



An HPLC method for the pharmacokinetic study of vincristine sulfate-loaded PLGA–PEG nanoparticle formulations after injection to rats

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ABSTRACT

The aim of this study is to develop a simple and applicable HPLC method for the detection of vincristine in rat plasma after administration of poly(lactic-co-glycolic acid)–poly(ethylene glycol) (PLGA–PEG) nanoparticles loaded with vincristine sulfate (VCR). Vincristine was extracted from rat plasma and vincristine sulfate was chosen as the internal standard (IS). Chromatographic separation of VCR and IS was achieved by a Dikma Dimonsil C₁₈ column (200 mm × 4.6 mm) with the mobile phase consisting of 0.02 M sodium dihydrogen phosphate–methanol (36:64, v/v, pH = 4.7) at a flow rate of 1.0 mL/min. The ultra-violet detection wavelength was set at 276 nm. The calibration curve was linear over a concentration range of 0.05–5.0 µg/mL. The intra-day and inter-day accuracy for three quality controls (QC) samples was 93.48–107.74% and 92.61–96.58%, respectively; the precision was less than 9%. The average method recoveries for vincristine from spiked plasma at all QC levels were over 83%; and extraction recoveries were between 66 and 70%. Vincristine was stable in rat plasma for one month at –80 °C, for 8 h at room temperature, as well as during three freeze–thaw cycles. This HPLC method was applied successfully to the pharmacokinetic study of vincristine in rats after a single intravenous injection of VCR in physiological saline (F–VCR) solution, VCR-loaded PLGA–mPEG nanoparticles with (NP1) and PLGA–PEG–folate nanoparticles (NP2) suspension, respectively. There were significant differences in main pharmacokinetic parameters between F–VCR and the nanoparticles. Both kinds of VCR-loaded nanoparticles displayed improved pharmacokinetic profiles.

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1. Introduction

Vincristine sulfate (VCR), extracted from the plant *Vinca rosea* [1], is an effective chemotherapeutic agent that has been used extensively for the treatment of a number of human carcinomas including acute leukemia, malignant lymphoma, and breast cancer. The accepted mechanism by which VCR exert its antitumor activity is by interacting with tubulin which causes disruption of microtubules of the mitotic apparatus, thereby arresting cell division in metaphase [2]. Dose-dependent and cumulative peripheral neuropathy, however, is the main dose-limiting side effect of chemotherapy [3]. Changed formulations, such as liposome delivery systems loaded with VCR are considered options that can reduce these side effects of VCR. Early studies by Mayer et al. [2,4] suggested that the administration of VCR liposome could protect vincristine from rapid elimination *in vivo*, increased plasma concentrations, and prolonged retention time of vincristine, resulting in improved pharmacokinetic profiles in comparison with

the unencapsulated drug. Poly(lactic-co-glycolic acid) and polylactide (PLGA and PLA), both approved by the FDA, are extensively used as pharmaceutical materials because of their biocompatibility and biodegradability [5]. Poly(ethylene glycol)-modified PLGA (PLGA–PEG) nanoparticles have been developed over the years due to their tremendous potential as long circulating systems [6].

There have been several methods described previously to analyze vincristine in plasma after the administration of VCR injection, from HPLC–UV [1,2,7–9], HPLC combined with electrochemical detection method [10], to LC–MS [11,12], then to LC–MS/MS [13–20]. However, these assay methods are comparatively complex; generally the mobile phases consist of several components and the pH values are required to be adjusted. In the last two methods, MS equipment is required which leads to the expensive test cost. Moreover, the sample handling process is more stringent than traditional HPLC–UV method to remove the endogenous compounds which may be responsible for a matrix effect and have negative effect on the quantitative MS measurement. Up to date, few assay methods cover the pharmacokinetic assay after the administration of PLGA–PEG nanoparticles loaded with VCR. The aim of this study is to develop a simple and applicable HPLC method for the analysis of vincristine in rat plasma after tail vein administration of VCR-loaded PLGA–mPEG nanoparticles

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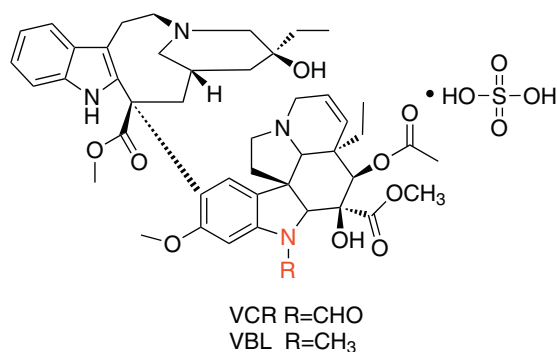


Fig. 1. Chemical structures of VCR and VBL.

