HPLC METHOD DEVELOPMENT FOR SIMULTANEOUS ESTIMATION OF ATOVAQUONE AND PROGUANIL IN TABLET DOSAGE FORM

SURAJ SAHOO*, PRASANNA KUMAR PANDA, SAGAR KUMAR MISHRA, SABUJ SAHOO

*School of Pharmaceutical Education & Research, Berhampur University, Berhampur 760007, University Department of Pharmaceutical Sciences, Utkal University, Bhubaneswar751004 Email: surajuniversity@rediffmail.com

ABSTRACT

This research article describes simple, accurate and precise, RP-HPLC procedure for the estimation of Proguanil and Atovaquone in tablet dosage form. Gradient separation was achieved using YMC Pack Pro, C8, 150*4.6mm, 5μ column with a flow rate of 2.0 ml/min using UV detection at 254nm. The mobile phase A consisted of water (pH 2.0) and mobile phase B consisted of acetonitrile: methanol in the ratio 50:50 v/v. The retention times of Proguanil and Atovaquone were about 6.0 and 12.0 min, respectively. The method was statistically validated for linearity, accuracy and precision. The linearity of Proguanil and Atovaquone shows a correlation coefficient of 0.999 and 0.999. The method was reproducible with intra and inter-day variations. The simplicity and accuracy of the proposed method ensures its use in routine quality control analysis of pharmaceutical formulations. The method established is robust, resisting small deliberate changes in flow rate and the ratio of the organic components in the mobile phase which is useful for the simultaneous determination of Proguanil and Atovaquone in its pharmaceutical tablet dosage form.

Keywords: Atovaquone, Proguanil, HPLC, Validation.

INTRODUCTION

Atovaquone is *Trans*-2-[4-(4-chlorophenyl) cyclohexyl]-3-hydroxy-1, 4-naphthalenedione. Atovaquone is a yellow crystalline solid that is practically insoluble in water. It has a molecular weight of 366.84 and the molecular formula C22H19ClO3.

The chemical name of proguanil hydrochloride is 1-(4-chlorophenyl)-5-isopropyl-biguanide hydrochloride. Proguanil hydrochloride is a white rystalline solid that is sparingly soluble in water. It has a molecular weight of 290.22 and the molecular formula C11H16ClN5•HCl. Both the drugs are manufactured in combined pharmaceutical formulation, profound search from data and literature available, it reveal that many methods have been reported including LC-MS, ultraviolet spectrophotometry, high performance liquid chromatography, TLC method for the analysis of Proguanil and Atovaquone either alone or in combinations with others[1,2,3,4,5,6].

Very few reports have appeared dealing with the estimation of Proguanil and Atovaquone by HPLC method in combination so far. Taking simplicity, cost-effectiveness, in the sector of chromatographic techniques for pharmaceutical analysis into account, HPLC method was developed.

Therefore, this paper proposes a RP-HPLC procedure for the assay of Proguanil and Atovaquone in combination tablet dosage form. The present RP-HPLC method was validated following the ICH guidelines[7].

MATERIALS AND METHODS

Proguanil and Atovaquone were obtained as a memento sample from Cellogen pharma Ltd., Navi Mumbai. Methanol, Acetonitrile, Tetrahydrofuran and water used were of HPLC grade. Perchloric acid used was of AR grade. The analysis was carried out on HPLC (make Shimadzu) configured with dual mode pump (model 10 AT VP) and detector (model SPD -10AVP), to monitor and integrate output signal.

Chromatographic Conditions

Column: YMC Pack Pro 150*4.6mm

Particle size: packing 5μ

Stationary phase: **C**8

Column Temperature: 30°C

Mobile phase A: Water (pH 2.0±0.05 adjusted by perchloric acid)

Mobile phase B: Acetonitrile: Methanol in the ratio 50:50 v/v

Diluent: Mixture of Tetrahydrofuran: Methanol: Water in the ratio 40:50:10

Flow rate: 2.0 ml/min

Detection: 254 nm

Retention time: About 6.0 and 12.0 min for Proguanil and Atovaquone respectively

Injection Volume: 10 μl.

Preparation of Mobile Phase

To optimize the HPLC parameters several mobile phase compositions were tried. Satisfactory peak symmetry (Tailing factor and theoretical plates as shown in Table 1) were obtained with mobile phase as mentioned above. The mobile phase was filtered through 0.45μ filter and degassed by ultrasonication for 20 min.

