Rapid Determination of Methadone in Plasma, Cerebrospinal Fluid, and Urine by Gas Chromatography and Its Application to Routine Drug Monitoring

Norbert Schmidt,[1] Reinhard Sittl,[2] Kay Brune,[1] and Gerd Geisslinger[1,3]

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Determination of methadone (MET) in biological fluids can serve to adjust dosages in patients suffering from cancer pain or participating in methadone maintenance programs. We developed a gas chromatographic assay using nitrogen-phosphorus detection. The method involves a single-step extraction from alkalized plasma, cerebrospinal fluid, or urine into *n*-hexane/isoamylalcohol (99/1, v/v). Dextropropoxyphene was used as internal standard. Separation was achieved with a silica SE-52-CB column (13 m × 0.25-mm I.D.). The method was validated for the determination of MET in plasma, urine, and cerebrospinal fluid with a quantification limit of 0.5 ng/mL. The coefficients of variation for within-day and between-day precision were within 10.2 and 14.1%, respectively. Approximately 100 samples can be analyzed by one person in the course of a working day, making the method applicable to routine drug monitoring. The method was demonstrated to be sensitive and accurate for pharmacokinetic studies in plasma, urine, or cerebrospinal fluid.

KEY WORDS: methadone; gas chromatography with nitrogen–phosphorus detection; plasma; urine; cerebrospinal fluid.

INTRODUCTION

Since its introduction into clinical practice in 1946, methadone (MET) [(±)-6-dimethylamino-4,4-diphenylheptan-3-one] acquired a unique place in cancer pain therapy (1) and in maintenance treatment of patients dependent upon narcotics such as diacetylmorphine (2). The pharmacokinetic parameters of MET differ from that of other opioids (3). Its almost complete oral bioavailability (4,5) favors its use by the oral route. Pharmacokinetic studies in normal subjects (6), postoperative patients (7), and MET maintenance patients (8) indicate that a potential of accumulation exists due to a wide interindividual range of the terminal half-life. This variability may require dose adjustment on the basis of individual pharmacokinetic parameters.

Quantitation of MET in biological fluids requires a sensitive and specific analytical method (9). Previously published analytical assays include thin-layer chromatography (10,11) immunoassay techniques (12,13), UV spectrophotometry (14,15), gas chromatography (GC) using either flame ionization (16,6), electron capture detectors (17), or nitrogen–phosphorus (NP) detection (18,19), high-performance liquid chromatography (20,21,22), and gas chromatography–mass spectrometry (GC-MS) using electron impact (23) or chemical ionization (24–26). Combined GC-MS is a highly satisfactory method for the quantification of MET, but the expensive instrumentation required is not available in all laboratories.

The purpose of this report was to develop a highly sensitive and specific method which is easy to handle and allows on-line analysis of plasma samples of addicts or of patients suffering from chronic pain in order to achieve an optimal dosage regimen.

MATERIALS AND METHODS

Materials

The enantiomers of MET as the pure compounds (as HCl salts) were donated by Hoechst (Frankfurt/Main, Germany). Dextropropoxyphene (HCl salt), used as the internal standard (IS) was a gift from the Department of Forensic Medicine (University of Erlangen, Nuernberg, Germany). All other chemicals and solvents were of HPLC or reagent grade.

Standard Solutions

Stock solutions (100 μg free base/ml) of R-MET and the IS were prepared in distilled water or isopropylalcohol. Working standards were prepared in drug-free plasma, cerebrospinal fluid (CSF), and urine, respectively, from the stock standard to yield concentrations of 0.5–400.0 ng/mL of the biological fluid and were stored frozen.

Apparatus and Chromatographic Conditions

The gas chromatographic analyses were performed on a Hewlett–Packard Model 5890 II instrument (Palo Alto, California) equipped with a nitrogen–phosphorus detector, a Hewlett–Packard 7673 A automatic injector with a split-splitless capillary inlet system operating in the splitless mode. The columns were 13 m × 0.25-mm I.D. and packed with silica SE-52-CB [0.25-μm film thickness] (CS Chromatography Service, Langerwehe, Germany). Column head pressure of the carrier gas (helium) was 50 psi. The nitrogen, air, and hydrogen flow rates were 28, 110, and 4 mL/min, respectively. The column oven was heated to 70°C, followed by a temperature gradient of 20°C/min to 240°C, the injection port to 220°C, and the detector to 280°C. The GC parameters and data were controlled by HP Chemstation software.

