Asian Journal of Chemistry Vol. 18, No. 4 (2006), 2475–2478

New Spectrophotometric Methods for Sildenafil Citrate

P. VENKAT RAO*, K. VASU‡, S. SUJATHA,
K. KISHORE KUMAR† and G. VIDYA SAGAR
Department of Pharmaceutical Sciences, Andhra University, Visakhapatnam- 530 003
E-mail: venkat771@indiatimes.com

Two simple, rapid and sensitive spectrophotometric methods for the determination of sildenafil in bulk samples and in formulations is described. The method A is based on the reaction between sildenafil citrate and diazotized dapsone in sodium hydroxide media and method B is based on the reaction between sildenafil citrate and diazotized *p*-sulphanilic acid in sodium hydroxide media. The graphs of absorbance *vs.* concentration show that Beer's law is obeyed over the concentration range 30–80 μg/mL for method A at 475 nm with molar absorptivity 0.7674×10^3 mol^{-1} cm^{-1} and for method B it was 20–100 μg/mL at 420 nm with molar absorptivity 9.461×103 mol^{-1} cm^{-1}. These methods were applied for the analysis of pure drugs and commercial formulations containing sildenafil citrate. The commonly encountered excipients and additives do not interfere with the determination. These methods offer the advantages of simplicity, rapidity and sensitivity without the need for extraction or heating.

Key Words: Sildenafil citrate, Beer's law, Spectrophotometry, Diazotised dapsone, Diazotized *p*-sulphanilic acid.

INTRODUCTION

Sildenafil, [(1-[4-ethoxy-3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1H-pyrozolo[4,3-d]pyrimidin-5-yl)phenyl sulphonyl]-4-methyl piperazine[1–3] (Fig. 1) is indicated for the treatment of erectile dysfunction in men. Literature survey reveals that there are a few methods[4–8] for the assay of drug in human plasma, *i.e.*, automated sequential trace enrichment of dialysates[9–11] by using RP-HPLC. No spectrophotometric method has so far been reported for the estimation of sildenafil.

The proposed methods involve utilization of diazotized dapsone and diazotized *p*-sulphanilic acid as chromogenic agents. The present work was undertaken to develop simple, rapid and sensitive spectrophotometric methods for the determination of sildenafil using the above reagents. The reaction conditions were thoroughly studied and the methods were applied successfully for the determination of the drug in the formulations.

EXPERIMENTAL

A Shimadzu 1601 UV spectrophotometer with 1 cm matched glass cells was used for all the absorbance measurements. Sildenafil citrate was obtained from Aristo Pharmaceuticals and dapsone 0.1% solution was prepared from commercial

†Department of Chemical Engineering, Andhra University, Visakhapatnam-530 003, India.
‡Department of Pharmaceutical Sciences, Annamalai University, Chidambaram-630 054, India.

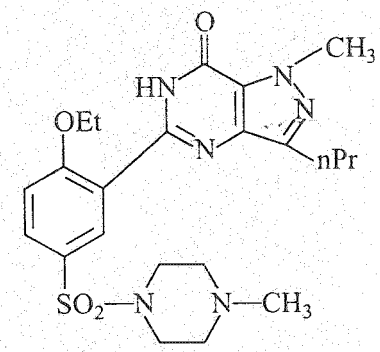

Fig. 1. Structure of sildenafil citrate

tablets. Sodium nitrate and sodium hydroxide were obtained from S.D. Fine Chemicals, Mumbai. *p*-Sulphanilic acid (1%) was obtained from CDH Limited, Mumbai. Standard solution of sildenafil citrate was prepared by dissolving 100 mg of drug in 100 mL calibrated flask to obtain a final concentration of 1000 µg/mL. Stock solutions of sodium nitrite (2%) and sodium hydroxide (4%) were prepared separately in distilled water.

