Application of High Field and Cryogen-Free Bench-Top NMR Platforms to the Monitoring and Quantitation of PDE5 Inhibitor Adulteration of Male Sexual Enhancement Supplements

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Abstract

For the past decade it has been well documented that many over-the-counter dietary supplement products that claim to enhance sexual performance are in fact adulterated with undeclared phosphodiesterase type 5 (PDE5) inhibitors[1]. Examples of these PDE5 inhibitors are sildenafil (Viagra®) and tadalafil (Cialis®) and a large number of analog materials. In a recent "raid" by Pfizer Global Security which obtained 91 products from convenience stores and filling stations in two U.S. metropolitan areas, it was found that 81% of the products (74) were adulterated with PDE5 inhibitors[2]. HPLC, NMR, and mass spectrometry have dominated screening and quantitation studies that have been reported in the literature[3-6]. Initiatives are under way under the auspices of AOAC and USP to establish the appropriate analytical methodologies to identify adulterated products. Adulterants are usually present at dosages typical of Viagra® (25-100 mg) and Cialis® (5-20 mg). The drug, or an analog, is customarily spray dried onto the herbal substrate. In order to avoid detection, adulterators have even been known to incorporate the drug material into the capsule shell itself. In this study, we investigated the ability of low field NMR (60 MHz) to identify and quantify PDE5 inhibitors in a number of over-the-counter products sold as sexual enhancement supplements. ^{1}H qNMR of a series of herbal supplements was performed on a 300 MHz NMR, as well as on a bench-top 60 MHz NMR, in order to determine the presence of drug adulteration by PDE5 inhibitors such as sildenafil, tadalafil, and their synthetic analogues. It was determined that simple sample preparation techniques (extraction with CD_3CN or CD_3CN/D_2O) are adequate, and subsequent ^{1}H NMR analysis can yield a rapid screening test. Identification of PDE5 inhibitors was found to be plausible from both the 60 and 300 MHz NMR data. Quantification by internal standards was also readily performed. Though the 300 MHz instrument yielded higher resolution spectra that were easier to assign in the aliphatic region, it was the aromatic proton region of the spectrum that allowed an easy direct spectral fingerprint comparison to be performed across magnetic field strengths.

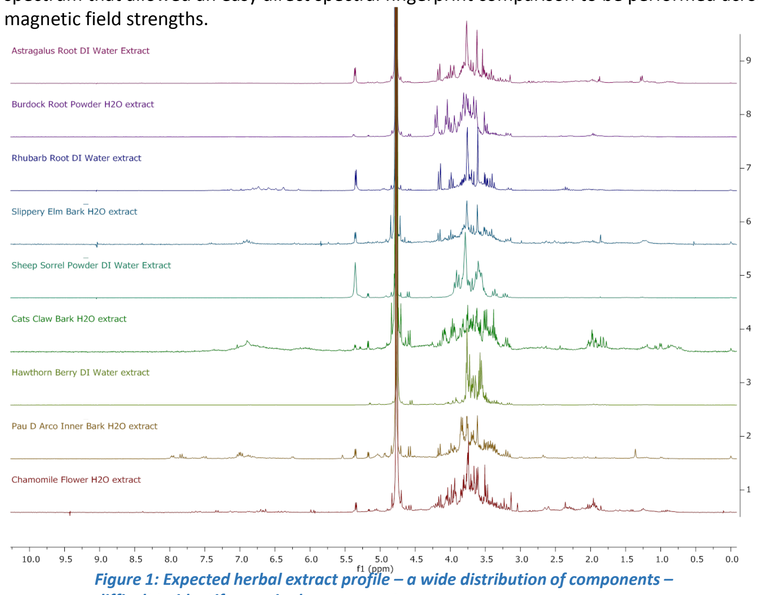


Figure 1: Expected herbal extract profile – a wide distribution of components – difficult to identify any single component

Experimental:

Sample Preparation

Qualitative NMR:

Open capsule or grind tablet
Place ~200 mg sample in 2 dram vial
Add 700 µl 99.8% CD_3CN or 20:80 D_2O/CD_3CN
Mix thoroughly (eg. vortex). Allow to settle.
Pipet supernatant into an Economy
5mm tube and allow solids to settle.

