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Determination of isoprene in human breath by thermal desorption gas chromatography with ultraviolet detection

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Abstract

We describe a new, highly sensitive and specific method for the analysis of isoprene (2-methyl-1,3-butadiene) in human breath. A known volume of expired air (150 ml) was drawn through a solid sorbent material to capture trace organic substances, followed by thermal desorption at 200°C and subsequent determination of isoprene by gas chromatography with diode-array ultraviolet detection. The calibration plot was linear ($r = 0.99$) over a wide range of breath isoprene concentrations (0–12 nmol/l), and levels down to 0.10 nmol/l were easily measurable. In sixteen healthy subjects (six men and ten women), all of whom were non-smokers, the mean, median and spread of breath isoprene concentrations were 3.73, 3.36 and 1.60–10.33 nmol/l, respectively. No statistically significant differences in the concentrations of breath isoprene were observed between the sexes. The mean (± S.D.) concentration of breath isoprene in nine consecutive tests with the same subject was 3.69 ± 0.60 nmol/l, and the coefficient of variation was 16.3%. Much larger variations in exhaled isoprene were seen during the day and also between days when the same subject was tested repeatedly. The excretion patterns of isoprene in human breath can be investigated with high selectivity and sensitivity with this new analytical method.

1. Introduction

Isoprene (2-methyl-1,3-butadiene) has been identified as the most prominent volatile hydrocarbon in human expired air [1–3]. Although the exact physiological role of isoprene is unknown, studies in animals and plants suggest that this hydrocarbon is produced through the mevalonic acid pathway [4,5]. The low boiling point (34°C) and negligible solubility in water means that isoprene is excreted as a waste product with the exhaled air [6,7]. Previous analytical methods for measuring isoprene, as well as other low-molecular-mass breath volatiles, have involved the use of gas chromatography (GC) with flame ionization detection [8–10]. However, quantitative analysis of breath isoprene is difficult, in part because of the low concentrations present and the fact that isoprene is an unstable oxidizable substance.

In this paper we describe a highly sensitive method for the qualitative and quantitative analysis of isoprene in human breath. The basic principle of this new method has been described in more detail elsewhere and involves GC separation and UV detection and identification

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[11,12]. Because isoprene is a conjugated diene the molecules absorb ultraviolet radiation at easily accessible wavelengths producing a well defined and characteristic gas-phase spectrum. We took advantage of the specific UV absorption spectrum of isoprene to develop a new method for measuring this endogenous volatile hydrocarbon with thermal desorption GC and diode-array detection.

2. Experimental

All chemicals and solvents were of analytical grade and were supplied by Merck (Darmstadt, Germany). Isoprene (99% purity) was purchased from Sigma (St. Louis, MO, USA) and was stored protected from light at 4°C.

2.1. Procedure for sampling and analysis of breath

The volunteer subjects were recruited from among workers at the laboratory. After holding their breath for 30 s, each subject exhaled into a 5-l PTFE bag which was kept at 35°C with an electrically heated blanket. Aliquots of breath (150 ml) were drawn from the collecting bag and the organic constituents were trapped on an absorbent tube containing three different chemicals: Tenax TA, HayeSepQ and Carbosphere (Alltech, Deerfield, IL, USA). An electronic bubble flow meter (Gilibrator, Gillian, W. Caldwell, NJ, USA) was used to measure the volumes of breath analysed. The precision of this device, expressed as coefficient of variation, was ± 2% for flow-rates between 20 and 80 ml/min,

Fig. 1 shows a block diagram of the equipment used for analysis of breath isoprene. This arrangement consists of a thermal desorption oven (e), a gas flow-cell built into a micro gas chromatograph (a) and a UV spectrograph with a photodiode-array detector (c). This method of analysis has been described in detail elsewhere [10] but has now been modified to include a spectrophotometer constructed from individual optical parts and flushed with nitrogen. This

