



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.

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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 nico-

tine 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 (C₇–C₃₀) 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 (Borgwaldt KC, GmbH, Germany). The

generation of such smoke sample and related sample preparation are described in detail elsewhere [11].

2.3. Headspace solid-phase microextraction procedures

The initial phase of SPME fiber selection was based on extraction capacities, expressed as the number of detected analytes (duplicate analysis). Three different SPME fiber coatings, medium polar (DVB/CAR/PDMS, PDMS/DVB) and highly polar (PA), were examined. All fibers were analysed using the following conditions: an incubation time of 1 min at 50 °C and an extraction time of 10 min performed at 50 °C. Fibers were desorbed in a CIS4 Cooled Injection System (Gerstel, Kortrijk, Belgium) using the following temperature program: isothermal period at –20 °C for 0.5 min, a ramp of 12 °C s^{–1} to 250 °C and kept for 2 min. After desorption, fibers were reconditioned for 40 mins according to manufacturer's guidelines to eliminate possible carry-over.

The experimental design for the optimisation of HS-SPME included screening and optimisation to obtain the most efficient extraction conditions for analysis of the volatiles from the PP fraction of the aerosol. A non-regular fractional factorial (FF) 2-level screening design was applied to establish the most relevant factors for HS-SPME factors [31]. The selected factors were particulate phase sample mass (CFP), sample incubation time, sample incubation temperature, sample extraction time, sample extraction temperature, fiber desorption time and fiber desorption temperature. The screening phase consisted of 19 experiments performed in random order. Details of the factors and levels established for each variable for screening experiments are provided in Table S-1. A 2² factorial circumscribed central composite design (CCD) with four axial points ($\alpha = 1.414$) and five central point replicates and response surface methodology [32] was used in order to determine the optimum values for the two most significant factors that emerged from screening experiments. The factors and levels selected for each factor (optimised from the screening design) are listed in Table S-2. Thirteen duplicate experiments were performed in random order. Both screening and CCD designs were generated using the commercial software package 'The Unscrambler® X version 10.3' (CAMO Software AS, Oslo, Norway). Peak area values were used to compare fiber efficiencies. A set of 4 external standards (toluene-D8, ethylbenzene-D10, 1,2-dichlorobenzene-D4, and phenanthrene-D10) was considered prior and after injections series to ensure constant performance of the GC × GC-TOFMS system during the comparison exercise. The liquid standard solution was spiked on the CPFs and SPME fibers were exposed to the headspace.

2.4. Instrumental analysis

The GC × GC-TOFMS system consisted of an Agilent 7890 (Agilent Technologies, Palo Alto, CA, USA) gas chromatograph and a Pegasus 4D TOFMS (LECO Corp., St. Joseph, MI, USA) with quad jet thermal modulator. The first dimension (¹D) column was a low-polarity 5% phenyl polysilphenylene-siloxane phase (Rtx®-5MS; 30 m × 0.25 mm i.d. × 0.25 μm film thickness) connected by means of a SilTite™ μ-Union (SGE International, Victoria, Australia) to a second dimension (²D) midpolarity Crossbond® silarylene phase column which has similar selectivity to 50% phenyl/50% dimethyl polysiloxane (Rxi®-17SilMS; 1.0 m × 0.15 mm i.d. × 0.15 μm film thickness). Both columns were from Restek Corporation (Restek Corp., Bellefonte, PA, USA). A similar column and stationary phase set was successfully used in previous studies of mainstream cigarette smoke PP [11,17]. The ²D column was installed in a separate oven located inside the main GC oven, providing more flexible temperature control. The system was equipped with a Ger-

