Research Paper

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Determination of sildenafil, vardenafil and tadalafil in dietary supplements sold in the Yemeni market

Pharma

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ABSTRACT

There have been a number of reports of dietary supplements contaminated with illegal adulterants that threaten consumer's health for their adverse pharmacological effects worldwide. In this study, our main aim was to investigate different dietary supplements of different forms present in the Yemeni market for the presence of sildenafil, vardenafil and tadalafil. An HPLC method was adopted for this study to investigate for their presence simultaneously. The mobile phase consisted of acetonitrile and aqueous solution containing ammonium acetate and formic acid using the gradient elution system at a detection wavelength of 292 nm. All of the samples were found to contain at least one of the investigated medicines. The confirmatory studies were performed by thin layer chromatography. Results obtained confirmed the presence of the investigated drugs in the dietary supplements tested.

Introduction

Male erectile dysfunction (ED), the persistent inability to achieve or maintain an erection for satisfactory sexual performance, is a common medical problem (Jeffecoate, 1991). According to a random study, over half of men between 40 and 70 years age have experienced erectile dysfunction (Goldstein, 2000). It is also estimated that the number of men who have erectile dysfunction will double in the next 25 years, ultimately affecting more than 330 million men worldwide (Goldstein, 2000).

Synthetic phosphodiesterase-5 (PDE-5) inhibitors, such as sildenafil citrate, vardenafil hydrochloride, and tadalafil have been widely used for the treatment of ED. Despite the efficacy of PDE-5 inhibitors for treatment of ED, their drawbacks are also significant. Adverse effects such as headache, facial flushing, dyspepsia, visual disturbances and muscle aches have been reported (Wespes et al, 2002). Furthermore, reports of blindness have been linked to the use of Viagra (Bella et al, 2006).

The popularity of these medications has expanded the market for ED treatments. Viagra, Levitra and Cialis are marketed as prescription drugs and must be used under medical supervision. Many patients resort to herbal alternatives, as herbal medicines are believed to be safer than synthetic ingredients. A dietary supplement is a product that contains a "dietary ingredient" intended to supplement the diet. These ingredients, generally undeclared in the labeling, can pose considerable dangers to consumers who may take these products without knowing that the ingredients are present. Furthermore, under the false assumption that these products are safe, patients may be tempted to administer larger doses than the one recommended by the manufacturer.

The use of herbal remedies and dietary supplements for treatment for ED has greatly increased. Several pure compounds derived from natural products such as yohimbine, citrulline, and forskolin have been reported to improve sexual performance (Drewes et al, 2003). However, some herbal products advertised as "all natural" had been found to be adulterated with synthetic PDE-5 inhibitors. Researchers identified the presence of sildenafil or tadalafil in 17 herbal products and quantified the two compounds with LC–UV (Gratz et al, 2004).

In this study, dietary supplements of different forms collected from the Yemeni market (pharmacies and stores) were analyzed for the presence of one of more of the approved synthetic PDE-5 inhibitors.

Methods

Materials ad reagents

The standards of the Vardenafil, Sildenafil, Tadalafil were gifts from Sheba Pharma, Yemen. Acetonitrile and methanol were purchased from Schlarlau Chemie (Spain). All other materials used were of analytical grades. The assayed products were purchased from pharmacies and stores in Sana'a and Aden. Twenty one dietary supplements of different forms were investigated. The samples included capsules (16 products), tablets (3 products), honey (1 product), and chocolate (1 product).

Preparation of standards

The standard solutions were prepared by weighing 20 mg from each standard. The powders were dissolved in 20 ml of methanol with sonication and solutions were then filtered using 0.45 µm nylon membrane filters under vacuum. One milliliter aliquots of each stock solution were transferred into a 10-ml volumetric flask, mixed and diluted to volume to yield a mixed standard solution. Serial dilution was carried out to obtain five working standards.

