CHINESE GROWTH HORMONE

BY RONNY TOBER In the last summer issue of Body of Science we published an extensive article about growth hormone. On our forums there is a lot discussion on the brands, the prices, and the quality of GH, especially that of Chinese origin. Generic Supplements, a Dutch underground laboratory, had done several analyses through us to find a constant and reliable API (raw powder) manufacturer. When they asked me to try and find a laboratory that was able and willing to analyse complex peptide hormones, because they wanted to analyse a generic Chinese GH version, I was very sceptical. From the research of the previous growth hormone article I knew that GeneScience had the patent in China for the manufacture of GH by secretion technology. And this method is the only one to produce 191 amino acid GH with high purity. When they offered us a sample in January 2006, we took it, and were very curious to find out the content. We ended up being greatly surprised by the results.

There are about 7 places making HGH powder in a lab in China, mostly the 192 amino acid variant known as somatrem. On the discussion boards there is much arguing about blue tops or red tops, but this means nothing as no one is sure which one you are getting from, nor where it was packed. These vials and tops are readily available and used for the packaging of all sorts of peptides. Everybody speaks about GeneScience Jintropin and its quality, but their main technical man left GenSci a year ago. He still owns 6% of the company, but he reportedly left due to a dispute. He has since started –producing HGH generic powder, which is the correct 191 amino acids growth hormone (somatropin), although it is made without GMP license. This individual is selling the technology and powders to many people, and subsequently there are many new brands

There is only one thing worse than buying poor quality steroids....

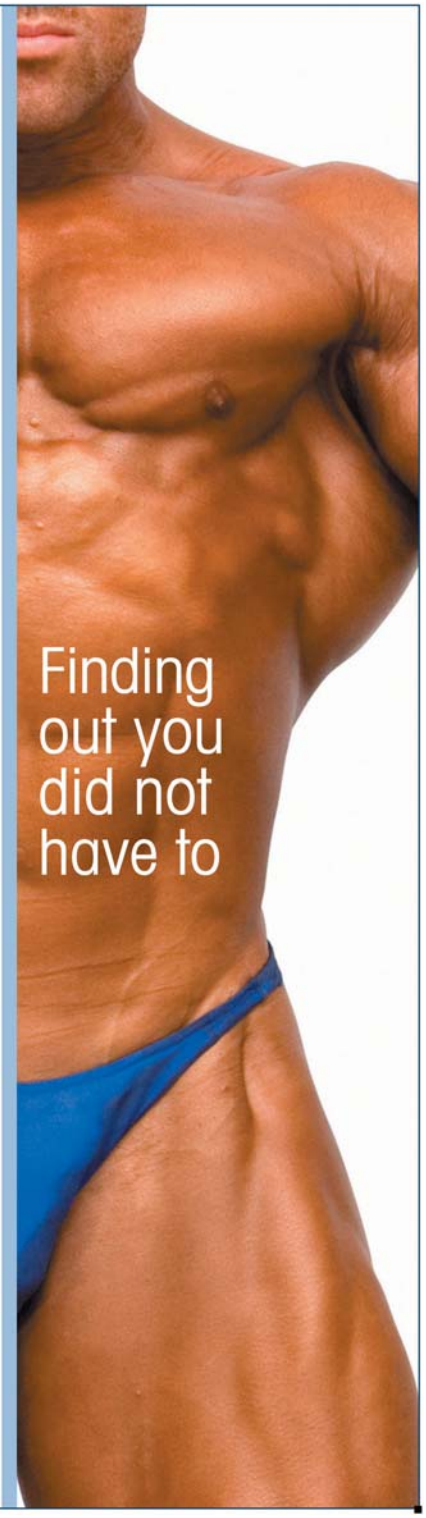

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Figure 1

of HGH now in circulation. Much of it makes use of this legitimate powder, but are all the products made of it of acceptable quality? The answer, of course, is no.

