Urinary volatile fingerprint based on mass spectrometry for the discrimination of patients with lung cancer and controls

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A B S T R A C T

Profile signals of urine samples corresponding to patients with lung cancer and controls were obtained using a non-separative methodology. The method is based on the coupling of a headspace sampler, a programed temperature vaporizer and a mass spectrometer (HS-PTV-MS). With only a centrifugation step as prior sample treatment, the samples were subjected to the headspace generation process and the volatiles generated were introduced into the PTV where they were trapped in the Tenax® packed liner while the solvent was purged. Finally, the analytes were introduced directly, without separation, into the mass spectrometer which allows obtaining the fingerprint of the analyzed sample. The mass spectrum corresponding to the mass/charge ratios (m/z) ranging between 35 and 120 amu (amu) contains the information related to the composition of the headspace and is used as the analytical signal for the characterization of the samples.

Samples of 14 patients with some type of cancer and 24 healthy volunteers were analyzed and the profile signals were subjected to different chemometric techniques, including support vector machines (SVM), linear discriminant analysis (LDA) and partial least squares- discriminant analysis (PLS-DA), with the aim of differentiating the samples of patients with cancer from those of control. Values of 100% were obtained both in sensitivity and specificity in most cases.

This methodology has been used previously, as described later, for the analysis of the fingerprint corresponding to saliva samples of patients and controls. However, up to date, the method has not been used in urine samples with the aim of fast discrimination between patients with cancer and controls. The advantages and disadvantages of using urine versus other types of matrices such as saliva are stated.

In view of the results obtained in this work, the use of pattern recognition techniques with data corresponding to HS-PTV-MS profile signals is highly suitable as a first screening step to differentiate samples. In addition, it could be applied to a high number of samples in a relatively short period of time due to its high throughput.

1. Introduction

The work developed by Paul et al. [1] on the detection of volatile organic compounds (VOCs) in human breath opened a new scientific field in the analysis of certain analytes for clinical diagnosis. Presence of biomarkers in body fluids, such as saliva, urine, breath or sweat, can provide important information about the state of health of an individual since their occurrence is mainly related with oxidative stress [2]. Therefore, biomarkers are compounds whose presence or absence is associated to a certain disease or whose concentration level differentiates between healthy and diseased individuals.

Gas chromatography coupled to mass spectrometry is the preferred method for the chemical analysis of volatile biomarkers in biological fluids [3–6]. In addition to the above, other techniques such as liquid chromatography [7] and capillary electrophoresis [8] have been also used. However, chromatographic procedures are generally slow and time consuming. As an alternative to solve these drawbacks several screening methods have been developed. An appropriate screening method must be rapid, reliable, easily accessible to a large number of patients, non-invasive, inexpensive and it must provide the diagnosis of an early stage cancer [9]. Early detection can improve outcomes and reduce mortality, sequelae, complications, and costs associated with health care [10]. Many screening methods including low dose spiral computer tomography, radiography and positron emission tomography

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are used in hospitals every day. However, they are not sufficiently cost-effective for large scale screening purposes and can be sometimes inappropriate to be applied in a wide-range population due to the side effects related to radiation exposure.

The development of analytical non-separative methods is of great interest due to their fast analysis speed. In addition, they usually use non-invasive matrices and have no side effect on the individual as in the previous case. Sometimes, to resolve the analytical problem in hand, it is not necessary to separate the individual compounds of the sample and it is enough with a profile signal formed by all the components in the sample. In this context, different techniques based on mass spectrometry, such as membrane inlet mass spectrometry (MIMS) [11,12], proton reaction mass spectrometry (PTR-MS) [13,14], selected ion flow tube mass spectrometry (SIFT-MS) [15,16] and ion mobility mass spectrometry (IM-MS) [17], have been used for bioanalysis. Another alternative is the one known by the name of electronic nose based mass spectrometry (HS-MS). The analysis procedure of an electronic nose based on direct coupling of a headspace sampler (HS) to a mass spectrometer involves the introduction of only the volatile compounds in the sample into the ionization chamber of the mass spectrometer. HS is a very attractive sample treatment technique for the analysis of volatile analytes [18] owing to ease of sample preparation, automation, speed and the absence of interferences from non-volatile compounds in the matrix. In addition, HS can be also considered within the green analytical procedures. Although the most used coupling in electronic noses based on MS is HS-MS, sometimes, to increase sensitivity, an additional step based on the use of programmed temperature vaporizers (HS-PTV-MS) is included. The main advantage of a PTV injector over others is that its use does not involve time-consuming steps. In addition to devices based on mass spectrometry, sensors using a metal oxide semiconductor (MOS) have also provided useful information and fast responses in the detection of a broad range of volatile compounds in biological samples [19–22].

