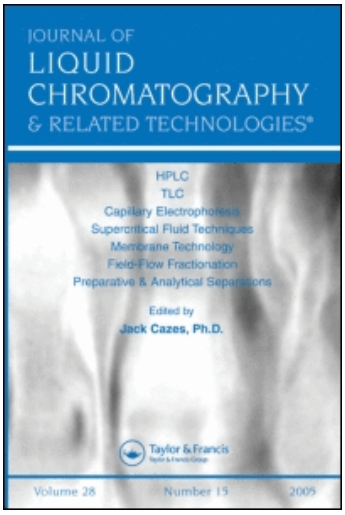

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

High Performance Liquid Chromatographic Determination of Tramadol in Pharmaceutical Dosage Forms

Iman Y. Zaghloul[a]; Mahasen A. Radwan[a]
[a] Department of Clinical Pharmacy, College of Pharmacy University Center for Women Students King Saud University, Riyadh, Saudi Arabia

To cite this Article Zaghloul, Iman Y. and Radwan, Mahasen A.(1997) 'High Performance Liquid Chromatographic Determination of Tramadol in Pharmaceutical Dosage Forms', Journal of Liquid Chromatography & Related Technologies, 20: 5, 779 — 787
To link to this Article: DOI: 10.1080/10826079708014142
URL: http://dx.doi.org/10.1080/10826079708014142

J. LIQ. CHROM. & REL. TECHNOL., 20(5), 779–787 (1997)

HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF TRAMADOL IN PHARMACEUTICAL DOSAGE FORMS

Iman Y. Zaghloul, Mahasen A. Radwan

Department of Clinical Pharmacy
College of Pharmacy
University Center for Women Students
King Saud University
PO Box 22452
Riyadh 11459, Saudi Arabia

ABSTRACT

A simple, specific and accurate high performance liquid chromatographic (HPLC) method for determination of tramadol in pharmaceutical dosage forms has been developed. Reversed phase chromatography was conducted using μ-Bondapak C_{18} column (3.9 x 150 nm) with an isocratic mobile phase consisting of 0.005 M triethylamine in 0.01 M sodium phosphate buffer (pH 5.5) containing 17% acetonitrile. The effluent was monitored on a UV detector at 230 nm. Each analysis required no longer than 8 minutes. Quantification was achieved by the measurement of the peak-area ratio of the drug to the internal standard (metoclopramide) and the detection limit was 75 ng/mL. Linear response ($r > 0.999$) was observed over the range of 0.1 - 10 μg/mL and was run on 6 different occasions. There was no significant difference ($p < 0.05$) between inter- and intra- day studies for tramadol determined for two different concentrations

(0.5 and 5.0 mg/mL). The mean relative standard deviations (RSD%) of the results of within-day precision and accuracy of the drug was < 7%. The stability of tramadol at different temperatures indicated that the drug is stable at 4, 25, and 50°C for at least 4 weeks. The effect of light, 1 N HCl, and 1 N NaOH on the stability of tramadol has also been investigated.

INTRODUCTION

Tramadol hydrochloride ((±)-trans-2-(dimethylaminomethyl)-1-(m-methoxyphenyl)- cyclohexanol hydrochloride; Tramal®) is shown in Figure 1. It is a centrally acting analgesic drug.[1] Tramadol has opioid agonist properties and activates monoaminergic spinal inhibition of pain. In healthy volunteers with experimentally induced pain, oral tramadol exhibited analgesic activity similar to that of dextropropoxyphene and was more effective than flupirtine[2] or dipyrone (metamizole)[3] but less effective than tildine.[4] Tramadol could be administered orally, rectally, intravenously or intramuscularly. Intravenous Tramadol 50-100 mg was equivalent in analgesic efficacy to morphine 5 to 15 mg/kg in patients with moderate pain following surgery.[5] Orally administered tramadol 100 mg was superior to that of placebo in a double blind crossover study in 12 healthy volunteers with pain induced by selective transcutaneous stimulation of the sural nerve.[6]

Most of the analytical methods available to quantitate tramadol utilize Gas Chromatographic assays with mass spectrometric detection (GC-MS).[7-8] To our knowledge, there is no up to date HPLC method available for the quantitation of tramadol in dosage forms for quality control purposes. Bioanalytical assays of tramadol in plasma and urine are available in the literature, however, they are written in Chinese.[9-10] This investigation describes a simple, specific, and sensitive HPLC method for the determination of Tramadol in pharmaceutical dosage forms.

EXPERIMENTAL

Chemicals and Reagents

Tramadol hydrochloride was a gift from Grunenthal (Stolberg, Germany). Metochlopramide was purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A). All other reagents and chemicals were of HPLC grade and used as received.

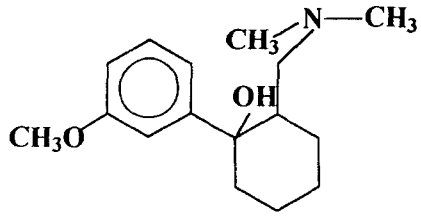

Figure 1. Structural formula of tramadol.