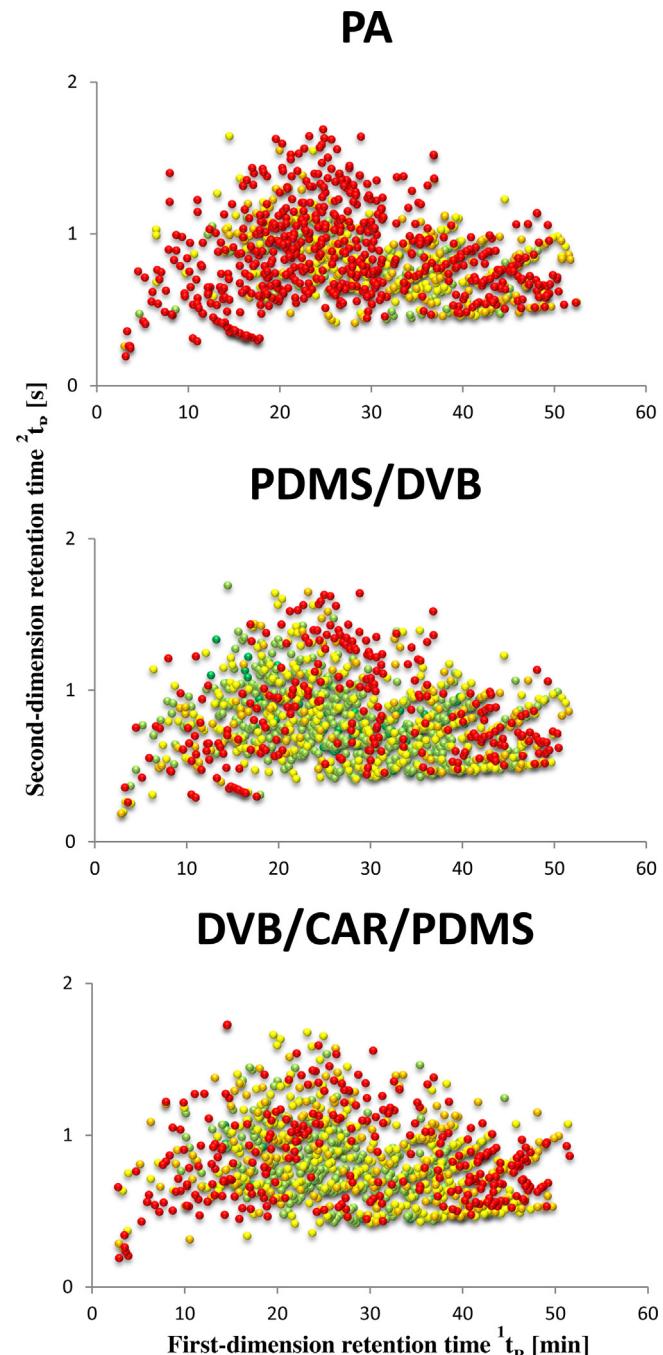


Fig. 1. GC × GC-TOFMS apex plots of THP PP volatiles obtained for three different SPME fibers. Coloured dots are representing %RSD of peak areas: ● <5, ▲ 5–15, △ 15–25, ■ 25–35, ▨ >35. (For interpretation of the references to colour in the text, the reader is referred to the web version of this article.)

stel MultiPurpose Sampler (MPS 2XL), SPME option for procedural automation, and the CIS4 Cooled Injection System. The carrier gas was helium at a corrected constant flow rate of 1 mL min^{–1} and the injector were set to splitless mode. The main oven temperature program comprised an isothermal period at 35 °C for 5 min, a ramp of 4 °C min^{–1} to 250 °C followed by a ramp of 20 °C min^{–1} to 300 °C and a final isothermal period at 300 °C for 1 min. The secondary oven was programmed with a 15 °C offset above the primary oven temperature. Modulation parameters consisted of 2 s modulation period (0.4 s hot pulse and 0.6 s cold pulse time) and a temperature offset of 20 °C above the secondary oven. Mass spectra was acquired in the range *m/z* 40–400 at the acquisition rate of 100 spectra s^{–1}.

