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Research Article

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Development and validation of first order derivative spectrophotometric method for simultaneous estimation of tramadol hydrochloride and diclofenac sodium in bulk and synthetic mixture

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ABSTRACT

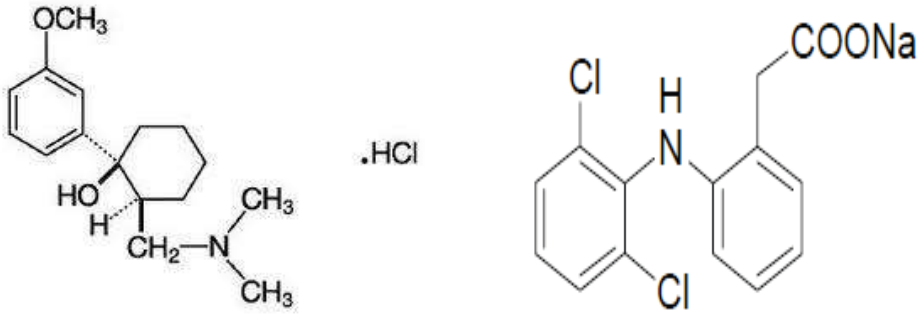
A simple, precise, accurate and reproducible spectrophotometric method has been developed for simultaneous estimation of Tramadol hydrochloride (TRA) and Diclofenac Sodium (DIC) by employing first order derivative method by using methanol as solvent. The first order derivative absorption at 251.13 nm (zero cross point of Diclofenac sodium) was used for quantification of Tramadol hydrochloride and 273.75 nm (zero cross point of Tramadol) for quantification of Diclofenac sodium. The linearity was established over the concentration range of 5-25 µg/ml in and 5-45µg/ml for Tramadol and Diclofenac sodium respectively. The correlation coefficient (R^2) for Tramadol 0.9998 and for Diclofenac sodium 0.9991 .The mean % recovery was found to be in range of 99.00% and 99.68% for TRA and DIC, respectively. The proposed method fulfils the validation parameter as per ICH guidelines and was successfully applied to the estimation of TRA and DIC in bulk as well as in synthetic mixture.

Key words: Tramadol Hydrochloride, Diclofenac Sodium, Spectroscopy, First order derivative, Validation.

INTRODUCTION

Tramadol Hydrochloride (TRA) is chemically cis 2[(dimethylamino) methyl]-1-(3 methoxyphenyl) cyclohexanol hydrochloride (Figure 1A). TRA is synthetic centrally acting opioid analgesics and used in treatment of moderate or severe acute or chronic pain.

Diclofenac sodium (DIC) is phenyl acetic acid derivative, chemically, 2{2[(2,6dichlorophenyl) amino] phenyl} acetic acid (Figure 1B). DIC is used in treatment of acute and chronic pain. It is also used for the treatment of sign and symptoms of osteoarthritis and rheumatoid arthritis. Tramadol Hydrochloride and Diclofenac sodium both are official in I.P., B.P. and U.S.P [1, 2, 3].



[A] Tramadol hydrochloride [B] Diclofenac sodium
Figure 1: Chemical Structure of Tramadol HCL (A), Diclofenac Sodium (B)

The literature survey indicates that various analytical methods involving spectrophotometry, TLC, HPLC, HPTLC, UPLC and Dual wavelength have been reported for TRA in single and in combination with other drugs [5-10]. Several analytical methods have been reported for DIC in single and in combination with other drugs involving UV spectroscopy, HPLC, LC-MS, RP-HPLC [11-15]. The literature review prompted to develop an accurate, precise and simple simultaneous method for the estimation of TRD and DIC in bulk form. The work was extending to synthetic mixture and shows no interference with the excipients.

EXPERIMENTAL SECTION

2.1 Reagents and Chemicals

Analytically pure Tramadol hydrochloride and Diclofenac sodium were used. Tramadol hydrochloride was procured from Comed chemical Ltd, Baroda, Gujarat, India. All the reagents used in method development were Analytical Grade from SRL.

2.2 Instruments

A shimadzu UV/Vis 1800 double beam spectrophotometer is used with wavelength accuracy (± 0.3 nm), 1 cm matched quartz cells and UV probe 2.35 software was used for all the spectral measurements. Calibrated analytical Balance Denver SI234, Germany, was used for weighing purpose.

