Writing the Perfect Method

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Analytical Method Determination of

Introduction

We visit too many labs where the previous analyst knew all the "secrets" to getting the method to work, but they were never written into the method. Now that analyst is gone, and the lab is starting over. You don't want to be put in that situation so you should make sure that you don't put others in that situation with the methods that you write. Over the next several weeks we will provide a summary of what makes one method better than another. Your goal should always be to write a perfect method, even if you aren't always successful!

We will consider the following sections that are common in many chromatographic methods:

- Scope/Purpose
- Reagents, Solutions, and Supplies
- Equipment
- Columns
- Operating Conditions
- Preparation of Standards
- Preparation of Samples
- Analysis Procedure
- Quality Control
- Calculations
- Reporting
- Being a Good Chromatographer and Other Things

Let's start with

Part 1: Scope and/or Purpose:

Yes, this is a GC or LC method, but there is more information that the user (and an auditor) needs to know. Are there limits to what this method can do, or only certain products that can be analyzed? What kind of results will be generated?

Do:

- List all the samples/products that were evaluated during development and/or validation.
- Specify the range of analyte concentrations that can be determined (purity, minor component, trace level, etc.)
- List restrictions if they are known (samples that cannot be analyzed with this method)

Do Not:

- List other samples or analytes if you have not evaluated them. Methods are not suggestions for future work, but summaries of past successes (and failures).
- Assume anything about who reads and uses the method. If they need to know it, you need to put it in the method.

Before we wrap up this section, a comment about how you write methods is important. Above we mentioned that you should consider how an auditor would read the method. Now, our focus is not on regulatory issues here, but they are important in many labs. More importantly, many of those same issues that a good auditor looks for are the same issues that differentiate a great method from a bad one - can anyone use this method, do we know what samples can be analyzed, do we know all the procedures needed for success, etc?

Quote for the Day

"Mistakes are, after all, the foundations of truth, and if a man does not know what a thing is, it is at least an increase in knowledge if he knows what it is not."

– Carl Jung

Writing the Perfect Method!

Part 2 - Reagents, Solutions, Supplies

Your goal should always be to write a perfect method, even if you aren't always successful! Can you answer "yes" to the following questions?

- Can anyone use this method as written?
- Do you know what samples can and cannot be analyzed, and what kind of information will be produced?
- Do users know ***all*** the procedures needed for success?

In the previous section, we started with the "Scope" section of your method. Now we move on to the next section.

Part 2: Reagents, Solutions, and Supplies:

It is important to list everything needed to perform the analysis. Nothing is more frustrating than having to stop in the middle of the procedure to find something that wasn't on the list. The same idea applies to any solutions that must be prepared, especially calibration standards. If they should be prepared in a certain order, or the order of reagent addition is important, that must be in the method.

Step back from your own experience and ask yourself - "have I included everything that I would do if I were in the lab following the method for the first time?" The fact that you know what to do does not guarantee that someone else will already have that information.

Do:

- List all reagents in their proper chemical form (hydrate, anhydrous, etc.) as they would be purchased and used.
- List the required purity or grade if it is important. In important analytical methods, it almost always is critical.
- Provide suggested suppliers (e.g., USP), only if that is important to method success. Be careful with the ubiquitous “or equivalent.” We will discuss this option later.
- Indicate what grade of glassware should be used. Most analytical methods require Class A volumetric containers. TC or TD should also be specified. If you aren’t familiar with these options, then a quick review can help. (At the risk of being self-serving, we cover this topic in our Analytical Laboratory Techniques seminar.)

- Carefully describe preparation of pre-mixed solutions or mobile phases. A solution of 50 % methanol in water can be prepared at least two different ways. Adding two equal volumes together or adding one solution and diluting to volume with the other will not produce the same concentration in the two mixtures! Describe which procedure must be used. Note that 50 %, 50:50, 50/50, and 50+50 are all ways to describe this solution. There are some industry-specific definitions for some of these abbreviations. Will the user know what this means?
    - An additional comment is needed regarding the use of units. <u>Always use units.</u> Is this solution v/v, w/v, or w/w? Unless you are using all aqueous solutions, the value for the solution may be different for each set of units.

Do Not:

- Specify a single supplier unless you are certain that only one source is acceptable.
- Specify part/product numbers in the method, unless necessary. These numbers can change (as can suppliers) and then you must change the method. Accurate descriptions should be adequate to avoid errors.
- Allow choices or options for critical processes and components unless you have evaluated them all. If it is important to success, it must be performed a certain way.
- Assume that a future reader will understand what you are thinking or doing. You may not have control over who is assigned to follow this method, or how much experience that they have. Returning to the Scope section from last time, if you think this method requires a specific level of experience or training that should have been listed.

