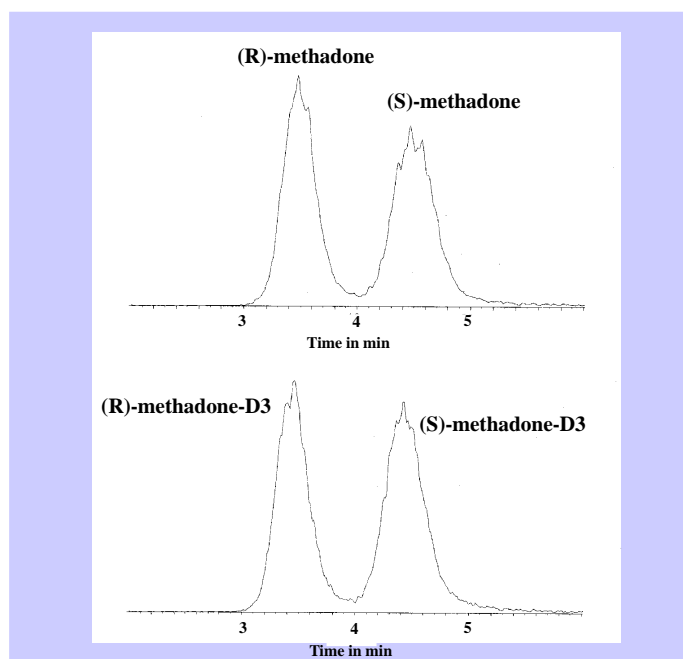


CHIRAL-AGP™ excellent choice for MS/MS detection - No matrix effects or ionization suppression

A rapid method for quantitative determination of methadone enantiomers in human plasma was developed by H.R. Liang et al. The results were published in J. Chromatogr. B 806 (2004) 191-198. The method offers high-throughput and improved specificity, sensitivity, linear range and ruggedness over previous published methods. No ionization suppression or matrix effects could be observed.

The effects of pH and the type and the concentration of organic modifier on the retention and the enantioselectivity were investigated using a 50x2.0 mm CHIRAL-AGP column. Baseline separation of the methadone enantiomers was achieved with a retention time of less than 5 min., see chromatograms below. Ionization suppression and other matrix effects were evaluated but no ionisation suppression or other matrix effects were observed. The high-throughput method showed excellent reproducibility, accuracy and ruggedness. All studies were conducted within the guidelines of the US FDA Good Laboratory Practice Regulations for Nonclinical Laboratory studies (Title 21 CFR Part 58).



Chromatograms obtained using CHIRAL-AGP 50x2.0 mm with a mobile phase containing 12% 2-propanol in 10 mM ammonium acetate. Flow rate 400 ul/min.. Temperature 45 degrees Celcius. The mass spectrometer was used in the multiple-reaction monitoring (MRM) mode, positive ions formed by TurbulonSpray™ ionization.

Methadone is an important drug for the treatment of severe pain and drug addiction. R-methadone is pharmacologically more active than the S-enantiomer. The aim of the referred study was to develop and validate a rapid, specific, sensitive, robust and reliable method for the quantification of methadone enantiomers in human plasma by LC/MS/MS.

Matrix effects or ionisation suppression

It is very important to investigate the matrix effects in order to develop a reliable and reproducible LC/MS/MS method. Ionization suppression using the CHIRAL-AGP column was studied by post-column infusion of R- and S-methadone or methadone-D3 and on-column injection of extracted blanks. No ionisation suppression or other matrix effects were observed during the retention times of the enantiomers even though a mobile phase containing a very high percentage of water was used on the CHIRAL-AGP column. The results are presented in the Table below.

(n=10)	Peak areas in human plasma (1000 ng/ml)	Peak areas in water (1000 ng/ml)
R-Methadone	222000	189400
S-Methadone	234000	206100
R-Methadone-D ₃	207000	215200
S-Methadone-D ₃	218000	224000

Evaluation of ionization suppression by comparing areas of R- and S-methadone and methadoneD3 extracted from human plasma and water

Note: It is recommended to use a CHIRAL-AGP guard column to protect the analytical column. This is of special importance in bioanalytical work..