Who Developed This £!!*% Method?

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Your mission, should you accept it, is to establish this chromatographic method in your laboratory. The method report will self-destruct in the next 30 seconds…

Mission: Impossible?

Technology transfer rides again! Have you ever drawn the short straw in your laboratory? You know the problem, don't you? In walks your boss who appears to be practising for an audition as a stand-up comedian by asking, "have you got any spare time?" Then comes the punch line you've been anticipating with dread, "I'd like you to establish this method". Politeness and the censor prevent me from printing your exact thoughts, but it's something like "Oh…", in combination with whichever expletive you would like deleted.

If you're lucky it may be a method developed within your organization or, if not, it may have been developed by an analyst somewhere in the world and you have to establish this from the literature. (Vice versa may be appropriate, depending on your viewpoint).

Forcing a smile on your face, you say to your boss that you'd like to do nothing better. If you read the last sentence correctly — *do nothing* is the option you would prefer. Anyway, semantics over, you now have the poisoned chalice in your hand and start to understand the magnitude of the problem. Let's start with the method report: noted for its comprehensiveness it stretches to a magnificent two pages, including the validation data. The method instructions include typical gems of scientific writing, such as:

- mix tube for 10 minutes
- prepared from potassium hydrogen phosphate solution
- use an ODS column that is 25 cm long.

How many times have we faced this problem of method or technology transfer? Moreover, how much time have we wasted in trying to get methods working that have been developed by other chromatographers? You'll be happy to know that you're not alone. Virtually every laboratory and organization has problems when it comes to establishing methods developed elsewhere. In fact, establishing another chromatographer's method could be viewed as more difficult than developing a method from first principles.

Defining Terms

Before we proceed much further, two terms are defined here:

(1) Developing Laboratory: the laboratory in which the method was initially developed, validated and reported. This is where the core analytical expertise should reside.

(2) Establishing Laboratory: the laboratory (or laboratories) which establishes the method.

It is important to realize the roles and responsibilities that each laboratory should have during technology transfer and afterwards in maintaining the method and developing it further.

NIH Syndrome

The NIH (not invented here) syndrome affects us all, especially when you see a method that was developed in another laboratory. Just imagine the thoughts going through your head as you read through the method report.

- I wouldn't have done it that way.
- That's too much solvent to wash the phase.
- I don't have the same equipment. I'll have to improvise.
- I can improve this method easily by…
- This is too complex, we need a simpler approach for routine operation.
- Who is this guy?
- This method is… (again fill in the expletive of your choice).

'Not invented here' syndrome can strike anyone anywhere as we as chromatographers are conditioned by our training and personal views. This is especially true if we have used a technique before with low success or consider ourselves 'an expert' in a particular technique. The temptation to change a 'poor' method is irresistible for some of us and here is the start of some of the problems of method transfer. In contrast, there are some chromatographers who develop more complex methods than are necessary to demonstrate their scientific prowess at the expense of their colleagues.

Into the Quagmire

The starting point (problem?) in establishing any method is usually either a written company report or a published scientific paper.

The majority of established methods are based on methods published in the scientific literature: these can be variable in their quality and description. Furthermore, the extent of the application of any method can be extremely variable. Some methods may only have a relatively small number of samples analysed on one or two days before publication of the manuscript. In contrast other methods may have several thousand samples before the manuscript is written and submitted. The methods in the former case have virtually no robustness data and knowledge of operation compared with the latter one. Which method would you like to establish in your laboratory?

The amount of information in a modern scientific paper is generally less than needed to establish effectively the method described within it. There are some exceptions to this statement, but these depend initially on how the author wishes to describe the method and how the paper is reviewed before being accepted by the journal. Also, the establishing laboratory is less likely to contact the developing laboratory to ask for clarification and advice with a published paper unless the establishing scientist knows the author. Here the likelihood for further modification is greater and the scientific paper could be looked at as the basis for developing the required method. Looking on the bright side, the establishing laboratory can always publish the new method to further our scientific knowledge.

Within most organizations, establishing a method developed in another laboratory of the group can be seen, on one hand, as an opportunity for developing contacts and the furtherance of the informal company network. On the other hand, it can be seen as an excuse to engage in undeclared guerilla warfare: this being the pinnacle of attainment of the NIH syndrome.

Personalities notwithstanding, the company method report should be of better quality with more experimental detail than the published scientific paper. This should be based on standards of report writing including contents required and degree of validation data to support the method's application and the style of writing.

Company-specific methods may be used to ensure uniform product quality in either development or manufacturing. Involvement of several sites with the same product requires standardization and this includes analytical methods. As chromatography is a major analytical technique, many methods to be transferred will use this technique. However, the quality of the writing of these reports can be very variable, which leads to problems in establishing laboratories.

It Only Happens to Me

Let's consider what happened during my first external method establishment within an organization. The report looked complete but the method, when established, did not achieve its quoted limit of quantification (LOQ). Contacting the method developer by phone, we spent some time discussing the experimental procedures in some detail. The unofficial method followed the report procedure until we came to a section describing how the analyte (bound to a solid phase) was washed. The report stated that the sorbent was washed and after centrifugation the wash solvent was removed by aspiration.

