plasmadrug concentration determination of both drugs in rabbit plasma. In vivo study of the optimized formulation was carried out and the samples were analyzed using the method developed by us. Further in vivo in vitro correlation was carried out using PKPD software.

The pharmacokinetic study showed prolonged Tmax, lowered Cl and reduced fluctuations in plasma concentrations. It exhibited good controlled-release properties. Result indicated that a good correlation exist between the two data’s. This new method will be of immense help for carrying out a pharmacokinetic study of these drugs in laboratories that lacks a sophisticated analytical instrument like LC/MS/MS.

The author concludes that IPN microbeads containing diclofenac sodium was developed by using ionotropic gelation method with sodium carboxymethyl locust bean gum and sodium carboxymethyl cellulose using aluminium chloride as the crosslinker. The effect of the above independent variable on the different physicochemical and physical mechanical properties of the developed IPN was evaluated by using various analytical tests. Out of six formulations, the optimized formulation was determined using the results obtained from the analytical studies. The goal of the research work was achieved through well-designed plan of experimentation and well-thought of physicochemical concept with support from statistical software and sophisticated instrumentation and at the same time without causing any environmental hazard. Finally, from these studies it appeared that, developed IPN beads are superior in terms of particle size, high drug entrapment efficiency and sustained release property. Lastly, it can be concluded that IPN beads made up of sodium carboxymethyl locust bean gum and sodium carboxymethyl cellulose with minor structural modification using aluminium chloride as the crosslinker can be conveniently prepared in the industry either by encapsulating in capsules or by preparing matrix tablets and can be commercialized as sustained release medication of diclofenac sodium for better drug therapy in nociception cases.
8.1 MATERIALS AND METHODS

- Diclofenac Sodium, used as a reference and in formulation was supplied by Yarrow Chemicals Products, Mumbai, India.
- Loratadine, used as an internal standard (IS) was supplied by Yarrow Chemicals Products, Mumbai, India.
- Acetonitrile (HPLC Grade) was received from Merck Specialities Private Limited, Mumbai, India.
- Methanol (HPLC Grade) was received from Merck Specialities Private Limited, Mumbai, India.
- Water (HPLC Grade) was procured from what was procured from Spectrochem Pvt. Ltd, Mumbai, India.
- Locust bean gum was purchased from Hema Laboratories Pvt. Ltd, Mumbai, India.
- Sodium carboxymethyl cellulose was purchased from Hema Laboratories Pvt. Ltd, Mumbai, India.
- Aluminium Chloride was purchased from Hema Laboratories Pvt. Ltd, Mumbai, India.
- Ammonium acetate was obtained from Qualigens Fine Chemicals, Mumbai, India.
- Xylocaine (2%) was taken from Astra Zeneca Pharma India Limited, Bangalore, India.
- Heparin Sodium was from Gland Pharma Limited, Hyderabad, India.
- Xylene was received from Merck Specialities Private Limited, Mumbai, India.
- Rectified Spirit was collected from Bengal Chemicals and Pharmaceuticals Limited, Kolkata, India.
- All reagents and solvents used were of analytical grade.

Cellulose and sodium carboxymethyl locust bean gum and its feasibility to control the release of model drug diclofenac sodium.

In Chapter 5, the aim and objectives of the research work along with the rationale behind the study were explained and specific objectives of the research work were highlighted.

In Chapter 6, development and in-vitro evaluation of different formulations along with a thorough investigation the drug sample (Diclofenac sodium) using UV - VIS spectrophotometer in different solvents and its standard curve was prepared. Tailored natural polyelectrolyte IPN beads composed of sodium carboxymethyl locust bean gum (SCMLBG) and sodium carboxymethyl cellulose (SCMC) were prepared by single water-in-water (w/w) emulsion gelation method from an aqueous environment containing SCMC as an emulsion stabilizer and AL3+ ions as a cross-linking agent for SCMC. This carbohydrate beads microbeads was characterized by SEM, particle size analysis, FTIR, DSC and XRD technique. SEM micrographs exhibited a spherical morphology of the prepared beads. XRD results confirmed the amorphous distribution of the drug molecules in the prepared hydrogel network beads. The swelling studies of beads have shown that with an increasing crosslinker concentration in the beads, water uptake has decreased. Complete drug release was achieved in alkaline medium at different periods of time depending upon the process variables and the release followed non-Fickian type transport mechanism. The results of the study indicate that drug loaded microbeads could be used to minimize the release of diclofenac sodium (DS) in acidic medium and to modulate the drug release in alkaline medium, which would help to minimize the gastrointestinal effects of DS.

