Drug Delivery and Translational Research (2023) 13:795–810
https://doi.org/10.1007/s13346-022-01239-x

ORIGINAL ARTICLE

Patient-friendly extemporaneous formulation of bisoprolol: application to stability and bioavailability studies

Soha M. El-Masry[1] · Sally A. Helmy[1,2] · Soha A. M. Helmy[3,4] · Eman A. Mazyed[5]

Accepted: 7 September 2022 / Published online: 3 October 2022

Abstract

Community and hospital pharmacists always face the challenge to prepare oral liquid extemporaneous formulations to fit the needs of a specific patient population when commercial forms or the required strength is unavailable. This study was performed to prepare a stable patient-friendly oral liquid extemporaneous formulation of bisoprolol. Eight different extemporaneous formulations were prepared using various suspending agent(s). The in vitro dissolution of all extemporaneous formulations was examined. A comprehensive accelerated stability study was carried out to evaluate the adequate beyond-use date of the most optimized extemporaneous formulation. A validated ultra-performance liquid chromatography method was used for the analysis and quantification of bisoprolol in the accelerated stability and bioavailability studies. A group of eight healthy volunteers was enrolled in a two-way cross-over experimental design to study the bioavailability of the most optimized extemporaneous formulation. The pharmacokinetic parameters of bisoprolol were estimated. Extemporaneous suspension containing 0.5% w/v xanthan gum was easily prepared with a simple, natural, safe, sugar-free excipients. It achieved the best dissolution behavior among other extemporaneous suspensions. It was an easily pourable viscous suspension with no sedimentation. At least 98% of the initial concentration of bisoprolol remained throughout the 6-month study period in the selected suspension regardless of the storage conditions. There was no perceptible change in color, odor, or taste, and no noticeable microbial growth was observed in any sample. The selected formulation was bioequivalent to the commercial tablet in terms of the rate and extent of absorption. This research may be of great help during development of appropriate extemporaneous formulation of bisoprolol fumarate. The simple preparation method could be utilized to draw up a standard operating procedure (SOP) easy to use by different types of pharmacy settings.

Keywords Bisoprolol · Extemporaneous suspension · Stability · Bioavailability · SOP

Introduction

The lack of the required strength or commercially available oral liquid dosage forms authorized for pediatric and geriatric patients with special needs is a challenging issue for health care providers in pediatric and geriatric settings [1, 2]. Extemporaneous oral liquids would be advantageous for these patients who cannot swallow solid dosage forms or receiving medicines via nasogastric or gastrostomy tubes [3].

✉ Sally A. Helmy
sallyhelmy@pharm.dmu.edu.eg; shelmy@taibahu.edu.sa; sallyhelmy@yahoo.com

Soha M. El-Masry
Elmasry_soha@pharm.dmu.edu.eg

Soha A. M. Helmy
drsohahelmy@yahoo.com

Eman A. Mazyed
eman_mazyad@pharm.kfs.edu.eg

[1] Department of Pharmaceutics, Faculty of Pharmacy, Damanhour University, Damanhour, Egypt

[2] Department of Clinical and Hospital Pharmacy, College of Pharmacy, Taibah University, AL-Madinah AL-Munawarah, Kingdom of Saudi Arabia

[3] Department of Languages and Translation, College of Arts and Humanities, Taibah University, AL-Madinah AL-Munawarah, Kingdom of Saudi Arabia

[4] Department of Foreign Languages, Faculty of Education, Tanta University, Tanta, Egypt

[5] Department of Pharmaceutical Technology, Faculty of Pharmacy, Kafrelsheikh University, POB 33516, Kafrelsheikh, Egypt

Hypertension is one of the most predominant chronic diseases in adult and children. Bisoprolol is a cardioselective β1-blocker used in treatment of hypertension and cardiovascular diseases in adults, children, and adolescents [4–7]. Bisoprolol is a BCS Class I drug (highly soluble and highly permeable drug) [8–11]. Its balanced hydrophilic and lipophilic properties make it a great competitor over other β-blockers, and give it ideal pharmacokinetic profile [12, 13]. The high absorption of bisoprolol after oral administration reflects a high bioavailability of approximately 90% [13]. It has a long half-life of 10–12 h, subsequently once daily administration is adequate to elicit the therapeutic effect of bisoprolol, which in turn has a beneficial effect on patient compliance [4, 13].

Bisoprolol is commercially available as 2.5-mg, 5-mg, and 10-mg heart-shaped film coated tablets. There are neither age-appropriate, nor commercial liquid bisoprolol formulations. Furthermore, splitting of bisoprolol commercial tablets (Concor®) may cause dosing inaccuracies and impair bioavailability due to the heart shape of tablets [14]. To our knowledge, bisoprolol is not available in any dosage form that could be suitable for patients with swallowing problems. Accordingly, a liquid extemporaneous formulation is highly desirable not only for use in pediatrics where the dosages are exceptionally small but also for adults who experience difficulty in swallowing and are typically fed by means of a feeding tube.

The stability of a pharmaceutical product during its entire shelf life is an important matter. The importance of stability is highlighted by regulations prescribed by the International Conference of Harmonization (ICH) and the European Medicines Agency (EMA) [15, 16]. Any change in the physical, chemical, microbiological, and therapeutic properties of any ingredient of the medication will affect the stability of the medication [17]. Accelerated stability testing, also known forced degradation study, can predict the stability of an active substance or finished pharmaceutical product in a short period via subjecting it to condition that accelerates its degradation [18]. The obtained stability information is helpful in the prediction of the shelf life and expiration dates of pharmaceuticals as well as establishing proper packaging and appropriate storage conditions. This will lead to the release of safe and effective pharmaceutical products to the market.

A search in Web of science, Springer, Embase, PubMed, and International Pharmaceutical Abstracts revealed no publications about extemporaneous formulations of bisoprolol. Accordingly, the aim of this research was to develop a safe, stable, palatable, and economic oral liquid extemporaneous formulation of bisoprolol at a target concentration of 0.5 mg/mL without using potentially harmful excipients. The stability of liquid extemporaneous formulations is limited and uncertain, and their bioavailability may be variable and/or unknown [19, 20]. Accordingly, a comprehensive accelerated stability study was carried out by subjecting the drug to various stress conditions in order to evaluate the adequate shelf life of the most optimized extemporaneous formulation. Consequently, a validated stability indicating ultra-performance liquid chromatography (UPLC) method was a prerequisite for this purpose. The conditions utilized for conducting the accelerated stability study were selected according to ICH guidelines [15]. The study was supported by physical and chemical stability on storage. Moreover, the research was extended to evaluate the bioavailability of the selected extemporaneous formulation in humans.

Material and methods

Material

Bisoprolol fumarate (99.9%) was supplied by Sigma-Aldrich Co. (St. Louis, USA). Concor® tablets *(*bisoprolol fumarate, anhydrous calcium hydrogen phosphate, maize starch, magnesium stearate, crospovidone, anhydrous colloidal silica, and microcrystalline cellulose) (MERCK Pharma GmbH, Darmstadt, Germany) were purchased from local market. Xanthan gum, Carbopol®, sodium alginate, sodium benzoate, strawberry flavor, and glycerol were obtained from Loba Chemicals, Mumbai, India. Sorbitol and carboxymethylcellulose (CMC) were purchased from Sigma-Aldrich Co. (St. Louis, USA). Ammonium dihydrogen orthophosphate was supplied by Riedel-de Haën, Germany. Methanol and acetonitrile HPLC grade were supplied by MERCK, Germany.

Methods

Preparation of bisoprolol extemporaneous suspensions

Eight different formulations (F1–F8) were extemporaneously prepared utilizing various suspending agents as listed in Table 1. Stock dispersion of each suspending agent (xanthan gum, Carbopol, sodium alginate, and CMC) was prepared by dispersing the required amount of one or two suspending agent(s) in 75 mL of distilled water using a magnetic stirrer (Stuart, Stone, Staffordshine, UK) at 500 rpm. Then, the volume of the dispersion was completed to 90 mL with distilled water. The resultant dispersions were allowed to hydrate for 24 h before use [21]. Bisoprolol suspensions (each contains 0.5 mg/mL) were prepared by crushing commercially available 5-mg Concor® tablets (R) and resuspending the resultant powder in glycerol and a small amount (5 mL) of the previous dispersion of suspending agent(s) [21]. The mixture was triturated until a smooth paste was formed. With continuous trituration, the paste was diluted with the remaining

Table 1 Composition of different extemporaneous suspensions of bisoprolol (0.5 mg/mL)

Ingredients	F1	F2	F2-unprocessed	F3	F4	F5	F6	F7	F8
Bisoprolol	0.05	0.05	0.05*	0.05	0.05	0.05	0.05	0.05	0.05
Xanthan gum	0.1	0.5	0.5	–	–	0.3	–	–	–
Carbopol	–	–	–	–	–	–	0.1	0.5	–
Sodium alginate	–	–	–	–	–	–	–	–	0.1
CMC	–	–	–	0.1	0.5	0.1	–	–	–
Sodium benzoate	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Glycerol (mL)	5	5	5	5	5	5	5	5	5
Sorbitol (mL)	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Strawberry flavor	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Water (mL)	To 100	To 100	To 100	To 100	To 100	To 100	To 100	To 100	To 100

All formulations were represented as % (w/v)

*F2-unprocessed is formulated using pure bisoprolol fumarate powder instead of crushed tablets

amount of the dispersion of the suspending agent(s). Finally, the required amounts of sodium benzoate, sorbitol (2.5 mL of solution contains 0.693 g of the sugar substitute sorbitol), and strawberry flavor were added to the mixture and the volume completed to 100 mL with shaking. For compounding formulation (F2-unprocessed), the same excipients and procedures were utilized as F2 except that the drug used was pure bisoprolol powder, as reported in Table 1.

