

## EVIDENCE FOR CONTAMINATION OF HERBAL ERECTILE DYSFUNCTION PRODUCTS WITH PHOSPHODIESTERASE TYPE 5 INHIBITORS

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### ABSTRACT

**Purpose:** We determined if pharmacological dosages of phosphodiesterase type 5 inhibitors (PDE5) inhibitors were present within a group of natural products marketed for the treatment of erectile dysfunction.

**Materials and Methods:** Seven herbal products marketed for the treatment of erectile dysfunction were purchased via the Internet or at local health food stores. Specimens were batched, relabeled and blindly analyzed for contamination with PDE5 inhibitors. High performance liquid chromatography and mass spectrometry were used to detect evidence of contamination with sildenafil, tadalafil or vardenafil.

**Results:** Of the 7 tested products 2 contained pharmacological dosages of sildenafil and tadalafil. Contamination with vardenafil was not identified. Mean dosages of sildenafil and tadalafil were 30.2 and 19.7 mg, respectively.

**Conclusions:** A significant proportion of natural products marketed for erectile dysfunction contains PDE5 inhibitors. Although marketed as natural products devoid of adverse effects, these agents are known to have potentially fatal drug interactions with nitrates. Better regulation of the natural health products industry is urged.

**KEY WORDS:** impotence, complementary therapies, government regulation, phosphodiesterase inhibitors, contraindications

Complementary and alternative medicine (CAM) continues to grow as a form of treatment for a variety of medical conditions.<sup>1</sup> Treatment of urological diseases has a long history of CAM practices. For instance saw palmetto has been used to treat lower urinary tract symptoms (LUTS) for more than a century and remains a top selling herbal preparation.<sup>2</sup> In addition, more than 42% of men with or at risk for prostate cancer use vitamin and mineral supplements.<sup>3</sup> Similarly erectile dysfunction (ED) remains a common societal problem. During the last 7 years the addition of phosphodiesterase type 5 (PDE5) inhibitors to the treatment regimen for men with ED has been nothing less than revolutionary. Sildenafil and related compounds are now the most frequently prescribed drugs for the treatment of ED.<sup>4</sup> Despite unproven efficacy natural products have long been touted as a method of treating ED, with aphrodisiacs having been available for millennia and remaining a significant part of many cultures.<sup>5</sup>

Herbal products are treated as foods by regulatory agencies in North America. As such they are not subject to the rigorous validation required for the approval of pharmaceutical agents. Production quality of herbal products has only recently become regulated. As of January 1, 2004 in

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Canada the *Natural Health Products Regulations* were enacted and applied to all natural health products.<sup>6</sup> Similar measures are being enacted in Europe as well.<sup>7</sup> The recent scandal surrounding the prostate cancer herbal product PC-SPES highlights this necessary regulatory enforcement. The PC-SPES product, known to induce castrate levels of testosterone and decrease prostate specific antigen levels,<sup>8</sup> was later discovered to contain pharmacological dosages of diethylstilbestrol,<sup>9</sup> indomethacin<sup>8</sup> and warfarin.<sup>8,10</sup> Given the size of the market for ED drugs, it is not surprising that many marketed herbal products for ED claim to have efficacy similar to that of PDE5 inhibitors without side effects or need for medical assessment. Given the history of some herbal products containing pharmacological agents, we determined whether herbal ED products contain PDE5 inhibitors.

### MATERIALS AND METHODS

**Product procurement.** We purchased 7 brands of marketed herbal products for the treatment of ED. Purchases were limited to oral tablets or capsules. Teas, tonics and foods were excluded from this analysis. Purchases were also limited to products with claims of efficacy if taken before sexual activity and not on a routine basis. Of the brands 6 were purchased via the Internet and 1 via a local health food specialty store. The Appendix lists the names and web sites of the tested preparations.

