Non-targeted differential screening of complex matrices using GC×GC-TOFMS for comprehensive characterization of the chemical composition and determination of significant differences

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Introduction

Non-Targeted Differential Screening (NTDS) represents a key methodology to not only comprehensively characterize the chemical composition of aerosol derived from different tobacco products, but also to determine the most relevant differences between complex matrices. Therefore, a complementary NTDS workflow has been developed using two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC×GC-TOFMS) in parallel with liquid chromatography coupled to high resolution accurate mass spectrometry (LC-HRAM-MS, see Poster TP 549, Arndt et al.). The approach maximizes the chemical space coverage with multiple analytical methods and determines significant differences based upon semi-quantitative information for all compounds. Together with a Computer-Assisted Structure Identification platform (CASI; in-house development[1]), which enhances the accuracy of compound structure identification and accelerates and standardizes the identification process, this approach facilitates the detection of novel compounds and differences in concentration.

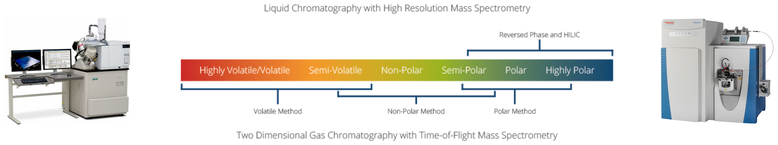
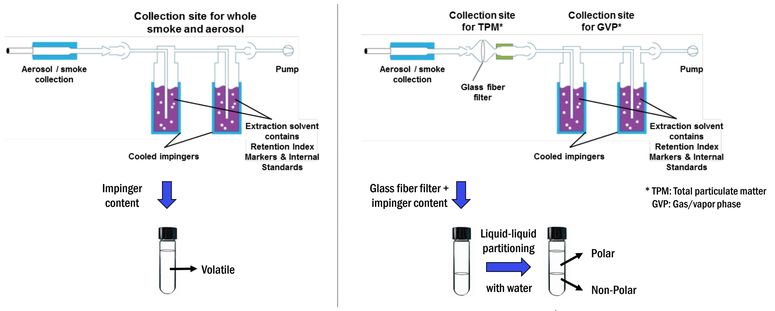


Figure 1. Analytical Coverage of Non-Targeted Differential Screening.

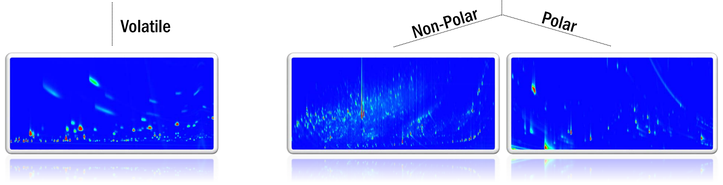
Experimental & Analytical Design

The experimental NTDS workflow comprises:

- Two type of sample collections for constituents that are highly volatile/volatile and non-polar/polar…



- and three analytical methods with direct cool on-column injection…



- …that are fully complementary and maximize the coverage of all GC amenable compounds.

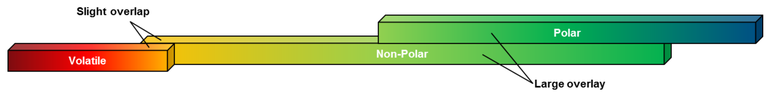


Figure 2. Experimental & Analytical Design.

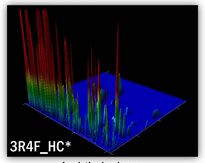
Advantages:

- Sample preparation is minimized to prevent changes in chemical composition
- Derivatization of polar constituents is avoided by introduction of a novel approach that enables direct injection of aqueous phases
- Compounds that are present in the non-polar and polar fractions can be summed as they derive from the same sample
- Each method contains a dedicated set of retention index markers and stable isotope-labeled internal standards
  → Extensive coverage of the chemical functionalities and separation space

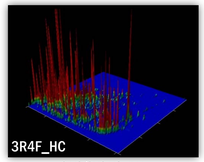
Analytical Methods

Volatile Method | Non-Polar Method | Polar Method

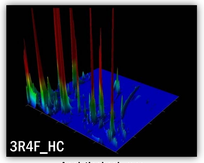
1. Chromatography and Instrumental Parameters



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
Temperature range
1D: 20°C → 230°C
2D: 0°C → 235°C



Analytical columns
DB-5MS 30m×0.25mm ID×0.25µm d_f
DB-17HT 2.2m×0.10mm ID×0.10µm d_f
Temperature range
1D: 30°C → 320°C
2D: 35°C → 340°C



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: 35°C → 250°C
2D: 55°C → 285°C

* 3R4F: Reference cigarette[2]
HC: Health Canada smoking regimen

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

Figure 3. Chromatography and Instrumental Parameters.

