Research Paper

Sensitive Titrimetric and Spectrophotometric Assay of Tramadol Hydrochloride in Pharmaceuticals using N-Bromosuccinimide and Indigocarmine as Reagents

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Two indirect methods based on titrimetric and spectrophotometric techniques are described for the determination of tramadol hydrochloride (TDH) in bulk drug and in tablets using N-bromosuccinimide (NBS) and indigocarmine as reagents. In titrimetry, a measured excess of NBS is added to an acidified solution of TDH in the presence of potassium bromide and the unreacted NBS is determined iodometrically. Spectrophotometry involves the addition of a known excess of NBS to TDH in acid medium followed by estimation of residual NBS by reacting with a fixed amount of indigocarmine (IC) and measuring the absorbance at 610 nm. The working conditions of the methods have been optimized.Titrimetric procedure is applicable over the range 2.5-25.0 mg of TDH, and the reaction stoichiometry is found to be 1:1 (TDH: NBS). In the spectrophotometric method, the absorbance is found to increase linearly with the concentration of TDH, which is corroborated by the correlation coefficient of 0.9997 (n=8). The system obeys Beer's law in the range 0.75-15.0 μg ml^{-1} with an apparent molar absorptivity of 1.74 x 10^4 l mol^{-1} cm^{-1} and Sandell sensitivity of 0.0172 μg cm^{-2}. The limits of detection (LOD) and quantitation (LOQ) are calculated to be 0.11and 0.34 μg ml^{-1}, respectively. Within-day variation determined on three concentrations showed accuracies ranging from 0.20 to 3.35%. The RSD was determined to be ≤ 3.45%. Day to day variation presented accuracies ranging from 0.67 to 3.60% with an RSD of <3.0%. The methods were successfully applied to the determination of TDH in tablet formulation and the results were compared with those of a reference method by applying Student's t-test and F-test. No interference was observed from common tablet adjuvants. The accuracy and reliability of the methods were further ascertained by recovery experiments via standard addition procedure.

Key Words: Tramadol Hydrochloride; N-bromosuccinimide; Titrimetry; Spectrophotometry; Pharmaceuticals

Introduction

Tramadol hydrochloride (TDH), chemically known as (1*R*,2*R*)-*rel*-2-[(dimethylamino)methyl]- 1-(3-methoxyphenyl)cyclohexanol (Fig. 1), is a synthetic analogue of codeine and is a centrally acting analgesic agent [1]. It is metabolized by the cytochrome P450 enzyme system in the liver to form eleven metabolites of which o-desmethyltramadol (M1) predominates, and has analgesic properties [2]. It has been used since 1977 for the relief of severe physical pain and has been the most widely sold opioid analgesic drug in the world [3]. Ultra-violet spectrophotometry [4, 5], high performance liquid chromatography [5-8], thin layer chromatrography-densitometry [9], capillary isotachophoresis [10], flow injection chemiluminescence spectrometry [11], voltammetry [12-14] and ion-selective based potentiometry [15-21] have been reported for determining TDH in pharmaceutical dosage forms.

Inspite of its inherent simplicity and excellent accuracy and precision, no titrimetric assay of TDH has been reported. Visible spectrophotometry, despite its fair selectivity and sensitivity, cost-effectiveness and easy accessibility has been sparsely employed in the assay of TDH. Rajput and Trivedi [22] have reported two methods based on dichloromethane soluble ion pair with bromocresol green measurable at 417 nm, and blue coloured chromogen formed on reacting TDH with Folin-Ciocalteu's reagent which was measured at 645 nm. The first method is critically

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dependent on the pH of the aqueous medium and involves the tedious extraction step whereas the second method is applicable over a narrow linear dynamic range. Both the methods are less sensitive with the molar absorptivity value less than 1×10^4 l mol^{-1} cm^{-1}. The method [23] based on the condensation of TDH with the mixed anhydrides of malonic and acetic acids though is reported to be sensitive with a linear range of 0.5-2.5 µg ml^{-1}, the reaction requires heating at 60°C for 40 min. Besides, the measurement is made at 330 nm where the interference from the tablet excipients is far more severe than at longer wavelengths. The kinetic spectrophotometric methods reported by Abdellatef [24] using permanganate and 4-chloro-7-nitrobenzofurazan are neither simple nor sensitive. The measurements were recorded in a thermostated water bath maintained at 90±1°C and are prone to imprecision. Kinetic methods, in particular, require judicial control of all experimental variables and the methods are deficient on reliability. The procedures are applicable over narrow linear ranges of 5-25 and 50-250 µg ml^{-1}.

