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Development and Validation of LC Method for Simultaneous Determination of Piroxicam and Paracetamol in New Pharmaceutical Formulation

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ABSTRACT

In this perspective, we present a reverse phase high performance liquid chromatographic method for simultaneous determination of piroxicam and paracetamol in commercial pharmaceutical dosage forms. The resulting contents of tablets were baseline resolved on a reverse phase Zorbax SB C18, (250 x 4.6 mm, 5 µm) analytical column. Mobile phase contains methanol, acetonitrile and 50 mM sodium dihydrogen orthophosphate (27:16:57 v/v) at a flow rate of 1 mL/min. UV detector was set at a wavelength of 254 nm. The resolution between paracetamol and piroxicam was less than five. Developed method was extensively proved to be robust for the titled drugs. This method was shown to be linear, correlation coefficient of paracetamol and piroxicam was 0.9986 and 0.9990 respectively. The percent recovery for paracetamol and piroxicam ranged between 100.5-101.3 and 100.6-100.8 respectively. The percent relative standard deviation for six replicates was less than 2 .The limit of detection and limit of quantification for paracetamol was 140 ng / mL and 400 ng / mL for piroxicam 29 ng / mL and 70 ng / mL . Sample concentrations were measured on weight basis to avoid internal standard. Proposed method is suitable for quantitative determination of the titled drugs in their commercial samples of tablet formulation with respect to assay.

Keywords : Piroxicam, Paracetamol, Liquid chromatography, Pharmaceutical formulation, Method validation

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INTRODUCTION

Piroxicam, 2*H*-1, 2-Benzothiazine-3-carboxamide, 4-hydroxy-2-methyl-*N*-2-pyridinyl - 1, 1-dioxide, Fig.1, is a non-steroidal anti-inflammatory drug with analgesic and anti-pyretic activity. It is widely used for the treatment of various inflammatory diseases and arthropathies, such as rheumatoid arthritis and osteoarthritis [1, 2]. Paracetamol, N-(4-hyroxyphenyl)-4'-Hydroxyacetanilide Fig.2, is commonly used as analgesic and antipyretic drug [3, 4]. An

increased interest of pharmaceutical company to develop formulation, contain both drugs as an active ingredient, in order to achieve more favorable effects in clinical trial. Literature survey revealed that, few analytical methods were reported in different pharmacopoeia for individual analysis of paracetamol and piroxicam [5-7]. Some methods were available for individual estimation of piroxicam in human plasma [8-9] and piroxicam in transdermal permeation studies [10]. Numerous methods have been reported for estimation of paracetamol in combination with other ingredients [11-16] and flurometric assay of paracetamol [17]. After method development, analytical method was validated to ensure their quality and suitability as per ICH guideline [18]. So far, to our present knowledge, no LC method has been reported for simultaneous determination of piroxicam and paracetamol in pharmaceutical formulation. In the present research work our objective was to develop a simple, specific, accurate, precise, reproducible and economical quality control method for simultaneous estimation of paracetamol and piroxicam as drug substances in their binary combination. Proposed method is highly sensitive and specific, which can be used for routine analysis of pharmaceutical formulations consisting of paracetamol and piroxicam as an active ingredient with short preparation and analysis time.

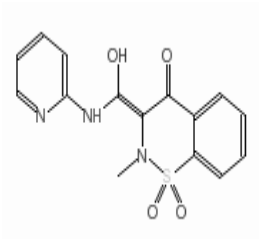

Fig.(1). Chemical structure of Piroxicam

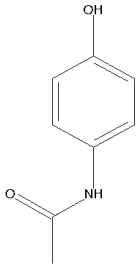

Fig.(2). Chemical structure of Paracetamol

MATERIALS AND METHODS

2.1 Chemicals and Regents

Piroxicam and paracetamol was used as working standard, kindly provided by Ranbaxy Laboratories Ltd., Goa, India. Analytical grade sodium dihydrogen orthophosphate and HPLC grade methanol were purchased from E. Merck (India). Highly pure water was prepared with Millipore Milli Q plus purification system. New formulation of tablet was purchased from Indian market. Tablet contains 325 mg of paracetamol and 20 mg of piroxicam as active ingredients per tablet.

