Policy article

Drug Testing and Analysis

Published online in Wiley Online Library

(www.drugtestinganalysis.com) DOI 10.1002/dta.1358

Analytical difficulties facing today's regulatory laboratories: issues in method validation

James D. MacNeil*

The challenges facing analytical laboratories today are not unlike those faced in the past, although both the degree of complexity and the rate of change have increased. Challenges such as development and maintenance of expertise, maintenance and up-dating of equipment, and the introduction of new test methods have always been familiar themes for analytical laboratories, but international guidelines for laboratories involved in the import and export testing of food require management of such changes in a context which includes quality assurance, accreditation, and method validation considerations. Decisions as to when a change in a method requires re-validation of the method or on the design of a validation scheme for a complex multi-residue method require a well-considered strategy, based on a current knowledge of international guidance documents and regulatory requirements, as well the laboratory's quality system requirements. Validation demonstrates that a method is 'fit for purpose', so the requirement for validation should be assessed in terms of the intended use of a method and, in the case of change or modification of a method, whether that change or modification may affect a previously validated performance characteristic. In general, method validation involves method scope, calibration-related parameters, method precision, and recovery. Any method change which may affect method scope or any performance parameters will require re-validation. Some typical situations involving change in methods are discussed and a decision process proposed for selection of appropriate validation measures. © 2012 John Wiley & Sons, Ltd.

Keywords: validation; multi-residue; scope; extension; change

Introduction

There are at least six critical areas which may be identified as posing challenges for regulatory laboratories in the current environment. These include recruitment and retention of staff, staff development, capital budgets for equipment purchase, operating budgets, facility infrastructure, and management of change. Failure to meet needs in any of these areas can lead to a serious weakness which, if uncorrected, may compromise the credibility of a laboratory and create a situation which will be difficult to correct in the short term.

Recruitment and retention of staff can involve significant amounts of time for a local manager in terms of preparation of work descriptions for positions, obtaining approval to fill new or vacated positions, preparation of materials for use in interviews, assessment of candidates and then introduction of new staff to the work environment. Retention of staff also requires significant effort, not only in building trust-based relationships with employees, but also in providing an environment where their career aspirations are addressed, including access to training that is directly related to current assignments, such as equipment-specific training, as well as more general training that enhances the overall knowledge of the employees and their ability to take on new tasks or roles within the organization. Purchase of capital equipment is also time-consuming, requiring time spent in determining the capabilities of equipment available from different manufacturers, assessment of requirements, preparation of specifications to meet the analytical needs, and then assessment of competing bids. Much of this work may be done in conjunction with professionals in a purchasing office, but the technical knowledge of the user is a critical element in ensuring that the best choice is made within the available budget. Planning for laboratory renovations and upgrades is also time-consuming, as is the time spent in balancing various needs within the available operational budget.

Unfortunately, many of the budgetary decisions that affect these five areas are made above local level, so the local manager does not always make the decision to staff vacant positions, to provide training, to purchase equipment, to increase spending on supplies and equipment maintenance, or to renovate the laboratory to meet new requirements. Instead, the local manager is charged with implementation of these decisions and finding ways to meet the operational needs within the budget limitations imposed by higher levels within the organization. While some aspects of these five critical areas will be discussed, there are limits as to how much influence the local manager can ultimately have on the decision-making process. The sixth area, management of change, is the area in which the local manager may be able to exert greater influence and therefore this area, specifically the challenges of maintaining method validation requirements within an accredited laboratory framework, is the main topic of the discussion which follows.

Personnel issues

People are perhaps the most critical element in determining whether a regulatory laboratory involved in the import and export testing of foods succeeds in its mandate, producing reliable

* *Correspondence to: James D. MacNeil, Scientist Emeritus, Canadian Food Inspection Agency, c/o Canadian Food Inspection Agency, Dartmouth Laboratory, 1992 Agency Drive, Dartmouth, NS B3B 1Y9, Canada. E-mail: jdm4533@eastlink.ca*

Department of Chemistry, St Mary's University, Halifax, NS, Canada

results and meeting international expectations for the operations of these laboratories, such as CAC/GL 27–1997.[1] Demographics can provide challenges in this regard. For example, the 5-Year Renewal Plan 2008–2013 for the Canadian Food inspection Agency projected retirement of up to 22.8% of staff during that 5-year period and stressed the importance of employee retention and recruitment activities, as well as staff development.[2] Loss of experienced scientists or analytical specialists from a laboratory creates knowledge gaps and loss of professional contacts which require time and resources to replace. When such individuals leave the laboratory, they take with them an intimate knowledge of the science supporting the regulatory issues with which they have been involved. In addition, they also frequently take with them access to a personal network of national and international contacts with other regulatory scientists in the field. While some of these contacts may be job-related and therefore remain in place for a new incumbent in the vacated position, many such contacts are more personal in nature, having been built through both common interests and confidence gained over years of work within scientific societies, committees, exchange visits, and cooperation on projects. Such networks of contacts are important sources of information on new developments in regulatory work and the maintenance of these networks is a challenge which must be faced when scientists vacate positions in which they have served for a number of years.

