



Development and validation of new analytical method for the simultaneous estimation of ibuprofen and diphenhydramine in bulk and pharmaceutical dosage form by RP-HPLC

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ABSTRACT

A simple, accurate, rapid and precise method was developed for the simultaneous estimation of Ibuprofen and Diphenhydramine in Pharmaceutical dosage form. Chromatogram was run through Inertsil ODS (250x4.6mm) 5 μ . Mobile phase used was Acetonitrile and Phosphate buffer (45:55) at a flow rate of 1.0 ml/min and detection wavelength was found to be 260 nm. The retention time was found to be 2.32 min and 2.93 min for Ibuprofen and Diphenhydramine respectively. The accuracy and reliability of the method was assessed by evaluation of linearity, precision (intra-day and inter-day % RSD >2), accuracy (98-102%), specificity, LOD, LOQ values in accordance with ICH guidelines. The developed method is applicable for routine quality control analysis of selected combined dosage forms.

Keywords: Ibuprofen; Diphenhydramine; RP-HPLC.

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INTRODUCTION

Development of simple and reproducible analytical methods for estimation of multicomponent drugs is very important part of quality control and for social awareness which is established in present work.^[1]

Ibuprofen^[3] is a prototypical Non-Steroidal anti-inflammatory agent with analgesic and antipyretic properties. Ibuprofen is a non-selective inhibitor of cyclooxygenase, an enzyme involved in prostaglandin synthesis via the arachidonic acid pathway^[2]. Its

pharmacological effects are believed to be due to inhibition cyclooxygenase-2 which decreases the synthesis of prostaglandins involved in mediating inflammation, pain, fever and swelling. Antipyretic effects may be due to action on the hypothalamus, resulting in an increased peripheral blood flow, vasodilation, and subsequent heat dissipation.

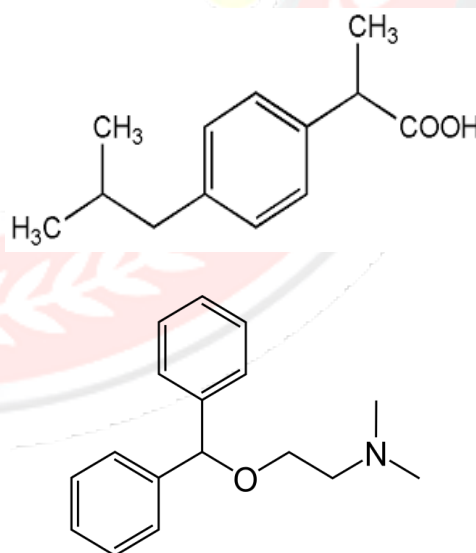


Figure 1: Ibuprofen and diphenhydramine

Diphenhydramine^[4] is a histamine H1 antagonist used as an antiemetic, antitussive, for dermatomes and pruritus, for hypersensitivity reactions, as a hypnotic, an antiparkinson, and as an ingredient in

common cold preparations^[2]. It has some undesired anti muscarinic and sedative effects.

MATERIALS AND METHODS

Chemical and Reagents

Ibuprofen and Diphenhydramine were kindly gifted by Nutech Biosciences Pvt Ltd, Hyderabad certified to contain 99.9% and 99.7% purity respectively. The drugs were used without further purification. All the solvents used in analysis were of HPLC grade.

HPLC method

Instrument

LC system used consists of Waters HPLC having Empower Software with 2690 separation module having PDA detector with universal loop injector of injection capacity 20 μ L. The column used was Inertsil ODS 5 μ (250x4.6mm) at ambient temperature. Different mobile phases were tested in order to find the best conditions, for separating both the drugs simultaneously.

Optimized Chromatographic conditions

The mobile phase having Acetonitrile and Phosphate buffer (45: 55) v/v was selected because it was found that it ideally resolve the peaks. The retention time was found to be 2.32 min and 2.93 min for Ibuprofen and Diphenhydramine respectively. Wavelength was selected by scanning all standard drugs over a wide range of wavelength 200nm to 350nm. Both the components showed reasonably good response at 260 nm.

Preparation of Phosphate Buffer

Accurately weigh 1.732g of Potassium Dihydrogen Ortho phosphate was taken in a 500ml volumetric flask, dissolved and diluted to 500ml with HPLC water and the volume was adjusted to pH 3.0 with Ortho Phosphoric Acid.

Preparation of Mobile Phase

Accurately measured 550 ml (55%) of above buffer and 450 ml of Acetonitrile (HPLC Grade) (45%) were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 μ filter under vacuum filtration.

Diluent Preparation: The Mobile phase was used as the diluent.

Standard Solution Preparation

Accurately weighed 20mg of Ibuprofen, 3.8 mg of Diphenhydramine and transferred to 10 ml individual volumetric flasks and 3/4th of diluents was added to these flasks and sonicated for 10 minutes. Flasks were

made up with diluents and labelled as Standard stock solution. (2000 μ g/ml of Ibuprofen and 380 μ g/ml of Diphenhydramine)

Sample Solution Preparation

5 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 100ml volumetric flask, 50ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (2000 μ g/ml of Ibuprofen and 380 μ g/ml of Diphenhydramine)

Recovery studies

To check the accuracy of sample by the developed methods and to study the interference of formulation additives, analytical recovery experiments were carried out by standard addition method at 50, 100 and 150% level. From the total amount of drug found, the percentage recovery was calculated.

