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ESTIMATION OF ORPHENADRINE CITRATE IN TABLET DOSAGE FORM BY RP-HPLC METHOD

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ABSTRACT

A sensitive, fast, precise, reversed phase- high performance liquid chromatography (RP-HPLC) method is developed for the determination of orphenadrine citrate in tablet dosage form. Linearity, precision, limit of detection (LOD), robustness and ruggedness, drug recovery and the system suitability parameters have been validated for the developed method. The HPLC conditions are methanol: acetonitrile: water (40:30:30, v/v/v) mobile phase, Zodiac C_{18} column (250x 4.6 mm x 5 µm), pump pressure (10.6 MPa), flow rate (1.0 ml/min) and the wavelength of detection is 217 nm. The measured retention time of orphenadrine citrate is 5.35 minutes. The limit of detection is 0.3 µg/ml. The linearity range measured is from 10-70 µg/mL with a correlation coefficient (R^2 = 0.998). The above measured parameters indicate the developed method is useful in determination of orphenadrine citrate in tablet dosage forms.

KEY WORDS

Orphenadrine citrate; RP-HPLC method; Tablet dosage form; UV detection.

1. INTRODUCTION

Anticholinergic drugs are usually prescribed for treating muscle pains and its infections, which activates muscular receptors. Of these, orphenadrine is one of the anticholinergic drugs that belongs to the ethanolamine category and antihistamine (allergic relief) class. It is used to treat painful muscle spasms (spasm of skeletal muscles), other similar conditions, as well as the treatment of some aspects of Parkinson's disease [1]. The salts of orphenadrine are available in two varieties, namely hydrochloride and citrate. The hydrochloride salt is used for the treatment of Parkinson's disease while the citrates form as a muscle relaxant. Here our interest is in orphenadrine citrate. The systematic (IUPAC) name of orphenadrine citrate is dimethyl [2-(2-methylbenz-hydrotoxy) - ethyl] dihydrogen citrate and the chemical formula is $C_{18}H_{23}NO$ (**Fig. 1**). Its molar mass is 269.38 gram/mol [1].

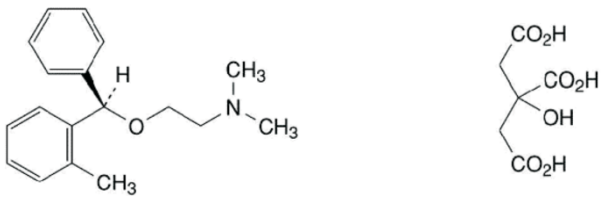

Fig. 1. The molecular structure of Orphenadrine citrate

From a detailed survey of literature it is observed that orphenadrine citrate is investigated in combination with other drugs in human serum as well as in pharmaceutical form. To mention, in combination with paracetamol [2, 3], caffeine and aspirin [4] and acetaminophen and ibuprofen [5] are reported. Analytical studies employing various methods are reported on orphenadrine citrate determination in pure form and in pharmaceutical preparations by spectrophotometry [5, 6], capillary electrophoresis [3] and capillary gas chromatography (CGC) [7], thin layer chromatography densitometry [8], gas chromatography mass spectrometry (GCMS) [9], gas liquid chromatography [10], HPLC/MS-MS method [11] and HPLC method [2, 4, 12, 13]. However, there is no report on the analysis of orphenadrine citrate individually as a single drug in tablet dosage form employing RP-HPLC method and UV detection to the best of our knowledge.

In this work, we present a method development of orphenadrine citrate in pharmaceutical form and validate the developed method based on the ICH guidelines [14] with parameters linearity, robustness, specificity, ruggedness and precision.

2. MATERIALS AND METHODS

2.1 Materials

The HPLC grade chemicals (methanol, water, acetonitrile) used in the current study are purchased from Merck Specialties private Limited, Mumbai, India.