2.3 Preparation of standard stock solutions

Accurately weighed 10 mg of TRA and DIC were transferred into separate 100 ml volumetric flask and dissolved in methanol and dilute upto the mark with methanol to get stock solutions containing 100 µg/ml TRA and 100 µg/ml DIC.

2.4 Selection of Analytical Wavelength

Solutions of TRA and DIC were prepared in methanol by appropriate dilution and spectrum was recorded between 200 – 400 nm. All zero order spectrums (D^0) were converted to first derivative spectrum (D^1) using delta lambda 10 and scaling factor 1.0. The zero crossing point (ZCP) were found to be 251.13 nm of DIC and 273.75 nm of TRD.

2.5 Method validation

The proposed method was validated according to the ICH Guideline Q2 (R1). The method has been validated in terms of Linearity, Precision, Accuracy, Limit of detection (LOD) and Limit of quantification (LOQ).

2.5.1 Calibration curve (Linearity)

Appropriate volume of aliquot from TRA and DIC standard stock solution was transferred to volumetric flask of 10 ml capacity. The volume was adjusted to the mark with methanol to give a solution containing 5 – 25 µg/ml TRD and 5 -45 µg/ml DIC. All D1 spectrums were recorded using above spectrophotometric condition. D1 absorbance at 251.13 nm and 273.75 nm were recorded for DIC and TRA respectively. Calibration curve were constructed by plotting average absorbance versus concentration for both drugs.

2.5.2 Accuracy

Accuracy was assessed by determination of the recovery of the method by addition of standard drug to the known amount of marketed formulation at 3 different concentration levels 80, 100, and 120% taking into consideration percentage purity of added bulk drug samples. Each concentration was analysed 3 times and average recoveries were measured.

2.5.3 Precision

The intraday and interday precision study of TRA and DIC was carried out by estimating different concentrations of TRA (5, 15, 25 µg/ml) and DIC (5, 25, 45 µg/ml), three times on the same day and on three different days and the results are reported in terms of % RSD.

2.5.4 Detection limit and Quantitation limit

ICH guideline describes several approaches to determine the detection and quantitation limits. These include visual evaluation, signal-to-noise ratio and the use of standard deviation of the response and the slope of the calibration curve. In the present study, the LOD and LOQ were based on the third approach and were calculated according to the 3.3 σ/S and 10 σ/S criterions, respectively;

Where; σ is the standard deviation of y-intercepts of regression lines and S is the slope of the calibration curve

2.5.5 Robustness

The sample solution was prepared and then analysed with change in typical analytical conditions like stability of analytical solution.

2.6 Analysis of Synthetic Mixture

Unavailability of the marketed formulation in local market, we had prepared the synthetic mixture for applicability of method to the routine analysis of the dosage form. For that as per the ratio of the drug, appropriate drug and excipients were mix together to form a uniform synthetic mixture. Form that mixture equivalent weight 0.2gm powder was taken and dissolves in 50 ml methanol and sonicated for 15 min. The solution was filtered and aliquot was taken to get 100 µg/ml stock solutions. Form that 10 µg/ml for TRD and 15 µg/ml for DIC solution was prepared for the analysis.

RESULTS AND DISCUSSION

A simple, economic, precise, accurate method for estimation of TRA and Diclofenac sodium was developed. This developed method was validated according to ICH guidelines.

For this method, 251.13 nm (Zero crossing point of DIC) and 273.75 nm (zero crossing point of TRD) of first order derivative spectra were selected for the analysis which shown in figure 2. Absorption of DIC at ZCP of TRD & absorption of TRD at ZCP of DIC and was taken.

