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IN VITRO AND *IN VIVO* EVALUATION OF ACECLOFENAC LYOPHILIZED ORALLY DISINTEGRATING TABLETS

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ABSTRACT

Aceclofenac, a non-steroidal anti- inflammatory drug, with poor solubility and bioavailability was taken as candidate for enhancement of *in vitro* dissolution and *in vivo* bioavailability. Development of Aceclofenac orally disintegrating tablet (ODT) using lyophilization technique was adopted. The ODTs were prepared by freeze-drying an aqueous dispersion of Aceclofenac, matrix former, filler (sugar alcohol), and an anti-collapse. The tablets were evaluated compendial (uniformity of weight, uniformity of content, friability, *in vitro* disintegration time and *in vitro* dissolution), together with wetting time, *in vivo* disintegration time, moisture analysis and scanning electron microscopy. The compendial results showed that lyophilized ODTs disintegrated within few seconds and showed significantly faster dissolution rate of Aceclofenac in comparison with commercially available immediate release tablet Aceclofenac tablet (Bristaflam®). *In vivo* evaluation for the best chosen Aceclofenac ODT formulation (LA#10) was done for determination of the drug pharmacokinetics in comparison with the immediate release tablet Aceclofenac tablet (Bristaflam® 100 mg). A randomized crossover design was adopted in the comparative bioavailability study done on four healthy volunteers. Statistical analysis revealed significant difference between the Bristaflam IR tablet and Aceclofenac ODT (LA#10) regarding the following pharmacokinetic parameters: C_{max}, T_{max}, $t_{1/2}$, $AUC_{(0-24)}$, $AUC_{(0-\infty)}$ ($p < 0.05$); while insignificant difference regarding Mean residence time (MRT) ($p > 0.05$). The relative bioavailability of the Aceclofenac ODT (LA# 10) was 186.12% relative to the IR tablet (Bristaflam®) taken as reference standard.

INTRODUCTION: Orally disintegrating tablets (ODTs) offer an advantage for populations who have difficulty in swallowing (Dysphagia). Dysphagia is common among all age groups and more specific with pediatric, geriatric population along with institutionalized patients and patients with nausea, vomiting, and motion sickness complications [1, 2]. ODTs with good taste and flavor increase the acceptability of bitter drugs by various groups of population. ODT is a single unit dosage form that disintegrates in the oral cavity rapidly, usually in few seconds to enhance compliance and overcome difficulty in swallowing by pediatric and geriatric patients [3].

Aceclofenac, the selected model drug, is poorly water soluble NSAID having poor bioavailability; hence the first aim of the present study was to enhance the aqueous solubility of drug. Aceclofenac shows high anti-inflammatory, antipyretic and analgesic activity with moderate incidence of gastric side effects and a high therapeutic index [4].

Several studies have been carried out to increase the aqueous solubility of Aceclofenac such as by complexation with b-cyclodextrin and solid dispersion. The main advantage of ODT is the capacity to disintegrate or dissolve rapidly in oral cavity with assessment of saliva within a minute without need of water. Thereafter this could enhance the bioavailability of drug through pre-gastric absorption from the mouth, pharynx and esophagus [5].

In this study, ODTs containing Aceclofenac were prepared by a freeze-drying technique in order to improve the dissolution rate and oral bioavailability of Aceclofenac. Aceclofenac is largely eliminated via metabolic transformation; therefore, an ODT of Aceclofenac that is partially absorbed through the oral mucosa directly enters the systemic circulation, bypassing the gastrointestinal tract and first-pass metabolism of the liver, which may result in an increase in the fraction of drug reaching the systemic circulation and also result in a rapid onset of action via a more comfortable and convenient delivery route than the intravenous route.

MATERIALS AND METHODS:

Materials: Aceclofenac was supplied by Sinochem, China. Mannitol was supplied by SPI Pharma Inc, USA. Lactose was supplied by Meggle GmbH, Germany. Maltodextrin was supplied by Grain Processing Corp., USA. Gelatin, glycine, sodium chloride and potassium chloride were received from Adwic, El-Nasr Pharmaceutical Chemicals Co., Egypt. The water used was distilled de-ionized water. All other chemicals were reagent grade and used as received. Bristaflam® 100 mg (Bristol Myers Squibb, Egypt) was used as a reference tablet in in-vivo studies.

Preparation of ODTs: A (3^3) Full factorial design for the freeze dried formulae was adopted to determine the effect of filler type, matrix former type and matrix former concentration. Aceclofenac ODTs were prepared using gelatin (hydroxypropylcellulose or xanthan gum) as a matrix former, a sugar alcohol (lactose monohydrate or mannitol or maltodextrin) and glycine as an anti-collapse The matrix former was used in three different concentrations (1%, 3% and 5% w/v), while the three sugar alcohols and glycine were used at a concentration of 2% w/v. The percentage of sugar alcohol and glycine used was optimized during the formulation process to result in a strong and elegant tablet that could be handled with ease. Gelatin (hydroxypropylcellulose or xanthan gum) was first dissolved in distilled water at about 40°C to obtain the required concentration. Mannitol or (lactose or maltodextrin) and glycine were then added to the gelatin solution in the pre-determined concentration. An accurately weighed amount of Aceclofenac powder was dispersed in the prepared aqueous solution using a magnetic stirrer to result in a dose of 100 mg Aceclofenac per 1 ml.

One milliliter of the suspension was then poured in each pocket of a PVC blister pack with a diameter of 13 mm and a depth of 3 mm resulting in a dose of 100 mg per tablet. The tablet blister packs were then transferred to a freezer at -22°C and kept in the freezer for 24 h. The frozen tablets were placed in a lyophilizer for 24 h using a Novalyphe-NL500 Freeze Dryer with a condenser temperature of -45°C and a pressure of 7 x 10^{-2} mbar. These formulations were evaluated compendial. The detailed composition of the prepared ODTs is presented in **table 1**. The prepared ODTs were kept in tightly closed containers in desiccators over calcium chloride (0% relative humidity) at room temperature until further use.

Characterization of ODTs:

- **Uniformity of Weight:** The test was carried out according to the British pharmacopoeia 2011 [6]. Twenty tablets, from each formula, were individually weighed and the mean of tablet weights was calculated. Results are presented as mean value ± standard deviation (SD).

- **Uniformity of content:** Ten randomly selected tablets from each formula were individually assayed for drug content uniformity. The drug in ODTs was assayed by dissolving each tablet in 250 ml simulated saliva fluid (pH = 6.8). The solution was then filtered, properly diluted, and the absorbance was spectrophotometrically measured at λ_{max} = 276 nm for Aceclofenac. Each individual tablet contents must be between 90 percent and 110 percent of the average content and the tablet

formula fails to comply with the test if more than one individual tablet content is outside these limits or if one individual content, is outside the limits of 75 percent to 125 percent of the average content.

- **Tablet friability:** Twenty tablets, from each formulation, were accurately weighed and placed in the drum of friabilator (Erweka type, GmbH, Germany). The tablets were rotated at 25 rpm for a period of 4 min and then removed, de-dusted and accurately re-weighed (BP 2011) [7]. The percentage loss in weight was calculated and taken as a measure of friability.

- ***In Vitro* Disintegration Time:** Disintegration times of the prepared ODTs were determined with six tablets in distilled water kept at 25 ± 0.5ºC using a ZT3-3 disintegration tester (Erweka, Germany) according to BP (2011) specifications [8]. The disintegration time was defined as the time necessary for the ODT to completely disintegrate until no solid residue remains or only a trace amount of soft residue remains on the screen. A digital stopwatch was used to measure the disintegration time to the nearest second. Only one ODT was analyzed at a time in order to ensure utmost accuracy. All results are presented as mean value ± SD (n = 6).

- ***In vivo* Disintegration Time:** The *in vivo* disintegration time of each of the prepared ODTs was evaluated in four human volunteers after giving informed written consent. The volunteers had no history of hypersensitivity to NSAIDs. Prior to the test, all volunteers were asked to rinse their mouth with distilled water. Each of the four subjects was given a coded tablet. Tablets were placed on the tongue and immediately the time was recorded. They were allowed to move the tablet against the upper palate of the mouth with their tongue and to cause a gentle tumbling action on the tablet without biting on it or tumbling it from side to side. Immediately after the last noticeable mass had disintegrated, the time was recorded. The subjects were asked to spit out the content of the oral cavity after tablet disintegration and rinse their mouth with distilled water. The swallowing of saliva was prohibited during the test, and also saliva was rinsed from the mouth after each measurement. The test results are presented as mean value ± SD [9].

- **Wetting Time:** Ten milliliters of distilled water containing eosin, a water-soluble dye was placed in a Petri dish of 10 cm diameter. Tablets were carefully placed in the centre of the Petri dish and the time required for water to reach the upper surface of the tablet was noted as the wetting time. The test results are presented as mean value of three determinations ± SD [10].

