RESEARCH ARTICLE

In Vivo Comparative Study of Different Dosage Forms of Baclofen

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Abstract

Aim: The aim of this study to compare the relative bioavailability of baclofen as a peroral tablet with the new formulations (transdermal and buccal films). **Materials and Methods:** Rapid and simple high-performance liquid chromatography/ultraviolet method has been adopted and validated for the determination of baclofen in human plasma with acceptable linearity, precision and accuracy. The study was performed on six human volunteers divided into three groups each contained two individuals. The first group was dosed with 20 mg of baclofen tablet (Lioraz®) as a reference, the second group received 40 mg baclofen transdermal film, and the third group received 20 mg baclofen buccal film. Transdermal and buccal films were subjected to *in vivo* study to evaluate the bioavailability parameters and then compared to the reference. **Results and Discussion:** Following Lioraz® tablet, the maximum concentration was achieved after 3.0 h unlike, after transdermal film with a double dose, it was at 6.0 h whereas, following buccal film, maximum time was 3.0 h post-dosing. Thus absorption from buccal films was faster, whereas transdermal spent longer times to reach the maximum concentration in systemic circulation. The mean values of pharmacokinetic parameters were significantly higher from transdermal than oral, demonstrating prolongation of baclofen action, whereas with buccal was lower. Taking Lioraz® as a reference, the % relative bioavailability from transdermal and buccal film was 78.99% and 89.22%, respectively. **Conclusion:** Study indicates that the absorption from buccal films was faster, whereas the transdermal spent longer times to reach the maximum drug concentration. Transdermal film of baclofen was successful in all the established studies by improving the prolongation of action and increasing the half-life of baclofen.

Key words: Baclofen, buccal films, high-performance liquid chromatography/ultraviolet detection, relative bioavailability, transdermal films

INTRODUCTION

Baclofen is a skeletal muscle relaxant, acting centrally by inhibiting transmission of reflexes at spinal level, possibly by acting on primary afferent fiber terminals resulting in the relief of muscle spasticity. It is used in the treatment of reversible spasticity, resulting from multiple sclerosis. Per-oral baclofen is the drug of choice for muscle spasm which is one of the common diseases especially in elderly women.[1,2] Baclofen is rapidly and completely absorbed from the gastrointestinal tract after an oral dose, with peak plasma concentrations occur about 0.5–3 h after ingestion, giving absolute bioavailability (compared to intravenous administration) of about 70–80%. About 30% of baclofen is bound to plasma proteins and about 70–80% of a dose is excreted in the urine mainly as unchanged drug; about 15% is metabolized in the liver mostly by deamination and the reminder amount excreted unchanged by feces. The elimination half-life of baclofen is about 3–4 h in plasma. The onset of action is highly variable and may range from hours to weeks.[3] Baclofen available in the market in limited formulations as an oral solution, oral suspension, tablets and intrathecal injection. Unfortunately, per-oral baclofen has a number of significant pharmacokinetic limitations as it is only absorbed in the upper small intestine by saturable active transport mechanisms and very limited in large intestine. In addition, baclofen has a short half-life

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of 3–4 h, and it is rapidly cleared from the blood that makes it needs to be administered frequently (4 times per day) to maintain therapeutic effects. Even with frequent dosing, there are fluctuations in circulating plasma drug levels. Frequent dosing is inconvenient and may lead to significant noncompliance. The most important dose-limiting adverse effects of baclofen therapy (e.g., somnolence, dizziness, motor weakness, and fatigue) are potentially related to peak drug concentrations, as indicated by their time course in preclinical studies with baclofen.[1,2] Due to limited absorption of baclofen in the large intestine attempts to develop an oral sustained release formulation of the drug but have not been successful due to dose dumping and intrapatient variations problems. Traditionally baclofen has been administered through intrathecal route which is difficult and hazardous to the patient. Due to all the previous drawbacks or limitations of available baclofen formulations, new drug deliveries including transdermal and transmucosal buccal films were formulated.

