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Short communication

Determination of fatty acid ethyl esters in hair by GC–MS and application in a population of cocaine users

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ABSTRACT

A gas chromatography–mass spectrometry method for the determination of ethyl myristate, ethyl palmitate, ethyl oleate, and ethyl stearate in hair samples was developed, validated and applied to real samples. Ethyl myristate, ethyl palmitate, ethyl oleate, and ethyl stearate are fatty acid ethyl esters (FAEE) which are known to be direct biotransformation products of ethanol. Their presence in the body fluids and tissue is therefore indicative of alcohol intake and, in particular, FAEE concentration in hair higher than 0.5 ng/mg is indicative of excessive chronic alcohol consumption. The method was applied to 80 hair samples formerly found positive for cocaine and FAEE analytical results were compared with the presence of cocaethylene, a cocaine metabolite formed only when alcohol and cocaine are used together. According to our data the two biomarkers (FAEE and cocaethylene in hair) are tools of great value in the assessment of the diagnosis of use of cocaine and ethanol. In fact, discrepancies were noted and might be related to various factors including differences in consumption habits and thus permitting to distinguish the use of both substances non-concurrently or concurrently. Also, the determination of both markers may, in some cases, discriminate the use of moderate or heavy alcohol amounts when associated with cocaine. Finally, in a population of non-cocaine-users our results support FAEE as valuable means in the assessment of excessive alcohol chronic use.

1. Introduction

The importance and diffusion of hair analysis in the diagnosis of alcohol heavy use and misuse have recently been establishing in the routine of analytical toxicology laboratories, following, in particular, the development of analytical procedures able to determine alcohol biotransformation products, such as fatty acid ethyl esters (FAEE) and ethyl glucuronide in hair samples. Both FAEE and ethyl glucuronide are recognized by the Society of Hair Testing as biomarkers of chronic excessive alcohol consumption [1].

FAEE have been known as biotransformation products of ethanol since the 1960s [2] and were proposed in the clinical practice as cardiomyopathy markers before [3] and as ethanol use/abuse markers later: in 2001 [4] FAEE started being used as markers of alcohol excessive use. Several different molecules formed from ethanol and fatty acids, phospholipids, or lipoproteins were identified and attributed to FAEE group, but only four of them were found to be correlated with alcohol use when retrieved in the keratinic matrices (ethyl myristate, ethyl palmitate, ethyl oleate, and ethyl stearate) [4,5].

Nearly all methods reported by the literature identify these analytes by head space-solid phase microextraction-gas chromatography–mass spectrometry (HS SPME GC–MS) [4–9] or, more recently, by GC–tandem mass spectrometry [10]. Both techniques performed satisfactorily and, on the basis of the analytical results a cut-off of 0.5 ng/mg for the sum of the 4 analytes was found to best reflect a chronic excessive alcohol consumption. In fact, as alcohol is a legal substance in most countries a cut-off is strictly needed in order to discriminate a moderate and an excessive use, i.e. more than 60 g of pure ethanol per day, according to the World Health Organization Guidelines and corresponding to roughly four alcoholic drinks per day [1].

The present study aimed at developing and validating a GC–MS method for FAEE in hair, which might be useful in laboratories whose instrumental equipment do not comprise HS-SPME or GC–MS–MS. As a matter of fact, a reliable (as ensured by full validation) GC–MS method will permit to extend and diffuse the analysis of excessive chronic alcohol use. Furthermore, the method was applied to hair samples previously analyzed and found positive for cocaine and either positive or negative to cocaethylene, a transesterification product of cocaine. Cocaethylene is a cocaine metabolite formed by carboxylesterases when cocaine and ethanol are consumed simultaneously and, therefore, can be used as ethanol intake indicator in cocaine users populations. For this

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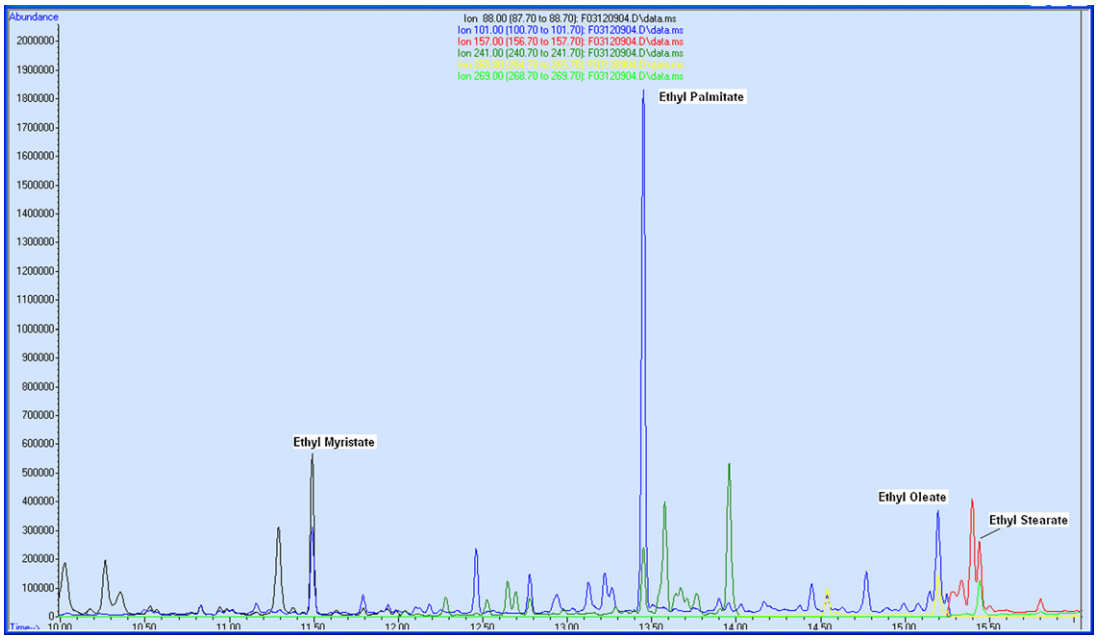

