DEVELOPMENT AND VALIDATION OF A STABILITY INDICATING ANALYTICAL METHOD FOR DETERMINATION OF RELATED SUBSTANCES BY RPHPLC FOR SOLIFENACIN SUCCINATE IN SOLIFENACIN SUCCINATE TABLETS

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ABSTRACT

Solifenacin succinate is a competitive muscarinic acetylcholine receptor antagonist. This article describes development and validation for the determination of related substances of Solifenacin succinate in Solifenacin succinate Tablets by using a high performance liquid chromatography. The high performance liquid chromatography resolution was achieved on a Waters Xterra RP-8 250 x 4.6, 5μ, column with a gradient elution at a flow rate of 1.2 mL/min. The detection was performed by a photo diode array Detector. The method was validated in the concentration range of Limit of quantitation to 150% of working concentration. The intra and inter-day precision and accuracy were within Limit. The overall mean recoveries of Solifenacin succinate impurities were in the range of 90.0% to 110.0% for Limit of Quantitation, 50%, 100% and 150%.

Introduction

Solifenacin succinate is a competitive muscarinic acetylcholine receptor antagonist. Muscarinic receptor antagonists are widely used for treatment of the syndrome of overactive bladder and urge urinary incontinence [1-4]. 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 [5-6]. 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 [7-8]. Analytical method is validated that allows the determination of Related Substances of Solifenacin succinate in Solifenacin succinate Tablets. The validation parameters, Specificity, forced degradation, linearity, repeatability, precision, Accuracy, Solution Stability and robustness were validated [9-10].

Patients and Methods

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Working standard and Impurity standard used in the Experiments are reported in Table No.1. Apparatus and instruments used in the experiment are listed in table No 2. Reagents and solvents used are: Water (HPLC grade, Milli Q), Di-sodium hydrogen phosphate anhydrous (AR grade), Acetonitrile (HPLC grade), Methanol (HPLC grade), and Orthophosphoric Acid (AR Grade).

S No.	Name
1	Solifenacin succinate
2	Impurity A
3	Impurity B
4	Impurity C

Sr No	Instrument	Make	Software	Detector/Model No
1	HPLC	Waters	Empower Software	2489 dual wavelength
2	HPLC	Waters	Empower Software	2998 PDA Detector
3	Sonicator	Lab India	NA	NA
4	Weight balance	Mettler Toledo	NA	ML204
5	Oven	Thermo lab	NA	GMP
6	Photolytic Chamber	Thermo lab	NA	GMP

Development Trials: Standard, impurities and spiked sample were injected in to HPLC using the following trials.

Table: 1 Development Trials 01 and 02

Chromatography Parameters	Trial 01	Trial 02
Column	X-Terra RP-8, 250 x 4.6mm, 5µm	X-Terra RP-8, 250 x 4.6mm, 5µm
Buffer	0.05M Ammonium Acetate in Water. Filter through 0.45µ Nylon membrane filter mixed and degas.	Weigh and transfer 1.42gm Disodium Hydrogen Phosphate Anhydrous in 1.0 litre water. Adjust its pH 6.8 with orthophosphoric acid. Filter through 0.45µ Nylon membrane filter mixed and degas.
Mobile phase	Mobile phase A: Buffer (100%)	Mobile phase A: Buffer :ACN (90:10)
	Mobile phase B: Acetonitrile (100%)	Mobile phase B: Acetonitrile : Methanol (70:30)
Diluent	Prepare a mixture of Buffer and Acetonitrile in the ratio of 50:50 v/v	Diluent 1: Buffer pH 6.8 PB Diluent 2: Methanol: ACN (40 :40) Diluent 3 : Buffer: Methanol : ACN (20 : 40 : 40)
Flow Rate	1.0 mL/min.	1.2 mL/min.
Injection Volume	20 µL	20 µL
Wavelength	215 nm	215 nm
Column Temp.	25°C	30°C
Elution	Gradient Elution	Gradient Elution
Standard Concentration	Solifenacin Succinate (2.5ppm)	Solifenacin Succinate (2.5ppm)
Sample Concentration	Solifenacin Succinate 400ppm	Solifenacin Succinate 400ppm

