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Research Article

# DEVELOPMENT AND VALIDATION OF FIRST ORDER DERIVATIVE SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF TRAMADOL HYDROCHLORIDE AND DICLOFENAC SODIUM IN TABLET DOSAGE FORM

**ANKIT PATEL*[1], JAIMIN PATEL[1], AMIT SHAH[2]**

**Department of Quality Assurance, Indubhai Patel College of Pharmacy and Research Centre, Dharmaj, Gujarat, India, Department of Quality Assurance, Indubhai Patel College of Pharmacy and Research Centre, Dharmaj, Gujarat, India, Department of Quality Assurance, Indubhai Patel College of Pharmacy and Research Centre, Dharmaj, Gujarat, India. Email: ankitpatel91289@yahoo.com**

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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 zero crossing method in methanol. The first order derivative absorption at 272.45 nm (zero cross point of TRA) was used for quantification of DIC and 282.81 nm (zero cross point of DIC) for quantification of TRA. The linearity was established over the concentration range of 2-18 µg/ml and 5-25 µg/ml for TRA and DIC with correlation coefficient $r^2$ 0.9941 and 0.9968, respectively. The mean % recoveries were found to be in the range of 99.2 – 99.73 % and 99.33 – 99.64 % for TRA and DIC, respectively. The proposed method has been validated as per ICH guidelines and successfully applied to the estimation of TRA and DIC in their combined Tablet dosage form.

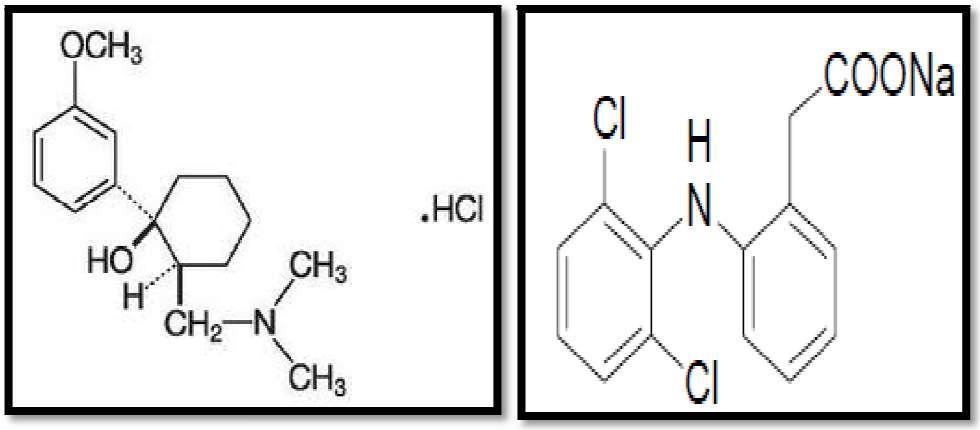
**Keywords:** Tramadol Hydrochloride, Diclofenac Sodium, First order derivative, Analytical Method validation.

## INTRODUCTION

Tramadol Hydrochloride (TRA) is chemically cis – 2 – [(dimethylamino) methyl] – 1 – (3methoxyphenyl) cyclohexanol hydrochloride[1] (Figure – 1a). TRA belongs to analgesic and narcotic category. TRA and its O-desmethyl metabolite (M1) are selective, weak OP3-receptor agonists. Indicated in the treatment of moderate to severe pain. TRA is used to treat postoperative, dental, cancer, and acute musculosketetal pain and as an adjuvant to NSAID therapy in patients with osteoarthritis. DIC is chemically, 2-{2-[(2,6-dichlorophenyl)amino]phenyl}acetic acid[2] (Figure – 1b). DIC is used in acute and chronic treatment of signs and symptoms of osteoarthritis and rheumatoid arthritis.

The review of literature revealed that various analytical methods involving spectrophotometry[3-10], TLC[11], HPLC[12-16], HPTLC[17-19], UPLC[20] have been reported for TRA in single form and in combination with other drugs. Several analytical methods have been reported for DIC in single form and in combination with other drugs including spectrophotometry[22-26], HPLC[27-33],HPTLC[34], LC – MS[35].

The present work describes the development of a simple, precise, accurate and reproducible spectrophotometric method for the simultaneous estimation of TRA and DIC in combined dosage forms. The developed method was validated in accordance with ICH Guidelines[40] and successfully employed for the assay of TRA and DIC combine dosage form.