**Product handling and shipping.** Twelve tablets or capsules were removed from the original bottle, placed into a light shielded plastic container and assigned a study letter (A through G) by 1 of the study investigators (MH). Products were then shipped at room temperature to the Prostate Cen-

tre at Vancouver General Hospital for high performance liquid chromatography analyses. Standards of PDE5 inhibitors for sildenafil, tadalafil and vardenafil were commercial pharmaceutical tablets.

**Sample preparation.** Each of the 7 herbal preparations was emptied from the capsules or tablets, crushed and transferred into 13 × 100 mm Pyrex® screw cap tubes, and weights determined. Standards for sildenafil were tablet samples of Viagra® (Pfizer Inc, 50 mg, Lot 2144), and for tadalafil and vardenafil tablets of Cialis® (Lilly, 20 mg, Lot A020228) and Levitra® (Bayer, 10 mg, Lot BXB7FT1), respectively. Standards were similarly transferred to Pyrex® tubes. Samples and standards were extracted with 5 ml of 50% reagent ethanol (Fisher Scientific A962-4) by vortexing until contents were fully disaggregated followed by sonication (VWR model 150T) for 30 minutes at room temperature. Aliquots of samples and standards were clarified by centrifugation at 10,000 g. Standards were serially diluted in 50% ethanol to bring them into the appropriate linear dynamic range for analysis. Viagra tablets are formulated as sildenafil citrate and values are given as sildenafil citrate equivalents to allow for direct comparison with these commercial products. Samples were diluted 10-fold in 50% ethanol and an initial set run to determine appropriate further dilutions.

**High performance liquid chromatography-mass spectrometry.** A Waters 2695 Separations Module equipped

with a Waters/Micromass ZQ2000® detector was used for quantitative analyses of sildenafil, tadalafil and vardenafil. The ZQ2000 detector settings for the scan function were capillary 3 kV, desolvation temperature 250C, source temperature 120C and cone temperature 20C. Extractor, RF lens and multiplier voltages were 3, 0.5 and 650 V, respectively, desolvation and cone gas flows were 300 to 50 l per hour, respectively, and cone voltage switched between 20 and 45 V. Scans were 0.5 seconds from 250 to 750 amu in electrospray ionization positive mode. For single ion recording (SIR) functions masses of 390.25, 475.26 and 489.28 with 0.3 seconds dwell, and cone voltages of 20, 45 and 45 V were used for tadalafil, sildenafil and vardenafil, respectively, in electrospray ionization positive mode. These molecular weights reflect the protonated parent molecule. Model and actual isotopic distributions obtained are noted in figures 1 and 2. Other parameters were like those of the scan mode.

Separation was performed using a Waters Nova-Pak® C18 3.9 × 150 mm 4 μm column at 25C. High performance liquid chromatography grade acetonitrile and methanol (Fisher Scientific), and formic acid (EM Science™, Analar grade), were used to prepare the mobile phase for liquid chromatography-mass spectrometry. The mobile phase consisted of 0.1% formic acid in Milli-Q® water (A), acetonitrile (B) and methanol (C) using a 20:10:70 ratio for A-to-B-to-C. Run times were 35 minutes per sample.