The present investigation aims to develop more sensitive and cost-effective methods for the determination of TDH in pure form and in dosage forms using titrimetric and spectrophotometric techniques. The methods employ N-bromosuccinimide which acts as an oxidizing as well as brominating agent and indigocarmine as auxiliary reagent. The proposed methods have been demonstrated to be superior to the reported methods with respect to speed, simplicity, sensitivity, cost effectiveness and eco-friendliness.

Experimental

Apparatus

A Systronics model 106 digital spectrophotometer with 1-cm matched quartz cells was used for all absorbance measurements.

Reagents and Materials

All the reagents used were of analytical-reagent grade and distilled water was used throughout the investigation.

N-bromosuccinimide (NBS): An approximately 0.01 M solution was prepared by dissolving about 1.8 g of NBS (SRL Research Chemicals, Mumbai, India) in water with the aid of heat and diluted to one litre with water. The solution was standardized iodometrically [25] and kept in an amber coloured bottle and stored in a refrigerator; and used in titrimetry. It was diluted appropriately to get 170 µg mL^{-1} NBS for use in spectrophotometric method.

Sulphuric Acid: Concentrated Sulphuric acid (Merck, Mumbai, India; sp. gr. 1.84) was diluted appropriately with water to get 1M.

Potassium Iodide (10 %): Prepared by dissolving 10 g of the chemical GR (Merck, Mumbai, India) in 100 mL of water. It was prepared just before use.

Starch Indicator (1 %): One gram of soluble starch GR (LOBA Chemie, Mumbai, India) was made into a paste with a few mL of water, and slowly poured with constant stirring into 100 mL boiling water; boiled for 2 min and cooled.

Sodium Thiosulphate (0.02 M): Prepared by dissolving about 5.0 g of the chemical (Sisco-chem Industries, Mumbai, India, assay 98 %) in one litre of water.

Hydrochloric Acid: Concentrated hydrochloric acid (Merck, Mumbai, India; sp. gr. 1.18) was diluted appropriately with water to get 5M HCl.

Indigocarmine: A 1000 µg mL^{-1} solution was prepared by dissolving 118 mg of dye (S.d fine-chem. Ltd., Mumbai, India, 85 % dye content) in water and made up to 100 mL in a volumetric flask with water. This was appropriately diluted to get a working concentration of 200 µg mL^{-1}.

Standard TDH Solution, Tablet and Preparations: Pharmaceutical grade TDH was received as gift from Jubiliant Orgonosys Ltd., Nanjangud, Mysore, India. A stock standard solution equivalent to 2.5 mg mL^{-1} of TDH was prepared by dissolving accurately weighed 625 mg of pure drug in water and diluted to the mark with water in a 250 mL calibrated flask. This solution was used in titrimetric work. Another stock solution equivalent to 100 µg mL^{-1} of TDH was prepared by dissolving accurately weighed 10 mg of pure drug in water and diluted to the mark in a 100 mL calibrated flask. The stock solution (100 µg mL^{-1} TDH) was diluted appropriately with water to get working concentrations of 25 µg mL^{-1} TDH for use in spectrophotometric method.

Procedure

Titrimetric Method (Method A)

A 10 mL aliquot of pure drug solution containing 2.5-25.0 mg of TDH was accurately measured and transferred into a 100 mL titration flask. The solution was acidified by adding 5 mL of 1 M sulphuric acid followed by 1 spatula of potassium bromide and 10 mL of 0.01 M NBS (using a pipette). The content was mixed well and the flask kept aside for 10 min with occasional swirling. Then, 5 mL of 10 % potassium iodide was added to the flask and the liberated iodine was titrated with 0.02 M sodium thiosulphate to a starch end point. A blank titration was run under same conditions. The drug content in the aliquot was calculated from:

$$Amount(mg) = (B\text{-}S) \times Mw \times R$$

where B = volume of thiosulphate solution consumed in the blank titration, mL; S = volume of thiosulphate solution consumed in the sample titration, mL; Mw = relative molecular mass of the TDH; R = molarity of NBS solution.

