

Chromatographic Determination of Milrinone Lactate by a Validated Stability-Indicating HPTLC-Densitometric Method

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ABSTRACT

Milrinone Lactate is a selective phosphodiesterase III (PDE-III) inhibitor widely employed in cardiovascular therapy. The availability of a reliable analytical procedure is essential for monitoring its content, purity, and stability in pharmaceutical preparations. The present investigation focused on the development and validation of a simple, sensitive, and stability-indicating high-performance thin-layer chromatographic (HPTLC) method for the quantitative determination of Milrinone Lactate. Chromatographic separation was performed on aluminium-backed silica gel 60 F254 plates following application of the test solutions as narrow bands. Ethyl acetate:methanol was employed as the developing solvent system and produced satisfactory migration and separation of Milrinone Lactate, with an R_f value of 0.68 ± 0.046. Quantification was carried out densitometrically in absorbance mode at 270 nm, at which the analyte produced a well-defined chromatographic response. The method exhibited linearity within the concentration range of 200–1200 ng/spot, with a coefficient of determination (R²) of 0.987. Validation was performed with respect to accuracy, precision, repeatability, robustness, and selectivity in accordance with applicable International Council for Harmonisation (ICH) recommendations. The calculated limit of detection (LOD) and limit of quantification (LOQ) were 144.9 ng/spot and 439.36 ng/spot, respectively. The method demonstrated satisfactory analytical performance and effectively differentiated the intact drug from degradation products generated under the investigated stress conditions. Its applicability was further established through the successful assay of Milrinone Lactate in a marketed pharmaceutical formulation. The developed procedure offers a straightforward and economical approach for routine quantitative analysis and stability assessment of Milrinone Lactate and may be suitable for quality-control applications in pharmaceutical laboratories.

Keywords: Milrinone Lactate, Stability indicating analytical method, HPTLC, Method Development, Validation, Degradation products

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INTRODUCTION

Milrinone Lactate is a synthetic bipyridine compound belonging to the class of phosphodiesterase type III (PDE-III) inhibitors and is employed primarily for the short-term treatment of acute heart failure and associated low-output states. Its therapeutic activity results from selective inhibition of PDE-III, which regulates the intracellular degradation of cyclic adenosine monophosphate (cAMP) in cardiac myocytes and vascular smooth muscle cells. By

suppressing cAMP hydrolysis, milrinone increases intracellular cAMP availability, producing a positive inotropic effect in the myocardium together with vasodilatory activity. These complementary actions enhance cardiac performance and decrease both ventricular preload and afterload, thereby contributing to improvement in cardiac output and overall hemodynamic status [1,2].

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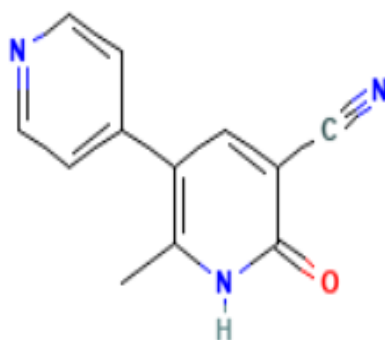


Figure 1: Structure of Milrinone Lactate

The availability of dependable analytical procedures is essential for establishing the identity, purity, and quality of pharmaceutical substances. Chromatographic techniques are particularly important for the quantitative assessment of drug substances because they can provide reliable separation and measurement with appropriate selectivity and sensitivity. Although several analytical approaches have been reported for the determination of milrinone, there remains a need for a convenient planar chromatographic procedure that can be applied for routine pharmaceutical analysis with reduced solvent consumption and operational requirements.

High-performance thin-layer chromatography (HPTLC) has evolved considerably from conventional thin-layer chromatography through advances in stationary-phase technology, sample application, chromatographic development, and densitometric measurement. The technique is now widely utilized in pharmaceutical analysis for the separation, identification, and quantitative estimation of active pharmaceutical ingredients. Its ability to process several samples simultaneously on a single chromatographic plate provides an important advantage for routine analytical laboratories. Moreover, HPTLC requires relatively small quantities of sample and mobile phase and generally involves straightforward sample preparation and chromatographic operation(3,4).

