To the Editor:
Therapeutic drug monitoring of immunosuppressive agents by liquid chromatography (LC) coupled with mass spectrometry (MS) can provide highly selective and sensitive quantification from biological fluids. Interlaboratory performance for cyclosporine, tacrolimus, and sirolimus, as assessed by LC/MS, in proficiency testing (PT) programs [e.g., College of American Pathologists (CAP)] has been poor, albeit improving, although individual laboratories report excellent interassay imprecision (CV <5.5%) at drug concentrations across analytical ranges (3–6). Lack of industry-wide standardization may contribute to variations in PT program performance across MS laboratories because it requires that those laboratories develop “home brew” assays, use reference materials with potentially different purities obtained from numerous sources, and prepare either whole-blood calibrator/quality-control (QC) materials in house or purchase commercial materials that may be verified only for a specific procedure. In addition, most PT samples are unlike routine specimens, being prepared from either artificial or whole-blood–like matrices (sometimes pooled patient blood) that are subjected to the rigors of batch preparation and shipping.

I describe here an experience of specific and unusual performance of PT samples, which likely resulted from instabilities of the internal standard (IS) in organic solvents and which may be of value to scientists struggling with the development or long-term performance of MS-based methods and to PT program providers who wish to help marginally performing laboratories improve.

My laboratory has single-quadrupole LC/MS and tandem LC/MS/MS systems with independent methods for tacrolimus. Both assays use ascomycin (Sigma-Aldrich) as IS and are based on protein precipitation with zinc sulfate and organic solvent. Tacrolimus is dissolved in acetonitrile (ACN) and stored at −40°C in glass is used for preparing whole-blood calibrators/QC materials. A common IS stock solution in ACN is stored at −40°C (~1 mg in a 3-mL glass volumetric flask) and used to make the working IS for each assay.

The LC/MS IS working solution is prepared by diluting the stock to 10 mL in glass with ACN (2 ng/10 μL) and storing at −20°C. For the LC/MS extraction, 10 μL of IS working solution, 250 μL of whole blood (0.5–40 μg/L tacrolimus), 250 μL of 0.1 mol/L sodium carbonate, 50 μL of 300 g/L zinc sulfate heptahydrate, and 600 μL of methanol are used.

RC/MS/MS IS working solution is prepared by diluting the LC/MS IS working solution to 50 mL in glass (0.2 ng/200 μL) with storage as above. For the LC/MS/MS extraction, 20 μL of whole blood, 80 μL of 0.1 mol/L zinc sulfate heptahydrate, and 200 μL of IS working solution are used.

Receipt of a “poor-quality” lot of ACN in which ascomycin was stable over several months had been identified. The LC/MS/MS IS working solution was therefore prepared by diluting the suspect methanolic LC/MS working solution with this new ACN. A series of PT samples analyzed with this solution showed a mean deviation of 10.5% (maximum, 21.1%; n = 27) with QC samples at 3.4% (n = 6), but all with normal IS peaks.

Preparation of the LC/MS IS working solution with the good-quality ACN corrected the problem, as evidenced by analysis of a series of 18 PT samples (deviation, 0.8%; deviation for QC samples, 3.2%; n = 6). Similarly, an LC/MS/MS IS working solution prepared from this ACN-based LC/MS working solution gave a mean deviation of 1.3% for a series of PT samples (n = 21; deviation for QC samples, 0.5%; n = 12). Similarly phenomena were observed with CAP samples (data not shown). Subsequent PT performance of both methods has been good: 3.7% deviation by LC/MS (n = 30) with −1.0% deviation for QC samples, and −2.6% (n = 36) by LC/MS/MS with −2.8% deviation for QC samples.

In addition to the original IS instability problem with ACN for the LC/MS/MS assay, these observations implicated the methanolic LC/MS IS working solution as the source of the aberrant performance of PT samples. Interestingly, caution has been urged against preparing methanolic...
Influence of Sampling and Storage Conditions on B-Type Natriuretic Peptide Immunoactivity for 3 Automated Assays

To the Editor:

B-Type natriuretic peptide (BNP) is a marker for diagnosis and prognosis of congestive heart failure (CHF). In response to the analytical uncertainties of automated BNP assays, the IFCC Committee on Standardization of Markers of Cardiac Damage published recommendations for the evaluation of in vitro BNP stability (1).