2.3 HPLC Measurements

A quantitative analysis was performed with high performance liquid chromatographic (HPLC) system , consisting of dual piston, reciprocating two 3500 pumps, equipped with AS 3000 auto sampler and PD 1000 detector of Jasco Corp. Japan. Chromatographic separation between two analytes was achieved by Zorbax SB C18 (250 x 4.6 mm, 5 µm) analytical column. The column oven temperature was maintained at 25º C and analyte detection was set at a wavelength of 254 nm. The peak purity was checked with the photodiode array detector. The flow rate of the mobile phase was set at 1 mL/min. Data acquisition was made with Borwin software. Mobile phase used throughout the experiment was a mixture of methanol, acetonitrile and 50 mM sodium dihydrogen orthophosphate (27:16:57 v/v). The mobile phase was filtered through a Millipore nylon membrane (pore size 0.45 µm) and degassed before use.

2.4 Preparation of Standard Solutions

A stock solution with paracetamol and piroxicam was prepared in mobile phase at about 3250 µg / mL and 200 µg / mL. Aliquots from each working solution were combined to yield a solution concentration of about 65 µg / mL of paracetamol and 4 µg / mL of piroxicam and diluted with mobile phase.

2.5 Preparation of Sample Solutions

Twenty tablets were accurately weighed and finely powdered. An accurately weighed portion of the powder equivalent to 325 mg of paracetamol and 20 mg of piroxicam were dissolved with 80 mL mobile phase in 100 mL calibrated flask. The mixture was extracted in ultrasonic bath for 10 minutes with continuous shaking at room temperature and completed to volume with mobile phase. Transfer 1 mL of filtered solution (through 0.45 µm nylon filter) into 50 mL calibrated flask and make up the volume with mobile phase to the mark. 20 µL solutions were injected in to chromatographic system, the chromatogram was measured at 254 nm, indicates excellent resolution between both peaks, shown in Fig.3.

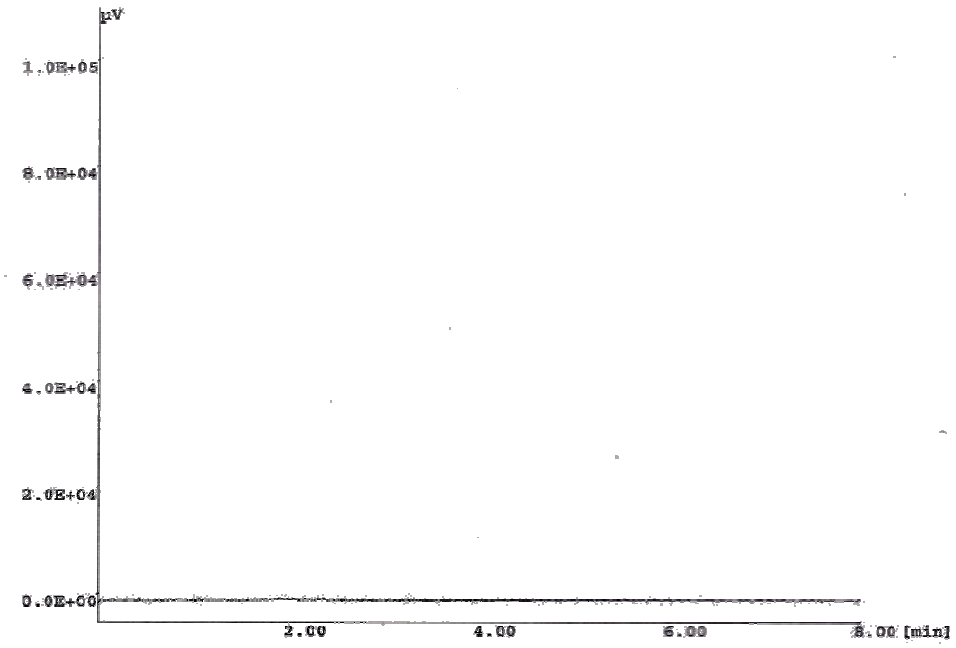

Fig.(3). A typical HPLC chromatogram of blank test

3. Method Validation

3.1 Specificity

Specificity is ability of method to measure the analyte response in presence of its excipients, impurities of the formulation. The specificity of the developed method was checked with peak purity test using photodiode array detector. The peak purity of paracetamol and piroxicam were 999 and 997 respectively in sample solution. The results of the peak purity analysis indicate, analyte peak was pure and no excipients interfering with analyte peak of sample in the tablet formulation.

