ORIGINAL ARTICLE

In vivo Pharmacokinetic Assessment of Lapatinib-Loaded Nanostructured Lipid Carriers Using High-Performance Liquid Chromatography Analysis

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Abstract

Background: Lapatinib, a tyrosine kinase inhibitor used for the treatment of human epidermal growth factor receptor 2-positive breast cancer, suffers from poor aqueous solubility and limited permeability, leading to suboptimal bioavailability. This study aimed to enhance its oral bioavailability through the formulation of lapatinib-loaded nanostructured lipid carriers (L-NSLCs) and to compare their pharmacokinetic performance with a conventional lapatinib suspension (L-SUS). **Materials and Methods:** L-NSLCs were developed using a lipid-based nanocarrier system and characterized for particle size, zeta potential, and drug entrapment efficiency. A pharmacokinetic study was conducted in male Wistar rats, where both L-NSLCs and L-SUS were administered orally at a dose of 50 mg/kg. Blood samples were collected at pre-determined intervals, and plasma drug concentrations were quantified using a validated high-performance liquid chromatography method with ultraviolet detection, employing gemcitabine HCl as the internal standard. **Results:** The pharmacokinetic analysis revealed a significant increase in systemic exposure with L-NSLCs compared to L-SUS. The maximum plasma concentration of L-NSLCs was 798.62 ± 26.14 ng/mL, approximately 2.9-fold higher than L-SUS (272.20 ± 16.12 ng/mL). The area under the curve ($AUC_{0-\infty}$) for L-NSLCs (92,903.96 ± 21,646.64 ng·h/mL) showed a marked enhancement compared to L-SUS (9,620.75 ± 781.21 ng·h/mL). In addition, the elimination half-life (t½) of L-NSLCs was prolonged to 59.62 ± 18.62 h, suggesting sustained drug release and extended circulation time. **Conclusion:** L-NSLCs significantly improved the bioavailability of lapatinib by enhancing its solubility, absorption, and sustained release profile. The lipid-based nanocarrier system offers a promising strategy for optimizing the oral delivery of poorly water-soluble anticancer drugs, potentially leading to better therapeutic efficacy and reduced dosing frequency. Further studies are required to evaluate the long-term stability and clinical benefits of L-NSLCs.

Key words: Bioavailability enhancement, *in vivo* studies, lapatinib, nanostructured lipid carriers

INTRODUCTION

Lapatinib, a dual tyrosine kinase inhibitor targeting both the epidermal growth factor receptor (EGFR)2 and human HER2, is utilized in the treatment of HER2-positive breast cancer. However, its clinical application is hindered by poor aqueous solubility and low permeability, classifying it as a biopharmaceutics classification system (BCS) Class IV drug. This classification indicates challenges in both solubility and intestinal permeability, leading to suboptimal oral bioavailability.[1]

Nanostructured lipid carriers (NLCs) have emerged as advanced drug delivery systems designed to enhance the solubility, stability, and bioavailability of poorly water-soluble drugs. NLCs are composed of a blend of solid and liquid lipids, creating a less ordered lipid matrix that can accommodate higher drug loads and improve drug release profiles. The lipid-based nature of NLCs facilitates

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lymphatic uptake, effectively bypassing first-pass hepatic metabolism and enhancing systemic absorption.[2]

High-performance liquid chromatography (HPLC) is a widely employed analytical technique for the precise quantification of drug concentrations in biological matrices.[3] Its sensitivity and specificity make it ideal for pharmacokinetic evaluations, enabling the assessment of parameters such as absorption, distribution, metabolism, and excretion of drug formulations.

Encapsulating lapatinib within NLCs is expected to enhance its pharmacokinetic profile by improving systemic absorption, prolonging circulation time, and potentially altering its metabolism and excretion pathways. *In vivo* pharmacokinetic studies are essential to assess key parameters such as maximum plasma concentration (Cmax), time to reach maximum plasma concentration (Tmax), area under the curve (AUC), half-life (t½), and clearance rate, which provide insights into the drug's absorption and bioavailability.[3,4] HPLC remains the gold standard for precise quantification of Lapatinib concentrations in biological matrices. This study aims to evaluate the *in vivo* pharmacokinetic performance of lapatinib-loaded NLCs using HPLC-based analysis. The pharmacokinetic parameters of the NLC formulation will be compared with those of the pure drug to determine the extent of improvement in bioavailability and systemic exposure. The findings from this research will contribute to the understanding of lipid-based nanocarriers as a strategy for optimizing the pharmacokinetics of BCS Class IV anticancer drugs, addressing their limitations in oral absorption and systemic circulation.[5]

