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Thermal desorption comprehensive two-dimensional gas chromatography coupled to time of flight mass spectrometry for vapour phase mainstream tobacco smoke analysis

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ABSTRACT

A thermal desorption comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (TD-GC × GC-TOFMS) method has been developed for the analysis of mainstream tobacco smoke (MTS) vapour phase (VP). The selection process of the sample introduction approach involved comparing the results obtained from three different approaches: a) use of gas sampling bag followed by SPME (Tedlar® -SPME), b) gas sampling bag followed by TD (Tedlar® -TD), and c) sampling directly on TD sorbents (Direct-TD). Six different SPME fibers and six different TD sorbent beds were evaluated for the extraction capacities in terms of total number of peaks and related intensities or peak areas. The best results were obtained for the Direct-TD approach using Tenax TA/Carbograph1TD/Carboxen1003 sorbent tubes. The optimisation of TD tube desorption parameters was carried out using a face-centered central composite experimental design and resulted in the use of the Tenax TA/Carbograph 1TD/Carboxen 1003 sorbent with a 7.5 min desorption time, a 60 mL/min tube desorption flow, and a 250 °C tube desorption temperature. The optimised method was applied to the separation of MTS-VP constituents, with 665 analytes detected. The method precision ranged from 1% to 15% for over 99% of identified peak areas and from 0% to 3% and 0% to 1% for both first (^1t_R) and second (^2t_R) dimension retention times, respectively. The method was applied to the analyses of two cigarette types differing in their filter construction. Principal component analysis (PCA) allowed a clear differentiation of the studied cigarette types (PC1 describing 94% of the explained variance). Supervised Fisher ratio analysis permitted the identification of compounds responsible for the chemical differences between the two sample types. A set of 91 most relevant compounds was selected by applying a Fisher ratio cut-off approach and most of them were selectively removed by one of the cigarette filter types.

1. Introduction

Mainstream tobacco smoke (MTS) is an aerosol containing an extremely complex mixture of chemicals. It consists of liquid/solid droplets, called the particulate phase (PP), suspended in a mixture of gases and semi-volatiles, the vapour phase (VP) [1]. When a smoking machine is used, the PP is retained by specially designed glass fiber filters (Cambridge filter pads) at room temperature, whereas the VP passes through the filter. More than 6000 compounds have been identified in MTS [2], some of which are considered to be toxic or carcinogenic [3]. Many additional cigarette smoke constituents are present at (ultra)trace levels and have not yet been properly separated nor identified, pushing the possible total number of cigarette smoke constituents to up to 100,000 [4]. Moving forward in the elucidation of MTS composition is a current challenge in Separation Science [1,5].

The VP, including air, represents 95% by weight of the whole tobacco smoke, with only 7% of tobacco-derived volatile organic compounds (VOCs), equivalent of 1.5% of the whole smoke [6,7]. It consists of complex mixtures of saturated and unsaturated hydrocarbons, aromatics, aldehydes, ketones, nitriles and miscellaneous VOCs [6,8]. The exhaustive collection of VP VOCs prior to gas chromatographic (GC) separation and mass spectrometric (MS) identification is a major challenge.

A common sampling approach is the use of gas sampling bags [7–9]. Dong and co-workers successfully applied this approach to

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the identification of a limited number of VOCs [7] and the specific analysis of sulphur containing compounds [10] in VP. However, because of the trace levels to consider, especially in the case of cigarette construction that include ultralow tar design with high ventilation and efficient charcoal filters, a pre-concentration step is necessary prior to GC–MS analyses [7]. Therefore, solvent-filled impingers and gas sampling bags are often used together for the collection of the chemical constituents of MTS-VP [8,11,12]. Darrall and co-workers reported on the use of several cold trap impinger solutions placed in series for the identification and quantitative analysis of benzene and 7 other VOCs using GC–MS [12]. Major drawbacks of such approaches are the delicate evaporation steps required for the concentration of the extracts, the need of large sampling devices to perform cryogenic cooling, and the resulting long sample preparation times. Another approach is to use gas sampling bags followed by other sample enrichment procedures such as solid-phase microextraction (SPME) [13] and thermal desorption (TD) [14]. Both approaches enable to routinely quantify selected VOCs in MTS-VP while ensuring higher sample throughput and virtually no solvent waste. The use of gas sampling bags is however constrained by the bag background compounds and possible sample carry over as well as possible sample integrity issues related to the permeability of the membrane of the bag [15,16].

Another sampling approach is to directly trap MTS-VP samples on sorbent beds. Adapted smoking machines can be designed for drawing air/vapour phase smoke at constant rate through TD tubes prior to GC–MS measurements [4]. More recently, cartridges with sorbent materials followed by multi-step solvent elution with various solvents have been used to collect and analyse VOCs from mainstream tobacco smoke [17].

To date, the majority of vapour phase studies focused on a relatively limited proportion of target analytes due to limitations related to peak capacity and sensitivity of classical single dimensional (1D)GC. Comprehensive two-dimensional GC (GC × GC) has emerged as an extremely powerful technique for the analysis of complex mixtures [1,18,19]. Main advantages of GC × GC over 1DGC are the increased peak capacity, the class-to-class separation from structured GC × GC chromatograms, and the cryogenic zone compression that enhance the overall sensitivity of the technique in case of thermal modulation [20]. Dallüge and co-workers were the first to illustrate the potential of GC × GC coupled with time-of-flight mass spectrometry (TOFMS) for the analysis of MTS aerosols [18]. It was later confirmed by several reports considering target/non-target analysis of MTS [1,21] and tobacco heating products [22]. Yet, to the best of our knowledge, there is no peer-reviewed scientific literature on the analysis of VP mainstream cigarette smoke based on a GC × GC-TOFMS approach.

The aim of the present work was to develop a new simple GC × GC-TOFMS method for MTS-VP sample analysis. To this end, different types of sample introduction approaches were investigated. The optimisation was carried out in terms of their extraction capacity and repeatability of results. The optimised method was applied for statistical comparison of two cigarette types that differed in their filter construction. The use of principal component analysis (PCA) and Fisher ratio [23] calculations was implemented for clear differentiation of the two sample types and to highlight the chemical compounds responsible for that differentiation.

2. Materials and methods

2.1. Analytical reagents and supplies

44 mm glass fiber filter pads (Cambridge filter pads) were purchased from Borgwaldt (Hamburg, Germany). Alkane standard solutions (C_6–C_{30}) were purchased from Sigma Aldrich (Diegem, Belgium). Tedlar® gas sampling bags (2L) were obtained from Sigma Aldrich (Diegem, Belgium). Commercially available SPME fibers and TD tubes were purchased from Sigma Aldrich (Diegem, Belgium) and Markes (Pontyclun, UK), respectively. The list of SPME fibers and TD sorbents is provided in Table 1. All fibers and TD tubes were conditioned prior to use according to manufacturer's instructions. A blank test was always performed before use for each SPME fibers and TD tubes to check for possible carry-over.

2.2. Samples

3R4F research reference cigarettes were acquired from the University of Kentucky College of Agriculture (Kentucky Tobacco Research & Development Center, USA). A classic 27-mm length cellulose acetate filter cigarettes (coded Type A) and a 27-mm length two-part filter (15 mm cellulose acetate section at the mouth end and a 12 mm cellulose acetate section containing 55 ± 4 mg of undispersed active carbon at the rod end) cigarettes (coded Type B) were provided by British American Tobacco (Southampton, UK). A and B cigarettes had a circumference of 24.6 mm and were made up of a 56-mm long tobacco rod containing a US style tobacco blend (tobacco rod density of 235 mg cm^{-3} at a moisture content of 13.5%). The carbon used in this study was a high activity, polymer-based material whose production, composition and performance have been described in detail previously [24].