The analytical signal generated by a MS-based electronic nose is known as profile or spectral fingerprint and it is characteristic of all the volatile compounds present in the sample. Generally, the mass spectrum that represents the sum of intensities of all the ions detected during the data-acquisition time is used as analytical signal. Chemometrics tools are used to extract the relevant information from the signal in order to separate it from noise and diverse information [23].

MS-based electronic nose has been used to solve problems in many different fields such as food [24,25], environmental [26,27] and pharmaceutical [28]. To date there are only four contributions in the biomedical field [29–32] using this approach. Three of them are focus on the semiquantitative determination of some volatile biomarkers related mainly to lung cancer in saliva [29,30] and urine [31]. In all three cases partial least square (PLS) regression was used. In the fourth application [32], the profile signals obtained from saliva samples using HS-PTV-MS were subjected to pattern recognition techniques with the aim to differentiate patients with cancer from healthy controls and satisfactory results were obtained.

In this work, urine samples corresponding to patients with lung cancer and controls are analyzed using a method based on HS-PTV-MS and the obtained profile signals are subjected to pattern recognition techniques in order to study the information contained in the fingerprint-type signal and discriminate between both types of samples. As far as we know, this is the first time that this methodology has been applied for the differentiation of patients with cancer from healthy individuals using urine samples and focusing on the entire volatile portion of the sample instead of individual compounds. Identification of the analytes which could be responsible of the separation between groups is beyond the scope of the work [6,31]. Finally, the advantages and disadvantages of using urine versus other types of matrices such as saliva are stated.

Table 1
General overview of the studied samples.

Training set	
Healthy individuals	
Samples 1–13	
Patients with cancer	
Sample	Pathology
25	Metastatic non-small cell lung cancer (adenocarcinoma)
26	Metastatic non-small cell lung cancer (squamous cell)
27	Metastatic non-small cell lung cancer (adenocarcinoma)
28	Metastatic non-small cell lung cancer (squamous cell)
29	Metastatic non-small cell lung cancer (adenocarcinoma)
30	Metastatic small cell lung cancer
31	Metastatic non-small cell lung cancer (adenocarcinoma)
32	Metastatic small cell lung cancer
External validation set	
Healthy individuals	
Samples 14–24	
Patients with cancer	
Patient	Pathology
33	Metastatic non-small cell lung cancer (adenocarcinoma)
34	Located lung cancer (squamous cell)
35	Located lung cancer (squamous cell)
36	Metastatic non-small cell lung cancer (adenocarcinoma)
37	Metastatic non-small cell lung cancer (adenocarcinoma)
38	Metastatic non-small cell lung cancer (squamous cell)

2. Materials and methdos

2.1. Samples

The urine samples used were obtained from 38 adults of both sexes and stored at –20 ºC. Samples nos. 1–24 (14 men, 10 women. Age range 38–75. pH range 5.3–8.0) were from healthy individuals unaffected by diseases; samples nos. 25–38 (12 men, 2 women. Age range 59–85. pH range 5.0–7.9) were from patients of lung cancer at the Internal Medicine Unit of the Virgen de la Vega Hospital in Salamanca. Table 1 shows a general overview of the studied samples. In order to perform the different supervised pattern recognition, the samples from healthy individuals and from patients with cancer were divided in two groups corresponding to a training set and a validation set. The study was authorized by the Ethics Committee Hospital.

For the analyses, the urine samples were thawed at room temperature and then they were centrifuged for 10 min at 5000 rpm in a 15-mL glass centrifuge tube with a screw cap (Scharlau, Spain). After centrifugation, 4.0 mL of each urine and 2.0g of NaCl were placed in a 10-mL headspace vial (Agilent Technologies, DE, Germany) which was sealed with a Teflon®/silicone septum (Agilent Technologies, DE, Germany).

2.2. HS-PTV-MS measurements

The instrumental configuration HS-PTV-MS, the method of analysis and the data acquisition software were equivalent to that described in a previous work [31].

2.3. Data analysis

The mass spectrum, which is the sum of the intensities of all the ions detected during the data acquisition time (35–120 m/z) was the analytical signal used.

A process of internal normalization was applied to the profile signals which consists on the expression of each variable as a percentage of the sum of all the variable values which are the different mass fragments measured.

The set of samples was divided into two groups: training and validation sets. The training set consisted of 13 urine samples (7 men and 6 women) from healthy individuals and 8 urine samples (7 men