International Journal for Pharmaceutical Research Scholars (IJPRS)

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

RESEARCH ARTICLE

RP-HPLC Methods Development for the Simultaneous Estimation of Quinine and Ciprofloxacin

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Manuscript No: IJPRS/V4/I2/00070, Received On: 25/04/2015, Accepted On: 03/05/2015

ABSTRACT

A simple, rapid, sensitive and specific liquid chromatographic method with UV detection for the simultaneous estimation of quinine and ciprofloxacin was developed. Separation was achieved with Hibar^R 250-4.6 HPLC column Purosphens^R STAR RP-18 and mobile phase containing acetonitrile: methanol: triethylamine (0.1%) in double grade distilled water in the ratio 1:6:3 (pH was adjusted with ortho-phosphoric acid as 3.8) at flow rate 1 ml/min. Quantitation was achieved with UV detector at 236 nm. The selected chromatographic conditions effectively separated quinine and ciprofloxacin with retention time of 4.383 and 3.042 min. respectively. The developed method is precise, accurate, reproducible and specific.

KEYWORDS

Ciprofloxacin Hydrochloride, Reverse Phase High Performance Liquid Chromatography, Quinine Sulphate

INTRODUCTION

Quinine sulphate is a white or almost white, needle like crystals or crystalline power. It is slightly soluble in water, sparingly soluble in boiling water and in alcohol, completely soluble in acetonitrile. The pH of a 10g/l suspension in water is 5.7 to 6.6. Store in air tight containers, Protected from light.

Drug Profile

Cinchonan-9-ol, 6'methoxy-(8α, 9R)-sulphate (2:1) (salt), dihydrate

Chemical Structure

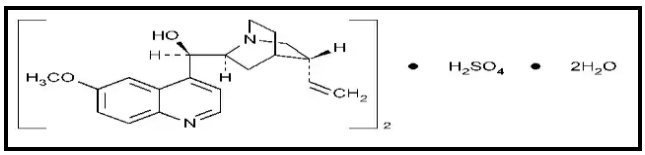

Figure 1: Structure of quinine sulphate

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Molecular Formula

$(C_{20}H_{24}N_2O_2).2H_2SO_4.2H_2O$

Molecular Weight

782.96g/mol[1]

Uses

Quinine is cinchona alkaloid that acts as a blood schizonticidal and weak gametocyte against *Plasmodium vivax* and *Plasmodium malariae*. As an alkaloid it is accumulated in the blood vacuoles of *plasmodium* species. Quinine is less effective and more toxic as a blood schizonticidal agent than chloroquine. However still it is very effective and widely used in the treatment of acute cases of severe *P. falciparum*. It is especially useful in the areas where there is known to be a high level of resistance to chloroquine[2].

Ciprofloxacin hydrochloride is a pale yellow, slightly hygroscopic, crystalline power. It is

soluble in water, slightly soluble in methanol, very slightly soluble in ethanol, practically insoluble in acetone, in ethyl acetate, and in methylene chloride. A solution has pH 3.5 to 4.5. Store in an air tight container, protected from light.

Drug Profile

1-cyclopropyl-6-fluoro-1,4-dihydro-oxo-7-(1-piperazinyl)-3-quinolinecarboxylicacid hydrochloride monohydrate

Chemical Structure

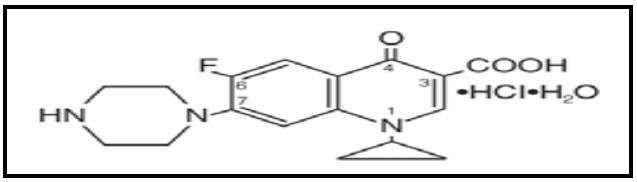

Figure 2: Structure of ciprofloxacin hydrochloride

Molecular Formula

$C_{17}H_{18}FN_3O_3$, HCl.H_2O

Molecular Weight

385.8 g/mol[3]

Uses

Ciprofloxacin has *in-vitro* activity against a wide range of gram-negative and gram-positive microorganisms. The bactericidal action of ciprofloxacin results from inhibition of the enzymes topoisomerase II (DNA gyrase) and topoisomerase IV, which are required for bacterial DNA replication, transcription, repair and recombination[4-5].

Quinine and ciprofloxacin both drugs are used for the treatment of malaria and typhoid fever respectively. There are so many methods described for the quinine and ciprofloxacin but there is no method estimated for RP-HPLC for both the drugs individually.

