Original Article

Enhancement of the dissolution profile of Tenoxicam by a solid dispersion technique and its analytical evaluation using HPLC

Manal K. Darwish[1,*], Manal M. Foad[2]

[1] *Department of Pharmaceutics, Faculty of Pharmacy (Girls), Al-Azhar University, Cairo, Egypt;*
[2] *Department of Analytical Chemistry, Faculty of Pharmacy (Girls), Al-Azhar University, Cairo, Egypt.*

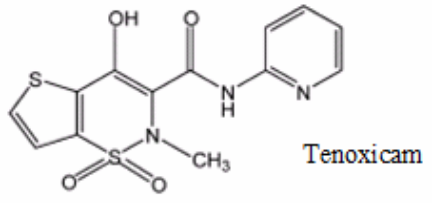
ABSTRACT: The aim of the present study was to improve the dissolution, and therefore the bioavailability, of poorly water-soluble tenoxicam. Solid dispersions consisting of tenoxicam with two different types of polymers were prepared. The first type were PVP_{30} and β-cyclodextrin and the second type were two superdisintegrants, explotab and croscarmellose sodium. A solid dispersion with an explotab ratio of 1:1 (F_8) had the best dissolution profile compared to all of the prepared solid dispersions as well as the pure drug, which was then formulated into tablets (T_2F_8). T_2F_8 had far better dissolution than commercial tablets, releasing only 28.3% of the drug, while T_2F_8 exhibited 96.5% drug release in 20 min. T_2F_8 was subjected to analytical validation as well as stability studies. The formulation was found to be stable after storage at 40°C for one month, 40°C and 75% relative humidity (40°C/75% RH) for one month, and 60°C for 15 days; this was confirmed by the absence of degraded product prepared in the laboratory by refluxing the drug with 1 N NaOH for 15 min. Infrared (IR) spectroscopy and differential scanning calorimetry (DSC) were performed on T_2F_8 to identify physicochemical interactions between the drug and carrier, hence its effect on dissolution. A simple and rapid HPLC method was also developed to determine tenoxicam in human plasma and was then used in a pharmacokinetic study. Plasma samples were analyzed on a C_{18} column with a mobile phase of 0.02 M sodium acetate:acetonitrile: methanol (7:2.5:0.5, v/v/v) and UV detection at 375 nm. The linear range of the plasma concentration was 1-16 µg/mL with a detection limit of 158 ng/mL. Within-day and between-day precision expressed as the relative standard deviation was less than 2%. The proposed method was successfully used in a bioequivalence study in healthy volunteers and mean pharmacokinetic parameters were calculated.

Keywords: Tenoxicam, Superdisintegrant, Pharmacokinetic, Solid dispersion, Dissolution enhancement

1. Introduction

Tenoxicam (4-hydroxy-2-methyl-*N*-(pyridine-2-yl)-2*H*-thieno-1,2-thiazine-3-carboxamide-1,1-dioxide) (*1*) is a nonsteroidal anti-inflammatory drug (NSAID) from the oxicam group that also has analgesic and antipyretic properties as a result of inhibiting prostaglandin synthesis (*2*). Like other oxicam derivatives, tenoxicam has been found to be about 99% protein-bound (*3*). Due to its accentuated hydrophilic character in comparison to other oxicams, tenoxicam is characterized by less penetration into tissues, explaining its lower incidence of adverse reactions (*4*). Tenoxicam has recently been studied using IR spectroscopy (*5*) and spectrophotometric (*6-9*), chromatographic (*10-12*), polarographic (*13*), and pharmacokinetic analysis (*14*).

Tenoxicam is a poorly water-soluble drug; for such drugs, dissolution plays an important role in their absorption (*15*). Although it has excellent oral bioavailability, its poor aqueous solubility limits its absorption dissolution rate and thus delays its onset of action. In order to enhance drug solubility in water and in biological fluids, many approaches have recently been devised and include salt formation, solubilization,



Tenoxicam

**To whom correspondence should be addressed:*
Dr. Manal K. Darwish, Pharmaceutics Department, Faculty of Pharmacy (Girls), Al-Azhar University, Cairo, Egypt;
e-mail: maboushady2000@yahoo.com

particle size reduction, solid dispersion (SD), self-dispersing liquid formulations (*16*), and the use of inclusion compounds based on cyclodextrin (*17*). Of these methods, solid dispersion is the most efficient. The technique provides a disposition of the drug on the surface of certain materials that can alter the dissolution properties of the drug. Once the solid dispersion is exposed to aqueous media and the carrier is dissolved, the drug is released as very fine colloidal particles (*18-20*). This results in a greatly enhanced surface area, thus prompting expectations of a high dissolution rate and level of bioavailability for poorly water-soluble drugs (*21*).

The aim of the present study was to formulate tenoxicam solid dispersions in order to improve dissolution and aqueous solubility to facilitate faster onset of action. Two groups of dissolution enhancers were used in the preparation of solid dispersions, the first group being PVP_{30} and β-cyclodextrin and the second group being two superdisintegrants, explotab and croscarmellose sodium. Solid dispersions with an improved dissolution profile were characterized using differential scanning calorimetry (DSC) and infra red spectroscopy (IR), and the one with the best dissolution profile was compressed into tablets. A simple and rapid method for the determination of tenoxicam in human plasma as well as in formulated tablets was developed and validated. Linearity range, limits of detection and quantitation, accuracy, precision, and specificity were determined in order to gauge the suitability of the method and confirm results. This method was used successfully in a pharmacokinetic study, revealing bioequivalence between tenoxicam tablets (T_2F_8) and commercial tablets.

2. Materials and Methods

2.1. *Materials*

Pure tenoxicam (TN) (purity 99.98%), explotab, and croscarmellose sodium (CS) were generously supplied by the Egyptian International Pharmaceutical Company (EIPICO; Tenth of Ramadan City, Egypt). β-Cyclodextrin was obtained from Fluka Chemical Corp., Buchs, Switzerland. Polyvinyl pyrolidone (PVP K_{30}) was generously supplied by El-Nile Pharmaceutical Co., Cairo, Egypt and Starch 1500 was from Colorcon, Shizuoka, Japan. HPLC-grade acetonitrile and methanol were purchased from Fisher Scientific, Pittsburgh, PA, USA. Sodium acetate and acetic acid were from El-Nasr, Kalubia, Egypt. Tenoxil tablets were obtained commercially.

2.2. *Instrumentation*

For HPLC, the Agilent-1100 series LC/DAD (Agilent Technologies, Böblingen, Germany) was used. For spectrophotometry, the Jasco FTIR-5300 spectrophotometer (Jasco Co., Tokyo, Japan) was used. A Shimadzu thermal analyzer (Shimadzu, Kyoto, Japan) was used Along with a United States Pharmacopeia (USP)-standard dissolution apparatus (Model DA-6D) (Veego, Bombay, India). Tabletting was done using an EK:O tabletting machine (Erweka, Frankfurt, Germany).

