UV Spectrophotometric/HPLC Method Development, Validation and f2 factor for Quantitative Estimation of Diclofenac Sodium with market product

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Abstract

For the quantification of Diclofenac Sodium in Pharmaceuticals dosage form by using High Performance Liquid Chromatography (RP-HPLC). Chromatographic separation was obtained by using chromatographic conditions on a system for obtaining the results according to USP specifications. The retention time (RT) was 7.6 min per analysis with mobile phase acetonitrile 40% and solution A 60%. The symmetry of column was 15cm x 4.6 mm with packing 5-µm L1 with flow rate 1.5mL/min and wavelength detected on 280nm by using Photodiode array detector. The validation has established the linearity, accuracy, precision, limit of detection (LOD), limit of quantitation (LOQ), and robustness of the test method. The calibration curve we obtained was linear with lowest concentration range 0.01mg/ml to highest concentration range 0.4mg/ml with same RT. The accuracy and precision were also within acceptable range ± 2.0 %. The behaviour of DS was different at room temperature and at freeze. All results were acceptable and this confirmed that the method is suitable for its intended use in routine quality control and assay of drugs.

Key words: Diclofenac sodium, UV spectrophotometric method, HPLC, Validation, f2 factor

Introduction

Diclofenac sodium is a non-steroidal anti-inflammatory medication (NSAID) or COX inhibitor. It is an anti-inflammatory, analgesic, and antipyretic drug that is highly effective. It is frequently used to treat acute and chronic pain, as well as rheumatoid and osteoarthritis. It is a low-molecular-

weight medication composed of 2-(2, 6- dichlorophenyl) amino benzeneacetic acid 4-(3H 1, 2, dithiol-3-thione-5-yl) phenyl ester (MWt: 318.13). The selection of nonsteroidal drug depends on the tolerability, therapeutic efficacy with clinical conditions. Diclofenac sodium [N-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-3-pyrazole-carboxamide] is one of the analgesic-antipyretic-nonsteroidal anti-inflammatory drug. Diclofenac is a widely used for the treatment of rheumatoid arthritis, osteoarthritis and ankylosing spondylitis osteoarthritis, musculoskeletal injuries, and post-surgery analgesia in human and veterinary medicine The molecule is practically water insoluble, but it is readily absorbed from the gastrointestinal tract as the salt form[1]. For the quantification of DS, various analytical techniques have been employed. The best method is Reverse phase High-Performance Liquid Chromatography (RP-HPLC-20AD Shimadzu) for the analysis of dosage or biological sample. The method should be reliable, selective, robust, and sensitive for the determination of DS in the dosage form for pharmaceuticals products. The assay method was validated using by USP 26[2]. The linearity, accuracy, precision, specificity, limit of detection (LOD)[3], and limit of quantification (LOQ) are used to determine the drug concentration of DS in various pharmaceutical commercial products. For the determination of Diclofenac sodium concentrations in pharmaceutical dosage forms, the present study presents the invention of a simple, precise, accurate, and reproducible spectrophotometric approach. The devised method was validated in accordance with the International Conference on Harmonization (ICH) Guidelines and was successfully used to the test Pharmaceutical preparation and dosage form[4]. Patients with chronic pain who score at least 50% on the visual analogue scale (VAS: 0–100 mm) are eligible for high-potency opioid treatment.

2. Materials and methods

2.1. Materials

Diclofenac Sodium was given as a gift by Variant Pharmaceuticals and was utilized as a working standard for raw material testing and stability studies. All additional chemicals and reagents were of analytical quality and were used exactly as they arrived. The water was deionized and purified used as Row water for the preparation of lab solutions. For the determination of the content of Diclofenac sodium in conventional tablets, randomly select from the market.

2.2. Liquid Chromatography Conditions

The HPLC system consisted of Shimadzu (21AD) with photodiode array detector for the detection and separation of components from the column at specific RT with flow rate 1.5ml/min. The column used dimensions 15cm x 4.6 mm with an Injection volume of 10µl at 280nm. Standard and samples was filtered with through a membrane filter of 0.45 µm (Sigma-Aldrich). The HPLC system was operated at 30°C. Before performing the analysis, degas the mobile phase using sonication for up to 5-10 minutes.

2.3. Preparation of Stock Solutions

The stock solution was prepared weight accurately DS or Working Standard, transfer to 100ml flask and dissolve it in 70 ml of diluent and dilute with diluent to 100 ml volume. Take 5ml of above solution in a 25 ml volumetric flask and dilute with diluent to the volume for final concentration 0.1mg/ml.

