Journal of Pharmaceutical and Biomedical Analysis 124 (2016) 149–156

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

Direct screening of tobacco indicators in urine and saliva by Atmospheric Pressure Solid Analysis Probe coupled to quadrupole-time of flight mass spectrometry (ASAP-MS-Q-TOF-)

Daniel Carrizo[a,1], Isabel Nerín[b], Celia Domeño[a], Pilar Alfaro[a], Cristina Nerín[a,*]

[a] Aragon Institute of Engineering Research (I3A), EINA, Department of Analytical Chemistry, University of Zaragoza, María de Luna 3, 50018 Zaragoza, Spain
[b] Smoking Cessation Unit, Department of Medicine, Psychiatry and Dermatology, Faculty of Medicine, University of Zaragoza, Domingo Miral s/n, 50009 Zaragoza, Spain

ARTICLE INFO

Article history:
Received 22 December 2015
Received in revised form 18 February 2016
Accepted 23 February 2016
Available online 26 February 2016

Keywords:
ASAP
Direct analysis
Tobacco exposure
Cancer
Biomarkers
Screening

ABSTRACT

A new screening method has been explored for direct analysis of tobacco smoke biomarkers in biological matrices (i.e., saliva and urine). Single run analysis using Atmospheric pressure Solid Analysis Probe (ASAP) and high resolution mass spectrometry with quadrupole and time of flight detector has been applied directly to some biological samples (i.e., urine and saliva), providing a fast, efficient and sensitive method of identification. The method has been applied to saliva and urine samples from heavy tobacco smokers for exposure studies. Nicotine itself, nicotine metabolites (i.e., cotinine, *trans*-3′-hydroxycotinine, nicotine-*N*-glucuronide) and other related tobacco smoke toxic compounds (i.e., NNK 4-[methyl(nitroso)amino]-1-(3-pyridinyl)-1-butanone, anatabine) were found in the analyzed samples. The identification of compounds was confirmed by ultrahigh performance liquid chromatography with MS-triple quadrupole detector after sample treatment. Different temporal trends and biomarkers behavior have been found in time series related samples. Both methods are compared for screening of these biological matrices.

1. Introduction

Tobacco use is an important cause of early death worldwide, causing a wide range of diseases and many types of cancer. Currently tobacco kills more than five million people and by 2030, the death toll will exceed eight million a year [1]. Recent predictions in China estimate a death toll higher than 2 million people in 2030 just because of tobacco. Cigarette smoke contains over 4000 different compounds, such as nicotine, hydrogen cyanide, carbon monoxide, nitrosamines and polyaromatic hydrocarbons [2]. Tobacco smoking is highly addictive, being nicotine, present in the cigarettes at relatively high concentration, the main responsible for that. Nicotine and other related alkaloids are absorbed in human beings through the skin and the lungs [3]. The primary precursors for the highly carcinogenic tobacco-specific nitrosamines are also at quite high concentration level and all together make these chemicals very important from a public health standpoint [4]. Tobacco also contains polycyclic aromatic hydrocarbons (PAHs), which are probably responsible as well for the cancer development in heavy smokers [5]. Nicotine and its major metabolite cotinine, used as tobacco biomarker, can be found after tobacco exposure in urine, blood and saliva samples [6–8] as well as in other non-conventional biological matrices like hair or meconium [9]. Although the concentration of these biomarkers can be very high in heavy smokers, the environmental and passive exposure to tobacco could be also measured in non-smokers with a sensitive analytical technique. Saliva and urine are important alternative matrices to blood for monitoring tobacco exposure, since collection is simple, non-invasive and can be performed by non-medical personnel. It is worth to emphasize that the assessment of tobacco smoke exposure is a major topic medical science, with important implications in public health and government policies.

A wide variety of analytical techniques have been applied to the analysis of nicotine, cotinine, *trans*-3-hydroxycotinine (3-HC) and related tobacco smoke biomarkers in various biological fluids. Those analytical methods include immunoassays [10,11], gas chromatography (GC) coupled to either flame ionization (FID) [12] or mass spectrometric (MS) detection [8,13,14] and high-performance

* Corresponding author.
E-mail address: cnerin@unizar.es (C. Nerín).
[1] Present address: Institute for Global Food Security, Queen's University, Belfast, United Kingdom.

http://dx.doi.org/10.1016/j.jpba.2016.02.033
0731-7085/

liquid chromatography (LC) coupled either to UV detector [15] or MS [16,17]. All of them have a mandatory extraction step followed by extensive clean-up and fractionation steps prior to instrumental analysis. These steps are tedious, time consuming and expensive, due to the amount and type of chemicals and materials required. Thus the direct analysis of samples without any prior sample treatment is an important advantage for any laboratory performing routine analyses of these types of contaminants. A direct approach for detecting the presence of these compounds without investing time and money in the sample treatment is an attractive option that should be explored in detail. Atmospheric-pressure solid analysis probe (ASAP), is a new method for rapidly analyzing volatile or semi-volatile liquid or solid materials, which has only a few applications reported to date [18–21]. Two ambient mass spectrometry techniques, desorption electrospray ionization (DESI) [22] and the direct analysis in real time (DART) [23] originated the ASAP technique by McEwen in 2005 [18]. An important advantage of ASAP technique is that the whole sample can be introduced into the ionization chamber, instead of only the ionized vapor released by the sample (e.g., DART and DESI). As vaporization and ionization with ASAP occur at atmospheric pressure a mass spectrum can be acquired in seconds from solid and liquid volatile or semi-volatile compounds. The non-volatile compounds which are not volatilized at about 500 °C cannot be analyzed using ASAP, as also occurs with DART and DESI. Thus, the ASAP technique extends the power of the analysis to unknown complex matrices. When ASAP is coupled to the high resolution -Q-TOF-MS technique the accurate mass of the fragments obtained facilitates the identification of the molecular structure of the compounds. This is an important advantage, especially when complex matrices (i.e., saliva or urine) without any prior treatment are involved. The identification of unknown compounds can be reached with the help of specific software tools such as MassLynx and ChemSpider chemical databases.

