Quantitation of Methadone and Metabolite in Patients under Maintenance Treatment

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Gas chromatography–mass spectrometry quantitative method was developed to monitor concentrations of methadone and its metabolite 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) in plasma and urine of patients. The developed method was simple, accurate and reproducible to quantify methadone and EDDP in plasma and urine samples in the concentration range of 15–1,000 and 50–2,000 ng/mL, respectively. The proposed analytical method was applied to plasma and urine samples obtained from 96 patients undergoing methadone maintenance treatment (MMT) with daily methadone doses of 2–120 mg/day. Urinary methadone excretion was observed to be significantly affected by pH, in which the ratio of methadone to EDDP was two times higher in acidic urine (P = 0.029). The findings of this study further enhance the guidelines for monitoring of methadone treatment among outpatients. Methadone-to-EDDP ratio in urine was found to be consistent at 24 and 4 h, hence suggesting the possibility that outpatients may be monitored with single urine sample in order to check for compliance. This study which provides data on peak concentrations of methadone and EDDP as well as the ratio of both compounds has added to the body of knowledge regarding pharmacokinetic properties of methadone among heroin-dependent patients under MMT.

Introduction

Methadone is a long-acting mu-opioid receptor agonist that is widely used in methadone maintenance treatment (MMT) for heroin addicts. There is large interindividual variability in response to methadone as demonstrated by a wide range of stabilized methadone doses that are required by patients under MMT, from as low as 10 mg/day to as high as 780 mg/day (1–3). Furthermore, patients taking the same dose of methadone had shown variable response towards methadone (4, 5).

The variability in the response to methadone is likely to be due to both pharmacodynamics and pharmacokinetics factors, with likely a larger contribution by the latter encompassing genetic, physiological, pathological and pharmacological factors (6). Therefore, methadone dosage should be individually determined based on the subjective and objective data obtained from each patient. For this reason, many analytical methods have been developed to quantify methadone and its metabolite in biological samples (urine, plasma, serum, saliva, hair, sweat and brain). These include capillary electrophoresis (7), radioimmunoassay (8), gas chromatography (9–11), liquid chromatography (12–14) and enzyme immunoassay (15).

In this study, a gas chromatography–mass spectrometry (GC–MS) analytical method was developed to quantitate methadone and 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) in plasma and urine samples, which can be applied for both individual dosage monitoring and compliance screening. Even though blood sampling is considered as invasive, it provides the most accurate measurement of drugs present in the body (16, 17). Urine specimens, on the other hand, may provide less accurate quantitative measurement of drugs; however, it is easily accessible. Furthermore, previous study showed that urine-estimated plasma concentrations of methadone can provide a more reliable guide for dose adjustment (17, 18). The GC–MS system was selected due to its ability for ready detection of compound without the need for derivatization (11). Furthermore, it is capable of separating complex chemical mixtures into individual components by the GC column, while identifying and providing quantitative information of each compound by the MS system (19).

Materials and methods

Chemicals and reagents

(±)-Methadone, (±)-EDDP and (±)-methadone-D3 were purchased from Cerilliant (Round Rock, TX, USA). Methanol, ethyl acetate, ammonium hydroxide, hydrochloric acid and phosphoric acid were purchased from Merck KGaA, and isopropanol was purchased from Fisher Scientific. Blank human plasma samples were obtained from pooled plasma samples of patients from the Laboratory, whereas blank human urine samples were contributed by staff members of the Laboratory of Pathology, Hospital Kuala Lumpur (HKL), Malaysia. All pooled blank plasma and urine samples were firstly screened to confirm that they are negative for methadone with no interference especially at the retention time of methadone and EDDP, before proceeding to method validation.

Preparation of stock solution and eluting solvent

Stock solutions were prepared by dissolving 10 and 100 μL of known amounts of reference materials (1 mg/mL) in 1,000 μL of methanol to obtain concentrations of 10 and 100 μg/mL, respectively, for methadone, EDDP and methadone-D3. Methadone-D3 was used as the internal standard for methadone and EDDP at concentrations of 500 and 1,000 ng/mL in plasma and urine, respectively. A 100 mM of phosphoric buffer at pH 5, 0.1 N of hydrochloric acid and mixture of eluting solvent with ethyl acetate : ammonium hydroxide : isopropanol : ammonium hydroxide (7 : 2 : 1) were prepared.

