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Non-targeted chemical characterization of complex matrices using nominal and high resolution accurate mass GC×GC-TOFMS

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2020 Online CORESTA Congress

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12 October - 12 November 2020

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Non-targeted Screening Talks by PMI R&D @CORESTA

ST 17 *1682*
The dos and don'ts of non-targeted screening by LC–HRAM-MS for chemical characterization of smoke-free products
WACHSMUTH C.; ARNDT D.; BUCHHOLZ C.; BENTLEY M.; GOUJON C.
Philip Morris Products S.A., PMI R&D, Quai Jeanrenaud 5, CH-2000 Neuchâtel, Switzerland

ST 18 *1683*
Computer-assisted structure identification (CASI) for high-throughput identification of small molecules by GC×GC–HRAM-TOFMS
KNORR A.; ALMSTETTER M.; MARTIN E.; CASTELLON A.; POSPISIL P.; BENTLEY M.; GOUJON C.
Philip Morris Products S.A., PMI R&D, Quai Jeanrenaud 5, CH-2000 Neuchâtel, Switzerland

ST 19 *1703*
Non-targeted chemical characterization of complex matrices by nominal- and high-resolution accurate-mass GC×GC–TOFMS
ALMSTETTER M.; KNORR A.; RHOUMA M.; MARTIN E.; CASTELLON A.; POSPISIL P.; BENTLEY M.; GOUJON C.
Philip Morris Products S.A., PMI R&D, Quai Jeanrenaud 5, CH-2000 Neuchâtel, Switzerland

ST 21 *1799*
Untargeted chemical characterization of the aerosol generated by a heated tobacco product
BENTLEY M.; ALMSTETTER M.; ARNDT D.; KNORR A.; MARTIN E.; POSPISIL P.; MAEDER S.
Philip Morris Products S.A., PMI R&D, Quai Jeanrenaud 5, 2000 Neuchâtel, Switzerland

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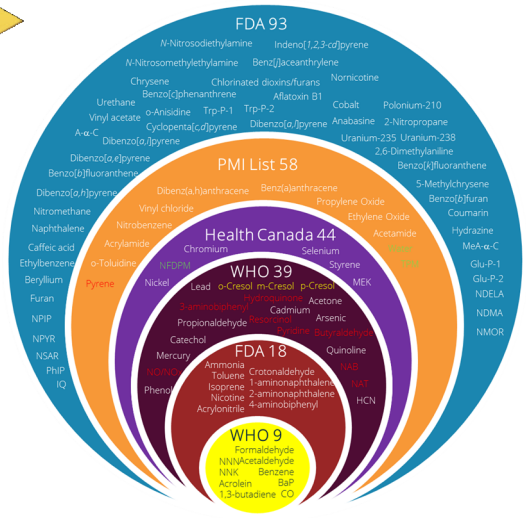
Why Non-Targeted Screening?

Quantitative analysis of HPHCs

PMI List 58 analyzed routinely in our labs by using validated and accredited methods in a GLP-certified environment



*HPHCs: Harmful and potentially harmful constituents

**THS 2.2: Tobacco Heating System 2.2, a heated tobacco product developed by Philip Morris Products S.A. and commercialized under the brand name *IQOS*®

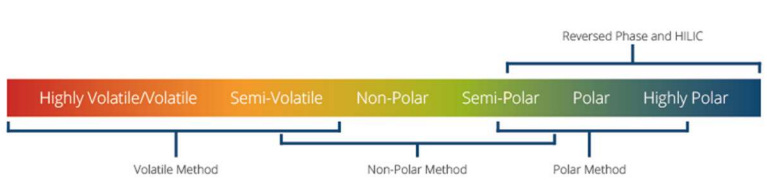
Reduction (%) of THS 2.2 vs. 3R4F

	Stick basis	
	Regular	Menthol
PMI 58 (54)	>92	>93
FDA 93 (107)	>90.5	>91.0

Schaller et al. *Regul Toxicol Pharmacol.* 2016

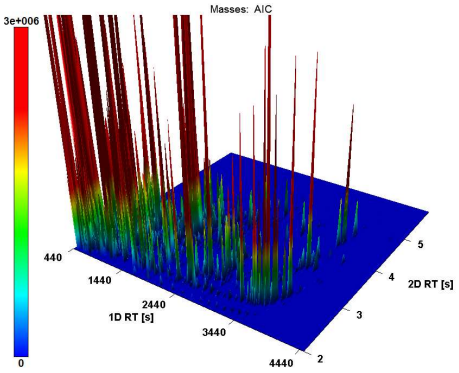
Non-Targeted Screening (NTS) of aerosol/smoke