2.4 Validation of Diclofenac HPLC assay

The RP-HPLC method was developed and validated in term of linearity, accuracy, precision, limit of detection, limit of quantitation, and robustness of the test method. Three standard calibration curves were created at different periods to assess linearity and accuracy.

2.4.1. Specificity

Drug	Sr.No	Concentration (mg/ml)	Injections	Retention time (RT)	Peak Areas
Diclofenac Sodium	**1**	0.01	1	7.689	240859
	2	0.02	1	7.682	472446
	3	0.05	1	7.677	1182884
	4	0.1	1	7.672	2404731
	5	0.2	1	7.681	4713802
	6	0.4	1	7.706	9593311

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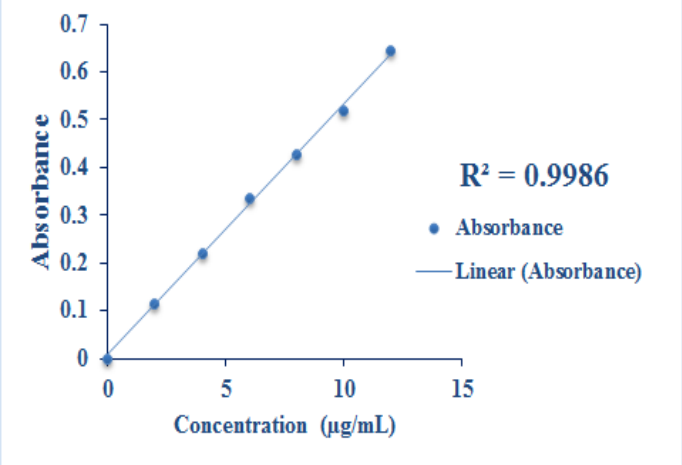

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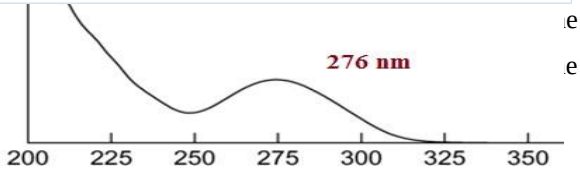


2.4.2. System Specificity

For examining the system suitability of DS, six replicate analyses were used at concentration 20 µm/ml. The acceptance criteria were ± 2.0 % for the relative standard deviation (%RSD) for retention time (RT) and peak area under curve.

Instrumentation:

The spectrophotometer utilised was a double beam UV-VIS spectrophotometer (UV-1900 I Shimadzu, Japan) connected to a computer running the spectra manager software, UV Probe. It was possible to acquire the spectra by using the following instrumental parameters: The wavelength range is 200–800 nanometers. An electronic balance was used to record all of the weights taken (Model Shimadzu AUX 120).

Results:

Method Development

that the solution of diclofenac sodium in methanol exhibits maximum absorption at 276 nm after scanning on a UV-Vis spectrophotometer. This absorption peak has been reported previously as max in the literature[5]. Furthermore, the obtained drug sample of diclofenac sodium conforms to the reference spectra (Figure 1).

Figure 1. UV spectra of Diclofenac sodium

3.1 Linearity and range

Figure.1: UV-VIS Spectrophotometer Spectra by using 1900i

Linearity tells about the direct relationship between results and analyte concentration. Six injections of the same medication at different concentrations were used to test linearity. Linearity experiments were conducted to identify the range over which Diclofenac sodium exhibit linear response. The stock solution of Diclofenac sodium was prepared by dissolving 100 mg of finely powder homogeneous sample of Diclofenac sodium into 100 mL of diluent for final concentration of 1.0 mg/ mL of Diclofenac sodium.

The stock solution was gravimetrically diluted in diluent to concentrations of 10ppm (0.01 mg/ml) ,20ppm (0.02 mg/ml) ,50ppm (0.05 mg/ml) ,100ppm (0.1 mg/ml) ,200ppm (0.2 mg/ml) and 400 ppm (0.4 mg/ml) respectively. The calibration graph is shown below, indicates linear relationship observed between the concentration and absorbance of the solutions. The R2 of calibration data point was calculated to 0.999. This indicates that the test procedure obeys Beer's law.

