The Analysis of Buprenorphine in Urine by Liquid Chromatography Tandem Mass Spectrometry

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Abstract
Buprenorphine is a semi-synthetic opioid derived from thebaine, a natural alkaloid in opium poppy. Buprenorphine is twenty-five to forty times more potent than morphine and is used for moderate to severe pain and opioid addiction treatment. Buprenorphine is given in very low doses, 0.3-0.8 mg per tablet. As a result, a highly sensitive method is required for its analysis in bodily fluids because it is found in low concentrations. Various methods have been used in the development of the method analysis of Buprenorphine, but LC/MSMS technology proved to be the best, most sensitive, and more efficient method compared to that of GC/MS. Due to instrumental complications results were never compiled. As a result, troubleshooting methods for LC/MSMS will be addressed.

Introduction
Buprenorphine (BUP) is a semi-synthetic opioid prepared from thebaine in a seven step sequence. Depending on the amount taken, BUP is 25 to 40 times more potent than morphine, which makes it a much more powerful analgesic. BUP is used to treat moderate to severe pain, along with aiding in the treatment in the dependence of opiates. BUP is a partial opioid receptor agonist and μ-opioid receptor agonist. Gas chromatography procedures with electro capture or mass spectrometric detectors, high performance liquid chromatography methods with UV, fluorescence, and electrochemical detectors have been reported. These methods all lacked sensitivity so they were unable to detect BUP and NBUP with concentrations of less than 1 ng/mL. The purpose of this experiment is to develop a rapid and efficient analysis of BUP, its metabolites, and glucuronides.

Results
Due to instrumental issues, the experiment was unable to be completed. Various troubleshooting aspects were addressed during the time of the experiment. The following scenarios are what were experienced along with what was done to fix them.

- Peaks of the retention times were shifting:
  - Purged the LC
  - Replaced the mobile phase manually
- Sensitivity was reduced over the course of the analysis:
  - Purged and changed the column
  - Ordered and made fresh calibrators
- Checks of status of MS/MS with polypropylene glycol and calibrated the MS/MS
- Checks status of auto-sampler with UV detector and replaced re-to-seal and injector

Other possible instrumentation issues and how to go about troubleshooting them include:

- Leaks
- To tighten the fittings and replace parts
- Pressure issues
- Replace column and/or mobile phase
- Baseline Noise and Drift
- Change mobile phase
- Adjust gradient system
- Purge column
- Peak Shape Problems
- Purge LC and column
- Change mobile phase
- Change guard column

Materials and Methods
- The reference materials BUP, NBUP, BUP-D2, NBUP-D3, BUP-3G, and NBUP-3G were purchased from Cerillan (Huntington, WV).
- The LC/MSMS analysis was performed on a Shimadzu LC-10AD liquid chromatography system consisting of a SCL-HT auto sampler, and an API 2000 MSMS instrument (Applied Biosystems, Toronto, ON, Canada) equipped with an electrospray interface.
- Flow injection analysis (FIA) was performed in order to optimize the parameters of the LC/MSMS for the analysis.
- Direct analysis of glucuronides and free BUP and NBUP ranging from 10-1,000 ng/mL.

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References

Figure 6: An example of a chromatogram with baseline noise.

Figure 7: An example of a fronting peak.

Figure 1: Molecular Structure of norbuprenorphine.

Figure 2: The basic metabolic pathway of Buprenorphine to Norbuprenorphine and its glucuronides.

Figure 3: The chromatogram on top shows greater sensitivity than the chromatogram at the bottom. The chromatograms were taken a month apart. Both samples are at the same concentrations and have the same parameters.

Figure 4: The LC/MSMS used during the course of the analysis.

Figure 5: The differences in separation based on column size. The 50 mm column shows greater separation than the 100 mm column. This is due to the different silicon bonded stationary phases in each column. Both samples are at the same concentration.