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Use of two sulfonthalein dyes in the extraction-free spectrophotometric assay of tramadol in dosage forms and in spiked human urine based on ion-pair reaction

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Tramadol is a centrally acting analgesic used in the prevention and treatment of moderate to severe pain. Two sensitive, selective, and rapid spectrophotometric methods are described for the determination of tramadol in its dosage forms and in spiked human urine. The methods are based on formation of yellow ion-pairs between tramadol and two sulfonthalein dyes; bromocresol purple (BCP) and bromocresol green (BCG) in dichloromethane medium followed by absorbance measurement at 400 and 410 nm, respectively. Under the optimum conditions, tramadol could be assayed in the concentration ranges, 1–15 and 1–16 μg ml^{-1} with correlation coefficient greater than 0.999 in both cases. The molar absorptivity values are calculated to be 1.84 × 10^4 and 1.97 × 10^4 l mol^{-1} cm^{-1} for BCP and BCG methods, respectively; and the corresponding Sandell sensitivity values are 0.0143 and 0.0134 μg cm^{-2}. The limits of detection (LOD) and quantification (LOQ) have also been reported. The stoichiometry of the reaction was found to be 1 : 1 in both cases and the conditional stability constant (K_f) values of the ion pairs have been calculated. The within-day and between-day RSD were 0.9–1.96% and 1.56–3.21%, respectively. The methods were successfully applied to the determination of tramadol in tablets and injections and also in spiked human urine with good recoveries. The procedures are simple, accurate, and suitable for quality control application.

Keywords: tramadol hydrochloride; visible spectrophotometry; assay; pharmaceuticals; spiked human urine

Introduction

Tramadol hydrochloride (TMDH), chemically known as (1*R*,2*R*)-*rel*-2-[(dimethylamino)methyl]-1-(3-methoxyphenyl)cyclohexanol, hydrochloride (Figure 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 11 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] A review of the literature revealed that several methods have been reported for the analysis of TMDH in pharmaceuticals. These methods include ultra-violet spectrophotometry,[4,5] high performance liquid chromatography (HPLC),[5–8] thin layer chromatography-densitometry,[9] capillary isotachophoresis,[10] flow injection chemiluminescence spectrometry,[11] voltammetry,[12–14] and ion-selective-based potentiometry.[15–21]

Visible spectrophotometry, despite its fair selectivity and sensitivity, cost-effectiveness, and easy accessibility has been sparsely employed in the assay of TMDH. 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 TMDH with Folin-Ciocalteu's reagent which was measured at 645 nm. The first method is critically 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 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 TMDH 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 thermo stated 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}.

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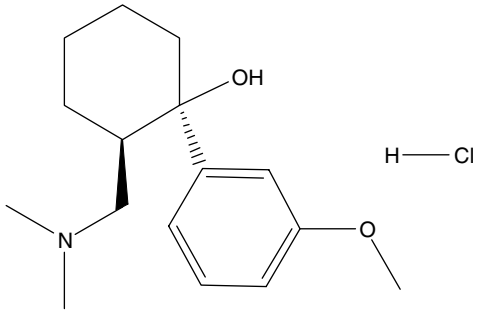


Figure 1. Structure of TMDH.

Ion-pair extractive spectrophotometry was used in the assay of pharmaceuticals[25–31] and different alkaloids[32,33] due to its sensitivity and selectivity. Though, ion-pair extractive spectrophotometry has several advantages, it has some difficulties and inaccuracies due to incomplete extraction or the formation of emulsions between organic and aqueous phase. The procedure involved in the assays is totally cumbersome. Few articles[34–38] were published for the analysis of pharmaceutical compounds through ion-pair formation without extraction and thereby overcoming all the problems encountered in extractive spectrophotometry.

In this piece of work, two accurate, precise, and extraction-free spectrophotometric methods for the determination of TMDH based on the interaction of its base with two sulfonthalein dyes namely, bromocresol purple (BCP) and bromocresol green (BCG) in dichloromethane are described. The proposed methods were demonstrated to be superior to the reported methods with respect to speed, simplicity, sensitivity, and cost effectiveness. The methods were successfully applied for the determination of TMDH in bulk drug, tablets, injections and spiked human urine with good recoveries. Also, the methods were demonstrated to be both robust and rugged, and found to be free from interference by adjuncts when applied to dosage forms.

Experimental

Apparatus

A Systronics model 106 digital spectrophotometer with 1 cm path length quartz cells was used to record the absorbance values.