(NP1) and PLGA–PEG–folate nanoparticles (NP2) suspension; and compare their pharmacokinetic behavior with that of VCR physiological saline (F-VCR) solution. Vinblastine sulfate (VBL) is chosen as an internal standard (IS). The chemical structures of VCR and VBL are shown in Fig. 1.

2. Materials and methods

2.1. Chemicals and reagents

For the synthesis of the polymer PLGA₁₅₀₀₀–PEG₃₃₅₀–folate (abbreviation: PLGA–PEG–folate) used in the preparation of NP2, PEG–bis–amine (NH₂–PEG–NH₂, average 3350 Da) was obtained from Biomatrik Inc. (Shanghai, China). Poly(lactide–co–glycolide) (PLGA–COOH, 50 mol% of lactide, average Mw 15 kDa) and PLGA₁₅₀₀₀–mPEG₂₀₀₀ (abbreviation: PLGA–mPEG) were purchased from Daigang Corporation (Shangdong Province, China). Polyvinyl alcohol (PVA, viscosity: 11–14 cp) was obtained from Shanghai Kayon Biological Technology Co., Ltd. (Shanghai, China). Vincristine sulfate (purity > 98%), the internal standard VBL (purity > 95%), and folic acid (purity ≥ 97%) were obtained from Aladdin Reagent Co., Ltd. (Shanghai, China). HPLC level methanol was purchased from J&K Scientific Ltd. (Shanghai, China). Distilled water was produced by a Milli-Q purification system (Millipore, Billerica, MA, USA). All other chemicals and reagents were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China), and were of analytical grade.

Polymer PLGA–PEG–folate was synthesized according to the previous report [21]. PLGA–mPEG nanoparticles loaded with VCR (NP1) and PLGA–PEG–folate nanoparticles loaded with VCR (NP2) were prepared using water–oil–water emulsion solvent evaporation method, which was described previously [22].

2.2. Animals

Healthy Sprague–Dawley (SD) rats (250 ± 20 g) were obtained from the Laboratory Animal Center, Shanghai Jiao Tong University, China. The animal experimental protocols were performed according to the guideline of the Experimental Animal Ethics Committee of Shanghai Jiao Tong University. Rats were kept in a room under a controlled temperature of 25 ± 1 °C and relative humidity of 50 ± 10%. After the collection of blood, animals were sacrificed by cervical dislocation during ether anesthesia.

2.3. Chromatographic and apparatus conditions

The chromatography system used was composed of a Shimadzu LC-20AT chromatographic system (Shimadzu, Kyoto, Japan) with a LC-20AT binary pump and a SPD-20A UV–vis detector. Data processing was performed with a LC Solution program. Analysis was

carried out on a Dikma Dimonsil C₁₈ column (200 mm × 4.6 mm, 5 μm, Dikma Technologies, China). The mobile phase was composed of 0.02 M sodium dihydrogen phosphate–methanol (36:64, v/v, pH = 4.7), and the flow rate was 1.0 mL/min. The column temperature was maintained at 25 °C, the UV–vis detection wavelength was set at 276 nm, and the injection volume was 20 μL.

2.4. Standard solutions and plasma samples

Stock solutions (1 mg/mL) of VCR and VBL in methanol were prepared and stored at –20 °C. The working solutions of VCR with 0.5, 1, 2.5, 5, 10, and 50 μg/mL were prepared by serial dilution of VCR stock solution. VBL working solution at a concentration of 10 μg/mL was also prepared by diluting VBL stock solution.

