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Research Article

Optimization of HPLC-FLD Conditions Using Analytical Quality by Design Approach for Quantification of Silodosin in Pharmaceutical Dosage Form

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Abstract

The objective of the present study was to develop a rapid and sensitive HPLC-florescence detection method for the quantification of silodosin in pharmaceuticals using "the Analytical Quality by Design (AQbD)" approach. For this purpose, at first, spectrofluorometric measurments were conducted to determine optimum excitation and emission wavelengths for silodosin, and they were found as 226 and 456 nm, respectively. A central composite design methodology was applied for optimization of critical method parameters. The parameters that have an impact on chromatographic separation of silodosin were selected as pH, column temperature, and organic content of the mobile phase (acetonitrile %) considering previous studies in the literature. A quadratic three-factor central composite design model consisting of 20 experimental observations was used for optimization of the parameters. According to the response surface methodology, the optimized conditions for the column temperature, acetonitrile percentage and the pH of the mobile phase were found as 36.8 °C, 28.2% and 3.3, respectively. The optimized method was validated according to ICH guidelines for accuracy, precision, working range, reproducibility, the limit of detection, the limit of quantification, and robustness. The method was linear in the range of 0.1–40 µg/mL, with a high correlation coefficient (0.9991) and acceptable precision (RSD<7.8%). Using the AQbD approach has provided advantages in terms of time consumption and costs. After validation studies, the developed method was successfully applied in the analysis of silodosin-containing tablet formulation indicating that the method could be used for routine quality control analyses.

Keywords: silodosin, analytical quality by design approach, central composite design, fluorescence detection, pharmaceutical analysis.

Farmasötik Dozaj Formlarında Silodosin Miktar Tayini için HPLC-FLD Yöntem Koşullarının Tasarım Yoluyla Analitik Kalite Yaklaşımıyla Optimizasyonu

Öz

Bu çalışmada "Tasarım Yoluyla Analitik Kalite (AQbD)" yaklaşımı kullanılarak farmasötiklerde silodosin miktarının belirlenmesi için hızlı ve hassas bir HPLC-floresans dedeksiyon yönteminin geliştirilmesi amaçlanmıştır. Bu kapsamda, öncelikle silodosin için optimum eksitasyon ve emisyon dalga boylarını belirlemek amacıyla spektroflorometrik ölçümler gerçekleştirildi ve dalga boyları sırasıyla 226 ve 456 nm olarak belirlendi. Kritik yöntem parametrelerinin optimizasyonu için bir merkezi kompozit tasarım metodolojisi uygulandı. Silodosinin kromatografik ayırımında etkili olan parametreler, literatürde daha önce yapılmış çalışmalar dikkate alınarak pH, kolon sıcaklığı ve mobil fazın organik içeriği (% asetonitril) olarak seçildi. Parametrelerin optimizasyonu için 20 deneysel gözlemden oluşan ikinci dereceden üç faktörlü bir merkezi kompozit tasarım modeli kullanıldı. Yanıt yüzeyi metodolojisine göre, kolon sıcaklığı, asetonitril yüzdesi ve mobil fazın pH'ı için optimize edilmiş koşullar sırasıyla 36,8 °C, %28,2 ve 3,3 olarak belirlenmiştir. Optimize edilen yöntem, doğruluk,

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kesinlik, çalışma aralığı, tekrarlanabilirlik, teşhis sınırı, tayin sınırı ve sağlamlık için ICH yönergelerine göre valide edildi. Yöntem, yüksek korelasyon katsayısı (0.9991) ve kabul edilebilir kesinlik değeri (RSD<%7,8) ile 0,1-40 µg/mL aralığında doğrusallık gösterdi. AQbD yaklaşımının kullanılması da zaman ve maliyetler açısından avantajlar sağlamıştır. Validasyon çalışmaları sonrasında geliştirilen yöntemin silodosin içeren tablet formülasyonunun analizinde başarıyla uygulanması, yöntemin rutin kalite kontrol analizlerinde kullanılabileceğini göstermektedir.