Table:2	Gradient	Time	MP-A	MP-B		Time	MP-A	MP-B
		0	75	25		0	70	30
		5	75	25		8	70	30
		30	50	50		28	45	55
		48	50	50		42	45	55
		50	75	25		50	70	30
		60	75	25		60	70	30
	Conclusion	Early elution of main peak and impurities were found with inadequate resolution and baseline noise was on higher side. So mobile phase and gradient were changed and injected as Trial 02.			Spiked sample injected in the above chromatographic system, though the main peak retention time enhanced in this trial upto 14.0mins, it has been observed that two peaks are merging so spiked sample solution was injected as trial 03 by slowing gradient programme.			

Development Trials 03 and 04

Chromatography Parameters	Trial 03	Trial 04
Column	X-Terra RP-8, 250 x 4.6mm, 5µm	X-Terra RP-8, 250 x 4.6mm, 5µm
Buffer	weigh and transfer 1.42gm Disodium hydrogen Phosphate Anhydrous in 1.0 litre water.adjust its pH 6.8 with orthophosphoric acid. Filter through 0.45µ Nylon membrane filter mixed and degas.	
Mobile phase	Mobile phase A: Buffer :ACN (90:10)	
	Mobile phase B: Acetonitrile : Methanol (70:30)	
Diluent	Diluent 1: Buffer pH 6.8 PB	
	Diluent 2: Methanol: ACN (40 :40)	
	Diluent 3 : Buffer: Methanol : ACN (20 : 40 : 40)	
Flow Rate	1.2 mL/min.	
Injection Volume	20 µL	
Wavelength	215 nm	
Column Temp.	30°C	
Elution	Gradient Elution	
Standard Concentration	Solifenacin Succinate (2.5ppm)	
Sample Concentration	Solifenacin Succinate 400ppm	
Gradient	Time MP- MP-B 0 78 22 10 78 22 25 60 40 40 40 60 50 78 22 60 78 22	Time MP- MP-B 0 80 20 12 80 20 28 60 40 42 60 40 50 80 20 60 80 20
Conclusion	Spiked sample injected in the above chromatographic system it has been observed that Imp-C was merging with the Gradient pattern inclination. So its peak shape was not found satisfactory. Further by slowing gradient programme injected as trial 04.	Spiked sample injected in the above chromatographic system, It was observed that all four peaks of Imp-A, Imp-B, Imp-C and Solifenacin succinate main peak are well separated.

Hence Trial 04 was considered as final optimised method and validation was performed on the following final methodology (Trail-04).

Preparation of Buffer:
Dissolve 1.42 g disodium hydrogen   phosphate anhydrous in 1litre of Water. Adjust pH to 6.8 with Ortho-phosphoric acid.

Mobile phase A   **:**   Buffer: Acetonitrile (90:10)
Mobile phase B   **:**   Acetonitrile: Methanol (70:30)
Diluent 1   **:**   Buffer
Diluent 2   **:**   Methanol: Acetonitrile (1:1)
Diluent 3   **:** Buffer: Methanol: ACN (20: 40: 40)
Chromatographic Conditions:
Column   :   Waters Xterra RP-8 250 x 4.6, 5μ
Flow Rate   :   1.2 mL / min.
Detection   :   215 nm.
Column Temp   :   30°C.
Injection Volume   :   20 µL.
Run Time   :   60 min.
Retention time   :   About 24 minutes

Gradient Program:

Time	Mobile Phase A	Mobile Phase B
0	80	20
12	80	20
28	60	40
42	60	40
50	80	20
60	80	20

Preparation of Diluted standard solution:
Weigh accurately about 25 mg of Solifenacin succinate working standard & transfer it into 100ml volumetric flask. Add 50ml of diluent 3 and sonicate for 5 minutes to dissolve and make up to the mark with diluent. Dilute 5.0 ml of this solution to 50 ml with the diluent 3 and mix. Dilute 5.0 ml of this solution to 50 ml with the diluent 3 and mix (2.5ppm).

Preparation of impurity stock solution:
Weigh accurately about 2.5 mg of Impurity A, B and C into 50 ml volumetric flask. Add 10ml of methanol and sonicate for 2 minutes to dissolve and make up to mark with methanol and mix well.

