Study on Metabolic Kinetics of Ampelopsin in Rat Liver Microsomes and Its Inhibition Effect by Pharmaceutical Excipients

HUANG Renjie[1,2], LIN Yannan[1], YAN Xueli[1], YI Tao[2], YANG Zhijun[2*], CHEN Hubiao[2*](*1.Department of Pharmacy, Fujian Health College, Fuzhou 350101, China; 2.School of Chinese Medicine, Hong Kong Baptist University, Hong Kong 999077, China*)

ABSTRACT: OBJECTIVE To investigate the metabolic regularity of ampelopisn in rat liver microsomes and the effect of pharmaceutical excipients on its metabolism. **METHODS** The UPLC-MS/MS method was established to screen metabolic conditions i*n vitro* and evaluate metabolic patterns by detecting the residual concentration of ampelopsin in the metabolic reaction system. **RESULTS** Ampelopisn metabolism was affected by incubation time, liver microsome concentration and initial ampelopisn concentration. Rat hepatocyte pigment P450 subenzyme CYP3A, CYP1A1/2 and CYP2E1 played a major role in serpentine metabolism. The inhibitory effect of pharmaceutical excipients on ampelopsin metabolism was dose-dependent manner. The metabolic kinetics studies revealed that Cremophor RH40, Tween 80, PVP K30, HPBCD and F68 exhibited significant inhibition metabolism in a mixed competition. **CONCLUSION** These pharmaceutical excipients are expected to improve the oral bioavailability of ampelopsin by inhibiting metabolism.

KEYWORDS: ampelopsin; pharmaceutical excipients; metabolic kinetics; metabolic inhibition

蛇葡萄素在大鼠肝微粒体中的代谢动力学及药用辅料对其抑制作用的研究

黄仁杰[1,2]，林燕喃[1]，鄢雪梨[1]，易涛[2]，杨智钧[2*]，陈虎彪[2*](1.福建卫生职业技术学院药学系，福州 350101；2.香港浸会大学中医药学院，中国香港 999077)

摘要：目的 研究蛇葡萄素在大鼠肝微粒体的代谢规律及药用辅料对其代谢的影响。**方法** 建立 UPLC-MS/MS 方法学体系，通过检测代谢反应体系的蛇葡萄素剩余浓度进行体外代谢反应条件的筛选和代谢规律的评价。**结果** 反应体系的孵育时间、肝微粒体浓度及蛇葡萄素浓度均对代谢产生影响，大鼠肝细胞色素 P450 亚酶 CYP3A、CYP1A1/2 和 CYP2E1 对蛇葡萄素代谢起主要作用。药用辅料对蛇葡萄素代谢的抑制作用呈剂量依赖性，其中聚氧乙烯氢化蓖麻油 40、吐温 80、聚维酮 K30、羟丙基β环糊精、普朗尼克 F68 以混合竞争模式显著抑制蛇葡萄素的代谢。**结论** 这些药用辅料有望通过抑制代谢作用来提高蛇葡萄素的口服生物利用度。

关键词：蛇葡萄素；药用辅料；代谢动力学；代谢抑制
中图分类号：R969.1 **文献标志码：**B **文章编号：**1007-7693(2021)03-0305-09
DOI: 10.13748/j.cnki.issn1007-7693.2021.03.009
引用本文：黄仁杰，林燕喃，鄢雪梨，等. 蛇葡萄素在大鼠肝微粒体中的代谢动力学及药用辅料对其抑制作用的研究[J]. 中国现代应用药学, 2021, 38(3): 305-313.

Ampelopsin(AMP), which was first isolated from *Ampelopsis meliaefolia* by Kotake and Kubota, has been reported as a major bioactive component in *Ampelopsis grossedentata* (Hand-Mazz) W.T. Wang, commonly known as vine tea[1]. In terms of its distribution in the plant, >27% of AMP is found in the leaves and tender stems, with >40% in the cataphylls. It has been reported that AMP possessed a variety of pharmacological activities, including hepatoprotective[2-3], hypoglycemic[4], antioxidant[3,5], anti-inflammatory[6], anti-tumor[7-9], and neuroprotective effects[10], et al. To evaluate its pharmacological potential and the possibility for drug product development, systematic research on AMP has been conducted over the last decades. Chemical structure of AMP was shown in Fig. 1.

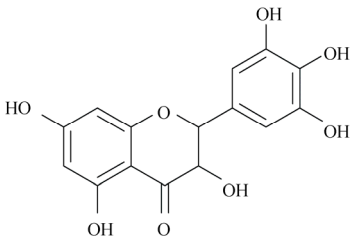

Fig. 1 Chemical structure of ampelopsin
图 1 蛇葡萄素的化学结构

Due to its poor water solubility and bioavailability, which result in the limited application of AMP in drug product development[11-12], various pharmaceutical excipients(PEs) and techniques have been adapted to solve this problem[11,13-14]. However, low bioavailability of drugs probably ascribes the poor

基金项目：福建省自然科学基金项目(2018J01118)
作者简介：黄仁杰，男，硕士，教授 Tel: 18950450855 E-mail: hrj2@163.com *__通信作者：__陈虎彪，男，博士，教授 Tel: (0852)34112060 E-mail: hbchen@hkbu.edu.hk 杨智钧，男，博士，教授 Tel: (0852)34112961 E-mail: yzhijun@hkhu.edu.hk

absorption or the extensive first-pass metabolism[15-16]. Cytochrome P450(CYP) enzymes are important roles to phase 1 metabolism. Furthermore, CYP activity may be affected by enzyme inducer or inhibitor. It has been demonstrated that many physicochemical properties and metabolism of drugs, especially those involved in hepatocytes and gastrointestinal membranes, are modulated by PEs[17-19]. The quantity and physicochemical properties of PEs effect on the magnitude of this inhibitory. Therefore, Generally Recognized as Safe(GRAS)-listed PEs has been used to modulate the drugs with low bioavailability due to metabolism inhibition. To our knowledge, how PEs modulates the metabolic behavior of AMP has not been studied.