An additional advantage of HPTLC is its flexibility in method optimization. Parameters such as stationary phase, mobile-phase composition, development distance, chamber saturation, and detection wavelength can be systematically adjusted to obtain suitable separation and quantification. Densitometric evaluation further enables quantitative measurement of separated analyte zones with satisfactory precision and reproducibility. Consequently, HPTLC can serve as a practical analytical platform when simplicity, throughput, reduced solvent usage, and economical operation are important considerations.

A review of the available literature revealed a limited number of specifically developed and validated HPTLC-densitometric procedures for the quantitative determination of Milrinone Lactate. This analytical gap provided the basis for the present investigation. Accordingly, the study was undertaken to establish a

simple, specific, accurate, precise, reproducible, and robust HPTLC method for the identification and quantitative estimation of Milrinone Lactate in bulk drug.

The developed chromatographic procedure was systematically optimized to obtain satisfactory separation and reliable densitometric response for Milrinone Lactate. Method performance was subsequently assessed in accordance with applicable International Council for Harmonisation (ICH) recommendations. The validation study included evaluation of linearity, accuracy, precision, specificity, robustness, ruggedness, limit of detection (LOD), and limit of quantification (LOQ) (5). The proposed method is intended to provide a practical and economical analytical procedure for routine quality-control assessment of Milrinone Lactate.

MATERIAL AND METHODS

Milrinone Lactate API and its marketed formulation, Milvas Injection USP (Celon Laboratories Pvt. Ltd., India), besides analytical-grade reagents like methanol, ethyl acetate procured from Loba Chemie Pvt. Ltd., Mumbai. All chemicals and reagents used were of analytical grade. and the volumetric glassware of class A grade were used all over the experimental work.

Instrumentation:

An automatic sample applicator (Camag Linomat) equipped with a Linomat 100-μL syringe 695.0014 was employed for samples application on the HPTLC plate. Chromatographic separations were performed on 20×20 cm aluminium packed plates pre-coated with 0.2 mm layers of silica. A Camag twin-trough chamber for 20×20 cm plates, with a stainless-steel lid, was used for ascending development of the plates. Densitometric scanning was performed on Camag TLC scanner III, operated with WINCAT software while the source of radiation was a D2lamp

HPTLC analysis

Pre-conditioning of HPTLC Plates

Following selection of the appropriate chromatographic layer, the HPTLC plates were initially rinsed with methanol to remove potential surface contaminants and impurities. The plates were subsequently subjected to activation in a hot-air oven at **70 °C for 60 min.** After

activation, the plates were allowed to attain room temperature before sample application.

Sample Application

Standard and sample solutions were applied to the pre-conditioned HPTLC plates using an automated sample applicator. The solutions were applied as narrow bands having a width of **6 mm**, with a separation of **9 mm between successive bands**. The application positions were maintained at **30 mm from the left edge** and **20 mm from the lower edge** of the plate to ensure consistent migration and scanning conditions. Application was performed under a continuous stream of nitrogen to facilitate rapid drying of the applied zones and minimize band broadening. The application rate was maintained at **150 nL/s**, while the instrument application setting was maintained at **5 s/ μ L**.

Selection and Optimization of the Mobile Phase

Selection of an appropriate mobile phase was considered a critical step in establishing the HPTLC method because the composition of the developing solvent directly influences analyte migration, zone compactness, resolution, and peak characteristics. During the preliminary optimization experiments, different solvent combinations and proportions were investigated to identify a system capable of producing a well-defined and symmetrical Milrinone Lactate zone with suitable migration characteristics.

Among the solvent systems evaluated, mixtures containing **ethyl acetate and methanol** were investigated at different proportions. The chromatographic performance of each composition was assessed on the basis of the position and shape of the analyte zone, compactness of the band, degree of separation, and reproducibility of the densitometric response. The solvent composition providing the most satisfactory chromatographic characteristics was subsequently selected for further optimization and validation of the proposed HPTLC method.

Chromatographic Development

Prior to chromatographic development, the developing chamber was equilibrated with the selected mobile phase for **20 min**. A volume of **10 mL of the mobile phase** was used for each development. The prepared HPTLC plate was carefully positioned in the saturated chamber, and chromatographic development was allowed to proceed to a distance of approximately **80 mm**. The development procedure was conducted under light-protected conditions to minimize any potential influence of light on the analyte. Throughout the development process, the laboratory conditions were maintained at **25 $\pm$ 3 $^{\circ}$ C**, with a relative humidity of **33 $\pm$ 3%**.

