

RESEARCH ARTICLE

RP-HPLC Method for Simultaneous Estimation of Paracetamol and Ibuprofen in Tablets

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ABSTRACT

A simple, selective, accurate high Performance Liquid Chromatographic (HPLC) method was developed and validated for the analysis of Paracetamol and Ibuprofen. Chromatographic separation achieved isocratically on a C₁₈ column [Use Inertsil C₁₈, 5 μ , 150 mm x 4.6 mm] utilizing a mobile phase of acetonitrile/phosphate buffer (60:40, v/v, pH 7.0) at a flow rate of 0.8 ml/min with UV detection at 260nm. Aceclofenac was used as an internal standard. The retention time of ibuprofen, paracetamol and aceclofenac was 2.48, 4.45 and 6.34 min respectively. The developed method was validated in terms of accuracy, precision, linearity, limit of detection, limit of quantitation. This study aimed at developing and validating an HPLC method, being simple, accurate and selective, and the proposed method can be used for the estimation of these drugs in combined dosage forms

KEY WORDS Paracetamol, Ibuprofen, RP-HPLC, Validation

INTRODUCTION:

Ibuprofen is chemically 2[4-(2-methyl propyl) phenyl] propanoic acid. The structural formula is C₁₃H₁₈O₂, and molecular weight is 206. It is non-steroidal anti inflammatory drug (NSAID). It is used for relief of symptoms of arthritis, primary dysmenorrhea, and fever and as an analgesic. Ibuprofen is known to have an ant platelet (blood-thinning effect). Paracetamol is chemically N-(4-hydroxyphenyl) acetamide. It is a centrally and peripherally acting non-opioid analgesic and antipyretic. Many methods have been described in the literature for the determination of paracetamol with other drugs individually and in combination¹⁻¹¹. However there is no RP-HPLC method reported for the simultaneous estimation of these drugs in combined dosage forms. Fixed dose combination containing paracetamol (400 mg) and ibuprofen (325 mg) is available in the tablet form in the market. The aim of this work was to develop an RP-HPLC method with ultraviolet detection for the simultaneous determination of paracetamol and ibuprofen in pharmaceutical dosage forms. The present RP-HPLC method was validated following ICH guidelines^{12,13}.

EXPERIMENTAL**Materials and Reagents:**

HPLC grade Sodium dihydrogen phosphate (NaH₂PO₄), disodium hydrogen phosphate Na₂HPO₄, acetonitrile- procured from Merck, India. High pure water was prepared by using Millipore Milli Q plus purification system.

Chromatographic Conditions:

A High Performance Liquid Chromatograph system, with LC solutions data handling system (Shimadzu-LC2010) with an auto sampler was used for the analysis. The data was recorded using LC 2010 solutions software. The purity determination performed on a stainless steel column 150 mm long, 4.6 mm internal diameter filled with Octadecyl silane chemically bonded to porous silica particles of 5 μ m diameter (Inertsil C₁₈, 5 μ , 150 mm x 4.6 mm, make: Shimadzu Ltd, Japan) with the mobile phase containing acetonitrile and phosphate buffer in the ratio of 60:40 (v/v pH 7.0) at ambient temperature. Flow rate was kept at 0.8 ml/min and the elution was monitored at 260 nm.

Standard stock solution (1mg/ml) of paracetamol and ibuprofen were prepared by dissolving 25 mg of drug in 25 ml of acetonitrile, separately. The solutions were suitably diluted with mobile phase to get mixed standard solution containing 25 μ g/ml of paracetamol and 20 μ g/ml of ibuprofen and 30 μ g/ml of aceclofenac as internal standard.

Received on 05.02.2009

Modified on 13.02.2009

Accepted on 22.02.2009

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Asian J. Research Chem. 2(1): Jan.-March, 2009; Page 70-72

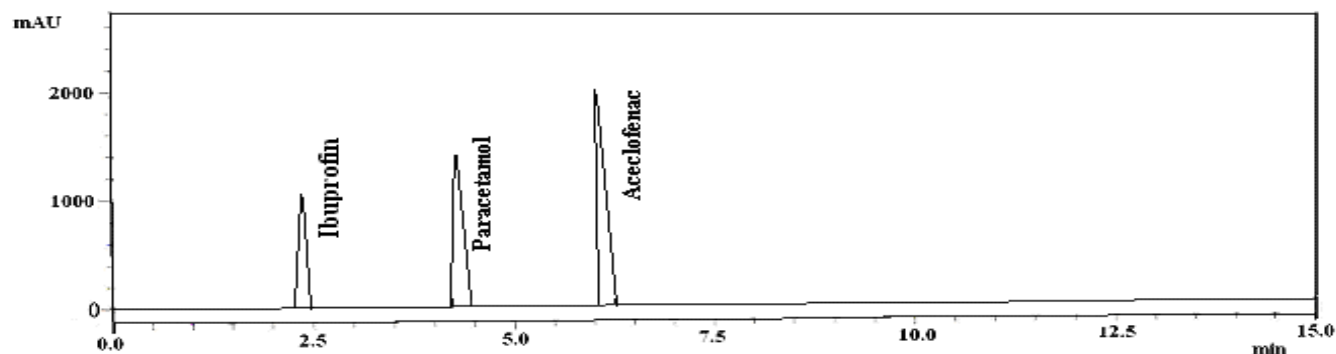


Figure 1: Typical Chromatogram of sample solution with internal standard

Table 1: RESULTS OF ANALYSIS OF FORMULATION AND RECOVERY STUDIES.