The primary objective of this study was to explain the metabolic behavior of AMP in the presence of PEs using rat liver microsomes. A secondary objective was to investigate how PEs concentration specifically affects the kinetics of AMP metabolism. Six excipients commonly used in improving the solubility of drugs, including polysorbate 80(Tween 80), poloxamer 188(F68), polyoxyl 40 castor oil (Cremophor RH40), PEG 400, hydroxypropyl-β-cyclodextrin(HPBCD), and PVP K30, which were selected as experimental excipients.

1 Materials and methods

1.1 Materials

AMP(batch number: 20150203), α-naphthoflavone (batch number: 20150821), fluvastatin(batch number: 20150617), 4-methylpyrazole(batch number: 20150805), quinidine(batch number: 20150722), and ketoconazole (batch number: 20150903) were obtained from Shanghai Yuanye Bio-Technology Co., Ltd. Internal standards(I.S) quercetin(batch number: 20150818) and ticlopidine hydrochloride(batch number: 20150506) were obtained from the National Institute for Food and Drug Control, with a purity of ⩾ 98.0%(HPLC). Rat mixed pool microsomes(batch number: 150915) were purchased from Research Institute for Liver Diseases(Shanghai) Co., Ltd. β-nicotinamide adenine dinucleotide phosphate (NADPH, batch number: 20150714) in the reduced form, tris(hydroxymethyl) aminomethane(Tris, ultra-pure grade, batch number: 20150408), Tween 80(batch number: 20151022), F68(batch number: 20151209), and HPBCD(batch number: 20151014) were purchased from Sigma. Cremophor RH40 (batch number: 20150911), PVP K30(batch number: 20151117) and PEG 400(batch number: 20151110) were obtained from BASF. HPLC-grade water(> 18 mΩ) was prepared by a Millipore water purification system(Millipore, USA). Both acetonitrile and methanol of HPLC-grade were purchased from RCI Labscan. All other reagents were of analytical grade.

1.2 Liquid chromatographic(LC)-mass spectrometry (MS) analysis

LC analysis was carried out on an Agilent 1290 UPLC system equipped with a 1290 binary pump solvent management system, 1290 TCC, and 1290 auto-sampler. The separation of the sample was conducted using a Waters ACQUITY BEH C_{18} column(100 mm×2.1 mm, 1.7 μm) with a column temperature of 40 ℃. Gradient elution was performed by using a mixture of A(0.1% formic acid) and B(0.1% formic acid in acetonitrile) as the mobile phase. The gradient elution program was set as follows: 10%→55% of B for 0–6 min, 55%→100% of B for 6–7 min, 100% of B for 7–9 min, 100%→10% of B for 9–9.1 min, 10% of B for 9.1–12 min and then returned to the initial condition. The flow rate was set at 0.35 mL·min^{-1}, while the auto-sampler was conditioned at 4 ℃, and the injection volume was 3 μL.

As for the MS detection, an Agilent 6460 Triple Quadrupole MS equipped with an Agilent Jet Stream electrospray ionization source (ESI) was operated in the negative ionization mode. The parameters of the source were set as follows: capillary voltage 3.5 kV; source temperature 300 ℃; drying gas temperature 300 ℃; drying gas flow 10 L·min^{-1}; nebulizer 45 psi; sheath gas temperature 350 ℃; sheath gas flow 10 L·min^{-1}. Quantitative analysis was performed using multiple-reaction monitoring(MRM) of the transitions of *m*/*z* 319.0>193.0 for AMP and *m*/*z* 301.0>179.0 for I.S, with a scan time of 0.10 s per transition. Mass Hunter software(Agilent Technologies) was used for the LC-MS/MS system control and data processing.

1.3 Method validation

Method validation followed the US Food and Drug Administration guidelines on validation of bioanalytical methods[20]. Specificity was validated by comparing the MRM ion chromatograms of blank microsomes, blank microsomes spiked with AMP and I.S and microsomes incubated with AMP(250 ng·mL^{-1}) and NADPH(1 mmol·L^{-1}) in Tris-HCl buffer for 10 min.

Calibration standards were prepared by spiking blank microsomes with AMP standard solutions to nine specific concentrations ranging in 1–1 000 ng·mL^{-1}. A standard curve was obtained by plotting the ratios of the chromatographic peak areas(AMP/I.S.) versus the concentration of these solutions. Quercetin was used as the I.S of AMP. The intercept, slope, and coefficient of determination were determined by weighted(1/conc^2) least-squares linear regression analysis.

To investigate the residual effect, two blank samples were injected continuously after the highest concentration point of the standard curve to observe whether there were residues of analyte and internal standard in blank samples.

To evaluate the accuracy and precision of the method, quality control(QC) samples of four concentrations[lower limit of quantification(LLOQ), low, medium, and high] were analyzed in six replicates on a single day and three consecutive days. The accuracy and precision were expressed by relative error(RE, %) and relative standard deviation (RSD, %), respectively. The accuracy and precision were defined as being within 80%–120%, and LLOQ was that at which the signal/noise ratio was ⩾10. The inter-day and intra-day accuracy and precision values for the lowest acceptable reproducibility concentrations were defined as being within ±15%.

