Journal of Pharmaceutical Innovation (2026) 21:541
https://doi.org/10.1007/s12247-026-10747-z

RESEARCH

Improved Bioavailability of Spironolactone–Hydroxypropyl-β-Cyclodextrin Complex Tablets Evaluated in Rat Model

Esra Demirturk[1] · Gulsum Gundogdu[2] · Meltem Cetin[3] · Erem Bilensoy[4]

Received: 10 February 2026 / Accepted: 10 May 2026
© The Author(s) 2026

Abstract

Purpose Spironolactone (SPL) is a mineralocorticoid receptor antagonist widely used in the management of hypertension and heart failure; however, its poor aqueous solubility and variable oral bioavailability limit its therapeutic performance. This study aimed not only to enhance the solubility and dissolution behavior of SPL through Hp-βCD complexation, but also to translate this approach into a scalable tablet dosage form and evaluate its in vitro and in vivo performance.

Methods An Hp-βCD: SPL inclusion complex (1:1 molar ratio) was prepared using the kneading method. The solid-state characteristics and complex formation were evaluated by Fourier transform infrared spectroscopy (FTIR), powder X-ray diffraction (PXRD), and differential scanning calorimetry (DSC). Solubility studies were conducted in acidic medium (0.1 N HCl, pH 1.2, 0.1% SLS). Tablets containing either pure SPL or the Hp-βCD: SPL complex were manufactured by direct compression and evaluated for flow properties, mechanical strength, and disintegration. In vitro dissolution testing and in vivo pharmacokinetic studies in rats were performed to assess drug release and oral bioavailability.

Results Spectroscopic and thermal analyses confirmed successful inclusion complex formation and transformation of SPL from a crystalline to an amorphous state. The Hp-βCD: SPL complex exhibited approximately a 6–7-fold increase in SPL solubility compared to pure drug. Dissolution studies demonstrated markedly enhanced drug release from Hp-βCD: SPL tablets, achieving 75% release within 10 min and 95% within 30 min, compared with 35% and 76%, respectively, for conventional SPL tablets. Pharmacokinetic evaluation revealed significantly higher plasma SPL concentrations, increased C_max, and elevated $AUC_{0-\infty}$ values for Hp-βCD: SPL tablets, indicating improved absorption and relative bioavailability.

Conclusion Inclusion complexation of spironolactone with Hp-βCD represents a simple yet effective formulation innovation to overcome solubility- and dissolution-related limitations of SPL. The developed Hp-βCD: SPL tablet formulation significantly improves oral bioavailability and offers a promising approach for achieving more consistent therapeutic outcomes in BCS Class II drugs.

✉ Esra Demirturk
demirturkesra@hotmail.com

[1] Faculty of Pharmacy, Department of Pharmaceutical Technology, Çukurova University, Adana, Turkey

[2] Faculty of Science, Department of Energy Science and Technology, Turkish-German University, Istanbul, Turkey

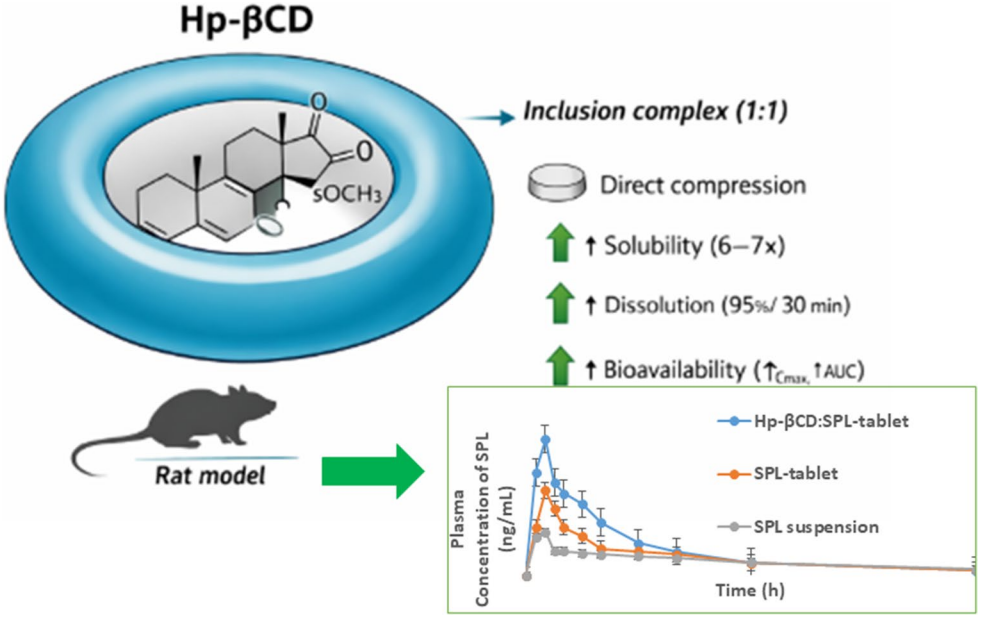
[3] Faculty of Pharmacy, Department of Pharmaceutical Technology, Atatürk University, Erzurum, Turkey

[4] Faculty of Pharmacy, Department of Pharmaceutical Technology, Hacettepe University, Ankara 06100, Turkey

Published online: 22 May 2026

Graphical Abstract

Hp-βCD inclusion complexation transforms crystalline spironolactone into an amorphous form, enhancing dissolution and oral bioavailability



Keywords Spironolactone · Hydroxypropyl-β-cyclodextrin · Inclusion complex · Solubility enhancement · Dissolution · Bioavailability · Pharmacokinetics

Introduction

Spironolactone (SPL; 7α-(acetylthio)−17α-hydroxy-3-oxopregn-4-ene-21-carboxylic acid γ-lactone) is a potassium-sparing diuretic with antihypertensive and anti-androgenic properties [1, 2]. Acting primarily as an aldosterone antagonist, SPL and its metabolites—canrenone and 7-α-thiomethyl-spironolactone—bind to mineralocorticoid receptors in the distal renal tubules, promoting potassium retention and enhancing sodium and water excretion [3, 4]. Clinically, SPL is indicated for conditions including hypertension, congestive heart failure, ascites, primary hyperaldosteronism, peripheral edema, and resistant hypertension, which is defined as uncontrolled blood pressure despite the use of three or more antihypertensive agents, including a diuretic [5, 6]. SPL has demonstrated notable efficacy in patients with resistant hypertension inadequately managed by standard therapy [6].

According to the Biopharmaceutics Classification System (BCS), SPL is a Class II compound, exhibiting low aqueous solubility (~ 27 µg/mL) and high intestinal permeability [1, 7]. Consequently, its oral absorption is dissolution-limited, leading to variable and incomplete bioavailability. SPL is lipophilic (logP ≈ 3), with limited pH-dependent solubility and susceptibility to hydrolytic degradation in gastrointestinal fluids, rapidly converting to active metabolites, which contributes to inter-brand and batch-to-batch variability in systemic exposure [8]. Commercially, SPL is available as tablets (25–100 mg) and suspensions (25 mg/5 mL), yet differences in dissolution and absorption among products underscore the need for more consistent formulations [1, 7]. Following oral administration, SPL is rapidly absorbed but extensively metabolized via first-pass effects, resulting in a short elimination half-life (~ 1.3 h), whereas its primary metabolite, canrenone, has a longer half-life (16–18 h) and sustains pharmacological activity [8, 9]. Both SPL and its metabolites are highly protein-bound (> 90%) and widely distributed due to their lipophilicity [1].

To overcome solubility-limited absorption, several formulation strategies—including particle size reduction, solid dispersions, lipid-based systems, and complexation—have been explored to improve SPL bioavailability [7]. Cyclodextrins (CDs), cyclic oligosaccharides derived from starch, are particularly effective for enhancing the oral bioavailability of poorly water-soluble drugs [10–13]. Because of their special structure, which has a hydrophilic exterior and a hydrophobic interior cavity, inclusion complexes that improve apparent solubility and dissolution can develop [14, 15]. Specifically, hydroxypropyl-β-cyclodextrin (Hp-βCD) has a well-established safety profile, enhanced

complexation efficiency, and excellent aqueous solubility [16, 17]. Although previous studies have reported cyclodextrin-mediated solubility enhancement of spironolactone, including Hp-βCD-based systems, these studies mainly focused on solution formulations, physicochemical characterization, or isolated pharmacokinetic observations. Limited information is available regarding the preservation of inclusion complex characteristics after incorporation into solid tablet matrices and the mechanistic relationship between solid-state modification, dissolution enhancement, and in vivo oral absorption. Furthermore, the translational applicability of Hp-βCD-based spironolactone systems to scalable tablet formulations remains insufficiently explored.

Previous studies have demonstrated the utility of CD complexes in enhancing SPL solubility and bioavailability. For instance, β-CD: SPL and γ-CD: SPL solid-state complexes improved water solubility up to 8- and 17-fold, respectively, with γ-CD: SPL exhibiting the fastest dissolution and highest plasma levels [18, 19]. Clinical pharmacokinetic evaluations also confirmed that β-CD complexation increased SPL's relative bioavailability to 233% compared to micronized powder [18]. Despite these advances, Hp-βCD-based SPL complexes formulated as tablets and systematically compared to conventional tablets and suspensions remain limited.

