Non-targeted screening using GC×GC-TOFMS for in-depth chemical characterization of aerosol from a heat-not-burn tobacco product

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Overview

Novel aspect: GC×GC-TOFMS-based non-targeted screening (NTS) applied for in-depth chemical characterization of aerosol from the Tobacco Heating System (THS) 2.2 (commercialized under the *IQOS*® brand name) using an automated data evaluation process that integrates structural identification, semi-quantification, and statistical comparison.

Results: A total of 384 compounds, excluding water, nicotine, and glycerin, were identified as being present in the aerosol of *IQOS* at a concentration of 100 ng/stick or greater (1,824 compounds in 3R4F[1]); of the 384 compounds, 220 (57.3%) were found exclusively in the nicotine-free dry particulate matter (NFDPM), 125 (32.6%) in the gas vapor phase (GVP), and 39 (10.2%) were found to be partitioned between both. More than 86% of all compounds identified were confirmed by reference standards.

Introduction and Objectives

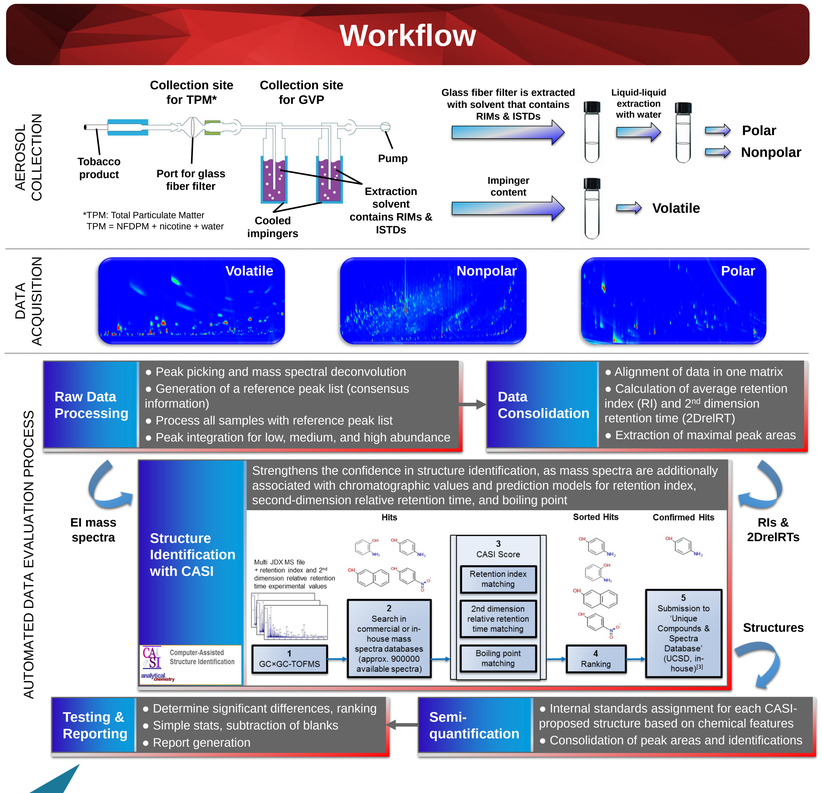
Comprehensive chemical characterization is key for the development of novel heat-not-burn tobacco products. Beyond the challenge of developing powerful analytical methods with sufficient spectral resolution, it is essential to have an automated data evaluation process in place.

GC×GC-TOFMS NTS uses multiple analytical methods to maximize the chemical space coverage. The structural identification process is streamlined with a computer-assisted structure identification (CASI)[2] platform, developed in-house, which improves the confidence level for compound identification and delivers semi-quantitative information for all compounds.