To investigate the extraction recovery, AMP at three concentrations(10, 100, 500 ng·mL^{-1}) was spiked to samples before and after extraction, respectively. The recovery of each QC level was calculated based on the peak area ratios of AMP added before and after extraction. For the evaluation of the matrix effect of three QC levels, the peak response from samples spiked post-extraction was designated as A, while the peak response from the pure standard solution containing an equivalent amount of AMP was as B. The ratio(A/B×100%) was used to evaluate the matrix effect. The extraction recovery and matrix effect of I.S were also simultaneously evaluated using the same method as described above.

In this study, freezing/thawing and long-term storage were not carried out during sample handling and analysis. For these experiments, QC samples were prepared in blank microsomes and stored at room temperature for 12 h. Auto-sampler stability was performed by setting pretreated QC samples in the auto-sampler (temperature, 4 ℃) for 48 h before analysis.

1.4 Stability of AMP in the buffer

AMP was incubated with water, 100 mmol·L^{-1} Tris-HCl buffer(pH 7.4) or 100 mmol·L^{-1} PBS buffer (pH 7.4) at 37 ℃ for 60 min, respectively. After incubation, samples were analyzed by LC-MS/MS analysis to determine the concentration of intact AMP to assess its degradation.

1.5 Effect of *in vitro* metabolic factors

The effects of the incubation time, the concentration of liver microsomes, and AMP concentration on the metabolism in rat liver microsomes were studied in detail.

AMP was pre-incubated at 37 ℃ for 5 min in an incubation system containing appropriate buffer(pH 7.4) and liver microsomal protein, followed by the addition of 100 μL NADPH(2.0 mmol·L^{-1}) to initiate the reaction in a 37 ℃ water bath with shaking at 100 r·min^{-1}. The concentrations of microsomes and NADPH were set at 1.0 mg·mL^{-1} and 1.0 mmol·L^{-1} in the final incubation system, respectively. To avoid potential effects on metabolism, the organic solvent volume was <1% of the total incubation system volume. The solutions were vortex for 1 min after ice-cold methanol containing 200 ng·mL^{-1} quercetin (I.S) was added to terminate the reaction. In the end, proteins were separated by centrifugation(13 000×*g*, 8 min, 4 ℃), and then the supernatant containing AMP and I.S was analyzed by UPLC-MS/MS.

1.6 Chemical inhibition

To confirm the CYP isoforms involved in the metabolism of AMP, the effects of CYP-selective inhibitors, including ticlopidine hydrochloride for CYP2B, quinidine for CYP2D6, ketoconazole for CYP3A1/2[21], α-naphthoflavone for CYP1A1/2[22], fluvastatin for CYP2C11[23], and 4-methylpyrazole for CYP2E1[24], on the metabolism of AMP in untreated rat liver microsomes were investigated. For each inhibitor, a triplicate test was performed with three rat liver microsomal samples randomly selected.

The inhibition study was performed with inhibitors of six different concentrations, and a single concentration of rat liver microsomes(1 mg·mL^{-1}) and AMP(80 μmol·L^{-1}). All inhibitors were dissolved in 50% methanol. To correct the effects of the solvent on microsomal activity, a blank sample with equivalent volume and concentration of methanol was included in the control incubations. The inhibitors were pre-incubated with rat liver microsomes and NADPH at 37 ℃ for 15 min, followed by the addition of AMP to initiate the reaction. The metabolism of AMP was assayed by UPLC-MS/MS and expressed as the disappearance rate of AMP.

1.7 PEs' effects on metabolic behavior of AMP and IC_{50} determination

To study the effects of PEs on the AMP metabolism in rat liver microsomes, two different concentrations(20 or 40 μg·mL^{-1}) PEs were added into the incubation system with AMP. The metabolic rate of AMP was characterized as above.

To determine the IC_{50} of PEs on microsomes, the metabolism of AMP was established by preincubating with the PEs for 15 min at 37 ℃. The concentration ranges of the PEs were as follows: Tween 80 (10.0–200.0 μg·mL^{-1}), F68(10.0–200.0 μg·mL^{-1}), Cremophor RH40(1.0–40.00 μg·mL^{-1}), PVP K30

(4.0–100.0 μg·mL^{-1}), and HPBCD(4.0–100.0 μg·mL^{-1}). And then, the incubation experiment was performed as described in "1.6".

1.8 PEs' inhibitory mechanism

To characterize the kinetics of AMP metabolism inhibition and estimate the K_i(inhibition constant), AMP(16–120 μmol·L^{-1}) was incubated in PEs with increasing concentrations. Final concentrations of the PEs were: Tween 80(0.00–40.00 μg·mL^{-1}), F68(0.00–100 μg·mL^{-1}), Cremophor RH40(0.00–10.00 μg·mL^{-1}), PVP K30(0.00–40.00 μg·mL^{-1}), and HPBCD(0.00–40.00 μg·mL^{-1}). Lineweaver-Burk analyses were performed to determine the nature of the inhibition and to further characterize any metabolic interaction. K_i values, the inhibitor constants, were determined by regression analysis of secondary plots(K_m/V_{max} ratio as a function of inhibitor concentration). The slopes were determined from linear regression analysis and converted to apparent K_m/V_{max}(K_m/V_{max}, app) values. Apparent K_m/V_{max} values were then plotted versus the concentration of PEs to generate K_i values(the x-intercepts of the linear regression line)[25-27].

