

MEASUREMENT OF SDZ RAD IN BLOOD BY HPLC-ELECTROSPRAY-TANDEM MASS SPECTROMETRY

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SDZ RAD [40-O-(2-hydroxy)ethyl-rapamycin, 958.3 Da] is a macrolide immunosuppressant drug currently under clinical investigation for the prophylaxis of acute rejection in solid organ transplant recipients. We report in this study a fully validated method for the quantification of SDZ RAD using HPLC-tandem mass spectrometry.

Whole blood samples (500 µl) were prepared by protein precipitation, using an organic solvent containing internal standard (40-O-(3'-hydroxy)propyl-rapamycin, 972.3 Da), followed by C18 solid phase extraction (300 mg, Isolute). A Novapak C18 column (100 x 2.1 mm i.d., Waters) was used as the analytical column and operated at 50°C. The mobile phase consisted of 80% methanol:20% ammonium acetate buffer (pH 5.1), pumped at flow rate of 0.2 ml/min (1:10 post-column split). Samples were submitted to an API III quadrupole mass spectrometer (PE SCIEX) via an ionspray (pneumatically assisted electrospray) interface operating in positive ionisation mode. Using an orifice potential of 40 V, the predominant precursor ion was the ammoniated species, $[M+NH_4]^+$. Mass spectrometric detection was by selected reaction monitoring using the mass transitions, m/z 975.7 \rightarrow 908.7 and m/z 989.8 \rightarrow 922.8 for SDZ RAD and the internal standard, respectively. The retention times of SDZ RAD and internal standard were 5.4 min and 6.0 min giving a total chromatographic run time of 8.0 min.

Specificity of the method was tested by analysing blood samples from 30 transplant recipients not receiving SDZ RAD therapy and blood supplemented with other commonly administered drugs. No interference was observed at the retention times of the analytes. The assay was linear from 0.5 µg/l to 100 µg/l ($r^2 > 0.996$, $n=9$). The intra- and inter-day inaccuracy and imprecision, determined using quality control samples at 0.5, 1.2, 20.0, and 75.0 µg/l, was $\leq \pm 5.4\%$ and $\leq 7.6\%$, respectively ($n=5$). The mean absolute recoveries (\pm SD) of SDZ RAD and the internal standard were $79.1 \pm 6.8\%$ and $83.4 \pm 5.9\%$, with a mean relative recovery (\pm SD) of $94.8 \pm 3.8\%$. Blood samples supplemented with SDZ RAD were found to be stable for up to 6 h on the bench. While extracted samples were stable in the autosampler for up to 24 h. No significant difference in SDZ RAD concentrations was observed for freshly prepared SDZ RAD supplemented samples and those taken through 3 freeze-thaw cycles ($p > 0.39$).

In conclusion, the reported method provides accurate, precise and specific measurement of SDZ RAD in human blood and is currently supporting pharmacokinetic studies in phase II and III clinical trials.

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