Journal of Pharmaceutical Analysis 7 (2017) 118–122

Contents lists available at ScienceDirect

Journal of Pharmaceutical Analysis

journal homepage: www.elsevier.com/locate/jpa

Original Research Article

Degradation kinetics of larotaxel and identification of its degradation products in alkaline condition

Xiaoming Liang[a], Zhenzhen Liu[b], Huiyan Shi[a], Yuanyuan Zhang[a], Shixiao Wang[a], Kaishun Bi[a], Xiaohui Chen[a,*]

[a] Department of Pharmaceutical Analysis, School of Pharmacy, Shenyang Pharmaceutical University, 103 Wenhua Road, Shenyang 110016, China
[b] Department of Functional Science, School of Medicine, Yangtze University, No.1 Nanhuan Road, Jingzhou City 434100, China

ARTICLE INFO

Keywords:
Larotaxel
Degradation kinetics
pH
Temperature
Degradation products

ABSTRACT

Larotaxel, a new taxane compound prepared by partial synthesis from 10-deacetyl baccatin III, is active against tumors. In this research, a selective LC–MS method was developed and validated for the study of degradation kinetics of larotaxel, which was carried out in aqueous solutions with different pH (1.5, 3.0, 5.0, 6.5, 7.4, 9.0, 10 and 11.0) and temperature (0, 25, 37 and 45 °C). The linear range was 0.5–25 μg/mL, the intra- and inter-day precisions were less than 7.0%, and accuracy ranged from 97.4–104.5% for each analyte. The observed rate obtained by measuring the remaining intact larotaxel was shown to follow first-order kinetics. The activation energies for degradation were 126.7 and 87.01 kJ/mol at pH 1.5 and 11, respectively. Although larotaxel was stable in pH 5, 6.5 and 7.4 buffers at 37 °C for 24 h during our study, increasing or decreasing the pH of the solutions would decrease its stabilities. Moreover, three main degradation products in alkaline condition were separated by HPLC and identified by Q–TOF–MS. The three degradation products were confirmed as 10-deacetyl larotaxel, 7, 8-cyclopropyl baccatin III and 10-deacetyl-7, 8-cyclopropyl baccatin III.

1. Introduction

Cancer is still the leading cause of death throughout the world. While improved prevention and screening programmes will most probably contribute to a reduction in the frequency of such deaths in the future, the development of new anticancer drugs will remain as a key component of integrated strategies to minimise the mortality associated with this disease [1]. Recently, several new compounds, such as taxanes and camptothecins, have demonstrated promising activities [2].

Larotaxel(4,10-β-diacetoxy-2-α-benzoyloxy-5-β,20-epoxy-1,13α-dihydroxy-9-oxo-19-norcyclopropa [g] tax-11-ene, 13 ester with (2R,3S)-N-tert-butoxycarbonyl-3-phenylisoserine, dihydrate) is a new taxane compound prepared as a single diastereomer by partial synthesis from 10-deacetyl baccatin III. The pharmaceutical mechanism of larotaxel is similar to that of the current taxanes which interact with polymerized tubulin to promote the formation of microtubules, prevent their disassembly, and thus block cell division at the G2-M phase [3–6]. Larotaxel has a much lower affinity for P-glycoprotein 1 than docetaxel and is shown to be active in cell lines resistant to doxorubicin, vinblastine, paclitaxel and docetaxel [7]. The antitumor activity in non-small cell lung cancer, the reproducible profile of toxicity and the ability to cross the blood-brain barrier make larotaxel worthy of further disease-oriented clinical development [8–10]. Since new molecular entities are evaluated in clinical development, the understanding, identification and control of degradation products or impurities in drug substances are essential, which can also assist the development of proper formulations and storage conditions of the drugs [11,12]. A very few reports are available in the literature on degradation kinetics of larotaxel and identification of its degradation products or impurities. The aim of the study was to determine the degradation rate of larotaxel in solutions with different pHs and temperatures by using liquid chromatography–mass spectrometry (LC–MS) to determinate the remaining intact larotaxel, to elucidate the degradation paths of the molecule and to identify its main degradation products in alkaline condition.

