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RP-HPLC ASSAY METHOD DEVELOPMENT AND VALIDATION OF MONTELUKAST AND DOXOFYLLINE IN BULK AND PHARMACEUTICAL DOSAGE FORMS

Shajan A[*1] and Narayanan N[2]

[1]Research Scholar, Karpagam University, Coimbatore, Tamilnadu, India- 641 021.
[2]Jaya College of Pharmacy, Thiruninravur, Chennai, Tamilnadu, India- 602 024.

ABSTRACT

A simple reverse phase high performance liquid chromatographic assay method was developed and validated for the simultaneous estimation of montelukast and doxofylline from bulk and pharmaceutical dosage forms. The chromatographic separation was achieved on phenomenex C18 (250 mm × 4.6 mm id, 5 µm particle size) column by using the mobile phase composition of acetonitrile: methanol: ammonium acetate buffer (70:10:20 v/v, pH 5.5), the detection of analyte was done at 274 and 347 nm for doxofylline and montelukast respectively by PDA detector. Montelukast and doxofylline obeys Beer Lambert's law in the concentration range from 3-9 µg/ml and 120-360 µg/ml. LOD was found to be 0.0025 µg/ml and 0.0078 µg/ml and LOQ was found to be 0.0076 µg/ml and 0.0235 µg/ml for montelukast and doxofylline respectively.

Key words: RP-HPLC, Doxofylline, Montelukast, validation.

INTRODUCTION

Montelukast is a potent, selective and orally active antagonist of the cysteinyl, CysTL1, leukotriene receptor used for the treatment of asthma in children and adults [1- 3]. It is a leukotriene modifier and has clearly demonstrated the ability to ameliorate bronchoconstriction and indices of airway edema and abnormal mucus production as observed in clinical trials [4].

Doxofylline (DX) is chemically 7-(1,3-dioxolan-2-ylmethyl)-3,7-dihydro-l,3-dimethyl-lH-purine-2,6-dione. It is used as a bronchodilator in asthma and chronic obstructive pulmonary disease [5,6]. Few methods have been reported in the literature for the estimation of montelukast [7-9] and doxofylline [10,11] individually and in combined dosage forms [12]. The objective of present work is to develop and validate [13] a simple, sensitive, rapid, accurate and precise RP-HPLC assay method that can be used for the routine analysis of the formulations containing these drugs.

MATERIALS AND METHODS

Reagents and chemicals

Doxofylline and montelukast were obtained as gift samples from Burgeon Pharmaceuticals Pvt. Ltd., Chennai. Methanol, acetonitrile used as a solvent was purchased from Merck (Mumbai, India). All other reagents and solvents used were of analytical grade.

Instruments

Shimadzu HPLC instrument equipped with a PDA detector, assisted with LC- Solution software

Preparation of buffer solution

3.85 g of ammonium acetate was taken in a 1000 ml volumetric flask, 1 ml of triethyl amine was added and volume was made up to the mark with water. The pH of this buffer solution was adjusted to 5.5 with glacial acetic acid.

Preparation of mobile phase

Mobile phase was prepared having composition of acetonitrile: methanol: ammonium acetate buffer (70:10:20 v/v, pH 5.5).

Corresponding Author :- **Shajan.A** Email:- shajpharma@yahoo.co.in

Asian J. Pharm. Res. Vol 3, Issue 2, 56-59, 2013.

Preparation of montelukast standard solution

Accurately weighed quantity of 10 mg montelukast was transferred to a 100 ml volumetric flask, dissolved in 25 ml of mobile phase and the solution was made up to the mark with mobile phase (Stock solution). From the above stock solution, 3 ml was transferred to 50 ml volumetric flask and the volume was made up with mobile phase. The solution was filtered with 0.45 µ filter and sonicated for 15 min.

