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# Determination of tramadol by capillary gas chromatography with flame ionization detection
## Application to human and rabbit pharmacokinetic studies

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**Abstract**

A rapid, sensitive, precise and accurate capillary gas chromatographic assay with flame ionization detection was developed for the determination of tramadol in human, rabbit, pig and dog plasma. It is comprised of only a one-step extraction procedure with dichloromethane at pH 11.15 and gas chromatography on a capillary column. The recoveries of tramadol and meperidine (internal standard) were greater than 88%. Calibration graphs were linear over the concentration range 12.5–10 000 ng/ml with a coefficient of variation, both within-day and between-day, of less than 10% at any level. The limit of detection was 8 ng/ml of plasma based on signal-to-noise ratio of 3. Six other clinically used analgesics were investigated to check for potential interferences and their analytical conditions. The specificity of this assay was checked with two major metabolites of tramadol ($M_1$: *O*-demethyltramadol; $M_2$: *N*-demethyltramadol). Tramadol in plasma did not decompose significantly at −20°C for 56 days. Pharmacokinetic application with intravenous tramadol in humans and rabbits revealed that tramadol followed a two-compartment open model with one distribution phase and one elimination phase. The distribution and elimination half-lives in humans were 1.02 and 141.9 min. The distribution and elimination half-lives in rabbits were 7.31 and 63.2 min, respectively. 

*Keywords:* Pharmacokinetics; Tramadol

## 1. Introduction

Tramadol is a centrally acting analgesic which acts at opioid receptors and also appears to modify the transmission of pain impulses by inhibition of norepinephrine and serotonin uptake [1]. The marketed preparation of tramadol is a racemic mixture which contains both the (+)- and (−)-enantiomers of tramadol. Both of these isomers exert their analgesic effect through different, but complementary and interactive pharmacologic mechanisms [2].

Tramadol may be administered orally, rectally, intravenously or intramuscularly [3]. In patients with pain, intravenous or intramuscular tramadol ex-

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hibited an analgesic activity equivalent to meperidine and one-tenth as potent as morphine [1–3]. Tramadol is well tolerated in short term use with the principal side effects of dizziness, nausea, vomiting, sedation, dry mouth and sweating. The tolerance and dependence potential of tramadol appears to be low. It is an useful alternative to the opioid analgesics currently available for the treatment of acute or chronic pain [3,4].

Tramadol undergoes extensive hepatic metabolism in humans and animals with phase I biotransformation of *O*- and *N*-demethylation followed by phase II biotransformation (glucuronidation and sulphation) of the *O*-demethylated compounds [5–7]. The major metabolites of tramadol in humans are *O*-demethyltramadol ($M_1$) and *N*-demethyltramadol ($M_2$). $M_1$ is pharmacologically active. Apart from $M_1$, all other metabolites are pharmacologically inactive [5–7]. Although $M_1$ has been shown to have four- to 200-times greater affinity for the μ-opioid receptor and a two- to fourfold higher analgesic potency than tramadol (in mice), the contribution of $M_1$ to pain relief in humans is unknown [8].

In the present report, we have developed a gas chromatographic method (GC) with flame ionization detection (FID). This method includes a one-step extraction procedure and gives good precision and accuracy with the low quantitative level of tramadol to 8 ng/ml. This sensitive method has been studied in human, rabbit, pig and dog plasma. Besides, pilot pharmacokinetic studies in patients and rabbits have been investigated to test the suitability of this method for clinical use.

## 2. Experimental

### 2.1. GC conditions

The HP 6890 series GC system (Hewlett-Packard, Wilmington, DE, USA) consisted of an automatic liquid sampler, an oven, a flame ionization detector and an integrator. The oven temperature was set up at 100°C, increased at a rate of 10 C°/min to 250°C and then held for 8 min. The injector temperature was 250°C and the detector temperature was 260°C. Pressure program of carrier gas for inlet: 123 kPa initial pressure, 123 kPa/min to 386 kPa, 0.2 min constant. Injection was performed in the splitless mode, purge off time 0.5 min. The carrier gas (nitrogen) flow was 1 ml/min. Separation was achieved using a capillary column (RTX 10024; 30.0 m×0.32 mm I.D., 0.25 μm film thickness) (Restek, Bellefonte, PA, USA). Data analysis was performed using the HP Chemstation software.

