

A New Era for Nutritional Supplement Manufacturers:

An Interview with Russell L. Rhines, PhD, Director of Technical Affairs, Innate Response

Interview by Matt Laughlin



Dr. Rhines has a diverse background in analytical testing, including peptide analysis at the University of Paris. He was research manager at Behring Diagnostics where his group developed immunoassays. As director of corporate technology at REVET Laboratories, Dr. Rhines consulted with high tech companies, providing specific analytical solutions to fit their needs. He also served as laboratory director at Microbac Laboratories Massachusetts Division. Currently, Dr. Rhines heads Delta Analytical and Research Laboratories as director QA/QC and laboratories, where he developed methods for testing BioSan's unique products and ensures stringent manufacturing requirements are met.

UH (Unified Health): Something that has always struck me about clinicians in holistic medicine is how much they value high quality supplements or botanicals. When I first heard you lecture I found your presentation illuminated the topic of what goes into manufacturing. It seems few clinicians really understand what is entailed in analyzing and testing products. Do you find that to be true in your experience?

RR (Russell L. Rhines, PhD): Absolutely. I found the same thing years ago when I worked in clinical diagnostics with MDs in hospital settings. The doctors that are prescribing the tests really don't know how they work, what their limitations are, or how quality is ensured. I see the same thing with clinicians in the natural health community. Many of the naturopathic physicians or chiropractors with whom I've spoken will recommend someone take a dietary supplement presuming it is what it's supposed to be, yet really have no idea about how it's shown to be so.

UH And it isn't for lack of seeking the information out; it just doesn't seem to be widely available. In your presentations to the clinical community, what are some of the things that most strike the clinicians in the audience?

RR I think many clinicians are surprised that the regulation in the supplement industry has been fairly poor until recently; the FDA previously required little testing. Supplements were treated more as food in the sense that you had to have a clean facility and you had to make sure there wasn't any microbial contamination. But prior to the new rule from the FDA, manufacturers really didn't have to test for potency of anything. A manufacturer simply bought a raw material from another source, and that source told the manufacturer the concentration of whatever was in there.

UH Before we get into the new regulations around manufacturing practices or your work with Innate Response Formulas, would you comment on your background in analytical testing?

RR Sure. Before coming here, I worked for a company called Microbac. They have 26 to 30 labs across the country, doing all sorts of analytical testing. I was the laboratory director for their Massachusetts division, and we did a lot of waste water testing for permitting purposes. We did drinking water testing, both for private homes as well as testing for MWRA, which is the water supply for Boston. And all large public water systems have to do this extensive testing once every two years, testing for synthetic or organic components (SOC). We were the first laboratory to receive full SOC certification in Massachusetts, and one of four in the country. Before that I worked for a company called REVET Laboratories, also doing analytical testing and waste water testing. We did custom testing, including working for a company in western Massachusetts who was the largest public water user in their city. They were looking to recycle their water; we went in, made suggestions, performed testing for them and designed new methods of testing to suit their purposes.

Prior to that, I worked for Behring Diagnostics, (now Dade Behring). I was a research manager and developed clinical tests for things like T3 and T4 (thyroid hormones), progesterone and digitoxin. We developed, for instance, a therapeutic drug monitoring test for digitoxin, which is one of those drugs where the therapeutic range and the lethal range are very, very close. Prior to that I received my PhD in 1991 from Baylor and immediately spent a year in Paris, France working in a laboratory synthesizing unnaturally occurring peptides and affinity chromatography.

UH Paris sounds like a fun place to start off your career.

RR Yes, it was fun. Paris was a great experience for me and my family.

UH In your present work, how would you describe the range of analytical testing you do for the Innate Response line?

RR The raw material and finished product testing we conduct is many and varied. Innate Response is produced by BioSan Laboratories, our parent company. We have two daughter companies; Durham Research, which manufactures the raw materials for most of the Innate Response products, and Delta, an analytical laboratory where we do all the testing for all the product lines. At BioSan Labs, we look at tablet weights and uniformity, thickness, hardness and friability (susceptibility to breaking); we ensure the tablets themselves are of the right quality, color, etc. The raw material testing is conducted at Delta; we test the minerals and the raw materials for heavy metal contamination, we test the products for vitamin potency, mineral potency and everything else that we claim.

I was hired to develop methods for testing the raw materials from Durham Research. When you manufacture products yourself you have to provide certificates of analysis, which requires testing. BioSan also had the foresight to realize more testing was on the horizon, because at that time this new FDA rule and guidelines were being discussed.

UH In terms of the Innate Response line as whole food supplements, you're not testing for pharmaceutical grade vitamins; you're testing for the potency of vitamins and minerals with a myriad of other food compounds and constituents. What kind of challenges does that present for you?

