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DEVELOPMENT AND VALIDATION OF A NOVEL STABILITY INDICATING RP-HPLC ASSAY METHOD OF SOLIFENACIN SUCCINATE IN TABLET FORMULATION

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ABSTRACT
A novel RP-HPLC assay method has been established for solifenacin succinate in pharmaceutical dosage forms. Successful separation of the solifenacin succinate was achieved on a Hiber R C-18 column (250mm × 4.6 mm i.d. with particle size of 5µm with potassium dihydrogen phosphate buffer (pH 2.5±0.05) and acetonitrile in the ratio of 60:40(%v/v) as mobile phase at a flow rate of 1.0ml/min . A calibration graph was obtained over six different concentrations in the range of 10-60µg/ml exhibiting regression equation of y = 3733.651x + 1358.6 (r^2= 0.9997) for solifenacin succinate that revealed an excellent correlation exists between peak area and concentration of drug within the concentration range indicated above LOD and LOQ for solifenacin succinate are found to be 0.54µg/ml and 1.80µg/ml respectively. The developed RP-HPLC method had effectively separated solifenacin succinate from their degradation products, making it stability-indicating. Suitability of this method for the quantitative determination of the solifenacin succinate was proved by validation in accordance with the requirements of International Conference on Harmonization (ICH) guidelines.

KEYWORDS: Solifenacin succinate, Stability Indicating Studies, Method Validation, HPLC Determination.

INTRODUCTION
Solifenacin succinate[1-3] (Fig.1) is a competitive muscarinic acetylcholine receptor antagonist used in the treatment of overactive bladder with or without urinary incontinence. Being an antagonist, it prevents the binding of acetylcholine to the M3 receptor subtype thereby reducing smooth muscle tone in the bladder, allowing the bladder to retain larger volumes of urine and reducing the number of incontinence episodes.

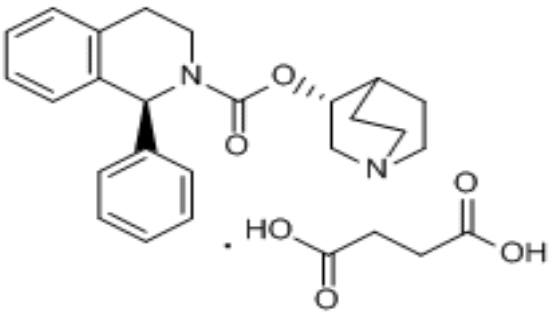

Figure 1: Chemical Structure of Solifenacin succinate

Monograph for solifenacin succinate is available only in European Pharmacopoeia[4]. Chemically, Solifenacin succinate is butanedioic acid (3R)-1-azabicyclo [2.2.2] octan-3-yl(1S)-1-phenyl-1,2,3,4-tetrahydroisoquinoline-2-carboxylate having an empirical formula of $C_{27}H_{32}N_2O_6$ and molecular weight of 480.5528gms/mol. It is freely soluble at room temperature in water, Glacial acetic acid, dimethyl sulfoxide and methanol. Presently six brands of generics of solifenacin succinate are available in local pharmacy that are formulated for oral administration. In the current study for one commercial formulation of solifenacin succinate in brand name of BISPEC (Dr. Reddy Laboratories Ltd; Dosage strengths -10mg) was used.

Very few HPLC methods[5-13] have been reported in the literature for the assay of solifenacin succinate in pharmaceutical dosage forms. To the best of our knowledge, the currently available analytical methods resulted in the drawbacks of long separation time, time-consuming and needed expensive extraction procedures. It was therefore, felt necessary to develop a new rapid, stability-indicating HPLC assay method for solifenacin succinate in oral dosage forms. The present paper describes the development and validation of a novel and rapid stability indicating RP-HPLC assay method for solifenacin succinate in oral dosage forms.

MATERIALS AND METHODS
Instrumentation
The present analysis was performed on a Shimadzu, a high pressure liquid chromatographic instrument, Japan was used for the present analysis. The instrument was

provided with a Hiber R C-18 column (250mm × 4.6 mm i.d. with particle size of 5 µm), an LC 20 AD pump and an SPD-M20A-PDA detector were L Hamilton syringe was used for sample injection. Data acquisition was done by using Winchrom software. Denver electronic analytical balance (SI-234) and pH of the mobile phase was adjusted by using Systronics digital pH meter. 100µL-1000µL micropipette (Eppendorf, Germany) was used for the mobile phase buffer preparation.

