Clinical Biochemistry 49 (2016) 1078–1091

Contents lists available at ScienceDirect

Clinical Biochemistry

journal homepage: www.elsevier.com/locate/clinbiochem

Alternative sampling strategies for the assessment of alcohol intake of living persons

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ARTICLE INFO

Article history:
Received 19 February 2016
Received in revised form 28 April 2016
Accepted 1 May 2016
Available online 18 May 2016

Keywords:
Alcohol
Review
Dried blood spots
Dried urine spots
Sweat
Skin surface lipids
Oral fluid
Exhaled breath
Hair
Nail
Meconium
Umbilical cord
Placenta

ABSTRACT

Monitoring of alcohol consumption by living persons takes place in various contexts, amongst which workplace drug testing, driving under the influence of alcohol, driving licence regranting programs, alcohol withdrawal treatment, diagnosis of acute intoxication or fetal alcohol ingestion. The matrices that are mostly used today include blood, breath and urine. The aim of this review is to present alternative sampling strategies that allow monitoring of the alcohol consumption in living subjects. Ethanol itself, indirect (carbohydrate deficient transferrin, CDT%) as well as direct biomarkers (ethyl glucuronide, EtG; ethyl sulphate, EtS; fatty acid ethyl esters, FAEEs and phosphatidylethanol species, PEths) of ethanol consumption will be considered. This review covers dried blood spots (CDT%, EtG/EtS, PEths), dried urine spots (EtG/EtS), sweat and skin surface lipids (ethanol, EtG, FAEEs), oral fluid (ethanol, EtG), exhaled breath (PEths), hair (EtG, FAEEs), nail (EtG), meconium (EtG/EtS, FAEEs), umbilical cord and placenta (EtG/EtS and PEth 16:0/18:1). Main results, issues and considerations specific to each matrix are reported. Details about sample preparation and analytical methods are not within the scope of this review.

1. Introduction

Alcohol is a legal psychoactive substance that has been widely used in many cultures for centuries. The severity of an alcohol intoxication is related to the volume of alcohol consumed and to the drinking pattern. Alcohol consumption can lead to impairment of physical coordination, consciousness, cognition, perception or behaviour [1] and is therefore not compatible with some professional activities or with driving a vehicle. Prenatal alcohol exposure, induced by alcohol consumption during pregnancy, can engender consequences on the newborn, such as distinctive craniofacial dysmorphology, growth retardation, common cognitive disorders, and social impairment [2]. Chronic misuse of alcohol can cause diseases, while acute intoxications may result in coma and death or be a contributing factor resulting in death. Moreover, alcohol use may induce social and economic problems.

After consumption, ethanol is readily absorbed from the stomach and from the small intestine (duodenum and jejunum) into the blood stream [3]. Ethanol is a small size (molecular weight (MW) = 46 g/mol) weak acid (pKa 15.9 at 25 °C), which can easily penetrate biological membranes by passive diffusion through aqueous channels. Ethanol is distributed into all body fluids and tissues, in proportion to their water content [4]. Between 2 and 5% of an ingested dose is excreted unchanged in the urine, breath and sweat. The ingested ethanol is mainly (about 95%) removed from the body by oxidative metabolism (phase I) and partially (<0.1%) by non-oxidative metabolism (phase II), i.e. via conjugation reactions. The non-oxidative metabolism of ethanol results in the formation of ethyl glucuronide (EtG), ethyl sulphate (EtS), phosphatidylethanol species (PEths) and fatty acid ethyl esters (FAEEs) (Fig. 1).

