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Synthesis of impurities of proguanil hydrochloride, an antimalarial drug

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ABSTRACT

Proguanil is a synthetic biguanide derivative of pyrimidine. 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. During the process development or degradation of Proguanil hydochloride, some of the related substances (impurities) were observed. These impurities were identified as 1-cyano-3-(1-methylethyl)guanidine (Impurity A), 1,5-bis(4-chlorophenyl)biguanide (Impurity C) and 1,5-bis(1-methylethyl)biguanide (Impurity D). Present work describes the synthesis and characterization of these impurities.

Keywords: Proguanil hydrochloride, impurities, synthesis.

INTRODUCTION

Proguanil, a biguanide derivative of pyrimidine is the most active of a series of synthetic aryl biguanide compounds tested for antimalarial activity in the mid-1940s. Proguanil was demonstrated to be both efficacious and nontoxic hence its wide use as a prophylactic anti-malarial. 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.

Most of the drugs in recent years are purely synthetically made. Unambiguously, the synthetic drugs certainly contain various impurities such as either chemical or microbial. But of course most of the impurities are chemical only. The presence of impurities, also called as, related substances in an active pharmaceutical ingredient (API) can have a significant impact on the quality and safety of the drug products. Therefore, it is necessary to study the impurity profile of any API and control it during the manufacturing of a drug product. As per ICH guidelines any impurities, which are forming at a level of $\geq$ 0.10% with respect to the API should be identified, synthesized and characterized thoroughly.

Chemical name of Proguanil hydrochloride is 1-(4-chlorophenyl)-5-isopropyl-biguanide hydrochloride. 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.

Refer Figure 1 for Molecular framework of Proguanil Hydrochloride.

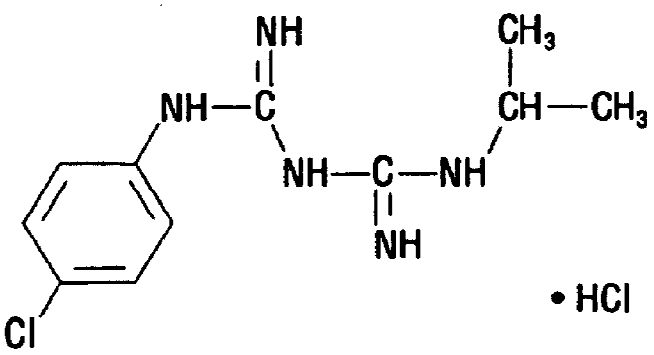


Figure 1:Molecular framework of Proguanil Hydrochloride

Proguanil,1-(4-chlorophenyl)-5-isopropyl-biguanide hydrochloride(Figure 1) is a prophylactic antimalarial drug, which works by stopping the malarial parasite, Plasmodium falciparum and Plasmodium vivax, from reproducing once it is in the red blood cells. It does this by inhibiting the enzyme, dihydrofolate reductase, which is involved in the reproduction of the parasite. The emergence of chloroquine resistant Plasmodium falciparum in our environment has led to a resurgence of interest in the use of Proguanil when daily prophylaxis of malaria is indicated. In addition Proguanil has found use in combination with other drugs such as atovaquone and dapsone in the treatment of resistant cases of falciparum malaria.

Here, we carried out the identification, synthesis, and characterization of 1-cyano-3-(1-methylethyl)guanidine (Impurity A), 1,5-bis(4-chlorophenyl)biguanide (Impurity C) and 1,5-bis(1-methylethyl)biguanide (Impurity D) of Proguanil Hydrochloride.

MATERIALS AND METHODS

Chemicals and reagents

Pure samples of Proguanil Hydrochloride were obtained from Ipca laboratories Limited as a gift. For synthesis of Impurities; Isopropyl Amine Hydrochloride, Sodium dicyanamide (Na–DCN), n-Butanol, Dioxane, P-chloropheny ldicyanamide (PCPD), p-chloro aniline (PCA), Hydrochloric acid; all were synthesis grade.

For Analysis by HPLC; methanol HPLC grade and hexane -1- sulfonic acid, sodium salt, AR grade andGlacial acetic acid HPLC grade were used. Highly purewater was prepared by double distillation and filtration through a 0.45 µm membrane filter.

Instrumentation

High performance liquid chromatography (analytical)

Shimadzu LC 2010 system equipped with a low pressurequaternary gradient pump along with a photo diodearray detector and auto sampler injectorwas used for analysis. The data was collectedand processed using LC Solution software. A Kromasil C18, 5 µm (150 mm x 4.6 mm) column wasemployed for the separation of impurity from Proguanil hydrochloride.

Mass spectrometry (LC/MS)

Initial LC/MS analysis was performed on a VarianInc, (USA) 410 Prostar Binary LC with 500 MS ITPDA detectors. The analysis was performed bydirect infusion mass with Electrospray Ionization (ESI) Negative & Positive mode ionization. Calculated the theoretical mass, mass which was obtained from the instrument and mass error.

NMR Spectroscopy

The 1H experiment was carried out for unknownimpurity at processional frequencies 400 MHz at25ºC on a Bruker Avance-300FT NMR spectrometer.^{1}H chemical shift was recorded on the δ scalein ppm, relative to $CDCl_3$or $DMSO_6$of δ 0.00 inppm.

FT IR Spectroscopy

The IR spectra were obtained using Shimadzu, FT IR spectrophotometer, with substances being pressed in a KBr pellet.