Chromatography

An ultra-performance liquid chromatography (UPLC) (Agilent, Malaysia) was employed for the detection of bisoprolol in stability and plasma samples. The mobile phase consisted of a mixture of 50 mM ammonium dihydrogen orthophosphate containing 0.2% triethylamine and acetonitrile at a ratio of (35:65 v/v). The flow rate was set at 1.0 mL/min on a Microsorb-MV 100–3 C_{18} column (100 mm × 4.6 mm, 5 µm; Agilent Technologies, Netherlands). The effluent was monitored using a UV Agilent detector 1290 DAD (Model: G4212A; Serial No. DEBAF04676, USA) set at 225 nm and a fluorescence Agilent detector 1260 FLD (Model: G7121A; Serial No. DEAE300893, USA) set at 215 nm for excitation and 298 for emission for the analysis of stability and plasma samples, respectively. Tinidazole was used as internal standard (IS). The method was validated in terms of linearity, precision, accuracy, sensitivity, and selectivity [22].

Preparation of stocks, samples, and calibration curves

Calibration curves were constructed in the range of 10–100 µg/mL and 1–100 ng/mL for stability and plasma samples, respectively. For stability samples, a stock suspension of bisoprolol 100 mg/mL was prepared in a mixture of 50:50 (v/v) of distilled water and compounded suspending vehicle that was previously prepared. A serial dilution was made to prepare five working solutions of bisoprolol in HPLC-grade water which were 10, 25, 50, 75, and 100 µg/mL. All standard solutions were passed through 0.45-µm Acrodisc microfilter before injection of 25 µL into UPLC. For plasma samples, a concentrated stock solution of bisoprolol 100 µg/mL was prepared in methanol. A 7-point calibration curve was prepared with concentrations of 10, 20, 50, 100, 200, 500, and 1000 ng/mL. The drug concentrations were calculated by comparing the peak areas of the samples with a calibration curve. The linearity of the calibration curves was confirmed by plotting the peak area ratios of drug/IS versus the corresponding bisoprolol concentrations with least-squares linear regression analysis.

The optimized extemporaneous suspension (F2) was used for stress and bioavailability studies. In case of stress studies, the selected extemporaneous suspension (100 mL) was diluted with 100 mL of each stress agent to obtain a final nominal bisoprolol concentration of 50 µg/mL and kept for stress studies. At a predetermined interval, 1-mL sample was withdrawn and filtered through a 0.45-µm Millipore filter and a 25-µL sample was injected into the UPLC. For the bioavailability study, the calibration standards of bisoprolol in plasma were prepared by transferring 25 µL from each working solution and IS to a set of clean test tubes. After evaporation of the methanolic solution, 0.25 mL of blank plasma was added to each tube to form a set of calibration standards with concentrations of 1, 2, 5, 10, 20, 50, and 100 ng/mL. After that, samples were treated with 100 µL of buffer solution (50-mM ammonium dihydrogen orthophosphate containing 0.2% triethylamine) and shacked for 30 s, and then 0.5-mL acetonitrile was added and shacked for another 30 s, then centrifuged at 3000 RCF for 7 min. The supernatant was evaporated under a gentle steam of nitrogen to dryness,

and then reconstituted with 150 µL of the mobile phase. Twenty microliters of the resulting solution was injected into the UPLC. The plasma samples obtained from volunteers were treated as calibration standards.

In vitro evaluation of extemporaneous suspensions

Appearance and pH measurement

All extemporaneous formulations were observed for appearance, smell, color, and texture. pH values of all extemporaneous suspensions were recorded using a pH meter (Thermo Scientific Orion Star A215, USA).

Viscosity measurement

Viscosity of all extemporaneous suspensions was recorded using a viscometer (Brookfield AMETEK, Inc., Middleborough, MA, USA) with spindle 3. The rotation speed was 10 rpm at 28.8 °C.

Sedimentation volume measurement

Five milliliters of each suspension was diluted to 10 mL with distilled water then shacked vigorously to ensure uniformity and left at room temperature. The sedimentation volume and the presence of floating particles were observed visually. The sedimentation ratio (F) could be calculated as F = Hµ/Ho, where Hµ is the ultimate height of the sediment as suspension settles in a cylinder, and Ho is the initial height of each suspension [23, 24].

Resuspendability of suspension

Resuspendability is a quantitative test to assess if a suspension is redispersed easily after a long period of standing [25]. Resuspendability of different suspensions was evaluated as resuspendable, resuspendable with difficulty, or not resuspendable [26].

Dissolution studies

No codified dissolution method was reported for bisoprolol extemporaneous suspensions accordingly; the dissolution studies were conducted according to FDA recommendations [27, 28]. The in vitro dissolution characteristics of all extemporaneous formulations (F1–F8) were conducted using USP-II dissolution apparatus (Copley DIS 8000, Nottingham, UK). The dissolution medium was 900 mL distilled water that maintained at 37 ± 0.5 °C and stirred at 100 rpm. A 5-mL sample from each suspension was taken using a 5-mL syringe [21]. Samples were collected at appropriate time intervals at 1, 3, 5, 10, 15, 30, 45, and 60 min. Bisoprolol concentrations were recorded using UPLC. The cumulative percentage of bisoprolol dissolved was calculated. The latter was plotted versus time to construct the dissolution profiles.

Data analysis

Model-dependent and model-independent approaches used to compare and fit the dissolution profiles of bisoprolol extemporaneous suspensions are listed in Table 2.

Model-dependent methods The dissolution data were fitted into different kinetic models such as zero-order, first-order, Higuchi, Hixson-Crowell, Weibull, and logistic [29, 30] (Table 2). The kinetic model with the highest determination coefficient (R^2) value was considered more appropriate. The model parameters were compared as extemporaneous suspension (T) versus Concor® tablets (R) using *t* test.

Table 2 Model-dependent and model-independent equations used to compare the dissolution profiles of bisoprolol extemporaneous suspensions

Function	Equation
Model-dependent equations	
Zero-order	$\%\ diss = k_0 t$
First-order	$\%\ diss = 100\left[1 - e^{-kt}\right]$
Higuchi	$\%\ diss = k_H t^{1/2}$
Hixson-Crowell	$\%\ diss = 100\left[1 - \left(1 - \frac{k_{HC} t}{4.6416}\right)^3\right]$
Weibull	$\%\ diss = 100\left[1 - e^{-\left(t/Td\right)\beta}\right]$
Logistic	$\%\ diss = 100\left[\frac{e^{(\alpha+\beta \log t)}}{1+e^{(\alpha+\beta \log t)}}\right]$
Model-independent equations	
DE	$DE = \frac{\int_{t_1}^{t_2} y.dt}{y_{100} \times (t_2 - t_1)}$
MDT	$MDT = \frac{\sum_{j=1}^{n} t_{jmid} x \Delta M_j}{\sum_{j=1}^{n} \Delta M_j}$
f_2	$f_2 = 50 \times log\left\{\left[1 + \frac{1}{n}\sum_{t=1}^{n}\left(R_t - T_t\right)^2\right]^{-0.5} \times 100\right\}$

% diss percent dissolved at time t, k_o zero-order release rate constant (mg%.h^{-1}), k first-order release rate constant (h^{-1}), k_H Higuchi release rate constant (mg.h$^{-1/2}$), k_{HC} Hixson-Crowell release rate constant (mg$^{1/3}$%.h^{-1}), α, scale factor, β shape parameter, *Td* time at which 63.2% of the material is dissolved, *DE* dissolution efficiency, *y %* dissolved at time *t*, *MDT* mean dissolution time, *j* release sample number, *n* number of dissolution sample times, t_{jmid} time at the midpoint between t_j and t_j^{-1} ΔM_j, the additional amount of drug released between *t* and t_j^{-1}, f_2 similarity factor, R_t and T_t the individual or mean percent dissolved at each time point, *t*, for the reference and test dissolution profiles, respectively

Model-independent methods The dissolution parameters were calculated from the dissolution profiles. They included Q5 and Q60 (% of drug liberated in the first 5 min and over 60 min, respectively), mean dissolution time (MDT), dissolution efficiency (DE), and similarity factor (f_2). The comparison of the dissolution profiles was conducted by statistical evaluation of MDT, DE, and f_2 [31, 32] (Table 2). Dissolution profiles were considered similar when the f_2 value was greater than 50 [8].

Calculations were performed by Excel add-in DDSolver [33]. The formulation that showed the best in vitro dissolution results was used for further investigation including forced degradation and bioavailability studies.

Forced degradation studies

Forced degradation studies were involved by subjecting bisoprolol to hydrolysis, oxidation, temperature, moisture, and light. Xanthan gum-containing extemporaneous suspension (F2) was used in the forced degradation studies. The conditions employed for performing stress studies were conducted according to ICH guidelines [15].

Chemical stability study

For hydrolysis (acid and alkaline) and oxidative degradation, the selected extemporaneous formulation (F2) was diluted with 0.1 M HCl, 0.1 M NaOH, and 3% H_2O_2, respectively, to achieve 50 µg/mL. The solutions were undergone with the described stress conditions for 1, 3, 24, 48, and 72 h. Also, testing was carried out under neutral conditions, wherein distilled water was used as a solvent. The blank samples were prepared by applying the selected stress factor in absence of the drug.

Photostability studies were done in both light and dark conditions. One set of the selected bisoprolol extemporaneous suspension (F2) was kept in a nature daylight (natural sunlight exposure by a laboratory window for the duration of experiment) or artificial white light during the night for 1 month and the second was kept in dark for the same period of time.

Stability of F2 toward temperature was performed during 6 months on samples stored under refrigeration (4 °C), at room temperature (25 °C), and under ICH accelerated aging conditions for (40 °C/ × 75% RH) in light-protected glass bottles using a stability cabinet (POL-EKD Climatic Chamber Apparatus, Poland) [15].

The concentrations of bisoprolol in stability samples were measured by UPLC. The retention times of bisoprolol in blanks and stability samples were compared to detect any endogenous and/or degradation peaks appeared at the same retention time of bisoprolol in the UPLC chromatograms. Percent drug degradation was calculated as $\%\ Degradation = (C0 - Ct/C0) \times 100$, where C_0 is the initial drug concentration at zero time, and C_t is the drug concentration after the specified experimental time. Bisoprolol extemporaneous formulation (F2) was considered stable when bisoprolol concentration remained above 90% of the initial concentration, without any change in physical properties. The results were compared statistically using ANOVA to demonstrate the effect of aging conditions on the chemical stability of bisoprolol in F2.

