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International Journal of Pharmaceutics 123 (1995) 229–235

international journal of pharmaceutics

Photochemical decomposition of midazolam IV. Study of pH-dependent stability by high-performance liquid chromatography

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Received 20 December 1994; revised 1 February 1995; accepted 13 February 1995

Abstract

A high-performance liquid chromatographic method was developed for the quantitation of midazolam in aqueous solutions exposed to light. Midazolam was separated from its main decomposition products on a Lichrosorb RP-18 column with a mobile phase consisting of methanol and 50 mM phosphoric acid with triethylamine, and the pH of the aqueous component adjusted to 3.5 with sodium hydroxide. The method showed good intra-day and inter-day precision and accuracy (RSD and RE < 2.5%). The quantitative assay of midazolam in photodecomposed citrate buffer solutions indicated the decomposition to be dependent on the pH of the aqueous solution; the rate of decomposition was lowest at very acidic pH and faster when the pH of the solutions was increased.

Keywords: Midazolam; Stability; Photochemical decomposition; HPLC

1. Introduction

Midazolam is a benzodiazepine derivative containing an imidazole ring fused to the 1,2-position in the benzodiazepine skeleton. The nitrogen in the imidazole ring is basic, having a pK_a value of about 6 (Walser et al., 1978; Vire et al., 1986; Andersin, 1991). Because of the basicity of the compound, water-soluble salts of midazolam can be prepared for aqueous injection.

Aqueous solutions of midazolam photodegrade both under irradiation from a high-pressure mercury lamp and in daylight. In artificially irradiated solutions the main products, which were identified by IR, ^{1}H-NMR, ^{13}C-NMR and mass spectrometry, are 6-(8-chloro-1-methyl-4,5-dihydro-2,5,10*b*-triazabenzo[*e*]azulen-6-ylidene)-cyclohexa-2,4-dienone (Fig. 1, 'dienone') and 6-chloro-2-methyl-4-(2-fluorophenyl)quinazoline (Fig. 1, I) (Andersin et al., 1994). The quinazoline derivative is also the main product in the daylight-exposed solutions and degrades further to 6-chloro-2-methyl-4(1H)-quinazolinone (Fig. 1, II) (Andersin and Mesilaakso, 1995). On examination by TLC the photodecomposition seemed to be dependent on the pH of the irradiated solutions; the number of photoproducts multiplied when the pH of the irradiated solution was in-

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SSDI 0378-5173(95)00064-X

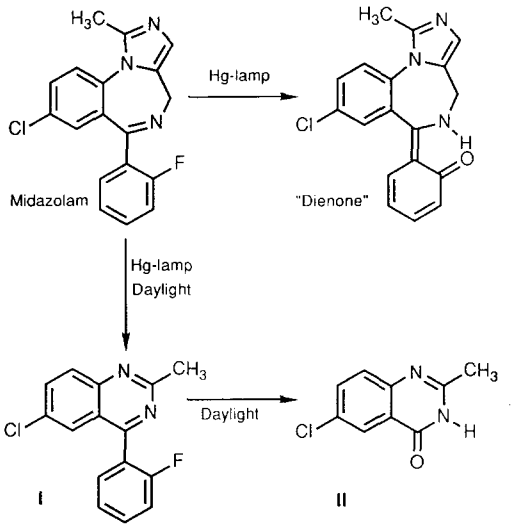

Fig. 1. Structures of midazolam and its main decomposition products, 6-(8-chloro-1-methyl-4,5-dihydro-2,5,10*b*-triazabenzo[*e*]azulen-6-ylidene)cyclohexa-2,4-dienone ('dienone'), 6-chloro-2-methyl-4-(2-fluorophenyl)quinazoline (I) and 6-chloro-2-methyl-4(1H)-quinazolinone (II).

creased, whereas only one photoproduct was found in 0.1 M hydrochloric acid.

Reports on the determination of midazolam have mostly been concerned with the quantitation of it and its hydroxy metabolites in biological samples. Both gas chromatographic (GC) and high-performance liquid chromatographic (HPLC) methods have been reported (e.g., De Kroon et al., 1989; Mastey et al., 1994). GC has also been used in quantitative studies of the photodecomposition kinetics of midazolam in ethanolic solutions (Andersin and Tammilehto, 1989), and quantitative HPLC methods have been developed for studies on the decomposition of midazolam due to the presence of various excipients in the formulations (Steedman et al., 1992, Bhatt-Mehta et al., 1993a,b). HPLC is a more convenient method for the quantitative assay of aqueous photodecomposed solutions because of the ease of sample preparation.