Chromatographic condition
The mobile phase A consisted of 4.0 g of Hexane-1-Sulphonic acid, sodium saltdissolved in a mixture of 790mL of water and 10mLof Glacial acetic acidand mobile phase Bconsisted of methanol, flow in ratio 45:55. A Kromasil C18column (150mm x 4.6 mm, 5 µ) was found to resolveProguanil hydrochloride. The mobile phase was filtered through a0.2µm membrane filter and then sonicated for 10min. The flow rate was set at 1.2mL/min. The drugshowed good absorbance at 235 and 254 nm, which were selectedas the wavelength for further analysis. All determinationswere performed at 30ºC column temperature.Mobile phase as buffer and methanol in the ratio of 45:55v/v, were used as sample diluent.

Preparation of solutions
Standard solution
Accurately weighed 10 mg of Proguanil hydrochlorideworking standard (WS)in 100mL volumetric flask, dissolve and dilute withdiluent (stock solution). The stock solution was furtherdiluted by using the same diluent to get concentrationof 0.2µg/mL of Proguanil hydrochloride.

Preparation of sample solution
Accurately weighed 10 mg of Proguanil hydrochlorideraw material in100mL volumetric flask dissolve and dilute withdiluent to get the concentration of 100 µg/mL of Proguanil hydrochloride

Preparation of System Suitability Solution
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 in 100 mL volumetric flask, dissolve and dilute 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

Detection of impurities by HPLC
Refer Figure 2 for HPLC chromatograms of Proguanil hydrochloride &impurity A, C& D at wavelength 254 & 235 nm.

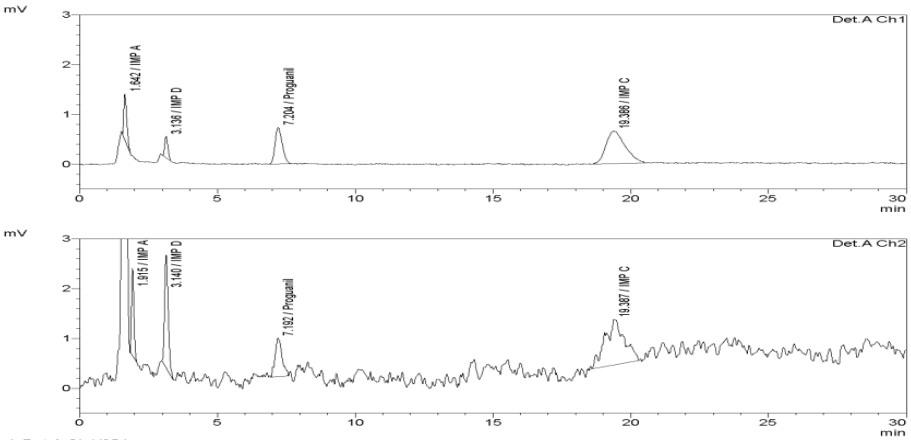


Figure 2: HPLC chromatograms of Proguanil hydrochloride and its impurities A,C& D

Synthesis of Impurities
Preparation of Proguanil hydrochloride Impurity A[1-cyano-3-(1-methylethyl)guanidine]

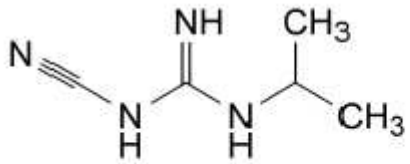
Raw Materials: Isopropyl amine hydrochloride, Sodium dicyanamide (Na–DCN), n-butanol, dioxane.

Procedure: Dissolved 500 mL of isopropyl amine hydrochloride in sufficient n-butanol, added about 500 mL of sodium dicyanamide and mixed well. Refluxed the reaction mixture for 4 h. Cooled the reaction mixture upto 60°C & filtered. Distilled out n-butanol under vacuum; sticky mass was obtained. To this sticky mass; 150 mL of

chloroform was added & stirred for 30 min. Filtered this solution & washed with chloroform. Distilled out chloroform under vacuum; sticky mass was obtained. In this sticky mass, dioxane was added, solid precipitate was obtained. Heated the reaction mixture upto 50°C to 55°C.Cooled to 0°C to 5°C. Filtered & washed the residue with chilled dioxane. Solid obtained was impurity A. Checked the purity.

RESULTS

Yield: 65 %
Refer figure 3 for Molecular framework of Proguanil Hydrochloride impurity A.
Refer figure 4 for Synthesis root of Proguanil Hydrochloride impurity A.



A. 1-cyano-3-(1-methylethyl)guanidine,

Figure 3: Molecular framework of Proguanil Hydrochloride impurity A

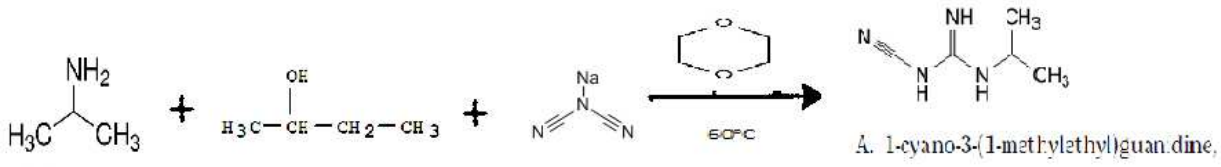


Figure 4: The synthesis root of impurity A.

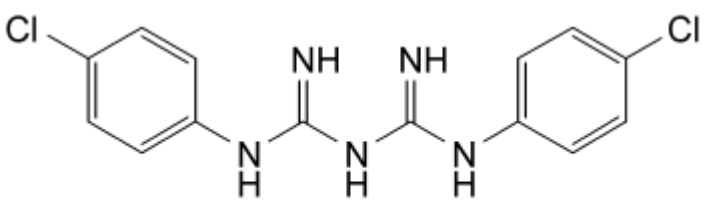
Preparation of Proguanil hydrochloride Impurity C [1,5-bis(4-chlorophenyl)biguanide]

Raw Materials:p-chloro phenyl dicyanamide (PCPD), p-chloro aniline (PCA), water, hydrochloric acid.

