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Assay method development and validation of ibuprofen tablets by HPLC

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ABSTRACT

A new simple, accurate, precise and reproducible a reverse phase high performance (RP-HPLC) method has been developed of ibuprofen in tablet dosage forms using C18 column (Hypersil BDS, 150 x 4.6 mm, 5 µm) in isocratic mode. The mobile phase contains a combination of Acetate buffer (triethylamine & ortho phosphoric acid) and acetonitrile in the ratio of 40:60% (v/v). The flow rate was 1.5 ml/min and detection wavelength was carried out at 220 nm. The retention times of ibuprofen was 3.2 min, respectively. The validation of method was carried out utilizing ICH guidelines. The described HPLC method was successfully employed for the analysis of pharmaceutical formulations containing Tablet dosage form.

Keywords: RP-HPLC, ICH guidelines, C18 column, ibuprofen.

INTRODUCTION

Ibuprofen is a nonsteroidal anti-inflammatory drug (NSAID) which is used in reducing inflammation and pain associated with many diseases like rheumatoid arthritis, osteoarthritis etc. It acts by inhibiting the cycloxygenase enzyme and thereby reducing the synthesis of prostaglandins. It is a racemic mixture of [+]S- and [-]R-enantiomers [1,2]. It is a white to off-white crystalline powder, with a melting point of 74° to 77°C. It is practically insoluble in water (<0.1 mg/mL), but readily soluble in organic solvents such as ethanol and acetone. Ibuprofen has a pKa of 4.43±0.03. The chemical name for ibuprofen is (*RS*)-2-(4-(2-methylpropyl) phenyl) propanoic acid [3,4]. The molecular weight of ibuprofen is 206.28. Its molecular formula is $C_{13}H_{18}O_2$ and it has the following structural formula:

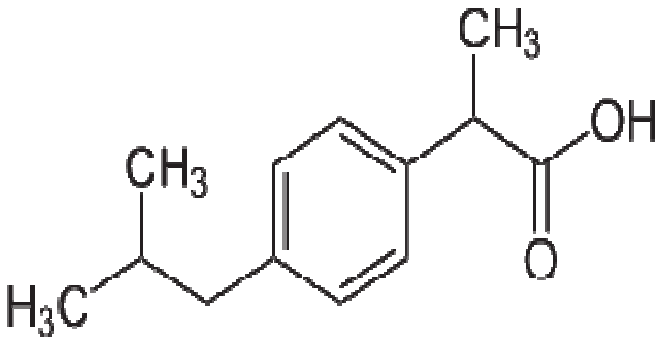


Figure-1: Chemical Structure of Ibuprofen

The main aim of the present work is to develop stability indicating RP-HPLC method for the quantitative estimation of Ibuprofen in pharmaceutical formulations such as tablet dosage forms etc. S (+) Ibuprofen form is not official in any pharmacopoeias but literature survey reveals that there are limited techniques for the estimation of ibuprofen in

tablet form using HPLC method [5-13]. Current research work mainly devoted to develop a simple, rapid, precise, accurate and reproducible isocratic RP-HPLC method for the determination of ibuprofen and the developed method is partialy validated with respect to specificity, linearity, precision, accuracy [14] .

MATERIALS AND METHODS

EXPERIMENTAL

I. Chemicals and Reagents:

Sample of ibuprofen pure drug was received from M/S Medreich Private Limited, Bangalore, India and tablet dosage form was purchased from market manufactured by M/S Genovo Development Services Ltd, Bangalore, India. HPLC grade acetonitrile, ortho-phosphoric acid were purchased from M/S Rankem , Mumbai, India.Triethylamine was purchased from M/S Rankem Mumbai, India.High pure water was prepared by using Millipore, Milli Q plus (TKA) purification system.

II. Instrumentation and Chromatographic Conditions:

The RP-HPLC method development and complete partial validation studies were performed using Alliance 2695 system (Waters, Shimadzu LC), comprising of a quaternary solvent delivery module, online degasser, column thermostat, auto sampler, photo diode array detector. Chromatogram output, integration of peaks and calculation of peak areas, retention times, system suitability parameters such as peak asymmetry column efficiency etc were obtained using the Empower software, version 2.6. An isocratic RP-HPLC method was achieved with Thermo Hypersil BDS, 150X4.6mm; 5µm column using a mobile phase consisting mixture of buffer and acetonitrile in the ratio 40:60. The buffer consists of HPLC grade water: Triethylamine: Orthophosphoric acid (1000ml: 1ml: 0.5ml). The method was carried out with the flow rate of 1.5ml/min and the column eluent was monitored at 220 nm with the injection volume of 10µl. The total Chromatographic runtime was 5 minutes and the column temperature was maintained at 250C.