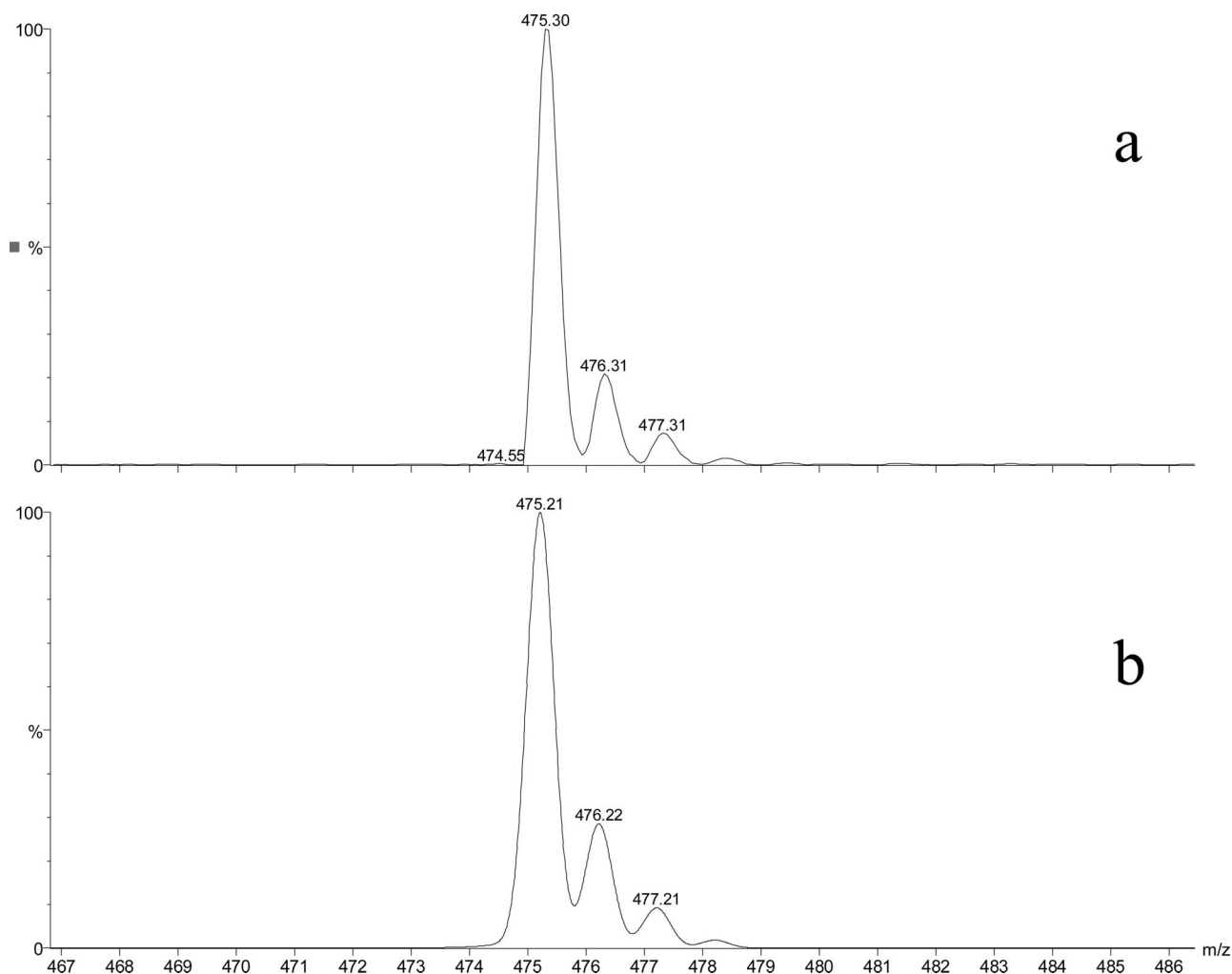


FIG. 1. Actual spectrum from peak at 25 minutes in sample B (a) compared with model of isotopic distribution for sildenafil (b)

