# Enantioselective quantification of methadone and its major metabolite (EDDP) in oral fluid by capillary electrophoresis

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## Abstract

Methadone is a synthetic analgesic widely used in methadone maintenance programmes. Methadone (MTD) is generally administered orally as a racemate, however the therapeutic effect is almost exclusively mediated by the (*R*)-enantiomer since its affinity for the μ-opioid receptor and its analgesic effect is up to 8 and 50 times, respectively, greater than for the (*S*)-enantiomer. Because of important inter-individual variability and in order to prevent withdrawal symptoms, an individual dose adjustment is often required.

To the best of our knowledge, only two analytical procedures using both liquid chromatography-mass spectrometry have been reported for the enantioselective quantification of MTD and/or its main metabolite, 2-ethylidene-1,5-dimethyl-3,3-diphenyl-pyrrolidine (EDDP) in oral fluid.

The purpose of the study was to develop a more rapid and reliable assay for the simultaneous quantification of MTD and EDDP in oral fluid using capillary electrophoresis (CE). Oral fluid specimens were collected before the daily MTD administration using Salivette devices (Sarstedt) and then centrifuged. After the addition of the internal standard, (*R*)-phenylethylamine, the mixture was alkalinized with 0.2 mL of an ammonia solution (25%), and extracted with 3 mL of cyclohexane. After centrifugation, the upper organic layer was evaporated to dryness. Baseline separations of MTD and EDDP enantiomers were obtained in 5 min using 0.2% highly sulphated γ-cyclodextrin as chiral selector and a 50 mM phosphate solution as background electrolyte. The extraction yields were between 77.4 and 92.9%, whereas the limits of detection ranged from 2.3 to 2.4 ng/mL. Intra- and inter-assay precision respectively accuracy were acceptable. The method was used for the analysis of oral fluid specimens obtained from patients enrolled in a MTD maintenance programme. Results showed MTD *R* vs. *S* ratios > 1 and EDDP *R*/*S* ratios < 1, confirming previous studies and/or plasma results.

## 1. Introduction

Methadone is a synthetic analgesic widely used in methadone maintenance programmes. Methadone (MTD) is generally administered orally as a racemate, however the therapeutic effect is almost exclusively mediated by the (*R*)-enantiomer since its affinity for the μ-opioid receptor and its analgesic effect is up to 8 and 50 times greater than for the (*S*)-enantiomer, respectively [1,2]. It has been largely demonstrated that there exists high inter-individual variability in the MTD metabolism [3-8].

Therefore, the enantioselective quantification of MTD is necessary in order to determine the level of the active (*R*)-MTD necessary to obtain maximum treatment efficacy, to prevent toxicity and to exclude non-controlled consumption (*R*)-MTD.

For some years, the interest in the use of oral fluid as an alternative specimen to therapeutic monitoring has increased because it offers several advantages: it is obtained by a non-invasive method of sampling, it is readily available, it can be often repeated and it contains the free fraction of drugs and therefore, is a better indicator of intoxication states. To the best of our knowledge, only two analytical procedures using both liquid chromatography-mass spectrometry have been reported for the enantioselective quantification of MTD (EDDP) in oral fluid (OF) [3,9]. Only one of them included the analysis of its major metabolite, 2-ethylidene-1,5-dimethyl-3,3-diphenyl-pyrrolidine (EDDP). The enantioselective separations were achieved on $\alpha_1$-acid glycoprotein columns which require frequent replacement, because they tend to gradually lose separation efficiency proportional to the number of injections [10-12].

The purpose of this study was to develop a rapid and specific method for the determination of enantiomeric ratios of MTD and EDDP in OF by capillary electrophoresis (CE) using highly sulphated γ-cyclodextrins (HS-γ-CD) as chiral selectors. The method applied to the enantioselective quantification of MTD and EDDP in OF of addicts under maintenance program for narcotic dependence.

## 2. Materials and methods

### *2.1 Reagents*

Racemic MTD and racemic EDDP were purchased from Cerilliant (Austin, TX, USA. (*R*)-(-)-MTD hydrochloride was a gift from the Unit of Medicinal and Drug Analysis the National Laboratory of Health (Luxembourg). HS-γ-CD (20% w/v) aqueous solution was obtained from Beckman (Fullerton, CA, USA). Phosphoric acid ($H_3PO_4$), ammoniac ($NH_3$, 25%), sodium hydroxide (NaOH), cyclohexane and *R*-(+)-1-phenylethylamine ((*R*)-PEA, $R{:}S \geq 99.5{:}0.5$) were purchased from Sigma-Aldrich (Bornem, Belgium). Ultrapure water ($H_2O$ and methanol HPLC grade (MeOH) were purchased from Lab Scan (Dublin, Ireland).

### *2.2. Specimen preparation*

#### *2.2.1. Collection of oral fluid*

Mixed saliva was obtained from patients undergoing a MTD. The study was approved by the Ethic Research Committee of Luxembourg. OF was collected just before the administration of the daily dose of MTD. Specimens were collected with Salivette devices (Sarstedt, Nümbrecht, Germany): after being soaked for 2 min by

OF, the cotton swab was placed back into the Salivette. The OF was recovered from the Salivette by centrifugation and the specimens were stored at -20°C until analysis.

