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Determination of vinflunine in rat plasma by liquid chromatography–electrospray ionization mass spectrometry for a pharmacokinetic study

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Abstract

A rapid, simple and sensitive high-performance liquid chromatography–electrospray ionization mass spectrometry (LC–ESI-MS) method for the quantification of vinflunine in rat plasma was developed and validated. After making alkaline with NaOH, plasma was extracted with ethyl acetate and determined by LC–MS. The analysis was carried out on a Shimadzu VP-ODS column (150 mm × 4.6 mm ID, packed with 5 μm C_{18} Silica RP particle). The mobile phase consisted of methanol–10 mM ammonium acetate buffer (80:20, v/v) with the flow rate of 1.0 ml/min. LC–MS was performed in the selected ion monitoring (SIM) mode using target ions at *m/z*: 817.3 for vinflunine and *m/z*: 373.2 for finasteride (IS). Chromatographic separation was achieved in less than 6 min and the calibration curve was linear over a concentration range of 0.025–6.25 μg/ml. The intra-assay and inter-assay variability values were less than 8.6%. The accuracy ranged from 91.5 to 105.6%. The established method has been successfully applied to a pharmacokinetic study of vinflunine in rats.

Keywords: Vinflunine; LC–MS; Pharmacokinetics; Rat

1. Introduction

The indole alkaloids vinblastine (VBL) and vincristine (VCR), initially extracted from the common Madagascar periwinkle, have been used in the clinic for over 40 years as anticancer agents [1]. These agents inhibit cell proliferation by interacting with the major component of microtubules, tubulin. Although both drugs have reasonable potency in the clinic, there are considerable toxic side effects associated with treatment with VCR (neurotoxicity) and VBL (myelosuppression). Therefore, much effort has been devoted to identifying novel synthetic analogs that have better activity with an associated reduction in toxicity. From these efforts two semi-synthetic vinca alkaloid derivatives, vindesine (VND) and vinorelbine (VRL), emerged and are currently used in the clinic. The success of VRL has resulted in further initiatives to develop analogues with further improvements in terms of activity and toxicity. Vinorelbine has now been further modified through super-acidic chemistry to generate more active derivatives. The process of production involves the insertion of two fluorine atoms at the 20′ position and reduction of the 3′4′ double bond to produce 20′,20′-difluoro-3′4′-dihydrovinorelbine, known as vinflunine (Fig. 1) [2].

Vinflunine, like the other vinca alkaloids inhibited microtubule assemble [3,4], but exerted markedly superior on antitumour activities against a screen of 13 murine and human tumour models compared to the parent compound, vinorelbine [5–7]. Differences in terms of the inhibitory effects of vinflunine on microtubules dynamics and its tubulin binding affinities have been identified, which appeared to distinguish it from the other vinca alkaloids. Vinflunine induced smaller spirals with a shorter relaxation time, which might be associated with reduced neurotoxicity [8]. Furthermore, although vinflunine appeared to participate in P-glycoprotein-mediated drug resistance mechanisms, it had proved only a weak substrate for this protein and a far less potent inducer of resistance than vinorelbine [9]. Vinflunine is presently in phase III clinical trials for treatment of bladder cancer and Non-Small-Cell lung cancer [10].

Compared to the extensive literatures on pharmacodynamic investigations of the other vinca alkaloids little data is avail-

