Asian Journal of Chemistry; Vol. 23, No. 2 (2011), 663-667

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in

Determination of Tramadol Hydrochloride by Using UV and Derivative Spectrophotometric Methods in Human Plasma

AYSEL KUCUK[1,*] and YUCEL KADIOGLU[2]

[1]Department of Chemistry, Faculty of Science & Arts, University of Sakarya, 54187 Sakarya, Turkey
[2]Department of Analytical Chemistry, Faculty of Pharmacy, University of Ataturk, 25240 Erzurum, Turkey

*Corresponding author: E-mail: ayselk@sakarya.edu.tr

(*Received*: 2 March 2010; *Accepted*: 24 September 2010) AJC-9133

Tramadol was analyzed by using zero-, first- and second-order derivative spectrophotometric methods in methanol and water media. The type of solvent, the degree of derivation, the range of wavelength and the range of concentration were chosen in order to optimise the conditions. The developed and validated method was successfully applied to the analysis of human plasma samples in methanol medium. Beer's law was obeyed in the linearity range of 10-100 μg mL^{-1} between the wavelength ranges of 240-290 nm. The solutions of standard were prepared in methanol and water media and the plasma samples were prepared in methanol medium. It was thought that methanol is more suitable solvent than water for tramadol analysis in human plasma. The standard calibration curves of tramadol were constructed by plotting absorbance *versus* concentration in the determined concentration range with the final dilution. Developed UV and derivative UV spectrophotometric methods in this study are accurate, sensitive, precise and reproducible and can be directly and easily applied to the plasma.

Key Words: Tramadol, UV Spectrophotometry, Derivative spectrophotometry.

INTRODUCTION

Tramadol hydrochloride, [(±)-*trans*-2-(dimethylaminomethyl)-1-(*m*-methoxyphenyl)-cyclohexanol hydrochloride, $C_{16}H_{25}NO_2$·HCl, f.w. = 299.84 g mol^{-1}] (Fig. 1), is a centrally acting analgesic agent which has been shown to be a synthetic analogue of codeine[1]. It is metabolised by the cytochrome P450 enzyme system in the liver to form 11 metabolites of which M_1 (O-desmethyltramadol) predominates and possesses analgesic properties[2]. It is used since 1977 for the relief of strong physical pain which is the opioid drug[3].

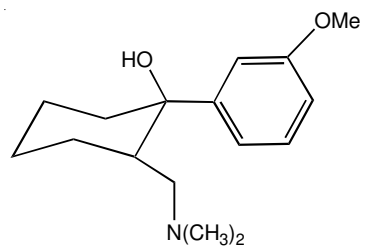


Fig. 1. Chemical structure of tramadol

Tramadol was determined by HPLC with UV detection[4-9], fluorescence detection[10] or electrochemical detection[11]. Capillary gas chromatography was also used for determining tramadol[12]. The literature has reported two spectrophotometric methods a kinetic investigated study of the oxidation reaction of the drug[13] and influence of substitution and solvent[14].

Tramadol has been generally analyzed by high performance liquid chromatographic methods in biological materials and no derivative spectrophotometric method in any biological materials has been reported in the literature. Therefore, the aim of the present work is to develop a simple and sensitive analytical method for the determination of tramadol by using UV and derivative spectrophotometry in methanol and water solvent media. This developed and validated method is applied to human plasma.

EXPERIMENTAL

The UV system consisted of Shimadzu UV-3101 PC Model, UV-vis-NIR scanning spectrophotometer, in a 10 mm quartz cells, was connected to PC Computer and Hewlett-Packard DeskJet Printer. The absorbance value of tramadol solutions was determined with a fixed slit width of 2 nm between wavelength range of 240-290 nm (n = 6).

Tramadol standard was a gift from Grünenthal (Aachen, Germany) and Abdi Ibrahim Ilaç San. ve Tic. A.S. (Istanbul, Turkey). The plasma samples were obtained from Ataturk

University-Hospital of Arastirma in Erzurum-Turkey. Purity of tramadol was tested by checking its melting point (181 ºC), UV, ^{1}H NMR, ^{13}C NMR spectra and by HPLC analysis using UV detection and no impurities were found. Tramadol solutions were prepared with distilled water and methanol of analytical grade (Merck). Ethyl acetate and sodium hydroxide were of Analytical reagent-grade from Merck.