2.3. *Preparation of tenoxicam physical mixtures and solid dispersions*

Solid dispersions of the drug with hydrophilic carriers were prepared in ratios of 1:2, 1:1, and 2:1 of drug: carrier. Two techniques used for preparation were either solvent evaporation or co-grinding. For solvent evaporation, an accurate weighed quantity of tenoxicam was dissolved in a minimum amount of ethanol in which PVP, explotab, and croscarmellose sodium were suspended, the suspension was transferred to a petri dish, the solvent was allowed to evaporate at room temperature for one hour, and the result was then dried in a hot air oven. The mass obtained in each case was crushed, pulverized, and sifted through 80 mesh. For co-grinding, ground mixtures of tenoxicam with β-cyclodextrin were prepared by grinding in a mortar for one minute.

Physical mixtures were formulated for comparison by mixing the drug and carriers in geometric proportions using a spatula without applying pressure. All solid dispersions were stored over anhydrous calcium chloride in a desiccator until further evaluation. The selected formulae are shown in Table 1.

Table 1. Composition of different tenoxicam formulations

Codes	Component	Ratio	Method
PD	Pure drug	–	–
$\mathbf{F_1}$	TN/PVP_{30}	1:2	SD/Co-evaporation
$\mathbf{F_2}$	TN/PVP_{30}	1:1	SD/Co-evaporation
$\mathbf{F_3}$	TN/PVP_{30}	2:1	SD/Co-evaporation
$\mathbf{F_4}$	TN/β-CD	1:2	SD/Co-grinding
$\mathbf{F_5}$	TN/β-CD	1:1	SD/Co-grinding
$\mathbf{F_6}$	TN/β-CD	2:1	SD/Co-grinding
$\mathbf{F_7}$	TN/Explotab	1:2	SD/Co-evaporation
$\mathbf{F_8}$	TN/Explotab	1:1	SD/Co-evaporation
$\mathbf{F_9}$	TN/Explotab	2:1	SD/Co-evaporation
$\mathbf{F_{10}}$	TN/CS	1:2	SD/Co-evaporation
$\mathbf{F_{11}}$	TN/CS	1:1	SD/Co-evaporation
$\mathbf{F_{12}}$	TN/CS	2:1	SD/Co-evaporation
$\mathbf{F_{13}}$	TN/PVP_{30}	1:2	Physical mixture
$\mathbf{F_{14}}$	TN/PVP_{30}	1:1	Physical mixture
$\mathbf{F_{15}}$	TN/PVP_{30}	2:1	Physical mixture
$\mathbf{F_{16}}$	TN/β-CD	1:2	Physical mixture
$\mathbf{F_{17}}$	TN/β-CD	1:1	Physical mixture
$\mathbf{F_{18}}$	TN/β-CD	2:1	Physical mixture
$\mathbf{F_{19}}$	TN/Explotab	1:2	Physical mixture
$\mathbf{F_{20}}$	TN/Explotab	1:1	Physical mixture
$\mathbf{F_{21}}$	TN/Explotab	2:1	Physical mixture
$\mathbf{F_{22}}$	TN/CS	1:2	Physical mixture
$\mathbf{F_{23}}$	TN/CS	1:1	Physical mixture
$\mathbf{F_{24}}$	TN/CS	2:1	Physical mixture

2.4. *Characterization of solid dispersions*

Solid dispersions were characterized by Fourier transform infrared (FTIR) spectroscopy; the scanning range was 400 to 4,000 cm^{-1} with a resolution of 2 cm^{-1} and differential scanning calorimetry (DSC) was performed at a rate of 5°C per min over the range of 30-300.

2.5. *In vitro dissolution studies*

Dissolution rate studies were performed in 900 mL of 0.1 N HCl (pH 1.2) at 37 ± 0.5°C, using a dissolution apparatus with paddles rotating at 50 rpm. Solid products, solid dispersions of the drug and carriers, and their physical mixtures containing 20 mg tenoxicam were subjected to dissolution; these results were then compared to those for the pure drug. At fixed time intervals, samples were withdrawn, filtered, and spectrophotometrically assayed for drug content at 375 nm. Dissolution efficiency (DE) was calculated from the area under the dissolution curve at time *t* and expressed as percentage of the area of the rectangle described by 100% dissolution in the same time (*22*).

2.6. *Tablet preparation*

Solid dispersions containing tenoxicam with explotab (1:1), croscarmellose sodium (1:2), and PVP_{30} (2:1) had the maximum *in vitro* dissolution of all prepared formulations, and this is why they were mixed with talc (2%), sodium lauryl sulphate (1%), and magnesium stearate (1%) and then compressed into tablets. The average weight of the tablets was adjusted to 250 mg using Avicel 102, and tablets were coded T_1F_3, T_1F_8, and T_1F_{10} for tenoxicam/PVP_{30}, tenoxicam/explotab, and tenoxicam/CS, respectively, as shown in Table 2. Starch 1500 was added to the aforementioned formulations as a disintegrating agent and the result was compressed into tablets (T_2F_3, T_2F_8 and T_2F_{10} in Table 2) to determine whether the presence or absence of a disintegrant would affect the dissolution of the prepared tablets. All ingredients were mixed and compressed in a tabletting machine using flat-tip punches and dies with an 8-mm diameter *via* a direct compression technique. *In vitro* dissolution studies for the prepared tablets and commercial tablets were conducted using 900 mL 0.1 N HCl as a dissolution medium at 50 rpm.

Table 2. Composition of tenoxicam tablets

Tablets code	Component	Starch 1500
T_1F_3	TN/PVP_{30}	–
T_1F_8	TN/Explotab	–
T_1F_{10}	TN/CS	–
T_2F_3	TN/PVP_{30}	5%
T_2F_8	TN/Explotab	5%
T_2F_{10}	TN/CS	5%

2.7. *Stability study of tablet*

In order to determine any changes in the *in vitro* drug release profile as a result of storage, a stability study of T_2F_8 tablets containing tenoxicam/explotab, which had the maximum dissolution release, was conducted at 40°C for one month, 40°C and 75% relative humidity (40°C/75% RH) for one month, and 60°C for 15 days.

2.8. *Validation of the HPLC method*

Tenoxicam was subjected to analytical validation in human plasma using an HPLC method according to USP guidelines, from which the recovery of the prepared T_2F_8 can be calculated.

