LC-NMR – Who in Their Right Mind Would Even Consider it?

"LC-NMR is a powerful technique to obtain detailed structural information on organic compounds in complex mixtures and/or at low level. The data obtained are complementary to LC/MS data."

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Bernie Monaghan (BM): Could you tell us a little about the group in which you work in Sunderland?

Nicolas Haroune (NH): Chemispec was established in 2000 when the University obtained a grant from the ERDF (European Research Development Fund) to purchase analytical equipment with the aim of helping small and medium enterprises in the North East of England. 2 NMR spectrometers (500 and 300MHz), 1 mass spectrometer (Apex II FT/MS) were purchased. More particularly, the 500MHz spectrometer was equipped with a flow probe for LC-NMR applications. We have now been offering LC-NMR analysis services nationwide for nearly 10 years to industries in the pharmaceutical and chemical sector. We have now been offering LC-NMR analysis services nationwide for nearly 10 years to industries in the pharmaceutical and chemical sector.

BM: Which manufacturers Instruments do you use currently? Are there any technological reasons for making these choices?

NH: These instruments were purchased from Bruker Ltd, a market leading manufacturer in this area.

BM: Can you give the readers some background into the interest and need for such a technique? What are the practical operating issues that have to be overcome to obtain high quality data?

NH: Our LC-NMR equipment allows the detection of only microgrammes quantities of unknowns. It also allows:

- The detection of all protons containing analytes
- It is a non invasive technique so it is possible to recover the sample after analysis
- It provides detailed structural information which are complementary to mass spectrometry analyses
- It represents a time saving since there is no need to collect fraction and as such the sample preparation minimised

These advantages make the LC-NMR suitable for the analysis of components in complex mixture at low concentrations, where difficult to purify or to unstable for isolation by Silica gel chromatography or preparative HPLC.

A schematic view of the LC-NMR system is shown in *Figure 1*.

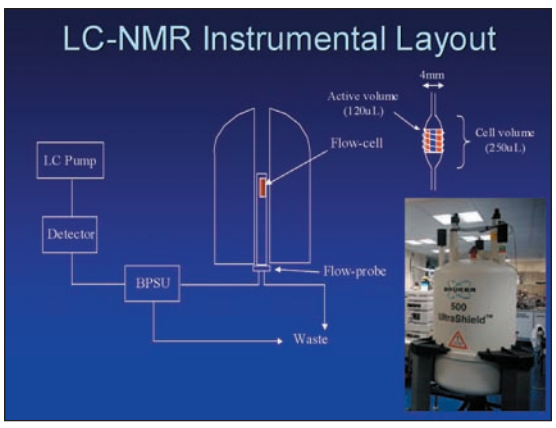

Figure 1. Schematic of Instrument layout

LC-NMR separation is usually carried out with a mixture of deuterated water and protonated organic solvent. It is fine to use these when the 5 to 10ppm region is of interest but since these solvents give rise to strong signals in the 1 to 5 ppm region, they can potentially overlap signals of interest.

This problem can be overcome by using fully deuterated organic solvents but these are expensive ~£650/500mL.

However, the enhancement of the quality data is spectacular as minimum background signals are observed; also these solvents are free from any impurities which can be found in organic solvents and again, can crowd the spectra with unwanted signals.

Finally, the peak of interest in the LC chromatogram should be well resolved and separated from major components, otherwise, severe cross contamination may occur in the NMR flow cell. To a certain extent, this can be overcome by storing a 'slice' of the LC peak in one of the loops of the BPSU (Bruker Peak Sampling Unit), and then eluting it towards the NMR at the end of the chromatography separation. However, this may not be suitable for unstable components, in which case the flow of the LC may be diverted to the waste using the switching valves of the BPSU, except when the compound of interest comes off the column.

BM: What advantages does NMR hold in identifying the active ingredients? Do you have examples where other detection methods alone were insufficient for your needs?

NH: NMR allows the scientist to obtain very detailed structural informations on the environment of the different protons in a given molecule, which are not easily obtainable by other techniques such as mass spectrometry (MS). For example, oxidation of an active ingredient is routinely identified by MS during stability trials of formulations. However, determining the exact position of this hydroxylation in the molecule is not always straightforward by MS/MS, especially when this takes place on aromatic rings. A number of these issues have been tackled by the use of LC-NMR.

BM: Are there other detection methods that you would like to include in your instrumentation? ELSD for example?

