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Headspace solid-phase microextraction coupled to comprehensive two-dimensional gas chromatography–time-of-flight mass spectrometry for the analysis of aerosol from tobacco heating product☆

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ABSTRACT

A method involving headspace solid-phase microextraction (HS-SPME) and comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC × GC-TOFMS) was developed and optimised to elucidate the volatile composition of the particulate phase fraction of aerosol produced by tobacco heating products (THPs). Three SPME fiber types were studied in terms of extraction capacity and precision measurements. Divinylbenzene polydimethylsiloxane appeared as the most efficient coating for these measurements. A central composite design of experiment was utilised for the optimization of the extraction conditions. Qualitative and semi-quantitative analysis of the headspace above THP aerosol condensate was carried out using optimised extraction conditions. Semi-quantitative analyses of detected constituents were performed by assuming that their relative response factors to the closest internal standard ($^{i}t_R$) were equal to 1. Using deconvoluted mass spectral data (library similarity and reverse match >750) and linear retention indices (match window of ±15 index units), 205 peaks were assigned to individual compounds, 82 of which (including 43 substances previously reported to be present in tobacco) have not been reported previously in tobacco aerosol. The major volatile fraction of the headspace contained ketones, alcohols, aldehydes, alicyclic hydrocarbons alkenes, and alkanes. The method was further applied to compare the volatiles from the particulate phase of aerosol composition of THP with that of reference cigarette smoke and showed that the THP produced a less complex chemical mixture. This new method showed good efficiency and precision for the peak areas and peak numbers from the volatile fraction of aerosol particulate phase for both THP and reference cigarettes.

1. Introduction

There is an ongoing interest for the development of new forms of tobacco products for the purpose of tobacco harm reduction. Such new products can vary significantly from regular combustible products and must be properly characterized, especially in terms of aerosol chemical composition. Over a decade ago a novel nicotine product called e-cigarette was introduced [1]. E-cigarettes are battery-powered devices that heat or vaporise a liquid containing nicotine, propylene glycol and/or glycerol and desired flavour blends to produce an aerosol which users inhale [2–4]. As the e-liquid composition is very different from the tobacco blends in a combustible cigarette, e-cigarettes produce aerosols that are less complex than smoke produced from conventional combustible tobacco products [5,6]. More recently, new generations of tobacco heating (heat-not-burn) products (THPs) were introduced [7,8]. Unlike e-cigarettes, the current commercial THPs operate in a number of different ways. One type uses a tobacco stick that looks like a regular cigarette and contains cut tobacco or reconstituted tobacco sheet that is heated inside a battery-powered device at temperatures below those at which pyrolysis occurs [7]. Another type of THP utilizes a charcoal tip that is lit before puffed air is heated

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through the tip and passes through the tobacco rod to release nicotine, added aerosol agent (glycerol), and tobacco flavours [9]. One common feature of all THPs is that the heating temperature should be sufficient to release nicotine and volatile tobacco constituents, but not high enough to initialize extensive pyrolysis or combustion of the tobacco, typically below 350 °C. So far, most of published chemical characterisations of THP aerosol are focused on those toxicants known to regulators based on cigarette smoke; the detailed chemical composition of wider chemical profile of THP aerosol remains unclear as no dedicated analytical methods have yet been developed for this purpose. To provide robust risk assessment of its aerosol, analysis and identification of both volatile organic compounds (VOCs) and other fractions of aerosols produced by THPs is very important, in addition to the identification and quantification of targeted toxicants.

It is well known that tobacco smoke is a dynamic aerosol containing an extremely complex and dynamic mixture made of up to 100,000 of individual compounds [10]. The aerosol produced by heating tobacco in THPs may contain less of pyrolysis and combustion derived substances, however, it may promote the release of those thermally distillable compounds into the stream of aerosol.

The chemical characterization of such a complex mixture using single-dimensional gas chromatography (1D-GC) is highly challenging. Comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC × GC-TOFMS) is known as a powerful tool for the analysis of complex mixtures of VOCs and semi-volatile organic compounds (SVOCs) in various applications [11–13]. The advantage of using GC × GC over 1D-GC is that it offers increased separation capacity due to consecutive separations performed on two different stationary phases. The higher dimensional structure-retention relationships provide class-type analyses and thermal modulation compresses the peak width, increases the signal-to-noise ratio (S/N), and thus enhances the sensitivity. The potential utility of GC × GC-TOFMS for tobacco smoke analysis from hand-rolled cigarettes was reported more than a decade ago [14], and more recently, the efficiency of GC × GC-TOFMS coupled to a dedicated data processing approach has been demonstrated for the characterisation of mainstream cigarette smoke particulate phase (PP) [11].