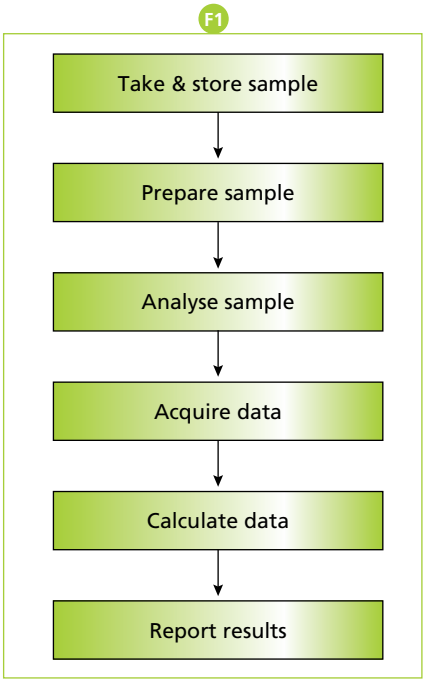

Figure 1: *The analytical process.*

In our laboratory, the procedure always had some excess solvent left, which was carried forward to the next stage of sample preparation.

During the course of our conversation, I discovered to my amazement that the method report was not quite accurate. After aspirating the solvent, the chromatographer from the developing laboratory took a tissue, rolled it into a long spiral and then proceeded to mop up the excess solvent before starting the next stage of the procedure. This was done as the solvent affected the LOQ of the assay, as was found after the method report was written and during the first routine application of the method to real samples. Suppressing my desire to strangle my colleague, I inquired why there was no update of the method report. We were too busy analysing samples was the reply. It would, dear reader, be rather impolite to communicate to you my thoughts at that precise time. Of course, I am confident this never happens in your laboratory.

Welcome to Excrement Creek…

Let's look more closely at the problem by considering the analytical process and some of the problems that could occur at each stage with transferring methods.

The analytical process consists of the following steps as shown in Figure 1 and listed below. Let's consider some of the problems of transferring methods that could occur at each stage.

Sampling and storage:

- sampling size different
- sampling protocol not defined correctly
- storage conditions not defined
- analyte stability under sampling and storage conditions not defined or assumed.

Sample preparation:

- precise description of the sample-preparation method not complete
- equipment used in sample preparation not fully defined
- preparation of standard stock solutions and analyte stability not described
- preparation of standard reference samples and calibration standards not described
- reagents are not equivalent between laboratories.

Chromatographic analysis:

- unqualified equipment used in one or both laboratories
- gradient equipment: high/low-pressure mixing or dead volumes are not similar
- columns are not the same type and from the same manufacturer
- detector performance is not similar: wavelength set, photometric accuracy, flowcell dimensions (partly related to unqualified equipment but also differences in performance between different makes/models of equipment)
- system suitability test is not defined
- reagents are not equivalent between all laboratories
- only the best and not typical chromatograms are included in the method report: axes and peaks not labelled.

Data acquisition:

- retention-time windows not set appropriately
- data-acquisition rate set incorrectly
- calibration model not defined.

Calculation and reporting results:

- post-analysis calculations defined incorrectly
- units of measurement or reporting not defined.

These are just some areas where poorly written instructions in the method report could result in problems in the establishing laboratory. I'm sure there are many potential problem areas, some mundane and others more exotic, that could be added to this list of problems for establishing chromatographic methods in other laboratories.

Finding the Paddle

How do we get out of this mess? The key is the quality of the documentation: the method report or the scientific paper. We must have improvements in the standards of scientific writing in both the scientific literature and within companies.

Within the scientific literature there can be the use of guidelines and checklists that can help authors write better quality papers and referees to ensure that key experimental conditions, procedures and equipment are adequately described to enable the method to be repeated in another laboratory. It is usually the experimental area of a paper that requires most improvement, in my experience.

In contrast, companies have the opportunity to develop policies and standards that can be enforced can save much time and effort in trying to establish methods that are poorly developed or are badly written.

General Responsibilities

Let's look at some of the responsibilities that the developing laboratory should perform during the course of developing a method.

- Develop the method for its intended use.
- Don't use method development as an excuse to incorporate the latest chromatographic toys and over-engineer an assay.
- Validate the method as appropriate for its intended use.
- Document the method requirements correctly; for example, unambiguous instrumentation, list the column supplier and batch where necessary etc.
- Write the analytical procedure so that it is the same as used in the laboratory.
- Define reference standards used: analyte, and related substances, metabolites and internal standard where used.
- Obtain purities, amounts and stability of all reference compounds.
- Provide typical chromatograms in the method report and not the best ones available.
- Define the system suitability test parameters and ranges where used.
- Ideally an independent analyst should establish the method using only the report.
- In organizations, communicate with the establishing laboratory.
- Assay transfer samples and report.

The responsibilities of the establishing laboratory can be outlined as:

- Pilot the method first to get the feel of it, then put more effort into meeting the acceptance criteria for transfer.
- Undertake a partial validation to confirm the performance of the method precision so that key parameters (e.g., accuracy, range and recovery) are equivalent to the developing laboratory.
- Check that the system suitability test, ideally using similar equipment, meets the set parameters.
- Do not modify or change the method (e.g., NIH syndrome). Some of these may be difficult in practice especially with availability of reagents and chemicals on a global scale.
- Use equivalent equipment and identical columns.
- Give feedback to developing laboratory about performance.

It's about communication, personalities and understanding when working in organizations with different sites. Easier method establishment can be achieved when

- chromatographers have a common understanding in both laboratories: meetings between sites and even exchange visits are useful ways of fostering this approach
- an agreement that if changes are needed to the method then the request goes back to the developing laboratory
- there is a common language, essential to understand approaches and help methods being transferred.

For further reading, I can recommend the article by Kirschbaum (1), which goes into more information about the interlaboratory transfer of high performance liquid chromatography methods by looking at the problems and outlining some solutions.

PS: Tell your boss not to give up his day job.

Reference

(1) J.J.Kirschbaum, *Journal of Pharmaceutical and Biomedical Analysis*, **7**, 813–833 (1989).