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STABILITY INDICATING HPTLC METHOD FOR THE ESTIMATION OF MIRABEGRON

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Abstract

In the pharmaceutical sector, a stability-indicating assay plays a critical role in analyzing samples for stability. Validation of such assays is essential to ensure precision, accuracy, reproducibility, selectivity, and robustness. Current guidelines for stability testing of drug substances and products mandate stress testing or forced degradation studies to validate stability-indicating assays. However, these guidelines do not specify how such studies should emphasize testing features prone to changes during storage, potentially affecting quality, safety, and efficacy.

Stability-indicating analytical methods are extensively utilized for active pharmaceutical ingredients, ensuring that allopathic medicines meet stringent quality standards even after prolonged storage and potential degradation. Recognizing the necessity for similar stability-indicating methods in herbal medicine, this study focuses on applying a developed and validated HPTLC method to detect products of forced degradation in Mirabegron.

Keywords

HPTLC, Stability Indicating, Mirabegron Degradation.

INTRODUCTION

Stability-indicating assays are crucial methods in the pharmaceutical industry for analyzing stability samples. Validation of these assays focuses on ensuring precision, accuracy, reproducibility, selectivity, and robustness. In pharmaceutical research, it is essential for an assay to be conclusively proven as stability-indicating, meaning it can accurately differentiate the active drug from its degradation products and impurities.

The genus Holarrhena, consisting of several species found across Asia, tropical Africa, Madagascar, India, the Philippines, and the Malayan Peninsula, holds significant importance in Indian medicine, particularly with its sole Indian species, H. antidysenterica.

Importance of Stability-Indicating Assays:

- Differentiation: They distinguish the active ingredient from related impurities and degradation products.
- Assurance: They ensure the detection of changes in the identity, quantity, and potency of drug substances or products.
- Stability Monitoring: They monitor the stability of drugs in their finished formulations.
- Shelf Life Confirmation: They establish and confirm the shelf life of drug substances and products.
- Other Uses: They are also applied in tasks like cleaning validation and performance testing, including dissolution testing.

Method Development: For Mirabegron, stock solutions were prepared by dissolving 11.50 mg in 10 ml of methanol, resulting in a concentration of 1.15 mg/ml. From this stock, various aliquots were applied to HPTLC plates, yielding concentrations ranging from 1.15 to 111.5 µg/spot using a LINOMAT 5 applicator with an 8 mm bandwidth.

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Chromatographic Conditions: The HPTLC parameters were optimized, utilizing precoated silica gel 60 GF254 plates with a mobile phase of chloroform:methanol

acid (8.2:1.5:1). Development occurred in a 20 cm x 10 cm twin trough glass chamber saturated with the mobile phase for 15 minutes. Detection was set at 254 nm using a deuterium lamp with a 7 mm bandwidth and an 8 cm chromatogram length. Densitometric scanning in reflectance-absorbance mode was performed with a Camag TLC scanner III controlled by Win CATS software (version 1.3.0 Camag). Quantification was based on the diffusely reflected light intensity, and Mirabegron concentrations were determined by comparing peak areas with linear regression.

The developed method demonstrated excellent resolution of standard Mirabegron (Rf value = 0.82) in tablet formulations, as depicted in Figures 3 and 4.

Method Validation of HPTLC Method as per ICH Guidelines

1. **Analytical Method Validation Overview:**
    - Ensures the suitability of an analytical test system for its intended purpose.
    - Validates the method's ability to provide accurate and reliable analytical data.
2. **Parameters Validated:**
    - **Linearity:**
        - Different concentrations of Mirabegron (10.35 - 109.50 µg/spot) were analyzed in triplicate.
        - Regression analysis showed a correlation coefficient (r^2) of 0.9997 in the range of 11.5 - 103.5 µg/spot (Figure 5).
    - **Limit of Detection (LOD) and Limit of Quantitation (LOQ):**
        - Determined using the standard deviation method.
        - Experimentally derived values for Mirabegron were found to be 3.45 and 10.35 µg/spot for LOD and LOQ, respectively.
    - **Precision Studies:**
        - Evaluated through repeatability (intra-day) and instrumental precision.

