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ANALYSIS OF DIFLUNISAL IN DENTAL FORMULATION

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ABSTRACT

A simple, accurate, specific, and precise RP-HPLC method has been developed and validated for the estimation of Diflunisal in bulk and tablet formulation. Chromatographic separation was achieved on Hypersil BDS, C_{18} 250 × 4.6 mm, 5µm column using potassium dihydrogen orthophosphate buffer pH-3.8 adjusted with 10% Orthophosphoric acid and Acetonitrile (55: 45 v/v) as mobile phase in isocratic mode. Flow rate of 1.0ml/min was optimized with detection wavelength at 254 nm. The retention time (Rt) was around 3.2180 min. The method was validated with respect to specificity, selectivity, linearity, accuracy, precision, and robustness as per ICH guidelines. The assay method was observed linear in the concentration range of 2-10µg/ml with a Correlation coefficient (r2) 0.999. The percentage recovery of active pharmaceutical ingredient from tablet formulation ranged from 99.10-99.75%. The Limit of Detection and Limit of Quantification were found to be 0.17µg/ml and 0.56µg/ml, respectively.

INTRODUCTION:

Diflunisal is chemically (2S, 3R, 4R, 5S,6R)-2-(3-{[5-(4-fluorophenyl) thiophen-2-yl] methyl}-4-methylphenyl)-6-(hydroxymethyl) oxane-3, 4, 5-triol.and belongs to the class of SGLT-2 inhibitors. Diflunisal is dental type of nonsteroidal anti-inflammatory drug. Diflunisal is used for pain control. Like all NSAIDs, diflunisal acts by inhibiting the production of prostaglandins and other inflammatory mediators which are involved in inflammation and pain. Diflunisal also has an antipyretic effect, but this is not a recommended use of the drug. It has an empirical formula of $C_{13}H_8F_2O_3$with a molecular weight 250.198 g/moles. It is freely soluble in DMSO, methanol and slightly soluble in water. Diflunisal is a prostaglandin synthase inhibitor and it decreases prostaglandin concenteration in peripheral tissues is marketed as tablet formand has bioavailability of 80-90% and is rapidly absorbed in the gastrointestinal (GI) tract. It has a relative oral bioavailability of 65% and reaches peak concentrations within 2 to 3 hours. Diflunisal is highly protein-bound, mostly to albumin at 99%. The present study was aimed for establishing a simple, accurate, and rapid RP-HPLC method for determination of Diflunisal in presence of

pharmaceutical excipients. The method was validated following analytical performance parameters suggested by ICH guidelines. (1-4)

2. MATERIAL AND METHOD

2.1 Materials: Instrument used in current research HPLC (Shimadzu) (model SPD-20A, LC-20AD). Diflunisalwas provided by Manus akktive Bio-pharmaceutical ltd, Ahmedabad, India as a gift sample. Diflunisal tablet (Dolobid, 500 mg, manufactured by Merck & co.) was purchased from local Pharmacy store.

2.2 Preparation of standard stock solution of Diflunisal: (100µg/ml): Accurately weighed 100 mg of Diflunisalwas transferred into 100ml volumetric flask, and then made up the volume upto the mark with Acetonitrile to give a stock solution having strength of 1000 µg/ml. 100µg/ml of Diflunisal solution was prepared by diluting 10ml of above standard stock solution with Acetonitrile in 100ml volumetric flaskup to the mark.

2.3 Selection of mobile phase: The mobile phase conditions were optimized so that drug would be separated in short run time. Various mobile phases, such as Methanol: Water, Acetonitrile: Water, Methanol: ACN: water, Phosphate buffer: Methanol in different proportion was tried. The combination of **Phosphate buffer: Acetonitrile (pH=3.8)** (**55:45%v/v**) provided optimum polarity for proper migration & separation and of Diflunisal. Under these conditions, the eluted peaks were well defined and resolved. **U**nder this optimized chromatographic condition the retention time of Diflunisal was 3.21 min. The retention time was confirmed by injecting working standard solution.