### 2.2. Chemicals and reagents

Tramadol and its major metabolites ($M_1$, $M_2$, Fig. 1) were kindly supplied by Grünenthal (Aachen, Germany). Meperidine (internal standard) was purchased from the National Bureau of Controlled Drugs, Department of Health, Taiwan. All chemicals and solvents were of analytical grade. All aqueous solutions were prepared using Mili-Q water (Milli-RO 60, Millipore, Bedford, MA, USA).

### 2.3. Standard solutions

#### 2.3.1. Tramadol

A stock solution of 1.0 mg/ml tramadol was prepared in water and seven standard solutions from 0.25 μg/ml to 200 μg/ml in water were made by a serial dilution. Aliquots of these standard solutions

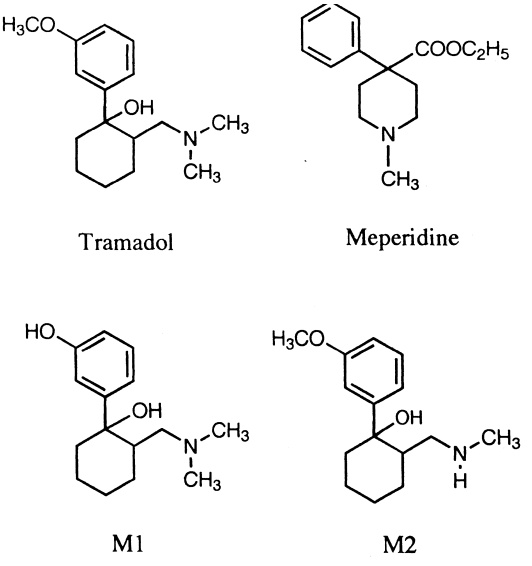


Fig. 1. Structures of tramadol, its metabolites ($M_1$: *O*-demethyltramadol and $M_2$: *N*-demethyltramadol) and meperidine (internal standard).

(5% of the final volume) were added to blank plasma to give final concentrations of 12.5, 25, 125, 250, 2500, 5000, 10 000 ng/ml.

#### 2.3.2. Internal standard

A 200 μg/ml meperidine stock solution was prepared in water and further diluted to give a working solution of 4 μg/ml in water. A 50-μl aliquot (200 ng) was added to every 1 ml aliquot of human, pig or dog plasma standard or specimen, and 25 μl (100 ng) to every 0.5 ml of rabbit plasma.

### 2.4. Sample preparation

To 1 ml of human, pig or dog and 0.5 ml of rabbit plasma placed in a 10 ml capacity glass tube, fitted with a PTFE-lined screw cap, 50 or 25 μl of internal standard solution and 1 or 0.5 ml of 0.5 *M* sodium carbonate buffer (pH 11.15) were added. The samples were extracted with 2 ml of dichloromethane by mixing for 30 s on a vortex shaker. After centrifugation at 1880 *g* for 10 min, the organic layer was poured into another 10 ml glass tube and evaporated to dryness by a refrigerated evaporator (models VLP 120, SC 110 and RVT 400, Savant, Holbrook, NY, USA). The samples were reconstituted by 50 μl of methanol. Aliquots of 1 μl were injected into the GC system.

### 2.5. Calibration

Calibration curves were prepared with blank plasma samples spiked with tramadol to cover the concentration range from 12.5 to 10 000 ng/ml and with the internal standard at the fixed concentration of 200 ng/ml. Calibration graphs were obtained by plotting drug concentrations against the peak-area ratio of tramadol/meperidine. The concentrations of unknown samples (tramadol) were determined by using the linear regression line (unweighted) of the calibration standard.

