Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Issue 8, 2015

Original Article

A NEW METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF PARACETAMOL IN PHARMACEUTICAL DOSAGE FORM BY REVERSE PHASE-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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Received: 07 Jan 2015 Revised and Accepted: 20 Jun 2015

ABSTRACT

Objective: An accurate, simple, reproducible and sensitive method for the determination of paracetamol in pharmaceutical dosage form was developed and validated using a reversed-phase C_{18} column (250 mm X 4.6 mm i. d, 5 µm particle size) with isocratic elution.

Methods: A mixture of Acetonitrile: 10 mM potassium dihydrogen orthophosphate buffer (15:85 v/v), pH 2.5 was used as a mobile phase at the flow rate of 1.0 ml/min and detector wavelength at 210 nm. The retention time of paracetamol was found to be 5.7 minutes (min). The method was statistically validated for the linearity, accuracy, precision and robustness.

Results: The linearity of paracetamol was in the range of 25.00 to 60.00µg/ml. This method showed an excellent linear response with the correlation coefficient (R^2) value of 0.999 for the paracetamol. The recovery of the drug was ranged from 99.51 to 100.68%. An intra-day and inter-day precision study of the new method was less than the maximum allowable limit (% RSD<2.0).

Conclusion: The proposed method was cost effective, which can be used for the estimation of paracetamol in bulk and in solid dosage forms.

Keywords: Paracetamol, RP-HPLC, Isocratic, Retention time, Validation.

INTRODUCTION

Paracetamol (PCM) is one of the most accepted analgesic and antipyretic drug and is used in the treatment of pain and fever in adults and children. PCM exists in varied dosage forms like tablets, capsules, suspensions and suppositories [1, 2]. PCM is the de-ethylated active metabolite of phenacetin, with molecular formula of $C_8H_9NO_2$ and molecular weight of 151.16. Its chemical structure is given in fig. 1.

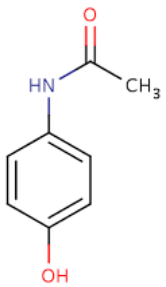

Fig. 1: Chemical structure of paracetamol

PCM has analgesic, antipyretic and weak anti-inflammatory activity. The exact mechanism by which, paracetamol produces its analgesic and antipyretic effects remains undefined. The primary mechanism of action is believed to be inhibition of cyclooxygenase (COX), with a predominant effect on COX-2. Inhibition of COX enzymes prevents the metabolism of arachidonic acid to prostaglandin H_2, an unstable intermediate byproduct which is converted to pro-inflammatory compounds. In the central nervous system, inhibition of COX enzymes reduces concentrations of prostaglandin E_2, which lowers the hypothalamic set-point to reduce fever, and activation of descending inhibitory serotonergic pathways to produce analgesia [3-5].

Development of analytical method for the assessment of drugs in pharmaceutical dosage form is of utmost necessity to confirm the quality of tablets with respect to assay. Various methods such as spectrophotometry and High Performance Liquid Chromatography (HPLC) methods have been reported for the analysis of paracetamol alone or in combination with other drugs in pharmaceutical preparations. But the reported methods were less sensitive with unsatisfied peak shape and less theoretical plates [6, 7]. The purpose of the current effort is to develop and validate a new analytical method for the determination of PCM in tablet dosage form. In this proposed method, we have put an effort to develop a cost-effective, rapid, and robust Reverse Phase High Performance Liquid Chromatography (RP-HPLC) method with enough data of validation parameters. However, to the best of our knowledge, using isocratic RP-HPLC method, we have used less organic phase for the estimation of PCM as compared to the reported studies [2, 8-11].

MATERIALS AND METHODS

All the chemicals and reagents were used in this method were analytical reagent grade and were obtained from Merck India. HPLC grade acetonitrile and potassium dihydrogen orthophosphate were obtained from S. D. Fine Chem Ltd. Deionized 18.2 MΩ water was used during the analysis was obtained by the water purification system (ELGA, UK). PCM Tablets IP 500 mg (Calpol Tablets) was obtained from our medical college Pharmacy. The HPLC-2010C_{HT} (Shimadzu, Japan) was equipped with UV detector, quaternary gradient pump and auto injector with 100 µl fixed loop and the analyte was monitored at 210 nm. Chromatographic analysis was performed on a phenomenex C18 column having 250 cmX4.6 mm i. d and 5 µm particle size. All the drugs and the chemicals were weighed on the electronic balance (BSA224S-CW, Sartorius, Germany). The mobile phase was filtered through 0.2µ membrane filter and degassed. The determination of PCM was performed at ambient temperature.