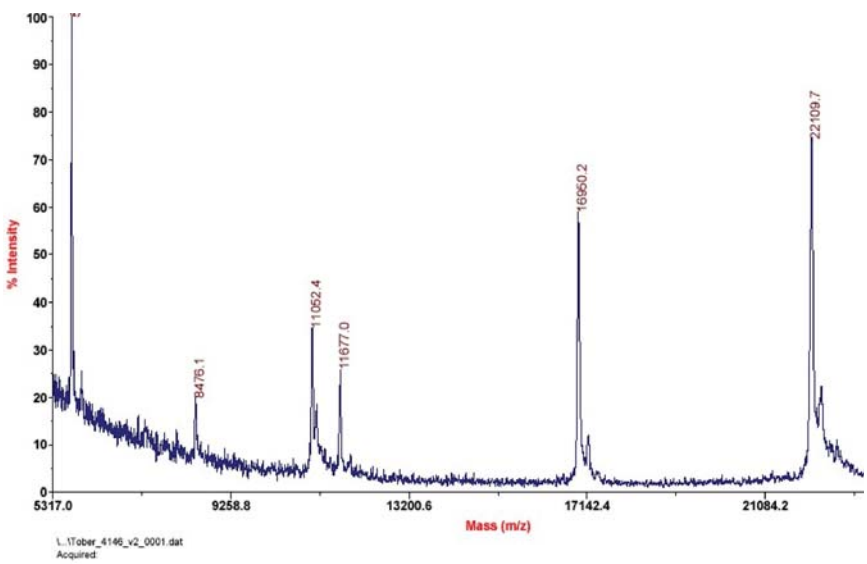
The key problem with HGH is packing. It cannot be packed in a kitchen. It cannot be packaged in a simple lab with a basic clean room or iso9001 (enough for tablet production) climate system. It must be done in a very specific controlled area with the right machinery. Otherwise, the powder, even if it is good itself, may degrade and cause welts or injection point problems when administered. The powder cannot sit for a long time when exposed to air, even the small amount trapped inside a vial when producing it. The problem with a GMP license in China is that it is not centralised. Every province has its own SFDA GMP personnel. Hygetropin, for example, has a real GMP certificate, which can be checked on the SFDA website. Chinese FDA allows only 8IU, so that would be the approved item. A form of Hygetropin is made with 10IU, but it is produced for export only without SFDA approval. This goes for all Chinese manufacturers.

So with all these different product and regulations, how can you tell you have a quality HGH product? One way to find out if the powder is correctly packed is to pay close attention when you first add the dilutent to the powder vial. When you pierce the needle (a small insulin needle is preferred) through the rubber septum, the water should be sucked into the vial without the need to push the plunger. This is because it was properly packaged without air, but a low-pressure vacuum, inside. It will literally suck the diluent in to fill the void. If you need to squeeze the syringe to inject your liquid in order to get any inside, it is not a good sign. It probably means there is air inside the vial, and your powder has been degraded to some degree.

On the forum these kind of issues where heavily discussed and we decided to perform more analyses on Chinese growth hormone. These are the most common:

- **Generic blue tops**
- **Kefei blue tops**
- **Hygetropin (Figure 1)**
- **Getropin**
- **Brown or yellow tops**
- **Jintropin**

Voyager Spec #1=>NF0.7=>MC[BP = 5734.6, 3118]



MALDI-spectra of sample, which was digested with trypsin. The Database search lead to the human growth hormone. The amino acid coverage for the protein reached values up to 95-98%, which–by the way–is extremely high for MALDI peptide mass fingerprint. Second I made the MALDI in linear-mode to determine the mass of the undigested sample. Calibration was made with an external standard. The mass accuracy for our MALDI lies in between 50-75 Da. We obtained very sharp signals. The mass with 22109 Da seems to be your protein ([M+H]+), whereas the double charged monomer is 11052 Da ([M+2H]2+). This mass would fit to the natural human growth hormone, as described with Swiss Prot PO1241. The mass with 16950 Da ([M+H]+) and the corresponding double charged monomer at 8476 Da remain unclear; also the masses at 11677 Da and 5738 Da. Perhaps they represent fragments of the growth hormone. I Don't know. To summarize, the clear identification of the peptide mass fingerprint and the mass at 22109 Da seem to represent the natural growth hormone. With the methods applied here, I don't find clear indications for the variations Met-hGh and des-Phe1-hGh are present.

More about these unexpected masses later in this article.

We left the choice to our members, and they chose for Hygetropin. We performed some analyses on this sample too and compared the samples together.

But first the Hygetropin :

Figure 2

This is the raw data of our analysis. The MW of the protein was determined as 22093 Da in MALDI-MS linear mode. This is a bit higher than the calculated MW (21880 Da). The difference might be due to oxydations at methionins. The amino acid sequence should be ok. This is also confirmed by the peptide mass fingerprint (PMF). We also measured the PMF in linear mode because some of the resulting peptides are quite long and are, therefore, not visible in MALDI-MS reflector mode.

