Novartis Crop Protection, Inc.
P.O. Box 18300
Greensboro, NC 27419-8300
www.cp.us.novartis.com

Tel 336 632 6000

NOVARTIS

October 27, 1999

Mr. Steven J. Rupp
ZirChrom Separations, Inc.
P.O. Box 868
Anoka, MN 55303

Dear Mr. Rupp:

After months of HPLC method development work with emamectin, I was out of ideas. I had tried many types of silica-based columns and many different mobile phases, but I could not find a system that would be suitable for scaling up to a preparative level. Every method that I had tested gave me broad, tailing peaks. Even highly endcapped, base-deactivated columns gave poor results. Knowing that the problem was with the NH-CH_3 of the hydrophobic emamectin molecule interacting with the Si-OH in the HPLC columns, I started looking for alternatives to silica-based chromatography. That was when I found the ZirChrom web site and read about their zirconia-based PBD column.

We purchased an analytical ZirChrom-PBD column, and I was really impressed by the improvement that it made in my chromatography. Although the concept of "tunable selectivity" was new to me, I soon found a system using an ammonium phosphate buffer that gave rapid resolution of the emamectin and sharp, symmetric peaks. Load testing revealed that this column could handle much higher amounts of emamectin than any silica-based column that I had worked with and still give baseline resolution.

With the success that we saw with the analytical column, we asked if a preparative HPLC column could be packed with the same 3µm particles. We wanted a column that was the same length as the analytical column (150 mm), but with a larger i.d. so that developed methods could be scaled up directly. Our goal was to find an efficient purification method for synthesized radiolabeled emamectin. After working closely with ZirChrom technical support, we ordered a 150 x 22 mm (i.d.) preparative column along with the recommended guard column.

The performance of the preparative column was even better than I had expected. We had hoped to be able to run about 10 mg of crude emamectin per injection, but I found that the column could still give baseline resolution when injecting as much as 25 mg. Our original method used a silica-based phenyl column and a multi-step solvent gradient. Using the phenyl column, we could purify 10 mg per run, and each run took 2 1/2 hours.

With the preparative ZirChrom-PBD column under isocratic conditions, I was able to purify 25 mg of crude emamectin in 30 minutes. The time required to purify 300 mg of crude emamectin was reduced from weeks to a day!

Sincerely,

David A. Hunt

David A. Hunt
Analytical Chemist