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**RESEARCH ARTICLE**

# A Validated Liquid Chromatographic Method for the Determination of Solifenacin Succinate (Urinary Antispasmodic) in Tablets

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**Abstract:** An isocratic reversed-phase liquid chromatographic method was developed and validated for the determination of Solifenacin succinate. Chromatographic separation was achieved on a $C_{18}$ column using an aqueous tetra butyl ammonium hydrogen sulphate (10 mM): acetonitrile (40:60, v/v), with flow rate 0.8 mL/min (UV detection at 254 nm). Linearity was observed in the concentration range of 20-200 µg/mL ($R^2$ = 0.999). The limit of quantitation was found to be 0.845 µg/mL and the limit of detection was found to be 0.0269 µg/mL. The method was validated as per ICH guidelines. The method is simple, precise, robust and accurate for the determination of Solifenacin in tablet dosage forms.

**Keywords:** RP-HPLC, Solifenacin succinate, Validation, Tablets

## Introduction

Chemically, Solifenacin[1] (SLFN) is 1-azabicyclo [2.2.2] oct-8-yl (1*S*)-1-phenyl-3,4- dihydro-1*H*-isoquinoline-2-carboxylate with an empirical formula of $C_{23}H_{26}N_2O_2.C_4H_6O_4$ (Figure 1) and a molecular weight of 480.55. It is generally used as a succinate. Solifenacin succinate is a white to pale-yellowish-white crystal or crystalline powder and freely soluble at room temperature in water, glacial acetic acid, dimethyl sulfoxide, and methanol. Solifenacin is a urinary antispasmodic (Anti-muscarinic class). It acts as a direct antagonist at muscarinic acetylcholine receptors in cholinergically innervated organs. Its anticholinergic-parasympatholytic action[2] reduces the tonus of smooth muscle in the bladder, effectively reducing the number of required voids, urge incontinence episodes, urge severity and improving retention, facilitating increased volume per void. Literature survey revealed that few HPLC[3-11], LC-MS[12-13], HPTLC[14-15] gas chromatography[16] and spectroscopic[17-18] methods have been reported for the determination of Solifenacin succinate in tablet dosage forms as well as in biological matrices. An attempt has been made to develop a simple and rapid reverse phase liquid chromatographic method for the determination of Solifenacin succinaten in tablet dosage forms which was validated according to ICH guidelines[19].

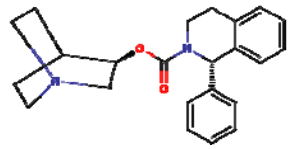

**Figure 1.** Chemical structure of Solifenacin

## Experimental

Solifenacin standard (purity 98.0-101.0) was obtained from Dr. Reddy's laboratories, Hyderabad. Acetonitrile and water (HPLC grade) were obtained from Merck (India). Solifenacin is available (Label claim: 10 mg) with brand names BISPEC (Dr. Reddy's laboratories, India) and SOLITEN (Ranbaxy laboratories Ltd., India). All chemicals were of analytical grade and used as received.

### *Preparation of tetra butyl ammonium hydrogen sulphate (10 mM) solution*

3.3954 grams of tetra butyl ammonium hydrogen sulphate (TBAHS) was transferred to a 1000 ml volumetric flask and dissolved in HPLC grade water (pH 3.37).

### *Preparation of solifenacin stock solution*

Solifenacin stock solution (1000 µg/mL) was prepared by accurately weighing 25 mg of SLFN in a 25 mL volumetric flask with mobile phase. Working standard solutions were prepared on a daily basis from the stock solution in a solvent mixture of TBAHS (pH 3.37) and acetonitrile (40:60, v/v). Solutions were filtered through a 0.45 µm membrane filter prior to injection.

### *Instrumentation and chromatographic conditions*

Chromatographic separation was achieved by using a Shimadzu Model CBM-20A/20 Alite HPLC system, equipped with SPD M20A prominence photodiode array detector with C18 (250 mm × 4.6 mm i.d., 5 µm particle size) column maintained at 25 ºC. Isocratic elution was performed using tetra butyl ammonium hydrogen sulphate (TBAHS) (pH 3.37) and acetonitrile (40:60, v/v). The overall run time was 10 min. and the flow rate was 0.8 mL/min. 20 µL of sample was injected into the HPLC system.

### *Method validation*

The method was validated for the following parameters: system suitability, linearity, limit of quantitation (LOQ), limit of detection (LOD), precision, accuracy and robustness.

### *Linearity*

Linearity test solutions for the assay method were prepared from a stock solution at different concentration levels (20–200 µg/mL) of the assay analyte concentration, 20 µL of each solution was injected in to the HPLC system and the peak area of the chromatogram obtained was noted. The calibration curve was plotted by taking the concentration on the x-axis and the corresponding peak area on the y-axis. The data was treated with linear regression analysis method.

