Research Article

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF PARACETAMOL AND IBUPROFEN IN FIXED DOSE COMBINATIONS

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ABSTRACT

In this study, high performance liquid chromatographic method have been developed and validated for the simultaneous determination of Paracetamol and Ibuprofen in combined pharmaceutical formulations. The chromatography was carried out on a C_{18} (250 mm x 4.6 mm, 10 µm) column with methanol and 0.05 M sodium dihydrogen phosphate (65:35 v/v) as mobile phase, at a flow rate of 1.0 ml/min, with detection at 230 nm. Separation was complete in less than 8 min. The calibration curves were linear in the concentration range of 50.00-400.0 µg/ml for paracetamol and 20.00-160.0 µg/ml for ibuprofen. The % recovery for paracetamol and ibuprofen is in the range between 99.53 and 99.83 with RSD values not greater than 0.62. The results of the studies showed that the proposed RP-HPLC method is simple, rapid, precise and accurate, which can be applied for the routine assessment of paracetamol and ibuprofen in pharmaceutical dosage forms.

Keywords: Paracetamol, ibuprofen, simultaneous determination, RP-HPLC, validation.

INTRODUCTION

The 2-arylpropionic acid derivative, ibuprofen [RS-2-(4-isobutyl-phenyl)propionic acid], is one of the most potent orally active antipyretic, analgesic and non-steroidal anti-inflammatory drug (NSAID) used extensively in the treatment of acute and chronic pain, osteoarthritis, rheumatoid arthritis and related conditions. The compound is characterized by a better tolerability compared with other NSAIDS[1]. Paracetamol (acetaminophen) is a widely used analgesic and antipyretic drug. It is well tolerated and lacks many of the side effects of aspirin, so it is commonly used for the relief of fever, headaches, and minor aches and pains as well as for the management of more severe pain, where it allows lower dosages of additional nonsteroidal anti-inflammatory drugs to be used, thereby minimizing overall side effects[2]. Taken together these differing modes of action and related therapeutic effects suggest that ibuprofen and paracetamol may complement each other and improved analgesia may be obtained using a combination, compared with individual administration. A fixed-dose combination is used with a view to simplifying prescribing, improve patient compliance, improve analgesic efficacy without possible increase in adverse effects or decrease adverse effects without loss of efficacy[3].

Many analytical methods were described for simultaneous determination of paracetamol and ibuprofen in pharmaceutical formulations, including: UV-spectrophotometry[4-9], high performance liquid chromatography[10-13], high performance thin layer chromatography[13-14] and gas chromatography[13].

The aim of the present study was to develop and validate a HPLC method for the simultaneous determination of paracetamol and ibuprofen in tablet dosage forms contained 500 mg paracetamol and 200 mg ibuprofen. The method described complied with validation requirements of ICH and could be used for routine quality control of pharmaceutical formulations in ordinary laboratories.

MATERIALS AND METHODS

Chemicals and reagents

Nuromol tablets, each containing 500 mg paracetamol and 200 mg ibuprofen, were supplied commercially. Paracetamol RS and ibuprofen RS were used as standards. LC-grade methanol was supplied from Merck (Germany). All other chemical reagents were of analytical grade.

Instrumentation and chromatographic conditions

Chromatographic separation was performed on modular HPLC system LC-10A Shimadzu (Japan) arranged with a LC-10A pump, solvent degasser DGU-3A, Rheodyne injector, column oven CTO-10A, SPD-M10A fixed wavelength detector and communication bus module CBM-10A. A LiChrosorb C18, 250 mm x 4.6 mm, 10 µm column was used as a stationary phase. The components were separated isocratically with a mobile phase consisting of 65 volumes methanol and 35 volumes 0.05 M sodium dihydrogen phosphate, adjusted to pH 7.00 with 10 M NaOH at a flow rate of 1.0 ml/min. The analysis was carried out at an ambient temperature and injection volume was 20 µl. The UV detector was set at 230 nm.

Preparation of reference solutions

Reference solution (a): The solution was prepared by dissolving of accurately weighed 50.0 mg paracetamol CRS and 20.0 mg ibuprofen CRS in methanol in a 50.0 ml volumetric flask.

Reference solution (b): The solution was prepared by diluting of 5.0 ml from reference solution (a) into a 25.0 ml volumetric flask with methanol.

Several serial dilutions from reference solution (a) were made for the preparation of calibration curves in the range of 50 to 400 µg/ml for paracetamol and 20 to 160 µg/ml for ibuprofen (25-200 % of the working concentrations).

Sample preparation

The homogenized powder from twenty tablets with average weight equivalent to amount of 500 mg paracetamol and 200 mg ibuprofen was transferred to a 100.0 ml volumetric flask. Approximately 70 ml methanol were added and the obtained mixture was ultrasonicated for 20 min. The contents were restored to room temperature and diluted to volume with methanol to furnish stock test solution. The stock solution was filtered through a 0.45 µm Nylon syringe filter and 2.0 ml was diluted into a 50.0 ml volumetric flask to give test solution containing 200 µg/ml paracetamol and 80 µg/ml ibuprofen.

RESULTS AND DISCUSSION

A reverse-phase HPLC method was developed for the simultaneous determination of paracetamol and ibuprofen in tablets. From the chromatogram shown in figure 1, it is evident that under the proposed chromatographic conditions both analytes were completely separated from each other, which indicated that the method is selective and could be applied for their identification and quantification simultaneously. No peaks were observed in the chromatogram of a blank sample, which showed that no interferences from the excipients occurred.

