MASS SPECTROSCOPY IN THE CRO WORLD

The rapid growth of the market for the outsourcing of the drug development pipeline to Contract Research Organisations (CRO) has been phenomenal since the turn of the century. Today it is estimated to be just below the $20 billion per annum mark and estimated to grow to $23.7bn by 2010[1]. Integral to the growth of this market place is the skill of Scientists such as the Principal Investigators (PI) who lead and are legally responsible for the conduct of the studies. They develop assays but also have to be extremely competent with high end Analytical Instrumentation such as LC-MS/MS, which is very much the workhorse of many CRO Laboratories. Bernie Monaghan Editor for the Separations Science and Spectroscopy sections of International Labmate, visited BioAnalytical Systems (BASi) based in Warwickshire, UK to speak with James Hillis and Mark Wareing the Operations and Managing Directors respectively of this medium sized CRO to discuss the current issues of workload, customer expectations and identifying and meeting technical challenges for the future.

Bernie Monaghan (BM) *- The CRO market is extremely dynamic with many of the large CRO's acquiring smaller laboratories across the globe and hence specialisations almost on a daily basis. How do you cope with this?*

James Hillis/Mark Wareing (JH/MW) - Having been in the business for over 30 years we realise the importance of partnering closely with our clients. Strong lines of communication are essential (Client to PI) and the ability to offer results from a wide variety of techniques performed by highly competent scientists using industry standard equipment is our forte. The ability to cope with high volumes of customer samples quickly is second nature to us. For example 8,000 samples were assayed using two separate LC-MS/MS methods and six instruments to generate 16,000 results over 365 analytical runs in 45 days. This is an example of the breadth and depth of our technology and scientific talent. This level of customer service does not come overnight.

BM *- What services do you offer to the customer base?*

JH/MW – BASi provides world-class research to the pharmaceutical industry worldwide. Established in 1974, we offer an extensive array of contract laboratory services and also manufacture more than 30 different scientific instruments.

The BASi business model is simple. Our unique role is our focus on developing innovative services and products that increase efficiency and reduce costs associated with taking new drugs to market. Each client has differing requirements and time lines so it is imperative that we offer a range of CRO services (Pharmaceutical Analysis, Bioanalytical Chemistry, Toxicology, Immunoassay, Stability Programmes and Non-GLP Research) that are fully compliant with all current and appropriate legislation (cGMP, GLP & GCP). As we have facilities in both UK and USA it is a seamless operation to move assays between labs in these countries, a benefit to multinational companies.

BM *- Clearly a wide range of services requiring an equally wide range of techniques and equipment. Expertise in specific areas is essential to successful CRO labs. What do you regard as your specialised areas and has this allowed you to develop assays for customers when this is necessary?*

JH/MW - We have excellent high level expertise in most aspects of the bioanalytical process but probably the skill set we do excel at is that of extracting the compound(s) of interest from the supplied matrix be it blood, plasma, tissue or urine. We have developed many procedures involving miniaturised and automated liquid/liquid extraction. We can however, use any other extraction technique. It is essential that in order to comply with customer requests for rapid turn around, specificity and ruggedness a high level of automation is involved and familiarity with robotics systems are essential.

Regarding assays developed over the years for customers we have many hundreds that can publicly be discussed although there are many more that are client confidential.

In the last 12 months alone over 80 methods were developed with over 50% being for proprietary drug candidates.

A more complete listing of those in the former category may be found at www.bioanalytical.com/services/assays

A typical assay that has been developed and used successfully is outlined below.

LCMS/MS Method for Quantitative Determination of Sildenafil and Desmethyl Sildenafil in Human Plasma

Sildenafil is used in the treatment of erectile dysfunction, acting as a potent and selective inhibitor of cGMP-specific PDE5. It is primarily metabolised by P450 3A4 to N-desmethyl sildenafil, an active metabolite with 50% of the parent's drug potency.

BASi has developed a LC-MS/MS method capable of rapid determination of both sildenafil and its metabolite down to 1 ng/mL in less than 0.5 mL of plasma. Chromatograms at the LLOQ (1.0 ng/mL) and 300 ng/mL (ULOQ for sildenafil) are shown in *Figure 1*.