- R.S.D. values were less than 1% for three different concentration levels (34.50, 57.50, and 80.50 µg/spot), indicating high precision (Table 1).

- **Accuracy Studies:**
  - Assessed by percentage recoveries of Mirabegron added to extracts and commercial products.
  - Recoveries ranged from 99.18% to 109.77%, demonstrating the method's accuracy (Table 2).
- **Robustness:**
  - Tested variations in mobile phase composition and chamber saturation time.
  - No significant changes observed in retention time, peak shape, or area under the curve of Mirabegron with ± 5% changes in toluene and diethylamine ratio, and saturation time variations from 15 to 30 minutes (Tables 3 and 4).

Stability Studies

The stability of Mirabegron in sample solutions was evaluated to check for spontaneous degradation over 3 days. Sample stability was tested after 24, 48, and 72 hours at 4.0°C and 25.0°C. Stability was assessed by comparing the chromatographic parameters of stored solutions with freshly prepared ones. The results were expressed as the percentage of non-degraded Mirabegron after 24, 48, and 72 hours. Methanol extract showed 1.89% degradation after 72 hours at 4°C and 2.99% at 25°C. In formulations, maximum degradation after 72 hours was 1.12% at 4°C and 2.02% at 25°C for vati, and 1.13% at 4°C and 1.45% at 25°C for capsules (Table 5).

Stability Studies of Mirabegron

Forced Degradation Studies of Mirabegron

Acid Degradation:

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- **Preparation:**
    - Diluted 8.5 ml concentrated HCl to 100 ml with distilled water (1N HCl).
    - Prepared 1 mg/ml Mirabegron solution.
    - Mixed 1 ml of Mirabegron solution with 4 ml of 1N HCl.
    - Refluxed mixture at 60°C for 3 hours.
- **Post-reflux:**
    - Cooled to ambient temperature.
    - Neutralized with 1N NaOH to pH 7.
    - Adjusted volume to 10 ml with methanol.
    - Applied final solution to TLC plates.
- **Results:**
    - Total degradation observed after 3 hours.
    - Reduced exposure to 1 hour showed similar degradation patterns.
    - Six peaks of degradants, highest at Rf 0.29 (64.99%).
    - Not stable under acidic conditions tested.

Base Degradation:

- **Preparation:**
    - Dissolved 4 g sodium hydroxide pellets in 100 ml distilled water (1M NaOH).
    - Mixed 1 ml Mirabegron solution (1 mg/ml) with 4 ml 1N NaOH.
    - Refluxed mixture at 60°C for 3 hours.
- **Post-reflux:**
    - Cooled to ambient temperature.
    - Neutralized with 1N HCl to pH 7.
    - Adjusted volume to 10 ml with methanol.
    - Applied final solution to TLC plates.
- **Results:**
    - Total degradation observed after 3 hours.
    - Reduced exposure to 1 hour showed similar degradation patterns.

- Four peaks of degradants, highest at Rf 0.22 (84.47%).
- Not stable under alkaline conditions tested.

Oxidative Degradation:

- **Preparation:**
  - Mixed 1 ml Mirabegron solution (1 mg/ml) with 9 ml 3% H2O2.
  - Refluxed mixture at 60°C for 3 hours.
- **Results:**
  - No degradation with 3% H2O2 for 3 hours.
  - Extended exposure to 8 hours showed no additional peaks.
  - Stable under oxidative conditions.

Wet Degradation:

- **Preparation:**
  - Refluxed 10 ml aqueous Mirabegron solution (1 mg/ml) at 60°C for 3 hours.
- **Results:**
  - Three degradant peaks at Rf values 0.34, 0.56, 0.59, and 0.76.
  - Mirabegron peak shifted from Rf 0.76 to 0.92.
  - Highest degradant peak at Rf 0.76 (51.81%).
  - 86.15% degradation observed.

