Original Research Article

Evaluation of Sildenafil as an Undeclared Adulterant in Herbal Aphrodisiac Preparations by HPLC

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Abstract

The success in the treatment of erectile dysfunction and subsequent improvement of quality of life by the three approved phosphodiesterase type-5 inhibitors (sildenafil, tadalafil and vardenafil) has led to an explosion of illicit sexual performance enhancement products. Several cases of adulteration of herbal aphrodisiac products with Phosphodiesterase type-5 inhibitors (PDE-5) and/or their unapproved analogues' have been reported worldwide. Therefore, the aim of this research is to evaluate herbal aphrodisiac preparations for the presence of sildenafil in northwest Nigeria. An isocratic Reverse Phase-High Performance Liquid Chromatography (RP-HPLC) for the determination of sildenafil was adopted and modified for this study. Fifty aphrodisiac herbal preparations were sampled and screened for the presence of sildenafil. Calibration curve was found to be linear within the concentration range of 10-50 µg/ml (r2 = 0.999); the method is precise (% RSD <2) and accurate (% recovery 98-100). Sixteen samples (32 %) were identified to be adulterated with sildenafil; the compounded concentrations in the products were found to be within the range of 0.45-39.8 mg of sildenafil per dose. Thus, consumption of these products could lead to serious health risks; hence the need for an immediate intervention by relevant stakeholders to safeguard public health.

Keywords: Adulteration, herbal aphrodisiacs, RP-HPLC, sildenafil.

INTRODUCTION

Despite advances and availability of allopathic medicines, traditional and herbal medicines are still popular in developing world [1]. This is sequel to the inherent notion that traditional and herbal medicines are safe coupled with the adverse effects and contraindication(s) of orthodox medicines. The quality control of traditional and herbal medicines, as well as their safety and efficacy have become important concern for both authorities and the public [1]. Adulterations of herbal medicines with orthodox drugs have been reported worldwide [2-6].

The success recorded with phosphodiestrate-5 (PDE-5) inhibitors in the treatment of erectile dysfunction has led to the increase in their popularity and thus accessibility. To this end, there are several reported cases of explosion in adulteration of aphrodisiac herbal preparations with PDE-5 inhibitors and/or their analogues [7-10]. Detection of sildenafil (Fig. 1), a prototype of PDE-5 inhibitors, as an illegally added substance in herbal products meant for improving sexual performance was also reported in Indonesia, Sudan and Bangladesh [8, 10, 11]. This drug has broad profile of interactions and adverse reactions including shortness of breath, angina, persistent headache, myocardial infarction, vaso-occlusive crisis (secondary to sickle cell anemia), non-arteritic anterior ischemic optic neuropathy (NAION) and priapism among others [12, 13]. The standard organization of Nigeria (SON) and National agency for food and drug administration (NAFDAC) warned that 'proliferation of adulterated products is a rapidly growing menace in Nigeria'[14]. Therefore, it is paramount to screen aphrodisiac herbal products marketed in Nigeria for the presence of sildenafil.
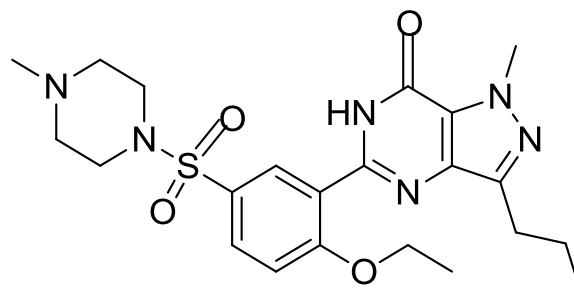

Figure 1: Chemical structure of sildenafil

METHODS

Materials

Sildenafil citrate was purchased from sigma-Aldrich, all solvents used were of HPLC grades; methanol (BPH Chemical, England), water (BPH Chemical, England). Fifty herbal aphrodisiac preparations marketed across four (Katsina, Kano, Kaduna, Zamfara) Northwestern Nigerian states, were sampled and randomly collected from streets, herbal centers, local markets, and stores. The samples were coded S1-S50.

