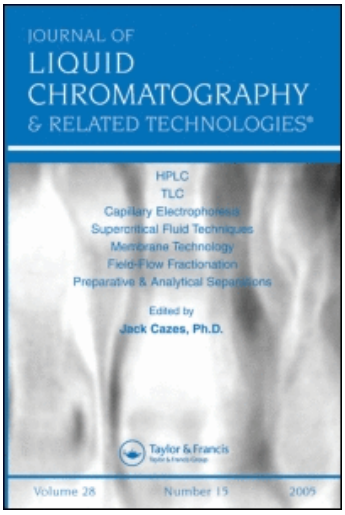

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Rapid Determination of Sildenafil Citrate in Pharmaceutical Preparations Using Monolithic Silica HPLC Column

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Rapid Determination of Sildenafil Citrate in Pharmaceutical Preparations Using Monolithic Silica HPLC Column

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ABSTRACT

A simple, rapid, and sensitive high performance liquid chromatography (HPLC) method has been developed and validated for the determination of sildenafil citrate in pharmaceutical preparations. The method was developed utilizing a 100×4.6 mm I.D. monolithic silica column and an isocratic elution of 60 : 40, v/v acetonitrile/water. The elution of the analyte was monitored at 292 nm with a flow rate of 2.0 mL/min. All analyses were conducted at ambient temperature. Linearity was observed in the concentration range from 50–3000 ng/mL, with a correlation coefficient (R^2) greater than 0.999. The limit of detection was 25 ng/mL. Parameters of validation prove the precision of the method

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and its applicability for the determination of sildenafil citrate in pharmaceutical tablet formulations. The method is fast (less than 1 min) and is suitable for high throughput analysis of the drug.

Key Words: Sildenafil; Viagra; Monolithic silica; Pharmaceutical analysis; Validation.

INTRODUCTION

Speed of analysis is becoming increasingly important in many application areas of HPLC, such as pharmaceutical analysis and toxicology laboratories, in order to increase throughput and reduce costs. Many drugs and biomedically important compounds are bases, the analysis of which remains problematic due to poor peak shapes which are often experienced in reversed phase chromatography.[1] In the past, efforts to decrease analysis times have focused on the use of short columns with particles that are smaller than the standard 5 μm. These columns offer good efficiency with higher flow rates, but also have a tendency to "plug" and back pressures tend to be high.[2] Many researchers have been trying to overcome the problem of high pressure drop associated with the use of small particles by employing ultra high-pressure liquid chromatography (UHPLC),[3] capillary electrochromatography (CEC),[4] or by open tube liquid chromatography.[5]

Recently, columns made of a single piece of monolithic silica were introduced as an alternative to particle-based columns. These columns possess a biporous structure consisting of larger macropores (2 μm) that permit high flow rates with low back pressure and smaller mesopores (13 nm) that provide a high surface area for high efficiency.[6] Therefore, it is possible to perform analyses with high linear flow velocity but without significantly reduced separation efficiency. The monolithic column has been used to analyze six-hydroxylated debrisoquine isomers,[7] ochratoxin A in different wines,[8] rofecoxib and its metabolite in human plasma,[9] and methylphenidate, with its de-esterified metabolite in rat plasma.[10]

Sildenafil citrate is a therapy used for the treatment of male penile erectile dysfunction through selective inhibition of phosphodiesterase type 5 to decrease the metabolism of cyclic guanosine monophosphate (cGMP), which induces smooth muscle relaxation in the corpus cavernosum with onset around 30–45 min.[11] Sildenafil is chemically known as 1-[[3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1H-pyrazolo[4,3-d]pyrimidine-5-yl)-4-ethoxyphenyl] sulfonyl]-4-methylpiperazine citrate (Fig. 1).

Sildenafil citrate in pharmaceutical formulations has been determined by voltammetric method.[12] Reversed phase HPLC methods have been utilized

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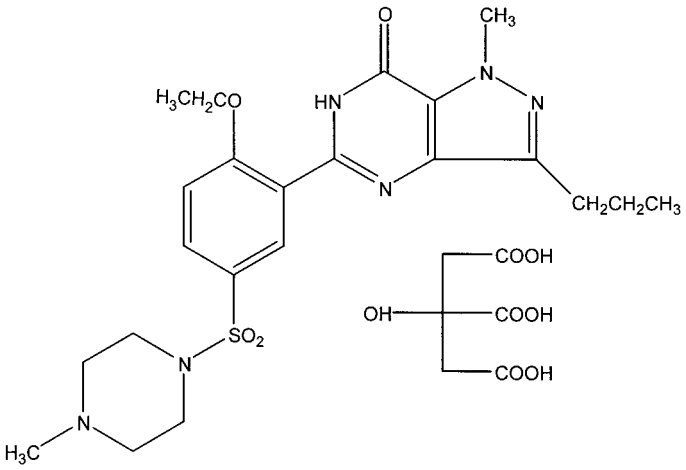

