

## Detection by gas chromatography-mass spectrometry of adulterated food supplements

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### Abstract

*The number of herbal food supplements on the Romanian market is continuously growing, with about 1500 new products every year, out of which approximately 15 products are aimed to enhance sexual performance. Phosphodiesterase type 5 (PDE-5) inhibitors such as sildenafil (Viagra®), tadalafil (Cialis®), vardenafil (Levitra®) and most recently avanafil (Stendra™) are authorized drugs used in the treatment of erectile dysfunction. Certain herbal food supplements which claim to enhance the sexual performance have been found to be adulterated with these drugs. Our study aims to develop an useful method of gas chromatography-mass spectrometry (GC-MS) for the efficient screening of herbal food supplements supposed to be adulterated with sildenafil, tadalafil, and vardenafil. The identification of pharmacological active principles was done by detection of specific molecular ions for each compound: sildenafil ( $m/z$  99, 404); tadalafil (389, 262) and vardenafil (113, 448). Sildenafil, tadalafil and vardenafil were successfully identified by GC-MS in different herbal food supplements. The results of this study show that six herbal food supplements were adulterated with PDE-5 inhibitors, which means 23% of the total 26 analyzed products.*

**Keywords:** herbal food supplements, pharmacological active principles, PDE-5 inhibitors, sildenafil, tadalafil, vardenafil.

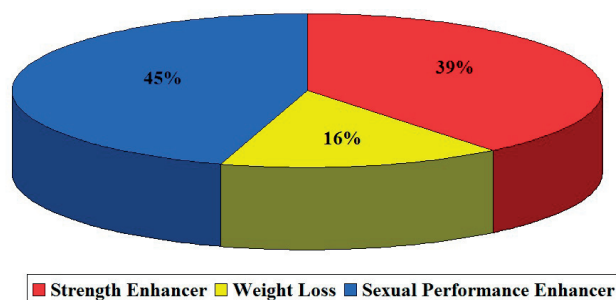
### 1. Introduction

Food supplements are a special category of products, placed between traditional herbal medicines and conventional food due to their composition, presentation and conditions of use. In recent years, the number of these products increased and their formula diversified (ingredients and combination) developing a huge market. The worldwide consumption of dietary supplements has also significantly increased, because consumers tend to trust natural products, believing they are safe and free of side effects (1).

Adulteration of herbal food supplements has become a problem all over the world, as well as in Romania during the last years. There are many reports about the adulteration of herbal food supplements with: steroids (strength enhancers); PDE-5 inhibitors or their analogues (sexual performance enhancers) and sibutramine and its analogues (weight loss products). In Figure 1 and Table 1 are shown 210 of products recalled by FDA in the period January 2010 to September 2013, which proves that adulteration of herbal dietary supplements is a major problem for all three categories of products presented below (2). The

general aim of food supplements adulteration is to enhance the physiological effects by using pharmacological active substances (3).

Many food supplements recommended as sexual performance enhancers are presented and advertised as “all natural” but they have been in contrast found to contain synthetic PDE-5 inhibitors. Thus commercially available herbal products have been „spiked” with legal drugs or their analogues, which have not been subjects to formal pharmacokinetic or other pharmacological tests (4, 5).



**Figure 1.** Food supplements recalled by FDA from January 2010, to September, 2013

**Table 1.** The products recalled by FDA from January 2010, to September, 2013

Total Products Recalled	Strength Enhancer	Weight Loss	Sexual Performance Enhancer
66210	82	33	95

