ijcrr
Vol 02 issue 12
Category: Research
Received on:28/10/10
Revised on:09/11/10
Accepted on:17/11/10

METHOD VALIDATION FOR ULTRAVIOLET SPECTROPHOTOMETRIC DETERMINATION OF DICLOFENAC SODIUM IN HUMAN STRATUM CORNEUM BY SKIN STRIPPING METHOD USING MARKETED DICLOFENAC SODIUM TOPICAL FORMULATIONS

S. S. Rawat[1], R. V. Mayee[2], V. A. Arsul[2]

[1]Shri. Bhagwan College of pharmacy, Aurangabad, Maharashtra, India
[2]Dr. Vedprakash patil Pharmacy College, Aurangabad, Maharashtra, India

E-mail of corresponding author: rahul.mayee@rediffmail.com

ABSTRACT

The Diclofenac sodium containing marketed gel, an emugel formulation and a spray formulation were applied on the skin. The release of Diclofenac sodium from transdermal gels and spray formulations was studied using the Tape stripping method. Diclofenac sodium exhibited distinct λmax in methanol at 285nm. A good linear relationship (r^2=0.9787) was observed between the concentration ranges of 5-25 µg/mL. The relative standard deviation (RSD) and assay values obtained by two analysts were 0.36 & 99.50, 0.31 & 99.60, for D1 formulation, 0.53 & 98.21, 0.45& 98.23 for D2 formulation and 0.54 & 97.76, 0.30 & 97.83 for D3 formulation respectively. The percutaneous absorption of D1 formulation was found to be more than other two formulations. The percentage recovery values indicates that there no interference from the excipients present in the formulation. This demonstrates that the developed spectroscopic method is simple, accurate and reproducible and can be easily used for the routine quality control of Diclofenac sodium in human stratum corneum by tape stripping method.

Keywords: Diclofenac gel formulations, Diclofenac spray formulations, Tape stripping method, Validation

INTRODUCTION

Diclofenac Sodium is sodium 2-[(2, 6-dichlorophenyl)- amino]phenylacetate. It is a non-steroidal anti-inflammatory drug applied to reduce inflammation and as an analgesic reducing pain in conditions such as arthritis or acute injury[1]. It is freely soluble in *methanol;* soluble in *ethanol (95%);* sparingly soluble in *water* and in *glacial acetic acid*; practically insoluble in *ether,* in *chloroform* and in *toluene*[2]. Literature survey revealed the availability of method of estimation of drug by Tape stripping method[3]. But a quantitative determination method of diclofenac sodium in various gel formulations and spray formulation and their comparison was not validated. The aim of present investigation is to validate UV

Spectrophotometric method for diclofenac sodium topical formulations and to develop a quality control tool for diclofenac sodium gel and spray formulation.

Experimental

Instruments

A UV Spectrophotometer (UV-1700): Chemito-Spectroscan UV 2600, Double beam UV Visible Spectrophotometer with a matched pair of 10 mm quartz cells were used for experimental purpose.

Materials

Diclofenac sodium API was procured as gift sample from Wockhardt Research Centre, Aurangabad. The obtained Diclofenac sodium was dissolve in methanol for experimental purpose. In present study, three commercial products of diclofenac, a commercially available marketed gel formulation (D1) containing diclofenac diethylamine 1% w/w which is equivalent amount of 1.6% w/w Diclofenac sodium, an emugel formulation (D2) containing 1% w/w diclofenac diethylamine which is equivalent amount of 1.6% w/w Diclofenac sodium and a spray formulation (D3) containing 1% w/w diclofenac diethylamine which is equivalent amount of 1.6% w/w Diclofenac sodium has been used for estimation. We compared their percutaneous absorption through skin from one of the topical preparations. The samples were procured from local market, Aurangabad.