Dry Heat Degradation:

- **Preparation:**
  - Heated 5 mg Mirabegron at 100°C for 3 hours, dissolved in 5 ml methanol.
  - Heated another 5 mg at 60°C for 24 hours, dissolved in 5 ml methanol.
- **Results:**
  - No degradation at 100°C for 3 hours.
  - Three peaks after 24 hours at 60°C.

- Confirmed Mirabegron peak at Rf 0.77.
- Total degradation of 5.22%.

Photostability Study:

- **Preparation:**
  - Exposed 5 mg Mirabegron to UV light (254 nm) for 24 hours, dissolved in 5 ml methanol.
- **Results:**
  - No degradation after 24 hours of UV exposure.
  - No degradation after 48 hours.
  - Stable under tested photostability conditions.

Result and Discussion:

Method Development

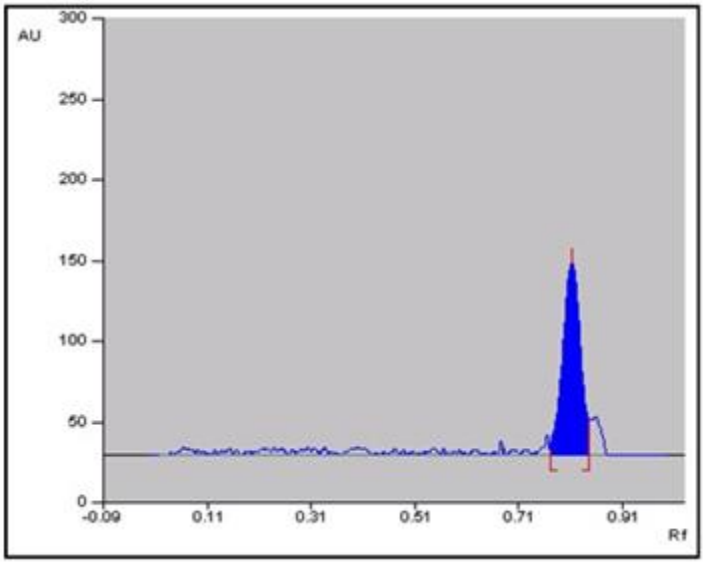

Fig. 3: HPTLC Chromatogram of Standard Mirabegron using optimized parameters

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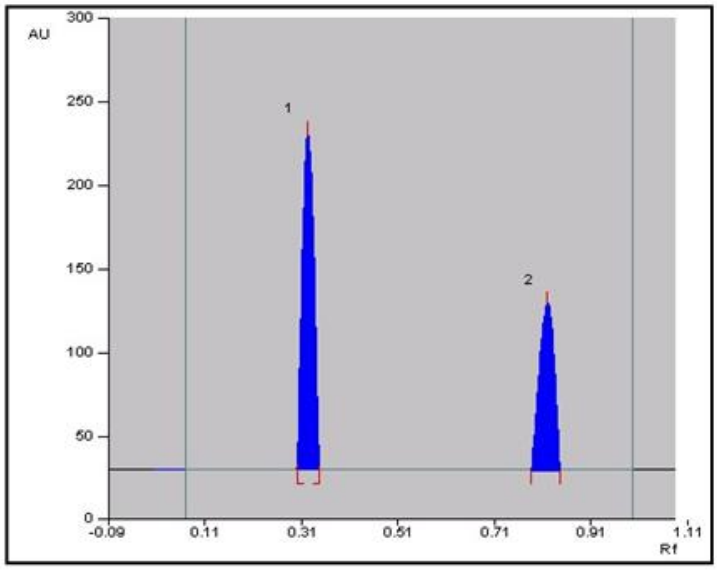