Physical stability study

Visual inspection of F2 was done weekly during the study period. The inspection included a change in odor or color as well as caking, redispersion, and precipitation problems. Additionally, microbiological stability was checked visually for possible cloudiness or possible mold formation [34]. Changes in the pH and viscosity of F2 were studied for 6 months at 25, 45, and 4 °C [35].

Fourier transform infrared spectrophotometry (FT-IR)

The physicochemical compatibilities of pure bisoprolol fumarate, R (Concor® tablets), and excipients used in the formulation of the most optimized suspension (F2) were evaluated by FT-IR (PerkinElmer, USA). Prior to scanning, samples were mixed separately with potassium bromide (1:10, w/w) and scanned from 4000 to 400 cm^{-1}.

Bioavailability study

Eight subjects of 23–38 years old and 69–85 kg weight participated in the in vivo bioavailability study. A randomized cross-over study design was performed under fasting conditions. Subjects received a single oral dose of the two treatments followed by 240 mL of water. The washout period between treatments was 2 weeks. The treatments were the R tablet (10-mg bisoprolol) and extemporaneous suspension (F2) (10-mg bisoprolol). Blood samples (5 mL) were collected prior to 0 h and at 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 24, and 48 h after F2 administration. Plasma samples were obtained after centrifugation of blood samples at 3000 RCF for 20 min and then stored at – 20 °C until analysis by UPLC. Pharmacokinetic analysis was performed by noncompartmental method [36, 37]. The pharmacokinetic parameters included peak concentration (C_{max}), T_{max} (time to reach peak concentration), elimination rate constant (k), half-life ($t_{1/2}$), area under concentration–time curve from zero to infinity ($AUC_{0-\infty}$), and mean residence time (MRT).

Bioequivalence assessment was based on predefined acceptance criteria of 80–125% for the 90% confidence interval for the ratio of the test (F2 suspension) and reference (Concor® tablet) products for the log-transformed data of AUC and C_{max} [8]. The statistical analysis was conducted using one-way ANOVA (Minitab, State College, PA, USA).

Results and discussion

Community and hospital pharmacists always face the challenge to prepare oral liquid extemporaneous formulations to fit the needs of a specific patient population when commercial forms or the required strength is unavailable. Accordingly, the primary aim of this research was to prepare a stable patient-friendly oral liquid extemporaneous formulation of bisoprolol with a 0.5 mg/mL as a target concentration. Furthermore, forced degradation studies were carried out to study the physical and chemical stability of bisoprolol and to evaluate the adequate shelf life of the optimized extemporaneous suspension. The research was extended to study the bioavailability of the optimized formulation in humans.

Preparation of bisoprolol extemporaneous suspensions

Several trials were conducted in order to obtain the most optimized formulation that met the desired quality requirements in terms of organoleptic properties, dissolution behavior, and stability, as depicted in Table 3. All formulations were prepared without the utilization of potentially unsafe excipients, and with a well-tolerable pH value. Formulation trials were carried out using various suspending agents to achieve homogeneity of redispersed suspensions. Xanthan gum and sodium alginate are natural polymers, whereas CMC and Carbopol are synthetic polymers. Xanthan gum is safe up to 15 g per day [38].

Sodium benzoate was involved in a small amount (0.05%w/v) in all formulations as a preservative to prevent microbial growth [34]. The U.S. FDA allows up to a 0.1% w/w of sodium benzoate in foods and beverages [39]. The WHO has set the acceptable daily intake level for sodium benzoate to 0–5 mg/kg [40, 41]. Glycerol is usually utilized in the preparation of liquid dosage forms as a solvent, thickening agent, lubricant, demulcent, sweetener, and humectant [42]. It is virtually non-toxic. Glycerol-containing pharmaceutical products are classified as "Generally Recognized as Safe" (GRAS) [42, 43].

Extemporaneous preparations should be esthetically appealing and efficacious, as well as fulfilling the quality requirements [44–46]. The poor palatability of extemporaneous formulations may have a negative impact on the patient compliance, and consequently the overall clinical outcomes. Sweetener in combination with flavoring agent was employed for improving patient adherence and providing safer and more palatable options for younger and elderly patients with dysphagia. Sorbitol is widely used as a low cariogenic and diabetic-friendly sweetener [47, 48]. The maximum daily intake of sorbitol in adults and neonates should be less than 20 g/day and 2 g/kg/week, respectively, to avoid gastrointestinal (GI) side effects [47, 48]. Red fruit (strawberry/raspberry) flavor is the most acceptable flavor in children [49]. Strawberry-flavored medications had the best tasting in comparison to the cherry-, bubble gum-, banana-, grape-, and peppermint-flavored formulations in children aged 3–12 years [50]. Accordingly, strawberry flavor was used in the preparation of bisoprolol-containing extemporaneous suspensions.

The selected extemporaneous formulation (F2) can be prepared easily in different types of pharmacy settings with a simple, natural, available, sugar-free excipients. It provides several advantages such as high safety, due to lack of possible harmful preservatives as parabens [51, 52]. It does not cause carcinogenicity problems due to the absence of

Table 3 pH, viscosity, sedimentation ratio, and resuspendability of the prepared extemporaneous formulations

Formulation	pH	V (mPa s)	Hμ (cm)	Ho (cm)	*F*	Resuspendability
F1	6.50	153	9.75	10	0.975	Easily resuspended
F2	6.70	217	10	10	1	No sedimentation
F2-unprocessed	6.80	205	NA	NA	NA	NA
F3	5.90	152	9.75	10	0.975	Easily resuspended
F4	6.33	157	9.70	10	0.970	Easily resuspended
F5	5.19	159	9.80	10	0.980	Easily resuspended
F6	5.32	145	9	10	0.900	Resuspended with difficulty
F7	5.20	164	6.50	10	0.650	Easily resuspended
F8	5.64	155	9.25	10	0.925	Easily resuspended

V viscosity, *Hμ* the ultimate height of the sediment as suspension settles in a cylinder, *Ho* the initial height of total suspension, *F* sedimentation ratio, and *NA* not applicable

sucrose [53]. Besides, it is appropriate additionally for diabetic patients. Furthermore, the absence of GI side effects was also demonstrated.

Chromatography

A stability indicating method was developed and validated to determine the selectivity of the method in stability and plasma samples in the presence of endogenous materials and/or degradation products. The UPLC method had a suitable level of precision, accuracy, and linearity. The calibration curves were linear with a correlation coefficient $R^2 > 0.999$ ($n = 6$) throughout the course of the assay (10–100 µg/mL and 1–100 ng/mL for stability and human plasma samples, respectively). The UPLC method was accurate and precise. Within-day percent coefficient of variation (%CV) ranged from 0.52 to 4.5% and 0.2 to 11.8%, while the between-day %CV ranged from 0.9 to 4.3% and 0.7 to 9.4% for stability and human plasma samples, respectively. Within-day accuracy ranged from 98.9 to 104.8% and 97.3 to 104.5%, while the between-day accuracy ranged from 97.7 to 105.1% and 96.2 to 103.8% for stability and human plasma samples, respectively. The retention time of bisoprolol and IS was 5.0 ± 0.25 min and 2.5 ± 0.1 min respectively. No peaks for any endogenous materials and/or degradation appeared at the same retention time for bisoprolol and IS indicating the selectivity of the assay method.

In vitro evaluation of extemporaneous suspensions

Appearance and pH measurement

All compounded extemporaneous formulations formulated from crushed Concor® tablets (5-mg bisoprolol fumarate) had a pink, milky color. However, the formulation (F2-unprocessed) prepared from pure bisoprolol was a clear liquid. Strawberry flavor along with sorbitol as a sweetener imparted pleasant organoleptic properties to the formulations. The resulting extemporaneous formulations were subjected to various assessments such as pH, viscosity, sedimentation ratio, and resuspendability measurements. Observations of these measurements were listed in Table 3. The pH values of all suspensions (F1–F8) were within the acceptable range (5–8) [54], ranged from 5.2 to 6.4, whereas the pH of the formulation (F2-unprocessed) was 6.8 (Table 3). The best pH values were noted in xanthan gum-containing extemporaneous formulations (F1, F2, F3, and F2-unprocessed) without the addition of any pH stabilizer. Xanthan gum is safe for oral utilization and supported by the regulatory authorities [38].

Viscosity measurement

The results showed that the viscosity of bisoprolol extemporaneous formulations was increased in the following order: F2 > F2-unprocessed > F7 > F5 > F4 > F8 > F1 > F3 > F6 (Table 3). Accordingly, xanthan gum-containing formulations (F2 and F2-unprocessed) possessed the most reasonable viscosity.

Sedimentation volume and resuspendability

The results indicated that the sedimentation ratios of xanthan gum-containing suspensions (F1, F2, F5) were 0.975, 1, and 0.98, respectively. F1 (0.1% w/v) and F5 (0.3% w/v) were easily resuspended; however, no sedimentation was occurred in case of F2 (0.5% w/v) during the test period (Table 3). Xanthan gum is often utilized as a flocculating agent to increase the homogeneity of suspension particles with no need to other adjuvants [21, 26]. The obtained results related to the loose and fluffy network of flocs that formed in the xanthan gum-containing suspension which can extend throughout the extra vehicle [55]. The increase in xanthan gum concentration in F2 (0.5% w/v) revealed a great flocculation and homogeneity of suspension compared to F1 and F5.

On the other hand, Carbopol-containing suspensions (F6 and F7) are considered pharmaceutically unacceptable. F6 (0.1% w/v Carbopol) had a sedimentation ratio of 0.9 and was resuspended with difficulty compared to other formulations. In case of F7 (0.5% w/v Carbopol), it was noticed that the increase in Carbopol concentration led to a reduction of the sedimentation ratio ($F = 0.65$); however, the suspension was resuspended easily by shaking (Table 3).