Preparation of doxofylline standard solution

Accurately weighed quantity of 400 mg doxofylline was transferred to a 100 ml volumetric flask, dissolved in 25 ml of mobile phase and the solution was made up to the mark with mobile phase (Stock solution). From the above stock solution, 3 ml was transferred to 50 ml volumetric flask and the volume was made up with mobile phase. The solution was filtered with 0.45 µ filter and sonicated for 15 min.

Preparation of sample solution

Bilayer tablets of doxofylline and montelukast were prepared as per the formula developed in our previous study [14]. 20 tablets were weighed and powdered and 902.52 mg of sample was transferred into 100 ml volumetric flask and mobile phase was added to dissolve the sample. The volume was made up to the mark using mobile phase and filtered with 0.45 µ filter paper. From the above stock solution 5 ml was transferred to 100 ml volumetric flask and the volume was made up with mobile phase. The solution was filtered with 0.45 µ filter and sonicated for 15 min.

Optimized chromatographic conditions

Stationary Phase : Inertsil C18 (4.6 x 250 mm, 5 µm)
Injector : Rheodyne
Flow rate : 1.5 ml/min
Operating temperature : Ambient temperature
Selected wave length : 274 and 347 nm for Doxofylline and montelukast respectively
Mobile phase ratio : Acetonitrile: methanol: ammonium acetate buffer (70:10:20 v/v, pH 5.5).
Injection Volume : 20µl
Run Time : 10 min

Validation

Linearity

The standard solution of montelukast and doxofylline was diluted with mobile phase to get concentration of 3-9 µg/ml and 120 – 360 µg/ml respectively. Then this solution was injected in HPLC and peak area was calculated, the calibration graph was plotted concentration versus peak area.

Accuracy

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out at 50%, 100% and 150% levels by standard addition method. Standard deviation and percentage recovery was calculated.

Precision

The precision of the method was demonstrated by interday and intraday variations studies. In this study repeated injections were made at 0 h, 8 h and 16 h on the same day and different days (3 days).

Robustness

Small deliberate changes were made in the method parameters and the peak area of sample solution was calculated.

Figure 1. Calibration graph for montelukast

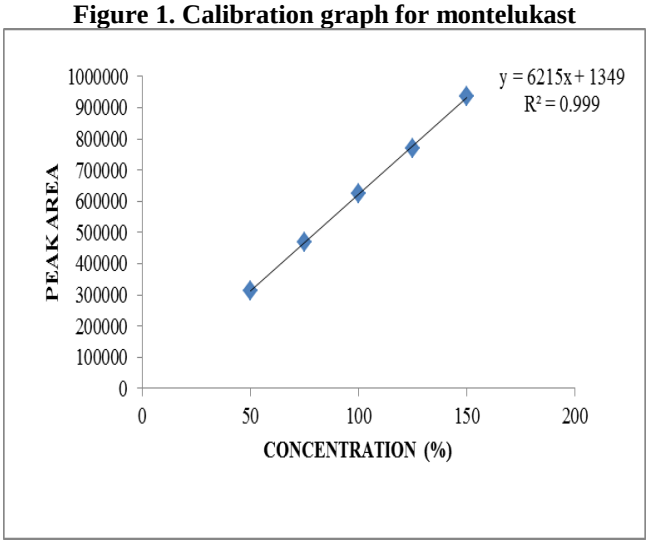
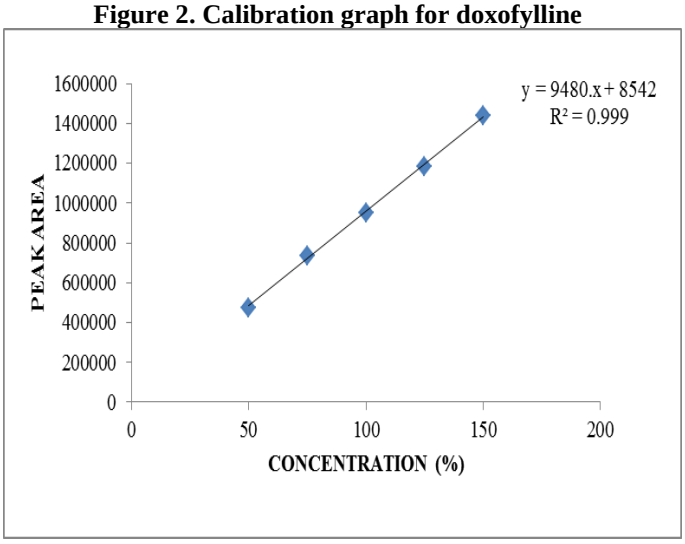


Figure 2. Calibration graph for doxofylline



Asian J. Pharm. Res. Vol 3, Issue 2, 56-59, 2013.

