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Development of a multi-omics sample preparation workflow for comprehensive Metabolomics, Lipidomics and Proteomics datasets using a single tissue sample

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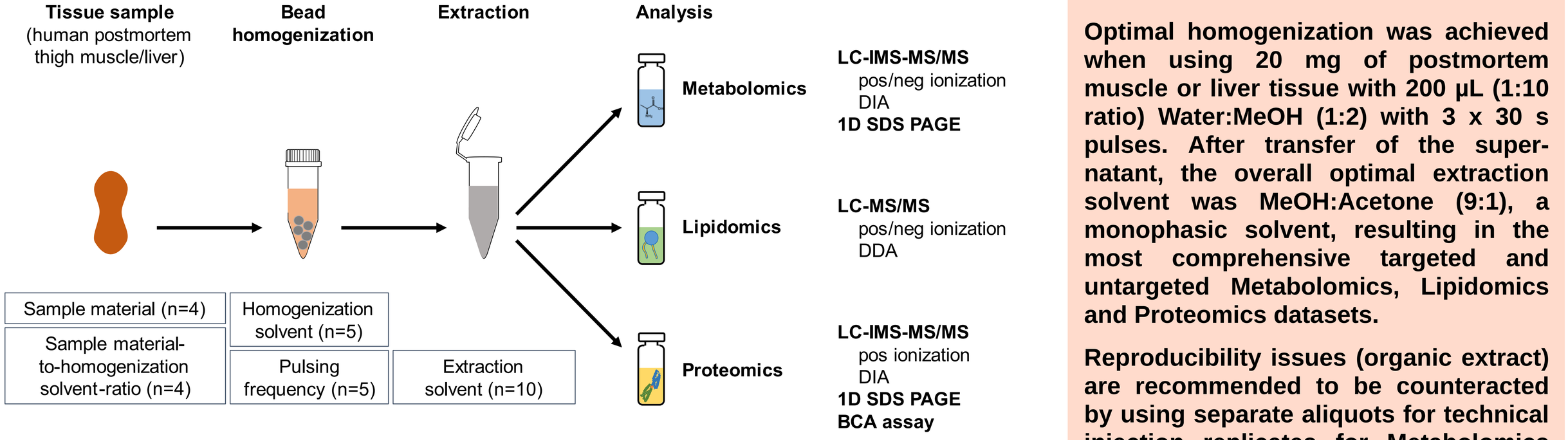

Figure 1: General sample preparation and analysis workflow for the evaluation of optimal homogenization and extraction parameters. Use of 3 mm zirconium beads; internal standards for Metabolomics and Lipidomics analysis were phenylalanine-d2 (2 µg/mL) pre-extraction, caffeine-d3 and palmitoleic-d2 (5 µg/mL each) post-extraction; for Proteomics analysis, the protein pellet was resuspended in 1 % SDC in HEPES containing TCEP (5 mM) and IAA (10 mM), trypsin-digested and de-salted/cleaned up using STAGE-tips.

Table 1: Tested homogenization and extraction parameters and corresponding conditions.

Parameter	Tested conditions
Sample material	5 mg, 10 mg, 20 mg, 40 mg
Sample material-to-homogenization solvent-ratio	1:5, 1:10, 1:20, 1:30
Homogenization solvent	Water, Water:MeOH (1:2), Water:ACN (1:2), MeOH, ACN
Pulsing frequency	1x30 s, 3x30 s, 5x30 s, 1x60 s, 1x90 s
Extraction solvent	MTBE:Water (1:1), MTBE:Water (2:1), DCM:Water (1:1), DCM:Water (2:1), Isopropanol:Water (1:1), Isopropanol:Water (2:1), ACN, MeOH:Acetone (9:1), ACN:Water (2:8), Butanol:ACN:Water (3:1:1)

Background/Aims

Multi-omics approaches have gained popularity aiming to wholistically investigate the biochemical processes in the human body. A frequent problem is the **requirement for specialized sample preparation for the different compound classes**. This leads to issues with the comparability between analyses (e.g. tissue homogeneity, additional freeze-thaw cycles) and difficulties combining results for biological interpretation. The **aim of the current study** was to **develop and thoroughly evaluate a multi-omics sample preparation workflow** that aids in **comprehensive Metabolomics, Lipidomics and Proteomics datasets** by comparing and adapting/modifying existing homogenization procedures along with monophasic (same extract for Metabolomics and Lipidomics analysis) and biphasic (dedicated extract for each "-omics" technique) extraction solvent mixtures. The focus was on postmortem human muscle and liver tissue samples.

Table 2: Parameters and corresponding decision criteria for ranking the different extraction solvents/mixtures; RSD: relative standard deviation.

Evaluation parameters	Decision criteria
1D SDS-PAGE – protein pellet	Number of protein bands (high) / band intensities
1D SDS-PAGE – metabolite fraction	Number of protein bands (low) / band intensities
BCA assay	Protein quantification results (high)
Metabolomics – targeted data processing	Variability btw. tech. reps (RSD, low)
Metabolomics – untargeted data processing, features	Number of features (high) / variability btw. tech. reps (RSD, low)
Metabolomics – untargeted data processing, compound class	Number of "organic acids and derivatives" (high)
Lipidomics – untargeted data processing, features	Number of features (high) / variability btw. tech. reps (RSD, low)
Lipidomics – untargeted data processing, compound class	Number of "lipids or lipid-like molecules" (high)
Proteomics – untargeted data processing	Number of identified peptides / variability btw. tech. reps (RSD)

Optimal homogenization was achieved when using 20 mg of postmortem muscle or liver tissue with 200 µL (1:10 ratio) Water:MeOH (1:2) with 3 x 30 s pulses. After transfer of the super-natant, the overall optimal extraction solvent was MeOH:Acetone (9:1), a monophasic solvent, resulting in the most comprehensive targeted and untargeted Metabolomics, Lipidomics and Proteomics datasets.

