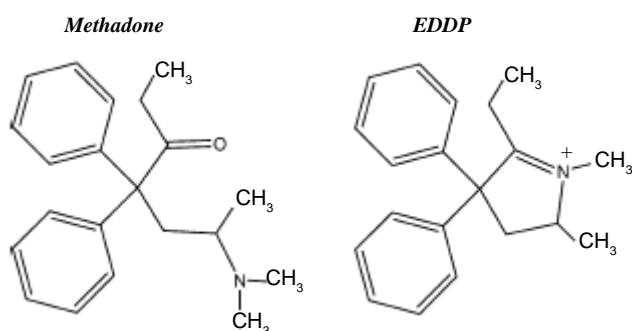


Separation of the enantiomers of methadone and its metabolite EDDP using CHIRAL-AGP and MS-detection

A sensitive stereoselective liquid chromatographic method with mass spectrometric detection (LC-MS) was developed and validated for the quantification of R- and S-methadone and the primary metabolite R- and S-2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) in human plasma. The method was published in *J. Chromatogr. B*, 809 (2004) 313-321 by Dale Whittington et al.

Methadone is a synthetic opioid agonist which is used in the prevention of opiate abstinence syndrome and as an analgesic for patients with moderate and severe pain. Methadone is chiral with a single asymmetric carbon. It is administered as a racemic mixture of (R)(-) and (S)(+) enantiomers. Methadone is mainly metabolized through N-demethylation to EDDP (2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine), containing a quaternary nitrogen. The metabolite is also chiral, see structures below:



There are stereoselective differences in methadone pharmacodynamics and pharmacokinetics. Due to variations in the pharmacokinetics of methadone between individuals and the risk for drug interaction, dose adjustments are often required. Analytical methods to be used in therapeutic monitoring as well as for investigations regarding methadone pharmacokinetics and drug interactions are important. The described method is a simultaneous stereoselective determination of the enantiomers of methadone and EDDP in human plasma.

Chiral separation of methadone and EDDP was achieved using a CHIRAL-AGP column, 100x2.0 mm, and a CHIRAL-AGP guard column, 10x2 mm. The mobile phase was a gradient consisting of:

Mobile phase A: 10% methanol in 20 mM ammonium formate pH 5.7

Mobile phase B: Methanol

Gradient:

0-4 min: Mobile phase A

4-6.5 min: Methanol conc. increased to 25%

6.5-9 min: Methanol conc. 25%

9-9.5 min: Methanol conc. increased to 30%

9.5-16 min: Methanol conc. 30%

16-21 min: Methanol conc. decreased to 10%

After each sample set (around 100 samples) the column was washed with 25% 2-propanol in deionized water in order to remove potential accumulation of interferences not removed by the sample preparation process.

Retention times:

| | R | S | R _s |
|-----------|------|------|----------------|
| EDDP | 12.5 | 16.2 | 3.8 |
| Methadone | 13.3 | 15.5 | 2.0 |

The mass spectrometer was operated in the positive electrospray ionization mode. All analytes were monitored in the same ion group:

| | <i>m/z</i> | <i>m/z</i> |
|------------------------|------------|------------|
| EDDP/d3-EDDP | 278.1 | 281.1 |
| Methadone/d9-methadone | 310.1 | 319.1 |

This method appears to be the first LC-MS assay for the simultaneous quantification of methadone and EDDP enantiomers in plasma. Detection limits for EDDP (0.1 ng/ml) are lower than other published methods and the assay is two to five times more sensitive than other LC-MS methods for methadone.

Total LC-MS runtime for 100 samples is approx. 36 hours.

The assay is sensitive, precise, accurate and robust and is well suited for chiral pharmacokinetic studies of methadone and its primary metabolite, EDDP.

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