In the present study, SPL–Hp-βCD inclusion complexes were prepared and characterized in vitro, followed by formulation into tablets. These tablets were compared with conventional SPL tablets and in-house-prepared suspensions to assess the effects of Hp-βCD complexation on dissolution, absorption, and overall pharmacokinetic performance. Accordingly, the present study was designed to establish a comprehensive formulation-development pathway, integrating inclusion complexation, solid-state characterization, tablet formulation, dissolution evaluation, and in vivo pharmacokinetic assessment. The aim of this study was not only to improve the solubility and dissolution behavior of spironolactone through Hp-βCD complexation, but also to translate this approach into a pharmaceutically applicable tablet dosage form and evaluate whether the improved in vitro performance leads to enhanced in vivo bioavailability [8, 9, 20–22].

Although cyclodextrin-based complexation approaches for spironolactone have previously been reported, the available studies mainly focused on physicochemical characterization or solution systems, with limited emphasis on translating these complexes into solid oral dosage forms. In particular, studies investigating Hp-βCD-based spironolactone inclusion complexes formulated as tablets and supported by in vivo pharmacokinetic evaluation remain extremely limited. Therefore, the present study aimed to develop and characterize a scalable Hp-βCD: SPL tablet formulation and evaluate its in vitro and in vivo performance.

Materials and Methods

Materials

SPL and Hp-βCD were obtained from Sigma-Aldrich (USA). Sodium lauryl sulfate (SLS) was purchased from Merck (Germany). Microcrystalline cellulose, lactose, and crospovidone were kindly provided as gift samples by İlko (Turkey). All other chemicals and reagents used in this study were of analytical grade and used as received without further purification.

Methods

Preparation of Hp-βCD: SPL Inclusion Complex

Hp-βCD: SPL inclusion complex was prepared using a kneading method (Salustio PJ, 2006). SPL and Hp-βCD (molar ratio 1:1) were transferred to a mortar and a small amount of warm water was added to the powder mixture to obtain a viscous slurry. Then, it was left to knead for 45 min at 45 °C. The dried matter was then ground to powder and sieved using sieve no. 80. Resulting complex was stored in airtight containers for further studies.

Although various molar ratios (e.g., 1:2 or 1:3 drug-to-cyclodextrin) are commonly explored in cyclodextrin complexation studies, a 1:1 ratio was selected in the present work for several reasons. First, phase-solubility studies reported in the literature for spironolactone and β-cyclodextrin derivatives typically demonstrate an A < sub> L-type profile, indicating the predominant formation of a 1:1 stoichiometric inclusion complex [23, 24]. Therefore, higher Hp-βCD ratios are not expected to yield additional thermodynamically stable complexes. For these reasons, and to maintain formulation efficiency and reproducibility, only the 1:1 molar ratio was investigated in this study.

Characterization of Hp-βCD: SPL Inclusion Complex

Structural, chemical and thermal analyses of the pure SPL, Hp-βCD, Hp-βCD: SPL physical mixture and Hp-βCD: SPL inclusion complex were performed using X-ray powder diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR) and Differential Scanning Calorimetry (DSC), respectively. XRD analysis was performed with a Rigaku Ultima-IV diffractometer employing Cu Kα radiation (λ = 1.5418 Å) in Bragg–Brentano geometry. Diffraction

patterns were recorded over a 2θ range of 3–50° at a scan rate of 0.02° (2θ)/min.

FTIR spectra were acquired using a Shimadzu IRTracerTM-100 spectrometer within the wavenumber range of 4000–400 cm^{-1}. The thermal properties of the samples were investigated using a differential scanning calorimeter (DSC, TA Instruments DSC250) under a continuous flow of nitrogen gas. Samples were heated from 20 °C to 350 °C at a controlled rate of 10 °C per minute. The instrument's temperature was calibrated to ensure an accuracy of ±0,1 °C throughout the measurements.

The solubility and in vitro dissolution samples of SPL were quantitatively analyzed using a validated UV–visible spectrophotometric method. The method was optimized to ensure accurate and reproducible determination of SPL concentrations in various dissolution and solubility media. The absorbance measurements were performed using a double-beam UV–visible spectrophotometer equipped with 1 cm quartz cells. The wavelength of maximum absorbance (λ_{max}) for SPL was determined to be 238 nm, and all analyses were carried out at this wavelength. Calibration curves were constructed by preparing a series of standard SPL solutions in the respective media within the concentration range of 2–20 µg/mL, showing good linearity with correlation coefficients (R^2) exceeding 0.999. For solubility studies, saturated solutions of pure SPL and the Hp-βCD: SPL inclusion complex were prepared in different media (0.1 N HCl pH 1.2; phosphate buffer (PB) pH 4.5; PB pH 6.8; and 0.1 N HCl pH 1.2 containing 0.1% SLS). After equilibrium, the samples were filtered through 0.45 µm membrane filters and suitably diluted before measurement. The method was validated in accordance with ICH Q2(R2) guidelines for parameters including linearity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ), selectivity, and stability. The low LOD and LOQ values confirmed the method's sensitivity, while the recovery results (98–102%) indicated good accuracy and reliability for quantitative analysis of SPL in dissolution and solubility samples [25].

Tablets Containing SPL or Hp-βCD: SPL Inclusion Complex

The compositions of tablets containing SPL (SPL-tablets) or Hp-βCD: SPL inclusion complex (Hp-βCD: SPL-tablets) presented in Table 1. We prepared the tablets using direct compression method. First, SPL (25 mg) or Hp-βCD: SPL inclusion complex (100 mg; equivalent to 25 mg SPL) and the excipients (microcrystalline cellulose and lactose as diluents, and crospovidone as a disintegrant) were mixed. Next, the powder mixture was blended with magnesium stearate as a lubricant. Then, we evaluated its flow properties before tablet compression.

Table 1 The compositions of tablets containing SPL or Hp-βCD: SPL inclusion complex

Ingredients	SPL tablets (mg)	Hp-βCD: SPL tablets (mg)
SPL	25	-
Hp-βCD: SPL inclusion complex	-	100*
Microcrystalline cellulose	150	110
Lactose	110	75
Crospovidone	12	12
Magnesium stearate	3	3

SPL Spironolactone, *Hp-βCD* Hdroxypropyl-β-cyclodextrin, *SPL-tablets* Tablets containing SPL, *Hp-βCD* SPL-tablets: Tablets containing Hp-βCD: SPL inclusion complex. *Equivalent to 25 mg SPL

The flow properties of the powder blends were evaluated by determining angle of repose, bulk density, tapped density, Carr's index, and Hausner ratio in accordance with USP<1174>and European Pharmacopoeia 2.9.34. The angle of repose was measured using a fixed funnel method. The funnel was positioned at a height of 5 cm above the flat surface and had an orifice diameter of 10 mm. Powder was allowed to flow freely through the funnel to form a cone, and the angle of repose was calculated from the height and radius of the powder heap. Bulk density was determined by gently filling a graduated cylinder with a known mass of powder, while tapped density was measured after 500 taps using a standardized tapping apparatus. Carr's index and Hausner ratio (HR= Tapped density/bulk density) were calculated from bulk and tapped density values and compressibility index [CI= ((tapped density-bulk density)/tapped density)x100] were determined. Flowability was interpreted according to USP<1174>classification criteria.

Evaluation of Tablets Containing SPL or Hp-βCD: SPL Inclusion Complex

For quality control of the prepared tablets, disintegration time, friability, crushing strength, diameter, thickness, weight uniformity, and dissolution were evaluated. The diameter and thickness of tablets ($n=10$) were measured using a digital tablet hardness tester (Erweka TBH 125, Germany). The resistance to crushing (hardness) of tablets ($n=10$) was determined using a tablet hardness tester (Erweka TBH 125, Germany). The friability of the tablets ($n=20$) was determined using a friabilator (Erweka TAR 220, Germany) operated at 25 rpm for 4 min. For the disintegration test, one tablet was placed in each of the six tubes of the basket, and the apparatus (Erweka ZT 220, Germany) was operated using 1000 mL of distilled water maintained at 37±1 °C as the immersion medium. The complete disintegration times of the tablets ($n=6$) were recorded.

Furthermore, dissolution test for tablets was performed. We performed the dissolution studies for pure SPL, Hp-βCD: SPL inclusion complex, and the tablets in 0.1 N HCl pH 1.2 with 0.1% SLS (1000 mL) kept at 37 ± 0.5 °C using the USP paddle apparatus (at paddle speeds 75 rpm) [26]. 10 mL of the sample was removed at regular intervals and replaced with fresh dissolution medium of the same temperature and volume (to maintain Sink Condition). These samples were filtered through a 0.45 µm pore-size filter and analyzed spectrophotometrically. For in vitro dissolution studies, aliquots withdrawn at predetermined time intervals were filtered and analyzed spectrophotometrically at 238 nm against corresponding blank dissolution media. The cumulative percentage of drug released was calculated using the calibration equation obtained for the medium.

In Vivo Studies

The pharmacokinetic investigation was approved by the Çukurova University Local Ethics Committee for Animal Experiments (permission date and number: 26.09.2024/9). Male albino Wistar rats (250–300 g, 10 months of age) were procured from the Medical Experimental Research and Application Center of Çukurova University. Experiments were conducted at the Department of Pharmacology, Faculty of Medicine, under standardized laboratory settings.

