



DEVELOPMENT & VALIDATION OF STABILITY INDICATING HPLC METHOD FOR DETERMINATION OF SOLIFENACIN IN BULK FORMULATIONS

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ABSTRACT

A stability indicating, isocratic, rapid, specific, and sensitive high performance liquid chromatographic (HPLC) method was developed and validated for determination of Solifenacin in bulk drug. The quantitative determination of SOLIFENACIN was performed on a Hypersil C₈ (250mm × 4.6 mm i.d., 5 μm) column with mobile phase consist of 10 mM potassium dihydrogen phosphate buffer with 0.1% v/v TEA in Milli-Q water (pH-3): Acetonitrile (60:40 %v/v) pumped at a flow rate of 1 mL min⁻¹ at 25 °C. The eluents were monitored at wavelength 210 nm using photo diode array (PDA) detector. The drug was subjected oxidation, hydrolysis and heat to apply stress conditions. The developed method was validated as per ICH guideline for the parameters such as specificity, linearity, precision, accuracy, limit of detection, limit of quantification and found to be satisfactorily. The drug was found to be hydrolyzed in acidic and alkaline conditions. We also found the degradation under oxidative stress condition. The developed method was able to separate all degradation product generated under forced degradation studies and drug peak was eluted at 7.82 min. The response was linear in the drug concentration range of 1–30 μg mL⁻¹ (r²=0.9991) with limit of detection and limit of quantification 0.019 μg mL⁻¹ and 0.063 μg mL⁻¹ respectively. The Relative Standard Deviation (RSD) values for intra- and inter-day precision studies were 0.50% and 0.62% respectively. Mean % recovery (mean ± SD) was found to be 100.51 ± 1.98.

Keywords: Solifenacin, Stability Indicating, HPLC, Photo diode array detector

INTRODUCTION

Solifenacin is a prescription drug used for the treatment of overactive bladder in adults with symptoms of urinary incontinence, urinary urgency and urinary frequency.

The chemical name of Solifenacin succinate is butanedioic acid, compounded with (1S)-(3R)-1-azabicyclo [2, 2, 2] oct-3-yl 3, 4-dihydro-1-phenyl-2(1H)-isoquinoline carboxylate (1:1) and the empirical formula is C₂₃H₂₆N₂O₂·C₄H₆O₄ and its molecular weight is 480.55 and structural formula of Solifenacin succinate is represented in figure 1.

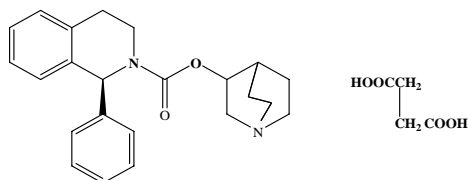


Fig. 1: Chemical structure of Solifenacin

Solifenacin is a competitive muscarinic receptor antagonist with selectivity for the urinary bladder over salivary glands. Solifenacin inhibits carbachol-induced intracellular calcium mobilization more potently in bladder smooth muscle cells than in salivary gland cells.

Solifenacin is approximately 98% (in vivo) bound to human plasma proteins, principally to α1-acid glycoprotein. Solifenacin is highly distributed to non-CNS tissues, having a mean steady-state volume of distribution of 600 L.

Solifenacin is extensively metabolized in the liver by CYP3A4 isoenzyme; however, an alternative metabolic pathway is also reported. The primary metabolic routes of Solifenacin are through *N*-oxidation of the quinuclidin ring and 4*R* hydroxylation of tetrahydro isoquinoline ring. One pharmacologically active metabolite (4*R*hydroxy Solifenacin), occurring at low concentrations and unlikely to contribute significantly to clinical activity, and three pharmacologically inactive metabolites (*N*-glucuronide and the *N*-oxide and 4*R*-hydroxy-*N*-oxide of Solifenacin) have been found in human plasma after oral dosing.

The major metabolites identified in urine were *N*-oxide of

Solifenacin, 4*R*-hydroxy Solifenacin and 4*R*-hydroxy-*N*-oxide of Solifenacin, and in feces 4*R*-hydroxy Solifenacin. The elimination half-life of Solifenacin following chronic dosing is approximately 45–68 hour¹.