RR Well, a big one. It's fairly easy to measure vitamin content in a pharmaceutical type vitamin, because there aren't many components in there. There are typically only vitamins, minerals, and excipients (inactives). But with our products, we have the vitamins and minerals, as well as herbs, botanicals and many thousands of other food compounds.

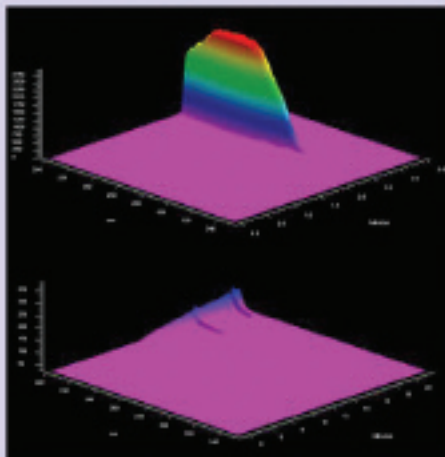
Imagine looking for vitamin C in a pharmaceutical grade vitamin. It would consist of taking some tablets and grinding them up and then extracting them with an aqueous solution. You would then inject it into the HPLC and you're pretty much finished. But if you were looking for vitamin C in an orange, you would have a lot more work to do, because there are a vast number of components, and they can potentially interfere with your analysis. You have to separate those things out and find a way to extract the vitamin C from the orange. A simple analogy is if your car is in a parking lot and there are only four other cars, it's pretty easy to find. But if you're looking around a parking lot with 4,000 other cars, it would be a whole lot more difficult. Because the vitamin C in our product is oranges, it's much more difficult.

UH So you have had to innovate different methods to accomplish that kind of analysis for whole food supplements. Could you offer an example?

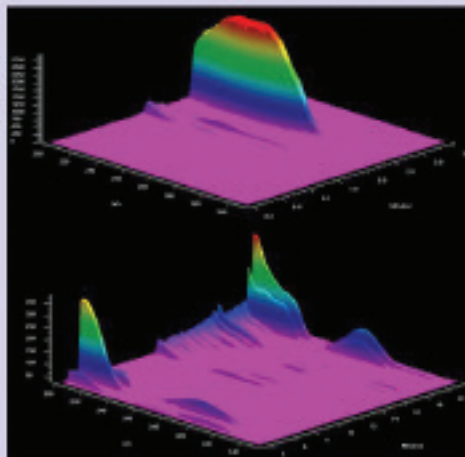
RR There are just so many, because no test works for everything. It's different for each product and each phase of testing. One example that comes to mind is the typical way vitamin A content is measured; vitamin A not as beta carotene, but as the typical retinol or one of the retinyl esters that are commonly used. The typical analytic testing for this is a long, complicated procedure that is very labor intensive. While I've been working for 30 years in the analytical industry I could only do maybe four samples in a day. There are different forms of vitamin A, whether retinol acetate, retinol palmitate, or even retinol if it is a topical application. The method that most people use involves breaking that vitamin A down into just plain retinol and then measuring retinol. The method I developed is a way to measure the ester directly.

To me, validation means: does it measure what it's supposed to measure? Does it pick up different concentrations? Do you get the same answer every time you keep testing the same sample? And the best thing you can do, if there's another method available, is compare the two. Take the samples and run them on the published method, and then run the same samples with the method you've developed.

Three Dimensional PDA Demonstrates Complexity of Whole Food Matrix



Pharmaceutical Vitamin



Whole Food Vitamin

Your method is valid if you get the same answers with both methods.

UH Here, you're referring to a test you would do on a finished product. In the case of a multivitamin, what kinds of tests would you do?

RR For a multi, the vitamin is the hard part. The minerals are comparatively simple. All vitamins have different physical properties, and roughly different classes, such as water soluble and fat soluble. For the water soluble vitamins, we grind up tablets as we do for any vitamin, and then the trick is to extract it with the appropriate solvent. There are different parameters; what pH does it need to be? What temperature? But once extracted, the stability profile may be very different than when in the product itself.

In other words, we can run our instruments and extract a lot of samples but that's not going to get the job done if the vitamin is going to break down in that solution. So one of the critical parts of method development is looking at those extracts and determining how long they're stable. For instance, if you wanted to extract vitamin C, you could use a pH 9 buffered water, and it would be easy to get all of the vitamin C out; however, it would oxidize and by the time you measured it wouldn't be there. You have to develop methods such that you can get what you want out and yet keep it stable in that form until it is analyzed.

