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Full Length Research Paper

Validation of analysis fatty acid ethyl esters as biomarkers of ethanol administration

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Abstract

To obtain an exact analytical method development validation should be performed to verify that the parameters are quite able to overcome the problem of performance analysis. Analysis of the chemical after drinking ethanol can be determined by analysis of ethanol in biological fluids such as blood, the next in a long time can not be detected because ethanol can form other metabolites. One of the metabolites that can form is Fatty acid ethyl esters (FAEEs) because ethanol reacts with fatty acids to produce neutral molecules known as esters. FAEE as a biomarker of ethanol is more sustainable than ethanol itself to analyse at any given time. The purpose of this experimental study is to examine the significance biochemical markers of alcohol given by mouth in the Wistar rats. The study design use "True randomize experimental post test only control group design." The rats are randomly distribute according to experimental design and are treated daily for one week (acute) with 20% alcohol. This study used 10 rats; with five rats for the treatment group with 20% alcohol acute and 5 rats treat as control group with distill water. The first analysis of ethanol and the second analysis of FAEE as biochemical markers of ethanol was done by gas chromatography. Blood samples are collected at 6 and 24 hours after the last oral intake of acute alcohol administration. The presence of FAEE shows that is persisting longer than ethanol and analysis by non-parametric test. At 6 hours after drinking 20% ethanol treatment, FAEE is significantly less detectable (1.8) than ethanol (3) ($p < 0.05$) but 24 hours after drinking 20% ethanol treatment, is more detectable FAEE (2.4) than ethanol (1.2) ($p < 0.05$).

Keywords: Fatty Acid Ethyl Ester, biomarkers, ethanol, gas chromatography, mass spectrometry.

INTRODUCTION

Persistent consumption of alcohol can cause increase in Blood Ethanol Concentration (BEC) and alcoholics disease for human beings (Wurst, 2006). BEC level to determine the level of ethanol consumption has a time limitation and necessary to find other biomarkers which persist longer in the body than ethanol.

One of the specific biomarkers of ethanol in hair is Fatty Acid Ethyl Ester (FAEE) as non-oxidative ethanol metabolites (Weinmann et al., 2004; Dahl, 2006). FAEE is stable marker than ethanol (Laposato, 1997; Bisaga et al., 2005). FAEE can also be detected in the blood for more than 24 hours after drinking alcohol (SOASAS, 2006).

Prolonged alcohol consumption for human being can cause liver disorder, and is known as serum glutamic piruvic transaminase (SGPT) and serum glutamic oxaloacetic transaminase (SGOT) were used as biochemical markers (Wallach, 2004 and POA, 2006). However, validation of FAEE standards compound seem to have a higher level by Gas Chromatography-Spectrometry Massa (GC-MS) before analyzing in biological fluid samples. Samples are taken from rats after repeating 20% ethanol consumption.

MATERIALS AND METHODS

Materials

Ethanol, Fatty Acid Ethyl Ester (FAEE): myristate, palmitate, oleate, hexanoate as internal standard, ten male Wistar rats with 5 rats are given distilled water as a

Figure 1. Centrifuge blood rats Wistar

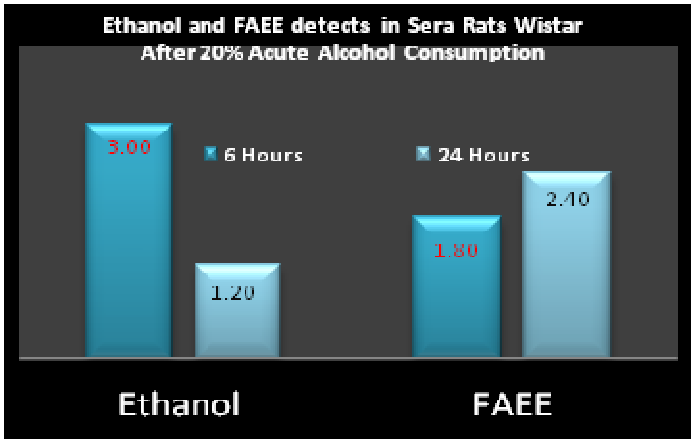

Figure 2. Data ethanol and FAEE after 6 and 24 hours 20% acute alcohol consumption

control and 5 rats are given repeated 20% ethanol for one week. Wistar rat blood was taken after 6 hours and 24 hours ethanol treatment.

Methods

Identification and separation alcohol by Gas Chromatography-Flame Ionization Detector (GC-FID), and FAEEs by Gas Chromatography-Mass Spectrometry (GC-MS) after centrifugation sample (Figure 1). Analysis ethanol in sera by dilute with aquades and analysis FAEE through solid phase extraction (SPE) column silica aminopropyl set in 10 kPa. The methods use "True randomize experimental post test only control group design."