(a) (b)

**Fig. 1: Chemical structure of (a) TRA and (b) DIC**

## MATERIALS AND METHODS

### Reagents and chemicals

Analytically pure TRA and DIC were kindly provided by Medico Lab, Ahmadabad as gratis samples. Analytical grade methanol was purchased from RFCL limited, New Delhi, India.

### Instruments

Two spectrophotometers were used for study, A Shimadzu UV/Vis 1800 double beam spectrophotometer with a wavelength accuracy (± 0.3 nm), 1 cm matched quartz cells and UV probe 2.32 software was used for all the spectral measurements and Shimadzu UV/Vis 1601 double beam spectrophotometer with a wavelength accuracy (± 0.3 nm) and 1 cm matched quartz cells was used for reproducibility study. Calibrated analytical balance K-EA 210 (K-Roy Instrument Pvt. Ltd) was used for weighing purpose.

### Spectrophotometric condition

All zero order spectrums ($D^0$) were converted to first derivative spectrum ($D^1$) using delta lambda 1.

**Preparation standard stock solutions**

Accurately weighed 100 mg of TRA and DIC standard were transferred to separate 100 ml volumetric flask and dissolved in 50 ml methanol. The flasks were shaken and volume was made up to the mark with methanol to give solutions containing 1000 µg/ml TRA and 1000 µg/ml DIC. From this solution 10 ml was transferred to volumetric flask of 100 ml capacity. Volume was made up to the mark to give a solution containing 100µg/ml of TRA and 100µg/ml DIC.

**Selection of Analytical Wavelength**

2-18 µg/ml solutions of TRA and 5 - 25 µg/ml solutions of DIC were prepared in methanol by appropriate dilution and spectrum was recorded between 200-400 nm and first derivative spectrums were obtained using above condition. The overlain first derivative spectrums of TRA and DIC at different concentration were recorded. The zero crossing point (ZCP) of TRA was found to be 272.45 nm and ZCP of DIC was found to be 282.81 nm.

**Method validation**

The proposed method has been extensively validated in terms of specificity, linearity, accuracy, precision, limits of detection (LOD) and quantification (LOQ), robustness and reproducibility. The accuracy was expressed in terms of percent recovery of the known amount of the standard drugs added to the known amount of the pharmaceutical dosage forms. The precision (Coefficient of Variation - C.V.) was expressed with respect to the repeatability, intra-day and inter-day variation in the expected drug concentrations. After validation, the developed methods have been applied to pharmaceutical dosage form.

**Specificity**

Commonly used excipients (starch, microcrystalline cellulose and magnesium stearate) were spiked into a pre weighed quantity of drugs. The $D^1$ spectrum was recorded by appropriate dilutions and the quantities of drugs were determined.

**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 solutions containing 2-18 µg/ml TRA and 5-25 µg/ml DIC. All $D^1$ Spectrum were recorded using above spectrophotometric condition. $D^1$ absorbance at 282.81 nm and 272.45 nm were recorded for TRA and DIC, respectively (n=6). Calibration curves were constructed by plotting average absorbance versus concentrations for both drugs. Straight line equations were obtained from these calibration curves.

**Accuracy**

Accuracy was assessed by determination of the recovery of the method by addition of standard drug to the pre-quantified placebo preparation at 3 different concentration levels 50, 100 and 150 %, taking into consideration percentage purity of added bulk drug samples. Each concentration was analyzed 3 times and average recoveries were measured.

**Precision**

The repeatability was evaluated by assaying 6 times of sample solution prepared for assay determination. The intraday and interday precision study of TRA and DIC was carried out by estimating different concentrations of TRA (6, 10, 14 µg/ml) and DIC (10, 15, 20 µg/ml), 3 times on the same day and on 3 different days (first, second, fifth) and the results are reported in terms of C.V.

**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 $3.3\sigma/S$ and $10\sigma/S$ criterions, respectively; where σ is the standard deviation of y-intercepts of regression lines and s is the slope of the calibration curve.

**Robustness**

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

**Reproducibility**

The absorbance readings were measured at different laboratory for sample solution using another spectrophotometer by analyst and the values obtained were evaluated using t- test to verify their reproducibility.

**Determination of Tramadol Hydrochloride and Diclofenac Sodium in their Combined Dosage**

**Sample preparation**

A powder quantity equivalent to 50 mg TRA and 75 mg DIC was accurately weighed and transferred to volumetric flask of 100 ml capacity. 60 ml of methanol was transferred to this volumetric flask and sonicated for 15 min. The flask was shaken and volume was made up to the mark with methanol. The above solution was filtered through Whattman filter paper (0.45µ). From this solution 2 ml was transferred to volumetric flask of 100 ml capacity. Volume was made up to the mark to give a solution containing 10µg/ml of TRA and 15µg/ml of DIC. The resulting solution was analyzed by proposed method. The quantitation was carried out by keeping these values to the straight line equation of calibration curve.