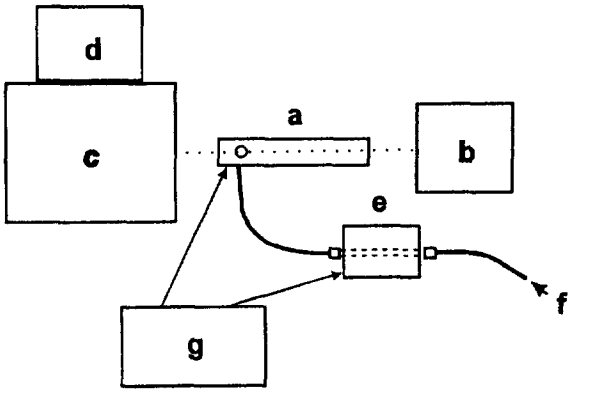

Fig. 1. Schematic diagram of equipment used for thermal desorption of breath volatiles and determination of isoprene: (a) gas flow cell with built in micro gas chromatograph, (b) deuterium lamp, (c) spectrograph, (d) photodiode-array detector, (e) oven for thermal desorption of trapped breath sample, (f) carrier gas line, (g) power supply and temperature regulation for the gas flow cell and the desorption oven.

modification allows quantitative measurements at UV wavelengths below 190 nm. The sorbent tube is connected to the carrier gas flow line (f) and placed in the preheated oven (e). After heating to 200°C for about 1 min the desorbed volatile compounds are flushed into a separation column in the gas flow line. The chromatographic operating conditions were: carrier gas flow-rate 25 ml/min, separating column 80 mm × 1.5 mm maintained at room temperature (21°C) and packed with 10-μm Hypersil WP C_8 and DCQF-1 as a stationary phase (Alltech).

2.2. Preparation of isoprene–air–vapour standards

A 0.02% (v/v) stock solution of isoprene was prepared in *n*-hexane. A standard curve was prepared by injecting exactly measured volumes of this solution (1, 2, 3 and 4 μl) into the gas chromatograph for analysis by diode-array UV detection. Fig. 2 gives an example of the calibration plot used for quantitative analysis of breath isoprene. The detector response was linear over the concentration range normally encountered in breath samples. Moreover, when ambient room air (150 ml) was analysed in

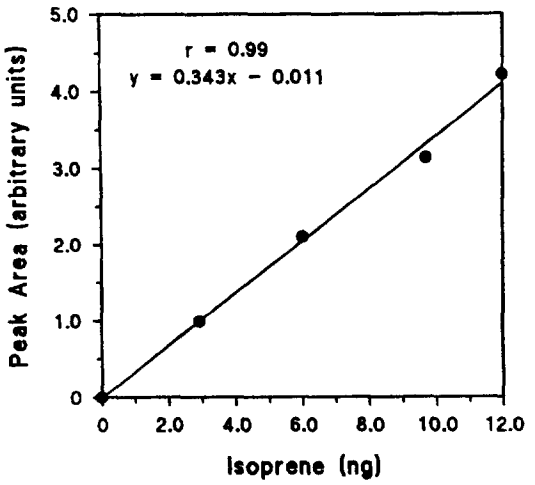

Fig. 2. Linear detector response relationship for analysis of isoprene by thermal desorption gas chromatography and diode-array UV detection. Known amounts of isoprene (in hexane solvent) were injected directly into the UV cell to produce this graph.

