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WHITE PAPER

The use of surrogate matrices in bioanalytical preclinical safety testing using chromatographic methods: a recommendation from the European Bioanalysis forum

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ABSTRACT
Within the bioanalytical community, the use of blank matrix from preclinical animals for bioanalytical method validation and sample analysis is common practice and required in the context of guidelines for bioanalytical method validation. At the same time, its use has been challenged by the scientific community for decades, since there is ample scientific evidence to allow the use surrogate matrices for this purpose. Nevertheless, legacy and current regulatory thinking continues to be reluctant to allow the use of surrogate matrices in bioanalytical testing except for so-called rare matrices. As part of ongoing discussions in relation to the ICH M10 Guideline, the European Bioanalysis Forum re-challenges the unnecessary use of blank matrices from preclinical animals and believes that, as part of community responsibility and ethical standards and when supported by data, the use of surrogate matrices should become widely accepted. It is in this context that targeted experiments were conducted within the European Bioanalysis Forum to gather additional data and re-open the discussions with all involved and that it should become acceptable to use surrogate matrices wherever possible.

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1. Introduction

The ethical need to ensure replacement, reduction, refinement and responsibility (3Rs) of animal use in safety testing is a key consideration in the development of new therapies, supported by both US FDA Center for Drug Evaluation and Research [1] and EMA under EU Directive 2010/63/EU [2]. Historical and current regulatory guidance or guidelines for bioanalytical method validation and sample analysis [3–5] outline that calibration samples, quality control (QC) samples and sample dilution integrity should be prepared or performed using the same matrix and species as that of the study samples. Although it is accepted that QC samples should mimic study samples by being prepared in the same matrix, the preparation of calibration samples and dilution of study samples in preclinical matrix is potentially in conflict with the 3Rs if alternatives are demonstrated to be suitable. The current ICH M10 guidance [5] already calls out that for 'rare' matrices, a surrogate matrix may be acceptable for analytical method validation, including dilution integrity, if it can be scientifically justified and demonstrated to be equivalent. A survey within the European Bioanalysis Forum (EBF) member companies [6] highlighted that due to the difficulty in obtaining some preclinical matrices, particularly non-human primate, several are already successfully using the surrogate matrix approach for calibration samples and dilution integrity for assays in these species under the 'rare' matrix caveat. However, the applicability of the surrogate matrix approach to all preclinical assays has the potential to significantly reduce

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the need for invasive blood draws across all preclinical safety testing without compromising data quality, if appropriately validated. At the same time, and aligned with current ethical standards, the industry should take responsibility to apply the principles of the 3Rs wherever possible and should not accept inappropriate use of laboratory animals when a valid alternative is available. To this end, the EBF formed a team to design and perform experiments to gather compelling and convincing data to re-open discussions across the Bioanalytical community, including the regulatory authorities, for the acceptance of replacement of preclinical matrix in assay validation and study sample analysis.

2. Experimental design

Seventeen EBF member companies tested existing, fully validated preclinical methods against two surrogate calibration lines, one prepared in human plasma and one prepared in a synthetic matrix; 2% Bovine Serum Albumin in Phosphate Buffered Saline (BSA-PBS). Existing QC samples that were already proven valid against the validated assay were tested at a minimum of Low, Medium and High levels (5 replicates each) against the surrogate matrix calibration lines on 3 separate occasions. A prerequisite when selecting the assay for testing was that a stable isotope labeled internal standard was available. Acceptance criteria was within ±15% for both precision and accuracy. Additional validation experiments, such as selectivity and specificity, were not assessed on the basis that if QC samples in preclinical matrix pass against a surrogate calibration line, the selectivity and specificity of the LC-MS assay was assured.

3. Results

Across the seventeen EBF member companies, 56 preclinical assays were tested against a human plasma calibration line and 40 were tested against a BSA-PBS calibration line. The assays tested were originally validated in either dog, rat, non-human primate, minipig, mouse, rabbit or hamster plasma (Table 1).

Across the assays tested were a variety of molecule types from low molecular weight small molecules (120 Da) through to acetylated peptides (5000 Da) and with a broad array of physico-chemical properties (logP, pKa). The results are presented in Table 2.

Of the 56 preclinical assays tested against a human plasma calibration line, 53 (94.6%) passed acceptance criteria for accuracy and 55 (98.2%) passed acceptance criteria for precision. Of the 40 preclinical assays tested against a BSA-PBS calibration line, 28 (70.0%) passed acceptance criteria for accuracy and 40 (100.0%) passed acceptance criteria for precision.