Spectrophotometric Method: Using Indigo Carmine (Method B)

Varying aliquots (0.3-6.0 mL) of a standard 25 µg mL^{-1} TDH solution were transferred into a series of 10 mL calibrated flasks by means of a micro burette and the total volume was brought to 6 mL by adding water. To each flask were added 1 mL each of 5 M hydrochloric acid and 170 µg mL^{-1} NBS solution (by means of a micro burette). The content was mixed well and the flasks were kept aside for 10 min with intermittent shaking. Finally, 1 mL of 200 µg mL^{-1} indigo carmine solution was added to each flask, the volume was diluted to the mark with water, mixed well and absorbance measured against a reagent blank at 610 nm after 10 min. A standard graph was prepared by plotting the absorbance *versus* the concentration of TDH. The concentration of the unknown was read from the calibration graph or computed from the regression equation derived using Beers' law data.

Assay Procedure for Tablets

Twenty tablets each containing 50 or 100 mg of TDH were separately weighed and ground into a fine powder. An amount of powder equivalent to 250 mg of TDH was weighed into a 100 ml calibrated flask, 40 ml of water added and the content shaken for 20 min; then the volume was made up to the mark with water, mixed well and filtered using Whatman No. 42 filter paper. The filtrate equivalent to 2.5 mg mL^{-1} TDH was used in titrimetric procedure and the same solution was diluted appropriately with water to get 25 µg ml^{-1} TDH for spectrophotometric method, and a convenient aliquot was subjected to analysis using the procedure described under spectrophotometric method.

Results and Discussion

The analytical applications of NBS are broadly based on bromination and oxidation reactions. These reactions have found extensive applications in the determination of a variety of organic compounds including those of pharmaceutical interest [26]. Ziegler and his coworkers [27] made detailed studies of NBS applications for allylic bromination. NBS can be considered a convenient source of molecular bromine or it can also act as a source of hypobromous acid which is the actual oxidizing agent. It is, therefore, used extensively as a brominating and also as an oxidizing agent.

Method Development

Direct titration of TDH with NBS was not feasible. In the proposed titrimetric procedure, the quantitative reaction between TDH and NBS was checked by treating (2.5-25.0 mg) of TDH with a measured excess of NBS in acid medium, in the presence of potassium bromide and determining the residual NBS iodometrically. In the range studied (2.5 to 25.0 mg), the reaction stoichiometry was found to be 1:1 (TDH: NBS). Sulfuric acid was found to be an ideal medium for the assay and the reaction stoichiometry was found to be unaffected in the presence of 1 to 5 mL of 1 M H_2SO_4 in a total volume of 25 mL, and 5 mL was chosen as the optimum volume. The reaction was found to be complete in 10 min and contact time upto 60 min had no effect on the stoichiometry or the

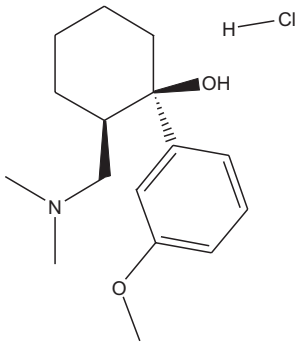

Fig. 1: Structure of Tramadol hydrochloride

results. A 10 mL volume of 0.01 M NBS was found adequate for quantitative bromination of TDH in the range investigated and one spatula of KBr (~1.5 g) was found sufficient.

In separate preliminary experiments indigo carmine was found to be destroyed irreversibly to the colourless species by NBS in the acid medium. The present spectrophotometric method is based on the bromination of the drug by measured excess of NBS and subsequent determination of the latter by reacting with indigo carmine, and measuring the absorbance at 610 nm (Fig. 2). TDH, when added in increasing concentrations to a fixed concentration of NBS, consumes the latter and there will be a concomitant decrease in the concentration of NBS. When a fixed concentration of dye is added to decreasing concentrations of NBS, a concomitant increase in the concentration of dye is obtained. As a result, the absorbance increased linearly with increasing concentration of drug (Fig. 5). Preliminary studies were performed to determine the upper Beer's law limits for indigo carmine in acid medium at 610 nm, and found to be 20.0 mg mL^{-1}. The blue colour due to 20 mg mL^{-1} indigo carmine was bleached by 17 mg mL^{-1} NBS. Hence, varying concentrations of TDH were reacted with 1 mL of 170 mg mL^{-1} NBS

