DETERMINATION OF UNDECLARED SILDENAFIL CITRATE AND TADALAFIL IN APHRODISIAC HERBAL PREPARATIONS BY TLC AND HPLC

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Abstract
Liquid chromatographic methods, namely TLC and HPLC, were developed for the qualitative and quantitative analysis of undeclared Phosphodiesterase type 5 inhibitors in aphrodisiac herbal preparations. Thirty aphrodisiac herbal preparations, marketed in Khartoum City, were screened for Sildenafil Citrate and Tadalafil. Nine of them were found to be adulterated with sildenafil citrate while tadalafil was detected in three preparations. HPLC analysis revealed that a single dose of the adulterated samples contain 165.46±0.79 mg of sildenafil or 60.76±0.60 mg of tadalafil. The use of such preparations could result in fatal side effects, knowing that the maximum daily dose for sildenafil and tadalafil is 100mg and 20mg, respectively.

Keywords: Herbal preparations; Adulteration; Sildenafil; Tadalafil; Liquid chromatography.

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INTRODUCTION

Despite the huge development and mass production of chemically synthesized drugs, large sections of the population in developing countries still rely on traditional practitioners and herbal medicines for their primary care [1]. This is, in part, due to the perceived effectiveness and safety of herbal medicines. Nevertheless, deleterious effects associated with the consumption of herbal products are disclosed from time to time. The majority of these adverse effects were attributed to different reasons including adulteration with drugs [2, 3]. To date, a plenty of studies worldwide have reported the adulteration of aphrodisiac herbal preparations with PDE-5 inhibitors and their analogues [4-9]. We herein report unprecedentedly detection and determination of undeclared Sildenafil (Figure 1) and tadalafil (Figure 2) in some aphrodisiac herbal preparations marketed in Khartoum City, Sudan.

MATERIALS AND METHODS

Materials

Thirty different herbal products, formulated as tablets, capsules, powder and honey samples, claimed to enhance sexual activity were collected from street, local practitioners (Ataras), honey centers, herbal centers and licensed companies in Khartoum city. Sildenafil working standard (as citrate) was kindly provided by General Medicines Co. (GMC), Sudan. Tadalafil working standard was kindly provided by Azal Pharmaceutical Industry, Sudan. The following chemicals were all obtained from Scharlau, Spain: Methanol Analytical grade; Methanol HPLC grade; Potassium dihydrogen phosphate; Chloroform; Ethyl acetate; N-Hexane; Acetonitrile HPLC grade; Acetone.

Instrumentation

HPLC Shimadzu – Japan consisted of Kromasil 100(4.6 mm × 250mm Column that contains 5 µm packaging L1), UV–Detector (SPD – 10AVP) adjusted at wavelength 290nm and Pump (LC-10Ai).

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Thin-layer chromatography plates (Aluminum sheets 20x20cm pre-coated with silica gel 60F254); Merck- Germany.

**Chromatographic Conditions**

HPLC separation was carried out using the mobile phase composed of a mixture of potassium dihydrogen phosphate (0.05M) pH adjusted to 5.8, acetonitrile and methanol (30: 50: 20) (v / v). The mobile phase was pumped through the column at a flow rate of 1 ml min⁻¹. The solvent systems used in TLC were: N-Hexane: Ethyl acetate: Methanol (8: 6: 2 v/v), Methanol: Chloroform (1: 4 v/v) and Methanol: Ethylacetate (1: 2 v/v). Visualization was accomplished under UV light (254nm-360nm).

**Preparation of Standard Solution for TLC Analysis**

An approximate quantity of 0.4g of either sildenafil citrate or tadalafil working standards was dissolved in methanol or acetone and sonicated for three minutes (solution A).

**Preparation of Sample Solution for TLC Analysis**

An approximate quantity of 0.4g of each sample was suspended in methanol or acetone, sonicated, centrifuged and filtered through 0.45µm syringe filter disc (solution B).

**Sample Preparation for Quantitative Analysis**

**Herbal Products Formulated As Powder and Honey**

An amount equivalent to 0.5g of each sample was suspended in methanol and the volume completed to 20 ml. The solution was sonicated for 30 minutes, centrifuged for 30 minutes at 3500 rpm and then filtered by using 0.45µm syringe filter disc (solution C).

**Herbal Products Formulated As Tablets and Capsules**

The average weight of five tablets or capsules was suspended in methanol and the volume was completed to 20 ml with methanol. The solution was sonicated for 30 minutes, centrifuged for 30 minutes at 3500 rpm and filtered using 0.45µm syringe filter disc. The filtrate was then diluted 5 to 10 v/v by methanol (solution D).