Figure 1. The chemical structure of sildenafil citrate.

for the determination of sildenafil citrate in dosage forms.[13–16] All these methods employed C18 columns and different composition of mobile phases and the retention time of the drug ranges from 10–15 min. In this study, a HPLC method for the quantitation of sildenafil citrate in pharmaceutical preparations is reported using a monolithic silica column with a retention time of the analyte less than 1 min.

EXPERIMENTAL

Chemical and Reagents

Sildenafil citrate was a gift from Pfizer Central Research (Sandwich Kent, NJ). HPLC-grade acetonitrile and methanol were purchased from Fisher Scientific (Fairlawn, NJ). Analytical reagent grade sodium dihydrogen phosphate and phosphoric acid were obtained from BDH Chemicals (Poole, UK). Viagra tablets® (containing 50 mg of sildenafil citrate/tablet) were obtained from Pfizer Scientific Office (Riyadh, Saudi Arabia).

Instrumentation and Chromatographic Conditions

The HPLC system consisted of a water solvent delivery pump (Model 510, Milford, MA), waters injector with a 20 μL sample loop (Model WISP 710B),

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Lambda max model 481 LC spectrophotometry UV, and a Hewlett-Packard 3394A integrator (Avondale, PA). Separations were performed on a reversed phase monolithic silica column [ChromLith Performance RP-18e, 100 × 4.6 mm I.D., Merck (Darmstadt, Germany)]. The mobile phase was acetonitrile : water (60 : 40, v/v). The mobile phase was filtered through a Millipore membrane filter (0.2 μm) from Nihon, Millipore (Yonezawa, Japan) and degassed before use. The flow rate was 2.0 mL/min and the chromatograms was monitored by UV detection at a wavelength of 292 nm.

Preparation of Standard Stock Solution

Stock solution of sildenafil citrate was prepared by accurately weighted 50 mg of the drug and dissolved in the mobile phase to 100 mL volumetric flasks, serial dilutions were carried out, using the mobile phase, to obtain the concentration ranges cited in Table 1.

Preparation of Standard Solution of Viagra

Four tablets were ground and powdered, an accurately weighted portion equivalent to 50-mg sildenafil citrate transferred to 100 mL volumetric flasks,

Table 1. Determination of sildenafil citrate in pure form by the proposed HPLC method and reference method.

	Proposed method			Reference method[13]		
Analyte	Nominal conc. (ng/mL)	Measured conc. (ng/mL)	Recovery[a] (%)	Nominal conc. (ng/mL)	Measured conc. (ng/mL)	Recovery (%)
Sildenafil citrate	50	50.90	101.8	50	51.75	103.5
	100	99.25	99.2	200	204.60	102.3
	200	195.60	97.8	500	503.50	100.7
	500	493.55	98.7	1,000	1009.00	100.9
	1,000	1027.90	102.8	3,000	3045.00	101.5
	2,000	2012.50	100.6	6,000	5910.00	98.5
	3,000	3040.70	101.4	7,500	7135.00	101.8
Overall recovery			100.3			101.3
RSD (%)			1.67			1.42

[a]The results are the average of three separate determination.

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mobile phase was added to the mark. The solutions were sonicated for 15 min, centrifuged at 3000 rpm for 10 min, and the supernatant was used to prepare solutions of various quantities of the analyte using the mobile phase as dilutent (Table 2).

Quantitation and Linearity

Equal volumes, (20 μL) of the standard preparation and the assay preparations that contain sildenafil citrate in the mobile phase were injected into the chromatograph and the chromatograms were recorded. Calibration standards of each concentration were analyzed in triplicate. Calibration curves of sildenafil citrate were constructed using the observed peak area vs. nominal concentrations of the analyte. Least squares linear regression analysis of

Table 2. Determination of sildenafil citrate in pharmaceutical preparation by the proposed method and the reference method.