The other formulations which contain CMC (F3–F5) and sodium alginate (F8) had a sedimentation ratio equal to 0.975, 0.970, 0.980, and 0.925, respectively. Besides, they were easily resuspended by shaking. This difference in the sedimentation ratios might be attributed to the type of the suspending agent used, since CMC and sodium alginate might form homogenous network in all concentrations used [21, 56].

The overall appeal and uniformity of the suspension relies upon the sedimentation ratio and the ease of redispersion. Accordingly, F2 suspension containing 0.5% w/v xanthan gum was the best one that offered homogeneity without any sedimentation ($F = 1$) with ease of pouring compared to other formulations which were less viscous and homogenous than F2.

Dissolution studies

The dissolution pattern of bisoprolol from R and different extemporaneous suspensions are illustrated in Fig. 1. Models utilized to describe the release kinetics of bisoprolol were

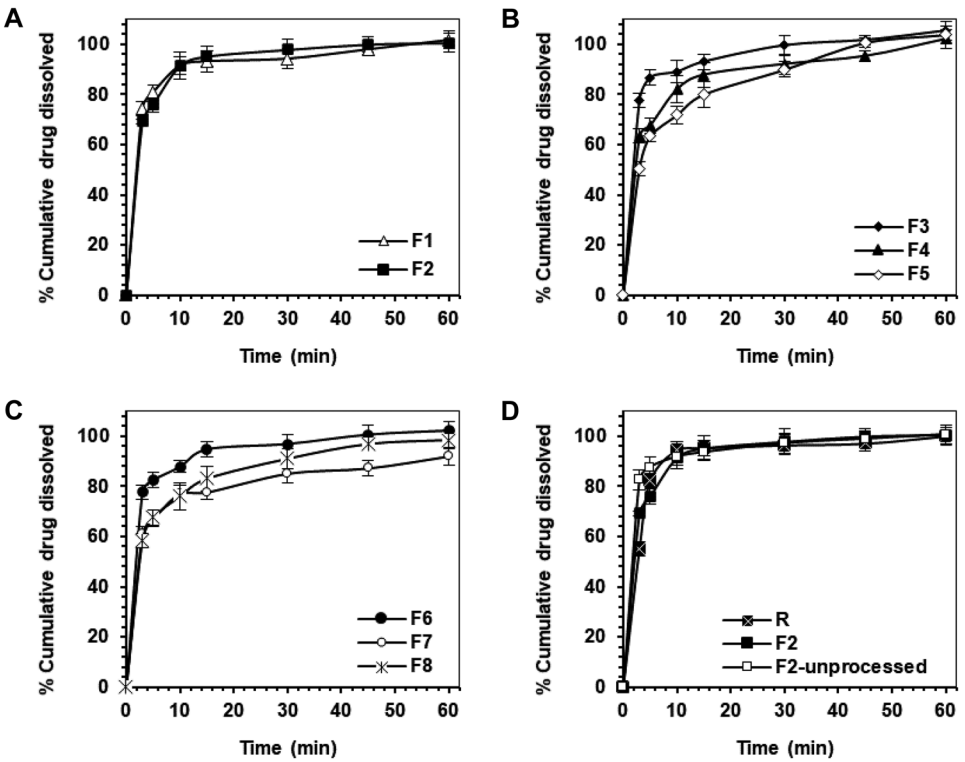

Fig. 1 Dissolution profiles of bisoprolol from R, different extemporaneous suspensions (F1-F8), and F2- unprocessed suspension (mean ± SE, $n = 3$)

zero-order, first-order [30], Higuchi kinetics [57], the cubic root law [58], Weibull distribution [29, 59], and the logistic model [29, 60, 61]. The derived parameters of model-dependent and model-independent in vitro characterization and other dissolution parameters are presented in Table 4.

The R tablet liberated 82.2% of its potency in the first 5 min (Q5) with complete bisoprolol dissolution after 60 min (Q60) (Table 4). This was concurred with the USP and FDA dissolution specification for Concor® tablets (R) where not less than 80% of bisoprolol should be dissolved within 20 min in water [62, 63]. Weibull distribution and logistic model can describe the type of dissolution profile and dissolution time as well [29]. The shape parameter (β) describes the shape of the dissolution profile, whereas the time interval necessary to dissolve 63.2% of the drug is represented by the time parameter (Td) [60]. Values for $\beta = 1$ indicates curve exponential, $\beta > 1$ shows a sigmoid curve, and $\beta < 1$ specifies to a parabolic curve with steeper initial slope that is consistent with the exponential [29, 60, 64, 65]. The dissolution data of R product was best fitted to the logistic model, while the second best fit was Weibull distribution. The β parameter of R product was > 1 (1.325), indicating a complex dissolution mechanism with a more pronounced sigmoid curve (S-shaped curve) with upward curvature followed by a turning point (Table 4). Also the first-order and Hixson-Crowell models fit gave the statistical parameters as being approximately the same as those of Weibull distribution for the R product (Table 4).

The effect of various types of suspending agents on the dissolution rate of bisoprolol was studied. All extemporaneous suspensions were obeyed logistic model and Weibull distribution, emphasizing the S-shaped dissolution profiles of all extemporaneous suspensions (Table 4). However, the preferred model that described drug release kinetics of the prepared suspensions was Weibull model. The β values calculated for all bisoprolol extemporaneous suspensions were < 1, which indicated parabolic curves with initial inflection (Table 4). The parabolic curves are common in suspensions that liberate the drug quickly in early times of dissolution and then remain at a constant dissolution [65, 66]. The Td values varied significantly (p<0.05) from one formulation to another with the recorded values being ranked as F5 > F7 > F8 > F4 > F3 > F6 > F2 > F1 (Table 4). However, the time necessary to dissolve 63.2% of bisoprolol from extemporaneous formulations F1 and F2 was shorter than the other suspensions (Table 4). The third best fit model was the first-order model in case of all extemporaneous suspensions, except F5, F7, and F8 followed by Higuchi kinetics (Table 4).

Xanthan gum was used as a suspending agent in concentration of 0.1% and 0.5% to prepare F1 and F2 suspensions, respectively. F1 and F2 achieved the best dissolution behavior among other extemporaneous suspensions (Fig. 1). The Q5, Q60, DE, and MDT values of F1 were 80.8%, 101.5%, 91.6%, and 4.86 min respectively (Table 4). A similar trend ($P > 0.5$) was noticed for the dissolution behavior of F2

Table 4 Model-dependent and model-independent in vitro characterization of bisoprolol extemporaneous suspensions

Model/parameter		R	F1	F2	F2-unprocessed	F3	F4	F5	F6	F7	F8
Zero-order (Z-O)	r^2	0.723	0.484	0.576	0.353	0.374	0.561	0.607	0.376	0.515	0.321
	k_0	11.35	11.51	11.21	11.96	11.61	10.05	8.850	11.36	9.620	7.010
First-order (F-O)	r^2	0.992	0.981	0.982	0.983	0.978	0.944	0.926	0.965	0.919	0.871
	k_1	0.290	0.39	0.33	0.519	0.450	0.250	0.18	0.420	0.230	0.210
Higuchi (H)	r^2	0.969	0.901	0.938	0.839	0.850	0.931	0.949	0.851	0.925	0.898
	k_H	32.12	33.28	32.17	34.94	33.87	28.88	25.34	33.13	27.76	24.67
Hixson-Crowell (H-C)	r^2	0.993	0.964	0.961	0.966	0.964	0.894	0.865	0.945	0.855	0.821
	k_{HC}	0.082	0.105	0.091	0.122	0.115	0.070	0.050	0.111	0.064	0.061
Weibull (W)	r^2	0.996	0.999	0.998	1	0.998	0.997	0.997	1	0.999	0.999
	α	5.192	1.259	1.678	0.794	0.931	1.695	2.303	0.907	1.605	1.784
	β	1.325	0.478	0.588	0.303	0.338	0.446	0.478	0.281	0.370	0.419
	T_d	3.467	1.918	2.413	1.824	2.966	3.265	5.725	2.730	4.189	3.983
Logistic (L)	r^2	0.999	0.999	0.996	1	0.998	0.996	0.997	1	0.999	0.999
	α	−2.469	−0.064	−0.507	0.760	0.447	−0.365	−0.774	0.585	−0.255	−0.459
	β	3.338	2.304	2.627	1.677	1.792	1.772	1.761	1.383	1.443	1.682
Best fit		L; W; H-C; F-O	L; W; F-O	L; W; F-O	L; W; F-O	L; W; F-O	L; W; F-O	L; W; H	L; W; F-O	L; W; H	L; W; H
Q5 (%)		82.2	80.8	76.0	87.6	84.7	67.5*	63.2*	82.44	67.0*	67.5*
Q60 (%)		100.0	101.5	100.5	100.5	105.3	102.0	103.7	102.1	92.0	98.4
DE		0.917	0.916	0.928	0.934	0.946	0.875*	0.856*[a]	0.932	0.804*	0.857*
MDT (min)		4.97	4.86	4.58	4.25	6.08*	8.47*	10.43*[a]	5.22	7.56*	7.69*

r2 determination coefficient, k_0 zero-order release rate constant ($mg\%.h^{-1}$), k_1 first-order release rate constant (h^{-1}), k_H Higuchi release rate constant ($mg.h^{-1/2}$), k_{HC} Hixson-Crowell release rate constant ($mg^{1/3}\%.h^{-1}$), α scale factor, β shape parameter, *Td* time at which 63.2% of the material is dissolved, *Q5* % of drug liberated in the first 5 min, *Q60* % of drug dissolved over 60 min, *DE* dissolution efficiency, *MDT* mean dissolution time

*Significant difference from R at $P < 0.05$

[a]Significant difference from F3 at $P < 0.05$

($f_2 = 74.5\%$) (Fig. 1 and Table 4) where the latter liberated 76.1% of bisoprolol after 5 min with 100% of the drug dissolved after 60 min. Moreover, a similar DE and MDT values ($P > 0.5$) of F2 (92.8% and 4.58 min) were observed in comparison to R and F1 (Table 4). F2 showed a high DE value (92.8%) concurred with the rapid dissolution rate observed. The dissolution rate of bisoprolol was not changed ($P > 0.5$) by increasing xanthan gum concentration (Table 4). Suspensions (F1 and F2) liberated bisoprolol at a rate similar to ($P > 0.05$) R product. This was further indicated from the values of the similarity factor which reflected the sameness of dissolution profiles of F1 and F2 and R. The values of f_2 were 57.4% and 62.1% for F1 and F2, respectively, confirming their similarity to R product. Xanthan gum is an effective flocculating agent at a relatively low concentration and has excellent suspending and wetting properties [21, 67]. Additionally, the rheological stability of xanthan gum toward pH changes is encountered during transit through the GI tract providing reasons for its use [21].

