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Validation of a GC/MS method for the determination of tramadol in human plasma after intravenous bolus☆

Veniero Gambaro *, Claudio Benvenuti, Lucia De Ferrari, Lucia Dell'Acqua, Fiorenza Farè

Istituto di Chimica Farmaceutica e Tossicologica, University of Milan, Viale Abruzzi 42, I-20131 Milan, Italy

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Abstract

Tramadol quantitative determination by gas chromatography–mass spectrometry (GC/MS) using nefopam hydrochloride as internal standard (IS) and two calibration curves because of the large range of concentration attended in the plasmatic samples is described. Plasma samples drawn from subjects in postoperative period treated with two different initial intravenous (iv) bolus of tramadol (50 and 100 mg) followed by tramadol at the same infusion rate (12 mg h^{-1}) are analysed. We operated for the qualitative analysis in Scan mode while for the quantitative analysis in SIM mode, selecting the ion *m/z* 58 for tramadol and *m/z* 179 for IS. The limit of detection (LOD) was 0.01 μg ml^{-1} and the limit of quantification was 0.04 μg ml^{-1}.

Keywords: Tramadol; Validation; GC/MS

1. Introduction

Tramadol is a synthetic 4-phenylpiperidine analogue of codeine (Fig. 1). Tramadol, like codeine, has a methyl substitution on the phenolic moiety of the morphine structure, which explains its relatively weak affinity for opiate receptors. Initially, it was thought that tramadol lacked selectivity for μ, k, or δ, opiate receptors, but the drug was recently found to have selectivity for the μ-receptor. Tramadol inhibits the reuptake of norepinephrine and serotonin, thus increasing the concentration of these two neurotransmitters in the central nervous system. Since endogenous norepinephrine and serotonin are involved in pain modulation, they may mediate the analgesic effect of tramadol [1].

When given in therapeutic doses, tramadol is well tolerated and does not produce respiratory depression or cardiovascular effects of clinical relevance. After oral administration, almost all the tramadol is completely absorbed from the gastrointestinal tract, reaching peak plasma concentration in 15–45 min [2].

This paper describes a specific, sensitive, precise and accurate gas chromatographic–mass spectrometric (GC/MS) method for determination of tramadol in human plasma after intravenous injection of 50 and 100 mg of tramadol hydrochloride, following by i.v. infusion of 12 mg h^{-1} of tramadol hydrochloride for 24 h. The levels of tramadol were quantified by modification of the method described by Merslavic and Zupancic-Kralij [2].

The aim of our work was to develop a simple method for analysis of tramadol in biological samples, especially important in bioequivalence and pharmacokinetic studies.

2. Experimental

2.1. Apparatus

- GC/MSA HP 5890 Series II gas chromatograph (Hewlett Packard, Palo Alto, CA), with a HP 7673 autosampler, electronic pressure control, split–splitless injector and a HP 5971 MSD mass selective detector with electronic impact was used.Manual

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* Corresponding author.
E-mail address: veniero.gambaro@unimi.it (V. Gambaro).

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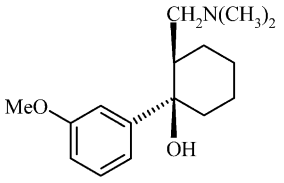

Fig. 1. Chemical structure of tramadol (1*RS*,2*RS*)-2-[(dimethylamino)methyl]-1-(3-methoxyphenyl)-cyclohexanol.

tuning of the MS with perfluorotributylamine (PFTBA) was used to adjust the relative abundance for *m*/*z* 69, 219 and 502.GC operating conditions were as follows: silica capillary column HP-1 (Hewlett Packard) cross-linked methylsilicone (12 m × 0.20 mm I.D., 0.33 μm film thickness); oven temperature was increased at a rate of 40 °C min^{-1} from 70 to 210 °C, 8 °C min^{-1} from 210 to 230°C and 10 °C min^{-1} from 230 to 300°C; the injector temperature was set at 280 °C; the flow rate of carrier gas (helium) was 1.2 ml min^{-1}; the injection was performed in splitless mode, purge off time 0.5 min.The MS detector parameters were: transfer line temperature 280 °C; solvent delay 3 min; electron energy 70 eV; the MS was run in Scan mode (*m*/*z* 50–550) for qualitative analysis and SIM mode (selected ion monitoring) for quantitative analysis (*m*/*z* 58 for tramadol and *m*/*z* 179 for internal standard (IS)).