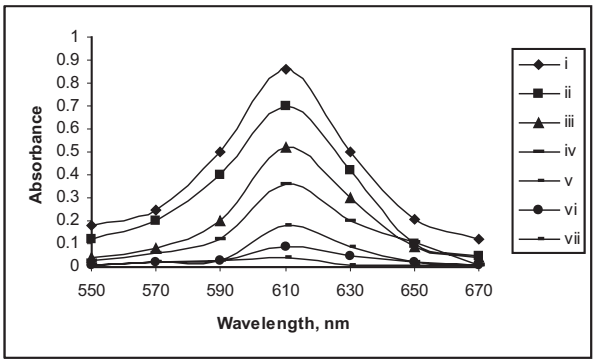

Fig. 2: Absorption spectra : (i. 15.0; ii. 12.0; iii. 9.0; iv. 6.0; v. 3.0; vi. 1.5 and vii. 0.8 mg mL^{-1} TDH)

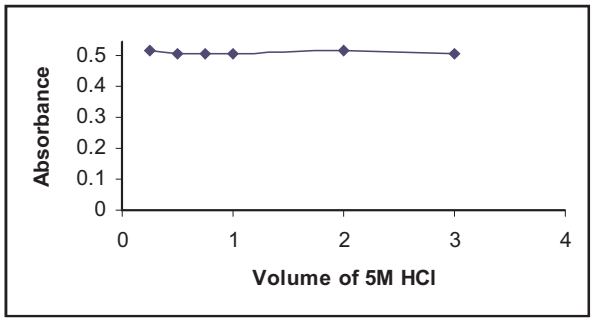

Fig. 3: Effect of 5M HCl

and the residual NBS was determined. This helped to fix the concentration range over which TDH could be determined. The reaction between TDH and NBS was found to be rapid and quantitative in HCl medium and 1 mL of 5M acid in a total volume of ~8 mL was found necessary. The bleaching of indigocarmine by unreacted NBS requires acid medium and bleaching was complete within 5 minutes. The effect of HCl was studied and 0.25-3 mL of 5M HCl in a total volume of 10 mL was found to produce constant effect on both the reaction between TDH and NBS, and residual NBS with Indigocarmine (Fig. 4). The possible reaction schemes for both the methods are as shown in the Fig. 4.

Method Validation

Analytical Data

A linear correlation was found (Fig. 5) between absorbance at λ_{max} and concentration of TDH in the ranges given in Table 1. The graph showed negligible intercept and is described by the regression equation:

$$Y = a + bX$$

(where Y = absorbance of 1-cm layer of solution; a = intercept; b = slope and X = concentration in µg ml^{-1}). Regression analysis of the Beer's law data using the method of least squares was made to evaluate the slope (b), intercept (a) and correlation coefficient (r) and the values are presented in Table 1. The optical characteristics such as Beer's law limits, molar absorptivity and sandell sensitivity values are also given in Table 1. The limits of detection (LOD) and quantitation (LOQ) calculated according to ICH guidelines [28] are also presented in Table 1. The significance of correlation coefficient of the spectrophotometric method was evaluated by calculating the t-value using the following formula [29]:

$$t \quad \frac{|r|\sqrt{n-2}}{\sqrt{1-r^2}}$$

The calculated t-value was compared with the tabulated value at the 95% significance level, using a two-sided t-test and (n-2) degrees of freedom. The null hypothesis in this case was that there was no correlation between the measured absorbance (Y) and concentration (X). Since the calculated t-values is 116.7 for spectrophtometric method, which is greater than the tabulated value (2.57), the null hypothesis