2.4 Analysis of tablet formulation: For analysis of Diflunisal in tablet, 20 tablets (Dolobid, 500 mg, manufactured by Merck & co) was accurately weighed and average weight was calculated. Tablet was finely powdered. Powder weight equivalent to 100mg of drug containing Diflunisal was dissolved in to a 100 ml volumetric flask made up the volume with Acetonitrile up to the mark. It was sonicated followed by filtration through whatmann filter paper. The filtrate was diluted up to the mark with Acetonitrile. The mixture contains 1000 µg/ml of Diflunisal. From above stock solution of Diflunisal formulation having 1000µg/ml aliquot 10 ml solution in 100 ml volumetric flask and made up the volume up to the mark with Acetonitrile to gives a Standard solution of Diflunisal formulation having concentration 100µg/ml. From above standard stock solution of Diflunisal aliquot 0.6 ml and transfer itinto a 10 ml volumetric flask and the volume was adjusted up to the mark with Mobile phase to attain final concentration 6µg/ml. (Table: 3)

2.5 ANALYTICAL METHOD VALIDATION:

The developed method was validated with respect to linearity, accuracy, and precision, limit of detection and limit of quantification in accordance with the ICH Q2 (R1) guideline.

2.5.1 Specificity: Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc. (fig: 2, 4)

2.5.2 Linearity (calibration curve): The linearity of Diflunisal was found to be in the range of 2-10µg/ml. Linearity of drug was checked in term of slope, intercept and correlation coefficient. (Fig: 5, 6)

2.5.3 Precision: The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: Intermediate (Intraday) precision, Reproducibility (Interday precision), Repeatability.

Table 1: Optical characteristic of diflunisal

S. no.	Parameters	Diflunisal
1	Wavelength	254
2	Linearity range (µg/ml)	2-10
3	Regression equation (y = mx +c)	y = 39281x-559.6
4	Correlation Coefficient (r^2)	0.999
5	Repeatability (% RSD, n=6)	0.5652
6	Intraday Precision (%RSD, n=3)	0.5232 – 0.6098
7	Interday Precision(% RSD, n=3)	0.7463 – 0.7789
8	Accuracy (% Recovery, n=3)	99.10-99.75
9	LOD (µg/ml)	0.17
10	LOQ (µg/ml)	0.56

Table 2: Result of recovery study

Name of Drug	% Level of recovery	Amount of drug Sample (µg/ml)	Spiked Amount Taken (µg/ml)	Total amount found (µg/ml)	% Recovery ±S.D.(n=3)
Diflunisal	50	4	2	5.95	99.16 ± 632.384
	100	4	4	7.98	99.75 ± 536.52
	150	4	6	9.91	99.10 ± 142.25

Table 3: Analysis of marketed formulation by proposed method

Brand Name	Drug Name	Amount taken (mg)	Amount found (mg)	% Assay ± S.D. (n=3)
Invokana	Diflunisal	6	5.964	99.40±0.78

Table 4: Robustness data for diflunisal

Condition	Variation	Diflunisal
		%Assay ± SD (n=3)
Flow rate (1 ml ± 0.1 ml/ min)	0.9 ml/min	97.55 ±269.02
	1.0 ml/min	99.53 ±115.24
	1.1 ml/min	98.31±328.11
Detection wavelength (257 nm ± 2 nm)	270 nm	98.59±583.67
	254 nm	99.76 ±112.24
	274 nm	97.95 ±335.68
Change in Mobile Phase Composition (%V/V/V)	57:43	97.36 ±245.43
	55:45	99.98±589.24
	53:47	98.11±353.74

