cMC-assist
Version 1.0

CMC-assist

Bruker User Tutorial

Version 001

This tutorial was written by

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i Introduction

The software package CMC-assist is designed for processing and analysis of acquired 1D ^{1}H NMR data. Its user-friendly and well structured interface enables new CMC-assist users as well as experienced Bruker software users to benefit from the comprehensive range of functionalities.

About Bruker‘s CMC-assist User Tutorial

Bruker‘s CMC-assist User Tutorial guides one through the analysis of different example data. Each chapter of this tutorial addresses a different aspect of the automated analysis in order to point out the major features of the software package CMC-assist.

- **Chapter ii** demonstrates the typical workflow of CMC-assist including data processing, spectra analysis and result reporting.
- **Chapter iii** deals with the definition of impurities and how to incorporate these in the automated spectra analysis.
- **Chapter iv** shows several options to manually refine the automatically generated results.
- **Chapter v** describes the modification of analysis settings in order to solve possible arising problems occuring during the automated spectra interpretation.
- **Chapter vi** comments on redrawing of molecular structures and rerunning the automated analysis.
- **Chapter vii** explains how to determine the concentration of the compound of interest.

About Bruker‘s CMC-assist User Manual

For further information on CMC-assist please refer to Bruker‘s CMC-assist User Manual which describes the main aspects of the software package in more detail. In order to facilitate navigation through this manual, the chapters are arranged according to a typical workflow:

- **Chapter 2** shortly describes the installation of the software package and the first steps to display and analyse a spectrum.
- **Chapter 3** addresses the handling of NMR data. This covers the different options of the *Data* menu as well as managing the data within the CMC-assist browser, including loading new data, saving or deleting data.
- **Chapter 4** explains all functionalities associated with the CMC-assist interface. This comprises the toolbar icons, the command line and the data window with its specific toolbar.
- **Chapter 5** deals with processing of NMR data. In addition to manually processing single datasets, the corresponding menu gives the opportunity to automatically process either single spectra or a batch of spectra in automation.
- **Chapter 6** describes all available functionalities regarding the analysis of NMR data. Besides carrying out a manual interpretation of the spectra, complete analysis of the spectra including integration, multiplet analysis, concentration determination and struc-

tural consistency check can be performed automatically.

- **Chapter 7** lists several possibilities to document the gained results, such as generating reports, printing data or exporting assignments in patent string format.

Font Conventions

- Commands that can be entered on the command line are in ***Arial bold italic***
- Menus, buttons and icons that can be clicked or selected are in *Arial italic*

Functionality

CMC-assist software package provides the following features:

- Processing of 1D ^{1}H NMR spectra
- Manual or automated analysis of 1D ^{1}H NMR spectra
- User-friendly front end for convenient result refinement
- Report generation that includes NMR assignment and patent strings in various formats

CMC-assist License

CMC-assist requires a license for startup. A license can be ordered online from: www.bruker-biospin.de/NMR/nmrsoftw/Licenses/index.html

A short instruction on updating the license file (for Windows operating systems: C:/flexlm/Bruker/licenses/license.dat and for systems running under Linux: /usr/local/flexlm/Bruker/licenses/license.dat) will be sent together with the license.

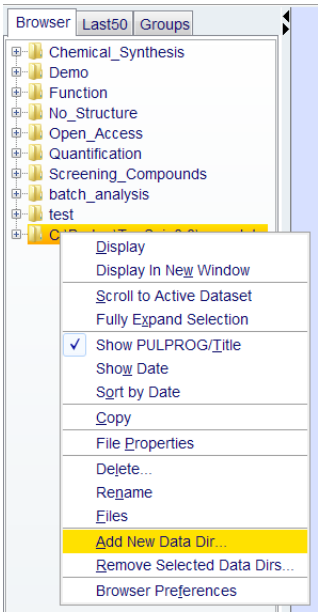
The following programs are distributed as part of CMC-assist but require a separate license:

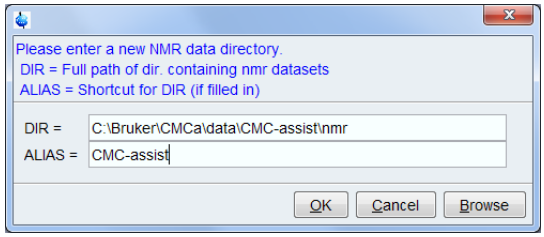
- PERCH NMR prediction (only for Windows operating systems)
- Line Shape Analysis (only for Windows operating systems)

ii CMC-assist Workflow

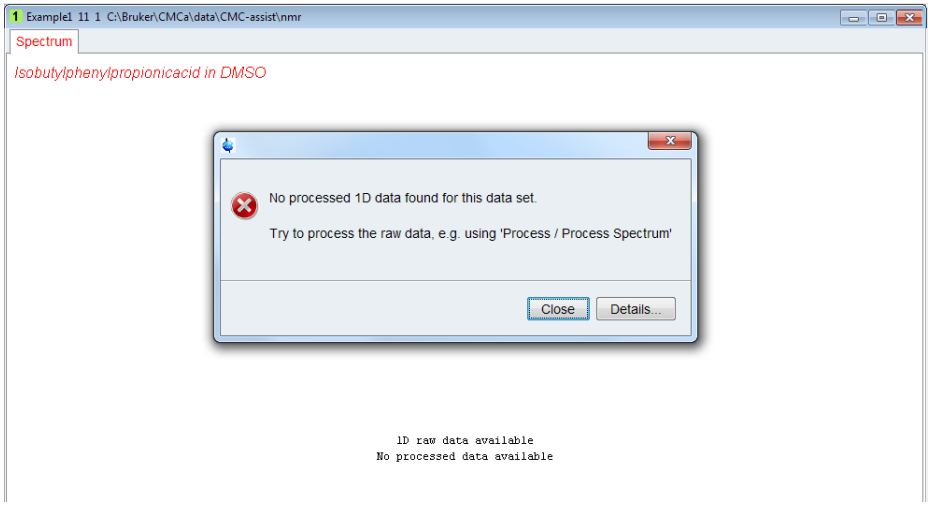
This first example illustrates a typical CMC-assist workflow including data processing, spectra analysis and results reporting.

First of all, the example data have to be added to the data broswer. Right click anywhere in the browser window, select *Add New Data Dir* and browse for the CMC-assist example data (please note that the path has to be defined including ‚nmr').

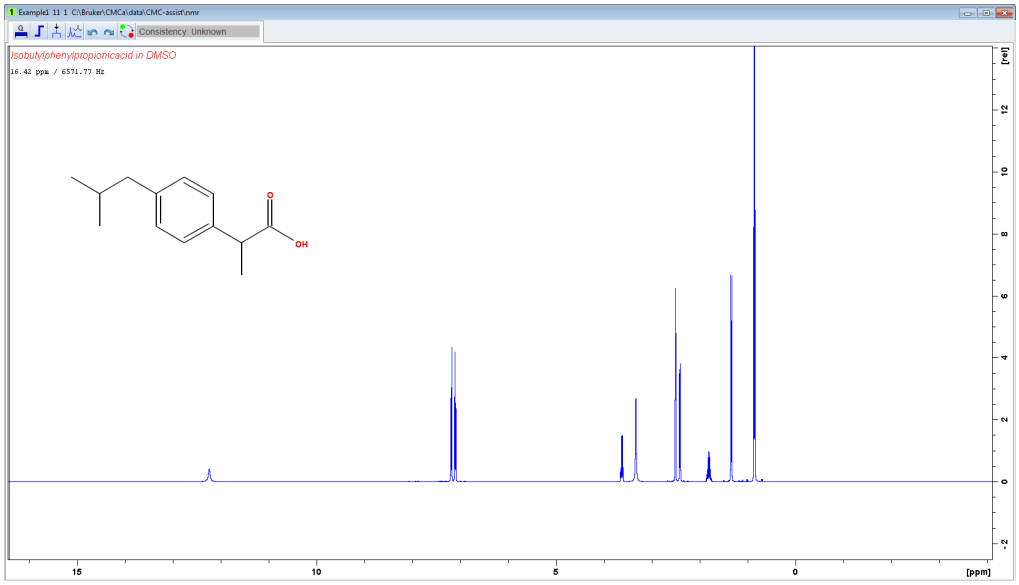




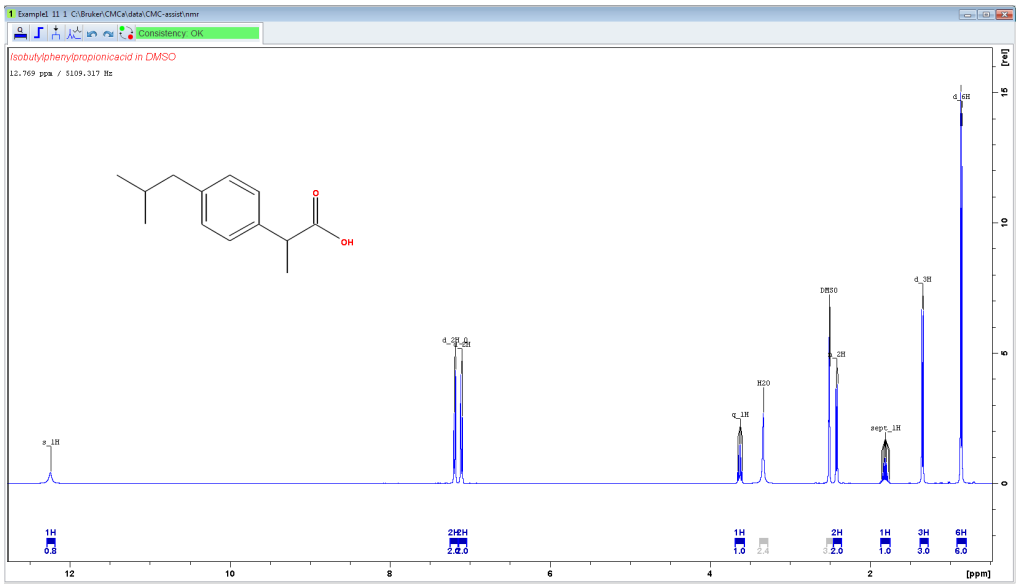
In order to open the data set Example1, expand the CMC-assist directory tree, left click on the data set Example1 and drag and drop this data set into the data window.