Instrumentation

The HPLC (Agilent technologies, 1260 infinity), is an auto sampler with an inline degasser, coupled with UV detector and an injector of 20 µL loop. UV detection was achieved by double beam Ultraviolet (UV) Spectrophotometer (Helios Zeta, Model 164617) with 10 mm matched quartz cell.

Chromatographic conditions

The method for sildenafil analysis previously reported was adopted and modified for this study [15]. Modifications were achieved by changing the column type; Prontosil C_{18} stationary phase (150 × 4.6 mm, 5 µm) with Techesphere 50 DS (250 × 4.6 mm) and introduction of extraction process with an organic modifier. Sildenafil was extracted from the samples using methanol. This is expected to increase selectivity, efficiency and shorten the total run time. Chromatographic separation was achieved at ambient temperature using methanol: water (85:15 v/v) as mobile phase at a flow rate of 1 ml/min and wavelength of 230 nm. The volume of injection was 10 µl.

Preparation of standard solution

A quantity (5 mg) of sildenafil citrate standard powder was weighed and dissolved in 5 ml methanol yielding a concentration of 1000 µg/ml stock solution. From this, working solutions (10-50 µg/ml) were prepared by serial dilution.

Accuracy was reported as the percent recovery (%

$$recovery = \frac{Concentration(spiked-unspiked)}{Concentration\ spiked} \times 100)$$

Detection and quantification of sildenafil adulterant in aphrodisiac herbal samples

Sildenafil peak retention time was used for confirmation of adulteration; the concentration of sildenafil was then extrapolated from the calibration curve.

RESULTS AND DISCUSSION

To best our knowledge, this is the first study that determines the presence of sildenafil as undeclared adulterant in herbal aphrodisiacs marketed in Nigeria using High Performance Liquid Chromatography. Our optimized method resulted in shorter retention time (3.4

Sample preparation

A quantity (0.3 g) of each sample was weighed and transferred in test-tubes containing 10 ml methanols. The mixture was centrifuged for 5 minutes at 3500 rpm. The supernatant was filtered through 0.45 µm filter paper, 1 ml of the filtrate was used for HPLC analysis.

Method validation
Calibration curve and Linearity

A calibration curve was constructed by serially diluting 1000 µg/ml stock solution of standard sildenafil to give five working solutions in the range of 10-50 µg/ml. Peak areas obtained were plotted against their corresponding concentrations using Microsoft excel 2007. Linearity was evaluated by least square method.

Sensitivity

Limit of detection (LOD) and limit quantification (LOQ) were calculated as ($3.3\sigma/S$) and $10\sigma/S$ respectively. Where σ = standard deviation at intercept on y-axis and S = slope of the curve of the calibration curve.

Precisions

Intra-day (repeatability) and inter-day (immediate) precisions were determined by analyzing three replicate solutions of the standard sildenafil (20, 30 and 40 µg/ml) three times within a day at one hour interval and three times for three consecutive days respectively. Precisions were expressed as percent relative standard deviation (% RSD).

Accuracy

Recovery experiment was conducted by standard addition method. The study was performed by spiking known concentrations of sildenafil standard solution in different volumes of herbal samples' supernatants to produce nominal concentrations of 10, 20 and 30 µg/ml of sildenafil standard solution.

and percent relative error (% Er = $\frac{amount(added - obtained)}{amount\ added} \times 100$).

minutes) for sildenafil (Fig. 2) as against 4 minutes from which the method was adopted from [15]; this method was later applied for determination of sildenafil as an illicit adulterant in herbal aphrodisiac preparations (Fig. 4).

