A Sensitive and Simple High Performance Liquid Chromatographic Method for Quantification of Tadalafil in Human Serum

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ABSTRACT

A simple, rapid, and sensitive high-performance liquid chromatographic method was developed and validated for determination of tadalafil, a selective and reversible phosphodiesterase inhibitor, in human serum. Methylparaben was used as the internal standard. Optimum conditions for tadalafil assay were investigated. The analyte and internal standard were extracted by a single-step liquid–liquid extraction with dichloromethane in alkaline serum. The chromatographic separation was performed on reverse phase LiChrospher 100, C18 column (Agilent Technologies, Palo Alto, CA) with a mobile phase consisting of 35% acetonitrile-65% water containing 0.1 mM glacial acetic acid (pH 2.5-2.7).

Ultraviolet detection was performed at 280 nm. Detection limit was 1.5 ng/mL, and limit of quantification was less than 10 ng/mL for tadalafil. The calibration curves were linear over the concentration range tested (10-800 ng/mL). Accuracy, precision, and stability studies were satisfactory. This analytical procedure is relatively inexpensive and simple and is particularly suitable when tandem mass spectrometric detection is not available. This method can be used to determine serum tadalafil concentrations in drug monitoring or in pharmacokinetic investigations.

INTRODUCTION

Tadalafil is a potent and selective phosphodiesterase-5 (PDE-5) inhibitor, a secondary messenger for the smooth muscle relaxing effects of nitric oxide, which plays an important role in the vasodilation of erectile tissues.[1-3] Oral PDE-5 inhibitors have become the preferred first-line treatment for erectile dysfunction worldwide.[4]

Tadalafil is well absorbed after oral administration with a mean maximum observed plasma concentration (C_{max}) of 378 ng/mL after oral administration of the 20-mg tablet.[5] C_{max} is achieved at a mean time of 2 hours after dosing.[5,6]

Table 1. Intraday Variability of the Assay for Tadalafil in Serum (4 series)

Concentration Added (ng/mL)	Concentration Found (ng/mL)	Standard Deviation	Coefficient of Variation (%)
10	10.03	0.48	3.50
20	19.69	0.63	2.25
50	50.93	0.56	1.75
100	96.11	0.85	4.00
200	209.52	3.72	4.75
300	310.78	1.69	3.75
400	393.12	7.38	2.00
600	569.39	4.25	5.00
800	820.44	5.34	2.75

Correlation coefficients are 0.9984, 0.9984, 0.9984, and 0.9993 for each of the 4 curves.

Table 2. Interday Variability of the Assay for Tadalafil in Serum (5 series)

Concentration Added (ng/mL)	Concentration Found (ng/ml)	Standard Deviation	Coefficient of Variation (%)
10	10.08	0.47	3.6
20	19.24	0.45	3.8
50	50.95	1.04	1.8
100	94.97	0.65	5
200	204.87	2.55	2.4
300	309.65	1.61	3.4
400	400.60	1.05	0.2
600	576.66	1.56	4
800	812.94	1.60	1.6

Correlation coefficients are 0.9993, 0.9993, 0.9991, 0.9993, and 0.9993 for each of the 5 curves

Absolute bioavailability following oral dosing has not been determined. Tadalafil mean volume of distribution is approximately 63 L, with a mean half-life of 17.5 hours in healthy subjects.[5-7]

The measurement of tadalafil in serum or plasma samples requires sensitive and specific methods. Tadalafil can be determined in biological samples, dietary supplements, or herbal matrices by liquid chromatography-tandem mass spectrometry with electrospray ionization[8-10] or micellar electrokinetic capillary chromatography.[11] A simple, sensitive, and less expensive high-performance liquid chromatographic (HPLC) method with ultraviolet (UV) detection is an alternative. Such a method was validated in the Bioequivalence and Quality Control Laboratory at Saint-Joseph University, Lebanon, and is described in this report.