Preparation of System suitability solution:
Weigh accurately about 100 mg of Solifenacin succinate working standard & transfer it into 250 ml volumetric flask. Add 200ml diluent 3 and sonicate for 10 mins to dissolve. Add 3 ml of impurity stock solution make up to the mark with diluent 3 and mix.

Preparation of sample solution:
Weigh and transfer 20 tablets in 250ml volumetric flask. Add 50ml diluent 1 and sonicate for 10minutes with intermittent shaking. Then add 150ml of diluent 2 and again sonicate for 20minutes with intermittent shaking. Cool and make up to the mark with diluent 2 and mix. Filter through 0.45µm nylon membrane filter (400ppm).

Preparation of Placebo solution:
Weigh and transfer placebo powder equivalent to 100 mg of Solifenacin succinate excluding the weight of API into 250ml volumetric flask. Add 50ml diluent 1 and sonicate for 10minutes with intermittent shaking. Added 150ml of diluent 2 and again sonicated for 20minutes with intermittent shaking. Cool and make up to the mark with diluent 2 and mix. Filter through 0.45µm nylon membrane filter.

Procedure:
Separately inject equal volumes of Blank (diluent) solution, System suitability solution, Placebo solution, 6 replicates of diluted standard solution and Sample preparation.

Sr. NO.	SAMPLE	RRT
1	Solifenacin succinate	1.0

2	Impurity A	0.5
3	Impurity B	0.84
4	Impurity C	1.78

Evaluation of system suitability:

% RSD of six replicate injections of diluted standard injections should not be more than 5.0.

Resolution Between Impurity B and Solifenacin succinate peak should not be less than 1.5.

Theoretical Plates for Solifenacin succinate peak should not be less than 1500.

Tailing factor for Solifenacin succinate peak should not be more than 2.0.

RESULT AND DISCUSSION:

Specificity: Specificity is the ability of the method to measure the analyte in the presence of process related and the degradation impurities. All known impurity solutions individually, sample solution and spiked sample solution with all known impurities at specification level were prepared and injected into the HPLC equipped with a photodiode array detector and analysed. Peak purity passed for Solifenacin succinate, Impurity A, Impurity B and Impurity C in control sample and spiked sample. Data is reported in Table no 3 and 4 and Figure No 1, 2, 3 and 4.
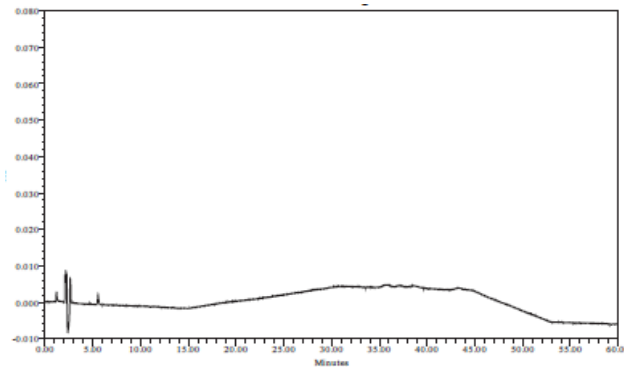
Table 3: Peak purity of standard and Control sample

Sample	Solifenacin succinate	
	Purity angle	Purity Threshold
Standard solution	2.683	4.059
Control sample	0.241	1.048

Table 4: Retention Time Table

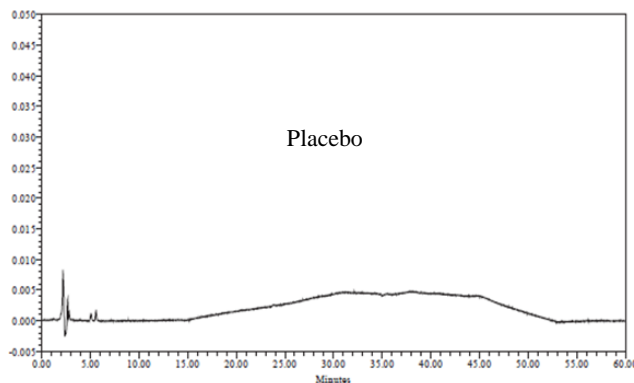| Name	Retention time (min)
Impurity A	12.009
Impurity B	20.097
Solifenacin succinate	24.192
Impurity C	47.296