1.9 Statistical analysis

All data were presented as $\bar{x} \pm s$. The statistical significance of differences was carried out using analysis of variance(ANOVA) test with a probability level at 1% or 5%.

2 Results

2.1 Method validation

The typical MRM chromatograms of blank microsomes(A), spiked microsomes containing AMP (250 ng·mL^{-1}) and quercetin I.S(200 ng·mL^{-1})(B), spiked microsomes containing AMP(80 μmmol·L^{-1}) incubated with microsomes(1.0 mg·mL^{-1}) and NADPH(1.0 mmol·L^{-1}) in Tris-HCl buffer(C) for 10 min were shown in Fig. 2. As shown in Fig. 2, the analysis of AMP and I.S could be performed without endogenous peaks interference.

The calibration curves exhibited good linearity over the tested concentration range(1–1 000 ng·mL^{-1}). The regression equation of the AMP in rat liver microsomes was: Y=0.022 3X–0.111 2(r^2=0.997 7). As shown in the data, the LLOQ for the AMP in rat liver microsomes was 1.0 ng·mL^{-1}.

After the standard sample with the highest concentration(1 000 ng·mL^{-1}) was analyzed, two blank samples were continuously injected and analyzed. The results showed that this method had no residual effect and did not affect the determination of AMP and internal standard.

For three QC concentrations of AMP, the intra-day precision(RSD, %) ranged between 1.3% and 4.7%, and accuracy(RE, %) ranged from -0.9% to 1.2%. Inter-day precision(RSD, %) ranged between 3.4% and 4.9%, and accuracy(RE, %) ranged from -4.6% to 2.9%, respectively. The precision and accuracy were acceptable in biological work media.

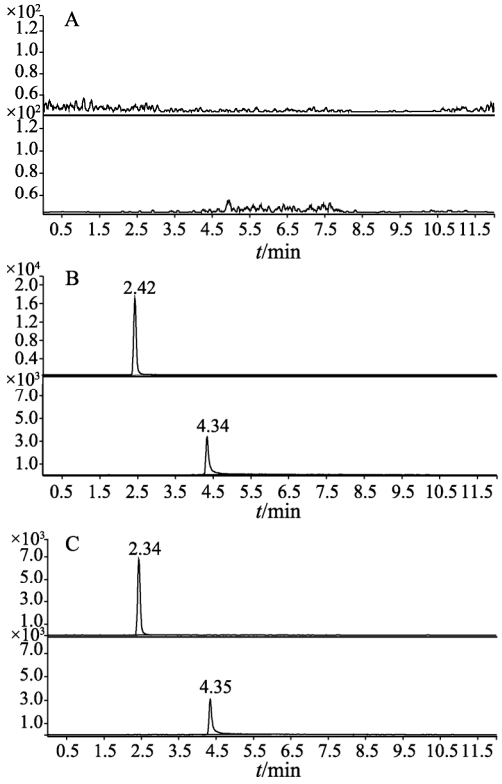

Fig. 2 Typical MRM chromatograms of ampelopsin and quercetin in rat liver microsomes.

A–blank liver microsome sample; B–blank liver microsome sample spiked with ampelopsin(250 ng·mL^{-1}) and quercetin I.S(200 ng·mL^{-1}); C–ampelopsin(80 μmol·L^{-1}) incubated with microsomes(1.0 mg·mL^{-1}) and NADPH(1.0 mmol·L^{-1}) at 37 ℃ for 10 min.

图 2 蛇葡萄素和内标槲皮素在大鼠肝微粒体中的 MRM 色谱图

A–空白肝微粒体；B–添加蛇葡萄素(250 ng·mL^{-1})和槲皮素内标(200 ng·mL^{-1})的空白肝微粒体；C–蛇葡萄素(80 μmol·L^{-1})在肝微粒体(1.0 mg·mL^{-1})和 NADPH(1.0 mmol·L^{-1})体系中 37 ℃孵育 10 min。

The average extraction recovery of AMP from QC samples ranged from (96.4±2.7)% to (103.2±3.5)%, whereas (96.7±2.3)% for I.S, which showed that the protein precipitation method acquired accurate and consistent data. In this study, the matrix effects derived from QC samples were between(98.4±3.7)% and (105.6±4.3)%, which were within the acceptable limits(85%–115%). Matrix effect on AMP was not significantly detected for the efficient sample treatment and high selectivity of MRM, indicating that ion suppression and enhancement from rat liver microsomes were negligible for this method.

The stability tests showed that AMP remain generally stable in QC samples at room temperature

for 12 h(RE: –2.0%–4.1%, RSD<3.1%), and in auto-sampler conditions for 48 h after preparation (RE: –2.3%–3.8%, RSD<2.5%).

2.2 *In vitro* metabolic conditions establishment

To investigate the potential chemical degradation of AMP in microsome-free solvent systems, AMP (80 μmol·L^{-1}) was incubated with water, PBS buffer, and Tris-HCl buffer(pH 7.4), respectively. The solvent effect on AMP was shown in Fig. 3A. The intact AMP concentration significantly decreased in PBS buffer during incubations up to 60 min as compared with in water, whereas there was no variation in Tris-HCl buffer. These results indicated that AMP was stable in Tris-HCl buffer, whereas no stable in PBS buffer. Therefore, Tris-HCl buffer(pH 7.4) was chosen for the subsequent *in vitro* metabolic experiment.