2.2 HPLC instrumentation

To develop a high performance liquid chromatographic method for quantitative estimation of orphenadrine citrate, an isocratic PEAK HPLC instrument with Zodiac C_{18} column (250 mm x 4.6 mm, 5 µm) is used. The instrument is equipped with a LC 20AT pump for solvent delivery and a variable wavelength programmable LC – 7000 UV-detector. A 20 µL Rheodyne injection port is used for injecting the samples. The mobile phase consisted of methanol, acetonitrile and water. Using the PEAK software the chromatograms are recorded and integrated and the results are analyzed with Microsoft Excel software. To determine the maximum absorption wavelength of orphenadrine citrate an ultraviolet (UV)-visible spectrophotometer (Techcomp UV 230D) with HITACHI software is used.

2.3 Preparation of standard solution

For the present studies, the standard (stock) solution is prepared from a 10 mg of weighed drug of orphenadrine citrate and is dissolved in 10 ml of acetonitrile in a 10 ml volumetric flask. For about two minutes the solution is sonicated in a sonicator to dissolve the drug completely and is left for cooling. Then it is filtered through a 0.45 µm nylon membrane millipore filter paper. A 1000 µg/ml of standard solution is prepared. From this solution required concentrations by proper dilution are obtained, which are used for the reported results.

3. METHOD DEVELOPMENT

As a first step of the method development, mobile phase volume ratio is developed. Firstly, standard organic solvents like methanol, acetonitrile along with water in pure form are tested separately as mobile phase. Acetonitrile and methanol are chosen because they have good properties like compatibility with HPLC systems, availability and safe to use. In addition, they have lower UV cut off and water miscible. From the observation, methanol showed better result; hence it is more in volume than acetonitrile. Then different volume ratios of methanol, acetonitrile, and water are tried and finally the 40:30:30 v/v/v showed a sharper chromatogram, high theoretical plates and low tailing factor. Thus, we choose this as an optimal mobile phase for the present study.

pH of the solution is checked after optimization of the mobile phase volume ratio with a pH meter and its value is 3.8. The solution containing orphenadrine citrate at different concentrations and at different wavelengths is used for determining the optimum wavelength.

Different flow rates are also checked to determine the optimum flow rate. This is also chosen keeping in mind, the recommended flow rate for the used column with a given internal diameter. The active pharmaceutical ingredient (API) concentration is chosen as 60 µg/ml after several iterations (trials/runs). Thus the finally optimized RP-HPLC conditions used for the present studies are: pH 3.8; detection wavelength-217 nm; mobile phase-

methanol: acetonitrile: water (40:30:30 v/v/v); pump pressure-10.6 MPa; flow rate -1.0 ml/min; run time-10 minutes; peak area-502387 mAU.

4. RESULTS AND DISCUSSION

The detection wavelength of orphenadrine citrate is obtained from spectrophotometric method. The spectrum of diluted solutions of the orphenadrine citrate in methanol is recorded separately on UV spectrophotometer. The wavelength is scanned from 200 nm to 400 nm. The absorption spectrum of orphenadrine citrate with X-axis as wavelength (nm) and absorbance (%) as Y-axis is shown in **Fig. 2**. The peak of maximum absorbance wavelength is observed, which is 217 nm. This value is in good agreement with the reported values [2, 4] within 2 nm corresponding to less than 1%. The difference in the wavelengths may be attributed to instrumental resolution and compositions of the solution.

There are nine parameters that have to be validated for the developed method in accordance with the ICH guidelines [14]. They are specificity, linearity, precision, accuracy, robustness, recovery, robustness, and ruggedness, limit of detection (LOD) and limit of quantification (LOQ). In the present work on orphenadrine citrate eight out of nine parameters have been checked. Further the system suitability is evaluated.