Glucuronidation of ethanol is a phase II conjugation reaction with UDPGA (uridine 5′-diphospho-β-glucuronic acid) through the action of endoplasmic reticulum UDP-glucuronosyltransferase enzymes [5]. About 0.02% of consumed ethanol is excreted in urine as EtG [6,7]. Sulfation of ethanol is a phase II conjugation reaction with PAPS

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http://dx.doi.org/10.1016/j.clinbiochem.2016.05.007
0009-9120/

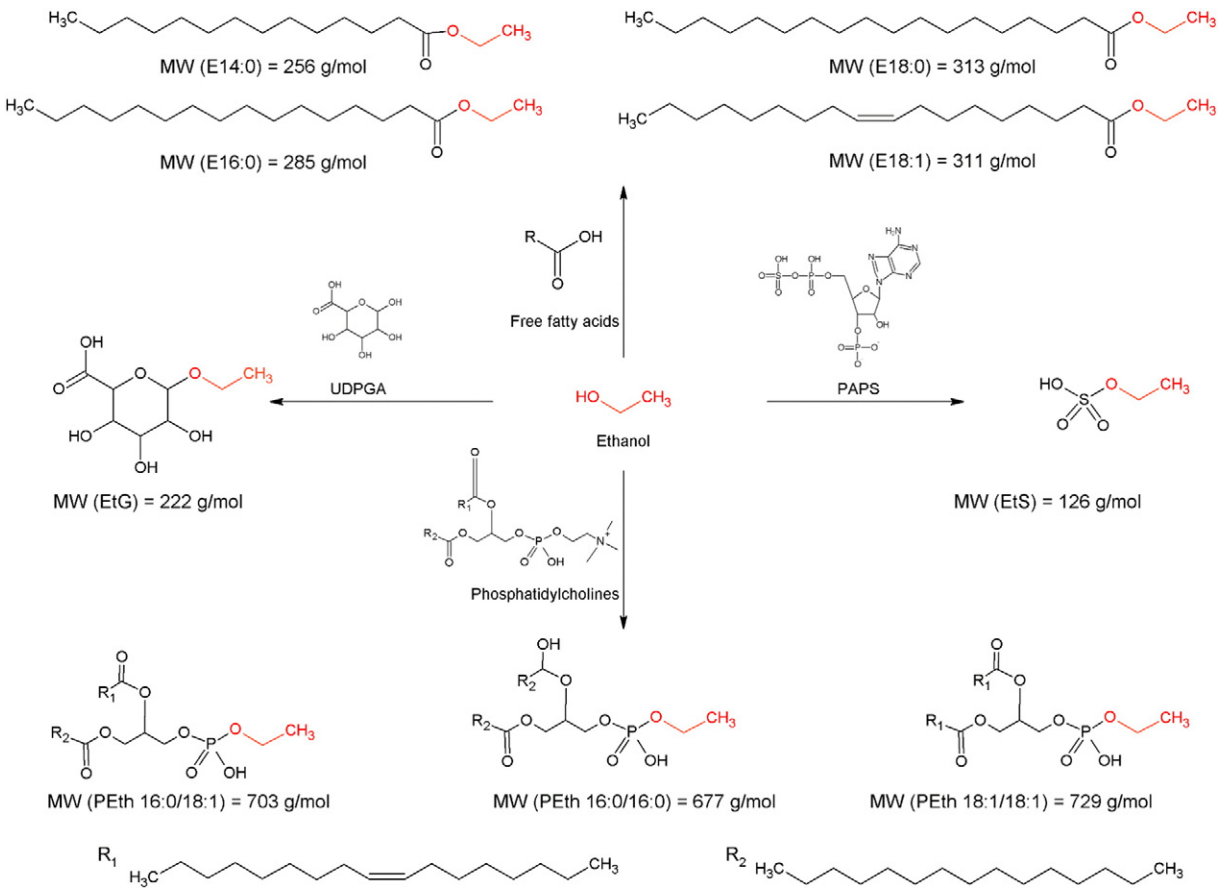

Fig. 1. Non-oxidative phase II metabolism of ethanol into EtG, EtS, PEths (PEth 16:0/18:1, PEth 18:1/18:1 and PEth 16:0/16:0) and FAEEs (ethyl myristate (E14:0), ethyl palmitate (E16:0), ethyl stearate (E18:0) and ethyl oleate (E18:1)), with indication of the molecular weight (MW).