- Non-targeted methods developed to deliver maximum coverage of the chemical space related to tobacco product aerosols
- Analytical methods, complementary by nature, are based on two-dimensional gas chromatography with time-of-flight mass spectrometry (GC×GC-TOFMS) and liquid chromatography with high-resolution accurate mass spectrometry (LC-HRAM-MS)

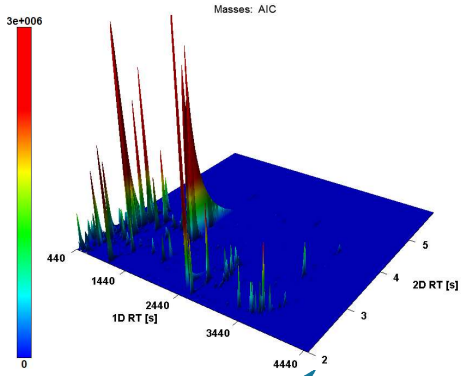


Heating rather than burning simplifies aerosol chemistry

Cigarette smoke (3R4F)



THS 2.2 aerosol



Example: GC×GC-TOFMS, Nonpolar method

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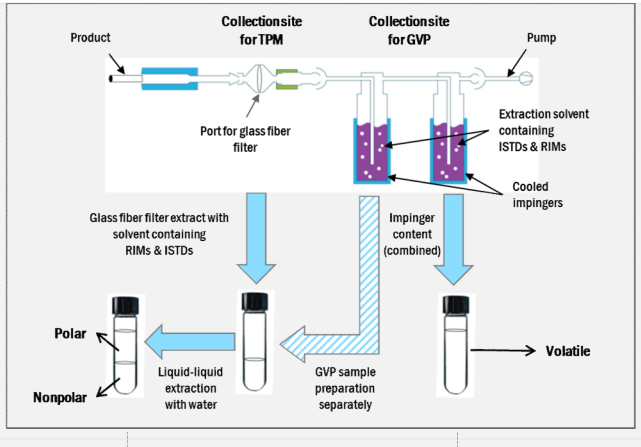
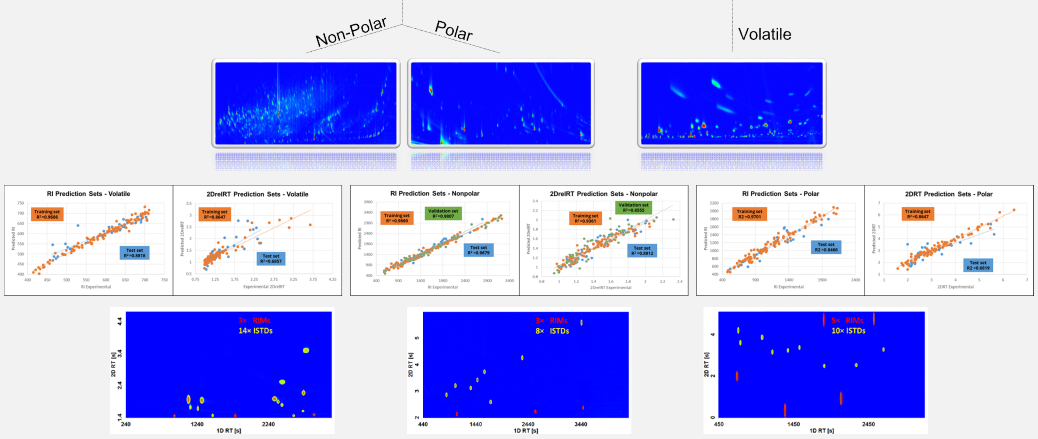
Overview of the Routine Workflow

