# A comparative study of frequency and time domain data analysis of HR-MAS $^1$H NMR data from *Apc*$^{Min/+}$ mouse gut tumours

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**INTRODUCTION:** Most HR-MAS tumour studies have used pattern recognition methods like Principal Component Analysis (PCA), Partial Least Square – Discriminant Analysis (PLS-DA) or neural networks to analyse the raw spectra, thus avoiding the problems of quantifying absolute metabolite concentrations (1). Although such strategies allow clustering and classification of the data, knowledge of the absolute concentrations of the metabolites is needed to make full use of HR-MAS $^1$H NMR spectroscopy for improving our understanding of tumour biology. In this study we compared time domain (ER-QUEST) and frequency domain (LCModel) methods in the estimation of the absolute concentration of metabolites from *Apc*$^{Min/+}$ mouse gut tumour tissue.

**METHODS: Animals and Sample collection**: *Apc*$^{Min/+}$ mice (2) were bred and maintained by backcrossing with a colony of C57BL/6J mice (Cancer Research UK Cambridge Research Institute, Biological Resources Unit, Cambridge, UK). HRMAS 1H NMR spectroscopy was performed on a Bruker 600MHz, with 4mm HRMAS probe. A CPMG pulse sequence with water pre-saturation and three echo times (50 , 100 and 200 ms) were used to obtain the $^1$H NMR data. The water signal observed in each individual experiment was used for estimation of the absolute metabolite concentrations (5).

**LCModel:** A modified LCModel basis set was used (4). Since these were not brain tumours, NAA & NAAG were omitted from the analysis. The phosphocreatine (PCr) signal was also simulated, along with a triplet at 2.64ppm (named "at264") and a singlet (named "at326") at 3.26ppm. **ER-QUEST:** Metabolite basis sets were simulated from the NMR-SCOPE programme available in the JMRUI 3.0 software package. In addition to the metabolites, which were fitted in a similar way to LCModel, we also simulated lipid signals at 0.9ppm, 1.30 ppm and 2.2ppm. An ER filter was used from 0.5 to 4 ppm in the spectra so as to compare these results to LCModel estimations. Similar to the data analysis of Rabeson et al (5), we also used background baseline subtraction in the ER-QUEST method. Fractional uncertainty a measure of precision in the data, was calculated by dividing the S.E.M. by the corresponding average value.

**RESULTS:** Metabolite concentrations estimated by the LCModel and ER-QUEST methods from $^1$H NMR data are presented in Table 1. Figure 1 shows the plot of fractional uncertainty in metabolite concentrations estimated by the two methods.

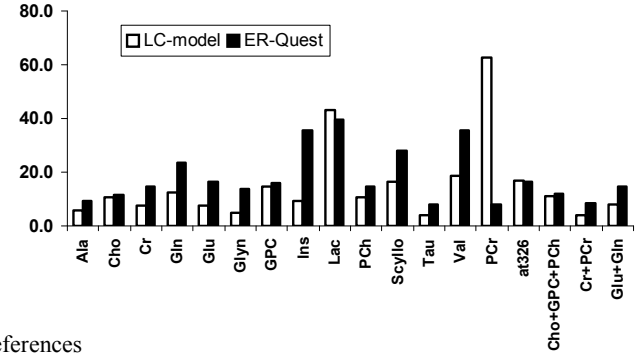
**DISCUSSION:** The metabolite concentrations estimated by the LCModel and ER-QUEST methods show significant differences (Table 1). For instance, the estimates of lactate were found to be lower by LCModel than by ER-QUEST (Table 1), although the fractional uncertainties of lactate were almost equal (Figure 1). On the other hand, the estimates of total choline-containing compounds (choline + PC + GPC) were significantly higher with LCModel than with ER-QUEST (Table 1). Fractional uncertainties of total choline were found to be equal (Figure 1) in the LCModel and ER-QUEST estimates. The fractional uncertainty of PCr was higher in the LCModel results, but the fractional uncertainty of total Creatine (Cr+PCr) was lower than with ER-QUEST. In most other cases (Figure 1) LCModel fitting resulted in either equal or smaller fractional uncertainties in comparison to ER-QUEST. In general, we found it easier to use the LCModel method as it was more user-friendly and robust. In contrast, the ER-QUEST method requires the movement of base sets by the user to match the observed peaks in the spectrum. We observed that the fittings will be completely inaccurate if the base sets of the ER-QUEST method are slightly misaligned with the observed peaks in the spectrum. Also lipid profiles and macromolecule profiles have not yet been designed into the programme. There were no significant differences in computational times between the LCModel and ER-QUEST methods.

**Conclusions:** We found that for the estimation of metabolite concentrations from HR-MAS $^1$H NMR spectral data, LCModel fitting resulted in either equal or smaller fractional uncertainties in comparison to fitting by the ER-QUEST method.

| | LCModel | | | ER-QUEST | | | |
|---|---|---|---|---|---|---|---|
| | mean | | s.e.m | mean | | s.e.m | p-value |
| Alanine | 0.81 | ± | 0.05 | 0.61 | ± | 0.06 | 0.0051 |
| Choline | 0.37 | ± | 0.04 | 0.14 | ± | 0.02 | 0.000013 |
| Creatine | 0.61 | ± | 0.04 | 0.09 | ± | 0.01 | 0.000001 |
| Gln | 0.32 | ± | 0.04 | 0.28 | ± | 0.06 | 0.5755 |
| Glu | 1.07 | ± | 0.08 | 2.50 | ± | 0.41 | 0.0055 |
| Glyn | 1.13 | ± | 0.06 | 1.97 | ± | 0.28 | 0.0051 |
| GPC | 1.72 | ± | 0.25 | 0.32 | ± | 0.05 | 0.0001 |
| Ins | 0.13 | ± | 0.01 | 0.30 | ± | 0.11 | 0.1444 |
| Lac | 3.32 | ± | 1.43 | 8.85 | ± | 3.50 | 0.0302 |
| PCh | 0.51 | ± | 0.05 | 0.16 | ± | 0.02 | 0.000013 |
| Scyllo | 0.09 | ± | 0.02 | 0.50 | ± | 0.14 | 0.0132 |
| Tau | 2.05 | ± | 0.09 | 1.90 | ± | 0.15 | 0.2523 |
| Val | 0.11 | ± | 0.02 | 1.92 | ± | 0.68 | 0.0263 |
| PCr | 0.46 | ± | 0.29 | 0.74 | ± | 0.06 | 0.2942 |
| at326 | 0.21 | ± | 0.04 | 5.02 | ± | 0.82 | 0.0002 |
| Cho+GPC+PCh | 2.58 | ± | 0.28 | 0.62 | ± | 0.07 | 0.000007 |
| Cr+PCr | 0.77 | ± | 0.03 | 0.83 | ± | 0.07 | 0.2036 |
| Glu+Gln | 1.38 | ± | 0.11 | 2.78 | ± | 0.41 | 0.0041 |

Table 1: Metabolite concentrations (in mmoles/L tissue water) estimated by the LCModel and ER-QUEST methods for Apc$^{/min}$ mouse gut tumours.

Figure 1 Plot of fractional uncertainty estimated by the LC-model and ER-QUEST methods

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