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DEVELOPMENT AND VALIDATION OF STABILITY-INDICATING RP-HPLC METHOD FOR ESTIMATION OF PROGUANIL HYDROCHLORIDE IN TABLET DOSAGE FORM

NANDINI R. PAI and SEEMA S. SAWANT*

Department of Chemistry, D. G. Ruparel College of Arts, Science & Commerce, Senapati Bapat Marg, Mahim (West), MUMBAI – 400016 (M.S.) INDIA

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ABSTRACT

A rapid RP-HPLC[1] method was developed for determination of Proguanil hydrochloride[1] in tablet dosage formulation. Proguanil hydrochloride was found to be degraded under different set of conditions as followed according to ICH guidelines[2] and the degradants so formed along with proguanil hydrochloride were separated using Kromasil C18, 150 mm × 4.6 mm × 5 µm column using Buffer : Methanol (45 : 55) as mobile phase, with a flow rate of 1.2 mL/min with a detection wavelength of 254 nm with injection volume of 20 µL. The method was validated[3,4] for specificity, linearity, accuracy, robustness, and precision. The obtained results indicated that the method is selective in analysis of proguanil hydrochloride in the presence of degradation products formed under various stress conditions.[2]

Key words: Proguanil hydrochloride, Stability-indicating, Validation.

INTRODUCTION

The chemical name of proguanil hydrochloride is 1-(4-chlorophenyl)-5-isopropyl-biguanide hydrochloride.[1] Proguanil hydrochloride is a white crystalline solid that is sparingly soluble in water. It has a molecular weight of 290.22 and the molecular formula $C_{11}H_{16}ClN_5.HCl$. It is official in British pharmacopeia.[1] This drug is manufactured in combined pharmaceutical formulation. After profound search from data and literature available, it was revealed that many methods have been reported including LC-MS[5], ultraviolet spectrophotometry[6], high performance liquid chromatography[7-9], TLC method[6] for the analysis of Proguanil either alone or in combination[10,11] with others.[12,13]

Very few reports have appeared dealing with the estimation of Proguanil hydrochloride by HPLC method in combination[11-13] so far. Taking simplicity, accuracy, 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 hydrochloride in tablet dosage form.[11] The present RP-HPLC method was validated following the ICH guidelines.[3,4]

Available online at www.sadgurupublications.com
*Author for correspondence; E-mail: ssawant_s@yahoo.com; Mo.: 098 21537004

EXPERIMENTAL

Material and methods

Reagents and chemicals

Proguanil hydrochloride was kindly provided by Ipca Laboratories Ltd. Methanol, glacial acetic acid and Hexane-1-sulphonic acid, sodium salt were of HPLC grade from Merck Ltd. Water used was of HPLC grade water from Millipore. Proguanil hydrochloride tablets claimed to contain 100 mg of the drug were manufactured by Ipca Laboratories Ltd.

Instrumentation

The HPLC system (Shimadzu, LC 2010) consisted of a UV-visible and Prominence PDA detector, a Kromasil C18 (150 x 4.6 mm), 5 µm column, with the LC solution software.

Chromatographic conditions

The chromatographic analysis was performed at 30ºC temperature on Kromasil RP-C18 analytical column with a mobile phase composed of Buffer : Methanol (45 : 55 v/v) and was isocratically eluted at a flow rate of 1.2 mL/min. A sample volume of 20 µL was used for each sample run, being injected into the HPLC system. The chromatogram was monitored with UV detection at a wavelength of 254 nm and the total run time was 30 min.

Preparation of buffer solution

4.0 g of Hexane-1-sulphonic acid, sodium salt was dissolved in a mixture of 790 mL water and 10 mL glacial acetic acid was added.

Preparation of standard solution

Proguanil hydrochloride reference substance was accurately weighed (10 mg) and dissolved in 70 mL quantity of mobile phase in 100 mL volumetric flask and diluted upto the mark and it generated a concentration of 100 µg/mL.

Analysis of tablet formulation

Sample preparation

Twenty tablets were accurately weighed and finely powdered. Tablet powder equivalent to 10 mg of proguanil hydrochloride was taken in 100 mL volumetric flask; 70 mL of mobile phase was added and sonicated for 15 min, cooled to room temperature. Made up the volume with mobile phase and mixed. It was filtered through 0.45 µ filter paper; it gave the concentration of 100 ppm.

