Development and validation of UPLC-MS/MS method for in vitro quantitative analysis of pyrazinamide in lipid core-shell nanoarchitectonics for improved metabolic stability

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ABSTRACT

Pyrazinamide (PZA), a medication for tuberculosis, has high aqueous solubility and low permeability, undergoes extensive liver metabolism, and exhibits liver toxicity through its metabolites. To avoid this, PZA in lipid core-shell nanoarchitectonics has been formulated to target lymphatic uptake and provide metabolic stability to the incorporated drug. The UPLC-MS/MS method for reliable in vitro quantitative analysis of pyrazinamide (PZA) in lipid core-shell nanoarchitectonics as per ICH guidance was developed and validated using the HILIC column. The developed UPLC-MS/MS method is a simple, precise, accurate, reproducible, and sensitive method for the estimation of PZA in PZA-loaded lipid core-shell nanoarchitectonics for the in vitro determination of % entrapment efficiency, % loading of pyrazinamide, and microsomal stability of lipid core-shell nanoarchitectonics in human liver microsomes. The % entrapment efficiency was found to be 42.72% (±12.60). Lipid nanoarchitectonics was found to be stable in human liver microsomes, where %QH was found to be 6.20%, that is, low clearance. Thus, this formulation is suitable for preventing PZA-mediated extensive liver metabolism. These findings are relevant for the development of other lipid-mediated, suitable, stable nanoformulations containing PZA through various in vitro methods.

KEYWORDS

pyrazinamide, validation, UPLC-MS/MS, HILIC column, in vitro, lipid core-shell nanoarchitectonics

INTRODUCTION

Tuberculosis is the deadliest disease worldwide. Drug targeting to Mycobacterium tuberculosis is challenging because of bacilli cloaking and thriving within alveolar macrophages [1]. Most first-line and combinational therapies for TB include pyrazinamide, isoniazid, ethambutol, and rifampicin. Pyrazinamide (PZA) plays a crucial role in first-line therapy and multidrug resistance therapy [2]. Being a BCS Class III drug, PZA possesses higher aqueous solubility and lower permeability [3] but undergoes extensive liver metabolism [4], which causes dose-dependent hepatotoxicity [4, 5], even though the dose has been reduced to 20–25 mg/kg from to 40–70 mg/kg [6]. PZA is metabolized in the liver by amidase and xanthine oxidase enzymes, and the resultant metabolites, such as pyrazinoic acid and 5-Hydroxy-pyrazinoic acid, exhibit potential liver toxicity [4, 5].
Considering the drawbacks mentioned above, it is necessary to avoid extensive liver metabolism in which lymphatic system uptake can possibly be executed. Lipid nanoarchitectonics are the most suitable approach for targeting lymphatic uptake and providing stability to incorporated drugs [7, 8]. The UPLC-MS/MS validated method is essential for quantifying PZA within lipid core-shell nanoarchitectonics. In vitro quantification studies, such as % entrapment efficiency (EE), % drug loading (DL), and in vitro microsomal stability in human liver microsomes (HLMs), were performed to validate the lipid core-shell nanoarchitectonics.

Some validated chromatographic methods have been reported for RP-HPLC combined with PZA from a formulation perspective [9, 10]. However, to the best of our knowledge, a validated UPLC-MS/MS method has not been reported to quantify PZA according to ICH guidelines through in vitro means. In addition, an in vitro assay of liver microsomal enzyme stability revealed the metabolic pattern of PZA-loaded lipid core-shell nanoarchitectonics.

Hence, we have developed and validated a sensitive, precise, and robust UPLC-MS/MS method in compliance with the ICH Q2 (R1) guidelines [11–13]. The method was further utilized to quantify the in vitro encapsulation behavior of lipid core-shell nanoarchitectonics with PZA incorporation. The metabolic pattern was analyzed in HLMs to assess the protection facilitated by lipid core-shell nanoarchitectonics.