Densitometric Detection and Scanning

Following completion of chromatographic development, the plate was removed from the chamber and dried for **1 min** using a plate dryer to eliminate residual mobile-phase solvent. The developed chromatographic zones were subsequently evaluated densitometrically in absorbance mode. Detection was performed at **270 nm**, corresponding to the maximum absorption wavelength (λ_{max}) of

Milrinone Lactate. A **D2 lamp** was used as the radiation source. The monochromator bandwidth was maintained at **6 mm**, while the slit dimensions were set to **6 $\times$ 0.30 mm**. The resulting densitograms were used for quantitative evaluation of the analyte.

Preparation of Standard Stock Solution

A primary standard stock solution of Milrinone Lactate having a concentration of **1000 μ g/mL** was prepared by accurately weighing **10 mg** of Milrinone Lactate and transferring it into a suitable volumetric flask. Methanol was added to dissolve the drug, followed by dilution to a final volume of **10 mL** with the same solvent. The resulting solution was subjected to sonication for **10 min** to ensure complete dissolution and uniformity of the prepared stock solution.

Preparation of Calibration Levels

The calibration study was performed by applying different volumes of the standard stock solution onto the HPTLC plate. Volumes of **0.2, 0.4, 0.6, 0.8, 1.0, and 1.2 μ L** were applied, with each level analyzed in **triplicate**. The corresponding chromatographic zones were developed under the optimized conditions and subsequently subjected to densitometric scanning at 270 nm.

The calibration relationship was established by plotting the **mean peak area** obtained from each concentration level against the corresponding amount of Milrinone Lactate applied to the plate. Linear regression analysis was subsequently performed to determine the calibration equation and associated regression characteristics. The resulting calibration curve was used to assess the linear response of the proposed HPTLC-densitometric method over the investigated concentration range.

RESULTS AND DISCUSSION

Development and Optimization of the HPTLC Method

The HPTLC procedure for Milrinone Lactate was established through sequential evaluation of the chromatographic variables that influence separation and densitometric measurement. The initial experiments focused on identifying a suitable support for the silica gel stationary phase. For this purpose, chromatographic performance was compared using silica gel layers supported on glass and aluminium substrates.^(6,7) The aluminium-supported plates produced chromatographic zones that were more compact and clearly resolved than those obtained with glass-supported plates. The improved zone characteristics were considered favorable for subsequent densitometric evaluation. Accordingly, **silica gel precoated aluminium plates** were selected for further method optimization. The mobile-phase system was then investigated to achieve reproducible migration of Milrinone Lactate together with a distinct and symmetrical chromatographic zone. The composition of the developing solvent was progressively examined during the optimization process, and the chromatographic response was assessed from the appearance and compactness of the analyte zone, migration behavior, and densitometric response. The optimized chromatographic conditions

obtained from these experiments were subsequently employed throughout the validation study(8,9). The developed HPTLC method produced a distinct chromatographic spot for Milrinone Lactate, with a mean R_f value of 0.68 ± 0.046 . This value indicates the migration of the analyte relative to the solvent front under the selected chromatographic conditions. The observed R_f value can be used as a characteristic parameter for the identification of Milrinone Lactate. Densitometric scanning at 270 nm enabled the detection and quantitative evaluation of the analyte.

Validation of the Analytical Procedure

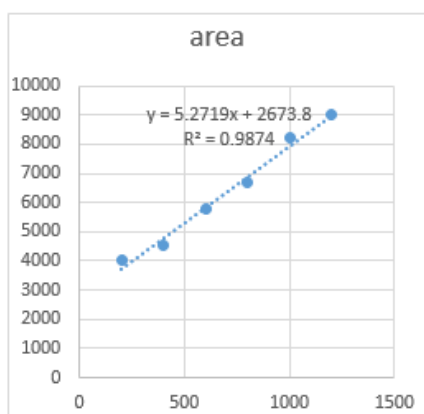
Following optimization, the analytical performance of the developed HPTLC-densitometric procedure was systematically investigated. Validation was undertaken with reference to the relevant requirements of the **ICH Q2 guideline** (10). The method was examined for its linear response, accuracy, precision, robustness, sensitivity, and related performance characteristics. In particular, **linearity, accuracy, precision, robustness, LOD, and LOQ** were investigated to establish the reliability of the procedure for quantitative estimation of Milrinone Lactate.