Anahtar Kelimeler: silodosin, analitik tasarım yoluyla kalite, merkezi kompozit tasarımı, floresans dedeksiyon, farmasötik analiz

1. Introduction

Benign prostatic hyperplasia (BHP) is a chronic disease in men with an incidence that is age-dependent resulting from lower urinary symptoms (Marks, Gittelman, Hill, Volinn, & Hoel, 2013; Yoshida, Kudoh, Homma, & Kawabe, 2011). α1-adrenergic receptor blockers (ABs) are effective pharmaceuticals, which represent the first-line of medical therapy for BPH. ABs are used by older men to eliminate the symptoms of an enlarged prostate, such as urinary compression, the frequency of urination, pausing during urination, poor flow, and incomplete bladder emptying (Er & Erk, 2016). Silodosin (SLD), is the last member of the AB ingredient family approved by the US Food and Drug Administration (FDA) for the treatment of BPE (October 2008) (Figure 1). It has been proven that SLD has higher selectivity for the dominant subtype of the α1A-adrenergic receptor in prostate tissues and inhibits the contraction of prostate smooth muscle. Therefore, it is widely used clinically in the treatment of BHP (Marks, Gittelman, Hill, Volinn, & Hoel, 2009; Roehrborn, Cruz, & Fusco, 2017; Yoshida et al., 2011).

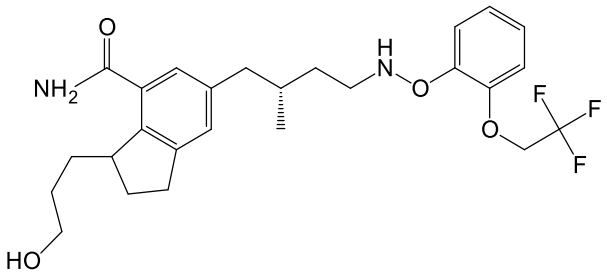

Figure 1. Chemical structure of Silodosin.

The use of the Analytical Quality by Design (AQbD) approach during the development of a new analytical method is an effective strategy in terms of time and quality (Kurmi, Singh, Bhutani, Singh, & Beg, 2014; Peraman, Bhadraya, & Reddy, 2015). "Quality by Design" has introduced by the FDA Office of Generic Drugs (OGD) for the purpose of "placing quality into pharmaceutical products to ultimately protect patient safety" (Vogt & Kord, 2011). The International Conference on Harmonization (ICH) has also defined QbD as a systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management (ICH, 2009). Analytical separation methods are essential for the quality control and analysis of active pharmaceutical ingredients and pharmaceutical products (Raman, Reddy Mallu, & Reddy Bapatu, 2015). Therefore, they are considered as part of QbD

concepts and expected to meet the stipulated quality requirements during production (Gavin & Olsen, 2008). The classical experimental procedure for method optimization by HPLC is performed by monitoring the effect of a factor on the experimental response under conditions where all other factors are kept constant. This optimization technique is time-consuming and costly. In addition, it ignores the interactions between the examined variables (Bezerra, Santelli, Oliveira, Villar, & Escaleira, 2008). AQbD approach has been used to provide fast and robust optimization of analytical methods by using multivariate statistical techniques. Response surface methodology (RSM) is one of the most widely-used statistical techniques in analytical optimization (Orlandini, Pinzauti, & Furlanetto, 2013). Central composite design (CCD) defines the design space used to assess the quadratic effects of factors in RSM (Gunst, 1996).

