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Research Article

Degradation Rate of Ebastine in an Aqueous Solution at pH 1.2 and the Effects of Cyclodextrins

Seiji Matsuyama, Takuro Kurita*, Tadakazu Tokumura
Laboratory of Pharmaceutics, Kagawa School of Pharmaceutical Sciences, Tokushima Bunri University, Shido 1314-1, Sanuki, Kagawa, 769-2193, Japan

***Corresponding author**
Takuro Kurita
Email: t-kurita@kph.bunri-u.ac.jp

Abstract: Ebastine (EBA), 4'-tert-butyl-4-(4-(diphenyl methoxy) piperidino) butyrophenone, is a second generation H1-antihistamine. The solubility of EBA is poor in aqueous solutions, but increases in acidic conditions. EBA is degraded in aqueous solutions. There are no reports about the degradation rate of EBA in acidic solutions. The aim of this study was to determine the degradation rate of EBA in an acidic solution and to stabilize EBA in the solution. The stability of EBA in a buffer solution at pH 1.2 with 1% methanol at 37, 50 and 60°C was examined. EBA in solution was determined by HPLC. α-, β- and γ-cyclodextrins (CDs) as stabilizers as well as the stability of the CDs in solution were examined. The degradation of EBA was considered to be a pseudo-first-order reaction. The apparent first-order rate constant of EBA in the pH 1.2 buffer solutions at 37°C was 14.9×10^{-3} $hour^{-1}$. When α-, β- and γ- CDs were added, the rate constant decreased to 12.2×10^{-3}, 0.83×10^{-3} and 7.20×10^{-3} $hour^{-1}$ for α-, β- and γ- CDs, respectively. β- CD was found to have the strongest stabilizing effect. The activation energy for the degradation of EBA under this condition was 106.9 kJ/ mol. The addition of CDs increased the value, and CDs were slowly degraded in the pH 1.2 solution.
Keywords: Ebastine, cyclodextrin, inclusion complex, stability, acidic condition, activation energy.

INTRODUCTION

Ebastine (EBA; 4'-tert-butyl-4-(4-(diphenyl methoxy) piperidino) butyrophenone) is a second generation histamine H_1-receptor antagonist used mainly for the treatment of allergic diseases [1]. The solubility of EBA in water is poor and its partition coefficient (logP) is 7.64 [2]. Therefore, EBA is in the class II group of pharmaceuticals in the biopharmaceutical classification system (BCS) [2, 3].

The solubility of EBA was thought to increase with a decrease in pH based on its chemical structure (Figure 1). In a paddle method dissolution test using 5 mg EBA tablets and 900 mL of dissolution test medium, the maximum dissolution percent at pH 1.2 was almost 100%, but decreased to about 80% at pH 5.0 and was <5% at pH 6.8 [4]. The poor solubility at neutral pH may cause a low bioavailability or variation in blood concentration of EBA in humans after oral administration of EBA preparations. It was reported that food affects EBA as the AUC value under fed conditions was higher than under fasting conditions [5]. The cause for this was not clear, but there is a possibility that it was due to poor solubility in aqueous solutions. Both improvement of solubility and enhancement of the dissolution rate are very important for EBA. However, reports for the solubility of EBA are not sufficient, and the effects of pH on the solubility have not been reported. We examined the solubility of EBA in buffer solutions at various pH values. EBA was degraded in a buffer solution at pH 1.2 and determination of the rate was required for consideration of the stability of EBA in the stomach.

Degradation of EBA in an aqueous solution was suggested [9], but the rate has yet to be reported. Cyclodextrin (CDs) are usually used for the stabilization of drugs. In the case of EBA, it has been reported that EBA forms inclusion complexes with β-CD derivatives [6]. Therefore, the effects of α-, β-, and γ-cyclo dextrins on the degradation rate were examined.

In this report, we describe the degradation rate of EBA in an acidic solution at pH 1.2, the effect of CDs on the degradation rate, and the change in CD concentration determined between the first and final sampling times when CDs were added to the EBA reaction system.

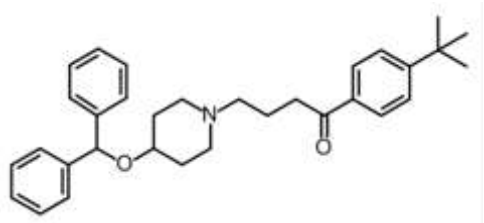

Fig-1: Chemical Structure of EBA (Ebastine; 4'-tert-butyl-4-(4-(diphenyl methoxy) piperidino) butyrophenone)

MATERIAL AND METHODS

Materials

Reagent grade EBA was purchased from LKT Laboratories, Inc. (St. Paul, MN, USA). α-CD and β-CD ware donated by Nihon Shokuhin Kako Co., Ltd. γ-CD was purchased from Wako Pure Chemical Industries, Ltd (Osaka, Japan). Other chemicals were reagent or HPLC grade.

