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Comparative Analysis of Different Brands of Diclofenac Sodium Extended Release Tablets Available in Pharmacies at Perambalur

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A B S T R A C T

The aim of the present study is to analyze the different brands of diclofenac sodium extended release tablets available in pharmacies at Perambalur. Diclofenac Sodium is an analgesic agent which is classified under Non steroidal anti-inflammatory drug (NSAIDs). It is the good drug of choice for the treatment of muscle aches, back aches, strains, sprains, dental pain, menstrual cramps, and sports injuries. It can also reduce pain, swelling, and joint stiffness caused by rheumatoid arthritis and osteoporosis. The diclofenac is the commonly prescribes drug to relieve pain. Currently various brands of diclofenac sodium are available in the local market. In case, there is lack in prescribed brand at the pharmacies in Perambalur, the alternative brand is essential to treat the patients. During this alternative, the efficiency and quality of the drug is important. Hence, there is the need to reveal the quality of different brands to ensure the safety of the patients. The comparative analysis was done under four different brands of diclofenac sodium available in pharmacies at Perambalur. These brands were tested through different parameters in accordance to the guidelines given in IP i.e. weight variation, hardness, friability, disintegration, dissolution, assay. The result shows that all the parameters (weight variation, hardness, friability, disintegration, dissolution, assay) are within the IP limits. We conclude that all the brands are in accordance with IP, therefore we can go for the alternative brand in diclofenac without any hesitation.

Keywords: Diclofenac sodium, enteric coated tablets, weight variation, hardness, friability, disintegration, dissolution, assay

A R T I C L E I N F O

CONTENTS

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1. Introduction

Analytical methods: The number of drugs introduced into the market is increasing every year. These drugs may be either new entities or partial structural modification of the existing one. Often a time lag exists from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias[1]. This happens because of the possible uncertainties in the continuous and wider usage of these drugs, reports of new toxicities (resulting in their withdrawal from the market), development of patient resistance and introduction of better drugs by competitors. Under these conditions, standards and analytical procedures for these drugs may not be available in the pharmacopoeias. It becomes necessary, therefore to develop newer analytical methods for such drugs[2]. Analytical methods should be used within good manufacturing practice (GMP) and good laboratory practice (GLP) environments, and must be developed using the protocols set out in the International Conference on Harmonization (ICH) guidelines (Q2A and Q2B). [3,4]

Physical methods of analysis involve the study of the physical properties of a substance; they include determination of the solubility, transparency or degree of turbidity, color, density or specific gravity (for liquid), moisture content and melting, freezing and boiling points. Physico-chemical methods are employed to study the physical phenomenon that occurs as a result of chemical reactions[5].

Among the physico-chemical methods the most important are optical (refractometry, polarimetry, emission and the fluorescence methods of analysis, photometry including photo colorimetry, and spectroflourimetry covering UV-Visible and IR regions, nephelometry and turbidimetry) electrochemical (potentiometry, colorimetry, amperometry and polarography) and chromatographic methods like (column, paper, thin layer, gas liquid, high performance liquid) etc. methods involving nuclear reactions such as nuclear magnetic resonance (NMR), paramagnetic resonance (PMR) are becoming more and more popular. The combination of liquid chromatography with mass spectroscopy is one of the most powerful tools available[6-9].

In this contrast, pharmaceutical analysis plays an important role in the quality assurance and quality control of bulk drug samples and pharmaceutical formulations. The spectrophotometric methods on the other hand are very simple and do not involve high cost[10]. They are easy to carryout, the instruments used need very little maintenance and no specially trained operators are necessary. Their drawback however is that they are not as sensitive as HPLC[11]. Once a stability-indicating method is in place, the formulated drug product can then be subjected to heat and light in order to evaluate potential degradation of the API in the presence of formulation excipients[12,13].

Metformin is a biguanide antihyperglycemic agent used for treating non-insulin-dependent diabetes mellitus (NIDDM). It improves glycemic control by decreasing hepatic glucose production, decreasing glucose absorption and increasing insulin-mediated glucose uptake. Metformin is the only oral antihyperglycemic agent that is not associated with weight gain[14].