Along with the developing technology, the determination of drugs in different matrices by bioanalytical methods has become possible. There are publications in the literature showing the determination of SLD in pharmaceutical preparations by different analytical methods. It has been previously reported that several analytical methods have been developed to identify SLD in drug preparations such as high-performance thin-layer chromatography (HPTLC) (S & Shankar Iyer, 2012), spectrofluorometry (Er & Erk, 2016; Rajput & Rajput, 2014), UV-spectrophotometry (Aneesh T P & Rajasekaran, n.d.), high-performance liquid chromatography (HPLC) methods coupled with ultraviolet (HPLC-UV) (Kishore, 2012), photodiode Array (HPLC-DAD) (Boltia, Abdelkawy, Mohammed, & Mostafa, 2018; Er & Erk, 2016) and mass spectrometry (Nair, Kumar, Sharma, & Karia, 2016; Zhao et al., 2009) detection methods. To the best of our knowledge, there are no studies showing the quantitative determination of SLD with high performance liquid chromatography with fluorescence detection in pharmaceutical preparations. In the present work, AQbD strategy was applied to develop an HPLC equipped with a fluorescence detector (HPLC-FLD) method for quantification of SLD in pharmaceuticals by using a CCD methodology. In this context, it is intended to improve and optimize the reported HPLC conditions for analysis of SLD in the literature.

2. Material and Methods

Chemicals and reagents

Silodosin (SLD) (purity >99%) and Carbamazapine (CRB) (as internal standard, purity >99%) were generously supplied by Novagenix Co. /Ankara/TURKEY). HPLC grade acetonitrile and methanol were obtained from Sigma Aldrich (St. Louis, MO, USA). sodium acetate, sodium phosphate and acetic acid used in buffer solutions to prepare mobile phases at different pH's were purchased from Sigma Aldrich (St.Louis, MO,USA). Hydrochloric acid (HCl, 37%) was purchased from Merck (Kenilworth, NJ, USA).

Instrumentation and chromatographic conditions

Liquid chromatographic experiments were performed using an HPLC system (Agilent 1200 Series) equipped with a fluorescence detector (Agilent, 1200), quaternary pump, solvent degasser, thermostatic column compartment and thermostatic automated injector. The separation was performed on a C18 column (5µ 4.6 mm × 150 mm, ACE, Aberdeen, Scotland). The pH values of the solutions were measured using a Mettler Toledo (Shangai, China) MP 220 pH meter. According to the conditions optimized by the response surface methodology, an isocratic elution system was used with a mobile phase mixture consisting of acetonitrile/methanol/buffer solution (28.2/56.8/15, v/v/v). The flow rate was 1.0 mL/min and the injection volume was set to 10 µL per sampling. During the determination of silodosin with a fluorescence detector, wavelengths of 226 nm for excitation and 456 nm for emission were used. The column temperature was set at 36.8 °C throughout the analysis.

Preparation of standard and sample solutions

Standard stock solutions of SLD and CRB (IS) were prepared by an accurate amount of pure SLD and CRB were weighed and dissolved in methanol (100 mL). Calibration standards and quality control solutions were prepared by diluting SLD and IS stock solutions in the methanol. The sample-set consisted of a pharmaceutical preparation that contained SLD (Urorec 8 mg Tablet, Turkey). Briefly, ten tablets of the pharmaceutical preparation purchased from the Turkish market were weighed and finely powdered. The amount corresponding to one tablet was weighed and dissolved in methanol. It was sonicated for 15 minutes, cooled to room temperature and filtered through a 0.45 µm membrane filter before the HPLC injection.

Method design and selection

To optimize the method parameters for determining SLD in pharmaceuticals, a central composite design was used. Thus, the three most effective parameters were selected and their combined effects were evaluated by surface response methodology. 20 different variations of these three factors (mobile phase pH value, column temperature and ACN composition in the mobile phase) were investigated. Six replicates of analysis on each method condition were performed to investigate experimental errors.

Software

The central composite design was performed and statistically evaluated by Design expert Software version 11 (Stat-Ease Inc., Minneapolis, MN, USA) and Excel 2016 (Microsoft, Redmond, WA, USA).