Preparation of stock and standard solutions: Stock solutions of tramadol were prepared at concentrations of 200 μg mL^{-1} in methanol and water and kept at room temperature. Working standard solutions were daily prepared by diluting of the stock solutions in the concentration range from 10-100 μg mL^{-1} for both solvent mediums. Five different concentrations of tramadol as the working standard solutions, chosen for the calibration curve were 10, 35, 65, 85 and 100 μg mL^{-1}. The standard solutions were prepared by dilution of different volumes of the stock solution to a constant volume with methanol and water. UV spectra were recorded against methanol and water as reference substances.

Preparation of plasma standard and samples: Frozen human plasma samples were left on the bench to thaw naturally and were vortexed prior to use. Samples were prepared by spiking to drug-free human plasma of working standard solutions in different concentrations.

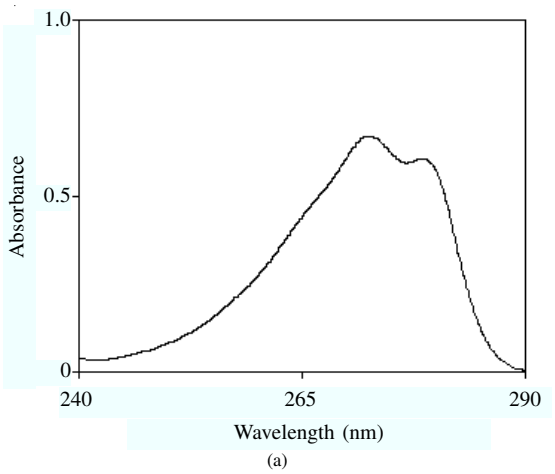
Extraction procedure: 1 mL of plasma was added into 8 clean tubes. The different concentrations of tramadol were then spiked into the plasma. The solutions were made alkaline by shaking on a vortex mixer, followed by addition of 1 drop of 0.1 M NaOH. The mixture was extracted with ethyl acetate by vortex mixing for an additional 10 min followed by centrifugation at 3500 g for 15 min. The organic layer was transferred into another tube and evaporated to dryness in a 40 ºC water bath under nitrogen gas. The residue was reconstituted with methanol of 4 mL and the absorbance of sample was determined in the UV system. Prepared samples were stored at -20 ºC until analysis.

RESULTS AND DISCUSSION

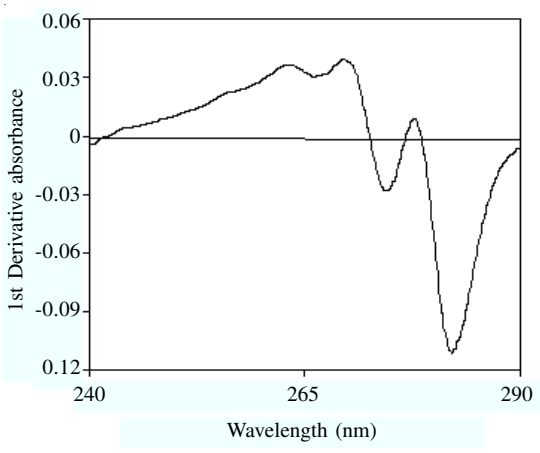
Method development: The UV absorption spectra of tramadol were monitored two broad shouldered peaks with maximum wavelengths at 273 and 279 nm in methanol medium and at 271 and 276 nm in water medium with band width 2 nm in the measuring wavelength range of 240-290 nm as shown in Fig. 2a and 2d, respectively. The maximum shoulders were showed to be more prominent the shoulder at left than that's at right[14]. However, these shoulders were not appeared, when the bandwidth was chosen as 5 nm and it was monitored a single well-defined maximum peak in UV spectrums as determined in previous report[15]. And these maximum peaks were broader at low concentrations so that analysis couldn't be performed. The peaks at higher concentrations were sharper but analysis couldn't be carried out because of the shoulder.

Derivative UV spectrophotometry was preferred for the analysis of tramadol. The peak shape was well defined and the separation of the shouldered peaks was better in this method. The zero-, first- and second-order spectra were obtained for tramadol in methanol and water at 240-290 nm with bandwidth 2 nm ($\Delta\lambda$ = 4). The developed method had been validated to determine tramadol concentrations in human plasma for methanol medium.