**MATERIAL AND METHODS**

**Materials**

Stearic acid (97% pure), PZA (≥98% purity), and isoniazid (INH) as an internal standard (IS) of ≥98% purity, and nicotinamide adenine dinucleotide phosphate (NADPH) were acquired from Sigma-Aldrich (St. Louis, MO, USA). Soybean phospholipids (Lipoid S-75) were obtained from Lipoid (Ludwigshafen, Germany). LC-MS grade methanol, water, and formic acid were procured from Merck Specialties Pvt. Ltd. (Mumbai, India). Triple distilled water was obtained through the filtration unit of the Milli-Q system (Millipore GmBH, Germany), and the remaining solvents were filtered through a 0.22 μm Millipore membrane filter (Merck, Darmstadt, Germany) and degassed in an ultrasonic bath for 30 min. Pooled HLMs were purchased from Gibco (Thermo Fisher Scientific, Waltham, MA, USA). All other chemicals, solvents, and reagents were either LC-MS grade or analytical grade and were used for method development, validation, and in vitro applications.

**Instrumentations**

Waters H-class UPLC (Waters, Milford, MA, USA) system with a column compartment and a quaternary pump was used for effective chromatographic separation. A Waters Xevo TQ-XS triple quadrupole mass spectrometer (Waters, Milford, MA, USA) fitted with an electrospray ionization (ESI) source was used to detect and quantify PZA in vitro. The system comprising UPLC coupled with a triple quadrupole mass spectrometer was commanded under the control of MassLynx software (Version 4.2). PZA and INH were precisely weighed using a pre-calibrated semi-analytical weighing balance (ME204/AD4, Mettler Toledo, Switzerland).

**Stock, quality control (QC), and sample solutions preparation**

PZA primary stock solution was prepared in LC-MS grade methanol at a concentration of 1.0 mg/mL. A secondary standard was prepared with 20% v/v methanol to attain working standard concentrations of 1.0–200 ng/mL. Nine calibration standards (1, 5, 10, 20, 40, 80, 100, 150, and 200 ng/mL) were prepared by further dilution of the secondary standard. The IS was prepared similarly and added to each calibration standard by maintaining the same diluent and a particular part of the volume (%v/v) to attain a final concentration of 5.0 ng/mL. Three levels of quality control samples, 1.0 ng/mL (low-level control (LQC)), 10 ng/mL (middle-level control, (MQC)), and 100 ng/mL (high-level control, (HQC)) of PZA, were prepared with the addition of IS (5.0 ng/mL) to examine the precision, accuracy, and robustness of the method. Sample solutions of lipid nanoarchitectonics were prepared to examine the in vitro determination of % drug entrapment efficiency (%DEE), % drug loading (%DL), and microsomal stability of PZA-loaded lipid nanoarchitectonics in HLMs using the validated UPLC-MS/MS method.

**Analytical method validation**

The developed UPLC-MS/MS method for evaluating and quantitative analysis of PZA was validated according to the International Council for Harmonization of Technical Specifications for Pharmaceuticals for Human Use (ICH) Guideline [11]. Validation parameters such as specificity, sensitivity, linearity, range, accuracy, precision, robustness, and system suitability were investigated to align the results with the ICH guidelines Q2 (R1).

**Specificity**

Quality control samples and PZA-loaded lipid nanoarchitectonic samples were subjected to the developed method to evaluate the specificity of the method for determining the interference of any other peaks at the same retention time (R,) of PZA and IS. Furthermore, the method was evaluated using stress-induced samples of PZA to determine the interference of the degradants due to hydrolysis and oxidative stress conditions. In addition, a blank matrix of lipid nanof ormulation and human liver microsomes was evaluated to determine the specificity of the developed UPLC-MS/MS method for the quantification of PZA.
Linearity and range

PZA was weighed accurately and dissolved in methanol to make a 1.0 mg/mL concentration primary stock solution. The secondary standard was prepared with 20% v/v methanol, and a calibration curve was constructed from a range of 1.0–200 ng/mL. The ratio of the peak areas of the analyte (PZA) to the IS was plotted against the nine-point concentrations of the analyte. A linear regression model was used to construct a standard curve. The weighting factor for linearity was set at 1.0.

Accuracy

The accuracy of the method was verified by determining the significance of the percentage recovery of PZA at three different concentrations: LQC, MQC, and HQC. The QC samples, LQC (1.0 ng/mL), MQC (10 ng/mL), and HQC (100 ng/mL), were chosen to cover the entire concentration range covered by the calibration curve. Triplicate concentration samples of LQC, MQC, and HQC were taken to assess the percent recovery, percent bias, and percent RSD.