Materials and reagents

Chemicals used were of analytical reagent grade. Tramadol hydrochloride (TMDH) was obtained from Jubilant Life Sciences Ltd (Nanjangud, Mysore, India). Dichloromethane (Sp. gr. 1.32), chloroform (Sp. gr. 1.474) and anhydrous sodium sulphate were purchased from Merck (Mumbai, India). Tramazac-TC 100 (100 mg TMDH) (Zydus Alidac Pvt Ltd, Bangalore, India), and Cemadol 50 CR (50 mg TMDH) (Life Medicare & Biotech Pvt. Ltd, Haridwar, India) tablets and Tramazac (50 mg mL^{-1} TMDH) injections were purchased from local commercial sources. Stock solutions of bromocresol purple (BCP) and bromocresol green (BCG) (both from Qualigens Fine Chemicals, Mumbai, India) each 0.1% were freshly prepared in dichloromethane. Kolthoff buffer solution of pH 9.0 was prepared by mixing 17.5 mL of 0.1 M potassium dihydrogen phosphate (S.D. Fine Chem, Mumbai, India) and 82.5 mL of 0.05 M sodium tetra borate (Merck, Mumbai, India) in a 100-mL volumetric flask.

Preparation of free base form (TMD) of the drug

An accurately weighed 57.92 mg of TMDH equivalent to 50 mg of base (TMD) was transferred into a 125 mL separating funnel, dissolved in 10 mL of water and added 25 mL buffer of pH 9.0. The content was mixed well and shaken for 15 min. Then, the solution was extracted with three 10-mL portions of dichloromethane and the organic layer was passed through anhydrous sodium sulphate. The organic extracts were then collected in a 100-mL volumetric flask and diluted to volume with dichloromethane. The resulting TMD solution (500 μg ml^{-1}) was then diluted stepwise to get a working concentration of 20 μg ml^{-1} with the same solvent.

General analytical procedures

Method A

Into a series of 5-mL calibration flasks, aliquots (0.25 to 3.75 mL) of TMD standard solution (20 μg ml^{-1}) equivalent to 1–15 μg ml^{-1} TMD were measured accurately and transferred; and to each flask 1 mL of 0.1% BCP dye solution was added and the content was diluted to 5 mL with dichloromethane. After 5 min, the absorbance of the yellow-coloured ion-pair complex was measured at 400 nm against a reagent blank similarly prepared.

Method B

Different aliquots (0.25 to 4.0 mL) of TMD solution (20 μg ml^{-1}) equivalent to 1.0–16.0 μg ml^{-1} were transferred into a series of 5-mL volumetric flasks. To each flask 1 mL of 0.1% BCG solution was added and the volume was brought to 5 mL with dichloromethane. The content was mixed well and the flasks were kept aside for 5 min. Then, the absorbance of the yellow ion-pair complex was measured at 410 nm against a reference solution similarly prepared but without adding the drug.

In both the methods, standard graphs were prepared by plotting the absorbance *versus* TMD concentration. The concentration of the unknown was read from the standard graph or computed from the regression equation derived using Beer's Law data.

Procedure for tablets

Twenty tablets were weighed and pulverised into a fine powder. The powder equivalent to 20 mg TMD was weighed out and transferred into a 125-mL separating funnel, the extraction procedure was followed and a 200 μg ml^{-1} TMD solution was prepared as described under the general procedure for pure drug. A suitable aliquot was diluted to get a working concentration of 20 μg ml^{-1} and used for the assay by following the general assay procedures described in method A and method B.

Procedure for injections

The declared concentration of TMDH in injection Tramazac was 50 mg mL^{-1} of solution. The contents of four injection tubes were pooled and an appropriate volume of the injection solution equivalent to 20 mg TMD was accurately transferred into a separating funnel and the general procedure was followed to prepare a 200 μg ml^{-1} TMD solution. This stock injection solution was further diluted to 20 μg ml^{-1} TMD and used for the assay by following the procedures described earlier.

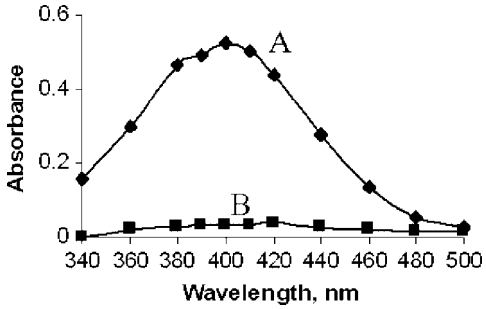


Figure 2. Absorption spectra of: A. TMD-BCP ion-pair complex (8.0 μg ml^{-1} TMD) and B. blank (method A).

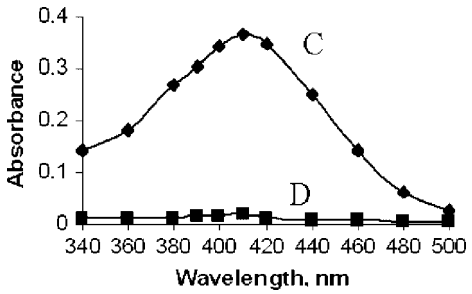


Figure 3. Absorption spectra of: C. TMD-BCG ion-pair complex (4.0 μg ml^{-1} TMD) and D. blank (method B).