EXPERIMENT

Reagents and Standards

All chemicals used were reagent grade. The following solvents and reagents were used: acetonitrile and water

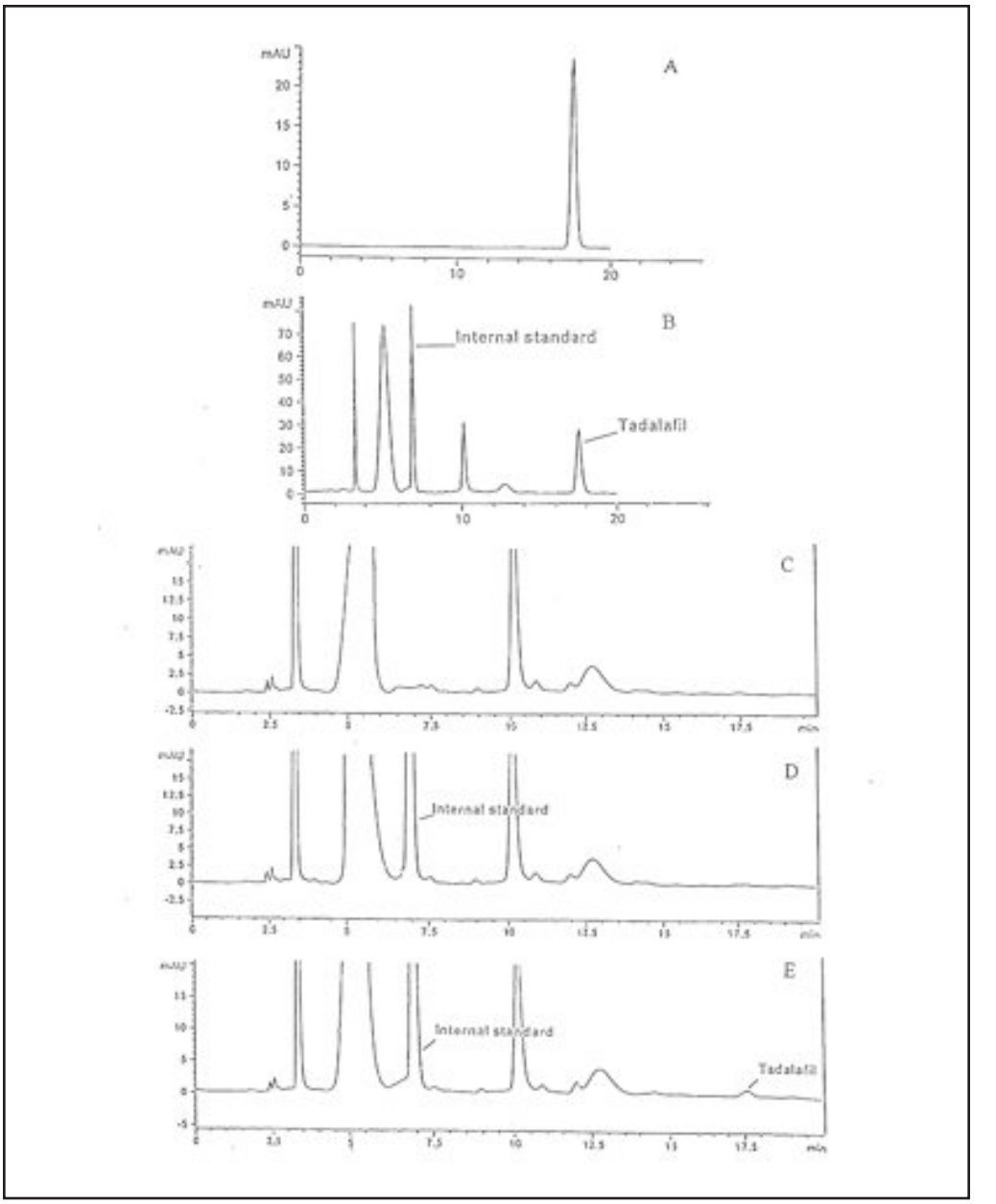

Figure 1. Chromatograms of (A) standard tadalafil solution, (B) extracted sample at 800 ng/mL, (C) blank serum, (D) blank serum with internal standard, and (E) extracted sample at 10 ng/mL.

