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Effect of temperature on chlorproguanil and proguanil hydrochloride solutions: a chemical stability study

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Abstract

Chlorproguanil and proguanil hydrochloride solutions in 0.5 M hydrochloric acid, water and 1 M ammonium hydroxide were subjected to different temperatures (22–80°C) for 68 h. The decomposition rate constants for chlorproguanil ranged from 1.60 to 47.6 × 10^3 h^{-1} in acid, 3.5 to 18 × 10^3 h^{-1} in water and 3.87 to 32.5 × 10^3 h^{-1} in base, between 50°C and 80°C. The activation energy E_a was 96.5, 52.12 and 62.1 kJ mol^{-1} in acid, water and base respectively. The proguanil decomposition rate constant ranged from 1.72 to 18.5 × 10^3 h^{-1} in acid, 1.58 to 9.67 × 10^3 h^{-1} in water and 2.34 to 15.77 × 10^3 h^{-1} in base, between 50°C and 80°C, with E_a values of 54.7, 73.3 and 62.5 kJ mol^{-1}. Three unidentified degradation products were separated in the acid solution for each of the compounds. Chlorproguanil and proguanil are stable ($t_{1/2}$ values over 30 days and up to 287 days respectively) in acid, water and base at temperatures below 22°C.

Keywords: Chlorproguanil; Proguanil; Chemical stability study; HPLC

1. Introduction

Chlorproguanil (CP) and proguanil (P) belong to the biguanide group of drugs used as antimalarial prophylactics. They are known to be active against malaria parasites in vivo with little activity in vitro [1,2]. This led to controversy over the active form of these compounds being either the main parent drug or the metabolities [3–5]. The main active metabolites for each drug, namely chlorcycloguanil and cycloguanil, have been identified and isolated [6,7].

Methods of analysis for these compounds in pharmaceutical dosage forms and in body fluids have been reported by some authors [8–10]. In the course of the analysis of Owoyale and Elmarakby [9] they observed some qualitative changes in the ultraviolet absorption of proguanil which was as a result of decomposition. Taylor et al. [10b] showed that the decomposition of

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proguanil gave four different products of which 4-chloroaniline has been identified. There is virtually no information on the chemical stability of chlorproguanil and limited information on proguanil and active metabolites especially at low concentrations as found in clinical samples. A knowledge of the kinetics of decomposition is essential for drugs such as these, in order to be able to assess the storage conditions of routine clinical or pharmacokinetics samples and for optimization of the sample pretreatment step during analysis. Pretreatment of biological fluids containing the biguanide antimalarials has been known to involve protein precipitation, liquid–liquid or solid phase extraction and reconstitution of analyte in mobile phase or water, with heat being applied in at least one of the steps.

Chemical stability studies may be conducted under normal storage or experimental operation conditions or under exaggerated conditions (accelerated stability testing) and the result extrapolated to normal conditions. The procedure usually involves the monitoring of the rate of decomposition of the parent compound or the rate of formation of the degradation products. The order of reaction and the rate constant for the decomposition at the respective temperatures and over a range of temperatures can then be determined and an Arrhenius plot made. The activation energy of the decomposition reaction can be calculated from the Arrhenius plot from which the stability at other temperatures can be predicted. This investigation is to extend the study on the pharmacokinetics of proguanil during which solid phase extraction of the biguanides was carried out in ammonium hydroxide, and elution of the analytes and evaporation to dryness was carried out in acidic medium. The purpose of this work is therefore to investigate the effect of temperature on the stability of proguanil, its in vivo metabolities [cycloguanil (C) and 4-chlorophenylbiguanide (4-CPB)] and chlorproguanil in acid, water and base with a view to suggesting storage and sample pretreatment conditions.

