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Development and validation of new RP-HPLC method for determining impurity profiling in proguanil hydrochloride drug as well as it's tablet dosage form

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ABSTRACT

A simple rapid, sensitive, accurate, precise and reproducible high performance liquid chromatographic method has been developed to estimate impurity profile for Proguanil hydrochloride in drug as well as in tablet dosage form. The HPLC analysis used a reversed phase Kromasil C18 (150 x 4.6mm, 5µm) column and mobile phase constituted of buffer and methanol (42:58 % v/v). The buffer was composed of 4.0g hexane-1-sulphonic acid, sodium salt and 10 mL of glacial acetic acid in 790 mL of water. The dual wavelengths of the detection were 235 nm& 254 nm. The validation data showed that the method was sensitive, specific and reproducible for the impurity determinations of proguanil hydrochloride in dosage as well as in bulk form. The method was linear from 0.075µg/mL to 0.75µg/mL for Proguanil hydrochloride and it's impurities A, C & D. The accuracy of the method was found to be 100.21% for impurity A, 99.76% for impurity C and 100.21% for impurity D. Inter and intraday assay relative standard deviation (RSD) were found less than 3.01% in drug form and 2.77% in tablet dosage form for impurity A, less than 3.03% in drug form and 3.03% in tablet dosage form for impurity C and less than 2.34% in drug form and 3.62% in tablet dosage form for impurity D. The proposed method provided an accurate and precise analysis of impurity A, C & D in Proguanil hydrochloride Drug form as well as in pharmaceutical dosage form

Keywords: Proguanil hydrochloride; antimalarial drug, validation

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INTRODUCTION

Proguanil hydrochloride is a chemically 1-(4-chlorophenyl)-5-isopropyl-biguanide hydrochloride.

It is widely used in chemoprophylaxis of malaria. It is chronically administered for malaria prophylaxis in sickle cell patients and in pregnant women in Nigeria.

The use of proguanil in the prophylaxis and treatment of malaria has increased recently due to the emergence of chloroquine resistant Plasmodium falciparum. The use of proguanil in combination with other antimalarial drugs has also been reported to possess synergic toxicity on the malaria parasite. Paludrine tablet manufactured by GlaxoSmithKline Ltd. was also used

Methods of analysis of proguanil hydrochloride in biological fluids such as human plasma and urine by LC-MS and LC-MS-MS were reported previously. Chloroaniline & cycloguanidine determination in proguanil hydrochloride in pharmaceutical dosage form was also reported. The method that identified the main degradation or process related products by using some other techniques had been reported. A thorough literature has revealed that very few methods were reported for the determination of impurities of proguanil hydrochloride. This method describes the analysis and identification ofimpurity A, C & Din Proguanil hydrochloride, API and it's tablet dosage form by

complementary use of the HPLC techniques. In the present study, we aim to develop and validate a RP-HPLC-DAD impurity study method that allows resolution, detection and quantitation of proguanil hydrochloride and it's impurities A, C & Din bulk substance and tablet dosage form.

We reported the development and validation of a simple HPLC impurity determination with UV detection for the quantitative determination of impurities in bulk substance as well as in tablet dosage form.

MATERIALS AND METHODS

Chemicals and reagents

All the reagents were of analytical-reagent grade. De-ionized water (Millipore), HPLC-grade methanol, hexane -1-sulfonic acid, sodium salt AR grade, Glacial acetic acid HPLC grade wereused

Instrumentation

The HPLC system was composed of LC 2010Shimadzu system fitted with Prominence PDA detector with LC Solution software. Analytical column used for this method is KromasilC18 (150 mm x 4.6 mm), 5µm

Buffer preparation

4.0 g of Hexane-1-Sulphonic acid, sodium salt dissolved in a mixture of 790 ml of water and 10 ml of Glacial acetic acid

Diluent preparation

Methanol and buffer in the ratio of 58 : 42

Standard Preparation

Proguanil hydrochloride reference substance was accurately weighed (10 mg) and dissolved in 70 mL quantity of diluent in 100 mL volumetric flask anddilutedupto the mark and it was further diluted to generate a concentration of 0.2µg/mL

