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Extraction Protocol for untargeted LC-MS/MS - Milk

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Protocol status: In development
We are still developing and optimizing this protocol

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Disclaimer

This is not a peer-review protocol and it has been created and edited based on inputs from several users and collaborators of the Dorrestein lab.

Abstract

This protocol details the extraction of milk small molecules for untargeted metabolomics analysis using liquid chromatography-tandem mass spectrometry (LC-MS/MS). This protocol can be adapted to other sample types. This is not a final document, not peer-reviewed and it can be updated.

Materials

Methyl tert-Butyl Ether (HPLC), Fisher Chemical™ **Fisher Scientific Catalog #**E127-4

Methanol Optima™ LC/MS Grade Fisher Chemical **Fisher Scientific Catalog #**A456-4

Extraction solvent: 75:25 Methanol:MTBE

Metadata - sample information

1 Prepare metadata table according to **ReDU2 metadata template** [for more information about the ReDU, see https://www.nature.com/articles/s41592-020-0916-7 and visit **https://redu.gnps2.org/selection/**].

2 Fill the metadata table with all the applicable information, we (Dorrestein Lab) will include all the information related to the MS side.

3 Specifically, from each sample we will need at least the NCBITaxonomy (e.g. **1313|Streptococcus pneumoniae**).

4 Additional columns can be created to include information related to the specificity of the experiments (e.g. microbial interactions: co-cultures vs monocultures).

5 In case of availability of reference compounds (e.g. synthetic peptides, isolated peptides), please include the corresponding information according to the **MSMS-chooser documentation** [see **MSMS-Chooser Template and Sequence Generator**].

6 For more information related to MSMS-chooser, see the manuscript from our lab: **Protocol for community-created public MS/MS reference spectra within the Global Natural Products Social Molecular Networking infrastructure**.

Notes, bottlenecks, and expected results

30m

7 This protocol calls for all samples to be at the same volume as variations in volume will affect extraction efficiency.

8 Store all extraction reagents at 4 °C or -20 °C (colder is better).

9 Adding 50 µL of milk into deep well plates is usually the longest step. Milk samples are often stored in 2 mL tubes, and can take up to a day to thaw at 4 °C.

10 Once milk is plated, the extraction process generally takes ~ 00:30:00. 30m

Centrifugation is the longest step.

11 This method allows for single-phase extraction of polar metabolites, lipids, and medications from milk. It is not optimised for any single class of molecule, but does provide good coverage of the milk metabolome. See references below for more information.

Sample preparation and microbial extraction

20m

12 Add 50 µL milk to 96-well deep well plate.

13 Add 400 µL cold 75:25 MeOH/MTBE + [M] 1 micromolar (µM) sulfadimethoxine to each sample.

13.1 [M] 1 micromolar (µM) sulfadimethoxine is an internal standard.

13.2 For smaller volumes, decrease MeOH/MTBE accordingly (eg, for 25 uL milk, add 200uL 75:25 MeOH/MTBE)

14 Sonicate 00:05:00 in a water bath sonicator.

5m

14.1 Deep well plates float in the sonicator, so you can just add them to the water bath.

15 Centrifuge for > 00:15:00 at 1800 rpm .

15m

15.1 A faster spin is ideal, but 1800 rpm is as fast as our centrifuge will go with 96 well plates.

15.2 Ideally at 4 °C to keep samples cold.

16 Transfer 200 µL to new shallow 96 well plate. This plate is now ready for LC-MS/MS data acquisition.

Note

Prepare a pool sample: take 20uL of each sample and combine it in a new vial or centrifugation tube. Then, add 200uL to one or several well in the same shallow plate used for LC-MS/MS. This sample will be used as PoolQC.

Note

If plate is not used immediately, store plate at -80 °C until LC-MS/MS acquisition.

16.1 Make sure you don't pull up any of the protein pellet with the supernatant - this can easily clog your column.

16.2 You can also store plates in -80 °C for a couple days, but they should be dried down for long-term storage.

Protocol references

References and additional information:

https://www.embl.org/groups/metabolomics/protocols-used-for-lc-ms-analysis/
https://redu.ucsd.edu/
https://ccms-ucsd.github.io/GNPSDocumentation/msmschooser/
https://pubs.acs.org/doi/abs/10.1021/ac501853d

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