2.3. Vapour phase smoke collection and sampling procedure

Cigarettes and Cambridge filter pads were conditioned for at least 48 h at 60% relative air humidity and 22 °C [25]. The MTS was generated using a Borgwaldt RM20D smoking machine (Borgwaldt KC GmbH, Germany). Smoking was conducted according to the relevant ISO standards applying a 35 mL puff of 2 s duration taken every 60 s with no blocking of filter ventilation holes [26]. Three sample collection methods were studied: a) sampling using Tedlar® bag followed by SPME (Tedlar®-SPME), b) sampling using Tedlar® bag followed by TD (Tedlar®-TD), and c) direct sampling on TD tube (Direct-TD). For each analysis five conditioned cigarettes were smoked and the PP was collected on Cambridge filter pads while the VP of mainstream smoke was collected on 2 L Tedlar® gas sampling bags or TD tubes. The volume of sample collected on Tedlar® gas sampling bags was 1.4 L. Tedlar® gas sampling bags were flushed with N_2 gas 5 times prior to use for sample collection. For all Tedlar®-SPME analysis, immediately on completion of smoking, the SPME fiber was inserted into the gas sampling bag through the dedicated septum. The SPME extraction was carried out for 30 min at room temperature (20 °C ± 2 °C). Fibers were analysed immediately after completion of SPME extraction process. For all Tedlar®-TD analysis, immediately on completion of smoking, the VP smoke was sampled onto pre-packed thermal desorption tubes using an ACTI-VOC low-flow sampling pump (Markes Ltd) operating at a flow rate of 50 mL/min. For all Direct-TD sampling, the prepacked TD tube was placed in between the Cambridge filter pad holder and the syringe pump of the smoking machine. The total volume of gas drawn through the sorbent for Direct-TD sampling was 1.4 L for each analysis. For both Tedlar®-TD and Direct-TD sampling, TD tubes were capped with DiffLok® caps (Markes Ltd) directly after the sampling procedure was completed to preserve the integrity of samples.

2.4. Instrumental analysis

2.4.1. GC × GC-TOFMS analyses

The GC × GC-TOFMS system consisted of an Agilent 7890 (Agilent Technologies, Palo Alto, CA, USA) gas chromatograph coupled

Table 1
Overview of SPME fibers and TD sorbents used in this study.

SPME fibers	Description	TD Sorbents	Description
Fiber A	100 μm Polydimethylsiloxane (PDMS)	Sorbent A	Carbograph 1TD
Fiber B	100 μm Polyethylene Glycol (PEG)	Sorbent B	Tenax TA
Fiber C	85 μm Polyacrylate (PA)	Sorbent C	Tenax GR/Carbopack B
Fiber D	85 μm Carboxen/Polydimethylsiloxane (CAR/PDMS)	Sorbent D	Tenax TA/Carbograph 5TD
Fiber E	65 μm Polydimethylsiloxane/Divinylbenzene (PDMS/DVB)	Sorbent E	Tenax TA/Sulficarb
Fiber F	50/30 μm Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS)	Sorbent F	Tenax TA/Carbograph 1TD/Carboxen 1003 (Universal)

to a Pegasus 4D TOFMS equipped with a quad jet LN_2 Cooled Thermal Modulator (LECO Corp., St. Joseph, MI, USA). The first dimension (^{1}D) column was a non-polar 5% diphenyl 95% dimethyl polysiloxane phase (30 m × 0.25 mm i.d. × 0.25 μm d_f) (Rtx-5MS, Restek Corp., Bellefonte, PA, USA) connected by means of a SilTite™ μ-Union (SGE International, Victoria, Australia) to a second dimension (^{2}D) midpolarity Crossbond® silarylene phase column exhibiting similar selectivity to 50% phenyl/50% dimethyl polysiloxane (1 m × 0.15 mm i.d. × 0.15 μm d_f) (Rxi® -17SilMS, Restek Corp.). The ^{2}D column was installed in a separate oven located inside the main GC oven, providing more flexible temperature control. The carrier gas was helium at a corrected constant flow rate of 1 mL min^{-1}. The main oven temperature program started with an isothermal period at 35 °C for 5 min, then a ramp of 4 °C min^{-1} up to 220 °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. The modulation parameters consisted of a 2 s modulation period (P_M) (0.4 s hot pulse and 0.6 s cold pulse time) and a temperature offset of 20 °C above the secondary oven temperature. The 2 s P_M was chosen to ensure sufficient sampling to minimise modulation-induced loss of first-dimension resolution [27], and maintain precision of peak heights that often distort with longer modulation period [28]. Mass spectra were acquired in the range m/z 29–500 at the acquisition rate of 100 spectra s^{-1}.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. Daily mass calibration and auto tuning were performed using perfluorotributylamine (PFTBA). Samples were acquired using ChromaTOF® (LECO Corp.) software version 4.50.8.0.

2.4.2. SPME procedure

For all SPME injections the GC × GC-TOFMS system was equipped with a Gerstel MultiPurpose Sampler (MPS 2XL), SPME option for procedural automation and the CIS4 Cooled Injection System (Gerstel, Kortrijk, Belgium). Each extracted SPME fiber was placed in the automated system and the injector was set to splitless mode. The CIS was programmed from −20 °C (0.5 min hold) and then ramped to 250 °C at 12 °C s^{-1} (hold for 120 s). The six types of fiber coatings investigated are listed in Table 1.

2.4.3. TD procedure

For all TD injections, the GC × GC-TOFMS system was equipped with an automated thermal desorber (TD-100, Markes Ltd.). Sample tubes underwent three stages of desorption process. The pre-desorption stage consisted of 1 min of dry purge of the sample tube at ambient temperature with a flow rate of 50 mL min^{-1}. During the tube desorption stage the sample tube was desorbed at 290 °C for 5 min with a flow rate of 50 mL min^{-1}. Samples were recollected at a temperature of −10 °C on a cold trap containing either a Tenax sorbent bed (general purpose carbon number C_4–C_{27}) or a proprietary sorbent from Markes ('Air Toxics' carbon number C_2–C_{32}). The cold trap with samples was placed for 2 min pre-trap fire purge time at flow rate of 50 mL min^{-1} to remove possible traces of undesirable water by diverting them to the vent port prior to desorption into the GC × GC-TOFMS instrument. The desorption of the cold trap was ramped at a heating rate of 24 °C s^{-1} from −10 °C to 300 °C where it was held for another 3.5 min. A transfer line/flow path with 1.5 m deactivated fused silica column was connected between the TD-100 and the GC ^{1}D column inlet maintained at 200 °C. Prior to the injection of the sample from the cold trap to the GC inlet, a split ratio of 1:100 was applied to prevent saturation of the MS ion source. Unless otherwise stated, all experiments were carried out with the aforementioned experimental parameters. The six types of TD sorbent beds investigated are listed in Table 1. For the optimisation of Direct-TD tube desorption conditions, a face-centered central composite design approach was utilised for the major tube desorption parameters: time, temperature and gas flow [29]. The experimental levels selected for all factors are listed in Table S-1 (supplementary material). All experiments were performed in random order with seven replicates of the central point experiments with the objective of estimating experimental error and detecting lack of fit. A total of twenty-one experiments were carried out using MATLAB 7.9.0 (R2009a) software.

2.5. Data processing

All data were acquired using ChromaTOF® (LECO Corp.) software version 4.50.8. These data were processed and summarised (retention times, peak areas, library search etc.) using the pixel-based GC Image™ (ZOEX Corp., Houston, TX, USA) software package version R2.5. Data processing for multivariate comparison of Type A and B samples was performed using Image Investigator™, part of the GC Image™ software package, used to analyse multiple chromatograms and examine statistical trends. For the analysis of MTS-VP and the comparison of samples, chromatograms were aligned following a procedure based on the creation of a template chromatogram that records peak patterns and carrying out resampling of the data to match retention times using GCproject™, part of the GC Image™ software package. Library searches of blobs/compounds were performed using NIST/EPA/NIH Mass Spectral Library (NIST 14) and Wiley Registry of Mass Spectral Data (9th edition) with a match factor threshold of >750. Further analyses of GC × GC–TOFMS results using interactive LRI filters (±10 range) were performed using NIST 14 Mass Spectral Library (NIST 2014/EPA/NIH) and Aroma Office ^{2}D Software (Gerstel K.K., Tokyo, Japan). Aroma office ^{2}D software is a searchable database with more than 73,000 entries, containing LRI information for a wide range of aroma compounds from many literature references [30].