Quantitative NMR:

Weigh Capsule contents to nearest 0.1mg
Weigh sample into vial to nearest 0.1 mg
Add appropriate internal standard weighed to nearest 0.1mg

300 MHz ^{1}H NMR – Varian Mercury 300 MVX

5 mm Varian ATB Probe
Pulse Width = 45° Tip Angle
Spectral Width = 8 kHz
Acquisition Time = 3.5 seconds
Relaxation Delay =7 Seconds (Qual)
=20 seconds (Quant)
Averages: 16-256 = S/N Decision

60 MHz ^{1}H NMR – Aspect Imaging 60 MHz NMR

5mm sample (can be 10 mm) – Experimental conditions are same as for 300 MHz except:
Spectral Width = 2 kHz
Pulse Averages = 8-64

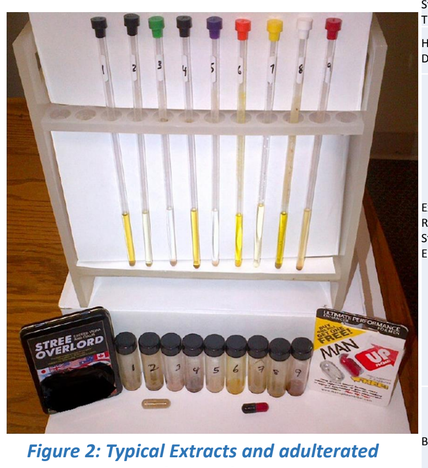
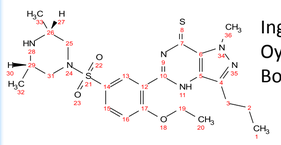


Figure 2: Typical Extracts and adulterated product packages.



Ingredients Declared on Label :
Oyster, Barberry, Dextrose, Snow Lotus, Bombyx Mori L, Ginger Root, Saffron Crocus

Figure 4: ^{1}H NMR spectra obtained at 60 MHz and 300 MHz on "Control" herbal supplement - received for analysis November 2012 – positive for sulfoaildenafil

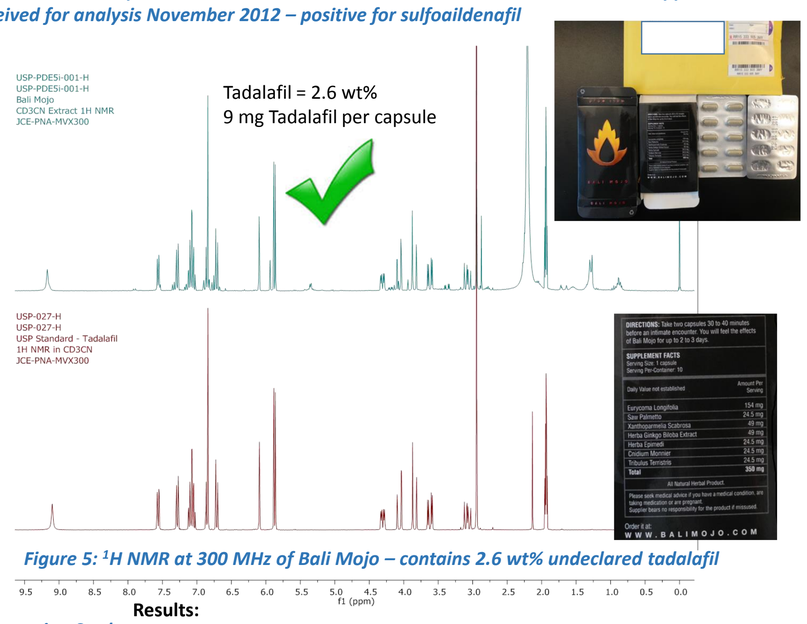


Figure 5: ^{1}H NMR at 300 MHz of Bali Mojo – contains 2.6 wt% undeclared tadalafil

