LMWMscale: A New Bioinformatics Tool for Low Molecular Weight Metabolites (LMWM) Quantification based on 1H-NMR spectroscopy

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Background

Metabolomics is a powerful tool to understand underlying pathophysiological mechanisms associated with metabolic disorders, proposed for biomarker discovery [1].

However, the current metabolomic approaches needs higher degree of automation and standardization for clinical applications [2].

The current study presents *LMWMscale® Test*, an automatic bioinformatics tool for high-throughput quantitative metabolic profiling based on ^{1}H-Nuclear Magnetic Resonance.

Results

The algorithm read and processed ^{1}H-NMR spectra in order to optimize, phase and baseline correct. The LMWM-associated regions were selectively batched in order to isolate, align and deconvolute each signal automatically.

The deconvolution approach used *Voigt* analytical functions (a mixture between lorentzian and gaussian functions) to reproduce the experimental curve minimizing the fitting error, to quantify the area of each signal proportional to the metabolite concentration. The deconvolution approach allowed the quantification of several LWMW signals with complex coupling patterns, even in highly overlapped spectral regions.

The resulting areas were transformed to concentration units by applying specific conversion factors. Consistency between standard techniques were evaluated for glucose and creatinine, the correlation coefficients were R^2>0.9.

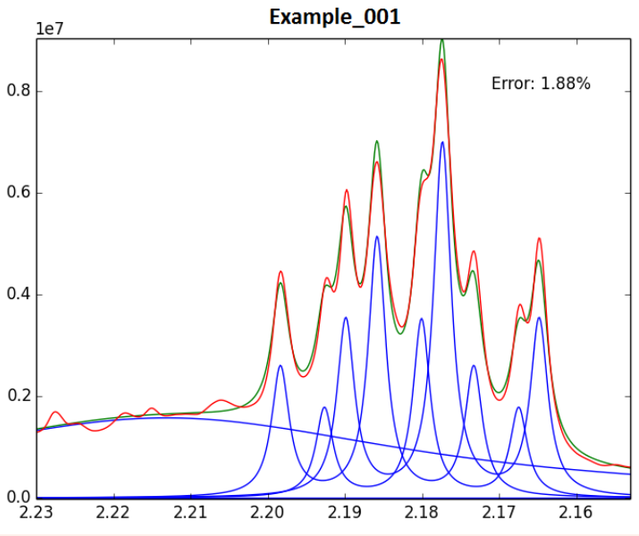


Figure 1. Image obtained from a urine sample showing the deconvolution of the specific region of azelate, pimelate and suberate.
The red line is the raw spectrum, the blue ones represent each individual analytical functions associated with each metabolite and the green one the resulting deconvolution.

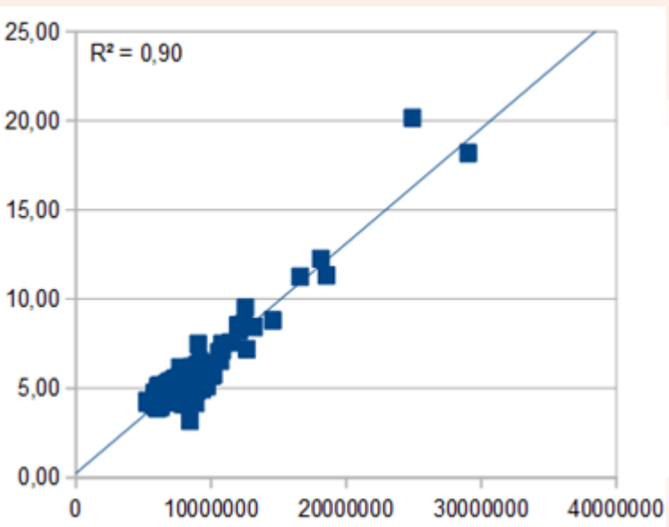


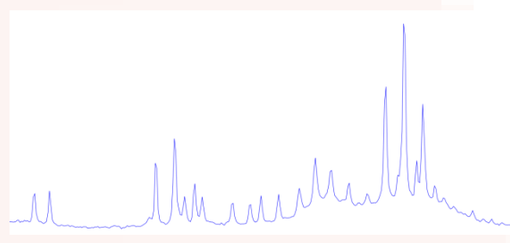
Figure 2. Linear regression of glucose concentration.
Scatter plot of the plasmatic glucose concentration of 184 subjects comparing enzymatic method and ^{1}H-NMR.

Materials and Methods

Study population

^{1}H-NMR spectra from different biological matrixes including 4.800 sera, 107 fecal extracts, 443 urines, 21 cell cultures and 21 culture medium samples.

LMWMscale Test



Our algorithm quantitatively profiled at least 10 low molecular weight metabolites for each biological matrix

Analytical validation

Analytical validation of ^{1}H-NMR glucose quantification was performed by linear regression and Pearson's correlation coefficient (r) with analogous measurements obtained with enzymatic methods.

Table 1. List of some of the most relevant metabolites profiled by LMWMscale.

Metabolite	Biological matrixes				
	Serum	Fecal extract	Urine	Culture medium	Cell culture
3-Hydroxybutyrate	✓				
Acetate	✓	✓	✓		
Acetone	✓				
Alanine	✓	✓	✓		✓
Creatine	✓	✓	✓		
Creatinine	✓	✓	✓		
Formate	✓	✓	✓		✓
Glucose	✓	✓	✓		✓
Glutamate	✓	✓			
Glutamine	✓	✓	✓		✓
Glycine	✓	✓	✓	✓	✓
Lactate	✓	✓	✓		✓
Methylhistidine	✓	✓	✓		✓
Tyrosine	✓	✓	✓		✓
Valine	✓	✓	✓	✓	✓
Isoleucine	✓	✓	✓	✓	✓
Leucine	✓	✓	✓	✓	✓
Choline		✓	✓	✓	✓
ATP				✓	
Lysine		✓	✓	✓	✓
Phenylalanine		✓	✓		✓
Pyruvate					✓
Butyrate		✓			
Malonate		✓			
Methanol		✓			
Propionate		✓			
Succinate		✓	✓		
Threonine		✓	✓		
Trimethylamine		✓	✓		

Conclusions

The *LMWMscale® Test* provides automatic quantitative screening of LMWMs present in biological matrixes from 1H-NMR spectra.

References

[1] C. B. Clish, "Metabolomics: an emerging but powerful tool for precision medicine," *Mol. Case Stud.*, vol. 1, no. 1, p. a000588, Oct. 2015.

[2] A. H. Emwas *et al.*, "Nmr spectroscopy for metabolomics research," *Metabolites*, vol. 9, no. 7. MDPI AG, 01-Jul-2019.