Reproducibility issues (organic extract) are recommended to be counteracted by using separate aliquots for technical injection replicates for Metabolomics and Lipidomics analyses.

Results Proteomics

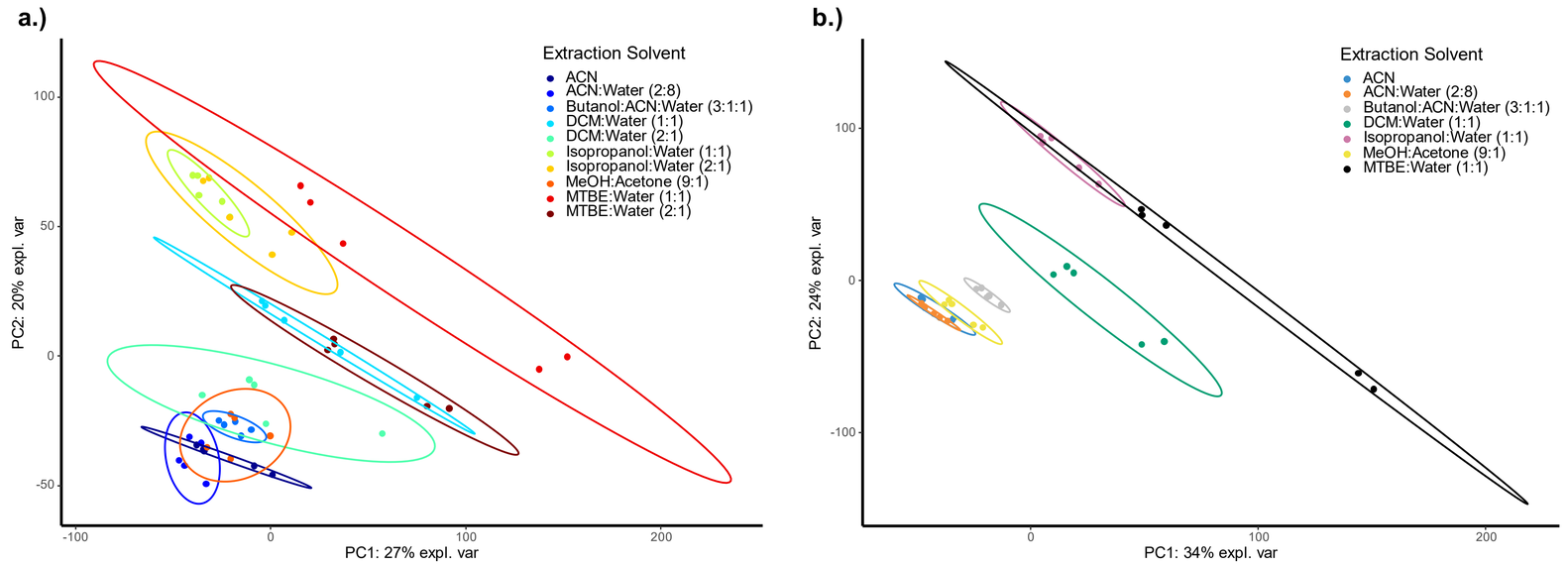

Figure 2: Principal component analysis of the untargeted proteomics data; underlying data includes all features (normalized abundance) with a positive peptide identification; a) muscle tissue samples; b) liver tissue samples.

Results Lipidomics

- Biphasic extraction solvents lead to the least number of processed features
- MeOH:Acetone (9:1) showed the highest number of total features extracted

Results Metabolomics

Use of monophasic extraction solvents lead to the greatest number of features extracted (number of features and compound class "organic acids and derivatives"), but also showed largest variability between technical injection replicates (most likely caused by evaporation of organic solvent with repeated injection).

Table 3: Number of peptides identified (human reviewed database) in the proteomics extracts using Progenesis QI (for proteomics); data is based on peptides with normalized peak area > 100; mean over biological and injection replicates (n=5) and corresponding relative standard deviations.

Extraction solvent	Number of peptides		RSD [%]	
	Muscle	Liver	Muscle	Liver
MTBE:Water (1:1)	3244	3578	1.6	9.5
MTBE:Water (2:1)	3573	-	2.7	-
DCM:Water (1:1)	3361	3661	3.1	2.1
DCM:Water (2:1)	3423	-	3.8	-
Isopropanol:Water (1:1)	2810	2901	3.4	6.1
Isopropanol:Water (2:1)	3080	-	7.0	-
ACN	3485	3624	2.5	1.8
MeOH:Acetone (9:1)	3447	3636	1.8	1.7
ACN:Water (2:8)	3207	3564	2.3	1.7
Butanol:ACN:Water (3:1:1)	3374	3549	0.5	1.4

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