Precision

The precision of the UPLC-MS/MS method was observed for intra-day precision (repeatability) and inter-day precision (intermediate precision). The intra- and inter-day precision of the developed method was investigated by evaluating three replicates of three quality controls at concentrations of 1.0, 10, and 100 ng/mL. On the same day, low, middle, and high QC samples were prepared and analyzed for intra-day precision. In contrast, three separate LQC, MQC, and HQC samples were prepared and analyzed for inter-day precision under identical experimental conditions over three consecutive days. By studying the percent recovery, percent bias, and percent RSD, intra- and inter-day precision were determined.

Sensitivity

The detection limit (LOD) was determined based on a visualization approach to assess the sensitivity of the UPLC-MS/MS method. The lowest quantitative concentration was selected based on precision and accuracy. The quantification limit (LOQ) was calculated by evaluating the lower concentrations between 0.5 ng/mL to 1 ng/mL, which showed acceptable precision and accuracy, denoted by the respective accuracy values and percent RSD.

Robustness

By intentionally adjusting the LC conditions, such as the composition of the mobile phase and column temperature, the robustness of the method was evaluated. The mobile phase composition and column temperature were varied between ±2.0% v/v and ±2°C, respectively. To determine the robustness, three QC samples in triplicate were evaluated to determine the influence of such variations in mobile phase composition and column temperature on the retention time and percent recovery of PZA.

System suitability

A system suitability test was conducted to evaluate the system performance before, after, and during the analysis. System suitability is a vital part of the chromatographic method and is performed by injecting the MQC (10 ng/mL) into six replicates. The percentage RSD of the peak area and retention time were evaluated.

Stability studies

PZA was exposed to different stress conditions to determine hydrolytic degradation behavior. PZA (5 mg/ml) was subjected to stress conditions using 0.1N HCl and 0.1N NaOH for 8 h. Oxidative experiments were conducted for 8 h in 15% (v/v) H2O2. Before UPLC-MS/MS analysis, samples were neutralized and subjected to prior dilution with mobile phase composition as a diluent, comprising an IS.

Application of the UPLC-MS/MS method

The validated UPLC-MS/MS method was successfully applied for the in vitro quantification of PZA in PZA-loaded lipid core-shell nanoarchitectonics. The in vitro percentage efficiency of drug entrapment (%DEE), percent drug loading (%DL), and microsomal stability were systematically evaluated in HLMs of PZA in PZA-loaded lipid core-shell nanoarchitectonics.

Preparation and estimation of PZA loaded lipid core-shell nanoarchitectonics

PZA-loaded lipid nanoparticles were fabricated by a homogenization technique using stearic acid and soy lecithin as shell and core-forming materials, respectively [14]. An aqueous solution of PZA containing soy lecithin as the core-forming material was emulsified with stearic acid (shell former) using the homogenization technique. Generated PZA-loaded lipid nanoparticles were further subjected to in vitro % DEE, % DL, and microsomal stability in HLMs to establish avoidance of extensive PZA metabolism through lipid core-shell nanoarchitectonics induced by the liver. The PZA-loaded lipid core-shell nanoarchitectonics were subjected to ultrafiltration through 3 KDa and centrifuged to separate free PZA. % DEE was evaluated by determining the quantity of PZA that trapped inside the matrix core of the developed lipid core-shell nanoarchitectonics. Samples were injected to determine the % DEE value using the validated UPLC-MS/MS method.

Stability of PZA loaded lipid core-shell nanoarchitectonics in HLMs

In 100 mM potassium buffer solution (pH 7.4), 150 µL of 3.33 mg/mL of HLMs was added and pre-incubated at 37°C for 10 min. Incubation was performed in duplicate. The PZA-loaded lipid core-shell nanoarchitectonics (10 µM) was...
applied to a regular preincubation blend of HLMs to give a final volume of 330 μL. A cofactor (2.5 mM) was added to the standard preincubation mixture of 330 μL of HLMs and incubated for 60 min at 37°C. Blank samples without cofactors and zero samples with cofactors were prepared and incubated at 37°C for 60 min. IS was added to blank and zero samples. At each time interval of 5, 10, 30, and 60 min, 50 μL of the incubation mixture was withdrawn, and IS solution was added, followed by the extraction solvent. All samples were vortexed and centrifuged for 10 min at 10,000 × g, and the supernatants were subjected to UPLC-MS/MS analysis. The half-life (T1/2) and intrinsic clearance (CLint) of verapamil, a positive control, were determined by non-linear regression analysis using a one-phase decay model (GraphPad Prism, version 5). The CLint of verapamil was calculated using the formula CLint = Kel/protein concentration, where Kel is the elimination rate constant.

