

Key to Outsourcing Method Development and Validation: A Pragmatic Approach

White Paper

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Introduction

In an industry that is seeing an increasing level of work being outsourced, the Contract Research Organisation (CRO) of choice needs to have proven experience in both the pragmatism and flexibility of the method developer’s mind set and a regulatory background in validation.

As companies are focussing on achieving ever shorter times of drug to market, it is vital that a tailored, pragmatic approach is adopted when engaging in both method development and validation activities for an Active Pharmaceutical Ingredient (API) or drug product (DP).

Although methods still require a high degree of robustness, the overall strategy should encompass a full evaluation of the regulatory requirements applicable

to the particular phase of the drug life-cycle; this is pivotal in order to ensure a successful regulatory submission, where the applicant must demonstrate suitable validation of all methods used to support the filing.

Successfully developed and validated analytical methods can reduce overall turnaround times spanning from pre-clinical right through to commercial release, with a well-developed method underpinning a robust product. Starting with the end point in mind, methods should have the desired flexibility built in during the early stages to allow easy translation from API to DP, thus potentially reducing costs throughout the product life-cycle.

Figure 1. below gives an overview of the drug life-cycle and illustrates typical validation parameters to be assessed at each stage.

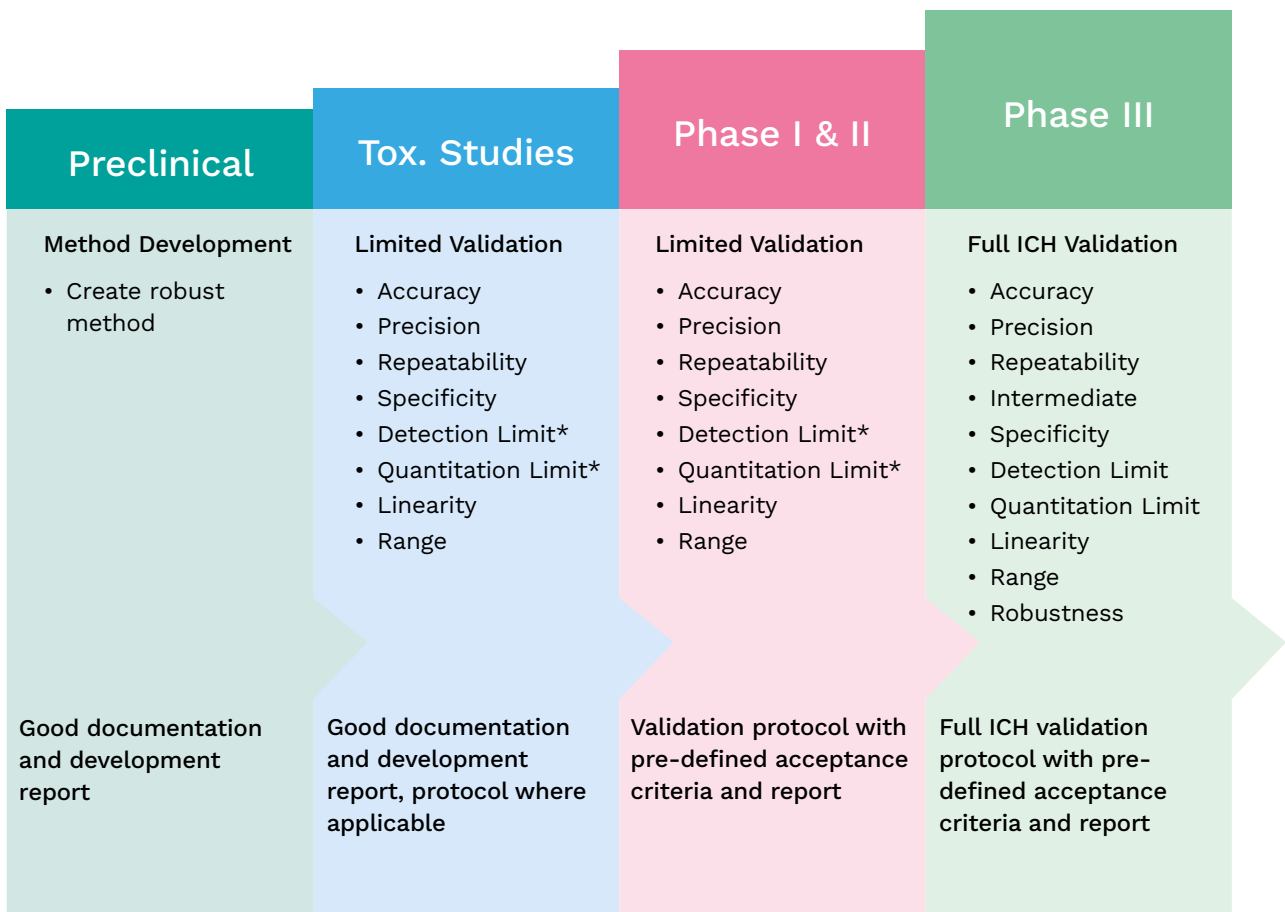


Figure 1: Drug life-cycle validation requirements

Method Development Strategy

Reliable and reproducible analytical methods are essential throughout the pharmaceutical development process and need to be capable of measuring the potency, purity and stability of the final drug product.

ICH Q2 (R1) - Validation of Analytical Procedures: Text and Methodology defines that “The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose”, therefore prior to initiation of any method development activity, the ‘purpose’ needs to be established. Some of the fundamental considerations are shown in Figure 2.



