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Analysis of Fatty Acid Ethyl Ester on Hair as a Diagnosis of Ethanol Abuse

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Abstract

Fatty Acid Ethyl Ester (FAEE) is one of ethanol consumption biomarker. The purpose of this research is to analyze qualitatively the presence of the FAEE compound in hair samples by GC-MS. This research has made a mixture of the FAEE standard (Ethyl Palmitate and Ethyl Oleate) 100 ppm with a 1:1 ratio then the variation of concentration made. The validation method of the FAEE standard was measured by GC-MS. Hair sample preparation consists of decontamination, continued by extraction and injected to the GC-MS system. The regression equation that got was y = -147600 + 481427,014x for ethyl palmitate and y= 200,00 + 116722,90x for ethyl oleate the correlation coefficient (r^2) value respectively that got was 0,9995 and 0,9984. Limit of detection using GC-MS respectively obtained by 1, 2759 ppm and 0,0095 ppm, limit of quantitation obtained by 42531 ppm and 0,0378 ppm. The Coefficient Variation (CV) value respectively obtained by 359% and 0, 001%, with %recovery value 100, 05%dan 100, 08%. The result showed that hair samples of all volunteers contain ethyl palmitate dan ethyl oleate. It means that hair samples can use to analyze human who suspects as alcohol abuse.

Keywords: Ethanol consumption • Hair samples • Ethyl oleate • Ethyl palmitate • Alcohol abuse

Introduction

Fatty Acid Ethyl Ester (FAEE) is one of the products produced from the esterification of fatty acids and ethanol which can be used as a biomarker of chronic alcohol consumption and is often used as a diagnostic tool in forensic and clinical sciences [1,2]. FAEE is the result of non-oxidative metabolites of alcohol formed in serum and distributed in all tissues after consuming alcoholic beverages. Some previous studies mention that positive FAEE was detected in samples of volunteers who consumed alcohol such as blood serum, even amniotic fluid [3,4]. Several different compounds which are products of esterification of alcohol with fatty acids or fatty acyl-CoA have been identified and classified as FAEE, but only four compounds are correlated with alcohol abuse such as ethyl palmitate (E16), ethyl oleate (E18: 1), ethyl stearate (E18), and ethyl myristate [1].

Based on previous studies that analyzed FAEE in blood samples after consuming alcohol showed that the concentration of FAEE in the blood of rats that consumed alcohol 20% more were detected 24 hours after consuming alcohol, compared to 6 hours after consuming alcohol. Unfortunately, FAEE compounds have a short half-life in blood (<36 hours) after consuming alcohol, therefore in FAEE blood can only be detected immediately after alcohol consumption [2].

In recent decades hair analysis has generally been used more to detect the history of drug use [5-9]. In hair samples in general, drug compounds that are detected in the form of derivatives after being metabolized by the body. Previous studies mentioned that the drug compound paracetamol was detected in hair samples as acetaminophen-TMS compounds. The advantage of hair analysis compared to blood lies in the presence of drugs in a long time (>3 months) compared to blood only (<36 hours), as with FAEE compound drugs which are the result of alcohol metabolism can also accumulate in the hair up to more than three months after consuming alcohol. Based on the description above, researchers are interested in qualitatively examining FAEE compounds in hair samples of four volunteers who have consumed alcohol in the past year. To guarantee that the analytical procedures used will provide valid and reliable results, then validation methods must be carried out, such as linearity, the Limit Of Detection /(LOD), Limit Of Quantitation / (LOQ), accuracy, and precision [3].

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Materials and Methods

Condition of gas chromatography mass spectrometry

Gas Chromatography-Mass Spectrometry (GC-MS) instrument Agilent 6980N Network. Gas Chromatography is equipped with Agilent 5973 mass detector with HP-5MS column 60 m × 0.25 mm × 0.25 µm. Helium (99%) is used as a carrier gas at a flow rate of 1.3 mL/min, the GC-MS sample is programmed as follows: First the temperature in the oven is maintained at 80°C for two minutes, then gradually increased by 10°C / min until reach 290°C, and be maintained for seven minutes so that a total time of 30 minutes is obtained. Injector temperatures and mass spectrometers are maintained at temperatures of 290°C and 230°C.

Production of standard solutions of ethyl palmitate and ethyl oleate

FAEE standards (standard solution of ethyl oleate and ethyl palmitate) are each weighed carefully 50 mg and placed in a 50 mL volumetric flask, then dissolved with ethanol PA to mark boundaries. The solution was shaken for 3 minutes to obtain an FAEE concentration of 1000 ppm.