2.8.1. *Preparation of standard solutions*

Different aliquots (0.01-0.16 mg) of the standard tenoxicam in methanol, 0.1 mg/mL were introduced into a series of 10-mL volumetric flasks and adjusted to volume with methanol. One mL of each solution was transferred to a series of 5-mL centrifuge tubes, each containing 1 mL blank plasma. Zero point three mL of acetonitrile/perchloric acid mixture (2:1) were added to each tube, vortexed for 30 sec, and then centrifuged at 5,000 rpm for 10 min.

2.8.2. *Linearity*

The linearity of the method was evaluated using a calibration curve in the range of 1-16 µg/mL tenoxicam. Twenty µL injections were made in triplicate for each concentration and chromatographed on a C_{18} column at ambient temperature using a mobile phase of 0.02 M sodium acetate (pH 2.7)-acetonitrile-methanol (70:25:5, v/v/v) at a flow rate of 1 mL/min and UV detection at 375 nm. The calibration curve was obtained by plotting the peak area as a function of drug concentration and the regression parameters were determined.

2.8.3. *Accuracy and precision*

The intraday and interday accuracy and precision were determined by replicate analysis of three sets of samples spiked with three different concentrations of tenoxicam (2, 8, and 16 µg/mL) within one day or on three consecutive days.

2.8.4. *Recovery*

Absolute recovery of tenoxicam was determined in triplicate by extracting blank human plasma samples spiked with tenoxicam; the mean peak area was compared to that obtained from the standard drug with the same concentration.

2.8.5. *Specificity*

The proposed method successfully quantitated tenoxicam even in the presence of degradation products. Forced degradation of the pure drug was performed in the laboratory where 10 mg of tenoxicam were transferred to a 100-mL conical flask. Twenty mL of 1 M NaOH were added and refluxed for 20 min. After cooling, 5 M HCl was added to the drug solution until the pH was adjusted to 8. The solution was then evaporated under a vacuum, extracted three times with 20 mL methanol, filtered into a 100-mL volumetric flask, and brought to volume with methanol. The obtained solution was labeled as contain degradates derived from 0.1 mg/mL tenoxicam. Laboratory-prepared mixtures containing different ratios of pure and degraded drug were analyzed using the proposed HPLC method.

2.8.6. *Analysis of tenoxicam in T_2F_8 tablets*

Ten prepared T_2F_8 tablets were weighed, finely ground, and mixed. The amount of fine powder equivalent to 10 mg tenoxicam was sonicated with 70 mL methanol for 15 min, filtered into a 100-mL volumetric flask, and brought to volume with methanol. The obtained solution was labeled as containing 0.1 mg/mL and analyzed after spiking by the proposed HPLC method, as described previously in the section on linearity.

2.9. *Bioavailability study*

The selected tablet formula T_2F_8 with the maximum dissolution profile (with a tenoxicam/explotab ratio of 1:1) and commercial tablets were subjected to a single-dose relative pharmacokinetic study. The study was a crossover bioavailability design that was performed using 6 healthy adult male volunteers between the ages of 22 and 30 years and weighing between 60 and 85 kg. The volunteers were prevented from taking any alcohol or drugs for 2 days prior to experiment and during the study. The volunteers fasted for 8 h before drug administration. Each subject ingested 2 compressed tablets of the two products (test and reference). An interval of 14 days was allowed prior to the next treatment. Whole blood samples were taken from a forearm vein pre-dose and at 1, 2, 3, 4, 5, 6, and 8 h post-dosing. The blood was centrifuged at 5,000 rpm for 10 min, and the plasma obtained was stored at -20°C until analysis. To compare the rate and extent of absorption of tenoxicam, the following pharmacokinetic variables were calculated for each volunteer using actual blood sampling times. The maximum plasma concentration (C_{max}) and the time required to reach this concentration (T_{max}) were read directly from the arithmetic plot of time *vs*. plasma concentration for tenoxicam. The overall elimination rate constant (k_e) was calculated from the slope of the terminal elimination phase of a semilogarithmic plot of concentration *vs*. time after subjecting it to linear regression analysis. The elimination half-life ($t_{1/2}$) was obtained by dividing 0.693 by k_e. The absorption rate constant (k_a) was calculated using the method of residuals (*23*). The area under the plasma tenoxicam concentration *vs*. time curve ($AUC_{0-\infty}$) was determined by means of the trapezoidal rule. The relative bioavailability of tenoxicam from matrix tablets in comparison to a reference formulation (commercial tablets) was calculated by dividing its $AUC_{0-\infty}$ by that of the commercial tablet dosage form.

3. Results and Discussion

3.1. *Characterization of solid dispersions*

3.1.1. *Differential scanning calorimetry*

Thermograms were carried out separately with the drug and carriers as shown in Figure 1. The DSC curve for tenoxicam had one endothermic peak at about 225°C, corresponding to its melting point, while crospovidone, explotab, and PVP had broad peaks at 80.8, 78.4, and 72.7°C, respectively. On the other hand, thermograms for all physical mixtures indicate that there was no appreciable shift in the melting peak of tenoxicam with all carriers. This was also true for

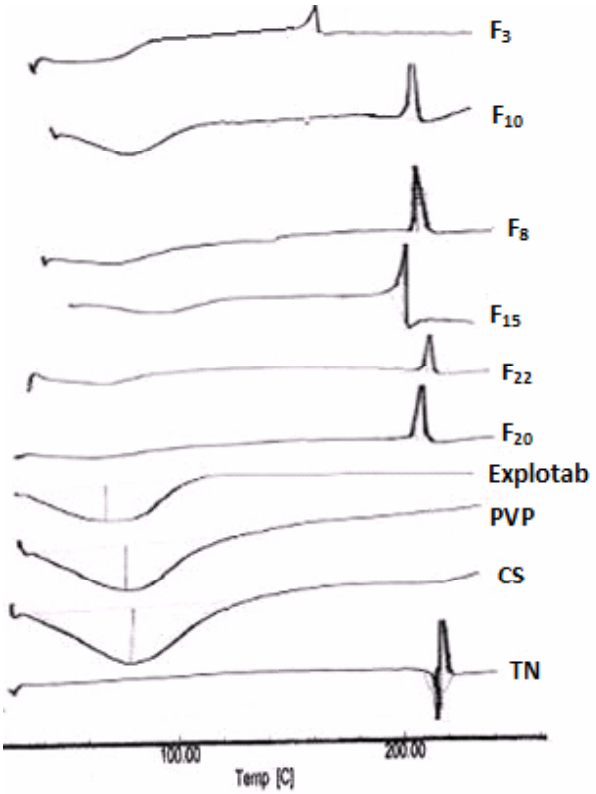


Figure 1. **DSC thermogram of tenoxicam, carriers, their physical mixtures, and corresponding solid dispersions.**

solid dispersions of the drug with croscarmellose and explotab, indicating the absence of strong interactions between the components. The increase in the dissolution rate was thus attributed to an increase in the available surface area of the drug due to improved wettability provided by the superdisintegrants. In the case of drug/PVP solid dispersion, the peak shifted slightly to a lower temperature (203°C); this may be due to a low carrier ratio in the solid dispersion, but a notable decrease in peak intensity is evidence of its formation.