Some labs will have a separate section, appendix, or document with "Notes" for the procedure. These notes are the little tricks and suggestions that make the method easier, or faster, or more reliable. Every good lab scientist knows the "secrets" to success – these are the “watch for …”, “make sure …”, “you can save time …”, “don’t …” tips that can really make a difference. Remember that your method is an opportunity to help train future scientists.

Quote for the Day

"In any science, then, one must present at the very outset evidence of repeatability, replication, reproducibility, accuracy, and precision ... repeatability or precision of analysis done in one laboratory by a single individual on a single day with one particular piece of equipment does not constitute science; that is a combination of science and art ... when we make a single observation without then proceeding to ensure it can be demonstrated again and again is when we start to fall off the cliff of reality and enter the

twilight zone of irreproducible results. Those are results that should not and must not be published ..."

– Professor Ira S. Krull,

"Guest Editorial — Reproducibility, Reproducibility, and Reproducibility,"

American Laboratory: Nov 2000, pp 7-8

Writing the Perfect Method!
Part 3: Equipment

It is important to list everything needed to perform the analysis, but nothing more. Maybe you did the development on a diode array detector or mass spectrometer. If that equipment is needed then, of course, it must be listed, but if you are not using the diode array's (spectral) capabilities, then an absorbance detector would probably be acceptable. If you think other users are likely to have a different detector type, you should consider at least a short evaluation on that type of equipment before completing the method.

Also, remember that different detector designs will have different performance characteristics and settings, so make sure your instructions will have enough information. For example, some diode array detectors allow you to set the collection bandwidth; an absorbance detector does not. If you want to use an absorbance detector later, then do your diode array development with a small bandwidth (2 or 4 nm) that would approximate the performance of the other instrument. If you want to use a feature that is only available on a diode array, then you may have to specify the use of a diode array in your method.

Finally, different manufacturers will have different names and settings for their instruments. Make sure your instructions are as simple and generic as possible.

Do:

- Include the type of instrument and general capabilities (e.g.., HPLC with gradient capabilities, GC with split/splitless injector, etc.).
- Include the features/modules required, but not anything extra.
- Include all instrument settings, remembering that there may be some differences in settings and terminology between different manufacturers. (More on this in the operating parameters section.)

Do Not:

- Specify a single instrument manufacturer and model unless the analysis must be performed on that exact model of equipment.
- Develop a method that uses the special features of one manufacturer unless that is the only way to produce acceptable results. The manufacturer can brag about their unique capabilities, but you should not.

- Do not use the "or equivalent" option for an instrument. This statement has very little meaning. List the capabilities needed as noted above. That will be enough.

Quote for the Day

"Without multivariate data analytic tools, the scientist will perceive the stream of data from the multi-channel instruments as annoying cacophony rather than sweet music. Consequently, they will play fancy new analytical instruments the good old way — one string at a time. For instance, they may choose to use only one of the available wavelengths or chromatographic peaks, ignoring the rest to avoid getting flooded with data. This forces the user to ignore a lot of potentially important information."

– Harald Martens and Tormod Naes, Multivariate Calibration, Wiley, New York, 1989, p. 23

Writing the Perfect Method!
Part 4: Columns:

If you are writing the perfect method (or at least trying), this should be the easy part, right? Just write down the description of the column and you are done. Except that we see many methods where some critical information is not included.

Internal diameter, length, and type (e.g.., C18, 5% phenyl) are the obvious items. But particle size (in LC) or film thickness (in GC) are also critical to producing the correct separation. Also, it isn't just a C18 column. Is it a Zorbax RX-C18, SB-C18, or XDB-C18? They are all different. And Agilent C18 columns are different than C18 columns from Waters, Restek, Supelco, or Phenomenex, or any other manufacturer. And the pore size (that's different than the particle size!) should also be specified. The values are usually unique to each manufacturer, but this can be an important variable when you are studying larger molecules.

The GC column manufacturers have done a good job of producing equivalent columns. Although they are not exactly equal, the results are often close to each other. The concept of "equivalent" columns in LC is far more complicated. There probably are no truly equivalent columns between different manufacturers. The old USP system of "L" columns was a convenient way to describe column chemistry, but it was not useful for predicting actual chromatographic behavior. As we discuss in our seminars on Introduction to LC and Reversed Phase the choice of stationary phase is important, especially for more polar analytes. Not all C18 columns are created equal.

There are some online tools now (search for "Hydrophobic Subtraction Model") to help with selecting similar (or not similar) columns. We cover some of this information in our Advanced LC topics and in the Best Practices in HPLC Column Selection webinar.

Do:

Include the following information for LC columns:

- Internal diameter (i.d.) in mm
- Length, in mm or cm (specify units)
- Particle size, in um
- Pore size, in Angstroms
- Stationary phase - manufacturer, brand/model, and any other unique descriptors

Include the following information for GC columns:

- Internal diameter, in mm or um
- Length, in m
- Film thickness, in um
- Stationary phase - manufacturer, brand/model, and any other unique descriptors.
- Use the term "or equivalent" for GC columns, but only if you have some evidence that a truly equivalent column exists, and you have tested it.
- Specify alternative columns if you have tested them. Show results.

