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Original Article

Development and validation of RP-HPLC method on Atovaqone and Proguanil in bulk and tablet dosage form.

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Abstract

A simultaneous RP-HPLC method is developed for the estimation of Atovaquone (ATV) and Proguanil (PRO) in tablet dosage form. Chromatography was carried on an Inertsil (ODS) 250×4.6mm, particle size 5µm, C18 column using isocratic composition of methanol as mobile phase A and 0.02M phosphate buffer as mobile phase B in ratio of 80:20 at a flow rate of 1ml/min with detection at 260 nm. The retention times of the ATV and PRO was about 10.62 and 3.83 min. respectively. The detector response is linear from 50-350µg/ml and 20-120µg/ml of test concentration for ATV and PRO respectively. The method was validated by determining its Linearity, accuracy and precision. The proposed method is simple, fast, sensitive, Linear, accurate, rugged and precise hence can be applied for routine quality control in bulk and in tablet dosage form.

Keywords: Stability indicating, RP-HPLC, Force degradation, Validation.

1. Introduction

Atovaquone is chemically, 2- [4- (4-chlorophenyl) cyclohexyl] – 3 – hydroxy - 1, 4-napthalenedione while Proguanil, is chemically 1-(4-chlorophenyl) -5 – isopropyl – biguanide hydrochloride, both these drug are employed as potent antimalarial drug. Both the drugs are marketed as combined dose tablet formulation (250:100mg ATV: PRO). Very few reports have appeared dealing with the estimation of Progaunil and Atovaquone by HPLC method in combination so far. our aim is to develop more accurate, precise, economic and simple method for these drugs. The present work describes the development of a validated analytical RP-HPLC method, which can quantify these components simultaneously from a combined dosage form.

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2. Materials and Methods

ATV and PRO was obtained as a sample from Glenmarks Generics Ltd Goa, & Siddhartha interchem Gujrat respectively. HPLC grade Methanol (Merck) and AR grade sodium dihydrogen orthophosphate (Research Lab) was used.

2.1. Instrument

Agilent 1120 compact LC, High Performance Liquid Chromatography with UV detector and Manual injector mode was used with EZ chrome software.

2.2. Chromatographic conditions

Chromatographic separations were achieved by using Inertsil (ODS) 250×4.6mm, particle size 5µm, C18 column. The mobile phase is consisting of Methanol as A and Buffer (0.02M Sodium dihydrogen phosphate monohydrate pH 6.0) as mobile phase B in the ratio of (80:20).

2.3. Preparation of Mobile phase

Buffer was prepared by dissolving 2.72 gm of Sodium Dihydrogen phosphate monohydrate (0.02mM) in 1000 ml of water and by adjusting the pH to 6.0 with dilute orthro phosphoric acid.

2.4. Preparation of Standard Stock solution

Accurately 10 mg of ATV and PRO standards were weighed and taken in 10ml volumetric flask. Dissolved by sonication in 5 ml of Diluent (in a ratio of 80:20 Methanol and Buffer) and then diluted to 10 ml with the Diluent to get 1000µg/ml standard stock solution.

2.5. Working Standard solution

2.5 ml and 1ml of the above standard stock solution was taken in 10 ml volumetric flask and made up to 10 ml with diluents to get a concentration of 250 and 100 µg/ml for ATV and PRO respectively.

2.6. Preparation of Sample solution

Ten tablets (ATV & PRO Tablet Glenmark generics) were accurately weighed and crushed into a fine powder. The powder equivalent to one tablet (250mg of ATV and 100 mg of PRO) was taken in 100 ml volumetric flask. About 75 ml diluents was added and sonicated for 20mins with intermediate shaking. Then the volume was finally made up to the mark (100 ml). Sample solution was filtered through whatman filter paper to get a clear solution. Then solution was used as final sample solution of a concentration of 250 and 100µg/ml.

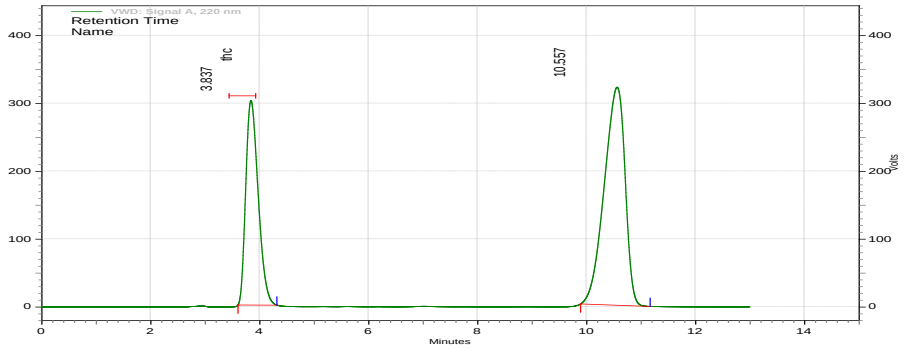

Fig. 1. Typical Chromatogram of Atovaquone (10.62) and Proguanil (3.83) by HPLC.