Procedure for spiked human urine

Into a 125-mL separating funnel, 25 mL of urine, 5.8 mg of TMDH and 5 mL of 0.1 M NaOH were transferred and mixed well. The resulting solution was extracted with three 5-mL portions of chloroform. The organic layer was passed over anhydrous sodium sulfate and collected into a 50-mL volumetric flask. The solvent was evaporated on a water bath and the resulting TMD residue was dissolved in dichloromethane and diluted to the mark with same solvent. A suitable aliquot of this solution (100 μg ml^{-1}) was further diluted to get a working concentration of 20 μg ml^{-1} TMD and subjected to analysis.

Results and discussion

Chemically, the structure of TMD features its basic nature and suggests the possibility of utilizing an anionic dye as a chromogenic reagent. In dichloromethane, TMD does not absorb in the visible region and even the dyes employed have insignificant absorbance (Figures 2 and 3). In contrast, when a solution of BCP/BCG in dichloromethane mixed with the drug solution also in dichloromethane, an intense yellow-coloured product is produced immediately. This is due to an opening of lactoid ring and subsequent formation of quinoid group.[39] It is supposed that the two tautomers are present in equilibrium but due to strong acidic nature of the sulfonic acid group, the quinoid body must predominate. Finally, protonated TMD forms ion-pair with the dye. The possible reaction mechanisms are shown in Scheme 1 and Scheme 2.

Optimization of variables and method development

Prepartion of free base form (TMD) of the drug

No ion-pair complex was formed when TMDH was reacted with either dye but the base form did form instantaneously. Hence, the first step is the conversion of the salt to its base forms. In order to convert the hydrochloride into free base and for its quantitative extraction into the organic solvent, a series of experiments was performed. To a fixed amount of TMDH, varying volumes of buffer were added and shaken for different lengths of time before extraction with three 5-mL portions of dichloromethane. The results of this study revealed that a complete conversion into base and quantitative extraction into dichloromethane layer was realized when the volume of buffer solution ranged from 20 to 30 mL with a shaking time of 10–20 min and after three extractions of 5-mL portions of dichloromethane. Therefore, 25 mL of buffer and 15 min shaking time were used in the extraction procedure in which 15 mL of dichloromethane was used as the extracting solvent.

Dye concentration and reaction time

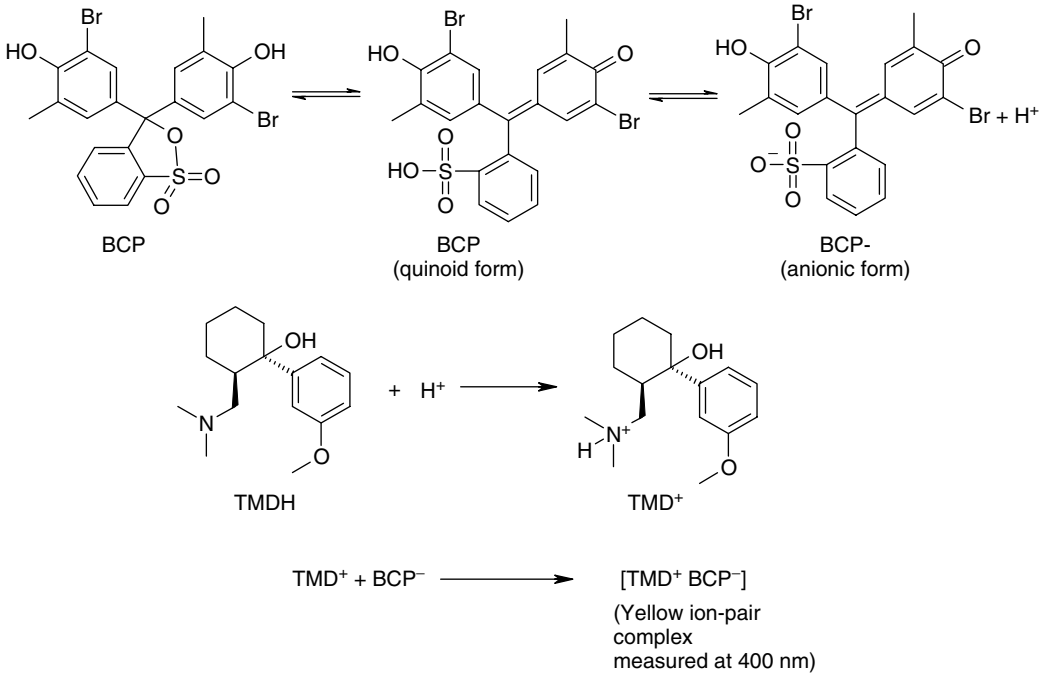
The experimental conditions were studied separately by measuring the absorbance of the final solution resulting from the reaction mixtures containing a fixed concentration of TMD (8.0 μg ml^{-1}) and various amounts of the dyes (0.0 to 2.0 mL of 0.1%). It was found that 1 mL of 0.1% dye (BCP in method A and BCG in method B) solution was sufficient to produce maximum and reproducible absorbance at 400 and 410 nm, for methods A and B, respectively. The reaction time or standing time after the addition of dye was also examined. It was found that 5 min standing time was sufficient for the complete formation of ion-pair complex. The absorbance of the ion-pair complex formed (in both methods) was observed to be stable for more than 24 h at room temperature.

