Precise High Performance Liquid Chromatography Technique for Simultaneous Quantification of Methadone and Cocaine in Rat Serum and Brain Tissue Samples

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Purpose
To develop and validate a sensitive and precise HPLC method for the simultaneous quantification of methadone and cocaine in rat serum and brain tissue samples. The method has been fully validated according to FDA guidelines and successfully applied on biological samples obtained from a drug-drug interaction study in rats.

Methods
The method was developed using a Waters® Alliance HPLC system equipped with 2695 module and 2487 photodiode detector. Samples were prepared by a liquid-liquid extraction procedure using hexanes and the analytical separation was performed using Symmetry C18 column (150 x 4.6 mm, 5 μm) (Waters®, Milford, MA) at a flow rate of 1 mL/min. Levo-tetrahydropalmatine (L-THP) was used as the internal standard. The mobile phase consisted of a mixture of (A) 5 mM potassium phosphate monobasic in water with 0.1% tri-ethyl amine (pH 6.5) and (B) acetonitrile. The initial gradient conditions were 50% B for 8 min, increased to 90% and maintained at this concentration for an additional 6 min. Detection was carried out using a dual UV detector at wavelengths 215 and 235 nm for methadone and cocaine; respectively. The method was validated with respect to specificity, linearity, accuracy, precision, recovery, and stability according to the FDA Guidelines.

Results
The quantification range was 0.05 to 10 μg/mL for both methadone and cocaine. The recovery of methadone and cocaine from serum and brain samples ranged from 88.84-108.89 %. Inter-day accuracy values of serum and brain samples ranged from 96.97-105.59 % while intra-day accuracy values ranged from 91.49 - 111.92 % for both methadone and cocaine. Stability assays showed that both methadone and cocaine were stable during sample storage, preparation and analytical procedures. There were no significant interfering peaks from endogenous substances in blank serum at the retention time of the analytes of interest (methadone and cocaine) and the internal standard. Quantification of methadone and cocaine samples from a drug-drug interaction study was successful. Serum concentrations ranged between 56.3 to 1400.85 ng/mL and 73.23 to 750.7 ng/mL for methadone and cocaine, respectively, while, brain concentrations ranged between 211.63 to 443.9 ng/g and 73.23 to 750.7 ng/g for methadone and cocaine; respectively.

Conclusion
A simple, sensitive, specific, accurate and reproducible HPLC-UV method for the simultaneous quantification of methadone and cocaine was developed and validated with a good sensitivity (LLOQ 50 ng/mL). This method can be applied to quantify methadone and cocaine samples from pre-clinical animal studies and can potentially be applied to human biological serum samples to monitor compliance to methadone maintenance treatment and to detect possible cocaine-methadone co-abuse.