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STABILITY INDICATING HPTLC METHOD FOR THE ESTIMATION OF SOLIFENACIN

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Abstract

The stability indicating assay is a method used in the pharmaceutical industry to analyze stability samples. It is crucial to validate the assay for precision, accuracy, reproducibility, selectivity, and robustness. Current guidelines for stability testing of drug substances and products require stress testing or forced degradation studies to be conducted for stability indicating assays. However, these guidelines do not specify how these studies should emphasize testing features susceptible to change during storage, which can affect quality, safety, and efficacy. Therefore, validated stability indicating testing methods must be used.

Stability indicating analytical methods are well-established for active pharmaceutical ingredients, ensuring that allopathic medicines meet quality standards despite product degradation. Given the need for stability indicating analytical methods in herbal medicine, this study aims to apply and validate an HPTLC method for detecting forced degradation products of Solifenacin.

Keywords

HPTLC, Stability Indicating, Solifenacin Degradation.

INTRODUCTION

The stability-indicating assay is a critical method employed for analyzing stability samples in the pharmaceutical industry. Validation of such assays is imperative to ensure precision, accuracy, reproducibility, selectivity, and robustness. Additionally, in pharmaceutical research, it is essential for an assay to unequivocally demonstrate its ability to be stability-indicating. This means it should accurately distinguish the intact drug from any potential degradation products.

The necessity for stability-indicating assays includes several key objectives:

- Differentiating the active ingredient from closely related impurities and degradation products.
- Ensuring the detection of changes in the identity, quantity, and potency of the drug substance or product.
- Monitoring the stability of a drug within its finished product.
- Establishing and confirming the shelf life of drug substances and products.
- Supporting various applications such as cleaning validation and performance testing, including dissolution testing.

Method Development:

Preparation of Standard Solutions: Stock solutions of Solifenacin were prepared by dissolving 11.50 mg in 10 ml of methanol, resulting in a concentration of 1.15 mg/ml. This solution was used to prepare various concentrations (ranging from 1.15 to 111.5 µg/spot) by applying 1, 3, 5, 7, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 µL using a LINOMAT 5 applicator with an 8 mm bandwidth.

Chromatographic Conditions: Several parameters of HPTLC were optimized:

- **Stationary Phase:** Silica Gel 60 GF254 pre-washed with methanol and activated at 60°C for 5 minutes.
- **Mobile Phase:** Toluene

acetate

(2.5:6.5:1) as per Indian Herbal Pharmacopeia, yielding a well-resolved band of Solifenacin at R_f 0.82.

- **Bandwidth:** Testing bandwidths of 6 mm, 7 mm, and 8 mm; 7 mm provided optimal separation without oversaturation of the sample spot, allowing for multiple tracks on a single TLC plate (20 x 10 cm).

Detection: Solifenacin's UV spectrum was observed, identifying λ_{max} at 254 nm. TLC plates were scanned at this wavelength using a deuterium lamp.

Densitometric Conditions for HPTLC Studies:

- **Stationary Phase:** Silica Gel 60 GF254 (Merck) pre-coated plates.
- **Mobile Phase:** Chloroform:methanol

acid (8.2:1.5:1).

- **Saturation Time:** 15 minutes.
- **Development Time:** 15 minutes.
- **Wavelength:** 254 nm.
- **Lamp:** Deuterium.
- **Band Width:** 7 mm.
- **Chromatogram Length:** 8 cm.

Experimental Setup: Linear ascending development was conducted in a 20 cm x 10 cm twin trough glass chamber saturated with the mobile phase. Densitometric scanning was performed

using a Camag TLC scanner III in reflectance-absorbance mode, controlled by Win CATS software (version 1.3.0 Camag). Compound concentrations were determined based on diffusely reflected light intensity, and evaluation involved comparing peak areas of Solifenacin via linear regression.

Results: The developed method demonstrated excellent resolution of standard Solifenacin (R_f value = 0.82) in tablet formulation extracts, as depicted in Figures 3 and 4.