III. Preparation of Standard Solution:

Weighed accurately about 40 mg of Ibuprofen working standard and transfered into a 100 ml volumetric flask. Added 70 ml of diluent-1 (buffer 20: acetonitrile 80), sonicate to dissolved and made up to volume with diluent-1 and mix well. Pipetted out 5 mL of the standard stock preparation and transfered into a 25 mL volumetric flask. Made of the volume upto the mark with diluent-2 (buffer 40: acetonitrile 60) and mixed well.

IV. Preparation of sample solution:

Weighed accurately not less than 20 tablets and note down the weight. Calculated the average weight. Crush the tablets into fine powder using motar and pestle.Weigh accurately the powdered sample equivalent to 100 mg of Ibuprofen and transfered into a 250ml of volumetric flask. Added 175ml of diluent-1 and sonicated for 30 minutes with intermittent shaking.Made of the volume upto the mark with diluent-1 and mixed well. Centrifuge a portion of the above solution at 3000 rpm for about 5 minutes. Pipette out 5ml of the above supernatant solution and transfered into a 25ml volumetric flask. Made up the volume upto the mark with diluent-2 and mixed well. Injected the clear solution into the HPLC system.

ANALYTICAL METHOD VALIDATION [15-25]:

The current RP-HPLC method was validated as per ICH guideline-Q2.

System suitability [15, 16]*:*

A system suitability test was an integral part of the method development to verify that the system is adequate for the analysis of IBU to be performed. System suitability test of the chromatography system was performed before each validation run. Six replicate injections of a system suitability standard (Ibuprofen) was made. Retention time (RT), tailing factor and theoretical plates for the six suitability injections was determined.

Precision [17, 18]:

The precision for the quantification of Ibuprofen by RP-HPLC was verified by repeatability (system precision & method precision).

System precision [19, 20]:

The system precision was performed by injecting six replicate injections of the standard solution in to the chromatography .The % RSD for the peak of interest was calculated and found less than 1% (should be less than 2%).

Method precision [21]:
The method precision was performed by preparing six replicate sample preparations as per testing procedure and injected in to the chromatography.The percentage w/w of ibuprofen was calculated from six replicates and %RSD.

Specificity [22,23]:
The specificity of the method was determined by analysing the interference of placebo was conducted. It was performed on Ibuprofen placebo in duplicate, equivalent to the weight of placebo present in portion of test preparation as per the test method and the impurity interference of Ibuprofen impurities by preparing individual impurity solution and mixed impurity solution at Specification level (0.2% for Ibuprofen impurities). Impurity spiked test solution of Ibuprofen tablet at 1% level of all impurities was injected into the HPLC. No interference was detected at the retention time of Ibuprofen in sample solution.

Linearity [23,24]:
Linearity was studied to determined by plotting a graph between concentration on X-axis and peak area on Y-axis, the correlation coefficient was determined. Five different concentrations of Ibuprofen working standard ranging from 4.06 µg/ml to 12.181 µg/ml were prepared and analyzed as per test method. Calibration curve was constructed by plotting average peak area versus concentrations and regression equation was computed for the drug.

Accuracy (Recovery) [25]:
The accuracy of an analytical method is the closeness of results obtained by that method to the true value for the sample. It is expressed as recovery (%), which is determined by the standard addition method. Samples were spiked with 50,100 and 150% of the standard and analyzed. The experiment was performed in triplicate. Recovery (%) and RSD (%) were calculated for each concentration.

RESULTS AND DISCUSSION

The current RP-HPLC technique is most accurate, reliable and precise method of analysis for the quantitative estimation of ibuprofen from its pharmaceutical formulations and dosage form. The specificity of the method was initially performed by injecting impurities and placebo in to the chromatography to check the contribution at the detection wavelength. The current method system suitability was accounted by measuring USP tailing factor, USP Plate count for the peak of Ibuprofen from Standard Solution and percentage RSD of Area for the peak of Ibuprofen from six replicate injection of Standard Solution. The obtained values are 1.0 as USP tailing factor (should be not more than 2.0), 8190 as USP Plate count (should be not less than 2000) and percentage RSD of area as 0.1(should be not more than 2.0). The typical ibuprofen retention time in the current developed method was about 5 minutes. All variations resulted with the very good percentage RSD of Assay i.e. less than 1.0%.

