International Journal for Pharmaceutical Research Scholars (IJPRS)

V-4, I-2, 2015 ISSN No: 2277 - 7873

RESEARCH ARTICLE

RP-HPLC Method for Simultaneous Determination of Paracetamol, Diclofenac Sodium and Famotidine in Tablet Dosage Form

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Manuscript No: IJPRS/V4/I2/00060, Received On: 14/04/2015, Accepted On: 24/04/2015

ABSTRACT

A simple, precise and accurate liquid chromatographic method was described for simultaneous determination of paracetamol, diclofenac sodium and famotidine in tablets. The chromatography was carried out on a C_{18} (250 mm x 4.6 mm, 10 µm) column with acetonitrile:water: 0.5% trimethylamine : o-phosphoric acid (60:20:5:15v/v) as mobile phase, at a flow rate of 1.0 ml/min, with detection at 240 nm. Separation was completed in less than 10 min. The calibration curves were linear in the concentration range of 125.0-1000.0 µg/ml for paracetamol, 25.00-200.0 µg/ml for diclofenac sodium and 5.00-40.00 µg/ml for famotidine. The intra- and inter-day relative standard deviations for both the components were <2.0 %. The results of the studies showed that the proposed RP-HPLC method is rapid, precise and accurate, which can be applied for the routine assessment of described drugs in pharmaceutical dosage forms.

KEYWORDS

Paracetamol, Diclofenac Sodium, Famotidine, Simultaneous Determination, RP-HPLC, Validation

INTRODUCTION

The combination of analgesics of proven efficacy is a strategy intended to achieve one or more therapeutic goals, such as facilitating patient compliance, simplifying prescribing, improving efficacy without increasing adverse effects or decreasing adverse effects without loss of efficacy[1,2]. In certain cases, the co-administration of antinociceptive agents results in synergistic effects and the doses of the individual drugs can be substantially reduced[3-5]. Non-steroidal anti-inflammatory drugs (NSAIDs) and paracetamol (PAR) are drugs widely used to treat moderate to mild pain, but they are often inadequate against severe pain.

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Clinical studies on patients with musculoskeletal conditions, dental pain or postoperative pain have shown that combinations of paracetamol and NSAIDs may provide additive pain-relief[6].

Diclofenac sodium (DIC) is a NSAID with pronounced anti-rheumatic, anti-inflammatory, analgesic and anti-pyretic properties. Diclofenac inhibits prostaglandin synthesis, which play a major role in the causation of inflammation, pain and fever. Paracetamol produces analgesia and antipyresis by a mechanism similar to that of salicylate, which involves inhibition of prostaglandin synthesis[7].

However, it is generally known that NSAIDs are associated with gastrointestinal complications, whereas paracetamol is associated with very few side effects. Famotidine (FAM), a histamine H_2–receptor antagonist, inhibits acid secretion and

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provides protection against mucosal injury in patients receiving NSAIDs. It is well known that famotidine was well tolerated and effective in preventing both gastric and duodenal ulcers in patients with arthritis receiving long-term NSAID therapy[8].

Certain successful attempts have been made for the determination of PAR[9-16], DIC[17-28] and FAM [28-37] in pharmaceuticals using different analytical techniques including spectrophotometry, spectrofluorimetry, high performance liquid chromatography, thin-layer chromatography, capillary zone electrophoresis, potentiometry, differential pulse voltammetry, polarography as alone or in combination with other drugs. Analytical method based on HPTLC for simultaneous determination of paracetamol, diclofenac and famotidine has been recently reported[38]. Based on our current and ongoing referencing work, till date, we have not come across any reported HPLC method for simultaneous estimation of drugs of interest in their combined dosage form. Present study emphasizes on the determination of paracetamol, diclofenac sodium and famotidine in their model drug dosage forms by high performance liquid chromatography.

MATERIAL AND METHODS

Materials

Paracetamol, diclofenac sodium and famotidine were supplied as standards. Commercial tablet formulations were purchased from the local market. HPLC grade acetonitrile was used to prepare the mobile phase. All other chemicals applied for the chromatographic experiments were of a reagent grade.

