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Research Article

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Development and Validation of RP-HPLC Method for the Determination of Potential Genotoxic Impurities m-Isophthalaldehyde and 3-(2-(7-Chloroquinoline-2-yl)-(e)-vinyl) Benzaldehyde in Montelukast Sodium

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ABSTRACT

The present paper describes a simple gradient reverse phase HPLC method for the determination of two potential genotoxic Benzene-1,3-dicarboxaldehyde or m-isophthalaldehyde (PHA) and 3-(2-(7-chloroquinoline-2-yl)-(e)-vinyl) benzaldehyde (BNA) in Montelukast Sodium (MNK). Good resolution between two aldehydes PHA, BNA and Montelukast Sodium was achieved with Zorbax SB phenyl (150 mm × 4. 6mm, 3.5 µ) column using a gradient of buffer 2% trifluroacetic acid, pH adjusted to 1.9 and acetonitrile. The flow rate was 1.5 ml/min and the elution was monitored at 238 nm. The factors involved in the method development are discussed. This method was validated as per International Conference on Harmonization (ICH) guidelines and is able to quantitate two aldehydes at 0.25 ppm levels each with respect to 1.7 mg/ml of MNK. The method is linear in range of 0.125–0.30 ppm, which matches the range of 50–120% of estimated permitted level (150 ppm) of PHA and BNA were not present in the three studied pure batches of MNK

Keywords: Development; Validation; Genotoxins

INTRODUCTION

Montelukast ([R-(E)]-1[[[1-[3-[2-(7-chloro-2-quinolinyl) ethyl] phenyl]-3-[2-(1-hydroxy-1-methylethyl) phenyl] propyl] thio] methyl] cyclo propane acetic acid, is a selective and orally active cysteinyl leukotriene receptor antagonist (Figure 1). It is used for prophylaxis and chronic treatment of asthma and its sodium salt is an anti asthmatic agent marketed as brand name telecast tab. Regulatory issues related to the presence of genotoxic impurities [1] have arisen with a greater frequency due to enhanced technological capability in identifying impurities and increased focus on their potential impact on human health. As per the guideline from the European Medicines Agency [2] on the limits of genotoxic impurities, a threshold of toxicological concern (TTC) value of 1.5 µg/day intake of a genotoxic impurity is considered to be associated with an acceptable risk for most of the pharmaceuticals. The concentration limit of permitted genotoxic impurity in ppm is the ratio of TTC in microgram/day and dose in gram/day. These impurities are likely to be formed from the raw material in the process. MNK is administered at a maximum daily dose of 10 mg/day in the form of tablets (5.0, 10.0 mg) with trade name as Telecast tab. Hence, the estimated permitted level of PHA & BNA in MNK is 150 ppm/day. Published literatures on monteklaust for drugs [3-6] using normal and other special techniques and few literatures reported on dosage form [7-9] using reverse phase chromatographic techniques and other papers using GC–MS and LC–MS methods were found for the determination of drug in human plasma[10-12]. However, most of the reported methods involve highly sophisticated instruments, which are not generally available in most of the up coming pharmaceutical industries and no literature are available on the analysis of PHA & BNA in MNK. Therefore, we have developed a simple gradient RP-HPLC method that can quantitate at permitted levels of PHA & BNA in MNK. This method is validated as per ICH guidelines in terms of limit of detection (LOD), limit of quantification (LOQ), linearity, precision, accuracy, specificity and robustness.

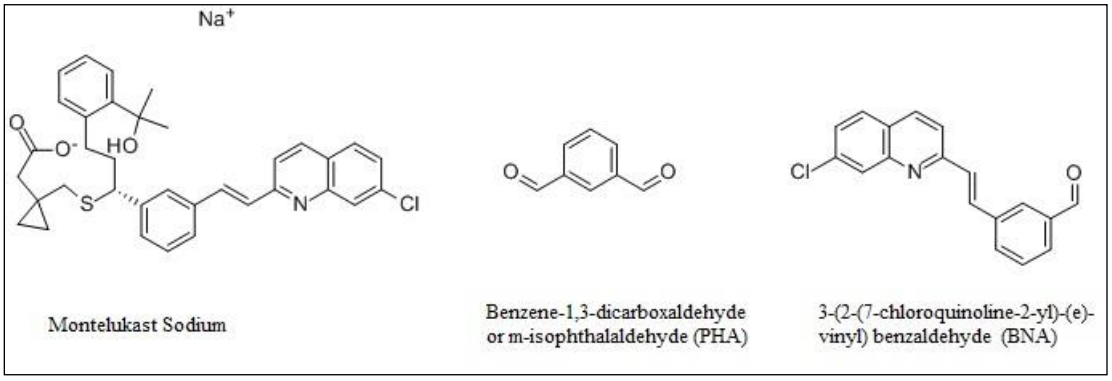

Figure 1: Structure of montelukast sodium and impurities

EXPERIMENTAL SECTION

Materials and Methods

All chemicals and solvents were of analytical grade. Trifluoro acetic acid and acetonitrile (HPLC grade) were procured from SRL. Benzene-1,3-dicarboxaldehyde (PHA) and 3-(2-(7-chloroquinoline-2-yl)-(e)-vinyl) benzaldehyde (BNA) were purchased from Sigma Aldrich Corporation, Bangalore, India.