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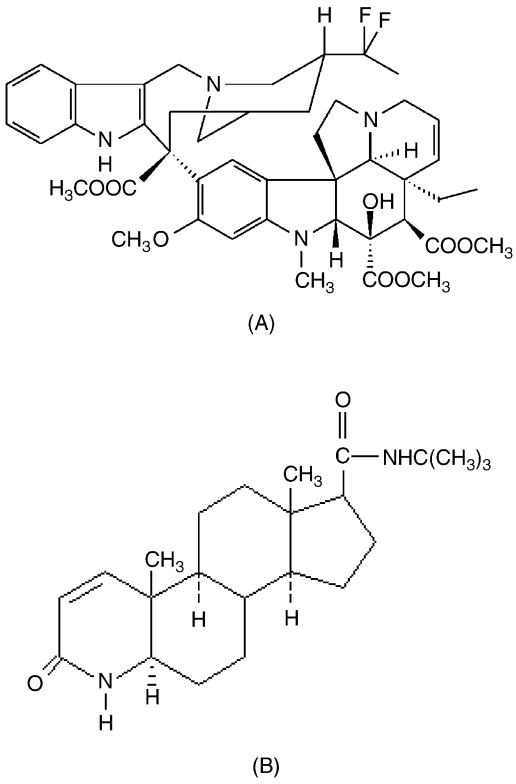

Fig. 1. Chemical structures of vinflunine (A) and finasteride (B).

able concerning the determination and pharmacokinetics of vinflunine. Recently, non-aqueous capillary electrophoresis and HPLC method were developed to separate and measure a group of 11 alkaloids including vinflunine [11]. Bennouna used HPLC to study the pharmacokinetics of vinflunine in human volunteers, in which vinflunine was extracted by diethyl ether under alkaline followed by a back-extraction in acidic conditions [8].

But the previous methods reported have limitations due to the complicated sample processing procedures or low sensitivity. Pharmacokinetic studies usually involve large numbers of biologic sample processing and quantification of small amount of drug in biologic samples, this highlighted the urgent need for a relatively simple and sensitive analytical method to quantify the compound of interest in biologic samples. This paper describes a relatively simple and sensitive liquid chromatography–mass spectrometry method suitable for the determination and pharmacokinetic evaluation of vinflunine in rat plasma. It was demonstrated that the method present here was simple, flexible and sensitive enough for the analysis of vinflunine in rat plasma samples with acceptable recovery and precision. This method has been successfully applied to the pharmacokinetic studies of vinflunine following a single i.v. dose of vinflunine to rats.

2. Experimental

2.1. Chemicals and reagents

Vinflunine (purity 99.87%) was supplied by Qilu Pharmaceutical Co. Ltd. (China). The internal standard (IS), finasteride (purity 99.10%, Fig. 1), was a gift from Organic Chemistry Laboratories of China Pharmaceutical University. Sodium hydroxide and ethyl acetate were of analytical grade, and methanol (Merck, Germany) of HPLC grade. All the other chemicals were purchased from Nanjing Chemical Reagent Co. (Nanjing, China). Purified water from a Milli-Q system (Millipore, Bedford, MA, USA) was used throughout the experiment.

2.2. Animal

Sprague–Dawley rats were obtained from the experimental animal center of China Pharmaceutical University, and the studies were approved by the Animal Ethics Committee of China Pharmaceutical University.

2.3. Liquid chromatograph–mass spectrometry (LC–MS)

HPLC analyses were performed using a Hewlett-Packard HP1100 LC system (Hewlett-Packard, USA) with a Shim-pack C_{18} column(150 mm × 4.6 mm ID, 5 μm, Shimadzu, Japan). The mobile phase was methanol–10 mM ammonium acetate (80:20, v/v), and the column temperature was maintained at 25 °C. A constant mobile phase flow rate of 1.0 ml/min was employed throughout the analyses. LC–ESI-MS was carried out using nitrogen to assist nebulization. A suitable single quadrupole mass spectrometer equipped with an electrospray ionization source was used in positive ion selected ion monitoring (SIM) mode, set with a drying gas (N_2) flow of 10 l/min, nebulizer pressure of 40 psig, drying gas temperature of 350 °C and capillary voltage of 4 kV. The fragmentor voltage was 120 V. Target ions were monitored at *m/z* 817.3 (Fig. 2) for vinflunine and *m/z* 373.2 (Fig. 2) for finasteride (IS) in the SIM mode.