The pop up window indicates that the raw data have not been processed yet. Processing can be performed automatically using the tab *Process Spectrum* (Process Spectrum) available within the *Process* menu.



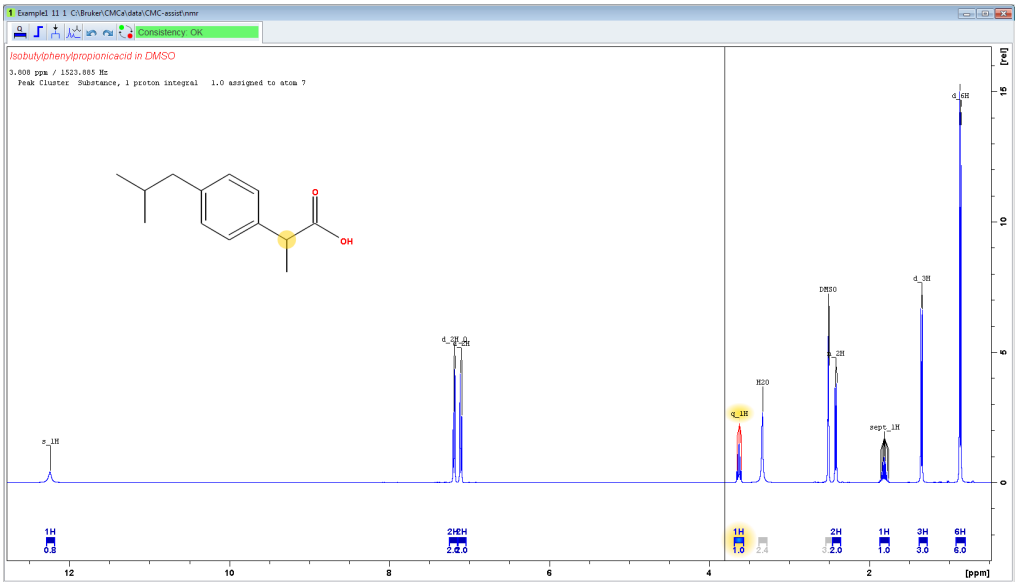
The processed spectrum is displayed together with the molecular structure and the consistency statement is defined as unknown as long as no automated spectra analysis has been performed or the statement has been modified manually. The spectrum will be analysed in automation by clicking on the tab *Analyse Spectrum* (Analyse Spectrum) which is a submenu of the *Analyse* menu.



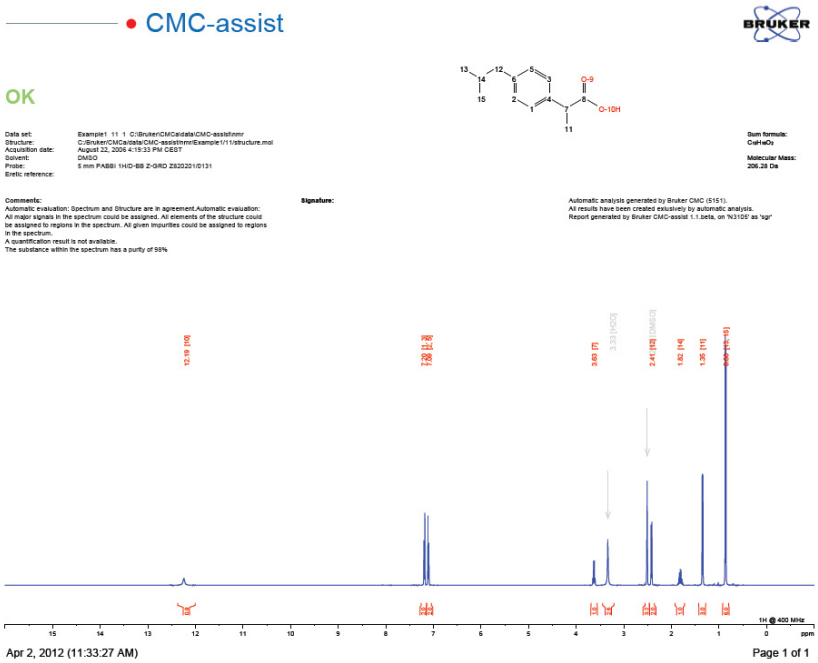
The automated spectra interpretation includes integration regions with their associated number of protons, multiplet definition for each peak cluster, peak assignment and the

consistency statement. The bars representing the integration regions are colored according to the type of signal. Blue bars label peaks that belong to the substance, whereas gray bars indicate solvent peaks or peaks originating from any known impurity.

The assignment can be visualized by placing the cursor on (or close to) a multiplet. The connected atom of the molecular structure will then be highlighted. Alternatively, the cursor can be moved over the different atoms of the molecular structure and the assigned multiplets in the spectrum will be highlighted.



These results can be documented as short report, full report and patent string by choosing the corresponding tab of the *Report* menu.

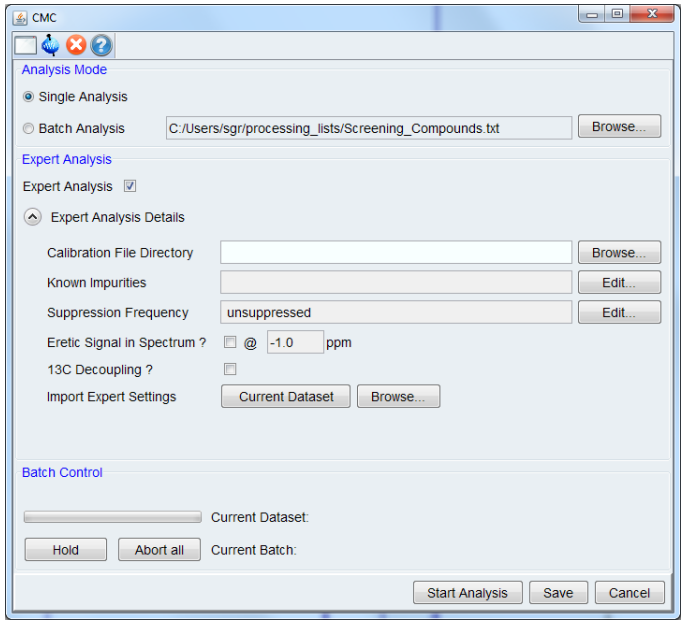


CMC-assist Workflow

iii Impurity Definition for Automated Spectra Analysis

The second example deals with the definition of impurities and how to incorporate these in the automated spectra analysis.

The data set Example2 is already processed and clicking on the *Analyse Spectrum* tab (available within the *Analyse* menu) opens the following dialog for analysis settings.

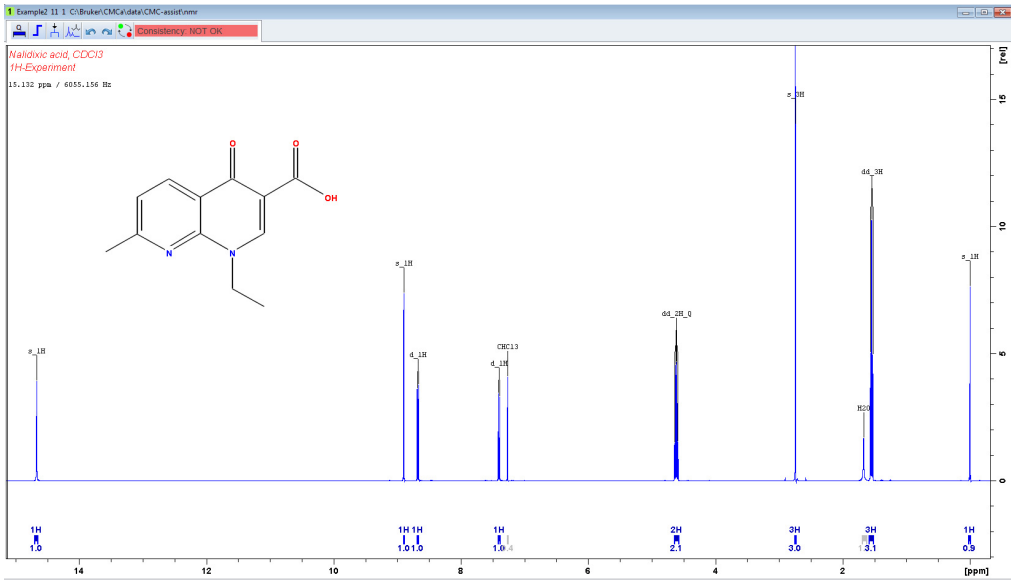


In order to reasonably analyse spectra in automation, some basic parameters need to be defined. These consist of the number of suppression frequencies, any known impurities that should be excluded from the analysis, possible ^{13}C decoupling, existence of an eretic signal including its position and for quantification purposes the path to the required calibration file.