3.1.2. *Fourier transform infrared (FTIR) spectroscopy*

Figure 2 shows the IR spectra for tenoxicam in physical mixtures and solid dispersions with different carriers. The IR spectrum of the plain drug had an absorption band at 3,395 cm^{-1} due to an O–H stretching vibration; the broadness of this band is indicative of hydrogen bonding. The strong band observed at 1,636 cm^{-1} is attributed to the carbonyl stretching vibration in the secondary amide group (CO–NH). The band located at 1,597 cm^{-1} is due to the stretching vibration of pyridyl nitrogen (C=N). Addition of the carriers studied to pure tenoxicam resulted in no shifting for any of these characteristic bands, indicating no chemical interaction between the drug and the polymers used.

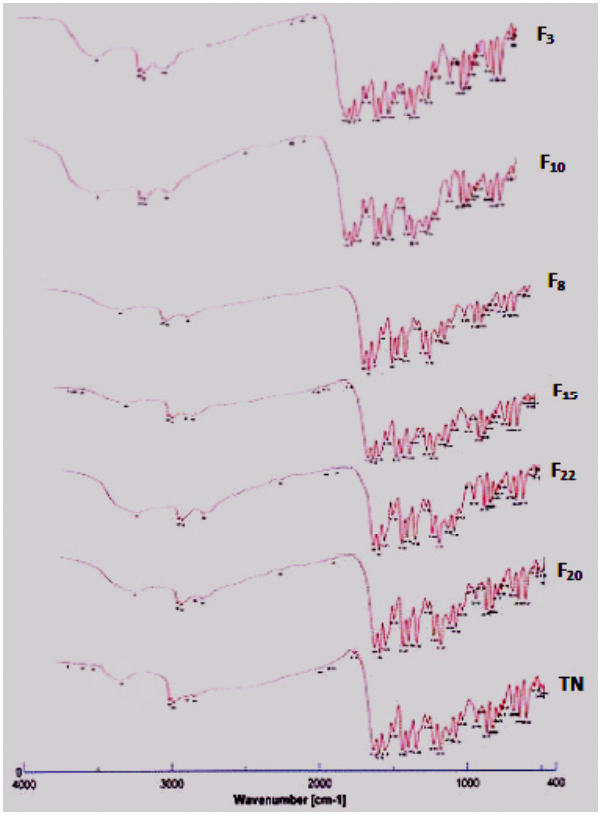


Figure 2. IR spectra of tenoxicam, physical mixtures, and corresponding solid dispersions for the chosen formulations.

3.2. *In vitro dissolution studies*

Dissolution profiles of the pure drug and drug-carrier binary systems are represented in Figures 3-6. As is apparent, the solid dispersion technique improved the dissolution rate of tenoxicam to a great extent. This is clearly evident from the % of drug dissolved in 20 min (DP_{20}) and dissolution efficiency at 60 min (DE_{60}) for

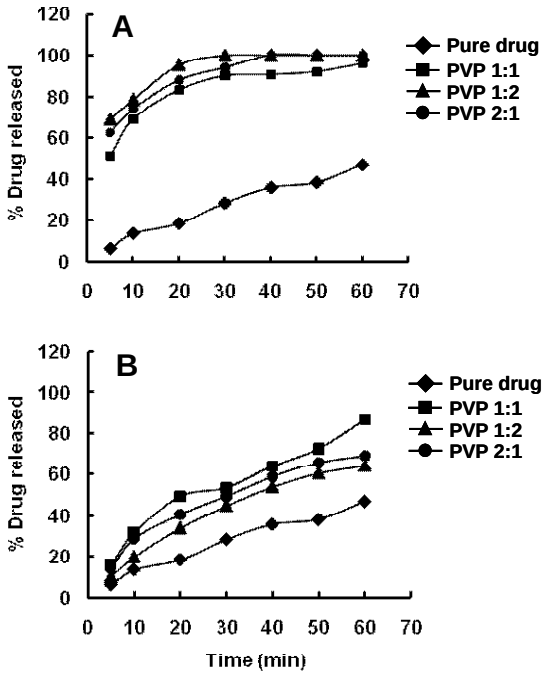


Figure 3. % drug released from solid dispersions (A) and physical mixtures (B) prepared with PVP_{30} in different ratios.

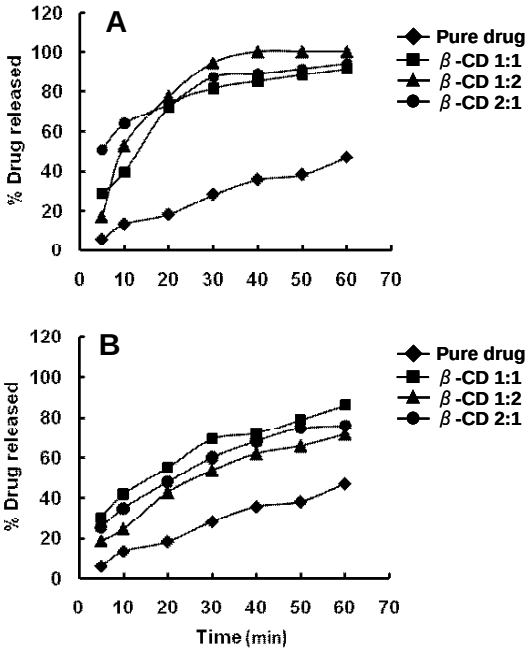


Figure 4. % drug released from solid dispersions (A) and physical mixtures (B) prepared with β-CD in different ratios.

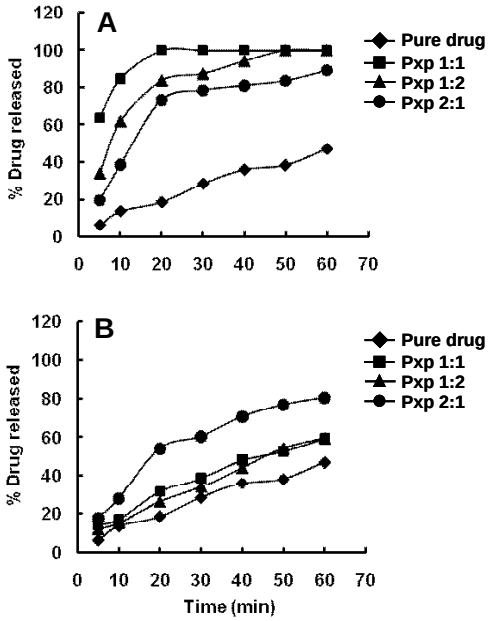


Figure 5. % drug released from solid dispersions (A) and physical mixtures (B) prepared with explotab in different ratios.

