

**VALIDATION AND APPLICATION OF A MODIFIED RP-HPLC METHOD  
FOR THE QUANTIFICATION OF MIDAZOLAM IN PHARMACEUTICAL  
DOSAGE FORMS**Sapna Desai<sup>1\*</sup>, Gali Vidyasagar<sup>2</sup> and Dhruv Desai<sup>3</sup><sup>1</sup>Department of Pharmaceutical Sciences. Pioneer Pharmacy Degree College, Vadodara, Gujarat.<sup>2</sup>Faculty of Pharmaceutical Sciences, Kskv, Kachchh University, Bhuj, Kutch, Gujarat.<sup>3</sup>Sun Pharmaceutical Industries Ltd. Vadodara. Gujarat.

**ABSTRACT:** The purpose of the study was to develop a simple, sensitive and rapid RP-HPLC method for the determination of Midazolam in marketed products. Chromatographic determination was performed in a reverse phase C<sub>18</sub> column (250 mm × 3.3 mm I.D. , 5µm particle size) using a mixture of acetonitrile: methanol: 0.065 M ammonium acetate buffer (50:20:30, v/v/v), final pH adjust to 5.5 ± 0.02 with ortho phosphoric acid as mobile phase and delivered at a flow rate of 1 ml/min. The UV detection was set at 220 nm. The calibration range was from 2.0 to 30.0 µg/ml. The method was validated in term of linearity (r<sup>2</sup>>0.98, RSD= 1.958%), precision (RSD=3.757 %) and accuracy. The limit of quantification was 2 µg/ml and the limit of detection was 0.1 µg/ml. The potency of midazolam in marketed products was determined by this method with acceptable precision and reproducibility.

**Keywords:** Midazolam, marketed products, RP-HPLC, development of a method.

**INTRODUCTION**

Midazolam (marketed in English-speaking countries under brand names Dormicum,<sup>[1]</sup> Hypnovel,<sup>[2]</sup> and Versed<sup>[3]</sup>) is a short-acting drug in the benzodiazepine class that is used for treatment of acute seizures and for inducing sedation and amnesia before medical procedures. It has potent anxiolytic, amnestic, hypnotic, anticonvulsant, skeletal muscle relaxant, and sedative properties.<sup>[4,5,6]</sup> Midazolam is a chemically 8-chloro- 6-(2-fluorophenyl)- 1-methyl- 4H-imidazo[1,5-a] [1,4] benzodiazepine. Its molecular formula is C<sub>18</sub>H<sub>13</sub>ClN<sub>3</sub>F and molecular mass is 327.78. It is available in different dosage forms: injection, tablet and syrup.

Midazolam is a short-acting benzodiazepine in adults with an elimination half-life of one to four hours. Midazolam is metabolized into an active metabolite alpha1 hydroxymidazolam. However, the active metabolite of midazolam is minor and contributes to only 10 percent of biological activity of midazolam. Midazolam is poorly absorbed orally with only 50 percent of the drug reaching the bloodstream. Midazolam is metabolised by cytochrome P450 (CYP) enzymes and by glucuronide conjugation. The therapeutic as well as adverse effects of midazolam are due to its effects on the GABA<sub>A</sub> receptors; midazolam does not activate GABA<sub>A</sub> receptors directly but, as with other benzodiazepines, it enhances the effect of the neurotransmitter GABA on the GABA<sub>A</sub> receptors resulting in neural inhibition.<sup>[7-9]</sup>

Literature survey reveals that HPLC, stability indicating HPLC and HPTLC methods are reported<sup>[10-17]</sup>. No one describes the comparison method (potency) between different pharmaceutical dosage form (e.g. tablet, syrup and injection). The present paper describes the Validation and application of a modified RP-HPLC method for the quantification of Midazolam in pharmaceutical dosage forms.

## Experimental

### Imstruments

The chromatography was performed on a Shimadzu (Columbia, MD) RP-HPLC instrument (LC-2010CHT) equipped with PDA detector and LC-solution software, Phenomenex (Torrance, CA) C18 column (250×4.6 mm id, 5 µm particle size) was used as stationary phase. Sartorius CP224S analytical balance (Gottingen, Germany) and ultrasonic cleaner (Frontline FS 4, Mumbai, India) were used during the research work.

### Reagents and materials

Standard sample of Midazolam was obtained from Sun Pharmaceutical Pvt Ltd, (Vadodara, Gujarat). Tablet, Syrup and injection formulation containing Midazolam was procured from local pharmacy. Triple distilled water, methanol, acetonitrile (S. D. Fine Chemicals) were used of HPLC grade.

### Preparation of mobile phase and chromatographic condition

RP-HPLC analysis was performed by gradient elution with a flow rate of 1 ml/min at ambient temperature. The mobile phase was prepared with 0.065 M ammonium acetate buffer, adjusted to pH  $5.5 \pm 0.02$  with orthophosphoric acid: methanol: acetonitrile (30:20:50, v/v/v). The mobile phase was filtered through 0.45 µm filter tips and was degassed. The  $\lambda_{\text{max}}$  for UV detection was set at 220 nm. AUFS (absorbance unit full scale) was kept at 0.002. The injection volume was 10 µl for all standards and samples. Before every injection every sample was filtered through 0.45 µm filter tips.

