The dos and don'ts of non-targeted screening by LC–HRAM-MS for chemical characterization of smoke-free products

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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
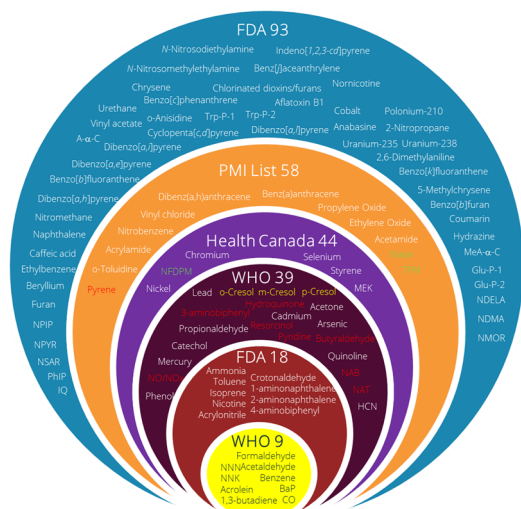BENTLEY M.; ALMSTETTER M.; ARNDT D.; KNORR A.; MARTIN E.; POSPISIL P.; MAEDER S.
Philip Morris Products S.A., PMI R&D, Quai Jeanrenaud 5, 2000 Neuchâtel, Switzerland

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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

*Harmful and potentially harmful constituents

**THS2.2: 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	>92	>93
FDA 93	>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 by using an unbiased approach
- 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)
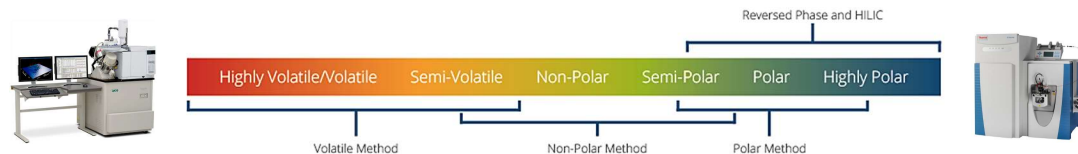
Automation by using chemoinformatics tools is highly important

Metabolomics Software (used with LC-HRAM-MS)

Unique Compound and Spectra Database

- Tobacco-specific in-house database (Martin et al. *J Cheminform* 2012)
- 630 MS[2] spectra of tobacco-related standards
- Determination of chemical classes
- Computation of physicochemical properties
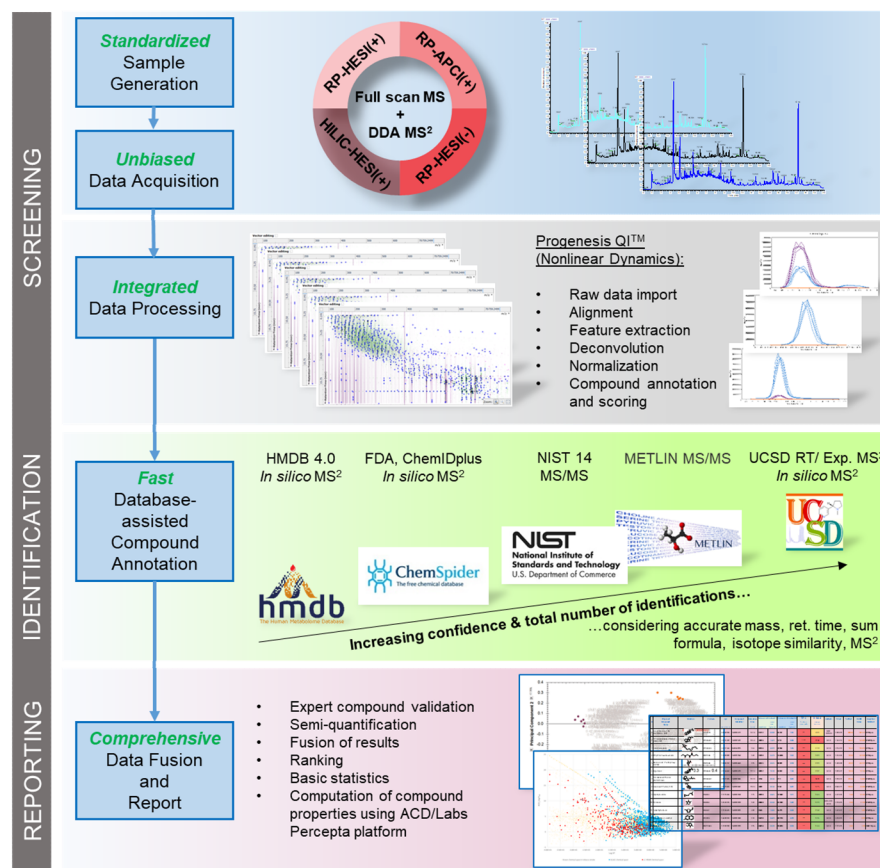- Link to other databases

