Validation of fatty acid ethyl esters analysis as biomarkers of ethanol with GC-MS

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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 (FAEE) because ethanol reacts with fatty acids produce neutral molecules known as esters. FAEE as a biomarker of ethanol is more sustainable than ethanol itself to analysis 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 use 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 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 that show 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 to increase Blood Ethanol Concentration (BEC) and alcoholics disease for human being (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 \textit{et al.}, 2004; Dahl, 2006). FAEE is stable marker than ethanol (Laposato, 1997; Bisaga \textit{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 disolder 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, validations of FAEE standards compound seem to have a higher level by Gas Chromatography – Mass Spectrometry (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 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 throught solid phase extraction (SPE) column silica aminopropyl set in 10 kPa. The methods use "True randomize experimental post test only control group design."