International Standard Serial Number (ISSN): 2319-8141
International Journal of Universal Pharmacy and Bio Sciences 8(2): March-April 2019
INTERNATIONAL JOURNAL OF UNIVERSAL PHARMACY AND BIO SCIENCES
*IMPACT FACTOR 4.018****
*ICV 6.16****
Pharmaceutical Sciences **RESEARCH ARTICLE……!!!**

SIMULTANEOUS DETERMINATION AND VALIDATION OF PARACETAMOL AND CHLOROQUINE PHOSPHATE BY RP-HPLC IN BULK AND PHARMACEUTICAL FORMULATION

Swarnalatha B M*, Manzoor Ahmed, A Satish Kumar Shetty

Department of Pharmaceutical Analysis, National College of Pharmacy, Shimoga - 577201, Karnataka, India.

KEYWORDS:

RP-HPLC, Paracetamol, Chloroquine Phosphate, UV detection, Validation.

FOR CORRESPONDENCE:

Swarnalatha B M*

ADDRESS:

Department of Pharmaceutical Analysis, National College of Pharmacy, Shimoga - 577201, Karnataka, India.

ABSTRACT

The simple, accurate, precise, and rapid RP-HPLC method was developed and validated as per the ICH guidelines for the determination of Paracetamol (PARA) and Chloroquine Phosphate (CHQ) in combined dosage form. The proposed RP-HPLC method utilizes a C18, 5µm, 250mm × 4.6mm i.d. column, using 0.05M Phosphate Buffer (pH 3.5) and Methanol as mobile phase in the ratio of 60:40, and UV detection at 283 nm. The retention time was 4.819 min and 6.165 min for PARA and CHQ at a flow rate of 1 mL/min. The described method was linear over a range of 10-50 µg/mL for both the drugs. The percentage mean recovery was found to be 99.95 for PARA and 99.98 for CHQ. The method was statistically validated for its linearity, accuracy and precision. Both interday and intraday precision was found to be showing less %RSD having high grade of precision of the method.

INTRODUCTION:

Paracetamol is used as an analgesic and antipyretic. It is official in the IP. Chloroquine phosphate is used mainly as antimalarial and anti-inflammatory. It is official in IP. Several methods are reported for individual estimation of PARACETAMOL and CHLOROQUINE. Combination of these two drugs used in the treatment of malaria.

Paracetamol (PARA), chemically know as N-(4-hydroxyphenyl)acetamide. It has weak activity on COX in the inflamed peripheral tissues which have high concentration of peroxides; however, it equals the blocking effect of asperin on this enzyme in the brain. Therefore, paracetamol is a potent antipyretic and is equi analgesic with asperin in therapeutic doses but devoid of significant anti-inflmmatory effect [1-4].

Chloroquine phosphate (CHQ), chemically known as 7-chloro-4[{4-(diethylamino)-1-methylbutyl} amino] quinoline, is a 4-aminoquinolein antimalarial drug. It is the prototype synthetic antimalarial drug most widely used to treat all types of malarial infections. The drug is also prescribed to decrease the symptoms of rheumatoid arthritis and to treat systemic and discod lupus erythematosus in adults [5-7].

The combination of Paracetamol and Chloroquine was significantly used in the treatment of malaria [8-10].

MATRIALS AND METHODS:

Instrument

A Shimadzu class HPLC unit accomplished with SPD-20AD UV-Visible detector; Enable C18(250*4.6*5) Column (Shimadzu);LC-20 AD Pump; Quantitative HPLC was performed on a isocratic mode with 20µl injection of sample loop. The output signal was monitored and integrated using software class LABSOLUTIONS (Shimadzu).

Chemicals

Standard Paracetamol and Chloroquine Phosphate were procured from yarrow chem products, Mumbai. Methanol (HPLC grade) was obtained from s d fine-chem limited. Potassium dihydrogen ortho-phosphate, Double distilled Water was obtained from Spectrochem pvt. limited.