Linearity Study

The relationship between the amount of Milrinone Lactate applied to the HPTLC plate and the corresponding

densitometric response was investigated over six levels. Standard solution volumes of **0.2, 0.4, 0.6, 0.8, 1.0, and 1.2 μL per spot** were applied to the plate, providing an analytical range of **200–1200 ng/spot**. The calibration levels were subjected to chromatographic development and densitometric measurement under the previously optimized experimental conditions.

For each level, the resulting Milrinone Lactate zone was scanned and the corresponding peak-area response was recorded. A calibration plot was subsequently generated by relating the measured peak area to the amount of drug applied per spot. Linear regression analysis was performed using **winCATS software** to obtain the calibration equation and correlation coefficient. The progressive increase in densitometric response with increasing amounts of Milrinone Lactate demonstrated a concentration-dependent analytical response within the investigated range. The regression characteristics obtained from the calibration study were used to establish the linearity of the proposed HPTLC method. The corresponding calibration data, regression parameters, and calibration plot are presented in **Table 1 and Figure 2**, respectively.



Sr. No.	Conc. (ng/spot)	Area
1	200	4006.41
2	400	4515.85
3	600	5769.33
4	800	6708.03
5	1000	8191.96
6	1200	8993.40

Figure2: Calibration curve for Milirinone Lactate Table 1: Linearity of Milirinone Lactate

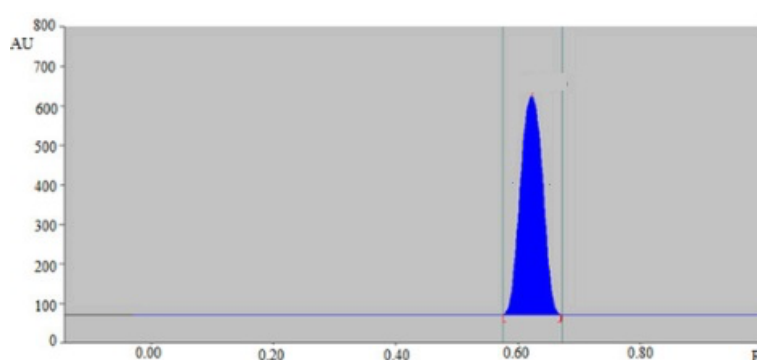


Figure 3: A typical Densitogram of standard Milirinone Lactate using mobile phase ethyl acetate-Methanol

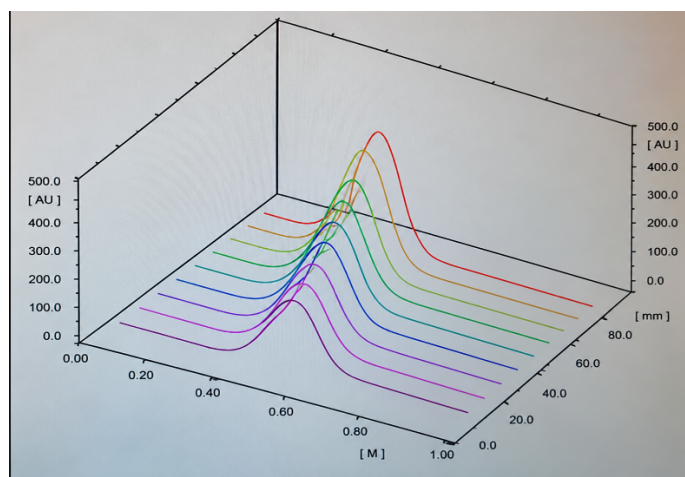


Figure 4: Linearity of Milrinone Lactate in 3D spectra

Precision

The precision of the developed HPTLC-densitometric procedure was investigated at three application levels corresponding to **0.6, 0.8, and 1.0 $\mu\text{L/spot}$** of the Milrinone Lactate standard solution. The study was designed to examine variability associated with densitometric measurement, sample application, and analysis performed on different days. For evaluation of measurement repeatability, the same chromatographic zone was scanned three consecutive times under identical instrumental conditions, and the resulting peak-area responses were compared. Application repeatability was subsequently assessed by applying the same standard solution three times at each selected level and determining the variation among the measured responses. Intermediate

precision was investigated by repeating the analytical procedure under the same experimental conditions on **two different days by the same analyst**. The precision data were expressed as the percentage relative standard deviation (%RSD) of the measured peak areas. The %RSD values obtained for the investigated concentration levels were **below 3%**, demonstrating low variability in both densitometric measurement and sample application. The results also indicated satisfactory consistency of the analytical response when the procedure was repeated on different days. Thus, the proposed HPTLC method exhibited satisfactory repeatability and intermediate precision for the quantitative determination of Milrinone Lactate. The detailed precision results are presented in **Table 2 and Table 3**

Intraday Precision Table 2.

