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Comparison of Spectral Data of Metabolites Collected from Bruker and Varian 600 MHz Spectrometers

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Abstract : The spectral data were collected from the two 600 MHz spectrometers from the two major manufacturers, Bruker and Varian. The samples were prepared to create standard curves for quantitative measurements of metabolite concentrations. Instead of employing one-dimensional ^{1}H experiments, the two-dimensional ^{1}H-^{13}C HSQC experiments were performed for better separation of resonances. For some resonances, the high salt condition hindered the linear correlation between the intensity and actual metabolite concentration. Excluding overlapped ones, most resonances showed good linearity. Although the Varian spectrometer showed better linearity, both spectrometers were able to generate acceptable standard curves. From this data, we could identify resonances that could be used to better quantify the concentrations of the particular metabolites. With these standard curves, the quantitative measurements of the metabolites from the real samples will be facilitated.

Keywords : metabolomics, NMR, quantitative, metabolite, concentration

INTRODUCTION

Metabolomics is the "systematic study of the unique chemical fingerprints that specific cellular processes leave behind" - specifically, the study of their small-molecule metabolite profiles.[1] The metabolome represents the collection of all end products of the gene expression in a biological organism. Thus, metabolic profiling can provide a snapshot of the system's physiology.

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One-dimensional (1D) ^{1}H nuclear magnetic resonance (NMR) spectroscopy has been used extensively as an analytical tool for identifying and quantifying small molecules.[2, 16-18] With the samples showing minimal peak overlap, 1D ^{1}H NMR can be employed because the peak intensity and concentration maintain a linear relationship. Recently, high-throughput analysis of complex biological processes at the metabolic level by NMR has been receiving a big attention.[3,4] These studies, however, relies on the 1D ^{1}H NMR, and inevitably suffer from the extensive peak overlap. Statistical techniques have been developed to interpret spectral data to a biological information.[5] Since the statistical method uses spectral density as a whole, it does not offer accurate quantification of each metabolite. Several methods were developed to overcome this problem,[7-11] but most of the published applications have remained qualitative.

As an alternative to the metabolomics by NMR, a two-dimensional ^{1}H-^{13}C HSQC has been proposed.[12] This method showed a promising result on getting quantitative data of metabolite concentrations. To get the accurate metabolite concentrations, we need to first set up a standard curve, and this is what this manuscript is about.

EXPERIMENTALS

Sample preparation

Four groups of samples were prepared for NMR experiments. Each group had three different levels of metabolite concentrations: 2, 5, and 10 mM. Group 1 contained 4-aminobutyrate, adenosine, alanine, AMP, asparagine, aspartate, betaine, choline, citrate, citrulline, creatine, ethanolamine, fructose, glutamate, glutathione-oxidized, histidine, homoserine, isoleucine, lactate, leucine, lysine, malate, methionine, myo-inositol, NAD, phenylalanine, proline, serine, tartarate, trans-4-hydroxyproline, trehalose, tryptophan, valine. Group 2 contained ADP, arginine, canavanine, carnitine, cysteine, galactose, glutathione-reduced, glycine, mannose, succinate, sucrose, taurine, threonine. Group 3 contained glycerol, glutamine, glucose, putricine (1,4 diaminobutane), acetic acid, uridine, betaine. Group 4 contained MES, orinithine, trehalose, maltose.

Each sample contained 5 mM HEPES, 0.5 mM DSS, and 0.5 mM sodium azide in 100 % D_2O in addition to the synthetic metabolite mixtures described above. pH was adjusted to 7.4 with NaOD or DCl.

NMR Experiments and Data Processing

All NMR spectroscopy was carried out at the National Magnetic Resonance Facility in Madison. NMR experiments were performed on either Bruker DMX 600 or Varian Unity Inova 600. Both spectrometers were equipped with a triple-resonance (^1H, ^{13}C, ^{15}N, ^2H lock) cryogenic probe. Sensitivity enhanced ^1H–^{13}C HSQC spectra were collected with 4 scans, 128 (or 512) increments for Varian (or Bruker), and GARP decoupling. The spectral widths were 13 ppm for ^1H and 100 ppm for ^{13}C. The carbon carrier frequency was set at 55 ppm.