### Preparation of the stock and the standard solutions

10 mg of Midazolam was accurately weighed and transferred to a 25 ml volumetric flask. 10 ml of mobile phase was added and sonicated to dissolve. The working standard solutions of various concentrations of 2, 4, 8, 12, 16, 20, 24, 30µg/ml were prepared by dilution of the stock solution with mobile phase.

### Assay of the tablet, syrup and injection products.

An average of ten tablets were accurately weighed and made into fine powder in a mortar with pastel. Accurately weighed powder sample equivalent to average weight of each tablet (7.5 mg) was taken in a 25 ml volumetric flask. 10 ml of mobile phase was added and sonicated to mix uniformly. The final volume was made by mobile phase and filtered through 0.45µm filter. The solution was diluted 10 times and 10 µl (filtered through 0.45 µ filter tips) of this solution was directly injected into the HPLC injector port. The average content of the tablets was determined using the calibration curve. 2.0 mg/2ml of Midazolam syrup, 2.0mg/2ml injection was transferred into a 100 ml volumetric flask. 60 ml of mobile phase was added and sonicated to dissolve. The final volume was made by mobile phase. The preparation was filtered through 0.45 µ filter tips before injection.

### Preparation of standard curve for tablet

Midazolam, 10 mg (standard, supplied by Sun Pharmaceutical Ltd) was dissolved in 25 ml mobile phase. The concentration of the solution was 400 $\mu$ g/ml. From this parent solution, solutions of various concentrations such as 2, 4, 8, 12, 16, 20, 24 and 30  $\mu$ g/ml were prepared using mobile phase acetonitrile: methanol: 0.065 M ammonium acetate buffer (50:20:30, v/v/v), final pH adjust to  $5.5 \pm 0.02$  with ortho phosphoric acid as mobile phase. 10  $\mu$ l of each of the solutions was injected into the HPLC system and the run time was 15 minutes for each injection. Before injecting the drug solution, a blank was also injected. The Area under the Curve (AUC) was plotted against concentration to get the standard curve.

### Preparation of standard curve for syrup and injection

30 mg of midazolam was weighed and transferred into a 100 ml volumetric flask. 60 ml of mobile phase was added and sonicated to dissolve. The volume was made 100 ml by mobile phase and mixed. Then it was filtered through 0.45 $\mu$  filter tips. Solutions of various concentrations such as 2, 4, 6, 8 and 10  $\mu$ g/ml were prepared using mobile phase. 10  $\mu$ l of each of the solutions was injected into the injector port and the run time was 15 minutes for each injection. Before injecting the samples, blank was also injected. The Area under the Curve (AUC) was plotted against concentration to get the standard curve.

## RESULTS AND DISCUSSION

### Linearity

Table 1 presents the equation of the regression line, correlation coefficient ( $r^2$ ), relative standard deviation (RSD %) values of the slopes. Excellent linearity was obtained for the compound between 2-30  $\mu$ g/ml with  $r^2$  values of 0.9820, 0.9984, 0.9990 and 0.9996.

**Table 1: Linearity of the method**

Compound	$\lambda_{max}$	Equation	$r^2$	Slope (RSD %)
	220	$Y = 24939 X + 4290.4$	0.9820	
Midazolam	220	$Y = 25338 X + 2816.9$	0.9984	1.958
	220	$Y = 26027 X + 4443.6$	0.9990	
	220	$Y = 25014 X + 4071.2$	0.9998	

X = concentration ( $\mu$ g/ml); Y = Area

RSD% = (standard deviation / mean) x 100

### Precision

The precision of the method (within-day variation of replicate determination) was checked by injecting midazolam for 10 times. The precision of the method, expressed as the RSD % is given in Table 2.

**Table 2: The Precision of the developed method**

Compound	$\lambda$ max	Peak Area (Mean $\pm$ SD) (n =10)	RSD %
Midazolam	220 nm	503490 $\pm$ 3015.5	3.757

**Reproducibility**

A standard working solution-containing Midazolam, producing final concentration of 8, 16 and 24  $\mu\text{g/ml}$  was prepared. The prepared mixture of standard solution was injected 10 times as a test sample. From the respective area counts, the concentration of the midazolam was calculated using the detector response Table 3.