- Centrifuge 4263A by A.L.C. s.r.l. (Milan, Italy);
- Balance MC1 by Sartorius (Goettingen, Germany);
- Centrifugal evaporator Maxy Dry Lyo by De Mori (Milan, Italy).

2.2. Chemicals and reagents

All reagents and solvents were of analytical-reagent grade.

Tramadol hydrochloride, batch no. 8C1160 purity 99.95%, was obtained from Formenti (Origgio, Varese, Italy) and the IS nefopam hydrochloride from Sigma Chemical (St. Louis, MO, USA).

Buffer solution pH 10 was purchased from J.T. Baker (Daventer, Holland), NaCl 0.9% from Baxter (Trieste, Italy), *n*-hexane from Merck (Darmstadt, Germany) and BSTFA+TMCS (99:1) from Supelco (Bellafonte, PA). Water was demineralized using an ELGA apparatus (Dasit).

2.3. Plasma sample collection

Plasma samples were collected from 14 patients treated, after orthopaedic or abdominal surgery, in two different ways: nine of them were treated with an initial 100 mg i.v. bolus of tramadol followed by an i.v. 12 mg h^{-1} infusion for 24 h, and five of them with an initial 50 mg i.v. bolus of tramadol followed by an i.v. 12 mg h^{-1} infusion for 24 h.

The plasma samples were collected just before the bolus administration and after the following times: 0.083 (5 min), 0.017 (10 min), 1, 3, 22 and 24 h. Plasma samples were kept at −20 °C until analysis.

2.4. Stock and standard working solutions

Separate stock solution of tramadol and internal standard were prepared at concentration 100.00 μg ml^{-1} in water and stored at +4 °C. Tramadol working solutions were prepared from stock solution up to final concentration of: 0.02, 0.20, 1.00, 10.00 μg ml^{-1}.

Internal standard working solution was prepared at the final concentration of 5.00 μg ml^{-1}.

2.5. Preparation of standard samples

Spiked plasma samples were prepared by adding aliquots of standard working solutions of tramadol to 0.5 ml of drug-free human plasma to a final concentrations from 0.04 to 10.00 μg ml^{-1} and 0.1 ml of standard working solutions of IS. All standard samples and the unknown samples were prepared according to the described method.

2.6. Quality control (QC) samples

QC were prepared by adding aliquots of standard working solution of tramadol, to a final concentrations of 0.10, 0.20 and 0.40 μg ml^{-1}, 0.1 ml of IS (5.00 μg ml^{-1}) and 0.5 ml of plasma blank. The QC were frozen, extracted following the procedure described at Section 2.7 and analyzed concomitantly with the unknown samples.

2.7. Extraction procedure

Tramadol was extracted from plasma by liquid–liquid extraction.

In 10 ml glass tube were placed 0.5 ml of plasma, 1.0 ml of physiologic solution, 0.1 ml of internal standard solution (5.00 μg ml^{-1}), 0.5 ml of buffer solution, and then extracted twice with 4 ml of *n*-hexane for 30 min. After centrifuging (30 min a 6000 rpm), the organic phase was isolated and evaporated to dryness with centrifugal evaporator.

The residue was reconstituted with 200 μl of *n*-hexane, transferred in microvial and evaporated to dryness with centrifugal evaporator. This step was repeated twice.

The final residue was reconstituted with 100 μl of BSTFA+TMCS (99:1) and heated in a thermostatic stove at 70 °C for 1 h; 1 μl was injected into the GC/MS system.