Table 1: Pharmacokinetic parameters data

Pharmacokinetic parameters	L-SUS	L-NSLCs
Cmax (ng/mL)	272.20±16.12*	798.62±26.14*
Tmax (h)	4.00	4.00
AUC_{0-t} (ng-h/mL)	8432.75±682.21*	32116.62±421.20*
$AUC_{0-\alpha}$ (ng-h/mL)	9620.75±781.21*	92903.96±21646.64*
Biological half-life (h)	13.20±2.42	59.62±18.62

L-SUS: Lapatinib suspension, L-NSLCs: Lapatinib-loaded nanostructured lipid carriers, Cmax: Maximum plasma concentration, AUC: Area under the curve. Data represented as mean±standard deviation (n=6) with a statistical difference at (*) P<0.001

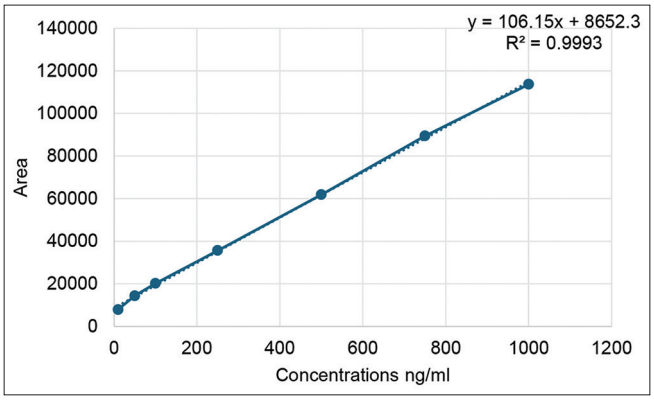

Figure 1: Calibration graph of lapatinib by high-performance liquid chromatography

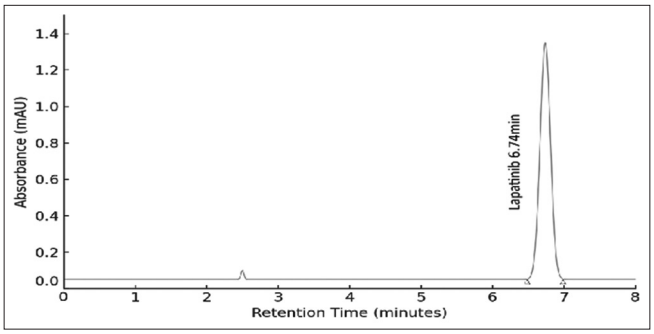

Figure 2: Standard high-performance liquid chromatography chromatogram of lapatinib in rat plasma

MATERIALS AND METHODS

Materials

The pharmacokinetic study utilized male Wistar rats aged 6–8 weeks, weighing approximately 250 g, sourced from a Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA)-approved animal facility. Lapatinib suspension (L-SUS) was formulated with 0.5% sodium carboxymethylcellulose (CMC), whereas lapatinib-loaded NLCs (L-NSLCs) were dispersed in distilled water. Acetonitrile served as a protein precipitating agent in the preparation of plasma samples. Blood samples were collected using heparinized microcentrifuge tubes, and plasma separation was conducted with a refrigerated centrifuge at 5,000 rpm and 4°C. Plasma samples were stored at −80°C in labeled eppendorf tubes. A nitrogen gas evaporator was employed to dry the plasma extracts before reconstitution in the mobile phase. The chromatographic analysis utilized an Agilent 1,200 series HPLC system with a C18 reversed-phase column (150 mm × 4.6 mm, 5 µm). The mobile phase comprised acetonitrile and phosphate buffer (pH 3.5) in a 50:50 v/v ratio, with a flow rate of 1.0 mL/min and detection wavelength set at 309 nm. Included in the laboratory equipment were a vortex mixer, micropipettes, and a pH meter for the preparation of the mobile phase.

