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DEVELOPMENT AND VALIDATION OF ULTRA VIOLET SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF ACID DISSOCIATION CONSTANT OF TRAMADOL HYDROCHLORIDE

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ABSTRACT

In the present study a simple, accurate, precise and economical UV spectrophotometric method for determination of acid dissociation constant (pKa) of tramadol hydrochloride has been developed. The wavelengths selected for ionized and unionized forms of tramadol hydrochloride were 209nm and 269nm respectively using buffer solutions as solvent. The relative concentrations of the ionized and unionized forms of the drug were determined by simultaneous equation method and determined the pKa value using Handerson – Hasselbalch equation. Using absorbance values, absorptivity values and simultaneous equation the concentration of ionized and unionized form of drug was determined and using Handerson-hasselbalch equation the pKa was found to be 9.3. The method utilizes easily available and cheap solvent for analysis hence the method was also economic. The method was validated statistically for accuracy, precision, reproducibility, linearity, LOD, LOQ and robustness & ruggedness. Hence the method could be conveniently adopted for routine analysis in quality control laboratories.

KEYWORDS: Tramadol hydrochloride, UV spectrophotometer, simultaneous equation method, method validation..

INTRODUCTION

Many drugs contain atleast one acidic and/or basic functionality and the ionization state of these groups plays an important role in determining the physicochemical properties of a

compound. A number of methods have been used for the determination of dissociation constant of weak acids and bases such as the potentiometric methods which are widely used, since they are fast and easy to study ionic equilibria in aqueous and non-aqueous solvents while other methods such as spectrophotometric and conductometric are time consuming but they are very accurate. The pKa value is a key parameter to predict the ionization state of a molecule with respect to pH. The pKa of a molecule is the pH at which the molecule is 50% protonated. Since most of the drug compounds have acidic and/or basic functionalities, their ionization state is controlled by both solution pH and acidic dissociation constants (i.e pKa values). [2,4] Tramadol hydrochloride (Fig.1) is a monoamine reuptake inhibitor, acts as muscle relaxant and show analgesic activity, used in the treatment of muscular pain and gout. Tramadol hydrochloride chemically is 2-[(dimethylamino) methyl]-1-(3-methoxyphenyl) cyclohexanol hydrochloride. This drug has a weak action on μ-opioid receptors and additionally inhibits reuptake of noradrenaline and enhances serotonin release within central pain pathways. [7-9]

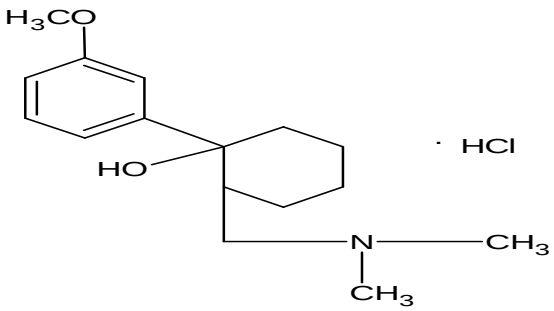

Figure 1: Structure of Tramadol Hydrochloride

Validation is the process used to confirm that the analytical procedure employed for a specific test suitable for its intended use. Results from method validation can be used to judge the quality, reliability and consistency of analytical result. It is an integral part of any good analytical practice. The USP has published specific guidelines for method validation and compound evaluation. USP defines eight steps for validation: Accuracy, Precision, Specificity, Limit of detection, Limit of quantitation, Linearity, Ruggedness and Robustness.

Literature survey revealed that some spectrophotometric and potentiometric methods have been reported for the determination of pKa of beta blockers and anti diabetic drugs. Hence, not even a single analytical method reported for the determination of pKa of tramadol hydrochloride. So, this study is useful because pKa is determined for tramadol hydrochloride by using UV spectrophotometric method as pKa is one of the important property used to estimate the absorption, distribution, metabolism and excretion of compounds in biological

systems. The UV spectrophotometric analysis is often preferred in quality control testing and ordinary laboratories due to its broader availability, suitability and ease of use. [1-3, 5, 6] The aim of the present study is to provide a simple, sensitive, accurate and reproducible UV spectrophotometric method for determination of pKa of Tramadol hydrochloride and hence an economical method was developed and validated according to the ICH guidelines.

MATERIALS AND METHODS

MATERIALS

A UV-VIS Spectrophotometer, model Shimadzu 1800 (Japan) was employed with spectral pair of matched quartz cells of 1cm optical path length. Buffer solution was used for preparing dilutions. Glassware used in each procedure were rinsed thoroughly with acetone and dried in hot air oven. All other reagents and chemicals were of analytical reagent grade.

METHODS

Selection of Solvent

For the selection of a solvent, many solvents have been used viz. ethanol, methanol, distilled water and buffer solution. Out of these the buffer solution used as a solvent for determination of pKa of Tramadol hydrochloride.

Preparation of stock solution of Tramadol Hydrochloride

Tramadol hydrochloride equivalent to 10mg was weighed accurately and transferred into a 100ml volumetric flask. About 50ml of solvent was added and sonicated to ultrasonic water bath for 5 minutes to dissolve the drug. The volume was made up to the mark with the solvent. The final solution contained concentration of 100µg/ml of Tramadol hydrochloride. Thus for the determination of pKa the wavelength should be selected for unionized and ionized forms of drug.

