Analysis of Mixtures by NMR Spectroscopy: Current Workflow Solutions using the ACD/Spectrus Platform

Dimitris Argyropoulos, Brent Pautler, Sergey Golotvin, Shahriar Jahanbakht, Vladimir Mikhalenko
Advanced Chemistry Development, Inc., 8 King Street East, Suite 107, Toronto, Ontario, Canada

Case A: Targeted Profiling.

In this case, one is interested in a small number of components that are to be identified in a mixture. The mixture has a very specific composition, general properties and the chemical shifts of the components are well known. This would be a typical case for the detection of metabolites in biological liquids/extracts [1] or the detection of specific components in foods/beverages [2].

The first step for this analysis consists of identifying the peaks of interest in the mixture and defining the range in which they appear. In most cases peak deconvolution will be needed for the extraction of accurate concentrations. Once this is achieved, the results can then be readily exported in tabular form. The process then continues for the next component. In principle only one or two characteristic peaks for each component are needed.

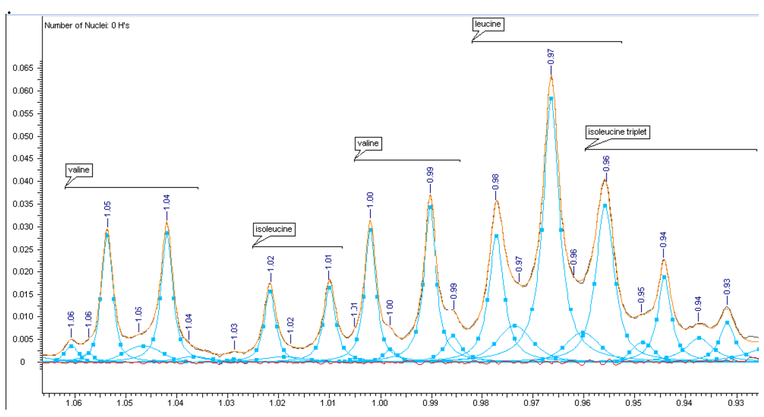


Figure 1: Deconvolution used to correctly identify the component peaks in the mixture.

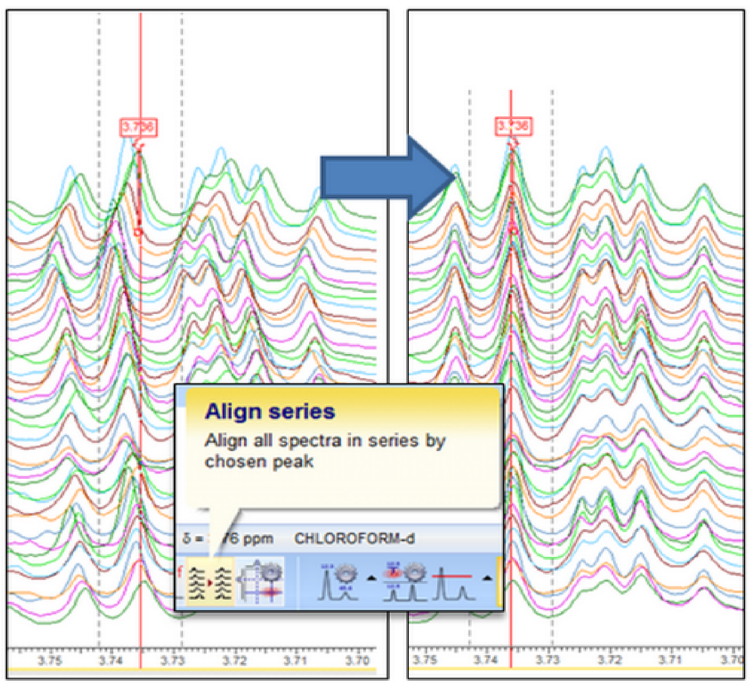


Figure 2: Alignment of multiple spectra prior to deconvolution.

The whole process can be fully automated in order to analyze several samples in one go. In this case a preparatory alignment step is required.

Concentrations can be calculated using either internal or external standards, where In the latter, running a spiked solution may be beneficial

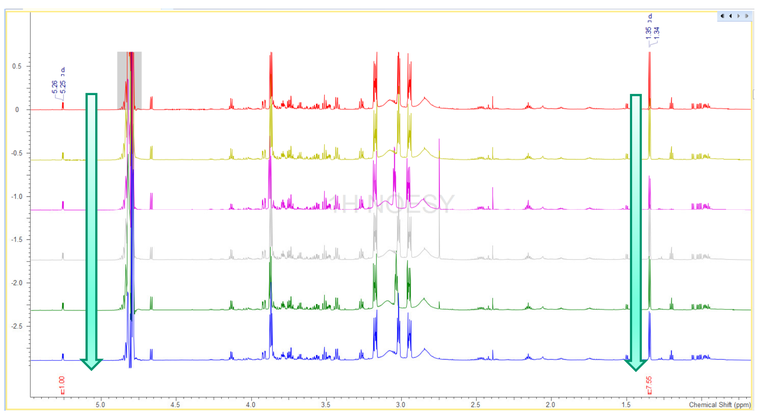


Figure 3: Batch processing setup of spectra. Once the peaks of interest have been selected, the peak deconvolution and quantitation can be performed across the entire series.

