

Gas Chromatography Tandem Mass Spectrometry for Biomarkers of Alcohol Abuse in Human Hair

Carolyn M. Zimmermann, BS and Glen P. Jackson, PhD

Abstract: We present the development of a new, faster analytical method for the determination of fatty acid ethyl esters (FAEEs) in human hair to detect potential alcohol abuse. FAEEs have been established as metabolites of ethanol consumption in humans and are embedded in the hair follicles during hair growth. The developed method has a total analysis time—including washing, extraction, concentration, separation, and detection—of less than 1 hour. Commonly used extraction procedures in the literature for these biomarkers are typically 15 hours. Analysis is performed using gas chromatography-tandem mass spectrometry (GC-MS/MS) with a GC separation time of less than 9 minutes. Using chemical ionization, mass spectrometric detection consists of selected reaction monitoring, which is widely considered to be one of the most selective and sensitive forms of mass spectrometric detection. Employing selected reaction monitoring helps to reduce interferences from the hair matrix, thereby making the method more selective for these biomarkers of interest. Limits of detection for each FAEE range from 0.002 to 0.030 ng/mg in hair. By using this faster extraction method, this research shows that this method could potentially be used to distinguish whether a person is a heavy drinker, moderate drinker, or nondrinker. The ability to rapidly analyze hair samples can be applied to a number of different areas, such as neonatal screening, parole violations, contributing factors in the cause of death, and any other application requiring the establishment of chronic versus acute alcohol abuse.

Key Words: fatty acid ethyl esters, hair analysis, alcohol, biomarkers, tandem mass spectrometry, MS/MS

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INTRODUCTION

Alcohol abuse is an ongoing problem throughout the United States. According to a survey taken in 1992 by the National Institute on Alcohol Abuse and Alcoholism, 14 million Americans met the diagnostic criteria for alcohol abuse or alcohol dependence.¹ For a variety of conscious and subconscious reasons most people do not accurately or honestly self-report alcohol consumption. Therefore, the ability to

objectively determine whether a person is a chronic or acute drinker is highly beneficial. Alcohol abuse can lead to short-term and long-term health effects in individuals, and causing justice and public safety issues. For example, it is known that between 14% and 20% of women in the United States drink while pregnant² and that this can lead to a variety of birth defects such as fetal alcohol spectrum disorder (FASD) and fetal alcohol syndrome (FAS).^{3,4} FAS affects 0.5–2 of every 1000 live births in the United States,⁵ and is diagnosed as causing head and facial abnormalities, restricted growth, and brain damage. FASD, affecting about 1% of live births in the United States,⁵ is identified as causing learning problems and attention deficits in children exposed to alcohol during pregnancy. Studies have shown that early detection of FASD can lead to a lower occurrence of learning disabilities by the time the child begins school, when proper care is taken.⁶ Therefore, a method to detect prenatal alcohol exposure would help in identifying FAS and FASD early so that proper attention can be given.

In addition to in utero alcohol exposure, alcohol abuse is frequently associated with crime. Almost half of all probationers in 1995 claim to have been under the influence of alcohol during their offense and one-third of probationers in 1995 received treatment for alcohol dependency while on parole.⁷ The Centers for Disease Control and Prevention (CDC) ranks excessive alcohol consumption as the third leading preventable cause of death in the United States. The National Highway Traffic Safety Administration reported that of the 1.6 million people arrested in the United States each year for driving under the influence or driving while intoxicated, one-third of these convictions are repeat offenders.⁸ Therefore, being able to determine whether a person is still a chronic alcohol abuser could be applied to license renewals of driving under the influence or driving while intoxicated offenders.

The close relationship between alcohol and health, justice or public safety makes the ability to detect and measure prior alcohol use in adults or newborns an important area for scientific study. Hair analysis is known to provide a longer history of substance abuse than biologic fluids such as blood or urine. Previous studies have shown that hair analysis can be used for the determination of alcohol abuse by detecting the presence of certain naturally occurring fatty acid ethyl esters (FAEEs).^{9,10} The detection of in utero alcohol exposure using FAEEs have been performed on the analysis of meconium^{4,11} or hair^{10,12} from newborns to help in the early detection of FAS or FASD. FAEEs are nonoxidative metabolites of ethanol and are quite stable at neutral pH values.¹³ These FAEEs are enzymatically formed from free fatty acids, triglycerides, or lipoproteins in

