

ABSTRACT

Method development and validation for quantitative determination of methadone enantiomers in human plasma by liquid chromatography/tandem mass spectrometry (LC/MS/MS)

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Introduction

Methadone plays important roles in the treatment of severe pain and drug addiction. (R)-methadone is pharmacologically more active than the (S)-enantiomer, and LC/UV and GC/MS have been used for determining the enantiomers. Due to various limitations of those methods, LC/MS methods have recently been reported; however, the latter methods are time-consuming and have a very narrow linear range and complicated chromatographic conditions. Therefore, we have developed a new LC/MS/MS method for high throughput, rugged and sensitive quantitation of the methadone enantiomers in human plasma.

Methods

Enantiomers of methadone were extracted from 0.1 mL aliquots of human plasma by an automated liquid/liquid extraction method. The (R) and (S) chromatographic peaks were baseline-resolved within 5 minutes on a chiral column (2 x 50 mm, 5 µm particle size) with an isocratic LC system and were detected by a Turboion Spray interface operated in the selected reaction monitoring mode.

Preliminary Data

The effects of pH and of types and concentrations of mobile-phase modifiers on the enantioselectivity of (R)- and (S)-methadone were investigated. Then the selectivity, linearity, precision and accuracy, and extraction recovery were fully evaluated. Matrix effects and ionization suppression were evaluated, but no matrix effects or ionization suppression were observed in those experiments. The method showed excellent reproducibility (overall CV <8%) and accuracy (overall Bias <2.7%) with a broad linear range. Each enantiomer was stable in human plasma after 5 freeze-thaw cycles, under bench-top storage at room temperature (RT) for six hours, in the reconstitution solution at RT for 17 hours, or in processed-extracts stored at RT for 142 hours. Narcotics, including morphine, cocaine, 6-monoacetylmorphine, ecgonine, and benzoylecgonine methyl ester did not interfere with the performance of the assay.