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Bioanalytical method development and validation for the pharmacokinetics and biodistribution study of pirfenidone-loaded solid lipid nanoparticles

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ABSTRACT

Pirfenidone (PRF) is a synthetic compound used to treat idiopathic pulmonary fibrosis, but its oral administration may cause systemic side effects. This study aimed to develop and validate a bioanalytical method to quantify PRF and evaluate the pharmacokinetic and biodistribution profile of PRF-loaded solid lipid nanoparticles (SLNs) delivered via the pulmonary route. A high-performance liquid chromatography (HPLC) method was validated following the Food and Drug Administration (FDA) guidelines, showing acceptable selectivity, accuracy, precision, and stability in plasma and lung tissue. PRF SLNs were prepared by solvent injection and had a mean particle size of 240.3 ± 3.57 nm and a polydispersity index of 0.386–0.392. Intratracheal administration of PRF SLNs resulted in higher maximum concentration and Area Under the Curve (AUC) values than oral PRF. Biodistribution studies showed that PRF SLNs achieved approximately fivefold greater AUC in lung tissue than free PRF, with lower distribution in the liver and kidneys. These findings indicate that pulmonary PRF SLNs enhance lung targeting and may reduce systemic exposure, while the validated HPLC method supports reliable pharmacokinetic and tissue distribution analysis.

1. INTRODUCTION

Idiopathic pulmonary fibrosis (IPF) is a long-term lung condition marked by the gradual formation of scar tissue in the alveoli. One of the pharmacological therapies recommended for the treatment of IPF is pirfenidone (PRF). PRF works by inhibiting the formation of scars in the lungs by suppressing fibroblast proliferation [1]. PRF can also inhibit the loss of lung function caused by scarring in the alveolar area of the lungs so that gas exchange in the alveoli is disrupted [2].

PRF is commercially available in tablet and capsule form at a dose of 801 mg/day. However, taking high doses of PRF can lead to various systemic side effects, including nausea, indigestion, diarrhea, and vomiting, as well as rash and hypersensitivity. To minimize gastrointestinal side effects, PRF is recommended to be taken with food. Food intake reduces the absorption rate and peak plasma maximum concentration (C_{max}), which may help reduce the adverse effects of PRF [3].

One alternative route for delivering PRF directly to the lungs in order to maximize IPF therapy is to use the pulmonary delivery route. Pulmonary delivery can be an alternative to systemic administration with the aim of achieving high local lung concentrations, thereby improving pharmacological therapy and reducing systemic side effects [4].

To achieve high local lung concentrations, various types of nanoparticles have been widely used in pulmonary delivery systems. One of them is solid lipid nanoparticles (SLNs). SLN was chosen as a carrier for pulmonary delivery because it has nano-sized particles that can reach the alveolar area of the lung, which is the location where progressive scarring occurs in IPF [5].

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A recent study by Kwok *et al.* [6] showed that PRF formulated into SLN had ideal physical characteristics with a particle size of 212.7 nm, a polydispersity index (PDI) of 0.39, and an entrapment efficiency of 95.02% [6]. The results also showed an increase in the cumulative dissolution of PRF SLN, which was 89.61% in the lung fluid medium. However, the results of this study do not yet show data from an *in vivo* study using the pulmonary delivery route regarding pharmacokinetic profiles and biodistribution tests.

Various analytical methods, such as high-performance liquid chromatography (HPLC), were used to quantify PRF in biological fluids. Although several methods for PRF analysis have been previously reported, studies applying validated HPLC methods to both plasma and lung tissue matrices in a pulmonary delivery context remain limited. Therefore, this study aims to develop and validate a bioanalytical method to quantify PRF and evaluate the pharmacokinetics and biodistribution of PRF SLN in rats using pulmonary administration.

2. MATERIALS AND METHODS

2.1. Materials

PRF was obtained from Accela Chembio (Shanghai, China), Glyceryl Monostearate (GMS) was sourced from Spectrum (New Jersey, USA), and Tween 80 was supplied by Gracefruit Ltd. (Bonnybridge, United Kingdom). Methanol, ethanol, and acetonitrile (CAN) were purchased from Merck (Darmstadt, Germany). Plasdone K-29/32 and Klucel LF were kindly provided by Ashland Pharmaceuticals (Wilmington, USA). A blood bank in Indonesia, called the Indonesian Red Cross Society, provided samples of human plasma. The study received approval from the Ethical Committee of Cipto Mangunkusumo Hospital, Faculty of Medicine, Universitas Indonesia, under Registration Number KET-161/UN2. F1/ETIK/PPM.00.02/2024.

2.2. Methods

2.2.1. Calibration standard solutions

Stock solutions of PRF were prepared at a concentration of 1 mg/ml in methanol. These stock solutions were then serially diluted with blank plasma to obtain calibration standard solutions at concentrations of 0.5, 1.0, 5.0, 10.0, 25.0, and 50.0 µg/ml. Each calibration standard was prepared fresh for each analytical run.

2.2.2. Quality control (QC) samples

QC samples were prepared at four concentration levels: lower limit of quantification (LLOQ, 0.5 µg/ml), low QC (LQC, 1.5 µg/ml), medium QC (MQC, 25 µg/ml), and high QC (HQC, 37.5 µg/ml). These samples were obtained by spiking blank rat plasma with PRF stock solution.

2.2.3. Chromatographic conditions

The HPLC system from Shimadzu (Japan) features a UV detector and utilizes a C18 column (4.6 × 250 mm, 5 µm) from Zorbax Eclipse Plus (Agilent, USA) for separation. The mobile phase was composed of phosphate buffer (pH 3.5) and acetonitrile (ACN) in a 60:40 v/v ratio, maintained at 30°C with a flow rate of 0.7 ml/min. An injection volume of 20 µl was employed, and the analysis was conducted at a wavelength of 310 nm.