Figure 2: Considerations for initiating method development

Although method development activities are applicable to a variety of analytical techniques, this paper focuses on High-Performance Liquid Chromatography (HPLC).

The development strategy can be exemplified as given in Figure 3, and is often cyclical in nature depending on the findings throughout the development itself.

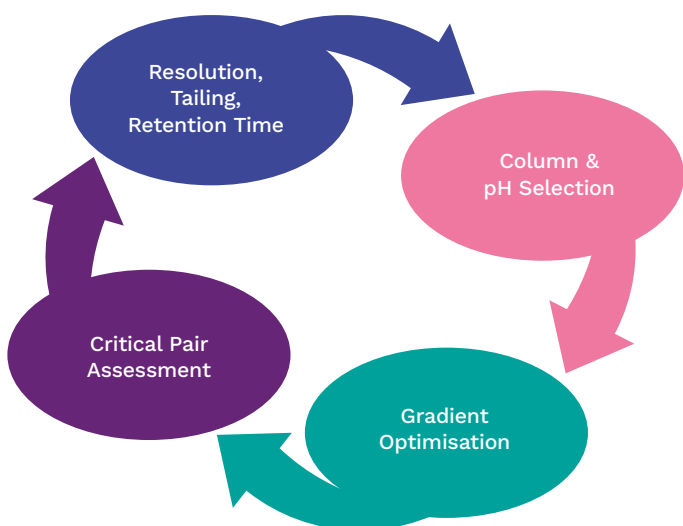


Figure 3: Steps to achieving a suitable chromatography

Some initial questions that the method developer should be asking are:

- What is the intended outcome for the method? Is it solely for determination of assay or will it be required for the measurement of related substances also? If the latter is also required, what level of sensitivity is needed (which would be driven by specification requirements)?
- From what matrix does the compound need to be extracted or analysed? The nature of this may well have a significant impact upon the desired sensitivity of the method.
- If the method is for DP, what is the intended dose(s) and presentation (i.e. capsule (soft/hard gel), tablet, i.v. solution or topical (cream/gel/ointment)?

Consider the scenario where a client has a requirement to develop a reverse phase (RP) HPLC method for the assay and related substance determination of an API (hydroxamic acid). The aim is then to eventually formulate this into a hard gelatin capsule (containing Swedish orange as a colourant).

Selecting appropriate samples for the method development is paramount since they should provide a ‘worst-case’ scenario in terms of reflecting all potential impurities. This allows the method developer to ensure (along with physical/chemical stressing), that the method is specific and stability-indicating, i.e. that all known related substances are sufficiently well resolved from one another and the active peak. Samples should ideally be impure development batches, representative of the final synthetic route (API) and/or manufacturing process (DP). The use of mother liquors, stressed samples, filtrates and stability samples is also recommended.

