



Achieving Reliable Mass Spectrometry Data: Back to Basics

Arranged by the Automation and Analytical
Management Group -
Royal Society of Chemistry

**Tuesday 6th June 2006
At the Society of Chemical Industry (S C I)**

London

Email: conference@aamg-rsc.org
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Achieving Reliable Mass Spectrometry Data: Back to Basics
Conference with Exhibition

Tuesday 6th June, 2006
At The Society of Chemical Industry (S C I)
London

- 10:00 **Registration and Coffee**
- 10:30 *Introduction and Welcome*
Mike Sargent
L G C, Teddington
- 10:35 *Data Reliability – The Wider View*
Mike Sargent
L G C, Teddington
- 11:00 *Common Problems with Mass Spectral Data – Recognition and Causes*
Bridget Stein
EPSRC National Mass Spectrometry Service Centre, Swansea
- 11:25 *Laboratory Performance and Problems in Making Accmass Measurements*
Tony Bristow
AstraZeneca, Macclesfield
- 11:50 *Meeting Training Needs for Mass Spectroscopists*
Andrew Reason
M-Scan Ltd, Wokingham
- 12:15 *DTI VAM Programme Training Guides on Mass Spectrometry for Beginners and Making Accmass Measurements*
Vicki Barwick
L G C, Teddington
- 12:45 Lunch and Exhibition
- 14:00 *False Results in Quantitative LC/MS – Understanding The Ionisation Process*
Mark Harrison
Thermo Electron, Hemel Hempstead
- 14:25 *Certainty of Identification in Pharmaceutical and Forensic Mass Spectrometry Applications*
Phil Teale
H F L, Fordham
- 14:50 *Instrumentation Issues for Reliable Mass Spectrometry Data*
Jackie Mosely
Durham University, Durham
- 15:15 *BMSS Training Activities*
Tony Bristow
BMSS Committee
- 15:40 *Panel Discussion*
Mike Sargent
L G C, Teddington

ABSTRACTS

DATA RELIABILITY – THE WIDER VIEW

Mike Sargent
LGC, Teddington

ABSTRACT

Reliable measurements based on mass spectrometry are essential for a wide range of applications such as the need to meet specific legal obligations, to ensure a product meets specification, or to assist in identification of a new, patented product. The cost of wrong data may be far more than the need to repeat sampling and analysis. For example, loss of business or customers, a requirement to replace products, health and safety implications, or damage to the environment. On the other hand, the cost of acquiring data is also high and it is essential to recognise that a valid measurement result does not have to be the ‘perfect’ answer. What is necessary is to obtain a result which is **fit for its intended purpose**.

In a world where quality systems, accreditation, GLP and regulatory requirements for measurement data have become the norm, it is easy to forget that the prime responsibility for reliable data still lies with the analyst. Moreover, it is no longer sufficient for the analyst or laboratory to be confident that their data is fit for purpose; they must be able to **demonstrate** a capability to produce valid results to the satisfaction of their customers, regulatory bodies, etc. The only way to meet all of these requirements is by ensuring that the analytical laboratory is able to undertake sound measurement science within the framework of a quality system. An organisation which does this should be able to ensure that their results are fit for purpose, easily demonstrate the validity of data to customers, and achieve consistency with results obtained elsewhere.

The basic principles needed to achieve valid analytical measurements are well recognised by the concept of ‘total quality management’ (TQM):

1. Measurements should satisfy a requirement agreed with the customer
2. Laboratories must use tested methods and equipment
3. All work should be carried out or supervised by qualified, competent staff
4. A laboratory should seek independent assessment of its performance
5. Results should be consistent with those obtained elsewhere
6. Recognised QC and QA procedures should be in place.

No matter how much effort is expended in achieving these principles, unreliable data will still occur unless all concerned with the measurement process also **use common sense**. This obvious requirement is often overlooked in the modern, regulated world. Thus it is essential that the responsible analyst talks to customers, takes note of “odd samples” or of instruments behaving differently, or acts on poor QC results whilst the work is still in progress. The laboratory which heeds all of this advice will produce reliable data at an acceptable cost, leading to better profitability and a successful business.

PROBLEMS WITH MASS SPECTROMETRY DATA - RECOGNITION AND CAUSES

Bridget K Stein

EPSRC National Mass Spectrometry Service Centre
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ABSTRACT

The tremendous growth in mass spectrometry over the past two decades has led to the technique being used in a wider range of applications and by more non-experts than ever before. The growth in the variety of techniques available has also made the application of mass spectrometry more complex and given more scope to the user to apply inappropriate conditions to their specific problem. Data interpretation has also become more complex. The combination of all these factors mean that poor quality mass spectrometric data may often be generated in ignorance, and lead to the wrong conclusions being drawn.

The aim of this presentation is to illustrate by example the range of problems that occur, grouping them under four main headings:

- Choice of method;
- The analytical process;
- Data representation;
- Data interpretation.