In Chapter 7, outcome of acute oral toxicity study of the optimized formulation is being presented. Experimental animals were basically divided into two groups i.e. control and test. Later on the test group was further sub divided into three groups (Low, Medium and High) based on the dose administered. Animals were subjected to controlled temperature and food consumption was monitored carefully. There were no significant changes in hematology, serum biochemistry, or terminal body weight following a 28-day observation period. No detectable abnormalities were found in the histopathology of the selected organs. These preliminary toxicological results suggest that, under the conditions of this study, short-term treatment of rat with this formulation does not have any detectable adverse effects. Hence, laboratory investigation revealed that our formulation was non-toxic and no significant changes were identified from histopathological evaluation.

In Chapter 8, a bio-analytical method for identification of diclofenac sodium was developed and the method was validated. The developed method was validated and can be used for the
8.2 CHROMATOGRAPHIC CONDITIONS

- **Instrument**: Analytical Technologies Limited, Gujarat, India.
- **Column**: Hypersil BDS, C18, 250 x 4.6 mm, 5 µm particle size, Stainless steel.
- **Mobile Phase**: Acetonitrile : Methanol (70:30% v/v)
- **Flow rate of Mobile Phase**: 1 ml/minute
- **Wavelength of Detection**: 276 nm
- **Detector**: UV-VIS Spectrophotometer model 300-M by Analytical Technologies Limited, India.
- **Pump**: Model P 3000A by Analytical Technologies Limited, India.
- **Injector**: Fixed loop Rheodyne injector system fitted with a 20 µl Rheodyne loop.
- **Integrating Software**: Analchem Chromatography workstation V.2.22.
8.3 BIOANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR ANALYSIS OF DICLOFENAC SODIUM IN RABBIT PLASMA BY HPLC ANALYSIS*

8.3.1 Preparation of stock and working solutions:

Stock solutions of diclofenac sodium and IS were prepared at concentration of 100 µg/ml in mobile phase. Stock solutions were stored at -20°C until they were used for working solutions by adding the appropriate volume of mobile phase. Working solutions of different concentrations were prepared from above-mentioned stock solution by diluting with mobile phase fresh before use. Structure of diclofenac sodium (DS) and Internal standard (IS) are shown in Figure 8.1.

![Structural representation of (A) loratadine (IS) and (B) diclofenac sodium.](image)

Figure 8.1: Structural representation of (A) loratadine (IS) and (B) diclofenac sodium.

8.3.2 Preparation of calibration standards and quality control (QC) samples:

Six calibration standards in plasma at concentrations of 20, 50, 150, 300, 500 and 1000 ng/ml were prepared by spiking appropriate aliquots of working solution of diclofenac sodium in plasma. For internal standard the final concentration in plasma was 1000 ng/ml. Four levels of QC samples at a concentration of 20 ng/ml (lower limit of quantification, i.e. LLOQ), 60 ng/ml (low), 500 ng/ml (medium), and 800 ng/ml (high) were also prepared following the above method. QC samples were prepared in a 50 ml pool, then aliquoted into pre-labeled 2 ml polypropylene vials and stored at -20°C.

8.3.3 Collection of blood samples:

Rabbits (New Zealand, Adult, White) of either sex weighing (1.5-2.0 kg) were chosen in this study and were given access to a normal standard diet and tap water ad libitum. Food was

SUMMARY AND CONCLUSION

Disease is a common manifestation of human life. Great progress has been made in the management of diseases through the intervention of drugs over the past fifty years. Today, drugs are almost never administered to a patient in an unformulated state and development of appropriate dosage form to deliver these drugs is a meaningful challenge for the pharmaceutical scientists. Now, these drugs are formulated in different types of dosage forms for the administration through various routes. Mainly two types of dosage forms are there, conventional dosage form and modified release dosage form. Among the modified release dosage forms, sustained release dosage forms are preferred due to their several inherent advantages like improved patient compliance, improve efficiency in treatment etc. Among the two types of sustained release dosage forms, multiple-unit dosage forms are much more advantageous than single unit due to their reliable biopharmaceutical behaviour. There are various methods for the preparation of multiple-unit dosage forms like spray drying, phase separation, solvent evaporation and ionotropic gelation method. Among these ionotropic gelation method has been selected for the study.