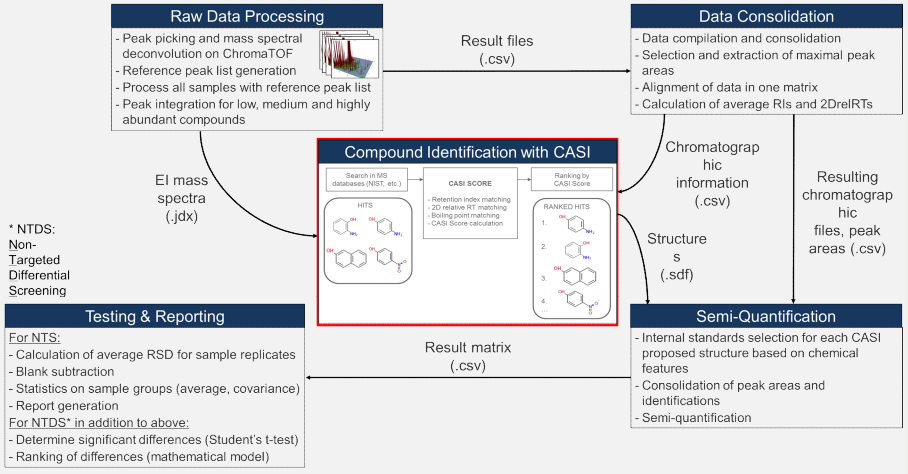
1. Aerosol Generation



2. Analytical Methods



3. Data Processing



Knorr, A., Almstetter, M. et al., "Performance evaluation of a non-targeted platform using GC×GC-TOFMS integrating computer-assisted structure identification and automated semi-quantification for the comprehensive chemical characterization of a complex matrix*", Analytical Chemistry, Jun 2019*
https://pubs.acs.org/doi/10.1021/acs.analchem.9b01659

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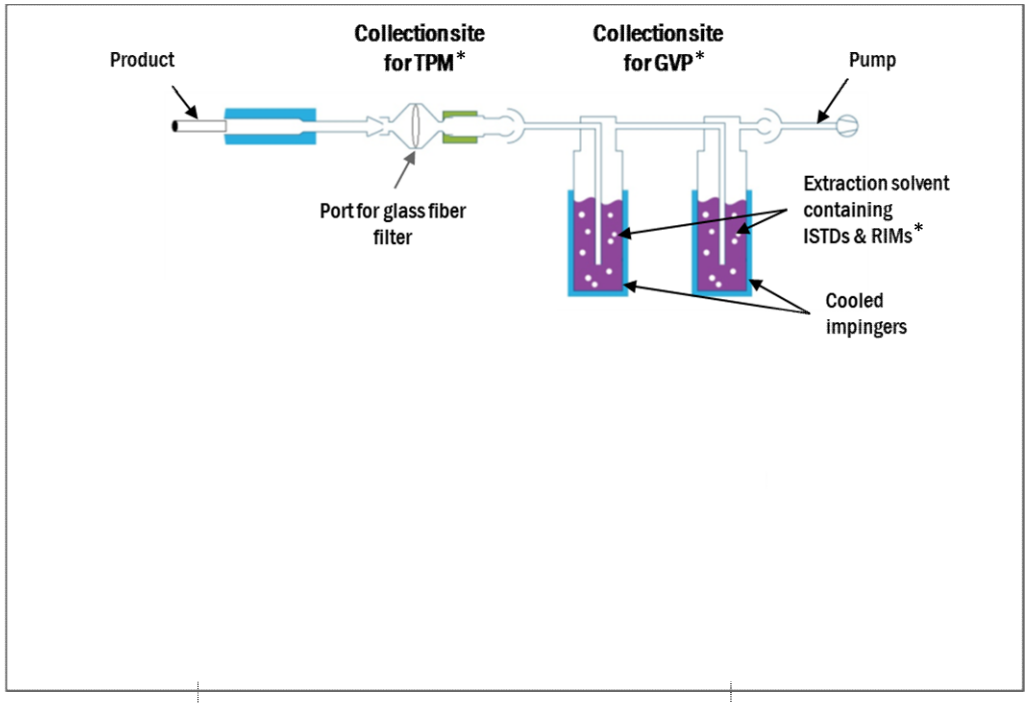
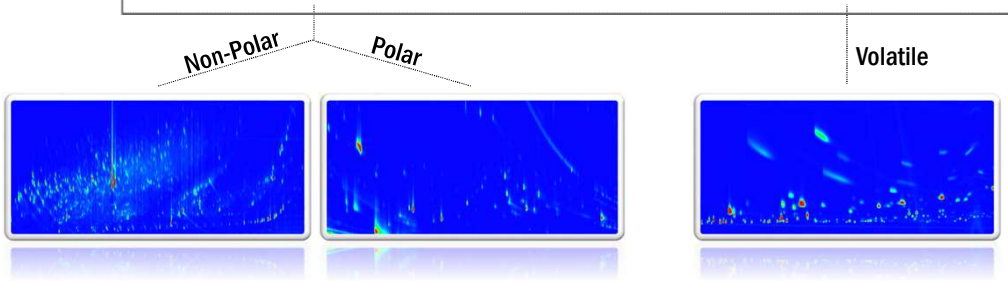
Routine Workflow