Do Not:

- Use the term "or equivalent" for LC columns (see discussion above).
    - There is one exception to this rule that can sometimes be valid. If you have very specific performance requirements (usually specified in a System Suitability section), then another column might be able to produce similar results. However, you must clearly and carefully specify these requirements so that there is no chance of a serious error resulting from a column change. The requirements should be, at a minimum, elution order, resolution, and peak shape.

      There is a maddening, and quite frankly, cheap/lazy habit of just using whatever column is in the instrument and assuming (hoping?) that it will produce the same results. No, … just no!
- Specify column types using USP designations (again, see above). Sorry USP, but this labeling system is just not an accurate way to choose an equivalent column.
- Specify alternative columns that you think will work. If it hasn't been tested, it should not be in the method.

Quote for the Day

"You can have it quick,

you can have it cheap,

you can have it good ...

pick any two."

– Anon.

Writing the Perfect Method!
Part 5: Operating Conditions

If you are writing the perfect method (or at least trying), this is the most important part, right? Just write down the instrument settings (assuming this is a chromatographic method), and you are done. But make sure that all critical information is included.

Each module/component has settings, and your method must include the important parameters for each. LC systems have pumps, autosamplers, column ovens, and detectors and maybe other things. GC systems have the same components, although the settings can be more complex when it comes to injection. The following checklist can be used to verify that you have everything. This list is easily prepared by having the instrument control software displayed when you write the method - if it is in the software, it needs to be in the method. You should be able to match up every setting in the instrument with a corresponding entry in the method.

Do:

You must include the following information for LC methods:

- Pump
  - Flow Rate, mL/min.
  - Run/Stop Time or Acquisition Time, min. Sometimes this is set in other modules.
  - Pressure limits – maximum and sometimes minimum, units vary by manufacturer.
  - Mobile Phase in % of each component, using the solutions specified in the Reagents section.
  - Gradient Table showing any changes in pump settings during the acquisition.
  - Other special settings, varying by manufacturer.
- Autosampler/Injector
  - Injection volume, in uL, including model-specific options like draw rate and other settings such as wash solvents, times, locations, etc., and any other special operating modes (low delay volume mode, overlapped injection cycles, injector cleaning, etc.). If you are not familiar with these options, then use this as an opportunity to learn more about your equipment and its capabilities.
  - Temperature, if you have a chiller in your unit.
  - If you are using manual injection, my first thought is “upgrade to an autosampler!” It is a good investment. For now, specify the order of events – valve position, load procedure, injection, and washing procedure.
- Column oven
  - Temperature, in degrees C.

    - Specify the minimum equilibration time required for the column to reach thermal equilibrium. This is not the time required for the thermostat to say that the heating unit is at the set temperature. The column is probably not completely at temperature yet. If you have not heard of this issue, here is a hint: start the system and monitor the pump pressure until it reaches a steady value. If your system is operating at more than ten degrees away from room temperature, then you may have to wait as long as 15 minutes. You can also make injections during this time. When the retention times finally stabilize, your system is ready.
- Detector
    - Absorbance Models: Wavelength in nm, bandwidth (for diode array), reference settings (for diode array if available/needed), slit width/band width.
    - Fluorescence: Excitation and Emission wavelengths.
    - Refractive Index: polarity.
    - ELSD: Evaporator temperature, nebulizer temperature (not always available), gas pressure or flow, detector settings (gain).
    - MS: Interface design type, all voltages, pressures, flows, etc. in the software method screens, monitor/scan type and settings.
    - ALL: data acquisition rate (in Hz or by peak width), and time constant/response time. These two are often not included in the methods that we review but can profoundly affect your results if not set correctly. Be aware that the names for each may vary across different manufacturers and software programs.
- Equilibration conditions and time at startup and between injections.

You must include the following information for GC methods:

- Carrier gas (we covered purity in a previous section)
- Inlet temperature (liner size and style comes under Equipment)
- Injection volume in uL along with pre- and post-wash settings and solvents, number of pumps, delay/equilibration times (as needed)
- Injection technique (split, splitless, cold on-column, pulsed, etc) and all settings for that technique (times, flows, pressures, etc.). If it is in your method setup screens, it must be in the written method.
- Detector temperature and gas flows, and any other voltage/current settings in the instrument method.
- Oven temperature and complete details for temperature programming.
- Carrier gas settings: control type (flow/pressure), value for gas flow through column, and any changes during analysis.
- Equilibration time and conditions at startup and between injections.

Do Not:

- Specify instrument-specific settings unless the method is validated for and expected to be only used on that brand of equipment.
- Offer alternative settings or ranges for settings unless you have evaluated these

Quote for the Day

"Everybody is ignorant only on different subjects."

