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Kinetic Spectrophotometric Determination of Sulbutiamine in Pharmaceutical Preparations and in Human Serum

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A kinetic spectrophotometric method was developed for the determination of sulbutiamine based on the catalytic effect on the reaction between sodium azide and iodine in an aqueous solution. Sulbutiamine at concentrations of 0.5 to 20 μg/ml was determined by measuring the decrease in the absorbance of iodine at 348 nm by a fixed-time method. The decrease in the absorbance in the first 5 min from the initiation of the reaction is related to the concentration of the drug. The detection limit of the procedure was 0.2 μg/ml. The relative standard deviation for the replicate determination (n = 10) of 4 μg/ml was 1.05%. The proposed procedure was successfully utilized in the determination of the drug in pharmaceutical preparations and human serum.

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Introduction

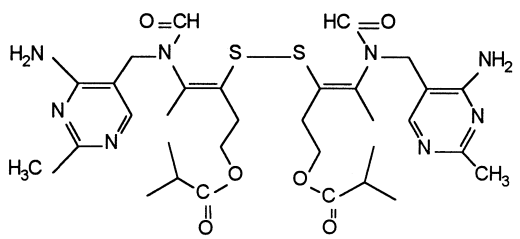
Sulbutiamine (Arcalion®) is a thiamine derivative (Scheme I) that has a specific neurotropic action. It is used to improve the intellectual performance and physical balance, and in the treatment of memory disorders, especially in elder patients.[1-3] The *in vitro* conversion of thiamine disulfide to free thiamine has been known.[4] The drug is obtained from free thiamine after three structural modifications:[5,6] I) opening of the thiamine ring, II) dimerization to a disulfide compound, and III) esterification of the two alcoholic functions. These modifications enhance its lipophilic properties relative to free thiamine.[5,6]

Although numerous methods for the determination of thiamine and its phosphoric esters have been published,[7,8] only a few procedures have been reported for the determination of thiamine disulfide derivatives including sulbutiamine. This is because thiamine disulfides are not easily oxidized to thiochromes.[9] Published methods for thiamine disulfides in general include precolumn high-performance liquid chromatography (HPLC),[10-12] polarography,[13,14] and colorimetric[15] techniques. The poor sensitivity was the main disadvantage of these methods.[9] Sulbutiamine, itself, is being determined by HPLC in dosage forms[16] or in human plasma.[9]

On the other hand, the catalytic-kinetic spectrophotometric method[17] is one of the most attractive approaches for the ultra-trace determination of certain chemicals. Its advantage is that only a spectrophotometer is necessary.

An example of such a procedure is the catalytic effect of sulfur-containing compounds on the well-known iodine-azide reaction, which has been widely applied to the determination of the compounds.[18-27]

The aim of the present study was to establish a catalytic-kinetic spectrophotometric procedure for the determination of sulbutiamine in pharmaceutical dosage forms and in human serum, based on its catalytic effect on an iodine-azide reaction.



Scheme 1 Sulbutiamine.

Experimental

Equipment

A Shimadzu (Model 1601PC) UV-Visible spectrophotometer (Shimadzu, Kyoto, Japan) was used to measure the absorbance at 348 nm. The cell chamber of the spectrophotometer was kept at a specified temperature using a Shimadzu thermostat Model TCC controller.

Chemicals and reagents

Sulbutiamine and un-coated Arcalion® tablets were kindly offered by Servier Egypt Industries Limited, Egypt. Commercial sugar-coated Arcalion® tablets were purchased at local markets. All other reagents were of analytical-reagent grade. Human serum was obtained from Blood Bank, Mansoura University Hospital, Egypt.

A sulbutiamine stock solution was prepared by dissolving 100 mg of sulbutiamine in 100 ml of 0.01 M phosphoric acid. Sulbutiamine standard solutions were prepared by the diluting this stock solution with the same solvent.

An iodine solution (0.01 M) was prepared by dissolving 0.254 g of I_2 in 100 ml of water containing 4.5 g of potassium iodide.

