RESEARCH PAPER

Determination of methadone and EDDP in oral fluid using the dried saliva spots sampling approach and gas chromatography-tandem mass spectrometry

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Abstract

The present work describes the development and validation of a novel approach to determine methadone (MTD) and its main metabolite (EDDP) in oral fluid samples, using the dried saliva spots (DSS) sampling approach and gas chromatography-tandem mass spectrometry (GC-MS/MS). Oral fluid samples (50 μL) were applied into Whatman™ 903 protein saver filter paper cards and were allowed to dry overnight. The extraction was carried out by immersion of the spot in 1 mL of isopropyl alcohol with agitation for 1 min. Afterwards, the extract was centrifuged for 15 min at 3500 rpm and the supernatant evaporated to dryness and reconstituted with 50 μL of methanol. The procedure was considered linear in the range of 10 to 250 ng/mL for both compounds, with determination coefficients greater than 0.99. Intra- and inter-day precision and accuracy revealed coefficients of variation (CVs) lower than 15% at the studied concentrations, with mean relative errors within ± 15% of the nominal concentrations. Recoveries ranged from 45 to 74%. The limits of detection and quantification were 5 and 10 ng/mL respectively for both analytes. All studied parameters complied with the defined criteria and the method enabled the successful determination of MTD and EDDP in oral fluid samples from patients undergoing opiate substitution/maintenance therapy.

Keywords Methadone · EDDP · Oral fluid · Dried saliva spots · GC-MS/MS

Introduction

Methadone (MTD) is the most commonly used drug in opiate substitution therapy [1]. Although heroin still prevails as the most widely consumed illicit opiate, several studies are now pointing to an increase in the consumption of synthetic opioids, such as MTD, buprenorphine, and fentanyl. Despite the fact that these substances are associated with a substantial proportion of overdose deaths, only a few countries, such as Denmark and the Czech Republic, have monitoring systems capable of conducting an analysis of trends in acute intoxications caused by their consumption [2].

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MTD hydrochloride is used as a racemic mixture, with the L-isomer being the most potent compound [3–5].

The *N*-demethylation catalyzed by the cytochrome P450 isoenzyme CYP3A4 occurs at the hepatic level, producing its major metabolite (inactive), 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), which subsequently undergoes demethylation to form the also inactive compound 2-ethyl-5-methyl-3,3-diphenyl-1-pyrroline (EMDP) [1].

Several publications have reported the determination of MTD and metabolites in traditional biological matrices, such as urine [5–7], blood [8, 9], serum [10, 11], and hair [12]. However, a non-conventional matrix such as oral fluid (OF) is also used, presenting a simple and non-invasive sampling procedure associated with an easy transportation and handling. Nevertheless, regarding drug monitoring, plasma remains the most applied biological matrix, since OF analysis still presents some drawbacks. OF samples may be affected by physiological variables such as pH and viscosity; the target analytes may be present in relatively low concentrations compared to other biological specimens, and might exist some difficulty in obtaining sufficient quantities of material for analysis [13–17].

In addition, in what concerns drug monitoring, there is a considerable interest in finding alternatives to plasma or blood, and OF has been increasingly studied, due to the possibility of developing more sensitive and reliable methods, but also due to its many advantages.

The correlation between opioid concentrations (MTD, EDDP) in plasma and OF has been evaluated and reported in the literature [18]. The precision of the comparison of plasma/OF concentrations presents values above 90% [19]; the concentrations were found very similar with a OF/plasma ratio of 1.3/1.0, respectively [20]. However, according to the literature, the concentrations of MTD in the OF may be higher than those found in plasma. This is due to the physicochemical properties of the compound (pKa, protein binding, liposolubility, molecular weight) and, as already mentioned, the influence of pH and salivary flow itself, which may vary from person to person [21].

The classic techniques used in the preparation of samples are solid-phase extraction (SPE) and liquid-liquid extraction (LLE). However, in current days, there is the need to resort to simple and inexpensive techniques with good recovery and selectivity, in order to minimize the amount of time spent in sample preparation, as well as the volume of sample and organic solvents required. In this context, miniaturized techniques such as solid-phase microextraction (SPME), microextraction in packed syringe (MEPS), and dispersive liquid-liquid microextraction (DLLME) have gained attention [22].

In the same sense, more recently, the dried matrix spots (DMS) sampling approach has attracted great interest, particularly in the analytical and forensic toxicology fields, since it requires reduced sample volumes and presents a great long-term stability. Dried blood spots (DBS) have been the most common DMS approach used for the analysis of drugs of abuse and metabolites [23]. However, few studies are starting to evaluate the application of DMS to other biological matrices, namely OF, often named dried saliva spots (DSS). DSS have been shown as a promising sampling and extraction procedure [24], with literature reporting its usefulness in the determination of D- and L-lactic acid in diabetic patients [25], anticonvulsant drugs [26] and lidocaine in saliva, and a candidate drug for the treatment of migraine as well [27].