Drug-free rat plasma containing sodium heparin as the anticoagulant was obtained from SD rats. Plasma calibration standards of VCR (0.05, 0.1, 0.25, 0.5, 1.0, and 5.0 μg/mL) were prepared by adding 40 μL of working solution (composed of 20 μL VCR and 20 μL IS working solutions) into 200 μL drug-free rat plasma. The following procedures were described in Section 2.5. Quality control (QC) samples were separately prepared in a similar way as calibration standard, and the concentrations of 0.1, 0.5, and 4.0 μg/mL of VCR in plasma samples were corresponding to low, medium, and high QC, respectively.

2.5. Sample preparation

A 200 μL of plasma sample was mixed with 20 μL of the IS working solution. Then, 1000 μL acetonitrile was added to precipitate protein. After centrifuging at 13,400 × g for 10 min, the supernatant was collected, transferred into a tube and dried under a stream of nitrogen. The residue was reconstituted in 200 μL of the mobile phase, filtered using a 0.45 μm Millipore filter, and then 20 μL of the sample was injected into the HPLC for analysis.

2.6. Method validation

2.6.1. Specificity

The specificity of the method was investigated by comparing the chromatograms of the following plasma samples after they were collected from the SD rats and treated according to the procedures described in Section 2.5: blank plasma, plasma sample spiked with VCR and IS, and plasma sample at 1 h after intravenous injection of NP2 suspension spiked with IS. There should be no interference from endogenous or exogenous materials observed at the retention time of vincristine and the IS. The plasma samples in each group were obtained from six different SD rats.

2.6.2. Calibration curves and linearity

Every calibration standard concentration was assayed six times. After injecting all the processed calibration standard samples of various concentrations covering the working range of the assay, the calibration curves were established in the range of 0.05–5.0 μg/mL. The peak areas of vincristine (A_{VCR}) and vinblastine (A_{VBL}) were recorded; the values of A_{VCR}/A_{VBL} and the concentrations of VCR were used to plot the calibration curve ($y = ax + b$).

2.6.3. Accuracy and precision

In order to assess the intra- and inter-day precision and accuracy of the assay, the three VCR QC samples at low, medium and high concentrations were prepared according to the steps described in Section 2.4 and analyzed six times for each concentration using HPLC. The precision of the assay was assessed by calculating the relative standard deviation (R.S.D.) for each concentration level; and inter-day precision was determined by the analysis of QC samples

on three consecutive days. Accuracy was calculated by comparing the average measurements with the nominal values, and was expressed in percent.

2.6.4. Recovery

The recoveries of vincristine were determined by the analysis of the above three VCR QC samples. A 40 μL of the standard solution (composed of 20 μL VCR and 20 μL IS working solutions) was transferred into 200 μL of blank rat plasma. Then the samples were treated according to the method described in Section 2.5 and the peak area ratio ($A_{\text{VCR}}/A_{\text{VBL}}$) was obtained. For the reference material, the same concentration standard solution in mobile phase was injected directly to the HPLC system and the peak area ratio ($S_{\text{VCR}}/S_{\text{VBL}}$) was also gained. The results of $[(A_{\text{VCR}}/A_{\text{VBL}})/(S_{\text{VCR}}/S_{\text{VBL}})]$ were defined as the method recoveries and expressed in percent. The extraction recoveries (%) of vincristine were determined using the following method: The peak area of vincristine (A_{VCR}) was compared to that of VCR directly dissolved in mobile phase and assayed by HPLC at the same concentration (S_{VCR}); the extraction recoveries of internal standard were obtained by analyzing plasma samples spiked with VBL at medium level of QC (0.5 $\mu\text{g}/\text{mL}$) using the same method. The experiments were repeated six times for each concentration.

2.6.5. Stability

The stability of vincristine in plasma was assessed by analyzing six replicates of QC samples at concentrations of 0.1, 0.5 and 4.0 $\mu\text{g}/\text{mL}$ during the sample storage and processing procedures. Freeze/thaw stability was determined after three freeze/thaw cycles (-80°C to room temperature, here the room temperature was 25°C). Then benchtop stability was assessed: After the addition of acetonitrile to precipitate protein in the plasma samples and centrifugation, the supernatant collected was exposed to room temperature for 8 h, which exceeded the residence time of the sample processing procedures. The stability was determined by comparing the calculated concentration of the test samples with the nominal concentration of VCR. Long-term stability was evaluated of the samples at -80°C for one month. Analytes were considered stable at each concentration when the concentration differences between the freshly prepared samples and the test samples were found to be not exceeding 15%.