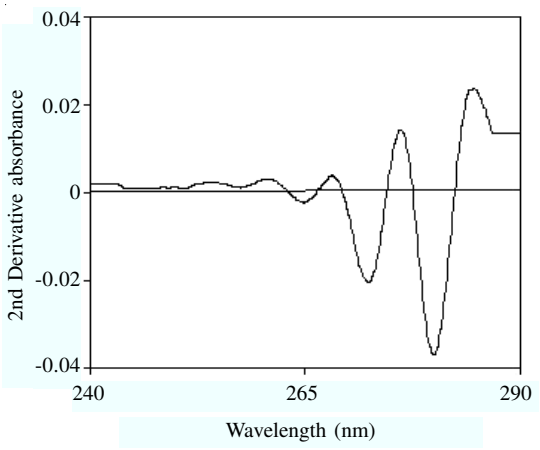
The first-order curve was displayed two maxima at 269 and 277 nm and two minima at 274 and 282 nm (Fig. 2b), while the second-order curve was showed three maxima at 268, 276 and 284 nm and two minima at 272 and 280 nm in methanol (Fig. 2c). The first-order curve was displayed two maxima at 268 and 276 nm and two minima at 273 and 281 nm (Fig. 2e), while the second-order curve was showed three maxima at 266, 274 and 283 nm and two minima at 271 and 278 nm in water (Fig. 2f).



(a)



(b)



(c)

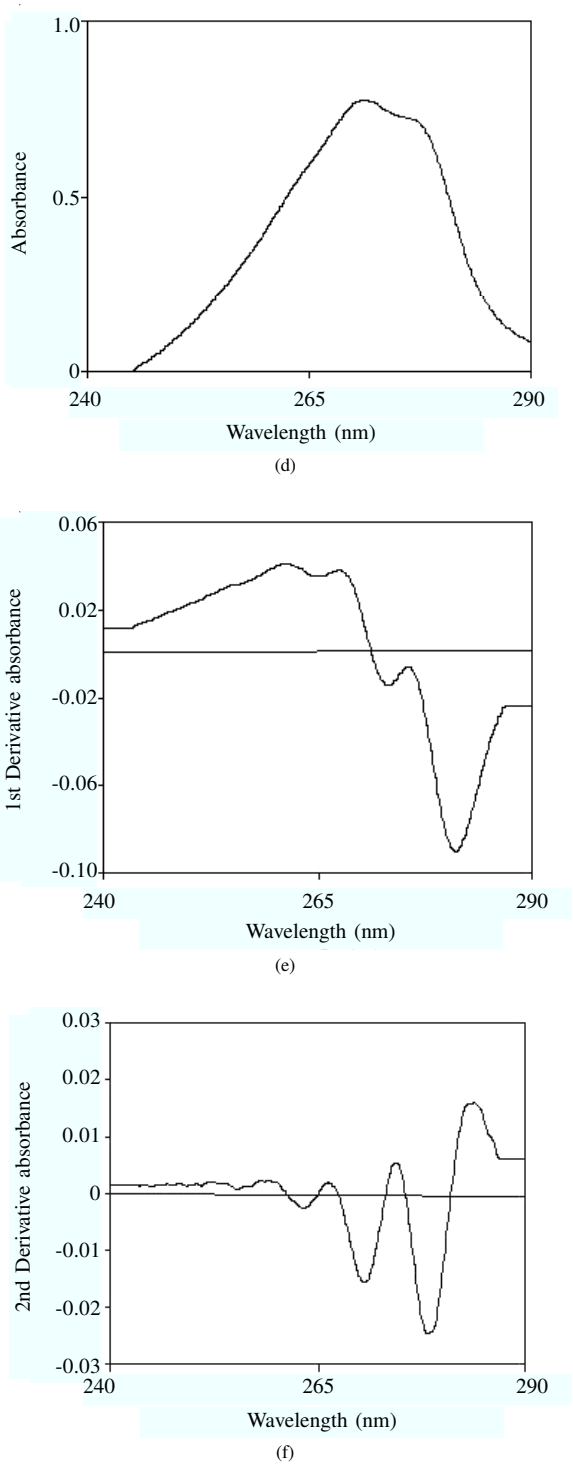


Fig. 2. Zero-, first- and second-order derivative spectrums of tramadol (100 μg mL^{-1}); in methanol medium (a-c) and in water medium (d-f)

