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Short Communication

Spectrophotometric determination of molsidomine in pharmaceutical formulations using bromocresol green*

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Introduction

Molsidomine (*N*-ethoxycarbonyl-3-morpholinosyndonimine, SIN-10) is a syndnonimine derivative with a mesoionic structure, which possesses a sustained coronary vasodilatation and antihypertensive effect following oral administration [1]. Monitoring of molsidomine and its metabolites in biological fluids has been carried out mostly by HPLC [2, 3]. UV spectrophotometric methods [4, 5] have been used for the determination of bulk molsidomine. No colorimetric procedure for the determination of molsidomine in the pure state or in dosage forms has been reported.

The present paper describes a simple and reproducible colorimetric assay for the determination of molsidomine in dosage forms. The method is based on the formation of a coloured ion-pair complex of the drug with a bromocresol green (BCG) as a reagent.

Experimental

Instruments

A spectrophotometer (Specord M40, Carl-Zeiss, Jena) with 1-cm cells and a pH meter (MA 5705, Iskra, Kranj) were used.

Materials

Molsidomine (Hoechst, Germany) was used as the working standard. Lopion^R tablets containing 2 mg of molsidomine were obtained from Jugoremedia (Zrenjanin). Bromocresol green (BCG), disodium phosphate, sodium hydroxide, anhydrous sodium sulphate (all Merck), citric acid monohydrate, chloroform and methylene chloride (all Fluka) were used. All reagents were of analytical grade.

Solutions

Molsidomine stock solutions: 1.0325×10^{-3} M and 2.065×10^{-4} M (A) freshly prepared solutions in water were used. McIlvaine's buffers (pH 2.40, 2.60, 2.80, 3.00 and 3.20; 0.25 M) were used [6]. Buffered BCG solution was 5.73×10^{-3} M in McIlvaine's buffer (pH 6.00; 0.25 M) (B); buffered molsidomine solution 3.10×10^{-4} M (C) and buffered BCG solution 3.10×10^{-4} M (pH 2.80; 0.25 M) (D) were used. All solutions of molsidomine were stable for 10 days when stored in the dark at 4°–8°C.

Preparation of sample solution

An appropriate amount of tablet powder (from Lopion^R tablets) containing 5 mg of molsidomine was transferred to a 100-ml calibrated flask and 75 ml water was added. The mixture was shaken for 5 min, diluted to 100 ml with water and filtered through Schleicher 8c. Schull 5981 Schwarzband filter-

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paper. The concentration of this final solution was 2.064×10^{-4} M molsidomine.

Procedure

To 5 ml of molsidomine solution (A) placed in an Erlenmeyer flask fitted with a ground-glass stopper, 3 ml BCG solution (B) and 5 ml of McIlvain's buffer solution (pH 2.80; 0.25 M) were added; finally, 10 ml of chloroform was added. The Erlenmeyer flask was stoppered and the mixture was gently shaken for 5 min by means of a shaking-machine. The yellow chloroformic layer was separated in the separation funnel and filtered through anhydrous sodium sulphate. The absorbance of the chloroformic phase was measured at 421 nm against a reagent blank.

Calibration curve

A series of eight solutions containing 0.50, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0 and 7.0 ml of molsidomine solution (A) were treated by the described procedure. For each concentration three experiments were performed and the absorbance was measured at 421 nm.

Investigation of the molsidomine–BCG ion pair

The composition of the molsidomine–BCG ion-pair complex was determined by Job's method of equimolar solutions [7] and Bent–French's method [8]. Experiments were conducted according to the described procedure using buffered molsidomine solution (C) and buffered BCG solution (D). Nine mixtures molsidomine and BCG with the addition of 5 ml of buffer solution (pH 2.80; 0.25 M) were prepared. The volumes of molsidomine solution used varied from 0.5 to 4.50 ml and those of BCG solution (D) from 4.50 to 0.5 ml; the total volume was always 5 ml. The extraction was performed with 10 ml of chloroform and the absorbance was measured at 421 nm. The dependence of absorbance on the concentration of the BCG reagent at pH 2.60–3.20 was also investigated. In these experiments different volumes (0.5–4 ml) of BCG solution (B) and different pH values were used in the formation of molsidomine–BCG ion-pair complex.

Results and Discussion

The absorption spectrum of the ion-pair complex formed between molsidomine and BCG was measured at 350–550 nm against the blank reagent (Fig. 1, curve 1).

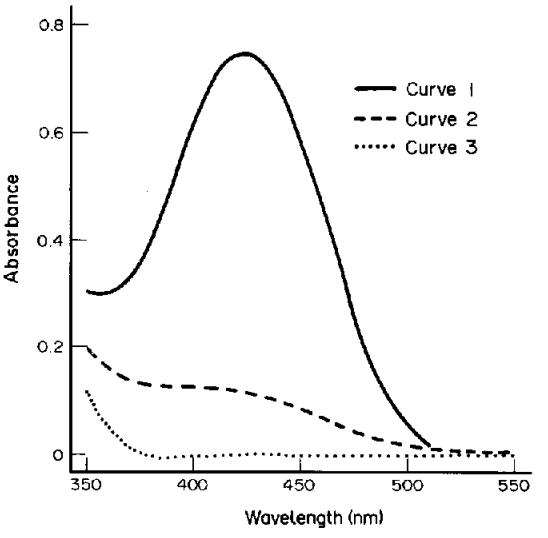

Figure 1
Absorption spectra of chloroformic extract of the ion-pair complex molsidomine–BCG (curve 1), chloroformic extract of BCG reagent (curve 2) and chloroformic extract of molsidomine (curve 3) (pH 2.80; 0.25 M).