2. Materials and method

CP, P, C and 4-CPB were obtained from ICI. Acetonitrile was supplied by Rathburn chemicals (Walkerburn, UK) and sodium lauryl sulphate (SLS) by Fisons (Loughborough, UK). Water was distilled and further purified by using a Millipore Milli-Q system. All other reagents were of AnalaR or equivalent grade. A Jasco liquid chromatograph consisting of a Jasco 980 intelligent pump and a Jasco UV 975 variable wavelength detector fitted with a nominal 18 μl flow cell was used. Injection was by a Rheodyne 7125 valve incorporating a 20 μl loop. The column was 100 mm × 2 mm i.d. (stainless steel) packed in the laboratory with a slurry of 3 μm ODS Hypersil (Shandon HPLC, Macclesfield, UK).

2.1. Sample preparation

Separate aqueous stock solutions were prepared for chlorproguanil, proguanil, cycloguanil and 4-CPB. To 50 ml volumetric flasks 0.1 ml of the chlorproguanil, 0.2 ml of the proguanil, 0.4 ml of the cycloguanil and 0.5 ml of the 4-CPB stock solutions were added followed by (1) water to 50 ml volume, or (2) concentrated hydrochloric acid and water to produce a 0.5 M HCl solution in 50

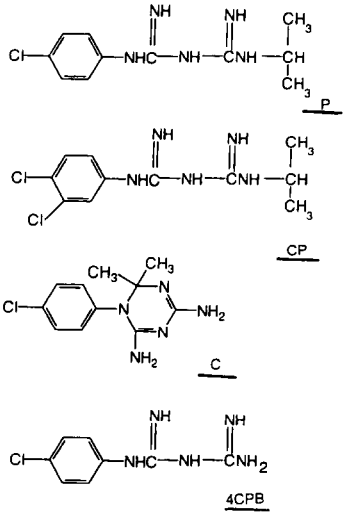

Fig. 1. Structural formula of proguanil (P), chlorproguanil (CP), cycloguanil (C) and 4-chlorophenylbiguanide (4CPB).

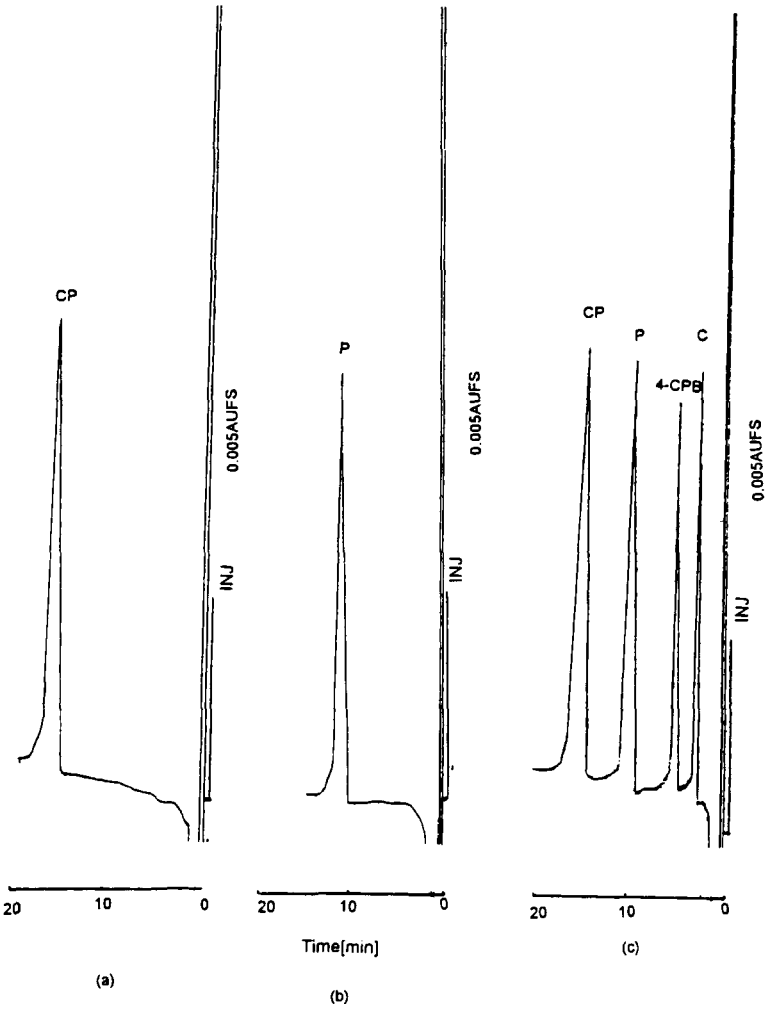

Fig. 2. Representative chromatogramms showing aqueous solutions of (a) CP 824 ng ml $^{-1}$, (b) P 435 ng ml $^{-1}$, (c) C 370 ng ml^{-1} and 4-CPB, before decomposition.

