Development of two different methods to characterize the chemical composition of cigarette mainstream smoke.

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Abstract

In order to characterize the main stream smoke while preserving its chemical compositions, two different approaches were developed. The first method is based on the trapping of the whole smoke at -183°C followed by the quantification of several smoke components using selected analytical techniques. The trapping is performed with a cryogenic instrument enabling a precise control of the temperature down to values as low as -190°C. After the trapping, the condensed smoke is diluted with a solvent and selected smoke components can be quantified using Gas Chromatography-Mass Spectrometry and Liquid Chromatography. The results obtained when trapping the main stream smoke of the 2R4F reference cigarette at -183°C were compared with those obtained by the standard methods. A good agreement was observed between the two approaches for 17 analytes, including some aldehydes, olefins, aromatic compounds and poly-aromatic compounds.

The second method was designed to analyze the chemical composition of a single puff using Gas Chromatography-Mass Spectrometry. The smoke is sampled with a gas syringe, while the puff is being drawn. In order to evaluate the maximum number of puffs during one smoking run, an Ultra-Fast Gas Chromatograph-Time of Flight-Mass Spectrometer was used to analyze the smoke composition. The Ultra-Fast module enabled to analyze VOCs from isoprene to diterpenes in 1 minute. The evolution of the puff composition in the course of a smoking run was studied for the 2R4F reference cigarette, on components of both the gas and the particulate phases.

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Quantitative analysis of the whole cigarette smoke trapped at -183°C

Approximately 4000 different compounds are found in mainstream smoke[1]. Mainstream smoke is distributed between the particulate phase and the vapor (gas) phase. In conventional analytical procedures, a quartz glass Cambridge pad is used to trap the smoke. It is generally accepted that the fraction of the mainstream smoke which is retained in the Cambridge filter (particles larger than 0.1µm) is the particulate phase, while the smoke which passes through the filter is defined as the gas-phase[2]. The Cambridge pad is often used to collect the particulate phase of mainstream smoke whereas an impinger is used for selective component determination in both particulate and vapor phases[3,4]. Other techniques such as electrostatic precipitation and jet impaction are also used to collect tobacco smoke[1,2]. These trapping techniques, which use a Cambridge pad and an impinger, often require a large number of cigarettes and cannot trap the whole smoke (vapor and particulate phase) when used separately. Furthermore, the use of a combined technique might induce a change in the puff profiles during smoking, which may modify the chemical composition of the mainstream smoke. An additional difficulty with these trapping techniques is the formation of artifacts[5]. The presence of many reactive compounds in the smoke might lead to the formation of new chemicals which were not present in the fresh smoke. To avoid these problems, the trapping of the mainstream smoke using liquid nitrogen was already tested in the 1950's[1]. However, when the trap was placed in liquid nitrogen, it acted as a pump and started to draw and condense oxygen. Therefore, the puff volume cannot be controlled and an accurate quantification was very difficult to achieve.

We wanted to develop a trapping method:

- Which avoids an uncontrolled modification of the chemical composition of the aerosol,
- Which is efficient for the particulate phase and for the gas phase,
- Which allows a good estimation of the concentration of the smoke components.

We designed a cryogenic instrument (see figure 1) which overcomes all the issues observed with a liquid nitrogen bath such as increase in the puff volume and oxygen condensation in the trap. This was achieved by decreasing the temperature to -183°C, which is just above the temperature of oxygen condensation. This temperature was obtained by heating the trap placed over liquid N_2. This permitted control of the puff volume and prevented oxygen condensation.

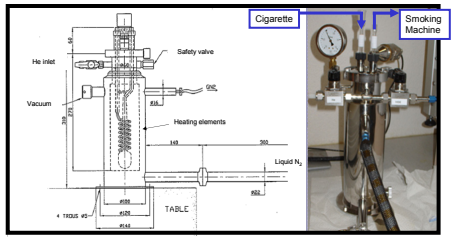

Figure 1: Cryogenic instrument

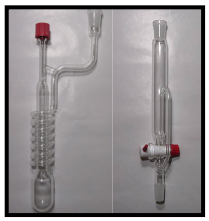

Figure 2: Trap and bubbler

At -183°C, the CO_2 and some other gases contained in the smoke are condensed. When reheating the trap after the smoking to collect the condensed smoke, some Volatile Organic Compounds (VOCs) such as acetaldehyde were carried away together with those gases.
In order to collect the VOCs a bubbler (see figure 2) containing a trapping solution (e.g. 2-diphenylacetyl-1,3-indandione-1-hydrazone (DPIH)) was connected to the spiral tubing part of the trap while the other exit of the trap was closed with a stopper. At the end of the bubbling, the trapping solution (5ml) was introduced into the trap to collect the remaining non-volatile fraction, and was collected for analysis.