Blank

Placebo
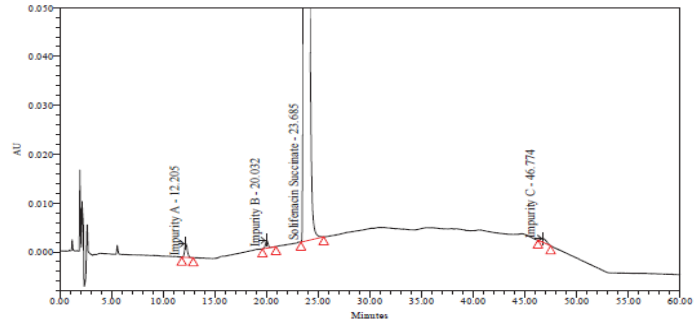
Figure no. 1: Blank & Placebo Chromatograms

Figure no. 2: System Suitability Chromatogram

PA: 2.683 TH: 4.059
— Peak: Solifenacin Succinate
— Purity
— Noise+Solvent (1.00)

PA: 2.683 TH: 4.059
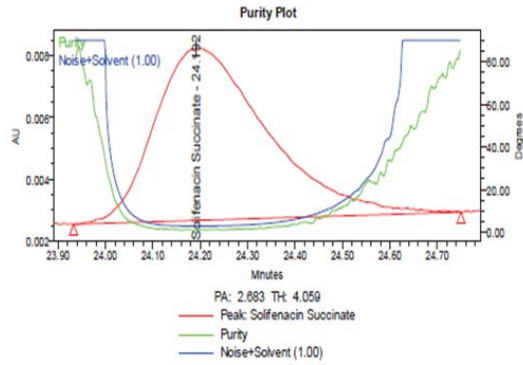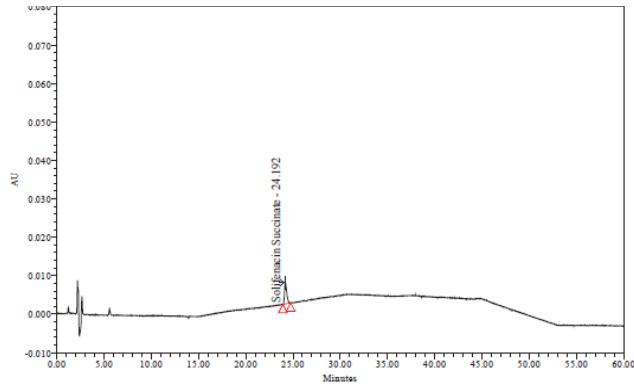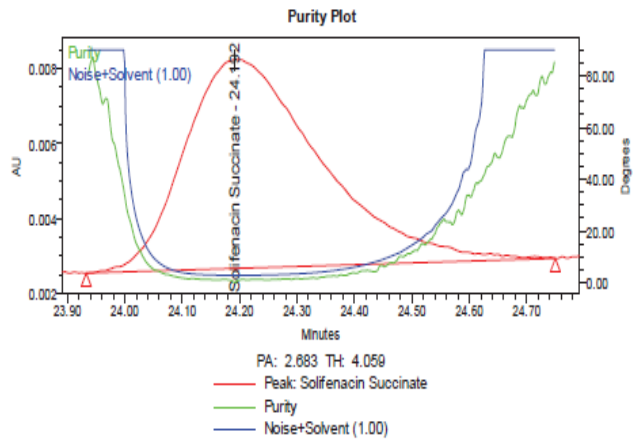— Peak: Solifenacin Succinate
— Purity
— Noise+Solvent (1.00)