In addition, DSS is described as a simple, accurate, robust, fast, inexpensive, and non-invasive alternative, being used for both collection and preservation of OF samples [27–29]. The procedure begins with the deposition of a 50 to 100 μL sample volume onto a filter paper [24, 30]. Thereafter, the filter paper is left to dry at room temperature. After this process, using a small quantity of the appropriate organic solvents, the target analytes are extracted and subsequently analyzed by chromatographic analysis.

The aim of the present work is the development and validation of a method for the determination of MTD and its metabolite EDDP in OF samples using the DSS sampling approach and GC-MS/MS. Its application will result in a simple, sensitive, and cost-effective procedure to determine these compounds enabling their monitoring.

Materials and methods

Reagents and standards

All analytical standards were purchased from Sigma-Aldrich (Sintra, Portugal) at the concentrations of 1 mg/mL (EDDP) and 100 μg/mL (MTD, MTDd3, and EDDPd3) in methanol. Methanol LiChrosolv® and acetonitrile Prolabo®, both provided by VWR International (Carnaxide, Portugal), were of HPLC grade. Ethyl acetate from VWR International (Carnaxide, Portugal) was of analytical grade. Isopropanol, dichloromethane, and hexane both provided by Fisher Scientific UK (Loughborough, UK) were all of analytical grade.

Deionized (DI) water was obtained from a Milli-Q System (Millipore, Billerica, MA, USA). Formic acid (Panreac Química SA, Barcelona, Spain) was also used with pro-analysis grade. Whatman™ 903 protein saver cards were acquired from Sigma-Aldrich (Sintra, Portugal). Working solutions were prepared by serial dilutions with methanol to final concentrations of 1000 and 2500 ng/mL. Internal standards (IS) were used at the concentrations of 100 ng/mL (MTD-d3) and 1 μg/mL (EDDP-d3). All the standard solutions were stored at − 10 °C and protected from the light.

Biological specimens

Drug-free OF samples were provided by laboratory staff and were used during all experiments. Authentic OF samples were received by the Farmaco-Toxicologia laboratory and were sent from Centro de Atendimento ao Toxicodependente - Casas de Santiago (Belmonte). These samples were collected by spitting and were subsequently stored at − 10 °C until analysis.

Gas chromatographic and mass spectrometric conditions

An HP 7890A gas chromatography system, equipped with a model 7000B triple quadrupole mass selective detector, from Agilent Technologies (Soquimica, Portugal) was used. This system was coupled to an automatic MPS2 autosampler injector from Gerstel (Mülheim an der Ruhr, Germany). The separation of the analytes was achieved using a capillary column of fused silica (30 m × 0.25 mm, I.D., 0.25 μm) with 5% phenylmethylsiloxane (HP-5 MS) supplied by J & W Scientific (Folsom, CA, USA). The oven temperature started at 150 °C for 2 min, followed by an increase of 20 °C per minute until 300 °C and was maintained for 3 min. The total time of the chromatographic run was 12.50 min. The injection volume of the sample was 2 μL in split mode (3:1), and the injector and transfer line temperatures were set at 220 and 280 °C, respectively. The carrier gas, helium, was set at a constant flow rate of 0.8 mL/min. In the mass spectrometer, the helium flow was 1.5 mL/min and that of nitrogen was 2.5 mL/min in the collision cell in electron impact mode, with a current of 35 μA and electron energy 70 eV. Data were acquired on multiple reaction monitoring (MRM) mode, using the MassHunter WorkStation Rev. B.02.01 acquisition software (Agilent Technologies). It was possible to choose two transitions for each compound (one quantitative and one qualitative) based on the highest masses and the most abundant mass peaks to maximize signal-to-noise ratio of the compounds in matrix extracts (Table 1).

Sample preparation

Of samples were thawed at room temperature, after which 50 μL was applied to the DSS card. The extraction procedure was optimized (see section Optimization of the extraction procedure), and the final conditions were as follows. After overnight drying, 1 mL of isopropanol and 20 μL of IS were added to the DSS cards, according to Jager et al. [31]. The mixture was agitated for 1 min at 70 rpm on a roller-mixer shaker. Thereafter, centrifugation was performed at 3500 rpm at 4 °C for 15 min. Subsequently, the supernatant was collected into glass tubes and evaporated to dryness under a gentle stream of nitrogen. The resulting residue was reconstituted in 50 μL of methanol and 2 μL was injected into the GC-MS/MS system.

Validation procedure

The described method was fully validated according to the guiding principles of the Food and Drug Administration (FDA) [32], the Scientific Working Group for Forensic Toxicology (SWGTOX) [33], and the specific recommendations from the EBF (European Bioanalysis Forum) [34] for the validation of methodologies when DBS procedures are involved.

