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Short communication

Spectrophotometric determination of midazolam in pharmaceutical formulations

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1. Introduction

Midazolam is 8-chloro-6-(2-fluorophenyl)-1-methyl-4*H*-imidazo(1,5-a)(1,4)benzodiazepine and belongs to a newer class of benzodiazepine derivatives [1]. The presence of an imidazole ring in the 1,2-position induces some changes in the properties characteristic of classical benzodiazepines such as basicity, stability in aqueous solution and rate of metabolism [2].

In aqueous solution midazolam behaves as a sparingly soluble diprotic base. At pH < 4 it under goes relatively slow reversible hydrolysis with opening of the benzodiazepine ring at the azomethine bond. The determination of acidity constants, mechanism of hydrolysis and hydrolysis constants, equilibrium constants in a heterogeneous system and solubility, as well as buffer characteristics in homogeneous and heterogeneous systems are described in an earlier paper [3].

The many reported analytical methods [4] for the determination of midazolam in various media indicate a broad interest in this compound. However, an official method for the assay of midazolam either in the British Pharmacopoeia (BP) or in the United States Pharmacopeia (USP) has not yet been proposed. Although spectrophotometric methods, owing to their simplicity and speed, occupy an important place in the analysis of pharmaceutical formulations, only one spectrophotometric method for the assay of midazolam has been developed so far [5,6]. This method is very sensitive but not universal, since it can be applied only to formulations containing midazolam in the form of the free base or the hydrochloride.

Since in aqueous solution midazolam undergoes various protolytic reactions [3], the aim of this work was to examine the extent to which a quantitative knowledge of the corresponding equilibria is necessary for a proper choice of optimum conditions for the development of a selective analytical method. In contrast to the current spectrophotometric method, a new spectrophotometric method should be applicable to the analysis of pharmaceutical formulations which contain midazolam not only in the form of the free base or the hydrochloride, but also in the form of the maleate.

2. Experimental

2.1. Apparatus

All spectrophotometric measurements were carried out on a GBS 911A spectrophotometer in 1-cm silica cells over the spectral range 210–320 nm at a scanning rate of 500 nm min^{-1}, against a corresponding blank.

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2.2. Standard solutions

A stock midazolam solution (5×10^{-4} M) was prepared by dissolving midazolam free base (Hoffman La Roche, Basle) in 0.1 M HCl. Aliquots of 0.25, 0.50, 1.00, 2.00, 3.00 and 3.50 ml, respectively, of this solution were transferred into 25-ml volumetric flasks and to each was added 5 ml of 5 M HCl; each solution was then diluted with double-distilled water to 25 ml. The absorbance of each solution was measured at 269 nm against 1 M HCl.

2.3. Sample solution

Ten Flormidal tablets (ICN Galenika, Yugoslavia) were weighed and powdered; 0.2086 g of the powder corresponding to the average weight of one tablet was transferred quantitatively to a 100-ml volumetric flask to which was added about 60 ml of 0.1 M HCl. After treatment in an ultrasonic bath for 10 min the flask was filled to 100 ml with 0.1 M HCl. The resultant suspension was filtered through a filter-paper (Schleicher & Schüll, blue strip No. 589[1]) and the first 10 ml of the filtrate was discarded. Different aliquots of the filtrate were transferred in to 25-ml volumetric flasks and further treated as described for standard solutions. The midazolam concentration of the working solutions ranged from 9.210×10^{-6} to 7.368×10^{-5} M, calculated with respect to the labelled midazolam content.

3. Results and discussion

3.1. Development of the method

In a previous paper [3] from this laboratory, using the acidity constants ($pK_{a1} = 1.37$, $pK_{a2} = 5.90$) and hydrolysis constants ($pK_{h1} = -2.59$, $pK_{h2} = -1.22$) of midazolam, the distribution of midazolam species in solution at equilibrium was calculated as a function of pH. The absorption specta of theses species are shown in Fig. 1. The spectrum of midazolam free base, B, was obtained by the usual procedure from NaOH solution (pH > 8). For recording the spectrum of the monoprotonated midazolam species, BH^+ (pH 4), and particularly that of the diprotonated species BH_2^{2+} (1 M HCl), it was necessary to apply a rapid working procedure in order to avoid the effects of hydrolysis. It was not possible to obtain the spectrum of the pure hydrolysis product $B'H_2^{2+}$ since this product is never present as the only species in the solution; at pH < 1 it occurs in equilibrium with the diprotonated form (hydrolysis is not complete). Therefore the spectrum of the equilibrium state $BH_2^{2+} \rightleftharpoons B'H_2^{2+}$ attained in 1 M HCl after 1 h equilibration was recorded. This spectrum intersects the spectrum of the diprotonated form at three isosbestic points (222, 248 and 269 nm). Besides, Fig. 1 shows the absorption spectra of maleic acid ($pK_{a1} = 1.92$, $pK_{a2} = 6.22$) [7] and its protolysis products, recorded from solutions in which the individual species were dominant.