2. Materials and methods

2.1. Chemicals and reagents

Larotaxel (purity > 98%) was synthesized in School of Pharmacy,

Peer review under responsibility of Xi'an Jiaotong University.
* Corresponding author.
E-mail address: cxh_syphu@hotmail.com (X. Chen).

http://dx.doi.org/10.1016/j.jpha.2016.11.002
Received 9 July 2016; Received in revised form 17 November 2016; Accepted 18 November 2016
Available online 19 November 2016

Fudan University (Shanghai, China). Phosphoric acid of HPLC grade was obtained from Kermel Chemical Reagent (Tianjin, China). Methanol and acetonitrile of HPLC grade were purchased from Fisher Scientific (NJ, USA). Distilled water was prepared from demineralized water throughout the study. Other chemicals were of analytical grade.

2.2. Apparatus and analytical conditions

The LC–MS analyses were performed on a Shimadzu LC–MS 2010 A system (Japan). Liquid chromatographic separation was performed on a Kromasil C_{18} column (150 mm×4.6 mm, 5 μm) and preceded by a C_{18} guard column (4.0 mm×2.0 mm, 5 μm, Phenomenex, Torrance, CA, USA) using methanol-water (85:15, v/v) as mobile phase at the flow rate of 0.8 mL/min with 25% of the eluent split into the inlet of the mass spectrometer. The injection volume was 10 μL. The column and autosampler tray temperature were kept constant at 25 °C and 4 °C, respectively. The analytes were ionized with an electrospray ionization (ESI) source in positive ion mode under the following source conditions: nebulizing gas 1.5 L/min; drying gas 1.5 L/min; curved desolvation line (CDL) temperature 250 °C; heat block temperature 200 °C; detector voltage 1.75 kV; and the other parameters were fixed for the tuning file. Analysis was carried out by selected ion monitoring mode at *m/z* 854.3 for larotaxel $[M+Na]^+$ and at *m/z* 876.3 for Paclitaxel $[M+Na]^+$(Internal standard (IS)).

The HPLC system consisting of two LC-10AT vp pumps and an SPD-M10A vp ultraviolet absorption (UV) detector (Shimadzu, Japan) was used. The degradation products in alkaline condition were separated on a Kromasil C_{18} column (150 mm×4.6 mm; 5 μm) with the following gradient: starting with acetonitrile-water (45:55, v/v) for 3 min, increasing linearly to acetonitrile-water (90:10, v/v) over 20 min, and then keeping acetonitrile-water (90:10, v/v) until 60 min. The flow rate was kept constant at 1.0 mL/min and column was maintained at 35 °C. The injection volume was 20 μL and the detector was set at 230 nm.

Quadrupole–time-of-flight–mass spectrometry (Q–TOF–MS) experiment was performed on a Bruker Daltonics micrOTOF-Q mass spectrometer (Billerica, MA, USA). For Q–TOF–MS experiment, the capillary voltage of the ion source was set at 4,500 V and nitrogen was used as the desolvation and nebulizing gas at a constant flow of 4.0 L/min. For collision-induced dissociation (CID) experiments, argon was used as the collision gas and the collision energy was set to 25 eV. The scan range was set at *m/z* 100–2000. Mass spectrometer was equipped with an ESI source and operated in positive ion mode.

For kinetic studies a water bath set from 0 to 45 °C was used. A pH meter was utilized for determining pH value of the phosphate buffers.

2.3. Degradation kinetics study and sample preparation

2.3.1. Calibration curves

Calibration curves for larotaxel was prepared by analyzing calibration samples prepared by putting the blank buffer solution (100 μL), the standard solutions (100 μL) and the internal standard (50 μL, 5 μg/mL) into 900 μL methanol and mixed well. Quality control (QC) samples were similarly prepared by spiking QC solutions from a different weighing of the reference substance. Each analytical run included a set of calibration samples, a set of QC samples in duplicate and experimental samples.