According to the logistic model, suspensions (F1 and F2) had the best results of the β (shape factor) parameter where the values were 2.304 and 2.627, respectively, compared to R (3.338), indicating that the dissolution profiles of F1 and F2 were similar to the profile of R. This was coincided with the previous results (Table 4). To sum up, F1 and F2 gave the best dissolution behavior among other extemporaneous suspensions; however, F2 had a higher viscosity (217 mPa s) compared to F1 (153 mPa s) (Table 3). Furthermore, F2 maintained its flocculated state and poured easily.

F2-unprocessed formulation was prepared using the pure powder of bisoprolol instead of crushed Concor® tablets. F2-unprocessed formulation was a clear viscous solution with a viscosity of 205 mPa s (Table 3). F2-unprocessed formulation liberated 86.7% of its potency in the first 5 min with complete bisoprolol dissolution after 60 min (Fig. 1d and Table 4). The DE and MDT values of F2-unprocessed formulation were 93.4% and 4.25 min, respectively, indicating the sameness between F2-unprocessed and F2 which was further confirmed with f_2 results ($f_2 = 59.76\%$) (Fig. 1d and Table 4).

CMC was used as a suspending agent in concentration of 0.1% and 0.5% to prepare F3–F5, respectively. Referring to the results of CMC-containing extemporaneous suspensions, the increase in the concentration of CMC resulted in a slower drug release. Extemporaneous suspensions with 0.1% CMC (F3) showed a comparable ($P > 0.05$) Q5 (84.7%) and DE (94.6%) to that of R, but the MDT (6.08 min) was increased significantly ($P < 0.05$) (Fig. 1 and Table 4). The similarity in the overall dissolution of F3 to R was further confirmed by the similarity factor test ($f_2 = 52.7\%$). Moreover, dissolution profile of F3 (0.1% CMC) was similar to that of F1 (0.1% xanthan gum) ($f_2 = 71.1\%$) where its aqueous dispersion has nearly the same viscosity (152 mPa s) and produced sediment layer that was easily redispersed by shaking (Table 3). In case of F4, the values of Q5, DE, and MDT were significantly changed compared to both R and F3. The increase in CMC from 0.1 (F3) to 0.5% (F4) resulted in a significant decrease in Q5 (67.5%), and the overall DE (87.5%). The increase in CMC concentration to 0.5% (w/v) (F4) resulted in the increase in viscosity of the F4 (157 mPa s) compared to F3 (152 mPa s) (Table 3). This was evidenced by the similarity factor test ($f_2 = 49.1\%$) and the significant increase in the MDT (8.47 min) (Fig. 1 and Table 4).

In case of F5, the addition of xanthan gum (0.3%) to CMC (0.1%) liberated bisoprolol at significantly lower rate compared to F3 which contains the same amount of CMC (0.1%). The combination of xanthan gum and CMC led to a slightly more viscous suspension (159 mPa s) which significantly ($P < 0.5$) reduced the dissolution rate of bisoprolol (Table 4). The Q5, DE, and MDT values of F5 were 63.2%, 85.6%, and 10.43 min, respectively, indicating the difference between F3 and F5 ($f_2 = 40.9\%$) (Fig. 1 and Table 4).

Another pattern was shown with Carbopol-containing suspensions (F6 and F7) (Fig. 1 and Table 4). In case of F6, a low concentration of Carbopol (0.1%) did not significantly change the dissolution parameters of bisoprolol ($P > 0.05$), compared to R product ($f_2 = 53.6\%$) (Fig. 1 and Table 4). However, the increase in Carbopol to 0.5% (w/v) (F7) retarded the dissolution rate of bisoprolol as indicated from the calculated dissolution parameters (Fig. 1 and Table 4). This was concurred with the viscosity results where F7 was more viscous suspension (164 mPa s) than F6 (145 mPa s). The Q5, Q60, DE, and MDT values were 67.0%, 92.0%. 80.4%, and 7.56 min, respectively (Fig. 1 and Table 4), indicating the difference between F6 and F7 ($f_2 = 44.4\%$).

Sodium alginate-containing extemporaneous suspension (F8) liberated 67.5% of bisoprolol in the first 5 min and > 98% after 60 min with the calculated DE and MDT being 85.7% and 7.69 min, respectively. This was further indicated from the similarity factor test ($f_2 = 49.1\%$) which reflected the difference of the dissolution profile of bisoprolol of F8 compared to R (Fig. 1 and Table 4). This could be attributed to the hydrated polymer surrounding bisoprolol particles which resulted in the formation of higher viscosity regions that encountered high resistance to the drug release [56].

Accordingly, from the dissolution, viscosity, and sedimentation results, the most satisfied and optimized extemporaneous suspension was xanthan gum-containing suspension (F2; 0.5%). The latter gave the best dissolution profile among all the extemporaneous formulations. It was an easily pourable viscous suspension with no sedimentation. Therefore, xanthan gum-containing extemporaneous suspension (F2) was used in the forced degradation and bioavailability studies.

Forced degradation studies

Forced degradation studies facilitate development, manufacturing, and packaging of pharmaceutical products [15]. A stress or accelerated stability study was done to study the impact of several environmental factors such as temperature, humidity, light, and oxidizing agents on the selected extemporaneous bisoprolol suspension (F2) as well as to predict its beyond-use date.

Chemical stability

Changes in temperature, moisture, and light have sometimes drastic effect on the stability of drugs [68]. Chemical interactions between drug and excipients may decrease the drug stability [69–71]. Therefore, stress testing should include the impact of variety of environmental factors such as temperature, humidity, light, and oxidizing agents as well as susceptibility across a wide range of pH values. The degradation behavior of bisoprolol under different stress conditions was studied by analyzing the tested samples at different time intervals.

For each stress condition, three chromatograms are shown in Fig. 2. Bisoprolol was quite stable in water for 6 months despite higher temperature, since UPLC chromatograms showed almost no change in peak area (Fig. 2a). Degradation of bisoprolol was more affected by 0.1 N HCl, 0.1 N NaOH, and 3% H_2O_2 (Fig. 2b and c). After exposure to long period of time, more than 15% and 25% of the bisoprolol were degraded by keeping it in 0.1 N HCl and 0.1 N NaOH for 72 h. Bisoprolol showed high degree of instability toward oxidation at room temperature (Fig. 2d). A significant decrease in bisoprolol concentration was obtained in 3% H_2O_2 (v/v) all over the period of the experiment (72 h). Drug degradation was clearly observed where more than 60% of the drug was decomposed by 3% H_2O_2 after 72 h. For photostability study, bisoprolol was stable in dark and light. The % degradation was 0.98% when suspension was subjected to light for 72 h. There were no changes in drug concentration or retention time in different samples kept in the dark or subjected to light for the period of the experiment (1 month). A similar result was reported and explained for the same drug [72, 73].

Physical stability

The results of changes in pH and viscosity of F2 stored for 6 months at 25, 45, and 4 °C are shown in Fig. 3. No apparent change in mean pH or viscosity occurred, regardless of whether they were stored at room temperature or in the refrigerator. The sedimentation, precipitation, and floating of suspensions were noted. No visible changes in color,