Challenges also must be faced when bench-level expertise is lost. Regulatory work requires some specialized knowledge and training which is not always found in new graduates from universities and technical colleges. It is an unfortunate reality that educational institutions often do not have equipment available that is used on a daily basis by staff in a regulatory laboratory. While a graduate may have had some theoretical training in chromatography and mass spectrometry, access to instruments to gain practical experience may be, at best, limited, unless it is gained through an assignment in a cooperative programme, where several terms are spent in work placements, or during summer employment. Thus, when an analyst expert in liquid chromatography-tandem mass spectrometry (LC-MS/MS) is lost to retirement or change of employment, a replacement may need not only some specific training in operation of the instrument used in the laboratory, but also some general training not provided as part of their academic experience. In addition, recent graduates in North America typically receive little exposure to the quality assurance issues which are part of the work environment in a regulatory laboratory. Each time an employee ends employment at a regulatory laboratory as a scientist, analyst, or technician, they take with them years of experience and knowledge that must be somehow transferred or re-acquired by the new employee who replaces them. While in some cases, this may be achieved through the development of other staff prior to the departure, in other cases there may be no opportunity for knowledge transfer and mentoring. When demographics or other factors result in the departure of a number of key individuals within a relatively short timeframe, the challenges faced by the laboratory in maintaining expertise, networks of contacts, and delivery of work commitments can be significant.

Financial resources

This is where another major factor, financial resources, can come into play. There are generally three distinct resource areas which can effect the operations of a regulatory laboratory – operating funds for materials, supplies and staff development; funds to purchase equipment; and funds to maintain and, when needed, renovate and upgrade the laboratory facility. The recent recession has not made things easier in terms of resource availability, as governments worldwide are generally trying to reduce costs following expenditures over the past two years to counter the recession. This may have an effect on laboratory budgets, putting pressure on staffing, operations, equipment replacement, and facility upgrades. When the operating budget does not increase at the same rate as the increase in the cost of laboratory supplies, for example, then the only means by which a government regulatory laboratory can deal with this are to either find a cheaper way to perform some tests or reduce the number of tests delivered. There may be pressure on salary budgets which delay or prevent staffing to replace employees who have retired or resigned, again not only leading to reductions in test or project commitments, but also causing longer-term problems with the transfer and maintenance of knowledge and expertise mentioned above. Delays in staffing of vacant positions or lack of training funds both contribute to the loss of knowledge and expertise, with the result that it may take some years to fully recover from any staff turnover in key positions.

Maintenance of a laboratory's equipment base, with replacements on a planned life cycle basis and additions of new technologies as they become the accepted approach for a particular analysis, is expensive. Indeed, it has been suggested that equipment maintenance is the most costly item in a laboratory's budget other than personnel.[3] In my experience, a typical life cycle for chromatographs and mass spectrometers under routine use in a regulatory laboratory is about seven years, although mass spectrometers are frequently operated for longer periods due to the costs of replacement. However, there are usually capital costs involved in extending the life cycle of an instrument, such as updates to software, addition of accessory devices (such as computer upgrades, new LC-MS interfaces or MS detectors), as well as increasing costs for maintenance and repair. When an LC-MS/MS instrument is required to deliver a specific number of tests on a monthly or annual basis, and that instrument is subject to increasing downtime for maintenance and repair, then delivery of test commitments is compromised, particularly if there is no time available on a similar instrument to maintain the regular flow of analyses.

The addition of modern equipment can pose other problems for a regulatory laboratory, as both the footprint of the instrument and the services required for operation may be different from equipment in use when the laboratory was designed and built. For example, when an analysis previously conducted using LC/UV is replaced with a method based on LC-MS or LC-MS/MS, not only may the equipment require more bench space, bit also the power requirements may be different, a supply of nitrogen may be required that was not previously needed, additional heat may be produced that must be managed and therefore the result may be a laboratory renovation project which requires both planning and budget authorization prior to the work being done to accommodate the new instrument. With the changes in instruments typically used in residue analysis shifting from LC to LC-MS and LC-MS/MS over the past decade, a laboratory may find that it is undergoing almost continuous renovations or trying to accommodate new instruments in areas not ideally suited for their use, both of which are disruptive and result in loss productivity.

Management of change

These challenges are not necessarily different from those faced by regulatory laboratories in past decades, although the convergence of events (demographics, changing technologies, recession) may be particularly challenging at this time for some laboratories. However, all regulatory laboratories face a common challenge of managing change, particularly in the area of scientific and technical requirements. A guideline issued by the Codex Alimentarius Commission[1] recommends that laboratories involved in the import and export testing of foods should be accredited under ISO-17025[4] or equivalent, should have a QA/QC system in place which meets international guidelines,[5] should participate in proficiency testing programmes (when available) which meet international guidelines[6] and should use suitably validated methods. It is this latter requirement which proves most challenging for many laboratory staff. Basically, the questions posed relate to how much validation is needed and in what circumstances validation is required.