Pharmaceutical preparation	Nominal conc. (ng/mL)	Measured conc. (ng/mL)	Recovery (%)
		Proposed method	
Viagra tablet[a]	50	50.75	101.5
(50 mg sildenafil	150	152.25	103.0
citrate/tablet)	300	303.50	101.2
	900	927.50	103.0
	1,500	1537.50	102.5
	2,500	2545.00	101.1
Overall recovery			102.2
RSD (%)			1.67
		Reference method[13]	
Viagra tablet[a]	50	52.00	104.0
(50 mg sildenafil	400	410.00	102.5
citrate/tablet)	900	931.50	103.5
	1,500	1477.50	98.5
	4,000	4068.00	101.7
	7,000	7287.00	104.1
Overall recovery			102.4
RSD (%)			1.42

[a]Product of Pfizer Co. (New York, NY).

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the data gave slope, intercept, and correlation coefficient data. From this data, a first order polynomial model was selected for the analyte.

Specificity

The specificity of the method was investigated by observing any interference encountered from excipients present in the formulations. It was shown that these components do not interfere with the proposed method (Fig. 2).

Validation

The limit of detection (LOD) and the limit of quantitation (LOQ) were determined as 3 and 10 times the baseline noise, respectively, following

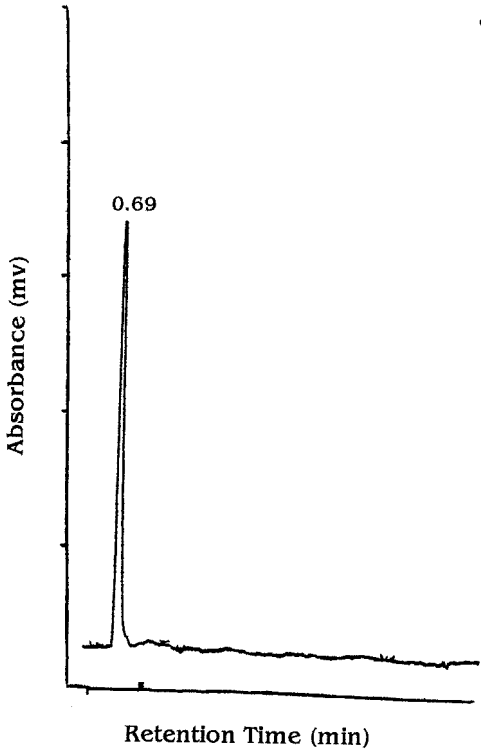

Figure 2. Typical chromatogram of sildenafil citrate in standard preparation. Chromatographic conditions: see Experimental.

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Table 3. Validation parameters for the determination of sildenafil citrate using the proposed method.

Parameter	Sildenafil citrate
Concentration range (ng/mL)	50–3000
Intercept (a)	0.3689
Slope (b)	0.0053
Correlation coefficient (R^2)	0.9997
$S_{y/x}$	0.4265
S_a	0.2245
S_b	0.3612
LOD (ng/mL)	25.0

the United States Pharmacopoeia.[17] The results of the statistical analysis of the experimental data, such as the slopes, the intercepts, the correlation coefficients obtained by the linear squares treatment of the results along with standard deviation of the slope (S_b) and intercept (S_a) on the ordinate and the standard deviation of the residuals ($S_{y/x}$) was shown in Table 3.

The good linearity of the calibration graphs and the negligible scatter of experimental points are clearly evident by the values of the correlation coefficient and standard deviation.[18] The robustness of the method is demonstrated by the versatility of the experimental factors that affect the peak area.

RESULTS AND DISCUSSION

Chromatography

The HPLC method carried out in this study, aimed at developing a fast chromatographic system, capable of eluting and resolving sildenafil from pharmaceutical preparations and that complies with the general requirements for system suitability. The preliminary investigations were directed toward the effect of various variables on the system suitability of the method. The parameters assessed include the detection wavelength, the type and quantity of the organic modifier, the salt concentration, and the pH of the mobile phase. Sildenafil citrate showed two UV-absorption maxima at 220–292 nm. The 292-nm wavelength showed a better resolution with steady baseline. The first trial was carried out by using mobile phase consisting of methanol–water (60 : 40, v/v). This system was found to be suitable to elute sildenafil but the retention time was long and a high tailing factor. As a mean to enhance the peak symmetry and reduce the tailing factor, the percentage of methanol

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portion in the mobile phase was decreased up to 30%. The results showed that the retention time increased but the tailing factor was so high for the sildenafil peak. Replacing methanol with acetonitrile was associated by enhancement of the peaks symmetry, a reduction in the tailing factor, and the retention time was short. Changing the water with phosphate buffer or acetate buffer of pH 5.5 and concentration from 0.1 to 0.5 M have no effect.

Consequently, the optimum chromatographic conditions with a mobile phase consisting of acetonitrile : water (60 : 40, v/v) were applied for all measurements. Figure 2 shows typical HPLC chromatograms for sildenafil using the optimized chromatographic system. The response factor R_f, (concentration/peak area), for the analyte under study was determined at 292 nm using a concentration of about 1000 ng/mL.

