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 poppies. Buprenorphine is twenty-five to forty times more potent than morphine and is used for moderate to severe pain and opiate addiction treatment. Buprenorphine is given in very low doses, 0.3-0.6 mg per tablet. As a result a highly sensitive method is required for its analysis in bodily fluids because it 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 κ-opioid receptor antagonist and μ-opioid receptor agonist. Gas chromatography procedures with electron capture or mass spectrometric detectors, high performance liquid chromatography methods with UV, fluorescence, and electrochemical detectors have been reported. These methods all lacked sensitivity as 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, it's metabolite, and glucuronides.

Materials and Methods

- The reference materials BUP, NBUP, BUP-D_4, NBUP-D_3, BUP3G, and NBUP3G where purchased from Cerillant (Austin, TX).
- The LCMSMS analysis was performed on a Shimadzu LC-10AD liquid chromatography system consisting of a SIL-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 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.

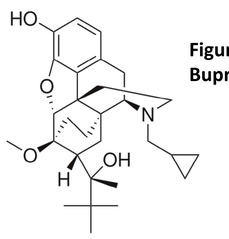

Figure 1: Molecular Structure of Buprenorphine.

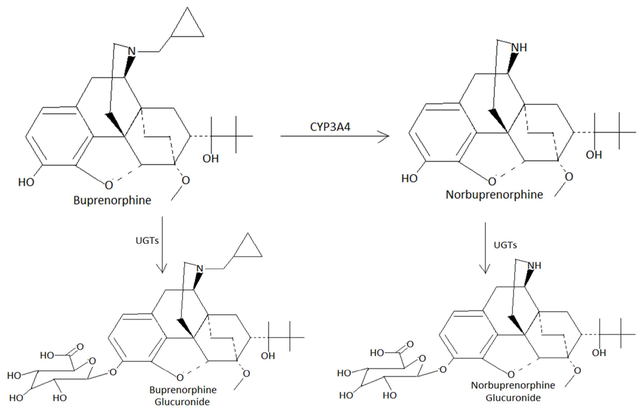

Figure 2: The basic metabolic pathway of Buprenorphine to Norbuprenorphine and its glucuronides.

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
  - Degassed the mobile phase manually
- Sensitivity was reduced over the course of the analysis:
  - Purged and changed the column
  - Ordered and made fresh calibrators
  - Checked status of MSMS with polyproplyene glycol and calibrated the MS/MS
  - Checked status of auto-sampler with UV detector and replaced roto-seal and injector

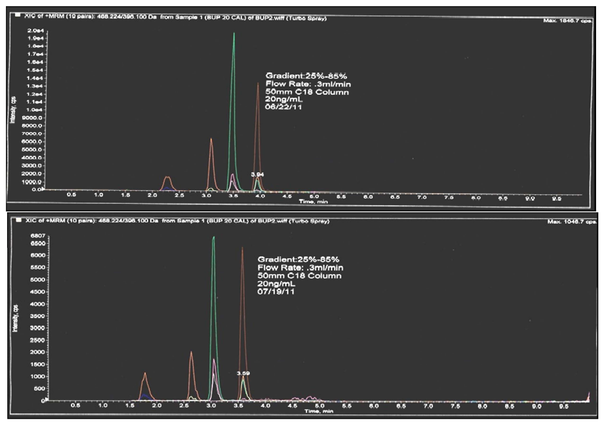

Figure 3: The chromatogram on top shows greater sensitivity than the chromatogram the bottom. The chromatograms were taken a month apart. Both samples are at the same concentrations and have the same parameters.

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

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

- Leaks
  - 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

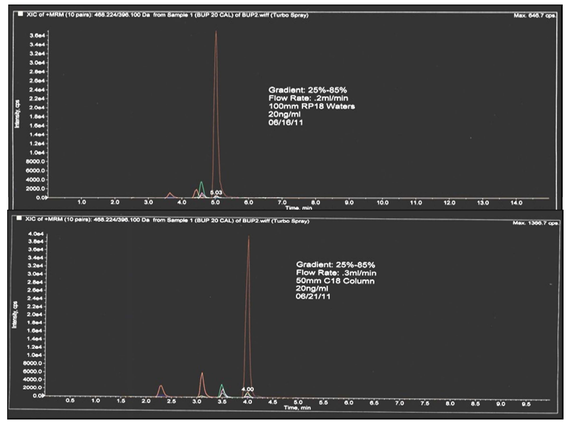

Figure 5: The differences in separation based on column size. The 50 mm column shows greater separation than the 100 mm column. This is due to the different silicon based stationary phases in each column. Both samples are the same concentration.

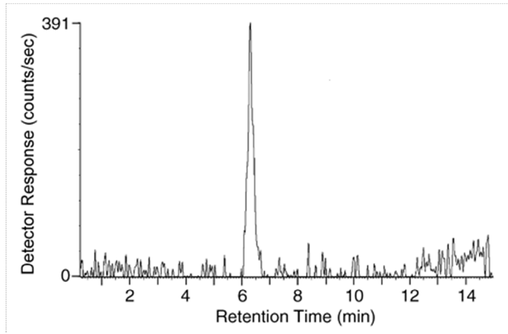

Figure 6: An example of a chromatogram with baseline noise.

Conclusions

Due to lack of results from the experiment because of instrumentation issues, options for continuing the experiment have been proposed: (1) transfer existing methods to the newer LC/MSMS system that is present in the lab, (2) trade in the API 2000 for a newer model and pay the difference, or (3) continue to troubleshoot the API 2000.

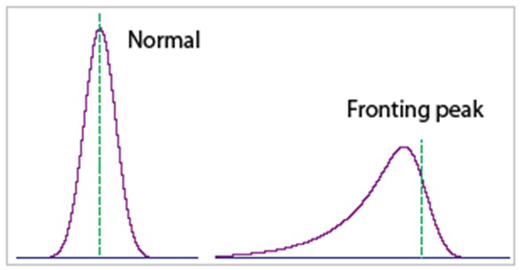

Figure 7: An example of a fronting peak.

References

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Acknowledgments

I would like to thank the Ventura County Crime Lab, Dr. Nunes-Daniel and Dr. Boehme for being my internship supervisor. I would also like to thank Dr. Rankin for being my MU topic advisor.