– Will Rogers, in P. M. Love, The Will Rogers Book, p. 199 (1972).

Writing the Perfect Method!
Part 6: Preparation of Standards

If you are writing the perfect method (or at least trying), this should be easy, right? List the standards that you use and their concentration. "Prepare standards at 0.1, 0.5, 1.0, and 2.0 mg/mL" should cover all the details you need. Except, ... there may be several questions that a new user might ask if they aren't included in the details.

Do:

- List all concentrations in clear units.
    - The term "ppm" or "ppb" can have three different meanings, and three different actual concentrations depending on the solvent. Similarly, "%" can mean w/w, w/v, or v/v. Use actual mass and/or volume units.
- Describe or list the mass and volume needed for each standard.
    - Some methods allow the use of "proportional volumes" in methods. This is acceptable if there are clear guidelines. For example, "Dissolve 500 mg in 500 mL of HPLC grade methanol using a volumetric flask" might be one example, with the qualifier that "proportional masses and volumes may be used." However, I would also add the following additional limitation, "... but a minimum volume of 50 mL must be used to ensure acceptable accuracy." This last statement is necessary because the relative errors in using glassware and balances will vary with the mass and volume. Generally, smaller volumes mean more error.
    - Remember that we specified the quality of glassware (e.g., Class A) in a previous section.
- Specify required accuracy and volumetric ranges.
    - "Dissolve 50.0 +/- 0.5 mg in 50.0 mL of HPLC grade methanol" makes it clear that you must use a four-place balance and a volumetric flask. It may also be desirable to specify the balance type and type of volumetric container or specify "record the actual mass to four decimal places" when you want a four-place balance used.
    - For volume measurements and dilutions, remember that the accuracy decreases in the following order: Class A pipets, Class B pipets, pipettors, serological pipets, syringes, graduated cylinders. If the highest accuracy is necessary, you will need to use Class A pipets.
    - The precision obtained with each option will vary with the operator's technique. Proper training is required. We give some examples in our seminar on Analytical Laboratory Techniques.
    - When preparing standards at multiple levels, clearly specify when one standard is a dilution of a more concentrated standard. There are different strategies for these dilutions (e.g., serial and parallel) which we discuss in the Analytical Laboratory Techniques seminar.

    - We recently developed an automated procedure for preparing dilutions using your programmable autosampler. Click here to download a copy.
- Specify the solvent(s) to be used.
    - The user should not have to guess which solvent is required. If mixtures are needed, specify how to prepare them. A "50% water/methanol" solution can be prepared at least two different ways with many different levels of accuracy, depending on the procedure and glassware that are used. We discussed this in an earlier section, but we mention it again to remind you of the importance of this information.

Do Not:

- Assume that the user will or should know what you mean. There should be no confusion.
- Step back from your method and ask yourself if you could properly follow this procedure if you were seeing it for the first time.
- One test of this idea might be to make a list of every piece of equipment and glassware that you used (look in the sink before you begin cleaning), and make sure that each is mentioned in the procedure.

Write It Down Somewhere (Please)!

If there is a consistent theme in this series on Writing the Perfect Method, it is "attention to detail." A recent laboratory visit made this idea all too clear. This laboratory was using written and approved methods for their analyses, but a new analyst immediately discovered some problems with the methods, including:

- Standard preparation procedures had missing or incorrect information because the standard would not dissolve using the procedure in the method or the resulting solution was cloudy.
- Sample preparation procedures were not correct because the final sample solution could not be filtered as described in the method.
- Calculations could not be verified against stated concentrations. (It wasn't clear if this was a procedural or mathematical error!)
- Instrument settings were not accurate or not complete.

Unfortunately, the laboratory notebooks were not much help. Clearly, the previous analyst knew something that was not written in the method (or documented anywhere else). The lab will probably have to start over with some of these methods. This problem could have been avoided with proper writing, review, approval, and auditing.

Would you be interested to see if we could improve your procedures? There is only one way to find out. Contact us for details.

Quote for the Day

"All scientific progress is progress in method."

and

"Unfortunately, technique is not uncommonly the weakest part of scientific investigations."

– M. S. Tswett*, 1910

*Tswett invented chromatography.

Writing the Perfect Method!
Part 7: Preparation of Samples

In an ideal situation, samples and standards are prepared in the same way, using exactly the same procedures and solutions. In the real world, things may be different.

If your method uses a “process standards" method (usually because it is a derivatization procedure), then both samples and standards must be treated the same way. In most other methods, however, samples will require additional steps.

Oh, the lucky ones who have a "dilute and shoot" method. Dissolve in mobile phase, filter (don't forget that), and you are ready. Unless your sample has insoluble components that float up to the top in your volumetric flask. Where do you "fill to the mark," at the top of the 'floaties' or at the bottom where the bulk liquid starts? I have heard analysts defend either method. Without taking sides on this one, be consistent; always do it the same way.

