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Simultaneous Estimation of Desloratadine and Montelukast Sodium by HPTLC

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Research Article

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ABSTRACT

High performance thin layer chromatographic method has been developed for the bioestimation of Desloratadine (DSLR) and Montelukast (MON) in their combined dosage form. Merck HPTLC aluminum plates of silica gel G_{60} F_{254} was used for separation of combined with 250 µm thickness using ethanol: methanol: ammonia formate solution: ammonia (9:1:0.5:0.5 v/v/v/v) as mobile phase. HPTLC separation of both drugs were carried out and followed by densitometric measurement was performed in the absorbance mode at 287 nm. The drugs were resolved satisfactorily with R_f values of 0.19 ± 0.03and 0.86 ± 0.03 for DSLR and MON, respectively. A Calibration was curve obtained from 0.06 µg/spot-0.36 µg/spot of DSLR (r^2>0.996) and 0.12 µg/spot-0.72 µg/spot of MON (r^2>0.999). The developed method was validated in terms of accuracy, precision, linearity, limit of detection, limit of quantification. The developed HPTLC method can be applied for the estimation of DSLR and MONT in bulk drug and drug formulation.

INTRODUCTION

Combination drug therapy is the use of two or more pharmacologic agents administered separately or in a fixed dose combination of two or more active ingredients in a single–dosage formulation. Drug combinations offering increased therapeutic efficacy and reduced toxicity, play an important role in treating multiple complex diseases. The therapy is used appropriately by screening for potential drug-drug interaction, contraindication or both and by making therapeutic recommendation aimed at achieving optimal response without increasing the potential for adverse drug interactions. It is estimated that 20% of the world population suffer from allergic diseases may be treats in the combination therapy [1]. Asthma is an inflammatory disorder of the bronchial airways produced by allergies, viral respiratory infections and airborne irritants, while

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genetic factors also develop asthma. Globally the prevalence of asthma and allergies has increased over the last few decades [2].

Montelukast is a Leukotriene Receptor Antagonist (LTRA) used for the treatment of asthma and to relieve symptoms of seasonal allergies. It is usually administered orally. Montelukast is a $CysLT_1$ antagonist; that it blocks the action of leukotriene D4 (and secondary ligands LTC4 and LTE4) on the cysteinyl leukotriene receptor $CysLT_1$ in the lungs and bronchial tubes by binding to it. This reduces the bronchoconstriction otherwise caused by the leukotriene and results in less inflammation [3]. The chemical structure of MON is shown in Figure 1. Desloratadine (descarboethoxyloratadine) is a non-sedative metabolite of loratadine, a second generation long-acting antihistaminic drug with selective peripheral H_1 receptor antagonistic activity. It has demonstrated anti allergic properties by inhibiting the release of pro-inflammatory cytokines such as IL-4, IL-6, IL-8 and IL-13 from human mast cells/basophilic as well as inhibition of the expression of the adhesion molecule P-selection on endothelial cells [4]. The chemical structure of DSLR is shown in Figure 2.

Nowadays Combination therapy directed at blocking the effects of both mediators might protect against the early asthmatic response better than either therapy alone. A combination of Desloratadine and Montelukast provided superior efficacy to either blocker administered alone.

A thorough literature survey reveals that there only few analytical methods available for the detection and quantification of combination of montelukast sodium (10 mg) +desloratadine (5 mg) [5-22].

However as per bibliographical revisions performed, no HPTLC analytical method has been reported for this combination. The present study was aimed at developing simple, specific, accurate and precise HPTLC method for the determination of montelukast sodium and Desloratadine in combination.

Figure 1. Structure of Montelukast sodium.

