146–254 μg/L. As long as more valid data are lacking, serum concentrations of 146–254 μg/L can be considered as a preliminary target range for TDM of aripiprazole. Moreover, it should be stressed that because pure and authentic reference materials for aripiprazole and its metabolite were not available, the accuracy of the method described here remains uncertain.

We thank Sandra Heller for skillful technical assistance.

Note Added in Proof: The manufacturer (Bristol-Myers Squibb) has subsequently provided us with pure aripiprazole. We prepared samples containing 50, 250, 500, and 1000 μg/L and assayed them with our method. The results obtained were 49, 240, 488, and 995 μg/L.

References


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The macrolide immunosuppressants sirolimus (Rapamycin; SRL) and its 40-O-(2-hydroxyethyl) derivative everolimus (Certican®; SDZ-RAD) are approved immunosuppressive agents for organ transplantation, but there is evidence that they can interfere with co-medications, including other immunosuppressive agents (1).

SRL and SDZ-RAD are metabolized in humans by the hepatic and intestinal cytochrome P450 3A subfamily (2,3), which also metabolizes many other agents. Both have the same mechanism of action but differ in their half-lives and treatment regimes with co-medications. Because of their narrow therapeutic spectrums, the clinical application of SRL and SDZ-RAD, like other immunosuppressants, is complicated by substantial intra- and inter-individual variations in drug absorption, distribution, metabolism, and elimination (4–6). Their concentrations must be carefully monitored in posttransplantation regimens, and dosage adjustment to reduce the risk of toxicity and rejection depends on the analytical method used for monitoring (7–9).

Several HPLC methods with ultraviolet (UV) detection or mass spectrometry (MS) are available for measurement of SRL and SDZ-RAD individually (10–16), and liquid chromatography (LC) methods using MS or tandem MS allow simultaneous measurement of these compounds in combination with other immunosuppressants (17–22). The purported advantage of LC-MS methods over LC-UV methods is the ability to monitor multiple drugs within the same analytical run. As the number of monitored compounds increases, however, instrument dwell time or the number of data points acquired for each mass transition may decrease and negatively affect the limit of quantification or precision (6,23). Furthermore, these methods are expensive and not readily available in most laboratories.

The presented reversed-phase HPLC-UV method for the simultaneous measurement of SRL and SDZ-RAD in whole blood was designed to allow easy sample preparation, to be simple to perform, to be cost- and time-effective, and to have low limits of detection. Separation and quantification of 2 drugs in parallel are achieved by isocratic elution. Both drugs can be detected in a single run, which is a great advantage when transplant patients are switched from one drug to the other.

The SRL was kindly supplied by Wyeth-Ayerst Research (Princeton, NJ), the SDZ-RAD by Novartis Pharmaceuticals Corporation (Basel, Switzerland), and the internal standard (IS), ascomycin, by Sigma-Aldrich (Vienna, Austria). Stock solutions of each compound were prepared in methanol at concentrations of 1 mg/L for SRL and SDZ-RAD and 20 mg/L for ascomycin; all stock solutions were stored at −20 °C. The solutions were stable at −70 °C for at least 16 months. All analytical- or HPLC-grade reagents were purchased from Merck.

Calibrators and in-house control samples were prepared in separate drug-free human whole-blood stock solutions: 6 non-zero concentrations (nominal values of 2.5, 5.0, 10.0, 20.0, 40.0, and 80.0 μg/L) and 4 quality controls with different concentrations (nominal values of 2.5, 7.5, 20.0, and 40.0 μg/L) for SRL and SDZ-RAD. Calibrators and controls were aliquoted into polypro-
Fig. 1. Representative chromatograms of SRL, SDZ-RAD, and the IS (ascomycin) in whole blood.
Retention times for the IS, SRL, and SDZ-RAD were 12.2, 17.3, and 19.2 min, respectively. (A), drug-free blank human blood; (B), blood samples from a patient receiving SDZ-RAD (7.4 μg/L) after kidney transplantation; (C), blood samples from a patient receiving SRL (20.8 μg/L) after heart transplantation; (D), drug-free human blood with both drugs added as internal quality control (7.6 μg/L SRL and 7.3 μg/L SDZ-RAD); (E), pooled samples from patients receiving SRL (11.6 μg/L) and SDZ-RAD (2.7 μg/L); (F), commercially available control used as external quality control (level III from ClinChek; 12.9 μg/L SRL and 17.8 μg/L SDZ-RAD).
Potential metabolites in pure form, they were not checked. For sample preparation, 500 μL of the calibrator, quality control, or patient sample (EDTA-anticoagulated whole blood) was placed in a screw-top test tube and mixed with 500 μL of NaCl (9 g/L) solution, followed by addition of 50 μL of IS (ascomycin) and 1.5 mL of 0.1 mol/L HCl. The mixture was vigorously vortex-mixed for 20 s, after which ~6 mL of a mixture of diethyl ether and 1-chlorobutane (75:25 by volume) was added. The tubes were capped and centrifuged at 3200g for 7 min at room temperature. The upper phase was transferred to another screw-top test tube containing 2.5 mL of 0.1 mol/L NaOH, agitated for 3–5 min, and then centrifuged at 3200g for 5 min. The upper organic phase was transferred to a conical tube and vortex-mixed for 30 s and then centrifuged at 3200g for 7 min at room temperature. The hexane layer was pipette off and discarded. The residue was transferred to autosampler vials, and 90 μL was injected into the HPLC system.

