Targeted Profiling of Common Metabolites in Urine

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Urine is a readily-collected, information-rich biofluid that can provide insight into the metabolic state of an organism. As a result, urine is often a focus in metabolomics investigations using NMR and MRI spectroscopy, in both diagnostic and monitoring applications. Targeted profiling is a powerful tool that can drive such studies, allowing you to directly identify and quantify various metabolites [1]. In this note we present several strategies for targeted profiling of such spectra with Chenomx NMR Suite.

Introduction

Fitting compounds to a urine spectrum (see Figure 1) can be difficult to generalize, since every spectrum can be affected by subtly different matrix effects, including varying pH, divalent cation concentration, dilution effects, and so on. However, some general strategies can help you to get more accurate results from targeted profiling in Chenomx NMR Suite.

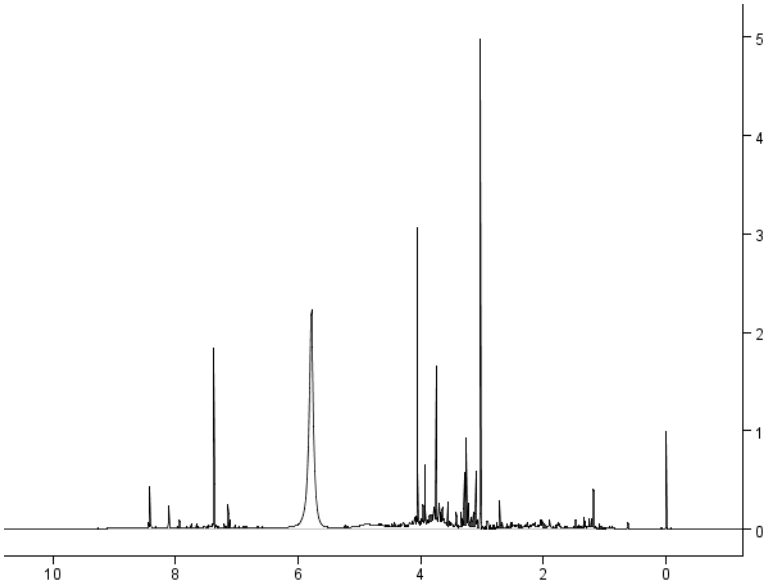

Figure 1. Real urine spectrum acquired at 800 MHz

General Techniques

Fit large, obvious signals quite early in your analysis. These signals tend to dominate the spectrum in the regions in which they appear, and fitting them first allows you to quickly fit a large amount of the total area of the spectrum with relatively little effort. Also, fitting the obvious compounds can help you to more confidently fit some of the more subtle patterns in the spectrum by effectively subtracting out the larger signals. Compounds most likely to contribute large, obvious signals to a human urine spectrum include creatinine, urea and trimethylamine N-oxide. Compounds contributing smaller signals that should still be easy to pick out include citrate, hippurate, alanine, trigonelline and 1-methylnicotinamide.

Effects of Water Suppression

Most methods of acquiring NMR spectra of aqueous samples such as urine involve some form of water suppression pulse. This has several effects, aside from the most apparent effect of reducing the size of the water peak. One of these is that clusters

appearing close to the water peak, that is, those resonating at a similar frequency, also experience a damping effect. These clusters appear smaller than you might expect, based on the number of protons responsible for the signal and the multiplicity of the signal. As a result, estimating the concentration of a multi-cluster compound based on clusters close to water can give artificially low results.

Compound-Specific Techniques

When you fit a compound with multiple clusters, use simple, isolated clusters, where available, to estimate the concentration of the compound. In any case, use clusters farther from the water peak in preference to those closer to the water peak. When necessary, overfit clusters near water slightly to compensate for the damping effect. For clusters farther away from water, fit them so that the sum line (appearing in red by default) best approximates the actual spectrum in the vicinity of the individual clusters.