Solifenacin exerts greater selectivity for urinary bladder over salivary gland than tolterodine, oxybutynin, darifenacin and atropine, and may consequently provide symptomatic benefit in the treatment of overactive bladder with less dry mouth than currently used anti-muscarinic drugs².

Solifenacin succinate is a novel muscarinic receptor antagonist, approved for the treatment of overactive bladder with affinity for muscarinic M₃ receptor subtype, high degree of selectivity and to the fact that most tissues or organs express multiple muscarinic receptors.

M₂ and M₃ receptors are mainly distributed in the bladder while M₃ subtype is distributed predominantly in the salivary gland and that M₃ subtype plays a major role in the physiological function of both organs. Solifenacin compared with oxybutynin binds to a greater extent to bladder M₃ muscarinic receptors in the bladder while it may exert a relatively little activity to bind exocrine M₃ muscarinic receptors^{3,4}.

Various methods are available for the analysis of Solifenacin in literature like LC-ESI-MS/MS, semi-micro high performance liquid chromatography. Analytical method for the estimation of Solifenacin in bulk drug was not reported by HPLC method or HPTLC method^{5,6}.

The aim of this work was to develop an accurate, specific, precise stability-indicating HPTLC method for the determination of Solifenacin. The developed method was validated as per the International Conference on Harmonization (ICH) guideline⁷.

Stability testing of new drug substances and products requires the stress testing to carry out and to elucidate the inherent stability characteristics of the active substance^{8,9}.

EXPERIMENT

Materials and methods

Solifenacin succinate (99.1 % as on dried basis) standard was kindly supplied by Cadila health care Ltd, Ahmadabad, India. HPLC grade methanol and acetonitrile were purchased from Merck chemicals,

Mumbai. The water for HPLC was prepared by double glass distillation and filtered through Milli Q water purification system. Potassium dihydrogen phosphate and orthophosphoric acid (OPA) were obtained from SD fine chemical Ltd. Mumbai, India.

HPLC consists of Shimadzu LC-10 ADVP solvent delivery system, SIL-10ADVP auto-injector, CTO-10ASVP column oven, SPD M-10AVP photo diode array detector, SCL-10 AVP System controller. The analytical column used was Hypersil C₈ column (250 mm × 4.6 mm i. d., 5 μm particle size). All analysis was carried out at a temperature of 25°C under isocratic conditions. The mobile phase consisted of a mixture of 10 mM potassium dihydrogen phosphate buffer with 0.1% TEA v/v at pH 3 (pH 3, adjusted with OPA): acetonitrile (60:40, v/v). The flow rate was 1 mL min⁻¹, the volume of injection was 20 μL, total run time was 12 min, and the detection was made at 210 nm. The mobile phase was filtered through Millipore filter (0.45 μm) and was degassed prior to use. All data integration was performed using class VP software.

Standard preparation

The assay concentration of Solifenacin was selected as 10 μg mL⁻¹. It was prepared by weighing 10 mg of Solifenacin and it was dissolved in 10 ml methanol to get concentration of 1 mg mL⁻¹ of Solifenacin. It was diluted with methanol to get a concentration of 100 μg mL⁻¹ (working stock). Further dilutions were carried out using the mobile phase (acetonitrile: 10 mM potassium dihydrogen phosphate buffer (pH 3) in the ratio of 40:60) to get resultant concentration of 10 μg mL⁻¹.

Linearity

From the working stock solution (100 μg mL⁻¹), the serial dilutions were done to get the concentration of 1, 5, 10, 15, 20, 25, and 30 μg mL⁻¹ respectively. A volume of 20 μL from each concentration of the solution was injected and chromatograms were recorded under the optimized chromatographic conditions. The peak area of the drug was plotted against the concentration of the drug.

Repeatability

Repeatability is the result of the method operating over a short time interval (within a day) under the same conditions. The peak area of assay concentration of the drug (10 μg mL⁻¹) was analyzed six times on the same day. The percentage relative standard deviation (% RSD) was calculated for the resultant peak area.

Intermediate precision

To assess the degree of reproducibility of the method, assay conc. (10 μg mL⁻¹) was analyzed on different day. The assay procedure was repeated six times and the chromatogram was recorded and the % RSD was calculated.