Conversion to unionized form (T. HCl)

By dissolving of the drug in acidic state i.e buffer pH 2.0, the drug was converted to its unionized form. For preparation of stock solution of unionized form, phosphate buffer pH 2.0 was prepared. The aliquot portion of standard stock solution of unionized form was diluted appropriately with solvent to obtain concentration 2-10 µg/ml of drug. The solution was scanned in the range of 200-400nm in 1cm cell against blank. The UV absorbance spectrum of the drug was recorded. From the spectrum the wavelength selected was 269nm for unionized form.

Conversion to ionized form (T. H+)

By dissolving of the drug in basic state i.e buffer pH 10.0, the drug was converted to its ionized form. For preparation of stock solution of ionized form, ammonia buffer pH 10.0 was prepared. The aliquot portion of standard stock solution of ionized form was diluted appropriately with solvent to obtain concentration 2-10 µg/ml of drug. The solution was scanned in the range of 200-400nm in 1cm cell against blank. The UV absorbance spectrum of the drug was recorded. From the spectrum the wavelength selected was 209nm for ionized form.

Comparison of linearity and reproducibility for the selection of lambda max

The wavelengths which show linearity and reproducibility in a solvent system were selected. For this, the linearity and reproducibility of the lambda max obtained with different solvent systems were compared. The linearity and reproducibility of lambda max for unionized form of drug was compared separately and that of ionized was compared separately. The lambda max for unionized form of drug was found to be 269nm and that of ionized from of drug was 209nm.

Comparison of linearity and reproducibility for the selection of solvent

The solvent which exhibits both ionized and unionized form of the drug was selected. For this, linearity and reproducibility of different solvents were compared. The solvent with best linearity reproducibility was selected. The selected solvent was phosphate buffer pH 7.0.

Selection of Concentration:

From the above tested concentrations, one concentration has to be selected by comparing reproducibility and accuracy data of the all concentrations. The most accurate and reproducible concentration was found to be 4µg/ml.

METHOD VALIDATION FOR pKa

Accuracy

Five replicate dilutions of 4µg/ml were prepared with buffer pH 7.0 from the stock solution these dilutions were tested at 209nm and 269nm wavelengths. From the absorbance value the pKa, S.D and RSD was calculated for each dilution.

Precision

For determination of precision a single dilution was tested.

System precision

A single dilution of 4µg/ml was prepared with pH 7.0 buffer from the stock solution; this dilution was tested 5 times at 209nm and 269nm wavelengths. From the absorbance value pKa, SD & RSD were calculated.

Method precision

Five replicate dilutions of 4µg/ml were prepared with buffer pH 7.0 from the stock solution these dilutions were tested at 209nm and 269nm wavelengths. From the absorbance value the pKa, S.D and RSD was calculated for each dilution.

Linearity

Several serial dilutions ranges from 2-100 µg/ml were prepared with pH 7.0 buffer from the stock solution; these dilutions were tested at 209nm and 269nm wavelengths. From the absorbance value the slope, intercept and coefficient of correlation was calculated.

Specificity

Different dilutions ranges from 2-10 µg/ml were prepared with buffer pH 7.0 from the stock solution these dilutions were tested at 209nm and 269nm wavelengths. From the absorbance value, the pKa was calculated. A graph was plotted between the pKa and concentration of dilutions.

LOD

Several serial dilutions ranges from 2-10 µg/ml were prepared with buffer pH 7.0 from the stock solution these dilutions were tested at 209nm and 269nm wavelengths. From the absorbance value the slope, standard deviation and LOD was calculated.

LOQ

Several serial dilutions ranges from 2-10 µg/ml were prepared with buffer pH 7.0 from the stock solution these dilutions were tested at 209nm and 269nm wavelengths. From the absorbance value the slope, standard deviation and LOQ was calculated.

Robustness

Robustness of the method was determined by varying the pH of the phosphate buffer. Dilutions were prepared with buffer of pH 6.8, 7.0 and 7.2. From the absorbance values pKa, S.D and RSD was calculated.

Ruggedness

Ruggedness of the method was determined by varying the parameters i.e. interday, intraday and inter analysts. Dilutions were prepared with buffer pH 7.0 at different parameters. From the absorbance values pKa, S.D and RSD was calculated.

RESULTS AND DISCUSSION

The solvent which exhibits both ionized and unionized form of the drug was selected. For this, linearity and reproducibility of different solvents were compared. The solvent with best linearity and reproducibility was selected. The selected solvent was phosphate buffer pH 7.0. From the above tested concentrations, one concentration has to be selected and that was selected by comparing reproducibility and accuracy data of the all concentrations. The most accurate and reproducible concentration was found to be 4µg/ml.

BUFFER pH 7.0 AS SOLVENT

When buffer pH 7.0 and its dilutions with drug were scanned throughout the UV region, the results obtained with blank i.e without drug buffer pH 7.0 were shown in Figure 2.