METHODS

Chemicals and Reagents

Acetonitrile (HPLC grade), double distilled water, methanol (HPLC grade) was obtained from s d fine-chem. limited, worli Mumbai. Triethylamine (AR grade) was obtained from s d fine-chem. limited, worli Mumbai. Ortho-phosphoric acid (85% pure) was obtained from Merck specialties private limited, worli, Mumbai.

Instrumentation

A Jasco LC-Net-II/ADC with intelligent UV/VIS detector (UV-2075 plus), intelligent HPLC pump (PU-2080 Plus) and RP-C18 column (Purospher^R STAR) was used. A manual syringe injector was used for the injection of sample. The HPLC system was equipped with Borwin software for data processing.

Determination of Appropriate UV Wavelength

The appropriate wavelength for the detection of the drug in mobile phase was determined by wavelength scanning over the range of 200-400 nm. After scanning appropriate wavelength was selected.

Chromatographic Condition

The mobile phase was prepared using acetonitrile: methanol: 0.1% triethylamine in water: (1:6:3), pH of which was adjusted to 3.8 with ortho-phosphoric acid was found to resolve quinine and ciprofloxacin. The degassing of mobile phase was done by sonication for 60 min.

The flow rate was set to 1.0 ml/min. Both drugs showed good absorbance at 235 nm, which was selected as wavelength for further analysis. The column temperature was maintained at room temperature.

Preparation of Stock Solutions

Standard stock solutions containing quinine sulphate and ciprofloxacin hydrochloride were prepared individually by dissolving 2 mg of drug in 10 ml volumetric flask using mobile phase and volume is make up to the mark.

This will give 200 µg/ml of solution. From this 5 ml of solution was pipette out and added in a 10 ml volumetric flask and diluted up to the mark with mobile phase which will give 100 µg/ml solutions respectively for both drugs and used for sample injection.

Retention Time (tR)

Retention time (tR) is the time it takes a solute to travel through the column. The retention time is assigned to the corresponding solute peak. The retention time is a measure of the amount of time a solute spends in a column. It is the sum of the time spent in the stationary phase and the mobile phase[6].

Resolution Factor (R)

Resolution factor shows the accuracy of the quantitative analysis and resolution factor should be greater than 1.5 or specified in the individual monograph.

Resolution factor can be calculated by the formula:

$$R = 2 (t_2-t_1) / 2.70 (W1_{h/2} + W2_{h/2}),$$

Where, W= Peak width, h= Height, t_1= Retention time of first peak, t_2= Retention time of second peak.

Tailing Factor (T)

It should meet the requirements of the individual monograph and can be calculated by the formula:

$$T= W 0.05 / 2F,$$

Where, W 0.05= Peak width at 5 % high, F= Leading edge of peak[7].

Capacity Factor (k')

The capacity factor (also called "capacity ratio") is symbolized by k' (USP terminology) or **k** (IUPAC/ASTM terminology). It is a measure of the retention of a peak that is independent of column geometry or mobile phase flow rate.

The capacity factor is calculated as: k' = (tR - t0) / t0. Where tR is the retention time of the peak, and t0 is the dead time of the column[8].

Asymmetry Factor

The asymmetry factor is a measure of peak tailing. It is defined as the distance from the center line of the peak to the back slope divided by the distance from the center line of the peak to the front slope, with all measurements made at 10% of the maximum peak height. The asymmetry factor of a peak will typically be similar to the tailing factor for the same peak, but the two values cannot be directly converted[9].

Theoretical Plates

Theoretical plate represents the column efficiency. It should meet the value given in the monograph. It can be calculated by the following formula:

$$N_a = N_t / E,$$

Where, N_a = is the number of actual, physical plates or trays, N_t is the number of theoretical plates or trays and E is the plate or tray efficiency[10].

RESULTS AND DISCUSSION

Wavelength Selection

The ultraviolet spectra of quinine sulphate and ciprofloxacin hydrochloride showed at 233 nm and 272 nm respectively. Therefore, 236 nm was wavelength selected to achieve the highest sensitivity for the study.

