



Research Article

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Development and Validation of Stability-Indicating HPTLC Method for Determination of Solifenacin Succinate as bulk Drug and in Tablet Dosage Form

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ABSTRACT

A new simple, stability- indicating high performance thin layer chromatographic (HPTLC) method has been developed and validated for estimation of Solifenacin succinate in bulk and in tablet dosage form. The optimized mobile phase was Methanol: Water: Glacial acetic acid (9:1:0.1v/v/v) with UV detection at 216 nm. The retention factor for Solifenacin succinate was found to be 0.49 ± 0.03 . The drug was subjected to stress conditions of hydrolysis under different pH conditions, oxidation, photolysis and thermal degradation as per ICH guidelines. Results were found to be linear in the concentration range of 2000-10000ng band⁻¹.

Keywords: Solifenacin succinate, HPTLC, Forced degradation, Validation.

INTRODUCTION

Solifenacin succinate, a muscarinic receptor antagonist, chemically is (3R)-1-Azabicyclo [2.2.2] oct-3-yl (1S)-1-phenyl-3,4 -dihydroisoquinoline-2(1H)-carboxylate compound with butanedioic acid (1:1).^[1] Muscarinic receptors play an important role in several major including contractions of urinary bladder smooth muscle. Solifenacin succinate is used in the treatment of overactive bladder with symptoms of urge urinary incontinence, urgency, and urinary frequency.^[2] Literature survey indicates a bioanalytical method^[3], some HPTLC methods^[4-5] reported for estimation of Solifenacin succinate. There were few stability indicating HPLC methods^[6-9] and stability indicating HPTLC methods^[10-11] reported for Solifenacin succinate

but stress degradation conditions and results did not match. The aim of the work was to confirm behavior of Solifenacin succinate under stress conditions.

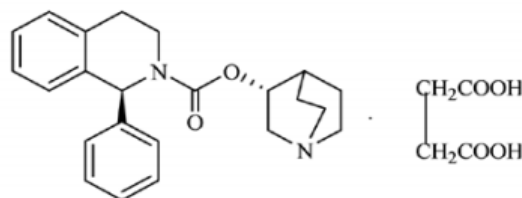


Fig. 1: Chemical structure of Solifenacin succinate

MATERIALS AND METHODS

Instrumentation and chromatographic conditions

Chromatographic separation of drug was performed on TLC plate precoated with silica gel 60 F₂₅₄ (10 × 10) with 250µm thickness (E. MERCK, Darmstadt, Germany) using a CAMAG Linomat 5 sample applicator (Switzerland). Samples were applied on the plate as a band with 6 mm width using Camag 100µL sample syringe (Hamilton, Switzerland).

Linear ascending development was carried out in 10 x 10 cm twin trough glass chamber (CAMAG, Muttentz,

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Switzerland) by using Methanol: Water: Glacial Acetic Acid (9:1: 0.1v/v/v) as mobile phase. The optimized chamber saturation time for mobile phase was 15 min. The length of chromatogram run was 9 cm and development time was approximately 20 min. TLC plates were dried in a current of air with the help of a drier. Densitometric scanning was performed on CAMAG thin layer chromatography scanner at 216 nm for all developments operated by WINCATS software version 1.4.2. The source of radiation utilized was deuterium lamp emitting a continuous UV spectrum between 200 to 400 nm.

Preparation of Standard Solution

Working standard solution of Solifenacin succinate was prepared by dissolving 10 mg of drug in 10 mL of methanol to get concentration of 1000 mcg mL⁻¹.

Selection of Detection Wavelength

Standard solution of Solifenacin succinate was diluted to concentration of 100 mcg mL⁻¹ and scanned in UV range of 200-400 nm. The UV spectrum is as shown in the Fig. 2. For HPTLC method 216 nm was selected as the wavelength for detection.

Stress degradation studies of bulk drug ^[12-16]

The forced degradation studies were carried out on bulk drug substance in order to confirm the stability-indicating property and selectivity of the developed method. The degradation was carried out under acid/base/ neutral hydrolytic, oxidative, thermolytic, and photolytic stress conditions.

Acid treatment

5 mL working standard solution of Solifenacin succinate (10000 mcg mL⁻¹) was mixed with 5 mL of 1 N HCl and volume was made up to 50 mL by using methanol. Solution was refluxed for 2 hours then solution was cooled and volume was adjusted to 50 mL. Then 6 µL of resulting solution was applied on TLC plate and developed under optimized chromatographic conditions.

