ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
25 (2001) 339–342

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

www.elsevier.com/locate/jpba

Short communication

FIA of sildenafil citrate using UV-detection

G. Altıokka *, Z. Atkosar, E. Sener, M. Tunçel

Department of Analytical Chemistry, Faculty of Pharmacy, Anadolu University, 26470 Eskisehir, Turkey

Received 19 August 2000; received in revised form 12 October 2000; accepted 22 October 2000

Abstract

A flow injection analysis (FIA) of sildenafil citrate (SLD) using UV detection is described in this study. The best solvent system was found to be consisting of 0.2 M phosphate buffer at pH 8 having 10% MeOH. A flow rate of 1 ml. min^{-1} was pumped and active material was detected at 292 nm. The calibration equation was linear in the range of 1×10^{-6}–5×10^{-6} M. Limit of detection (LOD) and limit of quantitation (LOQ) were calculated to be 3×10^{-7} and 8.9×10^{-7} M with a R.S.D. 1.9 and 0.6% ($n = 7$), respectively. The proposed method was applied to the determination of SLD in VIAGRA® tablet, containing 50 mg active material. The results were compared with those obtained from UV–Spectrophotometry. The results showed that there is a good agreement between FIA method and the UV–Spectrophotometry. The validation studies were realised by the related applications and the results were evaluated statistically. According to the results, insignificant difference was observed between the methods.

Keywords: Determination of sildenafil citrate; Flow Injection Analysis; Pharmaceutical application

1. Introduction

Sildenafil citrate (SLD), 1-[[3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-5-yl)–4-ethoxyphenyl] sulfonyl] -4-methyl piperazine citrate (Fig. 1) is a novel orally active inhibitor of the type V-cyclic guanosine monophosphate (cGMP)-specific phosphodiesterase (PDE5) on penile erectile activity in patients with male erectile dysfunction, which causes cGMP to accumulate corpus cavernosum [1–4].

There are a few studies in literature for the determination of SLD in plasma samples using high performance liquid chromatography (HPLC) method [5,6] and voltammetric techniques in pharmaceutical preparations [7]. The aim of this study was to investigate the SLD determination by flow injection analysis (FIA) method and apply to the pharmaceutical preparations.

* Corresponding author. Tel.: +90-222-3350580/3635; fax: +90-222-3350127.

0731-7085/01/$ - see front matter
PII: S0731-7085(00)00509-4

2. Experimental

2.1. Apparatus and chemicals

The HPLC apparatus used a Model LC 6A pump equipped with a 20 μl manual loop injector, a Model SPD-A10 UV variable wavelength detector and a Model C-R7A integrator (all Shimadzu, Japan). Spectrophotometric studies were done using a Model UV-2401 PC (Shimadzu, Japan). A Model WTW Multiline P4 Universal pH-meter cabled Sen-Tix 92T pH electrode (Germany) was employed for measuring and adjusting the pH of the solution.

Standard SLD (99.9%) and VIAGRA® tablets containing 50 mg active material were kindly supplied from Fako Ilaçları A.S. Istanbul, Turkey and Pfizer Ilac San. Tic. A.S. Istanbul, Turkey, respectively. Standard SLD was used without further purification. Other chemicals were of analytical grade of Merck (Germany).

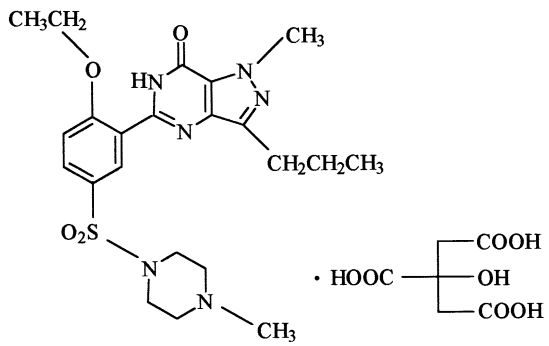

Fig. 1. The chemical structure of SLD.