ml volume, or (3) concentrated ammonia and water to produce a 1 M NH_4OH solution in 50 ml volume. Separate dilutions containing only chlorproguanil in one case and only proguanil in another were made in these three media. Final concentrations of compounds were: chlorproguanil 824 ng ml $^{-1}$, proguanil 435 ng ml $^{-1}$, cycloguanil 371 ng ml^{-1} and 4-CPB 210 ng ml^{-1}.

2.2. Effect of temperature

Samples (10 ml) of the above solutions in well-stoppered glass sample bottles were stored in the oven at temperatures of 37, 50, 60, 70 and 80°C, and at room temperature (15–22°C). Samples (0.250 ml) for analysis were taken on an hourly basis up to 8 h and then at 24, 48, and 68 h. Samples were analysed immediately or stored at −20°C until analysed. All samples were protected from light.

2.3. Analysis

The chromatography used was adopted from a

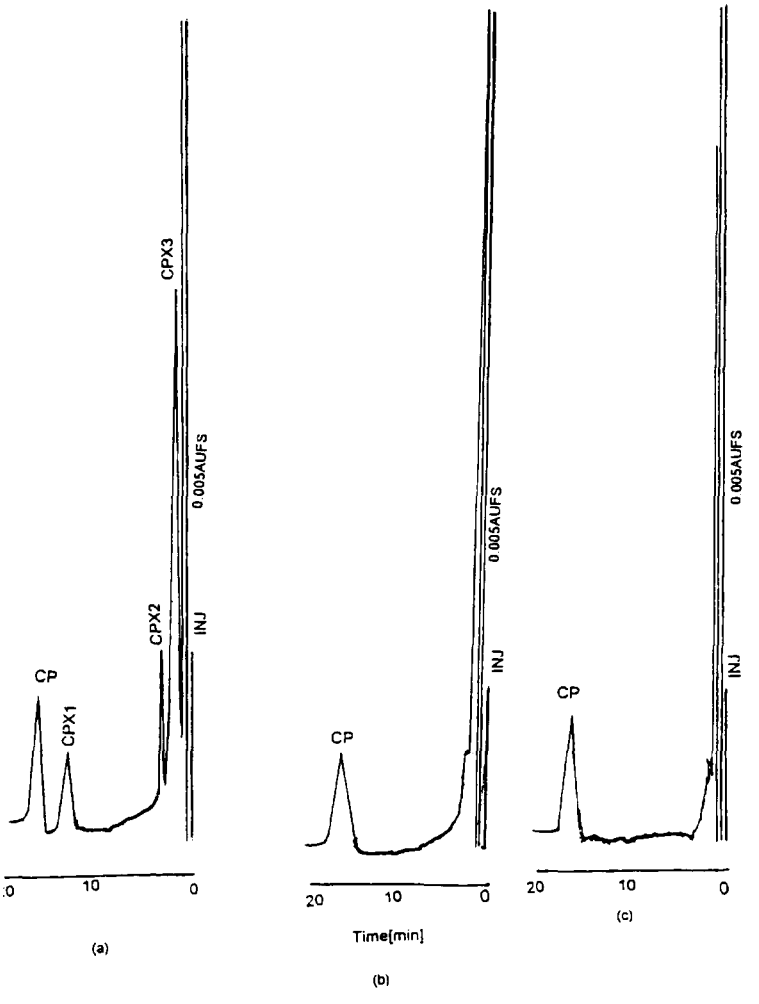

Fig. 3. Representative chromatograms showing CP in (a) 0.5 M HCl solution, (b) aqueous solution and (c) 1 M NH_4OH after decomposition at 80°C for 24 h. Peaks CPX1, CPX2 and CPX3 are decomposition products.

previously described procedure by Taylor et al. [10a]. The chromatographic solvent used was acetonitrile: 10 mM phosphate buffer pH 2.0 (50:50) containing 200 mM SLS. A flow rate of 0.4 ml min^{-1} was used. A 20 μl sample was injected into the chromatograph at the stated time intervals, and peak heights were measured at a detector wavelength set at 254 nm and a sensitivity range of 0.005 AUFS.