### 2.6. Repeatability, precision and accuracy

The repeatability of the method was estimated by comparing the linear regression slopes, intercepts and correlation coefficients of the calibration curves from human plasma. Precision and accuracy were determined on spiked human samples at seven concentrations (12.5–10 000 ng/ml) with respect to a calibration graph prepared every day. The precision of the method was expressed as the within-day and day-to-day coefficient of variation (%). The accuracy of this analytic method was determined by a method calculated as percent of mean deviation from known concentration [(concentration found−known concentration)×100/known concentration]. All samples for these purposes were freshly prepared and processed daily, including preparing the standard solution from the same stock solutions (1.0 mg/ml).

### 2.7. Selectivity

To determine the selectivity of this GC system, two major metabolites of tramadol ($M_1$, $M_2$) were studied, as well as drugs commonly used during anesthesia and in the postoperative period. These drugs included thiopentone, propofol, midazolam, etomidate, ketamine, succinylcholine, pancuronium, vecuronium, atracurium, diazepam, atropine, ephedrine, epinephrine, phenylephrine, glycopyrrolate, propranolol, dexamethasone and furosemide. Naloxone and analgesics, such as buprenorphine, codeine, fentanyl and morphine were also investigated. A 5-μg amount of the above drugs was added into 1 ml plasma. After extraction, these drugs were injected into the GC system to check their potential interference with the assay.

### 2.8. Stability and recovery

To determine the stability of tramadol in frozen human plasma, aliquots of seven standard tramadol solutions were added to a batch of blank human plasma (12.5–10 000 ng/ml), and then stored under −20°C freezing. The concentration was determined at 8 weeks after freezing. The tramadol calibration graph was constructed for each experiment. The results of the stability studies were analyzed using a 90% confidence interval approach [18].

The extraction recovery of tramadol and the internal standard in human plasma was determined at all levels of the calibration graph by comparing the data obtained by the direct injection of standard

aqueous solutions to those obtained after the whole extraction procedure.

### 2.9. Pharmacokinetic studies

#### 2.9.1. Humans

After institutional approval and informed consent, three patients (aged 38±4) receiving general anesthesia for gall bladder surgery enrolled into the study. Tramadol 100 mg was given intravenously at the induction of general anesthesia. A 7-ml of blood was obtained from a vein into a heparinized tube just before dosing and 2 ml at 1, 2, 3, 4, 5, 10, 20, 30 min and at 1, 2, 4, 6, 8 h after dosing.

#### 2.9.2. Animals

Following the guidelines of the American Association for the Accreditation of Laboratory Animal Care, four 5-month-old male New Zealand white rabbits weighing between 2.6 and 3.1 kg were used. After placing the animal in a restraining box, cannulations of the auricular artery and vein in the opposite ears were done. A 5-ml volume of blood was obtained from the artery at time zero and 1 ml at 2, 5, 10, 15, 30 min and at 1, 2, 3, 4, 5, 8 h after intravenous administration of tramadol (30 mg/kg). The blood was collected into heparinized tubes (50 μl heparin, 1000 I.U./ml).

Both human and rabbit plasma were obtained by centrifugation and immediately frozen at −20°C until assay. Plasma concentration–time profiles of tramadol in humans and rabbits were fitted by using the computer program PCNONLIN (version 3.0, Statistical consultants) [9]. Akaike information criteria, weighted residual sum of squares, and residual plots were used to judge the goodness-of-fit of the model to data. A C-strip computer program was used to obtain the initial parameter estimations, which were required for nonlinear regression analysis by the computer program PCNONLIN [10]. Pharmacokinetic parameters such as intercepts, the first-order rate constants, half-lives, clearance, and area under the plasma concentration time graph were calculated for patients and rabbits by standard formulae [11].

## 3. Results

### 3.1. Chromatography

As the chromatograms of extracts from humans, rabbits, pigs and dogs were similar, only the typical chromatograms of extracts from human plasma are shown (Fig. 2). No interfering peaks were detected in the blank plasma or in samples from the human subjects or animals. Although, a ghost peak appeared between 10.5 and 11.0 min, it did not interfere with the resolution of tramadol.