**Procedures**

**Qualitative Analysis of Prepared Sample Solution Using Developed TLC Method**

The three mobile phases were prepared and transferred into different chromatographic tanks; each tank was tightly closed and allowed to equilibrate for one hour. Each TLC plate was spotted with solutions A & B at a distance of 1cm from the bottom of the plate and allowed to dry .The plates were allowed to run upto 8cm height using the selected mobile phases, then dried and
examined under UV at $\lambda_{\text{max}254}$ nm. The retardation factor ($R_f$) for each working standard was used as an identification parameter for any similar $R_f$ values from the samples spots.

**Quantitation of Detected Sildenafil and Tadalafil in the Samples Solution**

**Calibration Curve**

A mixture of sildenafil standard (0.02g) and tadalafil standard (0.02g) was dissolved in methanol and the volume was completed to 100ml. Serial dilutions were made using methanol to obtain 6 solutions of concentrations range (0.064 – 200 µg/ml for each standard). The HPLC procedure was carried out using the mobile phase composed of a mixture of 0.05M potassium dihydrogen phosphate (pH adjusted to 5.8): acetonitrile: methanol (30: 50: 20) (v / v / v). The resultant peak areas were plotted against the standard concentrations to check the linearity.

In order to determine the content of sildenafil or tadalafil in the adulterated samples, solutions C and D were treated as under calibration curve. The resultant peak RT corresponding to either sildenafil or tadalafil were used for the confirmation of their presence as adulterants; on the other hand the peak areas were used for the quantitative estimation of the content of each adulterants depending on either direct sample/standard comparison or from the regression analysis data.

**Validation of the adopted HPLC method**

**Precision**

The Within-day and between-days data were determined for three concentrations within the linearity range. RSD % values were calculated to assess method precision which should be < 2.

**RESULTS AND DISCUSSION**

People, worldwide, hold a false perception about the safety of natural products and therefore prefer the use of herbal preparations rather than the marketed medicines. Several reports have disclosed the adulteration of herbal preparations with one or more of the medicines that are clinically used for the same therapeutic indication of the herbal preparation. In Sudan, the numbers of local practitioners and herbal companies have dramatically increased over the last decade and there have been an increasing demand of herbal products for sexual enhancement. This is greatly evidenced by the large number of different herbal preparations sold in the Sudanese market as alternatives to the PDE-5 inhibitors sildenafil and tadalafil. Thirty different aphrodisiac herbal products, marketed in Khartoum City were collected equally, from streets, local practitioners (Ataras), Honey centers, herbal centers and some companies specialized in herbal preparations. It was first planned to screen those thirty samples for the presence of
sildenafil citrate and/or tadalafil using TLC. Therefore, mixtures of solvents with different polarity and percentages were investigated. A mixture of N-Hexane: Ethyl acetate: Methanol (8:6:2 v/v/v) was first tried for analysis of the 30 samples. Three samples developed spots of a similar retardation factor to that of standard tadalafil (Rf = 0.63), nine samples developed spots of a smaller retardation factor (Rf = 0.16) and no spots were observed for the rest of the samples. Based on standard sildenafil retardation factor ((Rf = 0.16), the second set of above samples were proposed to be adulterated with sildenafil citrate. It was, thus, decided to try more polar system to bring the standard sildenafil citrate spot to a higher Rf. A mobile phase consisted of methanol: chloroform (1:4 v/v) separated sildenafil citrate and tadalafil with Rf values 0.85 and 0.88 respectively. The third mobile phase consisted of methanol: ethylacetate (1:2 v/v) showed the best separation for the developed spots with Rf values 0.43 and 0.83 for sildenafil citrate and tadalafil respectively. We hereby recommended this solvent system for routine TLC analysis of mixtures containing both sildenafil citrate and tadalafil. It is worth noting that TLC spiking test had further confirmed these preliminary findings. Having determined the samples that are adulterated with sildenafil citrate or tadalafil, we next directed our attention to further confirm the presence of these adulterants by HPLC method. To best of our knowledge, there are two reported HPLC methods for analysis of mixtures containing both sildenafil citrate and tadalafil [10, 11]. Out of the mobile phases cited in the reported methods, the mobile phase composition in [11] was found more promising to resolve both analytes from herbal matrix. Therefore, we decided to adopt the reported method [11] using the same mobile phase but changing the column length and introducing an extraction process with methanol. This adoption was expected to improve the resolution of the system through increasing the column efficiency (N), selectivity (α) and capacity factor [12]. Good resolution of both analytes from each other and from herbal matrix was obtained where sharp peaks at retention time 4.2 and 5.8 minutes were assigned to tadalafil and sildenafil citrate respectively (Figure 3).