Impurity Standard Preparation

Impurity A: Impurity A was accurately weighed (5 mg) and dissolved in70 mL quantity of diluent in100 mL volumetric flask anddilutedupto the mark and it was further diluted to generate a concentration of 0.5µg/mL

Impurity C: Impurity C was accurately weighed (5 mg) and dissolved in 70 mL quantity of diluent in 100 mL volumetric flask and dilutedupto the mark and it was further diluted to generate a concentration of 0.5 µg/mL

Impurity D: Impurity D was accurately weighed (5 mg) and dissolved in 70 mL quantity of diluent in 100 mL volumetric flask and diluted upto the mark and it was further diluted to generate a concentration of 0.5 µg/mL

System Suitability Solution Preparation

Accurately weighed 10 mg of proguanil hydrochloride and dissolved in 100 ml volumetric flask with diluent. This standard stock 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, Impurity C and Impurity D and dissolved in 100 ml volumetric flask with diluent (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 diluent to get the concentration of 0.2 µg/ml for proguanil hydrochloride and 0.5 µg/ml of each of proguanil hydrochloride impurity A, impurity C & impurity D

Sample Preparation

Raw Material: Proguanil hydrochloride raw material was accurately weighed (10 mg) and dissolved in 70 mL quantity of diluent in100 mL volumetric flask and diluted upto the mark and it was diluted to generate a concentration of 100 µg/mL

Tablet: Twenty tablets of proguanil hydrochloride (100 mg of proguanil hydrochloride) were separately weighed and grounded to fine powder. An amount equivalent to 10 mg of proguanil hydrochloride was transferred into 100 mL volumetric flask and dissolved in 70 mL quantity of diluent and made up volume to 100 mL to generate a concentration of 100 µg/mL

Chromatographic conditions
Before the mobile phase was delivered into the system, buffer and methanol were filtered through 0.2µm, PVDF membrane filter and degassed using vacuum. The chromatographic conditions used for the analysis are given below
Column: KromasilC18 (150 mm x 4.6 mm) 5µm

Wavelength: 235 nm& 254 nm, dual mode
Injection volume: 20 µl
Flow rate:1.2 mL/min
Column temperature:30ºC
Run time: 30 min

Method development
Detection wavelengths for the HPLC study were selected as 235 & 254 nm after recording the UV spectrum from 190 to 800 nm of the drug and representative sample from standard, impurity 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 & it's impurities A,C & Din 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 impurity study of Proguanil hydrochloride drug form and its tablet dosage form

Method validation
Method validation was conducted according to published guidelines. Impurity profiling was evaluated by intraday and inter day (two different days) precision and determined from replicate analysis of samples (100µg/mL). Analysis of six different sample solutions was performed in the same day for intraday precision. The precision were expressed in terms of RSD from mean intra and inter day sample analysis.

Accuracy of the method was tested by adding a known amount of proguanil hydrochloride impurities A, C & D (0.4, 0.5 and 0.6µg/mL for each) in three sample solutions. Calculating the percent recovery from the peak areas obtainedfor diluted solutions

Signal-to-noise ratios were employed to estimate limits of detection (3:1) and limits of quantitation (10:1) for each proguanil hydrochloride impurity A, C & D

The specificity of a method is its suitability for analysis of a substance in the presence of impurities. Specificity of the method was established through the study of the resolution (Rs) of proguanil hydrochloride samples. Overall selectivity was established through determination of drug purity and Rs peak area RSD each time.

Various system suitability parameters were also evaluated on a mixture sample on different days using freshly prepared mobile phase each time

Robustness was tested by analysis of variations in analytical condition. Influence of mobile phase flow rate, filter paper change and column make were evaluated. The chromatographic parameters monitored were peak retention time, tailing factor and theoretical plate number

