Utility of Ion-associate Formation Reactions for the Spectrophotometric Determination of Sildenafil Citrate in Pure form and in Virecta Tablets

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Abstract

A simple, rapid and sensitive extractive spectrophotometric method has been developed for the assay of sildenafil citrate (SILC) in pure and pharmaceutical formulations (Virecta tablets). This method is based on the formation of chloroform soluble ion-pair of SILC with bromothymol blue (BTB) and methylene chloride soluble ion-pair of SILC with bromophenol blue (BPB) and eriochrome blue black R (EBBR) in borax buffer of pH 3 and volume 1mL for BTB while acetate buffer of pH 3 and volume 1mL for BPB and universal buffer of pH 2 and volume 1.5 mL for EBBR with absorption maximum at 415, 420 nm and 510 nm for BTB, BPB and EBBR reagents, respectively. Reaction conditions were optimized to obtain the maximum colour intensity. The absorbance was found to increase linearly with the increase in SILC concentration, which was corroborated by the calculated correlation coefficient values (0.9909, 0.9901 and 0.9917 for BTB, BPB and EBBR reagents, respectively). The systems obeyed Beer’s law over the concentration range of 1-40, 1-50 and 3-70 μg mL$^{-1}$ for BTB, BPB and EBBR, respectively. Various analytical parameters have been evaluated and the results have been validated by statistical data. No interference was observed from common excipients present in pharmaceutical formulations.

Keywords: Virecta tablets; Extractive spectrophotometric determination; Ion-association complex

Introduction

Sildenafil, (5-[2-ethoxy-5-(4-methylpiperazin-1-ylsulfonyl) phenyl]-1-methyl-3 propyl-1, 6-dihydro-7H-pyrazolo [4, 3-d] pyrimidin-7-one), Figure 1 [1], is a potent and competitive inhibitor of the type-V cGMP specific phosphodiesterase enzyme, the predominant isoenzyme in the human corpus cavernosum. Its formula is C$_{28}$H$_{38}$N$_{6}$O$_{11}$S and its molecular mass: base: 474.6 g mol$^{-1}$; citrate: 666.7 g mol$^{-1}$. Sildenafil enhances relaxation of the corpus cavernosal smooth muscle, which in turn increases blood flow into the cavernosal spaces, thus leading to increased intracavernosal pressure, a key factor in producing an erect penis [2,3]. SILC; sold under the names Viagra and Revatio and under various other names, was a drug used to treat male erectile dysfunction (impotence) and pulmonary arterial hypertension (PAH). However, the introduction of sildenafil resulted to its widespread use as well as its abuse. Therefore, precise, sensitive and simple method for the determination of this drug is widely required. Several methods have been developed for this purpose. Pitos et al. [4] have proposed a HPLC method for determination of sildenafil and its active metabolite (N-desmethyl sildenafil) in human blood. Determination of sildenafil citrate in human plasma [5-8] and in pharmaceutical formulations [9-14] using chromatographic methods have been reported.

No official or pharmacopoeial method has been reported for the assay of sildenafil citrate in its formulations. Reports have been appeared describing accurate electroanalytical [15-23] and spectrophotometric [24-32] techniques for quantification and stability assay of SILC. Most of these methods are expensive, suffer from lack of selectivity and require careful control of conditions and considerable time for routine control analysis.

Therefore, precise, sensitive and simple method for the quantification of SILC in pharmaceutical preparations is required. Extractive spectrophotometric procedures are popular for their sensitivity in the assay of drugs and, therefore, ion-pair extractive spectrophotometry has received considerable attention for the quantitative determination of many pharmaceutical compounds. The present communication describes an extractive spectrophotometric procedure for the assay of SILC in pure form and in its formulations, which are based on the formation of ion-pairs with bromothymol blue (BTB), bromophenol blue (BPB) and eriochrome blue black R (EBBR) in acidic buffer.