Preparation of Standard Stock Solution

The standard stock solution was prepared by dissolving 0.2gm Diclofenac sodium in 200ml methanol to make final concentration of 100µg/ml. Different aliquots were taken from stock solution and diluted with methanol separately to prepare series of concentrations from 2-24 µg/ml. The λ_{max} for Diclofenac sodium in methanol was found to be 285 nm. The calibration curve was prepared by plotting absorbance versus concentration of Diclofenac sodium[4, 6].

Method

Three Diclofenac sodium formulations (D1, D2 & D3) were applied on the skin. The diethylamine acts as penetration enhancer. The release of Diclofenac sodium from transdermal gels and spray formulations was studied using the skin stripping method. Tape stripping is a simple and efficient method for the assessment of quality and efficacy of cosmetical and dermatological formulations. The tape stripping method in its standardized form is well-suited to determine the dermatopharmacokinetics of topically applied substances.

Before sampling the drug (D1) remaining on the site was removed by three cotton swabs to ensure complete removal of residual drug from the site. The pre-cut (1 sq. cm) adhesion tape was applied on site and mild force was applied to ensure the proper adhesion. The tape was removed and discarded.

Eight adhesion tape pieces were applied on the site area in same manner and each tape were removed using forceps so that complete removal of stratum corneum occurs. All 8 samples were collected in a single test tube with 60 ml of *methanol* in a 200-ml volumetric flask and dilute to volume with *methanol*. From this solution, suitable aliquots were prepared, then these dilutions were scanned in UV region and absorbances were noted at 285 nm and concentration was determined by linear regression equation. This method was repeated for D2 & D3 samples[5, 8, and 9].

RESULTS AND DISCUSSION

The tape stripping method for diclofenac sodium topical formulations was validated by studying the following parameters as ICH guide lines (ICH guide lines 1995) for method validation[7].

Linearity

The linearity of the response of the drug was verified at 1 to 100 µg/ml concentrations, but linearity was found to be between 5-25 µg/ml concentrations. The equation of the calibration curve for Diclofenac sodium obtained y = 0.0038x, the calibration curve was found to be linear in the aforementioned concentrations. The correlation coefficient (r^2) of determination was $R^2 = 0.9787$(Table 1)

Precision

Assay of method precision (intraday precision) was evaluated by carrying out six independent assays of test samples of D1, D2 and D3 formulations. The intermediate precision (interday precision) of the method was also evaluated using two different analysts, systems and different days in the same laboratory. The relative standard deviation (RSD) and assay values obtained by two analysts were 0.36 & 99.50, 0.31 & 99.60, for D1 formulation, 0.53 & 98.21, 0.45& 98.23 for D2 formulation and 0.54 & 97.76, 0.30 & 97.83 for D3 formulation respectively. (Table 2)

Accuracy (Recovery test)

Accuracy of the method was studied by recovery experiments. The recovery experiments were performed by adding known amounts of the drug in the placebo. The recovery was performed at two levels, 50 and 100% of Diclofenac sodium standard concentration. The recovery samples were prepared in before mentioned procedure. Three samples of each marketed formulation (D1, D2 & D3) were prepared for each recovery level. The solutions were then analyzed, and the percentage recoveries were calculated from the calibration curve. The result of analysis the recovery studies presented in Table 3& 4.

The percentage recovery values indicates that there no interference from the excipients present in the formulation that developed method is found to be sensitive, accurate, precise and most reproducible.

CONCLUSIONS

The equation of the calibration curve for Diclofenac sodium obtained y =

0.0038, the calibration curve was found to be linear in the aforementioned concentrations. The correlation coefficient (r^2) of determination was R^2 = 0.9787. The relative standard deviation (RSD) and assay values obtained by two analysts' shows that there is no major variation in the results. The recovery was performed at two levels, 50 and 100% of Diclofenac sodium standard concentration. The percentage recovery values indicates that there no interference from the excipients present in the formulations. The percutaneous absorption of D1 formulation was found to be more than other two formulations. This demonstrates that the developed spectroscopic method is simple, accurate and reproducible, can be easily used as the routine quality control tool for Diclofenac sodium gel formulations.