RESULTS AND DISCUSSION

Validation of FAEE as a biomarker of ethanol were analyzed by GC-MS is essential before they are implemented in applications such as blood samples in alcohol abuse. FAEE standards are mirystate, palmitate, and oleate calibration curve obtained in succession by a line equation: $y = 0.059$ x with $R^2 = 0.9938$; $y = 0.0568$ x with $R^2 = 0.9939$; and $y = 0.0701$ x with $R^2 = 0.989$ respectively. However, with these standards using blood samples obtained from rat studies of qualitative data in the form of chromatogram peak FAEE that can not be converted into FAEE levels using the standard equation above.

After repeated acute alcohol administration in rats, ethanol level in blood analyzed by GC-FID. Data on FAEE were not quantitative but as detectability category 1 if there was no peak, category 2 if there was only 1 or 2 peaks and category 3 if there were 3 peaks or more occurring at the same retention time for comparing FAEE myristate, palmitate, and oleate standards.

In general, FAEE persisted longer than ethanol in the blood of rats after alcohol treatment. After acute alcohol treatment (Figure 2), in 6 hours after 20% alcohol treatment, FAEE was significantly less detectable (1.8) than ethanol (3) ($p < 0.05$). However at 24 hours after

Table 1. Differences significant (*) traceability between Ethanol and FAEE after administration of 20% ethanol acutely

	Ethanol		FAEE		
	6hours	24hours	6hours	24hours	pValue
20 %	3.00	1.20	1.80	2.40	<0.05*

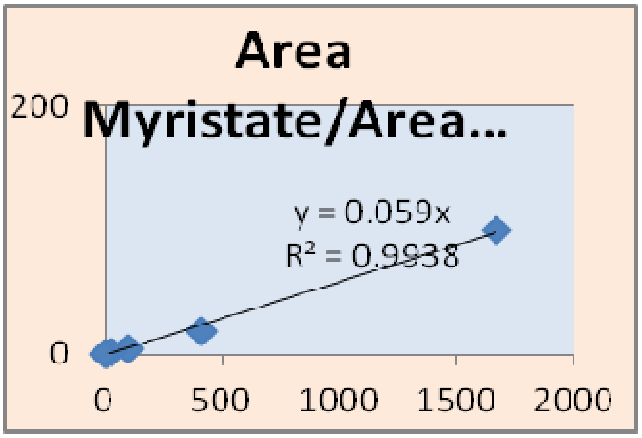

Figure 3. Calibration curve of myristate with ethyl hexanoate as standard internal

20% alcohol treatment, FAEE was also more detectable (2.4) than ethanol (1.2) ($p < 0.05$).

The presence of ethanol and FAEE then catagorize to be analyzed statistically using non-parametric statistics. Different data rates traceability Ethanol and FAEE as shown in Table 1 above.

Chromatogram using some standard results above are consistent with research Wurst et al., 2004, which found that the cut-off value of FAEE palmitic amounted to 0.40 ng / mg with $R^2 = 0.945$ in the hair and have been able to be used as biologycal markers of alcohol consumption 30-60 g/week. Some of the factors that cause can not be converted to chromatogram peak FAEE levels are low levels of FAEE in the blood samples.

Sample preparation of biologycal fluids by GC and GC-MS as an initial condition can do the analysis in forensic cases (Chamberlain, 1985; BMU, 2009). FAEE analysis in this study began with the isolation and purification of the blood of rats with SPE method and then injected into the column gas chromatography, GC-MS Varian DB-5% column with Phenyl Methyl Polysiloxane as stationary phase, calibrated with internal standards ethyl hexanoate. This method has been used by several previous investigators (Catherine et al., 2003) for human blood samples with the level of extraction efficiency of 40 to 73% and the limit of detection / LOD 0.008 to 0.084 pmol/mg.

FAEE levels in hair can also be determined by binding of albumin carrying fatty acids (Best et al., 2006) and obtained levels are between 0.1 and 2.0 mol per mole of protein. Detection of FAEE in hair samples for the purpose of a retrospective at the tested alcohol abuse during pregnancy have also been done by Pragst and Yegles, 2008. Necessary to study further whether the detection of FAEE in hair better when compared to the blood is done in this research needs to be done further research.

FAEE analysis by GC-MS as a biomarker specific ethanol can be obtained through a process of solid phase microextraction n-heptana/dimetil sulfoxide as the solvent mixture with a limit of detection is 1-10 pg/mg (Pragst and Yegles, 2008). However, Wurst et al., 2004 stated that FAEE are new markers that are sensitive and specific for knowing misuse of alcohol when compared with other alcohol metabolites. The presence of FAEE in hair has been used as biologycal markers for prenatal diagnosis of fetal abnormality rate due to alcohol (Fetal Alcohol Spectrum Disorder/FASD) in pregnant women who suffer from alcoholism (Caprara, 2003 and 2006; Kulaga et al., 2009).

One of the calibration curve of fatty acid ethyl myristate with ethyl hexanoate as internal standard is shown in Figure 3 above.

CONCLUSIONS

Fatty Acid Ethyl Ester persisted longer than ethanol in the blood of Wistar rats after peroral administration of alcohol for one week. It looks at the provision of 20% alcohol in the blood taken after 24 hours of treatment.

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