Preparation of mobile phase :

The mobile phase 1000 mL was prepared by mixing the Phosphate buffer and Methanol solution in the ratio of 60:40, pH was adjusted to 3.5 with Ortho-phosphoric acid. The mobile phase was filtered through a 0.45 µm filter paper and then sonicated for 10 min.

Preparation of Buffer solution

6.8 g of Potassium dihydrogen O-phosphate was dissolved in 1000ml of HPLC water (0.05M)

Preparation of standard solutions

100 mg each of PARA and CHQ were weighed separately and transferred in two different 100 mL volumetric flasks. Both the drugs were dissolved in 50 mL of mobile phase by sonication and then volume was made up to the mark with mobile phase to get a concentration of 1000 µg/mL of each component (stock A and A' solution).

From the above stock solution further dilutions were made to get the concentration range of 10-50µg/mL for both the drugs.

Preparation of sample solution:

B. Analysis of tablet formulation.

Twenty tablets of PARA and CHQ in combination were weighed and their average weight was determined. The tablets were crushed to fine powder and a tablet powder equivalent to 100 mg of PARA was weighed which also contains 100 mg of CHQ and transferred to 100 mL volumetric flask, dissolved in sufficient quantity of mobile phase. The solution was filtered through 0.4 µm membrane filter paper. The contents were sonicated for 20 minutes and the final volume was made up to the mark with mobile phase (sample stock "A" solution).

Appropriate aliquots were pipetted out from the sample stock "A" solution (1000 µg/mL) in to a series of 10 mL volumetric flask. The volume was made up to the mark with the mobile phase to get a set of solutions having the concentration range of 10 - 50 µg/mL of PARA and 10 - 50 µg/mL of CHQ.

A 20 µl volume of each sample mixture was injected in to the sample injector of HPLC system and their chromatograms were recorded under the same chromatographic conditions as described above. The area of each peak was determined at 283.0 nm and the amount of drug present in the sample mixture was determined.

Method Validation:

The developed analytical method was subjected to validation with respect to various parameters such as linearity, limit of detection (LOD), limit of quantification (LOQ) accuracy, precision, recovery studies and reproducibility as per the ICH guidelines

RESULTS AND DISCUSSION :

The proposed method was found to be simple and linear in the concentration range of 10-50 µg/ml for Paracetamol and 10-50 µg/ml Chloroquine Phosphate respectively. The method was found to be accurate and precise as indicated by recovery studies and % RSD not more than 2. Moreover LOD and LOQ for Paracetamol were found to be 0.33µg/ml and 0.99 µg/ml, respectively and for Chloroquine Phosphate were 0.102 and 0.309µg/ml, respectively. Thus the method is specific and sensitive.

Linearity:

Linearity was established by least square regression analysis of the calibration curve. The constructed calibration curve was linear over the concentration range of 10-50 µg/ml for PARA and 10-50 µg/ml for CHQ. Peak area of PARA and CHQ were plotted versus their respective concentration and linear regression analysis was performed on the resultant curves (fig.3 & fig.4). The regression equation was found to be y = 13176x + 2380.8 (r^2 = 0.9999) for PARA and y = 12127x+1428.3 (r^2 = 0.9999) for Chq. Summary of Validation and System Suitability Parameters of Paracetamol and Chloroquine Phosphate are shown in Table (1).

Accuracy (Recovery studies)

To check the degree of accuracy of the method, recovery studies were performed in triplicate by standard addition method at 80%, 100% and 120%. Known amounts of standard mixture of Paracetamol and Chloroquine Phosphate was added to pre-analyzed samples and was subjected to the proposed HPLC method. Results of recovery studies are shown in Table 2.

Precision

Precision was evaluated by carrying out six independent sample preparation of a single lot of formulation. The sample solution was prepared in the same manner as described in sample preparation. Percentage relative standard deviation (%RSD) was found to be less than 2% for within a day and day to day variations, which proves that method is precise.