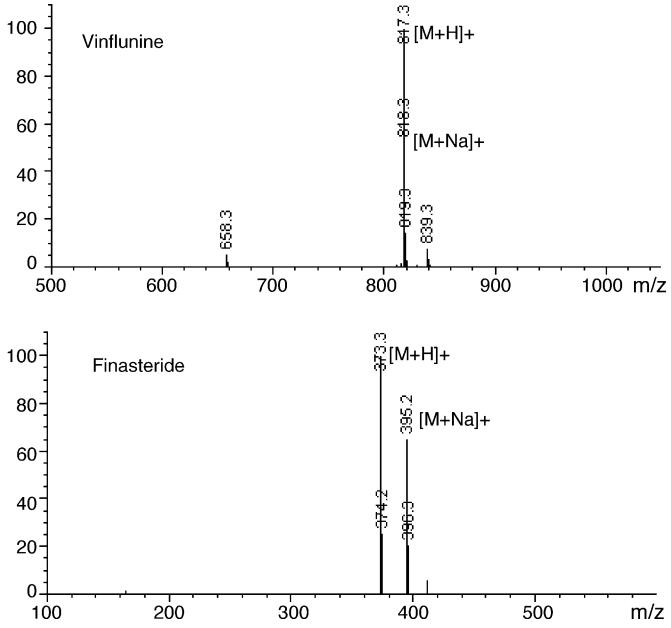

Fig. 2. Positive ion electrospray mass spectra obtained in a full scan mode from using authentic of vinflunine and finasteride.

2.4. Preparation of standard and quality control solution

A standard stock solution of vinflunine (1 mg/ml) was prepared by dissolving the drug in purified water. The solution was then successively diluted with purified water to prepare working standard solutions in the concentration range of 0.25–62.5 μg/ml for analyte. The IS stock solution (1 mg/ml) was prepared with methanol as solvent. Working solutions were prepared by diluting the stock solution of finasteride to 1 μg/ml with methanol. All the solutions were stored at 4 °C and were brought to room temperature before use.

For preparation of plasma calibration curves samples, 10 μl each of working solution within the concentration range of 0.25–62.5 μg/ml were evaporated in tubes under gentle stream of nitrogen at 50 °C. After addition of 0.1 ml blank rat plasma and mixing for 10 s on a vortex mixer, the samples were subjected to extraction and analysis. Plasma concentrations were 0.025, 0.05, 0.1, 0.19, 0.39, 0.78, 1.56, 3.12, 6.25 μg/ml for analyte. Plasma samples were quantified using the ratio of peak area of vinflunine to that of the IS. Peak area ratios were plotted against concentrations equation of the least squares regression line calculated.

All the quality control samples (QCs) used in the validation and during the pharmacokinetic study were prepared in the same way as the calibration standards before analysis. Plasma concentrations of QCs were 0.050, 0.39, 3.12 μg/ml for vinflunine.

2.5. Sample preparation

Plasma samples were thawed in a water-bath at 37 °C. 0.1 ml volume of the plasma sample was transferred to a 15 ml plastic test tube together with 20 μl of IS solution and 20 μl of 0.25 M sodium hydroxide solution. After vortex shaking for 1 min (Eppendorf, 5432 vortex mixer, Germany), 2 ml of ethyl acetate was added and the mixture was vortexed for 3 min. After centrifugation at 3500 rpm for 10 min (TGL-16G, Shanghai, China), the upper organic layer was quantitatively transferred to a 10-ml glass tube and evaporated to dryness using evaporator at 50 °C. The residue was reconstituted in 150 μl of the mobile phase, and then vortex-mixed. After centrifugation at 14 000 rpm (Thermo IEC, Micromax, USA) for 10 min, 90 μl supernatant was drawn and a 40-μl aliquot of the solution was injected into the LC/MS system for analysis.