As a significant factor of the drug metabolism *in vitro*, incubation time effects on metabolic behaviors of AMP in rat liver microsomes as well. As show in Fig. 3B, AMP was rapidly metabolized in the initial phase(10 min), and then the intact AMP concentration slightly decreased following incubation time for the remainder of 60 min. Consequently, an incubation time of 10 min was chosen for subsequent experiments.

Fig. 3C showed the effect of microsome concentrations on the metabolism of AMP. In the selected microsome concentrations ranging from 0.3 to 1.0 mg·mL^{-1}, the metabolic rate of AMP dramatically increased from 0.72 to 2.08 μmol·L^{-1}·min^{-1}·mg^{-1} protein and demonstrated first-order kinetics characteristics(r^2=0.985 4). Results indicated that higher microsome concentration lead to a greater metabolism capacity. However, at concentrations above 1.0 mg·mL^{-1}, microsome protein-binding also increased, resulting in decreased sample extraction recovery. Therefore, 1.0 mg·mL^{-1} was selected as the reaction concentration.

Fig. 3D showed the effect of AMP concentration on metabolism. It showed that as AMP concentration increased from 10 to 120 μmol·L^{-1}, the rate of AMP metabolism rapidly increased from 0.29 to 1.79 μmol·L^{-1}·min^{-1}·mg^{-1} protein. But a further increase in AMP concentration resulted in only a slight increase in the metabolism rate to 1.98 μmol·L^{-1}·min^{-1}·mg^{-1} protein. These results suggested that the metabolism capacity approaches its maximum at an AMP concentration of 120 μmol·L^{-1}.

2.3 Effects of CYP inhibitors on AMP metabolism

In order to confirm the CYP isoforms involved in AMP metabolism, the effects of CYP-selective inhibitors on AMP(80 μmol·L^{-1}) metabolism were investigated. With an inhibitor concentration of 50 μmol·L^{-1}, the inhibition ratios of >50%, 20%–50% and <20% were taken to indicate strong inhibitory effects, moderate inhibitory effects, and no inhibitory effects, respectively[28-29]. Fig. 4 showed the inhibitions for quinidine, fluvastatin and ticlopidine were all <20%, which was interpreted as

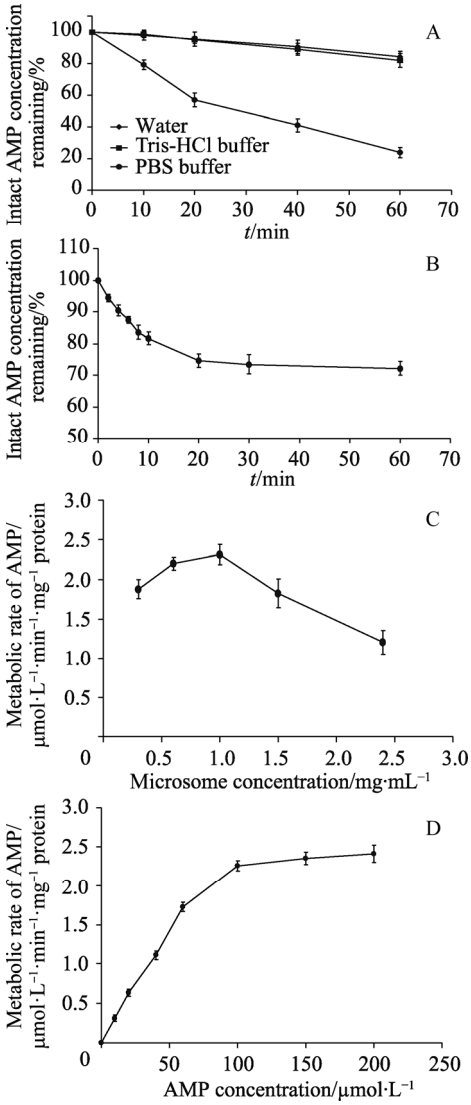

Fig. 3 Effect of buffer and *in vitro* metabolic factors on AMP in rat liver microsomes($\bar{x} \pm s$, n=3)

A–effect of buffer composition on incubation of ampelopsin for 60 min at 37℃; B–effect of incubation time on the metabolism of ampelopsin(80 μmol·L^{-1}) in rat liver microsomes(1.0 mg·mL^{-1}) containing NADPH(1.0 mmol·L^{-1}); C–effect of liver microsome concentration on the metabolism of ampelopsin(80 μmol·L^{-1}); D–effect of ampelopsin concentration on the metabolism of ampelopsin.

图 3 缓冲体系和体外代谢条件对蛇葡萄素在大鼠肝微粒体中反应的影响($\bar{x} \pm s$, n=3)

A–缓冲体系组分对蛇葡萄素在 37 ℃孵育 60 min 过程中的影响；B–在含 NADPH(1.0 mmol·L^{-1})的大鼠肝微粒体(1.0 mg·mL^{-1})中的孵育时间对蛇葡萄素(80 μmol·L^{-1})代谢的影响；C–肝微粒体浓度对蛇葡萄素(80 μmol·L^{-1})代谢的影响；D–体系中蛇葡萄素的浓度对代谢的影响。

