



## SEARCH FOR SIMPLE MOBILE PHASES IN RAPID LC SEPARATIONS-ANALYSIS OF DRUGS IN COMPLEX MATRIX (DICLOFENAC GEL AND INJECTIONS)

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### ABSTRACT

A novel, simple, rapid RP-HPLC method was developed for analysis of Diclofenac Diethylamine in Gel formulation. The same method was also used for injectable formulations. Unlike the existing methods, the proposed method selects the mobile phase excluding buffer. Use of buffer not only emerges with time consuming process but also makes the method more expensive along with reducing the column longevity. The present article is devoid of all the referred problems, It has selected a mobile phase consisting of methanol : acetonitrile ( 80 : 20 )v/v which gives a retention time of 2.453 min and 2.467 min respectively for Diclofenac gel and injection. The elution was carried out isocratically at flow rate of 1 ml / min. detected at  $\lambda_{max}$  of 284 nm . The limits of detection and limit of quantification were 13.994 and 42.408  $\mu\text{g/ml}$  respectively. The intraday and interday precision (measured by coefficient of variation, COV%) was always less than 2.0% with S.D. less than 1.0. The accuracy (measured by relative error %) was always less than 1%. The other validation parameters were performed and calculated statistically as per ICH guidelines.

**Keywords:** RP-HPLC, Diclofenac Gel, Diclofenac Injection, Validation

### INTRODUCTION

Diclofenac is a non-steroidal anti-inflammatory drug (NSAID) taken to reduce inflammation and as an analgesic reducing pain in conditions such as arthritis or acute injury. It can also be used to reduce menstrual pain, dysmenorrhea. The name is derived from its chemical name: 2-(2,6-dichloranilino)phenylacetic acid<sup>1-2</sup>. Optimization of HPLC method development of Diclofenac in its bulk and different pharmaceutical dosage forms has been discussed extensively in many standard articles.

Existing literature involve method development of gel formulation without extraction but practically it has been noticed that without extraction of gel formulation gives a number of intense peaks probably may be of the excipients without better resolution<sup>3-6</sup>. B.P.-2009 has optimized the method of Diclofenac gel by extracting the analyte using acetone and methanol and using the mobile phase consisting of a mixture of 34 volumes of a mixture of equal volume of a 0.1%w/v solution of orthophosphoric acid and 0.16%w/v solution of sodium dihydrogen orthophosphate adjusted o pH 2.5 and 66 volumes of methanol detected at a wavelength of 254nm which is particularly appreciable for QC analysis<sup>7</sup>.

Use of buffer again has been a custom in many method development processes to get a sharp and distinct peak without tailing and fronting and moreover it also suppresses the extra peaks which may be the result of degraded products or impurities present in the analyte ultimately eliminates the solvent effect. Buffer used in the mobile phase may give a sharp peak but simultaneously it reduces the longevity of column life and also considerable time is spent for washing the column to eliminate the buffered material and subsequent equilibration of the column<sup>8</sup>. Additionally considerable cost is involved in the way of using HPLC grade solvents. Hence, a more economical and rapid chromatographic method could be a matter of potential choice for method development. In order to fulfill those objectives the present study involves a development of a RP-HPLC method for the analysis of Diclofenac present in different dosage forms (gels & injectables) using mobile phase excluding the use of buffers. The method has been found to be very rapid, meeting all standard parameters of validation and capable of analyzing a large number of samples in a single day. The cost of the solvents required for analysis, column washing and equilibration is kept at a bare minimum.

### EXPERIMENTAL

#### Reagents and materials

Reference standard of Diclofenac sodium was procured from M/s C.I Laboratories, Kolkata. The gel formulation (Voveran 1% w/w Gel)

of M/S, Novartis was used. Diclofenac sodium Injections of Novartis, Troika & Lupin were used. Acetonitrile, methanol, were of HPLC grade and purchased from Merck, India. Membrane Filters P/N - M47N45, 47 mm, 0.45 $\mu$  were obtained from MZ ANALYSENTECHNIK.