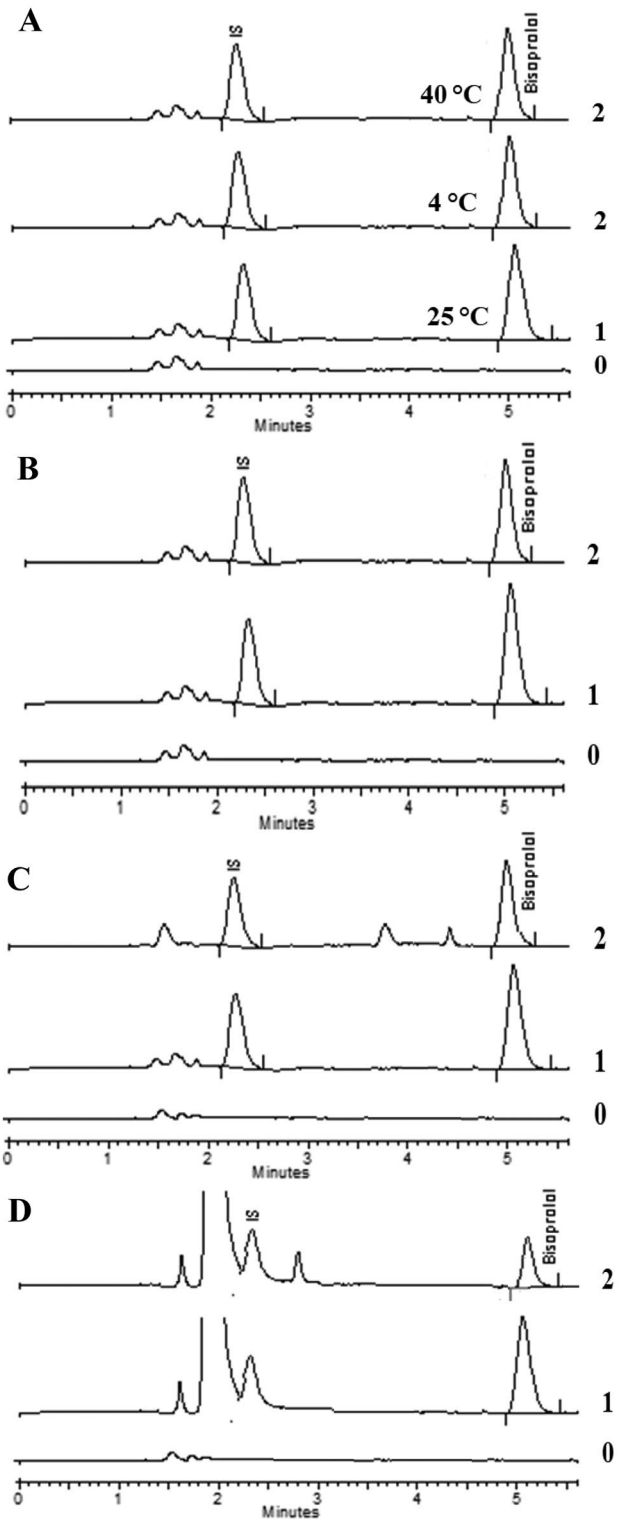

Fig. 2 Degradation of bisoprolol in **a** temperature (25 °C, 4 °C, and 40 °C); **b** 0.1 N HCl for 72 h; **c** 0.1 N NaOH for 72 h; and **d** 30% H_2O_2 for 72 h (0 is a blank sample; 1 is a sample without degradation in normal conditions of temperature and humidity (24 ± 2 °C and 50 ± 10%); and 2 is a sample under degradation conditions)

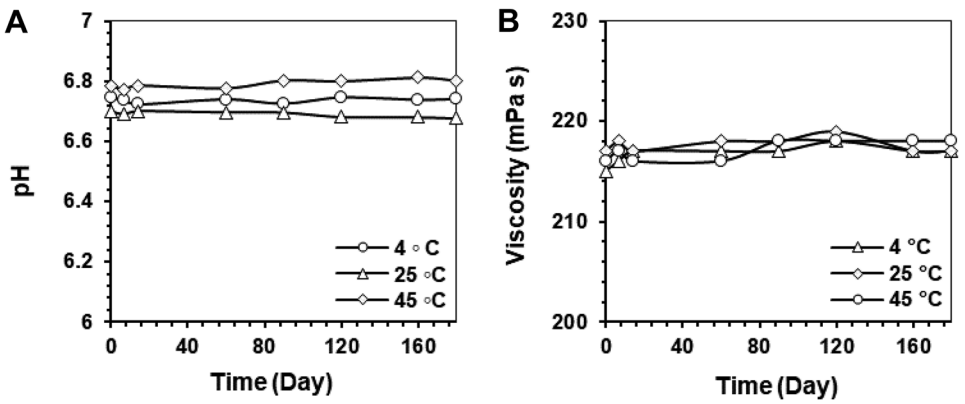

Fig. 3 Changes in **A** pH and **B** viscosity of the selected bisoprolol extemporaneous suspension (F2) stored for 6 months at 25, 45, and 4 °C

odor, taste, or smell were noticed during the study period. No sedimentation was noticed in F2 suspension throughout the study period, since sediment was prevented by the high viscosity value (217 mPa s) (Table 3).

Fourier transform infrared spectrophotometry (FT-IR)

FT-IR spectra of pure bisoprolol fumarate, xanthan gum, glycerol, F2 suspension prepared from commercial tablets, and pure bisoprolol are shown in Fig. 4. The pure bisoprolol fumarate spectrum showed several absorption bands (Fig. 4a). The peaks at 3505 and 3418 cm^{-1} were assigned for aromatic C-H stretching, while the peak at 2973 cm^{-1} was attributed to aliphatic C-H stretching vibration. A biforked absorption band for C = O stretching vibration was shown at 1612 cm^{-1}. The peak corresponding to the aromatic C = C stretching vibration was shown at 1550 cm^{-1}. The symmetric CH_3 and CH_2 bending vibrations were clear at 1350 and 1400 cm^{-1}. Peaks at 950–630 cm^{-1} revealed a bending vibration for skeletal vibration bands, out-of-plane aromatic C-H. Similar absorption bands for bisoprolol fumarate were reported previously [74].

FT-IR spectrum of xanthan gum revealed a vibration bands at 3413 cm^{-1} that was specified to OH group. Peaks at 1621 and 1407 cm^{-1} were specified for asymmetric– and

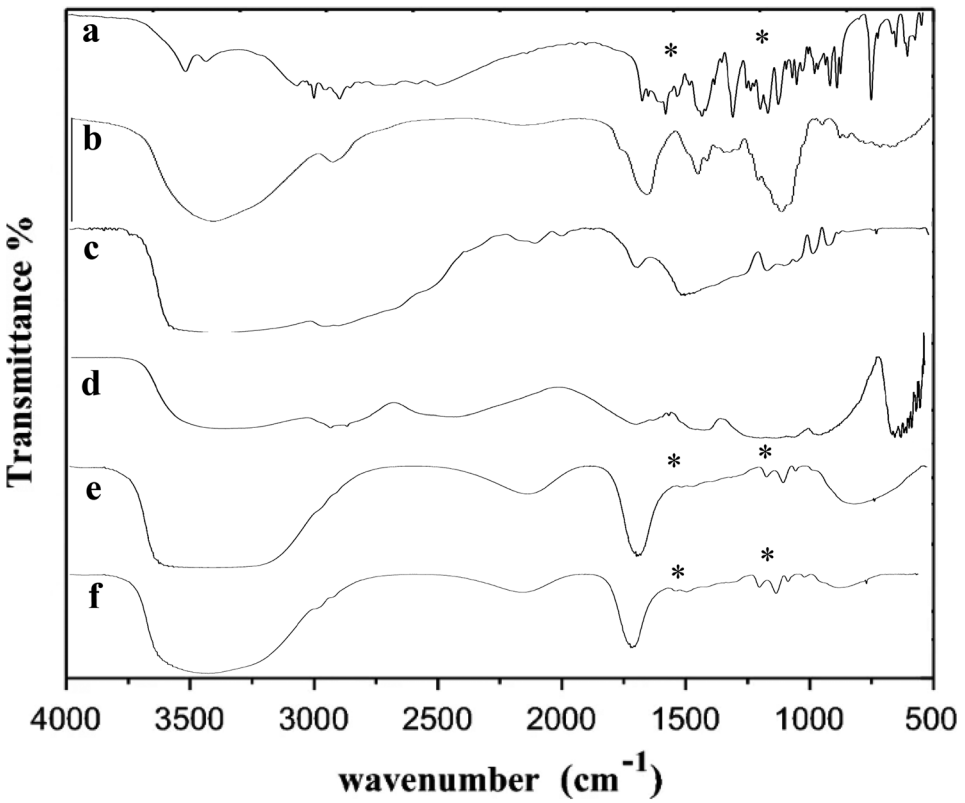

Fig. 4 FT-IR spectra of **a** pure bisoprolol fumarate, **b** xanthan gum, **c** glycerol, **d** Concor® tablet, **e** F2-unprocessed, and **f** F2

Table 5 Pharmacokinetic parameters of bisoprolol after a single oral dose administration of 10 mg to 8 healthy male volunteers

Parameters	Bisoprolol suspension (F2)	Bisoprolol tablet (R)	90% confidence interval, point estimate (lower limit–upper limit)
C_{max} (ng/mL)	52.3 ± 3.4	47.6 ± 2.6	0.954 (0.87–1.01)
t_{max} [a] (h)	1.5 (1.5–2.0)	2.5 (2.0–2.5)	
k_a (h^{-1})	2.2 ± 0.8	2.0 ± 0.9	
$AUC_{0\rightarrow t}$ (ng.h/mL)	354.5 ± 25.2	324.7 ± 20.8	0.98 (0.86–1.04)
$AUC_{0\rightarrow\infty}$ (ng.h/mL)	371.4 ± 26.3	344.8 ± 21.9	0.99 (0.87–1.0)
MRT (h)	7.8 ± 0.2	8.4 ± 0.3	
$t_{½}$ (h)	11.7 ± 0.9	12.7 ± 1.3	

Values are presented as mean ± SD

C_{max} peak plasma concentration, t_{max} time to reach peak plasma concentration, k_a absorption rate constant, $AUC_{0\text{-}t}$ area under the concentration–time curve from zero to the last measurable plasma concentration, $AUC_{0\text{-}\infty}$ area under the concentration–time curve from zero to infinity, *MRT* mean residence time, $t_{1/2}$ elimination half-life

[a]Median (minimum to maximum)

symmetric–COO–stretching vibrations of pyruvate and glucuronate groups, respectively, whereas the peak at 1060 cm^{-1} was related to stretching vibration of C–O–C group (Fig. 4b). Similar absorption bands concurred with those published earlier for the same polymer [74, 75] (Fig. 3b). In case of glycerol, the spectrum showed OH stretching frequency at 3391 cm^{-1}, while C-H stretching was due to the peak at 2935 cm^{-1} (Fig. 3c). Bending of the C-O–H group was revealed by the peak at 1454 cm^{-1} of the C-O stretching of the primary alcohol, which was shown at 1115 cm^{-1} (Fig. 3c). The absorption bands were similar to those published by Danish et al. [76]. Similar absorption bands were shown in the spectra of xanthan gum-containing extemporaneous suspensions prepared from crushed tablets (F2) and pure bisoprolol powder (F2-unprocessed), which clearly indicated that no interaction coexisted between bisoprolol and additives for both extemporaneous suspensions prepared from crushed tablets (F2) or pure drug powder (F2-unprocessed).

