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Contributed Paper

# High Performance Thin Layer Chromatographic Method for the Determination of Diclofenac Sodium in Pharmaceutical Formulations

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## ABSTRACT

A high performance thin layer chromatographic method for the determination of diclofenac sodium in pharmaceutical formulations was developed. The drug was extracted from the sample (emulgel) then various aliquots of this solution were spotted automatically by means of Camag Linomat IV (Switzerland) on a silica gel 60 $F_{254}$ aluminium plate, using a mixture of toluene : ethyl acetate : glacial acetic acid (60:40:1, v/v/v) as mobile phase. The spot areas were quantified by densitometry at 282 nm. Linear calibration curve was obtained over the range 5-80 $\mu g.mL^{-1}$ ($r^2$ = 0.9993). The method was applied to the determination of diclofenac sodium in Diclogel®, Voltaren® emulgel and Dosanac® emulsiongel with the average percentage recoveries of 104.21±1.59, 112.41±1.93 and 101.27±4.59, respectively. The standard deviation of diclofenac sodium in Dosanac® emulsiongel was higher than those obtained from Diclogel® and Voltaren® emulgel, probably owing to the matrices present in the sample. But the recoveries of the added diclofenac sodium in the samples are quite good. The average percentage labelled amount of diclofenac sodium in Diclogel®, Voltaren® emulgel and Dosanac® emulsiongel were 94.61±0.06, 97.87±0.11 and 94.81±0.03, respectively. They are not exceed the percentage labelled amount claimed, (not less than 90% and not more than 110%, USP 28) [1]. The proposed method is simple, rapid, sensitive, reproducible and accurate. It also consumed less reagents compared with the HPLC method. Therefore this method is suitable for routine analysis of this drug in raw materials and formulations.

**Keywords:** diclofenac sodium, high performance thin layer chromatography, pharmaceutical formulations.

## 1. INTRODUCTION

Diclofenac sodium [Sodium (*o*- {(2,6-dichlorophenyl) amino} phenyl) acetate] (Figure 1) is a synthetic nonsteroidal anti-inflammatory Drug (NSAID), has been proven as a safe and efficacious drug in the treatment of a variety of inflammatory and rheumatoid disorders [2]. The pharmacological effects of this drug are thought to be related to the inhibition of the conversion of arachi-

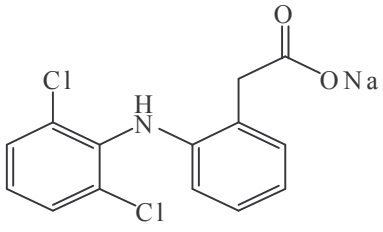


**Figure 1.** Structure of diclofenac sodium.

donic acid to prostaglandins, which are the mediators of the inflammatory process [3]. It is employed mainly in oral formulations, and the some extent, also for intramuscular injection and topical formulation [4]. For topical formulations, one of the most wildly used is Voltaren® emulgel, Diclogel® and Dosanac® emulsiongel. The advantage for this type of dosage form found to be effective for the treatment of local inflammatory [4]. However, the disadvantage was also observed due to careless of the patients which may wipe the drug out shortly after applied to the skin.

For the analysis of diclofenac, several spectrophotometric methods have been reported. Bucci *et al.* [5] described a UV ($\lambda$ = 276 nm) and UV first derivative ($\lambda$ = 240-340 nm) spectrophotometric methods for the determination of diclofenac sodium or diethylammonium in tablets, suppositories and gel with the limit of detection (LOD) of 4 $\mu$mole.$L^{-1}$. Sena *et al.* [6] determined diclofenac in pharmaceutical formulations containing vitamin B by using UV spectrophotometry and partial least squares regression. Bottello *et al.* [7] presented a sensitive spectrophotometric method for determining diclofenac sodium using methylene blue as colorimetric reagent in the pH range 9.2-9.4 to form a chloroform-extractable blue ion-association complex which gives maximum absorption at 653 nm. Agrawal *et al.* [8] reported two colorimetric methods for determining diclofenac sodium in tablets. The first method is based on the reaction of diclofenac with ferric chloride and 2, 2′- bipyridine to form a complex having maximum absorption at 520 nm. The second method is based on the reaction of diclofenac with methylene blue in phosphate buffer pH 6.8 forming a complex which gives maximum absorption at 640 nm. Damiani *et al.* [9] reported a spectrofluorometric method for quantitative analysis of diclofenac in 0.01 N HCl and measured the fluorescence intensity at $\lambda_{em}$ = 362 nm with the $\lambda_{ex}$ = 287 nm. Shafiee *et al.* [10] determined diclofenac sodium in injectable solution by gas chromatography (GC) and high performance liquid chromatography (HPLC). The ester was extracted and subjected to gas liquid chromatography with flame ionization detector, 5% SE-30/chrom W-HP (80–100 mesh) was used as a column in GC. For reversed phase HPLC, MeOH : $H_2O$ (55:45, v/v) was used as mobile phase. The separation was performed on a $\mu$-bondapak phenyl column using UV detector at 274 nm. Gonz´alez *et al.* [11] determined diclofenac sodium in the presence of cyanocobalamin and betamethasone in tablets by high performance liquid chromatography using RP 18 column and acetonitrile : water (40:60, v/v) (pH 3.45) as mobile phase with UV detection at 240 nm. Segura *et al.* [12] described a quantitative analysis of diclofenac in plasma using gas chromatography-mass spectrometry. Sioufi *et al.* [13] determined diclofenac in plasma and urine by using capillary gas chromatography-mass spectrometry (GC-MS).