Assuming a solubility screen has been performed to determine a suitable injection solvent, the first step is to evaluate the chemistry of the analyte molecule. This would include scrutiny of any potential ionisable groups, basic functionality (in this case, the hydroxamic acid), together with an evaluation of the pKa data, to determine if pH control is necessary (e.g. the use of buffers). An appropriate column technology should then be selected for initial screening; if screening with in-house columns, which have been used for previous analyses, ensure that the usage history is appraised (i.e. assess the impact of previous use of ion-pair reagents, which alter the nature of the stationary phase irreversibly). Since the compound is a hydroxamic acid, consideration should be given to the potential for secondary retention arising from interaction between acidic silanols within the column stationary phase and the nitrogen atom from the hydroxamic acid moiety of the API molecule. This particular type of interaction would manifest itself as broad tailing peaks. Adaption of a combination of the column chemistry, pH control and addition of a low level modifier such as triethylamine (TEA) may assist in reducing this secondary analyte retention.

Initiation of the development would typically include the use of scouting gradients using a simple mobile

phase composition (e.g. acetonitrile/water), not adding modifiers or buffers, unless absolutely necessary. A 'keep it simple' approach is always advisable to maintain future robustness of the method. 'In silico' modelling can also be used, but is not essential.

The use of a scouting gradient offers a number of advantages in the early stages of the development as it enables the potentially wide-range of polarities of analyte and related substances to be suitably resolved, as well as eluting the more non-polar components in a reduced run-time.

It is pivotal from the very early stages that flexibility/robustness is maintained in order for the method to encompass any changes that may occur with the dose and/or the type of presentation. Take the instance whereby an API assay and related substances method is being optimised to reduce the run time; whilst it is ideal to have as short a run time as possible, removing too many portions of the redundant baseline leaves far less scope for synergy in the future; if/when the API is formulated into DP, the presence of multiple excipients could pose issues if the API method is refined too much. Incorporating flexibility/synergy in the analytical method will offer multiple cost savings throughout the drug development life-cycle, including lower overall costs for development and QC release (where a single method could be employed for both API and product), both of which will also reduce analyst training needs.

In comparison to establishing the chromatographic conditions (Figure 3), not enough emphasis is placed on optimising the sample preparation. In DP method development this is often under-estimated and can ultimately lead to a less than robust analytical procedure in the longer term.

Before commencing experiments to determine the intended sample preparation procedure, the overarching principal underpinning all activities should be 'keep it simple'. More often than not, the laboratory developing an analytical procedure may not consider the fundamental project milestones/requirements. A method should not only be fit for successful validation and transfer, but also be able to robustly measure key stability characteristics to support API/DP shelf-life evaluation.

An initial evaluation of the UV spectral profiles for actives and key related substances should be performed. There are fundamental criteria that should be considered when carrying out this evaluation as this will essentially impact upon the overall robustness of the method. Taking the example given in Figure 4:

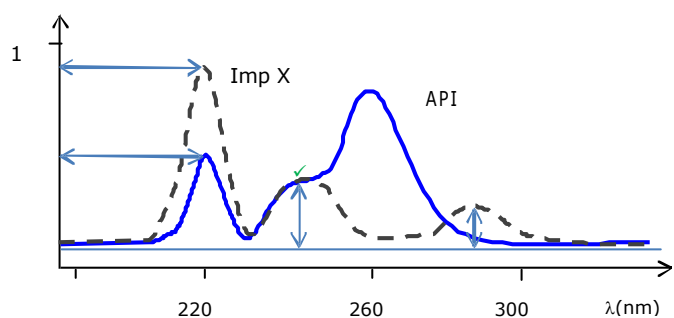


Figure 4: Example UV traces for API impurity X

Figure 4 illustrates the UV spectra for an API and its main impurity. When selecting an appropriate detection wavelength, the primary initial focus would centre around maximising sensitivity. At first glance this may suggest that in order to achieve maximal sensitivity, a detection wavelength of 260nm should be selected since this coincides with the I_{max} of the API. Alternatively, 220nm could be selected (although this would only give approximately half of the sensitivity for the API).