Chemicals and Solvents

Potassium dihydrogen ortho phosphate, Ortho Phosphoric acid (AR- Grade), Triethyl amine (AR-Grade), Hydrochloric acid (AR- Grade), Sodium hydroxide (AR-Grade), and Hydrogen peroxide (AR-Grade) and Acetonitrile of HPLC grade used were purchased from Merck Chemicals Ltd., Ahmadabad, India. High purity water was prepared by using Milli-Q Plus water purification system (Millipore USA). Solifenacin succinate (API-99.9% Pure) was provided by Dr Reddy Laboratories Ltd, Hyderabad as gratis sample and its commercial tablets in the brand name **BISPEC-10mg** was purchased from local Mediplus pharmacy.

Preparation of Mobile Phase

Mix potassium dihydrogen phosphate buffer (pH 2.5±0.05) and acetonitrile in the ratio of 60: 40 %v/v, degassed and filtered through 0.45 µ membrane filter.

Buffer preparation

Weigh and dissolve 6.8grams of potassium dihydrogen phosphate in 1000 ml of water, add 1.0mL triethyl amine mix well. Adjust pH with ortho phosphoric acid to 2.5±0.05.

Diluent

Acetonitrile and methanol in the ratio of 50:50%v/v

Preparation of stock and working standard solutions

Standard stock solution of concentration 1000µg/ml was transferring accurately weighed 10mg of solifenacin succinate in 100ml clean and dry volumetric flask containing 25 ml of diluent, sonicated for 2 minutes and later the solution was made up to the mark using the same diluent. Further, pipette 5.0mL of the above standard stock solution into a 25mL clean dry volumetric flask and dilute to volume with the same diluent. Linearity test solutions for the assay method of solifenacin succinate were prepared from the above stock solution at 6 concentration levels from 25% to 150% (5.0 - 30µg/mL) of assay concentration respectively.

Preparation of Sample Solution

Ten tablets of market formulations of solifenacin succinate (BISPEC -10mg; Dr Reddy Laboratories Ltd) were weighed separately and the average weight was determined. Accurately weigh and transfer a quantity of powder sample equivalent to 10mg of solifenacin succinate into a 100 mL clean dry volumetric flask, add about 25mL of diluent and sonicate to dissolve it for about 30 minutes and cool the solution to room temperature and dilute to volume with diluen. Filter the above sample solution through 0.45 µ membrane filter. From this sample stock solution, pipette out 5.0mL into a 25mL clean dry volumetric flask and dilute to volume with diluent. Further, dilutions of this solution were carried out with the same diluent in separate 10ml volumetric flasks to reach the final concentrations of 5.0-30µg/ml of solifenacin succinate respectively. 20µL of these solutions were injected in prescribed HPLC system under described conditions.

RESULTS AND DISCUSSION

A. Method development

To develop an efficient and simple RP-HPLC method for the analysis of the solifenacin succinate in pure and in its tablet dosage forms, various preliminary tests were conducted using different parameters such as mobile phase composition, wavelength of detection, type of the column, column temperature and pH of mobile phase respectively. Preliminary development trials with columns of different types, configurations and from different manufacturers revealed good separation of solifenacin succinate was achieved with analytical column, Hiber R C-18 column (250mm × 4.6 mm i.d. with particle size of 5 µm). 210nm was selected as the optimum wavelength for detection, as such best detector response for solifenacin succinate was obtained.

Secondly, different composition of mobile phases containing a mixture of potassium dihydrogen phosphate buffer (pH 2.5±0.05) and acetonitrile was tried and the best results were achieved on using the mixture of potassium dihydrogen phosphate buffer (pH 2.5±0.05) and acetonitrile in the 60:40 %v/v as mobile phase. Using the above mentioned column and mobile phase at a flow rate of 1.0ml/min, resulted in the elution of solifenacin succinate at the retention time of 5.743min respectively. Under the described experimental conditions the analyte peak area of solifenacin succinate was well defined and free from tailing.