Methods

Instrumentation and Chromatographic Conditions

Chromatographic separation was performed on a modular HPLC system LC-10AShimadzu (Japan) comprising a LC-10A pump, solvent degasser DGU-3A, Rheodyne injector with 20 µl loop, column oven CTO-10A, SPD-M10A UV detector with fixed wavelength and communication bus module CBM-10A. A LiChrosorb C_{18} (250 mm x 4.6 mm, 10 µm) column was used as a stationary phase. The components were separated isocratically using a mobile phase consisting of acetonitrile: water: 0.5% triethylamine: o-phosphoric acid (60:20:5:15 v/v) at a flow rate of 1.0 ml/min. The mobile phase was filtered through a 0.45 µm membrane filter and degassed. The analysis was carried out at an ambient temperature and the injection volume was 20 µl. The UV detector was set at 240 nm.

Preparation of Reference Solutions

Reference solution (a): The solution was prepared by dissolving 25.0 mg of accurately weighed diclofenac sodium in methanol in a 100.0 ml volumetric flask (C=250 µg/ml).

Reference solution (b): The solution was prepared by dissolving 10.0 mg of accurately weighed famotidine in methanol in a 100.0 ml volumetric flask (C=100 µg/ml).

Working reference solution: The solution was prepared by dissolving of accurately weighed 25.0 mg paracetamol in the first step and in the second step by diluting 20.0 ml of reference solution (a) and 10.0 ml of reference solution (b) with methanol into a 50.0 ml volumetric flask. The concentrations of the investigated compounds in working reference solution were as follows: paracetamol – 500 µg/ml, diclofenac – 100 µg/ml and famotidine – 20 µg/ml, respectively.

Sample Preparation

Twenty tablets were weighed and their average weight was calculated. These tablets were powdered and weight equivalent to half tablet containing 250 mg of paracetamol, 50 mg of diclofenac and 10 mg of famotidine was taken in a 100 mL dilution flask. Then about 50 mL of methanol was added to it. The solution was sonicated for 20-25 min at an ambient temperature with intermittent swirling, cooled and diluted up to the mark with diluent, mixed well. The stock solution was filtered through a 0.45 µm Nylon syringe filter and 5.0 ml of the filtrate was diluted into a 25.0 ml volumetric flask to give a test solution containing 500.0

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µg/ml PAR, 100.0 µg/ml DIC and 20.00 µg/ml FAM.

RESULTS AND DISCUSSION

In this work an LC method with UV detection for analysis of PAR, DIC and FAM in a tablet formulation was developed and validated. From the chromatogram shown in Fig. 1, it is evident that, under the proposed chromatographic conditions, the analytes of interest are completely separated, which indicates that the method is selective and could be applied for their simultaneous identification and quantification.

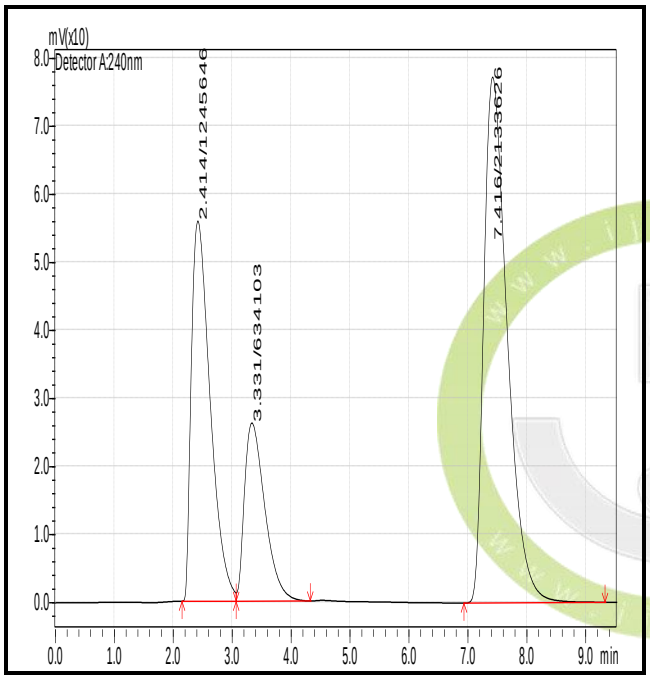

Figure 1: Chromatogram of Mixture of Standards – Paracetamol, Diclofenac Sodium and Famotidine

Method Validation

The proposed method was validated as per ICH guidelines[39] with respect to specificity, linearity, precision, accuracy, limit of quantitation (LOQ) and limit of detection (LOD).