2.7. Application to pharmacokinetic study of VCR-loaded nanoparticles

An *in vivo* pharmacokinetic study was undertaken to investigate whether there was improved pharmacokinetic behavior after the administration of the nanoparticles suspension, compared to that of F-VCR solution.

2.7.1. Animal administration and sampling

18 healthy SD rats were randomly divided into three groups, and fasted overnight with free access to water before drug administration. Rats in control group were tail vein injected with F-VCR solution (1.2 mg of VCR/kg). The other two groups were injected with 14.9 mg NP1 or 14.4 mg NP2 formulations which were prepared by suspending freeze-dried powders of the nanoparticles in 2 mL saline solution at the same dose of the control group. After administration, 200 μL of blood samples collected from the retro-orbital plexus at the predetermined time points of 0.083, 0.25, 0.5, 1, 2, 4, 8, 12, and 16 h were placed into heparinized micro-centrifuge tubes (100 IU/mL blood). The blood sample was centrifuged at $2300 \times g$ for 5 min and the separated plasma (100 μL) was stored at -80°C until analysis. The following procedures were similar to those which were described in Section 2.5. The difference was that 10 μL of the IS working solution was added to the above plasma

before the addition of acetonitrile (500 μL) to precipitate protein and the residue was reconstituted in 100 μL of the mobile phase. All the plasma samples were assayed within two days after the administration.

2.7.2. Calculations and statistics

HPLC results of samples were analyzed with the LC solution software (Shimadzu, Kyoto, Japan). Kinetica 4.0 (Thermo Electron Corp., USA) computer program was employed to analyze the plasma concentration–time data. The area under the concentration–time curve (AUC_{0-t}) from zero to the last time point, mean residence time (MRT), distribution phase half-life ($T_{1/2\alpha}$), elimination phase half-life ($T_{1/2\beta}$), and total body clearance (CI) of the drug were all obtained.

Data were presented as mean \pm standard deviation (S.D.). One-way analysis of variance (ANOVA) test was performed on the data to assess the impact of the formulation variables on the results ($n \geq 3$). Differences were considered statistically significant at $p < 0.05$.

3. Results and discussion

3.1. HPLC method development and sample handling

VCR and VBL share a very similar chemical structure, with only a minor difference at the N1 position (Fig. 1) that has either a $-\text{CHO}$ group or a $-\text{CH}_3$ group, respectively. In previous reports, several mobile phases were used to determine vincristine in plasma by HPLC, but they were comparatively complex, and in many cases, pH values must be adjusted. In fact, a very simple composition consisting of 0.02 M sodium dihydrogen phosphate–methanol (36:64, v/v, pH = 4.7) provided good separation of these two compounds on a Dikma Dimonsil C_{18} column. Moreover, the pH value of the mobile phase did not need to be adjusted because the pH value of the mixed solution consisting of the above two components was 4.7. Under the experimental conditions described above, the lowest limit of detection (LOD) and the lower limit of quantification (LLOQ) of VCR in plasma were 0.015 and 0.05 $\mu\text{g}/\text{mL}$, respectively, which met the requirement of the assay. A protein precipitation method using acetonitrile as the precipitating solvent was adopted for the plasma sample preparation, and proved to be simple and reliable in this work.

3.2. HPLC method validation

3.2.1. Specificity

Typical HPLC chromatograms of VCR and IS samples are shown in Fig. 2 and they represented the following cases, respectively: (A) VCR and IS dissolved in mobile phase and injected to HPLC system directly; (B) blank plasma sample; (C) blank plasma sample spiked with VCR (1.0 $\mu\text{g}/\text{mL}$) and IS (1.0 $\mu\text{g}/\text{mL}$); (D) plasma sample from a rat at 1 h after a single intravenous administration of NP2 suspension and spiked with IS. The blood samples collected were treated on the basis of the procedures covered in section 2.5. In Fig. 2C, VCR and the IS VBL were added simultaneously to the plasma; but in Fig. 2D, only VBL was added. From the results, vincristine peaks were well shaped in both blending VCR plasma samples and endogenous VCR plasma samples, no interfering peaks were observed at the retention times. The average elution time of vincristine and vinblastine was 6.6 min and 8.7 min, respectively. These observations indicated that the assay had adequate specificity.

