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Proposal of a new degradation mechanism of enalapril maleate and improvement of enalapril maleate stability in tablet formulation with different stabilizers

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Enalapril maleate (EM) is unstable in poorly designed tablet formulations. To improve the stability of EM, the degradation mechanism should be elucidated. In this study, we found that several commonly used excipients promoted the degradants of EM, particularly a diketopiperazine derivative (DKP). We propose two degradation pathways in which both acid and alkali can promote the formation of DKP, although previous reports suggested that DKP is produced mainly in acidic media. Based on the degradation pathways, we believe that subtle control of the microenvironmental pH can inhibit the formation of DKP. This was confirmed by the observation that the degradation rate became slower when certain organic acids were added to the binary mixtures of EM and excipient. The data showed that the stability of EM in the ternary mixtures was much higher than that in binary mixtures. It was further proved that tablets containing these organic acids produced less DKP after the accelerated test. We also found that the formation of DKP in tablets varied with different ratios of tartaric acid, which was used as a model organic acid. This illustrated that an optimum ratio of tartaric acid is required. These results indicated that the stability of EM in tablet formulation is closely associated with microenvironmental pH and the addition of a suitable organic acid based on the reaction mechanism is an effective strategy for improving the stability of EM.

1. Introduction

The selection of excipients is very important in drug formulation design. The designer should consider not only the functionality of the excipients, but also the compatibility between active ingredient and excipients. Incompatibility between the active ingredient and excipients can influence the stability and bioavailability of the drug. Chemical instability of the active ingredient involves hydrolysis, hydration, oxidation, intramolecular cyclization, photolysis, and racemization (Di and Kerns 2009). Excipients in the formulation can induce instability of the active ingredient by changing the water content, microenvironmental pH, and the reaction with active pharmaceutical ingredients (API) (Narang et al. 2012)

There are two strategies to overcome incompatibility between the drug and excipients. One is to replace the reactive excipients. However, some excipients are unique to the formulation and it is difficult to find substitutes. This method would limit the flexibility of the formulation. The other strategy is the addition of a stabilizer to the formulation based on the chemical mechanism (Fujita et al. 2010; Ren et al. 2008; Thumma et al. 2008). The stabilizer can improve the stability of the active ingredient without changing the original formulation. Despite this, it is necessary to understand the chemical mechanism thoroughly in order to select a suitable stabilizer.

Enalapril maleate (EM) is an oral angiotensin-converting enzyme inhibitor (ACEI) and executes its effect as enalaprilat (ET) *in vivo* (Mannisto et al. 2001; Wolfel 1998). The influence of temperature and relative humidity on the stability of EM in the solid phase has been investigated (Stanisz 2003; Wang et al. 2001). EM itself is quite stable in the solid state, but becomes unstable in some tablet formulations (Al-Omari et al. 2001; Cotton et al. 1987; Lima et al. 2008; Simoncic et al. 2007). The main degradants are diketopiperazine (DKP) and enalaprilat (ET). Previous research suggested that the degradation pathway of EM was pH dependent and that the degradants, DKP and ET, were mainly associated with the acidic matrix and basic matrix, respectively (Al-Omari et al. 2001). According to this theory, an acid excipient would increase the production of DKP. However, maleic acid was reported to improve the stability of EM in tablets (Bhushan and Somani; Toth et al.). Therefore, we speculated that another pathway is involved in DKP formation.

The aim of this paper was to study the chemical mechanism of DKP formation in the presence of excipients and to choose a suitable stabilizer for the tablet formulation of EM. We used UPLC-QTOF to analyze the degradation products of EM in the drug-excipient compatibility study. We then proposed two different pathways for the formation of DKP. In addition, several types of stabilizers were selected based on the degradation mechanism. The stabilizing effect was also tested in both the drug-excipients compatibility study and in different EM tablet formulations. The stabilizing effect was also tested in both the drug-excipients mixture and different EM tablet formulations.