The aim of the present study was to develop an HPLC method allowing the quantitation of midazolam in photodecomposed solutions in the presence of its decomposition products, and to use this method in the study of the effect of the pH on the photochemical stability of midazolam solutions. The effect of normal daylight was investigated by determining midazolam in daylight-exposed commercial Dormicum injections.

2. Materials and methods

2.1. Chemicals

Midazolam and desalkylflurazepam were kindly supplied by Hoffmann-La Roche (Basle, Switzerland). Reference substances, 6-chloro-2-methyl-4-(2-fluorophenyl)quinazoline and 6-(8-chloro-1-methyl-4,5-dihydro-2,5,10*b*-triazabenzo-[*e*]azulen-6-ylidene)cyclohexa-2,4-dienone, were isolated in qualitative photodecomposition studies of midazolam (Selkämaa and Tammilehto, 1989; Andersin et al., 1994). Potassium ferrioxalate was synthesised according to Hatchard and Parker (1956). Triethylamine (TEA) was obtained from Fluka (Buchs, Switzerland) and all other analytical grade reagents were purchased from Merck (Darmstadt, Germany).

HPLC grade methanol from Rathburn (Walkerburn, UK) and purified water from an Alpha-Q water purification system (Millipore, Molsheim, France) were used in HPLC assays.

2.2. Apparatus

The radiation source was a high-pressure mercury lamp Original Hanau TQ 718 (500 W) (Hanau, Germany). pH values were measured with a PHM 83 Autocal pH meter (Radiometer, Copenhagen, Denmark). Spectrophotometric measurements were performed with a Philips PU 8740 UV-Vis spectrophotometer (Cambridge, UK).

The chromatographic equipment consisted of two Waters 501 HPLC pumps coupled to a Waters automated gradient controller, a Rheodyne 7125 manual injector with a 20 μl loop, a Waters 991 photodiode array detector with NEC Power Mate 386/25 computer with PDA (photodiode array) software, and a Waters 5200 printer/plotter (all from Waters Associates, Milford, MA, USA).

2.3. Chromatographic conditions

Separations were performed at room temperature on a Lichrosorb RP-18 (125 × 4 mm i.d., 5 μm) column (Merck, Darmstadt, Germany) equipped with a Lichrosorb RP-18 (10 μm) precolumn (Merck, Darmstadt, Germany). Aqueous and organic phases were pumped separately and mixed in a ratio of 35:65 with the gradient controller. The aqueous phase consisted of 50 mM phosphoric acid with 14.4 mM TEA and was adjusted to pH 3.5 with NaOH prior to filtration through an HVLP 0.45 μm filter (Millipore, Watford, Ireland). The organic mobile phase component was methanol. The eluents were degassed separately with helium for about 15 min. The eluent flow rate was 1 ml/min. The absorbances in the chromatogram were measured at 220 nm.

The hold-up time was determined with disodium citrate, which coeluted with sodium nitrite (disodium citrate was present as a buffer component in each sample).

2.4. Calibration curve

Five standard solutions were prepared for the calibration curve in the concentration range 0.02–0.06 mM midazolam. Equal amounts (1 ml)

