White Paper

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Bioanalysis

# 2014 White Paper on recent issues in bioanalysis: a full immersion in bioanalysis (Part 1 – small molecules by LCMS)


The 2014 8th Workshop on Recent Issues in Bioanalysis (8th WRIB), a 5-day full immersion in the evolving field of bioanalysis, took place in Universal City, California, USA. Close to 500 professionals from pharmaceutical and biopharmaceutical companies, contract research organizations and regulatory agencies worldwide convened to share, review, discuss and agree on approaches to address current issues of interest in bioanalysis. The topics covered included both small and large molecules, and involved LCMS, hybrid LBA/LCMS, LBA approaches and immunogenicity. From the prolific discussions held during the workshop, specific recommendations are presented in this 2014 White Paper. As with the previous years' editions, this paper acts as a practical tool to help the bioanalytical community continue advances in scientific excellence, improved quality and better regulatory compliance. Due to its length, the 2014 edition of this comprehensive White Paper has been divided into three parts for editorial reasons. This publication (Part 1) covers the recommendations for small molecule bioanalysis using LCMS. Part 2 (Hybrid LBA/LCMS, Electronic Laboratory Notebook and Regulatory Agencies' input) and Part 3 (Large molecules bioanalysis using LBA and Immunogenicity) will be published in the upcoming issues of *Bioanalysis*.

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## Background

The 8th WRIB was hosted in Universal City, California, USA on March 10–14, 2014. The workshop included three sequential core workshop days and six training courses that together spanned an entire week in order to allow exhaustive and thorough coverage of all major issues in bioanalysis. This gathering brought together close to 500 professionals, representing over 200 companies, to share and discuss current topics of interest in the field of bioanalysis. Attendance included a wide diversity of industry experts from pharmaceutical and biopharmaceutical companies, CROs and multiple international regulatory agencies.

The actively contributing chairs in the 2014 edition of the WRIB were Eric Fluhler (Pfizer, USA), Olivier Le Blaye (ANSM, France), Dawn Dufield (Pfizer, USA), Lakshmi Amaravadi (Biogen Idec, USA), Lauren Stevenson (Biogen Idec, USA) and Fabio Garofolo (Algorithme Pharma, Canada).

The numerous regulatory agency representatives who contributed to the 8th WRIB included Sam Haidar (US FDA), Amy Rosenberg (US FDA), Susan Kirshner (US FDA), Laura Salazar-Fontana (US FDA), Mark Bustard (Health Canada), Jan Welink (Dutch Medicines Evaluation Board [MEB] and European Medicines Agency [EMA]), Olivier Le Blaye (French National Agency for Medicines and Health Products Safety [ANSM], France), Ronald Bauer (Agency for Health and Food Safety [AGES], Austria), Katalina Mettke (Federal Institute for Drugs and Medical Devices [BfArM], Germany), Emma Whale (Medicines and Healthcare products Regulatory Agency [MHRA], UK), Jason Wakelin-Smith (MHRA, UK), Noriko Katori (Ministry of Health, Labor, and Welfare – National Institute of Health Sciences [MHLW-NIHS], Japan) and Akiko Ishii-Watabe (MHLW-NIHS, Japan).

As with prior WRIB editions [1–6], a substantial number of topics were addressed

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| Acronyms | |
|---|---|
| **Abbreviation** | **Definition** |
| ADME | Absorption, distribution, metabolism, and excretion |
| BMV | Bioanalytical method validation |
| CRO | Contract research organization |
| CoA | Certificate of Analysis |
| DBS | Dried blood spots |
| GCC | Global CRO Council for Bioanalysis |
| ISR | Incurred sample reanalysis |
| LCMS | Liquid chromatography mass spectrometry |
| LLOQ | Lower limit of quantification |
| MIST | Metabolites in safety testing |
| MD | Method development |
| MOA | Mechanism of action |
| NTP | Nucleoside triphosphate |
| PK | Pharmacokinetic |
| QC | Quality control samples |
| SIL IS | Stable isotope-labeled internal standard |
| SPE | Solid phase extraction |
| WRIB | Workshop on Recent Issues in Bioanalysis |

during the workshop and distilled into a series of relevant recommendations. In the present White Paper, the exchanges, consensus and resulting recommendations on 36 recent issues ('hot' topics) in bioanalysis are presented. These 36 topics are distributed within the following areas:

- Small molecules by LCMS:
  - Emerging technologies (three topics);
  - Bioanalytical challenges (eight topics);
- Hybrid LBA/LCMS:
  - Large molecules by LCMS (five topics);
  - Antibody–Drug Conjugates (three topics);
  - Protein biomarkers by LCMS (five topics);
- Large molecules by LBA:
  - Immunogenicity (five topics);
  - PK LBA bioanalytical challenges (six topics);
- Electronic Laboratory Notebook (one topic).