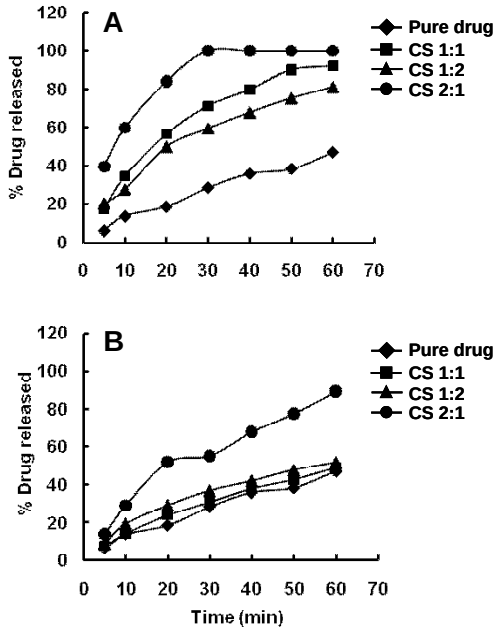


Figure 6. % drug released from solid dispersions (A) and physical mixtures (B) prepared with croscarmellose in different ratios.

the pure drug and its binary systems with carriers, as presented in Table 3. Solid dispersions formulated with a drug/PVP ratio of 2:1 had a DP_{20} and DE_{60} of 95.4% and 91.1%, respectively, in comparison to 18.5% for pure drug powder. Decreased crystallinity and increased wetting of the particles may be considered major contributors to the enhanced dissolution of tenoxicam from a solid dispersion system containing PVP (*24*). Solid dispersions with β-cyclodextrin as a carrier had an increase in drug release in comparison to the pure

Table 3. Dissolution parameters for different tenoxicam formulations

SSD codes	Component	Ratio	DP_{20} (%)*	DE_{60} (%)**
PD	Pure drug	–	18.5 ± 1.04	29.67
F_1	TN/PVP_{30}	1:2	88.0 ± 3.50	88.80
F_2	TN/PVP_{30}	1:1	83.2 ± 0.45	82.16
F_3	TN/PVP_{30}	2:1	95.4 ± 2.06	91.10
F_4	TN/β-CD	1:2	73.9 ± 1.34	79.90
F_5	TN/β-CD	1:1	72.3 ± 1.67	73.26
F_6	TN/β-CD	2:1	77.7 ± 1.55	82.49
F_7	TN/Explotab	1:2	73.3 ± 3.09	69.70
F_8	TN/Explotab	1:1	99.8 ± 2.44	91.52
F_9	TN/Explotab	2:1	83.7 ± 2.98	82.95
F_{10}	TN/CS	1:2	84.3 ± 2.76	86.15
F_{11}	TN/CS	1:1	56.7 ± 2.44	68.60
F_{12}	TN/CS	2:1	49.9 ± 1.34	58.70
F_{13}	TN/PVP_{30}	1:2	40.8 ± 1.32	50.68
F_{14}	TN/PVP_{30}	1:1	49.4 ± 1.98	57.18
F_{15}	TN/PVP_{30}	2:1	34.0 ± 1.22	45.39
F_{16}	TN/β-CD	1:2	47.9 ± 1.55	58.84
F_{17}	TN/β-CD	1:1	55.0 ± 2.49	64.72
F_{18}	TN/β-CD	2:1	42.7 ± 1.27	54.06
F_{19}	TN/Explotab	1:2	54.2 ± 3.22	59.77
F_{20}	TN/Explotab	1:1	31.4 ± 1.87	40.61
F_{21}	TN/Explotab	2:1	26.3 ± 1.54	38.89
F_{22}	TN/CS	1:2	51.9 ± 1.76	59.49
F_{23}	TN/CS	1:1	24.1 ± 1.23	32.43
F_{24}	TN/CS	2:1	29.3 ± 2.55	36.53

* DP_{20}: Percent drug dissolved in 20 min. ** DE_{60}: Dissolution efficiency at t = 60 min. (calculated from the area under the dissolution curve at t = 60 min and expressed as a % of the area of the rectangle described by 100% dissolution in the same time). Each value is the average of three determinations.

drug, as shown in Figure 4. Table 3 represents DP_{20} and DE_{60} of 77.7% and 82.49%, respectively, for TN/β-CD (2:1-SD). This enhancement can be attributed to the greater hydrophilic character of the systems due to the presence of the carrier, which can reduce interfacial tension between a poorly water-soluble drug and dissolution medium (*25*).

Moreover, in the case of β-cyclodextrin the carrier dissolves more rapidly than the drug in the early stage of the dissolution process. Hence, it can act on the hydrodynamic layer surrounding the drug particles, resulting in an *in situ* inclusion process that improves the dissolution of the drug. In fact, systems containing a larger amount of β-cyclodextrin had faster drug dissolution. The addition of the two superdisintegrants markedly improved the dissolution rate of the formulated solid dispersions in comparison to the pure drug; even their physical mixtures displayed an increase in drug release, as shown in Table 3 and Figures 5 and 6. Formulations prepared with explotab had a great increase in dissolution behavior as noted from DP_{20} values; a formulation with a 1:1 ratio had the highest DP_{20} value (99.80%) and a DE_{60} of 91.52%. Explotab was expected to absorb a large amount of water when exposed to dissolution medium and swell, thus resulting in the wetting of small drug particulates deposited on the surface of explotab. The carrier swelling would cause deaggregation of clusters of small drug particles and facilitate their dissolution. The improved dissolution in formulations with croscarmellose (Figure 6) could also be due to a reduction in the particle size of

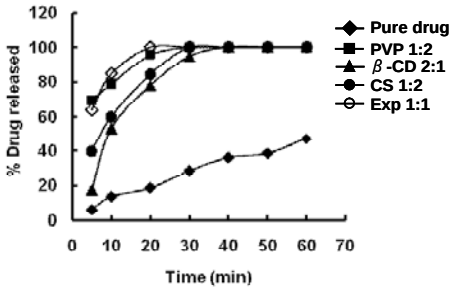


Figure 7. % drug released from solid dispersions exhibiting maximum release in comparison to the pure drug.

