

Automated Gas Chromatography for Studies of Midazolam Pharmacokinetics

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Plasma midazolam concentrations following single therapeutic doses can be quantitated using electron-capture gas-liquid chromatography. After addition of a benzodiazepine analogue as an internal standard, samples are extracted with benzene-isoamyl alcohol (98.5:1.5). The organic extract is evaporated to dryness, reconstituted, and chromatographed on an SP-2250 liquid phase. The automatic sampler allows up to 100 chromatographic analyses per 24-h period. The sensitivity limits are 2-3 ng of midazolam per ml of plasma, and the variation of identical samples is 7 per cent or less. The method was used in a study of six females who received single intravenous doses of midazolam for purposes of anesthetic induction prior to minor gynecologic procedures. Mean (\pm SE) kinetic variables for midazolam were: volume of central compartment, 0.37 (\pm 0.06) l/kg; total volume of distribution, 1.72 (\pm 0.05) l/kg; initial distribution half-life, 7.2 (\pm 1.6) min; elimination half-life, 2.5 (\pm 0.2) h; total clearance, 8.1 (\pm 0.52) ml \cdot min⁻¹ \cdot kg⁻¹. (Key words: Anesthetics, intravenous: midazolam. Hypnotics: benzodiazepines, midazolam. Measurement techniques: gas chromatography. Pharmacokinetics.)

MIDAZOLAM (fig. 1) is an imidazole benzodiazepine derivative under study as a short-acting anesthetic induction agent.¹⁻⁴ Evaluation of the pharmacokinetic properties of midazolam is important for an understanding of the time course of its clinical action. As such, there is a need for a reliable and sensitive method

for quantitation of midazolam in body fluids to facilitate pharmacokinetic studies. The present report describes an automated gas chromatographic method for analysis of midazolam in human plasma following therapeutic doses. The use of the method in a clinical pharmacokinetic study is illustrated.

Methods

INSTRUMENTATION

A Hewlett-Packard Model 5830A[®] gas chromatograph equipped with a 15-mCi ⁶³Nickel electron-capture detector, an electronic data processor-integrator, and a Model 7672A automatic sampler was used for the analysis. The column was 1.83 m in length by 2 mm internal diameter coiled glass packed with 3 per cent SP-2250 (50:50 methyl-phenyl silicone) on 80/100 Supelcoport (Packing 1-1767, Supelco, Inc.). A 95/5 mixture of argon/methane (Matheson) was the carrier (30 ml/min). Operating temperatures were: oven, 265° C; injection port, 310° C; and detector, 320° C.

REAGENTS AND STANDARDS

A 100 μ g/ml stock solution of midazolam base was prepared by dissolving 13.57 mg of midazolam maleate[¶] in 100 ml of distilled water. A working standard (0.5 μ g/ml) was prepared by dilution with distilled water. An iodinated benzodiazepine analogue[¶] (Ro 7-9749, fig. 1) served as an internal standard, of which stock and working solutions were prepared using benzene or toluene as the diluent.

Buffer was prepared by mixing 50 ml of 0.05 M NaHCO₃ with 23 ml of 0.1 M NaOH, and adjusting the pH to 11 by dropwise addition of 0.1 M NaOH.

PROCEDURE

Internal standard (25 ng) was added to a series of 16 \times 125 mm culture tubes. The solvent was evapo-

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¶ Kindly supplied by Dr. W. E. Scott, Hoffmann-LaRoche, Inc., Nutley, New Jersey.

rated to dryness at 40°C under mildly reduced pressure. Calibration standards were prepared by addition of 5, 10, 25, 50, 75, and 100 ng of midazolam together with 0.5–1.0 ml of drug-free plasma to a series of six of these tubes. To the remaining tubes, 0.1–1.0 ml of “unknown” plasma was added. One ml of pH 11 buffer and 2.5 ml of benzene (containing 1.5% per cent isoamyl alcohol) were added to each tube. The tubes were then covered with Teflon® coated screw-top caps, agitated for 30 s using a Vortex-type mixer, and centrifuged for 10 min at 400 × g. The organic phase was transferred to standard 2-ml Wheaton automatic sampling vials, and evaporated to dryness. The residue was reconstituted with 150–200 μl of toluene (containing 15 per cent isoamyl alcohol) and covered with aluminum foil. The automatic sampler was adjusted to deliver 6 μl per injection.