The rats were randomly assigned to three experimental groups (n=8 per group). To minimize the potential effect of food on drug absorption, animals were fasted overnight prior to administration, with free access to water. All formulations were administered under fasting conditions, and food was returned 30 min post-dosing. SPL suspension (prepared by dispersing SPL powder in 0.5% sodium carboxymethyl cellulose solution), SPL tablets, and Hp-βCD: SPL tablets, each dispersed in 0.5% sodium carboxymethyl cellulose solution prior to administration, were orally administered to rats via gavage at a dose of 25 mg/kg (based on SPL). Blood samples (~300 µL) were collected from each animal at predetermined time points (0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 h) via a polyethylene (PE-50) cannula inserted into the femoral vein. Samples were collected into heparinized microcentrifuge tubes and centrifuged at 5000 rpm for 5 min. The plasma was isolated and stored at −80 °C for further examination.

HPLC Method

This study employed a modified HPLC–UV method, initially established by Chaure et al., for the quantitative analysis of SPL in rat plasma samples within in vivo pharmacokinetic investigations [27]. The method underwent comprehensive validation in alignment with ICH Q2(R2) guidelines, evaluating parameters such as selectivity, linearity, precision, accuracy, and sensitivity. Table 2 summarizes the optimized chromatographic conditions. The analysis utilized a Shimadzu Nexera 2 system (Japan) with a diode array detector (PDA/DAD) calibrated to 240 nm. Separation was performed using an ODS-4 C18 column (4.6 × 250 mm, 5 µm; GL Sciences) at a constant temperature of 25 °C. A sample volume of 20 µL was injected for each run. Under these chromatographic conditions, well-defined and symmetrical peaks with satisfactory retention and resolution were obtained, demonstrating high reproducibility and sensitivity for the quantitative determination of SPL in rat plasma. A working solution of SPL (1 µg/mL) was prepared by diluting the SPL stock solution (100 µg/mL in acetonitrile) with the mobile phase for sample preparation. Calibration standards were produced by spiking blank rat plasma, devoid of SPL, with specified volumes of the working solution to attain final concentrations between 100 and 1000 ng/mL. Calibration curves were developed by plotting SPL concentrations against the corresponding peak area ratios of SPL, followed by linear regression analysis utilizing the least-squares method (n = 6). A dilution factor of 1:10 was utilized to guarantee that all measurements remained within the linear dynamic range.

Table 2 Optimum HPLC conditions for the analysis of SPL

Instrument:	Shimadzu Nexera 2 (Japan)
Wavelength	240 nm
Detector	Diode array detector (PDA/DAD)
Column	ODS-4 C18 (4,6 × 250 mm,5 µm, GL Sciences)
Mobile Phase	Methanol: Ultra pure water (70:30)
Flow rate	1 mL/min
Injection volume	20 µL
Temperature	25 °C

The regression parameters, comprising slope, intercept, and coefficient of determination (R^2), were established. The method's selectivity was assessed by comparing chromatograms of blank plasma to those of plasma samples spiked with SPL at concentrations of 100, 120, 140, 160, and 250 ng/mL, with six replicates for each concentration. An internal standard was unnecessary due to the consistent sample injection and detector response observed throughout all runs. This study quantified only the parent compound, SPL, excluding its metabolites from the analysis. This methodological choice was made to prioritize the bioavailability and plasma levels of the unchanged drug, as a comprehensive metabolite analysis would necessitate further sample preparation and analytical techniques.

Additionally, LOD (the lowest SPL concentration detectable but not quantifiable in the sample) and LOQ (the lowest SPL concentration measurable in the sample with appropriate precision and accuracy) were determined using the following equations (σ = the standard deviation (SD) of the intercept; S = the slope of the calibration curve) [25]:

$$LOD = 3.3x\left(\frac{\sigma}{S}\right)$$

$$LOQ = 10x\left(\frac{\sigma}{S}\right)$$

Additionally, the precision and accuracy for both intra-day and inter-day assessments were determined using standard SPL solutions at three concentrations within the calibration range: 100, 140, and 250 ng/mL. Each concentration underwent six analyses on the same day to evaluate intra-day variability, and six analyses over consecutive days to assess inter-day variability. Accuracy was quantified using relative error (RE), and precision was indicated by the percentage relative standard deviation (RSD%).

Plasma Sample Pretreatment

The plasma sample preparation procedure was modified from the method described by Kaukonen et al. with minor adjustments [28]. This method was chosen for its effectiveness in isolating the active compound from plasma and its practicality concerning the necessary solvents and equipment. One hundred microliters of acetonitrile were combined with one hundred microliters of plasma, and the resulting mixture was vortexed for five minutes. The sample underwent centrifugation at 12,000 rpm for 10 min, after which the supernatant was evaporated using a nitrogen stream to obtain a residue. The residue was reconstituted with 100 µL of the mobile phase, and the concentration of SPL in the solution was quantified using a validated HPLC-UV method.

The Analysis of Pharmacokinetic Data

Pharmacokinetic parameters for each experimental animal were assessed after the oral administration of SPL suspension, SPL tablet, and Hp-βCD: SPL-tablet formulations to rats. Blood samples were collected at specified time intervals, and plasma concentrations of SPL were measured using the validated HPLC method. The plasma concentration–time data were analyzed using a non-compartmental approach with Kinetica software (version 4.4; Thermo Fisher Scientific, Waltham, MA, USA). The concentration–time profiles provided essential pharmacokinetic parameters, such as elimination half-life ($t_{1/2}$), time to reach maximum plasma concentration (t_{max}), maximum plasma concentration (C_{max}), and the area under the plasma concentration–time curve from zero to infinity ($AUC_{0-\infty}$). The parameters were derived from actual experimental observations, independent of any specific compartmental model assumptions. Mean pharmacokinetic values for each formulation were obtained by averaging the results for each animal. The relative bioavailability (Frel) of the SPL-tablet and Hp-βCD: SPL-tablet formulations was determined in relation to the SPL suspension (reference formulation) using the subsequent equation.

$$F_{rel}\,(\%) = \frac{AUC_{\text{SPL tablet formulations}}}{AUC_{SPL\ suspension}} x100$$

This evaluation allowed the comparison of the extent and rate of absorption among the different SPL formulations, providing insight into the enhancement of oral bioavailability achieved through inclusion complexation with Hp-βCD.

Statistical Analysis

The obtained experimental data were expressed as the mean±standard error (SE) or standard deviation (SD), as appropriate. Linear regression analysis was performed to evaluate the linearity and precision of the HPLC-UV analytical method used for quantification. The area under the plasma concentration–time curve from time zero to the last measurable concentration ($AUC_{0-tlast}$) was calculated using the linear–log trapezoidal rule, which applies the linear trapezoidal method during the ascending phase and the logarithmic trapezoidal method during the descending phase of the concentration–time profile. The total area under the curve from zero to infinity ($AUC_{0-\infty}$) was determined as the sum of $AUC_{0-tlast}$ and the extrapolated terminal area. The extrapolated portion was calculated by dividing the last measurable plasma concentration (C_{last}) by the terminal elimination rate constant (λz). The λz value was obtained from the slope of the terminal log-linear portion of the plasma concentration–time curve using least-squares linear regression analysis. This approach enabled accurate estimation of the pharmacokinetic parameters reflecting the systemic exposure and elimination characteristics of SPL.

Statistical analyses were performed using GraphPad Prism software (version 9.0, GraphPad Software, San Diego, CA, USA). Data were expressed as mean±standard deviation (SD) or standard error (SE), as appropriate. Comparisons among multiple groups were conducted using one-way analysis of variance (ANOVA), followed by Tukey's multiple-comparison post hoc test for pairwise group comparisons. A p value of less than 0.05 was considered statistically significant. Prior to ANOVA, data distribution and homogeneity of variance were evaluated using appropriate statistical tests.