Preparation and evaluation of LNSLCs

LNSLCs were formulated using the microemulsion technique. The solid lipid was melted and combined with the liquid lipid to facilitate drug dissolution. This phase is identified as the lipid phase. The surfactant and lipids were heated to the same temperature to create the aqueous phase. To obtain

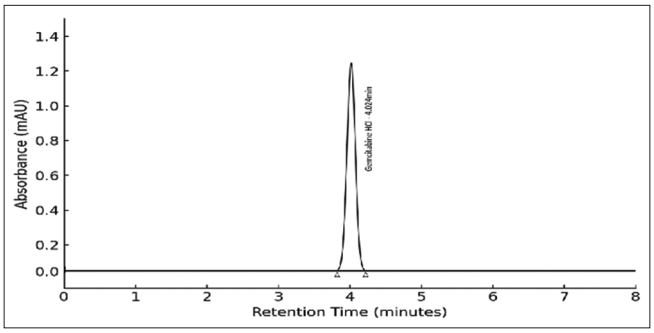

Figure 3: Standard high-performance liquid chromatography chromatogram of internal standard gemcitabine HCl in rat plasma

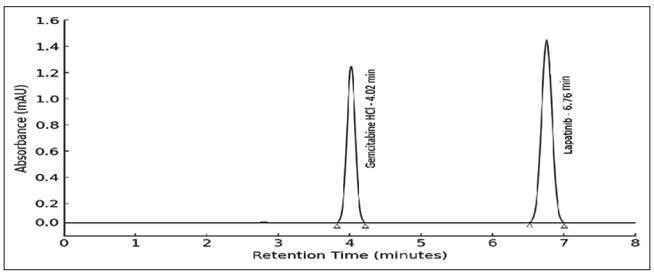

Figure 4: Standard high-performance liquid chromatography chromatogram of lapatinib and internal standard gemcitabine HCl in rat plasma

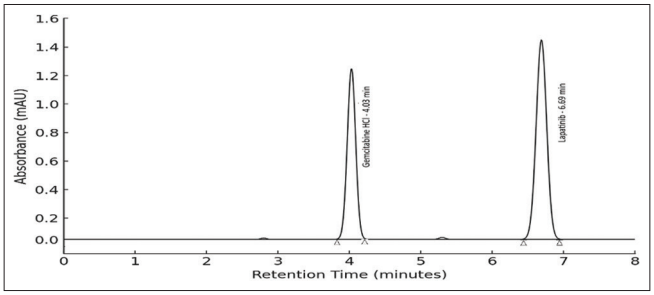

Figure 5: Formulation of high-performance liquid chromatography chromatogram of lapatinib and internal standard gemcitabine HCl in rat plasma

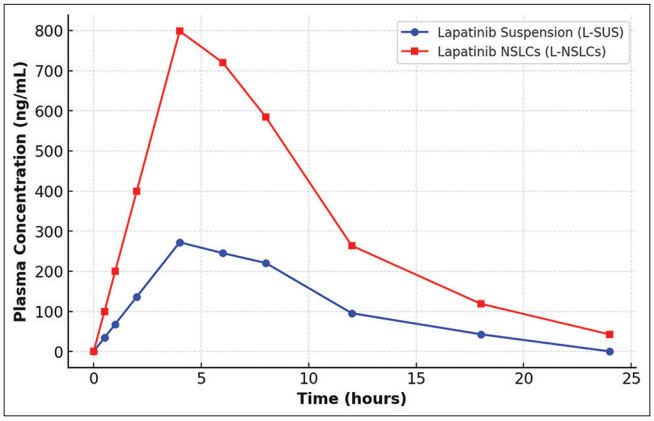

Figure 6: Plasma concentration time profile of lapatinib and nanostructured lipid carriers

a thermodynamically stable oil-in-water hot emulsion, the two phases were combined and subjected to gentle agitation. The mixture is rapidly dispersed in a substantial volume of cold water (0–4°C) while undergoing agitation, sonication, and subsequent filtration. Evaluations included assessments of particle size, cumulative percentage drug release, compatibility studies utilizing Fourier transform infrared and differential scanning calorimetry, and scanning electron microscopy analysis.[6]

