CHAPTER II

Bromocresol purple as an analytical reagent for spectrophotometric determination of sildenafil citrate (Viagra) in pharmaceutical formulations

Abstract

A simple, rapid and sensitive extractive spectrophotometric method has been developed for the assay of sildenafil citrate (SC) in pure and pharmaceutical formulations. The method is based on the formation of chloroform soluble ion-association complex of SC with bromocresol purple (BCP) in NaOAc- HCl buffer of pH 3.5 with absorption maximum at 406 nm. Reaction conditions were optimized to obtain the colored species of maximum stability and intensity. The absorbance was found to increase linearly with increase in concentration of SC, which was corroborated by the calculated correlation coefficient value (0.9993). The system obeyed Beer's law in the range of 0.9-15 μg/ml. 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. The utility of proposed method was examined. The results were compared by means of Student t-test and F-test with those of the reported method.

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GENERAL DRUG PROFILE

Sildenafil citrate (SC)

Chemical name
1-{[3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1H-pyrazole [4,3-d] pyrimidin-5-yl)-4-ethoxyphenyl] sulfonyl}-4-methylpiperazine citrate

Structure

Molecular formula
C_{22}H_{30}N_{6}O_{4}S.C_{6}H_{9}O_{7}

Molecular weight
666.7

Melting point
189 – 190 °C

Description
Crystalline, white to faint tan powder. May discolor on exposure to air and light.

Solubility
Readily soluble in water (3.5 mg/ml) and sparingly soluble in organic solvents

Category
Anti-impotent
INTRODUCTION

SC was synthesised by Teritt. Many workers have reported the activity of SC as an efficacious, orally active agent for the treatment of male erectile dysfunction. The physiological mechanism of erection of the penis involves release of nitric oxide (NO) in the corpus cavernosum during sexual stimulation. Nitric oxide then activates the enzyme guanylate cyclase, which results in increased levels of cyclic guanosine monophosphate (cGMP), producing smooth muscle relaxation in the corpus cavernosum and allowing inflow of blood. SC has no direct relaxant effect on isolated human corpus cavernosum, but enhances the effect of NO by inhibiting phosphodiesterase type 5 (PDES), which is responsible for degradation of cGMP in the corpus cavernosum. When sexual stimulation causes local release of NO, inhibition of PDES by SC causes increased levels of cGMP in the corpus cavernosum, resulting in smooth muscle relaxation and inflow to the corpus cavernosum.

SC is not official in any of the pharmacopoeia. Literature mentions only a few spectrophotometric methods for the determination of SC. Amin and El-Beshbeshy have described a spectrophotometric method of determination of SC in pharmaceutical formulations. This method involves heating at 60 ± 2 °C for 10-15 min, applicable for its assay in the concentration range of 10-260 μg/ml and has low sensitivity (ε =2.25-3.75 x 10³ l/mol/cm). Dinesh et al have reported extractive spectrophotometric methods for the assay of SC in dosage forms. These methods have low sensitivity (ε=0.979-1.58 x10⁴ l/mol/cm) and are stable for 2 h only. Recently, Salem has proposed three spectrochemical
methods for the assay of SC in bulk powder and in pharmaceutical 
formulations. \textsuperscript{6} Electrochemical oxidation of SC on carbon electrodes has been 
studied and the measurement of the peak current enabled for rapid 
determination of SC in pharmaceutical preparations. \textsuperscript{7} In addition to these, 
several HPLC methods \textsuperscript{8-15} have also been reported for the assay of SC. The 
thorough literature survey indicated that no attempt has been made to study the 
reaction of SC with bromocresol purple (BCP). The proposed method is more 
sensitive ($\varepsilon = 3.28-12.08 \times 10^4 \ \text{mol/cm}$) and stable (for more than 8 h) 
compared to reported spectrophotometric methods. The method is based on the 
formation of chloroform soluble ion-association complex of SC with BCP in 
NaOAc-HCl buffer of pH 3.5.

**EXPERIMENTAL**

**Reagent**

Aqueous solution of BCP (0.1 %) was employed in the study.

**Standard solution of drug**

A stock solution of SC containing 250 $\mu$g/ml was prepared in distilled 
water. The solution was observed to be stable at room temperature.

**Buffers**

The following buffers were prepared using the standard methods \textsuperscript{16-18}

1. KCl-HCl buffers of pH 1.0-2.2 (by mixing appropriate volumes of 0.2 M 
each of KCl and HCl).
2. NaOAc-HCl buffers of pH 0.65-5.2 using 1M each of NaOAc and HCl.
3. NaOAc-AcOH buffers of pH 3.6-5.6 (by mixing appropriate volumes of 0.2 M each of NaOAc and AcOH).
4. Potassium hydrogen phthalate-HCl buffers of pH 2.2-3.6 from 0.1 M each of potassium hydrogen phthalate and HCl.