Linearity was established within the concentration range of 10-50 µg/ml for sildenafil as indicated by its coefficient of determination (r^2= 0.999) which was close to unity (Fig. 3); previous study reported results with an excellent of coefficient of determination (r^2 = 0.9996) over a concentration range of 0.1-6 µg/ml for sildenafil [15]. The method was found to be sensitive as indicated by low values of LOD

and LOQ (Table 1). Repeatability and intermediate precisions were found to be within the official limits (Table 2), as indicated by % RSD < 2. Accuracy was established as shown by % recovery and % relative error of 98.07-100.8 and 0.03-1.93 respectively (Table 3) which is in accordance with the ICH guidelines [16].

Of the 50 herbal aphrodisiac preparations randomly collected across four Northwestern Nigerian states; sildenafil was identified in sixteen samples. The adulterated samples were found to contain doses in the range 0.45-39.8 mg of sildenafil (Fig. 5). Previous studies reported nine sildenafil positive samples out of 30 selected, with the adulterated samples containing 165.4 ± 0.79 mg of sildenafil per dose [10]; similarly, 5

traditional medicines preparations for enhanced sexual performance and 7 dietary supplements were also found to contain sildenafil [17, 18]. Consumptions of these products put the public health in a state of serious threat; major concerns are the cardiovascular risks, potentially fatal hypotension secondary to synergistic effect(s) with nitrates and alpha blockers, priapism, which could lead to permanent penile tissues damage. Moreover, even at extremely low dose of sildenafil, herbal-drug interaction or drug-drug interaction may be significant; protease inhibitors, clarithromycin, and erythromycin are known to increase the level or effect of sildenafil by hepatic enzyme inhibition [19].Therefore, all relevant stakeholders need to rise to the occasion to stem this growing menace.
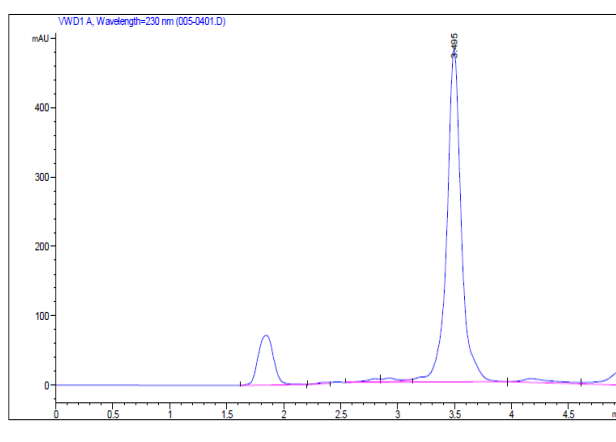

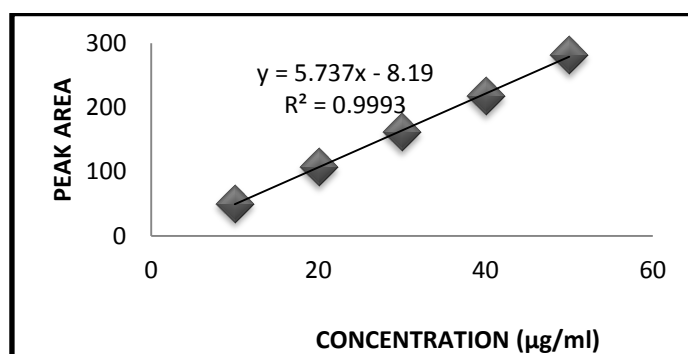**Figure 2: HPLC chromatogram of sildenafil citrate standard powder solution in methanol**

