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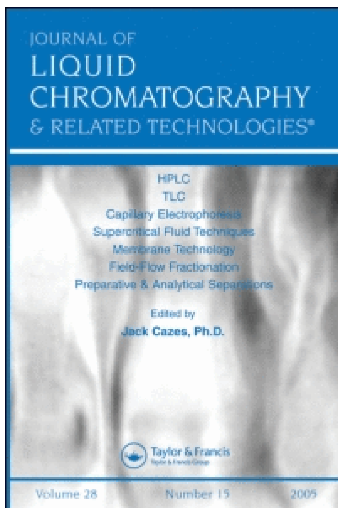

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A High Performance Liquid Chromatographic Method for the Determination of Rapamycin (Sirolimus) in Rat Serum, Plasma, and Blood and in Monkey Serum

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A HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF RAPAMYCIN (SIROLIMUS) IN RAT SERUM, PLASMA, AND BLOOD AND IN MONKEY SERUM*

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ABSTRACT

A high performance liquid chromatographic (HPLC) method was developed for the quantitation of rapamycin, an immunosuppressive agent, in biological specimens. The method employs a 15 cm Supelco LC-18 column (5 µm) interconnected to a 25 cm Supelco LC-18 column (5 µm). The mobile phase is a methanol/water gradient system. The flow rate is 0.51 ml/min and detection is by ultraviolet (UV) absorption at 276 nm. The method was validated for its specificity, precision, linearity and sensitivity in rat serum. Endogenous compounds in rat serum did not interfere with the detection of rapamycin or the internal standard (β-estradiol-3-benzoate). Based on a 1.0 ml serum sample, the assay was linear from 5 to 500 ng/ml. The intra-day coefficients of variation were below 10% and independent of concentration. Inter-day precision values ranged from 5.5 to 13.6%, the difference being independent of concentration. The specificity, linearity and sensitivity of the method was also demonstrated in cynomolgus monkey serum, rat plasma and hemolyzed rat whole blood. In each case, the method was specific, with no endogenous interferences. Furthermore, the method showed no interference from two newly reported rapamycin degradation products.

*This work was presented at the North New Jersey ACS Drug Metabolism Discussion Group Annual Symposium, Somerset, NJ, September, 1990.
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INTRODUCTION

Rapamycin (Figure 1a), an antitumor and antifungal agent isolated from the fungus *Streptomyces hygroscopicus* is under development as an immunosuppressant (1-2). Rapamycin has been found to have potent immunosuppressive activity while exhibiting little toxicity in primates (3-4). In order to carry out pharmacokinetic and drug disposition studies, we developed and validated a specific high performance liquid chromatographic method for the analysis of rapamycin in rat serum, plasma and whole blood and monkey serum.

EXPERIMENTAL

Chemicals and Reagents

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Rapamycin was obtained from Wyeth-Ayerst Research, Rouses Point, NY. A stock solution was prepared by dissolving 10.45 mg of rapamycin in 50 ml of methanol. Standard solutions were made by diluting the stock solution with methanol to obtain rapamycin concentrations of 10, 5, 2, 1, 0.5, 0.2 and 0.1 μg/ml. The internal standard (I.S.,β-estradiol-3-benzoate) was purchased from Aldrich (Milwaukee, Wisconsin). An I.S. stock solution was prepared by dissolving 18.0 mg of β-estradiol-3-benzoate in 50 ml methanol. Working I.S. solution was prepared by diluting 1.0 ml of stock solution to 50 ml with methanol to give a final concentration of 7.2 μg/ml. Two rapamycin degradation products A and B (Figures 1b and 1c) were prepared in house (5). HPLC grade methanol and water were obtained from EM Science (Gibbstown, NJ). Aluminum oxide (Neutral, Brockmann Activity Grade I) was purchased from J.T. Baker (Phillispsburg, NJ). Isopropyl ether was purchased from Fluka Chemical (Ronkonkoma, NY). Isopropyl ether was purified before each analysis by passing it through neutral alumina (Brockmann activity grade I). Before use, purity was checked by HPLC as described in the HPLC analysis procedure.

Instrumentation

The HPLC system consisted of a Waters Model 600E pump (Waters Associates, Milford, MA), a Waters WISP Model 712 autosampler, a Waters programmable multiwavelength detector set at 276 nm and a Hewlett Packard Model 3390A integrator (Hewlett-Packard, Avondale, PA). Separation of rapamycin was achieved using a Supelcosil LC-18 column (150 mm x 4.6 mm i.d., 5 μm particle size) interconnected to a Supelcosil LC-18 column (250 mm x 4.6 mm i.d., 5 μm particle size) and a Supelguard LC-18

FIGURE 1 Chemical Structures of Rapamycin (1a), Rapamycin Degradation Product A (1b) and B (1c)

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TABLE 1

HPLC Gradient Program for the Analysis of Rapamycin[a]

Time	Mobile Phase (%)	
(min)	Methanol	Water
Initial	87	13
25	92	8
36	92	8
40	87	13
55	87	13

[a]Linear gradient was used between each time point and the flow rate was 0.51 ml/min.

