Determination of solifenacin succinate in human plasma and pharmaceutical formulations using HPLC-UV

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Abstract: An accurate, rapid and precise high performance liquid chromatographic coupled with ultraviolet detector method has been developed for the determination of solifenacin succinate in pharmaceutical formulation and human plasma. Quantitative analysis of solifenacin succinate was performed using a C18 column at wavelength at 210 nm. The mobile phase consisted of 10 mM ammonium formate buffer (pH 4.0), acetonitrile and methanol (52.5:32.5:12.5 v/v/v). The method showed linearity in the concentration range of 3-60 μg/mL and the correlation coefficient was 0.9998 and 0.9994 for the standard and human plasma solutions, respectively. The proposed method was within acceptable limits in terms of accuracy, precision, selectivity, linearity, sensitivity and recovery parameters. This method is readily applicable for the determination of solifenacin succinate in human plasma and pharmaceutical formulations.

1. Introduction

Lower urinary tract symptoms are the most common health problems of men in elders. Lower urinary tract symptoms; may develop due to reasons like nervous system diseases, aging, bladder diseases as nocturnal polyuria and bladder tumors, urethral diseases, ureter lower end diseases (distal ureteral stones), prostate diseases: overactive bladder and benign prostatic hyperplasia. In order to evaluate the condition of male patients suffered from these symptoms are nominated under the term lower urinary tract symptoms lower urinary tract symptoms[1]. Benign prostatic hyperplasia, a histopathological entity associated with lower urinary tract symptoms, does not cause Lower urinary tract symptoms alone in older men, but contributes significantly to the occurrence of symptoms[2].

One of the factors causing lower urinary tract symptoms is overactive bladder syndrome. Overactive bladder is a syndrome where symptoms such as sudden and severe urination, urinary incontinence, frequent urination (2-8 times a day) and urge to wake up at overnight are observed together[1]. Overactive bladder develops as a result of hypersensitivity of afferent nerves, disabling

inhibitory mechanisms and contractions similar to voiding reflexes or increasing intercellular connections between detrusor myocytes[3]. Overactive bladder with increasing incidence in later ages causes many problems in daily activities, work and sexual life, sleep patterns and therefore affects patients psychologically, physically and economically[4,5]. Benign prostatic hyperplasia causes bladder dysfunction for several reasons such as increased bladder internal pressure, denervation of bladder smooth muscle and bladder enlargement. These events develop hypersensitivity to acetylcholine in the bladder and it is accepted that the increased amount of acetylcholine leads to overactive bladder by increasing bladder contraction. Therefore, overactive bladder is observed in severe benign prostatic hyperplasia cases[6]. Recently, anticholinergic drugs have been frequently used together with alpha-blocker drugs in the treatment of benign prostatic hyperplasia or benign prostatic hyperplasia patients with overactive bladder syndrome. Studies support the good results of combination of anticholinergic drugs with alpha blockers[7-9].

Solifenacin succinate, which is classified as anticholinergic drugs, has the chemical formula $C_{23}H_{26}N_2O_2 \cdot C_4H_6O_4$ (Figure 1). Its molecular weight is 480.561 g/mol. In particular, the succinate salt of solifenacin with anticholinergic activity is preferred for overactive bladder treatment because of its very low efficacy for different ion channels and other receptors. Solifenacin succinate, a muscarinic M3 subtype receptor blocker; It inhibits the frequent and uncontrolled contractions of detrusutor and peripheral muscle fibers by inhibiting neuromuscular regions that provide bladder contraction through acetylcholine. Thus, symptoms of overactive bladder syndrome such as incontinence and frequent urination, as well as mental, social and physical limitations in quality of life are eliminated[10]. Different analytical methods for the determination of solifenacin succinate alone or in combination with other drugs, are available in the literature. These methods are as follows; spectrophotometry[11-15], HPLC[16-23] and LC-MS-MS[24-26] methods. Therefore, in this study; It is aimed to develop an accurate, sensitive and simple method for determination of solifenacin succinate in spiked human plasma and pharmaceutical formulations.
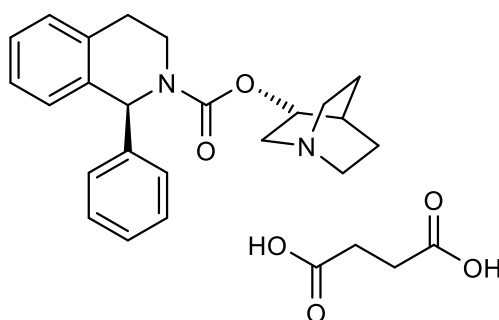

Figure 1. Chemical structure of solifenacin succinate

2. Experimental

2.1. Chemicals and Reagents

Solifenacin succinate and zolmitriptan (IS, purity>99%) were supplied by Zentiva (Ankara, Turkey) and Astra Zeneca (Istanbul, Turkey). Kinzy® tablets containing 5 mg solifenacin succinate per tablet are manufactured by Abdi İbrahim (Turkey). Phosphoric acid, sodium carbonate, tert-butyl-methyl ether, high purity methanol and acetonitrile, dichloromethane, hexane, ammonium formate and formic acid were purchased from Merck (Darmstadt, Germany). Since all chemicals were analytical grade, purification was not performed.

