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Technical Note

Analysis of Diltiazem and Desacetyldiltiazem in Plasma Using Modified High-Performance Liquid Chromatography: Improved Sensitivity and Reproducibility

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INTRODUCTION

Diltiazem is a commonly used calcium-channel blocking agent for the treatment of angina pectoris (1). The drug is also effective as an antiarrhythmic and antihypertensive agent (2,3). Various methods for determination of diltiazem and its major metabolite desacetyldiltiazem (DAD) using thin-layer chromatography (TLC) and gas chromatography (GC) have been published (4–6). More recently, several high-performance liquid chromatographic (HPLC) procedures have been reported (7–10). While these HPLC methods give a higher sensitivity than the TLC method and are less complicated than the GC method, they still suffer from either poor reproducibility (CV > 10%) at lower concentrations or the requirement for special conditions such as a heated column and ion-pairing reagent. We report a modified HPLC method which is simple to perform and produces a higher sensitivity and better reproducibility than those reported by others.

MATERIALS AND METHODS

The HPLC system consisted of a Model 6000A solvent delivery system (Waters Associates), a Spectro-Monitor 3000 variable-wavelength detector (LDC-Milton Roy Co.) operating at a 237-nm wavelength, and a fixed-loop injector fitted with a 20-μl loop (Model 7125, Rheodyne Inc. Cotati, Calif.). A 5 mm · 10 cm Radial-PAK reversed-phase column (CN 10μ Waters Associates) and a guard column were used.

The mobile phase consisted of a 55:45:0.25 (v/v/v) mixture of 0.06 *M* potassium dihydrogen phosphate buffer, methanol, and triethylamine (adjusted with phosphoric acid to pH 4.85). The flow rate was 1.0 ml/min with ambient column temperature. These conditions gave a retention time of 5.6 min for desacetyldiltiazem, 7.0 min for diltiazem, and 9.2 min for verapamil (as internal standard).

Stock standard solutions (500 μg of free base/ml) of diltiazem, DAD, and the internal standard, verapemil, were prepared in distilled water. Standard solutions were made by sequential dilutions to 50 and 5 μg/ml for both diltiazem and DAD and 20 and 4 μg/ml for the internal standard. All solutions were stored in total darkness at −70°C.

The extraction procedure involved first adding 1.0 or 0.5 ml (for drug concentrations higher than 250 ng/ml) of plasma sample to a 10-ml glass tube (fitted with a plastic cap) followed by 100 μl of internal standard solution. The sample was extracted with 6 ml of hexane–isopropanol (98:2, v/v) by rocking for 10 min and then centrifuged for 10 min at 2000 rpm. The organic layer was transferred to a 10-ml glass tube containing 100 μl of 0.025 *M* sulfuric acid, and the mixture was agitated on a Vortex mixer for 45 sec. The mixture was then centrifuged for 5 min and the organic supernatant was discarded. A 20-μl portion of the aqueous layer was then injected. A typical chromatogram is shown in Fig. 1.

Two standard curves were made, one covering the lower concentration range (10–250 ng/ml) and another the 200–3000 ng/ml concentration range.

RESULTS AND DISCUSSION

The maximum UV absorbance of diltiazem in the mobile phase is at 237 nm. The peak height at 254 nm is 70% of that at 237 nm under the present chromatographic condition.

We have made several modifications in the present assay, which is simple to perform but produces a high sensitivity and reproducibility. In previous diltiazem assays (7–10), either a μBondapak C18 column (10 μm, 30 cm · 3.9-mm i.d.) or a Zorbax CN column (6 μm, 25

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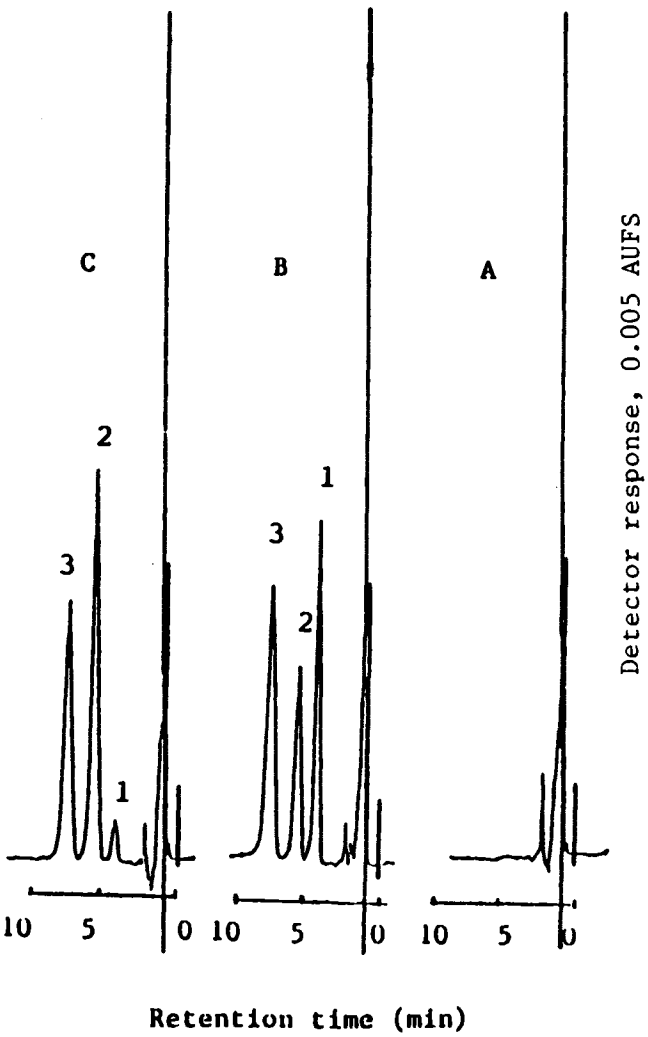

Fig. 1. Chromatograms of extracted plasma: (A) drug-free plasma; (B) spiked 1-ml sample from standard curve, 100 ng/ml of (1) DAD and (2) diltiazem, and 400 ng/ml of (3) internal standard, verapamil; (C) 1-ml sample from an anesthetized dog 40 min after 0.5 mg/kg of diltiazem HCl by 10-min infusion.

cm · 4.6-mm i.d.) was used at a flow rate of 1.5 or 1.8 ml/min, respectively. A Radial-PAK reversed-phase CN column (10 μm, 5 mm · 10 cm) was used in this assay with a flow rate of 1.0 ml/min, which gives better separation and a higher resolution.