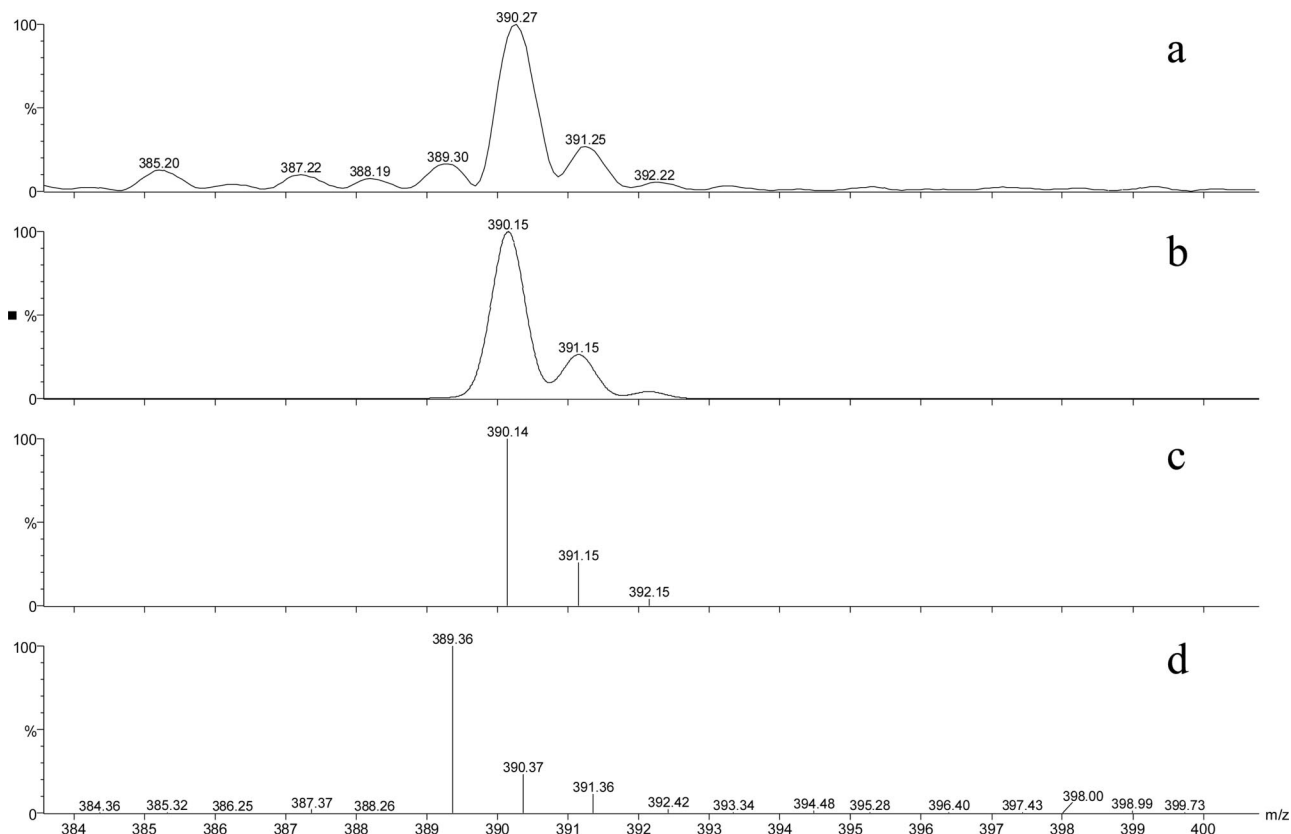


FIG. 2. Actual tadafafil spectrum from peak at 6.2 minutes for 100 $\times$  dilution of sample F (a) and comparison with computer generated model of isotopic distribution of tadafafil (b), definitively identifying presence of tadafafil in sample F. Equivalent of b is represented as centroided model (c). Also shown is lack of alignment of standard with artifact peak observed at 16.4 minutes in sample A (d).

## RESULTS

Retention times for tadafafil, sildenafil and vardenafil were 6.2, 25 and 27 minutes, respectively, and calibrations were run with each sample set. Figure 3 demonstrates the representative chromatograms of all herbal specimens with a sildenafil control. Significant adulteration with sildenafil was noted in sample B (Super-X). Figure 1, a is the actual sildenafil mass spectrum from the peak at 25 minutes in sample B. Figure 1, b compares this with the computer generated model of isotopic distribution for sildenafil, definitively identifying the presence of sildenafil in sample B. Measured average sildenafil levels in sample B (Super-X) were 30.2 mg per capsule, ranging from 27.6 to 31.3 (as sildenafil citrate equivalent).