2.2. Instrumentation and Chromatographic Conditions

The HPLC system of the Agilent 1200 series consists of a G1311A/Quat Pump model, an Agilent G1329A/ALS automatic injector, Agilent G1322A degaszer, Agilent® 5 µm x 150 x 4.6 mm C18 column and G1314B/VWD model UV detector. Chromatographic separation was implemented at 30 °C column temperature using a mobile phase consisting of 10 mM ammonium formate buffer (pH 4.0), methanol and acetonitrile (52.5:32.5:12.5 v/v/v) at a flow rate of 1 mL/min. The absorbance wavelength used in the detector was 210 nm. The injection volume was 10 µL.

2.3. Preparations of Stock, Standard Working and Quality Control Solutions

Stock solutions of solifenacin succinate and IS were gently weighed and dissolved in methanol. Final concentration of each compound were arranged to be 500 µg/mL. Several different concentration of solifenacin succinate (3, 5, 10, 20, 30, 40, 45, 50 and 60 µg/mL) were prepared as standard working solutions. Each aliquots contained 50 µg/mL of IS.

Quality control samples were diluted via spiking equivalent amount of standard working solutions to 0.1 mL of blank plasma whose final concentrations set to be 15, 35 and 55 µg/mL for solifenacin succinate including 50 µg/mL IS. Liquid-liquid extraction procedure were applied onto spiked plasma samples. Analyte recovery of plasma samples were calculated from these solutions.

2.4. Preparation of Pharmaceutical Formulation

10 Kinzy tablets containing 5 mg solifenacin succinate were weighed separately and averaged. After the tablets were pulverized in mortar, the amount corresponding to 1 tablet was weighed and dissolved in an ultrasonic bath with methanol in a 10 mL flask. The solution was allowed to stand for 10 min and filtered through 12 mm filter paper. The prepared solution was diluted with water to the samples with an solifenacin succinate concentration of 10, 30 and 50 µg/mL samples containing 50 µg/mL IS. After the prepared samples were analyzed, standard solifenacin succinate solutions were added with a final concentration of 17.5, 37.5 and 57.5 µg/mL of the same samples to determine recovery from the pharmaceutical formulation by the standard addition method.

2.5. Preparation of Plasma Samples

The plasma removed from -20 °C was allowed to dissolve at room temperature. An appropriate amount of solifenacin succinate solutions at final concentrations of 3-60 µg/mL and IS solution at a final concentration of 50 µg/mL in each sample were spiked in 0.1 mL of blank plasma.

Solifenacin succinate was extracted from human plasma by the liquid-liquid extraction procedure. Appropriate amounts of solifenacin succinate (3, 5, 10, 20, 30, 40, 45, 50 and 60 µg/mL) and 50 µL IS were added in 100 µL plasma. Plasma samples were vortexed for 60 sec. 300 µL of 3 M Na_2CO_3 was added to precipitate the plasma proteins. 1 mL of Methyl tert-butyl ether *(*MTBE*):*hexane:dichloromethane mixture (2:1:1, v/v/v) was added to the plasma samples. All plasma samples were mixed for 20 minutes at 2000 rpm and then centrifuged at 8600 rpm for 25 minutes. The organic layers obtained from each sample were transferred to 2 ml eppendorf tubes and evaporated under room conditions using nitrogen gas. The mixture was stirred for 5 min and completed to 1 mL by ultra-purified water containing 0.5 M H_3PO_4. It was filtered through vials with a 45 µm pore size filter. 10 µL was injected into the HPLC-UV system.

2.6. Method Validation and Statistical Analysis of the Results

To illustrate the applicability of the proposed analytical method, the analytical method was validated according to the International Conference on Harmonization Conference (ICH) for approval of analytical procedures (ICH) using several parameters[27]. All statistical calculations were performed with Excel Functions and Formulas.