Selection of Mobile Phase

Different combinations of acetonitrile, methanol and water with 0.1% triethylamine with pH adjusted to 3.8 by using ortho-phosphoric acid were tested and the optimum condition at acetonitrile: methanol: 0.1% triethylamine in water (1:6:3 v/v/v). The obtained chromatograph showed a retention time for quinine sulphate 4.383 (Figure 5 and Table 1) and ciprofloxacin hydrochloride 3.042 (Figure 6 and Table 2) respectively. In the synthetic mixture of quinine sulphate and ciprofloxacin hydrochloride shows rapid separation with retention time 4.383 and 3.042 respectively (Figure 7 and Table 3).

An RP-HPLC method for the simultaneous estimation of quinine sulphate and ciprofloxacin hydrochloride was developed. The peaks of quinine sulphate and ciprofloxacin hydrochloride are found to be well separated at 4.383 and 3.042 respectively using acetonitrile: methanol: 0.1% triethylamine in water (1:6:3 v/v/v) pH of which was adjusted to 3.8 with ortho-phosphoric acid as mobile phase at a flow rate of 1.0 ml/min with wavelength set to 236 nm.

Selection of HPLC Stationary Phase

The best results were obtained by using Hibar^R 250-4.6 HPLC column Purosphens^R STAR RP-18 as compared to other different stationary phase.

CONCLUSION

The developed RP-HPLC method is simple, accurate, sensitive, unique, precise, eco friendly, cost effective, fast and reproducible for simultaneous estimation of quinine sulphate and ciprofloxacin hydrochloride in bulk mixture. The method utilizes simple sample preparation, short analysis time and elution is done by isocratic method. It is concluded that this method can be adopted by the industries and academic institutions for their combination drug estimation. It shows novelty and utility of the overall work.

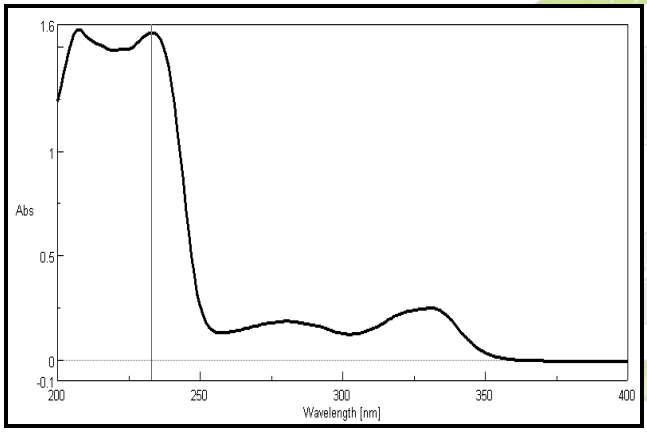

Figure 3: Spectra of quinine sulphate in distilled water

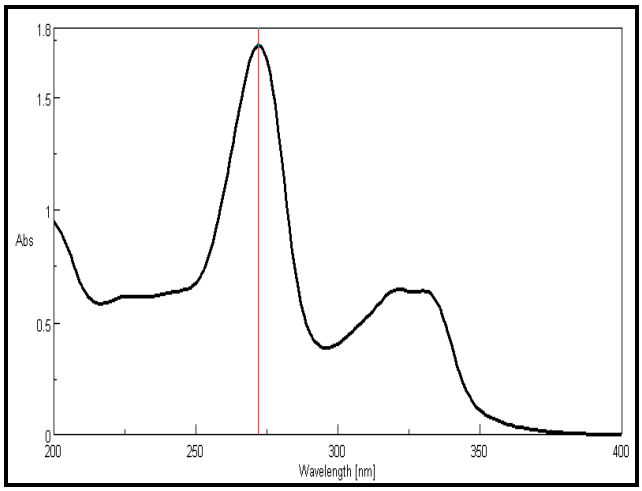

Figure 4: Spectra of ciprofloxacin hydrochloride in distilled water

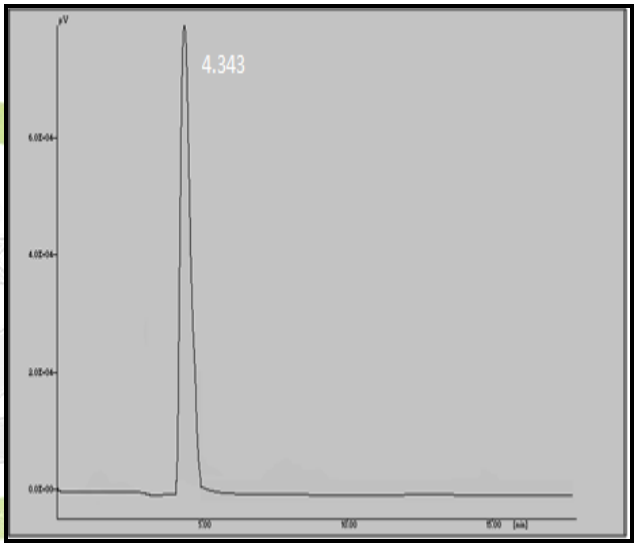

Figure 5: Chromatogram of quinine sulphate sample at λmax (236 nm)

