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AN ISOCRATIC RP-HPLC METHOD DEVELOPMENT AND ITS VALIDATION FOR SIMULTANEOUS ESTIMATION OF BUDESONIDE AND DICLOFENAC SODIUM IN PHARMACEUTICAL FORMULATION

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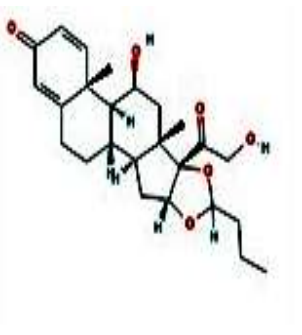
ABSTRACT

A unique, discriminative, simple, rapid, specific, cost-effective and isocratic reverse phase high performance liquid chromatographic method was developed and validated for the model drugs chosen in the study viz., Budesonide and Diclofenac in a novel pharmaceutical formulation. The chromatographic elucidation for the method development was carried out with YMC column of 4.6X 150 mm and 5 µm i.d using pH 3.0 phosphate buffer solution and acetonitrile in 30:70v/v ratio as mobile phase at a flow rate of 1 ml/min. The isocratic wavelength for both the drugs used in the study was set to 264 nm and detection was carried out using PDA detector at ambient temperature. The developed method was validated for precision, specificity, ruggedness, accuracy, linearity, LOD and LOQ. The calibration curves for Budesonide and Diclofenac were found to be linear. The retention times for Budesonide and Diclofenac were found to be 1.6 and 2.3 min respectively. Thus, the method developed in the current study can be adopted for quality control analysis of selected drug candidates.

INTRODUCTION

Budesonide, a non-halogenated glucocorticoid (Naikwade SR et al., 2008) is chemically a 17-[(1RS)-butylidenebis(oxy)]-11 ß, 21-dihydroxy pregna-1,4-diene-3, 20-dione as mentioned in figure 1b. It is used as a prime line drug to treat asthma and also colonic diseases like ulcerative colitis and chron's disease at dfferent doses. While its side effects and lack of maintenance therapy limits its use (Varshosaz J et al., 2011). Diclofenac Sodium, a nonsteroidal phenyl acetic acid cyclooxygenase (COX) inhibitor, chemically represented as 2-(2-(2,6-dichlorophenylamino)phenyl) acetic acid is popular as a front line drug to reduce joint pains observed in arthritis or other acute injuries.

1a. Diclofenac Sodium



1b. Budesonide

A Comprehensive survey of literature had revealed quite a lot of analytical techniques viz., high-performance liquid chromatography (Klencsar, 2017), Capillary gas chromatography, gas chromatography, micellar electro kinetic chromatography, capillary gas chromatography were reported for quantification of budesonide (BD) (Ryrfeldt A et al., 1984) and diclofenac (DC) (Patel YP et al., 1998) individually and/or in combination with other drugs (Krishna Sankaa et al., 2014). Nevertheless, the exhaustive literature review has revealed that most of the proposed methods present laborious procedures which are tedious and uneconomical along with the lack of selectivity and specificity (Blewett AJ et al., 2011).

Hence, the current work focuses on the development of a novel, simple, rapid, economical and a precise reverse phase HPLC method for the simultaneous estimation of budesonide and diclofenac sodium.

EXPERIMENTAL:

Chemicals and reagents

Budesonide and Diclofenac were gratis from pharmatrain laboratories, Hyderabad, India. Acetonitrile (HPLC grade) was procured from Molychem, India. Potassium dihydrogen phosphate, water and methanol (HPLC grade) and Orthophosphoric acid were procured from Finer chemical Ltd and Lichrosolv (MERCK), India respectively. Reference standards for budesonide and Nifedipine were obtained from Unichem pharmaceuticals, Mumbai, India.