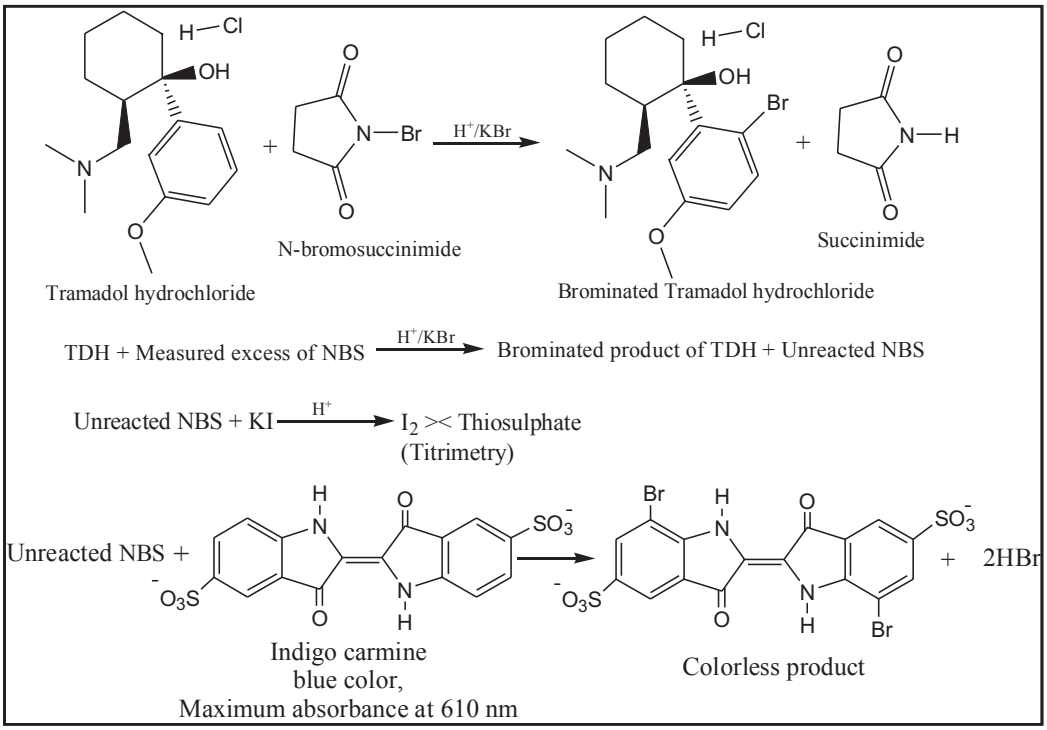

Fig. 4: Probable reaction scheme

Table 1: Analytical and regression parameters of spectrophotometric method

Parameter	Spectrophotometric method(n=8)
λ_{max}, nm	610
Beer's law limits, µg mL^{-1}	0.75-15.0
Molar absorptivity, L mol^{-1} cm^{-1}	1.74×10^4
Sandell sensitivity*, µg cm^{-2}	0.0172
Limit of detection, mg mL^{-1}	0.11
Limit of quantification, mg mL^{-1}	0.34
Regression equation, Y**	
Intercept, (a)	0.0048
Slope, (b)	0.0575
Correlation coefficient, (r)	0.9998
Standard deviation of intercept (S_a)	0.34671
Variance (S_a^2)	0.12048
$\pm tS_a/\sqrt{n}$	14.3051
Standard deviation of slope (S_b)	0.02344
$\pm tS_b/\sqrt{n}$	0.96712

*Limit of determination as the weight in µg per mL of solution, which corresponds to an absorbance of A = 0.001 measured in a cuvette of cross-sectional area 1 cm^2 and l = 1 cm.

$Y^{**} = a + bX$, where Y is the absorbance and X is the concentration in µg mL^{-1}, $\pm tS_a/\sqrt{n}$ = confidence limit for intercept, $\pm tS_b/\sqrt{n}$ = confidence limit for slope.

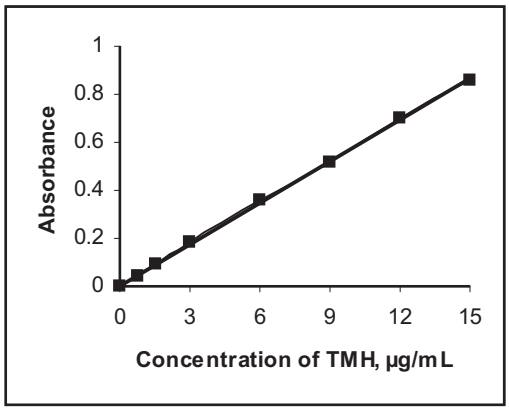

Fig. 5: Calibration graph

was rejected and it was concluded that a significant correlation did exist between Y and X. As expected, the closer | *r* | is to 1, i.e. as the straight-line relationship becomes stronger, the larger the value of t that is obtained.