LOD and LOQ :

The LOD was determined as the lowest amount of analyte that was reproducibly quantified above the baseline noise following triplicate injection. LOD was determined on the basis of signal to noise ratios and was determined using the analytical response of three times the background noise. Both LOD and LOQ were calculated on the peak area using the following equation:

LOD = 3.3 × SD/Slope

LOQ = 10 × SD/Slope

Where, SD – Standard deviation of the peak areas (triplicate injection) of the drug,

The limit of detection and Limit of quantification of PARA and CHQ was found to be 0.33 µg/mL and 0.102 µg/mL and 0.99 µg/mL and 0.309 µg/mL, respectively

Robustness :

The Robustness was evaluated by analysing the samples by varying few parameters like wavelength and flow rate. The validation results obtained confirm the suitability of the proposed method for simple, accurate and precise analysis of PARA and CHQ in pharmaceutical preparations. The degree of

reproducibility of the results obtained as a result of small deliberate variations in the method parameters and by changing analytical operators had proved that the method was robust and the data is summarized in table 5 and 6.

Ruggedness:

The evaluation of robustness should be considered during the development phase and depends upon the type of procedure under study. It should show the reliability of analysis with respect to deliberate variations in analyst or instrument. The solution containing 30µg/ml of PARA and 30µg/ml of Chloroquine Phosphate was injected into sample injector of HPLC two times by different analysts. Ruggedness result for variations in Analyst as shown in table (7).

