*International Journal of Pharmaceutics*, 60 (1990) 185–90
Elsevier



IJP 02037

# A chemical stability study of proguanil hydrochloride

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(Received 20 October 1989)
(Accepted 7 November 1989)

*Key words*: Proguanil; HPLC; Stability; Tablet

## Summary

A stability-indicating assay method is described for proguanil based on reversed-phase ion-pairing HPLC. Using this method a study of the stability of proguanil in solution is made. In solution, proguanil decomposes by a first-order reaction. It is shown that the major decomposition product is 4-chloroaniline and that two other minor products can be detected but not identified. First-order rate constants over the temperature range 40–90°C are determined and an activation energy of 90.8 kJ $mol^{-1}$ derived. The log $k$-pH profile is determined and indicates maximum stability in the range pH 6–8. From the magnitudes of the rate constants obtained, it is evident that proguanil is a very stable drug in solution. The effect of ultraviolet radiation on proguanil is to produce the same decomposition products. The effects of thermal stress and ultraviolet on a tablet formulation of proguanil (Paludrine *) are also studied and these further confirm the stability of this compound to both thermal and photochemical stress.

## Introduction

Proguanil has been used as an antimalarial prophylactic for many years. It is widely believed to act as a prodrug producing the putatively active metabolite cycloguanil. In spite of its extensive use, few reports appeared in the early literature on the analysis, pharmacokinetics or stability of this drug.

Early methods of analysis in body fluids were developed based upon colorimetric reactions (Spinks and Tottey, 1945a). A microbiological assay has also been proposed (Smith et al., 1961). Relatively recently, due to the resurgence of interest in proguanil as a consequence of chloroquine resistance, several assay methods for the determination of this drug and its metabolites in biological fluids based on HPLC have been published (Moody et al., 1980; Edstein, 1986; Kelly and Fletcher, 1986; Taylor et al., 1987). These assay methods were developed particularly with a view to undertaking pharmacokinetic studies. Reports of such studies are now appearing in the literature (Wattanagoon et al., 1987; Owoyale and Elmarakby, 1989).

There remains at present in the literature an almost complete absence of information on the stability of the biguanide drugs, proguanil and chlorproguanil. The assay method of Spinks and Tottey (1945b) relies upon the decomposition of proguanil and any related compounds to 4-chloroaniline. To investigation has reported data on the kinetics of the biguanide decomposition. A recent paper (Owoyale and Elmarakby, 1989) indicated some qualitative changes in the ultraviolet absorp-

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tion of proguanil as a result of decomposition and indicated a need for a full kinetic study of proguanil decomposition.

Such a study is required firstly in order to assess storage conditions of clinical samples. This is particularly important where the samples may be stored for appreciable times before assay and may require to be transported internationally. Secondly data are needed concerning the degradation of the drug formulated as a tablet particularly under the influence of heat and ultraviolet radiation.

It is the purpose of the present work to extend our study of the assay and pharmacokinetics of this drug by investigating its stability and to report data concerning its decomposition products and first-order rate constants as a function of temperature and also pH. An indication will also be given as to the effect of heat and ultraviolet radiation on Paludrine tablets.

Such a study is now possible because of the availability of an assay method which can be classed as stability indicating. That is, it is capable of specific assay of the biguanide in the presence of its decomposition products and also allows the quantitation of the major decomposition product at low extents of decomposition.

## Materials and Methods

Proguanil and Paludrine tablets were obtained as a gift from ICI plc. 4-Chloroaniline was obtained from BDH Laboratory Chemicals Division (Poole, U.K.). Acetonitrile was supplied by Rathburn Chemicals and sodium lauryl sulphate by FSA (Loughborough, U.K.). Water, used as a chromatographic solvent, was distilled and further purified using a Millipore Milli-Q system. All other reagents were of AnalaR or equivalent grade.

A variety of modular liquid chromatography equipment was used in the course of this work. This included Waters Associates M6000A pumps and M440 and M441 fixed-wavelength detectors. Varian Associates 2510 pumps and a 2550 variable-wavelength detector were also used. Diode array detectors, Varian Polychrom 9060 and Shimadzu SPDM6A were also used as required. Chromatographic columns were 100 × 2 mm slurry packed in the laboratory with 5 or 3 μm ODS Hypersil.