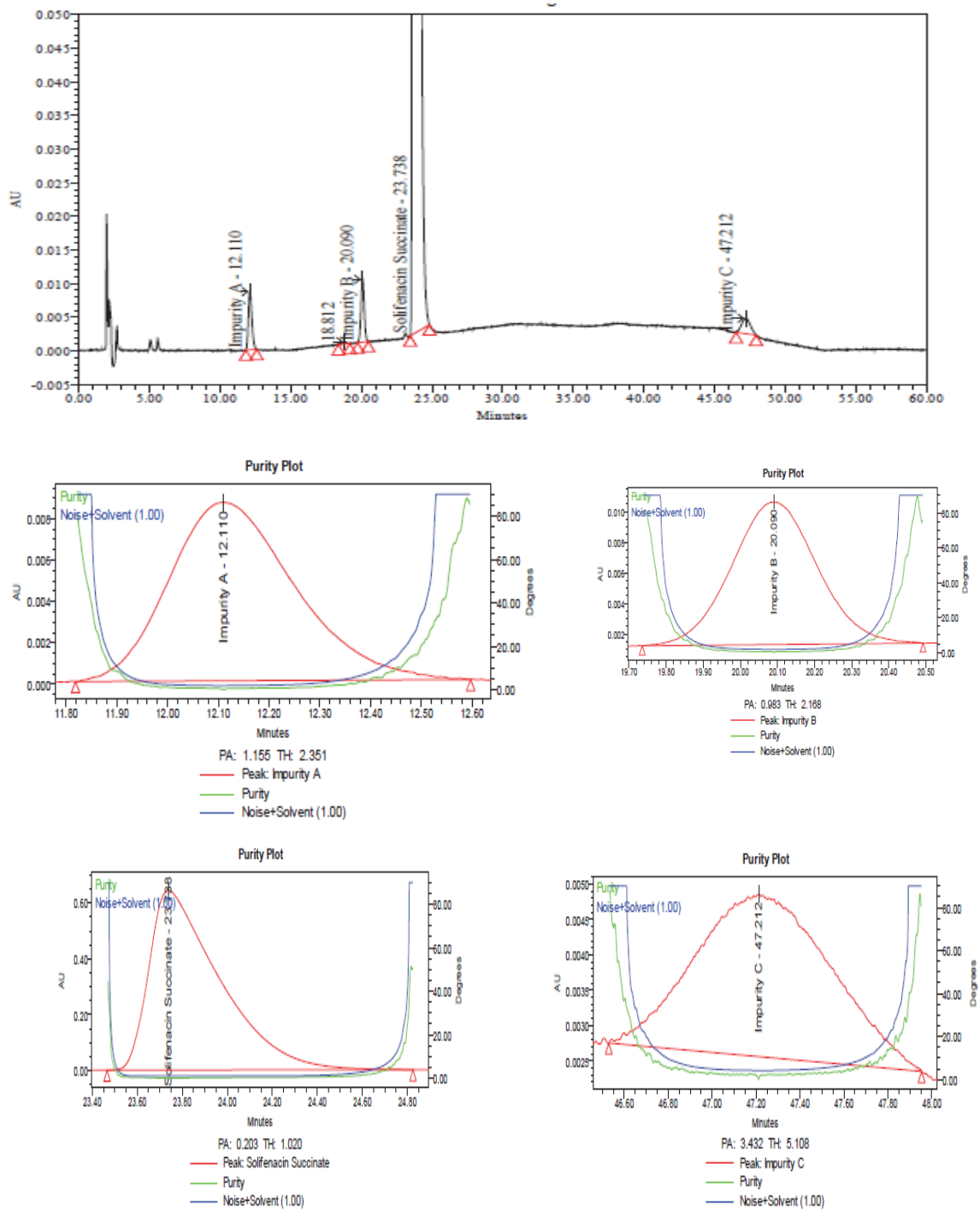Figure no. 3: Standard Chromatogram

Figure no 4: Spiked Sample Chromatogram

Forced Degradation Studies: Summary of Forced degradation data is reported in Table no 5.