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Correspondence: Glen P. Jackson, PhD, 175 Clippinger Laboratories, Ohio University, Athens, OH 45701-2979 (e-mail: jacksong@ohio.edu).
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the presence of ethanol by FAEE synthase. There are 3 different mechanisms by which FAEEs are believed to be integrated into the hair matrix⁹: (1) ethanol diffusion into the hair root cells where it is then processed by FAEE synthase; (2) esterification into the sebaceous gland due to the presence of ethanol from where esters are then released into the sebum and diffuse into the hair matrix; (3) ester synthesis in other organs and transportation occurs to the hair root cells via blood circulation. For newborns, it is suggested that ethanol from the mother can be transferred to and metabolized by the fetus.¹⁴ Prenatal hair starts to grow in the third or fourth month of fetal life, potentially allowing for the detection of alcohol exposure during the last trimester of pregnancy.¹⁵

The current state of the art method for detecting FAEEs in hair requires extensive washing and extraction procedures (>15 hours), followed by a 20- to 40-minute GC/MS analysis method.^{9,10,16} Because the sample preparation and extraction procedures currently make up ~99% of the total analysis time, these steps are the limiting factors and key targets for further reductions in analysis times. Our studies have reduced the total analysis time, including washing, pulverization, extraction, separation, and detection to 1 hour resulting in a greater than 90% reduction in total analysis time. To help avoid interferences from the hair matrix, our studies apply tandem MS and use selected reaction monitoring (SRM) to provide better selectivity and signal to noise ratios for these FAEEs.

MATERIALS AND METHODS

Subjects

The collection of hair samples for the following experiments was approved by the Ohio University Institutional Review Board (IRB # 06X096). Volunteers donated hair samples and were required to fill out a questionnaire, based on the alcohol use disorders identification test.¹⁷ Based on the questionnaire we were able to determine if a person was an abstainer (nondrinker), moderate drinker, or a heavy drinker. Definitions of these terms are defined by the CDC, and can be seen in Table 1.¹⁸

Hair was collected by cutting off small amounts of head hair, as close to the scalp as possible, from different areas

around the head. The sample amount taken from each donor varied. The hair that was collected from each donor was stored in a labeled envelope until it was tested. A few samples were tested from each category to include: heavy drinkers, moderate drinkers, and nondrinkers.

Reagents and Standards

The 5 FAEEs of interest in this study are ethyl laurate (12:0), ethyl myristate (14:0), ethyl palmitate (16:0), ethyl stearate (18:0), and ethyl oleate (18:1) purchased from VWR International (West Chester, PA) and Sigma-Aldrich (St. Louis, MO). Previous studies have shown that these 5 FAEEs are the most relevant biomarkers for the intake of alcohol in both hair and biologic fluids.^{4,9,10} Only even numbered fatty acids are naturally occurring in the human body, which makes ethyl margarate (17:0) an ideal internal standard (IS; VWR International). Solvents used were American Chemical Society (ACS) grade, and greater than 99% purity (Sigma-Aldrich).

Preparation of Stock and Working Solutions

FAEE standards were prepared by taking the solid standard and dissolving it in hexane to prepare a 10,000 ppm standard stock solution for each FAEE. A standard mixture solution of 1000 ppm in hexane of all 6 FAEEs (12:0, 14:0, 16:0, 17:0, 18:1, and 18:0) was then prepared from the individual stock solutions. For the liquid calibration curve, the 1,000 ppm mixture was diluted to 15 different concentrations in hexane including 0, 1, 5, 10, 15, 20, 25, 30, 35, 40, 50, 75, 100, 250, and 500 ppb. Injections of 1 μL of each calibration standard were run 5 times each in a random block design to establish the calibration curve via gas chromatography-tandem mass spectrometry (GC-MS/MS).

A separate 1000 ppm standard containing 5 FAEE (12:0, 14:0, 16:0, 18:1, and 18:0), excluding the IS (17:0) was used for the hair calibration. Bulk scalp hair from an abstainer collected over a 6-month period was used for the hair calibration. Thirty-milligram aliquots of clean, dry hair was spiked with different amounts of FAEEs to establish a calibration curve for the extraction procedure. Each hair sample was spiked to the appropriate concentration with the 1000 ppm mixture. The concentrations used for the hair calibration were 0, 0.05, 0.2, 0.5, 1, 1.5, and 2 ng/mg with each concentration