Table.1: Linearity of Diclofenac Sodium at different ppm concentration and peak areas

Figure.2: Calibration Curve for Diclofenac Sodium

The calibration graph is showing linear relationship between the concentration and absorbance of the solutions. There by indicating that the test procedure follows Beer's law.

Figure.3: Relationship between concentration and peak areas

Repeatability Diclofenac sodium:

It was calculated from the results of six consecutive determinations. To check repeatability, six samples were drawn and test solutions were prepared and tested according to the test procedure. Weight of 50 mg working standard having potency 99.8% for comparing the peak area of standard and samples. Weight of reference standard by using butter paper on analytical balance is 50.3mg with respect to potency of standard 99.8%**.** After preparing the reference standard and solutions for repeatability, we divide each solution into vials for the tray of HPLC. To prepare HPLC for analysis, we begin by cleaning it with filtered water and HPLC grade methanol. After a washing period of up to 25-30 minutes, purge the auto sampler using a purging solution composed of 40% water and 60% methanol. Now we start the saturation of column with mobile phase. If the mobile phase is more polar, peaks will appear first. Peak retention time (RT) is dependent on the polarity of the mobile phase in comparison to the stationary phase adsorbent in the column. At first we start from single run to judge the peak retention time, after single run we become able to add RT for other analysis. We start from 5 standard samples at first and then we start our sample analysis which depends on you, how much injections are needed to explore the results. We have got certain peak areas after analysis with respect to standard are 1-2290444, 2-2289550, 3- 2288859, 4-2288364, 5- 2287524 with average peak area is 2288948.

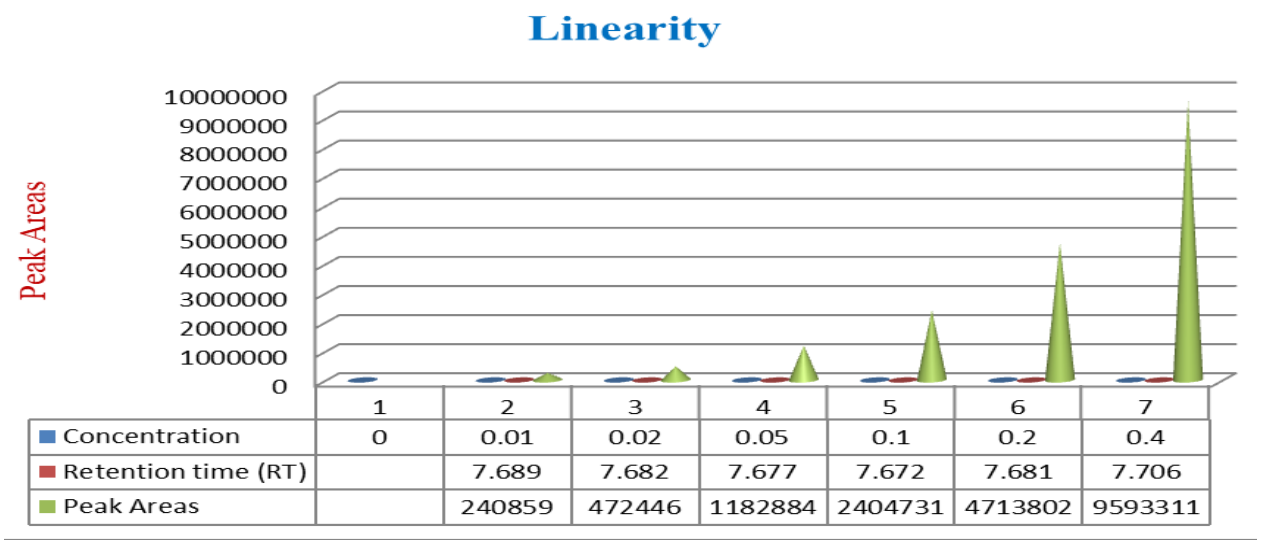


Table.2: Repeatability of Diclofenac Sodium

Sr. No.	Concentration (mg/ml)	Injections	Retention time (RT)	Peak areas	Average peak areas of test solution	% age Results of HPLC	Variation from theoretical Results
1	0.1mg/ml	1	7.656	2406613	2408238	101.66%	**1.66%**