3.2 System suitability

System suitability parameters like, relative standard deviation, column efficiency, resolution and tailing factor of the peaks were taken in to the consideration. Five replicates of mixed standard solution were injected; the percent relative standard deviation was less than 2 in both the analyte, shown in Table 1.

Table 1. System Suitability Report.

Parameters	Paracetamol	Piroxicam
Theoretical plates [l]	3692	9035
Resolution	0	12.9
Tailing factor	1.55	1.14
% RSD	1.11	1.46

[l]: per column length
RSD: relative standard deviation

3.3 Linearity

The linearity response of two analytes was established with series of working solutions. Linearity test solutions were prepared by diluting the stock solution to the required concentration with mobile phase. Each concentration was injected in triplicate, average of peak area was considered for calibration curve. The calibration graph was constructed by plotting the peak area response against concentration of each drug. Seven concentration levels were prepared for linearity study, ranged from 25 to 175 % of the analyte concentration of assay analysis. Linear response of peak area were observed, over the concentration range of 16.25 to 113.75 µg / mL of paracetamol and 1.0 to 7.0 µg / mL of piroxicam. The correlation coefficient for paracetamol was y = 104532x + 1858.2 = 0.9986 and y = 34294x +1763 = 0.9990 for piroxicam. The results indicate an excellent correlation between concentration and peak area response of each drug within the concentration range tested.

3.4 Limit of Detection and Limit of Quantification (LOD and LOQ)

The LOD and LOQ of the developed method were determined by injecting progressively low concentration of both the analyte. The LOD is the smallest concentration of analyte that gives measurable response (signal to noise ratio of 3). The LOD of paracetamol and piroxicam was 140 ng/mL and 29 ng / mL respectively .The LOQ is the smallest concentration of analyte that can be accuracy quantified, (signal to noise ratio of 10). The LOQ of paracetamol and piroxicam was 400 ng / mL and 70 ng / mL.

3.5 Method Reproducibility

The system precision is measured of method variability, by injecting five replicates of the same working solution. The percent relative standard deviation for paracetamol and piroxicam was 1.11 and 1.46. The inter-day precision was determined by assaying the tablets in triplicate per day for two consecutive days. The relative standard deviation was expressed in percentage of the label claim in assay estimation. The method was found to be precise for paracetamol and

piroxicam, the percent relative standard deviation was within 1.1-1.7 and 1.3-1.9 respectively, for inter-day assay. The intra-day precision of method was determined by preparing solution of tablets with three concentrations and three replicates each. For intra-day assay percent relative standard deviation was within 1.1-1.6 and 1.3-1.8 for paracetamol and piroxicam respectively. The results indicate an excellent precision between the analyte of the developed method, shown in Table 3.

Table 3. Intra-day and Inter-day Precision of Assay.

Level of Addition (%)	Ingredient	Amount Added (mg)	Amount found (mg)	*Average Recovery (%)
80	Paracetamol	260	262.9	99.9
	Piroxicam	16	16.1	99.1
100	Paracetamol	325	326.6	100.4
	Piroxicam	20	20.2	99.7
120	Paracetamol	390	390.3	100.7
	Piroxicam	24	24.2	100.1

[1]: (µg/ml) n = 6

3.6 Accuracy and Recovery

Accuracy of the method was studied by performing the recovery experiment. The recoveries of those drugs were obtained in laboratory prepared dosage formulation, containing 80, 100 and 120% of the labeled amount of active ingredients. At each level three samples were prepared. The recovery values for paracetamol and piroxicam ranged from 99.9 -100.7 % and 99.1 -100.1 % respectively, shown in Table 2. The average recovery at three levels (nine determinations) for paracetamol and piroxicam were 100.3 % and 99.6 % respectively.

Table 2. Results of the Recovery Test of Drugs in Tablets.