Figure 4 demonstrates representative chromatograms of the herbal preparations with a tadafafil control. Significant adulteration with tadafafil was noted in sample F. Figure 2, a shows an actual tadafafil spectrum from the peak at 6.2 minutes for a 100 $\times$  dilution of sample F. Figure 2, b compares this with the computer generated model of isotopic distribution of tadafafil, definitively identifying the presence of tadafafil in sample F. Measured average tadafafil levels in sample F (Stamina-Rx<sup>TM</sup>) were 19.77 mg per tablet ranging from 18.0 to 22.0. Triplicate samples analyzed in duplicate were examined as minimum criteria for each. Duplicate repeatability was 3.0%, 1.7% and 7.1% relative standard deviation for sildenafil, and 4.6%, 4.1% and 4.8% relative standard deviation for tadafafil. Means of the duplicates were used in the calculation of average adulterant levels. Significant levels were not detected in any other samples. Vardenafil was not detectable in any of the samples.

Figure 2, c is the equivalent of figure 2, b represented as a centroided model. Figure 2, d has been included to demon-

strate the lack of alignment of the standard with the artifact peak observed at 16.4 minutes in sample A from figure 1. The artifact at 16.2 minutes is due to the spillover of the second isotopic peak into the 390.25 amu SIR function used for tadafafil data collection.

## DISCUSSION

The use of CAM practices continues to gain acceptance among the public to treat and palliate a variety of medical conditions. Recent data from Gordon and Lin indicate that CAM practices continue to become increasingly popular.<sup>11</sup> It is important to point out that most CAM practices have not been validated using the principles of medical evidence such as randomized clinical trials, although there seems to be improvement in this area.<sup>1</sup>

Given their high prevalence, it is not surprising that many herbal products are being marketed to treat urological conditions. Saw palmetto, an herbal product to treat LUTS, is one of the top selling herbal products and has some evidence of proven efficacy.<sup>2</sup> A randomized study is currently under way assessing this product in comparison to pharmacological agents commonly used to treat LUTS.

ED is a common problem of aging males. As many as 50% to 70% of men 75 years old or older experience some degree of ED and the prevalence of this condition increases with age.<sup>12,13</sup> The notion of using natural products to treat ED is not new. Aphrodisiacs have been touted in many cultures for thousands of years to improve sexual function and desire. Yohimbine, initially derived from the Corynanthe yohimbe tree, is an example of such a product that has worked its way into the pharmacological industry.<sup>5</sup> During the last 7 years the addition of PDE5 inhibitors to the