- **Moisture Analysis:** The tablets were analyzed for their residual moisture content after lyophilization using Karl Fischer titrator (Metrohm, Switzerland). Each tablet was pulverized, inserted in the titration vessel containing dried methanol (Karl–Fischer grade) and titrated with Kombititrant reagent (Merck, Germany) after a stirring time of 3 min. Results are presented as mean value ± SD (n = 3).

- ***In vitro* Dissolution Studies:** The dissolution profiles of Aceclofenac in ODTs compared with the plain drug were determined in a dissolution tester (Erweka DT-700 Dissolution Tester, Germany) following the USP paddle method. All tests were conducted in 900 ml simulated saliva fluid without enzymes (SSF) at pH = 6.8. The dissolution medium was maintained at a temperature of 37 ± 0.5°C with a paddle rotation speed at 50 rpm. The amount of drug used was equivalent to 100 mg. At specified time intervals (1, 2, 5, 7, 10, and 15 min), 3 ml of dissolution medium was withdrawn and replaced with an equal volume of fresh medium to maintain a constant total volume.

  Samples were filtered through 0.45 µm Millipore filter and assayed for drug content spectrophotometrically at 276 nm after appropriate dilution. Cumulative amount of drug dissolved in the preparations was calculated using calibration equation. Dissolution tests were performed in six vessels per formulation (n = 6). The market product, Bristaflam, was also tested in the same way for comparison purposes using simulated intestinal fluid without enzymes at pH 6.8.

- **Scanning Electron Microscopic (SEM) Analysis:** Surface morphology and cross-sections of selected tablet formulations were examined using Jeol JSM-6400 scanning electron microscope (Tokyo, Japan). Cross-section samples were prepared by cutting a thin slice of the tablet using a scalpel.

***In Vivo* Absorption Studies:**

- **Study Design:**

- **Subject Population:** Four healthy male volunteers (ages between 25 and 35 years; mean age, 27 ± 1.7 years) participated in the study. All were within 10% of their ideal body weights (weights, 72 to 95 kg; mean weight 89 ± 14.1 kg and heights, 165 – 179 cm, mean height, 172 ± 6.8 cm). Health status of the volunteers was confirmed by complete medical history, physical examination and laboratory analysis for complete hematological and biochemical examination, all these were carried out at baseline. None of the volunteers had any history of drug or alcohol abuse, nor did they have any acute or chronic gastrointestinal, cardiac, vascular, hepatic or renal disease.

  The subjects were instructed to take no drugs for 1 week prior to and during the course of the study; no concurrent medication was allowed during the course of the study. No consumption of nicotine was permitted 12 hours before and 24 hours after drug intake, moreover, on each test day, coffee, tea, and cola beverages were withheld from subjects 12 hours before the administration and till the blood sampling was completed. Each subject read, understood, and signed an informed written consent. All subjects were informed about the risks and objectives of the study.

- **Study Design:** The study was performed to compare the pharmacokinetics of Aceclofenac 100 mg ODT formula (LA #10) and the reference, Bristaflam® 100 mg (Bristaflam, Bristol Mayers Squibb) using non – blind, two treatments, two periods, randomized cross over design. Under this design half of the subjects were given the immediate release treatment first and the ODT treatment second and the other half were given the treatments in the opposite order.

  The study was approved by the Research Ethics Committee at Faculty of Pharmacy, Cairo University and the protocol complies with Helsinki and Tokyo declarations for humans. Health status of the volunteers was confirmed by complete medical history, physical examination and laboratory analysis for complete hematological and biochemical examination. The subjects were instructed to take no medicines for one week prior to and during the course of the study.

  The subjects were received in the facility at 7.00 am of the day of study after an overnight fast as instructed before the study. From this time on they remained at the study site under controlled dietary and liquid intake until the end of the study day. No food was allowed for four hours after dosing. The washout was one week. The subjects were under medical supervision during the study and were watched for any adverse events such as gastrointestinal disturbances, nausea, vomiting, diarrhea or allergic reactions.

  For the ODT, one tablet equivalent to 100 mg was given to each subject. The ODT was administered orally without water, and each subject was asked to keep the ODT in the mouth for few minutes until completely dissolved in the saliva, then water was allowed after 30 minutes (Treatment A). The immediate release tablet, Bristaflam® 100 mg tablet was ingested with 200 ml of water (Treatment B).

- **Collection of blood samples:** Venous blood samples were collected in heparinized glass tubes before administration of the dosage form (zero time), and at 15, 30 minutes, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12 and 24 hours after administration of the Aceclofenac ODT and Bristaflam tablet. All samples were collected and plasma was immediately separated from the blood cells by centrifugation at 6000 rpm for 10 minutes and stored at -20^{o}C until analysis.

- **Analytical procedure for determination of Aceclofenac in plasma:**

- **Assay Method Description and Chromatographic conditions:** A simple, rapid, specific and reliable high performance liquid chromatographic assay of Aceclofenac in human plasma has been developed. Reversed phase chromatography was conducted. The analysis was done on Shimadzu LC Prominence 20 connected with PDA detector; using column ODS 3, Prodigy, (250 x 4.6 mm, 5 µm). The mobile phase was isocratic consisted of acetonitrile: methanol: 50 mM potassium dihydrogen phosphate buffer, in ratio of (30: 30: 40 v/v) and was delivered to the system at a flow rate of 1.5 ml/min, with an injection volume of 20 µl and the detection wavelength (λ_{max}) was 275 nm. Oxazepam was used as internal standard. All assays were performed at ambient conditions.

- **Preparation of Stock and Working Standard Solution:** Stock solution of Oxazepam (internal standard) solution was prepared by dissolving 50 mg of Oxazepam in 100 ml acetonitrile; then sonication for 5 minutes (500 µg/ml). The working internal standard was prepared on each day of analysis by diluting the stock solution to contain (5 µg/ml). Stock solution of Aceclofenac was prepared by dissolving 50 mg Aceclofenac in 100 ml acetonitrile; then sonication for 5 minutes (500 µg/ml). The working solution was prepared on each day of analysis by diluting the stock solution with a mixture of (Acetonitrile : Water [1:1]) to give serial dilutions containing 0.5, 1, 2, 3, 4, 5 and 7 µg/ml of Aceclofenac; which were shaken well and filtered over 0.45 µm syringe filter and injected onto HPLC.

- **Plasma sample preparation for determination of Aceclofenac:** The extraction procedure was applied in the preparation of plasma samples and standards, where 1 ml of each human plasma was transferred into 15 ml tube fitted with polyethylene cap. 1 ml of internal standard working solution and 1 ml acetonitrile were added. The mixture was vortexed for 2 minutes and centrifuged at 6000 rpm for 30 minutes. The upper layer was transferred to another tube and filtered through 0.45 µm syringe filter. A 20 µl volume of the supernatant was injected onto the HPLC column. Concentrations of Aceclofenac in unknown samples were calculated with reference to the prepared calibration curve. Retention time of Aceclofenac was 5.8 minutes.

- **Preparation of *in-vivo* Standard Calibration Curve:** For calibration curve, plasma standards were prepared by spiking 1 ml of blank plasma with 1 ml of the internal standard working solution and appropriate volumes of Aceclofenac working solution to produce concentrations ranging from (0.25, 0.5, 2, 4, 6, 8, 10 µg/ml). The spiked plasma standards were processes as described above. The calibration curve was obtained by plotting chromatographic peak area ratios (Aceclofenac /Oxazepam) against the corresponding nominal Aceclofenac concentration added. Samples were prepared and injected on the same day.

- **Sample Calculation:** The unknown sample concentration was calculated from the following formula: $C = [(R + Y) / S]$*; w*here C is Aceclofenac concentration, R is the peak area ratio (drug/internal standard), S is the slope of the calibration curve and Y is the Y-intercept.

- **Assay Method Validation:**

  - **Selectivity:** Plasma samples (four blanks) were chromatographed prior to use to check for endogenous components, which might interfere with Aceclofenac or (Internal standard) Oxazepam. Spiked plasma samples representing a low, medium and high Aceclofenac concentration were also analyzed to verify the selectivity of the method of analysis.
  - **Linearity and Linear Working Range:** The linearity and linear working range were determined from the constructed standard calibration curve.
  - **Intraday Accuracy and Precision**: sThe intraday accuracy and precision evaluations were assessed by repeated analysis and human plasma samples containing different concentrations of Aceclofenac on separate occasions in the same day (3 times on the same day). The analytical precision of the method was determined by the relative standard deviation of the percentage drug recovered.

- **Inter-day Reproducibility**: The day to day reproducibility of the assay for plasma samples was evaluated by comparing the least squares linear regression analysis of three standard plots obtained from spiked plasma standard at three different days over three different weeks period.
- **Freeze and Thaw Stability in Human Plasma:** Aceclofenac stability in human plasma was determined after three freeze-thaw cycles. In order to determine the effect of storage on the stability of frozen samples, three concentrations were selected representing the low, medium and high spiked plasma samples of the calibration curve. These concentrations were analyzed when fresh and after storage just prior to analysis (storage was at -20°C for 24 hours). The freeze-thaw cycle were repeated two more times, and then analyzed using the previously validated method.
- **Extraction recovery:** Relative recoveries of all Aceclofenac concentrations used in the plasma standards were evaluated by comparing their peak area ratios with those obtained from direct injection of unprocessed reference solutions of the same concentrations.

