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Original Article

UV SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF TRAMADOL HYDROCHLORIDE AND ACECLOFENAC IN BULK AND TABLET DOSAGE FORM

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ABSTRACT

Objective: To develop a simple, economic and validated UV spectrophotometric method for the simultaneous estimation of aceclofenac and tramadol hydrochloride in bulk and tablet dosage form.

Methods: The UV spectrophotometric method for simultaneous estimation of aceclofenac and tramadol hydrochloride has been developed. Methanol and water in the ratio 60:40 was used as the solvent. Tramadol hydrochloride and aceclofenac showed maximum absorbance at wavelength 214.8 nm and 275.6 nm respectively.

Results: The developed method showed tramadol hydrochloride and aceclofenac to be linear in the concentration range of 5–30 µg/ml with a correlation coefficient of 0.998 and 0.999 respectively. The result of recovery studies for the tablet was found to be in the range of 98.125%-101.0% and 98.01%-98.36% for tramadol hydrochloride and aceclofenac respectively.

Conclusion: The results show that the developed UV spectrophotometric method is simple, economical, accurate, precise and repeatable.

Keywords: UV spectrophotometric method, Tramadol Hydrochloride, Aceclofenac, Simultaneous estimation, Tablet dosage form, Validation

INTRODUCTION

Tramadol is an opioid pain medication which is used for moderate to moderately severe pain [1]. Tramadol is chemically known as (1R, 2R)-2-[(dimethylamino) methyl]-1-(3-methoxyphenyl) cyclohexan-1-ol (fig. 1) [2, 3].

Following parenteral administration, the analgesic potency of tramadol is about 10% of that of morphine. Postoperative pain relief provided by tramadol is comparable with that of pethidine. The combination with a non-opioid analgesic can further improve the analgesic efficacy of tramadol. Tramadol may prove particularly useful in patients with a risk of poor cardiopulmonary function when non-opioid analgesics are contraindicated and after surgery of the upper abdomen or thorax. Tramadol is a well tolerated and effective agent to reduce pain resulting from trauma, labor, and renal or biliary colic, and also for the management of chronic pain of nonmalignant or malignant origin, particularly neuropathic pain. Tramadol appears to produce less dependence and constipation than equianalgesic doses of strong opioids [4].

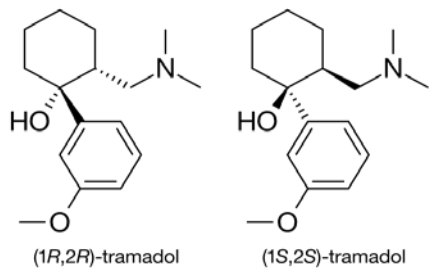

Fig. 1: Chemical structure of tramadol hydrochloride

Aceclofenac is a non-steroidal anti-inflammatory drug (NSAID) analog of Diclofenac [5]. It is chemically known as 2-[2-[2-(2,6-dichloroanilino) phenyl] acetyl] oxyacetic acid (fig. 2) [6]. It is prescribed for the relief of inflammation and pain in ankylosing spondylitis, osteoarthritis and rheumatoid arthritis [7].

It should not be given to breastfeeding mothers or people with porphyria, and is not recommended for children.

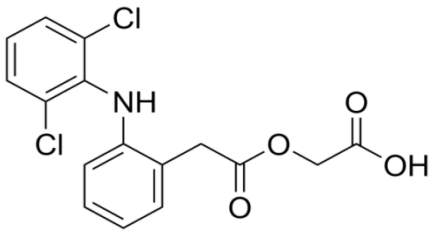

Fig. 2: Chemical structure of aceclofenac

The literature survey revealed that many analytical methods like UV spectrophotometry [8-14], Visible spectrometry [15-22], Spectro fluorimetry [23], HPTLC [24-26], UPLC [27], HPLC [28-31], GC-MS [32] and Cyclic Voltammetry [33] were reported for the estimation of tramadol hydrochloride and methods like UV spectrometry [34-42], HPLC [43-45] and HPTLC [46-48] were reported for the estimation of aceclofenac in bulk drug and pharmaceutical dosage form. However, only one UV method was reported for the estimation of aceclofenac and tramadol hydrochloride in combined dosage form by Second order Derivative Method [49]. Hence, we here report a new, simple and economical method for the simultaneous estimation of aceclofenac and tramadol hydrochloride by using Simultaneous Equation method [50].