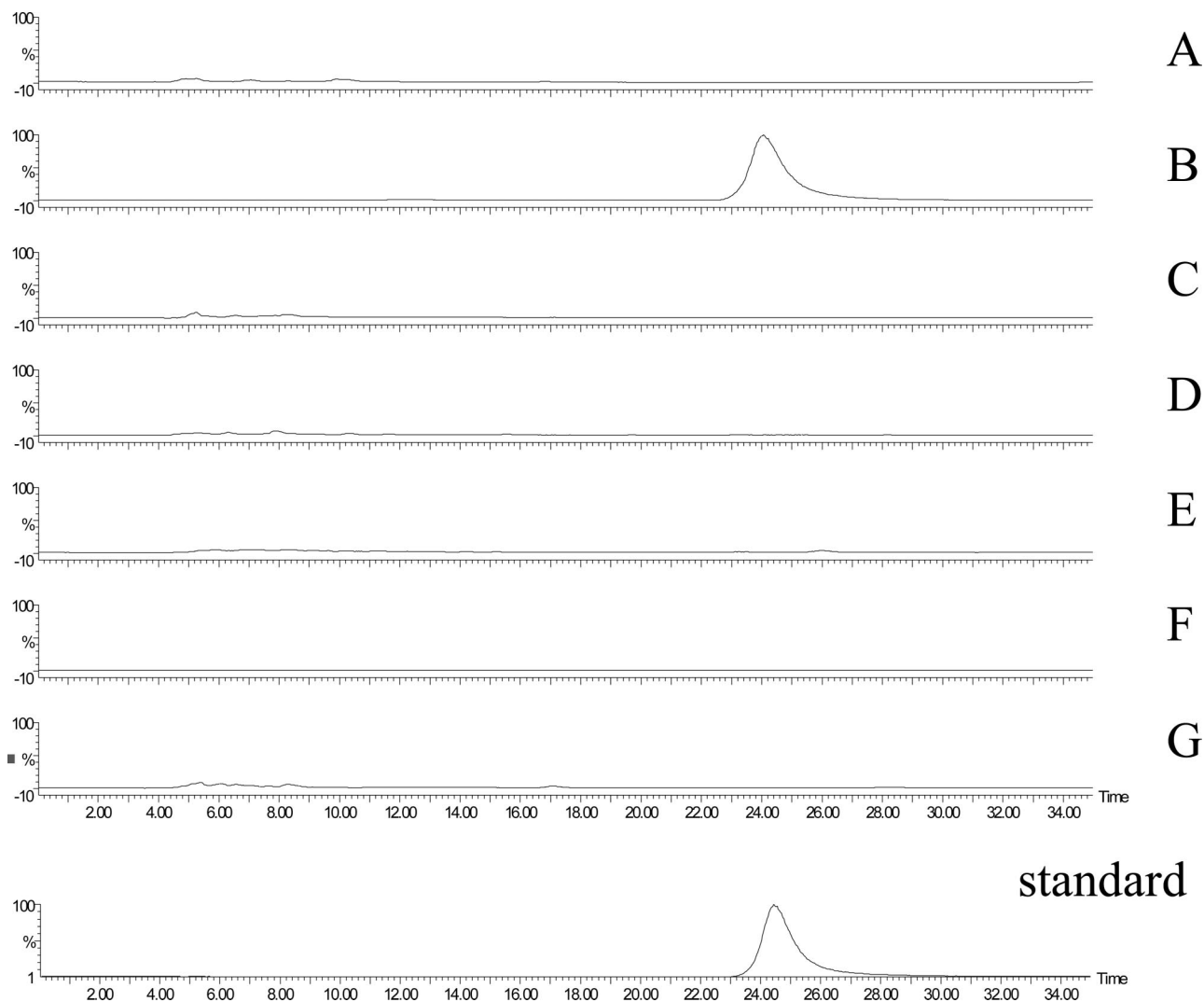


FIG. 3. Representative chromatograms for herbal samples A through G monitored by SIR 475.26 amu (sildenafil). All are  $10\times$  dilutions of 5 ml 50% ethanolic extract except for samples B and F which are  $10,000\times$ . Standard is from 250 ng/ml sildenafil calibration sample.

treatment regimen for men with ED has been nothing less than revolutionary. Sildenafil and related compounds are now the most frequently prescribed drugs for the treatment of ED.<sup>4</sup>

Herbal products are treated as foods by regulatory agencies in North America. As such they are not subject to the rigorous validation required for the approval of pharmaceutical agents. Production quality of herbal products has only recently become regulated. As of January 1, 2004, in Canada the Natural Health Products Regulations were enacted and applied to all natural health products. Given the size of the market for ED drugs, it is not surprising that many marketed herbal products for ED claim to have efficacy similar to that of PDE5 inhibitors without side effects or need for medical assessment.

To our knowledge this study represents the first to assess systematically a host of products marketed for the treatment of ED. Of the 7 tested products 2 demonstrated evidence of pharmacological doses of PDE5 inhibitors. Since these are not naturally occurring compounds, one can only conclude that these products have been deliberately contaminated with these agents. These data are also consistent with periodic reports from the Food and Drug Administration. The clinical implications of these data are significant. Fatal interactions with PDE5 inhibitors and commonly consumed drugs have been reported, and are well accepted. For example, use of PDE5 inhibitors and nitrates can lead to hypoten-

sion, shock and death. Nitrate usage is generally regarded as an absolute contraindication to PDE5 inhibitor prescription.<sup>14</sup> However, these agents purchased over-the-counter at local stores or via the Internet are touted as safe and devoid of adverse side effects. To our knowledge case reports of fatal interaction with these natural products have not occurred. However, it is possible that they are not being recognized due to the presumed safety of these products. Many spouses may also not be aware that their sexual partners have used these agents.