NH: Evaporative light-scattering detection (ELSD) is regarded as a valuable alternative to UV detection for liquid chromatographic analysis of substances that do not contain a chromophore. A key feature of ELSD is that - unlike refractometry - it can operate in gradient mode, thus allowing application of more selective liquid chromatographic methods. However, LC-NMR analysis requires the sample to be liquid when reaching the flow probe so, unless the flow is split prior the NMR probe, the destructive nature of light scattering detection prior NMR detection would be inappropriate.

BM: Do you have examples separations run by the system that the readers would find impressive.

NH: We have several excellent examples but unfortunately they are subject to confidentiality agreements but we hope to print and share these results some time soon.

Meantime Dr Karine Ndjoko Ioset from the Laboratoire of Pharmacognoxy and Phytochemistry from the University of Geneva, Switzerland has published work and presented an overview of the technique of LC-NMR at the spring symposium of The Chromatographic Society [1] held in Sunderland, UK in May 2009. Here she concentrated on extracts from natural products and the use of hybrid NMR techniques to identify previously unidentified (and often the most physiologically active) components of the extract.

The advantages of using are that the full structural and stereochemical information can be obtained (by the use of 2D NMR) but also it is a highly non-selective detection technique. Several examples were shown where assisted in the identification of unknown compounds such as those shown in *Figure 2*.

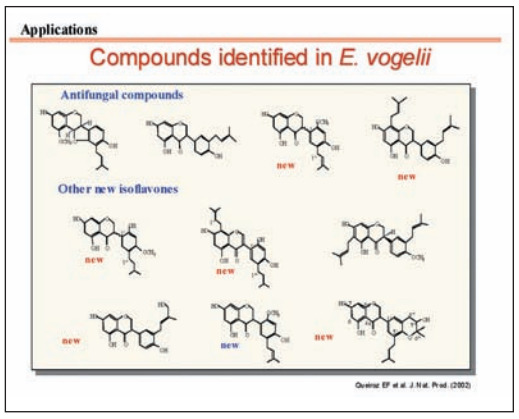

Figure 2. Compounds previously unknown, identified by LC-NMR

Furthermore, the complexity of the structure of extracts from H. mantegazzianum are shown in *Figure 3* illustrates the ability of to identify these components.

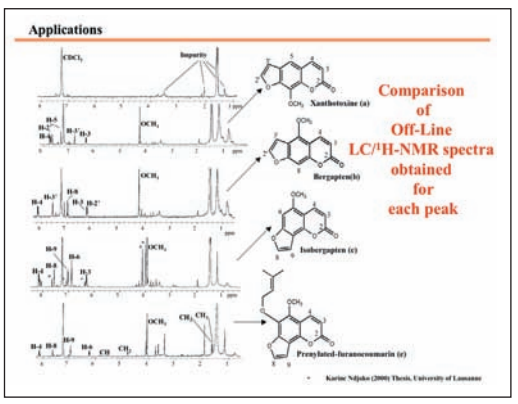

Figure 3. Extracts from H. mantegazzianum

BM: How do you see the spectroscopic methods of detection advancing in, say, the next 3-5 years? What room for improvement is there still to be made in the hyphenated sequence you have used? What is holding advancement back?

NH: Magnets are now available up to 1GHz. Combined with cryoprobe, these provide exceptional sensitivity and resolution. The construction of bigger magnets will require new material to be found because the higher the field at which a magnet operates, the more complicated the stress and energy management techniques that must be used to ensure its physical integrity and the safety of those who work with it. All aspects of magnet design are affected, including the choice of materials for electrical conductors and structural components, the electrical insulation systems employed, and so on. In the case of superconducting magnets, field stability and quench protection pose additional issues.

BM: Are there any alternative techiques for the analysis of components in mixtures?

NH: DOSY can appear as a very useful alternative technique to LC/NMR, in that it allow a mixture of components to be separated according to their diffusion rate in the NMR tube, without the need of additional expensive chromatography equipment, solvents and LC methods to be developed. In order to be resolved, the diffusion rates must differ by 2% for fully resolved resonances, but 30% for overlapped signals. The quality of the data is very much dependent on the acquisition conditions but also the software used to process the data and is not a trivial task from our point of view.

Furthermore, obtaining good quality data from DOSY experiments appears to be a challenging problem when:

- Signals of a degradation product at a level of 0.5% are likely to be overlapped by those of the parent compound which is present in much larger quantities.
- the degradation product is not much different from the parent compound in terms of molecular radius.
- Conformers having the same diffusion rate have to be separated and characterised.