Sr. no.	Conc.(ng/spot)	Area	Mean	S.D.	%RSD
1	600	6442.9	6518.266	85.2220	1.3
2	600	6474.5			
3	600	6637.4			
4	800	8576.1	8579.7	112.133	1.3
5	800	8444.2			
6	800	8718.8			
7	1000	10265.8	10196.56	57.590	0.56
8	1000	10199.1			
9.	1000	10124.8			

Interday Precision Table 3.

Sr. no.	Conc.(ng/spot)	Area	Mean	S.D.	%RSD
1	600	6386.7	6383.6	20.00	0.3
2	600	6406.4			
3	600	6357.7			
4	800	8351.7	8465.7	84.17	0.9
5	800	8461.0			
6	800	8589.4			
7	1000	9754.5	9623.56	101.7	1.05

8	1000	6506.3			
9.	1000	9609.9			

Accuracy (Recovery studies)

Recovery studies were carried out to assess accuracy of the method. These studies were carried out at three levels.

The percentage recovery was found to be within the limits and shown in Table-4

%Recovery	Standard API	Admixture	Area	Mean	%Recovery.
50	0.4µl(400ng)	0.2 µl(200 ng)	5973.2	6015.43	105
50	0.4µl(400ng)	0.2 µl(200 ng)	6012.5		
50	0.4µl(400ng)	0.2 µl(200 ng)	6060.6		
100	0.4µl(400ng)	0.4 µl(200 ng)	7192.0	7145.5	106
100	0.4µl(400ng)	0.4 µl(200 ng)	7013.2		
100	0.4µl(400ng)	0.4 µl(200 ng)	7231.5		
150	0.4µl(400ng)	0.6 µl(200 ng)	8120.5	8166.1	104.1
150	0.4µl(400ng)	0.6 µl(200 ng)	8104.3		
150	0.4µl(400ng)	0.6 µl(200 ng)	8273.5		

Robustness

Robustness was determined by altering chromatographic conditions like mobile phase composition. The low value

of RSD indicates robustness of the method. The results were shown in the Table 5

Table 5: Robustness

Conc.(µL)	Area	Mean	S.D.	R.S.D.
0.6 µL	6211.2	6329	100.7	1.65
0.6 µL	6457.4			
0.6 µL	6318.4			
0.8µL	8257.4	8188.3	55.8	0.6
0.8µL	8187.1			
0.8µL	8120.5			
1.0 µL	9311.0	9146.0	121.3	1.32
1.0 µL	9104.7			
1.0 µL	9022.4			

LOD and LOQ

Sensitivity of the proposed method was estimated in terms of Limit of Detection (LOD) and Limit of Quantitation. The ICH indicates that LOD (which they call DL, the detection limit) can be calculated as $LOD = 3.3\sigma / S$, and

the limit of quantification (which they call QL, the quantitation limit) $LOQ = 10\sigma / S$. Here σ is the standard deviation of the response and S is the slope of the calibration curve. S is estimated from the slope of the calibration curve for the analyte

Table 6: LOD and LOQ

Drug	LOD	LOQ
Milirinone Lactate	144.9ng	439.36ng

Stress Degradation Studies of Bulk Drug: Stability studies were carried out to provide evidence on how the quality of drug varies under the influence of various environmental conditions like hydrolysis, oxidation, temperature, etc. Dry heat and photolytic degradation were carried out in the solid state (11, 12)

Acid Hydrolysis: To 1 ml standard stock solution of drug (1000 µg/ml), 1 ml of 2 N HCl was added and volume made to 10 ml with methanol to get 100 µg/ml solution.

The solution was kept for 24 hr in dark, neutralized with 2 N NaOH and 10 µl of this solution was applied on TLC plate (1000 ng/band concentration). Acid degradation blank is prepared in the same way without using an analyte. Under acid hydrolysis, percent recovery obtained for Milirinone lactate was 83.74 % with one degradant peak at RF 0.22. The representative chromatogram is shown in Fig. 5.