Assay Precision and Accuracy

The precision of the methods was calculated in terms of intermediate precision (intra-day and inter-day) [30]. Three different amount/concentrations of TDH were analysed in seven replicates during the same day (intra-day precision) and on five consecutive days (inter-day precision). The RSD (%) values of intra-day and inter-day studies showed that the precision was good (Table 2). The accuracy of an analytical

Table 2: Intra-day and inter-day precision and accuracy studies

Method*	TDH taken	Intra-day (n=7)			Inter-day (n=5)		
		TDH found[a]	Precision[b]	Accuracy[c]	TDH found[a]	Precision[b]	Accuracy[c]
Titrimetry	7.50	7.53	3.45	0.44	7.77	1.26	3.60
	15.00	15.45	0.82	2.80	15.45	2.35	3.00
Spectrophotometric	22.50	22.87	0.35	1.65	22.13	2.22	1.64
	1.5	1.50	1.04	0.20	1.51	1.63	0.67
	6.0	6.20	2.02	3.35	6.18	1.56	3.00
	12.0	12.21	1.55	1.73	12.24	2.35	2.00

* TDH taken / found in titrimetric method is in mg and the same in spectrophotometric methods are in µg mL^{-1}, a. Mean, b. Relative standard deviation (%), c. Bias %: (found-taken/taken) x 100.

Table 3: Results of method robustness and ruggedness (all values in %RSD) studies

Method	Nominal amount concentration*	Reaction times (*n*=3)	Different analysts (*n*=4)	Different instruments (*n*=3)
Titrimetric	7.50	1.23	2.15	1.29
	15.00	1.10	2.23	2.23
	22.5	2.03	2.13	2.16
Spectrophotometric	4.00	0.89	1.06	2.13
	8.00	1.09	1.56	2.22
	12.00	1.95	2.06	2.56

*mg in titrimetry and µg mL^{-1} in spectrophotometry

method expresses the closeness between the reference value and the found value. Accuracy was evaluated as percentage relative error between the measured concentrations and taken concentrations for TDH (Bias %). The results obtained are compiled in Table 2 and show that the accuracy is good.

Method Selectivity

The proposed methods were tested for selectivity by placebo blank and synthetic mixture analyses. A placebo blank containing talc (250 mg), starch (300 mg), lactose (30 mg), calcium carbonate (50 mg), calcium dihydrogen orthophosphate (20 mg), methyl cellulose (40 mg), sodium alginate (70 mg) and magnesium stearate (100 mg) was extracted with water in titrimetric as well as spectrophotometric method and solution made as described under "assay procedure for tablets". A convenient aliquot of solution was subjected to analysis by titrimetry and spectrophotometry according to the recommended procedures. In all the cases, there was no interference by the inactive ingredients.

A separate test was performed by applying the proposed methods to the determination of TDH in a synthetic mixture. To the placebo blank of similar composition, 250 mg of TDH was added, homogenized and the solution of the synthetic mixture was prepared as done under "assay procedure for tablets". The filtrate was collected in a 100-mL flask. Five ml of the resulting solution was assayed (n=5) by titrimetry which yielded a % recovery of 101.40 ± 0.75. The synthetic mixture solution (2500 µg mL^{-1} in TDH) was appropriately diluted to get 25 µg mL^{-1} solutions and 3.0 mL was analysed by spectrophotometric method and the corresponding % recovery of TDH was 98.17 ± 1.42. These results demonstrate the accuracy as well as the precision of the proposed methods. These results complement the findings of the placebo blank analysis with respect to selectivity.

Robustness and Ruggedness

For the evaluation of the method robustness, two important experimental variables, volume of acid and reaction time were slightly varied deliberately. The analysis was performed with altered experimental conditions by taking three different concentrations of TDH and the procedure were found to remain unaffected as shown by the RSD values in the range of 0.92 to 2.17%. Method ruggedness was expressed as the RSD of the same procedure applied by four different analysts as well as using three different instruments, (burettes in titrimetry). The inter-analysts

Table 4: Results of assay of tablets by the proposed methods and statistical evaluation

Tablet Brand Name**	Nominal amount, mg	Found (% of nominal amount ± SD)*		
		Literature method	Titrimetric method	Spectrophotometric method
Tramazac TC[a]	100	101.3±0.78	102.50±1.46 **t** =1.68 **F** = 3.50	100.90±1.85 **t** = 0.48 **F** =5.62
Cemadol CR[b]	50	96.74±1.04	97.36±1.72 **t** = 0.70 **F** = 2.73	95.58±2.08 **t** = 1.17 **F** = 4.00