The HPLC system consisted of Hewlett-Packard (Agilent) 1100 Series components including a pump, autosampler, and UV wavelength detector set to a wavelength of 287 nm; the detector signals were recorded with an HP Chemstation (A.05.01) and integrator. Separation was on a Zorbax SB-C18 column [150 × 4.6 mm (i.d.); 3.5-μm bead size], with the thermostatic chromatograph oven set at 55 °C. The mobile phase, a mixture of methanol–water–acetonitrile (50:28:22 by volume), was delivered at 0.9 mL/min for 20 s, after which 500 μL of hexane was added to each tube. The tubes were then vortex-mixed for 30 s and centrifuged for 3 min at 3200g. The hexane layer was pipetted off and discarded. The residue was transferred to autosampler vials, and 90 μL was injected into the HPLC system.

The reliability and specificity of this method were further checked with 2 different commercial multi-immunosuppressant control samples (as external quality controls) of SRL and SDZ-RAD: 4 concentrations from ChromSystems and 3 from RECIPE ClinChek®. The method was free of interfering peaks from other immunosuppressant drugs. Fig. 1F shows a chromatogram of the level III external quality control from ClinChek (12.3 μg/L SRL and 17.8 μg/L SDZ-RAD).

The method was evaluated for linearity, specificity, and precision. The calibration curves showed excellent linear relationships between the peak-height ratios for the drugs and IS. The correlation coefficient (r) was >0.998 for all compounds (n = 5). The lowest quantifiable concentration for SRL and SDZ-RAD was 2.5 μg/L. The limit of detection, defined as the lowest concentration of drug giving a signal-to-noise ratio >3, was 1.0 μg/L for both. The lower limit of quantification, 2.5 μg/L, is sufficient to detect subnormal concentrations in whole blood; this value is suitable for the concentrations of SRL and SDZ-RAD found in transplant patients, in whom proposed trough concentrations would be >3 μg/L (24, 25). If 1 mL of blood is used, the lower limit of quantification can be lowered to 1.25 μg/L.

We evaluated intra- and interday precision by assaying quality controls containing 4 different concentrations of SRL and SDZ-RAD. For intraday precision, the samples were stored at −20 °C and measured on the same day; for interday precision, we used the mean values of quality controls measured on 6 different days. The intra- and interday imprecision (CVs) was 3.2%–10% and 2.0%–9.6%, respectively, for SRL and 4.0%–9.3% and 3.4%–8.3% for SDZ-RAD; the results are summarized in Table 1.

In addition to in-house control samples, we also used 2 commercial multi-immunosuppressant control samples containing SRL and SDZ-RAD. The interday CVs for these commercial quality controls were compared with the manufacturer-stated values for SRL and SDZ-RAD. The total CVs for the commercial quality controls for SRL at concentrations of 2.48, 9.00, 19.2, and 38.6 μg/L were 4.5%–10% and for SDZ-RAD at concentrations of 2.47,
4.38, 8.82, and 30.1 µg/L were 4.2%–11%. These data are not statistically different from the CVs for the in-house quality controls and from LC-tandem MS data reported by Annesley (26) for commercial quality controls from ChromSystems for SRL. Similar results were also obtained with other quality controls (levels I–III) from ClinChek (data not shown).

Extraction with a mixture of diethyl ether and 1-chlorobutane before HPLC analysis gave high and reproducible recovery of both drugs. To determine recovery, we added known concentrations of SRL and SDZ-RAD (2.5, 7.5, and 40.0 mg/L) to the blood samples and measured 3 replicates of each sample. We compared the response obtained for the extracted samples to which the drug had been added before extraction with the response of extracted blank blood samples to which the drug was added immediately before injection. The mean recoveries were 93.6% for SRL and 96.0% for SDZ-RAD.

We compared the stabilities of SRL and SDZ-RAD in whole-blood samples by measuring, in triplicate assays, samples to which the drugs had been added at 4 different concentrations on the day of the analysis and samples to which the same concentrations had been added in advance and stored under different conditions. There was no significant loss after storage at ambient temperature (25 °C) for 24 h, at 4 °C over a period of 7 days (days 1, 3, 5, and 7), and at −20 °C for 6 months. We tested the stability of the sample extracts in the autosampler tray by extracting 5 aliquots of the quality-control samples; after extraction; they were stable for 36 h in the tray.

In conclusion, we present a method incorporating liquid–liquid extraction and isocratic chromatography with UV detection for the measurement of SRL and SDZ-RAD concentrations in whole blood. Unlike previously reported HPLC assays, this method uses an IS (ascomycin) that is readily available commercially. The process, a modified version of an HPLC method for determination of cyclosporin A (27), shows reproducible recovery of SRL and SDZ-RAD. Numerous multi-immunosuppressant external quality controls (or calibrators) are available, and this HPLC-UV method can also use these external quality controls without interference. The advantage is that 2 different drugs can be analyzed quickly and economically with the same sample preparation and same chromatographic conditions in a single run.

References


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