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Article

High-Performance Liquid Chromatographic Analysis of Felotaxel, a Novel Anti-Cancer Drug, in Rat Plasma and in Human Plasma and Urine

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A high-performance liquid chromatographic method was developed for the determination of a new derivative of docetaxel, felotaxel, in rat plasma and human plasma and urine. The separation of felotaxel was performed on a Dikma C18 column with 0.2% formic acid and acetonitrile (50:50) as the mobile phase. The flow rate was 0.8 mL/min and the column effluent was monitored by an ultraviolet detector set at 275 nm. The method was validated and found to be linear in the range of 5–1,000 ng/mL. The lower limit of quantification was 5 ng/mL based on 100 μL of plasma. The variations for intra-day and inter-day precision were less than 6.9%, and the accuracy values were between 87.3 and 107.4%. The extraction recoveries were more than 80.5%. These data confirm that the developed method has satisfactory sensitivity, accuracy and precision for the quantification of felotaxel in rat plasma and in human plasma and urine. The method was successfully applied to a pharmacokinetics study of felotaxel after intravenous doses of 5 mg/kg in rats.

Introduction

Docetaxel promotes tubulin assembly in microtubules, stabilizes microtubules and inhibits microtubule depolymerization to free tubulin. This leads to disruption of the equilibrium within the microtubule system and ultimately to cell death (1). Recently, it has been found that 9-β-dihydro-9, 10-O-acetal taxanes are more active than docetaxel against resistant cancer cell lines that express P-glycoprotein tumor cell lines (2). Felotaxel (SHR110008) (Figure 1) has been found to be one of the most promising compounds in this new class of derivative. In nude mice bearing A549 human lung cancer xenografts, the antitumor activity of SHR110008 was 20% higher than that of docetaxel, even at a lower dose (3). Additionally, felotaxel has high potency against docetaxel-resistant cell lines, which is consistent with reports about the activity of the class of 9-β-dihydro-9, 10-O-acetal taxanes (4).

Recently, felotaxel has been under clinical development by Hengrui Pharmaceutical (Shanghai, China). The analysis of felotaxel in rat and dog plasma using liquid chromatography–tandem mass spectrometry (LC–MS-MS) has been validated (3). Lower limits of quantification (LLOQ) of this method in rat and dog plasma were 5 and 2 ng/mL using liquid–liquid extraction (LLE). The authors have previously established a modified LC–MS-MS method and applied it in pharmacokinetics research in rats (5). However, because LC–MS-MS is not available in most hospital laboratories, high-performance liquid chromatography (HPLC) may be used instead to measure drug concentration in plasma.

In the present study, the authors have developed a simple HPLC method, which uses an LLE procedure and still retains similar sensitivity to the previously reported method. It has an LLOQ of 5 ng/mL and is applicable for the determination of the felotaxel levels in rat plasma and in human plasma and urine. This method was used in a pharmacokinetics study of rats receiving low doses of treatment.

Experimental

Chemicals and reagents

Felotaxel (purity > 98.0%) was provided by Shanghai Hengrui Pharmaceutical. Tinidazole [internal standard (IS), purity > 98.0%] was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). HPLC-grade methanol was purchased from Fisher Scientific (Pittsburgh, PA). HPLC-quality water was prepared using a Milli-Q plot water purification system (Millipore, Bedford, MA). Formic acid was of analytical grade purity and purchased from Nanjing Chemical Reagent Co. (Nanjing, China). Ethyl acetate of HPLC grade was from Tianjin Baishi Chemical industry Co. (Tianjin, China). Drug-free plasma was provided by the Blood Center of Xijing Hospital (Xi'an, China).

Instrumentation and chromatographic conditions

The chromatography system consisted of an Agilent 1200 Series LC system equipped with an ultraviolet (UV) detector, an autosampler and an Agilent chromatography workstation (Agilent Technologies, Palo Alto, CA). Chromatographic separation was achieved on a Dikma Inspire-C18 column (Dikma, Beijing, China; 150 × 4.6 mm, 5 μm) associated with a guard column packed with the same bonded phase. The composition of the mobile phase was 0.2% formic acid and acetonitrile (50:50). A flow rate of 0.8 mL/min was used throughout the 11-min run. Chromatography was performed at 35°C. The column effluent was detected by UV absorbance at a wavelength of 275 nm.