The validation was performed following a 5-day validation protocol, and the studied parameters included selectivity, linearity, lower limits of quantification (LOQs), limits of detection (LODs), intra- and inter-day precision and accuracy, recovery, stability, and dilution integrity. The entire validation procedure was performed by spiking blank oral fluid samples at the required concentration levels.

Results and discussion

Optimization of the extraction procedure

Four parameters were considered for the optimization process, and these were extraction solvent, solvent volume, extraction time, and drying time. These experiments were performed in triplicate ($n = 3$) in order to minimize interferences and

Table 1 Mass spectrometric conditions

Analyte	Transitions (m/z) Precursor ion–product ion	Retention time (min)	Collision energy (eV)	Dwell time (ms)
Methadone	222.1–105.1*	8.46	20	50
	222.2–117.0		20	50
Methadone-d3	225.0–105.1*	8.46	10	50
EDDP	275.4–232.2*	7.94	20	50
	275.4–247.2		15	50
EDDP-d3	237.2–220.2*	7.93	10	50

*Quantifying transition

systematic errors. The entire optimization study was performed using MTD and EDDP at a concentration of 100 ng/mL.

The first step was the selection of the most suitable organic solvent that would allow greatest recoveries without simultaneously extracting interferences present in the biological matrix (Fig. 1a). This figure shows isopropanol as the solvent which resulted in better recoveries for both target analytes. However, in the case of EDDP, both isopropanol and methanol provided good recoveries.

After selecting the most appropriate solvent, the volume of isopropanol to be used in the extraction was evaluated (Fig. 1b). One milliliter of solvent appeared to be the most appropriate, since no statistical differences were observed between all studied volumes ($F(1,4) = 3.29$, $p < .05$) for EDDP and ($F(1,4) = 3.18$, $p < .05$) for MTD. In addition, this volume seemed to be in agreement with the new trends of lowering the consumption of organic solvents and the toxicity associated to their use, minimizing also the costs.
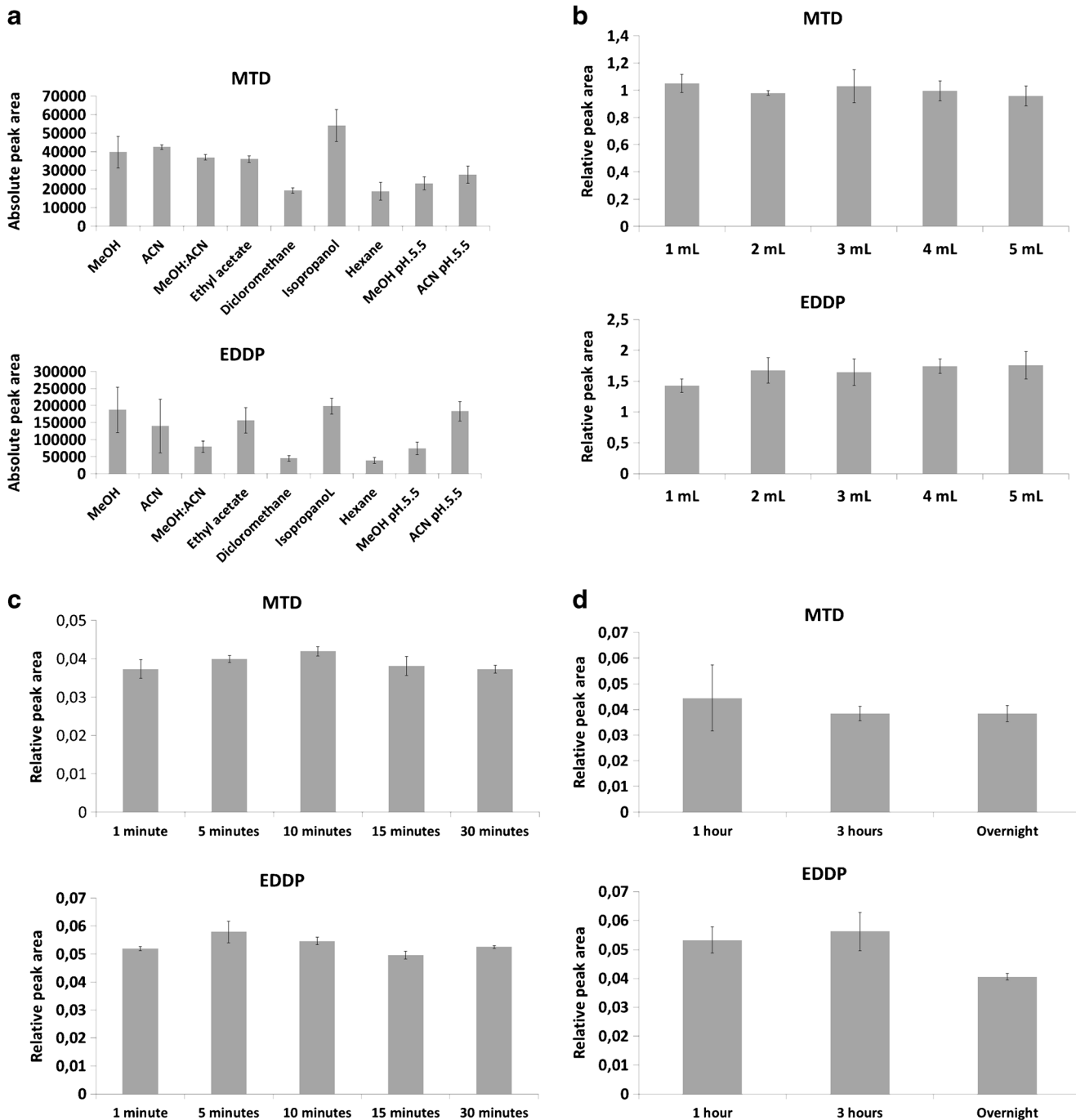
Fig. 1 Effects of the different organic solvents and/or mixtures in the extraction process (a), effects of the different solvent volumes (b), extraction time (c), and time of drying (d) in the extraction process