*Decomposition*

(a) Preliminary measurements indicated that proguanil in solution was relatively stable. For this reason the temperature dependence of the decomposition was determined in 0.25 M HCl solution. A $2 \times 10^{-3}$ M solution of proguanil hydrochloride in water was prepared and diluted with an equal volume of 0.5 M HCl. Samples (10 ml) of the resulting solution were placed in stoppered flasks and immersed in thermostatted water baths at the following temperatures: 40, 50, 60, 70, 85, and 90 °C. At various time intervals over a time scale of 360 h, 250-μl samples were withdrawn and 20 μl of each was injected into the chromatograph. The pH dependence of the decomposition of proguanil in solution was determined at 85 °C. McIlvaine's citric acid-phosphate buffer was used to prepare solutions of pH 2.3, 3.0, 4.0, 5.0, 6.0 and 7.0. Sorensen's glycine II buffer was used to prepare solutions of pH 8.0, 9.0, 10.0, 11.0, and 12.3. In each of these solutions the proguanil concentration, as above, was $1.0 \times 10^{-3}$ M.

(b) Solutions, made up as above in water were subjected to ultraviolet radiation in a MRQ125 Immersion Well Reactor (fitted with a 6 W mercury lamp) for various lengths of time.

(c) Tablets: Batches of 20 Paludrine tablets were stored in desiccators at temperatures of 55, 62, 78 and 98 °C. Corresponding batches were also stored at these temperatures in desiccators containing water such that 100% humidity was obtained. Samples of three tablets were removed at 29, 63 and 98 days for analysis. A three-tablet batch was analysed before storage.

*Analysis*

The assay method used to follow the decomposition of proguanil was similar to that peviously published for the determination of proguanil and its metabolites in biological fluids (Taylor et al., 1987). In the present work the solid-phase extraction stage was not required. Degraded solutions were injected directly and tablet assays were car-

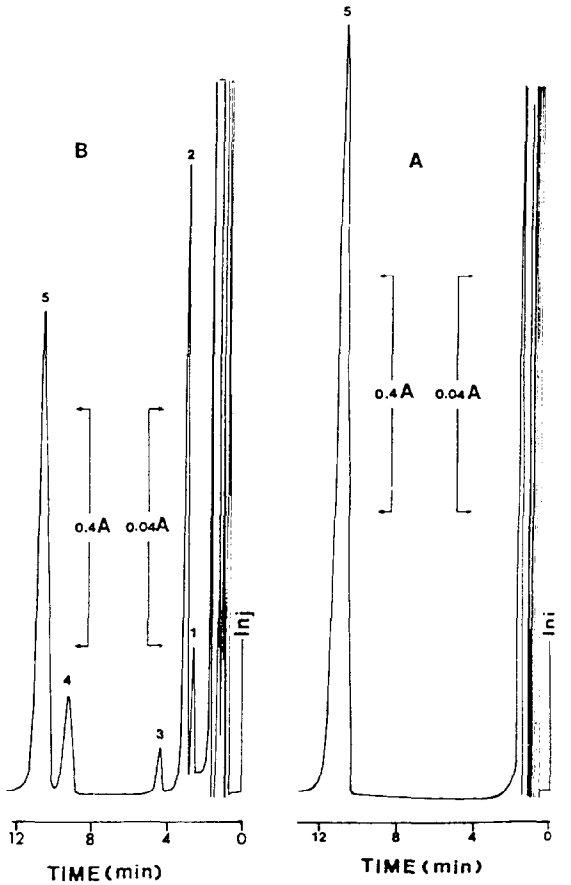


Fig. 1. Representative chromatograms showing solutions of (A) proguanil before undergoing decomposition and (B) proguanil after partial decomposition at 75 °C for 26 h. Peaks 1, 3 and 4 are decomposition products, peak 2 is 4-chloroaniline and peak 5 is proguanil.

ried out after solution of the drug and its decomposition products in water. The mobile phase of acetonitrile, 20 mM phosphate buffer containing 200 mM sodium lauryl sulphate (40 : 60) was capable of resolving the parent drug and all UV-absorbing decomposition products. This was established by examining peak purity by diode array spectrophotometry and also by progressively weakening the mobile phase. A typical chromatogram of degraded proguanil solution is shown in Fig. 1 together with that of an undegraded sample. It was possible to quantitate both parent drug and what was found to be the major decomposition product 4-chloroaniline using this stability indicating assay by comparing peak areas with regression lines of the peak areas of aqueous standards against concentration. An internal standard was not found to be necessary to establish adequate precision.

