



SMA Manual

© 2015 MESTRELAB RESEARCH □

Last Revision: 08-Jan-2015

SMA 1.0

by MESTRELAB RESEARCH

This is the manual of SMA 1.0

SMA Manual

© 2015 MESTRELAB RESEARCH

All rights reserved. No parts of this work may be reproduced in any form or by any means - graphic, electronic, or mechanical, including photocopying, recording, taping, or information storage and retrieval systems - without the written permission of the publisher.

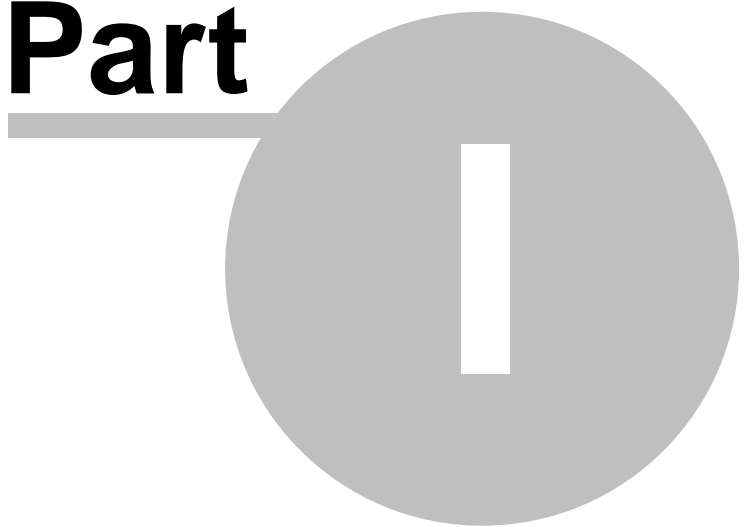
Products that are referred to in this document may be either trademarks and/or registered trademarks of the respective owners. The publisher and the author make no claim to these trademarks.

While every precaution has been taken in the preparation of this document, the publisher and the author assume no responsibility for errors or omissions, or for damages resulting from the use of information contained in this document or from the use of programs and source code that may accompany it. In no event shall the publisher and the author be liable for any loss of profit or any other commercial damage caused or alleged to have been caused directly or indirectly by this document.

Table of Contents

Part I Simple Mixture Analysis	6
Part II SMA Panel	12
1 Creating Experiments.....	14
2 Analysis Data.....	20
3 Results Table.....	20
Part III Batch SMA and Listeners	25

Part



1 Simple Mixture Analysis

This chapter describes the functionality required to perform the quantitation of simple mixture components by NMR. Although the usage is quite general, the usual requirement is that a component can be described by at least one peak area or a multiplet area in a prescribed region of a spectrum, and that the number of Hs (nuclides) for that is known. This information will be determined in the sample spectrum, using the integral value to quantify the species.

The SMA plugin is applicable to a wide range of real-world mixtures such as foods, nutraceuticals, *etc.* Typical usage will be in the Quality Control (QC) of *foods*, in that these samples typically show a relatively small number of compounds of interest (ca. 10-20), and a unique resonance can usually be found for each small constituent compound. More complex mixtures such as oils can sometimes be analyzed in SMA based on empirically-derived relationships based on region integrals. This is the important distinction between this method and the much more elaborate “mixtures” project currently under development.

The sample may have more than one spectrum; a compound quantitation may be derived by a simple mathematical function defined by the user.

Example

The analysis of Aloe vera is performed very well using ^1H NMR, and ca 12 compounds' concentrations are measured, with quantitation achieved in this case by addition of weighed amounts of a Certified Concentration Standard. This shortened description is a worked example of the functionality, and sample data are provided from this [link](#).

Characteristic chemical shift values, peak multiplicity, protonated carbon type and N values used for detection and quantitation of the major natural components of aloe vera leaf juice

Substance	Signal Type and N Parameter	Chemical shift, ppm
Acetylated Polysaccharides	Broad Group of CH_3 Singlets (N=3)	2.0-2.3
Isocitric acid	CH , Doublet (N=1)	4.25
Malic acid	CH , 4 peak multiplet (N=1)	4.45
α -Glucose	CH Doublet (N=1)	4.6
β -Glucose	CH Doublet (N=1)	5.2
Isocitric lactone	CH Doublet (N=1)	5.05

The area for each compound is extracted from a spectrum in D_2O or the same sample acidified with mineral acid. Furthermore, the concentration of acetylated polysaccharides (AP) is calculated using this empirical, validated equation:

$$C_{AP} = \frac{W_{Nic}}{AI_{Nic}} * \frac{AI_{AcMann}}{W_{sample}} * 3.06 * 100\%$$

where W represents weights, and AI represents integrals. Nic (Nicotinamide) is the concentration reference compound added to the analyte, and AcMann is an AP, Acyl Mannose. AI_{AcMann} is the integral for the region 2.0-2.3ppm.

**Commonly-used Abbreviations**

AI	Absolute Intensity: the area of a multiplet
NN	The Number of Nuclides (Hs) corresponding to a multiplet
CCF	Concentration Conversion Factor, used to derive concentrations from AI and NN
IR_x	A specific Integral region for a compound
MW	Molecular Weight of a compound
MWR	Molecular Weight of the concentration reference compound
SW	Sample Weight
RW	Reference compound Weight
RC	Reference Concentration
DF	Dilution Factor

Compound concentrations may be expressed in mM units relative to the internal concentration reference (Nicotinamide, in this example), or wt% according to this general formula:

$$C_x \text{ (wt\%)} = 100 * (W_{\text{Nic}} * I_x * N_{\text{Nic}} * MW_x) / (I_{\text{Nic}} * N_x * MW_{\text{Nic}} * W_{\text{Sample}})$$

Where:

C_x = concentration of component

W_{Nic} = weight of added nicotinamide internal standard (mg)

W_{Sample} = weight of sample (mg)

I_x = integration area of unique proton resonance from spectrum of component

I_{Nic} = sum of integration areas of 4 aromatic CH peaks of the nicotinamide standard

MW_x = Molecular Weight of Component

MW_{Nic} = molecular weight of nicotinamide standard (122.1 g/mol)

N = number of protons in peak group - molar conversion factor, (CH=1, CH₂=2, CH₃=3), for nicotinamide

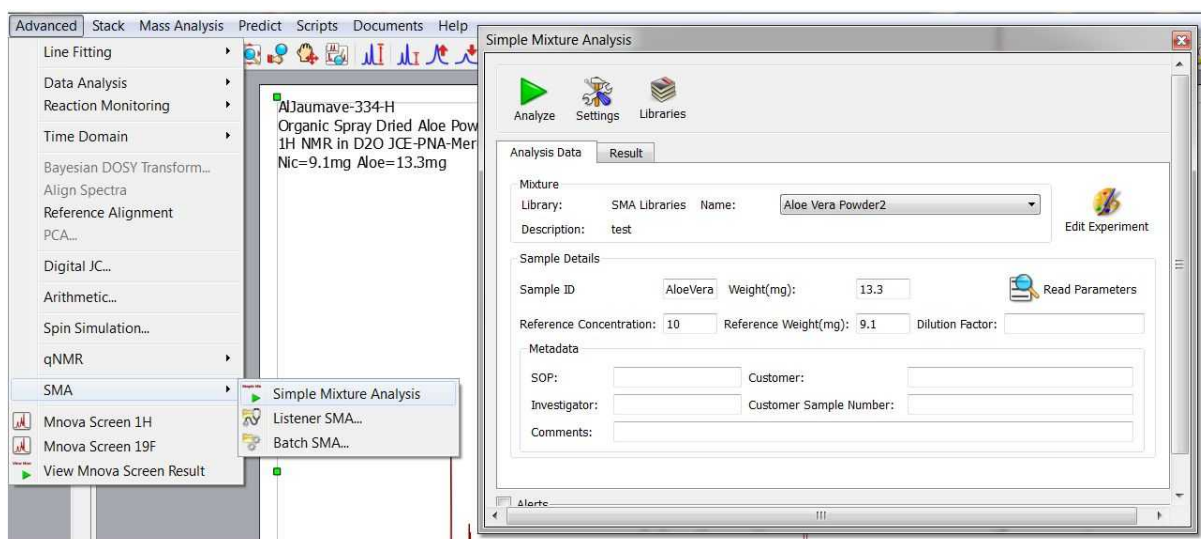
$N = 4$ (4 aromatic CH resonances) – or 3 if one CH resonance is overlapped by other components such as benzoate or formic acid and is therefore not utilized in the qNMR calculation.

