Organ transplantation

Determination of sirolimus and tacrolimus with HPLC/MS in whole blood

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Sirolimus (molecular mass 913.6 atomic mass units (amu)) is a 16-membered triterene macrolide lactone with a hemiketal-masked α,β-dioxocarboxamide in a 23-membered ring (figure 1). Both macrolides sirolimus (SRL) and tacrolimus (TRL) are used as immunosuppressants in patients after organ transplantation. The combination of SRL and TRL results in synergistic immunosuppression. Several drugs often used after organ transplantation (not only SRL and TRL) which are cytochrome P4503A inducers (e.g. rifampicin), affect blood concentrations of immunosuppressants and therefore require dose adjustment. As a result, regular therapeutic drug monitoring and blood concentrations guided dosing regimes have been recommended.

The development of simultaneous assays for therapeutic drug monitoring of immunosuppressants such as SRL and TRL has a distinct economic advantage, since only SRL and TRL pre-dose (trough level) concentrations are targeted in the range of 4 - 12 µg/L; TRL concentrations are usually slightly higher (6 - 15 µg/L). In comparison with TRL, an FDA-commercially-approved immunosuppressant drug such as SRL and TRL (0.1 - 1.5 µg/L) is a mixed substrate of SRL and TRL. The combination of SRL and TRL results in synergistic immunosuppression. To demonstrate the observed range of concentrations, the last point curve of SRL and TRL (0, 1, 2.5, 5, 10, 20, 30 and 40 µg/L) was constructed. Each calibration was linear in the range tested (all r² > 0.998). The lower limits of quantification (LLOQ) were 0.5 µg/L (TRL) and 1 µg/L (SRL). Precision and accuracy were determined by analysis of QC samples prepared at four concentrations spanning the calibration range. All values met the pre-defined acceptance criteria of ± 15 %.

Replacing the common vacuum manifold (BAKER) by an automated extraction procedure improved the reproducibility. Moreover, reducing the incubation time of samples to 15 minutes eliminated the need for longer extraction procedures.

A carry-over effect, especially during the automated extraction step was excluded by alternately extracting spiked blood samples containing 200 µg/L (out-of-range samples) and blank samples (c = 0 µg/L). The values of the intraday coefficient of variation (CV, n = 20) for QC samples for automated extraction were 12.5 % at 3 µg/L for SRL, 9.7 % at 6 µg/L, 6.8 % at 15 µg/L and 10.5 % at 3 µg/L for TRL, 7.0 % at 6 µg/L, 5.0 % at 15 µg/L. The accuracy was additionally monitored by monthly participation in the International Proficiency Testing Scheme. The results of the presented method are given in figures 3 and 4.

The relatively low column temperature of 40 °C significantly improved the shelf-life of the analytical column. We were able to carry out approx. 100 samples without the necessity of altering column or mobile phase. More than 1000 SRL/TRL samples were analysed using the same guard column and analytical column with no loss in sensitivity, accuracy or precision.

More than 1000 blood samples (trough level samples) of kidney, liver and other transplant patients (18-66 years of age) were analysed for SRL and/or TRL. All patients underwent transplantation of at least one organ. To demonstrate the observed range of concentrations, the last 3 months of the same six month period were analysed.
Organ transplantation

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Sirolimus (molecular mass 913.6 atomic mass units (amu)) is a 13-membered triene macrolide lactone with a hemiketal-masked α,β-unsaturated lactam in a 23-membered ring (figure 1). Both macrocycles sirolimus (SRL) and tacrolimus (TRL) are used as immunosuppressants in patients after organ transplantation. The combination of SRL and TRL results in synergistic immunosuppression.

Several drugs often used after organ transplantation (not only SRL and TRL) which are cytochrome P450 substrates, inhibitors (e.g. ketoconazole) and/or inducers (e.g. rifampicin), affect blood concentrations of immunosuppressants and therefore require dose adjustment. As a result, regular therapeutic drug monitoring and blood concentrations guided dosing regimes have been recommended.

The development of simultaneous assays for therapeutic drug monitoring of immunosuppressants like SRL and TRL has a distinct economic advantage, since the sample requirements (i.e. matrix and time collection) are the same for both drugs of interest. Whole blood has been recommended as the sample matrix because of high concentrations of SRL and TRL in the erythrocytes.

To assure the safety of SRL (and TRL) for patients receiving the drug, it is important that the laboratories participate in a proficiency testing scheme. In order to attain the status of a "reference laboratory", the participant must analyse a set of approx. 120 samples which will demonstrate the accuracy, reproducibility and reproducibility of the measurement of SRL. In March 2002, we successfully passed the testing scheme with the HPLC-MS method reported.

Results and Discussion

The specific semi-automated HPLC-MS assay presented allows the simultaneous quantification of sirolimus (SRL) and tacrolimus (TRL). The limit of quantification (LLOQ) and the accuracy of the HPLC-MS method reported.

To demonstrate the observed range of concentrations, the last point curve of SRL and TRL (0, 1, 2, 5, 10, 20, 30 and 40 µg/L) was constructed. Each calibration was linear in the range tested (all r² > 0.998). The lower limits of quantification (LLOQ) were 0.5 µg/L (TRL) and 1 µg/L (SRL). Precision and accuracy were determined by analysis of QC samples prepared at four concentrations spanning the calibration range. All values met the pre-defined acceptance criteria of ± 15 %. Replacing the common vacuum manifold (BAKER) by an automated extraction procedure improved the reproducibility.

A carry-over effect, especially during the automated extraction step was excluded by alternately extracting spiked blood samples containing 200 µg/L (out-of-range samples) and blank samples (c = 0 µg/L). The values of the intraday coefficient of variation (CV, n = 20) for QC samples for automated extraction were 12.5 % at 3 µg/L for SRL, 7.7 % at 6 µg/L, 6.8 % at 15 µg/L and 10.5 % at 30 µg/L for TRL, 7.0 % at 6 µg/L, 5.0 % at 15 µg/L. The accuracy was additionally monitored by monthly participation in the International Proficiency Testing Scheme

The relatively low column temperature of 40 °C significantly improved the shelf-life of the analytical column. We were able to carry out approx. 120 samples without the necessity of altering column or mobile phase. More than 1000-SRL/TRL-samples were analysed using the same guard column and analytical column with no loss in sensitivity, accuracy or precision.

More than 1000 blood samples (rough level samples) of kidney, liver and other transplant patients (18-66 years of age) were analysed for SRL and/or TRL. All patients underwent transplantation of at least one organ. To demonstrate the observed range of concentrations, the last

Figure 2: GCMS-2010 single quadrupole with GCMSsolution software

Figure 3: Measured sirolimus concentrations (µg/L) within International Proficiency Testing Scheme – a representative six month period

Figure 4: Calculated z-values during the same six month period