The other studied parameters were extraction and drying times. Regarding extraction time (Fig. 1c), no significant differences were observed for both EDDP and MTD $(F(1,4) = 6.93, p < .05)$ and $(F(1,4) = 3.11, p < .05)$ respectively. For this reason, the shortest period of time was selected. The studied drying times were 1 h, 3 h, and overnight. Despite the fact that a drying time of 1 h seemed to provide the best response (Fig. 1d), we have chosen to dry the spots overnight, thus assuring that all spots were completely and reproducibly dry before extraction (since with 1 h, some of the spots were not totally dry, and 3 h would be worse concerning laboratory workflow).

Method validation parameters

Selectivity

The method's ability to identify and quantify EDDP and MTD in the presence of endogenous compounds from the matrix was evaluated [35]. For this, blank OF samples from ten different origins were analyzed and checked for interferences at the retention times and selected transitions of the analytes of interest.

Additionally, a confirmation of the relative intensities (relative abundances) presented by each target analyte was performed as well [36]. Also, other compounds like
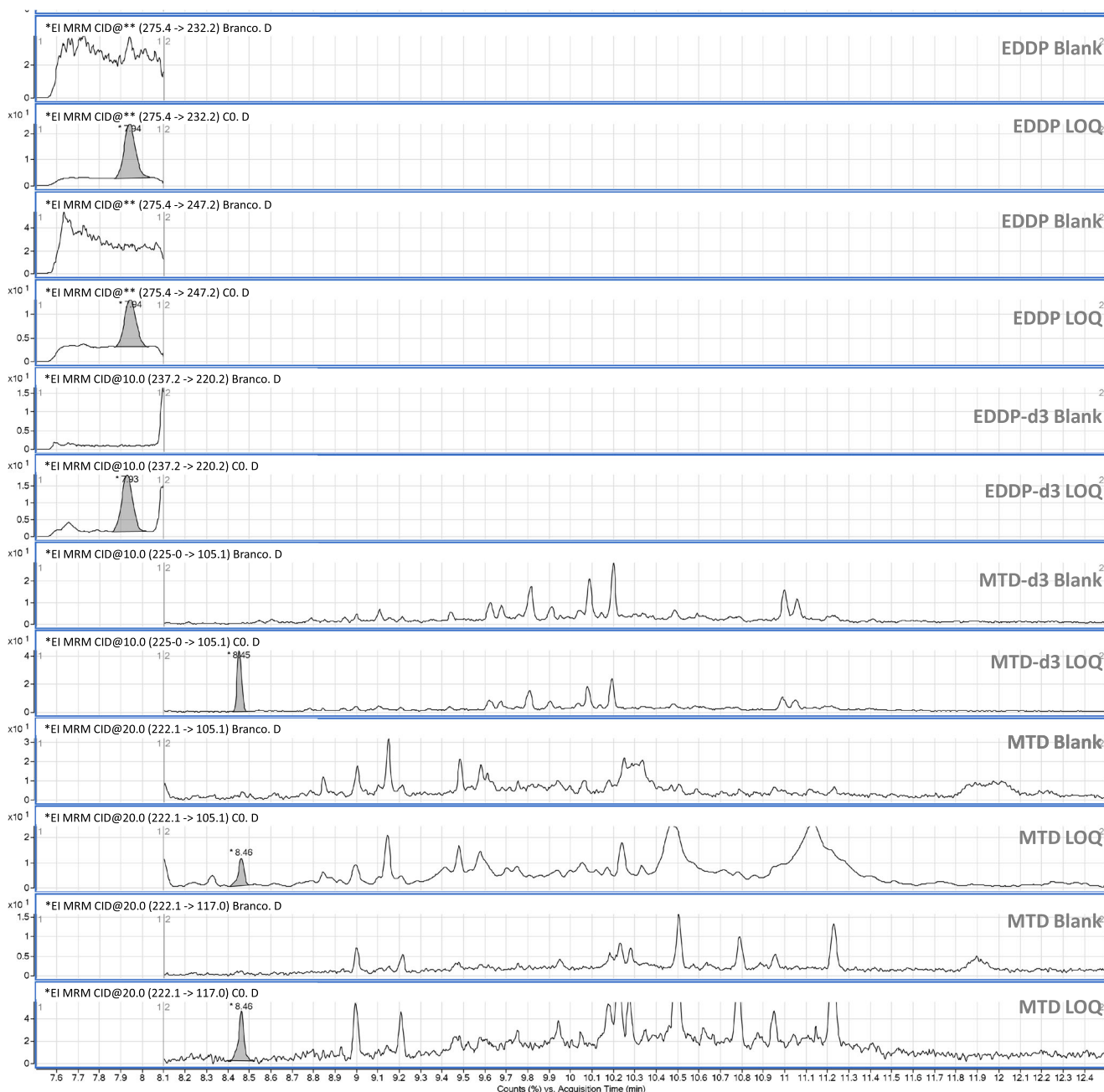