See [this poster](#) for further information.


The user can generate any number of *experiments*, and organize them by *libraries*.

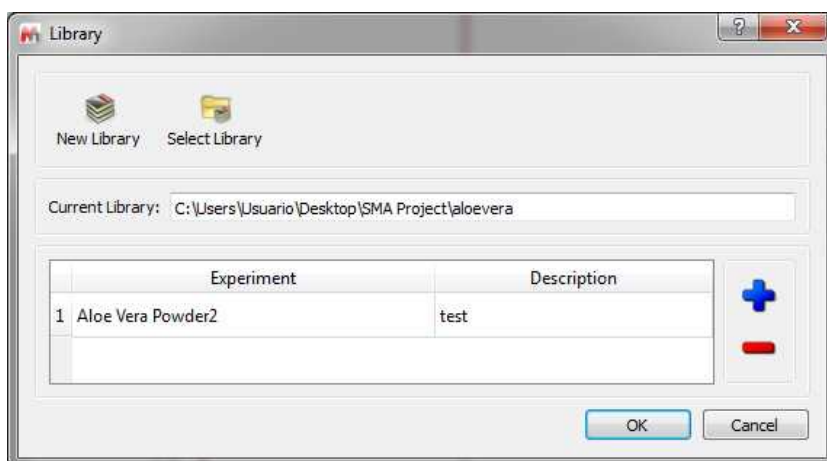
A Library is simply a specified file folder. Experiments are saved as readable files having an EXP extension. Once generated/validated they can be shared with other users simply by copying into a library folder.


Following the menu 'Advanced/Simple Mixture Analysis' will display the SMA panel.

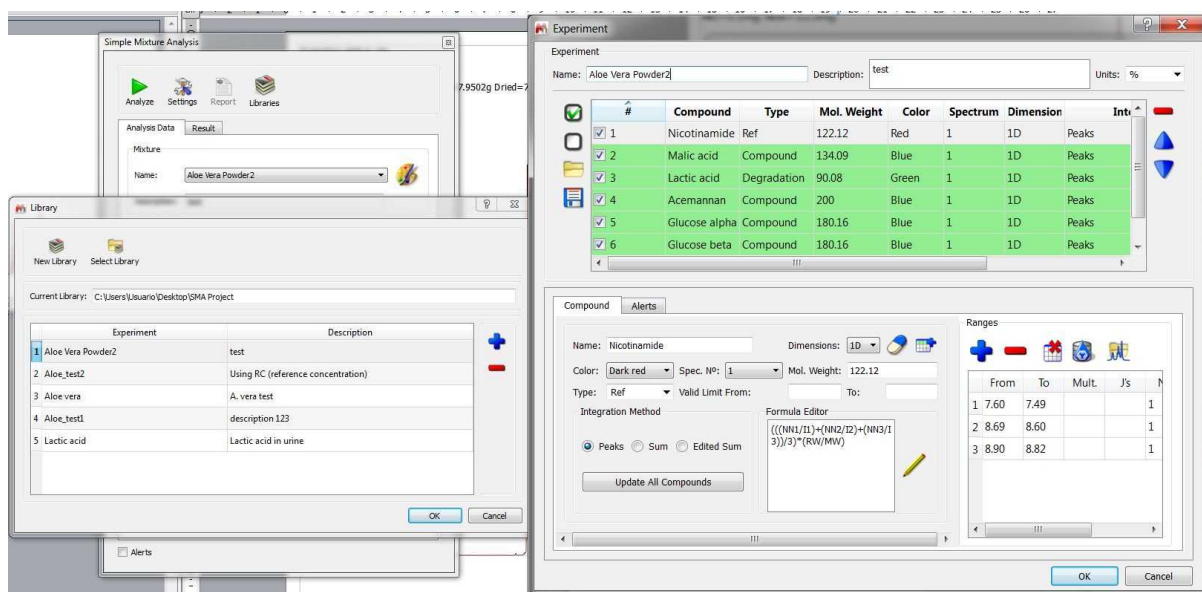


From this panel, you can load the 'experiments' library and fill the sample mass details. To load the

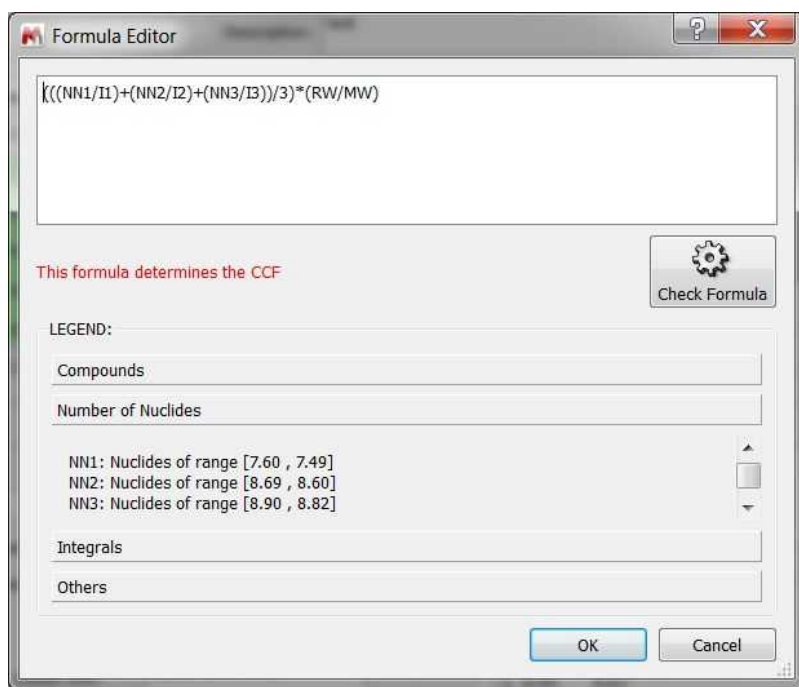
experiment, unzip [this file](#) and save the .exp file to your hard disk. Next, click on this button  of the SMA panel to display the 'Library' dialog box. Once there, click on the 'Select Library' button to select the folder containing the previously saved .exp file.