3.2.2. Linearity of calibration curve

The method was validated for vincristine in mouse plasma over the concentration range of 0.05–5.0 $\mu\text{g}/\text{mL}$ for VCR. Calibration curves prepared over these concentration ranges were linear with

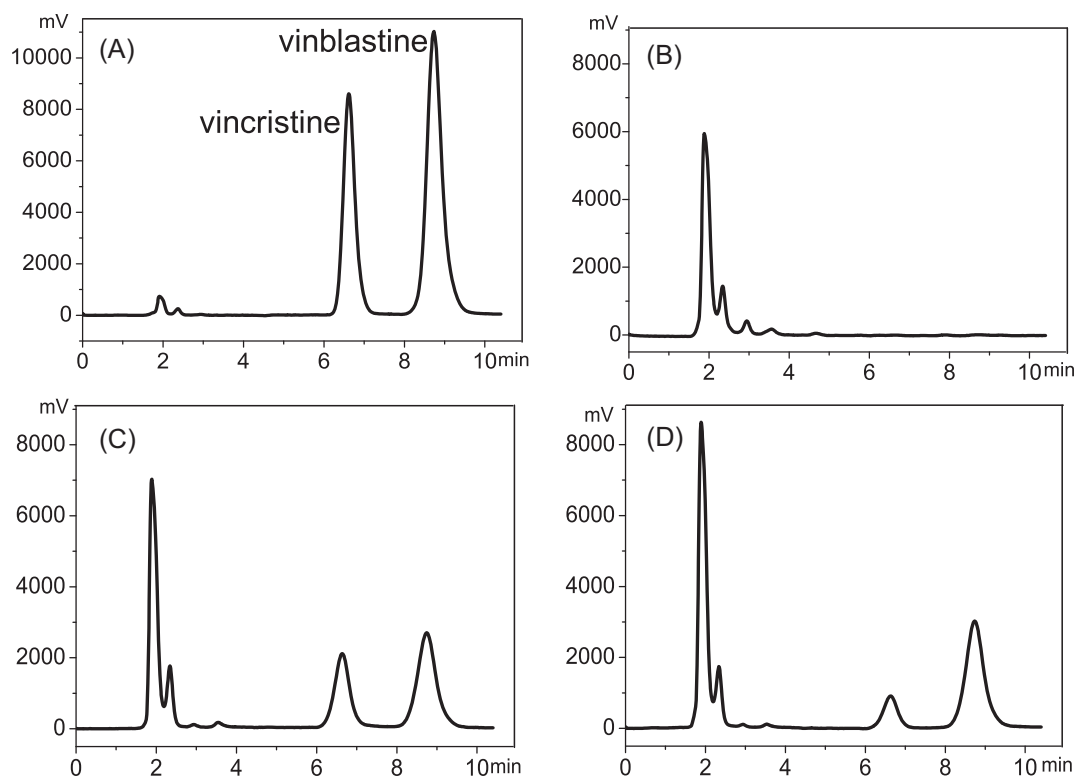


Fig. 2. Representative chromatograms of vincristine and IS samples: (A) VCR and IS dissolved in mobile phase and injected to HPLC system directly; (B) blank plasma sample; (C) blank plasma sample spiked with VCR (1.0 µg/mL) and IS (1.0 µg/mL), and (D) plasma sample from a rat at 1 h after a single intravenous administration of NP2 suspension and spiked with IS.

Table 1
The validation of intra- and inter-day precision and accuracy with QC samples ($n = 6$).

| Concentration (µg/mL) | Precision (% R.S.D.) | | Accuracy (%) | |
|-----------------------|----------------------|-----------|--------------|-----------|
| | Intra-day | Inter-day | Intra-day | Inter-day |
| 0.1 | 6.09 | 7.47 | 93.48 | 92.61 |
| 0.5 | 5.69 | 8.98 | 107.74 | 96.58 |
| 4.0 | 4.38 | 6.95 | 95.41 | 94.48 |

average correlation coefficients greater than 0.99 in plasma. The typical regression equation was $y = 0.804x - 0.031$ ($r = 0.9937$), y representing the peak area ratio of vincristine to vinblastine and x representing the concentration of VCR in plasma.

