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Lab Highlights
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LC IDENTIFIED AS MAJOR CONTRIBUTOR TO BIOTECHNOLOGY SEPARATIONS

Human insulin is the first commercial health care product produced by recombinant DNA technology. Eli Lilly, the producer of this synthetic insulin, often relies on HPLC to confirm the structure and to determine the potency of synthetic human insulin.

The story behind Lilly's recombinant DNA produced insulin is described in a recent issue of Science magazine (1). The article states,

> "High performance liquid chromatography (HPLC) techniques developed at Lilly can detect proteins that differ by a single amino acid, and HPLC tests show human insulin (recombinant DNA) is identical to pancreatic human insulin... A chromatogram of human insulin (recombinant DNA), pancreatic human insulin, and a mixture of the two, showed that they were superimposable and identical."

The article also states LC "...was useful for ensuring that we had the appropriate disulfide bonds and lacked other types of protein or peptide contaminants of a specifically degraded sample." The peptide maps following enzymatic digestion are identical for human insulin (recombinant DNA) and pancreatic human insulin.

The article concludes,

> "HPLC has now become an important analytical tool to determine structure and purity and is now considered to be a more precise measurement of potency than the rabbit assay, although most government regulatory agencies around the world still emphasize the rabbit potency assay... In the end, we employed 12 different tests to establish that what we had produced was human insulin. We believe that correlation among three of the tests was particularly important - the radioreceptor assay, radioimmunoassay, and HPLC."

In a separate article in Genetic Engineering News on interferon (2), Dr. Sidney Pestka of the Roche Institute of Molecular Biology says,

> "Just a few years ago, it was an enormous struggle to obtain a few micrograms of any interferon in pure form. With the rapid application of new technologies, many interferons have been purified and produced in relatively large amounts. These new technologies consist of three general areas: high performance liquid chromatography, monoclonal antibodies, and gene splicing. These three areas have merged to provide major achievements in interferon research."

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Dr. Pestka concludes that HPLC was "instrumental in achieving the first purification of human leukocyte interferon and in providing the initial surprising result that the leukocyte interferons were a family of closely related proteins, not a single species. HPLC has been used to purify other interferons as well."

The scientists at Alpha Therapeutics, a Los Angeles Biotechnology Company, are also working to look for new subspecies of naturally occurring leuckocyte interferon and are using size exclusion and reverse phase HPLC "with great success." Dr. Steve Herring, a Senior Research Scientist, in another article in Genetic Engineering News (3) concurs with Dr. Pestka,

> "...it was found that several classes of human interferon exist (leukocyte, fibroblast, immune), and that numerous interferon 'subspecies' may be present within each of these classes. Because these different interferon species can be quite similar, showing between 70 to 95 percent homology in amino acid sequence, it has been difficult until recently to separate them from each other."

Dr. Herring concludes the article by saying, "HPLC continues to be used in the purification of bacterially produced interferons" and that "columns and equipment are now available which would allow researchers to carry out the same separations to produce gram quantities of the interferon in a matter of minutes."

At the Schering Corporation in New Jersey, the work of Drs. Alan Levine and Paul Reichart demonstrates the advantage of HPLC. Their research groups have successfully isolated, purified, identified, and quantitated genetically engineered biologically-active proteins from fermentation broths. They use a Waters gradient LC to develop the separation on a Prep C_{18} Radial-PAK cartridge and then scale the separation up for use on a Waters PrepLC^{TM} 500. With the PrepLC^{TM} system they are able to take a 1.5 g protein sample with 30% biological activity and in 45 minutes obtain .5 g of 99% biologically active protein (4).

From these articles it is very evident that HPLC is a major contributor in biotechnology research.

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1. I. S. Johnson, Science, 219 (1983) 632
2. S. Pestka, Genetic Engineering News, (Sept/Oct. 1982)
3. S. Herring, Genetic Engineering News, (Mar/Apr. 1983)
4. Information courtesy of Drs. Levine and Reichart. Obtained by Kathy Robison, Waters sales representative at Schering.