### 3.2. Limit of detection, column retention times, retention factors and resolution

The limit of detection, retention times, capacity factors, resolution of tramadol and its metabolites, and other analgesics, which are widely used, were demonstrated in Table 1. No interfering peak to tramadol was found during this detection. The low detecting limit of tramadol, defined as a signal-to-noise ratio greater than 3, was 8 ng/ml of plasma, respectively. Certain drugs which are commonly used during anesthesia and in the postoperative period were also selected to check for their potential interference in the assay of tramadol. These included thiopentone, propofol, midazolam, etomidate, ketamine, succinylcholine, pancuronium, vecuronium, atracurium, diazepam, atropine, ephedrine, epinephrine, phenylephrine, glycopyrrolate, propranolol, dexamethasone and furosemide. No interfering peaks were found during the analysis.

### 3.3. Repeatability, precision and accuracy

Over a period of 84 days, the calibration graphs ($n$=32) were linear in the concentration range 12.5–10 000 ng/ml with correlation coefficients of 0.998±0.001 (mean±SD) and with a minimum intercept of 0.08±0.08 (peak area ratio; mean±SD) The slopes averaged 2.21±1.21 ml/ng with a coefficient of variation of 9.1%. Precision and accuracy studies in plasma showed an acceptable coefficient of variation (<10%) and high accuracy for both within-day ($n$=5) and day-to-day ($n$=20) studies (≤9%), as shown in Table 2.