Fig. 2 Chromatogram of selected fragments obtained after extraction of a blank sample and a sample spiked at the LLOQ

Table 2 Linearity data ($n = 5$)

Compound	Linear range (ng/mL)	Linearity		R^2	LLOQ (ng/mL)	LOD (ng/mL)
		Slope	Intercept			
Methadone	10–250	0.007 ± 0.001	0.336 ± 0.101	0.998 ± 0.002	10	5
EDDP	10–250	0.069 ± 0.024	0.863 ± 0.823	0.997 ± 0.002	10	5

amphetamine-type stimulants, cocaine, cannabinoids, opiates, caffeine, and nicotine metabolites were also studied in order to assure the selectivity of the present method.

Considering the WADA criteria for acceptance [36], no interferences were identified in the blank OF samples and EDDP and MTD were successfully identified in all the analyzed samples; hence, the described method was considered selective (Fig. 2).

Calibration curves and limits

The method was considered linear in the range of 10 to 250 ng/mL for both EDDP and MTD. No weighting factors were applied. The acceptance criteria included a determination coefficient (R^2) of at least 0.99 and the accuracy of the calibrators within $\pm 15\%$, except at the LOQ, where $\pm 20\%$ was considered acceptable (Table 2).

The method's LOQ was defined as the lowest concentration of analyte that could be determined with adequate precision and accuracy, i.e., with a coefficient of variation (CV, %) of less than 20% and a relative error (RE, %) within a $\pm 20\%$ interval of the nominal concentration.

The LOD is the lowest concentration reliably distinguished from the blank, showing a discrete peak presenting a signal-to-noise ratio of at least 3 [37]. LOD was determined by analyzing 10 replicates of spiked samples at 5 ng/mL.

The LOD and LOQ obtained for both analytes were 5 and 10 ng/mL, respectively.

The literature reporting the application of OF for MTD and EDDP determination is not vast. Nevertheless, when comparing the present results to those reported in the literature, one can assume that the limits obtained can be considered more than satisfactory, considering that a sample volume as low as 50 μL was used.

Concheiro et al. [38] reported LOQs of 1 ng/mL and LODs of 0.2 ng/mL for both compounds, yet using greater volumes of OF (250 μL) using a LC-MS/MS system. Also, Ya-Ching et al. [39] obtained LOQs for MTD and EDDP of 20 and 3 ng/mL, respectively, using 1 mL of sample by GC-MS.

The results obtained with the present method are surpassed by Hsiu-Chuan et al. [40], who analyzed 40 μL of OF by LC-MS/MS, reaching a LOQ of 0.25 ng/mL for both MTD and EDDP and a LOD of 0.1 ng/mL for MTD and 0.25 ng/mL for EDDP. The use of a LC-MS/MS system and a direct injection of the specimen with no extraction can explain the authors' results.

It is important to emphasize that the comparison of the present study with others published where the same preparation technique was used is not possible, since this is the first work that uses DSS as extraction technique for the determination of MTD and EDDP in OF samples.