Polymers have become an indispensable part of the drug delivery systems, be it conventional drug delivery or novel drug delivery. They have drastically changed the mode of drug delivery by introducing a lot of flexibility. Polymers have gained importance in the pharmaceutical industry as a dual drug encapsulants and vehicles of drug carriage either protecting an active agent during its passage through the body until its release or in storage by preventing moisture ingress. Polymers may be of synthetic or natural origin and should degrade in vivo to produce biocompatible or nontoxic byproducts along with the progressive release of dissolved or dispersed drug. These can be further metabolized or excreted via normal physiological pathways. In addition hydrophilic nature of the polysaccharides makes them to absorb water and swell. These properties have been used to prepare hydrogels which are three-dimensional hydrophilic networks capable of absorbing water without dissolving. The water absorption and swelling properties of the hydrogels can be controlled by physical or chemical crosslinking method. Hydrogels are three-dimensional, hydrophilic, polymeric networks capable of swelling in water or biological fluids, and retaining a large amount of fluids in the swollen state and release the drug at controlled rate. In general, hydrogel exhibits good biocompatibility.

Carboxymethylation of polysaccharides is a widely studied conversion since it's simple and leads to products with a variety of promising properties. Quite often, a single polymer may not provide desired properties and performance. One of the simple methods to improve the
withdrawn from the rabbits 12 hours before the initiation of experiments, but animals had free access to drinking water. Throughout the experiment, the animals were housed, two per cage, maintained at 22°C temperature (±2°C) and 50-60% relative humidity. The institutional animal ethical committee (UIEM, Miranda, Uttar Pradesh, India. Protocol approval no. 2012/07tae Ph.D./02) approved the protocol for this study.

Rabbits were divided into two groups of one each; one was the control group and second was the test group. A dose of 3 mg and 17 mg of pure diclofenac sodium and best optimized drug-loaded microspheres (E2) were administered by gastric intubation respectively. Blood samples (0.5 ml) were collected from right marginal ear vein at defined time intervals into centrifuge tubes containing heparin sodium. The blood samples were immediately centrifuged at 6000 rpm for 10 min to obtain plasma. The plasma samples were separated into eppendorf tubes and stored frozen at -20°C. The tubes were labeled with sample code number along with sampling time. This code did not reveal the formulation identity.

8.3.4 Sample preparation:-

For calibration standards, an aliquot quantity of 0.9 ml plasma sample was taken in a 10 ml stopper test tube and the 0.1 ml internal standard was added and mixed. To it 8 ml mobile phase was added. The mixture was then mixed for 10 min followed by centrifugation at 6000 rpm for 12 min. 5ml of organic layer was separated and evaporated to dryness at room temperature under reduced pressure. The residue was reconstituted in 200 ml of mobile phase, filtered through 0.22 μm membrane filter and 20 μl was injected into the HPLC system.

8.3.5 Validation of methods:-

The method was validated for specificity, linearity, accuracy, precision, recovery and stability according to the principles of the FDA industry guidelines29.

8.3.5.1 Specificity:-

The specificity is the ability of an analytical method to differentiate and quantify the analyte in the presence of other components in the sample. This test was performed by analyzing the blank plasma samples from 6 different sources to test for interference at the retention time of diclofenac sodium and loratadine (IS).

8.3.5.2 Linearity:-

The linearity of calibration curve for diclofenac sodium was assessed in the range of 20–1000 ng ml in plasma samples. Standard samples of DS and IS over the concentration range of 20–1000 ng ml and 1000 ng ml was prepared as described in Section 2.5, respectively. Peak area ratios of each analyte to IS were measured and the calibration curve was obtained from the least-squares linear regression (no weighting factor) presented with their correlation coefficients.
8.3.5.3 Accuracy and precision:

Accuracy and precision were determined by analyzing six replicates of three QC samples (low, medium, and high concentration) for consecutive 3 days. Accuracy (DEV) was determined as the percent difference between the mean observed concentration and the nominal concentration for each QC sample:16:

\[
\text{DEV} (\%) = \left[ \frac{\text{mean (observed)} - \text{mean (nominal)}}{\text{mean (nominal)}} \right] \times 100
\]

The precision of the assay was assessed by observing the concentration of 6 replicates of each QC sample for within-run precision (WRF) and 18 replicates of each QC sample for between-run precision (BRP). The between-run precision was defined as:

\[
\text{BRP} (\%) = \left[ \frac{\sqrt{\text{mean (within groups mean square)}}}{\text{mean (grand mean)}} \right] \times 100
\]

The within-run precision was calculated as

\[
\text{WRF} (\%) = \left[ \frac{\sqrt{\text{mean (within groups mean square)}}}{\text{mean (grand mean)}} \right] \times 100
\]

Where MS<sub>we</sub> = between groups mean square, MS<sub>we</sub> = within groups mean square, GM = grand mean, and n = represents the number of replicates within each validation run.

