Determination by Converting Menadione Sodium Bisulphite to Menadione in Pharmaceutical Preparation by Two Derivative Spectrophotometric Methods

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Running title: Menadione sodium bisulphite analysis by derivative spectrophotometric methods in pharmaceutical.

ABSTRACT

First- and second-order derivative spectrophotometric methods for the quantitative determination by converting menadione sodium bisulphite (MSB) to menadione (MN) with sodium carbonate were developed and validated in pharmaceutical preparation. The quantitative determination of MSB was carried out by using wavelengths 350 nm (for first-order; 1D) and 355 nm (for second-order; 2D) with the linearity ranges 0.5-40 µg/mL MN (i.e.0.82-65.6 µg/mL MSB). The percentages of conversion MSB to MN obtained with methods were more than 97.1 %. The proposed derivative spectrophotometric methods easily applied to commercial ampoule containing MSB and were statistically compared.

Keywords: Menadione sodium bisulphite, spectrophotometry, pharmaceutical

1. INTRODUCTION

Menadione (MN) and its derivatives called as vitamin K_3 are antihemorrhagic compounds and they are used as a required cofactor in the synthesis of blood clotting and in bone metabolism (Fauler et al. 2000; Shearer 2000). In addition, vitamin K_3 at relatively high dose has an antitumor activity against various human cancer cells (Lamson and Plaza 2003; Taper et al. 2004). Menadione sodium bisulfite (MSB) is a water-soluble

form of menadione. MSB addition products are used extensively as synthetic vitamin K_3 supplements in feed and pharmaceutical (Fauler et al. 2000; Lamson and Plaza 2003)

Variety of analytical methods include spectrophotometry (Helaleh 1997; Nagaraja et al. 2002; Sastry et al 1987), spectrofluorimetry (Ruiz et al. 2004; Gil-Torró et al. 1997; Nevado et al. 2001), voltammetry (Vire et al. 1988), chemiluminescence (Huang et al. 1999; Pérez-Ruíz et al. 1999) potentiometry (Rizk 2002), liquid chromatography (Laffi et al. 1988; Pérez-Ruiz et al. 2007; Liu et al. 1997) and gas chromatography (Castello et al. 1977) reported related to the quantitation of MN or MSB in pharmaceuticals. Each has some advantages and disadvantages. All of the spectroscopic determination of MN or MSB needs derivatization based on color-reaction with different chromophoric reagents ((Helaleh 1997; Nagaraja et al. 2002; Sastry et al 1987). In these studies; The method developed by Sarsty et al (1987) was based on the reaction of MN reduction product with 3-methyl-2-benzothiazolinone hydrozone (MBTH) in the presence of ferric chloride with a maximum absorption at 650–670 nm. The method developed by Helaleh et al (1997) was based on the reaction between MN and sodium hydroxide to form a colored complex with a strong absorbance at 450 nm. Finally, the determinations of MN and MSB have been carried out by Nagaraja el al (2002). The first method involves the reaction of MN and MSB with 3-methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH) in alkaline medium to give blue coloured product having maximum absorption at 625 nm. The second method proposes the reaction of MN and MSB with resorcinol in acidic medium to give red colored product having maximum absorption at 520 nm. There are no derivative spectrophotometric methods reported for the analysis of MN or MSB in pharmaceuticals. Derivative spectrophotometry is an analytical technique of great utility for extracting both qualitative and quantitative information from spectra composed of unresolved bands, and for eliminating the effect of baseline shifts and baseline tilts. It consists of calculating and plotting one of the mathematical derivatives of a spectral curve (Ojeda and Rojas 2004).

This paper reported a simple, rapid and sensitive method by first- and second-derivative spectrophotometry in pharmaceuticals for determination of MSB. The method is based on the analysis of MN by converting MSB to MN.

2. MATERIALS AND METHODS

2.1. Apparatus

A Thermospectronic double-beam UV-Visible spectrophotometer (HEλIOSβ) with a data processing system was used. First- and second-order derivative spectra of reference and sample solutions were recorded in 1 cm quartz cells at a scan speed of 600 nm/min, a scan range of 290-390 nm, fixed slit width of 2 nm and derivation interval (Δλ) 21.0 nm.

2.2. Reagents and Solutions

MSB and MN reference material and anhydric sodium carbonate were purchased from Sigma Company (USA). Hexane and concentrated HCl were obtained from Merck Company (Germany). Libavit K® ampoule (Mefar Drug Company, Turkey; in solution, per 1 mL: 10 mg MSB and excipients: potassium meta bisulphate (6 mg), sodium chloride (12.6 mg) and injection water (2 mL)) was obtained from the local market.

