International Journal of Pharmaceutics, 7 (1980) 177–178

Short Communication

An HPLC assay for the determination of proguanil hydrochloride in tablets

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(Received October 3rd, 1980)
(Accepted October 21st, 1980)

Proguanil hydrochloride is widely used as a prophylactic agent against malaria. It is usually administered in the form of 100 mg tablets. A specific, sensitive, simple procedure of short analysis time for the assay of the tablets has proved difficult to obtain. Turbidimetric (Spinks, 1946) and colourimetric methods (Spinks and Tottey, 1946), a GLC method involving electron capture (Holmes, 1979) and the BP gravimetric method have all been used as assay procedures, but only the BP method has specifically been applied to tablets. All are multistep and very time consuming.

A simpler, easily repeatable, less time consuming and highly specific HPLC method has been devised. The method is based on previously published work on the HPLC of proguanil and related compounds (Moody et al., 1980). For comparative purposes, results from the method outlined below have been compared with results using the BP assay.

The tablets and standard used were donated by ICI (Macclesfield, England). To ensure samples for all assays came from the same batch, three-hundred 100 mg tablets were powdered and bulked; all tablet assays were carried out using samples taken from this bulked mass.

The standard supplied was determined to be 99.7% pure with a standard deviation of 0.71 (4 assays) by the official BP method.

The HPLC method used was as follows: a sample of the powdered tablets containing the equivalent of 50 mg of proguanil hydrochloride was weighed out and dissolved as completely as possible in hot water, cooled and then filtered. The filtrate was made up to 50 ml with water. One ml of this solution plus 2 ml of 0.1% (w/v) phenylbenzoate in ethanol (as internal standard) were pipetted into a 25 ml volumetric flask and made up to the mark with water.

For preparation of the standard calibration graph, 5 mg of proguanil hydrochloride was weighed out, dissolved in hot water, cooled and then made up to 10 ml with water. From this solution, 1, 2 and 3 ml were made up to 25 ml with water including 2 ml of internal standard solution in each flask.

The liquid chromatograph used consisted of an Applied Chromatography System syringe pump linked to a Pye Unicam LC-3 ultraviolet detector. The chromatographic column (100 X 4.6 mm i.d.) was slurry packed at 200 bar with 5 μm Hypersil ODS (Shandon Southern, London, England). A Rheodyne Model 7120 injection valve fitted with a 20 μl loop was used. The wavelength of measurement was 254 nm and all measure-

TABLE 1

RESULTS OF THE ANALYSIS OF PALUDRINE TABLETS BY THE BP ASSAY METHOD AND THE HPLC METHOD

	Percentage of the stated amount *
BP assay	97.3 ± 1.27
HPLC assay	95.9 ± 1.69

* Mean and standard deviation of 9 determinations.

ments were made at ambient temperature. Acetonitrile was HPLC grade (Rathburn Chemicals, Walkerburn, England). All other chemicals used were obtained from BDH Chemicals (Poole, England).

The buffer used was 1.5% (w/v) sodium lauryl sulphate; 0.2% (w/v) sodium dihydrogen phosphate; 0.2 M orthophosphoric acid in water. The eluting solvent was 55% (v/v) acetonitrile and 45% (v/v) buffer. The pressure was 56 bar. Under these conditions the retention time of the internal standard was 3.5 min; that of the standard 5.5 min. The standard calibration graph had a correlation coefficient of 0.9936.

The assay results are shown in Table 1.

The HPLC method was found to be specific, and indeed could serve as an identity test, for proguanil. The resolution between proguanil and related biguanides has been shown to be very adequate (Moody et al., 1980). The most likely contaminant, 4-chloroaniline, has a retention time of 2 min and therefore will not interfere.

From Table 1, it is noticeable that the HPLC method gave a lower mean value than the BP method. The difference between the means was found to be significant at the 90% confidence limit level, but not significant for higher levels (Student's *t*-test; Langley, 1968). There is no significant difference in the precision of the two methods at the 5% level (*F*-test; Downie and Heath, 1970).

In conclusion this method offers a more rapid and more specific assay method than any currently available. It is of adequate sensitivity for unit dose assay and permits high sample throughput. Its limitation compared to the BP gravimetric method is the dependence on comparison with a standard substance.

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