3. Results and discussion

3.1. Analytical characteristics of method

Chromatographic separation was achieved using 50% acetonitrile in 10 mM phosphate buffer containing 200 mM SLS at pH 2.0 on a 100 mm × 2 mm RP [3 μm ODS] stainless-steel column. The system was specific for chloro-

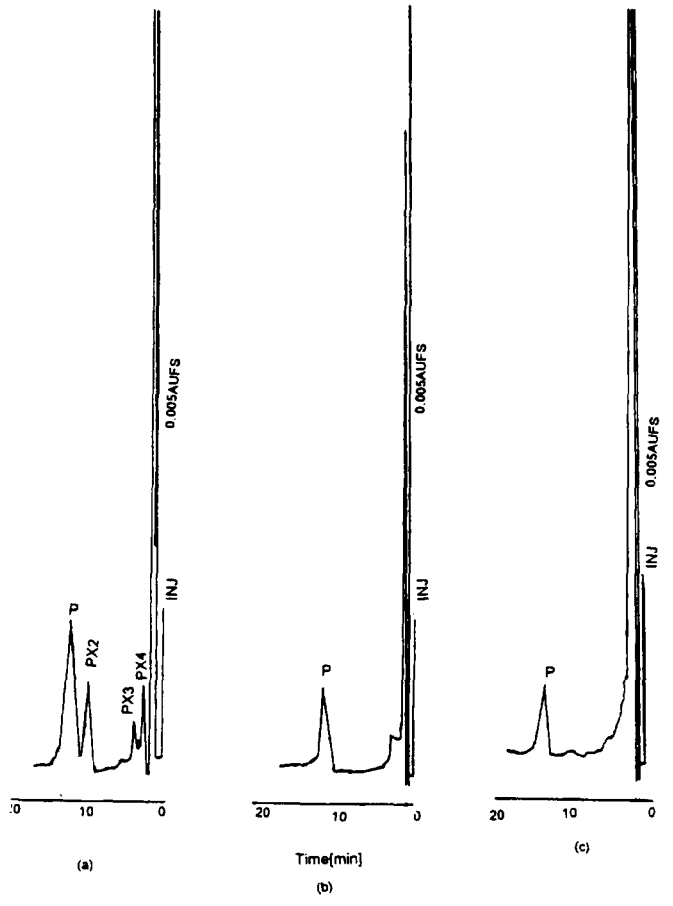

Fig. 4. Representative chromatograms showing P in (a) 0.5 M HCl solution, (b) aqueous solution and (c) 1 M NH_4OH solution after decomposition at 80°C for 24 h. Peaks PX2, PX3 and PX4 are decomposition products.

proguanil and proguanil (Fig. 1) with resolution (R) greater than one for parent drugs and degradation products (compare Figs. 2c and 5a). Linearity was good over the concentrations examined (CP, 824 ng ml^{-1}; P, 435 ng ml^{-1}), and the concentrations obtained during the decomposition. Typical regression equations for chlorproguanil and proguanil were $1.3743 \times 10^{-2}x - 0.30035$ with a correlation coefficient of 0.999 and $2.5297 \times 10^{-2}x - 0.236$ with a correlation coefficient of 0.998 respectively.