Fig. 4: HPTLC Chromatogram of chloroform extract of Tblet using optimized parameters

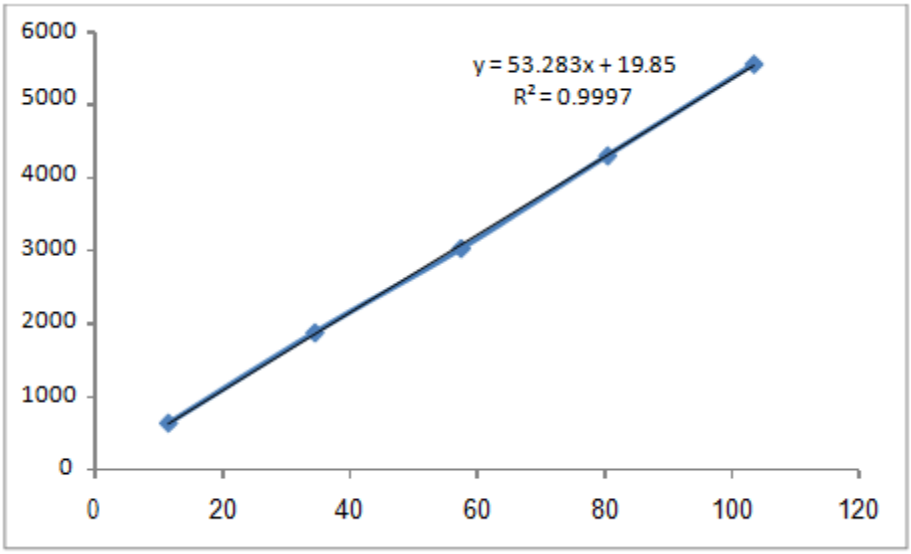

Fig 5: Calibration curve of Mirabegron

Table 1: Results of Precision Studies of Mirabegron

Type of	Intra-day	Inter-day

Precision	AUC for concentration of Mirabegron (µg/spot)			AUC for concentration of Mirabegron (µg/spot)		
S. No	**40**	**60**	**90**	**40**	**60**	**90**
1.	1825.62	3241.54	4235.02	1846.23	3245.26	4278.23
2.	1827.26	3237.83	4245.13	1871.22	3217.43	4245.78
3.	1831.03	3255.85	4278.14	1858.91	3265.22	4265.22
Mean	1827.95	3245.07	4252.72	1858.87	3242.67	4263.03
% RSD	0.41	0.47	0.58	0.52	0.49	0.50

Table 2: Recovery studies for Mirabegron in formulations containing Tablet

Extract & Formulation	Amount added in µg	AUC Formulation	AUC Standard	AUC Standard spiked formulation	Recovery ± S.D. (%)	S.D.
Methanol extract of Tablet	50.60	2150.775	2717.946	4919.838	111.155	0.264
Methanol extract of Tablet	63.25	2150.775	3391.982	5763.912	114.389	0.165
Methanol extract of Tablet	75.90	2150.775	4066.007	6327.431	111.958	0.187
Brand II	50.60	1730.641	2717.946	4173.213	103.191	0.099
Brand II	63.25	1730.641	3391.982	4969.448	106.711	0.715

Brand II	75.90	1730.641	4066.007	5616.369	106.579	0.099
Capsules	50.60	2150.775	2717.946	4781.568	108.031	0.154
Capsules	63.25	2150.775	3391.982	5491.761	108.988	0.033
Capsules	75.90	2150.775	4066.007	6141.553	108.669	0.077

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

Sr. No	Mobile phase composition (v/v)			Rf	AUC
	Toluene	Ethyl acetate	Diethyl-amine		
1.	2.5	6.5	1	0.82	3120.11±0.25
2.	2.625	6.5	0.5	0.82	3121.32±0.53
3.	2.375	6.5	0.5	0.82	3120.92±0.14
4.	2.5	6.5	1.05	0.82	3121.03±0.22
5.	2.5	6.5	0.95	0.82	3120.55±0.05
S.D.	-	-	-	0.0	0.45