All spectra were processed and visualized using nmrPipe[13] and Sparky software[14], respectively. Picked peaks were converted to a suitable format for MMCD[15] (http://mmcd.nmrfam.wisc.edu) to identify the metabolites using FMQ scripts (Ian Lewis, personal communication) written in R, a free statistics software package (http://www.r-project.org). This script, FMQ, provided suitable interface between MMCD and Sparky. FMQ generated a project for Sparky, and spectral comparison was facilitated by overlap function. The intensities of resonances of synthetic metabolites were measured using Sparky, and compared to the real concentrations. The standard curves were generate using Microsoft Excel.

RESULTS and DISCUSSION

Due to the patent to be submitted, we will consider HEPES and ADP in Group 2 samples only. Group 2 has 12 metabolites, which can be regarded neither too many nor too few compared to other groups. Other groups showed the similar features.

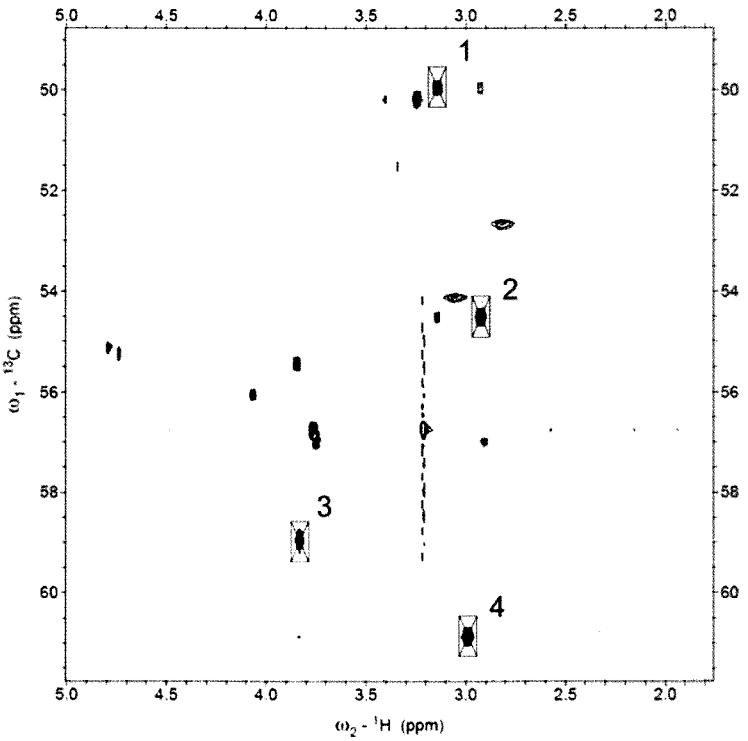

Fig. 1. Expanded region of ^{1}H-^{13}C HSQC spectrum of Group 2, 5 mM sample. HEPES resonances were denoted with boxes and numbers.

Internal consistency of NMR data

Since every sample contained the same concentration (5 mM) of HEPES, the intensities of HEPES resonances should be reasonably similar to one another. If we consider that many of the metabolites were ionizable so that the ionic strength of the sample increased, we may also expect some deviations. Figure 1 shows the HEPES resonances for internal consistency. Other than the peaks showing one-bond coupling, artifacts coming from the 2-bond coupling were also detected. As shown in Table 1, the resonances showing smaller variation were peaks #2 and #3 for Varian and the peak #3 for Bruker spectrometer. Peaks #1 and #4 are pH dependent, which is why they showed larger variations. The pH dependency of HEPES resonances can be viewed troublesome, but it is actually useful because it is an indicator about how well the sample was titrated. The most consistent peak in terms of the intensity was peak #3. We can use this peak of HEPES resonances for

internal standards when we calculate the concentration of metabolites from either Varian or Bruker spectrometer. However, the peaks from Varian spectrometer showed variations similar to or smaller than the ones from Bruker. Especially, variation of peak #2 from Bruker was nearly 6 times of that from Varian. This may be due to the fact that the Varian spectrometer was more up-to-date than Bruker one although they had the same field strength. The cryoprobes were known to be more sensitive to the salt concentration, but in the present study, they did not seem to be affected at all considering the near constant peak intensities.

Table 1. Concentration vs. peak intensity of HEPES. Relative variation was defined as percentage of standard deviation to average intensity.