Once in the Library dialog box, you can add new experiments by clicking on the 'blue plus button' or edit the existing ones by double clicking on the applicable row (you can also edit the experiment from the SMA panel, by clicking on this button ). The 'Experiment' dialog box will be displayed, allowing you to specify the reference- and component compound details (Units, molecular weight, colors, formula for the analysis, multiplets ranges, integration method, etc.):



It will be important for this example to define the suitable formula from the experiment dialog box. When a formula is entered for compound having Type = Ref, then it will be used to calculate the CCF. The enumerated value will be used when CCF is used in subsequent equations. That is, the correct concentrations will be determined for each unique sample.





Recall this fundamental equation that we will use to determine concentrations:

$$\text{Conc} = \text{CCF} * \text{AI/NN}$$

Where AI is the Absolute Integral for the multiplet, and NN is the number of nuclides (Hs) for that multiplet.

For further information about how to calculate CCFs with Mnova qNMR please take a look at this [document](#).

Finally, click on the 'Analyze' button to get the results (with the multiplet analysis boxes for each compound and reference in the selected colors):

	Compound	Result (%)	Annotation
1	<input checked="" type="checkbox"/> Malic acid	13.72	
2	<input checked="" type="checkbox"/> Glucose beta	5.04	
3	<input checked="" type="checkbox"/> Acemannan	4.19	
4	<input checked="" type="checkbox"/> Lactic acid	3.44	
5	<input checked="" type="checkbox"/> Glucose alpha	3.11	
6	<input checked="" type="checkbox"/> Nicotinamide	1.06e-6	

☒ Show All Results

Smart Cuts Report Clear Table Update

☐ Alerts

NOTE: if the spectrum has had Peak Picking applied, Mnova will keep these values. If not then peak picking will be performed automatically

Part



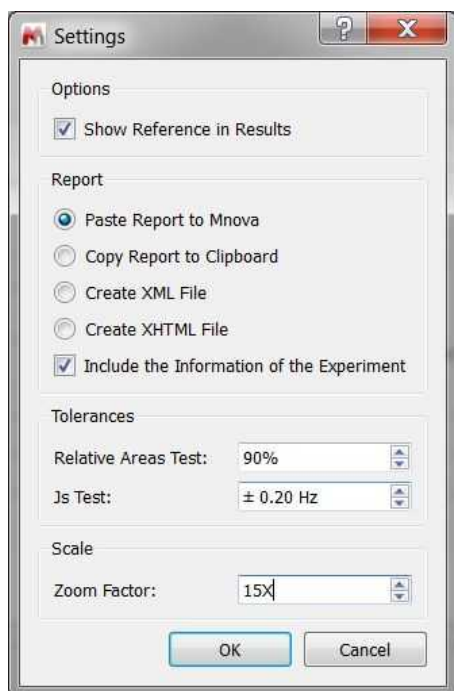
2 SMA Panel

The SMA panel contains two tabs; one for the sample-specific Analysis data, and the second to display and interact with the results.

The screenshot shows the 'Simple Mixture Analysis' window. At the top, there are three icons: a green play button labeled 'Analyze', a gear labeled 'Settings', and a stack of books labeled 'Libraries'. Below these is a tabbed interface with 'Analysis Data' and 'Result' tabs. The 'Analysis Data' tab is active and contains several input fields. Under the 'Mixture' section, there are fields for 'Library' (set to 'SMA Libraries'), 'Name' (set to 'Aloe Vera Powder2'), and 'Description' (set to 'test'). An 'Edit Experiment' button is to the right. The 'Sample Details' section includes 'Sample ID', 'Weight(mg)' (13.3), 'Reference Concentration' (10), 'Reference Weight(mg)' (9.1), and 'Dilution Factor'. A 'Read Parameters' button is next to the weight field. The 'Metadata' section has fields for 'SOP', 'Investigator', 'Customer', and 'Customer Sample Number', along with a 'Comments' text area. At the bottom left, there is an 'Alerts' checkbox.

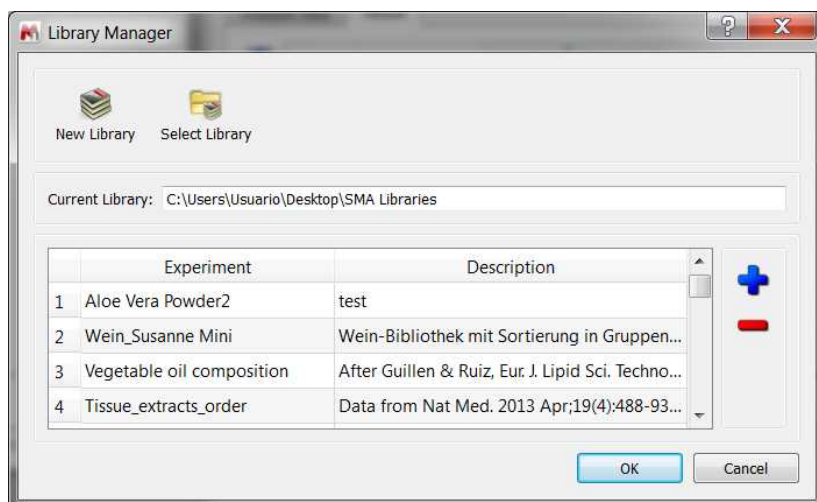
Additionally you will find the 'Analyze' button to launch the SMA analysis (or to recalculate the analysis after having applied changes in the spectrum). When pressed the first time, Analysis will consist of multiplet determination, enumeration of the prescribed equations, and notification of inconsistencies ("Alerts"). Subsequent Analyze operations will use the existing peaks and multiplets. This approach gives the user the opportunity to interact manually with the data and, if necessary, make changes that will be used for the final analysis.

You will also find a button to customize your settings:



From here you can select how to export your results (into Mnova, just in the clipboard, as an XML or HTML file) and what information will be included (reference, experiment, etc.). In addition, you can set the tolerance for the relative areas and coupling constants tests and to select the zoom factor (that will be applied when you double click on a compound from the 'results table')

Clicking on the 'Libraries' button will display the existing experiments of the current library (directory):



From here, you will be able to create a new library or to select the folder of any other existing one. This allows you, for example, to separate a test environment from a production one.

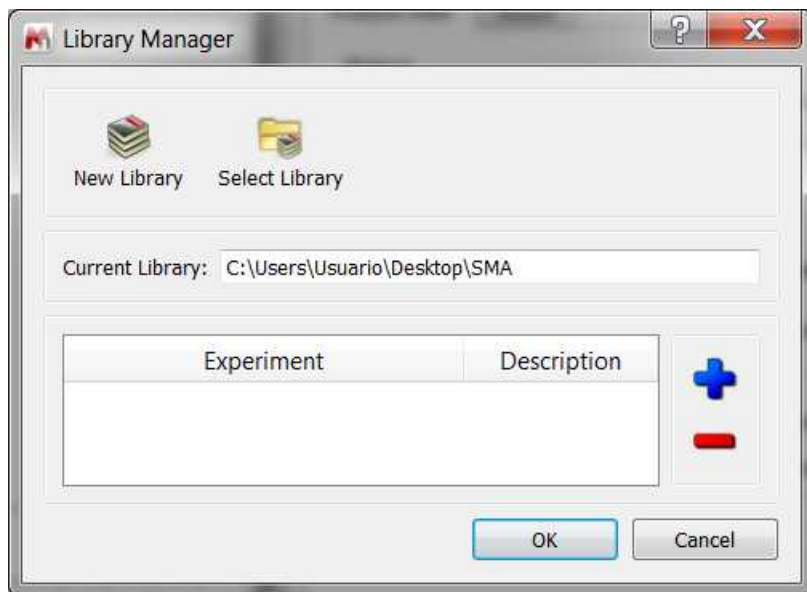
Left clicking on any experiment followed by OK, will load the applicable experiment. Double clicking on it, will allow you to edit the experiment.