Figure 2: RP-HPLC chromatogram of blank solution at 220 nm

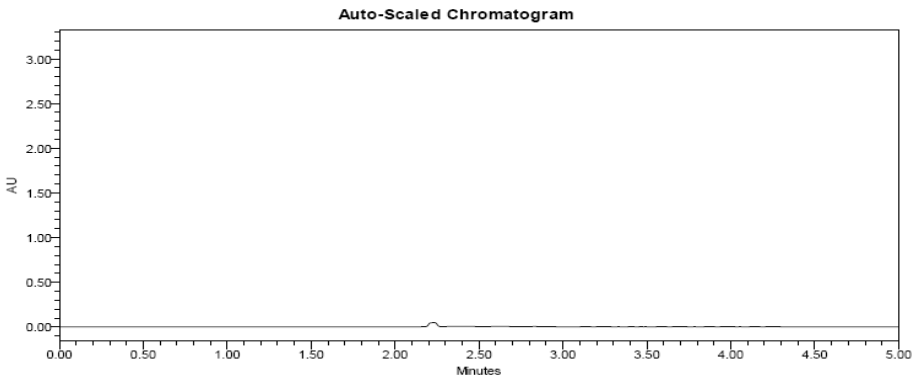


Figure 3: RP-HPLC chromatogram of standard solution at 220 nm

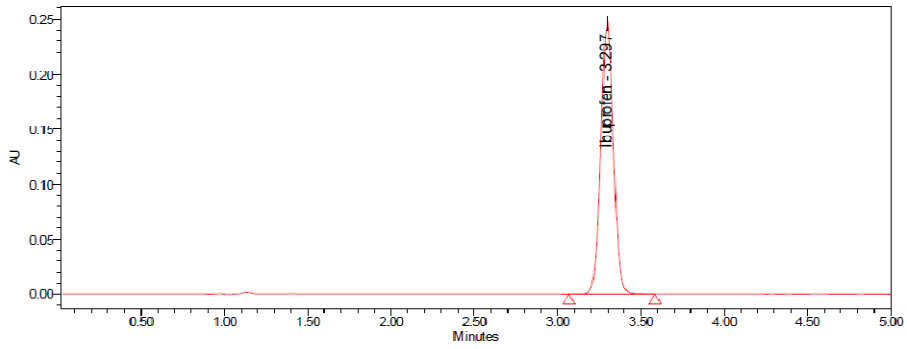


Figure 4: RP-HPLC chromatogram of ibuprofen and impurities at 220 nm

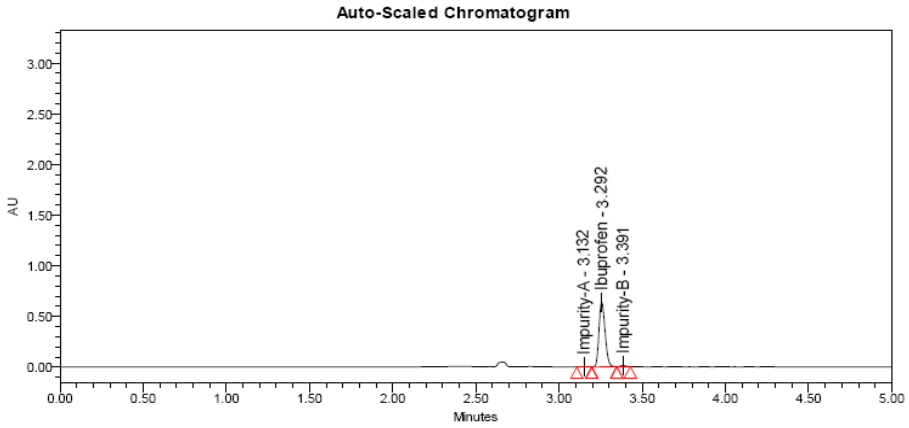


Table 1: System suitability test parameters for Ibuprofen

System Suitability Parameters	Observed value	Acceptance Criteria
Theoretical plate count for Ibuprofen peak from first injection of standard solution.	8190	NLT 2000
The tailing factor for Ibuprofen peak from first injection of standard solution.	1.0	NMT 2.0
The Relative standard deviation for Ibuprofen peak response from five replicate injections of standard solution.	0.1	NMT 2.0