*2.2.2. Extraction procedure*

The samples were worked up as described in reference [13]. Briefly, 200 µL of an aliquot of OF was alkalinized with 0.2 mL of an ammonia solution (25%), and extracted with 3 mL of cyclohexane. (*R*)-PEA was used as internal standard (IS).

### *2.3. Instrumentation and electrophoretic conditions*

All CE separations were carried out on a Beckman P/ACE System MDQ equipped with a photodiode array detector (Beckman Coulter, Fullerton, CA, USA). The 32 Karat software from Beckman was used for data acquisition. Uncoated fused silica capillary of 50 µm inside diameter (ID) and 40.2 cm total length were used for separations. The running buffer consisted of 50 mM $H_3PO_4$ buffer, pH 4.5 and contained 0.2% HS-γ-CD. The separation was achieved at 20°C using a voltage of +20 kV and the detection wavelength was set at 200 nm.

## 3. Results and discussion

### *3.1. Separation of MTD and EDDP enantiomers using HS-γ-CD as chiral selectors*

The use of HS-γ-CD resulted in good separations for the enantiomers of EDDP and MTD. Figure 1 shows an example of an electropherogram of MTD and EDDP separated into their enantiomers by CE in presence of HS-γ-CD. The enantiomeric elution order for MTD was established by injecting a solution containing a concentration of (*R*)-MTD: (*S*)-MTD in a ratio of 10:1. As optically pure EDDP enantiomers are not commercially available, (*R*)-EDDP was prepared from (*R*)-MTD using a procedure described previously [9]. For MTD, the enantiomeric elution order was *S* < *R* while the (*R*)-EDDP migrated faster than the (*S*)-EDDP.

### *3.2. Method validation*

The calibration curves were generated using a weighted (1/x) least-square regression model. Standard curve plots for MTD and EDDP enantiomers were linear in the range of 7.6- 625 ng/mL ($r^2 > 0.99$). The extraction yields were between 77.4 and 92.9%, whereas the limits of detection ranged from 2.3 to 2.4 ng/mL for EDDP and MTD respectively. Intra- and inter-assay precision respectively accuracy were acceptable for the purpose of our study.

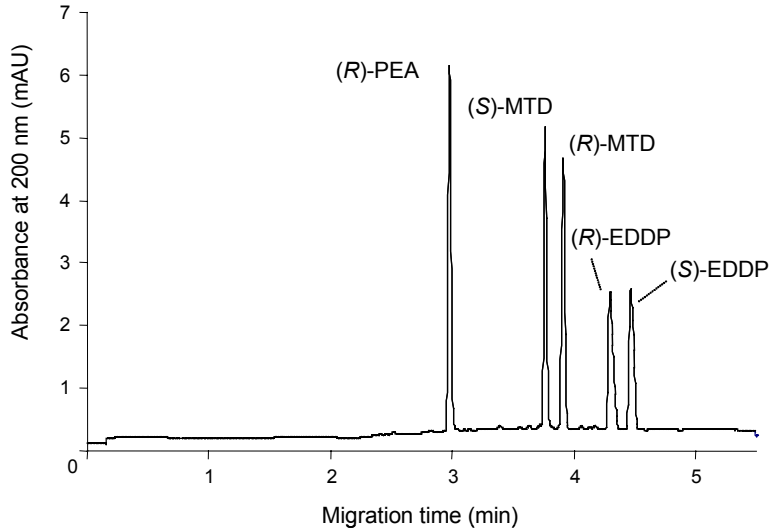


Figure 1. Electropherogram of an oral fluid sample spiked with 50 ng/ml of (*R*,*S*)-MTD, (*R*,*S*)-EDDP and (*R*)-PEA (IS).

### *3.3 Application to clinical specimens*

The validated method was applied to OF specimens obtained from patients undergoing a MTD maintenance treatment. All OF were tested positive for both enantiomers of MTD and the R/S ratios varied between 1.00 and 3.13. These results are consisted with the previously reported data, as the ratios determined in saliva are representative of the free fraction of MTD in blood only. The EDDP enantiomers were detected in ten OF specimens and the enantiomeric ratios ranged from 0.70 to 0.94. Only one study [9] has quantified the enantiomers of EDDP in five OF specimens, but the data obtained from these restricted analyses showed higher concentrations of (*S*)-EDDP in three specimens, while two OF specimens presented slightly higher concentrations of (*R)*-EDDP.

## 4. Conclusions

A rapid and a validated for the enantioselective quantification of MTD and of its major metabolite EDDP in OF has been applied to the determination of enantiomeric ratios of MTD and EDDP in OF obtained from patients enrolled in a MTD maintenance programme. The higher concentrations of (*R*)-MTD in OF was in accordance with previous data. Furthermore, this study pointed out a predominance of (*S*)-EDDP in OF.

## 5. References


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