

Simultaneous HPLC determination of Paracetamol and Indomethacin in tablet dosage forms

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ABSTRACT

A simple, specific, precise and accurate reversed phase liquid chromatographic (RP-LC) method has been proposed for the simultaneous determination of paracetamol and indomethacin in tablet dosage forms. The chromatographic separation was performed on a LiChrosorb C₈, 250 mm x 4.6 mm, 5 µm column at a detector wavelength of 230 nm and a flow rate of 1.0 ml/min. The mobile phase was composed of 0.01M sodium phosphate buffer pH 7.0 and acetonitrile (35:75 v/v). The retention times of paracetamol and indomethacin were found to be 3.05 and 6.96 min, respectively. The method was validated for the parameters like specificity, linearity, precision, accuracy, limit of quantitation and limit of detection. The calibration curves were linear in the concentration range of 30.00-240.0 µg/ml for paracetamol and 2.50-20.00 µg/ml for indomethacin. The % recovery for paracetamol and indomethacin is in the range between 98.97% and 99.84% with RSD values not greater than 1.90. The presented method for the simultaneous determination of paracetamol and indomethacin in tablets is specific, rapid and simple with good sensitivity. The analytical method can be successfully adopted for quality control tests for these drugs in tablet dosage forms

Key words: paracetamol, indomethacin, liquid chromatography, validation, quality control

INTRODUCTION

A combination of analgesics from different classes may provide additive analgesic effects with fewer undesired drug reactions than when a single therapeutic drug is used. There has been a trend over recent years for combining non-steroidal anti-inflammatory drugs (NSAIDs) with paracetamol (acetaminophen) for the management of acute postoperative pain [1, 2]. Combination of paracetamol and indomethacin is prescribed as an analgesic and anti-inflammatory agent in rheumatoid arthritis [3]. Indomethacin (1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indole-3-acetic acid) is a non-steroidal anti-inflammatory drug exhibiting analgesic, anti-inflammatory and antipyretic effects. It is a potent inhibitor of cyclooxygenase, reducing prostaglandin synthesis, relieving pain and reducing temperature in febrile patients and often also used topically in the eye to reduce local inflammation. Despite its high toxicity, indomethacin is a primary medicine prescribed for the treatment of rheumatoid arthritis, gout and collagen disease [4]. Paracetamol 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 applied 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 NSAIDs to be used, thereby minimizing overall side effects [5]. Several methods for the determination of indomethacin in pharmaceuticals and biological fluids have been reported. They include chromatography [6-11], potentiometry [12, 13], fluorimetry [14-16], spectrophotometry [16-19] and colorimetry [20, 21]. Literature survey reveals that there are many analytical methods for assaying paracetamol in pharmaceutical dosage forms, such as chromatography

[22-32], titrimetry [33], spectrophotometry [34, 35], spectrofluometry [36], voltammetry [37], colorimetry [38] and Fourier transform infra red spectrometry [39]. The methods for simultaneous determination of paracetamol and indomethacin were based on liquid chromatography [40], spectrophotometry [41], electrophoresis [42] and potentiometry [43].

The aim of the present study was to develop and validate a HPLC method for the simultaneous determination of paracetamol and indomethacin in tablet dosage forms contained 300 mg paracetamol and 25 mg indomethacin. 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, reagents and chromatographic conditions

Tablets containing paracetamol (300 mg) and indomethacin (25 mg) were obtained commercially. Working standards of paracetamol RS (Purity 100.09) and indomethacin RS (Purity 99.81) were provided by (Sigma-Aldrich). LC-grade acetonitrile was supplied from Merck (Germany). All other chemical reagents were of analytical grade. 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 C₈, 250 mm x 4.6 mm, 5 µm column was used as a stationary phase. The separation was performed isocratically with a mobile phase consisting 0.01M sodium phosphate buffer pH 7.0 and acetonitrile (35:75 v/v) 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 120.0 mg of precision-weighed paracetamol RS and 10.00 mg indomethacin RS in methanol, in a 100.0 ml volumetric flask. Reference solution (b): The solution was prepared by diluting 5.0 ml of reference solution (a) with methanol, into a 50.0 ml volumetric flask.

Sample preparation

The homogenized powder from twenty tablets with an average weight equivalent to 120.0 mg paracetamol and 10.00 mg indomethacin was transferred into a 100.0 ml volumetric flask. Approximately 70 ml methanol was added and the obtained mixture was sonicated for 15 min with intermittent shaking. The content was restored to room temperature and diluted to volume with methanol to furnish a stock test solution. The stock solution was filtered through a 0.45 µm Nylon syringe filter and 5.0 ml of the filtrate was diluted into a 50.0 ml volumetric flask to give a test solution containing 120 µg/ml paracetamol and 10.00 µg/ml indomethacin.

RESULTS AND DISCUSSION

The proposed method was validated with respect to selectivity, linearity, precision, accuracy, limit of quantitation (LOQ) and limit of detection (LOD) according to ICH requirements [44] to show it could be used for simultaneous determination of paracetamol and indomethacin in pharmaceutical formulations.

Selectivity

From the chromatogram shown in Fig. 1, it is evident, that under the chosen chromatographic conditions paracetamol (Tr=3.05 min) and indomethacin (Tr=6.96 min) were completely separated. The specificity of the proposed method was confirmed by injecting blank sample. The specificity analysis revealed the HPLC method did not suffer interference by the formulation excipients, since there were not another peaks on the retention times of paracetamol and indomethacin.

