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Note

Determination of midazolam in serum by gas chromatography with a nitrogen-selective detector

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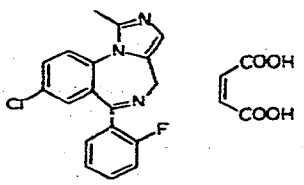
Midazolam maleate (I) is an investigational benzodiazepine which is similar in its clinical effect to diazepam. Because of its shorter half-life and water solubility, midazolam is especially suited for induction of anesthesia [1—3].

Using gas chromatography (GC) with an electron-capture detector, a procedure has been reported for the direct determination of midazolam in blood [4]. We report here a sensitive gas chromatographic procedure using a nitrogen-selective detector for the determination of midazolam in serum. Gas chromatography—mass spectrometry (GC—MS) was used to confirm the presence of the drug and the specificity of the procedure. Interferences and the use of different internal standards was also investigated.



(I)
Midazolam maleate

MATERIALS AND METHODS

Apparatus

We used a Hewlett-Packard Model 5840A gas chromatograph with dual nitrogen—phosphorus detectors. Coiled glass columns (1.2 m × 2 mm I.D.)

packed with 2% SP-2250 and 2% OV-101 on Chromosorb W HP (100—120 mesh) (Hewlett-Packard, Avondale, PA, U.S.A.) were used.

GC—MS analyses were performed on a Model 5985A quadrupole system (Hewlett-Packard, Palo Alto, CA, U.S.A.) used in the electron impact mode. The system consisted of a Hewlett-Packard 5840A gas chromatograph interfaced to the mass spectrometer. The glass column was 1.2 m × 2 mm I.D. packed with 2% SP-2250 Chromosorb W HP (100—120 mesh). The ionization energy was 70 eV. For selected ion monitoring (SIM) we used the gas chromatograph—mass spectrometer in the electron impact mode, with the electron multiplier voltage set at 3000 V and the dwell time for each ion being monitored was 100 msec.

The column temperature was 260°C for GC and 250°C for GC—MS. Helium was used as carrier gas with a flow-rate of 40 ml/min.

Reagents

All reagents were analytical reagent (AR) or spectral grade: *n*-heptane, isobutanol. Absolute ethanol was used. Anesthetic-grade diethyl ether (J.T. Baker, Phillipsburg, NJ, U.S.A.) was used. Sodium hydroxide, 0.5 and 4.4 mol/l; sulfuric acid, 1 mol/l; *n*-heptane—isobutanol (96:4, v/v); sodium sulfate, anhydrous.

Standards

Midazolam maleate and flurazepam dihydrochloride were obtained from Hoffmann-LaRoche (Nutley, NJ, U.S.A.).

Flurazepam, 1 mg/ml (internal standard). Dissolve 29.6 mg of flurazepam dihydrochloride in 25 ml of ethanol. Prepare a 20 μg/ml solution from the above.

Midazolam, 1 mg/ml. Dissolve 33.9 mg of midazolam maleate in 25 ml of ethanol. Prepare an intermediate solution of 0.1 mg/ml and aqueous standards from 0.1 to 5 μg/ml. Reconstitute lyophilized normal human serum to volume with the aqueous 0.1 to 5 μg/ml standards.

Procedure

A modification of our previously reported drug isolation procedure was used [5]. To 2 ml of serum containing midazolam, 0.3 ml of flurazepam internal standard (20 μg/ml) was added, and extracted at basic pH (4 ml of 0.5 mol/l sodium hydroxide) into 25 ml of *n*-heptane—isobutanol (96:4, v/v). The drugs were back-extracted into 4 ml of 1 mol/l sulfuric acid. The solution was made basic with 2.5 ml of 4.4 mol/l sodium hydroxide and back-extracted into 10 ml of diethyl ether. To the diethyl ether layer 1 g of anhydrous sodium sulfate was added. The mixture was shaken, filtered and then evaporated to dryness. The residue was dissolved in 25 μl of absolute ethanol and 1 μl was injected for analysis. The peak height ratio of midazolam to flurazepam (internal standard) was calculated for each sample, and then the concentration of the unknown was calculated using the closest standard. For GC—MS the ratios of the area counts at *m/e* 310/183, or 310/58 were used to calculate the midazolam concentration.