Case B: Unknown components, 1D spectra

It is not uncommon, in some cases, that one knows the possible components of a mixture but does not know whether they are actually present in the mixture or not. This is a different case than the previous one as now one has to first verify the presence of a component before defining its concentration.

The easiest way to do this is by recording 1D spectra (usually ^{1}H) and then searching for a match in a database containing standards [3]. The risk here is that some of the peaks may shift due to differences in pH, concentration, temperature etc. between the standards and the unknown. For this reason, it is generally better to follow this method in organic solvents. If this is not possible, then the search should be limited to the peaks that are less likely to have been affected.

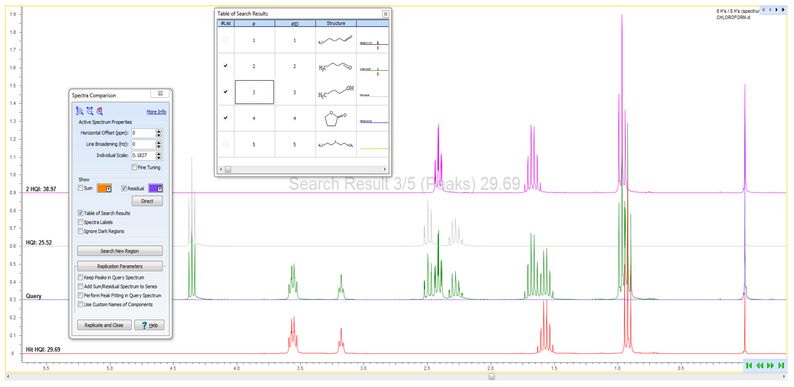


Figure 4: Result of database search and adjustment of the relative peak intensities, which aids in concentration determination.

The workflow for this case is to carefully select the peaks in the unknown first, and then do a database search for compounds containing these peaks. It is possible to filter the results by the compounds that do not have peaks in a particular range. The hits are ranked according to the Hit Quality Index (HQI) where 100% means that there was a perfect match. The HQIs in this case are going to be low since there will not be a single entry in the database with all the mixture peaks present.

The next step is to inspect each of the hits from the database and establish whether it is a true hit or not. Then, the relative concentration needs to be adjusted in such a way, as to minimize the residual signal. Finally the results are replicated to a new spectrum and the Table of Components is populated.

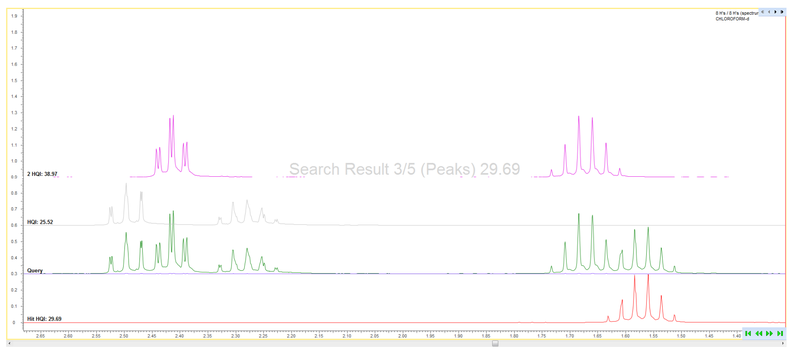
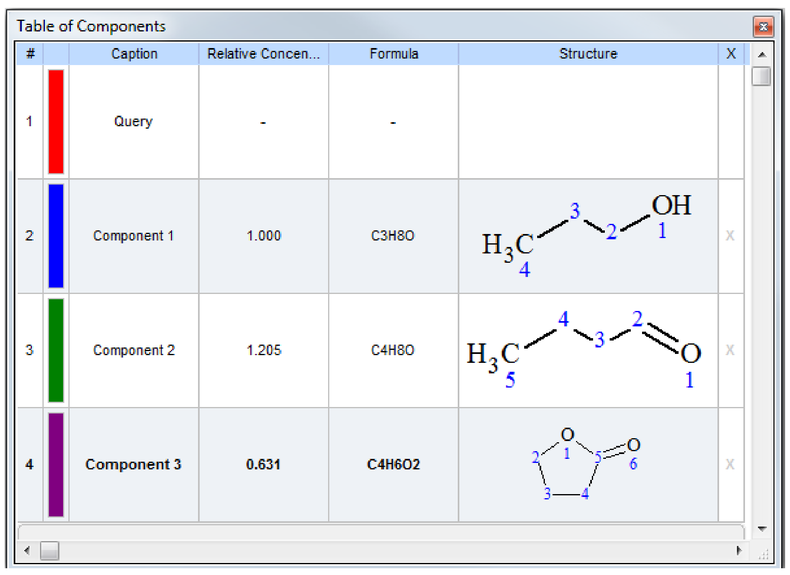


Figure 5: Detailed view of the Database search result. Note that the residual curve (dark purple) has been minimized, indicating that all of the components for those particular chemical shifts have been accounted for.



