

THERAPEUTIC DRUG MONITORING OF IMMUNOSUPPRESSANT DRUGS USING HPLC-MASS SPECTROMETRY

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There is currently an expanding range of new immunosuppressant drugs for the clinician to use for the prophylaxis of rejection in solid organ transplant recipients. Most of these drugs require the measurement of blood concentrations with subsequent dosage adjustment to maximise efficacy while minimising toxicity. Tacrolimus and sirolimus are two such drugs. They are administered in low dose and have low circulating blood concentrations (2 to 20 µg/L) and thus are a challenge to the analytical scientist. Providing a clinical service requires not only acceptable analytical performance, in terms of accuracy and precision, but also must meet the "clinical" expectation of timely result delivery. Consequently, methods must be rapid, reliable and robust. We have successfully utilised HPLC-mass spectrometry to provide a therapeutic drug monitoring service of these drugs. Further, several commercial immunoassays for tacrolimus and sirolimus have been evaluated against our methods.

Blood samples were prepared by protein precipitation followed by C18 solid phase extraction. Extracts were submitted to mass spectrometric detection (selected reactant monitoring) via an electrospray interface. The predominant precursor ion for these neutral drugs were the ammoniated species $[M+NH_4]^+$. The mass transitions monitored for tacrolimus and sirolimus were $m/z821.5_768.4$ and $m/z931.7_864.6$, respectively. Upper and lower limits of quantification, 0.2 µg/L and 100 µg/L, were well outside the expected therapeutic concentrations. Chromatographic analysis time were less than 10 min/sample for one alone and 12 min/sample for the simultaneous measurement of tacrolimus and sirolimus.

We currently analyse >5000 tacrolimus and >2000 sirolimus patient samples per year using these methods. Analytical performance can be evaluated by result from an external quality assurance scheme. Comparison of target concentrations against measured concentrations for external quality controls yielded the following equations: Target tacrolimus = $0.060 + 1.07 \times \text{measured tacrolimus}$ ($r^2=0.977$, $n=25$), Target sirolimus = $0.185 + 0.999 \times \text{measured sirolimus}$ ($r^2=0.990$, $n=19$).

In conclusion, we have shown that HPLC-mass spectrometry can be used in the clinic for therapeutic drug monitoring of immunosuppressant drugs and envisage the future acceptance of this technique.