Procedure: Dissolved p-chloroaniline in dilute hydrochloric acid solution. Added this solution volume/volume in a (1:1) mixture of p-chloro phenyl dicyanamide (PCPD) and water; mixed this solution at 70°C. This reaction mixture was heated for 65 h at 70°C; maximum reaction was completed. Stopped heating & cooled the reaction mixture up to 30°C. Filtered the reaction mixture & washed the residue obtained with water; Solid obtained was impurity C. Checked the purity.

RESULTS

Yield: 60 %
Refer figure 5 for Molecular framework of Proguanil Hydrochloride impurity C.
Refer figure 6 for Synthesis root of Proguanil Hydrochloride impurity C.



C. 1,5-bis(4-chlorophenyl)biguanide,

Figure 5: Molecular framework of Proguanil Hydrochloride impurity C

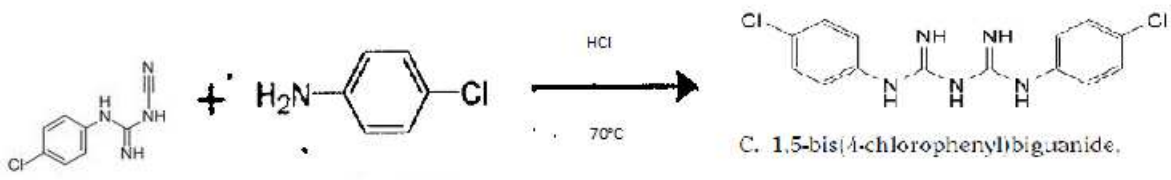


Figure 6: The synthesis root of impurity C

Preparation of Proguanil hydrochloride Impurity D [1,5-bis(1-methylethyl)biguanide]

Raw Materials: Sodium dicyanamide(Na–DCN), Isopropyl amine hydrochloride (IPA HCl), n-butanol.

Procedure: In a 100 mLfour necked round bottom flask, charged equal parts of isopropyl amine hydrochloride, n-butanol and sodium dicyanamide. Refluxed the mixture for 70 h by maintaining the pH from 2 to 3, after 70 h, stopped heating. Filtered this reaction mixture while hot (80°C) and washed the residue with hot n-butanol. Solid obtained was impurity D. Checked the purity.

RESULTS

Yield: 68 %
Refer figure 7 for Molecular framework of Proguanil Hydrochloride impurity D.
Refer figure 8 for Synthesis root of Proguanil Hydrochloride impurity D.

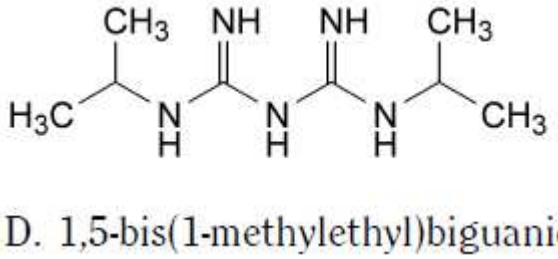


Figure 7: Molecular framework of Proguanil Hydrochloride impurity D

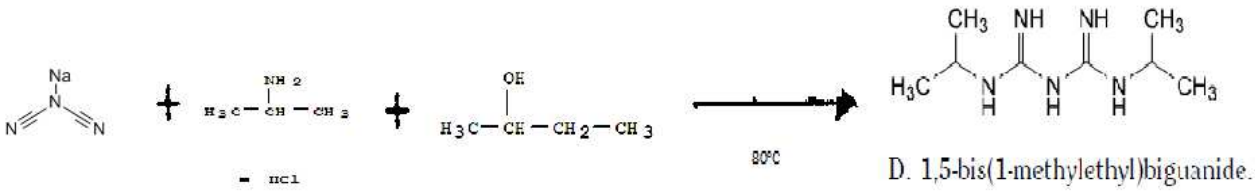


Figure 8: The synthesis root of impurity D.

RESULTS AND DISCUSSION

During the API process development or due to degradation of Proguanil hydrochloride various process-related or degraded impurities have been identified. The three known impurities were prepared by procedure of eachand characterized and confirmed. The structural data of the known impurities were confirmed with literature reported values.

A comprehensive study was undertaken to identify the unknown impurities by LC-MS followed by confirming through synthesis of this unknown impurities, followed by characterization based on spectroscopic techniques such as 1H NMR, IR Mass spectroscopy.

Presence of these known impurities was detected by HPLC in Proguanil hydrochloride. Proguanil hydrochloride impurity A, C & D as per BP were also used to compare with our synthesised impuritiesby using HPLC analysis and it was found comparable.

Characterisation of Impurity
The structure of impurities was confirmed by spectral analysis.

Proguanil hydrochloride impurity A
The structure of impuritywas confirmed by spectral analysis.

Peak purity index by HPLC 99.99%; FT IR (KBr) 3343, 3150, 2975 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) :1.210 (d, 3H), 1.227 (d, 3H), 3.706 (2, 2H), 5.394 (s, 1H); MS (EI) m/z 447.4 (M^+ + 1): calculated for $C_5H_{10}N_4$ molecular weight: 126.8; CHN Analysis: C, 47.60; H, 7.99; N, 44.41; Found: C, 47.71; H, 7.96; N, 44.33.