Table 1: System Suitability Test Parameters for the proposed method

Parameters	Proguanil	Atovaquone
Retention Time (min)	6.0	12.0
Theoretical plates	2135	2421
Tailing Factor	0.8	1.0

Assay Standard Preparation

Atovaquone standard stock solution

An accurately weighed quantity, 50 mg of Atovaquone was transferred in to a 50 ml volumetric flask, dissolved in a sufficient quantity of diluent. The volume was made up to the mark with diluent.

Proguanil standard stock solution

An accurately weighed quantity of 40 mg Proguanil was transferred in to a 100 mL volumetric flask and dissolved in a sufficient quantity of diluent. The volume was made up to the mark with diluent.

Standard solution

5.0ml each from Atovaquone and Proguanil standard stock solution were taken into 50 ml volumetric flask, dissolved well in sufficient quantity of diluent and make up to the mark with diluent.

Assay Sample Preparation

To get equivalent concentration with respect to standard preparation twenty tablets each containing 250 mg Atovaquone and 100 mg of Proguanil were accurately weighed and average weight was calculated triturated. Crushed fine powder equivalent to 250 mg Atovaquone and 100 mg of Proguanil were weighed accurately and transferred in to 250 ml volumetric flask, and dissolved, mixed and made up to the volume with diluent. From the above 5.0 ml was diluted to 50ml volumetric flask and volume was makeup to mark with diluent.

Procedure for analysis

Chromatographic conditions set for the analysis was optimized to get a study base line. After the stabilization of base line separate injections with equal volume of placebo, standard, and sample preparation were injected in to the HPLC system and chromatographed. Typical chromatogram obtained from standard injection is shown in figure 1. Similarly duplicate injections of sample were chromatographed and the average peak area of the drugs was computed from the chromatograms and the amount of the drugs present in the tablet dosage form was calculated. Assay for Proguanil and Atovaquone are 100.0 % and 99.8% respectively.
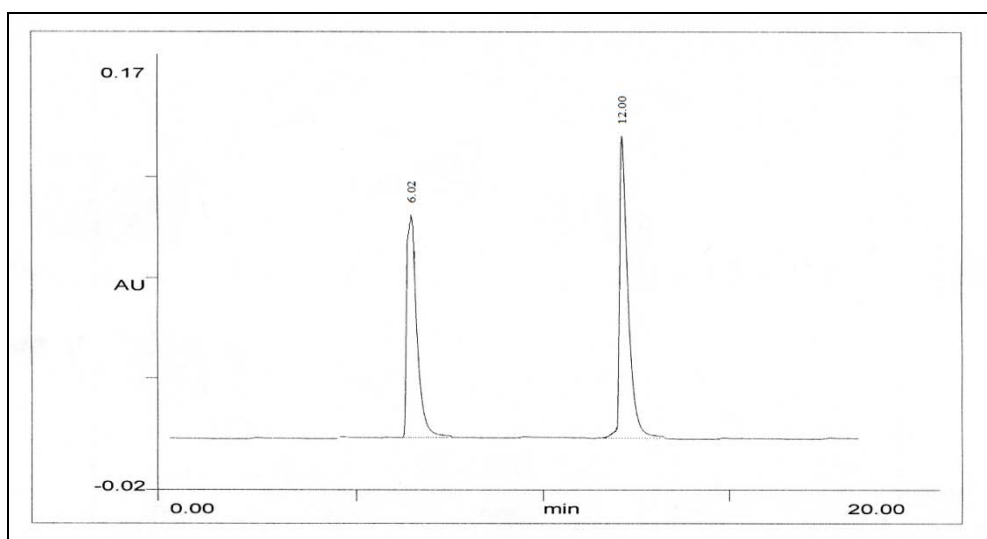

Fig. 1: Standard Chromatogram showing well separated peaks for Proguanil and Atovaquone.

Method validation

The developed analytical method was validated with respect to parameters which are specificity, linearity, precision, accuracy, robustness, and ruggedness and are executed as per the ICH guidelines.

RESULTS AND DISCUSSION

Linearity (Calibration curve)

The calibration curve was constructed with 5 concentrations ranging from from 50 to 150% of test concentration. The peak area ratio of the drug was considered for plotting the linearity graph. The linearity was evaluated by linear regression analysis, which was calculated as shown in Table 2.