We will not show all graphics in this article because this would work only confusing on the reader. What we did do was a comparison of the results of the Generic Supplements sample with the new sample from Hygene.

Figure 3

The peptide mass spectra of both hHGH products look similar. Figure 3 is an overlay of the two spectra separated into two different mass ranges. At the bottom you will see the new sample (Hygene), on top the old sample (Generic Supplements). Both, the N-terminal peptide (930.5 Da), and the C-terminal peptide at 842.4 Da are visible in both spectra. The last one is difficult to resolve because it is overlaid by a dominant signal at 844.5 Da. Therefore, we can say that at least a part of the molecule had the correct N- and C-terminus. An Met-hGh can be excluded since we did not see the N-terminal peptide at 1061.5 Da. Des-Phe1-hGh can also be excluded due to the lack of the N-terminal peptide at 783.4 Da. However, there are also differences within both samples. For example, the mass at 1628.9 Da is only present in the new sample, and is not explained by the theoretical digest. The mass 1274.6 Da is tremendously increased in the second sample. The mass 1438.7 Da is only present in the old sample, also the big masses, 3957.9 Da, and 4086.0 Da. All these masses are not explained by the theoretical digest. We could do MALDI-MS/MS to get the corresponding sequences, if you like? They can be miscleavages of the correct sequence, modifications of the expected sequence, or contaminations. The differential peptide at 1490.7 Da is derived from trypsin.

Voyager Spec #1=>SM35=>MC[BP = 4997.0, 6972]