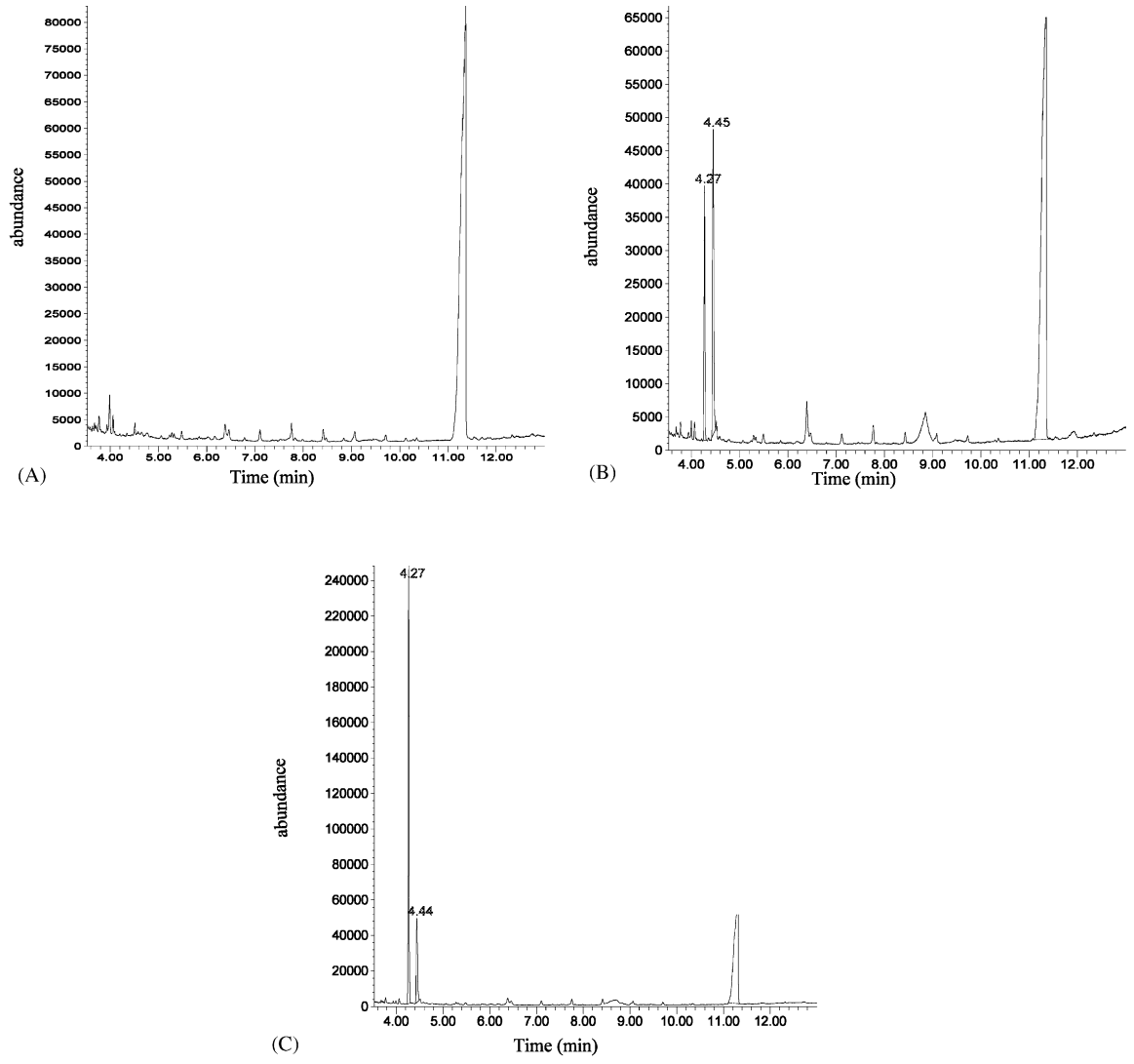

Fig. 2. Chromatograms of: (A) blank plasma; (B) blank plasma spiked with 0.100 μg ml^{-1} of tramadol ($t_R = 4.27$) and IS ($t_R = 4.45$); and (C) human plasma sample containing 0.504 μg $ml^{-1}$of tramadol and IS after bolus of 100 mg.

2.8. Pharmacokinetic parameters

The maximum plasma concentration (Cmax) of tramadol and the area under the plasma concentration curve (AUC_{0-t}) were calculated for each patient and expressed as mean, standard error of the mean (SEM) and range.

3. Results and discussion

3.1. Validation of the method

The validation program for the GC/MS method included specificity, linearity, LOD, LOQ, precision and accuracy studies of the tramadol in human plasma. The inter-day precision has been evaluated from QC samples' analysis. The validation data were obtained using a drug-free plasma.

3.1.1. Specificity

The specificity was studied by checking the chromatograms obtained from different blank plasma samples. As shown in Fig. 2, good separation of tramadol from internal standard was performed and no interfering peaks at the retention times of tramadol–TMS ($t_R =$ 4.27) and internal standard ($t_R = 4.45$) were observed.