2.3.2. Degradation kinetics of larotaxel in different pH solutions

An aliquot of 9 mL of 0.05 mol/L sodium phosphate buffer at different pHs of 1.5, 3.0, 5.0, 6.5, 7.4, 9.0, 10 and 11.0 adjusted with small volumes of phosphoric acid or 0.05 mol/L sodium hydroxide was pre-incubated at 37 °C in a water bath for 5 min, 1 mL of 200 μg/mL larotaxel solution was added to a final concentration of 20 μg/mL, and different pH values were set according to relative reference [13–16]. Each of these solutions was incubated separately at 37 °C in a water bath. According to an appropriate time schedule, typically at 0, 0.167, 0.333, 0.5, 0.75, 1, 2, 4, 8, 12, and 24 h, 100 μL of each larotaxel solution was transferred into a vial containing 1 mL methanol and 50 μL (5 μg/mL) of internal standard, then mixed, certificated and transferred to another vial. The solution was then stored at −20 °C until analysis, generally within 24 h. The experiment for each pH value was performed in triple. The injection volume was 10 μL for LC–MS analysis.

2.3.3. Degradation kinetics of larotaxel in different temperatures

Larotaxel solutions (20 μg/mL) in 0.05 mol/L Phosphate-buffered saline (PBS) at pH 1.5 and 11 were incubated at 0, 25, 37 and 45 °C in water bath, respectively. Then samples were prepared as described above. The experiment was performed in triple [17].

2.3.4. Degradation products in alkaline condition

For the degradation products study, 2 mL of 200 μg/mL larotaxel solution was mixing with 8 mL of 0.005 mol/L aqueous sodium hydroxide at room temperature, and then 20 μL of the solutions at the time 0.333, 0.667, 1, 2, and 4 h were used for HPLC analysis.

3. Results and disscusion

3.1. Assay validation for LC–MS

Linear calibration curve for larotaxel was obtained over the range of 0.5–25 μg/mL. The typical equation of the calibration curve was $y=0.2732x+0.0336$ (r =0.9991). Accuracy and precision were calculated based on the results of QC samples at three concentration levels of 1, 5 and 20 μg/mL (n=18). In this study, the intra- and inter-day precisions were less than 7.0%, and accuracy ranged from 97.4%–104.5% for each QC level. The post preparative samples with IS were stable at −20 °C for at least 24 h.

3.2. Degradation kinetics of larotaxel

3.2.1. Reaction order of larotaxel

Concentration (C), In(C) or $1/C$ was plotted against time (h) respectively to determine the reaction order of the degradation of larotaxel. Their corresponding correlation efficiencies (r) were calculated. It was found that the plot of In(C) against the reaction time (h) (first-order plot) showed a strong correlation under all experimental conditions with the correlation coefficients over 0.95.

3.2.2. Influence of pH

A typical first order of degradation of the drug profile is shown in different pH buffer solutions at 37 °C in Fig. 1. The half-life obtained from degradation of larotaxel in different pH buffer solutions at 37 °C

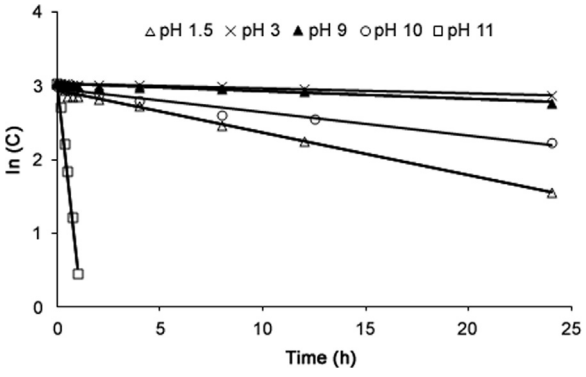

Fig. 1. First-order plots of the degradation of larotaxel in buffer solutions (0.05 M) at pH 1.5, 3, 9, 10 and 11.