TABLE 1. Definitions of Different Types of Drinkers Given by the CDC¹⁹

	Men	Women
Definition of One Drink		
12 oz of beer or wine cooler (~5% alc/vol = ~17 g alc.)*		
8 oz of malt liquor (~7% alc/vol = ~16 g alc)*		
5 oz of wine (~12% alc/vol = ~17 g alc)*		
1.5 oz of 80-proof distilled spirits or liquor (~40% alc/vol = ~17 g alc)*		
Types of drinkers		
Abstainer	<12 drinks in a lifetime or no drinks in the past year	<12 drinks in a lifetime or no drinks in the past year
Light Drinker	≤3 drinks/week	≤3 drinks/week
Moderate Drinker	>3 but <14 drinks/week	>3 but <7 drinks/week
Heavy Drinker	>14 drinks/week (more than 2 per day)	>7 drinks/week (more than 1 per day)

*Percentages of alcohol/volume (alc/vol) are approximate and vary slightly depending on the drink.

prepared and extracted in quadruplicate. Every hair sample was spiked to 0.8 ng/mg with the IS. After the hair samples were spiked and allowed to air-dry, the pulverization, extraction, and analysis procedure was then completed.

Sample Preparation

Each sample was washed in water (twice) and methanol (once) by vortexing the sample for about 1 minute to remove any external fatty acids. The hair was then dried under nitrogen.¹⁹ For each test, ~30 mg of washed and dried hair was weighed and spiked with 1 ng/mg of ethyl margarate (17:0) as the IS. After adding 6-8 stainless steel ball bearings to the sample, a Biospec Mini-Bead Beater 1 (Biospec Products Inc., Bartlesville, OK) was used at 4800 rpm for 3 minutes to pulverize the hair. Once samples were pulverized, the hair was separated from the beads, reweighed, and transferred to a 4-mL vial. The desired solvent was then added to the pulverized hair and the sample was either shaken or sonicated for a specified amount of time. Solvents tested were methanol, acetone, hexane, and hexane with dimethyl sulfoxide (DMSO). For the individual solvent tests, 2 mL of the chosen solvent (methanol, acetone, or hexane) was added. For the hexane with DMSO test, 2 mL of hexane and 0.5 mL DMSO was added. After shaking or sonication the solvent layer was then transferred to a 10-mL vial and dried under nitrogen. Following previous procedures, 1 mL of phosphate buffer (pH 7.6) and 0.5 g NaCl was usually added in our preliminary experiments. However, these experiments showed that extraction efficiencies were not significantly different whether buffer and salt were added or not (data not shown). Therefore, we chose not to add the phosphate buffer and NaCl to the final developed method.

Instrumentation

A TriPlus autosampler (Thermo Scientific, Waltham, MA) was used to handle head space solid-phase micro-extraction (HS-SPME) measurements which included

preheating the samples, HS adsorption with agitation and desorption into GC injection port. Each sample was preheated and agitated for 5 minutes at 90°C before sampling. HS-SPME was then performed on each sample for 30 minutes with agitation at a constant temperature of 90°C. The SPME fiber was exposed in the injection port at 260°C for 2 minutes. HS-SPME analysis was performed using a 65- μ m polydimethylsiloxane/divinylbenzene (PDMS/DVB) fiber (Sigma-Aldrich). GC-MS/MS analysis was performed using a Trace GC (Thermo Scientific) coupled to a Finnigan Polaris Q (Thermo Scientific) quadrupole ion trap in chemical ionization (CI) mode using isobutane (99%, Airgas, Radnor, PA) as the CI reagent gas at a constant flow of 0.6 mL/min. Samples were separated on a 28 m \times 0.25 mm \times 0.25 μ m RTX-5MS fused silica capillary column (Restek Cooperation, Bellefonte, PA). The carrier gas was ultrapure (99.999%) helium (Airgas) at a constant flow of 2.0 mL/min, which was purified in-line using an SGT triple gas filter (Thermo Scientific). The initial column temperature was 70°C for 1 minute. The temperature was then increased at 30°C/min to a final temperature of 280°C and held for 1 minute for a total separation time of 9 minutes. The transfer line temperature was 280°C with an ion source temperature of 225°C.

RESULTS

To demonstrate this GC-MS/MS method can be applied to a hair sample of an alcohol drinker, Figure 1A shows the reconstructed total ion chromatogram (TIC) from a heavy drinker where all 5 FAEE can be detected. Figure 1B is the TIC from an alcohol abstainer, showing a small amount of only 2 of the 5 FAEE present in low abundance. Above each figure shows the time in which each precursor ion is being isolated.