PHARMACOKINETIC STUDIES

Six otherwise healthy female patients, (age range, 20–47 years; body weight, 45–78 kg), participated in the study after giving informed consent. They were scheduled for elective diagnostic pelviscopy or cur-retage. A single 100-mg oral dose of phenobarbital was given the night before the procedure. Pre-anesthetic medication consisted of intramuscular promethazine (50 mg) and atropine (0.5 mg). Patients received a single rapid intravenous injection of 12.5 mg of midazolam base (as the maleate salt) for purposes of anesthetic induction. General anesthesia was then maintained with enflurane (1.0–2.5 per cent), nitrous oxide (60–70 per cent), and oxygen. The mean duration of the surgical procedure was 17 min.

Eighteen venous blood samples were drawn from the contralateral arm during the 8 hours after midazolam dosage. After centrifugation, the plasma was separated and frozen until the time of assay.

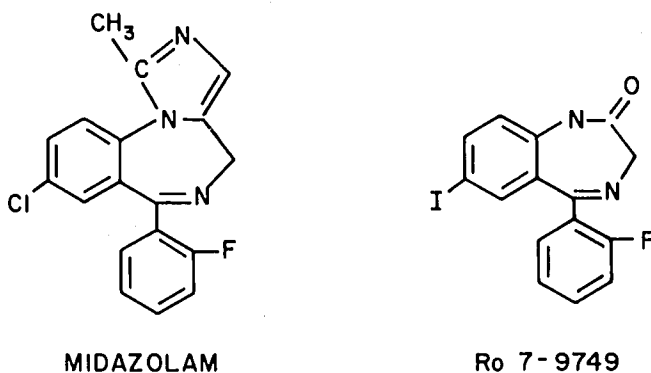


FIG. 1. Structural formula of midazolam and of Ro 7-9749, the internal standard used in the analysis.

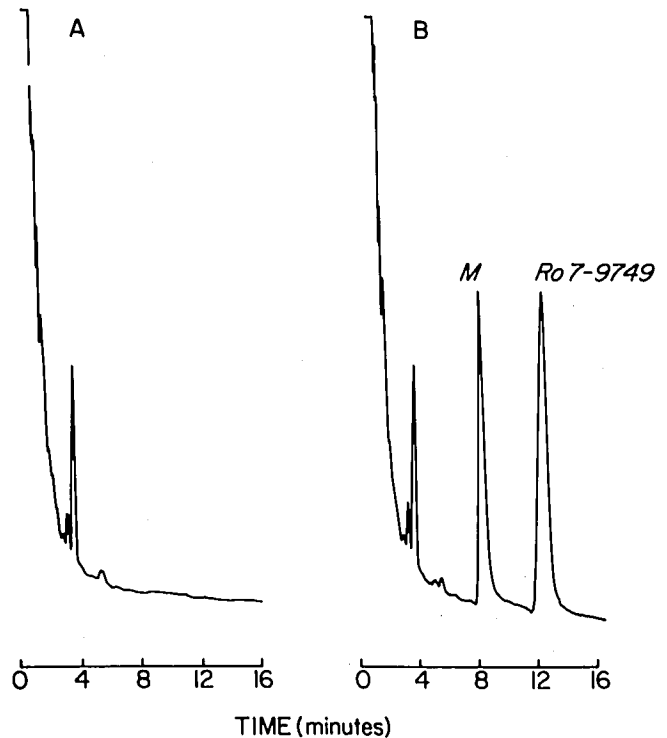


FIG. 2. A. Chromatogram of a drug-free control plasma extract. B. Chromatogram of the same plasma sample to which 50 ng/ml midazolam (M), and 25 ng/ml the internal standard, Ro 7-9749, was added.

Plasma midazolam concentrations in all samples were determined as described above.

Midazolam plasma levels were analyzed by weighted iterative nonlinear least squares regression techniques as described in detail previously.^{5,6} Data points were fitted to a linear sum of exponential terms. Coefficients and exponents from the fitted function were then used to calculate the following kinetic parameters for midazolam: volume of the central compartment (V_1), total volume of distribution using the area method (V_d), initial distribution half-life ($t_{1/2\alpha}$), elimination half-life ($t_{1/2\beta}$), and total clearance.

Results

EVALUATION OF THE METHOD

Under the described chromatographic conditions, approximate retention time for midazolam was 8.5 min and for Ro 7-9749, 12.5 min (fig. 2). The automated sampling system allows up to 100 chromatographic analyses per 24-h period.