Standard and working solutions

The primary stock solutions were prepared in methanol. The stock solutions of felotaxel and IS (tinidazole) were stored at

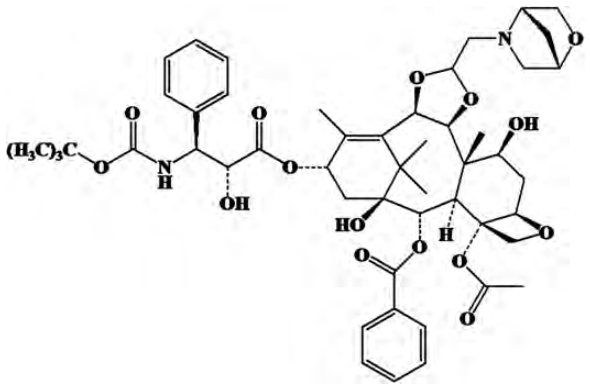

Figure 1. Chemical structure of felotaxel.

4°C, and were found to be stable for one month (data not shown).

Calibration standards were prepared by spiking an appropriate amount of concentrated stock solutions into blank control plasma and urine. The calibration ranges were 5.0–1,000.0 ng/mL (5, 10, 25, 50, 100, 250, 500 and 1,000 ng/mL) for plasma and urine. Three levels of quality control (QC) samples were prepared at 10, 100 and 500 ng/mL. QCs were stored at −20°C with the samples to be analyzed.

Sample preparation

The IS stock solution (30 μL tinidazole, 50 ng/mL in methanol) were added to 100 μL of plasma or urine. After brief vortex-mixing, the mixture was then precipitated with 500 μL ethyl acetate. After vortexing for 2 min, the samples were centrifuged for 10 min at 9,000 × g to separate the aqueous and organic layers. The upper organic layer was decanted into a glass tube. After evaporation at 40°C, the residue was reconstituted in 150 μL acetonitrile–water (50:50, v/v) by vortexing for 30 s. The sample was then centrifuged and placed in the HPLC autosampler.

Method validation

Selectivity

To assess the selectivity of the assay, drug-free human plasma and urine from six healthy donors and rat plasma from untreated rats were processed and analyzed to determine whether endogenous peaks co-eluted with felotaxel or IS. Any apparent response at the retention times of felotaxel and IS was compared to the response at the LLOQ for felotaxel and to the response at the working concentration for IS.

Extraction recovery

Replicate ($n = 3$) biological specimens (100 μL/sample) were spiked with known amounts of felotaxel to yield final concentrations of 10, 100 and 500 ng/mL; the spiked plasma was treated and analyzed as described previously. The extraction recoveries were calculated by comparing the chromatographic peak areas obtained from the extracts of the spiked sample with those obtained by direct HPLC injection of non-extracted compounds dissolved in the mobile phase. The efficiencies were determined at three different concentrations of felotaxel.

Calibration and sample quantification

Duplicate eight-point standard curves ranging from 5 to 1,000 ng/mL of felotaxel were run on three separate days for rat plasma and tissue samples, but only one day for human plasma. Data was collected and integrated using the Drugs and Statistics (DAS) program (Version 2.0, Chinese Pharmacological Society, China). Eight calibration standards were prepared at 5, 10, 25, 50, 100, 250, 500 and 1,000 ng/mL and the calibration curve plotted the peak area ratio (drug/IS) versus the concentration in ng/mL using a least squares linear regression model, weighted $1/y2$. Actual QC and stability samples were calculated from the resulting area ratio of the analyte to IS and the regression equation of the calibration curve.

Precision and accuracy

To assess the accuracy, within-day precision and between-day precision of the assay, replicate measurements of the QC samples were performed in biological samples at 10, 100 and 500 ng/mL over three different analytical runs. Values within ±15% for precision and accuracy were considered acceptable, except for concentrations at the LLOQ, at which 20% was accepted.

Stability

The QC samples at three different concentrations were stored at −20°C for 24 h and thawed at room temperature. When completely thawed, the samples were refrozen for 24 h under the same conditions. After three cycles, the percent loss of the analyte was determined by comparing the concentrations with those obtained before freezing.

Short-term temperature stability

The QC samples at different concentrations were thawed at room temperature, kept at this temperature for 24 h, and analyzed.

Long-term stability

The QC plasma and tissue distribution samples at different concentration levels kept at low temperature (−20°C) were studied for a period of two months.