Placebo sample preparation

Twenty placebo tablets were accurately weighed and finely powdered. Tablet powder equivalent to 10 mg of proguanil hydrochloride was taken in 100 mL of volumetric flask; 70 mL of mobile phase was added and sonicated for 15 min, cooled to room temperature. Made up the volume with mobile phase and mixed. It was filtered through 0.45 µ filter paper.

System suitability solution preparation

Accurately weighed 10 mg of proguanil hydrochloride and dissolved in 100 mL volumetric flask with mobile phase. This standard solution was further diluted to get the concentration of 2 µg/mL of proguanil hydrochloride (standard solution).

Accurately weighed each of 5 mg of proguanil hydrochloride impurity A, C & D and were dissolved in 100 mL volumetric flask with mobile phase (impurity stock solution).

Further pipetted out each from standard solution and impurity stock solution in one volumetric flask and was further diluted by using the mobile phase to get the concentration of 0.2 µg/mL for proguanil hydrochloride and 0.5 µg/mL of each of proguanil hydrochloride impurity A, C & D.

Figs. 1 and 2 are shown for HPLC chromatogram of Proguanil hydrochloride and system suitability solution, respectively.

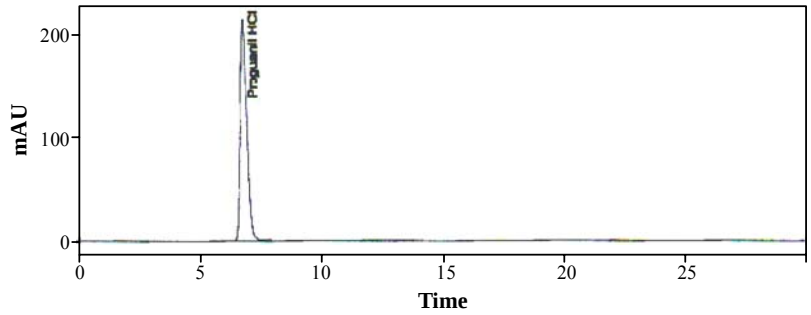

Fig. 1: HPLC chromatogram of proguanil hydrochloride

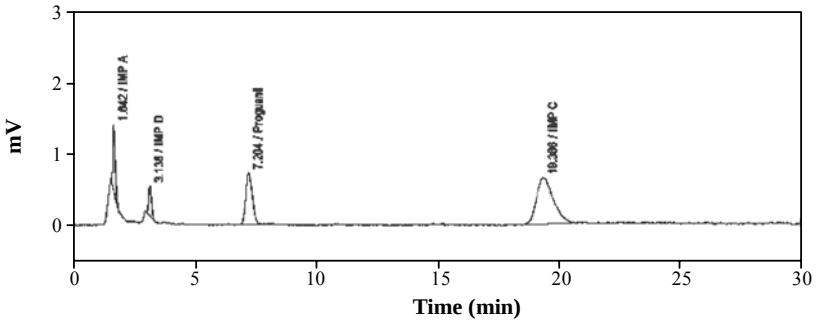

Fig. 2: HPLC chromatogram of proguanil hydrochloride system suitability solution

RESULTS AND DISCUSSION

Method development

Detection wavelengths[7,15] for the HPLC study were selected as 235 and 254 nm after recording the UV spectrum from 190 to 800 nm of the drug and representative sample from standard and sample solution by using PDA detector HPLC. The suitable area and peak selectivity of proguanil hydrochloride and it's impurities A, C & D were observed at these wavelengths. The chromatographic conditions were optimized for resolution of the peak of the drug and it's impurity under each condition by varying the stationary phase, proportion of methanol/acetonitrile/water in the mobile phase and the flow rate using representative samples. Several trials using various proportions of methanol and water as mobile phase were carried out. Subsequently, a mixture of different mobile phase composition was used to optimize the chromatographic conditions for resolving proguanil hydrochloride and it's impurities A, C & D in a single run. An appropriate blank was injected before the analysis of all the samples. Such an optimized method was then used to study the assay[14] determination of Proguanil hydrochloride's tablet dosage form. Figs. 3 and 4 are shown for standard and sample chromatograph, respectively.