Chromatographic conditions were optimized by experimentally evaluating various mobile phase compositions, pH values, and flow rates. The combination of ACN and phosphate buffer (60:40, v/v) at pH 3.5 was selected based on its ability to produce symmetrical, well-resolved peaks with minimal tailing. These conditions consistently provided optimal separation of PRF in both plasma and lung tissue samples.

2.2.4. Preparation of rats plasma and tissue homogenate

The blood was withdrawn from the rat's sinus retro-orbital and collected into a vial containing anticoagulant [10% Ethylenediamine tetra acetic acid (EDTA)]. For the separation of plasma, the collected blood was centrifuged at 10,000 rpm for 10 minutes at 25°C using a microcentrifuge (The Spectrafuge TM, USA), after which plasma was separated carefully and stored at −20°C. For a collection of the tissue, rats were anesthetized using diethyl ether and sacrificed humanely, followed by dissecting the animals to collect different organs such as the lung, liver, and kidney. The isolated organs were weighed and then homogenized in 0.9% NaCl. The final volume of the homogenate was 10 ml. The whole tissue was collected carefully and stored at −20°C till further use.

2.2.5. Sample extraction method

PRF was extracted from plasma utilizing the protein precipitation method. PRF with a certain concentration was spiked into 100 µl of human plasma in a microtube, followed by the addition of 50 µl of carbamazepine as the internal standard (IS) [9]. The resulting mixture was vortexed for 30 seconds. Next, 100 µl of ACN was added to precipitate proteins, followed by an additional vortex for 2 minutes. The samples were then centrifuged at 10,000 rpm for 10 minutes at 25°C. After centrifugation, the supernatant was carefully transferred to a clean vial and directly injected into the HPLC system for analysis [7].

2.2.6. Bioanalytical method validation

The procedure for quantifying PRF was validated in accordance with the guidelines established by the Food and Drug Administration [8] for bioanalytical method validation.

2.2.6.1. Calibration curves

Calibration curves were developed by analyzing spiked calibration samples over a period of three different days. The ratios of the peak areas of PRF to the IS were graphed against their corresponding analyte concentrations, and standard curves were established through linear regression analysis. The LLOQ was determined as the lowest concentration that could be quantified with acceptable accuracy and precision based on calibration curve validation.

2.2.6.2. Selectivity

To assess the method's selectivity and detect potential interference from components in plasma, six different blank

plasma samples were injected. The retention times for the peaks of PRF and the IS were examined.

2.2.6.3. Precision and accuracy

Precision and accuracy were determined by evaluating QC standards with the LLOQ, 0.5 µg/ml, LQC, 1.5 µg/ml, MQC, 25 µg/ml, and HQC, 37.5 µg/ml. This assessment aimed to measure both intra-day and inter-day precision and accuracy, requiring six replicates of each concentration to be analyzed over three consecutive days.

2.2.6.4. Recovery

QC standards (LQC, MQC, and HQC) were employed to determine plasma recovery. The effectiveness of PRF recovery was assessed by comparing the peak areas of each extracted QC standard to those of the standard solutions that were not extracted. This evaluation of recovery was performed in triplicate, and the IS was assessed using the same methodology.

2.2.6.5. Carryover

The carryover of PRF was evaluated by injecting the Upper Limit of Quantification (ULOQ) standard extract immediately before the plasma blank extract. A minimum of five replicates is required to ensure accurate measurements within the calibration range.

2.2.6.6. Stability study

The stability of PRF was evaluated throughout sample collection, handling, and after both short-term and long-term storage. Freeze-thaw stability was tested by subjecting LQC and HQC samples to three freeze-thaw cycles in triplicate. Each concentration was also maintained at room temperature for 24 hours. For long-term stability, the samples were stored at −20°C for 14 days, after which the analyte concentration was measured.

2.2.7. Partial validation of PRF in rat plasma

A PRF solution in rat plasma was prepared using the concentrations established in the calibration curve, including LLOQ, LQC, MQC, and HQC. Following the sample extraction method, the supernatant was collected and analysed by HPLC with an injection volume of 20 µl. The parameters evaluated included selectivity, LLOQ, and intra-day precision and accuracy tests.

2.2.8. Partial validation of PRF in rat lung tissue

In addition to plasma, a partial validation of PRF quantification was conducted in lung tissue to ensure the suitability of the extraction method in this matrix. The validation parameters included selectivity, LLOQ, and intra-day precision accuracy tests, confirming the reliability of the method for lung tissue analysis. Following the tissue preparation and extraction method, the supernatant was collected and analysed by HPLC with an injection volume of 20 µl.

2.2.9. Preparation of PRF-SLN

PRF SLN containing PRF and lipid (GMS) in a ratio of 1:6 w/w was prepared using the solvent injection method, as done by Kwok *et al.* [6]. However, in this research, the PRF SLN was made without lyophilization [6].

2.2.10. Particle size and PDI measurements

Particle size and PDI were measured using a dynamic light scattering instrument (Malvern Zetasizer Nano ZS, UK). Before measurement, the 10 ml samples were diluted tenfold with distilled water to ensure optimal particle dispersion and minimize multiple scattering effects [6].

2.2.11. Pharmacokinetic study

Pharmacokinetic studies were conducted on 18 male Sprague Dawley rats with body weight (BW) ranging between 200 and 250 g, divided into three groups ($n = 6$). The first group received a 10 mg/kg BW dose of free PRF solution (10 mg/ml) orally. The second group was administered the same free PRF solution at 10 mg/kgBW via the intratracheal route, while the third group received a 10 mg/kgBW dose of PRF SLN suspension (10 mg/ml) intratracheally. For the second and third groups, rats were anesthetized with an intraperitoneal injection of ketamine (75 mg/kgBW) and xylazine (12 mg/kgBW). PRF SLN was then administered intratracheally by surgically exposing the trachea and inserting a 30-gauge needle through an incision between the tracheal rings. Air was used to help distribute the PRF SLN into the lung tissues, and the rats were shaken several times to ensure proper distribution.