RESULTS AND DISCUSSION

Analytical method validation

Initially, the UPLC-MS/MS method was developed with various mobile phase ratios comprising buffer and acetonitrile in the reverse-phase column. Finally, effective chromatographic separation of PZA and IS was observed on the Waters H-class UPLC (Waters, Milford, MA, USA) system with the BEH HILIC column (100 × 2.1 mm, 1.7 μm, Waters, Milford, MA, USA) [15]. The column temperature was maintained at 45°C, and the injection volume was 2.0 μL. An isocratic elution of water: methanol (60:40 v/v) with 0.1% v/v formic acid was optimized with a flow rate of 0.2 mL/min and run time of 6.0 min. A Waters Xevo TQ-XS triple quadrupole mass spectrometer (Waters, Milford, MA, USA) equipped with an electrospray ionization (ESI) source was used for detection and quantification. The positive-ion ESI mode was employed for ionization of PZA and IS. MS/MS data were acquired using multiple reaction monitoring modes (MRM). The MRM transitions for PZA and IS were m/z 124.08 > 81.06 (Fig. 1A) and m/z 138.01 > 121.05 (Fig. 1B), respectively. The cone voltage and collision energy of 21 V and 19 eV for PZA and 12 V and 15 eV for INH, respectively, and a dwell time of 0.63 s. The following ESI inlet conditions were used: capillary 0.5 kV, cone 3 V, source temperature 150°C, desolvation temperature 500°C, cone gas flow 150 L/h, and desolvation gas flow 1000 L/h. Instrument control and data acquisition were performed using the MassLynx software (version 4.2). PZA and IS were eluted at retention times of 1.53 min and 1.43 min, respectively. Representative chromatograms of the LQC and IS are shown in Fig. 2. The target Lynx application manager was used for the quantitative data analysis.

Method validation

Specificity. Specific chromatograms of PZA quality control samples and PZA-loaded lipid nanoarchitectonics showed no interference with 1.53 and 1.43 min retention times of PZA and IS, respectively (Fig. 2). Furthermore, no interference with the degradants was observed at the retention time of PZA. Thus, the UPLC-MS/MS method proved to be an efficient method for evaluating samples containing PZA.

Linearity and range

The linearity of the UPLC-MS/MS method for PZA determination was carried out by considering calibration standard sets over the 1.0–200 ng/mL concentration range for PZA with a y = 0.0125x + 0.0138 linear regression equation and a coefficient of determination (R²) of 0.998. The SD values of the slope and intercept of the calibration curves were 0.0002 and 0.005, respectively.

Precision

The precision of the UPLC-MS/MS method specifies the degree of agreement between each identical sample subjected to intra-day and inter-day analyses for repeatability. The accuracy of the UPLC-MS/MS system was expressed as percent accuracy, percent RSD, and percent bias. Three replicates of QC samples at three different concentration levels (LQC, MQC, and HQC) were used to determine intra- and inter-day accuracy on the same day and on three consecutive days. A systematic representation of the respective QC samples and precision results is presented in Tables 1 and 2. The variation of the established UPLC-MS/MS system values in intra (Table 1) and inter-(Table 2) days was reflected in the percent RSD and was found to be within the limit in accordance with ICH Q2 (R1) guidelines.

Accuracy

The accuracy of the developed and validated UPLC-MS/MS method dictates the degree of closeness between the true values and the obtained values, expressed in terms of % recovery. The % recovery was determined using three replicates of LQC, MQC, and HQC. The % recovery of the developed method was 98.0 ± 2% for PZA and well within the acceptance criteria. The results are presented in Table 3. The developed UPLC-MS/MS method is accurate for estimating the PZA.

Sensitivity

The limit of detection (LOD) was used to assess the sensitivity of the developed UPLC-MS/MS method. It was determined based on a visualization method in compliance with the ICH guideline Q2 (R1). Therefore, the LOD of the method was found to be 0.5 ng/mL, whereas the LOQ was found to be 1 ng/mL. Furthermore, to evaluate the lower concentration detectability, the concentrations of 0.5 ng/mL, 0.6 ng/mL, 0.8 ng/mL, and 1 ng/mL were analyzed in triplicate, and the reliability of detection level was expressed in the % bias and % RSD of the LOQ, which showed acceptable accuracy and precision. The % bias, % RSD, %
accuracy of concentrations were determined, whereas the lower concentration (0.5 ng/mL) was shown <2% RSD. Subsequently, with an increase in the concentration from 0.5 ng/mL to 1 ng/mL, the % bias, % RSD was reduced, and % accuracy was increased (Table 4), and at the LQC of 1 ng/mL, %RSD, % bias, and accuracy were found to be 1.56, -1.30, and (101.30 ± 1.58), respectively, and were within the acceptable limits.
Robustness