2. Prediction Models for Retention Index (RI) and 2nd Dimension (relative) Retention Time (2D(rel)RT)

→ based on a total of approx. 500 compounds across all methods

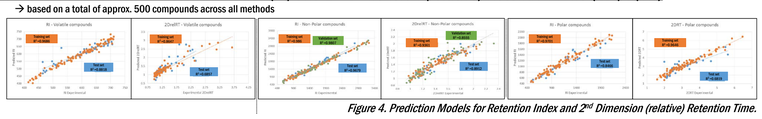


Figure 4. Prediction Models for Retention Index and 2nd Dimension (relative) Retention Time.

3. Retention Index Markers (RIMs) and Isotope Labeled Internal Standards (ISTDs)

→ RIMs are indicated in red, ISTDs in yellow

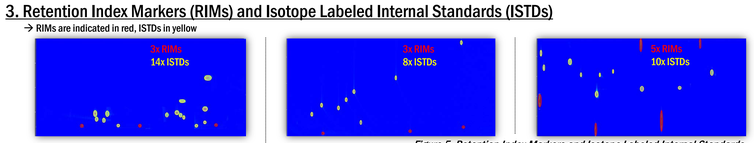
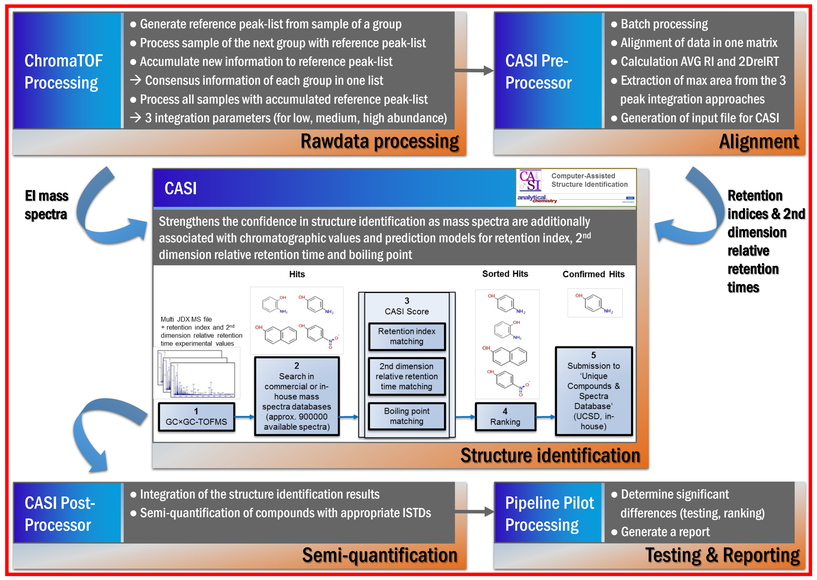


Figure 5. Retention Index Markers and Isotope Labeled Internal Standards.

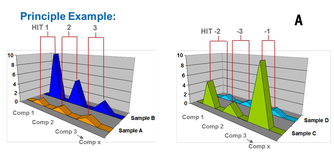
Automated Data Processing Workflow



Validation

Concept

- Addition of selected compounds in different amounts to TPM of a cigarette
- NTDS of fortified TPM vs. non-fortified TPM
- Comparison of experimentally generated HIT-list with theoretical HIT-list
- HIT-list is sorted by RANK formula[3] → considers relative differences in abundance of each compound as well as the absolute abundance



Validation Acceptability Criterion

- Correlation coefficient for the reciprocal theoretical HIT numbers and the experimentally found reciprocal HIT numbers > 0.98

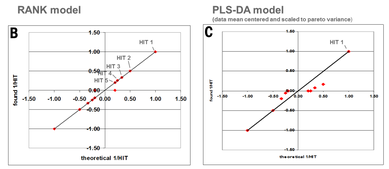


Figure 6. Principle of the Spike-in Experiment (A). Results of an in-house developed RANK Model (B) compared to a conventional PLS-DA Model (C).

