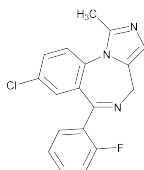


## Midazolam



$C_{18}H_{13}ClFN_3$  325.77  
 4-*H*-Imidazo[1,5-*a*][1,4]benzodiazepine, 8-chloro-6-(2-fluorophenyl)-1-methyl.  
 8-Chloro-6-(*o*-fluorophenyl)-1-methyl-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine [59467-70-8].

» Midazolam contains not less than 98.5 percent and not more than 101.5 percent of  $C_{18}H_{13}ClFN_3$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—*USP Midazolam RS*.

### Identification—

**A:** *Infrared Absorption* (197K).

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Loss on drying** (731)—Dry it at 105° for 2 hours; it loses not more than 0.5% of its weight.

**Residue on ignition** (281): not more than 0.1%.

### Change to read:

### Chromatographic purity—

*Ammonium acetate buffer* and *Mobile phase*—Prepare as directed in the *Assay*.

*Standard solution*—Prepare as directed for *Standard preparation* in the *Assay*.

*Sensitivity check solution*—Dilute the *Standard solution*, quantitatively and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.2 µg per mL.

*Test solution*—Dissolve an accurately weighed quantity of Midazolam in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a concentration of about 0.2 mg per mL.

*Chromatographic system* (see *Chromatography* (621))—Proceed as directed in the *Assay*, except to inject the *Standard solution* instead of the *Standard preparation*. In addition, chromatograph the *Sensitivity check solution* and the *Standard solution*, and record the peak responses as directed for *Procedure*: the ratio of the area of the midazolam peak of the *Standard solution* to the area of the midazolam peak of the *Sensitivity check solution* should be within 160 to 240.

*Procedure*—Inject a volume (about 25 µL) of the *Test solution* into the chromatograph, record the chromatogram, and measure the peak responses. Calculate the percentage of each impurity in the portion of Midazolam taken by the formula:

$$100(r_i / F) / [(\sum r_i / F) + r_T]$$

in which *F* is the relative response factor for each impurity, as shown in *Table 1*; *r<sub>i</sub>* is the peak response for each individual impurity in the *Test solution*; and *r<sub>T</sub>* is the peak response for midazolam in the *Test solution*: the impurities meet the requirements specified in *Table 1*.<sup>•</sup> (RB 1-Dec-2009)

### Assay—

*Ammonium acetate buffer*—Dissolve 7.7 g of ammonium acetate in water, dilute with water to 1000 mL, and mix. Adjust with glacial acetic acid to a pH of 5.5 ± 0.1.

**Table 1**

Compound	Relative Retention Time	Relative Response Factor ( <i>F</i> )	Acceptance Criteria, NMT (%)
Reduced midazolam <sup>1</sup>	0.20	1.0	0.1
Reduced reduced midazolam <sup>2</sup>	0.24	1.0	0.1
Amino compound <sup>3</sup>	0.25	0.5	0.1
Oxide midazolam <sup>4</sup>	0.46	1.3	0.1
Nitromethylene compound <sup>5</sup>	0.76	1.0	0.1
Dihydromidazolam <sup>6</sup>	0.83	0.5	0.1
Midazolam	1.0	—	—
Desfluorimidazolam <sup>7</sup>	1.14	1.0	•0.2 <sup>•</sup> (RB 1-Dec-2009)
6 <i>H</i> -isomer <sup>8</sup>	2.48	0.7	0.1
Unknown impurity	—	1.0	0.1
Total impurities	—	—	0.5

<sup>1</sup> 8-Chloro-3a,4-dihydro-6-(2-fluorophenyl)-1-methyl-3*H*-imidazo[1,5-*a*][1,4]-benzodiazepine.

<sup>2</sup> 8-Chloro-6-(2-fluorophenyl)-3a,4,5,6-tetrahydro-1-methyl-3*H*-imidazo[1,5-*a*][1,4]-benzodiazepine.

<sup>3</sup> 2-Aminomethyl-7-chloro-2,3-dihydro-5-(2-fluorophenyl)-1*H*-1,4-benzodiazepine.

<sup>4</sup> 8-Chloro-6-(2-fluorophenyl)-1-methyl-4*H*-imidazo[1,5-*a*][1,4]-benzodiazepine-5-oxide.

<sup>5</sup> 7-Chloro-1,3-dihydro-2-nitromethylene-5-(2-fluorophenyl)-2*H*-1,4-benzodiazepine-4-oxide.

<sup>6</sup> 8-Chloro-6-(2-fluorophenyl)-5,6-dihydro-1-methyl-4*H*-imidazo[1,5-*a*][1,4]-benzodiazepine.

<sup>7</sup> 8-Chloro-6-phenyl-1-methyl-4*H*-imidazo-[1,5-*a*][1,4]-benzodiazepine.

<sup>8</sup> 8-Chloro-6-(2-fluorophenyl)-1-methyl-6*H*-imidazo[1,5-*a*][1,4]-benzodiazepine.

## 2 Midazolam

*Mobile phase*—Prepare a filtered and degassed mixture of *Ammonium acetate buffer* and acetonitrile (2 : 1). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard preparation*—Dissolve an accurately weighed quantity of USP Midazolam RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.04 mg per mL.

*Assay preparation*—Transfer about 20 mg of Midazolam, accurately weighed, to a 100-mL volumetric flask. Dissolve in and dilute with *Mobile phase* to volume. Dilute quantitatively with *Mobile phase* to obtain a solution having a concentration of about 0.04 mg per mL.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains 5-μm packing L60. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Proce-*

*dure*: the column efficiency is not less than 10,000 theoretical plates; the tailing factor for midazolam is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 25 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the midazolam peaks. Calculate the percentage of C<sub>18</sub>H<sub>13</sub>ClFN<sub>3</sub> in the portion of Midazolam taken by the formula:

$$100(C_S / C_U)(r_U / r_S)$$

in which  $C_S$  and  $C_U$  are the concentrations, in mg per mL, of midazolam in the *Standard preparation* and the *Assay preparation*, respectively; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.