Talanta 184 (2018) 42–49

Contents lists available at ScienceDirect

Talanta

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

Determination of eight carbonyl compounds in aerosols trapped in phosphate buffer saline solutions to support *in vitro* assessment studies

Roberto Buratto, Daniela Correia*, Monique Parel, Maude Crenna, Mickaël Bilger, Audrey Debrick

PMI R&D (Part of Philip Morris International Group of Companies), Philip Morris Products S.A., Quai Jeanrenaud 5, CH-2000 Neuchâtel, Switzerland

ARTICLE INFO

Keywords:
LC-MS/MS
Carbonyl compounds
Aerosol
Tobacco Heating System 2.2
In vitro assessment
Validation

ABSTRACT

When investigating the toxicological impact of aerosols using *in vitro* systems like cell cultures, it is essential to have a quantitative measurement of the chemicals that the cells are exposed to.

Carbonyl compounds represent an important class of marker compounds for *in vitro* and *in vivo* exposure to different toxicological agents, including cigarette smoke (CS). A new LC-MS/MS method that quantifies eight of these analytes in aerosols trapped in phosphate-buffered saline solutions has been developed to measure exposure. During the method development phase, particular attention has been paid to the efficient derivatization of the target compounds in the trapped aerosols and to avoid the formation of poly-derivatized molecules, which could lead to inaccurate quantifications.

The method has been successively validated using the accuracy profile procedure. Selectivity, detection limits, precision, and accuracy have been evaluated for Vitrocell®, Gas Vapor Phase (GVP), and Whole Smoke (WS) matrices of smoke generated by 3R4F cigarettes and aerosol generated by the Tobacco Heating System (THS) 2.2, a heat-not-burn tobacco product developed by Philip Morris International (Smith et al., 2016) [1]. Validation results confirmed that the established working ranges also allow the analysis of THS aerosols, where the concentrations of carbonyl compounds are substantially lower than those generated by 3R4F cigarettes. Moreover, data gathered on 3R4F aerosol samples trapped with DNPH in acetonitrile solutions have been compared to the quantification given by an in-house UHPLC-MS/MS and reference values from the literature.

1. Introduction

Cigarette smoke contains more than 6000 compounds [2], of which 93 are classified as harmful and potentially harmful components (HPHC) by Food and Drug Administration (FDA) [3]. The chemical constituents of smoke can be split in two main components: the nicotine free dry particulate material and the gas phase. Carbonyl compounds represent an important category of chemicals in the gas phase. Stable, hydrophilic, chemically reactive, these compounds increase oxidative stress and oxidative damage [4], and they contribute to carbonylation of proteins with consequent protein unfolding and loss of function [5]. It has been reported that chronic exposure to pure carbonyl compounds, such as formaldehyde, augments the risk of asthma and cancer [6,7].

In this context, therefore, the quantification of these compounds in Cigarette smoke (CS) is extremely important. Nevertheless, their high volatility makes these harmful constituents difficult to analyze. The most common approach is to prepare stable derivatives by collecting the CS into acidic solutions of 2,4-dinitrophenylhydrazine (DNPH) [8] or using acidified DNPH-coated cartridges [9]. In acidic conditions, the derivatization reaction produces the corresponding 2,4-dinitrophenylhydrazones, which can be analyzed by GC or HPLC via UV [9] or mass spectrometry detection [8,10–12]. In the case of unsaturated aldehydes such as acrolein and crotonaldehyde, the derivatization reaction might produce bi- and tri-derivatized adducts [11,13]; in order

Abbreviations: LOD, limit of detection; LLOQ, lower limit of quantification; THS, Tobacco Heating System; FDA, Food and Drug Administration; DNPH, 2,4-dinitrophenylhydrazine; HPHC, Harmful and potentially harmful components; HPLC, High Performance Liquid Chromatography; GC, Gas Chromatography; PFBOA, O-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine; PBS, Phosphate Buffered Saline; MEK, Methyl-Ethyl-Ketone; ACN, acetonitrile; HC, Health Canada; GVP, Gas Vapor Phase; CFP, Cambridge Filter Pad; WS, Whole Smoke; VT, Vitrocell; LC, Liquid chromatography; STD, standard; MS, Mass Spectrometry; ICH, International Conference on Harmonization; UPLC, Ultra Performance Liquid Chromatography; LWRL, lower working range limit; UWRL, Upper Working Range Limit; CV_{IP}, Coefficient of Variation for Intermediate Precision; CV_r, Coefficient of Variation for Repeatability; AE, Aerosol extract; Pyr, pyridine; ISO, International Organization for Standardization; CORESTA, Cooperation Centre for Scientific Research Relative to Tobacco; SD, standard deviation; UV, Ultra Violet spectroscopy; CS, cigarette smoke