1. Aerosol Generation & Sample Preparation





*TPM: Total Particulate Matter

*GVP: Gas Vapor Phase

*ISTD: Internal Standard

*RIM: Retention Index Marker

Advantages:

- Sample preparation is minimized to prevent changes in chemical composition
- Direct injection of aqueous phases, thereby negating the requirement for derivatization of semi-polar/polar constituents
- Compounds that are present in the nonpolar and polar fractions can be summed as they derive from the same sample
- Each analytical method contains a dedicated set of retention index markers and stable isotope-labeled internal standards, which are representative of the anticipated range of chemical species present within the defined separation space

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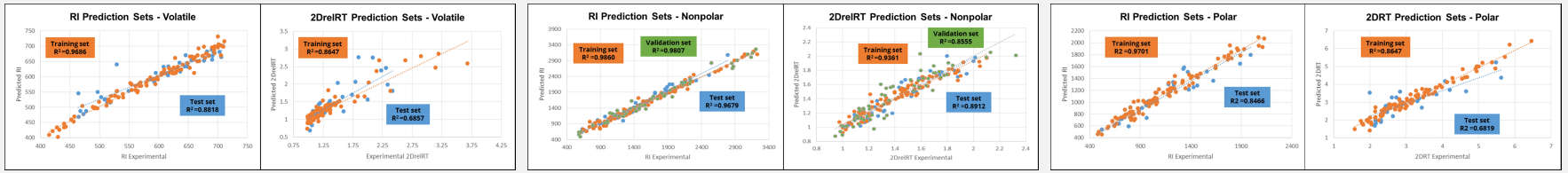
Routine Workflow

2. Analytical Methods

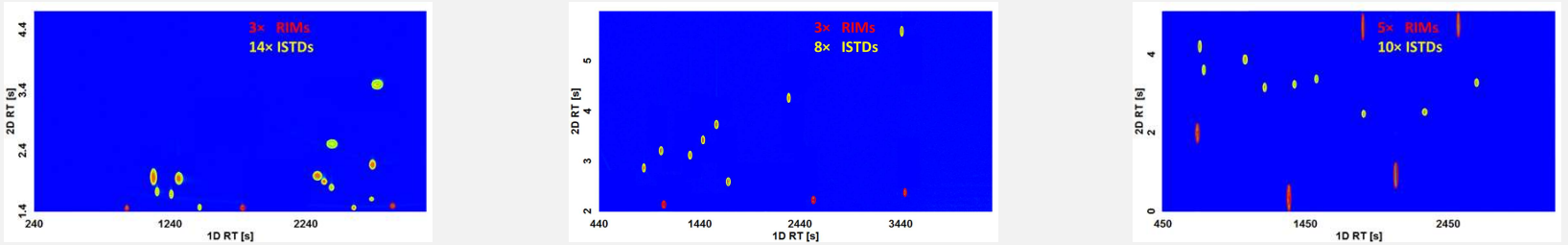
	Volatile	Nonpolar	Polar
Analytical columns			
	1D: DB-624UI 30m×0.25mm ID×1.40µm d_f 2D: DB-FFAP 2.4m×0.10mm ID×0.10µm d_f	DB-5MS 30m×0.25mm ID×0.25µm d_f DB-17HT 2.2m×0.10mm ID×0.10µm d_f	SLB-IL60/DB-FFAP 32m×0.25mm ID×0.25µm d_f VF-624MS 1.9m×0.15mm ID×0.84µm d_f
Temperature range			
	1D: -20°C → 230°C,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