There are various approaches to method validation in different disciplines, such as pharmaceutical analysis and food analysis, which have been reviewed in a recent publication.[7] The Codex Alimentarius Commission has adopted[8] the guidance issued by the International Union of Pure and Applied Chemistry (IUPAC) with respect to the validation of analytical methods within a single laboratory.[9] Additional guidance on the 'criteria approach' for validation of analytical methods within a single laboratory is contained in the Codex Alimentarius Commission Procedural Manual.[10,11] However, these guidance documents are basically directed at the initial validation of an analytical method. Practical issues arise which include the interpretation of the guidance to the validation of methods applicable to multiple analytes and matrices, the extension of methods to include new analytes and/or matrices and the analysis of matrices which are received infrequently and therefore not included in the initial method validation scheme. Another question which frequently arises is whether a change in a method is sufficiently significant to require re-validation of the method. The focus of this discussion is on these latter issues, leading to the development of a practical approach which may be taken to determine what should be included by a laboratory in a validation plan for:

a) a multi-residue method;
b) an extension of scope for a validated method; and
c) a change in a method.

Validation of a multi-residue method (MRM)

A multi-residue method (MRM) is one which incorporates an undefined number of analytes (pesticides, veterinary drugs, and/or contaminants), typically three or more,[12] and frequently also more than one sample matrix. It may be used as a method for detection of the target analytes, quantification of target analytes, and/or confirmation of the presence of target analytes. Thus, the validation design should be appropriate to the intended use. An additional consideration is one of practicality.[13] The development of an MRM frequently requires some compromises, so it is unrealistic to expect that all analyte/matrix combinations included within the scope of the method will meet a common performance standard. Since MRMs are frequently used as screening methods,[14] the first consideration therefore is demonstrating that the method is capable of detecting all target analytes in all target sample matrices at concentrations below a required action level with a specified degree of confidence. Different performance should be expected for the various analyte/matrix combinations included in the scope of the MRM. For analyte/matrix combinations which appear during method evaluation to meet recovery and precision requirements for quantitative analysis,[11] validation experiments are required to demonstrate quantitative performance. The detection system used may also provide sufficient information to enable the identification (confirmation) of specific analytes, when present. For these analytes, the validation scheme should incorporate selectivity experiments to demonstrate the suitability of the method for confirmation of analyte identity.[14–18]

Some issues discussed in the validation of a multi-residue method for veterinary drug residues in multiple matrices have been discussed in a recent paper,[19] particularly validation to the requirements for official residue control laboratories subject to the requirements of European Commission Decision 2002/657/EC.[14] Different requirements laid down in the EU requirements for validation of methods used for permitted drugs as opposed to prohibited substances, lack of emphasis on the dynamic range of the instrument, as well as lack of clear guidance on the number of replicates required for MRMs were all identified as problems related to this specific guidance document. These illustrate the problems associated with preparation of a validation guidance document if it is written in a prescriptive manner, based on the state of knowledge and techniques in common usage at the time of writing. Such documents are overtaken by rapid changes in technology and therefore require a regular schedule for revision to reflect more contemporary practices.

More generic recommendations on the validation of MRMs, based on the use of representative analytes and representative matrices, were made by an expert consultation sponsored by the Food & Agriculture Organization of the United Nations (FAO), the International Atomic Energy Agency (IAEA), the International Union of Pure & Applied Chemistry (IUPAC), and AOAC International.[20] The consultation recognized that most laboratories do not have the resources available to fully validate methods intended to be used for the detection of large numbers of analytes, such as pesticide MRMs used in food testing which typically target in excess of 100 compounds and are applied to diverse matrices. The consultation recommended that full validation should be conducted for selected analytes representative of the analytes included in the method and for matrices typical of the sample materials received routinely for analysis. Selection of representative analytes would be based, in large measure, on the expectation that those analytes were most likely to be encountered in typical samples, while representative matrices would be chosen from commonly received sample materials, based on the food triangle approach.[21,22] Thus, a leaf lettuce might be chosen to represent green, leafy vegetables and an orange might be chosen to represent citrus fruit. The approach suggested by the Miskolc Consultation was subsequently adopted by the Codex Alimentarius Commission[23] and the European Commission[24] in guidelines for the validation of MRMs for pesticides and is also being considered in guidance being drafted for the validation of multiresidue methods for veterinary drugs.[12]

Using the representative analyte/representative matrix approach to validation of MRMs, full method validation for quantitative analysis is conducted on the analytes considered to be of highest risk to consumers and those most likely to be encountered in typical samples, while the representative matrices are chosen to cover the range of commodities which may be received for analysis, again

with a focus on those considered most likely to contain residues in excess of permitted concentrations. The general approach taken in such a validation scheme is:

1. Identify the analytes and matrices to be included in the scope of the MRM.
2. Conduct initial experiments to demonstrate the chromatographic separation and identify detection capabilities (target ions for mass spectrometry or target wavelengths for detection) of all analytes to be included in the method.
3. Identify the representative analytes and matrices to be used for full validation.
4. Determine the relative response factors for the represented analytes relative to the analytes chosen to represent them. For example, if an MRM included all members of the nitroimidazole class, metronidazole might be selected as the representative analyte from that class and the relative response of other members of the class would be determined so that quantities present could be estimated based on a calibration for metronidazole.
5. Demonstrate that all target analytes can be detected in the various representative matrices selected at or above a specified concentration. For example, it might be decided that to be included in the scope of the method, authorized compounds should be detected at a concentration of one-half the MRL or banned substances should be detected at concentrations exceeding 0.1 ng/g.
6. Conduct a full quantitative validation for all representative analytes and matrices.
7. Conduct a full validation for an analyte when it is detected in a sample which may result in regulatory action or when it is noted that such an analyte is being detected routinely in typical samples.
8. Conduct confirmatory analysis using calibration and/or quality control materials prepared from authentic blank material of that matrix when an analyte is detected in a represented matrix and regulatory action may result. Full validation should be conducted for that matrix if it has become a significant part of the routine samples received or if any results are obtained which suggest that the representative analyte used in validation may not provide results which reflect the analytical performance obtained for the represented analyte. For example, the recoveries obtained for an analyte may be observed to be higher in a specific (represented) matrix, when tested, than those obtained using the representative matrix.

In summary, the intent is to demonstrate that an MRM is broadly applicable, but not to overstate the extent of the validation work conducted. For the analytes and matrices for which full validation has been conducted, quantitative results can be reported with a known confidence. For all other analytes and matrices, it can be stated with confidence that the method can detect those analytes above known threshold concentrations if they are present and can also provide an estimate of the quantity present. However, additional work is required to provide quantitative results with the same confidence that can be provided for those analyte/matrix combinations for which full validation has been conducted. It can be described as a risk-based approach to method validation, where the focus is on the analytes considered most likely to be detected or to cause potential harm to consumers if present, while the matrices selected are those most likely to be analyzed and those considered, based on known industry practices and other information sources, most likely to contain residues.

Extension of scope of a previously validated analytical method

Guidance on the extension of a validated method to include additional analytes and/or matrices was also provided by the Miskolc Consultation.[20] There are two possible situations which may occur in extending the scope of a validated method to include new analytes or matrices. In the simpler situation, no changes in the method are required to incorporate the new analytes or matrices. In these situations, the existing validation is not affected, so validation experiments focus on the method performance for the new analytes or matrices. The work should include an evaluation of analyte stability and all other performance criteria typically evaluated in a method validation, including calibration experiments and an assessment of method selectivity, recovery, and precision for the new analytes or matrices.

However, there are situations when a method extension becomes more complex and modifications to the original method are required. These are dealt with in the following section, where the effects of method change on validation are discussed.

Changes in a method and their effect on validation

There are various situations which may be envisaged in which a change in a method may be required. These include changes in a supplier of materials or instruments used, method modifications to address changes such as the unavailability of a material normally used in the method, modifications to improve sample throughput or method modifications to increase the scope in terms of analytes and/or matrices or to otherwise improve method performance.

It is not uncommon to make what appear to be simple changes in a method due to the availability of supplies, such as solvents or chromatography columns. It cannot be assumed, however, that such changes are trivial and can be made without an investigation of their impact on method performance. The usual approach to be taken when such changes are required is to conduct several sets of analyses comparing performance for replicate sample materials using both the original materials and the replacement materials. When no statistical difference in results is observed using statistical tools such as Students *t*-test or ANOVA, it can be considered that the change has no effect on method performance. For laboratories accredited under ISO-17025 or equivalent, the requirement is to fully document the comparison and to include the supplier of the new material in the laboratory's list of approved suppliers for that material for that method.[4] If the method standard operating procedure (SOP) has specified a particular supplier, this requires amendment either to list the additional supplier or, preferably, by writing a generic specification for the material. For example, instead of stating a particular chromatographic column and supplier, the method might specify the column dimensions, type of packing material, and required resolution for the analytes.

A similar approach may be taken when a suitable replacement for a material used in a method must be found due to a lack of availability of the material used in the initial method validation. For example, a shortage of supply of a solvent may require substitution of an alternative solvent. A manufacturer of an LC column or a solid-phase extraction (SPE) cartridge used in the method may discontinue the product or go out of business, requiring that a suitable replacement be found. As above, the

method performance with the replacement material should be compared with the original method by analysis of a suitable number of replicates (typically 10–20 replicates in 1–2 analytical runs) to determine if any significant difference in results is observed between the original and modified methods. If no significant change is observed, the new suppliers are added to the laboratory's list of approved suppliers and no further validation is required unless subsequent QC results indicate a change in method performance that was not detected in the original verification experiments comparing the changed method with the original. If the verification experiments or QC results subsequent to implementation of the change demonstrate that performance of the method has changed, then re-validation of any performance parameters affected may be required.

