Letters to the Editor

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References

More on Methanol-Associated Matrix Effects in Electrospray Ionization Mass Spectrometry

To the Editor:

Based on the observations reported by Annesley regarding methanol-associated matrix effects (1), I changed from the use of Burdick and Jackson (B&J)1 to EMD Chemicals (EMD) methanol in the mobile phases for tacrolimus, cyclosporine, and sirolimus procedures that used electrospray ionization on single LC-MS and tandem quadrupole LC-MS (LC-MS/MS) instrument systems. Presented herein are observations that supplement Annesley’s observations regarding the influence that brand of methanol can have on analytical performance.

I routinely performed tacrolimus and cyclosporine procedures using LC-MS/MS and sirolimus using LC-MS. The tacrolimus and cyclosporine procedures have been described previously (2, 3). The sirolimus procedures (unpublished data) were similar to the others. The same sample preparation was used for both LC-MS and LC-MS/MS analysis. Importantly, methanol supplemented with ammonium acetate and formic acid (prepared in and used directly from the manufacturer’s bottle) was the LC-MS/MS mobile-phase organic component, in which ammonium adducts were monitored. For LC-MS methods we used methanol (directly from the manufacturer’s bottle), and the aqueous component, water supplemented with sodium formate and formic acid, ensured sodium adduct signals for monitoring.

The change in brand of methanol from B&J to EMD did not affect the performance of the tacrolimus or cyclosporine LC-MS/MS assay, although cyclosporine and cyclosporin D (internal standard) peak areas increased moderately (15%). However, there was an immediate adverse effect on the sirolimus LC-MS procedure; performance worsened progressively until, on the sixth analytical run, the assay failed. Of note was the effect on the internal standard (ascomycin), for which the mean within-run peak height unexpectedly decreased between 10% and 36% from values in previous runs with B&J methanol. Peak height was used for tacrolimus and sirolimus LC-MS procedures because it reduced the need for manual reintegration, especially for samples with low concentration of analyte. Peak height was shown to provide performance specifications equivalent to peak area during method validation.

The within-run variation, a criterion for analytical run acceptance (i.e., ≤5%), was 9.1% initially, then 4.5%–7.3% subsequently. Fresh whole blood standard curve parameters were acceptable; however, the accuracy of the QC materials (fresh whole blood supplemented, divided

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1 Nonstandard abbreviations: B&J, Burdick and Jackson; EMD, EMD Chemicals; LC-MS/MS, tandem quadrupole LC-MS.
Table 1. Influence of source of methanol in mobile phase on mass spectrometer response.

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<th>Sample, parameter</th>
<th>Exp SRL Conc&lt;sup&gt;c&lt;/sup&gt;</th>
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<th>IS peak area</th>
<th>Calc SRL Conc&lt;sup&gt;c&lt;/sup&gt;</th>
<th>SRL peak area</th>
<th>IS peak area</th>
<th>Calc SRL Conc</th>
<th>SRL area ratio</th>
<th>IS area ratio</th>
<th>P&lt;sup&gt;e,f&lt;/sup&gt;</th>
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</table>

Standard curve slope

QC: 3.76 76 2370 3.59 35 1273 3.37 2.17 1.86
Mean 2441 1354 2.12 1.81 0.014

P<sup>e,f</sup> 0.003 0.007 0.678 0.134

**Letters to the Editor**

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*a* Omnisol® high-purity solvent for gas chromatography, HPLC, spectrometry, and gradient analysis.

*b* High purity solvent for HPLC, gas chromatography, pesticide residue analysis and spectrometry; B&J.

*c* Expected (Exp) sirolimus (SRL) concentration (Conc) and calculated (Calc) SRL Conc (µg/L).

*d* IS, internal standard.

*e* P value determined by independent sample t-test.

**f** Bold italicized type used for QC value exceeding established range, P value <0.05.

* SRL peak area, IS peak area × 10<sup>-15</sup>.
into aliquots, and frozen until use) decreased progressively. On the second run the high-QC material (mean 32.6 μg/L ± 4%) exceeded the established range, and on subsequent runs both medium (mean 11.9 μg/L ± 5%) and high QC failed. By the sixth run, all materials failed (low, mean 3.76 μg/L ± 5%). Importantly, reanalysis of the samples from each of these runs using LC-MS/MS (with EMD in the mobile phase) met the established acceptance criteria. Subsequent reversion to B&J for the sirolimus LC-MS procedure normalized assay performance immediately!

To document this phenomenon more clearly, a sirolimus analytical run, consisting of a standard curve and QC materials, was analyzed on the LC-MS/MS system by using EMD and then B&J. Another set of samples was analyzed on the LC-MS system in similar fashion.

When EMD was compared to B&J, peak areas for sirolimus increased more than 2-fold, whereas those of the internal standard increased somewhat <2-fold (P < 0.05) with the use of LC-MS/MS. In calibrator and QC materials, peak areas were increased similarly (Table 1, upper panel). Thus the EMD methanol appeared to improve assay sensitivity.

With the use of LC-MS with EMD compared to B&J, sirolimus and internal standard peak areas were decreased, except for sirolimus in QC materials (Table 1, lower panel). Interestingly, the EMD-to-B&J peak-area ratios for sirolimus in the standard materials decreased in relation to concentration. Moreover, with EMD but not B&J methanol, the internal standard peak areas were significantly higher in QC than calibrator materials. These adverse responses of sirolimus and internal standard with the EMD methanol, as well as calibrator and QC materials, lead to procedural failure (all QC values exceeded the established ranges).

After these procedures were performed, EMD was applied routinely to LC-MS/MS procedures and B&J to LC-MS. This protocol provided acceptable performance of all procedures until similar, but not identical, phenomena were observed when an old lot of B&J methanol was used inadvertently. Failure of a sirolimus LC-MS run was due to a concentration-dependent effect on the peak responses for sirolimus in the calibrator but not QC materials. The poor quality of the old B&J methanol was confirmed through separate infusions of old and more recent B&J with acquisition of mass scans (m/z 15–1600). Old B&J methanol evidenced strong signals at approximately 290, 440, and 600 m/z and background noise across the range was approximately 10-fold higher than the more recent lot of B&J methanol. Reanalysis of the samples using the recent B&J methanol produced a successful run.

In summary, differential responses of sirolimus and ascomycin, but not tacrolimus, to methanol in the mobile phase in fresh and previously frozen whole blood matrices affected control of analytical procedures. In no case did methanol compromise the linearity of the calibration, although the slopes certainly differed. Moreover, sirolimus exhibited an unexpected and unacceptable concentration-dependent response. These experiences support the need for continual assessment of analytical performance, with the expectation that aberrations may arise from unexpected sources. Also, as recommended by Annesley and others (1, 4, 5), rigorous evaluation of method performance (including internal standard) is required for procedures that are intended for clinical service.

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