Method validation

Method validation was conducted according to ICH guidelines. Assay performance was evaluated by intra day and inter day (two different days) precision and determined from replicate analysis of samples (100 ppm) in two analytical runs and was expressed in terms of RSD from mean intra and inter day assays. Analysis of six different sample solutions was performed in the same day for intra day precision. Linearity was carried out for proguanil hydrochloride over the concentration range of 15 to150 ppm. Accuracy of the method was tested by adding a known amount of proguanil hydrochloride (80, 100 and 120 ppm) in three placebo solutions. Determined the recovery of the theoretical concentration. Robustness was tested by analysis of variations in analytical condition. Influence of mobile phase flow rate, filter paper and column make were evaluated. The chromatographic parameters monitored were % Assay, peak retention time, tailing factor and theoretical plate number. Force degradation study was carried out.

Specificity

Specificity of an analytical method is its ability to measure accurately and specifically the analyte of interest without interference from the blank and placebo. Specificity of the peak purity of proguanil hydrochloride was assessed by comparing its retention time in standard and sample and good correlation was obtained. Injecting the individual identification standard solution of proguanil hydrochloride. The peak found pure in both standard and sample solution. Also there were no peaks when the placebo and blank were injected and no interferences, hence the method is specific. System suitability parameter also satisfied with respect to % RSD for replicate injection of standard, tailing factor and theoretical plates for proguanil hydrochloride peak.

Table 1 is given for specificity results and Figs. 3, 4, 5 and 6 are given for standard, sample, blank and placebo solution chromatograph, respectively. Figs. 7 and 8 are shown for proguanil hydrochloride UV Spectra graph and PDA purity graph, respectively.

Table 1: Specificity results

	Proguanil hydrochloride standard solution	Proguanil hydrochloride sample solution
Retention time in minute	7.030	7.052
Tailing factor (NMT 2.0)	1.504	1.522
Theoretical plates (More than 2000)	2638.74	2624.05
Peak Purity	Peak purity index : 0.999	Peak purity index : 0.999
Blank/Placebo Interference	Not detected	Not detected
% RSD peak area (NMT 2.0 %)	0.31 %	-

