THE Q&A

Matteo Villain

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This is a look into the Peptide Purification Industry from the eyes of Matteo Villain, Vice President of Research and Development at Bachem Americas, Torrance, USA. Matteo joined Bachem in 2004. In the capacity of Vice President of R&D, Matteo is in charge of introducing new peptide synthesis technologies to Bachem Americas. Under his supervision, the R&D group is responsible for custom pre-GMP and early GMP API development, which includes synthesis and purification strategies, analytical method development, and LC/MS method development. In the Research Department special emphasis is given to the use of LC/MS analysis to support process development, purification development, and characterization of impurities.

Matteo graduated from the University of Milan, Italy, in 1994; thereafter he collaborated as Post Doctoral fellow with different Universities and Research Organizations, both in the USA and in Switzerland. His main focus during his fellowship was the development of peptide therapeutics and solid-phase peptide synthesis (SPPS) strategies.

PHENOMENEX: As an expert in peptide chemistry would you agree that reversed phase high performance liquid chromatography (RP-HPLC) is the method of choice for peptide purification?

MV: Definitely yes. Reversed phase chromatography represents the state of the art technique for the final stage of the peptide purification process which allows us to reach the high level of purity required for active pharmaceutical ingredients (APIs).

PHENOMENEX: What are the main advantages of reversed phase HPLC compared to other purification techniques?

MV: Very often the selectivity that can be achieved with reversed phase chromatography cannot be achieved with other methods, for example ion-exchange chromatography. Ion-exchange chromatography remains a method orthogonal to reversed phase, but with reversed phase we can modulate the selectivity of the media by changing the buffer systems and achieve an effective purification that we cannot obtain with ion-exchange chromatography without using two purification medias. Reversed phase chromatography is a very flexible purification technique. The other advantage of reversed phase chromatography compared to ion-exchange chromatography is that we can achieve high resolution especially for synthetic peptides which contain impurities with retention characteristics very similar to the target molecule. And finally, when we use reversed phase chromatography, the final isolation of

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the solid product in the salt form is simplified by the fact that we can select volatile solvent and volatile counter ion. On the other hand, with ion-exchange

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chromatography, we still have the problem of removing the elution salt. So reversed phase chromatography gives us a very simple process which is easy to scale up.

PHENOMENEX: What are some other peptide purification techniques and how do they compare to purifying peptides with reversed phase chromatography?

MV: Countercurrent distribution has been used, and it is still used to purify peptides at large scale. This technique utilizes a system of different extraction solvents to perform the purification. The problem with this purification technique is that it does not offer the high resolution of reversed phase chromatography. Other purification techniques often applied for small organic molecules, like crystallization, are very complicated for long peptides and generally do not allow us to obtain the final high purity required for APIs. Others have also tried to purify peptides by size exclusion chromatography, but this technique is not very efficient and offers low loading capacities. It is not as cost efficient as reversed phase chromatography.

PHENOMENEX: What is your general approach in peptide purification development?

MV: At the very early stages in the developmental process, we identify the critical impurities in our product and develop a purification method that is able to separate the product from all the closely eluting impurities. We use a screening panel of different chromatography medias that we believe are appropriate for the product that needs to be purified. We only screen media that can meet our production scale-up needs at later stages of development and eventually commercialization. We then evaluate which media gives us the best selectivity for the impurities we are dealing with. We believe that the media selection is one of the critical steps in the developmental process since once multiple lots of a certain product have been produced, it is very difficult and complex to change over to a new media. This can lead to challenges with regulatory requirements along with a major investment from our customers. We are very confident with the media offered by Phenomenex.

PHENOMENEX: What type of stationary phase (particle size, pore size, and ligand) would you recommend for reversed phase HPLC peptide purification?

MV: It is very difficult to generalize because peptides are amphipathic heteropolymers and each peptide possesses some characteristic of hydrophobicity, hydrophilicity, and difference in charges. It is very difficult to single out one particular stationary phase. We know that except for the extreme case of hydrophilicity or with the extreme case of hydrophobicity, the optimal media for peptides is either a 10 µm C8 or 10 µm C18 stationary phase. A more important aspect in developing a purification process is the selectivity of the media. If the stationary phase doesn't provide the selectivity required by the nature of the impurities we need to separate, then it is not appropriate. The media pore size can also have an effect on the chromatography. There is a tradeoff between a larger pore size media like 200 Å and 300 Å, which should be more suitable for large peptides, and one with a 100 Å pore size. Typically, we've observed that media with larger pore sizes have reduced loading capacities and they tend to have a lower mechanical strength compared to smaller pore size media. At the end of the day, media selection comes down to selectivity and loading capacity, and is very dependent on the individual peptide.

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PHENOMENEX: Can you describe how you choose your panel of chromatography media?

MV: For us, the selection of media we include in the screening panel is extremely important. We are making a commitment to our customers that we are developing a long term viable process that they are most likely not going to use at commercial scale until 7-10 years from now. So, when we select media for large scale purification, there are multiple aspects we should consider. We have to take into account not only the media selectivity but we must also consider the bulk price and the reliability of the media supplier. We will never propose a media with great selectivity from a company which we are not convinced will still be in business in 10 years. If the selected media supplier went out of business, it would require a lot of costly process changes. This is why we select our media manufacturers very carefully.

PHENOMENEX: How many media phases do you typically screen?

MV: We will typically screen 3-5 different 10 µm medias per each application.

PHENOMENEX: What do you expect from a stationary phase media manufacturer in terms of availability, choice of phase, and delivery time?

MV: We need to know that the supplier can deliver within a very short lead time, or that they are able to produce the amount that we require very quickly. During the developmental process we expect the media manufacturer to supply between 1 kg and 10 kg.