4. Discussion & EBF recommendations

The data generated by the EBF member companies generally demonstrated good correlation between surrogate calibration lines and QC samples in all tested preclinical species. However, in the context of this data, it needs to be highlighted that there were no attempts made to modify the methods to overcome the matrix differences seen between the surrogate calibration standards and QC samples. With this in mind, the high pass rates observed using either surrogate matrix demonstrate the viability of the approach from a scientific perspective as passing QC samples are reflective of accurate quantitation in incurred samples. Furthermore, based on the experience within the EBF community this was an expected result for methods using a stable isotope labeled internal standard, that is known to compensate for differences in ionization and other matrix effects, and the basis for this approach. The assays that did not pass the acceptance criteria had no particular pattern in term of species, molecule type, molecule size or assay range and generally failed due to accuracy rather than precision. Given that the accuracy failures were more pronounced in PBS-BSA than in plasma, matrix composition and related effects is clearly a consideration when selecting an appropriate surrogate matrix. However, in the context of this data, it needs to be highlighted that there were no attempts made to modify the methods to overcome the matrix differences seen between the surrogate calibration standards and QC samples. Further development of the methods would undoubtably have resulted in acceptable performance given that in most instances, the accuracy failures were relatively minor at <5% outside acceptance criteria with acceptable precision.

The ethical advantages of using the surrogate matrix approach become clear when considering the volume of matrix used on just calibration samples and for sample dilution. If assumed in rodent species that in exsanguinating a rat and mouse, 8 ml and 0.8 ml of plasma can be collected, respectively, the animal savings are quickly apparent across just the assay validation, 28 day and 13-week regulated toxicology studies. If assumed 0.25 ml spiking volume for each calibration point, eight calibration points per assay and 12 batches (3 precision and accuracy batches, 1 additional validation experiments batch, 2 long term stability batches and 3 sample analysis batches in each of the 28-day and 13-week toxicology studies to cover original analysis, repeat analysis and incurred sample reanalysis) and 5 ml matrix for sample dilutions then this requires approximately 29 ml of matrix. This equates to 4 rats and 32 mice saved across validation and first 2 toxicology studies alone. Even for larger species that are not euthanized for

Table 1. Number of assays tested per species against human plasma and agains PBS-BSA.

Assay matrix	Assays tested vs human plasma	Assays tested vs PBS-BSA
Rat	23	15
Dog	18	13
Non-human primate	5	4
Minipig	1	0
Mouse	5	3
Rabbit	4	3
Hamster	0	2
Total	56	40

Table 2. Number of assays (and % of total number of assays) passing the acceptance criteria per surrogate matrix.

Surrogate matrix	Assays tested	Assays passing accuracy acceptance (%)	Assays passing precision acceptance (%)
Human plasma	56	53 (94.6%)	55 (98.2%)
PBS-BSA	40	28 (70.0%)	40 (100%)

matrix, the requirement for less matrix means a reduction in invasive blood draws, also aligned with the 3Rs philosophy.

In conclusion, based on the high level of acceptability of QC sample data when using surrogate calibration lines, even without specific development of the assays to address any potential matrix difference, and the ethical savings that the approach delivers from a 3Rs perspective, the EBF recommends that the surrogate matrix approach should be utilized wherever possible. Only in cases where the validation does not pass acceptance criteria when using this approach, and when also additional method development does not yield acceptable validation results, is the use of the same preclinical matrix recommended for calibration samples and for sample dilution. The development of assays with surrogate matrix for both calibration lines and sample dilution can be demonstrated in validation to have no impact on the data generated using the assay and has significant ethical advantages in reducing the use of animals in drug development.

The EBF would welcome further discussions with the regulatory authorities in support of bringing the issue to a question and answer (Q&A) on ICH M10, aiming at giving additional support for the use of surrogate matrix in bioanalytical testing. The approach is scientifically sound and is also part of an evolving community responsibility to support the 3Rs when bringing safe medicines to patients.

The discussions within this manuscript are limited to chromatographic assays. Within the EBF, a different team is generating experimental data for ligand binding assays and is planning to publish these data at a later time.

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

Disclaimer

The views and conclusion presented in this paper are those of the European Bioanalysis Forum and do not necessarily reflect the representative affiliation or company's position on the subject.

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