Fig 1: Chemical Structure of PARA

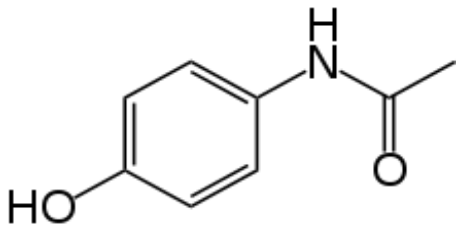

Fig 2: Chemical Structure of CHQ

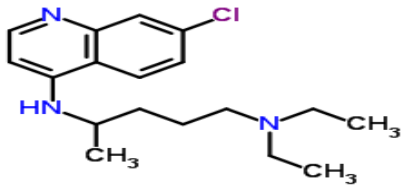

Fig 3 : Resolution Time of Paracetamol

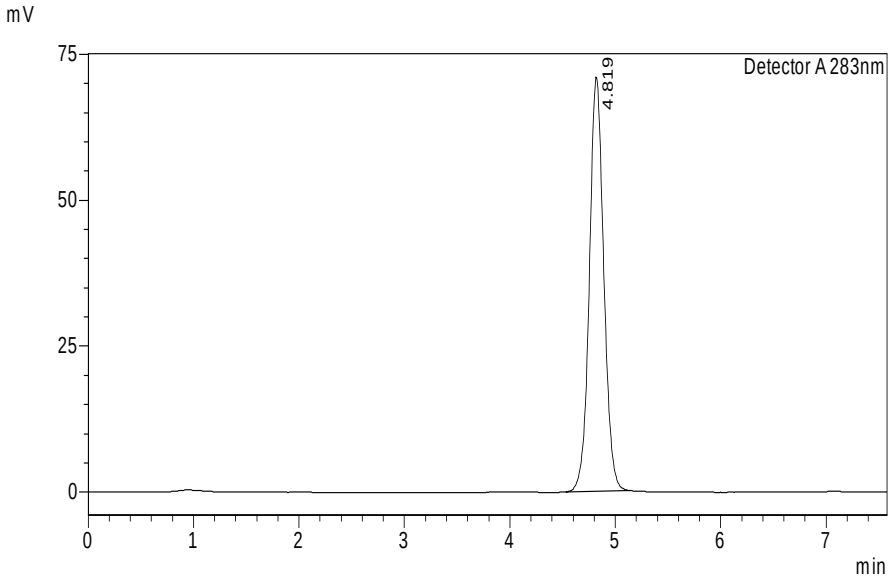

Fig 4 : Resolution time of Chloroquine Phosphate

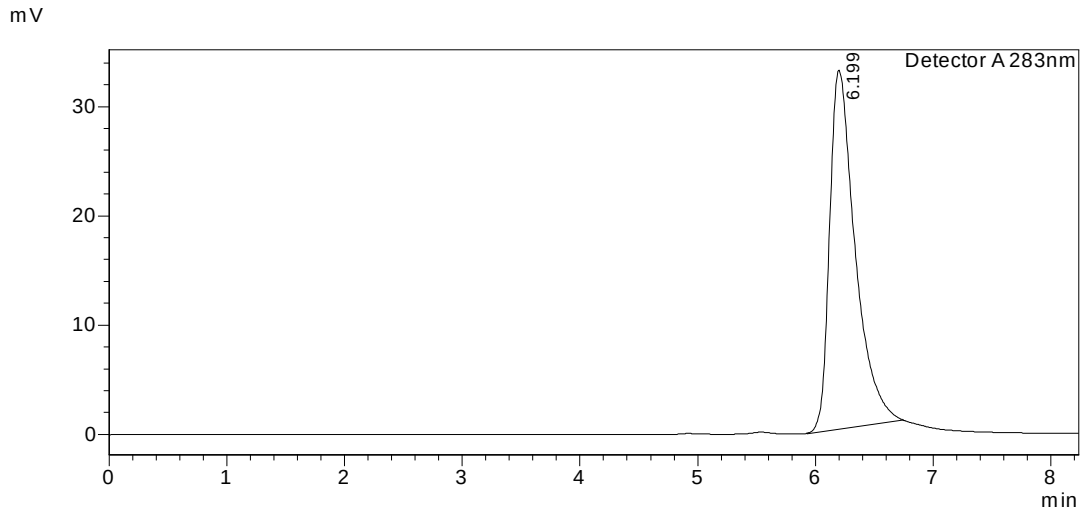

Fig 5 : Resolution Time Of PARA and CHQ

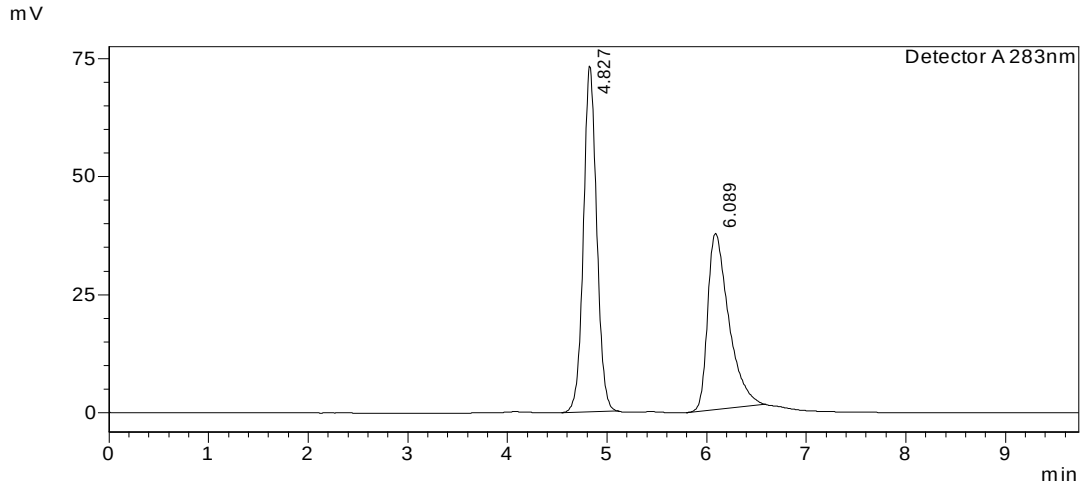

Fig 6 : Calibration curve for PARA at 283.0 nm by RP-HPLC Method

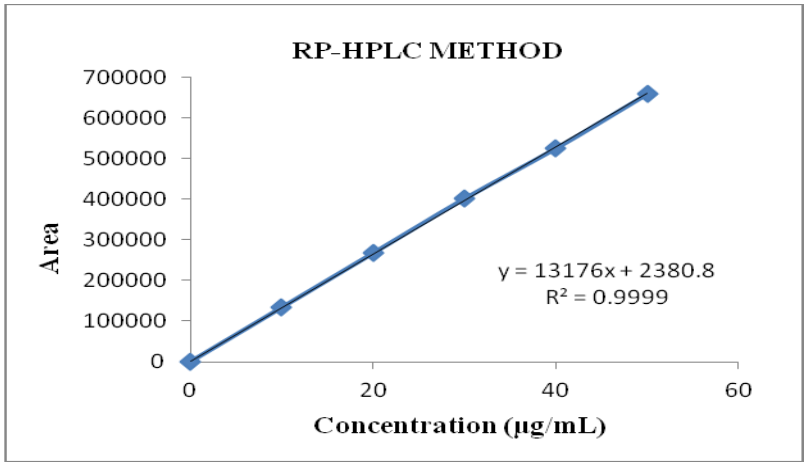

Fig 7 : Calibration curve for CHQ at 283.0 nm by RP-HPLC Method

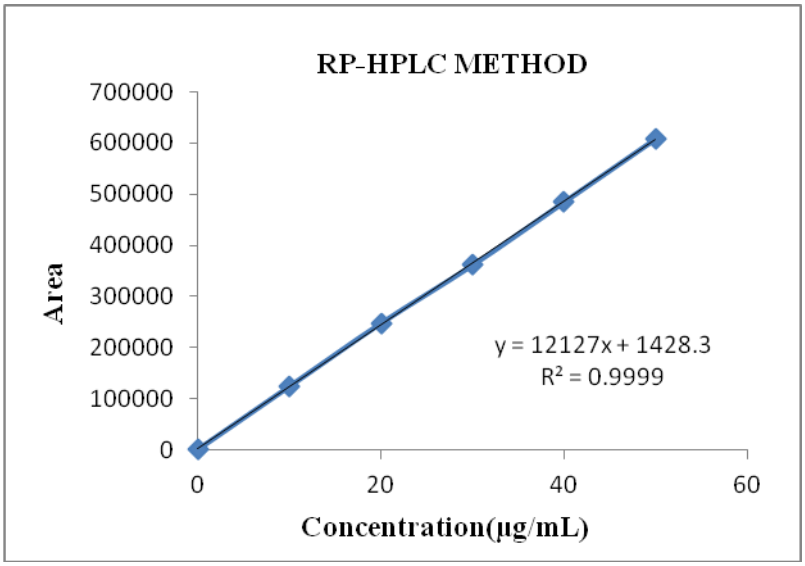

Table 1: Summary of validation Parameters by Developed Methods

Parameters	PARA	CHQ
Linearity Range µg/mL	10-50	10-50
Slope	13176	12127
Intercept	2380.8	1428.3
Regression Coefficient (r^2)	0.999	0.999
Limit of Detection (µg/mL)	0.33	0.102
Limit of Quantification µg/mL	0.99	0.309
Retention time (min)	4.819	6.165
Tailing factor	1.055	1.665
Resolution factor	3.996	
Theoretical plate	5777	4212

Table 2: Statistical Validation Data for Accuracy Determination

Level of % Recovery	Components	Amount present (µg/ml)	Amount of Standard drug added (µg)	Total amount recovered (µg)	% Recovery	RSD
80%	PARA	20	16	35.98	99.94	0.0424
	CHQ	20	16	35.97	99.91	0.1155
100%	PARA	20	20	39.96	99.9	0.0804
	CHQ	20	20	40.01	100.02	0.0381
120%	PARA	20	24	43.98	99.95	0.0860
	CHQ	20	24	43.97	99.93	0.0227