Pharmacokinetics Calculations: Pharmacokinetic parameters from plasma data following administration of the two treatments were estimated for each subject by using a computer program, WinNonlin® software (version 1.5, Scientific consulting, Inc., NC). The plasma concentration – time data were evaluated, and the following pharmacokinetic parameters were calculated:

C_{max} (µg/ml): it was determined as the highest observed Aceclofenac concentration during the 24 hours study.

T_{max} (hours); it was taken as the time at which C_{max} occurred.

AUC_{0-24} (µg.hr/ml)**;** was determined as the area under the plasma concentration – time curve up to the last measured time point calculated by the trapezoidal rule.

$AUC_{0-\infty}$ (µg.hr/ml): it was determined as the area under the plasma concentration – time curve up to the last measured time point calculated by the trapezoidal rule plus the residual area calculated as the concentration of the last measured time point divided by the elimination rate constant. Where $AUC_{0-\infty} = AUC_{0-24} + C_t/k$; and C_t is the last measured concentration at the time t, and k is the terminal elimination rate constant estimated by log-linear regression analysis on data visually assessed to be a terminal log-linear phase.

$t_{1/2}$ is apparent terminal half life and was calculated as **$t_{1/2}$ =** 0.693/k plasma half life.

MRT is the mean residence time and was calculated AUMC/AUC

f_{rel} is the relative bioavailability and was calculated as (AUC_{ODT} / AUC_{IR}) X 100.

Statistical analysis of the Pharmacokinetic Parameters: Statistical evaluation of C_{max}, t_{max}, $t_{1/2}$, AUC_{0-24}, and $AUC_{0-\infty}$ data by one way ANOVA statistical test using SPSS® 11.0 software (SPSS Inc., Chicago).

RESULTS AND DISCUSSION:

Characterization of Aceclofenac ODTs: All the prepared tablets showed acceptable weight variation with relative standard deviation ranged from 1 - 5% for the different formulations and the mean percent of Aceclofenac content in ODTs was found to be more than 92% from all formulations. All tablets showed residual moisture content of no more than 3%, indicating that the lyophilization process was efficient in removing water from the tablets.

Friability studies showed that tablets formulated with 3% and 5% matrix former showed acceptable percentage weight loss, within the acceptable range for tablets (less than 1%) except (LA#11) containing lactose and 3% hydroxypropylcellulose.

On the contrary, all the formulations containing 3% xanthan gum were friable. On the other hand, tablets formulated with 1% matrix former were friable and showed percentage weight loss that exceeded pharmacopoeial limits. The decreased mechanical properties of ODTs formulated with 1% matrix former could be attributed to the fewer number of crosslinks

formed between the matrix former strands as the concentration decreases. It has been reported that increasing the matrix former concentration usually results in a more extensive and rigid 3D network after freeze-drying due to increase in the number of matrix fibers forming crosslinks and interchain H-bonds, thereby resulting in an increase in the overall hardness of the tablets [11]. The percentage of relative standard deviation of the mean drug content, percentage of weight loss from friability studies, wetting time, *in-vitro* disintegration time and *in-vivo* disintegration time for the prepared ODTs is listed in **table 1**.

TABLE 1: CHARACTERIZATION OF THE 3^3 FULL FACTORIAL DESIGN ACECLOFENAC FREEZE DRIED FORMULAE

Formula	X_1	X_2	X_3	Friability (%)	Wetting time (Seconds) *	*In vitro*-Disintegration time (Sec) *	*In vivo*-Disintegration time (Sec) *	Residual moisture (%) ***	Drug content (%) **
LA #1	-1	-1	-1	4.78%	15 ± 1.34	10 ± 0.68	5 ± 0.64	1.43 ± 0.41	92.8% ± 1.76
LA #2	0	-1	-1	10.825	23 ± 0.24	18 ± 0.74	12 ± 0.62	1.74 ± 0.34	94.5% ± 1.46
LA #3	+1	-1	-1	3.61%	28 ± 0.78	23 ± 1.24	16 ± 0.61	1.16 ± 0.94	95.9% ± 1.43
LA #4	-1	0	-1	2.76%	100 ± 0.47	90 ± 0.68	80 ± 0.6	1.13 ± 0.35	98.17% ± 1.64
LA #5	0	0	-1	2.51%	112 ± 0.68	100 ± 0.67	92 ± 0.68	1.26 ± 0.48	97.02% ± 1.27
LA #6	+1	0	-1	0.82%	28 ± 0.41	22 ± 1.34	17 ± 0.41	0.68 ± 1.56	100.4% ± 1.98
LA #7	-1	+1	-1	100%	30 ± 0.73	25 ± 0.24	20 ± 0.73	1.34 ± 0.87	97.96% ± 3.17
LA #8	0	+1	-1	100%	37 ± 1.28	32 ± 0.78	27 ± 1.28	1.49 ± 1.69	98.42% ± 2.35
LA #9	+1	+1	-1	10.83%	46 ± 0.27	41 ± 0.47	36 ± 0.27	1.22 ± 0.35	95.74% ± 1.40
LA #10	-1	-1	0	0.7%	112 ± 1.34	100 ± 0.00	88 ± 0.39	1.24 ± 1.48	92.82% ± 1.46
LA #11	0	-1	0	2.61%	200 ± 0.24	180 ± 0.00	160 ± 0.64	0.99 ± 0.56	94.88% ± 2.68
LA #12	+1	-1	0	Zero	420 ± 0.78	409 ± 0.00	340 ± 0.62	1.54 ± 1.87	103.6% ± 1.43
LA #13	-1	0	0	0.9%	162 ± 0.47	145 ± 0.68	123 ± 0.61	2.94 ± 1.69	93.37% ± 2.64
LA #14	0	0	0	0.91%	170 ± 0.41	152 ± 0.72	140 ± 0.6	1.63 ± 1.35	97.82% ± 1.27
LA #15	+1	0	0	0.71%	52 ± 0.73	44 ± 0.64	30 ± 0.68	1.86 ± 1.48	100.2% ± 1.98
LA #16	-1	+1	0	23.65%	90 ± 1.28	80 ± 0.62	67 ± 0.41	1.68 ± 2.56	104.99% ± 3.1
LA #17	0	+1	0	20.21%	112 ± 0.27	100 ± 0.61	87 ± 0.73	1.34 ± 0.87	92.49% ± 2.35
LA #18	+1	+1	0	5.23%	115 ± 0.39	100 ± 0.6	90 ± 1.28	1.94 ± 0.69	100.74% ± 1.4
LA #19	-1	-1	+1	Zero	165 ± 0.41	154 ± 0.68	130 ± 0.27	1.73 ± 0.35	104.8% ± 1.76
LA #20	0	-1	+1	Zero	184 ± 0.73	175 ± 0.41	152 ± 0.39	1.66 ± 0.48	92.54% ± 1.46
LA #21	+1	-1	+1	Zero	522 ± 1.28	510 ± 0.73	495 ± 0.64	0.88 ± 0.56	93.94% ± 1.94
LA #22	-1	0	+1	Zero	223 ± 0.27	210 ± 1.28	190 ± 0.62	1.34 ± 0.79	97.76% ± 1.68
LA #23	0	0	+1	Zero	234 ± 0.39	225 ± 0.27	210 ± 0.61	0.54 ± 0.69	92.8% ± 1.75
LA #24	+1	0	+1	Zero	619 ± 0.41	600 ± 0.39	580 ± 0.6	1.26 ± 0.84	103.1% ± 1.98
LA #25	-1	+1	+1	Zero	764 ± 9.67	745 ± 8.91	730 ± 7.16	0.68 ± 0.69	92.11% ± 2.98
LA #26	0	+1	+1	Zero	893 ± 7.23	755 ± 7.98	742 ± 9.67	1.34 ± 0.83	94.38% ± 2.98
LA #27	+1	+1	+1	Zero	932 ± 10.3	900 ± 9.79	880 ± 11.88	2.84 ± 0.69	107.44% ± 2.9

*Data are mean values (n = 6) ± S.D. **Data are mean values (n = 10) ± S.D. ***Data are mean values (n = 3) ± S.D

Independent variables	Levels		
	Low	**Medium**	**High**
X_1 = Filler Type	Mannitol	Lactose	Maltodextrin
X_2 = Matrix former type	Gelatin	Hydroxypropylcellulose	Xanthan gum
X_3 = Matrix former conc.	1	3	5
Transformed values	**-1**	**0**	**+1**

It was also observed that tablets formulated with mannitol showed lower weight losses compared to tablets formulated with lactose and maltodextrin. This is in accordance with previously reported results in which mannitol as filler produces a stiff homogenous cake that improves the appearance and the hardness of the lyophilized product [12]. *In vitro* disintegration studies showed that ODTs containing lactose and maltodextrin showed longer disintegration times compared to ODTs containing mannitol. This may be attributed to the matrix forming effect of maltodextrin that synergies the binding property of the matrix former, so hindering the rate of disintegration [13, 14, 15]. Although, both mannitol and lactose have same solubility in water: 1 gm in 5.5 and 5 ml of cold water respectively, it was noted that lactose dissolve at a slower rate than mannitol.

