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AN ISOCRATIC, VALIDATED RP-HPLC TECHNIQUE FOR SIMULTANEOUS ESTIMATION OF DICLOFENAC AND NIFEDIPINE IN PHARMACEUTICAL FORMULATION

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ABSTRACT

A simple, rapid, and highly economical RP-HPLC method was developed for the simultaneous determination of model drugs chosen in the present study viz., diclofenac and nifidipine in their novel combined pharmaceutical formulation. The chromatographic elucidation for the method development was carried out with Phenominex (4.6 x 250mm, 5µm i.d) using pH 4.5 phosphate buffer solutions and acetonitrile in 40:60v/v ratio as mobile phase at a flow rate of 1 ml/min. The isocratic wavelength for quantification of both the analytes used in the study was set to 265 nm with PDA detector at ambient temperature. The developed method was validated for precision, specificity, ruggedness, accuracy, linearity, LOD and LOQ. The calibration curves for diclofenac and nifedipine were found to be linear in the concentration range of 25-125 µg/ml and 30-150 µg/ml respectively. The retention times for diclofenac and nifedipine were found to be 6.4 and 2.9 min respectively. Thus, the method developed in the current study can be adopted for quality control analysis of selected drug candidates.

INTRODUCTION:

The first analyte under analytical estimation in the current study is diclofenac Sodium, a nonsteroidal phenyl acetic acid cyclooxygenase (COX) inhibitor, chemically represented as 2- (2- (2, 6-dichlorophenylamino)phenyl) acetic acid (1) is popular as a front line drug to reduce joint pains observed in arthritis or other acute injuries. The second analyte under examination is 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 (2) is used as an anti anginal drug to treat angina pectoris. The chemical structures for both analytes quantified in this paper are depicted in figure 1a and 1b.

To date, scientific literature found in various databases were reported the use of well known analytical techniques viz., high-performance liquid chromatography, capillary gas chromatography, gas chromatography, micellar electro kinetic chromatography, capillary gas chromatography for quantification of nifedipine (NF)(3) and diclofenac either individually and/or in combination with

other drugs (4). Most of these published works were primarily revealed the tedious procedures used thereof with the lack of selectivity and specificity. Moreover there have been no published reports about the simultaneous quantification of diclofenac Sodium (5,6) and nifidipine by HPLC. With this background, the present work aims to focuses on the development of validated, novel, reliable and a precise reverse phase HPLC method for the simultaneous estimation of proposed analytes.

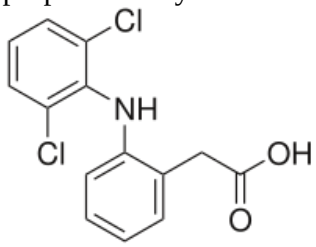

Fig A: Diclofenac

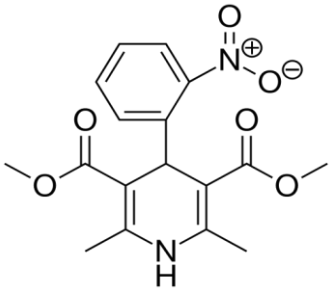

Fig B: Nifedefine

METHOD DEVELOPMENT

Reagents and chemicals

Diclofenac and nifedipine were procured as gift samples from Lee pharma, Visakhapatnam, India. Acetonitrile was procured from Molychem, India. Potassium dihydrogen phosphate, water (HPLC grade), methanol (HPLC grade) and Orthophosphoric acid were procured from Finer chemical Ltd and Lichrosol, India respectively. All other chemicals and reagents used in the process were of HPLC grade and are purchased from Merck Chemicals, India.

Instrumentation and Chromatographic conditions

Quantification of diclofenac and nifedipine was performed using RP- HPLC system of Waters, equipped with 2487 separation module employing UV detector with 10 µl injection volume. Phenominex C_{18} column of 4.6 x 250mm dimensions with 5µm i.d was used for the separation process. Mobile phase was prepared by mixing pH 4.5 phosphate buffers and acetonitrile of HPLC grade in 40:60 v/v ratios, filtered through 0.45 µm membrane filter, processed and detected at a flow rate of 1.0 ml/ min at 265nm wavelength as maximum absorbance respectively. The complete analysis was conceded at ambient temperature.

Preparation of mobile phase (Blank/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 4.5. This results in the formation of 0.1% OPA buffer.

Preparation of Mobile phase

400 ml of 0.1% OPA buffer solution was mixed with 600 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 40: 60v/v mobile phase of pH 4.5 phosphate buffer and acetonitrile respectively.

Preparation of standard stock

10mg and 12mg of accurately weighed quantities of diclofenac 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 120µg/ml concentrations of diclofenac and nifedipine respectively (Stock A). Later, 0.75 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 required concentrations of DF and NF respectively.