Method changes may also be required to incorporate new analytes or matrices or to address other concerns, such as increasing method throughput or replacement of a material with one considered less hazardous or one which reduces environmental concerns. For example, the original method may be based on the formation of a fluorescent derivative for fluorescence detection, but the analytes to be added do not form the required fluorescent derivatives. Therefore, the addition of these analytes requires a change from a fluorescent detector to a mass spectral detector, potentially changing the performance of the method for all analytes. As a second example, a method initially validated for matrices with low lipid content must be extended to include matrices with high lipid content, requiring the addition of steps to remove the lipids prior to the collection of the final extract for analysis. In both cases, the changes required in the method may affect the performance of the method for the previously validated analyte/matrix combinations. Other examples of change include removal of a solvent such as carbon tetrachloride or benzene to reduce risks to workers or reduction of test portion mass and solvent volumes to reduce the amount of waste material requiring disposal. All such modifications or changes have a potential impact on the validation status of the method and the potential impact on the method performance should be carefully assessed. It should not, however, be automatically assumed that such changes require a complete re-validation of the method, as the change may affect only certain performance characteristics, while others may remain unaffected.

A systematic approach to determining the impact of a change in a method on validation status

Rather than assuming that a method change requires complete re-validation of an analytical method, a more scientifically based approach is to evaluate the potential effects of the change on the different parameters usually considered in the validation of a method. The following series of questions may be considered as a basis for such an evaluation:

1. Does the change affect the scope of the method (analytical range, analytes, matrices)?
2. Could the change affect analyte stability, method robustness or critical control points?
3. Could the change affect method selectivity?
4. Could the change affect calibration parameters (analytical range, linearity, sensitivity, LOD, LOQ, CCα, CCβ) or system suitability requirements?
5. Could the change affect precision, recovery or measurement uncertainty?

A step-by-step evaluation using this approach will provide a reasonable and defensible basis on which to determine which performance parameters or other performance criteria associated with the method require re-validation or amendment. Additional guidance on the identification of performance factors which may require consideration in the validation design and common errors made in use of terminology in validation experiments are the topic of a recent paper.[25]

Does the change affect the scope of the method (analytical range, analytes, matrices)?

A typical statement of the scope of the method includes the analytes and matrices to which the method is applicable and the analytical range over which the method has been validated. Other than the changes in scope discussed above, in which the scope was extended to include new analytes and/or matrices, the most common change which could affect the method scope is a change in the analytical range over which the method is considered applicable. When a change in analytical range occurs, such as may happen when the introduction of a newer instrument results in an improved detector response (greater signal-to-noise obtained for a specified quantity of analyte), the extended analytical range should be validated, along with any other parameters affected by the change. These would most probably include the limits of detection and quantification. For laboratories validating methods under the 2002/657/EC criteria, the decision limit (CCα) and the detection capability (CCβ) should be re-validated.[14]

The situation becomes more complex when the change of scope requires modifications to the original method. In this case, the changes made may be such that the original validation experiments are not applicable to the revised method and all performance parameters potentially affected by the change may require re-validation. The exception may be analyte stability data previously obtained, if the changes are not considered such that changes in the stability would occur. For example, if there are no changes in the preparation of analytical standard solutions or their use, there is no reason to repeat these stability experiments. If there are no changes in solvents and reagents used in the method or in the processes used in the extraction and clean-up, there is probably no reason to repeat the 'stability in processing' experiments (simply monitor for indications of change in stability during the re-validation experiments for recovery and precision). Any new analytes or matrices added to the scope of the method must, however, be fully validated, as previously discussed under method extensions.

Could the change affect analyte stability, method robustness or critical control points?

The stability of analytical standards may be affected if, for example, the solvents used for preparation of analytical standards are changed. Such a change requires new experiments to demonstrate the stability of the standards unless stability information is already available from a reliable (citable) source. Changes in solvents, reagents, or other materials used in sample analysis may also affect analyte stability (during processing) and therefore experiments may be required to demonstrate that there is no loss in stability resulting from such changes. A change in analyte stability will typically be revealed as a change in analyte recovery. When such a change is observed, it should be determined if this is a result of a change in analyte stability or

whether it is linked to other causes, such as a failure to fully elute the analyte from an SPE cartridge or loss during a partitioning step. If no other cause can be identified, stability of the analyte during processing should be studied to determine if the change results from a change in stability. A method change that results in a loss of analyte stability may not be considered acceptable. However, if the change is considered necessary, the stability during processing should be carefully documented to control such losses of analyte during routine use of the method. This may include the identification of a critical control point. Changes in a method will not typically affect stability during storage, so these experiments should only be repeated if there is a change in storage conditions for sample material. However, should such studies prove necessary, a discussion of statistical methods to be used in such investigations can be found in a recent paper.[26]

When the method change is in a factor involved in the original ruggedness test, the ruggedness test should be repeated if inconsistent results are observed during performance verification. If the change could affect method ruggedness although no factors from the original ruggedness test design are involved, a ruggedness test should be conducted which includes the new factor(s) related to the change if anomalies are observed during verification. Similarly, if a change involves a critical control point, the effects of the change should be assessed to determine if the critical control point has been affected. This may lead to an amendment of the statement in the method with respect to the critical control point. For example, if the change in the method removes the conditions which required inclusion of a critical control point in the method, that critical control point may no longer be required.