Refer figure 9, 10,11, 12, 13& 14 for NMR, LC-MS Spectrum, FT IR Spectrum, FT IR Purity Index graph, UV Spectrum Index & HPLC Purity Graph of Proguanil hydrochloride impurity A respectively.

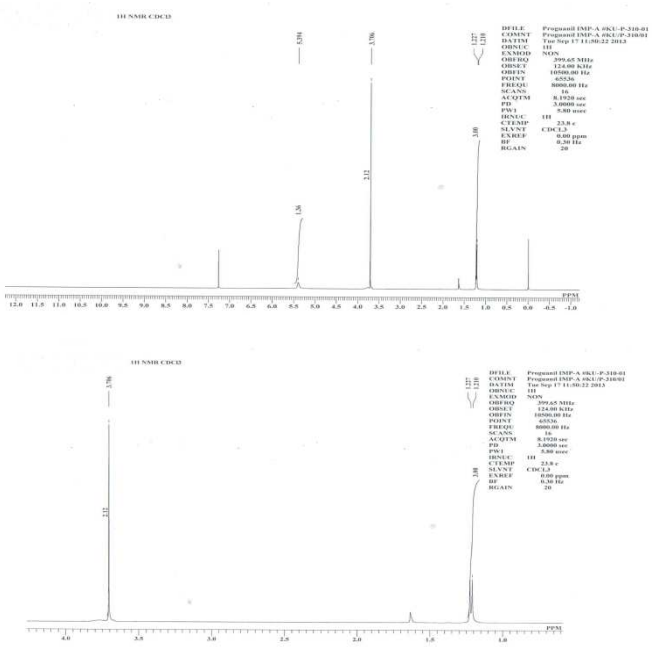


Figure 9: NMRgraphs of Proguanil hydrochloride impurity A

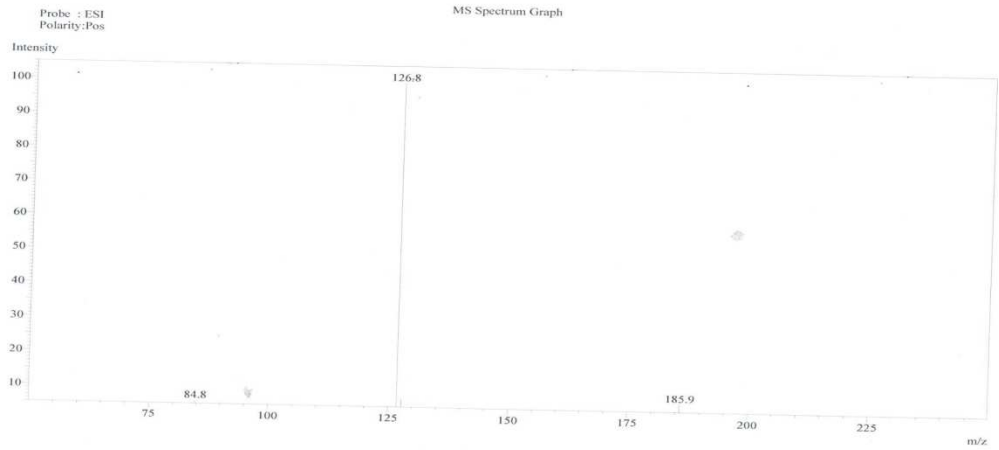


Figure 10: LC-MS Spectrum of Proguanil hydrochloride impurity A

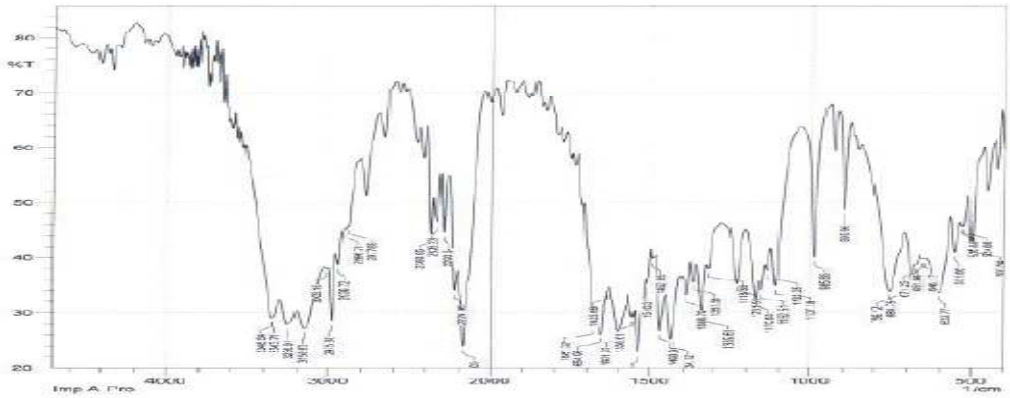


Figure 11:FT IR Spectrum of Proguanil hydrochloride impurity A

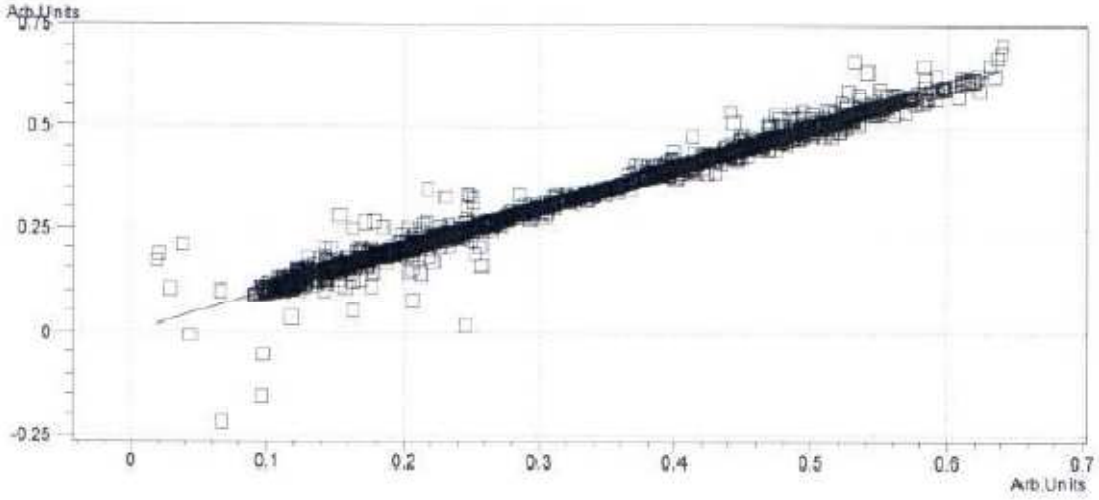


Figure 12:FT IR Purity Index graph of Proguanil hydrochloride impurity A

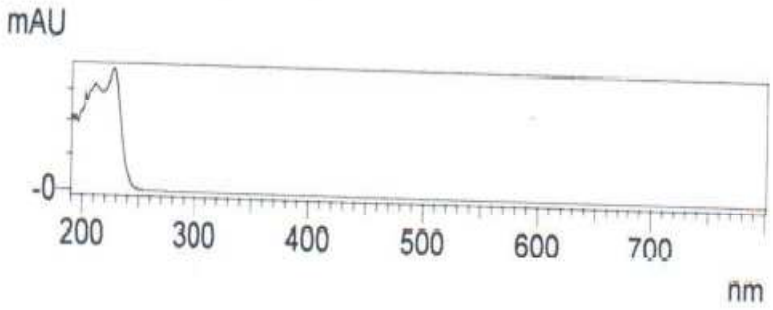


Figure 13:UV Spectrum Index of Proguanil hydrochloride impurity A

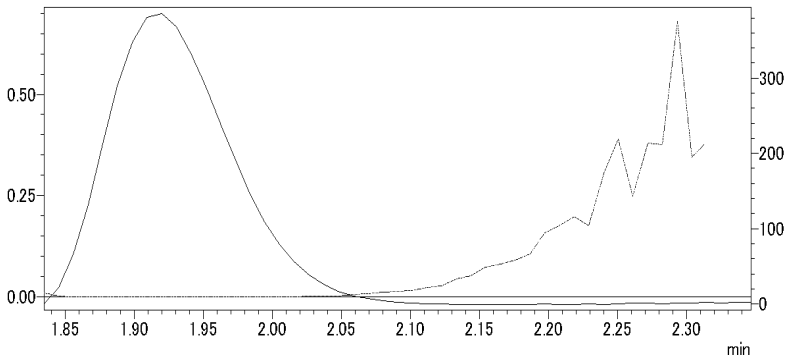


Figure 14:HPLC Purity Graph of Proguanil hydrochloride impurity A

Proguanil hydrochloride impurity C
The structure of impuritywas confirmed by spectral analysis.

Peak purity index by HPLC 99.99%; FT IR (KBr) 3307, 3197, 2992, 1623, 820 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) :7.3446 (3H, ddd), 7.33756 (4H, ddd), 7.32176 (5H, ddd), 7.31317 (4H, ddd,), 7.29042 (4H, ddd); MS (EI) m/z 447.4 (M^+ + 1) : calculated for $C_{14}H_{13}Cl_2N_5$ molecular weight: 321.6.; CHN Analysis: C, 52.19; H, 4.07; N, 21.74; Cl, 22.00 Found: C, 52.16; H, 4.04; N, 21.70;Cl, 21.93.

Refer figure 15, 16, 17, 18, 19&20for NMR, LC-MS Spectrum, FT IR Spectrum, FT IR Purity Index graph, UV Spectrum Index &HPLC Purity Graph of Proguanil hydrochloride impurity C respectively.