Table 5: Table for impurities in Forced Degradation Studies

Sr. No	Experiment	Degradation Condition	% Impurity A	% Impurity B	% Impurity C	% Single max	% Total imp
1	Control	--	ND	0.374	ND	0.023 RRT:0.79	**0.397**
2	Acid Degradation	5N HCl – RT/0 hr	ND	0.177	ND	0.021 RRT:0.79	**0.198**
		5N HCl – RT/24 hr	ND	0.166	ND	0.020 RRT:0.79	**0.186**
		5N HCl – 70°C/3 hr	ND	0.398	ND	0.337 RRT:0.87	**0.766**
3	Base Degradation	2N NaOH– RT/0 hr	ND	0.348	ND	0.157 RRT:0.94	**0.533**
		2N NaOH– RT/24 hr	ND	0.451	ND	0.047 RRT:1.79	**0.519**
		2N NaOH– 70°C/3 hr	ND	0.380	ND	0.034 RRT:0.97	**0.487**
4	Peroxide Degradation	30% H_2O_2 – RT/0 hrs_15ml	ND	21.549	ND	0.025 RRT:0.77	**21.574**
5	Thermal Degradation	105°C – 72 hours	0.037	1.119	ND	0.131 RRT:1.16	**1.538**
6	Photolytic Degradation	1.2 million lux hours	ND	0.207	ND	0.015 RRT:0.78	**0.222**
7	Humidity Degradation	25°C/92%RH – 72 hours	ND	0.368	ND	0.031 RRT:0.79	**0.399**

RT: Room Temperature, ND: Not Detected

Limit of Detection and Limit of Quantification: Based on determination of Prediction linearity, six replicate injections were made for LOD and LOQ precision. Data is summarized in the given Table no 6.

Table 6: Limit of Detection and Limit of Quantitation

	Solifenacin succinate	Impurity A	Impurity B	Impurity C
LOD				
% conc.	0.006	0.006	0.006	0.006
Conc.(µg/mL)	0.024	0.024	0.022	0.023
%RSD	11.56	11.30	10.65	12.79
LOQ				
% conc.	0.020	0.020	0.019	0.019
Conc.(µg/mL)	0.081	0.080	0.075	0.077
%RSD	6.08	3.65	7.41	3.26

Linearity: Excellent correlation was achieved for the regression line of Solifenacin succinate and its related impurities over a range from LOQ to 150 % of the limit level. The correlation coefficient obtained for all the plots was greater than 0.99. The linearity results are tabulated in Table No. 7 and 8 and Figure No.5.

Table 7: Table for Linearity of Solifenacin Succinate and Impurity A

Level	Concentration(µg/ml)	Solifenacin Succinate	Concentration(µg/ml)	Impurity A
LOQ	0.081	3312	0.080	6412
Lin-1	0.504	15756	0.402	33515
Lin-2	1.261	42613	1.004	82145
Lin-3	2.018	68557	1.606	132456
Lin-4	2.522	87956	2.008	166381

Level	Concentration(µg/ml)	Solifenacin Succinate	Concentration(µg/ml)	Impurity A
Lin-5	3.027	105781	2.410	201592
Lin-6	3.784	137050	3.012	248330
	Slope	**35991**	**Slope**	**82916**
	Intercept	**-1992**	**Intercept**	**-231**
	Correlation	**0.99926**	**Correlation**	**0.99993**