RESULTS AND DISCUSSION

HPLC Method Validation

As per the ICH guidelines, the method validation parameters checked were linearity, accuracy, Specificity, precision, limit of detection, limit of quantitation

Specificity

The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The specificity was performed by Injecting blank.

Table 1: Precision Results for Ibuprofen and Diphenhydramine

Injection	Ibuprofen Area	Diphenhydramine Area
Injection-1	1119086	381723
Injection-2	1101903	381976
Injection-3	1107719	382641
Injection-4	1106714	381996
Injection-5	1105937	385760
Injection-6	1105131	384071
Average	1107748	383028
Standard Deviation	5997.7	1587.0
%RSD	0.6	0.5

Linearity

The linearity of the proposed HPLC method for determination of Ibuprofen and Diphenhydramine was evaluated by analyzing a series of different concen-

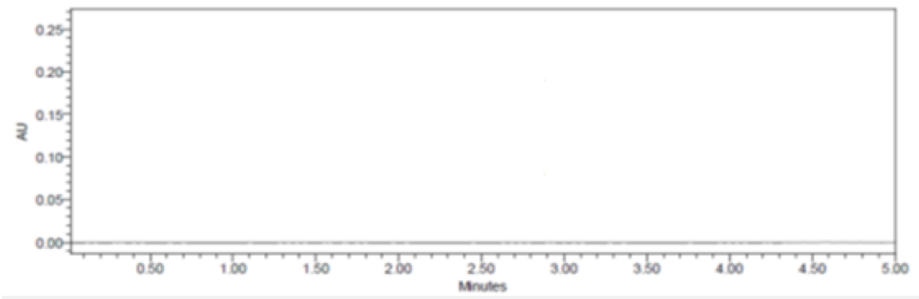


Figure 2: Blank Chromatogram

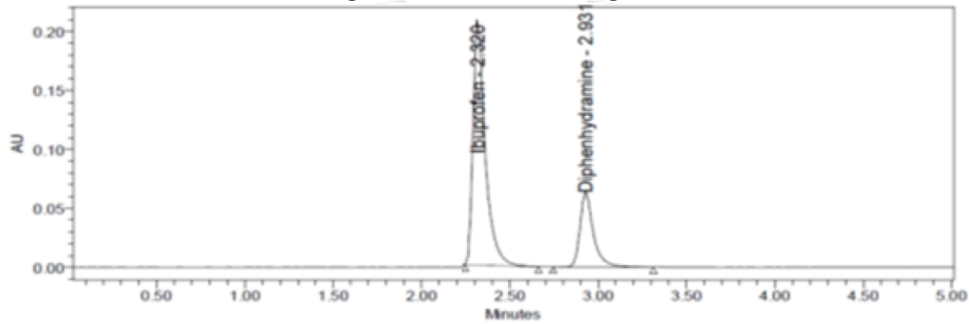


Figure 3: Chromatogram of Standard solution 1. Ibu (Rt 2.32), 2.Ten (Rt 2.931)

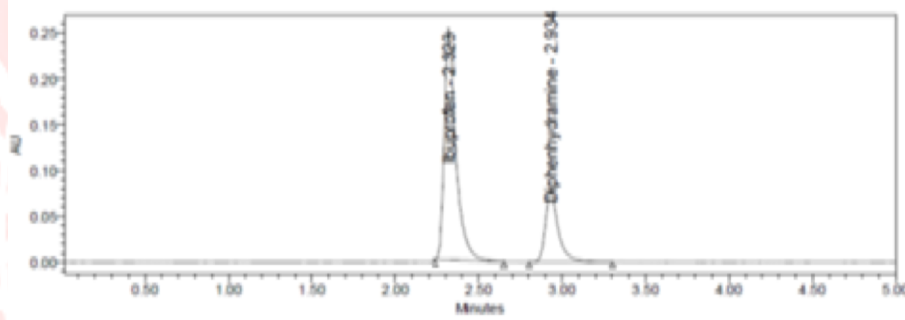


Figure 4: Chromatogram of Sample solution 1. Ibu (Rt 2.323), 2.Ten (Rt 2.934)