MATERIALS AND METHODS

Materials

Instrument

A UV-visible spectrophotometer (Shimadzu, Model No: 1800) with 1 cm light path and loaded with UV probe software (version 2.43) was used for recording of spectra and measurement of absorbance. An electronic weighing balance (1 mg sensitivity, Contech CA 123) and a sonicator (Expo-Hi-tech, Sr. No. 2K810011) were used.

Chemicals and reagents

Analytically pure sample of tramadol hydrochloride and aceclofenac were obtained from Yarrow Chem, Mumbai, India and the tablet formulation (TAXIDOL) was purchased from local markets with labeled amount 37.5 mg of tramadol hydrochloride and 100 mg of aceclofenac. Methanol (AR Grade) was obtained from Thomas Baker, Mumbai.

Methods

Solvent

A solvent used was prepared by adding methanol and water in the ration of 60:40.

Selection of suitable wavelength for detection

Suitable wavelength was obtained by recording UV spectra in the range of 200–400 nm for tramadol hydrochloride and aceclofenac respectively. The wavelength of maximum absorbance for tramadol hydrochloride and aceclofenac was found to be 214.8 nm and 275.6 nm respectively.

Preparation of stock and working standard solution

100 mg of tramadol hydrochloride working standard and 100 mg of aceclofenac working standard were weighed accurately in a 100 ml volumetric flask respectively. 80 ml of solvent was added, sonicated to dissolve and diluted to volume with the solvent. Further, 1 ml of this solution was diluted to 10 ml, respectively with the solvent. Then, 0.94 ml of tramadol hydrochloride and 2.5 ml of aceclofenac solution were diluted to 10 ml, respectively, with the solvent to get a final concentration of 9.4 µg/ml and 25 µg/ml respectively.

Preparation of stock and working sample solution

20 tablets were weighed and powdered. The quantity of powder containing the equivalent of about 37.5 mg of tramadol hydrochloride and 100 mg of aceclofenac was weighed accurately into a 100 ml volumetric flask. 50 ml of the solvent was added, sonicated for 20 min with intermediate shaking, diluted up to the mark with the solvent and mixed, and filtered through 0.45 µ PVDF filter. Further, 1 ml of this solution was diluted to 10 ml with the solvent. Then, 2.5 ml of this solution was diluted to 10 ml with the solvent to get a final concentration of 9.4 µg/ml and 25 µg/ml for tramadol hydrochloride and aceclofenac respectively.

Validation of methods [51]

Method validation is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Results from method validation can be used to evaluate the quality, consistency, and reliability of analytical results.

The method was validated for parameters like accuracy, linearity, interday precision, intraday precision, robustness, ruggedness, limit of detection (LOD) and limit of quantification (LOQ).

Linearity

The linearity of tramadol hydrochloride and aceclofenac was performed by using the standard solutions in the concentration range of 5–30 µg/ml. Calibration curve was acquired by plotting concentration versus absorbance at 214.8 nm for tramadol hydrochloride and at 275.6 nm for aceclofenac. The correlation coefficients were greater than 0.995.

Intraday precision

Six similar recordings of absorbance at 214.8 nm and 275.6 nm of a standard solution of tramadol hydrochloride and aceclofenac respectively at working concentration were obtained on the same day. The results showed % Relative Standard Deviation (% RSD) less than 2%.

Interday precision

Six similar recordings of absorbance at 214.8 nm and 275.6 nm of a standard solution of tramadol hydrochloride and aceclofenac respectively at working concentration were obtained on three consecutive days. The results showed % RSD less than 2%.

Accuracy

Three different determinations of absorbance at three different levels (80%, 100% and 120%) of a standard solution of tramadol hydrochloride and aceclofenac respectively at 214.8 nm and 275.6 nm were performed. Similarly, three different determinations of absorbance at three different levels (80%, 100% and 120%) of a sample solution containing both tramadol hydrochloride and aceclofenac at 214.8 nm and 275.6 nm were performed. The concentration of tramadol hydrochloride and aceclofenac in the sample solution was determined by substituting the absorbance values in the following equation.

$$C_x = \frac{A_{2a_{y1}} - A_{1a_{y2}}}{a_{x2}a_{y1} - a_{x1}a_{y2}} \qquad C_y = \frac{A_{1a_{x2}} - A_{2a_{x1}}}{a_{x2}a_{y1} - a_{x1}a_{y2}}$$

C_x = concentration of Tramadol Hydrochloride A_1 = absorbance of samples at 214.8 nm

C_y = concentration of Aceclofenac A_2 = absorbance of samples at 275.6 nm

The experiment was repeated thrice.