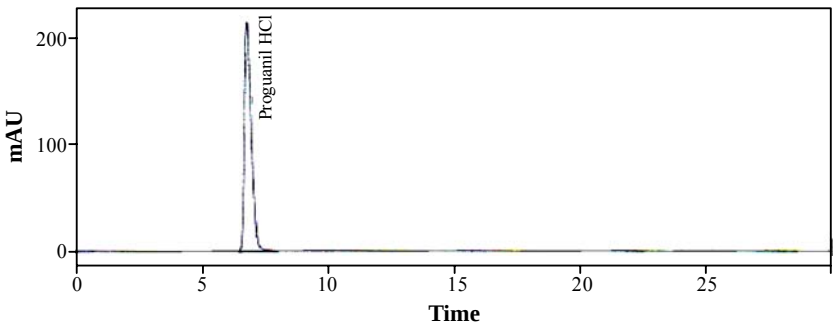

Fig. 3: HPLC chromatogram of proguanil hydrochloride in standard solution

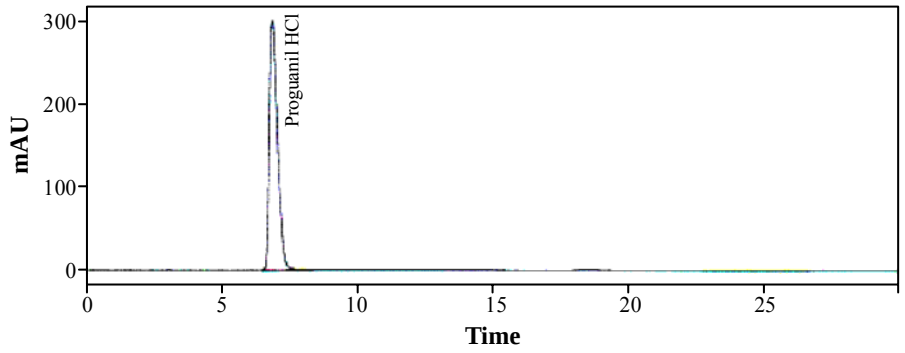

Fig. 4: HPLC chromatogram of proguanil hydrochloride in sample solution

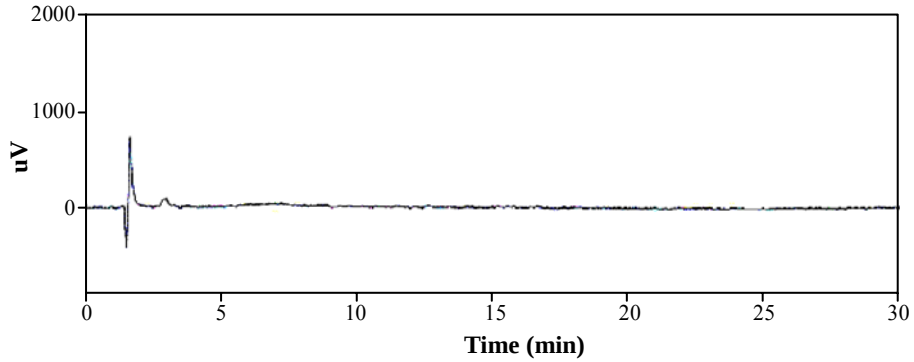

Fig. 5: HPLC chromatogram of blank solution

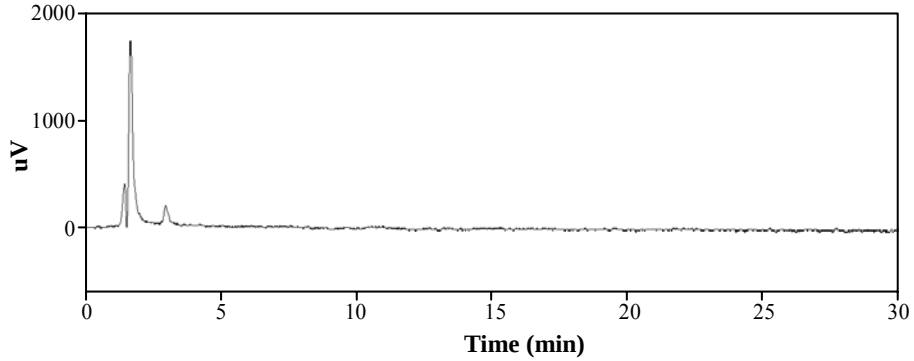

Fig. 6: HPLC chromatogram of placebo solution

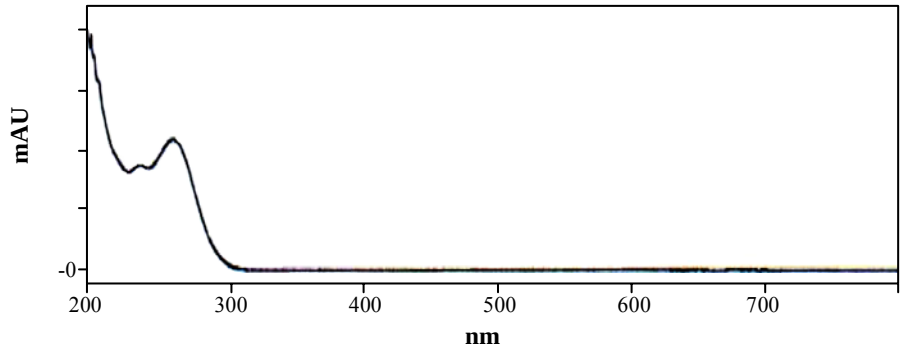

Fig. 7: UV Spectra graph for proguanil hydrochloride

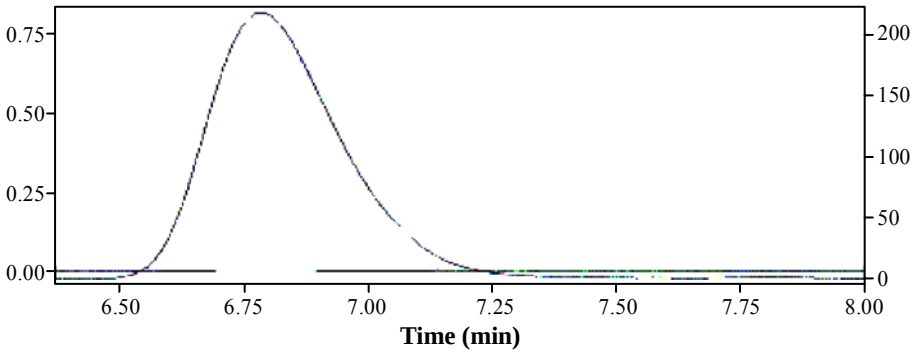

Fig. 8: HPLC PDA peak purity graph for proguanil hydrochloride

Linearity

Linearity was assessed with the aid of serially diluted calibration solutions from range of 15-150 ppm. The standards were injected separately. Calibration graphs were plotted on the basis of triplicate analysis of each calibration solution.

