INTRODUCTION

Liquor chromatography combined with atmosphere pressure mass spectrometry (LC-MS) has made a substantial contribution to the identification and quantification of drugs of abuse in clinical and forensic toxicology. The sensitivity and selectivity of LC-MS allow for the accurate determination of trace amounts of analytes in complex biological fluids. In recent years, oral fluid has been getting a lot of attention as an alternative matrix for drug testing. An advantage of oral fluid is the non-invasiveness of collection and availability in the field. The primary form of the drug found in oral fluid is a parent drug, permitting a direct comparison of pharmacokinetic data and drug concentration to observed effects. However, a disadvantage to monitoring drug use with oral fluid testing is the short detection window compared to urine.

The method described here, LC-APCI-MS/MS, is designed to determine the pharmacologically active drug concentration to observed effects. An advantage of oral fluid is the non-invasiveness of collection and availability in the field. The primary form of the drug found in oral fluid is a parent drug, permitting a direct comparison of pharmacokinetic data and drug concentration to observed effects. However, a disadvantage to monitoring drug use with oral fluid testing is the short detection window compared to urine.

MATERIALS AND METHODS

Materials

Compounds of interest

- Acetylcodeine
- Codeine
- Codeine-d6
- Norcocaine-d3
- EDDP-d3
- Benzoylecgonine-d8
- Methadone
- Heroin
- Heroin-d
- Normorphine
- 6-acetylmorphine
- Methadol
- Acetylmorphine-d
- Ecgonine
- Cocoaethylene
- Papaverine
- Morphin
- Propoxyphene
- 6-hydroxybenzylecgonine
- Ecgonine
- Norcocaethylene
- Norcodeine
- Methadone
- Methamphetamines

Methods

- Liquid chromatography coupled with atmospheric pressure ionization mass spectrometry (LC-APCI-MS/MS)
- Protein precipitation
- Matrix effect
- Relative recovery

RESULTS AND DISCUSSIONS

The preconcentration strategy for each analyte of interest was determined by desorption/ionization experiments. After optimization of the LC separation (column, mobile phase composition, gradient, flow rate, and selection of the optimum precursor product ion combination), each drug was quantified LC-APCI-MS/MS method was developed by LCMS/MS. To ensure optimal extraction conditions and optimal scan number across the peak, the chromatographic ion was divided in 7 segments, each segment a different tune file and different MRM scan parameters were applied, according to the compounds eluting at that time (Table 1).

Matrix effect

The matrix effect (blank oral fluid minus spiked oral fluid) of each drug was determined by direct infusion of single analyte solutions. The matrix effect was calculated by dividing the peak area of the sample with the peak area of the standard. The matrix effect for each drug is shown in Figure 6. The relative recovery for each compound was determined by investigating the effect of oral fluid on the matrix. The relative recovery of each compound was determined by investigating the effect of oral fluid on the matrix.

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