A list of retention times and mass-to-charge ratios for each FAEE is given in Table 2. After determining the retention times for each analyte using liquid injections of solutions

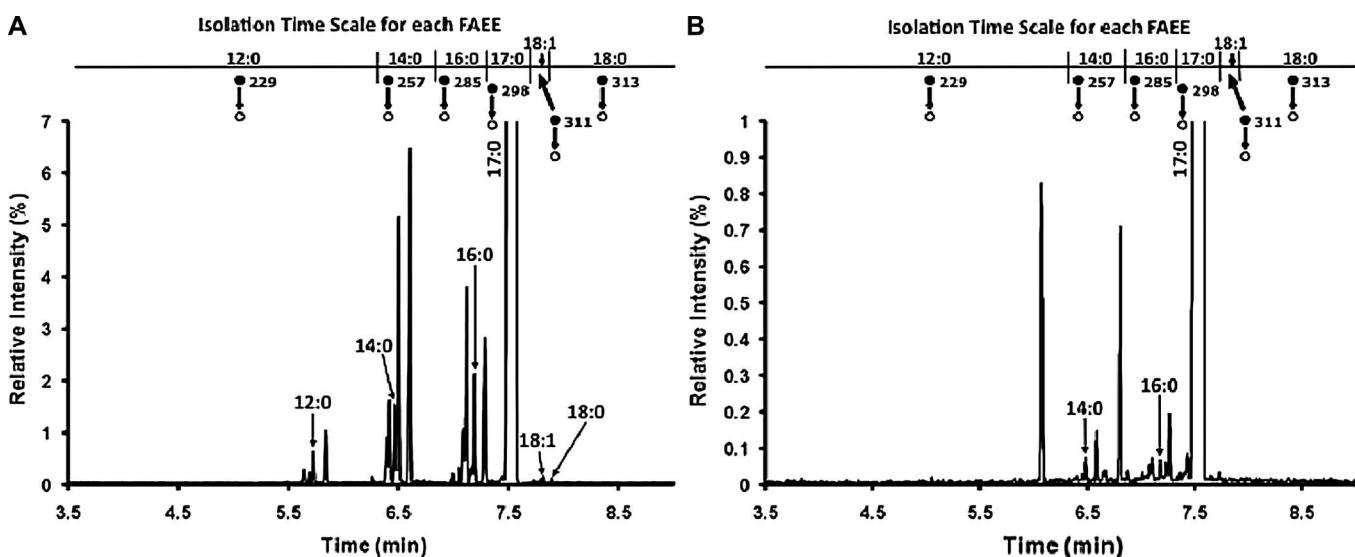


FIGURE 1. TIC chromatograms of conventional GC/MS spectra obtained using different preprogrammed SRM schemes for each FAEE. (A) Hair sample from a heavy drinker; (B) hair sample from a nondrinker.

TABLE 2. FAEE Retention Times and Mass-to-Charge Ratios Used for the GC-MS/MS Analysis

FAEE	Retention Time (min)	m/z, SRM
Ethyl laurate (12:0)	5.71	229 → 201
Ethyl myristate (14:0)	6.46	257 → 229
Ethyl palmitate (16:0)	7.15	285 → 257
IS ethyl margarate (17:0)	7.47	299 → 271
Ethyl oleate (18:1)	7.71	311 → 265, 311 → 247
Ethyl stearate (18:0)	7.78	313 → 285

containing standards of each FAEE, the quadrupole ion trap mass spectrometer was programmed through the commercial software to perform a segmented analysis in which to obtain product ion spectra of each ethyl ester. For each ethyl ester, the precursor ion [M + H]⁺ was isolated and consequently fragmented via collisional activation with the helium bath gas to yield the product ion spectra. The segmented analysis routine was programmed to change to the next precursor ion mass at the mid-point between the retention times of each analyte. Postacquisition extracted ion currents of selected product ions were then used to obtain SRM chromatograms. The SRM transitions selected in this work are shown in Table 2. With the exception of ethyl oleate, the loss of the ethyl-ester moiety (-28 u) was the dominant fragmentation pathway.

Scalp hair of a heavy drinker was collected from 4 to 5 months of growth, from multiple haircuts, and was pooled together and mixed. This pooled hair from this single consistently heavy drinker served as a control by which the different extraction methods could be compared. Each sample was spiked with 0.8 ng/mg of IS (17:0), so that samples could be evaluated against one another. Previous methods used a process of cutting the hair into fine small pieces, called mulching, to break up the hair matrix.^{12,16,20} In trying to achieve better extraction efficiencies in this method, a pulverization process was chosen to expose a larger portion of the hair matrix than the mulching process might provide.²¹ All of the following tests were performed with this pulverization step.