"Sample preparation" to separate the analytes from other parts of the sample may be required, using such techniques as liquid-liquid, liquid-solid, or sold phase extraction. As with any other part of the method, you must be as precise as necessary in describing these processes, including reagents, supplies, and procedures.

Do:

- Remember to list all reagents, accessories, and solutions in the appropriate section.
- Describe or list the masses and volumes needed for each sample. As most samples are usually solids, measuring out an adequate mass (approximate target amount) on an appropriate balance (specify number of decimal places) will be required. See the last topic for details.
- The method should prepare the sample for whatever final analytical technique is used. Ensure that your final dilution solvent is compatible with the instrument and operating mode.
    - Reversed-phase methods will require a solution which is aqueous-organic, at the proper pH. Note that the organic solvent must match the LC method’s mobile phase. If you have acetonitrile in your mobile phase, you should also have some in your injected solution. A failure to match the chemistry between mobile phase and injection solvent can lead to poor peak shape or even precipitation inside the instrument.
    - Use the maximum concentration of aqueous solution that will dissolve the components.
    - HILIC methods have the same requirements, but you should maximize the concentration of the organic phase.
- GC methods require a single solvent, which is similar in polarity to the column being used.

    - GC methods are less sensitive to solvent composition, but mixtures are rarely used. If you are using splitless injection techniques, a change in solvent can have a disastrous effect on your peak shape.
- Other analytical instrumentation will have their own unique requirements, but in every case, the solvent environment for both samples and standards should be as similar as possible.

Do Not:

- Assume other users will understand how to do a procedure, unless it is described in a document somewhere, or unless you require that they be trained in that technique in order to complete the procedure (update Scope as required).
- Specify that an exact mass be measured. This is nearly impossible to achieve with any practical accuracy or effort, so you should specify a range only (e.g., 100 +/- 2 mg).
- Assume that the user will or should know what you mean. There should be no confusion.
- Step back from your method and ask yourself if you could properly follow this procedure if you were seeing it for the first time.
- One test of this idea might be to make a list of every piece of equipment and glassware that you used (look in the sink before you begin cleaning), and make sure that each is mentioned in the procedure.
    - *Yes, I used this last month, but it was such a good suggestion that I decided to keep it for this month too!*

Quote for the Day

"All good science is accumulative; no one can get everything right the first time."

– Stephen Jay Gould,

Eight Little Piggies: Reflections in Natural History,

Norton, New York, 1993, p. 32

Writing the Perfect Method!
Part 8: Analysis Procedures

Your perfect method now must instruct the user on how to actually analyze samples. Current practice in regulated laboratories generally requires a far more formal set of procedures. In other environments, the procedures have historically been less demanding - e.g.., a standard followed by your samples. However, with the availability of autosamplers and other automation, it is usually advisable to do more to improve the integrity of the data.

All methods should discuss the following activities and provide some guidance on frequency and injection order. The differences between regulated and non-regulated labs will usually be the amount of effort required for each step, and whether some of the activities can be combined in a single injection.

- Instrument equilibration
- Method verification/system suitability
- Blanks
- Calibration
- Samples
- Continuing/check standards
- End of sequence verification/calibration
- Instrument/column cleaning and/or shutdown

Specific comments are listed below. Most of these topics are covered in more detail in our Best Practices in HPLC webinar.

Do:

- Describe instrument equilibration, which is important for all analytical instruments. However, longer is not always better. Provide general guidance for time (e.g., a minimum of 15 minutes).
- Check the LC system with injection of "conditioning injections" before you begin the formal analysis. Typically, you want to inject one or more standards to verify that retention times and peak size are consistent. It is very common for the first one or two injections to be "different" than the rest. Avoid this problem. (Note: this does not need to be a formal procedure as this information is never reported. This is an informal activity as part of equilibration.)
- System suitability is an important part of validated methods since this step verifies the performance of the instrument. The amount of effort required will be

related to the importance of the results, but at least some evaluation is required, even if it is only related to retention times.

- Blanks and calibration injections demonstrate the response of the system, and both must be included. Blanks are especially important for trace analysis methods. There are some known situations where there is some background quantity of analyte or interference. You must know how much is present. Do specify exactly how many standards are required and at what levels. I personally believe that we spend too much time calibrating in modern methods, but once the method is finalized, it is too late to change the procedures.
- Indicate whether samples are always analyzed singly or in duplicate or triplicate. Specify when you run check standards and spikes, and how often, or under which conditions they are included.
- You must always end the sequence of injections with a calibration verification. More on this next month when we discuss QC.
- Instrument shut down and column storage are not critical for method performance, but they will improve both instrument and column lifetimes if they are performed correctly.