Chromatographic Parameters

Analysis was carried out on Agilent 1100 series module equipped with PDA detector. However, for convenience, the elution was monitored at 238 nm. Separation was achieved by using Zorbax SB phenyl (150 mm × 4.6 mm, 3.5 µ) column using a gradient of buffer of trifluoroacetic acid pH adjusted to 1.9 and acetonitrile. The flow rate was 1.5 ml/min. Methanol was used as diluent. Ten micro liters of sample solution is injected each time.

Sample preparation:

Individual stock solutions of PHA and BNA were prepared by dissolving 2.5 mg each separately in 100 ml of methanol. This solution was further diluted to get a solution of 0.25 ppm of solution which was injected for identifying retention times and for resolution studies. For validation studies, required volumes of PHA and BNA stock solutions and 17 mg of MNK were taken in 10 ml volumetric flask. The contents were dissolved and diluted up to the mark with methanol. Since MNK and the two aldehydes are soluble in methanol, they were sonicated well and the clear solution was used for analysis.

Method validation:

The method validation is initiated by injecting 0.25 ppm of individual solutions of PHA and BNA with respect to 1.7 mg/ml of MNK and determining their S/N (signal to noise) ratio. To determine LOD and LOQ values, aldehydes concentrations were reduced sequentially such that they yield S/N ratio as 3 and 10 for LOD and LOQ respectively. The determined LOD and LOQ values were presented in Table 1. Linearity for two aldehydes was fixed in the range of 50-120% of the estimated permitted level (*viz.* 0.25 ppm with respect to 1.7 mg/ml of MNK solution).

Table 1: LOD, LOQ and linear regression analysis and precision data

Parameter	PHA	BNA
LOD (ppm)[a]	0.062	0.062
LOQ (ppm) [a]	0.125	0.125
Linear range(ppm) [a]	0.062 – 0.3	0.062 – 0.3
Slope	62.272	17.724
Intercept	0.329	0.267
Correlation coefficient	0.998	0.999
Precision (% RSD) [b]	0.171	0.168
Intermediate precision (% RSD) [b]	0.234	0.325

[a]LOD, LOQ and linearity range are given in ppm with respect to 1.7 mg/ml of MNK; [b]Six determinations using 0.25 ppm of aldehydes with respect to 1.7 mg/ml of MNK

Hence, 0.125, 0.15, 0.2, 0.25, 0.3 ppm solution mixture of two aldehydes were prepared and injected individually. The calibration curves were drawn between the peak areas versus concentration of aldehydes. The slope, intercept and correlation coefficient values were derived from linear least-square regression analysis and the data presented in Table 1 and reveals that an excellent correlation existed between the peak areas and the concentrations of PHA and BNA (Figures 2-4).