Stock solution

EBA (30 mg) was dissolved in 10 mL of methanol. This solution was stored at room temperature for 1 month.

Determination of EBA by HPLC

The concentration of EBA in a sample solution was determined by HPLC with a Model LC-20AD pump, equipped with a Model CBM-20A system controller, a Model SPD-20A UV spectrophotometric detector, a Model CTO-20A column oven and a Model SIL-20AC auto injector, all from Shimadzu Corporation (Kyoto, Japan). The mobile phase was acetonitrile-water-perchloric acid (60%)-sodium perchlorate monohydrate (700:300:1:5, v/v/v/w). The chromatographic column was a YMC Pack AM12S05 ODS (150 mm×6 mm I.D., particle diameter 5 μm) obtained from YMC Co., Ltd. (Kyoto, Japan). The flow rate, wavelength for determination, and temperature of the column were 1 mL/minute, 254 nm and 40°C, respectively. The injection volume into the column was 10 μL.

Calibration Solutions of EBA

One ml of the stock solution was diluted with methanol to make a 30 μg/mL EBA solution. The solution was further diluted with methanol to prepare solutions of EBA at concentrations of 2.5, 5, 10, 9, 15, and 20 μg/mL. A 500 μL aliquot of each EBA-methanol solution at 2.5 - 30 μg/mL was added to 500 μL of purified water containing 1% methanol. After the mixture was well stirred on a vortex mixer, the mixture was injected into the chromatograph.

Determination of CDs by HPLC

Concentrations of CDs in sample solutions were determined by HPLC with a Model LC-20AD pump, equipped with a Model CBM-20A system controller, a Model ELSD-LTII evaporative light scattering detector, a Model CTO-20A column oven and a Model SIL-20AC auto injector, all from Shimadzu Corporation (Kyoto, Japan). The mobile phase was methanol-water (70:930, v/v). The chromatographic column was a Cadenza CD-C18 CD005 (150 mm×4.6 mm I.D., particle diameter 3μm) obtained from Imtakt Co. (Kyoto, Japan). The flow rate, temperature of the column, pressure of the ELSD and temperature of the ELSD were 0.5mL/minute, 40°C, 345kPa and 40°C, respectively. The injection volume into the HPLC column was 10 μL.

Calibration Solutions of CDs

A 750 mg sample of α-CD was added to purified water containing 1% methanol to obtain a solution of α-CD at 15.0 mg/mL. The solution was diluted with purified water containing 1% methanol to prepare standard solutions of α-CD with concentrations of 1.5, 3, 6, 9, and 12 mg/mL. A 200 μL standard solution of α-CD was added to 800 μL of purified water. After the mixture was well stirred on a vortex mixer, the mixture was injected into the chromatograph. The solutions for calibration curves of β-CD and γ-CD were prepared by the same method.

Measurement of Stability of EBA and CDs in a pH 1.2 solutions containing 1% methanol

Five mL of the stock solution was diluted with methanol to obtain a solution of EBA at 1.5 mg/mL. A 49.5 mL sample of the 1st fluid for the dissolution test of JP XVI with or without 10 mM CDs was pre-incubated for 15 minutes at 37, 50 and 60°C. The experiments were initiated by adding 500 μL of the EBA solution at 1.5 mg/mL to the pre-incubated solution. The final concentration of EBA in the stability studies was 15 μg/mL (32 μm). Five hundred μL and 200 μL aliquots were removed from the reaction solution at the first and final sampling time. At another sampling time, a 500 μL aliquot was removed from the reaction solution. For EBA detection, the solution (500 μL) was added to 500 μL of methanol, and for detection of CDs, the solution (200 μL) was added to 800 μL of purified water. All samples were mixed well and immediately cooled down in a refrigerator (4°C) to disturb degradative reactions. EBA and CDs were stable for at least 2 weeks in a pH 1.2 solution at 4°C (data not shown).