Post-preparative stability

The autosampler stability was conducted by reanalyzing extracted QC samples kept under autosampler conditions (25°C) for 12 h.

Application to pharmacokinetic study

Approval was obtained from Animal Care and Ethics Committee of the Fourth Military Medical University (Xian, China). Adult male Sprague-Dawley rats (190–220 g, $n = 5$), purchased from the Laboratory Animal Center of the Fourth Military Medical University, were fasted for 12 h with free access to water. The minimum curative dose of 5 mg/kg for intravenous injection was obtained from a previous study in rats (3). Felotaxel solution formulated in 50% alcohol and 50% Cremophor EL was intravenously injected into the rats' lateral tail vein. Serial blood samples were collected in heparinized eppendorf tubes from the retro-orbital plexus before administration and after intravenous injection at time-points of 5, 10 and 30 min, and 1, 2, 3, 4, 6, 8, 12 and 24 h. After each sampling, the removed volume

of blood (0.4 mL) was supplemented with an equal volume of normal saline.

Results and Discussion

Method development

The mobile phase aqueous component, 0.2% formic acid in water, provided a good peak shape for felotaxel using the C18 column. In early studies of the pharmacokinetics of drugs of the taxane class, SPE was usually adopted for sample preparation (6-8). This is costly and time-consuming for the extraction of many samples in a pharmacokinetics study without automation. In recent years, LLE with *tert*-butyl methyl ether has been used for sample preparation for the analysis of taxanes (9–11). In this study, diethyl ether, ethyl acetate, cyclohexane, dichloromethane and *n*-butanol were evaluated.