Table 8: Table for Linearity of Impurity B and Impurity C

| Level	Concentration(µg/ml)	Impurity B	Concentration(µg/ml)	Impurity C
LOQ	0.075	3300	0.077	6668
Lin-1	0.373	16333	0.383	32663
Lin-2	0.933	42860	0.957	71980
Lin-3	1.493	69621	1.531	116802
Lin-4	1.866	87174	1.914	148987
Lin-5	2.239	104124	2.297	172621
Lin-6	2.799	127098	2.871	217582
	Slope	**46071**	**Slope**	**75147**
	Intercept	**4**	**Intercept**	**1940**
	Correlation Coefficient	**0.99971**	**Correlation Coefficient**	**0.99968**
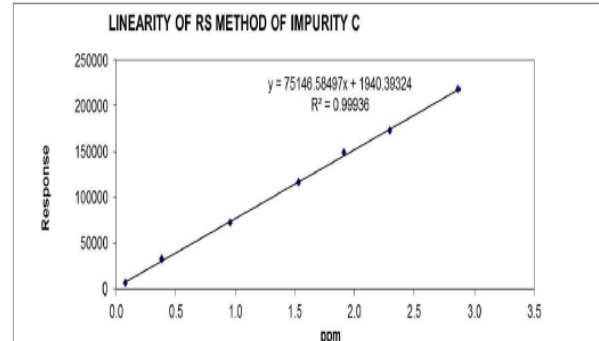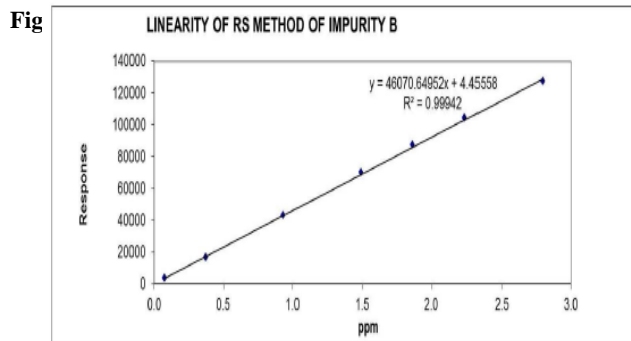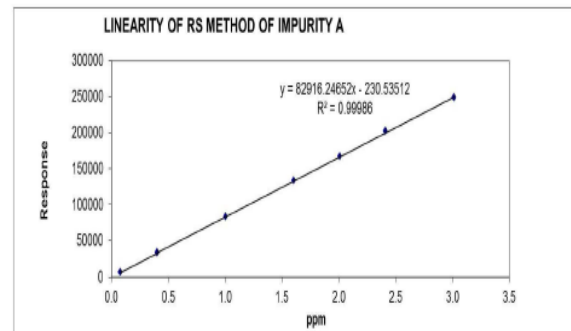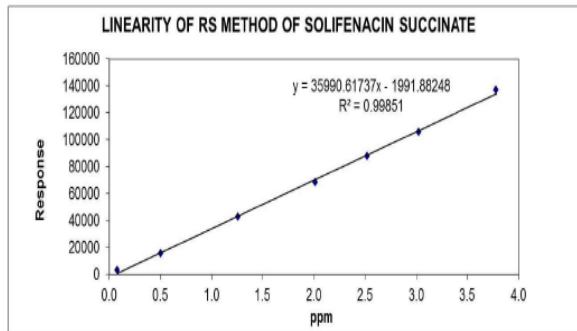
Figure no. 5: Linearity Plots

Accuracy: The studies were carried out at four different levels: LOQ, 50%, 100%, and 150% of limits. The percentages of recoveries of all known impurities were calculated with respect to amount spiked and amount recovered. The percentage recovery at each level was calculated against the Solifenacin succinate standard. Mean recovery should be in the range of 90.0% to 110.0% for 50%, 100% and 150% levels and 85% to 115% for LOQ level. Mean recovery in percentage is reported in Table no.9.

Table 9. Accuracy of Impurity of Solifenacin succinate Tablets

Name of Impurity	Mean Recovery (%)			
	LOQ	50%	100%	150%
Impurity A	99.9	98.7	98.3	99.0
Impurity B	96.1	100.6	98.2	101.5
Impurity C	98.9	104.2	96.1	96.0

Precision: Precision is the closeness of agreement between a series of measurements obtained from multiple sampling of same sample under the prescribed conditions. Quantification of individual impurities and Solifenacin succinate Tablets was performed for each of the preparations and the percent relative standard deviation (RSD) was determined for the content of the impurities.

 To evaluate the intermediate precision, the same experiment was repeated with a different analyst, different lot of column and a different instrument in the same laboratory. Precision and Ruggedness data are reported in Table no.10.