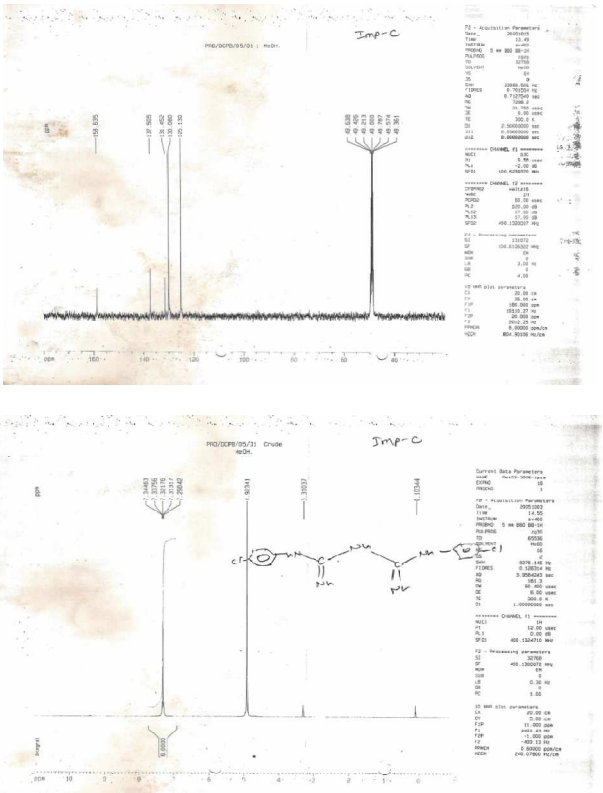


Figure 15: NMR graphs of Proguanil hydrochloride impurity C

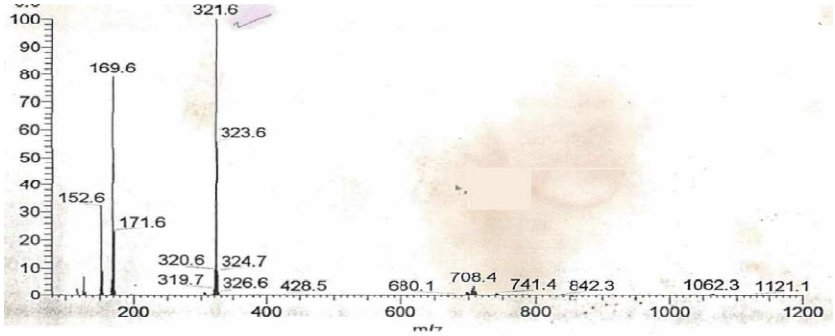


Figure 16: LC-MS Spectrum of Proguanil hydrochloride impurity C

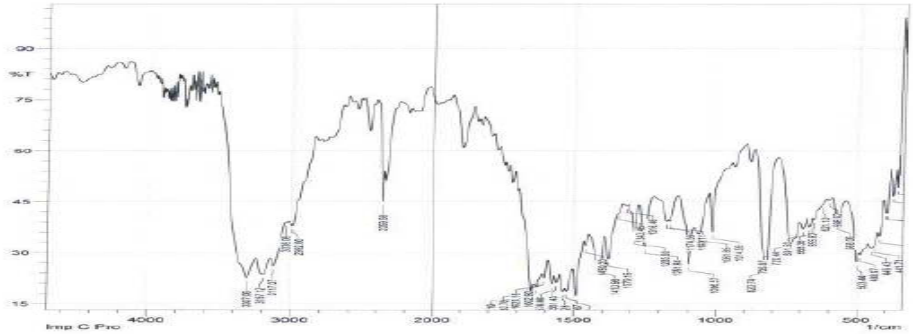


Figure 17: FT IR Spectrum of Proguanil hydrochloride impurity C

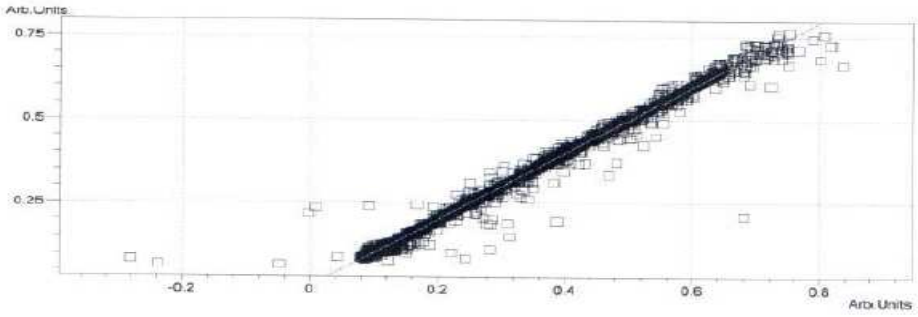


Figure 18: FT IR Purity Index graph of Proguanil hydrochloride impurity C

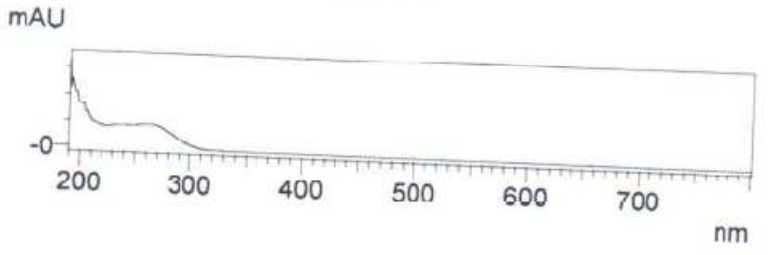


Figure 19: UV Spectrum Index of Proguanil hydrochloride impurity C

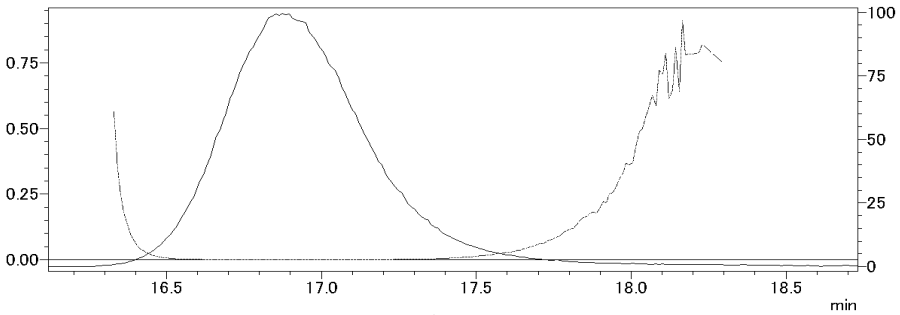


Figure 20: HPLC Purity Graph of Proguanil hydrochloride impurity C.

Proguanil hydrochloride impurity D

The structure of impuritywas confirmed by spectral analysis.

Peak purity index by HPLC 99.99%; FT IR (KBr) 3393, 3366, 2971 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): 1.54532 (m, 2H), 1.25431 (m, 3H), 1.10667 (d, 3H), 0.88095 (t, 3H),0.06924 (s,1H); MS (EI) m/z 447.4 (M^{+} + 1); calculated for $C_8H_{19}N_5$ molecular weight: 185.9; CHN Analysis:C, 51.86; H, 10.34; N, 37.80; Found: C, 51.82; H, 10.29; N, 37.84.