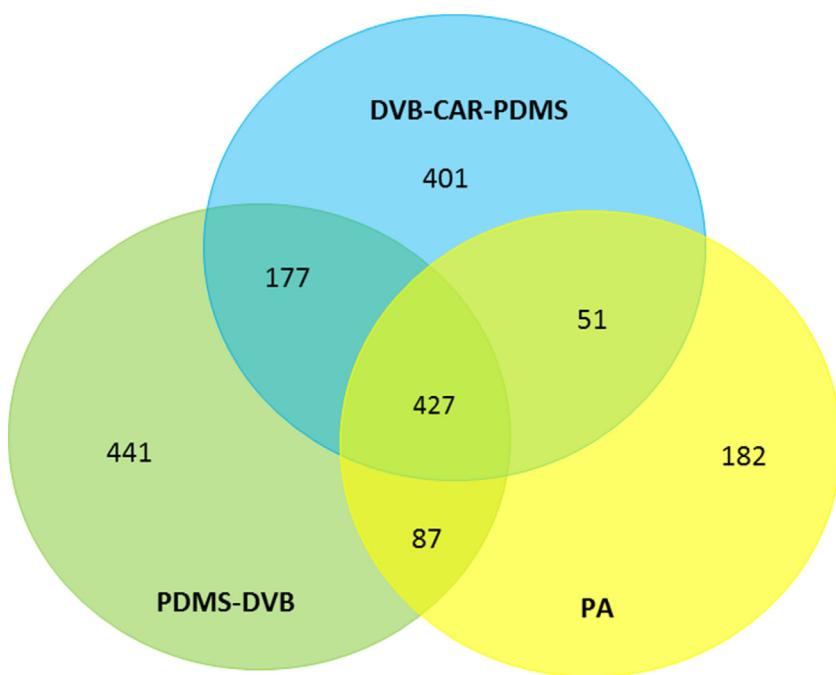


Fig. 2. Specific interactions of THP analytes with the different fibers.

The ion source temperature was set at 230 °C and the transfer line temperature was set at 250 °C. The detector voltage was 1500 V and the ionization electron energy (EI source) was set at 70 eV. Samples were acquired using LECO ChromaTOF® software version 4.50.8.

2.5. Data processing

The data processing for fiber selection was performed using the pixel-based GC Image™ software package version 2.5b6, 64-bit (Zoex, Houston, TX, USA), following minimum blob detection criteria: area 15, volume 300,000. Data processing for comprehensive evaluation of fiber precision was performed with Image Investigator™, part of the GC Image™ software package. Chromatograms were aligned following a procedure based on the creation of a template chromatogram that record peak patterns and carry out resampling of the data to match retention times using GC project™, part of the GC Image™ software package. Data processing for the characterisation of the PP fraction of THP aerosol replicates was performed on a matrix of data containing all calculated peak regions (i.e. every single peak found in any of the sample replicates). Reliable identification of analytes was performed using MS spectral match and Linear Retention Indices (LRI) information. Spectral library matching was carried out using NIST 14 EI Mass Spectral Database (NIST 2014/EPA/NIH) and Wiley Registry of Mass

Spectral Data (9th edition). A minimum similarity value of >750 for both similarity and reverse library match were applied. LRIs were verified using values available in the NIST 14 Mass Spectral Library (NIST 2014/EPA/NIH). If not available in the NIST 14 database, Comprehensive RI databases (AromaOffice²D version 4.00.00, Gerstel K.K., Tokyo, Japan) [33], WEB based RI collections (Flavornet, Cornell University [34], Flavor Database (Citrus Research and Educational Center, Florida State University [35])) and recent scientific literature RI information was used [36–38]. A retention index window of ±15 was applied to MS peak identification assignment. ChromaTOF® software version 4.50.8 was used for the data collection and processing of all chromatograms for the comparison of THP with the reference combustible cigarette.

3. Results and discussion

3.1. Selection of the SPME fiber

Each tested SPME fibers was exposed to the headspace of a quarter of a CFP for each analysis of the particulate phase fraction of aerosols from THP samples. All corresponding GC × GC-TOFMS chromatograms were aligned and processed with automated background correction, blob detection (minimum peak volume value set at 30,000) and phase shift (500 ms). Related peak tables were further manually filtered to remove column bleed and system artifacts. Retention time values (first (1t_R) and second (2t_R) dimension) of compounds were used to construct apex plots for fiber comparisons (Fig. 1).

As the same GC × GC column set was used for each injection, peak distribution patterns and space occupation were similar for each fiber. The difference was in the number of peaks detected where only 747 peaks were detected for the PA fiber, whereas 1056 and 1132 peaks were detected for DVB/CAR/PDMS and DVB/PDMS fibers, respectively. A Venn diagram is presented to reveal specific interactions of analytes with the different fibers (Fig. 2). The consistency of the results was studied by means of repeatability measurements. For each fiber, replicate analyses ($n=6$) were carried out under identical conditions to estimate the repeatability of the extraction efficiency. Each replicate analysis represents a

Table 1

Relative standard deviation (%RSD) values calculated for replicate ($n=6$) extractions of THP aerosols by SPME. Values were calculated on the basis of normalized peak areas in cumulative images produced after GC × GC-TOFMS analyses. Numbers of detected peaks are also given.