2. Investigations and results

2.1. First stage compatibility studies

After exposure to 60 °C for two weeks, the samples were analyzed by HPLC. The results are shown in Table 1. There were two

Table 1: Results of HPLC analysis of EM and the main degradation products after exposureto 60 °C for two weeks (n = 3)

Excipients	EM%	Impurity-1(%)	Impurity-2(%)
Talc	99.211 ± 0.063	0.097 ± 0.011	Bld[a]
Magnesium stearate	97.212 ± 0.206	0.425 ± 0.041	1.597 ± 0.256
Silicon dioxide	98.439 ± 0.321	0.299 ± 0.061	0.894 ± 0.099
Hydroxypropyl methylcellulose	96.225 ± 0.193	0.134 ± 0.026	2.396 ± 0.428
Starch	99.344 ± 0.109	0.105 ± 0.010	0.418 ± 0.006
Low-substituted hydroxypropyl cellulose	98.357 ± 0.215	0.106 ± 0.012	0.795 ± 0.104
Sodium starch glycolate	94.701 ± 0.305	0.109 ± 0.012	5.808 ± 0.645
Microcrystalline cellulose	99.38 ± 0.268	0.129 ± 0.02	0.434 ± 0.029
Dextrin	99.431 ± 0.023	0.12 ± 0.008	0.143 ± 0.011
Lactose	99.426 ± 0.031	0.139 ± 0.024	Bld[a]
Pregelatinized starch	98.895 ± 0.498	0.121 ± 0.01	0.576 ± 0.012

[a]Bld – below limit of detection

major degradation impurities of different degrees. In particular, impurity-2 significantly increased in the mixtures containing sodium starch glycolate, hydroxypropyl methylcellulose and magnesium stearate.

2.2. Identification of impurities using UPLC-QTOF MS

The sample mixed with hydroxypropyl methylcellulose as the excipient was selected for further analysis by UPLC-QTOF MS. The mass spectra and structures of enalapril, impurity-1 and impurity-2 are shown in Fig. 1.

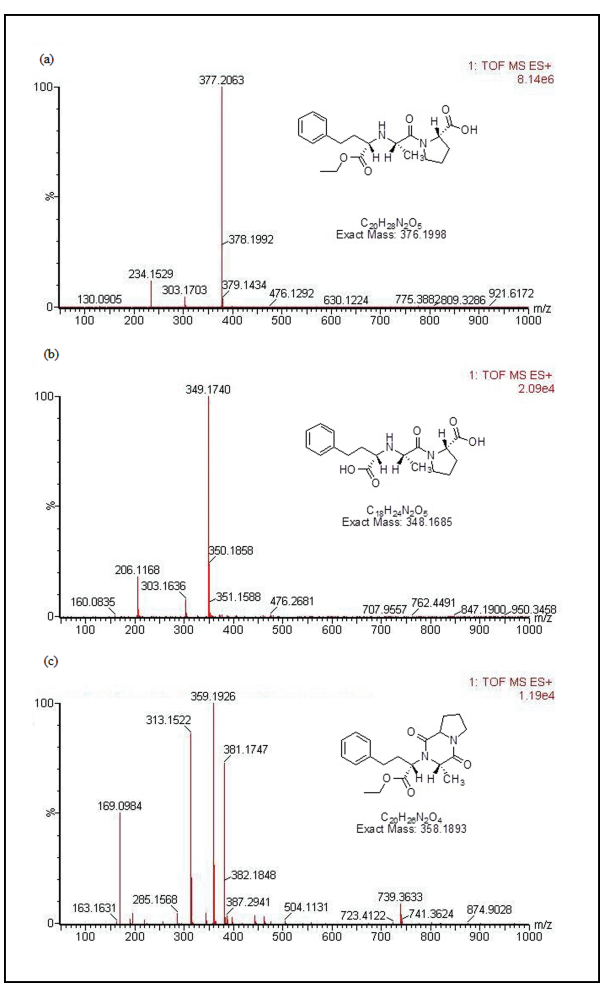

Fig. 1: The mass spectra of enalapril (a), impurity-1(b) and impurity-2 (c).

Using the UPLC-QTOF assay, the structures of the two major degradation impurities of EM were confirmed. They were enalaprilat (ET, degradation impurity-1) and a diketopiperazine derivative (DKP, degradation impurity-2). These results were similar to those reported in earlier studies (Bhardwaj and Singh 2008; Shiromani and Bavitz 1986; Stanisz 2003).