2.6. Method validation

The precision and accuracy of the assay were obtained by comparing the predicted concentration (obtained from the calibration curve) to the actual concentration of vinflunine spiked in blank plasma. Intra-day precision was determined by repeated analysis of each QC sample on one day ($n = 5$), and inter-day precision was determined by repeated analysis on five consecutive days ($n = 1$ series per day). The precision was expressed as the inter-day and intra-day coefficient of variation [CV = (S.D./mean of the recoveries) × 100%]. Accuracy was defined as the relative deviation in the computed value (E) of a standard from that of its true value (T) expressed as a percentage (RE%). It was calculated using the formula $RE\% = (E - T)/T \times 100$. The limit of quantitation (LOQ) was considered as the concentration of vinflunine that produced a signal-to-noise (S/N) ratio of 10.

2.7. Freeze and thaw stability

The freeze and thaw stability study samples were obtained by adding the standard solution in the blank rat plasma at three QC levels. These samples were frozen at −20 °C for 7 days, and then thawed at room temperature. After being allowed to completely thaw, the samples were refrozen for 24 h under the same conditions. This freeze-thaw cycle was repeated three times before these samples were analyzed.

2.8. Pharmacokinetic study

18 Sprague–Dawley rats (both sexes) weighing 180–220 g were assigned randomly into three groups for intravenous (i.v.) administration of 3.5, 7 and 14 mg/kg vinflunine. A cannula was inserted into the carotid artery of a rat under ether anesthesia. Blood samples were collected immediately before and at 0.033, 0.25, 0.75, 1, 2, 4, 8, 12, 24, 36, 48, 72 h after drug administration. The blood samples were withdrawn into heparinized Eppendoff tubes, and were centrifuged at 2000 rpm for 10 min at 4 °C. A 0.2 ml volume of plasma was obtained and stored at −20 °C until analysis.

Pharmacokinetic parameters were calculated from the plasma concentration-time data. The elimination half-life ($t_{1/2}$) was determined by linear regression of the terminal portion of the plasma concentration-time data. The area under the plasma concentration-time curve from zero to the last measurable plasma concentration point ($AUC_{0-\tau}$) was calculated by the linear trapezoidal method. Extrapolation to time infinity ($AUC_{0-\infty}$) was calculated as follows: $AUC_{0-\infty} = AUC_{0-\tau} + C_t/k_e$, where C_t is the last measurable plasma concentration and k_e is the terminal elimination rate constant.

3. Result and discussion

3.1. Method development

Different solvent mixtures were tested for extraction of vinflunine from plasma, such as ethyl acetate, ether, chloroform and ether–chloroform. All were evaluated for recovery and sample clean up. The best extraction was obtained using ethyl acetate.

The signal intensity obtained in the positive mode was much higher than that in the negative mode. The ESI mass spectrum showed that the protonated molecular ion $[M + H]^+$ of vinflunine was at m/z 817.3 (Fig. 2). The intensity of ion at m/z 817.3 was compared at fragmentor voltages of 70, 80, 100, 120, 130 and 150 V in order to determine the optimal collision energy. The result showed that the highest sensitivity was obtained using a fragmentor voltage of 120 V which was therefore used to carry out LC–ESI-MS in the SIM mode. At this collision energy the