SPME fiber	DVB/CAR/PDMS	PA	PDMS/DVB
RSD < 5%	14	0	53
RSD < 10%	133	10	251
RSD < 15%	310	62	471
RSD < 20%	455	139	635
RSD < 25%	589	217	754
RSD < 30%	688	311	822
RSD < 35%	769	410	877
Average%RSD	29.3 ($n=1056$)	42.5 ($n=747$)	24.8 ($n=1132$)

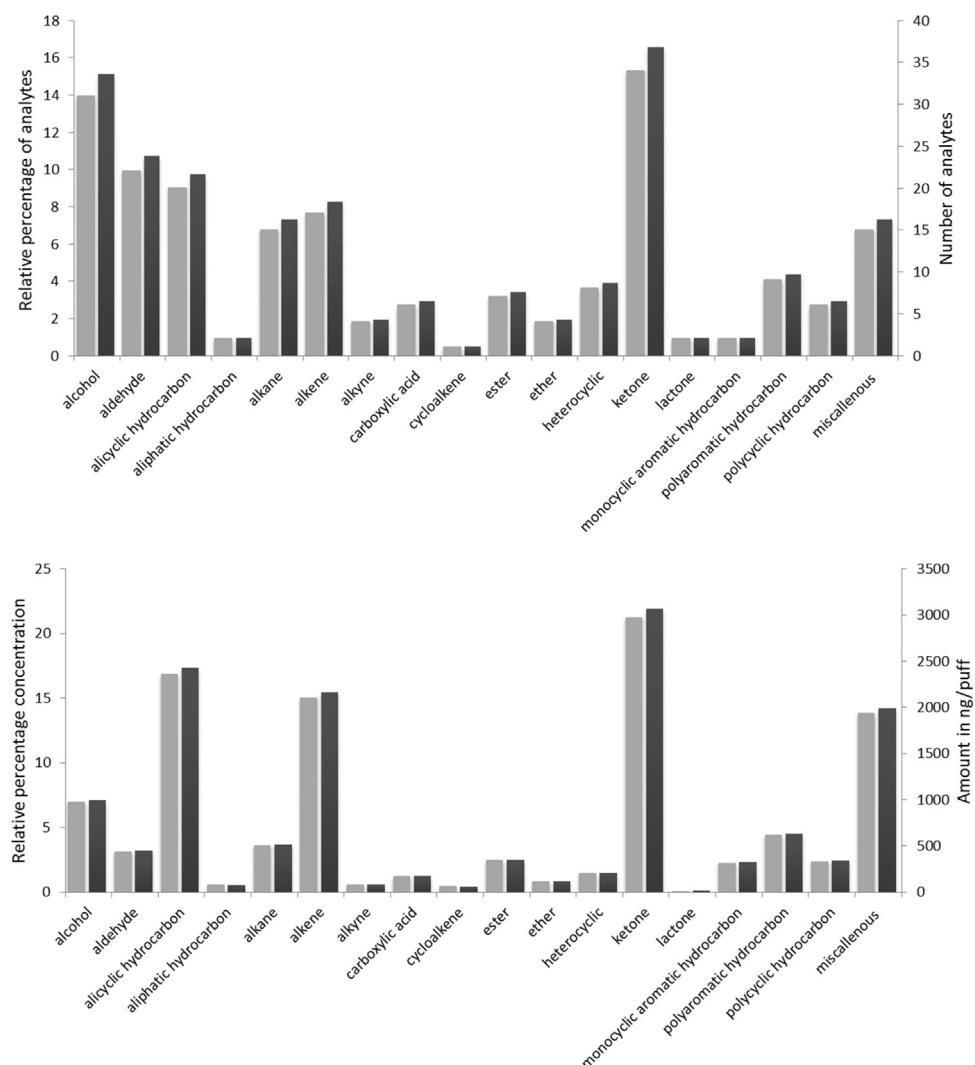


Fig. 3. Distribution of the major chemical classes identified in THP aerosol samples using HS-SPME GC × GC-TOFMS. Top: numbers of analytes (grey) and relative percentage of analytes (black); Bottom: concentrations (grey) and relative percentage concentrations (black).

