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Stability-Indicating Method Development and Validation of Solifenacin Succinate by RP-HPLC

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ABSTRACT

The present study describes a stability indicating reversed phase High Performance Liquid Chromatographic (RP-HPLC) method development and validation of Solifenacin succinate API. Phenomenex Luna C18(150x4.6mm)5µm column was used as stationary phase while mobile phase used is pH 3.0 1-octane sulphonic acid with OPA: Acetonitrile in the ratio of 60:40. Method was developed in isocratic mode with 10 minutes run time, at a flow rate of 1.0 mL/min. Eluent was monitored at 220 nm. The method was validated for specificity, linearity, accuracy, precision, robustness and solution stability. Linearity was conducted in the concentration range of 10-80µg/ml and the correlation coefficient was found to be more than 0.999. Recovery was found to be in the range of 70-130%. Degradation studies were performed by subjecting the sample for various stress conditions like acid, base, peroxide, photolytic, thermal and the % degradation was found to be satisfactory. The proposed method was successfully applied for the quantitative determination of Solifenacin succinate API and in formulations.

Keywords: Solifenacin succinate, RP-HPLC, Method Development, Validation, Stability-indicating.

1. Introduction

Solifenacin is indicated for the treatment of patients with over active bladder such as urinary urgency and high urinary frequency. The antispasmodic effect is thought to be

mediated through antagonist activity at muscarinic receptors. It binds competitively at M3 receptors and reduces of smooth muscle tone thus retaining the larger volumes of urine and reduce the number of urgency and incontinence episodes. Solifenacin is approximately 98% (in vivo) bound to human plasma proteins principally to acid glycoprotein. Solifenacin[1-3] is highly sensitive to non-CNS tissues having the mean steady volume of distribution of 600L. Metabolism: Solifenacin is extensively metabolized in the liver. The elimination is by the way of CYP3A4. The primary metabolic route of Solifenacin are through N-oxidation of quinuclidine ring and 4R- hydroxylation of tetra hydroisoquinoline. One primary active metabolite (4R- hydroxy Solifenacin) contribute to clinical activity. The inactive metabolites are N-glucuronide- oxide and 4R-hydroxy-N-oxide of Solifenacin. It is used in the treatment of overactive bladder such as urgent urination and incontinence.

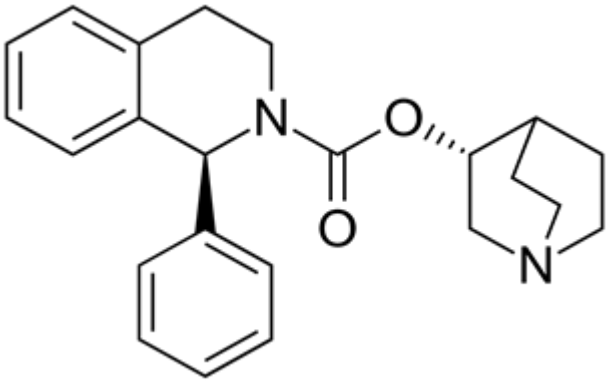

Figure 1. Chemical structure of Solifenacin succinate

Extensive literature review was conducted and an attempt was made to develop an unambiguous, valid method for the estimation of solifenacin succinate. Few of spectroscopic, chromatographic, and other analytical methods [4-19] have been reported for the estimation of solifenacin succinate individually and or along with drug combinations in pharmaceutical preparations. The aim of this study is to develop and validate a new simple, accurate and economic

stability-indicating RP-HPLC method with less runtime, which would be able to separate and quantify solifenacin succinate in a single run. The developed method was validated as per ICH guidelines [20-21] and can be applied lucratively to quality control purposes.

