# LCMS: From Pharmacokinetics to Metabolomics

> ***"My team is involved in drug metabolism and pharmacokinetics of novel chemical entities and in the clinical pharmacokinetic evaluation of some of our agents, which have reached Phase I/II clinical trials at The Royal Marsden Hospital..."***

**Author Details:**

Dr. Florence Raynaud,
The Institute for Cancer Research,
Sutton UK
Group Leader Pharmacokinetics and Metabolomics
Cancer Research UK Centre for Cancer Therapeutics
The Institute of Cancer Research
Sutton, Surrey, UK
Email: florence.raynaud@icr.ac.uk

**Bernie Monaghan (BM): Could you tell us a little about the unit and group in which you work at Sutton?**

**Florence Raynaud (FR)**: I work at The Institute of Cancer Research (ICR) in the Section of Cancer Therapeutics. Our mission is to discover and develop new drugs to treat cancer. We have an impressive track record in drug discovery in oncology as four compounds (melphalan, chlorambucil, carboplatin and ralitrexed) originating from the ICR have gained regulatory approval. In addition, we have several compounds in late clinical development (abiraterone, satraplatin and picoplatin). Six of our compounds are in early phase I/II clinical evaluations. They include conventional antiproliferative agents that are selectively transported to tumours and molecularly targeted agents such as HSP90, HDAC and PI3K inhibitors. These compounds have been licensed to pharmaceutical companies such as Novartis, Roche, Genentech and Onyx pharmaceuticals and some of them have been developed in partnership with biotechnology companies. We have an exciting pipeline which includes PKB inhibitors already licensed to Astra Zeneca, while B-RAF inhibitors, CHK1 and Aurora kinase inhibitors are all at licensing stage.

*Chelsea Site, Central London*

*Sutton Site, Surrey*

*Research Sections: Basic, Translational and Clinical*

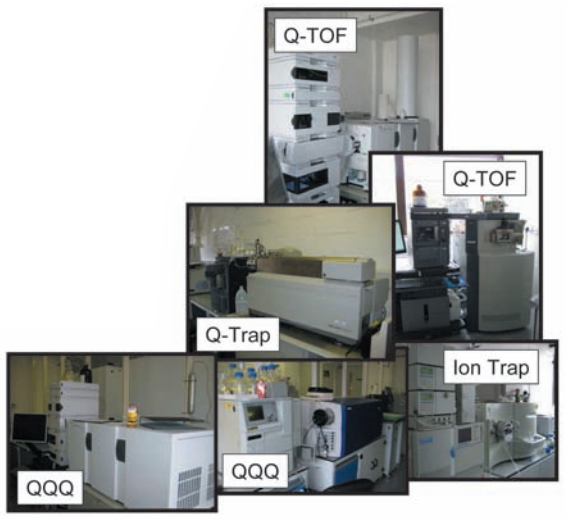
*Biochemistry, Breakthrough Breast Cancer Centre, Cancer Genetics, Cancer Therapeutics, Cell and Molecular Biology, Clinical Research, Clinical Magnetic Resonance Research, Gene Function and Regulation, Epidemiology, Haemato-oncology, Medicine, Molecular Carcinogenesis, Paediatric Oncology, Physics, Radiotherapy, Structural Biology*

We are at a really exciting crossroad in oncology drug development as many compounds are targeting molecular abnormalities that drive cancer. This means that drug administration can be optimised by evaluating the target modulation rather than the maximum tolerability. In addition, it may be possible to select the patients that are most likely to respond to treatment, which leads to personalised therapy. This requires a large investment in biomarker development and analysis.

