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HOW GOOD IS YOUR METHOD?

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Q: How do you demonstrate how good your chromatographic method is?

A: Method validation.

How do you define horror? One definition may be the sight of a manila coloured envelope on your door mat in the morning. No it's not your tax demand! Its much worse than that - you've been asked to review a manuscript for publication!! Are you sure you wouldn't like the tax demand?

Recovering from the shock, you can play a game as you review the manuscript. Imagine two containers or crocks. The contents of the Type 1 crock can be described as elemental, metallic and yellow. So far so good. The contents of a Type 2 crock are brown, high in organic matter and can be best described, dear reader, as agricultural fertiliser. Lets go through the manuscript for review and see which crock best fits it.

Start reading the manuscript, there is a good introduction that is well written and well referenced. We can classify this as a Type 1 crock. Read the experimental section next: sample preparation is outlined well, chromatographic equipment described well, solution preparation described well, a few minor details may be missing but nothing important. Looking good: Type 1 crock. Turning to the results and discussion, we read how selective the chromatographic separation is and how the method can be used. But where are the method validation data to support it's use? Nowhere! Oh dear….. Warning! Crock Type 2 alert!!

IMPACT OF SEPARATION METHODS

In the last decades, separation methods have been of utmost importance in the analysis of complex materials or in studies of analytes at trace concentrations. The development of both separation efficiency and new detection systems has led to continuous improvements of selectivity and

sensitivity, two integrated parameters in chromatography. Hyphenated systems like GC-MS, LC-MS and LC-MS-MS comprise a very powerful combination and are close to the ideal method with few interferences and with a high sensitivity that enables a low limit of quantification in the assay.

Accompanying this improvement in sensitivity and need to show that the data generated by these methods is reliable and is generally trustworthy. Hence the need for method validation as well as ongoing control of the method in routine use.

This Questions of Quality column concentrates on an introduction to method validation and the terminology involved.

TOP FIVE WORST ERRORS

We have been looking forward to this section: here the manuscript reviewers strike back. Our top five worst validation errors are:

1. A relative standard deviation calculated on two results (it's true - honest!)
2. No application data to illustrate the use of the method
3. No justification of the calibration model used
4. No sample chromatograms
5. Unsorted and bulky validation data to support the method (death by data drowning)

The names of the offending parties will, of course, be kept confidential - providing huge sums of money in large denomination used notes are sent to us at LC-GC International. Otherwise the names will go no further then the Internet.

But seriously, when you are writing think of your reader. You may have the attitude that the chromatographic literature is variable and poor, think of that brave band of reviewers who have weeded out all the Type 2 crocks and imagine what it would look like!

WHAT PARAMETERS CAN I MEASURE?

Having said that the way to demonstrate that method produces good quality data is through validation, what you we do? A good starting point for the validation of chromatographic methods can be found in the Eurachem / WELAC document on Accreditation for Chemical Laboratories [1]. This is a well written document that describes the different parts that constitute a good validation exercise for analytical chemical methods. This approach is in principle applicable to all kinds of analytes and in whatever

matrix that has to be analyzed. The following table gives the parameters that are found in the WELAC document as well as some others.

There are a number of parameters to consider and report when validating a chromatography method:

- Calibration
- Selectivity and specificity
- Range
- Linearity
- Sensitivity
- Limit of detection
- Limit of quantification
- Accuracy
- Precision
- Ruggedness

Each parameter will be discussed briefly.

Calibration: The heart of a chromatographic method is the daily calibration used to calculate the analyte(s) you are measuring. The calibration model is described in the analytical method document.

Daily calibration is often made from a couple of standard samples either at different concentration levels covering the range of assay or at 1 - 3 concentrations. The choice of calibration method has to be selected relative to the analysis. Is this done? Usually not! Type 2 crock?

There are a number of calibration options, especially on the larger data systems:

- average by amount,
- multilevel
- linear regression (with and without weighting)

more esoteric and chromatographically unsound calibration routines are also found:

- quadratic
- cubic
- spline (what's this? Not a clue but it looks good on the menu!)

Whichever calibration method is selected, it's choice must be validated. Without this approach, the rest of the data generated by the method may be best filed in the Type 2 crock with the organic matter.

Selectivity: The selectivity of an assay is a measure of the extent to which your method can determine a particular compound in the matrices you are analysing without interference from matrix components. If a method is perfectly selective for an analyte or group of analytes it can be considered to be specific. A common and serious mistake is calling a method specific when it is only selective. This is especially true when dealing with chromatographic methods which are not absolute only relative methods of analysis. Specific analytical methods are very rare.

Range. Quantitative analyses should have the working range of the method stated. We need to state the working range of the method, this is where the precision and accuracy of the method are acceptable - acceptable is defined by the use to which the data generated by the method are used.