Validation of HPLC method

The optimized chromatographic method has been validated according to the International Harmonization Conference (ICH) Q2 (R1) (Borman & Elder, 2017) using accuracy, precision,

working range, reproducibility, the limit of detection, the limit of quantification, and robustness parameters.

System suitability tests

SLD's system suitability standard solutions were prepared in methanol to a concentration of 1 µg/mL. Prior to sample analysis, system suitability standard solutions were injected 6 times. The RSD (relative standard deviation) of the peak areas obtained was less than 2% and it was determined that the system was suitable for the analysis of SLD by USP.

Linearity and working range

In order to plot the calibration curve, standard working solutions (0.1, 0.5, 1, 5, 15, 20, 25, 40 µg/mL) were prepared by diluting the SLD stock solution with methanol to 7 different concentrations. In order to determine linearity and working range, solutions at seven different concentration levels were prepared and measured three times. By analyzing the standard working solutions with the optimized method, the resulting peak areas were plotted against the solution concentration and the calibration curves were derived. The linearity of the method was indicated by the slope of the calibration curve and the correlation coefficient. The linearity was found between 0.1-40 µg/mL.

Accuracy and precision

To determine the accuracy and precision of the method, quality control solutions with 3 different concentrations (7.5, 15, 25 µg/mL) in the working range were prepared from the SLD stock solution. Each QC solution was analyzed by the method developed 6 times on the same day and three consecutive days. The intra- and inter-day precision and accuracy of the method were determined using peak areas obtained against each concentration.

Limit of detection (LOD) and limit of quantitation (LOQ)

Standard solutions were prepared at concentrations smaller than 0.1 µg/mL, which is the lowest value of the calibration curve. The chromatograms of these solutions were taken and the signal/noise (S/N) ratio of these chromatograms was determined as the concentration limit (LOD) at which the concentration was 3 and the concentration limit for the concentration of 10 (LOQ). The LOD value for silodosin was 0.03 µg/mL and the LOQ was 0.1 µg/mL.

Robustness

After changing the operating parameters such as flow rate, mobile phase ratio and column temperature, the stability of the method was determined as a result of the evaluation of the system suitability parameters. The method parameters investigated in this study are Flow rate (± 10%), mobile phase ratio (± 2%) and column temperature (± 2 °C). In spite of the small changes in these parameters, the analysis was carried out with appropriate accuracy and precision.

Application of the method to pharmaceuticals

Analysis of SLD from the pharmaceutical preparation was performed at the respective wavelength and the results were evaluated. Recovery studies from the pharmaceutical preparation were performed by the standard addition method. Urorec capsules (Recordati SpA, Turkey) a pharmaceutical preparation containing 8 mg silodosin, were used for analyzes. Content of capsule (0.3 g) was weighed precisely and dissolved 10 mL of methanol solution. Then the solution was sonicated with an ultrasonic bath for 30 min, filtered with a 0.45 µm membrane filter and diluted to a final volume of 50 mL. 5 µg/mL diluted samples from the drug solution were injected into the HPLC system. Subsequently, the standard working solutions were added individually at three different concentrations (5, 10 and 15 µg/mL) on these solutions and HPLC runs were repeated. From the peak area of total concentration, the concentrations of the added standard solutions (5, 10 and 15 µg/mL) were determined and the analytical recovery values were determined by proportioning to the concentration of the drug solution (5 µg/mL).

3. Results and Discussion

Development of chromatographic conditions

In order to determine the most effective chromatographic parameter on the fluorescence response of SLD, a few experiments were performed. The preliminary traits and our knowledge from the literature were suggested that the use of mobile phase with organic content in chromatographic separation and column temperature have a corrective effect on the retention time and peak shape of SLD. Another important factor was the pH value of the mobile phase. It is known that the pH can be used to control the retention in reversed phase liquid chromatography because of the relationship between the pH of the mobile phase and the degree of ionization of the analyte molecule (RLoBrutto, Jonesa & MMcNairb, 2001). In addition, pH changes in the medium cause changes in the fluorescence properties for many fluorescent compounds (Williams & Bridges, 1964). Figure 2 shows the fluorescence responses obtained from buffer solutions with different pH values in the same mobile phase composition. After choosing the best conditions in which was obtained the highest detector response for SLD, optimization of these conditions was determined by the CCD experiment.