My team is involved in drug metabolism and pharmacokinetics of novel chemical entities and in the clinical pharmacokinetic evaluation of some of our agents, which have reached Phase I/II clinical trials at The Royal Marsden Hospital. In addition, we are interested in the identification of novel biomarkers by LCMS metabolomics. We are focusing on the exploration of the exo-metabolome to circumvent difficulties in obtaining tumour specimens. Tumours are often not accessible, serial biopsies are invasive and, at best, only pre-treatment samples and post-treatment biopsies are obtainable. Circulating biomarkers are, therefore, highly desirable. We have so far initiated preclinical studies to evaluate the potential of metabolomics in identifying metabolomic biomarkers of signal transduction inhibitors. This is essential if you are developing a targeted agent as you have to prove, in the clinical context, that the compound modulated its target. We focus particularly on the inhibitors that we have developed in the Cancer Research UK Centre for Cancer Therapeutics at the ICR and which we have in clinical trials at The Royal Marsden hospital.

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

**FR**: We have seven liquid chromatography mass spectrometry instruments in the laboratory, from a range of manufacturers (Agilent Technologies, AB-Sciex, Thermo-Scientific and Waters Corporation). We have four triple quadrupoles (including one hybrid triple quadrupole linear ion trap), one ion trap and two quadrupole time of flight.




*Pharmacokinetic and Metabolomic Laboratory*

Our major source of research funding is through a Cancer Research UK program grant to the Centre for Cancer Therapeutics. This grant is peer reviewed every five years and has provision for some capital equipment purchase and associated maintenance costs. We operate in a cost contained environment and have a duty to deliver value for money from all our equipment. Our choice of instrumentation is therefore crucial to our work and to assist us in the delivery of future clinical candidates. Our instruments are extensively tested for all our applications (quantitative and qualitative) during demonstrations by manufacturers and evaluated for affordability. We keep our instruments running until they are no longer functional or no longer supported; we are therefore looking for robustness, in addition to low maintenance costs. As an example, we recently donated a 17-year-old instrument to a university for student training.

**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?**

**FR**: Liquid Chromatography-Mass spectrometry is a complete tool in drug discovery and development. Our routine in vitro assays of compound membrane permeability rely on generic separation methods and automated method optimisations. Pharmacokinetic analyses require better separation and better optimised and generally more sensitive methods. All these assays are performed on triple quadrupole instruments. In the past, the triple quadrupoles have been used to study compound percentage metabolised and microsomal clearance, followed by metabolite identification on ion traps. Recently, however, our quadrupole-time of flight instruments have been utilised to perform both of these activities. Technological developments allow simultaneous collection of full scan and MSMS spectra, providing quantitation of the parent and potential information on the metabolites formed within a single analytical run. This feature saves analytical time as the data is collected for metabolite identification should it be required. Metabolomic work relies on highly reproducible chromatography and high mass accuracy on the quadrupole time of flight instruments, in order to compare different experimental conditions. The problem is much more complex when comparing groups of samples containing thousands of metabolites to assess which metabolites from this complex mix vary under different conditions. This is not trivial and relies on sophisticated statistical analysis. Once the ions of interest are identified, metabolite identification needs to be performed. The fragmentation pattern and mass accuracy are of great help in the metabolite deconvolution and the available databases are a great support.

**BM: Are there other detection methods that you would like to include in your biomarker assay and metabolite identification work?**

**FR**: NMR has not, to date, been utilised in our in house metabolism work but we have collaborated with other groups. Similarly, we have collaborated with our biomarker NMR group here at the ICR for metabolomic analysis. GC-MS is able to detect metabolites that cannot be detected by LCMS and this is attractive, however the primary need for DMPK support has driven us toward LCMS technologies which are applicable to both business needs. There are new generation of LCMS instruments offering improved ionisation technologies and separation, which will no doubt have a significant impact in the field of metabolomics.

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

**FR**: We have recently evaluated the impact of the 1290 LC system from Agilent technology when compared with the 1200 and shown that we can perform analytical runs at much higher pressure and increase the number of features detected in a metabolomic plasma extract by 40 per cent using an identical gradient. Under the analytical conditions that we are using, over half of the features measured by the mass spectrometer in full scan analysis in ESI are detected with high reproducibility (>25%).

This is quite impressive given the number of features monitored (>1000) and their chemical diversity. In addition, we have shown that using the Jet Stream technology, we can also increase the number of features detectable without compromising their integrity.