Metformin may induce weight loss and is the drug of choice for obese NIDDM patients. When used alone, metformin does not cause hypoglycemia; however, it may potentiate the hypoglycemic effects of sulfonylureas and insulin. Its main side effects are dyspepsia, nausea and diarrhea. Glibenclamide[15] is an oral antihyperglycemic agent used for the treatment of non-insulin-dependent diabetes mellitus (NIDDM). It belongs to the sulfonylurea class of insulin secretagogues, which act by stimulating β cells of the pancreas to release insulin. Sulfonylureas increase both basal insulin secretion and meal-stimulated insulin release. Medications in this class differ in their dose, rate of absorption, duration of action, route of elimination and binding site on their target pancreatic β cell receptor.

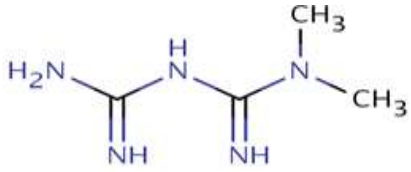


Figure 1: Metformin

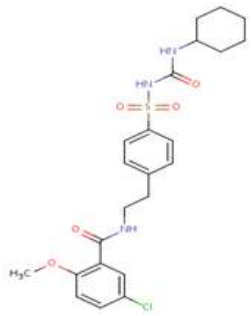


Figure 2: Glibenclamide

2. Materials and Methods

Apparatus

The instrument used for the study was Shimadzu HPLC with PDA detector having Spinchrome software version.

Reagents and Materials

The solvents used were Methanol, Acetonitrile, Potassium dihydrogen ortho phosphate, Dipotassium hydrogen phosphate, Orthophosphoric acid, Ammonium acetate and HPLC Water.

Selection of detection wavelength:

The sensitivity of method that uses PDA detector depends upon the proper selection of wavelength. An ideal wavelength is that gives maximum absorbance and good response for both the drugs to be detected. Standard solutions of Metformin and Glibenclamide were scanned in the UV range (200-400nm) and the spectrums obtained were overlaid and the overlain spectrum was recorded. From the overlain spectrum 210 nm was selected as the detection wavelength for the present study.

Selection of mobile phase

Initially the mobile phase tried was Methanol and water, Methanol, Buffer and water in various proportions. Finally, the mobile phase was optimized to Phosphate buffer (KH_2PO_4) of pH5.8: Acetonitrile (70:30 v/v) respectively.

Chromatographic trials for Simultaneous Estimation of Metformin and Glibenclamide by RP- HPLC.

Trial-1 Chromatographic Conditions:

Column: Inertsil ODS 3V (250×4.6mm,5µ)
Flow rate: 1ml/min
Injection volume: 20 µl
Wavelength: 210nm
Column temperature: 25 ºc
Mobile Phase: Potassium phosphate: Methanol: ACN (50:40:10)
pH : 3

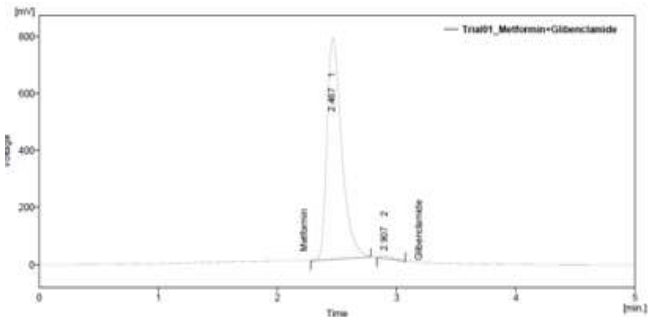


Figure 3: Trial 1 chromatogram

Observation:

The peak response of Glibenclamide was less .i.e.10mV. The Theoretical plate count of Metformin was less than 2000 and hence this trial was not optimized.

Trial 2 Chromatographic Conditions:

Column: Inertsil ODS 3V (250×4.6mm,5µ)
Flow rate: 1.0ml/min
Wavelength: 210nm
Injection volume: 20 µl
Column temperature: 25 ºc
Mobile Phase: (Na_2HPO_4) Buffer: Methanol (30:70)
pH : 7.0

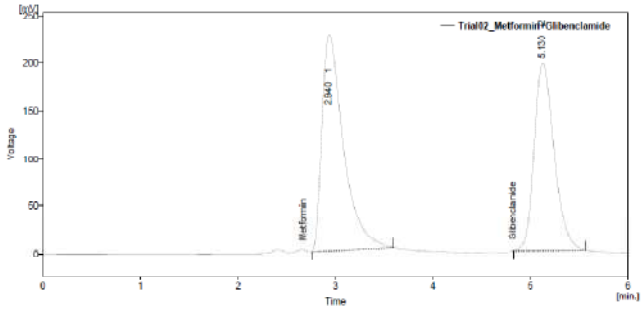


Figure 4: Trial 2 chromatogram

Observation:

Asymmetry was more than 2.0 and efficiency was less than 2000 for Metformin. This peak cannot be considered. So this trial was not optimized.