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

Instrumental parameters



Prediction Models



Retention Index and Semiquantification

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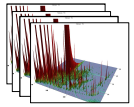
Routine Workflow

3. Data Processing

Raw Data Processing

- Peak picking and mass spectral deconvolution on ChromaTOF
- Reference peak list generation
- Process all samples with reference peak list
- Peak integration for low, medium and highly abundant compounds



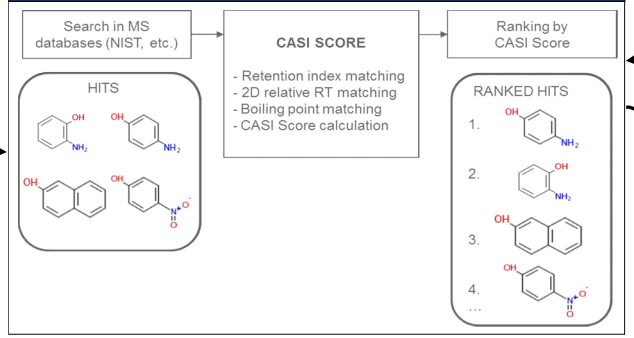
Result files (.csv)

Data Consolidation

- Data compilation and consolidation
- Selection and extraction of maximal peak areas
- Alignment of data in one matrix
- Calculation of average RIs and 2DrelRTs

EI mass spectra (.jdx)

Compound Identification with CASI



Chromatographic information (.csv)

Resulting chromatographic files, peak areas (.csv)

Structures (.sdf)

* CASI: Computer-assisted structure identification

* NTDS: Non-Targeted Differential Screening

Semi-Quantification

- Internal standards selection for each CASI proposed structure based on chemical features
- Consolidation of peak areas and identifications
- Semi-quantification

Result matrix (.csv)

Testing & Reporting

For NTS:
- Calculation of average RSD for sample replicates
- Blank subtraction
- Statistics on sample groups (average, covariance)
- Report generation

For NTDS* in addition to above:
- Determine significant differences (Student's t-test)
- Ranking of differences (mathematical model)

Knorr, A., et al., "Computer-Assisted Structure Identification (CASI)…", Analytical Chemistry, Oct 2013 https://pubs.acs.org/doi/abs/10.1021/ac4011952

Knorr, A., Almstetter, M. et al., "Performance evaluation of a non-targeted platform using GC×GC-TOFMS….", Analytical Chemistry, Jun 2019 https://pubs.acs.org/doi/10.1021/acs.analchem.9b01659

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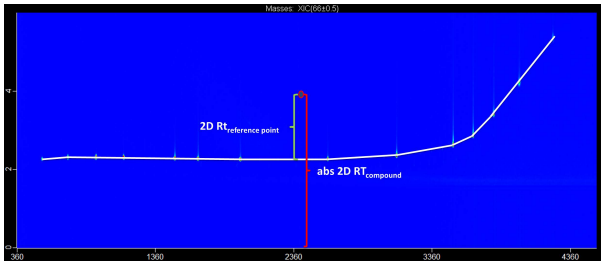
Method Development

- Aim is to continuously improve our methodologies and workflows

→ Transfer of the routine workflow to state-of-the-art high resolution accurate mass instrumentation