Chromatography

A Waters chromatographic system was used in the current method and consisted of the following: Waters 501 solvent delivery system, Waters 717 autosampler, Model 484 tunable absorbance UV detector set at 230 nm. Chromatograms were recorded on a Waters 746 Data Module integrator chart. The analytical separation was achieved on a μBondapak C-18 (5 mm particle size, 3.9 x 150 mm ID) equipped with a guard column (5 μm particle size, 25 x 4 mm ID).

The mobile phase consisted of 17% acetonitrile in 0.01 M sodium phosphate buffer (pH 5.5) containing 0.005 M triethylamine. Degassing of mobile phase was achieved by filtration through a 0.22 μm membrane filter.

The mobile phase was pumped isocratically at a flow rate of 1.2 mL/ min. The HPLC system was operated at ambient temperature. The chart speed was 0.25 cm/min. The injection volume ranged from 25 to 50 μL. Each sample run time required no longer than 8 min.

Standard Solutions

Stock solution of tramadol HCl (as 1 mg/mL tramadol) and metochlopramide (1 mg/mL), internal standard (IS), were prepared in deionized water (HPLC quality water). Tramadol stock solution was diluted to 100 and 10 mμg/mL to be used as working solutions. Weekly dilutions were made in deionized water to give tramadol concentrations of 0.1 - 10 μg/mL, and a constant concentration of 0.5 μg/mL of the IS. An aliquot of each standard was injected onto the HPLC in triplicates to be used as the standard curve.

Analysis of Dosage Forms

Capsules

The content of each capsule (50 mg labeled) was emptied in 200 mL of HPLC deionized water. Following vigorous shaking for 10 min, the undissolved contents were allowed to settle down. A ten mL of the supernatant was transferred to a 15 mL screw capped tube and centrifuged at 6000 rpm for 5 min. A one mL of the above solution was diluted with water to a final concentration of 10 μg/mL. An aliquot of 150 μL was mixed with 150 μL of the internal standard and a volume of 50 μL was injected in triplicates onto the HPLC for chromatography. Six replicates of commercial capsules were analyzed using the same method.

Recovery was calculated using a reference standard containing 50 mg (n = 3) subjected to the same treatment as described above for the capsules.

Intravenous ampoules

An aliquot of 100 μL from the ampoule (100 mg/2 mL ampoule) was diluted 100 fold with deionized water for a theoretical final concentration of 50 μg/mL. In a ten mL volumetric flask, a one mL of the above solution was mixed with 2.5 mL of the IS and brought to volume with deionized water. Aliquots of 75 μL of the solution were injected in triplicates onto the HPLC. A total of 6 vials were analyzed using this method.

Stability Study

The stability of tramdol solution was determined under different conditions of temperature, light, and pH for a period of 4 weeks. Different amber vials of tramadol solution at a concentration of 5 μg/mL were kept at 4, 25 and 50 ^{o}C and the stability was determined on day 0, 7, 14, 21, and 28.

Also the effect of extreme pH conditions on the stability of tramdol was investigated by using 1 N HCl and 1N NaOH. The effect of light was examined by exposing clear glass vials containing the drug solution to the day light for 4 weeks. Samples were taken for analysis once a week for the different conditions.

Drug Analysis

The peak area ratios of tramadol to IS were plotted against tramadol concentrations. To assess the accuracy and precision of the within-day and between-day assay, aqueous samples of tramadol at two different concentrations of 0.5 and 5 μg/mL on six different occasions over a four weeks period. Least square linear regression analysis was used to determine the slope, the intercept, and the correlation coefficients (r) of the standard curves. Each point on the calibration curve was based on at least 6 determinations.

The standard curves were constructed over a three month period to determine the day to day variability of the slopes and the reproducibility of the assay. The amount of tramadol in each dosage form was determined after considering the dilution factor for each dosage form.

Statistical Analysis

All results are expressed as mean ± standard deviations (SD). The relative standard deviation (RSD%) was calculated for all values. The *t*-test was used to examine the concentration difference at each day, and one-way analysis of variance (ANOVA) was employed to evaluate the reproducibility of the assay. The level of confidence was 95%.

RESULTS AND DISCUSSION

Figure 2 shows a typical chromatogram obtained following the analysis of tramadol capsule. Using the above chromatographic conditions, tramdol and metoclopramide were eluted at about 6.2 and 4.4 minutes, respectively. The current method provided optimum resolution of the drug and the internal standard.

The quantitation of the chromatograms were performed using peak-area ratios (Y) of the drug to the IS *versus* drug concentrations (X). Least-squares regression calibration curves were found to be linear over the serum concentration range of 0.1 to 10 μg/mL of tramadol. The mean linear regression equation was as follows: $Y = 0.58 X + 0.011$. The mean correlation coefficients, r, was generally > 0.999. The detection limit of the assay was 100 ng/mL at a signal to noise ratio of >3.