Choice of the solvent as the reaction medium

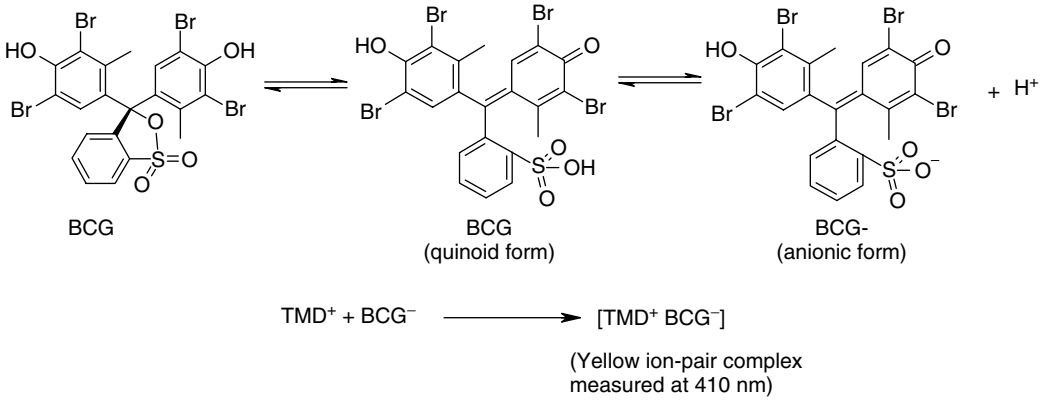
The effect of several organic solvents viz., chloroform, carbon tetrachloride, ethyl acetate, dichloromethane and 1,2-dichloromethane was studied as the medium for effective formation of coloured species. Dichloromethane was found to be the most suitable solvent for the formation of coloured complex for all reagents, yielding maximum sample absorbance and lower blank absorbance. Therefore dichloromethane was used as the reaction medium.

Investigation of composition of ion-pair complex

The reaction stoichiometries between TMD and BCP/BCG to form an ion-pair complex were established by Job's method of continuous variations[40] using equimolar concentrations of the free base form of the drug (TMD: prepared by following the general procedure) and the dye. The concentrations of TMD and dye solutions were 9.634×10^{-4} M in method A and 1.131×10^{-4} M in method B. Eight solutions containing TMD and the dye solution in various molar ratios, with a total volume of 3.0 and 4.0 mL with respect to method A and method B, were prepared. The volume was made up to 5 mL using dichloromethane and the absorbance was subsequently measured at 400 and 410 nm, for methods A and B, respectively. The results as shown in Figures 4 and 5 indicated the formation of a 1 : 1 TMD : BCP/BCG complex. The conditional



Scheme 1. Possible reaction mechanism for the formation of TMD-BCP ion-pair complex in dichloromethane.



Scheme 2. Possible reaction mechanism for the formation of TMD-BCG ion-pair complex in dichloromethane.

stability constant (K_f) of the ion-pair complex was calculated from the continuous variation data using Equation (1):[41]

$$K_f = \frac{A/A_m}{[1 - A/A_m]^{n+2} C_M(n)^n} \quad (1)$$

where A and Am are the observed maximum absorbance and the absorbance value when all the drug present is associated, respectively. C_M is the mole concentration of drug at the maximum absorbance and n is the stoichiometry which BCP/BCG ion-pair with TMD. The log K_f values were found to be 4.78 and 7.72 for methods A and B, respectively.

Method Validation

Linearity, sensitivity, limits of detection and quantification

A linear correlation was found between absorbance at λ_{max} and concentration of TMD in the ranges given in Table 1. The linear regression equations were Y = 0.0345 + 0.0694 X and Y = 0.0345 + 0.0680 X for methods A and B, respectively (where Y = absorbance and X = concentration of TMD in μg ml^{-1}). Regression analysis of the Beer's Law data using the method of least squares was made to evaluate the slope (m = 0.0694 and 0.0680 for methods A and B, respectively), intercept (b = 0.0039 and 0.0345 for methods A and B, respectively) and correlation coefficient (r) for each system and the values obtained from this investigations are presented in Table 1. The standard deviations of y-axis (S_y), slope (S_m) and intercept (S_b) are also calculated by using the formulae:[42]

$$S_y = \sqrt{\frac{[\sum y_i^2 - (\sum y_i)^2/N] - m^2[\sum x_i^2 - (\sum x_i)^2/N]}{N-2}},$$