If the data have been acquired in automation using the software package CMC-q available under TopSpin or the feature Fast Lane NMR integrated within IconNMR, all this information already exists within the data set. Thus, clicking the tab *Analyse Spectrum* directly interprets the current data set (like Example1).

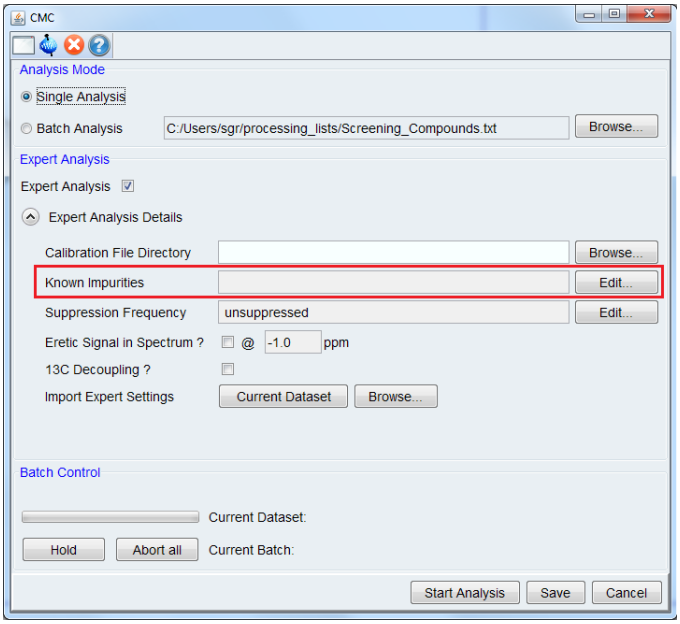
In the case the data have not been acquired with respective Bruker software packages plus the analysis runs for the first time on a data set, these parameters have to be specified for the automatic analysis. Hence, clicking the tab *Analyse Spectrum* opens a dialog that enables editing of the required information.

The pop up window shown above displays the default settings. Clicking on the corresponding button will start the automated analysis. Example2 gives the following result:



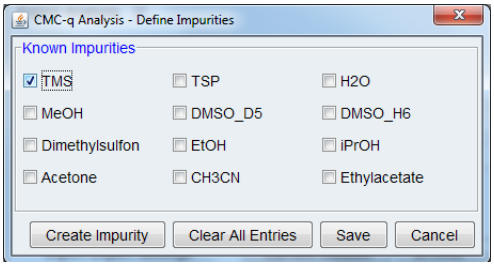
Although the software comes to the conclusion that the spectrum is not consistent with the provided structure, the spectrum gets analysed with respect to integration regions, plausible number of protons for these integration ranges, multiplet interpretation, and recognition of solvent peaks.

On closer inspection of the spectrum one identifies a peak at around 0 ppm that integrates to one proton. This peak does probably not belong to the substance and could also originate from TMS added to the solvent.



In order to define TMS as an impurity occuring in the spectrum, one has to reopen the dialog that enables modification of the required analysis information. All these settings

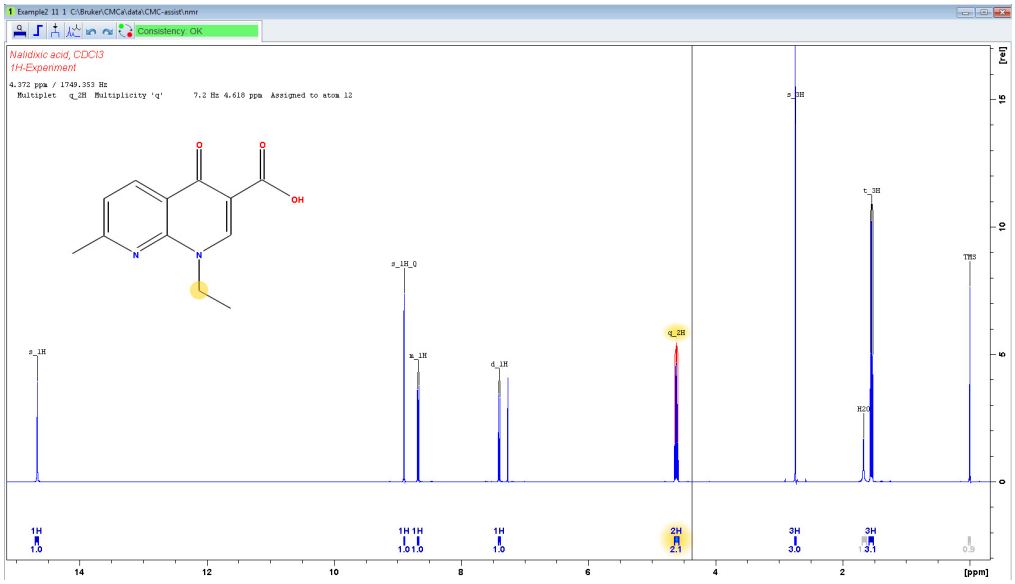
can be edited any time by the submenu *Analysis Settings*, accessible by the down arrow on the right side of the tab *Analyse Spectrum*. Clicking the button *Edit* on the right side of the field *Known Impurities* opens a new window where these impurities can be defined.



On the one hand, some frequent impurities found within spectra of chemically synthesized compounds can be selected by just ticking the corresponding boxes. In addition, special or rarely appearing impurities can be defined individually. In order to add an impurity, click the tab *Create Impurity* and fill in any known information. Please note that the individually entered impurities have to be activated the same way as the predefined ones by ticking the respective boxes. Before leaving this dialog any changes should always be saved.

For declaring TMS as a known impurity, one has to tick the corresponding box and afterwards save this setting. The field *Known Impurities* now contains TMS. By clicking on *Start Analysis* the software will interpret the spectrum with the additional information that the signal at around 0 ppm must not be assigned to the molecule.

By defining TMS as an impurity, the program finds no inconsistencies between spectrum and structure anymore and therefore it correctly assigns all analyte signals in the spectrum.