3.1. Linearity

Several aliquots of standard stock solution (5, 10, 15, 20, 25, 30, 35ml) of ATV and (2,4,6,8,10, 11, 12ml) of PRO respectively were taken in different 10 ml volumetric flask and diluted up to the mark with Diluents. Evaluation was performed with UV detector at 260 nm and Peak area was recorded for all the peaks and a calibration graph was obtained by plotting peak area versus concentration of ATV (Fig. 2 A),PRO (Fig. 2 B) respectively. The plot of peak area of each sample against respective concentration was found to be linear in the range of 50-350 and 20 - 120 µg/ml with correlation coefficient of 0.998 and 0.998 and linear regression equation being Y=61594x-16263543, and Y=1464942x +12357012 for ATV and PRO respectively.

3.2. Accuracy and Precision

The recovery experiment was carried out by spiking the already analyzed sample of the tablets with their different known concentration of standard ATV and PRO. The result is summarized in Table.1. The percent recovery for ATV ranges from 99.25% to 100.52 % and PRO ranges from 100.87 to 100.06 %. The reproducibility of the proposed method was determined by performing tablet assay at different time intervals on the same day (Intra-day assay precision) and on three different days (Inter-day precision). Results of intra-day and inter-day precision are expressed in % RSD. % RSD for Intraday assay precision was found to be 0.3519 (for ATV) and 0.2493 (for PRO) and that for Inter-day assay precision was found to be 0.9449 (for ATV) and 0.1660 (for PRO).

3.3. System Suitability

The system suitability test was applied to a representative chromatogram to check the various parameters such as column efficiency, resolution, precision and peak tailing. The result obtained is shown in Table 2.

3.4. Robustness

In all deliberately varied conditions, the RSD of contents of ATV and PRO were found to be well within the acceptable limit of 2%. The tailing factor for both the peaks was found to be 1.5.

3.5. Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ were determined based on the standard deviation of the y-intercept and slope of the calibration curves. Limit of detection and limit of Quantitation values for ATV were found to be 0.800 µg/ml and 2.420 µg/ml and that for PRO were found to be 1.500 µg/ml and 4.700 µg/ml.

3.6. Assay

The content of ATV and PRO found in the tablets by the proposed method are shown in Table 3. The low R.S.D indicates that the method is precise and accurate.

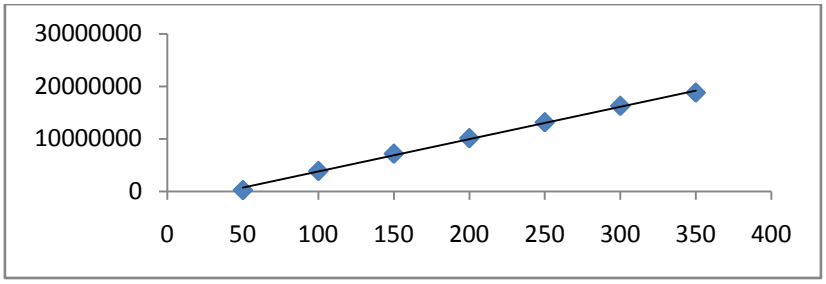

Fig. 2A. Linearity of ATV.

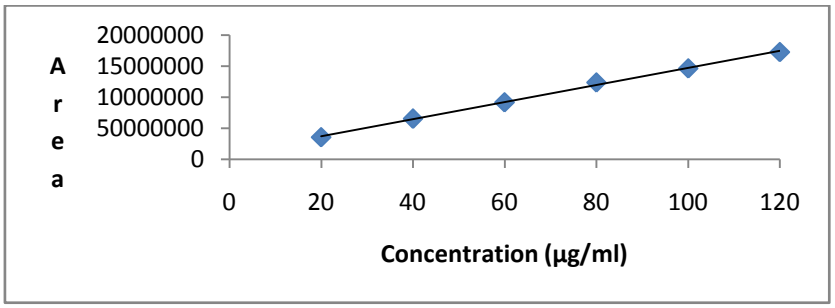

Fig. 2B. Linearity of PRO.

Table 1. Result of recovery studies.

Level of accuracy (%)	Amount of pure drug added(mg)		Percent recovery	
	ATV	PRO	ATV	PRO
50	125.01	50.04	102.45	101.57
100	250.07	100.06	100.03	100.69
150	375.05	150.08	100.09	100.51
	Mean % recovery		100.85	100.92
	S.D.		0.2275	0.1925

Table 2. System Suitability Parameters.

SYSTEM SUITABILITY PARAMETER	COMPONENT	
	ATV	**PRO**
Retention time	3.837	10.620
Theoretical plates	2152	3887

Table 3. Assay Results of Marketed Formulation.

Component	**Label claim (mg/tablet)**	**Amount found * (mg/tablet)**	**percent label claim ***	**S.D**	**%RSD**
ATV	250	247.86	99.14	0.057	0.055
PRO	100	99.98	99.84	0.705	0.705

Results and Discussion

The proposed chromatographic system was found suitable for effective separation and Retention time of ATV (10.6 min) and PRO (3.83 min). Chromatograms of mixed standard solutions which contained ATV and PRO were recorded. The method was statistically validated for linearity, accuracy and precision. The linearity of Proguanil and Atovaquone shows a correlation coefficient of 0.998 and 0.998. The method was reproducible with intra and inter-day variations. 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.

Conclusion

Finally we came to the point of conclusion that the standard deviation and % RSD calculated for all the methods were low, indicating high degree of precision of the methods. The results of the recovery studies show high degree of accuracy of the proposed methods. Comparative statistical study shows good correlation between methods. Hence the developed RP- HPLC methods for ATV and PRO are sensitive, accurate precise and reproducible and can be employed successfully in both bulk and pharmaceutical dosage form.

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