*Journal of Chromatography*, 413 (1987) 355–362
*Biomedical Applications*
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 3348

## Note

# High-performance liquid chromatographic method for the determination of carvedilol and its desmethyl metabolite in body fluids

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(First received May 16th, 1986; revised manuscript received July 11th, 1986)

Carvedilol, 1-(4-carbazolyloxy)-3-[2-(2-methoxyphenoxy)ethylamino]-2-propanol (I, Fig. 1), is a new $\beta$-blocking and vasodilating agent [1,2]. One of its metabolites is desmethylcarvedilol (II, Fig. 1), which has an equal $\beta$-blocking activity but only one tenth the vasodilating activity. It was necessary to determine the plasma levels and urine excretion of carvedilol and its metabolite for pharmacokinetic studies in man.

We have developed a simple, sensitive method for the determination of both compounds by high-performance liquid chromatography (HPLC). The method employs an internal standard (naftopidil, III, Fig. 1), liquid–liquid extraction and re-extraction and reversed-phase HPLC with fluorimetric detection.

## EXPERIMENTAL

### *Reagents*

Analytical-reagent grade chemicals were used as supplied by the manufacturers. Carvedilol, desmethylcarvedilol and naftopidil were synthesized in our laboratories.

*Britton–Robinson buffer, 0.1 mol/l (pH 8.0).* Weigh 6.175 g of boric acid into a 1000-ml flask, add 5.70 ml of glacial acetic acid, 6.825 ml of phosphoric acid and approximately 800 ml of deionized water. Adjust the pH to 8.0 with 5 mol/l sodium hydroxide solution and dilute to volume with deionized water.

### *Apparatus and HPLC conditions*

The HPLC system consisted of a Model 110A solvent delivery system (Beckman, Munich, F.R.G.), an ISS 100 autosampler (Perkin-Elmer, Überlingen,

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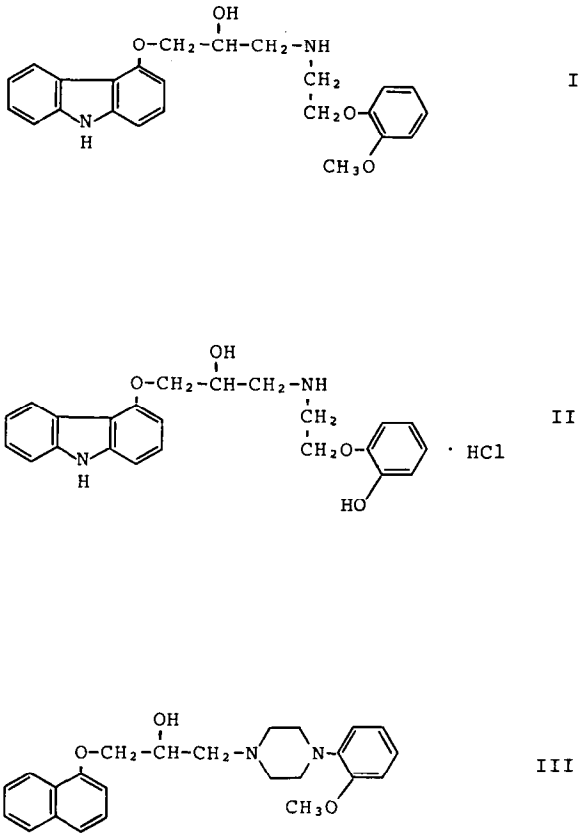


Fig. 1. Chemical structures of carvedilol (I), desmethylcarvedilol (II) and naftopidil, the internal standard (III).

F.R.G.), a Perkin-Elmer 650-10 LC fluorescence detector and a Kompensograph III recorder (Siemens, Erlangen, F.R.G.). The fluorimeter was set at an excitation wavelength of 285 nm, slit 5, and an emission wavelength of 340 nm, slit 10, and was operated at a sensitivity range of 3.