The terms ruggedness and robustness are considered as equivalent within the terminology approved by the Codex Alimentarius Commission for laboratories involved in the import and export testing of foods,[27] but may be defined differently in other areas of work, such as testing of drug formulations. This topic has been discussed in a review, which includes a consideration of the approaches and examples of typical factors included in experimental designs.[28]

Could the change affect method selectivity?

When a change in a method may affect method selectivity, an initial investigation is required to characterize potential interferences. A change in a method may be found to have no affect on method selectivity, which requires no re-validation, it may increase method selectivity or it may reduce method selectivity. The latter two situations may affect the status of the original validation. A change which increases method selectivity, such as the use of a more selective detector, may not require additional validation if verification experiments show no significant changes in method recovery and precision. While such a change may result in a lower LOD and LOQ, it may not be necessary to immediately re-validate these parameters if the concern is performance at an action limit where verification experiments have shown that no significant change in performance has occurred. In such situations, it may be more practical to include additional QC samples with subsequent routine analytical runs to generate the data to re-establish the analytical range, LOD and LOQ. However, laboratories conducting work under the provisions of 2002/657/EC may wish to re-validate the analytical range, CCα and CCβ before resuming analysis of routine samples.[14]

Another typical method change which may result in increased selectivity is the transfer of a method from a conventional LC separation to separation using ultra performance liquid chromatography (UPLC) technology. Such a change may have a greater impact on method performance, as the sharper peaks obtained may affect method calibration factors (analytical range, sensitivity, LOD, LOQ), as well as recovery (separation from co-eluting peaks in LC) and precision. Other factors, such as stability, critical control points and method ruggedness, would not typically be affected. In general, a change from LC to UPLC will require re-validation of the factors related to calibration (analytical range, sensitivity, etc.) and reliability of results (selectivity, accuracy/recovery, precision, measurement uncertainty). Similarly, a change in a method which results in reduced selectivity (such as changing from a mass spectral detector to a UV-detector) requires re-validation of all parameters affected, which will include both those related to method calibration and method reliability.

Could the change affect calibration parameters (analytical range, linearity, sensitivity, LOD, LOQ, CC_α, CC_β) or system suitability requirements?

The basis for a quantitative method is the reliability of the calibration curve and the parameters associated with that curve, such as the analytical range, sensitivity and the LOD and LOQ. For validations conducted according to the requirements of 2002/657/EC,[14] the parameters CC_α (the decision limit) and CC_β (the detection capability) are calculated instead of the LOD and LOQ. Typical changes which could affect these parameters are a change in the calibration procedure, such as switching from a calibration curve using pure standards to a matrix-matched calibration or introduction of an internal standard, or a change in the detector response. A change from external standard calibration to matrix-matched calibration or calibration via internal standard will not affect the method recovery, but it does introduce an automatic recovery correction. In such cases, although the absolute method recovery is unchanged, precision may be affected and require re-validation under the new calibration conditions.

As discussed, there may be instances where changes to LOD and LOQ which could result from a change in the calibration procedure are of less relevance, particularly if they fall one or more orders of magnitude below a regulatory limit that is the primary target of the method. These parameters may be re-established, if the circumstances warrant, by using QC data generated during routine use of the method, rather than by a separate set of validation experiments prior to implementation of the change. However, a change in the sensitivity of the calibration curve may adversely affect the ability of the method to discriminate between concentrations; for example, a loss in sensitivity may mean that a method which previously could distinguish between concentrations in increments of 1 ng/g now can only discriminate between concentrations which differ by 3 ng/g. In such a case, the performance of the method will be significantly affected, requiring a re-assessment of the sensitivity (and whether the method is still fit for purpose) and other parameters, such as precision and measurement uncertainty may also be affected, requiring re-validation for these parameters. When the method is applied to the detection of a banned substance for which no regulatory limit has been established, then the method performance becomes the *de facto* regulatory limit. In such a situation, re-validation of the analytical range, sensitivity, LOD, LOQ (CC_α, CC_β, where applicable) is required, as well as other factors that

may be affected. For example, interferences which were below the previous detection threshold may appear and affect method selectivity. The changes in the calibration curve may also affect other factors; for example, recovery, precision and measurement uncertainty may require validation at the concentrations added to the analytical range.

