K18 Postmortem Analysis of Buprenorphine/ Norbuprenorphine From Whole Blood by GC/MS

Ridhima D. Rao, BS, Sam Houston State University, Box 2525, 1003 Bowers Boulevard, Huntsville, TX 77341, and Dan T. Anderson, MS, Los Angeles County, Department of Coroner, 1104 North Mission Road, Los Angeles, CA 90033*

After attending this presentation, attendees will understand the general principles of burprenorphine, the prevalence and use of the drug in society, and the importance of analyzing for it at the Los Angeles County Department of Coroner.

The presentation will impact the forensic community by providing information on how to extract Buprenorphine and Norbuprenorphine from postmortem specimens with detection by gas chromatography/mass spectrometry (GC/MS).

Buprenorphine, is a semi-synthetic chemical derivative of thebaine which is used to relieve moderate to severe pain. As of 2002, the FDA approved the use of buprenorphine tablets for treatment of opioid addiction. The number of cases seen at the Los Angeles County Department of Coroner involving buprenorphine has slowly increased in the past few years because of their use in addiction clinics. The current literature describes methods for buprenorphine detection in various matrices such as hair, urine, and whole blood by LC/MS/MS; however, most do not require a comprehensive sample preparation necessary for GC/MS detection. Given the fact that most forensic toxicology laboratories are equipped with the GC/MS rather than the LC/MS/MS the object of this study was to develop and validate a method for the extraction of buprenorphine and its active metabolite, norbuprenorphine from postmortem blood with detection by GC/MS. The analysis consisted of a protein precipitation with acetonitrile, solid phase extraction, and silylation derivitazation with MSTFA. Quantitation was performed with the use of deuterated internal standards, d4- Buprenorphine and d3-Norbuprenorpine and the instrument was operated in the selected ion monitor (SIM) mode with the following ions:

d4-Buprenorphine	Buprenorphine	d3-Norbuprenorphine	Norbuprenorphine
454 *	450 *	527 *	524 *
486	482	509	506
510	506	528	525

* Quantitation ion

Linearity was achieved over a concentration range of 2.0 – 25 ng/ml for both drugs supplemented in porcine whole blood with a correlation coefficient exceeding 0.99. The percent recovery of buprenorphine (83%) and norbuprenorphine (68%) was determined at three concentrations (2.0, 5.0, and 10 ng/ml) over four separate days. Limit of quantitation was 2.0 ng/ml and the upper limit of linearity (beyond 25 ng/ml) was not explored as casework would be repeated at a dilution to be within the curve. The intra-assay reproducibility (n=4) was determined for buprenorphine 2.0 ng/ml (CV 11.53%), 5.0 ng/ml (CV 7.86%), 10 ng/ml (CV 4.81%) and norbuprenorphine 2.0 ng/ml (CV 11.78%), 5.0 ng/ml (CV 7.93%), and 10 ng/ml (CV 4.73%). The inter- assay reproducibility (n=12) was determined for buprenorphine 2.0 ng/ml (CV 8.30%), 5.0 ng/ml (CV 7.05%), and 10 ng/ml (CV 4.37%) and norbuprenorphine 2.0 ng/ml (CV 9.47%), 5.0 ng/ml (CV 7.74%), and 10 ng/ml (CV 4.05%). The method was determined to be free from matrix interferences (liver, bile, and urine) by the supplementation of buprenorphine and norbuprenorphine, in duplicate, at 10 ng/ml. Quantitation of both drugs were not affected by any matrix when compared with a blood calibration curve; however, the recovery of norbuprenorphine was severely diminished in the liver specimen, whereas, all the others had no effect. Lastly, the method was successfully verified by the comparison of three different external controls as well as casework that had previously been outsourced.

Buprenorphine is increasing in popularity both on the streets as well as being used in addiction clinics. Therefore, the analysis of buprenorphine and metabolite needs to be a common practice amongst postmortem toxicology laboratories.

Buprenorphine, Analysis, Postmortem

 ** Presenting Author*