The data processing of the MTS-VP sample replicates for the two cigarette types (a total of 24 runs; 12 for sample Type A and 12 for sample Type B) was performed on a matrix of data containing all calculated peak regions (i.e. every single peak found in any of the sample replicates). It consisted of a set of 1000+ compounds (variables) that were detected in each of the analysed MTS-VP samples. The matrix was submitted to principal component analysis (PCA) with mean-centering pre-treatment in order to elucidate clustering tendencies. A 24 × 1480 data matrix was calculated for PCA using chemometrics package Unscrambler® X version 10.3 (Camo, Norway).

Table 2
List of reliably identified MTS-VP constituents using TD-GC × GC–TOFMS.

Peak No.	Compound Name	CAS No.	Average $^{1}t_R$ [min]	RSD%	Average $^{2}t_R$ [sec]	RSD%	LRI $^{1}t_R$	NIST LRI	MS forward match	MS reverse match	Average peak area $\times 10^7$	RSD%	[c]Dong et.al [7]
1	2-butanone[a]	78-93-3	3.67	0.00	1.35	1.22	600	600[d]	782	893	19.64	4.14	×
2	2-pentene, 3-methyl-	922–62-3	3.72	0.46	0.80	0.94	602	609	776	827	27.11	17.95	
3	propanenitrile, 2-methyl-	78-82-0	4.07	0.00	1.07	0.84	621	623	754	819	13.18	9.24	×
4	1,3-pentadiene, 3-methyl-	2787–45-3	4.27	0.00	0.88	1.40	631	640	792	812	14.45	2.45	×
5	2-butenal[a]	4170–30-3	4.57	0.00	1.16	2.28	646	648[d]	758	809	17.51	1.52	×
6	hexane, 3-methyl-	589–34-4	5.00	0.00	0.79	1.04	669	676	763	880	4.86	13.9	
7	3-buten-2-one, 3-methyl-	814–78-8	5.03	0.00	1.05	1.98	671	671[d]	780	924	12.07	12.41	×
8	cyclohexene	110–83-8	5.14	0.49	0.86	1.86	676	679	806	875	13.98	12.31	
9	2-pentanone	107–87-9	5.40	0.00	1.15	1.98	690	685	760	825	22.14	4.53	
10	1-heptene	592–76-7	5.45	0.51	0.82	1.42	690	685	807	824	19.01	5.66	
11	2-hexene, 3-methyl-	10574–36-4	5.82	0.30	0.84	2.45	706	701	813	866	12.52	12.52	
12	furan, 2,5-dimethyl-	625–86-5	5.94	0.29	1.04	1.33	711	707	843	894	25.54	3.59	
13	2-pentene, 3-ethyl-	816–79-5	6.06	0.81	0.84	0.61	712	706	800	861	11.11	4.59	
14	2,4-dimethylfuran	3710–43-8	6.20	0.00	0.98	0.65	716	708	788	807	9.44	4.53	
15	thiocyanic acid, methyl ester	556–64-9	6.23	0.22	1.48	0.55	718	723[d]	900	929	5.07	8.68	
16	1,4-heptadiene	5675–22-9	6.38	0.27	0.89	2.41	723	715	750	886	13.38	7.91	
17	2-heptyne	1119–65-9	6.67	0.55	0.87	1.45	733	743	755	802	14.67	5.51	
18	butanenitrile, 3-methyl-	625–28-5	6.76	0.20	1.26	2.07	733	731	848	880	14.44	7.23	
19	3,4-heptadiene	2454–31-1	6.84	0.20	0.94	2.93	739	746	776	839	3.56	11.4	
20	cyclopentene, 3-ethenyl-	26727–45-7	7.06	0.24	1.06	2.10	741	732	809	814	15.61	6.13	
21	pyridine[a]	110–86-1	7.40	0.00	1.31	2.62	753	746	763	902	13.92	16.05	×
22	1-hexene, 3,5,5-trimethyl-	4316–65-8	7.90	0.27	0.85	2.45	767	769	776	808	9.81	13.95	
23	1,3,5-cycloheptatriene	544–25-2	7.93	0.00	1.17	1.86	768	772	854	877	21.35	12.8	
24	toluene[a]	108–88-3	8.04	0.34	1.17	2.58	771	763	862	896	11.45	4.78	×
25	3-octene	14919–01-8	8.32	0.21	0.83	0.62	780	789	763	815	4.26	9.72	
26	2-pentenenitrile	13284–42-9	8.34	0.16	1.32	1.03	780	772	752	819	7.41	2.99	
27	1,7-octadiene	3710–30-3	8.43	0.00	0.87	1.78	783	776	832	838	3.1	14.77	
28	2-hexanone	591–78-6	8.83	0.15	1.11	0.46	794	790	806	888	7.33	4.09	
29	cyclopentanone	120–92-3	8.83	0.00	1.41	0.70	794	791	841	859	9.84	4.31	
30	4-methyl-1,3-heptadiene	17603–57-5	9.06	0.5	0.87	2.71	798	792	767	825	2.4	14.31	
31	1-ethyl-5-methylcyclopentene	97797–57-4	9.43	0.00	0.85	1.56	810	815	831	902	6.42	8.94	
32	1,4-pentadiene, 2,3,3-trimethyl-	756–02-5	9.63	0.14	0.85	2.08	815	817	772	777	12.71	6.71	
33	3-octyne	15232–76-5	9.84	0.14	0.89	2.44	820	818	807	849	3.98	12.04	
34	cyclohexene, 1,6-dimethyl-	1759–64-4	10.03	0.36	0.89	1.10	826	838	818	863	15.58	7.74	
35	pyridine, 2-methyl-	109–06-8	10.11	0.17	1.22	0.42	828	818	784	877	5.26	14.09	×
36	heptane, 2,6-dimethyl-	1072–05-5	10.16	0.13	0.77	0.98	829	828	825	923	2.38	13.51	
37	1,3,6-octatriene	929–20-4	10.43	0.00	0.94	0.43	836	825	817	835	3.15	6.58	
38	2-cyclopenten-1-one	930–30-3	10.60	0.00	1.58	0.66	841	831	846	875	7.38	9.41	
39	2-hexenal	505–57-7	10.74	0.16	1.30	0.40	844	851	856	902	8.48	13.1	
40	2,6-dimethyl-1,3,6-heptatriene	928–67-6	10.77	0.00	0.95	0.58	844	858	773	823	9.54	11.27	
41	pentanenitrile, 4-methyl-	542–54-1	10.87	0.00	1.29	1.55	847	847	789	800	7.2	8.46	
42	2,4,6-octatriene	15192–80-0	10.87	0.00	0.93	0.68	847	840	824	841	5.87	10.79	
43	2-heptene, 2,6-dimethyl-	5557–98-2	11.03	0.00	0.84	1.90	851	850	764	792	11.65	10.76	
44	2-pentenal, 2-methyl-	623–36-9	11.03	0	1.31	0.39	851	852	769	847	8.12	7.7	
45	1-octene, 4-methyl-	13151–12-7	11.25	0.16	0.80	0.65	857	846	764	806	1.83	12.57	
46	3-octyne, 7-methyl-	37050–06-9	11.40	0.00	0.86	0.00	860	869	820	857	5.32	9.83	
47	cyclohexane, 1,2-bis(methylene)-	2819–48-9	11.38	0.16	0.97	1.02	860	853	783	801	2.46	11.91	
48	ethylbenzene	100–41-4	11.50	0.00	1.12	0.92	863	855	811	902	12.34	9.13	×
49	p-xylene	106–42-3	11.84	0.15	1.13	0.72	872	865	822	904	16.89	13.9	×
50	pyridine, 3-methyl-	108–99-6	11.93	0.11	1.28	0.64	874	863	775	884	5.09	7.87	
51	1,5-heptadiene, 2,6-dimethyl-	6709–39-3	12.43	0.00	0.87	0.97	886	882	815	834	4.23	14.99	

Table 2 (*Continued*)

