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Development and Validation of Reverse Phase High Performance Liquid Chromatographic Method for Simultaneous Estimation of Paracetamol and Piroxicam in Tablet

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Abstract: A simple, rapid and precise Reverse Phase High Performance Liquid Chromatographic method was developed for simultaneous estimation of Paracetamol and Piroxicam in Tablet. Eurosphere- 100 C_{18}, 250 x 4.6 mm, 5 µm particle size column, in isocratic mode with mobile phase Methanol: Water (70:30 v/v), pH was adjusted to 4.0 with acetic acid. The flow rate was 1.0 mL/min and individual component were measured at 227 nm. The retention time of piroxicam and paracetamol was 2.52 and 5.48 min respectively. The method was validated in terms of accuracy, precision, linearity, limit of detection, limit of quantitation, robustness and ruggedness as per ICH and USP guidelines. The assay and recovery studies show paracetamol and piroxicam in the range from 99 to 101 % were obtained at various added concentrations. The procedures were successfully applied for simultaneous determination of both drugs in laboratory prepared mixtures as well as commercial tablet dosage form.

Keywords: RP-HPLC; Validation; Paracetamol; Piroxicam

Introduction

Paracetamol is acetanilide derivative having analgesic, antipyretic and weak anti-inflammatory action [1-3]. Piroxicam is oxicam derivative having analgesic and anti-inflammatory action [4-6]. Various analytical methods had reported in literature for estimation of paracetamol [7-25] and piroxicam [26-32] individually and in combination with other drugs.

Fixed dose combination containing paracetamol and piroxicam in tablet dosage form is recently introduced in the market and no method is reported for the simultaneous estimation of both these drugs. The aim of present work is to develop a simple, rapid, precise and selective RP-HPLC method for the estimation of paracetamol and piroxicam in tablet dosage form.

Material and Method

Chromatographic system

Chromatographic separation was performed on a High Performance Liquid Chromatography system Systronic LC-6600 equipped with a LC 6600 solvent delivery system (pump), universal injector with injection volume 20 µL, and Ultra-visible (UV-VIS) detector. A Eurosphere C_{18} (KNAVER, Berlin, Germany) column (25cm × 4.6mm, 5 µm particle size) was used for the separation.

The finally selected optimized conditions were as follows: injection volume 20 µl, the mobile phase was isocratically pumped at 1 mL/min at ambient temperature, and the detection wavelength was 227 nm.

Chemicals and reagents:

The gift sample of Paracetamol was obtained from IPCA Laboratories Ltd., Mumbai and Piroxicam was obtained from Red Cross Pharmaceutical Ltd., Aurangabad. Tablet of brand name (Progesic plus, BIPL Pharma.) was procured from local pharmacy containing paracetamol (325 mg) and piroxicam (20mg). Acetic acid was of analytical grade, HPLC grade methanol and water was procured from Qualigens Ltd., Mumbai.

Mobile phase:

Methanol: Water (70:30 v/v), pH was adjusted to 4.0 with acetic acid.

Preparation of standard stock solutions:
Reference standard of paracetamol 10 mg and piroxicam 10 mg was transferred to 10 mL volumetric flask separately and dissolved in methanol. The flask was shaken for 30 min and the volume was made up to the mark with mobile phase to obtain standard stock solution of paracetamol and piroxicam 1000 µg/mL each. Stock solution was filtered through a 0.2 µm membrane filter. The working standard solution of paracetamol and piroxicam was prepared from suitable aliquots of stock solution.