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value, which is accepted either as conventional true value or accepted reference value and the value found. The accuracy was done by recovery studies. The known amount of standard drug was spiked to the pre-analyzed samples and the recovery of the drug was calculated. Accuracy was performed at 3 levels of 80, 100 and 120% of standard concentration. A solution containing 10 μg mL⁻¹ of the sample was spiked with 80, 100, and 120% of the standard Solifenacin solution (8, 10, 12 μg mL⁻¹) and analyzed in same chromatographic condition. The percentage recovery was calculated from the peak area of the drug.

Robustness

It is the capacity of a method to remain unaffected by small deliberate variations in method parameters:

Influence of variations in flow rate (±0.1 mL min⁻¹)

Influence of variations of pH of mobile phase (± 0.2)

Influence of change in temperature (± 5°C)

Influence of variations in composition of mobile phase (± 2%)

Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD is ability of analytical method to detect the lowest conc. of the analyte. LOQ is the lowest conc. of the analyte, which can be quantitatively analyzed with acceptable precision and accuracy. It can be calculated based on signal to noise ratio.

Assay of formulation

The tablets were purchased from a local drug store (Solifenacinten 10 mg Tab) supplied by Ranbaxy pharmaceuticals, India. 10 Tablets were weighed and average weight was calculated. An amount equivalent to 10 mg of Solifenacin was weighed and dissolved in methanol. Then the solution was sonicated for 30 min and filtered. The filtrate was further diluted to get a concentration of 10 μg mL⁻¹ (theoretical value) and was injected to the HPLC system.

System suitability parameters

These parameters include plate number (N), capacity factor (k'), asymmetry factor, and relative standard deviation (RSD) for peak area for repetitive injections. These parameters were within the acceptable limits.

Stock solution stability

The freshly prepared standard stock solution of Solifenacin 10 μg mL⁻¹ was stored in the refrigerated temperature. The stability of the stock was analyzed after one month duration and compared with the initial concentration.

Specificity

Specificity was established by determination of purity of the drug peak using a PDA detector.

Degradation studies

Alkali hydrolysis

25 mg of Solifenacin was weighed accurately and transferred to 25 mL volumetric flask and dissolved in 1M sodium hydroxide solution. Immediately after making up the volume, the solution was transferred to round bottom flask and kept for refluxing at 80 °C, 1 mL sample was taken at 48 hrs and the sample was neutralized with 1 M hydrochloric acid solution. It was further diluted to 10 ml with mobile phase and loaded into HPLC system.

Acid hydrolysis

25 mg of Solifenacin was weighed accurately and transferred to 25 mL volumetric flask and dissolved in 1 M hydrochloric acid solution. Immediately after making up the volume, the solution was transferred to round bottom flask and kept for refluxing at 80 °C, 1 mL sample was taken at 48 hrs and the sample was neutralized with 1M sodium hydroxide solution and diluted to 10 ml with mobile phase and loaded into HPLC system.

Oxidative degradation

25 mg of Solifenacin was weighed accurately and transferred to 25 mL volumetric flask and dissolved in 3% v/v hydrogen peroxide solution. Immediately after making up the volume, the solution was transferred to round bottom flask and kept at room temperature, 1 mL samples was withdrawn at 48 hrs and diluted to 10 mL with mobile phase and loaded into HPLC system.

Thermal degradation

Powdered API was kept in a hot air oven at 65°C for 3 days. The drug was found to be stable to dry heat.

RESULTS AND DISCUSSION

Method development

The chromatographic conditions were adjusted in order to provide a good assay performance. Initial trials were done on Supelco C₁₈ column (250 mm × 4.6 mm i.d., 5 μm), but the peak was distorted. Finally, Hypersil C₈ column (250mm × 4.6 mm i. d., 5 μm particle

size) was selected. Optimization of mobile phase was performed based on peak parameters (resolution of drugs and degradation products, tailing), sensitivity of the assay, ease of preparation, readily available, and run time. The mobile phase consisted of a mixture of 10 mM potassium dihydrogen phosphate buffer with

0.1% TEA v/v at pH 3 (pH 3, adjusted with OPA): acetonitrile (60:40, v/v) was selected which gave sharp and very well resolved peaks. The volume of injection was 20 µL and detection was made at 210 nm. The retention time for Solifenacin was 7.82 min and the total run time was 12 min (Fig.2).

