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# QUANTITATIVE ANALYSIS OF PARACETAMOL AND DICLOFENAC IN COMBINED DOSAGE FORM BY FIRST DERIVATIVE AND SIMULTANEOUS EQUATION METHOD IN APPLICATION TO THE DETERMINATION OF DISSOLUTION STUDY

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## ABSTRACT

Two simple, economical, precise, and accurate methods are described for the simultaneous determination of Paracetamol and Diclofenac in combined tablet dosage form. The first method is first- order derivative zero crossing method. The first order derivative absorption at 275.6 nm (zero cross point of Diclofenac) was used for Paracetamol and 242.69 nm (zero cross point of Paracetamol) for Diclofenac. The second one is simultaneous equation method in application to dissolution study. Simultaneous equation method was successfully applied to carry out dissolution study of commercial tablet formulation by using USP type II dissolution test apparatus. The solvent used is pH 6.8 phosphate buffer. Linearity range was 2-10µg/ml and 5-25 µg/ml for Paracetamol and Diclofenac respectively. The methods were validated with respect to linearity, precision and accuracy.

## INTRODUCTION:

**Paracetamol:** Paracetamol (PARA) is widely used as counter analgesic (pain reliever) and anti-pyretic (fever reducer). It is chemically N-(4-hydroxy phenyl) ethanamide. It acts by reducing the production of prostaglandins. It can be used for the treatment of pains of all kinds (head ache, dental pain, etc.,) in cancer patients. PARA is used for mild pain or it can be administered in combination with opoids (e.g.: codeine) and it has a little anti-inflammatory activity. PARA should not be used in severe liver diseases. From the literature survey Paracetamol can be estimated by different analytical methods they are HPLC [1, 2, 3], simultaneous method [4] and UV spectrophotometry [5, 6, 7].

**Diclofenac:** Diclofenac (DICLO) is a non- steroidal anti-inflammatory drug (NSAID) taken to reduce inflammation and as an analgesic to reduce pain in certain conditions. It is chemically 2-(2, 6-dichloranilino) phenyl acetic acid. The mechanism responsible for its anti-inflammatory, anti-pyretic and analgesic action is inhibition of prostaglandin synthesis by inhibiting the action of cyclo oxygenase (COX). It exhibits bacteriostatic activity by inhibiting the bacterial DNA synthesis. From the literature survey Diclofenac can be estimated by different analytical methods they are HPTLC [8], HPLC [9, 10], spectroflurometry [11], spectro-photometric [12, 13, 14] and UV absorptiometry [15].

Literature survey reveals that PARA and DICLO can be estimated by analytical and bio-analytical methods but these methods are more precise, economical and accurate.

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## MATERIALS AND METHODS:

**Instrumentation:** LABINDIA UV 3092 UV- Visible double beam spectrophotometer with a fixed slit width 1 nm and 1 cm matched quartz cells was used for all the spectral measurements, LABINDIA DS 8000 Dissolution test apparatus.

**Chemicals:** Phosphate Buffer pH 6.8 and Distilled water

## RESULTS AND DISCUSSION:

### METHOD A: First Order Derivative Spectrophotometry

**Standard stock solution:** Standard stock solutions of PARA and DICLO (1000µg/ml) were prepared by using pH 6.8 phosphate buffer and were further diluted with pH 6.8 phosphate buffer to obtain a final concentration of 100µg/ml (working standard solution).

**Procedure:** From standard stock solutions of PARA and DICLO (100 µg/ml), a suitable aliquots were diluted with phosphate buffer pH 6.8 to obtain a solutions of PARA (6µg/ml) and DICLO (15 µg/ml) and scanned in the range of 200-400 nm. The absorption spectra thus obtained were converted to first order. The first order derivative spectrum was selected for the analysis of both drugs. From the overlain spectrum of both the drugs (**Fig. 1**) wavelengths selected for quantization were 275.6nm (zero cross point of DICLO) for PARA and 242.69 nm (zero cross point of PARA) for DICLO.