RESULTS AND DISCUSSION

Degradation Rate of EBA in a pH 1.2 Solution and the Effect of CDs

The relationship between residual EBA (percent) on a logarithmic scale and time for the degradation of EBA at 37, 50, and 60°C in a pH 1.2 solution is shown in Figure 2. Each plot in Figure 2 is linear. These results indicate that the degradation reactions of EBA at 37, 50, and 60°C are apparent first-order reactions. The apparent first-order rate constant, k, was calculated from the slop of each straight line shown in Figure 2. The apparent first-order rate constants of EBA in a pH 1.2 solution at 37, 50, and 60°C were 14.9×10^{-3} ±

0.03×10^{-3}, $67.1\times10^{-3} \pm 5.03\times10^{-3}$, and $265.3\times10^{-3} \pm 12.3\times10^{-3}$ $hour^{-1}$ (mean ± SD, n=3), respectively. The apparent first-order rate constant for each plot is summarized in Table 1. Two peaks of EBA degradation products were observed on the HPLC chart at a retention time of 3.4 and 4.5 minutes when the retention time of EBA was 7.2 minutes. It was reported that when EBA was suspended in 0.1 M HCl and stored for 24 hours at 100°C, three degradative products, 1-[3-(4-tert-butylbenzoyl)propyl]-4-hydroxypiperidine, benzhydrol and benzophenone, were observed [8, 9]. The degradation products observed were considered to be 2 compounds of the 3 degradation products reported.

The effects of α-CD, β-CD and γ-CD on the degradation rate of EBA in pH 1.2 solutions at 37, 50, and 60°C are also shown in Figure 2. The apparent first-order rate constant, k, was calculated from the slop of each straight line shown in Figure 2. From the results of Figure 2 and Table 1, α-CD and γ-CD delayed the EBA degradation reaction. However, the effects were weak. From these findings, β-CD had the strongest effect on the degradation rate of EBA. It was reported that the β-CD cavity best fit the dimensions of the terminal benzene rings[7]. EBA has terminal benzene rings in its chemical structure, thus, β-CD had a stronger effect compared with the other CDs on the EBA degradation rate.

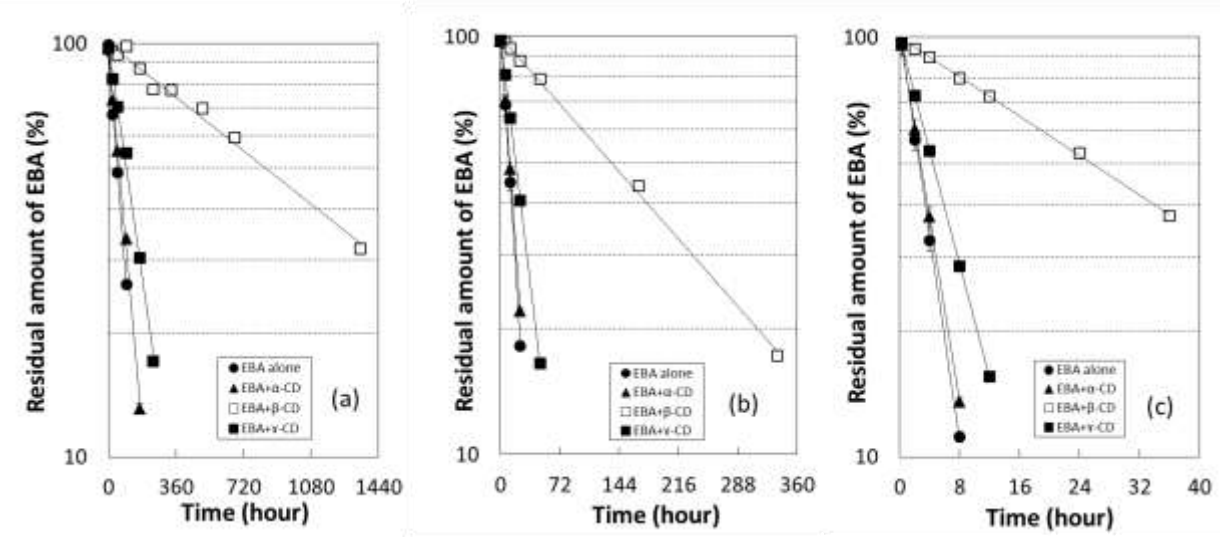

Fig-2: Degradation of EBA in a pH 1.2 Solution at 37 (a), 50 (b), and 60°C (c) and Effects of α-CD, β-CD, and γ-CD

●; EBA alone, ▲; α-CD, □; β-CD, ■; γ-CD
Each value represents the mean ± S.D. of 3 determinations.

