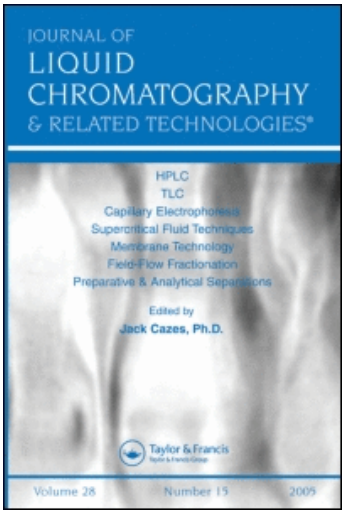

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

REVERSED-PHASE HPLC DETERMINATION OF SILDENAFIL CITRATE IN THE PRESENCE OF ITS OXIDATIVE-INDUCED DEGRADATION PRODUCTS

A. I. Segall[a]; M. F. Vitale[a]; V. L. Perez[a]; M. L. Palacios[a]; M. T. Pizzorno[a]
[a] Universidad de Buenos Aires, Buenos Aires, Argentina

Online publication date: 18 May 2000

To cite this Article Segall, A. I. , Vitale, M. F. , Perez, V. L. , Palacios, M. L. and Pizzorno, M. T.(2000) 'REVERSED-PHASE HPLC DETERMINATION OF SILDENAFIL CITRATE IN THE PRESENCE OF ITS OXIDATIVE-INDUCED DEGRADATION PRODUCTS', Journal of Liquid Chromatography & Related Technologies, 23: 9, 1377 — 1386
To link to this Article: DOI: 10.1081/JLC-100100421
URL: http://dx.doi.org/10.1081/JLC-100100421

J. LIQ. CHROM. & REL. TECHNOL., 23(9), 1377–1386 (2000)

REVERSED-PHASE HPLC DETERMINATION OF SILDENAFIL CITRATE IN THE PRESENCE OF ITS OXIDATIVE-INDUCED DEGRADATION PRODUCTS

A. I. Segall, M. F. Vitale, V. L. Perez, M. L. Palacios, M. T. Pizzorno*

Cátedra de Control de Calidad de Medicamentos
Facultad de Farmacia y Bioquímica
Universidad de Buenos Aires
Junín 956
1113 Buenos Aires, Argentina

ABSTRACT

The determination of sildenafil citrate in the presence of its oxidative-induced degradation products by reversed-phase HPLC is described. The method was validated as stability-indicating by forced decomposition of sildenafil citrate in acid, base, oxidative, thermal, and photochemical media. The chromatographic conditions employed a reversed-phase C_{18} column (LiChrospher, 5 μm, 25 cm x 4.6 mm) isocratic elution with 70 mM potassium phosphate monobasic containing 100 mM triethylamine (pH 3.0)-ACN (70:30, v/v) and ultraviolet (UV) detection at 225 nm. The peak area versus sildenafil citrate concentration proved linear over the 10-160% range of the working analytical concentration of 0.5-mg/mL. Mean absolute recovery of sildenafil citrate using the described method was 100.9 ± 1.1 % (mean ± SD, n = 9). The precision, expressed as relative standard deviation (RSD), of ten replicate injections of sildenafil citrate reference solution, remained below 0.51 %.

INTRODUCTION

Sildenafil [5- [2 - Ethoxy-5- (4 - methylpiperazin - 1 - ylsulfonyl) phenyl]-1-methyl-3-propyl-6,7-dihydro-1*H*-pyrazolo[4,3-*d*]pyrimidin-7-one] is used for the treatment of erectile dysfunction.[1] Sildenafil is a potent selective inhibitor of type V $_{C}$GMP phosphodiesterase (PDE-V) found in human corpus cavernosum.[2] The recommended dosage of sildenafil citrate is 50 mg taken about one hour before anticipated sexual activity.[1]

A computer search (Medline, Analytical Abstracts and International Pharmaceutical Abstract) disclosed only one HPLC method for the assay of sildenafil citrate in biological fluids.[3] The described method fails to provide selectivity data for various stress conditions of the finished product.