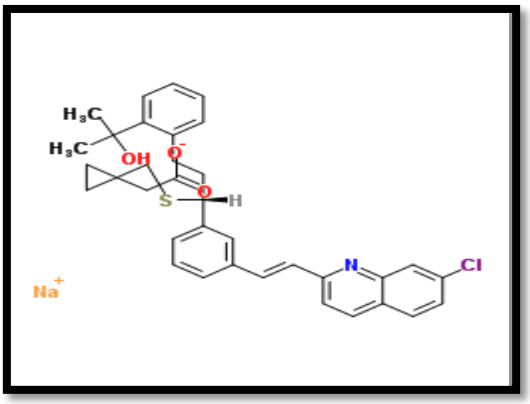
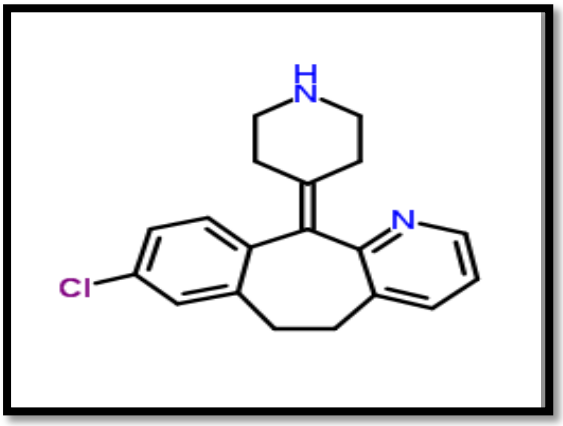


Figure 2. Structure of Desloratadine.



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EXPERIMENTAL

Instrumentation

HPTLC with Camaglinomat 5 Applicator, TLC Scanner 3 controlled by Wincats–planar chromatography manner, version-1.2.6 (Camag, Muttenz, Switzerland) and Merck TLC Plate coated with silica gel $G_{60}F_{254}$ on aluminum sheets (Merck Chemical Ltd, darmstadt, Germany), was used as stationary phase.

Reference substances, reagents and chemicals

Montelukast Sodium and Desloratadine were obtained as a gift sample. Methanol AR grade was purchased from Merck Lab and Qualigens Fine Chemicals Pvt. Ltd., India.

Chromatographic conditions

Before analysis HPTLC plates of silica gel $G_{60}F_{254}$ were cleaned by pre-development with methanol and activated at 110°C for 5 min for solvent removal. The sample Solutions of MONT and DSLR were spotted to plates (10 × 10 cm) by means of a Linomat-5 automatic spotter equipped with a 100 microlitre sample syringe and operated with settings of band length 6 mm, the migration distance 85 mm and the slit dimension 5 × 0.45 mm. The plate was developed in a twin trough chamber previously saturated for 20 min with the mobile phase, ethanol: methanol: ammonia formate solution: ammonia (9:1:0.5:0.5 v/v/v/v). The source of radiation used was a deuterium lamp emitting a continuous UV spectrum between 200 nm and 400 nm. All determinations were performed at ambient temperature with a detection wavelength of 287 nm.

Optimization of chamber saturation time

The fixed mobile phase system was added to one side of the twin trough chamber and effects of different saturation times from 5-20 minutes were evaluated. It was found that a saturation time up to 15 minutes caused changes in the R_f value and also edge effects. Whereas 20 min saturation time gave compact spots hence the saturation time of 20 minutes was fixed for further studies.

Solution preparations

Preparation of standard stock solution of DSLR: A quantity of about 10 mg of DSLR transferred to 10 ml of volumetric flask to obtain 1000 mcg/ml was prepared with methanol. From the above concentration take 1 ml make up to 10 ml to obtain concentration 100 mcg/ml.

Preparation of standard stock solution of MON: A quantity of about 10 mg of MON transferred to 10 ml of volumetric flask to obtain 1000 mcg/ml was prepared with methanol. From the above concentration take 1 ml make up to 10 ml to obtain concentration 100 mcg/ml.

Preparation of mixture of Standard solutions: From the above solutions, 3 ml of DSLR and 6 ml of MON transferred to 10 ml of volumetric flask to obtain mixture of solution with concentration of 30 mcg/ml of DSLR and 60 mcg/ml of MON.

Estimation from Formulation: Not less than ten tablets were taken and after calculating average weight, they were powdered. A quantity of powder equivalent to 5 mg of DSLR and 10 mg of MON was transferred individually to 100 ml volumetric flask dilute with methanol and sonicated for 45 min.The solution was extracted through whattman filter paper. From above solution 6 ml taken make up to 10 ml with methanol and 8 µl volume spotted on chromatogram plate, developed, scanned and integrated peak area was noted.