Standard working solutions (0.5-40 µg/mL) and quality control (QC) solutions (1, 5 and 30 µg/mL) was prepared by diluting with hexane from stock solution (100 µg/mL). A 0.01 M hydrochloric acid solution was made by dilution of concentrated hydrochloric acid. An anhydric sodium carbonate solution was prepared at % 10.6, w/v concentration in deionized water.

2.3. Recommended Procedures for the Determination of MSB in Drug

The contents of five ampoule (Libavit K®), each containing 20.0 mg of MSB (i.e 12.2 mg MN), was transferred to a 100 mL volumetric flask. 0.01 M HCl solution was added and the flask was shaken for 30 min. The volume was completed. Obtained drug stock solution which yield a 3.05 µg/mL and 12 µg/mL MN was transferred to a centrifuge tubes and then 0.5 mL anhydric sodium carbonate solution (10.6 %) (to shift the MSB to the water insoluble MN) and 2 mL n-hexane (to extract MN from the water phase) were added. This solution was vortexed for 5 min and centrifuged at 3000 g for 30 min. The supernatant were transferred and analyzed for obtained first- and second-order derivative spectra against blank prepared similarly.

2.4. Statistical analysis of the results obtained from proposed methods

All statistical calculations were performed with the Statistical Product and Service Solutions (SPSS) for windows, version 11.5. If calculated P values are 0.05 or less, correlations were considered statistically significant.

3. RESULTS AND DISCUSSION

3.1. Optimization of conditions

To develop a sensitive the first- and second order derivative spectrophotometric method, the experimental conditions as the degree of derivation, the wavelength range and smoothing were optimized. Optimum results were obtained in the measuring wavelength range 290-390 nm, high smoothing ($\Delta\lambda$=21.0) for first- and second-order derivative spectrophotometric methods. Figure 1 presents the overlay of first- and second order spectra of MN in the concentration of 0.5-40 µg/mL. The single peak at 350 nm was observed in first-order derivative spectra (Figure 1A). A maximum peak at 355 nm and two minimum peaks at 330 nm and 347 nm were observed in the second-order derivative spectra of MN (Figure 1B).

MSB converted MN at pH>11 as shown a reaction in Figure 2 (10 mg MSB is equivalent 6.1 mg MN (Huang et al. 1999). The effect of pH on conversion of MSB to the water insoluble MN was examined at various pH values at 9.50, 10.50, 11.10, 11.20, 11.30 and 11.50. The best condition for conversion was obtained at pH= 11.30.

To determine the conversion rate of MSB to MN, MSB and MN were added in 0.01 M HCl solution at calibration concentration range, separately. The MSB was converted to MN as described above section and then extracted with n-hexane. For first-order spectrophotometric method, the recoveries of MN and MSB added to 0.01 M HCl were 105.8 % (RSD %: 3.50 n=6) and 104.0 % (RSD %: 6.31 n=6), respectively. Consequently, the conversion rate of MSB to MN was determined to be 98.3 % (RSD: 3.38, n=6). For second-order spectrophotometric method, the recoveries of MN and MSB added to 0.01 M HCl were % 98.4 (RSD %: 2.88 n=6) and 95.3 % (RSD %: 2.16 n=6) for 355 nm. Consequently, the conversion rate of MSB to MN at mentioned wavelength was determined to be 97.1 % (RSD: 1.58, n=6).

3.2. Method Validation

3.2.1. Linearity and Sensitivity

The 350 nm wavelength for first-order derivative method and the 330 nm, 347 nm and 355 nm wavelengths for second-order derivative method were used for calibration curves. Eight-level calibration series with six analyses at each concentration level were measured. The standard calibration curves were constructed by

plotting the $\frac{d\,A}{d\,\lambda}$ (1D, for first-order derivative) and $\frac{d^2\,A}{d\,\lambda^2}$ (2D, for second-order derivative) versus MN concentrations. For all calibration curves, a good linearity within the concentration range of 0.5-40 µg/mL was showed for first- and second-order derivative spectrophotometric methods. The regression equations were obtained by the least-square regression method. The calibration curves, regression equations and correlation coefficients found for first- and second-order derivative methods were given in Table 1.

In the assay of other validation parameters, it was used wavelengths 350 nm for first-order derivative and 355 nm for second-order derivative to be high to correlation coefficient.