This comprehensive method development and validation process ensure the robustness and reliability of the HPTLC method for detecting forced degradation products of Solifenacin, crucial for pharmaceutical quality control and stability assessment.

Method Validation of HPTLC Method as per ICH Guidelines

1. Analytical Method Validation Overview:

- Validates the suitability of an analytical test system for its intended purpose.
- Ensures the method can provide accurate and reliable analytical data.

2. Parameters Validated:

- **Linearity:**
 - Solifenacin concentrations (10.35 - 109.50 $\mu\text{g}/\text{spot}$) were analyzed in triplicate.
 - Linear regression analysis showed a correlation coefficient (r^2) of 0.9997 in the range of 11.5 - 103.5 $\mu\text{g}/\text{spot}$ (Fig 5).
- **Limit of Detection (LOD) and Limit of Quantitation (LOQ):**
 - Determined using the standard deviation method.
 - Experimentally derived values for Solifenacin were found to be 3.45 and 10.35 $\mu\text{g}/\text{spot}$ for LOD and LOQ, respectively.
- **Precision Studies:**
 - Evaluated by repeatability (intra-day) and instrumental precision.
 - R.S.D. values were less than 1% for concentrations of 34.50, 57.50, and 80.50 $\mu\text{g}/\text{spot}$, demonstrating high precision (Table 1).
- **Accuracy Studies:**
 - Assessed through percentage recoveries of spiked Solifenacin in extracts and commercial products.
 - Recoveries ranged from 99.18% to 109.77%, within the acceptable range of 80-120%, indicating the method's accuracy (Table 2).
- **Robustness:**
 - Varied chromatographic parameters like mobile phase composition and chamber saturation time.
 - No significant changes observed in retention time, peak shape, or area under the curve with $\pm 5\%$ changes in toluene and diethylamine ratio, and saturation time variations from 15 to 30 minutes (Tables 3 and 4).
- **Stability Studies:**

- Evaluated the stability of Solifenacin in sample solutions over 72 hours at 4.0°C and 25.0°C.
- Minimal degradation observed in methanol extracts and formulations at both temperatures, confirming stability under storage conditions (Table 5).

Stability Studies of Solifenacin

Forced Degradation Studies

1. Acid Degradation

- Prepared 1M hydrochloric acid (HCl) and mixed with Solifenacin solution (1 mg/ml).
- Refluxed for 3 hours at 60°C, neutralized with 1 N NaOH to pH 7, and made up to 10 ml with methanol.
- TLC analysis after 1 hour reflux showed degradation peaks (Rf 0.11, 0.17, 0.34, 0.36, 0.49) alongside Solifenacin (Rf 0.19).
- Reduced HCl concentration to 0.1N showed minimal change, indicating instability under acidic conditions.

2. Base Degradation

- Prepared 1M sodium hydroxide (NaOH) and mixed with Solifenacin solution (1 mg/ml).
- Refluxed for 3 hours at 60°C, neutralized with 1 N HCl to pH 7, and made up to 10 ml with methanol.
- TLC analysis after 1 hour reflux showed degradation peaks (Rf 0.09, 0.14, 0.23, 0.35, 0.54) alongside Solifenacin (Rf 0.22).
- Reduced NaOH concentration to 0.1N showed minimal change, indicating instability under alkaline conditions.

3. Oxidative Degradation

- Used 3% hydrogen peroxide (H₂O₂) with Solifenacin solution (1 mg/ml).
- Refluxed for 3 hours at 60°C, then analyzed on TLC.
- Detected three additional degradation peaks, with a 35.66% decrease in Solifenacin peak area under oxidative stress.

4. Wet Degradation

- Refluxed aqueous Solifenacin solution (1 mg/ml) for 3 hours at 60°C.
- TLC analysis showed four degradation peaks (Rf 0.06, 0.36, 0.61, 0.80) alongside Solifenacin (Rf 0.21).
- Confirmed Solifenacin stability with 22.76% degradation.