BM: Is there any way to enhance the sensitivity for the detection of very low level of analytes?

Dr Ndjoko spoke about other hyphenation techniques which will allow improved sensitivity such as SPE-NMR and CAP-NMR by allowing the analytes to be concentrated in a minimum volume of solvent [2]. Highly impressive example of the analysis of isomers of Tropane alkaloids are shown in *Figures 4 and 5*. Techniques such as these are becoming more widely used and offer great potential for the future.

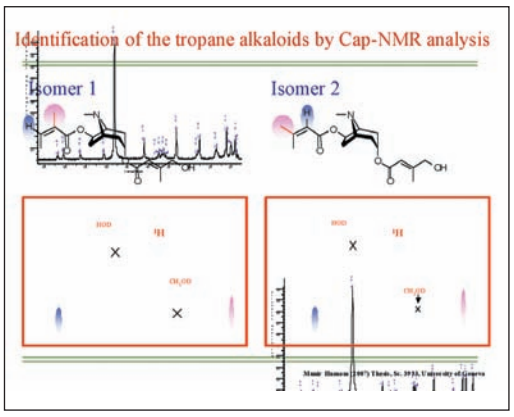

Figure 4. Example of CAP-NMR analysis

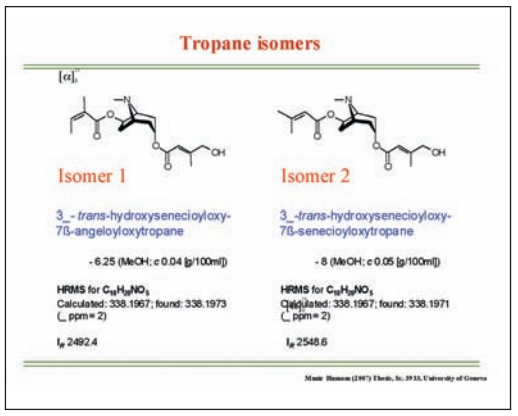

Figure 5. Example of isomeric identification enabled by CAP-NMR

ABOUT THE AUTHOR

Following completion of his PhD at the University of Clermont-Ferrand Nicolas moved to a postdoctoral position at Glasgow University where he studied the modification of proteins during oxidative stress.

As an integral part of his research projects, he has extensively used NMR, HPLC and Mass Spectrometry to identify the structure of various biomolecules, ranging from chemicals, metabolites to peptides and proteins.

Nicolas has been working in chemiSPEC for 4 years. Beside his Laboratory Management role, he focuses on implementing new NMR and MS methods so as to help clients solve their scientific and technical problems.

REFERENCES

1. www.chromsoc.com/features

2. Jaroszewski JW (2005) Hypehenated NMR methods in natural products research, Part 1: Direct Hyphenation, Planta Med 71(8), 691-700

ACKNOWLEDGEMENTS

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Swap'n Go

Joint Analytical Systems GmbH has introduced a new product - a user-friendly GC/MS interface which enables a 100% tool-free column exchange without MS venting. A narrow, special deactivated transfer tube is used as a connection between the analytical column and the MS source. Swap'n Go is an easy-to-use, self-installable robust device, which enables the operator to exchange capillary columns fast and simple - completely tool-free and without MS venting. Unscheduled disruption in analytical work due to column exchange is avoided.

The analytical column (200, 250 and 320 µm ID) is fixed only hand-tight to the Swap'n Go adapter by a special designed JAS-Tight nut sealed with a JAS-Tight ferrule. The column is also connected only hand-tight to the S/SL injector by means of a special JAS injector adapter. Instrument-specific systems are available for Agilent and Thermo instruments.

Benefits of using JAS Swap'n Go GC/MS-Interface System include: keeps MS-vacuum intact during column exchange and allows restart after 3-4 minutes; time-consuming venting of the MS is not required; enables fast, simple and 100% hand-tight exchange of analytical columns; avoids the use of additional He-purge/make-up gas flow, which might cause sample dilution; allows easy user-operated (also Retro-fit) installation for Agilent GC/MSD and Thermo

GC/MS systems; and avoids time-consuming venting of the MS and improves laboratory productivity.

JAS Swap'n Go GC/MS interface is 100% hand-tight tool and therefore unique in the market. This powerful device is delivered in a box including required ferrules and installation instruction (installation video and manuals in English and German).

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