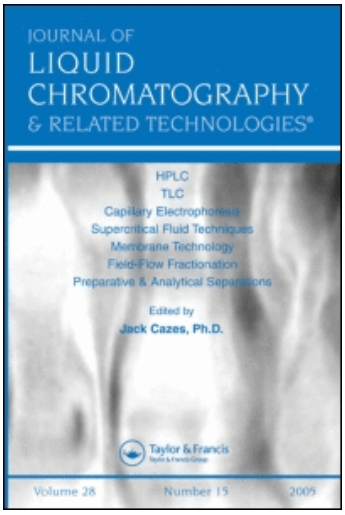

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

A Rapid Method For Cyclosporine A Determination By Hplc

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To cite this Article Shihabi, A. K. , Scaro, J. and David, R. M.(1985) 'A Rapid Method For Cyclosporine A Determination By Hplc', Journal of Liquid Chromatography & Related Technologies, 8: 14, 2641 — 2648
To link to this Article: DOI: 10.1080/01483918508076595
URL: http://dx.doi.org/10.1080/01483918508076595

JOURNAL OF LIQUID CHROMATOGRAPHY, 8(14), 2641-2648 (1985)

A RAPID METHOD FOR CYCLOSPORINE A DETERMINATION BY HPLC

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ABSTRACT

A rapid method is described for the determination of cyclosporine in whole blood by HPLC. The cyclosporine is extracted with an acetonitrile-isopropanol mixture, purified on a C_{18} minicolumn, and finally injected on a CN column. Recovery of the drug is greater than 90%.

INTRODUCTION

Cyclosporine A, an immunosuppressive agent, has greatly improved the survival rate of solid transplants (1,2). However, many side effects such as renal failure and hyperbilirubinemia necessitate careful monitoring of the therapeutic blood level.

Two characteristics of cyclosporine make the blood levels difficult to monitor. First, two-thirds of cyclosporine A is tightly bound to the red blood cells (3). This binding is

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temperature dependent (3). Second, cyclosporine has many metabolites which interfere with measurement by RIA. These metabolites do not interfere with the HPLC method; however, sample purification, with solid phase (4,5) or ether extraction (6,7), is required. Ether extraction yields clean chromatograms with recoveries of over 90%. In addition to being dangerous for routine clinical laboratories, the extraction is time-consuming, leading to very high cost per test. Direct solid-phase extraction using disposable columns is more suited for serum; however, the drug level in serum is only one-third of that in whole blood (3).

Here, we describe a method for cyclosporine A determination in whole blood which is based on pre-extraction of the drug with an acetonitrile-isopropanol mixture, solid-phase extraction, and finally injection on a Cyano-column. The recovery is over 90%. The solvent and the pre-extraction columns are recycled, reducing the cost of the test. The method is rapid and suited for routine laboratory use.

MATERIALS AND METHODS

Instrument

The HPLC instrument consisted of a Model 110A pump (Beckman Instruments, Fullerton, CA, U.S.A.) set at 1.3 ml/min. The column used for routine work was a DuPont CN 5 μm (150 mm X 4.6 mm i.d.) maintained at 56°C. The detector was an ultraviolet

Spectro Monitor Model III (Laboratory Data Control, Riviera Beach, FL, U.S.A.) set at 210 nm and 20 mA.

Reagents

1. Cyclosporine A stock standard: 50 mg/L of acetonitrile (stable).

2. Cyclosporine A working standard (500 µg/L): dilute the above standard 100-fold with a mixture of 10% acetonitrile in water (prepare fresh).

3. Stock internal standard: cyclosporine D, 50 mg/L of acetonitrile (stable).

4. Extraction solvent: 15 ml of stock internal standard/L of 10% isopropanol in acetonitrile (stable).

5. Test mixture: 1.2 ml of stock internal standard and 0.6 ml of stock standard were mixed with 8 ml of 50% acetonitrile (stable). This solution (100 µl) is injected on the column to check the column performance before the patients are injected.

6. Pump solvent: 45% acetonitrile in water. The solvent is collected and recycled 5-10 times. The separation is not affected by recycling the solvent. From a practical point of view, using the same solvent gives more reproducible results than preparing a fresh one each time.

Preparation of the Extraction Columns

Baker-Bond C_{18} extraction columns, 1 ml size (J.T. Baker Chemical Co., Philipsburg, NJ, U.S.A.) were washed initially with

2 ml of acetonitrile followed by 2.5 ml of 70% methanol under reduced pressure. After this step the columns were kept wet with 70% methanol.

Procedure

1. Thoroughly mixed whole blood (0.5 ml) and 1.0 ml of the extraction solvent were mixed in a 1.5 ml centrifuge tube for 15 sec, incubated for 5 min at room temperature, and mixed again for 15 sec. The tubes were centrifuged at 10,000 x g for 15 sec.