Each determination was performed on one single 2R4F cigarette. The extract obtained at the end of the trapping process was shared between different analytical methods to quantify selected smoke components. In this case 3 different analytical methods were used to quantify these compounds: A GC-MS method to quantify the volatile and semi-volatile compounds, A GC-MS/MS method to analyze the PAHs and a LC-Fluorescence method to analyze the derivatized carbonyl compounds.

The results obtained when trapping the main stream smoke of the 2R4F reference cigarette at -183°C were compared with those obtained by the standard methods. A good agreement was observed (see Table 1).

compounds	Cryo-trapping 1st tests (n=5x1cig) Average µg/cig	CV %	Cryo-trapping 2nd tests (n=5x1cig) Average µg/cig	CV %	Standard methods Inter-laboratory test[6] Average µg/cig	CV(r) %	CV(R) %	p labs x q replic x n cig
Pyridine (GC-MS)	5	21	5.6	18	7.02	10	36	5x5x(5-20)
Styrene (GC-MS)	4.9	15	6	18	5.11	9	45	5x5x(5-20)
Nicotine (GC-MS)	775	7	695	11	750	5	6	6x8x5
o-cresol (GC-MS)	1.9	9	1.8	23	1.89	7	14	6x5x(5-20)
Phenol (GC-MS)	6.4	8	6.9	21	7.32	7	42	6x5x(5-20)
m+p-cresol (GC-MS)	5.7	9	5.2	19	5.84	15	25	6x5x(5-20)
1,3-Butadiene (GC-MS)	29.8	6	26.7	33	29.94	5	25	5x5x(5-20)
Isoprene (GC-MS)	364.8	11	291.3	17	297.68	4	26	5x5x(5-20)
Benzene (GC-MS)	48.6	2	37.4	10	43.39	3	17	5x5x(5-20)
Toluene (GC-MS)	73.6	5	68.9	12	64.91	4	33	5x5x(5-20)
Benzanthracene (GC-MS/MS)			0.013	24	0.0145	6	N/A	1x5x20
Benzo[a]pyrene (GC-MS/MS)			0.009	9	0.007	8	27	6x5x(5-20)
Formaldehyde (LC-Fluo)	24.7	21	22.2	23	21.61	10	14	5x5x(2-5)
Acetaldehyde (LC-Fluo)	513.3	15	459.2	14	560.48	5	15	5x5x(2-5)
Acetone (LC-Fluo)	308.3	4	244.6	11	264.74	5	5	5x5x(2-5)
Acrolein (LC-Fluo)	36	33	34	9	58.77	7	14	5x5x(2-5)
Propionaldehyde (LC-Fluo)	47	13	40.2	8	43.92	5	13	5x5x(2-5)

Table 1: Analysis of the 2R4F reference cigarette

Analysis of the chemical composition of a single puff using GC-MS

The understanding of the smoking process requires to follow the evolution of the puff composition within a smoking run and to compare different runs. It was shown[7] that the direct injection of cigarette smoke into a GC-MS enabled the characterization of a wide range of Volatile Organic Compounds (VOCs). However, due to the time required by the chromatography, it was not possible to analyze independently several puffs during the same smoking run. Recently, Ultra-fast Gas Chromatography was developed. This technique shortens considerably the analysis time and we decided to use it to characterize the puff composition.

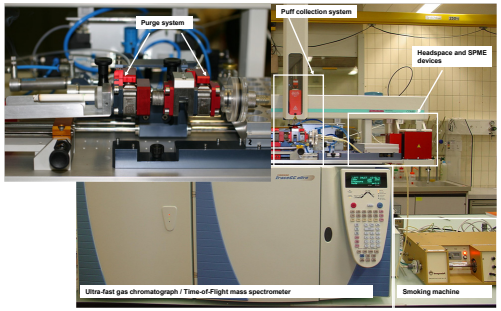

Figure 3: Ultra-fast GC-ToF-MS instrument

The ThermoFinnigan ® Ultra-fast GC-ToF-MS instrument used for this application presents the following characteristics:

- Reduced GC column dimensions (5m x 0.1mm i.d. x 0.1µm film thickness)
- Increased oven heating ramp (up to 1200°C/min.)
- Fast scanning MS instrument (up to 60 scans/s.)