Similar results were obtained by Windholz *et al*., (1976) and Rupal Patel, (2005). The more rapid disintegration rates of formulae containing mannitol can be directly attributed to its better solubility than lactose [16, 17]. ODTs prepared using 5% matrix former showed statistically significantly longer disintegration times compared to ODTs prepared using 3% and 1% matrix former. These results indicate that increasing the matrix former concentration in the tablets results in the formation of more cohesive and stable gels that are less likely to break up or dissolve easily in water.

These results are further confirmed by wetting time experiments in which tablets containing higher matrix former concentration shows significantly higher wetting times compared to tablets containing of lower matrix former concentration. Short wetting time is indicative of the highly porous nature of the tablet matrix. In vitro disintegration results are also in accordance with friability results in which harder tablets showed longer disintegration times.

Results show that *in vivo* disintegration times were shorter when compared to corresponding in vitro disintegration times for all formulations. This may be probably because of the gentle movement of the tablet in the mouth and hence gentle mechanical stress on the tablet. This is in accordance with the results obtained by Ciper and Bodmeier in a study on the preparation of a fast disintegrating capsule for administration in the oral cavity [18].

***In vitro* Dissolution Studies:** The cumulative Aceclofenac dissolved as a function of time from ODTs compared to the market product Bristaflam® is illustrated in **table 2**. Remarkable differences in the shape of the dissolution profiles of the prepared ODTs and the commercial tablet were observed. The percentages of drug dissolved from the best freeze dried ODT formula (LA# 10) was 79.17%, compared to only 1.78% for Bristaflam, after 1 minute.

These results indicate that the process used to prepare the ODTs greatly enhanced the extent and rate of dissolution of Aceclofenac from the prepared tablets. (LA# 10) ODTs containing 3% gelatin and mannitol showed faster dissolution rates when compared to the other ODTs formulations. These results correlate well with disintegration and wetting time testing results, where increasing the gelatin concentration resulted in longer disintegration and wetting times. Also (LA# 10) tablet formulation containing mannitol showed faster drug release than the corresponding formulations containing lactose and maltodextrin.

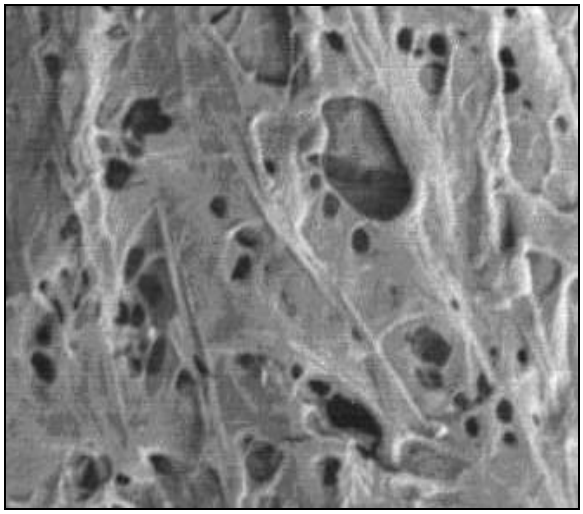
Similar results were obtained in a study on improving the dissolution rates of hydrocortisone and prednisone utilizing solid sugar dispersion systems [19]. The study revealed that the mannitol system had the fastest dissolution rate followed by the lactose system, and then finally the maltodextrin dispersion system. The percentage of drug dissolved from all ODT formulations was almost 100% after 15 min compared to only 38.69% for Bristaflam tablets.

TABLE 2: PERCENTAGE OF ACECLOFENAC DISSOLVED FOR THE FREEZE DRIED ODTS FORMULAE

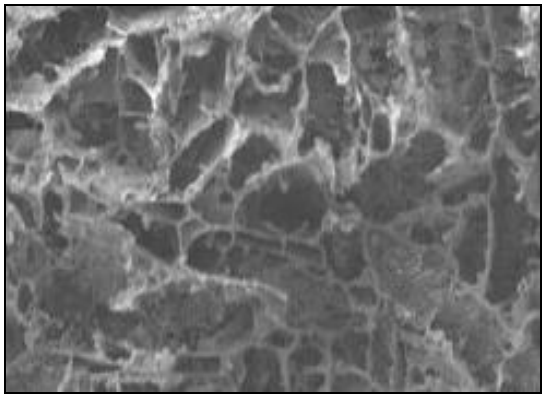
Time minute	Percentage Aceclofenac dissolved													
	Bristaflam	LA # 1	LA # 2	LA # 3	LA # 4	LA # 5	LA # 6	LA # 7	LA # 8	LA # 9	LA # 10	LA # 11	LA # 12	LA # 13
1	1.78	78.12	75.55	70.67	18.64	13.22	10.71	9.21	8.18	7.22	79.71	63.04	12.49	7.26
2	3.26	90.22	89.24	89.10	22.11	19.99	16.82	12.54	11.01	11.12	92.18	81.53	74.23	10.11
5	8.21	91.54	94.63	96.88	30.26	26.18	22.12	21.21	18.24	18.13	100.81	101.10	101.99	12.44
7	13.91	100.12	98.92	101.00	37.99	34.81	30.89	29.24	24.98	25.68	100.58	100.71	100.73	17.29
10	18.89	100.31	100.24	101.25	43.24	40.71	35.77	34.98	31.77	31.18	100.51	101.57	100.93	25.11
15	38.96	100.01	100.21	100.51	53.11	49.82	41.94	41.12	38.23	37.24	100.72	101.90	101.10	32.22
30	52.23	100.24	100.2	101.06	81.18	78.92	71.01	68.92	67.11	62.29	100.33	101.76	101.04	71.11

Time minute	Percentage Aceclofenac dissolved													
	LA # 14	LA # 15	LA # 16	LA # 17	LA # 18	LA # 19	LA # 20	LA # 21	LA # 22	LA # 23	LA # 24	LA # 25	LA # 26	LA # 27
1	5.24	3.31	2.63	2.12	2.01	15.78	12.22	5.23	5.19	4.91	2.81	0	0	0
2	7.12	5.00	5.23	4.11	3.82	51.83	49.23	42.18	5.68	5.21	4.11	0	0	0
5	9.21	7.97	7.12	5.23	5.12	64.89	62.11	54.38	6.23	6.01	5.21	0	0	0
7	12.11	12.76	7.89	5.98	6.23	71.01	69.18	62.01	8.46	7.11	5.48	1.22	0.91	0.87
10	20.89	17.35	8.91	6.91	7.28	75.24	72.83	70.12	11.79	9.22	7.21	2.15	2.01	1.09
15	31.24	27.00	11.22	7.22	9.11	98.34	95.61	95.22	15.43	12.38	10.5	3.77	3.00	2.13
30	68.49	64.34	13.68	10.44	11.22	101.11	100.33	100.43	28.86	24.14	19.19	4.91	4.09	4.12

Scanning Electron Microscopic (SEM) Analysis: Scanning electron micrographs of the surface and cross-section views of ODTs (LA# 10) is shown in figure (1). The micrographs show the highly porous nature of the prepared lyophilized tablets, which appears in both the surface and the inner structure. The highly porous nature of the tablets explains the rapid penetration of water, which results in rapid wetting, disintegration, and dissolution in the oral cavity. The micrographs show that ODT (LA# 10) contains larger and more diffuse pores (especially from the cross-section view) which might explain the very fast *in vitro* and *in vivo* disintegration as well as short wetting time.



(a) SURFACE VIEW



(b) CROSS SECTION VIEW

FIGURE 1: SEM GRAPH OF ACECLOFENAC FREEZE DRIED FORMULA (LA # 10)

Assay Method Validation of Aceclofenac:

Selectivity: Chromatograms obtained at the lower limit of sensitivity for drug free plasma showed no interfering peaks at the retention times of Aceclofenac and the internal standard. Typical chromatograms obtained from a processed blank human plasma sample alone, with Aceclofenac and with Aceclofenac /Oxazepam are illustrated in **figures** (**2, 3 and 4**) respectively. The retention times of Aceclofenac and the internal standard (Oxazepam) are approximately 5.8 and 7.25, respectively. Both peaks were sharp with good baseline resolution and minimal tailing, thus facilitating accurate measurements of the peak area ratios. No interferences by the metabolites or normal constituents of plasma were observed. The overall chromatographic time is 10 minutes.