		2	7.654	2408713			
		3	7.647	2409390			
2	0.1mg/ml	1	7.647	2361534	2361323	101.59%	**1.59%**
		2	7.649	2361420			
		3	7.653	2361015			
3	0.1mg/ml	1	7.658	2353305	2352768	100.58%	**0.58%**
		2	7.669	2353400			
		3	7.676	2351601			
4	0.1mg/ml	1	7.672	2293291	2296691	101.39%	**1.39%**
		2	7.675	2297969			
		3	7.667	2298813			
5	0.1mg/ml	1	7.663	2301922	2301554	100.95	**0.95%**
		2	7.660	2301037			
		3	7.657	2301702			
6	0.1mg/ml	1	7.655	2368037	2367802	100.59%	**0.59**
		2	7.655	2367828			
		3	7.652	2367540			

Drug **Repeatability**	**Range**	**Mean**	**Standard Deviation (SD)**	**Relative Standard Deviation (RSD)**
Diclofenac Sodium	100.58%---- 101.66%	101.12%	0.487%	0.481% ± 2.0 %

Reproducibility of Diclofenac sodium

To check the repeatability; 2 sets of five samples equivalent to 100 % of label claim were prepared and assayed by two analysts individually.

Analyst 01

Table.3: Reproducibility-1or analyst 1 of Diclofenac Sodium

Sr. No.	Concentration (mg/ml)	Injections	Retention time	Peak areas	Average peak areas of test solution	% age Results of HPLC	Variation from theoretical Results
1	0.1mg/ml	1	7.664	2368817	2364788	101.15%	**1.15%**
		2	7.670	2365317			

		3	7.675	2360231			
2	0.1mg/ml	1	7.676	2296347	2297708	99.55%	**-0.45%**
		2	7.675	2298194			
		3	7.670	2298582			
3	0.1mg/ml	1	7.666	2369169	2368967	101.33%	**1.33%**
		2	7.667	2368991			
		3	7.662	2368742			
4	0.1mg/ml	1	7.662	2290219	2292073	99.31%	**-0.69%**
		2	7.659	2290208			
		3	7.660	2295791			
5	0.1mg/ml	1	7.658	2353331	2353186	101.30%	**1.30%**
		2	7.657	2352459			
		3	**7.656**	**2353767**			

Drug **Analyst-1**	**Range**	**Mean**	**Standard Deviation (SD)**	**Relative Standard Deviation (RSD)**
Diclofenac Sodium	99.31%--101.33%	100.52%	1.008%	1.00% ± 2.0 %

Analyst 02

Table.4: Reproducibility-1or analyst 1 of Diclofenac Sodium

Sr. No.	Concentration (mg/ml)	injections	Retention time	Peak areas	Average peak areas of test solution	% age Results of HPLC	Variation from theoretical Results
1	0.1mg/ml	1	7.773	2376973	2377465	100.65%	**0.65%**
		2	7.776	2377780			
		3	7.773	2377642			
2	0.1mg/ml	1	7.771	2319169	2318808	100.08%	**0.08%**

		2	7.768	2318914			
		3	7.764	2318340			
3	0.1mg/ml	1	7.762	2316768	2317004	99.0%	**-1.0%**
		2	7.756	2316682			
		3	7.757	2317561			
4	0.1mg/ml	1	7.748	2369231	2371982	101.04%	**1.04%**
		2	7.741	2373041			
		3	7.744	2373673			
5	0.1mg/ml	1	7.748	2438961	2438894	101.28%	**1.28%**
		2	7.754	2439276			
		3	**7.760**	**2438444**			

Drug **Analyst-2**	**Range**	**Mean**	**Standard Deviation (SD)**	**Relative Standard Deviation (RSD)**
Diclofenac Sodium	98.4%--101.71%	100.05%	0.227%	0.2272% ± 2.0 %

Robustness of Diclofenac sodium

It is the measure how stable the test procedure is under slight variation in test procedure. Robustness can be defined as the ability to replicate the (analytical) method in different laboratories or under different conditions without encountering unexpected differences in the obtained result(s), and robustness test can be defined as an experimental setup used to assess the robustness of a method. The phrase ruggedness is frequently used interchangeably with robustness[6]. Robustness testing is most well-known and widely used in the pharmaceutical industry today, owing to the stringent rules imposed by regulatory agencies in that domain, which demand fully verified procedures. As a result, the majority of definitions and existing procedures, such as those from the ICH, may be found in that field, as demonstrated above. This has no bearing on the robustness testing of analytical methods used in other sectors, and so this guidance is not limited to pharmaceutical approaches[7]. The following changes were made deliberately in testing procedure. The test solution prepared according to the test procedure and kept at 15°C , 25° C and 35°C for 24 hours and assayed according to the test procedure. The results are compound with initial results and tabulated below. Weight of Reference Standard = 50.2 mg, Purity 99.8%

