Decoding Dietary Signatures in Human Fecal Metabolomes Using a Novel timsTOF Platform

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Introduction

NIST RM 8048 is a recently released human fecal reference material intended to support method development, benchmarking, and interlaboratory harmonization. The material consists of pooled fecal samples from vegetarian and omnivorous donors, providing a biologically relevant and chemically complex matrix for evaluating analytical workflows. Human fecal metabolomes encompass a broad dynamic range of endogenous metabolites, microbial products, dietary components, and host–microbiome co-metabolites, presenting substantial analytical challenges. Here, we apply complementary reversed-phase (RP) and hydrophilic interaction liquid chromatography (HILIC) separations, coupled with a high-resolution TIMS-MS platform, to achieve comprehensive metabolomic and lipidomic coverage. This integrated workflow enables systematic characterization of polar, semi-polar, and lipid species, generating digital metabolome archives that collectively define a comprehensive metabolome landscape:

An initial deep profiling benchmark for this newly available reference material.

Fig. 1 NIST Reference Material 8048 was used of this study. Different extraction methods and LC-TIMS-ToF-MS methods were combined for a comprehensive description of the metabolite and lipid content of this material to build a digital metabolome landscape from digital metabolome archives of single samples.

Methods

Metabolite extraction

Fecal samples were either extracted with 20% ACN / 80% H_2O (HILIC), 25% MeOH / 25% iPrOH / 50% H_2O (RP) or 50% MeOH / 50% MTBE (lipidomics) to cover different polarities.

Polar Metabolomics (HILIC)

Waters BEH Z-HILIC (100 mm x 2.1 mm ID, 1.7 µm)

A^+: 100% H_2O + 10 mM ammonium formate + 0.1% formic acid

B^+: 90% ACN / 10% H_2O + 10 mM ammonium formate + 0.1 formic acid

A^-: 100% H_2O + 10 mM ammonium acetate, pH 9

B^-: 90% ACN / 10% H_2O + 10 mM ammonium acetate pH 9

Detection in +/- using MoRE (*m/z* range 20 – 1300)

Non-Polar Metabolomics (RP)

Phenomenex Kinetex C18 (100 mm x 2.1 mm ID, 1.7 µm)

$A^{+/-}$: 100% H_2O + 0.1% formic acid

$B^{+/-}$: 100% ACN + 0.1% formic acid

Detection in +/- using PASEF (*m/z* range 20 – 1300)

Lipidomics (RP)

Waters Cortecs UPLC C18 (150 mm x 2.1 mm, 1.6 µm)

$A^{+/-}$: 40% H_2O / 60% ACN + 10 mM ammonium formate + 0.1% formic acid

$B^{+/-}$: 10% ACN / 90% iPrOH + 10 mM ammonium formate + 0.1% formic acid

Detection in +/- using PASEF (*m/z* range 100 – 1500)

Peak detection, recalibration and initial annotation was performed in MetaboScape 2026b (Bruker). Further analysis has been performed in R using packages from the RforMassSpectrometry ecosystem, Sirius with CSI:FingerID and MetaboScape 2026b.

Results

- Several thousand features were detected across all LC methods and ion modes
- Initial annotation was performed using multiple external libraries as well as rule-based lipid annotation and CCS matching and prediction
- Several suspected and several new metabolites/lipids were putatively annotated (on-going work)

Table 1: Number of detected features per method and ionization mode. Features were counted after blank correction (minimum 1.5 times higher than in blank and detected in all three replicates of either omnivore or vegetarian fecal samples).

Method	No. of features (with MSMS)
Metabolomics HILIC(+) (MoRE)	3569 (2764)
Metabolomics HILIC(-) (MoRE)	3002 (2253)
Metabolomics RP(+) (PASEF)	3255 (2594)
Metabolomics RP(-) (PASEF)	6645 (4701)
Lipidomics RP(+) (PASEF)	8404 (6755)
Lipidomics RP(-) (PASEF)	5194 (4328)

Initially, metabolite features present in the omnivore and vegetarian fecal samples were compared. Only features present in 2/3 or more in each group respectively were counted. Dependent on the LC method and ionization about 70-80% of all features were detected in both sample types.