Transdermal drug delivery offers many potential advantages as avoid gastrointestinal drug absorption difficulties, avoid the first-pass effect, improves the bioavailability that reduces the total daily dose, avoiding the inconvenience of parenteral therapy, provide extended therapy with a single application, suitable for drugs having short half-life, may be terminated rapidly, increased patient compliance (by considering simplified therapeutic regimen, painless delivery of drug, no chances of forgetting the dose once the device is applied on skin and easy to carry a patch in wallet, or ladies purse), can be taken without any aid and finally problem of dose dumping is least, because stratum corneum is more resistant than the inner membranes.[4-6]

The buccal cavity was found to be the most convenient and easily accessible site for delivery of drugs for both local and systemic delivery as retentive dosage forms.[7,8] Oral controlled release drug delivery has achieved many improved therapeutic advantages, such as ease of dosing administration, patient compliance and flexibility in formulation. As a site for drug delivery, the oral cavity offers several advantages over other alternative routes of drug administration.[9,10] The membranes that line the oral cavity are readily accessible and exhibit fast cellular recovery following local damage.[11] The oral transmucosal absorption is generally rapid because of the rich vascular supply to the mucosa and the lack of a stratum corneum epidermis. Furthermore, any drug diffusing into the oral mucosa membranes has direct access to the systemic circulation through capillaries and venous drainage, allowing for an increased bioavailability of the drug with a smaller dosage and less frequent administration, decrease toxicity and wastage of expensive drug because of reduction in initial drug loading concentration.[12] These factors make the oral mucosal cavity a very attractive and feasible site for systemic drug delivery.

This is an attempt to compare the bioavailabilities of baclofen transdermal (40 mg) and buccal films (20 mg) with the oral commercially available product in the market, namely, Lioraz® tablets (20 mg) as well as assessing the pharmacokinetics parameters of these formulations. Furthermore, to develop and validate an high-performance liquid chromatography (HPLC)/ultraviolet (UV) method for monitoring baclofen in plasma. The developed HPLC method will be bioanalytically validated to be sensitive, selective, specific, accurate, and precise.

MATERIALS AND METHODS

Baclofen powder was kindly supplied as a gift from Al-Fath Group (Pharo Pharma, Egypt). Acetonitrile, HPLC grade (Merck, Germany), and Methyl alcohol, HPLC grade (Euro lab, UK) were purchased from Bait Al-Khebrah Chemicals Co. Sana'a, Yemen. Test formulations (baclofen transdermal film and mucoadhesive buccal film); reference product (the oral commercially available product of baclofen in the market, namely, Lioraz® 10 mg tablets). All other reagents were of analytical reagent grade. All aqueous solutions were prepared from double–distilled water.

The quantitative determination of baclofen was performed by HPLC (HPLC gradient Breeze system, Waters, USA) using of a 1525 Binary pump, a 2489 UV/Vis detector and a OctaDeca steel C-18 column (300 mm × 4.6 mm ID; particle size 5 µm)(Waters, USA). Peak areas were calculated with Breeze-2 HPLC System software.

Development and validation of HPLC/UV method for monitoring baclofen in plasma

Preparation of standard solutions and internal standard

Stock solutions of 0.2 mg/ml were prepared and then diluted further with distilled water to yield appropriate working solutions for the preparation of the calibration standards. Five calibration solutions of baclofen were prepared by making serial dilutions of stock solution and spiking them into drug-free plasma in the range from 9524 to 46154 ng/mL to give concentrations of 9524, 18181, 26087, 33333, and 46154 ng/mL. Linearity, recovery, precision, and accuracy studies were conducted using these plasma standards. To decrease the interaction with components of plasma and to prevent the confused results of baclofen, it was advised to add an internal standard of the same drug with predetermined concentration (9524 ng/mL) to clear the appearance of spiked peak to make it easy to read.

Sample treatment

The plasma samples were taken in a centrifuge tube and subjected to centrifugation procedure at 8000 rpm for 15 min to obtain plasma. Then, access acetonitrile was added and shaken to precipitate the proteins in plasma (deproteinized

plasma). Then, centrifugation at 8000 rpm for 15 min and then the supernatant liquid was collected and finally filtered through a 0.45 µ millipore membrane filter.[13] The resultant plasma solution (200 µT) followed by adding 10 µ0 of internal standard and then 20 µl of the final mixture (210 µo) was injected into the HPLC column.

