

# Spectroscopy Modeling and analysis.

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## Introduction.

Spectroscopy Modeling and analysis is a very important part of Magnetic Resonance Spectroscopy (MRS). Major systematic differences between results from different groups are in fact introduced by their choices of Spectroscopy Modeling and analysis. But Spectroscopy Modeling and analysis should not only be of interest to the specialized user. The basic user of MRS should also understand the basic elements of Spectroscopy Modeling and analysis including both those of quantitation and those of metabolite identification.

In the interest of time Spectroscopy Modeling and analysis questions will be addressed using the example  $^1\text{H}$  MRS of the brain. The method can however easily be extrapolated to MRS of other tissues as well as to X nuclei MRS.

A whole host of methods that skip or in part skip the classical analysis will not be addressed in this talk. These include statistical classification strategies, principal component analysis, linear discriminant analysis, optimal discriminant vector, cluster analysis and artificial neural networks.

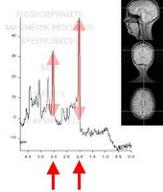
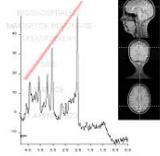
In order to save space, references are not given here. The book chapter "Spectroscopy:  $^1\text{H}$  Metabolite Concentrations", in *Quantitative MRI of the Brain* edited by Paul Tofts and published by Wiley 2003, provides a detailed overview of this topic and contains all the relevant references.

Spectroscopy Modeling and analysis is a mix of metabolite identification and quantification. In the present abstract it will be described in terms of a primary and a secondary analysis. The primary analysis lead to a value  $A_m$  for each metabolite, as for example peak area. The primary analysis is followed by a secondary analysis that calculates the quantity  $Q_m$  from the value  $A_m$ . An example of the quantity  $Q_m$  could be a peak area ratio.

## 1) The primary analysis of the data. - Levels of sophistication of the MRS modeling and analysis.

### a) Visual inspection

MRS has become easily accessible and many radiologists read the MRS data by visual inspection of the "images". This visual inspection without assistance from advanced modeling or analysis often is sufficient in a clinical setting. Such "reading" of MRS data however does involve some of the more advanced modeling steps. The peaks are identified and the sizes of the peaks are estimated consciously or unconsciously. In just looking at a spectrum and giving an opinion about the peaks, one does actually estimate peak positions and peak height ratios. This method has many limitations but is in practice used by many, so will be addressed below. The simple steps of "reading a spectrum" are often combined with a pattern recognition strategy used to identify different patterns of disease.

	- peak ratio estimation	- pattern recognition
		
Visual inspection inherently involves elements of more advanced analysis:	- identification	

b) A more precise measure of a *number for each metabolite* ( $A_m$ ).

Various methods are applied directly to the data to get a *number for each metabolite* ( $A_m$ ), this could be called the primary analysis.  $A_m$  is used to calculate a *quantity* ( $Q_m$ ),  $Q_m$  relates to the amount of the metabolite in different ways depending on the choice of  $Q_m$ . The methods used to find  $A_m$  may or may not use prior knowledge, as for example where to expect a resonance, which metabolites to expect, or in which approximate relative amounts to expect the signals. The methods used to find  $A_m$  may be more or less automated and thus more or less objective. The following is a list of typical ways to arrive at  $A_m$ .

i) Peak heights:

Peak heights are not the ideal measure of a metabolite, it is in fact not correct to use peak heights. The metabolite concentration is proportional to the peak area but not to the peak height. The peak heights change with the magnetic field homogeneity and relaxation properties, whereas peak areas are independent of these. Relying on peak height ratios may lead to pitfalls especially for partially overlapping peaks, when comparing a reference spectrum with high resolution to a spectrum with poor resolution. The peak height ratios are mentioned nevertheless, because estimation of peak height ratio is in fact what one does when “looking” at a spectrum, and if the resolution of the different resonances and in between individual spectra are very similar, as is often the case, then the first visual analysis of the peak height ratios will prove quite useful.

ii) Peak areas:

The peak area of a resonance is proportional to the concentration of the corresponding metabolite, peak areas are therefore often used in spectroscopy analysis. Problems of peak areas include those of defining the boundaries of where to measure the area, which become more problematic the broader the peaks are, if they overlap, and when the resonances are not simple singles.

iii) Fitting of single resonances:

Fitting the data to a set of peaks of an assumed shape is better than just measuring the peak area.

iv) Fitting of the full data-set in the time domain.

The most frequently used method of this type is MRUI.

v) Fitting of the full data-set in the frequency domain.

The most frequently used method of this type is LCModel.

## 2) The secondary analysis. – The calculation of $Q_m$ .

a) The calculated *quantities* ( $Q_m$ ).