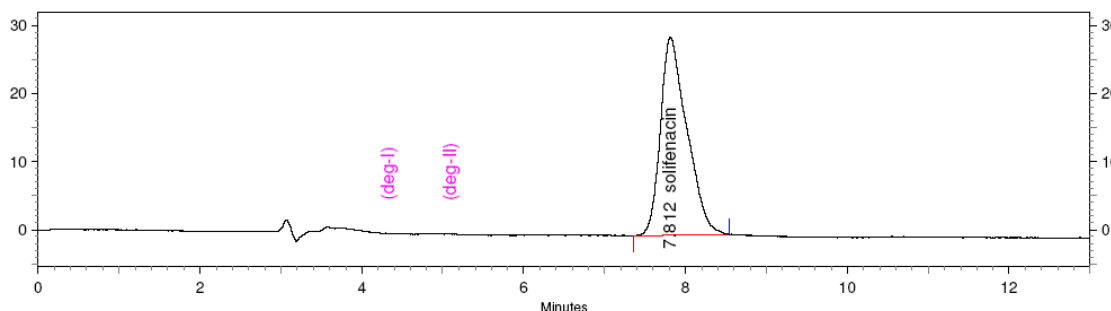


Fig. 2: Chromatogram of Solifenacin standard

Linearity

A linear calibration plot for Solifenacin was obtained at seven concentration levels (50- 150 % of assay concentration) in duplicate. Peak area of Solifenacin was plotted versus their respective concentrations and linear regression analysis performed on the resultant curves. Correlation coefficient was found to be more than 0.999.

Precision

The precision of the assay method was evaluated as repeatability and intermediate precision. For intra-day precision and inter-day precision, the % relative standard deviation (%RSD) of Solifenacin was found to be 0.50% and 0.62% respectively. These % RSD values were well within the generally acceptable limit of 2%, confirming good precision of the assay method. Result is given in table 1.

Table 1: Intra-day and inter-day precision of the method (n=6).

Amount added (µg/ml)	Peak area	SD	%RSD
Intra-day 10	743403	3738	0.50
Inter-day 10	755019	4753	0.62

LOQ and LOD

Limit of detection and limit of quantification was found to be 0.019 µg mL⁻¹ and 0.063 µg mL⁻¹ respectively.

Accuracy

The known amount of standard was spiked (80,100,120%) in triplicate to test solution and recovery of drug was calculated. The accuracy of method was calculated at three concentrations such as 8, 10 and 12 µg/mL of Solifenacin standard. The recoveries at three different concentrations were found to be within the range of 98 to 102 % as per ICH guidelines. Mean % recovery (mean ± SD) was found to be 100.51 ± 1.01. The results indicated that the recovery of Solifenacin in three different concentrations was more than 98%. Result is given in table 2.

Table 2: Recovery studies (n=6)

Amount added (µg/ml)	Amount recovered (µg/ml)	% Recovered
8	8.18	102.37
10	9.84	98.43
12	12.07	100.75

Mean % recovery =100.51

Robustness

The robustness of assay method was studied by incorporating small but deliberate changes in the analytical method (e.g. variations in flow rate, column temperature, mobile phase composition, pH of buffer, etc.) and also by observing the stability of the drugs for 24 hr at room temperature in the dilution solvent. In all the varied chromatographic conditions, there was no significant change in chromatographic parameters. Result is given in table 3.

Table 3: Robustness of the method (n=6).

Condition changed	SD of mean peak area	% RSD
Mobile phase composition	2836	0.47
pH of mobile phase	2199	0.30
Temperature	2514	0.64
Flow	3288	0.47

Assay of marketed formulation

Solifenacin R_t value of 7.89 min was observed for the chromatogram obtained from the drug samples extracted from the tablets formulation. There was no interference from excipients. The assay of Solifenacin was found to be 98.79%.

