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ESTIMATION OF MIDAZOLAM IN PARENTERALS BY RP – HPLC METHOD

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ABSTRACT

A rapid and reproducible reverse phase high performance liquid chromatographic method has been developed for the estimation of midazolam in its pure from as well as in parenteral dosage forms. Chromatography was carried out on an ODS column using a mixture of acetonitrile, methanol and water (40 : 20 : 40 v/v) as the mobile phase at a flow rate of 1.0 mL/min. Naphthalene was used as an internal standard and the detection was done at 254 nm. The retention time of the drug was found to be 7.84 min. The method produced linear responses in the concentration range of 0.1 to 16 µg/mL of midazolam. The method was found to be reproducible for analysis of the drug in injections.

Key words: Midazolam, Estimation, Injections, HPLC.

INTRODUCTION

Midazolam, 8-chloro-6-(2-fluorophenyl)-1-methyl-4H–imidazo [1, 5-a] [1, 4] benzo-diazepine is a short acting hypnotic- sedative drug with anxiolytic and amnestic properties. It interacts with γ – amino butyric acid (GABA) benzodiazepine receptor in the central nervous system, which is responsible for its pharmacological properties[1, 2]. A literature survey revealed that only few HPLC methods are available for the estimation of midazolam[3-5]. An attempt has been made to develop a rapid sensitive and validated HPLC method for the estimation of midazolam. The applicability of this method in determining the drug in commercial dosage forms was also studied.

EXPERIMENTAL

Chromatographic conditions

A Shimadzu LC 2010 CHT high performance liquid chromatographic instrument

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provided with a C18 Kromacil column (4.6 mm I.D x 25 cm) , a 20 µL Hamilton syringe, LC 2010 CHT VP series HPLC pumps , SPD 10A VP UV-Visible detector and Shimadzu CLASS-VP Version 6.12 SPl software was employed in the study.

HPLC grade acetonitrile (Qualigens), methanol (AR Grade, Qualigens) and water for HPLC (E.Merck India Ltd) were used for preparing the mobile phase. A freshly prepared 40 : 20 : 40 v/v mixture of acetonitrile, methanol and water was used as the mobile phase. The solvents and water were filtered through a 0.45µ membrane filter and sonicated before use. The flow rate of mobile phase was maintained at 1 mL/min. The column temperature was maintained at 25^0 C. The detection was carried out at 254 nm.

Drug and internal standard solutions

A pure sample of midazolam (M/s Gland Pharma Limited, Hyderabad) was used as the reference standard in this study. About 100 mg of midazolam was weighed accurately and transferred into a 100 mL volumetric flask and dissolved in 50 mL of the mobile phase. The solution was sonicated for 20 min and then the volume made up with a further quantity of the mobile phase to get a 1 mg/mL solution. Subsequent dilutions of this solution ranging from 0.1 – 32 µg/mL were made in 10 mL volumetric flasks after addition of 1.0 mL of naphthalene solution (200 µg/mL) as an internal standard to each dilution. Twenty microlitres of the dilutions was injected each time into the column at a flow rate of 1 mL/min. Each dilution was injected 5 times into the column and the corresponding chromatograms were obtained. From these chromatograms, the ratio of the area under the peak of the drug to that of the internal standard for each dilution was calculated. The regression of the drug concentrations over the ratios was computed. The regression equation obtained was used to estimate the amount of midazolam in pharmaceutical dosage forms.

Solutions containing 4 to 12 µg/mL of midazolam were subjected to the proposed HPLC analysis to check the inter – day and intra – day variation of the method by adding known amounts of midazolam to the pre – analyzed samples and then analyzing them by the proposed method.

Estimation of midazolam in injections

The commercial samples of injections containing the drug (Midzee of Gland Pharma and Shortal of Themis) were chosen for testing the suitability of the proposed method to estimate midazolam in the injections. For this, twenty injections were taken and the contents were pooled up. An accurately measured portion of this liquid equivalent to 100 mg of midazolan was transferred to a 100 mL volumetric flask and mixed with 50 mL of the mobile phase. The contents of the flask were allowed to stand for 6 hrs with intermittent

sonication to ensure complete solubility of the drug and then filtered through 0.45 µ membrane filter. From the filtrate, different aliquots were taken in separate 10 mL volumetric flasks. These solutions were spiked with suitable volume of the internal standard solution, such that the concentration of the internal standard in each solution was 200 µg/mL. The contents of the flasks were made up to the volume with mobile phase and mixed well. Twenty µL of each of these solutions was then injected 5 times into the column. The mean peak area ratios of the drug to the internal standard of 5 such determinations were calculated and the drug content in the injections was quantified using the regression equation obtained for the pure sample.

RESULTS AND DISCUSSION

The present study was carried out to develop a sensitive, precise and accurate HPLC method for the analysis of midazolan in pharmaceutical dosage forms. A mixture of acetonitrile, methanol and water in 40 : 20 : 40 v/v proportion was proved to be the most suitable of all combinations since the chromatographic peaks were better defined and resolved and almost free from tailing. Though the structure of naphthalene is not similar to midazolam, it was chosen as the internal standard because it showed better peak shape and peak location compared to other potential internal standards under the above mentioned chromatographic conditions. The retention times obtained for midazolam and the internal standard were 7.84 and 14.03, respectively. A model chromatogram is shown in Fig. 1.