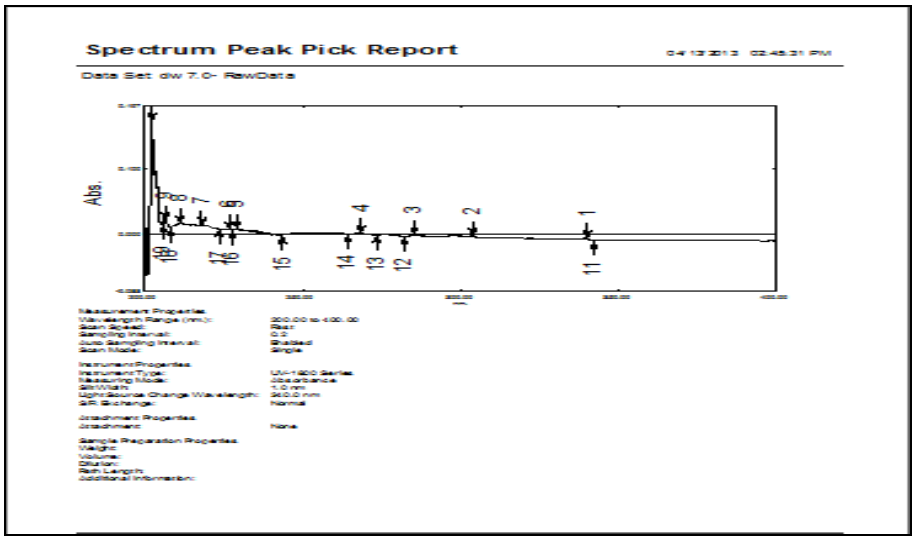

Figure 2: Spectrum of Buffer pH 7.0 (Blank)

The table 1 shows the spectral peaks with corresponding absorbance present in UV spectrum of buffer pH 7.0 without drug when it was scanned between190 to 400nm.

Table 1: Absorbance of Buffer pH 7.0 (Blank)

No.	5	6	7	8	9	10	16	17	19
Wavelength	229.60	227.20	218.00	211.00	206.60	202.60	228.60	223.20	205.00
Absorbance	0.007	0.008	0.013	0.016	0.021	0.173	0.006	0.007	0.015

Spectrum of 2µg/ml solution of Tramadol hydrochloride in buffer pH 7.0

When 2µg/ml solution of drug in buffer pH 7.0 was scanned throughout the UV region, the following spectrum was obtained as shown in Figure 3.

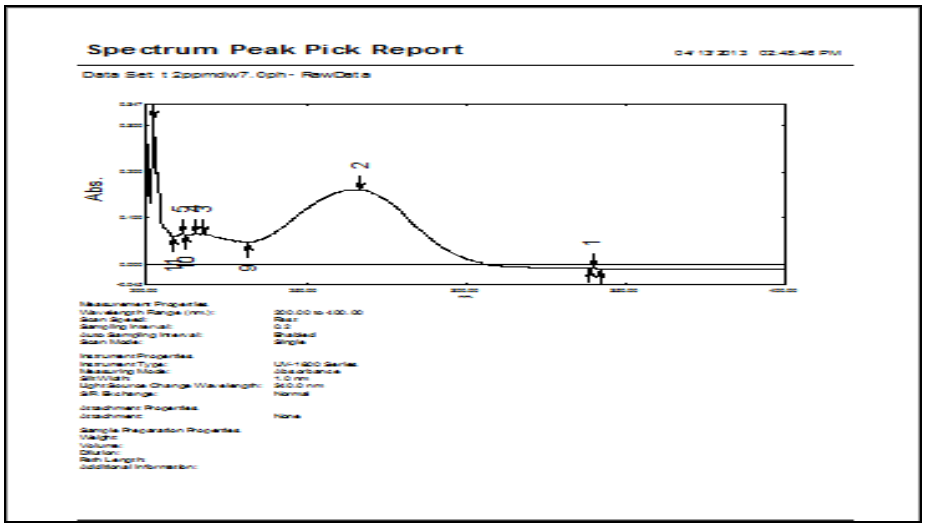

Figure 3: Spectrum of 2µg/ml of T. HCl in Buffer pH 7.0

The table 2 shows the spectral peaks with corresponding absorbance present in UV spectrum of drug having conc. of 2µg/ml in buffer pH 7.0.

Table 2: Spectral peaks present in spectrum of 2µg/ml of T. HCl with buffer pH 7.0 solvent

No.	**2**	**3**	**4**	**5**	**6**	**9**	**10**	**11**
Wavelength	268.80	218.20	215.20	211.60	202.20	232.00	212.40	208.60
Absorbance	0.131	0.065	0.065	0.064	0.046	0.063	0.058	0.094

After neglecting the spectral peaks due to the blank, the peaks left was shown in table 3 which indicates the actual spectral peaks with corresponding absorbance present in UV spectrum of drug having concentration of 2µg/ml in buffer pH 7.0.

Table 3: Spectral peaks present in 2µg/ml of drug without buffer pH 7.0 solvent.

Wavelength	268.80	215.20	232.00	212.40	208.60
Absorbance	0.131	0.065	0.063	0.058	0.094

Spectrum of 4µg/ml solution of Tramadol hydrochloride in buffer pH 7.0

When 4µg/ml solution of drug in buffer pH 7.0 was scanned throughout the UV region, the following spectrum was obtained as shown in Figure 4.