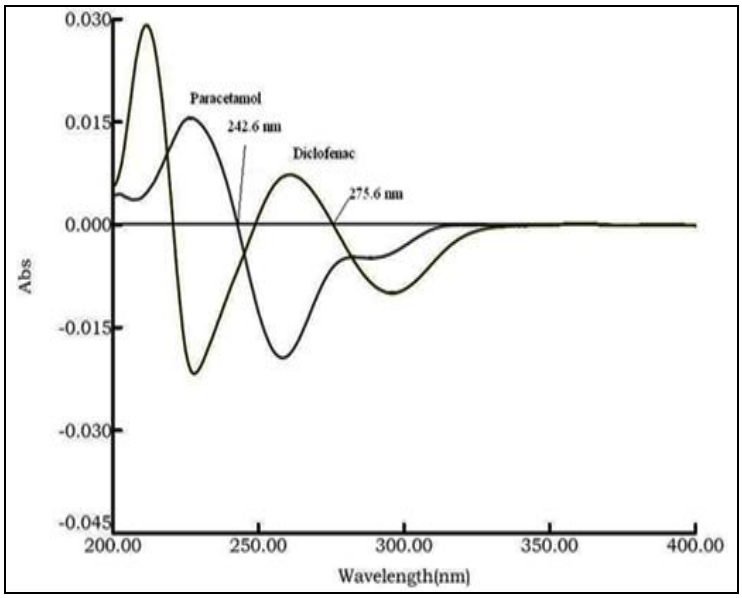


**FIG 1: FIRST ORDER DERIVATIVE OVERLAIN SPECTRA OF PARACETAMOL (PARA, 10 µg/ml) AND DICLOFENAC (DICLO, 10 µg/ml)**

Standard stocks solutions were diluted with phosphate buffer of pH 6.8 to obtain a concentration range of 2-10 µg/ml for PARA and 5-25µg/ml for DICLO. Derivative spectra were obtained over the range of 200-400nm for all the solutions. At 242.69 nm, first order derivative absorption spectra were developed for varying concentrations of PARA for its determination (**Fig. 2a**) and no interference of DICLO was observed as D1=0 (**Fig. 2b**). There is no change in the concentration of DICLO and has no effect on quantitative determination of PARA.

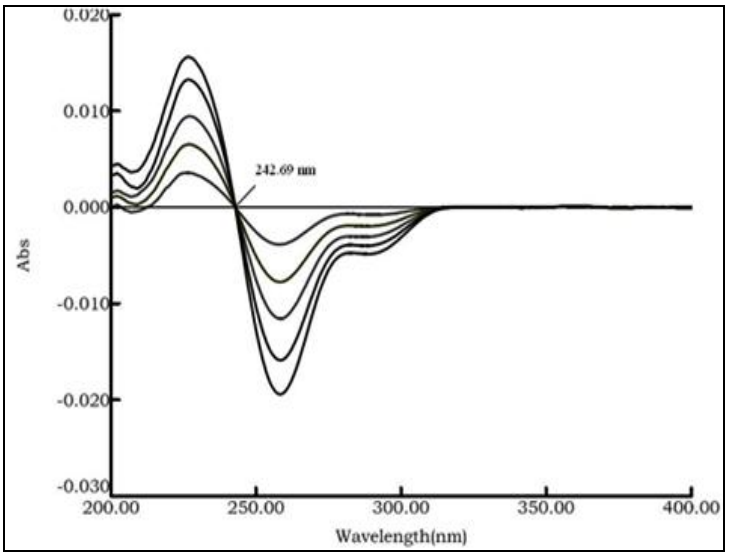


**FIG 2A: FIRST ORDER DERIVATIVE SPECTRA FOR PARA, (2, 4, 6, 8, 10µg/ml) AND AT 242.69 nm**

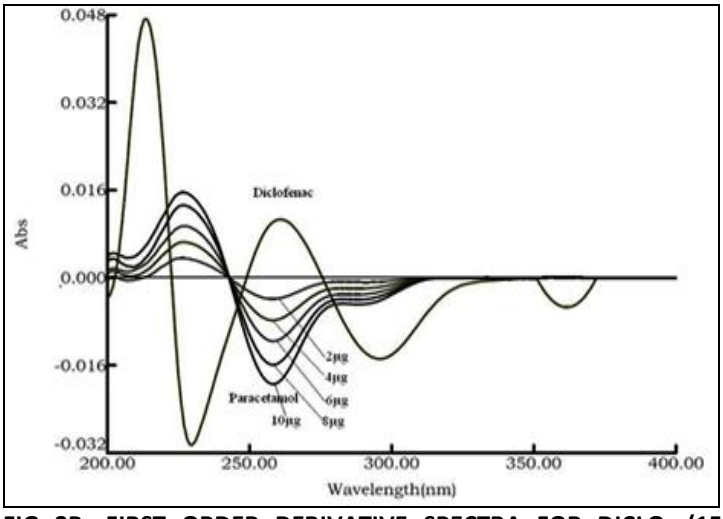


**FIG 2B: FIRST ORDER DERIVATIVE SPECTRA FOR DICLO, (15 µg/ml) AND PARA, (2, 4, 6, 8, 10µg/ml)**

To determine DICLO, the first order derivative spectra were used by making measurements at 275.6 nm (**Fig. 3a**) at which D1=0 for PARA. For quantitative determination of DICLO, no interference of PARA was found even at different concentrations (**Fig. 3b**).