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

S.No	Chamber saturation time (min)	R_f	AUC
1.	15	0.82	3120.12±0.25
2.	20	0.82	3120.19±0.12
3.	25	0.82	3121.66±0.23
4.	30	0.82	3120.38±0.25
S.D.	-	0.0	0.71

Table 5: Stability Studies of Mirabegron in Formulations Containing Tablet

	Percentage of non-degraded Mirabegron

Extracts & Formulations	Temperature					
	4 °C			25 °C		
	24hrs	48hrs	72hrs	24hrs	48hrs	72hrs
Methanol extract from Tablet	99.07	98.59	98.11	98.78	97. 45	97. 01
Brand II	99.85	99.14	98.88	99.13	98.52	97.98
Capsule	99.67	99.08	98.87	99.01	98.96	98.55

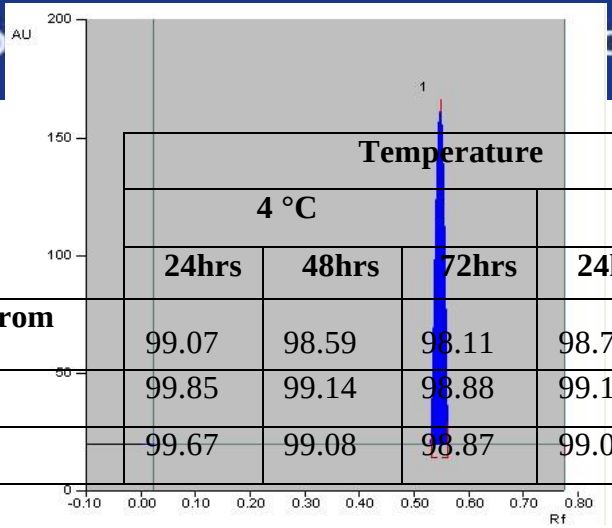