RESULTS

Chromatograms for a serum blank, standards, and patients' samples are given in Fig. 1. GC—MS of serum extracts confirmed the presence of midazolam *m/e* 310, 325, 312, 311 and the internal standard, flurazepam, *m/e* 86, 87, 58, 183. At 260°C the retention times for midazolam and flurazepam were 1.60 and 2.10 min respectively.

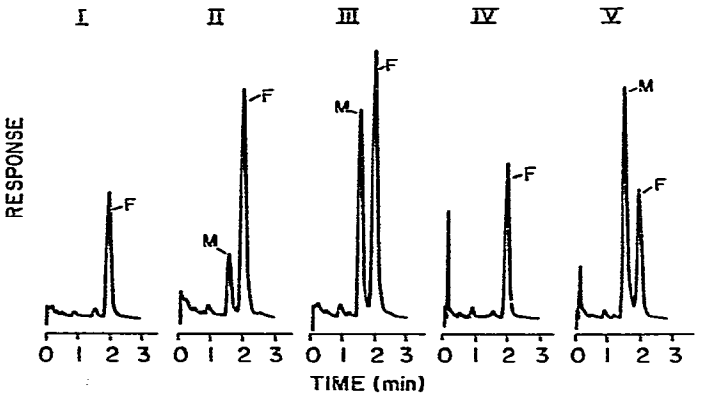


Fig. 1. Chromatograms of a serum blank, I; serum standards, II, III, and patient samples, IV, V, taken through the extraction procedure. Midazolam: II, 1 μg/ml and III, 3 μg/ml; patient serum: IV, prior to induction and V, with midazolam after induction. F = Flurazepam (internal standard) 2.6 μg/ml, M = midazolam. Conditions as in Procedure.

When the peak height value of sera containing 0.5, 1.0 and 3.0 μg of midazolam per ml, determined in duplicate, were plotted against concentration the resulting line had a slope of 1.54, a *y*-intercept of —0.27, a standard error of estimate of *Syx* of 0.07 and a correlation coefficient of 0.99. Sera containing 1, 3 and 5 μg/ml of midazolam determined on separate days ($n = 4$) gave a slope of 0.28, *y*-intercept of —0.03, *Syx* = 0.09 and a correlation coefficient of 0.98. The absolute recovery of sera containing 0.5, 1, 3, and 5 μg of midazolam per ml was 82 ± 7 ($n = 2$), 63 ± 7 ($n = 5$), 68 ± 6 ($n = 6$), and 70 ± 10% ($n = 5$) respectively. The absolute recovery of sera extracts in the 0.1 to 0.8 μg/ml range averaged 55 ± 9%. The relative within-run percent recovery using serum standards of 0.5, 1, 3, and 5 μg of midazolam per ml was 96 ± 20 ($n = 4$), 94 ± 8 ($n = 7$), 104 ± 16 ($n = 9$), and 106 ± 6% ($n = 5$) respectively. The within-run precision of sera standards containing 3 and 5 μg of midazolam per ml was 6% ($n = 7$ in each). The between-run precision at the 3 and 5 μg/ml concentration was 6 and 7% ($n = 5$ in each).

Prazepam and *p*-chlorodisopyramide were not well enough separated from midazolam, using either an SP-2250 or OV-101 column, to be used as internal standards. Although nitrazepam is well separated from midazolam, it did not chromatograph well. Flurazepam was therefore chosen as the internal standard since it is well separated from midazolam and shows good detector sensitivity. Since flurazepam is a commonly used benzodiazepine, patient serum blanks were run to verify the absence of flurazepam (Fig. 2).

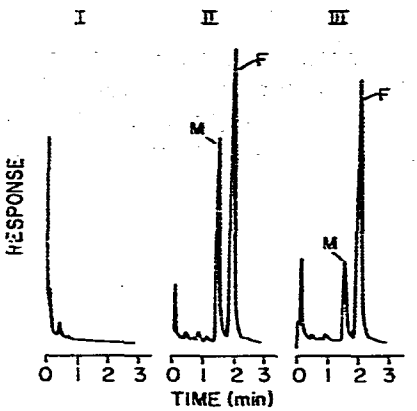


Fig. 2. Chromatograms of a patient's serum extracts before induction (I) and at 1—2 min (II) and 4—5 min (III) after induction. Midazolam concentration: (II) 1.8 and (III) 0.81 μg/ml. F = Flurazepam 2.6 μg/ml, M = midazolam.