Following the recommendations on the above topics, an additional section of this White Paper focuses specifically on several key inputs from regulatory agencies.

Due to its length, the 2014 edition of this comprehensive White Paper has been divided in three parts for editorial reasons. This publication (Part 1) covers the recommendations for small molecule bioanalysis using LCMS. Part 2 (Hybrid LBA/LCMS, Electronic Lab Notebooks and Input from Regulatory Agencies) and Part 3 (Large molecules bioanalysis using LBA and Immunogenicity) will be published in the upcoming issues of Bioanalysis.

## Small molecules by LCMS discussion topics

### Emerging technologies

#### Capillary microsampling

What additional validation is required for plasma microsampling? What is the current status in the industry on using plasma microsampling for Good Laboratory Practice (GLP) safety assessment studies? Is the physical site of sampling on the animal relevant? How to apply plasma microsampling in the clinical setting?

#### Microflow LCMS

What is the current industry status on the expectation that greater sensitivity can be achieved using microflow LCMS for more complex biological matrices such as tissue homogenates? Based on present data and industry standards, is it expected that the increase in detection response by going to low flow will overcome the limitations in the amount of sample that can be injected onto a small column and eluted at low flow rates in a reasonable time frame? Is it expected that microflow LCMS will be sufficiently advanced, rugged and consistent for wider use in regulated bioanalysis?

#### Emerging technologies in LCMS regulated bioanalysis

Which recommendations should be taken into consideration during the implementation of new technologies in support of regulated bioanalysis? What is the industry experience on the use of ion mobility in routine regulated bioanalysis? What is the additional instrument qualification needed for the use of ion mobility? What are the barriers in the introduction of new versus established LCMS vendors in a regulated environment, and how to overcome them?

## Bioanalytical challenges

### Stable isotope-labeled IS variability & acceptance criteria

Should prescribed acceptance criteria on IS response be established? Should IS response criteria be established by a general SOP for all methods, or by validation/study plans that are method-specific? Should outliers be excluded from the data? What methodology is acceptable? What should the criteria define? What criteria would trigger a formal scientific investigation when outliers are identified in a batch? Should the IS response relationship for specific subjects or doses be identified?

### Challenges in NTP analysis

What are the current status and future direction and improvement of ion-paring, ion-exchange and multimode LCMS analysis of NTPs? What is the best practice for evaluating the stability of NTPs in tissue samples? What are the recommended procedures for tissue sample collection and processing for the analysis of NTPs?

### Method transfer from sponsors to CROs

Regarding industry practice, should stabilities be repeated? How much change at CRO from sponsor method should be allowed? Do changes in automation require full validation or only partial validation? For cross-validations, should incurred samples (pooled or individual) be used? Are there blinding demographic information concerns? Is a two-way exchange of QC samples necessary? Is the blinding of the second laboratory necessary? For a method used at two sites within the same CRO, is a full validation required, or is cross-validation sufficient?

### Partial validation

What evaluations should be performed for partial validations associated with changes in systems/instruments? LC pumps? Autosamplers? Is system suitability testing sufficient when the changes in instrumentation are minor? For a change in species and matrix, is a full validation required, as prescribed by EMA? Is partial validation needed in changing from healthy volunteers to patients? Co-administered drugs? Change of strain and breed? What should be included in these partial validations?

### Stability & metabolite issues

Should freeze–thaw stability at -70°C be performed when samples are shipped on dry ice but kept at -20°C for long-term storage? Should stability at the highest subject sample concentration (above the curve range) be demonstrated? For the investigation of unstable metabolites that could impact analyte quantification, is there a minimum cut-off level of unstable metabolite(s), reported in the literature, where back/interconversion testing would not be required? For methods using DBS, should room temperature and shipping conditions be controlled and humidity be considered?

**Key term**

**Microflow LCMS:** An LCMS system operating at reduced flow rates (e.g., 1–50 µl/min) compared with conventional high flow LCMS that can significantly improve sensitivity with less sample volume and solvent consumption, without compromising method robustness, throughput, efficiency or data quality.

### Matrix co-stability assessment for fixed-dose combinations

Is there a final industry and regulators' consensus with the co-stability work required if stability data are already available for the individual analytes? If co-stability work is needed, which stabilities should be performed in such cases? Are short-term and freeze–thaw stabilities sufficient, as recommended by the GCC? Is long-term stability also needed? Why conduct co-stability experiments if there are no theoretical grounds to assume that there could be an issue with co-stability? What is the industry experience with co-stability? Do cases exist where instability was observed and attributed to the presence of co-medications?