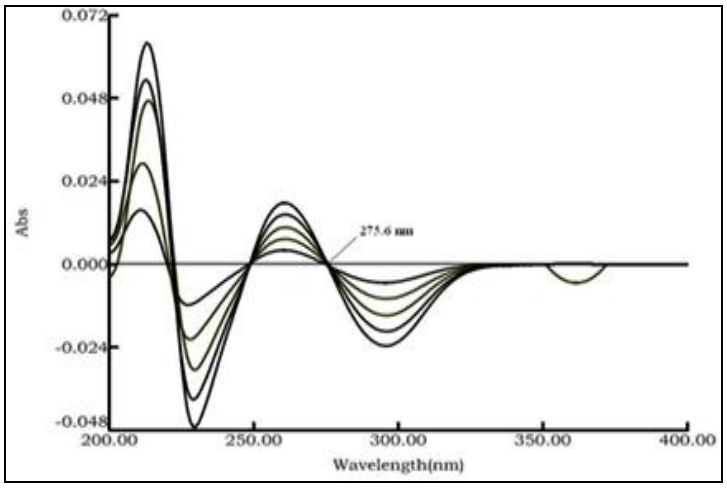


**FIG 3A: FIRST ORDER DERIVATIVE SPECTRA FOR DICLO (5, 10, 15, 20, 25µg/ml) AND AT 275.6 nm**

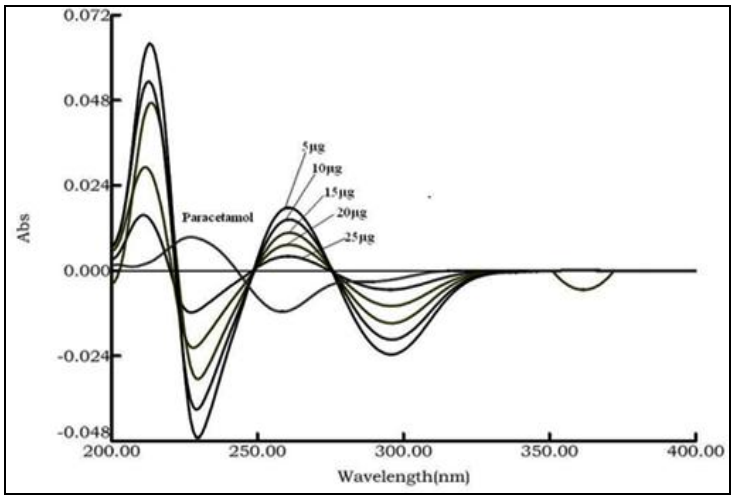


**FIG. 3B: FIRST ORDER DERIVATIVE SPECTRA FOR PARA, (6 µg/ml) AND DICLO, (5, 10, 15, 20, 25µg/ml)**

The overline spectra of both the drugs (**Fig. 4**). The calibration curves were constructed by plotting the drug concentration versus the absorbance values of first derivative spectrum (D1) 275.6 nm for PARA and 242.69 nm for DICLO. Statistical data for calibration curves is depicted (**Table 1**). The concentration of individual drugs present in the mixture was determined from the calibration curves in quantization mode.

**Sample preparation:** 20 tablets were powdered and weight equivalent to 10 mg was taken. The required concentrations were prepared with phosphate buffer of pH 6.8. The concentrations of both PARA and DICLO were determined by measuring the absorbance at 275.6nm and 242.69 nm in first order spectrum mode and the results of tablets analysis were calculated from the calibration curve in quantization mode.

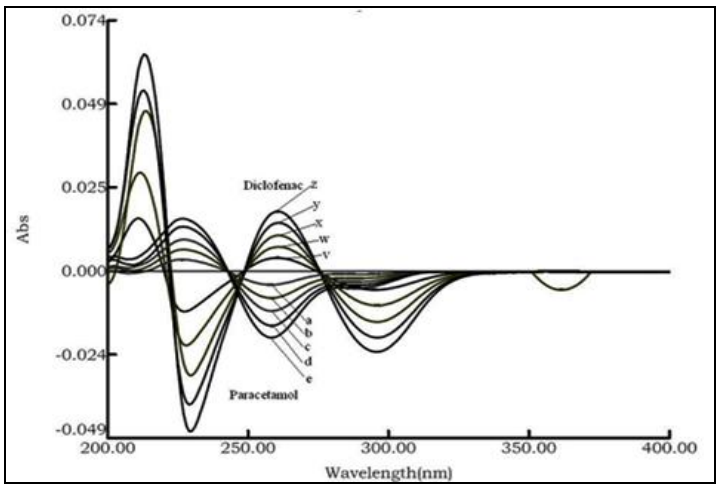


**FIG. 4: FIRST ORDER DERIVATIVE SPECTRA OF (A) 2, (B) 4, (C) 6, (D) 8, AND (E) 10 µg/ml SOLUTION OF PARA AND (V) 5 (W) 10, (X) 15, (Y) 20, And (Z) 25 µg/ml SOLUTION OF DICLO**