8.3.5.4 Extraction recovery:

The extraction recovery of analyte at three QC samples was determined by measuring the peak area responses from plasma samples spiked with particular standard working solution of analyte before extraction with these from drug-free plasma samples extracted and spiked with same concentration of analyte after extraction. The recovery of IS at concentration of 1000 ng/ml was determined in the same way. The recovery of diclofenac sodium was determined using six replicates of each QC sample.

8.3.5.5 Stability:

The stability of diclofenac sodium in plasma was evaluated with four studies: short-term, long-term and freeze-thaw stability study as well as stability in ambient temperature. Six replicates of three QC samples at concentration of 60 ng/ml (LQC), 500 ng/ml (MQC) and 800 ng/ml (HQC) was prepared and then subsequent HPLC analysis was carried out as described previously.

The QC samples were kept at room temperature for 24 h, extracted and then analyzed for the short-term stability study.

Evaluation and Research (CDER), Center for Veterinary Medicine (CVM), May 2001 (http://www.fda.gov/cder/guidance/index.htm).


8.8 References


The long-term stability study was carried out with plasma samples spiked with diclofenac sodium which were stored at -20 °C for 1 and 3 months, then extracted and analyzed.

The freeze-thaw stability study was evaluated by comparing the QC samples that had been frozen and thawed three times, with the plasma samples thawed once.

8.4 CHARACTERIZATION OF IN-VIVO STUDY PROTOCOL

8.4.1 Evaluation of pharmacokinetic parameters:

The analytical method proposed and validated here was successfully applied in a pharmacokinetics study of diclofenac sodium in rabbits. The institutional animal ethical committee (IFTM, Meerut, Uttar Pradesh, India, Protocol approval no - 2012/8534 PhD 02) approved the protocol for this study. Estimation of pharmacokinetic parameters was obtained from the plasma concentration vs time data. The maximum plasma drug concentration (Cmax) and the time required to reach (tmax) were directly read from the plasma concentration vs time data. The overall elimination rate constant (K0), was obtained from the slope of the terminal elimination phase of a semilogarithmic plot of concentration vs time after subjecting it to linear regression analysis. The half-life (t1/2) can be calculated by dividing 0.693 with K0. Method of residuals was used to calculate absorption rate constant (Ka). The area under the concentration time curve (AUC) up to the last sampling point was determined by the trapezoidal method using Prism 6 software. The relative bioavailability of DS from IPN microparticles in comparison to reference DS solution was calculated by dividing its AUC by that of the reference DS solution.

8.4.2 In-vivo invitro correlation (IVIVC):

To assess the viability and validity of the sustained nature of IPN microparticles, IVIVC study is essential since prolonged-release products may be specially suited for this kind of study. A high value of correlation coefficient suggested good correlation between in vitro and in vivo data.
8.5 RESULTS:

Table 8.1: Summary of calibration standards.

<table>
<thead>
<tr>
<th>Concentration (ng/ml)</th>
<th>Mean</th>
<th>S.D.</th>
<th>CV (%)</th>
<th>RE (%)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>19.62</td>
<td>0.84</td>
<td>4.28</td>
<td>+1.90</td>
<td>6</td>
</tr>
<tr>
<td>50</td>
<td>50.83</td>
<td>1.45</td>
<td>2.85</td>
<td>+1.66</td>
<td>6</td>
</tr>
<tr>
<td>150</td>
<td>151.15</td>
<td>2.15</td>
<td>1.42</td>
<td>-0.76</td>
<td>6</td>
</tr>
<tr>
<td>500</td>
<td>498.17</td>
<td>1.98</td>
<td>0.66</td>
<td>+0.61</td>
<td>6</td>
</tr>
<tr>
<td>1000</td>
<td>996.95</td>
<td>3.45</td>
<td>0.34</td>
<td>-0.30</td>
<td>6</td>
</tr>
</tbody>
</table>

Note: s.d. = standard deviation, c.v. (%) = coefficient of variation(standard deviation * 100)/mean, RE (%) = relative error, n = number of replicates.