Plasma midazolam concentration was linearly related to the peak area ratio (or peak height ratio) of midazolam to internal standard (fig. 3). Relative standard deviations for replicate samples ranged from 6–7 per cent at plasma levels of 25 ng/ml or

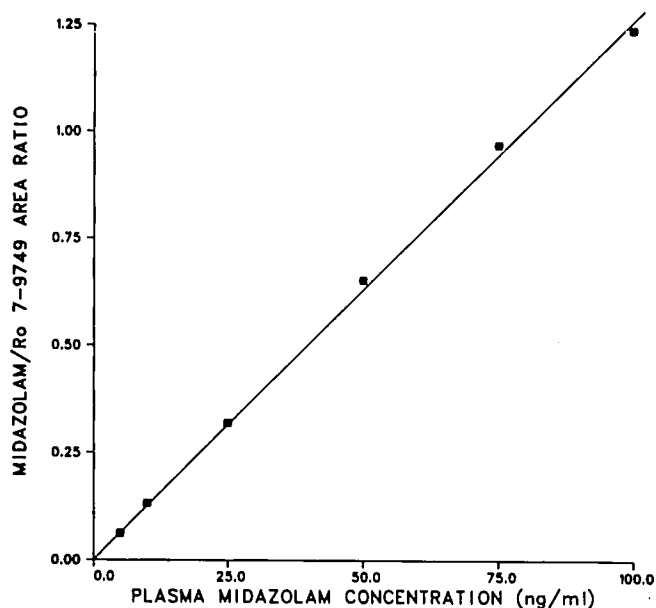


FIG. 3. Representative calibration curve showing the linear relation between plasma midazolam concentration and area ratio of midazolam to the internal standard. Solid line represents the function of best fit (passing through the origin) determined by least squares regression analysis.

below, and 3–5 per cent at levels of 50 to 100 ng/ml. The sensitivity limits of the method were approximately 2–3 ng of midazolam per ml of original sample. Recovery of midazolam and the internal standard was greater than 95 per cent complete.

Concentrations of midazolam in “unknown” samples from the pharmacokinetic study were determined using the slope of the calibration curve together with the area ratio of midazolam to that of the internal standard in each “unknown” sample.

METABOLITES AND INTERFERENCES

A chromatographic peak well-resolved from both midazolam and Ro 7-9749 (approximate retention time: 10 min) appeared in plasma extracts of patients who received midazolam. This peak corresponds to the hydroxylated metabolite of midazolam (Ro 21-6347); since it has negligible pharmacologic activity,** quantitation was not attempted. Desmethylmidazolam (Ro 21-5259), a second metabolite of midazolam, is not resolved from the parent drug using the SP-2250 liquid phase. However, reanalysis of plasma samples using a more polar liquid phase (OV-225) capable of resolving midazolam and desmethylmidazolam indicated that detectable amounts of desmethylmidazolam were not present.

**Limjuco RA (Hoffmann-LaRoche, Inc.): Personal communication, 1980.

Desmethylmidazolam, a major metabolite of diazepam and a minor metabolite of chlordiazepoxide, is incompletely resolved from midazolam and hence interferes with the assay. Desmethylmidazolam also appears in plasma following ingestion of its two precursor benzodiazepines, clorazepate, and prazepam. Other commonly administered benzodiazepines (oxazepam, lorazepam, and desalkylflurazepam formed from its precursor flurazepam) do not interfere with the assay.

PHARMACOKINETIC RESULTS

In all subjects, disappearance of midazolam from plasma was consistent with the sum of two or three exponential terms (fig. 4 and table 1). The initial distribution half-life of midazolam was rapid, with a mean value of 7.2 min (table 1). The elimination half-life ($t_{1/2\beta}$) averaged 2.5 h, with a range of 2.1–3.4 h. Midazolam distribution was reasonably extensive, with V_d values averaging 1.72 l/kg. Mean total clearance was 8.1 ml·min⁻¹·kg⁻¹, with a range of 5.8–9.0.