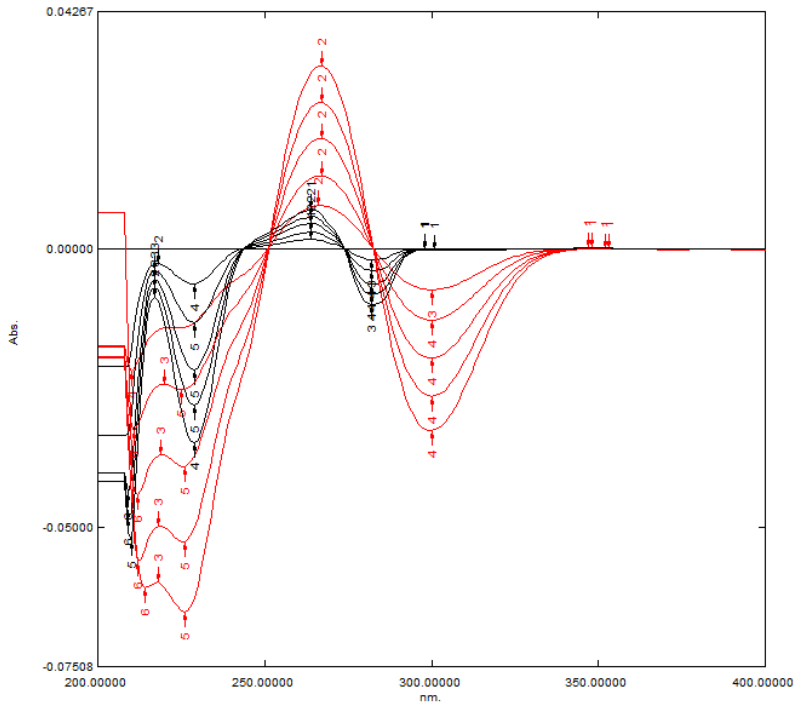


Figure 2: Overlain D^1 spectrum of TRD (5-25 ppm) and DIC (5-45 ppm) in Methanol. ZCP of DIC (151.13 nm) and ZCP of TRD (273.75 nm)

The linearity range of 5-25 µg/ml & 5-45 µg/ml for TRD and DIC was taken respectively. Straight line equations were obtained from mean of five sets and the calibration curves were shown in figure 3 and 4. The Correlation Coefficients (r^2) for TRD and DIC was found to be 0.9998 & 0.9991 respectively.

