Journal of Chromatography, 427 (1988) 195–198
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 4102

Letter to the Editor

Monitoring of cyclosporin in whole blood by reversed-phase liquid chromatography on a butyl column

Sir,

Immunosuppression with cyclosporin A (CsA) has led to major advances in organ and bone-marrow transplantations [1,2]. However, the very narrow therapeutic range requires a careful monitoring of the CsA levels in blood [3]. Two methods are currently available for CsA monitoring in biological fluids: radioimmunoassay (RIA) and high-performance liquid chromatography (HPLC) [4,5]. In contrast to HPLC, RIA is more appropriate for analysis of a large number of samples in one run. However, it is well known that cyclosporin concentrations are overestimated by RIA in a non-linear way by cross-reaction of the polyclonal antibodies with various metabolites of CsA [6].

To overcome the problem of temperature-dependent distribution of CsA between erythrocytes and plasma [7], we analysed whole blood samples. Furthermore, solvent extraction was preferred to solid-phase extraction as the latter procedure often resulted in higher background and irreproducible recoveries.

We report here HPLC conditions involving a Hypersil-WP-300 butyl column, together with a saturation column installed between the pump and the injector. Under these HPLC conditions very clear chromatograms are obtained and, even after injection of over 1800 extracts of whole blood samples, no loss in efficiency was noticed.

We used a Model LKB 2150 solvent-delivery system (LKB, Bromma, Sweden), a Valvo CV-6-UHPa-N60 injection valve with a 50-μl loop (Valco, Houston, TX, U.S.A) and a Pye Unicam variable-wavelength detector (Pye Unicam, Cambridge, U.K.) set at 214 nm. Chromatograms were recorded on a Philips PM 8251 recorder (Philips, Eindhoven, The Netherlands). Analyses were carried out on a 10 cm$\times$0.46 cm I.D. column packed with Hypersil-WP-300 C_4 5-μm particles from Shandon (Shandon Southern Instruments, Sewickley, PA, U.S.A.). Between the solvent-delivery system and the injector a 15 cm$\times$0.46 cm I.D. RSIL C_{18} 10-μm column was installed (Alltech, Europe, Eke, Belgium). Both columns were maintained at 70°C in a silicone oil bath. Columns were eluted isocratically with acetonitrile–methanol–water (40:15:45, v/v/v) at a flow-rate of 0.7 ml/min, resulting in a back-pressure of 70 bar (6.90 MPa)

All samples were collected on potassium EDTA in Sarstedt tubes just before

0378-4347/88/$03.50

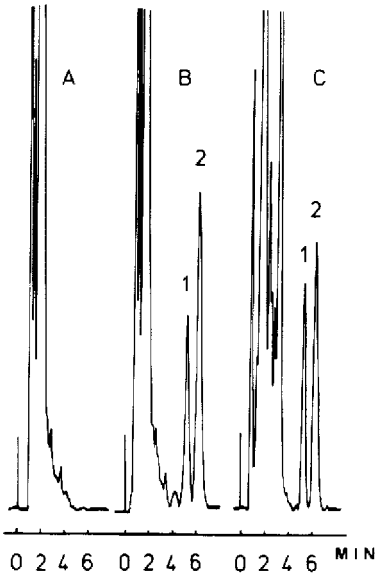

Fig. 1. Chromatograms of whole blood samples. (A) Total blank, no CsA, no internal standard (CsD); (B) standard containing 75 ng/ml; (C) patient sample containing 100 ng/ml. For chromatographic conditions, see text. Peaks: 1 = cyclosporin A, retention time (t_R) = 5 min, capacity factor (k') = 6.2; 2 = cyclosporin D, t_R = 6.5 min, k' = 7.6.