Equipment

Agilent Technologies 7890A Gas Chromatograph connected to 5975c inert mass selective detector (MSD) (Santa Clara, CA, USA).
USA), 48-well-positive pressure manifold (UCT), nitrogen generator (Imatronic), Type 16500 Dri-Bath thermo block (Thermolyne), GPT nitrogen (MOX Gases Sdn Bhd) and centrifuge (Kubota 5420) were used.

Solid-phase extraction
Strata™-X-Drug B column (Phenomenex, Inc., Torrance, CA, USA) with dimension 60 mg/6 mL was used for solid-phase extraction (SPE). To each 1 mL of sample, 1 mL of 100 mM of phosphoric acid (pH 5.0) was added. The pH was verified to be between 4.0 and 6.0 using pH indicator sticks. The columns were placed on 48-well-positive pressure manifold, and the pre-treated samples were loaded into the columns followed by two washing steps with 1 mL of 0.1 N of hydrochloric acid and 1 mL of methanol. Nitrogen gas with pressure of 20 psi was applied in between each step. The columns were then left for 10 min to dry under nitrogen gas at a pressure of 100 psi. Following that, the analytes were eluted with 1 mL of eluting solvent. The eluates were then evaporated and dried under nitrogen gas (100 psi) with gentle heat (50°C) in thermo block. Finally, the dried analytes were reconstituted with 100 μL of methanol for GC–MS analysis.

GC–MS parameters
GC–MS analysis was performed on Agilent Technologies 7890A Gas Chromatograph connected to 5975c inert MSD with Triple-Axis Detector equipped with a Zebrón™ ZB Drug-1 column (10 m × 0.18 mm × 0.18 μm film thicknesses). Temperature of the injection port in split mode (1 : 10) was set at 180°C. The oven temperature was programed as follows: the initial temperature was set at 200°C, held for 3 min and ramped at 25°C/min to 225°C, where it was further ramped at 45°C/min to 300°C and held for 1 min. The equipment was operated in selective ion monitoring (SIM) mode, and the following ions were monitored: methadone: m/z 294, 223 and 309; methadone-D3: m/z 297, 226 and 312 and EDDP: m/z 277, 262 and 276.

Method validation
Method validation was carried out by establishing specificity, limit of detection (LOD), limit of quantitation (LOQ), calibration curve, selectivity, accuracy, intra- and interassay precision, recovery and stability. The same validation methods were applied for both plasma and urine matrix. The validation of the method closely followed the guidelines of FDA (20).

Samples from patients
The SPE method described previously was applied to urine and plasma samples that were collected from patients under MMT at the University Malaya Medical Centre (UMMC), HKL, University Malaya Centre for Addiction Sciences (UMCAS) and Rehabilitation Centre of Al-Rahman Mosque, Kuala Lumpur, Malaysia. Written informed consents were obtained from all patients. This study was approved by the Medical Ethics Committee of UMMC and Medical Research and Ethics Committee of National Medical Research Register. Two samples were collected from each patient: the first sample was collected prior to methadone administration (trough), that is, at 24 h after the previous dose and the second sample was collected at 4 h after methadone administration (peak). Urine samples were collected in urine containers, and blood samples were collected in ethylenediaminetetraacetic acid tubes. Blood samples were spun in a centrifuge at 3,500 rpm for 10 min, and plasma was separated and transferred into autoclaved microcentrifuge tubes. The pH of all collected urine samples was measured using pH indicator sticks. All of the urine and plasma samples were then kept in −20°C freezer before analysis.

Statistical analysis
Continuous data were presented as mean ± SD, and categorical data were presented as percentage. Normality test was performed with Kolmogorov–Smirnov test. Comparison of mean between three groups was performed using Kruskal–Wallis test for non-normally distributed variables. Multivariate analysis was performed to adjust for confounding factors. Analyses were performed using SPSS 16.0 (Chicago, IL, USA), and a P-value of <0.05 was considered statistical significant.

Results and discussions
GC–MS conditions
The GC–MS was first operated in scanning mode in order to determine the retention time and mass spectrum of both methadone and EDDP. The known mass spectra were then used to develop the chromatographic method in SIM mode which is higher in sensitivity (21). The total GC–MS run time for one sample was 6.67 min with a solvent delay of 2.50 min. Injection of spiked plasma or urine sample in SIM mode showed the retention time of EDDP at 3.80 min and the retention time of both methadone and methadone-D3 at 4.35 min as shown in Figure 1.