3. Results

3.1. Linearity

     In this study, the calibration graphs were plotted by plotting the ratio of the peak area of the internal standard to the peak area of the sample corresponding to each concentration. In the proposed method for the analysis of plasma and standard working solutions, a linearity in the range of 3-60 µg/mL was observed for solifenacin succinate. Calibration curves for solifenacin succinate was successfully plotted by evaluating the results of 9 different samples. Calibration curves was plotted by calculating the average value of three independent measurements for each predetermined concentration. Table 1 shows both the correlation coefficient and the regression equation for plasma and standard working solutions. In Figure 2 and 3, the calibration curves show that each point overlaps.

Table 1. The correlation coefficient and the regression equation of standard solutions and plasma samples of solifenacin succinate.

Solifenacin succinate	Range (µg/mL)	LR[a]	Sa	Sb	R
Standard work solutions	3-60	y= 0.1412x – 0.0176	4.3×10^{-4}	7.0×10^{-4}	0.9998
Plasma samples	3-60	y= 0.1097x – 0.0161	3.8×10^{-4}	3.0×10^{-4}	0.9994

R; Coefficient of correlation, LR; Lineer regression, Sa; Standard deviation of intercept of regression line Sb; Standard deviation of slope of regression line, [a] Based on six calibration curves, x; solifenacin succinate concentration (µg/mL)
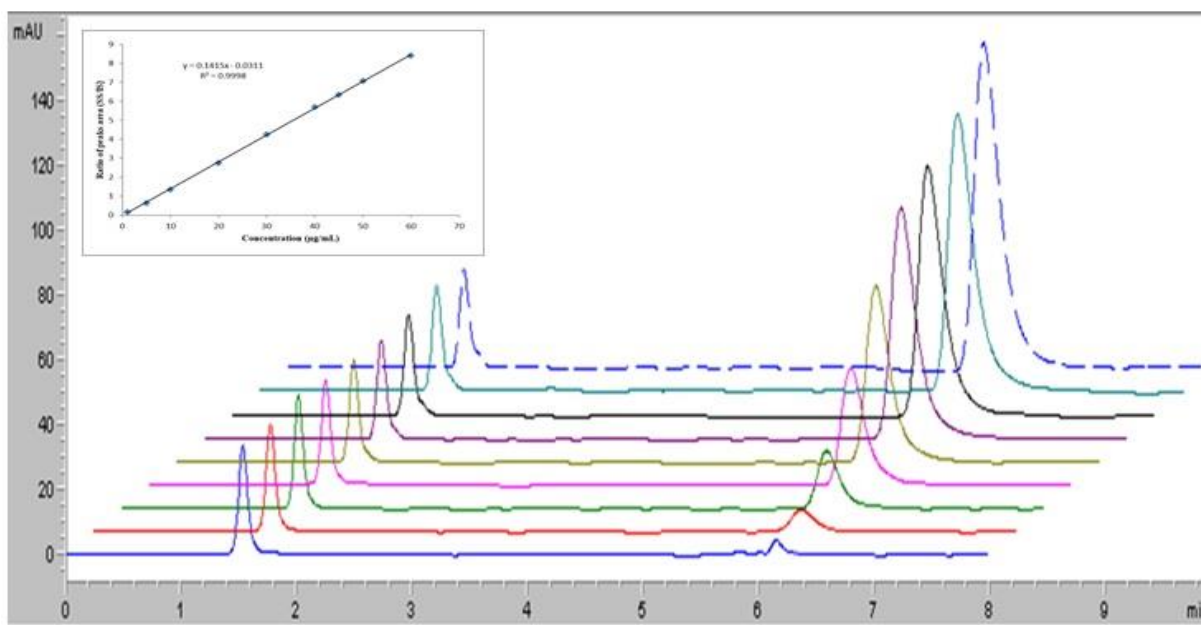