We analyzed the effect of exposure to room temperature for 24 h on whole-blood BNP immunoreactivity or the effect of 1 freeze–thaw (FT) cycle on plasma BNP immunoreactivity in 3 different assays. Blood samples from 4 CHF patients were collected on ice with Sarstedt tubes containing potassium EDTA (1.6 mg/mL EDTA), EDTA with aprotinin (500 kallikrein-inhibitory units/mL), and EDTA with benzamidine (80 μmol/mL). Whole blood was split into 2 aliquots: one was centrifuged (2400 g for 10 min) for immediate assay (baseline), with the remaining plasma stored at −20 °C; the other aliquot was allowed to stand at room temperature for 24 h under constant gentle mixing before centrifugation and assay. The procedure generated a total of 36 aliquots. BNP recoveries were calculated as percentage of baseline.

Baseline BNP values were 1133–4854 ng/L (mean, 1789 ng/L) with the AxSYM (Abbott Diagnostics), 593–3232 ng/L (mean, 1106 ng/L) with the ADVIA Centaur (Bayer Diagnostics), and 540–3839 ng/L (mean, 1359 ng/L) with the Beckman Coulter Access 2 (BioSite reagents). These values were not affected by protease inhibitors (one-way ANOVA for effect of protease inhibitors, P = 0.93, 0.92, and 0.95 for the AxSYM, Centaur, and Access 2, respectively). Exposure of whole blood to room temperature for 24 h led to a mean decrease of 21%. Neither the sampling conditions nor the assay affected BNP loss (Table 1). These data do not support the claims in the Access 2 and Centaur package inserts (BioSite technical bulletin, December 2004; Bayer 131075 Rev D, 2003), stating that BNP is stable in whole blood for 24 h at room temperature. After 1 FT cycle, the BNP loss averaged 10% and was equivalent for all assays, regardless of the presence of aprotinin or benzamidine, in contrast to one report showing that 1 FT cycle decreased BNP by 36% (2).

We also evaluated BNP stability during shorter storage times (room temperature and 4 °C), without agitation, using conditions close to those likely to be used with samples from outpatient clinics or physicians’ offices. EDTA samples from 5 CHF patients with baseline BNP values of 404–2746 ng/L (mean, 1623 ng/L) on the AxSYM, 232–1694 ng/L (mean, 933 ng/L) on the Centaur, and 234–1917 ng/L (mean, 985 ng/L) on the Access 2 were split and allowed to stand at room temperature and 4 °C for 4 and 8 h before centrifugation and assay. At room temperature, mean (SD) measured values after 4 h were 88 (8)%), 94 (6)%), and 94 (6)% of the baseline values on the AxSYM, Centaur and Access 2, respectively, and after 8 h, the measured concentrations were 85 (14)%), 91 (7)%), and 89 (6)% of baseline values. At 4 °C, after storage for 4 h, measured values were 95 (9)%), 90 (8)%), and 100 (17)% of baseline values on the AxSYM, Centaur, and Access 2, respectively, and after 8 h, they were 90 (10)%), 88 (10)%), and 99 (15)% of baseline values. No significant time or instrument effects were evidenced by ANOVA analysis for storage at room temperature (P = 0.37 and 0.46, respectively) and 4 °C (P = 0.48 and 0.22, respectively). The overall loss of BNP reactivity was 10%, a value comparable to the effect of 1 FT cycle. At 4 °C, loss of BNP immunoreactivity was somewhat lower, averaging 6%. Our results confirm the AxSYM claim, which was the only package insert (8G82-20) reporting the 4 h BNP stability at room temperature. In contrast, using the Biosite BNP POCT Triage® meter, Yeo et al. (3) reported a significant BNP loss after 4 h of storage at room temperature.

In conclusion, our data indicate that protease inhibition does not pro-

References

4. Immunosuppression, Louisville, Kentucky, April 18, 2005).
6. Keevil BG, McCann SJ, Cooper DP, Morris MR. Evaluation of a rapid micro-scale assay for tacrolimus solutions because an adduct forms that may or may not affect performance (6,7). I have observed this tacrolimus, but not ascomycin, adduct. However, in my laboratory methanolic ascomycin solutions are now being avoided.

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