67

The PDE-5 inhibitors that have been approved by both European Medicine Agency (EMA) and U.S. Food and Drug Administration (FDA) for the treatment of erectile dysfunction disease (ED) are: sildenafil citrate (Viagra<sup>®</sup>, manufactured by Pfizer in 1998), vardenafil hydrochloride (Levitra<sup>®</sup>, manufactured by Bayer, 2003), tadalafil (Cialis<sup>®</sup>, manufactured by Lilly, 2003), and most recently avanafil (Stendra<sup>™</sup>, manufactured by Vivus Inc., 2012) (6-13). These drugs are only available on doctoral prescription and must be used under medical control (14). PDE-5 inhibitors have not to be used in the same time with the treatment of diabetes, hypertension, hyperlipidemia and ischemic heart disease; they are not recommended for patients who use medicines based on organic nitrates (e.g. nitroglycerin, isosorbide dinitrate, isosorbide mononitrate, amyl nitrite, or nitrate) (15), because may result in severe and unpredictable decrease of blood pressure, accompanied by symptoms of hypotension (16). The most common adverse reactions induced by the three PDE-5 inhibitors are: headache, flushing, dyspepsia, nasal congestion, dizziness, myalgia, and back pain, abnormal vision (17).

The adulterated food supplements with analogues of PDE-5 inhibitors are a real threat to public health, because these compounds have not been drug-tested and more than this, they are not declared on the label (18).

The most dangerous is the intentional adulteration or ‘spiking’, which may be maliciously used to increase the effectiveness of the food supplement, and thus to enhance sales (7). Such type of adulteration was also detected in the preliminary tests performed by us.

Recently we have started in Romania to analyze the herbal food supplement composition; the main target was to detect the undeclared active pharmaceutical ingredients especially in the sexual performance products and weight loss. Our screening results were

surprising. There were found in so called natural products undeclared substances such as sildenafil, vardenafil, tadalafil, yohimbine and coffeine (in sexual performance enhancers) as well as sibutramine, fluoxetine or metronidazole (in weight loss products). From the point of view regarding the country of origin, most of the adulterated herbal food supplements entered on the Romanian market coming from China and other Asian countries, being imported by certain trade companies.

This study focused on sexual performance enhancer products, and a number of 26 products were analyzed on the first step.

## 2. Materials and method

Reference substances/pure samples were: tablets of Viagra<sup>®</sup> (Pfizer Limited, for sildenafil 50 mg), tablets of Cialis<sup>®</sup> (Lilly, for tadalafil 10 mg) and Levitra<sup>®</sup> (Bayer, for vardenafil 10 mg). Methanol and ethanol used as solvents were provided by Merck, Germany. Herbal food supplement samples were supplied by the National Office of Medicinal, Aromatic Plants and Bee Products, or have been bought from the Romania market.

All the analyzed products were solid powder encapsulated (17 products) or compacted in tablets (9 products). Each capsule was emptied and each tablet was crushed. Every 100 mg of fine powder was mixed with 1 ml of absolute methanol. Samples were mixed thoroughly by vortexing, followed by 15 minutes of sonication and 5 minutes centrifugation at 4000 rpm. The supernatant was collected and filtered by 0.2 µm membrane filters for GC-MS analysis. The tablets of Viagra, Levitra and Cialis, used as reference substances were crushed and every 100 mg of fine powder extracted with 5 ml diluting solvent (10% ethanol in methanol). The obtained standard solutions were mixed by vortexing followed by 30 minutes of sonication at room temperature and 5 minutes centrifugation at 4000 rpm. These solutions were serially diluted in diluting solvent up to 15, 7.5, 3.75 and 1.5 ppm to bring them into the linear dynamic range for analysis.

GC-MS system consists of HP6890 GC, HP5973 MS and fused silica capillary column, HP-5MS (30 m x 0.25 mm i.d. 0.25 µm film thickness). A 1 µL injection using splitless mode was performed on 280 °C injector port with helium flow at 1.2 ml/min. The oven ramping temperature was programmed at 250-320 °C (2 min hold) at a rate of 25 °C/min. The screening was performed on selected ion monitoring mode at *m/z* 99, 404 (sildenafil); 389, 262 (tadalafil); 113, 448 (vardenafil) while identification was done on full scan mode (40-500 amu). Compounds spectra obtained were compared to spectra of known compounds using the NIST Mass Spectral Search Program for the NIST/EPA/NIH Mass Spectral Library. This method used was adapted from C.N. Man & al. (19).

## 3. Results and discussions

The efficient chromatographic separation of sildenafil (SD), tadalafil (TD) and vardenafil (VD), at the retention time of 18.163, 15.876 and 20.226 min, was the evidence that this analysis do not require hydrolysis or derivatization procedure. The advantages of this type of analysis consist in a rapid sample preparation and a fast screen for PDE-5 inhibitors detection.