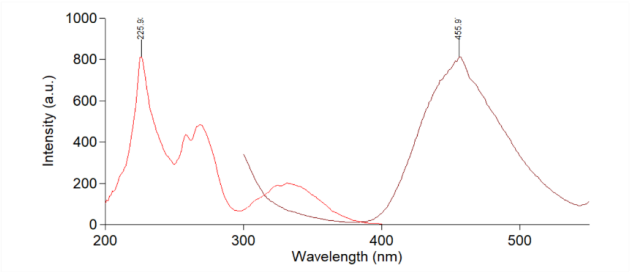

Figure 2. Fluorescence spectrum of silodosin comprising the successive excitation and emission scans. The wavelengths are shown maximum detector response was 226 and 456 nm for excitation and emission, respectively.

Central composite design

A central composite design was built using critical parameters (pH, organic content of mobile phase and column temperature) that are effective on fluorescence response. Before starting HPLC method development studies, silodosin standard solutions were measured with spectrofluorometry in order to identify excitation and emission wavelengths at which silodosin showed the highest fluorescence intensity and those are found as 226 and 456 nm for excitation and emission, respectively. It's well known that chemical parameters such as solvents, pH and temperature are highly effective on the relative fluorescence intensity of a chemical compound in the analytical methods (So & Dong, 2001). Preliminary studies on HPLC have shown that the changes made in the methanol composition did not have a large effect on the peak shape and resolution of SLD, but on the contrary, the changes in water and especially the acetonitrile composition, as well as the pH of the aqueous phase were influenced by the peak shapes and resolution obtained based on the quantum yield effects of the fluorescent compounds (Er & Erk, 2016). Moreover, the changes in the column temperature, which is another factor affecting the results in chromatographic analyzes, were found to be effective on retention times and peak area of SLD. Therefore, the experimental design studies were based to these three factors which seem to be effective on SLD HPLC-FLD analysis; acetonitrile composition of mobile phase, pH of mobile phase and column temperature.

In this study, a quadratic three-factor CCD model consisting of 20 observations was used. Experimental results obtained in the fluorescence responses to SLD runs under changed conditions are presented in Table 1. After experimental runs, the second-order polynomial response equation (equation 1) was used to identify between dependent and independent variables.

Table 1. 20 observation conditions determined by a three-factor CCD model and the resulting HPLC-FLD peak areas.

Run	Factor A (%ACN)	Factor B (pH)	Factor C (Col. Temp.)	Response (Peak Area)	Run	Factor A (%ACN)	Factor B (pH)	Factor C (Col. Temp.)	Response (Peak Area)
1	30	3	40	1308.8	**11**	30	3	20	1113.6
2	22.5	4	30	1279.4	**12**	22.5	4	46.817	1305.4
3	22.5	5.681	30	955.7	**13**	22.5	4	30	1272.8
4	15	5	40	1070.7	**14**	30	5	20	1118
5	22.5	4	13.182	1302.8	**15**	15	5	20	1068.3
6	22.5	4	30	1279.2	**16**	15	3	20	1076.1
7	22.5	2.318	30	963.7	**17**	35.113	4	30	1348
8	22.5	4	30	1274.5	**18**	9.886	4	30	1160.5
9	30	5	40	1117.2	**19**	22.5	4	30	1262.4
10	15	3	40	1087.9	**20**	22.5	4.	30	1264.4