The column (250 mm×4.6 mm I.D.) (Knauer, Bad Homburg, F.R.G.) was laboratory-packed by the conventional slurry method with Nucleosil 10 $C_{18}$ (Macherey & Nagel, Düren, F.R.G.). The mobile phase was 0.2% phosphoric acid–methanol (50:50, v/v) at a flow-rate of 2 ml/min. The total analysis time was 11 min.

*Procedure*

A 0.5-ml volume of plasma or urine was mixed with 0.05 ml of internal standard (2.5 μg/ml in deionized water) and 0.5 ml of 0.1 mol/l Britton–Robinson buffer (pH 8). After the addition of 5 ml of diethyl ether, the tubes were shaken in a test-tube rack on a reciprocating shaker with a frequency of 150 $min^{-1}$ and centrifuged for 10 min at 4000 *g*. The organic phase was transferred into a 10-ml conical centrifuge tube, taking care to avoid the interface, and re-extracted into

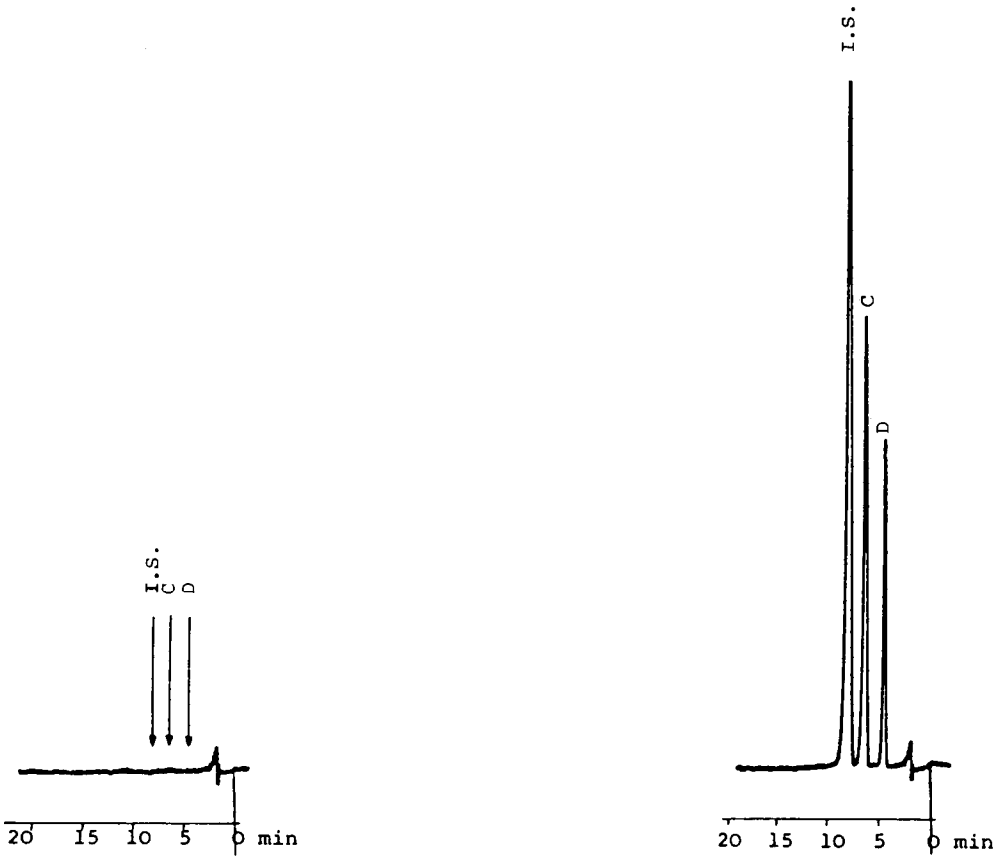



Fig. 2. Chromatogram of drug-free control human plasma sample.

