



Deciphering the urinary exposome of users of various nicotine and tobacco products by non-targeted LC-Orbitrap-MS analysis

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Introduction

Over the past decade, the tobacco landscape has changed dramatically with several new emerging nicotine-delivery products. Particularly in longitudinal cohort studies, compliance of subjects must be ensured by appropriate biomarkers. The exposure assessment today focuses on the comparison to smoking and the reduction in smoking-related harmful constituents. A comprehensive approach comprises a holistic view at the exposure profile in users of each of the new tobacco/nicotine products. Deciphering the urinary exposome may reveal suitable novel biomarkers specific to a certain nicotine-delivery product and improve our understanding of the absolute risk related to the use of these products.

OBJECTIVE

Identify **biomarkers** or **biomarker patterns** in urine to discriminate between different nicotine product user groups and non-users.

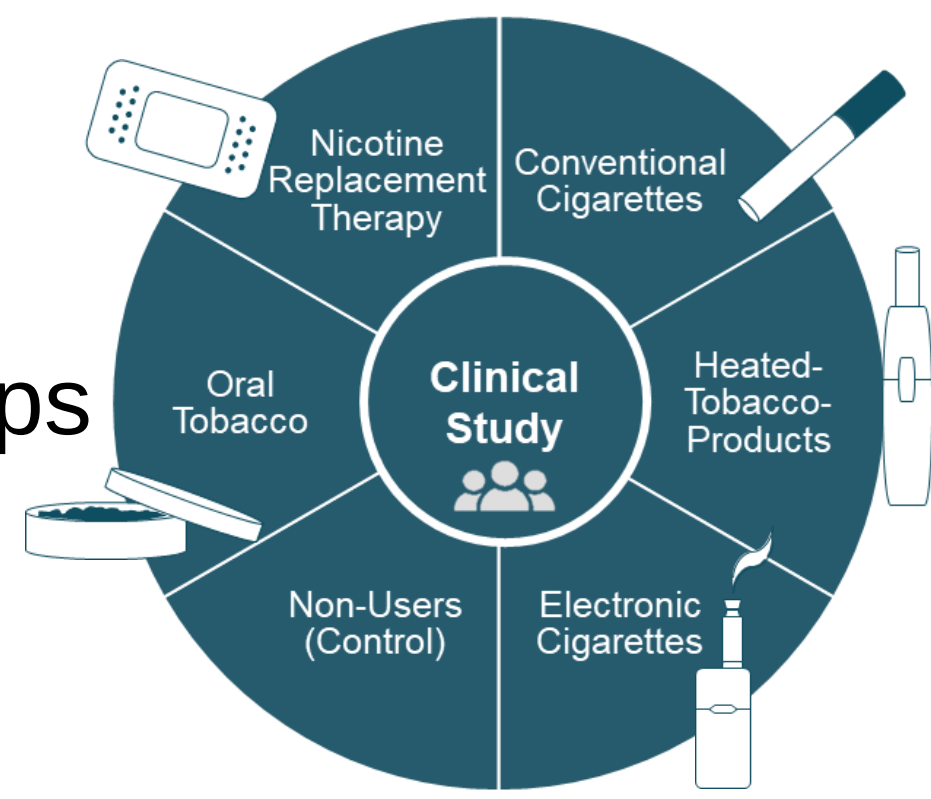
Summary & Conclusion

- Establishment of a **LC-Orbitrap-MS method** capable of detecting differences in the urinary exposome in the low ng/ml range ⇒ Identification of **more than 300 features significant to a specific nicotine product user group**
- Smoker exposome** shows by far the **highest burden** to harmful chemicals
- Minor alkaloids** and **TSNAs** indicate use of tobacco-derived products
- Electronic cigarettes (EC): Propylene glycol** in urine identified as **vaping-specific** biomarker of exposure
- NRT** and **snus (OT)** users show **unique flavor profile**
- Panel of **mercapturic acid (MA) VOC metabolites** show potential to discriminate **HTP** users from other products including cigarettes (CC)
- Non-targeted evaluation of urinary exposome identified specific biomarkers and adds important new insights into the absolute risk of emerging nicotine & tobacco products**