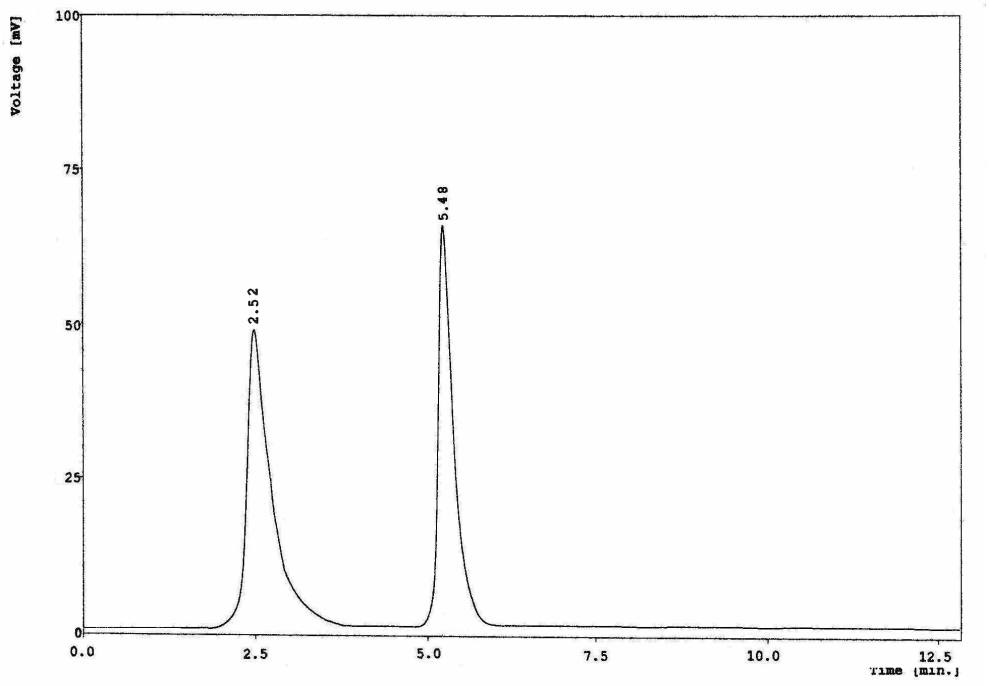
Optimization of the HPLC method
The pure drug solution of paracetamol and piroxicam were injected individually into HPLC system and allow run in different mobile phases like methanol: phosphate buffer, methanol: acetonitrile: water and methanol: water were tried in order to find the optimum conditions for the separation of paracetamol and piroxicam. It was found that mobile phase containing methanol and water (70:30, v/v), pH was adjusted to 4.0 with acetic acid, at a flow rate of 1.0mL/min with detection at 227 nm gave satisfactory results with sharp well defined and resolved peaks with minimum tailing as compared to other mobile phases. Under these conditions the retention times were typically 2.52 min for piroxicam and 4.41 min for paracetamol (Fig. 1).

Validation of the method [33]
Validation of the optimized HPLC method was carried out with respect to the following parameters.

Linearity and range
From Paracetamol standard stock solution, aliquots of 5, 10, 20, 30 and 40 µg/mL concentration was transferred to 10 mL volumetric flask and the volume was made up to the mark with mobile phase to obtain concentration of paracetamol 5-40 µg/mL. In the same way piroxicam aliquots of 1, 2, 4, 6 and 8 µg/mL was transferred to 10 mL volumetric flask from piroxicam standard stock solution and the volume was made up to the mark with mobile phase to obtain concentration of piroxicam 1-8 µg/mL. The solution of 20 µL was injected into column with the help of Hamilton syringe. All measurements were repeated six times for each concentration. The calibration curves of the area under curve Vs concentration were recorded for both drugs.

Precision
The precision of the method was verified by repeatability, interday and intraday precision. Repeatability studies were performed by analysis of three different concentrations of the drug in six times on the same day. Intra day precision was determined by analyzing tablet sample solutions at different time intervals on the same day and on different day for interday precision.

Figure 1.:Chromatogram of Piroxicam (t_R 2.52 min) and Paracetamol (t_R 5.48 min) in tablet formulation (Progesic plus) showing no interference of excipients in analysis.



Accuracy
Recovery studies were carried out by adding a known amount of standard solution of pure drug (paracetamol and piroxicam) to a preanalysed sample solution. These studies were carried out at 80%, 100% and 120% level.

Limit of detection and limit of quantitaiton
The LOD and LOQ were separately determined based on the calibration curves. The Standard Deviation of they – intercept and slope of the regression line were used.
The LOD and LOQ were calculated using the formulas,
LOD = 3.3 × D / S
LOQ = 10 × D / S
Where,
S = Slope of regression line
D = Standard deviation of y- intercept on the regression line

Robustness of method
To evaluate the robustness of the developed RP-HPLC method, minute variations in the optimized method parameters were done. The parameters such as, effect of change in pH of mobile phase, flow rate, effect of mobile phase ratio on the retention time, theoretical plates, area under curve and percentage content of paracetamol and piroxicam were studied. The solution containing, Paracetamol (32.5 µg/mL) and Piroxicam (2 µg/mL) was injected into sample injector of RP- HPLC three times under the varied conditions.