Table 1:
Herbal Supplements Included in PDE5i Adulteration Screening Study

Product Name	Supplier	Supplement Facts
Bulgarian Tribulus	Ultimate Nutrition, Inc	Tribulus Blend - Tribulus extract [fruit] and tribulus [aerial parts]
Men's Staminol Rapid Surge	General Nutrition Corporation	Niacin Sexual Health Circulatory Blend Maca Root Powder Horny Goat Weed Extract Damiana Leaf Extract Cayenne Fruit Powder Du-Zhong Bark Extract Yohimbe Bark Extract Caffeine Anhydrous Ginkgo biloba leaf Extract Res-Vida (trans-resveratrol) DHEA
Maca Complex	General Nutrition Corporation	Magnesium (as Mg Aspartate) Zinc (as Zn Algilane) Maca Roots/Tubers Powder MegaNutra Proprietary Grape Blend Yerba Mate Leaf Extract Rhodiola Rose Root Extract
Vigorexin	Vigorexin, LLC	Niacin Tribulus terrestris L-Citruline L-Arginine HCl Arginine AKG 2:1 Eurycoma longifolia Jack, 100:1 Maca Roots/Tubers Powder Avena sativa, 10:1 Grape Seed Extract Yohimbine HCl
Yohimbe Herbal Supplement Standardized Extract Traditional Male Herb	General Nutrition Corp	Yohimbe Bark Extract (2% Yohimbine Alkaloids = 9 mg)
Goldenseal Root Herbal Supplement Standardized Extract Traditional Male Herb	General Nutrition Corp	Goldenseal Root Extract (Minimum 5% Hydrastine = 10 mg)
Horny Goat Weed Dietary Supplement	General Nutrition Corp	Horny Goat Weed Extract Maca Root Extract Polypodium vulgare Root Powder
ExtenZe Extended Release Maximum Strength Male Enhancement	Global Products Management	Extenze Sexual Response Enhancement Blend Black Pepper extract (fruit) Cnidium Extract (seed) Damiana Extract (leaf) DHEA (98%) Gamma-Aminobutyric Acid (GABA-98%) Korean Ginseng (root) Barrenwort Extract (leaf) L-Arginine HCl (98%) Licorice Extract (root) Muira Puama Extract (bark) Pregnenolone (98%) Tribulus Extract (fruit) Folate (Folic Acid) Yohimbe HCl (98%) Astragalus Extract (root) Niacin Tongat Ali Extract (root) Velvet Bean Extract (seed) Chrysin (98%)
Bali Mojo	Bali Mojo, Inc.	Eurycoma longifolia Saw Palmetto Xanthoparmelia scabrosa Herba Ginkgo Biloba Extract Herba Epimedii Cnidium monnieri Tribulus terrestris

Results:

Samples obtained from local gas stations were found to contain undeclared sildenafil, tadalafil, or sulfoaildenafil at close to pharmaceutical dosages. Figure 1 shows what would be expected from a normal herbal supplement which shows a large complex mixture of components with very few identifiable components. Figure 3 shows the result of extracting "ManUp Now" and Stree Overlord products with 80:20 CD_3CN-D_2O. Specific chemistry is readily observed with both 300 MHz and 60 MHz spectrometers. ^{1}H qNMR internal standard protocols readily yield the quantitative content of the undeclared PDE5i adulterants.

Figure 4 shows the result obtained on another herbal supplement that was submitted for analysis in 2012. It's spectrum did not match that of sildenafil but was found to be sulfoaildenafil which is a well-known sulfur analog of sildenafil. Both 60 MHz and 300 Mhz results allowed quantitation of the PDE5i adulterant. Figure 5 shows the result obtained on Bali Mojo, a known adulterated product, that was bought on the internet and arrived discreetly from Malaysia. This supplement was found to contain about 9 mg of tadalafil per capsule.