Peak #	Varian				Bruker			
	Sample concentration			Relative Variation (%)	Sample concentration			Relative Variation (%)
	2mM	5mM	10mM		2mM	5mM	10mM	
1	13183	16358	17085	13.4	112555392	122620912	136036416	9.5
2	11351	11543	11007	2.4	93199664	84603776	71095064	13.4
3	10266	10472	10490	1.2	88698728	96028072	88479048	4.7
4	7447	11093	12227	24.4	62109316	89879480	96049864	21.9

An example of metabolite data: ADP

Figure 2 shows the expanded region covering resonances from ADP. Since the numbers of increments were different between two spectrometers, the peak shape of Bruker data looks more circular. Two spectra looked very similar other than the peak shape. When we analyzed the peak intensity, however, we could see the drastic differences. Figure 3 shows the concentration vs. peak intensity plot. Varian data showed nice linear regression lines, all pointing to the origin while Bruker data showed less ordered behavior. In fact, the average correlation values (R^2) of 6 resonances from Varian data were greater than 0.9995 while that from Bruker data were 0.9978. If we consider that the peak intensity should

converge to 0 when concentration goes to 0, then we can realize the Varian spectrometer provided much better data.

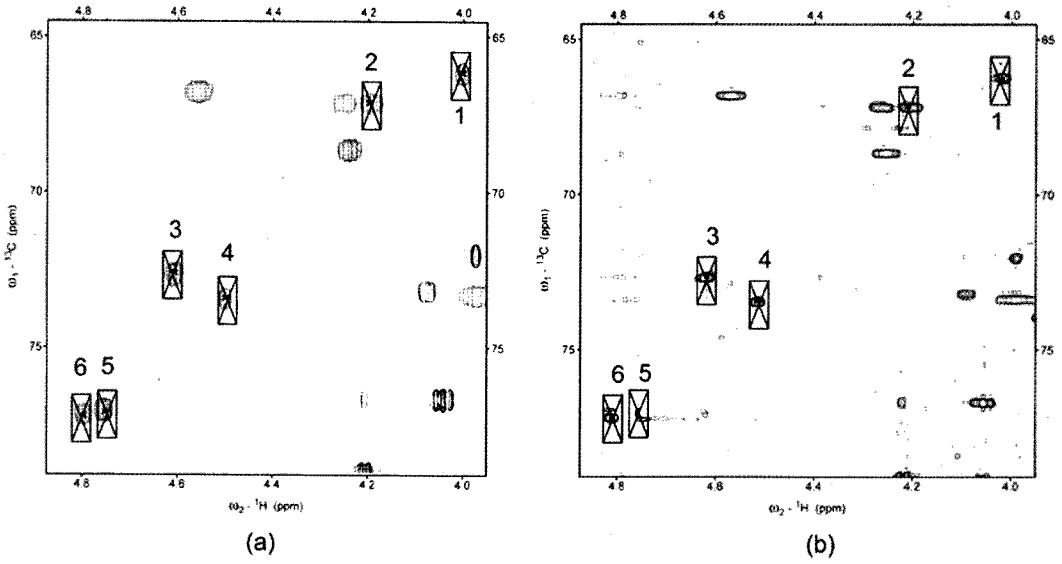

Fig. 2. Expanded region of ^{1}H-^{13}C HSQC spectrum of Group 2, 5 mM sample. (a) Varian data, (b) Bruker data. ADP resonances were denoted with boxes and numbers.

