ORIGINAL ARTICLE

Determination and stress studies on YK-1101, a potential histone deacetylase, by HPLC–UV and HPLC–TOF/MS methods

Chen Zhou[a], Wen-Yuan Liu[a,*], Feng Feng[b,**], Hai Ye[c], Chun-Yong Wu[a]

[a]Department of Pharmaceutical Analysis, China Pharmaceutical University, Nanjing 211198, China
[b]Department of Natural Medicine Chemistry, China Pharmaceutical University, Nanjing 211198, China
[c]Yoko Pharma Co., Ltd., Nanjing 210000, China

Abstract YK-1101, with its structure as S-((E)-4-((7S,10S,Z)-4-ethylidene-7-isopropyl-2,5,8,12-tetraoxo -9-oxa-16-thia-3,6,13,18-tetraazabicyclo[13.2.1]octadeca-1(17),15(18)-dien-10-yl)but-3-en-1-yl) ethanethioate, is synthesized as a potential histone deacetylase inhibitor. Its quality and stability under various stress conditions are not fully understood. In this study, a high performance liquid chromatographic (HPLC) method was established and validated for the analysis of YK-1101 bulk drug samples. The chromatographic separation was performed on a C_{18} column with acetonitrile and water as mobile phase in a gradient elution. Based on the established method, the stability studies of YK-1101 under various stress conditions were carried out. YK-1101 was shown to undergo degradation under basic and acidic stress conditions, while it was stable under oxidative, photolytic and thermal conditions. In addition, a time of flight mass spectrometer (TOF/MS) was coupled to HPLC for the characterization of major degradation products produced under basic and acidic stress conditions. Their degradation pathways were also discussed.

© 2013 Xian Jiaotong University. Production and hosting by Elsevier B.V

*Corresponding author. Tel.: +86 25 8327 1038;
fax: +86 25 8327 1269.
**Corresponding author.
E-mail addresses: liuwenyuan8506@163.com (W.-Y. Liu),
fengsunlight@163.com (F. Feng).
Peer review under responsibility of Xi'an Jiaotong University.

1. Introduction

YK-1101 is a novel depsipeptide with its chemical structure as S-((E)-4-((7S,10S,Z)-4-ethylidene- 7-isopropyl-2,5,8,12-tet-raoxo-9-oxa-16-thia-3,6,13,18-tetraazabicyclo[13.2.1]octadeca-1(17),15(18)-dien-10-yl)but-3-en-1-yl)ethanethioate (Fig. 1). It was designed and synthesized as a histone deacetylase (HDAC) inhibitor. HDACs are enzymes which catalyze the removal of the acetyl moiety from acetyl-lysine within histones to
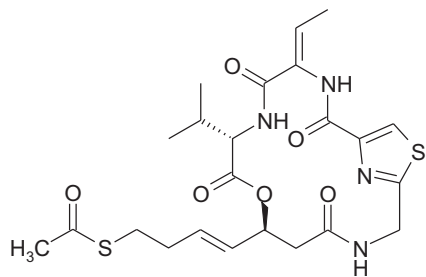
Fig. 1 Structure of YK-1101.

promote gene repression and silencing [1]. It was reported that HDAC inhibitors could inhibit proliferation, induce differentiation or apoptosis of tumor cells and activate gene transcription [2–4]. HDAC inhibition has been shown to be a successful strategy to treat certain forms of cancer. Two compounds, vorinostat [5] and romidepsin [6], are already on the market, while several others are in different stages of clinical trials, such as apicidin [7], dolastatin 10 [8], ET-747 and aplidine [9].

Although its pharmacological activity has been demonstrated, the quality and stability of YK-1101 are not fully understood. There has been no study reported on the determination and stability of this new medical compound. Hence, the aim of the study is to establish a rapid, specific and routine analytical method for its quality control. The established method was optimized and validated as per the guidelines of International Conference on Harmonization (ICH) [10,11]. The forced degradation studies, which could help to establish the degradation pathways and study the intrinsic stability of the molecule, were also carried out in this study. A high performance liquid chromatography with time of flight mass spectrometer (HPLC–TOF/MS) was established for the characterization of degradation products. Their degradation pathways were also discussed.