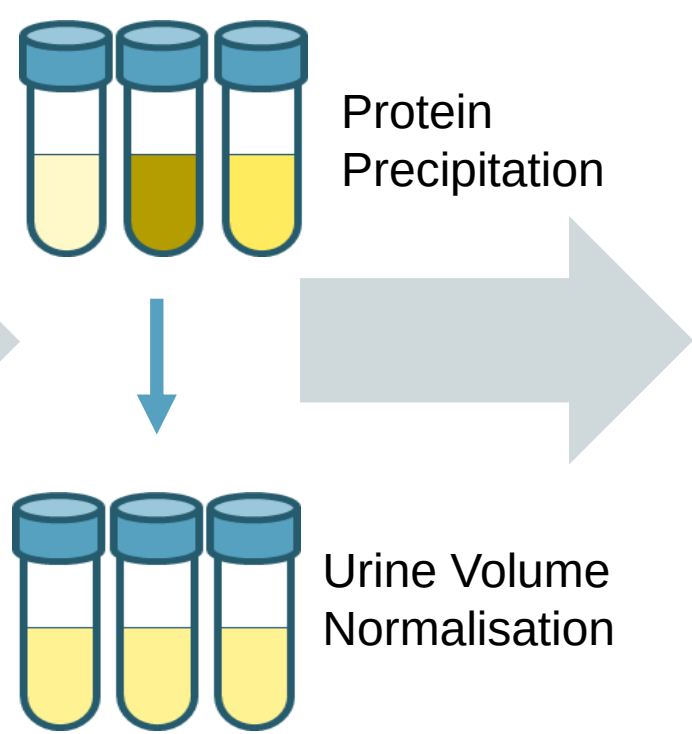
Experimental Design

Controlled Clinical Study

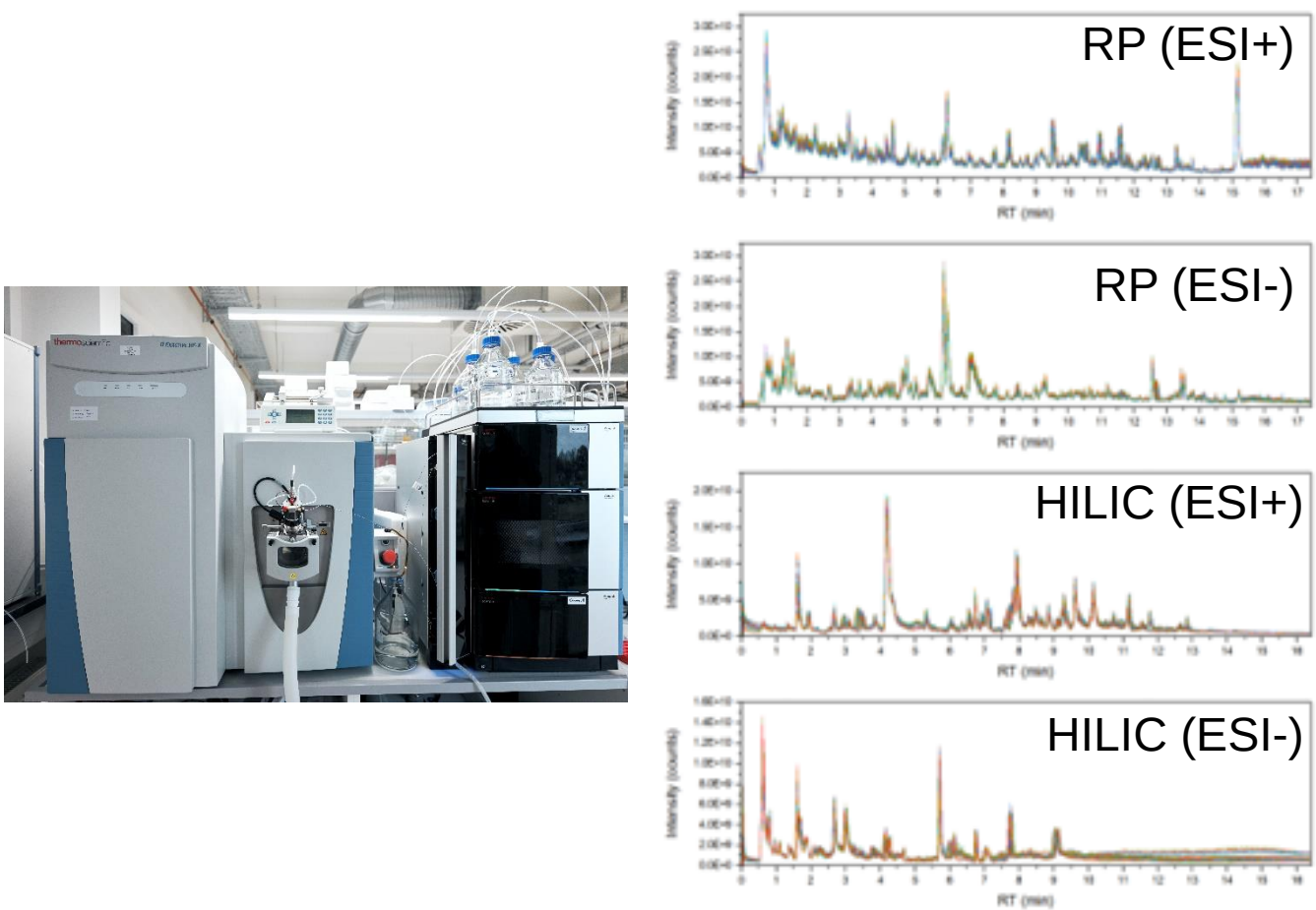
10 subjects per group
3 days of confinement
5 nicotine product user groups