Chromatographic conditions

From the method developmental studies the assay of solifenacin succinate in pharmaceutical formulations was carried by using an isocratic Shimadzu HPLC instrument with Hiber R C-18 column (250mm × 4.6 mm i.d. with particle size of 5µm), mobile phase of potassium dihydrogen phosphate buffer (pH 2.5±0.05) and acetonitrile in the ratio of 60:40(%v/v) at a flow rate of 1.0ml/min. Spectral analysis was carried using UV detector at a wavelength of 210nm. A sample volume of 20µL fixed loop at ambient temperature was material during the analysis (Table 1).

Table 1: Optimized Chromatography conditions

Parameter	Results
Mobile phase	Potassium dihydrogen phosphate buffer (pH 2.5±0.05): Acetonitrile in the 60:40 (%v/v)
Pump mode	Isocratic
pH	2.5
Column	Hiber R C-18 column (250mm × 4.6 mm i.d. with particle size of 5 µm),
Column Temperature	Ambient
Wavelength	210 nm
Injection Volume	20µL
Flow rate	1.0 ml/min
Run time	12 minutes
Retention Time	5.735 minutes
Area	76976

B.Forced Degradation Studies

The forced degradation or stress studies were performed at the concentration 20µg·mL^{-1} of solifenacin succinate (API) in presence of excipients to show the stability indicating property of the present proposed RP-HPLC method.

Intentional degradation was attempted to stress condition of acid hydrolysis (0.1N HCl at 70°C for 24hrs), base hydrolysis (0.1N NaOH at 70°C for 24hrs), oxidation (3% H_2O_2 at 50°C for 48hrs), thermal (at 80°C for 72hrs), and photolytic (1.2 million lux hours/ 200 watt hours) to evaluate the ability of the proposed method to separate solifenacin succinate from their degradation products. All stressed samples were analyzed by developed RP-HPLC method. Photodiode array (PDA) detector was employed to check and ensure the homogeneity and purity of the solifenacin succinate peak in all forced degradation sample solutions. For all the stability studies, the formation of degradable product was confirmed by comparing to chromatogram of the standard solution of solifenacin succinate kept under normal conditions.

Acid and base-induced degradation: This acidic and basic degradation was performed in the dark in order to exclude the possible degradative effect of light. 10ml of sample stock solution of solifenacin succinate was taken in a round bottom flask. 10mL of 0.1N HCl and 10mL of 0.1N NaOH were added separately to the stock solution. The reaction was carried out for 70^{o}C for 24hrs. Later the sample was cooled at room temperature and reaction was stopped, diluted with the mobile phase and mixed well. This solution was injected into the HPLC system.

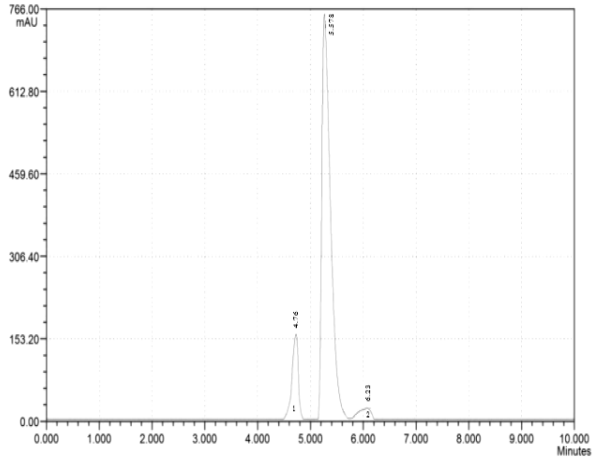

Figure B.1: Acid degradation

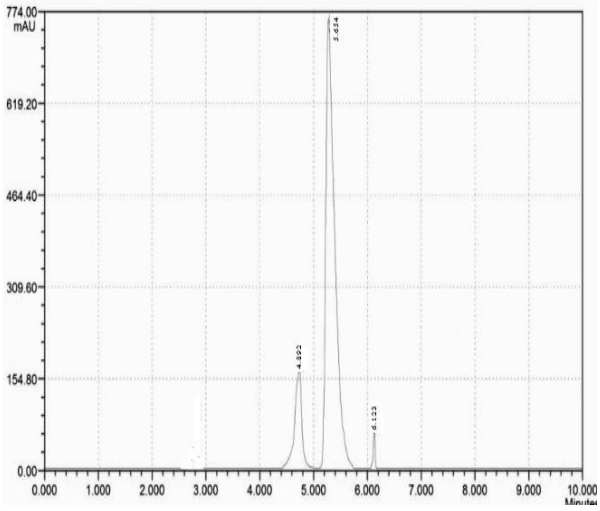

Figure B.2: Base degradation

Hydrogen peroxide-induced degradation: 10ml of sample stock solution of solifenacin succinate was taken in a round bottom flask. 10mL of 3% H $_2O_2$ was added separately to the stock solution. The reaction was carried out for 48 h at 50°C in the dark. After 4hr, sample was cooled at room temperature and reaction was stopped, diluted with the mobile phase and mixed well. This solution was injected into the HPLC system.