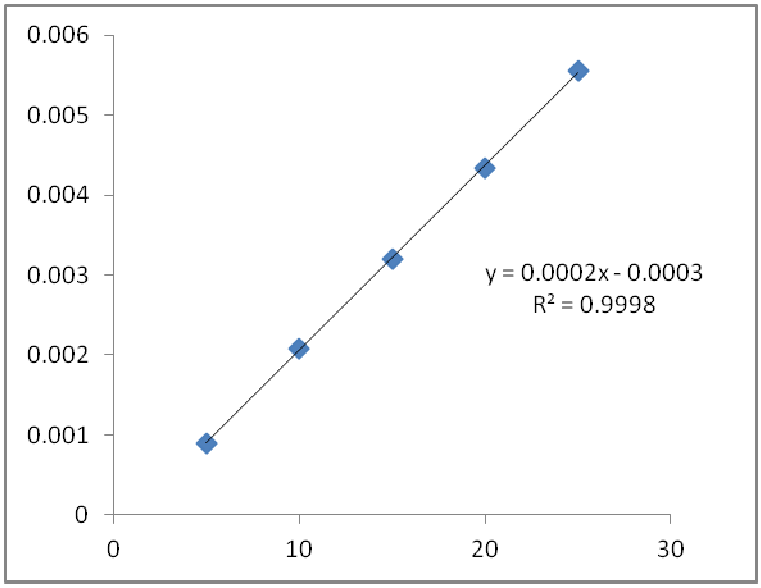


Figure 3: Calibration curve of Tramadol Hydrochloride

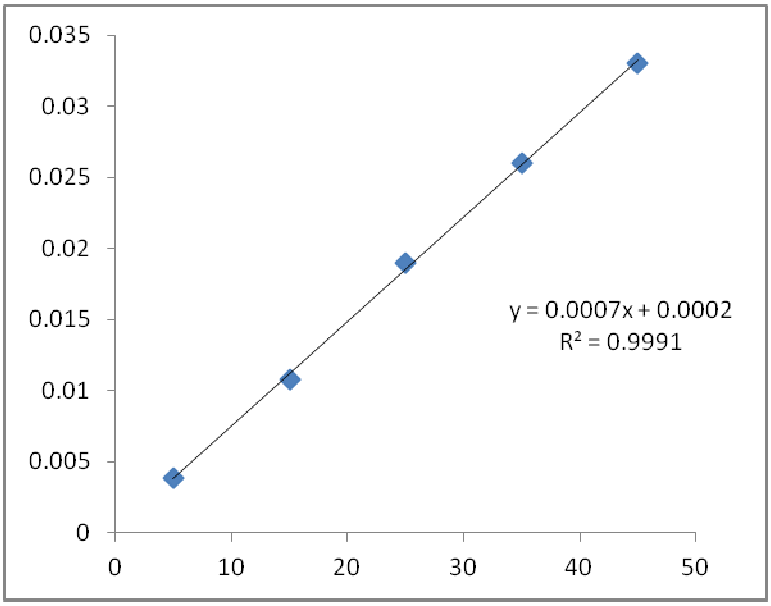


Figure 4: Calibration curve of Diclofenac Sodium

The accuracy of DIC & TRD was carried out as percent recovery shown in Table 1 and average recovery was found 99.85% & 98.43% respectively for DIC and TRD.

Table 1. Results of recovery studies of synthetic mixture

% Level	Amt. of DIC taken in µg/ml	Amt. of TRD taken in µg/ml	Amt. of Std DIC added in µg/ml	Amt.of Std TRD added in µg/ml	% Recovery (DIC)	% Recovery (TRD)	%RSD DIC	%RSD TRD
80%	15	10	12	08	99.47%	98.42%	1.89	0.77
100%	15	10	15	10	99.36%	98.25%	1.66	0.72
120%	15	10	18	12	100.72%	98.63%	1.30	0.99

The intraday precision and interday precision were expressed in terms of relative standard deviation (RSD). For intraday & interday precision, % RSD for TRD and DIC was found to be satisfactory shown in table 2 and 3.

Table 2. Intraday precision

SR NO.	DICLOFENAC SODIUM			TRAMADOL		
	Concentration	Mean absorbance	RSD	Concentration	Mean absorbance	RSD
1	5	0.0036±0.0004	0.9797	5	0.0006±0.0001	0.9865
2	25	0.0176±0.0001	0.8054	15	0.0026±0.0003	1.1152
3	45	0.0314±0.0003	0.9831	25	0.0046±0.0004	0.9394

Table 3. Interday precesion

SR NO.	DICLOFENAC SODIUM			TRAMADOL		
	Concentration	Mean absorbance	RSD	Concentration	Mean absorbance	RSD
1	5	0.0036±0.0004	0.9851	5	0.0006±0.0002	1.3570
2	25	0.0176±0.0001	0.8124	15	0.0026±0.0003	1.1385
3	45	0.0315±0.0003	1.0157	25	0.0046±0.0005	1.0729

The proposed method was evaluated statistically. The LOD was found to be 0.4575 µg/ml and 0.7742 µg/ml for DIC and TRD respectively. The LOQ for DIC 1.38 µg/ml and for TRD 2.34 µg/ml. The summary of validation parameter was shown in table 4.

Table 4. Summary of validation parameters

Parameter	**Diclofenac sodium**	**Tramadol Hydrochloride**
Linearity (µg/ml)	5 – 45 µg/ml	5 – 25 µg/ml
Co-relation coefficient(r^2)	0.9991	0.9998
Slope	0.00073	0.0002
Intercept	0.0002	0.0003
LOD (µg/ml)	0.4575	0.77421
LOQ (µg/ml)	1.3865	2.3461
Precision		
Intraday (n=3) RSD	0.922801	1.013879
Interday (n=3) RSD	0.937801	1.189528

The % assay was found to be 99% for TRD & 99.68% for DIC respectively Table 5. No interference was observed from the Pharmaceutical added excipients in synthetic mixture.

Table 5. Results of analysis of synthetic mixture

Drugs	Label claim (mg)	Amount of drug estimated (mg)	% label claim ± S.D.	% Recovery
DIC	75	74.76	99.68 ± 0.55	99.85%
TRD	50	49.5	99 ± 1.32	98.43 %

CONCLUSION

The proposed First order derivative spectroscopy method provides simple, specific, precise, accurate and reproducible quantitative analysis for simultaneous determination of TRA and DIC in combined dosage form. The method was validated as per ICH guidelines in terms of specificity, linearity, accuracy, precision, limits of detection (LOD) and quantification (LOQ), robustness and reproducibility. The proposed method can be used for routine analysis and quality control assay of TRA and DIC in bulk and synthetic mixture.

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