Table 2 is given for Linearity results.

Fig. 9 shows Linearity correlation graph.

Table 2: Linearity results

	Proguanil hydrochloride
Concentration range	15-150 ppm
Correlation coefficient	0.99998
Slope	65058.33
Y - Intercept	10386.98
R-square	0.99996

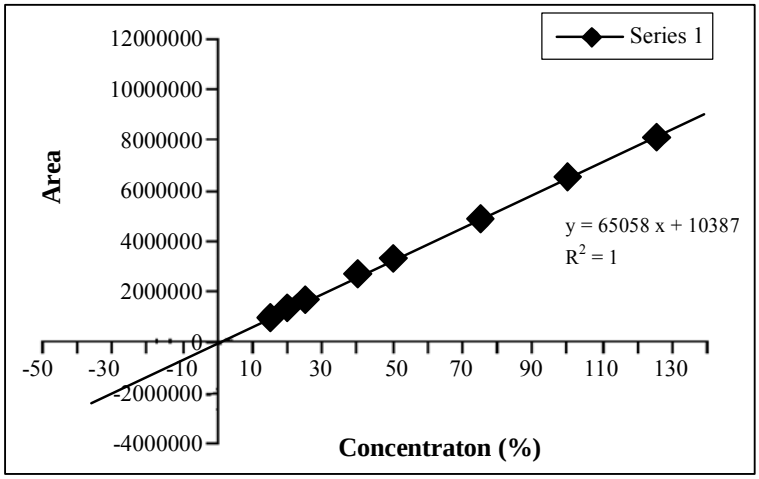

Fig. 9: Linearity graph for Proguanil hydrochloride

Precision

Precision was carried out for Inter and Intra day analysis for tablet dosage form.

Precision was evaluated by carrying out six independent sample preparations of a single lot of tablet. The sample preparation for tablet was carried out in same manner as described above.

Table 3 is given for observations of precision studies

Table 3: Precision studies observations

	S. No.	Proguanil hydrochloride in tablet (mg/tablet)	
		Method precision	**Intermediate precision**
	1	100.75	99.41
	2	99.16	100.22
	3	100.47	99.82
	4	99.14	99.14
	5	99.61	100.29
	6	99.53	99.02
	Mean	**99.83**	**99.78**
	SD	**0.7465**	**0.5004**
	RSD	**0.75**	**0.50**
Method precision - Intermediate Precision :	Mean	**99.80**	
	SD	**0.5997**	
	RSD	**0.60**	

Accuracy (recovery studies)

To check the degree of accuracy of the method, recovery studies were performed in triplet by adding known amount of placebo (about 20 mg) and standard addition method at 80, 100 and 120% concentration levels of standard (100 ppm). Known amounts of standard solution were added to the placebo (about 20 mg) and sample preparation method was carried out and was subjected to the proposed HPLC method.

Table 4 is given for Results of recovery studies.

Table 4: Results of recovery studies

Level	Proguanil hydrochloride
	% Recovery
80 %	99.81
100 %	100.25
120 %	99.81
Mean	99.96
% RSD	0.41

Robustness

To evaluate the robustness of the developed RP-HPLC method, small deliberate variations in optimized method parameters were done. The effect of change in flow rate, change in column make and

filter paper change were studied. The method was found to be unaffected by small changes like ± 0.1 mL in flow rate of mobile phase, column make change to phenomenox C18 and filter paper from 0.45 µ to whatmann 41 No. Table 5 shows Robustness study results.

Table 5: Robustness study results

	Filter paper 41 No.	Mobile phase flow rate 1.1 mL	Mobile phase flow rate 1.3 mL	Column make
Tailing Factor	0.878	0.899	0.867	0.849
Theoretical plates	9027.94	8352.32	40996.733	8671.73
% RSD for area	0.02	0.10	0.02	0.02
Retention time	6.857	7.905	6.045	6.877
% RSD for Assay	0.62	0.64	0.63	0.62

Stability of stock solution

During solution stability experiments, RSD for the Proguanil hydrochloride content was found 0.37% for tablet dosage form which was within 2% RSD. Results of the solution stability experiments confirmed that standard solutions and solutions in the mobile phase were stable for upto 12 hour during the analysis.