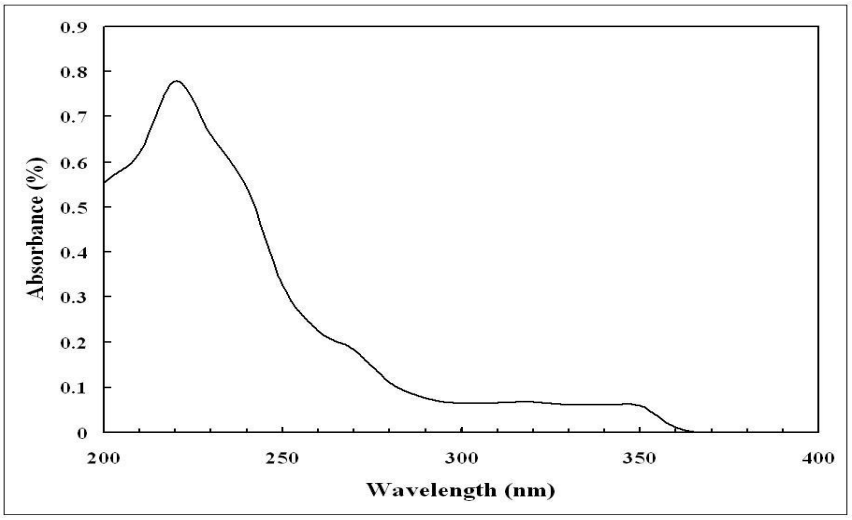

Fig. 2. Absorption spectrum of orphenadrine citrate.
The detection wavelength of orphenadrine citrate is at 217 nm.

4.1 Specificity

From the described above chromatographic conditions, the retention time of orphenadrine citrate is measured and no peak interferences are found in any of the studied samples. The mobile chosen depends mainly on the suitability of the drug under investigation. For orphenadrine citrate, in this work, we have chosen methanol, acetonitrile and water as mobile phase in the ratio (40:30:30 v/v/v). Shown in **Fig. 3** is the chromatogram of orphenadrine citrate obtained from the standard solution. The measured retention time is 5.35 minutes for a run time of ten minutes. The retention time reported in this work is a factor of 2 more than the reported value of 2.6 minutes [2] and is lesser than the reported value of 6.01 minutes [4]. The main difference is from the choice of mobile phase composition and the flow rate. Further, addition of water to mobile phase increases the retention time but it reduces the cost of the method. Further the system suitability is evaluated from the theoretical plate number and tailing factor. The theoretical plate (TP) numbers should be at least 2500 for each peak and in the present study it is 6071 that is a factor of 2.4 more than the recommended value and tailing factor has to be less than 2, which is 1.31 for the presented results. These conditions are fulfilled for orphenadrine citrate in the present study.

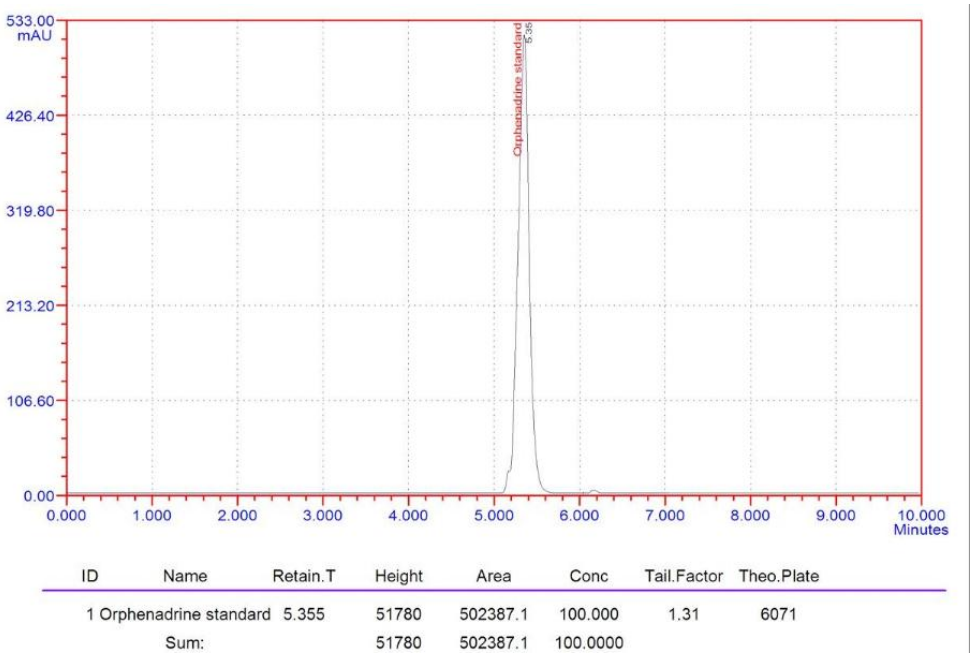

Fig. 3. Chromatogram of the standard solution of orphenadrine citrate. The retention time is 5.35 minutes.