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RESULTS AND DISCUSSION

Method development and optimization

For the development of HPTLC for both drugs, various mobile phase trials were made. Among various trials, the one containing a mixture of ethanol: methanol: ammonium formate solution: ammonia (9:1:0.5:0.5 v/v) gave compact dense band and it was chosen for further studies. The plate used was pre-coated silica gel G_{60} F_{254}. The fixed chromatographic conditions are given below.

Fixed chromatographic conditions:

Stationary phase: Pre coated silica gel 60 F_{254} aluminum sheets.

Mobile phase: Ethanol: methanol: ammonium formate.

Solution: ammonia (9:1:0.5:0.5 v/v/v/v).

Saturation time: 20 minutes.

Migration distance: 85 mm.

Band width: 6 mm

Slit dimension: 5 × 0.45 mm.

Source of radiation: Deuterium lamp

Scanning wavelength: 287 nm

R_f value: Desloratadine: 0.19 ± 0.03
Montelukast sodium: 0.86 ± 0.03

Method validation

Test method for the simultaneous estimation of DSLR and MON was validated under the ICH guidelines [22]. Parameters like linearity, precision, range, LOQ, LOD were examined.

Linearity and range: Different volumes of mixture of standard solutions (2,4,6,8,10,12 µl) were spotted with the help of Linomat V automatic sample applicator on the TLC plates. The plates were developed in 10x10 twin trough chamber saturated with the fixed mobile phase system and scanned using CAMAG TLC scanner 3. The R_f value was 0.19 ± 0.03 and 0.86 ± 0.03 of DSLR and MON respectively and the peak areas were noted.

The linear regression data showed a good linearity over a concentration range of 0.06 µg/spot - 0.36 µg/spot of DSLR and 0.12 µg/spot to 0.72 µg/spot of MON. The calibration data are shown in Tables 1 and 2, and the slope, intercept and correlation coefficient values were found are presented in Table 3. The calibration graph is shown in Figures 3 and 4. The standard densitograms obtained at different concentrations are shown from Figures 5-10.

Table 1. Calibration data for DSLR.

Concentration (µg/spot)	Peak area (AU)
0.06	371.6
0.12	696.52
0.18	951.87
0.24	1219.53
0.3	1392.3
0.36	1656.01

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Table 2. Calibration data for MON.

Concentration (µg/spot)	Peak area (AU)
0.12	2495.23
0.24	4294.64
0.36	5947.45
0.48	7938.05
0.6	9790.88
0.72	11510.04

Table 3. Regression data of DSLR and MON.

	DSLR	MON
Slope	8.58	8.66
Intercept	192.5	1361
Correlation coefficient	0.996	0.999

Figure 3. Calibration Graph of DSLR.

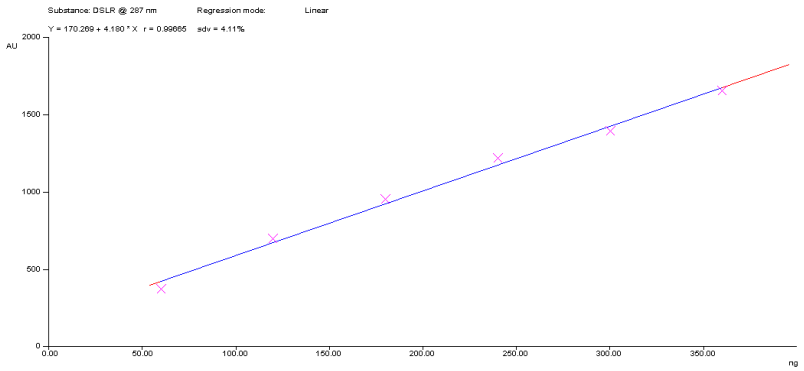

Figure 4. Calibration Graph of MON.