Robustness

Robustness of the method was evaluated by changing the wavelength by±2 nm. % RSD obtained was less than 2%.

Ruggedness

The ruggedness of the method was verified by analyzing six times the standard solution of tramadol hydrochloride and aceclofenac at working concentrations by a different analyst using different instruments, but operational and environmental conditions were maintained the same. From the results obtained, % RSD was found to be less than 2%.

Limit of detection (LOD) and Limit of quantification (LOQ)

The LOD and LOQ were calculated based on the calibration curves using the following formula.

$$\text{LOD} = 3.3 \times \frac{\sigma}{S} \quad \text{LOQ} = 10 \times \frac{\sigma}{S}$$

Where, σ = y-intercept S = slope of the regression line

RESULTS AND DISCUSSION

A simple, economical, precise and accurate method has been proposed in the article, for simultaneous estimation of tramadol hydrochloride and aceclofenac in bulk and tablet dosage form. Tramadol hydrochloride and aceclofenac showed maximum absorbance at wavelength 214.8 nm and 275.6 nm respectively. Hence, it was selected as the wavelength suitable for detection (fig. 3). The developed method was used for the simultaneous estimation of tramadol hydrochloride and aceclofenac in bulk and tablet dosage form. Solution stability was performed at different intervals using the same solution, and it was found that the solution is stable up to 7 h. Tramadol hydrochloride and aceclofenac were found to be linear in the concentration range of 5–30 µg/ml with a correlation coefficient of 0.998 and 0.999 respectively, the Beer's law was obeyed (table 1) (fig. 4 and 5). % Recovery study was performed in order to check the accuracy of the method, at three different concentrations (80%, 100% and 120%) of the standard drugs.

The result of recovery studies for the tablet was found to be in the range of 98.125%-101.0% and 98.01%-98.36% for tramadol hydrochloride and aceclofenac respectively (table 2). % RSD for intraday and interday precision were found to be less than 2%, which shows that the method is precise (table 3, 4, 5, 6). The LOD for tramadol hydrochloride and aceclofenac was found to be 2.412 µg/ml and 0.244 µg/ml respectively. The LOQ for tramadol hydrochloride and aceclofenac was found to be 7.308 µg/ml and 0.741 µg/ml respectively. The values showed that the method is sensitive and thus can be used for the determination of drugs, even

with lower concentrations (table 7). Robustness and ruggedness of the method were checked, and the % RSD was found to be less than 2%, which indicate that the method can be suitably used for estimation of tramadol hydrochloride and aceclofenac in combination and parameters like difference in days, instruments or analyst won't affect them (table 8, 9, 10). Optical characteristics and validation parameters of tramadol hydrochloride and aceclofenac are shown in table 11.

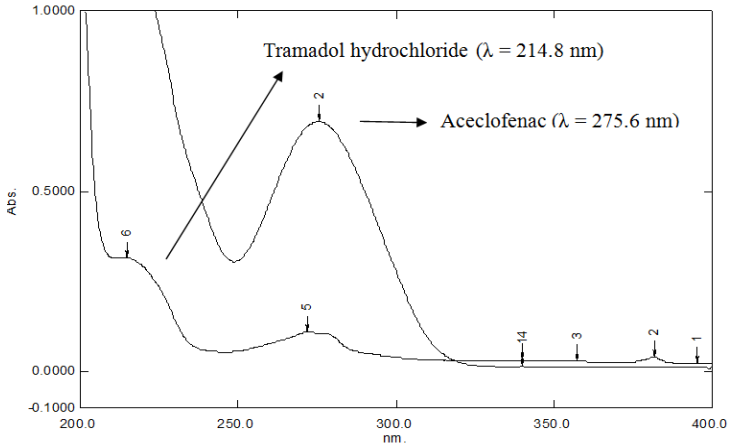

Fig. 3: Overlain spectra of tramadol hydrochloride (9.4 µg/ml) and aceclofenac (25 µg/ml) in the solvent