Fig. 3. Chromatogram of human plasma sample spiked with 45.8 ng/ml desmethylcarvedilol (D), 49.7 ng/ml carvedilol (C) and 250 ng/ml naftopidil (internal standard, I.S.).

300 $\mu$l of 0.05 mol/l sulphuric acid by shaking (150 $min^{-1}$) for 10 min and centrifugation for 10 min at 4000 *g*. The organic phase was discarded and the excess of diethyl ether in the aqueous phase was removed under a stream of nitrogen at room temperature (10 min). The solution was transferred into a sample vial and 100 $\mu$l were injected into the HPLC system.

Calibration graphs were prepared by assaying plasma or urine samples to which known amounts of carvedilol and desmethylcarvedilol had been added. Peak-height ratios of carvedilol and desmethylcarvedilol relative to the internal standard were plotted against the amounts of carvedilol and desmethylcarvedilol added.

## RESULTS

### *Selectivity*

Drug-free control human plasma shows no significant peaks at the retention times of desmethylcarvedilol, carvedilol or the internal standard when analysed by this method (see Fig. 2).

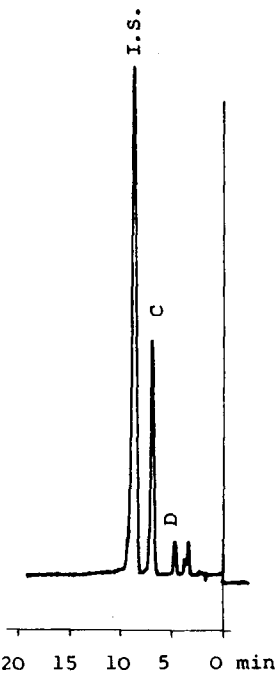



Fig. 4. Chromatogram of extract of a 500-$\mu$l human plasma sample from a volunteer taken 2 h after a single oral dose of 50 mg carvedilol. The concentrations are found to be 4.9 ng/ml desmethylcarvedilol (D) and 73.7 ng/ml carvedilol (C) (500 ng/ml I.S.).

Fig. 3 shows the chromatogram of an extract of 0.5 ml of human plasma spiked with 45.8 ng/ml desmethylcarvedilol, 49.7 ng/ml carvedilol and 250.0 ng/ml internal standard.

Fig. 4 shows the chromatogram of an extract of 0.5 ml of human volunteer plasma sample taken 2 h after an oral dose of 50 mg of carvedilol.

*Accuracy and precision*

To determine the between-run and within-run precision and accuracy, a plasma pool containing 47.64 ng/ml desmethylcarvedilol and 50.90 ng/ml carvedilol was

TABLE I

DESMETHYLCARVEDILOL IN PLASMA: DATA FOR PRECISION AND ACCURACY

| Day of evaluation | Concentration (mean ± S.D.) (ng/ml) | Relative standard deviation (%) |
|---|---|---|
| 1 | 44.05 ± 1.85 | 4.21 |
| 2 | 48.60 ± 2.60 | 5.35 |
| 3 | 49.32 ± 2.14 | 4.33 |
| 4 | 44.58 ± 1.22 | 2.74 |
| 5 | 44.58 ± 1.65 | 3.69 |
| 6 | 48.07 ± 1.36 | 2.82 |
| Mean | 46.53 ± 2.79 | 6.00 |

TABLE II

CARVEDILOL IN PLASMA: DATA FOR PRECISION AND ACCURACY

| Day of evaluation | Concentration (mean ± S.D.) (ng/ml) | Relative standard deviation (%) |
|---|---|---|
| 1 | 54.43 ± 2.06 | 3.79 |
| 2 | 57.78 ± 3.24 | 5.60 |
| 3 | 55.70 ± 1.77 | 3.19 |
| 4 | 48.93 ± 1.39 | 2.85 |
| 5 | 50.30 ± 1.36 | 2.70 |
| 6 | 53.03 ± 1.78 | 3.36 |
| Mean | 53.36 ± 3.61 | 6.76 |

prepared. On each of six different days six samples from this pool were analysed, giving a total of 36 samples. The values of the mean and the standard deviation (S.D.) calculated from the six measurements of each day represent the within-run accuracy and precision. The between-run accuracy and precision are indicated by the mean and S.D. of the six within-run calculations. This procedure