Table 1: Chromatographic data for quinine sulphate

Name	RT (min)	Area [µV.Sec]	Resolution	Plates	Asymmetry	Capacity
Quinine Sulphate	4.383	2288940.441	0.00	1525	2.71	852.63

Total Area of Peak = 2288340.441 [µV.Sec]

Table 2: Chromatographic data for ciprofloxacin hydrochloride

Name	RT (min)	Area [µV.Sec]	Resolution	Plates	Asymmetry	Capacity
Ciprofloxacin Hydrochloride	3.042	1161283.864	0.00	1435	1.72	364

Total Area of Peak = 1161283.864 [µV.Sec]

Table 3: Chromatographic data for quinine sulphate and ciprofloxacin hydrochloride in synthetic mixture

Name	RT (min)	Area [µV.Sec]	Resolution	Plates	Asymmetry	Capacity
Quinine Sulphate	4.383	2288340.441	2.09	2174.03	1.97	580
Ciprofloxacin Hydrochloride	3.042	1161283.864	0.00	1196.32	2.56	403.00

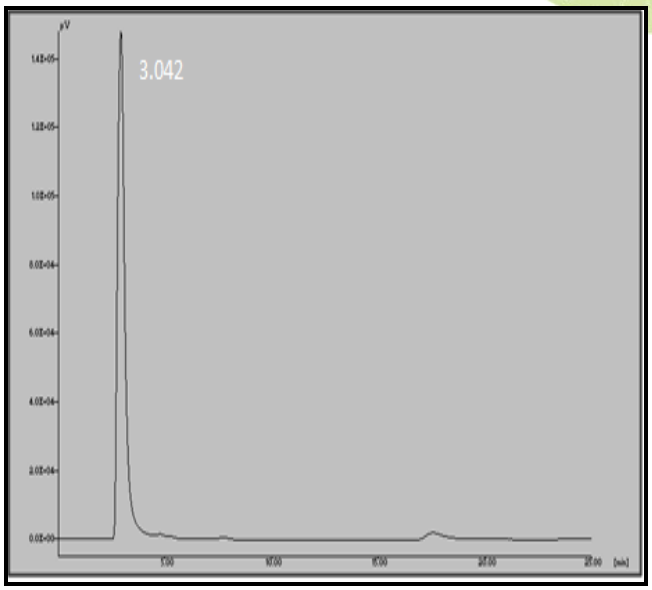

Figure 6: Chromatogram of ciprofloxacin hydrochloride sample at λmax (236 nm)

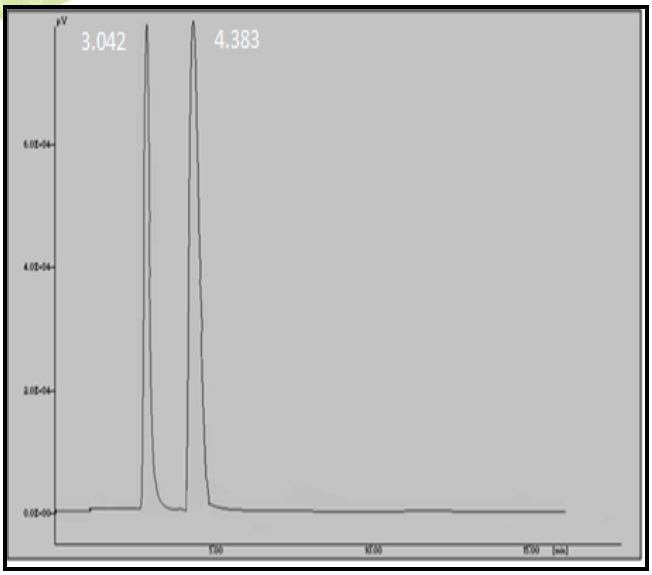

Figure 7: Chromatogram of quinine sulphate and ciprofloxacin hydrochloride synthetic mixture at λmax (236 nm)

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