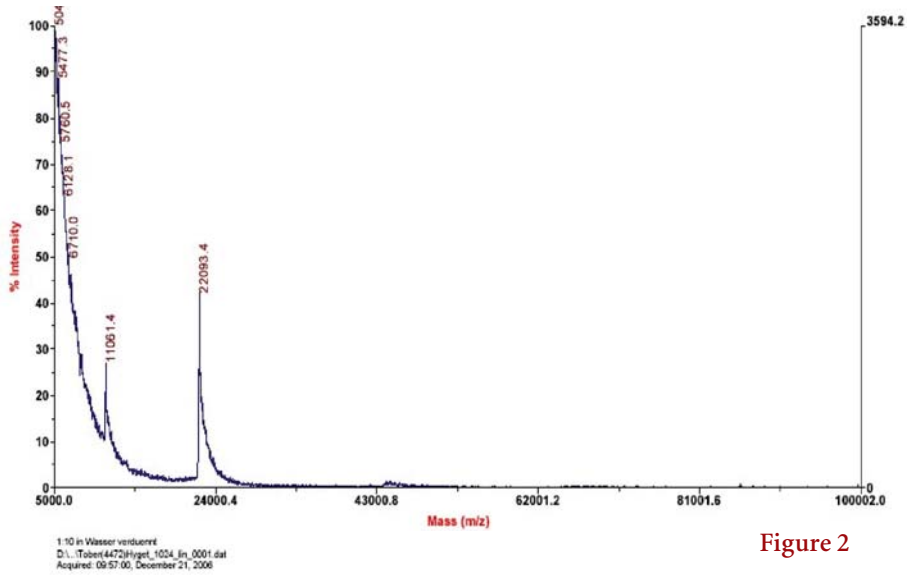


Figure 2

Sample Comparison

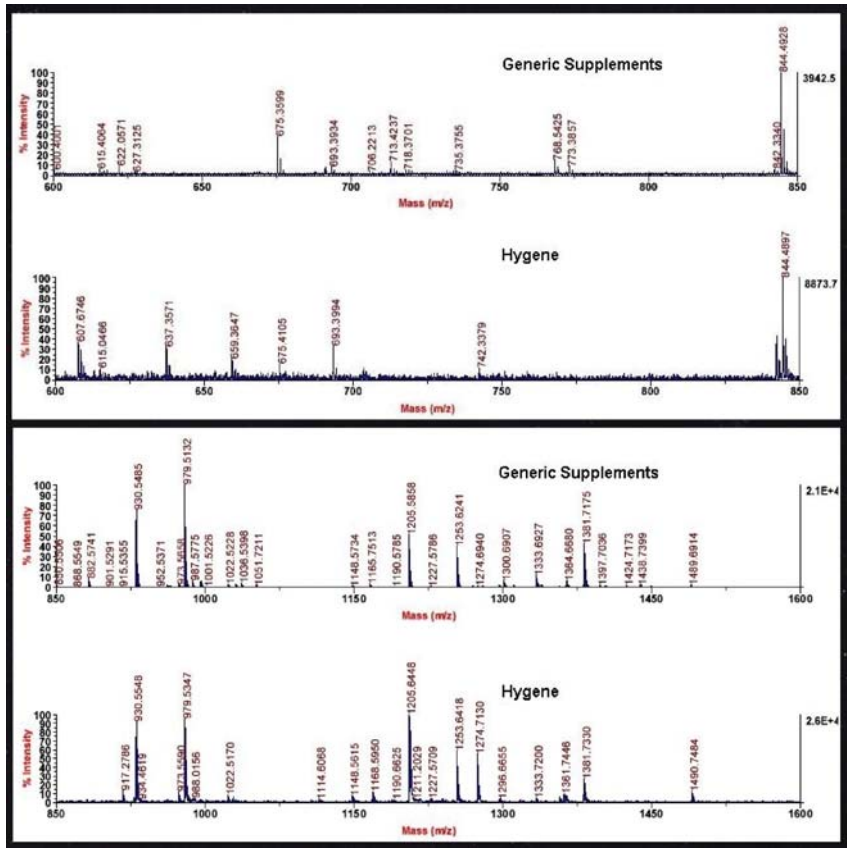


Figure 3

Theoretical Digest: Schematic amino acid of human growth hormone including the disulfide bonds