Method validation
The results of the method validation are summarized in Table I. From the analyses of extracted blank plasma samples, no interfering peaks were observed at the retention time of methadone, methadone-D3 and EDDP. Signal-to-noise ratios of 3 : 1 and 10 : 1 were calculated to determine the LOD and LOQ, respectively. For plasma, the LOD was 5 ng/mL for methadone and 2 ng/mL for EDDP and the LOQ was 15 ng/mL for methadone and 5 ng/mL for EDDP. For urine, the LOD was 10 ng/mL for methadone and 1 ng/mL for EDDP and the LOQ was 15 ng/mL for methadone and 2 ng/mL for EDDP.

The calibration curves of methadone and EDDP in plasma were constructed with six calibrators at concentrations of 15, 50, 100, 300, 500, and 1,000 ng/mL. The mean regression coefficient (r2) for methadone and EDDP was 0.999 and 0.996, respectively. On the other hand, the calibration curves of methadone and EDDP in urine were constructed with six calibrators at concentrations of 50, 100, 300, 500, 1,000 and 2,000 ng/mL. The mean r2 for methadone and EDDP was 0.999.

The presence of 19 potential interfering compounds (morphine, codeine, 6-acetylmorphine, 11-nor-9-carboxy delta-9-tetrahydrocannabinol, cocaine, ecgonine ethyl ester, benzoylecgonine, diazepam, nordiazepam, alprazolam, 7-amino-flunitrazepam,
phenobarbitone, amitriptyline, clomipramine, amphetamine, methamphetamine, MDMA, ephedrine and ketamine) at the concentration of 1,000 ng/mL in spiked plasma and urine samples (low concentration level) did not interfere with the quantitation of methadone and EDDP.

The intra- and interassay accuracy and precision for methadone and EDDP were <15%. Besides that, good recoveries of methadone and EDDP from both plasma and urine biological samples were observed (>95%). Methadone and EDDP were also found to be stable under short-term storage (4 h) in biological samples at room temperature and 1-day storage of postextracted samples in methanol at 4°C (%bias <15%).

Samples from patients under MMT

Demographic and toxicology characteristics of patients

The demographic and toxicology characteristics of patients are shown in Table II. The study group consisted of 96 patients who were under MMT for 1,322 days on average (SD ± 878, range 98–2,539). Methadone was administrated to patients daily in the form of syrup in the presence of a pharmacist. Of the total 96 patients, 76 were Malays, 4 were Chinese and 16 were Indians. All patients were males with average age of 47 years (SD ± 9, range 27–65). All patients were on single dose, and their average daily methadone dose was 61 mg/day.
The mean concentration of methadone in plasma at trough and peak was 197.14 ng/mL (SD ± 114.14, range 22.76–561.44) and 308.61 ng/mL (SD ± 157.40, range 63.38–766.14), respectively. The mean concentration of EDDP in plasma at trough and peak was 19.54 ng/mL (SD ± 10.63, range 5.75–82.20) and 32.07 ng/mL (SD ± 19.79, range 6.43–83.34), respectively. The trough plasma methadone concentration was consistent with those that have been previously obtained following administration of methadone doses between 20 and 200 mg/day. Bermejo et al. (11) obtained an average methadone concentration of 200 ng/mL (range 70–420 ng/mL) from 50 plasma samples that were collected at trough. Besides that, the trough plasma methadone concentration obtained in this study is also similar with those reported by Mohamad et al. (22) who obtained mean plasma methadone concentration of 235.26 ng/mL (range 269.0–708.50 ng/mL) from 49 patients with methadone dose range of 10–240 ng/mL; however, timing of sample collection was not mentioned. The methadone concentration at the peak was consistent with those of Jansson et al. (23) who obtained mean peak plasma methadone concentration of 337.30 ng/mL (SD ± 146.60, range 178.00–707.00) in 12 women patients.

There are a few previously reported studies on the plasma concentrations of EDDP from patients under MMT. The first study obtained a mean trough plasma EDDP concentration of 52 ng/mL (SD ± 37, range 14–211) from 93 male and female patients with methadone dose range of 10–235 mg/day (2). The second study obtained trough plasma EDDP concentration that ranged from 30 to 420 ng/mL from 10 patients with methadone dose range of 50–350 ng/day (24), whereas in the third study, plasma EDDP concentration ranged from <10 to 24 ng/mL from 22 patients without mention of the methadone dose and sample collection time (25). The trough EDDP plasma concentrations in this study were lower than previous studies (2, 24) which may be due to the lower methadone dose received by patients.