### What can we do less of during BMV for small molecules?

Do specific stability assessments (e.g., re-injection reproducibility, extract stability, etc.) need to be conducted as part of the initial set of validation experiments, or can they be conducted in the event that the situation which the assessment addresses actually occurs? Do assay validation reports need to be issued and approved prior to the initiation of sample analysis?

### LCMS MD versus BMV

Can MD data be used concurrently with method validation data to support regulated bioanalysis? If so, what is the appropriate delineation of MD and BMV (e.g., where does unstable metabolite testing reside)?

## Small molecules by LCMS discussions, consensus & conclusions

### Emerging technologies

#### Capillary microsampling

A microsample is typically defined as a target volume of sample to be collected of lower than 20 µl. The decision to use microsampling for the collection of bioanalytical samples is essentially driven by the scientific and ethical considerations of the application (e.g., toxicokinetic or pediatric studies). Scientifically, microsampling can enable toxicokinetic sampling from main study animals such that exposure can be directly related to a toxic effect. Ethically, microsampling can facilitate small volume sampling in pediatric clinical studies, plus it can improve animal welfare and be used to reduce the number of animals used in the toxicology setting. Due to uncertainty in the industry regarding regulatory acceptance of data, microsampling techniques are currently applied more frequently during the drug discovery phase rather than in a regulated environment.

Generally, any issues with the methodology are investigated during MD and additional validation assessments are conducted to address differences in sample handling compared with conventional sampling. Since capillary microsamples are still liquid samples (as opposed to e.g., DBS), no major differences are expected during these stages from a matrix perspective. However, one needs to evaluate each method's unique characteristics. One possible difference will be the need to perform the ISR evaluation on diluted samples instead of unprocessed samples. Moreover, regarding fixed volume capillaries, additional validation and verification may be required depending on the information provided by the vendor and how the devices are used (e.g., homogeneity of the plasma harvested from the capillary). Proper training for the critical steps in the collection and processing of microsamples is believed to be essential for successful implementation, especially in the clinical setting. Finally, uniformity in the collection procedure will be required in order to minimize any impact from differences in sampling sites [7].

#### Microflow LCMS

The decision to use microflow LCMS for a given bioanalytical method is mainly driven by the sensitivity needs of the assay and/or the available sample size (e.g., tissues, microsamples). The success of applying microflow is critically dependent on the sample clean-up efficiency. Microflow LCMS can lead to sensitive and robust methods with good throughput for both small and large molecules in a regulated environment. Nevertheless, microflow is predominantly used for protein biomarkers and therapeutic peptides where more interference is typically observed and greater sensitivity is needed.

#### Emerging technologies in LCMS regulated bioanalysis

To support the adoption of new technologies for regulated bioanalysis, experience within the discovery space is viewed as an important step to adding credibility and rationale for a particular application. The implementation of new technologies used for analytical objectives is of great interest and value to the bioanalytical community. It was agreed that cross-validation with an existing technology is only necessary if a change of platform occurs within a program or a study. Otherwise, a robust traditional method validation should suffice during the implementation of a new technology. Of course, the presence of unique variables that could impact the quality of the results (e.g., precision, accuracy, specificity, etc.) should be anticipated and considered. For example, in relation to ion mobility technology, the consensus was to view this technology as an additional separation mechanism within the LCMS instrument. This implies that it is validated during its use within a particular bioanalytical method. However, as this technology may be removed from the LCMS system, appropriate system suitability testing should be considered when adding or removing the device to/from an instrument.

In conclusion, the scientific community should be aware of its ability to influence regulatory agencies through the sharing of their knowledge and experience via publications, conferences and other means of communication. In order to minimize potential regional differences, great emphasis should be placed on educating regulators from all regions on new technologies, which are constantly evolving.

### Bioanalytical challenges

#### Stable isotope-labeled IS variability & acceptance criteria

Bioanalytical LCMS assays rely heavily on the use of stable isotope-labeled (SIL) IS in order to compensate for inherent assay variation due to, for example, matrix/ionization effects, extraction efficiency, transfer losses, and to improve the overall assay precision and accuracy. The variability and acceptance criteria of IS response has been extensively discussed in recent years, especially in the 2011 White Paper in Bioanalysis as part of the 2011 edition of the WRIB, as well as within the GCC, which led to specific recommendations on this topic [8]. Within the industry, various approaches are employed based on the method diversity, which ren-

ders the topic of IS acceptance criteria to be complex and challenging. Although IS variability is an indicator of assay performance, it is agreed that an assay may still provide reliable results while showing variable or trending IS responses. While bioanalytical scientists and regulatory authorities alike embrace rational and easy to apply algorithms for data approval and decision making, agreement on the definition of a single set of prescribed IS acceptance criteria that account for even the most commonly encountered situations has been difficult to achieve. The wide diversity of assay types, compound properties, inter-subject matrix effects and assay performance may not be adequately addressed via a single set of acceptance criteria. Several paradigms have been proposed to define IS acceptance criteria. These options include numerically comparing upper and lower boundaries to the mean IS response, trend analysis using the IS variation of known samples to define the acceptability of the IS variation for unknown samples, or establishing statistical methods to identify outliers within a data set.