Sample extraction and preparation

Twenty milligrams of each sample was used regardless its dosage form. Twenty five milliliters of acetonitrile was used for the extraction with sonication which varies in time according to the dosage form. After the sonication, samples were centrifuged to facilitate the filtration through the micro-membrane. A solution mixture of acetonitrile and water was made by mixing acetonitrile and distilled water in the ratio of 50:50. Twenty milliliters of the filtrates were diluted to 100mL with acetonitrile then injected in HPLC for analysis.

HPLC

The assay was performed using a Waters HPLC (15250 Binary HPLC Pump) with a 2489 Waters UV detector and a symmetry 3.5 µm C18 analytical column (Waters, USA). The method used was adopted from a previous study with minor modifications (Zhu et al, 2005). The mobile phase consisted of acetonitrile (A) and aqueous solution containing 0.3M ammonium acetate and 0.2% formic acid (v/v) (B). The gradient elution was programmed as follows: at time 0 minutes A was pumped at the rate of 5% after which it is increased linearly to 95% in the next 10 minutes. The system is kept constant as stated for the next 2 minutes then brought back to the initial ratio (i.e. A = 5%) in the next 2 minutes. The flow rate was 1 ml/min, injection volume was 10µL, and the detection wavelength was set at 292 nm.

Thin layer chromatography

The standards and sample solutions were applied to 0.20mm silica gel 60 with fluorescent indicator UV 254nm (Macherey - Nagel, Germany) with chloroform/ methanol/ 25% ammonia (90:1:5) as mobile phase. Spots were located under UV radiation at 254 nm (Mikami et al, 2002; Moriyasu et al, 2001).

Results

The standards and examined analytes were separated under the given chromatographic condition. Fig. 1 shows the chromatogram of mixed standards. The retention times for sildenafil, vardenafil, and tadalafil were 4.7, 3.9, and 6.1 min, respectively. The peaks were well-resolved with no overlapping. Surprisingly, the presence of the active substance has been confirmed in all tested samples. When compared to the standards, the all-natural supplement sample contained varying substances having similar retention times to one or more of the standards. Table 1

and Figures 2-3 show the composition in tested samples.

Table 1: Information and detected constituents of investigated dietary supplements.

No.	Form	Origin	Constituents Detected
1	Capsule	Egypt	Sildenafil
2	Capsule	China	Sildenafil
3	Capsule	China	Sildenafil
4	Capsule	Hong Kong	Sildenafil
5	Capsule	Hong Kong	Sildenafil
6	Capsule	China	Sildenafil
7	Tablet	China	Sildenafil
8	Tablet	Hong Kong	Sildenafil
9	Capsule	China	Sildenafil
10	Capsule	China	Tadalafil
11	Capsule	China	Sildenafil
12	Capsule	China	Sildenafil
13	Capsule	China	Sildenafil
14	Capsule	China	Sildenafil, Tadalafil
15	Capsule	Lebanon	Sildenafil
16	Soft gelatin capsule	China	Sildenafil
17	Capsule	China	Sildenafil
18	Tablet	Spain	Sildenafil
19	Capsule	China	Sildenafil, Tadalafil, Vardenafil
20	Honey	South Africa	Sildenafil
21	Chocolate	China	Sildenafil

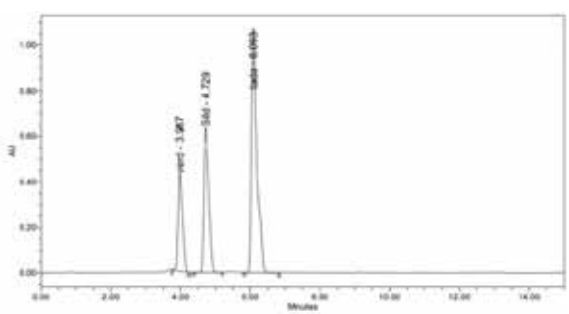

Figure 1: HPLC chromatogram of mixed standards.

