Estimation of Diclofenac Sodium in SEDDS Formulation by HPLC Method

A. V. Gotoskar¹, *J. Bhat M.²

¹. Department of Pharmaceutics, Indira College of Pharmacy, Tathwade, Pune-411 033, Maharashtra India.
². Institute of Pharmaceutical Sciences and Research Center, Bhagwant University, Ajmer-305004, India.

ABSTRACT
The present study was aimed to develop an analytical method for determination of Diclofenac Sodium SEDDS formulation in hard gelatin capsules by High Performance Liquid Chromatography (HPLC) method. The HPLC method was developed using chromatopack, Inertsil C18, 250x4.6mm, 5µm analytical column, simple mobile phase containing mixture of Methanol: Buffer in the ratio of 60:40 v/v, at flow rate of 1.0mL/min using wavelength at 254nm. The retention time of Diclofenac sodium was about 14 minutes. The method was validated for its specificity, accuracy, precision, and linearity, limit of detection (LOD), limit of quantification (LOQ), robustness and stability parameters. This method was applied to analyse the finished formulation without interference of excipients present in the formulation. The linear regression analysis data for the calibration plots showed a good linear relationship over the concentration range of 5-150 μg/mL for Diclofenac Sodium. The method showed good recoveries (98.0 to 101.5%). The proposed method was suitable for quantitative and entrapment efficiency determination of Diclofenac Sodium in SEDDS formulation in hard gelatin capsules. On the other hand, this HPLC method is allowed for elimination of the interference of the SEDDS lipophilic components dissolved in the buffer medium and no difference was observed between the results of this analysis in comparison with HPLC results.

Keywords: HPLC method, diclofenac sodium, NSAI drug, SEDDS formulation, hard capsules

Received 26 Jan 2014 Received in revised form 26 March 2014 Accepted 28 March 2014

*Address for correspondence:
J. Bhat M,
B-303, Plot-E, Rock Avenue, Hindustan Naka, Kandivali (west), Mumbai-400067, Maharashtra India.
E-mail: harish.bhat@rediffmail.com

INTRODUCTION
In-vitro testing of drug release from pharmaceutical dosage forms is a common step of a routine control, as well as an initial phase of a product development process. In addition, dissolution test and drug release profile provides valuable information on physicochemical stability of the final product. Although oral forms i.e. conventional tablets and capsules are mainly tested for drug release on a routine base, the test is also recommended for some other products including SEDDS formulated as liquid dosage form in hard capsules which is also an oral dosage form.

The in-vitro release test for SEDDS has always posed many problems, including choice of a suitable apparatus, test conditions and interpretation of the results (1). Even when the flow-through apparatus dedicated for this dosage form was introduced to European Pharmacopoeia, other methods like basket or beaker methods, with or without dialysis membrane, are still in use (2-3). During the test samples of the dissolution medium are collected and analyzed by a suitable analytical method, what can be a difficult step as the release of active substance from SEDDS is accompanied by dissolution of the components of a SEDDS. In SEDDS with lipophilic bases, besides lipids, also surfactants and antioxidants may be present and they can dissolve in the acceptor fluid during the test. Their release rate is unknown and their amount in the analyzed samples varies in an unpredicted manner. Thus it is important to choose a suitable method for the analysis of the active substance an instrumental method where these components do not interfere with the measurements.
Diclofenac Sodium (Benzene acetic acid, 2-[(2,6-dichlorophenyl)amino]-monosodium salt. Sodium [o-(2,6-dichloroanilino) phenyl]acetate). Diclofenac Sodium is phenyl acetic acid derivative. It is non-steroidal anti-inflammatory drug (NSAID) that exhibits anti-inflammatory, analgesic and anti-pyretic activities in both animals and human beings [4]. The apparent volume of distribution is 1.4L/Kg. It is 99% bound to human serum proteins [5-6]. It diffuses into and out of synovial fluid. It is eliminated through urinary and biliary excretion of the glucuronide and the sulphate conjugates of the metabolite [7]. It has little anti-microbial activity and is under investigation for the treatment of tuberculosis. Diclofenac Sodium is used for the relief of sign and symptoms of osteoarthritis, rheumatoid arthritis and ankylosing spondylitis. It is often used to treat chronic pain associated with cancer. It may prevent the development of Alzheimer disease if given daily in small doses for many years. It also acts as anti-uricosuric agent [8-9].