TABLE III

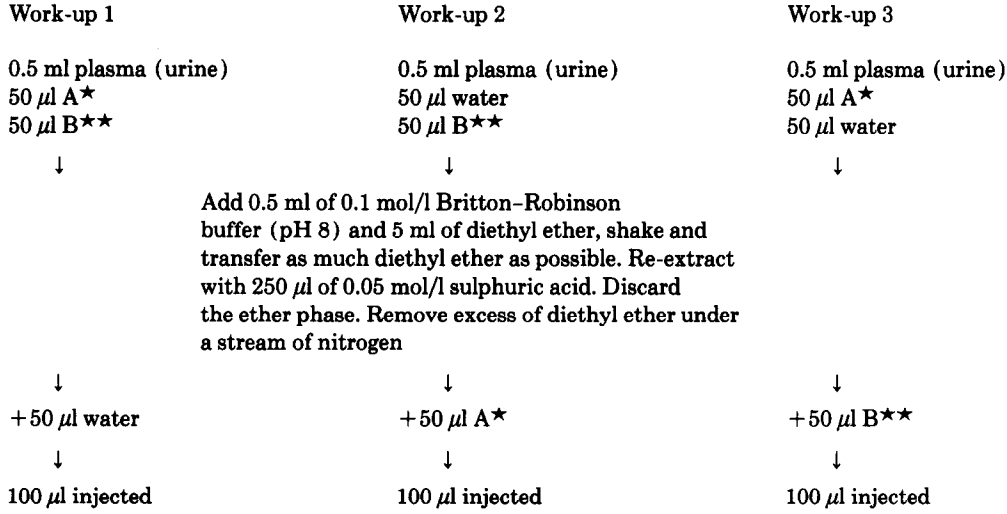
DESMETHYLCARVEDILOL IN URINE: DATA FOR PRECISION AND ACCURACY

| Day of evaluation | Concentration (mean ± S.D.) (ng/ml) | Relative standard deviation (%) |
|---|---|---|
| 1 | 41.95 ± 0.97 | 2.31 |
| 2 | 50.23 ± 1.51 | 3.01 |
| 3 | 51.50 ± 2.34 | 4.55 |
| 4 | 48.92 ± 0.92 | 1.87 |
| 5 | 47.72 ± 1.97 | 4.14 |
| 6 | 55.33 ± 2.59 | 4.67 |
| Mean | 49.27 ± 4.47 | 9.06 |

TABLE IV

CARVEDILOL IN URINE: DATA FOR PRECISION AND ACCURACY

| Day of evaluation | Concentration (mean ± S.D.) (ng/ml) | Relative standard deviation (%) |
|---|---|---|
| 1 | 48.20 ± 0.62 | 1.29 |
| 2 | 54.82 ± 1.26 | 2.29 |
| 3 | 57.33 ± 2.23 | 3.89 |
| 4 | 54.57 ± 1.27 | 2.33 |
| 5 | 55.12 ± 2.00 | 3.64 |
| 6 | 58.25 ± 2.03 | 3.48 |
| Mean | 54.71 ± 3.61 | 6.59 |




★A = Aqueous standard solution, 1000 ng/ml desmethylcarvedilol–1000 ng/ml carvedilol
★★B = Aqueous internal standard, 2500 ng/ml.

Fig. 5. Procedure used in the recovery study. Work-up 2 represents 100% recovery of desmethylcarvedilol and carvedilol and work-up 3 100% recovery of naftopidil. Each work-up was repeated twelve times for plasma.