To extract the ethyl esters from the physically degraded hair of this heavy drinker, different solvents were examined with 15 or 30 minutes of sonication. Solvents included 2 mL of acetone, methanol, or hexane or 2 mL of hexane with 0.5 mL DMSO (Fig. 2). Each test was performed in quadruplicate. It is important to note that there is some variation within these results. The total average error for the extraction of ethyl esters from the hair of a heavy drinker was ~50% relative standard deviation (RSD). As shown in the method validation section, the largest source of error is in the distribution of FAEES in the hair matrix itself. Due to the large variability of FAEES in the hair sample, no statistically significant difference is seen between the four different solvents used at the 95% confidence level (CL), as shown in Figure 2.

These results do show that comparable extraction efficiencies could be achieved at considerably shorter extraction times under sonication in compared with 15 hours of shaking. Therefore, we found no statistically significant difference in the extraction efficiencies between 15 minutes of sonication and 15 hours of shaking for any of the solvents or solvent combinations studied. Table 3 compares our developed method with the most commonly adopted method in the literatures, which we also ran here for comparison.

Method Validation

Using the modified extraction procedure and SRM, calibration curves were generated for both liquid and hair samples to determine instrument and method limits of detection (LOD). Instrument LODs were established using standard solutions for the five FAEES (12:0, 14:0, 16:0, 18:0, 18:1) and the IS (17:0), with concentrations ranging from 0 to 500 pg on column. For the liquid samples, the LODs for each FAEE ranged from 2 to 11 pg on column, with quantitation linear over 2 orders of magnitude. Hair from an abstainer was used for the hair calibrations; a calibration curve of the hair samples was generated from quadruplicate aliquots at each concentration by adding known amounts of each FAEE to 30-mg aliquots of clean dry hair. Concentrations for this calibration ranged from 0 to 2 ng/mg for each FAEE. To be able to

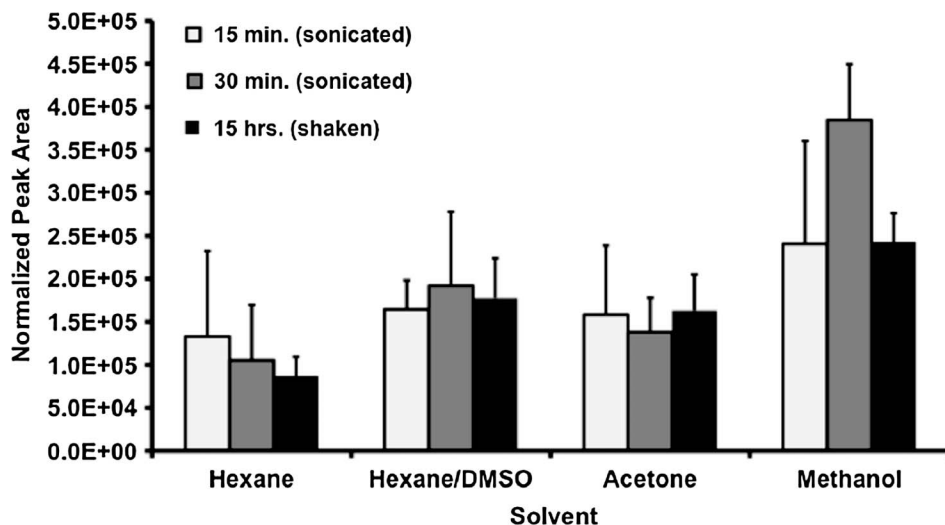


FIGURE 2. A comparison of the total FAEE concentration at different extraction solvents and extraction times from the hair of a heavy drinker. Error bars show 95% CI (N = 4).

TABLE 3. Comparison of the Most Commonly Used Method for Extracting and Analyzing Ethyl Esters With Our Preliminary Work

	Pragst et al ^{10,17}	This Work
Preliminary washing then drying	15 min	15 min
Pulverization	N/A	3 min
Liquid extraction	15 h	15 min*
Centrifugation	15 min	N/A
Evaporating to dryness	5 min	5 min
SPME extraction	30 min	30 min
Total extraction time	16.1 h	1.1 h
GC-MS†	17 min	9 min
Total analysis time	16.4 h	1.3 h (>90% reduction in analysis time)

*With sonication.