$$S_m = \sqrt{\frac{S_y^2}{\sum x_i^2 - (\sum x_i)^2/N}},$$

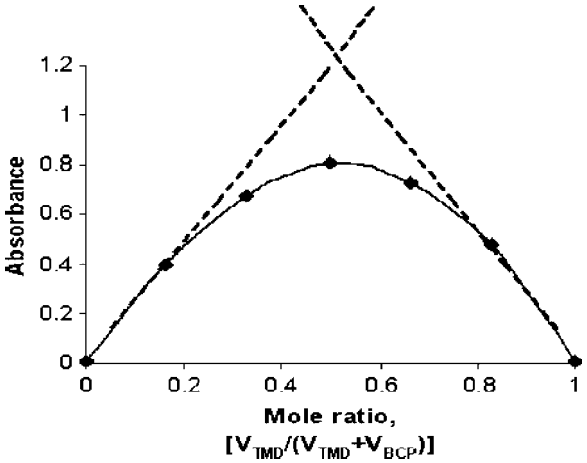


Figure 4. Job's plot obtained from equimolar solutions of TMD and BCP (9.634×10^{-4} M).

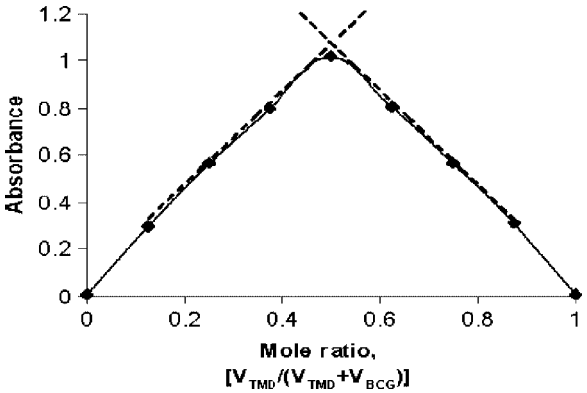


Figure 5. Job's plot obtained from equimolar solutions of TMD and BCG (1.131×10^{-4} M).

and $S_b = S_y \sqrt{\frac{1}{N - (\sum x_i)^2 / \sum x_i^2}}$, where y_i is the absorbance obtained for x_i concentration of drug, and N is the total number of measurements. A plot of *log* absorbance *versus log* concentration yielded a straight line in each case, with slope equal to 0.9397 and 1.0159 for methods A and B, respectively, further confirming the linear relationship between absorbance and concentration. The optical characteristics such as Beer's Law limits, molar absorptivity and Sandell sensitivity values[43] of both the methods are also given in Table 1. The LOD and LOQ calculated according to ICH guidelines[44] using the formulae:

$$\text{LOD} = 3.3\ \text{S/m and LOQ} = 10\ \text{S/m}$$

where S is the standard deviation of blank absorbance values, and m is the slope of the calibration plot. The high values of ε and low values of Sandell sensitivity and LOD indicate the high sensitivity of the proposed methods.

Intra-day and inter-day precision and accuracy

The precision and accuracy of the proposed methods were studied by repeating the experiment seven times within the day to determine the repeatability (intra-day precision) and five times on different days to determine the intermediate precision (inter-day precision) of the methods. These assays were performed for three levels of analyte. The results of this study are summarized in Table 2. The percentage relative standard deviation (%RSD) values were $\leq$ 1.96% (intra-day) and $\leq$ 3.21% (inter-day) indicating good precision of the methods. Accuracy was evaluated as percentage relative error (%RE) between the measured mean concentrations and taken concentrations for TMD. The %RE was $\leq$ 3% demonstrate the high accuracy of the proposed methods.

Table 1. Sensitivity and regression parameters

Parameter	Method A	Method B
λ_{max}, nm	400	410
Colour stability	>24 h	>24 h
Linear range, µg ml^{-1}	1-15	1-16
Molar absorptivity(ε), L mol^{-1} cm^{-1}	1.84×10^4	1.97×10^4
Sandell sensitivity*, µg cm^{-2}	0.0143	0.0134
Limit of detection (LOD), µg mL^{-1}	0.22	0.10
Limit of quantitation (LOQ), µg mL^{-1}	0.66	0.29
Regression equation, Y**		
Intercept (b)	0.0039	0.0345
Slope (m)	0.0694	0.0680
Standard deviation of Y-axis (S_y)	0.0116	0.0187
Standard deviation of b (S_b)	7.85×10^{-3}	0.011
Standard deviation of m (S_m)	7.75×10^{-3}	1.06×10^{-3}
Regression coefficient (r)	0.9996	0.9990

* 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=b+mX, Where Y is the absorbance, X is concentration in µg ml^{-1}, b is intercept, m is slope.

