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Short communication

Spectrophotometric methods for the rapid determination of menadione and menadione sodium bisulphite and their application in pharmaceutical preparations

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Abstract

Two simple, rapid and sensitive spectrophotometric determination of menadione and its sodium bisulphite derivative (MSB) have been carried out. The first method involves the reaction of menadione and its sodium bisulphite derivative with 3-methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH) is sodium hydroxide medium to give blue coloured product having maximum absorption at 625 nm and the coloured species is stable for more than 1 h. The Beer's law is obeyed in the range 0.4–16 μg ml^{-1}. The second method proposes the reaction of menadione and its sodium bisulphite derivative with resorcinol in concentrated sulphuric acid medium to give red coloured product having maximum absorption at 520 nm and is stable for 3 h. The Beer's law was obeyed in the range of 1–24 μg ml^{-1}. Molar absorptivity for the above two methods were found to be 7.6×10^3 and 4.5×10^3 l mol^{-1} cm^{-1}, respectively. All the measurements were carried out at room temperature. These two methods have been successfully applied for menadione and its sodium bisulphite derivatives in injections and tablets of pharmaceutical formulations. The results compare favourably with official method.

Keywords: Menadione; Menadione sodium bisulphite; MBTH; Resorcinol and spectrophotometry

1. Introduction

Menadione and its derivatives are antihemmorrhagic compounds associated with enzymes involved in blood clotting. They are helpful in blood coagulation by activating prothrombin, the precursor of thrombin, for the formation of blood clotting enzyme. Menadione sodium bisulphite addition products are used extensively as synthetic vitamin-K_3 supplements in poultry and swine feeds. Thus trace analytical techniques are necessary for the study of their biological actions, as well as pharmacalogical and toxicological studies. Many analytical techniques such as spectrofluorimetry [1–3], polarography [4], gas–liquid chromatography [5,6], titrimetry [7] are used. Some spectrophotometric methods have also been reported. Most of them are either time consuming [8–10] or tedious [11,12] or involve the use of

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reagents which are difficult to obtain [13]. The method proposed by Sastry et al. [14] reports the reaction of reduced form of menadione with MBTH in presence of iron(III) to give blue coloured product. However, this method is tedious, as it involves reduction of menadione with zinc and hydrochloric acid which takes 1 h to complete and involves extraction with chloroform. Absorption readings has to be taken within 20 min and vitamin-A seriously interferes.

The proposed methods involve only 5 min to produce the reaction product and allows the determination in low quantities.

2. Experimental

2.1. Instrumentation

A Jasco model UVIDEC-610 UV–VIS-Spectrophotometer with 1.0 cm matched cells were used for all absorption measurements.

2.2. Materials and reagents

Menadione, menadione sodium bisulphite, MBTH and resorcinol, all purchased from Sigma (USA) were used in the estimation. Analytical grade sodium hydroxide and concentrated sulphuric acid were used. Deionised water was used throughout the experiment wherever it was necessary.

2.3. Standard solutions

(a) A 1000 μg ml^{-1} stock solution was prepared by dissolving 100 mg of pure menadione in 100 ml of ethanol. Working standard solutions were prepared by suitable dilution of the stock solution with ethanol.
(b) Aqueous solution of 1000 μg ml^{-1} menadione sodium bisulphite solution was prepared by dissolving 100 mg of it in 100 ml of distilled water.
(c) A 0.5% of 3-methyl-2-benzothiazolinone hydrazone hydrochloride solution was prepared by dissolving 0.5 g of it in 100 ml of distilled water. A 0.2% resorcinol was prepared by dissolving 0.2 g of resorcinol in 100 ml of ethanol.

2.4. General procedure

2.4.1. Method A

Aliquots of standard solutions of menadione and MSB (10–400 μg) were transferred into a series of 25 ml calibrated flasks. 5.0 ml of 0.5% MBTH and 2.0 ml of 5 M sodium hydroxide were added and the mixture was set aside for 5 min. Then the contents of the flask were diluted upto the mark with distilled water and shaken well. The absorbance of the blue coloured product was measured at 625 nm against the corresponding reagent blank and the calibration graph was constructed.