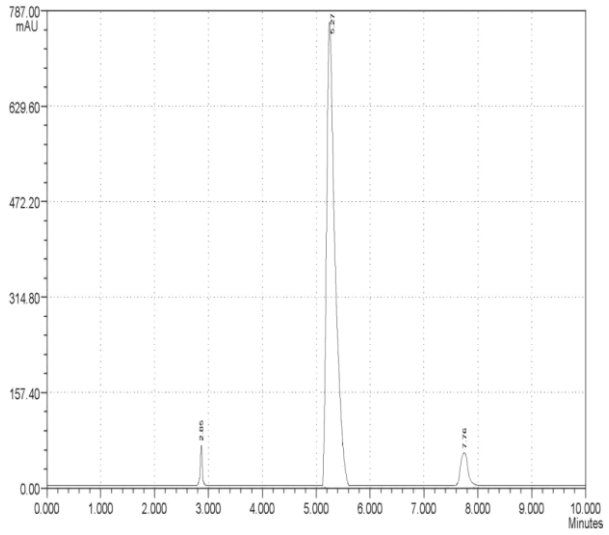

Figure.B.3: Peroxide degradation

Thermal stress: This study was performed by keeping powdered drug content at around 80˚C for 72 hours. After this it was allowed to come at room temperature. This powdered drug was used for prepare stock solution preparation as per procedure described in page and the same was used in this study.

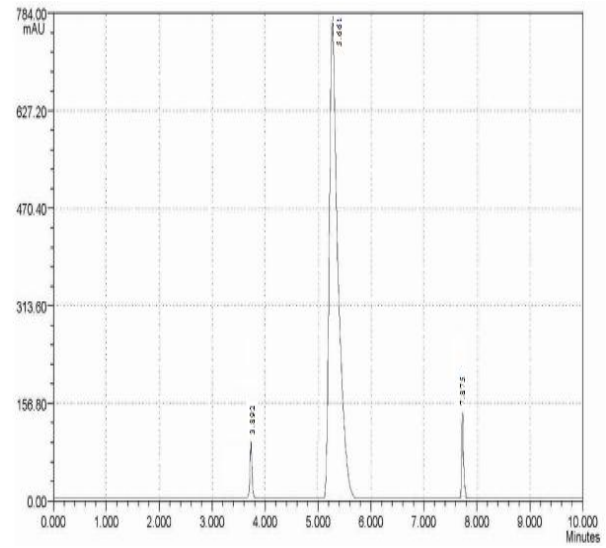

Figure B.4: Thermal degradation

Photolytic Stress: Photolytic degradation study was performed by exposing drug content in sun-light for 72 hour, further it diluted using the same diluent.

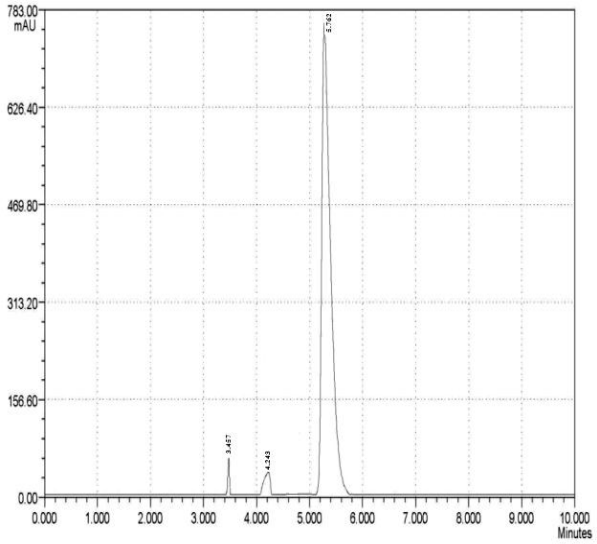

Figure. B.5: Photolytic degradation

Table 2: Results of stress degradation studies by the proposed method

Condition	No. of deg. Products obtained	Rt	% Degraded	% assay Recovered	Tf	Tp
Acid	03	5.578	13.78	87.2	1.96	4848
Base	03	5.654	10.59	90.4	1.38	4740
Peroxide	03	5.270	11.46	89.90	3.39	4855
Thermal	03	5.661	9.90	91.70	1.72	4970
Photolytic	03	5.762	6.17	94.45	2.12	4445

