# AssureNMR™: CRAFT, Biologics, HMDB

## Complete Reduction to Amplitude Frequency Table[1]

The CRAFT[1] processing approach was created by K. Krishnamurthy to address issues faced in quantification of components in complex mixtures, typically seen in metabolomics experiments. This approach converts time-domain data to frequency-amplitude tables. Combining frequency-amplitude tables offers an attractive workflow to the generation of a 'targeted fingerprint' that may be used to evaluate metabolomics data for contributions of specific metabolites to variance in the dataset. This approach is now available in AssureNMR, expanding capabilities for these complex mixtures.

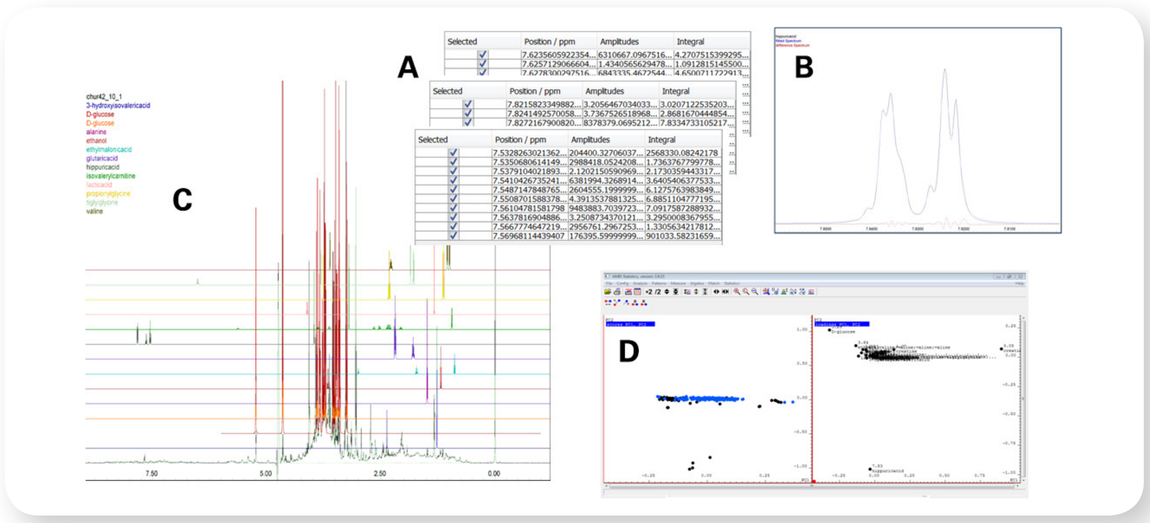



Fig. 1 Human urine data were used in AssureNMR with CRAFT to generate (A) frequency - amplitude tables, (B) modeled CRAFT results of hippuric acid peak at 7.83ppm showing fitted and difference results, (C) modeled CRAFT results of select resonances with the actual urine spectrum and (D) PCA scores and loadings plot of the targeted fingerprint from CRAFT tables on urine data with metabolites of distinction identified.

## Utilize HMDB[2-4] and DrugBank[5]

Identifying and quantifying small molecule metabolites in complex mixtures requires access to large and reliable databases. Therefore, access to public databases greatly assists evaluation of metabolomics data. The popular public NMR databases, Human Metabolome Database (HMDB) and DrugBank, with data on ~900 molecules each, may be used within AssureNMR with both 1D proton and 2D HSQC.

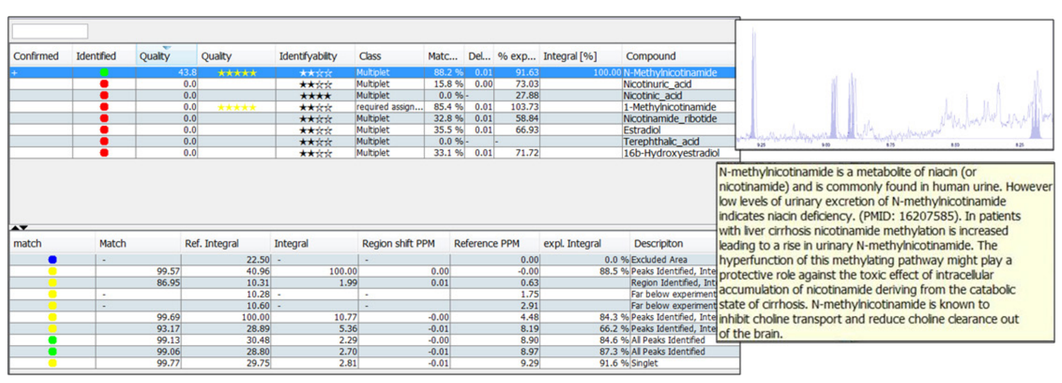
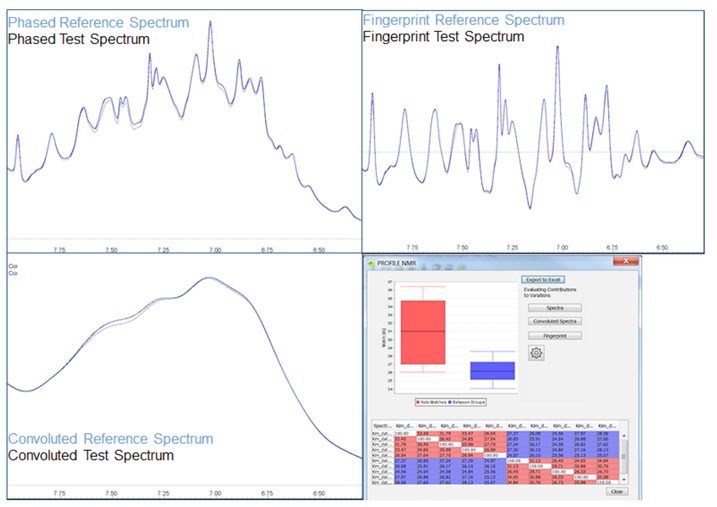