*Mean value of five determinations

**Marketed by: [a] Zydus Alidac Pvt. Ltd., Bangalore, India, [b]Life medicare and biotech Pvt. Ltd., Haridwar, India

Tabulated t-value at the 95% confidence level is 2.77; Tabulated F-value at the 95% confidence level is 6.39

Table 5: Results of recovery experiments by standard addition method

Formulation studied	Titrimetric method				Spectrophotometric method			
	TDH in tablet, mg	Pure TDH added, mg	Total found, mg	Pure TDH recovered*, Percent ± SD	TDH in tablet, µg mL^{-1}	Pure TDH added, µg mL^{-1}	Total found, µg mL^{-1}	Pure TDH recovered*, Percent ± SD
Cemadol CR 50	9.74	5.0	14.62	97.61± 1.20	4.78	2.50	7.19	96.33±1.58
	9.74	10.0	19.62	98.88 ± 1.46	4.78	5.00	9.50	94.52±1.76
	9.74	15.0	24.37	97.54 ± 1.55	4.78	7.50	12.14	98.14±1.84

*Mean value of three determinations

RSD were within 2.88% whereas the inter-instruments RSD for the same TDH concentrations ranged from 0.87 to 2.42% suggesting that the developed method were rugged. The results are shown in Table 3.

Application to Formulations

The proposed methods were applied to the determination of TDH in two representative tablets Tramazac-TC 100 (Zydus Alidac Pvt Ltd., Bangalore, India), and Cemadol 50 CR (Life Medicare & Biotech Pvt. Ltd., Haridwar, India) purchased from local stores and containing other inactive ingredients. The results in Table 5 show that the methods are successful for the determination of TDH and that the excipients in the dosage forms did not interfere. The same batch tablets were assayed by the reference method [5] which consisted of measurement of the absorbance aqueous solution of TDH at 271 nm. The results obtained by the proposed methods agree well with the claim and also are in agreement with those by the reference method. When the results were statistically compared with those of the reference method by applying the Student's t-test for accuracy and F-test for precision, the calculated t-value and F-value at 95% confidence level did not exceed the tabulated values of 2.77 and 6.39, respectively, for four degrees of freedom. Hence, it is implied that no significant difference exists between the proposed methods and the reference method with respect to accuracy and precision.

Recovery Study

To study the reliability and reproducibility of the proposed method, a standard addition technique was followed. A fixed amount/concentration of drug from preparations was taken and pure (standard) drug at three different levels was added. The total was found by the proposed methods. The determination with each level was repeated three times and the percent recovery of the added standard was calculated from:

$$\%\text{Recovery} \quad \frac{[C_F - C_T]}{C_P} \quad 100$$

where C_F is the total amount/concentration of the analyte found; C_T, amount/concentration of the analyte present in the formulation; C_P, amount/

Table 6. Comparison of the performance characteristics of the reported and proposed methods

S.No.	Reagents/reaction	λmax, nm	Linear range, μg mL^{-1}, (ε, L mol^{-1} cm^{-1})	Remarks	Reference
1	a) BCG/ion-pair	417	2-15 (9.92×10^3)	Requires rigid pH control and liquid-liquid extraction	22
	b) F-C reagent, redox	645	10-40 (3.03×10^3)	Less sensitive, narrow linear range	
2	Malonic and acetic acid anhydrides, condensation	330	0.5-2.5	Requires heating at 60ºC for 20 min, narrow linear range, measurement at shorter wavelength	23
3	a) $KMnO_4$, reduction	610	5-25	Kinetic measurements made at 90±1ºC, narrow linear ranges	24
	b) 4-Chloro-7-nitrobenzofuran	467	50-250		
4	**NBS-indigocarmine, bromination**	**610**	**0.75-15.0 (1.74×10^4)**	**No heating or extraction step required, highly sensitive**	**Present method**

concentration of analyte (pure drug) added to formulation. Results of this study presented in Table 5 and reveal that the accuracy of methods was unaffected by the various excipients present in the formulations.