RECOMMENDED PROCEDURES

After a detailed and systematic study of the various parameters involved in the formation of ion-association complex (as described under results and discussion), the following procedure was recommended for the determination of SC.

Analysis of pure drug (Bulk sample)

Aliquots of the solution containing 10-150 μg of SC were transferred into a series of 125 ml separating funnels. A volume of 1 ml of NaOAc-HCl (Walpole buffer) buffer of pH 3.5 and 5 ml of BCP were added. Chloroform (10 ml) was added to the separating funnels, the contents were shaken well and left at room temperature for a minute. The two phases were allowed to separate and the chloroform layer was passed through anhydrous sodium sulphate. The absorbances of yellow colored complex were measured at 406 nm against the reagent blank. A calibration graph was plotted.
Assay procedure for tablets

Six tablets were weighed and powdered. An amount of the powder equivalent to 25 mg of SC was weighed into a 100 ml beaker containing about 70 ml of distilled water. Using a mechanical stirrer, the powder was completely disintegrated. It was transferred into a 100 ml volumetric flask and diluted to the mark with distilled water. It was then filtered through a Whatman filter paper No. 40 to remove the insoluble. A volume of 25 ml of the filtrate was diluted to 100 ml and a suitable aliquot was taken and analyzed using the procedure given above.

RESULTS AND DISCUSSION

Extractive spectrophotometric procedures are popular for their sensitivity in the assay of drugs and hence, ion-pair extractive spectrophotometry has received a considerable attention for the quantitative determination of many pharmaceutical compounds. Since the analyte is a citrate salt of sildenafil, we have considered only sildenafil for further discussion. Sildenafil has a weak acidic moiety (pKₐ = 8.7). Protonation is very difficult in the substituted and fused rings of pyrimidine and pyrazol, due to resonance and steric effects. Hence, the site vulnerable for protonation in sildenafil is the nitrogen bonded to electron donating methyl group in the piperazine ring. It was observed that the anionic dye, BCP formed an ion-association complex with the positively charged drug. The drug-dye
stoichiometric ratio as determined by Job's method\textsuperscript{23} was found to be 1:1. The drug-dye complex, with two oppositely charged ions, behaves as a single unit held together by weak electrostatic forces of attraction. Based on these, we propose the probable reaction mechanism for the formation of the complex as shown in Scheme 1.

\textit{Spectral characteristics of ion-association complex}

In order to determine the wavelength of maximum absorption ($\lambda_{\text{max}}$) of ion-association complex formed in the proposed method, specified amount (within Beer's law limit) of SC was taken and the reaction product was developed following the procedure. The absorption spectrum was scanned on a spectrophotometer in the wavelength region of 300-600 nm against the reagent blank. The complex exhibited maximum absorption at 406 nm. The reagent blank showed a negligible absorbance at $\lambda_{\text{max}}$ (Fig. 1) thereby permitting good analytical conditions for the assay of SC. Hence, all the subsequent measurements were carried out at 406 nm.

\textit{Optimization of reaction conditions}

Optimum reaction conditions for quantitative formation of the ion-pair complex were established via a number of preliminary experiments by measuring the absorbances of a series of solutions at 406 nm by varying one parameter at a time and fixing the others. These optimum conditions were incorporated in the procedure.
**Optimization of pH of buffer**

It was observed that the effective extraction of the complex depended on the type of buffer used and its pH. The effect of pH was studied by extracting the coloured complex in presence of various buffers such as KCl-HCl (pH=1.0-2.2), NaOAc-HCl (pH=0.65-5.2), NaOAc-AcOH (pH=3.6-5.6) and potassium hydrogen phthalate-HCl (pH= 2.2-3.6). It was noticed that the maximum colour intensity and constant absorbances were observed in NaOAc-HCl buffer (Walpole buffer) of pH 3.5 (Fig. 2). Further, 1 ml of NaOAc-HCl buffer gave maximum absorbances and reproducible results. Low absorbance values were observed at pH values higher or lower than 3.5 of buffer medium. Hence, Walpole buffer of pH 3.5 was used for all subsequent measurements.

**Effect of reagent**

The effect of the reagent was examined by measuring the absorbances of solutions containing a fixed concentration of SC and varied amounts of the reagent. Maximum colour intensity of the complex was achieved with 5 ml of 0.1 % BCP (Fig. 3). Low absorbance values were noticed with the reagent more than or less than 5 ml.