Linearity, limit of detection and limit of quantification

Calibration curve was constructed in the range of 30.00-240.0 µg/ml for paracetamol and 2.50-20.0 µg/ml for indomethacin to encompass the expected concentration in measured samples. A calibration curves were plotted between the mean peak areas vs. respective concentrations. The corresponding linear regression equations were $y=11254.x-1420.1$ with square of correlation coefficient R^2 of 0.9999 for paracetamol and $y=16198.x-1103.6$ with square of correlation coefficient R^2 of 0.9998 for indomethacin, respectively. An excellent correlation existed between the peak areas and the concentrations of both compounds. The limit of quantitation and limit of detection were calculated from the standard deviations and slopes of the responses using a signal-to-noise ratio as per ICH guidelines [44]. The LOQs for paracetamol and indomethacin were found to be 1 µg/ml and 0.5 µg/ml, while the LODs were 0.2 µg/ml and 0.1 µg/ml, respectively.

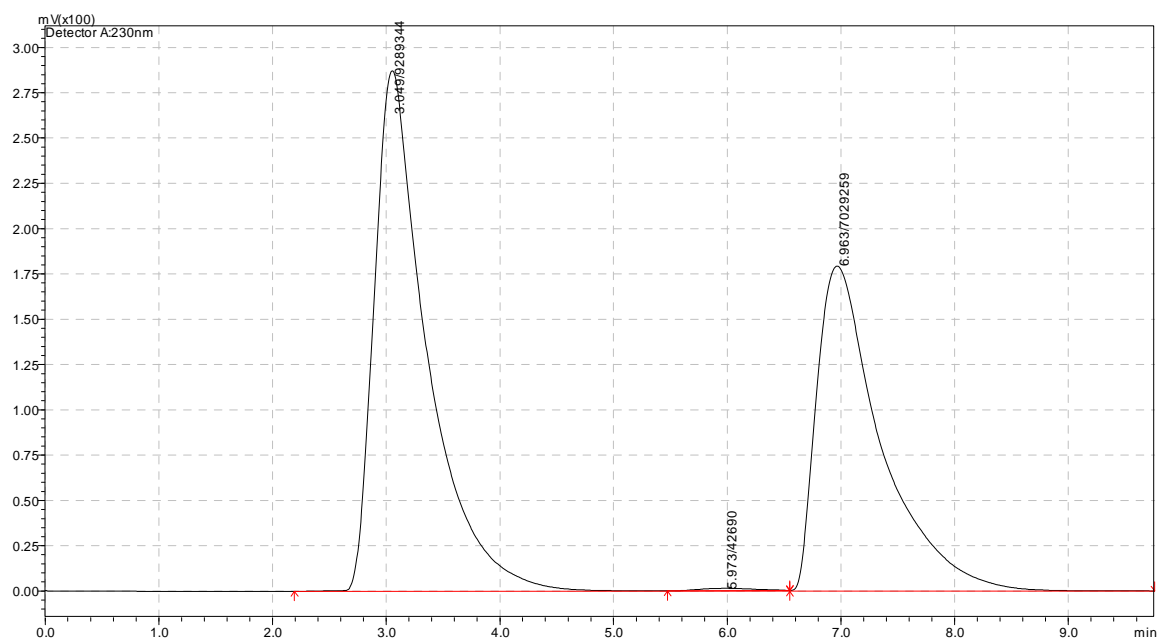


Fig.1. Chromatogram from analysis of sample

Precision

The precision of the method was evaluated by performing six independent determinations of the test sample preparation and calculating RSD (%). The RSD values measured during assessment of precision were <2.0% for both analytes, confirming the method is precise (Table 1).

Table 1. Precision of the method

paracetamol		indomethacin	
Amount claimed (mg/tablet)	Amount found (mg/tablet)	Amount claimed (mg/tablet)	Amount found (mg/tablet)
300.0	299.8	25.00	25.12
	299.2		24.92
	300.5		24.78
	299.1		25.21
	300.4		24.57
	300.1		24.81
Mean	299.6	Mean	24.90
S _d	0.596	S _d	0.235
%RSD	0.20	%RSD	0.94

Table 2. Results from study of accuracy

Amount of sample (µg/ml)		Sets	Amount drug of spiked (µg/ml)		Average amount recovered (µg/ml)		Mean recovery (%) ± SD		% RSD	
PAR	IND		PAR	IND	PAR	IND	PAR	IND	PAR	IND
60	5	1	0	0						
60	5	2	0	0	59.60	4.98	99.33±0.86	99.53±1.90	0.87	1.90
60	5	3	0	0						
60	5	1	30	2.5						
60	5	2	30	2.5	89.84	7.48	99.84±0.73	99.77±1.72	0.73	1.72
60	5	3	30	2.5						
60	5	1	60	5						
60	5	2	60	5	119.6	9.90	99.70±0.87	98.97±1.24	0.87	1.24
60	5	3	60	5						
60	5	1	90	7.5						
60	5	2	90	7.5	149.7	12.5	99.82±0.85	99.66±1.47	0.85	1.74
60	5	3	90	7.5						

Accuracy

The accuracy of the method was determined by calculating the recoveries of paracetamol (PAR) and indomethacin (IND) by the standard addition method. Known amounts of standard solutions of both PAR and IND (50, 100, and 150%) were added to prequantified sample solutions of tablets. The method was found to be accurate with

recoveries of 98.97%–99.84% and an acceptable RSD of not more than 2% at each level. The recoveries obtained by the proposed method for PAR and IND were shown in **Table 2**.

CONCLUSION

The newly developed RP-LC method for simultaneous determination of paracetamol and indomethacin in 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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