Table 8.2: Assessment of accuracy and precision from quality control samples.

<table>
<thead>
<tr>
<th>Nominal Concentration (ng/ml)</th>
<th>GM (ng/ml)</th>
<th>S.D. (ng/ml)</th>
<th>Dev (%)</th>
<th>WRP (%)</th>
<th>BRP (%)</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>58.56</td>
<td>1.12</td>
<td>2.40</td>
<td>1.12</td>
<td>0.78</td>
<td>18</td>
</tr>
<tr>
<td>500</td>
<td>497.25</td>
<td>3.35</td>
<td>0.55</td>
<td>2.07</td>
<td>0.11</td>
<td>18</td>
</tr>
<tr>
<td>800</td>
<td>796.17</td>
<td>2.98</td>
<td>0.47</td>
<td>1.25</td>
<td>0.15</td>
<td>18</td>
</tr>
</tbody>
</table>

Note: gm = grand mean, s.d. = standard deviation, dev = percent deviation from nominal value, wrp = within-run precision, brp = between-run precision, n = total number of replicate observation.

Table 8.3: Short-term and long-term stability data.

<table>
<thead>
<tr>
<th>Storage condition</th>
<th>LQC (60 ng/ml)</th>
<th>MQC (500 ng/ml)</th>
<th>HQC (800 ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 freeze-thaw cycle</td>
<td>92.45 (4.78)</td>
<td>96.87 (1.52)</td>
<td>98.75 (1.78)</td>
</tr>
<tr>
<td>24 h ambient</td>
<td>93.61 (5.64)</td>
<td>97.32 (1.32)</td>
<td>98.88 (2.48)</td>
</tr>
<tr>
<td>1 month frozen (-20 C)</td>
<td>95.69 (4.39)</td>
<td>97.62 (1.74)</td>
<td>97.92 (2.79)</td>
</tr>
<tr>
<td>1 month frozen (+20 C)</td>
<td>95.24 (4.36)</td>
<td>96.63 (3.51)</td>
<td>96.89 (3.25)</td>
</tr>
</tbody>
</table>

Note: The data presented in this table are the percentage of measured value vs. theoretical value with cv in parenthesis (n = 6).

8.6.7 In vitro in vivo correlation (IVIVC)-

To assess the viability and validity of the sustained nature of IPR microparticles, IVIVC study is essential, since prolonged-release products may be especially suited for this kind of study. When the fraction of drug released from our best optimized formulation in pH 7.4 was plotted against the fraction of drug absorbed, a linear correlation was obtained (Figure 8.4). A higher value of correlation coefficient suggested that a good correlation existed between in vitro-in vivo data.

8.7 Conclusion-

The HPLC method described here for analysis of diclofenac sodium in plasma is very simple, specific and sensitive. Few analytical methods using LCMSMS and HPLC have been reported for the determination of the same in renal plasma.

The proposed method to analyze diclofenac sodium in plasma by HPLC with UV detection (LLOQ: 20 ng/ml and run time: 30 min) happens to be first of its kind described so far in the literature. This new method will be of immense help for carrying out a pharmacokinetic study of diclofenac sodium in laboratories that lacks a sophisticated analytical instrument like LCMSMS. The process described above also has an added advantage due to the use of a simple mobile phase, as we know that use of buffer solutions can be harmful to some extent and may reduce the life of a column.
8.6.5 Stability:

The stability of diclofenac sodium in plasma was determined under various conditions according to the procedure described in the earlier section. Each stability test included six replicates of three levels of QC samples. All stability results for the analyte are presented in Table 8.3.

QC samples undergoing three freeze-thaw cycles gave coefficient of variance %CV = 4.78 and an accuracy of 92.45–98.75%. QC samples stored at ambient temperature for 24 h gave %CV = 5.64 and an accuracy of 93.61–98.88%.

Long-term frozen storage stability was tested at 1 and 3 months after QC sample pools were prepared and stored at −20 °C. The 1-month stability data of all three QC samples showed an accuracy of 95.69–97.92% with %CV = 4.39 and the 3-months stability data had an accuracy of 95.24–96.89% with %CV = 4.36 in plasma.