2.1 Creating Experiments

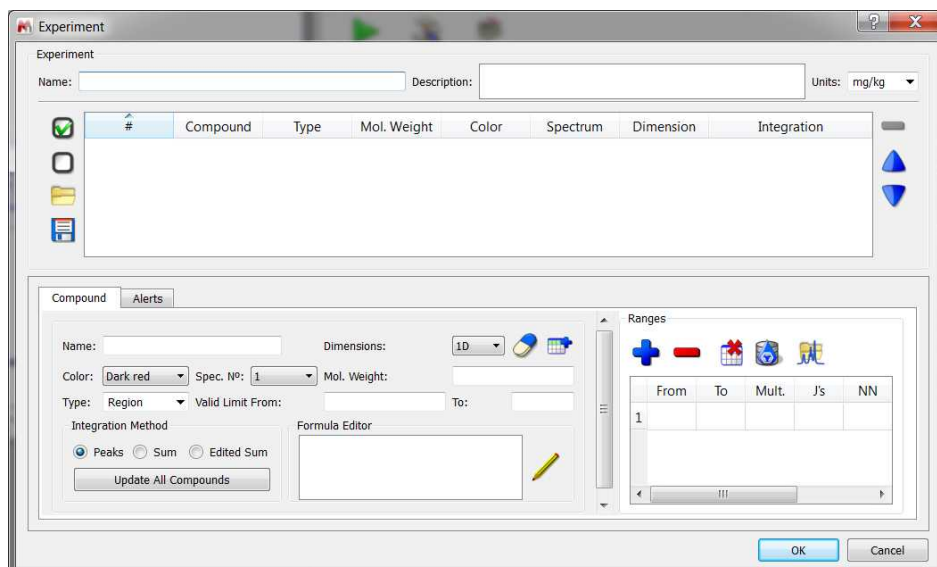
A protocol, or Method to measure component concentrations in a mixture must first be developed and validated by the user, using data from one or more 1D or 2D NMR experiments.

Application of this analysis to routine samples is then significantly expedited, using a highly efficient work-flow that combines full automatic software analysis, user checking, and reanalysis (if needed).

You can create experiments by clicking on the Libraries button of the SMA panel which will display the 'Library Manager' dialog box:



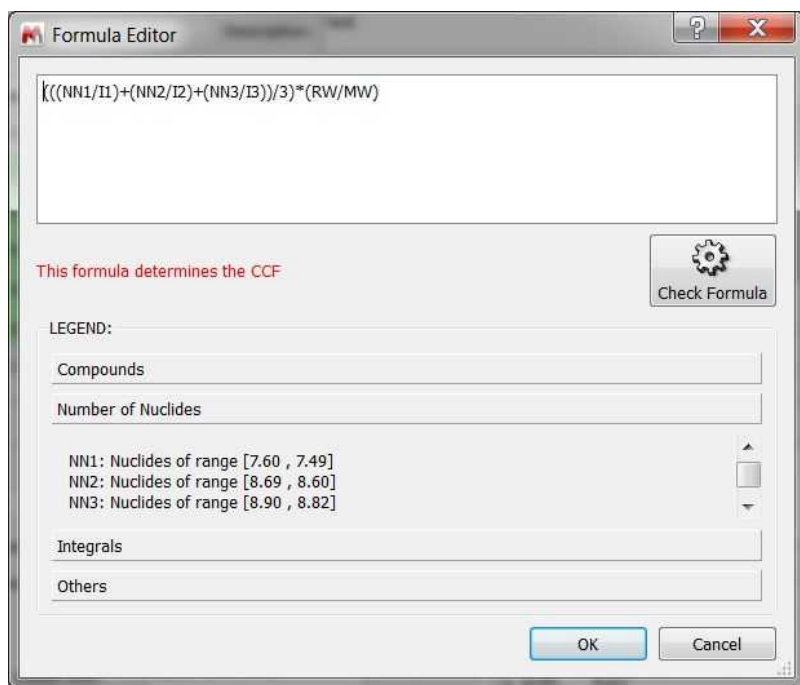
The 'blue plus' button + will allow you to create a new experiment:



You will find some edit boxes to include the experiment name, descriptions and units. The next step would be to specify the compounds' information by typing the applicable name, selecting the multiplet color, spectrum number (to be used in documents with spectra in different pages), molecular weight,

type (reference, compound, degradation, etc), valid limits for results (if the result is outside the limits, it will be showed in red), integration method to be used (Peaks, Sum or Edited Sum. There is also button to update the integration method for all the compounds of the library at once) and the formula editor. The ability to address data from >1 experiment for a sample is useful in this case because it allows us to perform the full analysis using data from the native and acidified sample spectra.

Clicking on the 'Edit Formula' button, will allow you to enter the formula for the applicable compound:

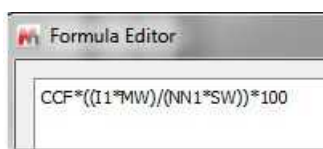


The *equation editor* flexibly specifies the calculation of a concentration

- A concentration reference compound 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 more than one experiment
- Use concentrations of other components, which is convenient when there is overlap
- 1D and 2D datasets
- Integral data from the spectra

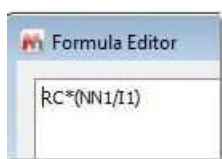
Note that the legend flexibly shows what can be included in the equation.

There could be cases in SMA where a reference signal of known concentration is used to calculate the CCF:

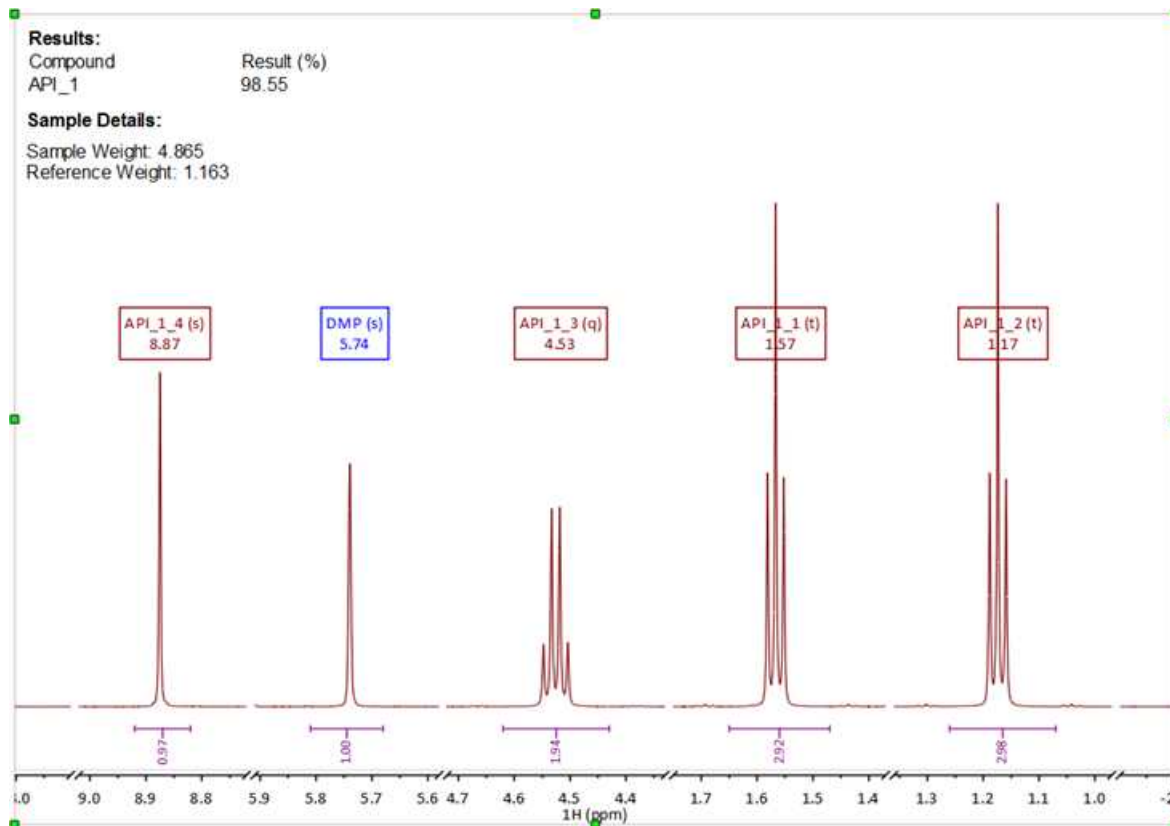


$$CCF = RC / (AI/NN)$$

where RC is the "Reference Concentration" - the concentration equivalent of the reference signal; which could be added to the formula editor if it is needed:



You can include advanced math functions (to get statistical analysis for example). You can find below an example where we calculated the purity based on four different multiplets for the same compound, **API_1_x** (x=1-4); using DMP as reference:



We then have the calculated concentrations for each of these by using in the equation editor:

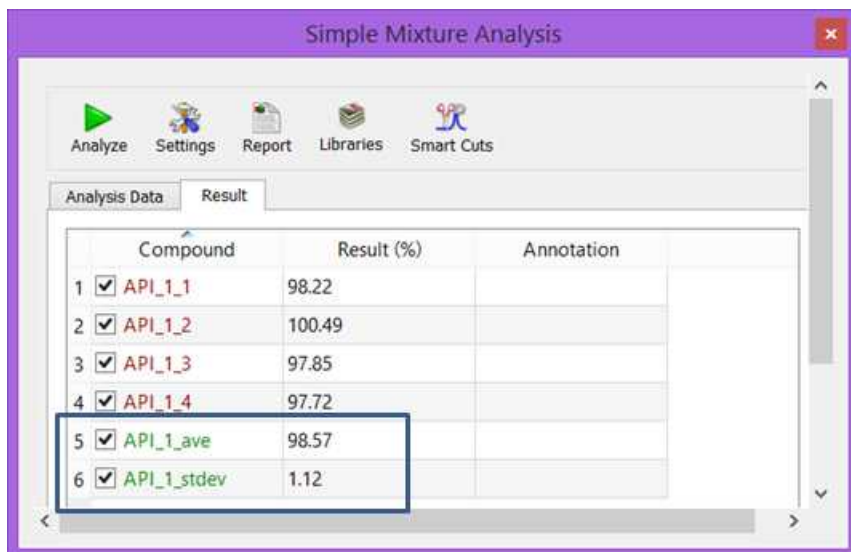
C_API_1_x

Then, you can get the 'Average' by using the formula below:

$$0.25 * (C_API_1_1 + C_API_1_2 + C_API_1_3 + C_API_1_4)$$

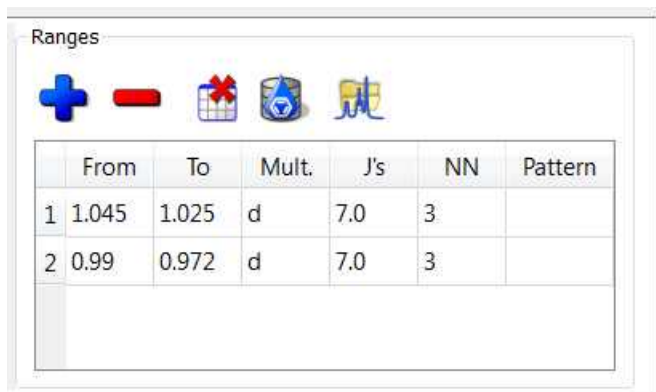
And the Standard deviation by using this formula:

$$\text{Math.sqrt}(0.25 * (\text{Math.pow}(C_API_1_ave - C_API_1_1, 2) + \text{Math.pow}(C_API_1_ave - C_API_1_2, 2) + \text{Math.pow}(C_API_1_ave - C_API_1_3, 2) + \text{Math.pow}(C_API_1_ave - C_API_1_4, 2)))$$




	Compound	Result (%)	Annotation
1	<input checked="" type="checkbox"/> API_1_1	98.22	
2	<input checked="" type="checkbox"/> API_1_2	100.49	
3	<input checked="" type="checkbox"/> API_1_3	97.85	
4	<input checked="" type="checkbox"/> API_1_4	97.72	
5	<input checked="" type="checkbox"/> API_1_ave	98.57	
6	<input checked="" type="checkbox"/> API_1_stdev	1.12	


In the right-bottom part of the 'Experiment Dialog', you will find the 'Ranges table' which will allow you to enter the chemical shift ranges of the compound signals, including information for multiplicity, coupling constants, number of nuclides and the path for the pattern (in the case that you are using it).



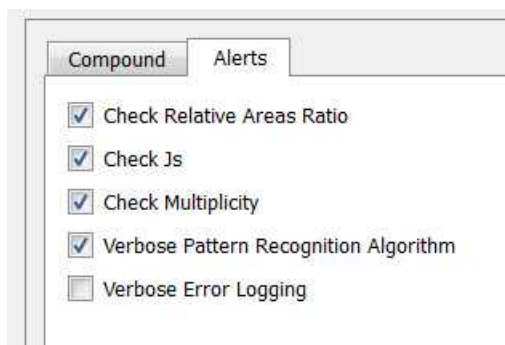
	From	To	Mult.	J's	NN	Pattern
1	1.045	1.025	d	7.0	3	
2	0.99	0.972	d	7.0	3	

Clicking on the 'blue plus' button will allow you to add a new range - which can also be deleted by using the 'red minus' button. There will be also a button to clear the table, and to import ranges from an

Mnova database . When this button is pressed, the Database will be searched for the compound name. If there is a match, the compound molecular weight and the multiplet chemical shift ranges for the ¹H-NMR spectrum will be read, populating the Compound Ranges.

You can import the ranges from a Mnova document containing the ¹H-NMR spectrum of a pattern just by clicking on this button .

Under the 'Alerts' tab, you can the checks made that lead to some warning messages:



- Check Relative Areas ratio: an alert is shown when the relative areas are not within the tolerance of the expected value. The result is shown in red (see Nicotinamide in the screenshot above). The NN ratios are compared with those of the experimental areas. The tolerance should be set considering the NMR spectrum acquisition parameters and whether or not perfect ratios can be expected.
- Check Js: an alert is shown when the found value is not within the tolerance of the expected value. The result is shown in red (see Lactic acid in the example above). The message shows: "The list of Js of the multiplet Lactic acid is not the expected". A tolerance of ca 0.5 Hz would be typical.
- Check Multiplicity: an alert is shown when expected and found multiplicities don't match. The result is shown in red (see Acemannan in the example above). The message shows: "The Multiplicity of the multiplet Acemannan is *m* and it was expected to be *l*". Compounds with several multiplets: an alert is shown if any of them is missing.

