Reproducibility of NMR Spectra in a Metabolomics Context

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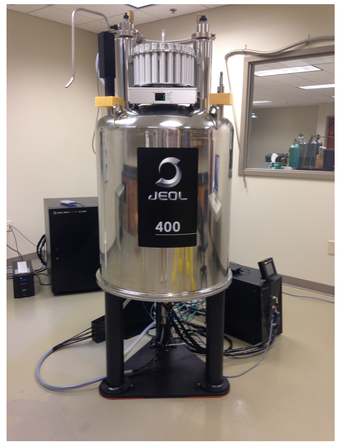
Background

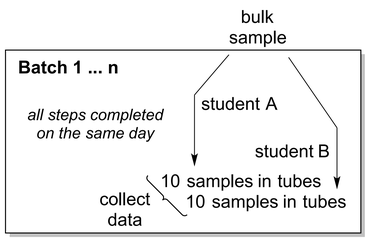
The Chemistry and Biochemistry Department of DePauw University recently upgraded to a JEOL ECS 400 MHz Nuclear Magnetic Resonance (NMR) Instrument. This machine applies an external magnetic field that interacts with the magnetic properties of active atomic nuclei, revealing the structures of compounds. A multitude of experiments are possible, including Correlation Spectroscopy (COSY) and Heteronuclear Single Quantum Coherence (HSQC) experiments. In the future we intend to use the NMR instrument in a metabolomic study of the medicinal plant *Portulaca oleracea*, commonly known as purslane. To ensure that our data is reliable and neither the NMR instrument nor the users affect the outcome, we conducted this reproducibility study. We hypothesized that any variation between the spectra would be insignificant.



NMR Instrument

Experimental Design

We prepared 80 replicate samples of the same solution to determine if there was any variation in the results between samples made on different days and prepared by different people. Ten samples per person were analyzed in four different batches, each on a different day.



different batches on different days

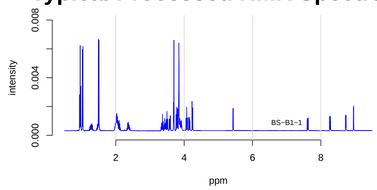
Sample Preparation

The samples consisted of 550 μL of phosphate buffer, 50 μL of a 1% weight per volume solution of trimethylsilyl propanoic acid (TSP) in 99.9% D D_2O, and 0.01 moles each of sucrose, L-proline, *dl*-alanine, L-isoleucine, and nicotinamide. Data was collected using a pre-saturation pulse sequence to supress the residual water peak.

Data Processing

The data were processed in Delta. The spectra were phased and referenced, then exported into the statistics software `R`. The package `ChemoSpec` was used first to align and normalize the data, and then to conduct hierarchical cluster analyses (HCA), principal component analyses (PCA), and ANOVA principal component analyses (AOV-PCA) to explore the amount of variation between each sample.

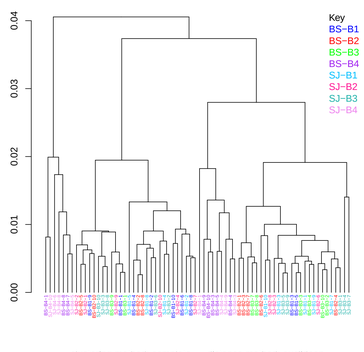
Typical Processed NMR Spectrum



Hierarchical Cluster Analysis (HCA)

HCA is a statistical tool used to show the distances and similarities between spectra. An algorithm is used to create a tree diagram which groups the spectra based on their similarity.

Hierarchical Cluster Analysis

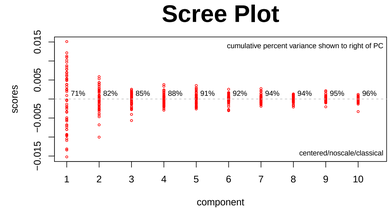


Careful inspection shows that samples in batch 4 cluster together. Further investigation revealed that these samples were contaminated with acetone.

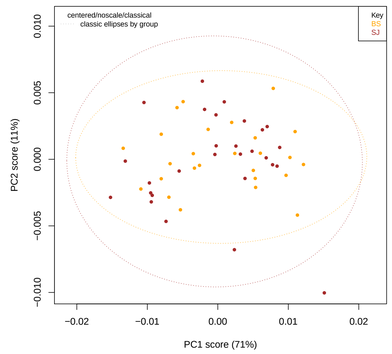
Principal Component Analysis (PCA)

PCA is a dimension reduction method that finds hidden components within the data to describe the essence of the data. The noise is removed and correlated peaks are collapsed. Initial PCA analysis indicated that Batch 4 was contaminated with acetone; we removed these data points before continuing.

Scree Plot

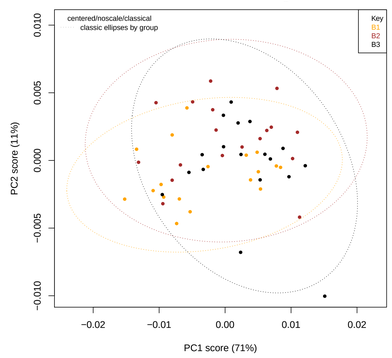


PCA Scores Plot by Person



This plot shows only trivial data variation due to different NMR users.

PCA Scores Plot by Batch

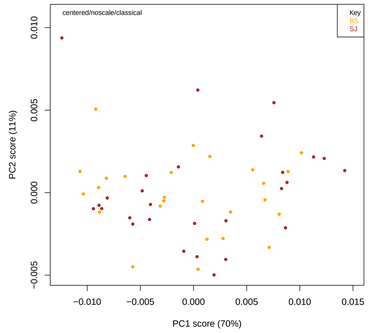


This plot shows very little separation by sample batch, though the confidence ellipse for one batch is oriented slightly differently.

AOV–PCA

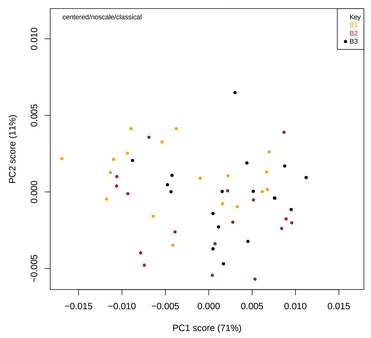
ANOVA PCA is a method that splits the data by experimental factor and then runs PCA seperately on each factor.

AOV PCA by Person

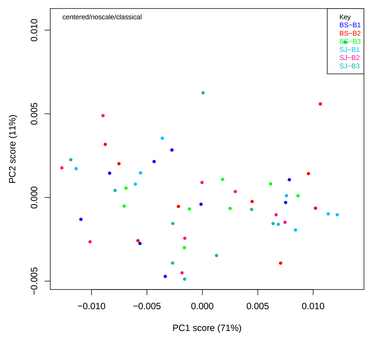


In an AOV–PCA plot, if a factor is significant, there will be separation along PC1. No such separation is evident in samples prepared by different NMR users (above) or different batches run on different days (below).

AOV PCA by Batch



AOV PCA Batch x Person



This plot is analogous to an interaction plot in ANOVA. It shows that the combination of batch and user factors is insignficant.

Conclusion

Our statistical analyses do not reveal any observable patterns within or between sample batches or NMR users after the removal of batch 4 due to contamination.

This confirms our initial hypothesis that the instrument is stable enough that the same sample run on different days or by different people gives a substantially identical NMR spectrum. This gives us confidence that differences which we expect to observe in our upcoming metabolomics studies will be due to planned, deliberate experimental conditions and not due to determinate error.

Acknowledgments

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