In an effort to investigate the occurrence of PDE5 inhibitors in male enhancement products that can be obtained at nutrition stores, we purchased a number of products (listed in Table 1) and analyzed them with 60 and 300 MHz NMR instrumentation. ^{1}H NMR spectra of 5 of the 8 products are shown to the right (Figures 8-12). None of these products were found to contain undeclared PDE5i adulterants. One sample was found to contain undeclared caffeine (Maca complex – Figure 11). The process did demonstrate how quick and straightforward the NMR screening analysis is. The fact that 60 MHz instrumentation can be readily utilized for screening makes it an ideal technique for enforcement of identity and anti-adulteration standards in the field or at distributed field laboratories where the only facility requirement is 110V power supply.

Finally in Figure 6 (below) we demonstrate the true resolution of 60 MHz data that in many of the comparisons in this poster are "stretched" by the use of the ppm scale to 5 times the original line-width. The figure shows the 60 and 300 MHz data for the "ManUp Now" product extract with the "frequency space" 60 MHz spectrum shown for comparison. The natural resolution of the 60 MHz NMR system utilized in this study is 1 Hz non-spinning while the 300 MHz NMR attains a resolution of about 0.5 Hz. Figure 7 demonstrates the ease of use of modern NMR software whereby qNMR calculations are performed automatically.

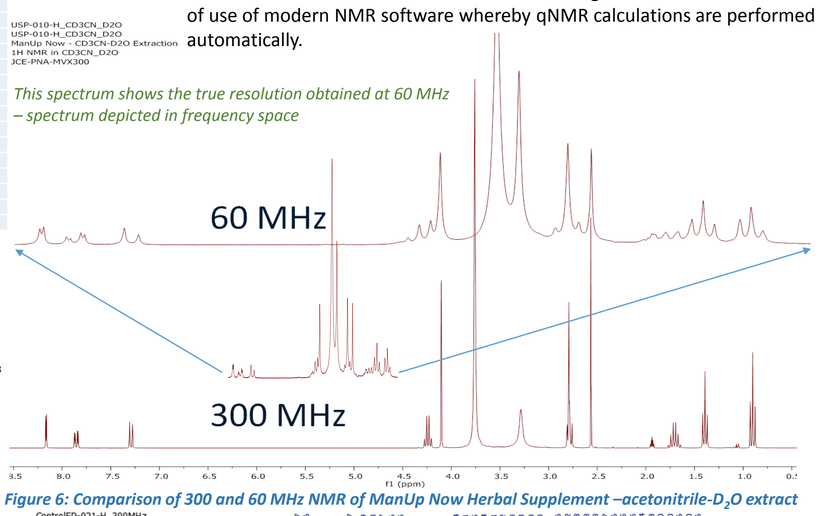


Figure 6: Comparison of 300 and 60 MHz NMR of ManUp Now Herbal Supplement –acetonitrile-D_2O extract