5. Dry Heat

- Heated Solifenacin at 100°C for 3 hours and 60°C for 24 hours.
- No degradation observed; extended exposure (48 hours at 60°C) also showed no degradation.

6. Photostability Study

- Exposed Solifenacin to UV short (254 nm) light for 24 hours.
- Detected two additional degradation peaks (Rf 0.12, 0.64) alongside Solifenacin (Rf 0.22).
- Showed 18.47% decrease in Solifenacin peak area, indicating UV-induced degradation.

Result and Discussion: Method Development

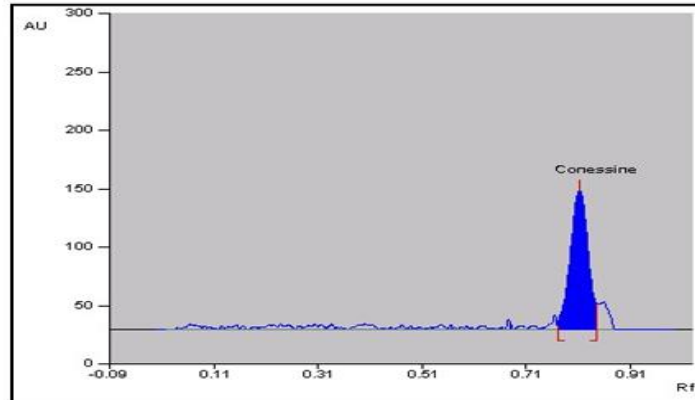


Fig. 3: HPTLC Chromatogram of Standard Solifenacin using optimized parameters

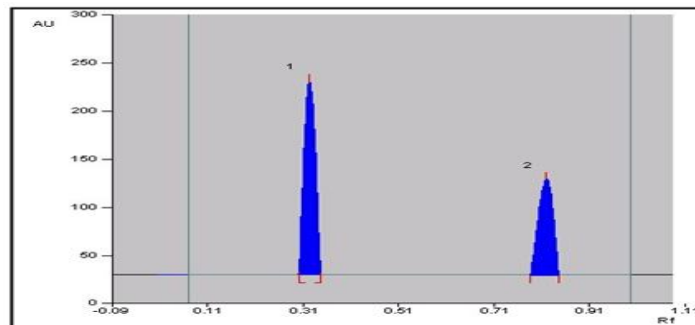


Fig. 4: HPTLC Chromatogram of chloroform extract of Tablet using optimized parameters

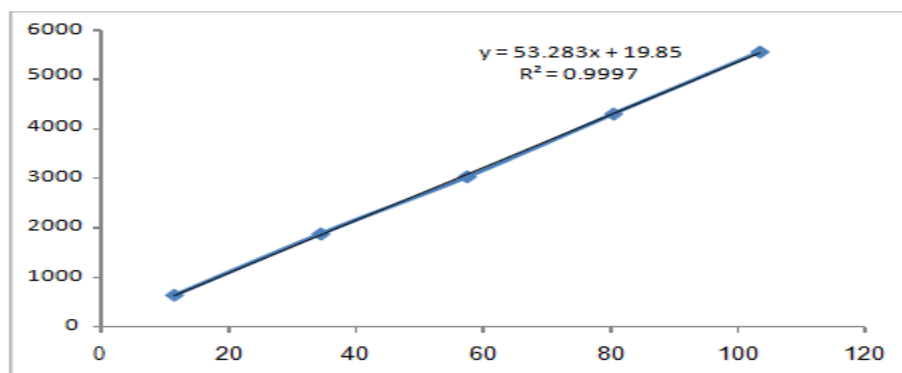


Fig 5: Calibration curve of Solifenacin

Table 1: Results of Precision Studies of Solifenacin

Type of Precision	Intra-day			Inter-day		
	AUC for concentration of Solifenacin ($\mu\text{g}/\text{spot}$)			AUC for concentration of Solifenacin ($\mu\text{g}/\text{spot}$)		
S. No	40	60	90	40	60	90
1.	2118.17	3675.72	4768.51	2140.88	3680.82	4816.09
2.	2119.01	3671.62	4779.65	2168.39	3649.19	4780.36
3.	2114.16	3691.46	4815.98	2154.83	3701.81	4801.73
Mean	2119.78	3679.60	4788.05	2160.699	3676.940	4799.391
% RSD	0.45	0.52	0.64	0.57	0.54	0.55