Fig. 3: Typical HPLC chromatogram for Tadalafil and Sildenafil citrate standards mixture (40µg/ml)
Successfully, the adopted HPLC method was proved to be suitable for separation of mixtures of sildenafil and tadalafil. The adopted HPLC method was then applied for the estimation of the content per dose of sildenafil or tadalafil in the adulterated samples. The adulterated samples showed intractable mixture of products eluted at retention times 2 – 3.5 minutes; peaks at 4.2 and 5.8 minutes which were assigned to the adulterant tadalafil and sildenafil citrate (Figure 4, 5).

At this time, the validation of the adopted method was carried following the ICH guidelines [13]. The adopted method was found to be precise (RSD% < 2) and free from interference from other components of the herbal preparations. Furthermore, linearity test revealed that the adopted method is linear for sildenafil citrate \( R^2 = 0.9998 \) and tadalafil \( R^2 = 1.00 \) at a concentration range of 0.064 – 200 µg/ml. The system suitability tests were greatly evidenced upon analysis of the standard sildenafil citrate and tadalafil chromatograms parameters (Table 1).

**Table 1: Calculated system suitability parameters for the HPLC method (ICH guidelines)**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Number of theoretical plates</th>
<th>HETP (mm)</th>
<th>peak symmetry factor</th>
<th>Capacity factor</th>
<th>chromatographic Resolution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tadalafil</td>
<td>5555.389</td>
<td>22.222</td>
<td>0.93</td>
<td>4.0</td>
<td>5.73</td>
</tr>
<tr>
<td>Sildenafil</td>
<td>5761.946</td>
<td>23.05</td>
<td>1</td>
<td>5.5</td>
<td></td>
</tr>
</tbody>
</table>
Finally, the adopted method was applied in the quantification of the adulterated samples. Our findings revealed that, these samples contained 38.0 – 165.0 mg/dose and 39.0 – 60.7 mg/dose of sildenafil citrate and tadalafil, respectively (Table 2).

**Table 2: Summary of the content of tadalafil and sildenafil per dose in some adulterated samples**

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Dose / g</th>
<th>Weight taken / g</th>
<th>Adulterant</th>
<th>Sildenafil</th>
<th>Content % (w/w) ± RSD%</th>
<th>Content / dose ± RSD%</th>
<th>Tadalafil</th>
<th>Content % (w/w) ± RSD%</th>
<th>Content / dose ± RSD%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.4026</td>
<td>0.5</td>
<td></td>
<td></td>
<td>2.22 ± 0.05</td>
<td>0.1197 ± 0.96</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>9.1533</td>
<td>0.5</td>
<td></td>
<td></td>
<td>0.42 ± 1.04</td>
<td>0.0384 ± 2.94</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>4.7059</td>
<td>0.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.83 ± 1.65</td>
<td>0.039 ± 4.65</td>
</tr>
<tr>
<td>13</td>
<td>20</td>
<td>0.5036</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.29 ± 0.02</td>
<td>0.058 ± 0.02</td>
</tr>
<tr>
<td>24</td>
<td>5</td>
<td>0.5185</td>
<td></td>
<td></td>
<td>1.36 ± 0.003</td>
<td>0.068 ± 0.003</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>0.7426</td>
<td>0.7426</td>
<td></td>
<td></td>
<td>22.3 ± 0.417</td>
<td>0.165 ± 0.79</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>13</td>
<td>0.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.47 ± 0.59</td>
<td>0.0607 ± 0.59</td>
</tr>
</tbody>
</table>

All the positive samples for tadalafil were found to contain over therapeutic dose (> 20 mg/dose) [14]. On the other hand, 56% of sildenafil-positive samples contained over therapeutic dose (> 100 mg/dose) [15]. It is worth noting that all preparations collected from licensed herbal companies (eight preparations) were found to be adulterated with sildenafil citrate or tadalafil. The serious side effects of sildenafil and tadalafil along with their potential drug-drug interactions and/ or drug-diet interactions render consumption of these adulterated herbal preparations very dangerous. It is worth noting that 67% of the adulterated samples are heavily advertised in the media. It, therefore, appeared that the channels delivering herbal preparations to the market are not strictly regulated by the authority body. An advantage of the adopted method is that it can be used for the routine analysis of sildenafil and tadalafil in pharmaceutical formulations using either drug as an internal standard for the other.

**CONCLUSION**

In this study, TLC and adopted HPLC method were used for identification and quantification of sildenafil citrate and tadalafil in 30 herbal preparations marketed in Khartoum City. Twelve preparations were adulterated with sildenafil citrate and tadalafil and 50% of these preparations were found to be overdosed, putting the health of patients at stake. The findings of the current studies clearly revealed that the manufacturers have clandestine intention to deceive and satisfy customers who are seeking for better sexual performance. These manufacturers also misguide public through objectionable advertisements via both print and electronic media. Thus, to protect
herbal consumers form unethical practicing, more effort should be elicited by the authorized persons to make the present regulations effective and to set new rules, if necessary.

REFERENCES