(HPLC grade, ROMIL-SpS, Cambridge, UK), dichloromethane stabilized with ethanol analytical grade (Lab-Scan, Hasselt, Belgium), and methylparaben (Sigma, Germany). NaOH 1N was prepared in the laboratory using concentrated NaOH (Merck, Whitehouse Station, NJ). Tadalafil standard was supplied by local manufacturer (Pharmaline, Lebanon).

Stock solution of tadalafil was prepared by dissolving 15 mg in 25 mL water/acetonitrile (50/50, v/v). Internal standard stock solution was prepared by

Table 3. Tadalafil Recovery After Extraction: Drug-Free Human Serum Was Spiked With Tadalafil at Different Concentrations and Extraction Coefficient Calculated

Concentration (ng/mL)	Average Extraction Coefficient (%) (n=3 for each level)
10	76.5
20	79.2
50	85.5
100	82.7
200	85.3
300	77.9
400	83.3
600	84.6
800	81.1

dissolving 50 mg in 20 mL of acetonitrile. All stock solutions were protected from light and kept at -20˚C. They were stable for at least 6 months.

Serum calibration samples were prepared using a 1/20 dilution of tadalafil stock solution with drug-free serum. Stability of the drug in serum at –20˚C over 6 months was documented. Internal standard solution was diluted 1/10 with acetonitrile and was used in samples preparation.

Instrumentation

The HPLC system consisted of a 1100 series quaternary pump, degasser, automatic injector, thermostatted column compartment, and diode array detector (Agilent Technologies, Palo Alto, CA); Vortex TecnoKartell TK3; shaker BIOSAN Multi Bio RS-24, and innovative mixing cycle (VWR international, USA). The data were collected using the system software (Chemstation 1990-2002, Agilent Technologies).

Chromatographic Conditions

The separation was achieved on an Agilent LiChrospher 100, C18 column, 5-µm particle size, 250 x 4 mm I.D., with a 2-µm precolumn filter. The mobile phase consisted of 65% water acidified with glacial acetic acid (0.1 mM, pH 2.5-2.7) and 35% acetonitrile.

The flow rate was 0.8 mL/min, and UV detection was performed at 280 nm. All analyses were made at room temperature. The injection volume was 25 µL, and a small volume of air was bubbled through each sample before injection.

Serum Extraction Procedure

One hundred microliters of internal standard solution were added to 500 µL of serum in 15 mL-polypropylene centrifuge tubes with flat caps (15 x 118 mm, Corning, NY) and mixed for 10 seconds on vortex. Fifty microliters of NaOH 1N was immediately added with a micropipette while gently vortexing the tubes. To each tube 6 cc of dichloromethane was added, and the tubes were vortexed for 30 seconds at high speed. The sample was finally shaken on a rotating shaker (30 rotations/minute) for 10 minutes.

After centrifugation for 10 minutes

Table 4. Drug-Free Human Serum Spiked With 3 Different Concentrations of Tadalafil and Stored at –20˚C Over a 6-Month Period

Concentration Added (ng/mL)	Concentration Obtained (ng/mL)			
	Day 1	Day 30	Day 90	Day 180
20	18.65	21.31	19.56	19.13
200	216.98	219.51	187.46	193.43
800	815.54	784.32	805.64	819.83

at 3500 rpm at 4°C, the clear dichloromethane layer was transferred with a calibrated pipette to a disposable glass tube (10 x 75 mm) and evaporated under a gentle stream of nitrogen. The dried residue was taken up with 30 µL of mobile phase, and 25 µL of this mixture were injected in the HPLC apparatus.

RESULTS

Assay Validation

For assay validation, tadalafil was mixed with drug-free human serum over the concentration ranges 10-800 ng/mL. Each mixture was divided into several portions. Intraday variability was examined using 4 series, and interday variability was examined using 5 series performed on 5 separate days. Tables 1 and 2 show the results of within-run and day-to-day precision studies. The precision was determined using coefficients of variation (CV) within the day and between days. The calibration curves were linear over the concentration range studied (correlation coefficients 0.998 to 0.999). Concentrations higher than 800 ng/mL were not tested for linearity.