Analysis Data Result


	Compound	Result (%)	Annotation
1	<input checked="" type="checkbox"/> Nicotinamide	762.62	
2	<input checked="" type="checkbox"/> Malic acid	142.68	
3	<input checked="" type="checkbox"/> Glucose beta	62.73	
4	<input checked="" type="checkbox"/> Acemannan	57.68	
5	<input checked="" type="checkbox"/> Lactic acid	43.77	
6	<input checked="" type="checkbox"/> Glucose alpha	40.00	
7	<input checked="" type="checkbox"/> Reference	1.35e-5	

☒ Show All Results

Smart Cuts Report Clear T

☒ Alerts

[13:08:42]: Missing multiplet for compound Glucose alpha
 [13:08:42]: Multiplet Glucose alpha_2 [6,5.8] is invalid and will not be used
 [13:08:42]: The list of Js of the multiplet Lactic acid is not the expected
 [13:08:42]: Relative areas ratio 1.95 between multiplet Nicotinamide_3 and multiplet Nicotinamide_2 is not the expected: Expected value in range [0.10-1.90]
 [13:08:42]: Relative areas ratio 1.92 between multiplet Nicotinamide_1 and multiplet Nicotinamide_2 is not the expected: Expected value in range [0.10-1.90]
 [13:07:40]: The Multiplicity of the multiplet Acemannan is m and it was expected to be t

Once you have finished with the description of your compound, just click on the 'Add new compound' button  to include it in your experiment and to continue adding additional compounds. N.B. If you do not add the compound details in this way then they will be lost.

Once you have built your experiment with the desired compounds, you can change the priority of any of them by using the blue arrow keys. The analysis will be performed in the #-number order. Compounds can be removed or added – usually temporarily – using the check box next to the number: compounds with a green background color will be enumerated.

Experiment

Name: Aloe Vera Powder2 Description: test Units: %

#	Compound	Type	Mol. Weight	Color	Spectrum	Dimension	Integration
<input checked="" type="checkbox"/> 1	Nicotinamide	Ref	122.12	Red	1	1D	Peaks
<input checked="" type="checkbox"/> 2	Malic acid	Compound	134.09	Blue	1	1D	Peaks
<input checked="" type="checkbox"/> 3	Lactic acid	Degradation	90.08	Green	1	1D	Peaks
<input checked="" type="checkbox"/> 4	Acemannan	Compound	200	Blue	1	1D	Peaks
<input checked="" type="checkbox"/> 5	Glucose alpha	Compound	180.16	Blue	1	1D	Peaks
<input checked="" type="checkbox"/> 6	Glucose beta	Compound	180.16	Blue	1	1D	Peaks

You can check/uncheck any existing compound to include it or not in your analysis and you can import/export the content of an experiment by using the applicable load&save buttons.

2.2 Analysis Data

The analysis data tab of the SMA panel will allow you to select the desired experiment from the current library and also to edit it.

This tab also includes the sample details and metadata information which can be read from the dataset just by clicking on the 'Read Parameters' button:

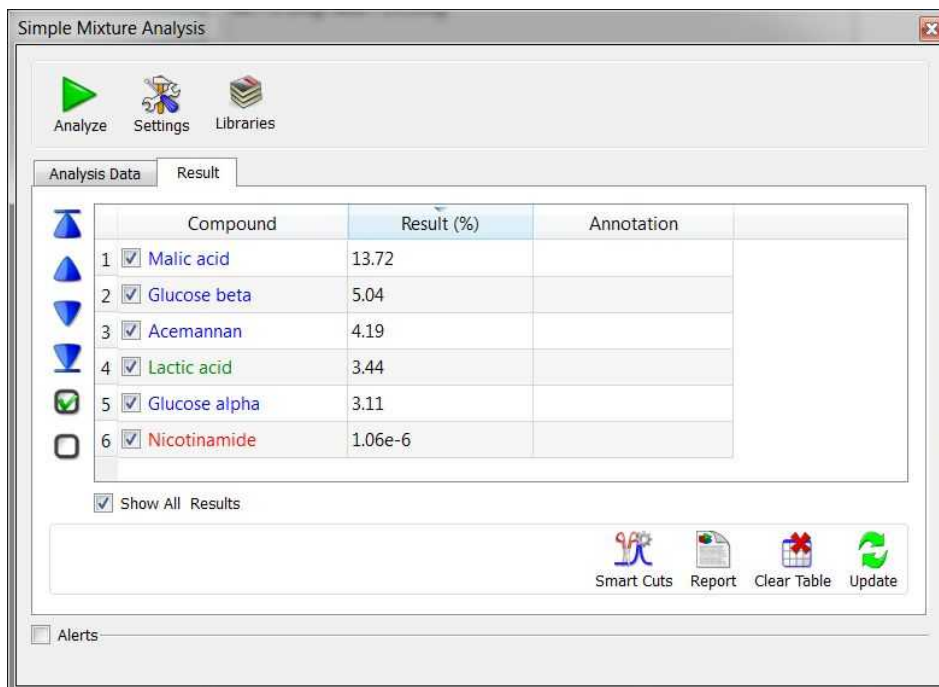
The screenshot shows the 'Analysis Data' tab of the SMA panel. At the top, there are three icons: a green play button labeled 'Analyze', a wrench and screwdriver labeled 'Settings', and a stack of books labeled 'Libraries'. Below these is a tabbed interface with 'Analysis Data' and 'Result'. The 'Analysis Data' tab is active and contains several sections:

- Mixture:** A form with 'Library:' set to 'SMA Libraries', 'Name:' set to 'Aloe Vera Powder2' (in a dropdown menu), and 'Description:' set to 'test'. To the right is an 'Edit Experiment' button with a colorful icon.
- Sample Details:** A form with 'Sample ID' set to 'Aloe3', 'Weight(mg):' set to '125.9', 'Reference Concentration:' set to '10', 'Reference Weight(mg):' set to '6.8', and 'Dilution Factor:' set to '1.2'. To the right is a 'Read Parameters' button with a magnifying glass icon.
- Metadata:** A form with 'SOP:' set to 'SOP data', 'Customer:' set to 'Aloe Analysers', 'Investigator:' set to 'EV', 'Customer Sample Number:' set to 'CoatsAloe-458-H', and 'Comments:' set to '1H NMR in D2O AJD-PNA-Merc300'.