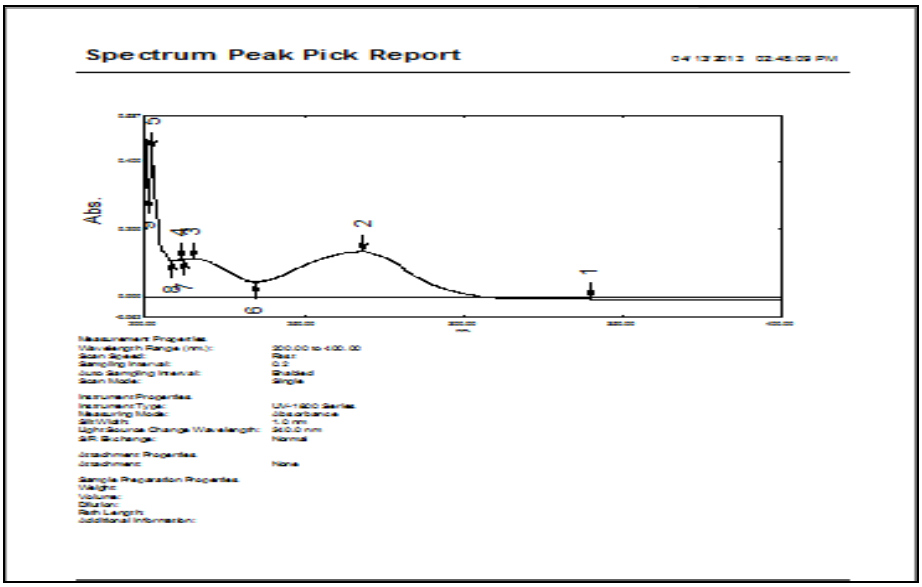

Figure 4: Spectrum of 4µg/ml of T. HCl in Buffer pH 7.0

The table 4 shows spectral peaks with corresponding absorbance present in UV spectrum of drug having concentration of 4µg/ml in buffer pH 7.0.

Table 4: Spectral peaks present in spectrum of 4µg/ml of T. HCl with buffer pH 7.0 solvent

No.	2	3	4	5	6	7	8	9
Wavelength	268.60	215.40	211.60	202.40	235.00	212.40	208.60	201.40
Absorbance	0.133	0.111	0.110	0.438	0.041	0.109	0.104	0.295

After neglecting the spectral peaks due to the blank, the peaks left was shown in table 5 which indicates the actual spectral peaks with corresponding absorbance present in UV spectrum of drug having concentration 4µg/ml in buffer pH 7.0.

Table 5: Spectral peaks present in 4µg/ml of T. HCl without buffer pH 7.0 solvent

Wavelength	268.60	215.40	235.00	212.40	208.60	201.40
Absorbance	0.133	0.111	0.041	0.109	0.104	0.295

Spectrum of 6µg/ml solution of Tramadol hydrochloride in buffer pH 7.0

When 6µg/ml solution of drug in buffer pH 7.0 was scanned throughout the UV region, the following spectrum was obtained as in Figure 5.

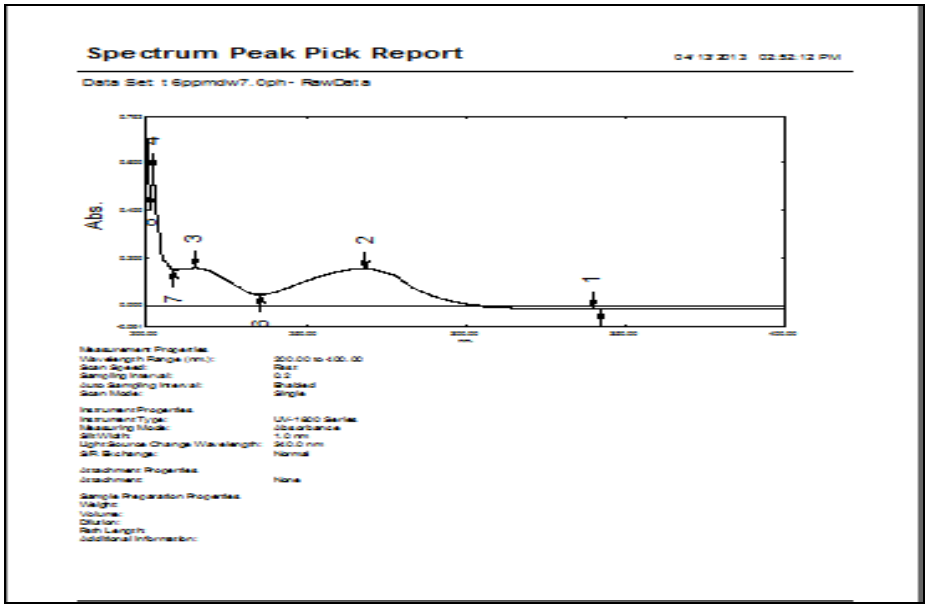

Figure 5: Spectrum of 6µg/ml of T. HCl in Buffer pH 7.0

The table 6 shows spectral peaks with corresponding absorbance present in UV spectrum of drug having conc. 6µg/ml in buffer pH 7.0.

Table 6: Spectral peaks present in spectrum of 6µg/ml of T. HCl with buffer pH 7.0 solvent.