Fig. 2 AssureNMR match of a human urine spectrum using HMDB. Biologic information in HMDB is particularly informative and assists in component identification.

## Biologics: 1D PROFILE[6] and Automated 2D HSQC Analysis

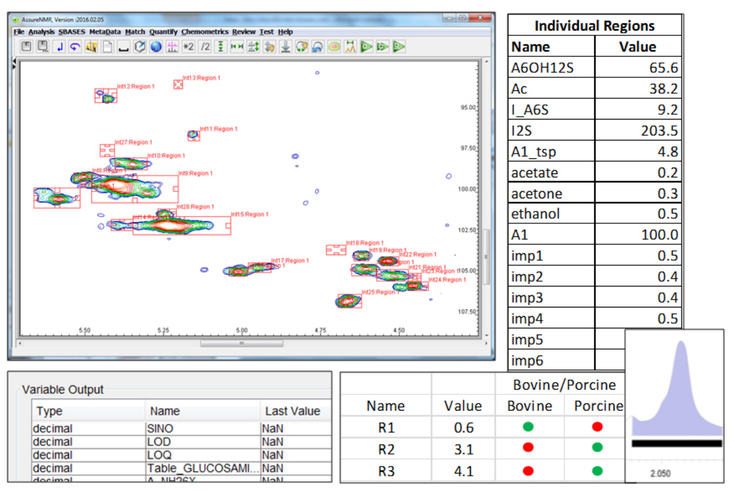
Biologics are products derived from living organisms and constitute a wide range of medicines (mAbs, proteins, polysaccharides, etc.) for treatment of many serious disorders such as cancer, diabetes and autoimmune diseases. NMR plays an important role in the evaluation of the quality of biologics and also in the approval of biosimilar versions of the brand biologics. Small changes in the higher-order structure, which may alter the biological activity, are readily detected and quantified by NMR. Tools for analysis of biologics are now included in AssureNMR:

- PROFILE[6] (PROtein FIngerprint by Lineshape Enhancement), a 1D $^{1}H$ approach that generates a high resolution structural fingerprint of the protein therapeutic (Figure 3).
- Automated 2D HSQC quantitative analysis (Figure 4) that may be used to detect structural changes and impurities and classify material. An equation builder enables custom analysis of the biopharmaceutical.




PROFILE6
1. Phases and aligns spectra
2. Calculates convoluted reference spectrum
3. Generates fingerprint spectrum
4. Performs similarity analysis

Fig. 3 Evaluation of mAb spectra using AssureNMR with 5 controls, 5 pass and 5 fail spectra. Data kindly provided by L. Poppe (Amgen).




Automated 2D HSQC & 1D analysis Heparin
1. Quantify and ID regions in HSQC
2. Apply Equations
3. Linefit 1D
4. Classify Material

Fig. 4 Automated 2D $^{1}H$, $^{13}C$-HSQC and 1D $^{1}H$ analysis[7] of heparin using AssureNMR at Ronzoni Institute. Customization of the AssureNMR method allowed implementation of the Ronzoni approach with automated reporting for quantification and material classification. Data kindly provided by M. Guerrini.


## Acknowledgements and References

We are grateful to our customers who shared in the development of AssureNMR 2.1 including Leszek Poppe, Marco Guerrini, Lucio Mauri, and Andrea Sefler. We thank Prof. David Wishart and his team for creating HMDB and DrugBank and allowing us to access these databases from our software.



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## Summary

- AssureNMR expands its use beyond quality control of small molecule APIs to include the evaluation of complex mixtures and biologics.
- Complete Reduction to Amplitude Frequency Table (CRAFT) offers an additional matching and quantification approach and enables targeted fingerprinting.
- HMDB and DrugBank is accessible directly from AssureNMR to assist in identifying components in complex mixtures.
- Evaluate biologics for small changes in higher order structure using PROFILE.
- Automate quantitative 2D HSQC analysis for evaluation of biologics and complex mixtures.