Table 4. Dissolution parameters for tenoxicam tablets

Tablets code	DP_{20} (%)*	DE_{60} (%)**
Commercial	28.3	42.82
T_1F_3	32.8	49.59
T_1F_8	86.8	84.53
T_1F_{10}	63.0	72.65
T_2F_3	53.4	74.23
T_2F_8	96.5	93.77
T_2F_{10}	86.6	86.70

* DP_{20}: Percent drug dissolved in 20 min. ** DE_{60}: Dissolution efficiency at t = 60 min. (calculated from the area under the dissolution curve at t = 60 min and expressed as a % of the area of the area of the rectangle described by 100% dissolution in the same time). Each value is the average of three determinations.

the drug, its deposition on the surface of the carrier, and improved hydrophilicity. Figure 7 shows the superior drug release rate for each carrier used. A PVP_{30} ratio of 1:2, a β-CD ratio of 2:1, an explotab ratio of 1:1, and a croscarmellose ratio of 1:2 resulted in maximum drug release in comparison to other formulations as well as the pure drug.

3.3. *In vitro dissolution studies for tablets*

Based on *in vitro* dissolution performance, solid dispersions with a PVP_{30} ratio of 2:1, explotab ratio of 1:1, and croscarmellose ratio of 1:2 were selected for compression into tablets since they had superior drug release rates. Table 4 shows a comparison of the percent drug released in 20 min (DP_{20}) as well as dissolution efficiency at 60 min (DE_{60}) in tablets prepared from the selected solid dispersions with different carriers and that of commercial tablets. As is apparent from the table, T_2F_3, T_2F_8, and T_2F_{10} had a DP_{20} of 53.4, 96.5, and 86.6%, respectively, while they had a DE_{60} of 74.23, 93.77, and 86.7%, respectively. They also had a higher drug release than tablets without disintegrant (T_1F_3, T_1F_8, and T_1F_{10}) in which the disintegration step was not enhanced. Figure 8 shows a delay in the release of the drug from tablets in comparison to their solid dispersions due to the time taken (several minutes) by the disintegration step in tablets.

3.4. *Stability study of tablets*

In order to determine any changes in the *in vitro* drug

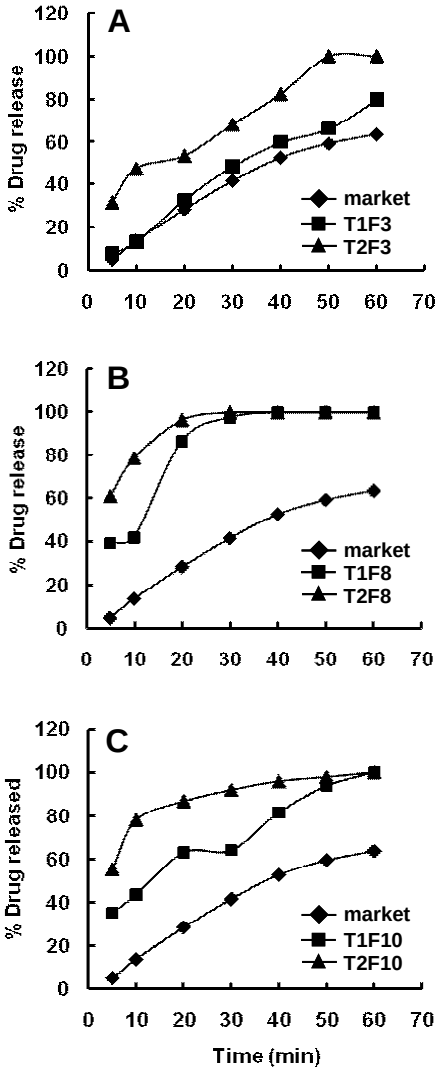


Figure 8. % drug released from commercial tablets and formulated tablets with and without disintegrant, for tablets prepared with PVP_{30} (A), tablets prepared with Explotab (B) and tablets prepared with croscarmellose (C) in comparison to commercial tablets.

release profile as a result of storage, a stability study of tablets containing tenoxicam/explotab, which had the maximum dissolution release, was conducted at 40°C for one month, 40°C and 75% relative humidity (40°C/75%RH) for one month, and 60°C for 15 days. As shown in Figure 9, under these conditions no significant changes in the tenoxicam release profile from T_2F_8 tablets were observed. This similarity in drug release was confirmed by data obtained from values for the difference factor (f_1), and similarity factor (f_2). These factors help to assure similarity in product performance according to the US Food and Drug Administration's guides for industry (*26*). Generally f_1 values up to 15 (0-15) and f_2 values greater than 50 (50-100) ensure sameness or equivalence of the two curves (*27*,*28*). Table 5 shows values for f_1 and f_2 obtained from *in vitro* dissolution data for tenoxicam-explotab tablets after storage for the aforementioned times. All values were within the accepted range, indicating the similarity of all tablets at all storage times.

Table 5. Results of similarity factors obtained after different storage times

Factors	T_2F_8 (15 days)	T_2F_8 (30 days) (40°C/75% RH)	T_2F_8 (30 days) (40°C)
f_1	3	8	10
f_2	71	68	64

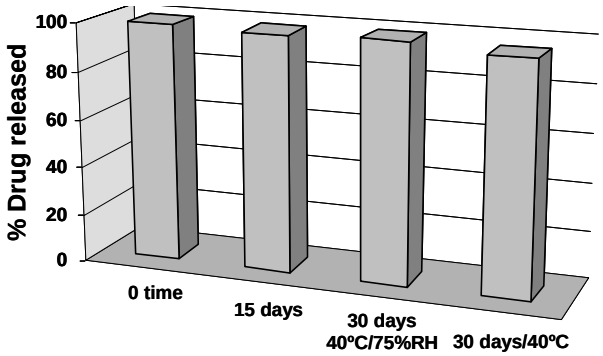


Figure 9. % drug release of (T_2F_8) tablet after different storage times.

3.5. *Validation data*

3.5.1. *Optimization of chromatographic conditions*

Different chromatographic conditions affecting the separation process were studied and optimized. Different compositions of the mobile phase, flow rates, and wavelengths were tried. Tenoxicam's peak was resolved by using a reversed-phase Nucleosil C_{18} column (particle size: 5 µm, 250 mm × 4.6 mm), a mobile phase of 0.02 M sodium acetate (pH 2.7) acetonitrile-methanol (70:25:5, v/v/v) at a flow rate of 1 mL/min, and UV detection at 375 nm. Under these conditions, the drug exhibited a sharply resolved peak at 8.2 min. A chromatogram of human blank plasma and blank plasma spiked with tenoxicam is shown in Figure 10.

3.5.2. *Linearity*

According to peak area-response at 375 nm, Beer's law was obeyed over the range of 1-16 µg/mL of tenoxicam, with a high correlation coefficient (0.9998). The limit of quantitation (LOD) was assessed using the slope of calibration curve and standard deviation of the blank and was found to be 526 ng/mL, as shown in Table 6.

3.5.3. *Accuracy and precision*

Inter- and intraday accuracy and precision of the proposed procedure were calculated. Inter- and intraday accuracy (expressed as R%) ranged from 97.1 to 98.3 and from 98.5 to 98.9, respectively. However, precision (expressed as RSD%) ranged from 0.21 to 0.95 and from 0.84 to 1.32, respectively, as shown in Table 6.