No.	2	3	4	6	7
Wavelength	268.60	215.40	202.20	236.00	208.60
Absorbance	0.155	0.155	0.568	0.043	0.147

After neglecting the spectral peaks due to the blank, the peaks left was shown in table 7 which indicates the actual spectral peaks with corresponding absorbance present in UV spectrum of drug having concentration of 6µg/ml in buffer pH 7.0 (blank).

Table 7: Spectral peaks present in 6µg/ml of T. HCl without buffer pH 7.0 solvent.

Wavelength	268.60	215.40	208.60
Absorbance	0.155	0.155	0.147

Spectrum of 8µg/ml solution of Tramadol hydrochloride in buffer pH 7.0

When 8µg/ml solution of drug in buffer pH 7.0 was scanned throughout the UV region, the following spectrum was obtained as in Figure 6.

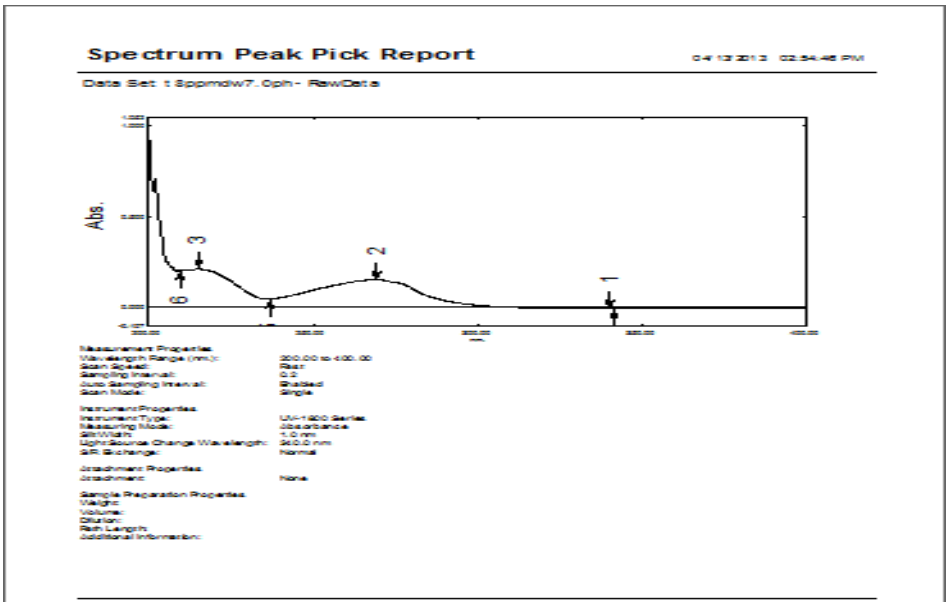

Figure 6: Spectrum of 8µg/ml of T. HCl in Buffer pH 7.0

The table 8 shows the spectral peaks with corresponding absorbance present in UV spectrum of drug having concentration of 8µg/ml in buffer pH 7.0.

Table 8: Spectral peaks present in spectrum of 8µg/ml of T. HCl with buffer pH 7.0 solvent

No.	**2**	**3**	**5**	**6**
Wavelength	269.60	215.40	237.00	209.60
Absorbance	0.157	0.209	0.043	0.198

After neglecting the spectral peaks due to the blank, the peaks left was shown in table 9 which indicates the actual spectral peaks with corresponding absorbance present in UV spectrum of drug having concentration of 8µg/ml in buffer pH 7.0 (blank).

Table 9: Spectral peaks present in 8µg/ml of T. HCl without buffer pH 7.0 solvent.

Wavelength	269.60	215.40	209.60
Absorbance	0.157	0.209	0.198

Spectrum of 10µg/ml solution of Tramadol hydrochloride in buffer pH 7.0

When 10µg/ml solution of drug in buffer pH 7.0 was scanned throughout the UV region, the following spectrum was obtained as in Figure 7.