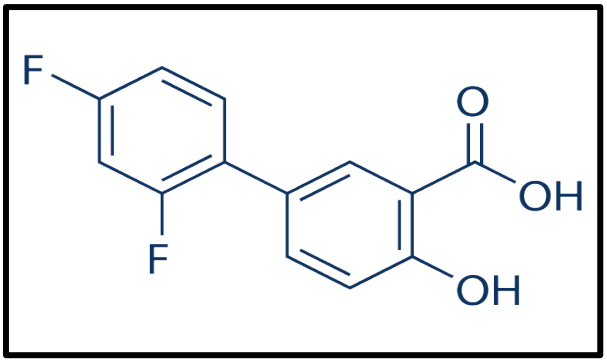

FIGURE 1: Structure of diflunisal

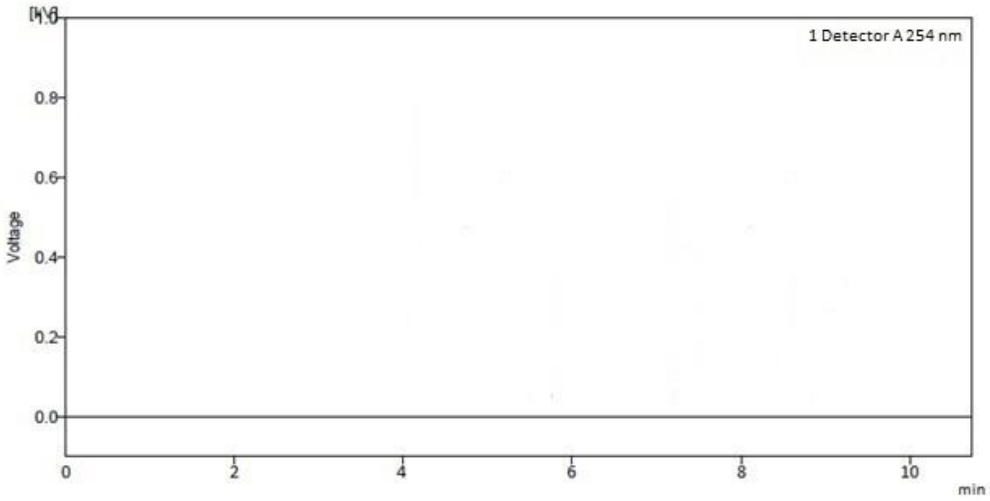

Figure 2: Chromatogram of blank

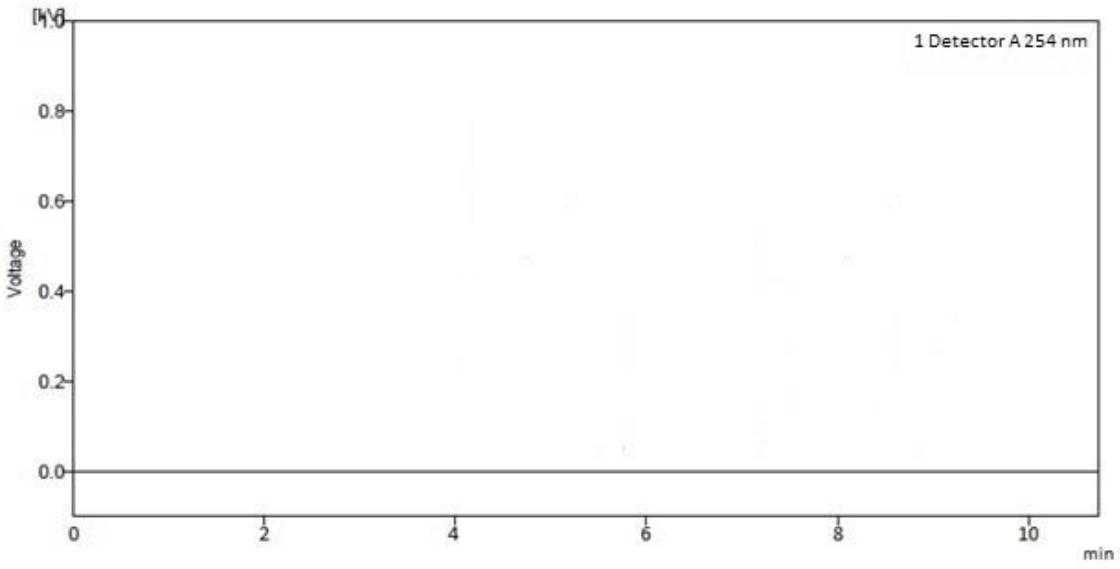

Figure 3: Chromatogram of placebo