The limit of detection (LOD) and limit of quantification (LOQ) were determined with ratio of signal to noise which were lower than 3 and 8. LOD and LOQ were 0.1 µg/mL and 0.2 µg/mL for first-derivate spectrophotometric method, respectively. These values for second-derivate spectrophotometric method were 0.2 µg/mL and 0.3 µg/mL, respectively.

3.2.2. Precision and Accuracy

The precision of the methods as the percent relative standard deviation (RSD %) and the accuracy of methods as relative error (RE) were evaluated with within-day and between-day measurement at QC solutions concentrations (1, 5 and 30 µg/mL) for MN. The RSD% values for intra-day and inter-day precision of proposed methods were found to be ≤7.28% and the RE values for the intra-day and inter-day accuracy studies of methods were found to be between -3.40 and 0.80. These results were given in Table 2.

3.2.3. Extraction Recovery

Extraction recovery was determined by adding standard solution of MN to drug solution separately. To determine recovery of MN, 1, 5 and 30 µg/mL standard MN (QC solutions) were also added to 10 µg/mL MSB (i.e 6.1 µg/mL MN) which was prepared from Libavit K® ampoule and then the quantification of MN was analyzed by proposed methods. Experiments of each level were repeated six times. The mean recoveries were found ranged 98.6-103.5 %. No interference from the common excipient was observed. The results showed in Table 3.

3.2.4. Application and Comparison of the Proposed Methods

The developed spectrophotometric methods were applied to determine the MSB in the Libavit K® (from Mefar Drug Company, Turkey) ampoule (Table 4). By each of two methods, quantitative analysis of MSB in commercial pharmaceutical was performed by converting MSB to MN. The first- and second-order derivative spectrum obtained from Libavit K® soft capsule are shown in Figure 3. It was obvious that the maximum and minimum wavelengths of drug solution are same with standard solutions. Inter-method comparisons were performed by *student-t* test (*p-value* is <0.05). According to data obtained in this analysis, there was no significant difference between first- and second-derivative spectroscopy methods (*p=0.178*). In addition, the MSB contents measured by the proposed methods were in good agreement with the values supplied by the manufacturers.

4. CONCLUSIONS

First- and second- derivative spectrophotometric methods were developed and valideted for analysis of MN by converting MSB to MN in commercial pharmaceutical containing MSB in this study. Simplicity, the short analysis time, low cost, low organic solvent consumption, long linearity range and low sensitivity are the advantages of the proposed derivative methods against some of the introduced methods. Additionally,

the proposed method should also be useful for accurate and precise routine analysis of MSB in pharmaceuticals.

5. REFERENCES

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TABLE 1: Regression analysis results of the proposed methods

Method	**Range (μg/mL)**	**LR[a]**	**Sa**	**Sb**	**R**
First-order Spectrophotometric Method	0.5-40	$1D_{350nm}$=0.0430x+0.0126	0.0009	0.0004	0.9997
Second-order Spectrophotometric Method	0.5-40	$2D_{330nm}$=0.0003x+0.0039	1.0E-06	0.0001	0.9997
	0.5-40	$2D_{347nm}$=0.0003x+0.0003	5.48E-05	5.16E-05	0.9997
	0.5-40	$2D_{355nm}$=0.0005x+0.0002	1.0E-06	1.0E-06	0.9998

[a] Based on six calibration curves LR: Linear regression Sa: Standard deviation of intercept of regression line, Sb:Standard deviation of slope of regression line R: Coefficient of correlation x: MN concentration (μg/mL), 1D: First order-absorbance, 2D: Second order-absorbance.

TABLE 2: Precision and accuracy results of the proposed methods

	Intra-Day			**Inter-Day**		
Added (μg/mL)	**Found±SD**	**RE**	**RSD%**	**Found±SD**	**RE**	**RSD%**
First-order Spectrophotometric Method						
1.0	0.974±0.012	-2.56	1.00	0.966±0.063	-3.40	7.18
5.0	4.997±0.028	-0.05	0.54	4.889±0.116	-2.22	2.24
30	29.48±0.072	-1.74	0.24	29.10±0.225	-2.98	0.77
Second-order Spectrophotometric Method						
1.0	0.973±0.068	-2.67	6.99	1.003±0.073	0.33	7.28
5.0	4.866±0.163	-2.66	3.35	5.040±0.273	0.80	5.42
30	28.93±0.602	-3.55	2.08	29.46±1.360	-1.78	4.62

SD: Standard deviation of six replicate determinations, RSD: Relative standard derivation, RE: Relative error