2. Experimental

2.1. Chemicals and reagents

Samples and reference standard of YK-1101 were supplied by Yoko Pharma Co., Ltd. (Nanjing, China). HPLC-grade acetonitrile was purchased from TIDEA Co., Ltd. (Fairfield, USA). Other analytical grade reagents were purchased from Nanjing Chemical Co., Ltd. (Nanjing, China). Water for HPLC analysis was purified by a Milli-Q water system (Millipore, USA).

2.2. Sample preparation and stress studies

A degassed mixture of acetonitrile–water (20:80, v/v) was used as diluent during preparation of the standard and test solutions. Stock solution of 1.0 mg/mL of YK-1101 sample was prepared in the diluent for the determination of related substances and stress studies. The stock solution was diluted to 180 μg/mL for assay. The corresponding reference standard solution of YK-1101 was also prepared in this diluent.

All the stress studies were carried out as per ICH requirement. Basic, acidic, oxidative, photolytic and thermal degradation was performed, respectively, in 0.01 M NaOH, 1 M HCl in 60 °C water bath, 30% hydrogen peroxide,

illumination of 1.2 million lx and 80 °C water bath. Samples were withdrawn at appropriate time, the pH was adjusted to neutral and the samples were subjected to HPLC–TOF/MS analysis. The stability of YK-1101 was observed and the resolution between YK-1101 and its degradation products was calculated to evaluate the potential impurities and the specificity of the proposed method.

2.3. Apparatus and conditions

HPLC analysis was performed on a Shimadzu LC-2010 system consisted of a quaternary pump, a vacuum degasser, an autosampler and a column heater-cooler. An Inertsil ODS-SP column (4.6 mm × 250 mm, 5 μm) purchased from GL Sciences Inc. (Japan) with a security guard was used for separation. Acetonitrile (solvent A) and water (solvent B) were used as mobile phase with the following gradient elution mode: 20–30% A in 0–10 min; 30% A in 10–35 min; 30–40% A in 35–40 min; 40% A in 40–60 min and re-equilibration duration was 5 min. The flow rate was 1 mL/min and the injection volume was 20 μL. The column temperature was maintained at 30 °C. Detection wavelength was set at 226 nm.

MS analysis was carried out using an Agilent 6224 TOF LC–MS system. An electrospray ionization (ESI) was employed and set in positive ion mode. The spray voltage was set to 5.0 kV and the capillary temperature was maintained at 350 °C. Nitrogen was used as both the drying and nebulizing gas. The scanning range was m/z 50–1000. Analytical data were acquired using Agilent Masshunter workstation software 4.0.

2.4. Validation of the method

2.4.1. Specificity
Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities and degradation products. Degradation studies were conducted by stressing under acidic, basic, oxidative, thermal and photolytic conditions according to option 2 of Q1B in ICH guidelines.

2.4.2. Limit of detection (LOD) and limit of quantification (LOQ)
The LOD and LOQ for YK-1101 were estimated at a signal-to-noise ratio of 3:1 and 10:1, respectively, by injecting a series of diluted standard solutions with known concentrations.

2.4.3. Linearity
Linearity for YK-1101 was prepared by diluting the stock reference standard solution to a series of required concentrations at 60, 120, 180, 240 and 300 μg/mL. The calibration curves were drawn by plotting the peak area of the target analyte versus its corresponding concentration. The linear range of YK-1101 was from 33.3% to 166.6% with respect to sample concentration of 180 μg/mL.

2.4.4. Precision
Precision of the method was verified by repeatability and intermediate precision studies. Repeatability studies were performed by analysis of test solution in hexaplicate on 3

days. Intermediate precision of the method was checked by different analysts on different days using different instruments.

2.4.5. Accuracy

The accuracy of the method was evaluated in triplicate by adding appropriate amounts of the reference standard solution of the target compound into the test solution of YK-1101 at three different levels (i.e., 80%, 100% and 120%) with respect to the concentration of test solution at 180 μg/mL, and the mean recoveries were calculated.
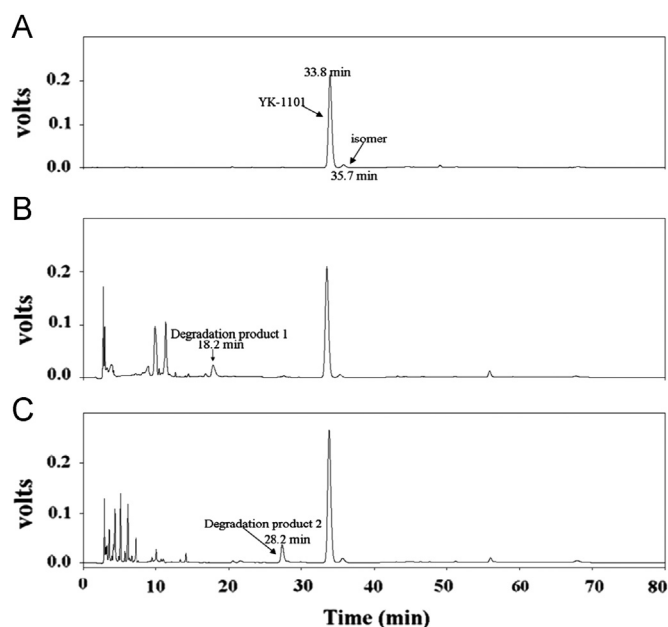
2.4.6. Robustness