2. Materials and methods

Equipment

The Method development and Validation was carried out using Waters Alliance-HPLC system equipped with waters 1525 binary HPLC pump, 2695-separation module connected to 2996-photo diode array detector, and Waters 2707 auto sampler. The data was acquired by Empower® version 2. The other equipment used were Ascoset Electronic balance, ADWA pH meter, heating mantle. Ultrasonic bath was used for sonication of the samples. Hot air oven was used to carry out thermal degradation studies. UV cross linker, with series of 23400 model UV chamber, equipped with a UV fluorescence lamp with the wavelength range between 200 & 300 nm was used for photo degradation studies.

Chemicals and Reagents

Solifenacin succinate working standards was kindly given as gift sample by Spectrum Pharma Limited, Hyderabad. HPLC grade solvents include acetonitrile, water and methanol. Analytical grade chemicals include sodium hydroxide, hydrochloric acid, 20% hydrogen peroxide, Ortho phosphoric acid, Triethyl amine and potassium dihydrogen phosphate were purchased from E. Merck Limited, Mumbai, India.

Chromatographic conditions

HPLC analysis was carried out on Waters Alliance-HPLC system equipped with 2695-separation module connected to 2996-photo diode array detector and the data was acquired by Empower® version 2. Separation was achieved using Phenomenex Luna C18(150x4.6mm)5µm as a column with mobile phase of pH 3.0 1-octane sulphonic acid with OPA: Acetonitrile in the ration of 60:40. The samples were analyzed using 20 µL injection volume, Flow rate was maintained at 1.0mL/min with runtime of 10 min and the temperature was maintained at 30°C throughout the analysis. Detection and purity establishment of the drugs were achieved using PDA detector at 220 nm wavelength.

Preparation of Working Standard Solution

Weigh and transfer about 50 mg of Solifenacin succinate working standard into 100ml volumetric flask, add about 50mlof diluent. Dilute to the volume with diluent and mix well. Pipetted 5ml of the above solution is transferred into a 50 ml of volumetric flask and dilute to volume and mix.

Preparation of Sample Solution

Weighed and transfer 10 tablets into a 200 ml volumetric flask. Add 140 mL of diluent and sonicated for 30 minutes with intermediate shaking. Allow the sample to cool to temperature and dilute to the volume with diluent and mix. Transferred the above solution sample solution into centrifuge and centrifuged at 2500 rpm for 10minutes. Pipette out 5.0 mL of above supernatant liquid in to 25 ml volumetric flask. dilute to the volume with the diluent.

Method Validation

The developed and optimized RP-HPLC method was validated according to international conference on harmonization (ICH) guidelines Q2(R1) in order to determine the system suitability, linearity, limit of detection (LOD), limit of quantification (LOQ), precision, accuracy, ruggedness and robustness.

System suitability

System suitability parameters were evaluated to verify system performance. 20 µL of standard solution was injected five times into the chromatograph, and the chromatograms were recorded. Parameters such as number of theoretical plates and peak tailing were determined.

Specificity

The specificity of the analytical method was established by injecting the solutions of diluent (blank), placebo, working standards and sample solution individually to investigate interference from the representative peaks.

Precision

Repeatability/ method precision was performed by injecting six replicates of same concentrations of Solifenacin succinate, calculated % assay and %RSD. Reproducibility/ Ruggedness/ Intermediate precision was performed using different analysts and a different instrument in the same laboratory.

Accuracy

Accuracy of the proposed method was determined using recovery studies by spiking method. The recovery studies were carried out by adding known amounts (50%, 100% and 150%) of the working standard solutions of Solifenacin succinate to the pre-analysed sample. The solutions were prepared in triplicates to determine the accuracy.

Linearity

Linearity was evaluated by analyzing different concentrations of the standard solutions of Solifenacin succinate. Six working standard solutions ranging between 10µg/mL-80µg/mL were prepared and injected. The response was a linear function of concentration over peak area and were subjected to linear least-squares regression analysis to calculate the calibration equation and correlation coefficient.

Limit of detection and Limit of quantification

Limit of detection (LoD) and limit of quantification (LoQ) of Solifenacin succinate were determined by calibration curve method. Solutions of Solifenacin succinate were prepared in linearity range and injected (n = 3).