$Y = b0 + b1X1 + b2X2 + b3X3 + b12X1X2 + b13X1X3 + b23X2X3 + b11X12 + b22X22 + b33X32$ (Equation 1)

where, Y is the peak area obtained from fluorescence response of SLD, Xi are the three independent variables (% acetonitrile content of mobile phase, pH of mobile phase and Column temperature) and b values are regression coefficients. The coefficients in Equation 2 represent the expected change in response per unit change in factor value when all remaining factors are held constant (Gunst, 1996). The final equation which is mentioned to the coefficients of the quadratic model were estimated by least squares regression as follow (Equation 2);

$Y = 1273.62 + 49.05A – 16.52B + 15.59C – 20.27AB + 22.53AC – 25.67BC – 16.15A2 – 120.28B2 + 1.48C2$ (Equation 2)

The coefficients obtained in equation 2 determine the quadratic model's predictions of the fluorescence response for SLD under certain conditions. However, the model found after pairing the function to the data can sometimes not sufficiently describe the experimental domain studied (Bezerra et al., 2008). Therefore, we applied the ANOVA (analysis of variance) for testing the significance and adequacy of the model. Statistical parameters obtained from the ANOVA for the quadratic model are given in Table 2. The large f-value (34.07) of the model implied the model is significant and the variation of the response was directly related to the variation of the factors. The compatibility quality of the quadratic model was expressed by R^2 (correlation coefficient). The adjusted R^2 was 0.9400, indicating a good fit of the model based on the suggested value limit of R^2>0.80 (Arslan-Alaton, Tureli, & Olmez-Hanci, 2009). The p-value less than 0.050 is another indication that the model is statistically significant. A, A^2, B^2 are the significant model terms according to ANOVA results.

Table 2. ANOVA results for quadratic model

Source	SS	DF	MS	F-value	p-value
Model	2.125E+05	9	23616.65	34.07	< **0.0001**
A-%Acn	11818.86	1	11818.86	17.04	**0.0020**
B-pH	1156.12	1	1156.12	1.66	0.2256
C-Col Temp	34.07	1	34.07	0.05	0.8290
AB	483.60	1	483.60	0.69	0.4231
AC	804.00	1	804.00	1.15	0.3068
BC	1388.64	1	1388.64	2.00	0.1873
A^2	45434.12	1	45434.12	65.53	< **0.0001**
B^2	165781.72	1	165781.72	239.13	< **0.0001**
C^2	647.74	1	647.74	0.93	0.3565
Residual	6932.63	10	693.26		
Cor Total	2.195E+05	19	24309.91		
R^2 =0.7609					
$R^2_{adj.}$ = 0.9400					

SS: Sum of Squares, MS: Mean Square, DF: degrees of Freedom

Three-dimensional surface response graphs are frequently used in order to interpret the interaction of variables with the coefficients obtained in the predicted model. This visualization is usually drawn in a two-dimensional representation of a three-dimensional graph (Bezerra et al., 2008). Figure 3 illustrates the profiles for the quadratic response 3D surface plot determined the influence of two factors interaction on the peak area in the optimization of two variables. Figure 3(a) represents surfaces where the maximum point is located inside the experimental region. The figure shows that both the percentage of Acetonitrile and pH of the mobile phase are effective on the SLD fluorescence response when the column temperature is held at 30 °C. The optimum points where the highest fluorescence response was obtained for the acetonitrile percentage and the pH of the mobile phase were 28.2% and 3.3, respectively. In Figure 3(b) and Figure 3(c), it is seen that there are relatively flat zones in relation to C variable (Column Temperature) indicating that variation of this factor has little effect or no effect on the chromatographic system. This is why no significant differences could be obtained for C and C2 factors in the ANOVA analysis of the quadratic model (Table 3). Nevertheless, the optimum value for the column temperature in the quadratic model was 36.8 °C. This is remarkable, because the temperature is known to be effective on the fluorescence response and keeping the temperature at a constant value will be useful for the robustness of the chromatographic method.