Results and Discussion

Characterization of Hp-βCD: SPL Inclusion Complex

FTIR spectroscopy (Fig. 1a) and PXRD (Fig. 1b) were utilized to verify the formation of an inclusion complex between

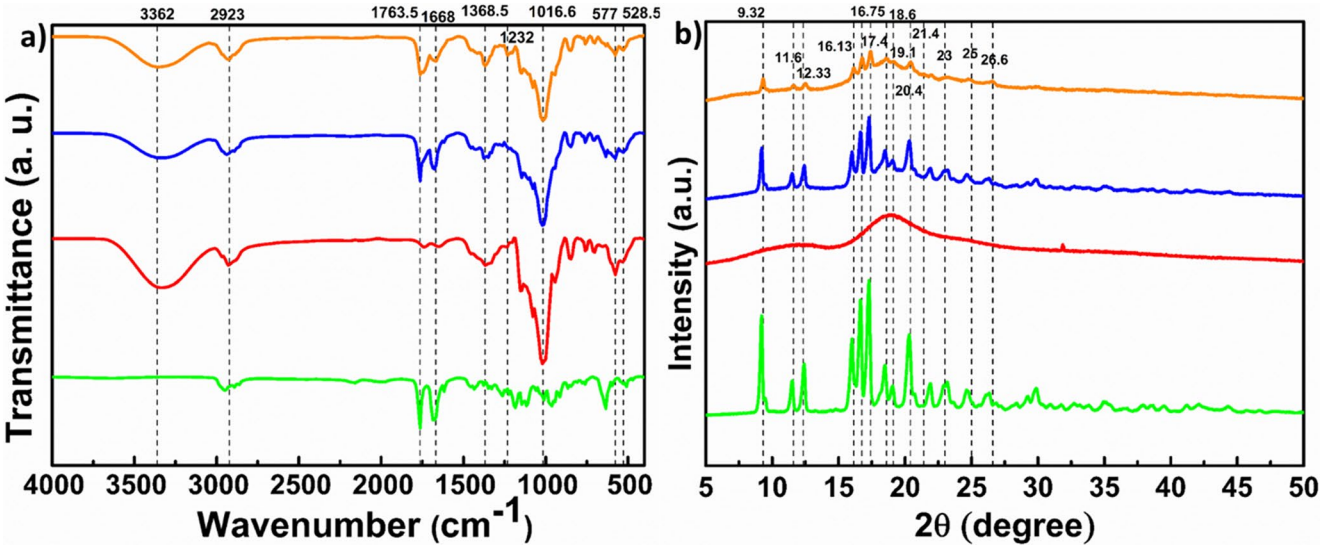


Fig. 1 **a** FTIR spectra **b** PXRD Pattern of Hp-βCD: SPL inclusion complex, physical mixture (SPL + Hp-βCD), Hp-βCD, SPL

SPL and Hp-βCD. In the FT-IR spectrum of pure SPL, the characteristic C–H stretching vibration at 2949 cm^{-1}, the lactone C = O stretching at 1763 cm^{-1}, and the thio-acetyl C = O stretching at 1681 cm^{-1} were observed, consistent with previous literature reports [29]. The FT-IR spectrum of Hp-βCD exhibited a broad O–H stretching band at 3331 cm^{-1}, C–H valence vibrations in the 2968–2930 cm^{-1} region, deformation vibrations at 1454 and 1368 cm^{-1}, and strong polysaccharide-related bands between 1204 and 1020 cm^{-1} corresponding to C–C–O, C–O and C–O–C asymmetric stretching. Additional bands at 943 and 856 cm^{-1} further confirmed the presence of glucopyranose units, in agreement with earlier reports [30]. The FT-IR spectra of SPL and Hp-βCD also aligned well with previously published findings [31].

Although the FT-IR spectrum of the physical mixture (SPL + Hp-βCD) resembled that of the inclusion complex, subtle shifts and peak intensity changes were noticeable, indicating minor interactions but insufficient to confirm complexation. In contrast, the inclusion complex exhibited attenuated or shifted SPL-associated vibrational bands, along with broadening typical of Hp-βCD, suggesting that SPL was partially or fully incorporated within the hydrophobic cavity of Hp-βCD. These spectral modifications collectively support the successful formation of the Hp-βCD: SPL inclusion complex.

The XRD data further substantiated these findings. Pure SPL displayed numerous sharp and intense diffraction peaks, characteristic of its crystalline nature. In the physical mixture, SPL's crystalline reflections were still present although at reduced intensity, indicating a simple superimposition of the individual components. Hp-βCD showed a broad amorphous halo centered near $2\theta \approx 18.9°$, consistent with literature descriptions of its non-crystalline structure [20, 32]. Importantly, the XRD pattern of the Hp-βCD: SPL inclusion complex demonstrated a marked reduction in the intensity of SPL's characteristic crystalline peaks, indicating a substantial loss of crystallinity. This transformation suggests that SPL was molecularly dispersed within the amorphous Hp-βCD matrix.

Taken together, the FT-IR peak shifts and attenuation, along with the XRD evidence of reduced crystallinity, confirm the successful formation of the Hp-βCD: SPL inclusion complex and support the conclusion that SPL is likely accommodated within the Hp-βCD cavity.

DSC was employed to investigate the thermal behavior and complementary evidence of the inclusion complex formation between SPL and Hp-βCD. Figure 2 presents the DSC thermograms of pure SPL, HP-βCD, their physical mixture, and the Hp-βCD: SPL inclusion complex. Pure SPL exhibits a distinct endothermic peak at approximately 207.9 °C, corresponding to its melting point [33, 34]. In contrast, the DSC thermogram of Hp-βCD does not display any thermal events in this region, although a broad endothermic peak splitting at 157 °C and 160.4 °C and a distinct peak 247.6 °C are observed. The DSC thermogram of physical mixture shows endothermic peaks at 167 °C, 193 °C and 258.4 °C. However, this thermal signal is absent in the DSC thermogram of the Hp-βCD: SPL inclusion complex, suggesting the successful formation of the complex. Altogether, especially the PXRD and DSC data strongly support the formation of the Hp-βCD: SPL inclusion complex, in agreement with previous reports on similar systems [35].

Evaluation of Hp-βCD: SPL Inclusion Complex and the Tablet Formulation Containing this Complex

Solubility is a critical factor for the absorption of an active substance from the gastrointestinal tract. The dissolution

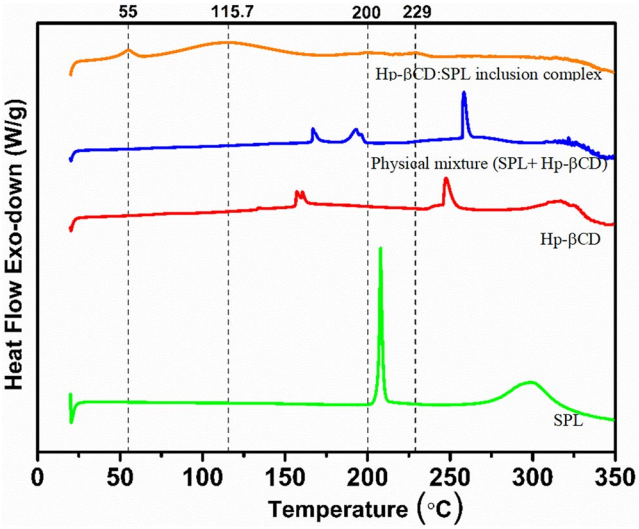


Fig. 2 DSC curves of Hp-βCD: SPL inclusion complex, physical mixture (SPL + Hp-βCD), Hp-βCD, SPL

process serves as the rate-limiting factor in the oral absorption of poorly water-soluble active substances [36].

SPL, categorized as a class II drug per the BCS, exhibits variable and incomplete gastrointestinal absorption [37], thereby impacting its therapeutic efficacy. Spironolactone demonstrates moderate intrinsic intestinal permeability attributed to its lipophilic steroidal structure; however, the restricted quantity of the drug dissolved in the intestinal lumen limits its effective membrane transport. Additionally, presystemic degradation and swift conversion to active metabolites within the intestinal wall further reduce the proportion of unchanged drug accessible for systemic circulation. The interplay of solubility and permeability limitations leads to significant variability in oral exposure. Appropriate formulations, such as cyclodextrin inclusion complexes and nanosized carriers, can optimize the dissolution rate, intestinal drug concentration gradient, and, consequently, the effective permeation and bioavailability of SPL.

In the solubility study performed for pure SPL and Hp-βCD: SPL inclusion complex, the media with different pH values (0.1 N HCl pH 1.2, PB pH 4.5, PB pH 6.8, and 0.1 N HCl pH 1.2 with 0.1% SLS) were used. The results of the solubility study are presented in Fig. 3. According to the solubility study results, after the formation of the inclusion complex, the solubilities of SPL in HCl pH 1.2 and HCl pH 1.2 with 0.1% SLS increased approximately six and sevenfold, respectively. However, the solubility of SPL increased very slightly in PB pH 4.5 and 6.8 media.

Jarho et al. investigating the complexation of SPL with CDs (β-CD, Hp-β-CD, sulfobutyl ether-β-CD, γ-CD, sulfobutyl ether-γ-CD) and the effect of pH on the CD-catalyzed deacetylation of SPL. Their study primarily focused on the chemical stability and degradation kinetics of SPL in the presence of CDs. They showed that the CD-catalyzed degradation of SPL decreased with low pH in the stability study. However, our study aims to develop and evaluate a solid inclusion complex formulation of SPL with Hp-β-CD, emphasizing improvements in aqueous solubility, dissolution rate, and oral bioavailability. Moreover, our work includes both in vitro and in vivo assessments, providing a comprehensive pharmaceutical evaluation that extends beyond the physicochemical stability aspects previously reported [38].

To prepare SPL-tablets or Hp-βCD: SPL-tablets by direct compression, we first prepared powder mixtures. After the addition of magnesium stearate to the powder mixtures, their flow properties were evaluated and the results were presented in Table 3. According to USP < 1174>, the obtained values indicate good flowability, supporting the suitability of the powder blends for direct compression without the need for additional flow-enhancing excipients. The angle of repose values of the powder mixtures prepared for SPL-tablets and Hp-βCD: SPL-tablets were 27.34 ± 0.88° and 24.10 ± 0.54°, respectively (Table 3). A range of 25–30° for the angle of repose indicates that the powder exhibits excellent flow properties [39]. Cohesiveness, shape, size, surface area and moisture content of powder etc. influence powder flow properties. We calculated compressibility index and Hausner ratio which are fast and simple methods used to predict powder flow properties (Table 3). Powders with compressibility index and Hausner ratio of 11–15% and 1.12–1.18, respectively show good flow properties. As a result, the powder mixture we prepared for SPL-tablets or the Hp-βCD: SPL-tablets had good flow properties (Table 3).