Preparation of Sample solution

Pellet formulations were accurately weighed and powdered in mortar. An equivalent weights of diclofenac (10 mg) and nifedioine (12 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.

PERCENTAGE ASSAY OF ANALYTES

Assay of prepared sample and standard solutions was performed using optimized chromatographic conditions viz., 40:60 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 DF and NF for standard and sample solutions was noted separately (n=3).

VALIDATION OF DEVELOPED METHOD

The simultaneous estimation technique established in the current study for two analytes viz., diclofenac and nifedipine was confirmed through validation procedures authenticated by ICH guidelines. The parameters tested in validation of developed method were linearity, specificity, accuracy, precision, intermediate precision (ruggedness), detection limits (LOD & LOQ) and stability studies through degradation conditions. The linearity of the analytes was measured at five concentrations with the combination of two drugs. Specificity was determined by analyzing blank with the sample and standard solutions, to check for the interference of excipients used in the formulation. Accuracy of the sample and standard solutions was estimated at low (50%), medium (100%) and high (150 %) concentrations of analytes and percentage mean recovery of analytes was calculated. While the precision was determined at five levels of analyte concentrations and %RSD was calculated. The interday precision (Ruggedness) was analyzed at 6, 12 and 24 hrs in a day for % RSD. The detection limits viz., LOD and LOQ for both the analytes was checked by calculating S/N ratio. The results for all the parameters were compared with the standard limits or acceptance criteria given by ICH guidelines. Stability of the sample solution containing both the drugs was tested at different conditions such as hydrolytic degradation in acidic media, alkaline media, ambient temperatures etc and checked for the degradation of drugs in the sample.

RESULTS AND DISCUSSION

The current study developed an isocratic RP-HPLC technique for the quantification of diclofenac and nifedipine as a combinatorial formulation to treat arthritis and angina pectoris. The method was optimized through validation protocol to confirm the better separation and resolution process by adopting various types and compositions of mobile phases, flow rates, columns etc. At the end of vast trials, better peak results were obtained with 0.1 % orthophosphoric acid buffer adjusted to pH 4.5 and acetonitrile in 40:60 v/v ratio as mobile phase and Phenominex C_{18} column of 4.6 x 250mm, 5µm i.d dimensions . Both the analytes had shown reliable and repeatable peak responses at absorption wavelength of 265nm using UV detector with the flow rate of 1.0 ml/min. The retention times of the analytes was observed at 6.4 and 2.9 for diclofenac and nifedipine respectively.

Discussion on validation parameters for method optimization

The current study developed a method for effective and reliable separation and resolution of diclofenac and nifedipine with in short elution time with simple and economical mobile phase composition. The appropriateness and reproducibility of the method was confirmed through the testing of system suitability parameters. Standard and sample solutions of analytes were tested for system suitability parameters viz., tailing factor, resolution and theoretical plate count as per ICH guidelines to confirm the reliability of developed method.

The standard and sample solutions were injected into system for six consecutive times to confirm the repeatability of results and the results were within the acceptance criteria. The linearity of analytes in standard and sample solutions were found to be linear in the concentration range of 20-120 µg/ml

for diclofenac and 25-150 µg/ml for nifedipine. The linearity of analytes was checked by correlation coefficient value and was found to be 0.999 and 0.998 for diclofenac and nifedipine respectively. The specificity of the proposed method had reported no significant interference of excipients used in the formulation with blank and analytes.

The % recovery of analytes estimated to know the accuracy of developed method was found to be 100.13 and 100.53 % for diclofenac and nifedipine respectively and the results were within the standard limits.

Table 01: System suitability parameters

Parameters	Diclofenac	Nifedipine	Standard Limits
Resolution factor	5.86	6.06	Not less than 2.0
Theoretical plate count	3415.94	2940.49	Not less than 2000
Tailing factor	1.84	1.87	Not more than 2.0
Assay (%)	100.17	100.24	98-102%

Table 02: Validation parameters

S.No.	Parameter	Validation method	Diclofenac	Nifedipine	Standard Limits
1	**Correlation coefficient**	Linearity	0.999	0.999	0.99
2	**Interference is checked**	Specificity	Not observed	Not observed	No interference
3	**Mean % Recovery at 50%, 100% and 150%**	Accuracy	100.13	100.53	98-102
4	**% RSD**	Precision	0.8	0.8	Not be more than 2.0
5	**% RSD (Interday)**	Ruggedness (Intermediate precision)	0.6	0.7	Not be more than 2.0
6	**S/N ratio**	LOD (ng/ml)	3.02	3.0	3.0
7	**S/N ratio**	LOQ (ng/ml)	10.0	9.98	10.0
8	**Peak purity**	Stability	Passed	Passed	No degradation

