Talanta 160 (2016) 21–27

Contents lists available at ScienceDirect

Talanta

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

Headspace-programmed temperature vaporizer-mass spectrometry and pattern recognition techniques for the analysis of volatiles in saliva samples

Ana Pérez Antón [a], Miguel del Nogal Sánchez [a,*], Ángel Pedro Crisolino Pozas [b], José Luis Pérez Pavón [a], Bernardo Moreno Cordero [a]

[a] Departamento de Química Analítica, Nutrición y Bromatología, Facultad de Ciencias Químicas, Universidad de Salamanca, 37008 Salamanca, Spain
[b] Servicio de Medicina Interna, Hospital Virgen de la Vega, Complejo Asistencial Universitario de Salamanca, 37007 Salamanca, Spain

ARTICLE INFO

Article history:
Received 26 April 2016
Received in revised form
24 June 2016
Accepted 28 June 2016
Available online 29 June 2016

Keywords:
HS-PTV-MS
Volatile biomarkers
Pattern recognition techniques
Saliva

ABSTRACT

A rapid method for the analysis of volatiles in saliva samples is proposed. The method is based on direct coupling of three components: a headspace sampler (HS), a programmable temperature vaporizer (PTV) and a quadrupole mass spectrometer (qMS).

Several applications in the biomedical field have been proposed with electronic noses based on different sensors. However, few contributions have been developed using a mass spectrometry-based electronic nose in this field up to date.

Samples of 23 patients with some type of cancer and 32 healthy volunteers were analyzed with HS-PTV-MS and the profile signals obtained were subjected to pattern recognition techniques with the aim of studying the possibilities of the methodology to differentiate patients with cancer from healthy controls. An initial inspection of the contained information in the data by means of principal components analysis (PCA) revealed a complex situation were an overlapped distribution of samples in the score plot was visualized instead of two groups of separated samples. Models using K-nearest neighbors (KNN) and Soft Independent Modeling of Class Analogy (SIMCA) showed poor discrimination, specially using SIMCA where a small distance between classes was obtained and no satisfactory results in the classification of the external validation samples were achieved. Good results were obtained when Mahalanobis discriminant analysis (DA) and support vector machines (SVM) were used obtaining 2 (false positives) and 0 samples misclassified in the external validation set, respectively. No false negatives were found using these techniques.

1. Introduction

According to the American Cancer Society guidelines [1], screening methods for the detection of cancer at the early stage satisfactorily influences the survival of patients. Methods such as radiography, low dose spiral computer tomography, magnetic resonance, positron emission tomography and fluorescence bronchoscopy are used to increase survival nowadays. However, they could be sometimes unsuitable to be applied in a wide-range population due to the side effects on health related to radiation exposure. In addition, it is not sufficiently cost-effective for large-scale screening purposes.

Analysis of volatile organic compounds (VOCs) in matrixes such as saliva [2,3], urine [4,5] and breath air [6–8] is gaining interest in the last years since the odor inspection can provide information about the state of health of an individual. Some investigations showed the ability of canine olfaction to discriminate patients suffering different types of cancer from healthy individuals [9,10].

Electronic noses are an attractive alternative for the analysis of volatile compounds by evaluating the total chemical profile of a sample rather than detecting each compound individually. Significant effort has been made in the development of electronic noses for biomedical application due to their potential in rapid detection and odor characterization. Their use in cancer detection is basically the adaptation of an ancient practice in medicine [11]. Some examples are those concerned with rotten stink of a lung abscess or the fishy odor of liver illness. Since Dodd and Persaud introduced the first electronic nose (1982) using a metal oxide sensor [12], several devices based on conducting polymer-based sensor and optical and piezoelectric sensors have been used to solve many problems satisfactorily in fields such as the medical and diagnostic [13–18], food [19,20], environmental and security

* Corresponding author.
E-mail address: mns@usal.es (M. del Nogal Sánchez).

http://dx.doi.org/10.1016/j.talanta.2016.06.061
0039-9140/

[21,22], among others. In addition, artificial olfactory sensors based on biomaterials such as mammalian cells or proteins have been developed to mimic the human olfactory system [23].

Mass spectrometry-based electronic nose has been also used to solve many problems successfully in fields such as food and environmental [24–27] but it has been hardly used in the biomedical field despite its advantages: higher number of sensor in each analysis, no poising effect and stability of the signal. To date there is only two contributions [28,29] using this approach for the semi-quantitative determination of volatile biomarkers related mainly to lung cancer. In both cases, methods for the determination of individual compounds were developed based on PLS models. In one of them [29] a PTV is incorporated between the HS and the qMS to improve the sensitivity. This new approach in the non-separative analysis allows reaching detection limits in the low ppb range which are typical in biological samples from patients with lung cancer [30]. To the best of our knowledge, no contribution using pattern recognition of HS-PTV-MS profile signals and focusing on the entire volatile portion of the sample instead of individual compounds has been developed up to date to check the possibilities of the methodology for the discrimination between patients and healthy controls.

Beside mass spectrometry-based electronic nose, other sensitive methods have been used for the monitoring of biomarkers such as ion mobility spectrometry (IMS) [31,33], proton transfer reaction mass spectrometry (PTR-MS) [32,33] and selected ion flow tube mass spectrometry (SIFT-MS) [4,33].