**BM: How do you see the Chromatographic/ 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?**

**FR**: The technological advances in the last decade have been phenomenal. Instrumentation is progressing all the time in sensitivity, mass accuracy dynamic ranges and separation technology.

There is no reason why these technological advances should halt, but the capabilities of data analysis are limiting. Processing data is time consuming; increasing sensitivities results in an increase in the number of ions collected, which increases file sizes in metabolic and metabolomic assays. We have to ensure that available bioinformatics tools are capable of analysing data efficiently and fully, producing useable end results.

The software has to deconvolute singly charged/multiply charged ions, handle saturation issues, normalise for drift and still be able to detect differences from one group to the other. The tools are advancing but there is still a wide scope for improvement.

**BM: There appears to be room for lots more research into improving the sensitivity and other aspects of the assay. What are you currently working on?**

**FR**: We are continually attempting to improve sensitivity, specificity, reproducibility and efficiency. Improving sensitivity allows the use of reduced sample volumes (for example dried blood spots). Better separation is advantageous as it increases assay specificity, which may decrease interference from other analytes. If there is no issue with interference, then better separation can increase the speed of the analysis. In some of our clinical studies our results will impact clinical decisions, we therefore have to completely eliminate the risk of interference. Reproducibility is essential in analytical work whether you are measuring discrete analytes in clinical trials or attempting to highlight differences between experimental conditions in metabolomic studies. At some point in the assay development and optimisation, a decision has to be made on a method, which is often a compromise between various parameters (cost, specificity. sensitivity, reproducibility, time etc.). Ultimately, the assay has to be fit for purpose and we are attempting to do this as efficiently and cheaply as possible.

**About the author**

Florence Raynaud graduated as a Pharmacist from the University of Paris 5. She has an MSc degree in pharmacochemistry from the same university. She has a PhD from the University of Strasbourg where she had her first post-doctoral position. She has been at The Institute of Cancer Research for the last 18 years and is the author of over 100 publications.

**Acknowledgements**

None of the work is possible without the help of my team and co-workers, many of which are shown here.

## New Light-Measurement System Ideal for Analysis of LEDs and Lamps

Now available from **Ocean Optics** is a light-measurement system for spectroradiometric analysis of LEDs, lamps, flat panel displays and other radiant sources, as well as solar radiation. With its small footprint, powerful microprocessor and low-power display, the new Jaz-ULM-200 is a convenient, versatile alternative to standard light meters and radiometers.

Jaz is family of stackable components that share common electronics and communications and are configurable for a variety of applications. Included in the Jaz-ULM-200 stack is a CCD-array spectrometer that can be optimised for a variety of radiometric measurements and a microprocessor with onboard display. Jaz provides functionality not found in traditional light meters, allowing users to capture, process and store full spectra without the need for a PC.

With only three pushes of a button, the system's irradiance-measurement software, which is stored on an SD card, collects full spectral irradiance information from the selected light source. This data can then be post-processed to give the intensity parameter of choice, including μW/cm$^2$, lumens, lux, PAR (photosynthetically active radiation) or any other light intensity parameter. The system's three-button wizard simplifies operation so that even non-spectroscopy experts are able to perform fast and accurate measurements.

The Jaz-ULM-200 includes much more than a spectrometer and microprocessor. Its Ethernet module has data storage capability via an SD card slot and allows users to connect to the Jaz unit via the Internet.

The Internet capability makes remote measurements such as solar irradiance possible and enables the creation of networked sensing modules. Also included are a rechargeable lithium-ion battery module (with SD card slot) for portability and a special mounting fixture for orienting the Jaz stack horizontally to facilitate hands-free operation.

Additional system components include a direct-attach cosine corrector for collecting radiation within a 180-degree Field of View, a carrying case with shoulder strap and a rugged Pelican case for storing all your gear. Software includes the Jaz system software and JAZ-A-IRRAD, an irradiance-measurement application that comes on an SD card.