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Research Article

A validated simultaneous estimation of Budesonide and Nifedipine in pharmaceutical formulation by RP-HPLC method

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ABSTRACT

A discriminative, easy, quick, precise, economical and isocratic reverse phase high performance liquid chromatographic method was developed and validated for the model drugs chosen in the study viz., Budesonide and Nifedipine in a novel pharmaceutical formulation. The chromatographic elucidation for the method development was carried out with Inspire (250 mm X 4.6 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 260 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 Nifedipine were found to be linear in the range of 10 -50 µg/ml and 25 -150 µg/ml concentration respectively. The retention times for Nifedipine and Budesonide were found to be 2.1 and 3.2 min respectively. Thus, the method developed in the current study can be adopted for quality control analysis of selected drug candidates.

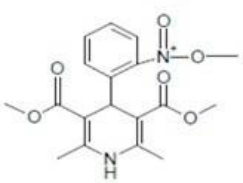
Keywords: budesonide, nifedipine, RP-HPLC, validation.

INTRODUCTION

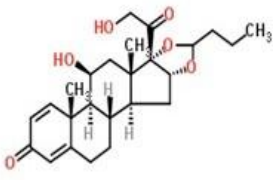
Budesonide, a non-halogenated glucocorticoid (Naikwade SR et al., 2008) is chemically a 17-[(1RS)-butylidenebis (oxy)]-11 ß, 21-dihydroxypregna-1,4-diene-3, 20-dione as mentioned in figure 1b. It is used as a prime line drug to treat asthma and also colonic diseases like ulcerative colitis and chron's disease at different doses. While its side effects and lack of maintenance therapy limits its use (Varshosaz J et al., 2011). Nifedipine is a dihydropyridine calcium channel antagonist, chemically known as 1, 4-dihydro-2, 6-dimethyl- 4-(2-nitrophenyl)-3, 5-pyridine dicarboxylic acid dimethyl ester as mentioned in figure 1a, majorly used to treat angina pectoris.

A Comprehensive survey of literature had revealed quite a lot of analytical techniques viz., high-performance liquid chromatography, Capillary gas chromatography, gas chromatography, micellar electro kinetic chromatography, capillary gas chromatography were reported for quantification of nifedipine (NF) (Patel YP et al., 1998) and budesonide (BD)(Ryrfeldt A et al., 1984) 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.

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 nifedipine.



1a. Nifedipine



1b. Budesonide

Experimental Method Development

Chemicals and reagents

Nifedipine and budesonide 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

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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 nifedipine 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. Inspire C_{18} column (4.6 X 250mm, i.d., 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 and operated and detected at a flow rate of 1.0 ml/ min and 260nm 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 30mg of accurately weighed quantities of budesonide and nifedipine 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 nifedipine 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 NF respectively.

Preparation of Sample solution

The pellet formulations were accurately weighed and powdered in mortar. An equivalent weights of budesonide (10 mg) and nifedioine (30 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, 260 nm wavelength, 10µl Injection volume and 10 min run time. 10µl of standard and sample solutions were injected separately into the system and the chromatogram was recorded. The retention times of BD and NF for standard and sample solutions was noted separately (n=3).

Experimental method validation

The above developed method for the simultaneous estimation of budesonide and nifedipine 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 NF. 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 nifedipine in a single dosage form for combinatorial therapy of asthma and angina was optimized to enable better separation and resolution using different mobile phases for trial. 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 ratio of pH 3.0 orthophosphoric acid buffer and acetonitrile were selected to be the better comination for effective, rapid and reliable separation process. The results have shown that a better peak symmetry and resolution were attained with Inspire C_{18} column (4.6 X 250mm, i.d., 5µm) compared to other columns used in trial. Both the analytes (BD and NF) in standard and sample preparations have presented better and reliable responses at 260 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 nifedipine and budesonide were found to be 2.1 and 3.2 min respectively and were not reported with any peak tailing.