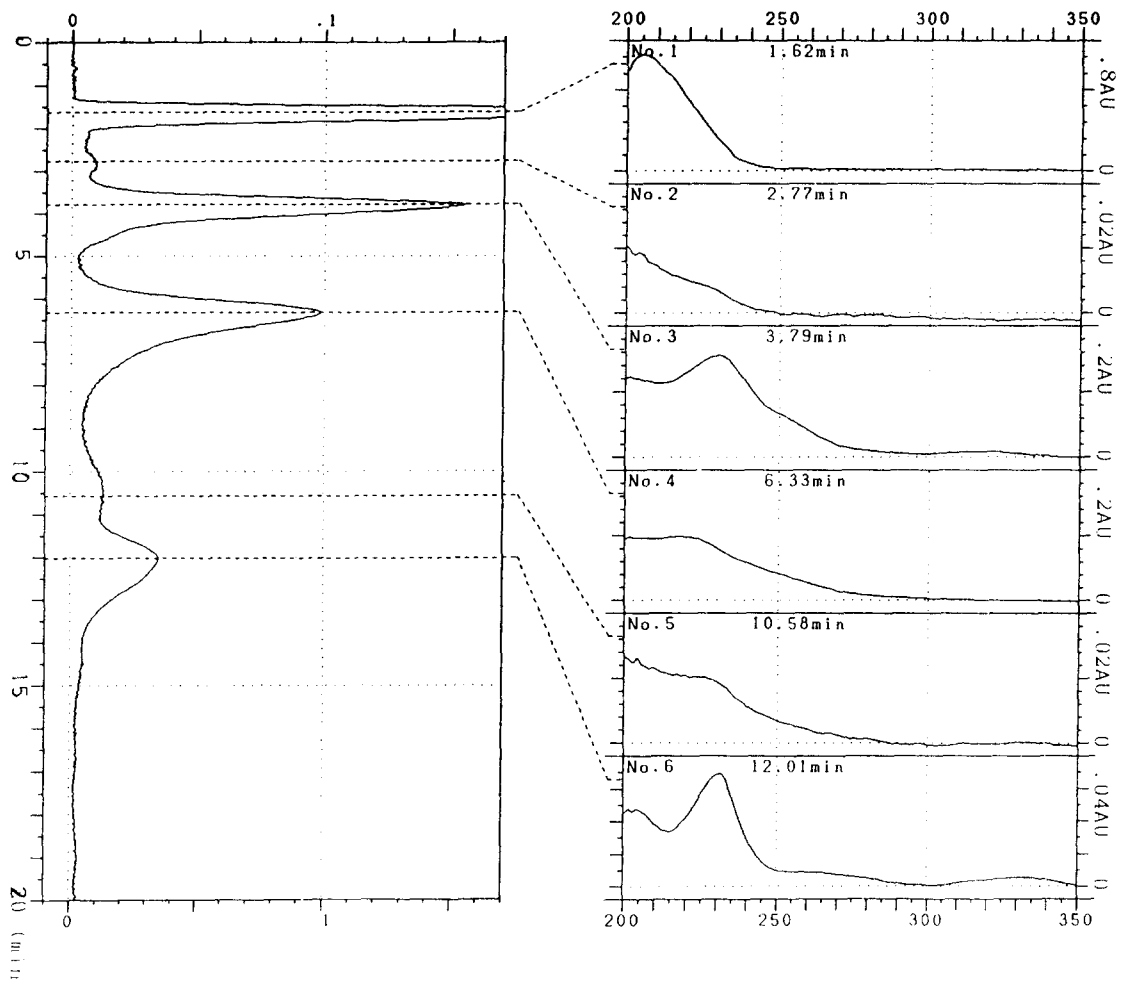

Fig. 2. HPLC chromatogram of a mixture of midazolam and its decomposition products, desalkylflurazepam (peak 3), midazolam (peak 4), quinazoline derivative (peak 5), dienone derivative (peak 6); peak 1, disodium citrate (buffer component of the solution); peak 2, impurity of desalkylflurazepam.

of water dilutions of a 5 mM stock solution of midazolam in citrate buffer pH 2.2 (Scientific Tables, 1971) and 0.1 M NaOH were allowed to stand for 2 h before adjustment of the volume to 5.0 ml with methanol. Six parallel samples were prepared and filtered through Acrodisc nylon bulk filters (Gelman Sciences, MI, USA). The calibration curve was constructed by plotting peak areas vs midazolam concentration.

2.5. Precision and accuracy

Intra-day precision and accuracy of the method were assessed from five standard solutions with six parallel samples of each. Inter-day precision and accuracy of the method were assessed from four freshly prepared 0.05 mM solutions of midazolam. Repeatability and reproducibility of the HPLC assay were determined by replicate analysis of 0.02 and 0.05 mM standard solutions six times in one day and on six different days.

2.6. Photodegradation of midazolam

Midazolam solutions (0.25 mM) in citrate buffers pH 1.3, 2.3, 3.0, 4.0, 5.0 and 6.4 were prepared from the stock solution of midazolam (5 mM in citrate buffer pH 2.2). A 3 ml aliquot of each solution ($n = 4$) was exposed to a high-pressure mercury lamp in a quartz cuvette, which was placed behind a Corning CS-7-54 filter and a filter solution of potassium chromate in a 1 cm quartz cuvette (0.27 mg/ml) at a distance of 4.5 cm from the lamp. The solution was stirred with a magnetic stirrer and the cuvette holder was cooled with running water (system designed by Ulvi, 1994). The intensity of light in the wavelength region 313 nm (separated from the mercury lamp by the above-mentioned filters) was measured by potassium ferrioxalate chemical actinometry (Hatchard and Parker, 1956).