Solifenacin succinate assay was dropped to 87.2%, 90.4%, 89.90%, 91.7% and 94.45 in acid hydrolysis, alkali hydrolysis, peroxide oxidation degradation, thermal degradation and photolytic stress. In all the above described stress conditions peak purity of solifenacin succinate peak was found greater than 990, which indicated that all peaks are well separate from solifenacin succinate peak and also found that, that there

is no interference from degradants, facilitating error-free quantification of solifenacin succinate. The chromatograms obtained after subjecting sample solution to acid hydrolysis, alkali hydrolysis, peroxide oxidation degradation, thermal degradation and photolytic degradation are presented in figures B.1-5 and the results obtained are summarized in Table 2.

C: Method validation

The method was validated according to International Council for Harmonization Q2 (R1) guidelines [14] for validation of analytical procedures in order to determine the specificity, linearity, limit of detection, limit of quantification, accuracy, precision, robustness and ruggedness respectively.

i. System Suitability

The HPLC system was stabilized for forty min. by following the chromatographic conditions as described in Table 3 to get a stable base line. Blank solution followed by standard solution was injected to check the system suitability parameters like symmetric factor and number of theoretical plates. To ascertain the system suitability for the proposed RP-HPLC method, a number of parameters such as relative retention, theoretical plates, peak asymmetry and peak areas of solifenacin succinate have been calculated with the observed reading and the results are recorded in Table 3& in fig.2 respectively.

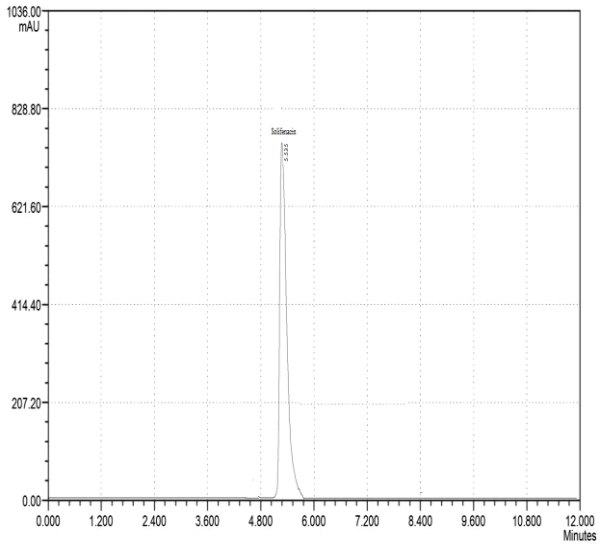

Figure 2: System suitable chromatogram

Table 3: System suitability conditions by the proposed method

Sample	Retention time	Area	Tailing factor	Theoretical plates
Solifenacin succinate	5.535min	76976	1.24	3754

ii. Specificity

Standard, blank and formulation solutions were prepared and were analyzed in the optimized method conditions. Base line obtained for the resultant chromatograms were compared and confirmed that no chromatographic interference was observed at the retention time of solifenacin succinate in the analysis of blank solution. In standard and formulation solution, similar retention time was observed, no other additional detections were found revealing specificity of the developed RP-HPLC method.

iii. Linearity

For linearity study working standard solution s of solifenacin succinate was prepared with mobile phase from the stock solution in the concentration range of 5.0, 10, 15, 20, 25 and 30µg/ml respectively. These solutions were injected in triplicates onto the HPLC system and the resulting chromatograms were recorded. A standard calibration curve for solifenacin succinate was constructed by plotting their response ratios (ratios of the peak area of the analyte) against their respective concentrations. Method of least square analysis was carried out for getting the slope, intercept and correlation coefficient values. The linearity of solifenacin succinate were found to be in the range of 5.0-30µg/mL with correlation coefficient greater than 0.9997, revealing an excellent correlation between the peak area and analyte concentration respectively. The results of linearity studies were given in table 4 and the calibration curve so obtained was presented in fig.2.E respectively**.**