Optimized Chromatographic Method

Flow rate: 1ml/min
Column: Inertsil ODS (250*4.6mm) 5µ
Wavelength: 210nm
Injection volume: 20µl
Column temperature: 25 ºc
Mobile Phase**:** Phosphate buffer (KH_2PO_4): Acetonitrile (70:30)
pH of buffer: 5.8

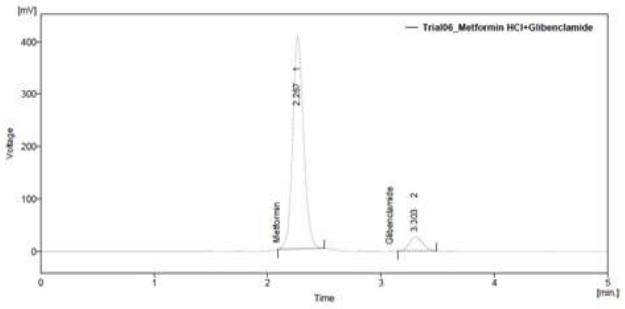


Figure 5: Optimized chromatogram

Observations:

There is good resolution (>1.5) between the peaks and USP Tailing is within the limit (Not more than 2) and USP plate count is > 2000, hence as the system suitability parameters passed as per ICH guidelines .Hence this trial was selected as optimized method.

Preparation of Phosphate (KH2PO4) buffer 20mM

2.72 gm. of Potassium dihydrogen phosphate was weighed and dissolved in 100ml of water and volume was made up to 1000ml with water. Adjust the pH to 5.8 using ortho phosphoric acid. The buffer was filtered through 0.45µ filters to remove all fine particles and gases.

Preparation of mobile phase

Mix a mixture of above buffer 700 ml (70%) and 300 ml of Acetonitrile (HPLC grade- 30%) and degassed in ultrasonic water bath for 5 minutes. Filter through 0.22 µ filter under vacuum filtration.

Diluents preparation

Mobile phase was used as the diluent.

Preparation of standard stock solution of Metformin

25 mg of Metformin was weighed and transferred in to 250ml volumetric flask and dissolved in methanol and then make up to the mark with methanol and prepare 10 µg /ml of solution by diluting 1ml to 10ml with methanol.

Preparation of standard stock solution of Glibenclamide

25 mg of Glibenclamide was weighed in to 250ml volumetric flask and dissolved in Methanol and then dilute up to the mark with methanol and prepare 10 µg /ml of solution by diluting 1ml to 10ml with methanol.

Preparation of standard solution

250 mg of Metformin and 2.5 mg of Glibenclamide was dissolved in 100 ml of Diluent and was further diluted to get stock solution of Metformin and Glibenclamide. From this 1mL of the solution was transferred to 10 mL volumetric flask and made up with diluent. (This solution contains 250µg/ml and 2.5µg/ml of Metformin and Glibenclamide Respectively).

Preparation of sample solution:

20 tablets (each tablet contains 500 mg of Metformin and 5 mg of Glibenclamide) were weighed and taken into a

mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of Metformin and Glibenclamide (µg/ml) were prepared by dissolving weight equivalent to 500 mg of Metformin and 5 mg of Glibenclamide and dissolved in sufficient mobile phase. After that filter the solution using 0.45-micron syringe filter and Sonicated for 5 min and dilute to 100 ml with mobile phase. The filtered solution was further diluted (1 to 10 ml) in the diluent to make the final concentration of working sample equivalent to 100% of target concentration.

Procedure

20µL of the blank, standard and sample were injected into t he chromatographic system and areas for the Metformin and Glibenclamide the peaks were used for calculating the % assay by using the formulae.

System suitability

- Tailing factor for the peaks due to Metformin and Glibenclamide in standard solution should not be more than 1.5.
- Theoretical plates for the Metformin and Glibenclamide peaks in standard solution should not be less than 2000.

Assay calculation

$$\textbf{Assay}\ \% = \frac{\textbf{sample area}}{\textbf{Standard area}} \times \frac{\textbf{dilution sample}}{\textbf{dilution of standard}} \times \frac{\textbf{P}}{\textbf{100}} \times \frac{\textbf{Avg.wt}}{\textbf{Lc}} \times \textbf{100}$$

Where:

Avg.wt = average weight of tablets

P= Percentage purity of working standard

LC= Label Claim of Metformin and Glibenclamide in mg/ml.