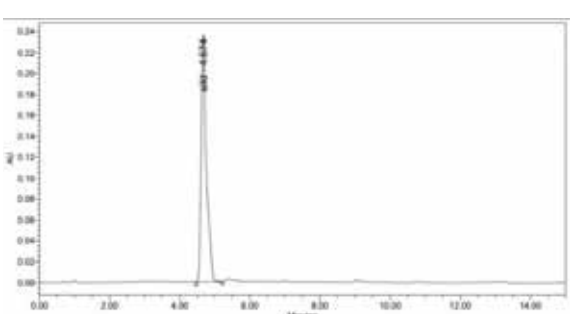

Figure 2: Chromatogram of product 14 extract. Peak corresponds to the peak of sildenafil.

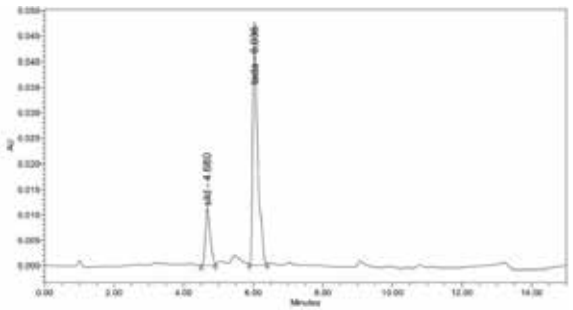

Figure 3: Chromatogram of product 1 extract. Peaks correspond to peaks of sildenafil and tadalafil.

TLC is widely used in pharmaceutical analytical laboratories throughout the world for identity testing, because it is simple, rapid, robust and inexpensive. In confirmation experiments, we conducted a qualitative analysis. The extracts of standards and samples were applied to Silica gel 60 F254 plates with chloroform/methanol/25% ammonia (90:1:5) as mobile phase. After development, dark spots corresponding to the standards used were observed under UV light (main wavelength: 254 nm), and were clearly resolved and well separated from the other components (Fig. 4). Some samples which expressed good HPLC peaks did not show TLC spots due to the high concentration needed in TLC studies.



Figure 4. TLC of the standards and samples.

Discussion

Many techniques have been employed in detection of sildenafil and related compounds in dosage forms. HPLC was found to be a useful instrument for determination of these compounds in pharmaceutical preparations (Daraghmeh et al, 2001; Dinesh et al, 2001). TLC methods are developed for identification of sildenafil as an illegal additive in soft drinks and foods (Mikami et al, 2002; Moriyasu et al, 2001).

Dietary supplements advertising nutrition or weight reduction abilities have gain popularity in many countries in recent years. Dietary supplements are very complex because they contain many kinds of compounds. Prolonged or excessive consumption of these supplements containing undeclared amounts of drugs may cause serious adverse health consequences.

The samples examined in present work included capsules, soft gelatin capsules, chocolate, and honey. The compositions of all these supplements are rather complicated. The presence of the active substance has been confirmed in all tested samples. In qualitative terms, there is a high incidence of adulteration of dietary supplements in the Yemeni market. The high incidence of adulteration with sildenafil, i.e. 20 samples either alone or mixed with other adulterants, is because it is the first to be discovered for that matter being the cheapest in procurement. This way the manufacturer will be able to reduce the cost of production as well as increase margin of profit.

Conclusions

With the advancement in development and production technology of sildenafil and its related compounds, the quantity of these compounds is becoming bigger and bigger and their prices are on decline. Therefore, more of these compounds are

being added to dietary supplements illegally. In this study, liquid chromatography was used for the simultaneous determination of three synthetic PDE-5 inhibitors. It was shocking that all tested samples were found positive for the inclusion of one or more of these substances. The adulteration of dietary supplement and their bulk powders by modified drugs appears to be a growing trend. Consequently, the authorities should have a program for screening for adulterants in supplements pending for registration.

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