To evaluate the robustness of the established method, the chromatographic conditions were deliberately altered and the resolutions were calculated. Column temperatures of 25 °C and 35 °C instead of 30 °C, detection wavelengths of 221 and 231 nm instead of 226 nm, and three different ODS columns were employed and the resolutions were studied.

3. Results and discussion

### 3.1. Optimization of HPLC condition

In order to achieve satisfactory separation, chromatography conditions, such as column types, mobile phase composition and time program, were optimized. Preliminary experiments were carried out by subjecting sample preparation to various types and brands of commercial HPLC columns (Kromasil KR60-5CN, BDS Hypersil Phenyl, Lichrospher C8, etc.), and an Inertsil ODS-SP column (4.6 mm × 250 mm, 5 μm) was selected. Buffers such as triethylamine solution and phosphate buffer at different pH, and organic modifiers, including methanol and acetonitrile, were tried. It turned out that acetonitrile and water with gradient elution (mentioned in Section 2.3) showed the best column efficiency and satisfactory resolution. The optimized chromatogram is presented in Fig. 2A.

Fig. 2 Typical HPLC chromatograms of: (A) YK-1101 test solution, (B) basic stress study under 0.01 M NaOH, and (C) acidic stress study under 1 M HCl in 60 °C water bath.

Table 1 Robustness testing for YK-1101.

Factor	Resolution	Tailing factor
Column temperature (°C)		
25	2.0	1.17
30	2.1	1.15
35	2.1	1.14
Detection wavelength (nm)		
221	2.1	1.15
226	2.1	1.15
231	2.1	1.16
Different columns		
Inertsil ODS-SP (4.6 mm × 250 mm, 5 μm)	2.1	1.15
Megres C_{18} (4.6 mm × 250 mm, 5 μm)	2.0	1.15
Hedera ODS-2 (4.6 mm × 250 mm, 5 μm)	1.9	1.00
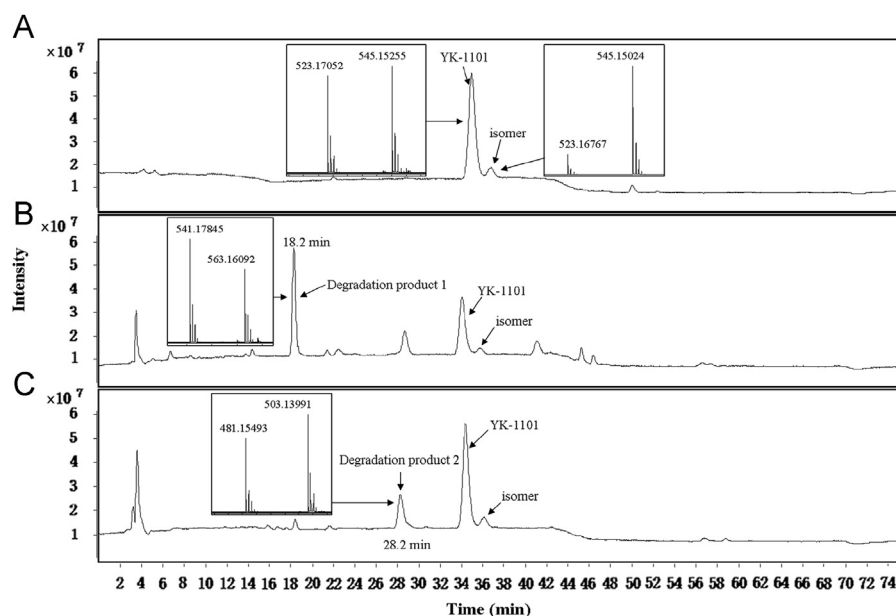
Fig. 3 Typical TIC and MS of: (A) YK-1101 sample, (B) degradation product 1, and (C) degradation product 2.