All the 26 products analyzed are described in Table 2. Out of these, 6 herbal food supplements were detected to be adulterated with PDE-5 inhibitors. The adulterated products could be divided into two categories, according to their country of origin as follows: Asia (China and Japan) and North America (Canada).

The adulterated herbal food supplements originated from Asia: three of them were manufactured in China and two in Japan. All the Chinese products (Fig. 2) were adulterated with vardenafil (samples CLV-15, CLV-25, CLV-26) while the two Japanese products (Fig.

3) were adulterated with sildenafil (sample CLV-5) or with a combination of sildenafil, tadalafil and vardenafil, respectively (sample CLV-17). The presence of all three PDE-5 inhibitors demonstrates that product adulteration is not an accident (contamination) and it was done intentionally by the manufacturer aiming to increase the pharmacologically effects of this product (deliberately adulteration).

A single dietary supplement originating from Canada was found to be adulterated with tadalafil, as it could be seen in Figure 4.

**Table 2.** Identification of sildenafil, tadalafil and vardenafil in herbal food supplements by GC-MS

Sample	Product description	Country of origin	PDE-5 inhibitors
CLV-1	capsules	China	-
CLV-2	tablets	Canada	Tadalafil
CLV-3	capsules	China	-
CLV-4	capsules	China	-
CLV-5	tablets	Japan	Sildenafil
CLV-6	tablets	United Kingdom	-
CLV-7	tablets	China	-
CLV-8	tablets	SUA	-
CLV-9	capsules	China	-
CLV-10	tablets	SUA	-
CLV-11	tablets	SUA	-
CLV-12	capsules	United Kindom	-
CLV-13	capsules	France	-
CLV-14	tablets	Romania	-
CLV-15	capsules	China	Vardenafil
CLV-16	capsules	India	-
CLV-17	tablets	Japan	Sildenafil, Tadalafil, Vardenafil
CLV-18	capsules	SUA	-
CLV-19	capsules	Romania	-
CLV-20	capsules	France	-
CLV-21	capsules	United Kindom	-
CLV-22	capsules	China	-
CLV-23	capsules	Canada	-
CLV-24	capsules	United Kindom	-
CLV-25	capsules	China	Vardenafil
CLV-26	capsules	China	Vardenafil

One of the most spectacular results was obtained by analyzing a Romanian product: different samples from the same batch suspected to be non-compliant. A sample was voluntarily offered to the control authority by the manufacturer, to be official tested and another one was bought from the market. Manufacturer's product proved to be compliant, while the purchased product was adulterated with yohimbine together with tadalafil. This test demonstrated that the producer deliberately adulterated the analysed herbal food supplement in order to enhance its effectiveness (by cumulating the effects of all substances) and, subsequently tried to hide the adulteration when an official control occurs. Combining more substances involved in the sexual activity but undeclared their presence on the labelling, could cause major health risks, especially if the consumer includes in its diet coffee, tea, alcohol, energy drinks or different drugs contain nitrates.

In recent years the distribution channels for sexual performance enhancers were more and more diversified, and included: sex shops, specialized stores for traditional herbal products, pharmacies, but also: on-line and telephone orders (direct sales).

One of the most serious problems in the surveillance activity of the market is the lack of specific legislation to regulate online trade. Thus, an increased number of consumers (especially young people) have access to these kinds of products. Sexual performance enhancers are sold as „dietary supplements, natural or safe products” and could be ordered from different specialised web-sites (virtual stores). This is comfortable for the client and ensures a greater level of confidentiality, but the products delivered could be unsafe, adulterated or mislabelled.