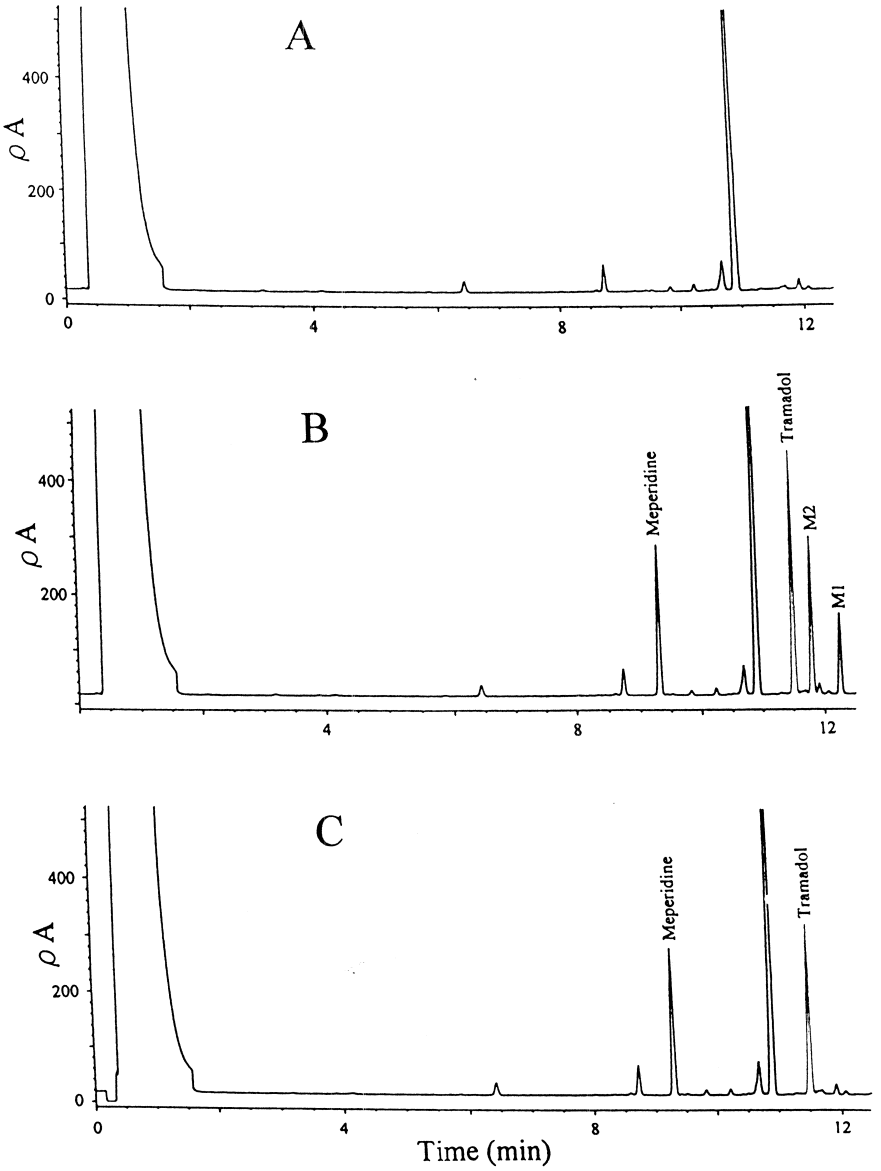


Fig. 2. Chromatograms of extracts from (A) blank plasma, (B) plasma spiked with tramadol (250 ng/ml) and its metabolites ($M_1$: *O*-demethyltramadol 250 ng/ml and $M_2$: *N*-demethyltramadol 250 ng/ml) and (C) sample from a patient (tramadol 210 ng/ml). Internal standard (meperidine 200 ng/ml).

### *3.4. Stability and recovery*

The stability of tramadol in plasma under freezing at −20°C for 8 weeks were also determined. The results indicate that no significant degradation occurred at 8 weeks after freezing.

Compared to other extracted organic solvents or mixtures tested for extraction (benzene, diethyl ether, chloroform, or hexane–isopropanol), the solvent dichloromethane gave the best recovery and chromatograms with less background noise for both human and animal samples. The absolute recoveries

Table 1
Retention times, retention factors, resolution and limit of detection of various drugs[a]

| Drug | $t_R$ (min) | $\kappa'$ | $R$ | Limit of detection (ng/ml) |
|---|---|---|---|---|
| Tramadol | 11.53 | 13.41 | [b] | 8 |
| $M_1$ | 12.27 | 14.34 | 8.41 | 32 |
| $M_2$ | 11.81 | 13.76 | 3.18 | 16 |
| Meperidine | 9.33 | 10.66 | 22.92 | 8 |
| Morphine | 16.4 | 19.50 | 55.32 | 2500 |
| Fentanyl | 20.8 | 25.00 | 105.31 | 90 |
| Codeine | 15.9 | 18.88 | 49.72 | 50 |
| Buprenorphine | 16.3 | 19.38 | 54.24 | 5000 |
| Naloxone | 20.09 | 24.11 | 97.32 | 125 |

[a] Conditions: see text; $t_R$ = retention time of peak; $\kappa'$ = retention factor = $(t_1 - t_o)/t_o$; $t_1$ = retention time of drug detected; $t_o$ = retention time of unretained peak; $R$ = resolution = $2|t_2 - t_1|/(W_2 + W_1)$.

[b] Tramadol is the standard drug for detection; $t_2$ = retention time of tramadol; $W_1$ = peak width of the drug detected; $W_2$ = peak width of tramadol. $M_1$: *O*-demethyltramadol, $M_2$: *N*-demethyltramadol.

of tramadol and the internal standard — meperidine — were between 88 and 93%, independently of the tramadol concentration (Table 3). The one-step extraction procedure is fairly rapid. This allows the analysis of 80 samples at least per day using an autosampler.

### *3.5. Pharmacokinetic studies*

The GC method has been used extensively in our laboratories. The observed plasma concentration–time curves for tramadol in three surgical patients and four rabbits are shown in Fig. 3. Both the data of humans and rabbits were successfully fitted to a linear two-compartment open model with one distribution phase and one elimination phase (Table 4). The distribution and elimination half-lives in human were 1.02 and 141.9 min, respectively. The distribution and elimination half-lives in rabbits were 7.31 and 63.2 min, respectively.