THP operation temperature does not exceed 350 °C, hence it is anticipated to emit minimal pyrolysis-derived compounds and no combustion-derived substances in their emissions. Being a novel tobacco-product category, thorough chemical characterization of its aerosol composition is necessary. Despite the importance of studying VOCs emitted from THPs, no standardised methods have yet been published for the generation, collection, and analysis of THP aerosols. For conventional combustible cigarettes, various sample preparation techniques, including solvent-filled impinger trains [15], adsorbent materials [16], cold traps, headspace analysis [17], and direct injection of gas sample [18,19] have been developed. Solid phase microextraction (SPME) is a simple, rapid, solvent-free, and sensitive technique for extracting chemicals directly from sample headspace for VOC analysis. The high accumulation capacity of SPME and the ease of automation have made it a technique of choice for measurement of VOCs by GC–MS. In tobacco chemistry research, SPME GC–MS has been applied to the characterisation of cigarette smoke [20], tobacco leaves [21], mainstream cigarette smokes PP [22], and mainstream cigarette smoke VP [23]. Ye [24] reported satisfactory SPME repeatability (precision in the range 2–11%) by analysing mainstream cigarette smoke aerosol from different cigarette types. This was confirmed in the qualitative and quantitative analysis of mainstream cigarette smoke samples [25].

The principal objective of the present study was to develop a HS-SPME GC × GC-TOFMS method for the analysis of volatile and semi-volatile compounds present in the PP of aerosol emitted from heating tobacco. Three different types of SPME fibers were studied prior to the optimisation of SPME extraction conditions using a response-surface design approach. Considering that the extraction efficiency of SPME depends on several factors [26], multivariate methods of optimization, including factorial designs and response-surface methods, have been used to evaluate the main and interactive effects of several variables simultaneously with a reduced number of experiments. Seven selected representative compounds naturally present in THP aerosol were monitored for extraction efficiency. The qualitative and semi-quantitative analysis of THPs was successfully carried out with the optimised method. The proposed method was applied to the comparison of aerosol particulate phase compositions of THPs to reference combustible cigarette.

2. Materials and methods

2.1. Materials and reagents

For particulate phase aerosol sampling, 44 mm glass fiber filter pads (Cambridge filter pads, CFP) were purchased from Borgwaldt KC GmbH (Hamburg, Germany). Saturated alkane standard solution (C_7–C_{30}) and Internal standards (toluene-D8, ethylbenzene-D10, 1, 2-dichlorobenzene-D4 and phenanthrene-D10) were purchased from Sigma-Aldrich (Diegem, Belgium). All reagents were of analytical standard grade with purity >98%. Standard solutions were prepared gravimetrically using methanol (Sigma–Aldrich) and stored at 4 °C. Commercially available SPME fibers in 23-gauge needle sizes suitable for autosampler: 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS), 65 μm polydimethylsiloxane/divinylbenzene (PDMS/DVB) and 85 μm polyacrylate (PA) were purchased from Sigma-Aldrich (Diegem, Belgium). Fibers were conditioned prior to use according to manufacturer's guidelines in a fiber bake-out station (Gerstel, Kortrijk, Belgium). To assure the integrity of results, fiber blanks, instrument blanks and instrument air blank tests were always performed to check for possible carry-over and laboratory cross-contamination. 20 mL headspace vials, PTFE septa magnetic crimp caps and an automated SPME holder were obtained from Gerstel (Kortrijk, Belgium).

2.2. Samples and sample preparation

Typical type of THP devices has been described in the literature [7,9,27]. To demonstrate the principle of heating tobacco rather than burning it, small samples of tobacco can also be heated in a research heating furnace to generate the heated tobacco derived aerosol [28]. A scheme of the THP device, sample, and sampling procedure used in this study is provided in the supplementary data (Fig. S-1). THP samples and Cambridge filter pads were conditioned for at least 48 h at 60% relative air humidity and 22 °C prior to testing [29]. Puffing of the THP was performed using a linear syringe drive system A14 (Borgwaldt KC GmbH, Germany). As no standard puffing regime has been defined for THP so far, all sample collections were conducted according to the Health Canada Intense (HCI) puffing regime for cigarettes that consisted of bell-shaped puffs, each of 55 mL with puff duration of 2 s and with 30 s intervals between puffs [30]. The particulate phase of the aerosol was collected on CFPs that were later divided in four equal pieces placed in separate 20 mL headspace vials. Vials were sealed with a magnetic crimp cap and analysed immediately after. 3R4F research reference cigarettes were obtained from the University of Kentucky College of Agriculture (Kentucky Tobacco Research & Development Centre, USA). 3R4F smoke samples were produced using a Borgwaldt RM20D smoking machine (Brogwaldt KC, GmbH, Germany). The