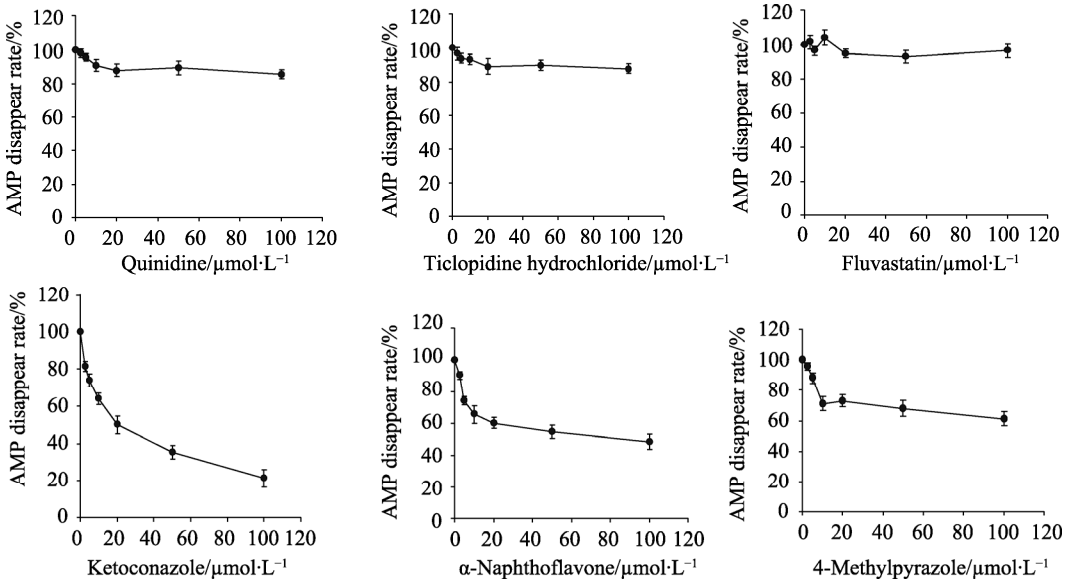

Fig. 4 Effects of the CYP-selective inhibitors on the metabolism of ampelopsin in rat liver microsomes($\bar{x} \pm s$, n=3)

图 4 细胞色素选择抑制剂对蛇葡萄素在大鼠肝微粒体中代谢的影响($\bar{x} \pm s$, n=3)

meaning no inhibitory effects. Ketoconazole had strong inhibitory effect with inhibition ratio of 65.5%, while α-naphthoflavone and 4-methylpyrazole had moderate inhibitory effects with inhibition ratios of 45.2% and 32.1%, respectively. This suggests that the CYP3A1/2, CYP1A1/2, and CYP2E1 inhibitors contributed to the *in vitro* metabolism of AMP, while CYP2C11, CYP2D6 and CYP2B inhibitors had no conspicuous effects on AMP metabolism in rat liver microsomes.

2.4 PEs modulation effects on metabolism

The effects of diversiform PEs on AMP metabolism *in vitro* were shown in Fig. 5. To avoid addition of PEs leading to difference in AMP solubility, the AMP concentration was controlled below saturation in each reaction mixture all along. In the absence of any PEs, 61.5% of AMP remained intact after incubation. The 20 μg·mL^{-1} of PEs significantly inhibited the metabolism of AMP in comparison with control(P<0.01). It indicated that these PEs were effective on inhibiting the metabolism of AMP in liver microsomes. Interestingly, the metabolic inhibition of Cremophor RH40, Tween 80, PVP K30, HPBCD, and F68 were significantly increased while the PEs concentrations were increased to 40 μg·mL^{-1}. At that level, PEG 400 did not significantly affect AMP metabolism. However, as PEG 400 concentrations greater than 100 μg·mL^{-1}, it induced AMP metabolism.

Although strongly inhibited metabolism in concentration-dependent method, five PEs relative inhibitory potency was very different(Fig. 6). The IC_{50} values *in vitro* were 3.69, 15.25, 25.35, 53.96, and 61.76 μg·mL^{-1} for Cremophor RH40, PVP K30, HPBCD, Tween 80, and F68, respectively.

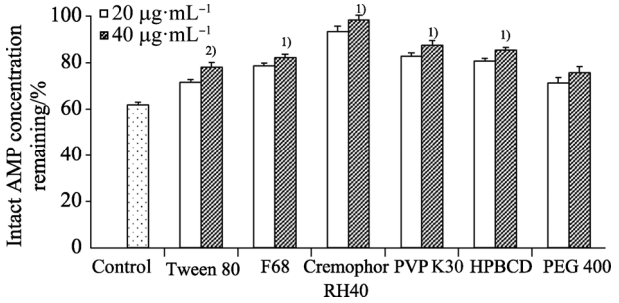

Fig. 5 Dose-dependent inhibition of the metabolism of ampelopsin by six pharmaceutical excipients in rat liver microsomes($\bar{x} \pm s$, n=3)

Compared with 20 μg·mL^{-1} concentration group, $^{1)}P$<0.05, $^{2)}P$<0.01.

图 5 6 种药用辅料对蛇葡萄素在大鼠肝微粒体中代谢的剂量依赖性抑制作用($\bar{x} \pm s$, n=3)

与 20 μg·mL^{-1} 浓度组进行比较，$^{1)}P$<0.05, $^{2)}P$<0.01。

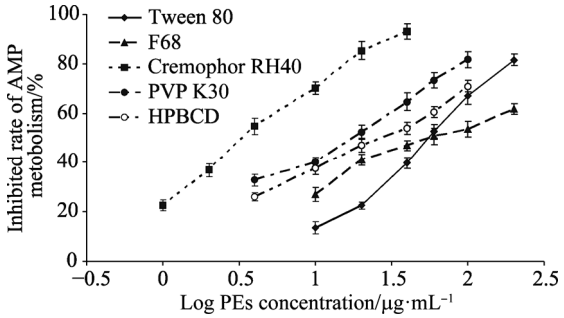

Fig. 6 IC_{50} determination of five pharmaceutical excipients in liver microsomes($\bar{x} \pm s$, n=3)