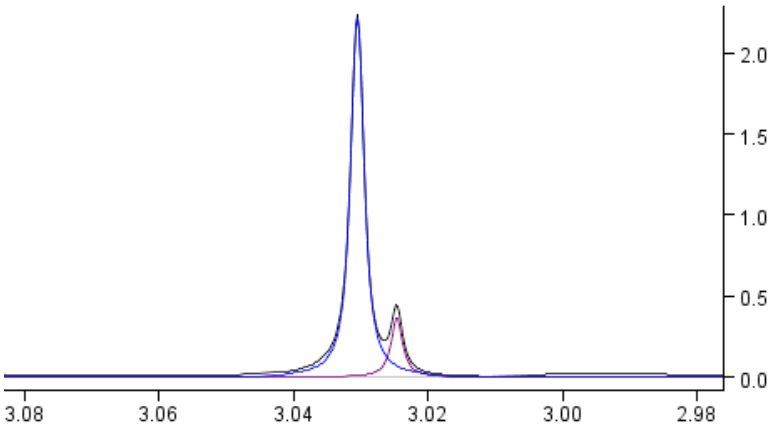

Figure 2. Overlap region of creatinine (blue) and creatine (purple)

Compounds that have only one signal can be difficult to fit, as it is common to see multiple signals of similar intensity in any given area in a urine NMR spectrum. Fitting single peaks to any one of these signals is simple enough, but determining which signal is the correct one for the compound in question poses an interesting challenge. In general, fit these compounds later in your analysis. Many of the other compounds that you fit will have clusters nearby, and fitting these overlapping clusters will reduce the number of candidate signals for the single-peak compounds.

Compound Groups

As you analyze a urine NMR spectrum, you will notice that a number of specific compounds have clusters that overlap. Such overlap is most common when there is a strong structural similarity among the compounds, as in the methyl groups of lactate and threonine, or the imidazole protons of histidine and the methylhistidines. However, coincidental overlap is also a distinct possibility (see Figure 1), as urine samples can contain several hundred metabolites detectable by ^{1}H-NMR. Fit these groups of compounds concurrently, as fitting them separately will result in inaccurate concentrations.

Exhaustively listing every possible combination of compounds that might overlap in a urine sample is well beyond the scope of this note. However, there are a number of combinations that to consider in any analysis of human urine samples.

Creatinine and creatine will always appear together in any aqueous sample containing either one (Figure 2), as they slowly interconvert in aqueous solution. They are structurally similar, and their methyl signals tend to overlap. In urine samples, creatinine is always be the larger of the two.

Lactate and threonine also show a degree of structural similarity, and their methyl signals tend to overlap. Relative concentrations vary, so verify any concentration that you estimate using the methyl signals with other clusters for both compounds.

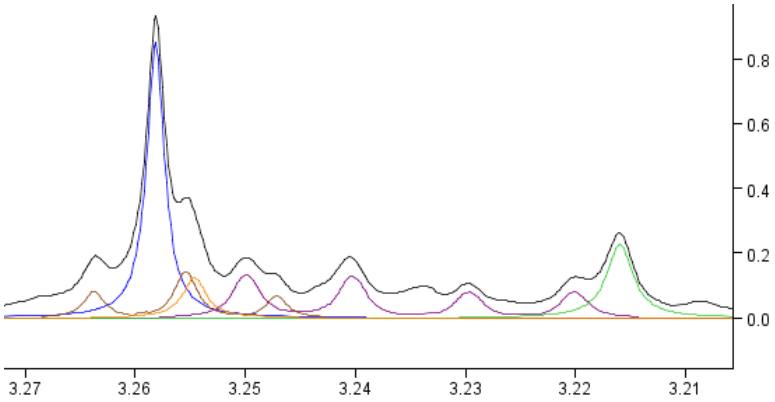

Figure 3. Overlap region of trimethylamine N-oxide (blue) and betaine (orange); several other compounds also overlap in the same region

Trimethylamine N-oxide and betaine each have a trimethylammonium group, so the large methyl signals for both appear very close together . Relative concentrations will vary, and several other compounds appear in the same region (Figure 3).