Alkali hydrolysis

Initially 0.5 M Sodium hydroxide concentration was used for the degradation of Solifenacin but it was found that the drug was stable and there was no degradation. So 1 M sodium hydroxide was used further and it showed that drug was degraded in 1M sodium hydroxide. The degraded peak was found at the retention time of 4.3 minutes. The spectrum of the degradation product was taken and found that spectra's were matching to the standard Solifenacin spectra. Peak purity index (0.9999) was greater than peak purity threshold (0.9991) which indicates that there is no merging of impurity peaks and peak is pure (fig.3, 4, 5).

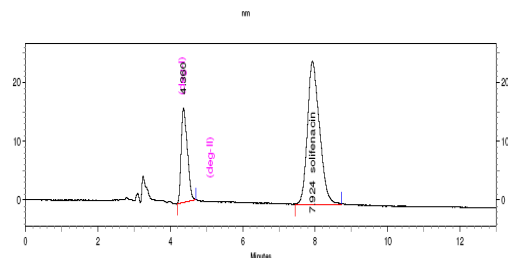


Fig. 3: Chromatogram of Solifenacin in 1N sodium hydroxide at 80 °C

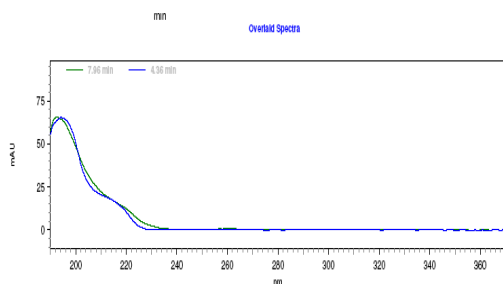


Fig. 4: Overlaid spectra of Solifenacin 1N sodium hydroxide at 80 °C

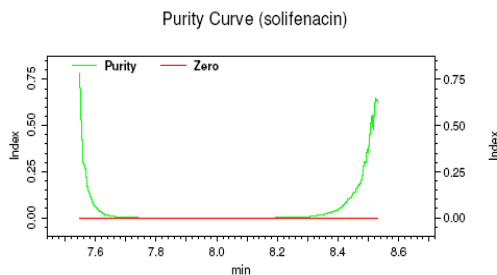


Fig. 5: Peak purity curve of Solifenacin in 1N sodium hydroxide at 80 °C

Acid induced degradation

Initially 0.5 M Hydrochloric acid concentration was used for the degradation of Solifenacin but it was found that the drug was stable and there was no degradation. So 1M hydrochloric acid was used further and it showed that drug degraded in 1M hydrochloric Acid. The degraded peak was at the retention time of 4.3 minutes. The spectrum of the degradation product was taken and found that spectra's were matching to that of standard Solifenacin spectra. Peak purity index (0.9999) was greater than peak purity threshold (0.9991) which indicated that there is no merging of impurity peaks and peak is pure (fig. 6, 7, 8).