Peak No.	Compound Name	CAS No.	Average $^{1}t_R$ [min]	RSD%	Average $^{2}t_R$ [sec]	RSD%	LRI $^{1}t_R$	NIST LRI	MS forward match	MS reverse match	Average peak area $\times 10^7$	RSD%	[c]Dong et.al [7]
52	3,4-octadiene, 7-methyl-	37050–05-8	12.55	0.15	0.87	1.34	890	891	840	870	1.11	7.16	
53	styrene[a]	100–42-5	12.74	0.26	1.19	1.91	895	893	759	821	10.61	3.23	×
54	o-xylene	95–47-6	12.78	0.14	1.08	0.75	896	887	773	895	5.67	14.69	
55	cis-4-nonene	10405–84-2	12.88	0.32	0.80	0.65	897	885	834	897	1.21	7.55	
56	2-cyclopenten-1-one, 2-methyl-	1120–73-6	13.41	0.13	1.47	2.75	912	913	760	896	4.71	13.85	
57	santolina triene	2153–66-4	13.56	0.25	0.86	0.95	915	908	787	840	7.47	4.49	
58	octane, 2,6-dimethyl-	2051–30-1	14.29	0.10	0.77	2.52	934	933	798	864	1.47	12.51	
59	camphene	79-92-5	14.76	0.12	0.86	1.40	947	952	775	800	10.91	12.41	
60	benzene, propyl-	103–65-1	15.11	0.09	1.07	1.09	956	953	797	877	3.77	10.94	
61	benzene, 1-ethyl-2-methyl-	611–14-3	15.41	0.09	1.12	0.75	964	969	771	815	7.54	6.76	
62	benzaldehyde	100–52-7	15.58	0.11	1.44	0.28	968	974[d]	851	910	0.35	11.86	
63	benzene, 1,3,5-trimethyl-	108–67-8	15.67	0.00	1.07	0.38	970	972	810	821	5.11	8.38	
64	benzene, 1,2,4-trimethyl-	95–63-6	16.11	0.08	1.12	0.75	982	978	806	836	3.98	9.84	
65	cis-2,6-dimethyl-2,6-octadiene	2492–22-0	16.42	0.11	0.89	0.58	990	985	792	838	7.84	11.37	
66	benzonitrile	100–47-0	16.53	0.00	1.52	0.27	992	994[d]	757	886	0.26	14.4	
67	1-decene	872–05-9	16.58	0.10	0.93	2.91	993	989	859	889	3.33	10.75	
68	ß-myrcene	123–35-3	16.60	0.00	0.92	2.02	994	993[d]	783	819	12.95	14.82	
69	benzene, 1,2,3-trimethyl-	526–73-8	16.67	0.00	1.10	1.37	996	1005	752	791	7.96	13.76	
70	decane	124–18-5	16.87	0.00	0.80	0.94	1001	1000	861	906	3.18	14.4	
71	benzene, 1-propenyl-	637–50-3	16.79	0.39	1.17	0.44	1001	1011	736	787	3.76	32.42	
72	(−)-ß-pinene	18172–67-3	16.96	0.08	0.91	1.20	1004	1002[d]	768	769	9.68	10.29	
73	bicyclo[3.2.1]oct-2-ene, 3-methyl-4-methylene-	49826–53-1	17.07	0.00	1.01	0.40	1006	1000	806	822	1.79	9.55	
74	2-decene, (Z)-	20348–51-0	17.10	0.00	0.84	1.00	1007	1002	829	867	1.28	12.63	
75	delta-3-carene	13466–78-9	17.13	0.00	0.92	1.31	1009	1011	822	838	8.03	13.37	
76	3,5-heptadien-2-ol, 2,6-dimethyl-	77411–76-8	17.18	0.10	1.02	0.54	1010	1001	774	826	4.19	11.59	
77	2-ß-pinene	127–91-3	17.56	0.10	0.97	0.57	1019	1029[d]	813	816	9.98	12.8	
78	cyclohexene, 1-methyl-4-(1-methylethyl)-	5502–88-5	17.73	0.00	0.97	1.02	1023	1025	864	885	8.18	11.26	
79	nonane, 2,6-dimethyl-	17302–28-2	17.81	0.08	0.77	0.82	1026	1018	829	868	1.31	14.6	
80	limonene	138–86-3	18.07	0.17	1.06	1.28	1032	1030	855	861	27.12	12.75	×
81	trans-ocimene	502–99-8	18.12	0.09	1.06	2.10	1034	1036	764	797	3.12	9.29	
82	ß-ocimene	13877–91-3	18.40	0.00	0.98	0.42	1041	1037	809	827	5.17	9.11	
83	2,3-dimethyl-2-cyclopenten-1-one	1121–05-7	18.61	0.09	1.48	0.51	1047	1040	764	841	1.14	10.85	
84	1H-indene	95–13-6	18.64	0.09	1.36	1.00	1047	1041	809	869	2.31	13.44	
85	trans-ß-ocimene	3779–61-1	18.78	0.09	0.99	0.83	1051	1049	820	839	5.26	7.18	
86	decane, 3-methyl-	13151–34-3	19.57	0.00	0.79	0.52	1071	1071	815	894	0.12	12.29	
87[b,a]	bicyclo[3.3.0]oct-2-en-7-one, 6-methyl-		19.80	0.0	1.33	0.48	1077	1076	768	770	1.29	13.5	
88	benzaldehyde, 4-methyl-	104–87-0	19.76	0.09	1.53	0.27	1077	1079	777	975	0.61	14.66	
89	3-undecene	821–97-6	19.81	0.07	0.81	0.51	1078	1086	792	819	0.22	11.03	
90	benzene, 1-methyl-4-(1-methylethyl)-	99–87-6	19.95	0.09	1.14	1.06	1081	1081	787	830	3.13	8.47	
91	4-undecene	821–98-7	20.34	0.67	0.88	0.86	1092	1092	823	853	3.24	8.68	
92	2,4-dimethylstyrene	2234–20-0	20.44	0.47	1.22	0.62	1094	1084	751	812	3.47	8.62	
93	cis-p-mentha-2,8-dien-1-ol	3886–78-0	20.59	0.33	1.10	0.50	1097	1102	774	794	0.37	9.51	
94	bicyclo[5.1.0]octane, 8-(1-methylethylidene)-	54166–47-1	20.63	0.00	0.98	0.56	1098	1099	821	866	1.62	7.44	
95	undecane	1120–21-4	20.71	0.07	0.84	1.39	1101	1100	839	873	2.26	12.62	