Table 1
The half-life obtained from degradation of larotaxel in different pH buffer solutions at 37 °C.

pH value	1.5	3	9	10	11
half-life (h)	11.8	108.3	68.6	21.8	0.27

Table 2
Effects of temperature on the degradation rates of larotaxel at different pH 1.5 and 11 (n=3).

pH value	Temperature (K)			
	273	298	310	318
1.5	-[a]	0.0064[b]	0.0584	0.1452
11	0.0264	0.5729	2.576	5.9351

[a] Below detection limit.
[b] The apparent degradation rate kinetic constant(k) of larotaxel was determined, under pH 1.5 and 11, at different temperatures (K): this was calculated from Arrhenius equation.

is shown in Table 1. The degradation rate increased with the pH increase when the pH of the solution was in basic part; conversely, decreasing the pH of the solution led to degradation rate increase when the solution is in acid part. All these results indicated specific basic or acidic catalyzed effects on the degradation of larotaxel. Larotaxel was stable in pH 5, 6.5 and 7.4 buffers for 24 h at 37 °C during our study, which indicated that larotaxel is stable in neutral and partial acidic solutions.

3.2.3. Influence of temperature

The temperature dependency of degradation rate constant of larotaxel was investigated at 0, 25, 37 and 45 °C with the pH values of the solutions maintaining at 1.5 and 11, respectively. The effect of temperature on the degradation of larotaxel is shown in Table 2, from which we can see that the apparent degradation rate kinetic constant (k) correspondingly increased with the increase of temperature. Linear curves were obtained by plotting the logarithm of apparent degradation kinetic constants (ln$k$) against $1/T$ in the solutions at pH 1.5 and 11 with the correlation coefficients over 0.99, which was mainly following Arrhenius equation ($k=A\ e^{-E_a/RT}$, where k is the reaction constant (h^{-1}), A is the frequency factor (h^{-1}), E_a is the activation energy (J/mol), R is the gas constant (8.314 J/mol K), and T is the temperature (K)). The activation energies (E_a) for degradation calculated from the slopes of the lines were 126.7 and 87.01 kJ/mol at pH 1.5 and 11, respectively [18]. The slopes of the lines are shown in Fig. 2. According to the rule that the higher E_a is, the less the degradation reaction is influenced by temperature, the result indicated that temperature made bigger contribution to the degradation of larotaxel in the solution of pH 11 than that of pH 1.5.

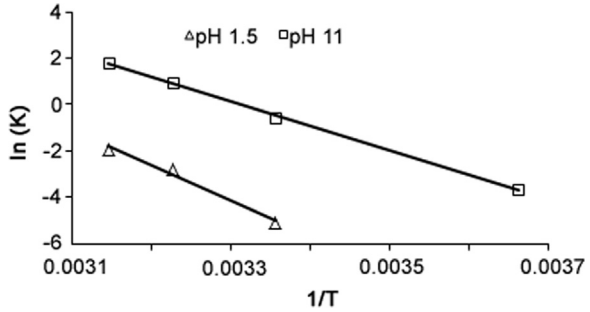

Fig. 2. Linear curves of the logarithm of apparent degradation kinetic constants (ln$k$) against $1/T$ in the solutions at pH 1.5 and 11.

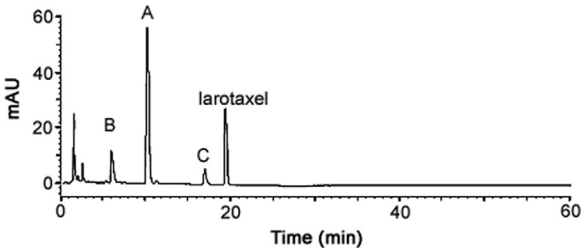

Fig. 3. HPLC chromatogram of larotaxel in 0.005 M NaOH for 1h.