†Pragst et al used electron ionization with selected ion monitoring, whereas this work uses CI with selected reaction monitoring.

compare samples and correct for variability in extraction efficiencies, 0.8 ng/mg of IS was added to each hair sample. For the hair calibration, the LODs for each FAEE ranged from 0.002 to 0.03 ng/mg, as shown in Table 4, and linear over 2 orders of magnitude.

The error in the liquid calibration study was 14% RSD ($n = 4$), whereas the error for the spiked hair calibration study using the SPME fiber was 19% ($n = 4$). These 2 studies indirectly establish that the major source of the error encountered with the analysis of hair from a heavy drinker (~50% RSD) can be attributed to the heterogeneity of FAEE distribution in the hair matrix. Using SPME, extraction efficiencies for the hair calibration were calculated by spiking teetotaler's hair to a concentration of 2 ng/mg and comparing the peak areas of each FAEE to the liquid-injection calibration curve. At this concentration, the extraction efficiencies were around 5% for ethyl laurate (12:0), ethyl myristate (14:0), and ethyl stearate (18:0), and around 3% for ethyl palmitate (16:0), and ethyl oleate (18:1).

When generating the liquid injection and SPME calibration curves, samples were analyzed in random block design. In cases where low concentrations (or blanks) were analyzed directly following a high concentration, no evidence of carry-over was observed. The 2-minute desorption time in the GC injection port was apparently long enough to prevent carryover between samples. Shorter desorption times were not studied.

Analysis of Different Hair Samples

To demonstrate this faster extraction method could be used on different samples different hair samples were tested. Three different samples of heavy drinkers were tested, including the heavy drinkers hair used in testing the extraction method. Two different samples were tested for both moderate drinkers and abstainers. All samples were run in triplicate. Table 5 shows the results of the 7 hair samples studied in this work from subjects characterized as heavy, moderate, and nondrinkers. After cleaning, each hair sample was spiked with 0.8 ng/mg of the IS before analysis. The concentrations of each FAEE in each sample were established from the linear regression lines of the spiked-hair calibration curve and are shown in Table 5, along with the total FAEE concentration.

DISCUSSION

Our results demonstrate how the extraction procedures could be radically reduced by performing physical degradation of the hair—through pulverization with a bead beater—prior to liquid extraction with sonication. Table 3 compares the time required for each step of the most commonly used procedure for extracting ethyl esters from human hair with the method tested in our laboratory. Our analysis can be completed 16 times faster than the currently used application with no measurable difference in extraction efficiencies.

Using SRM, we have demonstrated that the separation and detection of 5 FAEEs can be achieved in less than 9 minutes with method detection limits within 0.002–0.03 ng/mg. These detection limits are equal to or superior to existing technologies,^{10,22,23} but are also considerably more selective than previously published methods. The current method therefore has the added advantage of reduced interferences when analyzing real hair samples. Figure 3 demonstrates the advantage of SRM over selected ion monitoring (SIM). Figure 3A is a TIC of the product ion scan of ethyl palmitate (m/z 285). Because the ions of m/z 285 have been isolated throughout the window shown, this plot is analogous SIM. In SIM mode, ethyl palmitate appears as a small peak at 7.15 minutes surrounded by more abundant compounds also with m/z values of 285. The product ion mass spectra of ethyl palmitate is seen in Figure 3B, indicating that the fragment ion at m/z 257 is the dominant fragmentation pathway. By performing SRM (m/z 285 → 257), the signal to noise (S/N) for ethyl palmitate at 7.15 minutes can be

TABLE 4. Comparison of Our Method Detection Limits to Other Published Methods

	Our Work (SPME, GC/MS/MS, CI)		Pragst et al ¹⁶ (SPME, GC/MS, EI)		DeGiovanni et al ¹² (SPME, GC/MS, EI)		Caprara et al ²⁰ (SPE, GC/MS, CI)	
	LOD (ng/mg)	R ²	LOD (ng/mg)	R ²	LOD (ng/mg)	R ²	LOD (ng/mg)	R ²
Ethyl laurate (12:0)	0.002	0.997	—	—	—	—	0.005	—
Ethyl myristate (14:0)	0.004	0.998	0.015	0.999	0.02	0.994	0.0025	—
Ethyl palmitate (16:0)	0.030	0.998	0.02	0.999	0.01	0.994	0.04	0.999
Ethyl oleate (18:1)	0.021	0.991	0.04	0.999	—	—	0.0025	—
Ethyl stearate (18:0)	0.009	0.990	0.01	0.994	0.1	0.914	0.0025	—

SPE, solid phase extraction. Each calibration curve was accomplished using hair from a teetotaler spiked with each FAEE and an IS.