There are several limitations of this study that deserve mention. We limited our purchases to products that were specifically recommended for use before intercourse on an as needed basis. The majority of marketed products is recommended for daily use and perhaps is not as prevalently contaminated with PDE5 inhibitors. Similarly since we limited our analysis to capsules and tablets, we cannot generalize our findings to other products such as teas, tonics and raw extracts.

#### CONCLUSIONS

These products require regulation. Many pharmacological raw chemicals are easily purchased overseas, and can be added to any product and marketed as natural and safe.

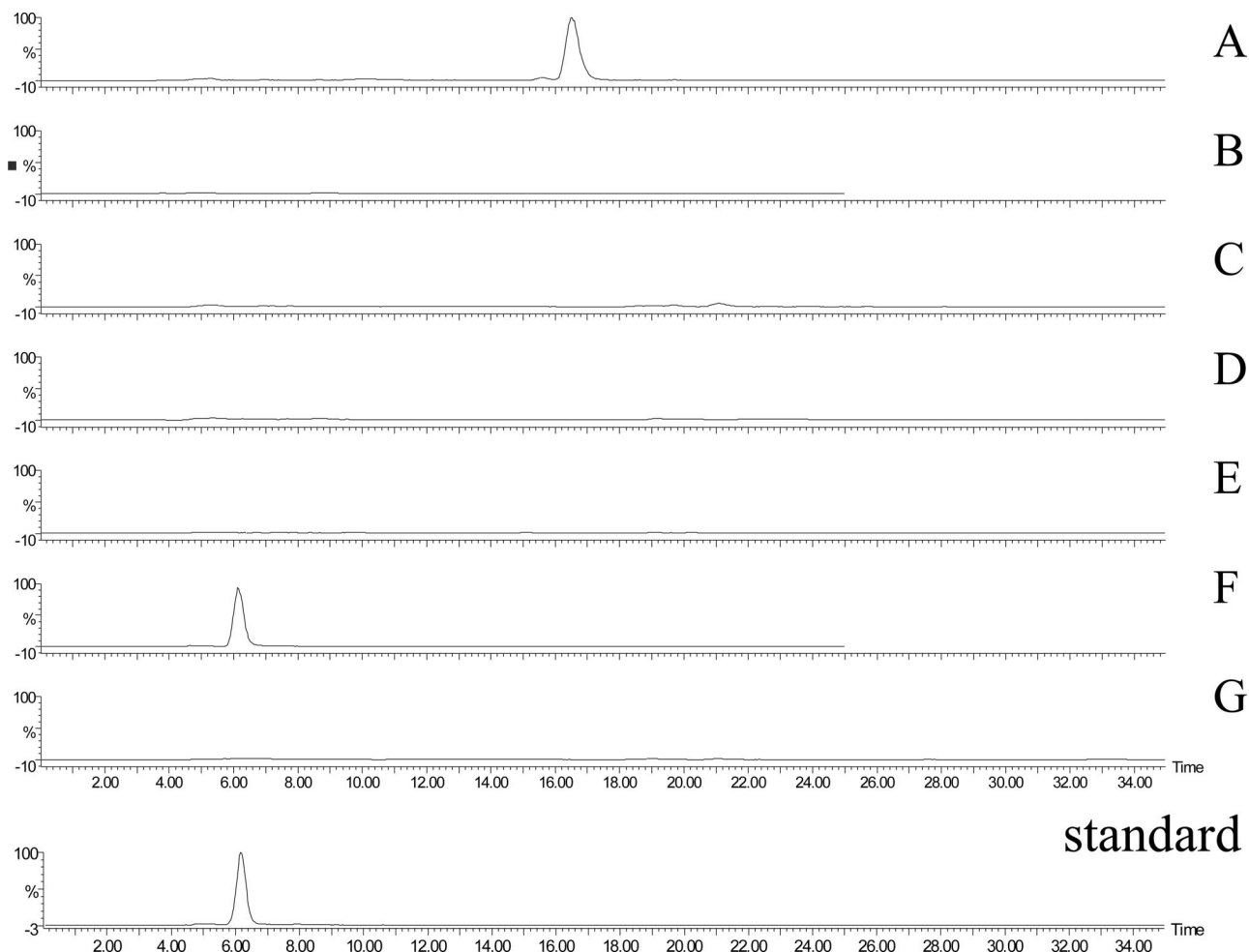


FIG. 4. Representative chromatograms for herbal samples A through G monitored by SIR at 390.25 amu (tadalafil). All are 10× dilutions of 5 ml 50% ethanolic extract except for samples B (1,000×) and F (100×). Standard is from 5 μg/ml tadalafil calibration sample.

PC-SPES, an herbal preparation touted for the treatment of prostate cancer, was known to induce castrate levels of testosterone and decrease prostate specific antigen levels.<sup>8</sup> PC-SPES had gained tremendous popularity among patients and was being evaluated in randomized trials. Later chemical composition analyses indicated that PC-SPES and its related compound SPES were contaminated with pharmacological dosages of warfarin,<sup>10</sup> diethylstilbestrol<sup>9</sup> and indomethacin.<sup>10</sup> PC-SPES was ultimately withdrawn from the market at the insistence of regulatory agencies. Serious medical complications had been reported from PC-SPES and likely could have been prevented had regulation existed. These data indicate that the same can now be said about certain herbal products used to treat ED.

Neil Fleshner had the main idea for this project and wrote the majority of the article. Melissa Harvey did the background research about the individual product selection, and also purchased, relabeled and shipped the products. Hans Adomat performed the mass spectrometry analyses. Catherine Wood performed sample preparation and extraction for mass spectrometry. Andy Eberding was involved in background work for method development with respect to chromatography and mass spectrometry. Karen Hersey provided input into study design and manuscript preparation. Emma Guns wrote the article with Doctor Fleshner and is head of the mass spectrometry laboratory at the Prostate Centre at Vancouver General.