The robustness of the developed UPLC-MS/MS method was determined by examining the effect of deliberate change in the mobile phase composition (40 ± 2% v/v) organic composition and column temperature (45 ± 2°C) at three QC samples. The slight changes in the mobile phase composition and column temperature did not show any significant changes in the retention time, peak area ratio of PZA and IS, and % recovery (Table 5). The % recoveries of all modified conditions were found to be between 80% and 120%. This suggests that the developed UPLC-MS/MS method is robust for the estimation of PZA.

System suitability

The system suitability was determined by injecting the MQC (10 ng/mL) into six replicates, and the % RSD of the peak area and retention time was evaluated. The retention time and peak area ratio of PZA were found to be within the acceptable limit of <2.0%; the results are presented in Table 6. Hence, the proposed validated UPLC-MS/MS method indicated a better performance of the system.

Stability studies

PZA was exposed to different stress conditions, such as hydrolysis and oxidative stress conditions, to evaluate its stability. The % recovery revealed no degradation under all stress conditions at room temperature (25 ± 3°C), such as hydrolysis and oxidation when exposed to 0.1N HCl, 0.1N NaOH, and 15% v/v hydrogen peroxide (Table 7). The percentage recovery was close to the initial value, and the loss was less than 5% (<5%), indicating the stability of PZA over 8 h under the stress condition of media comprising 0.1N HCl, 0.1N NaOH, and 15% v/v hydrogen peroxide. The chromatograms of each stress condition are presented in Fig. 3 for 0.1%, 0.1%, and 15.0% v/v H₂O₂. PZA was stable under all the aforementioned stress conditions.

Table 1. Intra-day precision values of developed UPLC-MS/MS method

| NC (ng/mL) | FC (ng/mL) | % Accuracy | Avg. % Bias | Precision (% RSD) |
|------------|------------|------------|-------------|-------------------|
| 1.0        | 0.98 ± 0.01| 98.32 ± 1.33| 1.68        | 1.35              |
| 10         | 9.82 ± 0.11| 98.16 ± 1.06| 1.84        | 1.08              |
| 100        | 100.12 ± 0.68| 100.12 ± 0.68| −0.12      | 0.68              |

*Data are expressed as mean ±SD, n = 3; NC = nominal concentration; FC = found concentration.
Applications of the validated UPLC-MS/MS method

% DEE and % DL from the encapsulated PZA from lipid core-shell nanoarchitectonics were extracted by liquid-liquid extraction method, and values were found to be 42.72% (±12.60) and 5.13% (±1.36), respectively. The chromatograms of PZA extracted from the lipid nanoarchitectonics and their respective blanks are shown in Fig. 2.

Table 3. Accuracy values of developed UPLC-MS/MS method

| QC Level | NC (ng/mL)* | FC (ng/mL) | % Accuracy | Avg. % Bias | % RSD | FC (ng/mL) | % Accuracy | Avg. % Bias | % RSD |
|----------|-------------|------------|------------|-------------|-------|------------|------------|-------------|-------|
| LQC 1    | 1.0         | 0.98 ± 0.01| 98.16 ± 1.63| 1.84 | 1.66  |
| MQC 10   | 9.80 ± 0.05 | 98.03 ± 0.46| 1.97 | 0.47  |
| HQC 100  | 98.12 ± 0.36| 98.12 ± 0.36| 1.88 | 0.37  |

*Data are expressed as mean ±SD, n = 3; NC = nominal concentration; FC = found concentration.

Table 4. % RSD and % bias of the samples in-between the LOD and LOQ range

| NC (ng/mL)* | FC (ng/mL) | % Accuracy | Avg. % Bias | % RSD | FC (ng/mL) | % Accuracy | Avg. % Bias | % RSD |
|-------------|------------|------------|-------------|-------|------------|------------|-------------|-------|
| 0.5         | 0.29 ± 0.02| 59.73 ± 5.61| 40.27 | 9.40  |
| 0.6         | 0.44 ± 0.04| 74.22 ± 6.71| 25.78 | 9.04  |
| 0.8         | 0.75 ± 0.05| 94.66 ± 6.42| 5.34  | 6.79  |
| 1.0         | 1.01 ± 0.01| 101.30 ± 1.58| -1.30 | 1.56  |

*Data are expressed as mean ±SD, n = 3; NC = nominal concentration; FC = found concentration.