Experimental

Reagents and materials

All chemicals and reagents used were of analytical reagent grade and some of them were used as such without any further purification. They included sildenafil citrate (SILC) which was provided by EVA

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Experimental

Reagents and materials

All chemicals and reagents used were of analytical reagent grade and some of them were used as such without any further purification. They included sildenafil citrate (SILC) which was provided by EVA.
Pharma Company for Pharmaceutical Industry. Dyestuffs included bromothymol blue (BTB), bromophenol blue (BPB) and eriochrome blue black R (EBBR) (were purchased from Win lab, U.K).

Hydrochloric acid was supplied from Merck. While chloroform, methanol, acetone, methylene chloride and ethylene chloride were supplied from El-Nasr Company, Egypt. Potassium chloride, borax, acetic acid, phosphoric acid and boric acid were supplied from El-Nasr Company for Chemicals (Egypt).

Virecta was manufactured by EVA Pharma for Pharmaceuticals and Medical Appliances, Egypt. Each F.C. tablet contains: Sildenafil (as citrate) (2.473 g) in one liter bidistilled water.

Universal buffer solutions of different pH values ranged from 2 to 6 were prepared by adjusting 100 mL solution of the acid mixture to the desired pH value using 0.1 N NaOH solution. Borax and acetate buffer solutions were prepared using the recommended method [33].

Apparatus

Prior to analysis, all glassware used were washed carefully with distilled water and dried in the oven before use.

The spectrophotometric measurements were carried out using the manual Unico 1200 spectrometer (United Products and Instruments, Inc.) in the wavelength range from 190-1000 nm and quarts cell of 1cm optical length was used. Small volumes were taken using automatic pipettes Socorex Swiss (50-200 µL).

Assay procedure for pure drug

In a 10 mL calibrated volumetric flask, 0.5-1 mL of 0.05% w/v BTB, BPB or EBBR solutions were added to 0.1- 0.5 mL of SILC solution (1 mg mL \(^{-1}\)), then 1 mL universal buffer (pH = 2), borax buffer (pH = 3) and acetate buffer (pH = 3) were added in case of BTB, BPB and EBBR, respectively. The volume was completed to the mark with distilled water. The mixture had been left for 10, 15-20 and 10 minutes for BTB, BPB and EBBR, respectively, and mixed well in a 30 mL separating funnel then shaken well with chloroform twice (in case of BTB or BPB) or once (in case of EBBR) with 5 mL portions for extracting the ion-pair after shaking well for 1 min. The organic layer was collected in 10 mL measuring flask and the absorption spectra of the resulting solutions were scanned in the wavelength range from 320-600 nm versus chloroform or methylene chloride as blank solution, from which the optimum wavelength for each ion pair was selected.

Assay procedure for virecta tablets

Ten tablets were ground well. A portion of tablets powder equivalent to 100 mg of SILC drug was weighed, then dissolved in the minimum volume of methanol. The solution mixture was shaked in a mechanical shaker and filtered and then transferred accurately to 100 mL measuring flask, completed to the mark with methanol.

Using different concentrations of SILC with BTB, BPB and EBBR reagents which were prepared and the procedure was carried out as mentioned before. The ion-pairs were collected in 10 mL measuring flask. Small volumes were taken using automatic pipettes Socorex Swiss (50-200 µL).

Results and Discussion

Since the analyte is a citrate salt of sildenafil, sildenafil was considered only for further discussion. Sildenafil containing basic functional groups with a pK\(_a\) value of 8.7 has a weak acidic moiety. In the substituted and fused rings of pyrimidine and pyrazol, protonation is very difficult due to resonance and steric effects. Therefore, the only site in sildenafil vulnerable for protonation is the nitrogen bonded to electron donating methyl group in the pipazenerine ring [24]. It was observed that the anionic dyestuff reagents namely BTB, BPB and EBBR, form ion-pairs with the positively charged sildenafil drug. The drug-dye stoichiometric ratio was determined by Job’s continuous variation method and molar ratio methods. It was found to be 1:1 with BTB, BPB and EBBR reagents (Figure 2). Each drug-dye complex, with two oppositely charged ions, behaves as a single unit held together by
weak electrostatic forces of attraction [24,36]. Based on these findings, we propose a probable reaction mechanism for the formation of the ion pairs as shown in Scheme 1.