2. The supernatant was mixed with 1.5 ml of water and applied to a prepared C_{18} extraction column. (The flow-rate was kept close to 1 ml/min under reduced pressure.) When the sample had drained from the column, 5 ml of 70% methanol was applied to the column. Finally, the column was allowed to drain dry.

3. Cyclosporines were eluted from the column with 1.3 ml of acetonitrile, under gravity, into an evaporation vial. (The extraction column may now be recycled by washing with 2.5 ml of 70% methanol. The column may be recycled 6 times.)

4. The acetonitrile was evaporated at 75°C under a gentle stream of air.

5. The vials were reconstituted with 100 µl of 50% acetonitrile, sonicated for 15 sec, incubated for 5 min at room temperature, and then the entire contents injected on the CN column (100-µL loop).

RESULTS AND DISCUSSION

Cyclosporine A binds thighly to red blood cells as well as to the C_{18} packing materials. An acetonitrile-isopropanol mixture was used in this study to extract cyclosporine A from whole blood. However, the extraction solvent cannot be applied directly to the column since the drug will elute without binding to the C_{18} column. This problem was overcome by diluting the acetonitrile-isopropanol mixture (containing the cyclosporine) with water to bring the acetonitrile concentration to approximately 40%. Under these conditions, cyclosporine A and the internal standard cyclosporine D bind tightly to the C_{18} packing of the minicolumn. Contaminants were then washed from the column with 70% methanol. Finally, the cyclosporines were eluted with acetonitrile, concentrated by evaporation, and injected on the Cyano-column.

Blood contains many contaminants which absorb at 210 nm. The extraction columns used in this method were effective in sample cleanup (Fig. 1). A great number of the peaks in the chromatograms were found to arise from the columns themselves. Furthermore, different C_{18} extraction columns from other manufacturers exhibited different peaks (Fig. 2).

Some of the previous methods used serum (5), or hemolyzed red blood cells (6) for cyclosporine determination. However, proteins clog the extraction columns reducing the recovery and more importantly, make the recovery from one sample to another

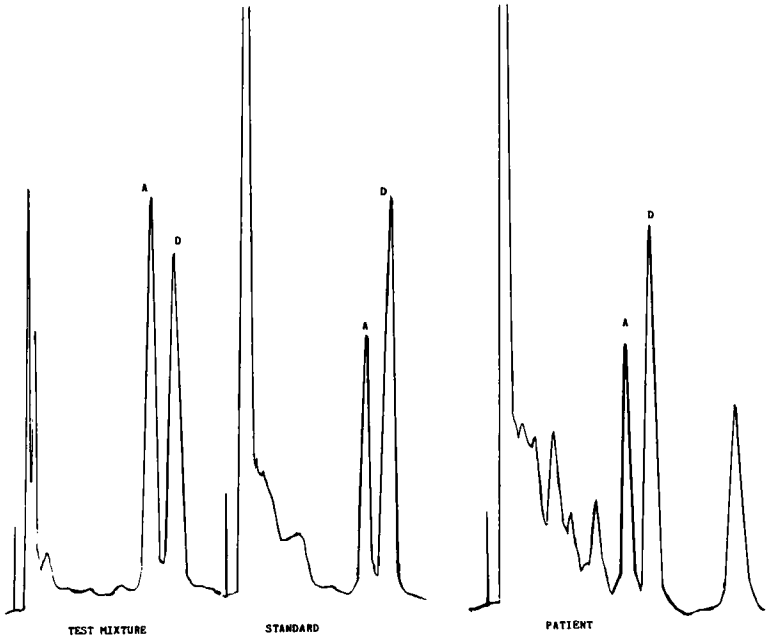

FIGURE 1: Chromatogram of the test mixture, standard and patient. (A = cyclosporine A, D = cyclosporine D). The capacity factors for cyclosporines A and D are 10 and 12 min, respectively.

rather variable. The columns for these extractions can only be used once. The isopropanol-acetonitrile extraction step in our method was quite effective in removing the proteins and increasing the recovery of the extraction. The average recovery of 500 μg of cyclosporine A added to 10 different samples of blood was 96% (range 86-108%). The extraction columns were used 6-10 times without loss of recovery. We tried extraction with methanol in place of the 10% isopropanol in the acetonitrile