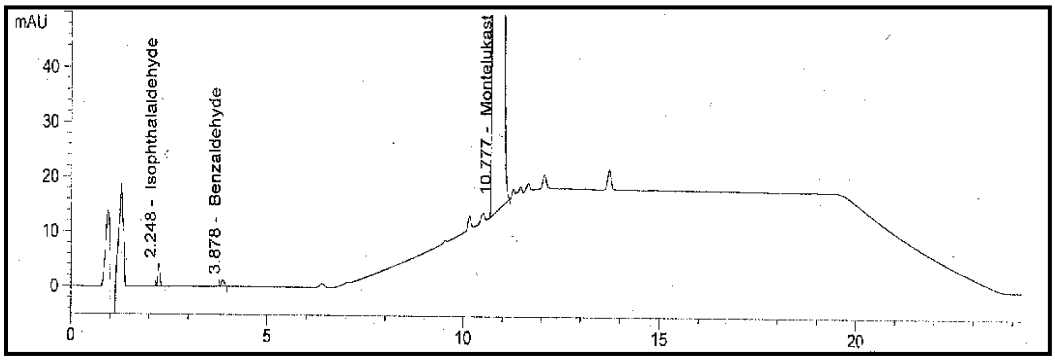

Figure 2: LC chromatogram of 1.7 mg/ml of MNK and 0.25 ppm of aldehydes

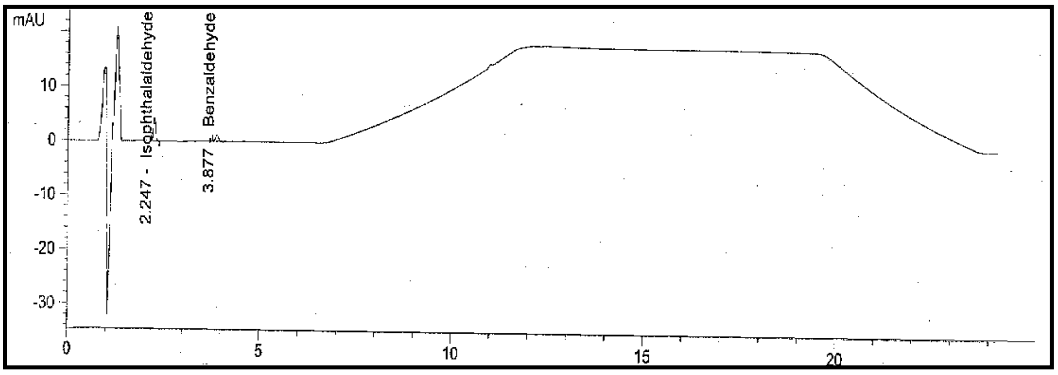

Figure 3: LC chromatogram of 0.25 ppm of aldehydes (PHA and BNA)

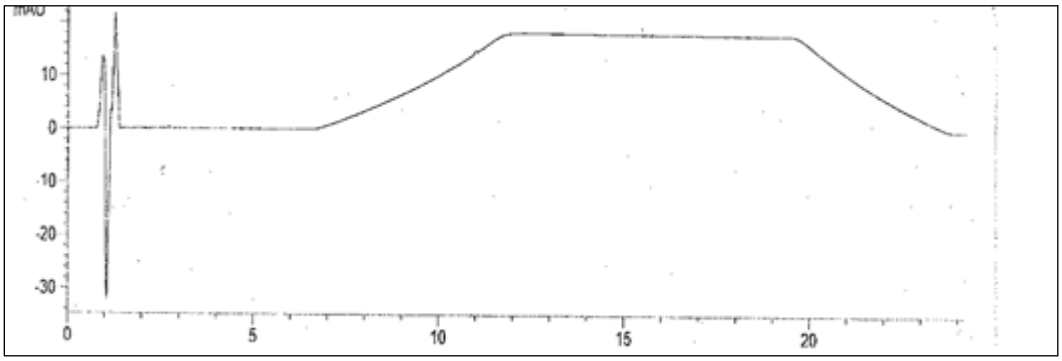

Figure 4: LC chromatogram of blank

The precision was evaluated at two levels *viz.* repeatability and intermediate precision. Repeatability was checked by calculating the relative standard deviation of six replicate determinations by injecting six freshly prepared solutions containing 0.25 ppm of each aldehyde on the same day. The same experiments were done on six different preparations on different instrument for evaluating intermediate precision. The low % RSD values confirm the good precision of the developed method. When three pure batch solutions of 1.7 mg/ml MNK were injected using this method and found to be absent of PHA and BNA in all the batches. Hence, recovery studies was performed using standard addition method to evaluate accuracy and specificity, therefore, the accuracy of the method was determined by spiking 0.125, 0.25, 0.30 ppm of aldehydes to a batches of pure MNK (1.7 mg/ml) and each determination was carried in triplicates and results of recovery indicates the method is accurate (Table 2). The specificity of PHA and BNA with respect to the MNK is determined by analyzing the batches of MNK. The robustness of the method was studied with deliberate modifications in flow rate of the mobile phase and column temperature. The flow rate of the mobile phase was 1.5 ml/min and the same was altered by 0.2 units i.e. from 1.3 ml/min to 1.7 ml/min and effect of column temperature on resolution were studied at 30°C and 40°C instead of 35°C and results indicate that these changes does not have any impact on suitability parameters. Stability of solution in diluent were studied by injecting solutions which is stored at room temperature at various intervals and results are recorded and concluded that PHA and BNA were stable up to 12 hrs respectively.

Table 2: Evaluation of accuracy and specificity of the proposed method

Sample	% Recovery of PHA (mean±% RSD)			% Recovery of BNA (mean±% RSD)		
	0.125ppm	0.25ppm	0.30ppm	0.125ppm	0.25ppm	0.30ppm
Preparation-1	99.8	99.5	99.9	98.8	99.2	99.1
Preparation-2	99.1	99.6	99.3	98.2	99.4	99.6
Preparation-3	99.3	99.1	99.8	98.8	99.8	99.9

RESULTS AND DISCUSSION

Based on validation study, the system suitability parameters like % RSD of retention time and area response are well within the acceptance criteria. The precision study has shown that test method is precise. The recovery study results indicating that the test method has acceptable level of accuracy and in Linearity study, the correlation coefficient was above the criteria and range was established for this method from 50% to 120% (0.125–0.30 ppm) of the target concentration and robustness study results were well within the acceptance criteria of system suitability parameters and it was established the method is robust in nature.

CONCLUSION

The evaluation of obtained values and results concludes that proposed RP-HPLC method provides simple, precision, rapid, accurate and robust quantitative analytical method for determination and estimation of aldehydes in Montelukast sodium. Hence the method can be easily adopted for routine estimation.

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