3.3. Degradation products and degradation paths

As larataxel is very unstable in alkaline condition, degradation products were investigated in 0.005 mol/L sodium hydroxide aqueous. The typical chromatogram is shown in Fig. 3. Three degradation products were found and identified. The same type of degradation products were found under acid condition (pH 1.5) since the 12 h, while the amounts were less than that found under alkaline conditions.

3.3.1. Larotaxel

The Q–TOF–MS spectrum of larotaxel showed a sodium adduct $[M+Na]^+$ at m/z 854.3711 and a protonated molecular ion $[M+H]^+$ at m/z 832.3892. The fragmentation pathway of the protonated molecular ion at m/z 832.3892 was obtained: m/z at 551.2634, 491.2428, 369.1712 and 291.1698, as shown in Fig. 3D.

3.3.2. Structure elucidation of larotaxel Impurity A

The Q–TOF–MS spectrum of Impurity A showed a sodium adduct $[M+Na]^+$ at m/z 591.2554 and a protonated molecular ion $[M+H]^+$ at m/z 569.2734, indicating that Impurity A has molecular mass less than that of larotaxel by 263.1283 Da. The fragmentation patterns of the molecular ion at m/z 569.2734 were obtained as follows: m/z at 491.2428, 369.1712, 351.1831 and 309.1212, as shown in Fig. 4A. These fragments ions are in agreement with the fragmentation pathway of the proposed structure of impurity C, so the Impurity A was deduced as 7, 8-cyclopropyl baccatin III.

3.3.3. Structure elucidation of larotaxel Impurity B

The Q–TOF–MS spectrum of Impurity B showed a sodium adduct $[M+Na]^+$ at m/z 549.2453 and a protonated molecular ion $[M+H]^+$ at m/z 527.2628, indicating that the Impurity B has molecular mass less than that of Impurity A by 42.0106 Da. As the ion intensity of $[M+H]^+$ was very weak, the fragmentation patterns of the molecular ion at m/z 549.2453 were obtained as follows: m/z at 489.2248, 427.1923 and 367.1526, as shown Fig. 4B. Impurity B was deduced as 10-deacetyl-7, 8-cyclopropyl baccatin III.

3.3.4. Structure elucidation of larotaxel Impurity C

The Q–TOF–MS spectrum of Impurity C showed a sodium adduct $[M+Na]^+$ at m/z 812.3616 and a protonated molecular ion $[M+H]^+$ at m/z 790.3803, indicating that the Impurity C has molecular mass less than that of larotaxel by 42.0089 Da. The fragmentation patterns of the molecular ion at m/z 790.3803 were obtained as: m/z at 509.2539, 491.2428, 351.1612 and 291.1698, as shown in Fig. 4C. These fragments ions are similar to the fragmentation pathway of the proposed structure of larotaxel. Further confirmation was given by the HPLC retention time. On the basis of the data, Impurity C was deduced as 10-deacetyl larotaxel.

The order of HPLC retention time, molecular mass and fragments of larotaxel and its degradation products are similar to those of the impurities of larotaxel found by Che, et al. [19] in alkaline condition.