Ruggedness of method
Standard solution containing mixture of paracetamol (32.5 µg/mL) and piroxicam (2 µg/mL) was prepared from stock solution and analyzed by two different analyst using same operational and environmental conditions. From the area, the amounts of both the drugs were calculated.

System Suitability Parameters
As per USP-24, system suitability tests were carried out on freshly prepared standard stock solution of paracetamol and piroxicam of both drugs 20 µL were injected under optimized chromatographic condition and following parameters were studied to evaluate the suitability of the system.

Analysis of a marketed formulation

Twenty tablets of paracetamol and piroxicam (Progesic plus*) were weighed accurately to determine average weight of tablets and then the tablets were crushed to fine powder. The tablet powder equivalent to Paracetamol (325 mg) and Piroxicam (20 mg) was weighed, transferred to a 100 mL volumetric flask and dissolved in methanol, shake for 30 min and the volume was made up to the mark with mobile phase. The content was ultrasonicated for 20 min. The solution was filtered through a 0.2 µm membrane filter paper. This tablet solution was further diluted with mobile phase to obtain mixed sample solutions in the Beer's and Lamberts range.

Results and Discussion

The results of validation studies on simultaneous estimation method developed for paracetamol and piroxicam in the current study involving methanol and water (70:30, v/v), pH was adjusted to 4.0 with acetic acid, as the mobile phase for HPLC are given below.

Linearity

The drug response was linear (r^2 = 0.9989 for paracetamol and 0.9995 for piroxicam) over the concentration range between 0-40 µg/mL for paracetamol and 1-8 µg/mL for piroxicam. The mean (± SD) values of the slope, intercept and correlation coefficient for paracetamol and piroxicam were 0.021 (± 0.14), 0.000741 (± 0.13), 0.9998 (± 0.0001) and 0.012 (± 0.10), 0.000163 (± 0.17), 0.9996 (± 0.0001), respectively.

Precision

The results of the repeatability, intra-day and inter-day precision experiments are shown in Table 1(a) and Table 1(b). The developed method was found to be precise as the RSD values for repeatability of intra-day and inter-day precision studies were < 2 %, respectively which is under limit as per recommendations of ICH guidelines.

LOD and LOQ

The LOD and LOQ were separately determined based on the calibration curves for paracetamol and piroxicam. The LOD and LOQ were found to be 0.118 µg/mL and 0.359 µg/mL for paracetamol and 0.046 µg/mL and 0.138 µg/mL for piroxicam respectively.

Robustness and Ruggedness

The standard deviation of the peak areas was calculated for each parameter and the % RSD was found to be less than 2 %. Results shows low values of % RSD, as shown in Table 2 (a) and Table 2 (b) signify the robustness and ruggedness of the method.

Recovery studies

As shown from the data in Table 3, good recoveries of the paracetamol and piroxicam in the range from 99 to 101 % were obtained at various added concentrations.

Analysis of a marketed formulation

Experimental results of the amount of paracetamol and piroxicam in tablets, expressed as a percentage of label claims were in good agreement, thereby suggesting that there is no interference from any of the excipients which are normally present in tablets. In the replicate analysis

(n=3) of paracetamol and piroxicam by proposed method showed that the content of paracetamol and piroxicam was 99.98% and 100.08% respectively. The retention times of piroxicam and paracetamol was found to be 2.52 and 5.48 min respectively and the result of the analysis of tablet are given in Table 4.

System suitability studies

The column efficiency, resolution and peak asymmetry were calculated for the standard solutions and the results are expressed in Table 5. The values obtained demonstrated the suitability of the system for analysis of this drug combination.

Conclusion

The proposed RP-HPLC method for the simultaneous estimation of paracetamol and piroxicam in combined tablet dosage forms is accurate, precise, linear, rugged, robust, simple, rapid, and selective. It can be adopted efficiently and easily for routine quality control (QC) analysis of raw materials, formulations and dissolution testing with accuracy and repeatability of results.

