World Inventia Publishers

Journal of Pharma Research

http://www.jprinfo.com/

Vol. 8, Issue 4, 2019 ISSN: 2319-5622 USA CODEN: JPROK3

Research Article

STABILITY INDICATING HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS DETERMINATION OF IBUPROFEN AND HYDROCODONE BITARTRATE IN THEIR COMBINED DOSAGE FORM

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Received on: 11-03-2019; Revised and Accepted on: 19-04-2019

ABSTRACT

A *simple, rapid, economical, precise and accurate HPLC method for simultaneous estimation of Hydrocodone Bitartrate and Ibuprofen in their combined dosage form has been developed. A reverse phase high performance liquid chromatographic method was developed for the simultaneous estimation of Hydrocodone Bitartrate and Ibuprofen In Their Combined Dosage Form has been developed. The separation was achieved by LC- 20 AT C18 (250mm x 4.6 mm x 2.6 µm) column and Buffer (Potassium Phosphate, pH 4.5) : Methanol (50:50) at a flow rate of 1 ml/min. Detection was carried out at 223 nm. Retention time 3.303 min and 5.740 for Ibuprofen and Hydrocodone Bitartrate respectively. The method has been validated for linearity, accuracy and precision. Linearity observed for Hydrocodone Bitartrate 1.5-3.75 µg/ml and for Ibuprofen 40-120 µg/ml. The percentage recoveries obtained for Hydrocodone Bitartrate and Ibuprofen were found to be in range of 99.478 ± 1.196 and 99.917 ± 0.897 respectively. Developed method was found to be accurate, precise and rapid for simultaneous estimation of Hydrocodone Bitartrate and Ibuprofen In Their Combined Dosage Form The proposed method was successfully applied for the simultaneous estimation of both the drugs in commercial Combined dosage form.*

KEYWORDS: *Hydrocodone Bitartrate, Ibuprofen, Simultaneous Estimation, HPLC Method, Validation.*

INTRODUCTION

Hydrocodone, sold under brand names such as Vicodin and Norco among many others, is a semisynthetic opioid derived from codeine one of the opioid alkaloids found in the opium poppy.

It is a narcotic analgesic used orally for relief of moderate to severe pain, but also commonly taken in liquid form as an antitussive/cough suppressant.

Ibuprofen is a medication in the nonsteroidal anti-inflammatory drug (NSAID) class that is used for treating pain, fever and inflammation. This includes painful menstrual periods, migraines, and rheumatoid arthritis.It may also be used to close a patent ductus arteriosus in a premature baby. It can be used by mouth or intravenously. It typically begins working within an hour.

Literature survey reveals that few spectrophotometric methods and high performance liquid chromatography (HPLC) methods have been reported for the estimation of Hydrocodone Bitartarate and Ibuprofen. The aim of this study is to develop a simple, precise and accurate reverse phase HPLC (RP-HPLC) method for the estimation of Hydrocodone Bitartarate and Ibuprofen in bulk forms as per ICH guidelines. The validation procedure done according to USP 30 guidelines [3-11].

MATERIALS AND METHODS

Instrumental and analytical conditions:

The HPLC analysis was carried out on Waters HPLC (2695) equipped with photodiode array (PDA) detector (2996) and auto sampler integrated with empower2 software. The column used is inertsil BDS C18 column (250 mm ×4.6 mm, 5 µ particle size) and detection was performed at 223nm. The injection volume of sample was 20 µl and the run time was 8.0 minutes. An isocratic mobile phase consisted of water and methanol in the ratio 40:60v/v was carried out with the flow rate at 1 ml/minute.

Preparation of stock solution:

Standard stock solutions were prepared by taking 80mg of Ibuprofen, and 3mg of Hydrocodone Bitartrate was transferred to a 100 ml volumetric flask, Add 60 ml Mobile phase and Shake for 15 min and make up volume with Mobile phase.

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DOI: https://doi.org/10.5281/zenodo.2647878

Preparation of working standard:

Working standards were prepared by taking 1 mL from standard stock solution and transferred to 10 ml volumetric flask and made up volume up to the mark with the mobile phase Inject above Solution 20 µl for Assay Analysis.

Reagents and Chemicals:

Hydrocodone Bitartarate and Ibuprofen were obtained as gift samples from Abbvie Pharmaceuticals.