Intra-day, inter-day, and intermediate (combined intra- and inter-day) precision and accuracy

Intra-day precision and accuracy were assessed by analyzing 6 replicates of blank OF samples spiked with EDDP and MTD at 3 concentration levels on the same day, treated according to the described DSS procedure. The obtained CVs were lower than 11% for all studied concentrations, with an inaccuracy within $\pm 6\%$.

Inter-day precision and accuracy were studied within a 5-day period at 7 concentration levels. The obtained CVs were lower than 15% for the target analytes at all tested concentrations, with a mean relative error within $\pm 11\%$.

Simultaneously with these studies, intermediate (combined intra- and inter-day) precision and accuracy were also evaluated by the analysis in triplicate of quality control (QC) samples at four concentration levels (15, 35, 125, and 225 ng/mL) during the 5-day validation period ($n = 15$). The obtained CVs were lower than 10% for the analytes, with a mean relative error within $\pm 5\%$. Table 3 shows the precision and accuracy data.

Dilution integrity

With the possibility of having authentic samples with concentrations above the upper limit of quantification (ULOQ), it is necessary to perform dilution integrity tests during validation [41].

To assess dilution integrity, two concentrations above the ULOQ were prepared and three dilution factors were studied (1:2, 1:5, and 1:10), all of them allowing the concentrations to fall within the linearity interval. This test was performed in triplicate. All dilutions were performed with blank OF and have allowed an accurate determination of EDDP and MTD after multiplying by the dilution factor. The obtained CVs, for both EDDP and MTD, were typically lower than 13%, with an inaccuracy within $\pm 12\%$ (Table 4).

Table 3 Precision and accuracy data

Compound	Inter-day precision and accuracy (n = 5)				Intermediate precision and accuracy (n = 5)				Intra-day precision and accuracy (n = 6)			
	Concentration (ng/mL)	Measured concentration (ng/mL)	CV (%)	RE (%)	Concentration (ng/mL)	Measured concentration (ng/mL)	CV (%)	RE (%)	Concentration (ng/mL)	Measured concentration (ng/mL)	CV (%)	RE (%)
Methadone	10	10.45 ± 1.03	9.84	4.46	15	15.36 ± 0.59	7.25	2.50	10	9.90 ± 1.09	10.99	− 1.04
	25	22.26 ± 1.12	5.05	− 10.94								
	50	52.31 ± 3.88	7.42	4.62	35	36.53 ± 2.21	7.50	4.25	100	105.71 ± 4.25	4.02	5.71
	100	108.13 ± 4.49	4.16	8.13								
	150	156.29 ± 8.14	5.21	4.20	125	136.32 ± 2.15	9.00	4.50	250	238.39 ± 11.20	4.70	− 4.64
	200	211.08 ± 9.21	4.36	5.54								
	250	255.77 ± 8.16	3.19	2.31	225	226.93 ± 1.39	1.50	1.14				
EDDP	10	10.89 ± 0.81	7.46	8.95	15	15.30 ± 0.27	6.00	1.75	10	10.55 ± 0.98	9.31	5.45
	25	23.66 ± 1.69	7.15	− 5.37								
	50	48.36 ± 2.94	6.09	− 3.28	35	34.78 ± 1.55	9.25	− 0.75	100	101.20 ± 10.72	10.60	1.20
	100	102.22 ± 7.79	7.62	2.21								
	150	155.09 ± 4.63	2.99	3.39	125	121.20 ± 5.27	5.75	− 2.75	250	264.95 ± 21.50	8.12	5.98
	200	204.74 ± 4.44	2.17	2.37								
	250	248.58 ± 5.49	2.21	− 0.57	225	226.20 ± 8.95	7.50	0.50				

Table 4 Dilution integrity ($n = 3$)

Compound	Dilution factor	Concentration (ng/mL)	Measured concentration (ng/mL)	CV (%)	RE (%)
Methadone	1:2	300	318.21 ± 15.34	4.82	6.07
		500	495.15 ± 29.38	5.93	− 0.97
EDDP		300	299.06 ± 12.08	4.04	− 0.31
		500	492.48 ± 62.78	12.75	− 1.50
Methadone	1:5	300	329.46 ± 9.69	2.94	9.82
		500	521.72 ± 35.23	6.75	4.34
EDDP		300	295.15 ± 29.54	10.01	− 1.62
		500	457.84 ± 8.09	1.77	− 8.43
Methadone	1:10	300	334.64 ± 2.72	0.81	11.55
		500	516.98 ± 46.18	8.93	3.40
EDDP		300	307.41 ± 14.00	4.55	2.47
		500	469.79 ± 40.67	8.66	− 6.04

Stability

By carrying out stability studies, it is possible to observe the behavior of the target analytes when the spiked samples are submitted to different storage conditions. With this, it was possible to evaluate the applicability of the method after different types of storage.