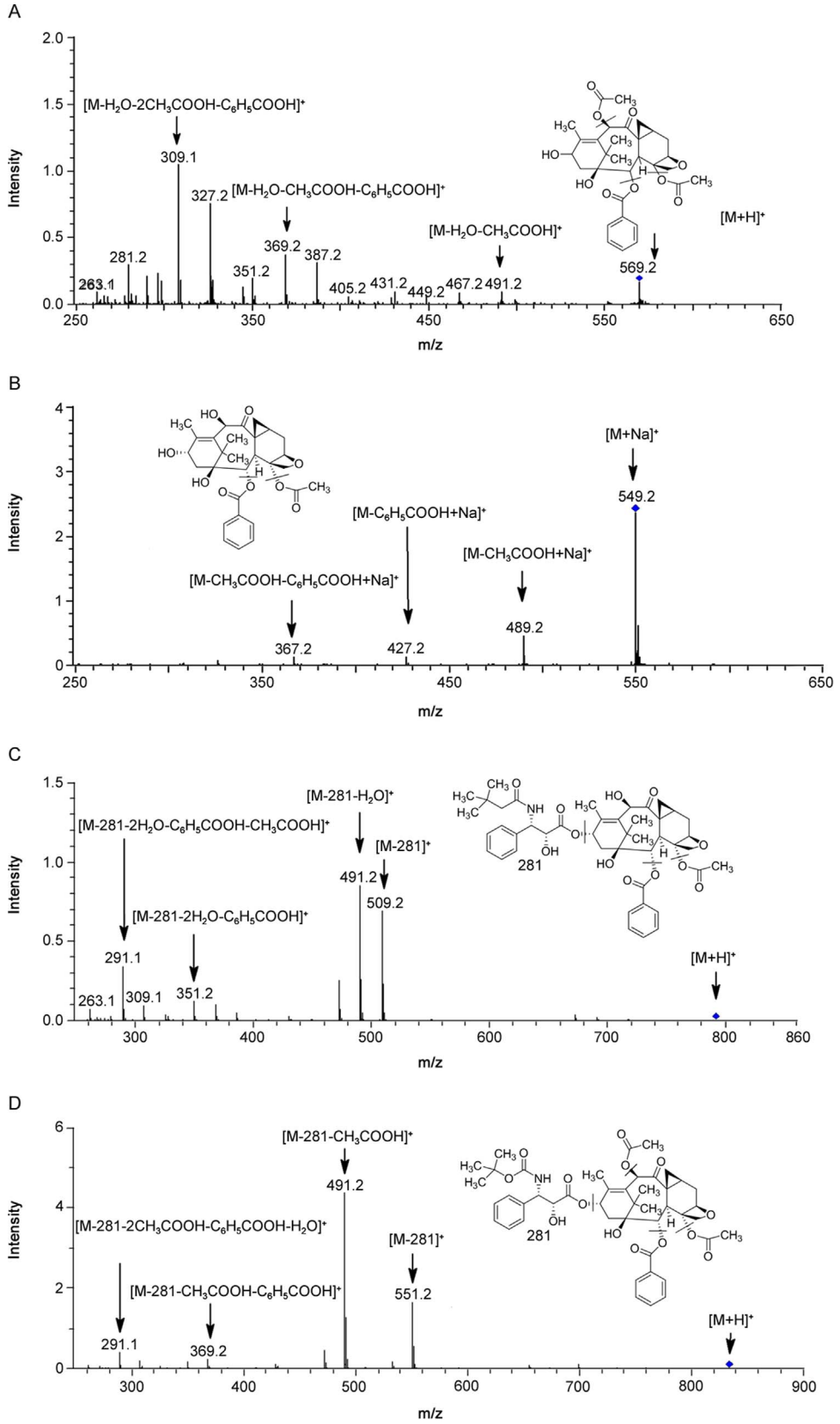

Fig. 4. The fragmentation pathway of 7, 8-cyclopropyl baccatin III (A), 10-deacetyl-7, 8-cyclopropyl baccatin III (B), 10-deacetyl larotaxel (C) and larotaxel (D).

Finally, Impurities A, B, and C were confirmed as 7, 8-cyclopropyl baccatin III, 10-deacetyl-7, 8-cyclopropyl baccatin III and 10-deacetyl larotaxel, respectively.

