# Using ASICS to quantify metabolites in 1D 1H NMR spectra: an application to perinatal survival in pigs

Gaëlle Lefort, Laurence Liaubet, Patrick Tardivel, Cécile Canlet, Marie Tremblay-Franco, Laurent Debrauwer, Nathalie Villa-Vialaneix, Rémi Servien

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G. Lefort[1], L. Liaubet[2], P. Tardivel[3], C. Canlet[3,4], M. Tremblay-Franco[3,4], L. Debrauwer[3,4], N. Villa-Vialaneix[1] and R. Servien[3]

[1] *MIAT, Université de Toulouse, INRA, Castanet-Tolosan, France*
[2] *GenPhySE, Université de Toulouse, INRA, ENVT, Castanet Tolosan, France*
[3] *Toxalim, Université de Toulouse, INRA, ENVT, INP-Purpan, UPS, F-31027 Toulouse, France*
[4] *Axiom Platform, MetaToul-MetaboHUB, National Infrastructure for Metabolomics and Fluxomics, F-31027 Toulouse, France*

1D $^1$H Nuclear Magnetic Resonance (NMR) is a high-throughput technology that allows to obtain metabolomic profile easily (*e.g.*, from fluids such as blood) at low cost. However, unlike other types of data, it is difficult to detect which metabolites are present from the 1D $^1$H NMR spectrum of a complex mixture. In 1D $^1$H NMR spectrometry, each generated spectrum is usually first divided into intervals called buckets. Then, the areas under the curve are computed for each bucket. These steps are repeated for each spectrum and multivariate statistical analyses or tests are used to provide a list of buckets that are significantly different between the studied groups but requires that 1D $^1$H NMR experts identify the metabolites involved in the significant buckets for interpretation. Not only is this identification step tedious, time consuming and expert dependent but it also leads to a serious loss of information since the identification of metabolites is restricted to significant ones. Another way to proceed would be to identify and quantify all the metabolites in each spectrum and to perform statistical analyses on these data but this problem is still an open issue.

In this communication, we present a new method, called ASICS (Automatic Statistical Identification in Complex Spectra), which allows the identification and the quantification of metabolites in a complex mixture obtained with 1D $^1$H NMR [1]. Based on a set of pure metabolite spectra, a linear model with a Lasso penalty is fitted. This model computes the proportions of each metabolites in the complex spectrum. Global or local chemical shift variations due to various experimental conditions or peak overlapping are also automatically handled by a specific warping strategy.

To asses the performance of ASICS, we used data from PORCINET (ANR-09-GENM-005). The project's goal is to understand the mechanisms of maturity and perinatal survival in pigs. Blood samples were collected on fetuses at 90 and 110 days of gestation. With these samples, metabolic profiles were obtained by 1D $^1$H NMR as well as biochemical dosages of three metabolites

(glucose, fructose and lactate). First, a comparison between ASICS quantification and dosages was performed. This analysis shows a correlation superior to 0.8 between ASICS quantifications and real dosages that assessed the good performances of the method. Then, we compared metabolome analyses based on classical buckets or on the relative concentrations provided with ASICS. Metabolites varying between the two stages of gestation were extracted. Similar results were obtained with both approaches for many metabolites such as glucose, creatinine or alanine that showed an increasing between 90 and 110 days of gestation. Furthermore, ASICS highlights new metabolites that had not been identified with buckets. In conclusion, although both approaches give quite similar results, ASICS allows a faster and simpler direct biological interpretation than the classical buckets approach.

This method is available in an R package, **ASICS**, or within the user-friendly Galaxy/W4M interface developed by the MetaboHUB infrastructure and IFB (French Institute of Bioinformatics).

# References

[1] Tardivel P., Canlet C., Lefort G., Tremblay-Franco M., Debrauwer L., Concordet D., Servien R. (2017). ASICS: an automatic method for identification and quantification of metabolites in complex 1D 1H NMR spectra. *Metabolomics*, **13**(10): 109. https://doi.org/10.1007/s11306-017-1244-5