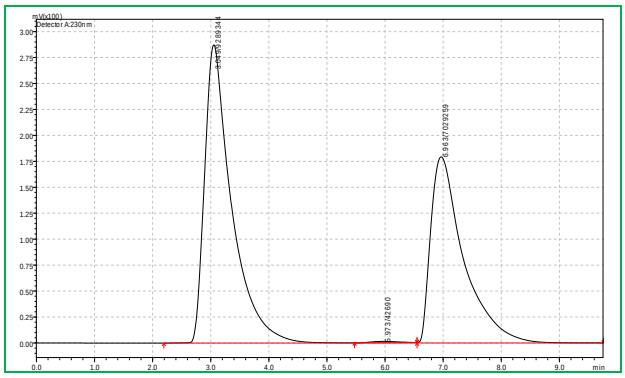

Figure 1: Chromatogram obtained from paracetamol RS and ibuprofen RS

The system suitability studies were carried out to determine theoretical plates, resolution and tailing factors. The results were given in table 1. The values obtained demonstrated the suitability of the system for the analysis of investigated drug combination, system suitability parameters may fall within ±3% standard deviation range during routine performance of the method.

Table 1: System suitability test parameters for paracetamol and ibuprofen

Parameter	Paracetamol	Ibuprofen
Retention time (min)	3.04	6.96
Resolution	2.68	-
Tailing factor	0.81	0.73
Theoretical plates	5486	4870

Validation of analytical procedure

The proposed method was validated with respect to specificity, linearity, precision and accuracy, limit of quantification (LOQ) and limit of detection (LOD).

Linearity

Linearity was evaluated by determining five different concentrations of the standard working solutions of paracetamol and ibuprofen in triplicate. The peak area and concentration of each drug was subjected to regression analysis to calculate the calibration equations and correlation coefficients. Calibration plot data slope (a), intercept (b), and correlation coefficients (R^2) were listed in table 2.

Table 2: Linear regression data for calibration curves

Drugs	Paracetamol	Ibuprofen
Concentration range (µg/ml)	50.00-400.0	20.00-160.0
Slope	118682.4	104160.2
Intercept	-2070.6	-1258.2
Correlation coefficient (R^2)	0.9999	0.9987

Limits of quantitation and limits of detection

The limit of detection (LOD) was calculated to be three times the standard deviation of baseline noise from analysis of each compound. The limit of quantitation (LOQ) was measured as the lowest of analyte that could be reproducibly quantified above the baseline noise, i.e. for which duplicate injection resulted in an RSD ≤ 2%. The LOQs for paracetamol and ibuprofen were found to be 2 µg/ml and 5 µg/ml, while the LODs were 0.4 µg/ml and 0.5 µg/ml, respectively.

Accuracy

Accuracy was determined by applying the proposed method to synthetic mixtures of the drug product components to which known quantities of each drug substance had been added (corresponding to 75, 100 and 125 % of the label claim of each drug). The accuracy was expressed as the percentage of analytes recovered by the assay. Mean recoveries for paracetamol and ibuprofen from the specific formulations were shown in table 3. The results indicated good accuracy of the method for the simultaneous determination of both drugs as revealed by mean recovery data.

Precision

The precision of analytical method was investigated by performing six consecutive replicate injections of the same standard solution. The standard deviation (S_d) and relative standard deviation (RSD) obtained are listed in table 4.

Table 3: Results from study of accuracy

Drug	Level (%)	Theoretical concentration (µg/ml)	Observed concentration (µg/ml)	Mean recovery (%) ± SD	RSD (%)
Paracetamol	75	148.7	147.5	99.65±0.455	0.46
			148.2		
			148.9		
	100	198.1	197.5	99.82±0.130	0.13
			197.7		
			198.0		
	125	246.2	245.1	99.83±0.275	0.28
			245.8		
			246.5		
Ibuprofen	75	59.85	59.23	99.60±0.621	0.62
			59.64		
			59.97		
	100	81.20	80.94	99.66±0.445	0.45
			80.56		
			81.27		
	125	100.4	99.94	99.53±0.370	0.37
			99.56		
			100.3		

Table 4: Values of S_d and RSD as confirmation of precision

Paracetamol		Ibuprofen	
Amount claimed (mg/tablet)	Amount found (mg/tablet)	Amount claimed (mg/tablet)	Amount found (mg/tablet)
500.0	497.2	200.0	198.5
	498.1		199.4
	499.3		199.1
	500.9		200.8
	498.9		199.2
	499.2		199.1
Mean	498.9	Mean	199.4
S_d	1.247	S_d	0.771
%RSD	0.25	%RSD	0.39

Table 5: Results from study of solution stability

Time (h)	Assay (%), test solution stored at 2-5°C		Assay (%), test solution stored at ambient temperature	
	Paracetamol	Ibuprofen	Paracetamol	Ibuprofen
Initial	99.96	98.87	99.91	98.91
12	99.87	98.54	99.98	98.90
24	99.80	98.30	99.59	98.13
36	99.91	98.81	99.60	98.80
48	99.68	99.10	99.32	98.67

Solution stability

Sample solution stability was evaluated by storing the solution at ambient temperature and at 2-5ºC and analysis after 12, 24, 36, and 48 h. The responses from the aged solutions were compared with those from freshly prepared standard solutions. The results showed that for the 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 occurred. Table 5 showed the results obtained from evaluation of stability.

CONCLUSION

The newly developed LC method for simultaneous determination of paracetamol and ibuprofen in combined dosage forms is specific, precise, accurate and rapid. Hence the proposed method is suitable for the quality control of the raw materials, formulations and dissolution studies.

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