3.2. Effect of 0.5 M Hydrochloric acid solution

The decomposition rate constants for both drugs (chloproguanil and proguanil) were obtained at different temperatures by plotting the logarithm of peak height against time. The rate of decomposition of chlorproguanil was generally found to be faster than that of proguanil. At 80°C, the rate constant k of decomposition of chlorproguanil ($k = 47.6 \times 10^3$ h^{-1}) is higher than that of proguanil ($k = 18.45 \times 10^3$ h^{-1} or 3.08×10^4 min^{-1}) which is similar to what Taylor et al. [10b] reported for proguanil (3.18×10^4 min^{-1} at 70°C). Plots of k for chlorproguanil and proguanil following the Arrhenius equation gave activation energies E_A of 96.5 kJ mol^{-1} and 71.13 kJ mol^{-1} (correlation coefficients of the slope being 0.990 and 0.991) respectively. The large values of E_a obtained with HCl, when compared with E_a values obtained in water and ammonium hydroxide showed

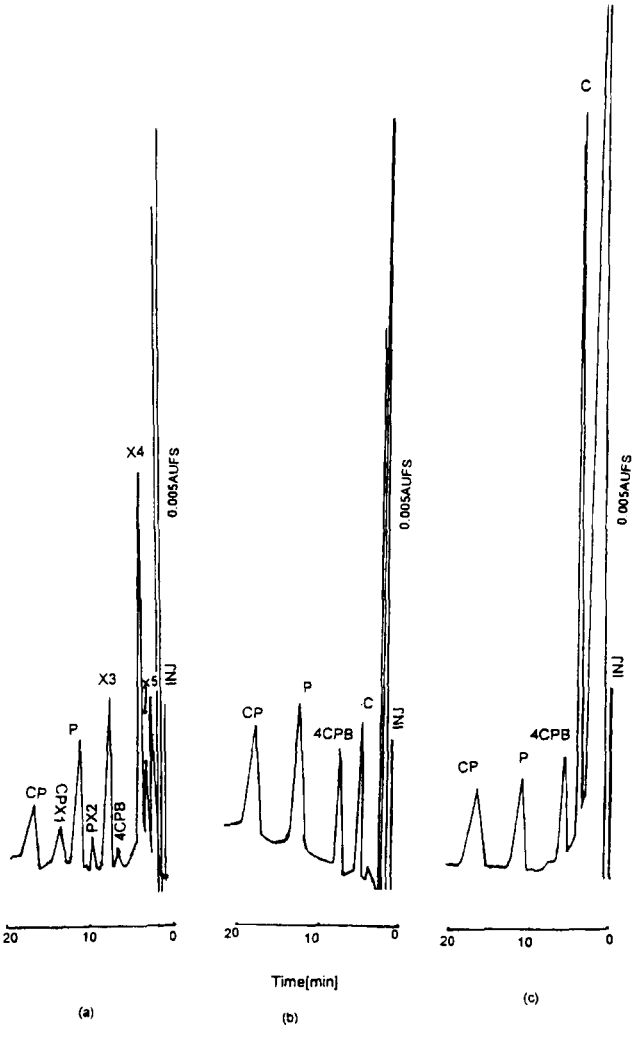

Fig. 5. Representative chromatograms of CP, P, C and 4-CPB after decomposition at 80°C in (a) 0.5 M HCl, (b) aqueous solution and (c) 1 M NH_4OH solution. CPX1, PX2, X3, X4 and X5 are decomposition products.

differences in reaction mechanism and pathway of decomposition. This was confirmed by the decomposition products obtained in HCl and those in water and ammonium hydroxide (see Figs. 2–5).

The formation of peak CPX1 for chlorproguanil and peak PX2 for proguanil (Figs. 3b and 4b) were similar to the formation of peak No4 (unidentified compound) in the report of Taylor et al. [10b] for proguanil. Both CPX1 and PX2 (see Figs. 6a and 6b) increase in peak height to a maximum plateau before declining to a minimum peak height of almost the same height as the parent drug. The initial rate of formation of CPX1 is 49×10^3 h^{-1} at 80°C within the first 12 h of the study and before its decomposition, while the initial rate of formation of PX2 is 11×10^3 h^{-1} at 80°C. Peaks X3 and X4 increaed in height throughout the study period while peak X5 (Fig. 5b) did not increase appreciably. The variation in peak height of these compounds with time and temperature showed that they are decomposition products while CPX1 and PX2 are intermediate