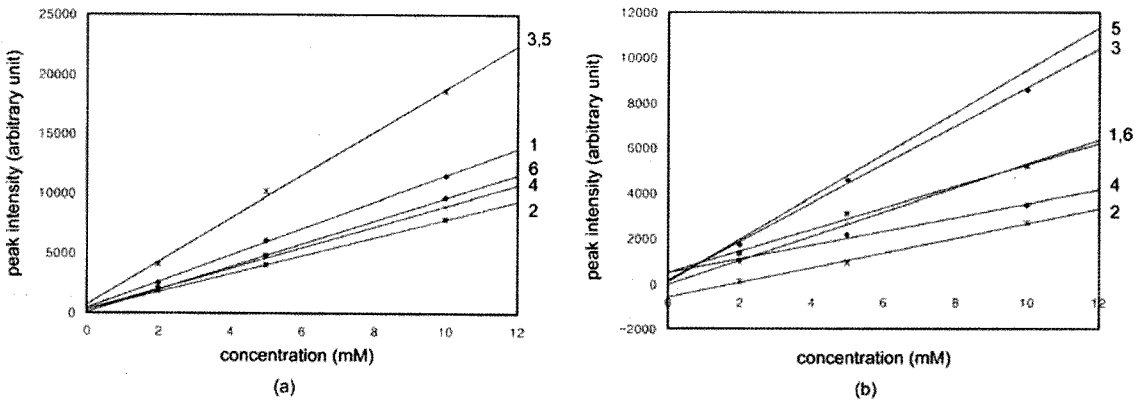

Fig. 3. Concentration vs. peak intensity of ADP. (a) Varian data, (b) Bruker data.

Nevertheless, if we choose the ones showing R^2 value greater than 0.999 and the y-intercept close to 0 in both Varian and Bruker spectrometers, then we can use data from either spectrometer to determine the concentration of ADP in the actual extract. With the above criteria, we can conclude that the most reliable candidate for quantification would be resonance #3. With this procedure, the critical resonance signals from other metabolites could be identified.

Since we determined which resonance to use to quantitatively estimate the concentration from the peak intensity, we can use only such peaks in the spectra of real extracts. Data from other resonances were also analyzed, but the detailed information is not provided at this moment due to the patent application.

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REFERENCES

1. B. Daviss, *The Scientist* **19**, 25-28 (2005).
2. G. F. Pauli, B. U. Jaki, and D. C. Lankin, *J. Nat. Prod.* **68**, 133-149 (2005).
3. J. C. Lindon, J. K. Nicholson, E. Holmes, and J. R. Everett, *Concepts Magn. Reson.***12**, 289-320 (2000).
4. J. C. Lindon, E. Holmes, and J. K. Nicholson, *Prog. Nucl. Magn. Reson. Spectrosc.* **39**, 1-40 (2001).
5. E. Holmes, O. Cloarec, and J. K. Nicholson, *Proteome Res.* **5**, 1313-1320 (2006).
6. M. E. Dumas, E. C. Maibaum, C. Teague, H. Ueshima, B. Zhou, J. C. Lindon, J. K. Nicholson, J. Stamler, P. Elliott, Q. Chan, and E. Holmes, *Anal.Chem.***78**, 2199-2208 (2006).
7. A. M. Weljie, J. Newton, P. Mercier, E. Carlson, and C. M. Slupsky, *Anal. Chem.* **78**, 4430-4442 (2006).
8. T. W. M. Fan, *Prog. Nucl. Magn. Reson. Spectrosc.* **28**, 161-219 (1996).

9. M. R. Viant, *Biochem. Biophys. Res. Commun.* **310**, 943-948 (2003).
10. J. Kikuchi, K. Shinozaki, and T. Hirayama, *Plant Cell Physiol.* **45**, 1099-1104 (2004).
11. T. W. M. Fan, A. N. Lane, M. Shenker, J. P. Bartley, D. Crowley, R. M. Higashi, *Phytochemistry* **57**, 209-221 (2001).
12. I. A. Lewis, S. C. Schommer, B. Hodis, K. A. Robb, M. Tonelli, W. M. Westler, M. R. Sussman, and J. L. Markley, *Anal. Chem.* **79**, 9385-9390 (2007).
13. F. Delaglio, S. Grzesiek, G. W. Vuister, G. Zhu, J. Pfeifer, and A. Bax, *J. Biomol. NMR* **6**, 277-293 (1995).
14. T. D. Goddard, and D. G. Kneller, SPARKY 3, University of California, San Francisco.
15. Q. Cui, I. A. Lewis, A. D. Hegeman, M. E. Anderson, J. Li, C. F. Schulte, W. M. Westler, H. R. Eghbalnia, M. R. Sussman, and J. L. Markley, *Nature Biotechnology* **26**, 62 (2008).
16. J. H. Lee, T.-K. Lee, R.-S. Kang, H. J. Shin, and H.-S. Lee, J. Kor. Magn. Reson. Soc. 11, 56 (2007)
17. S. Y. Park, B. S. Hwang, K. H. Ji, and J.-R. Rho, J. Kor. Magn. Reson. Soc. 11, 122 (2007)
18. B. S. Hwang, S. Y. Park, M. G. Park, and J.-R. Rho, J. Kor. Magn. Reson. Soc. 12, 33 (2007)