Table 10: Over all %RSD of 12 preparations Comparison for Impurities in Precision and Ruggedness study

Sr. No.	% Impurity A	% Impurity B	% Impurity C	%Single Max	% Total Imp.
Precisio	ND	0.383	ND	0.032	0.415
Precisio	ND	0.383	ND	0.031	0.414
Precisio	ND	0.383	ND	0.031	0.414
Precisio	ND	0.385	ND	0.032	0.417
Precisio	ND	0.383	ND	0.032	0.415
Precisio	ND	0.383	ND	0.033	0.416
Rugged	ND	0.398	ND	0.027	0.422
Rugged	ND	0.400	ND	0.027	0.427
Rugged	ND	0.397	ND	0.028	0.425
Rugged	ND	0.395	ND	0.028	0.423
Rugged	ND	0.397	ND	0.025	0.422
Rugged	ND	0.394	ND	0.028	0.422
Mean	**NA**	**0.390**	**NA**	**0.030**	**0.419**
% RSD	**NA**	**1.79**	**NA**	**10.00**	**1.19**

ND: Not detected, NA: Not applicable

Robustness: The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters. Deliberate changes were made from original experimental conditions to record the tailing factor and theoretical plates of the Solifenacin succinate Tablets to determine the robustness of the developed method. Data are reported in Table no.11.

Table 11: Robustness, RRT

Sr. no.	Parameters	Variations	RRT		
			Impurity A	Impurity B	Impurity C
1	Control-1	-	0.53	0.80	1.88
	Control-2		0.47	0.88	2.06
	Control-3		0.51	0.85	1.99
2	pH of Buffer	+ 0.2 units	0.64	0.77	1.79
		- 0.2 units	0.49	0.85	2.02

Sr. no.	Parameters	Variations	RRT		
			Impurity A	Impurity B	Impurity C
3	Flow rate	-0.1ml/min	0.50	0.86	2.02
		+0.1ml/min	0.46	0.85	2.01
4	Column Temp	+5°C	0.51	0.82	1.93
		-5°C	0.45	0.86	2.04
5	Wavelength	-5 nm	0.51	0.85	1.99
		+5 nm	0.51	0.85	1.99

Stability of Analytical solution: The solution stability of sample and standard solution provides an indication of the method's reliability in normal usage during the storage of the solutions used in the method. No significant changes were experienced in the content of any of the impurities during solution stability. The % Cumulative RSD of Standard solution and sample Solution are reported in Table No.12 and 13.

Table 12: Table for solution stability for diluted standard at room temperature

Sr. No.	Time (hrs)	Response (Area)
1	INITIAL	94348
2	28	93285
3	50	93814
4	67	93372
5	84	93747
6	104	92416
%RSD		0.70

Table 13: Table for solution stability for sample solution preparation at Room Temperature

Sr. No.	Time (hrs)	Area		
		Solifenacin	Impuri	Highest
1	INITI	14708579	72160	4759
2	33	14726379	72787	4647
3	43	14765971	72751	4638
4	60	14787204	72847	4741
5	77	14833584	72749	4677
6	97	14948871	72755	4874
%RSD		0.59	0.35	1.88

Standard Solution is stable for 104 hrs and Sample solution is stable for 97 hrs at room temperature.

Table 14: Table for System Suitability

Sr. No.	Experiment	% RSD	Resolution between impurity B and Solifenacin succinate peak	Tailing factor	Theoretical Plates
1	Forced degradation -1	1.31	6.6	1.5	49765
2	Forced degradation -2	1.38	8.2	1.3	53965
3	Prediction linearity	0.63	6.5	1.2	48608
4	Precision, Accuracy, Filter Equivalency, Solution Stability	0.88	4.9	1.3	43101
5	Ruggedness	1.35	7.2	1.3	29533
6	LOD & LOQ ,Linearity	0.52	5.9	1.3	42777

SUMMARY AND CONCLUSION: The Validated HPLC method for the related substance of Solifenacin succinate Tablets is linear, precise, accurate, Robust and specific. The results of the validation carried out for the method satisfied the ICH requirements. This method can be used for the detection and quantification of known, unknown and degradation impurities in the Solifenacin succinate tablets during routine analysis and also for stability studies in view of its capability to separate degradation products.

LIST OFABBREVIATIONS:

No.	Number
MP	Mobile Phase
RT	Room Temperature
LOD	Limit of Detection
LOQ	Limit of Quantitation
Imp	Impurity
Unk	Unknown
Max	Maximum
hrs	Hours
HPLC	High performance Liquid Chromatography
RSD	Relative Standard Deviation
RRT	Relative retention time
ND	Not Detected
NA	Not Applicable
min	Minutes

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