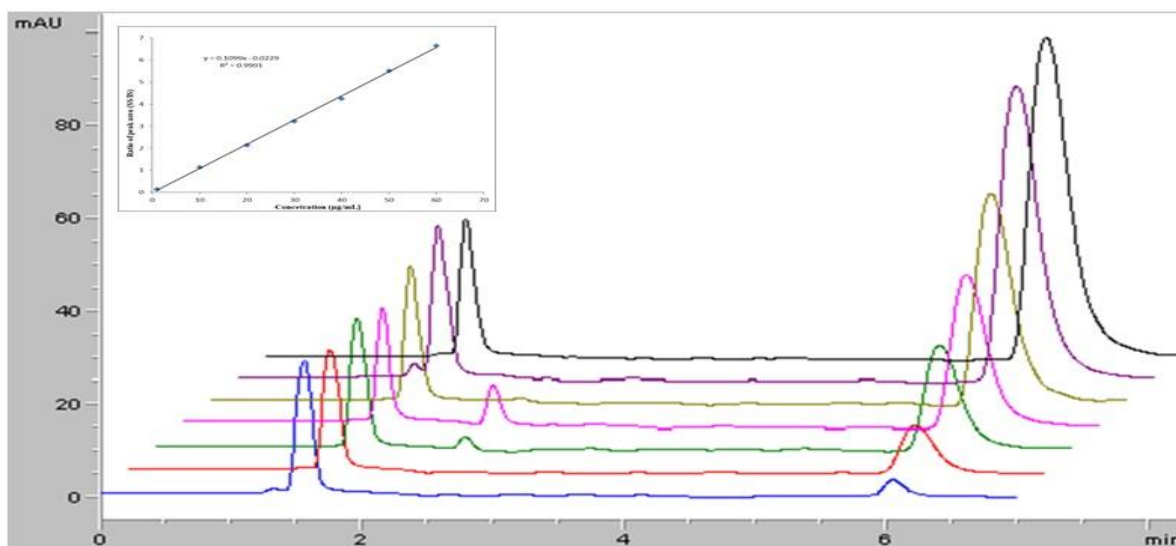**Figure 2.** HPLC chromatograms of standard solutions of solifenacin succinate (3, 5, 10, 20, 30, 40, 45, 50 and 60 µg/mL) and IS (50.0 µg/mL)

Figure 3. HPLC chromatograms of solifenacin succinate plasma samples (3, 5, 10, 20, 30, 40, 45, 50 and 60 µg/mL) and IS (50.0 µg/mL)

3.2. Selectivity and Specifty

The blank plasma solution, to which the extraction procedure was performed, was measured by the recommended method. Chromatograms of blank plasma and solifenacin succinate spiked plasma samples were compared. No interference was observed along the analysis at 210 nm. In addition, the major excipients in pharmaceutical formulation were dissolved in methanol and the proposed method was applied to the excipients, which did not interfere with the active pharmaceutical ingredients (API). In developed method, retention time for solifenacin succinate was 6.09 minute. IS was detected in 1.52 (Figure 2 and 3). These results show that; the method is specific and selectivity for the determination of solifenacin succinate in plasma samples and pharmaceutical preaparations.

3.3. Sensitivity

Limit of detection (LOD), defined as the concentration value corresponding to signal/noise (S/N) ratio 3 and the limit of quantification (LOQ, S/N: 10), defined as the lowest concentration that can be detected. The LOQ and LOD values of solifenacin succinate for the standard and plasma samples of proposed method was 3 µg/mL and 1 µg/mL, respectively.

3.4. Precision and Accuracy

Precision was determined by intra-day and inter-day precision. Intra-day precision; 3 different concentrations in the linear range were determined by measuring six replicates on the same day. Inter-day precision was determined by analyzing the same samples over the 3 following days. The concentrations of the quality control solutions prepared for accuracy and precision were 15, 35 and 55 µg/mL for solifenacin succinate. Relative standard deviation (RSD) values for precision and the relative error values for accuracy were calculated for solifenacin succinate. These results are given in Table 2. Obtained results for precision and accuracy were found to be acceptable.

Table 2. Precision and accuracy of the proposed method (n=6)

Added (µg/mL)	Intra-day			Inter-day		
	Found±SD (µg/mL)	Precision RSD%	Accuracy RE%	Found±SD (µg/mL)	Precision RSD%	Accuracy RE%
15	15.70±0.06	0.39	4.72	15.60±0.08	0.52	3.93
35	34.10±0.35	1.05	-2.64	33.90±0.95	-3.07	-3.07
55	55.60±0.83	1.50	1.19	53.40±0.88	-2.90	-2.92

SD; Standard Deviation, RSD; Relative Standard Deviation, RE; Relative Error

3.5. Recovery

The developed HPLC-UV method was applied directly to the drugs. The data obtained showed that the developed method can be safely applied for the quantitative evaluation of solifenacin succinate in pharmaceutical formulation. The standard addition technique was applied for recover in pharmaceutical formulation. For this, the quality control solutions were added to 7.5 µg/mL pharmaceutical formulation solutions and assayed with proposed method. The analytical recover was calculated [the total concentration of analyte-the concentration of pure analyte added)/the concentration of present analyte in formulation x 100] and founded as 100.2 ± 0.16% for solifenacin succinate (Table 3).