Areas of the UV curve to avoid are those which are positioned on a particularly sharp incline/decline since at these regions, only very small changes in the UV output would lead to significant changes in the peak response, potentially leading to a non-robust method. Therefore, in order to collect both the API and impurity peaks, much closer inspection of the UV curves would be needed; the wavelength selected should not only aim to give maximal response and sit on a shallow point of the slope, but also represent a point on the profile whereby the response of the active and impurity are closely matched. Scrutiny of the above would suggest a wavelength of 240nm would satisfy the aforementioned criteria. Any further refinement in sensitivity could then be sought via manipulation of solution concentration and/or injection volume.

If synergy in the API/impurity response is not achievable, then an alternative joint wavelength could be used. However, relative responses between active and impurity should always be assessed and where necessary, correction factors applied. Bear in mind that sufficient sensitivity in the impurity response is still required to meet the prescribed specification limits. If there is no possibility of a compromise with a single joint wavelength, multiple wavelengths could be used.

Sample preparation is crucial in building a platform for the overall method development process. If we take the example given here, there are a number of considerations that need to be assessed early on.

When preparing a sample solution, a decision needs to be made with regards to the number of dosage units to be incorporated: this is driven by the need to obtain a suitable sample solution concentration (within the solubility limits of the active/impurities), optimisation of column loading (in conjunction with injection volume) to obtain a peak that is within linear range of the detector and to provide adequate sensitivity of related substances.

All of these factors have to be balanced with the need to take a representative number of dosage units: this is essential to achieving a robust method as it will reduce the impact of any fill weight bias that may skew assay results. Additionally, taking the capsule example highlighted here, the actual sampling method needs to be carefully considered. For instance, is it more appropriate to transfer the entire capsule (shell and contents), or simply empty the contents (with washings), or perhaps take a representative weighing of the bulk fill? It is preferential to adopt as simple a sample preparation as possible, so the first option would be preferable. However, one must also consider the implication if the gelatin capsule is to be added

into an aqueous medium, whereby the capsule shells will dissolve and potentially cause interference with the extraction and chromatography. It would be ideal to use organic solvent in the 'stock', whereby the contents will dissolve but prevent the dissolution of the gelatin, and then take an aliquot of this into an aqueous medium to closely reflect the starting conditions of the gradient.

Where possible, sample preparations should avoid any lengthy dilution steps in order to minimise errors, maximise recovery and save analytical time. Adjustment of injection volume and UV wavelength could be used as alternative options when refining the column loading.

Another potential area for error when dealing with high levels of excipients in volumetric analysis is the impact of excluded volume: this can occur if the mass of powder blend taken into a volumetric flask is significant enough to displace volume that would otherwise be occupied by the sample solvent. In such instances it may be worth considering the addition of a fixed volume of diluent as opposed to diluting up to volume in a flask. Any issue with excluded volume would tend to manifest itself as a higher than expected assay due to the lower sample solvent volume (and hence stronger sample concentration).

It is worth noting that caution should be exercised when bulking the contents of capsules and then taking a weighing as, for early phase products where the formulation remains in the 'optimisation' phase, segregation of the components may occur leading to errors with assay results.

For the capsule example given in this article, an additional issue for the product pertains to the colour of the capsule shell. The UV absorption of the Swedish orange may interfere with the determination of the active and/or related substances, and therefore this would again need to be considered when optimising the sample preparation and HPLC method parameters.

Throughout the development, all findings should be continually evaluated in order to identify which parameters are particularly susceptible to minor adjustment, and ensure that these are experimentally assessed prior to moving into the validation phase. Typically, areas such as linearity, extraction efficiency and method repeatability should all be well characterised ahead of planning the validation in order to reduce any risk to the future robustness of the method (and any significant unwanted time and cost).

Scrutiny of the above should also enable a validation protocol to be produced that is far more representative of the specific API/DP.