The chloroformic extract showed maximum absorbance after shaking for 5 min. The shape of this absorption spectrum and the position of the absorption maximum of the ion-pair complex formed did not vary with pH; this result indicates that only one type of complex is formed. The absorbance of a repeated chloroformic extract of the molsidomine–BCG ion-pair complex was insignificant. In order to select the optimum pH, five experiments were performed with each of the following buffers (pH 2.60, 2.80, 3.00 and 3.20). The maximum absorption of the ion-pair complex at 421 nm was obtained in the buffered solution at pH 2.80 (0.25 M). The influence of BCG concentration at the same pH value was also investigated. The optimum ratio was: $C_{BCG} \times C_{molsidomine}^{-1} \geqslant 12$.

The composition of the molsidomine–BCG ion-pair complex was determined by Job's method of equimolar solutions and by Bent–French's method of logarithmic absorbance analysis. The ratio of molsidomine and BCG in the ion-pair complex, determined by Job's method is shown in Fig. 2. The reaction conditions were: $C_{molsidomine} = C_{BCG} = 3.10 \times 10^{-4}$ M (pH 2.80; 0.25 M).

The curve exhibits the maximum $X_{max} = 0.67$, which means that the components of the

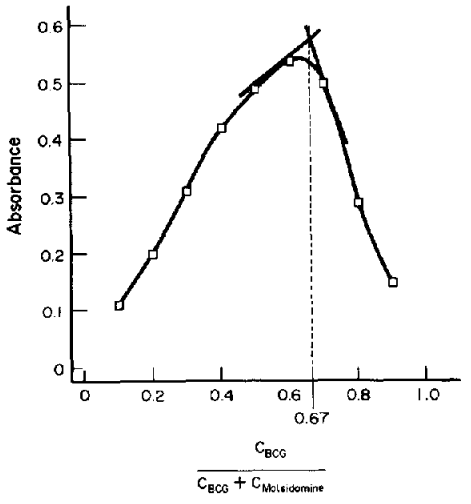

Figure 2
Job's curve of equimolar solutions for molsidomine–BCG ion-pair complex of chloroformic extract; $C_{molsidomine} = C_{BCG} = 3.10 \times 10^{-4}$ M; pH = 2.80; 0.25 M.

ion-pair complex react in a 1:2 stoichiometric ratio (molsidomine:BCG).

The number of molsidomine molecules in the ion-pair complex was determined by Bent–French's method. There was a linear relationship between log A (absorbance) and log $C_{molsidomine}$ for the range of concentrations (4.13×10^{-5} M to 1.44×10^{-4} M) investigated. The Bent–French's equation obtained was: $y = -4.066 + 1.076x$; correlation coefficient, $r = 0.9972$. The slope of the straight line was 1.076, which means that only one molsidomine molecule takes part in the formation of the ion-pair complex. Thus it was confirmed that the ratio of components in the molsidomine–BCG ion-pair was 1:2.

The conditional stability constant of the ion-pair complex was calculated by the method of Sommer [9] using Job's curves. The conditional stability constant of the molsidomine–BCG ion-pair complex was: log $K = 7.172$ (SD = 0.139, $N = 7$). The results suggest that morpholinyl- and imino-moieties take part in the formation of the ion-pair complex.

The calibration curve for molsidomine in the chloroformic extract showed a linear dependence of the absorbance on the concentration of molsidomine in the concentration range of 1.023×10^{-5} M to 1.45×10^{-4} M. Beer's law was obeyed up to 2.50 μg ml^{-1}. The regression equation was: $y = 0.016 + 0.023x$; $r = 0.9951$. The molar absorptivity was 9.34×10^3 l mol^{-1} cm^{-1}.

Table 1
Determination of molsidomine in the bulk drug and in Lopion^R tablets

Sample (n = 10)	Molsidomine bulk drug I	Molsidomine bulk drug II	Lopion^R tablets
Taken (mg)	1.50	2.50	2.00
Found (mg)	1.49	2.48	1.99
Mean (%)	99.33	99.20	99.50
SD	0.025	0.031	0.027
RSD (%)	1.68	1.25	1.36

The spectrophotometric method described for the determination of molsidomine was found to be very simple and sensitive and therefore was applied to the determination of molsidomine in the bulk drug and in Lopion^R tablets. The results are shown in Table 1.

The results obtained confirm the suitability of the proposed method for the accurate and precise analysis of molsidomine in the bulk drug and in tablets. The proposed method is rapid and simple and since no expensive laboratory technique for separation is needed, the method can be used for routine analyses.

References

[1] A. Takeshita, M. Nakamura, T. Tajimi, N. Matsuguchi, A. Kuroiwa, S. Tanaka and Y. Kikuchi, *Circulation* **55**, 401–412 (1977).
[2] D. Dell and J. Chamberlain, *J. Chromatogr.* **146**, 465–472 (1978).
[3] C. Dutot, J. Morean, P. Cordonnier, O. Spreaux-Varoquay, C. Klein, J. Ostrowski, C. Advenier, W. Gärtner and M. Paus, *J. Chromatogr.* **528**, 435–446 (1990).
[4] J. Kračmar and J. Kračmarova, *Českoslov. Farm.* **38**, 145–155 (1989).
[5] M. Pernarowski, in *Pharmaceutical Chemistry*, Vol. 2 (L. Chatten, Ed.). pp. 13–57. UMI, Michigan (1992).
[6] D.D. Perrin and B. Dempsey, in *Buffers for pH and Metal Ion Control*, pp. 153–155. Chapman and Hall, London (1974).
[7] H. Irving and T.B. Pierce, *J. Chem. Soc.* 2565–2571 (1959).
[8] N. Bent and C. French, *J. Amer. Chem. Soc.* **63**, 568–573 (1941).
[9] L. Sommer, *Chem. Listy* **55**, 574–581 (1961).

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