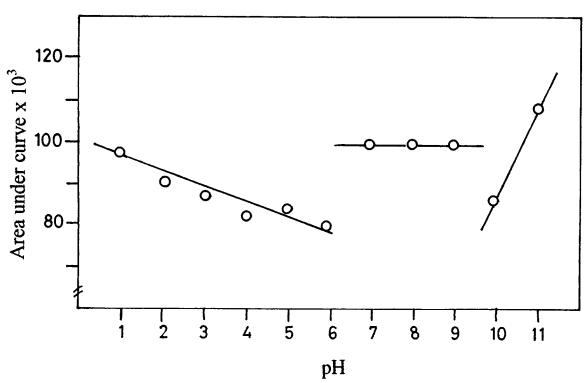

Fig. 2. Variation in the AUC values of SLD (1×10^{-6} M) in relation to pH.

2.2. Solutions

A stock solution of SLD (1×10^{-3} M) was prepared using bidistilled water and the dilutions were made in the range of 1×10^{-6}–5×10^{-6} M. As a mobile phase, an aqueous solution of MeOH (10%,v/v) was used. The buffer solutions were prepared using 1 M CH_3COONa (pH 1–6) and 1 M K_2HPO_4 (pH 7–11.02) and their pH values were adjusted in the range of 1–11.02 using 2 M HCl or 2 M KOH.

2.3. Application to the tablets

Ten VIAGRA® tablets were weighed and finely powdered in a mortar. The average weight of a tablet was calculated. A sample equivalent to one tablet was weighed and transferred to a 100 ml calibrated flask, 2 ml phosphate buffer (1 M, pH 8) was added, magnetically stirred for 20 min and made up to volume with bidistilled water. A sufficient amount of the solution was pipetted in a tube and it was centrifuged for 10 min. The supernatant was diluted to the predetermined values and injected in to sample loop by means of a syringe.

3. Results and discussion

To determine the parameters for the optimisation, an SLD solution having 5×10^{-6} M was used. The solvent system consisted of MeOH and bidistilled water. To investigate the percentage of MeOH, it was varied beginning from 10 to 50% (v/v). It was found that the optimum concentration of MeOH, in view of peak morphology, was 10% (v/v). To determine the optimum flow rate, the flow rate was changed from 0.5 to 3 ml. min^{-1} and the best flow rate was found to be 1 ml. min^{-1}. The final concentration of buffer in the test solutions was 0.2 M. When the base line

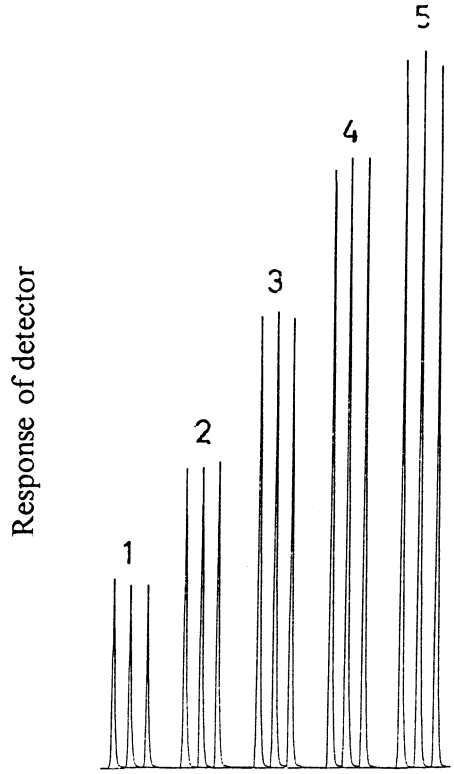

Fig. 3. The signals in the 1×10^{-6}–5×10^{-6} M concentration range of SLD.

Table 1
Linearity and accuracy of FIA method for SLD[a]

Parameters	Intra-day precision ($k = 1$; $n = 8$)	Inter-day precision ($k = 4$; $n = 32$)
Slope ± S.D.	$8.08 \times 10^{10} \pm 5693$	$8.37 \times 10^{10} \pm 6847$
Intercept	30 375	27 438
Correlation coefficient (r)	0.9996	0.9990
Slope ± CL ($P = 0.05$)	$8.08 \times 10^{10} \pm 4747$	$8.37 \times 10^{10} \pm 5710$

[a] S.D., standard deviation; CL, confidence limit; k, number of the set; n, number of the sample.

was reached, another sample was injected. The peak areas versus pH are illustrated in Fig. 2.