8.6.6 Pharmacokinetic Study:

Figure 8.3 shows the mean plasma concentration time profile obtained following oral administration of an aqueous solution of DS and IPN microbeads. The Cmax of diclofenac sodium from oral solution was 2 ± 0.1 h, and the peak concentration (Cmax) at that time was 62.2 ± 1.5 ng mL−1. In the case of IPN microbeads, the Cmax was 55 ± 1.7 ng mL−1, which was significantly different from that obtained from oral solution. The mean tmax value after administration of IPN microbeads was 4 ± 0.48 h, which was significantly different from oral solution of DS (Table 8.4).

The elimination rate constant (k0) of the drug from solution was 0.444 ± 0.01 h−1, and that obtained from the IPN microbeads was 0.227 ± 0.014 h−1. Thus, the lower Cmax prolonged tmax and decreased t1/2 of diclofenac sodium in rabbit is another important indication on the in vivo performance of sustained release IPN microbeads in providing a prolonged drug delivery. These in vivo pharmacokinetic parameters were in good agreement with the observed in vitro drug release rate of the drug from microbeads as per our previous in vitro study. The extent of absorption (bioavailability) was significantly higher (p < 0.05) with IPN microbeads, as seen from the AUC oral = 278.50 ± 27.2 μg h mL−1 followed by the solution of DS (180.6 ± 22.8). When the AUCs obtained from the microbeads were divided by the AUC

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Solution</th>
<th>IPN microbead</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax (ng mL−1)</td>
<td>62.2 ± 1.5</td>
<td>55 ± 1.7</td>
</tr>
<tr>
<td>tmax (h)</td>
<td>2 ± 0.1</td>
<td>4 ± 0.48</td>
</tr>
<tr>
<td>AUC (ng mL−1 h−1)</td>
<td>180.6 ± 22.8</td>
<td>278.50 ± 27.2</td>
</tr>
<tr>
<td>S.D (h−1)</td>
<td>0.444 ± 0.01</td>
<td>0.227 ± 0.014</td>
</tr>
<tr>
<td>t1/2 (h)</td>
<td>1.56 ± 0.05</td>
<td>4.87 ± 0.041</td>
</tr>
<tr>
<td>Relative bioavailability</td>
<td>1.00</td>
<td>1.54</td>
</tr>
</tbody>
</table>

Figure 8.2: Representative chromatogram of: (A) Blank plasma spiked with drugs (150 ng mL−1) and IS (B) Plasma sample containing diclofenac sodium (674.07 ng mL−1) spiked with IS.
8.6 Discussion

8.6.1 Specificity

Representative chromatograms of blank plasma spiked with diclofenac sodium and IS, and plasma sample after 2 h of administration of an oral 20 mg dose of diclofenac sodium is presented in Figure 8.2. The IS and analyte was well resolved in this particular bioanalytical method. Moreover, there is no chemical interaction between the analyte and IS. Total run time of the chromatogram is about 30 min and the retention time of drug and IS are 6.8 and 22.7 min, respectively. No interfering peaks at those retention times were found in the chromatogram obtained from blank plasma, as the area of analytic and internal standard did not differ in the case of successive analysis of matrix from different individuals.

8.6.2 Linearity

The equation of the calibration curve was obtained by least-squares linear regression analysis of the peak area ratio of diclofenac sodium to internal standard versus concentration. The calibration curve exhibits an excellent linearity with regression coefficient of 0.9951 (±0.0077, range: 0.9891-0.9979). The calibration equation shows with average slope 0.0023 (±0.0005, range: 0.0022-0.0027) and intercept 0.207 (±0.041, range: 0.185-0.257), respectively. Table 8.1 shows all back-calculated values with excellent accuracy and precision.

8.6.3 Accuracy and precision

The between-run and within-run precision values of diclofenac sodium for various concentrations ranged from 0.11% to 0.78% and 1.12% to 2.07%, respectively. At the same concentrations, the values for accuracy were also within acceptable limits. The precision and accuracy data of three QC samples are presented in Table 8.2.

8.6.4 Extraction recovery

The recoveries (mean) of diclofenac sodium at low (60 ng/ml), medium (500 ng/ml) and high (800 ng/ml) quality-control samples was 81.52%, 89.31% and 95.29%, respectively. The recovery (mean) of IS was very good by mix solvent of ethanol-methanol and its value were 92.58% of the concentration used in the assay.