Force degradation study

Photostability: Exposed about 1000 mg of powder in photostability for 1.2 million Lux hours. Weighed accurately this powder equivalent to 10 mg of proguanil hydrochloride into 100 mL volumetric flask added 60 mL of mobile phase and sonicated for 15 min with intermittent shaking and made upto the mark with mobile phase. Filtered the solution through 0.45 µ nylon filter.

Heat: Exposed about 1000 mg of powder in drying oven at 60ºC for 7 days. Weighed accurately this powder equivalent to 10 mg of proguanil hydrochloride into a 100 mL volumetric flask added 60 mL of mobile phase and sonicated for 15 min with intermittent shaking and made upto the mark with mobile phase. Filtered the solution through 0.45 µ nylon filter.

Acid degradation

Weighed accurately about 10 mg of proguanil hydrochloride and transferred into a 100 mL volumetric flask and added 75 mL of mobile phase and sonicated for 15 min and made up with mobile phase. Then added 10 mL of 0.1 N hydrochloric acid and sonicated, placed it aside for 2-3 hrs, then neutralize the solution with 10 mL of base. The solution was filtered through 0.45 µ nylon filter.

Base degradation

Weighed accurately 10 mg of proguanil hydrochloride and transferred into a 100 mL volumetric flask and added 75 mL of mobile phase and sonicated for 30 min and made up with mobile phase. Then added 10 mL of 0.1 N sodium hydroxide and sonicated, place it aside for 2-3 hrs, then neutralized the solution with 10 mL of acid. The solution was filtered through 0.45 µ nylon filter.

Peroxide degradation

Weighed accurately 10 mg of proguanil hydrochloride and transferred into 100 mL volumetric flask

and added 75 mL of mobile phase and sonicated for 30 min and made up with mobile phase. Then, 0.1 mL of 10% hydrogen peroxide was added and sonicated, placed it aside for 2-3 hrs, then neutralized. The solution was filtered through 0.45 µ nylon filter.

Result for force degradation studies

The study showed that slight degradation observed when treated with acid, base, peroxide after heating and photostability and heat conditions. The peak purity index for drug was found to be within acceptance criteria. i.e. not less than 0.990. From the above study, it was established that no other product of degradation was found to interfere with the retention time of proguanil hydrochloride. Alkali hydrolysis showed comparatively higher degradation of proguanil hydrochloride peak than acid hydrolysis. Maximum degradation of 13.87% with assay drop down to 86% of proguanil hydrochloride was achieved, the study of peak purity proved that the peak due to proguanil hydrochloride remains pure though there was decrease in peak area response over the period of hydrolysis, which very well establishes the stability indicating capacity of the method. No degradation was observed for proguanil hydrochloride tablets when exposed to photo-stability and heat. The drop down in assay may be due to absorption of moisture by the test sample after exposure. For all the degraded samples, the proguanil hydrochloride peak passed the peak purity testing, leading to a conclusion that the peak is spectrally homogeneous. In other words, none of the degradants formed during the stress study co-elute with the proguanil hydrochloride peak. The placebo kept in the oven and photostability chamber did not show any peak at the retention time of proguanil hydrochloride. Thus, the specificity of the method is confirmed and the method is proved to be stability indicating.

Table 6 is given for Force degradation study observations.

Figure 10, 11, 12, 13, 14, 15 and 16 show peak purity graph for proguanil hydrochloride in acid hydrolysis 0 hour degradation, acid hydrolysis 3 hour degradation, alkali hydrolysis 0 hour degradation, alkali hydrolysis 3 hour degradation, oxidation degradation, photostability degradation andheat degradation, respectively.