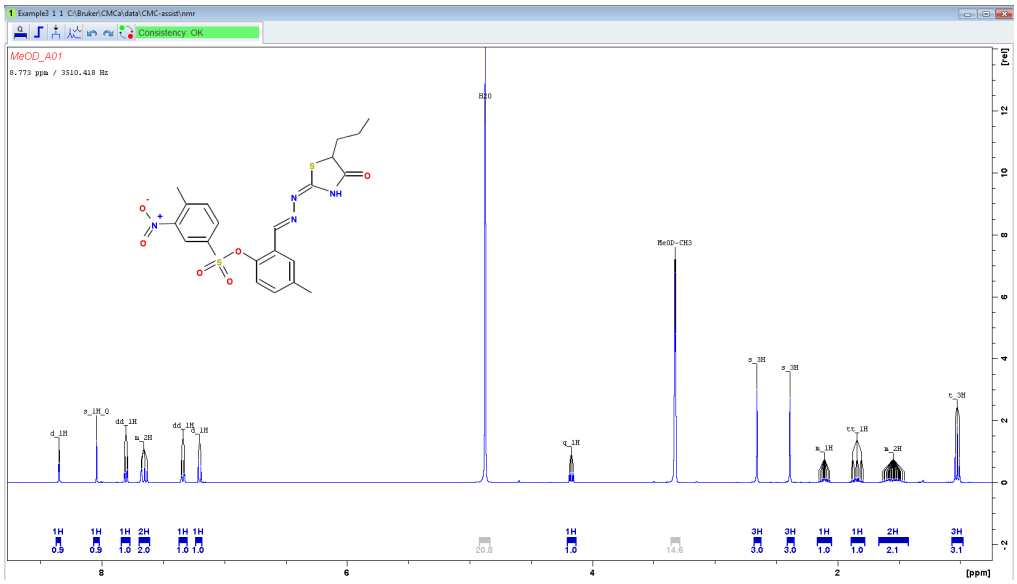


Impurity Definition for Automated Spectra Analysis

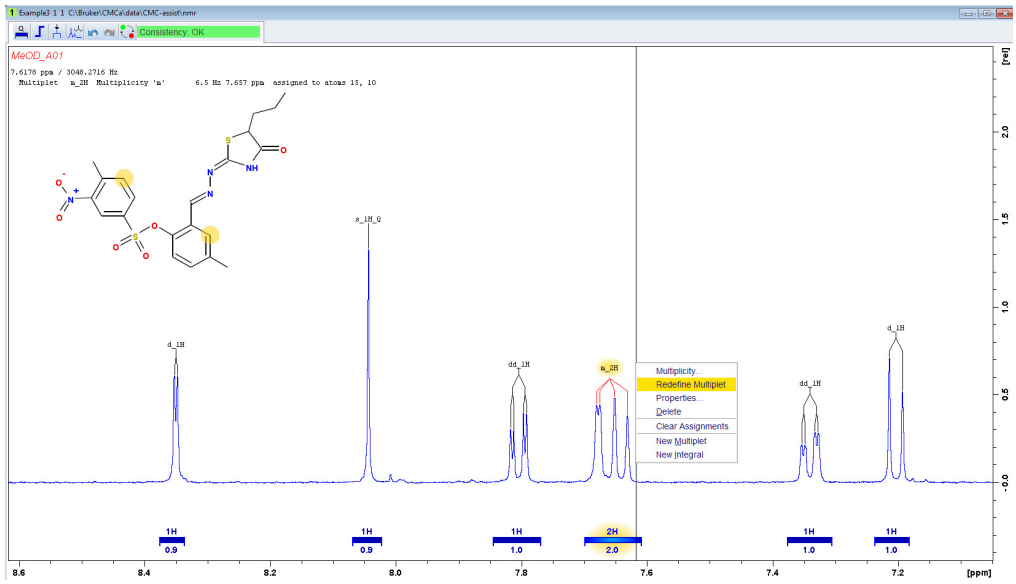
iv Manual Result Refinement

The third example shows several options to manually refine the automatically generated results.

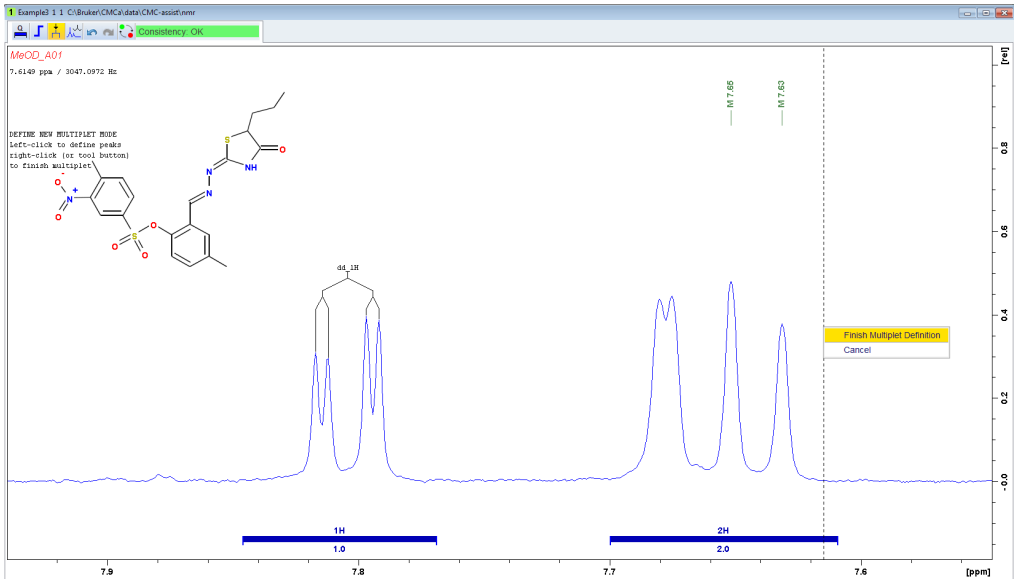
After analysing the preprocessed data set Example3, one should have a closer look at the proposed multiplets, integration regions and assignments.



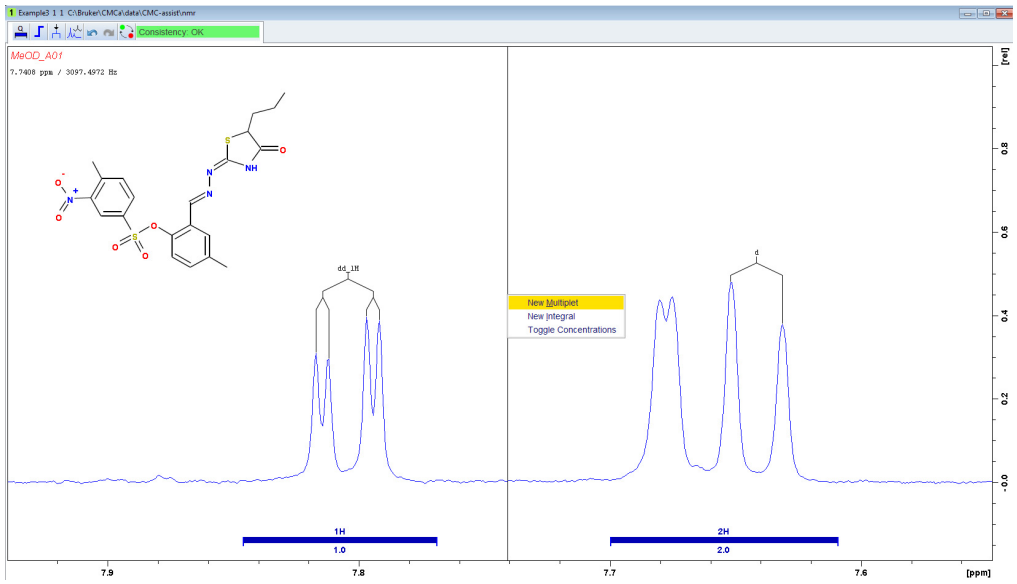
The peak cluster at around 7.66 ppm could be separated into two individual peak clusters. Therefore, the cursor line has to be close to the corresponding peak cluster with the cursor above the baseline. Right clicking offers the option *Redefine Multiplet*.



Left clicking on *Redefine Multiplet* deletes the current multiplet and enters the multiplet mode. In order to define a new multiplet, left click on the peaks that belong to one multiplet and subsequently right click anywhere and choose *Finish Multiplet Definition*.

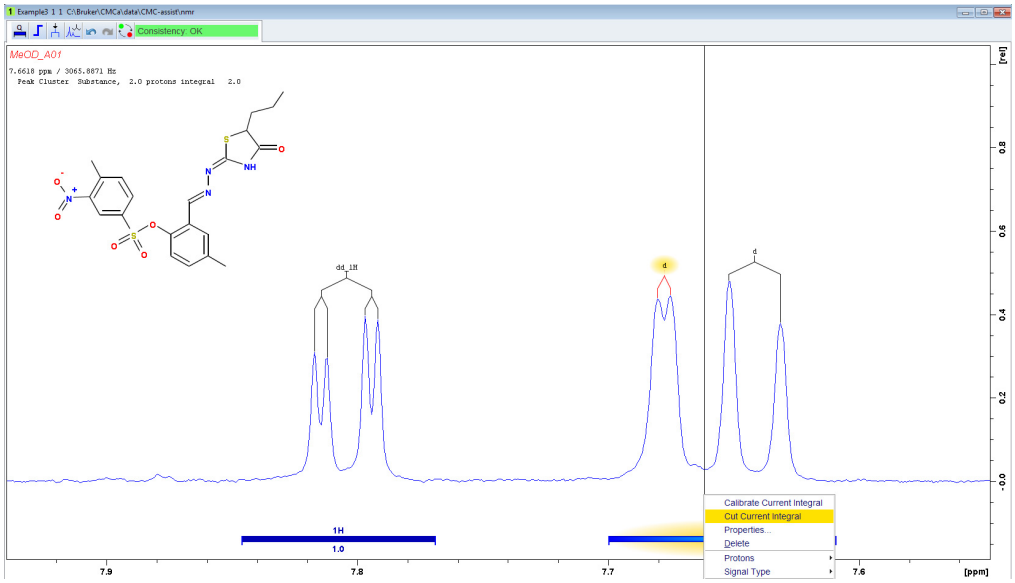


After defining this doublet the multiplet mode will be left again. The option *Redefine Multiplet* corresponds to the modification of one multiplet. For the definition of the second multiplet, right click anywhere and select *New Multiplet*. This general option allows the creation of one multiplet (exactly as described before) and afterwards also leaves the multiplet mode.