2.3. Proposed mechanisms of the formation of the main impurities of EM

DKP (degradation impurity-2) was the main degradation product of EM in the mixtures. The mechanism of formation of this impurity was proposed according to the molecular structure of the degradation product and the properties of the excipients. Enalapril dehydrates and cyclizes intermolecularly to form DKP (Lin et al. 2002). Previous reports have suggested that the degradation of EM is pH-dependent and the main degradation product in acidic media is DKP (Al-Omari et al. 2001; Ip and Brenner 1987). However, this does not explain the stabilization effect of maleic acid (Bhushan and Somani; Toth et al.). In contrast, we believe that both acid and alkali can promote the formation of DKP. According to our theory, catalytic mechanisms vary with pH (Fig. 2). At low pH, partial protonation of the carbonyl oxygen increases the electrophilicity of the carbonyl. At high pH, the amino group is exposed due to neutralization of partial maleate and its nucleophilicity is enhanced. Either condition can promote the cyclization reaction and DKP formation. That is why basic excipients such as magnesium stearate increased the ratio of DKP. Hygroscopic excipients such as sodium starch glycolate and hydroxypropyl methylcellulose can also promote this reaction.

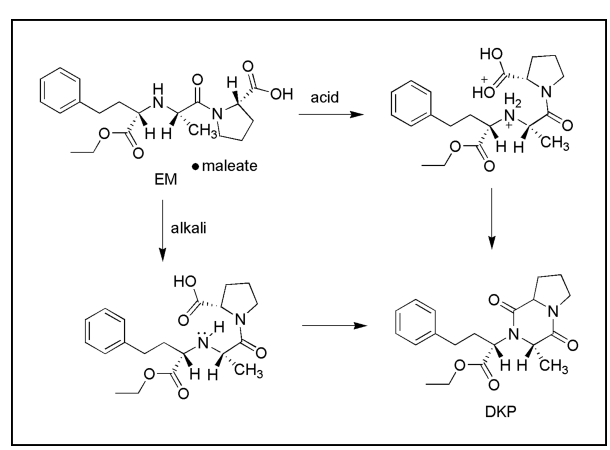

Fig. 2: Proposed mechanism of DKP formation.

Table 2: Ratio of DKP (%) in the ternary mixture after exposure to 60 °C for two weeks (n = 3)

Stabilizer	Magnesium stearate	Hydroxypropyl methylcellulose	Sodium starch glycolate
Without stabilizer	0.776 ± 0.118	1.262 ± 0.037	2.534 ± 0.192
Maleic acid	0.461 ± 0.077	0.334 ± 0.062	1.019 ± 0.100
L(+) tartaric acid	0.275 ± 0.034	0.984 ± 0.107	0.854 ± 0.120
Malic acid	0.139 ± 0.016	0.201 ± 0.044	1.133 ± 0.145
Taurine	0.099 ± 0.004	0.402 ± 0.018	1.193 ± 0.090
Citric acid	5.475 ± 0.662	0.547 ± 0.055	1.213 ± 0.058
Hexanedioic acid	0.989 ± 0.140	0.433 ± 0.063	3.300 ± 0.143
Lactic acid	8.971 ± 0.679	0.326 ± 0.018	14.786 ± 1.901
Na_3PO_4	37.454 ± 2.524	16.203 ± 3.242	51.071 ± 6.466
Na_2HPO_4	58.003 ± 9.692	45.840 ± 8.948	95.112 ± 10.007

2.4. Selection of stabilizers

Based on the proposed mechanism of degradation, the use of excipients such as sodium starch glycolate, hydroxypropyl methylcellulose and magnesium stearate should be avoided in the formulation of EM. However, these excipients are commonly used in tablet formulation and have excellent tableting properties. Therefore, the addition of a stabilizer should be considered if it improves drug stability in tablet formulations.

The formation of DKP is an intermolecular cyclization dehydration reaction. This cyclization reaction which is faster in a basic microenvironment may be inhibited by the addition of an acidic stabilizer. However, excess acid conditions could also promote the reaction. Therefore, care should be taken when choosing a suitable acid. Some commonly used acids and two kinds of sodium salts were added to the mixture with the same mole ratio of EM to determine if they increased the stability of the formulation. Following exposure to 60 °C for two weeks, the samples were analyzed by HPLC and the formation of DKP was compared to determine if they increased the stability of the formulation. The ratio of the peak area of DKP was chosen to indicate the degree of degradation. The results are shown in Table 2.