Precision

Demonstration of Method precision was done by taking 5 samples from the same batch of formulation and analyzed individually and the assay content of each sample was estimated. The average for the 5 determinations was calculated along with the RSD for the replicate

determinations. The Ruggedness of the method was demonstrated through the study of the variations which are day to day and analyst to analyst variation and results are shown in Table 3.

Accuracy

The accuracy of the HPLC assay method was assessed by adding known amount of the drug to a sample solution of known concentration and chromatographed the samples as per the proposed method. The % recovery and RSD of at each level was calculated as shown in Table 4.

Robustness

As defined by the ICH, the robustness of an analytical procedure describes to its capability to remain unaffected by small and deliberate variations in method parameters. Robustness was achieved by small variation in the chromatographic conditions and found to be unaffected by small variations like variation in volume of mobile phase composition, flow rate of mobile phase and is shown in Table 5.

Table 2: Regression analysis of the Calibration curve for the proposed method

Parameters	Proguanil	Atovaquone
Linearity range (µg/ml)	20-60	50-150
Slope	143	678
Intercept	122	668
Correlation coefficient	0.999	0.999

Table 3: Results for Precision with ruggedness

Drug	Intraday assay(Analyst 1)		Inter day assay(Analyst 2)	
	% Obtained	% RSD	% Obtained	% RSD
Proguanil	100.0	0.64	100.2	1.07
Atovaquone	99.8	0.14	100.2	1.06

Table 4: Results for Accuracy

Levels	Proguanil		Atovaquone	
	% Recovered	**%RSD**	**% Recovered**	**%RSD**
50%	99.4	0.6	99.5	0.5
100%	100.0	1.6	99.6	0.7
150%	100.0	1.4	99.9	0.7

Table 5: Results for Robustness

Factor	Change	Retention time		%RSD	
		Proguanil	**Atovaquone**	**Proguanil**	**Atovaquone**
Flow rate(ml/min)	-0.2	6.02	12.02	0.23	1.01
	+0.2	5.93	12.05	0.41	0.98
% of Acetonitrile	-0.5	6.05	12.00	0.56	0.87
	+0.5	6.08	12.05	0.78	0.94
% of Methanol	-0.5	5.99	11.92	0.58	0.65
	+0.5	6.04	12.06	0.57	0.87

Placebo injection was chromatographed at 254 nm which apparently depict non interference of excipients at the retention times of Proguanil and Atovaquone.

Results obtained with respect to individual parameter are within the acceptance criteria and as stated earlier are validated as per ICH guidelines.

CONCLUSION

Data indicates that the procedure for assay of tablet dosage form is validated which is simple as well. Therefore it can be used for the routine quality control analysis for the determination of the Proguanil and Atovaquone in their tablet dosage forms.

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REFERENCES

1. Eidstein MD Simultaneous measurement of Proguanil and Cycloguanil in human plasma by High Pressure Liquid Chromatography. J. Chromatogr. 1986; 380: 184-189.

2. Kelly JA, Fletcher KA High Pressure Liquid Chromatography method for the determination of proguanil and cycloguanil in biological fluids. J. Chromatogr. 1986; 381: 464-471.

3. Bergqvist Y, Hopstadius C Simultaneous Separation of Atovaquone, Proguanil and Its Metabolites on A Mixed Mode High Performance Liquid Chromatography Column. J. Chromatogr. 2000; 741: 189-193.

4. Pingale S G, Nerurkar K K, Padgaonkar A M, Pawar U D and Mangaonkar K V, Alternative LC–MS–MS Method for Simultaneous Determination of Proguanil, Its Active Metabolite in Human Plasma and Application to a Bioequivalence Study. Chromatographia 2009; 70: 1095-1102.

5. Gurule S, Goswami D, Khuroo A H, Monif T LC-APCI mass spectrometric method development and validation for the determination of atovaquone in human plasma Biomedical Chromatography. 2010; 24: 497-505.

6. Hund E, Massart DL, Verbeke J S Evaluation of the H-point standard additions method (HPSAM) and the generalized H-point standard additions method (GHPSAM) for the UV-analysis of two-component mixtures. J Pharm Biomed Anal.; 1999: 21, 23-42.

7. ICH Topic Q 2 (R1), ICH Harmonised Tripartite Guideline, validation of Analytical Procedures, 1995.