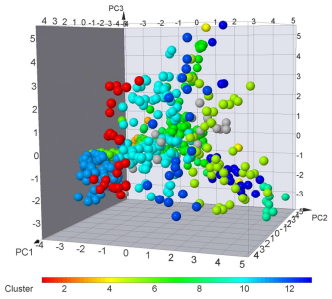
1. Expansion of the Retention Index System

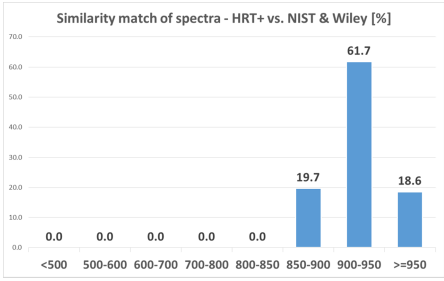
- Deuterated n-alkanes are used to generate a hypothetical second dimension retention time reference system compensating for systematic shifts
- Second dimension relative retention time (2DrelRT) is calculated from second dimension peaks in relation to hypothetical reference points based upon linear regressions of deuterated n-alkanes



2. Data for Retention Time Prediction models

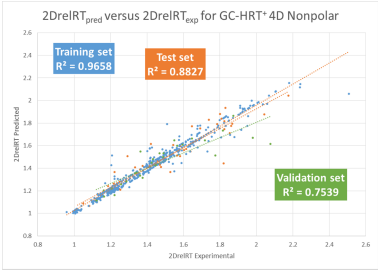
- Injection of reference material
- Registration of chromatographic and MS information under predefined quality criteria in UCSD*
- Clustering of compounds
- Enhancement of the clusters

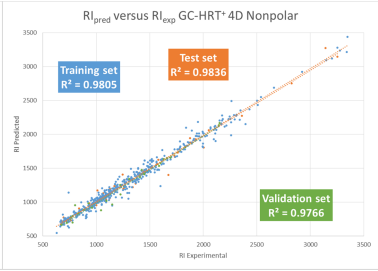


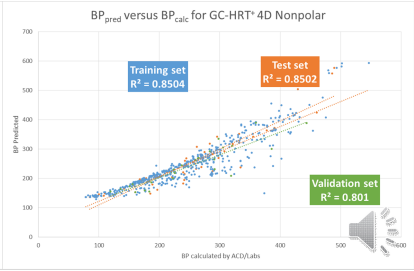


3. Retention Time Prediction

- Quantitative structure-property relationship (QSPR) models
- Compounds were split into training, test and validation set
- Descriptors for Retention Index (28), 2DrelRT (25) and Boiling Point (BP)
- Predicted parameters are enhancing the confidence for correct compound identification