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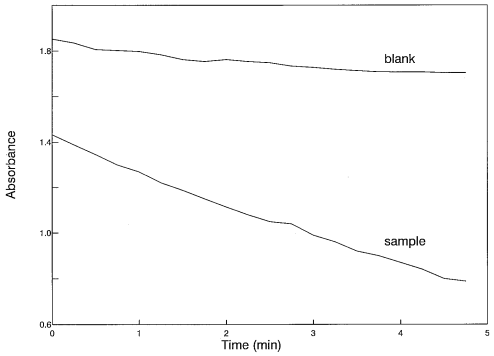

Fig. 1 Effect of the reaction time on the reaction between I_2 and azide at 35˚C in the absence (blank) and presence (sample) of 10 μg/ml sulbutiamine.

A 10% dilution of this solution was used for the experiment.

Sodium azide solution (1 M) was prepared by dissolving 6.501 g of sodium azide in 100 ml of distilled water. Phosphate buffer solutions in the pH range of 1 – 10 were prepared in distilled water. The pH of these buffer solutions was finally adjusted with HCl or NaOH solutions.

Procedures

Preparation of the calibration curve. First, 1 ml of the sulbutiamine standard solution containing 5 – 200 μg was transferred into10-ml volumetric flasks, followed by 1 ml of phosphate buffer (pH 3.0). Then, about 1 ml of a 1 M azide solution was added to the flask and the volume was made up to about 8 ml using distilled water. The solution was kept in a water bath (35˚C) for 3 min. Then, 1 ml of 0.001 M iodine solution was added and the volume was adjusted to the mark with distilled water and mixed. An aliquot of the reaction solution was quickly transferred into a quartz cell, and then it was held in the cell chamber (kept at 35˚C) of the spectrophotometer and the absorbance was recorded at 348 nm *versus* time (in a kinetic mode) for 4.8 min (5 min after the addition of iodine) against phosphate buffer solution (pH 3.0). A blank experiment was carried out under the same conditions without the drug, and its absorbance was recorded against a phosphate buffer solution (pH 3.0). The absorbances at 4.8 min (5 min after the addition of iodine) of the blank and the sample were obtained from the records, and were designated as A_0 and A, respectively. The difference between the absorbances ($A_0 - A$) was plotted against the drug concentrations. The calibration curve was prepared by a linear regression of the plots.

Determination of sulbutiamine in dosage forms. First, 10 Arcalion® tablets were weighed and pulverized. Then, the amount of the powder equivalent to about 100 mg of sulbutiamine was weighed accurately and then transferred to a 100-ml volumetric flask. Then, 50 ml of 0.01 M phosphoric acid was added to the flask. The mixture was then sonicated for 5 min at room temperature, and the volume was adjusted to the mark with the same solvent. The solution was centrifuged for 10 min at 3500 rpm. Then, 5 ml of the supernatant was transferred into another 100-ml volumetric flask and the volume was adjusted to the mark with 0.01 M phosphoric acid. One milliliter of this solution was used for the determination of drug content following the procedure described above.

Determination of sulbutiamine in serum. First, 2 ml of 10% trichloroacetic acid (TCA) was added to 1 ml of a human serum sample. The serum was homogenized for 1 min and the homogenate was centrifuged for 5 min at 3500 rpm. Then, 2 ml of the supernatant was used for the determination of the drug content by the procedure described above. The calibration curve for the determination of sulbutiamine in serum was prepared separately by using human serum samples spiked with sulbutiamine at a concentration level of 10 – 200 μg/ml by the procedure described above.

Table 1 Correlation coefficients for the calibration curves at different reaction times after the addition of iodine solution

Reaction time	Regression coefficient (r)
0.2	0.929
0.5	0.958
1.0	0.987
1.5	0.991
2.0	0.993
2.5	0.994
3.0	0.994
3.5	0.996
4.0	0.997
4.5	0.998
5.0	0.999
5.5	0.998
6.0	0.994
6.5	0.985
7.0	0.972
7.5	0.910
8.0	0.840

Quantification

The concentration of the sulbutiamine in the sample solution was calculated from the $A_0 - A$ value obtained for the sample solution by the procedure described above by using the slope and the intercept of the corresponding calibration curve.

Results and Discussion

Iodine oxidizes azide in an acid medium to form iodide and nitrogen. This reaction is very slow at low concentrations of the reactants, and in the absence of a sulfur-containing compound. This reaction, however, is very much accelerated in the presence of sulfide or thiol-containing compounds in the medium.[9,16-27] This reaction can be followed spectrophotometrically by monitoring the change in the absorbance at 348 nm.