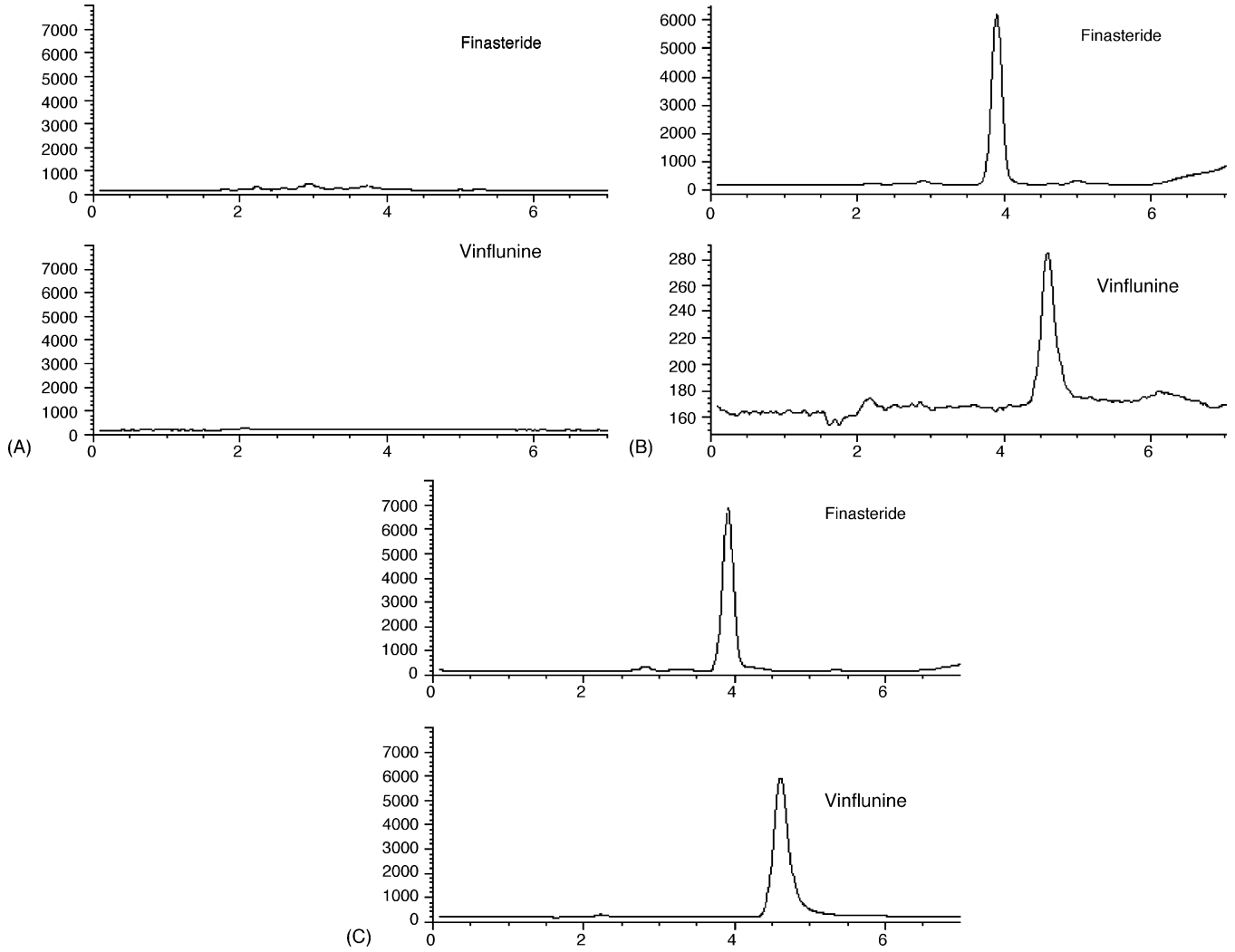

Fig. 3. Representative chromatograms of (A) blank rat plasma, (B) rat plasma spiked with vinflunine at LLOQ and finasteride (IS) at its nominal concentration, (C) plasma sample obtained at 0.75 h after a single i.v. dose of 7 mg/kg vinflunine from a rat.

most intense product ion of the IS was the protonated molecular ion at *m/z* 373.2. Finasteride was selected as an internal standard (IS) because of its similar ionization condition and appropriate retention time compared to vinflunine.

3.2. Method validation

3.2.1. Selectivity

Potential interference from endogenous compounds was investigated by analyzing rat plasma of six different sources. Typical chromatograms of a blank plasma sample, a blank plasma sample spiked with vinflunine at the LLOQ and the IS at its nominal concentration, and a plasma sample obtained at 0.75 h after a single i.v. dose of 7 mg/kg vinflunine from a rat are shown in Fig. 3. No significant interference or ion suppression from endogenous substances was observed at the retention time of the analyte and IS. The LC–MS method has high specificity because only the objective ions derived from the analyte of interest are monitored. This was expected given the high degree of specificity normally associated with LC–MS (SIM) methods.