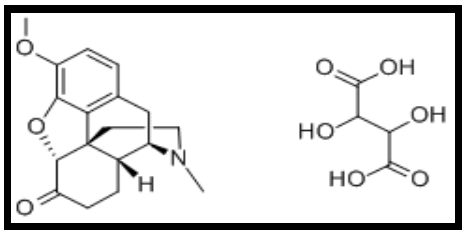

Fig. 1: Structure of Hydrocodone Bitartarate

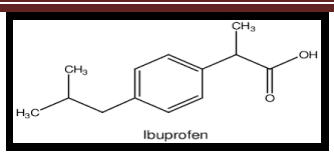

Fig. 2: Structure of Ibuprofen

Methods:

Initially various combination of mobile phases were tried systematically to separate Hydrocodone Bitartarate and Ibuprofen on Hypersil C18 column with a suitable run time. In order to get acceptable peak shapes. Mobile phase composed of water and methanol showed the increased resolution between Hydrocodone Bitartarate and Ibuprofen. Therefore this mobile phase combination was selected as optimized mobile phase in the ratio of 40:60v/v respectively. To improve resolution the stationary phase used was Hypersil BDS C18 column (250 mm ×4.6 mm, 5 µ particle size). Various wavelength detections were carried out to analyze both the drugs but maximum absorption showed at 223nm, so 223nm was selected as detection wavelength for both drugs. The retention times of Hydrocodone Bitartarate and Ibuprofen were found to be 5.74 and respectively. The obtained chromatogram was presented in Fig. 3. The system suitability parameters were given in Table 1.

Table No. 1: System suitability parameters

Parameters	Hydrocodone Bitartarate	Ibuprofen
Retention Time	5.740	3.303
Theoretical Plate count	8112	8048
Asymmetry	1.353	1.238
Resolution	-	12.117

USP: United States of Pharmacopoeia

Table No. 2: Precision results

Injections	Hydrocodone Bitartarate	Ibuprofen
1	1111.843	**3032.991**
2	1114.118	**3039.122**
3	1116.314	**3045.179**
4	1118.600	**3051.236**
5	1112.957	**3036.104**
6	1097.758	**3042.167**
Mean ± S.D (n=6)	1111.932 ±7.354	**3041.133±6.560**
%RSD	**0.661**	**0.216**

SD: Standard Deviation; RSD: Relative Standard Deviation

Method validation:

The purpose of the method validation is to demonstrate that the method is suitable for its intended use as per ICH guidelines. To establish the performance characteristics, the above method was validated according to ICH guidelines to meet the essentials for the intended purpose. The analytical parameters (performance characteristics) were tested by using the optimized conditions.

Specificity:

Hydrocodone Bitartarate and Ibuprofen chromatographic peaks were evaluated by testing the sample solution for the interference of any degradation components or the impurities due to the methodology, where any other peaks were not found in the chromatogram.

Linearity:

The linearity of an analytical procedure is its ability (within a given range) to obtain test results, which are directly proportional to the concentration of analyte in the sample. Six concentrations were prepared by taking serial dilutions of 40, 60, 80, 100 and 120µg/ml from working standard and were analyzed for detector of linearity. The linearity graphs for both the drugs were plotted by taking concentration (µg/ml) on X-axis and peak area (absorbing units) on Y-axis. They were shown in Figs. 4 and 5.

Precision:

Precision is a description of random errors, a measure of statistical variability it refers to the closeness of measurements to each other from multiple sampling of the

same homogenous sample under prescribed conditions. Repeatability was tested by injecting the 6 replicates of 40µg/ml solution on the same day as intra-day precision. The chromatogram was recorded and the results were given in Table 2. Concentrations and the percent recovery were calculated.

Accuracy:

The accuracy is a closeness of measured value to the known value. It was tested by taking triplicates that are 80%, 100% and 120%.

Robustness:

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. It was tested for the flow rate variations, it did not show any effect on method performance. Which indicates that the method was robust. The results were given in Table 4.

Ruggedness:

Different analysts on three different days performed the inter day variations by injecting 6 replicates of test solution. The percent RSD was calculated and the statistical analysis did not show any significant difference between the results acquired by different analysts.

Detection and quantitation limits:

According to the ICH guidelines by using the signal to noise ratio approach The limit of detection (LOD) and limit of quantification (LOQ) were tested. According to this, the LOD and LOQ of Hydrocodone Bitartarate were 0.276µg/ml and 0.837µg/ml respectively. The LOD and LOQ of Ibuprofen were 3.565µg/ml and 10.803µg/ml respectively.