Sildenafil has basic functional groups with a pK_a value of 8.7.[3] Difficulties may arise during the analysis of compounds with basic properties. Adsorption of such compounds by exposed silanols on C_{18} column materials may be avoided by working at acid pH, thus preventing dissociation and acid exchange. As a rule, the addition of a terciary amine such as 30 mM of triethylamine is enough to correct the chromatogram.

This report describes a sensitive, accurate, and reproducible reversed phase HPLC method for the determination of sildenafil citrate in tablets. In this method, which was found to be stability-indicating, the effect of mobile phase pH on resolution, tailing factor, and retention time were examined.

EXPERIMENTAL

Apparatus

The chromatographic system consisted of a dual piston reciprocating Spectra Physics pump **(**Model ISO Chrom**.** LC pump), a UV-Vis Hewlett Packard detector (Model 1050), a Hewlett Packard integrator (Series 3395) and a Rheodyne injector (Model 7125).

Chemicals and Reagents

Acetonitrile used in the mobile phase was HPLC grade. Distilled water was passed through a 0.45-micron membrane filter. Monobasic potassium phosphate, triethylamine, and phosphoric acid were AR grade. Solutions and mobile phase were prepared just before use and all solvents and solutions for HPLC analyses were filtered through a Micron Separations N04SP04700 nylon membrane filter (pore size 0.45 μm) and vacuum degassed before use.

Sildenafil citrate was kindly donated by Laboratorios Kampel Martian (Argentina) and used as standard without further purification.

A commercial local tablet formulation was used. Its composition was: Sildenafil citrate 70.24 mg, in a matrix of: microcrystalline cellulose, povidone, sodium crosscarmelose, hydroxypropyl methylcellulose, magnesium stearate, colloidal silicon dioxide, dibasic calcium phosphate, polyethylene glycol 6000, sodium saccharin, and sodium cyclamate.

Chromatographic Conditions

The mobile phase consisted of a buffer solution [70 mM monobasic potassium phosphate and 100 mM triethylamine in water (pH 3.0)-acetonitrile (70:30, v/v)], prepared by adding 9.7 g of monobasic potassium phosphate and 10.4 g of triethylamine to 1 L of water and the pH adjusted to 3.0 ± 0.1 with 85 % phosphoric acid. For mobile phase preparation, 700 mL of the buffer solution and 300 mL of acetonitrile were combined, mixed well, allowed to equilibrate to room temperature, and vacuum degassed before use.

The analytical column was a Merck LiChrospher^{R} 100 RP-18 (4 x 250 mm, 5 μm) column. All analyses were performed under isocratic conditions at a 1.0-mL/min flow rate, and 15-min run time, at room temperature. In these conditions sildenafil citrate retention time (t_R) was roughly 7 minutes. Detector sensitivity was set at 2 a.u.f.s. and eluents monitored at 225 nm. The volume of each injection was 20 μL.

Calibration Curve

Solutions ranging from 50 to 800 μg/mL of sildenafil citrate were prepared in the mobile phase from a methanol stock solution of sildenafil citrate standard. The calibration curve was constructed by plotting peak areas against micrograms injected.

Preparation of Solutions

A standard stock solution of Sildenafil citrate, 1 mg/mL, was prepared in methanol. The standard preparation was obtained by diluting the Sildenafil citrate stock solution with mobile phase to yield a concentration of 0.5 mg/mL.

Resolution Solution

Twenty five milligrams of Sildenafil citrate were dissolved in 10 mL of H_2O_2 30 vol, refluxed for 15 min, and suitably diluted to 50 mL with mobile phase.

System Suitability

System suitability results were calculated according to the USP 23 <621> from typical chromatograms.[4] Instrument precision as determined by ten successive injections of the standard preparation should provide a relative standard deviation (RSD) below 1.0%. The tailing factor should not exceed 1.4 at 5% peak height. Column efficiency should be greater than 900 theoretical plates. Finally, 20 µL of the Resolution Solution were injected. The resolution between sildenafil citrate and the nearest adjacent peak should be greater than 2.2 (Figure 1).