Table 3. Recovery values of standard solution spiked in drug

Method	Added (µg/mL)	Found±SD (µg/mL)	Recovery (%)	RSD %
First-order Spectrophotometric Method	1.0[a]	0.975±0.045	95.52	4.628
	5.0[a]	4.979±0.191	99.58	3.851
	30[a]	29.14±0.867	97.15	2.977
Second-order Spectrophotometric Method	1.0[a]	0.980±0.056	98.00	5.772
	5.0[a]	4.833±0.242	96.66	5.011
	30[a]	29.26±1.280	97.55	4.373

. [a]: MN standard solution. SD: Standard deviation of six replicate determinations, RSD: Relative standard derivation

Table 4. Determination of MSB in drug (20 mg MSB/ampoule)

Methods	n	Found[a]± SD (mg)	Recovery (%)	RSD (%)	Confidence Interval
First-order Spectrophotometric Method	12	19.0±0.85	95.0	4.48	90.3-104.5
Second-order Spectrophotometric Method	12	19.4±1.09	97.1	5.66	87.4-103.8

[a]: MSB concentration, n: number of determination, SD: Standard deviation of six replicate determinations, RSD: Relative standard derivation

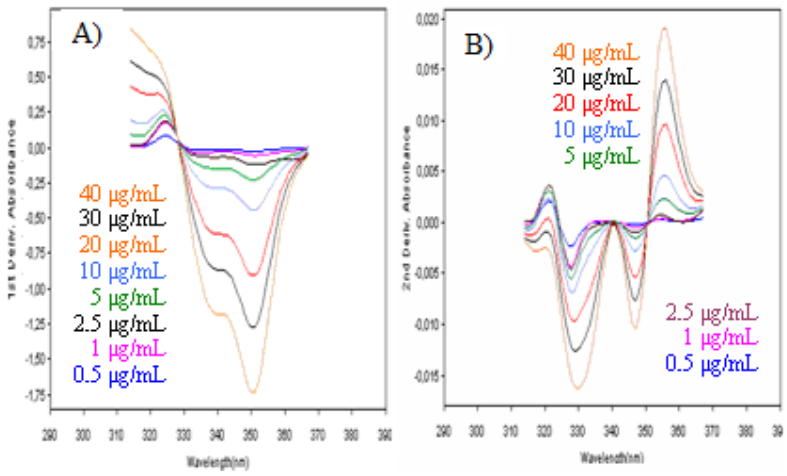

Figure 1. Spectrum of standard solutions of MN in calibration graph points: A) First-order derivative spectra, B) Second-order derivative spectra

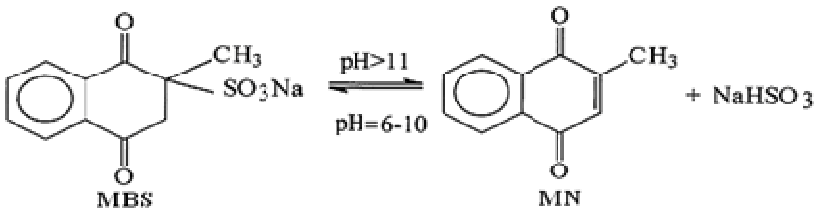

Figure 2. Reaction scheme for MSB in alkaline media.

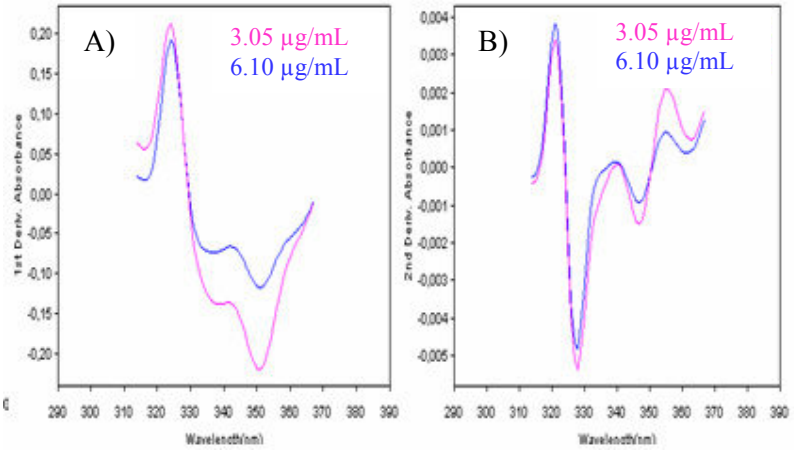

Figure 3. Spectra of solutions of Libavit K® ampoule containing MSB analyzed by converting MSB to MN: A) First-order derivative spectra, B) Second-order derivative spectra.