In vivo studies of lapatinib

Experimental animals

Male Wistar rats, weighing approximately 250 g and aged 6–8 weeks, were selected for the pharmacokinetic investigation of lapatinib. The rats were housed under controlled environmental conditions, characterized by a 12-h light/dark cycle, a relative humidity of 50–60%, and a temperature maintained at 22 ± 2°C. Unrestricted access to water and a conventional pelleted diet were provided. To ensure uniform absorption conditions, the animals were deprived of food for approximately 12 h and given continuous access to water. The study protocol received approval from the institutional animal ethics committee of St. Paul's College of Pharmacy, in compliance with the CPCSEA guidelines (Approval No.: SPCP/2021–22/CEU/01).

Calibration graph

A validated reverse-phase HPLC (RP-HPLC) method was employed to establish a calibration curve for lapatinib. Standard solutions of lapatinib were prepared in plasma at concentrations ranging from 10 to 1,000 ng/mL. To guarantee precision and accuracy, gemcitabine HCl was implemented as an internal standard. The peak area ratio of lapatinib to gemcitabine HCl was recorded after each solution was analyzed. The peak area ratio was plotted against the lapatinib concentration to construct the calibration curve [Figure 1].[7]

Study design

The study was designed as a randomized controlled trial involving 18 male Wistar rats, which were randomly divided into three groups (n = 6 per group): Control Group: Received no drug. Group 1 (L-SUS): Received lapatinib oral suspension at a dose of 50 mg/kg Group 2 (L-NSLCs): Received NSLCs, equivalent to 50 mg/kg of lapatinib. The pure L-SUS was prepared in 0.5% CMC solution, while the NLC formulation was dispersed in distilled water. The final dose volume was adjusted to 1 mL/200 g of body weight. Formulations were administered orally using a ball-tipped gavage needle to ensure precise and uniform dosing. Post-administration, animals were monitored for 1 h for any signs of distress, abnormal behavior, or adverse effects.[8]

Blood collection

Blood samples were collected at specific time intervals: 0 (pre-dose), 0.5, 1, 2, 4, 6, 8, 12, 18, and 24 h after administration to assess the absorption, distribution, and elimination phases of lapatinib. Blood sampling was conducted through the lateral tail vein, with 0.2–0.3 mL of blood collected into heparinized microcentrifuge tubes to inhibit coagulation. The samples were centrifuged at 5,000 rpm for 10 min at 4°C to isolate the plasma, which was subsequently stored at −20°C for future analysis.[9,10]

Mobile phase preparation

The mobile phase for HPLC analysis was formulated with acetonitrile and phosphate buffer (pH 3.5, 50:50 v/v) to optimize the separation of lapatinib and gemcitabine HCl (internal standard). A phosphate buffer (0.05M, pH 3.5) was prepared by dissolving 6.8 g of potassium dihydrogen phosphate (KH_2PO_4) in 1,000 mL of deionized water, with pH adjustment achieved through the addition of ortho-phosphoric acid. Both solvents underwent filtration using a 0.22 µm membrane filter and were degassed through ultrasonication for duration of 10–15 min to eliminate dissolved gases. The mobile phase was delivered at a flow rate of 1.0 mL/min through a C18 reversed-phase column, which was equilibrated for 30 min before injection. A blank plasma sample was initially injected to identify any endogenous peaks, followed by standard solutions of lapatinib and gemcitabine HCl to verify retention times. The final plasma samples were injected, resulting in distinct, well-resolved peaks with minimal interference. Equilibration and pH control were consistently maintained during the analysis to improve peak resolution and reproducibility.[8,9]

Pharmacokinetic analysis[11,12]

Cmax (max plasma concentration)

Plasma concentration-time data directly determined Cmax. The greatest plasma concentration at any moment was Cmax for each rat. We compared the mean Cmax for each formulation to L-SUS and NSLCs.

Time to maximum concentration (tmax)

The time to attain Cmax (Tmax) was determined by observing the Cmax. It was calculated from each rat's plasma concentration-time profile and given as a median for each formulation.

$t_{1/2}$ (elimination half-life)

The elimination half-life ($t_{½}$) was calculated using the equation:

$$t_{½}=0.693/k_{el}$$

where k_{el} is the elimination rate constant. k_{el} was determined by performing linear regression analysis on the log-transformed plasma concentration values in the terminal elimination phase of the concentration-time curve.