Fig 6: HPTLC Chromatogram of Mirabegron after acid degradation

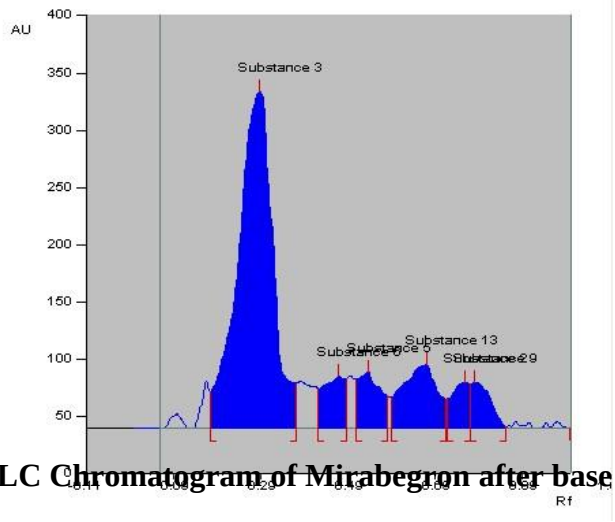

Fig.7: HPTLC Chromatogram of Mirabegron after base degradation

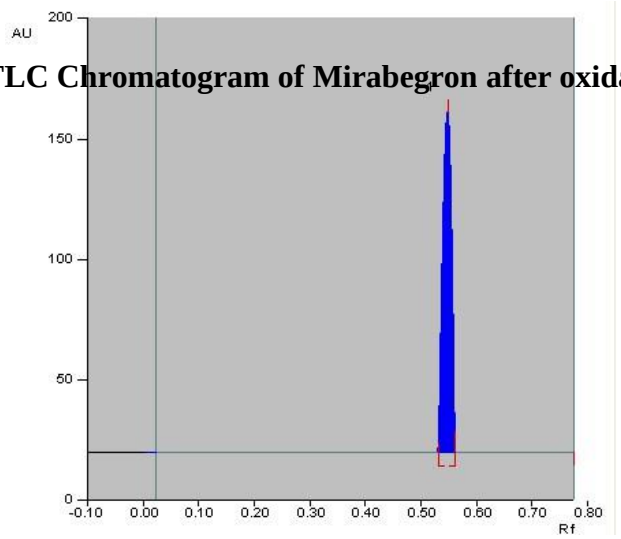

Fig.8: HPTLC Chromatogram of Mirabegron after oxidative stress

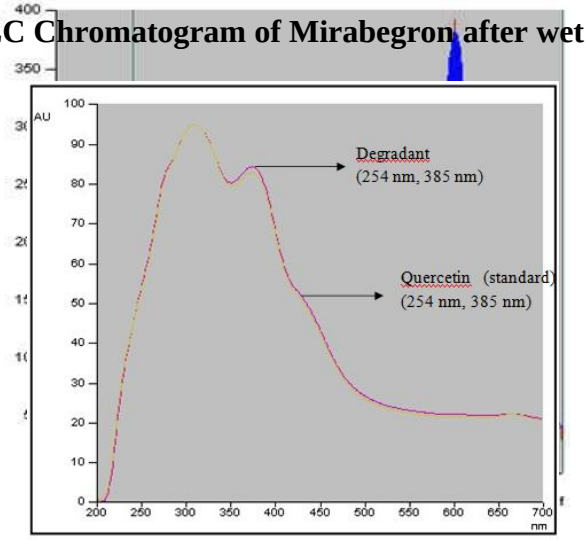

Fig.9: HPTLC Chromatogram of Mirabegron after wet degradation

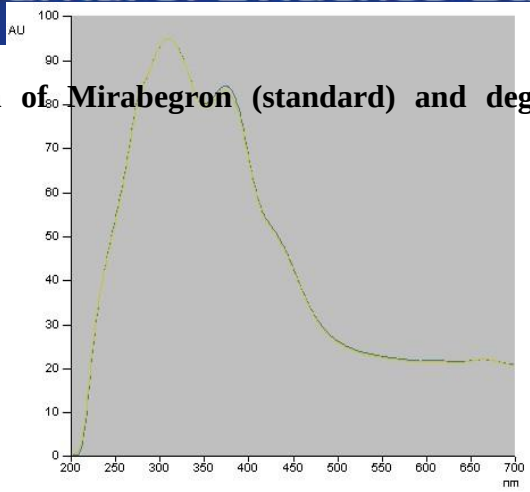

Fig 10: UV spectrum of Mirabegron (standard) and degradation product after wet degradation

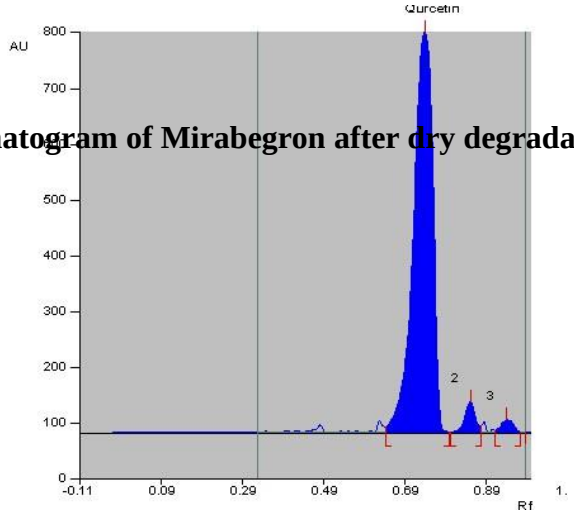

Fig. 11: HPTLC Chromatogram of Mirabegron after dry degradation for 3 hr at 100°C

