EXTRACTION OF BUPRENORPHINE AND NORBUPRENORPHINE FROM URINE SAMPLES USING NEW NBE™ (NARROW BORE EXTRACTION) COLUMNS: FULLY AUTOMATED SAMPLE PREPARATION

David Hall, Emmanuel Chanco, Phil Dimson

SPEware Corp., 14180 Live Oak Ave, Suite J, Baldwin Park CA. 91706

Sample preparation in clinical and toxicology laboratories has historically been very labor intensive. Endpoints of the analytical process, i.e., integration of sample accessioning and of instrumental results into LIMS software, have been streamlined and have improved the reliability of sample identification, but the intermediate steps of preparation of the samples for instrumental analysis have remained, to one degree or another, manual in nature.

In recent years, the use of robotic liquid handlers has done much to address the issues associated with the transfer of samples, buffers, internal standards, and other reagents, in preparation for extraction and purification. The use of barcode readers has been used to create worklists for transfer of sample batch information from LIMS to liquid handlers to GCMS and LCMS systems.

Similarly, automated solid phase extraction devices have addressed the purification steps used to prepare the samples for instrumental analysis. What were once purely multi-step manual processes on vacuum boxes or positive pressure manifolds (column conditioning, sample application, sorbent washing, sorbent drying, and elution) are now performed by devices built specifically for these purposes.

Here, we present data for the extraction and analysis of urine samples using a fully automated platform. The system runs unattended from the time the urine samples are placed on the deck until the sample extracts are ready for transfer to the LCMS autosampler.

The system consists of a SPEware ALD4 96-well automated solid phase extraction platform integrated with an 8-probe Hamilton Microlab® STARlet liquid robotic handler. The STARlet is equipped with a barcode reader and gripper arm capability. Samples in barcode-labelled test tubes are placed on the deck in strip racks. The samples are transferred via disposable tips to a 96-well SPE plate, along with buffer, enzyme, and internal standard, and the plate is heated on the deck of the robot to perform enzyme hydrolysis. The hydrolyzed samples in the SPE plate are then transferred by the gripper arm to the ALD4 for processing.

The ALD4 SPE platform features 96-well SPE capability, with microprocessor controlled positive pressure profiles for liquid application and a switching valve for access to up to 11 solvents. Column conditioning steps (when required), sample application, column washing, sorbent drying, extract collection, and solvent evaporation are all performed by the ALD4 without user intervention.

Buprenorphine and its metabolite norbuprenorphine were chosen as model compounds to demonstrate this process. Analyses for these compounds are required by pain management and rehabilitation management laboratories. Buprenorphine is a relatively potent opioid and LOQ requirements are commensurately low (1-10 ng/mL). Clean sample extracts are needed in order to meet these requirements.

SPEware NBE™ SCX (Narrow Bore Extraction) 96-well plates were used for the extractions. These plates feature reduced dead volume SPE columns, low bed mass, and sorbents optimized for this configuration. The columns lend themselves to the use of elute-and-shoot elution solvents such as methanol / ammonium hydroxide. For this work, however, a more selective elution solvent (ethyl acetate / methanol / ammonium hydroxide 93:5:2) was chosen in order to improve extract cleanliness. Elution solvent volume was optimized for maximum recovery.

Many selective elution solvents typically contain water immiscible, non-HPLC compatible components. Such solvents must be evaporated before dissolving the extracts in HPLC mobile

phase for analysis. This step requires transfer of the collection tubes or plates to a separate device designed for this purpose. The use of Narrow Bore columns, however, permits the use of very low elution volumes (25-100uL, depending on bed mass and the chemistry of the elution). These low volumes are evaporated using the positive pressure manifold on the ALD4; transfer to a separate device is not required. The resulting dried residues are dissolved in mobile phase, delivered by the ALD4, in preparation for transfer to the autosampler. Laboratory workflow is significantly improved.

In this work, the sample extracts are analyzed in MRM mode using an AB Sciex 5000 triple-quadrupole mass spectrometer interfaced with a Shimadzu liquid chromatograph. Data are presented showing limit of detection, limit of quantification, and linearity of the total process.