AUC (area under the concentration-time curve)

The area under the plasma concentration-time curve ($AUC_{0-\infty}$) was calculated using the trapezoidal rule to determine systemic drug exposure. It was estimated as:

$$AUC_{0-\infty}=AUC_{0-t}+C_tk_{el}$$

where AUC_{0-} is the total AUC from time 0 to the last measurable concentration (Ct), and k_{el} is the elimination rate constant. The AUC values were compared between L-SUS and Lapatinib NSLCs to evaluate bioavailability differences.

RESULTS AND DISCUSSION

Calibration graph

The calibration graph for lapatinib in plasma was successfully established using RP-HPLC with Gemcitabine HCl as an internal standard. The calibration curve exhibited a linear response over the concentration range of 10–1,000 ng/mL with a regression coefficient (R^2) of 0.9993, indicating a strong correlation between peak area ratio and concentration.

Pharmacokinetic study

The analysis utilizing HPLC was performed to develop a reliable method for quantifying lapatinib in plasma, employing gemcitabine HCl as an internal standard. The initial chromatogram illustrates the blank plasma sample, indicating no presence of interfering endogenous peaks at the retention times of lapatinib and the internal standard. The second chromatogram illustrates the internal standard (gemcitabine HCl), which exhibits a distinct peak at approximately 4.02 min, indicating its accurate separation from other plasma components [Figure 3].

The third chromatogram illustrates the chromatographic profile of a standard lapatinib sample that has been spiked with the internal standard. Two distinct peaks were identified at 4.02 min (gemcitabine HCl) and 6.76 min (lapatinib), demonstrating the method's efficiency in separation and reproducibility [Figure 4]. The fourth chromatogram, obtained from an extracted plasma sample, exhibited well-separated peaks for both lapatinib (6.69 min) and gemcitabine HCl (4.03 min), thereby confirming the suitability of the developed method for pharmacokinetic studies [Figure 5].

The findings validate the specificity, selectivity, and reproducibility of the HPLC method for quantifying lapatinib. The clearly defined peaks and stable retention times observed in multiple runs confirm the reliability of the analytical

method. This method is effective for the pharmacokinetic evaluation of lapatinib in biological samples.

Pharmacokinetic data of lapatinib

The plasma concentration-time profile of L-SUS and L-NSLCs illustrates the pharmacokinetic differences between the two formulations [Table 1]. The L-SUS demonstrated a peak plasma concentration (Cmax) of 272.2 ng/mL at 4 h, subsequently declining to negligible levels by 24 h. The L-NSLCs formulation demonstrated a markedly elevated Cmax of 798.62 ng/mL at 4 h, signifying enhanced systemic absorption. The sustained release characteristics of L-NSLCs are demonstrated by prolonged plasma exposure, with detectable levels maintained for up to 24 h, in contrast to the suspension formulation, which showed rapid elimination. The elevated AUC values for L-NSLCs relative to L-SUS indicate improved bioavailability attributed to enhanced solubilization and lymphatic transport. The extended presence of L-NSLCs in circulation indicates its potential to decrease dosing frequency and improve therapeutic efficacy, positioning it as a viable alternative to traditional L-SUS.

The pharmacokinetic analysis of L-SUS and L-NSLCs indicates a notable enhancement in drug absorption and systemic exposure with the NSLC formulation [Table 1]. The Cmax of L-NSLCs was 798.62 ± 26.14 ng/mL, which is approximately 3 times greater than that of L-SUS (272.20 ± 16.12 ng/mL), suggesting improved solubilization and absorption of the drug. The Tmax (time to reach Cmax) remained constant at 4 h, indicating that the rate of absorption was not significantly altered; instead, the extent of absorption was enhanced.

The AUC values, reflecting overall drug exposure, exhibited a significant improvement for the NSLCs. The AUC_{0-t} (restricted to the final sampling point) for L-NSLCs (32116.62 ± 421.20 ng-h/mL) was 3.8 times higher than that of L-SUS (8432.75 ± 682.21 ng-h/mL), indicating enhanced bioavailability. $AUC_{0-\infty}$, indicating total systemic exposure, was 9.6 times greater for L-NSLCs (92903.96 ± 21646.64 ng·h/mL) compared to L-SUS (9620.75 ± 781.21 ng-h/mL), indicating extended drug circulation.