the next dose of CsA. The extraction procedure applied is related to the one published by Annesley et al [8]. To 1 ml of whole blood, 30 μl of the methanolic CsD working solution, containing 8.044 μg/ml, was added as an internal standard. After mixing, 2 ml of a 90 m*M* hydrochloric acid solution in water were added to haemolyse the erythrocytes. Samples were vortexed several times over a period of 10 min. After addition of 7.5 ml of methyl *tert.*-butyl ether the samples were mixed at a rotary mixer for 9 min. Samples were centrifuged for 6 min at 1500 *g* and the organic layer was transferred with a pasteur pipette into a second glass tube containing 2 ml of 90 m*M* sodium hydroxide in water. The tubes were shaken vigorously by hand for 2 min and centrifuged as above. The organic (upper) layer was again transferred to another (conical) glass tube and evaporated to dryness under nitrogen at room temperature. The residue was reconstituted in 75 μl of the chromatographic solvent, and a final lipid extraction was performed with 200 μl of *n*-heptane by vortex-mixing. To separate the liquid phases, samples were centrifuged for 1 min, and 50 μl of the mobile phase (bottom layer) were injected into the chromatographic system. For calibration, 500-μl aliquots of packed cell material (obtained from the blood bank) were diluted with 500 μl of water, supplemented with 75–375 ng of CsA and analysed by the above described method. For internal quality control, 1.2-ml aliquots of a spiked pool were stored at −20 °C, and one of those samples was taken through the entire procedure every day. A standard curve was obtained by plotting peak-height ratios (CsA/CsD) versus the standard concentrations. Unknown concentrations are determined by comparing peak-height ratios with this calibration curve.

Fig. 1 illustrates chromatograms from a whole blood sample obtained from a

volunteer free of CsA, a standard containing 75 ng/ml and a renal transplant patient sample containing 100 ng/ml. As can be seen, resolution of CsA and CsD is excellent and both peaks are also well separated from the solvent front. Although transplant patients are also treated with a large number of other drugs, no interfering or late eluting peaks are observed. The concentration–response relationship is found to be linear between 30 and 1000 ng/ml. The limit of detection when analysing a 1-ml sample is ca. 30 ng/ml. The extraction recovery of CsA was 83.0 ± 2.1% and was shown to be independent of the concentration up to 375 ng/ml. At a concentration of 114 ng/ml a within-run coefficient of variation (C.V.) of 3.8% was found on ten replicate analyses. The between-run C.V. as obtained from the analyses of the internal quality control samples was 6.6% at the level of 124.5 ng/ml ($n = 20$) and 6.2% at the level of 115.8 ng/ml ($n = 25$).

By using a stationary phase more polar than the classical C_{18} an excellent peak shape is obtained and no peaks interfere with CsA and CsD, in contrast to the observations of Maguire et al. [9] after specimen collection in Sarstedt containers. The wide pore size (300 Å) makes this packing material especially suited for the chromatography of biopolymers [10]. Furthermore, the butyl phase clearly is very stable, as over 1800 whole blood extracts have already been injected with no observable loss of efficiency. The dimensions of the column (10 cm × 0.46 cm I.D.) result in a detection limit of 30 ng/ml, far below the 100 ng/ml level widely accepted to be necessary, for prevention of graft rejection. As one chromatogram is obtained in ca. 9 min, the HPLC procedure yields accurate whole blood results very rapidly, on the same day as specimen collection. The extraction is technically easy and the method is demonstrated to be reproducible and useful for a routine clinical laboratory allowing daily dosage adjustment if necessary.

Laboratoria voor Medische Biochemie
en voor Klinische Analyse,
Faculteit Farmaceutische Wetenschappen,
Rijksuniversiteit Gent, Harelbekestraat 72,
B-9000 Ghent (Belgium)

W.E. LAMBERT
A.P. DE LEENHEER*
H.R. WALGRAEVE

Faculty of Pharmaceutical Sciences,
Department of Toxicology, Khon Kaen University,
Khon Kaen 40002 (Thailand)

W. TASSANEEYAKUL

1 D.M. Canafax and N.L. Asher, Clin. Pharm., 2 (1983) 515.
2 J.M. Hows, S. Palmer and E.C. Gordon-Smith, Transplantation, 33 (1982) 382.
3 B.O. Myers, J. Ross, L. Newton, J. Luetscher and M. Perbroth, N. Engl. J. Med., 311 (1984) 699.
4 R.J. Sawchuk and L. L. Cartier, Clin. Chem., 27 (1981) 1368.
5 P.M. Kabra, J.H. Wall and N. Blanckaert, Clin. Chem., 31 (1985) 1717.

6 W.T. Robinson, H. F. Schran and E.P. Barry, Transplant. Proc., 15 (1983) 2403.
7 R.W. Yatscoff, D.N. Rush and J.R. Jeffery, Clin. Chem., 30 (1984) 1812.
8 T. Annesley, K. Matz, L. Balogh, L. Clayton and D. Giacherio, Clin. Chem., 32 (1986) 1407.
9 S. Maguire, F. Kyne and D. UaConaill, Clin. Chem., 33 (1987) 1493.
10 J.D. Pearson, N. T. Lin and F. E. Regnier, Anal. Biochem., 124 (1982) 217.

(First received October 22nd, 1987; revised manuscript received December 29th, 1987)