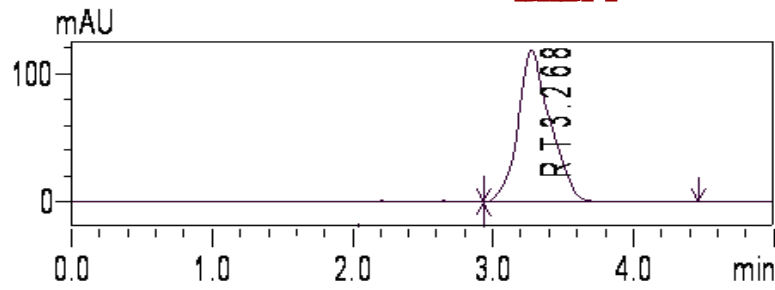
**Table 3: The reproducibility of the method**

Compound	Standard concentration $\mu\text{g/ml}$	Measured concentration $\mu\text{g/ml}$ , (n =10) (Mean $\pm$ SD)	RSD %	Deviation %
Midazolam	8	8.012 $\pm$ 0.0223	0.278	0.15
	16	15.984 $\pm$ 0.0145	0.090	0.10
	24	23.856 $\pm$ 0.0489	0.198	0.60

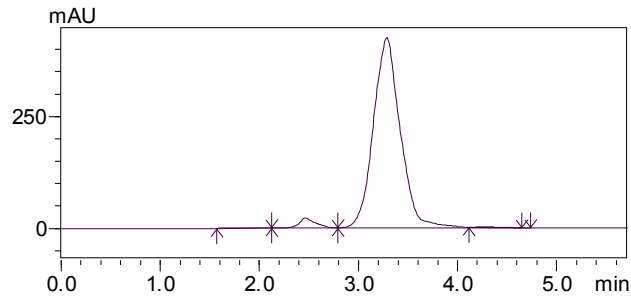
The potency was determined for tablets, injection and syrup of marketed preparations (Table 4). The potencies were found 101.45%, 99.15% and 99.17% for tablets, injection and syrup respectively figure-1 .

**Table 4: Determination of drug content present in tablet, injection and syrup (n = 10)**

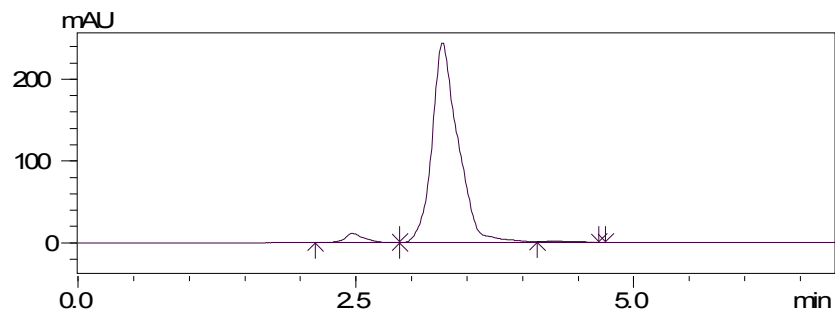
Dosage forms	AUC (Mean $\pm$ SD)	Equation	Amount (mg) per 7.5 mg tablet, injection 5 ml (2 mg/2ml) or 118 ml (2mg/2ml) syrup	Potency (%)
Tablets	501464 $\pm$ 9176.832	Y= 24939 X + 4290.4	7.492 $\pm$ 0.0495	99.89 $\pm$ 0.23 %,
Injection	652311 $\pm$ 56892.3	Y= 28932 X + 1198.1	1.985 $\pm$ 0.0214	99.25 $\pm$ 0.51%
Syrup	162915.5 $\pm$ 1300.369	Y = 25338 X+ 2816.9	1.998 $\pm$ 0.0141	99.90 $\pm$ 0.728 %



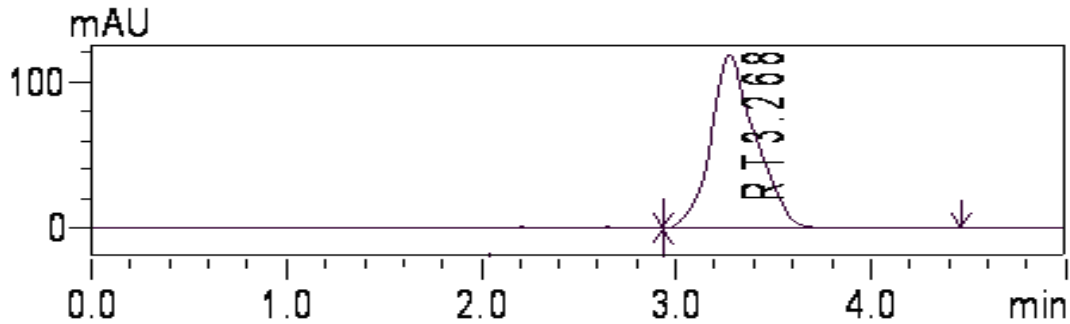
(I)



(II)



(III)



(IV)

**Figure 1:** Different chromatograms of Midazolam in standard solution (I), tablet (II), injection (III) and syrup (IV) with their retention time of 3.268, 3.215, 3.171 and 3.268 minutes respectively.

## Conclusion

The RP-HPLC method for the determination of midazolam is validated in this study has acceptable correlation coefficient, RSD (%) and deviation which makes it versatile and valuable in many applications, especially in pharmaceutical dosage form and drug concentration monitoring. The method can also be readily adapted for routine quality control analysis.

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