Discussion

The described method uses the sensitivity and selectivity of the electron-capture detector^{7–10} for quantitation of midazolam in human plasma following single therapeutic doses. Both midazolam and its internal standard are essentially completely extracted

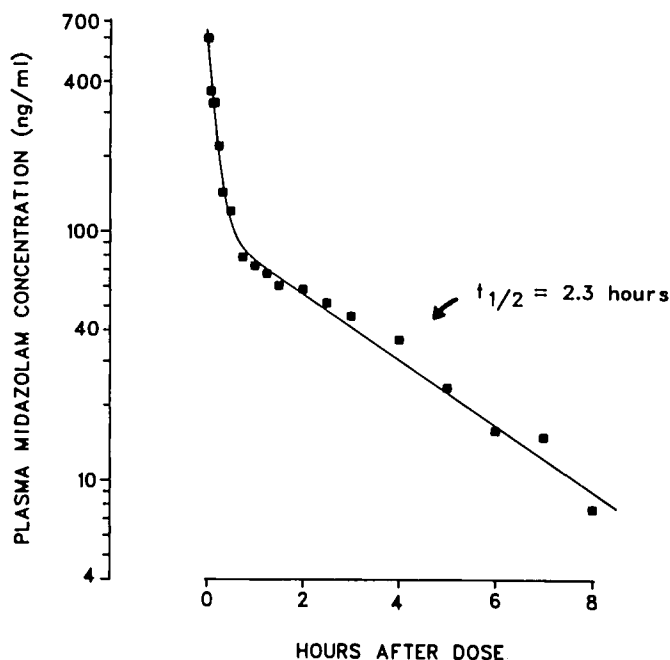


FIG. 4. Plasma midazolam concentrations following intravenous administration of midazolam to patient 905. Solid line represents the pharmacokinetic function determined by nonlinear least squares regression analysis. See table 1 for kinetic parameters.

TABLE 1. Pharmacokinetics of Midazolam

Subject	Age (yr)	Weight (kg)	Kinetic Variables for Midazolam					
			Number of Exponential Terms	$t_{1/2\alpha}$ (min)	$t_{1/2\beta}$ (h)	V_1 (l/kg)	V_d (l/kg)	Clearance (ml·min ⁻¹ ·kg ⁻¹)
901	31	72	2	10.3	2.9	0.50	1.90	7.46
902	20	52	2	12.2	2.3	0.53	1.78	8.91
903	38	78	3	4.3	3.4	0.21	1.69	5.76
904	47	63	3	1.5	2.1	0.19	1.60	8.98
905	23	55	2	6.4	2.3	0.35	1.76	8.92
906	30	45	2	8.4	2.2	0.46	1.62	8.57
Mean				7.2	2.5	0.37	1.72	8.10
SE				± 1.6	± 0.2	± 0.06	± 0.05	± 0.52

into a relatively nonpolar solvent without sample preparation or cleanup. Up to 100 samples can be extracted in a working day and chromatographed overnight using the automatic sampling system. The overall methodologic approach is similar to that described in an earlier report.¹⁰ However, we found that the internal standard described by Puglisi and associates¹⁰ did not extract from plasma replicably, and yielded coefficients of variation for identical samples that were unacceptably large (greater than 10 per cent). Use of Ro 7-9749 as internal standard eliminated these problems.

The pharmacokinetic study of midazolam illustrates the applicability of the method. Kinetic findings confirm that midazolam is a short-acting benzodiazepine derivative that is rapidly cleared from plasma. After an initial phase of distribution immediately after intravenous dosage, elimination proceeds with a half-life averaging 2.5 h in healthy individuals. Tissue distribution is reasonably extensive, with V_d values exceeding body weight. Mean total clearance (8.1 ml·min⁻¹·kg⁻¹) was considerably less than hepatic blood flow (approximately 21 ml·min⁻¹·kg⁻¹). Midazolam may therefore be effective when administered orally, since less than 50 per cent of the dose would be removed by first-pass hepatic extraction.

The kinetic profile of midazolam contrasts with that of diazepam. The elimination half-life of diazepam ranges from 20–70 h in young healthy persons, and may exceed 100 h in elderly patients.^{11,12} Furthermore, diazepam is biotransformed into an active metabolite, desmethyldiazepam, which is even more slowly eliminated. Thus, chemical persistence of diazepam and desmethyldiazepam well into the postoperative period is a certainty when diazepam is used as a premedicant or induction agent, whereas this is not the case with midazolam. Considerable further work is needed to elucidate variability between individuals in midazolam pharmacokinetics, as well as disease states and drug interactions that might alter its disposition.

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