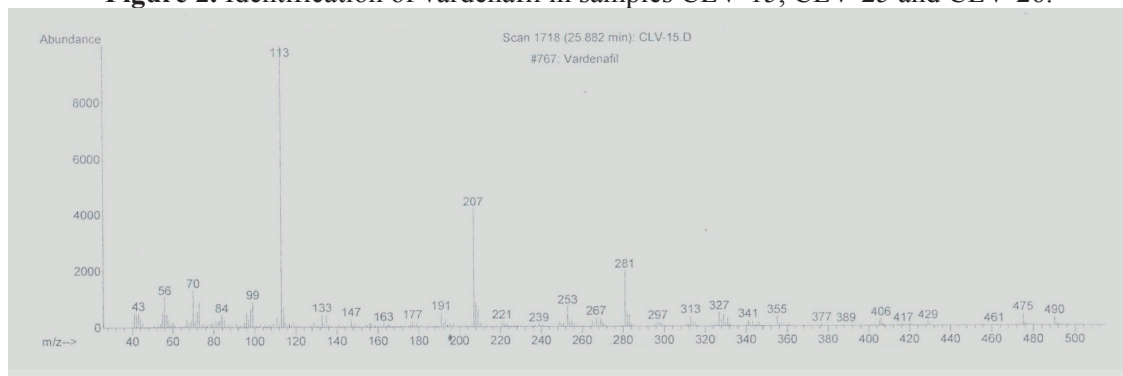
In our opinion, the adulteration of herbal food supplements with pharmaceutical substances may have two different sources: the first - unwittingly adulteration – by including in the products composition adulterated raw materials, or the second one, deliberately adulteration by voluntary including in food supplements different pharmacologically active substances, aiming to obtain spectacular effects by increasing the product efficiency. Imported raw material from other countries offers to the manufacturers’ whose products were detected to be adulterated a logic explanation for unwittingly adulteration of their final product. Generally speaking, both unwittingly and deliberately adulteration, have serious consequences for the consumer health because the undeclared pharmaceutical substances from adulterated products could interact with some medications (PDE-5 inhibitors have potentially fatal interaction with drug nitrates). On the other hand, the consumer becomes a true victim because he is not warned by the product label, because that does not contain recommendations for use, or medical advice for special categories of vulnerable persons.

As a result of other countries experience, literature reviewed and our own investigation, there was identified a significant number of adulterated products on the Romanian market. Under these circumstances, the control authority improved the procedure of notification requiring to all operators to analyze their products before enter on the market, in order to identify the potential adulteration of herbal food supplements with PDE-5 inhibitors. Introducing such compulsory tests would really diminish the number of non-compliant herbal food supplements from the market and, in the meantime, the level of consumers exposure, they will be better protected, having access to safer products.

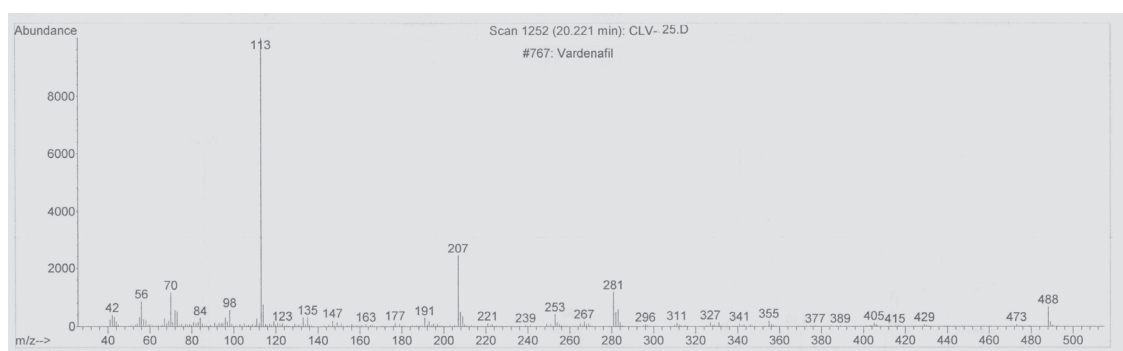
This study represents for Romania one of the first screening of the herbal food supplements recommended to be used for erectile functions improvement. The target of the study was simply to detect the PDE-5 inhibitors in the composition of the natural products that are advertised as sexual performance enhancers.