Preparation of ethyl palmitate and ethyl oleate standards

As much as 5.0 mL of ethyl oleate and ethyl palmitate standard solution with a concentration of 1000 ppm pipetted, then diluted with ethanol in a 50 mL volumetric flask to mark boundaries to obtain FAEE standard solution with a concentration of 100 ppm. All standard solutions are then ready to be injected into the GC-MS system after the condition of gas chromatography is selected and obtained. Each

Each standard solution was then mixed with ratio 1:1 so a mixture concentration of 50 ppm was obtained, then pipetted respectively 0.0 mL; 1.0 mL; 2.0 mL; 4.0 mL; 8.0 mL was put into a 10.0 mL volumetric flask and diluted using ethanol so that each standard solution was obtained with a standard solution concentration of 0 ppm, 5 ppm, 10 ppm, 20 ppm, and 40 ppm and then injected into the GC-MS system [4].

Method validation

Linearity: Linearity is done by measuring variations concentration of FAEE standard solutions namely 0 ppm, 5 ppm, 10 ppm, 20 ppm, and 40 ppm with triple repetitions, so that the calibration curve is obtained based on concentration and area, with the formula:

$y = a+bx$

Information

y=Peak Area (Peak Area)

x=Concentration of Substances

Limit of Detection (LOD) and limit Quantitation (LOQ) are determined based on linearity data then a standard deviation value (s) will be obtained. The s value is then used to determine LOD and LOQ using the formula:

$$s = \sqrt{\frac{\sum_i (X_i - \bar{x})^2}{n-1}} \qquad LOD = \frac{3\,x\,S}{b} \qquad LOQ = \frac{10\,x\,S}{b}$$

Information:

s = Deviation standard

xi =Concentration of substances

$\bar{x}$ =Average concentration

n = Amount of data

b = Gradient (Slope)

Accuracy and precision

The accuracy value is determined by measuring a 2.0 mL standard solution mixture with a concentration of 50 ppm, then dissolving using ethanol in a 10.0 mL volumetric flask to the mark boundaries so that a standard solution of 10 ppm is obtained with triple repetitions. Determination of accuracy is done by calculating percent recovery with the formula:

$$\%Recovery = \frac{\text{Concentration measured}}{Theoritical\ concentration} \text{x } 100\%$$

The precision is determined by calculating the Coefficient of Variation (CV) with the formula:

$$CV = \frac{s}{x} \text{ x } 100\%$$

s = deviation standard

$\bar{x}$ = Average value

Hair specimen extraction

Hair specimens were obtained from four volunteers, one volunteer who did not consume alcohol as a control, and three other volunteers were known to consume alcohol with varying frequency of alcohol consumption ranging from volunteers who consumed several times a year, several times a month and several times in a week. 150 mg of hair specimens were decontaminated first with diethyl ether+acetone in a ratio of 1:1 v/v 13 then extracted with a mixture of dichloromethane+ethanol in a ratio of 0.3 mL: 1 mL v/v then followed by centrifugation with a speed of 5000 rpm for 5 minutes, then the ethanol layer is taken as much as 1 µL and injected into the GC-MS system [5].

Results

Standard analysis of ethyl palmitate and ethyl oleate

Ethyl palmitate and ethyl oleate are one of the FAEE groups that have been validated using gas chromatography. The results of the analysis showed that on the blank chromatogram, peaks which were standard compounds of ethyl palmitate and ethyl oleate were not found, whereas in standard solutions with ethanol solvent showed a

single peak of ethyl palmitate and ethyl oleate compounds at retention times of 22.03 minutes and 23.86 minutes, respectively is shown in Figure 1. Based on the results of the chromatogram below, obtained a good separation between ethyl palmitate and ethyl oleate with resolution values above 2.00 (Figures 1-2) [6].

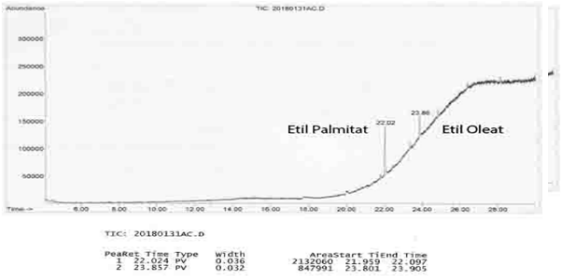

Figure 1. Peak area of 5 ppm ethyl palmitate and ethyl oleate using GC.