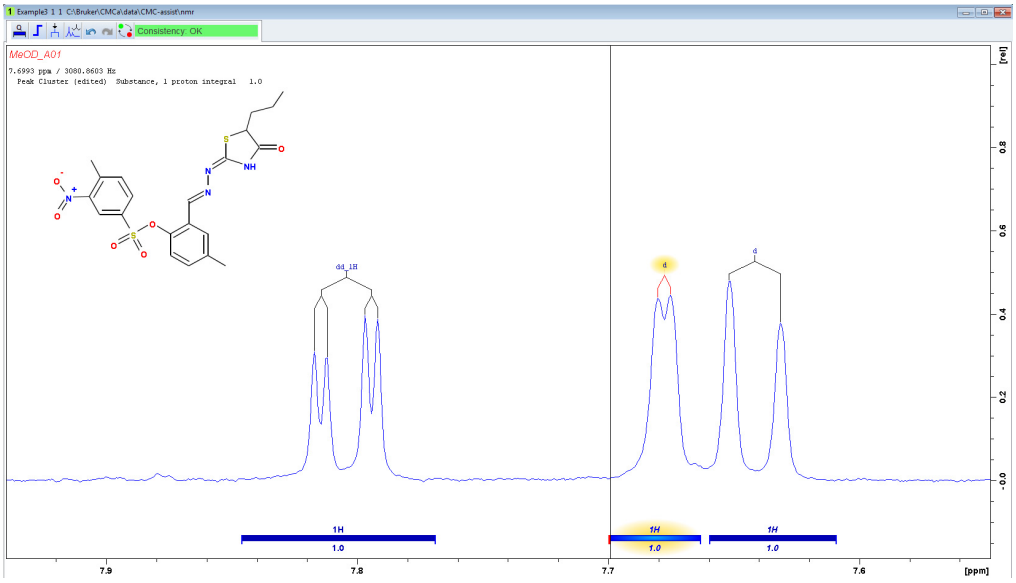


As this peak cluster consists of two multiplets now, one might want to cut the integration region and define one integral per multiplet. For any modifications on the integration

regions, the cursor line has to be positioned at the desired integral with the cursor underneath the baseline. Right clicking then shows all possible actions executable on the integral. The option *Cut Current Integral* will cut the integral at the position of the cursor.

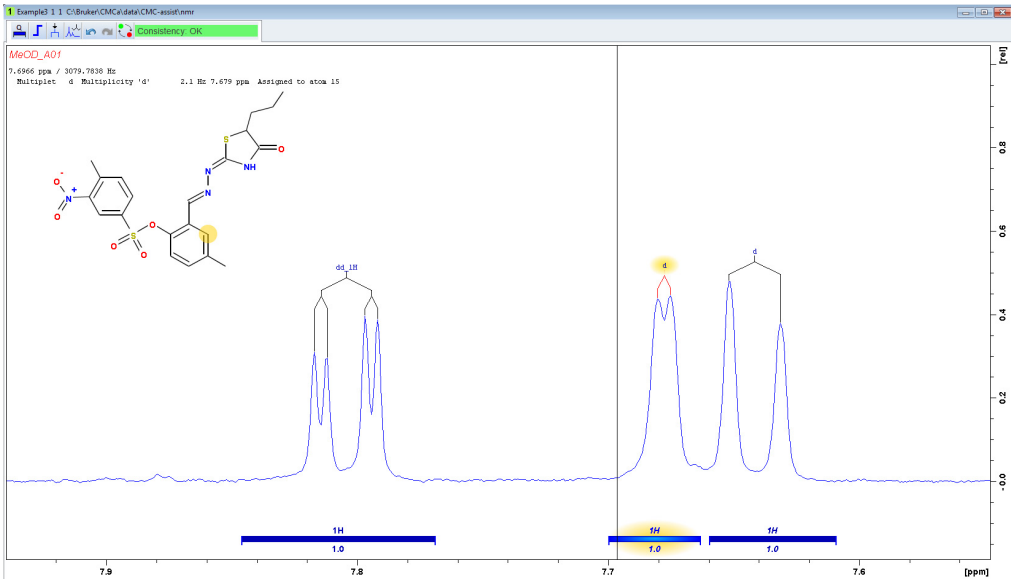


The size of the resulting integration regions can be easily modified by left clicking on the vertical bar when it turns red and moving the mouse to adjust the width of the integral.

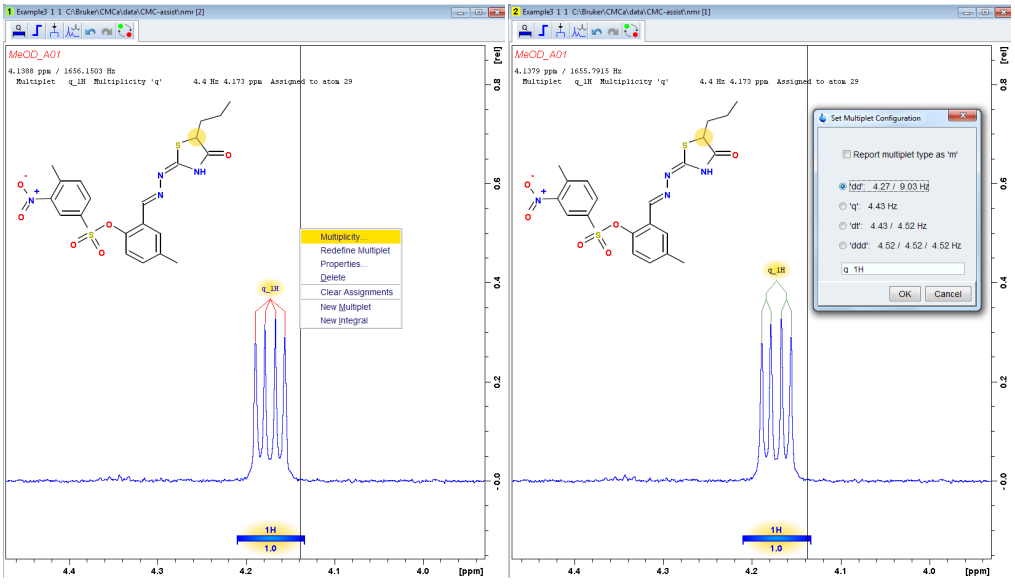


As the assignment displays the connection between the atom of the structure and the multiplet of the peak cluster, deleting the multiplet also deletes the assignment. Hence, these two redefined multiplets have to be newly assigned. In order to connect a multiplet to an atom of the molecular structure, left click on the multiplet and drag this multiplet to the appropriate atom of the displayed structure. Assignments can be viewed by moving

the cursor over the multiplets or the atoms of the molecule. Either way multiplet and atom that are linked together will be highlighted.



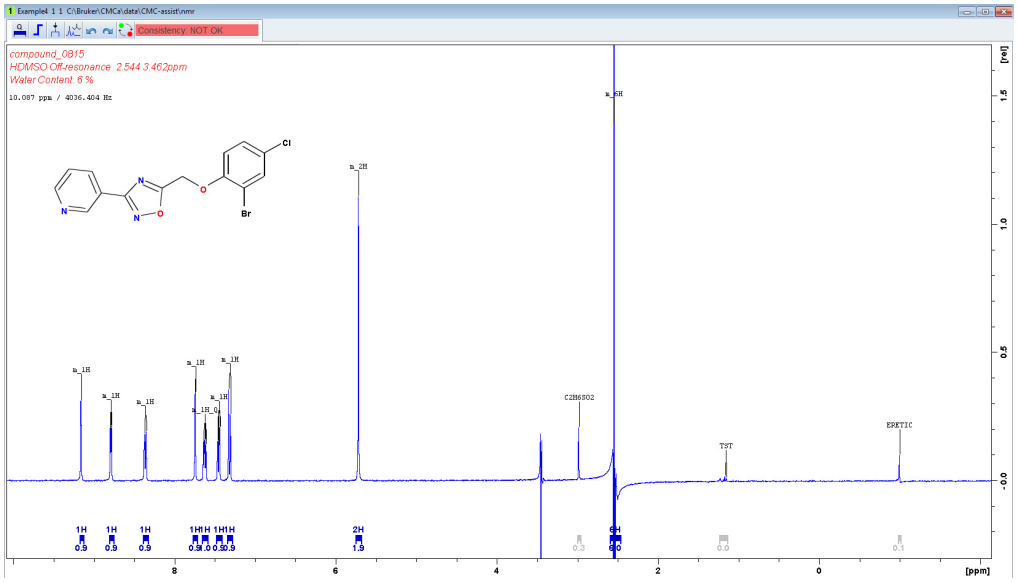
Furthermore, the automatic analysis of the peak cluster at around 4.17 ppm can be improved by manually defining the cluster as a doublet of doublet instead of a multiplet. Choose dd from the list of suggested multiplet possibilities, shown when right clicking on the multiplet and selecting the option *Multiplicity*.



v Analysis Settings

This example describes the modification of analysis settings in order to solve possible arising problems occuring during the automated spectra interpretation.

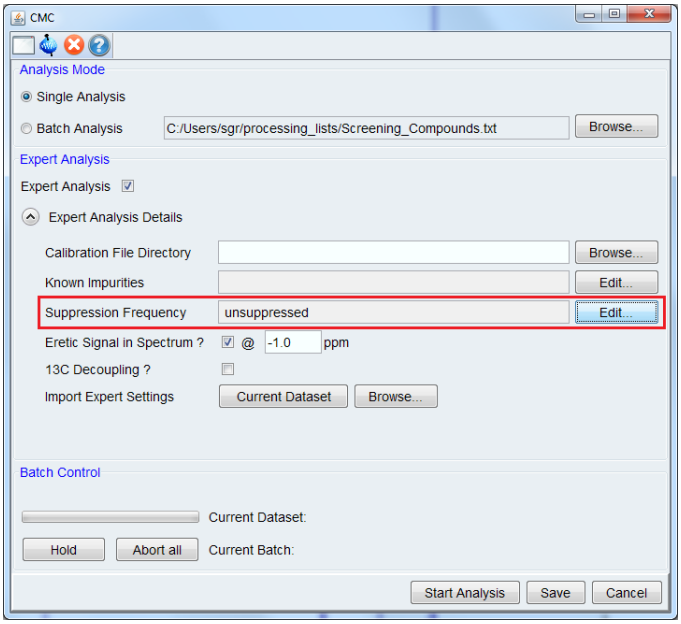
Analysing the spectrum of data set Example4 in automation results in inconsistent. One common reason for inconsistencies between spectrum and structure are undefined impurities that cause additional signals in the spectrum (see Chapter 3). Another frequent reason are incorrect settings for the automated spectra interpretation.