Rat's blood (0.5 ml) was taken from the retro-orbital sinus at intervals (10, 20, 30, 60, 90, 120, 150, and 180 minutes). These samples were transferred into Eppendorf tubes containing sodium tri-potassium EDTA as an anticoagulant. Plasma was separated by centrifugation, and the amount of the drug was quantified using the developed and validated method of HPLC. The HPLC system, produced by Shimadzu (Japan), featured a UV detector and a C18 column (4.6 × 250 mm, 5 µm, Zorbax Eclipse Plus, Agilent, USA) for separation purposes. The mobile phase utilized a 60:40 v/v combination of phosphate buffer (pH 3.5) and ACN at a flow rate of 0.7 ml/min and a column temperature of 30°C. An aliquot (20 µl) was injected volume, with the analysis performed at a wavelength of 310 nm.

2.2.12. Biodistribution test

The biodistribution study was conducted by dividing rats into two groups ($n = 15$). The first group received a 10 mg/kgBW dose of free PRF solution (10 mg/ml) via the intratracheal route, while the second group was administered PRF SLN suspension (10 mg/ml) at the same dose via the intratracheal route. Rats were sacrificed at time points of 10, 30, 60, 120, and 180 minutes. Blood samples were collected into Eppendorf tubes containing EDTA, and the amount of the drug was quantified using the developed and validated method of HPLC. The lungs, liver, and kidneys were harvested and rinsed with 0.9% saline. Each tissue was then homogenized with saline (5 ml per gram of tissue) at 12,000 rpm for 3 minutes. The tissue homogenates were then mixed with ACN (1:1 v/v) to precipitate proteins and extract the drug. The mixture was centrifuged at 10,000 rpm for 10 minutes at 25°C to collect the supernatant [10]. All samples were processed and analyzed

using the HPLC system, following the same procedures as in the pharmacokinetic studies.

2.2.13. Statistical analysis

Statistical analysis was conducted using SPSS version 26, and a significance level of $p < 0.05$ was used to determine statistical significance.

3. RESULTS AND DISCUSSION

3.1. Validation of the developed HPLC method

According to this study, the time retention, theoretical plate, and tailing factor achieved by PRF and IS fulfilled the necessary criteria for optimal system performance. The details of the system suitability are shown in Table 1.

3.1.1. Lower limit of quantification

The LLOQ value must be at least 1/20 of the C_{max} [11]. In this study, the LLOQ was set at 1 µg/ml based on previous research, where the C_{max} of PRF was 21.8 µg/ml [12]. A concentration of 1 µg/ml was prepared in plasma, and five replicates were performed. The results showed that the 1 µg/ml concentration met the required specifications, with a percentage difference (% diff) ranging from −16.042% to −19.840% and a coefficient of variation (% CV) of 0.812%, both of which were within the acceptable limits of < ± 20%. The LLOQ was then tested at a half dilution (0.5 µg/ml), with five replicates carried out. The results indicated that the 0.5 µg/ml concentration also met the criteria, with % diff ranging from −13.293% to −16.238% and a % CV of 0.424%. Further dilution to a quarter concentration (0.25 µg/ml) was then tested with five replicates. However, the 0.25 µg/ml concentration did not meet the required specifications, as the % diff ranged from −34.561% to −50.376%, and the % CV was 0.145%.

3.1.2. Calibration curve

The calibration curve was established over a concentration range of 0.5 to 50 µg/ml, which included blank samples, zero samples, and six distinct concentrations: 0.50, 1.00, 5.00, 10.00, 25.00, and 50.00 µg/ml. The calibration curve equation was y = 0.3741x + 0.0004, with the linear regression value obtained (R^2) = 0.9993 (Fig. 1), where the *x* value was the concentration of PRF, and the *y* value was the peak area ratio (PAR) between PRF and IS. The linear regression value obtained has met the requirements, namely ≥0.995 [8]. These results showed that the calibration curve was linear.

Table 1. Results of system suitability ($n = 6$).

Parameter	Acceptance criteria	Observed	
		PRF	IS
Tailing factor	<2.0	1.37 ± 0.11	1.53 ± 0.13
Theoretical plate count	>2,000	4,353 ± 72.0	4,733 ± 82.9

3.1.3. Accuracy and precision

The intra-day accuracy values were 84.00% (LLOQ), 114.00% (LQC), 113.92% (MQC), and 112.40% (HQC), with corresponding %RSD values of 2.38%, 0.58%, 1.09%, and 1.33%, respectively. Inter-day accuracy values were 84.00% (LLOQ), 113.33% (LQC), 107.00% (MQC), and 106.67% (HQC), with %RSD values of 2.38%, 0.59%, 5.68%, and 5.22%, respectively. All accuracy and precision values were within the acceptance limits as defined by the Food and Drug Administration [FDA (2018)] and EMA (2011), which recommend accuracy within ±20% for LLOQ and ±15% for LQC, MQC, and HQC, with precision (%RSD) not exceeding 15%. These results confirm that the method is accurate, reproducible, and suitable for the quantification of PRF in biological matrices. The precision and accuracy results are summarized in Table 2.

3.1.4. Recovery

The recovery was observed by comparing the peak area of the analyte and IS (PAR) obtained by extraction with the peak area of the analyte and IS (PAR) without extraction (actual peak area) carried out at each concentration. The recovery was conducted at three different concentrations, namely 1.5 µg/ml (LQC), 25.0 µg/ml (MQC), and 37.5 µg/ml (HQC), with three replicas carried out. The results showed the average PRF % recovery values for LQC, MQC, and HQC were, respectively, 80.86%, 84.38%, and 80.53% (Table 3). Recovery value does not have specific requirements; recovery does not have to be 100%, but the results obtained must be consistent and reproducible [8].