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

The parameters limit of detection and limit of quantification are calculated from the calibration equations obtained from the experiment. These are determined from the sensitivity during linearity measurements. These are calculated on the criteria LOQ=3.3LOD. For orphenadrine citrate, 0.3 µg/ml is the limit of detection and 1.00 µg/ml is the limit of quantification. The obtained results are compared with the values reported in literature. These values are relatively higher than those reported value [2] by a factor of 50.

4.3 Linearity

The linearity of the peak areas is determined for seven different concentrations of orphenadrine citrate in the range 10-70 µg/ml. By plotting the peak areas as Y-axis and the corresponding seven concentrations (µg/ml) as X-axis the calibration graph is constructed (**Fig. 4**). The data is fitted to a linear function and the linear regression is tested. It is found to be precise from the value of the correlation coefficient, R^2 = 0.998 and satisfies the ICH guidelines.

4.4 Precision

This is a commonly validated parameter for drugs under study through the intra-day and inter-day measurements. The repeatability of the orphenadrine citrate drug for the application and for the measurement is studied with six samples. Repeatability application is evaluated by measurement of the peak area for each sample and by comparing the relative standard deviation (RSD). Intra-day precision is studied at 60 µg/ml of orphenadrine citrate for all the six samples on the same day while for inter-day precision the same concentration is used but its peak area variation is studied for three successive days in a week. The data obtained from intra-day and inter-day measurements for precision checks are given in **Table 1**. The mean peak area given in the table is for six (n=6) different samples (all at 60 µg/ml). They are normalized with the peak area of one of the sample. The corresponding standard deviation (SD) and the relative standard deviation (RSD) are also given. The RSD of all the samples from intra-day measurements is 1.07 and for inter-day measurements is 0.73, which is less than 2%, respectively. This satisfies the ICH guidelines and hence the method can be said precise.

Table 1 Results of Intra-day and Inter-day precision and Ruggedness parameters of Orphenadrine citrate.

Parameter	Concentration (µg/ml)(n=6)	Normalized Area Mean±SD	RSD	ICH guidelines
Intra-day precision	60	1.018±0.011	1.07	<2%
Inter-day precision	60	0.987±0.072	0.73	<2%
Ruggedness	60	0.991±0.017	1.74	<2%

The ruggedness of the method is validated for three samples by two analysts separately. The results are given in **Table 1** with the mean normalized peak area, SD and the RSD obtained from the peak areas. The RSD is less than 2% fulfilling the ICH guidelines.

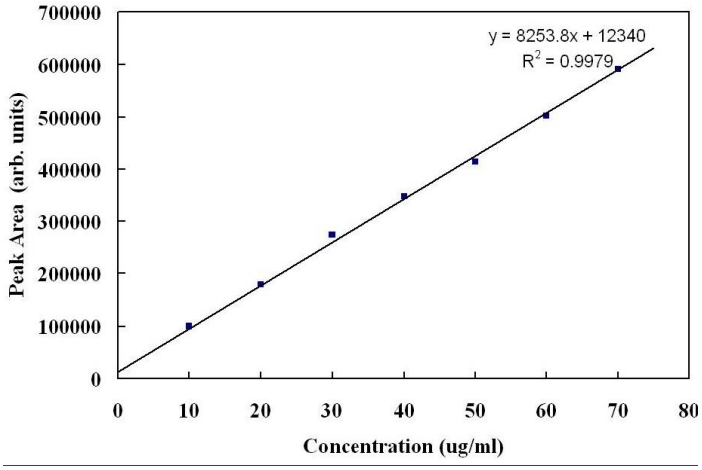

Fig. 4. Linearity results of orphenadrine citrate.
Orphenadrine citrate concentration (µg/ml) versus peak area (arb. units).