#### Preparation of the standards

For the determination of Diclofenac sodium the stock was prepared by dissolving 50 mg of Diclofenac sodium in 50 ml methanol. From the stock solutions 0.1 ml was taken and further diluted with methanol to 10 ml (concentration of 10  $\mu\text{g/ml}$ ); these were further diluted with methanol to produce the remaining 20  $\mu\text{g/ml}$ , 50  $\mu\text{g/ml}$ , 60  $\mu\text{g/ml}$ , 100  $\mu\text{g/ml}$ , 150  $\mu\text{g/ml}$  working standards.

#### Sample preparation and extraction

**Gel:** For the analysis of pharmaceutical formulations, a quantity equivalent to labeled amount was weighed and transferred into extraction flask, to this suitable amount of acetone was added and the mixture was subjected to vigorous shaking for 15 min for complete extraction of drugs, and then filter and evaporated<sup>7</sup>. Then the residue was dissolved in methanol and filtered, then injected to HPLC system for the analysis.

**Ampoules:** A quantity equivalent to 50 mg was transferred into the volumetric flask and volume was filled up to 50 ml with methanol. 0.67 ml was taken and further diluted with methanol to 10 ml, filtered and injected to HPLC system for the analysis.

#### Chromatography

Chromatographic separation was performed on a Jasco HPLC system consisting of Jasco PU - 2089 pump, Jasco UV 2010 plus photo diode array detector. Rheodyne injection syringe with 20  $\mu\text{l}$  loop volume and windows based chrompass software. An ODS C-18 RP- column ( Intersite 4.6 mm X 2.5 cm, 10  $\mu\text{m}$  ) was used for separation . The elution was carried out isocratically at flow rate of 1 ml / min using acetonitrile : methanol ( 20: 80 v/v) as mobile phase. The run time was 10 min. Before analysis both mobile phase and sample solutions were degassed by sonication and filtered through 0.2- $\mu\text{m}$  filter. The analytes were monitored at 284 nm. The analytes were identified by comparison of retention times obtained from sample and standard solutions. The work was performed in an air-conditioned room maintained at  $25 \pm 2^\circ\text{C}$ .

#### Construction of calibration plots

From the standard stock solution of Diclofenac sodium, different dilutions were prepared and from the chromatogram the peak areas

were measured. Calibration plots of concentration against peak area were then constructed for Diclofenac sodium.

From the calibration plots it was found that response to Diclofenac sodium was a linear function of concentration in the range 10–150  $\mu\text{g ml}^{-1}$ . Marketed formulation samples (gel and injections) were quantified by reference to these calibration plots.

#### Assay of formulation

For performing the assay of the gels and ampoules formulations, six replicates of the required dilutions were prepared from the gels and ampoules stock solution and sonicated for 10 min. The solutions (20  $\mu\text{l}$ ) were then injected for quantitative analysis. The amounts of Diclofenac sodium were calculated by extrapolating the peak area from the calibration curve. The results are reported in Table 1.

**Table 1: Results from assay of the formulation**

Drug	Label claim	Amount found	Drug content %	S.D	COV %	S.E
Diclofenac Gel	1% w/w in a gel	1.025 w/w	102.5 %	0.01449	1.433	0.0102
Diclofenac Injection X	Each ml contain 25 mg	25.432 mg	101.73 %	0.23349	0.9266	0.1651
Diclofenac Injection Y	Each ml contain 25 mg	25.25 mg	101 %	0.19	0.7581	0.1343
Diclofenac Injection Z	Each ml contain 25 mg	25.7 mg	102.8 %	0.1148	0.43567	0.0788

S.D., standard deviation; COV, coefficient of variance; S.E., standard error

#### Method validation

The method was validated for linearity, accuracy, precision, repeatability, selectivity, and specificity. All validation studies were performed by replicate injection of sample and standard solutions.

#### Linearity

Linearity was determined separately for Diclofenac sodium by plotting peak area against concentration. From these calibration plots it was clear that response was a linear function of concentration over the ranges 10– 150  $\mu\text{g mL}^{-1}$  for Diclofenac sodium. The linear regression equations for Diclofenac sodium was:

$$\text{Diclofenac sodium } y = 0.0012x + 0.0056 \quad (n = 6, r^2 = 0.997)$$

where  $y$  is response (peak area) and  $x$  the concentration.

