Research and Reviews: Journal of Pharmacy and Pharmaceutical Sciences

e-ISSN: 2347-226X

p-ISSN: 2319-9857

Bioestimation of Desloratadine and Montelukast Sodium in Spiked Human Plasma by High Performance Thin Layer Chromatography

Anvithaa Varghese*

Department of Pharmaceutical Analysis, Sriramakrishna College of pharmacy, Tamil Nadu, India

Research Article

Received: 10/08/2021

Accepted: 24/08/2021

Published: 31/08/2021

***For correspondence:**

Anvithaa Varghese, Department of Pharmaceutical Analysis, Sriramakrishna College of pharmacy, Tamil Nadu, India

E-mail: anvithavarghese@gmail.com

Keywords: Montelukast sodium, Desloratadine, HPTLC, Method validation

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 G60 F254 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 the 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 Rf values of 0.19 ± 0.03 and 0.86 ± 0.03 for DSLR and MON, respectively. 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 bio estimation of DSLR and MONT in bulk drug and drug formulation.

INTRODUCTION

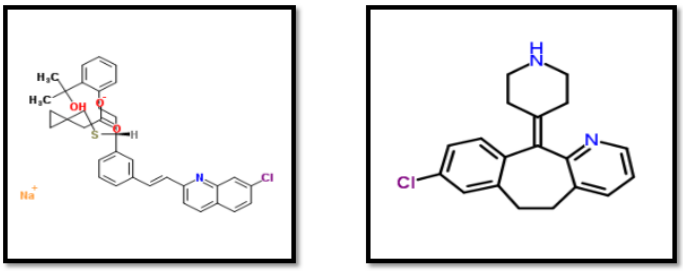
Combination drug therapy is the use of two or more pharmacologic agents administered separately or in a fixed dose combination of two or more active ingredients in a single-dosage formulation [1]. Drug combinations offering increased therapeutic efficacy and reduced toxicity, play an important role in 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 are may be treats in the combination therapy [2]. Asthma is an inflammatory disorder of the bronchial airways produced by allergies, viral respiratory infections and airborne irritants, while genetic factors also develop asthma. Globally the prevalence of asthma and allergies has increased over the last few decades.

e-ISSN: 2347-226X
p-ISSN: 2319-9857

Montelukast sodium is a leukotriene receptor antagonist (LTRA) used for the maintenance treatment of asthma and to relieve symptoms of seasonal allergies [3]. Desloratadine (descarboethoxyloratadine), invented in 2005, is a non sedative metabolite of Loratadine, a second generation long acting antihistaminic drug with selective peripheral H1 receptor antagonistic activity.

Development of validated analytical procedure is an essential part of research area to sure various purposes like qualitative and quantitative analysis, pharmacokinetics and pharmacodynamics study etc (Figure 1).

Figure 1. Structure of montelukast sodium and structure of desloratadine.



MATERIALS AND METHODS

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. The HPTLC instrument used was Camag HPTLC with Linomat-5 injection and camag TLC scanner-3. All the apparatus and instruments used were calibrated and validated.

Instrumentation and chromatographic conditions

Before analysis HPTLC plates of silica gel G60 F254 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 85mm 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 format 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 and 400 nm. All determinations were performed at ambient temperature with a detection wavelength of 287 nm.

Preparation of Standard solution

The stock solution of DSLR (500 mcg/ml) and of MON (1000 mcg/ml) was prepared by dissolving in methanol. From the above solution, 0.4 ml^{-1} ml of DSLR and MON transferred to 10 ml of volumetric flask to obtain mixture of solutions with concentrations of 20-50 mcg/ml of DSLR and 40-100 mcg/ml of MON.

Sample preparation

To clean up the plasma proteins, the sample preparation procedure is adopted. Here protein precipitation and Liquid-Liquid extraction technique was carried out using organic solvents.

In protein precipitation, to a clean and dry centrifuge tube, 0.5 ml of human plasma and 0.5 ml of standard drug solution were added and vortexes. To this solution 1 ml of acetonitrile added and vortexed. This solution was centrifuged at 3000 rpm for 15 min and 20 µl of this was collected and injected in optimized chromatographic condition. The same was repeated with methanol as protein precipitate agent.