Robustness

To examine the robustness of the developed method, experimental conditions were deliberately changed, resolution, tailing factor, and theoretical plates of Solifenacin succinate peaks were evaluated. To study the outcome of the flow rate on the developed method, it was changed ± 0.2mL/minute. The effect of column temperature on the developed method was studied at ± 5°C, organic phase composition in mobile phase was changed ±10% and pH of the buffer is changed ±0.2. In all the above varied conditions, the composition of aqueous component of the mobile phase was held constant.

Forced Degradation Studies

Stress studies were performed by considering Solifenacin succinate working standard solution to provide the stability-indicating property and specificity of the proposed method. Intended degradation was attempted by the stress conditions of exposure to photolytic stress (1.2 million lux hours followed by 200 Watt hours), heat (exposed at 105°C for 6 hours), acid (1N HCl for 6 hours at 60°C), base (1N NaOH for 6 hours at 60°C), oxidation (20% peroxide for 6 hours at 60°C), water (refluxed for 12 hours at 60°C), and humidity (exposed to 90% RH for 72 hours). The solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

3. Results and discussion

System Suitability

From the results in table 1, the column efficiency for Solifenacin succinate peak was identified from the theoretical plate count which is more than 3000, tailing factor less than 2.0, %RSD was found to be less than 2.0%.

Table 1. System suitability data

System suitability parameter	Observed value	Acceptance criteria
USP Tailing factor	1.41	NMT2.0
USP Theoretical Plate Count	5765	NLT 2000
% RSD	0.05	NMT2.0%

Specificity

From the obtained chromatograms in figures 2 to 4 it can be inferred that there were no co-eluting peaks at the retention time of Solifenacin succinate which shows that peak of analyte was pure and the excipients in the formulation did not interfere with the analyte of interest.

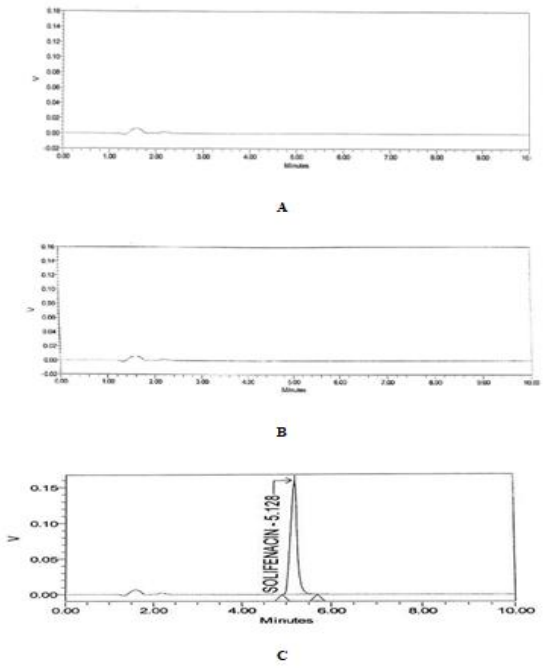

Figure 2. Chromatograms of (A) Blank, (B) Placebo, (C) Standard

Precision

From the results in table2, % Assay for Solifenacin succinate was found to be in the range of 98 – 102%, and the % RSD for Solifenacin succinate to be within 2%. Hence the method is precise, reproducible and rugged for 48 hours' study.

Table 2. Precision data

Injection	**Peak area (System precision)**	**%Assay (Method precision)**
01	1636895	98.4
02	1638092	98.1
03	1637238	98.2
04	1636328	98.1
05	1636388	98.4
06	1637666	98.1
Average	1637244	98.21
Standard deviation	**681.49**	**0.14**
%RSD	**0.04**	**0.1368**

Linearity

Linearity was evaluated by analysing different concentrations of Solifenacin succinate. From the results tabulated in table 3, it is inferred that the correlation coefficient was greater than 0.999. The slope and y-intercept values were also provided, which confirmed good linearity between peak areas and concentration.

