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Review

Determination of ethanol in human fluids — II. Determination of ethanol in urine, breath and saliva

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Abstract: A review is presented to continue with a previous one in which the methods for determination of ethanol in urine, breath and saliva are surveyed.

Keywords: *Ethanol; urine; breath; saliva; review.*

Introduction

Ethanol diffuses rapidly throughout the organism due to its solubility in water and lipids and thus it can be, and has been, determined in every type of biological fluid: blood, urine, saliva, etc. In the previous paper [1] methods for its determination in blood were reviewed. The present paper presents a review of methods for determination in other biological fluids, where these are intended as an alternative to measurement in blood. Here only the most useful techniques, namely those concerning analysis in urine, breath and saliva, will be considered.

Determination of Ethanol in Urine

The determination of ethanol in urine has been proposed as a substitute for measurement in blood. For them to be a correlation the subject must completely evacuate the contents of the bladder and perform a second micturition 30 minutes after the first; the latter portion is collected for analysis. The correlation has been studied by different authors, who have found contradictory results. Thus, Froentges [2] proposed the expression $Y = 0.6582\ X - 0.608$ (Y and X being the concentrations in urine and blood, respectively). The equation was applied in about 10 000 tests and provided an

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error of ±2.8%. Payne [3] analysed blood and urine samples from 35 persons under the influence of alcohol and found an average concentration of ethanol in urine 1.33 times higher than in blood. Morgan [4] considers the calculation of the ethanol content in blood from its concentration in urine unsatisfactory because he found the approximations used introduce very large errors. Owing to these shortcomings, the methods for determination of ethanol in breath have displaced those of urine in routine analyses.

Almost all the methods used for the determination of ethanol in blood may be adapted for its determination in urine (see Jain and Cravey [5, 6] for review). We shall adopt a similar classification to that used for the determination of ethanol in blood [1]; that is, the methods concerned will be grouped into chemical, enzymic and chromatographic, and a comparison will be established between the determination in both types of liquids where appropriate.

(a) *Chemical methods*

These are based on the reducing property of ethanol, which is separated from the sample — usually by distillation — and titrated with an oxidant such as dichromate, either directly [7, 8], by back titration [9] or by photometric measurements [10]. Vanadic acid [11] has also been used as an oxidant but less frequently.

(b) *Enzymic methods*

The high selectivity of these methods allows their application to all types of samples without modification. No pretreatment other than dilution is necessary for urine. The methods described to date are based on the oxidation of ethanol by nicotinamide adenine dinucleotide (NAD^+) in the presence of alcohol dehydrogenase (ADH), with photometric detection either of a dye (formazan) yielded in a coupled reaction [12] or of the reduced form of the coenzyme, NADH, using a centrifugal analyser [13], or differential voltammetric detection has been used for the reduced form of 2,6-dichlorophenolindophenol which is yielded in the coupled reaction, favouring the displacement of the reaction towards completion and increasing its sensitivity [14]. The differential nature of the measurement makes the use of a blank unnecessary.

(c) *Chromatographic methods*

These methods have been applied to the determination of alcohol in all types of biological materials: urine, saliva, viscera, etc. by direct injection of liquid samples, after a separation process (extraction or distillation) or by the head-space technique. Direct injection of the sample only requires addition of the internal standard as a prior step [15–19]. The major drawback lies in the risk of column contamination by compounds present in the sample.

All methods involving the previous extraction of ethanol use dioxane as solvent [20]. A chromatographic method with prior distillation of ethanol from urine has been suggested by Fox [21]. The head-space technique is the most commonly used chromatographic method for the analysis of ethanol in urine [22–25].

Determination of Ethanol in Breath

The determination of ethanol in breath dates from 1847 [26] and its official use was introduced in 1927 as the Bagen method [27]. During the interval 1930–1950 the main physiological, pharmacological and pharmacokinetic features of ethanol and its influence

on drivers were described [28]. In addition, several analysers were produced for measuring alcohol in breath such as the "drunkometer" [29], "intoximeter" [30] and "alcometer" [31]. The intention was to allow the measurement of alcohol in drivers at the roadside by nonspecialized staff. The ethanol contained in the blood plasma diffuses freely through the lungs following Henry's Law and establishes a ratio between the ethanol content of blood and breath. The ratio does not remain constant throughout a forced expiration owing to temperature changes from 37.5°C to 34°C. The level of agreement is only of the order of 0.15 g of ethanol per litre of blood [32–36]. Notwithstanding this error, the simplicity of these methods has given rise to their wide development, which allows their classification into chemical, voltammetric, conductimetric, thermochemical and infra-red absorption, dehydrogenation and chromatographic. The majority of the methods proposed so far have resulted in the commercialization of a device in each case.

(a) *Chemical methods*

These are based on the oxidation of alcohol by dichromate or permanganate in concentrated sulphuric acid. The percentage of ethanol in weight is calculated from the amount of oxidant consumed. Based on this principle, different devices have been commercialized, such as the detector tubes for ethanol in breath from Kitagawa [37], Reanal [38], Medicor Muvek [39], Kelly [40], Luckey [41], Ducie [42], the alcolyser and alcotest [43] and the breathanalyser which is the most commonly used [44–46]. The photoelectric intoximeter has undergone several modifications [48–50] since its invention by Forrester [47].