Table 5. Robustness values of developed UPLC-MS/MS method

| S.no | Parameters | Sample Conc. (ng/mL)* | Retention time (min)* | % Recovery* |
|------|------------|-----------------------|-----------------------|-------------|
| A. Change in mobile phase composition |
| 1    | Water: METHANOL (58:42) | 1.52 | 81.60 ± 0.00 |
| 1    | Water: METHANOL (62:38) | 1.52 | 81.60 ± 0.00 |
| 1    | Water: METHANOL (69:31) | 1.52 | 81.60 ± 0.00 |
| B. Change in Column oven temperature |
| 1    | Water: 43°C | 1.53 | 97.60 ± 1.13 |
| 1    | Water: 44°C | 1.53 | 108.26 ± 9.23 |
| 1    | Water: 47°C | 1.53 | 97.50 ± 0.50 |

*Data expressed as mean (±SD), n = 3.

Applications of the validated UPLC-MS/MS method
The liver is the main organ involved in drug metabolism. Liver microsomes are helpful in evaluating the microsomal stability of lipid nanoparticles in in vitro models, as liver microsomes contain many drug-metabolizing enzymes. PZA undergoes extensive liver metabolism to pyrazinoic acid and 5-Hydroxy pyrazinoic acid. Hence, lipid-based nanoformulations are the most suitable approach to target lymphatic uptake and provide stability to the incorporated PZA. The positive control drug, verapamil, underwent extensive metabolism in HLMs, $T_{1/2}$, $CL_{int}$ in vivo in the liver, and was found to be 9.27 min, 74.81 $\mu$L/min/mg protein, 2.39 mL/min/g/liver, respectively. According to a well-stirred model ($%QH$), the in vivo $CL$ of verapamil based on the whole blood drug concentration was found to be 74.76%. Whereas PZA lipid nanoformulation was found to be stable in HLMs and $T_{1/2}$, respectively, $CL_{int}$ in vivo in the liver was found to be 432.62 min and 1.67 $\mu$L/min/mg protein and 0.05 mL/min/g/liver. $%QH$ of PZA lipid-based nanoformulation was found to be 6.20%. The $%QH$ values of <30% and >70% were classified as low and high clearance, respectively. Hence, the PZA-lipid-based nanoformulation falls under the low-clearance mode. Thus, this formulation is suitable for preventing PZA-mediated extensive liver metabolism.

CONCLUSIONS

The proposed UPLC-MS/MS method for quantifying in vitro samples comprising PZA was developed and validated in compliance with ICH guidelines. The UPLC-MS/MS method was shown to be simple, precise, accurate, reproducible, and sensitive for use in the estimation of PZA in PZA-loaded lipid core-shell nanoarchitectonics for the in vitro determination of $%DEE$, $%DL$, and microsomal stability of lipid core-shell nanoarchitectonics in HLMs. Additionally, this established UPLC-MS/MS method of PZA may easily be applied for both the in vitro determination of PZA in other lipid-mediated nanoformulations.

Table 6. System suitability values of developed UPLC-MS/MS method

| Concentration (ng/mL)* | Retention time (min) | % RSD | Tailing factor | % RSD |
|------------------------|----------------------|-------|---------------|-------|
| 10 ng/mL               | 1.53 ± 0.0           | 0.00  | 0.97 ± 0.01   | 1.03  |

*Data expressed as mean ($±SD$), $n = 6$.

Table 7. Stability study of PZA in different media

| PZA Concentration | Stress Conditions | Exposure Time | % Recovery ($±SD$) |
|-------------------|-------------------|---------------|--------------------|
| 0.1 N HCl         | 5.0 mg/mL         | 96.51 ($±0.002$) |
| 0.1 N NaOH        | 8 h.              | 98.71 ($±0.000$)  |
| 15% v/v H₂O₂      |                   | 97.50 ($±0.004$)  |

*Data are expressed as mean ($±SD$), $n = 3$.