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precolumn with 2 cm x 4.6 mm Supelcosil cartridge purchased from Supelco (Bellefonte, PA).

Extraction of Rapamycin from Biological Fluids

Fifty μl of methanol were added to 1.0 ml of the experimental sample in a 16 x 125 mm disposable screw-cap culture tube and mixed by vortex. Fifty μl of the working standards were added to the control serum samples and mixed by vortex to prepare the standard curve. Next, 50 μl of I.S. working solution (β-estradiol-3-benzoate) were added to each tube and mixed by vortex. Two and a half ml of purified isopropyl ether were added and the tubes were vortexed, shaken for 15 min and then centrifuged at 2300 rpm for 10 min. The isopropyl ether phase was transferred to a clean conical screw-cap tube and evaporated to dryness under a stream of nitrogen gas.

HPLC Analysis

The mobile phase was composed of methanol and water. An HPLC gradient mobile phase was used for chromatography (Table 1). The isopropyl ether extract was reconstituted in 100 μl of mobile phase [methanol/water (87:13)] and 60 μl was injected onto the HPLC column. The column was maintained at room temperature and the detection wavelength was 276 nm.

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Specificity

The specificity of the method was evaluated with regard to interference due to the presence of endogenous substances in the extract of control rat serum. Ten random individual control sera and a control serum spiked with rapamycin and internal standard were processed by the method described above and analyzed. Specificity testing was also performed in four control monkey serum, two rat whole blood and four rat plasma, respectively. Since recently, two degradation products of rapamycin were identified (5), the chromatographic properties of these compounds were also measured using the HPLC system described above to characterize the specificity of the method.

Linearity, Precision and Accuracy

The linearity, minimum and maximum quantifiable concentrations, precision and accuracy of the method were evaluated in both intra-and inter-day studies. Intra-day data were obtained by analysis of four replicates of 5, 10, 25, 50, 100, 250 and 500 ng/ml concentrations of rapamycin in 1.0 ml of control rat serum. Inter-day data were obtained by analysis of five replicates of spiked 5, 50 and 500 ng/ml serum on three separate days using analytical standards independently prepared on each day of analysis. The linearity of the method was also performed in rat plasma and whole blood and monkey serum.

Calculations

Rapamycin concentrations in ng/ml were obtained from the regression line relating rapamycin/internal standard peak area ratios to the compound concentrations calculated using unweighted linear regression in a Hewlett-Packard Model 85 calculator or an IBM compatible computer.

RESULTS

HPLC Analysis

Typical HPLC chromatograms for control serum, control serum spiked with rapamycin and internal standard (β-estradiol-3-benzoate) and a serum sample from a rat given

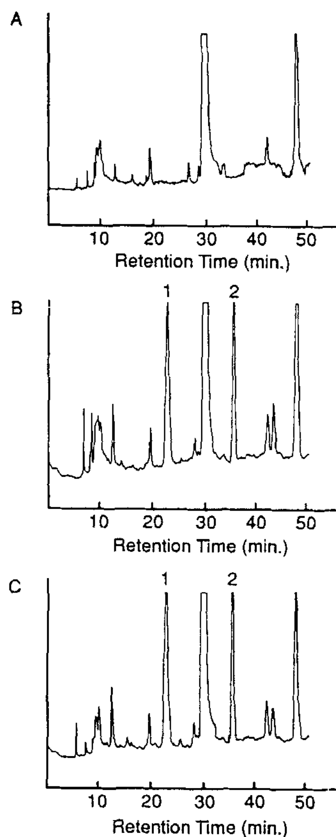

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FIGURE 2 Chromatograms of Control Rat Serum (A), Control Rat Serum Spiked with Rapamycin and β-estradiol-3-benzoate (B) and a Serum Sample from a Rat given Rapamycin (C). 1, Rapamycin; 2, β-estradiol-3-benzoate.

rapamycin intravenously are presented in Figure 2. Under the conditions specified, the retention times for rapamycin and β-estradiol-3-benzoate were 23.4 and 36.8 min., respectively. No endogenous interfering substances were detected in the extracts of ten random control rat serum samples. The endogenous interfering substances were not found in the control rat plasma, whole blood and monkey serum as well. The assay of rapamycin was not interferred by the two degradation products (A and B) of rapamycin; A and B showed very similar retention times in the chromatogram (Figure 3).

