VALIDATED SPECTROPHOTOMETRIC METHOD TO DETERMINE VARDENAFIL AND SILDENAFIL IN PHARMACEUTICAL FORMS USING POTASSIUM IODIDE AND POTASSIUM IODATE

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ABSTRACT

Objective: To develop and validate simple, sensitive, precise and free of organic solvents method for the determination of sildenafil (SIL) and vardenafil (VAR) in bulk and pharmaceutical formulation.

Methods: The method is based on the reaction of studied drugs with a mixture of potassium iodide and potassium iodate in an aqueous medium at (25±2 °C) to form yellow coloured triiodide ions (I$_3^-$) within 45 min. The reaction is followed spectrophotometrically by measuring the absorbance at 288, 351 nm and 285, 351 nm for sildenafil and vardenafil respectively.

Results: The effects of analytical parameters on the reported systems were investigated. Beer’s law of SIL was obeyed in the range of (0.4-12) μg ml$^{-1}$ and (0.6-16) μg ml$^{-1}$ . Molar absorptivity was found to be (67.659 ×10$^{3}$) l mol$^{-1}$ cm$^{-1}$ at 285 nm, 351 nm respectively. Beer’s law of VAR was obeyed in the range of (0.2-13) μg ml$^{-1}$ and (0.5-40) μg ml$^{-1}$ . Moreover, molar absorptivity’s were found to be (68.719 ×10$^{3}$) l mol$^{-1}$ cm$^{-1}$ at 285 nm, 351 nm respectively.

Conclusion: The proposed method has been applied to determine the components in dosage forms with an average recovery of 98.15% to 103.45% and the results have been found in good agreement with those results obtained by the reference methods.

Keywords: Sildenafil citrate, Vardenafil hydrochloride, Spectrophotometry, Potassium iodide, Potassium iodate, Triiodide ion

INTRODUCTION

Erectile dysfunction (ED) is one of the most common chronic diseases affecting men and its prevalence increases with aging. It is also the most frequently diagnosed sexual dysfunction in the older male population. Treatment of erectile dysfunction is based on phosphodiesterase type 5 (PDE-5) inhibitors including sildenafil (SIL), and vardenafil (VAR). PDE-5 inhibitors have high efficacy and safety rates, even in difficult-to-treat populations such as patients with diabetes mellitus [1]. Sildenafil citrate was the first drug approved for the treatment of ED in 1998. The United States Food and drug administration (FDA) approved tadalafl and vardenafil hydrochloride in 2003 [2].

There are several studies in medical literature reporting the determination of sildenafil citrate in pharmaceuticals, plasma samples, herbal drugs or dietary supplements using liquid chromatography method [1-7], electroanalytical methods [8, 9]gas chromatography [10], Thin layer chromatography [11], capillary electrophoresis [12] and capillary chromatography [13-15]. Many spectrophotometric Methods have also been reported [16-22].

As same as, determination of VAR in bulk, tablet dosage forms, and biological fluids were analyzed using different analytical systems, including HPLC [3, 23-25], capillary electrophoresis [26] capillary chromatography [27] LC-MS [28], and spectrophotometric methods [29, 30]. In the previous studies, the spectrophotometric determination of SIL and VAR depend on UV spectrophotometric method, oxidation of the drug and complex formation. Most of these methods demand organic solvents or organic reagents, unlike the proposed method.

The aim of the present study was to report a new, simple, and environment-friendly method to determination SIL and VAR as raw materials and in pharmaceutical preparations.

MATERIALS AND METHODS

Apparatus

Uv-visible spectrophotometer (JASCO, model V650, Japan) with 1.00 cm quartz cells. Ultrasonic processor (Power sonic, model 405, Korea) was used to sonicate the sample solutions. Adjustable micro-pipettes covering a volume range from 2 to 5000 μl (ISO-LAB, Germany), used for the preparation of the experimental solutions. Analytical balance (Crison, model 2474, Germany). pH meter (Crison, GLp21/Eu, Spain).