Alkali treatment

5 mL working standard solution of Solifenacin succinate (10000 mcg mL⁻¹) was mixed with 5 mL of 1 N NaOH and volume was made up to 50 mL by using methanol. The solution was refluxed for 18 hours then solution was cooled and volume was adjusted to 50 mL. Then 6 µL of resulting solution was applied on TLC plate and developed under optimized chromatographic conditions.

Oxidative degradation

1 mL working standard solution of Solifenacin succinate (10000 mcg mL⁻¹) was mixed with 1 mL of 3% v/v H₂O₂ and volume was made up to 10 mL by using methanol. The solution was kept at room temperature for 4 hours. Then 6 µL of resulting solution was applied on TLC plate and developed under optimized chromatographic condition.

Photo-degradation ^[15]

Photolytic studies were carried out by exposure of drug to UV light up to 200 watt hours square meter⁻¹ and subsequently to fluorescence light to achieve

illumination not less than 1.2 million lux hours. Sample was weighed, dissolved in methanol to get concentration of (1000 mcg mL⁻¹). 6 µL of the resulting solution was applied to HPTLC.

Neutral Degradation

1 mL working standard solution of Solifenacin succinate (10000 mcg mL⁻¹) was mixed with 1 mL distilled H₂O and volume was made up to 10 mL by using methanol. The solution was kept at room temperature for 24 hrs. Then 6 µL of resulting solution was applied on TLC plate and developed under optimized chromatographic condition.

Degradation under dry heat

Dry heat study was performed by keeping drug in oven at 80°C for period of 6 hours then sample was withdrawn, weighed and dissolved in methanol to get solution of (1000 mcg mL⁻¹). 6 µL of the resulting solution was applied to HPTLC.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

The primary objective in developing this stability indicating HPTLC method is to achieve the response of Solifenacin succinate free from interference by degradation products, if any. The chromatographic separation was achieved by linear ascending development in 10 cm × 10 cm twin trough glass chamber using Methanol: Water: Glacial acetic acid (9:1:0.1v/v/v) mobile phase and detection was carried out at 216 nm. The retention factor for Solifenacin succinate was found to be 0.49 ± 0.03.

Result of forced degradation studies

Acid treatment

Solifenacin succinate on treatment with 1N HCl for 2 hours reflux showed broadened peak with peak purity within limits, thus it is not possible to calculate % degradation.

Alkali treatment

Solifenacin succinate on treatment with 1N NaOH and kept for 18 hours reflux showed 14.20% degradation with no degradation product observed.

Peroxide treatment

Solifenacin succinate on treatment with 3%v/v H₂O₂ and kept for 4 hours at room temperature showed 10.30% degradation with no degradation product observed.

Neutral treatment

Solifenacin succinate on treatment with H₂O for 24 hours at room temperature it showed 1.99% degradation with no degradation product observed.

Photo degradation Studies

Solifenacin succinate when exposed to ultraviolet light (200 Watt hours/Square meter) and when exposed to fluorescence light (1.2 million lux hours) it showed 3.18 %degradation.

Dry Degradation Studies

Degradation was observed for Solifenacin when the bulk drug substance was exposed to dry heat at 80°C for 8 hours, it showed 2.06% degradation.

The forced degradation studies data are summarized in Table 1.

Table 1: Data of forced degradation studies of Solifenacin succinate

Stress conditions/ duration	% Assay of active substance
Acidic /1 N HCl/ reflux 2 hours	-
Alkaline /1 N NaOH/ reflux 18 hours	85.80
Oxidative / 3% H ₂ O ₂ / at room temperature 4 hours	89.70
Neutral/H ₂ O/at room temperature 24 hours	98.01
Photolysis UV/200 watt hours/square meter	96.82
Fluorescence / 1.2 million lux hours	96.01
Dry heat/ 80°C/ 8 hours	97.94

Table 2: Assay of tablet powder

S. No.	Peak area of Solifenacin succinate	Amount Recovered (ng/band)	% Assay
1	8870.5	6081.81	101.36
2	8652.3	5912.77	98.54
3	8680.2	5933.95	98.89
4	8752.3	5989.97	99.83
5	8655.2	5915.02	98.58
6	8810.2	6034.96	100.58
Mean	8736.7	5978.08	99.63
SD	90.006	69.751	1.163
%RSD	1.03	1.16	1.167

Method Validation [17]

The method was validated for specificity, linearity, range, accuracy, precision, detection limit, quantitation limit and robustness, in accordance with ICH guidelines.