RESULTS AND DISCUSSION

Method development and optimization
Using a mobile phase consisting of different buffers with methanol and acetonitrile at different ratios and at different mobile phase pH values was attempted. Changes in the analytical procedure were tested. Different mobile phases with different proportions of organic modifier (acetonitrile/methanol) were tried. The pH value of the mobilephase was checked over a wide range. The ion pairing reagent hexane-1-sulphonic acid, sodium salt was also used to obtain better peak shape, solution stability & resolution. It was observed that the peakshape and retention time of proguanil hydrochloride was found to be broad compared to the buffer-acetonitrile or methanol composition as mobile phase. After various trials of mobile phases, ion pairing reagent hexane-1-sulphonic acid, sodium salt with glacial acetic acid was selected as buffer, and buffer-methanol ratio was chosen to be 42:58. Chromatographic run was evaluated using Kromasil C18 column. After selecting the best conditions based on peak performance, the run

time of the proposed method was 30 min with isocratic elution. During injection of a standard and sample solution, the retention times found were about 7.2 min for Proguanil hydrochloride and about 1.8 min for impurity A, about 3.13 min for impurity D and about 20.0 min for impurity C respectively. It shows good resolution of chromatogram with symmetrical peak. The proposed chromatographic conditions were found to be appropriate for the quantitative determination. System suitability tests were carried out as per ICH guidelines and the parameters are summarized in Table 1

Refer figure 1, 2 & 3 for system suitability, standard &sample solution chromatograph respectively

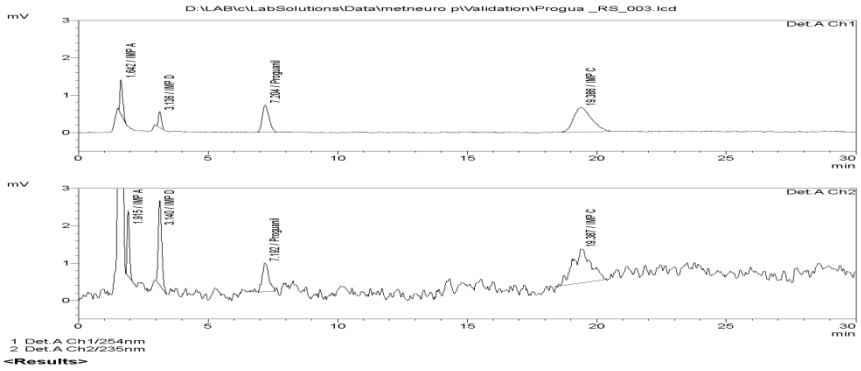

Figure 1: Chromatogram of the system suitability solution

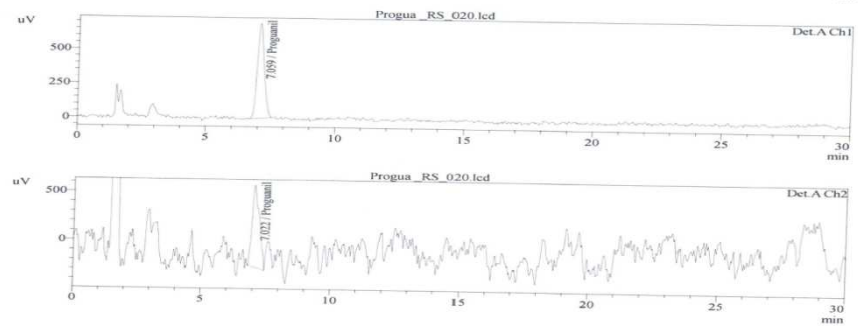

Figure 2: Chromatogram of the standard solution

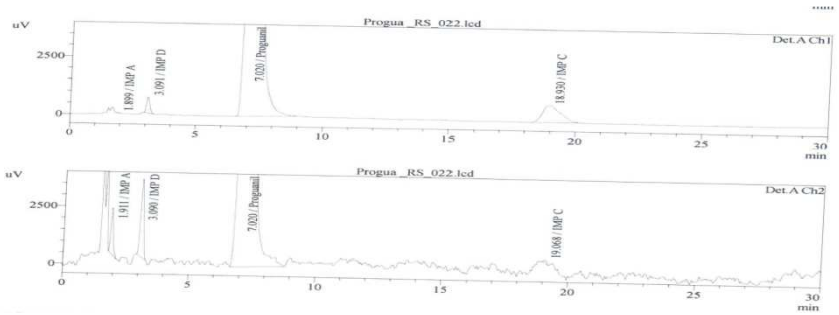

Figure 3: Chromatogram of the sample solution

Method Validation

Linearity: Linearity was studied by preparing standard solutions at different concentration levels for proguanil hydrochloride and it's impurities A, C & D. The linearity range was found to be 0.075 -0.75µg/ml for proguanil hydrochloride and it's impurities A, C & D

