HPLC Analysis of Rapamycin

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A fast gradient method was developed for the separation of rapamycin from an extracted human plasma matrix using a ZirChrom®-PBD column. The unique chemistry of this zirconia-based stationary phase allows for elution of the majority of the matrix interferences early in the gradient, while rapamycin elutes as a sharp, symmetrical peak in the middle of the gradient slope, free of interferences.

Introduction

The structure of rapamycin, also known as Sirolimus, is shown below in Figure 1. Rapamycin is one of a number of macrolide immunosuppresant drugs, and is commonly used in combination with other immunosuppresants such as cyclosporine (1). Previous reports have indicated that cis- and trans- isomer forms of rapamycin exist, often leading to split or double peaks in reversed-phase separations. This phenomenon has been observed by UV detection, and the identity of both peaks as rapamycin has been confirmed by LC/MS (1,2).

Previous reports of the analysis of rapamycin by UV detection have commented on overly long analysis times (45 minutes) mainly due to matrix interences (3). Several reports of detection by mass spectrometry have indicated analysis times of 10-15 minutes for plasma samples (1,4).

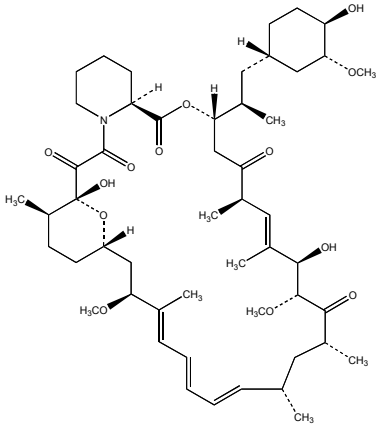

Figure 1. Structure of rapamycin

Experimental

The chromatographic conditions for the analysis of rapamycin were as follows:

Column:	50 mm x 4.6 mm id. ZirChrom®-PBD
Mobile phase:	Gradient elution from 5-95% B over 5 minutes A: 20mM Ammonium phosphate, pH 5.0 B: Acetonitrile
Flow Rate:	2.0 ml/min.
Temperature:	75 °C
Detection:	278 nm
Inj. Volume:	5 µl

Chromatograms obtained by injecting a rapamycin standard (blue trace), and an extracted plasma sample (red trace) are shown in Figure 2. We note that the column temperature in this separation is 75 °C, which is important to obtain good peak shape for this compound.

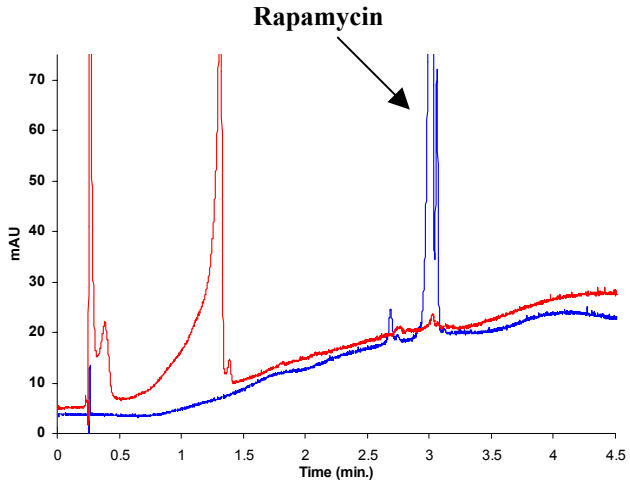

Figure 2: Analysis of rapamycin standard (blue trace), and rapamycin in extracted plasma sample (red trace).

In the chromatogram for the rapamycin standard, a small shoulder is often observed on the tail of the peak and is most likely an isomer of the main peak.

In the red trace it is clear that all of the matrix components are eluting very early in the gradient, eliminating any potential interferences with quantitation of the rapamycin.

ZirChrom columns combine the high efficiency usually associated with silica columns with unsurpassed chemical and thermal stability, resulting in this extraordinarily fast and highly selective analysis.

References

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