Chromatographic conditions

The phenomenex C-18 column was equilibrated with the mobile phase, acetonitrile: 10 mM potassium dihydrogen phosphate15:85

(v/v); pH 2.5. The active drug was monitored with UV detector at 210 nm, and the injection volume was 50 µl. The flow rate was maintained at 1.0 ml/min and the total run time was kept 8 min.

Preparation of standard stock solution

10.00 milligram (mg) of PCM was weighed and transferred to 10 mL volumetric flask and dissolved in the mobile phase (diluent) to get a solution containing 1 mg/ml.

Preparation of working standard solution

The stock solution was diluted with mobile phase to obtain the working standard of 50µg/ml.

Preparation of sample solution

Twenty tablets, each containing 500 mg of PCM weighed and finely powdered; a quantity of powder equivalent to 50 mg of PCM was weighed and transferred to 100 mL volumetric flask. To this 20 mL of diluent was added and kept for the sonication for 30 min, later made up to the volume by using diluent. The sample solution was filtered through 0.20 µm membrane filter, and then 5 ml of filtrate was further diluted to 50 mL volumetric flask by using diluent to get a concentration of 50µg/ml.

Method validation

All the method validation parameters such as accuracy, linearity, precision, detection limit, quantification limit and robustness were validated as per the International Conference on Harmonization (ICH) guidelines [12].

System suitability parameters

To evaluate system suitability parameters such as theoretical plates, tailing factor and retention time of six replicate injections of standard paracetamol of concentration 50µg/ml was used and the % RSD values were calculated.

Linearity and range

The linearity was analyzed through the standard curves ranging from 25.0µg/ml to 60.0µg/ml. The linearity was evaluated by linear regression analysis, which was calculated by the least-square regression analysis.

Accuracy

The truthfulness of an analytical method expresses the closeness between the expected value and the value found. In the present study, consecutive analysis (n=3) for three different concentration of the standard mixture (80, 100 and 120% of the nominal concentration) was carried out to verify the accuracy of the proposed method.

Precision

Precision of the method was determined by repeatability (intra-day precision) and intermediate precision (inter-day precision) of both standard and sample solutions. Precision was determined in six replicates of both standard and sample solutions. The results were expressed as % RSD of the measurements.

Sensitivity

Limit of Detection (LOD) and Limit of Quantification (LOQ) was determined using calibration curve method according to ICH Q2 (R1) recommendations [12]. The LOD (k = 3.3) and LOQ (k = 10) of the proposed method was calculated using the following equation:

$A = k\sigma/S$, where A = LOD or LOQ, σ is the standard deviation of the response, and S is the slope of the calibration curve.

Robustness

To determine the robustness of the current method, the effect of flow rate was studied at 0.9 mL and 1.1 mL/min instead of 1.0 mL/min. The effect of pH was studied at 2.4 and 2.6 instead of 2.5. The effect of mobile phase composition was assessed at (Buffer: ACN = 86.5:13.5, v/v) and (Buffer: ACN = 83.5:16.5, v/v) instead of (Buffer: ACN = 85:15 v/v). The % RSD of robustness testing under these conditions was calculated.

RESULTS AND DISCUSSION

The main objective of the chromatographic method was to develop a precise, specific RP-HPLC method for the estimation of PCM. In order to develop a suitable isocratic RP-HPLC method, different buffer pH, organic solvent concentration and column chemistry were applied to achieve the isocratic elution of paracetamol. The mobile phase acetonitrile: 10 mM potassium dihydrogen orthophosphate (15:85 v/v) adjusted pH 2.5 using orthophosphoric acid with the flow rate of 1.0 mL/min and detector wavelength at 210 nm was found to be satisfactory.