Refer Table1 for linearity study observations

Refer Figure 4, 5, 6 & 7for linearity graph of proguanil hydrochloride, impurity A, C&D respectively

Table 1: Linearity study observations

	Proguanil hydrochloride	Impurity A	Impurity C	Impurity D
Concentration range	0.075-0.75 µg/ml	0.075-0.75 µg/ml	0.075-0.75 µg/ml	0.075-0.75 µg/ml
Correlation coefficient	0.99969	0.99988	0.99904	0.99981
Slope	127.98	117.08	317.24	43.40
Y – Intercept	111.38	-109.15	-494.29	-85.04
R-square	0.99937	0.99976	0.99807	0.99962

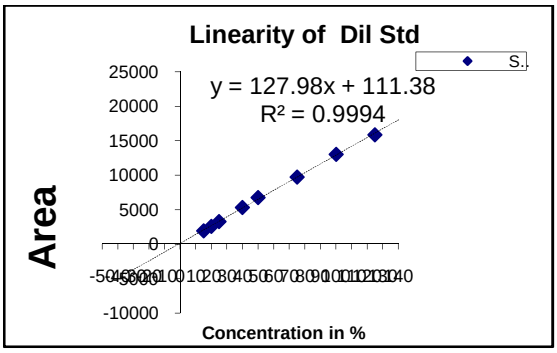

Figure 4:Linearity graph forproguanil hydrochloride standard

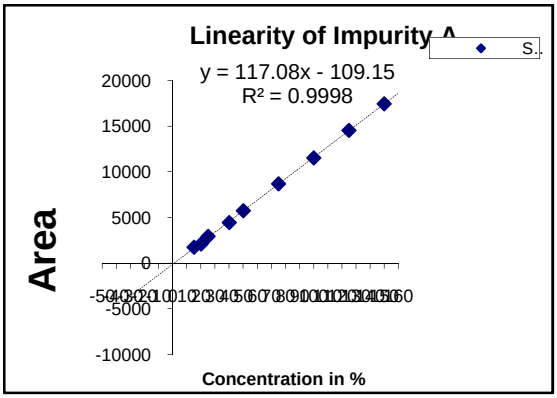

Figure 5: Linearity graph for proguanil hydrochloride impurity A

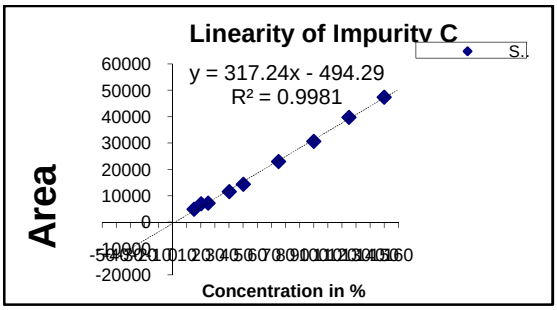

Figure 6: Linearity graph for proguanil hydrochloride impurity C

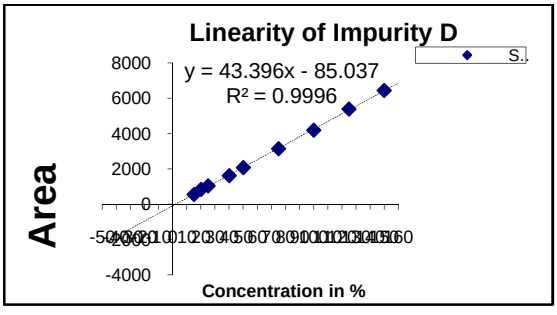

Figure 7: Linearity graph for proguanil hydrochloride impurity D

Specificity

Specificity is the ability to unequivocally assessthe analyte in the presence of components that maybe expected to be present. Typically, these might includeimpurities, degradants, matrix, etc. Specificity ofan analytical method is its ability to measure accuratelyand specifically the analyte of interest withoutinterference from the blank and placebo. Specificity ofthe peak purity of proguanil hydrochlorideand it's impurities A,C & D were assessed by comparingthe retention time of standard and the sampleand good correlation was obtained. Injecting the individual identification solution of Proguanil hydrochloride and it's impurities A, C & D for identification purpose. All the peaks were found pure in presence of each other. Also there were nopeaks when the placebo and blank were injected andno interferences, hence the method is specific. System suitability solution was injected to determine the resolution, tailing factor and theoretical plates for both the peaks. Refer Table 2 for Specificity study observations