Table 1. Validation results for Montelukast and Doxofylline

Parameters	Montelukast	Doxofylline
Specificity	No interference	
Linearity (µg/ml)	3-9	120-360
	R^2 - 0.999	R^2 - 0.999
Accuracy (%)	99.37	100.37
Precision – Repeatability*(%)		
0 h	99.83 ± 0.35	99.37 ± 0.24
8 h	99.36 ± 0.06	99.24 ± 0.12
16 h	98.7 ± 0.01	98.85 ± 0.03
Precision – Intermediate*(%)		
Day -1	100.07 ± 0.99	98.90 ± 0.57
Day - 2	99.63 ± 0.03	99.22 ± 0.20
Day - 3	98.67 ± 0.02	98.77 ± 0.02
Instrument - 1	99.60 ± 0.15	99.02 ±0.08
Instrument - 2	99.86 ± 0.58	98.98 ± 0.30
Analyst -1	99.60 ± 0.07	99.57 ± 0.01
Analyst -2	99.96 ± 0.27	99.60 ± 0.02
Column -1	99.61 ± 0.05	99.12 ± 0.19
Column -2	99.51 ± 0.08	99.13 ± 0.06
Robustness*(%)		
Flow rate	+ 10 %	99.57 ± 0.06	99.15 ± 0.09
	- 10 %	99.59 ± 0.08	99.24 ± 0.12
Mobile phase	+ 2 %	99.54 ± 0.03	98.85 ± 0.68
	- 2 %	99.57 ± 0.09	99.25 ± 0.01
Wavelength	+ 2 nm	98.02 ± 0.76	99.78 ± 0.10
	- 2 nm	97.94 ± 0.13	98.79 ± 0.16
Temperature	+ 2˚C	98.91 ± 0.03	99.67 ± 0.09
	- 2˚C	99.21 ± 0.03	99.81 ± 0.04
Assay	99.57 ± 0.04		99.33 ± 0.47

*Values are expressed as mean ± SD, n = 6

Asian J. Pharm. Res. Vol 3, Issue 2, 56-59, 2013.

RESULTS AND DISCUSSION

The method developed in the present study using Inertsil C18 (4.6 x 250 mm, 5 µm) as stationary phase and acetonitrile: methanol: ammonium acetate buffer (70:10:20 v/v, pH 5.5) as mobile phase gave good separation of montelukast and doxofylline at 3.314 and 6.14 min respectively. The detector response of doxofylline and montelukast was found to be linear in the range of 3-9 µg/ml and 120-360 µg/ml respectively. The R^2 values were found to be 0.999 for doxofylline and 0.999 for montelukast (Figure 1 and 2), which indicates good linearity between concentration and peak area. The interday and intraday assay variance indicated the precision and reproducibility of the proposed method. The mean % recovery was close to 100 which indicate the accuracy of the method. LOD was found to be 0.0025 µg/ml and 0.0078 µg/ml and LOQ was found to be 0.0076 µg/ml and 0.0235 µg/ml for montelukast and doxofylline respectively. The results are presented in table 1.

CONCLUSION

The proposed RP-HPLC method was found to be sensitive, accurate and reproducible for the quantification of doxofylline and montelukast in both bulk and solid dosage form. The excipients present in the dosage form did not interfere with the analysis. Hence the developed method can be useful for the routine quality control analysis and quantitative determinations of doxofylline and montelukast.

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