Table 3. Linearity data

S. No	**Concentration (µg/ml)**	**Average peak area**
01	10	346863
02	25	841841
03	40	1370439
04	50	1667597
05	60	2006178
06	80	2674198
Correlation coefficient	**0.9998**	

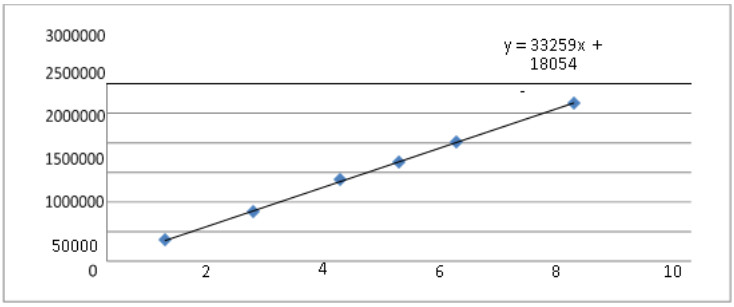

Figure 3. Calibration curve of Solifenacin succinate

Accuracy

From the results in table 4, the % recovery for Solifenacin succinate found to be in the range of 98 –102% and the % RSD for Solifenacin succinate is less than 2%. Hence the proposed method was accurate.

Table 4. Accuracy data

Sample no.	% spike level	Amount spiked	Amount recovered	%recovery	Average % recovery	
1	40%	9.82	9.60	97.8	99.1	99.46%
2		9.82	10.01	101.9		
3		9.82	9.61	97.8		
1	100%	24.55	24.59	100.3	100.2	
2		24.55	24.61	100.2		
3		24.55	24.60	100.0		
1	160%	39.28	39.16	99.7	99.1	
2		39.28	38.39	97.8		
3		39.28	39.20	99.8		

LoD and LoQ

The Limit of Detection and Limit of Quantification of Solifenacin succinate were calculated by using following equations (ICH, Q2 (R1)) and the LoD and LoQ values are reported in table 5. These LOD = 3.3 × σ/S and LOQ = 10 × σ/S

Where σ = the standard deviation of the response and S = slope of the calibration curve.

LOD was found to be 0.06µg/ml

LOQ was found to be 0.20µg/ml

Robustness

From the results in table 5, it is evident that the system suitability parameters such as resolution, RSD, tailing factor, and the theoretical plate count of Solifenacin succinate remained unaffected by deliberate changes. The results were presented along with the system suitability parameters of optimized conditions. Thus, the method was found to be robust with respect to variability in applied conditions.

Table 5. Robustness data

Parameters	System Suitability Parameters			
	Theoretical Plates	Peak Tailing	%RSD	RT (min)
Optimized method	6669	1.3	0.1	5.128
Flow rate (1.2 mL/min)	6207	1.3	0.1	4.474
Flow rate (0.8 mL/min)	7212	1.3	0.1	6.597
Temperature (35°C)	7063	1.3	0.1	5.109
Temperature (25°C)	6620	1.3	0.1	5.101
Organic phase composition (50%)	6162	1.4	0.1	4.244
Organic phase composition (30%)	7404	1.3	0.1	6.874
pH 3.2	6615	1.3	0.1	5.120
pH 2.8	6907	1.4	0.1	5.365

Forced Degradation Studies

The samples were analyzed with the above mentioned HPLC conditions using a PDA detector to monitor the homogeneity and purity of the Solifenacin succinate. The results which were shown in table 6 indicates that the degradation was not observed in photolytic stress, humidity, acid, base, water hydrolysis, and thermal stress studies. It was interesting to note that all the peaks due to degradation were well resolved from the peaks of Solifenacin succinate. Further, the peak purity of Solifenacin succinate was found to be homogeneous based on the evaluation parameters such as purity angle and purity threshold. Hence, the method is considered to be "stability-indicating."

