Pak. j. sci. ind. res., vol. 39, nos. 1-4, January - April 1996

COLORIMETRIC DETERMINATION OF MEFLOQUINE IN BIOLOGICAL FLUIDS AND PHARMACEUTICAL DOSAGE FORMS

S.A ADELUSI AND A.O. ONYEKWELI*

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

(Received August 31, 1995)

A rapid, accurate and simple colorimetric method is proposed for the determination of mefloquine in biological fluids and pharmaceutical dosage forms. The green complex (soluble in nitrobenzene) formed between mefloquine and cobalt/thiocyanate reagent has absorption maximum at 625nm. The percentage recoveries of 98-101% obtained in urine, plasma and serum and the limit of sensitivity of 0.1 ug/ml showed that the method is applicable to the determination of mefloquine in these biological fluids. The method also gave a percentage label strength of 98% for formulated mefloquine tablets and capsules.

Key words: Mefloquine, Biological fluid, Tablets and capsules.

Introduction

Mefloquine was developed as a results of drug resistant malaria parasite developed in chloroquine. Among the large number of arylcarbinol amines with the structure based on cinchona alkaloids screened for antimalarial activity, mefloquine was found to have a high effective antimalarial action against chloroquine resistant *Plasmodium falciparium* [1].

Presently this drug is still at experimental stage and it is controlled by the World Health Organization (WHO). The kinetics of this drug have been studied in healthy volunteers and the method of analysis for determination of this drug in plasma has been very costly High Performance Liquid Chromatography (HPLC). [2] Following the report [3] that certain aminoquinoline antimalarial react with cobalt and cyanate ions to from stable ternary complex, the present study has investigated this for the determination of mefloquine in biological samples and pharmaceutical dosage forms.

Experimental

Apparatus. Spectra were recorded on SP 1800 spectrophotometer using 1cm cells.

Sample. Mefloquine hydrochloride was a gift as a pure authentic sample from Army Walter Reed, Washington D.C. Blood bags from healthy volunteers were obtained from the University of Benin Teaching Hospital. Plasma and serum were obtained from this sample and absence of quinoline compound confirmed in them. Urine of healthy volunteers who have not taken quinoline containing antimalarial drugs for more than six months was collected and confirmed to show the absence of quinoline containing antimalarials.

Preparation of mefloquine tablets and capsules. Tablets and capsules each containing 250mg of mefloquine were formulated as follows:

Mefloquine tablets. Mefloquine granules were prepared by the conventional method of massing and screening [4] using 12.5g mefloquine and 6.25g of maize starch powder homogeneously mixed in a mortar and wetted with sufficient quantity of starch mucilage, the binding agent. Free-flowing granules with sizes ranging between 200 and 500 um and dried to a moisture level of 1% were intimately mixed with 1% w/w magnesium stearate, a lubricant and 5% w/w maize starch powder, the disintegrant 400mg of the granule was compressed at constant pressure of 6 units in a single punch machine (The killian and Co. GMBH, KOLN-NIETH Type 5) fitted with a 12.5mm diameter flat-faced punches and die previously polished with a 1% w/w suspension of magnesium stearate in chloroform to form tablets.

Mefloquine capsules. Powder blend containing 12.5g of mefloquine and 500g of lactose powder was produced by the method of serial dilution using a mortar and pestle. Hand filling method was employed to produce capsules containing 350mg of the powder blend, equivalent to 250mg of the active drug.

Reagents. Citrate buffer (pH 3.0) was prepared by adding 6ml of 2M sodium hydroxide solution and 10ml of 2M citric acid solution and making the solution up to 100ml with distilled water in a 100ml standard flask. pH of this solution was checked by a pye-Unican pH meter and the pH of the solution adjusted to the required value by adding a few milliters of sodium hydroxide solution when necessary.

The cobalt-thiocyanate reagent was prepared by dissolving 8.8g of cobalt -II chloride hexahydrate and 4.3g of ammonium thiocyanate in 100ml of water.

*Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Development of the complex. The method adopted for the developmet of the complex is the one described by Hassan *et al.* [3]. The complex which is soluble in nitrobenzene had maximum wavelength of absorption at 625cm. The absorbance of the solution was found to be proportional to the concentration of mefloquine in solution.