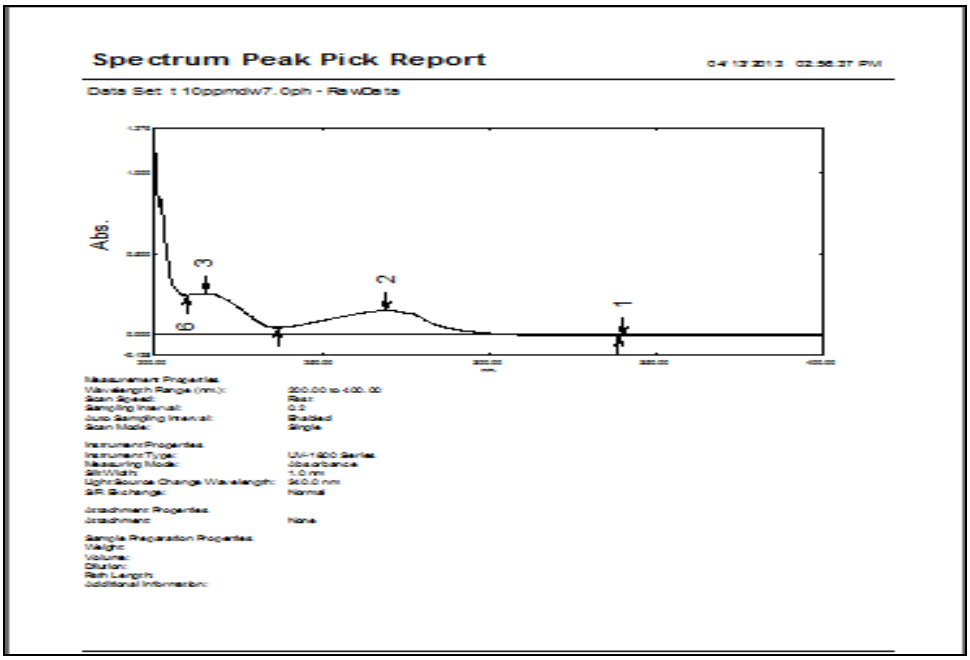

Figure 7: Spectrum of 10µg/ml of T. HCl in Buffer pH 7.0

The table 10 shows spectral peaks with corresponding absorbance present in UV spectrum of drug having concentration of 10µg/ml in buffer pH 7.0.

Table 10: Spectral peaks present in spectrum of 10µg/ml of T. HCl with buffer pH 7.0 solvent.

No.	**1**	**2**	**3**	**6**
Wavelength	340.20	269.40	215.40	209.60
Absorbance	-0.007	0.170	-0.253	0.242

After neglecting the spectral peaks due to the blank, the peaks left was shown in table 11 which indicates the actual spectral peaks with corresponding absorbance present in UV spectrum of drug having concentration of 10µg/ml in buffer pH 7.0 (blank).

Table 11: Spectral peaks present in spectrum 10µg/ml of T. HCl without buffer pH 7.0 solvent.

Wavelength	269.40	209.60
Absorbance	0.170	0.242

The table 12 shows the results for reproducibility of the T. HCl in buffer pH 7.0 solvent (2-10µg/ml). Maximum reproducibility was obtained at two wavelength i.e 209nm and 269nm.

Table 12: Reproducibility of T. HCl of the buffer pH 7.0 solvent

S.No.	Selected Wavelengths	Concentration(µg/ml)					Remarks	Reproducibility
		2	4	6	8	10		
1.	201.00		✓				1	Not considered
2.	209.00	✓	✓	✓	✓	✓	5	considered
3.	212.00	✓	✓				2	Not considered
4.	215.00	✓	✓	✓	✓		4	considered
5.	232.00	✓					1	Not considered
6.	235.00		✓				1	Not considered
7.	269.00	✓	✓	✓	✓	✓	5	considered

✓ - not considered

✓ ✓ - not considered

✓ ✓ ✓ - not considered

✓ ✓ ✓ ✓ - can be considered

✓ ✓ ✓ ✓ ✓ - considered

The table 13 shows the results for linearity of the T. HCl in buffer pH 7.0 solvent (2-10µg/ml). Results of linearity indicate that maximum linearity was attained only at the two wavelengths i.e 209nm and 269nm. Hence buffer pH 7.0 solvent does produce linearity and reproducibility at two wavelengths: 209nm for ionized form and 269nm for unionized form. So, this solvent was selected.

Table 13: Linearity of T. HCl of the buffer pH 7.0 solvent

S. No.	Selected Wavelengths	Concentration(µg/ml)					Linearity
		2	4	6	8	10	
1.	201.00		0.295				Not Applicable
2.	209.00	0.094	0.104	0.147	0.198	0.242	Applicable
3.	212.00	0.058	0.109				Not Applicable
4.	215.00	0.065	0.111	0.155	0.209		Not Applicable
5.	232.00	0.063					Not Applicable
6.	235.00		0.041				Not Applicable
7.	269.00	0.131	0.133	0.155	0.157	0.170	Applicable

Molar Absorption Coefficient of Unionized and Ionized form of drug

a) At acidic pH 2.0 (unionized form of drug)

Absorptivity of unionized form of drug at λ_{max} of unionized form of drug i.e 269nm.

From the linearity plot the absorbance for 4µg/ml obtained is 0.087

$ax_1 = 0.087/4*10^{-6}*100 \quad = 217.5$

Absorptivity of unionized form of drug at λ_{max} of ionized form of drug i.e 209nm

$ay_1 = 0.067/4*10^{-6}*100 \quad = 167.5$

b) At basic pH 10.0 (ionized form of drug)

Absorptivity of ionized form of drug at λ_{max} of unionized form of drug i.e 269nm

$ax_2 = 0.035/4*10^{-6}*100 = 87.5$

Absorptivity of ionized form of drug at λ_{max} of ionized form of drug i.e 209nm

$ay_2 = 0.146/4*10^{-6}*100 = 365$

Concentration of Unionized and Ionized form of drug

By using various absorbance values, absorptivity values and simultaneous equation the concentration of ionized and unionized form of drug was determined.