Serum concentrations of six patients who were induced for anesthesia with midazolam prior to open heart surgery are given in Table I. Blood samples were collected at 0, 1—2, 4—5, and 13—14 min after induction and analyzed by this procedure.

TABLE I

PATIENT MIDAZOLAM CONCENTRATIONS

Total dose (0.2 mg/kg) infused within 15 sec and samples collected at specified times using opposite arm.

Patient	Dose (mg)	Midazolam concentration (μg/ml) Time (min)		
		1—2	4—5	13—14
1	16	4.6	0.81	0.56
2	15.3	1.2	0.52	0.28*
3	19.6	1.2	0.28	—
		3.0	3.74	3.20**
4	20.8	1.8	0.81	0.31
5	12.4	0.66	0.44	—
6	14	1.1	0.81	0.3

*GC—MS analysis gave 1.3, 0.66 and 0.30 μg/ml, respectively.
**Patient was also given diazepam. Serum diazepam concentrations are as indicated.

DISCUSSION

Prazepam interference can be eliminated by first extracting at pH 7.4 and then following this procedure. Prazepam as well as diazepam will remain in the organic phase while midazolam and flurazepam are extracted into the

sulfuric acid. Nordiazepam, which is the active metabolite of diazepam will interfere. The following most commonly prescribed benzodiazepines and metabolites will not interfere on a 2% SP-2250 column: oxazepam, chlordiazepoxide, norchlordiazepoxide, and diazepam. The tricyclic antidepressants, amitriptyline, doxepin, imipramine, their metabolites, as well as the commonly prescribed antiarrhythmics, quinidine, procainamide and disopyramide will not interfere with this procedure. In the presence of interferences, sera extracts can be analyzed by another technique such as GC—MS [6]. Using GC—MS in the SIM mode, good agreement was observed between GC and GC—MS (Table I). Masses monitored were *m/e* 310 and 325 for midazolam and *m/e* 58, 86, and 183 for flurazepam. Fig. 3 shows a SIM run of a urine standard which is 100 ng/ml in midazolam. Urine determinations were performed using the present extraction procedure followed by GC or GC—MS for analysis. Using GC—MS in the SIM mode, the detection sensitivity is 2—4 ng/ml.

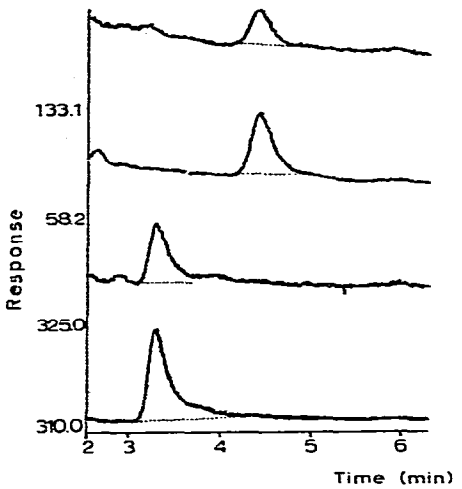


Fig. 3. GC—MS of a urine extract (SIM mode) containing 100 ng/ml of midazolam and 40 ng/ml of flurazepam as internal standard; *m/e* 310 and 325 for midazolam and 58 and 183 for flurazepam.

The results of six patients analyzed in this study (Table I) indicate that midazolam is rapidly distributed from the main compartment with the alpha phase half-life being less than 10 min. This results in low patient midazolam concentrations within a short period of time after induction. The sensitivity of the present GC procedure is less than 50 ng/ml with a relative recovery of 100% using serum standards.

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REFERENCES

1 C.R. Brown, G.H. Sarnquist, C.A. Canup and T.A. Pedley, Anesthesiology, 50 (1979) 467.
2 J.C. Reves, C. Guenter and C. Holcomb, Can. Anaesth. Soc. J., 25 (1978) 211.
3 R.J. Fragen, F. Gahl and N. Caldwell, Anesthesiology, 49 (1978) 41.
4 C.V. Puglisi, J.C. Meyer, L. D'Arconte, M.A. Brooks and J.A.F. de Silva, J. Chromatogr., 145 (1978) 81.
5 J. Vasiliades, C. Owens and D. Pirkle, Clin. Chem., 25 (1979) 311.
6 J. Vasiliades, C. Owens and F. Ragusa, Clin. Chem., 25 (1979) 1900.