Here we propose a rapid and simple method for the analysis of the profile signals corresponding to saliva samples of patients with cancer and healthy controls using an HS-PTV-MS electronic nose and pattern recognition techniques. Identification of the analytes present in the headspace which could be responsible of the separation between groups is beyond the scope of the work.

2. Material and methods

2.1. Samples

500 μL of unstimulated saliva samples were obtained from 55 adults of both sexes and placed directly into a 10.0 mL vial sealed with Teflon®/silicone septum caps (Agilent Technologies, DE, Germany). The saliva samples were collected and analyzed on the same day. Samples 1–23 were from patients at the Internal Medicine Unit of the Virgen de la Vega Hospital in Salamanca; samples 24–55 were from healthy volunteers apparently unaffected by diseases. Table 1 shows a general overview of the studied patients. The study was authorized by the Ethics Committee Hospital.

Table 1
General overview of the studied patients.

Sample	Pathology
1	Metastatic undifferentiated non-small cell lung cancer
2	Metastatic non-small cell lung cancer (adenocarcinoma)
3	Metastatic infiltrating urothelial carcinoma
4	Metastatic undifferentiated sarcoma
5	Metastatic non-small cell lung cancer (adenocarcinoma)
6	Metastatic pancreatic adenocarcinoma
7	Metastatic non-small cell lung cancer (squamous cell)
8	Located lung cancer (squamous cell)
9	Metastatic non-small cell lung cancer (adenocarcinoma)
10	Metastatic melanoma
11	Metastatic non-small cell lung cancer (adenocarcinoma)
12	Metastatic small cell lung cancer
13	Metastatic infiltrating urothelial carcinoma
14	Metastatic non-small cell lung cancer (adenocarcinoma)
15	Metastatic undifferentiated non-small cell lung cancer
16	Metastatic non-small cell lung cancer (squamous cell)
17	Metastatic multicenter hepatocellular carcinoma
18	Metastatic pancreatic adenocarcinoma
19	Metastatic non-small cell lung cancer (adenocarcinoma)
20	Metastatic non-small cell lung cancer (adenocarcinoma)
21	Located lung cancer (squamous cell)
22	Metastatic melanoma
23	Non-metastatic infiltrating ductal breast carcinoma

2.2. HS-PTV/MS measurements

HS sampling was performed with a PAL autosampler (CTC Analytics AG, Zwingen, Switzerland). The oven temperature and the equilibration time were adjusted at 70 °C and 10 min, respectively. During this time, the vials were shaken at 750 rpm. A 2.5 mL syringe at 120 °C was used.

A programmed temperature vaporizer (PTV) inlet (CIS-4: Gerstel, Baltimore, MD, USA), in the solvent-vent injection mode was employed. A liner (71 mm × 2 mm) packed with Tenax-TA® was used. In the first step, the sample from the headspace was injected into the PTV. During this step, the split valve is open, allowing solvent elimination. The solvent vent temperature and the vent flow were adjusted to 25 °C and 50 mL/min, respectively. A venting pressure to 5.0 psi (34,474 Pa) was imposed. The purge time was set at 0.1 min, after which the splitless mode was programed for 1.5 min while the temperature was increased by 12 °C/s to 250 °C. In this step, the analytes were desorbed from the liner and transferred to the column. Finally, the split valve was opened again to clean the system (purge flow 150 mL/min) and the liner temperature was held at 250 °C (0.5 min). Cooling was accomplished with liquid CO_2.

The interface between the PTV and the MS was a low-polarity DB-VRX capillary column (20 m × 0.18 mm × 1 μm) from Agilent J&W, which was maintained at 240 °C in an Agilent 6890 GC device along the time of analysis. In this way, the separation capacity of the column was removed and it behaved as a simple transfer line from the PTV to the mass detector.

The detector was a quadrupole mass spectrometer (HP 5973N) equipped with an inert ion source. It was operated in the electron-ionization mode using an ionization voltage of 70 eV. The ion source temperature was 230 °C, and the quadrupole was set at 150 °C. The analyses were performed in scan mode (3.46 scan/s). The *m/z* range was 25–125 amu. The following *m/z* ratios were removed from the profile signal since they are typical from gases in the headspace, N_2, O_2, Ar, CO and CO_2, which do not come from the sample and mask the minor components: 28, 32, 40 and 44.

The signal-recording time was 2.5 min. An interval of 6.0 min between sample injections was chosen in order to allow the PTV to cool from the final (250 °C) to the initial temperature (25 °C).

2.3. Data analysis

Data collection was performed with an Enhanced ChemStation [34] from Agilent Technologies. Pattern recognition techniques were performed using the Unscrambler® v10.2 statistical package [35] and the Pirouette 3.11 software from Infometrix Inc. (Woodinville, WA) [36].

3. Results and discussion

3.1. Study of the signals obtained

The analytical signal used was the mass spectrum of each sample which represents the sum of the intensities of all the ions detected during the data acquisition time. Fig. 1 shows the comparison between the average mass spectrum of all the patients and