From the results shown above, the addition of sodium phosphate and sodium dihydrogen phosphate greatly promoted the formation of DKP. Some types of organic acid such as maleic acid, tartaric acid and malic acid inhibited the formation of DKP to a degree, whereas other types of acid promoted the formation of DKP.

An early publication reported that basic agents could suppress formation of the cyclization product of moexipril hydrochloride during wet granulation (Gu et al. 1990). The authors believed that stabilization occurred because the basic excipients neutralized the acidic nature of the hydrochloride salt at the reaction site. Our research indicated that some organic acids, instead of basic excipients, stabilized EM. These results may be due to the difference in acidity between hydrochloric acid and maleic acid.

2.5. Optimization of tablet formulation

To verify whether the conclusion drawn above could be assumed with a real tablet formulation, we added the acids to an EM tablet formulation with the same weight ratio containing lactose, L-HPC, starch and magnesium stearate. The tablets were exposed to 60 °C for 10 days and analyzed by HPLC. The ratio of the peak area of DKP to that of enalapril is shown in Table 3.

The results were similar to those shown in Table 2. Hexanedioic acid and lactic acid increased the reaction rate in the tablet. The other acids decreased the reaction rate in the tablet. However, some did not have the same effect as they did in the ternary mixture. This discrepancy showed that the acceleration test of the tablets was necessary due to the complexity of the real formulation. Further studies on this phenomenon are needed.

Table 3: Ratio of DKP (%) and EM (%) in EM tablets after exposure to 60 °C for ten days (n = 3)

Stabilizer	DKP%	EM%
Without stabilizer	2.485 ± 0.794	97.299 ± 0.742
Maleic acid	0.237 ± 0.024	99.492 ± 0.181
Malic acid	1.184 ± 0.056	98.564 ± 0.150
Taurine	1.318 ± 0.084	98.481 ± 0.096
L(+) tartaric acid	0.788 ± 0.047	98.661 ± 0.026
Citric acid	0.966 ± 0.180	97.102 ± 0.421
Hexanedioic acid	2.700 ± 0.522	94.111 ± 0.669
Lactic acid	5.184 ± 0.618	98.979 ± 0.076

According to a previous report, the amount of excipients has a significant influence on stability properties. We hypothesize that the ratio of the drug to excipients may influence EM stability. Thus, we prepared tablets containing different ratios of L(+) tartaric acid and repeated the acceleration test. The results are shown in Fig. 3.

These findings showed that the degree of degradation changed with different ratios of L(+) tartaric acid. The production of DKP decreased when the ratio of L(+) tartaric acid was increased at the initial stage. However, DKP production increased with increasing ratio of L(+) tartaric acid when the ratio was over 1.0. These data prove that the correct acidity is important in obtaining the optimum formulation.

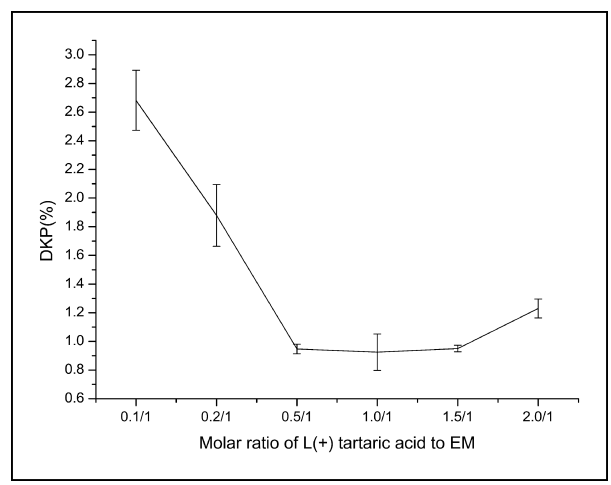

Fig. 3: Ratio of DKP (%) in EM tablets after exposure to 60 °C for 10 days (n = 3).