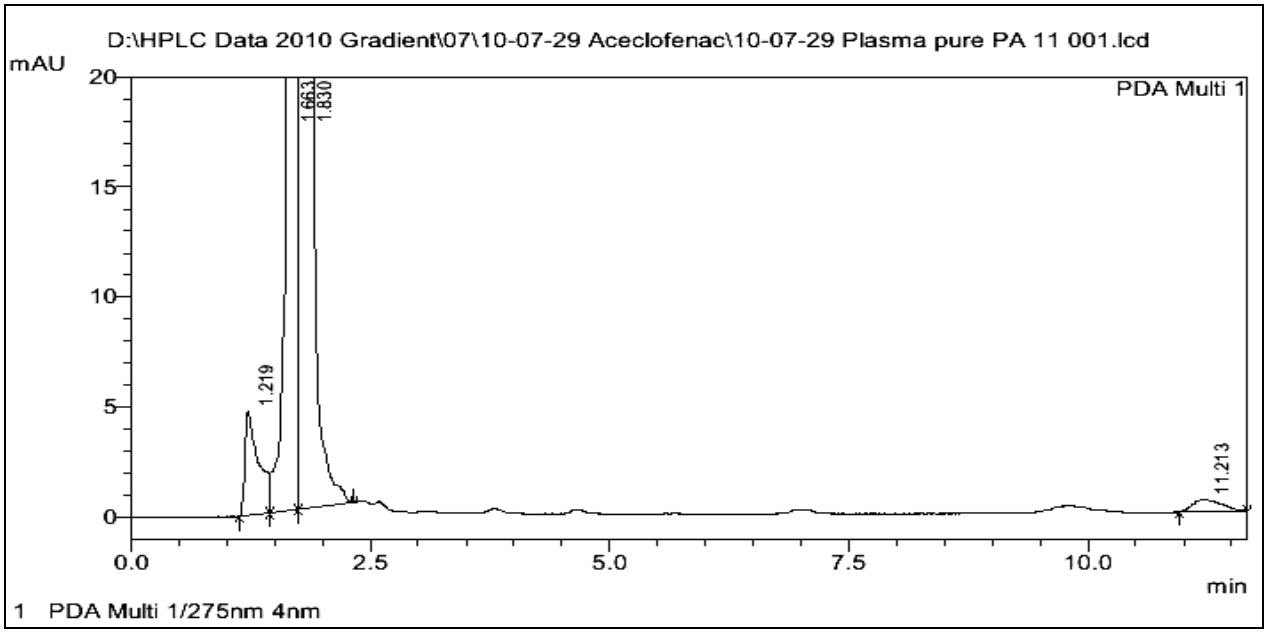


FIGURE 2: A TYPICAL CHROMATOGRAM OBTAINED FROM A PROCESSED BLANK HUMAN PLASMA SAMPLE

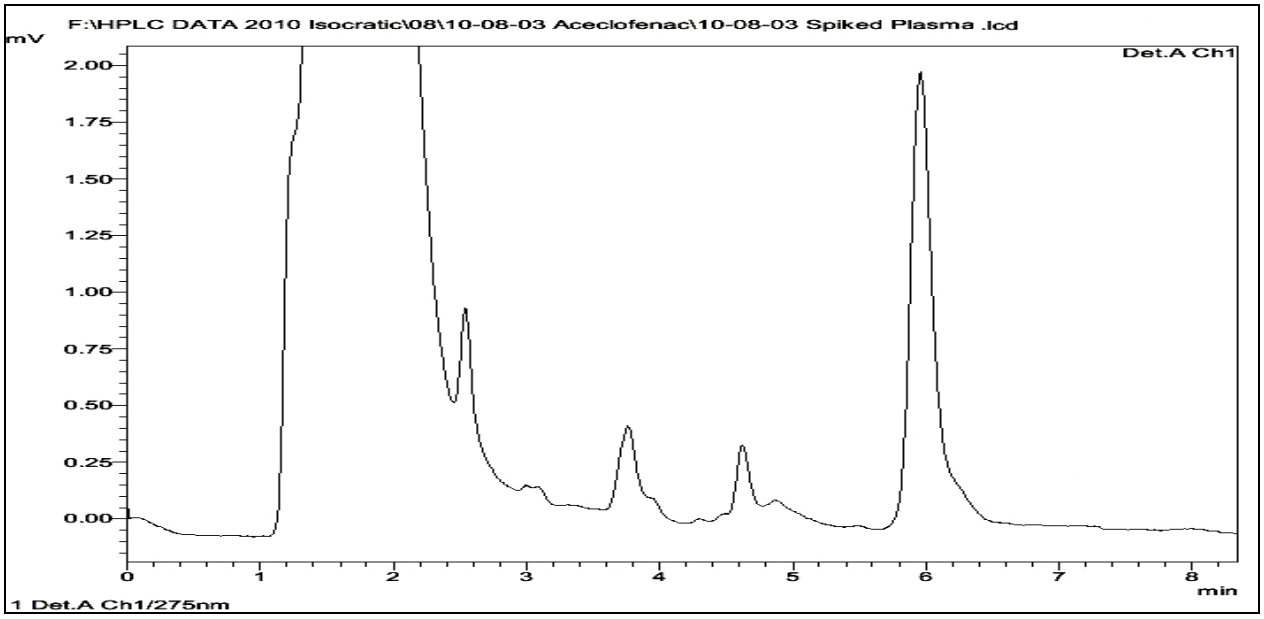


FIGURE 3: A TYPICAL CHROMATOGRAM OBTAINED FROM PROCESSED HUMAN PLASMA SPIKED WITH ACECLOFENAC

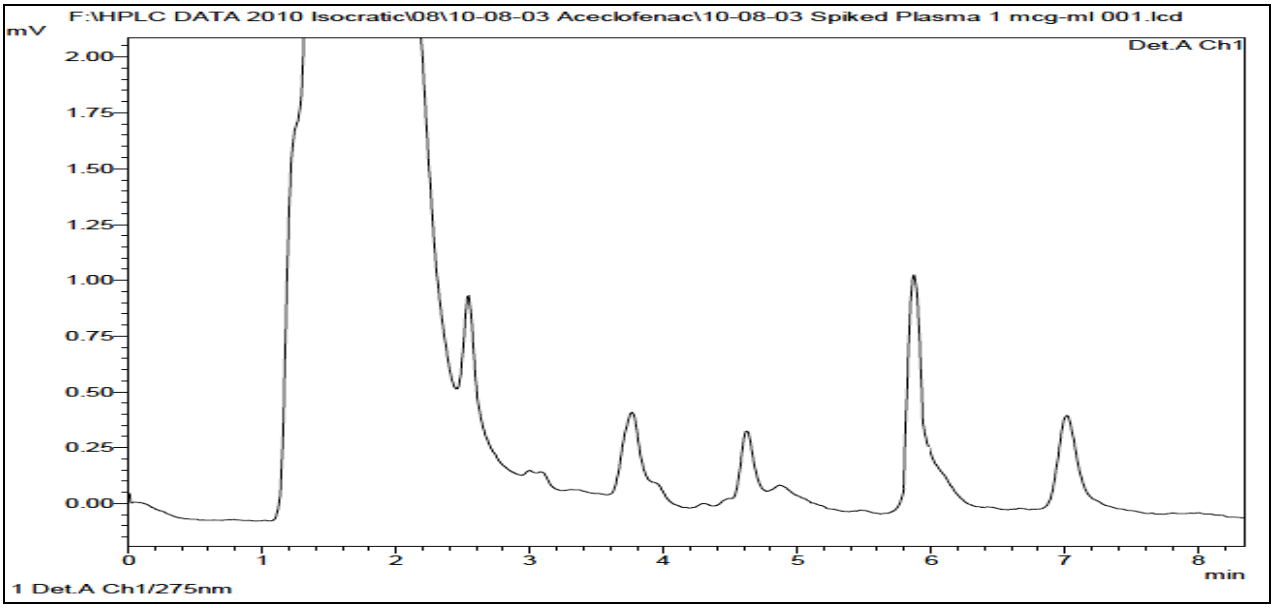


FIGURE 4: A TYPICAL CHROMATOGRAM OBTAINED FROM PROCESSED HUMAN PLASMA SPIKED WITH ACECLOFENAC AND OXAZEPAM

Linearity and Linear working range: Table 3 and figure 5 represent the standard curve of Aceclofenac using HPLC. The regression equation: Y = 0.2712x + 0.0015 (where Y is the peak area of Aceclofenac and x is the concentration of Aceclofenac in µg/ml. The lower limit of quantification was 0.5 µg/ml, with a linear response across the full range of concentrations from 0.5 to 7 µg/ml (R^2 = 0.9999). Standard curve obtained for Aceclofenac in plasma samples was highly linear in the concentration range of 0.25 - 10 µg/ml. Linear regression analysis of the standard calibration plot for human plasma was Y = 0.264x + 0.012, (R^2 = 1); where y and x are the peak area ratio and Aceclofenac concentration, respectively. This data was illustrated in **table 4 and figure 6**.