#### Accuracy

The accuracy of the method was confirmed by studying recovery at three different concentrations, 80, 100, and 120% of those expected, in accordance with ICH guidelines, by replicate analysis ( $n = 6$ ). Standard drug solutions were added to a pre-analyzed sample solution and percentage drug content was measured. The results from study of accuracy are reported in Table 2. From these results it was clear that the method enables very accurate quantitative estimation of Diclofenac sodium in gels and ampoules dosage form, because all the results were within acceptable limits, i.e.  $\text{COV} < 2.0\%$  and  $\text{S.D.} < 1.0$ .

**Table 2: Results from recovery study**

Drug	Amount taken ( $\mu\text{g ml}^{-1}$ )	Amount Added % $\mu\text{g ml}^{-1}$	Recovery ( % $\pm$ S.D )	COV %
Diclofenac Gel	50	80 40	100.62 $\pm$ 0.7788	0.774
		100 50	101.55 $\pm$ 0.8357	0.8229
		120 60	101.39 $\pm$ 0.4492	0.44304
Diclofenac Injection	50	80 40	100.374 $\pm$ 0.9488	0.94526
		100 50	99.426 $\pm$ 0.7543	0.7586
		120 60	100.366 $\pm$ 0.6612	0.6587

S.D., standard deviation; COV, coefficient of variance

#### Precision, LOD and LOQ

Precision was studied both intra-day and inter-day. Six replicate sample solutions were prepared from the stock solution. For study of intra-day precision the concentrations of the three drugs were measured three times on the same day at intervals of 1 h. In the

inter-day study the drug concentrations were measured on three different days. The limits of detection and quantitation, LOD and LOQ, were calculated by use of the equations  $\text{LOD} = 3.3\sigma/S$  and  $\text{LOQ} = 10\sigma/S$ , where  $\sigma$  is the standard deviation of the  $y$  - intercept and  $S$  is the slope of the calibration plot. The results are reported in Table 3.

**Table 3: Results from determination of intra-day and inter-day precision, and LOD and LOQ**

Drug	Slope S.D	Mean $\pm$	Intercept Mean $\pm$ S.D	Correlation coefficient
Diclofenac Sodium	0.0012 $\pm$	0.0000816	0.0032 $\pm$ 0.005089	$\geq 0.997$

Drug	Intra day precision COV %	Inter day precision			LOD $\mu\text{g mL}^{-1}$	LOQ $\mu\text{g mL}^{-1}$
		Day1a	Day2a	Day3a		
Diclofenac Gel	0.75168	0.86926	0.9652	0.82012	13.994	42.408
Diclofenac Injection	0.82238	0.49463	0.76589	0.37656	13.994	42.408

A mean from six determinations COV, coefficient of variance; LOD: limit of detection; LOQ: limit of quantitation.

#### Selectivity and Specificity

The selectivity of the method was checked by injecting solutions of the drug. It was observed that a sharp peaks for Diclofenac sodium gel and injection were obtained at retention times 2.453, and 2.467 min, respectively. The specificity of the method was assessed by

comparing chromatograms obtained from drug standards (Fig. 4) with that obtained from gel and injection solutions. The retention times of the drug standards and the drugs from sample solutions were same, so the method was specific. The method was also specific and selective because there was no interference from excipients in the gel and injection as evident from the chromatograms.