Specificity

The specificity of the method was determined by checking the interference of the components against placebo. No interference was observed for any of the excipients of the drugs.

Calibration and Linearity

Calibration curves were plotted in the range of 125.0-1000.0 µg/ml for PAR, 25.00-200.0 µg/ml for DIC and 5.00-40.0 µg/ml for FAM. The corresponding linear regression equations were y=165741.1x-2653.2 with a squared correlation coefficient R^2 0.9998 for PAR, y=23541.2x-2341.9 with a squared correlation coefficient R^2 0.9999 for DIC and y=56241.9x-4412.2 with R^2 of 0.9998 for FAM, respectively. An excellent correlation existed between the peak areas and the concentrations of investigated compounds.

Limit of Quantitation and Limit of Detection

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[39]. The LOQs for PAR, DIC and FAM were found to be 0.5 µg/ml, 0.25 µg/ml and 1 µg/ml, while the LODs were 0.1 µg/ml, 0.05 µg/ml, and 0.2 µg/ml, respectively.

Accuracy

Accuracy of the method was evaluated by standard addition technique, which was performed by addition of known amounts of pure PAR, DIC and FAM to known concentrations of tablet powder and analysed by proposed methods in triplicate. Results presented in Table 1 indicated good accuracy and showed no interference from tablet excipients.

Precision

Intraday precision (repeatability) was calculated using two concentrations of PAR (250, 750 µg/mL), DIC (50, 150 µg/mL) and FAM (10, 30 µg/mL) in triplicate using proposed methods. The interday precision (reproducibility) was repeated three times on three different days for analysis of two different concentration (250:50:10, 750:150:30 µg/mL) for analyzed drugs.

The values of % RSD (Table 2) for PAR, DIC and FAM were found to be in the range from 0.32 to 0.87 indicating good repeatability and reproducibility of the analytical procedure.

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Table 1: Recovery Studies of PAR, DIC and FAM

Drug	Amount taken (µg/ml)	Amount added (µg/ml)	Amount recovered ± SD* (µg/ml)	% RSD
PAR	250.0	62.50	311.9±0.91	0.30
		125.0	375.6±1.27	0.34
		187.5	436.9±1.15	0.26
DIC	50.00	12.50	62.47±0.49	0.78
		25.00	75.20±1.05	1.40
		37.50	86.9±1.05	1.21
FAM	10.00	2.50	12.67±0.05	0.39
		5.00	15.08±0.07	0.46
		7.50	17.69±0.09	0.51

**Average value of three determinations, RSD is relative standard deviation*

Table 2: Precision of the Method

Precision	Amount taken (µg/ml)			% Mean*			% RSD		
	PAR	DIC	FAM	PAR	DIC	FAM	PAR	DIC	FAM
Intra day	250	50	10	99.56	100.5	99.61	0.36	0.32	0.54
Intra day	750	150	30	100.1	100.2	100.9	0.65	0.61	0.61
Inter day	250	50	10	99.48	99.89	99.81	0.84	0.56	0.38
Inter day	750	150	30	99.75	99.52	99.62	0.78	0.87	0.42

**Mean of three determinations*

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CONCLUSION

The validated RP-LC method developed here proved to be simple, specific, accurate and precise. It can successfully used for routine analysis of paracetamol, diclofenac and famotidine in combined dosage form without any interference from common excipients.

AKNOWLEDGMENTS

The present study was kindly supported by Project No. 8/2014 from Medical Science Council.

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