TABLE 5. The Amount of Each FAE Detected for Different Hair Samples of Heavy, Moderate, and Nondrinkers

ng/mg Ethyl Esters in Hair	Heavy Drinker A0018, N = 3	Heavy Drinker A0020, N = 3	Heavy Drinker A0013, N = 3	Moderate Drinker A0009, N = 3	Moderate Drinker A0021, N = 3	Nondrinker A0008, N = 3	Nondrinker A0016, N = 1
Ethyl laurate (12:0)	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002
Ethyl myristate (14:0)	0.09	0.11	0.10	0.10	0.11	0.05	0.06
Ethyl palmitate (16:0)	0.82	0.80	0.67	0.33	0.17	0.06	<0.02
Ethyl oleate (18:1)	0.98	0.90	1.03	0.53	0.28	0.07	0.07
Ethyl stearate (18:0)	0.48	0.27	0.19	0.16	0.16	0.14	0.04
Total*	2.38	2.09	1.98	1.12	0.72	0.32	0.16

Concentrations are taken from an average of n aliquots.
 *Sum total does not include ethyl laurate.

increased significantly, as shown in Figure 3C. The SIM peak at 7.15 minutes is only 3% of the total area of all the peaks in the time period shown, whereas the SRM peak at 7.15 minutes is approximately 97% of the total area. In this comparison, SRM improved the selectivity and S/N ratios by more than 3000 times the SIM S/N ratios. Such selectivity is highly desirable for the analysis of FAEs in such complex matrices.

A comparison of detection limits with this method to other methods is shown in Table 4. In our method, ethyl palmitate (16:0) has a relatively higher detection limit compared with the other FAEs. This is most likely due to the surrounding noise from the hair matrix during the isolation of ethyl palmitate. Compared with other SPME methods, our method has better detection limits, presumably due to the