Table.5: Reproducibility-1or analyst 1 of Diclofenac Sodium

Storage Condition	15°C		25°C		35°C	
	Sample I	Sample II	Sample I	Sample II	Sample I	**Sample II**
Weight of Samples	157 mg	155 mg	155mg	155 mg	160 mg	**157 mg**
Peak area of test solution	2387468	2323634	2323947	2375139	2449200	**2396416**
% of Label Claim	100.73%	99.32%	99.32%	101.52%	100.78%	**100.48%**
Average	100.02 %		100.42 %		**100.63 %**	
Standard Deviation	**0.02%**		**0.42%**		**0.63 %**	

Sensitivity: Sensitivity of the proposed method was estimated in terms of Limit of Detection (LOD) and Limit of Quantitation (LOQ) (Table 6).

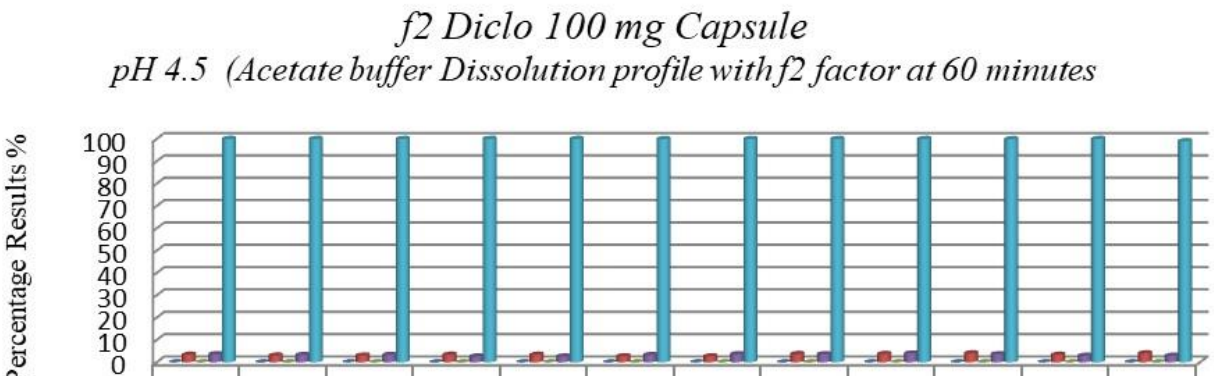
Table.6 Drug Sensitivity

Drug	LOD	LOQ
Diclofenac Sodium	0.19 ± 0.004	0.44 ± 0.011

F2 Factor at different P^H & Comparative Dissolution Profile:

There is a rising realization of the need of submitting a quality regulatory submission, especially when FDA refuses to file an application under 21 CFR314.10 because the application or abbreviated application is incomplete (does not on its face contain information necessary under section 505(b) and 21 CFR 314.50). This is what happened to Merck & Co when they first submitted their NDA, ezetimibe atorvastatin tablets. Pertinent to this commentary, two out of the four reasons cited for the filing deficiency[8]. At least three guidance documents contain information about f2: Guidance for Industry, Dissolution Testing of Immediate Release Solid Oral Dosage Forms (2), Guidance for Industry, Waiver of In Vivo Bioavailability and Bioequivalence Studies for Immediate Release Solid Oral Dosage Forms Using a Bio pharmaceutics Classification System (3), and Guidance for Industry, Immediate Release Solid Oral Dosage Forms[9]. While these three guidance's provide essentially the same information, the most comprehensive