Refer figure 8, 9, 10 & 11 for peak purity graph of Proguanil hydrochloride standard, impurity A, C & Drespectively

Table 2: Specificity study observations

	Proguanil hydrochloride	Impurity A	Impurity C	Impurity D
Retention Time in minute	7.204	1.642	19.386	3.136
Relative retention time	1.0	0.228	2.69	0.435
Resolution	11.107	-	14.377	6.988
Tailing Factor (NMT 2.0)	1.261	1.713	1.319	1.336
Theoretical plates (More than 2000)	3336.56	2145.45	4205.86	2881.01
Peak Purity	Peak Purity Index : 0.999	Peak Purity Index : 0.999	Peak Purity Index : 0.999	Peak Purity Index : 0.999
Blank/Placebo Interference	Not detected	Not detected	Not detected	Not detected
% RSD peak area (NMT 2.0 %)	0.78 %	0.68 %	0.57 %	0.73 %

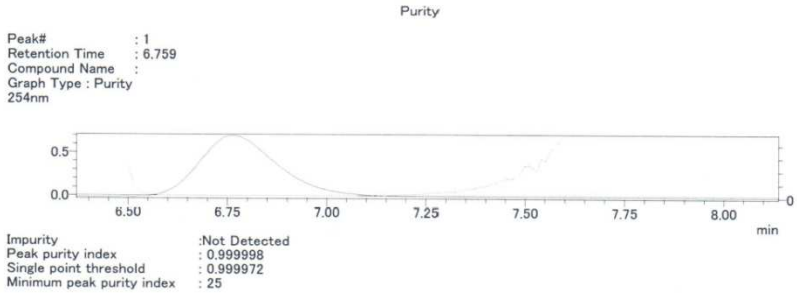

Figure 8: Proguanil hydrochloride standard peak purity graph

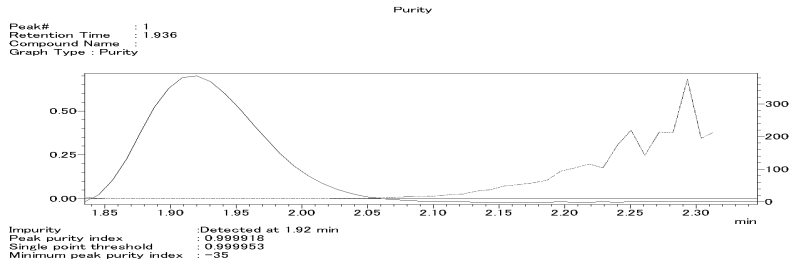

Figure 9: Proguanil hydrochloride Impurity A peak purity graph

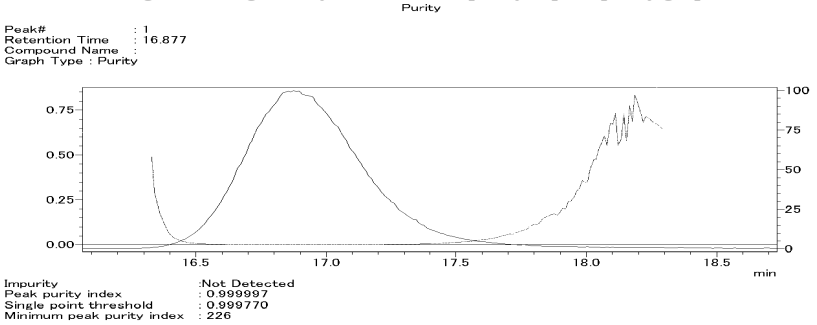

Figure 10: Proguanil hydrochloride Impurity C peak purity graph

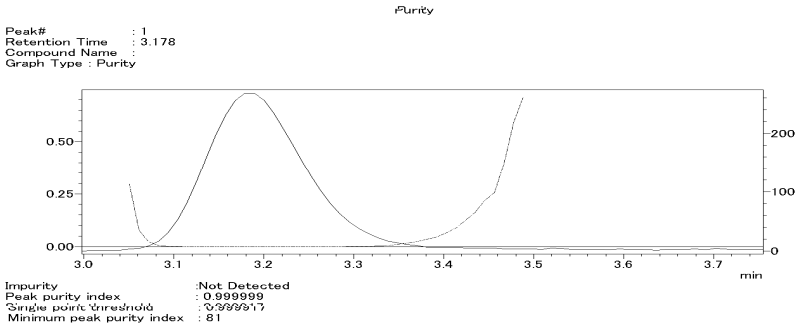

Figure 11: Proguanil hydrochloride Impurity D peak purity graph

Precision& Ruggedness

Precision was carried out for Inter and Intraday analysis for both drugas well as for tablet dosage form.Precision was evaluated by carrying out six independent sample preparations of a single lot of bulk drug and formulation.The sample preparation for bulk product was carried out insame manner as described in sample preparation for raw material.The sample preparation for tablet dosage form was carried out in same manner as described in sample preparation for tablet and spiking of impurity solutions to the concentration of about 0.5 µg/mL of each of impurity A, C & D.Relative standard deviation (% RSD) was found to beless than 5% for each impurity, which proves that the method is precise

Refer Table 3& 4 for precision study observations for Raw material&Tablet formulations respectively

Table 3: Precision study observations for Raw material

	Sr. No.	Impurities in RM (%)					