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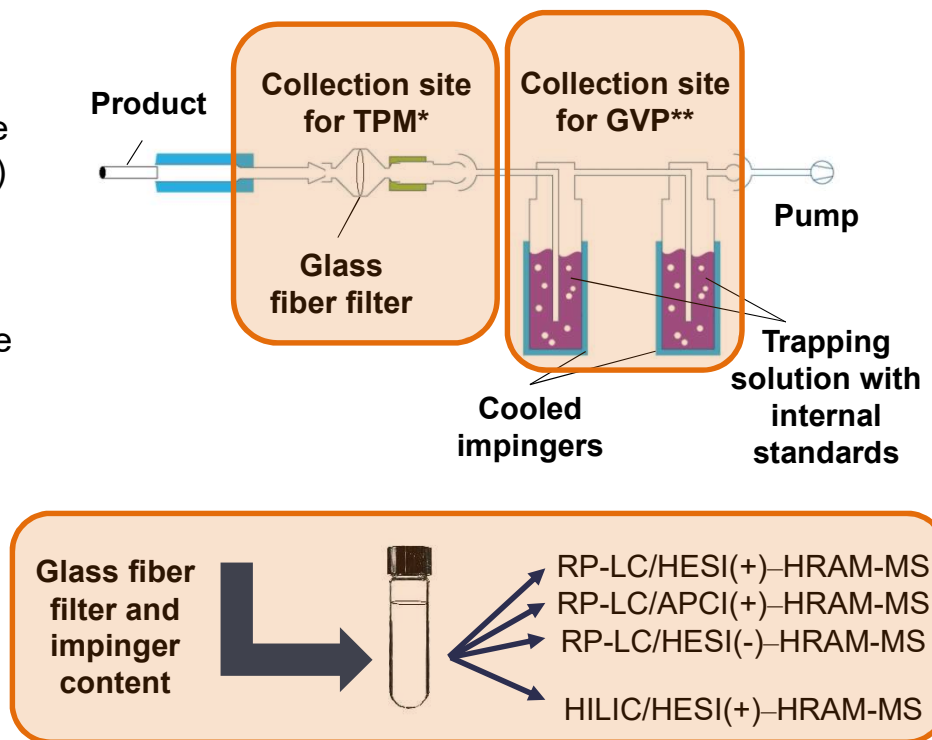
LC–HRAM-MS Routine Workflow

Arndt et al. *RCMS* 2019

Sample Preparation

- Aerosol/smoke generation by using a linear smoking machine in accordance with the Health Canada intense (HCI[†]) smoking regimen

- Harmonized approach was adopted that employed separate trapping of the particulate and gas–vapor phases (combined whole aerosol or smoke)

- Minimum sample preparation, MeOH and ACN containing a set of internal standards were used as trapping solutions for RP-LC and HILIC

- Three replicates were collected from THS 2.2 and/or 3R4F, reference samples and blanks

Product · **Collection site for TPM*** · **Collection site for GVP**** · **Pump**

Glass fiber filter

Cooled impingers

Trapping solution with internal standards

Glass fiber filter and impinger content →

RP-LC/HESI(+)–HRAM-MS
RP-LC/APCI(+)–HRAM-MS
RP-LC/HESI(-)–HRAM-MS

HILIC/HESI(+)–HRAM-MS

*TPM: total particulate matter   **GVP: gas/vapor phase

†Official Method T-115, Determination of "Tar", Nicotine and Carbon Monoxide in Mainstream Tobacco Smoke, Department of Health, Canada, 1999