3. Results and Discussion

Method Validation Parameters

1. Specificity: The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The specificity was performed by Injecting blank and sample.

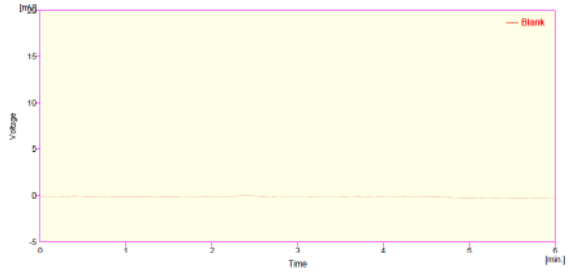


Figure 7: Chromatogram of Blank

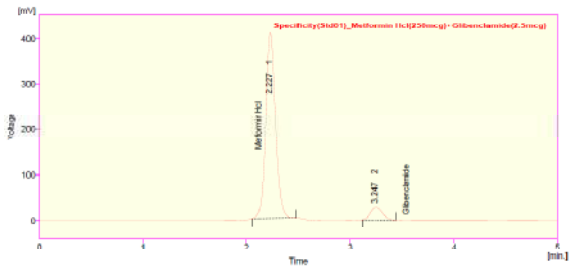


Figure 7: Chromatogram of Sample

2. Linearity: Linearity of the method was determined by constructing calibration curves. Standard solutions of Metformin and Glibenclamide of different concentrations level (50%, 75%, 100%, 125%, and 150%) were used for this purpose. Each measurement was carried out in six replicates and the peak areas of the chromatograms were plotted against the concentrations to obtain the calibration curves and correlation coefficients.

Acceptance criteria: Correlation coefficient should be not less than 0.999.

3. Range

Based on precision, linearity and accuracy data it can be concluded that the assay method is precise, linear and accurate in the range of 150 ppm to 350 ppm for Metformin and 1.5 ppm to 3.5 ppm for Glibenclamide

4. Accuracy: Accuracy of the method was determined by recovery experiments. There are mainly 2 types of recovery studies are there.

a) **Standard addition method:** To the formulation, the reference standard of the respective drug of known concentration was added, analyzed by HPLC and compared with the standard drug concentration.

b) **Percentage method:** For these assay method samples are prepared in three concentrations of 50%, 100%, and 150% respectively.

Acceptance criteria: The mean % recovery of Metformin and Glibenclamide at each level should be not less than 95.0% and not more than 105.0%.

Assay procedure

10µL of the standard and sample solutions of Metformin and Glibenclamide were injected into the HPLC system and the chromatograms were recorded. Amount of drug present in the Tablets were calculated using the peak areas.

5. Precision

Method precision also called as repeatability /Intra-day precision indicates whether a method gives consistent results for a single batch. Method precision was demonstrated by preparing six test solutions at 100% concentration as per the test procedure & recording the chromatograms of six test solutions. The % RSD of peak areas of six samples was calculated. The method precision was performed on Metformin and Glibenclamide formulation.

Acceptance criteria

The % RSD for the area of sample injections results should not be more than 2.

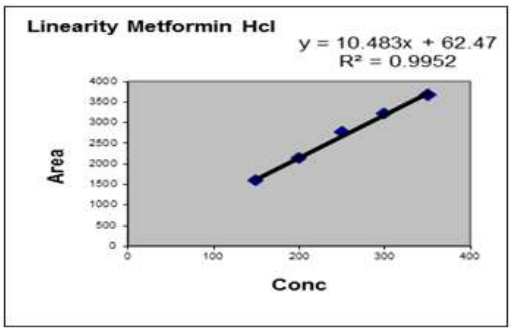
Selection of solvent

Solutions of Metformin and Glibenclamide were prepared by dissolving in mobile phase and UV spectrum of each was recorded by scanning between 200-400 nm.