Refer figure 21,22,23, 24, 25& 26for NMR, LC-MS Spectrum, FT IR Spectrum, FT IR Purity Index Graph, UV Spectrum Index&HPLC Purity Graph of Proguanil hydrochloride impurity D respectively.

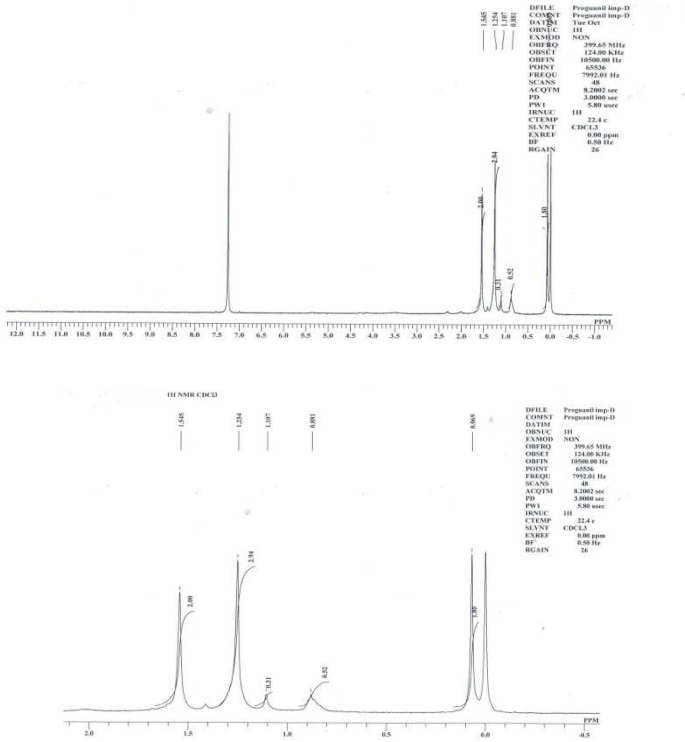


Figure 21: NMR graphs of Proguanil hydrochloride impurity D

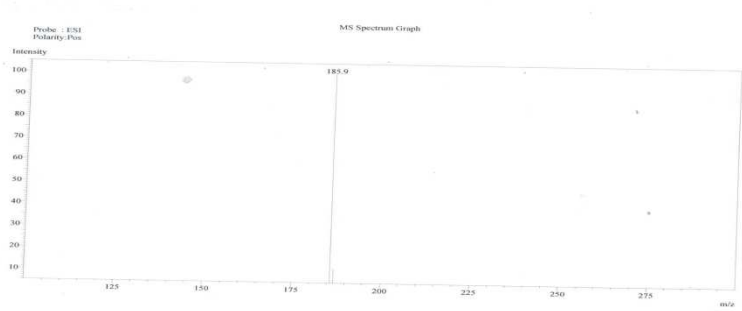


Figure 22: LC-MS Spectrum of Proguanil hydrochloride impurity D

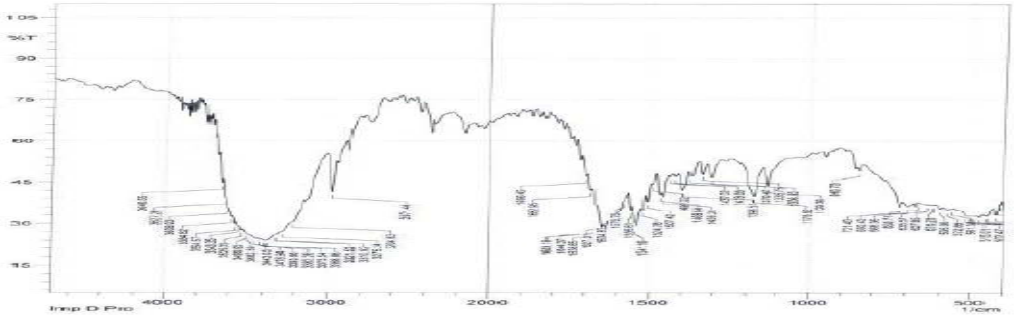


Figure 23: FT IR Spectrum of Proguanil hydrochloride impurity D

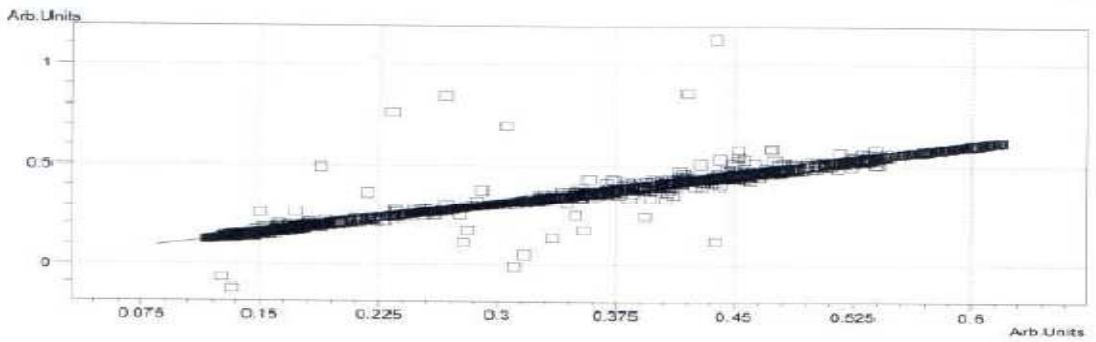


Figure 24: FT IR Purity Index graph of Proguanil hydrochloride impurity D

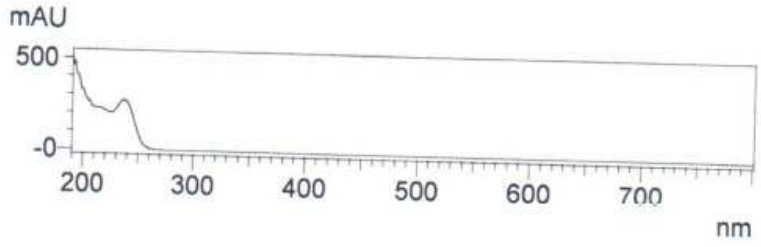


Figure 25: UV Spectrum Index of Proguanil hydrochloride impurity D

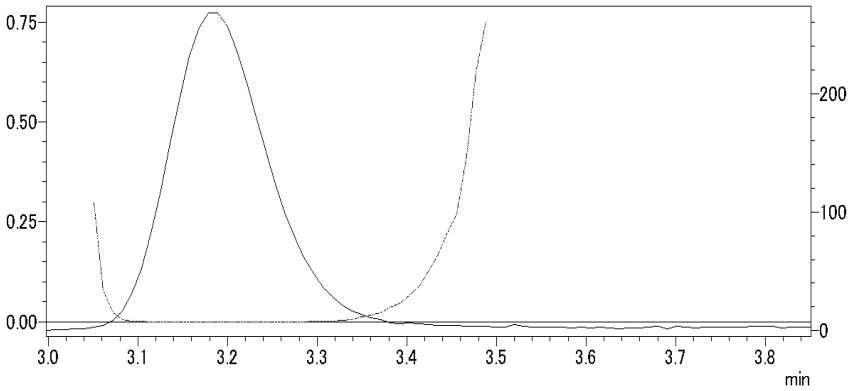


Figure 26: HPLC Purity Graph of Proguanil hydrochloride impurity D

Finally, Proguanil impurity A, C & D were individually injected with the Proguanil hydrochloride APIin the HPLC and the HPLC data was compared with that of the Proguanil impurity A, C & D as per BP by using spectral purity graph. As expected, by using FTIR purity graph (figure 24, 18&12 respectively); synthesised impurity was matching with the BP impurity purity graph.

CONCLUSION

We have demonstrated the synthesis and complete characterization of three1-cyano-3-(1-methylethyl)guanidine (Impurity A), 1,5-bis(4-chlorophenyl)biguanide (Impurity C) and 1,5-bis(1-methylethyl)biguanide (Impurity D) of Proguanil hydrochloride. This investigation helped us to establish the impurity profile of Proguanil hydrochloride.

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REFERENCES

[1] International conference on harmonization (ICH) Guidelines, Q_3A (R), February **2002.**