SILC reacts with BTB, BPB and EBBR reagents in acidic buffer to give chloroform soluble ion-pair with BPB and methylene chloride soluble ion-pairs with BTB and EBBR reagents. The ion-pairs exhibit absorption maxima at 415, 410 and 510 nm for BTB, BPB and EBBR reagents, respectively (Figure 3). Under the experimental conditions, the reagents blank showed negligible absorbance thereby permitting good analytical conditions for quantitative determination of SILC.

**Optimization of reaction conditions**

Optimum reaction conditions for quantitative determination of ion-pairs were established via various preliminary experiments.
has no effect on the formation and stability of the ion pairs using BPB and EBBR reagents. The absorbance is generally increased by increasing the temperature and reached a maximum value at 40 °C using BTB reagent. The temperature is slightly increased or decreased above this temperature. Therefore, the room temperature is chosen as the best temperature with BPB and EBBR, and 40 °C with BTB for determination of the drug under study in pure and in pharmaceutical formulations.

The effect of time on the formation and stability of the ion-pairs is studied carefully. The absorbance values remain almost unchanged with the increase of time. The optimum time for the completion of the reaction of SILC with BTB, BPB and EBBR reagents is 10, 15-20 and 10 minutes, respectively. The results indicate that ion–pairs need the mentioned time for their complete formation.

Validation of the method

Validity of beer’s law: Spectrophotometric determination of SILC drug is carried out under the favourable conditions of acidity, suitable buffer, buffer concentration, reagent concentration, time, temperature, ratios, wavelength and extracting solvent.

The results of determination of the drug under investigation are shown in Table (1). The validity of Beer’s law for the formed ion-pairs through the reaction of the drug under study with BTB, BPB and EBBR reagents is studied under optimum experimental conditions.

The calibration curves are rectilinear over the concentration range of 1-40, 1-50 and 3-70 µg mL⁻¹ for the drug using BTB, BPB and EBBR reagents, respectively. The mean recovery values obtained amount in the range of 100.0-101.0, 98.00-101.5 and 98.50-101.6% for SILC drug using BTB, BPB and EBBR reagents, respectively. The obtained results indicate the success of the applied procedure in the determination of the studied drug in pure form.

The analytical parameters namely the molar absorptivity (ε), Sandell sensitivity (S), limits of detection (LOD) and quantitation (LOQ), also the regression equation for the drug are summarized in (Table 1).

The low values of the calculated standard deviation and relative standard deviation (SD = 0.015-0.058, 0.04-0.062 and 0.02-0.06, RSD = 0.07-0.29, 0.20-0.31 and 0.10-0.30 % using BTB, BPB and EBBR reagents, respectively), indicate the high accuracy and precision of the proposed method. This is supported also by the calculated values of Sandell sensitivity of 0.0033, 0.0150 and 0.0277 µg cm⁻² using BTB, BPB and EBBR reagents, respectively. The limits of detection and quantitation are calculated and the data obtained are listed in Table (1). The obtained data reflect the sensitivity of dyestuffs reagents (BTB, BPB and EBR) to the SILC drug determination. The correlation coefficients of the data obtained are found to be 0.9909, 0.9901 and 0.9917 for SILC drug using BTB, BPB and EBBR reagents, respectively.

It is concluded that BTB, BPB and EBBR reagents can be applied successfully for the determination of SILC drug in the concentration ranges mentioned above with high accuracy, precision and sensitivity, as indicated by the values of SD, RSD and Sandell sensitivity.