3. Discussion

In this paper, we propose two mechanisms for DKP formation from EM. One mechanism concerns acid catalysis, and the other is base catalysis. Based on this theory, suitable organic acids can inhibit the formation of DKP by combining with the amino group in EM to decrease its nucleophilicity. Thus, the reaction rate of cyclization would be decreased. We screened various stabilizers of EM and found that some types of organic acid such as maleic acid, tartaric acid and malic acid reduced the formation of DKP. The stabilization effect was proved both in the drug and excipient compatibility tests and in the acceleration test of the real tablet formulation. Our theory was further verified by the phenomena that an optimum ratio of acid stabilizer was required in the tablets for EM stability.

The incompatibility of drug and excipients restrict the selection of excipients. Some excipients with excellent functionality cannot be used due to incompatibility. Our research indicated that the addition of stabilizer could prevent incompatibility between EM and the reactive excipients. This stabilization effect was achieved by controlling the microenvironmental pH. We also speculate that this strategy could be applied to improve the stability of other ACEIs with similar chemical structures.

4. Experimental

4.1. Materials

Enalapril maleate was obtained from Zhejiang Huahai Pharmaceuticals Co., Ltd. The following excipients which have been approved for use in pharmaceutical dosage forms were received from the inventory of the Guangdong Bidi Pharmaceutical Corporation: maize starch, lactose, microcrystalline cellulose, colloidal silicon dioxide, magnesium stearate, hydroxypropyl methylcellulose, talc, low-substituted hydroxypropyl cellulose, sodium starch glycolate, dextrin, and pregelatinized starch. HPLC grade acetonitrile was purchased from Merck (Germany). All other chemicals were analytical grade reagents.

4.2. Instruments

The chromatographic experiments were carried out using an 1100 series HPLC (Agilent Technologies, Germany) system consisting of an on-line degasser (G1379A), quaternary pump (G1311A), auto injector (G1329A), column thermostat (G1314A) and variable UV detector (G1316A).

Impurities were identified by high-resolution mass spectrometry (MS) using quadrupole-time-of-flight (QTOF) MS (Waters Corp., Milford, MA, USA) with the ACQUITY UPLC^{TM} system (Waters Corp., Milford, MA, USA).

4.3. HPLC and UPLC-QTOF MS conditions

The analytical separation was performed on a 150 mm × 4.6 mm, 5 μm Agilent SB-C8 column (Agilent, USA). The mobile phase was a 75:25 (v/v) mixed aqueous solution of 10 mM KH_2PO_4 adjusted with phosphoric acid to pH 2.2: acetonitrile. The flow rate of 1.2 mL/min was employed throughout the analysis. The analyses were performed at 50 °C and the volume of solution injected onto the column was 20 μL. The analyte was monitored by UV at 215 nm.

The UPLC-QTOF MS investigation of impurities was carried out on a ACQUITY BEH C18 UPLC column (2.1 × 100 mm, 1.7 μm, Waters Corp., Milford, MA, USA) at 25 °C with the flow rate set at 0.4 mL/min and using gradient elution with a mobile phase composed of solvent A (water) and solvent B (acetonitrile). The auto-sampler was conditioned at 4 °C. MS analyses were performed in ESI positive ionization mode in the mass range of m/z 50–1000. The ion source settings were as follows: capillary voltage: 3.0 kV; extraction cone voltage: 4.0 V; source temperature: 100 °C; desolvation temperature: 350 °C; desolvation gas flow: 700 L/h; cone gas flow: 50 L/h.

4.4. Compatibility studies

100 mg of both EM and a selected excipient were accurately weighed into a 2 mL glass vial in the first stage compatibility study. The mixture was thoroughly mixed on a vortex mixer for 5 min. The vials were sealed and heated in the oven at 60 °C for two weeks.

The second stage compatibility studies were carried out using 1:1:1 mass/mass/ratio ternary mixtures of drug, reactive excipient and selected stabilizer. Each component (100 mg) was mixed by accurately weighing and transferring to 2 mL glass vials. The mixtures were thoroughly mixed by vortexing for 5 min. The vials in the first group were sealed and heated in the oven at 60 °C for two weeks.

Third stage compatibility studies were carried out using a tablet formulation. Each tablet contained EM, lactose, L-HPC, maize starch, magnesium stearate and organic acid. The tablets were heated in an oven at 60 °C for ten days.

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