Method A: In a series of 10 mL volumetric flasks, 1 mL of dapsone solution and 0.6 mL of sodium nitrite (2%) were mixed and allowed to stand for 5 min. Then different concentrations of drug were added. It developed yellow coloured chromogen after 10–15 min. Then 0.2 mL of sodium hydroxide (4%) was added. The resultant solution was made up to 10 mL with distilled water. Then optical density was measured at 475 nm against reagent blank. The colour was stable for 45 min. The concentrations of unknown solutions were determined from calibration graph or from regression equation calculated from the Beer's law data.

Method B: Aliquots of standard drug solution containing 20–100 µg of sildenafil were transferred to a series of 10 mL calibrated flasks. The reagent was prepared by adding 1 mL of sodium nitrite (2%) to 5 mL of *p*-sulphanilic acid (1%) in hydrochloric acid (10%). Then 2 mL of reagent and 5 mL of sodium hydroxide (4%) were added to each of the flasks and diluted to the mark with distilled water. The absorbance of the yellow coloured chromogen was determined at 420 nm against the reagent blank.

Assay procedure for pharmaceutical samples: Ten tablets of sildenafil citrate were weighed, finely powdered and an amount equivalent to 100 mg of the drug was treated with distilled water and filtered. The filtrate was made up to 100 mL with distilled water in order to get 1 mg/mL solution. Aliquots of varied concentrations were prepared from the stock solution and the drug content was analyzed by using Method A and Method B.

RESULTS AND DISCUSSION

The effect of reagent concentration on colour intensity of the complex was studied. The absorbance decreased with higher and lower amounts of reagents shown in Figs. 2A and 2B.

The effects of time of reaction for colour development on the colour intensity of the complexes were studied. Results given in Figs. 3A and 3B show that maximum absorbance was found at 475 nm for Method A and at 420 nm for Method B after 15 min.

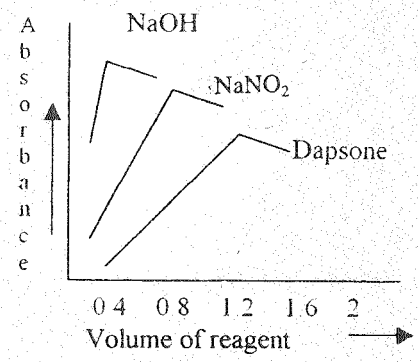

Fig. 2A

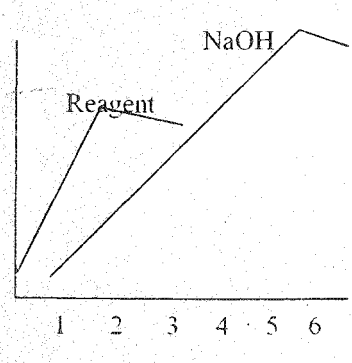

Fig. 2B

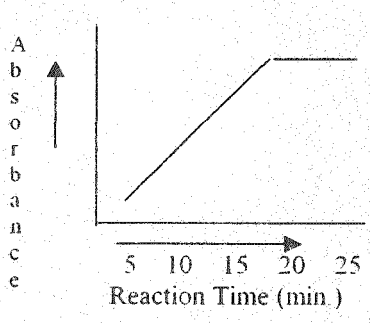

Fig. 3A

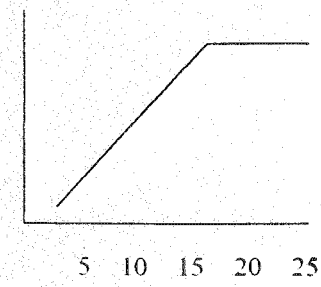

Fig. 3B

Stability of the coloured complexes was studied over a period of 2–3 h in light. It was observed that the colour was stable up to 45 min for Method A and up to 1.5 h for Method B. Absorbance was observed to decrease after this time. The yellow coloured complexes in Method A and Method B show maximum absorbance at 475 and 420 nm, respectively. The molar absorptivities were found to be 0.7674×10^3 and 9.46×10^3 mol^{-1} cm^{-1} for Method A and Method B, respectively. The solutions obeyed Beer's law at 475 and 420 nm in the concentration ranges of 30–80 and 20–100 μg/mL with Sandell's sensitivities 8.7×10^{-2} and 7.1×10^{-2} μg cm^{-2} per 0.001 absorbance unit. Five concentrations were made at each concentration level and the regression equations were

$$y = 9 \times 10^{-4} \times c + 0 \quad (1)$$

$$y = 0.01395 \times c + 0.0013 \quad (2)$$

Accuracy and precision: Five replicate determinations of each sample solution were carried out to test the accuracy and precision of the methods for the determination of sildenafil in its pure form and in tablets. The results of the methods in this investigation appeared (Table-1) to be highly satisfactory. The optical characteristics and precision data are shown in Table-1.