The dissolution studies for pure SPL, the Hp-βCD: SPL inclusion complex, SPL-tablets, and Hp-βCD: SPL-tablets were conducted in 1000 mL of 0.1 N HCl (pH 1.2) containing 0.1% SLS at 37 ± 0.5 °C using USP Apparatus II (paddle method, 75 rpm), in accordance with the USP 43 "Spironolactone Tablets" monograph. The selected dissolution medium was further supported by the solubility results obtained in this study, which demonstrated enhanced SPL solubility and maintenance of sink conditions in acidic medium containing 0.1% SLS. Sink conditions were quantitatively verified for the dissolution studies. The highest experimentally determined solubility of spironolactone was approximately 3.05 mg/mL in 0.1 N HCl containing 0.1% SLS. Considering the tablet dose equivalent to 25 mg SPL,

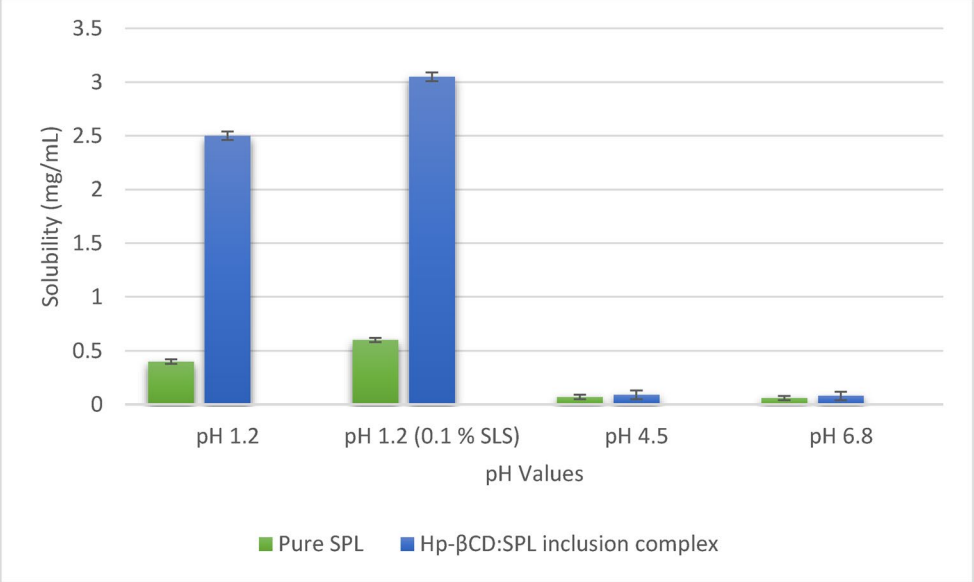


Fig. 3 The solubility study of pure SPL and Hp-βCD: SPL inclusion complex in media with different pH values (0.1 N HCl pH 1.2, PB pH 4.5, PB pH 6.8, and 0.1 N HCl pH 1.2 with 0.1% SLS)

approximately 8.2 mL of dissolution medium would be sufficient to dissolve the entire drug amount. According to compendial expectations, sink conditions require a medium volume at least three times greater than the saturation volume. Therefore, a minimum volume of approximately 24.6 mL would satisfy sink conditions, whereas the present study employed 1000 mL dissolution medium. These results confirm that sink conditions were maintained throughout the dissolution experiments. The dissolution sampling time points (5, 10, 15, 20, 30, 45, 60, 90, and 120 min) were selected to adequately characterize the initial, intermediate, and late phases of drug release from both conventional and Hp-βCD-based tablet formulations. Early sampling points were particularly included to capture the rapid dissolution behavior expected from the Hp-βCD inclusion complex formulation, while later time points enabled evaluation of the completeness of drug release and establishment of the dissolution plateau. The selected time intervals are consistent with compendial expectations for immediate-release dosage forms and allow appropriate comparison of dissolution profiles among formulations. According to USP 43, the dissolution conditions for SPL tablets are 1000 mL of 0.1 N HCl containing 0.1% SLS, 75 rpm (Apparatus 2), and a duration of 60 min, with ≥75% of the labeled amount of SPL expected to dissolve within this period. In our study, 18% of pure SPL and 85% of SPL in Hp-βCD: SPL inclusion complex dissolved within 60 min. For Hp-βCD: SPL-tablets, 75% and 95% of SPL were dissolved within 10 and 30 min, respectively. In contrast, SPL-tablets exhibited slower dissolution, with 35%, 54%, and 76% of SPL dissolved at 10, 30, and 60 min, respectively. These results indicate a significant enhancement in dissolution rate and extent for the Hp-βCD inclusion complex and its tablet formulation compared to the pure drug and conventional SPL-tablets.

Table 3 Flow characteristics of the powder mixtures prepared for tablet formulations (Mean ± SD)

Parameter	The powder mixture for SPL-tablets	The powder mixture for Hp-βCD: SPL-tablets
Angle of repose	27.34 ± 0.88°	24.10 ± 0.54°
Flow time (sec)	5.00 ± 1.01	4.00 ± 1.12
Bulk density (g/mL)	0.72 ± 0.07	0.63 ± 0.05
Tapped density (g/mL)	0.84 ± 0.06	0.73 ± 0.04
Compressibility index (%)	14.29 ± 0.06	13.70 ± 0.05
Hausner ratio	1.17 ± 0.06	1.16 ± 0.05

Resende et al. evaluated the impact of SPL'crystal forms (pure crystal form II and the form II contaminated with form I) obtained from different manufacturers on their aqueous solubility and their effect on tablet dissolution profiles. They performed solubility studies in different media (water; 0.01 mol/LHCl; 0.1 mol/L HCl; 0.1 mol/L HCl with 0.1% (w/v) SLS; 0.1 mol/L potassium PB-pH 7.2; 0.1 mol/L potassium PB-pH 6.2; 0.1 mol/L sodium acetate buffer-pH 4.5; 0.1 mol/L sodium citrate buffer-pH 3.0). They reported that the solubility of form II contaminated with form I and pure form II in 0.1 mol/L HCl with 0.1% SLS were 150.74 ± 3.78 µg/mL and 101.10 ± 5.20 µg/mL, respectively and SPL's solubility in 0.1 mol/L HCl with 0.1% SLS increased approximately 3-fold compared to the other aqueous media. They conducted a dissolution study for tablet formulations (containing form II or form II contaminated with form I)

in 0.1 mol/L HCl with 0.1% SLS. They determined that 79.55% (for form II) and 89.32% (for form II contaminted with Form I) of SPL were dissolved at 60 min [37] (Fig. 4).

According to USP < 1174>, powders exhibiting an angle of repose between 25–30°, Carr's index values between 11 and 15%, and Hausner ratios between 1.12 and 1.18 are considered to possess good flowability suitable for direct compression processes. Finished product quality control tests were carried out for the tablets. In this context, diameter, thickness, disintegration time, weight variation, friability and hardness values of the SPL-tablets and Hp-βCD: SPL-tablets are given in Table 4. The shape and diameter of a tablet depend on the punches and chosen for its compression [40]. The thickness and diameter values of SPL-tablets and Hp-βCD: SPL-tablets are close to each other (Table 4). Determination of tablet thickness is significant for the consistency in the manufacturing process, such as particle size and distribution, preparation of powder mixture. Compressibility of the powder, die's diameter and internal volume, applied force or pressure, etc. affect tablet thickness [41]. Tablet thickness should be controlled within ± 5% deviation of a standard value to facilitate packaging [42]. According to USP < 905 > and Ph. Eur. 2.9.5, tablets with an average weight above 250 mg should not deviate by more than ± 5% from the mean weight, and all tested tablets complied with this criterion. Acceptable friability for tablets is typically less than 1% (Table 4). The friability values of both formulations were below 1%, indicating adequate mechanical resistance according to USP < 1216 > recommendations for conventional tablets. The hardness values indicated sufficient mechanical strength while still allowing rapid tablet disintegration. The disintegration time values for of SPL-tablets and Hp-βCD: SPL-tablets are close to each other (Table 4). The rapid disintegration times (< 2 min) confirmed the suitability of the formulations as immediate-release tablets and were well within pharmacopeial limits.

Table 4 The quality control tests for the tablet formulations (Mean ± SD)

Parameter	SPL-tablets	Hp-βCD: SPL-tablets
Thickness (mm)	3.11 ± 0.01	3.12 ± 0.01
Diameter (mm)	12.02 ± 0.01	12.03 ± 0.01
Hardness (N)	120.00 ± 2.34	124.00 ± 2.45
Friability (%)	0.23 ± 0.05	0.24 ± 0.06
Average weight (mg)	300.00 ± 2.21	299.00 ± 2.13
Disintegration time (min)	1.10 ± 0.16	1.00±0.0.12
Assay (%)	96.23 ± 0.56	97.89 ± 0.66

HPLC Method

In this study, an HPLC-UV method for SPL analysis in rat plasma samples was developed and validated according to the ICH guideline [25]. The linearity of the method was evaluated within the range of SPL concentrations of 100–1000 ng/mL. The calibration curve obtained by plotting the peak areas (mAu) versus the concentrations of SPL (ng/mL) is shown in Fig. 5. The calibration curve equation calculated using linear regression analysis was y = 8262.2x + 20,220 (Fig. 5). A good linear relationship was established between the peak areas and the concentrations (100–1000 ng/mL) of SPL with the determination coefficient (R^2) 0.9997 (Fig. 6).