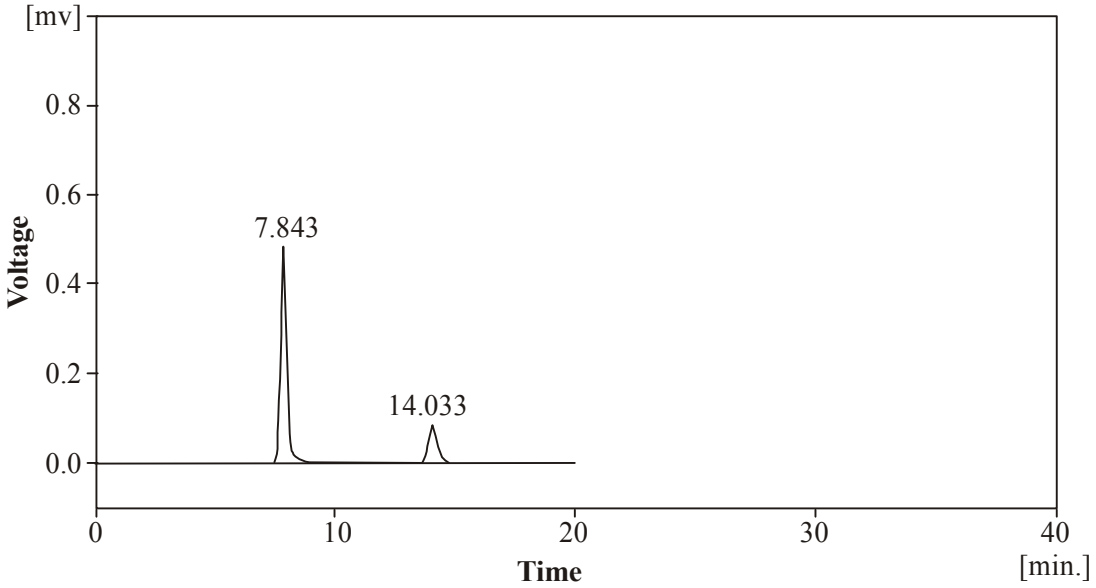

Fig 1: Typical chromatogram for the estimation of midazolam

Each of the samples was injected 5 times and the same retention times were observed in all cases. The ratios of peak area of midazolam to peak area of internal standard for different concentrations set up as above were calculated and the average value for 5 such determinations are shown in Table 1. The peak areas of both; the drug and internal standard were reproducible as indicated by low coefficient of variation. A good linear relationship (r=0.999) was observed between the concentration of midazolam and the respective ratios of peak areas in the concentration range of 0.1 to 16 µg/mL of the drug. The regression curve was constructed by linear regression fitting and its mathematical expression was y = 8902 x + 2046 (where y is the ratios of peak areas of the drug to that of internal standard and x is the concentration of midazolam). When midazolam solutions containing 4 to 12 µg/mL were analyzed by the proposed method for finding out intra – and inter – day variations, a low coefficient of variation was observed (Table 2). This shows that the present HPLC method is highly precise. The amounts of midazolam obtained from the pre – analyzed samples containing known amounts of added drug are shown in Table 3. About 99.89% of midazolam could be recovered from the pre-analyzed samples indicating high accuracy of the proposed method.

Table 1: Calibration of the proposed method

Concentration (µg/mL)	Mean peak area ratio (n = 5)	Coefficient of variance (%)
0.1	0.81	0.650
0.5	3.88	0.165
1.0	7.656	0.063
2.0	15.044	0.454
4.0	30.056	0.868
8.0	59.678	0.789
16.0	118.718	0.830
Regression equation from 0.1-16 (µg/mL): y = 8902 x + 2046 (r = 0.999)		

The drug content in the injection was quantified using the proposed analytical method. The injections were found to contain an average of 99.38% of the labeled amount of the drug. The low coefficient of variation indicates the reproducibility of the assay of midazolam in dosage forms. It can be concluded that the proposed HPLC method is

sufficiently sensitive and reproducible for the analysis of midazolam in pharmaceutical dosage forms within a short analysis time. The method was duly validated by evaluation of the required parameters.

Table 2: Precision of proposed method

Concentration of midazolam (µg/mL)	Observed concentration (µg/mL) of midazolam			
	Intra – day		Inter – day	
	Mean (n = 5)	Coefficient of variance (%)	Mean (n = 5)	Coefficient of variance (%)
4	3.97	0.67	3.92	0.06
8	7.95	0.07	7.95	0.09
12	11.97	0.08	11.95	0.09

Table 3: Recovery data of midazolam

Amount of drug (µg) added to pure drug / formulation	Recovery from drug solution		Recovery from injection	
	Mean (± s.d.) amount (µg) found (n = 5)	Mean (± s.d.) % recovery (n = 5)	Mean (± s.d.) amount (µg) found (n = 5)	Mean ± (s. d.) % recovery (n = 5)
10	9.99 ± 0.005	99.93 ± 0.09	9.99 ± 0.047	99.88 ± 0.48
50	49.93 ± 0.09	99.86 ± 0.18	49.44 ± 0.47	98.88 ± 1.13

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