#### APPENDIX: TESTED PRODUCTS FOR ERECTILE DYSFUNCTION

Product	Assigned Letter	Web Site
Biovigora™	A	www.oasisbiotech.com
Super-X	B	www.herbalhealer.com
Vip ViGa	C	www.herbalremedies.com
Vuka Vuka	D	www.vukavuka.com
Herbal Viaphrodisiac	E	www.lifesines.com
Stamina-Rx™	F	www.freestamina.com
Herbal Erotica™	G	www.thehealinglight.com

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#### EDITORIAL COMMENT

The findings of this study are, in effect, a compelling condemnation of the absence of regulation of herbal products and a harsh black mark on the nutraceutical industry. The government policy which allows for unrestricted and unsupervised manufacturing and marketing of nutraceuticals and food supplements has resulted in real,

present and possibly fatal dangers to consumers. Fleshner et al show that the over-the-counter products Super-X and Stamina-Rx™ are hardly harmless food supplements, having been secretly and intentionally contaminated with therapeutic doses of phosphodiesterase inhibitors. If these products continue to be available to unsuspecting consumers, sooner or later someone unwittingly taking one of these products with an organic nitrate drug for angina will die of a cardiovascular catastrophe. Such deaths may already have occurred without being recognized because these products are sometimes used by patients clandestinely.

Without regulation of the manufacturing and marketing of nutraceuticals, the public is at the mercy of unscrupulous nutraceutical businesses. There is no way for consumers to know which nutraceutical companies are reliable and ethical, and which are not. The nutraceutical industry has failed to police itself. Until there is governmental regulation of the nutraceutical industry, one must conclude from this study that nutraceuticals and other “natural” herbal products pose indeterminable and grave risks to public health. Consumers cannot trust that ANY herbal or nutraceutical product coming from this unregulated industry is safe to use. There is an urgent need for new government policy and/or legislation to protect consumers from dangerous practices of manufacturers and purveyors of nutraceutical and herbal products and food supplements. Much more strict oversight of this industry by the Food and Drug Administration and other government agencies would be an important step in the right direction.

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