ACKNOWLEDGEMENTS

The authors acknowledge Principal, Dr. Vedprakash Patil Pharmacy College Aurangabad, as well the Principal, Shri Bhagwan College of Pharmacy, N-6, Cidco, Aurangabad, India for providing facilities for conducting research. The authors also thanks to Wockhardt Research Centre, Aurangabad for providing the Diclofenac Sodium API.

REFERENCES

1. United States Pharmacopoeia, USP 24/NF 19, US Pharmacopoeial Convention Inc,2000, USA, pp.2149-2152.
2. 2. Indian Pharmacopoeia I (1996), Government of India Ministry of Human & Family Welfare Published by the Controller of Publication, Delhi.
3. 3. U.V.Sera, M.V.Ramana, *In vitro* skin absorption and drug release – a comparison of four commercial hydrophilic gel preparations for topical use. The Indian Pharmacist, 2006, 73**,** 356-360
4. 4. Agrawal, YK. and Shivramchandra K, Spectrophotometric determination of diclofenac sodium in tablets. *J. Pharm. Biomed. Anal*. 1991, 9: 97-100.
5. 5. Knudsen, EA. Isolation of dermatophytes from foot wear with adhesive tape strips. J, of Medical and veterinary Mycology 1986, 25; 59-61
6. 6. Flynn, G.L., Shah, V.P., Tenjarla, S.N., et al., Assessment of value and applications of in vitro testing of topical dermatological drug products. Pharm. Res. 1999, 16, 1325–1330.
7. 7. ICH-Q2R1, Validation of Analytical Procedures: Methodology International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use 1996, Geneva, Switzerland.
8. Pinkus H. Examination of the Epidermis by the strip method of removing horny layers. J Invest Dermatol 1951, 16; 383-386.
9. Mo, Z and Chen Z: UV spectrophotometric determination of compound diclofenac sodium injection. *Yaowu Fenxi Zazhi.*, 1988, 8: 314-316.

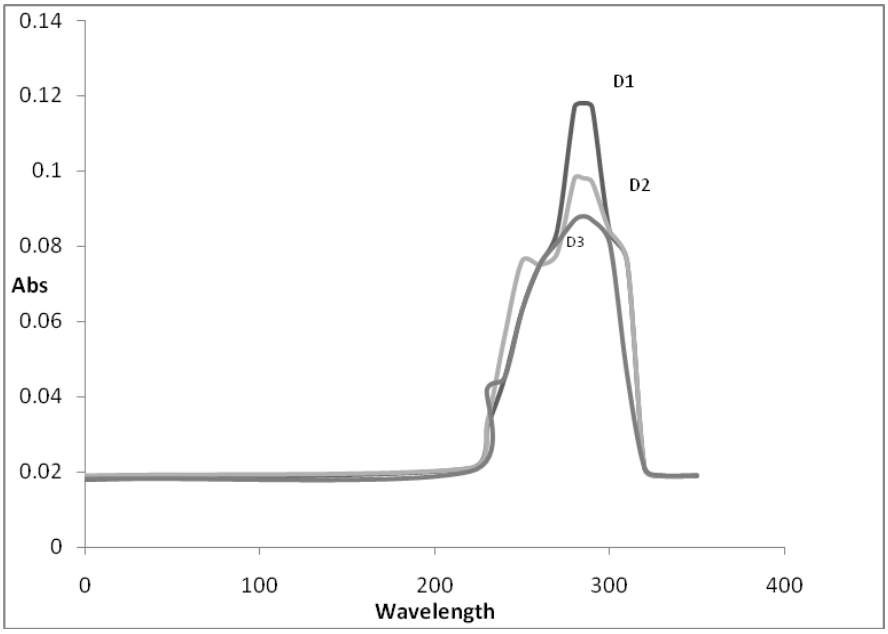

Figure 1: Selection of wavelength for Diclofenac sodium from marketed formulation D1, D2 and D3.