3.2.3. Precision and accuracy

The intra- and inter-day precision and accuracy of this method were evaluated using the above three QC samples, and the results are listed in Table 1. Intra-day accuracy ranged from 93.48 to 107.74%; and inter-day accuracy ranged from 92.61 to 96.58%. The intra- and inter-day assay precision (R.S.D.) for vincristine were $\leq 6.09\%$ and $\leq 8.98\%$, respectively.

Based on the "Guidance for Industry: Bioanalytical Method Validation (FDA, May 2001)", the mean value of the accuracy should

be within $\pm 15\%$ of the theoretical value, except at LLOQ, where it should not deviate by more than $\pm 20\%$; the precision around the mean value should not exceed 15% of the relative standard deviation (R.S.D.), except for LLOQ, where it should not exceed 20% of the relative standard deviation. The data of the accuracy and precision in the assay were within the recommendations. These results indicated that this method had good accuracy and precision.

3.2.4. Recovery

The method recovery was evaluated by comparison of the vincristine/vinblastine peak area ratios of the extracted samples at the three QC levels with the standard solutions of equivalent concentrations. The results are listed in Table 2. There was no significant difference in the method recoveries of vincristine at low, medium, and high concentrations. Simultaneously, the extraction recoveries of vincristine and vinblastine were determined by comparison of the peak area of the extracted samples at the three QC levels with the standard solutions of equivalent concentrations individually. The mean extraction recoveries for vincristine and vinblastine were between 66 and 70%. There was also no significant difference in the extraction recoveries of vincristine at the three QC levels indicating that the method for plasma handling was effective for extracting vincristine.

Table 2
Method recoveries and extraction recoveries of vincristine and vinblastine from rat plasma ($n = 6$).

| Analytes | Nominal concentration (µg/mL) | Mean \pm S.D. calculated (µg/mL) | Method recoveries (%) ^a | Extraction recoveries (%) ^b |
|----------|-------------------------------|------------------------------------|------------------------------------|--|
| VCR | 0.1 | 0.083 \pm 0.007 | 83.0 \pm 8.4 | 66.2 \pm 8.7 |
| | 0.5 | 0.42 \pm 0.03 | 84.0 \pm 7.1 | 67.4 \pm 8.1 |
| | 4.0 | 3.41 \pm 0.21 | 85.3 \pm 6.2 | 67.9 \pm 6.4 |
| VBL | 0.5 | – | – | 69.7 \pm 5.7 |

^a Expressed as [(mean calculated concentrations)/(nominal concentrations) \times 100].

^b Expressed as [(peak area of the sample in plasma after treatment)/(peak area of the standard solution at the same concentration which was directly injected to the HPLC system) \times 100].

Table 3
Stability data for vincristine in rat plasma samples under various conditions ($n=6$).

| Experimental conditions | Normal concentration ($\mu\text{g/mL}$) | Mean \pm S.D. calculated ($\mu\text{g/mL}$) | Precision (% R.S.D.) |
|--|---|---|----------------------|
| After three freeze–thaw cycles (%) | 0.1 | 0.0913 ± 0.0038 | 4.2 |
| | 0.5 | 0.468 ± 0.0267 | 5.7 |
| | 4.0 | 3.78 ± 0.18 | 4.8 |
| At room temperature for 8 h (%) | 0.1 | 0.0891 ± 0.0035 | 3.9 |
| | 0.5 | 0.453 ± 0.021 | 4.6 |
| | 4.0 | 3.61 ± 0.13 | 3.6 |
| At -80°C for one month (%) | 0.1 | 0.0964 ± 0.0047 | 4.9 |
| | 0.5 | 0.494 ± 0.019 | 3.8 |
| | 4.0 | 4.17 ± 0.18 | 4.3 |