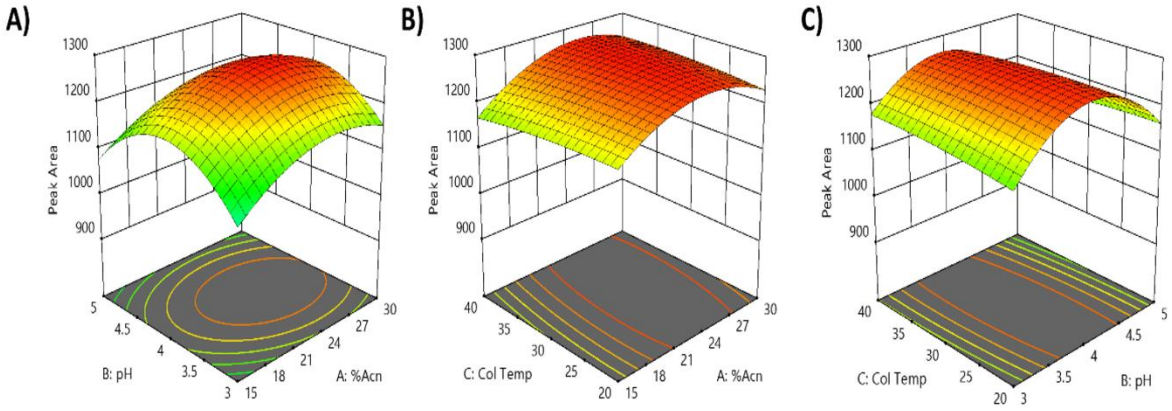

Figure 3. Quadratic 3D surface graphs constructed between two factors and peak area response A) pH and %ACN composition of mobile phase B) Column temperature and % ACN composition of mobile phase C) Column temperature and pH.

Table 3. The results of recovery experiments of pharmaceutical preparation analysis.

Drug	Added amount of drug (µg/mL)	SLD Standards (µg/mL)	Found (µg/mL)	Recovery (%)	Standard Deviation	RSD
Urorec Tablet 50 mg	5	5	5.22	104.4	0.35	7.08
		10	4.94	98.8	0.24	4.85
		15	4.98	99.6	0.45	9.03

Selection of internal standard

In order to determine the internal standard substance, HPLC runs were performed containing various drug-active substances one by one with SLD under optimized chromatographic conditions. CRB had a good resolution with symmetric peak shape and appropriate retention time which did not prolong analysis time (Figure 4).