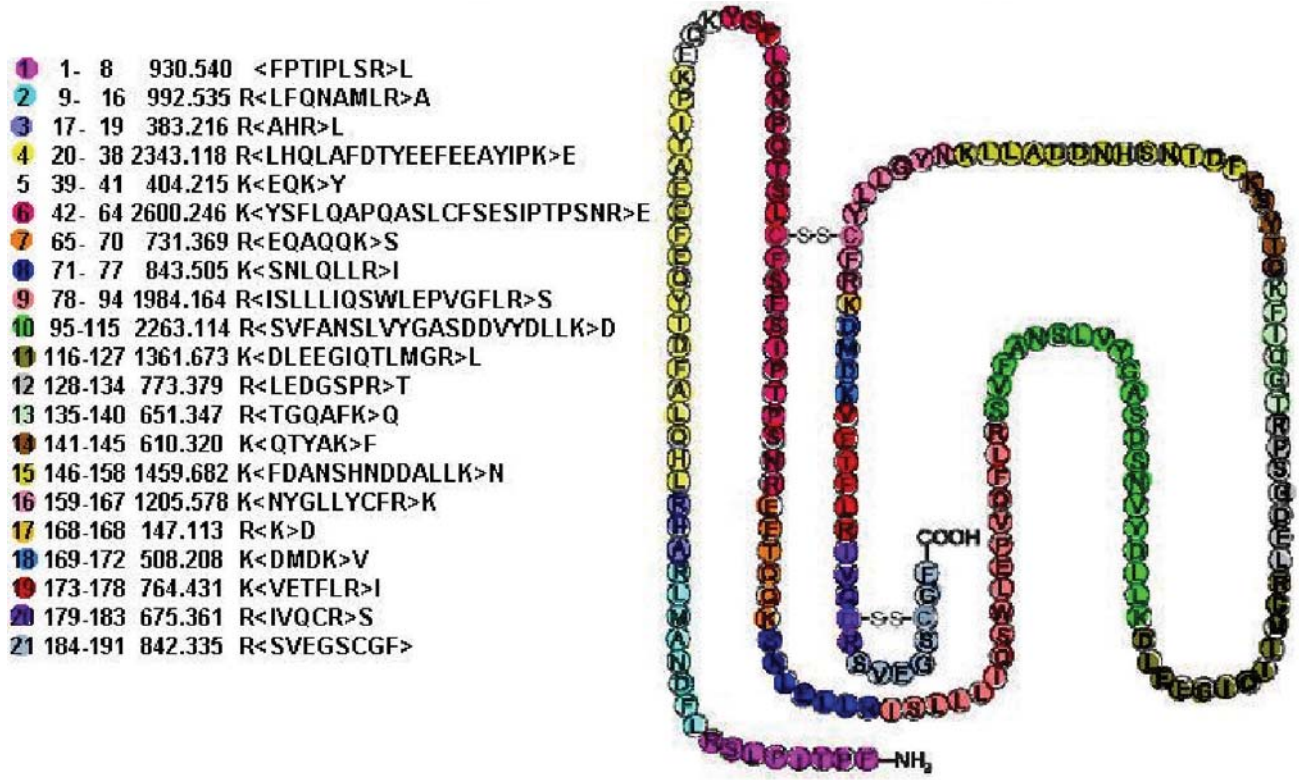


Figure 4

We have red about the theoretical digest, but do we understand what it means? In the body, this recombinant made human growth hormone is a 191 amino acids long chain, with a Molecular Mass or Molecular Weight of 22 KDa, 22000 Dalton or 22 KiloDalton. This hormone is enzymatically digested into different fragments. These fragments have a certain sequence of different amino acids and weight that relates with it. On the left you'll see a graphic of the human growth hormone. To make it easier to understand, we coloured the different fragments in different colours (Figure 4). It also shows the different masses of the different fragments. When the analysts find masses in the graphic that shouldn't be there due to the theoretical digest they refer to these masses as "not explained by the theoretical digest".

Are these results sufficient for you or do you want us to confirm the sequences of selected peptides by MALDI-MS/MS?

We wanted to know for sure that's why we asked the lab to identify these unexpected masses by means of tandem MS.

MALDI-MS/MS

Intact proteins are ionized by matrix-assisted laser desorption/ionization (MALDI), and then introduced into a mass analyser. In the second, proteins where enzymatically digested into smaller peptides using an agent (trypsin). The collection of peptide products then was introduced into the mass analyser. Whole protein mass analysis is primarily conducted using either a time-of-flight (TOF) MS, or Fourier transform ion cyclotron resonance (FT-ICR). These two types of instrument are preferable here because of their wide mass range, and in the case of FT-ICR, its high mass accuracy. Mass analysis of proteolytic peptides is a much more popular method of protein characterization, as cheaper instrument designs can be used for characterization. Additionally, sample preparation is easier once whole proteins have been digested into smaller peptide fragments. Tandem MS (MS/MS) is becoming a more popular experimental method for identifying proteins. Collision-induced dissociation is used in mainstream applications to generate a set of fragments from a specific peptide ion. The fragmentation process primarily gives rise to cleavage products that break along peptide bonds. Because of this simplicity in fragmentation, it is possible to use the observed fragment masses to match with a database of predicted masses for one of many given peptide sequences.

Here are the sequence coverage's of the spectra of the MALDI-MS/MS results of the unexplained peptide masses. Two of them (1252.6 and 2726.3) turned out to be miscleaved somatotropin peptides with nice scores. The third one (1380.7) was identified to be from ASAH protein (see the database query), but the score was very low, so I think this is a false positive result. Compare the sequence coverage (important are b- and y-ions!) of the two somatotropin peptides with that of the third peptide (1380.8 Da). In the first spectra nearly all signals are explained by y- or b-ions. In the third spectra many peaks are not explained at all...! The result is probably wrong! All the other peptides had low scores, and I would not believe Mascot scores of > 30. And a Mascot score lower then 30 is a limit for a threshold which IDs you can believe or not. They are considered to be false positives.

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Figure 5

The miscleaved peptides we identified, were very likely generated during the tryptic digest. They don't explain anything about the differences in quality in the different batches. They occur due to slightly different conditions during the tryptic digest. If we digest SDS-PAGE bands we don't know exactly the protein content of these bands. So, we add a standardized amount of trypsin to them. Depending on the real protein amount there are different protein/trypsin ratios in different samples. This can lead to the effect that you get more miscleavages (not cleaved sites at K or R) than in samples with a lower trypsin concentration. Also, temperature, pH, incubation time, etc. are other factors influencing the digest. So, even if we find differently cleaved peptides the original protein samples can be identical! The only thing that allows us to differentiate between samples would be peptides derived from other proteins (contaminations), or peptides that are different in their sequence compared to the original sequence. However, we have not been able to identify these kinds of peptides. This might indicate that both samples are indeed quite pure. Maybe you should send us a less pure sample, then we should easily see the differences, even in SDS-PAGE ;-)