complete and independent application of the method, including sample preparation. For each fiber replicate, a cumulative chromatogram was created by combining six chromatograms based on most reliable peaks that were detected and matched in all six samples [39,40]. Cumulative images apex plot were complemented by %RSD values to add the precision factor to the number of peaks in fiber comparisons. %RSD values were divided into five levels of precision that are represented by different colours in Fig. 1. Detailed and average %RSD values for each fiber are listed in Table 1. It clearly showed that the PDMS/DVB fiber not only provided the best extraction efficiency in terms of number of detected peaks, but also offered the best repeatability. As used earlier for SPME optimisation [24], a cut-off value of %RSD < 15% showed that 42% of the 1132 peaks were considered reproducible for the PDMS/DVB fiber. Hence, the PDMS/DVB fiber was selected for all subsequent analyses.

3.2. SPME optimisation strategy

Screening experiments were performed to confirm the most significant factors based on the peak area of the selected analytes from the selected seven factors (Table S-1). Peak area values were based on the use of the set of 4 external standards. Relative standard deviations (RSDs) for repeated injections ($n=8$) of

the standard solution ranged from 4% to 6%. Statistical analyses were performed using Fisher's F-test. For the model, $p < 0.1$ was regarded as significant and for the selected analytes $p < 0.05$ was considered as significant. The eight-fractional factorial design comprised a set of 19 experiments for THP particulate phase VOC/SVOC samples in random order. For the purpose of experimental design optimisation, seven representative compounds of relevant chemical classes (Table S-3) were selected from the sample, based on their concentration in cigarette smoke and differing volatilities and polarities: furfuryl alcohol, 2-cylcohexen-1-one, butanoic acid 4-hydroxy-, 2-furanmethanol 5-methyl-, benzene acetaldehyde, β -humulene and neophytadiene. These analytes were naturally present in THP samples. The statistical significance of models built describing the relationship between responses and the factors are available in Table S-3. All models were found statistically significant with ANOVA (p -values < 0.1). From the ANOVA results it can be seen that factor D (extraction time) and E (extraction temperature) were the most statistically significant with larger sets of analytes showing p -values < 0.1 (p -values of total peak areas were 0.01 and 0.03 respectively, for factor D and E).

A CCD was thus considered for the optimisation of SPME extraction time and temperature conditions. For the optimisation, peak area of aforementioned analytes was considered as responses separately. The aim was to search for extraction time and extraction