3.1.2. Linearity

Four standard samples at different concentration levels ranging 0.08–1.00 and five standard samples at different concentration levels ranging 0.50–10.00 μg ml^{-1}, were prepared and checked for linearity. The calibration curves were drawn between response ratio (area of tramadol sample/area of internal standard) and

Table 1
Validation results for tramadol

	Values	
Linearity		
Linear range (μg ml^{-1})	0.50–10.00	0.08–1.00
Calibration points	4	5
Equation of line	$y = 64.861x -$ 5.615	$y = 40.208x +$ 0.072
Slope	64.861	40.208
±SD of slope ($n = 4$)	0.886	2.109
CV% of slope ($n = 4$)	2.20	3.21
Intercept	−5.615	0.072
±SD of intercept ($n = 4$)	0.149	1.379
r	0.9986	0.9990
LOD		
Limit of detection (μg ml^{-1})	0.01	
σ ($n = 6$)	0.067	
b	40.208	
LOQ		
Limit of quantitation (μg ml^{-1})	0.04	
CV% ($n = 5$)	5.28	
Rec% ($n = 5$)	110.25	
Precision intra-day (CV%)		
0.20 μg ml^{-1} ($n = 5$)	4.38%	
1.00 μg ml^{-1} ($n = 5$)	0.87%	
Accuracy (Rec%)		
0.20 μg ml^{-1} ($n = 5$)	113.00%	
1.00 μg ml^{-1} ($n = 5$)	97.22%	

SD, standard deviation; CV%, coefficient of variation; r, correlation coefficient; σ, standard deviation of noise; b, slope; Rec%, Recovery.

concentrations using the least-squares method. The results are shown in Table 1.

3.1.3. Limit of detection and quantitation

The limit of detection (LOD) defined as LOD = $(3.3\sigma)b^{-1}$ where σ is the standard deviation of *noise* (a value of 6 times the SD of the blank) and b is the slope of the regression line, was found to be 0.01 μg ml^{-1}. The limit of quantification (LOQ) defined as the lowest concentration of the tramadol that can be measured with acceptable precision and accuracy, was 0.04 μg ml^{-1}. The results are shown in Table 1.

3.1.4. Precision and accuracy

To determine intra-day precision and accuracy of the assay, replicate set of spiked plasma samples containing known concentrations of the analytes at two different concentration (0.20 and 1.00 μg ml^{-1}) were analysed on the same day.

Inter-day precision has been evaluated from the values obtained in the daily analyses of the QC samples.

Precision has been determined as the percent of coefficient of variation (CV%), which is the ratio between SD and the average concentration found.

Accuracy has been calculated as the percent ratio between the found and the known concentrations (the results are show in Tables 1 and 2).

Table 2
Results of the inter-day precision test of the QC samples

Added concentration (μg ml^{-1})	Found concentration (μg ml^{-1}) (mean ± SD)	CV%
0.10 ($n = 11$)	0.01 ± 0.0054	5.53
0.20 ($n = 10$)	0.19 ± 0.0056	2.90
0.40 ($n = 11$)	0.41 ± 0.0221	5.40

4. Pharmacokinetic study

*C*max and AUC showed a clear difference for the two dosage schemes, being the mean values after the 100 mg bolus superior to the ones detected after the lower bolus (Table 3).

Table 3
Pharmacokinetic data after intravenous tramadol hydrochloride: 50 and 100 mg bolus followed by 12 mg h^{-1} infusion (mean and SEM, range in brackets)

i.v. bolus (mg)	AUC (μg ml^{-1} h)	*C*max (μg ml^{-1})
50	1.06 ± 0.21 (0.60–1.71)	0.51 ± 0.10 (0.28–0.82)
100	3.90 ± 1.30 (0.52–10.37)	2.28 ± 0.78 (0.20–5.89)

5. Conclusion

The experimental study was designed to allow the determination of tramadol plasma levels in patients treated with two different dosage schemes differing for the initial i.v. bolus (50 or 100 mg of active principle) followed by an i.v. infusion at the same constant rate. The analytical difficulties in determining the plasma concentrations scattered in a very wide range were overtaken preparing two calibration curves to appropriately monitor tramadol administered to patients in post-operative period.

The evaluation of the two dosage schemes was performed computing the relevant pharmacokinetic parameters. The findings evidenced that doubling the initial bolus and keeping constant the subsequent infusion rate, higher plasma levels and pharmacokinetic parameters are attained. Hence, facing severe or resistant pain, it is possible to approach an effective treatment through the administration of a greater starting bolus that guarantees a higher kinetic performance.

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