Statistical approaches are often perceived as a way to add consistency, but they may not always adequately capture all scenarios encountered. In some instances, trending and unique patient responses may be more relevant than individual outlier values. Whatever the approach adopted within an organization, whether a single set of criteria described in a general SOP for all methods or assay-specific criteria defined in the validation/study plan or specific method SOP, it does not eliminate the need to closely monitor the IS response in each analytical run, to examine the validity of the data obtained and to perform investigations as needed. Acceptance criteria for IS response variability should be set based on scientific judgment, and consideration should be given to the potential questions a regulator could ask in light of the data presented. The scope of the study in which the assay is to be employed is also to be kept in mind when evaluating IS response variability.

From a regulatory perspective, IS response variation should be monitored and criteria put in place in a written procedure or SOP with the goal of maintaining the integrity of the study data, with some form of pre-defined criteria or trend analysis deemed to be appropriate. Other approaches taken should be documented and defensible. The use of a SIL IS is favored over chemical analogs; however, it does not justify the use of less restrictive criteria for monitoring IS response. Regulators reiterated that significant variation in IS response should be investigated. Graphical presentation of IS response variation is not routinely needed in the bioanalytical report; however, regulators indicated that IS variation observations outside of SOP criteria should be referenced in the report, and investigations of IS variation should be summarized in the final report.

### Challenges in NTP analysis

NTP quantification presents unique challenges owing to their known instability, the variability of their extraction from the matrix (e.g., PBMC) and other analytical complexities, such as chromatographic retention and assay ruggedness due to the highly polar phosphate moiety. Multimode LC (ion exchange and reversed-phase) demonstrated promise in improving assay performance. To help mitigate the instability of NTPs in tissue samples, it is recommended to conduct the sample extraction at the clinical site in order to minimize the delay between the sample collection and its processing.

### Method transfer from sponsors to CROs

When transferring a bioanalytical method from the sponsor to the CRO, multiple questions arise with regard to the extent of validation required to support the transfer and maintain the integrity of the method. The main challenge faced with method transfers is the fact that differences can be seen when employing a given method between laboratories, which can impact data consistency and may be an indicator of method issues. Recommendations in relation to this topic were issued as part of a previous White Paper, where it was recommended that for method transfers between laboratories, a full validation should be performed, including stability reassessments [4].

If some conditions appear to vary between sites, a risk-based approach may be applied in determining whether it would be possible to successfully transfer the method and which assessments would be warranted, considering the nature and extent of variations in conditions (e.g., modifications in method steps, instruments, storage conditions, etc.). For example, as the analyte stability is molecule-dependent under specific handling and storage conditions, stability evaluations may not need to be re-performed if the conditions are known to be exactly the same. An agreement was also reached towards switching from manual to automatic aliquoting of samples (e.g., from a micropipette to an automated liquid handling system), where it is recommended to conduct a minimum of one precision and accuracy run to validate such a change.

If a single study is done at two different sites, a cross-validation is deemed necessary and should use spiked QC samples as well as incurred samples, in line with the current and the 2013 draft FDA Guidance documents on BMV [9,10]. For QC samples, a pre-defined criterion for accuracy (percent nominal) should be applied; a two-way exchange of QC samples is not mandatory. If the same assay (i.e. the same method SOP) is used at

two sites, one cross-validation run using QC samples is considered sufficient. However, if a change in country is involved where differences in SOP interpretation could be anticipated, more runs may be considered.

As a good practice from the sponsor, it is expected that the CRO be informed if issues were observed in critical tests done during MD and/or validation. This will help in establishing the required evaluations to be conducted by the CRO based on scientific observations for successful and reliable method transfer. In the case it is decided not to re-validate certain parts, it is expected that the method validation documentation (e.g., source data, equipment calibration/validation, etc.) be available for these parts performed earlier. It should also be kept in mind that the laboratory where these experiments were performed could then also be a site to be inspected.

### Partial validation

When modifications are made to a previously validated bioanalytical method, additional validation assessments may be needed to ensure suitable performance. Although regulatory guidance documents mention the need for partial validation when changes are applied to a method and provide examples of possible changes, they do not include recommendations on the tests to be conducted to validate these changes. Hence, different scenarios and their potential impact were discussed in order to propose the minimum assessments that would need to be included in the partial validation. The first change that was discussed was a switch in detection instrument to a different platform (e.g., from an AB SCIEX API3000 to an API5000). This type of change would minimally require that sensitivity, precision and accuracy (at least three runs), and matrix effects be re-assessed. However, stability assessments would not need to be re-evaluated. A change in autosampler model would minimally involve the evaluation of carryover, precision and accuracy. A change in LC pump model would not prompt a partial validation if the separation was isocratic; however, selectivity evaluations should be performed if a gradient separation or change in mobile phase composition was used.