Goal:

- To cover the broadest possible chemical space amenable to GC×GC separation
- To obtain accurate structural proposals that can subsequently be confirmed by reference standards
- To give a detailed overview of the chemical composition of the *IQOS* aerosol

Workflow



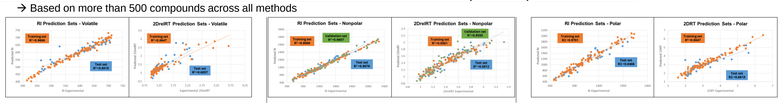
Analytical Methods

Volatile	Nonpolar	Polar
1. Chromatography and instrumental parameters		
Analytical columns 1D: DB-624UI 30m×0.25mm ID×1.40µm d_f 2D: DB-FFAP 2.4m×0.10mm ID×0.10µm d_f	Analytical columns DB-5MS 30m×0.25mm ID×0.25µm d_f DB-17HT 2.2m×0.10mm ID×0.10µm d_f	Analytical columns SLB-IL60/DB-FFAP 32m×0.25mm ID×0.25µm d_f VF-624MS 1.9m×0.15mm ID×0.84µm d_f
Temperature range 1D: -20°C → 230°C, 2D: 0°C → 235°C	Temperature range 1D: 30°C → 320°C, 2D: 35°C → 340°C	Temperature range 1D: 35°C → 250°C, 2D: 55°C → 285°C

Mass spectrometry: EI, full scan, data acquisition rate 200 spectra/s

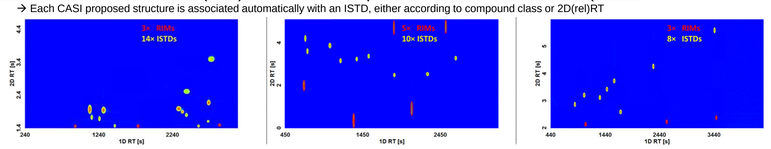
2. Prediction models for retention index and second-dimension (relative) retention time

→ Based on more than 500 compounds across all methods



3. Retention index markers (RIM) and stable isotope-labeled internal standards (ISTDs)

→ Each CASI proposed structure is associated automatically with an ISTD, either according to compound class or 2D(rel)RT



Results

1. Chromatography

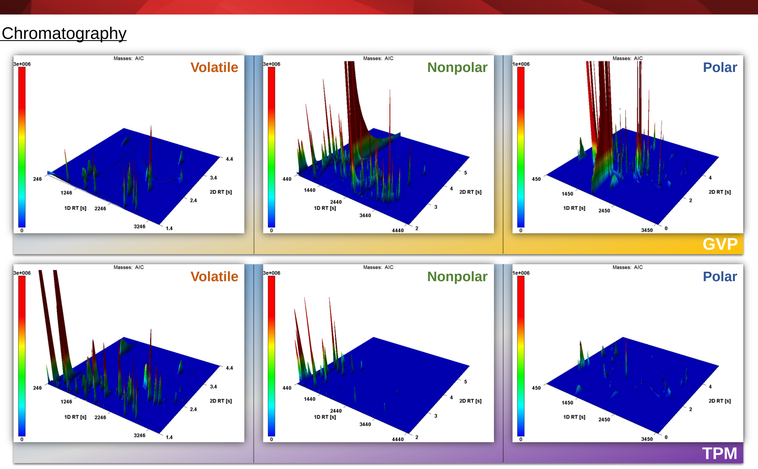
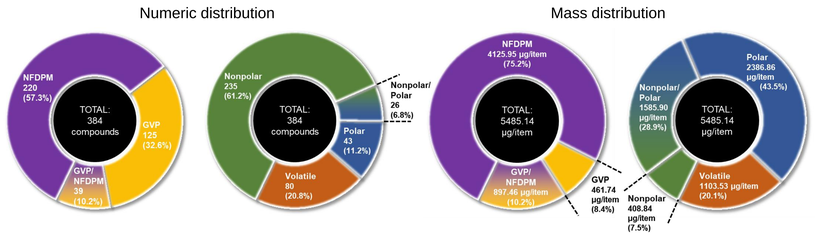