**Validation parameters:** The method was validated statistically as per ICH/USP 16 guidelines for all the parameters like accuracy, linearity, precision, ruggedness and specificity. Accuracy of the method was ascertained on the basis of recovery studies, carried out by standard addition method in which pre-analyzed samples were taken and standard drug was added at three different levels (80%, 100% and 120% of the test concentration). The % recovery ± SD was given (**Table 2**).

The linearity of the method was established from the first derivative spectra by the measurement of absorbance of standard solutions containing varying concentrations of each compound in the presence of constant concentrations of other one. Linearity range was 2-10µg/ml and 5-25 µg/ml for PARA and DICLO respectively ($r^2$<1).

Precision was studied by analyzing five replicates of sample solutions and concentrations were calculated.

Ruggedness was established by carrying out experiment at different conditions like intraday, interday and by different analyst. Specificity of the method was ascertained by analyzing the standard drug sample. There was no interference by the excipients present in the formulation.

**TABLE 1: OPTICAL CHARACTERISTICS AND RESULTS OF FORMULATION ANALYSIS**

| S. No | Parameter | | METHOD A | | METHOD B | |
|---|---|---|---|---|---|---|
| | | | PARA | DICLO | PARA | DICLO |
| 1 | Wavelength (λnm) | | 275.6 | 242.69 | 243 | 281 |
| 2 | Beers law limit (µg/ml) | | 2-10 | 5-25 | 2-10 | 5-25 |
| 3 | Regression equation (y=mx) | | y=0.001x | Y=0.001x | y=0.058x | y=0.029x |
| 4 | Correlation coefficient | | 0.997 | 0.996 | 0.999 | 0.998 |
| 5 | Precision | Intraday | 1.01 | 0.78 | 1.06 | 0.92 |
| | %RSD | Interday | 1.98 | 1.25 | 1.12 | 1.01 |

**TABLE 2: RESULTS OF RECOVERY STUDIES**

| S. No | Recovery level (%) | Amount spiked | | Amount recovered | | % Mean recovery | | %RSD | |
|---|---|---|---|---|---|---|---|---|---|
| | | PARA | DICLO | PARA | DICLO | PARA | DICLO | PARA | DICLO |
| Method A | 80 | 2 | 5 | 2.02 | 5.98 | 101 | 99.6 | 0.98 | 1.20 |
| | 100 | 4 | 10 | 4.01 | 10.10 | 100.25 | 101 | 1.30 | 1.12 |
| | 120 | 6 | 15 | 5.98 | 15.01 | 99.96 | 100.06 | 0.86 | 0.92 |
| Method B | 80 | 2 | 5 | 2.04 | 4.95 | 102 | 99 | 1.40 | 1.70 |
| | 100 | 4 | 10 | 3.96 | 10.25 | 99 | 102.5 | 0.96 | 1.20 |
| | 120 | 6 | 15 | 6.05 | 15.21 | 100.83 | 101.4 | 0.81 | 0.89 |

By observing validation parameters, the described method was found to be specific, accurate, precise and economical and can be successfully applied to analyze commercially available tablet contains PARA and DICLO. The results obtained are satisfied.

**Method B: Simultaneous equation method in application to Dissolution study:** For selecting the analytical wave lengths for simultaneous equation method, standard solutions of PARA (6µg/ml) and DICLO (15µg/ml) were scanned in the UV range. **Fig. 5** represents the overlain spectrum of both the combinations. Wavelengths of PARA and DICLO were found to be 243 nm and 281 nm respectively.