3.1.5. Selectivity

Selectivity was conducted to determine the possibility of interference in the analyte and IS. A selectivity test was conducted on blank plasma and an LLOQ concentration of 0.5 µg/ml using six different plasmas. The selectivity results showed that the percentage of interference components present in the retention time of PRF ranged from 0% to 6.87%, while no interference components were detected at the retention time

Table 2. Precision and accuracy of PRF.

Concentration	Intraday			Interday		
	Measured concentration (Mean ± SD; µg/ml)*	Accuracy (%)	RSD (%)	Measured concentration (Mean ± SD; µg/ml)*	Accuracy (%)	RSD (%)
LLOQ (0.5 µg/ml)	0.42 ± 0.01	84.00	2.38	0.42 ± 0.01	84.0	2.38
LQC (1.5 µg/ml)	1.71 ± 0.01	114.00	0.58	1.70 ± 0.01	113.0	0.59
MQC (25.0 µg/ml)	28.48 ± 0.31	113.92	1.09	26.75 ± 1.52	107.0	5.68
HQC (37.5 µg/ml)	42.15 ± 0.56	112.40	1.33	40.00 ± 2.09	106.67	5.22

*All values were represented as the mean ± SD ($n = 6$).

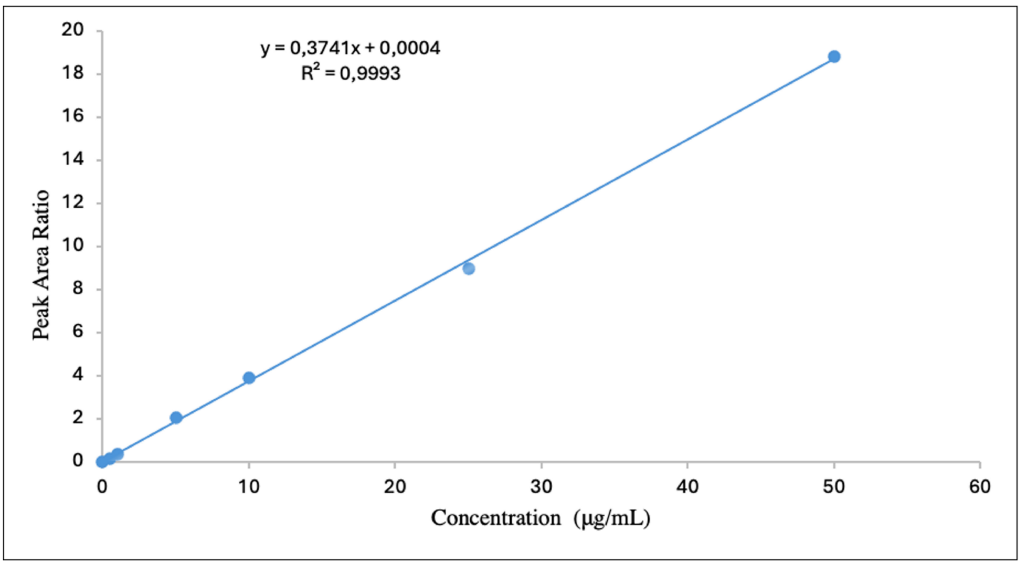

Figure 1. A representative calibration curve of PRF in human plasma.

Table 3. Recovery values of PRF in plasma samples.

Concentration	Extracted	Unextracted	CV (%)	PRF recovery (%)	IS recovery (%)
	Measured concentration (Mean ± SD; µg/ml)				
LQC (1.5 µg/ml)	1.66 ± 0.07	0.64 ± 0.01	4.79	80.86	80.68
MQC (25.0 µg/ml)	25.72 ± 0.17	9.40 ± 0.08	1.16	84.38	80.10
HQC (37.5 µg/ml)	46.99± 0.32	18.09 ± 0.12	0.26	80.53	80.52

%Recovery was calculated from peak area: (Extracted / Unextracted) × 100. All data were represented as the mean ± SD (n = 3).

of the IS (Figs. 2 and 3). According to the criteria, this method demonstrated selectivity since the percentage interference for all replicate analyses at LLOQ concentrations was less than 20%, while the IS showed interference below 5% [11].

3.1.6. Carry over

Carryover was conducted to determine the possibility of residual analyte participation after PRF was injected at the ULOQ. The results showed that carryover was 0% in the blank plasma after injecting the ULOQ of PRF, and the IS was 0%. Based on the results, the criteria were satisfied, as the PRF concentrations were below 20% and the IS was under 5%.

3.1.7. Stability

All results showed that PRF remained stable under the tested conditions, including freeze–thaw cycles, short-term exposure, long-term storage, and autosampler conditions (Table 4). The measured concentrations appeared slightly higher than the nominal concentrations, which may be attributed to matrix effects commonly observed in biological samples. Nevertheless, the results were highly reproducible, with %CV values ranging from 0.27% to 2.65%, which are well within the acceptable precision limits (<15%) set by regulatory guidelines (FDA, EMA). Therefore, the stability of PRF under all tested conditions was considered acceptable.

3.2. Partial validation of PRF in rat plasma and lung tissue

A partial validation of PRF in rat plasma and lung tissue was performed to confirm that the fully validated bioanalytical method remains applicable to a different biological source while maintaining accuracy, reliability, and consistency in its results. According to the FDA Bioanalytical Method Validation Guidance (2018), partial validation is permitted when applying an existing validated method to the same type of biological matrix (e.g., plasma or tissue) across different species, such as from human to rat plasma.

