A MODEL APPROACH TO METHOD VALIDATION

Single method validation is a complex process with endless variations - but what happens when the situation is further complicated by an extensive product portfolio and a limited timeframe? Here, Rachel Reid explains how this challenging set of circumstances led to a forward-thinking solution.

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There are many reasons for developing and validating methods for quantifying vitamins in supplements or food/drink products. From the need to comply with new or updated legislation, or substantiate health claims, to the adoption of new processing techniques or the latest on-trend ingredients, industry innovation drives change. This means product developers constantly need to ensure the analytical methods in place remain accurate and fit for purpose.

Whether the product in question is a brand new concept or a revamped version designed to widen its appeal, the basic principle remains the same; is the vitamin/active in the finished product still present in sufficient quantity at the end of its shelf life to satisfy the labelling claim?

A seemingly simple question, but one which raises a series of challenging issues. And this is largely due to the inherent complexity of vitamins and the infinite variations of the product formulations themselves.

There is no 'one size fits all' approach. So what's the answer?

Vitamin variation

The route to an effective method starts with understanding the chemistry of the chosen vitamin in the specific matrix type. A request for a method to validate vitamin A in a protein drink, for example, must be accompanied with a comprehensive ingredient list and recipe. This is because much of the development work needs to concentrate on how to actually extract the compound of interest (COI) from the product matrix, rather than the instrumentation used for the final analysis which is known to be technically accurate.

Some of the vitamins, for example, are light sensitive. Samples therefore need to be prepared in a dark room with the use of amber glassware, which provides some protection from ultraviolet light and so helps to limit degradation. Vitamin C degrades through exposure to heat, light or oxygen – so part of the method needs to focus on limiting the length of time from extraction of the sample to instrument analysis. The product format also has an influence. Extracting a vitamin from a supplement capsule, for example, will require a different approach to the one taken to isolate the same active from an energy bar.

While such steps provide a useful foundation to work from and are routinely adopted, they are by no means enough to deal with the sheer number of potential variables which must be addressed on a case-by-case basis. Vitamin forms are a case in point. Vitamin E, for example, is available in synthetic and natural form, with the latter also present is a further three possible chemical structures. Equally, vitamin D can be specified as either the more commonly used, animal-derived D3 or its vegan-friendly D2 alternative.

Different levels of solubility, encapsulation and even the potential for interference or loss during manufacturing must also be carefully considered. But it doesn't stop there. The method also needs to take into account a whole range of issues beyond the actual ingredient formulation such as packaging and storage conditions. Without forgetting the implications of geography; a product is likely to react very differently when subject to a hot and humid environment compared to cold temperatures; meaning there is considerable potential for change and loss of stability over time.

These diverse parameters dictate which extraction approach is taken. A range of in-house RSSL methods provide a sound basis, with adaptations - or optimisations - then made to tailor the final approach according to the individual product.

Viewed in this context, it's clear that the validation part of the process is equally important in order to ensure the chosen method is not only robust, but also provides the accuracy and repeatability required to meet the stringent demands of the relevant authorities as well as quality control.

Method validation in action

By necessity, this is a thorough process which - even with efficiencies built in - has the potential to be time consuming and costly. As an indication, single method development/optimisation and validation typically takes approximately ten days from brief to completion. If multiple validations are required, it would take significantly longer but efficiencies are built within the project. Given the level of complexity and potential for variable factors, this timeframe is generally planned into overall product development programmes.

But what if it can't be?

Sometimes it's not possible to work within these standard parameters. Commercial pressures and changing business strategies create new challenges and a different dynamic for product developers to manage.

This is certainly the case for a number of clients which require method development and validation for multiple products at the same time to ensure their production and label claim is substantiated.

An example of a brief to our technical team was to develop and validate two analytical methods suitable for the quantification of three vitamins. Not surprisingly, this was far from straightforward due to differences in ingredient formulation, the vitamin forms used and mixture of three different product formats e.g. powders, bars, tablets and syrups. This level of complexity meant it was not possible to validate all the products within the given timeframe; a single validation approach simply wasn't an option. So the RSSL team and the customer came out with a solution to develop a bespoke framework for this particular challenge, designed to balance commercial demands with scientific accuracy.

To reduce the number of validations required and meet the desired deadline, a risk-based approach was taken. This meant not only developing the right method, but also formulating a number of "worst case scenario" models for each of the ingredients in the products which were based on the highest concentration levels; those with strongest impact. For some products, the use of products with a more comprehensive list of ingredients was sufficient to represent a range. This ensured the subsequent models or selected products produced were accurate for the assay of the vitamins in the matrix and the other ingredients present did not interfere with the analysis.

Products were grouped according to their format type and then by similar ingredients and quantities. This meant client communication was a fundamental part of the process. For each product, we required a full ingredient listing and recipe breakdown with corresponding quantities to ensure that the proportions for each model or selected product was correct. So if the recipe demanded 60% bulking agent, this needed to be replicated along with the equivalent percentage of actives.

These models or selected products were then used to represent the product range and used for method validation. This significantly reduced the time and numbers involved in the project, from a high number of individual products to a quarter the amount of models requiring method development and validation.

Project overview

In summary, the project was split into four stages:

- **Phase 1**: Model preparation and selection of representative products: This involved collating all the product data from the client, developing a model on paper for approval before progressing to the physical preparation of each model. A blank was also prepared for each model to help with validation later on. This blank would contain all ingredients except the actives.
- **Phase 2**: Method feasibility: In-house methods were used to analyse all the models and blanks, while also providing some feasibility. Each was then assessed in terms of its suitability for validation.
- **Phase 3**: Method optimisation / development: In some instances, low level optimisation of the RSSL in-house method was required; which involved making adaptations to ensure the final method was suitable for validation. Remedial actions included optimising the extraction technique, for example, or modifying the chromatography conditions.
- **Phase 4**: Method validation: The final stage involved; specificity, linearity, accuracy, method precision (repeatability), robustness as well as limit of detection and limit of quantification.

By taking a pragmatic approach and breaking a complex situation down into bite size pieces, the RSSL team has successfully collaborated with a number of clients to achieve the desired outcome – analytical methods that were demonstrated to be fit for purpose for a wide range of vitamins and product types.

Looking ahead

These particular type of projects were focused on vitamins but it proved highly informative in that it opened up new ways of working which can now be translated to other actives in the food and drink categories.

But while advances in technology may mean the arrival of more sensitive instrumentation and sophisticated software packages, when it comes to the food and drink industry there is no substitute for practical expertise and knowledge. The complexity of new and improved recipes - across all categories - means that although such technical tools are undoubtedly a valuable support, they are only part of performing a method development and validation exercise. A solid understanding of chemistry, food formulation and instrumentation will always be required to reach a successful solution.