Alcohol 62 (2017) 41–47

Contents lists available at ScienceDirect

Alcohol

journal homepage: http://www.alcoholjournal.org/

Efficient determination of six fatty acid ethyl ethers in human whole blood by gas chromatography-mass spectrometry

Jiaolun Li [a, 1], Xinyu Zhang [a, b, 1], Zebin Lin [a], Zhibin Huang [a], Yulan Rao [a, *]

[a] Department of Forensic Medicine, School of Basic Medical Sciences, Fudan University, Shanghai, 200032, PR China
[b] Department of Pharmaceutical Analysis, China Pharmaceutical University, 24 Tongjiaxiang, Nanjing, 210009, PR China

ARTICLE INFO

Article history:
Received 9 September 2016
Received in revised form
22 February 2017
Accepted 22 February 2017

Keywords:
Fatty acid ethyl esters
Ethanol markers
Gas chromatography-mass spectrometry
Human whole blood

ABSTRACT

Fatty acid ethyl esters (FAEEs) have been widely studied as specific markers of ethanol intake and mediators of ethanol-induced diseases. In the present study, a simple and rapid gas chromatography-mass spectrometry (GC-MS) method was established for the qualitative and quantitative analysis of six fatty acid ethyl esters (FAEEs), including ethyl myristate, ethyl palmitate, ethyl stearate, ethyl oleate, ethyl linoleate, and ethyl arachidonate, in human whole blood. FAEEs were extracted from 200 μL of human whole blood by a modified liquid-liquid extraction, and the hexane layer was injected directly into GC-MS with ethyl heptadecanoate as the internal standard. The limits of detection (LODs) and limits of quantification (LOQs) were in the range of 5–50 ng/mL and 15–200 ng/mL, respectively. Linearity ranged up to 10 μg/mL with r^2 higher than 0.998. Accuracy was in the range of 90.3–109.7%, while intra-day and inter-day precision were 0.7–9.3% and 3.4–12.5%, respectively. This method was then applied to 38 real samples from forensic cases. Differences in the most common FAEEs between Chinese and Western subjects were discussed. The relationship of FAEE concentrations with age and gender was also investigated.

Introduction

Fatty acid ethyl esters (FAEEs), non-oxidative metabolites of ethanol, have been detected in serum as well as in a variety of tissues after ethanol ingestion (Doyle et al., 1994). Because FAEEs have longer detection windows than ethanol, they are viewed as markers for ethanol intake, especially in the fields of clinical and forensic toxicology. FAEEs have also received increasing attention because of their toxic effects in various tissues and organs. It has been proven that FAEEs are associated with mitochondrial dysfunction (Lange & Sobel, 1983) and hepatic and pancreatic injury (Bhopale et al., 2006; Haber, Wilson, Apte, & Pirola, 1993; Werner et al., 1997), and have effects on human platelets (Salem & Laposata, 2006) and peripheral blood mononuclear cells (Alhomsi et al., 2008). Thus, in addition to interpreting alcohol consumption, accurate quantitative analysis of FAEEs is also of important significance for diagnosis and treatment of ethanol-induced cytotoxicity, organ damage, and disease.

Some methods have been established for the determination of FAEEs in various biological samples (Auwärter et al., 2001; Bakdash et al., 2010; Cabarcos et al., 2012; Hutson, Aleksa, Pragst, & Koren, 2009; Hutson, Rao, Fulga, Aleksa, & Koren, 2011; Kwak et al., 2010; Politi, Mari, Furlanetto, Del Bravo, & Bertol, 2011; Pragst, Auwaerter, Sporkert, & Spiegel, 2001; Roehsig, de Paula, Moura, Diniz, & Yonamine, 2010). Among these samples, meconium probably has limited application for detection of ethanol intake during pregnancy, while hair can only reflect alcohol consumption that had taken place months previously. As for the detection of recent exposure to alcohol, blood is a more suitable specimen. So far, studies using blood to detect alcohol exposure have used mainly plasma, serum, and dried blood spots (Bernhardt, Cannistraro, Bird, Doyle, & Laposata, 1996; Kaphalia, Cai, Khan, Okorodudu, & Ansari, 2004; Luginbühl, Schröck, König, Schürch, & Weinmann, 2016; Moore & Lewis, 2001; Zybko, Cluette-Brown, & Laposata, 2001). In fact, whole blood is a much more available specimen for the detection of alcohol and its markers, especially in hemolytic samples. However, compared with plasma and serum, whole blood requires higher sample purification because of its much more complicated matrix. To the best of our knowledge, there have been no previous studies using human whole blood samples to detect the presence of FAEEs.

* Corresponding author. Fax: +86 21 5423 7404.
E-mail address: yulan_rao@fudan.edu.cn (Y. Rao).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.alcohol.2017.02.359
0741-8329/

The FAEE group contains more than 20 different substances, including ethyl laurate (E12), ethyl myristate (E14), ethyl palmitate (E16), ethyl palmitoleate (E16:1), ethyl stearate (E18), ethyl oleate (E18:1), ethyl linoleate (18:2), ethyl linolenate (E18:3), and ethyl arachidonate (E20:4). Previous studies showed that E16 and E18:1 were the predominant FAEEs in dried blood spots (Luginbühl et al., 2016) and plasma (Kaphalia et al., 2004). However, these studies mainly used specimens from Westerners. Because FAEEs are synthesized by ethanol and fatty acids (Politi, Leone, Morini, & Polettini, 2007), the types of FAEEs present were likely to be determined by fatty acids intake, which were strongly affected by dietary structures. Thus, it remains to be investigated whether the FAEEs present in Chinese subjects differ from FAEEs present in subjects from Western countries.