* Correspondence to: Philip Morris Products S.A., Quai Jeanrenaud 5, 2000 Neuchâtel, Switzerland.
E-mail address: daniela.correia@pmi.com (D. Correia).

https://doi.org/10.1016/j.talanta.2018.02.048
Received 1 June 2017; Received in revised form 11 February 2018; Accepted 12 February 2018
Available online 16 February 2018
0039-9140/

to avoid this side reaction, collection can be performed separately from DNPH derivatization [9] or pyridine can be added [12] to quench the poly-derivatization reaction.

When *in vitro* studies are performed to evaluate the effect of aerosol carbonyl constituents on cell cultures, levels of exposure should be determined directly in a cell-compatible medium. In this case, the aerosol constituents are trapped in a phosphate-buffered saline (PBS) solution and the derivatization reaction occurs after the aerosol collection, when an O-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine (PFBOA) [14] or a DNPH solution is added to the PBS trapping solution. When DNPH derivatization is chosen, the pH of the PBS trapping solution must be adjusted to match the ideal pH conditions for cell growth. Therefore, the kinetics of derivatization is not the same as when the reaction occurs directly in the DNPH trapping solution.

Here we report a new analytical method that has been developed to quantify eight carbonyl compounds, namely formaldehyde, acetaldehyde, acetone, acrolein, propionaldehyde, crotonaldehyde, Methyl-Ethyl-Ketone (MEK), and butyraldehyde in aerosols trapped in PBS solutions. Indeed, these compounds represent the main carbonyls present in the lists of HPHC, provided by regulatory bodies such as the FDA [3] and by the Health Canada [15]. During the study particular attention has been paid to the kinetics of derivatization of carbonyls compounds trapped in this type of solution, in order to optimize the time allowed for the derivatization reaction. Moreover, the kinetics of formation of the poly-derivatized adducts during the verification of stock standard solutions has been investigated to minimize the poly-derivatization of unsaturated aldehydes as acrolein and crotonaldehyde. The method has been validated over wide ranges of concentrations and has allowed the quantification of the carbonyl compounds also in aerosols generated by the Tobacco Heating System 2.2 (THS 2.2) [1,16,17], where the content of these harmful molecules is substantially reduced compared to smoke from cigarettes [18]. Moreover, this methodology has been compared with existing methods for the determination of the eight target compounds in aerosols trapped with DNPH in acetonitrile (ACN), showing a good agreement between different methods.

In conclusion, our results highlight a new method for the carbonyl quantification that has been successively developed and validated not only in CS but also in aerosol generated by THS 2.2, which is known to contain a minimal amount of these compounds [18].

2. Materials and methods

This section describes the final setup of the validated method. Development and validation tests are described in section “Results and discussion”.

2.1. Materials

The reference cigarettes (3R4F) were obtained from the University of Kentucky (Lexington, KY, USA; www.ca.uky.edu/refcig) and were conditioned for at least 48 h under controlled conditions of 22 ± 1 °C and relative humidity of 60 ± 3% before to be used for smoke generation, on accordance with ISO standard 3402 [19]; THS items were conditioned as for 3R4F cigarettes.

Dulbecco's Phosphate Buffered saline solution (modified, without calcium chloride and magnesium chloride, sterile-filtered, suitable for cell culture, Sigma-Aldrich) was used for smoke or aerosol trapping. 2,4-dinitrophenylhydrazine (DNPH, > 90.0% purity, Acros Organic) was used for the preparation of the 15 mM DNPH solution in acetonitrile (ACN, > 99.9% purity, Fluka), acidified with perchloric acid (60%, Sigma-Aldrich).