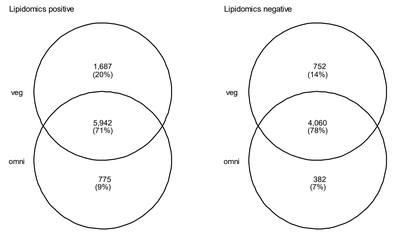

Fig. 2. Overlap of lipid features detected in omnivore or vegetarian fecal samples.

Beside features specific to each diet, several metabolites were detected in both but show specific trends (see Fig 3.). Several non-annotated features in the lipidomics data set show higher values in vegetarian fecal samples and represent interesting targets for further investigations (see Fig 4).

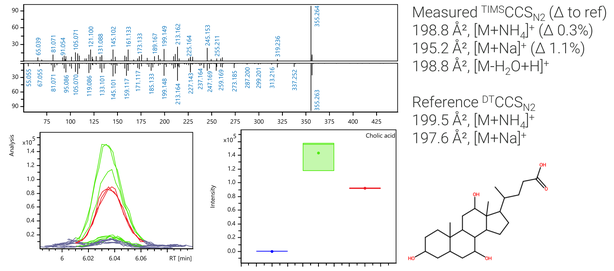

Fig. 3. Cholic acid detected in metabolomics (RP) as example for multidimensional annotation, using MS², RT and CCS. Reference CCS were derived from PubChem.

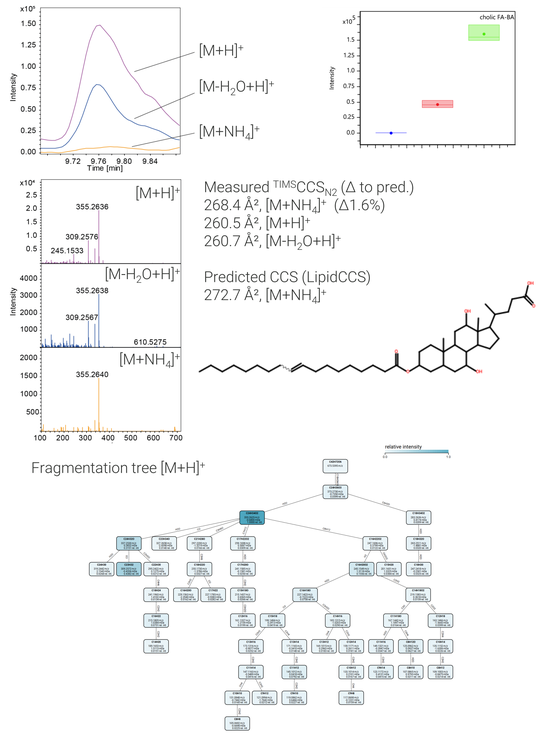

Fig. 4. Example of a novel bile acid detected in fecal samples. FA 16:1 ester of cholic acid or similar. Multiple adducts were detected and Sirius putatively annotated all of them as the same structure, increasing confidence.

Summary

NIST RM 8048 was profiled using different chromatographic and detection methods using the timsMetabo TIMS-QTOF. Besides known metabolites detected previously, e.g. by Cruz *et al.*, several additional features with putative annotations were detected representing ideal candidates for further investigations, e.g. fatty acid esters of 3-hydroxy bile acids.

Conclusion

- timsMetabo enables sensitive detection of thousands of features across complementary LC methods in NIST 8048, generating digital metabolome archives that together define a unified metabolome landscape.
- Integration of MS, MS/MS, RT, and CCS provides the confidence required to populate these archives with high-quality annotations and to explore unknowns.
- We present an initial deep profiling benchmark (a digital metabolome landscape) for NIST 8048 .

LC-TIMS-MS/MS

Note and Disclaimer:
Aiko Barsch and Matthew R. Lewis are employees of Bruker Corporation or one of its subsidiaries ("Bruker"). Bruker manufactures and sells analytical instruments including mass spectrometers, mass spectrometry consumables and software. Bruker mass spectrometers and software were used in this study.