High performance thin layer chromatography (HPTLC) can be used for identification and for control of batch-to-batch consistency in the stability testing of drugs and for purposes of control throughout the entire manufacturing process of drugs, as well as quality control of the finished product. It has the advantages of being sensitive, selective, rapid, accurate and reproducible.

The present paper reports the development and validation of a new high performance thin layer chromatography (HPTLC) method for determination of diclofenac sodium in Voltaren® emulgel, Diclogel® and Dosanac® emulsiongel.

## 2. Materials and Methods

### 2.1 Chemicals

Diclofenac sodium (Reference Standard No T191067, 99.9 %, Sigma, Switzerland), Ethyl acetate (BDH laboratory supplies, England), Toluene (Lab-scan analytical science, Ireland), Glacial acetic acid (Farmitalia Carlo Erba, Italy), Methanol (Lab-scan analytical sciences, Ireland). HPTLC precoated plates silica gel 60 $F_{254}$ 20×10 cm, layer thickness 0.2

mm (Merck, Germany). All other chemicals used were analytical grade.

**2.2 Samples**

Diclogel® (batch no. 30131; Polipharm Co., Ltd.), Voltaren® Emulgel (batch no. 2B101; OLIC (Thailand) Limited) and Dosanac® Emulsiongel (batch no. 925058; Siam Bheasach Co., Ltd.).

**2.3 Instruments**

- CAMAG Automatic developing system (Camag® Muttenz,Switzerland).
- CAMAG TLC Scanner and Computer Aided Testing Software (CATS) evaluation software, deuterium lamp, scanning by absorbance at 282 nm, evaluation via peak area.
- Centrifuge (SIGMA, Scientific, Germany).
- UV viewing system (Chromato-VUE® C 70G, USA).
- UV-visible spectrophotometer (Chromato-VUE® C 70G, USA).

**2.4 Standard Stock Solution**

A standard stock solution of diclofenac sodium (1,000 $\mu g.mL^{-1}$) was prepared in methanol. Working standard solutions in a range of 5-80 $\mu g.mL^{-1}$ were prepared by dilution from this stock solution.

**2.5 Sample Preparation**

One gram of sample was accurately weighed into a 100 mL beaker and dissolved in 10 mL methanol. Then the sample solution was transferred into a 25 mL volumetric flask and adjusted to volume with methanol. The solution was vortexed for 30 s then centrifuged at 4,000 rpm for 30 min. The supernatant containing diclofenac sodium was taken and filtered by membrane filter (90 mm Dia. Whatman®). The filtrated supernatant was used for further analysis.

**2.6 HPTLC Method for Determining Diclofenac Sodium**

A series of standard solutions containing 5-80 $\mu g.mL^{-1}$ of diclofenac and the sample solutions were applied respectively, applied the 3 mm band width by Camag 100 $\mu L$ sample syringe (Hamilton, Bonaduz, Switzerland) on a precoated silica gel aluminium plate 60 $F_{254}$ (20×10 cm) with 200 $\mu m$ layer thickness (E. Merck, Darmstadt, Germany). A linomat IV was employed with a constant rate of 3 $s.\mu L^{-1}$ and the space between two bands was 5 mm. Development was performed in an Automatic Multiple Development (AMD) system using toluene : ethyl acetate : glacial acetic acid (60:40:1, v/v/v) as mobile phase, [previous investigation has done as described in the Results and Discussion section 3.1]. Densitometric scanning was performed on Camag TLC scanner III in the reflectance-absorbance mode (Zig-Zag) at 282 nm for all measurements and operated by CATS software. Concentrations of the compound of chromatogram (spot) on the plate were determined from the intensity of diffusely reflected light. The spot areas were plotted against diclofenac sodium concentrations. Then the content of diclofenac sodium in each sample was calculated by reference to the calibration curve.