As seen in Fig. 2, at higher acid and base concentration, there are significant differences in peak area. However, these differences are minimum at pH values between 7 and 9. The ranges of these pH values are close to the pK_a value of 8.7 for SLD [5]. Therefore, the phosphate buffer of pH 8 was chosen as working pH. The signals for the SLD at concentrations ranging from 1×10^{-6} to 5×10^{-6} M were obtained under the conditions described above and they are given in Fig. 3.

Although the prepared solutions give the same signals during a week time, it is not always possible to obtain the true stability of the molecule. For this aim, HPLC or TLC methods are recommended.

The relationship between area under curve (AUC) against SLD concentration was found to be $AUC = 8.25 \times 10^{10}C(M) + 41655.5$, $r = 0.9997$. Limit of detection (LOD) ($S/N = 3.3$) and limit of quantitation (LOQ) were calculated to be 3×10^{-7} and 8.9×10^{-7} M with R.S.D. 1.9 and 0.6% ($n = 7$), respectively.

Linearity and accuracy in the concentration range of 1×10^{-6}–5×10^{-6} M were examined employing intra-day and inter-day studies for the determination of SLD. The results were evaluated statistically and these are demonstrated in Table 1.

Very accurate results were obtained for intra-day and inter-day experiments with a good correlation. These results indicate that the FIA method could be used for the analysis of SLD.

3.1. Application to the pharmaceutical dosage forms

The proposed technique was applied to the tablets containing 50 mg SLD. The absorbance was monitored at 292 nm. The AUC was used for calibration. UV–Spectrophotometry was chosen as a comparison method. The additives in the tablet have no interference effect. The absorbances of the same solutions were measured at 292 nm using quartz cells. The relationship between absorbance (A) and concentration of SLD (C) was found to be $A = 11807.5C(M) + 1.26 \times 10^{-3}$, $r = 0.9998$.

The validity of the method was examined by applying to the VIAGRA® tablets. All the results

Table 2
The assay results of SLD in VIAGRA® tablets[a]

	FIA	UV–Spectrophotometry
Mean	47.7	48.5
n	7	7
RSD%	1.90	1.05
CL	±0.20	±0.15
F-test of insignificant	3.30	$F_{0.05} = 4.28$ (table)
t-test of insignificant	0.85	$t_{0.05} = 2.18$ (table)

[a] Each tablet contains 50 mg SLD.

of the assays were evaluated statistically and presented in the Table 2.

High reproducibility was observed and insignificant differences between FIA and UV–Spectrophotometry at the 95% probability level. To conclude, the method proposed in this study is easy, accurate, precise and rapid. Therefore, it can be suggested for the routine analysis of SLD.

References

[1] M. Boolell, S. Gepi-Attee, J.C. Gingell, M.J. Allen, Br. J. Urol. 78 (1996) 257–261.
[2] I.V. Turko, S.A. Ballard, S.H. Francis, J.D. Corbin, Mol. Pharmacol. 56 (1999) 124–130.
[3] B.H. Lowentritt, P.T. Scardino, B.J. Miles, F.J. Orejuela, E.C. Schatte, K.M. Slawin, S.P. Elliott, E.D. Kim, J. Urol. 162 (1999) 1614–1617.
[4] G. Brock, Drugs Today 36 (2000) 125–134.
[5] J.D.H. Cooper, D.C. Muirhead, J.E. Taylor, P.R. Baker, J. Chromatogr. B 701 (1997) 87–95.
[6] A.I. Segall, M.F. Vitale, V.L. Perez, M.L. Palacios, M.T. Pizzorno, J. Liq. Chromatogr. Relat. Technol. 23 (2000) 1377–1386.
[7] J.J. Berzas, J. Rodriguez, G. Castaneda, M.J. Villasenor, Anal. Chim. Acta 417 (2000) 143–148.