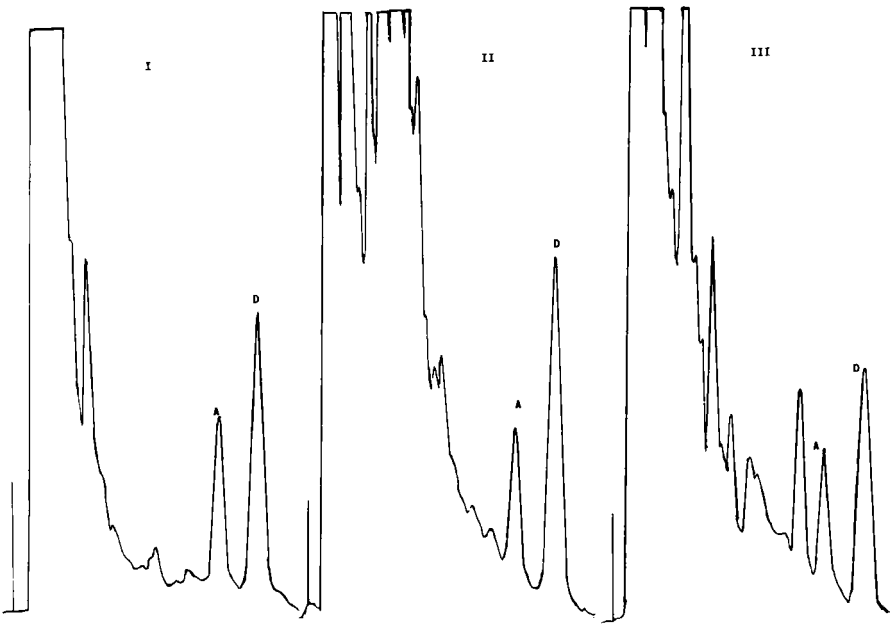

FIGURE 2: Effect of the extraction column on cyclosporine assay (same sample): I = Baker C_{18} columns, II = Brand A columns, III = Brand B columns

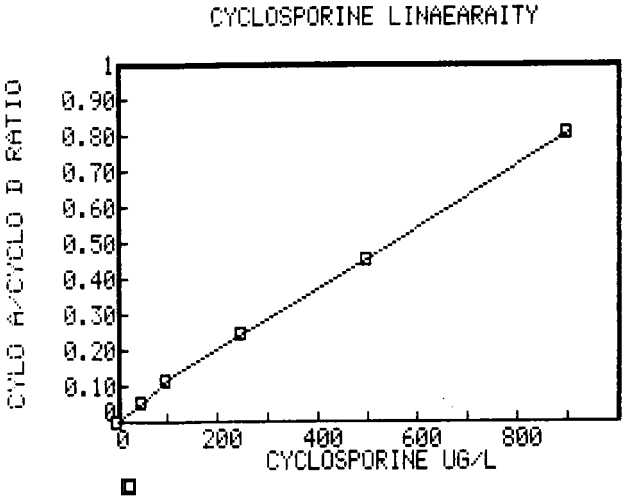

FIGURE 3: Linearity of cyclosporine A assay.

mixture. The peak heights for a 500 µg standard were about 25% higher in the isopropanol-acetonitrile mixture. In addition, methanol was not quite an effective deproteinizing agent as evidenced by the columns staining red when methanol was used for extraction.

The test is linear between 50-900 µg/L (Fig. 3). We tried to use different columns; e.g., butyl, C_{18}, and other CN columns. We found the DuPont CN column required the least amount of acetonitrile, less heating, and gave sharper peaks for cyclosporines A and D.

REFERENCES

1. Merion, R., White, D.J.G., Thiru, S., Evans, D.B., and Calne, R., Cyclosporine: Five years' experience in cadaveric renal transplantation. N. Engl. J. Med., 310, 148, 1984.

2. Stiller, C.R., The Canadian trial of cyclosporine. Transplant. Proc., 15, 2479, 1983.

3. Neideberger, W., Lemaire, M., Nussbaumear, K., Maurer, G., and Wagner, D., Distribution of cyclosporine in blood components and tissues. Transplant. Proc., 15, 2419, 1983.

4. Swachulk, R.J., and Cartier, L.L., Liquid chromatographic determination of cyclosporine A in blood and plasma. Clin. Chem., 17, 1368, 1981.

5. Yee, G.C., Gmur, D.J., and Kennedy, M.S., Liquid chromatographic determination of cyclosporine in serum with use of rapid extraction procedure. Clin. Chem., 29, 2269, 1982.

6. Lensmeyear, G., and Fields, B.L., Improved liquid chromatographic determination of cyclosporine with concomitant detection of cell bound metabolite. Clin. Chem., 31, 196, 1985.

7. Garro, R., and Lapalus, P., Simplified liquid chromatographic analysis of cyclosporine A in blood and plasma with the use of rapid extraction. J. Chromatogr., 337, 416 1985.