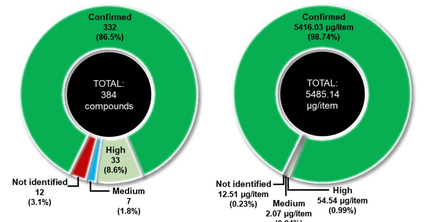
Figure 1. *GC×GC surface plots of the IQOS TPM (top) and GVP (bottom) aerosol fractions.*

2. The chemical composition of the *IQOS* aerosol – facts & figures

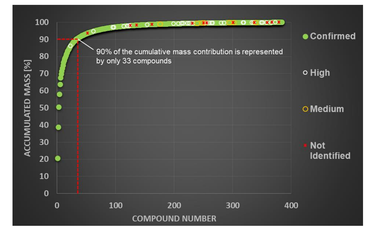
→ Aerosol was generated using the Health Canada Intense smoking regime[4]
→ Water and nicotine were extracted from the Cambridge filter pad, and their amounts were subtracted from the TPM to obtain a value for NFDPM
→ A 100 ng/stick cut-off limit was selected, which enabled an estimated 99.8% of the total aerosol mass determined by GC×GC-TOFMS to be evaluated



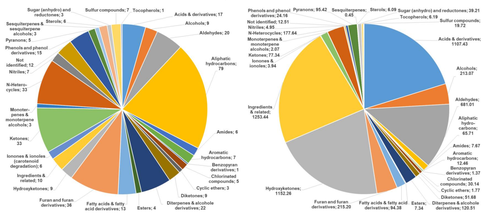
Compound identification distribution (numeric and mass)



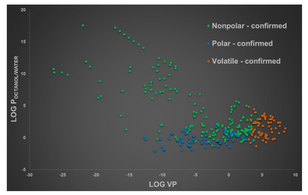
Cumulative mass of individual chemical constituents



Compound class distribution (numeric and mass [µg/item])



Log $P_{OW,\ predicted}$ versus log $VP_{\ predicted}$ plot for all confirmed compounds



3. Non-targeted differential screening (NTDS)[5] of *IQOS* aerosol versus 3R4F smoke

In a parallel investigation, the chemical composition of the *IQOS* aerosol was compared with 3R4F smoke using a NTDS approach. These data were reported to the FDA on December 8, 2017, as part of the Modified Risk Tobacco Product Application. A total of only 42 compounds (three unique) were significantly higher (p-value < 0.05) in the *IQOS* aerosol versus 3R4F smoke, whereas approximately 1,070 compounds were found to be elevated in 3R4F smoke compared with the *IQOS* aerosol.

Top 10 significantly elevated compounds in *IQOS* aerosol versus 3R4F smoke

Hit numbers, proposed compound names and structures found in the respective methods

CASI score, probability of the structure proposals and final confidence level

Semi-quantitative (N=3) values based on predefined rules, where ISTDs are allocated to corresponding compound classes or 2D(rel)RTs

Additional columns for more details on
CASI → RI/2DrelRT/BP deviation (pred vs exp/calc)
Structure → CAS/PMICODE/MW/Formula/Smiles
Significance → Rank, T-test
Compound present in Blank → Exclusion
Constituent Origin → Aerosol, Material, Plant, Flavor

