Identification of Individual Components Present in a Sample Mixture by ^{1}H NMR and ACD/NMR Workbook Suite

Brent G. Pautler[1] and J. Martin Faulkner[2]
[1] **Advanced Chemistry Development, Inc.** Toronto, ON, Canada, brent.pautler@acdlabs.com
[2] **Department of Chemistry, University of North Carolina Charlotte,** Charlotte, NC, USA, jmfaulkn@uncc.edu

Introduction

The management of an analytical chemistry facility in both academic and industrial environments has many challenges associated with it including, but not limited to, instrumentation operation and maintenance, sample preparation, user training, data analysis, report generation, as well as accounting and administrative duties. Facilities, such as the Regional Analytical Chemistry Laboratory (RAChel, http://rachelab.uncc.edu) at the University of North Carolina Charlotte, provide state-of-the-art analytical chemistry equipment and services for non-routine analysis. The establishment of RAChel was in response to the demand for an interdisciplinary facility that allowed the acquisition of analytical data for competitive analysis, deformulation, product comparison, identification of processing deposits, polymer structure elucidation, and characterization of monomers, additives, and catalysts.[1] In order to best serve the facility's users and customers, many instruments from several different vendors have been acquired and are used on a daily basis.

The analytical data desired for a particular sample/problem often requires multiple techniques; therefore a vendor-neutral software platform providing the necessary tools to aid in the data interpretation, management, reporting, and knowledge retention is required. The ACD/Spectrus Platform provides the ability to manage all of the raw data from a variety of instruments within or across facilities in a structured homogeneous environment, where it may be searched and re-used to help accelerate decision-making while reducing strain on time and resources.[2] ACD/NMR Workbook Suite, an extension of ACD/Spectrus Processor, provides advanced processing and interpretation tools for the NMR spectroscopists. Initially developed with the synthetic chemist in mind for the characterization/identification of "pure" samples, these tools have been extended to facilitate a mixture analysis workflow incorporating spectral database searching and reporting. This ultimately allows for the ability to analyze more complex samples in a fast, effective, and reliable manner.

Discussion

The traditional approach for mixture component identification usually involves a chromatographic separation and peak detection; however, NMR spectroscopy has become a popular alternative to provide in-depth information on mixture composition in a non-invasive manner without the need for a physical seperation.[3] The identification of individual components in a mixture by ^{1}H NMR spectroscopy requires the accurate assignment of resonances, which can be overwhelmingly complex, as illustrated in the example spectrum shown in Figure 1. The spectrum contains both fully-resolved and overlapping ^{1}H