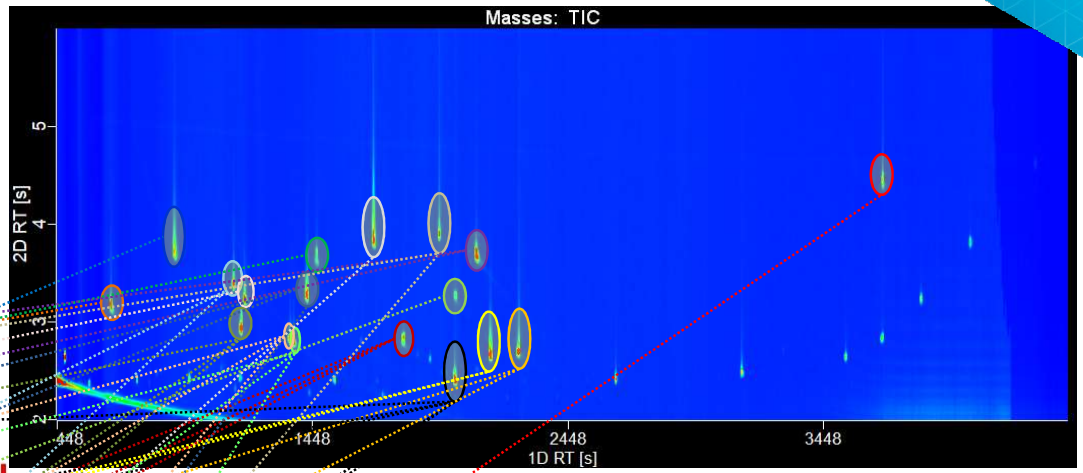


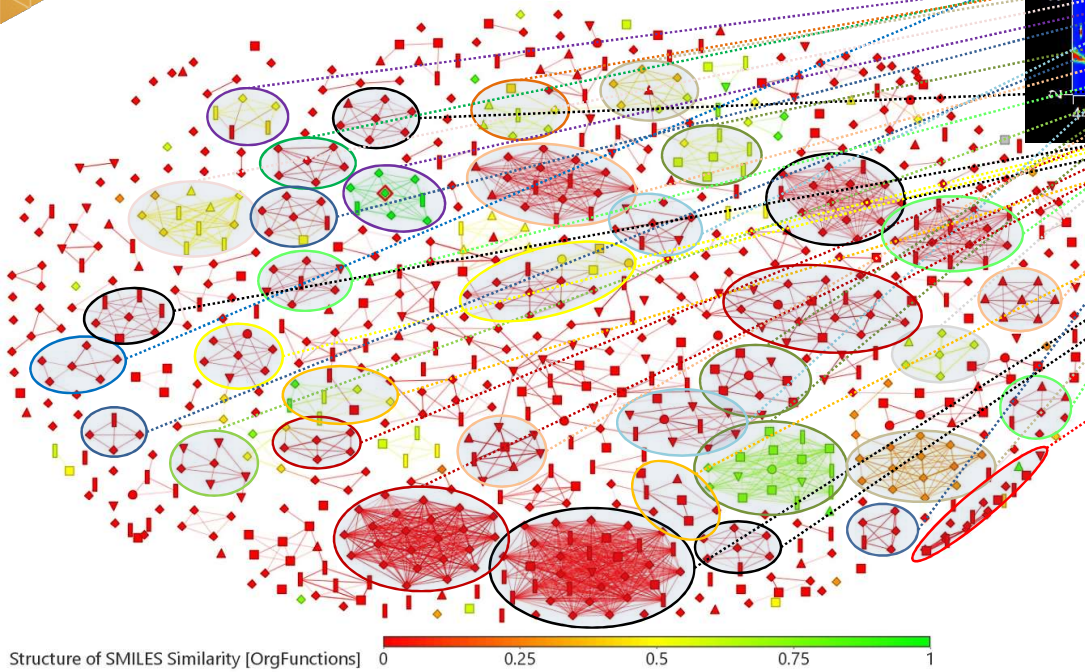


Method Development

4. Semiquantification

- Data from various aerosol matrices
- Cluster map of structural similarities
- Association of clusters to isotope-labeled internal standards
- Robustness of ISTDs





- Rules based on functional group priorities according to IUPAC
- CASI automatically assigns ISTDs based on structure proposal and calculates the semi-quantitative concentration

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Applications

Analytical and Bioanalytical Chemistry (2020) 412:2675–2685
https://doi.org/10.1007/s00216-020-02502-1

RESEARCH PAPER

Check for updates

Comprehensive chemical characterization of the aerosol generated by a heated tobacco product by untargeted screening