Linearity of calibration curves: Five-level calibration series with six analyses at each concentration level were measured. The standard calibration curves of tramadol were constructed by plotting absorbance *versus* concentration for zero-, first- and second-order derivative UV spectrums in both methanol and water media. The results were analyzed by linear simple regression model of y = mx + b by the least-squares method. For all calibration curves, a good linearity within the concentration range of 10-100 μg mL^{-1} was showed. The regression equations of calibration curves and correlation coefficients in methanol medium were found for zero-, first- and second-order as $y = 6.5 \times 10^{-3}x + 9.5 \times 10^{-3}$ (r = 0.9984), $y = 2.3 \times 10^{-4}x + 1.6 \times 10^{-3}$ (r = 0.9955) and $y = 1.2 \times 10^{-4}x + 1.1 \times 10^{-3}$ (r = 0.9932), respectively (Table-1). The regression equations of calibration curves and the correlation coefficients in water medium were obtained for zero-, first- and second-order as $y = 7.1 \times 10^{-3}x - 5.1 \times 10^{-3}$ (r = 0.9928), $y = 1 \times 10^{-3}x + 2 \times 10^{-3}$ (r = 0.9942) and $y = 1.2 \times 10^{-4}x + 1.7 \times 10^{-3}$ (r = 0.9903), respectively (Table-2). The regression equations of calibration curves and correlation coefficients for plasma samples in methanol medium were found for zero-, first- and second- order as $y = 6.3 \times 10^{-3}x - 1.1 \times 10^{-2}$ (r = 0.9927) and $y = 7.0 \times 10^{-4}x + 1.0 \times 10^{-2}$ (r = 0.9908) and $y = 1.9 \times 10^{-3}x - 2.9 \times 10^{-3}$ (r = 0.9910), respectively (Table-3).

TABLE-1
CALIBRATION CURVES OF TRAMADOL IN METHANOL

Features	Zero-order	First-order	Second-order
Regression equation	$y = 6.5 \times 10^{-3}x + 9.5 \times 10^{-3}$	$y = 2.3 \times 10^{-4}x + 1.6 \times 10^{-3}$	$y = 1.2 \times 10^{-4}x + 1.1 \times 10^{-3}$
RSD (%)	0.58-4.15	1.24-7.72	2.75-9.74
Correlation coefficient (r)	0.9984	0.9955	0.9932
Linear range (μg mL^{-1})	10-100	10-100	10-100

TABLE-2
CALIBRATION CURVES OF TRAMADOL IN WATER

Features	Zero-order	First-order	Second-order
Regression equation	$y = 7.1 \times 10^{-3}x - 5.1 \times 10^{-3}$	$y = 1.0 \times 10^{-3}x + 2.0 \times 10^{-3}$	$y = 1.2 \times 10^{-4}x + 1.7 \times 10^{-3}$
RSD (%)	0.76-4.45	1.99-7.23	3.22-10.90
Correlation coefficient (r)	0.9928	0.9942	0.9903
Linear range (μg mL^{-1})	10-100	10-100	10-100

TABLE-3
CALIBRATION CURVES OF TRAMADOL IN PLASMA FOR METHANOL MEDIUM

Features	Zero-order	First-order	Second-order
Regression equation	$y = 6.3 \times 10^{-3}x - 1.1 \times 10^{-2}$	$y = 7.0 \times 10^{-4}x + 1.0 \times 10^{-2}$	$y = 1.9 \times 10^{-3}x - 2.9 \times 10^{-3}$
RSD (%)	1.15-5.99	1.00-2.68	1.03-7.01
Correlation coefficient (r)	0.9927	0.9908	0.9910
Linear range (μg mL^{-1})	10-100	10-100	10-100

The correlation coefficient of standard calibration curves for zero-, first- and second-order derivative spectrophotometry of tramadol in methanol solvent was higher than those of in water. Thus, it had been found that the methanol solution was better than water for working in human plasma analysis. In addition, the tramadol contains a weakly absorbing chromophore in its molecule[13]. This is the reason why UV detection is not suitable for the determination of low tramadol concentrations[14]. Besides, the low concentrations indicated poor linearity in order that it was emerged the occurrence of scattering at these tramadol concentrations. Therefore, the concentration range of 10-100 μg mL^{-1} was chosen as the most suitable for all measurements in UV spectrophotometry.