Validation

Validation is more regimented than method development and there is also more guidance for the analyst on how to perform the work. The scope of the validation depends on the phase of the drug development; again 'fit for purpose' is essential. This is summarised in Figure 5.

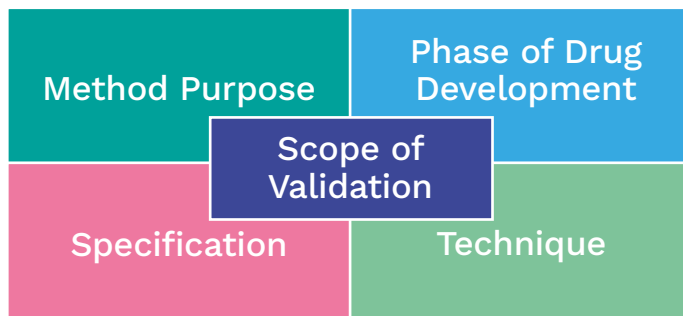


Figure 5: Considerations for validation

Pharmaceutical companies often use a phase or risk-based approach. Each company's phased method validation procedures and processes vary, but the overall philosophy is the same (see Figure 6).

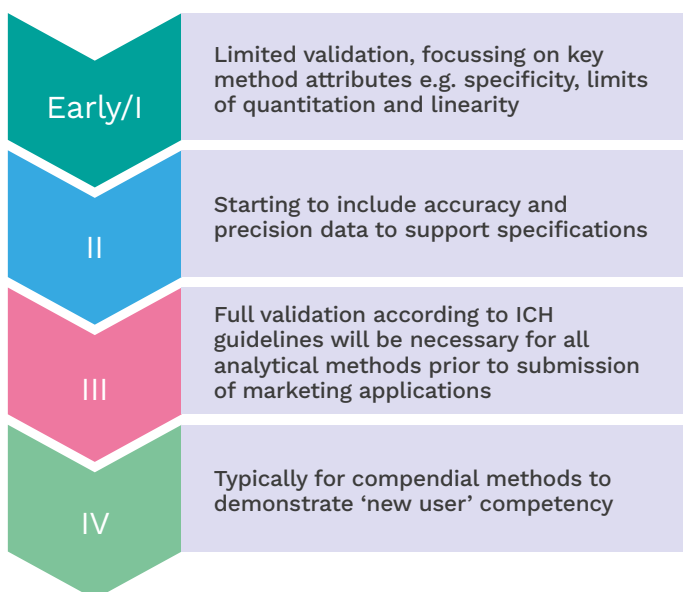


Figure 6: Phase-specific validation summary

As discussed previously, during method development and early stages, the development team should begin to develop an experience base, gathering information about which method parameters are important (see Figure 3) assessing what has the greatest effect on the analytical results and method performance (columns, temperatures, analysis time, and preparation of sample). At this stage, validating only the essential elements helps maintain flexibility during the very fluid stages of early development, but providing some confidence in the method.

Regardless of phase, a protocol is recommended (and required for later stage development). The method validation protocol should address the following:

- What will be done?
- Why it is being done?
- How it will be done?
- Who will be involved?
- Pre-defined acceptance criteria

The protocol should define the project owners and responsibilities and although it sounds obvious, it should also reference the intended analytical method or, ideally, attach a copy in an appendix. The objective

and rationale for the validation must be stated and include the method application, purpose and scope.

The materials to be used are also to be specified (or at least referenced to an appropriate document). Such issues are easily overlooked, but do act as a very useful aide-mémoire to make the author ensure that there are enough relevant samples, reference standard, placebo mix and reagents in sufficient quantity and within expiry date for use over the duration of the validation exercise.

Equipment (e.g. detector types) to be used should be stipulated, i.e. a variable wavelength detector (VWD)

will not be suitable for peak purity during specificity, as spectral data is needed to provide evidence for specificity.

The validation parameters should be listed (see Figure 7) and against each parameter, sufficient detail included so as to be able to carry out the experimental work to avoid ambiguity. This means consciously stipulating volumes, weights, amounts of excipients etc. for accuracy and linearity, especially where scaling sample preparations up and down to suit the required ranges may mean issues with extractable volumes.