The limits of detection (LOD) and quantification (LOQ) for the established HPLC-UV method were assessed by injecting low-concentration SPL standard solutions under the optimized chromatographic parameters. The LOD and LOQ values for SPL were calculated as 27.87 ng/mL and 84.46 ng/mL, respectively.

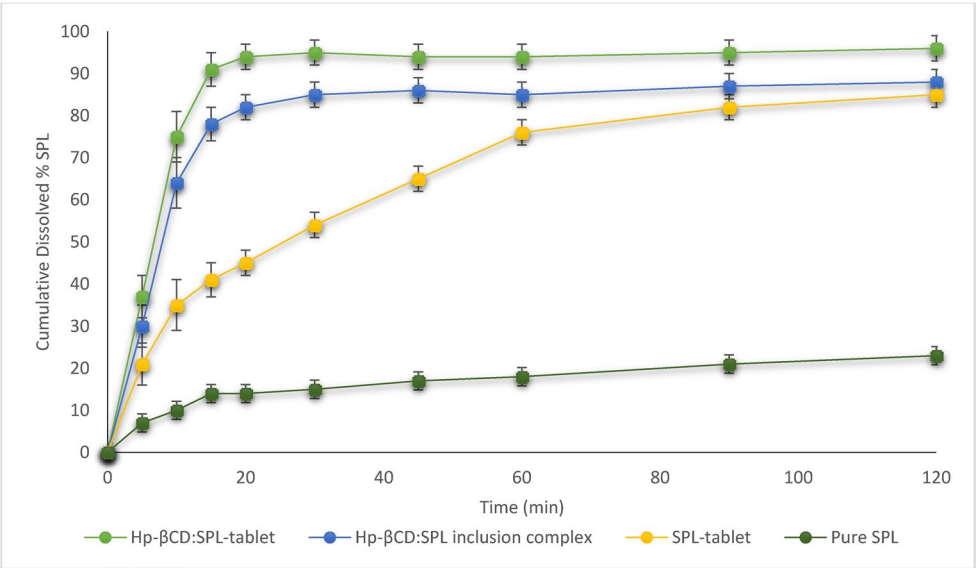


Fig. 4 In vitro dissolution profiles of pure SPL, Hp-βCD: SPL inclusion complex, SPL-tablets and Hp-βCD: SPL-tablets. (n = 6, Mean ± SD)

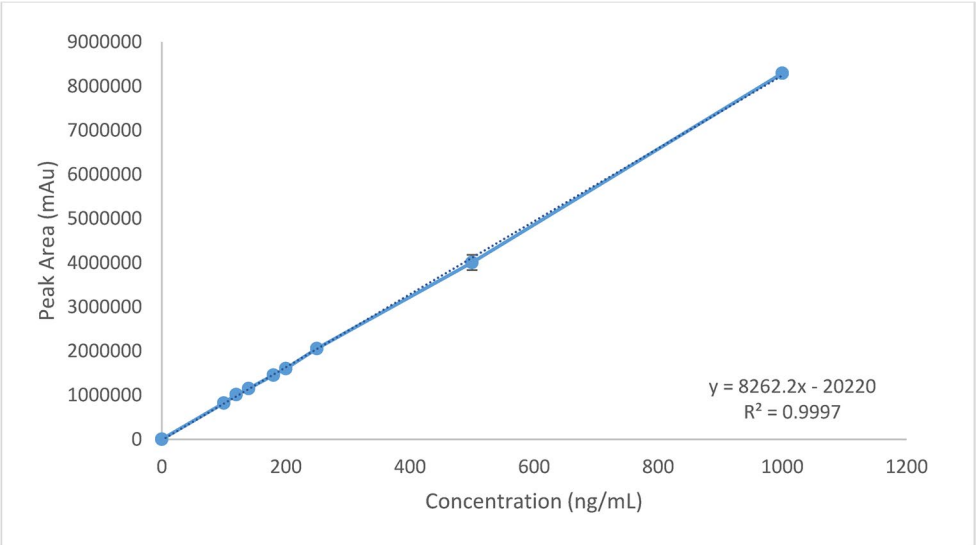


Fig. 5 Calibration curve of SPL (Mean ± SD; $n = 6$)

Method precision (expressed as %RSD) and accuracy (expressed as %RE) were examined at SPL concentrations of 100, 140, and 250 ng/mL ($n = 6$). The intra- and inter-day accuracy and precision results are presented in Table 5. All % RE and % RSD values were below 15% across the tested concentrations, confirming that the developed HPLC-UV method for SPL quantification in rat plasma is both accurate and precise [43].

Pharmacokinetic Parameters

The validated HPLC–UV method developed in this study was successfully applied to quantify SPL plasma concentrations and to evaluate its pharmacokinetic profile following oral administration of SPL suspension, SPL-tablet, and Hp-βCD: SPL-tablet formulations (each at a single dose of 25 mg/kg) in rats. The mean plasma concentration–time profiles for the different SPL formulations are shown in Fig. 7, while the pharmacokinetic parameters were calculated by non-compartmental analysis using Kinetica software (version 4.4; Thermo Fisher Scientific, Waltham, MA, USA) and are summarized in Table 6 (data expressed as mean ± SD; $n = 8$). As shown in Table 6 the $AUC_{o-\infty}$ and C_{max} values of the Hp-βCD: SPL-tablets were significantly higher than those of the SPL suspension, indicating enhanced systemic exposure.

Statistical analysis (one-way ANOVA followed by Tukey's post-hoc test) showed significant differences between the Hp-βCD: SPL-tablets and both other formulations in terms of $AUC_{0-\infty}$ and C_{max} ($p < 0.05$). Although minor variations were observed in t_{max} and $t_{1/2}$, these differences were not statistically significant.

The pharmacokinetic findings indicated that the SPL suspension yielded the lowest $AUC_{0-\infty}$ and C_{max} values compared to the other formulations. This discovery aligns with the established biopharmaceutical constraints of spironolactone, a BCS Class II drug noted for its inadequate water solubility and absorption restricted by dissolution rate (Lopalco et al., 2022). The rough characteristics of the suspension probably led to increased particle size and less effective surface area, thereby constraining the rate and degree of dissolution in the gastrointestinal tract. As spironolactone is predominantly absorbed in the upper small intestine, where transit time is brief, insufficient breakdown during this absorption period results in diminished systemic exposure. Conversely, the commercial SPL tablet exhibited elevated $AUC_{0-\infty}$ and C_{max} values, attributable to enhanced dissolving characteristics resulting from reduced particle size, the inclusion of disintegrants and wetting agents, and more effective drug dispersion following administration. The Hp-βCD: SPL complex pill demonstrated the most significant improvement in bioavailability. Cyclodextrin complexation enhances the apparent aqueous solubility and dissolution rate of hydrophobic drugs by forming inclusion complexes, thereby preserving the drug in a molecularly dispersed state during passage through the absorptive areas of the intestine [22, 44]. The significant rise in both AUC and C_{max} for the Hp-βCD formulation is ascribed to the enhanced solubilization ability of Hp-βCD and the effective delivery of dissolved medication inside the optimum absorption zone of the gastrointestinal tract.

Importantly, the enhanced dissolution performance observed in vitro was reflected in significantly increased