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Three Reverse Phase Methods

One HILIC Method

- Hypersil GOLD™ C18 column 150 × 2.1 mm i.d., 1.9 µm

- **RP-LC/HESI(+) & RP-LC/APCI(+):**
  MP A: 10 mM NH$_4$Ac in water, MP B: 1 mM NH$_4$Ac in MeOH,
  Internal Standard: D8-Isophorone (C$_9$H$_6$D$_8$O)

- **RP-LC/HESI(-):** MP A: 1 mM NH$_4$F in water, MP B: MeOH,
  Internal Standard: D19-Decanoic acid (C$_{10}$HD$_{19}$O$_2$)

| Time (min)	A (%)	B (%)	Flow (µL/min)
0	85	15	400
7.00	10	90	400
12.80	0	100	400
18.00	0	100	400
18.10	85	15	400
20.00	85	15	400

- Accucore™ HILIC column 150 × 2.1 mm i.d., 2.6 µm

- **HILIC/HESI(+):**
  MP A: 10 mM NH$_4$Ac in water,
  MP B: 10 mM NH$_4$Ac in ACN,
  Internal Standard:
  D4-Myosmine (C$_9$H$_6$D$_4$N$_2$)

Time (min)	A (%)	B (%)	Flow (µL/min)
0	2	98	500
7.00	25	75	500
8.00	2	98	500
15.00	2	98	500

Q Exactive™ Hybrid Quadrupole Orbitrap MS (Thermo Fisher): Full-scan mode (*m/z* 80–800) and MS^2 fragmentation

RP-LC/HESI(+)

RP-LC/APCI(+)

RP-LC/HESI(-)

HILIC/HESI(+)

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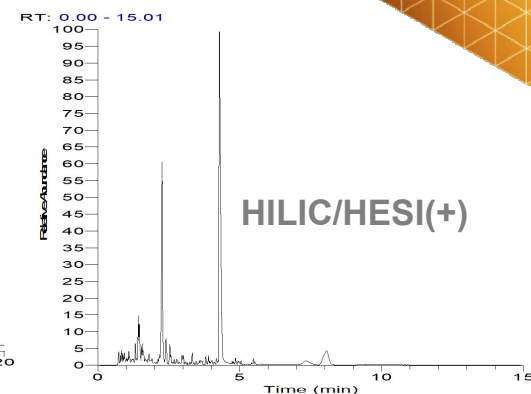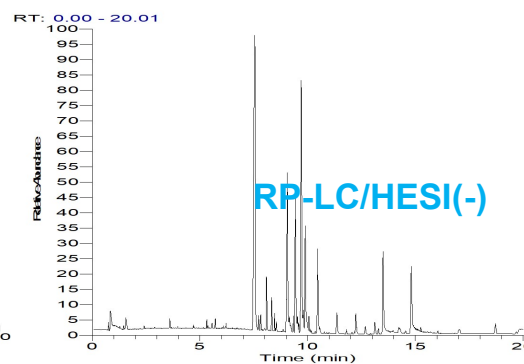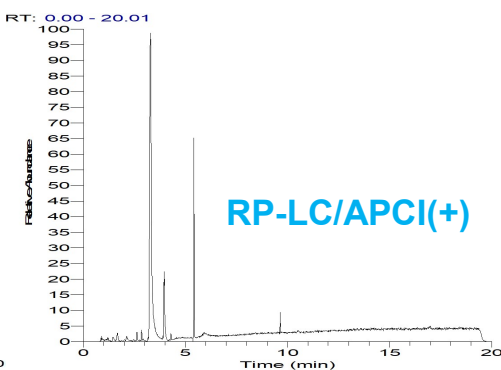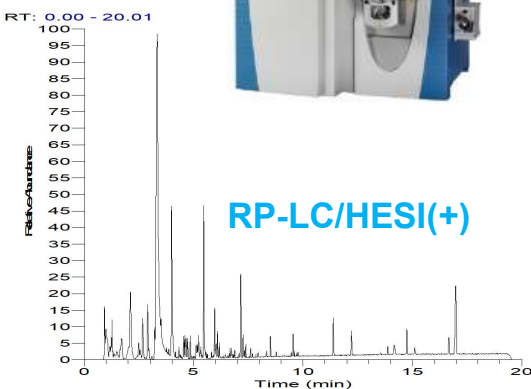
Analytical Methods