The calibration curves for the standard ethyl palmitate and ethyl oleate shown in Figure 2 are obtained from the calculation of standard measurement results with five variations of the concentration of ethyl palmitate and ethyl oleate namely 0 ppm, 5 ppm, 10 ppm, 20 ppm and 40 ppm, so that the regression equation y = -147600+481427, 014 x for ethyl palmitate and y=200.00+ 116722.90x for ethyl oleate.

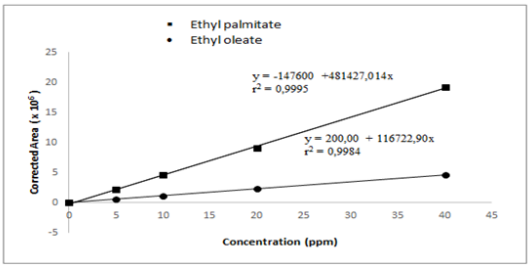

Figure 2. Standards calibration curve for ethyl palmitate and ethyl oleate in ethanol. **Note:** Ethyl palmitate; Ethyl oleate

Correlation coefficient (r_2) of the standard ethyl palmitate and ethyl oleate are 0.9995 and 0.9984, respectively. Linearity is allowed if the value of the correlation coefficient (r_2) is ≥ 0.997 14. The standard limit of detection of ethyl palmitate and ethyl oleate by GC-MS method were 1.2759 ppm and 0.0095 ppm, respectively, and for the quantitation limits were 4.2531 ppm and 0.0378 ppm respectively which means the gas chromatography is very sensitive to detect ethyl palmitate and ethyl oleate in the sample [7].

The accuracy and precision obtained using the calibration curve method is done by measuring the corrected area of ethyl palmitate and ethyl oleate standards at a concentration of 10 ppm with three repetitions. Based on the results of the calculation of the coefficient variation values of the standard ethyl palmitate and ethyl oleate, respectively obtained by 3.59% and 0.001%, the accuracy validity test qualified if the CV value ≤ 2% 14. The calculation results show the coefficient variation of the ethyl palmitate standard exceeds than the specified conditions that is equal to 3.59% while the coefficient of variation of the ethyl oleate standard suitable with the specified conditions that is equal to 0.0001%.

While the percent recovery value of the ethyl palmitate and ethyl oleate standards was obtained at 100.05% and 100.08%, respectively. Acceptable percent recovery values for analyte measurements in the range of 80% to 110% indicate a good level of accuracy [8-11].

Discussion

Analysis of FAEE compounds in hair sample

Hair analysis was performed on 4 volunteers who were known to have consumed alcohol over the past year, then 150 mg of hair specimens were taken as much as 150 mg for analysis. In this study

all hair samples showed positive results (+) on ethyl palmitate and ethyl oleate, with retention times of 22.00 min and 23.83 min, respectively. Reviewed from the retention time of the two peaks produced is the same as the retention time produced by the standard, while it is reviewed from ion fragmentation samples with the standard and the presence of Library C/Database 02\NIST. L shows the compound ethyl palmitate and ethyl oleate (Table 1) (Figure 3) [12,13].

Volunteer	Results (+)/(-)		Retention time (minutes)	
	Ethyl palmitate	Ethyl oleate	Ethyl palmitate	Ethyl oleate
I	(+)	(+)	22,00	23,83
II	(+)	(+)	22,00	23,83
III	(+)	(+)	22,00	23,83
IV (Control)	(+)	(+)	22,00	23,83

Table 1. Results of hair samples analysis

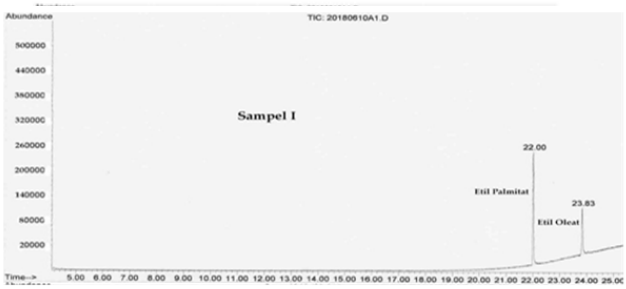

Figure 3. Chromatogram one of the volunteer hair samples.

Detection of ethyl palmitate and ethyl oleate compounds that are FAEE in 4th volunteer hair samples as a control can be caused by the use of hair care products containing ethanol either by only 10% or up to 60% such as hair oil, or hair spray products. The presence of ethanol content in these hair care products causes the synthesis of ethyl ester in the hair, so that even though it does not consume ethanol, FAEE compounds can be detected in very small concentrations [14,15].