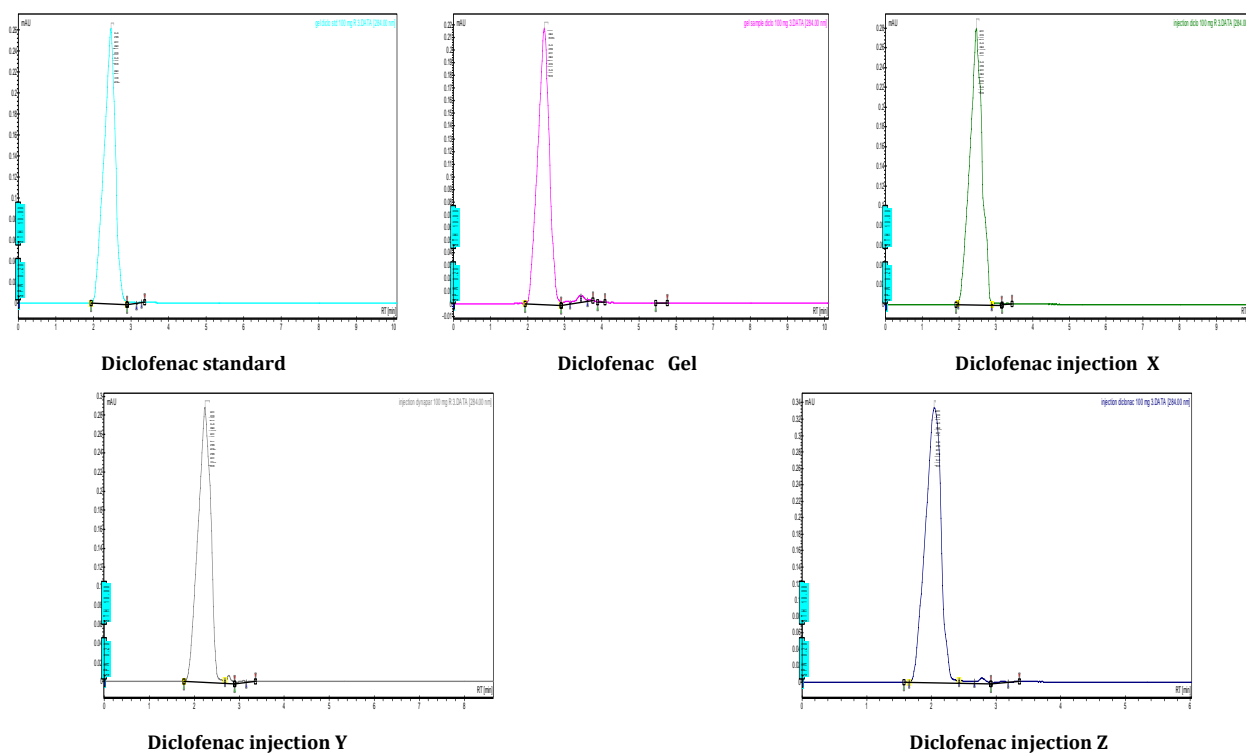


Fig. 4: Chromatograms obtained from gel and injection, with retention times.

## RESULTS AND DISCUSSION

The method approached gives a novelty to the development procedure of Diclofenac in its gel and injectables formulation. The proposed method indicates a comparative less time consuming method development by selecting a solvent system without buffer. In addition it also gives a more economical and allows more analysis to be performed with in very short time. The proposed method retorts with elution of the sample from column within 2.453 min and 2.467 min respectively for gel and injection.

The chromatogram of the gel formulation shows a very sharp peak with better resolution. The injectables were also proceeded with the very same approach and it was perceived from the chromatogram that an extra peak is eluted with better resolution from the main peak. It was further confirmed by injecting three different brand of injectable formulation and noticed that the extra peak is being eluted in the three brand formulation with different peak areas. It was reported that this impurity could have originated from the autoclave cycling during sterilisation<sup>9</sup>. It may be a matter of investigation that the impurity peak is found with different peak area and hence it may be taken into account for further quantification for routine analysis in QC laboratory .

## CONCLUSION

A new, reversed-phase HPLC method has been developed for simultaneous analysis of diclofenac gel and injection formulations. It is shown above that the method was cost effective and less time consuming, accurate, reproducible, repeatable, linear, precise, and selective, proving the reliability of the method. The run time is relatively short, i.e. 10 min, which enables rapid quantitation of many samples in routine and quality-control analysis of gel and injection formulations. The same solvent was used throughout the experimental work and no interference from any excipient was observed. These results show the method could find practical application as a quality-control tool for analysis of Diclofenac from

their different pharmaceutical dosage forms in quality-control laboratories. It may be a matter of further study to explore the use of simple mobile phases excluding buffer for the estimation of various classes of drugs in a variety of dosage forms.

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