Effect of variables on the reaction rate

The effect of such variables as for reaction time, pH, type of buffer, temperature, and azide concentration, were studied to establish the best reaction conditions to give the maximum sensitivity.

Reaction time. There are many methods, such as fixed-time, initial rate and rate-constant, for measuring the catalytic effects.[17,18] Among them, the fixed-time measurement is the most conventional and simplest.[17,18] Figure 1 shows that as the reaction time increased the difference in the absorbance of a blank (A_0) and a sample (A) at 348 nm increased. The most suitable reaction time was found to be 5 min after the addition of iodine (or 4.8 min after start the kinetic run), based on its correlation coefficient of the calibration curve, as shown in Table 1.

Effect of pH. The effect of the pH on the catalyzed (sample) and un-catalyzed (blank) reactions was studied by reacting 1 ml

Table 2 Assay of sulbutiamine in Arcalion® tablets by the proposed method and the published polarographic method[14]

Formulation	Claimed amount	Analytical results[a], %	
		Proposed method	Published method[b]
Arcalion®, un-coated	200 mg/tablet	99.35 – 101.09	98.05 – 103.29
Arcalion®, coated	200 mg/tablet	98.76 – 100.75	97.83 – 102.56

a. Number of experiements: 10. b. Ref 14.

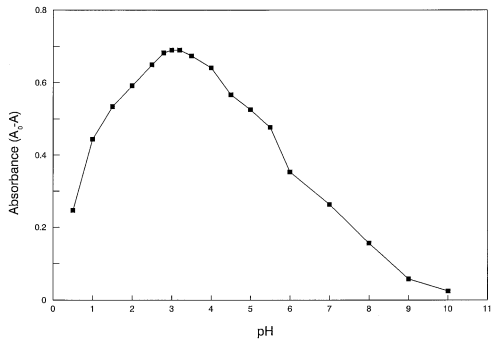

Fig. 2 Effect of the pH on the reaction between I_2 and azide at 35˚C in the presence of 10 μg/ml sulbutiamine.

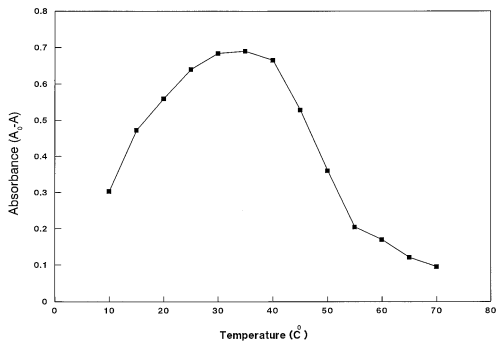

Fig. 4 Effect of the temperature on the reaction between I_2 and azide in the presence of 10 μg/ml sulbutiamine.

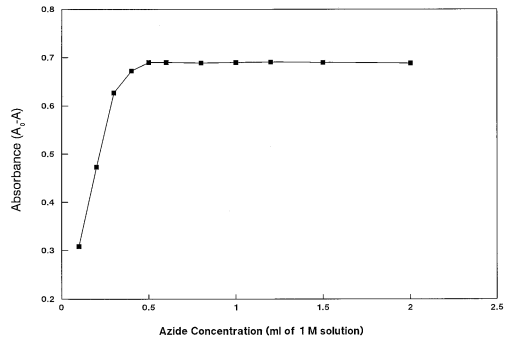

Fig. 3 Effect of the azide concentration on the reaction between iodine and azide at 35˚C in the presence of 10 μg/ml sulbutiamine.

of 0.001 M iodine solution and 1 ml of 1 M sodium azide solution at 35˚C with and without the addition of 40 μg/ml sulbutiamine solution. The rate of un-catalyzed reaction simply increased as the pH increased, while that of catalyzed reaction increased up to pH 4.0, and then declined. Since the maximum difference between both reactions was observed at around pH 3 (Fig. 2), a working condition of pH 3.0 was selected in this study.

Effect of the azide concentration. The effect of the azide concentration is shown in Fig. 3. It indicates that as the volume of 1 M azide solution increased the reaction rate increased up to 0.5 ml, whereas a greater volume of the solution had no effect. Therefore, 1 ml of 1 M azide solution was used in this study.

Effect of the iodine concentration. As the volume of 0.001 M iodine solution increased, the net reaction rate increased. However, volumes of more than 1 ml could not be used because iodine solution has a strong color.