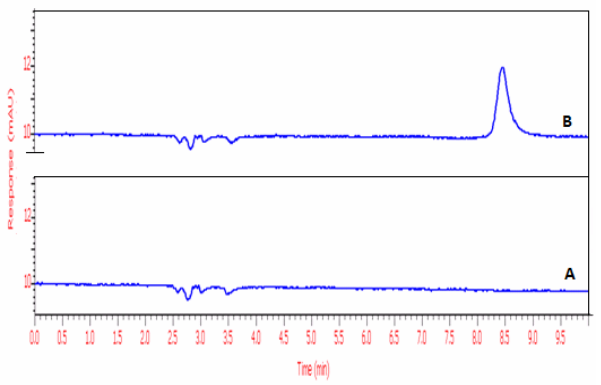


Figure 10. Chromatogram of human blank plasma (A) and blank plasma spiked with tenoxicam (B).

Table 6. Selected spectral data for the determination of spiked tenoxicam by the proposed HPLC procedure

Parameter	HPLC procedure
Linearity range (µg/mL)	1-16
λ_{max}	375 nm
LOD (ng/mL)	158
LOQ (ng/mL)	526
Regression parameters	
Slope ± SD (S_b)	9,842 ± 33.9
Intersept ± SD (S_a)	-108.6 ± 279.9
SD of residual (S_{xy})	413.6
Correlation coefficient (r^2)	0.9998
Accuracy (R%)	
Interday	97.1 - 98.3
Intraday	98.5 - 98.9
Precision (RSD%)*	
Interday	0.21 - 0.95
Intraday	0.84 - 1.32

*$n = 9$.

Table 7. Absolute recovery of tenoxicam from spiked human plasma

Taken conc. (µg/mL)	Found conc. (µg/mL)	Recovery (%)
2	1.73	86.5
6	5.29	88.2
10	8.66	86.6
14	12.21	87.2
16	13.83	86.4
Mean ± SD%	–	86.98 ± 0.75

3.5.4. *Absolute recovery*

Using the proposed HPLC method, absolute recovery of the drug ± SD was 86.98 ± 0.75% (Table 7).

3.5.5. *Specificity*

The pure drug was selectively determined using the proposed HPLC method in the presence of up to 95% of its alkaline induced degradation product with a mean recovery of 99.92 ± 1.01%. Peaks at 2.4 and 3.4 min for degradates were well resolved from the intact tenoxicam peak at 8.2 min (Figure 11). A suggested degradation pathway was proposed (Scheme 1) and verified by the IR spectra of both the drug and degradates.

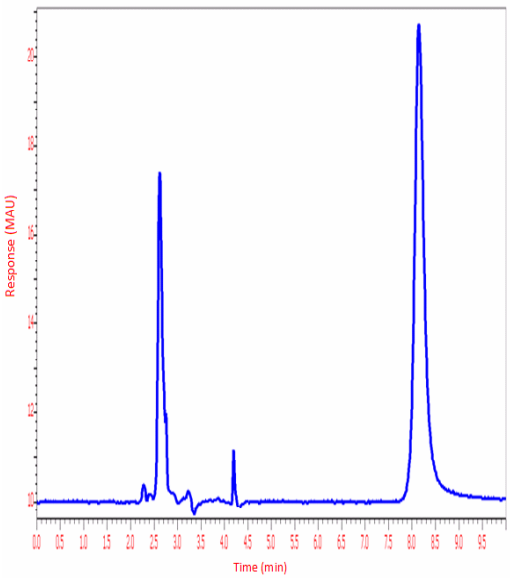
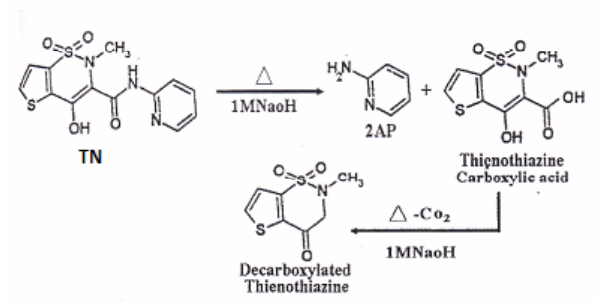


Figure 11. **HPLC chromatogram of a mixture of tenoxicam and its degradates.**



Scheme 1. **Degradation pathway of tenoxicam.**

Table 8. Statistical analysis of the results obtained by the proposed procedure for the determination of tenoxicam in the prepared T_2F_8 tablets in comparison to those from a conventional method

Parameter	Proposed HPLC procedure	Conventional method (*6*)
n	5	6
Mean recovery%	97.23	99.3
SD	0.69	0.51
Variance	0.47	0.26
t^*	0.552 (2.262)	–
F^*	1.83 (5.19)	–

* Values in parentheses are the theoretical *t* and *F* values at $P = 0.05$.

Appearance of a single peak at 8.3 min in the chromatogram of the prepared T_2F_8 tablets proved the successful evaluation of tenoxicam in the prepared tablets without interference from additives with a mean recovery of 97.23 ± 0.69%. This was then compared statistically to the results of a conventional method (*6*); no significant differences were found between the two methods at a probability of 95% (Table 8).

3.6. *Bioavailability studies*

The mean tenoxicam plasma concentration *vs*. time profiles is shown in Figure 12. The mean

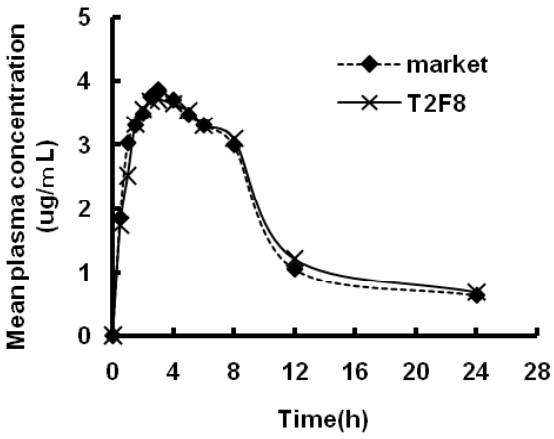


Figure 12. **Mean plasma concentration of tenoxicam after oral administration of T_2F_8 tablets in comparison to commercial tablets.**

Table 9. Mean pharmacokinetic parameters for tenoxicam after oral administration of T_2F_8 tablets in human volunteers

Pharmacokinetic parameters	Volunteers orally administered	
	T_2F_8 tablets	Commercial tablets
AUC (0-24)	25.62	26.05
$AUC_{0-\infty}$ (μg/mL/h)	90.71	83.56
$t_{1/2}$ (h)	0.653782	0.867316
k_a (h^{-1})	1.059986	0.799017
T_{max} (h)	2.5	3.0
C_{max} (μg/mL)	3.72	3.87
Relative bioavailability (%)	108.54	–

pharmacokinetic parameters calculated from individual plasma tenoxicam concentrations *vs*. time profiles are summarized in Table 9.