The *number for each metabolite* ( $A_m$ ) is used to calculate *quantities* ( $Q_m$ ), that could be ratios or other quantities that are more or less closely related to the metabolite concentration in the measured tissue, see the section below. In comparing analysis methods and understanding how

they differ, it is important to know which elements of the relation between  $Q_m$  and  $A_m$  that depend on the primary analysis and which ones are elements that can be applied independently of the choice of primary analysis.

b) Normal ranges.

For clinical practice it is recommended to have sets of normal ranges of the *quantities* ( $Q_m$ ) for each metabolite, for each type of tissue or location, and for different ages of the healthy individuals. Rather than reporting  $Q_m$  for each metabolite in a patient, it is much more convenient to report whether  $Q_m$  is within the normal range (mean  $\pm$  2 SD), or if outside the normal range, then to report that “ $Q_m$  is x % below or above the normal value”. The referring physician wants to know how abnormal  $Q_m$  is in % of normal, rather than getting a lot of absolute numbers.

Which of the *quantities* ( $Q_m$ ) that are appropriate to use (ratios, “absolute concentrations” or anything in between) depends on the MRS question, but the approach of comparing  $Q_m$  to the relevant normal value is essential and should be used, no matter whether “visual inspection” or a full blown quantitative analysis is the road to “ $Q_m$ ”.

c) Relative to internal reference ( $Q_m$  being a ratio).

The major advantage of the ratio is simplicity. The major disadvantage is that assumptions about the denominator under various conditions will not apply.

i) Metabolite ratios.

The denominator of the metabolite ratio is most frequently the  $A_m$  for total creatine. Total creatine is unaffected by many conditions, but in severely diseased or injured brain total creatine often deviate substantially from the normal values.

ii) Metabolite to internal water ratios.

The denominator could be chosen to be the  $A_m$  for the internal water. Internal water is unaffected by many conditions, but in severely diseased or injured brain as well as in newborns internal water deviate substantially from the normal values.

d) Quantification of metabolite concentrations ( $Q_m$  being an “absolute” number).

Quantification of concentrations takes more effort than calculation of ratios, but the result can be just as precise or even more precise, in terms of coefficients of variance. Often the term institutional unit is used rather than concentration, this is done to highlight that the measured quantity may be prone to systematic errors caused by the methods chosen for the experiment. The systematic error is likely to affect all individuals in a similar fashion, so when comparing patient data to healthy reference data, many effects will cancel out. In the following the term “concentration” will be used, but the reader should be reminded the only with caution and depending on the level of precision needed should this be understood as a true “absolute” concentration.

The concentration  $C_m$  of a metabolite  $m$  may be calculated as  $C_m = A_m f f_{rel} f_{comp} f_{scale}$ , where  $f_{rel}$ ,  $f_{comp}$  and  $f_{scale}$  are correction - and scaling factors that can be applied independent of which type of primary analysis that led to  $A_m$ . They can therefore be added to the analysis independently of whether  $A_m$  was found by cutting paper and weighing it to find peak areas numbers, whether peak areas were found using computerized peak fitting, or whether  $A_m$  was found using LCMoel, MRUI or any other of the newer MRS analysis tools.

i) The factor “f” is a scaling factor that depend in part on the primary analysis, it incorporates

(1) Signal loss due to coupling, some models as for example LCMoel automatically accounts for this

(2) Differing numbers of protons for the resonance in vivo compared to that of the chemical in the concentration standards, some models as for example LCMoel automatically accounts for this

(3) The concentration of the chemical in the concentration standard, and its corresponding “A”, some models as for example LCMoel automatically accounts for this.

- (4) Relative VOI sizes, some models as for example LCModel automatically accounts for VOI size.
  - (5) Absolute temperatures in Kelvin in vivo and in the concentration standard.
  - (6) Dry weight vs. wet weight corrections, if applied.
  - (7) RF field map corrections, if applied.
- ii) The correction factor “ $f_{rel}$ ” takes care of the effects of relaxation and saturation both in the measured individual and in the concentration standard.  
If TE is sufficiently short compared to  $T_2$  (at 1.5 Tesla TE < 20-30 ms will do) and TR is sufficiently long compared to  $T_1$  (at 1.5 Tesla TR > 3000 ms will do, but 5000-6000ms is better), then the effect of even substantially different relaxation times in normals as compared to patients will only have negligible effects on the correction factor  $f_{rel}$ , so it is in practice not worth the effort to measure  $T_1$ s and  $T_2$ s corresponding to each individual spectrum. Reference values of relaxation times suffice.
- iii) The value of  $A_m$  derived from the primary analysis is an average value from a given volume of interest (VOI). The correction factor “ $f_{comp}$ ”, is a compartment correction factor that scales as demanded by the compartment composition of the volume of interest. The most frequently used correction is the cerebrospinal fluid correction in brain MRS. Cerebrospinal fluid contains glucose and lactate, but most other metabolites are mainly present in the tissue. The average tissue concentration is calculated from the average volume concentration by applying the correction factor “ $f_{comp}$ ”. This correction factor is of major importance when substantial atrophy leads to large partial volumes of cerebrospinal fluid in the measured volumes.
- iv) The correction factor “ $f_{scale}$ ”, scales the signal according to the coil loading. The principle of reciprocity or other methods may be used for this. The most direct way to do this relies on a transmit/receive coil, but scaling via another transmit/receive coil (e.g. the body coil) is also feasible.