### *Precision*

The intra-day precision of the assay method was evaluated by carrying out 9 independent assays of a test sample of SLFN at three concentration levels (40, 80 and 100 µg/mL) (n=3) against a qualified reference standard. The RSD of three obtained assay values at three different concentration levels was calculated. The inter-day precision study was performed on three different days *i.e.* day 1, day 2 and day 3 at three different concentration levels (40, 80 and 100 µg/mL) and each value is the average of three determinations (n=3). The RSD of three obtained assay values on three different days was calculated.

### *Accuracy*

The accuracy of the assay method was evaluated in triplicate at three concentration levels (80 %, 100 % and 120 %) and the percentage recoveries were calculated. Standard addition and recovery experiments were conducted to determine the accuracy of the method for the quantification of SLFN in the drug product. The study was carried out in triplicate at 90, 100 and 110 µg/mL. The percentage recovery in each case was calculated.

### *Robustness*

The robustness of the assay method was established by introducing small changes in the HPLC conditions which included wavelength (249 and 259 nm), percentage of acetonitrile in the mobile phase (62 and 58) and flow rate (0.7 and 0.9 mL/min). Robustness of the method was studied using six replicates at a concentration level of 100 µg/mL of Solifenacin.

### *Analysis of marketed formulations*

The content of 25 tablets (each containing 10.0 mg of SLFN) was mixed and quantity equivalent to 25 mg of drug weighed accurately and dissolved in mobile phase in a 25 mL volumetric flask, sonicated and filtered. The filtrate was diluted as per the requirement and 20 µL solution of each of marketed formulations (BISPEC and SOLITEN) was injected in to the HPLC system for conducting the assay.

## Results and Discussion

A reversed-phase liquid chromatographic technique was developed to determine Solifenacin in tablet dosage forms. Satisfactory resolution was achieved with use of a mixture of TBAHS and acetonitrile (40:60, v/v) and C18 column was adopted (UV detection at 254 nm) (PDA detector).

### *HPLC method development and optimization*

Initially the samples were analyzed using a mobile phase consisting of TBAHS: acetonitrile (90:10, v/v) at a flow rate of 1.0 mL/min. Under these no drug peak was observed and so the mobile phase was changed to TBAHS: acetonitrile 70:30, 20:80, 30:70 and finally to 40:60, v/v with a flow rate 0.8 mL $min^{-1}$ has given a sharp peak at 3.07 min which was chosen as the best chromatographic response for the entire study. The typical chromatogram for Solifenacin was shown in Figure 2.

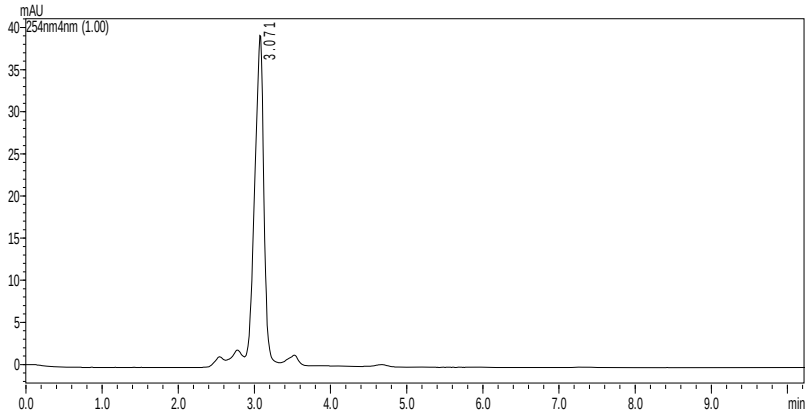


**Figure 2.** Typical chromatogram of Solifenacin succinate (200 µg/mL)

**Method validation**

*Linearity*

Solifenacin has shown linearity over the concentration range 20–200 µg/mL (Table 1). A graph was drawn by taking the concentration of the drug on the x-axis and the corresponding peak area on the y-axis (Figure 3). The linear regression equation was found to be y = 1644x-753.9 with correlation coefficient 0.999.