Specificity

The specificity of the method was ascertained by peak purity profiling studies. The peak purity values were found to be more than 0.9997, indicating the no interference of any other peak of degradation product, impurity or matrix.

Assay

Tablets were procured from local market. Five tablets, each containing 5 mg of Solifenacin Succinate were weighed and powdered. Powder equivalent to 10 mg of drug was transferred to 10 mL volumetric flask and was diluted with methanol, sonicated for 10 min, volume made to 10 mL by using methanol (1000µg/mL). Solution was filtered through whatman filter paper, 6µL volumes of filtrate was applied on plate. Analysis was repeated for six times. % assay was determined from linearity equation.

Linearity and range

The standard stock solutions of Solifenacin succinate (1000 mcg mL⁻¹) were applied on TLC plate in range of 2, 4, 6, 8 and 10 µL. Straight-line calibration graphs were obtained in the concentration range 2000-10000 ng band⁻¹ with high correlation coefficient > 0.999.

Accuracy

To check accuracy of the method, recovery studies were carried out by adding standard drug to sample at three different levels 80, 100 and 120 %. Basic concentration of sample chosen was 4000 ng band⁻¹ from tablet solution. The drug concentrations were calculated from respective linearity equation. The results of the recovery studies indicated that the method is accurate for estimation of drug in tablet dosage form. The results obtained are shown in Table 3.

Precision

Set of three different concentrations in three replicates of standard solutions of were prepared. All the solutions were analyzed on the same day in order to record any intraday variations in the results.

Table 3: linearity of Solifenacin succinate 2000- 10000 ngband⁻¹

Table 3: Linearity of Standard Solution 2000-10000 ng/mL						
Concentration (ngband ⁻¹)	Area					Mean Area ± SD
2000	3486.1	3352	3412.2	3470.5	3472.6	3438.68 ± 56.18351
4000	6025.2	6531.8	6390	6328.6	6470.3	6349.18 ± 196.8965
6000	8115.3	9374.2	8944.7	8869.8	9125.8	8885.96 ± 472.6336
8000	10454.6	11690.9	12226.5	11014.2	11298.2	11336.88 ± 670.9677
10000	13340.9	14344.4	14125.2	13719.8	13543.2	13814.7 ± 413.581

Table 4: Recovery Studies of Solifenacin succinate

Drug	Amount taken (ng band ⁻¹)	Amount added (ng band ⁻¹)	Total amount found (ng band ⁻¹)	% Recovery*	% RSD
Solifenacin succinate	4000	3200	7235.36	100.48	0.92
	4000	4000	7941.72	99.26	0.63
	4000	4800	8876.99	100.87	1.79

*Average of three determinations

Intra-day variation, as RSD (%), was found to be in the range of 0.38 to 1.15. For Inter day variation study, three different concentrations of the standard solutions in linearity range were analyzed on three consecutive days. Interday variation, as RSD (%) was found to be in the range of 0.69 to 1.63. The lower values of % R.S.D. obtained indicated that method is precise.

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

LOD and LOQ were calculated as $3.3\sigma/S$ and $10\sigma/S$, respectively; where σ is the standard deviation of the

response at lowest concentration in range and S is the slope of the calibration plot. The LOD and LOQ were found to be 144.05 and 436.51 ng band⁻¹, respectively.

Robustness Studies

Robustness of the method was determined by carrying out the analysis under conditions during which mobile phase saturation time, wavelength and the effect on the area of drug were noted. Robustness of the method checked after deliberate alterations of the analytical parameters showed that areas of peak of interest remained unaffected by small changes of the

operational parameters (% R.S.D. < 2). The results are given in Table 7.