The next step will be to analyze all the approximately 270 products from the Romanian market, and to improve the analytical investigation aiming to detect not only sildenafil, tadalafil, vardenafil but also their analogues. The most recently scientific results reported by different groups of researchers showed an increasing number of all the PDE-5 inhibitors analogues up to 57 (A.M. Popescu & al, 2014 under press, D.N. Patel & al. [20]).

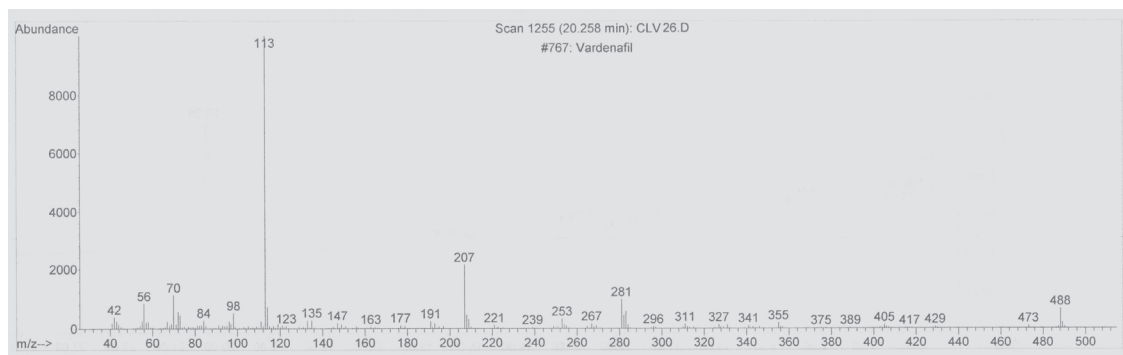
**Figure 2.** Identification of vardenafil in samples CLV-15, CLV-25 and CLV-26.