When there is a change in species and/or matrix, the EMA and Japanese guidelines on BMV state that a full validation is needed [11,12]. However, the 2013 draft FDA Guidance refers to partial validation [10]. From a regulatory standpoint, it was expressed that such decisions be left to the judgment of the organization. The consensus reached was to perform again all evaluations, except solution stabilities, which represents a nearly full validation. For other changes such as moving from healthy subjects to patients, inclusion of co-administered drugs, or change in animal strain and/or breed, when using a SIL IS, close monitoring of the IS response would help determine if partial validation is warranted. If consistent SIL IS response is observed, no partial validation would be required.

### Stability & metabolite issues

Several topics concerning the stability of analytes and supportive validation assessments were discussed in depth and consensus was reached on some key items. One of them pertained to whether freeze–thaw stability at -70°C is to be demonstrated when samples are shipped on dry ice but kept at -20°C for long-term frozen storage. It was agreed that it is not deemed required, especially when the bioanalytical laboratory has submitted data demonstrating long-term stability at both -20°C and -70°C, as well as freeze–thaw stability at -20°C.

As per current regulatory guidance on BMV, stability is to be demonstrated at low and high QC concentration levels. However, the industry is sometimes questioned about the need to demonstrate stability at the highest subject sample concentration (above the curve range). The feedback from regulators was that although the demonstration of such stability was said to be desirable, it was also mentioned that it could be assessed on a case-by-case basis, based on how much greater the subject concentrations were than the high QC used in the evaluation of the long-term stability. One should ask: is extrapolation to the observed level appropriate? Despite the fact that stability is often more of a concern at low concentrations, solubility issues may potentially arise at higher levels. As part of preclinical studies where very high levels may be encountered in study samples, stability at such high levels may need to be considered. However, in a clinical context where samples mostly fall within the analytical range and where samples above the range are not significantly above the ULOQ, it was concluded that stability would not be required.

For investigations of suspected unstable metabolites that could impact analyte quantification with the possibility of reversible or interconverting metabolites, the consensus was that one should not solely rely on a cut-off (% of parent) based on levels reported in literature to decide whether or not to conduct back/interconversion testing. Such literature levels often represent a mean level and may vary significantly, depending on the method employed and the samples used (where variations in PK profile come into play). Therefore, it is considered sound scientific practice to conduct back/inter-conversion testing on suspected unstable metabolites and not rely on literature alone.

Regulators conveyed that for metabolite testing, if authentic reference standards with full certificates of analysis are not commercially available for a specific

metabolite, a scientific justification for the use of other materials may be acceptable, and should include analytical method/physicochemical considerations (e.g., molecular weight, mass transitions, polarity, chromatographic conditions, etc.) of the analyte and metabolite(s), as well as the expected concentrations of the metabolite(s) in matrix. The use of incurred samples for this purpose (e.g., characterized by quantitative NMR or based on specific activity if radiolabeled) can be applied on a case-by-case basis but not routinely. As the demonstration of selectivity is fundamental to the validation of an assay, the validation report should include discussion on the investigation of possible interference from metabolites.

Stability issues as part of DBS assays were also discussed, and it was agreed that room (ambient) temperature should be controlled, with definition and assessment of temperature 'extremes', considering the fact that room temperature can vary widely (for example from 18°C to above 25°C). Alternatively, temperature could be monitored via data loggers to ensure samples will be covered by appropriate stability data. A similar approach would also apply for the control of shipping conditions in terms of humidity (e.g., the presence with the DBS samples of silica gel that changes color when humid).

### Matrix co-stability assessment for fixed-dose combinations

As a consequence of the current trend for the increased development of fixed-dose drug combinations in the pharmaceutical industry, some auditors from regulatory authorities started to request matrix stability data in the presence of all co-administered compounds. A request was also seen for compounds administered as separate doses (i.e. not co-formulated). Such requests pose significant challenges to both the applicant and the CRO, as it often happens that drugs, which may be owned by different companies, are analyzed in different laboratories, and performing these stabilities has a considerable impact on resources and workload for additional validation work. This topic has been debated at length at various discussion forums and at international scientific conferences pertaining to regulated bioanalysis. Discussion was initiated at the 2010 WRIB, and continued as part of the 2011 and 2012 editions [3–5]. The GCC also published their recommendations in a White Paper specifically dedicated to this topic, where it was suggested to conduct short-term and freeze–thaw stabilities for fixed-dose combinations [13]. Interestingly, the 2013 draft FDA Guidance on BMV does not explicitly mention the need to address stability in the matrix containing all the analytes, while the EMA Guideline does; although, only in general terms, without detailing which stability evaluations would be warranted. Some companies may decide to do short-term, freeze–thaw and long-term stability, with the aim of continuing to generate data to show the absence of impact from co-medications, or to simply ensure that no hurdles are encountered in the process of dossier submission and to thereby accelerate filing.