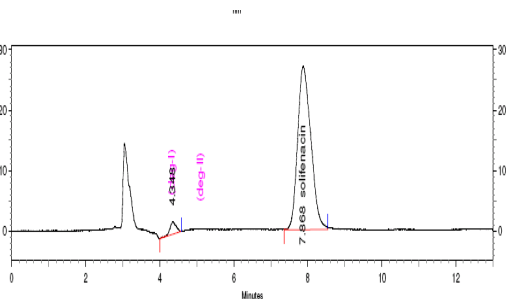


Fig. 6: Chromatogram of Solifenacin in 1 N hydrochloric acid at 80 °C

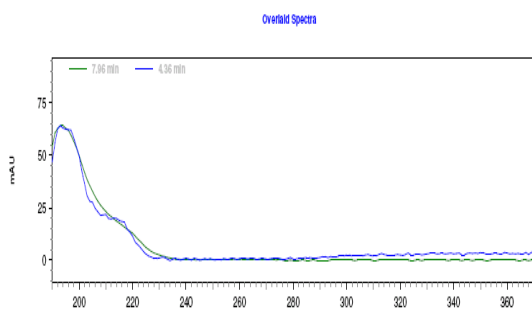


Fig. 7: Overlaid spectra of Solifenacin in hydrochloric acid at 80 °C

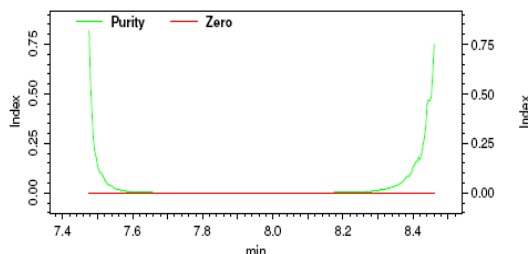


Fig. 8: Peak purity curve of Solifenacin 1N hydrochloric acid at 80 °C

Oxidative degradation

Initially 3% hydrogen peroxide was used for the degradation of Solifenacin; it showed that drug was degraded in 3% hydrogen peroxide. The degraded peak was found at the retention time of 5.3 minutes. The spectrum of the degradation product was taken and found that spectra's were matching to the standard Solifenacin spectra. Peak purity index (0.9999) was greater than peak purity threshold (0.9991) which indicated that there is no merging of impurity peaks and peak is pure (fig.9, 10, 11).

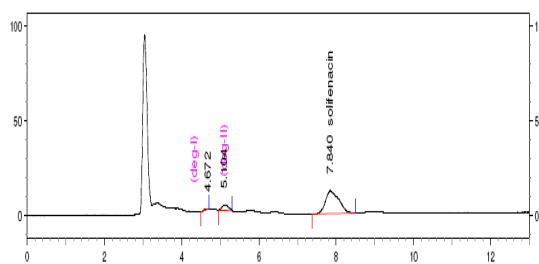


Fig. 9: Chromatogram of Solifenacin in 3% hydrogen peroxide at room temperature

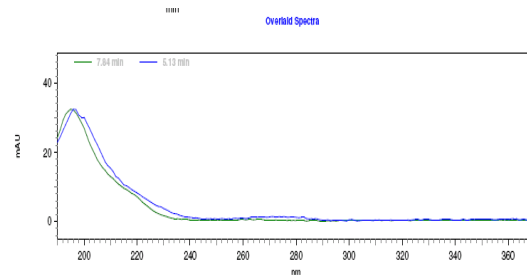


Fig. 10: Overlaid spectra of Solifenacin 3% hydrogen peroxide at room temperature

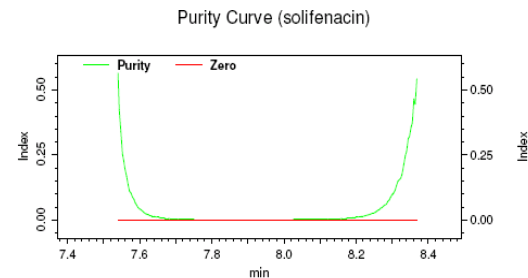


Fig. 11: Peak purity curve of Solifenacin in 3% hydrogen peroxide at room temperature

Thermal degradation

There was no degradation observed at 65 °C for 2 weeks. It indicated that the drug is stable to heat.

Degradation results are given table 4.

Table 4: Thermal degradation of the product

Stress condition applied	Retention times of degradation products
Alkaline hydrolysis	4.36 min.
Acid hydrolysis	4.35 min.
Oxidative degradation	5.10 min.

Table 5: Summary of validation data

Validation property	Data
Linearity range($\mu\text{g}/\text{mL}$)	1-30
Coefficient of determination	0.9991 \pm 0.01
Limit of detection ($\mu\text{g}/\text{mL}$)	0.019
Limit of quantification ($\mu\text{g}/\text{mL}$)	0.063
% Recovery (n = 6)	100.51
Precision (RSD [%])	
Intra-day precision (n = 6)	0.50
Intermediate precision(n=6)	0.62

CONCLUSION

This study is a typical example of development of a stability-indicating assay, established following the recommendations of ICH guidelines. It is one of the studies where forced decomposition studies were done under all different suggested conditions and the products were resolved in a single isocratic run. In literature, most of the studies on development of stability-indicating assays involve either forced decomposition studies under only one or two conditions or separation of drug from major degradation products whose standards were available. The developed method is simple, accurate, precise, specific, selective and rugged. It is proposed for analysis of the drug and degradation products in stability samples in industry.

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