Table No. 1: Precision studies. a) Repeatability (n= 6)

Component	Amount Present (mg)	Amount Found (%)	Standard Deviation	% Coefficient of Variation	Standard Error*
Para	325	99.96	0.489	0.480	0.289
Pix	20	100.00	0.526	0.527	0.194

b) Inter-day and intra-day precision

Parameter	Inter-day precision (n= 6)		Intra-day precision (n= 6)	
	Paracetamol	Piroxicam	Paracetamol	Piroxicam
% Mean	99.95	100.08	99.94	100
S.D.	0.065	0.045	0.687	0.598
% R.S.D.	0.065	0.045	0.688	0.599
S.E.	0.029	0.020	0.315	0.297

Table No. 2 : a) Robustness testing

Factor	Level	Retention time		Theoretical Plates		Area		% Content	
Flow Rate (mL/min)		PARA	PIX	PARA	PIX	PARA	PIX	PARA	PIX
0.90	-1	5.76	2.69	5758	8965	1625.21	170.35	99.81	99.91
1.0	0	5.48	2.52	5562	8535	1572.73	169.65	99.98	100.08
1.1	+1	5.41	2.47	5513	8496	1516.14	168.33	100.02	100.13
Mean ± S.D (n = 3)		5.55 ± 0.185	2.56 ± 0.115	5611 ± 129.64	8665.33 ± 260.25	1571.36± 54.548	169.44 ± 1.02	99.94 ± 0.112	100.04 ± 0.115
% of methanol in the mobile phase (v/v)									
69	-1	5.43	2.49	5498	8478	1531.86	169.83	99.93	99.94
70	0	5.48	2.52	5562	8535	1572.73	169.65	99.98	100.08
71	+1	5.51	2.57	5624	8618	1596.93	170.86	99.88	100.18
Mean ± S.D. (n = 3)		5.473 ± 0.029	2.526 ± 0.014	5561.3 ± 63.0	8543.67± 70.40	1567.17± 32.89	170.72± 0.65	99.93 ± 0.050	100.07± 0.121
pH of mobile phase									
3.9	-1	5.52	2.54	5589	8611	1456.00	168.35	99.79	99.92
4.0	0	5.48	2.52	5562	8535	1572.73	169.65	99.98	100.08
4.1	+1	5.45	2.46	5499	8512	1581.25	170.25	100.05	100.12
Mean ± S.D. (n = 3)		5.483 ± 0.035	2.506 ± 0.041	5550 ± 46.184	8552.67± 51.81	1536.66± 69.98	169.42± 0.971	99.94 ±0.135	100.04± 0.106

PARA = Paracetamol, PIX = Piroxicam

b) Ruggedness studies

Drug	Label Claim (mg/mL)	Amount Found (%)	
		Analyst I	Analyst II
Paracetamol	325	99.93	99.85
Piroxicam	20	100.02	100.12

Table No. 3: Recovery studies

Drug	Label claim (mg/tablet)	Amount added (mg)	Total amount (mg)	Amount recovered (mg) ± S.D.	Recovery (%)
PARA	325	260 (80 %)	585	585.02 ± 0.068	100.003
		325 (100 %)	650	650.08 ± 0.202	100.012
		390 (120 %)	715	715.07 ± 0.147	100.009
PIX	20	16 (80 %)	36	35.943 ± 0.0602	99.84
		20 (100 %)	40	39.776 ± 0.1939	99.44
		24 (120 %)	44	43.893 ± 0.0378	99.75

Table No. 4: Analysis of tablet formulation.

Drug	Labelled amount[a] (mg/tab)	Amount found (mg/tab)	Recovery (%) Mean ± S.D.
Paracetamol	325	324.97	99.99 ± 0.02
Piroxicam	20	19.99	99.97 ± 0.16

[a] Progesic plus Tablet, BIPL Pharma, India.

Table No. 5: System suitability studies

System Suitability Parameters	Proposed Method	
	Piroxicam	Paracetamol
Retention Time (t_R)	2.52	5.48
Capacity Factor (k)	1.65	3.41
Theoretical Plate Number (N)	8535	5562
Tailing Factor (T)	0.76	0.51
Resolution Factor (R)	3.172	-

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