- The four analytical LC–MS methods were clearly complementary to each other in the analysis of 3R4F-derived TPM

- RP-HESI(+) demonstrated the greatest coverage, with over 50% of the compounds being identified

- Recommendations: Generic and complementary methods to be preferred, optimization of ionization efficiency, QC samples to be included in sequence for quality checks and alignment of dataset
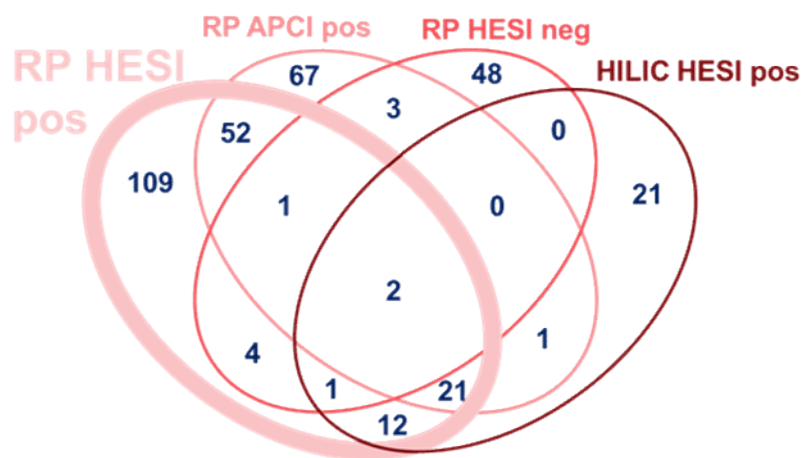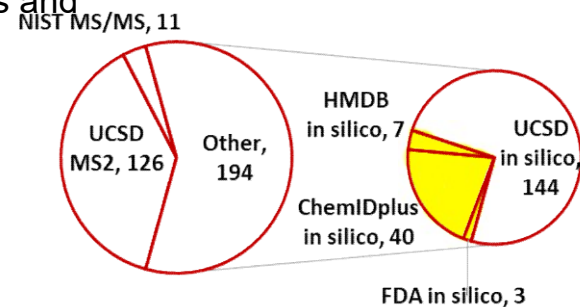

Coverage and overlap of compounds identified by the four separate chromatographic/ionization approaches in 3R4F-derived TPM (Arndt et al. 2019). A total of 331 major compounds above a threshold of 100 ng/item were identified.

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Compound Identification

- Three different identification strategies:
  - Experimental MS^2 spectra comparison by using UCSD (RT, exp. MS^2)
  - Experimental MS^2 spectra comparison by using NIST 14 MS/MS and METLIN MS/MS
  - *In Silico* MS^2 spectra comparison by using UCSD, HMDB 4.0, FDA, ChemIDplus, and other integrated databases

- Identifcation by using an overall score based on accurate mass and ret. time match, isotope similarity, and fragmentation score

- Confirmed compounds and three confidence categories for structural proposals: high, medium, and not identified

- The high coverage of chemical space is not only because of the comprehensive analytical methods used, but also because of the employed complementary compound ID strategies including multiple databases