**RESULTS AND DISCUSSION**

First order derivative spectrophotometric method was developed for determination of TRA and DIC. The proposed method has been extensively validated as per ICH guidelines. Summary of validation parameters for proposed method was given in Table 1.

The overlain $D^1$ spectrum of TRA and DIC at different concentrations revealed that at 282.81 nm (ZCP of DIC) TRA possesses significant $D^1$ absorbance and at 272.45 nm DIC possesses significant $D^1$ absorbance. Considering above facts, wavelength 282.81 nm and 272.45 nm were selected for the estimation of TRA and DIC, respectively (figure 2).

Linearity was assessed for TRA and DIC by plotting calibration curves of the $D^1$ absorbance versus the concentration over the concentration range 2-18 µg/ml and 5-25 µg/ml, respectively. The correlation coefficients ($r^2$) for TRA and DIC were found to be 0.9941 and 0.9968, respectively (Table 2). The following equations for straight line were obtained for TRA and DIC.

Linear equation for TRA, $y = 0.0052x + 0.004$

Linear equation for DIC, $y = 0.0608x - 0.0592$

The % recoveries were found to be in the range of 99.2 – 99.73 % for TRA and 99.33 – 99.64 % for DIC (Table 3). The precision of method was determined by repeatability, intraday and interday precision and was expressed as the C.V. (Table 1), which indicate good method precision.

The Limit of detection for TRA and DIC was found to be 0.144µg/ml and 1.60µg/ml respectively. Limit of quantification for TRA and DIC was found to be 0.43µg/ml and 4.86µg/ml at 282.81 nm and at 272.45 nm respectively (Table 1).

The method was also found to be specific, as there was no interference observed when the drugs were estimated in presence of excipients and robust, as there was no significant change in absorbance up to 24 hours of preparation of solution in methanol. The proposed spectrophotometric method was successfully applied to TRA and DIC combined dosage form. The results are shown in Table 6.

