A simple and sensitive HPLC method for determination of tacrolimus in pharmaceutical dosage forms

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1. Introduction

Tacrolimus, also known as FK506, is a potent immunosuppressive agent commonly used after allogeneic organ transplantation to reduce the risk of organ rejection and to treat autoimmune diseases. This is achieved by inhibiting the creation of the molecule interleukin-2, which promotes the development and proliferation of T cells, which are key to the acquired immunity response.

A simple and sensitive RP–HPLC method with UV detection was developed for determination of tacrolimus in pharmaceuticals.

2. Materials and methods

- The method was performed using Waters — Alliance HPLC system equipped with quadruple pump, separation module e-2695, and automatic sampler (Waters corporation, USA). The detection wavelength was optimized with Waters 2489 UV/Vis Detector. All data were processed with the Empower ® software.
- The separation was performed on a Watters ODS 2 column (125 mm x 4.0 mm, 5µm) with a mobile phase consisted of acetonitrile and water acidified to pH of 4.0, 45:55 (*V/V*). The flow rate was set at 1 mL min^{-1} and UV detection was performed at 210 nm.
- The temperature of the injector was set at 25 ºC and the run time was 15 minutes.
- The method was validated by determination of specificity, linearity, precision, accuracy, limit of detection and limit of quantitation and robustness, following the ICH Q2(R1) guidelines.[2]

3. Standard and sample preparation

- The standard solution was prepared by dissolving the API, TC Reference standard, in a mixture of equal volumes of ACN and H_2O. The working concentration of the standard and the sample solution was 0.15 mg/mL.
- An amount of tacrolimus containing the equivalent of 1.5 mg, was dissolved together with the solvent, added to a volumetric flask and treated on a vortex mixer and an ultrasonic bath.
- The solutions were cooled down to room temperature and the rest of the solvent was added.
- Before the injection in the HPLC system, the standard and sample solutions, were filtered through a 0.45 µm polytetrafluoroethylene (PTFE) filter.

4. Results and discussion

4.1. Specificity (selectivity)

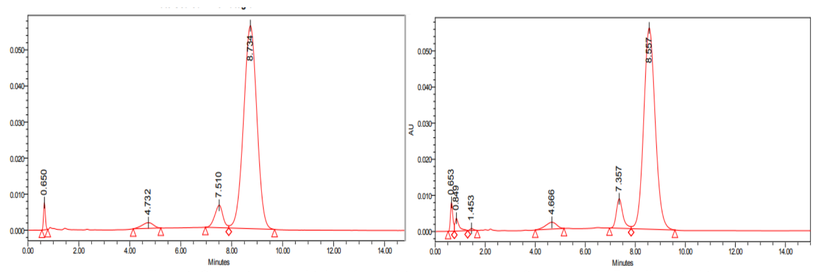
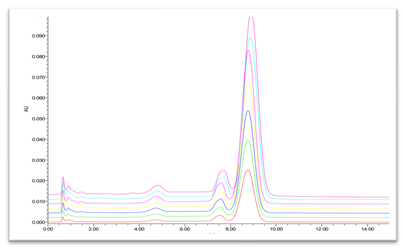


Figure 1. Chromatograms obtained from mixture of standards (left) and sample, Tacrolimus (right)

4.2. Linearity and range



50 % (0.0795 mg/mL)	70% (0.1193 mg/mL)	100% (0.1590 mg/mL)
125% (0.1988 mg/mL)	150% (0.2385 mg/mL)	175% (0.2783 mg/mL)
	200% (0.3180 mg/mL)	

Figure 2. Linearity: Overlay chromatograms of standard solutions ranging from 50—200%

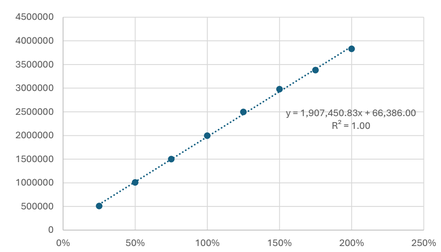


Figure 3. Graphic plot of regression analysis, least square method

4.3. Precision

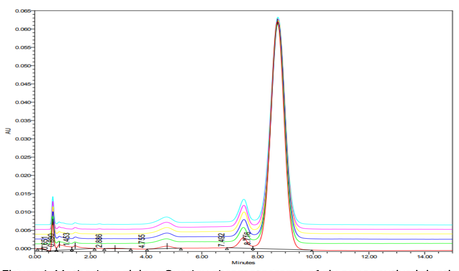


Figure 4. Method precision: Overlay chromatograms of six consecutive injections of standard solutions and samples