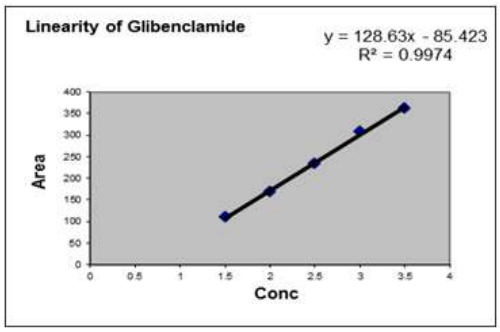
Validation of the Method

Linearity

Metformin and Glibenclamide: Serial dilutions of 150 ppm to 350 ppm for Metformin and 1.5 ppm to 3.5 ppm for Glibenclamide were injected into the column and detected at a wavelength set at 210 nm. The calibration curve was obtained by plotting the concentration vs. peak area and the correlation coefficient was found to be 0.995 and 0.997 respectively.



Linearity graph of Metformin



Linearity graph of Glibenclamide

Table 1: Linearity of Metformin and Glibenclamide

Metformin			Glibenclamide	
S.No.	**Conc. µg/ml**	**Peak Area**	**Conc. µg/ml**	**Peak Area**
1	150	1598.165	1.5	109.89
2	200	2141.807	2	167.979
3	250	2777.099	2.5	233.116
4	300	3218.275	3	308.562
5	350	3680.663	3.5	361.164
S.D.	79.06	831	0.79	102
Slope	10.48		128.60	

Table 2: Showing accuracy results for Metformin and Glibenclamide

Metformin				
Sample no.	**Spiked Amount (mcg)**	**Recovered Amount (mcg)**	**% Recovered**	**% Average recovery**
1	250	251.23	100.49	100.02%
2	300	302.48	100.83	
3	350	345.61	98.75	
Glibenclamide				
Sample no.	**Spiked Amount (mcg)**	**Recovered Amount (mcg)**	**% Recovered**	**% Average recovery**
1	2.5	2.52	100.90	100.24 %
2	3	2.96	98.63	
3	3.5	3.54	101.20	

Table 3: Result of Robustness study

Parameter	**Metformin**		**Glibenclamide**	
	Rt(min)	**Tailing factor**	**Rt(min)**	**Tailing factor**
Flow				
0.8mL/min	2.800	1.281	4.107	0.157
1.0 mL/min	2.337	1.259	3.413	1.219
Wavelength				
208nm	2.233	1.222	3.270	1.258
210nm	2.337	1.259	3.413	1.219
212nm	2.247	1.269	3.280	1.290

Table 4: % RSD results of Metformin and Glibenclamide

Drug	**%RSD**
Metformin	1.68
Glibenclamide	1.70

Table 5: Ruggedness Results of Metformin

S.No.	Sample name	Name	RT	Area	USP Tailing	USP plate count
1	**Analyst-1(Std)**	Metformin	2.263	2746.283	1.222	**2658**
2	**Analyst -2(Std)**	Metformin	2.240	2733.829	1.308	2603
3	**Analyst 1 (Spl)**	Metformin	2.240	2713.213	1.222	2443
4	**Analyst 2 (Spl)**	Metformin	2.263	2725.113	1.222	2658

Table 6: Ruggedness results of Glibenclamide

S.No.	Sample name	Name	RT	Area	USP Tailing	USP plate count
1	**Analyst-1(Std)**	**Glibenclamide**	3.303	222.491	1.355	3577
2	**Analyst -2(Std)**	**Glibenclamide**	3.283	223.091	1.281	3534
3	**Analyst 1 (Spl)**	**Glibenclamide**	3.283	214.437	1.290	3534
4	**Analyst 2 (Spl)**	**Glibenclamide**	3.303	220.047	1.323	3577

Table 7: Results for LOD & LOQ

Drug name	LOD (µg)	LOQ (µg)
Metformin	24.89	75.44
Glibenclamide	0.020	0.061

4. Conclusion

A new method was established for simultaneous estimation of Metformin and Glibenclamide by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Metformin and Glibenclamide by using C_{18} column (4.6×250mm)5µ, flow rate was 1ml/min, mobile phase ratio was (70:30 v/v) Phosphate Buffer: ACN and detection wavelength was 210 nm. Precision and recovery studies were also found to be with the range. The proposed HPLC method was found to be simple, specific, precise, accurate, rapid and economical for simultaneous estimation of Metformin and Glibenclamide in pharmaceutical dosage form. The developed method was validated in terms of accuracy, precision, linearity, robustness and ruggedness and results will be validated statistically according to ICH guidelines. The Sample recoveries in all formulations were in good agreement with their respective label claims. Hence the suggested RP-HPLC method can be used for routine analysis of Metformin and Glibenclamide in API and Pharmaceutical dosage form.

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