#	Proposed compound name	Structure	Method	CASI Score	Probability	Confidence		SEMI-QUANTIFICATION IQOS Sum conc. [µg/test item]	SEMI-QUANTIFICATION 3R4F Sum conc. [µg/test item]	X-fold change	+ Identification details CASI	+ Structure identifier	+ Ranking / testing	+ BLANK	+ Origin / probable source	
											Retention Index / measured	CAS	Rank	Presence / absence	Aerosol (from database)	
1	Propylene glycol		Polar	928	HIGH	IDENTIFIED	Confirmed by REF STD	174.92	23.73	7.4	996.70	57-55-6	43786.26	-	x	
2	1-Hydroxy-2-propanone		Nonpolar	858	HIGH	IDENTIFIED	Confirmed by REF STD	161.80	96.80	1.7	589.87	116-09-6	4656.56	-	x	
3	2-Furanmethanol		Nonpolar	861	HIGH	IDENTIFIED	Confirmed by REF STD	39.18	7.00	5.6	814.86	98-00-0	1602.12	-	x	
			Polar	862	HIGH	IDENTIFIED	Confirmed by REF STD				1053.41	98-00-0	703.97	-	x	
4	2-Furancarboxaldehyde, 5-methyl-		Nonpolar	923	HIGH	IDENTIFIED	Confirmed by REF STD	11.10	2.94	3.8	963.74	620-02-0	1393.53	-	x	
			Polar	886	HIGH	IDENTIFIED	Confirmed by REF STD				984.98	620-02-0	3.07	-	x	
5	cis-4-Hydroxymethyl-2-methyl-1,3-dioxolane		Nonpolar	846	HIGH	IDENTIFIED	Confirmed by REF STD	2.09	0.04	47.4	881.28	3773-93-1	83.00	-	x	
			Polar	835	HIGH	IDENTIFIED	Confirmed by REF STD				1006.05	3773-93-1	961.67	-	x	
6	Butyrolactone		Nonpolar	917	HIGH	IDENTIFIED	Confirmed by REF STD	4.08	0.73	5.6	898.62	96-48-0	764.63	-	x	
			Polar	942	HIGH	IDENTIFIED	Confirmed by REF STD				1066.73	96-48-0	53.08	-	x	
7	Furfural		Nonpolar	884	HIGH	IDENTIFIED	Confirmed by REF STD	31.08	25.91	1.2	787.36	98-01-1	767.93	-	x	
8	4-Cyclopentene-1,3-dione		Nonpolar	723	MEDIUM	IDENTIFIED	Confirmed by REF STD	3.80	0.76	5.0	856.11	930-60-9	670.29	-	x	
9	2-Propanone, 1-(acetyloxy)-		Nonpolar	891	HIGH	IDENTIFIED	Confirmed by REF STD	16.92	8.01	2.1	833.48	592-20-1	569.52	-		
10	1-Hydroxy-2-butanone		Nonpolar	852	HIGH	IDENTIFIED	Confirmed by REF STD	0.95	0.47	2.0	704.76	5077-67-8	473.50	-	x	

Table 1. *Top 10 compounds that are significantly elevated in IQOS aerosol versus 3R4F smoke; sorted according to Rank[6].*

Conclusion

The GC×GC-TOFMS non-targeted screening workflow has been applied successfully to characterize the chemical composition of the *IQOS* aerosol. In total, 384 compounds (corresponding to a mass of 5.485 mg/item) were found to be present at a concentration of 100 ng/stick or greater, 220 (57.3%) in NFDPM, 125 (32.6%) in GVP, and 39 (10.2%) were found to be partitioned between both NFDPM and GVP. A total of 332 (86.5%) could be confirmed by reference standards so far (corresponding to a mass of 5.416 mg/item, 98.7%).

References

[1] Roemer, E., et al., *Contributions to Tobacco Research*, **2012**, 25, 316
[2] Knorr, A., et al., *Anal. Chem.*, **2013**, 85(23), 11216
[3] Martin, E., et al., *J. Cheminform.*, **2012**, 4, 11
[4] Official Method T-115, Determination of "Tar", Nicotine and Carbon Monoxide in Mainstream Tobacco Smoke, *Department of Health*, Canada, **1999**
[5] Almstetter, M., et al., *Researchgate*, **2016**, DOI10.13140/RG.2.2.32692.55680
[6] Knorr, A., *International Patent*, WO 2013098169 A1, PCT/EP2012/076244, **2013**, Jul 4

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Competing Financial Interest

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