96	cyclohexene, 3-(3-methyl-1-butenyl)-	56030–49-0	20.80	0.00	0.97	0.65	1103	1104	804	806	0.68	13.44
97	1,3,8-p-menthatriene	18368–95-1	21.19	0.57	1.18	2.17	1115	1119	795	862	0.89	8.32
98	2-cyclohexen-1-ol, 1-methyl-4-(1-methylethenyl)-, trans-	7212–40-0	21.36	0.06	1.28	1.07	1118	1123	758	778	1.25	8.3
99	o-isopropenyltoluene	7399–49-7	21.80	0.00	1.23	0.42	1129	1117	783	873	0.53	11.39
100	2,6-dimethyl-1,3,5,7-octatetraene	460–01-5	21.94	0.16	1.13	2.80	1133	1131	822	870	0.44	10.83
101	4-acetyl-1-methyl-1-cyclohexene	01–09-6090	22.1	0.00	1.33	0.61	1137	1137	790	822	0.69	13.23
102	3-undecen-5-yne	74744–29-9	22.13	0	1.00	0.51	1138	1140	820	830	0.49	10.26
103	exo-7-(trans-1-propenyl)bicyclo[4.2.0]oct-1(2)-ene	107983–42-6	22.19	0.08	1.12	0.92	1139	1140	823	852	1.05	5.44
104	1H-indene, 2,3-dihydro-4-methyl-	824–22-6	22.30	0.00	1.29	0.40	1142	1145	751	768	1.5	11.81
105	benzene, 1,3-diethyl-5-methyl-	2050–24-0	22.47	0.00	1.14	0.45	1146	1147	783	831	0.79	12.4
106	1H-indene, 3-methyl-	767–60-2	22.70	0.00	1.39	0.40	1153	1155	773	841	3.07	11.38
107	1H-indene, 1-methyl-	767–59-9	22.93	0.00	1.39	0.54	1159	1160	813	862	2.62	6.56
108	8a-methyl-1,2,3,5,8,8a-hexahydronaphthalene	107914–93-2	22.97	0.00	1.13	0.79	1160	1168	803	813	1.32	10.46
109	benzene, 1-methyl-4-(1-methyl-2-propenyl)-	97664–18-1	23.83	0.00	1.21	0.43	1182	1190	764	803	0.24	9.27
110	1-dodecyne	765–03-7	23.90	0.00	0.92	0.44	1184	1191	781	886	0.35	13.6
111	naphthalene	91–20-3	24.03	0.00	1.58	0.26	1188	1182	821	932	1.05	9.95
112	3-dodecene	7239–23-8	24.19	0.06	0.89	0.00	1192	1185	834	908	1.73	10.53
113	dodecane	112–40-3	24.48	0.07	0.86	0.60	1200	1200	836	916	1.19	9.59
114	2-dodecene	7206–13-5	24.70	0.00	0.90	0.93	1206	1202	781	823	0.65	6.97
115	undecane, 2,6-dimethyl-	17301–23-4	25.00	0.00	0.84	0.61	1214	1210	834	901	1.18	9.94
116	(−)-myrtenol	19894–97-4	25.02	0.07	1.04	2.65	1215	1213	786	799	0.12	12.83
117	1-dodecen-3-yne	74744–36-8	25.44	0.05	0.96	0.43	1226	1222	800	810	0.27	14.83
118	1,12-tridecadiene	21964–48-7	27.53	0.00	0.95	0.55	1284	1275	792	814	0.13	13.42
119	3-tridecene	41446–57-5	27.80	0.00	0.91	0.57	1292	1285	816	834	0.78	14.81
120	tridecane	629–50-5	28.09	0.05	0.88	0.46	1300	1300	883	908	0.69	14.35
121	methylnaphthalene	90–12-0	28.13	0.00	1.57	0.57	1301	1301[d]	804	908	0.29	10.43
122	naphthalene, 2-methyl-	91–57-6	28.72	0.06	1.63	0.00	1318	1315	787	926	0.24	12.61
123	triacetin	102–76-1	30.10	0.00	1.48	0.74	1359	1344	780	872	1.67	7.83
124	nicotine[a]	54–11-5	30.56	0.16	1.47	0.51	1371	1361	758	761	0.69	14.01
125	ß-elemene	515–13-9	31.03	0.00	1.06	0.38	1386	1390[d]	757	845	0.14	14.1
126	4-tetradecene	41446–78-0	31.23	0.00	0.94	0.59	1392	1388	848	902	0.31	13.5
127	ß-longipinene	41432–70-6	31.47	0.00	1.14	0.36	1399	1403	785	809	0.21	10.43
128	tetradecane	629–59-4	31.50	0.00	0.90	0.57	1400	1400	854	916	0.31	10.62
129	gamma-elemene	29873–99-2	32.07	0.00	1.16	0.45	1418	1428[d]	788	869	0.17	14.51
130	aromandendrene	489–39-4	32.60	0.00	1.15	0.45	1434	1440	801	843	0.24	12.35

[a] Compounds identified from Hoffmann list.
[b] CAS number is not available – confirmed with NIST ID No: 150437.
[c] VP compounds tentatively identified in 1R4F reference cigarette type [7].
[d] Library Retention Indices confirmed with AromaOffice 2D database.

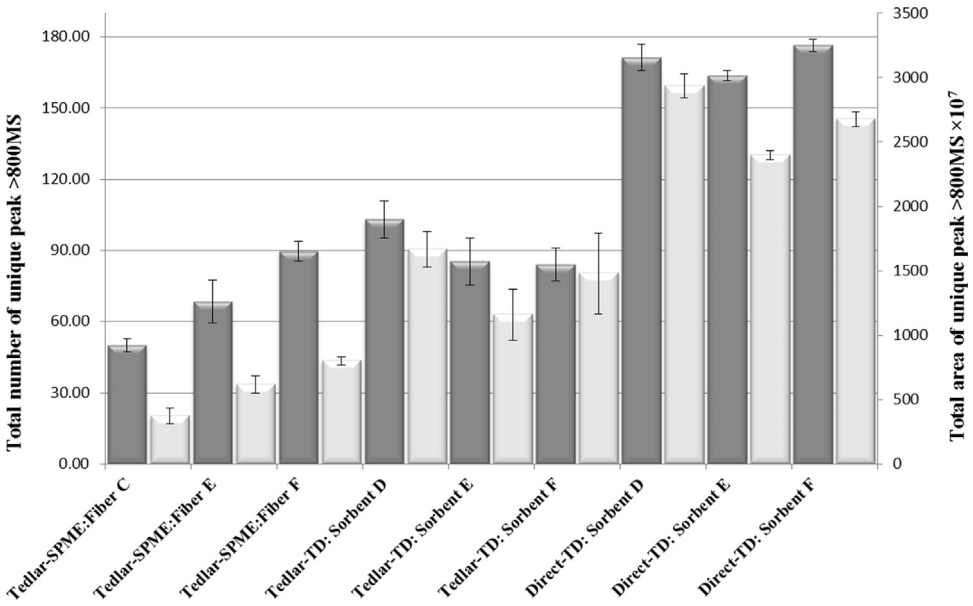

Fig. 1. Total number of unique analytes with a library match factor >800 (dark grey, left y-axis scale) and corresponding peak areas (light grey, right y-axis scale) obtained using selected fibers and sorbents for Tedlar®-SPME, Tedlar®-TD, and Direct-TD for sampling MTS-VP constituents in the reference cigarette by GC×GC-TOFMS.

3. Results and discussion

3.1. Selection of the sample collection/introduction approach

The optimisation of VP sampling techniques was carried out considering three different types of sample introduction approaches: sampling using Tedlar® bag followed by SPME (Tedlar®-SPME), sampling using Tedlar® bag followed by TD (Tedlar®-TD), and direct sampling on TD tube (Direct-TD) without the use of impingers or gas sampling bags. In order to determine the most suitable SPME fiber or TD sorbent bed for the subsequent analysis, we tested six different SPME fibers and six different TD sorbent beds in terms of extraction capacity of VP VOCs. The dispersion of the VP constituents over the 18 GC × GC chromatograms obtained by combining all SPME fibers and TD sorbents for the 3 approaches (Tedlar®-SPME, Tedlar®-TD, and Direct-TD) is provided in Figure S-1 (supplementary material). The bubble plots are presented in a 'contact sheet' format to allow quick visual interpretation of the relative peak numbers and distribution over the chromatographic plane. Each chromatogram was considered on the basis of the number of unique peaks (number of 'peaks' after manual removal of column bleed, multiple hits formed by peak tailing/fronting effect, fiber/sorbent bleed, etc.). This number ranged between 457 and 596 for TD sorbent A and SPME fiber E, respectively (see Figure S-2 in supplementary material for all values). From the six evaluated SPME fibers, the DVB/CAR/PDMS (Fiber F), PDMS/DVB (Fiber E), and PA (Fiber C) fibers provided the highest number of peaks, whereas for TD sorbents it was Tenax/Carbograph/Carboxen (Sorbent F), Tenax/Sulficarb (Sorbent E), and Tenax/Carbograph (Sorbent D).