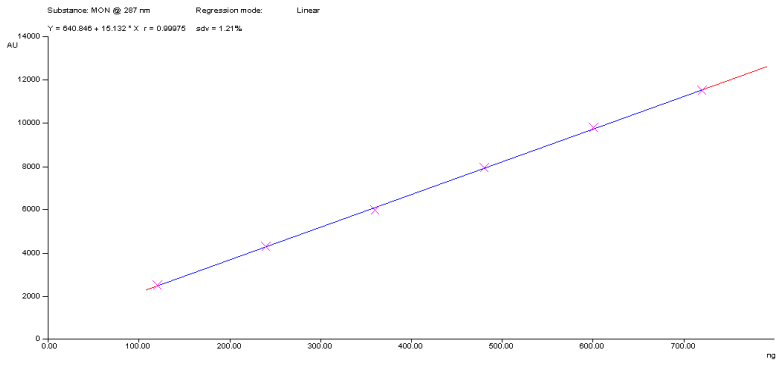

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Figure 5. Densitogram of DSLR 0.06 µg/spot and 0.12 µg/spot MON.

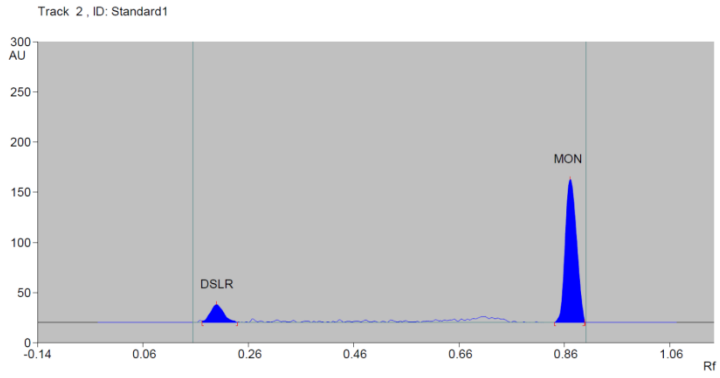

Figure 6. Densitogram of 0.12 µg/spot DSLR and 0.24 µg/spot MON.

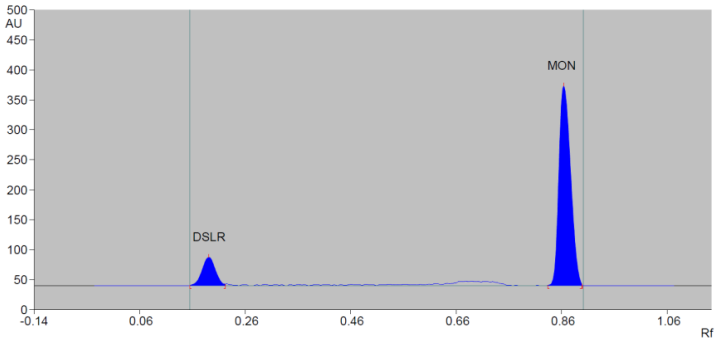

Figure 7. Densitogram of 0.18 µg/spot DSLR and 0.36 µg/spot MON.

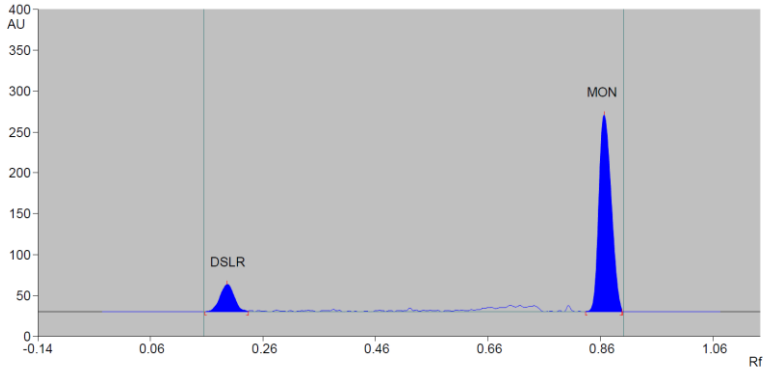

Figure 8. Densitogram of 0.24 µg/spot DSLR and 0.48 µg/spot MON.

