Comprehensive chemical characterization of complex matrices through integration of multiple analytical modes and databases for LC-HRAM-MS-based non-targeted screening

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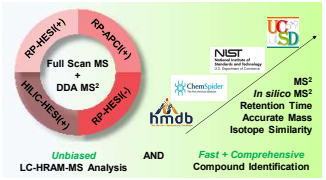
Overview



Novel aspect: Integration of multiple analytical methods, databases, and compound identification strategies into a single liquid chromatography coupled to high-resolution accurate mass spectrometry (LC-HRAM-MS) workflow

Results: 331 major constituents of 3R4F-derived smoke identified due to complementary

- Chromatographic/ionization methods
- Databases
- Compound identification strategies

Introduction and Objectives

Non-targeted screening (NTS) based on LC-HRAM-MS is a key methodology for characterizing the chemical composition of complex matrices. Given the great number and diversity of small molecules in tobacco smoke, several analytical approaches have to be combined to maximize coverage of the chemical space. The analytical workflow must be linked with a comprehensive data processing routine, including (semi-)automated compound identification, which is key to successfully handling the vast amounts of data. Together, comprehensive chemical characterization with high-confidence annotation of small molecules can be achieved. In this study, this analytical approach will be used to characterize the chemical composition of smoke samples from a 3R4F reference cigarette[1].

Goal:
Generic approach for comprehensive chemical characterization

- In an unbiased way (i.e., without any predefined target compounds)
- With maximum confidence and coverage of LC-amenable compounds

achieved with highest possible degrees of automation and standardization.

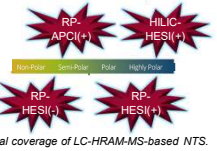
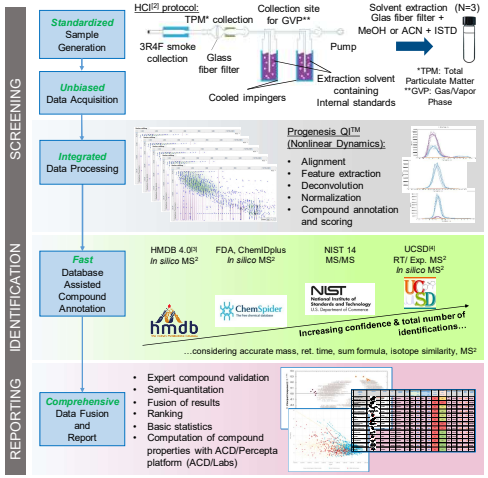


Figure 1. Analytical coverage of LC-HRAM-MS-based NTS.

Workflow

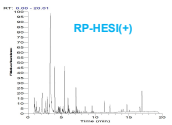


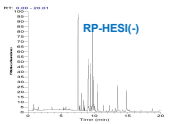
Analytical Methods

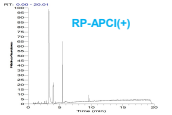
Four separate chromatographic/ionization approaches:

- Column oven at 50 °C
- Injection volume of 1.5 µL

Three RP methods at 400 µL/min:



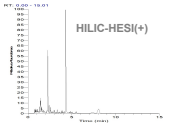




Time (min)	A (%)	B (%)
0	85	15
7.00	10	90
12.80	0	100
18.00	0	100
18.10	85	15
20.00	85	15

- Hypersil GOLD™ column 150 × 2.1 mm i.d., 1.9 µm
- **RP-HESI(+) & RP-APCI(+):** MP A: 10 mM NH_4Ac in water, MP B: 1 mM NH_4Ac in MeOH, Internal Standard: D8-Isophorone ($C_9H_6D_8O$)
- **RP-HESI(-):** MP A: 1 mM NH_4F in water, MP B: MeOH, Internal Standard: D19-Decanoic acid ($C_{10}HD_{19}O_2$)

HILIC method at 500 µL/min:



Time (min)	A (%)	B (%)
0	2	98
7.00	25	75
8.00	2	98
15.00	2	98

- Accucore™ HILIC column 150 × 2.1 mm i.d., 2.6 µm
- **HILIC-HESI(+):** MP A: 10 mM NH_4Ac in water, MP B: 10 mM NH_4Ac in ACN, Internal Standard: D4-Myosmine ($C_9H_6D_4N_2$)

Q Exactive™ Hybrid Quadrupole Orbitrap MS (Thermo Fisher): full scan mode and data-dependent first order fragmentation (MS^2) using HCD and stepped NCE ion activation modes

Results

The four analytical methods and compound identification strategies were highly complementary

- 331 major identified constituents above a semi-quantitative threshold of 100 ng per cigarette
- RP-HESI(+) proved as most universal (Fig. 2)
- HILIC-HESI(+) identified unique small and polar compounds (Fig. 3)

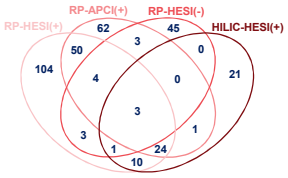


Figure 2. Coverage of compounds achieved by the four separate chromatographic/ionization approaches.

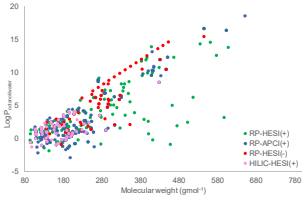


Figure 3. $LogP_{OW}$-versus-MW of identified compounds in LC-HRAM-MS-based non-targeted screening.

