



K16 A HPLC/MS/MS Method for Simultaneous Determination of Three Opiates and Three Benzodiazepines in Postmortem Blood

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After attending this presentation attendees will have new knowledge of a Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) method for simultaneous determinations of morphine, hydromorphone, hydrocodone, alprazolam, diazepam, and nordiazepam in postmortem blood using multiple reaction monitoring (MRM) techniques and corresponding deuterated internal standards. Simultaneous analysis of multiple opiates and benzodiazepines has not previously been reported in postmortem blood.

This presentation will impact the forensic community by demonstrating an additional application of LC/MS/MS to the analysis of complex drug mixtures in postmortem specimens.

Analyses were performed with an ABI 3200 Q-Trap instrument operating in a positive polarity mode. For these analyses, one mL specimens of blood were basified by addition of saturated sodium borate buffer, and then extracted once with four volumes of the mixture of 1-chlorobutane and 2-propanol. The organic layer was evaporated and the residue was reconstituted into 0.5 mL of reconstitution solvent (aqueous buffer/acetonitrile; 9:1). Reconstitution solutions were filtered and chromatographed in an acetonitrile/ammonium formate gradient. Instrument parameters were optimized by infusion of solutions of each drug. The chromatographic column was maintained at 25°C and the run time was 12.5 minutes.

The method was validated by examining selectivity, precision, accuracy, linearity, recovery, suppression, and limits of quantitation and detection. Calibration curves were quadratic for all analytes over the concentration range 10–1000 ng/mL, and correlation coefficients (R^2) were better than 0.999. Intraday and interday precision for all analytes at concentrations of 50, 200, and 500 ng/mL was between 4.1% and 10.6%, intraday and interday accuracy for all analytes at the three concentration levels was between 88% to 114%. Recoveries were between 13% and 52%. Limits of detection and quantitation were 3 and 10 ng/mL, respectively. Selectivity results demonstrate that the precision and accuracy of the analytes were not affected by the presence of 14 other common drugs. Only diazepam showed ion suppression in postmortem blood, and morphine and hydromorphone showed ion enhancement. Four postmortem blood specimens were analyzed by this method. The four specimens were also analyzed by alternate, individual LC/MS/MS opiate, and benzodiazepine methods. The results obtained by this new combined opiate/benzodiazepine method match well with the results run by the individual methods.

A method is described that is applicable to simultaneous determination of at least six opiates and benzodiazepines over a broad range of concentrations. Individual analytes were well separated, suggesting that the method is amenable to addition of other opiates or benzodiazepines.

LC/MS/MS, Benzodiazepines, Opiates