Specificity

The specificity of the method was investigated by observing any interference from excipients present in the pharmaceutical preparations. It was shown that these components do not interfere, thus, the HPLC method presented in this study is selective for sildenafil citrate. The specificity was also demonstrated by induced degradation of sildenafil citrate samples, by treating them either with 0.1 M HCl and storing the sample of room temperature for 24 h, or with 0.1 M NaOH and storing the sample at room temperature for 24 h. The recovery of sildenafil was 99.6% and 99.4% in the case of 0.1 M HCl and 0.1 M NaOH, respectively. The possible photodegradation of sildenafil citrate in solution of the mobile phase (sample of 2 mg/mL) was studied by exposing various samples of sildenafil citrate to direct sunlight and dark for 7 days. The samples kept in the dark showed full recovery without significant degradation. However, sildenafil citrate sample solutions showed a photodegradation of about 0.6%. Thus, the proposed method developed here is stability indicating, since it allows the separation of sildenafil from degradation products and it allows the quantitative determination of sildenafil in formulations.

Linearity

The linearity of calibration curves (peak area vs. concentration) for sildenafil citrate in pure solution, as well as in dosage forms, were checked over the concentration ranges of about 50–3000 ng/mL with correlation coefficient (R^2) of better than 0.999 as determined by least squares analysis.

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Limit of Detection, Limit of Quantitation, and Accuracy

The LOD and LOQ were calculated for the calibration graphs of sildenafil as 3 and 10 times of the noise level for LOD and LOQ, respectively. The values for LOD and LOQ are given in Table 3. The accuracy of the method was tested by analyzing different samples of sildenafil citrate at various concentration levels in pure solutions. The results were expressed as percent recoveries of the particular components in the samples (Table 1). Table 1 shows that the overall percent recoveries of sildenafil in pure solution by the proposed method were 100.3% and relative standard deviation (RSD) was 1.67, indicating that these values were acceptable and in agreement with the reference method.[13]

Stability of Analytical Solutions

The stability of sample solutions of sildenafil were tested by HPLC over 24 h. The freshly prepared and the 24 hour-stored samples were analyzed by the optimized proposed HPLC method. The percent difference observed was in the range of −0.28–0.26 (Table 4), indicating the possibility of using standard solutions of sildenafil citrate in pure solutions over a period of 24 h without degradation.

Robustness

The optimum HPLC conditions set for this method have been slightly modified for samples of sildenafil citrate as a means to evaluate the method ruggedness. The small changes made include the mobile phase ratio, the flow rate, and the detection wavelength (Table 5). Table 5 shows that the percent recoveries of sildenafil were good under most conditions and did not show a

Table 4. Stability of sildenafil citrate in standard solutions over a period of 24 h.[a]

Quantity added (ng/mL)	Quantity found (ng/mL)		Difference (%)
	Fresh solution	Stored solution	
100.25	100.63	100.91	−0.28
600.80	602.60	601.80	0.13
1500.00	1514.90	1511.70	0.21

[a]Difference (%) = {[(quantity found in fresh solution) − (quantity found in stored solution)]/(quantity found in fresh solution)} × 100.

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Table 5. Effect of experimental parameters on the percent recoveries of sildenafil citrate.

Parameters	Modification	Sildenafil recovery (%)
Mobile phase ratio (v/v)	Water : acetonitrile	
	40 : 60	100.3
	35 : 65	99.5
	45 : 55	100.4
Flow rate (mL/min)	1.5	99.4
	2.0	100.3
	2.5	99.8
Wavelength (nm)	295	99.8
	292	100.3
	285	99.2

significant change when the critical parameters were modified. Considering the modification in the system suitability parameters and the specificity of the method, as well as carrying the experiment at room temperature, would conclude that the method conditions are robust.

Application of the Analysis of Sildenafil Citrate in Pharmaceutical Formulations

The validity of the method developed here was applied to various concentrations taken from the pharmaceutical formulations (Viagra® tablets) for determining their content of sildenafil citrate. The values of the overall drug percentage recoveries with respect to the label claimed (Table 2) were 102.2% and RSD was 1.67, indicating that these values were acceptable and agreement with the reference method.[13]

CONCLUSION

A high-speed HPLC method has been developed and validated for the determination of sildenafil citrate in the tablet formulation. The method utilizes a new monolithic silica column technology and reversed phase HPLC analyses with UV detection. The method can be used for routine determination and chromatographic purity of sildenafil in bulk material and in dosage forms for quality control purposes.

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