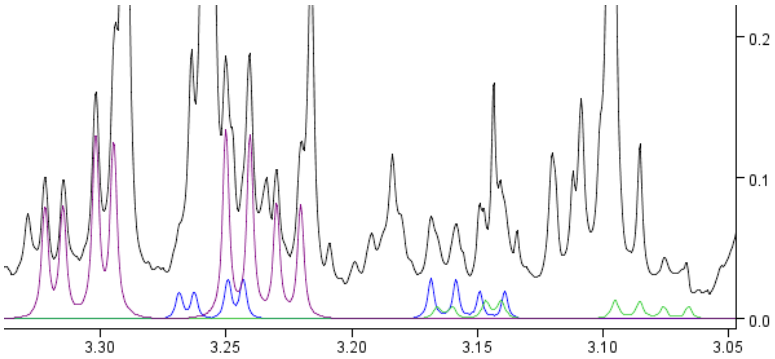

Figure 4. Aliphatic regions of histidine (blue) and the methylhistidines (purple and green)

Histidine, π-methylhistidine and τ-methylhistidine pose an interesting challenge (Figure 4), as their aliphatic proton signals overlap heavily. All three are frequently present in human urine.

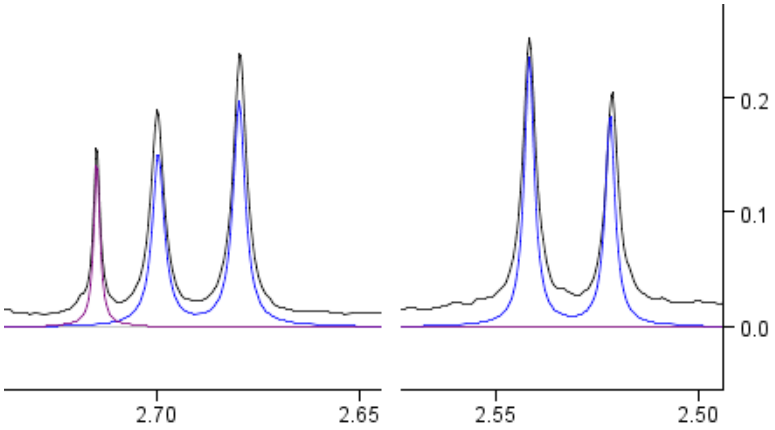

Figure 5. Overlap region of citrate (blue) and dimethylamine (purple)

Tryptophan and 3-indoxylsulfate also have similar, indole-based ring systems, as do trigonelline and 1-methylnicotinamide, which are pyridinium-based. As a result, the

associated signals from ring protons appear close together. Several clusters for each appear in otherwise quiet regions of the spectrum.

Citrate and dimethylamine appear frequently in human urine, and often appear together. Both have simple patterns, and appear in a relatively quiet region of the spectrum (Figure 5).

Succinate and pyroglutamate do not have any overt structural similarity, but the single peak of succinate overlaps one of the complex clusters of pyroglutamate. This can make arbitrarily identifying the signal associated with succinate difficult. Fitting pyroglutamate concurrently, using other clusters in the compound to aid in determining an appropriate concentration, can simplify the identification of the succinate signal.

Conclusion

Although applying targeted profiling to a urine spectrum appears complex, such analyses can be approached systematically. The simple techniques outlined above form a basis for systematically profiling most human urine spectra.

Acknowledgements

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References

[1] AM Weljie, J Newton, PM Mercier, E Carlson, and CM Slupsky. **2006.** *Targeted Profiling: Quantitative Analysis of 1H-NMR Metabolomics Data*. *Anal Chem.* 78(13):4430-4442

[2] R Rosewell and C Vitols. **March 2006.** *Identifying Metabolites in Biofluids*. *Chenomx Inc.*