3.2. Results of method validation

The LOD and LOQ for YK-1101 were 0.026 and 0.077 µg/mL, respectively. The linearity test for the assay of YK-1101 was carried out by injecting a series of YK-1101 solutions with concentrations at 60, 120, 180, 240 and 300 µg/mL and the peak areas were recorded. The values of slope, intercept and correlation coefficient were 42622, 150147 and 0.9999, respectively.

The RSD percentage for assay of YK-1101 during precision study was within 0.5%, and the RSD percentage in the intermediate precision study was within 1.0%, confirming the good precision of the established HPLC method. The accuracy of the assay method was evaluated in triplicate at three concentration levels. The percentage recoveries of YK-1101 ranged from 99.2% to 102.2%.

The robustness results (Table 1) showed that the resolutions between YK-1101 and its adjacent peaks were always greater than 1.5, and the tailing factors were within 1.18, confirming the robustness of the method.

3.3. Results of forced degradation studies

3.3.1. Thermal, photolytic and oxidative degradation
YK-1101 was found to be stable under thermal, photolytic and oxidative conditions. No significant degradation products were observed.

3.3.2. Basic degradation
YK-1101 was degraded under basic stress condition. The typical chromatogram under 0.01 M NaOH is presented in Fig. 2B. A major degradation product (degradation product 1) with its retention time at 18.2 min was detected. When the strength of NaOH solution was increased to 0.1 M, YK-1101 degraded completely. Under the conditions of phosphate buffer solution (pH 7.0–8.0), 0.001 M and 0.005 M NaOH, no significant degradation products were observed by the established method.
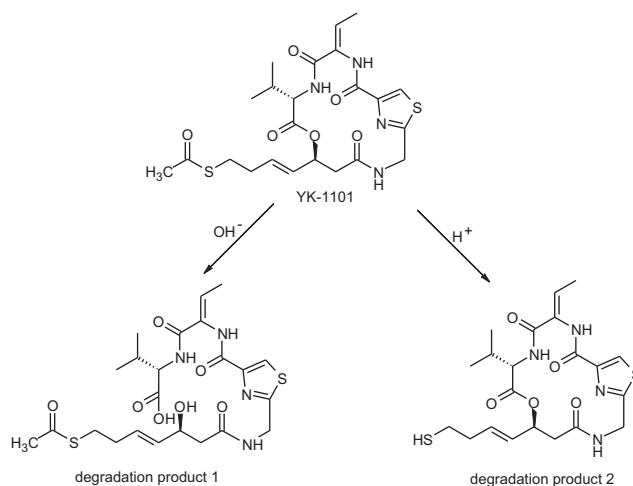
Fig. 4 Proposed degradation pathways of YK-1101 under stress conditions.

3.3.3. Acidic degradation
YK-1101 was stable when treated with 1 M HCl for 4 h at room temperature. However, when the temperature of water bath was increased to 60 °C, about 20% of YK-1101was degraded (Fig. 2C). The major degradation product (degradation product 2) with its retention time at 28.2 min was detected and further studied by LC–TOF/MS [12].

3.4. Structure prediction of the major degradation products

The typical (+)TIC of test solution is shown in Fig. 3. YK-1101 with its retention time at 33.8 min and a related compound with the retention time at 35.7 min showed the same [M+H]$^+$ ion at m/z 523.1. Therefore, the related compound was an isomer of YK-1101.

As shown in Fig. 3B, the $[M+H]^+$ ion for degradation product 1 was at m/z 541.17845 ($C_{23}H_{32}N_4O_7S_2$), which was 18u more than that of YK-1101. Therefore, it was the hydrolysis product of YK-1101. Regarding the structure of YK-1101, there are an ester and three amide bonds which might be hydrolyzed. As amides are generally more stable towards alkaline hydrolysis than esters [13–15], accordingly, the ester group in the structure of YK-1101 is preferred to undergo alkaline hydrolysis. As shown in Fig. 3C, the $[M+H]^+$ ion for degradation product 2 was at m/z 481.15493 ($C_{21}H_{28}N_4O_5S_2$), which was 42u less than that of YK-1101. It could be explained as the thioester being hydrolyzed under acidic condition and a sulfhydryl formed [16]. The proposed degradation pathways for the two compounds are presented in Fig. 4.