Figure 3: Calibration curve of sildenafil standard
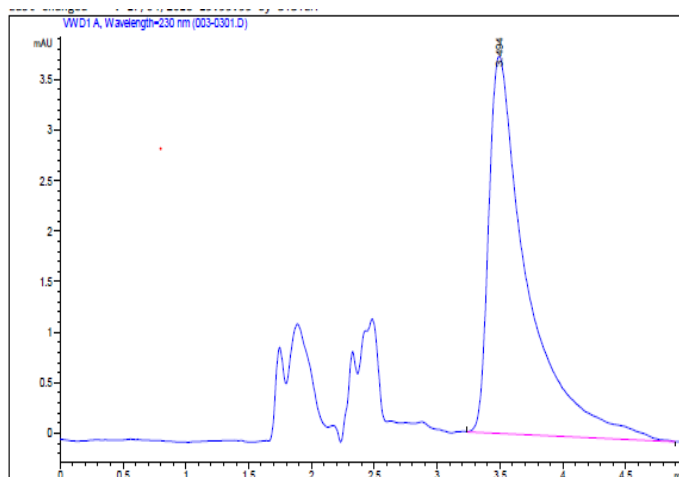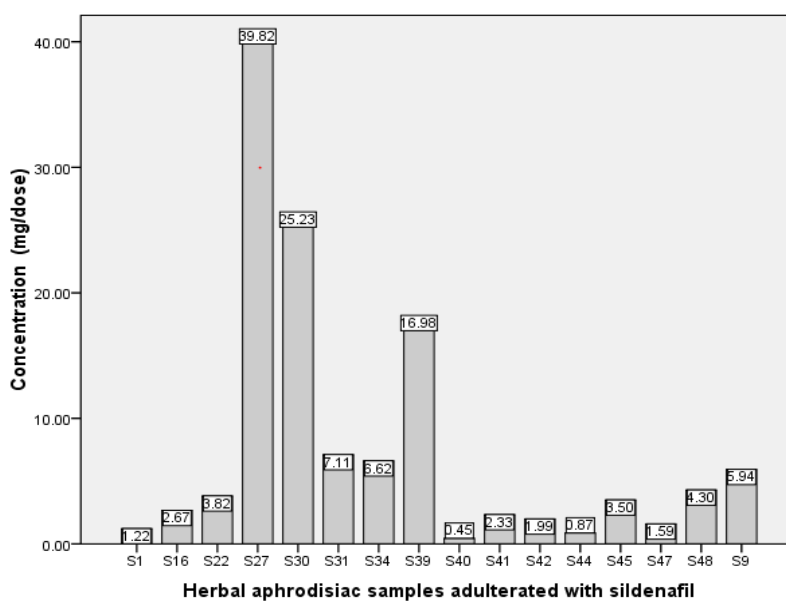
Table 1: Method sensitivity

Parameters	Values (µg/ml)
Range	10-50
Limit of detection (LOD)	1.61
Limit of quantification (LOQ)	4.89

Table 2: Results of precision studies (Intra-day and Inter-day) of sildenafil standard

S/NO.	Concentration (µg/ml)	Intra-day Mean ± SD	% RSD	Inter-day Mean ± SD	% RSD
1	20	106.1 ± 1.55	1.46	106.4 ± 1.86	1.75
2	30	159.4 ± 1.88	1.38	159.2 ± 2.95	1.85
3	40	216.8 ± 1.01	0.34	219.2 ± 2.71	1.17

Table 3: Accuracy and percentage recovery of sildenafil standard

S/No:	Concentration (µg/ml)		Percentage	
	Spiked	Recovered	Error	Recovery
1	10	10.003	0.03	100.03
2	20	19.956	0.22	99.78
3	30	29.421	1.93	98.07

Figure 4: HPLC chromatogram of one of the samples adulterated with sildenafil

Error bars: 95% confidence interval
Figure 5: Concentration of sildenafil in the adulterated herbal samples

CONCLUSION

In this study, sixteen herbal aphrodisiac preparations marketed in Northwestern Nigeria (32 %) were found to be compounded with sildenafil; a synthetic PDE-5 inhibitor used primary for erectile dysfunction, with a single dose of the adulterated sample containing sildenafil content within the range of 0.45-39.8 mg despite its well-known contraindications and interactions.

Conflict of interest

We declare that we have no conflict of interest.

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