**Table 1.** Linearity of Solifenacin succinate

| Conc. µg/mL | Mean peak area* ± SD | RSD |
|---|---|---|
| 20 | 32946±279.0 | 0.85 |
| 40 | 66195±337.4 | 0.51 |
| 50 | 80660±463.0 | 0.57 |
| 80 | 131779±455.3 | 0.35 |
| 100 | 160820±497.6 | 0.31 |
| 150 | 241640±1388.3 | 0.57 |
| 200 | 332079±1607.8 | 0.48 |

** Mean of three replicates*

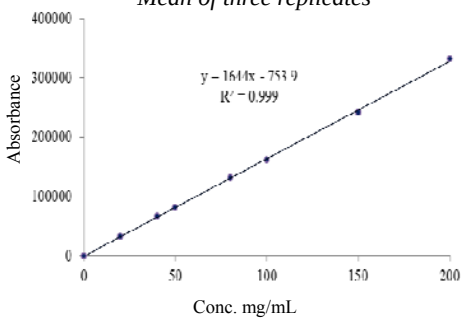


**Figure 3.** Calibration curve of Solifenacin succinate

*Precision*

The precision of the method was determined by repeatability (intra-day precision) and intermediate precision (inter-day precision) of the SLFN standard solutions. Repeatability was calculated by assaying three samples of each at three different concentration levels (40, 80 and 100 µg/mL) on the same day. The inter-day precision was calculated by assaying three samples of each at three different concentration levels (40, 80 and 100 µg/mL) on three different days. The RSD range was obtained as 0.39-0.66 and 0.41-0.53 for intra-day and inter-day precision studies respectively (Table 2).

*Accuracy/recovery studies*

The method accuracy was proven by the recovery test. Known amounts of SLFN standard was added to aliquots of samples solutions and then diluted to yield total concentrations as 90, 100 and 110 µg/mL as described in Table 2. The assay was repeated over three consecutive days. The resultant RSD was in the range 0.35-0.59 (< 2.0) with a recovery 99.09-100.08.

*Robustness*

The robustness of an analytical procedure refers to its ability to remain unaffected by small and deliberate variations in method parameters and provides an indication of its reliability for routine analysis. The robustness of the method was evaluated by assaying the same sample

under different analytical conditions deliberately changing from the original condition and the RSD was less than 2.0 (0.35-1.06) indicating that the proposed method was robust (Table 3).

**Table 2.** Precision and accuracy study of Solifenacin succinate

| Conc. µg/mL | Intra-day precision | Inter-day precision | |
|---|---|---|---|
| | *Mean peak area ± SD (RSD) | *Mean peak area ± SD (RSD) | |
| 40 | 66128±259.92 (0.39) | 66301±282.40 (0.43) | |
| 80 | 132519±879.43(0.66) | 132514±695.88 (0.53) | |
| 100 | 161154±920.23 (0.57) | 161514±655.05 (0.41) | |
| | Accuracy | | |
| Conc. µg/mL | *Mean peak area ± SD (RSD) | Drug found µg/mL | * Recovery |
| 90 | 145600±676.37 (0.46) | 89.21 | 99.09 |
| 100 | 161452±952.96 (0.59) | 100.08 | 100.08 |
| 110 | 177507±616.05 (0.35) | 109.82 | 99.84 |

**Mean of three replicates*

**Table 3.** Robustness study of Solifenacin succinate

| Parameter | Condition | Mean peak area ± SD (RSD) | Recovery |
|---|---|---|---|
| Mobile phase composition (± 2) | 38:62<br>40:60<br>42:58 | 161795±566.82 (0.35) | 99.8 |
| Flow rate (± 0.1 mL) | 0.7<br>0.8<br>0.9 | 162154±1712.38 (1.06) | 100.1 |
| UV detection (± 5 nm ) | 249<br>254<br>259 | 161191±996.03 (0.62) | 99.5 |

**Mean of three replicates*

### *System suitability*

The system suitability test was performed to ensure that the complete testing system was suitable for the intended application. The capacity factor was more than 2, theoretical plates were more than 2000 and tailing factor was less than 2 for the SLFN peak. The peak purity index was found to be 1.0000. The LOQ was found to be 0.845 µg/mL and the LOD was found to be 0.269 µg/mL.

### *Analysis of commercial formulations (Tablets)*

The proposed method was applied for the determination of Solifenacin in tablets (BISPEC and SOLITEN). The percentage of purity was found to be 99.11- 100.33 (Table 4) and no interference was observed from the excipients of the tablets.

**Table 4.** Assay of Solifenacin succinate (Tablets)

| Formulation | Labeled claim, mg | *Amount found mg | *Recovery(%) ± SD |
|---|---|---|---|
| BISPEC® | 10 | 10.03 | 100.33±0.75 |
| SOLITEN ® | 10 | 9.87 | 99.11±0.51 |

**Mean of three replicates*

## Conclusion

The proposed liquid chromatographic method for the determination of Solifenacin succinate

is precise, accurate, robust and can be applied for the determination of Solifenacin in pharmaceutical dosage forms.

*Acknowledgement*

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