Results

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

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

Semi-quantitative (N=3) values based on predefined rules, where internal standards are allocated to corresponding compound classes

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

							SEMI-QUANTIFICATION			Identification Details CASI	Structure Identifier	Ranking / Testing	BLANK	Origin / Probable Source
Hit #	Proposed compound name	Structure	Method	CASI Score	Probability	Confidence	Tobacco Product 1 Sum conc. [µg/test item]	Tobacco Product 2 Sum conc. [µg/test item]	X-fold change	Retention Index measured	PMICODE	Rank	Presence / Absence	Aerosol (from database)
1	Propylene glycol		Polar	927.53	HIGH	IDENTIFIED Confirmed by REF STD	23.73	174.92	7.4	996.70	PM0001952	43786.26	-	x
2	trans-4-hydroxymethyl-2-methyl-1,3-dioxolane		Polar	834.74	HIGH	HIGH Agreed	0.00	2.09	unique	1006.05		961.67	-	x
			Non-Polar	846.2	HIGH	HIGH Agreed				881.28		83.00	-	x
3	2-Furanmethanol		Non-Polar	861.1	HIGH	IDENTIFIED Confirmed by REF STD	1.12	8.79	7.8	814.86	PM0000003	1602.12	-	x
			Polar	862.38	HIGH	IDENTIFIED Confirmed by REF STD				1053.41	PM0000003	703.97	-	x
4	2(5H)-Furanone		Non-Polar	885.1	HIGH	IDENTIFIED Confirmed by REF STD	1.99	5.32	2.7	898.40	PM0000431	272.99	-	x
			Polar	931.19	HIGH	IDENTIFIED Confirmed by REF STD				1180.30	PM0000431	83.66	-	x
5	Butyrolactone		Non-Polar	917.2	HIGH	IDENTIFIED Confirmed by REF STD	0.73	4.08	5.6	898.62	PM0000396	764.63	-	x
			Polar	942.23	HIGH	IDENTIFIED Confirmed by REF STD				1066.73	PM0000396	53.08	-	x
6	2-Furancarboxaldehyde, 5-methyl-		Non-Polar	922.9	HIGH	IDENTIFIED Confirmed by REF STD	2.94	11.10	3.8	963.74	PM0000001	1393.53	-	x
			Polar	885.66	HIGH	IDENTIFIED Confirmed by REF STD				984.98	PM0000001	3.07	-	x
7	1-Hydroxy-2-propanone		Non-Polar	858.1	HIGH	IDENTIFIED Confirmed by REF STD	6.96	26.65	3.8	589.87	PM0000221	3374.74	-	x
8	Furfural		Non-Polar	883.7	HIGH	IDENTIFIED Confirmed by REF STD	26.86	47.21	1.8	787.36	PM0000002	767.93	-	x
9	2-Cyclopentene-1,4-dione		Non-Polar	723.0	MEDIUM	IDENTIFIED Confirmed by REF STD	0.76	3.80	5.0	856.11	PM0000429	670.29	-	x
10	2-Propanone, 1-(acetyloxy)-		Non-Polar	891.2	HIGH	HIGH Agreed	8.01	16.92	2.1	833.48	PM0003012	569.52	-	

Figure 7. Example and Description of a Fused Data Result Table.

Conclusion & Outlook

- The high quality EI mass spectra generated by GC×GC-TOFMS, combined with an enhanced and automated structural identification and semi-quantification workflow, presents a powerful and robust platform for non-targeted assessment of complex matrices
- In total, the NTDS workflow has tentatively identified and semi-quantified more than 3000 constituents in smoke of a cigarette across the different methods
- NTDS is mainly used to compare aerosol of tobacco products, but can also be applied to a variety of other matrices

Next steps:
→ Transfer existing methodologies to GC×GC-HR-TOFMS and establish a workflow for structure elucidation of unknown compounds

References

1) Knorr, A., Monge, A., Stueber, M., Stratmann, A., Arndt, D., Martin, E., Pospisil, P., Computer-Assisted Structure Identification (CASI) - An Automated Platform for High-Throughput Identification of Small Molecules by Two-Dimensional Gas Chromatography Coupled to Mass Spectrometry, Anal Chem. 2013, 85(23):11216-24.
2) University of Kentucky (www.3r4f.com), (http://www2.ca.uky.edu/refcig/3R4F%20Preliminary%20Analysis.pdf)
3) Knorr, A., A method of analyzing data from chemical analysis, International Patent WO 2013098169 A1, PCT/EP2012/076244, 2013, Jul 4.

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