## 3. Results and Discussion

**3.1 Selection of Mobile Phase**

During the development of the HPTLC method four different compositions of mobile phase including methanol : ethyl acetate, methanol : ethyl acetate : glacial acetic acid, toluene : ethyl acetate and toluene : ethyl acetate : glacial acetic acid were tested. It was found that the mobile phases, methanol : ethyl acetate and methanol : ethyl acetate : glacial acetic acid provided bad constituent separation with tailing occurred. When using toluene : ethyl acetate as mobile phase the spots of standard and sample moved slowly with tailing occurred but no separation occurred when using ethyl acetate as mobile phase. A mixture of toluene : ethyl acetate : glacial acetic acid (60:40:1, v/v/v) was proved to be the best because it gave good resolution for diclofenac sodium with the $R_f$ of 0.51.

**3.2 Method Validation**

The following parameters has been used to validate the developed HPTLC method for the estimation of diclofenac sodium in pharmaceutical formulations.

**3.3 Sensitivity and Linearity**

The sensitivity of the assay was determined in terms of limit of detection (LOD), limit of quantitation (LOQ), linearity range and correlation coefficient. The detection limit of the method was investigated by applying various concentrations of standard diclofenac sodium solution on the precoated HPTLC plate. After development, the spot areas were quantified by densitometry. Limit of detection (LOD) and limit of quantitation (LOQ) were calculated from the standard deviation (S.D.) of densitometric response and slope of curve (S) using the equation;

$$LOD = 3.3 (S.D. / S)$$
$$LOQ = 10 (S.D. / S)$$

After densitometric analysis of diclofenac sodium at 282 nm, the lowest amount of drug, which could be detected was found to be 1 $\mu g.mL^{-1}$ (3 $\mu mole.L^{-1}$, which is more sensitive than the UV spectrophotometric method [5] and the lowest amount of drug which could quantified was found to be 5 $\mu g.mL^{-1}$. The calibration curve of diclofenac sodium standard was found to be linear in the range of 5-80 $\mu g.mL^{-1}$ (Figure 2). The mean values (±S.D.) of correlation coefficient, slope and intercept are shown in Table 1.

**3.4 Accuracy**

The accuracy of the proposed method was verified by analyzing the aliquots of sample solutions equivalent to diclofenac sodium 2 $\mu g.mL^{-1}$ spiked with various concentrations of standard diclofenac sodium solutions 25, 30 and 35 $\mu g.mL^{-1}$ respectively, using the proposed procedure. The recovery of each spiked standard diclofenac sodium was calculated. Results are presented in Table 2.

Regression equation; $Y = 149.92C + 6808.10$ ($r^2 = 0.9994$) where $C$ is the concentration in $\mu g.mL^{-1}$ and $Y$ is the peak area.

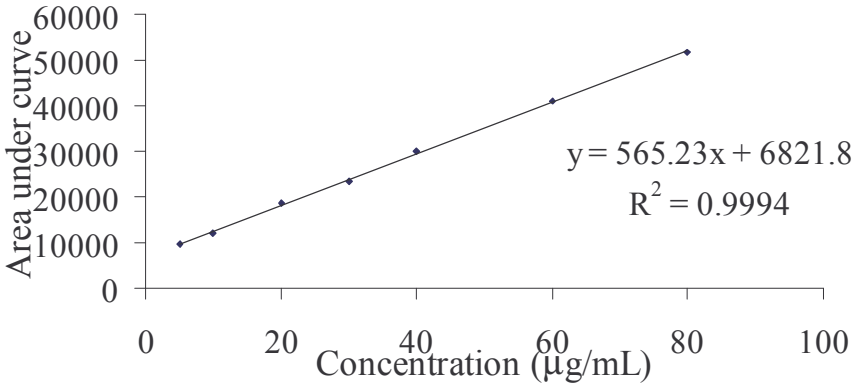


**Figure 2.** Linear calibration curve of diclofenac sodium 5-80 $\mu g.mL^{-1}$.

**Table 1.** Linear regression data for calibration curves (n=7) of diclofenac sodium obtained from HPTLC.

| Parameter | Value |
|---|---|
| Linearity range | 5-80 $\mu g.mL^{-1}$ |
| $r^2 \pm$ S.D. | 0.9993 ($\pm 7.00 \times 10^{-5}$) |
| Slope ± S.D. | 564.08 (± 30.08) |
| Intercept ± S.D. | 6838.63 (± 20.98) |