Although the industry still does not have reported cases of co-stability issues and thus no scientific rationale seems to exist on why co-administered drugs would impact stability, no consensus was reached among the regulators. An agency representative suggested that stability studies (including long-term stability) should be carried out in a relevant matrix containing all analytes, since the QC samples employed for subject sample analysis and for stability experiments should reflect as much as possible the study subject samples. Following this rationale, this would imply that co-stability would also need to be assessed in drug–drug interaction trials. Clearly, more discussion is needed between regulators before a final consensus on this highly debated topic will be reached.

### What can we do less of in BMV for small molecules?

The minimum requirements to fully validate bioanalytical methods for the quantification of small molecules in biological matrices are well established in existing regulatory guidance documents. The value and/or necessity of the experiments that are currently conducted during method validation were discussed, since some validation evaluations go beyond the primary focus of assay validation, which should serve to demonstrate that the assay is accurate and of sufficient sensitivity to measure a given analyte with appropriate precision. In principle, validation experiments need to be conducted to assess conditions that will arise during sample analysis. However, in practice, validation experiments are conducted not only to assess conditions that will take place during sample analysis, but also situations that might occur, without knowing in advance if they will really occur during sample analysis.

The general consensus reached was in favor of the assessment of such conditions using a risk-based approach; i.e. dependent on if the condition is actually encountered or is highly expected to be encountered. For example, one may decide not to perform re-injection reproducibility in the initial validation phase if no run will be re-injected because re-injection is not allowed (e.g., when the workflow is to re-extract and re-analyze samples in the event of instrumentation failure). Performing the assessment in such a case would not add any value to the method validation. Similarly,

extract stability is only relevant in the case of delayed analysis of extracts (considering that the laboratory is able to prove, with appropriate documentation, that samples are indeed always analyzed on the day of extraction). Other experiments that may not be relevant in certain situations include multiple freeze–thaw assessments for rodent plasma when microsampling procedures do not generate enough sample volume for multiple analyses; long-term stock solution stability when the stock solution is used fresh daily; and hyperlipidemic matrix test for preclinical species in the case of purpose-bred animals fed a standard diet.

With regard to the assay validation reports, it was agreed that they do not need to be issued and approved prior to the initiation of sample analysis. However, validation experiments must be conducted and results must be acceptable prior to the analysis of study samples. In any case, a formal confirmation of approval of the validity of the method is required prior to the start of sample analysis.

### LCMS MD versus BMV

Both the current 2001 FDA Guidance and the recently released draft FDA Guidance on BMV [9,10] make reference to the use of MD data. In these documents, reference is made to the concept of method establishment (i.e. 'development and validation'), which is then followed by the application of the validated method for sample analysis. In practice, many bioanalytical laboratories clearly separate MD and validation activities to allow efficiency and flexibility in the former, and ensure regulatory compliance during the latter. However, recent industry/regulator discussions on fit-for-purpose assays and the realization that using MD data in full conjunction with method validation can be scientifically sound, has many industry scientists reconsidering the use of MD data to support regulated bioanalysis. It was agreed that with appropriate documentation, the use of key MD experiments could reduce rework while maintaining a defendable application of the bioanalytical method. However, if a given experiment is deemed critical to the use of the assay (i.e. suitable for intended use), consideration should be given to perform it again as part of BMV.

The use of MD data to support specific aspects of BMV raises the question of whether, within the organization establishing an assay, such MD data is required to be audited by the internal QA group. The consensus on this subject was that MD data, by definition, is not intended to be reviewed by QA nor are there established standards to review bioanalytical MD data against. Hence, while potentially resource sparing, the use of certain MD experiments to support BMV (i.e. without repeating them during BMV) would likely reduce the amount of supporting documentation. The decision on which assessments could be restricted to the MD phase needs to be specific to the intended use of the assay. In practice there are core method characterizations associated with precision, accuracy, sensitivity, selectivity and analyte stability that will always be considered integral to method validation. Regardless of the decision taken, one must be prepared and able to defend the conclusions drawn to support the full validation of an assay, whether they are derived from MD or BMV data. It was also recommended that as a general approach, the testing of potentially unstable metabolites should be conducted in MD and then be confirmed as part of BMV.