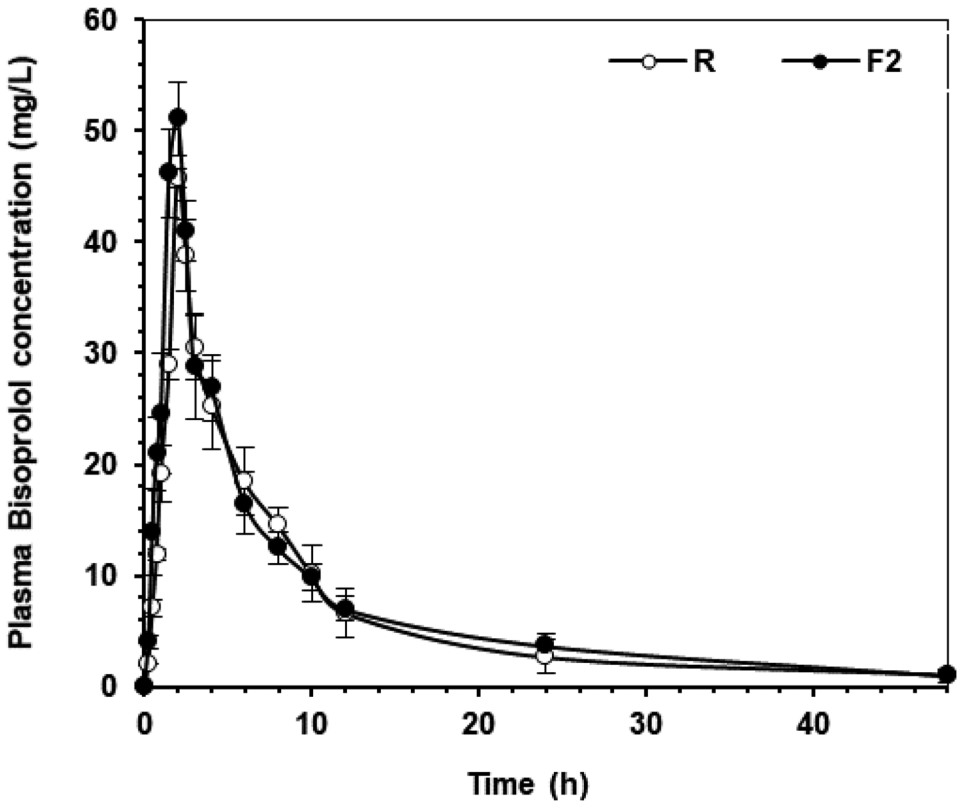

Fig. 5 Mean plasma concentration–time profiles of (○) R and (●) F2 after a single dose of 10-mg bisoprolol ($n = 8$, mean ± SE)

Bioavailability study

Based on the results of stability and dissolution studies, xanthan gum-containing extemporaneous suspension (F2) was used in the bioavailability studies. The absorption and therapeutic efficacy of a bisoprolol in a suspension compounded from crushed tablets is unlikely to differ from that of the commercial tablets used in its compounding. To confirm this, the bioavailability of the selected bisoprolol extemporaneous suspension (F2) was evaluated. The pharmacokinetic parameters obtained with R and F2 are shown in Table 5. Figure 5 shows the mean plasma concentration–time profiles of bisoprolol absorbed in humans. Two similar profiles were observed (Fig. 5). There was no significant difference in pharmacokinetic parameters between F2 and R (Table 5). The pharmacokinetic parameters were concurred with that published earlier [77]. The bioavailability of bisoprolol from F2 suspension was 107.7% compared to R tablets. The 90% CIs of the F2/R ratios for C_{max}, $AUC_{0\text{-}t}$, and $AUC_{0\text{-}\infty}$ of bisoprolol were within the acceptance range for bioequivalence.

Conclusion

This research may be of great help during development of appropriate extemporaneous formulation of bisoprolol fumarate. The simple preparation method could be utilized to draw up a Standard Operating Procedure (SOP) easy to use by different types of pharmacy settings. A stable patient-friendly oral liquid extemporaneous formulation of bisoprolol with a target concentration of 0.5 mg/mL was successfully prepared using commercial tablets as a source of active ingredient. Strawberry-flavored sugar-free suspension can be prepared easily in both community and hospital pharmacies with safe, simple, natural, and available excipients. Moreover, it is appropriate for patients with diabetic diseases without any cariogenicity or carcinogenicity problems. In our study, at least 98% of the initial concentration of bisoprolol remained throughout the 6-month study period in the selected suspension regardless of the storage conditions. No noticeable changes in color, odor, taste, or visible microbial growth were observed in any sample. The selected formulation (F2) was a well-distributed suspension after gentle shaking. Additionally, it was bioequivalent to the commercial tablet in terms of the rate and extent of absorption.

Author contribution All authors participate in the design, development of methodology, acquisition of data, analysis and interpretation of data, writing, and revision of the manuscript.

Availability of data and materials All data relevant to the study are included in the article.

Declarations

Ethics approval All described experiments, the use of human materials, and the research protocols were approved by the Ethics Committee of Damanhour University (no. 1022PT35F), and all the procedures were in accordance with Good Manufacturing Practice.

Consent to participate Not applicable.

Consent for publication All authors read and approved the final manuscript.

Competing interests The authors declare no competing interests.

References

1. Shah SS, Hall M, Goodman DM, et al. Off-label drug use in hospitalized children. Arch Pediatr Adolesc Med. 2007;161(3):282–90.
2. American Academy of Pediatrics. Off-Label use of drugs in children. Pediatrics 2014;133:563–567. https://pediatrics.aappublications.org/content/133/3/563. Accessed 15 Jul 2021.
3. Logrippo S, Ricci G, Sestili M, et al. Oral drug therapy in elderly with dysphagia: between a rock and a hard place! Clin Interv Aging. 2017;12:241–51.
4. Leopold G, Ungethüm W, Pabst J, et al. Pharmacodynamic profile of bisoprolol, a new beta 1-selective adrenoceptor antagonist. Br J Clin Pharmacol. 1986;22(3):293–300.
5. Rosenberg J. Gustafsson F Bisoprolol for congestive heart failure. Expert Opinion Pharmacother. 2008;9(2):293–300.
6. Patel AR, Shaddy RE. Role of β-blocker therapy in pediatric heart failure. Ped Health. 2010;4(1):45–58.
7. Alabed S, Sabouni A, Al Dakhoul S, et al. Beta-blockers for congestive heart failure in children. Cochrane Database Syst Rev. 2016;Issue 1. Art. No.: CD007037.
8. U.S. Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research (CDER). Guidance for industry: Waiver of in vivo bioavailability and bioequivalence studies for immediate-release solid oral dosage forms based on a Biopharmaceutics Classification System. 2017. https://www.gmp-compliance.org/files/guidemgr/UCM070246.pdf. Accessed 15 Jul 2021.
9. World Health Organization (WHO). Proposal to waive in vivo bioequivalence requirements for WHO Model List of Essential Medicines immediate release, solid oral dosage forms. Technical Report Series No. 937, 40th Report, Annex 8 of WHO Expert committee On Specifications for Pharmaceutical Preparations. 2006;391–437. https://www.gmp-compliance.org/files/guidemgr/WHO_TRS_937_annex8.pdf. Accessed 15 Jul 2021.
10. Committee for Medicinal Products for Human Use (CHMP). Guideline on the investigation of bioequivalence. 2010. http://www.ema.europa.eu/docs/enGB/documentlibrary/Scientificguideline/2010/01/WC500070039.pdf. Accessed 15 Jul 2021.
11. Ramirez E, Laosa O, Guerra P, et al. Acceptability and characteristics of 124 human bioequivalence studies with active substances classified according to the Biopharmaceutic Classification System. Br J Clin Pharmacol. 2010;70(5):694–702.
12. Haeusler G, Schliep HJ, Schelling P, et al. High beta 1-selectivity and favourable pharmacokinetics as the outstanding properties of bisoprolol. J Cardiovasc Pharmacol. 1986;8(Suppl 11):S2–15.
13. Leopold G, Pabst J, Ungethüm W, et al. Basic pharmacokinetics of bisoprolol, a new highly beta 1-selective adrenoceptor antagonist. J Clin Pharm. 1986;26(8):616–21.