		Impurity A		Impurity C		Impurity D	
		Method Precision	Intermediate Precision	Method Precision	Intermediate Precision	Method Precision	Intermediate Precision
	1	0.022	0.023	0.022	0.022	0.023	0.024
	2	0.022	0.022	0.021	0.022	0.023	0.023
	3	0.023	0.023	0.022	0.023	0.024	0.024
	4	0.022	0.023	0.022	0.021	0.023	0.023
	5	0.023	0.022	0.023	0.022	0.023	0.025
	Mean	**0.022**	**0.023**	**0.022**	**0.023**	**0.025**	**0.023**
	SD	**0.022**	**0.023**	**0.022**	**0.022**	**0.023**	**0.024**
	RSD	**0.0005**	**0.0005**	**0.0007**	**0.0007**	**0.0004**	**0.0008**
Method Precision - Intermediate Precision	**Mean**	**0.023**		**0.022**		**0.024**	
	SD	**0.0005**		**0.0007**		**0.0007**	
	RSD	**2.34**		**3.03**		**3.01**	

Table 4: Precision study observations for Tablet formulation

	Sr. No.	Impurities in Tablet (%)					
		Impurity A		Impurity C		Impurity D	
		Method Precision	Intermediate Precision	Method Precision	Intermediate Precision	Method Precision	Intermediate Precision
	1	0.021	0.022	0.022	0.022	0.023	0.023
	2	0.021	0.023	0.021	0.022	0.022	0.022
	3	0.022	0.023	0.023	0.023	0.024	0.023
	4	0.021	0.022	0.021	0.022	0.023	0.023
	5	0.021	0.022	0.022	0.022	0.023	0.022
	Mean	**0.020**	**0.023**	**0.021**	**0.023**	**0.021**	**0.022**
	SD	**0.021**	**0.022**	**0.022**	**0.022**	**0.023**	**0.023**
	RSD	**0.0004**	**0.0005**	**0.0008**	**0.0004**	**0.0007**	**0.0005**
Method Precision - Intermediate Precision	**Mean**	**0.022**		**0.022**		**0.023**	
	SD	**0.0008**		**0.0007**		**0.0006**	
	RSD	**3.62**		**3.03**		**2.77**	

Accuracy (recovery studies)

To check the degree of accuracy of the method, recovery studies were performed in triplet by impurities standard addition method at 80, 100 and 120% concentration levels of Impurity standard(0.5 µg/mL). Known amounts of standard solutions of impurity were addedto the pre-analyze draw material samples and were subjected to the proposed HPLC method. The % recovery wasfound to be within the limits of the acceptance criteria with average recovery of 100.21% for impurity A, 99.76% for impurity C and 100.21 for impurity D