The liquid-liquid extraction was carried out with the mixture of dichloromethane: Ethyl acetate (1:1 v/v). After centrifugation the organic layer was collected and evaporated to dryness under the stream of nitrogen gas .The

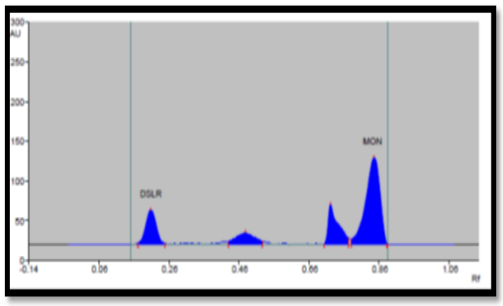
residue was reconstituted with 1 ml of methanol and a 20 µl of this was plotted on TLC plate and analyzed. The extraction procedure that gives highest recovery of two drugs was selected.

Linearity and range

Mixtures of standard solutions were spotted with the help of Linomat V automatic sample applicator on the TLC plates. The plates were developed in 10 × 10 twin trough chamber saturated with the fixed mobile phase system and scanned using CAMAG TLC scanner 3. The Rf 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 0.4 µg/spot-1 µg/spot of DSLR and 0.8 µg/spot-2 µg/spot of MON. The slope, intercept and correlation coefficient values were found are presented. The standard densitogram obtained at 0.4 µg/spot DSLR & 0.8 µg/spot MON (Figure 2).

Figure 2. Densitogram of 0.4 µg/spot DSLR & 0.8 µg/spot MON.



Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ were determined by applying decreasing amount of the drug in triplicate on the plate. The lower concentration at which the peak is detected is called limit of detection. The lowest concentration at which the peak is quantified is called limit of quantification.

RESULTS AND DISCUSSION

A new method has been developed for bioestimation of desloratadine and montelukast sodium in spiked human plasma by high performance thin layer chromatography [4]. The method was found to be linear concentration range of 0.4-1 µg/spot for DSLR and 0.8-2 µg/spot for MONT respectively. Prepared synthetic mixture was analyzed and amount of drug determined by proposed method [5]. The proposed method was validated as per ICH guideline. Precision was calculated as repeatability (% RSD is less than 1.0) and inter and intraday variations (%RSD is less than 1.0) for both drugs (Table 1).

Table 1. Summary of validation parameters for the proposed method.

Parameters	HPTLC	
Stationary phase	pre-coated silica gel 60F_{254} on aluminium sheets	
Mobile phase	Ethanol: methanol: Ammonium formate: Ammonia (9:1:0.5:0.5 v/v/v/v)	
Wavelength	λ= 287 nm	
Validation	DSLR	MON
Linearity	0.4-1 µg/spot	0.8-2 µg/spot

r2	0.997	0.9997
Recovery	50-85%	60-91%
Precision (%RSD)	Within limit	
LOD	0.3 µg/spot	0.4 µg/spot
LOQ	0.5 µg/spot	1 µg/spot

CONCLUSION

The bioanalytical methods developed in the current research work for the simultaneous estimation of DSLR and MON have 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 both tablets and spiked plasma.

REFERENCES

1. Sonia K, et al. HPTLC Method development and Validation: An Overview. J Phar Sci Res. 2017;9:652-657.
2. Ravi C, et al. Development and validation of stability indicating RP-HPLC method for Simultaneous determination of Desloratadine and Montelukast Sodium in pharmaceutical dosage form. Int J Phar Analyt Res. 2016;5:294-309.
3. Gandhi BM, et al. Method development and validation for simultaneous estimation of montelukast sodium and desloratadine by RP-HPLC. Am J Analyt Chem. 2015;6:651-658.
4. Ibrahim FA, et al. Simultaneous determination of desloratadine and montelukast sodium using second derivative synchronous fluorescence spectrometry enhanced by an organized medium with applications to tablets and human plasma. Luminescience. 2014;51:736-742.
5. Rima M, et al. Spectrophotometric determination of montelukast sodium and desloratadine in combined dosage. Int J Chem Tech Res. 2013;5: 136-141.