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A Primer on Bad Laboratory Practices (BLPs) for Bioanalytical Chemistry

How to do bioanalytical chemistry the wrong way...

We hear a lot these days about Good Laboratory Practices (GLP) as sanctified by the Food and Drug Administration as part of its mandate to assure that drugs are both safe and effective. One possible way to help reinforce what should be done is to examine a list of a few things that should *not* be done. I divide this abbreviated list into tactical (hands-on) and strategic (more philosophical and human resource-oriented) issues. These are listed in no particular order. Keep in mind that a large part of my teaching approach involves (1) humor and (2) stimulating discussion.

Tactical BLPs

1. Always include data falling outside the validated concentration range of the assay. This saves a great deal of time when overrange study samples are encountered. Why dilute such samples or extend the range when the data simply can be reported as observed?

2. When the reference standard is 98% pure, it is good practice to report the accuracy of the final results to at least five significant figures to placate the quality assurance group. What's really important is how many decimal places the software prints out, not the number of significant figures in the data. If it is printed, it must be significant. Every reader of the *New York Times* and the *National Enquirer* knows that.

3. Always believe the numbers on a mechanical pipette are accurate to better than 0.1%, even if they have never been calibrated. Remember, the manufacturers of such devices have impeccable quality control procedures and what they say in their advertising is absolutely guaranteed with no exceptions. At all. Ever.

4. It is most convenient to use a pH meter to measure or adjust the pH of LC mobile phases that are mixtures of water and acetonitrile or methanol. To make the measurement only with the aqueous components of the mixture is clearly inconvenient and unnecessary. Likewise, the date on which the calibrating buffers were purchased should not be marked on those bottles for fear of disturbing anyone who might use them. As for wine or scotch, the older the buffer, the better. Think "vintage buffer."

5. The temperature of an LC column need not be controlled for the best precision and accuracy. Everyone knows that temperature control is important in GC, but not in LC. This is among the best baloney that can be purchased.

6. When following a printed method, there is no reason to pay attention to the salt form of the reference standard.

Weigh out the same amount whether the salt is a hydrobromide or a bitartrate.

7. When taking a reference standard out of a deep freeze (e.g. -80° C), open the bottle immediately to enable moisture to condense on the standard, increasing its weight. That way the standard will last much longer.

8. If software can give a chromatographic peak area or peak height to 13 significant figures, this clearly shows that variation in the 10^{th} or 11^{th} figure will impact on whether an approved drug is really going to be safe and effective. Likewise, having records of the high school grades of the programmers who wrote the software is critical to understanding whether the data is good or not. Also, an electronic signature based on an 85-digit password is sure to overcome all possibility of fraud in drug development (just as collecting nail clippers will prevent aircraft hijackings).

9. Always pick an LC mobile phase pH as close as possible to the pK_a of the analyte. That way, if the pH varies slightly from one mobile phase batch to the next, the retention times will shift enough to keep you challenged and fascinated by how irreproducible chromatography can be.

10. Inject analytes into the liquid chromatograph using a solution that has a much stronger eluent strength than the mobile phase. This will help broaden the peaks and make them easier to see.

11. For gradient elution, it is best to form the gradient using 100% aqueous and 100% organic in each mobile phase bottle. This will assure that you get outgassing and form lots of pretty bubbles, producing a noticeably noisy baseline. This is more interesting visually than the standard approach.

12. When using a fixed loop LC injection valve, be sure to load the valve with a volume that matches the volume of the loop exactly so no sample is wasted.

13. Never do a bioanalytical experiment using a laboratory animal. It is totally clear from articles in airline magazines that the behavior of molecules in biological systems can be deduced *a priori* from computer simulations that make experiments totally unnecessary, and even enable the avoidance of synthesizing a compound that on a theoretical basis will not make it as a drug. Believe this stuff. It will facilitate your early retirement.

13a. If you're less than confident in theory, you might then try only *in vitro* experiments with cell cultures, which again enable you to avoid the messy reality of intact mammalian species from mice to monkeys to humans. Life is too complicated to have to deal with reality. Computer simulations are more convenient and there is no Animal Care and Use Committee (IACUC).

14. To determine the Lower Limit of Quantitation (LLOQ) for a derivatized analyte in a bioanalytical sample, prepare the derivative in mg quantities and then dilute it by 23 orders of magnitude.

15. Always wear white socks with dark pants and dark shoes in order to complement a white lab coat.

16. When studying protein binding for highly bound drugs using dialysis, always determine the drug on the protein side because the chromatographic peaks will be larger.

17. LC/MSMS is the ultra-selective analytical cure-all and you can forget the need to do sample cleanup or chromatography. Matrix effects, ion suppression, and labile metabolites that give the same m/z transitions as the parent compound are imaginary notions.

18. If a method works well in one species or matrix, run it with any kind of sample because they will all behave the same. Since the genome of a mouse, rat, and scientist are better than 98% similar, it's expected that their body fluids are close to identical too.