In vitro testing of drug release from pharmaceutical dosage forms is a common step of a routine quality control test, as well as an initial phase of a product development process before the optimization of the final formulation. In addition, HPLC test provides valuable information on physicochemical stability of the product with accuracy, without interference. Although oral forms i.e. tablets and capsules are mainly tested for drug release on a routine base, the test is also recommended for products including SEDDS as a liquid dosage form in hard capsules. The in-vitro release test for SEDDS has always posed many problems, including choice of a suitable apparatus, test conditions and interpretation of the results (10) due to the involvement of many excipients and complications in the formulations.

![Structure of Diclofenac sodium](image)

Figure 1: Structure of Diclofenac sodium (C14H10Cl2NNaO2 318.13)

**MATERIALS AND METHODS**

**Materials:**
Diclofenac Sodium (USP) was provided by Aarti Drugs Pvt Ltd (Bisar Thana), Sodium acetate trihydrate used were of AR grade of S.D.Fine. HPLC grade water was obtained from Milli Pore Merk water purification system.

Aconon was obtained from Abitec and Polysorbate 80(Tween -80) was purchased from Mohini Organics Pvt. Ltd (Mumbai).

**Apparatus:**
The analysis was performed by using the analytical balance GR-200 (AND), pH meter PHAN (Lab India), HPLC used was DG-U-20A Shimadzu. Column used in HPLC was Inersil C8, 250x4.6mm,5μm, with a flow rate of 1.0 mL/min.

**Chromatographic condition:**
The chromatographic condition for method was optimized is shown in (Table 1).

**Mobile Phase:**
The mobile phase consists of mixture of 40 volume of buffer and 60 volume of Methanol, which are filtered through 0.45μm filter and degassed in a sonicator for about 10 minutes and transferred to a solvent reservoir.

The permutation and combination of the mobile phase were used and finally Sodium acetate trihydrate and Methanol (40:60) v/v was selected as an appropriate mobile phase which gave good resolution and acceptable system suitability parameters.
Table 1: Optimized chromatographic conditions

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Optimized condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromatograph</td>
<td>HPLC Agilent-1100</td>
</tr>
<tr>
<td>Column used</td>
<td>Inersil C8, 250x4.6mm,5μm</td>
</tr>
<tr>
<td>Wavelength</td>
<td>254 nm</td>
</tr>
<tr>
<td>Flow rate</td>
<td>1.0 mL/min</td>
</tr>
<tr>
<td>Injection volume</td>
<td>20 μL</td>
</tr>
<tr>
<td>Mobile Phase</td>
<td>Sodium acetate trihydrate and Methanol (40:60)</td>
</tr>
<tr>
<td>Column Temperature</td>
<td>25°C</td>
</tr>
<tr>
<td>Run time(for assay)</td>
<td>20 minutes</td>
</tr>
<tr>
<td>Run time(RS)</td>
<td>20 minutes for standard and 40min for sample and blank</td>
</tr>
<tr>
<td>Diluent</td>
<td>100% Methanol</td>
</tr>
<tr>
<td>Retention Time</td>
<td>About 14 min</td>
</tr>
</tbody>
</table>

PROCEDURE:

Preparation of standard solution:
Standard preparation :(100ppm) for Assay :
Weighed accurately 10.0mg of Diclofenac Sodium standard in to a 20mL volumetric flask and made up to the volume with the diluents. Further pipetted 5mL and made up to 25mL with Methanol in a 25 mL volumetric flask.

Sample preparation for Assay :(in comparison to std area) :
Weighed about 130mg of capsule content into 20mL volumetric flask and made up to the volume with Methanol. Further pipetted 2mL into 20mL volumetric flask and made up to volume with Methanol.

Standard preparation :(10ppm) for Related substances :
Weighed accurately 10.0mg of Diclofenac Sodium standard in to a 20mL volumetric flask, and made up to the volume with the diluent. Further pipetted 2mL and made up to 100mL with Methanol.