Table 2. Evaluation of intra-day and inter-day accuracy and precision

		Intra-day accuracy and precision (n=7)			Inter-day accuracy and precision (n=5)		
Method	TMD taken, µg ml^{-1}	TMD found, µg ml^{-1}	%RE	%RSD	TMD found, µg ml^{-1}	%RE	%RSD
A	4.0	4.02	0.50	1.96	4.05	1.25	1.89
	8.0	8.03	0.38	1.32	8.12	1.50	1.65
	12.0	12.23	1.92	1.28	12.25	2.08	2.35
B	4.0	4.09	2.25	1.82	4.07	1.75	2.33
	8.0	8.12	1.50	0.90	8.10	1.25	1.56
	12.0	12.36	3.00	1.90	12.11	0.92	3.21

%RE. Percent relative error, %RSD. relative standard deviation.

Selectivity

A systematic study was performed to determine the effect of matrix by analyzing the placebo blank and synthetic mixture containing TMDH. A placebo blank of the composition: starch (10 mg), acacia (15 mg), hydroxyl cellulose (10 mg), sodium citrate (10 mg), talc (20 mg), magnesium stearate (15 mg), and sodium alginate (10 mg) was made and its solution was prepared as described under 'tablets', and then subjected to analysis. The absorbance of the reaction product with placebo solution in each case was almost equal to the absorbance of the blank

which revealed no interference. To assess the role of the inactive ingredients on the assay of TMD, a synthetic mixture was separately prepared by adding calculated amount of TMDH w.r.t. TMD to the placebo mentioned above. The drug was extracted and solution prepared as described under the general procedure for tablets. The solutions after appropriate dilution were analyzed (three different concentrations of TMD; in triplicate) following the recommended procedures. The percentage recovery of TMD (98.64–102.6) was almost same as obtained for pure drug of identical concentrations. The standard deviation values were 0.98–1.85%. These accurate and precise results demonstrated that the proposed methods are free from interferences due to the additives added to TMDH.

Robustness and ruggedness

The robustness of the methods was evaluated by making small incremental changes in the volume of dye (1 ± 0.25 mL of 0.1%) and contact time (5 ± 1.0 min), and the effect of these changes was studied on the absorbance of the ion-pair complex. The changes had negligible influence on the results as revealed by small intermediate precision values expressed as %RSD (1.12–2.13).

Method ruggedness was demonstrated by having the analysis done by four analysts, and also by a single analyst performing the analysis on four different instruments in the same laboratory. Intermediate precision values (%RSD) in both instances were in the range 0.89–2.65% indicating acceptable ruggedness.

Application to tablets and injections

The proposed methods were applied for the quantification of TMD in commercial tablets and injections. The same batch tablets were assayed by the reference method[5] which consisted of measurement of the absorbance aqueous solution of TMDH at 271 nm. The results obtained by the proposed methods agreed well with the label claim and also are in agreement with those by the reference method. Statistical analysis of the results did not detect any significant difference between the performance of the proposed methods and reference method with respect to accuracy and precision as revealed by the Student's *t*-value and variance ratio F-value.[45] The results of assay are given in Table 3.

Table 3. Results of analysis of TMDH formulations by the proposed methods and statistical comparison of the results with the reference method

Brand name	Nominal amount#	Percent TMD found±SD*		
		Reference method	Method A	Method B
Tramazac TC 100[a]	100	99.15 ± 0.96	98.36 ± 0.89	98.16 ± 1.02
			t=1.35	**t**=1.58
			F=1.16	**F**=1.13
Cemadol 50[a]	50	101.0 ± 0.89	100.20 ± 1.11	101.3 ± 1.23
			t=1.19	**t**=0.45
			F=1.56	**F**=1.91
Tramazac[b]	50	100.15 ± 1.16	101.55 ± 1.22	98.56 ± 1.08
			t=1.86	**t**=2.24
			F=1.11	**F**=1.15

[a] Tablet and [b] injection. # mg/tablet in tablets and mg/mL in injection.
* Mean value of 5 determinations.
(Tabulated t-value at the 95% confidence level and for four degrees of freedom is 2.77).
(Tabulated F-value at the 95% confidence level and for four degrees of freedom is 6.39).

Table 4. Recovery of TMD from spiked human urine

Method	TMD added, μg ml^{-1}	TMD found*, μg ml^{-1}	Recovery of TMD (Percent±SD)
A	4.00	3.83	95.75 ± 0.52
	6.00	6.05	100.8 ± 0.85
	8.00	8.40	105.0 ± 1.02
B	6.00	6.08	101.3 ± 0.55
	8.00	8.06	101.3 ± 0.26
	10.00	10.60	106.0 ± 1.06

* Mean value of three determinations.

Application to spiked human urine

The high sensitivity of the proposed methods allowed determination of TMDH in spiked human urine. The extraction procedure described earlier was followed prior to determination and the recovery studies were performed on three spiked concentrations. The results of these studies (Table 4) revealed that, other constituents present in the urine did not interfere in the methods. The concentration of TMDH in spiked urine was adjusted assuming that 50% of the dose is found in the urine unchanged.