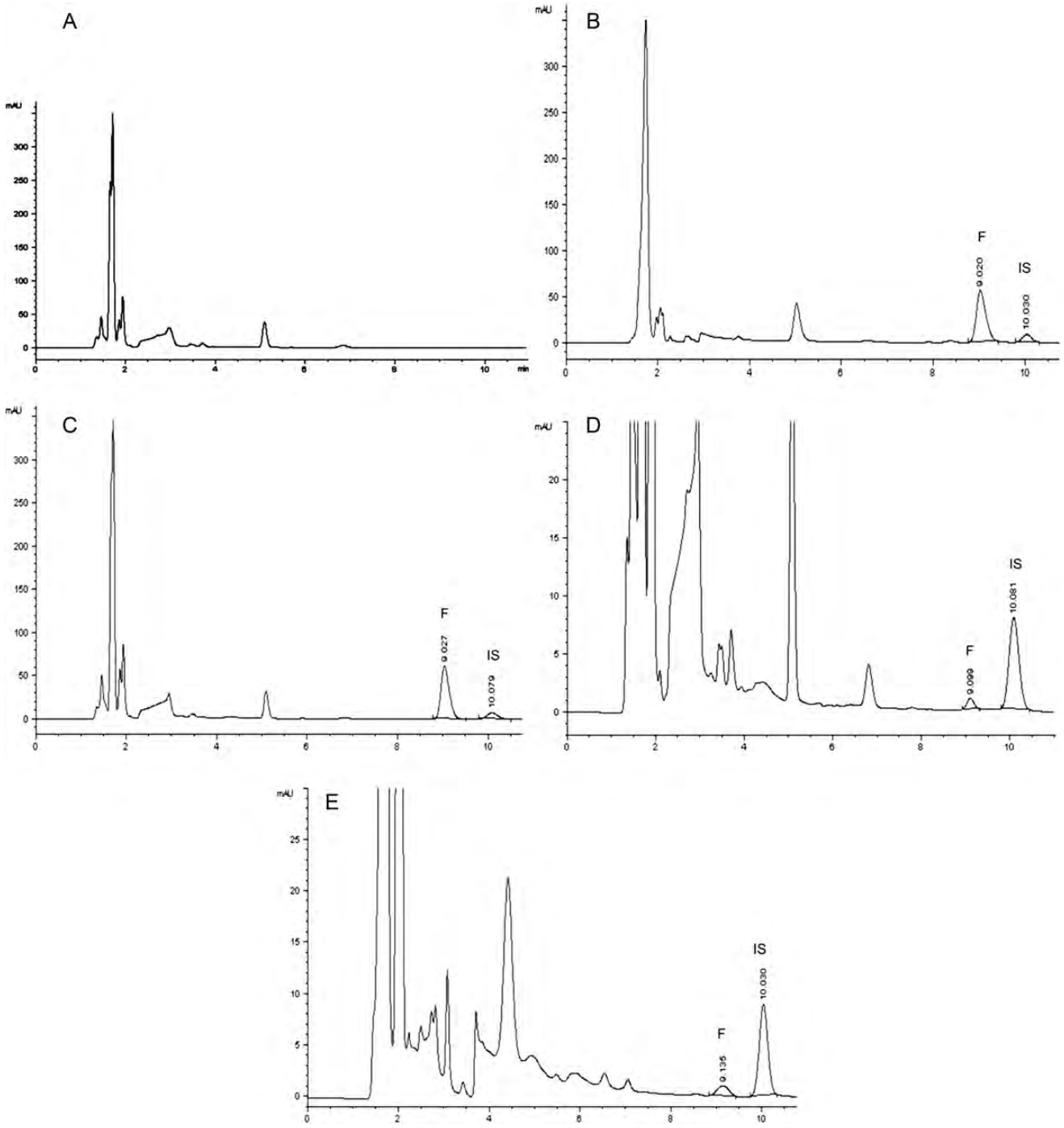

Figure 2. Typical chromatogram for felotaxel (F) and tinidazole (IS) in human plasma sample: blank rat plasma (A); blank rat plasma spiked with 300 ng/mL felotaxel (B); rat plasma collected from a male rat 30 min after intravenous administration of 5 mg/kg of felotaxel (C); human plasma spiked with LLOQ levels of felotaxel (D); human urine spiked with LLOQ levels of felotaxel (E).

Ethyl acetate proved the most efficient extraction solvent, with few interferences and constant recovery.

An appropriate IS is necessary for accurate quantitative analysis of analytes in biological fluids. In this study, tinidazole was chosen as the IS. Tinidazole is a stable chemical raw material that is widely used and commercially available, which displayed a similar solubility to solvent extraction and adsorbent function to the cartridge, compared with those of felotaxel. In addition, the retention times and extraction recoveries for felotaxel and tinidazole were very close, and the resolution between the peak of felotaxel and tinidazole was also good. Based on these observations, tinidazole was employed as the IS for the quantitative analysis of felotaxel in samples.

Selectivity

The extraction and HPLC assay resulted in symmetrical peak shapes and good baseline resolution of felotaxel and tinidazole. Plasma matrix components did not interfere with the analysis. Using this system, the retention times for felotaxel and tinidazole were 9.0 and 10.0 min. Figure 2 illustrates typical chromatograms of plasma and urine. The total analysis time for each run is 11 min. Peak shape and retention time were the same for the injection of pure standards in the mobile phase as for the extracted plasma standards and samples.

Recovery and linearity

Results of the recovery studies with the selected extraction procedure are summarized in Table I, showing that the recovery of felotaxel was more than 85% at all three nominal concentration levels for various samples.

The linearity of felotaxel in human plasma and urine and in rat plasma was in the range from 5 to 1,000 ng/mL. Mean calibration line parameters were found as follows: $Y = 0.02X + 0.00027$ ($r^2 = > 0.9990$), where Y equals the internal ratio (drug/IS) of the peak area response and X equals the concentration spiked. The non-zero standards showed less than 20% deviation at the 5 ng/mL concentration and less than 15% deviation at all other concentration levels.

Accuracy and precision

The results of accuracy and precision measurements assessed by analyzing QC samples at three concentrations are presented in Table I. Both the intra-day and inter-day precision in different matrices was less than 15%. The results are shown in Table I. The intra-day precision (RSD) ranged from 1.1 to 4.8% and the inter-day precision (RSD) ranged from 0.9 to 6.9%. The intra-day accuracy ranged from 87.3 to 107.4% and the inter-day accuracy ranged from 90.0 to 107.2%. The data indicated that the present method has satisfactory accuracy, precision and reproducibility.

Stability

Table II lists the data for the freeze-thaw stability, short-term stability under room temperature and the bench-top stability at three concentrations for samples. Stability was expressed as the percentage ratio of the measured concentration to the nominal concentration. The results showed that analytes were stable under conditions investigated in this study because the measured concentrations were all within 85–115% of the nominal concentrations.