Chemicals and reagents

Pharmaceutical grade sildenafil (99%) and vardenafil (99.5%) were received from XUHUANG, CHINA. Potassium iodate and potassium iodide (Panreac, Germany). All reagents and solvents were of analytical grade. Stock standard solution (1 mg ml$^{-1}$) of SIL and VAR was prepared by dissolving 25 mg from each of SIL and VAR in double distilled water and diluting to 25 ml with double distilled water. 0.15 M of potassium iodate and 0.2 M of potassium iodide solutions were prepared by dissolving the accurately weighed amount of the pure solid in double distilled water. All other chemicals and reagents were of analytical grade and all solutions were prepared with double distilled water. All solutions are stable for a period of 2 d when stored at (4 °C).

General procedure

Increasing volumes of SIL or VAR working standard solution were transferred into series of 10 ml volumetric flasks that contain 2.5 ml of KI (0.2 M) and 1 ml of KI03 (0.15 M) for SIL and 3 ml of KI (0.2 M) and 1.0 ml of KI03 (0.15M) for VAR. The volume was made up to the mark with distilled water and the absorbance was measured after 45 min at 288, 351 nm and 285, 351 nm against a similar reagent blank for SIL and VAR respectively. The standard calibration plot was prepared to calculate the amount of the analyzed drug in bulk samples. All measurements were carried out at room temperature (25±2 °C).
Procedure for pharmaceutical formulations

Twenty individual tablets were weighed and pulverized carefully. An accurately weighed amount of the powder equivalent to 50 mg of SIL or VAR was transferred into 50 ml volumetric flask and dissolve in 50 ml of water. The content of the flask was sonicated for 30 min then diluted to the volume with water. A portion of this solution was centrifuged for 15 min at 5000 rpm then a suitable volume of the supernatant was transferred into 10 ml volumetric flask, and the procedure continued to use for the analysis of SIL/VAR by the spectrophotometric method after 45 min.

RESULTS AND DISCUSSION

Absorption spectra

Iodide ions convert to free iodine in an acidic medium of VAR or SIL, the acidity comes from HCl or HClO3; then free iodine reacts with a surplus of iodide ions to form yellow complex from triiodide (I3-) [31].

\[ \text{IO}_3^- + 5 \text{I}^- + 6 \text{H}^+ \rightarrow 2 \text{I}_2 + 3 \text{H}_2\text{O} \]

Optimization of reaction conditions

The optimum conditions for the development of method were established by varying one parameter at a time and keeping the others fixed and observing the effect produced on the absorbance of the coloured products.

A volume of 2.5 ml of 0.2 M KI, 1.0 ml of 0.15 M KIO3 against reagent, Blank, (3) SIL against distilled water

Application to the pharmaceutical dosage forms

The proposed procedures were applied to determine the studied substances in their pharmaceutical formulations. The results in table 3 indicate the high accuracy and precision. As can be seen from table 3, the proposed method has the advantages of being virtually free from interferences by excipients and common degradation products. The results obtained were compared statistically by the student's t-test (for accuracy) and the variance ratio F-test (for precision) with those obtained by the reference methods [3, 23] on samples of the

Table 1: Analytical parameters of spectrophotometric methods

| parameters                          | SIL     | 351 nm | VAR     | 285 nm | 351 nm |
|------------------------------------|---------|--------|---------|--------|--------|
| Linear range μg ml⁻¹               | 0.4-12  | 0.6-16 | 0.2-13  | 0.5-40 |
| ε L mol⁻¹ cm⁻³                     | 67.659×10⁵ | 37.955×10³ | 68.719×10⁴ | 26.691×10³ |
| Detection limit μg ml⁻¹             | 0.07    | 0.09   | 0.04    | 0.08   |
| Limit of quantification μg ml⁻¹     | 0.25    | 0.3    | 0.13    | 0.25   |
| Regression equation                | *(A = m C + b)* | m=0.099 | m=0.052 | m=0.109 | m=0.044 |
|                                    | b=0.002 | b=0.011 | b=0.012 | b=0.005 |
| Correlation coefficient            | 0.9996  | 0.9988 | 0.9985  | 0.9994 |

*With respect to A=mC+b, where C is the concentration (μg ml⁻¹) and A is the absorbance.
same batch (table 3). The values of t- and F-tests obtained at 95% confidence level and four degrees of freedom did not exceed the theoretical tabulated value indicating that no significant difference between the proposed method and references method.