Stability of PZA loaded lipid core-shell nanoarchitectonics

The liver is the main organ involved in drug metabolism. Liver microsomes are helpful in evaluating the microsomal stability of lipid nanoparticles in in vitro models, as liver microsomes contain many drug-metabolizing enzymes. PZA undergoes extensive liver metabolism to pyrazinoic acid and 5-Hydroxy pyrazinoic acid. Hence, lipid-based nanoformulations are the most suitable approach to target lymphatic uptake and provide stability to the incorporated PZA. The positive control drug, verapamil, underwent extensive metabolism in HLMs, $T_{1/2}$, $CL_{int}$ protein, $CL_{int}$ in vivo in the liver, and was found to be 9.27 min, 74.81 $\mu$L/min/mg protein, 2.39 mL/min/g/liver, respectively. According to a well-stirred model ($%QH$), the in vivo $CL$ of verapamil based on the whole blood drug concentration was found to be 74.76%. Whereas PZA lipid nanoformulation was found to be stable in HLMs and $T_{1/2}$, respectively, $CL_{int}$ in vivo in the liver was found to be 432.62 min and 1.67 $\mu$L/min/mg protein and 0.05 mL/min/g/liver. $%QH$ of PZA lipid-based nanoformulation was found to be 6.20%. The $%QH$ values of <30% and >70% were classified as low and high clearance, respectively. Hence, the PZA-lipid-based nanoformulation falls under the low-clearance mode. Thus, this formulation is suitable for preventing PZA-mediated extensive liver metabolism.

Fig. 3. Stability of PZA in [A] 0.1 N HCl, [B] 0.1 N NaOH, [C] 15% v/v H₂O₂
Declaration of competing interest: The authors declare that there are no conflicts of interest.

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REFERENCES

1. Thalla, M.; Kant, K.; Dalchand; Rawat, R.; Banerjee, S. J. Biomol. Struct. Dyn. 2020, 38, 5195–203.
2. Millard, J. D.; Mackay, E. A.; Bonnett, L. J; Davies, G. R. Syst. Rev. 2019, 8, 329.
3. Becker, C.; Dressman, J. B.; Amidon, G. L.; Junginger, H. E.; Kopp, S.; Midha, K. K.; Shah, V. P.; Stavchansky, S.; Barends, D. M. J. Pharm. Sci. 2008, 97, 3709–20.
4. Tripathi, K. D. Essentials of Medical Pharmacology 6th ed. Jaypee Brothers Med. Publ. P Ltd. 2008, 188.
5. Goodman, L. S. Goodman and Gilman’s Manual of Pharmacology and Therapeutics; Mcgraw Hill Professional, 2008.
6. Shih, T.-Y.; Pai, C.-Y.; Yang, P.; Chang, W.-L.; Wang, N.-C.; Hu, O. Y.-P. Antimicrob. Agents Chemother. 2013, 57, 1685–90.
7. Banerjee, S.; Roy, S.; Nath Bhaumik, K.; Khetrapal, P.; Pillai, J. Artif. Cells Nanomedicine Biotechnol. 2018, 46, 540–58.
8. Pandit, S.; Roy, S.; Pillai, J.; Banerjee, S. ACS Omega. 2020, 5, 4433–48.
9. Momin, M. A. M.; Rangnekar, B.; Das, S. C. J. Liq. Chromatogr. Relat. Technol. 2018, 41, 415–21.
10. Calleri, E.; De Lorenzi, E.; Furlanetto, S.; Massolini, G.; Caccialanza, G. J. Pharm. Biomed. Anal. 2002, 29, 1089–96.
11. Abraham, J. Handbook of transnational economic governance regimes. In Brill | Nijhoff; Tietje, C., Brouder, A., Eds. 2010; pp. 1041–53.
12. Banerjee, S.; Chattopadhyaya, P.; Ghosh, A.; Kaity, S.; Veer, V. Drug Res. 2013, 63, 450–6.
13. Chaudhari, V. S.; Borkar, R. M.; Murty, U. S.; Banerjee, S. J. Pharm. Biomed. Anal. 2020, 186, 113325.
14. Thalla, M.; Gangasani, J.; Saha, P.; Ponneganti, S.; Borkar, R. M.; Naidu, V. G. M.; Murty, U. S. N.; Banerjee, S. ASSAY Drug Dev. Technol. 2020, 18, 249–60.
15. Niguram, P.; Goswami, A.; Kate, A. S. J. Pharm. Biomed. Anal. 2020, 186, 113279.