PHENOMENEX: Are Phenomenex media in your screening panel?

MV: Yes, they are. Phenomenex has started to reach critical mass in the industry and the company has 35 years of history in chromatography. This proves that Phenomenex will likely be here for many years to come while offering the same bulk media we are getting today well into the future.

PHENOMENEX: In terms of media selection, describe a media that you wish was commercially available?

MV: Sometimes we have a need for media with higher chemical resistance. There have been some situations where we would like to use more acidic or more basic conditions than what is the normal range of our regular chromatography media. So we think there is a future possibility to use higher chemically stable media. I know this media already exists but I would like to see a decrease in the media cost to make them more commercially viable.

PHENOMENEX: In your view what would be the most efficient way to scale up your purification process?

MV: We normally use a 250 x 4.6 mm ID, 10 µm analytical scout column to optimize the conditions of purification. We use this small column as a real purification system to collect and analyze micro fractions. We then confirm the analytical column conditions with a 5 cm ID column

prior to scale-up to the large diameter column used at final manufacturing scale.

PHENOMENEX: What are some of the challenges facing peptide purification and how can they be overcome in the future?

MV: There are many challenges in purifying peptides. In my opinion, one of the biggest challenges facing the peptide industry is the current expectation both by the authorities and by the sponsors to have a complete understanding and control of the impurity profile associated with a peptidic API. One key concept in this context is control. This can only be achieved by the use of highly sophisticated analytical tools. Bachem has recognized this change since the early 2000s. These expectations have prompted us to adopt the most advanced chromatographic analytical tools available. Since 2004 we have conducted significant investments in ultra-high performance liquid chromatography (UHPLC). UHPLC allows us to determine the true purity of the product with details that we have not been able to observe before. Use of UHPLC in combination with high resolution mass spectrometry currently allows us to really dissect and to resolve the majority of the impurities and to establish for most of them a potential identity.

While in preparative scale we do not yet have the equivalent resolution capacity of UHPLC, we realized over the years that by applying UHPLC analysis during the purification process we can obtain a clear understanding of the impurity profile. This understanding allows us to control in great detail the impurities level.

Just because we can see the impurities with UHPLC does not mean that we can economically resolve and completely reject them at preparative scale. On the other hand we can control them, and set realistic limits in accordance with regulatory expectations. Totally removing these impurities can come at a high cost due to an increase in product recovery loss. On the other hand controlling them allows us to produce material with a well-controlled and defined impurity profile.

The second challenge is the economic and ecologic aspect of chromatography. In the US and in Europe, there is a high price for operating an organic solvent system and remaining environmentally compliant. So we are constantly reducing the amount of our organic solvent consumption. This is achieved by leveraging the selectivity of the purification media with the objective of reducing the number of purification dimensions, and by maximizing column loading.

PHENOMENEX: Is solvent recycling an option to overcome your environmental challenge?

MV: There are ways to recycle solvents but they are very expensive. Working in a regulated environment, one of the draw backs of solvent recycling is that we have to verify that we are continuously able to recover the solvent with the same initial specifications. Solvent recycling works relatively well if we operate a single product facility, because the impurities that we are generating are all correlated to the product. Most of the facilities that are developing peptides at the intermediate scale are not single product facilities. Solvent recycling also gets very complicated when we try to recycle solvent mixtures used in gradient methods.

PHENOMENEX: At what point do you feel that your purification process is under control?

MV: My process is under control when I know the fate of my most relevant impurities. I also have to know in which part of the process the impurities are generated. Our objective is to be in control during what we call the qualification batch. The industry is changing and the FDA is changing its expectation from CMOs. In the past, if I could repeat a purification three times without deviation and with the same final material quality then I was in control of the purification process. The FDA and the EMEA are in the process of changing their expectations on the process validation approach. These agencies expect that we are in control when we totally understand our process. So I know my process is in control when I know the fate of my most relevant impurities.

PHENOMENEX: Do you see your work load increasing next year? In the next 5 years?

MV: Yes. There is always an interest in biotechnology and biopharmaceuticals, and we see companies making this reinvestment in the near future.

PHENOMENEX: Which types of peptide therapeutics do you think will increase in the coming years?

MV: We see a fast growing interest in pegylated peptides. Some people believe that some of the draw backs with peptides are their short half life *in vivo* or low bioavailability. Many times these drawbacks can be overcome by pegylation. At the experimental level, many peptides have a very short half life, in the range of minutes. When derivatized with PEG, some peptides exhibit a prolonged half life in the order of hours and even days. In the past there were many peptides with interesting in vitro activity that were abandoned due to poor bioavailability. I think we will see a rebirth of these peptides in a PEG form.

PHENOMENEX: What is one change or improvement you hope to see in the peptide separations industry over the next few years?

MV: I would like to see the combination of modern media with purification equipment that will withstand higher pressures at a preparative level. I would like to experiment and see a large scale purification apparatus with supercritical fluid chromatography (SFC) applied to peptides. Most of the purification methods I have seen with SFC are isocratic methods. Isocratic methods rarely apply to peptide purification, which normally employ gradients. And many times, after CO_2 evaporation, the product is left in a slurry of organic solvents. This may allow the product to be isolated by precipitation or solvent evaporation, but I still see some technical problems with this technique when applied to purifying peptides.

PHENOMENEX: Pharmaceutical companies, as well as contract manufacturing organizations (CMOs), increasingly outsource their phase screening, as well as purification development, to PhenoLogix™, an analytical support laboratory within Phenomenex that specializes in HPLC method development and optimization. Are such services relevant for the kind of work your company is doing?

MV: Historically we have preferred to keep all development in house, but I can understand the potential advantage of using this group to streamline purification optimization.