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## A Quantitative Determination of Methadone and its Metabolite (EDDP) in Dry Blood Spots Using LC-MSMS

### Introduction

The present research study provides a much simplified approach whereby a highly sensitive LC-MS/MS triple quadrupole mass spectrometer is used to measure directly the methadone and EDDP concentrations through the dried blood spot (DBS) samples.

## 2. Method

Fresh human whole blood was spiked with different concentrations of methadone and EDDP. About 30 µL of the blood were spotted to Whatman DMPK-C cards. Cards were air dried for about 2 hours and then stored desiccated at room temperature. The cards were punched with 6mm punch and placed into a vial containing 100 µL IS (0.01ng/mL) working solution. Samples were vortexed for 5 minutes and centrifuged for 5 minutes at about 4000 rpm. After centrifugation, supernatants were transferred into HPLC vials for analysis.

### 2.1. Mass Spectrometry Conditions

The QSight® 220 Mass Spectrometer system was used for the analysis. This instrument is equipped with heated coaxial flow ion source and “Hot Source-Induced Desolvation” (HSID™) interface, with a multi-orthogonal channel and laminar flow sampling. Electrospray voltage is set to 5000V, the source and HSID temperatures were set to 350 and 300°C, respectively.

Table 1: Operating conditions

Name	MRM	CE
EDDP	279.1/234.0	40
EDDP-d3	281.1/234.0	40
Methadone	310.1/265.0	20
Methadone-d9	319.1/265.0	20

### 2.2 LC Conditions

The samples were analyzed using HPLC conditions. The LC gradient elution is shown in Table 2 and the following conditions:

**Column:** Chromolith-RP18E column (100x3 mm, 2.6 µm)  
**Mobile phase:** A (0.1% formic acid 100% H2O)  
 B (0.1% formic acid 100% ACN)  
**Flow rate:** 0.5 mL/min  
**Injection volume:** 10 µL  
**Column temp:** 40°C

### Quick Facts:

- Quantitative method for methadone and EDDP in dried blood spots.
- LLOQ of 0.1 ng/mL for both compounds.
- Excellent accuracy from 0.1-100 ng/mL for both compounds.

Table 2: LC conditions

Time (min)	Solvent B %
0	10
0.5	30
1	50
1.3	80
1.5	98
1.8	98
1.9	10
3.5	10

## 3. Results

### 3.1. Preliminary Study of Methadone and EDDP in Solvent

Figure 1: Methadone

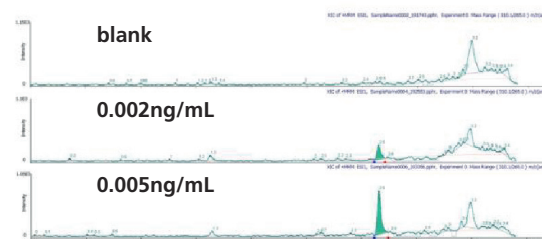
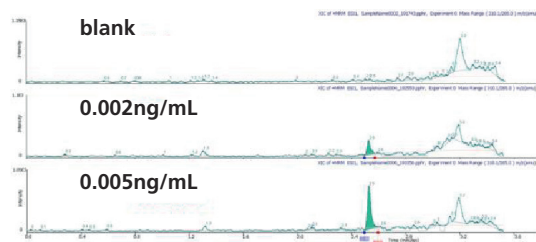
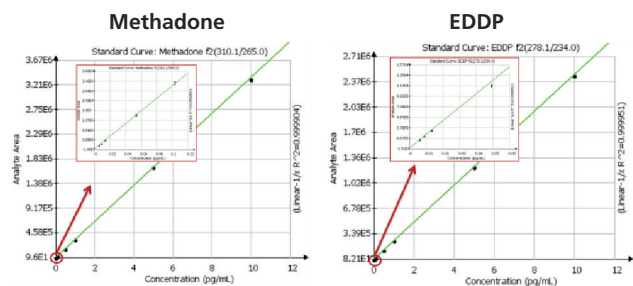


Figure 2: EDDP



Methadone and EDDP calibration curves in solvent from 0.002 to 10 ng/mL. The linear fittings using 1/x weighting are excellent, with a coefficient R2 of 0.997 and 0.998 for methadone and EDDP, respectively.