$0.133 = 217.5C_x + 87.5C_y$

$0.104 = 167.5C_x + 365C_y$

$217.5\ C_x + 87.5C_y = 0.133$

$87.5C_y = 0.133 – 217.5\ C_x$

$C_y = (0.133 – 217.5\ C_x)/87.5$

$167.5C_x + 365[(0.133 – 217.5\ C_x)/87.5] = 0.104\ C_x = 0.00061$

Put this value of C_x in eq.1

$C_y = (0.133 – 217.5*0.00061)/87.5\ C_y = 0.000003$

Now, the values of C_x and C_y in moles/lit

C_x = 0.00061 * 1000 / molecular weight of unionized form of drug *100 $= 2*10^{-5}$ mol/lit

C_y = 0.000003 * 1000 / molecular weight of ionized form of drug *100 $= 10^{-7}$ mol/lit

Determination of pKa

By using Handerson-Haselbalch equation the pKa was determined.

pKa = pH - log(ionized/unionized)

$= 7 – \log (10^{-7}/2*10^{-5})$

$= 7 – \log (0.005) = 9.3$

The obtained value for pKa of Tramadol hydrochloride was 9.3 and the theoretically calculated or value obtained from literature review was found to be 9.4. So the accuracy of the method was found to be 98.9%.

METHOD VALIDATION FOR pKa

The developed method of determining pKa was validated by determining SD, RSD for accuracy, precision, reproducibility, linearity, ruggedness and robustness, LOD, LOQ and specificity.

Accuracy

Five replicate dilutions of 4µg/ml were prepared with buffer pH 7.0 from the stock solution these dilutions were tested at 209nm and 269nm wavelengths. From the absorbance value the pKa, S.D and RSD was calculated for each dilution. (Table 14)

Table 14: Accuracy for pKa

S.No.	Absorbance (4µg/ml)		pKa	SD	RSD
	At Unionized λ_{max}(269nm)	At Ionized λ_{max}(209nm)			
1.	0.131	0.101	9.0	0.057	0.0062
2.	0.132	0.103	9.2		
3.	0.131	0.105	9.3		
4.	0.130	0.102	9.1		
5.	0.129	0.105	9.2		

Limit of SD and RSD for accuracy is 5, hence accuracy is acceptable.

Precision

For determination of precision a single dilution was tested.

System precision

A single dilution of 4µg/ml was prepared with pH 7.0 buffer from the stock solution; this dilution was tested 5 times at 209nm and 269nm wavelengths. From the absorbance value pKa, SD & RSD were calculated. (Table 15)

Table 15: System Precision for pKa

S.No.	Absorbance (4µg/ml)		pKa	SD	RSD
	At Unionized λ_{max}(269nm)	At Ionized λ_{max}(209nm)			
1.	0.133	0.104	9.3	0.025	0.0027
2.	0.131	0.105	9.3		
3.	0.134	0.102	9.2		
4.	0.134	0.103	9.3		
5.	0.131	0.103	9.2		

Limit of SD and RSD for system precision is 5, hence it is acceptable.

Method Precision

Five replicate dilutions of 4µg/ml were prepared with buffer pH 7.0 from the stock solution these dilutions were tested at 209nm and 269nm wavelengths. From the absorbance value the pKa, S.D and RSD was calculated for each dilution.(Table 16)

Table 16: Method Precision for pKa

S.No.	Absorbance (4µg/ml)		pKa	SD	RSD
	At Unionized λ_{max}(269nm)	At Ionized λ_{max}(209nm)			
1.	0.134	0.102	9.2	0.09	0.009
2.	0.130	0.101	8.9		
3.	0.128	0.097	8.8		
4.	0.133	0.102	9.2		
5.	0.131	0.100	9.0		

Limit of SD and RSD for method precision is 5, hence it is acceptable.

Linearity

Several serial dilutions ranges from 2-100 µg/ml were prepared with pH 7.0 buffer from the stock solution; these dilutions were tested at 209nm and 269nm wavelengths. From the absorbance value the slope, intercept and coefficient of correlation was calculated. Based on linear regression analysis, the responses at unionized λmax 269nm and at ionized λmax 209nm is related to concentration ranges were linear. Figure 8, 9 and table 17.

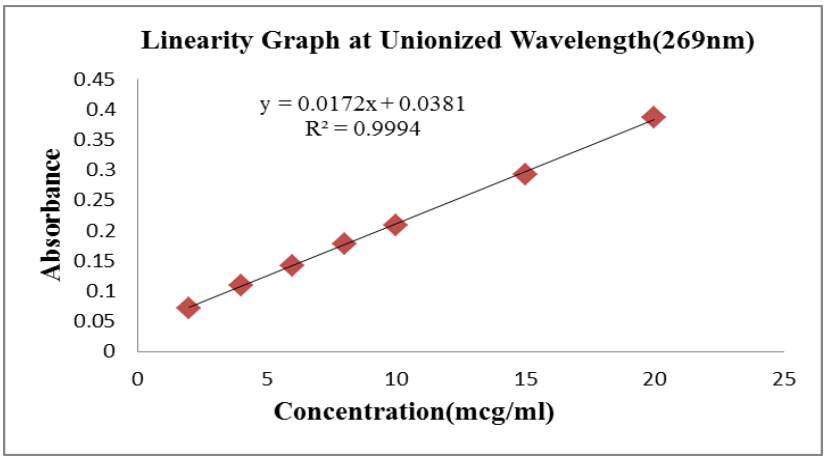

Figure 8: Linearity Graph for Unionized form of drug (λ_{max}269nm)