In this study, partial validation was conducted following the transfer of the method from human plasma to rat plasma and lung tissue. The parameters tested included selectivity, LLOQ, and intra-day accuracy and precision. The LLOQ concentration met the required specifications, with % difference and % CV values both below ±20%, and no interfering components were detected in rat plasma or lung tissue. Intra-day accuracy and precision were evaluated at four concentrations [0.5 µg/ml (LLOQ), 1.5 µg/ml (LQC), 25.0 µg/ml (MQC), and 37.5 µg/ml (HQC)] and also fulfilled the acceptance criteria, with % difference and % CV within ±20%.

3.3. Particle size and PDI of PRF SLN

The characterization of PRF SLN is presented in Table 5. The median particle size (Dv50) was 234.7 ± 3.30 nm.

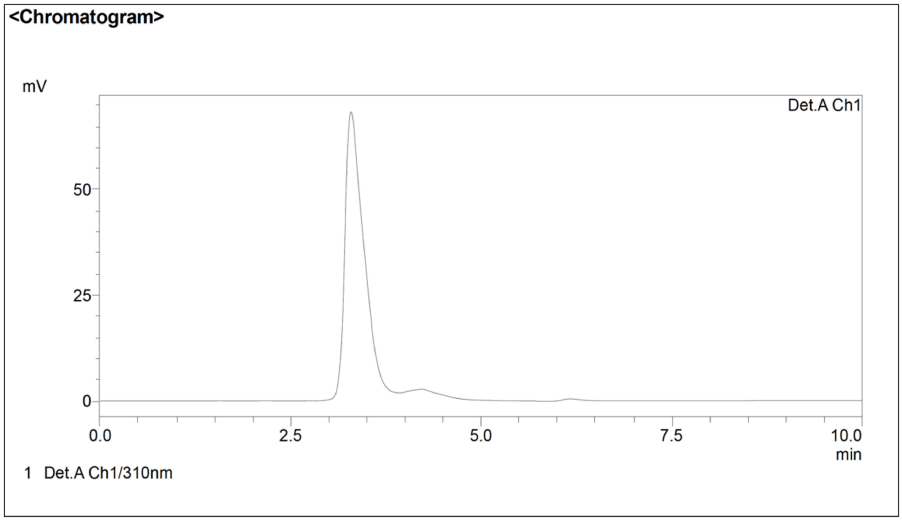

Figure 2. Chromatograms of blank plasma.

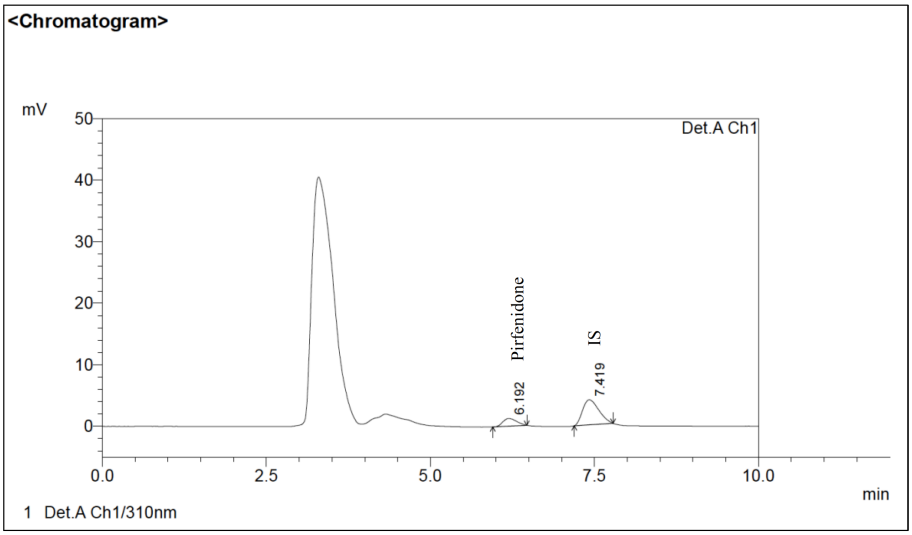

Figure 3. LLOQ chromatograms of PRF and IS in plasma.

The Dv mean and *Z*-average were 240.3 ± 3.57 and 327.5 ± 7.80 nm, respectively, and the PDI ranged from 0.386 to 0.392. These results are consistent with previous studies on SLN formulations. For example, Kwok *et al.* [6] reported a particle size of 212.7 nm for PRF-loaded SLNs. Particle size is an important determinant of pulmonary deposition, with particles smaller than 5 µm capable of reaching the alveolar region [13]. Clearance through alveolar macrophages is size-dependent, with optimal phagocytosis occurring for particles between 1.5 and 3 µm [14]. Therefore, PRF SLNs with a median size of 234.7 ± 3.30 nm are expected to evade macrophage clearance and prolong retention in the lung tissue.

3.4. Pharmacokinetic study

The average plasma concentration-time profiles following intratracheal and oral administration at a dose of 10 mg/kg BW are illustrated in Figure 4. The pharmacokinetic parameters were detailed in Table 6. Each of the three groups received the identical dose of 10 mg/kg BW. Results showed that both intratracheal routes resulted in higher plasma C_{max} than the oral group ($p < 0.05$). The intratracheal group (PRF SLN and free PRF) produced a C_{max} that increased 3 to 4 fold that of the oral group, namely 5.11 ± 0.04, 6.00 ± 0.05, and 1.50 ± 0.03 µg/ml, respectively. PRF SLN produced a lower C_{max} at time (T_{max}) 20 minutes compared to free PRF, which produced a higher C_{max} at time 10 minutes. The SLN system has a lipid matrix structure that allows slower drug release, while free PRF without a lipid matrix can easily enter the lung tissue and quickly distribute to the systemic circulation [15]. This allows PRF SLN to have a lower peak C_{max} and a slightly slower T_{max} compared to free PRF.

Comparable results were observed in the AUC0-∞. The findings indicated that the intratracheal group (PRF SLN and free PRF) exhibited higher values than the oral group, with

Table 4. Stability studies of PRF.