Inter-day Precision									
Paracetamol									
Parameters	**0 Day**			**1 Day**			**2 Day**		
Mean concentration[1]	48.82	64.40	80.56	48.82	64.40	80.56	48.82	64.40	80.56
% RSD	1.2	1.6	1.1	1.2	1.5	1.3	1.7	1.5	1.4
Piroxicam									
Mean concentration[1]	3.04	4.06	5.08	3.04	4.06	5.08	3.02	4.06	5.08
% RSD	1.9	1.6	1.3	1.5	1.7	1.5	1.6	1.7	1.4
Intra-day Precision									
Parameters	**0 Day**			**1 Day**			**2 Day**		
Paracetamol									
Mean concentration[1]	48.22			64.54			80.66		
% RSD	1.4			1.1			1.6		
Piroxicam									
Mean concentration[1]	3.05			4.07			5.02		
% RSD	1.7			1.8			1.3		

**: (n =3)*

Average recovery: the average of three levels, nine determinations

3.7 Solution Stability and Mobile Phase Stability

The solution stability of paracetamol and piroxicam were performed by leveling the solution of sample and standard in tightly capped volumetric flask at room temperature, at an interval of 12 hr. to the study period. Furthermore, mobile phase stability of method was performed by analyzing the freshly prepared sample solution against freshly prepared standard solution at an interval of 12 hr. to the 24 hr duration. The solution stability was determined in terms of the percent assay against the freshly prepared standard solution. The percent relative standard

deviation of paracetamol and piroxicam were calculated during mobile phase and solution stability experiment. The percent relative standard deviation was less than 2, for both ingredients up to 24 hr. The result indicates, the solutions were stable up to 24 hr. at an ambient temperature.

RESULTS AND DISCUSSION

4.1 Method Development and Method Optimization

The primary target was to achieve the better resolution between the analyte in compound formulation under common experimental conditions with isocratic LC method. In order to avoid derivatiztion of active ingredients used in formulation. The chromatographic method was optimized at various pH of the 50 mM sodium dihydrogen orthophosphate used in mobile phase. The optimal pH value of the 50 mM sodium dihydrogen orthophosphate was found to be 4 of the mobile phase. Several pH experiments were performed on 50 mM sodium dihydrogen orthophosphate ranged from 3.0 to 5.0. Both analyte exhibits significant maximum absorption at a wavelength of 254 nm in ultraviolet region. Throughout experiment mobile phase contains methanol, acetonitrile and 50 mM sodium dihydrogen orthophosphate (27:16:57 v/v). The 50 mM sodium dihydrogen orthophosphate having pH 4 without any adjustment, by using this mobile phase the retention time of paracetamol and piroxicam were observed at 2.37 minute and 4.38 minute respectively.