Sample preparation :(1000ppm) for Related substances :
Weighed accurately about 357mg of SEDDS formulation from capsule (equivalent 50mg of Diclofenac Sodium), into 50mL volumetric flask and made up to the volume with Methanol.

Calibration curve:
From the stock solution, measured volumes of working standards were prepared in the concentration range of 1- 5μg/ml. 20μL injections were made for each concentration in triplicate and were analyzed under optimized chromatographic conditions. A Calibration curve was plotted by using the response (peak area) versus concentration of drug. Regression equation was calculated.

Procedure for analyzing SEDDS formulation:
Diclofenac Sodium SEDDS formulation was weighed equivalent to 50mg of Diclofenac Sodium and was transferred to a 100mL volumetric flask containing 25mL of mobile phase. The content of the flask was allowed to stand for 15minutes with intermittent sonication to ensure complete solubility of the drug and later it was made up to volume with mobile phase. The test solution was filtered through 0.45μm membrane filter. The filtrate was finally diluted to 10X with mobile phase and appropriate dilutions were made to obtain concentration in the range of 1-5ug/mL. The steady base line was recorded by using the optimized chromatographic conditions. The assay was subjected for calculating regression equation. The procedure was repeated for 6 times and the percentage of drug in the formulation was calculated.

METHOD VALIDATION:

Linearity: The method was linear in the concentration of 1 to 5μg ml for Diclofenac sodium standard.
Figure 2: Linearity of diclofenac sodium

**System precision:**
The system precision was checked by using standard chemical substance to ensure that the analytical system is working properly. The retention time and the area response of five determinations should be measured and calculate the relative standard deviation (Table 2).

**Table 2: System precision**

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Diclofenac Sodium</th>
<th>Area Response</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Retention Time</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>14.575</td>
<td>1593001.270</td>
</tr>
<tr>
<td>2</td>
<td>14.564</td>
<td>1597998.766</td>
</tr>
<tr>
<td>3</td>
<td>14.545</td>
<td>1579163.523</td>
</tr>
<tr>
<td>4</td>
<td>14.549</td>
<td>1592380.090</td>
</tr>
<tr>
<td>5</td>
<td>14.547</td>
<td>1598147.927</td>
</tr>
<tr>
<td>6</td>
<td>14.535</td>
<td>1597549.096</td>
</tr>
<tr>
<td>Mean</td>
<td>14.5525</td>
<td>1593040.11</td>
</tr>
<tr>
<td>SD</td>
<td>0.01</td>
<td>7266.44</td>
</tr>
<tr>
<td>RSD</td>
<td>0.10%</td>
<td>0.46%</td>
</tr>
</tbody>
</table>

**Accuracy:**
Accuracy for the assay of diclofenac sodium SEDDS was determined by applying the method in triplicate samples of mixture of placebo to which known amount of diclofenac sodium standard. The sample were filtered through 0.45mm membrane filter and injected into the chromatographic system.

**Injection Profile:**
Inject blank (Dissolution Media) (1 injection) and Standard Preparation (5 injections) and check for system suitability.

**System suitability:**
The tailing factor for diclofenac sodium peak is 0.79. The % RSD of 5 replicate injections of standard preparation is 0.5%. The column efficiency for diclofenac sodium peak is 4350 theoretical plates.

**Data Interpretation:**
From the above results, it can be concluded that retention time and area response are consistent as evidenced by relative standard deviation. Hence it can be concluded that the system precision parameter meets the requirement of method development.
Table 3: System suitability parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Diclofenac sodium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calibration rang (µg/mL)</td>
<td>5-150</td>
</tr>
<tr>
<td>Theoretical plates</td>
<td>4350</td>
</tr>
<tr>
<td>Tailing factor</td>
<td>0.79</td>
</tr>
<tr>
<td>% Recovery</td>
<td>99.0% to 101.5%</td>
</tr>
</tbody>
</table>

Calculations and formula:

Avg. Sample area \( \times \) Standard weight \( \times \) Dilution \( \times \) Potency \( \times \) Avg. fill weight

Avg Standard area \( \times \) Dilution \( \times \) Sample weight \( \times \) 100 \( \times \) Label claim

RESULTS AND DISCUSSION

The self emulsifying drug delivery system (SEDDS) formulation of Diclofenac Sodium is a novel and versatile approach for overcoming the formulation difficulties. The present developed method is novel for the determination of Diclofenac Sodium in SEDDS formulations. The method was found to be specific as excipients in the formulation did not interfere in the estimation of Diclofenac Sodium in SEDDS formulation.