TABLE 3: RELATIONSHIP BETWEEN THE CONCENTRATION OF ACECLOFENAC AND THE MEAN RELATIVE PEAK AREA USING HPLC

Concentration (µg/ml)	Peak area	± S.D
0.5	0.1355	0.02
1	0.28	0.005
2	0.54	0.006
3	0.81	0.005
4	1.08	0.006
5	1.37	0.027
7	1.897	0.039

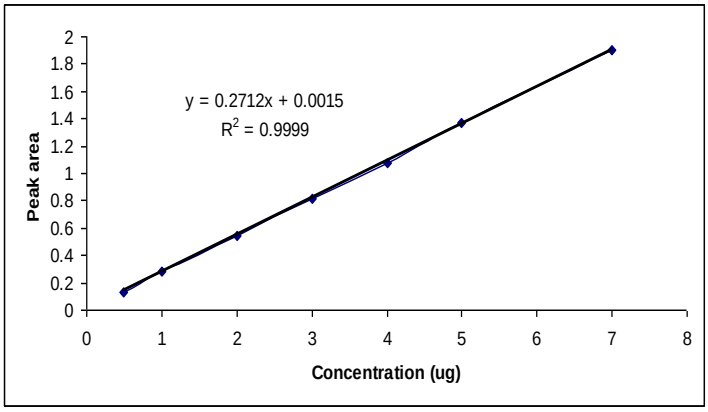


FIGURE 5: CALIBRATION CURVE OF ACECLOFENAC STANDARD

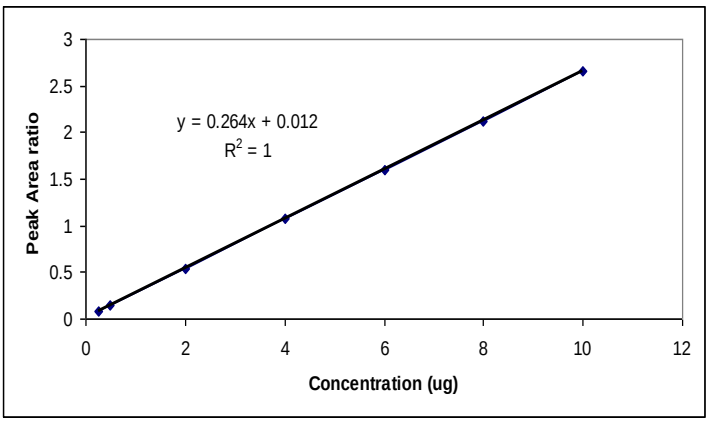


FIGURE 6: CALIBRATION CURVE OF ACECLOFENAC SPIKED HUMAN PLASMA

TABLE 4: RELATIONSHIP BETWEEN THE CONCENTRATION OF ACECLOFENAC IN HUMAN PLASMA AND THE MEAN RELATIVE PEAK AREA USING HPLC

Concentration (µg/ml)	Peak area ratio	± S.D
0.25	0.078	0.021
0.5	0.144	0.004
2	0.54	0.0084
4	1.068	0.010
6	1.596	0.034
8	2.124	0.046
10	2.652	0.05

Intraday (Within Day) Accuracy and Precision: The analytical precision of the method is determined by the relative standard deviation of the percent drug recovered as shown in **table 5**.

Inter-day (Day to Day) Reproducibility: Table 6 reveals the results of the inter-day evaluations. The results show that the correlation coefficients mean was higher than 0.99 and the coefficient of variance of the slope of the three trend lines was less than 2%. The analysis of variance of the data showed no significant difference in the slopes of the three standard plots. The results thus confirmed excellent linearity of the calibration lines and high reproducibility of the assay.

TABLE 5: ANALYTICAL PRECISION FOR THE ANALYSIS OF ACECLOFENAC PERFORMED ON 3 SETS OF STANDARD CURVES OF THE SAME DAY

Spiked conc. (µg/ml)	Peak area ratio			Mean	± S.D	RSD %
	1	2	3			
1	0.24873	0.25545	0.24904	0.25	± 0.0038	1.511
2	0.5117	0.52314	0.50671	0.51	± 0.008	1.639
3	0.79188	0.77211	0.78806	0.78	± 0.01	1.338
4	1.03958	1.04728	1.06024	1.05	± 0.01	0.995
5	1.30581	1.30253	1.29584	1.3	± 0.005	0.178
r^2	0.9997	0.9998	0.99919	0.9998		
Slope	0.26420	0.26183	0.26471	0.264		
Intercept	-0.01307	-0.00539	-0.01416	-0.014		

TABLE (6): INTRA-DAY REPRODUCIBILITY FOR THE STANDARD PLOT OF ACECLOFENAC IN PLASMA

Standard plot [i]	Slope [ii]	Intercept [ii]	Correlation Coefficient
1	0.26420	-0.01307	0.9997
2	0.26351	-0.0141	0.9992
3	0.26277	-0.01296	0.9991

i- Obtained in 3 different days

ii- Mean of 3 determinations at each drug concentration

Freeze and Thaw Stability in Human Plasma: Table 7 shows the peak area ratio of three Aceclofenac concentrations, namely 0.5, 5 and 10 µg/ml representing low, medium and high spiked plasma samples of the calibration curve (when fresh and stored at -20°C). Slight difference between spiked drug concentration in both fresh and stored samples were observed.

TABLE 7: THE PEAK AREA RATIO OF ACECLOFENAC TO THE INTERNAL STANDARD OF THREE DIFFERENT CONCENTRATIONS, (FRESH AND STORED AT -20°C)

Concentration (µg/ml)	Peak Area Ratio					
	Fresh Sample			Stored Sample		
0.5	0.144	0.136	0.151	0.143	0.1357	0.151
5	1.332	1.3299	1.3335	1.33	1.3297	1.3333
10	2.652	2.6497	2.6532	2.651	2.6495	2.6531

Extraction Recovery: Data of the extraction recovery of Aceclofenac from plasma samples fortified with the drug in the same concentrations of the drug used in the calibration curve in human plasma are shown in **table 8**. The extraction recovery ranged from (89.41 - 105.23%) with a mean percent recovery 96.708% and the average coefficient of variation was 0.6396%. The previous outcomes revealed the suitability of the method for the determination of Aceclofenac in the plasma samples.

TABLE 8: WITHIN DAY ACCURACY FOR DETERMINATION OF ACECLOFENAC IN PLASMA

Aceclofenac (µg/ml)	n = 3	Mean Recovery (µg/ml)	± S.D	Recovery %	CV%
1		0.8941	0.016	89.41	1.792
2		1.9042	0.007	95.21	0.343
3	3	2.900	0.001	96.68	0.040
4		3.8804	0.033	97.01	0.862
5		5.2615	0.008	105.23	0.161

Assessment of Pharmacokinetic Parameters: The study was completed by the four volunteers who were included in the pharmacokinetic analysis. The volunteers tolerated very well the two treatments and did not complain of any adverse effects during the course of the study. No signs of GI disturbances or allergic reactions were observed from any of the volunteers during the study.

***In-vivo* Absorption Studies:** The plasma mean concentrations of Aceclofenac following administration of an oral dose (100 mg) of the selected ODT formula (LA# 10) and Bristaflam® tablet to four healthy volunteers are shown in **tables 9 and 10 and figures 7 and 8**, respectively. **Figure 9** shows the comparative mean plasma concentration of Aceclofenac following administration of an oral dose (100 mg) of Bristaflam® IR tablets and ODT (LA# 10) to four healthy volunteers.

Peak Plasma Concentration (C_{max}): The peak plasma concentration (C_{max}) of Aceclofenac following the administration of Bristaflam® tablets has a mean of 3.758 ± 0.047 µg/ml ranged from 3.705 - 3.82 µg/ml. The peak plasma concentration (C_{max}) of Aceclofenac following the administration of selected ODT formula (LA# 10) has a mean of 7.0635 ± 0.178 µg/ml ranged from 6.815 - 7.224 µg/ml as shown in **tables 11 and 12**. ANOVA showed significant differences ($\rho < 0.05$) as shown in **tables 13**.

Time of Peak Plasma Concentration (T_{max}): Tables 11 and 12 showed that the mean time taken to peak plasma concentration for *(T_{max})* following administration of Bristaflam® tablets was 2.875 ± 0.25 hours, while it was 1 hour following administration of selected ODT formula (LA# 10). T_{max} is significantly different ($\rho < 0.05$).

Area Under the Curve (AUC_{0-24}) and ($AUC_{0-\infty}$): The area under the plasma concentration curve AUC_{0-24} and $AUC_{0-\infty}$ of Aceclofenac following the administration of Bristaflam® tablets have means of 20.5 ± 0.38 µg.hr/ml (ranged from 19.98-20.85 µg.hr/ml) and 21.41 ± 0.59 µg.hr/ml (ranged from 20.66 – 21.89 µg.hr/ml), respectively. On the other hand, the area under the plasma concentration curve AUC_{0-24} and $AUC_{0-\infty}$ of Aceclofenac following the administration of selected ODTs formula (LA# 10) have means of 38.98 ± 0.53 µg.hr/ml (ranged from 38.32 – 39.08 µg.hr/ml) and 39.85 ± 1.18 µg.hr/ml (ranged from 38.76 – 41.2 µg.hr/ml), respectively as shown in table (11 and 12). ANOVA showed a significant difference between the two formulae Bristaflam® and ODT (LA# 10) ($\rho < 0.05$) as shown in **table 14**.