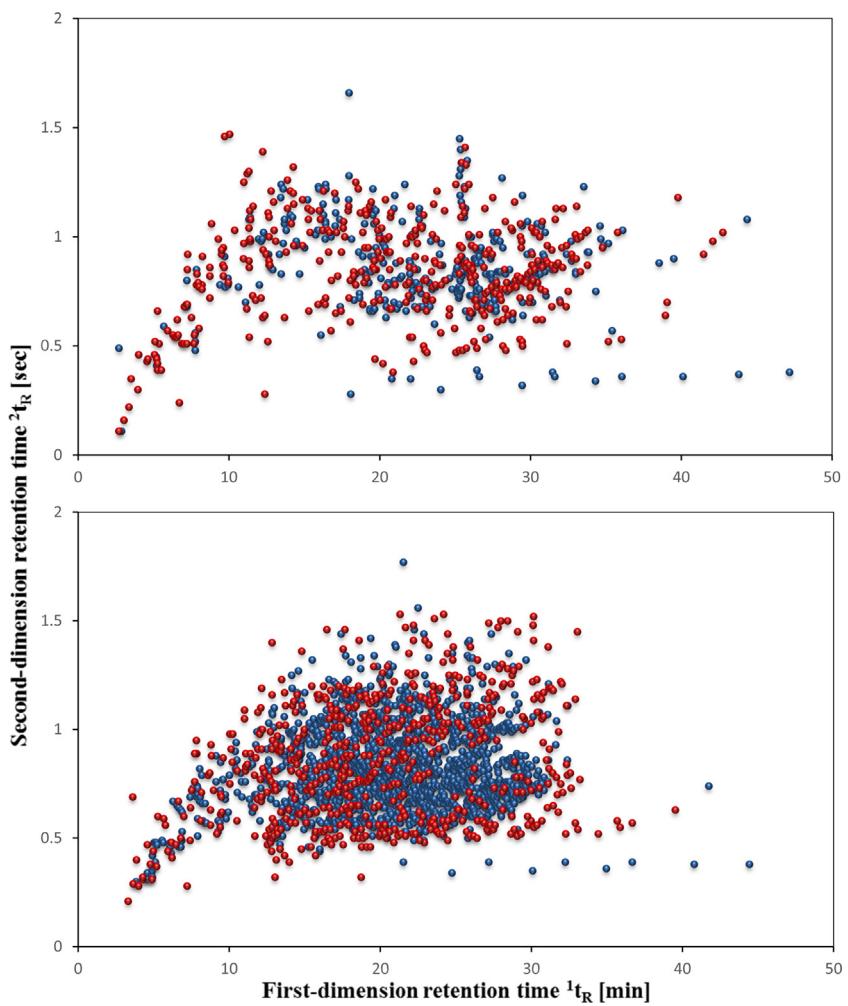


Fig. 4. GC \times GC-TOFMS apex plots of THP aerosol (top) and reference combustible cigarette product (bottom). Red blobs represent identified compounds (MS match factor >800); Blue blobs represent unknowns. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

temperature values (independent variables) for which the peak areas of the selected compounds (dependent variables) were maximized. Five replicates of the central point were performed with the objective of estimating experimental error and detecting any lack of fit. The matrix for CCD consisted of 13 duplicates experimental runs performed in random order (CCD levels and chromatograms are provided in Table S-4 and Fig. S-2, respectively). A second-degree polynomial model including main effects for the two factors – extraction temperature and extraction time, their interaction and their quadratic components – was used. Contour plots of the CCD models built for the responses of extraction temperature and extraction time for the representative compounds and the total peak area are provided in Fig. S-3. Each of these separately modelled responses were processed through a desirability function, rather than combining several elementary responses into a more complex objective function [32]. The extraction time and temperature that provided maximum peak areas for butanoic acid 4-hydroxy- and 2-furanmethanol 5-methyl-, were 10 min and 50 °C, respectively. Furfuryl alcohol also showed good extraction at temperature of 50 °C with lower extraction time of 7 min. Less volatile compounds such as β -humulene and neophytadiene required high extraction temperatures. In addition, the total peak area of THP constituents also increased when extraction time and temperature were increased (see Fig. S-2). However, if more low boiling analytes were extracted the quality of the peaks for the less volatile analytes was reduced. This later overloading fact was thus undesir-

able as it created poor peak finding results. A compromise situation was selected with an extraction time of 15 min and an extraction temperature of 50 °C.

3.3. Analysis of THP aerosol samples

For the qualitative and semi-quantitative analysis of VOCs/SVOCs from THP particulate phase fraction of aerosol constituents collected, a quarter of Cambridge filter pad was spiked with internal standard (concentration corresponding to 50 ng/puff). A total of 205 individual compounds were assigned using an interactive search including linear retention indices and mass spectral library search. To our knowledge this is the first time that such a list of THP aerosol constituents is reported. The detected constituents were semi-quantified assuming that their response factors relative to the closest internal standard (1t_R) were equal to 1. A total of 17 different chemical classes were found in THP aerosol. Compounds that did not belong to any assigned group or contained two or more functional groups were treated as miscellaneous compounds. The major identified THP aerosol constituents consisted of ketones (n = 34), alcohols (n = 31), aldehydes (n = 22), and alicyclic hydrocarbons (n = 20). Estimated concentrations, number of analytes identified, and their relative percentage values are provided in Fig. 3. Detailed information about all identified THP aerosol constituents is provided in Table S-5.