Table 2: Recovery studies for Solifenacin in formulations containing tablet

10 μl of each Extract & Formulation	Amount added in μg	AUC			Recovery \pm S.D. (%)
		Formulation	Standard	Standard spiked formulation	
Methanol extract of Tablet	46	2150.78	2718.95	4919.84	111.16 \pm 0.24
	57.5	2150.78	3391.98	5763.91	114.39 \pm 0.15
	69	2150.78	4066.01	6327.43	111.96 \pm 0.17
Brand II	46	1730.64	2718.95	4173.21	103.19 \pm 0.09
	57.5	1730.64	3391.98	4969.45	106.71 \pm 0.65
	69	1730.64	4066.01	5616.37	106.58 \pm 0.09
Capsules	46	2150.78	2718.95	4781.57	108.03 \pm 0.14
	57.5	2150.78	3391.98	5491.76	109.08 \pm 0.03
	69	2150.78	4066.01	6141.55	109.67 \pm 0.07

Table 3: Robustness (Mobile phase variation) studies of Solifenacin

Sr. No	Mobile phase composition (v/v)			R _f	AUC
	Toluene	Ethyl acetate	Diethylamine		
1.	2.5	6.5	1	0.82	3120.12±0.23
2.	2.625	6.5	0.5	0.82	3121.35±0.52
3.	2.375	6.5	0.5	0.82	3120.93±0.15
4.	2.5	6.5	1.05	0.82	3121.06±0.23
5.	2.5	6.5	0.95	0.82	3120.59±0.06
S.D.	-	-	-	0.0	0.45

Table 4: Robustness (Chamber saturation time variation) studies of Solifenacin

S. No	Chamber saturation time (min)	R _f	AUC
1.	15	0.83	3120.13±0.25
2.	20	0.83	3120.16±0.14
3.	25	0.83	3121.65±0.26
4.	30	0.83	3120.35±0.25
S.D.	-	0.0	0.73

Table 5: Stability Studies of Solifenacin in formulations containing Tablet

Extracts & Formulations	Percentage of non-degraded Solifenacin					
	Temperature					
	4 °C			25 °C		
	24hrs	48hrs	72hrs	24hrs	48hrs	72hrs
Methanol extract of Tablet	99.06	98.55	98.12	98.28	97.25	97.11
Brand II	99.82	99.13	98.86	99.23	98.32	97.68
Capsule	99.64	99.04	98.85	99.11	98.56	98.35