Stability-Indicating Validation

The HPLC method was validated as stability-indicating by forced degradation of sildenafil citrate. Samples were prepared by transferring approximately 25 mg of sample into 50 mL volumetric flask. Sildenafil citrate was subjected to thermal (in an oven at 110°C, 24 h) and photochemical degradation (in an open container exposed to daylight for 24 h). Intentional degradation was attempted using 10 mL of HCl 1N, NaOH 1N, H_2O_2 30 vol and refluxing for at least 15 minutes. After degradation treatments were completed, samples were allowed to cool to room temperature and diluted to the same concentration as the standard preparation, after being neutralized with acid-base if required. Samples were then analyzed against the standard.

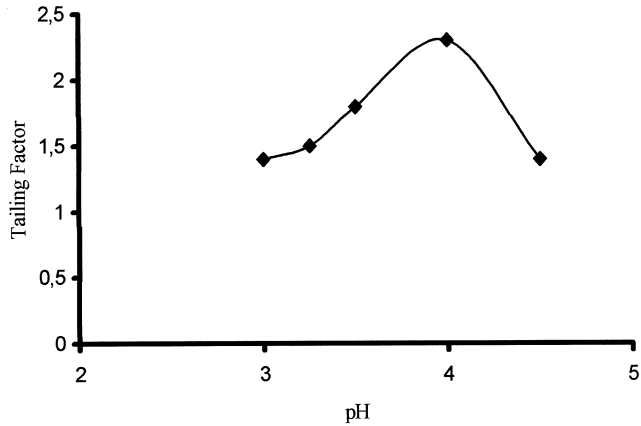

Figure 1. Effect of Mobile Phase Buffer pH on Sildenafil Citrate Tailing Factor.

Accuracy

Assay accuracy was assessed by fortifying placebo tablets with known amounts of sildenafil citrate at 80, 100, and 120% of sample solution concentration.

Procedure

Solutions were prepared on a weight basis and volumetric flasks used as suitable containers in order to minimize solvent evaporation.

Prior to injecting solutions, the column should be equilibrated for at least 30 minutes with the mobile phase flowing through the system. Quantitation was accomplished by using an external standard method. Each solution was injected in triplicate and the relative standard deviation (RSD) was required to remain below 1.5% on a sildenafil citrate peak area basis.

RESULTS AND DISCUSSION

Effect of Mobile Phase pH on Tailing Factor, Resolution, and Retention Time

Resolution solutions of sildenafil citrate at different pH values of buffer solution were analyzed. Figures 1 and 2 show the effect of mobile phase buffer pH solution on the tailing factor of sildenafil citrate peak and on resolution,

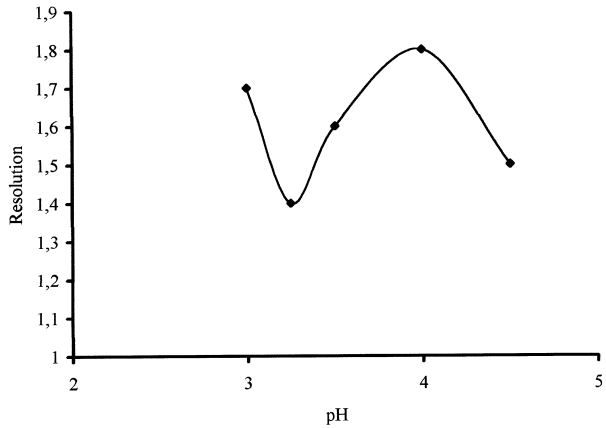

Figure 2. Effect of Mobile Phase Buffer pH on Sildenafil Citrate Resolution.

Table 1

Effect of pH of Buffer Solution on Tailing Factor, Retention Time, and Resolution for Sildenafil Citrate and its Oxidative-Induced Degredation Products

Buffer Solution pH	Sildenafil Citrate Tailing Factor	Sildenafil Citrate Retention Time	Resolution	Degradation Products Tailing Factor	Degradation Products Retention Time
3.0	1.4	7.335	1.7	2.1	9.475
3.25	1.5	8.564	1.4	2.1	11.223
3.5	1.8	8.240	1.6	2.3	11.544
4.0	2.3	8.484	1.8	3.1	13.294
4.5	1.4	7.343	1.5	2.2	10.090

both of which were significantly affected. Based on these results, a pH range of 3.0 ± 0.1 was selected (Table 1).