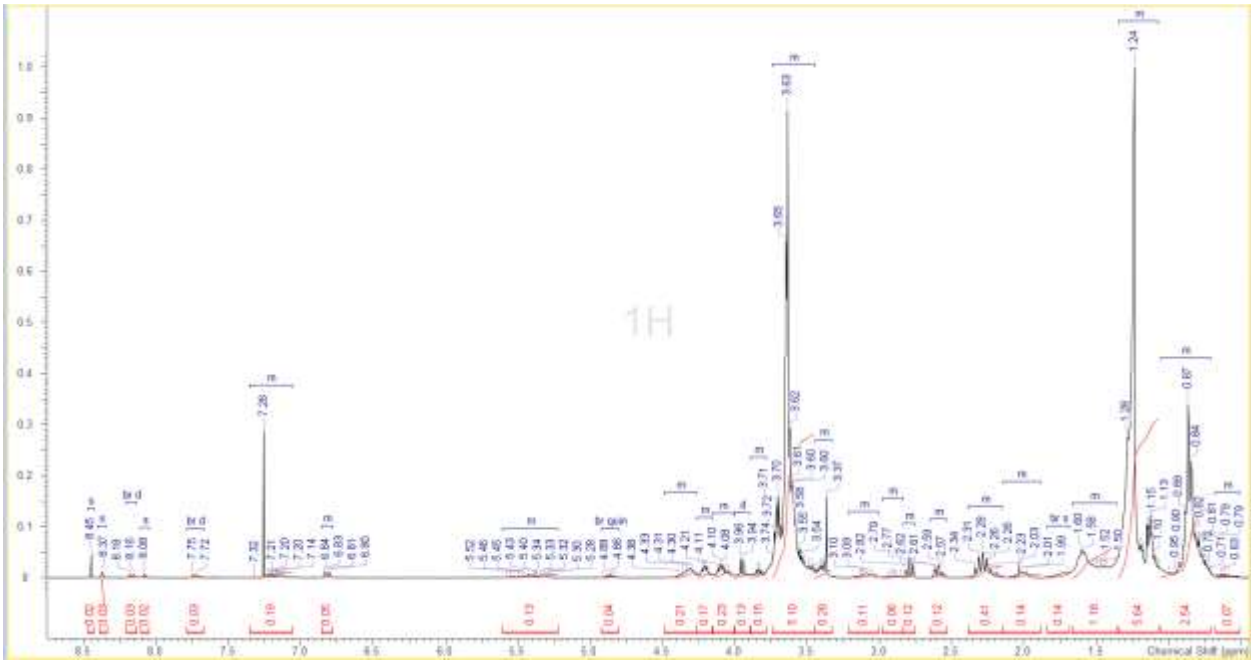

Figure 1. *A ^{1}H NMR spectrum of a sample mixture of lubricants and/or alkoxylates containing an unknown number of pure components. Resonances/groups of resonances have been peak picked to query against libraries of pure/known compounds to facilitate component identification.*

resonances that need to be taken into consideration for component identification. The new streamlined workflow developed in NMR Workbook Suite involves querying spectral libraries built in ACD/Spectrus DB that contain known components (either pure, or a defined mixture) in a sequential manner to reconstruct the mixture one component at a time in a bottom-up approach.

To facilitate the workflow, the spectral comparison tool within NMR Workbook Suite offers users the flexibility to search the entire spectral width or a defined region. Sub-region spectral searching can be advantageous when working with more complex mixtures, as the number of matches returned can be dramatically reduced. Typically, the aliphatic region of a mixture spectrum contains many overlapping ^{1}H resonances from multiple components limiting identification; therefore it is often advantageous to start in a less crowded region (such as the aromatic) to identify fully resolved signals first that could also contain ^{1}H resonances in the aliphatic region, simplifying the region as more components are identified and accounted for. Once the search is performed on a sub-region, the search result(s) are displayed below the query spectrum and a residual curve overlays the query spectrum (display can be on or off, Figure 2). The residual curve is easily minimized under the component signals by adjusting the chemical shift, intensity, and/or line broadening to allow for the analyst to decide if the search result is, in fact, correct. Once the analyst is satisfied with the search result, the component can be selected in the Table of Search Results and a new region queried. Upon searching a new region, the selected components in table of search results are stored and displayed above the query for reference (Figures 2B, C). In order to ensure identified components do not have additional ^{1}H resonances that are not present in the query spectrum, the entire spectral region should be checked by zooming out of the search window (Figure 2D).