- Algorithm for *in silico* prediction of MS spectra should consider adducts

NIST MS/MS, 11
HMDB in silico, 7
UCSD MS2, 126
Other, 194
UCSD in silico, 144
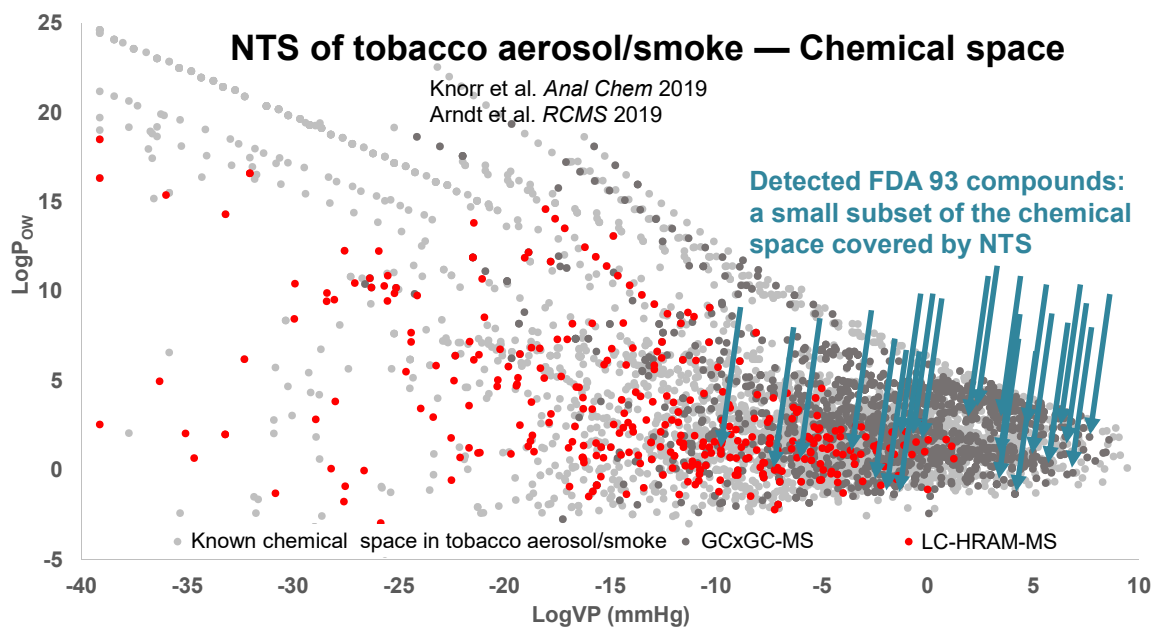ChemIDplus in silico, 40
FDA in silico, 3

Total number of compounds identified by the different ID strategies in TPM derived from a 3R4F reference cigarette (Arndt et al. 2019). Fifty new compounds that were not present in our application-oriented UCSD database could be identified, which demonstrates the versatility and potential applicability of our NTS workflow for other matrices

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Coverage of NTS Methods

- Complementary character and excellent coverage of known tobacco aerosol and smoke related chemical space by NTS methods demonstrated by means of more than 4,000 calculated compounds
- LC–HRAM-MS-based NTS (> 60%) and GC×GC–TOFMS-based platform (+30%) covered a very broad range that was almost fully representative of the known chemical space

NTS of tobacco aerosol/smoke — Chemical space

Knorr et al. *Anal Chem* 2019
Arndt et al. *RCMS* 2019

Detected FDA 93 compounds: a small subset of the chemical space covered by NTS

Known chemical space in tobacco aerosol/smoke • GCxGC-MS • LC-HRAM-MS

$LogP_{OW}$

LogVP (mmHg)

Log P_{OW}: logarithm of octanol/water partition coefficient values   Log VP: logarithm of vapor pressure

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Applications

Non-targeted differential screening (NTDS) of THS 2.2 aerosol versus 3R4F smoke with LC–HRAM-MS*

- NTDS is used to identify chemical constituents of higher concentrations in prototypes of novel products compared to a reference test item, followed by evaluation of the toxicological impact of these substances

- Differences were revealed by an empirically developed mathematical model that considered the relative abundance of all detected constituents as well as their semi-quantitative estimates of absolute abundance
(Knorr, A., International Patent WO 2013098169 A1, PCT/EP2012/076244, 2013.Jul 4)

THS 2.2 vs. 3R4F

### Comprehensive chemical characterization of THS 2.2 aerosol with LC–HRAM-MS

- A 100 ng/stick cutoff limit was selected

- Ca. 67% of the compounds identified by LC–HRAM-MS were confirmed by reference standards, representing 85% in terms of the total mass characterized
(Bentley et al. *Anal Bioanal Chem* 2020)

Confirmation Confidence Compound-Based
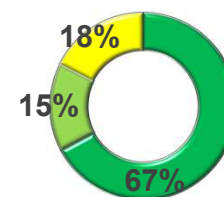
Confirmation Confidence Mass-Based

- Confirmed
- High
- Medium

18%
15%
67%

7%
8%
85%
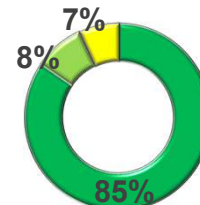
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