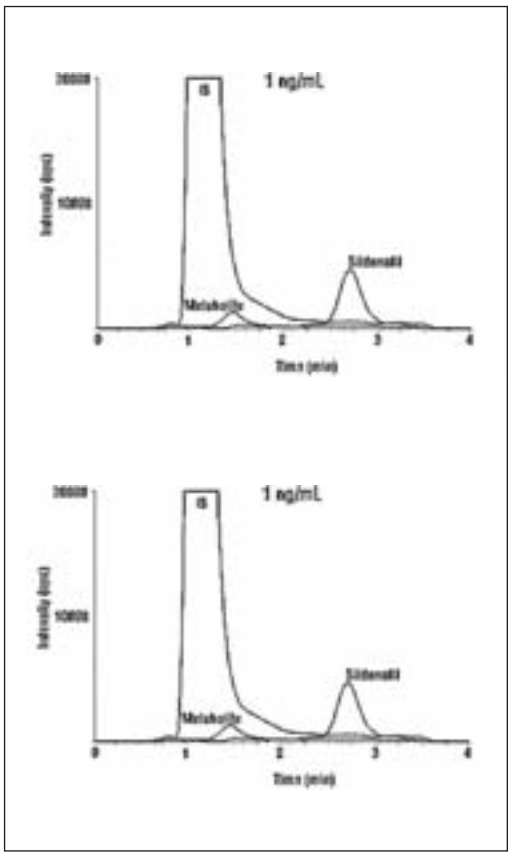

Figure 1. Chromatograms at the LLOQ (1.0 ng/mL) and 300 ng/mL (ULOQ for sildenafil).

This method involves liquid-liquid extraction and a post-column switching valve with a 6.5 minute chromatographic run time. It is robust with standard calibrator and quality control accuracies and precisions well within the required limits. The between-run accuracy and precision data for sildenafil and desmethyl sildenafil are shown in *Table 1* and *Table 2*, respectively.

Spectroscopy Focus

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Table 1. Between-run precision and accuracy for sildenafil.

Concentration	n	Mean	% CV	% Bias
1 ng/mL	18	0.99	11.2	-1
3 ng/mL	18	2.79	5.9	-7
30 ng/mL	18	2.94	4.4	-2
500 ng/mL	18	505	3.4	1

Table 2. Between-run precision and accuracy for desmethyl sildenafil.

Concentration	n	Mean	% CV	% Bias
1 ng/mL	18	0.98	9.1	-2
3 ng/mL	18	2.79	4.8	-7
30 ng/mL	18	26.7	7.9	-11
306 ng/mL	18	39.5	6.2	2

***BM** - Maintaining state-of-the-art top end Analytical Instrumentation with staff who are capable of extracting maximum scientific returns from this is clearly very important. Would you like to elaborate on the equipment you routinely use?*

JH/MW - We are very customer driven in Instrument purchases. Customers tend to like what they know and know what they like to see in an outsourcing CRO lab. If big Pharma invests in certain technologies the CRO industry generally follows. Since LC-MS/MS is the preferred method of choice for almost 65% of our 'public assays', we have standardised on the Sciex API 4000 and use Watson LIMS system. For the front end of the system we utilise CTC Analytics auto samplers and BAS pumping units. For assays involving LC and other detection systems we use BAS Electrochemical , Fluorescence and UV detectors

***BM** - Is the current technology platform available with top end MS sufficient for the demands placed on you by customers or does that area need improvements?*

JH/MW - There have been major developments in both the front ends and the MS systems available. The API5000 in our opinion appears to offer increased sensitivity but may be compound dependent. MS scanning times are now very fast and with faster scanning times we can still get enough points across very sharp chromatographic peaks from, for example UPLC separations. The advent of UPLC has made a difference with, increased sensitivity and specificity due to increased separation efficiencies. It is still chromatography but with the benefits come associated issues of which users are now more aware. For example, bacterial growth in mobile phases and unfiltered mobile phases quickly block the small particle columns.

The Spark Symbiosis (online SPE) is something I'm very excited about. In theory the rate limiting step becomes uncapping the sample tubes. This is above and beyond a normal chromatographic system. In my opinion, a lab needs to have an expert user to fully utilise this technology. There is also turbulent flow (turbo flow) technology. Uptake of this technology has been slow, but those labs that have it seem to like it, To our mind, however, it appears to be a complicated set-up.

***BM** - What areas do you need to see improving?*

JH/MW - Column technology has lagged a little. This is in part due to the slow uptake of new column technologies. Scientists can be very conservative.

The major headache is normally the software – in particular software integration and software validation requirements. For example, I believe the industry is struggling to properly implement electronic signatures outside the USA. Mass Spec software tends to be written to include the functionality for every possible application for every user which can complicate its regular day-to-day use. If the functions were simplified and written to freely talk to other software packages, life would be much simpler. You may not like Microsoft but we all know how to use Word and Outlook and it should be possible to be able to move across applications seamlessly thereby allowing easier and more accurate import and export of data and content.

***BM** - Do you use any particular types of ionisation modes for specific assays or is there one specific for all/most types of work?*

JH/MW - Many drugs are basic, so most things fly well in positive ionisation mode. Again, most things analyse well in electro spray (TurbolonSpray in Sciex speak) in my experience. Negative ionisation and APCI are much less common.