System suitability refers to the procedures that may be included in a method to demonstrate that the equipment is functioning properly and has been properly calibrated to perform the test.[29,30] The term 'system qualification' is also used in some publications.[25] System suitability statements typically are written for regulatory methods to identify a minimum acceptable chromatographic separation (retention time, with a ± specification, for each analyte and a minimum peak separation between analytes) and detector performance (a minimum signal-to-noise response for injection of a specified quantity of the analyte or a test material). A change in column may require a revision of system suitability requirements for the chromatographic separation, while a change in the detector may require a modification of the system suitability statement for detection. Such changes, while not strictly part of the method validation, should be included in the method SOP.

Could the change affect precision, recovery or measurement uncertainty?

Some method changes have been discussed where recovery, precision, and/or measurement uncertainty may also be affected due, for example, to a change in the analytical range or sensitivity of the calibration curve. There are other changes in a method that may not directly affect the calibration curve, but may directly affect method recovery or precision and, thereby, the reliability of the analytical result. Perhaps the most obvious example that could be considered is the need to change an extraction or partitioning solvent or the need to switch to a different solid phase extraction cartridge. In both cases, both the recovery and the consistency of recovery (precision) may be affected by the change and therefore these parameters will require re-validation once the change has been optimized. A change in the method which extends the analytical range will also require validation of analyte recovery and precision at representative concentrations within the extension of the range, but may not require re-validation of recovery and precision at points previously validated within the original range if the change should have no effect on method performance at those concentrations. When recovery and precision change, this also affects measurement uncertainty at the concentrations where this occurs, so measurement uncertainty should also be determined, based on the new data generated in the validation. In some circumstances, it may be appropriate instead to verify the continued performance of the method at those concentrations by the use of QC data.

Conclusions

Method validation should not be approached as some form of black box where a set of experiments are conducted by rote and results tabulated without full consideration of the circumstances and the requirements associated with them. The basic purpose of method validation is to demonstrate the fitness for purpose of a method to meet specific analytical requirements. When those needs change or the method itself is changed, additional validation or re-validation for certain performance factors may be required. The need for such validation experiments can be defined by a critical examination of what has changed, how this change may affect method performance and what performance factors require further validation. Complete re-validation of a method is not necessarily the outcome of every change or modification which may be made during the use of a method in a laboratory. For method extensions or the design of validation experiments for MRMs, a similar approach should also be considered which reflects the intended use and expected range of application of the method. An approach which attempts to cover every possible eventuality will result in an unnecessary expenditure of resources and frustrated staff. The validation design chosen should itself be fit for purpose and address the fundamental requirement that it supports the claims which the laboratory will make for performance of the method.

Conflicts of interest

The author received support from the OECD to cover cost of travel and attendance at the meeting where the paper was presented.

References

[1] CAC (1997) CAC/GL 27–1997, Guidelines for the Assessment of the Competence of Testing Laboratories Involved in the Import and Export Control of Food. Codex Alimentarius Commission, Joint FAO/WHO Food Standards. Available at: http://www.codexalimentarius.net/download/standards/355/CXG_027e.pdf [19 April 2010].
[2] CFIA. CFIA Renewal Plan 2008–2013, Canadian Food Inspection Agency. Available at: http://www.inspection.gc.ca/english/hrrh/renpla/renplane.shtml [5 May 2011].
[3] L.W Collins. Managing laboratory maintenance. *American Laboratory* **2006**, *38*, 20.
[4] ISO/IEC-17025(E), General Requirements for the Competence of Calibration and Testing Laboratories, 2nd Edition. International Organization for Standardization, Geneva, **2005**.
[5] M. Thompson, R. Wood. Harmonized guidelines for internal quality control in analytical laboratories. *Pure Appl. Chem.* **1995**, *67*, 649.
[6] M. Thompson, S.L.R. Ellison, R. Wood. The international harmonized protocol for the proficiency testing of (Chemical) analytical laboratories. *Pure Appl. Chem.* **2006**, *78*, 145.
[7] D. Stöckl, H. D 'Hondt, L.M. Thienpont. Method validation across the disciplines: Critical investigation of major validation criteria and associated experimental protocols. *J. Chromatogr. B* **2009**, *877*, 2180.
[8] GL 49–2003. Harmonized IUPAC Guidelines for Single-Laboratory Validation of Methods of Analysis, Joint FAO/WHO Food Standards Program. Available at: http://www.codexalimentarius.net/download/standards/10256/CXG_049e.pdf [19 April 2010].
[9] M. Thompson, S.L.R. Ellison, R. Wood. Harmonized guidelines for single-laboratory validation of methods of analysis. *Pure Appl. Chem.* **2002**, *74*, 835.
[10] Joint FAOWHO Food Standards Programme, General criteria for the selection of single-laboratory validated methods of analysis, in Codex Alimentarius Commission Procedural Manual, 20th edition. Available at: ftp://ftp.fao.org/codex/Publications/ProcManuals/Manual_20e.pdf [8 August 2011].
[11] Joint FAO/WHO Food Standards Programme, Working instructions for the implementation of the criteria approach in Codex in Codex Alimentarius Commission Procedural Manual, 20th edition. Available at: ftp://ftp.fao.org/codex/Publications/ProcManuals/Manual_20e.pdf [8 August 2011].
[12] Joint FAO/WHO Food Standards Programme, Discussion Paper on Methods of Analysis for Residues of Veterinary Drugs in Foods, CX/RVDF 10/19/6, Codex Alimentarius Commission. Available at: ftp://ftp.fao.org/codex/ccrvdf19/rv19_06e.pdf [15 June 2011].
[13] Joint FAO/WHO Food Standards Programme, General criteria for the selection of methods of analysis, in Codex Alimentarius Commission Procedural Manual, 20th edition. Available at: ftp://ftp.fao.org/codex/Publications/ProcManuals/Manual_20e.pdf [8 August 2011].
[14] Commission Decision of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results (2002/657/EC).*OJEC* **2002**, *L221*, 8.