19. Scientifically elegant methods are *always* to be preferred over simple, practical approaches. It is most important to impress your colleagues with the complexity of your methodology, rather than to come up with something that actually works. An assay with 25 steps is to be preferred over one that achieves the same end in five steps (particularly if one step is to stand on your left leg and tug your right ear while performing the method). Otherwise, people in the lab will not be challenged enough and get bored.

Strategic BLPs

1. The best way to work with bioanalytical chemists is to not tell them the context of the project or, in some cases, even the structure of the analyte or its pK_a. By following this rule, the analytical chemists will remain apathetic to the project and not feel like full members of the project team. After all, anyone can get good numbers, given the fancy instruments and software available today. It's simply a matter of pushing a few buttons. Bioanalytical people are in the trees; they should not be allowed to see the forest. Remember: Information is power and sharing it is giving up power.

2. The best way to outsource bioanalytical work is to hold an auction on the Internet where the various bidders are blind to one another and can't talk about details with the sponsor. In this way, the low bidder can be accepted without muddying the process with such details as quality, experience, trust, schedule and location. It really doesn't matter whether a lab has experience with molecules of similar structure, since this work has no intellectual content and is very similar to buying screws in bulk at a hardware store.

3. Be sure that only the outsourcing liaison at a pharma or biotech talks to his/her "customer relations" equivalent at a contract lab. To have the scientists at both institutions actually speak to one another is likely to be disruptive. Besides, *all* of the necessary details have been clearly written down in methods documentation. There is no

"art" in the process. It is all routine. Scientists simply don't understand the "big picture" and should be kept in the closet where they can do their work.

4. It is often true that bioanalytical projects involve multiple departments in a big pharma. For example, a preclinical pharmacokinetics department might design the experiment, an animal dosing group on a different floor, in a different building or in a different city might collect the samples to be sent to the bioanalytical group. The bioanalytical group, in yet another location, need not be involved at all in designing the experiment or facilitating the sample collection. One of the biggest problems with small companies is that these people all interact well with one another, and that just adds a bureaucratic impediment to progress. This lack of focus is one reason discovery of new drugs is much more efficient at very large companies who then must license their candidates to the small ones for further development.

5. Numbers supporting a hypothesis are clearly acceptable; any which do not must definitely be in error and can be blamed on a mistake involving a lab technician or an instrument. Those latter samples should be tested again and again until the "correct" answer is obtained.

6. "We've always done it this way at Company X. We can't accept doing it some other way even if it is much better. The paperwork to change would simply overwhelm us and the format of our documentation in the lab would suddenly not fit tradition." SOPs are SOPs and they must never be violated or changed. After all, good protocol documentation is far more important than good data efficiently obtained.

7. Don't send analytical chemists to meetings on pharmacology or drug metabolism or neuroscience or cancer where they might actually learn something of great importance that can help advance science. Instead, send them to meetings of other analytical chemists looking at toys.

8. The really important thing is to advance people according to the "degrees" they list after their name. It really doesn't matter that a smart and curious B.S. or M.S. person may know more of value to a company after four years of work than a Ph.D. knows after six years in school. They may also know less, but why take the chance? It's best just to assume they know less. Otherwise, it would be embarrassing when the director of a laboratory of ten Ph.D.s is a Mr. or Ms. instead of a Dr. It is ideal to use the UK as a model whereby multiple descriptors at both the beginning and end of a name are highly indicative of being beyond the need to accomplish anything of real importance. In any event, the only people who deserve real respect are those with the label M.D. (Many very capable people collect academic degrees and titles. The points I am making here are that (1) many others don't, and (2) labels can be very deceptive.)

9. Be sure that biologists and organic chemists actually believe what they read in advertisements and trade magazines about modern instrumentation. They will get the impression from these sources that LC/MS/MS, for one example, or biosensors, for another, actually work great, never encounter interferences, have linearity stretching from Boston to London and can be operated by an idiot.

What Have We Learned?

The above is presented with some small degree of cynicism for the purpose of stimulating thought and hoping that several points may have been made. These include:

1. In bioanalytical chemistry, the details must not be neglected.

2. Everyone else's profession in science looks simpler than your own, but this is never true. The more you know about a subject, the more complicated it gets.

3. Measurements made without attention to why they must be made are often bad measurements. Bioanalytical chemists should understand the "bio" as much as the "analytical."

4. Don't believe everything you read, whether it is published in a "peer-reviewed" journal, a trade magazine, or an advertisement.

5. Many components of regulatory affairs have nothing whatever to do with making drugs safer or more effective, but many other components do!

6. Arrogance in one profession flows from ignorance in another. Using the example of drug development, many professions are required. Organic chemistry, pharmacology, biochemistry, drug metabolism, toxicology, analytical chemistry, pharmaceutics, medicine and pharmacokinetics are a few of them. All are essential. If any one is missing, nothing will happen. Thus, all deserve equal respect.

7. A sense of humor is essential to lowering stress in drug and medical device development.

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This article first appeared in *Contract Pharma*, Vol.4, No.1, and is being reprinted with permission.