Do Not:

- Specify an exact equilibration time (e.g., 15 minutes), as this is not technically accurate and can cause problems with compliance auditors. Specify a reasonable range; do not burden the analyst with demanding requirements that do not help the quality of data.
- Ignore the need to verify system performance. This does not require extensive evaluation but should be present in every method. It can even be an informal review of the chromatogram, but the operator must take responsibility for noting that the system is working correctly.
- Assume that the blanks are negative. You must verify, even if it takes only one injection.
- Let the analyst decide on how many replicates to inject, or how often to run check standards. Demanding procedures are not required, but something must be specified in the method.
- Ignore the need to prepare both the instrument and column for storage/shutdown. Typically, you will want to remove any dissolved solids from the LC system (buffers, etc.) and clean the column before storage. Failure to do this will cause early failure of both column and instrument. GC systems normally benefit from a column “bake out” at the end, but you would not want your system to stay at those temperatures for storage.

Quote for the Day

"Statistics is like taking cross sections of the trunk of a tree. It can tell you a lot about its history. You can figure out how healthy it is, how much volume the whole tree has, how much is root and how much is branch. But what it can't tell you is where the tree will branch, and which branches will become major, which minor, and which will rot and fall off and die."

– Orson Scott Card

"The Originist," in Foundation's Friends,

M. H. Greenberg, Ed., Tor Books, New York, 1989.

Writing the Perfect Method!

Part 9: QC

I am often asked “how much QC is necessary” or “how often should I calibrate the method?” I usually reply, "enough" or "as much as you need to do" or “it depends.” Of course, these are worthless answers! But it does point out the fact that how much QC you do depends on several factors, including:

- How important is this analysis?
- How important is the answer (in terms of accuracy and precision)?
- How stable is the instrument response?
- Who will use the information, and do they have expectations on the value of the number?
- What is the risk if the analysis fails? Will there be scientific or economic consequences?
- Do you only have enough sample for one injection? Is the sample not stable?

Why is QC required? Without getting into a philosophical discussion, I will say that for me, you must calibrate or check your calibration at "regular" intervals. How often will depend on the factors listed above. However, remember these two concepts:

- Your results are only valid if you have verified that your system is within expected calibration limits.
- Calibration is about risk! If a calibration check fails, then every analysis since the most recent successful calibration is now suspect. If you only check calibration once a week, you could lose up to an entire week of data. Can you afford this risk?

I recently discussed this topic on our Facebook page. Click to see the three posts on “How Often Should I Calibrate My HPLC Method?”

Do:

- If this is a very important analysis, then you should probably update your calibration every day. This may include multiple injections at a single level or injections at multiple levels. This procedure takes longer, but is the most rigorous, since the calibration is updated for today's analysis. Most pharmaceutical formulations are analyzed this way.
- If this analysis is not as critical, or calibration is a lengthy procedure, then it is usually sufficient to check/verify your calibration. Inject a single standard, maybe in duplicate or triplicate, and verify that it analyzes at the expected amount (+/-

some expected tolerance). The tolerance should be less than the difference that you want to measure (e.g.., if a 20% difference is important (trace analysis situations), then the tolerance should be around 5-10%). You can also set this value based on injection reproducibility.

- Always include an injection of one or more blanks. Both reagent and system blanks may be necessary.
- If you have many samples, or are concerned about calibration stability, or if the analysis requires much time, then insert additional check standards during the analysis.
- Always end with injection of one or more calibration checks. You must verify the calibration at the conclusion of the analysis.
- System suitability is an important part of validated methods since this step verifies the performance of the instrument. The amount of effort required will be related to the importance of the results, but at least some evaluation is required, even if it is only related to retention times.

Do Not:

- Ignore the need to verify system calibration with every analysis set. It may not require more than one or two injections, but it can be a simple procedure to enhance the quality of your data.
- Forget to calibrate at the end of the analysis set. You run the risk of having to lose all of your data, if the next calibration check fails.
- Do not let the analyst decide on how many replicates to inject, or how often to run check standards. Demanding procedures are not required, but something must be specified in the method.

Quote for the Day

"Ascertaining if a process is capable of meeting standards is nothing more than using collected data to decide if the calculated upper and lower control limits of a process, which is stable and in control, lie within product specifications. If a process is not capable, then before SPC [Statistical Process Control] can be implemented, management, MANAGEMENT, must take action to correct the process. If management cannot or will not act to correct the process then they can expect no improvement in the quality of output from any quality improvement program, much less from SPC."

– Roger W. Berger and Thomas H. Hart,

Statistical Process Control: A Guide for Implementation,

ASQC Quality Press, Milwaukee, WI (1986)

Writing the Perfect Method!

Part 10: Calculations and Reporting

As we finish up this series on the perfect method, we want to cover the last main issues – how to calculate results, and what numbers to put in your report. Like every other topic that we have discussed, these are just as important as the rest. A poorly written section here could result in wasted time or incorrect results.