During degradation products study, Impurities A and C were first increased and then decreased in 0.005 mol/L aqueous sodium hydroxide, indicating that they were also unstable in base, which can be

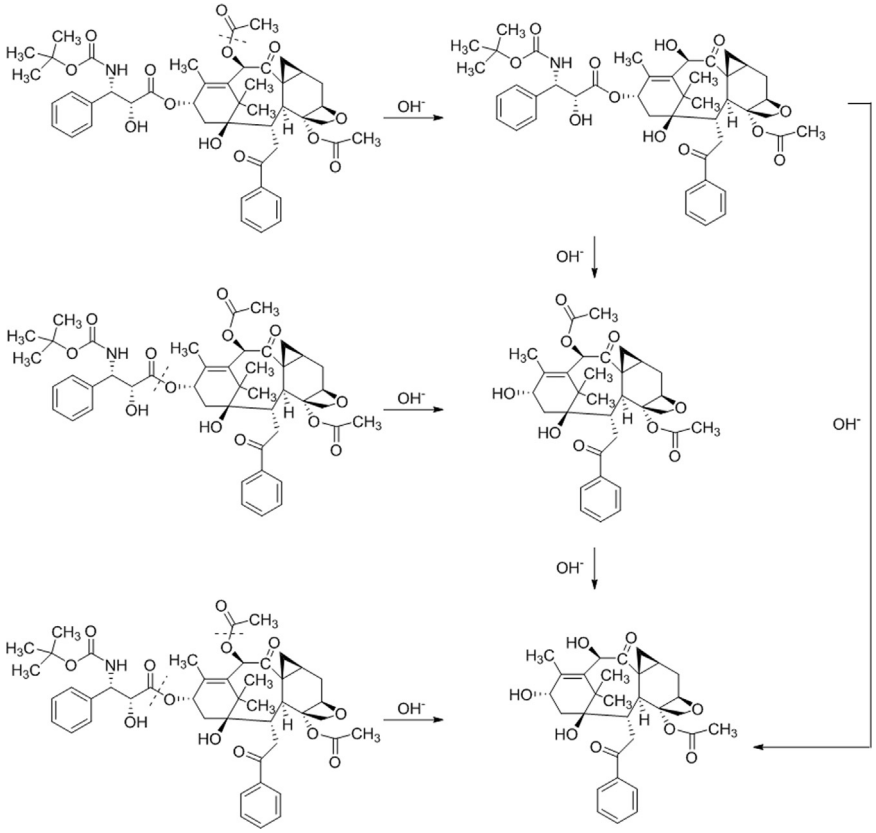

Fig. 5. Degradation paths of laraotaxel in alkaline condition.

interpreted by the ester bond easily broken in alkaline condition. The formation of the three impurities was deduced as shown in Fig. 5. The mechanisms and methods for reducing the degradation of larotaxel need to be investigated in the future.

4. Conclusion

In present study, to investigate the stability of larotaxel in buffers with different pHs and temperatures, an LC–MS method was developed and validated for the determination of larotaxel. With this method, the degradation of larotaxel was found to be pH- and temperature-dependent and more stable in acid than in base. Three degradation products were found and identified by Q–TOF–MS, and they were confirmed as 7, 8-cyclopropyl baccatin III, 10-deacetyl larotaxel and 10-deacetyl-7, 8-cyclopropyl baccatin III. These results may be helpful for the storage condition of larotaxel and also be of some practical utility for other taxanes, such as paclitaxel and docetaxel.

Acknowledgments

The authors would like to thank Dr. Xing Tang (Shenyang Pharmaceutical University, China) for making valuable suggestions on this manuscript.