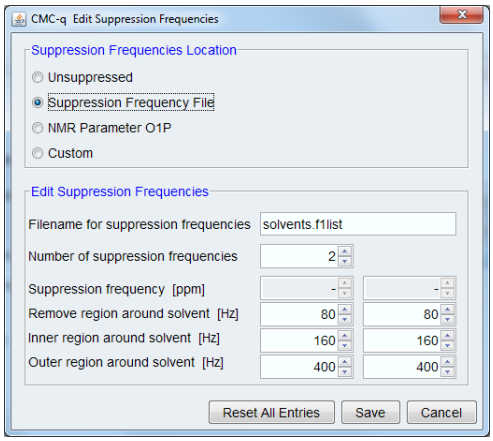
In order to get any indication of what might have caused the inconsistency, one can have a look at the cmcqs file. Type *expl* on the command line and open the file cmcqs with any text editor. This file summarizes the current analysis settings such as known impurities that were taken into account for the spectra interpretation, information on an existing eretic signal, if the spectrum was acquired with decoupling or not, etc. A very important setting is the information on the number of suppression frequencies. As stated in the cmcqs file this spectrum was analysed under the assumption of an unsuppressed spectrum. However, the experimental data clearly show two suppression frequencies.

```
##$VERSION= <4955>
##$COMPOUND= <Example4>
##$NMR= <C:/Bruker/CMCa/data/CMC-assist/nmr/Example4/1/pdata/1/1r>
##$MOL= <C:/Bruker/CMCa/data/CMC-assist/nmr/Example4/1/structure.mol>
##$SJRACK_NO=
##$HOLDER=
##$PANEL_COL= <>
##$PANEL_ROW= <>
##$RACK_ID= <>
##$IMPURITIES= <Dimethylsulfon/TST>
##$CUSTOM_IMPURITIES= <>
##$SUPPFREQ_SOURCE= <UNSUPPRESSED>
##$SUPPFREQ_NO= 0
##$SUPPFREQ_FILE= <>
##$SUPPFREQ= <>
```

To edit these settings open the submenu *Analysis Settings*, accessible by the down arrow on the right side of the tab *Analyse Spectrum*. By clicking on the *Edit* button next to the field *Suppression Frequency* another window appears.



This pop up window allows modification of the number of suppression frequencies (unsuppressed, single suppressed or double suppressed) and the type of suppression (suppression frequency identical to O1P, suppression frequencies stored in a suppression frequency file or manually edited suppression frequencies).



• Unsuppressed

In the case the data were acquired unsuppressed select this option and no further specification is required.

• Suppression Frequency File

Suppressed spectra can be acquired in various ways, depending on the number and

position of the solvent signals. Consequently, the information about the suppression frequencies can be stored in different parameters or files.

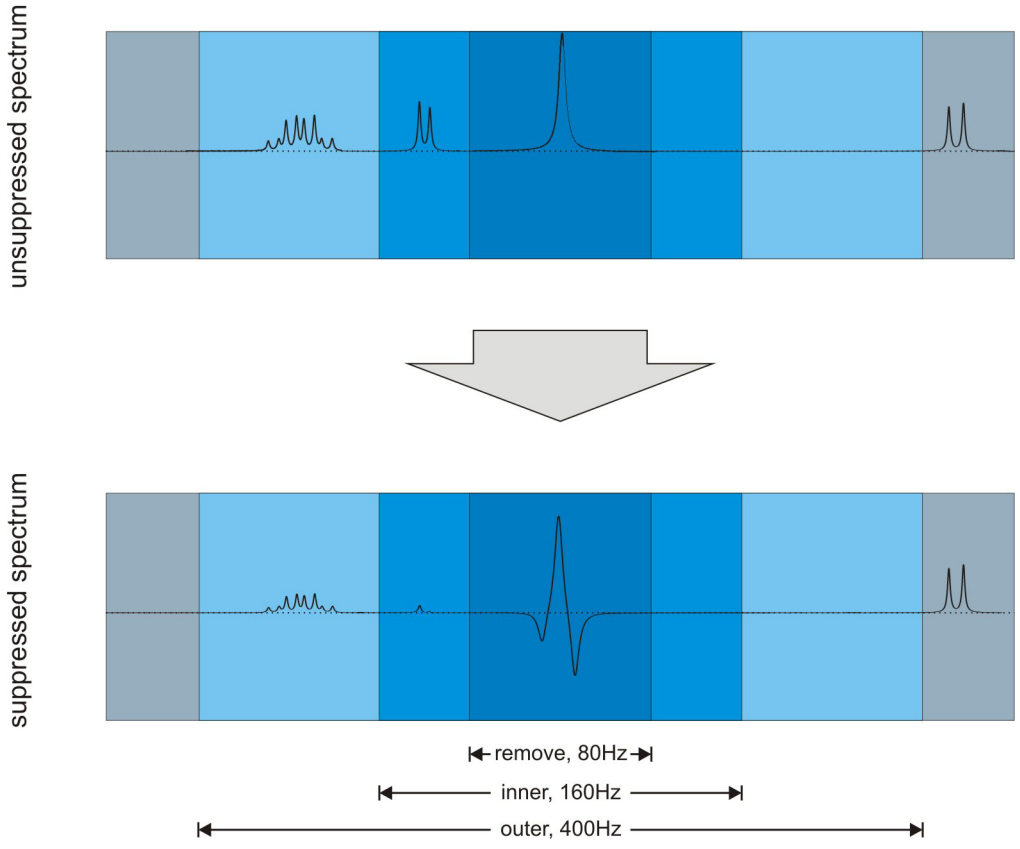
If several solvent signals have to be suppressed or the suppression frequency is off-resonant, all the information about number and position of the suppression frequencies is typically stored in a frequency file. In this case, the filename has to be specified in the lower part of the dialog (this file should be automatically generated during acquisition and located at the level of the expno within the dataset). In addition, the field *Number of suppression frequencies* has to be edited and must match with the number of entries in the frequency list.

- NMR Parameter O1P

  Choose this option for on-resonant solvent suppression. By definition, this type of suppressed spectra possesses only one suppression frequency whose frequency corresponds to the NMR parameter O1P.

- Custom

  In the unlikely case that none of the above mentioned variations of suppressed spectra matches the type of acquired data, the number and position of the suppression frequencies can be specified individually.



- Remove region around solvent

  The range in Hertz (Hz) defined in this field is completely ignored for spectra analysis.

Typically, it covers the suppression frequency itself including the nearby suppression artefacts. Please note that a setting of 60 Hz means a region of ±30 Hz around the suppression frequency. Besides, each suppression frequency requires one entry, where the left entry in the dialog is related to the suppression frequency with the lower ppm value.

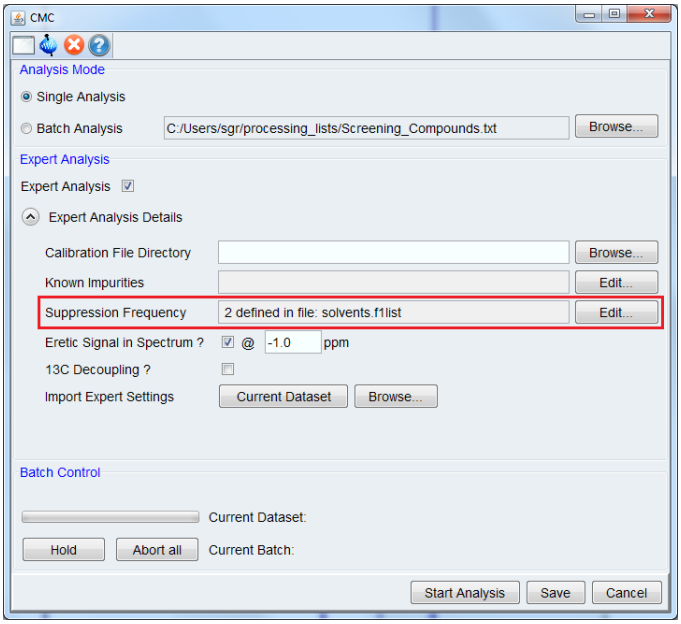
- Inner region around solvent

  This region refers to the area close to the suppression frequency, that is still significantly influenced by the suppression. Peaks within this specified range are taken into account for the analysis, however their multiplicity and proton number is not interpreted.