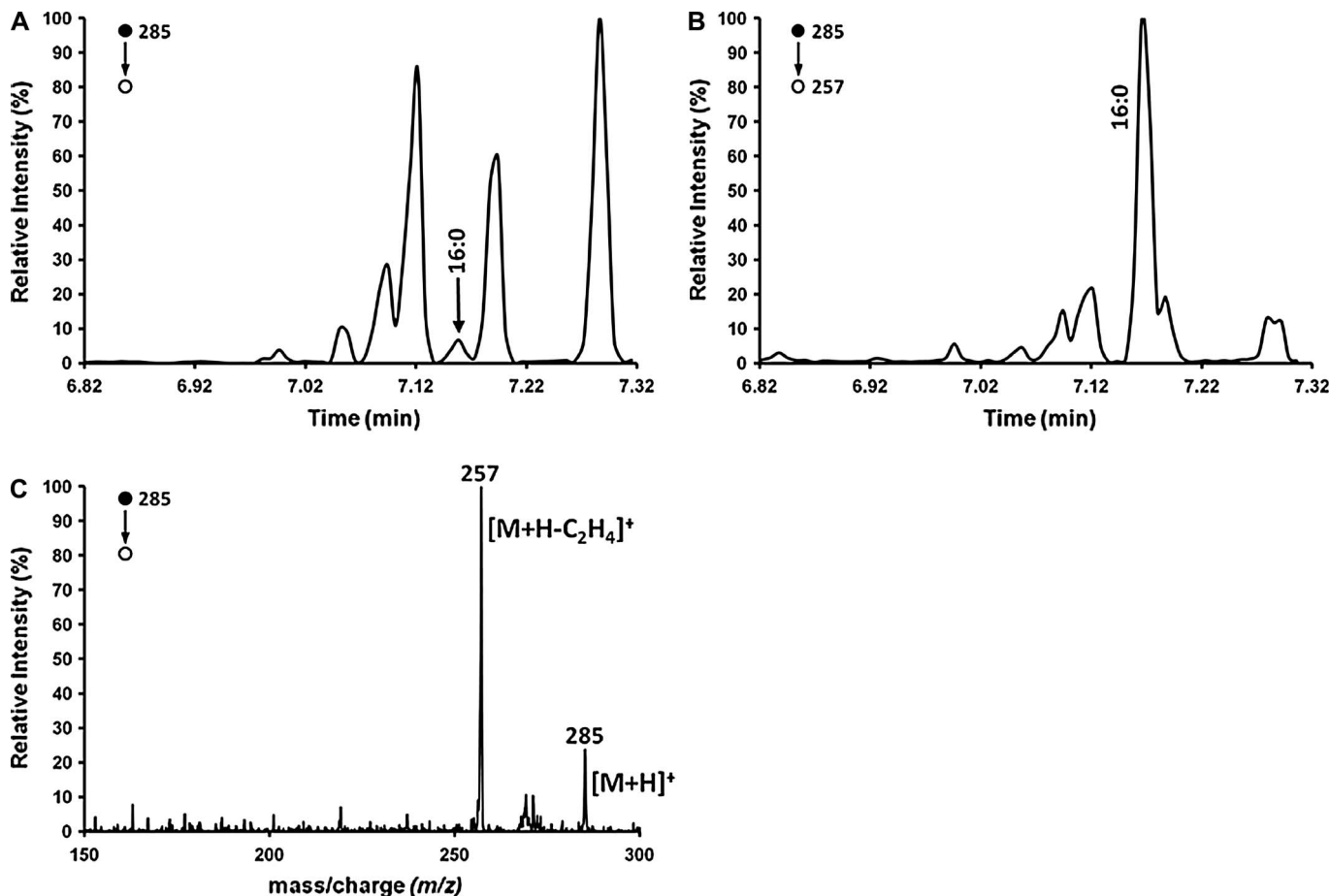


FIGURE 3. Product ion scanning and selected reaction monitoring of a heavy drinkers hair sample. A, Product ion scanning m/z 285 (ethyl palmitate); B, product ion mass spectrum obtained from the fragmentation of m/z 285 at 7.15 minutes (ethyl palmitate); C, SRM of the fragmentation of the molecular ion ethyl palmitate m/z 285 \rightarrow 257.

increased selectivity. Our method detection limits are typically not as good as the solid phase extraction method, which suggests that solid phase extraction has significantly better extraction efficiencies than SPME. Percent recoveries for SPME extractions range from 3% to 5% for each FAEE as established from comparing peak areas of spiked hair with peak areas from liquid injections. Therefore, improvements in SPME extraction efficiencies could significantly improve detection limits even more.

It can be observed in Figure 1 that small amounts of these FAEEs are seen in the hair of a nondrinker. This is because small amounts of ethanol can be ingested from our diets or derive from hair products (shampoos, dyes, etc.).²⁴ Pragst and coworkers have already demonstrated that the sum of 4 FAEEs can serve as a marker to distinguish between nondrinkers, moderate drinkers, and heavy drinkers.^{16,25} The quantities of each FAEE in hair derived from this current study could be used in a similar manner to protocols established by Pragst and coworkers. Table 5 demonstrates that this method can be applied to hair samples from multiple donors. Although this only compares 7 different samples, we can see a distinction in the total FAEE concentration of heavy, moderate, and nondrinkers. Individual concentrations for each FAEE are shown in table 5 for each sample. It is important to note that ethyl laurate was not one of the FAEE tested in Pragst et al's experiments,^{10,16} but was tested in some other methods.^{4,20} Therefore, we included ethyl laurate in this testing to see if it was detectable. Looking at the individual concentration of each FAEE in Table 5, it can be seen that ethyl laurate (12:0) was not detected in any of these hair samples. As Pragst et al has also determined,¹⁰ it seems that ethyl laurate is not directly indicative of alcohol consumption. The total FAEE concentrations are also given in this table; showing that total FAEE concentrations for heavy drinkers are around 2 ng/mg, moderate drinkers are around 1 ng/mg, and nondrinkers are under 0.4 ng/mg. Given that a distinction in total FAEE concentration is observed between the different levels of alcohol consumption, this method could be equally well suited to clinical trials. Our calculated total concentrations for the 4 FAEEs are consistently slightly higher than the typical values given by Pragst et al.¹⁶ The reason for this small systematic difference has yet to be established. Presumably, a more thorough clinical trial including many more samples would help validate this considerably faster procedure and better establish the accuracy of the method.

CONCLUSIONS

This research describes a method to detect biomarkers in human hair for the determination of alcohol usage, which offers improved selectivity and quicker analysis time than current methods. Although more testing from a larger pool of samples needs to be done before being able to use the described method in a standard laboratory setting, this method seems promising in being able to determine whether a person is a heavy, moderate, and nondrinker. Once cut-off levels are established for this method to distinguish between heavy, moderate, and nondrinkers we see this method being applicable to clinical applications such as neonatal screenings

and corrections/jurisprudence applications such as parole violations.

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