Multi-channeling LC-MS/MS Forensic Methods for High-Throughput Urine Screening to Detect Buprenorphine and Ethanol Use.

Joe Di Bussolo[1], Catherine Lafontaine[1], Mercedes Castillo [2], Thomaskutty Thomas[2], Hashim Othman[2]

[1]Thermo Fisher Scientific, 101 Constitution Blvd., Franklin, MA 02038
[2]Bio-Reference Laboratories, 481 Edward H. Ross Dr., Elmwood Park, NJ 07407

Two forensic LC-MS/MS methods used to detect buprenorphine and/or ethanol use were run on a multichannel UHPLC system utilizing positive-displacement pumps. A maximum throughput of 34 urine samples per hour was achieved when batches were submitted across three channels. Since the data windows of both methods were a little more than 1/3rd of the total run time, adding the fourth channel did not increase sample throughput. However, using all four channels provided assurance that the total throughput would not be compromised in the event of one channel shutting down because of a leak or a column reaching its maximum pressure.

Urine specimens and corresponding calibrators and QCs to be analyzed for buprenorphine and norbuprenorphine were hydrolyzed by incubation with β-glucuronidase solution and then mixed with cold methanol containing buprenorphine-D_3 and norbuprenorphine-D_4 internal standards. After centrifugation, 10 uL injections of supernatants from each preparation provided the desired quantitation range from 5 to 500 ng/mL, which was consistently linear ($r^2 > 0.995$ with 1/X weighting) whether the calibrators were injected into one channel or across all channels.

Urine specimens and corresponding calibrators and QCs to be analyzed for ethyl-glucuronide and ethyl-sulfate were diluted 1:10 with water containing internal standards EtG-D_3 and EtS-D_3. Using 10 uL injections, the desired quantitation range from 100 to 5000 ng/mL was consistently linear ($r^2 > 0.995$ with 1/X weighting) whether when calibrators were injected into one channel or across all channels.

For both methods, internal standard peak areas showed less than 25% coefficient of variation (CV) among calibrators, QCs and specimens (n = 20) on any of the four channels. Retention time variations throughout these batches were less than 3% CV. Results were within +/- 15% of those determined on a conventional multichannel system using reciprocating pumps. Comparatively, the multichannel system with positive-displacement pumps reduced solvent consumption by at least 65%.