2.4.2. Method B

Aliquots of standard solutions of Menadione and its sodium bisulphite derivative (25–600 μg) were pipetted out into series 25 ml calibrated flasks. An appropriate volume of ethanol was added to make the volume to 2.5 ml in the flask. 1.0 ml of 0.2% alcoholic resorcinol and 5.0 ml of concentrated sulphuric acid were added and set aside for 5 min. Reaction mixture in the flasks were cooled and diluted with distilled water upto the mark. The absorbance of the resulting red coloured product was measured at 520 nm against the corresponding reagent blank. Calibration graph was constructed.

2.5. Assay of vitamin-K compounds in pharmaceutical formulations

2.5.1. Tablets

The powdered tablet equivalent to 192.3 mg of menadione sodium bisulphite (i.e. 100 mg of menadione) was dissolved in distilled water and filtered if necessary. Working standards were prepared and the same procedures for methods A and B were followed as in case of pure compound.

2.5.2. Injections

1.0 ml of injection vial of menadione sodium bisulphite containing 10.0 mg of it (i.e. 5.2 mg of

menadione) was dissolved in 10 ml of alcohol. Working standards were prepared by suitable dilution and same procedures were followed as in the case of pure compound.

3. Results and discussion

The method A involves the reaction of Menadione and MSB with MBTH in sodium hydroxide medium to form blue coloured product having the absorption maximum at 625 nm. In method B, Menadione and MSB were treated with resorcinol in concentrated sulphuric acid medium to form red coloured product showing the absorption maximum at 520 nm. The colourless reagent blanks of both methods have negligible absorptions at these wavelengths. The absorption spectra for both blue and red coloured products are shown in Fig. 1.

3.1. Optimum reagent concentration

In method A, a 0.5% MBTH in the range 4.0–8.0 ml and 5 M sodium hydroxide in the range 1.0–5.0 ml were used to get the stable blue product. It was found that 5.0 ml of MBTH and 2.0 ml of sodium hydroxide were necessary to form the blue coloured product with maximum intensity and stability. For method B, a 0.2% resorcinol in the range of 0.5–3.0 ml and concentrated sulphuric acid in the range 3.0–7.0 ml were used to get the required red product. 1.0 ml of resorcinol and 5.0 ml of concentrated sulphuric acid were selected to obtain the red coloured product with maximum intensity and stability.

3.2. Reaction sequence

The probable mechanisms are being suggested for both methods as it was not possible to isolate