3.2.5. Stability

The stability experiments were carried out by analyzing QC samples to test the samples under all possible conditions that they might experience after collecting and prior to analysis. The results are summarized in Table 3. It was found that vincristine was stable in plasma for three cycles when stored at -80°C and thawed to room temperature, which was evidenced by the facts that the calculated concentration after treatment was very near to the normal concentration and the precision (%R.S.D.) ranged from 4.2 to 5.7%. Benchtop stability was evaluated at room temperature because the post-treatment of the samples was carried out under this condition. Data showed that vincristine in rat plasma was stable at ambient temperature for 8 h, as well as after storage at -80°C for 1 month. Moreover, it was found that VCR and VBL stock solutions were stable at least for two months at or below -20°C (the loss of VBL was $\leq 10\%$). These results suggested that vincristine and vinblastine were stable under the experimental conditions of the analytical runs.

3.3. Application of the developed HPLC method to pharmacokinetics study

The method was successfully applied to the determination of vincristine in plasma obtained from the rats following a single intravenous administration of 1.2 mg/kg VCR. The acetonitrile protein precipitation method was used in the plasma treatment as described above. A comparative pharmacokinetic study was performed by determining drug levels in plasma up to 16 h after tail-vein administration. The plasma concentration–time curves are shown in Fig. 3. The relevant pharmacokinetic parameters, including AUC, MRT, C_{max} , $T_{1/2\alpha}$, $T_{1/2\beta}$, and CI were determined using two-compartmental analysis of a Kinetics program and listed

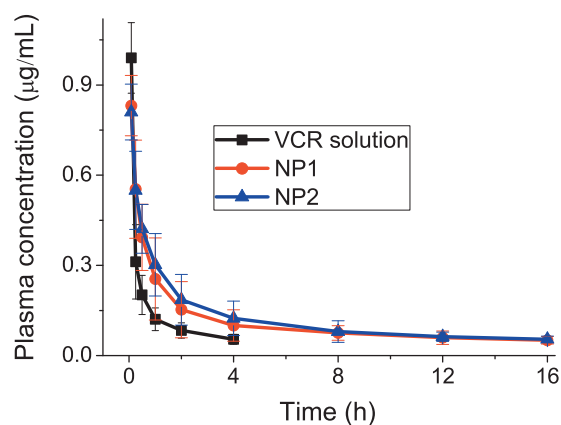


Fig. 3. Mean plasma concentration–time profiles of vincristine in rats ($n=6$) after a single intravenous injection of F-VCR solution and VCR-loaded nanoparticles (NP1 and NP2) suspension at the dose of 1.2 mg/kg, respectively.

in Table 4. There was a significant difference ($p < 0.05$) in the pharmacokinetic parameters between the groups which were injected with VCR-loaded nanoparticles (NP1 and NP2) suspension and the control group. After intravenous injection of F-VCR solution, though the concentration of vincristine in the plasma within the first 10 min was higher than the other two groups which were administered VCR-loaded nanoparticles, vincristine was eliminated rapidly from the circulation and its concentration was below LLOQ beyond 4 h. $T_{1/2\alpha}$ and $T_{1/2\beta}$ calculated were 0.08 ± 0.02 and 2.26 ± 0.59 h, respectively. By contrast, after the nanoparticles suspension was administered at the same dose (according to the amount of VCR), vincristine could still be detected in the plasma for up to 16 h. The concentration–time profiles were biphasic with a rapid distribution phase and a slow elimination process [23]. $T_{1/2\alpha}$ for NP1 and NP2 were 0.41 ± 0.06 and 0.50 ± 0.05 h, respectively; $T_{1/2\beta}$ were 11.31 ± 1.18 h (for NP1) and 10.69 ± 0.94 h (for NP2), respectively. It was believed that sustained release profiles of vincristine from nanoparticles led to longer circulation time in plasma [24]. The area under the plasma concentration–time curve (AUC_{0-16}) was significantly increased by 3.39 times for NP1 and 3.61 times for NP2, respectively, compared with F-VCR solution. However, the maximum plasma concentrations (C_{max}) of vincristine encapsulated into the nanoparticles were slightly lower than the control group; and the elimination rate of vincristine was significantly reduced to about one-third than that of F-VCR solution. From the above results, VCR-loaded PLGA–PEG nanoparticles displayed improved pharmacokinetic profiles, compared with F-VCR solution *in vivo*.