## Results and Discussion

*Reaction scheme*

Examination of the chromatogram of partially decomposed proguanil showed the presence of four decomposition products. The variation in the peak heights of these compounds with time is shown in Fig. 2. From this it is seen that peaks 1–3 increase uniformly with time which suggests that these compounds are products in the reaction. Peak 4 appears to reach an almost steady-state condition which indicates it to be an intermediate in the decomposition. All peaks were found to have different ultraviolet spectra when examined by diode array spectrophotometry.

The spectrum of peak 2 was found to be identical with that of 4-chloroaniline. Also, 4-chloroaniline was found to have a retention time on this system identical with that of peak 2. Using molar concentrations obtained by comparison with aqueous standards of both proguanil and 4-chloroaniline it was established that the sum of these two compounds was greater than 90% of the original amount of proguanil after 50% decomposition. This showed that 4-chloroaniline is the major de-

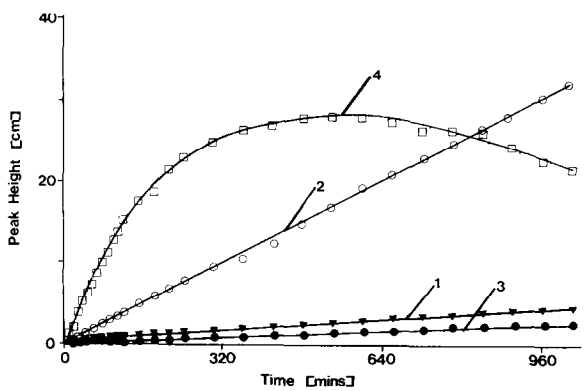


Fig. 2. Variation of peak height for the decomposition products, 1–4, during proguanil decomposition at 85 °C.

composition product and that peaks 1 and 3 represent the products of much slower alternative decomposition pathways. This conclusion was confirmed by comparing the first-order rate constants obtained by following the decrease of proguanil concentration and the increase of 4-chloroaniline concentration under the same conditions. The rate constant obtained by following proguanil decomposition was $8.87 \times 10^{-4}$ $min^{-1}$ and the mean value of calculating the first-order rate constant by monitoring the production of 4-chloroaniline resulting from three different initial proguanil concentrations was $8.16 \times 10^{-4}$ $min^{-1}$. These values indicate that 4-chloroaniline accounts for about 92% of proguanil decomposed.

*Solution decomposition*

*Order of reaction.* The reaction order of proguanil decomposition was determined in two ways. The initial rate of 4-chloroaniline was determined over a period of 2 h for four initial proguanil concentrations ranging from $1.3 \times 10^{-4}$ to $1.04 \times 10^{-3}$ mol $dm^{-3}$. A plot of log(initial rate 4-CLA) vs log(initial proguanil concentration) gave the following regression equation:

$$\log(\text{initial rate}) = 0.987 \log[\text{proguanil}]_0 - 3.06.$$

The slope of this line indicates that the reaction is first order. Also, when the decomposition was studied over large extents of reaction good linearity was obtained when first-order plots were used to establish rate constants, correlation coefficients greater than 0.995 being regularly obtained.

*Effect of temperature.* The first-order rate constants at temperatures over the range 40–90 °C were determined by plotting the logarithm of proguanil concentration vs time and measuring the slope. At all temperatures used, the reaction was followed to greater than 25% completion. These measurements were carried out in 0.25 M HCl solution in order to achieve adequate extents of decomposition. The rate constants obtained at the various temperatures together with their standard deviations and the correlation coefficients of the regression lines are shown in Table 1. These values when plotted according to the Arrhenius equation showed good linearity and yielded an activation energy of 90.8 kJ $mol^{-1}$.

TABLE 1

*Rates of proguanil decomposition in 0.25 M HCl at different temperatures*

| Temperature (°C) | $k'$ ($min \times 10^{-1}$) | S.D. ($\times 10^5$) | $R$ |
|---|---|---|---|
| 40 | 0.16 | 0.028 | 0.955 |
| 50 | 0.33 | 0.047 | 0.967 |
| 60 | 1.03 | 0.039 | 0.997 |
| 70 | 3.18 | 0.248 | 0.992 |
| 85 | 8.87 | 0.720 | 0.992 |
| 90 | 19.60 | 1.483 | 0.997 |

*Effect of pH.* First-order rate constants at a temperature of 90 °C over the range pH 2–12 in the buffer solutions described and including the value determined in 0.25 M acid are shown as a log($k$)-pH profile in Fig. 3. It appears that the hydrolysis reaction is catalysed by both acid and base. If the anomalously low value at pH 3 is ignored the drug appears to be most stable from about pH 6 to 8.