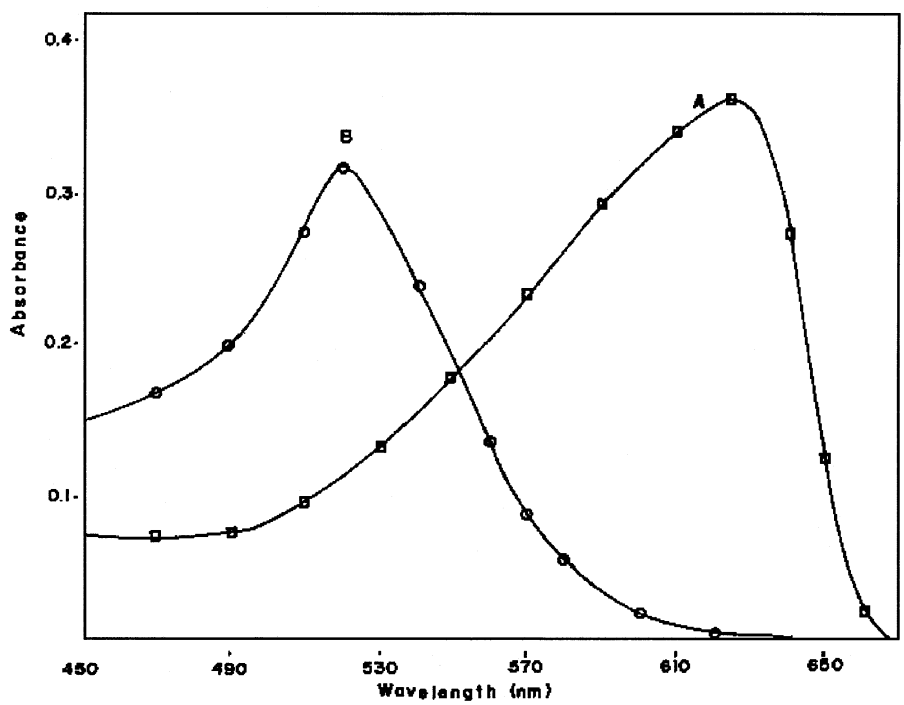
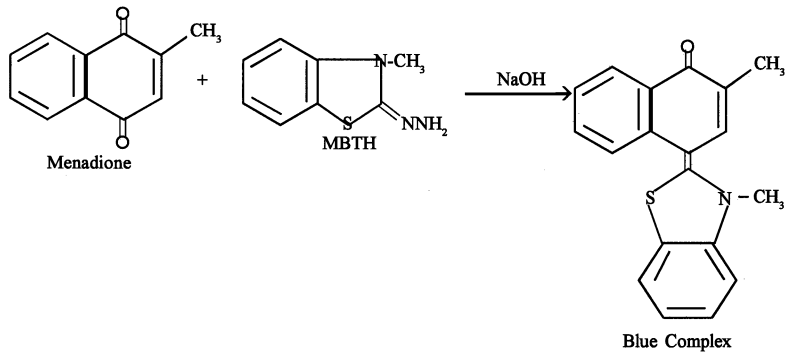
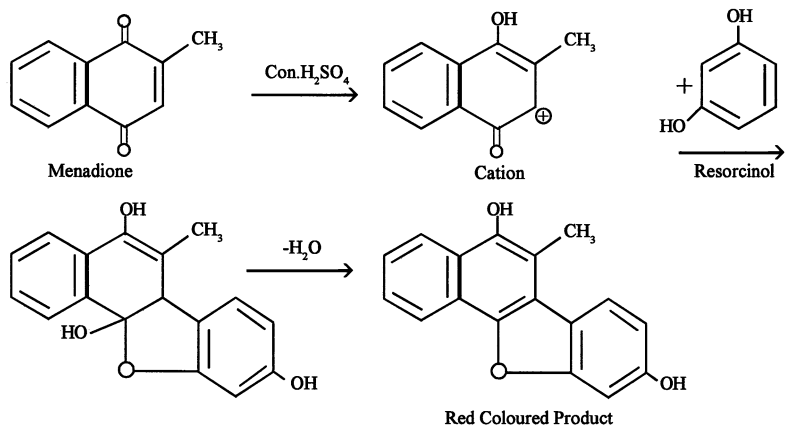


Fig. 1. Absorption spectra of: (A) Menadione (8 μg ml^{-1}) + MBTH + NaOH; (B) Menodione (12 μg ml^{-1}) + H_2SO_4 medium.



Scheme 1.



Scheme 2.

these products in solid state. In method A, Menadione and MSB may form diazo products with MBTH in alkaline medium, which readily lose a molecule of Nitrogen to form the coupled blue product, as shown in Scheme 1. In method B, it is supposed that the oxygen atom of Menadione and MSB get protonated in presence of concentrated sulphuric acid to form a carbocation which may undergo electrophyllic addition with electron rich resorcinol to form the product which loses a molecule of water to form the red coloured product as given in Scheme 2.

3.3. Quantification

Table 1 shows linear caliberation ranges and equation parameters for the proposed methods. Validation for the proposed methods were done by determining parameters like detection limit, limit of quantification range of error etc. To determine the detection limit, the least concentrate of the analytes (0.1–0.2 μg ml^{-1} for method A and 0.03–0.1 μg ml^{-1} for method B) were determined without change in λ_{max}. Limit of quantification of the least concentrate of the analytes was determined for both the methods (0.1 μg ml^{-1} for method A and 0.03 μg ml^{-1} for method B) and was found to be three times the limit of detection.