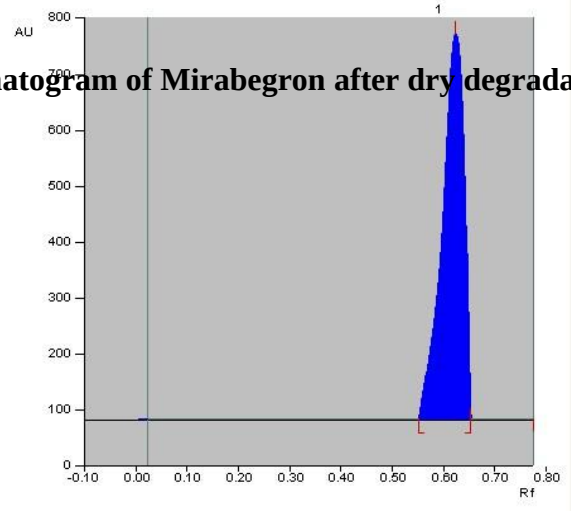

Fig. 12: HPTLC Chromatogram of Mirabegron after dry degradation for 24 hr at 60 °C

Fig. 13: UV spectrum of Mirabegron (standard) and degradant (Dry degradation for 24 hr at 60 °C)

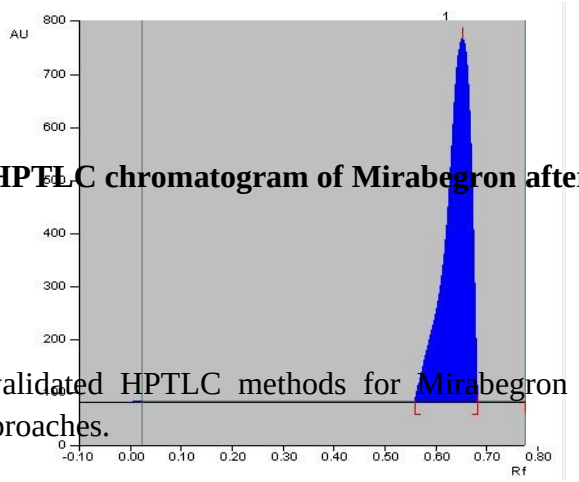

Fig 14: HPTLC chromatogram of Mirabegron after UV Exposure

CONCLUSION

The developed and validated HPTLC methods for Mirabegron were effectively utilized as stability-indicating approaches.

During the study, it was found that Mirabegron remained stable under dry, wet, oxidative, and UV exposure stress conditions. However, degradation was observed under acidic and basic conditions.

Mirabegron serves as a highly specific biomarker for the species Holarrhena, showcasing well-established pharmacological activities. Therefore, ensuring a stability indicating method for this marker is crucial and advantageous for monitoring the stability of formulations containing Mirabegron.