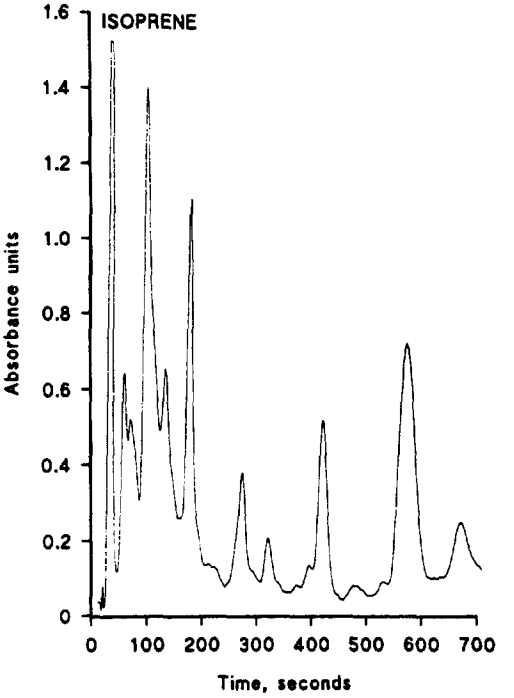

Fig. 3. Gas chromatogram obtained from analysis of human breath by thermal desorption and diode-array UV detection operating at a wavelength of 195.0 nm. Note the presence of other endogenous volatiles also absorbing radiation at this wavelength.

control experiments, isoprene could not be detected.

3. Results

Fig. 3 shows a chromatogram obtained from analyzing human expired air by thermal desorption GC with the UV detector set at a wavelength of 195 nm. Besides a prominent peak corresponding to isoprene, several other endogenous breath volatiles were seen at this wavelength. Fig. 4 shows the same gas chromatogram monitored at a higher wavelength (215.4 nm) which corresponds to the UV absorption maximum for isoprene. The presence of the other breath volatiles was now much less noticeable, and quantitative analysis of isoprene was possible with high selectivity. To identify unequivocally the presence of isoprene in breath, we compared the absorption spectrum obtained from the biological sample with the spectrum obtained from analysis of an authentic isoprene standard. As shown in Fig. 5, the agreement between the two spectra between 180 and 280 nm was excellent. To avoid an overlap, the ordinate scale was displaced slightly when preparing this figure.

Fig. 6 demonstrates the time-to-time biological variation in breath isoprene concentration for nine separate exhalations from one healthy male subject. The mean, S.D. and coefficient of variation (C.V.) were 3.69 nmol/l, 0.60 nmol/l, and 16%, respectively. Both the analytical and intra-individual variations are included in this C.V. of 16% which can be compared with a C.V. of 2.3% when eight consecutive samples were analyzed from one single exhalation. The mean concentration of isoprene in breath samples collected from sixteen healthy individuals was 3.73 nmol/l (S.D. = 1.86 nmol/l) and the median concentration was 3.36 nmol/l (range 1.60–10.33). No statistically significant differences in concentration of breath isoprene were evident between men and women (Table 1).

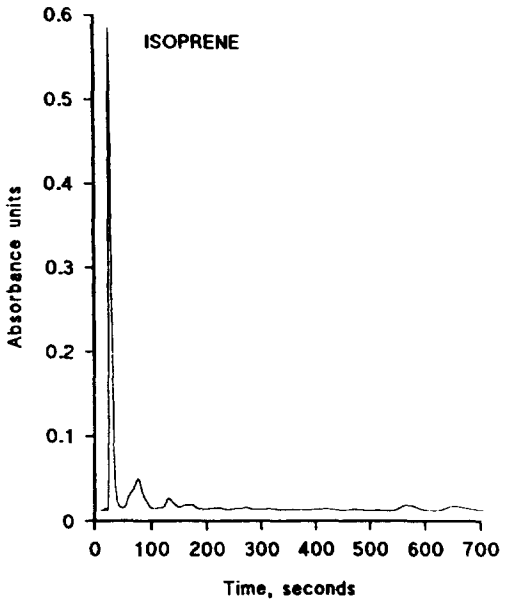

Fig. 4. Gas chromatogram obtained from analysis of human breath by thermal desorption and diode-array UV detection operating at a wavelength of 215.4 nm which corresponds to the absorption peak maximum for isoprene.

