UNIFYING SAMPLE PREPARATION FOR METABOLOMIC AND PROTEOMIC ANALYSIS

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1. INTRODUCTION

- The integration of proteomic and metabolomic technologies is gaining interest in diverse fields of life sciences and systems biology.
- Proteomic and metabolomic studies, however, typically require different sample preparation procedures.

2. GOAL

The aim of this work is to explore compatible sample preparations that can extract polar and non-polar metabolites with minimal degradation, and efficiently precipitate proteins for metabolomics and proteomics analysis of the same sample, respectively.

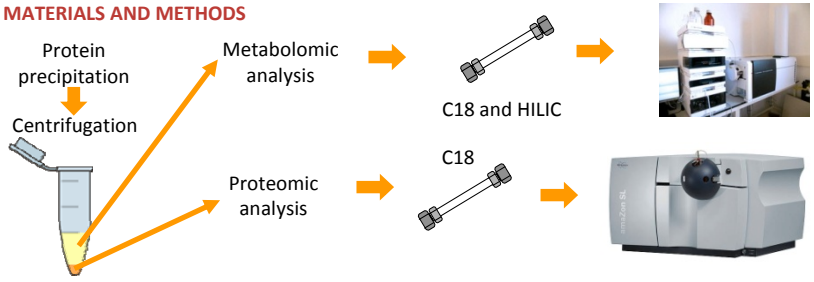
3. EXPERIMENTAL DESIGN

Eighteen aliquots of HepG2 cells (2M cells) were used.
Metabolites and proteins were isolated using 6 different protocols (n=3) combining different organic solvents and additives at different temperatures

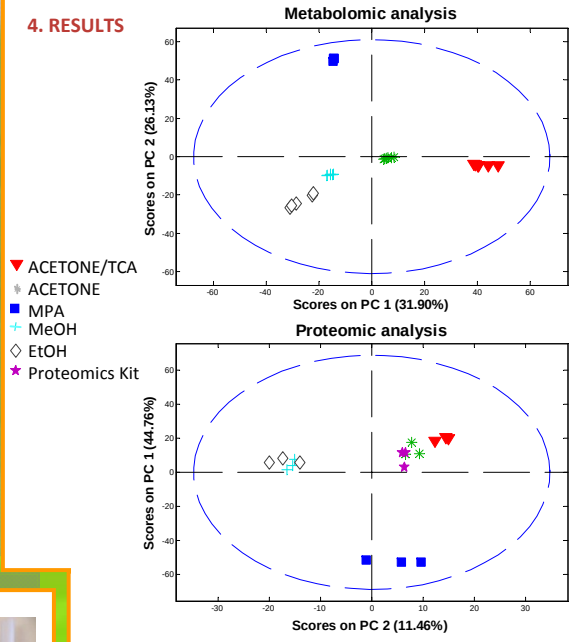
	Solvent	Analysis
Protocol 1	Acetone/TCA	Proteomics/Metabolomics
Protocol 2	Acetone	Proteomics/Metabolomics
Protocol 3	MPA	Proteomics/Metabolomics
Protocol 4	Hot MeOH	Proteomics/Metabolomics
Protocol 5	Hot EtOH/H2O	Proteomics/Metabolomics
Protocol 6	Proteomics Kit	Proteomics

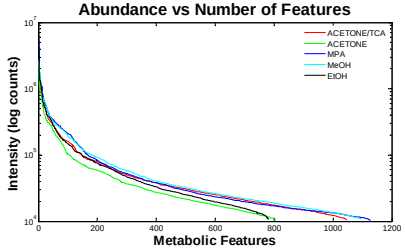
Table 1. Protocols used. TCA: trichloroacetic acid MPA: metaphosphoric acid MeOH: methanol EtOH: ethanol.

4. MATERIALS AND METHODS



4. RESULTS

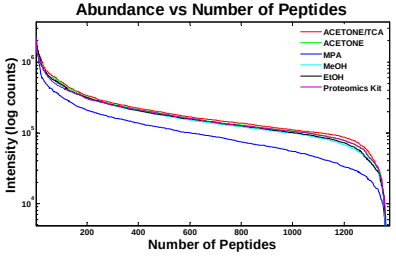




MPA and MeOH protocols produce the highest number of features.

In contrast, MeOH and EtOH do not extract so many metabolites efficiently.

PCA shows large distances between protocols, of which MPA is the most distinctive protocol.



The number of detected peptides is similar for all protocols used, although with an overall lower intensity when using the MPA protocol

PCA shows a clear separation of the MPA protocol with respect to other protocols. The two protocols based on acetone extraction and the standard proteomics method show similar properties. The same is observed with EtOH and MeOH protocols.

5. CONCLUSIONS

Multivariate data analysis of metabolomic and proteomic datasets shows a separation of different extraction procedures used in our study. MPA seems to provide particularly different extraction/precipitation properties relative to other methods explored.

Acid-containing protocols are far apart from each other, of which MPA shows the largest number of metabolite features detected.

In Proteomics analysis three clusters are observed: (i) MPA; (ii) MeOH/EtOH and (iii) acetone-based protocols, although the number and identity of peptides detected by each protocol do not differ considerably .

We present complementary experimental conditions that allow extensive metabolome and proteome coverage. Further work is in progress to identify metabolites and proteins, and integrate the results in a systems biology approach.