Table 6: Force degradation study observations

Proguanil hydrochloride	Acid hydrolysis		Alkali hydrolysis		Oxidation	Photostability	Heat
	0 hr.	3 hrs.	0 hr.	3 hrs.	H_2O_2	UV	60°C, 7 days
Retention time in minute	6.781	6.864	6.782	6.894	6.795	6.897	6.888
% Assay	95.32	91.59	94.51	86.13	97.10	97.30	95.27
Peak purity peak purity index	0.9999	1.0000	0.9999	0.9999	0.9999	0.9999	0.9999

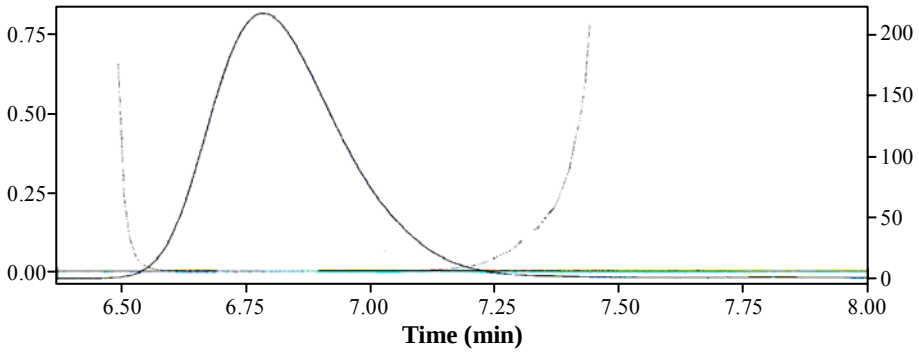

Fig. 10: Peak purity graph for acid hydrolysis, 0 hour degradation

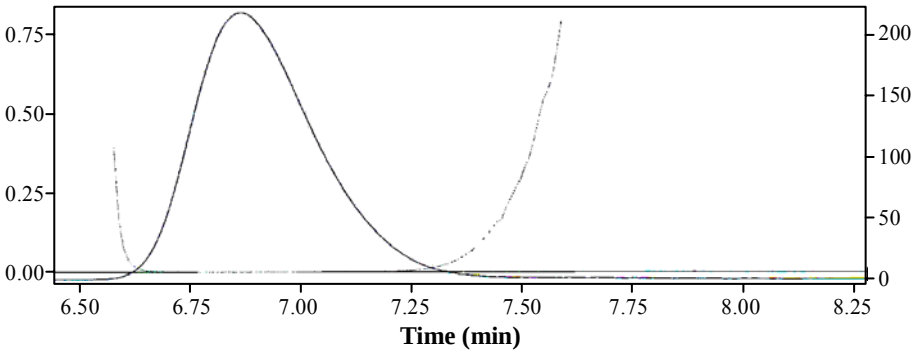

Fig. 11: Peak purity graph for acid hydrolysis, 3 hour degradation

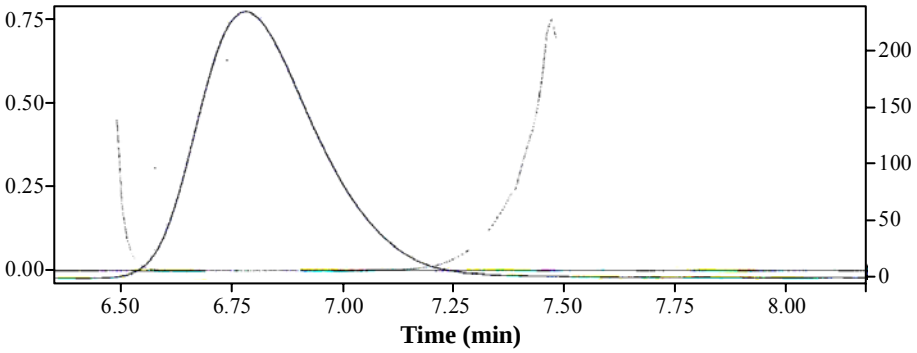

Fig. 12: Peak purity graph for alkali hydrolysis, 0 hour degradation

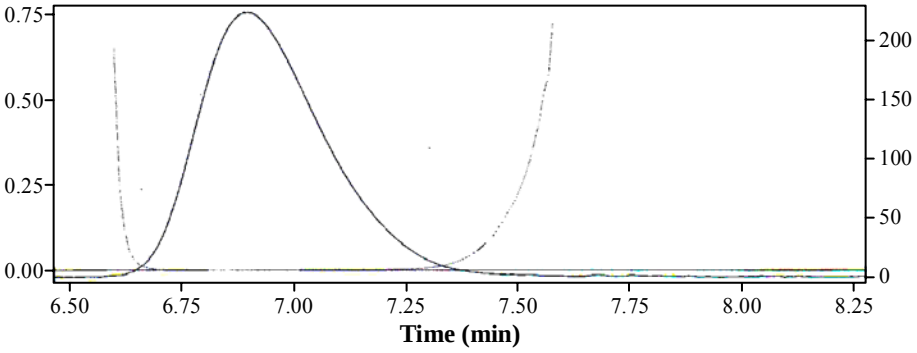

Fig. 13: Peak purity graph for alkali hydrolysis, 3 hour degradation

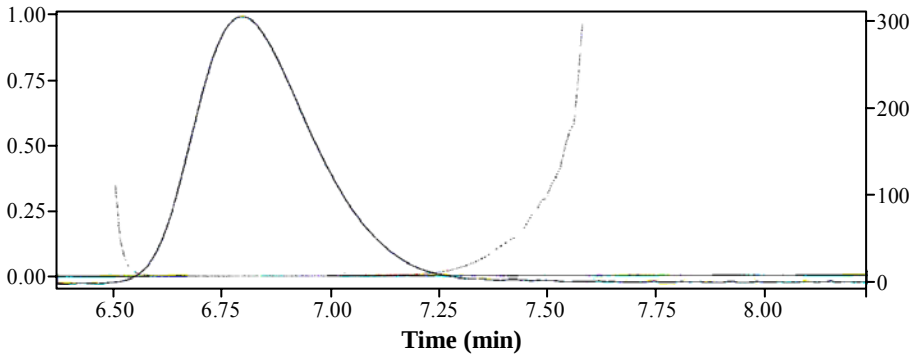

Fig. 14: Peak purity graph for oxidation degradation

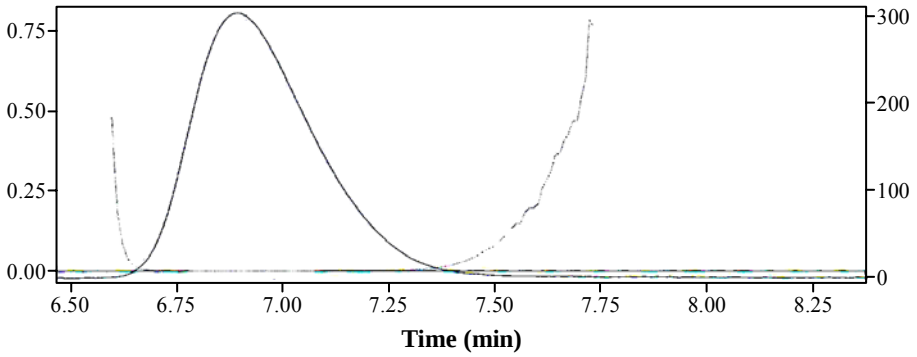