To account for the precision and accuracy of the methods, five separate determinations for pure samples and commercial samples by both the methods were carried out for the concentration, within Beer's law range. The values of relative standard deviation (R.S.D.), range of error at 95% confidence limit are listed for both methods in Table 1. The regression equation $y = ax + b$, was used to calculate slope a and intercept b

Table 1
Optical characteristics

Parameters	Method A	Method B
Colour	Blue	Red
λ_{max} (nm)	625	520
Stability (h)	1	3
Beer's law range (μg ml^{-1})	0.4–16	1–24
Detection limit (μg ml^{-1})	0.1381	0.0325
Limit of quantification (μg ml^{-1})	0.4603	0.1084
Photometric range (μg ml^{-1})	1–15	2–22
Molar absorptivity (1 mol^{-1} cm^{-1})	7.63×10^3	4.5×10^3
Sandell's sensitivity (μg cm^{-2})	0.0232	0.0382
Regression equation[a]		
Slope (a)	0.0378	0.241
Intercept (b)	0.0019	0.0007
Correlation coefficient (r)	0.9993	1.10
R.S.D. (%)	0.4790	0.5864
Range of error (at 95% confidence level)[b]	±0.6649	±0.8139

[a] $y = ax + b$ where x is the concentration of menadione in μg ml^{-1}.
[b] Calculated from five determinations ($n = 5$).

where y represents optical density and x the concentration of the drug.

3.4. Interference

For methods A and B a detailed study on the interference of different excipients were made. For this, absorbance values of the solutions containing

Table 2
Effect of interfering ions and excipients on the determination of 10 μg ml^{-1} of menadione

Ions/compounds added	Tolerance limit (ppm)	
	Method A	Method B
Glucose	400	400
Sucrose	500	500
Lactose	400	400
Starch	500	500
Talc	450	500
Vitamin-A	1000	1000
Vitamin-B_1	1000	980
Vitamin-B_2	2500	2400
Vitamin-B_6	300	300
Vitamin-D	1000	1000
Vitamin-E	1500	1200
Ascorbic acid	2000	2000
Tartarate	500	500
Oxalate	500	500
Phosphate	450	500
Chloride	350	300
Fluoride	500	500
Sulphite	300	350
Sulphide	450	400
Alginate	500	500
Thiocyanate	500	500
Sulphate	400	350
Nitrite	500	10
Nitrate	800	20
Sodium	350	300
Aluminium	300	300
Iron(III)	20	20
Iron(II)	10	10

Table 3
Determination of menadione in pharmaceutical preparations

Drug (MSB)	Normal amount (mg)	Found (mg)	Recovery (%)	Recovery[a] ± R.S.D.	USP method
Method A					
Injection[b]	10 ml^{-1}	9.8	98.0	98 ± 0.1517	96.8 ± 0.82
Injection[c]	10 ml^{-1}	9.78	97.8	97.8 + 0.3349	96.8 ± 0.82
Tablet[d]	20	19.6	98.0	98 ± 1.40	–
Method B					
Injection[b]	10 ml^{-1}	9.9	99.0	99 ± 0.8752	96.8 ± 0.82
Injectjon[c]	10 ml^{-1}	9.89	98.9	98.9 ± 0.1834	96.8 ± 0.82
Tablet[d]	20	19.4	97.0	97.0 ± 0.8686	–

[a] Average of five determinations.
[b] Marketed by N.I. Pharmaceuticals Ltd.
[c] Marketed by Montari Pharmaceuticals Ltd.
[d] Marketed by Grandix Pharmaceuticals Ltd.