Extraction Procedure

For the determination of MET a 1.00-mL aliquot of plasma, CSF, or urine (standard, quality control, or a sample from a dosed patient) and 50 μl of the prepared IS solution (10 μg/mL) were transferred to a conical glass tube and alkalized with 0.20 mL of 1 *M* K_2CO_3. It was then extracted into 2.50 mL of *n*-hexane/isoamylalcohol (99/1, v/v) by agi-

[1] Department of Experimental and Clinical Pharmacology and Toxicology, University of Erlangen-Nuernberg, Universitaetsstr. 22, D-8520 Erlangen, Germany.
[2] Department of Anesthesiology, University of Erlangen-Nuernberg, Maximiliansplatz 1, D-8520 Erlangen, Germany.
[3] To whom correspondence should be addressed.

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tation for 30 min at room temperature. The organic layer was evaporated to dryness under a gentle stream of dry nitrogen. The dry residue was redissolved in 100 μL of *n*-hexane and was transferred to a microinsert and dried again. After the addition of 4 μL isopropylalcohol and vortexing, aliquots of 1 μL were injected into the GC system.

Calculations

Standard curves were obtained by adding IS and known amounts of MET in distilled water to a drug-free plasma, CSF, or urine sample. Extraction was carried out as described above. Standard curves were constructed for plasma, CSF, and urine, respectively. The method was calibrated for each run by regressing MET/IS peak area ratios against methadone concentrations in the calibration standards. Power regression ($y = A \cdot x^B$) was found best to represent the data in the concentration range of 0–50 ng/mL; above this range, linear regression ($y = am + b$) was employed. MET concentration in quality controls and unknown samples was calculated using the regression equations.

Validation of the method was performed by assaying quadruple sets of calibration and quality control standards on 3 separate days. The daily calibration curves were used to calculate the concentration of MET in standards and controls, and these data were pooled across experimental days to evaluate precision and accuracy. The method was validated in human plasma, human CSF, and human urine on three occasions, respectively. Quality control samples were run with each batch of unknowns and showed no significant change in the concentration over a 3-month period.

Extraction recovery of MET and IS was determined by spiking plasma, CSF, or urine to contain 1.0, 5.0, 10.0, 50.0, 100.0 ng/mL MET and 500.0 ng/mL IS and were extracted as described above. Peak areas from extracted samples ($n = 4$) were compared to peak areas from injection of appropriate standard solutions.

APPLICATION TO DRUG MONITORING

Patients suffering from severe cancer pain were treated with R-MET employing individual drug monitoring. Initially a bolus of 5 mg R-MET was administered. Venous blood samples (3 mL) were collected over approximately 100 hr to determine the pharmacokinetic parameters needed to calculate the i.v. loading dose and the oral maintenance dose (27) (Fig. 1).

RESULTS AND DISCUSSION

Chromatographic Separation and Validation

Chromatographic separation was completed within 14 min and no interfering peaks were observed with either 2-ethyl-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), the main metabolite of MET, or other analgetics such as morphine, tramadol, and arylpropionic acids. Typical chromatograms of a blank human plasma and a real sample obtained from a patient after administration of 5 mg R-MET are depicted in Fig. 2. The retention times were 11.9 and 12.2 min for MET and the IS, respectively. The limit of quantification (lowest concentration that could be determined during the between-day validation with either precision or an accuracy of less than or equal to 15%) was 0.5 ng/mL. The recovery data for MET and IS in human plasma, CSF, and urine are listed in Table 1. The peak-area ratios of MET were best fitted ($r > 0.998$) using power regression to the amount of MET added to blank human plasma, CSF, or urine in the