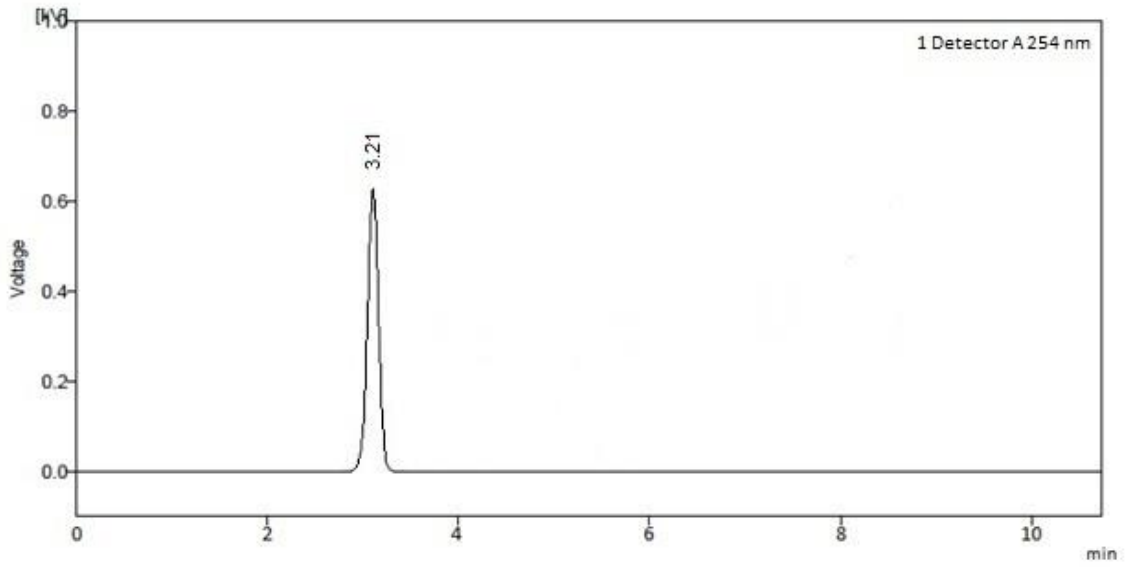

Figure 4: Chromatogram of diflunisal (6µg/ml) in acetonitrile: phosphate buffer (45:55)

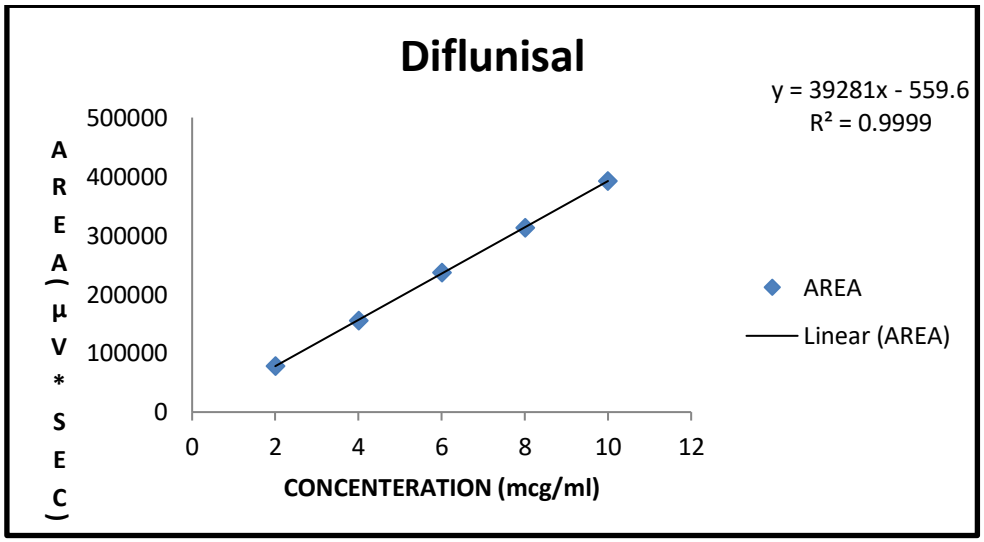

Figure 5: Calibration curve of diflunisal (2-10 µg/ml)