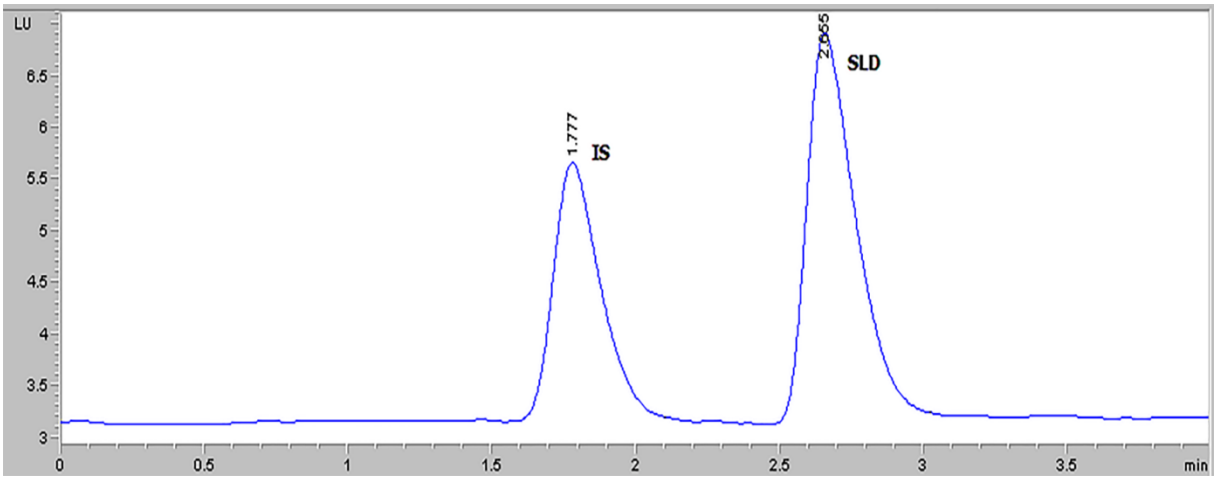

Figure 4. HPLC-FLD chromatogram of SLD and internal standard (Carbamazapine) in optimized HPLC conditions.

Analytical quality control and method validation

The method was validated for specificity, selectivity, sensitivity, linearity, precision, accuracy, robustness and recovery according to the International Conference on Harmonization (ICH) guidance Q2(R1) Validation of Analytical Procedure: Text and methodology (ICH , 2014). The results of the validation study are summarized in Table 4.

Table 4. Validation study results for silodosin analysis in optimized HPLC-FLD conditions.

Parameters	**Findings**
Linearity range (ng/ml)	0.1-40
Correlation coefficient (R^2)	0.9991
LOD (µg/ml)	0.03
LOQ (µg/ml)	0.1
Precision (CV)	
Intra-day	≤7.8%
Inter-day	≤6.8%
Accuracy (RE%)	(-6.2) to (8.6)

For specificity of the method, the blank solution containing the components which may be expected to be present in the pharmaceutical analysis of SLD (corn starch, Mannitol (E421), Magnesium stearate, Sodium lauryl sulfate, Gelatin and Titanium dioxide (E171)) was

injected to HPLC-FLD system under optimized conditions. No interfere effect was observed in SLD and IS chromatographic regions.

The calibration curve generated using the ratio of the SLD peak area to the IS peak area was linear in the range of 0.1-40 µg/ml. The mean slope of the calibration curve was "y = 136.89x + 12.738" and the mean correlation coefficient was 0.9991. The limit of quantification (LOQ) is 0.1 µg/ml and the detection limit of the method (LOD) calculated by the signal-to-noise ratio of the SLD peak was obtained as 0,03 µg/ml. Accuracy and precision studies were evaluated with HPLC injections on the same day and on different days of quality control samples at concentrations of 0.2, 1 and 3.75 µg/ml by having six replicates. Precision values were given by the coefficient of variation (CV). The highest CV values for intra- and inter-day precisions were determined as 7.8% and 6.8%, respectively. Intra- and inter-day accuracy were determined in terms of relative error; both were ranged from -6.2 to 8.6.

Recovery experiments were done by the standard addition method. The known amounts of SLD and IS mixtures at different concentrations (5, 10 and 15 µg/ml) were spiked with the pre-analyzed SLD commercial tablet solutions (Urorec 10 mg) at 5 µg/ml. The mixtures were reanalyzed by the developed method as described in the sample preparation section. Six different solutions were prepared for each recovery level. The results obtained are shown in Table 4, quite high recovery values have been obtained.

4. Conclusion

An HPLC-FLD method optimized for statistical tools was developed for analysis of SLD pharmaceutical products. Using the AQbD approach has provided advantages in terms of time consumption and costs. The CCD experimental method was used in the implementation of the required procedures in AQbD, such as detections of interactions between factors and simultaneously optimization of multiple responses. Thus, the critical factors affecting the process and their impact strength were also determined. The resulting method is fast with low analysis time and quite sensitive with the advantage of fluorescence detection.

The validation study under optimized conditions confirmed the method is specific, accurate, linear, reproducible and robust. Furthermore, the recovery study performed with SLD-containing commercial preparation showed high accuracy. Therefore, this HPLC-FLD method is suitable for use as a routine quality control analysis in a pharmaceutical environment.

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Conflict of interest

All authors declare no conflict of interest.

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