	ICH Analytical Parameters			
	Identification	Impurity Quantitative	Impurity Qualitative	Assay
Accuracy	No	Yes	No	Yes
Precision: repeatability	No	Yes	No	Yes
Precision: intermediate	No	Yes	No	Yes
Reproducibility	*	*	*	*
Specificity	Yes	Yes	Yes	Yes
Limit of detection	No	No	Yes	No
Limit of quantitation	No	Yes	No	No
Linearity	No	Yes	No	Yes
Range	No	Yes	No	Yes

**Reproducibility data (inter-lab method assessment) are not part of the marketing authorisation dossier. However, it is advisable to perform such experiments in order to ensure methods are standardised.*

Figure 7: ICH validation parameters

Note: Acceptance criteria for each parameter should be pre-defined in the protocol and ideally based on knowledge gained during the drug/method development stages and pre-validation work. They should be relevant and achievable. An assay at ppb will most likely have a wider tolerance than an HPLC assay of an active!

In terms of GMP compliance, it is recommended to define the procedures to be applied in the instance of out of specification (OOS) results and planned/unplanned deviations and always include a revision history section. Signatories for author and reviewer/approvers should be evident before any lab work is initiated. Any working copies of the protocol used by the analyst(s) should include these to ensure that the analysts are working to the correct version.

Again for GMP validation it is an expectation that verification of equipment performance is demonstrated (e.g. calibration and qualification status) and also that analysts are all trained in the technique used.

A report needs to be written up on completion of the validation in order to allow traceability to the data and provide comments on the acceptance criteria status (pass or fail) and any required discussion. Again, ensure there is a reference to validation protocol and the version number, if there have been any new versions issued. Any deviations or OOS investigations that were carried out during the validation should be also detailed. The report should also be signed and approved.

Revalidation

Revalidation is necessary whenever a method and/or product has changed, however the extent of validation required is very specific to the extent of the change and the particular product.

If the formulation is modified, a revalidation may be necessary, e.g. if a 10 mg capsule and a 100 mg capsule have been validated and now a 50 mg version is needed, consider the impact of the change and revalidate, where applicable. It might be that the formulation is exactly the same as before (just varying amounts of excipient fill, and importantly no colour change) then one could argue that the previous validation is still valid. However if the first step of the extraction needs to be carried out at a different concentration then this might mean a different 'challenge' to the method. Assuming the final concentration and sample diluent are the same as before, precision and accuracy may then need repeating, although linearity might not. As with the initial validation a protocol and report would need to be generated.

It is also important to highlight that if a synthetic route of an API itself changes, then the related substances profile may well be very different and hence not only a revalidation would be necessary, but possibly also redevelopment. This would again highlight the importance of building in sufficient flexibility to the original method to encompass such change.

Conclusion

In conclusion, having significant previous experience in the area of method development and validation is central in selecting an appropriate CRO; they need to possess the ability to work in a pragmatic, GMP compliant manner to achieve a solid method that will ultimately support a successful DP filing and also serve to be reliable and robust in its future use.

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Linda is a “Chemistry with German” graduate from Kingston Polytechnic. “Lin” started out her professional analytical career with Scotia Pharmaceuticals. After 7½ years carrying out analytical method development, Lin then moved onto Prova, where she worked as an Analytical Projects Manager for 10 years. Lin joined RSSL in 2009 and heads up the Pharma Development team, specialising in HPLC and dissolution analysis in the development and stability testing of New Chemical Entities and formulations mainly in Early Phase.



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Steve has over twenty years’ industry experience, having graduated in 2000 from Kingston University with a Bachelor’s Degree in Pharmaceutical Science. He has spent his entire career to date working in the pharmaceutical sector and has acquired all of his experience within a CRO environment, gaining exposure to a wide range of analytical techniques and product matrices. Steve’s main area of expertise is Early Phase Development, Validation and Stability Testing of New Chemical Entities and Formulations mainly in Pre-clinical Development or Phases I and II.

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