a) The mass spectra of samples CLV-15 with peaks at retention time 25.882

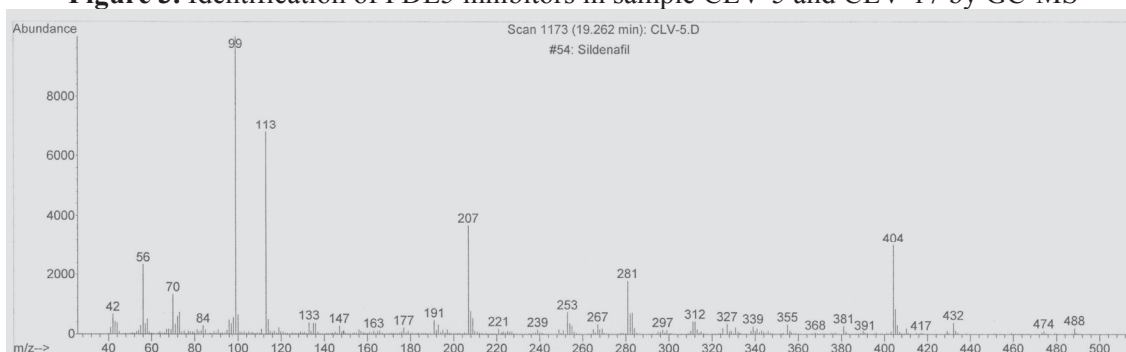


b) The mass spectra of samples CLV-25 with peaks at retention time 20.221

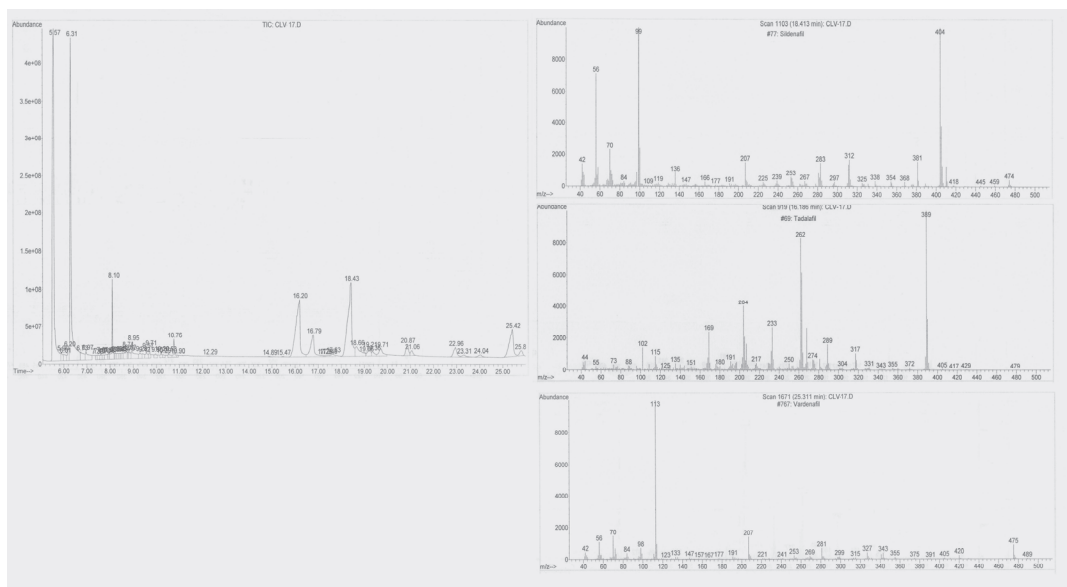


c) The mass spectra of samples CLV-26 with peaks at retention time 20.258.

**Figure 3.** Identification of PDE5 inhibitors in sample CLV-5 and CLV-17 by GC-MS

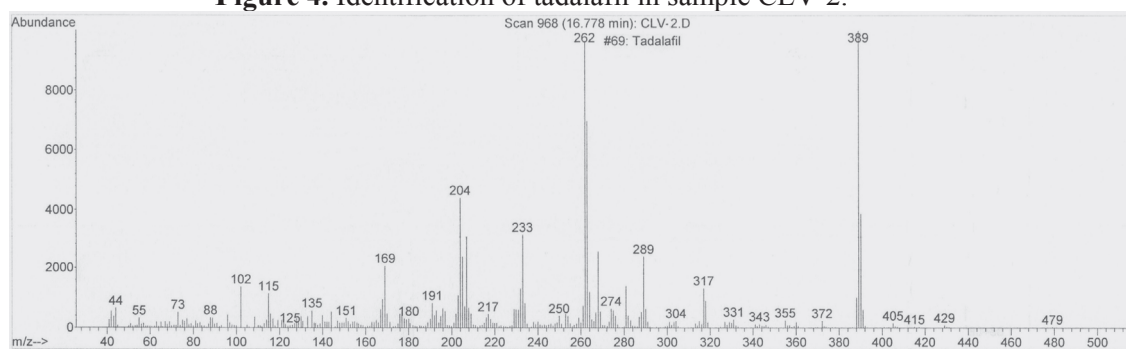


a) The mass spectra of sample CLV-5 at retention time 19.262;



b) **left** - The gas chromatogram of sample CLV-17 with peaks at retention times 18.413 (sildenafil), 16.186 (tadalafil) and 25.311 (vardeafil); **right** – The mass spectra of sample CLV-17 at retention times 18.413 (sildenafil), 16.186 (tadalafil) and 25.311 (vardeafil)

**Figure 4.** Identification of tadalafil in sample CLV-2.



The mass spectra of samples CLV-2 with peak at retention time 16.778

#### 4. Conclusions

As result of the first screenings of 26 dietary supplements used for sexual activity enhancement we have been detected 6 products adulterated with sildenafil, tadalafil or vardenafil. One of them has been adulterated with all three substances.

Sildenafil, tadalafil and vardenafil were successfully identified by gas chromatograph-mass spectrometer without derivatization or hydrolysis procedure. The reported MS data was helpful in rapid detection of sildenafil, tadalafil and vardenafil in herbal food supplements.

Herbal food supplements could be dangerous for consumers when drug substances, undeclared on the label, are hidden in their composition. Pharmaceutical substances may interfere with the consumer's diet and could result in adverse reaction and side effects, because the consumer it is not warned about the presumptive risks of the so called “natural” product consuming.

The screening of all herbal food supplements advertised as sexual performance enhancers proved to be necessary for the detection of PDE-5 inhibitors before the products entries on the market.

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