References

[1] N. Yamamoto, N. Boku, H. Minami, Phase I study of larotaxel administered as a 1-h intravenous infusion every 3 weeks to Japanese patients with advanced solid tumours, Cancer Chemoth. Pharm. 65 (2009) 129–136.
[2] T. Kurata, Y. Shimada, T. Tamura, et al., Phase I and Pharmacokinetic Study of a New Taxoid, RPR109881A, given as a 1-Hour Intravenous Infusion in Patients With Advanced Solid Tumors, J. Clin. Oncol. 18 (2000) 3164–3171.
[3] O. Metzgerfilho, C. Moulin, E.D. Azambuja, et al., Larotaxel: broadening the road with new taxanes, Expert. Opin. Inv. Drug 18 (8) (2009) 1183–1189.
[4] P.B. Schiff, J. Fant, S.B. Horwitz, Promotion of microtubuleassembly in vitro by Taxol, Nature 277 (1979) 665–667.
[5] F. Gueritte-Voegelein, D. Guenard, F. Lavelle, et al., Relationships between the structure of taxol analogues and their antimitotic activity, J. Med. Chem. 34 (1991) 992–998.
[6] E.K. Rowinsky, R.C. Donehower, R.J. Jones, et al., Microtuble changes and cytotoxicity in leukemic cell lines treated with taxol, Cancer Res. 48 (1988) 4093–4100.
[7] P. Zatloukal, R. Gervais, J. Vansteenkiste, et al., Phase II Study of Larotaxel(XRP9881) in combination with cisplatin or gemcitabine as first-line chemotherapy in nonirradiable stage IIIB or stage IV non-small cell lung cancer, J. Thorac. Oncol. 3 (2008) 894–901.
[8] C. Sessa, C. cuvier, S. Caldiera, et al., Phase I clinical and pharmacokinetic studies of the taxiod derivative RPR 109881 A administered as a 1-hour or a 3-hour infusion in patients with advanced solid tumors, Ann. Oncol. 13 (2002) 1140–1150.
[9] Z. Liu, L. Zhang, P. Ju, et al., Determination of larotaxel and its metabolites in rat plasma by liquid chromatography–tandem mass spectrometry: application for a pharmacokinetic study, J. Chromatogr. B. 947 (2014) 132–138.
[10] W.C. Liu, T. Gong, P. Zhu, Advances in exploring alternative Taxol sources, RSC Adv. 6. 54 (2016) 48800–48809.
[11] M.D. Argentine, P.K. Owens, B.A. Olsen, Strategies for the investigation and control of process-related impurities in drug substances, Adv. Drug. Deliv. Rev. 59 (2006) 12–28.
[12] X.B. Suo, Y.J. Deng, H. Zhang, et al., Degradation kinetics of water-insoluble Lauroyl-indapaminde in aqueous solutions: prediction of the stabilities of the drug in liposomes, Arch. Pharm. Res. 30 (2006) 876–883.
[13] Z. Hou, P. Qin, Y. Zhang, et al., Identification of anthocyanins isolated from black rice (Oryza sativa L.) and their degradation kinetics, Food Res. Int. 50 (2) (2013) 691–697.
[14] S.M. Mitchell, J.L. Ullman, A.L. Teel, et al., pH and temperature effects on the hydrolysis of three β-lactam antibiotics: ampicillin, cefalotin and cefoxitin, Sci. Total. Environ. 466 (2014) 547–555.
[15] J.A. Khan, X. He, N.S. Shah, et al., Kinetic and mechanism investigation on the photochemical degradation of atrazine with activated H_2O_2, $S_2O_8^{2-}$ and HSO_5^-, Chem. Eng. J. 252 (2014) 393–403.
[16] T.Y. Min, Y. Hong, F.Y. Li, et al., Improvement of avermectin crystallization technology, Chin. J. Pharm. 9 (2002) 014.
[17] S.F. Qi, L.Q. Gao, W.J. Shu, et al., Study on degradation kinetics of amorolfine hydrochloride in aqueous solution, Prog. Pharmacol. Sci. 11 (2010) 010.
[18] X. Jiang, X. Shao, Z. Yan, et al., Degradation kinetics of Chuanhuning injection, Chin. J. Drug 5 (2006) 017.
[19] X. Che, L. Shen, H. Xu, et al., Isolation and characterization of process-related impurities and degradation products in larotaxel, J. Pharm. Biomed. 55 (2011) 1190–1196.