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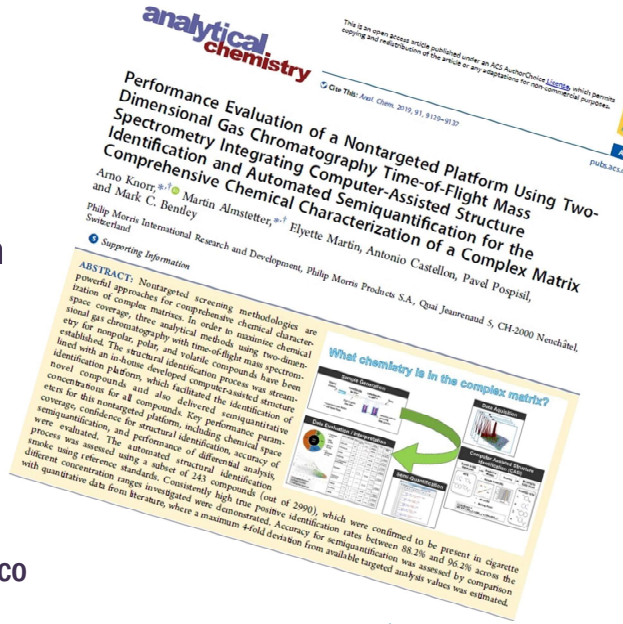
Abstract
A suite of untargeted methods has been applied for the characterization of aerosol from the Tobacco Heating System 2.2 (THS2.2), a heated tobacco product developed by Philip Morris Products S.A. and commercialized under the brand name IQOS®. A total of 529 chemical constituents, excluding water, glycerin, and nicotine, were present in the mainstream aerosol of THS2.2, generated by following the Health Canada intense smoking regimen, at concentrations ≥ 100 ng/item. The majority were present in the particulate phase (*n* = 402), representing more than 80% of the total mass determined by untargeted screening; a proportion were present in both particulate and gas-vapor phases (39 compounds). The identities for 80% of all chemical constituents (representing > 96% of the total determined mass) were confirmed by the use of authentic analytical reference materials. Despite the uncertainties that are recognized to be associated with aerosol-based untargeted approaches, the reported data remain indicative that the uncharacterized fraction of TPM generated by THS2.2 has been evaluated to the fullest practicable extent. To the best of our knowledge, this work represents the most comprehensive chemical characterization of a heated tobacco aerosol to date.

Comprehensive Chemical Characterization of THS2.2 aerosol with GC×GC-TOFMS

- Aerosol was generated using the Health Canada Intense smoking regime
- Water, nicotine, and glycerin were determined by using separate quantitative methods
- Three confidence categories - high, medium and not identified
- A 100 ng/stick cut-off limit was selected



analytical chemistry

Performance Evaluation of a Nontargeted Platform Using Two-Dimensional Gas Chromatography Time-of-Flight Mass Spectrometry Integrating Computer-Assisted Structure Identification and Automated Semiquantification for the Comprehensive Chemical Characterization of a Complex Matrix

Arno Knorr,*[,†] Martin Almstetter,*[,†] Elyette Martin, Antonio Castellon, Pavel Pospisil, and Mark C. Bentley

Philip Morris International Research and Development, Philip Morris Products S.A., Quai Jeanrenaud 5, CH-2000 Neuchâtel, Switzerland

Supporting Information

ABSTRACT: Nontargeted screening methodologies are powerful approaches for comprehensive chemical characterization of complex matrices. In order to maximize chemical space coverage, three analytical methods using two-dimensional gas chromatography with time-of-flight mass spectrometry for nonpolar, polar, and volatile compounds have been established. The structural identification process was streamlined with an in-house developed computer-assisted structure identification platform, which facilitated the identification of novel compounds and also delivered semiquantitative concentrations for all compounds. Key performance parameters for this nontargeted platform, including chemical space coverage, confidence for structural identification, accuracy of semiquantification, and performance of differential analysis were evaluated. The automated structural identification process was assessed using a subset of 245 compounds (out of 2990), which were confirmed to be present in cigarette smoke using reference standards. Consistently high true positive identification rates between 88.2% and 96.2% across the different concentration ranges investigated were demonstrated. Accuracy for semiquantification was assessed by comparison with quantitative data from literature, where a maximum 4-fold deviation from available targeted analysis values was estimated.

Non-targeted Differential Screening (NTDS) of THS 2.2 aerosol versus 3R4F smoke with GC×GC-TOFMS

- Chemical composition of the THS 2.2 aerosol was compared with 3R4F smoke
- Differences were revealed by an empirically developed mathematical model that considers relative abundance of all detected constituents as well as their semi-quantitative estimate of the absolute abundance (Knorr, A., International Patent WO 2013098169 A1, PCT/EP2012/076244, 2013.Jul 4)
- These data were reported to the FDA on December 8, 2017, as part of the Modified Risk Tobacco Product Application

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Non-Targeted Screening

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