Chromatographic Conditions and Instrumentation

Separation of budesonide and diclofenac was performed on WATERS reverse phase high performance liquid chromatographic system equipped with 2695 separation module employing Photo diode array (PDA) detector with 210 µl injection volumes. YMC column (4.6 x 150, 5µm) was used for the separation process. Mobile phase was prepared by mixing pH 3.0 phosphate buffers and acetonitrile of HPLC grade in 30:70 v/v ratios, filtered through 0.2 µ membrane filter, operated and detected at a flow rate of 1.0 ml/ min and 264nm wavelength respectively. The separation process was carried out at ambient temperature.

Preparation of mobile phase (Diluent)

Preparation of 0.1% Orthophosphoric acid (OPA) buffer

1ml of OPA was diluted in 1000ml of water (HPLC grade) to prepare 0.1% OPA solution. Required quantity of sodium hydroxide solution was added to the above solution to adjust the pH to 3.0. This results in the formation of 0.1% OPA buffer.

Preparation of Mobile phase

300 ml of 0.1% OPA buffer solution was mixed with 700 ml of acetonitrile of HPLC grade, sonicated for thorough mixing and degassing for 10-15 min and filtered through 0.45 µm membrane filter under vacuum. This gives 30: 70v/v mobile phase of pH3.0 phosphate buffer and acetonitrile respectively.

Preparation of standard stock

10mg and 100mg of accurately weighed quantities of budesonide and diclofenac were transferred into a clean and dry volumetric flask of 10 ml capacity. To the above mixture of drugs, few ml of methanol was added to dissolve the ingredients, diluted with diluents (mobile phase) and made up the final volume with diluents after sonication to ensure complete solubility of drugs in solvent. This results in the formation of 100 µg/ml and 300µg/ml concentrations of budesonide and diclofenac respectively (Stock A). Later, 0.3 ml of stock A was pipette out into a 10 ml volumetric flask and diluted with diluents to make up the required volume to obtain 10 µg/ml and 30 µg/ml concentrations of BD and DC respectively.

Preparation of Sample solution

The pellet formulations were accurately weighed and powdered in mortar. An equivalent weights of budesonide (10 mg) and diclofenac (100 mg) were taken into a volumetric flask of 10 ml capacity; few ml of diluents was added and was subjected to sonication at ambient temperature for 20 – 25 min with irregular swirling, cooled and made up to the required volume with the same diluents. The solution was further diluted to desired concentration before subjecting to analysis.

Assay

Assay of prepared sample and standard solutions was performed using optimized chromatographic conditions viz., 30:70 v/v OPA and acetonitrile as mobile phase, 1 ml/min flow rate, 264 nm wavelength, 10µl Injection volume and 10 min run time. 20µl of standard and sample solutions were injected separately into the system and the chromatogram was recorded. The retention times of BD and DC for standard and sample solutions was noted separately (n=3).

Experimental Method Validation

The above developed method for the simultaneous estimation of budesonide and diclofenac was validated according to the protocol mentioned in ICH guidelines for specificity, accuracy, precision, linearity, LOD, LOQ and stability. Specificity was obtained by analyzing blank and sample solutions (at 100% level), to check for the interference of excipients used in the preparation of formulation at their respective retention times. Linearity of the method was confirmed at five concentration levels of mixed solutions of BD and DC. Accuracy and precision of standard and sample solutions was investigated from recovery studies and % RSD obtained from six repeated injections respectively.

RESULTS AND DISCUSSION:

Optimization of developed method

An isocratic RP-HPLC technique for the quantification of budesonide and diclofenac in a single dosage form for combinatorial therapy of asthma and rheumatoid arthritis was optimized to enable better separation and resolution using different mobile phases for trials. The results complied that 0.1% orthophosphoric acid buffer adjusted to pH 3.0 was gave acceptable peak shape than other buffer solutions used as trials. Different compositions of buffer and acetonitrile were tried to exhibit the better separation of analytes used in standard and sample preparations. Finally, a mobile phase composition of 30:70 v/v ratios of pH 3.0 orthophosphoric acid buffers and acetonitrile were selected to be the better combination for effective, rapid and reliable separation process. The results have shown that a better peak symmetry and resolution were attained with YMC column (4.6 X 150mm, i.d., 5µm) compared to other columns used in trial. Both the analytes (BD and DC) in standard and sample preparations have presented better and reliable responses at 264 nm as shown in Figure.1. using UV detector, while the flow rate of mobile phase was maintained as 1.0 ml/min throughout the study. The retention times for budesonide and diclofenac were found to be 1.6 and 2.3 min respectively and were not reported with any peak tailing.