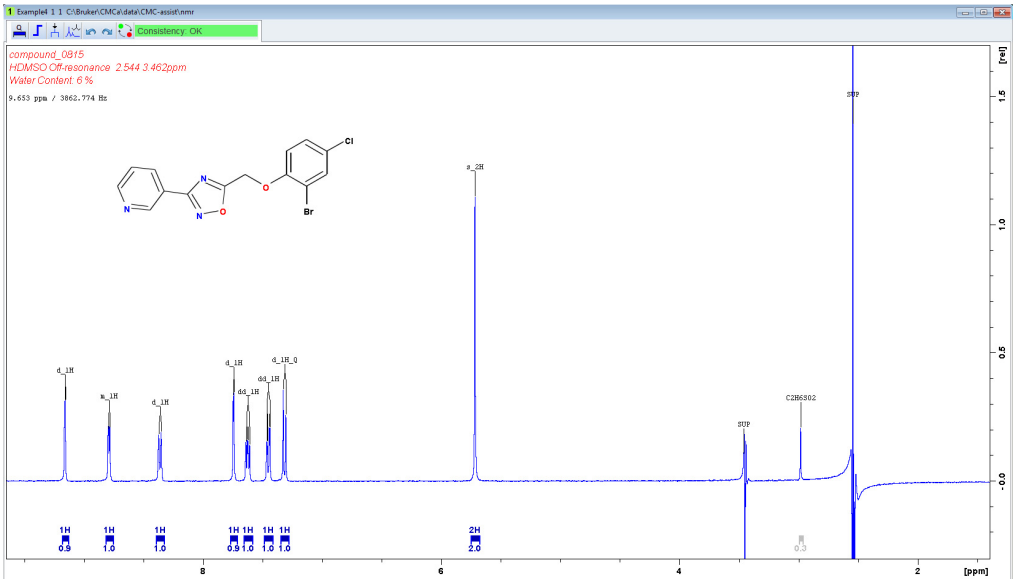
- Outer region around solvent

  Peaks within this outer region only experience minor disturbances from the suppression. For the automated spectra interpretation, these peaks are analysed with respect to their multiplicity, however their integrated proton numbers are classified as not reliable.

The spectrum of Example4 has been acquired with solvent and water suppression. Information on the position of the suppression frequencies is automatically stored in the file solvents.f1list by the parameter set WET. Consequently, tick the option *Suppression Frequency File* and accept the default settings for the filename, the number of suppression frequencies and the ranges for the different regions around the solvent. After saving these settings the information on the suppression frequencies will appear in the *Analysis Settings* dialog.



Reanalysing the spectrum with these settings leads to the statement ‘consistent‘, spectrum and structure are in agreement.

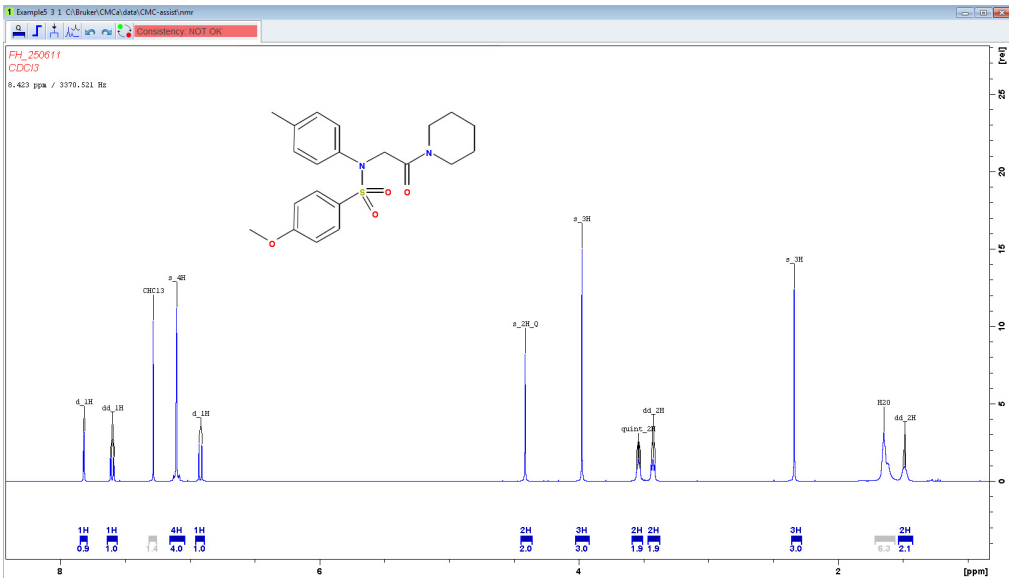

Example1 1 1 C:\Bruker\CMCa\data\CMC-assist\nmr
Consistency: OK
compound_0815
HDMSO Off-resonance 2.544 3.462ppm
Water Content: 6 %
9.653 ppm / 3862.774 Hz
Cl
Br
N
O
d_1H
m_1H
dd_1H
d_1H_Q
a_2H
SUP
C2H6SO2
1H
2H
8
6
4
2
[ppm]
[rel]

Analysis Settings

vi Modifying Molecular Structures

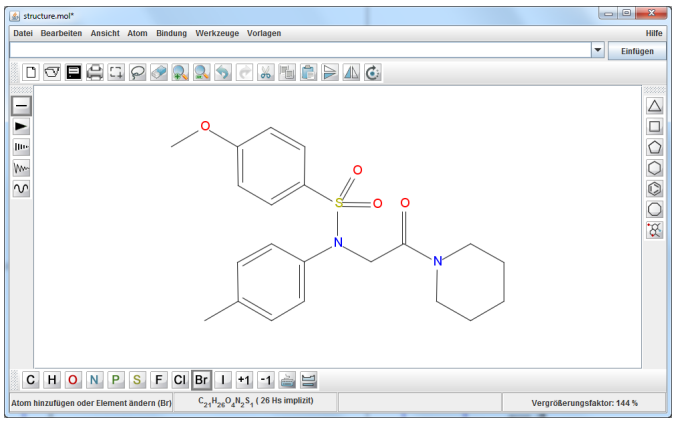
This example illustrates how to reanalyse a spectrum with a modified molecular structure.

The automated analysis of data set Example5 indicates that the provided structure is not consistent with the experimental data.

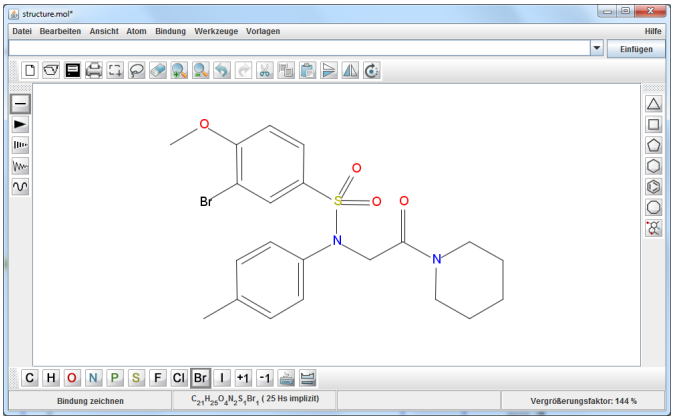


Assuming that one has an alternative structure proposal in mind or has an indication from additional experimental data, the molecular structure can be modified and the spectrum can be reanalysed with the newly defined molecule in order to find out if this compound matches the experimental data.

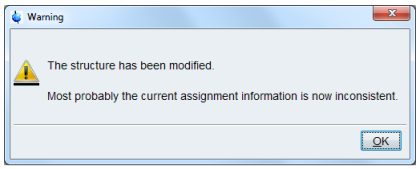
The program JChemPaint, an editor for 2D molecular structures, is an integrated part of CMC-assist that enables drawing and modifying chemical structures. Clicking on the tab *Editor* within the *Structure* menu starts the structure editor in a separate window.



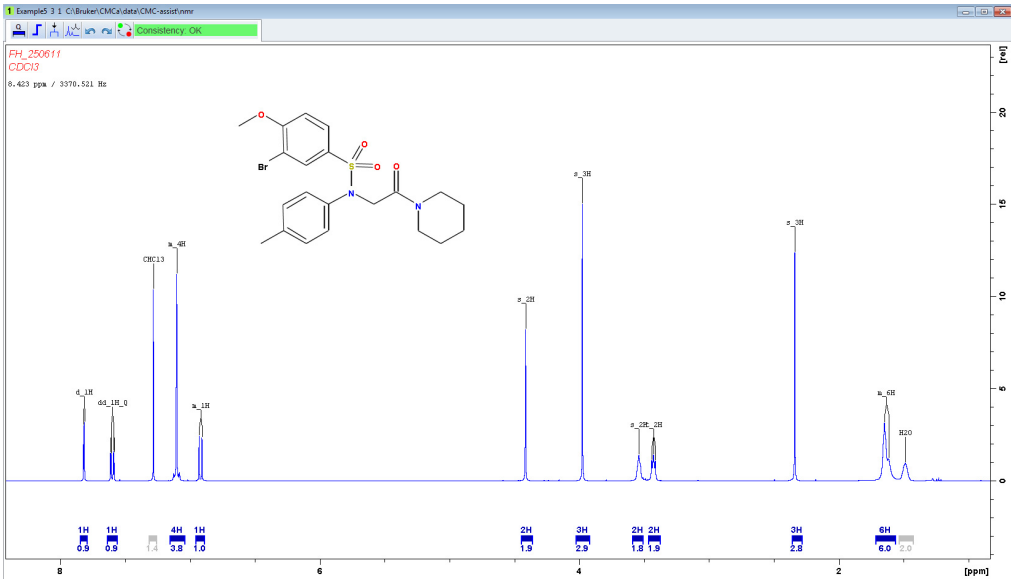
Add a bromide in ortho position to the methoxy group of the aromatics; save the modified structure (file/save) and close the JChemPaint editor. The structure displayed with the spectrum will be updated. Note that saving modified structures will overwrite already existing .mol files.