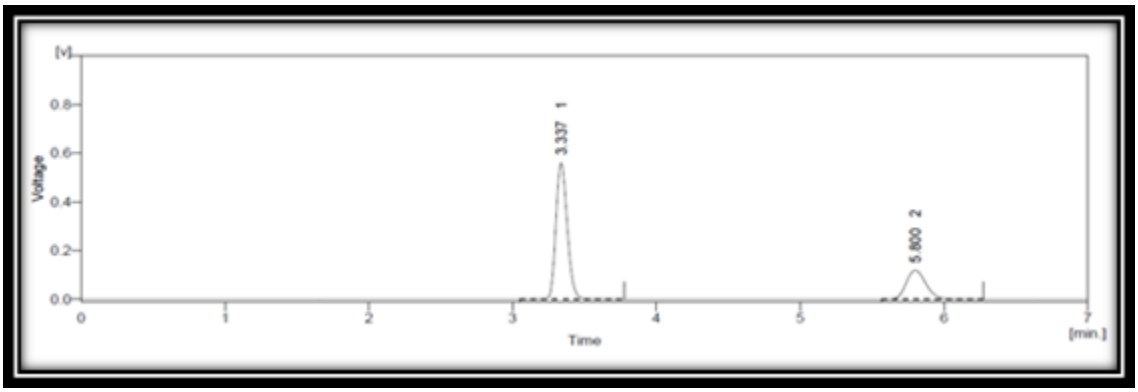

Fig 3: Standard chromatogram of Ibuprofen and Hydrocodone Bitartrate

Table No. 3: Accuracy results for Hydrocodone Bitartarate and Ibuprofen

Analyte	%Concentration	Amount added(µg/ml)	Amount found (µg/ml)	% Recovery	Mean % recovery
Ibuprofen	80	32	32.073	100.230	**99.97**
	100	40	39.966	99.917	
	120	48	47.883	99.757	
Hydrocodone Bitartrate	80	1.2	1.205	100.436	**99.82**
	100	1.5	1.492	99.478	
	120	**1.8**	**1.791**	**99.532**	

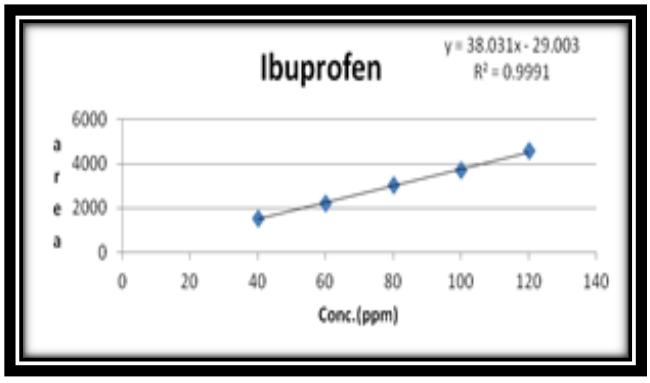

Fig. 4: Linearity graph of Ibuprofen

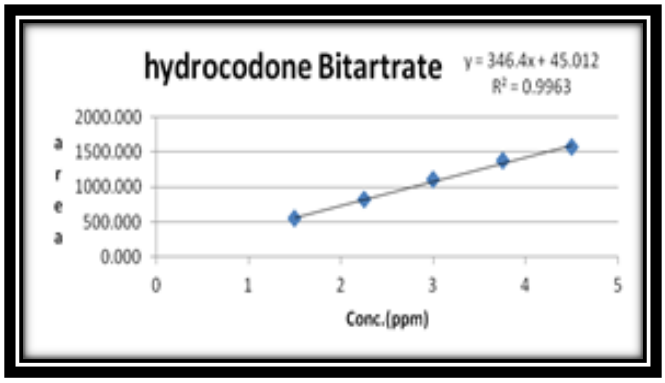

Fig. 5: Linearity graph of Hydrocodone Bitartrate

Table No. 4: Robustness results of Ibuprofen and Hydrocodone Bitartrate

Parameters	Ibuprofen		Hydrocodone Bitartrate	
	Area	%RSD	Area	%RSD
Flow rate -0.2ml/min	3157.78	0.383	1155.46	**0.191**
Flow rate +2.0 ml/min	**2972.20**	**0.635**	**1090.64**	**0.613**

Stability Indicating Method:

Acid degradation:

Accurately pipette out 1 ml of stock solution each 10 ml volumetric flask. Add 1 ml of 0.1 N Hydrochloric acid to each flask. Store flask at 40 ˚C for 4 hours.

