Measurement of Nifedipine in Plasma by Gas–Liquid Chromatography and Electron-Capture Detection

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In this method for measuring nifedipine, a calcium-channel inhibitor now widely used in the treatment of cardiovascular disease, the drug is extracted from plasma under basic conditions into toluene, and an aliquot of the extract is injected directly into a gas chromatograph equipped with an OV-101 column and an electron-capture detector. The standard curve is linear between 1 and 100 μg/L; the assay measures not only nifedipine, but also a major metabolic product (found only after oral administration of the parent drug) and photodegradation products. The procedure is rapid and adequately sensitive for routine use in clinical monitoring of nifedipine in plasma.

**Additional Keyphrases:** calcium-channel antagonist • drug assay • photodegradation • monitoring therapy • heart disease • drug metabolite

Nifedipine, a 1,4-dihydropyridine derivative, recently was introduced into clinical use in the United States as an antianginal agent. By reversibly blocking the cell membrane sites through which calcium moves, the drug inhibits transmembrane calcium ion flux and is known as a “calcium-channel antagonist” (1). Its major actions in vivo derive from its affinity for vascular tissue, where it blocks excitation–contraction coupling. It has therefore been remarkably effective in control of the variant form of angina pectoris that is thought to be due to abnormal vasconstriction in the coronary arterial circulation (2). It has also been widely used to treat chronic stable angina (3).

Very little information is available concerning its pharmacokinetics or pharmacodynamics, either in normal subjects or in patients. The compound is light-sensitive, which presents difficulties in developing valid assay techniques for its measurement in plasma. Ramsch developed a fluorescent assay method (4) but this was subsequently shown to be nonspecific. Other techniques used in assay of the drug include gas chromatography (5, 6), “high-performance” liquid chromatography (7), and mass spectrometry (8). None of these methods has been adapted for convenient use in clinical monitoring of the drug in plasma.

Here we describe a simple gas-chromatographic method for measuring plasma nifedipine concentrations, and for detecting its photodegradation products and a major metabolite. This approach has been used in preliminary studies of nifedipine pharmacokinetics in normal subjects, and it can be used to monitor drug concentrations in patients after therapeutic doses.

**Materials and Methods**

**Principle.** Nifedipine and an added internal standard (diazepam) partition rapidly into toluene from plasma at an alkaline pH. An aliquot of the toluene phase may be injected directly into a gas chromatograph, equipped with a 3% OV-101 column and an electron-capture detector. The drug’s concentration in plasma is evaluated by comparing the peak-area ratio (nifedipine:internal standard) with that found for standards of known concentrations.

**Procedure.** Pure nifedipine powder (Pfizer Inc., New York,
NY 10017) dissolves readily in methanol for preparation of stock solutions, which are stable for at least one month if refrigerated (4–7 °C) and protected from light. When added to plasma, the drug is stable for at least four months if the specimen is stored frozen, protected from light. Nifedipine is quite light-sensitive and breaks down rapidly on exposure to daylight, tungsten-bulb light, or standard fluorescent light. It is, however, stable when “gold” fluorescent light is used. Therefore, all procedures described are best done in a laboratory area in which only gold fluorescent bulbs (General Electric, model no. F40G0) are used.

To separate glass centrifuge tubes, add: (a) 1.0 mL of Tris buffer (50 mmol/L, pH 9.0); (b) 0.5 mL of toluene; (c) 10 µL of internal standard (diazepam, 10 µg/mL); and (d) 1.0 mL of plasma (as a blank reference sample, standard, or unknown sample). Agitate the tubes for 50 s, then centrifuge for 10 min at 2500 rpm. Aspirate 2 to 3 µL directly from the toluene layer and inject into the gas chromatograph.

For the studies described here we used a Model 3700 gas chromatograph with a 63Ni electron-capture detector and a CDS-111 electronic integrating recorder unit (Varian Corp., Palo Alto, CA 94303). The following settings were used:

| Column: OV-101, 3% on 100/120 mesh WHP; 185-cm glass column (2 mm i.d., 6 mm o.d.) |
| Column temp: 215–220 °C |
| Injector temp: 240 °C |
| Detector temp: 280 °C |
| Carrier gas: nitrogen, flow rate = 30 mL/min |

For plasma standards containing nifedipine in concentrations ranging between 1.0 and 100 µg/L, the ratio of peak areas of nifedipine:internal standard was plotted versus known drug concentration for each sample, and the best-fit line was determined by standard linear regression analysis. The concentration of nifedipine in unknown samples was determined by reference to such a standard curve.

Results

Figure 1 shows typical chromatograms. Panel A shows the pattern for a sample of fresh blank plasma to which internal standard (IS) was added; no interfering peaks are seen. However, use of old plasma for blank or standard preparation commonly results in an interfering peak appearing between the internal standard and nifedipine peaks, so only fresh plasma should be used. Panel B shows the chromatogram for a plasma sample taken after the intravenous administration of 1.0 mg of nifedipine. Panel C shows a chromatogram of plasma taken after a 10-mg oral dose of nifedipine. The last panel shows evidence of a major metabolite (OM), which we have seen only in plasma after oral drug dosing. The structure of this metabolite is not completely established, but several possibilities have been suggested (5–8).