In this way it is hoped to alert the audience to the ubiquitous nature and scale of the problem, as well as to help them recognise where it could be affecting their own systems. The audience should also be alerted to some of the subtleties of mass spectrometry as a technique and the subsequent need for more mass spectrometry education in the community.

LABORATORY PERFORMANCE AND PROBLEMS IN MAKING ACCMASS MEASUREMENTS

Tony Bristow

AstraZeneca, Macclesfield

ABSTRACT

In October 2003 the results from an intercomparison study on accurate mass measurement mass spectrometry were published.¹ The data demonstrated the capability of the variety of mass spectrometers used to make such measurements. The most common type of mass spectrometer used in the study was TOF based, be that stand alone TOF or the hybrid QTOF. Much of the data produced using this type of mass spectrometer was of high accuracy and precision. However, the greatest degree of variability was observed with TOF based instruments. Of greatest concern was the variability in precision and accuracy where the participants had described the use of the same experimental protocols.

In response to these observations a second and more detailed intercomparison study, focussing solely on TOF and QTOF mass spectrometers is underway. A variety of experimental parameters are being investigated in a systematic manner. These included ion abundance or intensity, the m/z range of the ions measured, the m/z difference between a single lock mass ion and the analyte ion and data processing.

In the first part of this presentation, the findings of the study will be reported and the parameters that produced the greatest contribution to the measurement uncertainty will be described. The application of the data to provide increased confidence for the selection of an elemental formula, based on validated instrument capability and known measurement uncertainty, will be discussed

In the second part of this presentation the factors that influence the mass accuracy and precision on Fourier Transform Ion Cylotron Resonance (FT-ICR) mass spectrometers will be described. Initially, experiments were carried out with only an external calibration of the m/z scale used to make accurate mass measurements. Known ion abundance ratios and absolute ion abundances were used to establish the confidence in the mass measured and the elemental formula determined. Internal calibration was also used to correct for frequency shifts (mass shift) experienced by the ions inside the FT-ICRMS cell due to ion space charge effects. The use of a second ESI sprayer for the separate and controlled introduction of a calibration mixture for HPLC-FT-ICRMS accurate mass measurement was employed. This approach produced a significant improvement in the mass accuracy achieved and greater confidence in the elemental formulae determined.

References:

1. A. W. T. Bristow and K. S. Webb, *J. Am. Soc. Mass Spectrom* **14**, 1086 (2003).

MEETING TRAINING NEEDS FOR MASS SPECTROSCOPISTS

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ABSTRACT

M-Scan (Mass Spectrometry Consultants and Analysts) offers training courses which cater for the rapid growth and interest in mass spectrometry for investigation of a wide range of problems in chemistry, biochemistry, pharmacology, forensic science and environmental analysis. These courses have developed over the last 20 years of training Mass Spectroscopists in these various fields.

M-Scan has identified the training requirements for Mass Spectroscopists and has implemented a range of courses to meet these needs. Examples taken from M-Scan's MS introductory course (which is designed for scientists who have little or no experience of mass spectrometry but wish to take advantage of the technology for their investigations, either using in-house facilities or the services of a contract laboratory such as M-Scan) plus further more in-depth courses targeted towards major MS applications will be used to discuss M-Scan's course design and execution.

DTI VAM PROGRAMME TRAINING GUIDES ON MASS SPECTROMETRY

Vicki Barwick

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ABSTRACT

Since 1988 the Valid Analytical Measurement (VAM) programme has been working with UK industry to help ensure that analytical measurements are carried out competently and accurately. Analytical science plays a pivotal role in the UK and the competence of the analytical workforce is therefore essential. Knowledge transfer activities form a key part of the VAM programme, the aim being to raise awareness of the importance of producing valid results and to encourage the adoption of best practice. These activities include the development of training guides, books and software and the organisation of seminars and workshops. Many of the guidance documents produced can be downloaded free of charge from the VAM website (www.vam.org.uk).

Two documents are of particular interest to this meeting. The guide, 'Methodology for Accurate Mass Measurement of Small Molecules' can be downloaded from the VAM website. Accurate mass measurement is used to determine elemental formulae. The better the accuracy of the measurement, the less the ambiguity in assigning the elemental formula. The aim of the guide is to provide those carrying out accurate mass measurements with a clear summary of the essential steps in obtaining reliable data. Topics covered include: tuning and peak shape; ion abundance; resolving power; calibration; sample introduction; data manipulation; and validation and quality control checks.

A second document, 'Best Practice Guide for Generating Mass Spectra' will be available to download from the VAM website in September. The guide is aimed at novice users of mass spectrometry who need to generate mass spectra for compound identification purposes. Poor quality spectra can be misleading and result in incorrect conclusions. The guide will help users to generate spectra that are fit for purpose and to recognise poor quality spectra and some of the common causes. It will cover the selection of an appropriate ionisation mode, key aspects of instrument set-up that need to be addressed before generating a mass spectrum, how to evaluate spectral quality and molecular ion recognition.