The biological half-life ($t_{½}$) of L-NSLCs (59.62 ± 18.62 h) was significantly longer than that of L-SUS (13.20 ± 2.42 h), suggesting sustained drug release and delayed elimination. The prolonged half-life may result in decreased dosing frequency and improved therapeutic outcomes. The enhanced bioavailability of L-NSLCs results from improved drug solubilization, extended systemic circulation, and possible lymphatic uptake, thereby circumventing first-pass metabolism.

The pharmacokinetic data reveal a significant 3.8–9.6-fold increase in bioavailability with NSLCs, indicating enhanced absorption and prolonged drug release, which may result in improved therapeutic efficacy when compared to the traditional suspension formulation.

CONCLUSION

The pharmacokinetic evaluation of L-NSLCs indicated a notable enhancement in drug absorption and systemic exposure relative to L-SUS. The formulation of L-NSLCs demonstrated an increased Cmax, extended half-life, and significantly elevated AUC values, suggesting improved bioavailability. The prolonged drug release and extended circulation duration of L-NSLCs indicate their potential to enhance therapeutic efficacy and decrease dosing frequency. This study demonstrates the efficacy of lipid-based nanocarriers in addressing the solubility and permeability challenges associated with lapatinib, presenting a viable strategy for enhancing the oral delivery of BCS Class IV anticancer drugs.

REFERENCES

1. Khan S, Sharma A, Jain V. An overview of nanostructured lipid carriers and its application in drug delivery through different routes. Adv Pharm Bull 2023;13:446-60.
2. Li M, Zheng Y, Shan FY, Zhou J, Gong T, Zhang ZR. Development of ionic-complex-based nanostructured lipid carriers to improve the pharmacokinetic profiles of breviscapine. Acta Pharmacol Sin 2013;34:1108-15.
3. Brito Raj S, Chandrasekhar KB, Reddy KB. Formulation, *in-vitro* and *in-vivo* pharmacokinetic evaluation of simvastatin nanostructured lipid carrier loaded transdermal drug delivery system. Future J Pharm Sci 2019;5:9.
4. Rangaraj N, Pailla SR, Shah S, Prajapati S, Sampathi S. QbD aided development of ibrutinib-loaded nanostructured lipid carriers aimed for lymphatic targeting: Evaluation using chylomicron flow blocking approach. Drug Deliv Transl Res 2020;10:1476-94.
5. Beloqui A, Del Pozo-Rodríguez A, Isla A, Rodríguez-Gascón A, Solinís MÁ. Nanostructured lipid carriers as oral delivery systems for poorly soluble drugs. J Drug Deliv Sci Technol 2017;42:144-54.
6. Haritha PN, Janakiraman K, Madhav KV, Chaitanya PS. QBD-powered design and optimization of nanostructured lipid carriers loaded with BCS class IV anticancer drug. Ind J Pharm Educ Res 2024;58:s828-36.
7. Rangaraj N, Pailla SR, Chowta P, Sampathi S. Fabrication of ibrutinib nanosuspension by quality by design approach: Intended for enhanced oral bioavailability and diminished fast fed variability. AAPS PharmSciTech 2019;20:326.
8. Fda.Gov. Available from: https://www.fda.gov/media/72286/download [Last accessed on 2025 Mar 10].
9. Mordenti J. Man versus beast: Pharmacokinetic scaling

in mammals. J Pharm Sci 1986;75:1028-40.
10. Li X, Jia X, Niu H. Nanostructured lipid carriers co-delivering lapachone and doxorubicin for overcoming multidrug resistance in breast cancer therapy. Int J Nanomedicine 2018;13:4107-19.
11. Kumar KK, Nagoji KE, Nadh RV. A validated RP-HPLC method for the estimation of lapatinib in tablet dosage form using gemcitabine hydrochloride as an internal standard. Indian J Pharm Sci 2012;74:580-3.
12. Mondal S, Biswal S, Bhar K. Determination of lapatinib in bulk and tablet dosage form using ultraviolet spectrophotometric and RP-HPLC analytical methods. Int J Pharm Investig 2021;11:208-13.

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