(3′-phosphoadenosine 5′-phosphosulphate) through the action of cytosolic sulfotransferase [8]. Only 0.01–0.02% of the consumed ethanol is excreted in urine as EtS on a molar basis [9,10].

EtG and EtS are two small, polar and acidic metabolites of ethanol [11]. The pKa of EtG was estimated between 2.84 and 3.21 [11–13] and of EtS at −3.14 [11].

PEths are a group of abnormal phospholipids formed in the presence of ethanol, via the action of phospholipidase D, which normally hydrolyses phosphatidylcholine into phosphatidylic acid and choline in cell membranes [14]. Up to 48 different PEths have been detected in blood collected during autopsy of heavy drinkers [15]. All species have a common phosphoethanol head onto which two fatty acids of variable chain length and degree of saturation are attached. Although analysis of blood from heavy drinkers shows a huge interindividual variation of the distribution of the different PEths [16], PEth 16:0/18:1 and PEth 16:0/18:2 are the two predominant PEths detected [15–18]. Preliminary studies suggest that PEth 16:0/18:1, PEth 16:0/18:2, PEth 18:1/18:1, PEth 16:0/20:4 and PEth 18:1/18:2 could constitute together >80% of total PEths, whereas PEth 16:0/16:0 alone could represent about 1–5% [19]. While some methods (e.g. HPLC coupled to light-scattering detection [20–23] and non-aqueous capillary electrophoresis coupled to UV detection [24]) measure the total amount of PEths, other methods (such as LC–MS/MS) are able to identify and quantify individual molecular species [16–18,25–28]. The three commercially available PEths are presented in Fig. 1.

FAEEs are a group of >20 substances formed by enzymatic esterification of ethanol and free fatty acids. Ethyl myristate (E14:0), ethyl palmitate (E16:0), ethyl stearate (E18:0) and ethyl oleate (E18:1) (Fig. 1) are the most studied FAEEs.

Different enzymes (i.e. FAEE synthase, acyl-CoA: ethanol *O*-acyltransferase, lipoprotein lipase, cholesterol esterase, carboxylesterase and carboxylester lipase) catalyse the esterification of ethanol to free fatty acids. More information about the biochemistry of FAEEs can be found in an article published in 2003 [29]. Politi et al. in 2007 and Cabarcos et al. in 2015 have published interesting reviews about the detection of FAEEs in biological samples [2,30].

After excessive and chronic alcohol consumption, ethanol can induce indirect effects on the body via its interference with liver function (increased carbohydrate deficient transferrin (CDT%), gamma-glutamyltransferase (GGT), aspartate aminotransferase/alanine aminotransferase (AST/ALT)) and its effect on the size of red blood cells (increased mean corpuscular volume (MCV)).

Up to now, indirect biomarkers of alcohol measured in whole blood (MCV) or serum (CDT%, GGT, ALT, AST) are traditionally used to detect alcohol dependence. Monitoring of alcohol consumption is most often performed in breath, blood and urine. Blood alcohol concentration (BAC) is of particular interest, due to its correlation with the effect of alcohol. To avoid venepuncture sampling and to allow the evaluation of alcohol consumption in traffic situations, breath sampling has since long been introduced to detect persons under the influence of alcohol. Then, application of a factor, based upon the blood-breath concentration ratio, allows the conversion of the ethanol concentration in breath (BrAC) to the BAC [31]. Analysis of ethanol and/or EtG/EtS in urine allows a longer detection window, but does not lead to information concerning the status of the person e.g. at the time of an accident.

Besides breath, blood and urine for the determination of ethanol itself and blood and urine for the detection of direct and indirect markers of ethanol use, the alcohol consumption of a living person can also be monitored via so-called ‘alternative sampling strategies’. These will be the subject of this review, which will discuss ‘classical’ samples obtained from a living person via an alternative way (e.g. dried blood spots (DBSs)), as well as ‘alternative’ samples (i.e.