International Journal of Pharmaceutics, 39 (1987) 137–140
Elsevier

IJP 01318

Spectrophotometric determination of menadione and menadione sodium bisulfite in pharmaceutical preparations

C.S.P. Sastry, N. Rajendraprasad Singh, M. Narayana Reddy and D.G. Sankar

Department of Organic Chemistry, Foods, Drugs and Water, School of Chemistry, Andhra University, Waltair (India)

(Received 7 October 1986)
(Modified version received 9 March 1987)
(Accepted 27 April 1987)

Key words: Spectrophotometry; Menadione; Menadione sodium bisulfite; 2-Methyl-1,4-naphthalene diol; Routine analysis

Summary

A simple, selective and sensitive spectrophotometric method is described for the determination of menadione (or menadione sodium bisulfite) in bulk samples and pharmaceutical preparations. The method is based on the reaction of its reduction product with 3-methyl-2-benzothiazolinone hydrozone (MBTH) in the presence of ferric chloride whereby intense blue colour with maximum absorption at 650–670 nm is developed. The reaction is sensitive enough to permit the determination of 0.6–7.5 μg menadione/ml with an average recovery of 99% and a standard deviation of 1.7%. There is no interference from other vitamins or from any common pharmaceutical diluents and additives.

Introduction

Menadione is a synthetic derivative (2-methyl-1,4-naphthoquinone) with the physiologic properties of antihemmorrhagic vitamin K. Its water-soluble derivative, menadione sodium bisulfite, exhibits greater activity. A sensitive method would greatly aid in determining menadione and menadione sodium bisulfite in a variety of preparations.

Standard methods recommended (U.S. Pharmacopeia, 1970; National Formulary, 1970) are titrimetric procedures with ceric sulfate. Polarographic measurements in non-aqueous or aqueous media, coulometric, spectrophotometric and gas chromatographic (Hashmi, 1973) methods have also been reported. Spectrophotometric determination of menadione by reactions with, 2,4-dinitrophenylhydrazine (Sathe et al., 1956), concentrated hydrochloric acid–zinc chloride (Bandelin et al., 1959), 3-methyl-1-phenyl pyrazole-5-one (Patel et al., 1975), ethyl cyanoacetate, dimethylmelonate and acetyl acetone (Hassan et al., 1975), *p*-phenylene diaminedihydrochloride (Sane et al., 1983), and thiosemicarbazide (Reddy et al., 1983) have been suggested. Although many of these spectrophotometric procedures are selective for menadione, they are not satisfactory for estimating low quantities of menadione (< 2 μg/ml) or its derivatives.

The present investigation was undertaken to develop a sensitive and selective spectrophotomet-

Correspondence: C.S.P. Sastry, Department of Organic Chemistry, Foods, Drugs and Water, School of Chemistry, Andhra University, Waltair 530 003, India.

ric method for the determination of menadione. The reaction of the reduced form of menadione with MBTH in the presence of ferric chloride proved to be suitable for this purpose.

Materials and Methods

Preparation of 2-methyl-1,4-naphthalene diol (reduced menadione) standard solution

From menadione. A 1000 μg/ml stock solution was prepared by dissolving 100.0 mg of pure menadione in 20 ml of methanol. To that 10 ml of dilute hydrochloric acid (methanolic) and 1.5 g of zinc dust was added in portions. After standing for one hour at room temperature, the solution was filtered through cotton wool, the residue was washed with three 5-ml portions of methanol and the filtrate was diluted to 100 ml with methanol. Working standard solution 25 μg/ml was prepared by appropriate dilution of the stock solution with methanol.

From menadione sodium bisulfite. Aqueous solution of menadione sodium bisulfite equivalent to 100 mg of menadione was treated with 3 ml of 4% sodium hydroxide and precipitated menadione was extracted with chloroform (2 × 10 ml). The chloroform in the extract was evaporated under N_2 and the resulting residue was dissolved in 20 ml of methanol and treated as in menadione.

3-Methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH), 2 mg/ml (8.5×10^{-3} M) solution, was prepared by dissolving 200 mg of MBTH in 100 ml of distilled water. Ferric chloride, 5 mg/ml (1.85×10^{-2} M) solution was prepared by dissolving 500 mg of ferric chloride hexahydrate in 100 ml of 0.1 M HCl solution.

Instrumentation

Elico spectrophotometer model CL 24 was used for all absorbance measurements.

Procedure

Sample preparation: preparation of 2-methyl-1,4-naphthalene diol

From menadione. An accurately weighed amount of powder obtained from tablets containing about 100 mg of menadione was transferred into a 50 ml conical flask and menadione was extracted with three 10-ml portions of chloroform. During each extraction, the mixture was shaken vigorously for 2 min and filtered. The chloroform extract, protected from light, was evaporated to dryness under N_2 in a water bath and the resulting residue was treated as described for the standard menadione solution (see Materials and Methods).