Table 2 : Standard injections responses for System precision test

Injection No.	Response
01	2690227
02	2686108
03	2687578
04	2689836
05	2688685
06	2688824
Mean	2688543
Standard deviation	1514.416
% Relative standard deviation	0.1

Table 3: % Assay and %RSD values of Ibuprofen for Method Precision test

Sample No.	% Assay of Ibuprofen
01	100.0
02	101.3
03	100.4
04	101.0
05	100.9
06	100.4
Mean	100.7
Std Dev	0.4803
% RSD	0.5

Table 4: Placebo Interference in specificity of Ibuprofen

Sample No:	Interference found (Yes/No)
1	NO
2	NO

Table 5: Impurity Interference – Retention times in specificity of Ibuprofen

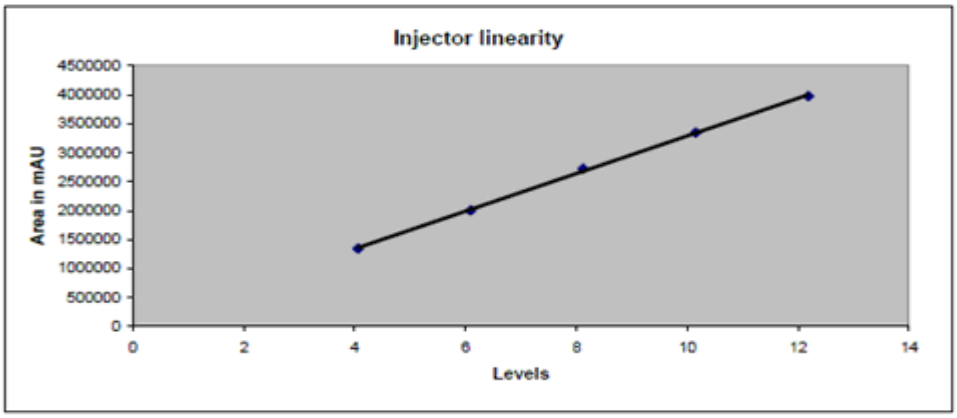
S.NO.	PEAK	Retention Time in Minutes
1	Ibuprofen	3.292
2	Impurity-A	3.132
3	Impurity-B	3.391

Table 6: Concentration of Ibuprofen stock solution

Stock weight taken (mg)	40.70	Volume Taken (ml)	10	Potency (%) (as such basis)	99.76
Volume (ml)	100	Dil. To (ml)	50	Stock (µg/ml)	81.2046

Fig 5: Linearity graph of Ibuprofen

linearity Sample



Slope : 162552.2661
Intercept : 40687.55606
Residual Sum of Squares : 965667915.9019
Corelation Coefficient : 0.99965

Table 7: Response of Ibuprofen at various linearity levels

Level	Stock solution (ml)	Dilution (ml)	Concentration in µg/ml	Response
50%	2.50	50	4.060	1347986
75%	3.75	50	6.090	2005201
100%	5.00	50	8.120	2725814
125%	6.25	50	10.151	3347545
150%	7.50	50	12.181	3976786
			Slope	162552.2661
			Y-Intercept	40687.55606
			Residual Sum of Squares	965667915.9019
			Correlation Coefficient	0.99965

Table 8: % Recovery of Ibuprofen at various spiked levels

Sample No.	Level	'mg' added	'mg' Found	% Recovery	Mean % Recovery	%RSD
1	50%	100	101.989	101.60	101.1	0.7
2	50%	100	101.88	100.26		
3	50%	100	101.933	101.33		
4	100%	100	101.230	101.231	101.5	0.2
5	100%	100	101.570	101.575		
6	100%	100	101.710	101.708		
7	150%	100	101.79	101.04	101.7	0.5
8	150%	100	101.88	101.97		
9	150%	100	101.95	101.96		

CONCLUSION

The main focus of this research article was developed and validated for the quantification of ibuprofen in bulk drug and pharmaceutical formulations such as tablet. The method gave good resolution for the drug with a short analysis time 5 minutes. The developed method was validated by using various validation parameters like accuracy, precision, linearity, specificity. All the validation parameters were found to be well within the acceptance criteria. It is shown that the method was accurate, reproducible, repeatable, linear, precise, and selective, proving the reliability of the method [26,27,28]. These results show the method could find practical application as a quality control tool for analysis of the drug in its Tablet dosage forms in quality control laboratories.