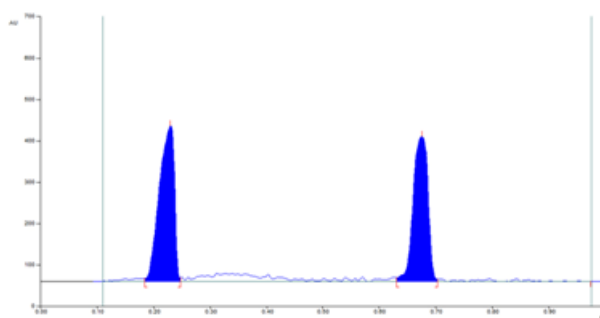


FIG. 5: Densitogram of Milirinone Lactate after Acid Degradation

Alkaline Hydrolysis: To 1 ml standard stock solution of drug (1000 µg/ml), 1 ml of 1N NaOH was added and volume made to 10 ml with methanol to get 100 µg/ml solution. The solution was kept for 24 hr in the dark, neutralized with 1 N HCl and 2 ml diluted to 10 ml with methanol to get 20 µg/ml solution. Alkali degradation

blank is prepared in the same way without using analyte. Under alkaline hydrolysis, the percent recovery obtained for Milirinone lactate was 79.58 % with one degradant peaks at RF 0.23. The representative chromatogram is shown in **Fig. 6**.

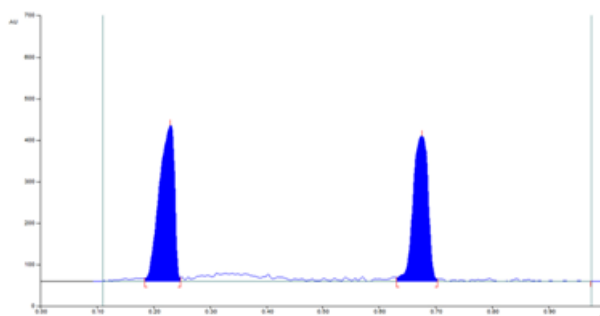


FIG. 6: Densitogram of Milirinone Lactate after Alkaline Degradation

Degradation under Oxidative Condition: To 1 ml standard stock solution of the drug (1000 µg/ml), 1 ml of 30% H₂O₂ was added and volume made to 10 ml with methanol to get 100 µg/ml solution. The solution was kept for 24 hr in dark and 2 ml diluted to 10 ml with methanol

to get 20 µg/ml solution. The blank is prepared in the same way without using an analyte. Under oxidative degradation, the percent recovery obtained for Milirinone Lactate was 97.86 % with no degradant peak. The representative chromatogram is shown in **Fig. 7**.

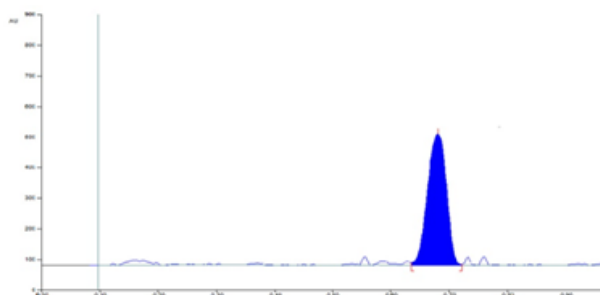


FIG. 7: Densitogram of Milirinone Lactate after Oxidation With 30% V/V H₂O₂

Degradation under Dry Heat: Dry heat study was performed by keeping the drug sample in an oven (100° C) for a period of 2 hour. A sample was withdrawn 10 mg was dissolved in methanol to get a solution of 1000 µg/ml and further diluted with methanol to get 20 µg/ml as final

concentration and injected. Under dry heat degradation conditions, the percent recovery obtained for Milirinone lactate was 99.28 % with no degradant peak. The representative chromatogram is shown in **Fig. 8**.