图 6 肝微粒体中 5 种药用辅料的 IC_{50} 值测定($\bar{x} \pm s$, n=3)

In general, the PEs inhibitory effects on the metabolism of a drug appear to involved disruption of enzyme activities, drug solubility, and micellar formation[19, 30-31]. In this study, the effect of different PEs on AMP metabolism was mostly likely on account of disruption of enzyme activities because the AMP solution was always unsaturated state and because the PEs concentration was below critical micelle concentrations. The dose-dependent inhibition effect of PEs on drug metabolism is similarly meaningful in dosage form design. Including these PEs in AMP pharmaceuticals can be an effective means to prevent phase 1 metabolism existing in the liver and gastrointestinal tract, and thus improve oral bioavailability of AMP.

2.5 Mechanism of PE inhibition

To understand the inhibit metabolism mechanism of PEs, their metabolic kinetics according to PEs concentration were studied. The reaction rates were calculated as the amount of AMP reacted per minute of microsome incubation time and per milligram of microsomal protein. The Michaelis-Menten parameters, K_m and V_{max} for AMP were 737.7 μmol·L^{-1} and 158.7 μmol·L^{-1}·min^{-1}·mg^{-1} protein, respectively.

A Lineweaver-Burk plot was used to describe the AMP metabolic kinetic profiles in the presence of five PEs(Fig. 7). In the present of increasing concentrations of Tween 80, F68, Cremophor RH40, PVP K30, and HPBCD, an increase in K_m values and a decrease in V_{max} values was investigated, respectively. This was consistent with a mixed-type inhibition. The curves of Tween 80, F68, PVP K30, and HPBCD intersect in the third quadrant, and it could be preliminarily judged that the inhibition of PEs on rat liver microsomes was a noncompetitive-anticompetitive inhibition. The value of K_i, means inhibition constant, would take up 50% of the binding sites. The K_i values of Tween 80, F68, Cremophor RH40, PVP K30 and HPBCD were computed as approximately 119.26, 176.03, 6.56, 55.47, and 90.93 μg·mL^{-1}, respectively(Fig. 8), indicating a high affinity for enzymes for AMP metabolism.

3 Discussion

Using rat liver microsomes, AMP metabolism *in vitro*, particularly in the presence of PEs was studied. The AMP metabolism in rat liver microsomes was under the influence of buffer composition, incubation time, the concentrations of liver microsome and AMP. It was found that CYP3A1/2, CYP1A1/2, and CYP2E1 inhibitors significantly inhibit the metabolism of AMP in rat liver microsomes. These results also demonstrate that PEs readily inhibits AMP metabolism via a mixed-type inhibition mechanism. This study is an initial attempt to predict the possible variations in AMP pharmacokinetics caused by interactions with some PEs. PEs' effects on bioavailability of AMP need to be further confirmed by *in vivo* studies directly. However, these *in vitro*

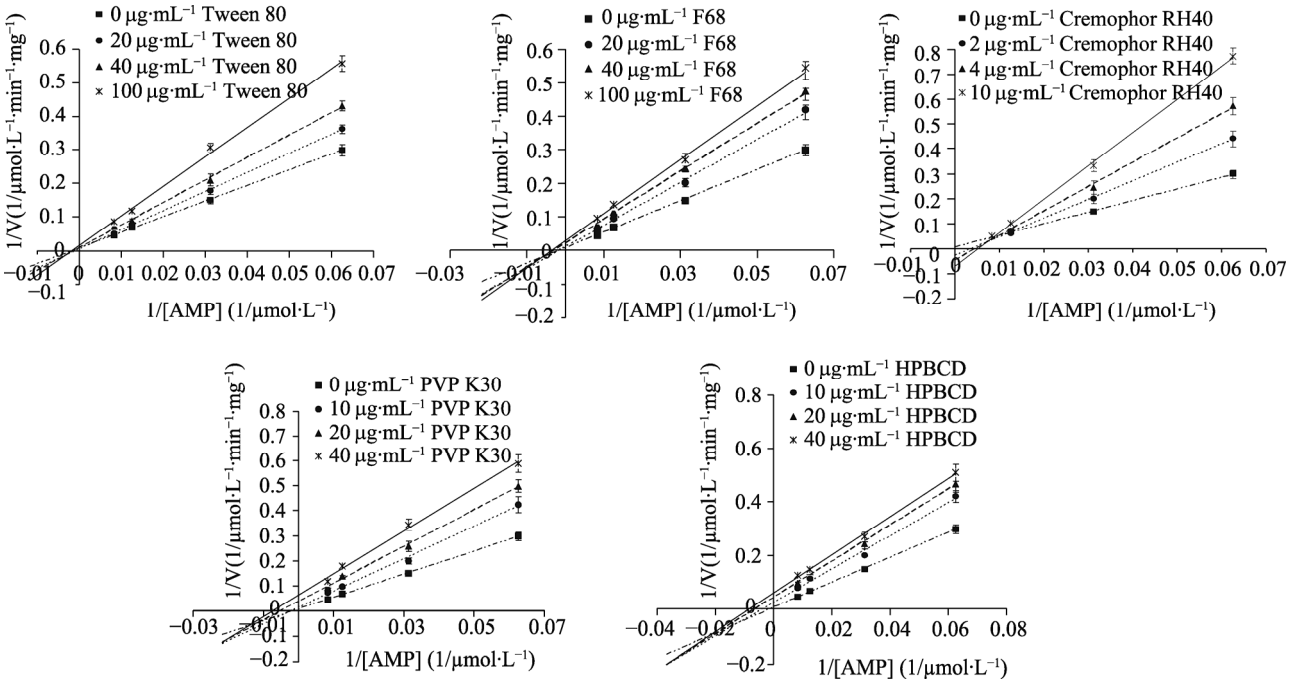

Fig. 7 Lineweaver-Burk plots of 5 kinds of pharmaceutical excipients to inhibition the metabolism of ampelopsin in rat liver microsomes($\bar{x} \pm s$, n=3)