Between day measurements: The validity and applicability of the proposed dyestuff reagents and the reproducibility of the results obtained can be further proved by carrying out four replicate experiments at three concentrations of SILC drug. Table (2) shows the values of between-day relative standard deviations for different concentrations of the drug obtained from experiments carried out over different concentrations of the reagents.
Table 3: Determination of SILC in Virecta tablet using BTB, BPB and EBBR reagents.

| Reagent | Solvent | A

max (nm) | Concentration range (µg mL

-1) | ε L mol

-1 cm

1 | Reference |
|---|---|---|---|---|---|
| BTB | Chloroform | 415 | 1.2–25.0 | 1.58×10

4 | [24] |
| CCR | Chloroform | 460 | 1.5–60.0 | 9.79×10

3 | [24] |
| chromotrope 2B | Methylene chloride | 540 | 3.3–67.0 | 1.02×10

4 | [25] |
| chromotrope 2R | Methylene chloride | 520 | 3.3–96.0 | 8.30×10

3 | [25] |
| bis-3,6-(o-carboxyphenylazo)-chromotropic acid | Methylene chloride | 540 | 5.0–115.0 | 6.83×10

3 | [25] |
| bis-3,6-(oxydihydroxyphenylazo)-chromotropic acid | Methylene chloride | 570 | 2.5–125.0 | 5.42×10

3 | [25] |
| bis-3,6-(p,N,N-dimethylphenylazo)-chromotropic acid | Methylene chloride | 600 | 8.3–166.7 | 3.35×10

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| 3-phenylazo-6-o-hydroxyphenylazo-chromotropic acid | Methylene chloride | 575 | 0.8–15.0 | 2.32×10

3 | [25] |
| Iodine | 1,2-Dichloroethane | 366 | 15–160 | 3.75×10

3 | [26] |
| TCNQ | Acetonitrile | 841 | 15–220 | 2.58×10

3 | [28] |
| DDQ | Methanol | 460 | 20–260 | 2.41×10

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| TCNE | Acetonitrile | 415 | 10–210 | 3.05×10

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| TNF | 1,2-Dichloroethane | 412 | 15–240 | 2.29×10

3 | [28] |
| Chloranilic acid | Acetonitrile | 529 | 20–180 | 3.26×10

3 | [28] |
| Chloranil | Acetonitrile | 550 | 28–150 | 3.42×10

3 | [28] |
| Bromalin | Methanol | 455 | 15–170 | 2.90×10

3 | [28] |
| Congo Red | | 523 | 0.2-7.0 µg/mL | | [31] |
| Sudan 11 | | 554 | 0.2-7.0 µg/mL | | [31] |
| Gentian Violet | | 569 | 0.2-7.0 µg/mL | | [31] |
| Methylene blue | Aqueous medium | 600 | Up to 10.6 | 3.00×10

4 | [40] |
| Ethyl eosin | Aqueous medium | 520 | 1.3–3.3 | 2.44×10

4 | [41] |
| BTB | Methylene chloride | 415 | 1–40 | 2.01×10

3 | Proposed method |
| BPB | Chloroform | 410 | 1–50 | 4.44×10

4 | Proposed method |
| EBBR | Methylene chloride | 510 | 3–70 | 2.40×10

4 | Proposed method |

Table 4: Comparative studies between the proposed and previously reported spectrophotometric methods for determination of SILC.

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Conclusion

Unlike the gas chromatographic and HPLC procedures, the
instrument is simple and affordable. The importance lies in the chemical reactions upon which the procedures are based rather than upon the sophistication of the instrument. This aspect of spectrophotometric analysis is of major interest in analytical pharmacy since it offers distinct possibility in the assay of a particular component in complex dosage formulations. The reagents utilized in the proposed methods are cheaper, readily available and the procedures do not involve any critical reaction conditions or tedious sample preparation. The method is unaffected by slight variations in experimental conditions such as pH and reagent concentration. Moreover, the methods are free from interference by common additives and excipients. The wide applicability of the new procedures for routine quality control is well established by the assay of SLC in pure form and in pharmaceutical preparations.

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