Effect of the temperature. The effect of the temperature on the reaction rate was studied in the range of 10 – 70˚C (Fig. 4). It was observed that as the temperature increased, the rate of both catalyzed and un-catalyzed reactions increased up to 35˚C. In this temperature range, the rate of the catalyzed reaction was accelerated more than that of the un-catalyzed reaction, as was indicated by the increase in the $A_0 - A$ value. Above 35˚C, the $A_0 - A$ value decreased, which suggested that either the rate of un-catalyzed reaction was also accelerated, or iodine became lost because of volatilization due to heating. Thus, a temperature of 35˚C was used in this study.

Beer's law and sensitivity

Based on the $A_0 - A$ values of sulbutiamine standard solutions, the proposed method was found to give a rectilinear calibration curve over the concentration range of 0.5 – 20 μg/ml with a regression coefficient (r) of 0.999 (n = 13). The detection limit of the method was 0.2 μg/ml. The repeatability of the method was studied by replicate analysis of a standard sulbutiamine solution (4 μg/ml). The standard deviations estimated from the results of 10 replicate analyses were 1.05% for the proposed method and 2.23% for the published polarographic method.[14]

Analysis of pharmaceutical preparations

The proposed method was successfully applied to the determination of sulbutiamine in tablets. Since commercial sulbutiamine tablets were sugar coated, it was essential to analyze un-coated tablets separately in order to verify whether the coating materials would interfere with the analysis. Table 2 shows that the proposed method gave mean recoveries in the range of 98.76 to 101.09% for both coated and un-coated tablets, and that the published polarographic[14] gave those in the range of 97.83 to 103.29%. These results indicated no interference from the coating materials, excepients or additives, such as starch, gum, and lactose. The high sensitivity of the proposed method suggests its applicability to routine dissolution studies.

The accuracy was estimated by recovery studies of the sulbutiamine added to its tablets by the proposed method. Plots of the amount added *vs*. the amount recovered gave a regression line with a slope of near unity (0.997 ± 0.005), an intercept of near zero (0.033 ± 0.02) and a correlation coefficient of near

Table 3 Recovery of sulbutiamine spiked to human serum

Added/μg ml^{-1}[a]	Recovered sulbutiamine, %				
	Exp-1[b]	Exp-2	Exp-3	Avg.[c]	S.D.
0.67	96.2	96.8	102.7	98.6	2.933
1.0	95.9	100.2	101.4	99.2	2.361
2.0	102.2	97.2	96.01	98.5	2.685
4.0	100.9	96.9	101.2	99.7	1.960
8.0	95.8	97.9	99.3	97.7	1.438
10.0	102.2	100.2	98.7	100.4	1.434
13.3	96.7	98.9	100.2	98.6	1.445

a. Concentration was calculated according to final dilutions.
b. Exp: experiment.
c. Avg.: average.

unity (0.999). These data indicate that the proposed method is both sensitive and selective.

Assay of sulbutiamine in human serum samples

The applicability of the proposed method for the assay of sulbutiamine in serum was tested by analyzing human serum samples spiked with sulbutiamine. The calibration curve for sulbutiamine in serum ($Y = -0.15 + 0.241X$) was found to be linear at a spiking range of 10 – 200 μg/ml. The recoveries of sulbutiamine at different concentrations were studied; and the results are given in Table 3. The proposed method gave recoveries of 98.929 ± 2.272 % (95.82 to102.7 %). The presence of indigenous compounds in serum samples did not interfere with the analysis.

General consideration

Because there is a possibility that the decrease in the absorbance 348 nm could have been due to the reaction of iodine with sulbutiamine, experiments were conducted where iodine was allowed to react with sulbutiamine in the absence of azide and the results were compared with experiments without sulbutiamine. These experiments were conducted at different concentrations of sulbutiamine. No difference was found between the absorbances at 348 nm from experiments with and without sulbutiamine. This result suggests that the observed decrease in the absorbance at 348 nm was mainly due to the catalytic effect of sulbutiamine on the reaction between iodine and azide, rather than a direct reaction between iodine and sulbutiamine.

In conclusion, the proposed method was accurate, precise and sensitive and was successfully utilized in the determination of the sulbutiamine in dosage forms as well as in human serum samples.

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