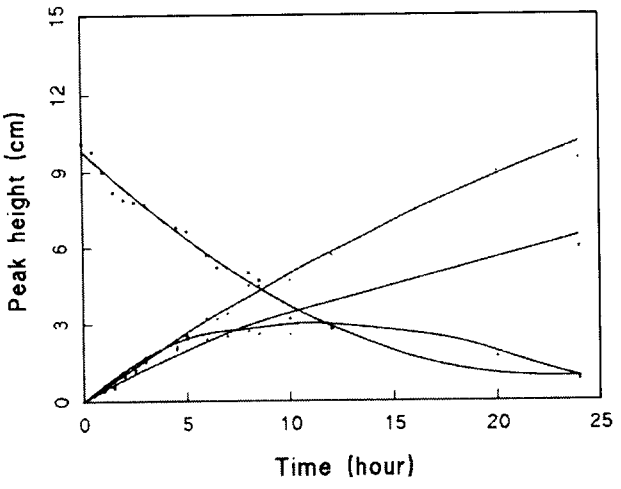

Fig. 6. Plot of the decomposition of CP over a period of 24 h in 0.5 M HCl at 80°C. The variation in peak height is shown for CP and its decomposition products (CPX1, CPX2 and CPX3).

products, possibly with close structural relationships with the parent drugs. The chromatogram of chlorproguanil (CP, with a retention time of 14.5 min) alone showed three decomposition products (CPX1, CPX2 and CPX3) in this system with retention times of 12.2, 2.8 and 1.6 min respectively (see Fig. 3).

3.3. Effect of aqueous solution

The decomposition rate constant k for chlorproguanil in aqueous solution ranged between 3.5 (50°C) and 18.0 (80°C) $\times 10^3$ h^{-1} with a correlation coefficient of 0.950 or better while those of proguanil ranged from 1.58 (50°C) to 9.67 (80°C) $\times 10^3$ h^{-1} (see Tables 1a and 2a). The decomposition followed first order reaction kinetics in all cases. A plot of the rate constants following the Arrhenius equation yielded activation energies $E_a = 52.12$ kJ mol^{-1} and 54.9 kJ mol^{-1} for chlorproguanil and proguanil (with correlation coefficients of the plot being 0.991 and 0.960) respectively. The aqueous solutions of both chlorproguanil and proguanil were found to show no significant change in stability at room tempera-

Table 1
Rate of chlorproguanil decomposition in aqueous, acidic and basic solutions with temperature

Temperature (°C)	k ($\times 10^3$ h^{-1})	Standard deviation of slope ($\times 10^{-5}$)	Correlation coefficient
(a) 0.5 M hydrochloric acid solution containing 824 ng ml^{-1} chlorproguanil			
15–22	–	–	–
37	0.48	4.92	0.990
50	1.60	37.00	0.900
60	8.63	97.00	0.987
70	18.15	95.50	0.990
80	47.60	17.00	0.993
(b) Aqueous solution containing 824 ng ml^{-1} chlorproguanil			
15–22	–	–	–
37	–	–	–
50	3.50	3.37	0.991
60	6.44	57.50	0.970
70	13.60	170.00	0.941
80	18.00	24.00	0.950
(c) 1 M ammonia solution containing 824 ng ml^{-1} chlorproguanil			
15–22	–	–	–
37	0.474	0.238	0.999
50	3.87	85.90	0.911
60	11.37	121.00	0.970
70	28.14	130.00	0.994
80	32.50	34.60	0.960

Table 2
Rate of proguanil decomposition in aqueous, acidic and basic solutions with temperature

Temperature (°C)	k ($\times 10^3$ h^{-1})	Standard deviation of slope ($\times 10^{-5}$)	Correlation coefficient
(a) 0.5 M hydrochloric acid solution containing 435 ng ml^{-1} proguanil			
15–22	–	–	–
37	–	–	–
50	1.72	37.57	0.940
60	4.96	12.04	0.999
70	9.84	32.58	0.996
80	18.50	88.00	0.998
(b) Aqueous solution of 435 ng ml^{-1} proguanil			
15–22	–	–	–
37	–	–	–
50	1.58	4.74	0.999
60	2.45	38.20	0.942
70	3.92	54.40	0.951
80	9.67	36.50	0.995
(c) 1 M ammonia solution containing 435 ng ml^{-1} proguanil			
15–22	–	–	–
37	–	–	–
50	2.34	18.77	0.987
60	3.17	60.10	0.964
70	8.89	90.00	0.960
80	15.77	65.86	0.993