3.2.2. Calibration curve and sensitivity

Excellent linearity was obtained over the concentration range of 0.25–6.25 μg/ml for analyte in rat plasma. The correlation coefficients for the calibration regression curves were greater than 0.999. The equations of the representative calibration curves was as follow: $y = 1.21x + 0.03836$ ($n = 5$).

The current method had an LLOQ of 0.025 μg/ml (accuracy and precision less than 20%) for analyte, which is sufficient for pharmacokinetic studies following i.v. administration of vinflunine. The detection limit for vinflunine was 5 ng/ml with a signal-to-noise ratio (S/N) = 3.2.

3.2.3. Precision and accuracy

The method showed good precision and accuracy. Table 1 summarizes the intra- and inter-day precision and accuracy for

Table 1
Intra-day and inter-day coefficients of precision and accuracy for determination of vinflunine in rat plasma ($n = 5$)

Added concentration (μg/ml)	Intra-day			Inter-day		
	Measured concentration (μg/ml) (mean ± S.D.)	CV%	RE%	Measured concentration (μg/ml) (mean ± S.D.)	CV%	RE%
0.05	0.052 ± 0.003	4.8	3.2	0.046 ± 0.002	3.6	−7.5
0.39	0.40 ± 0.02	5.3	1.5	0.38 ± 0.03	7.3	−8.5
3.12	3.3 ± 0.06	6.0	5.6	3.0 ± 0.3	8.6	−3.2

Table 2
Freeze and thaw stability for vinflunine in rat plasma ($n = 5$)

Added concentration (μg/ml)	Measured concentration (μg/ml) (mean ± S.D.)	Precision (CV%)	Accuracy (RE%)
0.05	0.047 ± 0.005	9.6	−6.1
0.39	0.35 ± 0.02	5.9	−11
3.12	3.1 ± 0.2	7.8	0.00

Table 3
The pharmacokinetic parameters of vinflunine after i.v. administrations to rats

Dose (mg/kg)	$t_{1/2}$ (h)	V_d (l/kg)	MRT (h)	CL (ml/h)	$AUC_{0-\tau}$ (μg h/ml)
3.5	18.29 ± 1.87	19.60 ± 2.39	26.40 ± 2.70	146.9 ± 12.8	4.61 ± 0.26
7.0	19.36 ± 1.43	23.61 ± 2.76	27.94 ± 2.07	188.0 ± 27.3	7.28 ± 0.52
14	18.67 ± 2.06	23.84 ± 4.12	26.94 ± 2.97	237.6 ± 84.9	12.78 ± 2.99

vinflunine from the QC samples, respectively. In this assay, the intra-run precision was less than 6.0%, and the inter-run precision less than 8.6%. The accuracy was from 91.5 to 105.6%.

3.2.4. Stability

The freeze and thaw stability was determined by analyzing vinflunine samples at three concentrations after 3 freeze-thaw cycles. The coefficients of variation were all within 10% for analyte and the deviation from the expected concentration ranged from 0 to −10.7 for vinflunine. These results (Table 2) show that vinflunine are stable in the frozen rat plasma and during the freeze-thaw cycles. QC samples of vinflunine obtained by extraction showed no significant degradation after at least 24 h at room temperature (8.4% deviation of the spiked values). Standard stock solution of vinflunine was shown to remain stable for at least 2 months at 4 °C.