Calculations

I was reading a method recently because I was asked to create a new report template to replace the current spreadsheet. There were several equations listed, using a mixture of written descriptions and abbreviations. Some made sense; some did not. I could not find definitions for some of the abbreviations, so it was not always clear how to actually calculate the results. An example spreadsheet printout was available, but was no help, because the labels did not match the names in the method. Despite looking over the method for about 10 minutes, I still had no idea how to duplicate the calculations. Studying the formulas in the actual spreadsheet file would be the only way to duplicate the results.

Reporting

For most people outside the lab, the purpose of the lab is to generate numbers – numbers in a report. We might imagine that we are actually generating “data” or “information,” but most readers of our reports will have no idea how we got the results, and they will rarely appreciate how much skill and effort that it requires. Still, this is what we do, so we need to do it the right way.

The purpose of your report is to communicate results to the user.

But remember that results are more than just a number from the spreadsheet. You are also communicating information about how “good” this number is – does it reflect an estimate of a trace level analysis or is it an accurate and precise value to be used in a certificate of analysis or manufacturing release document. Does this number indicate whether it comes from a single measurement or multiple replicates? Is there any indication of the variability in the number? That is, can I estimate if two numbers are essentially the same? Will the reader be able to answer any of these questions?

For Calculations and Reporting sections,

Do:

- Define all abbreviations and acronyms.
- Include the units for all numbers, either individually, or in the column header of tables.
- Show example calculations if the calculations are complex.
- Report the proper number of significant figures. Most chromatographic methods will generate two or three significant figures. Your method should specify the number of significant figures, or the decimal level for reporting (e.g., report to the first decimal place). If you are not sure, consider reviewing our free on-line course on Significant Figures and Rounding.
- For more than two replicate measurements, you must report the average, standard deviation, and the number of data points used to calculate the values. Without the number of data points, the standard deviation will have no real meaning. For more information, consider ordering our seminar on Analytical Lab Techniques, which also includes sections on other lab skills.

Do Not:

- Assume that the reader knows abbreviations or can “figure them out.”
- Forget the units. The reader should know the units for every number in the report.
- Copy values from a spreadsheet without considering significant figures.
- Assume the user will understand the variability of the method and therefore know how to interpret the numbers.

Quote for the Day

"People, including managers, do not live by pie charts alone — or by bar graphs or three-inch statistical appendices to 300 page reports. People live, reason, and are moved by symbols and stories."

– Tom Peters,

Thriving on Chaos, Knopf, 1987

Writing the Perfect Method!

Part 11: Integration Settings

Several sections earlier we talked about settings that are to be included in the method. Clearly, you need to give the user advice on how to integrate the chromatograms to generate the most appropriate results. The integration baseline determines the "bottom" of the peak, and its location will determine the size of the peak (in area and height units). That size value is what we use for calibration and analysis, so this really is an important step in generating an analytical result.

The question is – "What integration advice do I include? "

I read many different methods from many different laboratories. Most do not even mention integration. Those that do, usually have what I consider to be the wrong approach. So, this month I am going to switch the format and start with **what you should NOT do:**

Do Not:

- Simply list integrator settings with fixed parameters and values.

This is what most methods include, but this is a bad idea. Why? Because it reflects the current obsession of compliance with specific rules and procedures. While I do not philosophically disagree with this idea, there are cases where rigid implementation leads to bad science, and bad science usually leads to bad results. The intent is that more control means more consistent results. However, such an idea only applies if the process is stable and reproducible and responds the same way in all situations. Real-world chromatography isn't like that.

Baselines, peak shape, noise, retention time, and interfering peaks can all vary between over time. Certainly, if there are major changes, that suggests a problem that must be fixed. But many changes are small and within the normal range of variability, yet they may require slightly different integration settings to properly define the peak.

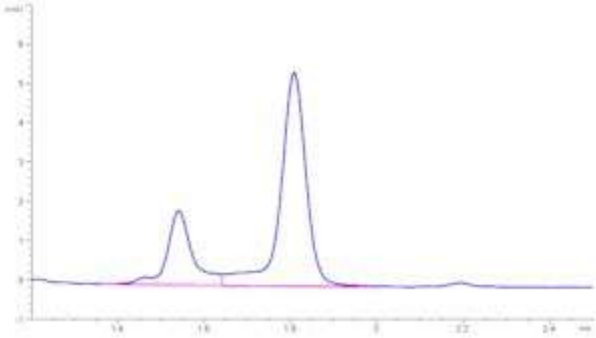
Remember, the settings don't magically create the data that represent peak size. The settings are the instructions to the program for where to draw the integration baseline. It is the position of the baseline that matters. **You need to be able to adjust whatever parameters are necessary to ensure that the integration baseline is created the same way for each sample for as long as you use this method.** Fixed settings will not always satisfy this requirement.

So, what should you do?