Linearity: Determined by analysing samples of varying concentration and establishing the relation between response and concentration. It is highly demanded that a method is linear over a substantial range but it is not an absolute requirement, in particular if the deviation from linearity can be explained.

Limit of Detection (LOD): This is determined experimentally for each analyte in a method by repeatedly analysing blank matrix and one with the analyte present a concentration whose response in the chromatographic system is equivalent to the mean blank response plus three standard deviations. It is no use to determine the LOD in reagent blanks but the LOD must be measured in the matrix to give the correct background.

The usefulness of this parameter will depend on the nature of the work that you are performing. For environmental analysis where you are measuring the presence or absence of an analyte, this is a vital parameter of any method, qualitative and quantitative. Other methods may find that the LOD may only be useful as a measure of the condition of the chromatographic system.

Sometimes, LOD and also LOQ (see below) can be difficult to measure when the matrix can vary, such as in biological materials or environmental samples such as river water. In this case, more work is needed to determine an average or range of values for these parameters.

Limit of Quantification (LOQ): This is the lowest concentration of an analyte that can be measured with an acceptable level of precision and accuracy. The LOQ should be measured using analyte in matrix and is usually the lowest point of the calibration curve. This value must be determined by

experimentation and never by extrapolation. An acceptable level is in general considered to be around 10 to 20 % relative standard deviation with the aims of your method in mind.

Ruggedness (Robustness): Variations that are introduced when a method is set up under other conditions may result in a different performance. Such influences can most easily be tested for by using experimental design to elucidate those factors that are most crucial for a successful execution of a method. In liquid chromatography for instance such tests could comprise the influence of type and concentration of modifiers and additives, pH, temperature, ion strength etc. It is also important to establish that the analyte is stable during storage of the sample, during the work-up procedure and in the prepared sample. Most of this should be included in the method development but completed in the method validation.

Accuracy: Accuracy is the closeness of the obtained value to the true value which, no doubt, is the most difficult parameter to validate. One has to consider the influences of sampling and work-up procedure as well as interferences in the separation and the detection systems. Reference materials truly representing the unknown sample are not always available and solid samples and complex biological matrices are especially tricky to mimic. The analyte can be added to liquid samples and recovery studies may give an idea of where your method stands with respect to accuracy in the steps following. The ideal situation is to have a totally independent method, even as regards sample treatment, to compare with. Accuracy has to be evaluated both at a low and a high concentration level of the analyte.

Precision: Precision of a method is a measure of how close independent test results agree with each other and is expressed in terms of standard deviation. When measurements have been performed under repeatable conditions (same method, material, operator, laboratory and in a narrow time period) we can talk about Repeatability. When measurements have been performed under conditions with the same method and material, but by a different operator or in another laboratory or during a time period of days or weeks we talk about Reproducibility.

Sensitivity: The difference in analyte concentration corresponding to the smallest difference in the response of the method that can be detected. In other words, can your method distinguish analyte concentrations between 1 and 2, 1 and 10 or only 1 and 100 units. This parameter has a direct impact on how you report your results which should be done appropriately.

How many results do we see with results reported like 10.00. This implies that the method is capable of sufficient sensitivity of distinguishing between 10.00 and 10.01. A type 2 crock without any doubt, as many chromatographic methods have difficulty distinguishing between 9 and 10!

Sensitivity of a method can be measured from the slope of the calibration curve or can be determined experimentally by measuring closely related analyte concentrations over the whole range of the assay.

HOW MUCH WORK SHOULD I DO?

Much has been written about validation of analytical methods but it is not clear if there is only one validation exercise to be performed. Ideally validation criteria should be compiled at different steps in the development of an analytical procedure, but to a different extent. Some parameters are quite time consuming and laborious and cannot be reported until there is a long-term experience of the overall performance (such as analyte stability studies). Feedback during the development is needed and a well executed test of ruggedness before entering a full validation.

The best advice to determine the extent of method validation is to assess how critical are the data generated by the method and balance them with the method validation data needed to support them. For example, are we looking at qualitative data for a method, then the main thrust of the method validation would be selectivity with some work on the limit of detection.

REPORTING THE METHOD

Scientific papers in the chromatographic literature often contain elaborate studies of different aspects of validation. Which data are the important ones to report in order to avoid making the papers crowded with all kinds of stuff found experimentally on one or more occasions? Preferably one should be able to summarize the validation results without the need to publish all individual results.

The input and comments from the readers about method validation is most welcome.

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Bob McDowall - usual stuff!!

REFERENCES

[1] Accreditation for Chemical Laboratories: Guidance on the interpretation of the EN45000 series of standards and ISO Guide 25. Section 15: Validation
Eurachem Guidance Document No.1
WELEC Guidance Document No. WGD2
April 1993.