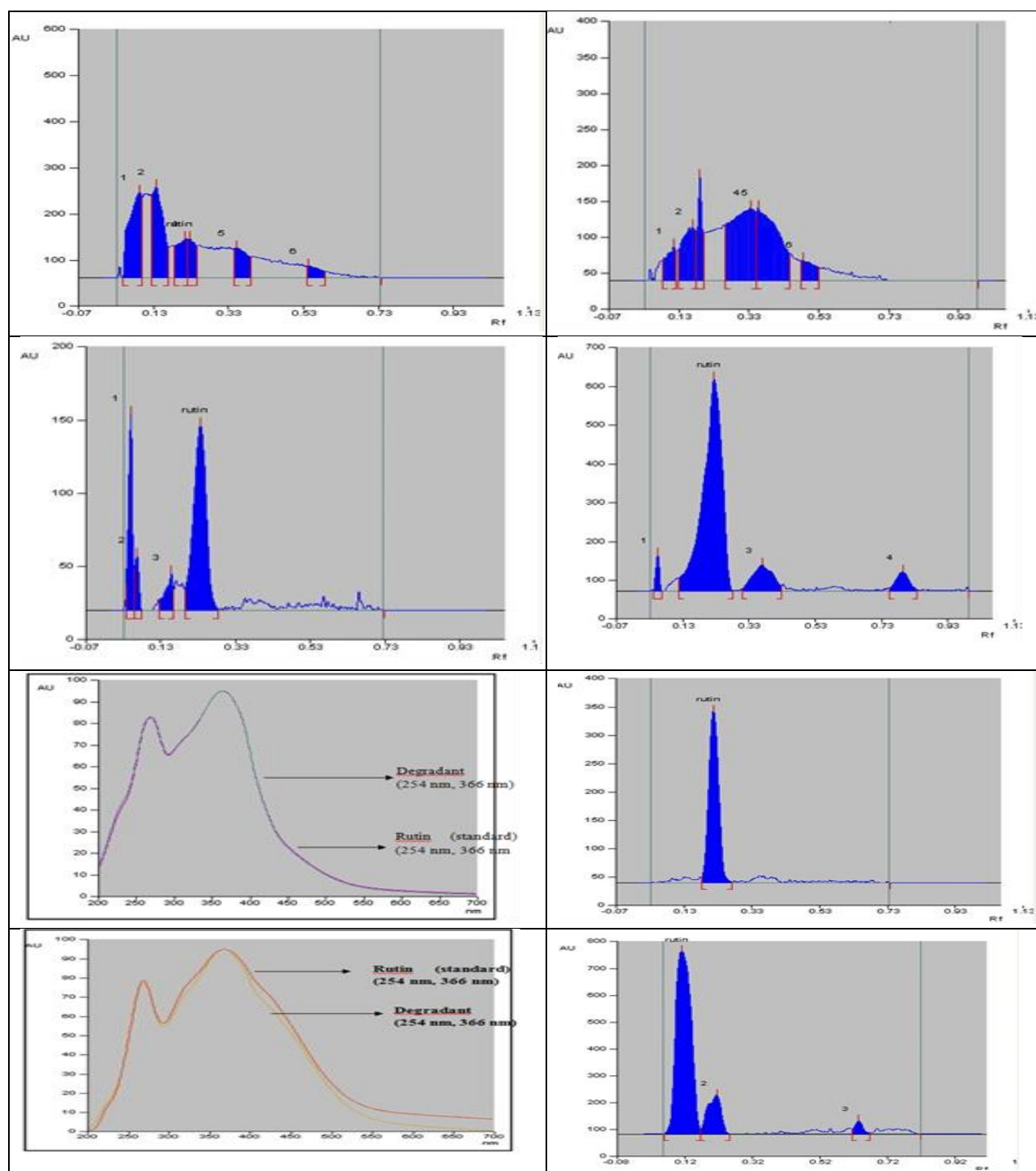


Fig 6: HPTLC Chromatogram of Solifenacin after acid degradation

Fig.7: HPTLC Chromatogram of Solifenacin after base degradation

Fig.8: HPTLC Chromatogram of Solifenacin after oxidative stress

Fig.9: HPTLC Chromatogram of Solifenacin after wet degradation

Fig 10: UV spectrum of Solifenacin (standard) and degradation product after wet degradation

Fig. 11: HPTLC Chromatogram of Solifenacin after dry degradation for 3 hr at 100°C

Fig.12: UV spectrum of Solifenacin (standard) and degradant (dry degradation for 3 hr at 100°C)

Fig. 13: HPTLC chromatogram of Solifenacin after UV Exposure

CONCLUSION

Developed and validated HPTLC methods were effectively applied to determine the stability of Solifenacin, demonstrating their utility as stability-indicating methods. The study revealed that Solifenacin remained stable under dry, wet, oxidative, and UV exposure stress conditions. However, degradation was observed under acidic and basic conditions. Solifenacin is a specific biomarker for the species *Holarrhena* and possesses established pharmacological activities. Therefore, ensuring the stability of formulations containing Solifenacin through reliable stability-indicating methods is crucial and advantageous.

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