Fig. 15: Peak purity graph for photostability degradation

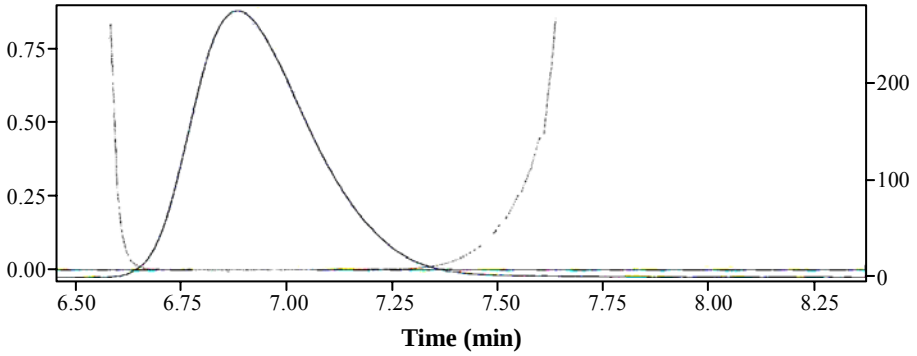

Fig. 16: Peak purity graph for heat degradation

CONCLUSION

Considering the efficiency of HPLC, attempt has been made to develop simple, accurate, precise and rapid method for simultaneous estimation of proguanil hydrochloride in a tablet dosage form. Thus method described enables the quantification of proguanil hydrochloride. All the analytical parameters studied for the method are within the acceptance limit set for the method. Therefore the analytical method of stability indicating assay of proguanil hydrochloride in its tablet formulation is considered to be validated for use. Hence, this method can be used for analysis of solid dosage form in quality control department. The methods described for the determination of Proguanil hydrochloride in marketed tablet formulations can be successfully employed for the determination of Proguanil hydrochloride

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