Refer Table 5 for results of recovery studies

Table 5: Results of recovery studies

	Impurity A	Impurity C	Impurity D
Level	% Recovery	% Recovery	% Recovery
80 %	100.39	100.66	99.60
100 %	99.79	99.24	101.34
120 %	100.44	99.39	99.70
Mean	**100.21**	**99.76**	**100.21**
% RSD	**0.80**	**0.81**	**0.97**

Limit of quantification and limit of detection

LOQ and LOD can be determined based on visual evaluation, signal-to-noise approach, standard deviation of the response and slope (calibration curve method). LOQ and LOD were calculated as LOD= 3.3×N/B and LOQ = 10×N/B, where N is the standard deviation of the peak areas of the drugs(n = 3), taken as a measure of noise, and B is the slope of the corresponding calibration curve. Limit of detection of proguanil hydrochloride was found to be 0.06µg/ml and the limit of quantification of impurity A, C & D were found to be 0.07µg/ml, 0.10 µg/ml & 0.15µg/ml respectively. Limit of detection of proguanil hydrochloride was found to be 0.03µg/ml and the limit of detection of impurity A, C & D were found to be 0.04µg/ml, 0.04µg/ml & 0.1 µg/ml respectively

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 makeand filter paper change was carried out. Tailing factor and theoretical plates were studied. The method was found to be unaffected by small changes like ±0.1 ml in flow rate, column make change to Phenomenox C18, 150 X 4.6 mm and filter paper from 0.45 µto whatmann 41 no

Refer Table 6 for the results of Robustness parameter

Table 6: Robustness parameter results

	Content	Column make	Filter paper 41 no.	Flow rate 1.1 mL/min	Flow rate 1.3 mL/min
Tailing Factor	ProguanilHCl	1.494	1.301	1.55	1.587
Theoretical plates	ProguanilHCl	2393.80	3073.46	2272.46	2046.36
Resolution	ProguanilHCl	9.119	10.292	8.906	8.332
	Impurity A	-	-	-	-
	Impurity C	11.914	13.088	10.660	9.311
	Impurity D	4.898	5.894	4.702	5.141
% RSD	ProguanilHCl	0.742	0.823	0.887	0.777
Retention Time	ProguanilHCl	6.892	6.921	7.526	6.326
Retention time for impurity	Impurity A	1.923	1.911	2.080	1.777
	Impurity C	18.53	18.53	20.28	16.972
	Impurity D	3.034	3.046	3.302	2.791
% RSD for Impurity content in RM	Impurity A	2.22	2.47	3.09	2.36
	Impurity C	2.32	2.48	2.66	3.14
	Impurity D	2.86	2.86	3.01	2.88
% RSD for Impurity content in Tablet	Impurity A	2.55	2.53	2.56	2.95
	Impurity C	3.07	2.83	2.81	3.30
	Impurity D	3.53	3.37	3.39	3.26

Stability of stock solution

During solution stability experiments, RSD for the impurity A, C & D content were found 3.52%, 4.29% and 4.13%respectively for raw material and 2.79%, 3.50% and 2.09% respectively for tablet dosage form which were within 5 % RSD. Results of the solution stability experiments confirmed that standard solutions and solutions in the diluent were stable for upto 12 hour during the analysis

CONCLUSION

As described in ICH guidelines, the identification and isolation of impurities is a very important task during drug synthesis and storage. It can provide crucial toxicology and safety data of the final drug and dosage forms. We have identified three impurities in samples of proguanil hydrochloride drug substance and drug product, characterized by HPLC analytical data. The HPLC method developed and validated allows a simple and fast quantitative determination of impurityA, impurity C and impurity D from bulk drug and its formulation. A mobile phase

composed of solvent A and acetonitrile with a short run time (30 min) and isocratic elution used were found to be advantageous and made the routine analysis easy. Among the significant advantages of this method are simplicity, selectivity, accuracy and precision ensuring that it is suitable for determining the impurity content of proguanil hydrochloride in tablet dosage form.

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