Bioanalysts have a vested interest in whether regulatory inspectors consider the MD phase of method establishment as part of their investigations. This relates to interpretation of the current draft BMV Guidance from the FDA that makes reference to MD activities and reporting. In response, it was shared that the regulators' main interest in relation to MD is in understanding MD evaluations that might not have been included in the method validation experiments and report. Moreover, regulators are also interested in tracking MD changes in bioanalytical methods (e.g., a method that fails during validation and is re-developed before attempting a new validation). However, it was confirmed that the inclusion of a formal MD report as part of submissions is not necessary; a concise summary providing historical perspective and context for changes to the method is considered sufficient for reporting purposes.

## Conclusion

Below is a summary of the recommendations made during the 8th WRIB.

### Small Molecules by LCMS recommendations

#### Emerging Technologies

- Microsampling should be applied based on scientific, ethical and regulatory considerations. The conduct of plasma microsampling itself presents no major differences when compared with traditional plasma sampling. However, additional consideration may be required for ISR which will often be conducted on diluted samples, and additional testing may be required when using fixed volume capillaries. Appropriate training is crucial in its successful implementation.

- The decision to use microflow LCMS is mainly driven by the sensitivity needs of the assay and/or the available sample size, and its success is critically

dependent on the efficiency of sample clean-up. Although this technology can lead to sensitive and robust methods with good throughput, at present it is used mainly for biomarkers, proteomics and therapeutic peptides where more interferences are typically observed and greater sensitivity is needed.

- Emerging technologies are of interest and add value in the evolving field of regulated bioanalysis. Cross-validation with an existing technology is necessary only if the change of platform occurs within a program or a study; however, the presence of unique variables that could impact quality should be considered. The bioanalytical community should take advantage of their opportunity to influence authorities by regularly sharing their knowledge and experience on new technologies.

## Bioanalytical challenges

- IS response evaluation is a complex topic with many scenarios to consider. A single set of criteria for IS acceptance or triggering an investigation may not be practical. A variety of approaches have been employed/discussed as appropriate acceptance criteria for IS response variation. These include numerical boundaries based on the IS variation of known samples, trend analysis and statistical methods to identify outliers. Whatever the approach taken, and whether a general set of criteria or assay-specific criteria are established, the IS response should be closely monitored; abnormal variability and outliers should be investigated.

- NTPs represent a modality with unique bioanalytical challenges owing to their inherent chemical properties (polarity), known instability (enzymatic susceptibility) and the associated complexity of extraction from the matrix (tissues and PBMCs). Overcoming these challenges is best accomplished by performing the sample extraction with an appropriate enzyme inhibitor at the clinical site to minimize the delay between sample collection and processing.

- When transferring a bioanalytical method from the sponsor to the CRO, a risk-based approach may be applied to determine the evaluations required, considering the nature and extent of variations in conditions. If a single study is done at two different sites, a cross-validation using spiked QC samples and incurred samples is recommended. If the same method SOP is used at the two sites, one cross-validation run using QC samples may be sufficient.

- When a validated bioanalytical method has a change in detection instrument to a different platform, minimally sensitivity, precision/accuracy on at least three runs and matrix effect should be re-assessed during partial validation. Partial validation for a change in autosampler model would involve, at minimum, the evaluation of carryover, and single run precision and accuracy. A change in LC pump model may not prompt any partial validation if the chromatography is isocratic, while evaluations may be more involved if a gradient of mobile phase is used. If switching from manual pipetting to automated pipetting, a minimum of one precision and accuracy run is recommended. Regarding a change in species and matrix, all evaluations should be re-performed, except solution stabilities. For changes such as moving from healthy subjects to patients, inclusion of co-administered drugs, or change in animal strain and/or breed, when using a SIL IS, a close monitoring of the IS response would help define if partial validation is warranted.

- Demonstrating freeze–thaw stability at -70°C is not mandatory when samples are shipped on dry ice but kept at -20°C for long-term storage, given that long-term stability at both -20°C and -70°C, as well as freeze–thaw stability at -20°C, are proven. Matrix stability at concentrations above the calibration curve range may be conducted on a case-by-case basis. For DBS assays, room (ambient) temperature and humidity should be controlled.

- Although no specific industry cases where stability issues have been associated with co-medications have been reported, no clear consensus was reached by the regulatory agencies regarding the need to demonstrate stability in matrix containing all co-administered compounds for fixed-dose combinations.

- The decision to perform validation assessments for conditions that may not occur during sample analysis may be made using a risk-based approach. It was also agreed that final validation reports do not need to be approved and issued prior to the initiation of sample analysis; however, validation experiments must be conducted and results must be acceptable before proceeding with the analysis of study samples.

- It was agreed that when based on sound science and appropriately documented, the use of key MD experiments to support validation was acceptable. However, if a given experiment is deemed critical

to the assay, consideration should be given to re-perform it as part of BMV. MD data, by definition, is not meant to be reviewed by QA.