Drug	Amount mg/tab		% Label claim	% Recovery
	Labeled	Found		
Paracetamol	400	399.06± 1.045	99.80± 1.020	98.90± 0.815
Ibuprofen	325	324.01± 1.135	96.05± 1.095	96.01± 0.580

*Average of 6 determinations deviation. (Combiflam, Aventis ltd, Mumbai) each tablet containing 400 mg of paracetamol and 325 mg of ibuprofen.

Twenty tablets (Combiflam, Aventis ltd, Mumbai) each containing 325 mg of ibuprofen and 400 mg of paracetamol were weighed, and powder equivalent to 25 mg of paracetamol was weighed accurately and taken into 25 ml volumetric flask. The drugs were extracted into acetonitrile, volume was adjusted to 25 ml, vortexed and then filtered through 0.45 μ membrane filter. From this solution, further dilutions were made using mobile phase to get a final concentration of 25 μ g/ml of paracetamol and 20 μ g/ml of ibuprofen, to this 30 μ g/ml of aceclofenac as internal standard and this solution was used for the estimation. The concentration of the drugs were calculated (Table 1).

With the optimized chromatographic conditions, a steady baseline was recorded. The retention time of ibuprofen, paracetamol and aceclofenac was found to be 2.48, 4.45 and 6.34 min respectively. A typical chromatogram of sample solution is given in figure-1. Detection was done at 260 nm. The assay procedure was repeated for six times and mean peak area ratio and mean weight of standard drugs were calculated. The percentage of individual drugs found in formulation, mean, standard deviation in formulation were calculated and presented in table 1. The results of analysis shows that the amount of drugs was in good agreement with the label claim of the formulation.

The method was validated as per ICH guidelines^{14,15}. The accuracy of the method was determined by recovery experiments. The recovery studies were carried out six times and the percentage recovery were calculated and presented in table 1. From the data obtained, added recoveries of standard drugs were found to be accurate.

The precision of the method was demonstrated by inter day and intra day variation studies. In the intra day studies, six repeated injections of standard and sample solutions were made and the response factor of drug peaks and percentage

RSD were calculated. In the inter day variation studies, six repeated injections of standard and sample solutions were made for three consecutive days and response factor of drug peaks and percentage RSD were calculated. From the data obtained, the developed HPLC method was found to be precise.

Table 2: VALIDATION AND SYSTEM SUITABILITY STUDIES.

Parameters	Paracetamol	Ibuprofen
Linearity range	20 to 80 μ g/ml	10 to 70 μ g/ml
Regression equation $Y = mx + c^+$	$y = 0.0071x - 0.001$	$y = 0.0061x + 0.002$
Correlation coefficient	0.999	0.998
Theoretical plate/meter	26458	28764
Resolution factor	1.30	1.30
Asymmetric factor	0.90	1.01
Tailing factor	1.2	1.0
LOD (ng/ml)	6	10
LOQ (ng/ml)	15	25

* $Y = mx + c$, m : slope, c : y axis intercept

The linearity of the method was determined at seven concentration levels ranging from 20 to 80 μ g/ml for paracetamol and 10 to 70 μ g/ml for ibuprofen. The calibration curve was constructed by plotting response factor against concentration of drugs. The slope and intercept value for calibration curve was $y = 0.0071x - 0.001$ ($R^2 = 0.999$) for paracetamol and $y = 0.0061x + 0.002$ ($R^2 = 0.998$) for ibuprofen. The results show that an excellent correlation exists between response factor and concentration of drugs within the concentration range indicated above.

The Limit of Detection (LOD) and Limit of Quantification (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method. The LOD is the smallest concentration of the analyte that gives a measurable response (signal-to-noise ratio of 3). The LOD for paracetamol and ibuprofen was found to be 6ng/ml and 10ng/ml respectively. The LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified (signal-to-noise ratio of 10). The LOQ was 15 ng/ml and 25ng/ml for paracetamol and ibuprofen respectively (Table-2).

The ruggedness of the method was determined by carrying out the experiment on different instruments like Shimadzu HPLC (LC-2010), Agilent HPLC by different operators using different columns of similar type Intersil C₁₈, Hypersil C₁₈. Robustness of the method was determined by making slight changes in the chromatographic conditions. No marked changes in the chromatograms demonstrated that the HPLC method developed are rugged and robust.

In order to demonstrate the stability of both standard and sample solutions during analysis, both solutions were analyzed over a period of 5 hours at room temperature. The results show that for both solutions, the retention time and peak area of paracetamol and ibuprofen remained almost unchanged(%RSD <2) and no significant degradation within the indicated period, thus indicated that both solutions were stable for at least 5 hours, which was sufficient to complete the whole analytical process.

The system suitability studies were carried out to determine theoretical plate/meter, resolution factor, asymmetric factor and tailing factor. The results were given in the Table 2. The values obtained demonstrated the suitability of the system for the analysis of this drug combinations, system suitability parameters may fall within $\pm 3\%$ standard deviation range during routine performance of the method.

Thus the proposed RP-HPLC method for the simultaneous estimation of paracetamol and ibuprofen in combined dosage forms is accurate, precise, linear, rugged, robust, simple and rapid. Hence the present RP-HPLC method is suitable for the quality control of the raw materials, formulations and dissolution studies.

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