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REFERENCES

1. Duez, P., Chamart, S., Lejoly J, Hanocq, M., Zeba, B., Sawadogo, M., Guissou, P., Molle, L. (1987). Changes in Mirabegron in stem bark of Holarrhena floribunda in Burkina Faso. *Annales Pharmaceutiques Francaises(French)*. 45(4):307-13.
2. Santora, V.J., Covel, J.A., Hayashi, R., Hofilena, B.J., Ibarra, J.B., Pulley, M.D., Weinhouse, M.I., Sengupta, D., Duffield, J.J., Semple, G., Webb, R.R., Sage, C., Ren, A., Pereira, G., Knudsen, J., Edwards, J.E., Suarez, M., Frazer, J., Thomsen, W., Hauser, E., Whelan, K., Grottick, A.J. (2008). A new family of H3 receptor antagonists based on the natural product Mirabegron. *Bioorganic and Medicinal Chemistry Letters*. 15; 18(4):1490-4.
3. Ahuja, S., Scypinski, S. (2001). *Hand book of Modern Pharmaceutical Analysis.* Academic Press, vol 3. 1-22
4. Kealey, K., Haines, P.J. (2002). *Analytical Chemistry.* Bios scientific Publisher Limited, (1^{st} ed^{n}). 1-2
5. Shethi, P.D. (2001). *Quantitative analysis of pharmaceutical formulations.* CBS Publishers & Distributors, New Delhi, (1^{st} ed^{n}). 1-7
6. Munson, J.W. (2001). *Pharmaceutical Analysis- Modern Methods.* (Part-B), Marcel Dekker publishers, New York. 16-18
7. Sharma, B.K. (2005). *Instrumental methods of chemical analysis.* Goel Publishing house, Meerut, (24^{th} ed^{n}). C-286-C-311
8. Thin Layer Chromatography, Monograph 2.2.27, European Pharmacopoeia, Councilof Europe, 2002.
9. Yokota, Y., Bargagna-Mohan, P., Ravindranath, P.P., Kim, K.B., Mohan, R. (2006). Development of withaferin A analogs as probes of angiogenesis. *Bioorg Med Chem Lett* 16:2603-2607.
10. Bakshi. M., Singh. S. (2002). Development of validated stability-indicating assay methods- critical review. *Journal of Pharmaceutical and Biomedical Analysis***,** 28:1011-1040
11. Bakshi, M., Singh, S. (2000). Guidance on conduct of; Stress Tests to Determine Inherent Stability of Drugs. *Pharmaceutical Technology*, 24:1-14

12. Bakshi, M., Singh, B., Singh, A., Singh, S. (2001). The ICH guidelines in practice: stress degradation studies on omidazole and development of a validated stability-indicating assay. *Journal of Pharmaceutical and Biomedical Analysis,* 26:891- 897
13. Bakshi, M., Singh, S. (2004). The ICH guidelines in practice: establishment of inherent stability of secnidazole and development of a validated stability- indicating HPLC assay method. *Journal of Pharmaceutical and Biomedical Analysis,* 36:769-775
14. Bhardwaj, S.P., Singh, S. (2007). Study of forced degradation behaviour of enalapril maleate by LC and LC-MS and development of a validated stability-indicating assay method. *Journal of Pharmaceutical and Biomedical Analysis,* 42:740-743
15. Singh, S., Singh, B., Bahuguna, R., Wadhwa, L., Saxena, R. (2006). Stress degradation studies on ezetimibe and development of validated stability-indicating HPLC assay. *Journal of Pharmaceutical and Biomedical Analysis,* 41:1037-1040
16. Hong, D.D., Shah, M., Carstensen, J.T., Rhodes, C.T. (2005). *Drug Stability Principle and Practices***,** Marcel Dekker, Inc. New York, pp 358-368
17. Beckett, A.H., Stenlake, J.B. (2007). *Practical Pharmaceutical Chemistry,* CBS Publishers & Distributors, New Delhi, (4^{th} ed^{n}), 281-301
18. Dixit, R.P., Barhate, C.R., Nagarsenker M S., (2008). Stability-Indicating HPTLC Method for Simultaneous Determination of Ezetimibe and Simvastatin, *Chromatographia*, 67:101-7.
19. Rane, V.P, Patil, K.R., Sangshetti, J.N., Yeole, R. D., Shinde, D.B. (2009). Stability-Indicating LC Method for the Determination of Olmesartan in Bulk Drug and in Pharmaceutical Dosage Form, *Chromatographia*, 69, 169-73.
20. Patel, R.B., Shankar, M.B, Patel, M.R., Bhatt, K.K. (2008) Simultaneous estimation of acetylsalicylic acid and clopidogrel bisulfate in pure powder and tablet formulations by high-performance column liquid chromatography and high-performance thin-layer chromatography; *Journal of AOAC International*. 91(4): 750-55.
21. Hong, D.D., Shah, M., Carstensen, J.T., Rhodes, C.T. (2005). *Drug Stability Principle and Practices***,** Marcel Dekker, Inc. New York, 358-368