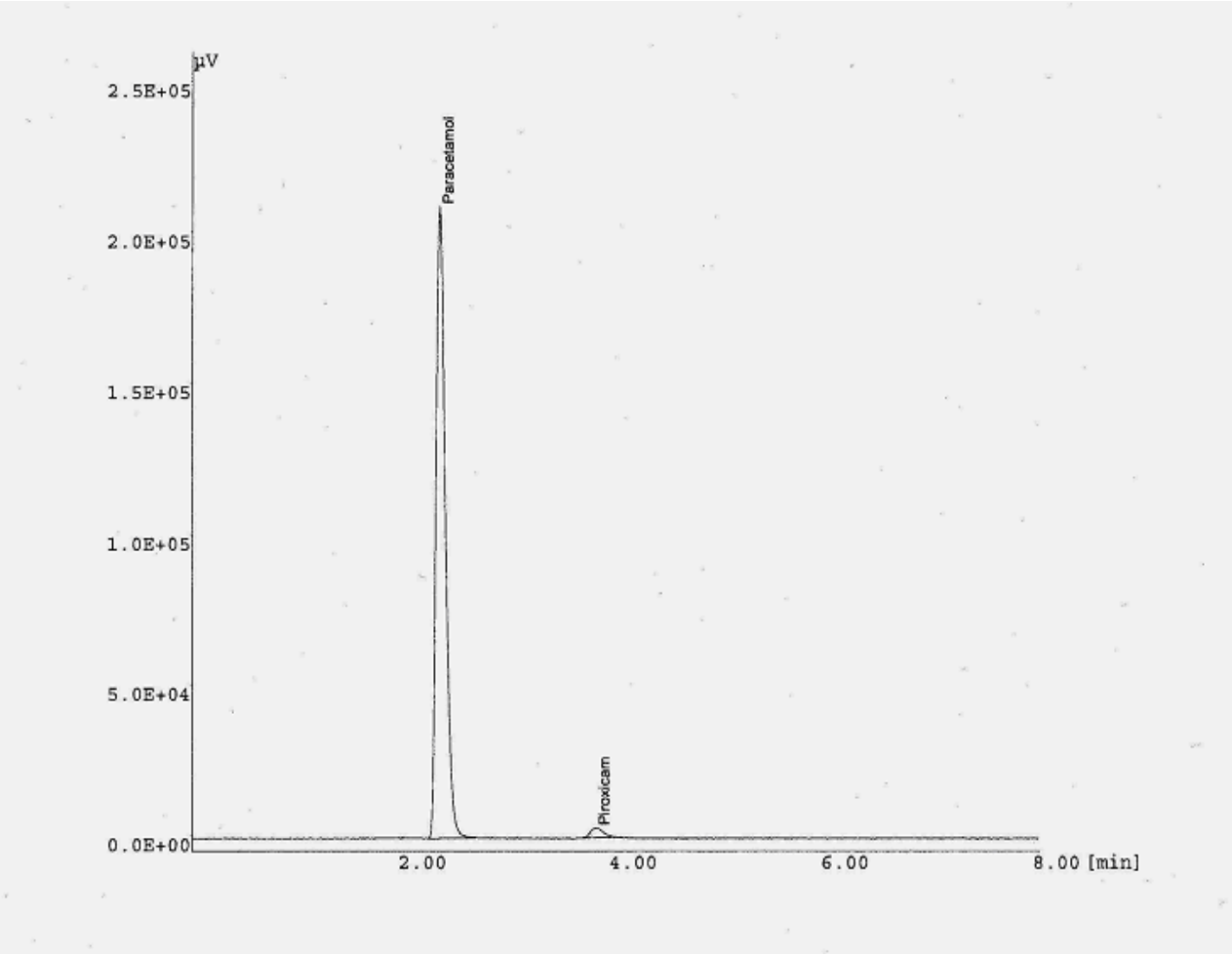

Fig.(4).A typical HPLC chromatogram of the tablet sample solution containing 65 µg/ml of Paracetamol and 4 µg/ml of Piroxicam.

Experiments were performed by changing the pH of the 50 mM sodium dihydrogen orthophosphate, without changing composition of methanol, acetonitrile and 50 mM sodium dihydrogen orthophosphate. When pH 3 of the 50 mM sodium dihydrogen orthophosphate was adjusted with 10% v/v orthophosporic acid solution, the sequence of peak elution was paracetamol at 2.41 minute and piroxicam at 5.53 minute. There was slight change in retention time, without affecting any elution order of paracetamol and piroxicam. It indicates, at pH 3 of 50 mM sodium dihydrogen orthophosphate, ionic strength concentration of the mobile phase can affect slightly on retention time of the analyte peak. When pH 5 of 50 mM sodium dihydrogen orthophosphate was adjusted with 10 % w/v sodium hydroxide solution the sequence of elution was paracetamol at 0.92 minute and piroxicam at 3.97 minute, paracetamol elute one minute before than previous experiment and ionic strength concentration can affect more with paracetamol than piroxicam. Mobile phase containing methanol, acetonitrile and 50 mM sodium dihydrogen orthophosphate (27:16:57 v/v) gave better resolution between paracetamol and piroxicam shown in Fig.4.Also there is no interference blank and excipients with analyte peak shown in Fig.3 The chromatographic runtime was less than 10 minutes. Resolution between the peaks were checked at a high concentration of one analyte and low concentration of other i.e. paracetamol at 200 µg/ mL) and piroxicam at 2 µg/ mL with the same mobile phase. In this case the resolution between two analyte peaks was more than 5, which indicates that there is better resolution between paracetamol and piroxicam with extremely high and low concentration of analyte

CONCLUSION

A simple, specific, liner, precise and accurate, rapid and suitable RP-LC method has been developed and validated in routine analysis for quantitative determination of piroxicam and paracetamol in tablet formulation. . Method is simple and specific, as all peaks are we resolved from each other and excipients with total run tome less than 10 min. This method can be applicable in the quality control department for the assay analysis of the titled drug in tablet formulation.

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