Daylight-induced photodegradation of midazolam was studied by exposing vials of commercial Dormicum solutions 5 mg/ml (Roche, B1713MFD1093) to daylight on a southern windowsill in the laboratory for 1, 2, 3 and 4 months, from May to September.

HPLC samples of the photodecomposed solutions were prepared in duplicate in the same way as standard solutions.

3. Results and discussion

An HPLC method was developed to separate midazolam from its main decomposition products (Fig. 2). A mobile phase consisting of phosphoric acid (pH adjusted to 3.5) and methanol in the proportion 35:65 (1 ml/min) gave optimum resolution of the midazolam peak from the peaks of the decomposition products in the chromatogram. Retention factors of the compounds were 2.9 for midazolam, 0.7 for desalkylflurazepam, 5.5 for the quinazoline derivative and 6.4 for the dienone derivative. When the pH of the aqueous component was raised the retention time of midazolam became longer. If the aqueous component is too acidic, midazolam will exist partly in the open-ring form (Bhattacharyya and Grant, 1982, Orive et al., 1989, Andersin, 1991), eluting as a separate peak in the HPLC assay, with retention factor 0.6. For this reason the addition of base (0.1 M NaOH) to the samples was essential in the quantitation, base addition converting the open-ring form to the closed one. When the organic component of the mobile phase was changed to acetonitrile with the same solvent strength (55%), the peaks of desalkylflurazepam and midazolam merged in the chromatogram, while the quinazoline and dienone derivatives were better separated. Since only the quantitation of midazolam was of interest, methanol was preferred for the organic mobile phase component. Triethylamine was added to the mobile phase to prevent tailing of the basic compounds. Peak purity analysis proved the midazolam peak to be spectroscopically homogeneous in the chromatogram of the irradiated solutions.

The calibration curve for midazolam was linear in the concentration range studied, with the regression equation $y = -0.00133 + 0.893 \times$ ($r^2 = 0.997$). 95% confidence limits were for the intercept -0.00631 to $+0.00365$ and for the slope 0.878–0.916. The within-day relative standard deviation (RSD) of six standard solutions at each

Table 1
Within-day precision and accuracy of the assay ($n = 6$)

Concentration (mM)	RSD%	RE% [a]
0.060	1.1	−0.31
0.050	2.0	+1.8
0.040	2.3	−0.16
0.030	2.5	−2.0
0.020	0.7	+1.3

[a] RE% = (Exp. − Theor.)/Theor. × 100%.

concentration level varied between 0.7 and 2.5% (Table 1). The method was accurate, the within-day relative errors (RE) being < 2.0%. The inter-day precision and accuracy of the method were also good, showing an RSD of 1.9% and an RE of 1.2%. The repeatability and reproducibility of the HPLC assay were good (Table 2).

The Corning CS-7-54 filter and the potassium chromate solution were used to isolate the 313 nm line from the mercury lamp (Nicoderm and Aquilera, 1983). At this wavelength the quantum yield of the ferrioxalate actinometer is constant and the intensity of light can be determined. The absorption of midazolam is low at this wavelength (Andersin, 1991), which should lead to uniform absorption of light over the whole reaction volume (Calvert and Pitts, 1966).

The intensity of the light of the high-pressure mercury lamp was measured by ferrioxalate chemical actinometry to be 1.7×10^{16} photons/s (RSD 4.1%, $n = 12$). The intensity was measured at the beginning and end of each irradiation series and decreased by not more than 10% of the initial value during the day. Between days the lamp sleeve was purified with 10% acetic acid.

According to Carstensen (1990), stability tests require only about 15% degradation, whereas for exact chemical kinetic data degradation needs to be followed through several half-lives. The secondary reactions are also at a minimum when the degradation is followed over a short time period. To allow comparison of the data obtained, the slopes of the degradation curves were calculated, from the mean concentration of midazolam remaining ($n = 4$) according to zero- and first-order kinetics. There was no difference in the r^2 values calculated: 0.89–0.99 for zero-order and 0.88–0.99 for first-order. Because the differences were extremely small in the log scale based on the small proportion of midazolam decomposed, zero-order kinetic slopes were used in the statistical comparison of the results.