From menadione sodium bisulfite. The powered dosage form equivalent to 192.3 mg of menadione sodium bisulfite (i.e. 100 mg of menadione) was initially treated with 3 ml of 4% sodium hydroxide and subsequently extracted with 3 × 10 ml portions of chloroform. The residue resulting after the evaporation of chloroform was dissolved in 20 ml methanol and treated as in menadione.

The extracts from either menadione or menadione sodium bisulfite (0.25–3.0 ml) after reduction were added to 10 ml graduated tubes. Reduced menadione working solution was added to another set of tubes. 2 ml of MBTH solution was added, diluted to 5 ml with methanol and heated for 2 min in a boiling water bath. After that 1 ml of ferric chloride solution was added and allowed to

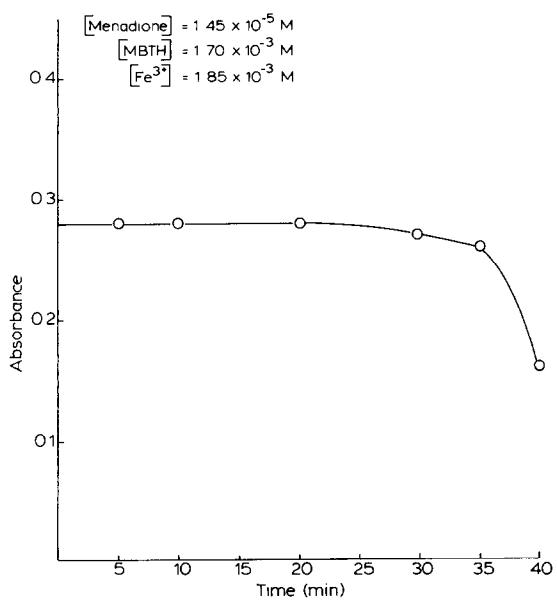

Fig. 1. Stability of the coloured species.

stand for 1 min. After cooling to room temperature, the solution was diluted to 10 ml with methanol. Absorbance was measured at 650 nm within 20 min against reagent blank. The calibration graph was prepared similarly.

Results and Discussion

Reduced menadione in slightly acidic conditions reacts with MBTH in the presence of ferric chloride to form a complex with a maximum absorption at 650–670 nm (Fig. 2). The mechanism of the reaction between MBTH and reduced menadione (2-methyl-1,4-naphthalene diol) in the presence of ferric chloride is supposed to be oxidative coupling reaction as in the case of other phenolic compounds reported (Friestad et al., 1969).

The concentration of MBTH, ferric chloride and hydrochloric acid, sequence of addition of reagents, the reaction temperature and time and the final dilution were selected as a compromise between optimum sensitivity, stability and minimum blank reading. The absorbance of reaction product at 650 nm remains stable (Fig. 1) for 20 min after final dilution during which the absorbance must be measured. Under the experimental conditions described, the absorbance (A) in a 1-cm cell is a linear function of the concentration (c, μg/ml) over the range 0.6–7.5 μg/ml. The regression equation, $A = 0.00066 + 0.1122c$ was obtained by the linear least-squares treatment of the results. The apparent molar absorptivity was found to be 1.93×10^4 l · mol^{-1} · cm^{-1}. The precision of the method was evaluated by the analysis of 6 replicate samples containing 25

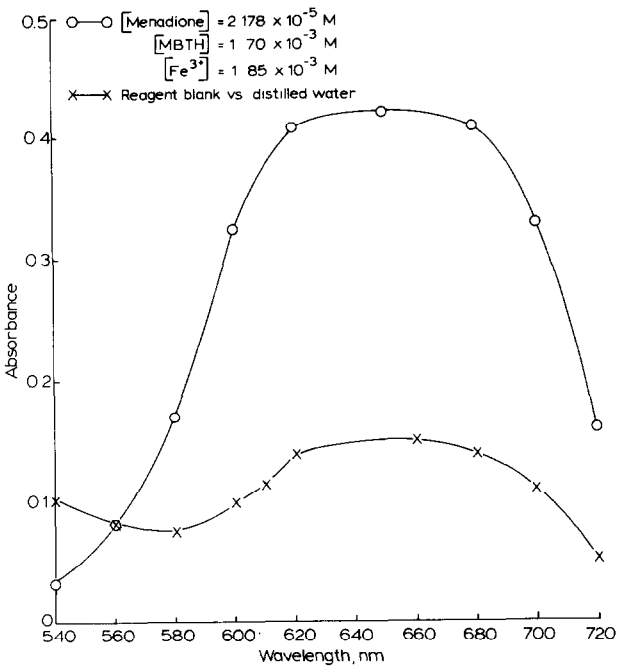

Fig. 2. Absorption spectra of reduced menadione–MBTH–Fe^{3+} system.