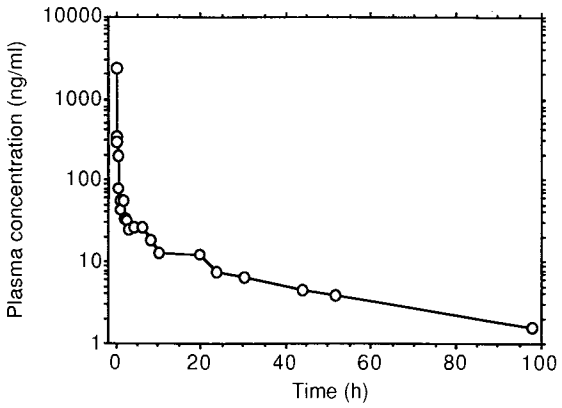

Fig. 1. Plasma concentration vs time profile of R-MET in a patient following iv administration of 5 mg of the R-enantiomer.

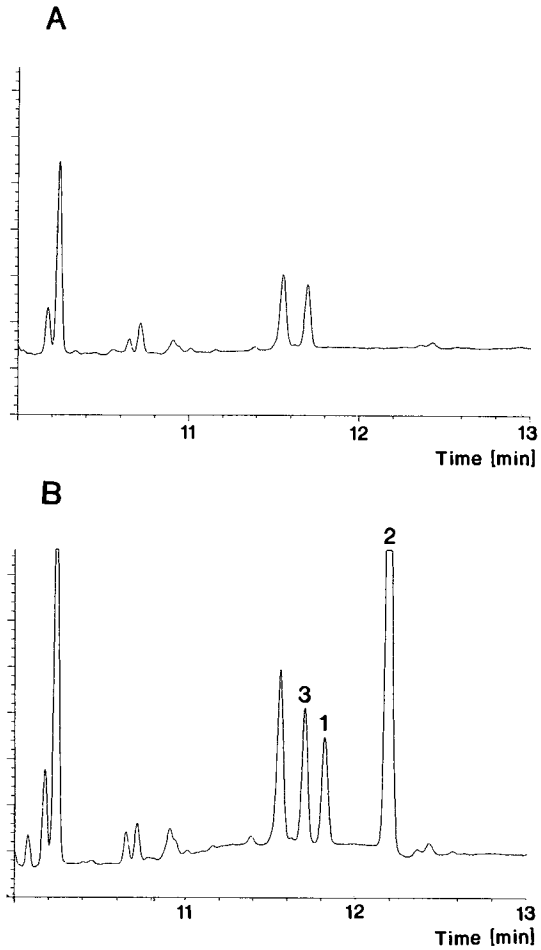

Fig. 2. Typical GC chromatograms of blank plasma (A) and of a real sample of plasma (15 ng/mL) (B) obtained from a patient 8 hr following administration of 5 mg R-MET iv. Peaks: 1, *R*-MET; 2, dextropropoxyphene (IS) (500 ng/mL); 3, unknown peak.

Table I. Recovery Data for Methadone and the IS in Human CSF, Plasma, and Urine

	Concentration (ng/mL)		Recovery (mean ± SD, n = 4) (%)
	MET	IS	
CSF	1.0		100.5 ± 1.7
	5.0		97.0 ± 2.3
		500.0	74.8 ± 5.1
Plasma	1.0		102.8 ± 3.8
	5.0		100.3 ± 5.1
	10.0		98.9 ± 4.2
	50.0		98.5 ± 4.9
	100.0		95.2 ± 5.2
	400.0		97.6 ± 5.5
		500.0	78.6 ± 6.0
Urine	1.0		92.3 ± 4.0
	5.0		96.6 ± 7.1
	10.0		83.8 ± 5.9
	50.0		108.2 ± 6.1
	100.0		91.2 ± 7.0
		500.0	75.5 ± 4.7

given concentration range (Table I) below 50 ng/mL (typical equation parameters: A = 0.00507, B = 1.0505); above this concentration range linear regression showed the best fit (typical equation parameters: A = 0.0504, B = 0.0123). The within-day and between-day precisions over 4 days are given in Tables II and III, respectively. A major advantage of the method we have developed is that it requires no reextraction. Other previous methods require reextraction (18,19) and show a lower sensitivity, 1.0 or 2.5 ng/mL, with a coefficient of variation of 22 (19) or 11.8% (18), respectively.