The mean concentration of methadone in urine at 24 h was 8,180.73 ng/mL (SD ± 7,592.36, range 101.33–32,003.60) and 12,397.41 ng/mL (SD ± 10,346.60, range 532.32–37,105.00),

(SD ± 25, range 2–120). The trough blood and urine samples were collected at a mean of 24 h (SD ± 2, range 17–27), and peak blood and urine samples were collected at a mean of 4 h (SD ± 0, range 3–4). The pH of all collected urine samples ranged from 5 to 9.

Of the 96 recruited patients, 34 patients consented for both trough and peak samples. The other 62 patients consented only for trough sample collection due to the reason of being unable or unwilling to return to or stay in the clinic for 4 h duration. A total of 96 plasma samples and 64 urine samples were collected at 24 h, whereas 54 plasma samples and 21 urine samples were collected at 4 h. Urine samples were not collected from all of the recruited patients since some did not agree to do so. All the collected 130 plasma samples and 85 urine samples were analyzed with GC–MS with the validated method as described previously. Dilution (1 : 10) of urine samples were carried out for those samples with concentrations higher than the linearity limit (2,000 ng/mL).

<table>
<thead>
<tr>
<th>Table I</th>
<th>Validation Results of Methadone and EDDP</th>
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<tr>
<td>Parameters</td>
<td>Plasma</td>
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<tr>
<td>Calibration curve</td>
<td>15–1,000</td>
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<tr>
<td>R²</td>
<td>0.999</td>
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<tr>
<td>LOD (ng/mL)</td>
<td>5</td>
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<td>LOQ (ng/mL)</td>
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<tr>
<td>Selectivity</td>
<td>Accuracy (%bias)</td>
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<td>Recovery (%)</td>
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<td>Stability</td>
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<th>Table II</th>
<th>Demographic and Toxicological Characteristics of Patients Included in the Study</th>
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<tr>
<td>Variables</td>
<td>Values</td>
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<tr>
<td>Age (years)</td>
<td>47 ± 9 (range 27–65)</td>
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<tr>
<td>Length of MMT (days)</td>
<td>1,322 ± 878 (range 98–2,539)</td>
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<tr>
<td>Methadone dose (mg/day)</td>
<td>61 ± 25 (range 2–120)</td>
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<td>Trough sample collection time (h)</td>
<td>24 ± 2 (range 17–27)</td>
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<tr>
<td>Peak sample collection time (h)</td>
<td>4 ± 0 (range 3–4)</td>
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<tr>
<td>Methadone plasma concentration at trough (ng/mL)</td>
<td>197.14 ± 114.14 (range 22.76–561.44)</td>
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<tr>
<td>Methadone plasma concentration at peak (ng/mL)</td>
<td>308.61 ± 157.40 (range 63.38–766.14)</td>
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<tr>
<td>EDDP plasma concentration at trough (ng/mL)</td>
<td>19.54 ± 10.63 (range 5.75–82.20)</td>
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<tr>
<td>EDDP plasma concentration at peak (ng/mL)</td>
<td>32.07 ± 19.79 (range 6.43–83.34)</td>
</tr>
<tr>
<td>Methadone urine concentration at 24 h (ng/mL)</td>
<td>8,180.73 ± 7,592.36 (range 101.33–32,003.60)</td>
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<tr>
<td>Methadone urine concentration at 4 h (ng/mL)</td>
<td>12,397.41 ± 10,346.60 (range 532.32–37,105.00)</td>
</tr>
<tr>
<td>EDDP urine concentration at 24 h (ng/mL)</td>
<td>7,416.42 ± 6,129.04 (range 66.01–27,006.30)</td>
</tr>
<tr>
<td>EDDP urine concentration at 4 h (ng/mL)</td>
<td>14,684.42 ± 9,788.99 (range 537.89–37,443.20)</td>
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</table>
respectively. The mean concentration of EDDP in urine at 24 and 4 h was 7,416.45 ng/mL (SD = 6,129.04, range 66.01–27,006.30) and 14,684.42 ng/mL (SD = 9,788.99, range 537.89–37,443.20), respectively. There have been increasing numbers of studies to determine the data on methadone and EDDP urinary excretion (26–29). However, no clear correlation between methadone doses and its concentration in urine was identified. One previous study determined that methadone concentration in urine samples collected 24 h after previous methadone intake from 50 patients with methadone doses of 20–200 mg/day. A methadone concentration range of 100–13,250 ng/mL was reported (11). Another study reported methadone and EDDP concentration ranging from 126 to 3,873 ng/mL and 3,600 to 12,910 ng/mL, respectively, from five urine samples without information about methadone dose and timing of sample collection (25).