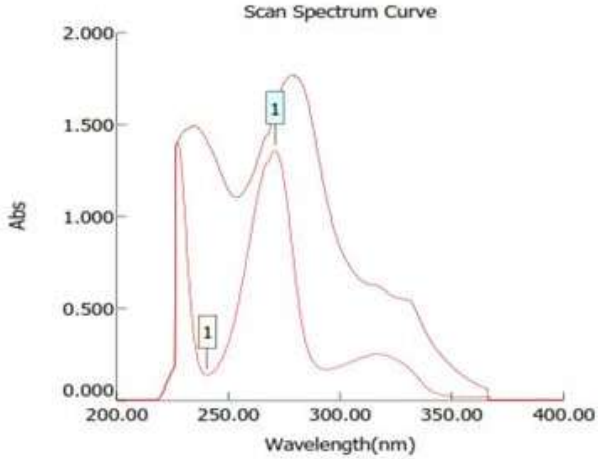


Figure 1: Selection of wavelength for BD and DC

Optimization of validation procedures

The method proposed for effective separation of budesonide and diclofenac had shown short elution time with good separation between BD and DC. The system suitability parameters were tested as per ICH guidelines to confirm the suitability and reproducibility of the developed method. Standard sample was injected for six consecutive times to ensure the repeatability of theoretical plate count, tailing factor and resolution and are found to be absolutely within the limits.

Table. 1. System suitability parameters of BD & DC

Parameters	Budesonide	Diclofenac	Acceptance criteria
Theoretical plate count	2935.56	4800.35	Not less than 2000
Tailing factor	1.52	1.46	Not more than 2.0
Resolution factor	6.50		Not less than 2.0
Assay (%)	100.12	99.93	98-102%

The method was found to be linear at 10-30 µg/ml for budesonide and 50 – 150 µg/ml for diclofenac and the linearity were inveterate by regression values that were found to be 0.999 for both BD and DC which confirms the linearity of the results. The study also reported no interference of blank (diluent) with analytes which confirms the specificity of blank with analytes. The mean % recovery of analytes at low, medium and high concentrations of samples was analyzed to determine the accuracy of the proposed method and the results

Table 02: Validation parameters

Validation method	Parameter	Budesonide	Diclofenac	Acceptance criteria
Linearity	Correlation coefficient	0.999	0.999	0.99
Specificity	Interference is checked	No interference	No interference	No interference
Accuracy	Mean % Recovery at 50%, 100% and 150%	100.38	100.38	98-102
Precision	% RSD	0.5	0.1	Not be more than 2.0
Ruggedness (Intermediate precision)	% RSD (Interday)	0.6	0.2	Not be more than 2.0
LOD (ng/ml)	S/N ratio	3.00	3.02	3.0
LOQ (ng/ml)	S/N ratio	9.98	10.0	10.0
Stability	Peak purity	Passed	Passed	No degradation

Table 03: Results showing Robustness for BD and DC

Flow rate (ml/min)	System suitability results for BD		System suitability results for DC		USP resolution
	USP plate count	USP tailing	USP plate count	USP tailing	
0.9	3013.80	1.57	4951.17	1.46	6.64
1.0	2935.56	1.52	4800	1.46	6.50
1.1	2845.18	1.43	4596.34	1.42	6.34

