ANALYSIS OF OPIATES AND THEIR GLUCURONIDE METABOLITES IN BIOLOGICAL FLUIDS USING MIXED-MODE SOLID PHASE EXTRACTION AND ULTRAPRIMARY LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY

OBJECTIVE

Develop a method to simultaneously extract and quantify morphine and five of its metabolites from plasma.

INTRODUCTION

Morphine is the most commonly used opioid analgesic for the treatment of severe pain (1). In humans, the major metabolites of morphine are morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G) (2). Both of these metabolites are pharmacologically active, but have opposite effects. The effects of M6G have been reported to be equal to or greater than morphine (3), while M3G counteracts morphine’s analgesic activity (4). It is possible for morphine to form freebase, hydroxyl, and acid metabolites, the last of which is structurally similar to heroin. Quantification of all metabolites in biological fluids is critical for applications such as drug metabolism, metabolite profiling, abuse detection, and veterinary medicine. Therefore, the need for a rapid and accurate method to analyze morphine and all of its metabolites in one analysis is clear.

Both the extraction and analysis of morphine and its related metabolites present several challenges using LC-MS and SPE. First, these compounds are extremely polar and are poorly retained on traditional reversed-phase columns. Furthermore, adequate LC separation of all analyzed opiates in a short analysis time is difficult. Second, the presence of slightly different functional groups between the metabolites may cause different retention behaviors on mixed-mode SPE sorbents, which can result in lower recoveries for some or all of the analytes. In addition, some morphine metabolites are not stable at the high pH extremes that are employed for eluting in traditional mixed-mode SPE protocols. Finally, a fast-scanning MS instrument is required to properly sample each of the peaks (typical peak width of 2-3 seconds) exiting the UPLC column in order to obtain accurate and precise quantitation.

This work describes a method for the simultaneous extraction and analysis of morphine and five of its related metabolites from plasma using mixed-mode SPE in the ultrafast format and UPLC-MS/MS. The SPE protocol employs an elution into a neutralizing solvent to maintain analyte stability prior to UPLC-MS/MS analysis. Separation of all six analytes was achieved in 2 minutes with R2 ≥ 0.996, neutralizing solvent prior to evaporation. Analyte degradation was minimized by eluting into a neutralizing solvent prior to evaporation. SPE recovery was between 84.9% and 111.0%, depending on the analyte, and inter-day variation was less than 10% (≤ 20%) for 6-acetylmorphine. The method was linear over three orders of magnitude with S/N ≥ 1,000.

CONCLUSIONS

OUTLINE

- A method for the simultaneous extraction and quantitation of morphine and five of its metabolites from plasma was developed using UPLC®-MS/MS detection and SPE-UPLC®-MS/MS.
- Total run time was less than 11 minutes.
- Calibration degradation was minimized by eluting into a neutralizing solvent prior to evaporation.
- SPE recovery was between 84.9% and 111.0%, depending on the analyte, and inter-day variation was less than 10% (≤ 20%) for 6-acetylmorphine.
- The method was linear over three orders of magnitude with S/N ≥ 1,000.

LOQ values ranged from 0.1 to 0.5 ng/mL with S/N ≥ 15 for all analytes.

REFERENCES