The retention time of PCM was 5.6 min. Our proposed method has good symmetrical peak shape, theoretical plates and tailing factor as compared to reported studies [6, 7]. The mobile phase used in the present method has less organic phase as compared to other studies [2, 6-9, 13]. This may decrease cost of analysis, which may be economical to quality control labs. The typical chromatogram of the assay solution is shown in fig. 2.

System suitability

The results (mean±%RSD of six replicates) of the chromatographic parameters are shown in table 1, indicating the good performance of the system.

Linearity and range

The linearity of the method was determined at seven concentration levels ranging from 25.0µg/ml to 60.0µg/ml for PCM.

The calibration curve was constructed by plotting response factor against the concentration of drugs. The slope and the intercept value for the calibration curve was, y = 15839x+15476 (r^2= 0.999) for the PCM. The result show that an excellent correlation exists between the response factor and the concentration of drug. The linearity range was comparatively better than the reported studies [7, 11], which may help in detecting lower concentration of the drug. The calibration curve is shown in fig. 3.

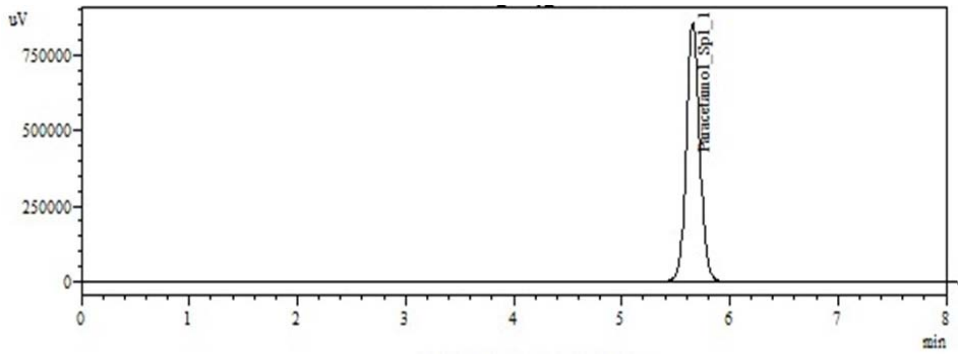

Fig. 2: Typical chromatogram of assay solution

Table 1: Results of system suitability parameters

S. No.	Parameter	Value (mean±% RSD)*
1	Retention Time	5.363±0.009
2	Peak Area	7809879±0.119
3	Theoretical plates	8976±0.897
4	Tailing factor	1.02±0.173

***** Mean and % relative standard deviation of six replicates.

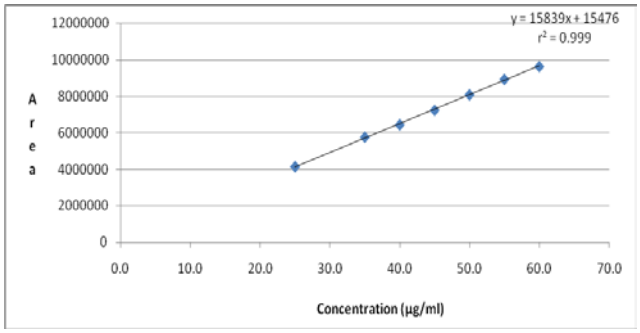

Fig. 3: Calibration curve for Paracetamol

Accuracy

Accuracy of the developed method was determined by the method of standard addition. Known amount of PCM solution was added to a pre quantified sample solution, and the amount of PCM was estimated by measuring the peak areas. The percentage recovery varied from 99.90 to 100.58 for PCM, indicating good accuracy of the method. Percentage recovery of PCM drug was calculated and shown below in the table 2.

Precision

The precision of the method was determined by intra-day precision and inter-day precision of both standard and sample solutions. Precision was determined in six replicates of both standard (50µg/ml) and sample solutions (50µg/ml). The results were expressed as %RSD of the measurements. % RSD values were below 2% signifies a good precision. The repeatability, intra-day and inter-day results are shown in the table 3(A-D).

Sensitivity

The LOD and LOQ were established by evaluating the minimum level at which the analyte could be readily detected and quantified with accuracy, respectively. The LOD and LOQ of PCM by the proposed method were found to be 0.02µg/ml and 0.06µg/ml, respectively as shown in table 4.