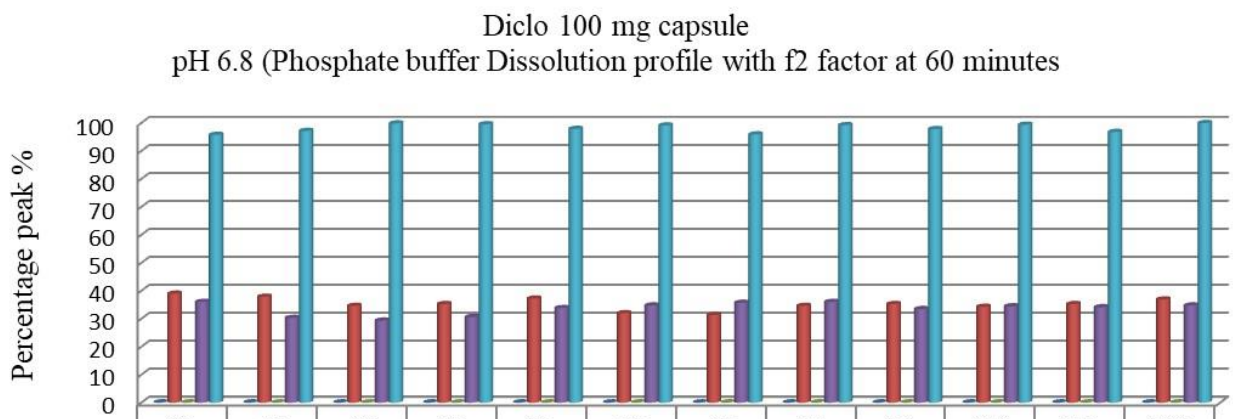
discussion of the problem is found in the Guidance for Industry, Dissolution Testing of Immediate Release Solid Oral Dosage Forms[10].



	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
abs.	0.01	0.009	0.009	0.01	0.01	0.008	0.008	0.011	0.011	0.012	0.01	0.012
Result	3.38	3.04	3.04	3.38	3.38	2.7	2.7	3.71	3.71	4.05	3.38	4.05
abs.	0.011	0.01	0.01	0.008	0.008	0.01	0.011	0.011	0.012	0.011	0.009	0.009
Result	3.63	3.3	3.3	2.64	2.64	3.3	3.63	3.63	3.96	3.63	2.97	2.97
f2 (in %)	100.00	99.99	99.99	100	100	99.94	99.94	99.97	99.97	99.9	100	99

Figure.4: The dissolution profiles of diclofenac sodium and the commercial product Mobikare were compared at pH 4.5 in an acetate buffer at 60 minutes

One limitation of current criteria is that in vivo release may be multiphasic, reducing the f2 metric's ability to predict in vivo product. For instance, if an oral product is developed to act locally and is designed to remain intact until it reaches a pH of 6.8 or greater (reaches the distal GI tract), and then a burst of drug (greater than 80%) is released 15–30 minutes later, one could argue that this profile is comparable to that of an immediate-release product dissolution and that the f2 rules would apply. It may be prudent to define the criteria as >85% dissolved in 60 or 90 minutes at a pH 6.8 phosphate buffer[11].



	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
abs.	0.119	0.116	0.106	0.108	0.114	0.098	0.096	0.106	0.108	0.105	0.108	0.113
Result	38.98	37.87	34.61	35.26	37.22	32	31.34	34.61	35.26	34.28	35.26	36.89
abs.	0.113	0.095	0.092	0.096	0.106	0.109	0.112	0.113	0.105	0.108	0.107	0.109
Result	36.03	30.29	29.33	30.61	33.8	34.75	35.71	36.03	33.48	34.44	34.12	34.75
f2 (in %)	95.74	97.13	99.82	99.52	97.87	99.11	95.89	99.2	97.78	99.34	96.78	99.98

Figure.5: The dissolution profiles of diclofenac sodium and the commercial product Mobikare were compared at pH 6.8 in Phosphate buffer at 60 minutes

Range:

Based on linearity results test range of analytical control procedure for assay of Diclofenac sodium is conformed as 0.01 to – 0.4 mg/ml

Specificity:

Specificity of the analytical control procedure of assay of Diclofenac sodium in Diclo 50 mg capsule is not determined by taken peak area of the placebo sample. The peak area obtained is 0.

Dosage:

In adults, the initial dosage is 25 to 50mg three times daily, depending on the severity of the condition. The maintenance dose should be adjusted to the minimum that will provide continuous therapeutic control. The tablets should ~ swallowed whole, with or after a meal. The dosage in children is 2 to 3mg per kilogram bodyweight daily

Conflict of Interest: The authors have declared that they have no conflicts of interest.

Conclusion:

The proposed UV spectrophotometric and HPLC approach was found to be extremely simple, quick, and cost-effective. With excellent recovery precision, f2 factor for the comparative dissolution with market product Mobikare, linearity, repeatability, analyst 1, analyst2 and robustness this method has been verified in accordance with ICH recommendations and is acceptable for the measurement of Diclofenac sodium.

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