

Assessment of compatibility between extraction methods for NMR and LC/MS-based metabolomics



INTRODUCTION

Metabolomics is focused on the profiling and quantification of small, naturally occurring compounds that collectively constitute the so-called metabolome and, as such, serve as direct signatures of biochemical activity in cells. In this context, two different technologies have arisen in the field: nuclear magnetic resonance (NMR) and mass spectrometry (MS). However, due to the wide range of chemically and structurally diverse metabolites there is not a unique NMR or MS analytical platform able to reliably measure such a diversity of compounds, especially for untargeted metabolomic studies. Therefore, the implementation of multipatform approaches has been used to expand coverage of the metabolome.

The main drawback for this approach is the sample preparation disparities between NMR and MS. Specifically, deuterated solvents used in NMR strategies can complicate LC/MS analysis due to potential mass shifts, whereas acidic solutions typically used in LC/MS methods to enhance ionization of metabolites can severely affect reproducibility of NMR measurements. These intrinsically different sample preparation requirements result in the application of different procedures for metabolite extraction, which involve additional sample and unwanted variability.

In the present study, we use liver tissue to optimize metabolite extraction conditions for NMR analysis followed by LC-ESI MS by using the same sample extract with no need for solvent exchange or further pre-treatment.

OBJECTIVE

To develop several extraction protocols for liver tissue samples involving different aqueous/organic solvents and temperatures to obtain a suitable extract to be analysed by NMR and LC-MS simultaneously.

SAMPLE MEASUREMENT

- 1- Rat liver was ground on a mortar with liquid N₂.
- 2- 100 mg aliquots were homogenized with 2 mL (x 3) of each solvent system used in the study at the three different temperatures (-20° C, room temperature, 60° C)
- 3- The supernatant was evaporated to dryness under a stream of O₂-free N₂ and further reconstituted in 500 µL of deuterated acetonitrile and deuterated water (Z:8).
- 4- The reconstituted extracts were analysed first by NMR and LC-MS afterwards.

EXTRACTION PROTOCOLS

Table 1: Temperatures and extraction solvent combinations and used in the study.

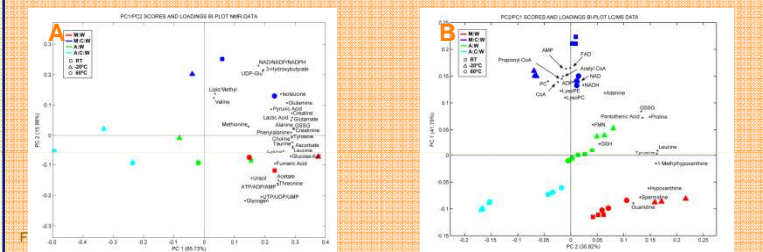
	-20° C	Room Temperature (25° C)	60° C
Methanol:Chloroform:Water (M:C:W)	▲	■	●
Methanol:Water (M:W)	▲	■	●
Acetonitrile:Chloroform:Water (A:C:W)	▲	■	●
Acetonitrile:Water (A:W)	▲	■	●

BIPLLOT ANALYSIS

The biplot is a scatter plot that graphically displays loadings and scores from the principal component analysis (PCA) of a given data. In this study, each biplot displays the scores on the twelve extraction conditions and loading on the metabolites identified by either NMR (A) or LC-MS (B). In both cases, the biplot shows that solvent combination (color code) has a greater effect in the extraction of metabolites than solvent temperature (shape code).

The solvent combinations which gave the best extraction yields for the metabolites used in the study were M:W and M:C:W for both NMR and LC-MS.

For LC-MS, important cofactors such as coenzyme A (CoA), acetyl-CoA, propionyl-CoA, malonyl-CoA and key oxidizing and reducing agents such as NAD⁺, NADH and FAD were extracted more efficiently using M:C:W.



EXTRACTION EFFICIENCY

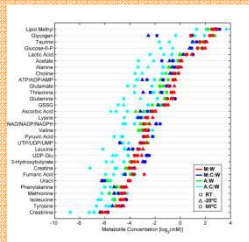


Fig. 3: Quantification by NMR of the 30 metabolites extracted with the different extraction protocols.

VARIATION OF THE EXTRACTION METHOD

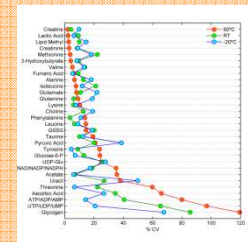


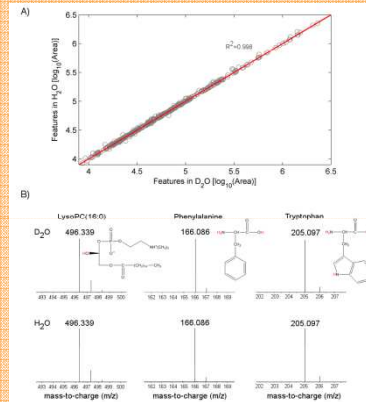
Fig. 4: Coefficient of variation for metabolites extracted using M:C:W at different temperatures. Metabolite extraction were performed by triplicate.

D₂O AND H₂O CORRELATION

Figure 5:

(A) Scatter plot representing the area of each feature from the XCMS matrix of LC/MS data of liver samples reconstituted in H₂O and D₂O. A correlation coefficient (R²) of 0.997 indicates a high linear regression, which demonstrates insignificant differences between the number and abundance of features detected in liver extracts reconstituted in H₂O and D₂O.

(B) Mass spectra of LysoPC (16:0), phenylalanine and tryptophan reconstituted in D₂O (top), and H₂O (bottom). Labile hydrogens are marked in red. Mass spectra show that the isotopic distribution of the compounds are not altered by D₂O, indicating a quick back-exchange of labile ²D to ¹H in aqueous LC/MS buffers due to a total solvent accessibility of small molecules structures.



CONCLUSIONS

- ❖ The distinctive physico-chemical properties involved when measuring by NMR or LC/MS make these two technologies highly complementary for metabolomic studies.
- ❖ The use of deuterated solvents required for NMR is not a limitation for the analysis by LC-MS.
- ❖ The choice of solvents largely dominate the extraction efficiency of the method.
- ❖ The temperature used during the extraction has a minor effect in the extraction efficiency.
- ❖ Metabolites difficult to detect (or even impossible, such as glycogen) by reverse phase LC-MS can easily be detected by NMR.
- ❖ Protein removal is more effective in methanol containing methods than those containing acetonitrile (data not shown).