Four types of stability were studied: processed samples, short-term, freezing/thawing, and long-term. This assay was performed with samples spiked at the QC concentration levels (15, 35, 125, and 225 ng/mL).

Regarding processed samples stability, the previously analyzed QC samples were re-analyzed after 24 h in the autosampler. The obtained CVs were lower than 13% with mean relative errors within a ± 12% interval.

For short-term stability study, the OF samples were left at room temperature for 24 h, after which 50 μL was applied to the DSS cards. The CVs obtained were lower than 15% for all concentrations, with a mean relative error within ± 13%. Regarding the stability during freeze/thaw cycles, the samples

were frozen for 24 h, then allowed to thaw at room temperature, and then frozen again. A total of 3 cycles were performed. The obtained CVs were lower than 15% for all concentrations, with an inaccuracy within 6%.

Finally, for long-term stability, the prepared QC samples were applied on the DSS cards and left on bench-top unassisted for 2 months. It was possible to observe that EDDP was stable for 2 months; however, MTD was only stable for 2 weeks. In all concentrations, the CVs obtained were lower than 14% at all studied concentrations, with a mean relative error within ± 15%. Table 5 shows the stability data.

Recovery

In order to study the recoveries obtained with the present extraction procedure, two sets of samples were prepared in triplicate at three concentration levels (25, 100, and 250 ng/mL). The first set was prepared by spiking the analytes post-DSS procedure (representing 100% efficiency), while the

Table 5 Stability data ($n = 3$)

Compound	Concentration (ng/mL)	Processed sample stability		Short-term stability		Freeze/thaw stability		Long-term stability	
		CV (%)	RE (%)	CV (%)	RE (%)	CV (%)	RE (%)	CV (%)	RE (%)
Methadone	15	8.83	− 4.75	0.81	5.81	9.44	2.37	11.11	0.42
	35	12.56	5.03	5.71	5.21	5.30	− 1.75	10.90	1.12
	125	1.68	11.39	1.72	12.92	5.20	6.04	5.53	− 8.44
	225	2.03	9.03	5.60	2.12	4.52	4.28	11.55	4.15
EDDP	15	4.18	2.80	7.69	0.27	7.02	3.44	8.75	− 4.13
	35	7.76	− 0.19	2.82	− 3.91	14.60	0.57	7.31	3.74
	125	9.56	2.93	2.72	− 4.64	11.11	0.74	11.71	− 4.84
	225	11.56	4.90	9.49	7.37	1.47	5.56	3.45	− 10.85

Table 6 Extraction efficiency (%) of the compounds under the optimized extraction conditions ($n = 3$)

Compound	Efficiency (%)		
	25 ng/mL	100 ng/mL	250 ng/mL
Methadone	60.5 ± 13.3	74.2 ± 13.7	66.7 ± 7.1
EDDP	63.3 ± 4.2	54.2 ± 3.2	45.3 ± 2.9

second set was prepared by spiking pre-DSS procedure. The recovery results were obtained by comparing the relative peak areas obtained by both sets.

Table 6 resumes the obtained results. An average recovery of 67% was obtained for MTD, and 54% for EDDP.

A recovery close to 100% for MTD and EDDP was reported by dos Santos et al., using 0.1 mL of OF and liquid-liquid extraction. The authors have obtained a LOD of 32 ng/mL and LOQ of 99 ng/mL for MTD and a LOD of 29 ng/mL and a LOQ of 88 ng/mL for EDDP. Also in this same work, but using SPME, no great differences were observed for these results [42].

In the work of Damns et al. [43], using 200 µL of OF, the recoveries of MTD and EDDP were also close to 100%, however using protein precipitation.

Regarding DSS applications and recoveries obtained, Abdel-Rehim A and Abdel-Rehim M [24] showed that the DSS analysis of the spiked saliva sample compared to the direct injection of the same concentration of lidocaine resulted in a recovery close to 100%.

In the case of antiepileptic drugs determined in OF using DSS, Carvalho et al. [26] reports recoveries ranging from 41

Table 7 Comparison DSS procedure with solid-phase extraction as sample preparation

	DSS		SPE	
	EDDP	MTD	EDDP	MTD
Sample #1	54.48	243.67	57.60	269.61
Sample #2	38.75	150.29	37.06	152.31
Sample #3	65.21	210.85	62.39	201.08
Sample #4	47.89	198.91	40.73	190.03
Sample #5	15.12	70.86	19.25	81.11
Sample #6	27.30	178.29	32.71	184.15

to 61% which can be compared with the ones obtained with the present method. Nevertheless, in these last cases, a proper comparison cannot be performed since recoveries will also depend on the compounds affinity to the filter paper and on the sample preparation procedures.