Table 1: Calibration data for tramadol hydrochloride and aceclofenac

S. No.	Concentration (µg/ml)	Absorbance of tramadol hydrochloride at 214.8 nm	Absorbance of aceclofenac at 275.6 nm
1	5	0.1645	0.1365
2	10	0.2974	0.2727
3	15	0.4082	0.4040
4	20	0.5588	0.5296
5	25	0.6783	0.6930
6	30	0.8028	0.8222

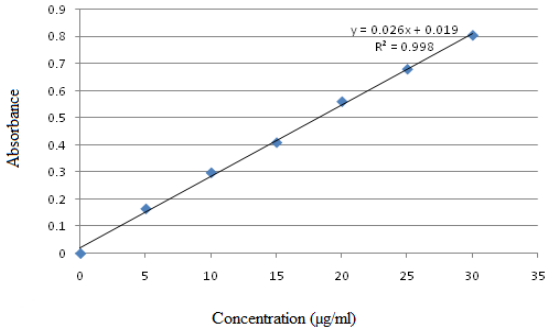

Fig. 4: Calibration curve of tramadol hydrochloride at 214.8 nm

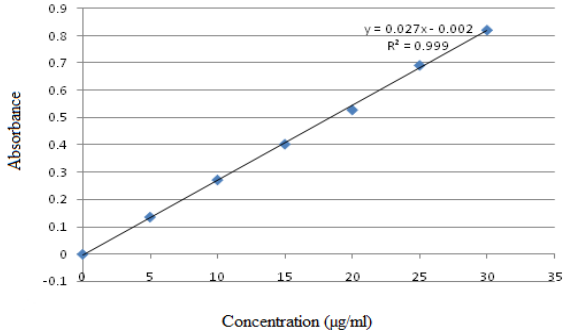

Fig. 5: Calibration curve of aceclofenac at 275.6 nm

Table 2: Result of accuracy for tramadol hydrochloride and aceclofenac

Concentration level	% Mean recovery of tramadol hydrochloride (n=3)	% Mean recovery of aceclofenac (n=3)
80%	99.95%	98.01%
100%	101.0%	98.36%
120%	98.125%	98.17%

Table 3: Results for intraday precision of tramadol hydrochloride

S. No.	Concentration	Absorbance at 214.8 nm
1		0.3148
2		0.3147
3		0.3147
4	9.4 µg/ml	0.3148
5		0.3148
6		0.3146
Mean*		0.314733
SD**		0.00008
% RSD***		0.025

* mean of six determinations ** standard deviation *** % relative standard deviation

Table 4: Results for intraday precision of aceclofenac

S. No.	Concentration	Absorbance at 275.6 nm
1		0.6930
2		0.6930
3		0.6929
4	25 µg/ml	0.6928
5		0.6929
6		0.6927
Mean*		0.692883
SD**		0.000117
% RSD***		0.0169

* mean of six determinations ** standard deviation *** % relative standard deviation

Table 5: Results for interday precision of tramadol hydrochloride

S. No.	Concentration	Absorbance at 214.8 nm		
		Day 1	Day 2	Day 3
1		0.3148	0.3146	0.3147
2		0.3148	0.3146	0.3146
3		0.3147	0.3144	0.3148
4	9.4 µg/ml	0.3146	0.3145	0.3147
5		0.3147	0.3145	0.3145
6		0.3145	0.3144	0.3147
Mean*		0.314683	0.3145	0.31467
SD**		0.000117	0.00008	0.00010328
%RSD***		0.037	0.025	0.033

* mean of six determinations ** standard deviation *** % relative standard deviation

Table 6: Results for interday precision of aceclofenac

S. No.	Concentration	Absorbance at 275.6 nm		
		Day 1	Day 2	Day 3
1		0.6930	0.6927	0.6925
2		0.6929	0.6928	0.6926
3		0.6929	0.6928	0.6928
4	25 µg/ml	0.6930	0.6926	0.6928
5		0.6930	0.6927	0.6926
6		0.6928	0.6927	0.6928
Mean*		0.69293	0.6927167	0.692683
SD**		0.00008	0.00007	0.000132916
%RSD***		0.012	0.010	0.019

* mean of six determinations ** standard deviation *** % relative standard deviation

Table 7: Results of LOD and LOQ

Active Ingredient	LOD (µg/ml)	LOQ (µg/ml)
Tramadol Hydrochloride	2.412	7.308
Aceclofenac	0.244	0.741

Table 8: Robustness result of tramadol hydrochloride (variation parameter: wavelength±2 nm)