Standard solutions were prepared using formaldehyde-DNPH (99.9% purity), acetaldehyde-DNPH (99% purity), acetone-DNPH (99.9% purity), acrolein-DNPH (98% purity), propionaldehyde-DNPH (99.9% purity), crotonaldehyde-DNPH (99.9% purity), MEK-DNPH (99% purity) and butyraldehyde-DNPH (99.9% purity) from Sigma-Aldrich. The internal standard solution was prepared using acetone-d6 (purity 99%, isotopic purity 99.85%), MEK-d5 (purity 99%, isotopic purity 98%) and propionaldehyde-d2 (purity 99%, isotopic purity 98%) from Sigma-Aldrich, previously derivatized with DNPH (> 90.0% purity, Acros Organic). Pyridine (> 90% purity, Sigma-Aldrich) was used to quench the DNPH-derivatization reaction during aerosol samples and internal standard solution testing procedure. Acetonitrile (ACN, > 99.9% purity, Fluka), water (H_2O, > 99.9% purity, Fluka), acetic acid (> 99.7% purity, Sigma-Aldrich) and ammonium acetate (> 98% purity, Fluka) were used for mobile phase preparation.

2.2. Aerosol generation and sample measurement solutions preparation

Smoke from 3R4F cigarettes and aerosol from THS items were generated under Health Canada (HC) regimen conditions [20], using a 30-port carousel smoking machine. THS aerosols were generated by a modified version of this machine, where THS items are puffed through THS 2.2 devices integrated into the system.

The aerosol trapping was performed in absence of cell cultures, with the smoking machine running under the same configuration used for *in vitro* experiments. Different types of aerosol fractions [21] were trapped in PBS solutions: for WS type of samples, the undiluted aerosol was trapped in a bottle filled with phosphate buffer saline solution; for GVP type of samples, the particulate phase from the undiluted aerosol was trapped on a glass fiber Cambridge Filter Pad (CFP), while the GVP was trapped in a bottle filled with phosphate buffer saline solution placed after the filter (CFP is discarded after the aerosol collection); for Vitrocell® (VT) type of samples, the whole aerosol was delivered to the Vitrocell® 24/48 system [22] by applying different levels of aerosol dilution.

For 3R4F cigarettes, GVP and WS fractions of the smoke generated by 10 items were accumulated and collected in 36 mL of PBS, while VT smoke collections were obtained using 18.5 mL of PBS, 10 items and with a 69% smoke dilution. For THS items, GVP and WS fraction of the aerosol generated by 15 items were accumulated and collected in 25 mL of PBS, while VT aerosol collections were obtained using 18.5 mL of PBS and 10 items. In total, five aerosol collections were gathered for GVP and WS matrices, while six aerosol collection were gathered for VT matrix.

Right at the end of the process, aerosol extracts (AE) were prepared by mixing 8 mL of the aerosol collection with 12 mL of a 15 mM DNPH, 30 mM $HClO_4$ solution, in order to derivatize the trapped carbonyl compounds. After 30 min, 1 mL of pyridine was added to quench the derivatization reaction and avoid the formation of poly-derivatized carbonyl adducts.

Before injection, GVP and WS aerosol extracts generated by 3R4F cigarettes were diluted 10 times using a DNPH/PBS/Pyr solution (refer to following paragraph for details), while GVP and WS aerosol extracts generated by THS items were diluted 2 times with the same solution. Aerosol measurement solutions were then prepared by mixing 200 µL of diluted or undiluted AE with 300 µL of water and 700 µL of internal standard solution (0.2 µg/mL acetone-d6-DNPH, 0.2 µg/mL propionaldehyde-d2-DNPH, and 0.2 µg/mL MEK-d5-DNPH in ACN) in a LC-vial.

2.3. Standard measurement solutions preparation

Individual 675 µg/mL formaldehyde-DNPH, 1159 µg/mL acetaldehyde-DNPH, 879 µg/mL acetone-DNPH, 265 µg/mL acrolein-DNPH, 339 µg/mL propionaldehyde-DNPH, 462 µg/mL crotonaldehyde-DNPH, 411 µg/mL MEK-DNPH and 331 µg/mL butyraldehyde-DNPH stock solutions were prepared by dissolution of the compounds in ACN.

The working solution A was prepared by combination and dilution of the individual stock solutions in a DNPH/PBS/Pyr solution. The latter consists of a mixture of 57% of a 15 mM DNPH, 30 mM $HClO_4$