Mixtures Analysis of Complex Mixtures

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qNMR is the “gold standard” for compound quantification and has wide application. The quantitative analysis of mixtures poses special difficulties, but is a very worthwhile objective.

We describe a simple, robust approach to mixtures analysis where each component can show a distinct NMR signal, or one related to another component. The method is applicable to a wide range of foods, cosmetics, body fluids, *etc*. This is called *Simple Mixtures Analysis* (SMA).

SMA - Overview

Library experiments

A *protocol*, or *Method* to measure component concentrations in a mixture must first be developed and validated by the user. Data from 1D or 2D experiments can be used.

Application of this analysis to routine samples is then significantly expedited, using a work-flow that combines full automatic software analysis and user checking.

Integration data is extracted from multiplet peaks in each new spectrum, and a user-specified equation determines the concentration or weight%.

The workflow improves speed and quality

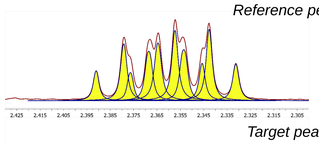
Error checking
Multiplet type, *J*s, and integral ratios can be specified. If outside acceptable ranges, a warning is produced

Manual refinement
Incorrectly selected multiplets can be manually adjusted for a correct analytical result, and the concentrations corrected

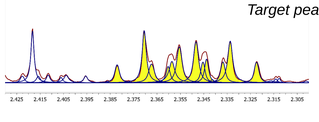
Reporting
A simple output summary can be pasted on the spectrum, or an XML file written for customized output or DB inclusion.

Component peaks

Reference peak pattern



Target peak pattern

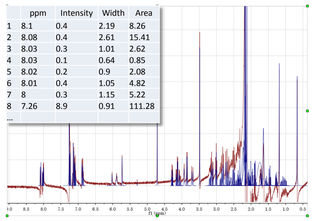


Peak Pattern Recognition

Component multiplets can be recognized using conventional methods, or with a novel pattern recognition algorithm.

A Reference peak pattern is found, and searched for in the mixtures spectrum. A scoring system is used, which also allows inexact but correct matches to be found.

Basic processing: GSD



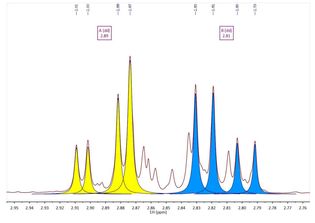
	ppm	Intensity	Width	Area
1	8.1	0.4	2.19	8.26
2	8.08	0.4	2.61	15.41
3	8.03	0.3	1.01	2.62
4	8.03	0.1	0.64	0.85
5	8.02	0.2	0.9	2.08
6	8.01	0.4	1.05	4.82
7	8	0.3	1.15	5.22
8	7.26	8.9	0.91	111.28

This trial NMR spectrum (red) is challenging for deconvolution

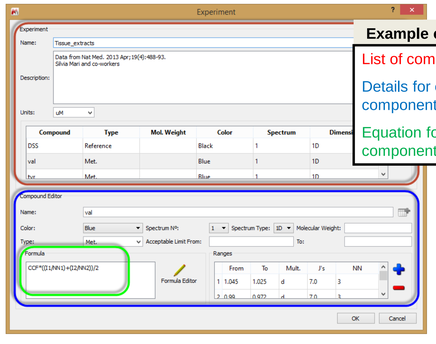
GSD still deconvolutes the peaks (blue) and determines areas

The two doubleted-doublets of Citric Acid here overlap heavily with other mixture components in wine

Multiplet areas differ only by <0.5% using GSD



Method development: Experiments



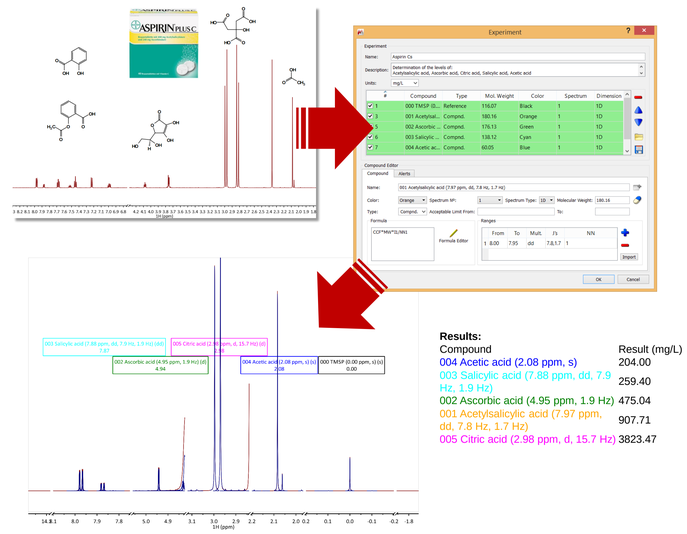
An *Experiment* is made up of a definitions for a sample reference, and known components:

- PPM ranges and specified multiplets define each species by its area
- If target multiplets are not correctly found then an error message can be shown

The *equation editor* flexibly specifies calculation of a concentration

- A mixture reference can be used to derive a *CCF*, which can then be used to determine other component concentrations
- Spectral math: use areas and sample-specific data from >1 experiment
- 1D and 2D datasets
- Integral data from the spectra

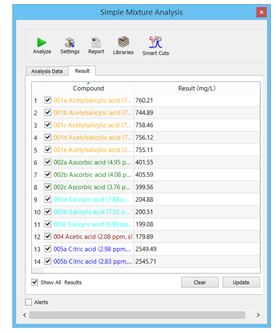
SMA examples



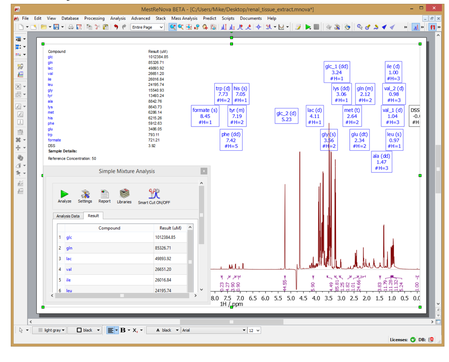
Results:

Compound	Result (mg/L)
004 Acetic acid (2.08 ppm, s)	204.00
003 Salicylic acid (7.88 ppm, dd, 7.9 Hz, 1.9 Hz)	259.40
002 Ascorbic acid (4.95 ppm, 1.9 Hz)	475.04
001 Acetylsalicylic acid (7.97 ppm, dd, 7.8 Hz, 1.7 Hz)	907.71
005 Citric acid (2.98 ppm, d, 15.7 Hz)	3823.47

The Experiment was redesigned now to individually assess the concentration based on all component multiplets in Aspirin Plus C



SMA of amino acids in serum



We have designed a versatile, functional program for the quantitation of simple mixture components by NMR. The user can use a flexible equation editor to have access to integrations from one or more 1D or 2D spectra, and thereby design an analysis. Multiplets can be automatically checked and the analysis adjusted manually. Reporting can be fully customized.

The software is applicable to a wide range of real-world mixtures such as foods, neutraceuticals, *etc*.

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