Method validation: The precision of the method, expressed as the relative standard deviation (RSD = 100.SD/mean) was evaluated by determining the inter-day and intra-day RSD of the measured peak absorbance for different concentrations. Accuracy was expressed as the mean percentage of analyte recovered in the assay. Both statistical parameters were calculated in each concentration level for the sensitivity of the method. The limit of quantification (LOQ) was determined as the lowest concentration on the standard calibration curve that was measured routinely with acceptable precision (RSD < 20 %) and accuracy (80-120 %)[4,8]. The limit of detection (LOD), determined as the lowest concentration of analyte which was distinguished from the blank with reasonable confidence was also calculated[16]. The all RSD values were found as lower than 10 %.

Sensitivity: Spectrophotometrically, the limit of quantitation (LOQ) was found as 5 μg mL^{-1} (zero-order), 7 μg mL^{-1} (first-order) and 7 μg mL^{-1} (second-order) in methanol and 6 μg mL^{-1} (zero-order), 6.5 μg mL^{-1} (first-order) and 7.5 μg mL^{-1} (second-order) in water. The limit of detection (LOD) was 1.5 μg mL^{-1} (zero-), 3.5 μg mL^{-1} (first-) and 4 μg mL^{-1} (second-) in methanol (S/N = 3.4) and 2.5 μg mL^{-1} (zero-), 5.5 μg mL^{-1} (first-) and 6 μg mL^{-1} (second-) in water (S/N = 2.2), respectively.

The limit of quantitation (LOQ) for tramadol determined in plasma was 10 μg mL^{-1} (zero-), 9 μg mL^{-1} (first-) and 7 μg mL^{-1} (second-) in methanol. And the limit of detection (LOD) was found as 9 μg mL^{-1} (zero-), 9 μg mL^{-1} (first-) and 6 μg mL^{-1} (second-) in methanol (S/N = 2).

Repeatability: Repeatability is given as inter-day and intra-day precision and accuracy where evaluated by analyzing three different concentration of tramadol on three different day. Six replicate determinations at three different concentrations (25, 50 and 75 μg mL^{-1} for tramadol in methanol and water) were carried out to test the precision of these methods. The RSD values from tramadol were found to be 1.77-3.54 % (zero-), 2.32-5.65 % (first-) and 4.35-8.31 % (second-) for intra-day precision and 0.89-3.64 % (zero-), 2.02-4.22 % (first-) and 3.33-7.23 % (second-) for inter-day precision in methanol. The RSD values of the evaluated intra-day precision were showed as 0.76-4.40 % (zero-), 2.47-3.07 % (first-) and 3.22-7.80 % (second-) and inter-day precision values were 1.959-5.70 % (zero-), 3.30-3.85 % (first-) and 7.78-8.83 % (second-) in water medium. These data indicated that the developed methods had a good repeatability. It was showed to be a difference between the RSD values and that the RSD values of methanol were smaller than the RSD values of water as given in Tables 4-6.

TABLE-4
INTRA-DAY AND INTER-DAY PRECISION FROM ZERO-ORDER UV SPECTROPHOTOMETRIC METHOD OF TRAMADOL IN METHANOL AND WATER MEDIA

Zero-order (n = 6)		Intra-day			Inter-day		
Sample	Conc. (μg mL^{-1})	$\overline{X}$	SD	RSD	$\overline{X}$	SD	RSD
Tramadol (methanol)	25	0.1736	6.14×10^{-3}	3.54	0.1711	6.23×10^{-3}	3.64
	50	0.3322	6.95×10^{-3}	2.09	0.3392	3.02×10^{-3}	0.89
	75	0.4995	8.86×10^{-3}	1.77	0.4946	9.50×10^{-3}	1.92
Tramadol (water)	25	0.1759	7.75×10^{-3}	4.40	0.1839	9.39×10^{-3}	5.11
	50	0.3157	5.94×10^{-3}	1.88	0.3080	17.6×10^{-3}	5.70
	75	0.5368	$4.06 \times .10^{-3}$	0.76	0.5020	9.78×10^{-3}	1.95