Chromatographic conditions

To achieve the most effective chromatographic separation and analysis, the mobile phase was optimized by examining the effect of pH and varying the percentage of methanol and water content.[13] There are several small unknown peaks of plasma and the retention time of an unknown peak was near to the baclofen peak that not affect the separation. To make them far separated, the separation was studied carefully by increasing the content of methanol in methanol-water mixture from 50% to 55% and using a column of 300 mm instead of 200 mm. It was demonstrated that 55% of methanol in mobile phases could obtain the best separation. It was demonstrated that the retention time of unknown peaks decreased and the resolution was improved with the increasing of buffer pH. When pH was above 4.5, the retention time was almost stable, and the best resolution was obtained at pH 5.0.5 mM sodium citrate buffer (pH 5.0), and 2.0 ml/min of flow rate was selected for further investigation. The mobile phase was filtered through a 0.45 µ millipore membrane filter and was then degassed by ultrasonication. Analysis was run at a flow rate of 2.0 ml/min for 10 minutes, the column was kept at room temperature, and the detection wavelength was 220 nm.[13]

Human volunteers

Six healthy, non-smoking, non-chewing Qat, male volunteers (with age range 25–27) years old and weight range (60–75) kg were selected for this study. Subjects did not take any other medications for at least 1 week before and throughout the entire study. The study was approved and conducted as per guidelines prescribed by Institutional Ethics Committee (Human Ethical Committee (MECA NO.: [2017/06]).

Administration and blood sampling

The volunteers were divided into three equal groups each having two volunteers. The first group was dosed with 20 mg of oral commercial marketed baclofen tablet (Lioraz®) as a reference formulation, while the second group of volunteers received 40 mg baclofen transdermal film. The third group members received 20 mg baclofen mucoadhesive buccal film. On the acceptance of volunteers, two oral tablets (of 10 mg) were taken with a drought of water, transdermal films were placed on the arm area and adhering using plaster and the last one, buccal formulations were applied to oral cavity, on the buccal mucosa between the cheek and gingiva in the region of the upper canine and gently pressed against the mucosa for about 30 s to ensure adhesion. A drop of water was placed on the one face of the transdermal and buccal formulation before it was applied to the skin or buccal mucosa.

Blood samples (5 ml) were withdrawn from the vein of volunteers using a venous cannula 24 G needle. Samples were withdrawn at zero time (before drug administration) and at 1.0, 2.0, 3.0, 4.0, 6.0, 12.0, and 24.0 h after drug administration and collected in heparinized centrifuge tubes. Blood samples were centrifuged at 8000 rpm for 15 min to separate the plasma. The clear supernatant serum layer was transferred into labeled tubes and stored immediately at −20°C, pending baclofen assay.

Samples preparation

Frozen plasma samples were thawed at ambient temperature (25°at ambient temperatmin, and the baclofen was assayed using the developed HPLC/UV method as previously described. The method was fully validated with respect to adequate sensitivity, linearity, recovery, accuracy, and precision (within-day and between-day).[13]

Pharmacokinetic analysis

The plasma concentration-time data for baclofen following peroral, transdermal and buccal administration were evaluated to assess the bioavailability of baclofen and the following pharmacokinetics parameters were calculated.[14-16]

The maximum plasma concentration (C_{max}) and the time required to reach C_{max} (T_{max}) were directly read from the arithmetic plot of time versus plasma concentration of baclofen. The area under the plasma concentration versus time curve ($AUC_{0-\infty}$) was determined by means of trapezoidal rule. The relative bioavailability of baclofen from tested transdermal and buccal films in comparison to reference formulation (Lioraz®, oral tablets) was calculated by dividing its $AUC_{0-\infty}$ with that of Lioraz®. Relative bioavailability of baclofen from the tested transdermal and buccal formulations in comparison to reference formulation (Lioraz®, oral tablets) was calculated.

RESULTS AND DISCUSSION

Analytical method validation

Selectivity and resolution

Chromatograms obtained from blank plasma sample [Figure 1a], blank plasma sample spiked with an internal standard [Figure 1b] showed no interfering with the determination of baclofen. Under the chromatographic conditions used, the baclofen represented by internal standard peaks were well resolved, and the retention time was approximately 2.77 min. It can be seen from the figure that good separation and detectability of baclofen in plasma were obtained and no endogenous components from plasma were found to interfere with any of the analyzed compounds which indicates a good resolution and selectivity.