Storage condition	LQC (1.5 µg/ml)		HQC (37.5 µg/ml)	
	Measured concentration (Mean ± SD; µg/ml)	CV(%)	Measured concentration (Mean ± SD; µg/ml)	CV(%)
Short term (25°C, 24 hours)	1.85 ± 0.02	1.09	45.03 ± 0.2	1.16
Long term (−20°C, 14 days)	1.81 ± 0.03	1.93	43.71 ± 0.85	1.95
Freeze-thaw three cycles (−20°C)	1.85 ± 0.01	0.27	46.10 ± 0.58	1.25
Autosampler stability (25°C, 24 hours)	1.80 ± 0.05	2.65	41.09 ± 0.62	1.51

All data were represented as the mean ± SD (n = 3).

Table 5. PRF SLN characterization.

Parameters	Average ± SD
Dv 10 (nm)	164.0 ± 3.70
Dv 50 (nm)	234.7 ± 3.30
Dv 90 (nm)	328.7 ± 7.91
Dv mean (nm)	240.3 ± 3.57
Z-avg (nm)	327.5 ± 7.80
PDI	0.389 ± 0.03

All values were represented as the mean ± SD (n = 3).

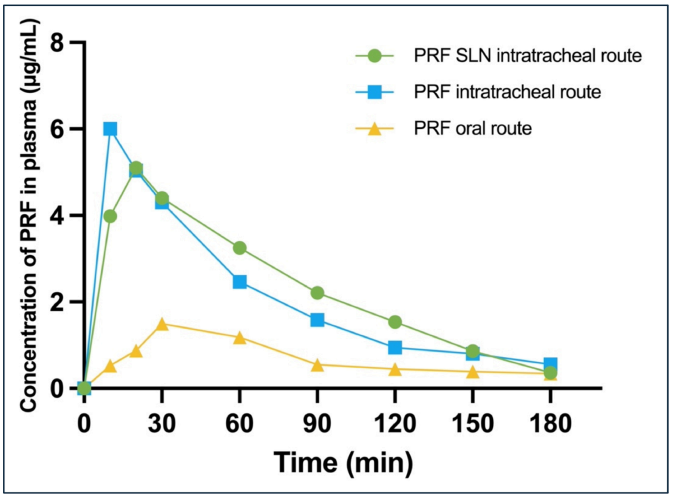

Figure 4. Mean plasma concentration-time profiles for PRF after administration to rats. Each point represents the mean ± SD (n = 6).

measurements of 7.26 ± 0.05, 7.22 ± 0.24, and 3.39 ± 0.41 µg.h/ml, respectively (p < 0.05). The intratracheal group will more quickly reach the lung organ, which has a large surface area and extensive blood vessels that allow rapid absorption of drugs into the bloodstream [16]. In addition, the lungs have lower enzymatic activity than the digestive tract, resulting in less drug degradation and higher bioavailability [17]. This may increase the amount of drug in the systemic circulation over time, resulting in high Area Under the Curve (AUC) values in the intratracheal group compared to the oral group.

The intratracheal group (PRF SLN and free PRF) had a faster elimination half-life ($t_{1/2}$) compared to the oral group. PRF SLN was cleared from plasma faster with a $t_{1/2}$ of 0.48 ± 0.02 hours, which is 2.7-fold lower than the $t_{1/2}$ of the free PRF group and 5.7 fold than the oral group, where the $t_{1/2}$ of free PRF was 1.33 ± 0.29 hours, and the oral group was 2.74 ± 0.68 hours. These results indicate that the intratracheal group, especially PRF SLN, showed faster drug elimination in the systemic circulation compared to the oral group (p < 0.05). This result correlates with the results of the elimination rate constant (Ke), which shows that PRF SLN has a Ke value that is 4.3-fold higher than that of the free PRF and oral groups. The Ke result correlates with $t_{1/2}$, which indicates a rapid rate of elimination from the systemic circulation. This is in accordance with the aim of pulmonary drug delivery, which favors local drug action in the lungs with reduced systemic exposure; it can also be advantageous in reducing systemic side effects of PRF [4].

3.5. Biodistribution test

The deposited PRF concentrations after intratracheal administration are presented in Figure 5. The C_{max} and AUC0-∞ parameters are shown in Table 7. Biodistribution tests were conducted to compare PRF SLN and free PRF formulations administered intratracheally. Results showed that the largest C_{max} and AUC0-∞ values were found in plasma. The C_{max} and AUC0-∞ values between PRF SLN and free PRF in plasma were consistent with the results of the pharmacokinetic study. The amount of PRF SLN retained in the lungs progressively decreased with time. After 3 hours, the concentration of PRF in the lung tissue decreased to <1 µg/ml. A high distribution of PRF was achieved in plasma rather than lung organs in both PRF SLN and free PRF. Abundant pulmonary capillaries can lead to the rapid absorption of drugs into the blood circulation, especially particles that tend to be smaller (<0.5 µm) when entering the lungs and can be easily absorbed into the systemic circulation [14]. Particles smaller than 200 nm will not be recognized by macrophages due to their small size or faster absorption by lung epithelial cells [18]. In a different study, rats administered intratracheally with curcumin nanocrystals (NC) showed that NC-S, with a particle size of 246.16 ± 21.98 nm, exhibited reduced lung retention and enhanced systemic absorption [19]. Additional research has indicated that exposure to cerium oxide nanoparticles through intratracheal administration or inhalation can lead to their translocation into the bloodstream and accumulation in various organs, such as the liver, facilitated by their small size, which allows them to bypass the body's defense mechanisms that typically filter larger particles in the lungs [20].

Table 6. Pharmacokinetic parameters from the pharmacokinetic study.