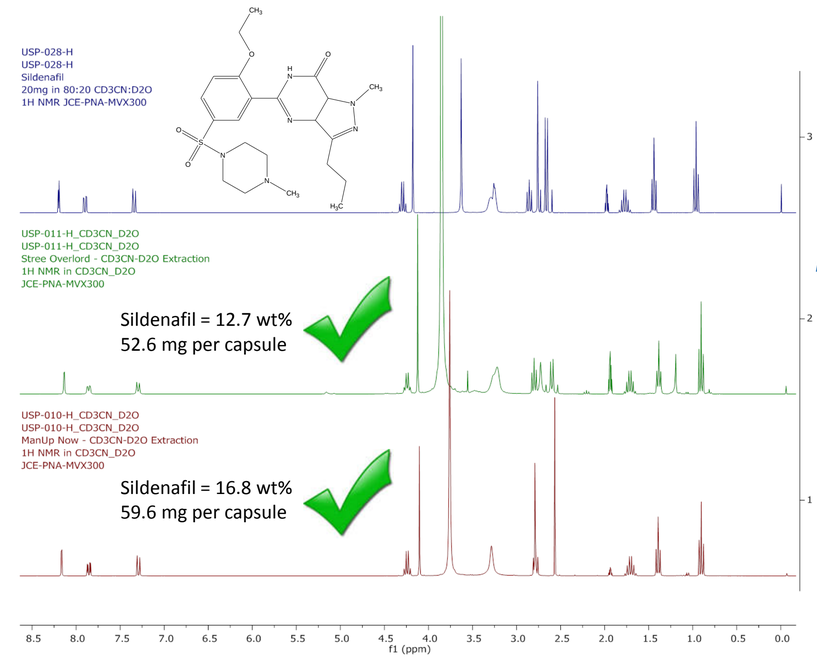


Figure 3: 300 MHz ^{1}H data of Stree Overlord and ManUp Now herbal supplements purchased at a local Danbury, CT gas station in February 2014 - compared to USP sildenafil standard . Both are positive for sildenafil

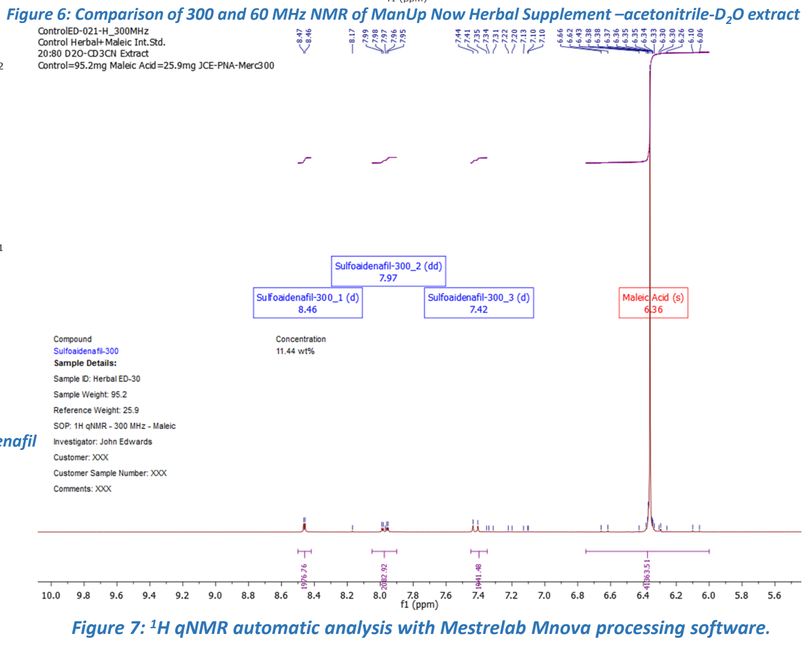


Figure 7: ^{1}H qNMR automatic analysis with Mestrelab Mnova processing software.

References:

1. Venhuis, B.J., de Kaste, D., J. Pharm. Biomed. Anal., 69 (2012), pp 196-208
2. Campbell, N. et al., J. Sex Med., 10(7) (2013), pp 1842-1849
3. Singh, S. et al., Trends Anal. Chem., 28(1) (2009), pp 13-28.
4. Vaysse, J. et al, J. Pharm. Biomed. Anal., 59 (2013), pp 58-66.
5. Venhuis et al., Forensic Sci. Int., 214, (2012), pp e20–e22
6. Mustazza et al., J. Pharm. Biomed. Anal., 96 (2014), pp 170–186