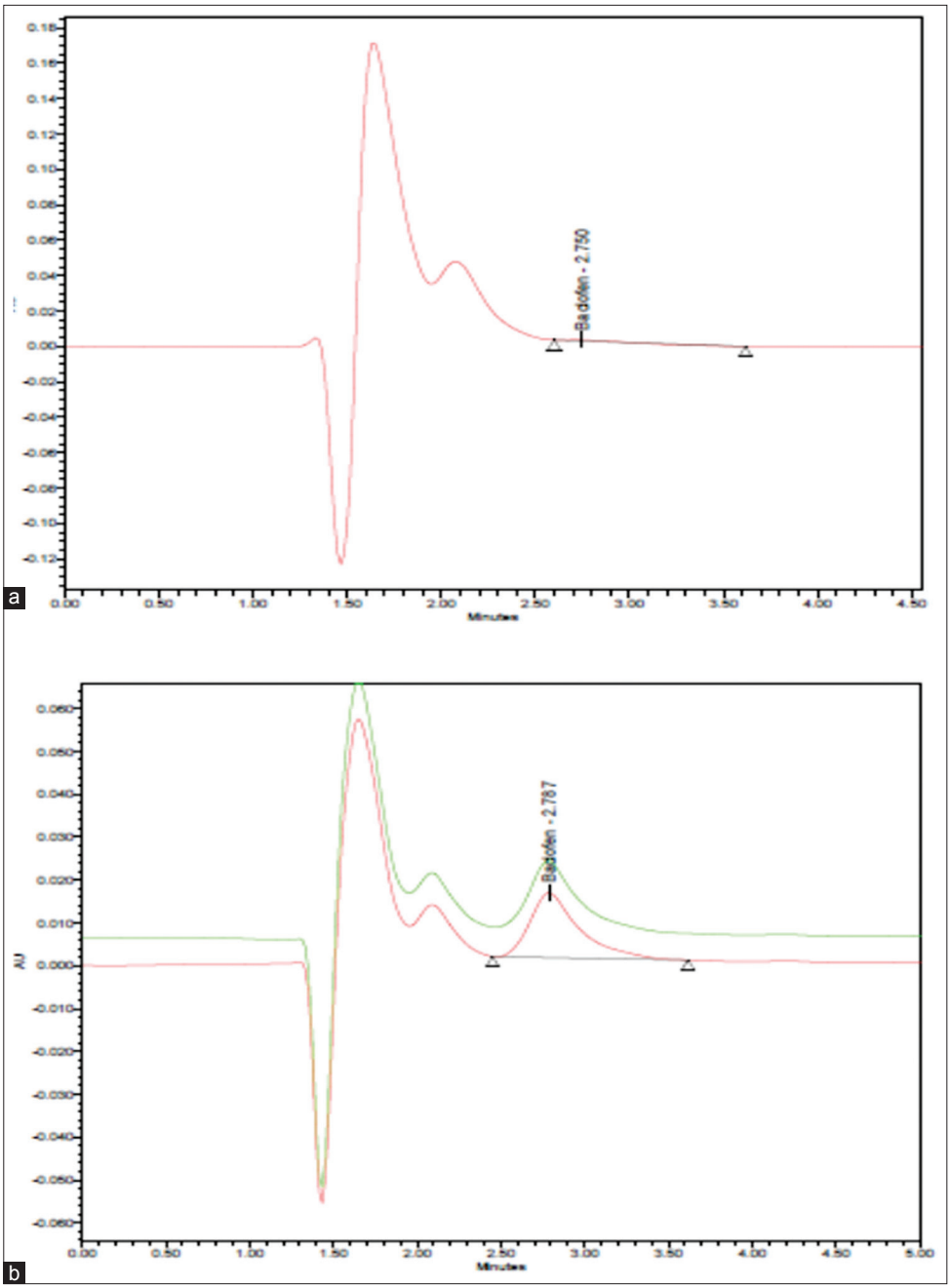

Figure 1: High-performance liquid chromatography of human plasma, (a) blank human plasma; and (b) plasma spiked with baclofen (internal standard)