Table-1: Apparent EBA Degradation Rate Constant in a pH 1.2 Solution at 37, 50 and 60°C and Effects of α-CD, β-CD, and γ-CD on Those Rate Constants

EBA : CD	37°C		50°C		60°C	
	Rate Constant ($10^{-3}\times hour^{-1}$)	$t_{1/2}$ (hour)	Rate Constant ($10^{-3}\times$ $hour^{-1}$)	$t_{1/2}$ (hour)	Rate Constant ($10^{-3}\times hour^{-1}$)	$t_{1/2}$ (hour)
EBA alone	14.9 ± 0.30	46.7 ± 0.94	67.1 ± 5.03	10.3 ± 0.78	265.3 ± 12.3	2.61 ± 0.12
EBA+α-CD	12.2 ± 0.11	57.0 ± 0.53	61.7 ± 0.35	11.2 ± 0.06	245.9 ± 2.45	2.82 ± 0.03
EBA+β-CD	0.83 ± 0.01	838.3 ± 13.4	5.16 ± 0.06	134.2 ± 1.46	27.0 ± 0.67	25.6 ± 0.63
EBA+ɤ-CD	7.20 ± 0.11	96.3 ± 1.52	37.5 ± 0.27	18.5 ± 0.13	155.8 ± 0.50	4.45 ± 0.01

Each value represents the mean ± S.D. of 3 determinations.

Arrhenius plots and activation energy of EBA degradation

Figure 3 shows Arrhenius plots for the degradation of EBA with or without CDs. All plots showed good linearity. The degradation activation energy was calculated from the slope of plots. The results are shown in Table 2. The activation energy of EBA alone, and those solutions containing α-CD, β-CD, and γ-CD were 106.9, 111.8, 129.4, and 114.2 kJ/mol, respectively. Activation energy values increased in the presence of CDs, and when β-CD was added, the EBA degradation activation energy was the highest.

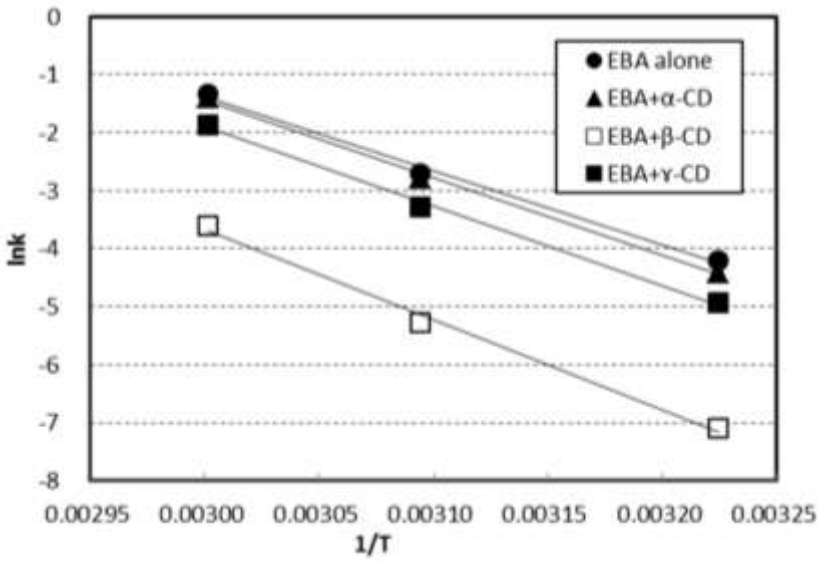

Fig-3: Arrhenius Plots Based on the Rate Constants Measured in a pH 1.2 Solution

●; EBA alone, ▲; α-CD, □; β-CD, ■; γ-CD

Each value represents the mean ± S.D. of 3 determinations.

Table-2: Effects of α-CD, β-CD, and γ-CD on the Activation Energy for the Degradation of EBA in a pH 1.2 Solution

	Ea (kJ/mol)
EBA alone	106.9
EBA+α-CD	111.8
EBA+β-CD	129.4
EBA+γ-CD	114.2

Each value is calculated from a data set as shown in Fig-3.

Stability of CDs in pH 1.2 solutions

Each residual percent (% of initial concentration) of CDs at each final sampling time is shown in Table 3. The residual percent of α-CD was over 90% under all conditions. In the case of β-CD, the residual percent was about 80% due to the longer storage period compared with α-CD and γ-CD and the stabilizing effects of β-CD on EBA. The residual percent of γ-CD was about 90% under all conditions. The degradations of CDs in acidic solution were previously reported [10, 11], but recently not reported. The present study was not focused on determining the degradation rates of α-CD, β-CD, and γ-CD in pH 1.2 solutions. Additional studies using recent technology are needed.