Figure 8: 60 and 300 MHz ^{1}H NMR of Goldenseal Root

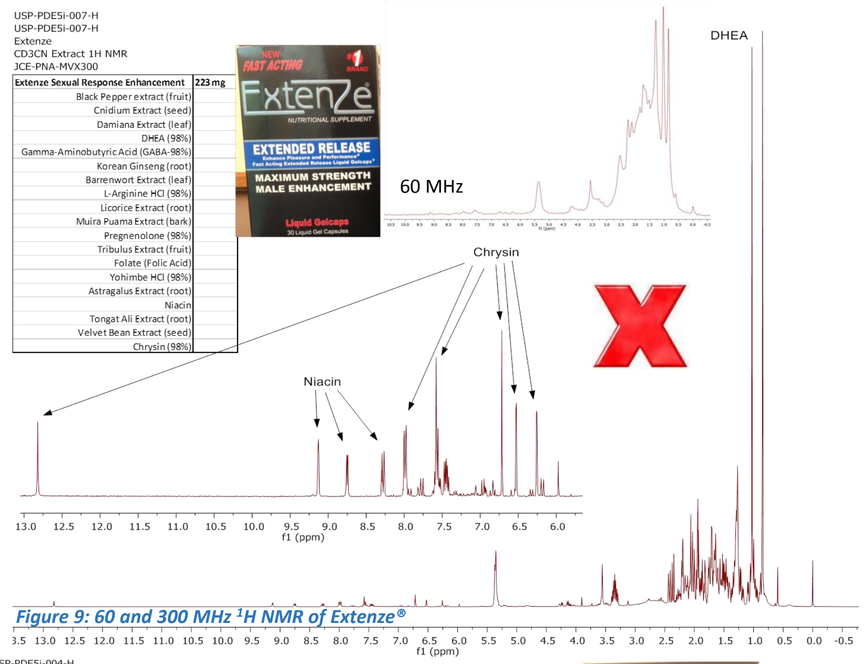


Figure 9: 60 and 300 MHz ^{1}H NMR of Extenze®

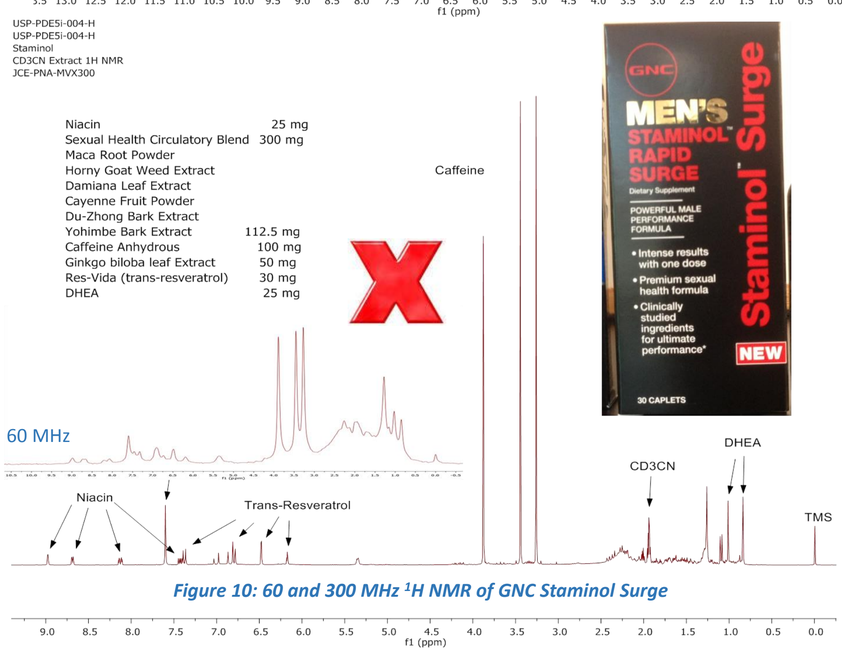


Figure 10: 60 and 300 MHz ^{1}H NMR of GNC Staminol Surge