[2] International patent of publication number WO 2009/113092 A2, September **2009**, 17.
[3] Taylor RB, Moody RR, Ochekpe NA. *J. Chromatogr. Amsterdam*. **1987**,416: 394-399.
[4] Kalpesh N. Patel, Jayvadan K. Patel, *J. Chem. Pharm. Res*, **2010**; 2(1): 5-14.
[5] Patent application number: 20120283299, patent publication date:**2012**-11-08.

[6] Olugbenga et al., *AJPSR*,Nov. **2011**, volume 1 issue 6.
[7] Paul Hommerson,ISBN **2009,** 978-90-393-5101-7, NUR 913.
[8] Eidstein MD. *J. Chromatogr*. **1986**; 380: 184-189.
[9] DebjitBhowmik, *J. Chem. Pharm. Res*., **2010**, 2(1): 83-90.
[10] T. B. Patrudu, Der ChemicaSinica, **2011**, 2 (2):283-285.
[11] British pharmacopeia monograph, **2012**.
[12] Deepnandan S. Dubhashi, Nandini R. Pai, *Der Pharmacia Lettre*, **2010**, 2(4): 1-10.
[13] DeeptaunshuAtulPusalkar, Nandini R. Pai, *Der Pharmacia Lettre*, **2012**, 4(6):1657-1664.
[14] ICH Harmonized tripartite guideline, Q2B, **1996.**
[15] Rashid R Munjewar**,** *Der Pharmacia Lettre*, **2010,** 2(6):244-251.
[16] Vekariya N. A., *Der Pharmacia Lettre*, **2011,** 3(6):240-249.
[17] Seema S Sawant, Nandini R. Pai, *Der Pharmacia Chemica*, **2013**, 5(4): 274-281.
[18] MithunRudrapal, *Der Pharma Chemica*,**2010**, 2 (1): 194-203.
[19] Yogesh Murti, *Der Pharma Chemica*, **2010**, 2 (4): 271-277.
[20] SwarajPatil, *Der Pharma Chemica*, **2013**, 5 (4):80-86.
[21] SoumendranathBhakat, *Der Pharma Chemica*, **2012**, 4 (3):1247-1263.
[22] K. Siddappa, *Der Pharma Chemica*, **2012**, 4 (3):1206-1213.
[23] Nitish Gupta, *Der Pharma Chemica*,**2009**, 1 (2): 133-144.
[24] D Bhowmik, *Der Pharmacia Lettre*,**2009**, 1 (2) 262-276.
[25] M. Rudrapal, *Der Pharmacia Lettre*, **2011**, 3(3):29-36.
[26] G O Avwioro, *Arch. Apll. Sci. Res*., **2010**, 2 (3):112-116.