**Concentration ratio of methadone to EDDP**

The ratios of methadone to EDDP at trough and peak were calculated for each individual and shown in Table III. The mean plasma methadone-to-EDDP ratio at trough was 12.67 (SD = 7.00, range 3.92–53.95, median = 11.01), whereas the mean plasma methadone-to-EDDP ratio at peak was 11.29 (SD = 5.34, range 4.06–30.04, median = 10.70). This suggested that methadone-to-EDDP ratio in plasma was generally similar at trough and peak in each particular individual on regular methadone intake, in which methadone concentration is ~12 times higher than the EDDP concentration. These findings were similar with a previously reported ratio of methadone to EDDP that ranged from 5.6 to 15.1 (25).

Meanwhile, the mean urine methadone-to-EDDP ratio at 24 and 4 h was 1.17 (SD = 0.69, range 0.14–3.64, median = 1.08) and 0.95 (SD = 0.60, range 0.06–2.00, median = 0.79), respectively. The mean urine methadone concentrations were slightly higher than EDDP at 24 h. However, ~50% of the patients had methadone concentrations higher than EDDP concentrations and vice versa for the other 50% of the patients. Previous studies that reported on urine samples collected at 24 h stated a higher EDDP concentrations compared with methadone concentrations, however, median ratios of EDDP to methadone in each individuals were <1.00, which indicated that methadone concentration in urine collected at 24 h was higher than EDDP concentration in most of the patients (28, 30). In contrast, another study reported consistently higher methadone concentrations in five analyzed urine samples. However, the timing of specimen collection was unspecified (25). On the other hand, the mean methadone-to-EDDP ratio in urine collected at 4 h was <1.00; however, ~50% of the urine samples had higher methadone concentrations and vice versa for the other 50% of the patients. This suggested that methadone-to-EDDP ratio in urine is generally similar at any time point of sample collection (within 24 h) in most patients on regular methadone intake, which is close to 1.00. Besides that, another study in outpatients reported no change in the ratio from stabilization to maintenance phase (28). Thus, the present findings suggest the possibility of monitoring outpatients with single urine sample.

The estimate of methadone-to-EDDP ratio in plasma and urine is useful as it can be applied to investigate short-time abstinence, in which a high methadone-to-EDDP ratio would indicate a recent consumption compared with a continuous intake (31). Besides that, methadone-to-EDDP ratio or vice versa is useful in explaining the activity of methadone metabolic enzymes in each individual (28). It was suggested that urine of patient which detected EDDP without methadone or with low concentration of methadone can be suspected as being a 'fast metabolizer’ (28). Furthermore, estimation of methadone-to-EDDP ratio is helpful in monitoring compliance of patients with prescribed methadone. Urine sample with only methadone without EDDP could be an indication of non-compliance. Evidence from previous case study reported that patients attempted to pass the compliance test by adding a portion of their prescribed methadone to their urine and diverting the remainder of their methadone (32). Non-compliance should only be confirmed after multiple monitoring to avoid misinterpretation of results due to intraindividual variation in the excretion patterns.

**Peak-to-trough ratio of methadone and EDDP**

Peak-to-trough concentration ratios of plasma methadone and EDDP were calculated from 34 patients with both trough and peak plasma samples collected. The results are shown in Table IV. The mean ratio was 1.79 (SD = 0.56, range 1.00–3.97) for methadone and 1.78 (SD = 0.75, range 0.62–3.64) for EDDP. A similar peak-to-trough ratio of methadone and EDDP was observed, which could be due to the comparable long elimination half-lives of both methadone and EDDP, which is 24–36 versus 40–48 h (33).

