastically 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.


## 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

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[17], LLE is accredited as the standard technique to pretreat whole blood from forensic cases for drug and poison detection in China.

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 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-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA), ketamine, methadone, pethidine, secobarbital, lidocaine, clenbuterol, benzhexol, carbamazepine, diazepam, chlorpromazine, olanzapine, 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.

**Table 1**
Retention time and fragment ions of the 19 drugs chosen to be spiked into human whole blood.

| 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-methylenedioxyamphetamine; MDMA, 3,4-methylenedioxymethamphetamine.

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