(b) *Voltammetric methods*

These are based on the oxidation of ethanol to acetic acid at a controlled potential. The current intensity yielded [51] is proportional to the concentration of ethanol in the breath sample at levels equal to or lower than 3 g of ethanol per litre of blood [52]. This method is used in the portable alcolimiter designed by Jones *et al.* [53, 54]. Its precision has been tested against the ADH/NAD^+ enzymic method, giving a standard deviation of ± 0.0525 g l^{-1} for an average concentration of ethanol in blood of 0.5 g l^{-1}. The alcolmeter APIS-MI [56] is based on a similar principle and provides standard deviations of ± 0.045 g l^{-1} for concentrations of 0.8 g l^{-1}, with a coefficient of variation of 6.4%.

(c) *Conductimetric methods*

These rely on the measurement of the conductivity of ethanol in a conductimetric cell. In the portable device ALERT™ [57] a type-N semiconductor measures conductance increments proportional to the concentration of ethanol in the breath sample. This device requires complicated handling, and is sensitive to pressure and temperature changes and to other conductors present in the sample. Similar instruments have been designed by Vysok-Skola Chemik-Technologika [58] and by Morand [59].

(d) *Thermochemical methods*

The instrument designed by Burroughs *et al.* [60], based on this principle, uses a Wheatstone bridge to measure the heat of oxidation of ethanol as the sample passes through a catalytic resistance. Sensitivity is improved by passing the sample through a second non-catalytic resistance which compensates for the effect of changes in temperature, thermal conductivity and convection.

(e) *Methods of infra-red absorption*

These are based on the infra-red absorption of energy by ethanol vapour in breath samples. There are two such commercial devices. The Intoxilyzer, described by Harte [61, 62] measures the absorption band of the C–H bond stretching vibration (λ = 3.39μ). A specially designed detection system [63] is used and the relative standard deviation for the analysis of ethanol in breath vs that in blood is 16%. A Japanese analyser [64] operates with a double beam, and features response times shorter than 1 s. It has a linear range starting from 2 mg of ethanol per litre of breath (4 mg ml^{-1} blood). Results are in agreement with gas–liquid chromatography.

(f) *Dehydrogenation methods*

The instrument designed by Kamei and Koezuka [65] electronically measures the protons (from the hydroxyl group) generated in the catalytic dehydrogenation of ethanol. In a modification introduced by these authors the hydrogen produced by passing the sample through a catalytic filter is measured with the aid of a vacuum ionization manometer, ionic pump or diode [66]. The method is sensitive to 0.1 mg of ethanol per litre of breath.

(g) *Chromatographic methods*

Gas chromatography offers several advantages over other conventional methods, such as simplicity, rapidity, accuracy and specificity. Its most outstanding features are accuracy and the separation of ethanol from other alcohols, aldehydes and ketones.

In some methods alcohol in the breath samples is absorbed into a solution of Mg $(ClO_4)_2$ [67] or dry isopropanol, then chromatographed and detected by flame ionization. Chromatographs specially designed for this type of analysis are the Alco-analyzer [69] used for the analysis of ethanol in breath and blood (breath samples are collected with the so-called Mobat Sober-Meter [39]), and the Intoximeter (GCI) [49, 70], whose specificity and easy operation have extended its use. The latter performs an assay in 90 s and displays the results either digitally or via a recorder. Monis *et al.* [71] have used the GCI for analysis of breath trapped into indium tubes with excellent results.

Determination of Ethanol in Saliva

The possibility of calculating the content of ethanol in blood from its concentration in saliva has been studied as an alternative to the analysis of ethanol in breath. The ratio between the concentration of ethanol in blood and saliva is altered by the contamination of saliva, introducing serious errors in the determination. This accounts for the low interest and development in this aspect of ethanol analysis in biological fluids.

Feller and Le Petit [72] have studied the ethanol/drug concentration ratios for several drugs (guinidine, sulfamerazine, paracetamol, diazepan and ethanol) in saliva and blood, and have shown that some drugs, including ethanol, are equally distributed between saliva and blood plasma.

A device designed for the analysis of ethanol in saliva — the Alcolmeter AE-D1 [73] — is easy to handle and may be used by nonspecialists. It employs an electrochemical fuel cell [74] to sense and measure the concentration of ethanol in the sealed head-space vapour above the fluid sample. The fuel cell is housed in the 'sensor head' and is an integral part of the aspirating sampling system which automatically introduces a fixed volume (1 ml) of head-space into the detector. The alcohol in the sample is captured by

the platinum electrode in the fuel cell and electrochemically oxidized to acetic acid. The reaction releases electrons from the alcohol molecule, producing an electron flow which gives a voltage change across an external resistance. This voltage is directly proportional to the concentration of ethanol in the head-space vapour which, in its turn, is in equilibrium with the fluid ethanol concentration, in agreement with Henry's Law. The calibration curve is linear up to 2.5 g of ethanol per litre. A comparison between the results obtained from the analysis of saliva with this instrument and those obtained simultaneously on blood by gas chromatography (111 experiments) show that the following equation applies: $Y = 0.018 - 0.985\,X$ (where Y and X are the concentrations of ethanol in blood and saliva, respectively) [74].

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