*Effect of UV.* From the high stress required to induce experimentally measurable rates of decomposition it is evident that proguanil, in solution, is

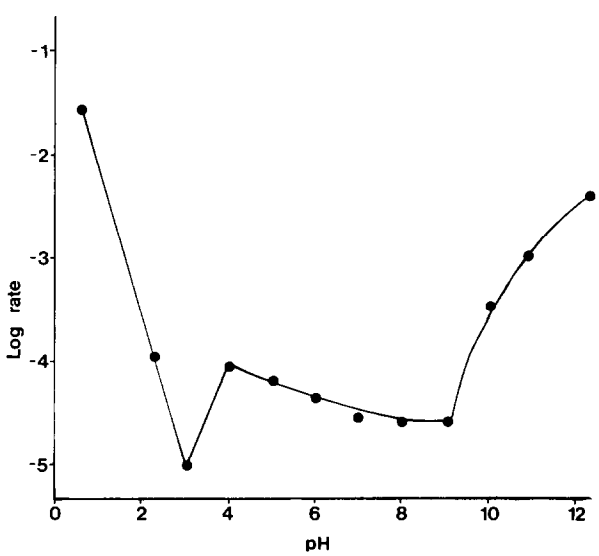


Fig. 3. Log $k'$-pH profile for proguanil decomposition at 90 °C.

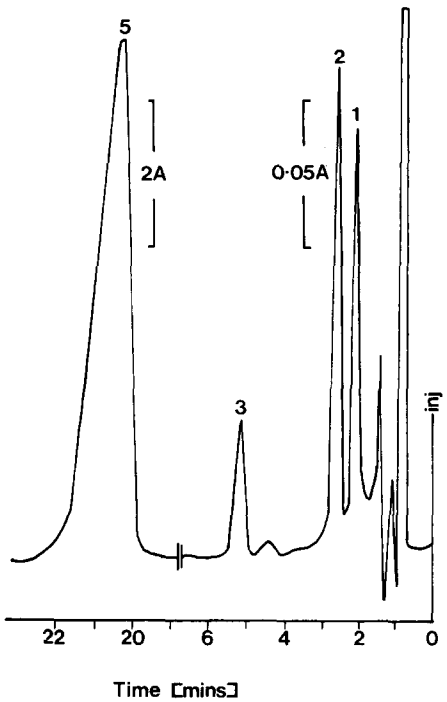


Fig. 4. A representative chromatogram of partially decomposed proguanil solution after irradiation with UV light for 5 h. Peak identification as in Fig. 1.

a stable compound. This stability was also evident when solutions of the drug were subjected to ultraviolet radiation in a photochemical reactor.

A typical chromatogram of proguanil solution degraded in this way is shown in Fig. 4. The major product was again found to be 4-chloroaniline. Fig. 4 represents a separation using a chromatographically weaker solvent than that described above for the purpose of ensuring optimum resolution. Such photochemical stress did not appear to have a marked effect in accelerating the reaction. Under the experimental conditions used rate constants of about $8.34 \times 10^{-5}$ $min^{-1}$ were obtained.

*Tablet decomposition*

*Effect of temperature.* The general stability of proguanil is further shown by the decomposition rate constants determined for this drug in tablet form. These values ranged from $0.8 \times 10^{-9}$ to $6.0 \times 10^{-9}$. No clear trend with temperature or humidity could be determined. The amplitudes of these rate constants do not differ statistically from zero. This is a result of the low extents of reaction achieved over this time scale together with the inherent lower precision associated with tablet sampling (Dihuidi et al., 1982). No evidence of 4-chloroaniline was observed in the chromatograms of degraded proguanil tablets which would have allowed application of the initial rate method (Taylor and Shivji, 1987). It was assumed that this was due to loss of the decomposition product by evaporation, although the possibility exists that different products are being produced during the solid state decomposition. No evidence of loss of proguanil was observed when tablets were irradiated under ultraviolet radiation for relatively short times.

## Conclusions

From the above results the assay method described appears to be adequate as a stability-indicating method in that it can quantitate proguanil specifically and can also detect and quantitate the major decomposition product.

The stability results confirm, quantitatively, some of the suggestions made concerning proguanil in a previous publication (Owoyale and Elmarakby, 1989) in terms of its photochemical stability and the low concentrations of decomposition product detected.

The overall conclusion from the present study is that proguanil is a very stable compound with respect to both temperature and photochemical stress and has, as its major detectable decomposition product, 4-chloroaniline.

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