Linearity

The calibration curve for baclofen was constructed from measurements of five concentrations in the range of 9524-46154 ng/mL in spiked plasma. A series of plasma samples containing 9524, 18181, 26087, 33333, and 46154 ng/mL of baclofen were measured. The calibration curve was obtained by plotting the peak area of baclofen (μV*sec) versus concentration of baclofen in ng/mL; data were presented in Table 1. The mean plasma standard curve (n = 3) was found to be linear over the concentration range used as in Figure 2 with a correlation coefficient (r^2) of 0.9997. However, the detection limit was estimated to be 1000 ng/mL.

Extraction recovery

To establish the extraction recovery, an internal standard was added to drug-free plasma and then spiked with 2000, 2500, 3000, 4000, and 5000 ng/mL of baclofen. Recovery (%) assessed from the replicate analysis (n = 3) for 3 days. The peak area of baclofen extracted was compared with the peak area of baclofen at same concentrations in mobile phase injected directly into the HPLC/UV system. The data obtained for different concentrations of baclofen are summarized in Table 2. The absolute recovery of baclofen at 2000, 2500, 3000, 4000, and 5000 ng/mL was 101.18, 99.48, 99.73, 103.19, and 97.67%, respectively, and the percentage

coefficient of variation (CV%) was 2.06, 4.59, 2.70, 3.26, and 2.43, respectively, with a mean percent recovery of 100.25 ± 3.00. Thus, the overall recovery was >91% indicating that the developed HPLC/UV method was highly accurate.

Precision and accuracy

Within-day and between-day precision and accuracy were evaluated by spiking blank plasma (internal standard added) with baclofen at three concentration levels: 2000, 3000, and 5000 ng/mL. Within-day and between-day precision and accuracy are summarized in Tables 3 and 4, respectively. The within- and between-day precision expressed as coefficients of variation (CVs%). As shown in Table 3, the coefficients of variation for the within-day precision were: 1.23% at 2000 ng/mL, 1.25% at 3000 ng/mL, and 2.19% at 5000 ng/mL. The CVs % for between-day precision were: 3.48% at 2000 ng/mL, 2.77% at 3000 ng/mL, and 1.53 % at 5000 ng/mL. The values for the CV% were all <10% at the concentration range determined indicating good precision of proposed method. An indication of within- and between-day accuracy was based on a calculation of the mean percentage recovery, and the relative error of the mean measured concentrations compared with the nominal or added concentrations. The accuracy was within (90.15 ± 3.48–104.41 ± 1.31%) at the selected concentration values, and the relative error was <10%. This method was found to be precise and accurate and considered suitable for the pharmacokinetic study of baclofen.

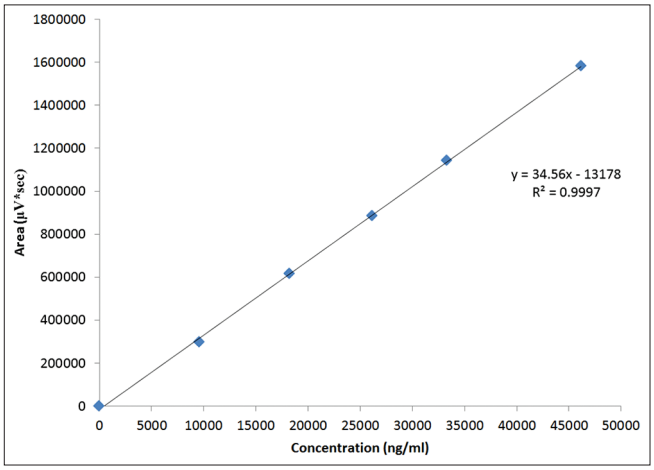

Figure 2: Calibration curve of baclofen in human plasma at 220 nm using the adopted high-performance liquid chromatography/ultraviolet detector method

Table 1: Relation between concentration of baclofen in human plasma and peak area using the adopted HPLC/UV detector method

Concentration (ng/mL)	Area(µV*sec)
0	0
9524	298919
18181	616629
26087	883997
33333	1143807
46154	1583753
Correlation coefficient (r^2)	0.9997

y=34.56x–13178. HPLC: High-performance liquid chromatography, UV: Ultraviolet

Comparative bioavailability studies

Pharmacokinetic analysis data

Transdermal and buccal formulations were designed to be applied to the human volunteers on the skin and buccal mucosa to be remained continuously attached to them during the period of study. The volunteers exposed to application had not any problems or disturbances during film applications. There were no signs of local irritation observed in any volunteer and no trouble at all of them was complained.

