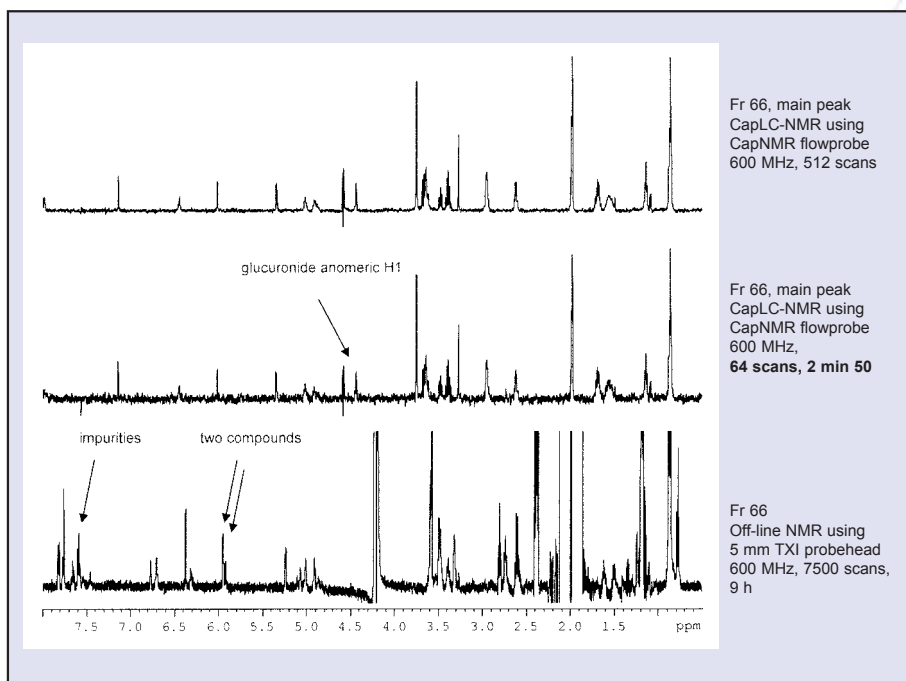


## Metabolite Analysis: Hepatocyte Incubations

Hepatocyte incubations are gaining more and more importance in the early stages of metabolite investigations. Due to small incubation sizes though, the absolute amount of metabolite is limited and such samples suggest themselves for CapLC-NMR analysis using the Protasis/MRM CapNMR™ FlowProbe.

The figure shows spectra of a fraction that was obtained from a preparative separation of a rat hepatocyte incubation of GW433737 (50 mM). This type of sample usually contains only small amounts of metabolites and thus requires long acquisition times using standard NMR procedures (lower spectrum). The two upper spectra show the results of the capillary LC-NMR experiments: a significant increase in sensitivity (less than 3 minutes acquisition time compared to 9 hours before) and the absence of endogenous impurities, as well as the separation from a further co-eluting metabolite, due to the additional separation step.



Data courtesy of Dr. Martin Sandvoss,  
GlaxoSmithKline, Ware, Herts., UK.  
Bruker DRX Spectrometer and  
Waters® CapLC system  
0.3 mm x 100 mm, 3 mm Targa C-18 CapLC column  
[A] 0.1 % formic acid-d3 in D<sub>2</sub>O  
[B] acetonitrile - d3  
1-90 % [B] gradient in 30 mins.

Capillary LC-NMR shows excellent sensitivity that is about an order of magnitude higher than conventional NMR set-ups. The Protasis/MRM CapNMR flowprobe opens up fields of research for direct LC-NMR analysis that have not been accessible so far.

Apart from the gain in sensitivity, the main advantage of CapLC-NMR over LC-NMR is the use of fully deuterated solvents which results in higher spectral quality without the need to set up suppression sequences and without the loss of important parts of the NMR spectrum.

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