**Selection of organic solvent**

Several organic solvents viz., chloroform, carbon tetrachloride, ethyl acetate, xylene, diethyl ether, butyl acetate, toluene, dichloromethane and chloro benzene were tried for effective extraction of the coloured species from aqueous phase. Quantitative extraction of the complex was achieved only with
chloroform. Further, it was observed that only one extraction was adequate to achieve a quantitative recovery (99.11-100%) of the complex with chloroform. Shaking times of 0.5 to 2 min produced constant absorbances and hence a shaking time of 1 min was maintained throughout.

Order of addition of reagents

Experiments were carried out to investigate the effect of order of addition of reagents on stability of ion-association complex. From the results, it was concluded that there was no appreciable change in sensitivity or stability of colored complex even if the order of addition was changed.

Precision and accuracy

The precision of the proposed method was ascertained from absorbance values obtained by actual determination of six replicates of fixed amount of the bulk sample of the drug. The low values of percent relative standard deviation recorded in Table 1 indicated good precision of the method.

To determine the accuracy of the proposed method, different amounts of bulk sample were taken within the Beer's law limits and analyzed. The percentage errors were calculated and are given in Table 1. These values revealed that the proposed method was reasonably accurate.

Optical characteristics of ion-pair complex

In order to examine the range in which the yellow colored ion-association complex obeyed Beer's law, the absorbances of a series of solutions containing increased amounts of SC were measured at $\lambda_{\text{max}}$ against the reagent
Regression analysis of Beer's law plot revealed a good correlation between absorbance and concentration of colored product in the concentration range of 0.9-15 μg/ml. The graph of absorbances versus concentration showed zero intercept and is described by regression equation, \( Y = bX + c \) (where \( Y \) is the absorbance of a 1 cm layer, \( b \) is the slope, \( c \) is the intercept and \( X \) is the concentration of each of the selected drug in μg/ml) obtained by least-squares method. The results are summarized in Table 1. Molar absorptivity and Sandell's sensitivity values were calculated and are given in Table 1.

**Effect of temperature on the colored complex**

The effect of temperature on colored complex was studied in the temperature range of 15-40 °C. It was found that the colored complex was stable upto 34 °C. At higher temperature, the drug concentration was found to increase due to volatile nature of chloroform. As a result, the absorbance of the colored complex increased. However, the complex was stable for more than 8 h at room temperature.

**Detection and quantification limits**

According to the Analytical Methods Committee, the detection limit (LOD) is the concentration of SC corresponding to a signal equal to the blank mean \( (Y_B) \) plus three times the standard deviation of the blank \( (S_B) \). Quantification limits (LOQ) is the concentration of SC corresponding to the blank mean plus ten times the standard deviation of the blank. The LOD and
LOQ values for the proposed method were calculated and found to be 0.28 μg/ml and 0.93 μg/ml, respectively.

**Studies on interference of excipients and other ions**

The possible analytical application of the proposed method was examined by studying the effects of excipients and other substances, which often accompany with SC in pharmaceutical products. This was carried out by adding different amounts of foreign substances to SC and color was developed following the procedure described earlier. A substance was considered to be interfered with the determination if the observed absorbance values differed by more than ± 2 % from that for the drug alone. It was noticed that the talc, glucose, starch, acetate, phosphate, lactose, sulphate, dextrose and magnesium stearate did not interfere in the assay of SC at the levels found in dosage forms. Thus, the proposed method was observed to be free from interferences by various substances.

**Recovery Studies**

Recovery studies were carried out by standard addition method. For this, known quantities of pure SC were mixed with definite amounts of pre-analyzed formulations and the mixtures were analyzed as before. The total amount of the drug was then determined and the amount of the added drug was calculated by difference. The average percent recoveries ranged from 98.92-101.86 % showed that the commonly encountered excipients in the formulation did not interfere in the proposed method.
**Ruggedness**

To ascertain the ruggedness of the method, four replicate determinations at different concentration levels of the drugs were carried out. The within-day RSD values were found to be less than 1.1%. The values of between-day RSD for the assay of different concentrations of drug are given in Table 2, and these values indicated that the proposed method has reasonable ruggedness.

**Stoichiometry of the complex**

The drug to dye stoichiometric ratio was determined by Job’s method of continuous variation using $1.0 \times 10^{-4}$ M solutions of each of drug and BCP. The results indicated that the ratio of drug to reagent to be 1:1 (Fig. 5).