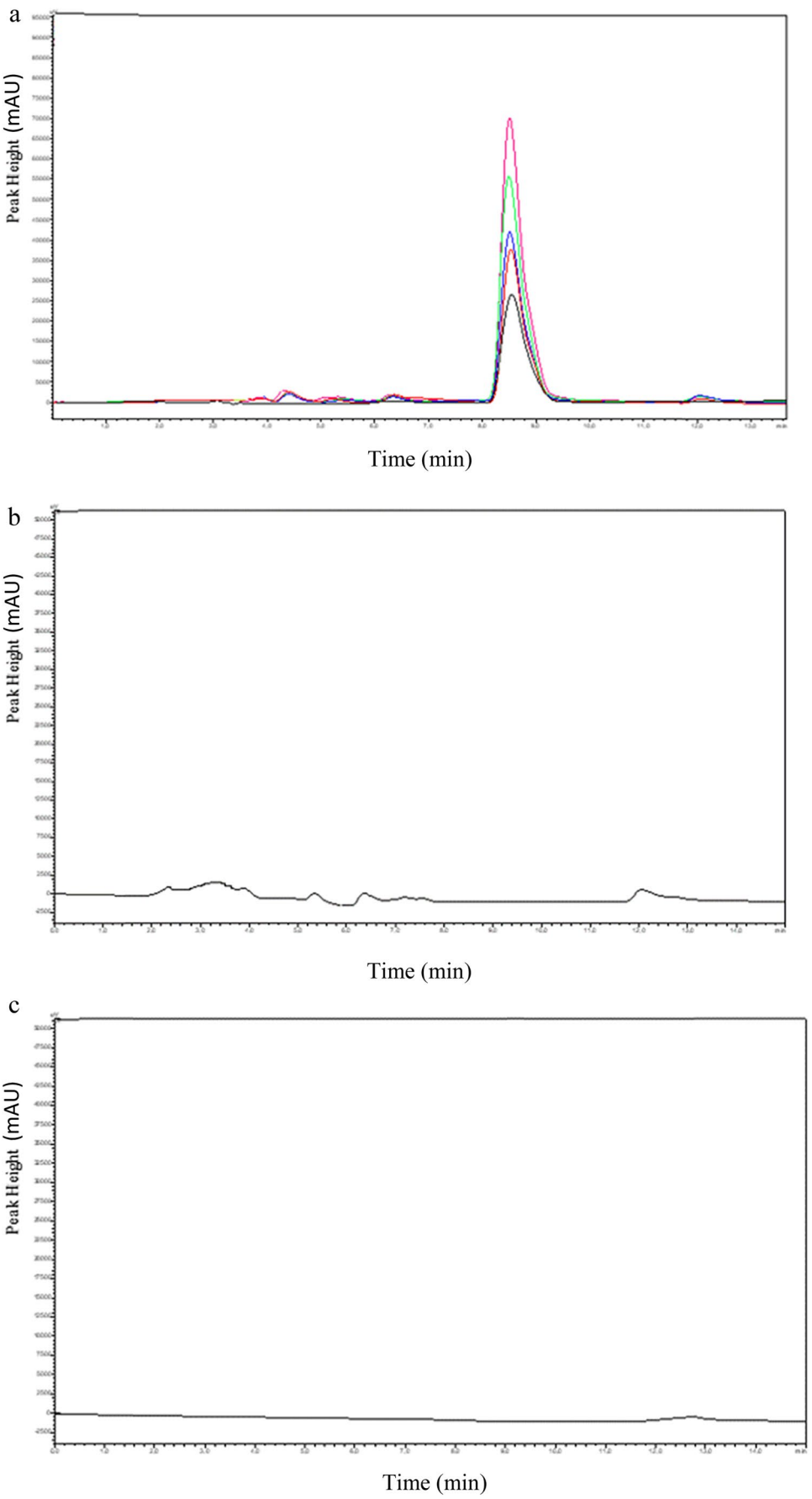


Fig. 6 Representative HPLC chromatograms for spironolactone (SPL): (**a**) SPL standard solutions at concentrations of 100, 120, 140, 160, and 250 ng/mL, (**b**) blank rat plasma sample, and (**c**) chromatogram of the mobile phase (blank injection) under the optimized chromatographic conditions. Chromatographic conditions: detection at 240 nm; mobile phase methanol: water (70:30); flow rate 1 mL/min

Table 5 The results of intra- and inter-day accuracy and precision of the developed HPLC-UV method (mean ± SD)

Added Concentrations (ng/mL)	Intra-day ($n=6$)		Inter-day ($n=6$)	
	Accuracy (RE, %)	Precision (RSD, %)	Accuracy (RE, %)	Precision (RSD, %)
100	0.032	4.55	0.067	7.87
140	0.025	2.37	0.055	4.31
250	0.024	2.22	0.035	3.67

RE relative error, *RSD* relative standard error

Table 6 The pharmacokinetic parameters obtained for different SPL formulations (Mean ± SD, $n=8$)

Parameters	SPL suspension	SPL-tablet	Hp-βCD: SPL-tablets
$AUC_{0-\infty}$(ng.h/mL)	2945.65 ± 210	3365.21 ± 230[a]	4554.23 ± 278[a]
C_{max}(ng/mL)	280.23 ± 80	550.00 ± 85[a]	881.67 ± 86[a]
t_{max}(h)	1.05 ± 0.22	1.09 ± 0.26[a]	1.05 ± 0.21[a]
$t_{1/2}$(h)	7.24 ± 1.23	5.81 ± 1.37[a]	5.15 ± 1.21[a]
F_{rel}%	-	114.24	154.61

[a] $p<0.05$ compared with coarse SPL suspension group. Statistical significance was evaluated using one-way ANOVA followed by Tukey's post-hoc test

$AUC_{0-\infty}$ area under the plasma concentration time curve from time zero to extrapolation to time infinity, C_{max} maximum plasma concentration, t_{max} time to reach maximum plasma concentration, $t_{1/2}$ elimination half life, F_{rel} relative bioavailability

systemic exposure in vivo, supporting the translational relevance of the formulation strategy. Overall, the present findings suggest that Hp-βCD complexation improves spironolactone bioavailability through a multifactorial mechanism involving reduced crystallinity, improved wettability, enhanced dissolution kinetics, and maintenance of the drug in a molecularly dispersed state. These mechanistic insights extend current understanding beyond conventional solubility enhancement approaches.

Conclusion

The present study demonstrates that Hp-βCD complexation can be successfully integrated into a scalable immediate-release tablet formulation capable of significantly improving the dissolution behavior and oral bioavailability of spironolactone. The enhanced systemic exposure observed for the Hp-βCD tablets, together with the improved in vitro dissolution performance, supports the translational potential of this formulation strategy for poorly water-soluble BCS Class II drugs.

Importantly, the findings provide mechanistic insight into how cyclodextrin-mediated amorphization and improved wettability can be preserved within a solid tablet matrix and translated into enhanced oral absorption. Overall, the study extends current understanding beyond conventional solubility enhancement approaches and highlights the pharmaceutical applicability of Hp-βCD complexation as a practical strategy for improving spironolactone delivery.

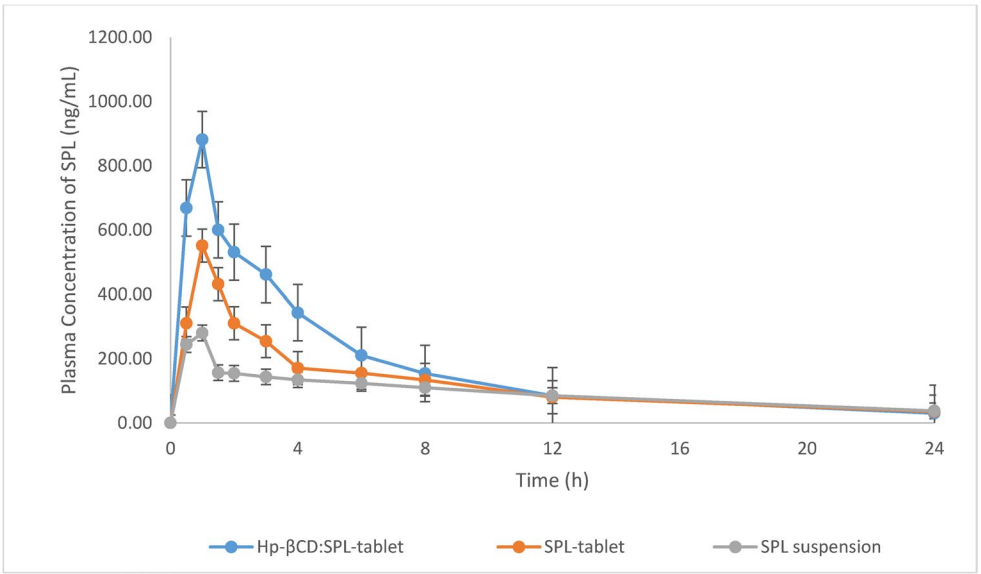


Fig. 7 The mean concentration–time profiles obtained the different SPL formulations in rats after oral administration (Mean ± SD; $n=8$)

Acknowledgements The authors would like to thank the laboratory and animal research center staff for their technical assistance throughout the experimental studies.

Author Contributions E.D.— Conceptualization, supervision, formal analysis, review, and final editing of the manuscript. G.G. — Data curation, writing—original draft preparation, and figure preparation. M.C. and E.B. — Methodology, investigation, data curation, and writing—original draft preparation.

Funding Open access funding provided by the Scientific and Technological Research Council of Türkiye (TÜBİTAK). This research did not receive any specific funding from governmental or non-governmental agencies.

Data Availability No datasets were generated or analyzed during the current study.