## 4. Discussion

Following oral or parenteral administration, tramadol 100 mg exhibits an analgesic activity of 4 to 6 h [1–4] with minimal effective concentration of around 200–300 ng/ml in plasma [12–14]. Several methods have been reported to determine the plasma concentration of tramadol. These include GC–mass spectrometry [12,15], GC with nitrogen-selective detection [16], and high-performance liquid chromatography with UV detection [17] or with fluorescence detection [13,18,19]. All these methods are sensitive enough with a detecting limit of tramadol below a

Table 2
Precision and accuracy of tramadol determined by the GC-FID method

| Known concentration (ng/ml) | Concentration found (mean±SD; ng/ml) | Coefficient of variation (%) | Accuracy (% mean deviation) |
|---|---|---|---|
| Within-day ($n$=5) | | | |
| 12.5 | 13.6±1.3 | 9.6 | 8.9 |
| 25 | 27.1±2.3 | 8.5 | 8 |
| 125 | 121±11 | 9.1 | −3.2 |
| 250 | 262±22 | 8.4 | 4.8 |
| 2 500 | 2398±146 | 6.1 | −4.1 |
| 5 000 | 4920±192 | 3.9 | −1.6 |
| 10 000 | 9680±397 | 4.1 | −3.2 |
| Day to day ($n$=20) | | | |
| 12.5 | 13.5±1.2 | 8.9 | 8 |
| 25 | 27.2±2.2 | 8.1 | 8 |
| 125 | 120±9 | 7.5 | −4 |
| 250 | 242±15 | 6.2 | −3.2 |
| 2 500 | 2463±131 | 5.3 | −1.5 |
| 5 000 | 5134±186 | 3.6 | 2.7 |
| 10 000 | 10310±381 | 3.7 | 3.1 |

Table 3
Absolute recoveries of tramadol and meperidine (internal standard) from spiked plasma samples ($n=8$)

| Drug | Concentration (ng/ml) | Recovery (%) (mean±SD) | Coefficient of variation (%) |
|---|---|---|---|
| Tramadol | 12.5 | 88.4±7.2 | 8.1 |
| | 25 | 90.6±8.2 | 9.1 |
| | 125 | 92.3±7.0 | 7.6 |
| | 250 | 89.7±4.8 | 5.4 |
| | 2 500 | 89.9±3.9 | 4.3 |
| | 5 000 | 90.7±8.2 | 9.0 |
| | 10 000 | 91.3±5.4 | 5.9 |
| Meperidine | 200 | 89.4±4.6 | 5.1 |

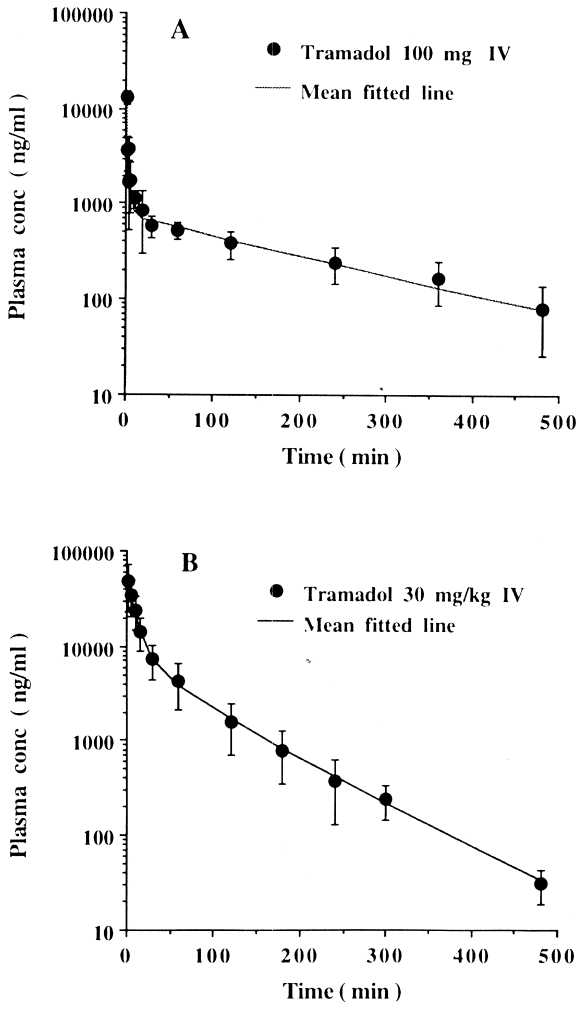


Fig. 3. Plasma concentration–time profiles of tramadol in three patients (A) and four rabbits (B) receiving intravenous tramadol.