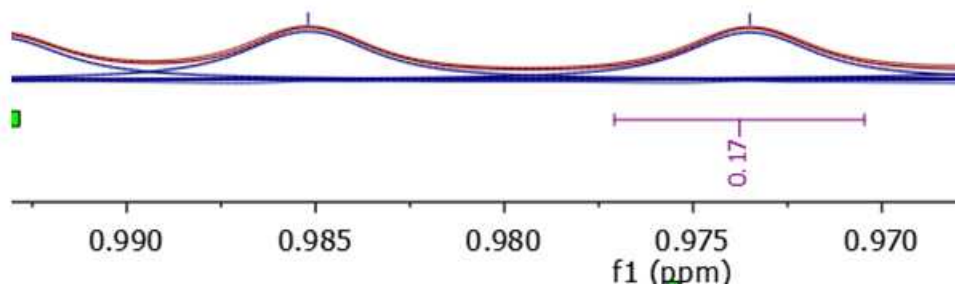
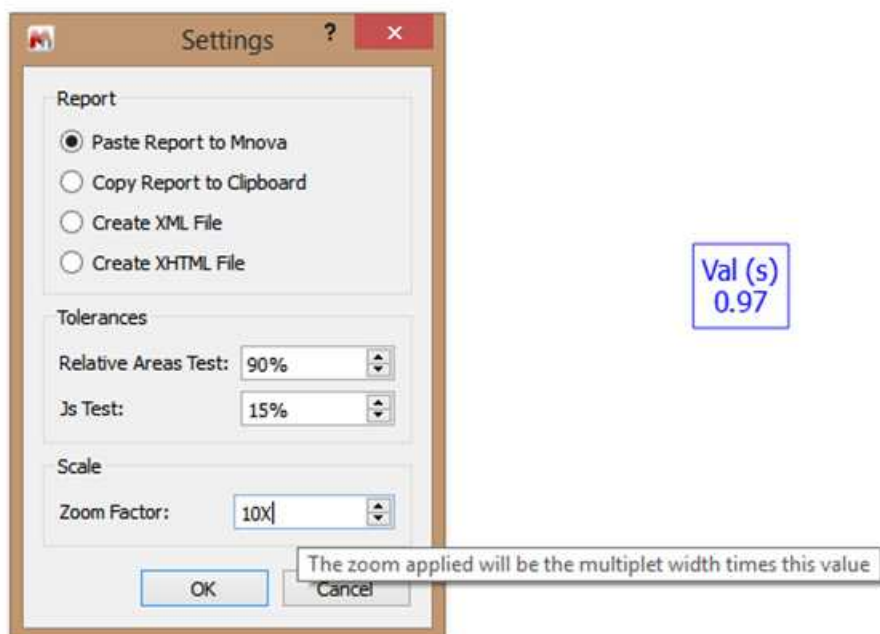
If the dataset does not include the information, the user will be required to fill the desired fields (sample ID, sample weight, reference concentration, reference weight, dilution factor, etc).

2.3 Results Table

The results table will display the concentration values (in the units selected in the applicable experiment) for each compound (and reference, if the option was checked under the settings).



If you double click on any of the components from the Result tab the spectrum is zoomed and the centered multiplet of this component is shown for inspection. The zoom applied when double-clicking a component is fixed by the multiplet range. The zoom factor applied can be customized via Settings:



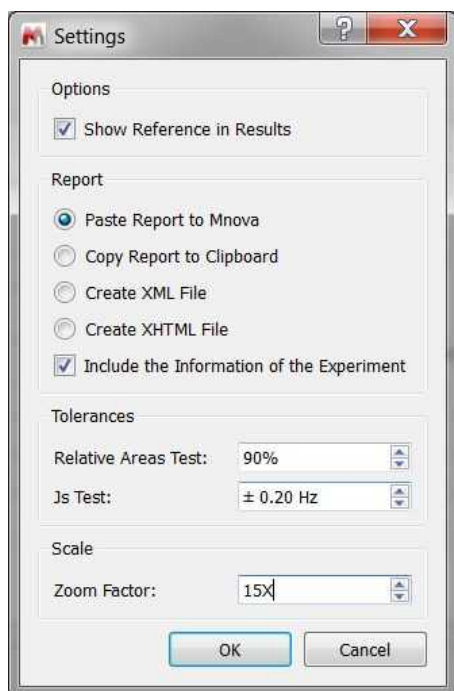
If you click on 'Smart Cuts' button, the expected cuts are made and a fit to highest intensity will be applied. All cuts are restored when pressing again the 'Smart Cuts' button:

There are also Next/Previous/Last/First buttons to move through the list of compounds in the results table, showing each multiplet in turn. When navigating through the compounds by clicking the arrow buttons, the zoom view of the first multiplet in each compound is obtained. Alternatively, to navigate randomly through the different multiplets for a compound you need to double click the row of this compound in the SMA table

You will be allowed to make some changes in the spectrum (for example with the multiplet ranges, or peaks used for the multiplet) and click on the 'Update' button to recalculate the analysis.

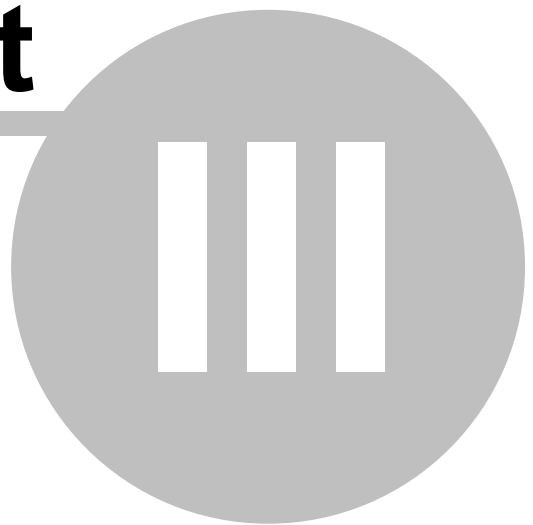
Reporting

Clicking on the Report button, will generate a report according to the selected settings. Only the compounds checked in the 'Results Table' will be reported.



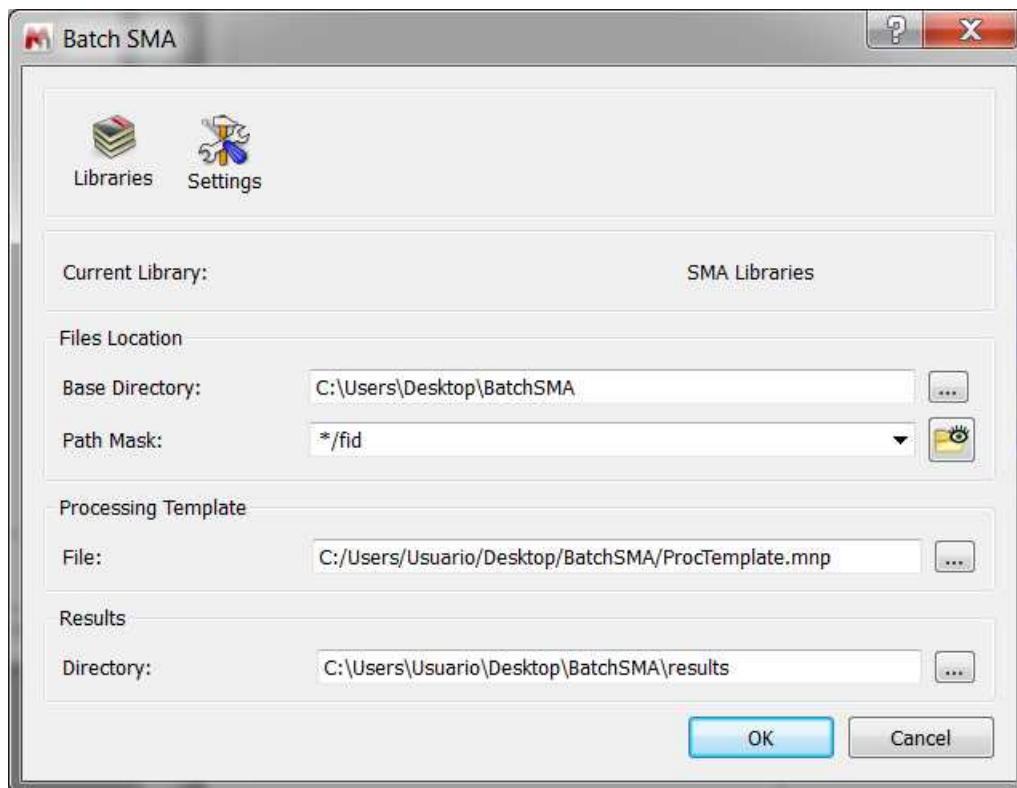
As you can see in the screenshot above, you can choose to report the results directly in Mnova, copy the results to clipboard (to be pasted afterwards) and export them as XML or HTML files.