Parameters	PRF oral route	Free PRF intratracheal route	PRF SLN intratracheal route
t_{max} (min)	0.50 ± 0.00	0.17 ± 0.00	0.33 ± 0.00
C_{max} (µg/ml)	1.50 ± 0.03	6.00 ± 0.05*	5.11 ± 0.04*#
$AUC_{0-\infty}$ (µg.h/ml)	3.39 ± 0.41	7.22 ± 0.24*	7.26 ± 0.05*
$t_{1/2}$ (h)	2.74 ± 0.68	1.33 ± 0.29	0.48 ± 0.02*#
K_e (/h)	0.33 ± 0.22	0.66 ± 2.21	1.44 ± 0.05

All values are represented as the mean ± SD (n = 6). * Intratracheal routes are compared to oral routes (p-value < 0.05). #Intratracheally PRF SLN is compared to Free PRF(p-value < 0.05).

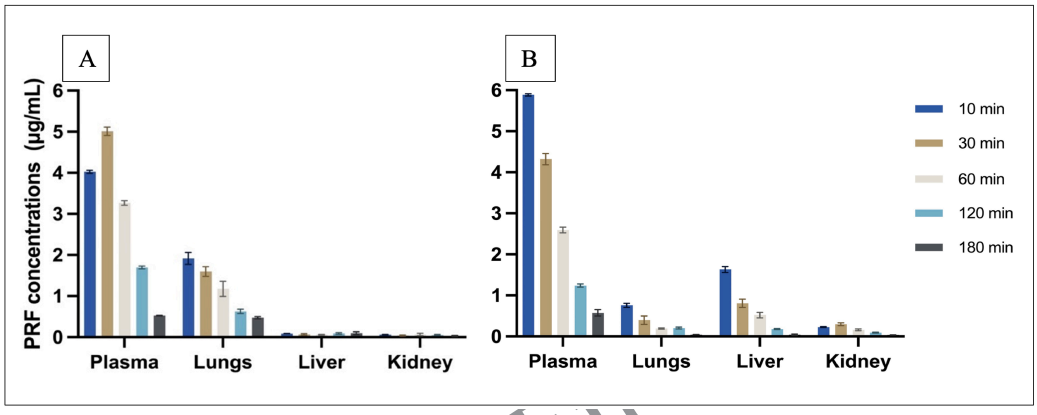

Figure 5. The deposited PRF concentration (µg/ml). (A) PRF SLN (10 mg/ml) via the intratracheal route. (B) Free PRF (10 mg/ml) via the intratracheal route. All values are presented as mean ± SD (n = 3).

Table 7. C_{max} and $AUC_{0-\infty}$ in the plasma, lung, liver, and kidney after intratracheal administration of the PRF SLN and free PRF to rats.

		Parameters		
		C_{max} (µg/ml)	AUC_{0-t} (µg.h/ml)	$AUC_{0-\infty}$ (µg.h/ml)
Plasma	PRF SLN	5.01 ± 0.10	7.50 ± 0.07	8.10 ± 0.06
	Free PRF	5.89 ± 0.03	6.74 ± 0.05	7.50 ± 0.15
Lung	PRF SLN	1.92 ± 0.15*	2.89 ± 0.17*	3.93 ± 0.15*
	Free PRF	0.76 ± 0.05	0.72 ± 0.03	0.77 ± 0.03
Liver	PRF SLN	0.11 ± 0.01	0.24 ± 0.22	0.26 ± 0.22
	Free PRF	1.63 ± 0.07	1.30 ± 0.11	1.37 ± 0.04
Kidney	PRF SLN	0.08 ± 0.02	0.36 ± 0.19	0.31 ± 0.16
	Free PRF	0.30 ± 0.04	0.56 ± 0.11	0.46 ± 0.03

All values were represented as the mean ± SD (n = 3). * PRF SLN is compared to Free PRF (p-value < 0.05).

Therefore, PRF SLN, with a particle size of 240.3 ± 3.57 nm, is expected to be quickly absorbed into the systemic circulation.

Despite the higher concentration of PRF in plasma, PRF SLN showed higher concentrations with C_{max} 1.92 ± 0.15 µg/ml, which was 2.5-fold higher compared to free PRF 0.76 ± 0.05 µg/ml (p < 0.05). The AUC0-∞ results also showed that PRF SLN was 5-fold higher than free PRF with 3.93 ± 0.15 and 0.77 ± 0.03 µg.h/ml, respectively (p < 0.05). The SLN carrier system can increase the penetration and accumulation of drugs in the lung targeting compared to free drugs [21]. In addition, the liver and kidney organs showed lower C_{max} and AUC0-∞ values of PRF SLN compared to free PRF. This indicates that PRF SLN is distributed less towards the liver and kidney organs.

4. CONCLUSION

The study demonstrated that the developed HPLC method for analyzing PRF is accurate, precise, and reliable, meeting regulatory requirements. PRF-loaded SLNs showed optimal particle size (240.3 ± 3.57 nm) for pulmonary delivery. Pharmacokinetic studies revealed higher systemic

drug exposure AUC and prolonged drug release for PRF SLNs compared to free PRF, with faster elimination, favoring localized lung action. Biodistribution analysis showed that PRF SLNs achieved higher concentrations in plasma than in the lungs, indicating rapid absorption into systemic circulation, but still demonstrated greater lung retention and reduced liver and kidney distribution compared to free PRF, supporting effective lung targeting.

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6. AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

7. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

8. ETHICAL APPROVAL

Ethical approvals details is given in the 'Material and Methods' section

9. DATA AVAILABILITY

All data generated and analyzed are included in this research article.