At 1, 2, 3 and 4 hour time interval, remove the flask from water bath and cool the content. Add 1 ml of 0.1 N Sodium hydroxide in each flask, Dilute up to volume with mobile phase, mix evenly. Prepare blank preparation simultaneously without sample.

Base degradation:

Accurately pipette out 1 ml of stock solution each 10 ml volumetric flask. Add 1 ml of 0.1 N Sodium hydroxide to each flask. Store flask at 40 ˚C for 4 hours.

At 1, 2, 3 and 4 hour time interval, remove the flask from water bath and cool the content. Add 1 ml of 0.1 N Hydrochloric acid in each flask, Dilute up to volume with mobile phase, mix evenly. Prepare blank preparation simultaneously without sample.

Oxidative degradation

Accurately pipette out 1 ml of stock solution to each 10 ml volumetric flask. Add 1 ml of 3 % Hydrogen peroxide to each flask. Store flask at 40 ˚C for 4 hours.

At 1, 2 and 3 hour time interval remove the flask from water bath and cool the content. Dilute up to volume with mobile phase, mix evenly. Prepare blank preparation simultaneously without sample.

Photo degradation

Drugs for photo stability testing was placed in a photo stability chamber and exposed to direct UV light for 24 hrs. Following removal from the photo stability chamber, the sample was taken at 6hr, 12hrs and 24hrs and prepared for analysis as previously described. Prepare blank preparation simultaneously without sample.

Thermal degradation

Accurately weight tablet powder equivalent to 4mg Hydrocodone Bitartarate and 40mg Ibuprofen and transfer to 100 ml volumetric flask. Exposed under heat at 80 ºC for 4 hours. After 1, 2 and 4hour time period, remove the flask from water bath and cool the content. Add about 5 ml of mobile phase and sonicate to dissolve it completely and make volume up to the mark with methanol. Taken 1ml from this solution and transferred to 10ml volumetric flask and volume was made up with mobile phase.

Calculation for Stability:

Table No. 5: Hydrocodone Bitartrate and Ibuprofen std for stability

Drugs	Area
Ibuprofen	3119.95
Hydrocodone Bitartrate	1145.437

Table No. 6: Hydrocodone Bitartrate % Degradation

Ibuprofen				
Parameter	**Standard**		**Sample**	
	Area	**%Degradation**	**Area**	**%Degradation**
Acid	2016.263	35.375	2046.895	34.393
Base	2082.288	33.259	2011.164	35.539
Thermal	2008.656	35.619	2004.011	35.768
Oxidation	2704.053	13.330	2714.979	12.980
Photo	2747.448	11.939	2712.114	13.072

Table No. 7: Ibuprofen % Degradation

Ibuprofen				
Parameter	**Standard**		**Sample**	
	Area	**%Degradation**	**Area**	**%Degradation**
Acid	941.177	17.832	925.209	19.227
Base	879.883	23.184	858.514	25.049
Thermal	764.220	33.281	787.653	31.236
Oxidation	794.933	30.600	776.791	32.184
Photo	833.647	27.220	811.399	29.162

CONCLUSION

In this present work a new simple, selective, linear, precise, accurate and robust HPLC method was developed and validated for the simultaneous estimation of Hydrocodone Bitartarate and Ibuprofen in bulk form in accordance with the ICH guidelines. This method gives good resolution between both the drugs. Linearity was observed in the concentration range of 40 - 120µg/ml for both the drugs. The wavelength detection at 223nm. The system suitability tests revealed that numbers of theoretical plates were above 2000 and the tailing factor is >2. The percentage recoveries of Hydrocodone Bitartarate and Ibuprofen were 100.75% and 100.96% respectively, which shows the accuracy of the method. Precision values were within the acceptability limit, which indicates that the method is precise. Specificity experiment shows that there is no interference of degradation components with the main peaks, which confirmed the specificity of the method. The lowest values of LOD and LOQ, as obtained by the method, indicate the sensitivity of the method. Thus, this methodcan be useful for the routine analysis of Hydrocodone Bitartarate and Ibuprofen in combined bulk form.

ACKNOWLEDGMENTS

The authors are thankful to Abbvie pharmaceuticals and pharma lab for providing samples and chemicals.

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How to cite this article:

Akshay V, et al. STABILITY INDICATING HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS DETERMINATION OF IBUPROFEN AND HYDROCODONE BITARTRATE IN THEIR COMBINED DOSAGE FORM. J Pharm Res 2019;8(4):186-190. **DOI: https://doi.org/10.5281/zenodo.2647878**

Conflict of interest: The authors have declared that no conflict of interest exists.

Source of support: Nil