Table 2
Repeatability and reproducibility of the HPLC assay ($n = 6$)

Concentration (mM)	Intra-day		Inter-day	
	RSD%	RE%	RSD%	RE%
0.05	1.6	+0.51	1.8	−0.14
0.02	0.81	+1.6	0.76	+0.82

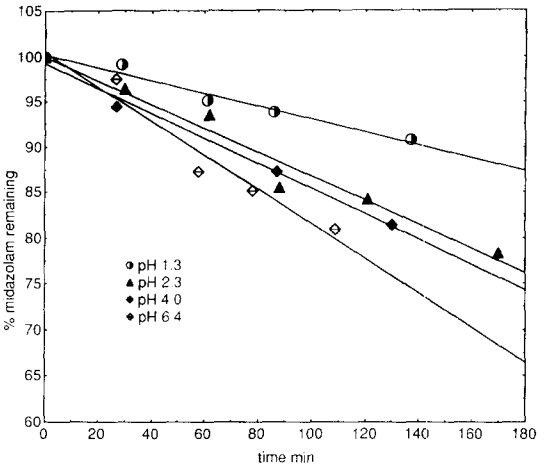

Fig. 3. Photodegradation time course of midazolam in four different citrate buffer solutions (pH 1.3, 2.3, 4.0 and 6.4). Intensity of light in wavelength region 313 nm: 1.7×10^{16} photons/s.

The photodecomposition of midazolam was studied in citrate buffers of six different pH values (Fig. 3; pH 1.3, 2.3, 4.0 and 6.4). No degradation of midazolam was observed in samples stored in the dark. The mean relative standard deviation of midazolam concentrations after a certain exposure time ($n = 4$) within each irradiation series was 2.1% (0–4.9%). The decomposition rate was significantly lower at pH 1.3 (two independent groups; t-test for the slopes, $p > 0.05$). Between solutions of pH 2.3 and 3.0 and 4.0 there was no statistical difference in the slopes, but the solution of pH 2.3 was more stable than solutions of pH 5.0 and 6.4 showing equality of slopes. The difference in the photostability of midazolam in solutions of different pH could be due to the

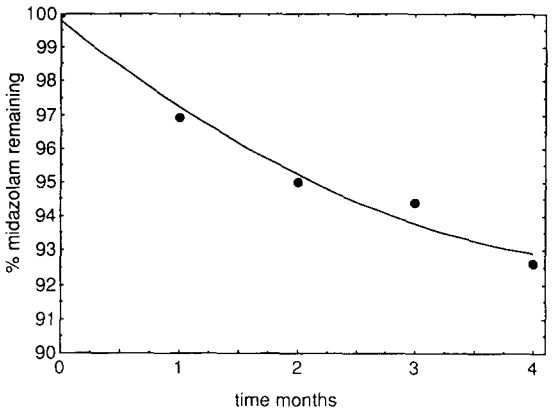

Fig. 4. Photodecomposition of midazolam in Dormicum injections in normal daylight on a southern windowsill.

formation of the open-ring form, which may stabilize the molecule against degradation. At pH 1.3, midazolam exists over 90% in the open-ring form. In the pH range 2.3–4.0 an equilibrium exists between open- and closed-ring forms; the open-ring form dominates in the more acidic solution (about 70% open-ring form at pH 2.3) and the decomposition is appreciably less than in the solutions of pH 3.0 and 4.0, where only a small proportion of midazolam is in the open-ring form. At near neutral pH values, midazolam is totally in the closed-ring form, and the decomposition is considerably faster.

The photostability of midazolam in normal daylight in commercial Dormicum solutions is shown in Fig. 4. During a 4 month period the concentration of midazolam decreased to about 92% of the initial value. Formation of yellow colour in the solutions was evident on visual inspection even after 1 month exposure. No degradation of midazolam was observed in Dormicum solutions stored in the dark for 4 months.

4. Conclusions

Aqueous midazolam solutions photodegrade both under irradiation from a high-pressure mercury lamp and in daylight. To prevent photodegradation of the drug, midazolam solutions should be stored in the dark. The pH of the solutions should be as low as possible, depending on the intended use, to minimize the rate of photodegradation, which increases with the pH.

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