- More identifications due to the employed complementary compound ID strategies
- 50 compounds not present in UCSD (in-house database) but identified by means of *in silico* prediction of MS^2 spectra based on ChemIDplus, HMDB, and FDA databases (Table 1)

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- *In silico* fragmentation appeared promising for compound class ID in the absence of reference MS^2 spectra (Figs. 4 and 5)
- Based on reference MS^2 spectra of commercial standards, our approach was able to differentiate between isomers (Fig. 5)

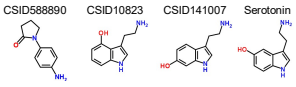


Figure 4. Chemically similar structural proposals to cotinine (correct match) revealed by in silico fragmentation.

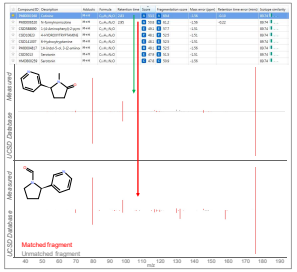
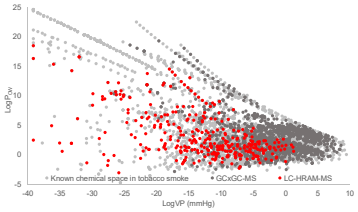


Figure 5. Differentiation of structural isomers in tobacco smoke.

LC-HRAM-MS-based NTS covered a large part of the chemical space of tobacco smoke



- LogVP (vapor pressure) and $logP_{OW}$ values for 4141 known compounds of tobacco smoke predicted
- 331 compounds identified by LC-HRAM-MS were spread over > 60% of the logVP-versus-$logP_{OW}$ plot (Fig. 6)

Figure 6. Coverage of known chemical space of tobacco smoke by LC-HRAM-MS NTS.

Table 1. Top 10 major chemical constituents identified in 3R4F-derived smoke by LC-HRAM-MS NTS[a]

#	Name[b]	Identifier	Formula	MW[b] (gmol⁻¹)	Ret. Time (min)	RSD[c] (%)	Score	Frag. Score	Δm[d] (ppm)	Isotope Similarity	ΔRet. Time[e] (min)	Method[f]	ID Basis
1	Solanesol	PMI0000409	C45H74O	631.07	17.08	2.7	71.0	91.3	-2.4	99.8	0.0	**RP-HESI(+)**, RP-APCI(+)	UCSD MS²
2	Nicotine	PMI0004286	C10H14N2	162.23	3.32	2.9	59.3	98.9	-1.9	89.7	-0.1	**RP-HESI(+)**, RP-APCI(+), HILIC-HESI(+)	UCSD MS²
3	Bombiprenone	PMI0006795	C43H70O	603.02	16.76	3.3	42.7	15.3	-1.0	99.4	na	**RP-HESI(+)**, RP-APCI(+)	UCSD *in silico* MS²
4	Triacetin	PMI0000113	C9H14O6	218.20	4.03	5.2	76.0	100.0	-2.2	99.3	0.0	RP-HESI(+)	UCSD MS²
5	7-Ketositosterol	PMI0009304	C29H48O2	428.69	12.30	7.3	44.9	30.3	-1.1	95.5	na	**RP-HESI(+)**, RP-APCI(+), HILIC-HESI(+)	UCSD *in silico* MS²
6	Pytoene Carotenoid	HMDB39093	C40H64	544.94	15.51	4.2	41.8	11.7	-0.9	98.5	na	RP-HESI(+)	HMDB *in silico* MS²
7	5,9,13,17,21,25,29-Hentriacontaheptaen-2-ons, 6,10,14,18,22,26,30-heptamethyl	PMI0006129	C38H62O	534.90	15.19	3.7	46.0	33.7	-0.9	97.5	na	RP-HESI(+)	UCSD *in silico* MS²
8	(3β)-3-Methylandrost-5-en-17-one	CSID114234	C20H30O	286.45	8.39	3.1	50.4	56.9	-2.0	97.4	na	RP-HESI(+)	ChemIDplus *in silico* MS²
9	Palmitic acid	PMI0000164	C16H32O2	256.42	9.78	4.0	56.6	59.2	1.4	98.1	0.1	RP-HESI(-)	UCSD MS²
10	N-Octanoylnornicotine	PMI0001863	C17H26N2O	274.40	7.21	4.4	62.8	58.2	-1.4	97.5	0.0	**RP-HESI(+)**, RP-APCI(+), HILIC-HESI(+)	UCSD MS²

[a] Compounds are sorted in descending order of yield. [b] Confidence levels: green, confirmed: retention time and mass spectra within specified tolerance ranges in comparison to a standard that was analyzed under the same experimental conditions; black, high: score > 50 or score > 45 and fragmentation score > 45; brown, medium: score < 45 or score between 45-50 and frag. score < 45. [c] MW, weight of neutral molecule. [c] RSD, relative standard deviation (N = 15 total observations from three sample replicates that were injected fivefold). [d] Δm, difference between experimental and theoretical mass. [e] ΔRet. Time, difference between retention time in a sample and standard in database. [f] na, not available. [g] In cases of compounds that were detected with multiple analytical methods, it is highlighted in bold from which method the information on the analytical figures of merit were extracted.

Conclusions

- Our developed LC-HRAM-MS-based NTS approach has been successfully applied for in-depth chemical characterization of 3R4F-derived smoke samples
- Analytical coverage could be increased by integration of multiple chromatographic/ionization approaches and compound identification strategies into a single workflow
- Unexpected compounds beyond those in tobacco-specific databases were identified, demonstrating the versatility and potential applicability of our NTS workflow for other matrices

References

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Competing Financial Interest
The research described in this poster was sponsored by Philip Morris International.

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