- **Intraday Precision (n=3):** Solutions containing 4, 6, and 8µg/ml of Diflunisal was analyzed three times on the sameday and %R.S.D was calculated. (Table: 1)
- **Interday Precision (n=3):** Solutions containing 4, 6, and 8µg/ml of Diflunisal was analyzed on three differentsuccessive days and %R.S.D was calculated. (Table: 1)
- **Repeatability (n=6):** Solutions containing 6µg/ml of Diflunisal was analyzed for six times and %R.S.D. was calculated. %R.S.D was calculated. (Table: 1)

2.5.4 Accuracy (n=3):

- The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. Accuracy of the

developed method was confirmed by doing recovery study as per ICH guideline at three different concentration levels 50%, 100%, 150% and the values were measured at all wavelengths for 4µg/ml of Diflunisal. (Table: 2)

2.5.5 Limit of detection and Limit of quantification:

Limit of detection (LOD) and limit of quantification (LOQ) of the drug were estimated from the standard calibration curve. The std. deviation of Y- intercept of regression line was used to calculate LOD and LOQ. (Table: 1)

$$LOD = 3.3 \times (\sigma / S)$$
$$LOQ = 10 \times (\sigma / S)$$

Where, σ = standard deviation of the Y intercept of calibration curve

S = Mean slope of the corresponding calibration curve.

2.5.6 Robustness:

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. It should show the reliability of an analysis with respect to deliberate variation in method parameter. (Table: 4) In case of liquid chromatography, examples of typical variations are:

- Influence of variations of pH in mobile phase;
- Influence of variations in mobile phase composition;
- Different columns (different lots and/or suppliers)
- Flow rate

3. RESULT AND DISCUSSION

In the present work a simple reverse phase high performance liquid chromatographic method has been developed, optimized and validated for the estimation of Diflunisal in pharmaceutical formulations. The work is carried out on Hypersil BDS, C18 (100mm x 4.6 mm, 5µ.) in the isocratic mode using a mobile phase consisting of buffer and ACN taken in the ratio 55:45v/v. The analysis performed at ambient temperature using a flow rate of 1.0 ml/min with a run time of 10 min. The eluent was monitored using at a wavelength of 254 nm. The developed chromatographic method was validated for specificity, linearity, precision, accuracy, and robustness. The method was linear in the range of 2-10µg/ml for Diflunisal with correlation coefficient of 0.999. The % Recovery of Diflunisal was found in the range of 99.10-99.75% which indicated that the method is accurate. The % RSD for intraday precision and inter-day precision for Diflunisal were found to be 0.5232 – 0.6098 and 0.7463 – 0.7789, respectively, which indicate the method is precise. (Tab :1) The retention time of Diflunisal was 3.2180 min, the number of theoretical plates was 8445 and tailing factor was 1.22 for Diflunisal, which indicates efficient performance of the column. Selectivity of the method was demonstrated by the absence of any interfering peaks from other coexisting excipient substances at the retention time of the drug. The limit of detection and limit of quantification for Diflunisal were found to be 0.17µg/ml and 0.56µg/ml, which indicate the sensitivity of the method. Validated method was applied for the determination of Diflunisal in commercial formulations. The % assay was found to be 99.40% for Diflunisal. (Table: 1)

4. CONCLUSION

A simple, rapid, sensitive, accurate and precise RP-HPLC method has been developed and validated for routine analysis of Diflunisal. The proposed RP-HPLC method is suitable for estimation of Diflunisal. The developed method was successfully applied in marketed tablet formulation and it can be utilized for the routine analysis of Diflunisal in dental formulation.

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