Conclusion

FAEE compounds that can be detected in hair samples using GC-MS are ethyl palmitate and ethyl oleate which have retention times of 22.00 and 23.83 minutes, respectively. It means that hair samples can use to analyze human who suspects as alcohol abuse.

Conflict of Interest

Authors declare that there is no conflict of interest in this study.

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References

1. Crunelle, Cleo, L, Yegles M, Nuijs ALN, and Covaci A, et al. "Hair Ethyl Glucuronide Levels as a Marker for Alcohol Use and Abuse: A Review of the Current State of the art." *Drug Alcohol Depend* 134 (2014): 1-11.
2. Dumitrascu, C, Paul R, Kingston R, and Williams R. "Influence of Alcohol Containing and Alcohol Free Cosmetics on FAEE Concentrations in hair. A Performance Evaluation of Ethyl Palmitate as Sole Marker, *versus* the Sum of four FAEEs." *Forensic Sci Int* 283 (2018): 29-34.
3. Liu, Y, Zhang X, Li J, and Huang Z, et al. "Stability of Ethyl Glucuronide, Ethyl Sulfate, Phosphatidylethanols and Fatty Acid Ethyl Esters in Postmortem Human Blood." *J Anal Toxicol* 42 (2018):352.
4. Cabarcos, Pamela, Tabernero J, Otero JL and Míguez M, et al "Quantification of Fatty Acid Ethyl Esters (FAEE) and Ethyl Glucuronide (EtG) in Meconium for Detection of Alcohol Abuse During Pregnancy: Correlation Study Between both Biomarkers." *J Pharm Biomed Anal* 100 (2014): 74-78.
5. Suaniti, Ni Made, Djelantik AAGS, Suastika K, and Astawa INM. "Validation of Analysis Fatty Acid Ethyl Esters as Biomarkers of Ethanol Administration."*J Med Med Sci* 5 (2012): 330-333.
6. Dasgupta, Amitava. "Alcohol and its Biomarkers: Clinical Aspects and Laboratory Determination." *Elsevier* 2015.
7. Thieme, Detlef, Sachs H, and Uhl M. "Proof of Cannabis Administration by Sensitive Detection of 11-nor-Delta (9)-tetrahydrocannabinol-9-carboxylic acid in Hair Using Selective Methylation and Application of Liquid Chromatography-Tandem and Multistage Mass Spectrometry." *Drug Test Anal* 6 (2014): 112-118.
8. Maublanc, Julie, Dulaurent S, Imbert L, and Kintz P, et al. "Unusual Pattern in hair After Prazepam Exposure." *Toxicol Anal Clin* 26 (2014): 24-26.
9. Chatterton, Craig, and Kintz P. "Hair Analysis to Demonstrate Administration of Amitriptyline, Temazepam, Tramadol and Dihydrocodeine to a Child in a Case of Kidnap and False Imprisonment." *J Forensic Legal Med* 23 (2014): 26-31.
10. Darmapatni, Gunapria KA. "Pengembangan Metode GC-MS Untuk Penetapan Kadar Acetaminophen Pada Spesimen Rambut Manusia." *J Biosains Pascasarjana* 18 (2016): 255-266.
11. Best, Catherine A., and Laposata M. "Fatty Acid Ethyl Esters: Toxic Non-Oxidative Metabolites of Ethanol and markers of Ethanol Intake." *J Biosains Pascasarjana* 5 (2003): 202-217.
12. Bossers, LCAM, Paul R, Berry AJ and Kingston R, et al. "An Evaluation of Washing and Extraction Techniques in the Analysis of Ethyl Glucuronide and Fatty Acid Ethyl Esters From Hair Samples." *J Chromatogr B* 953 (2014): 115-119.
13. Pragst, F, Auwaerter V, Sporkert F, and Spiegel K. "Analysis of Fatty Acid Ethyl Esters in Hair as Possible Markers of Chronically Elevated Alcohol Consumption by Headspace Solid-Phase Microextraction (HS-SPME) and Gas Chromatography-Mass Spectrometry (GC-MS)." *Forensic Sci Int* 121 (2001): 76-88.
14. Pragst, Fritz, and Yegles M. "Determination of Fatty Acid Ethyl Esters (FAEE) and Ethyl Glucuronide (EtG) in hair: A promising way for Retrospective Detection of Alcohol Abuse During Pregnancy?" *Ther Drug Monit* 30 (2008): 255-263.
15. de Giovanni, Nadia, Donadio G, and Chiarotti M. "The Reliability of Fatty Acid Ethyl Esters (FAEE) as Biological Markers for the Diagnosis of Alcohol Abuse." *J Anal Toxicol* 31 (2007): 93-97.

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