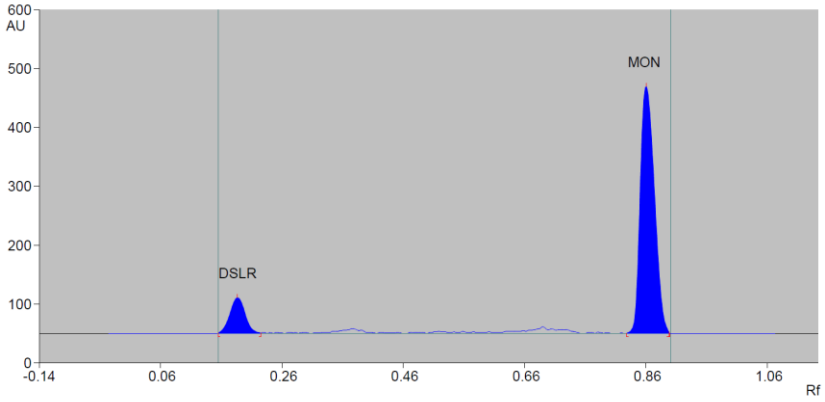

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Figure 9. Densitogram of 0.3 µg/spot DSLR and 0.6 µg/spot MON.

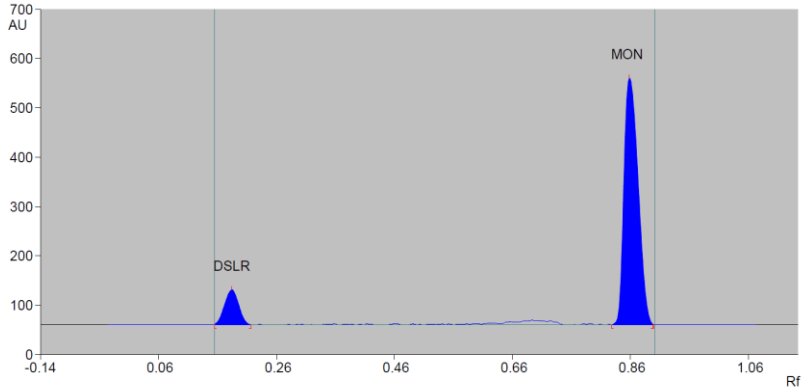
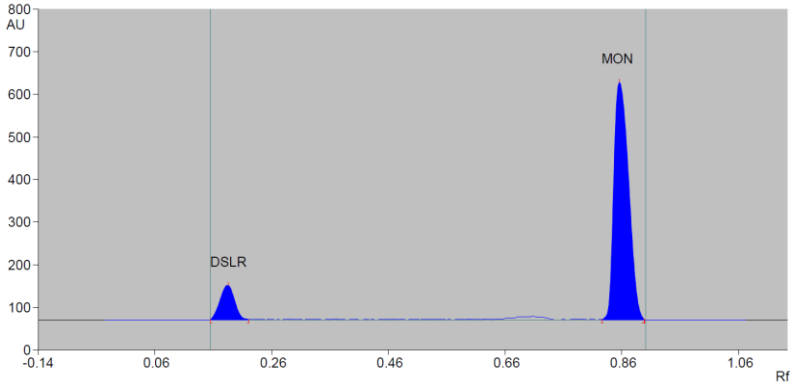


Figure 10. Densitogram of 0.36 µg/spot DSLR and 0.72 µg/spot MON.



Precision: The method precision was obtained by repeating the determination of mixture of standard solutions of DSLR and MON of two selected concentrations 0.12 µg/spot and 0.18 µg/spot for DSLR and 0.24 µg/spot and 0.36 µg/spot**.** The % RSD calculated for inter-day, intra-day and repeatability (repeatability of sample measurement and sample application respectively) were found on Tables 4-7.

Table 4. Intraday and interday precision for DSLR.

Concentration (µg/spot)	Peak area		%RSD*	
	Intraday	Interday	Intraday	Interday
0.12	1137.1	1011.4	0.4	0.4
	1145.8	1017.5		
	1158.8	1021.2		
0.18	1546.2	1225.75	1.5	0.9
	1572.3	1236.51		
	1596.4	1248.01		
*Average of 3 value				

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Table 5. Intraday and interday precision for MON.

Concentration (µg/spot)	Peak area		%RSD*	
	Intraday	Interday	Intraday	Interday
0.24	4285.2	3945.2	0.1	0.3
	4293.5	3956.1		
	4296.1	3972.1		
0.36	5725.9	4423.3	0.1	0.2
	5735.7	4432.2		
	5744.8	4442.2		
*Average of 3 values				

Table 6. Repeatability sample measurement and sample application for DSLR.