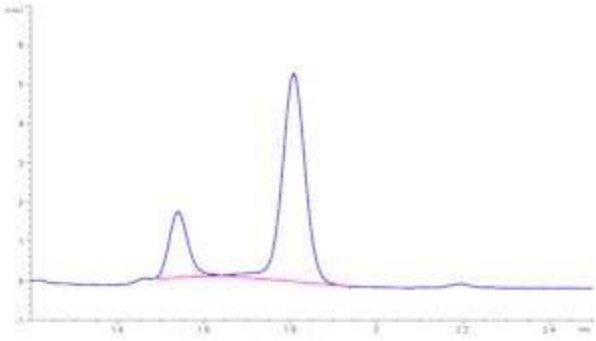
Do:

- Include a chromatogram with the "correct" integration baseline displayed.
- If certain problems occur with some regularity, you may want to include an example of "incorrect" integration. Such an example might show how to deal with a small change in the baseline or common interference that can lead to integration errors.
- If necessary, include a range of integrator settings that will produce the correct result.

For example, if you have an unusual chromatogram, you might include a figure like this with the caption, "Correct integration settings."



If necessary, add an example of "Incorrect integration settings."



Finally, include a range of settings, such as:

Peak Threshold = 0.1 - 1.5

Add the following instructions:

"Adjust the settings as needed over the indicated range to produce an integration baseline similar to the 'good' example. If you cannot produce acceptable integration using these values, contact your supervisor for instructions."

Finally, implementing these changes means that you must understand how to adjust the parameters in your software. Manual integration is not an acceptable option in many situations so you must know how to get the integrator to produce the correct results. And don't be fooled into thinking that these differences are not significant. In our seminar on Integrating Chromatographic Peaks, we show common examples where the measured peaks areas are wrong by more than 100 %!

It's Over

Well, we finally reached the end of this series. I hope you found it to be helpful, or at least stimulated some thought and discussion about your methods. Any discussion is always welcome, and if you would like us to look over your methods, don't hesitate to ask.

Be Safe, Write Well!

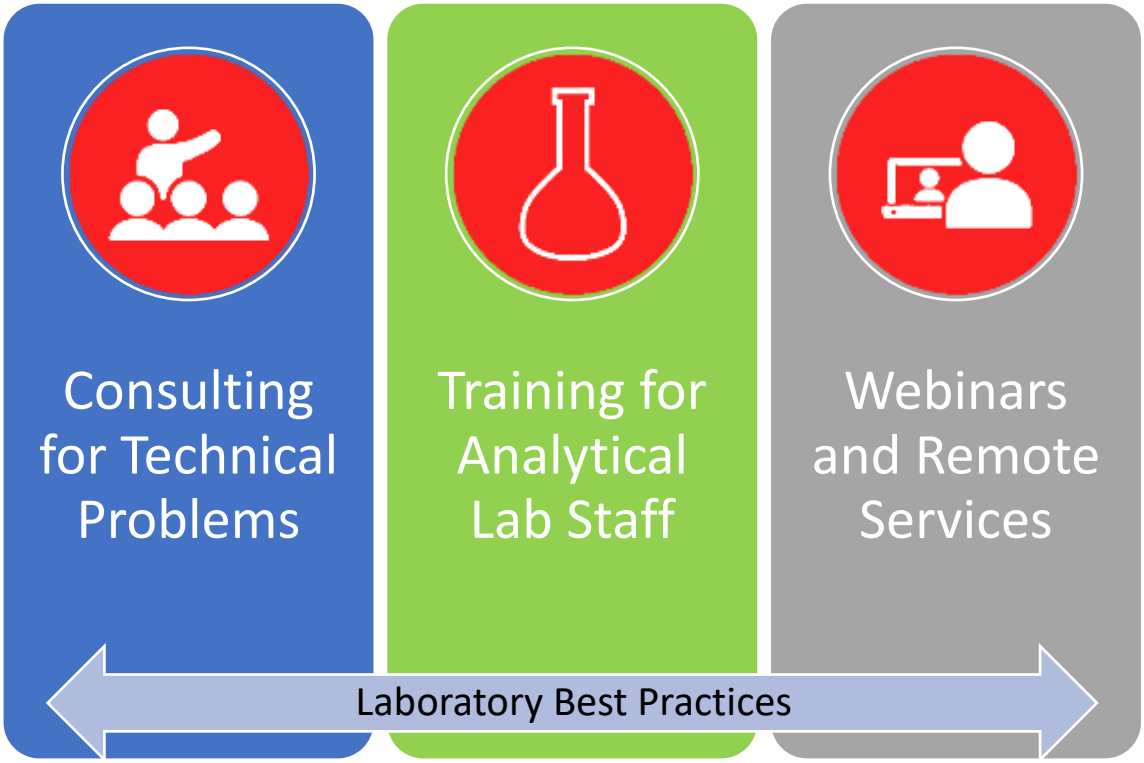
Quote for the Day

"Always expect to find at least one error when you proofread your own statistics. If you don't, you are probably making the same mistake twice."

Cheryl Russell,

quoted in Tom Parker, p. 124.

About Us



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