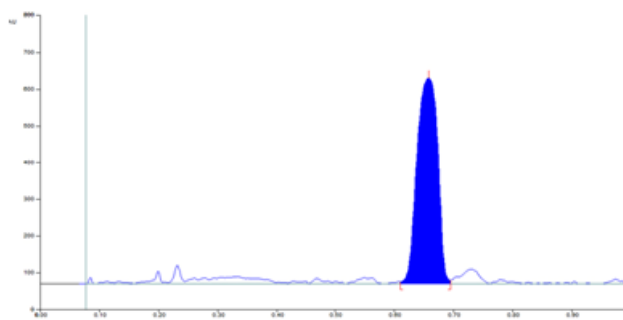


FIG. 8: Densitogram of Milirinone Lactate after Dry Heat Degradation

Photodegradation Studies: The photodegradation stability study of the drug was studied by exposing the drug to UV light providing illumination of NLT 200 watt hr/m² and exposure to cool white fluorescence light of NLT 1.2 million Lux-Hr. After exposure and accurately weighed 10 mg of drug was transferred to 10 ml of volumetric flask; the volume was made up with methanol. Further dilution was made with methanol to get 20 µg/ml

as final concentration and injected. An average 98.19.74 % of milirinone lacatate was recovered with no peak of degradant after exposure to UV light. An average 98.62 % of milirinone lacatate was recovered with no degradant peak after exposure to fluorescence light. The representative chromatogram is shown in **Fig. 9** and **Fig. 10**, respectively (13).

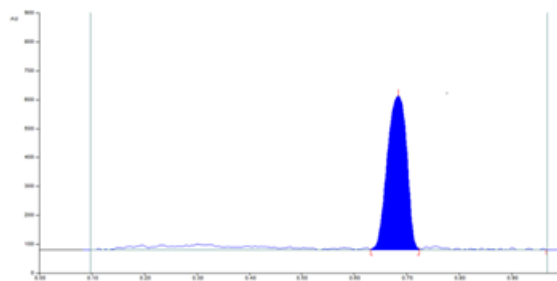


FIG. 9: Densitogram of Milirinone Lactate after Uv Illumination Exposure

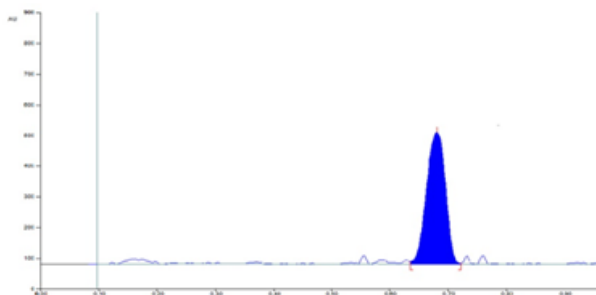


Fig. 10: Densitogram of Milirinone Lactate after Fluroscent Light Exposure

Table 7: Summary of Degradation Parameters

Stress condition/Duration	% Recovery of Analyte	RF of degraded products
Acidic/ 2 N HCl for 24 hours	83.74 %	Degradant at Rf 0.22
Alkaline/ 1 N NaOH for 24 hours	79.58 %	Degradant at Rf – 0.23
Oxidative/ 30 % H ₂ O ₂ at room temperature 24 hours	97.86 %	-
Dry heat / 100°C/ 24 hours	99.28 %	-
UV illumination NLT 200 watt hours/square meter	98.19 %	-
Fluroscent light NLT 1.2 X 10 ⁶ Lux hr	98.62 %	-

CONCLUSION

For the investigation of Milirinone Lacatate, a novel HPTLC densitometric technique was created, verified by

ICH criteria, and found to be repeatable, precise, accurate, and robust. In compared to other analytical techniques, the process is quick, easy, and affordable. It is also sufficiently feasible enough to be recommended for the regular analysis of Milrinone Lactate. For the safety of patients, the quality of pharmaceutical goods is crucial. The presence of degradation chemicals or contaminants may impact the effectiveness and safety of medications. Milrinone Lactate forced degradation research was conducted under the ICH-required circumstances to examine its degradation profile and clarify the structures of the degradation products. An environmentally friendly, sensitive, exact, precise, affordable, time- and cost-efficient, and repeatable stability-indicating HPTLC method is developed. The validation experiments demonstrated that the methods were linear, precise, accurate, specific, and selective to the medication in the presence of degradation products. The drug has been shown to be susceptible to photolytic and alkali hydrolytic degradation at room temperature. Research on stability indications was conducted in compliance with ICH Q1A (R2) guidelines. The method has been applied to evaluate tablet items that are currently on the market. The presented method may be used frequently in quality control laboratories to analyse Milrinone Lactate for pharmaceutical tablet dosage.

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Conflicts Of Interest: Authors declare that there is no conflict of interest

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