By using hexane–isopropanol (98:2) instead of methyl-butyl ether, which a previous assay used as the extraction organic solvent (7), the extraction tube does not need to be immersed in a dry ice–acetone mixture. This makes the extraction procedure simpler without affecting the extraction recovery. Further, neutral pH extraction with acidic back-extraction greatly decreased the chromatographic interference from plasma and produced a good recovery. Altering the plasma pH was unnecessary since the addition of 0.1 ml sodium borate buffer (pH 9) did not increase drug recovery. Among four different acids for back-extraction, sulfuric acid was chosen, as it gave less interference and the best recovery from human or dog plasma. The 0.025 *M* sulfuric acid concentration for back-extraction was lower than in previous diltiazem assays (7,10), which preserves column performance while still yielding a good recovery of all three components.

The sensitivity limit of the assay for both diltiazem and DAD was 10 ng/ml for a 1-ml extracted plasma sample (at a minimum signal-to-noise ratio of 4 with a CV within 5%). The lower limit of this assay can be improved to 5 ng/ml or less by increasing the injection volume. In our present study we used only 20 μl of a 100-μl sample for injection, while in the previous assay either 50 μl of an 80-μl sample or 80 μl of a 100-μl sample was injected.

To determine the assay precision, five concentrations (10–2000 ng/ml) were each measured six times within 1 day. The results are shown in Table I. The coefficient of variation (CV) was always less than 5% for either drug.

To determine sample recovery, drug-free plasma was spiked with drug concentrations ranging from 50 to 1000 ng/ml. Compared to aqueous solutions without extraction, the average recovery for four concentrations was 98.4% for diltiazem and 96% for DAD, with a CV of 1.76 and 2.90%, respectively.

A linearity study was performed by analysis of 20 standard curves over a 1-month period. The concentration ranges were 10–250 and 200–3000 ng/ml for diltiazem and 10–200 and 150–1600 ng/ml for DAD. The correlation coefficient of each standard curve was always greater than 0.999. The day-to-day CV in the slope of the standard curves was within 5% for both diltiazem and DAD.

The specificity of this assay was evaluated in the presence of other cardiovascular drugs such as amiodarone, procainamide, theophylline, lidocaine, flecainide, disopyramide, quinidine, propafenone, caffeine, and cimetidine. None interfered with this assay except quinidine and disopyramide, which have retention times close to that of DAD.

Diltiazem is light sensitive. The aqueous standard solution, when stored in total darkness at 4°C, was stable for at least 2 months. When exposed to light, the diltiazem concentration decreased in a time-related manner and produced another peak which had the same chromatographic retention time as DAD. Therefore, sample exposure to light must be minimized.

Table I. Within-Day Reproducibility (Precision and Accuracy)

Drug	Concentration (ng/ml)	Measured (Mean ± SD)[a]	CV (%)
Diltiazem	10	10.1 ± 0.4	4.50
	25	23.8 ± 0.6	2.58
	100	97.7 ± 3.2	3.25
	500	489.0 ± 4.4	0.90
	2000	1984.0 ± 63.2	3.20
DAD	10	10.2 ± 0.4	4.00
	25	18.8 ± 0.7	3.85
	100	98.6 ± 3.9	3.96
	400	400.4 ± 3.3	0.82
	1600	1521.6 ± 55.8	3.67

[a] $N = 6$.

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REFERENCES

1. R. Zelis. *N. Engl. J. Med.* 306:926–928 (1982).
2. J. J. Rozanski, L. Zaman, and A. Castellanos. *Am. J. Cardiol.* 49:621–628 (1982).
3. K. Maeda, T. Takasugi, Y. Tsukano, Y. Tanaka, and J. Shiota. *Int. J. Clin. Pharmacol. Ther. Toxicol.* 19:47–55 (1981).
4. K. Kohno, Y. Takeuchi, A. Etoh, and K. Noda. *Arzneim.-Forsch. Drug Res.* 27:1424–1428 (1977).
5. V. Rovei, M. Mitchard, and P. L. Morselli. *J. Chromatogr.* 138:391–398 (1977).
6. J. P. Cloxel, G. Caille, Y. Taeymans, P. Theroux, P. Biron, and J. G. Besner. *J. Pharm. Sci.* 73:207–209 (1984).
7. C. Verguese, M. S. Smith, L. Anonsen, E. L. C. Pritchett, and D. G. Shand. *J. Chromatogr.* 272:149–155 (1983).
8. R. E. Wiens, D. J. Runser, J. P. Lacz, and D. C. Dimmit. *J. Pharm. Sci.* 73:688–689 (1984).
9. J. P. Clozel, G. Gaille, Y. Taeymans, P. Theroux, P. Biron, and F. Trudel. *J. Pharm. Sci.* 73:771–773 (1984).
10. D. R. Abernethy, J. B. Schwartz, and E. L. Todd. *J. Chromatogr.* 342:216–220 (1985).