Table II. Within-Day Coefficients of Variation (CV) in Determination of Methadone

	Concentration (ng/mL)		CV (%)	Mean percentage difference[a]
	Added	Found (mean ± SD; n = 4)		
CSF	1.0	0.98 ± 0.10	10.0	2.0
	10.0	10.10 ± 0.84	8.4	1.0
Plasma	0.5	0.49 ± 0.05	10.2	2.0
	1.0	1.04 ± 0.07	6.8	4.0
	5.0	5.30 ± 0.26	4.9	6.0
	10.0	10.0 ± 0.68	6.7	0.1
	25.0	25.7 ± 1.27	4.9	2.8
	50.0	51.5 ± 2.78	5.4	3.0
	100.0	100.5 ± 5.36	5.3	0.5
	200.0	201.7 ± 12.94	6.4	0.9
	400.0	399.5 ± 13.10	5.5	1.1
Urine	0.5	0.49 ± 0.02	6.4	2.0
	1.0	0.98 ± 0.03	5.2	2.0
	5.0	4.75 ± 0.35	7.4	5.0
	10.0	9.5 ± 0.49	5.2	5.0
	25.0	24.9 ± 2.50	10.0	0.4
	50.0	46.3 ± 7.51	1.6	7.4
	100.0	100.9 ± 7.10	7.1	0.9

[a] Mean percentage difference: (found − added)/added × 100.

Table III. Between-Day Coefficients of Variation (CV) in Determination of Methadone

	Concentration (ng/mL)		CV (%)	Mean percentage difference[a]
	Added	Found (mean ± SD; n = 4)		
CSF	1.0	1.00 ± 0.10	14.1	0.1
	10.0	10.4 ± 0.79	7.6	4.0
Plasma	0.5	0.49 ± 0.06	12.6	2.0
	1.0	0.99 ± 0.08	7.9	1.0
	5.0	5.2 ± 0.30	5.8	4.0
	10.0	10.3 ± 0.66	6.4	3.0
	25.0	25.4 ± 1.79	7.1	1.6
	50.0	50.9 ± 2.24	4.4	1.8
	100.0	101.9 ± 6.66	6.5	1.9
	200.0	201.2 ± 11.48	5.7	1.8
	400.0	398.1 ± 13.90	5.1	1.4
Urine	0.5	0.50 ± 0.03	6.8	0.1
	1.0	0.99 ± 0.04	4.5	0.1
	5.0	4.9 ± 0.40	8.2	2.0
	10.0	9.5 ± 0.38	3.9	5.0
	25.0	24.5 ± 1.5	6.2	2.0
	50.0	48.5 ± 4.6	9.5	3.0
	100.0	100.4 ± 5.1	5.0	0.4

[a] Mean percentage difference: (found − added)/added × 100.

Application to Therapeutic Drug Monitoring

About 100 samples can be analyzed by one person in the course of a working day using an automatic sampler running overnight. The whole procedure from extraction to determination of MET in plasma, CSF, or urine of patients was completed within 4 hr, which is suitable for therapeutic drug monitoring. Additionally, we have validated the method for the determination of MET in urine, making the method applicable to patients participating in methadone maintenance programs. One site of action of opioids is in the dorsal horn of the spinal cord. In order to determine the CSF distribution of MET, the method was also validated in CSF. However, this GC assay, like all other GC and GC-MS assays published so far, is not able to determine the two enantiomers of MET stereoselectively. This disadvantage appears acceptable because no inversion of R-MET into the less analgesic S-MET was found during therapeutical treatment with MET. These data were obtained using a stereospecific high-performance liquid chromatographic MET assay, which will be published elsewhere (submitted for publication). The stereoselective HPLC method is, however, neither sensitive nor easy to handle for on-line drug monitoring.

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