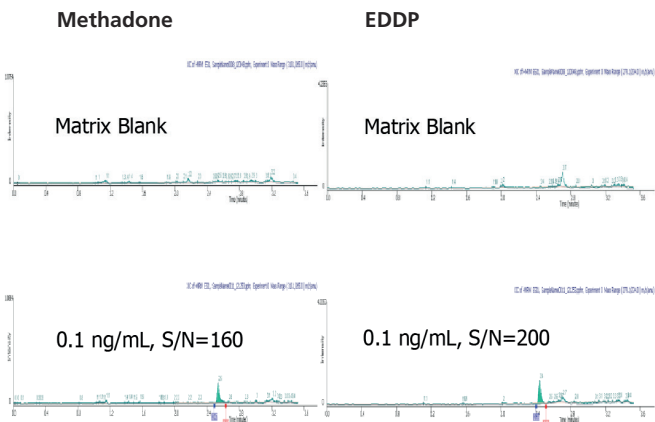
Figure 3: Calibration curves of methadone and EDDP



### 3.2. Extracted Ion Chromatograms (EICs) of Analytes

EICs of Methadone and EDDP in a human DBS blank and in DBS samples containing methadone and EDDP. No interference was observed.

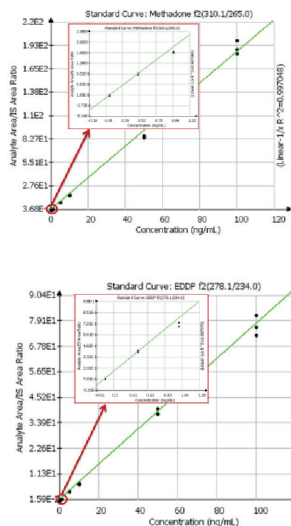
Figure 4: EICs of Methadone and EDDP



### 3.3. Calibration Curves of DBS Extracted Analytes

Methadone and EDDP calibration curves in DBS for the range of 0.1 to 100 ng/mL with a 10 µL injection. The curves were in good linearity with 1/x weighting. The fitting coefficient was 0.997 and 0.998 for methadone and EDDP, respectively.

Figure 5: Calibration Curves of DBS Extracted Analytes



Methadone DBS Calibration Curve:  
 $y=1.857x-0.00132$   
 with 1/x weighting  
 $R^2=0.997$

EDDP DBS Calibration Curve:  
 $y=0.7787x-0.00855$   
 with 1/x weighting  
 $R^2=0.998$

Table 3: Summary of LOQ, Precision and Accuracy

Analyte	LOQ (ng/mL)	CV%	%Accuracy
Methadone	0.1	9.1	108
EDDP	0.1	8.3	99

Table 4: Summary of QC Accuracy and CV

Analyte		(0.25 ng/mL)	(5 ng/mL)	(80 ng/mL)
Methadone	Accuracy (%)	106.2	104.1	107.3
	CV (%)	5.6	2.5	4.4
EDDP	Accuracy (%)	95.8	89.1	94.7
	CV (%)	3.2	2.1	6.6

## 4. Conclusion

The results in this study illustrate a fast, accurate and precise LC-MS/MS method using the QSight® 220 mass spectrometer for quantifying methadone and EDDP in DBS samples. The LOQs for both methadone and EDDP are 0.1 ng/mL with an excellent signal to noise ratio. Good linearity from 0.1 to 100 ng/mL is observed for both analytes. The sample preparation procedure is simple and rapid with no SPE or LLE clean up required.

## 5. Contact information

To learn more about PerkinElmer Mass Spectrometry, our products or services, please visit our website or contact us directly.

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