In the current study, a simple, rapid, and reliable method for the determination of six major FAEEs in human whole blood using gas chromatography-mass spectrometry (GC-MS) was established. The method was then applied to the measurement of FAEEs in a variety of human whole blood samples, in order to determine the predominant FAEEs in Chinese human whole blood, as well as to investigate the relationship of FAEE concentrations with age and gender.

Methods and materials

Reagents and standards

E14, E16, E18, E18:1, E18:2, and ethyl heptadecanoate (E17, as the internal standard) were purchased from TCI Chemical Industry Co., Ltd (Tokyo, Japan). E20:4 was obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). HPLC-grade methanol, ethanol, isopropanol, acetonitrile (ACN), and hexane were purchased from Sigma-Aldrich (Chemie GmbH, Steinheim, Germany). Analytical grade heptane was obtained from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China).

Each stock standard solution of FAEE was prepared at 1 mg/mL in isopropanol. Working solutions were prepared by mixing and diluting the stock standard solutions using isopropanol. All the stock solutions were stored at −20 °C.

Human whole blood samples used in the current study were provided by the Department of Forensic Medicine, Fudan University (138 Yixueyuan Road, Shanghai, PR China) from forensic cases.

Sample preparation

A 200-μL aliquot of human whole blood was placed in a 2-mL Eppendorf tube and spiked with a 10-μL aliquot of internal standard (IS) working solution (30 μg/mL). Then 1 mL of ethanol and 300 μL of hexane were added and vortex-mixed for 5 min. The sample was centrifuged at 12,000 rpm (a *g* value of 13,523) for 5 min, and the upper hexane phase was transferred to a 150-μL glass insert. Then 3 μL of the sample was injected directly.

GC-MS analysis

GC-MS conditions

GC-MS analyses were performed on an Agilent (Palo Alto, CA, USA) gas chromatograph 7890 equipped with a 5975 C mass selective detector (MSD) with an electron ionization (EI) chamber. Injection was carried out in split mode (2:1) at an injector temperature of 250 °C. The ionizing energy was set at 70 eV. The temperature of inlet, quadrupole, and ion source were held at 250 °C, 150 °C, and 230 °C, respectively. The gas chromatographic separation was performed on an HP-5MS capillary column (30 m × 0.25 mm × 0.25 μm) (Agilent Technologies, Palo Alto, CA, USA), using helium as a carrier gas at 1 mL/min. The temperature of the column was held at 100 °C initially for 1 min and increased by 15 °C/min to 210 °C, held for 7 min, then increased to 280 °C at a rate of 20 °C/min and held for 3 min. The ion source was turned on after a solvent delay of 5 min. The MS system was set in full scan mode in the mass range of 50–350 m/z. The retention times and the m/z of the target analytes are given in Table 1.

Screening for the target analytes

In order to investigate the most common FAEEs in Chinese subjects, six different sources of whole blood samples with blood alcohol concentrations (BAC) higher than 2.0 mg/mL were investigated. BAC was determined by a headspace gas chromatograph with a flame ionization detector (GC-FID) according to a previous method, with LOQ of 0.05 mg/mL (Rao et al., 2013). GC-MS operating in the full-scan mode was employed for the initial screening for FAEEs. FAEEs were detected by searching the standard spectrogram libraries and then identified by comparison with standards according to the retention time and mass spectrum. E16, E18:1, and E18 were found in each sample, while E14, E18:2, and E20:4 were detected in part of the samples. Thus, our target analytes for the method development were chosen to be E14, E16, E18:2, E18:1, E18, and E20:4.

Method validation

The method was validated for selectivity, linearity, precision, and accuracy according to the US Food and Drug Administration (FDA) (US Department of Health And Human Services, 2013).

Selectivity was verified by 10 different sources of control human whole blood from non-alcohol consumers.

The limit of detection (LOD) was calculated based on a signal-to-noise ratio (S/N) of 3. The limit of quantification (LOQ) was calculated based on an S/N ratio equal to or greater than 10 with satisfactory precision and accuracy.

Linearity was carried out using control human whole blood samples from non-alcohol consumers, spiked with different concentrations of FAEEs, considering the peak areas relative to the internal standard. E14, E16, and E18 were spiked at 0.015, 0.025, 0.1, 0.3, 1, 3, and 10 μg/mL, while E18:1, E18:2, and E20:4 were spiked at 0.1, 0.2, 0.4, 0.8, 1.5, 3, and 10 μg/mL. Standard curves were generated by 1/X weighing factor.

Precision and accuracy were determined by five replicates of three different levels. Intra-day precision was evaluated by one run and inter-day precision was evaluated on three successive days.

Application to real samples

The proposed method was applied to the determination of 38 real samples from forensic cases, including 34 male samples and 4 female samples. BAC of these samples ranged from 0 to 3.5 mg/mL.

Table 1
Retention times and *m/z* values for the GC-MS analysis of six FAEEs and IS.

Analyte	t_R (min)	*m/z*
E14	9.2	**88**,101
E16	11.5	**88**,101
E18:2	14.6	**67**,81
E18:1	14.8	**88**,101
E18	15.4	**88**,101
E20:4	17.4	**79**,91
E17:0 (IS)	13.1	**88**,101

Ions presented in **bold type** were used for quantification.