TABLE V

PARAMETERS OF CALIBRATION GRAPHS

| Compound | Parameter | Plasma | Urine |
|---|---|---|---|
| Desmethylcarvedilol | Slope | 53.81 | 67.19 |
| | Intercept | 1.55 | 0.82 |
| | Correlation coefficient | 0.9971 | 0.9969 |
| Carvedilol | Slope | 74.48 | 102.30 |
| | Intercept | 0.89 | 1.47 |
| | Correlation coefficient | 0.9983 | 0.9971 |

TABLE VI

INFLUENCE OF VOLUME SIZE ON STANDARD DEVIATION (S.D.)

| Sample volume (µl) | Desmethylcarvedilol | | | Carvedilol | | |
|---|---|---|---|---|---|---|
| | Mean (ng/ml) | S.D. (ng/ml) | R.S.D. (%) | Mean (ng/ml) | S.D. (ng/ml) | R.S.D. (%) |
| 100 | 101.25 | 7.09 | 7.0 | 101.33 | 5.65 | 5.5 |
| 250 | 104.07 | 3.81 | 3.7 | 103.73 | 3.60 | 3.5 |
| 500 | 101.86 | 3.19 | 3.1 | 102.56 | 2.11 | 2.1 |

was also used for the analysis of urine samples containing 45.81 ng/ml desmethylcarvedilol and 49.70 ng/ml carvedilol.

Tables I–IV show the results of these measurements.

*Recovery study*

The recovery of desmethylcarvedilol, carvedilol and naftopidil from plasma and urine was studied. The procedure used for this study is shown in Fig. 5.

For each set, mean peak heights were calculated and percentage recoveries of desmethylcarvedilol, carvedilol and naftopidil were determined by comparing samples 1 and 2 and samples 1 and 3, respectively. The recoveries from plasma samples were 96.5% for desmethylcarvedilol, 95.8% for carvedilol and 103.8% for naftopidil and from urine samples 93.2% for desmethylcarvedilol, 93.4% for carvedilol and 95.2% for naftopidil.

*Linearity*

For plasma and urine, a concentration range from 0 to 100 ng/ml was chosen and samples were analysed in triplicate. The parameters of the resulting calibration graphs are given in Table V.

To facilitate the analysis of plasma samples with higher concentrations without preparing another calibration graph, the influence of variable volume size was evaluated. The concentrations of the samples were 101.86 ng/ml desmethylcarvedilol and 102.56 ng/ml carvedilol. Each volume size was analysed six times (Table VI).

*Detection limit*

Six individual standards in the lower range of the calibration graph (2.5, 5 and 10 ng/ml) were analysed and the value of the mean and the standard deviation were calculated. The detection limit (DL) was obtained according to the following equation:

$$DL = X_B + \frac{3s_n + 3s_{n-1} + \ldots + 3s}{n}$$

where $X_B$ = mean value of the blank signal (if any), $s_n$ = standard deviation of an individual concentration range assayed and $n$ = number of concentration ranges assayed.

Based on reference calibration graphs from 0 to 100 g/ml, the detection limit for desmethylcarvedilol was 0.46 ng/ml in plasma and 1.07 ng/ml in urine and that for carvedilol was 0.38 ng/ml in plasma and 0.82 ng/ml in urine.

## CONCLUSION

A sensitive and selective HPLC method has been developed for the determination of carvedilol and desmethylcarvedilol in plasma (serum) and urine samples. This extraction–re-extraction method permits the analysis of the large numbers of samples required for pharmacokinetic studies. Up to 100 samples a

day can be analysed and the method has been used successfully for thousands of analyses.

ACKNOWLEDGEMENTS

The author is grateful to Mrs. B. Auer, V. Adamczak and I. Hintzen for excellent technical assistance and Mrs. D. Hildebrand for preparing the manuscript.

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