14. Helmy SA. Tablet splitting: is it worthwhile? Highlight of 16 commonly used medications in the outpatient settings. J Manag Care Spec Pharm. 2015;21(1):76–68.
15. International Conference on Harmonization (ICH). Stability testing of new drug substances and products, Q1A(R2); ICH Harmonized Tripartite Guideline; Geneva, Switzerland. 2003. [Accessed 15th June 2021 at].
16. European Medicines Agency (EMA). Guideline on pharmaceutical development of medicines for pediatric use. 2013. https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-pharmaceutical-development-medicines-paediatric-use_en.pdf. Accessed 15 Jun 2021.
17. United States Pharmacopeia (USP). Stability considerations in dispensing practices 1191. Rockville MD. 2006.
18. Küpper TE, Schraut B, Rieke B, et al. Drugs and drug administration in extreme environments. J Travel Med. 2006;13(1):35–47.
19. Glass BD, Haywood A. Stability considerations in liquid dosage forms extemporaneously prepared from commercially available products. J Pharm Pharm Sci. 2006;9:398–426.
20. Skwierczynski C, Conroy S. How long does it take to administer oral medicines to children. Paediatr Perinat Drug Ther. 2008;8:145–9.
21. Yousif HS, Khalil YI. Some Variables Affecting the Formulation of Oral Loratadine Suspension. Iraqi J Pharm Sci. 2008;17(2):18–25.
22. U.S. Food and Drug Administration Centre for Drug Evaluation and Research (FDA). Guidance for Industry- Analytical Procedures and Methods Validation for Drugs and Biologics. Silver Spring, MD: Center for Drug Evaluation and Research, US Department for Health and Human Services. 2015. https://www.fda.gov/media/87801/download. Accessed 24 Jul 2021.
23. Lachman L, Lieberman H, Kanig J. The theory and practice of industrial pharmacy, LeaandFebiger, 3rd edn., 1986;chapter 16, 18: 480–488, 535.
24. Aulton ME. Powder flow. In: Aulton ME, Taylor KM, editors. Aulton's Pharmaceutics, The design and manufacture of medicines. 4th ed. China: Churchill Livingstone; 2013. p 187–99.
25. Martin A. Physical pharmacy, Leaand Febiger, Philadelphia, London, 4th ed., 2000; chapter 12, 18, 287: 477–486.
26. Tempio JS, Zatz JL. Flocculation effect of xanthan gum in pharmaceutical suspensions. Pharm Res. 1980;69:1209–14.
27. The United States Pharmacopeia and National Formulary USP 30–NF 25.; The United States Pharmacopeial Convention, Inc.: Rockville, MD. 2007.
28. Dissolution Methods Database. U.S. Food and Drug Administration Web site. 2016. https://www.fda.gov/drugs/drug-approvals-and-databases/dissolution-methods-database. Accessed 24 Jul 2021.
29. Yuksel N, Kanık AE, Baykara T. Comparison of in vitro dissolution profiles by ANOVA-based, model-dependent and - independent methods. Int J Pharm. 2000;209(1–2):57–67.
30. Costa P, Lobo JMS. Modeling and comparison of dissolution profiles. Eur J Pharm Sci. 2001;13(2):123–33.
31. Khan KA. The concept of dissolution efficiency. J Pharm Pharmacol. 1975;27(1):48–9.
32. Polli JE, Rekhi GS, Augsburger LL, et al. Methods to compare dissolution profiles and a rationale for wide dissolution specifications for metoprolol tartrate tablets. J Pharm Sci. 1997;86:690–700.
33. Zhang Y, Huo M, Zhou J, et al. DDSolver: an add-in program for modeling and comparison of drug dissolution profiles. AAPS J. 2010;12(3):263–71.
34. Cirri M, Maestrelli F, Mennini N, et al. Development of a stable oral pediatric solution of hydrochlorothiazide by the combined use of cyclodextrins and hydrophilic polymers. Int J Pharm. 2020;587:119692.
35. Cárdenas RHL, Cortés AAR, Argotte RR, et al. Investigation of dissolution profiles from suspensions containing benzoyl metronidazole using a statistical model with repeated measurements. Drug Dev Ind Pharm. 1994;20(6):1063–73.
36. Hedaya MA. Basic pharmacokinetics. In: Taylor and Francis, editor. Taylor and Francis group; 6000 Broken Sound Parkway NW, Suit 300, Boca Raton, FL 33487–2742, England. 2012.
37. Helmy SA, El-Bedaiwy HM. Simultaneous determination of paracetamol and methocarbamol in human plasma by HPLC using UV detection with time programming: Application to pharmacokinetic study. Drug Res. 2014;64(07):363–7.
38. Daly J, Tomlin J, Read NW. The effect of feeding xanthan gum on colonic function in man: correlation with in vitro determinants of bacterial breakdown. Br J Nutr. 1993;69(3):897–902.
39. Del Olmo A, Calzada J, Nuñez M. Benzoic acid and its derivatives as naturally occurring compounds in foods and as additives: Uses, exposure, and controversy. Crit Rev Food Sci Nutr. 2017;57(14):3084–103.
40. World Health Organization (WHO). Benzoic acid and sodium benzoate. 2000. https://www.who.int/ipcs/publications/cicad/cicad26_rev_1.pdf. Accessed 26 Jul 2021.
41. Lazarević K, Stojanović D, Rancić N. Estimated daily intake of benzoic acid through food additives in adult population of South East Serbia. Cent Eur J Public Health. 2011;19(4):228–31.
42. Eccles R, Mallefet P. Soothing properties of glycerol in cough syrups for acute cough due to common cold. Pharmacy (Basel). 2017;5(1):4. https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=SCOGS&sort=Sortsubstance&order=ASC&startrow=1&type=basic&search=glycerin. Accessed 26 Jul 2021.
43. Brion F, Nunn AJ, Rieutord A. Extemporaneous preparation of oral medicines for children in european hospitals. Acta Paediatr. 2003;92(4):486–90.
44. Kairuz TE, Gaugiulo D, Bunt C, et al. Quality, safety and efficacy in the off-label use of medicines. Cur Drug Safety. 2007;2:89–95.
45. Nahata MC, Allen LV. Extemporaneous drug formulations. Clin Therap. 2008;30(11):2112–9.
46. Whittaker A, Currie AE, Turner MA, et al. Toxic additives in medication for preterm infants. Arch Dis Child Fetal Neonatal Ed. 2009;94:F236–40.
47. Birkhed D, Edwardsson S, Kalfas S, et al. Cariogenicity of sorbitol. Swed Dent J. 1984;8(3):147–54.
48. Thompson A, Reader S, Field E, et al. Open-label taste-testing study to evaluate the acceptability of both strawberry-flavored and orange-flavored amylmetacresol /2,4-dichlorobenzyl alcohol throat lozenges in healthy children. Drugs R D. 2013;13:101–7.
49. Angelilli ML, Toscani M, Matsui DM, et al. Palatability of oral antibiotics among children in an urban primary care center. Arch Pediatr Adolesc Med. 2000;154(3):267–70.
50. Tolia V, Han C, North JD, et al. Taste comparisons for lansoprazole strawberry-flavored delayed-release orally disintegrating tablet and ranitidine peppermint-flavored syrup in children. Clin Drug Invest. 2005;25(5):285–92.
51. Nowak K, Ratajczak-Wrona W, Górska M, et al. Parabens and their effects on the endocrine system. Mol Cell Endocrinol. 2018;474:238–51.
52. Paes Leme AF, Koo H, Bellato CM, et al. The role of sucrose in cariogenic dental biofilm formation—new insight. J Dent Res. 2006;85(10):878–87.
53. Jones DS. Pharmaceutics - dosage form and design. London: Pharmaceutical Press; 2008.
54. Liu Z, Li J, Nie S, et al. Study of an alginate/HPMC-based in situ gelling ophthalmic delivery system for gatifloxacin. Int J Pharm. 2006;315:12–7.
55. Shah MB, Sheth BB. Effect of polymers on dissolution from drug suspension. J Pharm Sci. 1979;65(11):1618–23.
56. Gibaldi M, Feldman S. Establishment of sink conditions in dissolution rate determinations. Theoretical considerations and

application to non-disintegrating dosage forms. J Pharm Sci. 1967;56:1238–1242.
57. Higuchi T. Mechanism of sustained-action medication: theoretical analysis of rate of release of solid drug dispersed in solid matrices. J Pharm Sci. 1963;52:1145–9.
58. Hixson AW, Crowell JH. Dependence of reaction velocity upon surface and agitation I—theoretical consideration. Ind Eng Chem. 1931;23:923–31.
59. Langenbucher F. Linearization of dissolution rate curves by the Weibull distribution. J Pharm Pharmacol. 1972;24:979–81.
60. Rawlings JO. Applied regression analysis: a research Tool. 2nd ed. Belmont, CA: Wadsworth; 1988.
61. United States Pharmacopoeia and National Formulary (USP 36-NF 31). The United States Pharmacopoeial Convention Inc, 12601 Twinbrook Parkway. Rockville, Maryland. 2013;20852:5825.
62. Charoo NA, Shamsher AAA, Lian LY, et al. Biowaiver monograph for immediate-release solid oral dosage forms: bisoprolol fumarate. J Pharm Sci. 2014;103:378–91.
63. Adams E, Coomans D, Smeyers-Verbeke J, et al. Nonlinear mixed effects models for the evaluation of dissolution profiles. Int J Pharm. 2002;240(1–2):37–53.
64. Papadopouloi V, Kosmidis K, Vlachou M, et al. On the use of the Weibull function for the discernment of drug release mechanisms. Int J Pharm. 2006;309(1–2):44–50.
65. da Silva AS, da Rosa Silva CE, Paula FR, et al. Discriminative Dissolution Method for Benzoyl Metronidazole Oral Suspension. AAPS PharmSciTech. 2016;17(3):778–86.
66. Aulton ME. Pharmaceutics, The science of dosage form design, Chirchill Livingston, London, Newyork, 2nd ed., 2002;chapter 23:334.
67. Naveed S, Basheer S, Qamar F. Stability of a dosage form and forced degradation studies. J Bioequiv Availab. 2016;8(4):191–3.
68. Singh S, Junwal M, Modhe G, et al. Forced degradation studies to assess the stability of drugs and products. TrAC, Trends Anal Chem. 2013;49:71–88.
69. Brümmer DH. How to approach a forced degradation study. Technical Bulletin. Life Sci. 2011;31.
70. Singh R, Rehman ZU. Current trends in forced degradation study for pharmaceutical product development. J Pharm Edu Res. 2012;3:54.
71. Vujanović IK, Jelić D, Antunović V, et al. Stability study of amlodipine besylate and bisoprolol fumarate in aqueous solutions. Contemporary Mater. 2014;V−2:212–221.
72. Kurbanoglu S, San Miguel PR, Uslu B, et al. Stability-indicating UPLC method for the determination of bisoprolol fumarate and hydrochlorothiazide: application to dosage forms and biological sample. Chromatographia. 2014;77:365–71.
73. Jagdale SC, Bari NA, Kuchekar BS, et al. optimization studies on compression coated floating-pulsatile drug delivery of bisoprolol. BioMed Res Int. 2013;Article ID 801769:11.
74. Bhat V, Shivakumar HR, Sheshappa RK, et al. Preparation and study on miscibility, thermal behavior of biocompatible polymer blends of xanthan Gum-polyacrylamide. Int J Plast Technol. 2014;18:183–91.
75. El-Masry SM, Helmy SA. Hydrogel-based matrices for controlled drug delivery of etamsylate: prediction of in-vivo plasma profiles. Saudi Pharm J. 2020;28:1704–18.
76. Danish M, Mumtaz MW, Fakhar M, et al. Response surface methodology based optimized purification of the residual glycerol from biodiesel production process. Chiang Mai J Sci. 2016;43(X):1–13.
77. Tjandrawinata RR, Setiawati E, Yunaidi AD, et al. Bioequivalence study of two formulations of bisoprolol fumarate film-coated tablets in healthy subjects. Drug Des Devel Ther. 2012;6:311–6.