Specificity

Sildenafil citrate was stressed by thermal, acidic, basic, oxidative, and photochemical degradation for up to 24 hours. No interfering peaks at the retention time of sildenafil citrate were observed in any of the stressed sample (Table 2).

Stability determinations were conducted to assess method specificity for the assay of sildenafil citrate without interference from the oxidative-induced

Table 2

Degradation of Sildenafil Citrate

Condition	Time (h)	Recovered (%)	RRT Degrad.[a] Products
Acid 1N HCL, refl.[b]	0.25	2.0	0.36, 0.48
Base 1N NaOH, refl.[b]	0.25	2.0	0.39
H_2O_2 30 vol., refl.[b]	0.25	43.8	0.27, 0.30, 1.29
Dist. H_2O, refl.[b]	0.25	52.2	0.49
Dry heat, 110°C	24	23.5	None detected
Daylight	24	62.8	0.49

[a] RRT: relative retention time; degrad.: degradation. [b] refl.: refluxed.

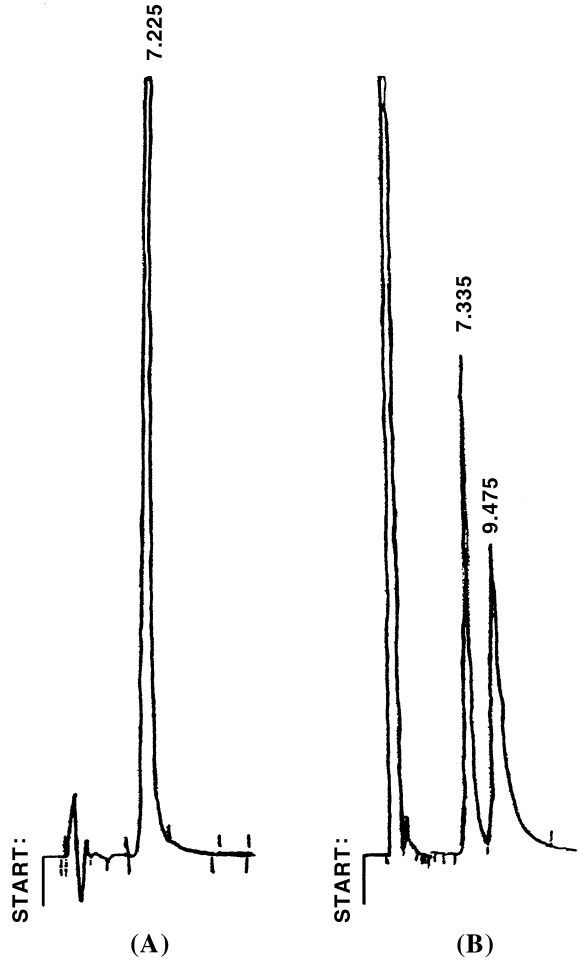

Figure 3. Chromatograms of Sildenafil Citrate Standard (A) and Oxidative Degradation (B).

degradation products. The forced degradation experiment described above yielded a reduction in intact sildenafil citrate (retention time 7.335 minutes) with the formation of a new peak eluting at circa 9.475 minutes (Figure 3).

Precision

The precision of the results, reported as the relative standard deviation (RSD%), was 0.51 % as determined on 10 replicate injections of standard preparation.

Table 3

Linearity of Sildenafil Citrate Response

Injected (µg)Response	Average Peak Area (%)	RSD
1	9279521	1.60
5 48449269	0.61	
8 77690005	1.28	
10 97298517	0.19	
12115901000	0.25	
16157597680	1.44	

Slope[a] = 9841153 ± 203969
Intercept[b] = -920663 ± 2022620

[a] Confidence limits of the slope (p = 0.05). [b] Confidence limits of the intercept (p = 0.05).