Table 4
Pharmacokinetic parameters of tramadol in three surgical patients and four rabbits after intravenous tramadol injection

| Parameter[a] | Unit | Human | Rabbit |
|---|---|---|---|
| Dose | | 100 mg | 30 mg/kg |
| A | μg/ml | 36.2±32.6 | 50.6±29.0 |
| B | μg/ml | 0.76±0.06 | 7.74±4.42 |
| $\alpha$ | 1/min | 1.029±0.856 | 0.107±0.041 |
| $\beta$ | 1/min | 0.005±0.002 | 0.012±0.004 |
| $T_{1/2\alpha}$ | min | 1.02±0.68 | 7.31±2.90 |
| $T_{1/2\beta}$ | min | 141.9±50.0 | 63.2±24.2 |
| $AUC_{0-\infty}$ | μg min/ml | 192.0±65.0 | 1071.7±459.1 |
| $CL_t$ | ml/min | 55.6±16.2 | 3.3±1.7 |
| Body weight | kg | 68±3 | 2.8±0.2 |

[a] Equation: plasma concentration ($C_p$) = $A\ e^{-\alpha t} + B\ e^{-\beta t}$; $A$, $B$, intercepts; $\alpha$, $\beta$ are the first-order rate constants for the central and tissue compartments; $T_{1/2}$: half-life of the first-order rate constant; $AUC_{0-\infty}$, area under the time–concentration graph to time infinity; $CL_t$, total plasma clearance.

level of 20 ng/ml. However, some of these methods involve a time-consuming multiple extraction procedure [13,16], others need relatively costly equipment (mass spectrometry) [12,15]. Besides, some did not challenge their selectivity by using the metabolites of tramadol [12,13,17].

In our method, the extraction procedure included only one step and the instruments used were relatively inexpensive (HP 6890 series GC-FID system). The recoveries of tramadol and internal standard were greater than 88% and the limit of detection of tramadol was 8 ng/ml in plasma. Besides, this sensitive method has high selectivity and has been successfully applied to both human and rabbit pharmacokinetic studies.

From the pharmacokinetic point of view, although $M_1$ and other metabolites were presented at high cumulative concentrations in urine after tramadol administration [4,20], their concentrations in plasma were low. The reported maximum plasma concentration of $M_1$ following a 100 mg tramadol intravenous injection was around 30 ng/ml [18]. It was just below the low detection limit of our method. This is the reason why $M_1$ and $M_2$ were not determined in our assay following a single dose of tramadol 100 mg. However, higher concentrations of $M_1$ and $M_2$ were added into the assay as references to challenge the selectivity of this GC method. The results demonstrate that tramadol can easily be separated from its metabolites.

It is found that the elimination half-life of tramadol in humans in our study (141.9 min) was different from that of previous reports (5–6 h) [4,15,18]. This discrepancy may be due to a difference in the duration of data collection for curve fitting. Because the clinical duration of tramadol is around 4–6 h in humans, an 8-h plasma data of tramadol was used for curve fitting in our study. In contrast, a 12-to-24-h plasma data of tramadol was used for curve fitting in previous reports and a longer terminal half-life was obtained [4,15,18]. In a phamacokinetic–pharmadynamic point of view, an application of this longer terminal half-life which obtained from a period out of clinical effect to explain the clinical effect of tramadol is worth determination.

In conclusion, we have developed a rapid, sensitive, precise and accurate GC-FID method for determination of tramadol in humans and animals. It may become an alternate to previous reported methods for determination of tramadol in pharmacokinetic studies.

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