This Ultra-Fast module enabled to separate VOCs from isoprene to neophytadiene in 1 minute (see Figure 4). The complete analytical process, including the purge of the system and the column cooling, took less than 3 minutes. Therefore, 1 puff out of 3 can be analyzed when using the ISO smoking regime (1 puff/min).

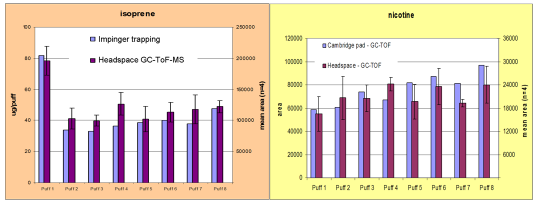

Figure 5: Puff profiles of the 2R4F reference cigarette; Comparison with reference methods.

We wanted to develop an analytical method:

- Enabling the analysis of very volatile products such as isoprene or acetaldehyde and of semi-volatile products such as nicotine or neophytadiene (a diterpene of tobacco),
- Which is fast enough to analyze several puffs during the same smoking run,
- Which can be used to analyze the whole smoke or the gas phase only.

In order to enable the on-line collection of the smoke during the smoking process, the ultra-fast GC-MS instrument was interfaced with a smoking machine. Both instruments were interfaced with a computer enabling the synchronization of the sampling during the smoking process (see Figure 3). The Cambridge filter holder could be placed either before or after the sampling system. This enabled the sampling of the gas phase only or of the whole smoke. A purge system was also installed in order to diminish the carry-over. The carry-over observed from one puff to the next for nicotine was about 10% only.

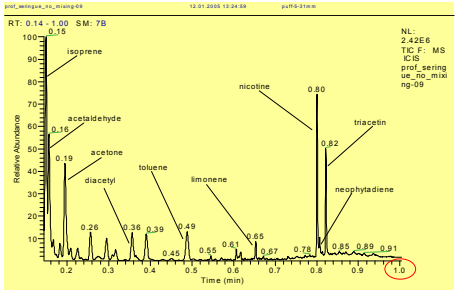

Figure 4: T.I.C. of the 5th puff of a 2R4F cigarette (DB-WAX column)

The sampling speed of the headspace syringe was adjusted in order to collect the smoke during all the puffing time (2 seconds). In order to check that the sampling did not bias the puff composition, the results obtained with this method were compared with those obtained by standard methods for the 2R4F reference cigarette: impinger trapping for the isoprene[8] (gas phase) and Cambridge filter for nicotine (particulate phase). A good correlation was observed with the standard methods (see Figure 5).

This analytical method was used to monitor more than 50 compounds present in cigarette puffs. The rapidity of the analysis enabled to analyze 1 puff out of 3 when using an ISO smoking regime. A minimal carry-over was observed and the sampling system did not bias significantly the composition of the gas phase and of the particulate phase of the smoke.

1) Dube, M.F. & Green, C.R., *Recent Advances in Tobacco Science*, **1982**, *8*, 42. *2) Tobacco, production, chemistry and technology, Edited by* D. Layten Davis and Mark T. Nielsen, **1999**, *Blackwell science, pp 412. 3)* Sakuma, H., Kusama, M., Shimokima, N., Sugawara, S., *Tobacco Science.* **1978**, *22*, 158. *4)* Vekris, S.L. & Hook, P.G., *20th Tobacco Chemistry Research Conference, Winston-Salem, North Carolina*, **1966**.
5) Schonherr, H., Klimisch, H.-J., Harke, H.-P., *Beitrage zur Tabakforschung Int*, **1973**, *7*, 18. *6)* Chen P .X., Moldoveanu, S., *Beitrage Zur Tabakforshung int.* **2003**, *20*, 448. *7)* Xue L., Thomas C.E, Koller K.B., *Beitrage zur Tabakforschung Int* **2002**, *20*, 251. *8)* Wagner K.A., Higby R., Stutt K., *Beitrage zur Tabakforschung Int* **2005**, *21*, 273.