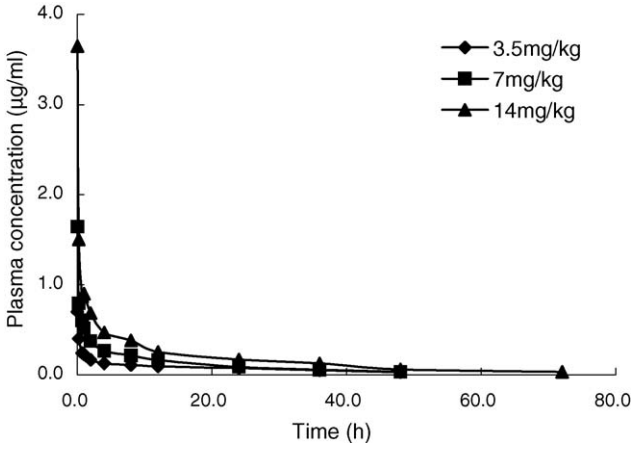

Fig. 4. Mean plasma concentration-time profile of vinflunine after i.v. administration of 3.5, 7 and 14 mg/kg vinflunine to rats.

3.3. Application

This validated analytical method was used to study pharmacokinetic profiles of vinflunine in rat plasma after i.v. 3.5, 7, 14 mg/kg vinflunine to 18 rats. The mean plasma concentration-time profiles of vinflunine are shown in Fig. 4. A diexponential decay was observed with a sharp decrease during the first phase. The pharmacokinetic parameters of vinflunine after i.v. administrations are listed in Table 3. Volume of distribution (V_d) is large (approximate 22.3 l/kg), suggesting an extensive distribution in rat tissues. The resulting mean half-life value was assessed at 18.8 h based on a 72-h sampling period. A dose-proportional increase of blood exposure concentration was demonstrated on AUC, indicating that the pharmacokinetics of vinflunine in rat was linear in the investigated dose range (3.5–14 mg/kg).

4. Conclusion

In the present study, a simple, rapid, sensitive, accurate and precise LC/MS method was developed and validated for the determination of vinflunine in rat plasma. Because of the relatively short chromatographic run time and straightforward sample preparation procedure, a sample throughput of about 150 per day was achieved. The present method has been successfully applied to the pharmacokinetic study of vinflunine in rats.

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References

[1] R.C. Donehover, E.K. Rowinsky, V.T. Devita, S. Hellman, S.A. Rosenberg, Cancer Principles and Practice of Oncology, J.P. Lippincott, Philadelphia, PA, 1993, pp. 409–417.
[2] J. Fahy, A. Duflos, J.P. Ribet, J. Am. Chem. Soc. 119 (1997) 8576–8577.
[3] M.A. Jordan, L. Wilson, Biochemistry 29 (1990) 2730–2739.
[4] A. Kruczynski, J.M. Barret, C. Etievant, F. Colpaert, J. Fahy, B.T. Hill, Biochem. Pharmacol. 55 (1998) 635–648.
[5] A. Kruczynski, F. Colpaert, J.P. Tarayre, P. Mouillard, J. Fahy, B.T. Hill, Cancer Chemother. Pharmacol. 41 (1998) 437–447.
[6] B.T. Hill, H.H. Fiebig, W.R. Waud, M.F. Poupon, F. Colpaert, A. Kruczynski, Eur. J. Cancer 35 (1999) 512–520.
[7] A. Kruczynski, B.T. Hill, Oncol. Hematol. 40 (2001) 159–173.
[8] J. Bennouna, P. Fumoleau, J.P. Armand, E. Raymond, M. Campone, F.M. Delgado, C. Puozzo, M. Marty, Ann. Oncol. 14 (2003) 630–637.
[9] C. Etiévant, J.M. Barret, A. Kruczynski, et al., Invest. New Drugs 16 (1998) 3–17.
[10] J.C. Jacquesy, M.P. Jouannetaud, Ann. Pharm. Fr. 63 (2005) 28–34.
[11] L. Barthe, J.P. Ribet, M. Pelissou, M.J. Degude, J. Fahy, A. Duflos, J. Chromatogr. A 968 (2002) 241–250.