[15] R. Bethem, J. Boison, P. Gale, D. Heller, S. Lehotay, J. Loo, *et al*. Establishing the fitness for purpose of mass spectrometric methods. *J. Am. Soc. Mass Spectrom*. **2003**, *14*, 528.
[16] USFDA (**2003**) Guidance for Industry 118: Mass Spectrometry for Confirmation of the Identity of Animal Drug Residues. Final Guidance. US Department of Health and Human Services, Food and Drug Administration, Center for Veterinary Medicine. Available at: http://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/UCM052658.pdf [25 March 2011].
[17] Joint FAO/WHO Food Standards Program, CAC/GL 56–2005, Guidelines on the Use of Mass Spectrometry (MS) for Identification, Confirmation and Quantitative Determination of Residues, Codex Alimentarius Commission. Available at: http://www.codexalimentarius.net/download/standards/10185/cxg_056e.pdf [9 August 2010].
[18] Joint FAO/WHO Food Standards Program. CAC/GL 71–2009, Guidelines for the Design and Implementation of National Regulatory Food Safety Assurance Programme Associated with the Use of Veterinary Drugs in Food Producing Animals. Available at: http://www.codexalimentarius.net/download/standards/11252/CXG_071e.pdf [19 April 2010].
[19] A. Kaufmann. Validation of multiresidue methods for veterinary drug residues: Related problems and possible solutions. *Anal. Chim. Acta* **2009**, *637*, 144.
[20] L. Alder, P.T. Holland, J. Lantos, M. Lee, J.D. MacNeil, J. O'Rangers, *et al*. Guidelines for single-laboratory validation of analytical methods for trace-level concentrations of organic chemicals, in Principles and Practices of Method Validation, (Eds: A. Fajgelj, A. Ambrus), The Royal Society of Chemistry, Cambridge, UK, **2000**, pp. 179–248.
[21] D.A.T. Southgate. Reference material for improving the quality of nutritional composition data for foods. *Fresenius Z Anal. Chem*. **1987**, *326*, 660.
[22] W.R. Wolf. History of reference materials for food and nutrition metrology: As represented in the series of BERM symposia. *Anal. Bioanal. Chem*. **2010**, *397*, 413.
[23] Joint FAO/WHO Food Standards Program. CAC/GL 40–1993, Rev.1-2003: Guidelines on Good Laboratory Practice in Residue Analysis. Available at: http://www.codexalimentarius.net/download/standards/378/cxg_040e.pdf [15 June 2011].
[24] Directorate General for Health and Consumers, European Commission Method Validation and Quality Control Procedures for Pesticide Residues Analysis in Food and Feed, Document No. SANCO/10684/2009 (Supersedes Document No. SANCO/3131/2007, Implemented by 01/01/2010). Available at: http://ec.europa.eu/food/plant/protection/pesticides/docs/qualcontrol_en.pdf [12 July 2011].
[25] P. Araujo. Key aspects of analytical method validation and linearity evaluation. *J. Chromatogr. B* **2009**, *877*, 2224.24.
[26] D. Hoffman, R. Kringle, J. Singer, S. McDougall. Statistical methods for assessing long-term analyte stability in biological matrices. *J. Chromatogr. B* **2009**, *877*, 2262.
[27] Joint FAO/WHO Food Standards Program, CAC/GL 72–2009, Guidelines on Analytical Terminology. Codex Alimentarius Commission. Available at: http://www.codexalimentarius.net/download/standards/11357/cxg_072e.pdf [20 April 2010].
[28] B. Dejaegher, Y. Vander Heyden. Ruggedness and robustness testing. *J. Chromatogr. A* **2007**, *1158*, 138.
[29] International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, Validation of analytical procedures: Text and methodology Q2(R1). Available at: http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1__Guideline.pdf [20 March 2011].
[30] International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medicinal Products, Secretariat c/o COMISA, Validation of Analytical Procedures: Methodology, VICH GL2. Available at: http://www.vichsec.org/pdf/gl02_st7.pdf [20 March 2011].