A comprehensive literature review was carried out for all identified THP aerosol constituents to classify them in terms of their possible origin and properties. Among the 205 identified THP aerosol constituents, 144 were previously reported in tobacco leaves [10,41], while 122 were previously found in cigarette mainstream smoke [10,11,14]. This overlapping of THP aerosol composition with tobacco leaf and cigarette smoke may reflect the fact that the THP heating process is capable to trigger the release of, at least, a portion of the tobacco leaf constituents directly into the aerosol. Nevertheless 82 compounds found in THP here for the first time reporting their presence in smoke or aerosol related samples, including 43 that were previously reported in tobacco leaves [10,41]. This possibly highlights the preservation of certain molecules during the heating procedure, compared to the combustion and pyrolysis processes observed in regular cigarette products, or the enhanced efficiency of our approach to isolate analytes that originally remained hidden in sample noise. Among the 124 THP aerosol constituents, 60 of them were belonging to terpenes and their derivatives could be part of flavour and fragrance substances, and 156 could be part of natural products and extractives.

Finally, the HS-SPME GC × GC-TOFMS developed here was applied for the analysis of particulate phases fraction of 3R4F reference cigarette smoke to compare the VOC/SVOC profile of the PP headspaces of 3R4F cigarette smoke and THP aerosol. For the comparison of THP and reference cigarette a minimum signal-to-noise ratio of 1000 (S/N-1000) was applied. Such an approach was successfully used previously to detect relevant peaks at low concentrations in the analysis of mainstream cigarette smoke PP from 3R4F reference cigarette [11]. Results obtained after automated peak finding were manually filtered to exclude column bleed and artifacts. The 1t_R and 2t_R values were used to build apex plots obtained for the two types of products (Fig. 4). The THP aerosol sample chromatogram was much less complex than the chromatogram of the reference cigarette smoke. The average total number ($n=6$) of peaks for the THP aerosol and 3R4F reference cigarette smoke were 723 and 1995, respectively. For peak assignments, peak identification was carried out when mass spectral similarity and reverse match values were above 800 [14,17]. For lower match values, peaks were labelled as 'unknown'. Under these conditions, 56% and 31% of detected analytes were identified with reasonable confidence for the THP aerosol and 3R4F reference cigarette smoke, respectively. The larger percentage of unknowns found in combustible sample was partly due to the large number of peak produced, thus overloading the chromatogram and affecting the peak finding algorithm and the quality of mass spectra. This result highlighted the current limitation of GC × GC-TOFMS and the need to continue to develop the separation and/or mass identification capability of the method and maybe consider additional sample fractionation prior to GC × GC-TOFMS measurements.

The precision of the analytical methodology was estimated from repeated measurements ($n=6$). %RSD values for the total peak area for THP and reference cigarette were 10% and 2%, respectively. For the total number of peak these values became 6% and 2%. Furthermore, %RSD values for the area of assigned peaks with $>800MS$ matchs for THP and reference cigarette were 13% and 2%, correspondingly. For the total number of assigned peaks with $>800MS$ these values become 4% and 2%. This demonstrated that the HS-SPME GC × GC-TOFMS method developed in the present study offered reasonable precision for the two sample types. The large number of remaining unknown peaks was the main limiting factor for the detailed comparison of the sample types. Both the use of high-resolution mass spectrometry (HRMS) for more accurate mass measurement, and the use of, yet to come, more selective stationary phases for separating unresolved peaks are possible actions to take to enhance the capability to identify larger numbers of unknowns.

4. Conclusions

A HS-SPME GC × GC-TOFMS method was developed for the analysis of VOCs emitted from the PP fraction of THP aerosols. A set of 205 individual compounds of which the major contributors originated from possible flavour chemicals, and natural substances and extracts. For the 82 compounds reported for the first time in THP aerosol, 43 compounds were reported previously in tobacco leaves. Major contributors of THP constituents were ketones, alcohols, aldehydes, alicyclic hydrocarbons, and alkenes. Compared to combustible product PP, THP sample chromatograms were significantly less complex, illustrating the greater chemical complexity of volatiles and semi-volatiles emitted from combustible products and the associated technical challenge of characterizing whole smoke emissions. Nevertheless, full characterization of THP PP is not yet achieved as a significant part of the compounds present in the aerosol have not yet been identified. The full description of the THP PP will require the use high resolution/high accuracy MS and possible fractionation of the aerosol prior analysis.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.chroma.2017.09.014>.

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