FALSE RESULTS IN LC/MS – UNDERSTANDING THE IONISATION PROCESS

Mark Harrison

Thermo Electron, Hemel Hempstead

ABSTRACT

LC/MS and LC/MS/MS are widely used for quantitative analysis in many different industries. LC/MS/MS is often chosen for its sensitivity, selectivity and robustness. However the biggest unknown variability in the analysis is often the chemistry of the ionisation process.

In theory the atmospheric pressure ionisation techniques are simple solution, or gas phase processes. In reality this is can be quiet different. As well as the expected protonated or deprotonated molecular ions, adducts ions are often seen. The origin of these ions will be discussed with data from deuterium exchange experiments to support the hypothesis.

Some of the source parameters which need to be considered when undertaking quantitative analysis will be discussed. The effects of pH, mobile phase composition and matrix components, and how they can affect the results obtained will be shown.

These issues will be illustrated with examples from real analyses from our laboratories. Some suggestions will also be given as to how to identify, diagnose and rectify problems occurring in the ionisation process.

CERTAINTY OF IDENTIFICATION IN PHARMACEUTICAL AND FORENSIC MASS SPECTROMETRY

Phil Teale

HFL Ltd

ABSTRACT

Certainty of identification of analytes in the areas of pharmaceutical, forensic and sports testing clearly have important legal repercussions. However, the approach to the generation of mass spectrometric data and the use of defined criteria to support the identification of an analyte is different in each case.

Within the pharmaceutical industry qualitative analysis is largely used at the chemistry stage, be it synthetic or combinatorial, in metabolite identification, impurity identification and patent protection. Although there is obviously wide application of qualitative mass spectrometry within the pharmaceutical industry its application is very much the realm of the expert mass spectrometrist and there are no widely agreed industry standard criteria related to mass spectral quality.

By contrast the use of qualitative mass spectrometry for forensic and sports testing tends to be more tightly constrained. Samples are derived from subjects and situations over which the analyst has no control they must therefore be considered unique. The methods used must therefore provide sufficient structural information to ensure the identification will withstand legal scrutiny. In these areas generic criteria for the generation and interpretation of mass spectrometric data have been developed. In this presentation we will contrast the approaches taken in the pharmaceutical industry with those of the forensic / sports testing. The rationale behind generic criteria applied to sports testing and their utility will be discussed using real examples to highlight their usefulness and limitations.

INSTRUMENTATION ISSUES FOR RELIABLE MASS SPECTROMETRY DATA

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ABSTRACT

Modern mass spectrometers come in a variety of configurations with differing levels of performance and functionality. Add to this the ever increasing range and diversity of applications requiring mass spectrometry and suddenly the demands on any instrument becomes quite high. Consequently, often after constantly changing the set-up of an instrument, there is a resulting pressure on any operator to generate good quality, reliable data.

Reliable mass spectrometric data can be obtained by being aware of the limits of the mass spectrometer in question, having knowledge of current levels of performance for that instrument and matching that performance to the requirements for the application. Validation procedures are of particular importance as many laboratories rely increasingly on automated techniques.

As a means to address instrumentation issues when acquiring mass spectra, the following factors will be considered in detail:

- Choosing the right instrumentation for a desired application by giving thought to performance of the hardware components.
- Qualifying and optimising instrument set-up.
- Validate and maintain that level of instrument performance over time.

BMSS TRAINING ACTIVITIES

Tony Bristow

AstraZeneca, Macclesfield
& BMSS Education Officer

Education of the membership of the BMSS is a key role of the society. Therefore, in 2004, the BMSS committee made the decision to focus more of its efforts in this specific area with the establishment of the BMSS Education Initiative. To support this initiative a new BMSS officer role specific to education was also established, along with the formation of an education sub-committee within the BMSS committee.

The education officer and the sub-committee have identified a number of areas within mass spectrometry education on which to focus their efforts. The output of this process was a four-year education plan and the main themes of the plan are shown below.

1. The establishment of a BMSS Lecturer.
2. The production of a MS for schools talk.
3. The production of a MS Course for Undergraduates and Industry.
4. Web based training packages.
5. A survey of MS training courses, MS training packages and MS books and the production of a database containing this information.
6. The production of a MS training course for the non-specialist MS user.
7. Training material available through the BMSS newsletter, Mass Matters.
8. Funding for travel grants.

This presentation will describe the progress of the education initiative to date. A mass spectrometry presentation for schools covering a wide variety of applications has been developed. So far this has been presented to a number of schools and also at the Royal Society of Chemistry's, Chemistry: The Next Generation, Spectroscopy day.

A number of surveys are underway to look at the MS training requirements of academia and industry. Once this is complete, training modules will be designed and produced and then advertised. Another major activity is web-based training. The options for web-based training are being assessed and following this subject modules will be defined. One final area of significant activity is the identification of sources of funding to support the many education activities. BMSS has also received support from other training providers, such as Crawford Scientific.