Mean values for the two preparations were close together. Based on statistical data, pharmacokinetic parameters of the two preparations indicated bioequivalence. The relative bioavailability of tenoxicam tablet was found to be 108.54 %.

4. Conclusion

Based on the current study, improvement in the dissolution of the water-insoluble drug tenoxicam was achieved through solid dispersion using different carriers, the best of which was explotab, which exhibited complete drug release in 20 min. A tenoxicam/explotab solid dispersion was then compressed into tablets but its enhanced dissolution profile was maintained. Moreover, a sensitive and rapid method was provided for the analysis of tenoxicam in human plasma either in bulk or in pharmaceutical formulations. Validation of the proposed method was carried out according to USP guidelines; results were precise and accurate. The short duration of the assay and its specificity allowed for the use of the method in routine analysis and in a pharmacokinetic study, where the formulated tablet was found to be bioequivalent to commercial tablets when taken by human volunteers.

Acknowledgments

The authors would like to thank the International Pharmaceutical Company (EIPICO), Egypt, for their donation of tenoxicam.

References

1. British Pharmacopoeia Commission. British pharmacopoeia. 2008; vol. II, Crown copyright. 2007; pp. 2089-2090.
2. Hye SG, In KC. Effect of vehicles and penetration enhancers on the *in vitro* percutaneous absorption of tenoxicam through hairless mouse skin. Int J Pharm. 2002; 236:57-64.
3. Sora L, Toma G, Stefan U, Jean N, Victor D, Andrei M. Fast RPLC-UV method on short sub-two microns particles packed column for the assay of tenoxicam in plasma samples. J Pharm and Biom Anal. 2007; 43:1437-1443.
4. Nilson OG. Clinical pharmacokinetics of tenoxicam. Clin Pharmacokinet. 1994; 26:16-43.
5. Atay O, Dincol F. Quantitative determination of tenoxicam by infra-red spectrophotometry. Anal LeH. 1997; 30:1675-1684.
6. El-Walily AFM, Blaih SM, Barary MH, El Sayed MA, Abdine HH, El-Kersh AM. Simultaneous determination of tenoxicam and 2-aminopyridine using derivative spectrophotometry. J pharm Biomrd Anal. 1997; 15:1923-1928.
7. El-Ries MA. Spectrophotometric determination of piroxicam and tenoxicam in pharmaceutical preparations using uranyl acetate as a chromogenic agent. Anal LeH. 1998; 31:793-807.
8. Vi jayaraghavan C, Vi jayaraj R. Spectrophotometric determination of tenoxicam in plasma and urine samples. Indian-Drugs. 1997; 34:604-605.
9. Amin AS. Spectrophotometric determination of tenoxicam in pharmaceutical formulation using alizarin. J Pharm Biomed Anal. 2002; 29:729-736.
10. Taha EA, Salama NN, Fattah LE. Stability indicating chromatographic methods for the determination of some oxicams. J AOAC-Int. 2004; 87:366-373.
11. Joseph J, Bertucat M. Selective assays for quantitation of tenoxicam in presence of its photodegradation products. J Liq Chromatogr Relat Technol. 1999; 22:2009-2021.
12. Mason JL, Hobbs GJ. Simple method for the analysis of tenoxicam in human plasma using high performance liquid chromatography. J Chromatogr B Biomed Appl. 1995; 665:410-415.
13. Atkopar Z, Tuncel M. The polarographic determination of tenoxicam in pharmaceutical preparations. Anal Lett. 1996; 29:2383-2397.
14. Gao LQ, Wu D, Tao XL, Gang YY. Study on the relative bioavailability of tenoxicam capsules. Yaowu Fenxi Zazhi. 2006; 26:589-591.
15. Amidon GL, Lennemas H, Shah VP, Crison JR. A theoretical basis for a biopharmaceutic drug classification: the correlation of *in vitro* drug product dissolution and *in vivo* bioavailability. Pharm Res. 2005; 12:413-420.
16. Gershanik T, Benita S. Self dispersing liquid formulations for improving oral absorption of lipophilic drugs. Eur J pharm. 2000; 50:179-188.
17. Dixit RP, Nagaresenker MS. *In vitro* and *in vivo* advantage of celecoxib surface solid dispersion and dosage form development. Ind J Pharm Sci. 2007; 69:370-377.
18. Vippagunta SR, Maul KA, Tallavajhala S, Grant DJW. Solid-state characterization of nifedipine solid dispersions. Int J Pharm. 2002; 236:111-123.
19. Devi VK, Vijayalakhmi P, Avinash M. Preformulation studies on celecoxib with a view to improve bioavailability. Indian J Pharm Sci. 2003; 65:542-545.
20. Zedong D, Ashish C, Harpreet S, Duk SC, Hitesh C, Navnit S. Evaluation of solid state properties of solid dispersions prepared by hot-melt extrusion and solvent co-precipitation. Int J Pharm. 2008; 355:141-149.
21. Goldberg AH, Galbaldi M, Kanig KL. Increasing dissolution rates and gastrointestinal absorption of drugs *via* solid solutions and eutectic mixtures III. Experimental evaluation of griseofulvin-succinic acid solution. J Pharm Sci. 1966; 55:487-492.
22. Khan KA. The concept of dissolution efficiency. J Pharm Pharmacol. 1975; 27:48-49.
23. Gibaldi M, Perrier D. Pharmacokinetics. New York, NY: Marcel Dekker; 1990.
24. Sethia S, Squillante E. Solid dispersion of carbamazepine in PVP K30 by conventional solvent evaporation and supercritical methods. Int J Pharm. 2004; 272:1-10.
25. Mura P, Faucci MT, Parrini PL. Effects of grinding with microcrystalline cellulose and cyclodextrins on the ketofen physicochemical properties. Drug Dev Ind Pharm. 2001; 27:119-128.
26. Costa P. An alternative method to the evaluation of similarity factors in dissolution testing. Int J pharm. 2001; 220:77-83.
27. FDA. Guidance for industry: Immediate release of oral solid dosage forms–Scale up and post approval changes: chemistry, manufacture, controls, *in vitro* dissolution testing, and *in vivo* bioequivalence documentation. Centre of Drug Evaluation and Research, Rockville, MD, USA, 1995.
28. Shah VP, Tsong Y, Sathe P, Liu JP. *In vitro* dissolution profile comparison--statistics and analysis of the similarity factor, f_2. Pharm Res. 1998; 15:889-896.

(Received January 6, 2009; Accepted January 12, 2009)