Sample Preparation



Sample Analysis



Data Evaluation

Compound discoverer 3.3
mass detection, peak extraction, RT alignment, deconvolution, integration, feature alignment

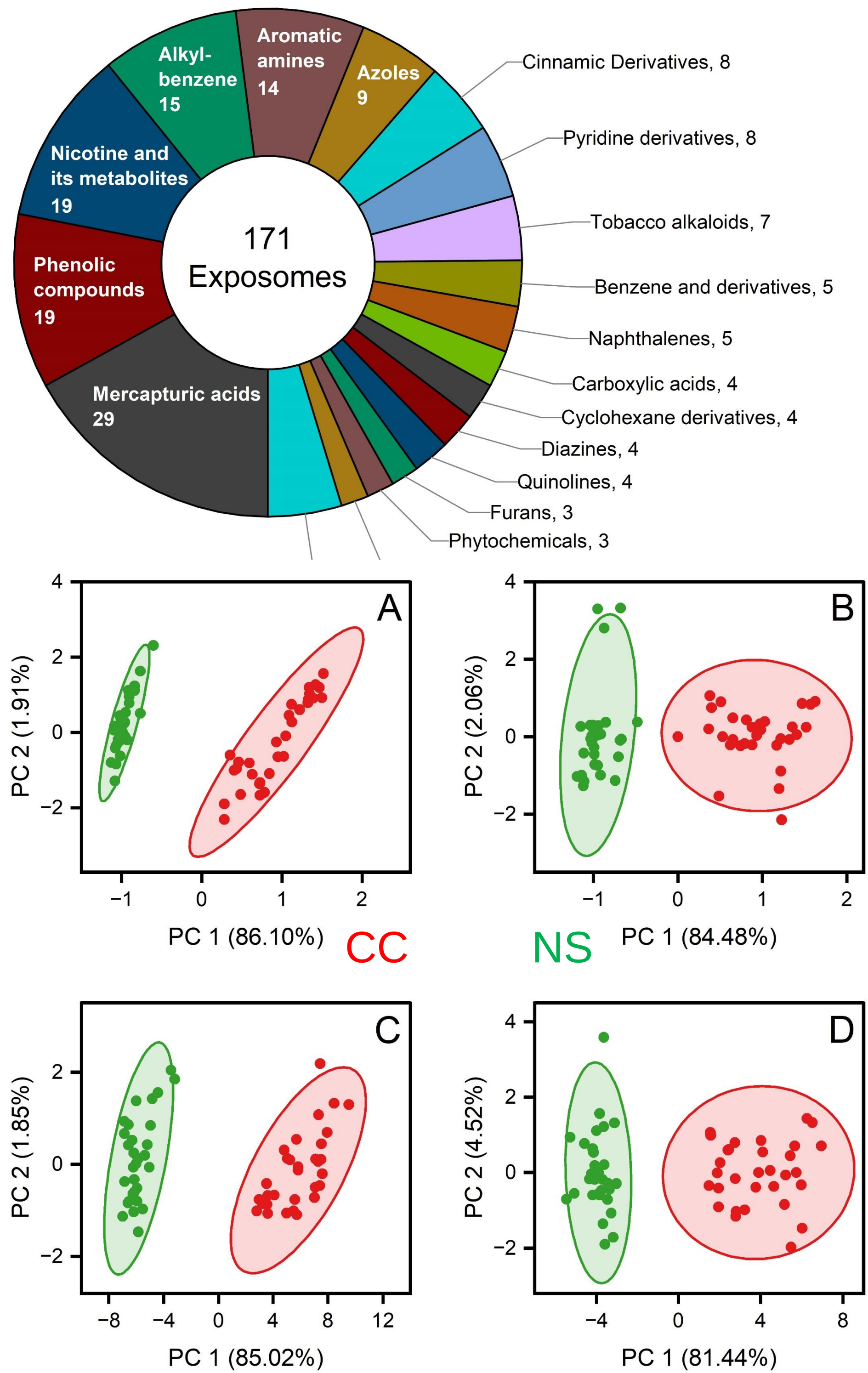
Statistical Analysis
ANOVA and post-hoc Tukey's honest sign test;
Fold change ≥ 2; p ≤ 0.05