In the plasma recovery study, The quality control solutions of solifenacin succinate was added to the plasma with internal standards. After that, plasma samples were extracted by the proposed extraction method. Recovery of the method was calculated by the following formula: [(Peak area ratio of spiked plasma samples of API/IS)/(Peak area ratio of standards of API/IS) x100] and plasma recovery value is % 95.60±0.69.

Table 3. Recovery values of solifenacin succinate in samples (n=6)

Samples	Amount Taken (µg/mL)	Amount added (µg/mL)	[1]Amount Found ±SD (µg/mL)	Recovery % (RSD%)
Pharmaceutical Formulation: Kinzy 5 mg Tablet	7.5	10	9.91±0.10	99.2 (1.04)
		30	30.15±0.13	100.5 (0.44)
		50	50.57±0.24	101.1 (0.47)
Plasma Samples		10	9.31±0.25	93.1 (2.68)
		30	28.89±0.84	96.3 (2.91)
		50	48.7±0.98	97.4 (2.01)

SD; Standard deviation of six replicate determinations, RSD; Relative standard deviation,
[1]amount found for pharmaceutical formulation: [total amount found-amount taken]

3.6. Application of the Proposed Methods in Pharmaceutical Formulation

The developed and validated HPLC-UV method were applied to determine solifenacin succinate in Kinzy tablet (5 mg) and the quantitative analysis of solifenacin succinate was performed directly without any derivatization. For evaluate the amount of solifenacin succinate in tablet, the tablet samples were analyzed six times after preparation of tablet as mentioned in "preparation of pharmaceutical formulation" section. The results obtained by proposed method are given in Table 4.

Table 4. Determination with proposed method of solifenacin succinate in pharmaceutical formulations

Drug	Label claim (mg per tablet)	Amount Found (mg)±SD	Mean recovery (%)	RSD (%)	Confidence interval
Kinzy	5	4.98±0.26	99.94	3.55	98.55-101.28

SD; Standard deviation of six replicate determinations, RSD; Relative standard deviation

4. Discussion

The effectiveness of lower urinary tract symptoms, which adversely affects the quality of life of elders, is tried to be reduced by trying the last different treatment methods. The drug groups used for lower urinary tract symptoms in the clinic are now preferred more often than not alone, but in combination. For this purpose, anticholinergic drugs are used in the treatment of both overactive bladder and benign prostatic hyperplasia. Different analytical methods are available in the literature for the determination of solifenacin succinate alone or in combination with other drugs. The reported spectrophotometric methods do not allow a wide working range for the determination of solifenacin succinate[11-15]. In addition, these methods include complex sample preparation and derivatization steps[11,13,15]. The HPLC methods in the literature, on the other hand, lead to a loss of time and solvent consumption due to long analysis periods of approximately 30 minutes[17,18,21,22]. The total analysis time of the present method is 7 minutes. It is shorter than the methods in the literature. In addition, in our method, solifenacine succinate is eluted in less time than the reported methods[19,21-23]. The low injection volume[17, 16] and mobile phase flow rate[18] of the method we developed provide an advantage for less chemical consumption than the reported methods. The fact that our method has a lower LOQ value than the HPLC study reported[18] in the literature shows that our method is more sensitive. When the recoveries from the pharmaceutical preparation are compared with the studies in the literature, our method is superior[18, 20]. The extraction procedures used in the LC-MS / MS methods reported for the determination of solifenacin succinate in human plasma showed recovery from human plasma lower than the extraction procedure we developed. In addition, more biological material is used in these methods for the extraction of solifenacin succinate[24-26]. In our extraction procedure, a very low human plasma, such as 100 μL, is sufficient for high efficiency recovery of solifenacine succinate. Solifenacin succinate is commonly used in pharmaceutical formulation for overactive bladder and this proposed method could be applied in drug monitoring studies of solifenacin succinate without time consuming and avoiding batch interferences. Determination of solifenacin succinate provides lower chemical and biological material usage. In addition to this, quality control studies could be succeeded in shorter period with lower chemical usage. Our method differs from the methods reported in the literature[7-17] considering the chromatographic parameters used such as UV wavelength, column, temperature, detector, flow rate, preparation procedures of standard and plasma samples.

5. Conclusion

In summary, a rapid, sensitive and convenient HPLC-UV method has been developed that allows the determination of solifenacin succinate in human plasma and pharmaceutical formulation. A novel extraction method were applied that provides good recovery for spiked human plasma samples. The method was validated according to ICH guidelines. Results showed that the method is suitable for accuracy, precision, sensitivity, recovery, linearity, specifity and selectivity parameters. The method can be applied for routine analysis of solifenacin succinate in quality control assays and pharmaceutical formulation. Proposed method could also be used in drug monitoring studies of each drug.

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