Table 6. Forced degradation studies at different stress conditions

S. No	Stress condition	% degradatio n	Purity angle	Purity threshold
1	Unstressed Sample	0.0	0.221	0.343
2	Acid	1.95	0.166	0.306
3	Base	0.83	0.179	0.320
4	Thermal	2.6	0.376	0.544
5	Peroxide	0.2	0.164	0.315
6	Photolytic	2.0	0.166	0.303
7	Humidity	0.4	0.200	0.354

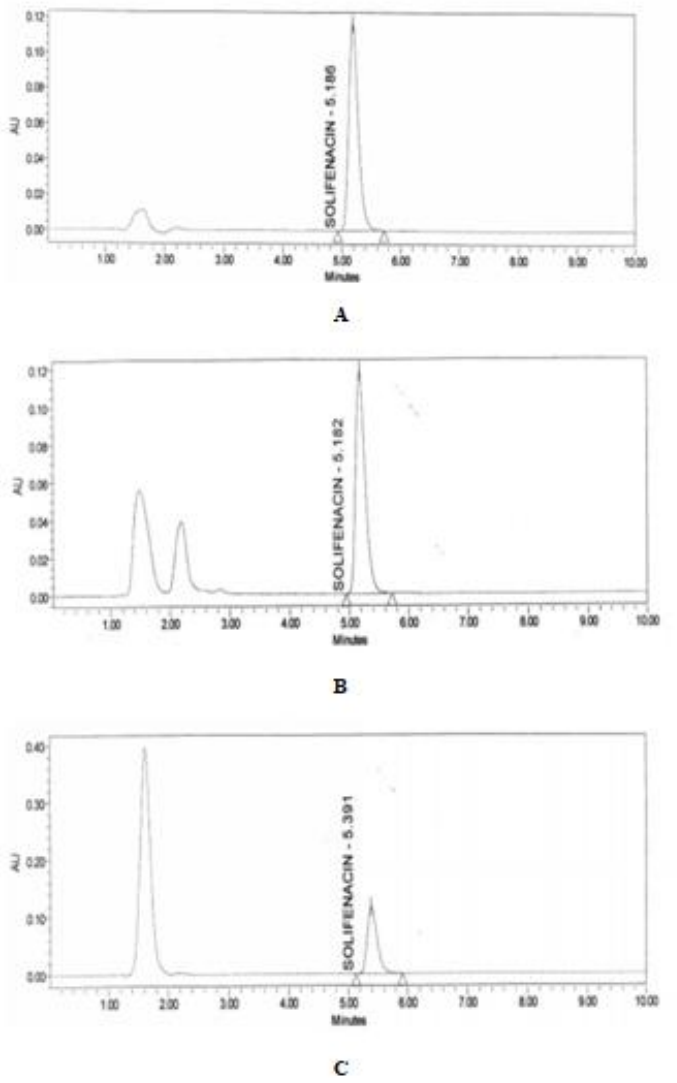

Figure 4. Degradation plots of (A) Acid stressed sample, (B) Base stressed sample, (C) Peroxide stressed sample