Table-3: Residual Percent (%) of α-CD, β-CD, and γ-CD and EBA in a pH 1.2 Solution at 37, 50, and 60°C

		Time (hour)[1)]	Residual EBA (%)	Residual CD (%)
	EBA+α-CD[2)]	168	13.1 ± 0.32	101.2 ± 0.96[3)]
37°C	EBA+β-CD	1344	31.8 ± 0.79	88.6 ± 0.91
	EBA+γ-CD	240	17.1 ± 0.48	98.8 ± 1.95
	EBA+α-CD	24	22.0 ± 0.17	95.3 ± 1.37
50°C	EBA+β-CD	336	17.2 ± 0.28	76.5 ± 7.12
	EBA+γ-CD	48	16.5 ± 0.28	88.1 ± 0.34
	EBA+α-CD	8	13.5 ± 0.47	92.1 ± 2.32
60°C	EBA+β-CD	36	37.6 ± 0.69	82.9 ± 1.60
	EBA+γ-CD	12	15.5 ± 0.09	88.4 ± 0.47

1) Time shows the final sampling time.

2) The concentrations of EBA and CDs were 32μM and 10mM.

3) Each data shows the mean ±SD of n=3.

CONCLUSION

The degradation of EBA in pH 1.2 solutions was considered as a pseudo- first-order reaction. The apparent first-order rate constant of EBA in the solution at 37°C was 14.9×10^{-3} $hour^{-1}$. When α-, β- and γ- CDs were added, the rate constants decreased to 12.2×10^{-3}, 0.83×10^{-3} and 7.20×10^{-3} $hour^{-1}$ for α-, β- and γ- CDs, respectively. β-CD had the strongest stabilizing effect. Activation energy for the degradation of EBA under this condition was 106.9 kJ/mol. The addition of CDs increased the value, and the CDs were slowly degraded in the pH 1.2 solution.

CONFLICT OF INTEREST (COI)

The authors declare no conflict of interest.

REFERENCES

1. Ibrahim F, EI-Din MKS, Eid MI, Wahba ME; Validated stability indicating liquid chromatographic determination of Ebastine in pharmaceuticals after precolumn derivatization, application to tablets and content uniformity testing. Chem. Cent. J., 2011; 5: 24–37.
2. Gerebtzoff G, Seelig A; In silico prediction of blood-brain barrier permeation using the calculated molecular cross-sectional area as main parameter. J. Chem. Inf. Model., 2006; 46: 2638–2650.
3. Loftsson T, Brewster ME, Ma´sson M; Role of cyclo dextrins in improving oral drug delivery. Am. J. Drug Deliv, 2004; 2: 261–275.
4. Ebastine tablet "EBASTINE" Interview form, 3rd ed., Sawai Pharmaceutical Co. Ltd., 2014.
5. Pentikis HS, Huang MY, Dorr MB, Heald DL; The effect of food on the bioavailability of ebastine. Am. J. Ther., 1997; 4: 80-84.
6. Maddens T, Ve´laz I, Machı´n R, Isasi JR, Martı´n C, Martı´nez-Oha´rriz MC, Zornoza A; Complexation of ebastine with β-cyclodextrin derivatives. J. Incl. Phenom. Macrocycl. Chem., 2011; 70: 415–419.
7. Uekama K; Design and Evaluation of Cyclodextrin-Based Drug Formulation. Chem. Pharm. Bull., 2004; 52: 900–915.
8. Arend MZ, Cardoso SG, Hurtado FK, Ravanello A, Lanzanova FA; Development and Validation of a Stability-Indicating LC Method for Determination of Ebastine in Tablet and Syrup. Chromatographia, 2009; 69: 195–199.
9. Ebastine tablet "EBASTEL" Interview form, 13rd ed., Sumitomo Dainippon Pharma Co., Ltd., 2009.
10. Szejtli J, Budai Zs; Acid hydrolysis of β-cyclodextrin. Acta Chem. Acad. Sci. Hung., 1979; 99: 73.
11. Myrbäck K, Järneström T; Über die Schardinger-Dextrine. I. Oxydation des β-Dextrine mit HJO_4. Saure Hydrolyse. Arkiv Kemi., 1949; 1: 129.