Accuracy evaluation by recovery study (standard addition method)

To further assess the accuracy of the methods, recovery experiments were performed by applying the standard addition technique. The recovery was assessed by determining the agreement between the measured standard concentration and added known concentration to the sample. The test was done by spiking the pre-analyzed tablet powder with pure TMDH at three different levels (50, 100, and 150% of the content present in the tablet powder (taken) and the total was found by the proposed methods after the necessary sample treatment steps. Each test was repeated three times. In both cases, the recovery percentage values ranged between 94.0 and 107.3% with standard deviation in the range 0.07–1.56%. Closeness of the results to 100% (Table 5) corroborates the findings of the study with pure drug.

Conclusions

Two spectrophotometric methods for the determination of tramadol hydrochloride in bulk drug, tablets, injections, and spiked human urine sample were developed and validated. The results obtained demonstrate the analytical potential of extraction-free spectrophotometry using dyes as ion-pair reagents. As the most relevant features of the proposed methods, one should emphasize the absence of pH control, liquid-liquid extraction, or heating steps. The methods are more precise than kinetic spectrophotometric procedures since the latter are prone to imprecision; the rate of the reaction is critically dependent on a number of experimental variables. When compared to the existing non-spectrophotometric

Table 5. Results of recovery study for tablet *via* standard addition method

Tablet studied	Method A				Method B			
	TMD in tablet, μg ml^{-1}	Pure TMD added, μg ml^{-1}	Total found, μg ml^{-1}	Pure TMD recovered (Percent±SD*)	TMD in tablet, μg ml^{-1}	Pure TMD added, μg ml^{-1}	Total found, μg ml^{-1}	Pure TMD recovered (Percent±SD*)
Cemadol 50	4.0	2.0	5.99	99.5 ± 0.07	4.05	2.0	5.93	94.0 ± 0.86
	4.0	4.0	7.93	98.3 ± 0.10	4.05	4.0	8.34	107.3 ± 1.56
	4.0	6.0	9.80	96.7 ± 0.08	4.05	6.0	9.78	95.5 ± 0.56

* Mean value of three determinations.

and spectrophotometric methods, the proposed methods exhibit better characteristics regarding accuracy, precision, sensitivity, robustness, ruggedness, low reagent consumption, and low waste generation; furthermore, they use a very easily accessible instrument and do not require skilful operators. The proposed methods were successfully applied for the assay of TMD in tablets, injections and spiked human urine without any interference. Hence, the methods can be used in routine analysis of drug in quality control laboratories.