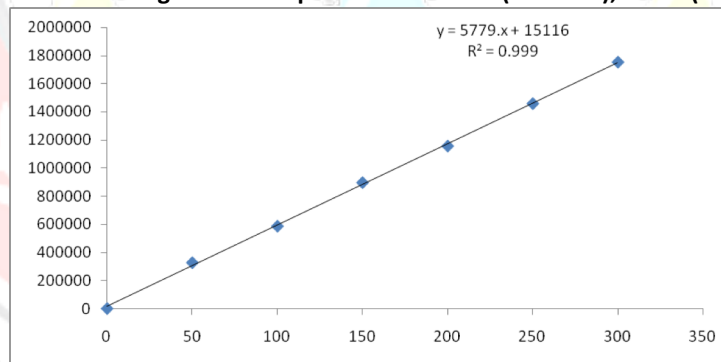


Figure 5: Calibration graph of Ibuprofen

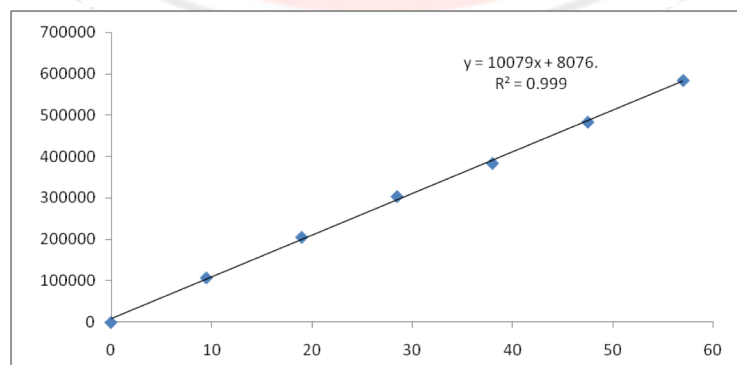


Figure 6: Calibration graph of Ibuprofen

trations of standard drug. In this study five concentrations were chosen, ranging between 50-300 µg/ml for Ibuprofen and 9-55 µg/ml for Diphenhydramine. Each concentration was repeated three times. The linearity of the calibration graphs was validated by the high value of correlation coefficient, slope and the intercept value

The equations of the regression lines obtained are

For Ibuprofen: $R^2 = 0.999$; For Diphenhydramine: $R^2 = 0.999$

Acceptance criteria: Correlation coefficient should be not less than 0.999.

Precision

Precision of the analytical method was studied by analysis of multiple sampling of homogeneous sample. Precision was demonstrated by repeatability and intermediate precision measurements of peak area and peak symmetry parameters of HPLC method for each title ingredients. The repeatability (within-day in triplicates) and intermediate precision (for 2 days) were carried out at five concentration levels for each compound. Triplicate injections were made and the obtained results within and between the days of trials were in acceptable range. The value of %RSD for Ibuprofen and Diphenhydramine were found to be less than 2 indicates that the developed method is precise.

Acceptance criteria: The % RSD for the area of six sample injections results should not be more than 2.

Accuracy

Accuracy of an analytical method is the closeness of test results obtained by that method to the true value. The accuracy of an analytical method should be established across its linearity range. Accuracy was performed in three different levels, each level in triplicate for Ibuprofen and Diphenhydramine using standards at 50%, 100% and 150%. Each sample was analysed in triplicate for each level. The mean recoveries were found in the range of 98 – 102% by which we can say the method was accurate.

Recovery studies

To check the accuracy of sample by the developed methods and to study the interference of formulation additives, analytical recovery experiments were carried out by standard addition method at 50, 100 and 150% level. From the total amount of drug found, the percentage recovery was calculated. The results are reported below

Acceptance criteria: The mean % recovery of Ibuprofen and Diphenhydramine at each level should be not less than 98.0% and not more than 102.0%.

Table 2: Analysis data of formulation

Parameter	Ibuprofen	Diphenhydramine
Label claim (mg)	200	38
Drug found	200.01	37.96
% Accuracy	100.03%	99.99%

Limit of detection (LOD)

It is calculated according to ICH recommendations where the approach is based on the signal-to-noise ratio. Chromatogram signals obtained with known low concentrations of analytes were compared with the signals of blank samples. A signal-to-noise ratio 3:1 was considered for calculating LOD respectively.

Table 3: LOD Results for Ibuprofen and Diphenhydramine

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Ibuprofen	78	189	3.02
Diphenhydramine	78	188	3.00

Limit of quantitation (LOQ)

It is calculated according to ICH recommendations where the approach is based on the signal-to-noise ratio. Chromatogram signals obtained with known low concentrations of analytes were compared with the signals of blank samples. A signal-to-noise ratio 10:1 was considered for calculating LOQ respectively.

Table 4: LOQ Results for Ibuprofen and Diphenhydramine

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Ibuprofen	78	650	10.00
Diphenhydramine	78	649	9.98

SUMMARY AND CONCLUSION

A new method was established for the simultaneous Estimation of Ibuprofen and Diphenhydramine by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Ibuprofen and Diphenhydramine by using Inertsil ODS (250x4.6mm) 5µ. Mobile phase used was Acetonitrile and Phosphate buffer (45: 55) at a flow rate of 1.0 ml/min and detection wavelength was found to be 260 nm. Precision and recovery studies were also found to be with the range. The proposed HPLC method was found to be simple, specific, precise, accurate, rapid and economical for estimation of Ibuprofen and Diphenhydramine in Pharmaceutical dosage form. The developed method was validated in terms of accuracy, precision, linearity, robustness and ruggedness, and results will be validated statisti-

cally according to ICH guidelines. The Sample recoveries in all formulations were in good agreement with their respective label claims. Hence the suggested RP-HPLC method can be used for routine analysis of Ibuprofen and Diphenhydramine in API and Pharmaceutical dosage form.

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