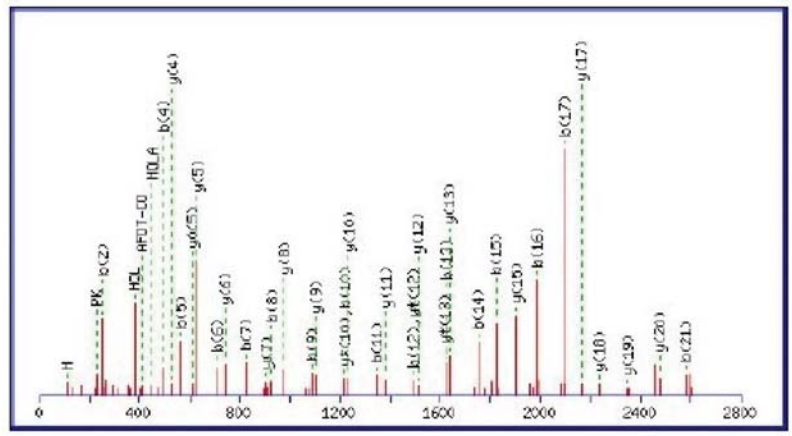
The SDS-PAGE shows no pollutions or contaminations [in Hygetropin].

Independent Informative Growth Hormone Websites

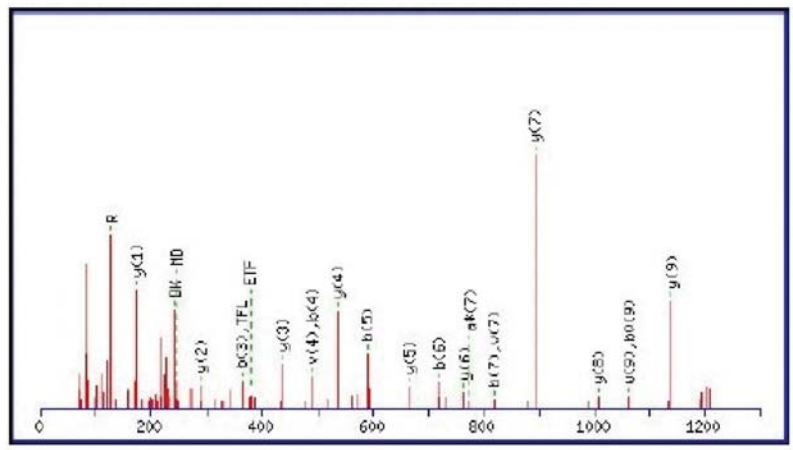
A new phenomenon seems to be informative research websites regarding human growth hormone. These websites often come from the Chinese manufacturers, for example www.hgh-research.com and www.somatropin.net. It appears that a group of Chinese manufacturers of growth hormone actually launched the latter website. This website wants *"to inform users and oppose abuse of growth hormone"*, according to a press message. But the website is in reality an ingenious marketing-instrument of GeneScience, the manufacturer of the growth hormone brand Jintropin. The press message that announced the launch of somatropin.net is surprisingly honest about who are the most important users of growth hormone. It is not the children with a deficiency of growth hormones, but athletes, bodybuilders, and anti-agers.

The explanation about growth hormone use emphasizes the positive effects, and spends comparatively little attention to the side effects: *"HGH can promote lean muscle growth and weight loss, can increase energy, shorten recovery time between workouts, heal damaged tissue, and strengthen joints and ligaments. HGH is also commonly used as an anti-aging drug to prevent and repair the damage responsible for wrinkled skin, osteoporosis, and memory loss."* That is not information. That is advertising, and an oversimplification in its purest form. Everyone knows aging comes with a decrease in hormone levels. Just to supplement growth hormone until the youthful levels are reached will not undo the signs of aging. That would make growth hormone the fountain of youth, which it is not.