Declarations

Competing Interests The authors declare no competing interests.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

References

1. Resende RC, de Oliveira ARM, Fernandes GFS, da Silva CF, de Oliveira MAL, da Silva DL. Biopharmaceutical properties and dissolution profile of spironolactone tablets. J Appl Pharm Sci. 2016;6(7):1–7. https://doi.org/10.7324/JAPS.2016.60701.
2. Cadegiani FA, Wambier CG, Goren A. Spironolactone. An antiandrogenic and anti-hypertensive drug that may provide protection against SARS-CoV-2–induced ARDS in COVID-19. Front Med. 2020;7:453. https://doi.org/10.3389/fmed.2020.00453.
3. Carone L, Oxberry SG, Twycross R, Charlesworth S, Mihalyo M, Wilcock A. Spironolactone. J Pain Symptom Manage. 2017;53(2):288–92. https://doi.org/10.1016/j.jpainsymman.2016.12.320.
4. Hsueh TP, Tsai TH. Exploration of sodium homeostasis and pharmacokinetics in bile duct-ligated rats treated by anti-cirrhosis herbal formula plus spironolactone. Front Pharmacol. 2023;14:1092657. https://doi.org/10.3389/fphar.2023.1092657.
5. Filippone EJ, Naccarelli GV, Foy AJ. Controversies in hypertension V: Resistant and refractory hypertension. Am J Med. 2024;137(1):12–22. https://doi.org/10.1016/j.amjmed.2023.08.018.
6. Acelajado MC, Hughes ZH, Oparil S, Calhoun DA. Treatment of resistant and refractory hypertension. Circ Res. 2019;124(7):1061–70. https://doi.org/10.1161/CIRCRESAHA.118.312156.
7. Lopalco A, Manni A, Keeley A, Haider S, Li W, Lopedota A, et al. In vivo investigation of HPβCD-based formulation of spironolactone in aqueous solution for paediatric use. Pharmaceutics. 2022;14(4):780. https://doi.org/10.3390/pharmaceutics14040780.
8. Murai T, Yasuno H, Shibasaki M, Imai H. Quantitative evaluation of spironolactone degradation to canrenone in gastrointestinal conditions. Biol Pharm Bull. 2019;42(3):431–7. https://doi.org/10.1248/bpb.b18-00689.
9. Kagawa T, Morita S, Okada A, Takahashi H. Pharmacokinetic characterization of Spironolactone and Canrenone after oral administration. Eur J Drug Metab Pharmacokinet. 2017;42(6):905–12. https://doi.org/10.1007/s13318-017-0414-4.
10. Loftsson T, Duchêne D. Cyclodextrins and their pharmaceutical applications. Int J Pharm. 2007;329(1–2):1–11. https://doi.org/10.1016/j.ijpharm.2006.10.044.
11. Özcan Bülbül E, Eleftheriadou K, Üstündağ Okur N, Siafaka P. Cyclodextrins as drug vehicles for antimicrobial applications. J Pharm Technol. 2020;1(1):18–24. https://doi.org/10.37662/jpt.2020.3.
12. Cirri M, Maestrelli F. Cyclodextrin inclusion complexes for improved drug bioavailability and activity. Pharmaceutics. 2023.
13. Agüeros M, Zabaleta V, Espuelas S, Campanero MA, Irache JM. Cyclodextrin–poly(anhydride) nanoparticles for oral drug delivery. J Control Release. 2010;145(1):2–8. https://doi.org/10.1016/j.jconrel.2009.11.002.
14. Saokham P, Muankaew C, Jansook P, Loftsson T. Solubility of cyclodextrins and drug/cyclodextrin complexes. Molecules. 2018;23(5):1161. https://doi.org/10.3390/molecules23051161.
15. Esteso MA, Romero CM. Cyclodextrins. Properties and applications. Int J Mol Sci. 2024;25(8):4547. https://doi.org/10.3390/ijms25084547.
16. European Medicines Agency. Questions and answers on cyclodextrins used as excipients. EMA/CHMP/495747/2013. 2017.
17. European Medicines Agency. Cyclodextrins used as excipients. EMA/CHMP/333892/2013. 2017.
18. Yusuff N, York P. Spironolactone–cyclodextrin complexes: phase solubility and ultrafiltration studies. Int J Pharm. 1991;73(1):9–15. https://doi.org/10.1016/0378-5173(91)90094-5.
19. Seo H, Tsuruoka M, Hashimoto T, Fujinaga T, Otagiri M, Uekama K. Enhancement of Spironolactone oral bioavailability by β- and γ-CD complexations. Chem Pharm Bull. 1983;31(1):286–91. https://doi.org/10.1248/cpb.31.286.
20. Loftsson T, Brewster ME. Pharmaceutical applications of cyclodextrins. J Pharm Sci. 1996;85(10):1017–25. https://doi.org/10.1021/js950534b.
21. Saokham P, Loftsson T, Hedges J. Cyclodextrins in drug delivery. J Pharm Pharmacol. 2018;70(10):1235–50. https://doi.org/10.1111/jphp.12906.
22. European Food Safety Authority. Re-evaluation of β-cyclodextrin (E459). EFSA J. 2016;14(12):4628. https://doi.org/10.2903/j.efsa.2016.4628.
23. Salustio PJ, Feio G, Figueirinhas JL, Pinto JF, Cabral Marques HM. Effect of preparation methods on ibuprofen–βCD complexation. Eur J Pharm Biopharm. 2006;62(3):241–6. https://doi.org/10.1016/j.ejpb.2005.08.004.
24. Lopalco A, Cutrignelli A, Laquintana V, Lopedota A, Franco M, Denora N, et al. Spironolactone: biopharmaceutics, pharmacokinetics and strategies. Pharmaceutics. 2022;14(8):1581. https://doi.org/10.3390/pharmaceutics14081581.
25. International Council for Harmonisation. ICH Q2(R2): Validation of analytical procedures. 2022.
26. United States Pharmacopeial Convention. USP–NF Online. Rockville, MD: USP. 2020.

27. Chaure PP, Deshmukh TA, Tajane MA. RP-HPLC estimation of losartan and spironolactone. J Pharm Sci Res. 2019;11(8):2866–71.
28. Kaukonen AM, Lennernäs H, Mannermaa JP. Water-soluble β-CDs in paediatric spironolactone solutions. J Pharm Pharmacol. 1998;50(8):871–6. https://doi.org/10.1111/j.2042-7158.1998.tb03324.x.
29. El-Nabarawi MA, Shamma RN, Farouk F, Abdelkader H. Spironolactone-loaded LeciPlexes for topical delivery. Pharmaceutics. 2020;12(1):25. https://doi.org/10.3390/pharmaceutics12010025.
30. Nastasović A, Jovičić M, Janković B, Milutinović-Nikolić A, Grujić S, Onjia A. Structural characterization of glycidyl methacrylate–based macroporous copolymers. Iran Polym J. 2013;22(2):85–96. https://doi.org/10.1007/s13726-012-0122-4.
31. Yadav E, Gupta A, Kumar A. Formulation and characterization of spironolactone inclusion complexes. Mater Today Proc. 2022;51:2539–49. https://doi.org/10.1016/j.matpr.2021.11.122.
32. Jambhekar SS, Breen P. Cyclodextrins in pharmaceutical formulations II. Drug Discov Today. 2016;21(2):363–8. https://doi.org/10.1016/j.drudis.2015.11.017.
33. Tiwari G, Tiwari R. Spironolactone–βCD complexation: characterization and release. AAPS PharmSciTech. 2011;12(2):482–9. https://doi.org/10.1208/s12249-011-9615-4.
34. Parmar KK, Tank HM. Inclusion complexes of spironolactone with β-cyclodextrin. Int J Pharm Sci Res. 2018;9(6):2367–75. https://doi.org/10.13040/IJPSR.0975-8232.9.
35. Rajabi O, Tayyari F, Salari R, Tayyari SF. Interaction of spironolactone with HPβCD in solution and solid state. J Mol Struct. 2008;878:78–83. https://doi.org/10.1016/j.molstruc.2007.08.039.
36. Savjani KT, Gajjar AK, Savjani JK. Drug solubility: importance and enhancement. ISRN Pharm. 2012;2012:195727. https://doi.org/10.5402/2012/195727.
37. Resende RC, Viana OMMS, Freitas JTJ, Bonfilio R, Ruela ALM, Araújo MB. Analysis of spironolactone polymorphs. Braz J Pharm Sci. 2016;52(4):1–10. https://doi.org/10.1590/S1984-82502016000400005.
38. Jarho P, Vander Velde D, Stella VJ. Cyclodextrin-catalyzed deacetylation of spironolactone. J Pharm Sci. 2000;89(2):241–9.
39. United States Pharmacopeial Convention. Flow properties. USP 43–NF38. Rockville, MD: USP. 2020.
40. Gülsün T, Akdağ Caylı Y, İzât N, Çetin M, Öner L, Şahin S. Terbutaline sulfate ODTs by direct compression and freeze-drying. J Drug Deliv Sci Technol. 2018;46:251–8. https://doi.org/10.1016/j.jddst.2018.05.014.
41. Ghimire P, Shrestha AC, Pandey S, Chapagain B, Dhakal S. Pharmacopoeial comparison of tablet QC tests. GSC Biol Pharm Sci. 2020;11(3):155–65. https://doi.org/10.30574/gscbps.2020.11.3.0147.
42. Mekasha YT, Chali BU, Feissa AB, Godena GH, Hassen HK, Wega SS. Quality evaluation of azithromycin tablets. PLoS One. 2023;18(3):e0282156. https://doi.org/10.1371/journal.pone.0282156.
43. Qin C, Feng W, Chu YJ, Lee JB, Berton M, Bettonte S, et al. Bioanalytical HPLC-UV method for lopinavir. Biomed Chromatogr. 2020;34:e4934. https://doi.org/10.1002/bmc.4934.
44. Salustio PJ, Cabral Marques HM, Veiga FJB. Spironolactone and its complexes with β-CD: NMR and DFTB-SCC characterization. J Incl Phenom Macrocycl Chem. 2006;56(3):293–302. https://doi.org/10.1007/s10847-005-9030-9.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.