As the proper identification of analytes is of prime interest, replicates of the most promising fibers and sorbents were performed and further compared based on the number of reliable peaks. The average (n = 3) values of numbers of unique peaks exhibiting a library match factor >800 as well as their peak area are shown in Fig. 1. The average value of unique peaks exhibiting high quality MS spectra (>800 MS match factor) observed for the Direct-TD approach is more than twice the value observed for Tedlar®-SPME and Tedlar®-TD. This is most probably because of the contamination, background levels of gas sampling bags, sorption to the Tedlar bag surface, as well as possible sample integrity issues related to the permeability of the bag membrane [15,16]. Furthermore, peak area data indicated that both Tedlar®-TD and Direct-TD approaches had better extraction capacity compared to Tedlar®-SPME. This was to be expected as SPME is an equilibrium extraction method [31], whereas, TD is an exhaustive extraction method that depends on the properties of the analytes sorption to the selected sorbent combination and sampling conditions [29]. In addition, Direct-TD systematically provided more consistent results compared to both sampling bag approaches. Total numbers of unique peaks with >800 MS match value and corresponding peak areas were more than twice higher in the Direct-TD analysis compared to the two other approaches. In short, among the three approaches evaluated, Direct-TD is the best available approach for MTS-VP analysis as it eliminates requirements of any additional consumables and provides a chromatogram with high quality MS spectra and more consistent results. Moreover, the approach offers collection, extraction, and concentration of MTS-VP constituents in a single step. Among the studied TD sorbents, Tenax/Carbograph/Carboxen (sorbent F) provided the larger number of unique peaks with >800 MS match value with good precision. Additionally, this sorbent combination has the unique extraction selectivity and the ability of trapping analytes with carbon number in the range of C_2–C_{30}. Sample recollections were carried out on a C_2–C_{32} cold trap. This combination of TD sorbent and trap ensured proper sampling of low boiling compounds so that the limitations of the global method in terms of low boiling compound analyses was based on the limitations introduced by the GC oven operating temperature and the liquid nitrogen cryogenic modulation.

3.2. Experimental design approach for direct TD trapping

Tube desorption parameters were optimised following a face-centered central composite experimental design approach. The response surface with a second-order quadratic model was used to correlate between responses and changeable parameters. For the optimisation of experimental design, a set of six different classes of organic compounds (styrene, pyridine, decane, ethylbenzene, o-xylene and naphthalene) were selected amongst all compounds present in MTS-VP samples collected from the reference material [1,32]. Their selection was based on their significance in tobacco

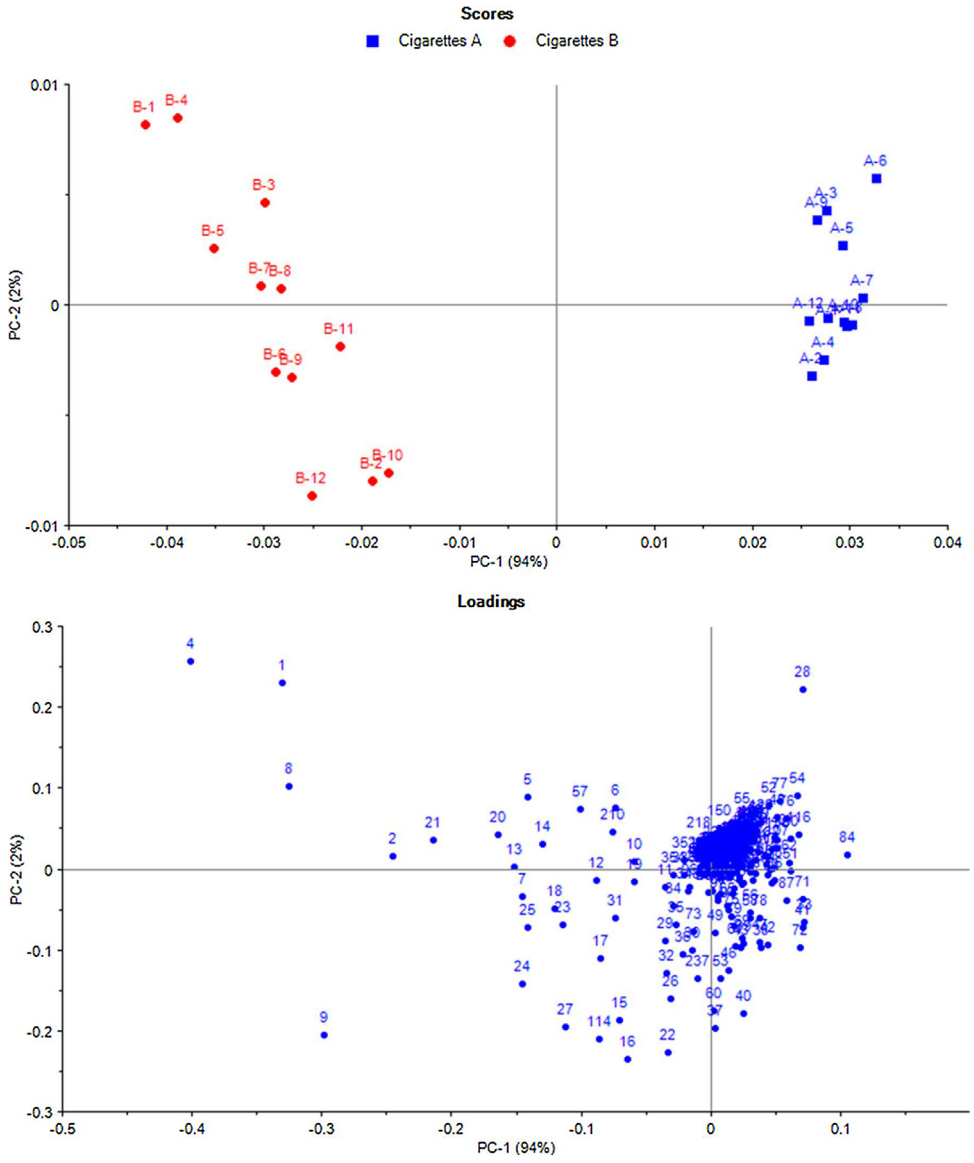

Fig. 2. Principal component analysis Score (top) and Loading (bottom) plots for MTS-VP constituents of two cigarette types A and B analysed by TD-GC×GC-TOFMS.

smoke and to cover a large range of volatilities and polarities. The aim was to search for desorption time, desorption temperature and desorption flow (independent variables) for which peak areas of the aforementioned compounds (dependent variables) were maximised. Response surface plots obtained in the CCF in terms of peak areas for the six selected compounds but also for all unique peak analytes with a library match factor >800 are provided in Figure S-3.

The maximum responses for all classes of compounds were obtained with tube desorption time near the center points. All compounds had small increase of response with the function of tube desorption flow. In addition, decane showed the maximum response around the center points for tube desorption flow, whereas naphthalene maximum response was increasing with tube desorption time and tube desorption flow. Temperature had no effect on the response of the peak area of selected analytes nor all unique peak analytes with a library match factor >800 (this high matching factor was used to limit the number of analytes to be considered for this comparison exercise). Tube desorption temperature should be set as the function of TD sorbent rather than analyte and it should not exceed the maximum operating temperature of the sorbent as it can cause sorbent bleeding and impact chromatogram integrity. Furthermore, by nature, MTS-VP does not contain high molecular weight compounds to elute at higher temperatures. In order to transform a multiple response problem to an overall solution for the optimisation process, each of these separately modelled responses were processed through a Desirability function, rather than combining several elementary responses into a more complex objective function [33]. In practice, a composition function was created to provide the best compromise of a joint response and approach the optimal value for all evaluated variables. In conclusion, the overall optimum of TD experimental conditions, identified by maximised desirability, were a 7.5 min desorption time, a 60 mL min^{-1} tube desorption flow, and a 250 °C tube desorption temperature.