4. Discussion

The presence of isoprene in human expired air has been investigated by several research groups after the first report by Jansson and Larsson in 1969 [1]. Current interest in breath isoprene is associated with studies of other volatile hydrocarbons in breath such as ethane and *n*-pentane, compounds originating from lipid peroxidation [13]. Most analytical methods for measuring isoprene as well as other volatile hydrocarbons make use of GC with packed columns and flame ionization detection [8–10]. Occasionally, the presence of isoprene has been confirmed by gas chromatography–mass spectrometry (GC–MS) [14–16]. Many of these early reports dealing with the analysis of volatile hydrocarbons in breath were considered suspect because breath isoprene was not well resolved from breath pentane with the chromatographic systems used [16].

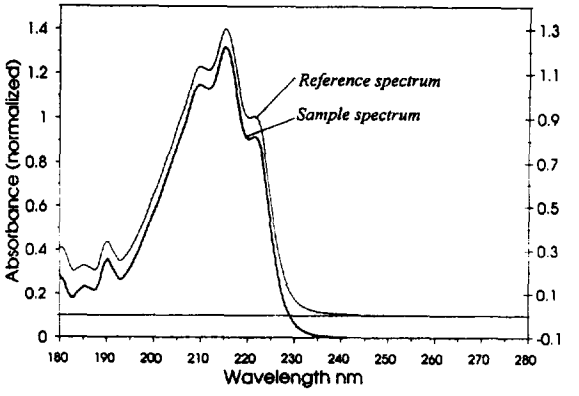

Fig. 5. Comparison of the UV absorption spectra of endogenous breath isoprene (sample spectrum) with an isoprene reference substance (reference spectrum). The ordinate scale for the reference spectra was shifted for clarity.

GC–MS is a well established method for the analysis of volatile organic compounds in the expired air and this technique combines good sensitivity with high specificity [15,17]. The GC–UV absorption method described here represents a new approach for biochemical analysis of organic volatile substances and this combination of technology is not yet commercially available. The gas-phase UV spectrum represents a fingerprint of the molecule and it is possible to distinguish and identify structural isomers with this method [12]. Unequivocal identification of isoprene was possible by matching the UV absorption spectrum of the biological sample with an authentic isoprene standard. Moreover, with our method of analysis, breath isoprene cannot be confused with breath pentane because saturated hydrocarbons such as methane, ethane and *n*-pentane do not absorb UV radiation in the same range of wavelengths as isoprene.

Different investigators have reported widely different concentrations of isoprene in human breath. Mendis et al. [17] suggested that these discrepancies can be attributed to the kind of methodology being used and the standardization procedure used for making quantitative measurements. The method of sampling breath is an important variable when endogenous volatiles are analyzed and the phase of exhalation captured should be carefully considered. We collected mixed expired air and not alveolar air or breath samples obtained after a rebreathing manoeuvre. In a recent study, the mean breath isoprene concentration was reported as 7.05 nmol/l (S.D. = 3.53 nmol/l) when GC was used