## Acknowledgements

The authors would like to acknowledge the US FDA, Europe EMA, Health Canada, France ANSM, The Netherlands MEB, Austria AGES, Germany BfArM, Japan MHWL and UK MHRA for supporting this workshop. L Stevenson (Biogen Idec), L Amaravadi (Biogen Idec), H Myler (Bristol-Myers Squibb), D Dufield (Pfizer), L Salazar-Fontana (FDA), H Neubert (Pfizer), L Kirkovsky (Pfizer), F Garofolo (Algorithme Pharma), I Dumont (Algorithme Pharma), B Gorovits (Pfizer), E Fluhler (Pfizer), R Hayes (MPI Research), K Xu (Genentech), M Arnold (Bristol-Myers Squibb), S Bansal (Roche), T Verhaeghe (Janssen), A Bergeron (Algorithme Pharma), O Laterza (Merck), SC Alley (Seattle Genetics), M Szapacs (GlaxoSmithKline), A Wilson (AstraZeneca), S Kaur (Genentech) and A Joyce (Pfizer), for their major contribution in this White Paper. E Fluhler (Pfizer), O Le Blaye (France ANSM), D Dufield (Pfizer), L Amaravadi (Biogen Idec), L Stevenson (Biogen Idec) and F Garofolo (Algorithme Pharma) for chairing the workshop. E Fluhler (Pfizer), D Dufield (Pfizer), L Amaravadi (Biogen Idec) and L Stevenson (Biogen Idec) for chairing the White Paper discussions. All the workshop attendees and members of the bioanalytical community who have sent comments and suggestions to complete this White Paper. W Garofolo, L Lu, X Wang, E Petrova, M Losauro and B Felcyn for the logistic assistance of the event. Future Science Group as a trusted partner.

## Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

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## References

1 Savoie N, Booth BP, Bradley T *et al.* 2008 White Paper: the 2nd calibration and validation group workshop on recent issues in good laboratory practice bioanalysis. *Bioanalysis* 1(1), 19–30 (2009).

2 Savoie N, Garofolo F, van Amsterdam P *et al.* 2009 White Paper on recent issues in regulated bioanalysis from the 3rd calibration and validation group workshop. *Bioanalysis* 2(1), 53–68 (2010).

3 Savoie N, Garofolo F, van Amsterdam P *et al.* 2010 White Paper on recent issues in regulated bioanalysis and global harmonization of bioanalytical guidance. *Bioanalysis* 2(12), 1945–1960 (2010).

4 Garofolo F, Rocci M, Dumont I *et al.* 2011 White Paper on recent issues in bioanalysis and regulatory findings from audits and inspections. *Bioanalysis* 3(18), 2081–2096 (2011).

5 DeSilva B, Garofolo F, Rocci M *et al.* 2012 White Paper on recent issues in bioanalysis and alignment of multiple guidelines. *Bioanalysis* 4(18), 2213–2226 (2012).

6 Stevenson L, Rocci M, Garofolo F *et al.* 2013 White Paper on recent issues in bioanalysis: 'Hybrid' – the best of LBA and LC/MS. *Bioanalysis* 5(23), 2903–2918 (2013).

7 Mohammed BS, Cameron GA, Cameron L *et al.* Can finger-prick sampling replace venous sampling to determine the pharmacokinetic profile of oral paracetamol? *Br. J. Clin. Pharmacol.* 70(1), 52–56 (2010).

8 Lowes S, Jersey J, Shoup R *et al.* Recommendations on: internal standard criteria; stability; ISR and recent 483s by the Global CRO Council (GCC) for Bioanalysis. *Bioanalysis* 3(12), 1323–1332 (2011).

9 US Department of Health and Human Services, US FDA, Center for Drug Evaluation and Research, Center for Veterinary Medicine. Guidance for Industry, Bioanalytical Method Validation, Rockville, MD, USA (2001).

10 US Department of Health and Human Services, US FDA, Center for Drug Evaluation and Research, Center for Veterinary Medicine. Draft Guidance for Industry, Bioanalytical Method Validation, Rockville, MD, USA (2013).

11 EMA. *Guideline on Bioanalytical Method Validation.* Committee for Medicinal Products for Human Use (CHMP), London, UK (2011).

12 Guideline on Bioanalytical Method Validation in Pharmaceutical Development. Japan Ministry of Health, Labour, and Welfare (MHLW), Japan (2013).

13 Lowes S, Boterman M, Doig M *et al.* Recommendations on bioanalytical method stability implications of co-administered and co-formulated drugs by Global CRO Council for Bioanalysis (GCC). *Bioanalysis* 4(17), 2117–2126 (2012).