Table 4: Results of Linearity by the proposed method

Level	Concentration (µg/ml)	Peak area
Level – 1(25%)	5	19672
Level – 2(50%)	10	38488
Level – 3(75%)	15	57456
Level – 4(100%)	20	76976
Level – 5(125%)	25	95136
Level – 6(150%)	30	112457
Range: 5.0 to 30µg/ml		Slope :3733.651 Intercept :1358.60 Correlation coefficient: 0.9997

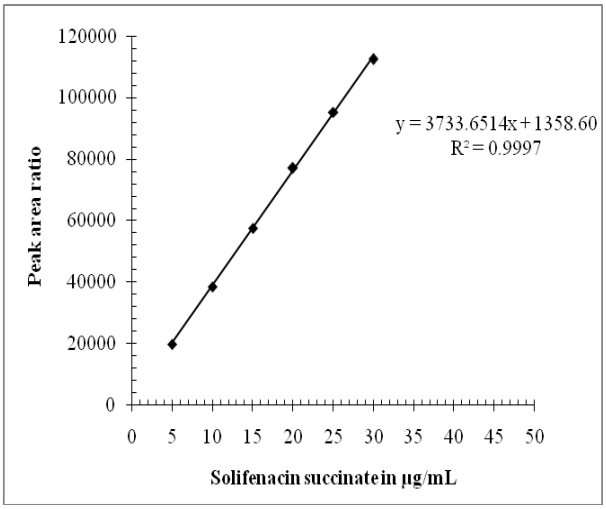

Figure 3: Calibration curve of solifenacin succinate

iv. LOD & LOQ

The LOD and LOQ for solifenacin succinate were determined at a signal to-noise ratio of 3:1and10:1, respectively, by injecting a series of dilute solutions with known concentrations. The LOD and LOQ values for solifenacin succinate by the proposal method were 0.54 & 1.80µg/ml that indicated good sensitivity of the developed RP-HPLC method (Table 5).

Table 5: Limit of detection and limit of quantification by the proposed method

Parameter	Measured Value
Limit of Quantification	0.54µg/ml
Limit of Detection	1.80µg/ml

v. Precision

The precision of the present proposed method was demonstrated by the inter-day and intra-day variation studies. In intra-day studies, six repeated injections of standard solutions of solifenacin succinate were made and response factor of the drug peak, and the % RSD were calculated. In the inter-day variation studies, six repeated injections of standard solutions of solifenacin succinate were injected were made for three consecutive days and response factor of the drug peak, the % RSD were calculated. From the results it was found that the developed HPLC method found to be precise. Results of the precision studies are presented in table 6**.**

Table 6: Results of Precision studies by the proposed method

Concentration(100%)	Intraday precision	Interday precision
20µg/ml	76142	76787
	76175	76448
	76525	76331
	76976	76856
	76826	76528
	76005	76778
%RSD*	0.520	0.279

*Average of six determinations

vi. Accuracy

Recovery studies were conducted by analyzing known amounts of pure drug added to each of the previously analyzed formulation and the total amount of drug within the linearity limits. 50%, 100% and 150% recoveries were tested for standard drug solution and the peak areas were compared with the linearity results. Results of recovery studies were given in table 7 revealing that the proposed RP-HPLC method is accurate for determination of solifenacin succinate.

Table 7: Results of recovery studies by the proposed method

Spike Level	Target Conc.(µg/ml)	Spiked conc.(µg/ml)	Final Conc.(µg/ml)	Conc. Obtained	% Assay
50%	10	10	20	19.79	98.95
	10			19.51	97.55
	10			19.86	99.30
100%	20		30	29.92	99.73
	20			29.73	99.10
	20			29.80	99.33
150%	30		40	39.89	99.72
	30			39.86	99.30
	30			40.08	100.20

vii. Robustness

The robustness of the method, the experimental conditions was deliberately changed. During the study, other chromatographic conditions were kept the same as per the experimental section and the resolution of solifenacin succinate was evaluated. The effect of change in flow rate ± 0.2mL/min (0.8 and 1.2mL/min), column oven temperature ± 5°C (20 and 30°C) and wavelength ±

2.0 (208nm and 212nm) were studied.. From these studies it was observed that slight deliberate changes did not have any significant change on the intensity of the peak or on chromatographic suitability of this method, concluding that the method is highly robust .The results of robustness were given in table 8.