Elimination Half Life ($t_{1/2}$): The elimination half life ($t_{1/2}$) of Aceclofenac following the administration of Bristaflam® tablets has a mean of 4.36 ± 0.21 hours ranged from 4.16-4.56 hours, where the elimination half life ($t_{1/2}$) of Aceclofenac following the administration of selected ODT formula (LA# 10) has a mean of 6.64 ± 1.11 hours ranged from 5.01-7.53 hours as shown in table (11 and 12). The elimination half life of the ODT was determined to be higher and statistically different relative to the mean half-life estimate following administration of IR tablets (p = 0.007) as shown in **tables 15**.

Although, the increase in Aceclofenac half-life from ODT is inconsistent with the pharmacokinetic theory, in which an increase in absorption should not affect elimination, this result could be attributed to the small number of subjects and/or the high variability associated with the mean half-life parameter from the ODT. This variation in half-life may be due to genetic differences in intrinsic hepatic enzyme activity along with some other factors such as age and nutrition [20].

Mean Residence Time (MRT): The mean residence (MRT) of Aceclofenac following the administration of Bristaflam® tablets has a mean of 5.76 ± 0.24 hours ranged from 5.52 – 6.06 hours, where the mean residence (MRT) of Aceclofenac following the administration of selected ODT formula (LA# 10) has a mean of 5.88 ± 0.04 hours ranged from 5.83 – 5.91 hours as shown in table 11 and 12. Statistical comparison of MRT parameter indicated insignificant difference (p = 0.366) as shown in **tables 16**. According to the mean plasma levels of 4 subjects completing the study, the relative bioavailability (f_{rel}) of the test formula was found to be 186.12% based on the mean ($AUC_{0-\infty}$) compared to that of the reference standard product**.**

Based on these results, it can be concluded that the improved bioavailability obtained from ODT (LA# 10) may be due to rapid and efficient absorption of Aceclofenac from buccal mucosa resulting in a decreased pre-systemic transformation due to first pass hepatic extraction or metabolism in the epithelium and/or lumen of GI tract or by combination of these processes. Because of the nature of the study design and the small number of subjects in this study, the results can only be considered preliminary and further studies with a larger number of subjects under different conditions such as varying conditions of ingesting ODTs with water and food intake should be conducted.

TABLE 9: PLASMA CONCENTRATION OF ACECLOFENAC FOLLOWING ADMINISTRATION OF AN ORAL DOSE (100 mg) OF THE SELECTED ODT FORMULA (LA# 10) TO FOUR HEALTHY VOLUNTEERS

Time (hour)	Plasma concentration of Aceclofenac (µg/mL)					
	V1	V2	V3	V4	Mean	± SD
0	0	0	0	0	0	0
0.25	5.49	5.27	5.72	5.21	5.42	0.23
0.5	6.49	6.221	6.712	6.37	6.44	0.20
1	7.065	6.815	7.224	7.15	7.06	0.17
1.5	5.238	5.096	5.445	5.21	5.24	0.14
2	4.86	4.294	4.623	4.71	4.62	0.23
2.5	4.242	4.025	4.115	4.493	4.21	0.20
3	3.765	3.543	3.645	3.55	3.62	0.10
4	3.118	3.076	3.11	3.008	3.07	0.05
6	2.484	2.457	2.463	2.473	2.46	0.01
8	1.352	1.335	1.33	1.343	1.34	0.009
10	1.212	1.208	1.222	1.218	1.21	0.006
12	0.701	0.806	0.809	0.808	0.78	0.053
24	0.32	0.274	0.31	0.285	0.29	0.021

V: volunteer

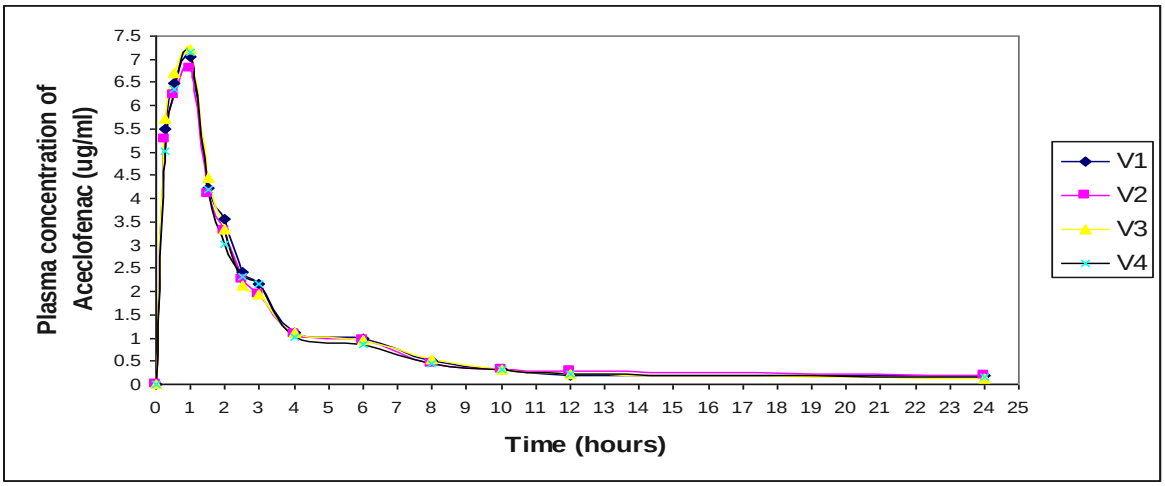


FIGURE 7: INDIVIDUAL PLASMA CONCENTRATION OF ACECLOFENAC FOLLOWING ADMINISTRATION OF AN ORAL DOSE (100 mg) OF THE SELECTED ODT FORMULA (LA# 10) TO FOUR HEALTHY VOLUNTEERS

TABLE 10: PLASMA CONCENTRATION OF ACECLOFENAC FOLLOWING ADMINISTRATION OF AN ORAL DOSE (100 mg) OF THE BRISTAFLAM® TABLET TO FOUR HEALTHY VOLUNTEERS

Time (hour)	Plasma concentration of Aceclofenac (µg/mL)					
	V1	V2	V3	V4	Mean	± SD
0	0	0	0	0	0	0
0.25	0.36	0.28	0.32	0.3	0.315	0.034
0.5	0.79	0.66	0.72	0.67	0.71	0.059
1	1.35	1.28	1.37	1.33	1.333	0.038
1.5	2.28	2.015	2.32	2.45	2.266	0.183
2	3.354	3.12	3.44	2.95	3.216	0.223
2.5	3.63	3.54	3.72	3.82	3.678	0.12
3	3.75	3.705	3.76	3.68	3.724	0.037
4	2.64	2.61	2.68	2.57	2.625	0.046
6	1.28	1.3	1.33	1.16	1.268	0.074
8	0.95	0.99	0.98	0.9	0.955	0.04
10	0.5	0.504	0.47	0.49	0.491	0.015
12	0.28	0.301	0.21	0.285	0.269	0.04
24	0.16	0.174	0.132	0.112	0.145	0.028

V: volunteer

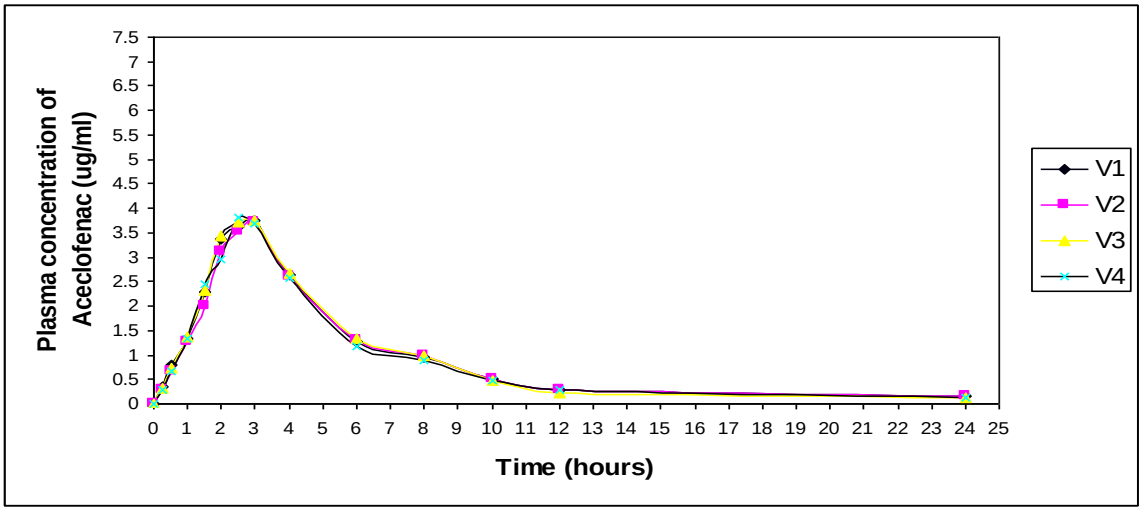


FIGURE 8: INDIVIDUAL PLASMA CONCENTRATION OF ACECLOFENAC FOLLOWING ADMINISTRATION OF AN ORAL DOSE (100 mg) OF THE SELECTED MARKET PRODUCT, BRISTAFLAM®, TO FOUR HEALTHY VOLUNTEERS