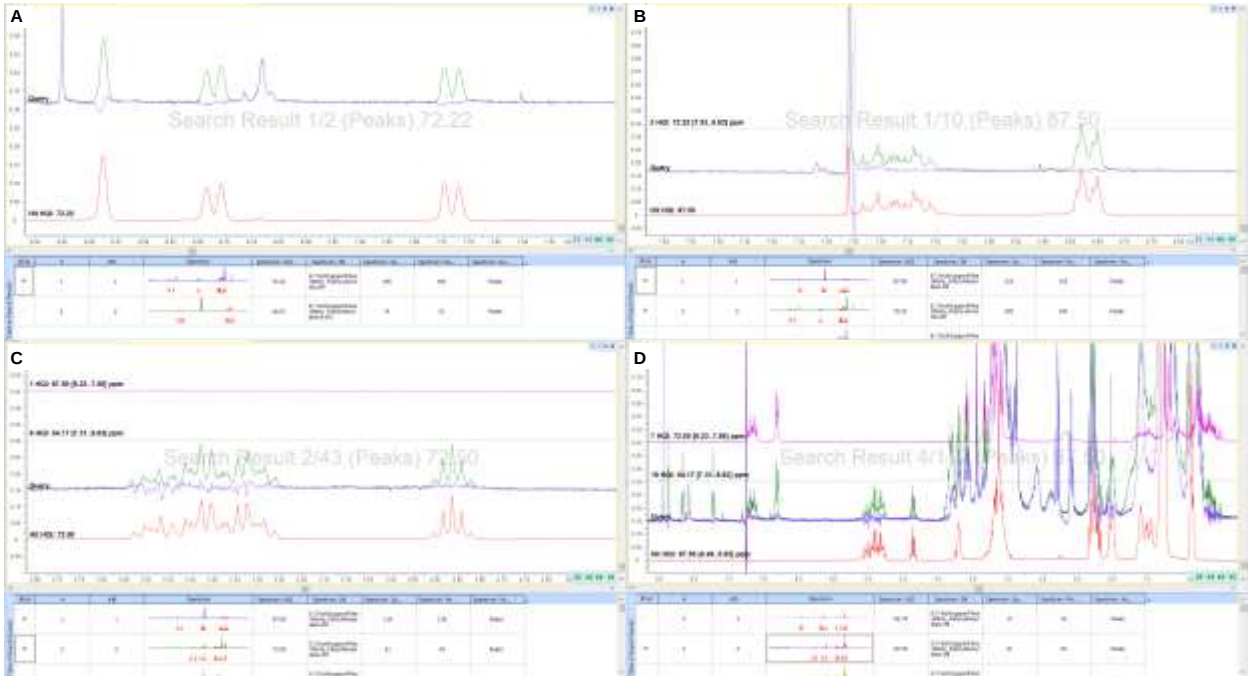

Figure 2. *The sequential workflow for component identification from a sample containing an unknown lubricant/alkoxylate mixture by ^{1}H NMR Spectroscopy and NMR Workbook Suite. (A) Search was first performed on the 7.53–8.62 ppm region that contains resolved signals. The "Hit Spectrum" (red) is displayed below the query (green) that has the residual curve (purple) overlaid (display can be on or off) and can be adjusted (chemical shift, intensity, line broadening) in order to effectively minimize the residual curve. Once the analysis is satisfied with the search result, the component is selected in the Table of Search Results and a new region can be searched. (B) The search results of the second spectral region (6.23–7.56 ppm) with the second component identified and residual minimized. Upon searching the second region, the first component selected in Table of Search Results is displayed above the query for the analyst's reference. (C) The search results for the third query region (4.49–5.82 ppm) displaying the identified component and the minimized residual curve. (D) The same result as in (C) zoomed out to highlight that the component fits with the entire query spectrum and that "extra" peaks are not present which would be easily observed by deviations in the residual curve.*

Upon completion, the final results are automatically tabulated and the identified components are presented in a stacked series below the mixture query spectrum for review (along with the residual curve if desired, shown above the query, Figure 3A). The component series displays the component number from the database, the Hit Quality Index (HQI), along with the sub-spectrum search region that was used to identify the component, if applicable. In addition, the relative concentrations of each component in the query spectrum are automatically calculated in the Table of Components. Additional information can be displayed on the spectra and the table (if present in the database) such as component names, chemical structures, and ^{1}H signal assignments. Integral as well as multiplet information is also automatically transferred to the query spectrum and can be displayed (Figure 3B). The integral/multiple information is color coded to match the color of its corresponding component spectrum. The table of integrals can be further used for quantitation, if the query spectrum contains a known amount of internal standard, and the identified components contain structural information, provided that the data was acquired with the appropriate delays.[4] The results can then be reported using the standard reporting functionality and/or databased for future reference.