Determination of the recoveries of mefloquine in plasma serum and urine. Concentrations of mefloquine in the range 5-50 ug/ml, were prepared in plasma, serum and urine. The amount of mefloquine in each of these biological fluids was determined as follows:

2ml of each of the samples was taken and transfered to 100ml separating funnel and 3ml of the cobalt-thiocyanate reagent added followed by 10ml of the citrate buffer (pH 3.0). 10ml of nitrobenzene was then added and shaken for one minute after which the entire solution was centrifuged at 8000 revolutions per minute to clarify the nitrobenzene layer. 5ml of the supernatant layer was carefully pipetted into a 10ml volumetric flask and the volume adjusted to 10ml with nitrobenzene. The absorbance of the solution was read on a spectrophotometer in the visible region using the above mixture (without the drug as a blank reagent). The concentration in the sample was then determined using calibration curve.

Determination of percentage strength of mefloguine in the capsule and table formulations. Contents of 10 capsules were emptied into a weighing bottle and the average weight of the contents of 10 capsules determined. For the tablets, 10 tablets were powdered in a mortar. 100mg of this powder was accurately weighed into a 100ml volumetric flask and 25ml of ethanol was added to dissolve the mefloquine content. Distilled water was then added up to the 100ml mark, then the flask was shaken thoroughly and the contents were allowed to settle. 2ml of the supernatant layer was analysed as described under recoveries of mefloquine in plasma, serum and urine.

Results and Discussion

The absorption spectrum of green complex showed a maximum absorption at 625nm which coincided with the one reported by Hassan *et al.* [3] for chloroquine, primaquine and amodiaquine respectively. The determination of mefloquine at various stages in this study was therefore carried out by determining the absorbance value of mefloquine cobalt-thiocyanate complex at this wavelength (625nm).

By modifying the procedure of Hassan *et al.* [3], it was possible to determine mefloquine by this method. One of the advantages of this method is that it is not necessary to extract mefloquine from the body fluid or from tablets and capsules before carrying out its determination.

The effect of the reaction time of green complex was investigated by measuring the absorbance at various times. It was found to be stable for several hours, that is, from 15 minutes to 60 hrs.

Application to mefloquine determination. The method has been applied for the quantitative determination of mefloquine in formulated forms (tablets and capsules) and in body fluids (plasma, serum and urine). In tablets and capsules, the percentage label strength of 98.0% and 98.6% were obtained respectively. These results indicate the suitability of the method for routine quality control analysis. The percentage recoveries of mefloquine from plasma, serum and urine were between 98.98 to 100.20% when mefloquine 5-50μg/ml mefloquine were contained in these media. In a separte experiment, we were able to get a recovery of 97.50-98.60% for 0.1μg/ml mefloquine in plasma, serum and urine. Below o.1 μg/ml mefloquine concentration in these media, no reproducable results were obtained. This shows that the method has a limit of detection of 0.1ug/ml which is two times the detection limit of 0.05ug/ml for the HPLC method [2]. The method is therefore applicable for the determination of low concentration of mefloquine in the body fluids.

For quantitative analysis of mefloquine in the blood and urine samples, it will be necessary to separate the mefloquine and its metabolite using TLC chromatography (5). The spots when detected can be scrapped, dissolved in ethanol, centrifuged and the supernatant layer taken for analysis

Interference. This method is useful to determine the concentration of mefloquine where there is no quinoline containing compounds, Since the quinoline containing compounds gave similar reaction as mefloquine, the presence of quinoline ring will cause some interference. However, in pharmaceutical dosage forms, mefloquine is unlikely to be formulated with another quinoline containing drug.If it is suspected that mefloquine and another quinoline containing compounds have been administered together, separation of the compounds using appropriate method such as Thin Layer Chromatography (TLC) is necessary before the application of this new method.

Acknowledgement. The authors are grateful to the Director , Army Walter Reed, Washington D.C. for providing the drug, Mefloquine hydrochloride powder in pure form; to Mr. Aayabene and Mr. J. Adebayo for their technical assistance and particularly for the research grant from URPC, University of Benin.

References

1. J.Y. Mu, Z.H. Israili, and P.G. Dayton, (1975). Am. Soc. Pharm. Exp. Therap. **3,** 198-205.

2. J.M. Kapetanovic, T.D. DiGiovanni,T. Bartosevich, V. Melendez, J. Von-Bredow and Helfler, M. (1983). J. Chromat. 277, 209-215.
3. S.N. Hassan, E.S. Metwelly and A.A. Abou (1982). Analyst 107, 1235-1240
4. A.O. Onyekweli, (1985), J. Appl. Sci., 3, 33-34.
5. D.E. Schwartz, W. Welsea, D. Richard-Lenoble, and M. Gen, (1980). Acta Tropica 37, 238-242.