Concentration (µg/spot)	Peak area		%RSD*	
	Sample measurement	Sample application	Sample measurement	Sample application
0.18	1526.3	1516.2	0.5	0.9
	1533.2	1523.7		
	1539.1	1544.6		
	1546.3	1535.8		
	1554	1552.9		
	1531.2	1542.8		
*Average of 3 value				

Table 7. Repeatability sample measurement and sample application for MON.

Concentration (µg/spot)	Peak area		%RSD*	
	Sample measurement	Sample application	Sample measurement	Sample application
0.36	5402.2	5412.1	0.1	0.3
	5412.1	5424.7		
	5420.3	5436.3		
	5428.3	5447.2		
	5437.2	5456.2		
	5448.4	5468.9		
*Average of 3 value				

Limit of Detection (LOD) and Limit of Quantification (LOQ): The lowest concentration of the analyte detectable was found to be 0.03 µg/spot for DSLR and 0.06 µg/spot for MON. The lowest concentration of the analyte at which it is quantifiable was found to be 0.06 µg/spot for DSLR and 0.12 µg/spot for MON.

Stability of chromatographic plate: When the developed chromatographic plate was exposed to atmosphere, the analytes are likely to get decomposed. Hence it was necessary to conduct stability studies of the plate. Stability of the analyte on the plate was studied at different time intervals and peak areas were compared with peak area of freshly scanned plate. The developed plate was found to be stable for 1 hr.

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Application of the proposed method

The developed method was applied for determination of DSLR and MON content in tablets. Sample solution (0.24 µg/spot and 0.48 µg/spot) was injected, densitogram was recorded, shown in Figures 11 and 12 and the quantification was done by using peak area. The results shown in Table 8.

Table 8. Analysis of formulation.

Drug	Amount of drug (mg/tablet)				Label claim	
MONDESLOR	DSLR		MON		DSLR	MON
	Labeled	Found	Labeled	Found		
	5 mg	5.3 mg	10 mg	10.6	106%	106%
*Average of six observation						

Figure 11. Densitogram of API: 0.24 µg/spot DSLR and 0.48 µg/spot MON.

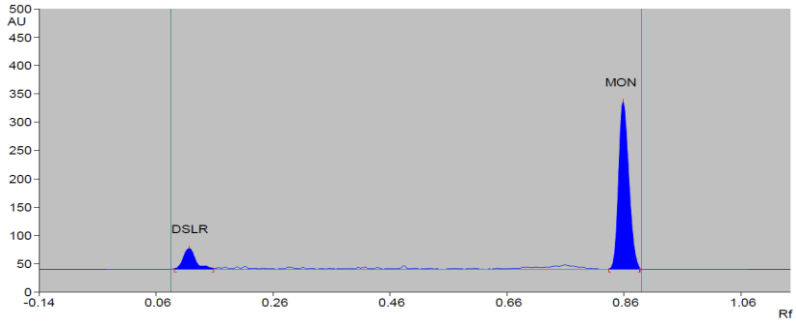
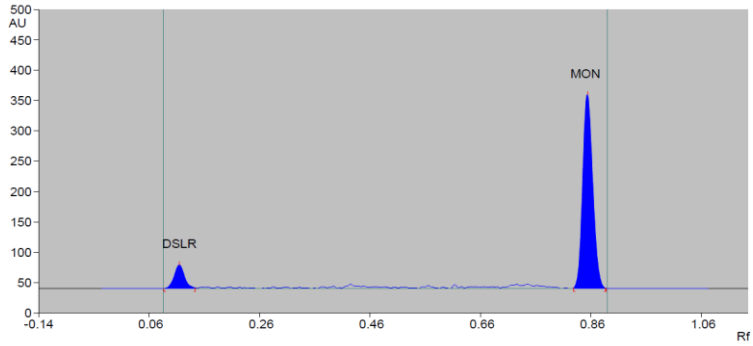


Figure 12. Densitogram of TAB: 0.24 µg/spot DSLR and 0.48 µg/spot MON.



CONCLUSION

The method developed in the current research work for the simultaneous estimation of DSLR and MON has laid down quantification method as per ICH guidelines. The high-performance Thin Layer Chromatography method is the first one of its kind and offers good range of analysis of two drugs in tablet.

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