TABLE 1

Estimation of menadione and its analogues in pharmaceutical preparations

Sample	Labelled amount (mg)	Amount found by:	
		Proposed method	Reported method
MSB: 10 mg	10	9.85	9.92
MSB: 10 mg; R: 50 mg AdMs: 0.33 mg; CP: 125 mg Cal: 500 IU; AA: 100 mg	10	9.95	9.98
MSB: 10 mg; R: 50 mg AdMs: 0.35 mg; CP: 125 mg Cal: 500 IU; AA: 100 mg	10	9.90	9.86
MSB: 20 mg; R: 50 mg; AA: 100 mg MH: 40 mg; AdMs: 1 mg CP: 100 mg	20	19.90	20.10
MSB: 20 mg; R: 100 mg AdMs: 2.5 mg; AA: 50 mg	20	19.80	19.70

MSB = menadione sodium bisulfite; R = rutin; AdMs = adrenochrome monosemicarbazone; CP = calcium (dibasic) phosphate; Cal = Calciferol; AA = Ascorbic acid; MH = methyl hespiridin.

μg/ml of menadione or 50 μg/ml of menadione sodium bisulfite in the final assay solution. At these concentration levels, the calculated % RSD and % range of error (95% confidence limits) for menadione and menadione sodium bisulfite are (1.7, 1.8) and (2.76, 2.89), respectively.

The method has been applied to the determination of menadione and its water-soluble derivative, menadione sodium bisulfite, in commercial tablets. The results are compared with those obtained by the traditional 2,4-dinitrophenyl hydrazine (Sathe et al., 1956) and found to be in good agreement (Table 1). The other components usually present in dosage forms such as rutin, adrenochrome monosemicarbazone, calcium dibasic phosphate, calciferol, vitamin E, ascorbic acid, methyl hespiridin in excess of their normal occurrence do not interfere. However, vitamin A shows positive interference due to the development of turbidity after ferric chloride addition and so needs preliminary separation.

From the data obtained, it is evident that the proposed method is simple and sensitive with reasonable precision and accuracy when compared to many of the reported methods and so it can be used for the routine determination of menadione or its water-soluble derivative menadione sodium bisulfite (after converting it initially to menadione).

References

Bandelin, F.J. and Penkratz, R.E., The colorimetric determination of menadione. *Drug Standards*, 27 (1959) 36.

Friestad, H.O., Ott, D.E. and Gunther, F.A., Automated colorimetric microdetermination of phenol by oxidative coupling with 3-methyl-2-benzothiazolinonehydrazone. *Anal. Chem.*, 41 (1969) 1750.

Hashmi, M., *Assay of Vitamins in Pharmaceutical Preparations*, Wiley, London, U.K., 1973, pp. 391–415.

Hassan, S.S.M., El-Fattah, M.A. and Zaki, M.T., Spectrophotometric determination of vitamin K_3 (menapthrone), *Fresenius Z. Anal. Chem.*, 275 (1975) 115–117.

Hassan, S.S.M., Vitamins and coenzymes, part F. In S. Colowick and N. Koplan (Eds.), *Methods in Enzymology, Vol. 67*, Academic, New York, 1980, pp. 125–128.

Narayana Reddy, M., Prakasa Sastry, C.S. and Viswanadham, N., Determination of menadione and its analogs, *Ind. J. Pharm. Sci.*, 45 (1983) 255–257.

National Formulary, 13th Edn. Am. Pharma. Assoc. Washington, DC, 1970, 405.

Patel, J., Mehta, R. and Shastri, M., Colorimetric method for the estimation of menadione and menadione sodium bisulfite in pharmaceutical dosage forms, *Ind. J. Pharm.*, 37 (1975) 141–143.

Sathe, V., Deve, J. and Ramakrishnan, C., Spectrophotometric method for the estimation of vitamin K_3. *Nature (London)*, 177 (1956) 276.

Sane, R.T., Joshi, V.J., Jukar, S.R., Joshi, S.K. and Sawant, S.V., Simple colorimetric methods for the determination of vitamin K_4 (dibutyrate) from pharmaceutical preparations. *Ind. Drugs*, 21 (1983) 30–31.

U.S. Pharmacopoeia, 18th revision, U.S. Pharmacopoeial Convention, Rockhill, MD, 1970, 395.