MONISOTOPIC Mass of neutral peptide Mr (calc) : 2726.3227



MONISOTOPIC Mass of neutral peptide Mr (calc) : 1252.6121



MONISOTOPIC Mass of neutral peptide Mr (calc) : 1380.7224

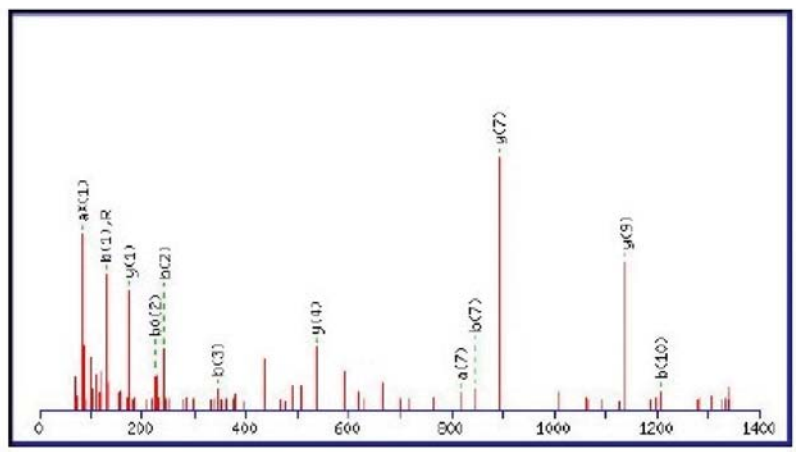


Figure 5

It is not clear who the people are behind somatropin.net. In a press message on the site are the names of three companies: GeneScience Pharmaceuticals, Neogenica BioScience and AnkeBio. Neogenica is the most prominent present in the press message, for the message quotes 'a spokesman' of Neogenica, while after this nobody else is being quoted. ***"We basically want to stop HGH scams, prevent abuse and educate people on what HGH can and cannot do for them"***, according to the information from Neogenica. For the rest you'll find no information about Neogenica on www.somatropin.net. There is little wonder why. Neogenica sells no growth hormone, but only IGF-1. Neogenica launched its IGF-1 LR3-preparation Revitropin in March 2006. And there is little doubt it is a product meant especially for bodybuilders.

"IGF is more effective at directly causing muscle growth and density increases", claims Neogenica. ***"IGF is also much more cost effective"***. Neogenica sends its products *"in a discrete manner"*. Also a nice fact to know. But how is Neogenica involved in somatropin.net? Well, it appears that Neogenica is directly tied to GeneScience. The press message about Neogenica's introduction of Revitropin was written by David Garcia. That name is the person that registered somatropin.net. The information can be verified by a quick search on whois.org. But this David also has an email address, that ends up going to an account at genescience.cn. GeneScience used to sell LR3-IGF-1, under the brandname Igtropin. It is my belief that Igtropin is the same as Revitropin, only now because it is a bodybuilder-targeted product is not associated with GeneScience, at least publicly. No matter what, once you know who controls the website somatropin.net, you realise how cleverly this website is set up and what the real intention of this site is. Somatropin.net is a marketing-instrument, which puts the competition in a bad light and leads readers/customers to buy GeneScience products.

Domain name: somatropin.net

Registrant Contact	David Garcia
Email	info@genescience.cn
Fax	+1.8326153423
Address	1 Remnim Street, Changchun, Jilin 130012, CN

Price comparison

Blue tops	1 USD per IU
Brown tops	1,4 USD per IU
Hygetropin	1,6 USD per IU
Jintropin	3,3 USD per IU
Nutropin	22 USD per IU
Genotropin	13 USD per IU
Humatrope	18 USD per IU
Saizen	13 USD per IU
Serostim	10 USD per IU
Norditropin	18 USD per IU
Zomacton	20 USD per IU

On somatropin.net you will find a price comparison. A quick glance and you see that the European brands are way too expensive. If you see the price difference, the message is clear, "you must be insane to buy those instead of the Chinese products". Ansomone from AnkeBio, or Fitropin from Kexing, are cheap also. But on the first page the website tells visitor that those compounds, just like Tev-Tropin of Teva Pharmaceuticals, contain no real somatropin – but only somatrem. Again, somatrem is an older variant of growth hormone, which contains one amino acid extra than endogenous growth hormone, and is considered less effective. Furthermore, the website reveals that Fitropin by Kexing is not somatropin. ***"Because GeneScience Pharmaceutical Co., Ltd. has the patent for manufacturing HGH by secretion technology in China, Kexing cannot manufacture their product through such a pure and effective means."*** If you want the real 191aa growth hormone, and do not want to pay more then a few dollar for an IU, then you only have one choice according to the website, Jintropin of GeneScience.