Recovery experiments: In order to assess the possible analytical applications of the method, recovery experiments were performed. Recovery experiments gave values of 99.8% (Table-2). The formal excipients and additives in the formulation do not interfere in proposed methods. These methods can be employed for the routine determination of the drug in pure and dosage forms.

TABLE-1
OPTICAL CHARACTERISTICS AND PRECISION DATA

Parameters	Method A	Method B
Absorption maxima (nm)	475	420
Beer's law limits (mcg/mL)	30–80	20–100
Sandell's sensitivity (mcg/cm^2/0. 001 AU)	8.7×10^{-2}	7.1×10^{-2}
Molar absorptivity (mol^{-1} cm^{-1})	$0.76\,74 \times 10^3$	9.461×10^3
Correlation coefficient (r)	1	0.9999
Regression equation (y)*:		
Slope (b)	9×10^{-4}	1.39×10^{-3}
Intercept (a)	0	0.0013
% RSD	2.87	2.260
Range of error:		
Confidence limit with 0.05 level	3.013	2.3725
Confidence limit with 0.01 level	4.713	3.711

Application to pharmaceutical samples: To enhance the usefulness of the methods, some pharamaceutical formulations (tablets) containing sildenafil were analyzed. The results of the methods appeared to be satisfactory.

TABLE-2
RECOVERY OF PURE DRUG ADDED TO FORMULATIONS

Drug and formulations	Drug initially present (μg) (formulation)	Pure drug added (μg)	Amount of pure drug recovered (μg)	Recovery (%)
Juan 25	300	500	499.0	99.8
Juan 50	300	400	398.0	99.5
Juan 100	300	300	297.0	99.0
Exix 25	300	300	299.9	99.9
Exix 50	300	400	399.9	99.9
Exix 100	300	300	299.9	99.9
			Average	99.8

REFERENCES

1. T.F. Loe, *N. Engg. J. Med.*, **342**, 1802 (2000).
2. K. Anderson, *Physiol. Rev.*, **75**, 326 (1995).
3. M. Boolel, M.J. Allen and G.J. Muirhead, *Indian Drugs*, **38**, 8 (2001).
4. R.A. Hall and T.P. Whitehead, *J. Clin. Pathol.*, **23**, 328 (1970).
5. J.D.H. Cooper, D.C. Turnell, B. Green and F. Verillon, *J. Chromatogr. A*, **456**, 53 (1986).
6. X. Zhu, S. Xiao, B. Chen, F. Zhang, S. Yao, Z. Wan, D. Yang and H.W. Han, *J. Chromatogr. A*, **1066**, 89 (2005).
7. P. Zao, S.S.-Y. Oh, P. Hou, M.-Y. Low and H.-L. Koh, *J. Chromatogr. A*, **1104**, 113 (2006).
8. M. Boolel, M.J. Allen and G.J. Muirhead, *Inst. J. Impotence Res.*, **8**, 47 (1996).
9. J.D.H. Cooper, D.C. Muihead and J.E. Taglor, *J. Chromatogr. B*, **701**, 87 (1997).
10. B. Green , J.D.H. Cooper and D.C. Tunnel, *Ann. Clin. Biochem.*, **26**, 361 (1989).
11. K. Larsson, W. Hermann, P. Moller and D. Sanchez, *J. Chromatogr. A*, **450**, 71 (1988).

(*Received*: 10 January 2005; *Accepted*: 21 March 2006) AJC-4747