4.5 Robustness

The robustness of the method is verified by deliberate changes made to some parameters such as the mobile phase volume ratio, pH of the solution and to detection wavelength. They are given in **Table 2** along with percentage change in peak areas with respect to the standard. orphenadrine citrate concentration (60 µg/ml) is used for these studies. The influence of the changes to the above parameter values and the corresponding change in peak area is evaluated. This is checked by consciously changing the mobile phase volume ratio (>10%), pH value (10%) and wavelength (<1%) from the optimized conditions of the above parameters. The percentage change in peak area is calculated for each parameter and is found to be less than 2% satisfying the ICH guide lines. The values shown in **Table 2** indicate robustness of the method. Though the method is robust at 10% variation of the chosen parameters; mobile phase composition variation is found to be a less sensitive from the standard peak area. The influence of the pH value is less than 1% from the standard peak area. A less than one percent change in wavelength from the central value showed less than 1% peak area change. Furthermore, factor of three increase in percentage change in peak area is observed for more than 25% increase of methanol concentration and greater than 25% decrease in acetonitrile concentration of mobile phase without changing the water concentration. In the case of pH, a ten percent (10%) decrease in pH resulted in a factor three increase in change in peak area and for less than 2% increase in wavelength also showed a factor 3 increase in peak area percentage change.

Table 2 Robustness results from the data of orphenadrine by varying the parameters mobile phase, pH and wavelength.

Parameter	Parameter Change	Area (mAU)	% of Change in peak area
Standard	----	502387	----
Mobile Phase (MP)	Methanol: Acetonitrile: water		
	MP-1 45:25:30 (v/v/v)	499023	0.66
	MP-2 35:35:30 (v/v/v)	501255	0.22
pH	4.0	503877	0.29
	3.6	506513	0.82
Wavelength	219 nm	497669	0.93
	215 nm	500870	0.30

4.6 Recovery

The accuracy of the method is generally verified by recovery experiments carried out by standard addition technique, internal standard method or external standard method by adding known amount of the standard to the sample. Here the recovery study on orphenadrine citrate is done by standard addition method to which known amount of orphenadrine citrate is added. Three different percentage determinations (50%, 100%, and 150%) are used to study the recovery of the drug. The analysis of each percentage level is repeated three times (n = 3). Finally, the percentage recovery of orphenadrine citrate is compared with the actual amounts. The results are given in **Table 3**. Shown in the table are the mean concentrations obtained and their standard deviation (SD). The coefficient of variation (CV) or the relative standard deviation (RSD) in percentage is also given. The mean recovery of the drug and its corresponding SD along with the calculated error percentage is also given in the table. The good recovery of the product in the range of 99.67% (lower limit) to 101.17% (upper limit) suggests the high accuracy of the method. This is in accordance with the ICH guidelines.

4.7 Formulation analysis

This analysis is carried out on orphenadrine citrate tablets (Orphipal – 50 mg). The procedure is repeated two times, separately by weighing the tablet in powder form. Twenty tablets are powdered and weighed; the average weight (124 mg) is noted. The powder equal to 1mg of the drug is taken and is dissolved in 10ml of methanol. From a concentration of 100 µg/ml solution, 60 µg/ml is prepared and this is used for formulation assay studies. The results are given in **Table 4** that are in good agreement with ICH guide lines.

Table 3 Results of the recovery studies of Orphenadrine citrate by standard addition method at different concentrations.

Recovery	Target conc. (µg/ml) n=3	Spiked conc. (µg/ml)	Final conc. (µg/ml)	Concentration Obtained Mean±SD	CV or RSD (%)	Recovery (%) Mean±SD	Error (%)
50%	20	10	30	30.3±0.1	0.3	101.17±0.51	0.29
100%	20	20	40	40.0±0.44	1.0	100.07±1.01	0.58
150%	20	30	50	49.9±0.4	1.0	99.67±0.51	0.30

Table 4 Formulation analysis results of orphenadrine citrate.

Formulation	Form	Dosage	Concentration	Amount found	% Assay
ORPHIPAL	Tablet	50 mg	60 µg/ml	59.25	98.75

5. CONCLUSION

In conclusion, orphinadrine citrate in tablet dosage form using RP-HPLC method is investigated quantitatively as a single drug for the first time to the best of our knowledge. The developed method is simple, precise and accurate for the determination of orphenadrine citrate in tablet dosage form. This method is validated by checking the parameters such as linearity, precision, ruggedness and robustness. In this method, the recovery of the drug is good and is verified from the statistical analysis of the parameters.

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