**Analysis of tablets and statistical comparison of the results with reported method**

The utility of the proposed method for the assay of pharmaceutical preparations marketed under different trade names was examined. The results of assay of tablets containing SC are summarized in Table 3. These results were compared statistically by Student t-test and by the variance ratio F-test with those obtained by reported method. The Student t-values at 95% confidence level did not exceed the theoretical value indicating that there was no significant difference between the accuracy of the proposed and reported methods. It was also observed that the variance ratio F-values calculated for $p= 0.05$ did not exceed the theoretical value indicating that there was no significant difference between the precision of the proposed and reported methods.
CONCLUSIONS

Unlike the gas chromatographic and HPLC procedures, the spectrophotometric method is simple and of not high cost. The importance lies in the chemical reaction upon which the procedure is based rather than upon the sophistication of the instrument. This aspect of spectrophotometric analysis is of major interest in analytical pharmacy as it offers distinct possibility in the assay of a particular component in complex dosage formulations. The reagent utilized in the proposed method is cheaper, readily available and the procedure does 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 method is free from interference by common additives and excipients. The wide applicability of the new procedure for routine quality control is well demonstrated by the assay of SC in pure form and pharmaceutical preparations. Hence, the proposed method could be employed for quality assurance.
REFERENCES


<table>
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<tr>
<th>Parameter</th>
<th>BCP</th>
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<tr>
<td>$\lambda_{\text{max}}$ (nm)</td>
<td>406</td>
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<tr>
<td>Beer’s law limit ($\mu$g/ml)</td>
<td>0.9-15</td>
</tr>
<tr>
<td>Molar absorptivity (l/mol/cm)</td>
<td>$3.28 \times 10^4$</td>
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<tr>
<td>Sandell’s sensitivity (ng/cm$^2$)</td>
<td>20.32</td>
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<td>Stability (h)</td>
<td>8.5</td>
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<tr>
<td>Correlation coefficient (r)</td>
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<td>Regression equation ($Y$) $^a$</td>
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<td>Slope, b</td>
<td>0.0424</td>
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<tr>
<td>Intercept, c</td>
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<td>Relative standard deviation (%) $^d$</td>
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<td>% Error $^d$</td>
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<td>Limit of detection ($\mu$g/ml)</td>
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<td>Limit of quantification ($\mu$g/ml)</td>
<td>0.93</td>
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$^a Y = bX + c$, where $X$ is the concentration of drug in $\mu$g/ml

$^d$ Average of six determinations
Table 2. Between-day precision of the assay of SC by the proposed method

<table>
<thead>
<tr>
<th>Drug</th>
<th>Amount taken/µg</th>
<th>Amount found*/µg</th>
<th>RSD, %</th>
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<tr>
<td>Edigra</td>
<td>25</td>
<td>24.52</td>
<td>0.87</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>48.86</td>
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<tr>
<td></td>
<td>100</td>
<td>100.41</td>
<td>0.19</td>
</tr>
<tr>
<td>Silighra</td>
<td>50</td>
<td>49.58</td>
<td>0.39</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>100.77</td>
<td>0.34</td>
</tr>
<tr>
<td>Penegra</td>
<td>50</td>
<td>50.26</td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>98.78</td>
<td>0.55</td>
</tr>
<tr>
<td>Caverta</td>
<td>25</td>
<td>25.41</td>
<td>0.72</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>50.72</td>
<td>0.63</td>
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</table>

* Average of four determinations

Table 3. Determination of SC in pharmaceutical preparations by the proposed method and its comparison with reported method

<table>
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<tr>
<th>Formulation</th>
<th>Label claim (mg per tablet)</th>
<th>Recovery *± SD, % and its comparison with reported method</th>
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<tr>
<td></td>
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<td>Reported method</td>
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<tr>
<td>Edigra</td>
<td>25</td>
<td>99.28 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>100.2 ± 0.61</td>
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<td></td>
<td>100</td>
<td>99.51 ± 0.4</td>
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</tr>
<tr>
<td></td>
<td>100</td>
<td>101.0 ± 0.48</td>
</tr>
<tr>
<td>Penegra</td>
<td>50</td>
<td>100.7 ± 0.68</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>99.47 ± 0.27</td>
</tr>
<tr>
<td>Caverta</td>
<td>25</td>
<td>99.04 ± 0.72</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>102.1 ± 0.62</td>
</tr>
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* Average of six determinations
Scheme 1. Probable reaction mechanism for the formation of SC-BCP (1:1) complex
Fig. 1. Absorption spectra of (a) reagent blank and (b) the ion-association complex of SC (12 ppm) with BCP

Fig. 3. Effect of volume of 0.1 % BCP on the absorbances of ion-association complex of SC (10 ppm)
Fig. 4. Beer's law plot of SC

Fig. 5. Composition of SC-BCP complex by Job's method using 1x 10^{-4} M each of drug and BCP