Peak-to-trough concentration ratio of methadone was used as surrogates of methadone elimination half-life in which a high ratio indicates a shorter elimination half-life (34). This ratio was suggested to be most clinically useful that can determine the appearance of withdrawal symptoms. It was estimated that an ideal methadone peak-to-trough ratio was 2.00 or less, in which the concentration at peak should be no more than twice the concentration at trough (35). From the result of this study, although the mean peak-to-trough ratio of methadone was <2.00, ~15% of the patients had ratios of >2.00. There is a high possibility that these patients may experience withdrawal symptoms before the consumption of the next methadone dose; therefore, splitting of daily methadone dose should be considered instead of increasing once-a-day dose. Previous studies reported that among patients with methadone peak-to-trough ratio of >2.00, increase in the

### Table III

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<thead>
<tr>
<th>Variables</th>
<th>Mean ± SD (range)</th>
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<tr>
<td>Plasma</td>
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<tr>
<td>Trough</td>
<td>12.67 ± 7.00 (3.92–53.95), median = 11.01</td>
</tr>
<tr>
<td>Peak</td>
<td>11.29 ± 5.34 (4.06–30.04), median = 10.70</td>
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<tr>
<td>Urine</td>
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<tr>
<td>24 h</td>
<td>1.17 ± 0.69 (0.14–3.64), median = 1.08</td>
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<tr>
<td>4 h</td>
<td>0.95 ± 0.60 (0.06–2.00), median = 0.79</td>
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### Table IV

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean ± SD (range)</th>
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<tbody>
<tr>
<td>Plasma</td>
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<tr>
<td>Methadone</td>
<td>1.79 ± 0.56 (1.00–3.97)</td>
</tr>
<tr>
<td>EDDP</td>
<td>1.78 ± 0.75 (0.62–3.64)</td>
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</table>
once-a-day methadone dose was not able to maintain the methadone level for a longer period in the body, in which it would only elevate the methadone concentration at peak but not at trough. Thus, it may result in overmedication several hours after dosing but is likely to result in withdrawal symptoms later (36, 37).

For the urine samples, peak-to-trough ratio was not calculated as the estimation of methadone or EDDP elimination in urine required total volume of urine voided. Furthermore, its excretion in urine is affected by urinary pH, glomerular filtration rate, chronic renal or liver disease, concomitant medication or any psychological condition (24). The limitation of this study is that the total volume of urine voided could not be determined from patients as all of them were outpatients and most of the patients were wage earners and, thus, it was troublesome for the patients to remain in the methadone clinic during the sampling period.

Effects of urinary pH on concentration ratio of methadone to EDDP
The influence of urinary pH on methadone-to-EDDP ratio was identified, and a bar chart of this ratio against urinary pH was plotted. However, there were fluctuations in the methadone-to-EDDP ratios across pH (Supplementary data, Figure S1). Thus, another bar chart (Figure 2) was plotted by grouping them into acidic, neutral and basic pH groups. There was statistical significant difference between methadone-to-EDDP ratio, and the three pH groups after adjustment of methadone dose received ($P = 0.029$). The mean methadone-to-EDDP ratios of acidic, neutral and basic urine were 1.21 (SD ± 0.64, range 0.14–3.64, median = 1.10), 0.83 (SD ± 0.61, range 0.16–2.50, median = 0.48) and 0.54 (SD ± 0.67, range 0.06–1.54, median = 0.30), respectively. Methadone-to-EDDP ratio was ~2 times lower in acidic group as compared with basic group, which indicated that the excretion of methadone was higher in the acidic group and lower in the basic group. This finding was found to be consistent with a previous study on complete 24 h urine samples, which reported that a 3-fold increase in methadone renal clearance at low pH was associated with decreased EDDP-to-methadone ratio (38). The increase in methadone excretion in acidic urine was due to the shorter plasma half-lives, decreased volume of distribution and increased body clearance as compared with alkaline urine (38, 39).

Conclusion
The GC–MS analytical method developed in this study was found to be a simple, accurate and reproducible quantitative method to quantify methadone and EDDP in plasma and urine samples. Screening of urine samples obtained at 24 and 4 h from patients under MMT revealed the possibility of monitoring outpatients with single urine sample. Besides that, the peak concentrations of methadone and EDDP as well as the ratio of both compounds that we obtained in this study have added to the body of knowledge regarding pharmacokinetic properties of methadone among heroin-dependent patients under MMT. Urinary methadone excretion was found to be significantly affected by pH; therefore, pH of urine samples obtained from patients should be determined especially while monitoring compliance to treatment. The proposed GC–MS method can be applied as a screening tool to monitor compliance, hence providing the means to identify individuals with subtherapeutic or toxic levels, and also to identify ‘fast’ metabolizer so that the adjustment or splitting of dosage could be carried out for a more effective treatment outcome.

Supplementary data
Supplementary data are available at Analytical Toxicology Journal online.

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