Table 3: Statistical Validation Data for Intra-day Precision

Components	Mean	Std.deviation	Co-efficient of variation	Standard error
PARA	100.00	0.0894	0.0894	0.0366
CHQ	100.00	0.0988	0.0988	0.0405

$n^* = 6$

Table 4 : Statistical validation data for Interday precision

Components	Mean*	Standard Deviation*	Co-efficient of Variation	Standard Error*
PARA	99.98	0.0981	0.0981	0.0402
CHQ	100.0	0.0894	0.0894	0.0366

Table 5: Robustness result for variations in Flow Rate (mL/min)

Method Parameter	Level	Retention Time		Tailing factor	
Flow rate (mL/min)		PARA	CHQ	PARA	CHQ
0.9	-1	4.899	6.253	1.098	1.698
1.0	0	4.819	6.199	1.055	1.665
1.1	+1	4.632	6.029	1.015	1.651

Table 6: Robustness result for variations in Wavelength (nm)

Method Parameter	Level	Retention Time		Tailing factor	
Wavelength(nm)		PARA	CHQ	PARA	CHQ
281	-2	4.792	6.157	1.034	1.653
283	0	4.819	6.199	1.055	1.665
285	+2	4.753	6.253	1.085	1.699

Table 7: Ruggedness result for variations in Analyst.

Method Parameter	Retention Time		Tailing Factor	
Analysts	PARA	CHQ	PARA	CHQ
Analyst 01	4.819	6.199	1.055	1.665
Analyst 02	4.782	6.078	1.037	1.661

CONCLUSION :

The proposed RP-HPLC method for the simultaneous estimation of Paracetamol and Chloroquine Phosphate in combined dosage forms was found to be sensitive, accurate, precise, simple and rapid. Hence the present RP –HPLC method may be used for routine analysis of the raw materials and formulations.

AKNOWLEDGEMENT:

Authors express sincere thanks to the principal and staff department of pharmaceutical analysis of National College of pharmacy, Shimoga for guidance, encouragement and providing laboratory facilities.

REFRENCES:

1. Satoskar R S, Nirmala N Rege, Bhandarkar S D. Pharmacology and pharmacotherapeutics. 23rd ed.2013:p.11-7.
2. Indian pharmacopoeia. Indian Pharmacopoeia commission Ghaziabad, 2010(3):1861-2.
3. Sharma H L, Sharma K K. Principle of pharmacology. 1st ed.2007:p.836
4. https://pubchem.ncbi.nlm.nih.gov/compounds.
5. Laurenc LB, John SL, Keith LP. Goodman and Gilman's. The pharmacological basis of therapeutics; McGraw Hill, Newyork, U.S.A. 2005: 1119-21.
6. https://en.wikipedia.org/wiki.
7. Palled P J, Dushyanth Reddy V, Mannor VS and Bharat Chowdary. Validated isocratic/gradient RP-HPLC for simultaneous estimation of Paracetamol Ibuprofen and Caffeine in marketed formulations using diclofenac as internal standard. Ana chem: an indian J, 2017;17(1):1-20.
8. Deepali Gharge, Pandurang Dhabale. Simultaneous estimation of Nimesulide and Paracetamol in solid dosage form by RP-HPLC method. Int J of pharmaTec Res, 2010;2(2):1330-3.
9. Samson Israel D, Krishna Chaitanya K, Gowri Sankar D, Vijayalakshmi A. Method development and validation for simultaneous determination a multiple drug dosage form of paracetamol, Orphenedrine, Ibuprofen by RP-HPLC. J of global trends in ph sci,2013;4(3):1153-62.
10. Lawal A, Umar R A, Abubakar M G, Faruk U Z. Development and validation of UV spectrophotometric and HPLC methods for quantitative determination of Chloroquine and Amodiaquine in pharmaceutical formulations. Int J of chemTec Res,2012;4(2):669-76.