

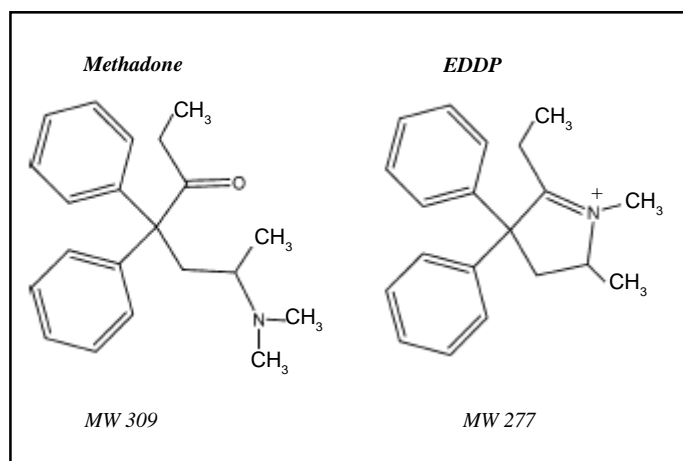
A robust and reliable isocratic LC/MS-method for the separation of the enantiomers of methadone and its metabolite EDDP using CHIRAL-AGP

A rapid, sensitive and highly specific method for quantification of free and total R- and S-enantiomers of methadone and its metabolite EDDP (2-ethylidine-1,5-dimethyl-3,3-diphenylpyrrolidine) was developed and published by M.L. Etter et al (*Clinical Biochemistry*, vol.38, 2005, 1095-1102). The method was used in a patient study, published by D.C. Lehotay et al in the same volume of *Clinical Biochemistry*, page 1088-1094.

The objective of the study was to develop a method by which free and total fractions of both enantiomeric forms of methadone and EDDP could be quantified in clinical samples. It is only the free or non-protein bound fraction of the drug that is available to bind to the opioid receptors and to be metabolized.

The requirements on the method was as follows:

- It had to be able to differentiate between the enantiomers for both methadone and the metabolite EDDP
- It had to be sensitive enough to monitor metabolism of methadone in patients taking different doses
- It had to be sensitive enough to quantitate the expected low levels of non-protein bound EDDP



The column chosen to fulfil the above requirements was CHIRAL-AGP 100x3.0 mm (+ CHIRAL-AGP guard 10x3 mm). The mobile phase was 2.5% acetonitrile in 5 mM ammonium acetate buffer pH 4.1. Flow rate was 0.5 ml/min and injection volume 10 µl.

MS/MS with chemical ionization was chosen as detection method, as this "soft" fragmentation results in the molecular ion and characteristic fragment ions.

In order to separate non-protein bound fractions of both analytes from the protein bound fractions, serum was centrifuged using selective ultrafiltration membranes with a molecular cut-off of 10000 Da. The ultrafiltrate was then extracted using liquid-liquid extraction. The residue after evaporation was then reconstituted in the mobile phase and injected.

With the use of tandem mass spectrometry as detection it was possible to differentiate between two co-eluting peaks. Although S-EDDP coeluted with both enantiomers of methadone, simultaneous quantification of all four enantiomers was possible. On the CHIRAL-AGP column R-enantiomers of methadone and EDDP elutes before the corresponding S-enantiomer.

<i>Retention times</i>	
	<u>min</u>
R-EDDP	3.5
S-EDDP	5.4
R-methadone	4.9
S-methadone	6.0

Total run-time was 10 minutes. Under the conditions used, R- and S-EDDP was base-line resolved, while R- and S-methadone was not fully base-line resolved. d9-Methadone was used as internal standard.

<i>Validation data</i>		
	<u>Methadone</u>	<u>EDDP</u>
Linearity	0.9998	0.9996
Precision	3.1-4.7%	4.5-12.9%
Accuracy	97.9-102.2%	84-100.5%

The method is robust, reliable and provides precise and accurate results with detection limits that provide meaningful data in order to study the pharmacokinetics of methadone in patients. The method is well suited for use in clinical laboratories, as LC/MS/MS is now becoming more used in such laboratories, in order to achieve better specificity for a number of assays.

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