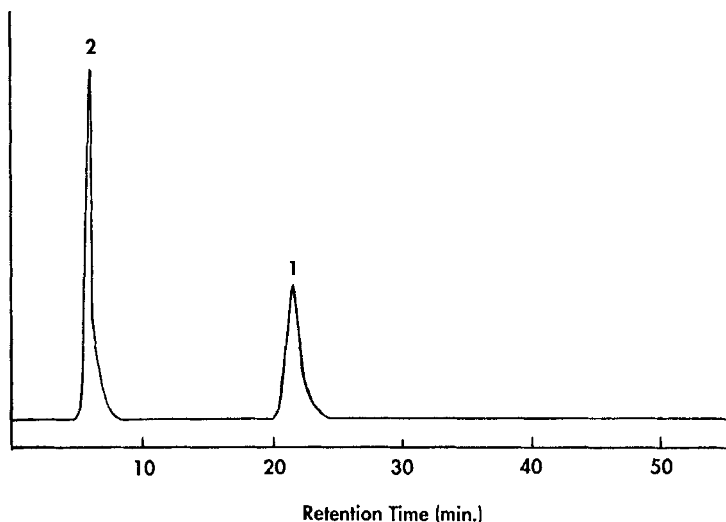

FIGURE 3 Chromatograms of Rapamycin and its Degradation Products A and B. 1. Rapamycin; 2. Degradation Products A and B.

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Linearity and Sensitivity

The linearity of the peak area ratio to the concentration of rapamycin was calculated using linear regression analysis. The method was linear for concentrations between 5 and 500 ng/ml in rat serum and whole blood and monkey serum with a correlation coefficient (R^2) of >0.99. For the rat plasma, it was linear from 10 to 500 ng/ml.

Precision and Accuracy

The intra-day precision of the method was assessed by calculating the coefficient of variation for replicate samples (N=4) at 5, 10, 25, 50, 100 (N=3), 250 and 500 ng/ml. The coefficients of variation were 2.7, 7.5, 3.3, 0.6, 5.2, 9.8 and 4.9%, respectively, and were independent of concentration. Analytical bias, based on the percent difference between found and theoretical concentrations, was within ±13% and was not concentration dependent. The coefficients of variation in the inter-day precision study were 5.5, 8.4 and 13.6% at 500, 50 and 5 ng/ml, respectively, the differences being independent of concentration.

DISCUSSION

An HPLC method for the quantitation of rapamycin in rat serum has been developed and validated. The method is specific with no endogenous interferences. The assay is linear in the range of 5-500 ng/ml with the minimum quantifiable concentration of 5 ng/ml. It is important with this method to check the isopropyl ether, the mobile phase and control sera for possible interfering peaks before use.

This method has also been validated for use in the measurement of rapamycin in monkey serum, rat whole blood and rat plasma. In each case, the method was shown to be specific, with no endogenous interferences. For monkey serum and rat whole blood, the method was linear from 5 to 500 ng/ml. For rat plasma, it was linear from 10 to 500 ng/ml. These results indicate that this method may be applied to the analysis of rapamycin in these matrices.

Yatscoff et al (6) reported an HPLC method to measure rapamycin in whole blood. Desmethyl rapamycin was used as the internal standard. In our method, the more readily available internal standard, β-estradiol-3-benzoate has been shown to provide satisfactory results.

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REFERENCES

1. S.N. Sehgal, H. Baker, C. Vezina. Rapamycin (AY-22989), a new antifungal antibiotic. Fermentation, isolation and characterization. J Antibiot, 28: 729-32 (1975).
2. C.P. Eng, S.N. Sehgal, C. Vezina. Activity of rapamycin (AY-22989) against transplanted tumors. J Antibiot, 37: 1231-37 (1984).
3. R.Y. Calne, S. Lim, A. Somaan, D. St. J. Collier, S.G. Pollard, D.J.G. White. Rapamycin for immunosuppression in organ allografting. The Lancet, 227 (1989).
4. P.M. Kimball, R.H. Kerman, B.D. Kahan. Production of synergistic but nonidentical mechanisms of immunosuppression by rapamycin and cyclosporine. Transplantation, 51: 486-90 (1991).
5. C.P. Wang, K. Chan, R. Schiksnis, J.A. Scatina, S.F. Sisenwine. High performance liquid chromatographic isolation, spectroscopic characterization and immunosuppressive activities of two rapamycin degradation products. J.Liquid Chrom., 17 (16): 3383-3392 (1994).
6. R.W. Yatscoff, C. Faraci, P. Bolingbroke. Measurement of rapamycin in whole blood using reverse-phase high-performance liquid chromatography. Ther. Drug Monit., 14: 138-141 (1992).

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