Table 8: Robustness results by the proposed method

S.No.	Parameter	Condition	Mean area	% difference
1	Flow rate	0.8mL/min 1.2mL/min	76915 76280	1.82 1.11
2	Column temperature	20°C 30°C	76186 76404	1.00 1.24
3	Wavelength in nm	208nm 210nm	76120 76156	-0.18 1.48

*Average of six determinations

viii. Ruggedness

Inter-day precision at person to person variation was measured to express the ruggedness of the developed method. Standard drug solution of solifenacin succinate at concentration of 20µg/ml was prepared by 6 different persons and the solution was injected in to HPLC system. Peak area response was noted and %RSD was calculated. Results of the ruggedness were shown in the table 9.

Table 9: Ruggedness results by the proposed method

Concentration(100%)	Peak Area
20µg/ml	76692
	76619
	76591
	76567
	76306
	76082
%RSD*	0.305

ix. Assay of solifenacin succinate in pharmaceutical formulations

The proposed RP-HPLC method was applied for the determination of solifenacin succinate in **BISPEC** tablets. The results of this assay was 99.80% (n=3) (RSD = 0.21%) of the label claim for the formulation. The results of the assay indicated that the proposed RP-HPLC method is selective for the assay of solifenacin succinate without interference from excipients used in the tablets (Table 10 &Fig.4).

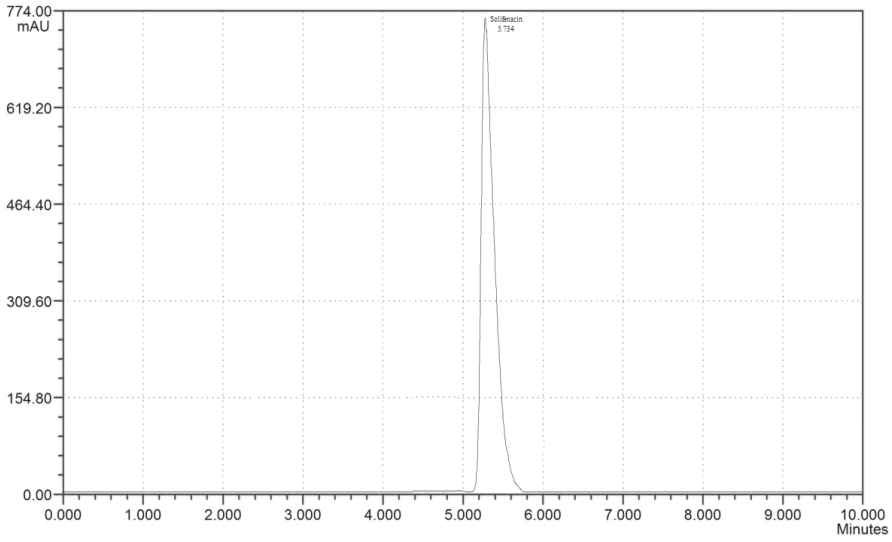

Figure 4: Chromatogram of formulation

Table 10: Results of assay of solifenacin succinate

S.No	Brand name	Available form	Label claim	Concentration	Amount found*	% Assay
1.	BISPEC	Tab	10mg	20µg/ml	19.92 µg/ml	99.80

*Average of three determinations

CONCLUSIONS

A new analytical stability indicating RP-HPLC method is developed and validated for the determination of solifenacin succinate in bulk and dosage forms on Shimadzu HPLC instrument with Hiber R C-18 column (250mm × 4.6 mm i.d. with particle size of 5µm) as stationary phase with mobile phase of potassium dihydrogen phosphate buffer (pH 2.5±0.05) and acetonitrile in the 60:40 (%v/v) with UV detection of 210nm respectively. The peak of solifenacin succinate was distinguishable and quantifiable among the peaks of degradation products under tested stress conditions, making the present RP-HPLC method is stability indicating. The amount of the solifenacin succinate in dosage formulations was found to be in good agreement with label claim. The major advantage of this proposed RP-HPLC method is that it requires limited pretreatment of the sample and is sufficiently sensitive to be an alternative method to that of European Pharmacopoeia.[4] reported in the literature.

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