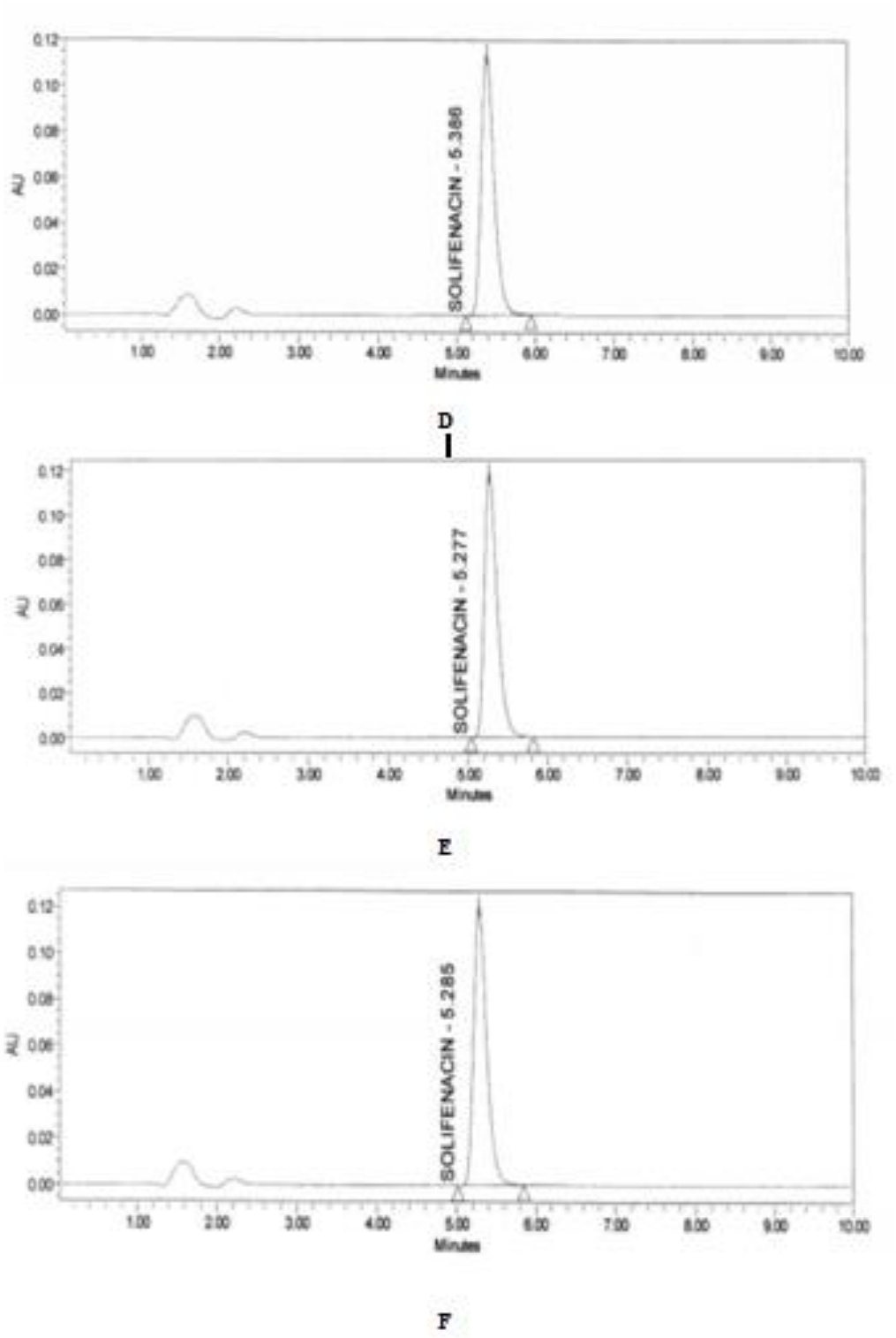

Figure 5. Degradation plots of (D) Dry heat stressed sample, (E) Photolytic stressed sample, (F) Humidity stressed sample

4. Conclusion

A simple and rugged RP-HPLC method has been developed for the determination of Solifenacin succinate in active pharmaceutical ingredients and sample. The proposed method was validated in accordance with ICH guidelines by testing its parameters which include system suitability, specificity, precision, linearity, LOD, LOQ, accuracy and robustness. The method was very specific to separate the peaks of active pharmaceutical ingredients from the degradation products which were obtained with good resolution after forced degradation studies. Thus, stress induced studies prove the effectiveness of the proposed stability-indicating RP-HPLC method which can be adopted in routine analysis in pharmaceutical industries.

Conflicts of Interest

The authors declare that they have no conflict of interest.

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