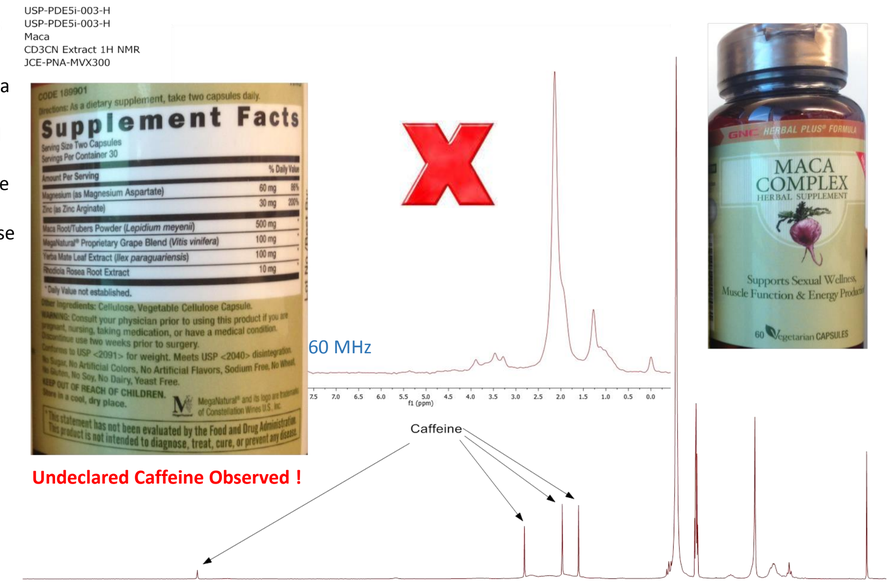


Figure 11: 60 and 300 MHz ^{1}H NMR of Maca Complex

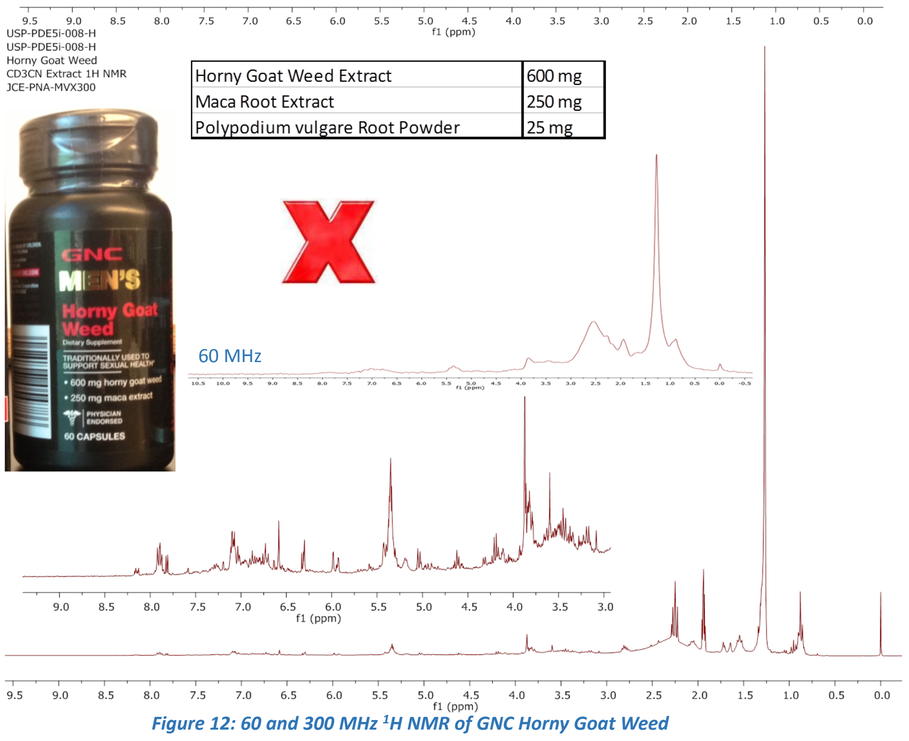


Figure 12: 60 and 300 MHz ^{1}H NMR of GNC Horny Goat Weed