REFERENCES

[1] Budavari S., The Merck Index, 12th Edn., Merck Research Lab., Division of Merck and Co., Inc.,Whitehouse Station , N.J., **1996**
[2] Sweetman and Sean, Martindale, The complete drug reference, 37th Edition, April **2011**.
[3] Lee EJD, Williams K, Day R,Graham G, Champion D. *B. J. Clin. Pharmacol*.; 19, 669-74, **1985**.
[4] Cheng H, Rogers JD, Demetriades JL, Holland SD, Depuy E. Pharmacokinetics and bioinversion of ibuprofen enantiomers in humans. 11:824-30, **1994**.
[5] Muralidharan S, Meyyanathan SN *Jr of Planar Chromatography – Modern TLC*. **2009,** 22,Suppl. 3, 207-10.
[6] Dhartarkar PG, Kalamkar RV, Saoji SD, Ingle G, Atram SC, Madhuri D. *Der Pharma Chemica*, **2011,** 3, 361-66.
[7] Hulst AD, Augustijns P, Arens S et al., *Journal of Chromatographic Science*, **1996,** 34, Suppl. 6, 276-81.
[8] Krzek J, Starek M, and Jelonkiewicz D, *Chromatographia*, **2005,** 62, Suppl. 1112, 653-57.
[9] Sethi PD, Identification of Drugs in Pharmaceutical Formulations by Thin Layer Chromatography, CBS Publisher, New Delhi, India, **1992**.
[10] Thomas CJT and Savage S, *Drug Development and Industrial Pharmacy*, **1985**, 11, Suppl. 5, 1123-31.
[11] Lau YY, *Journal of Liquid Chromatography and Related Technologies*, **1996**, 19,Suppl. 13, 2143-53.
[12] Karnes HT, Rajasekharaiah K, Ralph E, and Farthing D, *Journal of Liquid Chromatography and Related Technologies*, **1988**, 11, Suppl. 2, 489-99.
[13] Mandal U, Das A, Chakraborty SAU, Nandi U, Chattaraj TK, Tapan KP, *Arzneimittel-Forschung*, **2008**; 58, Suppl. 7, 342-47.
[14] International Conference on Harmonization (ICH). Q2 (R1),Text on Validation of Analytical Procedures, Geneva, Switzerland **2005**.
[15] Menddham, J., Denney, R. C., Barnes, J. D., and Thomas, M. J. K., Vogel's Textbook of Quantitative Chemical Analysis, 6th Edn.; India ; Pearson Education (Singapore) P. Ltd., **2003**.
[16] International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, ICH Harmonised Tripartite Guideline – Validation of Analytical Procedures:Text and Methodology Q2(R1), Current Step 4 version, London **2005**.
[17] "ICH guidelines, validation of analytical procedure: Methodology Q2B"; I.C.H. Harmonized Tripartite Guidelines, **1996**.
[18] ICH, Q2A "Text on validation of analytical procedures", Int. Conference of Harmonization, Oct. **1994**.
[19] ICH, Q3B "Text on validation of analytical procedures": methodology, Int. Conference of Harmonization, Nov. **1996**.
[20] Dinc, E., Yucesoy, C and Onu, F, *J. Phar. Biomed. Anal*., **2002**, 15, 1091.
[21] ICH, 3QB Validation of Analytical Procedures: Methodology, International Conference on Harmonization. November **1996**.
[22] ICH, Q1A Stability testing of new drug substances and products, in: Proceedings of the international conference on harmonization, Geneva, Switzerland,October, **1993**.
[23] ICH, Q2B, Harmonised tripartite guideline, Validation of analytical procedure: Methodology, International conference on harmonization, Geneva, Switzerland, March **1996**.
[24] ICH Guidance on analytical method validation, International convention on quality for the pharmaceutical industry, Toronto, Canada, September **2002**.
[25] ICH, Q1B, Stability testing: photostability testing of new drug substances and products. In: International Conference on Harmonization, IFPMA, Geneva,
Switzerland, **1996**.
[26] S. Pattanaik, S. C. Si, S. S. Nayak, *Asian Journal of Pharmaceutical and Clinical Research*, **2012**, 5, 168-171.
[27] Mahendra K. Patil,et.al, *Der Pharmacia Sinica*, **2011**, 2 (2):368-374.
[28] Shalu Rani, Kamal Saroha, Navneet Syan, Pooja Mathur, *Der Pharmacia Sinica*, **2011**, 2(5),17-29.