Plasma baclofen concentrations obtained following a single peroral dose administration of 20 mg of the available market product (Lioraz®, oral tablet), the transdermal film and the transmucosal buccal film; each to two volunteers is compiled in Table 5. Figure 3 shows the individual plasma concentration-time curves of baclofen for volunteers after peroral administration of the market product (Lioraz®) and after application of transdermal and transmucosal buccal films.

The individual and the mean pharmacokinetic parameters (C_{max}, T_{max}, K_e, $t_{1/2}$, AUC_{0-24}, $AUC_{0-\infty}$ and mean residence time [MRT]) calculated from baclofen plasma concentration-time

Table 2: Recovery of baclofen from spiked plasma using the developed HPLC/UV method (*n*=3, mean±SD)

C_{added} (ng/mL)	$C_{measured}$(ng/mL)±SD	Recovery(%)±SD	CV(%)
2000	2023.58±41.21	101.18±2.06	2.04
2500	2486.92±114.21	99.48±4.57	4.59
3000	2992.04±80.83	99.73±2.69	2.70
4000	4127.75±134.42	103.19±3.36	3.26
5000	4883.65±118.73	97.67±2.37	2.43
Mean % recovery±SD		100.25±3.10	3.00

C added: Nominal (added) concentration, C measured: Measured (found) concentration, SD: Standard deviation. CV%: Percentage coefficient of variation (precision), HPLC: High-performance liquid chromatography, UV: Ultraviolet

Table 3: Within-day precision and accuracy of the developed HPLC/UV method (*n*=3, mean±SD)

C_{added}(ng/mL)	$C_{measured}$(ng/mL)±SD	Recovery (%)±SD	CV (%)	RE
2000	1973.28±24.26	98.66±1.23	1.23	1.34
3000	3131.92±39.23	104.41±1.31	1.25	–4.40
5000	4713.56±103.46	94.27±2.07	2.19	5.73

C added: Nominal (added) concentration, C measured: Measured (found) concentration, SD: standard deviation, RE% (relative error): Relative deviation from the nominal concentration (accuracy), CV%: Percentage coefficient of variation (precision), HPLC: High-performance liquid chromatography, UV: Ultraviolet

Table 4: Between-day precision and accuracy of the developed HPLC/UV method (*n*=3, mean±SD)

C_{added} (ng/mL)	$C_{measured}$ (ng/mL)±SD	Recovery (%)±SD	CV (%)	RE
2000	1802.96±69.62	90.15±3.48	3.86	9.85
3000	2906.42±80.55	96.88±2.68	2.77	3.12
5000	4615.39±70.50	92.31±1.41	1.53	7.69

C added: Nominal (added) concentration, C measured: Measured (found) concentration, SD: Standard deviation, RE% (relative error): Relative deviation from the nominal concentration (accuracy), CV%: Percentage coefficient of variation (precision), HPLC: High-performance liquid chromatography, UV: Ultraviolet

Table 5: Mean plasma concentrations of baclofen following administration of Lioraz® oral tablets, transdermal and transmucosal buccal films to human volunteers (*n*=2, mean±SD)

Time (h)	Mean plasma concentration (ng/mL)±SD		
	Oral	Transdermal	Buccal
0	0.00±0.00	0.00±0.00	0.00±0.00
1	112.16±11.09	62.91±22.43	433.43±235.57
2	1021.83±110.55	179.96±42.36	1825.70±670.76
3	2050.00±70.71	754.71±119.80	2450.00±353.55
4	1865.00±91.92	1104.55±218.56	1476.16±65.27
6	878.29±101.41	1200.00±141.42	319.50±156.27
12	291.56±82.64	470.31±188.16	54.31±34.37
24	0.00±0.00	0.00±0.00	0.00±0.00