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References

[1] G. Grosa, E. D. Grosso, R. Russo, G. Allegrone, *J. Pharm. Biomed. Anal.* **2006**, *41*, 498.
[2] K. Budd, R. Langford, *Brit. J. Anaesth.* **1999**, *82*, 493.
[3] W. Lintz, H. Barth, G. Osterloh, E. Schmidt Bothelt, *Drug Res.* **1999**, *48*, 889.
[4] F. W. Mang, W. Liu, J. N. Y. Ang, *Yaowu Fenxi Zazhi* **1994**, *14*, 42.
[5] K. Aysel, K. Yücel, *Il Farmaco* **2005**, *60*, 163.
[6] W. F. Kartinasari, T. Palupi, G. Indrayanto, *J. Liq. Chromtogr. R. T.* **2004**, *27*, 737.
[7] I. Y. Zaghloul, M. A. Radwan, *J. Liq. Chromtogr. R. T.* **1997**, *20*, 779.
[8] Y. L. Zhong, *Yaowu Fenxi Zazhi* **1997**, *17*, 279.
[9] J. Krzek, M. Starek, *Biomed. Chromatogr.* **2004**, *18*, 589.
[10] M. Pospisilova, M. Polasek, V. Jokl, *J. Pharm. Biomed. Anal.* **1998**, *18*, 777.
[11] J. K. Zhang, J. G. Li, Y. F. Tu, *Fenxi Kexue Xuebao* **2009**, *25*, 173.
[12] E. M. P. J. Garrido, J. M. P. J. Garrido, F. Borges, C. Delerue-matos, *J. Pharm. Biomed. Anal.* **2003**, *32*, 546.
[13] P. Norouzi, R. Dinarvand, M. R. Ganjali, A. S. E. Meibodi, *Anal. Lett.* **2007**, *40*, 2252.
[14] E. M. P. J. Garrido, J. M. P. J. Garrido, F. Borges, C. Delerue-Matos, *J. Pharm. Biomed. Anal.* **2003**, *32*, 975.
[15] M. R. Ganjali, Z. Memari, F. Faridbod, R. Dinarvand, P. Norouzi, *Electroanal. (New York)* **2008**, *20*, 2663.
[16] Y. J. Wu, J. Shi, J. H. Zhang, K. L. Zhao, Z. P. Zhang, *Fenxi Shiyanshi* **2005**, *24*, 49.
[17] C. L. Huang, R. Xiu, Z. Y. Liu, Y. C. Li, C. J. Meng, *Fenxi Kexue Xuebao* **2005**, *21*, 48.
[18] C. L. Huang, R. Xiu, Q. X. Dong, X. Z. Chen, H. Q. You, *Fenxi Ceshi Xuebao* **2005**, *24*, 35.
[19] M. Wang, Y. L. Long, D. H. Li, W. B. Zhou, G. Z. Zhao, Z. Zhang, *Fenxi Huàxué* **1997**, *25*, 448.
[20] H. Hopkala, G. Misztal, A. Wieczorek, *Die Pharmazie* **1998**, *53*, 869.
[21] J. L. Yue, Q. H. Yu, D. H. Li, G. Z. Zhao, W. Shi, L. R. An, *Fenxi Huàxué* **2000**, *28*, 84.
[22] R. J. Sadhana, P. D. Trivedi, *Indian Drugs* **2001**, *38*, 100.
[23] E. A. Hisham, M. E. Magda, M. E. Heba, M. A. Magda, *Spectrochim. Acta A* **2006**, *65*, 1087.
[24] H. E. Abdellatef, *J. Pharm. Biomed. Anal.* **2002**, *29*, 835.
[25] A. Onal, S. E. Kepekci, A. Oztunc, *J. AOAC Int.* **2005**, *88*, 490.
[26] N. Rahman, N. Ahmad Khan, S. N. Hejaz Azmi, *IL Farmaco* **2004**, *59*, 47.
[27] K. C. Ramesh, B. G. Gowda, M. B. Melwanki, J. Seetharamappa, J. Keshavayya, *Anal. Sci.* **2001**, *17*, 1101.
[28] N. Rahman, S. N. Hejaz-Azmi, *J. Pharm. Biomed. Anal.* **2000**, *24*, 33.
[29] N. Silva, E. E. S. Schapoval, *J. Pharm. Biomed. Anal.* **2002**, *29*, 749.
[30] T. Perez-Ruiz, C. Martinez-Lozano, V. Tomás, A. Sanz, E. Sahuguillo, *J. Pharm. Biomed. Anal.* **2001**, *26*, 609.
[31] K. Basavaiah, V. S. Charan, *IL Farmaco* **2002**, *57*, 9.
[32] H. Maghsoudi, A. Fawzi, *J. Pharm. Sci.* **1978**, *67*, 32.
[33] H. Farsam, H. H. Yahya-Saeb, A. Fawzy, *Int. J. Pharm.* **1981**, *7*, 343.
[34] M. M. El-Kerdawy, M. A. Moustafa, S. M. El-Ashry, D. R. El-Waseef, *Anal. Lett.* **1993**, *26*, 1669.
[35] H. H. Abdine, *Alex. J. Pharm. Sci.* **2000**, *14*, 75.
[36] H. Abdine, F. Belal, N. Zoman, *IL Farmaco* **2002**, *57*, 267.
[37] D. H. Manjunatha, S. M. T. Shaikh, K. Harikrishna, R. Sudhirkumar, P. B. Kandagal, J. Seetharamappa, *Ecl. Quim. Sao Paulo* **2008**, *33*, 37.
[38] K. Basavaiah, A. M. Sameer, K. B. Vinay, *J. Food Drug Anal.* **2009**, *17*, 434.
[39] S. Ashour, M. Fawaz Chehna, R. Bayram, *Int. J. Biomed. Sci.* **2006**, *2*, 273.
[40] D. A. Stooge, D. M. West, *Principals of Instrumental Analysis,* Holt, Rinhart, Winston: New York, **1971**.
[41] N. Erk, *Anal. Lett.* **2003**, *36*, 1183.
[42] G. D. Christian, *Analytical Chemistry*, 6th edn, John Wiley and Sons (Asia) Pvt. Ltd.: Singapore, **2004**, pp. 104–105.
[43] H. Zavis, D. Ludvik, K. Milan, S. Ladislaw, V. Frantisck, *Handbook of Organic Reagents in Inorganic Analysis.* (Eds: University of Aberdem, Ellis Horwood Limited), John Wiley & Sons IC: New York, London, Sydney, Toronto, **1976**.
[44] International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, ICH Harmonized Tripartite Guideline, *Validation of Analytical Procedures: Text and Methodology,* Q2(R 1), Complementary Guideline on Methodology dated 06 November 1996, ICH, London, **2005**.
[45] J. Inczedy, T. Lengyel, A. M. Ure, *IUPAC Compendium of Analytical Nomenclature: Definitive Rules,* Blackwell Science Inc.: Boston, **1998**.