10. PUBLISHER'S NOTE

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11. USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declare that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

REFERENCES

1. Hazem RM, Antar SA, Nafea YK, Al-Karmalawy AA, Saleh MA, ElAzab MF. Pirfenidone and vitamin D mitigate renal fibrosis induced by doxorubicin in mice with Ehrlich solid tumor. Life Sci. 2022;288:120185. doi: https://doi.org/10.1016/j.lfs.2021.120185
2. Ruwanpura SM, Thomas BJ, Bardin PG. Pirfenidone: molecular mechanisms and potential clinical applications in lung disease. Am J Respir Cell Mol Biol. 2020;62:413–22. https://doi.org/10.1165/rcmb.2019-0328TR
3. Barranco-Garduño LM, Buendía-Roldan I, Rodriguez JJ, González-Ramírez R, Cervantes-Nevárez AN, Neri-Salvador JC, *et al.* Pharmacokinetic evaluation of two pirfenidone formulations in patients with idiopathic pulmonary fibrosis and chronic hypersensitivity pneumonitis. Heliyon. 2020;6:e05279. doi: https://doi. org/10.1016/j.heliyon.2020.e05279
4. Shi C, Ignjatović J, Wang J, Guo Y, Zhang L, Cvijić S, *et al.* Evaluating the pharmacokinetics of intrapulmonary administered ciprofloxacin solution for respiratory infections using *in vivo* and *in silico* PBPK rat model studies. Chin Chem Lett. 2023;34:107463. doi: https://doi. org/10.1016/j.cclet.2022.04.061
5. Weber S, Zimmer A, Pardeike J. Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) for pulmonary application. Eur J Pharm Biopharm. 2014;86:7–22. https://doi.org/10.1016/j.ejpb.2013.08.013
6. Kwok K, Suhariyono G, Surini S. Response surface methodology-aided development of pirfenidone-loaded solid lipid nanoparticles for intrapulmonary drug delivery system. Int J Appl Pharm. 2024;16:283–90. https://doi.org/10.22159/ijap.2024v16i4.50231
7. Cheruku S, Darna B, Medipalli V, Nekkalapudi AR, Sadasivam RK. Bioanalytical method development and validation for the quantitation of larotrectinib in human plasma: application to pharmacokinetics in healthy rabbits. J Appl Pharm Sci. 2023;13:111–8. https://doi.org/10.7324/JAPS.2023.127799
8. Food and Drug Administration (FDA), Center for drug evaluation and research. Bioanalytical method validation: guidance for industry. Silver Spring, MD: Food and Drug Admnistration, 2018.
9. Meng H, Xu Y. Pirfenidone-loaded liposomes for lung targeting: preparation and *in vitro*/*in vivo* evaluation. Drug Des Devel Ther. 2015;9:3369–76. https://doi.org/10.2147/DDDT.S84046
10. Togami K, Kanehira Y, Tada H. Pharmacokinetic evaluation of tissue distribution of pirfenidone and its metabolites for idiopathic pulmonary fibrosis therapy. Biopharm Drug Dispos. 2015;36:205–15. https://doi.org/10.1002/bdd.1932
11. Harahap Y, Saputra R, Kuswardani. Development and validation of methamphetamine analysis methods in dried blood spot sample using gas chromatography - mass spectrometry. Int Res J Pharm. 2019;10:18–24. https://doi.org/10.7897/2230-8407.1008240
12. Kang JH, Yang MS, Kim DW, Park CW. *In vivo* pharmacokinetic and pharmacodynamic study of co-spray-dried inhalable pirfenidone microparticles in rats. Drug Deliv. 2022;29:3384–96. https://doi.org/10.1080/10717544.2022.2149899
13. Huang Z, Huang Y, Wang W, Fu F, Wang W, Dang S, *et al.* Relationship between particle size and lung retention time of intact solid lipid nanoparticle suspensions after pulmonary delivery. J Control Release. 2020;325:206–22. https://doi.org/10.1016/j.jconrel.2020.06.004
14. Chaurasiya B, Zhao YY. Dry powder for pulmonary delivery: a comprehensive review. Pharmaceutics. 2021;13:1–28. https://doi.org/10.3390/pharmaceutics13010031
15. Maboos M, Yousuf RI, Shoaib MH, Nasiri I, Hussain T, Ahmed HF, *et al.* Effect of lipid and cellulose based matrix former on the release of highly soluble drug from extruded/spheronized, sintered and compacted pellets. Lipids Health Dis. 2018;17:136. doi: https://doi. org/10.1186/s12944-018-0783-8
16. Price DN, Stromberg LR, Kunda NK, Muttil P. *In vivo* pulmonary delivery and magnetic-targeting of dry powder nano-in-microparticles. Mol Pharm. 2017;14:4741–50. https://doi.org/10.1021/acs.molpharmaceut.7b00532
17. Magramane S, Pápay Z, Turbucz B, Antal I. Formulation and characterization of pulmonary drug delivery systems. Acta Pharm Hung. 2019;89:63–83. https://doi.org/10.33892/aph.2019.89.63-83
18. Huang L, Huang XH, Yang X, Hu JQ, Zhu YZ, Yan PY, *et al.* Novel nano- drug delivery system for natural products and their application. Pharmacol Res. 2024;201:107100. doi: https://doi.org/10.1016/j.phrs.2024.107100

19. He Y, Liang Y, Mak JCW, Liao Y, Li T, Yan R, *et al.* Size effect of curcumin nanocrystals on dissolution, airway mucosa penetration, lung tissue distribution and absorption by pulmonary delivery. Colloids Surf B Biointerfaces. 2020;186:110703. doi: https://doi.org/10.1016/j. colsurfb.2019.110703
20. Nemmar A, Al-Salam S, Beegam S, Yuvaraju P, Ali BH. The acute pulmonary and thrombotic effects of cerium oxide nanoparticles after intratracheal instillation in mice. Int J Nanomed. 2017;12:2913–22. https://doi.org/10.2147/IJN.S127180
21. Schneider CS, Xu Q, Boylan NJ, Chisholm J, Tang BC, Schuster BS, *et al.* Nanoparticles that do not adhere to mucus provide uniform and long-lasting drug delivery to airways following inhalation. 2017;3(4):e1601556. doi: https://doi.org/10.1126/sciadv.1601556

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