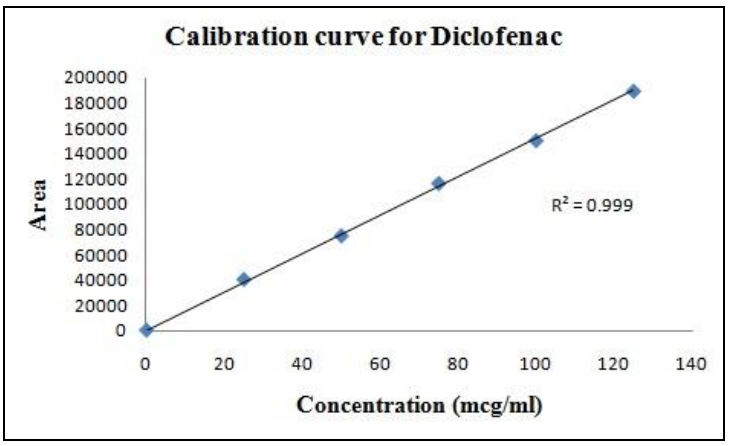

Figure 1: Calibration curve for Diclofenac

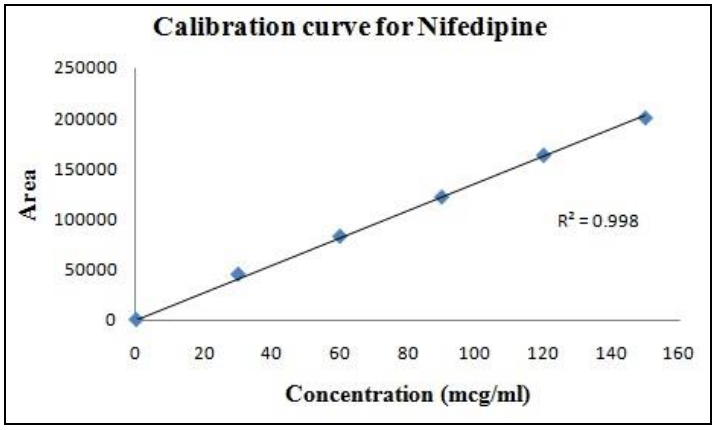

Figure 2: Calibration curve for Nifedipine

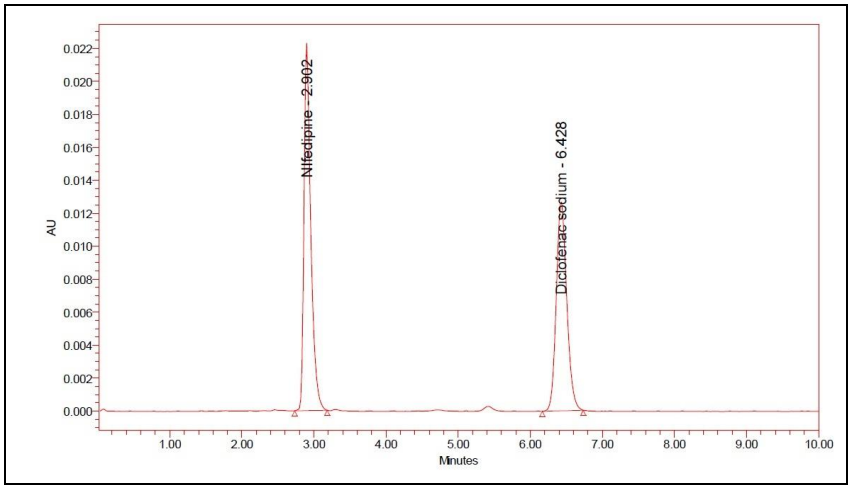

Figure 3: Chromatogram for simultaneous estimation of sample for analytes

The reproducibility of accuracy results was confirmed through precision studies by calculating % RSD and was reported to be 0.8 for both the drugs while the ruggedness was observed to be 0.6 and 0.7 for diclofenac and nifedipine respectively. S/N ratio values for both the analytes to determine LOD was 3.02 and 3.0 ng/ml and LOQ was 10.0 and 9.98 for diclofenac and nifedipine respectively.

The validation protocol and testing procedures adopted in this study was according to ICH guidelines and the results were observed to be within the acceptable standard limits. Therefore, the projected method in the current paper was identified to be reliable. Stability of analytes in sample had presented peak purity which reflects that there was no significant degradation of analytes even after exposure to variable chemical and thermal conditions.

CONCLUSION:

The isocratic RP-HPLC technique, developed and validated in this paper was confirmed to be an easy, quick, steadfast and precise method. Thus, it is suitable for the simultaneous assessment of diclofenac and nifedipine. High resolution, good theoretical plate count, low tailing factor and high percentage recovery and detection values suggested the method to be a dependable technique for the analytes used in the formulation.

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