UH This is done in order to document the potency of a particular vitamin or mineral in a finished product?

RR Exactly. We're analyzing our products so we know the form that's in the finished product. It's a trade off, because most of the work is in the extraction part of the process, yet many of the published USP methods or AOAC methods simply don't work.

UH They work for isolates, or pharmaceutical grade vitamins.

RR Yes, and some of them do work on whole food supplements, but not all of them. Often we may start with one of those methods and then modify it. We may vary the pH on the water soluble vitamins, or sometimes we sonicate the material with sound waves. When you're first starting to develop a method, for instance, you might extract for 10, 20 and 30 minutes each under different conditions and com-

pare all those to determine how long you have to do this to arrive at a viable extract. After that, you look at the stability of what you've extracted.

UH You mentioned you might run extracts through an "HPLC." What is that?

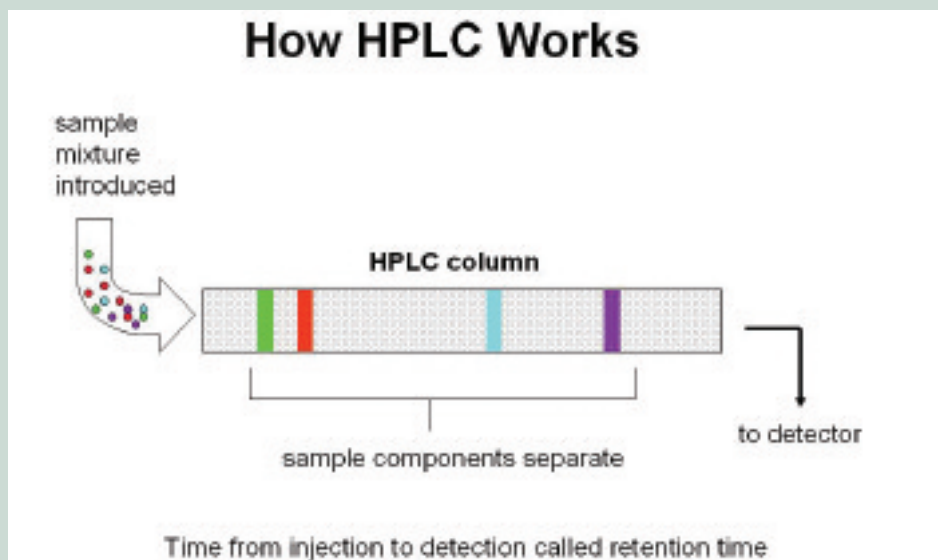
RR HPLC stands for high performance liquid chromatography. The HP part is self-evident – high performance. The sample has to be dissolved in a liquid, which is what I was describing with regard to extraction methods. You have to get the vitamin out of the tablet and into a liquid form in order to analyze it, all the while maintaining its stability. Chromatography simply means separation of components. So, once you've got this liquid and you have your vitamin of interest among thousands of other things, you want to separate out what you're looking for and quantitate it.

Basically this liquid extracted sample is injected into the HPLC; as it goes through the column, the things that are dissolved in that extracted liquid interact with the material in the column to different extents. Each component's progress through the column is impeded depending on this interaction. They emerge at the end of the column at different times; vitamin C might take four minutes, for instance, while vitamin B1 may take ten minutes. The HPLC essentially gets the components in liquid form and separates them along the column. Of course, there's a whole lot more to that and different types of columns, etc. For example, after it comes out of the column, you have to have a way to detect each component, and there are various methods we use to detect these vitamins as they elute.

UH Sounds like you can test for multiple vitamins with one extraction.

RR Yes, to an extent. Ideally, I would like to take a tablet and test every single vitamin with one extraction and one HPLC run. But that is not possible, because you're never going to get the water soluble vitamins and the fat soluble vitamins out at the same time; the fat soluble vitamins don't like water and they'll just stay in the tablet where they're happy. (Laughter) For fat soluble vitamins we'll use various organic solvents to extract them.

UH How are the minerals tested?



RR We take the tablets again and grind them into a powder, and conduct what's called an acid digestion, where we add strong acids and heat them. What that does is destroy all of the other vitamins and other organic compounds in the sample and gets rid of all or most of interferences. What you're left with is an acidic solution with all the minerals dissolved in it. It's a whole lot easier than the vitamin part because the minerals pretty much have the same properties. We then take that and analyze it by ICP.

UH What does ICP stand for?