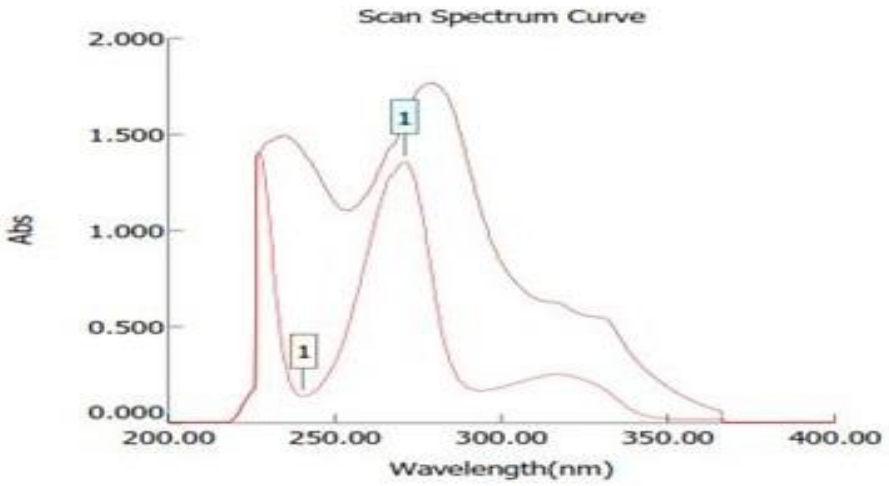

Figure 1: Selection of wavelength for BD and NF

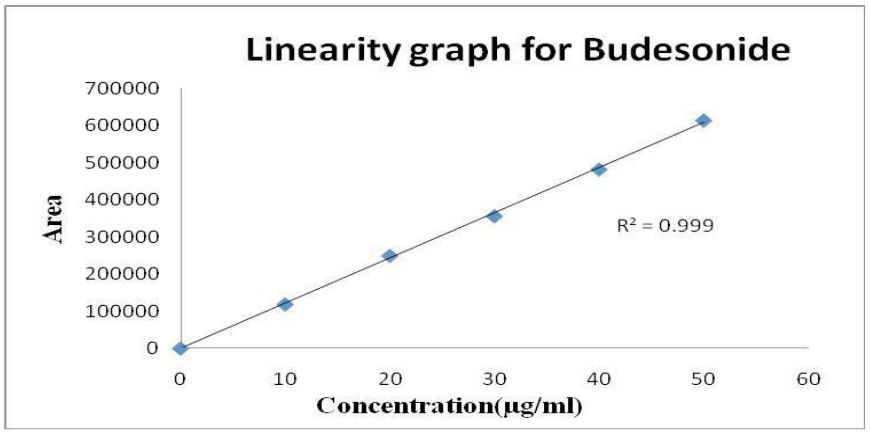

Figure 2: Calibration curve for budesonide

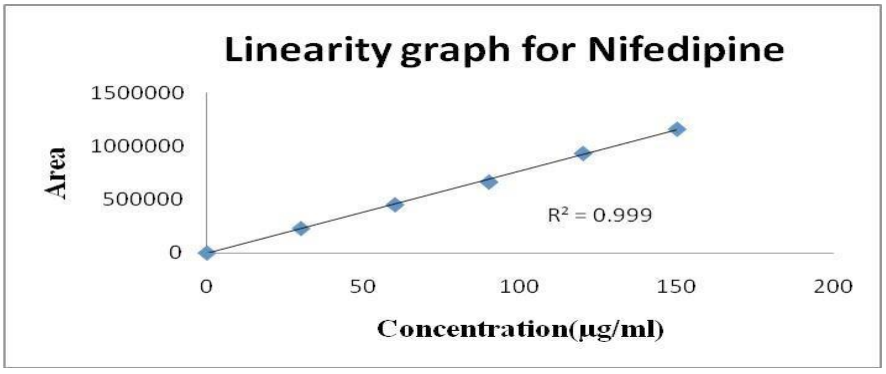

Figure 3: Calibration curve for nifedipine

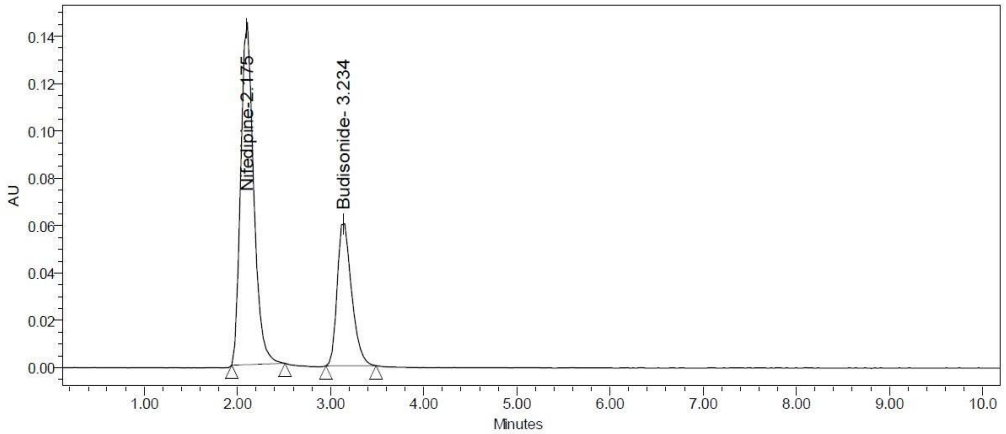

Figure 4: Chromatogram for simultaneous estimation of sample for analytes

Table 1: System suitability parameters

Parameters	Budesonide	Nifedipine	Acceptance criteria
Theoretical plate count	3468.62	3215.24	Not less than 2000
Tailing factor	1.32	1.17	Not more than 2.0
Resolution factor	5.11	4.17	Not less than 2.0
Assay (%)	100.95	98.38	98-102%

Table 2: Validation parameters

Validation method	Parameter	Budesonide	Nifedipine	Acceptance criteria
Linearity	Correlation coefficient	0.999	0.999	0.99
Specificity	Interference is checked	Not observed	Not observed	No interference
Accuracy	Mean % Recovery at 50%, 100% and 150%	100.14	100.06	98-102
Precision	% RSD	0.2	0.7	Not be more than 2.0
Ruggedness (Intermediate precision)	% RSD (Interday)	0.9	0.6	Not be more than 2.0
LOD (ng/ml)	S/N ratio	3.0	2.98	3.0
LOQ (ng/ml)	S/N ratio	10.0	9.98	10.0
Stability	Peak purity	Passed	Passed	No degradation

Optimization of validation procedures

The method proposed for effective separation of budesonide and nifedipine had shown short elution time with good separation between BD and NF. 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.

The method was found to be linear at 25 – 150 µg/ml for nifedipine and 10-50 µg/ml for budesonide and the linearity were inveterate by regression values that were found to be 0.999 for both BD and NF 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 (100.14 and 100.06 % for BD and NF respectively) were found to be within the acceptance criteria of 98 – 102 %. The precision for analytes was measured in % RSD and was reported as 0.2 and 0.7 for BD and NF 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 2.98µg/ml while that of LOQ values were found to be were within the standard limits of 10.0 and 9.98µg/ml for BD and NF 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 nifedipine. 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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