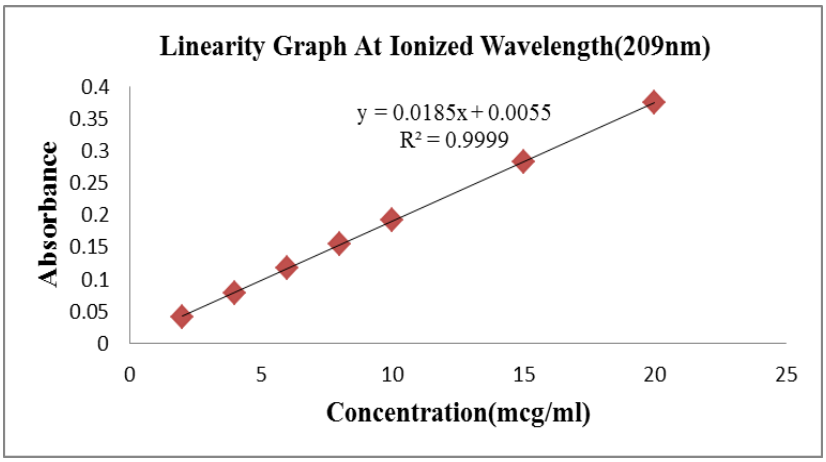

Figure 9: Linearity Graph for Ionized form of drug (λ_{max}209nm)

Table 17: Results of Linearity for pKa

Wavelength	Regression Equation	Slope	Intercept	Coefficient of Correlation
269nm	y = 0.017x+0.038	0.017	0.038	0.999
209nm	y = 0.018x+0.005	0.018	0.005	0.999

Coefficient of Correlation for linearity should be close to 1 and the obtained value for r^2 is 0.999, hence it is acceptable.

Specificity

Different dilutions ranges from 2-10 µg/ml were prepared with buffer pH 7.0 from the stock solution these dilutions were tested at 209nm and 269nm wavelengths. From the absorbance value, the pKa was calculated. A graph was plotted between the pKa and concentration of dilutions. (Figure 10)

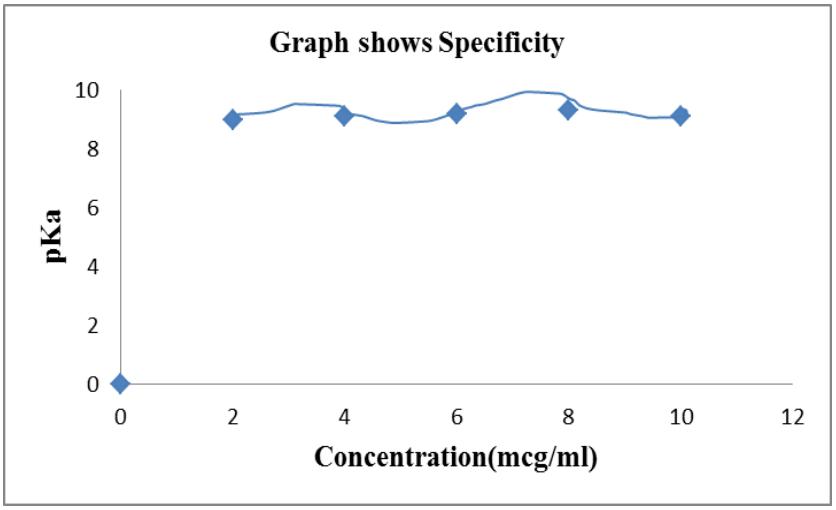

Figure 10: Results of Specificity for pKa

The pKa calculated at various concentrations should be in range when compared with corresponding standard.

LOD and LOQ

Several serial dilutions ranges from 2-10 µg/ml were prepared with buffer pH 7.0 from the stock solution these dilutions were tested at 209nm and 269nm wavelengths. From the absorbance value the slope, standard deviation, LOD and LOQ was calculated. (Table 18).

Table 18: LOD and LOQ for pKa

Wavelength	LOD	LOD
209nm	0.24µg/ml	0.72µg/ml
269nm	0.27µg/ml	0.81µg/ml

Robustness and Ruggedness

Robustness of the method was determined by varying the pH of the phosphate buffer. Dilutions were prepared with buffer of pH 6.8, 7.0 and 7.2. From the absorbance values pKa, S.D and RSD was calculated. Ruggedness of the method was determined by varying the parameters i.e. interday, intraday and inter analysts. Dilutions were prepared with buffer pH 7.0 at different parameters. From the absorbance values pKa, S.D and RSD was calculated. (Table 19)

Table 19: Robustness and Ruggedness for pKa

S.No.	Validation Steps	SD	RSD
1.	Robustness	0.04	0.0043
2.	Ruggedness	0.053	0.0056

CONCLUSION

The proposed spectrophotometric method was found to be simple, sensitive, accurate, precise and economic method for determination pKa (i.e acid dissociation constant) of Tramadol hydrochloride. Using absorbance values, absorptivity values and simultaneous equation the concentration of ionized and unionized form of drug was determined and using Handerson-hasselbalch equation the pKa was found to be 9.3. The method utilizes easily available and cheap solvent for analysis hence the method was also economic. Method was further validated for accuracy, precision, reproducibility, linearity, LOD, LOQ and robustness & ruggedness. Hence the method could be conveniently adopted for routine analysis in quality control laboratories.

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