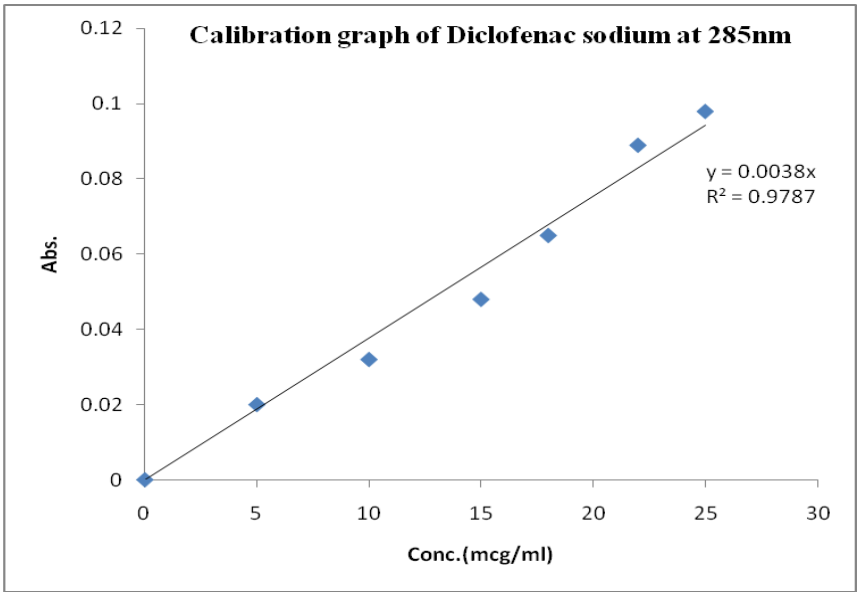

Figure 2: Calibration graph of Diclofenac sodium at 285nm

Table 1: Linearity of Diclofenac sodium

S. No.	Concentration (µg/ml)	Absorbance at 285 nm
1	5	0.02
2	10	0.032
3	15	0.048
4	18	0.065
5	22	0.089
6	25	0.098

Table 2- Determination of Precision

Sample number	Assay of Diclofenac sodium as % of labeled amount					
	D1 formulation		D2 formulation		D3 formulation	
	Analyst-I (Intra-day precision)	Analyst-II (Inter-day precision)	Analyst-I (Intra-day precision)	Analyst-II (Inter-day precision)	Analyst-I (Intra-day precision)	Analyst-II (Inter-day precision)
1	99.42	99.70	98.25	98.22	97.74	97.81
2	99.63	99.23	98.13	98.44	97.73	97.83
3	99.58	99.57	98.18	98.23	97.76	97.88
4	99.10	99.88	98.19	98.46	97.83	97.84
5	100.12	99.98	98.22	98.43	97.71	97.79
6	99.20	99.25	98.28	98.21	97.84	97.84
Mean	99.50	99.60	98.21	98.23	97.76	97.83
RSD	0.36	0.31	0.53	0.45	0.54	0.30

Table 3: Recovery Studies

Formulation	Level	%Recovery	%RSD*
D1	50%	99.7	0.2534
	100%	99.5	0.3050
D2	50%	99.4	0.2531
	100%	99.5	0.3121
D3	50%	99.3	0.3142
	100%	100.2	0.4124

* **Relative Standard Deviation** of six observations

Table 4: Analysis of formulation

Formulation	Amount(mg/ gel)		% label claim	%RSD*
	Labeled	Found		
D1	1.6% w/w	1.59% w/w	98.50	0.1843
D2	1.6% w/w	1.57 % w/w	98.21	0.2432
D3	1.6% w/w	1.56% w/w	97.83	0.1431

* **Relative Standard Deviation** of six observations