The method developed by Gallo and coworkers [5,6] for the determination of midazolam in pharmaceutical formulations is based on measurements of the absorbance of the solution (pH 7) at the wavelength of the absorption maximum (225 nm) of the free base. Although it is very sensitive, that method can be applied only to formulations in which midazolam occurs in the form of the free base or hydrochloride. From Fig. 1, it is clearly seen that maleic acid and its protolysis products absorb at the wavelength of the absorption maximum of midazolam free base.

On the basis of the analysis of the absorption spectra of midazolam it may be concluded that optimum conditions for the determination of midazolam in the presence of maleic acid comprise: an acidic solution (1 M HCl) in which the equilibrium $BH_2^{2+} \rightleftharpoons B'H_2^{2+}$ exists; and the wavelength of the isosbestic point at 269 nm. At this wavelength maleic acid does

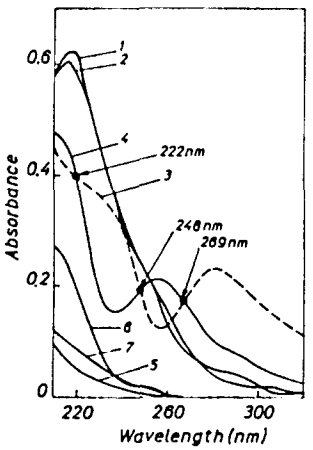

Fig. 1. Absorption spectra of equilibrium species in solutions of midazolam and maleic acid. (1) B (pH 11); (2) BH^+ (pH 4); (3) BH_2^{2+} (1 M HCl); $BH_2^{2+} \rightleftharpoons B'H_2^{2+}$ (1 M HCl); (5) H_2A (maleic acid, pH 0); (6) HA^- (pH 4); (7) A^{2-} (pH 11). $c = 1.8 \times 10^{-5}$ M.

Table 1
The content of midazolam in Flormidal tablets [a]

Taken [b] concentration (M)	Found mg per tablet	RSD ($n = 6$)	Percentage of label claim
9.210×10^{-6}	14.69	0.41	97.93
1.842×10^{-5}	14.91	0.42	99.40
4.605×10^{-5}	14.89	0.47	99.27
7.368×10^{-5}	14.99	0.58	99.93

[a] Label claim: midazolam 15 mg (in the form of maleate) per tablet.
[b] Midazolam concentration in the working solution calculated relative to the label claim.

not absorb and, since $\varepsilon_{BH_2^+} = \varepsilon_{B \cdot H_2^+}$, there is no change in absorbance with time.

3.2. Validation

Beer's law was checked by measuring the absorbance of acidic midazolam solutions (1 M HCl) at 269 nm. The midazolam concentrations ranged from 5×10^{-6} to 7×10^{-5} M. The corresponding linear regression equation was $A = 9831c + 0.0063$ and the correlation coefficient was 0.9999.

The precision of the method determined by analyzing midazolam solution at four different concentrations (six determinations for each), experssed as the relative standard deviation, ranged from 0.45 to 0.70%, indicating very good reproducibility of the method.

3.3. Assay of midazolam tablets

The applicability of the method to the analysis of pharmaceutical formulations was checked by determining midazolam in Flormidal tablets which contain midazolam in the form of the maleate.

In order to examine the selectivity of the proposed method with respect to the tablet excipients, spectra of standard solutions of midazolam and of a tablet extract were analyzed. The consistency of λ_{max}, λ_{min}, wavelengths of isosbestic points as well as of the spectra, which followed the hydrolysis of the drug with time in the region where maleic acid and its protolysis products exhibit no absorption ($\lambda > 260$ nm), show that these substances do not interfere.

The results of the determination of midazolam in Flormidal tablets are presented in Table 1. The mean value obtained per tablet was 14.87 mg (99.13% of the labelled claim) with an RSD of 0.56%.

The proposed method is specific, simple, fast and accurate. The possibility of the spectrophotometric determination of midazolam in pharmaceutical dosage forms, in which it is present not only in the form of the free base or hydrochloride but also in the form of maleate, makes the proposed method superior to the spectrophotometric method previously reported for the assay of midazolam [5,6].

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References

[1] A. Walser, L.E. Benjamin, Sr., T. Flynn, C. Mason, R. Schwartz and R.I. Fryer, J. Org. Chem., 43 (1978) 936–944.
[2] M. Gerecke, Br. J. Clin. Pharmacol., 16 (1983) 11S–16S.
[3] L.B. Pfendt, T.J. Janjić and G.V. Popović, Analyst, 120 (1995) 2145–2151.
[4] A.J. Ribes and J. Osteryoung, Anal. Chem., 62 (1990) 2632–2636.
[5] M.M. Orive, B. Gallo, R.M. Alonso, F. Vicente, J.C. Vire and G. Patriarche, J. Microchim. Acta., 1 (1989) 181–190.
[6] B. Gallo, R.M. Alonso, F. Vicente, J.C. Vire and G.J. Patriarrche, Anal. Lett., 21 (1988) 1211–1220.
[7] N.G. Topp and C.W. Davies, J. Chem. Soc., Part I, (1940) 87–93.