What they don't tell you is that there are more generic Chinese growth hormones, and cheaper brands, for example like Hygetropin. This one appears to be just as good according to our analyses. Some members of bodyofscience.com have even claimed that the effect of Jintropin was sometimes lower then other brands, even doubting the quality. That is why we have sent a sample from Jintropin and a sample of Generic Supplements to the toxicological department of a very famous university, where a professor doctor will analyse the samples carefully for peptide contaminations and purity. You should find the results in our next issue. Until then we are making no negative comments about Jintropin. But it is why we added Hygetropin to our price comparison. Since John left GeneScience, it is my opinion that a product using his powder such as Hygetropin is as good as that from any other certified manufacturer. ■

Website research: www.ergogenics.org

Special thanks to Dr. Michael Halder from Germany for sharing his time and knowledge with us.

Counterfeit HGH of the Month

Normally Jintropin 4IU has a green top. These confused counterfeits actually have Kexing tops, but are in vials supposedly produced by GeneScience (GenSci). The colors on the labels are wrong too. This is a very weird product.

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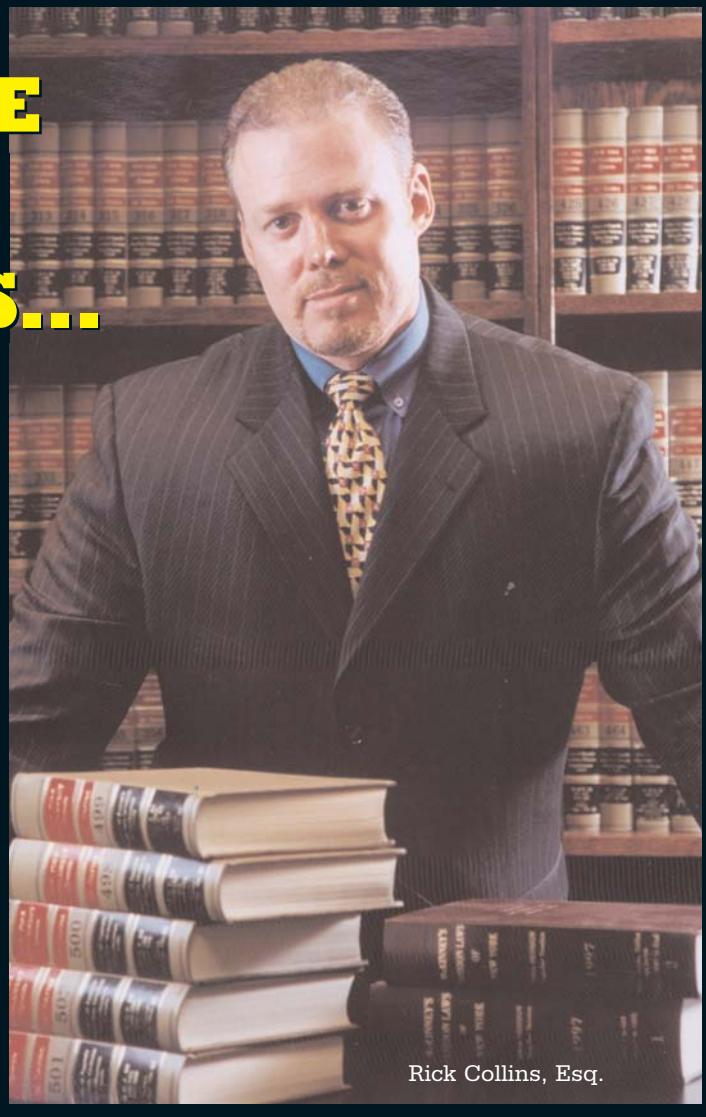
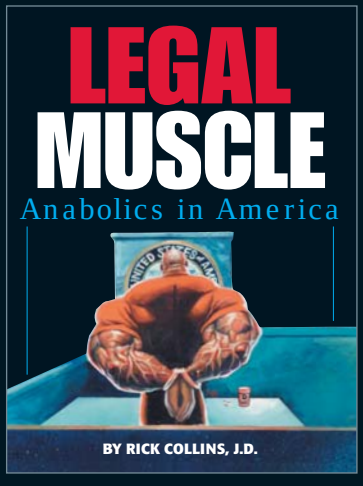

Rick Collins, Esq.

Rick Collins, Esq., is the nationally recognized legal defender of bodybuilders and bodybuilding itself, and one of the most trusted names in the health and fitness community. This straight-talking lawyer and former competitive bodybuilder is consulted daily on sports nutrition issues and anabolic steroid cases by clients from coast to coast (Rick is available for consultations through www.cmgesq.com or 516-294-0300). His firm serves some of the top names in the sports supplement industry.



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