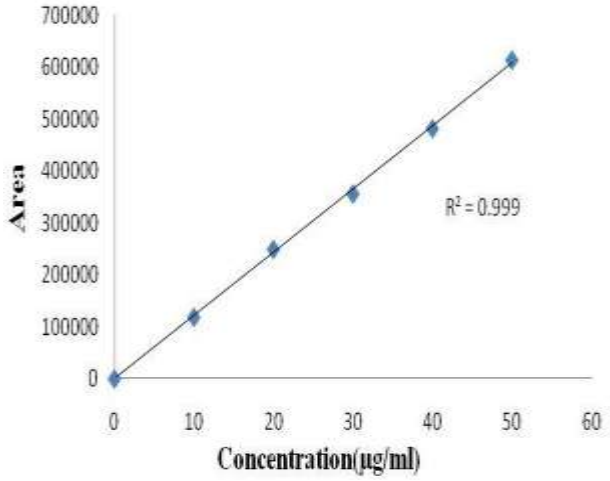


Figure 2: Calibration curve for budesonide

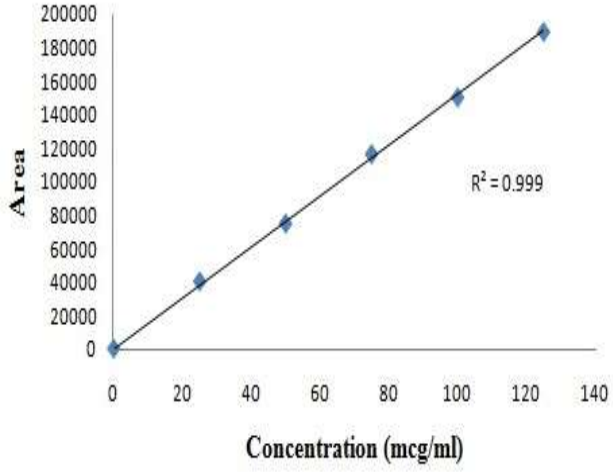


Figure 3: Calibration curve for Diclofenac

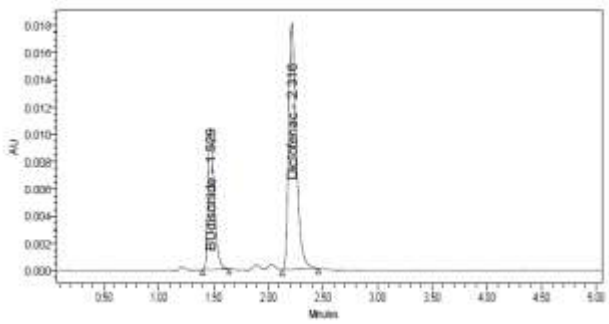


Figure 4: Chromatogram for simultaneous estimation of sample for analytes

(100.38 and 100.38 % for BD and DC respectively) were found to be within the acceptance criteria of 98 – 102 %. The precision for analytes was measured in % RSD and was reported as 0.5 and 0.1 for BD and DC respectively and were within the acceptable limits of not more than 2.0. Limit of detection was expressed in terms of S/N ratio and the results for LOD were found to be 3.0 and 3.02µg/ml while that of LOQ values were found to be were within the standard limits of 9.98 and 10.00µg/ml for BD and DC respectively.

All the validation parameters evaluated are within the acceptable limits mentioned in ICH guidelines and hence the proposed method in the current study is found to be accurate. The degradation studies for the standard and sample solutions were also estimated to determine the peak area responses for hydrolytic degradation in acidic, alkaline medium and in room temperature at 6, 12 and 24 hrs. The results for stability studies had exhibited no significant differences.

CONCLUSION

The proposed isocratic RP-HPLC technique in the current study had proved to be simple, rapid, reliable, accurate and precise. Hence it is suitable for simultaneous estimation of budesonide and diclofenac. High resolution and high percentage recovery values proved the method to be free from interference of analytes with excipients used in the formulation.

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