The aim of this work was to explore a direct method for the screening of nicotine and their major metabolites as well as other highly toxic tobacco biomarkers in biological fluids. The results will be compared to the conventional sample treatment followed by UPLC–MS-TQ. From this study additional biomarkers will be proposed for studying the tobacco exposure and evaluate the risk for consumers. This article represents the first study using direct analysis of this kind of compounds in one single run through ASAP-MS-Q-TOF in biological matrices. This way, fast identification of toxic compounds and new markers from tobacco could be possible.

2. Materials & methods

2.1. Chemicals and reagents

Nicotine (>98%) cotinine (98%), 1-hydroxypyrene (98%), 9-hydroxyphenanthrene (technical grade), ammonium acetate and formic acid (98%), methanol (reagent grade) and acetonitrile (LC–MS quality) were purchased to Sigma (Madrid, Spain). Stock solutions of nicotine and cotinine at a concentration of 1.0 mg/ml, were prepared separately in methanol. Stock solutions of 1-hydroxypyrene and 9-hydroxxphenanthrene at a concentration of 1.0 mg/ml were prepared separately in acetonitrile. All stock solutions were stored at −20 °C until analysis.

2.2. ASAP-Q-TOF-MS analysis

Samples were directly introduced into the ASAP-Q_TOF_MS Xevo G2 QTOF (Waters Corporation, Manchester, UK) dipping a solid glass capillary in the liquid samples. Then, the samples wet the exterior of the glass capillary. Two dips were used for each analysis. Nitrogen was used as a desolvation gas at 450 l h^{-1} flow. No cone flow was needed for this technique. Optimization of key ion source parameters, corona current (μA), sample cone (V) and desolvation gas temperature (°C) were carried out using nicotine as a reference standard. The voltage of the sampling cone was varied from 30 V to 80 V and the voltage of the extraction cone was fixed at 0.1 V. Target samples were analyzed in continuous mode (3 min) with a cone voltage ramp (30–80 V) and desolvation gas temperature ramp (200–500 °C). Atmospheric Pressure Ionization (API) in positive polarity was selected, source temperature was 120 °C. The parameters of the XEVO G2 QTOF were: scan time 1s and the mass range considered was m/z 130–1000. Each sample was analyzed in triplicate. A blank sample was also analyzed under the same experimental conditions.

In addition to the high resolution mass achieved, isotopic ratios (C^{12}/C^{13}, N^{14}/N^{15}, O^{16}/O^{18}) and software tools were used to confirm the target compounds. MassLynx software from Waters was used, which considers the isotopic model and the elemental composition. The first one generates an isotopic model for a specific compound of interest, while the elemental composition gives an idea of an elemental composition, which is a priori known. Another used tool is ChemSpider (www.chemspider.com), which was used to confirm and support the obtained mass spectra.

After the identification, quantitative analysis was performed using saliva and urine spiked samples with two pure standards, 1-PYR and 9-PHE, and analytical features were obtained.

2.3. UPLC–MS/MS analysis

The conventional method was carried out using an Acquity UPLC–MS-TQ (triple quadrupole) system from Waters (Milford, MA, USA). Chromatographic separation was performed on a Waters Acquity UPLC@ BEH C18 (1.7 μm, 2.1 mm × 100 mm) at 28 °C. Samples were filtered by 0,2 μm previously injection. Mobile phases were: eluent A (acetonitrile with 0.3% formic acid) and eluent B (water with 0.3% formic acid). Flow rate was 0.25 ml min^{-1} and injection volume 10 μl. The time program for multi-step gradient was 0–6 min, 35% A—65% B to 60% A—40% B, 6–9 min, 60% A—40% B to 100% A—0% B;, 9–10 min, 100% A—0% B to 35% A—65% B. Run time was 10 min and sample temperature was set at 7 °C.

The MS equipment consisted of a Waters Micromass Quattro Micro™ triple-quadrupole system (Manchester, UK). The MS system was controlled by MassLynx Software, Version 4.0. The APCI+ (positive Atmospheric Pressure Chemical Ionization) interface consisted of a heated nebulizer probe and a standard atmospheric pressure source equipped with a corona discharge pin. The source and probe temperatures were set to 100 °C and 550 °C, respectively. The corona current was 6.0 μA; the cone voltage was 35 V; the extractor voltage was 5 V, and the RF lens voltage was set to 0.1 V. The desolvation and cone flow gases were 600 and 40 l/h, respectively. Analysis was performed in selected ion recording (MRM), selected m/z was 194.23 with a transition to m/z 165.34 for 9-phenantrol and for 1-hydroxypyrene m/z 218.25 with a transition to m/z 189.333.

The analytical features included intra-day precision, dynamic range and accuracy for quantitative purposes. A representative family of analytes (i.e., hydroxy-PAHs), thus, two hydroxy-PAHs, as representative compounds, 9-phenantrol (9-PHE) and 1-hydroxypyrene (1-PYR) were used.

Calibration curves were obtained by plotting the experimental concentration of the 9-PHE and 1-PYR against the theoretical concentration of each compound, using a least-square regression. The limit of detection (LOD) was determined as the concentration corresponding to a peak height that was three times the baseline noise. A 10:1 ratio of peak height to baseline noise was used to determine the limit of quantification (LOQ).