***BM** - Clearly a situation as with all areas of analytical instrumentation and business where fresh challenges are constantly there to be met and overcome. What is on your list in this respect?*

JH/MW – The list is a living document, as soon as you cross some items off as 'accomplished' then more take their place!

It is probably easy to categorise them into four main areas:

Firstly moving to increased sensitivities in detection systems. Low sample volumes can restrict the sensitivities that can be obtained yet increasing sample volumes is a retrograde step. Manufacturers of the equipment used in the assay (from sample preparation to mass spectrometers) must develop next generation instrumentation, which is capable of handling ever-decreasing sample volumes without compromising the ability of the method to record improved sensitivities.

Secondly the question of sample throughput must be addressed and this should not compromise any benefits arising from the first point above. There is a need to reduce analysis times by exploiting advances in column technology using small particle columns. This drives down sample times and hence from an economic viewpoint, analytical costs which all lead to improved instrument efficiencies for the CRO's who can move to 24/7 up time.

The linking of improvements in points one and two cannot be accomplished with similar advancements in the Robotics and Automation industry.

Thirdly we have the question of increasing regulation which not only leads to increasing levels of administration on projects but different interpretations of the legislation which can also lead to problems. CRO's of long standing, such as ourselves, have greater degrees of understanding here and often the clients themselves may not be fully au fait with the latest regulatory requirements.

For example there is now an intended FDA requirement for incurred sample reproducibility (ISR) data that means up to 10% of samples should be re analysed and results included in submissions to the FDA. Our Chief Scientific Officer, Dr Ron Shoup has been leading opinion in this area in the US.

Finally there is a requirement within the CRO industry as a whole to expand its skills base. In particular many of the analysts are familiar working with molecules of around 600 amu ('small' molecules) but the future pharmaceutical molecules they will be required to analyse could be regarded as bio-molecules around the 10,000 amu region.

***BM** - What is your current major challenge?*

JH/MW – Following recent investment by BASi in the European facility, we plan to build on our expertise already incumbent in the UK and ally this to that resident in the USA We have recently moved into a new 9000 sq. ft facility in the UK and intend to further invest to grow the services offered. As anyone who has been through the experience of moving equipment and support services, whilst at the same time continuing to offer a top-line level of customer service can testify, it has been be a difficult time. We have now managed to to successfully move all our equipment and people into a fully commissioned facility and in the future this will also allow growth to accommodate many more LC-MS/MS systems. and expansion of our proven range of both pre-clinical and clinical services. Overall we now offer over 40 LC-MS/MS systems as well as pharmaceutical analysis, immunochemistry and biomarker analysis as well as a wide range of PK/PD capabilities.

We have a successful track record and history of using Industry standard equipment allied to a forward looking philosophy and envisage continuing to build on these to the benefit of our customers and with continued investment in the skills of our people.

References

[1]. The CRO Market Outlook: Emerging Markets, Leading layers and Future Trends (Business Insights, May 2007).

Imported Seafood Contamination Testing Methods

Thermo Fisher Scientific, Inc recently announced that the company has developed three food testing methods in response to recent concerns over contamination of imported seafood. The three methods utilise the advanced analytical technology of the Thermo Scientific TSQ Quantum™ family of triple quadrupole mass spectrometers with Highly Selective Reaction Monitoring (H-SRM), enabling the trace-level analysis of complex samples such as animal tissue.

Thermo Fisher has developed a sensitive and reliable liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) method using H-SRM and the Thermo Scientific TSQ Quantum Discovery MAX triple quadrupole mass spectrometer for the detection of nitrofuran metabolites, which are veterinary antibiotics banned in many countries because of health concerns for humans. The method demonstrates a limit of quantitation (LOQ) as low as <0.05 µg/kg in fortified crawfish meat for all four nitrofuran metabolites.

A triphenylmethane dye used as a fungicide in aquaculture, malachite green and its metabolite leucomalachite green have demonstrated putative carcinogenic activity and have been banned by both the FDA and the EU. Thermo Fisher's LC-MS/MS method detected both contaminants in roasted eel meat using the TSQ Quantum Discovery MAX triple quadrupole mass spectrometer. The method showed excellent linearity, accuracy and reproducibility. Additionally, H-SRM was shown to reduce the chemical noise effectively in a complicated sample matrix, further improving the method sensitivity and specificity.

Government agencies worldwide have prohibited the use of chloramphenicol for the treatment of animals producing food products for human consumption due to the risk of a lethal condition called aplastic anemia in humans. Thermo Fisher has developed an LC-MS/MS method for chloramphenicol on the Thermo Scientific TSQ Quantum Discovery that yields subpicogram limits of quantitation, which translates to sub-parts-per-billion sample concentrations as well as accuracy and precision values well within the guidelines of the US government for analytical method development and validation.