SD: Standard deviation

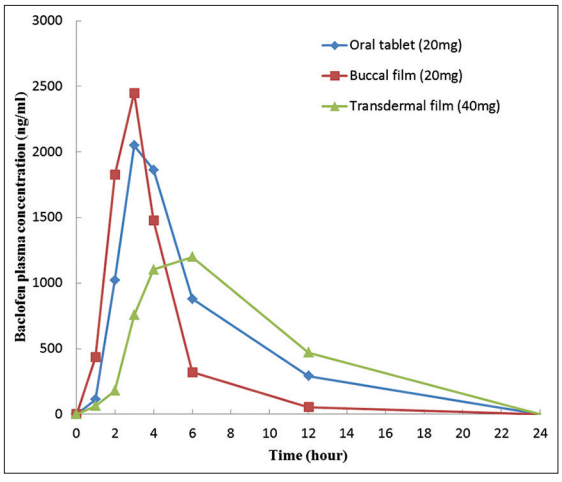

Figure 3: Mean plasma concentration-time profiles of baclofen following a single oral dose administration (20 mg/body) of Lioraz® tablet, after the application of transdermal film (40 mg/body) and transmucosal buccal film (20 mg/body)

data of the two volunteers following the administration of each product, in addition to the relative bioavailability of prepared transdermal and transmucosal buccal formulations with respect to Lioraz® oral tablets are shown in Table 6. The mean plasma level profiles (mean ± standard deviation) of baclofen obtained following the administration of each the tested formulae are compared in Figure 3. Following peroral administration of the reference product (Lioraz® tablet), the mean plasma concentration of baclofen quickly increased to reach a C_{max} of 2050.00 ± 70.71 ng/mL after 3.00 h of peroral dosing. The $AUC_{0-\infty}$ after peroral dosing was found to be 13364.18 ± 1264.96 ng.h/mL with MRT of 5.80 ± 0.64 h. Unlike the peroral administration, after the application of the transdermal film, C_{max} of 1200.00 ± 141.42 ng/mL was achieved 6.00 h after dosing. The AUC_{0}-∞ was found to be 16220.46 ± 6401.90 ng.h/mL with MRT of 13.85 ± 4.62 h. Whereas following the buccal application of the transmucosal buccal film, C_{max} of 2450.00 ± 353.55 ng/mL was achieved after 3.00 h of dosing. The $AUC_{0-\infty}$ was found to be 10860.49

± 843.49 ng.h/mL with MRT of 3.52 ± 0.55 h.

Referring to Table 6 and Figure 3, it can be seen that T_{max} values revealed that the transmucosal formulation spent the same time to reach the maximum drug concentration in the systemic circulation as the oral dose (Lioraz® tablet) by time of 3.00 h whereas, the time required to reach to maximum concentration from transdermal films was slower than reference product in which reaching peak plasma concentration in 6 h. The mean value of C_{max} was higher for drug administration from transmucosal buccal bioadhesive device than from oral tablet whereas the transdermal device was lower than oral tablet and this may due to the large hydration of buccal by saliva that fasten the dissolution of films and so increase erosion of drug. On the other hand, a higher prolongation in the $t_{1/2}$ for the tested transdermal formulations (9.27 h) than the reference product (3.13 h) was obvious in Table 6 and Figure 3 whereas the transmucosal buccal films showed shorter $t_{1/2}$ of baclofen than the reference product (1.62 h). Thus, in case of transdermal films, prolonged T_{max}, increased MRT and decreased K_e of baclofen indicated that the drug release from transdermal formulations is slow thereby providing a prolonged and controlled *in vivo* delivery of the drug and this was not correct with the transmucosal buccal films that means no prolonged action and so make it good application for the emergency state. The overall mean values of $AUC_{0-\infty}$ by transdermal route for film was 1.21 times, higher than that of the oral route at the doubled dose, and in another way, the buccal is lower than oral route by 0.81 times than oral at the same dose of the drug.