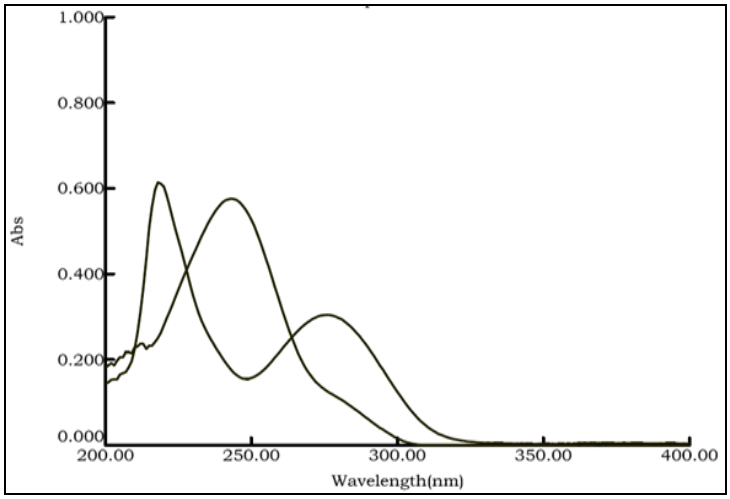


**FIG. 5: OVERLAIN SPECTRUM OF PARA AND DICLO**

Working standard solutions (2-10µg/ml) and (5-25µg/ml) for PARA and DICLO were prepared respectively. All the solutions were measured at both the wavelengths and four calibration curves were constructed.

Absorptivities at each wavelength for PARA and DICLO were determined and were used to form the equation. The absorbance and absorptivity values at particular wavelengths were submitted in the following equation to obtain concentration.

CX = (A2ay1-A1ay2)/ (ax2ay1-ax1ay2)

CY = (A1ax2-A2ax1)/ (ax2ay1-ax1ay2)

CX = concentration of PARA

CY = concentration of DICLO

A1=absorbance of samples at 243nm.

A2= absorbance of samples at 281 nm.

ax1 is the absorptivity of PARA at 243nm.

ax2 is the absorptivity of PARA at 281 nm.

ay1 is the absorptivity of DICLO at 243nm.

ay2 is the absorptivity of DICLO at 281 nm.

**Dissolution study:** The dissolution study was carried out for both PARA and DICLO combination and was validated. A calibrated dissolution apparatus (USP type II) was used at 50 rpm and bath temperature was maintained at 37±0.5°C. Phosphate buffer of pH 6.8 was used as a dissolution medium. Six tablets were evaluated and dissolution samples were collected at 5, 10, 15, 20, 25, 30, 45, 60 min time interval. At each time point, a 5 ml sample was removed from each sample and replaced, filtered through a Whatmann Filter (0.45µm, 25mm). 0.1 ml of filtrate was diluted to 10 ml with phosphate buffer pH 6.8 and analyzed by simultaneous equation method, and percentage release of PARA and DICLO was calculated by using eq (3) and (4) respectively.

**Paracetamol % release= (C para*900*100*100)/1000*500…… (3)**
**Diclofenac % release = (C diclo*900*100*100)/1000*500…….. (4)**

**Validation parameters:** Under experimental conditions described, the calibration curve, assay of tablets and recovery studies were performed. A critical evaluation of proposed method was performed by statistical analysis of data where slope, intercept, correlation coefficient was shown in (Table 1). As ICH guidelines, the method validation parameters checked were linearity, accuracy and precision. Beers law was obeyed in the range of PARA (2-6µg/ml) DICLO (5-25 µg/ml).

Results of recovery studies were shown in (Table 2). The accuracy and reproducibility is evident from the data as results are close to 100% and standard deviation is low. Percentage release during dissolution study was always greater than 80% for PARA within 30min and greater than 75% for DICLO within 60 min (**Fig. 6**)

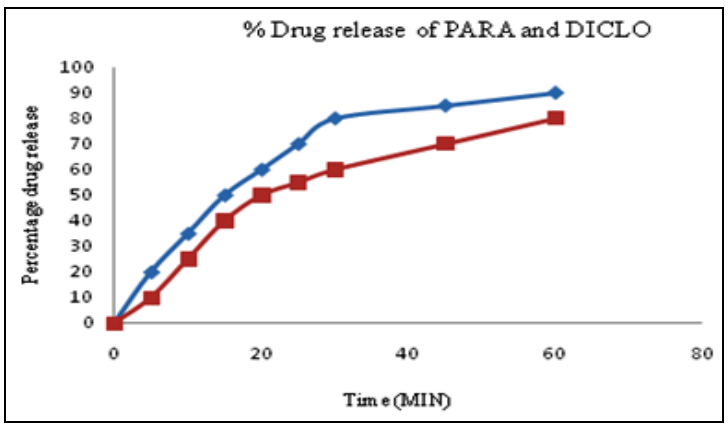


**FIG. 6: % DRUG RELEASE OF PARA AND DICLO**

**CONCLUSION:** The validated spectrophotometric methods employed here proved to be simple, economical, precise and accurate. Simultaneous equation method can be used to carry out dissolution study in combination tablet formulation.

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