### 3) Metabolite identification.

The recurring problem of metabolite identification is over enthusiastic assignments. Lactate has for example often been assigned without proper evidence. One must learn systematic rules and apply common sense when assigning metabolites. In the basic analysis such rules include to know the chemical shift, and expected shape of the resonances. One very important example of this is lactate. The doublet must have a 7 Hz splitting, the peaks must point up or down as decided by the pulse sequence, the position of the center of the peak must be 1.33 ppm, and the splitting must be visible if it should be. Examples of errors include confusion with broader signals from lipid or macromolecules as well as confusion with other doublets as those of alanine at 1.48 ppm or propylene glycol at 1.14 ppm. See the table below for further reference.

The use of computerized fitting is helpful, but cannot be used in isolation. The correct estimation of the values  $A_m$  in the primary analysis, in fact relies totally on correct assignments of all major resonances in the spectrum. An example of this is that if a short TE spectrum contains a large mannitol resonance and is analyzed with LCModel without mannitol in the basis set, then the estimates of the values  $A_m$  will be incorrect, this especially counts for glutamine and glutamate.

### Summary

Spectroscopy Modeling and analysis is a mix of metabolite identification and quantification. In the present abstract it has been described in terms of a primary and a secondary analysis. The primary analysis lead to a value  $A_m$  for each metabolite. The primary analysis could be more or less sophisticated and the values  $A_m$  could be peak heights (with extreme caution), peak areas, fitted areas or output from models as MRUI or LCModel. The primary analysis is then followed by a secondary analysis that calculates the quantity  $Q_m$  from the value  $A_m$ . The quantity  $Q_m$  could be a metabolite ratio, institutional unit, or a metabolite concentration depending on what level of sophistication is needed to answer a given question.

<b>Metabolite</b>	<b>Chemical shift / ppm</b>  (only the main resonances of the metabolites as they appear @ 1.5 T are listed, complex multiplet may change appearance as function of B <sub>0</sub> )	<b>Resonance type</b>  (singlet, doublet, triplet, complex multiplet, ...)	<b>Abbreviation</b>  (other may be found in the literature)
<b>N-acetylaspartate</b>	<b>2.02</b>	<b>singlet ... complex multiplet ...</b>	<b>NAA</b>
<b>Glutamine+Glutamate</b>	<b>2.05-2.5</b>	<b>complex multiplet</b>	<b>Glx</b>
<b>Total Creatine</b>	<b>3.03 3.9</b>	<b>singlet singlet</b>	<b>Cr</b>
<b>Total Choline</b>	<b>3.22</b>	<b>singlet</b>	<b>Cho</b>
<b>Myo-Inositol</b>	<b>3.56</b>	<b>complex multiplet complex multiplet</b>	<b>mI</b>
<b>Scyllo-Inositol</b>	<b>3.36</b>	<b>singlet</b>	<b>sI</b>
<b>Glucose</b>	<b>3.43 3.8</b>	<b>complex multiplet complex multiplet</b>	<b>Glc</b>
<b>Water</b>	<b>4.7 temperature dependent</b>	<b>singlet</b>	<b>H<sub>2</sub>O</b>
<b>Lactate</b>	<b>1.33 4.11</b>	<b>doublet quartet</b>	<b>Lac</b>
<b>Succinate</b>	<b>2.4</b>	<b>singlet</b>	<b>-</b>
<b>Acetate</b>	<b>1.9</b>	<b>singlet</b>	<b>-</b>
<b>Alanine</b>	<b>1.48</b>	<b>doublet</b>	<b>-</b>
<b>Propylene glycol</b> Part of the vehicle for drugs like phenobarbiturate or pentobarbiturate	<b>1.14</b>	<b>doublet</b>	<b>-</b>
<b>Ethanol</b>	<b>1.16</b>	<b>triplet</b>	<b>-</b>
<b>Mannitol</b> a drug that acts as a vasodilator, it is used mainly to reduce pressure in the cranium	<b>3.78</b>	<b>complex multiplet</b>	<b>-</b>
<b>Methylsulfonylmethane</b> a dietary supplement	<b>3.15</b>	<b>singlet</b>	<b>MSM</b>