The assay curve is linear between 1.0 and 100 µg/L for known concentrations of nifedipine added to fresh plasma, analyzed as described (r = 0.9961, p < 0.01). The y-intercept is not significantly different from zero (0.026). Estimates of within- and between-assay precision are shown in Table 1. For within-run studies we measured nifedipine in aliquots of frozen plasma samples to which the drug had previously been added; five aliquots were evaluated at each of six different nifedipine concentrations across the assay range. Between-run evaluation was carried out by processing aliquots from the same frozen plasma samples, to which nifedipine had been earlier added, on each of eight separate days.

No interference peaks were detected for plasma to which we added digoxin, quinidine, procainamide, furosemide, or propranolol, in accepted therapeutic concentrations.

As others have emphasized (5–7), nifedipine is light-sensitive. Figure 2 illustrates the appearance of photodegradation products when nifedipine in toluene is exposed to the usual laboratory light for 45 min. The rate of photodegradation is similar for various drug concentrations, 25, 50, and 100 µg/L, with a mean first-order rate constant of 0.0149 (SD 0.001) min⁻¹. In plasma, however, this process was slower and degradation products were seen only after 1.5 to 2.0 h of exposure to ordinary fluorescent lighting.

Figure 3 shows the applicability of this assay technique to the measurement of plasma nifedipine concentrations. Data are shown for 12 normal subjects given a single 1.0-mg dose, intravenously. Analyzed by a model-independent approach, these data revealed an elimination-phase half-time of 1.77 (SD 0.25) h (range, 0.89–2.89 h) and a total clearance of 0.62 (SD 0.01) L/h per kilogram body wt (range, 0.37–1.01 L/h per kilogram body wt). Preliminary studies have shown substantial variation in the rate of appearance of nifedipine in plasma after similar oral doses to different individuals.

Discussion

This technique is rapid, simple, and appears to be adequately sensitive to provide data for both therapeutic drug monitoring and pharmacokinetic studies (Figure 3). In addition, nifedipine can be clearly separated from its metabolites and (or) photodegradation products without cumbersome derivatization procedures or time-consuming differential

| Table 1. Intra- and Inter-Assay Variation in Gas-Chromatographic Measurement of Plasma Nifedipine |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                 | Intra-assay     |                 |                 |                 |                 |                 |                 |                 |
|                 | (n = 5)         |                 |                 |                 |                 |                 |                 |                 |
| Mean            | 1.06            | 2.40            | 5.75            | 20.98           | 56.22           | 98.12           |
| SD              | 0.58            | 0.22            | 0.355           | 1.879           | 3.63            | 6.73            |
| CV, %           | 5.51            | 9.10            | 6.17            | 8.96            | 6.96            | 6.86            |
|                 | Inter-assay     |                 |                 |                 |                 |                 |                 |                 |
|                 | (n = 8)         |                 |                 |                 |                 |                 |                 |                 |
| Mean            | —               | —               | 6.12            | 22.52           | 50.75           | 99.97           |
| SD              | —               | 0.61            | 2.01            | 3.58            | 6.82            |
| CV, %           | —               | 10.00           | 8.93            | 7.05            | 6.82            |
extraction approaches. Previous assay methods have been either nonspecific (4) or insufficiently sensitive (6, 7) to support meaningful studies in human subjects.

Jacobsen et al. (5) used a similar analytical approach in preliminary studies. Our report extends and expands their findings. We report greater assay sensitivity, a spurious (and unidentified) component in old plasma which may interfere with measurement of nifedipine, and the appearance of a metabolite after oral drug administration that is not seen after intravenous administration. In a study in seven normal subjects, Ramsch (9) also used electron-capture detection to measure plasma nifedipine concentrations, but did not detail his experimental method.

Kondo et al. (10) reported that nifedipine oxidation occurred on the column of their gas chromatograph, resulting in two peaks; we have not found this in our studies, possibly because we have used lower temperatures in the injector port (240 vs 260 °C).

We confirmed that nifedipine in toluene breaks down rapidly when exposed to standard laboratory fluorescent light (Figure 2), with the reaction following first-order kinetics in the initial concentrations studied here. This observation of a first-order relationship for photodisappearance is inconsistent with a unimolecular photochemical reaction, which should be zero-order (11). This finding needs elaboration, but either differential light absorption at low nifedipine concentrations or the intervention of an as-yet-undefined biomolecular process could be the explanation.

This assay is currently used in our laboratories, both as a research tool and for monitoring drug concentrations in the plasma of patients who do not appear to respond clinically to the usual doses of nifedipine. It can easily be instituted in a clinical laboratory equipped for gas–liquid chromatography.

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References