Table 2: Results of recovery studies

Concentration of Paracetamol (%) (n=3)	Added amount (mg)	Amount recovered (mg)	Amount recovered (%)(mean±SD)*	RSD (%)
80	3.512	3.508	99.90±0.357	0.36
100	4.390	4.409	100.43±0.145	0.14
120	5.268	5.299	100.58±0.119	0.12

***** mean±Standard Deviation of three replicates.

Table 3: Results of method precision

A. Repeatability

S. No.	Sample Area	Amount Recovered (in mg)	% Recovery
1	7759330	497.96	99.59
2	7773639	499.42	99.88
3	7770331	498.51	99.70
4	7765042	498.71	99.74
5	7774421	499.08	99.82
6	7776524	498.75	99.75
Mean	7769881	498.74	99.75
Std Dev	5969.4	0.4981	0.0999
% RSD	0.08	0.10	0.10

B. Intermediate precision (Day 1)

S. No.	Sample Area	Amount Recovered (in mg)	% Recovery
1	7762565	498.78	99.76
2	7783404	500.35	100.07
3	7781845	499.86	99.97
4	7761433	499.41	99.88
5	7786419	500.08	100.02
6	7784785	500.28	100.06
Mean	7776742	499.79	99.96
Std Dev	10520.7	0.6012	0.1202
% RSD	0.14	0.12	0.12

C. Intermediate precision (Day 2)

S. No.	Sample Area	Amount Recovered (in mg)	% Recovery
1	7752615	499.41	99.88
2	7774855	500.76	100.15
3	7768489	500.98	100.2
4	7751219	499.63	99.93
5	7772985	501.58	100.32
6	7777442	501.16	100.23
Mean	7766268	500.59	100.12
Std Dev	10500.2	0.8718	0.175
% RSD	0.14	0.17	0.17

D. Comparison between Intermediate Precision Day 1 (Analyst 1) and Intermediate Precision Day 2 (Analyst 2)

Sample	Taken amount of sample Paracetamol (mg)	Analyst-1		Analyst-2	
		Amount found(mg)	% Recovery±SD*	Amount found(mg)	% Recovery± SD*
Calpol 500 mg Tablet	500	499.79	99.96±0.120	500.59	100.12±0.175

* % of Recovery±Standard Deviation of six samples (assay).

Table 4: Results of LOD and LOQ

S. No.	Parameters	Paracetamol
1	LOD	0.02 (µg/ml)
2	LOQ	0.06 (µg/ml)

Table 5: Robustness of the method

Parameter		Amount of paracetamol added (µg/ml)	Amount of paracetamol detected (mean±SD)*	% RSD
Change in mobile phase composition	Buffer: ACN = 86.5:13.5	50	49.80±0.19	0.13
	Buffer: ACN = 85:15	50	50.30±0.28	0.08
	Buffer: ACN = 83.5:16.5	50	50.60±0.14	0.37
Change in pH mobile phase	2.40	50	50.90±0.53	0.05
	2.50	50	50.30±0.28	0.08
	2.60	50	51.30±0.64	0.01
Change in flow rate	0.9 mL/min	50	50.50±0.32	0.34
	1.0 mL/min	50	50.30±0.28	0.08
	1.1 mL/min	50	51.20±0.64	0.37

* mean±Standard Deviation of six replicates.

Robustness

The %RSD of robustness testing under different altered conditions is indicating that the current method is robust. Slight deliberate changes were made from the optimized parameters to check the robustness of the method. The method was found to be rugged and the results are shown in the table 5

CONCLUSION

The proposed study describes HPLC method for the identification and quantification of PCM. The method was validated and found to be simple, sensitive, rapid, accurate and precise. The developed method was cost effective as compared to the reported methods. The high percentage of recovery shows that the method can be successfully used for routine analysis. Hence the present RP-HPLC method is suitable for the quality control analysis of raw materials, formulation and stability studies.

ACKNOWLEDGEMENT

The Authors are grateful to the Chairman Sri A. C Shanmugam, Vice-chairman Sri ACS Arunkumar and Executive Director of RRMCH, Bangalore for providing all the facilities to carry out this research work.

CONFLICT OF INTERESTS

Declared None

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