#		Caption	Relative Concen...	Formula	Structure	X
1		Query	-	-		
2		Component 1	1.000	C3H8O		X
3		Component 2	1.205	C4H8O		X
4		Component 3	0.631	C4H6O2		X

Figure 6: The final table of components containing the identified components with their relative concentrations.

Case C: Unknown components, 2D spectra

The previous case is straightforward when there are relatively few number of components within a very simple matrix. Unfortunately, this is not always the case as mixtures tend to be more complicated, containing a large number of severely overlapping peaks.

The solution for such complex spectral mixtures in NMR was to use multidimensional spectroscopy. This "spreads" the overlap to two or more dimensions thus greatly reducing it and therefore aiding in the identification and assignment of the mixture components. Two dimensional NMR spectroscopy is by far the most popular due to its relative ease of acquisition and understanding.

The same can be applied to mixture analysis. With careful experimental selection, the mixture peaks can all be isolated and the overlapping signals greatly reduced or eliminated [4]. A modern 2D experiment will not take significantly longer to record with most requiring no more than 5 to 10 minutes.

An interesting complication may be that one would think that mixture searching with 2D spectra would require a database of 2D spectra. Although this is correct it is not obligatory. The 2D spectrum of any compound can be very accurately predicted if one knows the 1D spectra and the structure. This means that if databases with 1D spectra already exist then these can be used for mixture searching using 2D spectra. The only additional requirement is to combine the 1D spectra on-the-fly and see if they match the peaks in the 2D query spectrum.

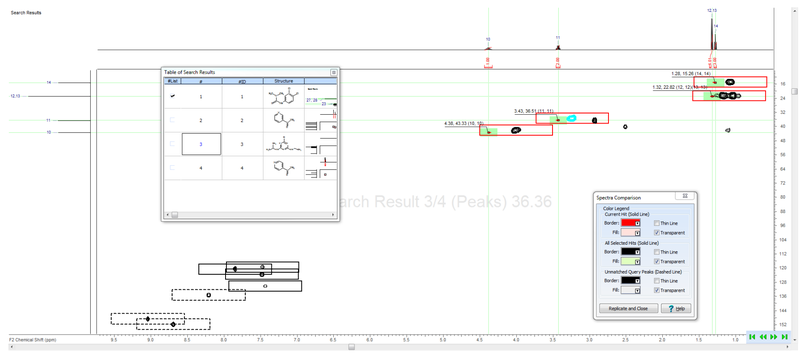
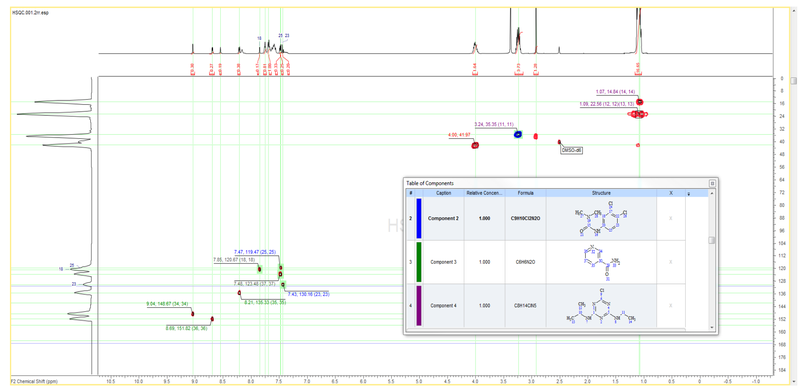


Figure 6: 2D NMR spectrum mixture search. The HSQC spectrum of a pesticides mixture is searched against a database of 1D spectra. Peaks with a solid black border are already assigned peaks (component 1), peaks with a dotted black border are unassigned peaks while the ones with a solid red border are the peaks corresponding to the current hit.

The workflow is similar to the previous case. One starts by the careful selection of the peaks to be searched. The 2D spectra could have artifact peaks, so these need to be cautiously excluded. The next step is the actual search of the database. Since 2D spectra are of inherently lower resolution than 1D spectra, a looseness factor needs to be considered for both dimensions. The results are presented in the same format, highlighting the unassigned and matched peaks. Finally the result is replicated and a new spectrum with the components coded with different colours is presented.



#		Caption	Relative Concen...	Formula	Structure	X
2		Component 2	1.000	C3H8O2		X
3		Component 3	1.000	C6H6N2O		X
4		Component 4	1.000	C8H14CN5		X

Figure 7: Result of the 2D mixture searching after replication. The component peaks are colour coded according to the legend in the inset Table of Components. Peaks coloured gray are peaks that appear for more than one component.

References

[1] Nagato, E. G., *et al.*, *Aquat. Toxicol.* **2016,** 170, 175-186.
[2] Kew, W., *et al.*, *Magn. Reson. Chem.* **2017**, 55, 785-796.
[3] Pautler, B. G., *et al.*, *Environ. Sci. Technol.* **2012**, 46, 3753-3761.
[4] Woods, G. C., *et al.*, *Water Res.* **2012,** 46, 3398-3408