Table I
Precision, Accuracy and Recovery of Felotaxel Assay in Rat Plasma and in Human

		Intra-day precision		Inter-day precision		Recovery	
Biological matrix	QC (ng/mL)	Accuracy (Mean, %)	Precision (RSD, %)	Accuracy (Mean, %)	Precision (RSD, %)	Mean (%)	RSD (%)
Human plasma	10	88.6	1.9	94.3	0.9	86.7	6.2
	100	107.4	4.8	101.2	3.5	87.4	5.3
	500	96.7	1.5	90.7	2.7	81.2	1.6
Human urine	10	92.8	1.2	92.3	5.8	80.5	3.5
	100	87.3	2.3	88.5	1.5	86.3	1.6
	500	93.5	1.1	97.4	2.0	87.9	4.1
Rat plasma	10	101.7	3.3	106.3	6.9	89.3	3.0
	100	89.9	2.0	94.1	4.1	86.1	2.3
	500	93.5	1.4	93.5	1.7	88.4	1.8

Table II
Stability Tests of Felotaxel Assay in Rat Plasma and in Human Plasma and Urine

		Remaining (mean ± SD, %)			
Biological matrix	QC (ng/mL)	Freeze-thaw stability	Short-term stability	Long-term stability	Post-preparative stability
Human plasma	10	94.1 ± 2.9	92.9 ± 2.6	87.3 ± 3.1	86.3 ± 4.5
	100	93.4 ± 2.7	95.1 ± 1.9	104.7 ± 2.5	101.9 ± 3.2
	500	86.8 ± 4.3	92.6 ± 2.8	96.5 ± 1.7	91.2 ± 1.7
Human urine	10	93.9 ± 2.7	87.9 ± 4.0	94.1 ± 5.1	96.9 ± 3.0
	100	91.5 ± 3.4	94.7 ± 3.3	92.2 ± 5.5	94.7 ± 4.3
	500	86.6 ± 3.1	96.3 ± 2.4	87.8 ± 6.0	105.3 ± 3.0
Rat plasma	10	93.5 ± 3.4	91.9 ± 5.3	92.5 ± 2.6	91.0 ± 2.5
	100	91.2 ± 3.6	91.4 ± 2.1	89.3 ± 3.0	91.4 ± 3.7
	500	90.7 ± 2.3	93.7 ± 2.2	94.8 ± 3.3	92.4 ± 3.6

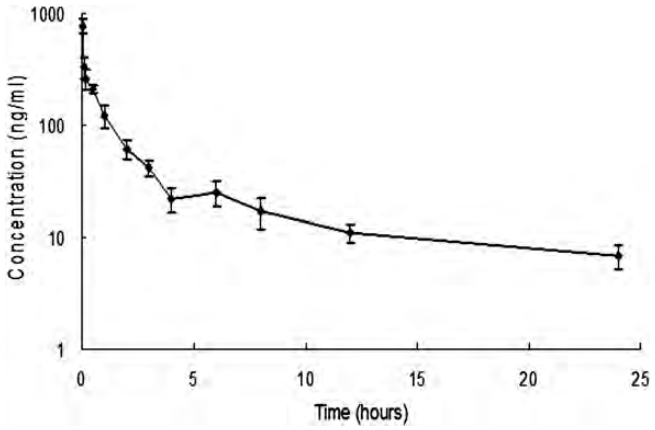

Figure 3. Plasma concentration-time profile of felotaxel from rats after intravenous administration of 5 mg/kg of felotaxel (data are shown as the mean ± SD, $n = 5$).

Application to pharmacokinetic study

The developed assay was applied to a pharmacokinetic study after intravenous administration of felotaxel to rats at a dose of 5 mg/kg. A two-compartment model was proposed and validated through WinNonlin programs (Version 6.2; Pharsight, CA). The mean plasma concentration-time profiles of felotaxel following intravenous administration are shown in Figure 3.

The plasma level of felotaxel declined rapidly, with a half-life of 6.27 ± 2.50 h, which was consistent with the result found by Hu *et al.* (3).

Conclusion

A sensitive, selective and reliable HPLC method using sample preparation by LLE separation on an HPLC column was developed and validated for the quantification of felotaxel in rat plasma and in human plasma and urine.This is the first full validation of an HPLC procedure capable of determining the concentrations of felotaxel in biological samples. The assay has been successfully used in the routine analysis of rat plasma in a preclinical study.

Acknowledgments

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