RR ICP stands for inductively coupled plasma. This involves a quartz tube, with argon passing through that tube; subject it to a very high radio frequency energy and it strips the electrons off the argon to form plasma; hence the name. If you look at an ICP plasma it looks like a little candle. But there's nothing burning there; it's just bare argon atoms, and it's very, very hot. The center of the plasma can be 15,000 to 20,000 degrees. At that temperature it's hotter than the surface of the sun. What's nice about this technique is it allows you to analyze many, many minerals at the same time. When you take a mineral and you excite it somehow – in this case we're using heat from the plasma – they give off wavelengths at very discrete energies. Every mineral, and every element which is part of the mineral, has a set of atomic lines associated with it in actual nanometers. You can pick a wavelength for each element and you can find one in the presence of twenty others.

UH That makes it easier.

RR It makes it a whole lot easier. We could analyze for 25 minerals at once.

UH Is ICP also used to test for heavy metals?

RR Yes it is. We test for four heavy metals; lead, cadmium, mercury and arsenic, which are the most common heavy metals you might find, especially in botanicals. The type of ICP we use is called OES – optical emission spectroscopy. As I said previously, it's the light coming from these excited minerals that we detect. For most elements you can see down to the low part per billion range. With cadmium, for instance, you can easily see two parts per billion in a solution.

UH Wow, it sounds very precise.

RR It's an amazing instrument; it really is. I remember when I saw one of the first ones ever made. It was a monstrous thing. (Laughter)

UH They're a little sleeker these days. (Laughter)

RR They are. The one we presently use is probably six years old and they just keep getting smaller and smaller. Soon enough you'll be able to stick one in your pocket. (Laughter)

UH How do you go about testing botanicals, given their complex nature?

RR It's a very, very difficult thing, and we continue to work on methods to do that. We're pursuing HPTLC, or high performance thin layer chromatography. This is very similar to the HPLC, with regard to the chromatography. It's just a matter of how it's done. In this case, a botanical is extracted,

providing many components in solution, and then spotted on a plate coated with silica. That spot dries out and then the plate is placed in a solvent; as the solvent runs up the plate it takes that spot with it and the different components react with the plate and migrate up that plate at different rates. What you're left with is a complex finger print, if you will.

UH What makes botanicals more challenging?

RR The big challenge with botanicals is that they're so different. It depends on where they're grown and the time of the year they're harvested. You'll never see two plants that look exactly alike.

UH When testing, you're paying attention, primarily, to the known constituents in the botanicals?

RR Yes, we'll run standards of the known constituents. We'll purchase a certified material that is what it's supposed to be and compare against that. Another popular testing method for botanicals is organoleptic assessment, meaning the taste, color, smell and appearance of the product. If it's supposed to be apple powder and it's blue and it tastes like cranberry, it's not apple powder. It's fairly reliable; the wine industry has been reliably using organoleptic testing for years.

UH Could you comment on the variations to the kinds of testing and methods used, and the costs involved?

RR The equipment we have – the four HPLCs, the ICP, the mass spectrometer – probably cost a little over a million dollars alone. And that doesn't include the cost of having all of those installed in your laboratory, which involves getting the electricity, the water, the gas, other ancillary equipment, and so on. A lot of these smaller companies do very little testing. For a company our size, we have an impressive lab.

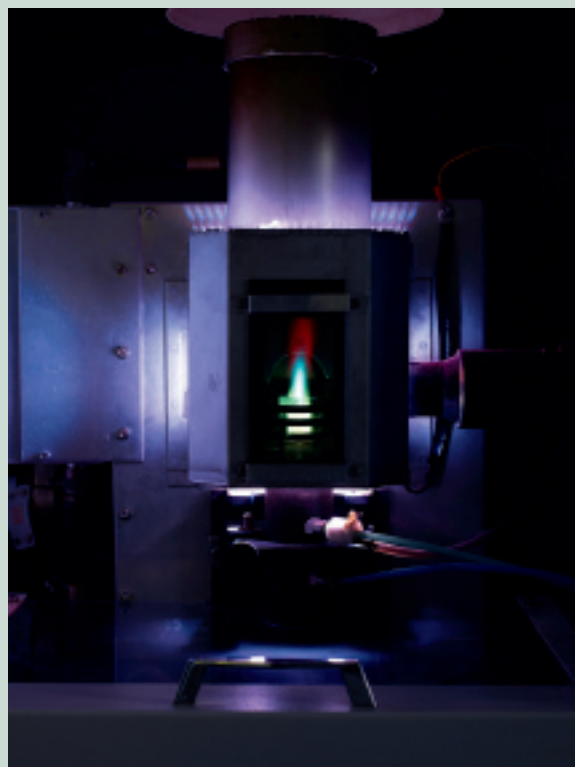


Figure 1: Photograph of ICP (Inductively Coupled Plasma)

UH Does the testing for the Innate Response line meet the current FDA regulations?