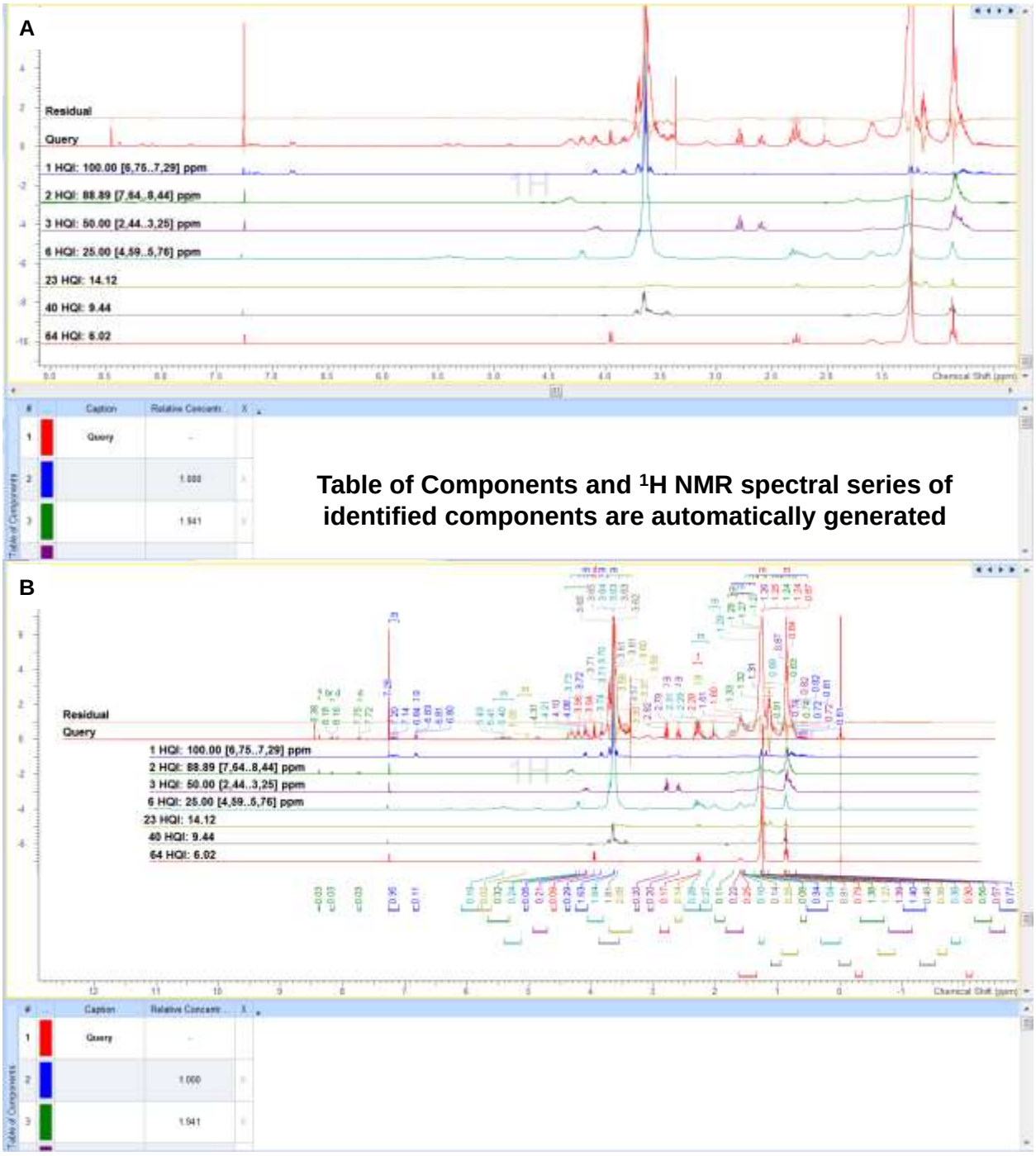

Figure 3. *The final search results for the example query spectrum where seven components were identified as the primary components of the mixture. (A) Upon identification of the major component in the query spectrum, closing the searching functionality, NMR Workbook Suite automatically generates a series containing the identified components (below the query spectrum), residual curve (above the query spectrum) along with the Table of Components that displays spectral information stored in the database. (B) The same search results illustrating that the integral and multiplet information is retained, and transferred to the query mixture spectrum. Each label is color coded to its corresponding component spectrum.*

Conclusions

The improvements of the mixture search workflow has greatly enhanced the usability of ACD/NMR Workbook Suite for the identification of components in a mixture. Developed to facilitate the rapid identification of components from a mixture using 1D NMR Spectroscopy, its goal to ease the strain on the analyst was achieved. The workflow was designed for the general analyst and can be applied to many types of samples including formulated products, petroleum, food and beverage, and biological samples to name a few. Future extensions to this tool include the expansion to include 2D NMR spectroscopic techniques (such as COSY, HSQC) as well as automatic quantitation if internal standard information is included.

References

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