Identification
MZCloud, mzVault, HMDB, KEGG, MoNA,
NIST MS 2020, Reference standards
In-silico fragmentation (Sirius, MetFrag)

Results & Discussion

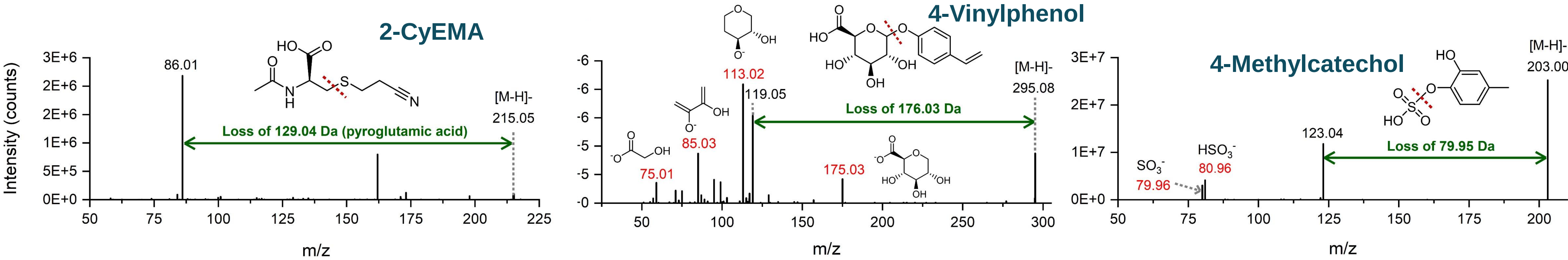
Urinary exposome of smokers

171 metabolites significantly elevated in smokers



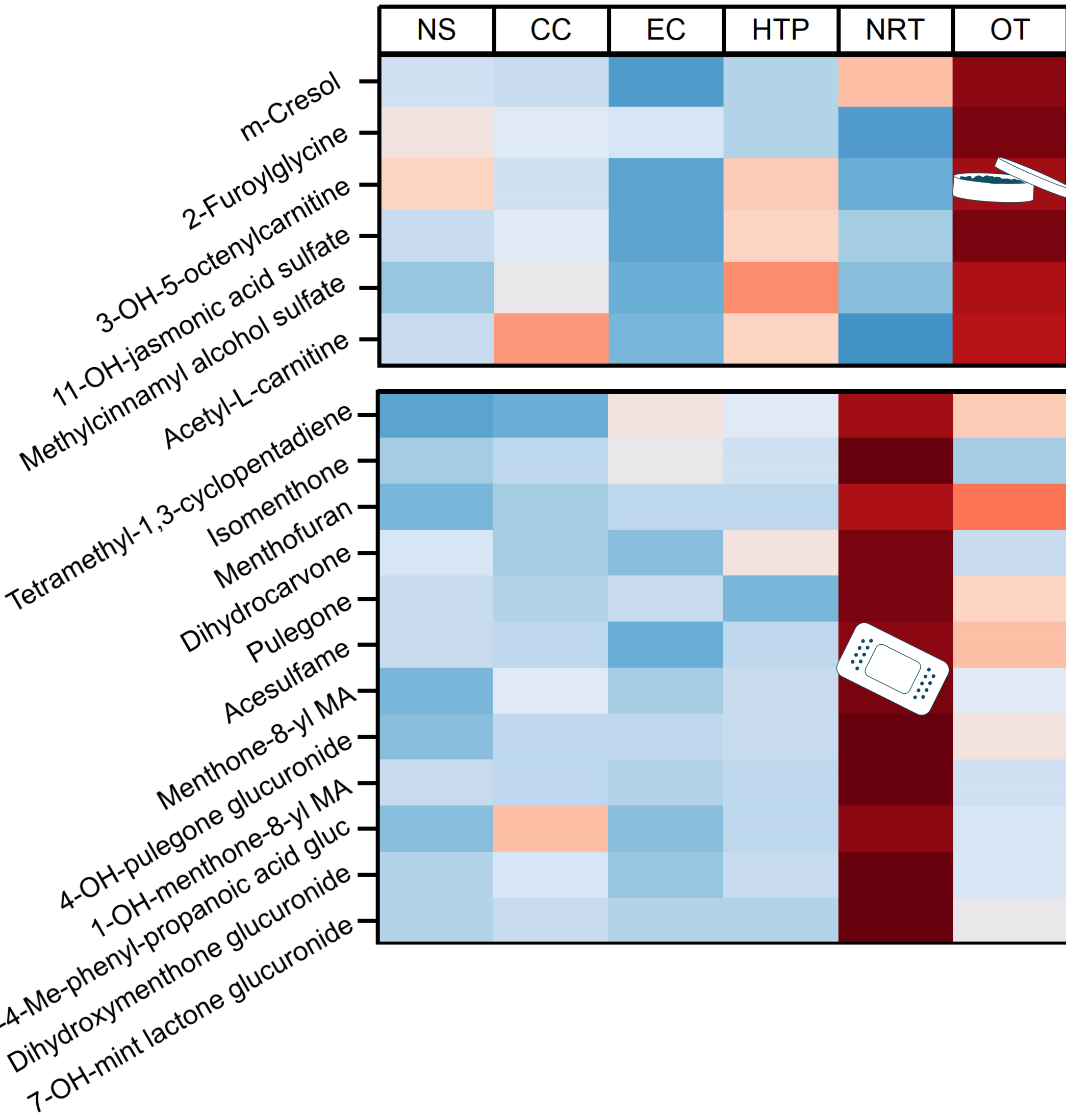
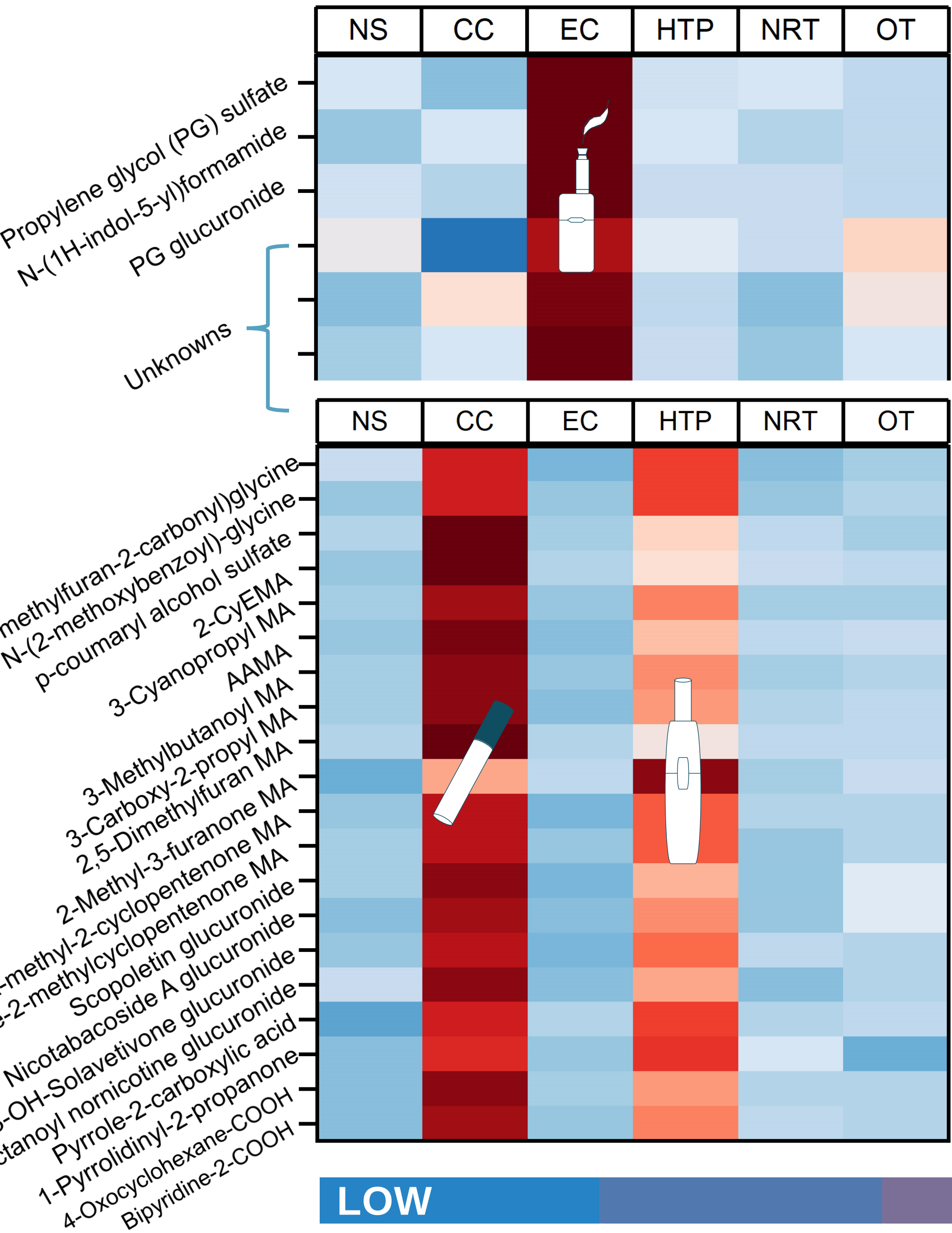
PCA score plot of urinary metabolites elevated in **smoker vs non-smoker**.
A) Reversed-phase (+); B) Reversed-phase (-); C) HILIC (+); D) HILIC (-)

Identification strategy for phase II metabolites



Mass spectra of phase II conjugates: Neutral loss (NL) depicted by green arrows; Characteristic fragment ions (CFI) in red

- Mercapturic acids** display **NL of 129 Da** in negative ion mode
- Glucuronides** exhibit **NL of 176 Da** and generated **CFIs at m/z 75, 85, 113, 175** in negative ion mode
- Sulfate** conjugates show **NL of 79 Da** and produced **CFIs at m/z 79, 80** in negative ion mode



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