3.3. Application of the TD-GC × GC-TOFMS method

3.3.1. Analysis of vapour phase mainstream tobacco smoke

The method was applied to the qualitative analysis of VOCs in MTS-VP from reference material. Twelve replicates of analysis were performed and, after proper alignment, a cumulative image was

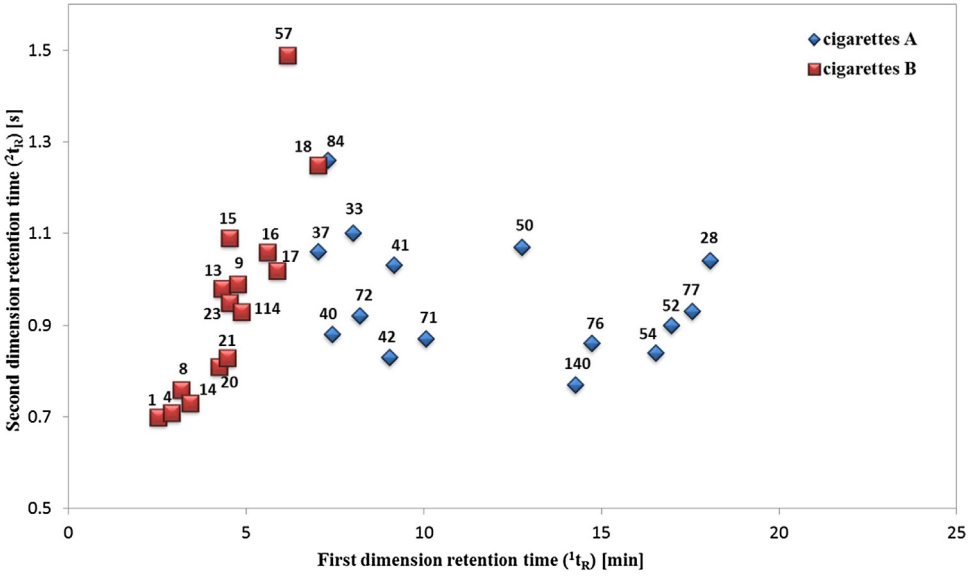

Fig. 3. Apex plot of the most important compounds for the differentiation of the two cigarette types (sample type A and B in blue and red, respectively) analysed by TD-GC×GC-TOFMS. (37: cyclopentene, 3-ethenyl-; 84: pyridine; 40: 1,4-hexadiene, 4-methyl-; 33: toluene; 72: 2,3-hexadiene 2-methyl-; 42: 6-methyl-2-heptyne; 41: 4-methyl-1,3- heptadiene; 71: pyridine, 2-methyl-; 50:o-xylene; 140:octane, 2,6-dimethyl-; 76: bicyclo[4.1.0] heptane, 7-(1-methylethylidene)-; 54: 1-decene; 52: beta-myrcene; 77: 1,3,6-octatriene, 3,7-dimethyl-; 28: limonene; 1: cyclopropane, 1,1-dimethyl-; 4: 1,3-Butadiene, 2-methyl-; 8: iso- butyraldehyde; 14: 1-hexene; 20: 3-hexyne; 21: 1,3,5-hexatriene; 23: 1,3-pentadiene,3-methyl-; 13: butanal, 3-methyl-; 15: 2-butenal); 9: benzene; 114: 2,4-hexadien-1-ol; 16: 2-penten-1-ol; 17: furan, 2,5-dimethyl; 57: thiocyanic acid, methyl ester; 18: disulfide, dimethyl). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

created by combining all chromatograms [1]. A total of 655 reliable peaks were detected after manual cleaning of column bleeding signals, TD sorbent bleeding signals, artifacts and peak tailing-related hits. Amongst these quality signals, a total 130 peaks were identified using both interactive search of LRI (±10) and mass spectral library searching (>750 MS library match). The main analytical results are shown in Table 2. They include retention time information and mass spectral library match values as well as average peak areas. The precision of the method was studied by analyses of six replicates and it was expressed by RSD% values of the all identified peak absolute area and retention times (Table 2). For peak areas, fifty-five peaks showed excellent precision in the range of 1%-10%, 72 peaks showed acceptable precision in the range of 10%-15%, and only three peaks had RSD% above 15%. The RSD% values for both first dimension and second dimension retention times ranged from 0% to 3% and 0% to 1%, respectively. This demonstrated the high precision of the TD-GC × GC-TOFMS method. To the best of our knowledge, this is the first time such a large number of VOC constituents are reported and identified in MTS-VP.

Because of the use of a regular GC oven and liquid nitrogen as the cryogenic fluid for modulation, our approach did not allow us to consider a significant part of the MTS-VP VOCs that comprise highly volatile compounds (below butane). The use of lower trapping temperatures during TD sampling and trapping, as well as a dedicated negative temperature GC oven fitted with a flow modulator would be of interest to extend the present work to low boiling compounds. Nevertheless, analytes listed in Table 2 significantly enlarge the list of the few previous reports on MTS-VP components and offer a more comprehensive overview as the majority of the previous studies relied on target analysis and were limited in terms of the description of the VOC profile. Only one study previously reported 92 tentatively identified compounds in MTS-VP from 1R4F reference cigarette material using a dedicated GC–MS technique capable to isolate molecular weights in the range from 28 to 136 (C1–C10) [7]. Our approach was limited to the range from 69 to 218 (C4–C14) but we attempted to make a fair comparison with the study of Dong et al. [7] by considering the retention time window from 3-methyl-3-buten-2-one to limonene. Dong et al. were able to identify a total of 37 compounds, while our GC × GC approach was able to identify a total of 80 compounds. Fourteen identified compounds were present in both studies. Out of 130 identified MTS-VP constituents, only five compounds (Table 2) were present in the sub-sets of the so-called 'Hoffmann list', a list comprising 44 constituents of prime interest in terms of toxicity.

3.3.2. *Comparison of two different cigarette types*

The comparison of two different cigarette types that differ only in their filter construction was carried out by performing twelve replicates of injections for each cigarette type A and B. The detailed description about these two cigarette types and their PP chemical compositions have been reported previously [1], but limited data are currently available regarding the real chemical impact of such filter on the VP composition [24]. Samples were pre-processed by mean of background correction and blob detection. Each single TD-GC × GC-TOFMS chromatograms of both cigarette types A and B were used to build a cumulative image by combining all chromatograms into a single image after carrying out chromatogram alignment. This image represents all the constituents present in all samples. This template creation approach [34] and its successful application for MTS-PP was reported elsewhere [1]. The present template was made of a total of 1480 regions and a 24 × 1480 matrix containing the relative percent responses of peak areas submitted to PCA analysis after mean-centering pre-treatment. Fig. 2 shows the corresponding PC1 vs. PC2 score (top) and loading (bottom) plots. The score plot clearly shows the relationships among the samples and account for more variation in the data set than any other pair of components with PC1 describing 94% of the explained variance. This further demonstrates the ability of the TD-GC × GC-TOFMS method to separate and highlight MTS-VP constituents responsible for the differentiation between the two sample types (see the loading plot). The apex plot in Fig. 3 displays the chromatographic positions of the most relevant compounds (15 compounds from each cigarette types excluding unknowns) for the differentiation of the two cigarette types. Their localisation in the 2D space