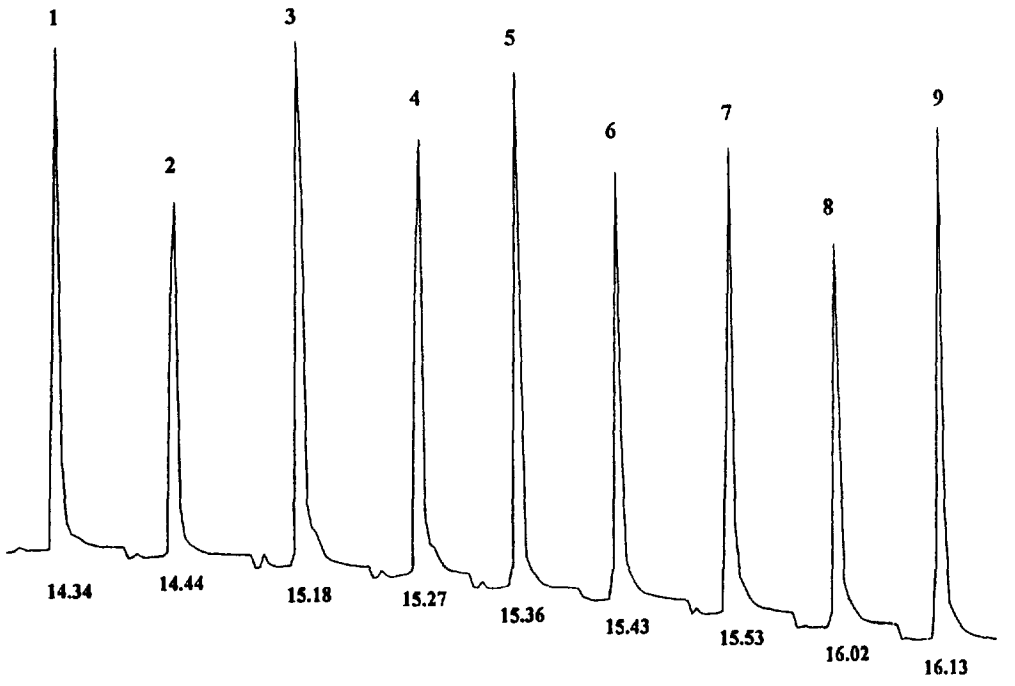

Fig. 6. Intra-individual variations in the concentration of breath isoprene as demonstrated by nine consecutive tests in one subject over about 100 min. The times of sampling breath are marked on the plot.

for analysis [17]. However, Kohlmüller and Kochen [13] criticized many of the earlier reports dealing with the analysis of volatile hydrocarbons exhaled in breath because of a failure to distinguish between *n*-pentane and isoprene. These hydrocarbons were thought to co-elute under the GC conditions used in some early studies. With improved GC separation conditions the concentration of isoprene in breath ranged from 0.27 to 3.13 nmol/l, being considerably lower than values previously reported [13]. The concentrations of *n*-pentane in breath were between six and fifty times less than the concentration of isoprene [13].

Several research groups have shown that breath isoprene concentrations exhibit a circadian variation, seemingly depending on the state of sleep and wakefulness [16–18]. In fifty fully awake healthy subjects, the concentrations of breath isoprene were 14.6 ± 6.4 nmol/l [19], being much less than levels observed in the same individuals immediately after awaking. DeMaster

Table 1
Variations in the concentration of isoprene in human breath in healthy male and female subjects

Test subjects	*n*	Concentration (mean ± S.D.) (nmol/l)	Coefficient of variation (%)	Span (nmol/l)
Males	6	3.46 ± 0.84[a]	24	2.42–4.65
Females	10	3.89 ± 2.43	62	1.60–10.3

[a] No statistically significant differences between breath isoprene concentration in men and women according to the Mann–Whitney *U*-test.

and Nagasawa [6] confirmed this diurnal variation in the excretion pattern of breath isoprene but the concentrations they reported (mean 28 nmol/l) were considerably higher than those reported by Cailleux and Allain [19].

A link between breath isoprene and cholesterol biosynthesis was suggested by Stone et al. [8] who showed that isoprene levels dropped appreciably when subjects were treated with lovastatin, a well known inhibitor of sterol biosynthesis. Feeding a cholesterol-rich diet for several weeks lowered the concentration of isoprene in breath and this was thought to reflect an altered rate of synthesis of this sterol. Whether breath isoprene is generated locally in the lungs and upper airways or reflects the concentration in pulmonary blood remains to be elucidated. The blood–air partition coefficient for this volatile hydrocarbon and correlation and comparison between breath and blood isoprene concentrations do not seem to have been published.

The biosynthesis, turnover and excretion pattern of isoprene might reflect some fundamental biochemical process that still remains to be explored. Isoprenylation of proteins is a recently discovered mechanism in the biosynthesis of cholesterol by the mevalonate pathway, and this reaction appears to be involved in membrane binding of a number of proteins [20,21]. In this context, the fact that isoprene is the most abundant biogenic hydrocarbon exhaled in human breath should not be overlooked.

Acknowledgement

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