Table 4
Comparison of visible spectrophotometric methods for the determination of menadione

Sl. no.	Reagent	Coloured species	Drug analyzed	λ_{max} (nm)	Range of determination (ppm)	ε (l mol^{-1} cm^{-1})	Remarks	Reference
1.	Ammonium molybdate	Molybdenum blue complex	Menadione and Menadione sodium bisulphite	535	1–200	Not reported	Reaction is carried out with reduced Menadione	[15]
2.	2,4-Dinitro phenylhydrazine in 2NHCl	Condensation Product	Menaphthone	635	2–300	Not reported	Mixture is incubated at 35 °C for 10 min and extracted with amyl alcohol	[10]
3.	2,4-Dinitro Phenylhydrazine	Condensation product	Menaphthone	635	5–68.8	Not reported	Reaction mixture is incubated at 35 °C for 2 h and tedious method	[16]
3a	2,4-Dinitro phenylhydrazine	Condensation product	Menadione	635	2–30	Not reported	Involves extraction	[19]
4.	Reductive coupling with titanium	Blue Complex	Menaphthone and napthaquinone	505–510	20–100	Not reported	Reaction mixture is kept in dark for 30–45 min before measuring absorption	[17]
5.	With isoniazid in NH_3	Yellow Green hydrazone	Menaphthone	600	200–600	Not reported	Heated on water bath to complete the reaction	[18]
6.	With thiosemi carbazide in alkaline medium	Condensation Product	Menadione	540	4–40	3.6×10^3	Mixture is kept for 30 min and the reaction is carried out in propanolic medium	[8]
7.	Aqueous dimethyl amine in ethanol medium	Violet Red Complex	Menadione and Menadione sodium bisulphite	510	0.2–300	2.8×10^3	Less sensitive requires high concentration of regent (33%)	[20]
8.	With NEDA in sodium hydroxide medium	Coupled Product	Menadione	450	10–30	Not reported	Kept in dark for 30 min	[9]
9.	(A) With MBTH in sodiun hydroxide medium	Condensation reaction	Menadione and Menadione sodium bisulphite	625	0.4–16	7.63×10^3	Requires 5 min to form stable coloured species in both the cases	Proposed methods
	(B) With resorcinol in presence of concentrated sulphuric acid	Electrophyllic addition of resorcinol with cation of Menadione		520	1–24	4.5×10^3		

10 μg ml^{-1} of pure compounds and various amounts of diverse ions were measured. In the method A, to 10 μg ml^{-1} of the pure sample, a known amount of interfering ion was added, followed by the addition of MBTH and sodium hydroxide. After 5 min absorbance values were measured. In the method B, to 10 μg ml^{-1} of the solution of the pure compound added 2.15 ml of ethanol, followed by the addition of resorcinol and concentrated sulphuric acid. After 5 min the readings for absorbance are taken. It was found for both these methods, many of the cations, anions and other concomitant substance such as glucose, lactose, alginate, talc etc. do not interfere in the analysis and the tolerance limits of these and other ions are listed in Table 2. The highlight of these methods is that vitamins such as A, C, B_1, B_2, B_6, D and E do not interfere in the analysis. However, Nitrite, Nitrate, iron(II) and iron(III) were found to interfere seriously.

3.5. Stability

To asses the stability of the reaction product in each method.

3.5.1. Method A

To the known concentration of Menadione and MSB, added MBTH and 5 M sodium hydroxide, left the mixture for 5 min for the reaction to get completed. Solution in the 25 ml flask was made upto the mark with distilled water, shaken well and immediately the absorbance was measured (i.e. at zero time). At regular intervals of 5 min absorbance values were measured. It was found that after 60 min there was continuous decrease in the absorbance values indicating that the coloured species was stable for 1 h.

3.5.2. Method B

To the known concentration of Menadione and MSB, added resorcinol and concentrated sulphuric acid, left the reaction mixture for 5 min. Then the mixture is cooled rapidly and made upto 25 ml with distilled water in a calibrated flask. Immediately the absorbance measurement was made (i.e. at zero time). It was found that absorbance measurements made at regular intervals of 5 min till 3 h shows no change and after that there was continuous decrease indicating that the coloured species was stable upto 3 h (Tables 3 and 4).

3.5.3. Effect of temperature on stability

Effect of temperature on stability of the products of both the methods were studied between 5 and 65 °C. The results obtained indicated that the absorbance values remain constant in this range. Further increase in temperature resulted in the decrease in the absorbance values indicating the dissociation of coloured species.

4. Conclusions

The proposed method is simple, rapid, fairly selective and sensitive compared to already reported methods. Thus our method can be adopted as an alternative to the already existing methods.

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