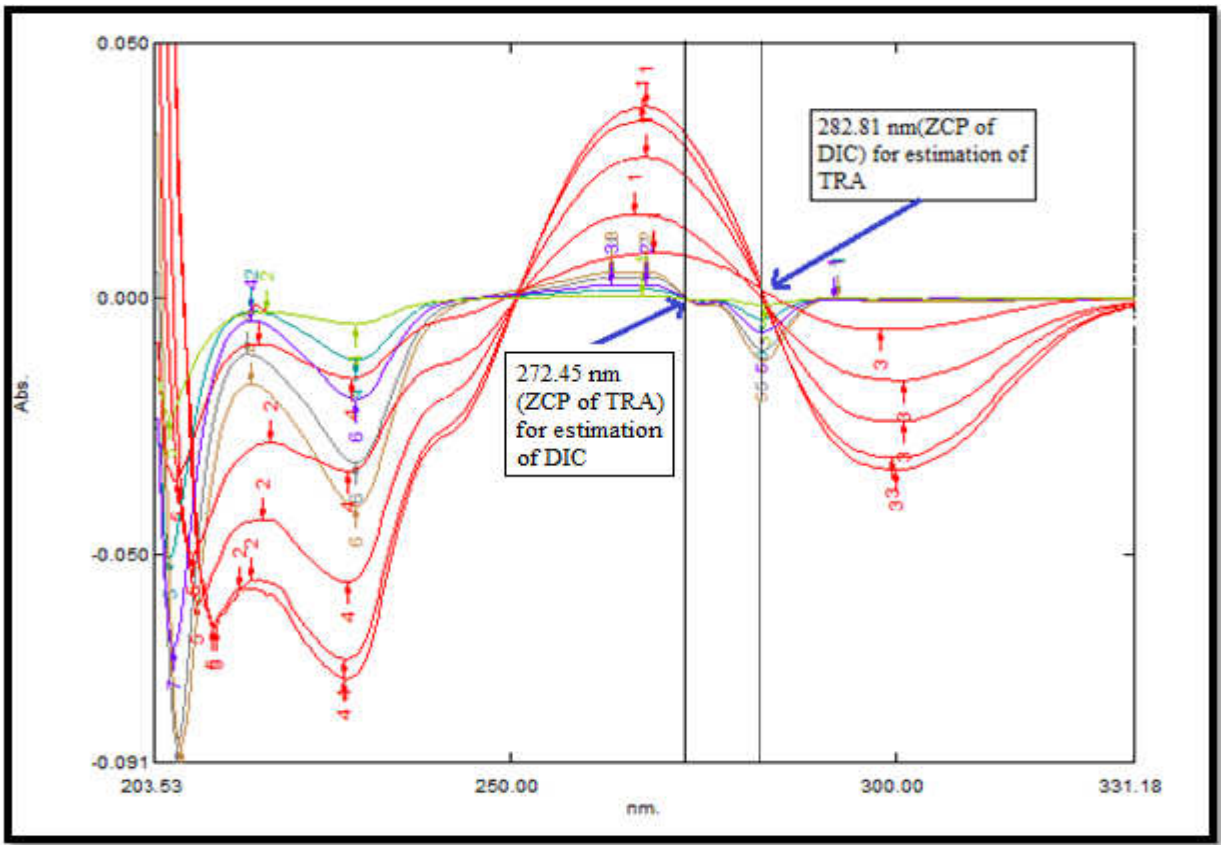



**Fig. 2: Overlain D¹ spectrum of TRA (2-18µg/ml) and DIC (5-25 µg/ml) in methanol**

**Table 1: Summary of Validation Parameters of derivative spectrophotometric method**

| Parameters | TRA | DIC |
|---|---|---|
| Recovery % | 99.2 – 99.73 % | 99.33 – 99.64 % |
| Repeatability ( n=6) | 0.792 | 0.442 |
| Precision(C.V.) | | |
| Intra-day (n=3) Inter-day (n=3) | 0.72 – 1.82 | 0.3 – 0.92 |
| | 0.1 – 0.72 | 0.06 – 0.95 |
| Limit of Detection (µg/ml) | 0.144 | 1.60 |
| Limit of Quantitation (µg/ml) | 0.43 | 4.860 |
| Specificity | Specific | Specific |
| Robustness | Robust | Robust |
| Solvent suitability | Suitable for 24 hrs. | Suitable for 24 hrs. |

**Table 2: Statistical data for TRA and DIC by derivative spectrophotometric method**

| Parameter | TRA at 282.81 nm | DIC at 272.45 nm |
|---|---|---|
| Linear Range (µg/ml) | 2 – 18 | 5 – 25 |
| Slope | 0.0052 | 0.02833 |
| Intercept | 0.004 | 0.02021 |
| Standard deviation of slope | 0 | 0.0000547 |
| Standard deviation of intercept | 0.000228 | 0.02959 |

**Table 3: Accuracy data for TRA and DIC by derivative spectrophotometric method**

| % Level | Amount of drug added (µg/ml) | | Amount recovered (µg/ml) | | % Recovery | |
|---|---|---|---|---|---|---|
| | TRA (µg/ml) | DIC (µg/ml) | TRA (µg/ml) | DIC (µg/ml) | % TRA | % DIC |
| 50 % | 5 | 7.5 | 4.9633 | 7.45 | 99.2 | 99.33 |
| 100 % | 10 | 15 | 9.96 | 14.9266 | 99.6 | 99.51 |
| 150 % | 15 | 22.5 | 14.9633 | 22.4266 | 99.73 | 99.64 |

**Table 4: Reproducibility data for TRA at 281.81 nm (10 µg/ml)**

| Instrument 1 Mean ± S.D. (n=3) | Instrument 2 Mean ± S.D. (n=3) | Result of t test* | Inference |
|---|---|---|---|
| 0.05566 ± 0.0000577. | 0.054 ± 0.0008164 | 0.129 | Not significant difference |

* At 95% confidence interval, (t-Tabulated = 4.30)

**Table 5: Reproducibility data for DIC at 272.45 nm (15 µg/ml)**

| Instrument 1 Mean ± S.D. (n=3) | Instrument 2 Mean ± S.D. (n=3) | Result of t test* | Inference |
|---|---|---|---|
| 0.1916 ± 0.000577 | 0.19 ± 0.001 | 0.199 | Not significant difference |

* At 95% confidence interval, (t-Tabulated = 4.30)

**Table 6: Assay Results of Marketed Formulation**

| Formulation | Actual concentration (µg/ml) | | Amount obtained (µg/ml) | | % TRA ± S.D. | % DIC±S.D. |
|---|---|---|---|---|---|---|
| | TRA | DIC | TRA | DIC | | |
| Tablet | 10 | 15 | 9.96 | 14.9266 | 99.6 ± 0.01 | 99.51 ±0.03055 |

n=3 determination

## CONCLUSION

The proposed first order derivative method provide 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 combined dosage form.

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