3.4. Discussion

Favorable chromatography conditions at least include the baseline separation of the analyte with the internal standard and other exogenous materials, a mobile phase with simple composition and an appropriate pH value to obtain good peak shape of the analyte during the analysis. Embree et al. [8] reported the assay of vincristine in human plasma following administration of VCR liposome

Table 4

Pharmacokinetic parameters (mean \pm S.D., $n=6$) of vincristine after a single intravenous injection of F-VCR solution, NP1 suspension, and NP2 suspension at the dose of 1.2 mg/kg, respectively.

| Parameters | VCR formulations | | |
|--|--------------------|----------------------|----------------------|
| | F-VCR solution | NP1 | NP2 |
| AUC_{0-16} ($\text{h } \mu\text{g/mL}$) | 0.75 ± 0.11 | $2.54 \pm 0.22^*$ | $2.71 \pm 0.26^*$ |
| MRT (h) | 2.49 ± 0.68 | $13.24 \pm 1.15^*$ | $12.46 \pm 0.96^*$ |
| C_{max} ($\mu\text{g/mL}$) | 0.99 ± 0.06 | 0.83 ± 0.04 | 0.81 ± 0.03 |
| $T_{1/2\alpha}$ (h) | 0.08 ± 0.02 | $0.41 \pm 0.06^{**}$ | $0.50 \pm 0.05^{**}$ |
| $T_{1/2\beta}$ (h) | 2.26 ± 0.59 | $11.31 \pm 1.18^*$ | $10.69 \pm 0.94^*$ |
| CI (mL/h) | 397.79 ± 52.39 | $120.60 \pm 27.88^*$ | $105.33 \pm 35.64^*$ |

* $p < 0.05$, compared with F-VCR solution (control group).

** $p < 0.01$, compared with F-VCR solution (control group).

injection, the mobile phase was comparatively complex consisting of diethylamine aqueous solution–acetonitrile–methanol, and its pH value was required to adjust 7.0. In the experiment, various mobile phases were used to determine vincristine in the plasma samples after the administration of its nanoparticle suspension, and finally a mobile phase consisting of 0.02 M sodium dihydrogen phosphate–methanol (36:64, v/v, pH = 4.7) was found to be appropriate which could achieve the baseline separation of vincristine with the IS. The composition was simple without the adjustment of the pH value and the chromatography conditions were easily controlled. According to the results of the HPLC method validation and material selection, vincristine and IS peaks eluted within 10 min. Besides, the favorable recoveries indicated that the method for plasma handling was effective for extracting vincristine and removing plasma proteins and other impurities. In sum, the assay method of vincristine with high accuracy and good precision was simple and easily controlled.

The results of pharmacokinetic study *in vivo* clearly showed that there was a significant difference in the pharmacokinetic profiles after the administration of two kinds of nanoparticles (NP1 and NP2) suspension, compared to those after the administration of F-VCR solution. Plasma concentration of F-VCR solution administrated group (the control group) decreased rapidly while those of the NP1 and NP2 administrated group decreased slowly. This phenomenon could be explained by the different drug release properties after administration. Compared to the rapid release of nonencapsulated vincristine after intravenous injection, vincristine released slowly from the two kinds of VCR-loaded nanoparticle suspension, and these formulations protected vincristine from rapid elimination in the early phase after administration, resulting in significantly elevated vincristine plasma concentrations beyond 0.25 h. According to the results and references, we conjectured that after the administration of VCR nanoparticle formulation in which vincristine was entrapped into PLGA–PEG nanoparticles, the *in vivo* pharmacokinetics profiles in rats could be improved.

4. Conclusions

In this study, a simple and effective HPLC method was provided for the determination of vincristine with good accuracy and precision. This method was successfully applied to the pharmacokinetic analysis of F-VCR solution and VCR-loaded PLGA–PEG nanoparticle suspension in rats. The results showed that, compared with F-VCR, both NP1 and NP2 could prolong the residence time of vincristine in the plasma, enlarge the AUC, and lower systemic clearance. In conclusion, compared to the administration of F-VCR, the phar-

macokinetic profiles *in vivo* were improved after an intravenous administration of VCR-loaded nanoparticles in SD rats.

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