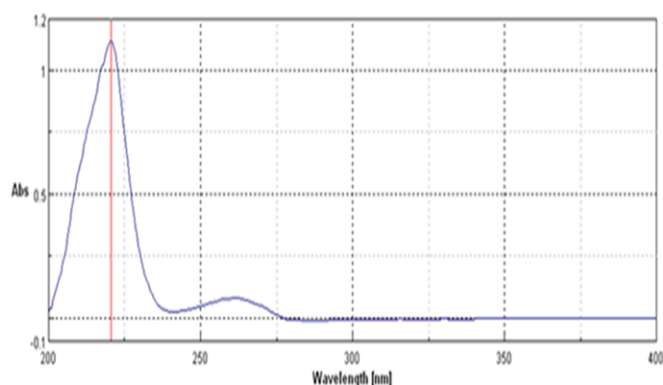


Fig. 2: UV Spectra of standard solution of Solifenacin succinate 100 mcg mL⁻¹

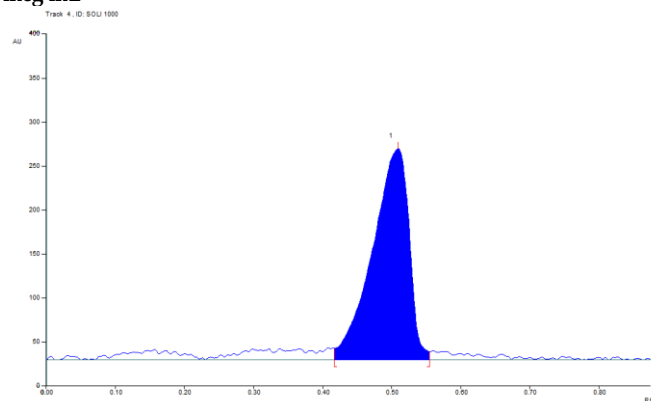


Fig. 3: Representative densitogram of standard solution of Solifenacin succinate (6000 ng band⁻¹, RF = 0.51)

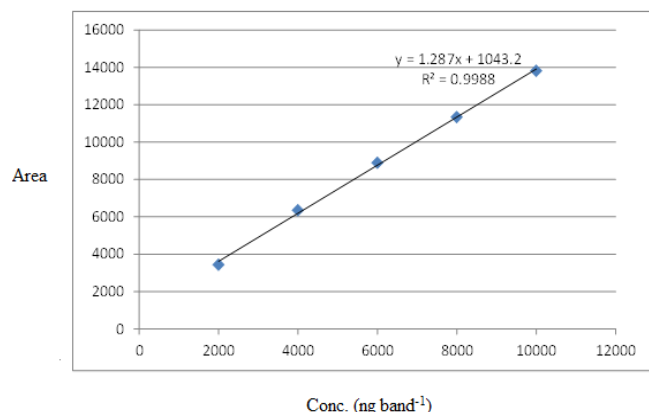


Fig. 4: Calibration curve of Solifenacin succinate of range 2000-10000 ng band⁻¹

DISCUSSION

Some research papers reported that Solifenacin succinate degrades under acid and alkali catalyzed hydrolytic conditions using 0.1N HCl and 0.1N NaOH where as some research papers reported stringent conditions of 5N HCL and 5N NaOH for forced degradation study of Solifenacin succinate. Current study confirmed that Solifenacin succinate does not degrade at mild conditions and by using 1N HCl and 1N NaOH the drug showed degradation but no degradation product was observed. Solifenacin succinate was found to be stable at thermal, neutral and photo degradation study which resembled previous research work.

Table 5: Intra-day precision of Solifenacin succinate

Concentration (ng/band)	Area	Mean Area	SD	% RSD
2000	3600.30	3600.53	0.38	0.38
	3590.70			
	3610.60			
4000	6212.60	6234.70	1.16	1.15
	6302.40			
	6189.10			
6000	8852.30	8865.30	0.41	0.40
	8901.20			
	8842.40			

Table 6: Inter-day precision of Solifenacin succinate

Concentration (ng/band)	Area	Mean Area	SD	% RSD
2000	3600.25	3645.18	1.653	1.63
	3550.20			
	3685.10			
4000	6219.30	6210.70	1.626	1.62
	6123.20			
	6289.60			
6000	8860.50	8808.73	0.701	0.69
	8752.30			
	8813.40			

Table 7: Robustness Data in Terms of Peak Area (% RSD)

S. No.	Parameter	(% RSD)
1	Mobile phase saturation time (± 10 %)	1.39
2.	Mobile phase variation	1.40

*Average of three determinations

The developed HPTLC method is precise, specific, accurate, reproducible, and stability-indicating, without interference from the excipients or from degradation products resulting from treatment with acid, alkali, oxidizing agent, or from UV irradiation and can be used for quantitative analysis of Solifenacin succinate in pharmaceutical dosage form. This method is used to monitor stability of solifenacin succinate.

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