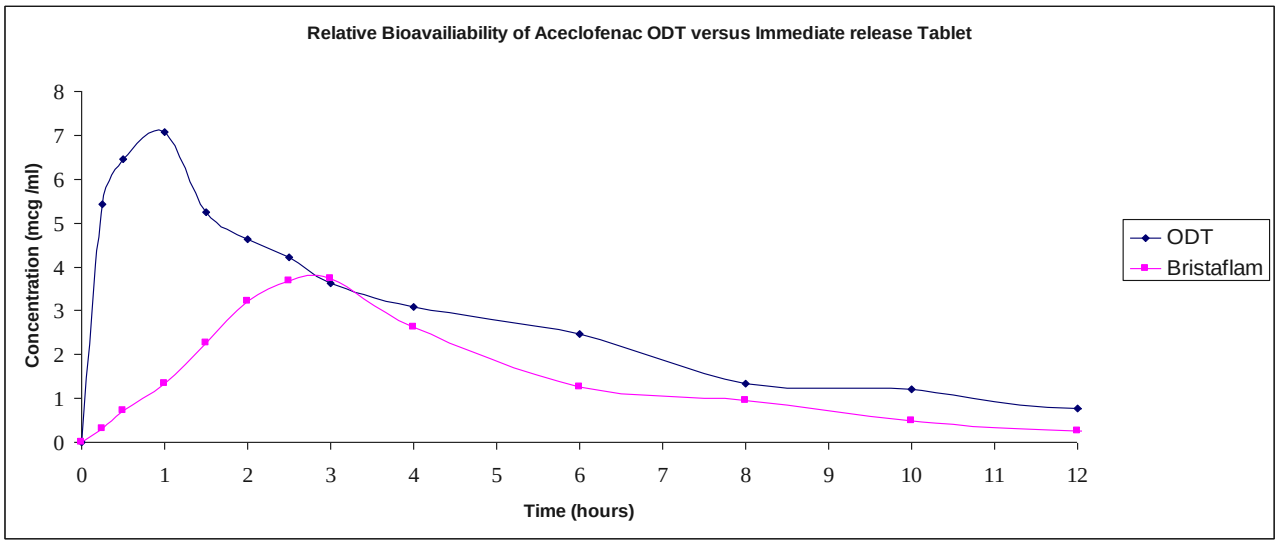


FIGURE 9: MEAN PLASMA CONCENTRATION OF ACECLOFENAC FOLLOWING ADMINISTRATION OF AN ORAL DOSE (100 mg) OF BRISTAFLAM® IR TABLETS AND ODT (LA# 10) TO FOUR HEALTHY VOLUNTEERS

TABLE 11: SUMMARY OF ACECLOFENAC ODT 100 mg TABLET *IN–VIVO* INTERPRETATION

	V1	V2	V3	V4	Mean	SD
T_{max}	1	1	1	1	1	0
C_{max}	7.065	6.815	7.224	7.15	7.0635	0.177948
AUC_{0-24}	38.9325	38.3226	39.6023	39.0813	38.98468	0.526581
$AUC_{0-\infty}$	41.2048	38.7643	40.4738	38.9718	39.85368	1.179624
$t_{1/2}$	5.0186	6.9223	7.5397	7.081	6.6404	1.112441
MRT	5.8308	5.9141	5.904	5.8801	5.88225	0.037145

TABLE 12: SUMMARY OF BRISTAFLAM 100 mg TABLET *IN–VIVO* INTERPRETATION

	V1	V2	V3	V4	Mean	SD
T_{max}	3	3	3	2.5	2.875	0.25
C_{max}	3.75	3.705	3.76	3.82	3.75875	0.047324
AUC_{0-24}	20.8458	20.7278	20.437	19.9833	20.49848	0.384018
$AUC_{0-\infty}$	21.8896	21.8742	21.2347	20.6556	21.41353	0.590282
$t_{1/2}$	4.5219	4.5669	4.189	4.1608	4.35965	0.21443
MRT	5.8451	6.064	5.5242	5.6177	5.76275	0.241853

TABLE 13: ANOVA TEST FOR C_{MAX} OF THE SELECTED ACECLOFENAC ODT FORMULA (LA# 10) IN COMPARISON TO COMMERCIAL TABLET BRISTAFLAM®

ANOVA: SINGLE FACTOR α0.05

SUMMARY				
Groups	**Count**	**Sum**	**Average**	**Variance**
ODT	4	28.254	7.0635	0.031666
BISTAFLAM	4	15.035	3.75875	0.00224

ANOVA Reject Null Hypothesis because p < 0.05 (Means are Different)						
Source of Variation	SS	df	MS	F	P-Value	F critical
Between Groups	21.84275	1	21.84275	1288.458	0.000	5.987378
Within Groups	0.101716	6	0.016953			
Total	21.94446	7				

TABLE 14: ANOVA TEST FOR $AUC_{(0-\infty)}$ OF THE SELECTED ACECLOFENAC ODT FORMULA (LA# 10) IN COMPARISON TO COMMERCIAL TABLET BRISTAFLAM®

ANOVA: SINGLE FACTOR α0.05

SUMMARY				
Groups	**Count**	**Sum**	**Average**	**Variance**
ODT	4	159.4147	39.85368	1.391512
Bristaflam	4	85.6541	21.41353	0.348433

ANOVA Reject Null Hypothesis because p < 0.05 (Means are Different)						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-Value*	*F crit*
Between Groups	680.0783	1	680.0783	781.7242	0.000	5.987378
Within Groups	5.219833	6	0.869972			
Total	685.2981	7				

TABLE 15: ANOVA TEST FOR T ½ OF THE SELECTED ACECLOFENAC ODT FORMULA (LA# 10) IN COMPARISON TO COMMERCIAL TABLET BRISTAFLAM®

ANOVA: SINGLE FACTOR α0.05

SUMMARY				
Groups	**Count**	**Sum**	**Average**	**Variance**
ODT	4	26.5616	6.6404	1.237524
Bristaflam	4	17.4386	4.35965	0.04598

ANOVA	**Reject Null Hypothesis because p < 0.05 (Means are Different)**					
Source of Variation	SS	df	MS	F	P-Value	F crit
Between Groups	10.40364	1	10.40364	16.21131	0.007	5.987378
Within Groups	3.850512	6	0.641752			
Total	14.25415	7				

TABLE 16: ANOVA TEST FOR MRT OF THE SELECTED ACECLOFENAC ODT FORMULA (LA# 10) IN COMPARISON TO COMMERCIAL TABLET BRISTAFLAM®

ANOVA: SINGLE FACTOR α0.05

SUMMARY				
Groups	Count	**Sum**	**Average**	**Variance**
ODT	4	23.529	5.88225	0.00138
Bristaflam	4	23.051	5.76275	0.058493

ANOVA	**Accept Null Hypothesis because p > 0.05 (Means are the same)**					
Source of Variation	SS	df	MS	F	P-Value	F crit
Between Groups	0.028561	1	0.028561	0.954042	0.366	5.987378
Within Groups	0.179618	6	0.029936			
Total	0.208178	7				

TABLE 17: THE MEAN PHARMACOKINETIC PARAMETERS OF ACECLOFENAC AFTER ADMINISTRATION OF 100 mg ODT (LA# 10) AND IMMEDIATE RELEASE (IR) TABLET TO FOUR VOLUNTEERS

Parameter	ODT (LA# 10)	IR tablet	Statistical test
C_{max} (µg/ml)	7.064 ± 0.178	3.76 ± 0.473	*p* = 0.00
T_{max} (h)	1 ± 0.00	2.875 ± 0.25	*p* = 0.00
$AUC_{(0-24)}$ (µg*h/ml)	38.98 ± 0.53	20.5 ± 0.384	*p* = 0.00
$AUC_{(0-\infty)}$ (µg*h/ml)	39.85 ± 1.18	21.41 ± 0.59	*p* = 0.00
$T_{1/2}$ (h)	6.64 ± 1.11	4.36 ± 0.214	*p* = 0.007
MRT (h)	5.88 ± 0.03	5.76 ± 0.242	*p* = 0.366
	Relative Bioavailability (f_{rel}) = 186.12%		

Data are mean value ± S.D

CONCLUSIONS: We demonstrated that an orally rapidly disintegrating tablet of is a promising formulation resulting in more rapidly dissolved Aceclofenac and more effectively absorbed into the blood stream with significantly higher bioavailability when compared to standard immediate release oral dosage form. The study suggests that ODT (LA# 10) formulation developed in this work may be an alternative to conventional formulations of Aceclofenac.

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