ture (15–22°C) and 37°C over the course of the study. Proguanil is more stable in aqueous solution at the elevated temperatures than chlorproguanil. The chromatogram showed that the decomposition products were different from those reported in dilute hydrochloric acid by Taylor et al. [10b] and those in the present study under the effect of 0.5 M hydrochloric acid (see Figs. 3a and 4a).

3.4. Effect of 1 M ammonium hydroxide

The rate constant of decomposition ranged from 0.47 (37°C) to 32.5 (80°C) $\times 10^3$ h^{-1} for chlorprogaunil as compared with 2.34 (50°C) to 15.77 (80°C) $\times 10^3$ h^{-1} for proguanil. The rate of decomposition of both compounds at high temperatures followed the first order kinetics and a plot of the rate constants following the Arrhenius equation yielded activation energies E_a of 62.1 kJ mol^{-1} and 62.5 kJ mol^{-1} (with correlation coefficients of the plot being 0.993 and 0.950) for chlorproguanil and proguanil respectively. The E_a values were close to those of aqueous solutions, therefore suggesting a similar decomposition process (rate and mechanism of decomposition). The compounds decomposed as indicated by the reduction in peak height for chlorproguanil, proguanil and 4-CPB over the temperature range, but the decomposition products, being close to the solvent front, and not separated in this system. The transformation however might be cycloguanil (and its analog, chlorcycloguanil) as its peak height increased while others decrease (see Figs. 3c, 4c and 5c). The rate constant and $t_{1/2}$ values between 50 and 80°C showed proguanil to be more stable than chlorproguanil. From the above results, chlorproguanil and proguanil are stable in acid, water and base at room temperatures (22°C) as shown by the extrapolated rate constants and $t_{1/2}$ values (see Table 3). This further confirms the findings of Taylor et al. [10b] on the stability of proguanil at low temperatures and at neutral pH.

Table 3
Extrapolated rate constants for chlorproguanil and proguanil

Temperature (°C)	k in 0.5 M HCl ($\times 10^3$ h^{-1})	$t_{1/2}$ in 0.5 M HCl (days)	k in aqueous solution ($\times 10^3$ h^{-1})	$t_{1/2}$ in aqueous solution (days)	k in 1 M NH_4OH ($\times 10^3$ h^{-1})	$t_{1/2}$ in 1 M NH_4OH (days)
(a) Extrapolated rate constants h and $t_{1/2}$ for chloroproguanil at 22, 30 and 37°C						
22	0.097	297	0.555	52	0.510	56.6
30	0.274	105	0.972	30	0.991	29.13
37	–	–	1.55	18.6	1.728	16.7
(b) Extrapolation rate constants k and $t_{1/2}$ for proguanil at 22, 30 and 37°C						
22	0.165	175	0.227	127	0.257	112.4
30	0.363	79.6	0.410	70.4	0.493	58.6
37	0.699	41.3	0.671	43.03	0.863	33.5

4. Conclusion

The decomposition pattern as shown in the chromatograms produced with this chromatographic system and the activation energies obtained in ammonia and water were found to be similar (E_a50–62 kJ mol^{-1}) but different from those in acid medium (E_a73–96.5 kJ mol^{-1}). This suggests a change or a different mechanism of reaction, with different decomposition products. Decomposition products produced needed to be identified so as to prevent possible interference with the parent compounds during chromatographic or chemical analysis. At low concentrations as found in clinical samples, these compounds are stored at low temperatures (< 20°C). Pretreatment of samples involving heating should be at maximum temperatures of <35°C and such heating should be for a short time. The in vitro decomposition products are different from the in vivo metabolic products. Chlorproguanil and proguanil are stable compounds with respect to the experimental solution media (acid, water and base) at 15–22°C but not stable at high temperatures in any of the media.

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