Conclusions

Two procedures for the determination of tramadol based on in its analytical properties are proposed. The procedures are useful for the assay of the active compound in bulk drug and in tablets. The titrimetric method is the first ever reported for tramadol and is applicable over a wide dynamic semi-micro range. It is clear from the performance characteristics of the proposed spectrophotometric method with those of the reported methods presented in Table 6 that the present method is free from such disadvantages as rigid pH control, extraction and heating steps and use of organic solvents. With a detection limit of 0.11 μg mL^{-1} and molar absorptivity value of 1.74×10^4 L mol^{-1} cm^{-1}, the method is the most sensitive ever reported for tramadol. Both the methods are simple, rapid and cost-effective and usefully employed in quality control laboratories.

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References

1. Grosa G, Grosso Ed, Russo R Allegrone G *J Pharm Biomed Anal* **41** (2006) 498
2. Budd K and Langford R *British J Anaesth* **82** (1999) 493
3. W Lintz, H Barth, G Osterloh and E Schmidt Bothelt *Drug Res* **48** (1999) 889
4. Mang FW, Liu W and Yang JN *Yaowu Fenxi Zazhi* **14** (1994) 42
5. Aysel K and Yücel K *Il Farmaco* 60 **(2005) 163**
6. Kartinasari WF, Palupi T and Indrayanto G *J Liq Chromtogra Rel Tech* **27**(2004) 737
7. Zaghloul IY and Radwan MA *J Liq Chromatogra Rel Tech* **20** (1997) 779
8. Zhong YL *Yaowu Fenxi Zazhi* **17** (1997) 279
9. Krzek J and Starek M *Biomed Chromatogra* **18** (2004) 589
10. Pospisilova M, Polasek M and Jokl V *J Pharm Biomed Anal* **18** (1998) 777
11. Zhang JK, Li JG and Tu YF *Fenxi Kexue Xuebao* **25** (2009)173-176
12. Garrido EMPJ, Garrido JMPJ, Borges F and Delerue-Matos C *J Pharm Biomed Anal* **32** (2003) 546
13. Norouzi P, Dinarvand R, Ganjali MR and Meibodi ASE *Anal Lett* **40** (2007) 2252
14. Garrido EMPJ, Garrido JMPJ, Borges F and Delerue-Matos C *J Pharma Biomed Anal* **32** (2003) 975

15. Ganjali MR, Memari Z, Faridbod F, Dinarvand R and Norouzi P *Electroanalysis (New York)* **20** (2008) 2663
16. Wu YJ, Shi J, Zhang JH, Zhao KL and Zhang ZP *Fenxi Shiyanshi* **24** (2005) 49
17. Huang CL, Xiu R, Liu ZY, Li YC and Meng CJ *Fenxi Kexue Xuebao* **21** (2005) 48
18. Huang CL, Xiu R, Dong QX, Chen XZ and You HQ *Fenxi Ceshi Xuebao* **24** (2005) 35
19. Wang M, Long YL, Li DH, Zhou WB, Zhao GZ and Zhang Z *Fenxi huàxué* **25**(1997) 448
20. Hopkala H, Misztal G and Wieczorek A *Die Pharmazie* **53** (1998) 869
21. Yue JL, Yu QH, Li DH, Zhao GZ, Shi W, An LR *Fenxi huàxué* **28** (2000) 84
22. Sadhana RJ and Trivedi PD *Indian Drugs* **38** (2001) 100
23. Hisham EA, Magda ME, Heba ME and Magda MA *Spectrochim Acta Part A: Molecul Biomolecular Spectro* **65** (2006) 1087
24. Abdellatef HE *J Pharm Biomed Anal* **29** (2002) 835
25. Mathur NK and Narang CK The determination of organic compounds with N-bromosuccinimide and allied reagents, Academic Press: New York, 1975, p. 13.
26. Berka A, Vulterin J and Zyka J (1965) Newer Redox Titrants, 1st Edition, Pergamon Press: London, p 38.
27. Ziegler K, Spath A, Schaaf E, Schumann W and Winkelmann E *Ann Chem* **551** (1942) 80
28. International Conference on Hormonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, ICH Harmonised Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology Q2(R 1), Complementary Guideline on Methodology dated 06 November 1996, incorporated in November 2005, London.
29. Miller JN and Miller JC (2000) "Statistics and chemometrics for analytical chemistry", Pearson Education Limited, England. GA Shabir *J Chromatogr A* **987** (2003) 57