**Table 2: Accuracy and precision of the determination of SIL and VAR in bulk powder by the proposed method**

| Drug | $\lambda_{max}$ (nm) | Mg/ml | Found | Found±S. D* | RSD (%) | Recovery % |
|------|----------------------|-------|-------|-------------|---------|------------|
| SIL  | 288                  |       |       |             |         |            |
|      | 0.4                  | 0.403 | 0.403±0.015 | 3.69 | 100.80          |
|      | 2                    | 2.014 | 2.014±0.039 | 1.94 | 100.70          |
|      | 8                    | 8.056 | 8.056±0.113 | 1.40 | 100.70          |
|      | 12                   | 12.130| 12.130±0.140 | 1.15 | 101.08          |
|      | 0.6                  | 0.595 | 0.595±0.025 | 4.21 | 99.16           |
|      | 4                    | 4.067 | 4.067±0.112 | 2.75 | 101.67          |
|      | 10                   | 10.044| 10.044±0.192 | 1.91 | 100.44          |
|      | 16                   | 16.050| 16.050±0.250 | 1.56 | 100.31          |
|      | 351                  |       |       |             |         |            |
|      | 0.2                  | 0.207 | 0.207±0.027 | 4.40 | 103.45          |
|      | 1                    | 1.026 | 1.026±0.047 | 4.58 | 102.60          |
|      | 8                    | 8.132 | 8.132±0.130 | 1.60 | 101.65          |
|      | 13                   | 12.950| 12.950±0.209 | 1.61 | 99.615          |
|      | 0.5                  | 0.501 | 0.501±0.021 | 4.26 | 100.29          |
|      | 4                    | 4.092 | 4.092±0.125 | 3.05 | 102.00          |
|      | 20                   | 19.631| 19.631±0.228 | 1.16 | 98.15           |
|      | 40                   | 40.307| 40.307±0.363 | 0.90 | 100.76          |

*Average of five determinations, **Standard deviation, ***Relative standard deviation.

**Table 3: Determination of SIL and VAR in their pharmaceutical preparations using the proposed and reference methods**

| Formula     | Drug | Claim (mg/tab) | Found%±SD* | Proposed method $\lambda_{max}$ (nm) | Reference method [3] |
|-------------|------|----------------|-------------|--------------------------------------|----------------------|
| VIGRAVID    | SIL  | 25             | 25.14±1.17  | 25.06±1.92                          | 25.19±0.52           |
|             |      | 50             | 50.14±0.90  | 50.75±1.45                          | 50.33±0.92           |
|             |      | 100            | 101.98±0.49 | 101.15±1.92                         | 101.35±0.89          |
| FASTFIX     | VAR  | 10             | 9.93±0.67   | 9.96±1.97                           | 9.96±0.72            |
|             |      | 20             | 19.86±1.17  | 20.17±1.03                          | 20.01±0.40           |

* Average of five determinations, (four degrees of freedom), At 95% confidence level-value is 2.776 and F-value is 6.26, **Supplied by NCPI products- Syria, ***Supplied by UNIPHARMA products, Syria.

**DISCUSSION**

In this study, the developed spectrophotometric method is free of organic solvents [16-20, 22, 29], rapid, and has more sensitivity than previous methods [16-20, 22, 29, 30]. The proposed method can be carried out without any organic solvents or reagents in contrast with many previous spectrophotometric methods.

A comparative summarized study between the proposed methods and previous spectrophotometric methods for determination of SIL and VAR has been provided in table 4 and table 5 respectively.