4. Conclusion

In this work, a stability-indicating HPLC method was established to investigate the effects of pH, temperature, light and hydrogen peroxide on the stability of YK-1101. As the result, YK-1101 was shown to undergo degradation under basic and acidic stress conditions, and two degradation products were proposed by the established HPLC–TOF/MS method. The method was also validated with respect to specificity, linearity, accuracy, precision and robustness, and found to be suitable for checking the quality of bulk samples of YK-1101 at the time of batch release and also during its stability studies.

References

[1] R. Marmorstein, Structure of histone deacetylases: insights into substrate recognition and catalysis, Structure 9 (12) (2001) 1127–1133.

[2] R. Glauben, E. Sonnenberg, M. Zeitz, et al., HDAC inhibitors in models of inflammation-related tumorigenesis, Cancer Lett. 280 (2) (2009) 154–159.

[3] P. Bertrand, Inside HDAC with HDAC inhibitors, Eur. J. Med. Chem. 45 (6) (2012) 2095–2116.

[4] Y. Wang, X. Wang, L.M. Liu, et al., HDAC inhibitor trichostatin A- inhibited survival of dopaminergic neuronal cells, Neurosci. Lett. 467 (3) (2009) 212–216.

[5] V.M. Richon, J. Garcia-Vargas, J.S. Hardwick, Development of vorinostat: current applications and future perspectives for cancer therapy, Cancer Lett. 280 (2) (2009) 201–210.

[6] R. Vinodhkumar, Y.S. Song, T. Devaki, Romidepsin (depsipeptide), induced cell arrest, apoptosis and histone hyperacetylation in lung carcinoma cells (A549) are associated with increase in p21 and hypophosphorylated retinoblastoma proteins expression, Biomed. Pharmacother. 62 (2) (2008) 85–93.

[7] M.Y. Ahn, S.G. Ahn, J.H. Yoon, Apicidin, a histone deaceylase inhibitor, induces both apoptosis and autophagy in human oral squamous carcinoma cells, Oral Oncol. 47 (11) (2011) 1032–1038.

[8] M.A. Hoffman, J.A. Blessing, S.S. Lentz, A phase II trial of dolastatin-10 in recurrent platinum-sensitive ovarian carcinoma: a gynecologic oncology group study, Gynecol. Oncol. 89 (1) (2003) 95–98.

[9] B. Albella, G. Faircloth, L. López-Lázaro, et al., In vitro toxicity of ET-743 and aplidine, two marine-derived antineoplastics, on human bone marrow haematopoietic progenitors: comparison with the clinical results, Eur. J. Cancer 38 (10) (2002) 1395–1404.

[10] ICH. Topic Q1A (R2): stability testing of new drugs substances and products, in: Proceedings of the International Conference on Harmonization (ICH), IFPMA, Geneva, Switzerland, 2003.

[11] ICH. Topic Q2 (R1): validation of analytical procedures: text and methodology, in: International Conference on Harmonization (ICH), IFPMA, Geneva, Switzerland, 2005.

[12] X.F. Chen, H.T. Wu, G.G. Tan, et al., Liquid chromatography coupled with time-of-flight and ion trap mass spectrometry for qualitative analysis of herbal medicines, J. Pharm. Anal. 1 (4) (2011) 235–245.

[13] E.A. Gadkariem, F. Belal, M.A. Abounassif, et al., Stability studies on diloxanide furoate: effect of pH, temperature, gastric and intestinal fluids, Farmaco 59 (4) (2004) 323–329.

[14] Y.X. Guo, Z.L. Xiu, D.J. Zhang, et al., Kinetics and mechanism of degradation of lithospermic acid B in aqueous solution, J. Pharm. Biomed. Anal. 43 (4) (2007) 1249–1255.

[15] J.C. Waterval, J.C. Bloks, R.W. Sparidans, et al., Degradation kinetics of aplidine, a new marine antitumoural cyclic peptide, in aqueous solution, J. Chromatogr. B 754 (1) (2001) 161–168.

[16] J.B. Tong, S.W. Zhang, J.R. Kou, et al., On quantitative relationship between hydrolysis rate with structure parameters of ester and thiol esters, Comput. Appl. Chem. 23 (5) (2006) 436–439.