Diclofenac Sodium standard having concentration 50 mg/ml was scanned in UV-region between 200-400 nm. \( \lambda \) max of diclofenac sodium was found to be at 254 nm. The diclofenac sodium peak in the sample was identified by comparing with the Diclofenac Sodium standard and the retention time was found to be around 14 minutes. The estimation Diclofenac Sodium was carried out by RP-HPLC using Mobile phase having a composition volumes of buffer, 60 volumes of Methanol and 40 volumes of Sodium acetate trihydrate (60: 40 v/v). Then finally filtered using 0.45µ nylon membrane filter and degassed in sonicator for 10 minutes. Column used in HPLC is Inersil C8, 250x4.6mm, 5µm, with a flow rate of 1.0 ml/min. The run time for assay is 20min and run time for RS is 20 min for standard and 40min for sample and blank, RT is about 14min. System suitability parameters such as the tailing factor for Diclofenac Sodium peak is 0.79, the %RSD of 5 replicate injection of standard preparation is 0.5%, the column efficiency for diclofenac sodium peak is 4350 theoretical plates.

The results of analysis showed that the amount of drug was in good agreement with label claim of developed SEDDS formulation. It was observed that there were no marked changes in chromatogram; the results indicated that the developed formulation was stable up to 12-14 hours which was sufficient for completing the analytical procedures. The developed method was specific and reproducible for the quantitative determination of Diclofenac in SEDDS formulation with a good resolution and high sensitivity.

The Accuracy limit is the % recovery should be in the range of 99.0% to 101.5%. The developed method shows that the accuracy is well within the limit, which shows that the method is capable of showing good accuracy.

CONCLUSION

SEDDS formulation prepared shows release intended NSAID. There are no methods developed for quantitative determination of 99.0% to 101.5% in SEDDS formulation. Precision and accuracy for 99.0% to 101.5% were comparable with other HPLC.

ACKNOWLEDGMENT

The author is grateful to the ACG Associated Capsule Pvt Ltd for providing the necessary infrastructural facilities to carry out the study. We would like to thanks Dr. Avinash R. Tekade for his guidance in the publication of article.
Figure 3: Chromatograph representing the blank
Figure 4: Chromatograph of Diclofenac Sodium standard

- Product Name: Diclofenac Sodium
- Sample Name: Standard
- Sample No: 2
- Program File: ASD_AMDDIC_CAP_ASY_1
- Method File: ASD_AMDDIC_CAP_ARS_1
- Date Acquired: 07.01.2013 19:01:49
- Instrument ID: 1080-HPLC-0021
- Column No: 1
- Sequence Name: DICASY_1_1080-MS-1300007_0701
- Sequence Path: STARDEVELOPMENTSDICLOFENAC SODIUM CAPSULESEQUENCE2013DICASY_1_1080-MS-1300007_0701
- Parameters: Linearity

<table>
<thead>
<tr>
<th>Peak No.</th>
<th>Ret. Time (min)</th>
<th>Peak Name</th>
<th>Area μ AU*sec</th>
<th>Area %</th>
<th>Plates (USP)</th>
<th>Rel. Ret. Time</th>
<th>Asym (USP)</th>
<th>Capacity factor (USP)</th>
<th>Resolution (USP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9.867</td>
<td>DICLOFENAC SODIUM</td>
<td>1900148.279</td>
<td>100.00</td>
<td>3077</td>
<td>1.00</td>
<td>0.82</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
</tbody>
</table>

*Area: Area under the curve, Ret. Time: Retention Time, Asym: Asymmetry Factor, Capacity Factor: Capacity Factor, Resolution: Resolution*
Figure 5: Typical chromatograph of Diclofenac Sodium in SEEDS formulation (50ppm)
Figure 6: Typical chromatograph of Diclofenac Sodium in SEEDS formulation (100ppm)
Figure 7: Typical chromatograph of Diclofenac Sodium in SEEDS formulation (150ppm)
REFERENCES