Fig. 1. Gas chromatogram of a positive sample (ethyl myristate: 0.13 ng/mg, ethyl palmitate: 0.61 ng/mg, ethyl oleate: 0.71 ng/mg, ethyl stearate: 0.25 ng/mg, total FAEE: 1.87 ng/mg) acquired in SIM mode.

reason, 80 cocaine positive hair samples were selected and analytical results of FAEE and cocaethylene were compared in order to evaluate their reliability as markers of excessive and/or chronic drinking.

2. Materials and methods

2.1. Hair samples

Samples of head hair were chosen among those found positive to cocaine in individuals controlled for drugs of abuse for regranting driver's license. Samples were admitted to the study after complete anonymization. The same length (between 3 and 5 cm) was used for both cocaine and FAEE analysis.

2.2. Standards and reagents

Ethyl myristate, ethyl palmitate, ethyl oleate, and ethyl stearate were acquired from Sigma–Aldrich (Milan, Italy), cocaine and its main metabolites (benzoylecgonine, ecgonine methylester, and cocaethylene) were purchased from LGC Standards (Milan, Italy). All reagents were of HPLC grade or higher and acquired from Sigma–Aldrich.

2.3. Sample preparation for FAEE determination

All hair samples were accurately double washed with methanol (2 ml for about 2 min each). The four FAEE analytes were extracted from the keratinic matrix finely cut with scissor (about 100 mg, lower amounts were considered acceptable down to 30 mg) by overnight incubation in n-hexane/dimethylsulphoxide (4 and 0.5 ml respectively) with alpha-colestane as internal standard (50 ng, purchased from Sigma–Aldrich). The following morning, the incubation layer of n-hexane was extracted on aminopropyl-NH_2 solid phase extraction (SPE) cartridges (Varian, Harbor City, CA) that were initially conditioned with dichloromethane followed by n-hexane (3 ml each). After elution of the incubation solvent, the analytes were extracted by n-hexane (3 ml) and, subsequently, by dichloromethane (3 ml) and the resulting extract was evaporated under a stream of nitrogen. The residue was dissolved in 50 μl of n-hexane and injected in the gas chromatograph–mass spectrometry (GC–MS) apparatus in selected ion monitoring (SIM) mode.

2.4. Sample preparation for cocaine and its metabolites

Samples were finely cut with scissor, and, after nalorphine addition (50 ng, internal standard), overnight incubated with hydrochloric acid 0.1 N, solid phase extracted (Bond Elut Certify LRC cartridges, Varian, Harbor City, CA) using the method proposed by the manufacturer for basic drugs (cartridges conditioned with methanol and phosphate buffer, addition of the sample, rinsed with water, hydrochloric acid and methanol and eluted with dichloromethane–isopropanol (8:2) with 2% ammonium hydroxide), derivatised with 50 μl of N-methyl-N-(trimethylsilyl)trifluoroacetamide (75 °C for 15 min) and injected in the GC–MS apparatus.

2.5. Gas chromatography–mass spectrometry

The analysis was developed, validated and performed on an Agilent GC–MS with Inert MSD (GC 7890A, MS 5975C equipped with a 7693 autosampler) equipped with a phenylmethylsilicone 5% (HP-5MS) capillary column (30 m × 0.25 mm I.D. × 0.25 μm film thickness). The injector was set at 300 °C and splitless injection was performed. The column oven temperature was programmed at 100 °C for 0.5 min, then increased to 200 °C at 12 °C/min, to 300 °C at 8 °C/min, and held at 300 °C for 3 min. Identification was performed in selected ion monitoring mode with at least three ions for each analyte: *m/z* 88, 213, 256 for ethyl myristate (*m/z* 88 used for quantification); 101, 241, 284 for ethyl palmintate (quantifier: 101); 101, 265, 310 for ethyl oleate (quantifier: 101); 157, 269, 312 (quantifier: 157); and 217, 357, 372 for internal standard (217 used for quantification).

For cocaine analysis the same instrument was used. Main parameters were the same described above except for oven temperature that was maintained at 100 °C for 1 min, increased then to 300 °C at 20 °C/min, and hold at 300 °C for 3 min. For identification purposes, three ions were monitored for each analyte: *m/z*