Chemometrics

Application Note

Toxicity Effects by NMR-Based Metabonomics and Mixture Analysis

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Summary

In toxicology studies, alterations to the intrinsic *in vivo* biochemical profile can be monitored via NMR spectroscopic analysis of urine. Chemometric processing of the NMR data can enhance understanding of the toxic effects, such as by examination of the PCA scores. However, the scores view of the data can be complex. And, the underlying eigenvectors, based on variance in the data, may have little discernible relationship to actual spectral patterns.

Mixture analysis algorithms estimate the shapes of contributing source materials and infer the contribution of each source profile to the mixture. Thus, mixture analysis can assist in interpretation of complex systems. This note demonstrates application of mixture analysis to a complex metabonomics study.

^{1}H NMR spectra of urine samples from laboratory study animals were obtained at 600 MHz on a Bruker NMR spectrometer (Bruker Biospin, Germany). The data were truncated to a range of 10 to 0.2 ppm and a subset was made that excluded the range of 4.5 to 6.1 ppm so as to remove variation due to solvent suppression.

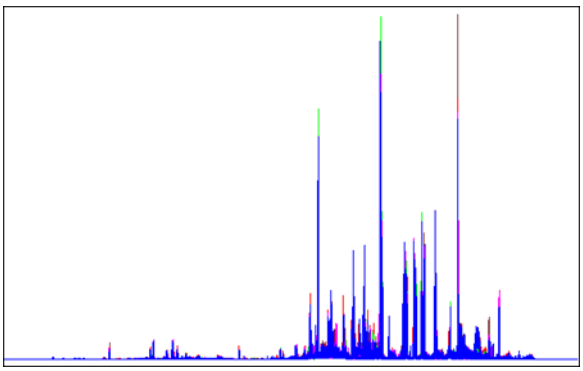

Figure 1: Overlay of the NMR Traces

Data analysis was performed with Pirouette® and included a normalization step as a pretreatment to minimize variation in urine concentration.

Figure 2 shows results of a PCA on the full collection of data including control, low and high doses. Intermediate exposure times of high dose samples align with factor 1 while the control and low dose samples vary in an orthogonal direction. Colors in the plot were assigned via HCA .

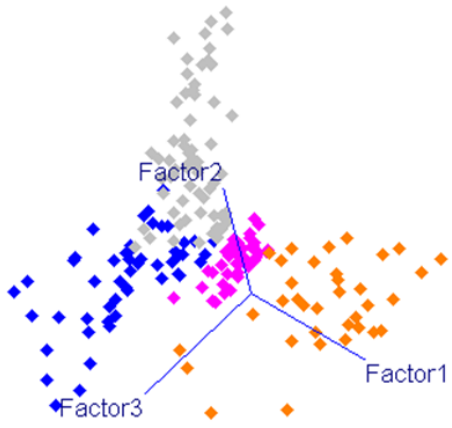

Figure 2: PCA scores plot for all specimens indicating 3 different effects based on dosage

Mixture analysis was run on these data using the ALS algorithm in Pirouette. The software allows you to choose different numbers of sources to be considered in the mixture solution. Figure 3 is a view of the NMR "pure source profiles" if we consider only three effects.

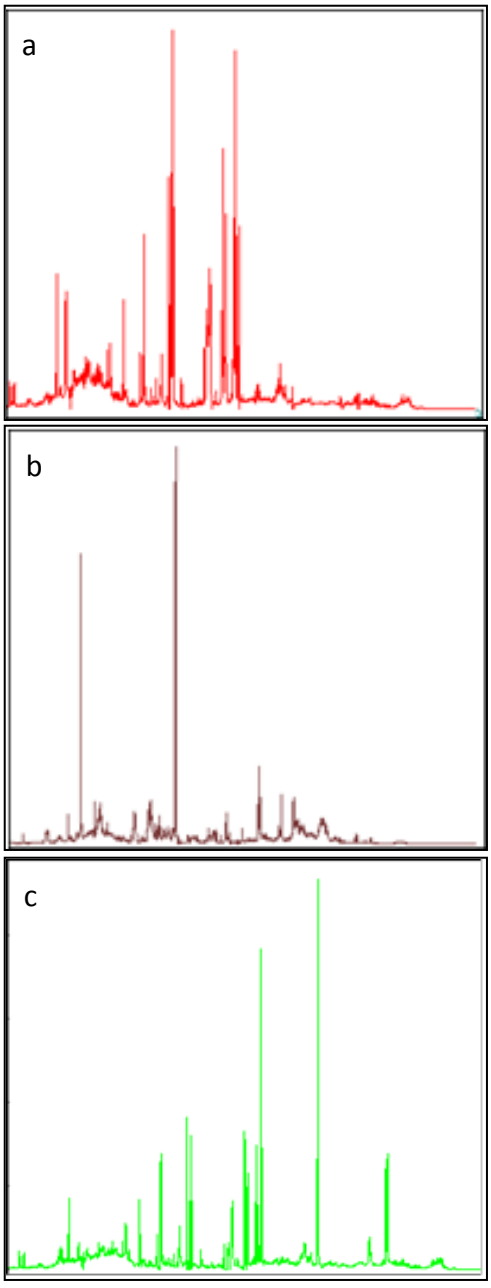

Figure 3: Graphical summary of the ALS result; (a) predose, (b) intermediate, (c) after-effect

After extracting the pure NMR spectra for the principal effects, we can use them to explain

the primary driving forces in the experiment. Thus, the reconstructed NMR trace on the top of Figure 3 contributes most greatly to the predose samples. Similarly, the center NMR trace contributes to the greatest extent those samples from the intermediate exposure times, while the bottom spectrum relates to the after effect samples, that is, day 7 and some day 5 and 6.

The first profile shows a typical control sample with the resonances from citrate, 2-oxoglutarate and succinate present in the center. From the second source profile this shows the classic pattern of markers for hydrazine hepatotoxicity, namely taurine, creatine and 2-aminoadipate. Finally, the last profile shows the classic pattern of contamination with the largest peak being acetate, and the two peaks to the right are lactate and ethanol.

We subsequently looked at the outcome of a 6-source solution and see interesting results. Basically, the 6 sources show samples of:

1. *the effect of high dose*
2. *the effect of control and low dose*
3. *the after effect from all samples*
4. *the pre-exposure time*
5. *a second group of pre-exposure*
6. *a group of early exposure control and low dose*

Conclusion

The results from the ALS analysis concur with previous data and visual inspection of the NMR spectra. Thus, mixture analysis provides a method of inferring the key profiles representing biochemical events (control, toxicity, time-related change, clinical monitoring, etc.). Because the ALS source profiles retain characteristics of NMR spectra, results are relatively easy to interpret. Study of classic toxins or drugs in development might benefit from such a mixtures approach.