**Table 4: Comparison of the proposed methods with the existing spectrophotometric methods for the determination of Sildenafil citrate**

| Reagent          | Spectrophotometric Method | Solvent       | Temp. °C | Concentration range, µg/ml | Extinction, ε mol/cm | Reference |
|------------------|---------------------------|---------------|----------|---------------------------|----------------------|-----------|
| iodine           | direct                    | dichloroethane| 25±1     | 15.0-160                  | 3.75×10<sup>2</sup>  | [16]      |
| TCNQ             | direct                    | acetonitrile  | 50±2     | 15.0-220                  | 2.58×10<sup>4</sup>  | [16]      |
| DDQ              | direct                    | methanol      | 50±2     | 20.0-260                  | 2.41×10<sup>4</sup>  | [16]      |
| TCNE             | direct                    | acetonitrile  | 50±2     | 10.0-210                  | 3.05×10<sup>4</sup>  | [16]      |
| TFN              | direct                    | dichloroethane| 60±2     | 15.0-240                  | 2.25×10<sup>4</sup>  | [16]      |
| chloranilic acid | direct                    | acetonitrile  | 60±2     | 20.0-180                  | 3.26×10<sup>4</sup>  | [16]      |
| chloranil        | direct                    | acetonitrile  | 60±2     | 28.0-150                  | 3.42×10<sup>4</sup>  | [16]      |
| bromanil         | direct                    | methanol      | 60±2     | 15.0-170                  | 2.90×10<sup>4</sup>  | [16]      |
CONCLUSION

The proposed analytical procedures were simple, rapid, accurate and precise, so it can be used for the routine analysis of SIL and VAR in bulk and pharmaceutical formulations. The sample recoveries from all formulations have good agreement with their respective label claims, which suggested non-interference with formulations excipients in the assay. Moreover, the present method is environment-friendly because it does not need any organic reagents or solvents; it is free extractive and very sensitive comparing with other spectrophotometric methods.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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Table 5: Comparison of the proposed methods with the existing spectrophotometric methods for the determination of vardenafil hydrochloride

| Reagent | Spectrophotometric method | Solvent | Temp. °C | Concentration rang. µg/ml | ε lmol/cm | Reference |
|---------|--------------------------|---------|----------|---------------------------|-----------|-----------|
| BCG     | extractive               | chloroform | 25±2     | 1.2-25.0                  | 1.58×10⁴  | [17]      |
| CCR     | extractive               | chloroform | 25±2     | 1.5-60.0                  | 9.79×10³  | [17]      |
| chromotrope 2B | extractive       | Methylene | 25±2     | 3.3-87.0                  | 1.02×10⁴  | [17]      |
| chromotrope 2R | extractive           | Methylene | 25±2     | 3.3-96.0                  | 8.30×10³  | [18]      |
| 3-phenylazo-6-o-carboxyphenylazo-chromotropic acid | extractive | Methylene | 25±2     | 5.0-115.0                 | 6.83×10³  | [18]      |
| bis-3,6-(o-hydroxyphenylazo)-chromotropic acid | extractive | Methylene | 25±2     | 2.5-125.0                 | 5.42×10³  | [18]      |
| bis-3,6-(p,N,N-dimethylphenylazo)-chromotropic acid | extractive | Methylene | 25±2     | 8.3-166.7                 | 3.35×10³  | [18]      |
| 3-phenylazo-6-o-carboxyphenylazo-chromotropic acid | extractive | Methylene | 25±2     | 0.8-15.0                  | 2.32×10⁴  | [18]      |
| BTB     | direct                   | chloroform | 40±2     | 1.0-40.0                  | 20.12×10³ | [19]      |
| PBP     | direct                   | chloroform | 25±2     | 1.0-50.0                  | 44.40×10³ | [19]      |
| EPPR    | direct                   | chloroform | 25±2     | 3.0-70.0                  | 24.00×10³ | [19]      |
| MCP     | direct                   | dichloromethane | 25±2 | 1.3-50.0                 | 2.28×10⁴  | [22]      |
| BCG     | direct                   | dichloromethane | 25±2 | 0.8-27.0                 | 3.05×10⁴  | [22]      |
| BTB     | direct                   | water     | 25±2     | 0.4-12                   | 6.76×10⁴  | [22]      |
| Proposed method | direct          | water     | 25±2     | 0.4-16                   | 3.795×10⁴ | [22]      |

We can see this is the unique colorimetric method does not need any organic compound to determine SIL and VAR.
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