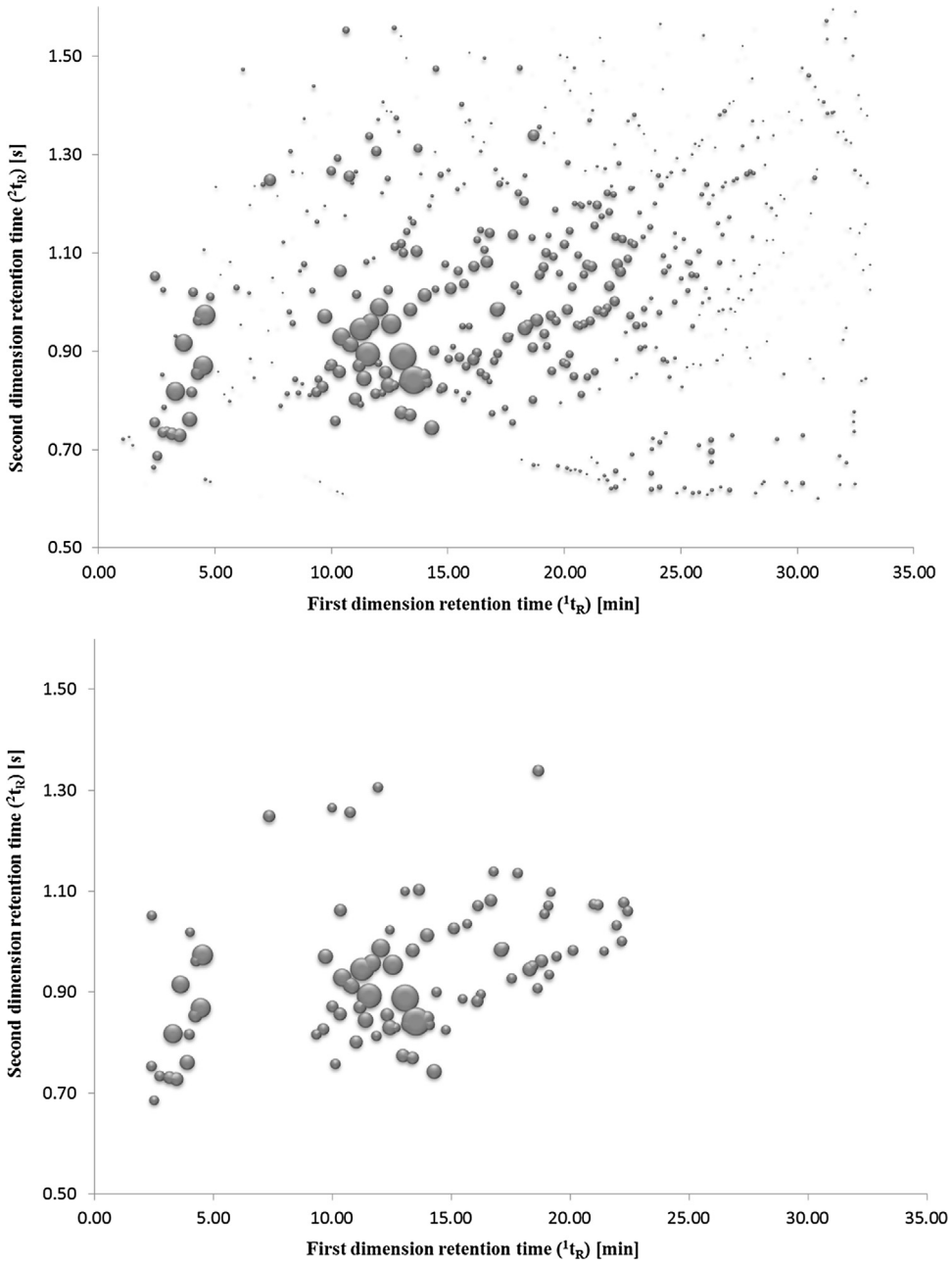

Fig. 4. TD-GC×GC-TOFMS two-dimensional Fisher ratio plots showing the biggest class-to-class differences between cigarette type A and B (top) and for the most influencing (F>300) compounds (bottom). The sizes of the bubbles are proportional to the Fisher ratio values.

allows to demonstrate that the compounds of the VP generated by cigarette A (cellulose acetate filter) are in volatile range equivalent to C_8–C_{11}, while the compounds of the VP generated by cigarette B (active carbon/cellulose acetate) are in volatile range equivalent to C_5–C_8.

The supervised fisher ratio (F) calculation is a very useful tool to identify the potential chemical difference between sample classes. This approach has been successfully applied in various fields [1,23]. Fisher ratios were calculated for all the 1480 regions generated from the template for the two cigarette types. The F value was based on the percent response determined by calculating a mean area and further normalised against the sum of all areas. Fig. 4A shows the two-dimensional (mean ^1t_R vs. mean $^2t_{R)}$ Fisher ratio plot for percent responses of compounds detected in the 24 GC × GC chromatograms. The sizes of the bubbles are proportional to the Fisher ratio values for detected compounds; the higher the F value, the greater the differences between the two classes. Based on a cut-off strategy we previously reported [1], we decided to apply a high F value threshold of 300 (F_{crit} value of 7.95 at significance level $\alpha = 0.01$) in order to reduce the data set and, hence find the compounds with the biggest influence on differences between the two cigarette types. A total of 91 compounds showing the biggest influence on the difference between the two cigarettes types (Fig. 4B) were thus extracted from over 1000 compounds (see Table S-2).

As part of the quest for developing visualisation strategies to highlight reduced or increased analyte concentrations when different classes of samples are considered, we also report the results under the form of bubble plots that represent the difference in peak area means for classes. In this case, areas of analytes from the type B class were subtracted to areas of analytes from the type A class

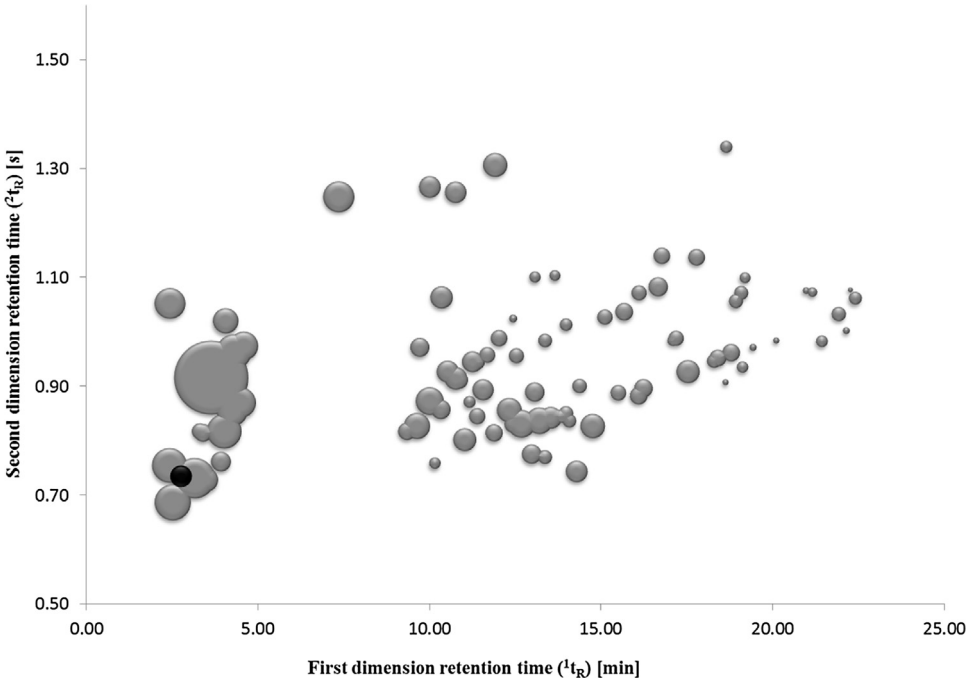

Fig. 5. TD-GC×GC-TOFMS two-dimensional plot showing differences in peak area means for the selected 91 compounds of the two classes of samples investigated. Mean differences are proportional to the size of the bubbles; positive differences are shown as grey bubbles while negative are shown as black ones.

(Fig. 5). For 90 of the 91 compounds with an F value >300, this difference was positive (grey bubbles). This clearly illustrates the effect of using different construction of filters on the composition of the MTS-VP, confirming the trapping capacity of carbon-based materials for selected volatile analytes. Table S-2 lists the aforementioned (91) tentatively identified compounds in terms of their F-value, molecular weight, and mean peak areas for both classes of cigarettes and their ratios. Out of the 91 compounds 74 compounds were tentatively identified and the remaining compounds were unassigned due to the poor MS library match.

4. Conclusions

A thermal desorption comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (TD-GC × GC-TOFMS) method was developed for the analysis of mainstream tobacco smoke vapour phase (MTS-VP). To our best knowledge this is the first report on the use of a TD-GC × GC-TOFMS method for the analysis of MTS-VP samples. It was applied for the qualitative analysis of MTS-VP constituents of a reference cigarette type where a total of 130 peaks were reliably identified with high precision using interactive LRI and MS library matching. This approach shows that collection, extraction and concentration of MTS-VP constituents can reproducibly be carried out in a single step and yields high quality chromatographic data and mass spectra that allow to gain confidence in the identification of the isolated analytes. To demonstrate the application of the method and expand on previous PP study [1], our method was used for the analysis of two cigarette types that differed in their filter construction. Supervised statistical analyses permitted to clearly differentiate the two types of samples based on their MTS-VP compositions. The refinement of the Fisher ratio values using the $F_{critical}$ cut-off approach allowed to highlight 91 compounds having the biggest influence on the differences between the two types of cigarettes. The use of various bubble plots that represent the difference in peak area means for classes allowed to clearly visualise variations of analytes quantities between the different classes of samples.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.chroma.2017.10.013.

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