RR It goes beyond. This might be a good place to comment on the new FDA rule. When the FDA publishes a new rule, such as the new regulations being enacted, it's almost 900 pages long. There are a lot of things in there that are vague, and a lot depends on the interpretation of it; a manufacturer can read it and still not understand exactly what it is they're specifically required to do. So, over time, answers to those specific questions tend to come out in specific guidelines that the FDA publishes later. For instance, it took ten years to get the current rule published from the time they began this process.

UH What's the core of the new rule?

RR In June of 2007, a new FDA rule – regulations and good manufacturing practices – was enacted for and applies to nutritional supplement manufacturers only. Suppliers of dietary ingredients and raw materials were initially included, but that was removed by the time the final rule was published. What this means as a manufacturer is that if somebody sells you vitamin C and it's really vitamin A, it's the responsibility of the manufacturer; you have to test those raw materials to ensure they're what they're supposed to be. The FDA has put most of the emphasis on raw material testing by the manufacturer, the idea being that if you test your raw materials before you mix them up into a tablet, that tablet is going to be fine because you have tested all the ingredients. The new rule was ten years in the making, due in part to the enormous financial impact it had on the industry. Over that period, the initial proposed rule went through a great deal of revision.

UH Can you explain the rules regarding expiration dates? As a clinician, you'd want to know that your product is still potent after purchasing it and using it in practice at a later date.

RR That's another important aspect of this new rule. The regulations do not require manufacturers to put an expiration date on products. However, if you do put an expiration date on finished products – as we do – then you have to show at the end of the expiration that product still has 100% or more of everything you claim is there. We've been doing this for years, since we first began manufacturing our raw material.

The products must be tested for the actual expiration; each of our 150 products has to go into long-term stability testing. This requires a great deal of space because you have to do the testing in finished packaging; you can't just take a few tablets. This is done for every scale of the formula, whether 30, 60 or 90 count – all must be tested.

Of course, if you bring a product to market with a 3-year expiration date, you're not going to wait three years for stability testing data. So, in addition to a 3-year test under normal conditions we also conduct accelerated stability testing using a method from the pharmaceutical industry guidelines. For instance, we keep a formulation at 45 degrees C at 75% relative humidity in a chamber; at the end of three months, if you have 100% or more of everything you've put in the product, this roughly translates to two years of shelf life in the real world.

UH What can you say about documenting all of these various kinds of testing methods?

RR Documentation is essential. Not just to comply, but

as good practice. In my experience in clinical diagnostics, of all FDA inspections, 70 to 80% of compliance had to do with documentation. They'll come in and see if the facility is clean, that sort of thing and then look at what you've documented. They are very precise about this. The way they look at it, for example, is if someone added an ingredient into the mix and 400 people saw them do it, if it wasn't documented, it just didn't happen.

UH What kinds of things do Innate Response document?

RR Everything from beginning to end. We have the specifications for our raw materials, and we have documentation that shows that the raw materials we purchased passed those specifications. All of the manufacturing steps are documented. We have a master batch record; this is how one makes it, these are the steps of how to do it. Each and every step is documented. Every time someone weighs an ingredient, one person weighs and records while another signs off that they witnessed this. At the end of the day when we have a finished product, we can document every raw material that went into that product. This is valuable, too, because if something went wrong anywhere during that process you can trace it. Traceability is very important and this is something we've been doing forever.

UH It seems as though this rule is going to impact the industry significantly, for manufacturers and clinicians alike. What does this mean for other companies?

RR With this new regulation coming into play, I think you'll see a lot of companies are just not going to be able to afford this kind of testing. If you don't have your own lab and you have to send materials to other laboratories for testing, it is extraordinarily expensive. It could cost you \$10-15,000 to test a single lot of a multivitamin product.

As for clinicians, I think they'll be more assured that what they are using is what it says on the label now that the manufacturers are required to test those products. We've tested competitor products that don't meet their claims; they may have not tested their materials and some things are inherently unstable and one just needs to change the formulation to make them stable. This new regulation will force that. Slowly but surely I think you'll find higher quality products on the market.

UH Given the complexities and costs involved, while BioSan Labs and the Innate Response line is ready, it sounds as though many of the other mid-size or smaller companies in the industry may be more hard-pressed to comply.

RR Exactly. BioSan Labs has myself and a team of chemists, but a lot of manufacturers don't have PhD chemists on site and they may not be aware of a lot of these problems or where to turn. We've been preparing for several years before the rule was published, and before that the company was sending out samples to various laboratories. We're in really good shape.

UH Thanks for your time, Dr Rines.

RR My pleasure; thank you.