The bioavailability of the selected transdermal and transmucosal buccal formulations containing 40 mg of baclofen in case of transdermal and 20 mg in case of buccal formulations was determined. The results were compared with reference oral tablet (Lioraz® tablet) containing 20 mg of baclofen. The transdermal and buccal films showed relative bioavailability of 78.99% and 89.22%, respectively, with respect, to Lioraz® oral tablets [Table 6]. The relative bioavailability from transdermal and transmucosal buccal route is a quick absorption in case of buccal and a very slow absorption in case of the transdermal route. The relative bioavailability for transdermal film was lower than that for buccal film with respect to double dose. Buccal films adhered to high permeable buccal membrane compared to a transdermal film that was adhered to low permeable membrane (skin) and resulted in a higher bioavailability.

The disadvantages of erratic oral absorption and interaction with food can also be potentially overcome by designed transdermal and buccal drug delivery systems. Moreover, the introduced formulations effectively sustained the release of baclofen and also maintained plasma concentrations during the entire application period. The transdermal film particularly offered more sustained delivery profile than buccal film and oral tablet with the absence of sharp peaks. This sustained-release behavior fulfills the criteria required for a sustained-release preparation, i.e., smooth plasma level profile and long duration of effect.

According to the previous data, transmucosal buccal films failed to improve the peroral tablet in prolong the action and improve patient compliance. Thus, only the transdermal dosage forms designed in the present study was found to provide a prolonged steady-state concentration of baclofen with minimal fluctuations and improved bioavailability. Further clinical trials in humans of the introduced preparations are also encouraged.

CONCLUSION

The developed HPLC/UV method of analysis was suitable for the determination of baclofen in human volunteer plasma and showing low retention time, good linearity, acceptable precision, and accuracy over the concentration range used. The relative error, determined by comparing the measured concentrations to the expected concentrations, was within the limit with a mean extraction recovery of 100.25% ± 3.10. MRT, T_{max} and $t_{1/2}$ values revealed that the tested formulations spent a longer time to reach the maximum drug concentration in the systemic circulation and effectively sustained the

Table 6: Mean pharmacokinetic parameters of baclofen following administration of Lioraz® oral tablets, transdermal and transmucosal buccal films (*n*=2, mean±SD)

Parameters	Lioraz® tablet 20 mg (reference product)	Transdermal film 40 mg (F13)	Transmucosal buccal film 20 mg (B4)
C_{max} (ng/mL)	2050.00±70.71	1200.00±141.42	2450.00±353.55
T_{max} (h)	3.00±0.00	6.00±0.00	3.00±0.00
K_e (h^{-1})	0.23±0.04	0.08±0.03	0.43±0.08
$t_{1/2}$ (h)	3.13±0.47	9.265±3.26	1.65±0.33
AUC_{0-24} (ng.h/ml)	12018.49±695.11	9492.84±1674.44	10722.88±951.24
$AUC_{0-\infty}$ (ng.h/ml)	13364.18±1264.96	16220.46±6401.90	10860.49±843.49
MRT (h)	5.80±0.64	13.85±4.62	3.52±0.55
Relative bioavailability (%)		78.99	89.22

$AUC_{0-\infty}$: Area under the plasma concentration versus time curve, MRT: Mean residence time, SD: Standard deviation

absorption of baclofen and also maintained an elevated plasma baclofen concentration during the entire application period. The mean value of C_{max}, MRT, AUC_{0-10}, and $AUC_{0-\infty}$, was significantly higher for a drug administered from transdermal formulations than oral tablet, demonstrating improved prolongation of action of baclofen from tested transdermal formulation whereas the buccal film was lower in all pharmacokinetic parameters. No statistically significant differences were observed between the two transdermal and buccal films with regard to all pharmacokinetic parameters. Taking Lioraz® oral tablets as a reference product, the percentage relative bioavailability values of baclofen from the selected transdermal and transmucosal buccal films were calculated and found to be 78.99 and 89.22%, respectively. The absorption from buccal films was faster, whereas the transdermal spent longer times to reach the maximum drug concentration in the systemic circulation. Thus, the transdermal and buccal delivery of baclofen using these designed bioadhesive formulations showed low bioavailability compared to the oral delivery. Transdermal film of baclofen was successful in all the established studies by improving the prolongation of action and increasing the half-life of baclofen.

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