Part



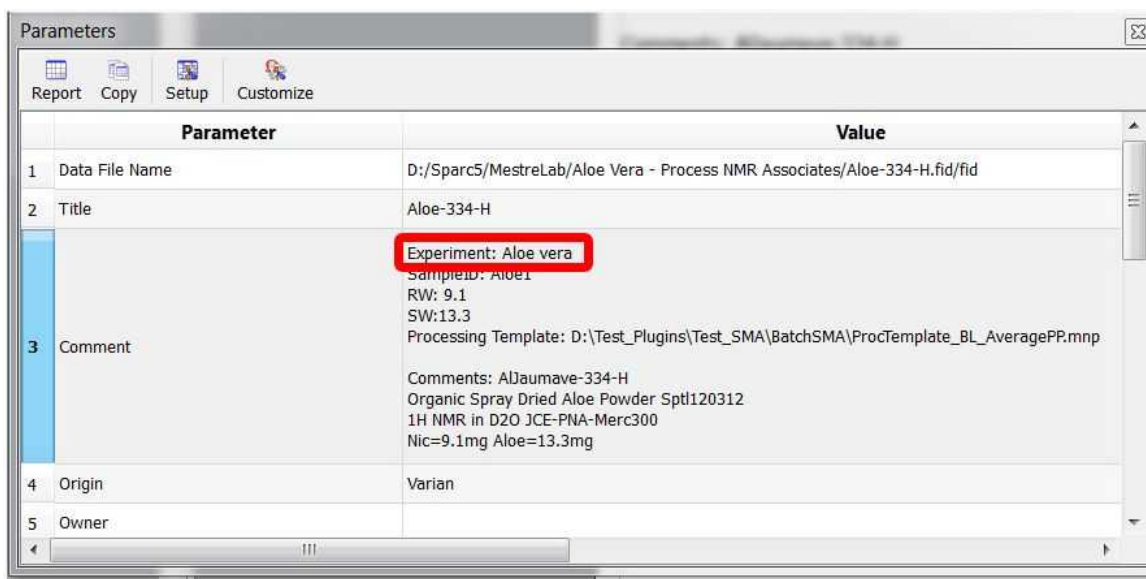
3 Batch SMA and Listeners

You can run the analysis in batch mode by following the menu 'Advanced/SMA/Batch SMA':

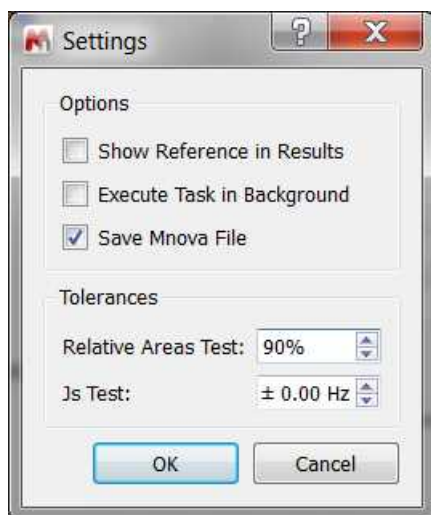


You would need to select the desired experiment under the current library, the path of the folder containing all the datasets to be analyzed and a path mask (to load those datasets into Mnova. All Operating System standard conventions can be used for this specification (*, ?, !, etc.), and the files that will be processed can be previewed by clicking on the button next to the path mask box. It is possible to select a predefined processing template. Finally, select the folder to save your results, and click on the OK button.

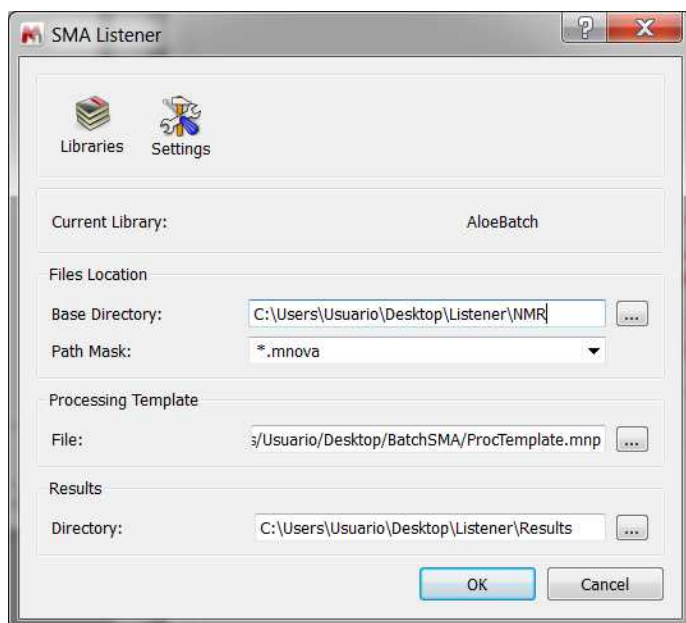
The spectra must contain a parameter (or a line in the comment parameter) with the name of the applicable experiment (to allow Mnova to find the experiment in the library):



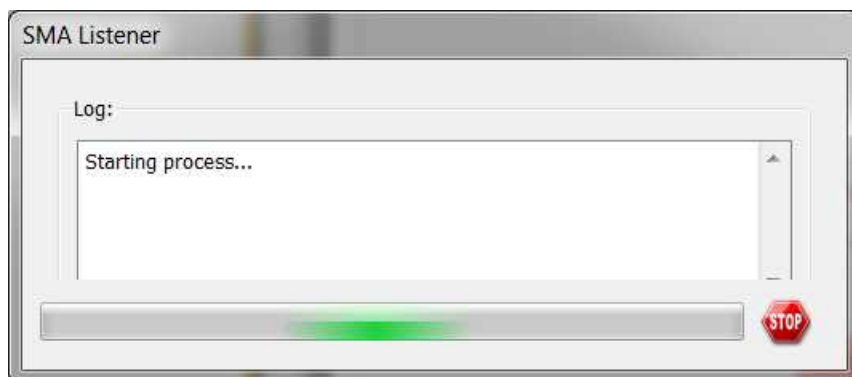
Clicking on the Settings button will allow you to include the reference in the result, execute the batch mode analysis in background and specify whether or not to save the results as Mnova files. From here you can also select the tolerances for the relative area and coupling constants tests:



You can run Batch SMA as a **listener**. Mnova can 'listen' for the appearance of data in a series of folders or drives, grab data as is acquired and run the SMA analysis automatically, saving the result in the selected folder. The settings for the SMA listener can be selected under the 'Advanced/SMA/Listener SMA' menu:



When the listener is working, Mnova will display a dialog box like this:



Thank you!

Thank you for reading this manual, and for purchasing this release version of Mnova SMA. We will be very keen to read your feedback on the application, to hear about any bugs you may find and to also listen to any additional ideas or suggestions you may have.

Please remember that you can send all those, and any queries about the software, or requests for help, to:

support@mestrelab.com

Keep checking our web site (www.mestrelab.com) for additional information on our range of software packages, and for news on our company.