It should be noted that the differences between the extraction procedures may influence the recovery of compounds. It is also noted that with these recovery values, the LODs and LOQs achieved are within the range of concentrations commonly used for monitoring of MTD consumption in real samples, and are even lower than those published by other authors; therefore, the herein proposed method has been shown to be sensitive for application in the laboratory routine.

Method applicability

The present method was successfully applied in routine analysis of MTD and EDDP with DSS sampling. As an example,
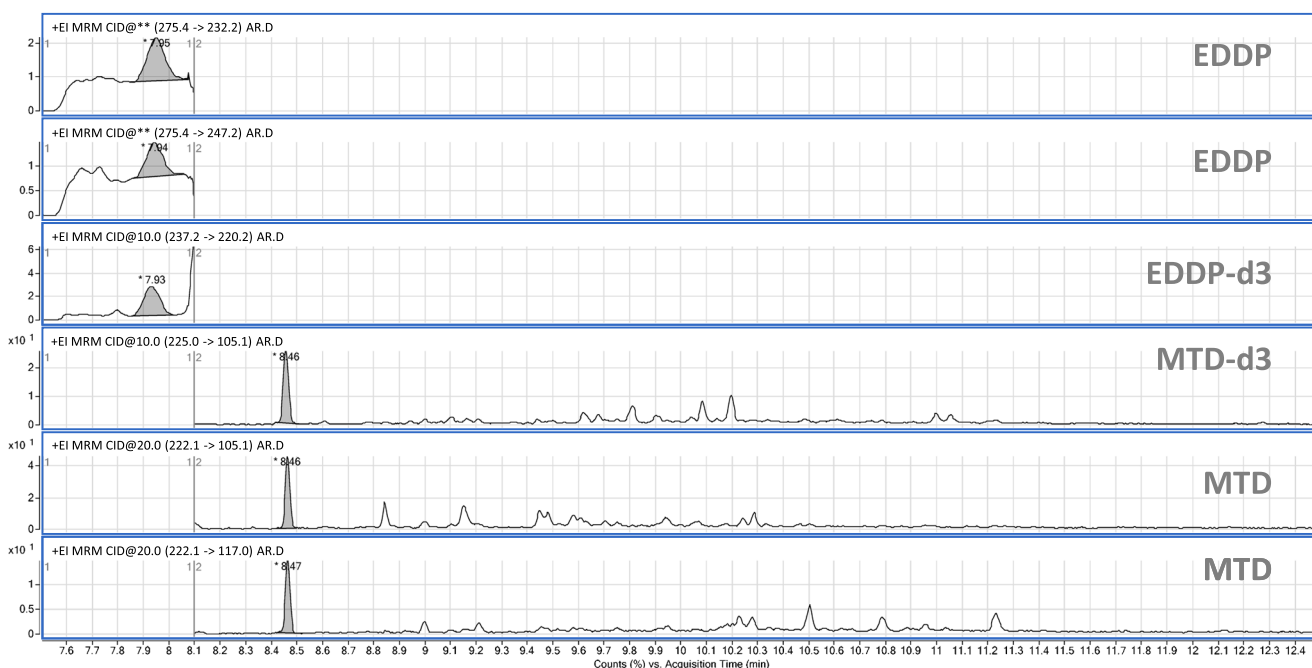

Fig. 3 Chromatogram obtained after analysis of an authentic sample

Fig. 3 shows a chromatogram of an authentic sample sent from Centro de Atendimento ao Toxicodependente - Casas de Santiago (Belmonte) which is responsible for the treatment of opiate addicts. The concentrations of MTD and EDDP were 246.56 ± 7.53 and 20.65 ± 3.08 ng/mL, respectively.

In addition, these results were compared to those obtained by analyzing neat oral fluid samples and extraction by SPE [12], and the obtained concentrations were perfectly overlapping (BIAS < 15%), which demonstrates de adequacy of this new sampling approach (Table 7).

Conclusions

With the use of DSS and GC-MS/MS, it was possible to develop and validate a method to determine MTD and its major metabolite EDDP in OF samples. The extraction procedure was proven to be simple, sensitive, and cost-effective and allowed to obtain great stability of the samples in diverse conditions, not compromising the transport and storage after sampling. The present method was linear in the range of 10–250 ng/mL, with low detection and quantification limits as well as adequate precision and accuracy values using reduced small sample volume (50 µL). This reduced sample volume can be extremely advantageous, since sample amount is often limited.

The applicability of the method was proven using real OF samples, demonstrating the usefulness of this method in the therapeutic monitoring of these drugs.

Compliance with ethical standards

The present study was approved by the ethics committee from Casas de Santiago and has been conducted according to ethical standards. The analyzed samples belonged to individuals who gave an informed consent for their use (including the drug-free samples used in the validation experiments, provided by laboratory staff), and all analyses were carried out according to the ethical standards of the institution.

Conflict of interest The authors declare that they have no conflicts of interest.

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