The limit of quantification (LOQ) was calculated using the standard deviation of the intercepts and the mean slope of the calibration curves (LOQ = 3 x standard deviation of the intercepts/mean slope), and it was 5 ng/mL (however, concentrations less than 10 ng/mL showed CVs more than 10% and were not incorporated in the curves). The detection limit (3 x signal-to-noise ratio) was 1.5 ng/mL.

Figure 1 shows typical chromatograms of standard tadalafil solution, extracted sample at 800 ng/mL, blank serum, blank serum with internal standard, and extracted sample at 10 ng/mL.

Extraction Yield

Several extraction solvents were attempted: diethylether, ethylacetate, chloroform, n-hexane, n-pentane, and dichloromethane. Best results were obtained with dichloromethane. Extraction recoveries determined by comparing the peak heights obtained by direct injection of standard tadalafil solution with those obtained after dichloromethane extraction of serum samples were not less than 75% over the 10 to 800 ng/mL concentration range (Table 3).

Stability

The amount of tadalafil recovered over a 6-month period in serum samples stored at –20°C did not show significant differences from the initial concentrations (Table 4).

DISCUSSION AND CONCLUSION

A sensitive, reliable, and accurate reversed-phase HPLC method was described in this report for the determination of tadalafil in human serum. This analytical procedure is relatively inexpensive and simple and is particularly suitable when tandem mass spectrometric detection is not available. The assay was unaffected by the presence of heparin or sodium ethylenediaminetetraacetic acid in the collection tube. The method described here can be used for determination of tadalafil concentrations in drug monitoring or for pharmacokinetic analysis purposes.

REFERENCES

1. Pomerol JM, Rabasseda X. Tadalafil, a further innovation in the treatment of sexual dysfunction. *Drugs Today (Barc)*. 2003;39:103-113.
2. Francis SH, Corbin JD. Molecular mechanisms and pharmacokinetics of phosphodiesterase-5 antagonists. *Curr Urol Rep.* 2003;4:457-465.
3. Seftel AD. Phosphodiesterase type 5 inhibitor differentiation based on selectivity, pharmacokinetic, and efficacy profile. *Clin Cardiol.* 2004;27(4 suppl 1):I14-I19.

4. Bella AJ, Brock GB. Tadalafil in the treatment of erectile dysfunction. *Curr Urol Rep.* 2003;4:472-478.

5. Gupta M, Kovar A, Meibohm B. The clinical pharmacokinetics of phosphodiesterase-5 inhibitors for erectile dysfunction. *J Clin Pharmacol.* 2005;45:987-1003.

6. Meuleman EJ. Review of tadalafil in the treatment of erectile dysfunction. *Expert Opin Pharmacother.* 2003;4:2049-2056.

7. Corbin JD, Francis SH. Pharmacology of phosphodiesterase-5 inhibitors. *Int J Clin Pract.* 2002;56:453-459.

8. Ramakrishna NV, Vishwottam KN, Puran S, et al. Quantification of tadalafil in human plasma by liquid chromatography-tandem mass spectrometry with electrospray ionization. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2004;809:243-249.

9. Gratz SR, Flurer CL, Wolnik KA. Analysis of undeclared synthetic phosphodiesterase-5-inhibitors in dietary supplements and herbal matrices by LC-ESI-MS and LC-UV. *J Pharm Biomed Anal*. 2004;36:525-533.

10. Zhu X, Xiao S, Chen B, et al. Simultaneous determination of sildenafil, vardenafil and tadalafil as forbidden components in natural dietary supplements for male sexual potency by high-performance liquid chromatography-electrospray ionization mass spectrometry. *J Chromatogr A*. 2005;1066:89-95.

11. Rodriguez Flores J, Berzas Nevado JJ, Castenada Penalvo G, Mora Diez N. Development of a micellar electrokinetic capillary chromatography method for the determination of three drugs employed in the erectile dysfunction therapy. *J Chromatogr B Analyt Technol Biomed Life Sci.* 2004;811:231-236.