Linearity

Six solutions containing sildenafil citrate at concentrations ranging from 50 to 800 µg/mL were analyzed. The curve of peak area versus micrograms injected proved linear. Response linearity was evaluated by regression analysis and the regression equation (Y = 9841153X – 920663) presented a correlation coefficient (r) of 0.99988 while intercept values were not significantly different from zero, (p = 0.05) (Table 3).

Microsoft Excel software was used to plot peak areas versus micrograms injected (Figure 4).

Accuracy

Placebo tablets were spiked at different levels with known amounts of sildenafil citrate at 80, 100, and 120%. Individual recovery ranged from 99.1 to 102.3 (Table 4). Recovery at all levels was 100.9 % with an RSD value of 1.1%.

Recovery linearity was evaluated by plotting the amount recovered versus the amount spiked. Linear least squares analysis of the data yielded a correlation coefficient (r) value and slope of 0.9983 and 0.9764 respectively. The

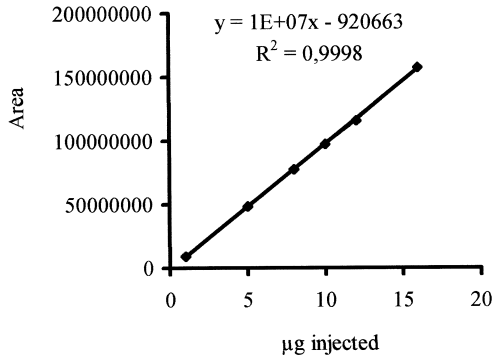

Figure 4. Peak Area Response Versus µg Injected of Sildenafil Citrate.

r value indicates that the method is linear over the concentration range investigated. The slope value was close to unity and the intercept was not significantly different from zero (t test, $p = 0.05$) which confirmed the accuracy of the method over the range investigated.

Table 4

Assay Accuracy*

% w/w	Amount Added (mg)	Amount Determined (mg)	Amount Recovered (%)	Average Recovered (n = 3)	RSD (%)
	40.5	40.8	100.7	---	---
80	40.6	40.9	100.6	101.2	0.9
	40.6	41.6	102.3	---	---
	49.9	50.8	101.7	---	---
100	49.7	50.9	102.3	101.3	1.2
	50.6	50.6	100.0	---	---
	60.4	61.0	101.0	---	---
120	60.5	60.7	1002.	99.9	0.9
	60.8	60.3	99.1	---	---
Overall Recovery (n = 9)				100.9	1.1

* Accuracy acceptance criteria, 97.0 to 103.0; precision acceptance criteria, 3% within each level.

Sildenafil citrate recovery achieved showed that there was no interference from excipients present in the tablets.

Range

Assay method range was set at 80 to 120% of the finished product label claim, since the method proved precise, accurate, and linear within these limits.

CONCLUSION

The HPLC method described in this work is simple, precise, accurate, and useful for stability studies on sildenafil citrate. It is also suitable as an alternative for routine analysis and quality control of sildenafil citrate in tablets.

ACKNOWLEDGMENT

The authors thank Laboratorios Kampel Martian (Argentina) for assistance and donation of drugs and reactives.

REFERENCES

1. "FDA Approves Oral Therapy for Erectile Dysfunction," an editorial in Am J. Health-Syst Pharm., **55**, 981-984 (1998).

2. A. M. Martel, A. Graul, R. Rabasseda, R. Castañer, "Sildenafil: Treatment of Erectile Dysfunction, Phosphodiesterase V Inhibitor," Drugs-Future, **22(2)**, 138-143 (1997).

3. J. D. H. Cooper, D. C. Muirhead, J. E. Taylor, P. R. Baker, "Development of an Assay for the Simultaneous Determination of Sildenafil (Viagra) and its Metabolite (UK-103,320) using Automated Sequential Trace Enrichment of Dialysates and High Performance Liquid Chromatography," J. Chromatogr. B, **701**, 87-95 (1997).

4. **The United States Pharmacopeia 23**, U. S. Pharmacopeial Convention, Rockville, MD, 1995, pp. 1768-1779.

Received September 29, 1999 Author's Revisions December 5, 1999
Accepted October 31, 1999 Manuscript 5180