S. No.	Absorbance at 212.8 nm	Absorbance at 216.8 nm
1	0.3144	0.3148
2	0.3143	0.3150
3	0.3144	0.3149
4	0.3143	0.3148
5	0.3143	0.3149
6	0.3145	0.3149
Mean*	0.314367	0.314883
SD**	0.00008	0.00007
% RSD***	0.025	0.022

* mean of six determinations ** standard deviation *** % relative standard deviation

Table 9: Robustness result of aceclofenac (variation parameter: wavelength±2 nm)

S. No.	Absorbance at 273.6 nm	Absorbance at 277.6 nm
1	0.6925	0.6926
2	0.6924	0.6924
3	0.6924	0.6926
4	0.6926	0.6925
5	0.6925	0.6925
6	0.6925	0.6924
Mean*	0.692483	0.6925
SD**	0.00007	0.00009
% RSD***	0.010	0.013

* mean of six determinations ** standard deviation *** % relative standard deviation

Table 10: Data of ruggedness of tramadol hydrochloride (9.4 µg/ml) and aceclofenac (25 µg/ml)

S. No.	Absorbance of tramadol hydrochloride at 214.8 nm				Absorbance of aceclofenac at 275.6 nm			
	Instrument 1	Instrument 2	Analyst 1	Analyst 2	Instrument 1	Instrument 2	Analyst 1	Analyst 2
1	0.3148	0.3138	0.3148	0.3105	0.6930	0.7034	0.6975	0.7133
2	0.3129	0.3008	0.3149	0.3148	0.6859	0.7231	0.6853	0.6936
3	0.3128	0.3038	0.3108	0.3149	0.7034	0.6922	0.6911	0.6924
4	0.3149	0.3048	0.3138	0.3148	0.6872	0.6877	0.7126	0.7140
5	0.3048	0.3140	0.3148	0.3104	0.6918	0.7018	0.7002	0.7202
6	0.3108	0.3114	0.3148	0.3128	0.6928	0.6935	0.6998	0.6951
Mean *	0.3118	0.3081	0.3140	0.3130	0.6924	0.7003	0.6978	0.7048
SD**	0.0038	0.0057	0.0016	0.0022	0.0062	0.0127	0.0093	0.0124
%RSD***	1.21	1.85	0.51	0.703	0.895	1.81	1.33	1.76

* mean of six determinations ** standard deviation *** % relative standard deviation

Table 11: Optical characteristics and validation parameters of tramadol hydrochloride and aceclofenac

Parameters	Results of tramadol hydrochloride	Results of aceclofenac
Detection wavelength	214.8 nm	275.6 nm
Beer's law limits	5–30 µg/ml	5–30 µg/ml
Regression equation (y = mx+c)	y = 0.026x+0.019	y = 0.027x–0.002
Correlation coefficient (r^2)	0.998	0.999
Slope (m)	0.026	0.027
Intercept (c)	0.019	0.002
Intraday precision (% RSD)	0.025	0.0169
Interday precision (% RSD)	<2	<2
Accuracy (% Recovery)		
80%	99.95%	98.01%
100%	101.0%	98.36%
120%	98.125%	98.17%
LOD	2.412 µg/ml	0.244 µg/ml
LOQ	7.308 µg/ml	0.741 µg/ml
Robustness		
Wavelength (±2 nm) (% RSD)	<2	<2
Ruggedness (Using different instrument and different analyst) (% RSD)	<2	<2

The results obtained prove that the method is useful for simultaneous estimation of tramadol hydrochloride and aceclofenac in bulk and tablet dosage form. The developed method used very economical solvent and hence can be performed easily. Simultaneous estimation of the drugs can be achieved by various techniques like derivative spectroscopy or absorbance correction method, but simultaneous equation method was found to be easily performable for this combination of drugs as both the drug shows absorbance at each other's wavelength of maximum absorbance. Estimation of tramadol hydrochloride and aceclofenac can thus be economically and simply done by simultaneous equation method.

CONCLUSION

The results indicate that the developed UV spectrophotometric method is simple, economical, precise and accurate. The developed method was found to be suitable for determination of tramadol hydrochloride and aceclofenac as a bulk drug and in marketed tablet dosage form without the interference of excipients. Statistical analysis proves that the method was repeatable. Hence, this method can be used for the simultaneous estimation of tramadol hydrochloride and aceclofenac in bulk and tablet dosage form.

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CONFLICT OF INTERESTS

Declared none

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