**Table 2.** Recovery study of diclofenac sodium by TLC densitometric, standard addition technique for determination of diclofenac sodium (n = 7).

| Pharmaceutical sample (marketed formulation) (2 μg.mL$^{-1}$) | Concentration of addition standard (μg.mL$^{-1}$) | % Recovery (±S.D.) | %CV |
|---|---|---|---|
| Diclogel® | 25 | 106.24 ± 0.32 | 0.29 |
| | 30 | 108.04 ± 1.98 | 1.82 |
| | 35 | 98.36 ± 2.49 | 2.54 |
| Voltaren® emulgel | 25 | 120.32 ± 0.36 | 0.30 |
| | 30 | 108.34 ± 4.50 | 4.26 |
| | 35 | 108.58 ± 0.94 | 0.82 |
| Dosanac® emulsiongel | 25 | 99.54 ± 3.21 | 3.37 |
| | 30 | 101.11 ± 4.83 | 4.78 |
| | 35 | 103.18 ± 5.74 | 5.89 |

### 3.5 Precision

The intra-day reproducibility was evaluated by analyzing the sample repeatedly and inter-day reproducibility was evaluated by analyzing the sample of diclofenac sodium over a period of three days. Good precision were obtained with %CV of 2.13 and 2.46 for intra-day and inter-day, respectively.

### 3.6 Application

The propose method was applied to the determination of diclofenac sodium in pharmaceutical preparation (emulsion gel form). A comparative determination in the same samples were also investigated by spectrophotometric method [5]. The results are summarized in Table 3. Excellent correlation between the two methods was obtained. The results were obtained from the proposed method compared with those obtained by using the spectrophotometric method with the student *t*-test. The calculated *t*-value in all cases were less than the tabulated value (2.44 for 6 degree of freedom at 95% confidence level). These results indicate that the proposed method does not differ significantly from known method.

**Table 3.** Comparison between the HPTLC method and UV spectrophotometric method for the determination of diclofenac sodium in dosage forms.

| Pharmaceutical Sample | % Labelled amount of diclofenac sodium found (±S.D.) * | | Calculated *t*-value [b] |
|---|---|---|---|
| | HPTLC method [a] | UV spectrophotometric method [a] | |
| Diclogel® | 94.61 ± 0.06 | 96.26 ± 0.98 | 1.47 |
| Voltaren® emulgel | 97.87 ± 0.11 | 96.78 ± 0.48 | 1.30 |
| Dosanac® emulsiongel | 94.81 ± 0.03 | 95.31 ± 0.91 | 0.47 |

*S.D. = standard deviation

[a] Each value is the average of 7 determinations,

[b]Tabulated t-value for $p = 0.05$ and six degrees of freedom is 2.44

## 4. CONCLUSION

The HPTLC method for determining diclofenac sodium in pharmaceutical formulations was developed using toluene : ethyl acetate : glacial acetic acid (60:40:1, v/v/v) as mobile phase. The peak areas of the densitogram were quantified by densitometer at 282 nm. The limit of detection and limit of quantitation were found to be 1 and 5 $\mu g.mL^{-1}$, respectively. The calibration curve was linear over the range of 5-80 $\mu g.mL^{-1}$. The mean values (±S.D.) of correlation coefficient, slope and intercept were found to be 0.9993 ($\pm 7.0\times10^{-5}$), 564.08 (±30.08) and 6838.63 (±20.98), respectively. The method was applied to the determination of diclofenac sodium in Diclogel®, Voltaren® emulgel and Dosanac® emulsiongel with the average percentage recovery of 104.21±1.59, 112.41±1.93 and 101.27±4.59, respectively and the % labelled amount of 94.61±0.06, 97.87±0.11 and 94.81±0.03, respectively. The proposed method is simple, sensitive and accurate with good precision is suitable for routine analysis of this drug in formulations.

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