Whenever the molecular structure is modified, a pop up window appears, indicating that the current spectrum assignment is based on the analysis with a different structure.



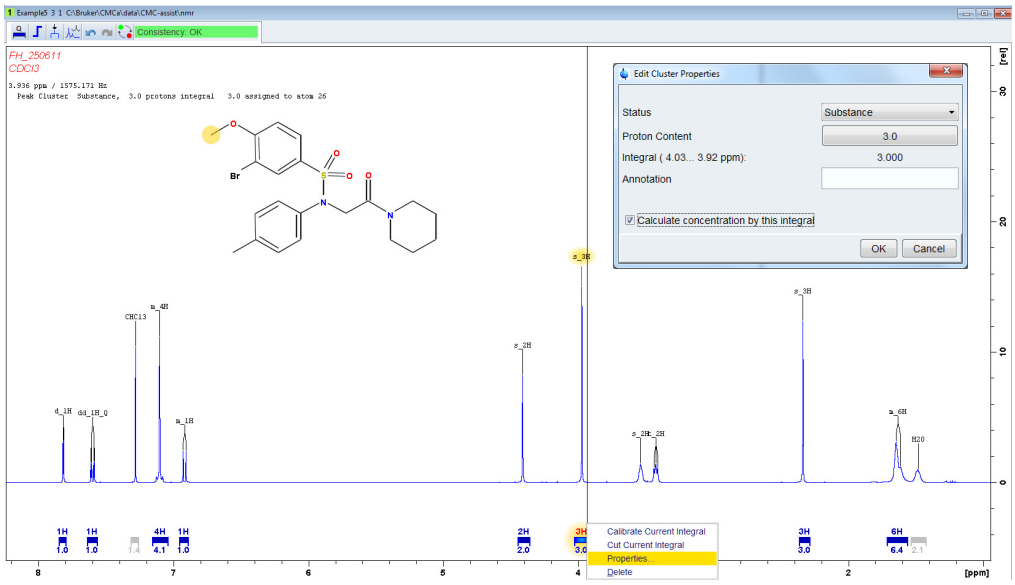
Reanalysing the spectrum with the modified chemical structure shows that this compound matches with the experimental data.



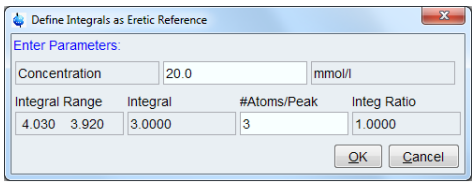
vii Concentration Determination

This example explains how to determine the concentration of the compound under investigation using an external standard of known concentration.

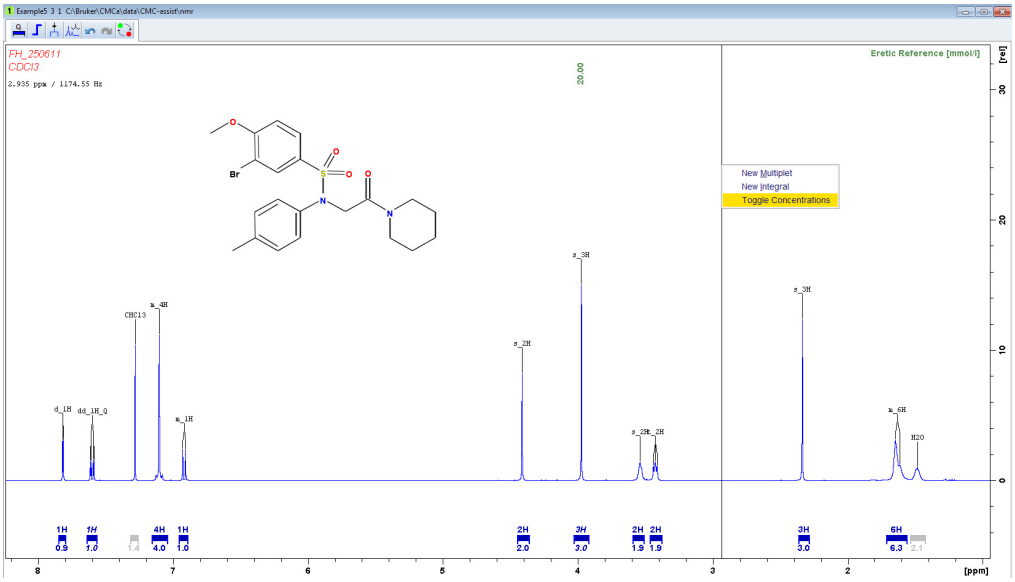
The concentration of the compound of interest will be calculated based on at least one signal from the reference spectrum. In this tutorial the reference spectrum is Example5. After choosing a suitable peak (e.g. the one at around 3.98 ppm) and integrating it, right click on its corresponding integral and select *Properties*. A pop up window will appear. Tick *Calculate concentration by this integral*.



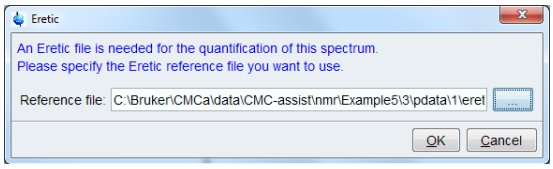
Afterwards, the down arrow on the right side of the tab *Quantification* offers the possibility to define the marked and integrated signal(s) as reference. Clicking the submenu *Define as Eretic Reference* opens up a dialog, where the assigned proton number for each labeled and integrated signal has to be entered as well as the exact concentration of the reference substance (all the given information will be saved in a file called eretic).



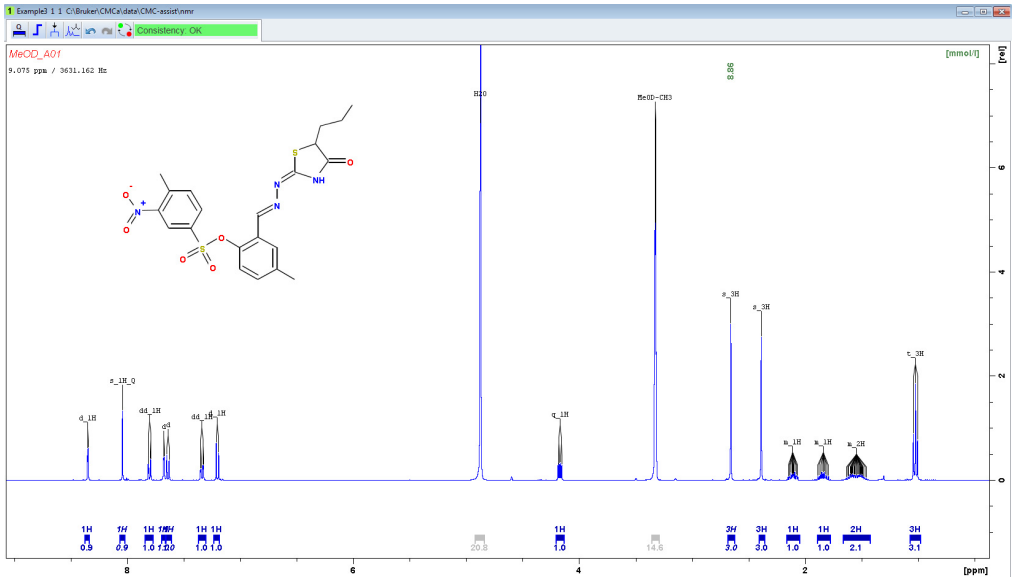
Once the spectrum is defined as reference spectrum this information together with the appropriate concentration will be indicated in the data window. Right clicking anywhere except on an integral or multiplet provides the opportunity *Toggle Concentrations* to show or hide this information.



In this tutorial the concentration of the compound of Example3 will be calculated. After opening the data set Example3, specify the reference spectrum on which the concentration determination should be based. The down arrow on the right side of the tab *Quantification* offers the option *Browse for Reference* which opens a dialog that allows browsing for the quantification reference. Please note that the full path (including procno) to the reference spectrum is required.



For quantification of the compound of interest, right click on the desired integral (e.g. the integral around 2.66 ppm) on which the concentration determination should be based, select *Properties* and tick the box for *Calculate concentration by this integral*. The calculated concentration will be displayed in green numbers above the selected integral(s).


MeOD_A01
[mmol/l]
HDO
MeOD-CH3
NH
[ppm]

viii Contact

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