TABLE-5
INTRA-DAY AND INTER-DAY PRECISION FROM FIRST-ORDER UV SPECTROPHOTOMETRIC METHOD OF TRAMADOL IN METHANOL AND WATER MEDIA

First-order (n = 6)		Intra-day			Inter-day		
Sample	Conc. (μg mL^{-1})	$\overline{X}$	SD	RSD	$\overline{X}$	SD	RSD
Tramadol (methanol)	25	0.0083	4.71×10^{-4}	5.65	0.0088	3.73×10^{-4}	4.22
	50	0.0157	4.70×10^{-4}	3.00	0.0155	5.00×10^{-4}	3.23
	75	0.0215	5.00×10^{-4}	2.32	0.0233	4.71×10^{-4}	2.02
Tramadol (water)	25	0.0243	7.45×10^{-4}	3.07	0.0247	8.16×10^{-4}	3.30
	50	0.0457	1.37×10^{-3}	3.00	0.0466	1.63×10^{-3}	3.50
	75	0.0765	1.89×10^{-3}	2.47	0.0740	2.85×10^{-3}	3.85

TABLE-6
INTRA-DAY AND INTER-DAY PRECISION FROM SECOND-ORDER UV SPECTROPHOTOMETRIC METHOD OF TRAMADOL IN METHANOL AND WATER MEDIA

Second-order (n = 6)		Intra-day			Inter-day		
Sample	Conc. (μg mL^{-1})	$\overline{X}$	SD	RSD	$\overline{X}$	SD	RSD
Tramadol (methanol)	25	0.0057	4.71×10^{-4}	8.31	0.0052	3.73×10^{-4}	7.23
	50	0.0105	5.00×10^{-4}	4.76	0.0112	3.70×10^{-4}	3.33
	75	0.0158	6.87×10^{-4}	4.35	0.0167	9.43×10^{-4}	5.65
Tramadol (water)	25	0.0065	5.07×10^{-4}	7.80	0.0057	4.99×10^{-4}	8.83
	50	0.0100	8.16×10^{-4}	8.16	0.0088	6.87×10^{-4}	7.78
	75	0.0155	5.00×10^{-4}	3.22	0.0135	1.12×10^{-3}	8.30

TABLE-7
INTRA-DAY AND INTER-DAY PRECISION FROM DERIVATIVE UV SPECTROPHOTOMETRIC METHOD OF TRAMADOL IN PLASMA FOR METHANOL MEDIUM

Plasma (n = 6)		Zero-order			First-order			Second-order		
Sample	Conc. (μg mL^{-1})	$\overline{X}$	SD	RSD	$\overline{X}$	SD	RSD	$\overline{X}$	SD	RSD
Intra-day	25	0.1602	5.41×10^{-3}	3.38	0.0270	2.98×10^{-4}	1.10	0.0456	7.74×10^{-4}	1.70
	50	0.2936	7.84×10^{-3}	2.67	0.0454	7.68×10^{-4}	1.69	0.0810	1.15×10^{-3}	1.42
	75	0.4599	10.9×10^{-3}	2.37	0.0670	6.68×10^{-4}	1.00	0.1430	3.22×10^{-3}	2.25
Inter-day	25	0.1598	5.93×10^{-3}	3.71	0.0286	8.55×10^{-4}	2.99	0.0464	1.22×10^{-3}	2.63
	50	0.2928	6.99×10^{-3}	2.39	0.0428	1.20×10^{-3}	2.80	0.0714	7.53×10^{-4}	1.05
	75	0.4770	13.9×10^{-3}	2.91	0.0571	8.34×10^{-4}	1.46	0.1680	9.05×10^{-3}	5.39

For the plasma samples, the RSD values were found 2.37-3.38 % (zero-), 1.0-1.69 % (first-) and 1.03-4.78 % (second-) as intra-day precision and 2.37-3.38 % (zero-), 1.0-1.98 % (first-) and 1.42-2.25 % (second-) as inter-day precision in only methanol solvent (Table-7). And the good recovery values were found between 86.3 and 97.2 % in plasma samples. The mean recovery and RSD values were determined to be 91.7 and 3.9 % (Table-8).