Table 1. Results from intermediate precision

Variance 1st analyst (SD2)	0.14
Variance 2nd analyst (SD2)	0.06
F (larger variance/smaller variance)	2.33
Arithmetic mean (12 samples, 2 analyst)	99.06
Standard deviation (12 samples, 2 analyst)	0.505
Total Relative Standard Deviation (12 samples, 2 analyst)	0.63

4.4. Accuracy

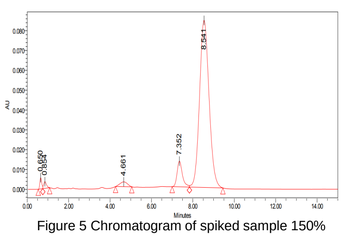


Figure 5 Chromatogram of spiked sample 150%

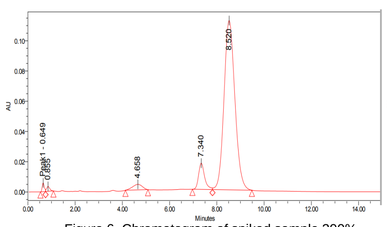


Figure 6. Chromatogram of spiked sample 200%

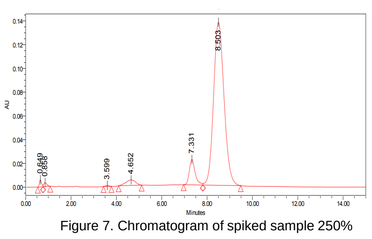


Figure 7. Chromatogram of spiked sample 250%

4.5. Robustness

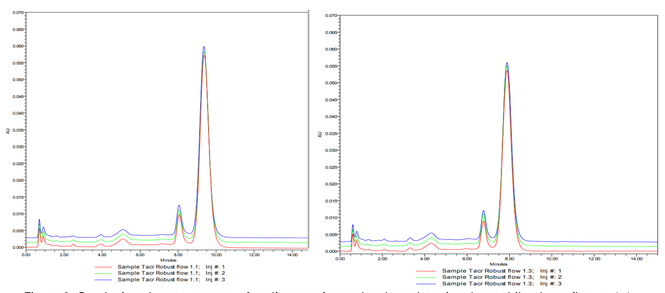


Figure 8. Overlaying chromatograms of replicates of sample when changing the mobile phase flow at 1.1 mL/min and 1.3 mL/min

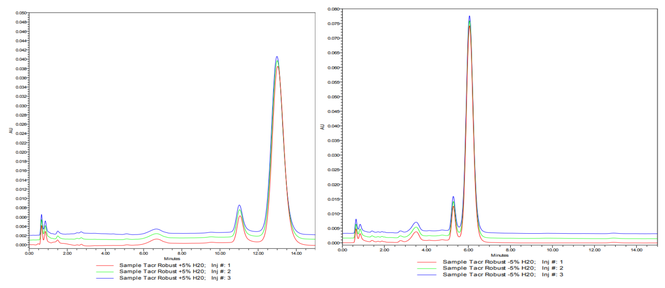


Figure 9. Overlaying chromatograms of replicates of sample when changing the mobile phase component ratio (+5 and -5%)

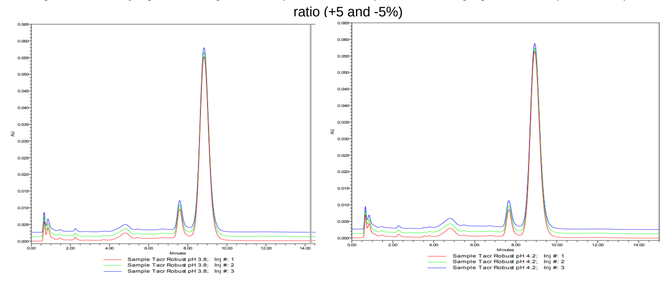


Figure 10. Overlaying chromatograms of replicates sample when changing the mobile phase pH at 3.8 and 4.2

System suitability

- System suitability test is designed to evaluate the components of the analytical system to show that the performance of the system meets the standards required by the method. In our case, the system suitability was evaluated through these parameters: capacity factor, symmetry factor and number of theoretical plates.
- Each of these test parameters was determined by injecting six consecutive replicas of the standard solution, prepared in the working concentration, and after calculating the arithmetic mean of the results obtained for each test parameter, we concluded that this HPLC system was suitable for content determination of tacrolimus in pharmaceuticals.

5. Conclusion

Based on the obtained results of this specialist paper, it can be concluded that the method, based on the use of an HPLC system with a UV/Vis detector, was successfully developed and validated as a simple isocratic chromatographic method.

Due to the lower consumption and easy availability of the constituent components of the mobile phase, as well as the solvent used for the preparation of the standard solution and the samples for analysis, this analytical method is also considered economically viable.

Our method has been properly validated according to the ICH Q2(A1) guide and can be applied in practice. It is selective, linear in the given range, accurate, precise and robust.

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