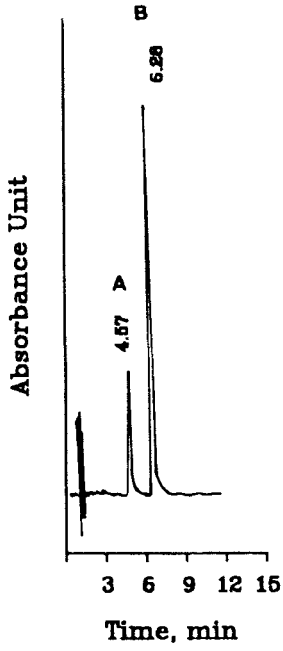

Figure 2. A typical chromatogram of tramadol capsule. A: metoclopramide and B: tramadol

Table 1 represents the results obtained for intra- and inter-day variability study of tramdol samples. The within-day precision for the studied concentrations (0.5 and 5 μg/mL) showed RSD% of 4.3 and 6.4%, respectively. The evaluated between-day precision evaluated over a 4 weeks period varied from 5.3 to 6.9%. This outcome shows the accuracy of the assay.

The reproducibility was evaluated by comparing the linear regressions of six standard plots carried out on 6 different occasions over a three month period. The mean correlation coefficient was > 0.999. The RSD% of the slopes, of the six lines, was 6.8%. Analysis of variance of the data indicated no significant difference in the slopes ($p < 0.05$), within-day and between-day, of the calibration curves of tramadol. The results confirmed the reproducibility of the assay method.

The stability of tramadol (5 μg/mL) was studied under extreme conditions of temperature and pHs. Table 2 represents the percentage recovery of tramadol at different sampling time throughout the study. Tramdol was shown

Table 1

Intraday and Interday Precision of Tramadol Standards

Theoretical Concentration (ug/mL)	Intraday[a] Measured Concentration (μg/mL) (Mean SD)	RSD%	Interday[b] Measured Concentration(μg/mL) (Mean SD)	RSD%
0.5	0.51 (0.02)	4.3	0.48 (0.03)	6.3
5.0	5.32 (0.34)	6.4	5.40 (0.37)	6.9

[a] Mean values represent six different tramadol standards for each concentration.
[b] Interday reproducibility was determined from six different runs over a 4 week period.

Table 2

Stability of Tramadol Solution Under Different Storage Conditions (at 5μg/mL)

Condition	Percent Recovery Days 0	7	14	21	28
Temperature °C					
4	102	98	99	101	96
25	103	102	97	95	93
50	98	101	99	102	99
1N HCl	103	102	104	98	101
1N NaOH	99	101	103	95	100

to be stable for at least 4 weeks at different temperatures even up to 50°C. Although extreme pH's conditions were used, the drug seemed to be stable for up to 4 weeks. When the drug was exposed to the light (in clear glass vials) at room temperature, tramdol did not show any sensitivity to light.

The amount of tramadol in each dosage form was calculated after using the dilution factor. Statistical analysis was used to detect any significant differences between capsules. One-way analysis of variance of six replicates of each capsule (six capsules were analyzed). Results indicated that no significant difference exists between the six capsules ($p < 0.05$). The same trend was observed for the intravenous dosage form ($n = 6$) subjected to the same treatment. The measured amount of each of the studied dosage forms was within the manufacturer's claimed one.

CONCLUSION

The HPLC method described herein provides a simple, rapid, and reproducible determination of tramadol in dosage forms which makes it potentially valuable in quality control of the drug's dosage forms.

REFERENCES

1. C. R. Lee, D. M. Tavich, M. Sorkin, Drugs, **46**, 313 - 40 (1993).

2. P. Rhodewald, H. W. Granitzki, E. Nedderman, Pharmacology, **37 (4)**,209-17 (1988).

3. E. Feingold, W. Classen, C. H. Tille, P. Netter., Abstract No. 12., Arzeneimittel-Forsheng/Drug Research, **32**, 901 (1982).

4. B. Brommet, W. M. Herrmann, E. Scharein, Fortschritte der Medizine, **107**, 385-9 (1989).

5. R-J. M. Houmes, M. A. Voets, A. Verkaaik, W. Erdmann, B. Lachmann, Anesthesia and Analgesia, **74**, 510-14 (1992).

6. L. Collart, C. Luthy, P. Dayer, Abstract, Br. J. Clinical Pharmacology, **35**, 73P (1993).

7. W. Lintz, H. Uragg, J. Chromatogr., **341**, 65-79 (1985).

8. R. Becker, W. Lintz, J. Chromatogr., **377**, 213-20 (1986).

9. C. Yu, Zhongguo-Yiyao-Gongye Zazhi, **21**, 408-9 (1990).

10. Y. Xu, Y. Q. Xu, C .J. Zhang, L. Shen, Acta-Pharm-Sinica-Yao-Hsueh-Pao, **28 (5)**, 379-83 (1993).

Received May 13, 1996
Accepted June 5, 1996
Manuscript 4184