TABLE-8
ACCURACY AND RECOVERY DATA OF TRAMADOL WHICH WERE OBTAINED FROM SPECTROSCOPIC METHOD FOR ADDED CONCENTRATIONS (*IN VITRO*)

Added conc. (μg mL^{-1})	Measurement conc.* (μg mL^{-1})	Accuracy** (relative error) (%)	Recovery (%)
10	9.2	-8.0	92.0
25	23.4	-6.4	93.6
35	30.2	-13.7	86.3
50	44.1	-11.8	88.2
65	57.6	-11.4	88.6
75	69.0	-8.0	92.0
85	81.5	-4.1	95.9
100	97.2	-2.8	97.2
Mean		91.7	
RSD (%)		3.9	
SD		3.6	

*Mean values, **Accuracy = [(found - added)/added] × 100.

Conclusion

The liquid-liquid extraction method used in this study has been simplicity used and also successfully applied to human by UV method and rabbit plasma samples[17] by HPLC-DAD method. It hasn't been described the comparison of methanol and water solvent media for the determination of tramadol by UV and derivative spectrophotometric methods in the literature except for previous report[15]. To our best of knowledge, there is no UV and derivative spectrophotometric method for determination of tramadol in human plasma. This is the first description of tramadol determination in human plasma in methanol by UV method as to literature. The investigation can be given an idea in order to choose the optimum solvent medium for UV and derivative determination of tramadol in human plasma.

The analytical UV and derivative spectrophotometric methods were developed and validated for quantitative determination of tramadol in human plasma. The proposed methods have good reproducibility and the results show good recoveries of tramadol from the spiked samples. These methods can be potentially useful in a routine laboratory because of its simplicity, rapidity, sensitivity, precision and accuracy.

ACKNOWLEDGEMENTS

The authors would like to thank Grunenthal GmbH (Germany), Abdi Ibrahim Ilaç San. ve Tic. A.S. (Turkey) for her invaluable help in providing drug standard.

REFERENCES

1. K.S. Lewis and N.H. Han, *Am. J. Health Syst. Pharm.*, **54**, 643 (1997).
2. K. Budd and R. Langford, *Br. J. Anaesthesia*, **82**, 493 (1999).
3. W. Lintz, H. Barth, G. Osterloh and E. Schmidt-Böthelt, *Arzneim.-Forsch./Drug Res.*, **48**, 889 (1998).
4. S.H. Gan, R. Ismail, W.A.W. Adnan and Z. Wan, *J. Chromatogr. B*, **772**, 123 (2002).
5. I.Y. Zaghloul and M.A. Radwan, *J. Liq. Chromatogr. Rel. Tech.*, **20**, 779 (1997).
6. M. Nobilis and J. Kopecky, *J. Chromatogr. A*, **949**, 11 (2002).
7. G.C. Yeh, M.T. Sheu, C.L. Yen, Y.W. Wang, C.H. Liu and H.O. Ho, *J. Chromatogr. B*, **723**, 247 (1999).
8. M. Nobilis, J. Kopecky, J. Kvetina, J. Chladek, Z. Svoboda, V. Vorisek, F. Perlik, M. Pour and J. Kunes, *J. Chromatogr. A*, **949**, 11 (2002).
9. M. Nobilis, J. Pastera, P. Anzenbacher, D. Svoboda, J. Kopecky and F. Perlik, *J. Chromatogr. B*, **681**, 177 (1996).
10. P. Overbeck and G. Blaschke, *J. Chromatogr. B*, **732**, 185 (1999).
11. M. Valle, J.M. Pavon, R. Calvo, M.A. Campanero and I.F. Troconiz, *J. Chromatogr. B*, **724**, 83 (1999).
12. S.T. Ho, J.J. Wang, W.J. Liaw, C.M. Ho and J.H. Li, *J. Chromatogr. B*, **736**, 89 (1999).
13. H.E. Abdellatef, *J. Pharm. Biomed. Anal.*, **29**, 835 (2002).
14. J. Kracmar, J. Kracmarova and Z. Stejskal, *Die Pharm.*, **42**, 18 (1987).
15. A. Küçük and Y. Kadioglu, *IL Farmaco*, **60**, 163 (2005).
16. A.C. Metha, *J. Clin. Pharm. Therap.*, **14**, 465 (1989).
17. A. Küçük, Y. Kadioglu and F. Çelebi, *J. Chromatogr. B*, **816**, 203 (2005).