图7 5种药用辅料抑制蛇葡萄在大鼠肝微粒体中代谢的Lineweaver-Burk 模型拟合($\bar{x} \pm s$, n=3)

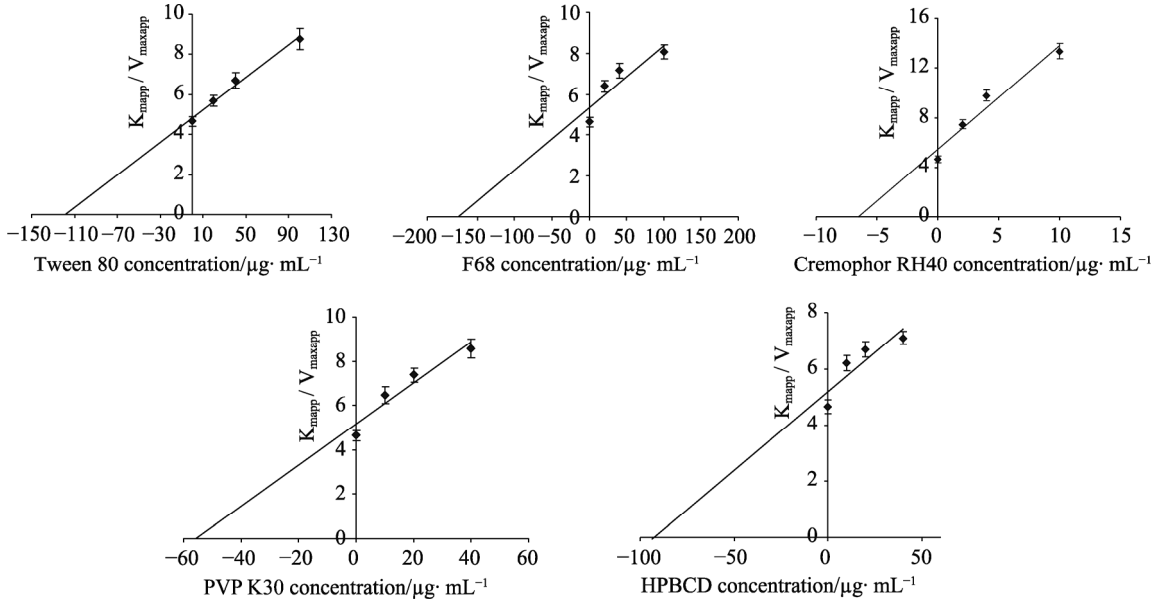

Fig. 8 Secondary conversion plot using the slopes of the primary Lineweaver–Burk plots versus the concentrations of 5 kinds of pharmaceutical excipients($\bar{x} \pm s$, n=3)

图8 Lineweaver–Burk 拟合直线的斜率对5种药用辅料浓度进行二次变换拟合($\bar{x} \pm s$, n=3)

metabolic behaviors might be useful to the selection of suitable PEs in the early stages of AMP pharmaceuticals development. In addition, inclusion of appropriate PEs in pharmaceuticals could improve the availability of drugs having low bioavailability like AMP.

REFERENCES

[1] LIU D, YANG J S. A study on chemical components of *Tetrastigma hemsleyanum* Diels et Gilg. Native to China[J]. China J Chin Mater Med(中国中药杂志), 1999, 24(10): 611-612, 638.

[2] HASE K, OHSUGI M, XIONG Q B, et al. Hepatoprotective effect of *Hovenia dulcis* Thunb. on experimental liver injuries induced by carbon tetrachloride or D-galactosamine/ lipopolysaccharide[J]. Biol Pharm Bull, 1997, 20(4): 381-385.

[3] MURAKAMI T, MIYAKOSHI M, ARAHO D, et al. Hepatoprotective activity of tocha, the stems and leaves of *Ampelopsis grossedentata*, and ampelopsin[J]. BioFactors, 2004, 21(1/2/3/4): 175-178.

[4] ZHONG Z X, QIN J P, ZHOU G F, et al. Experimental studies of hypoglycemic action on total flavone of *Ampelopsis grossedentata* from Guangxi[J]. China J Chin Mater Med(中国中药杂志), 2002, 27(9): 687-689.

[5] HE G X, DU F L, YANG W L, et al. Effects of Tengcha flavonoids on scavenging oxygen free radicals and inhibiting lipid-peroxidation[J]. J Chin Med Mater(中药材), 2003, 26(5): 338-340.

[6] QI S, XIN Y, GUO Y, et al. Ampelopsin reduces endotoxic inflammation via repressing ROS-mediated activation of PI3K/Akt/NF-κB signaling pathways[J]. Int Immunopharmacol, 2012, 12(1): 278-287.

[7] LIU D Y, LUO M. Study on inhibitory effect of ampelopsin on melanoma by serologic pharmacological method[J]. J Chin Med Mater(中药材), 2001, 24(5): 348-350.

[8] ZENG S, LIU D Y, YE Y L, et al. Anti-tumor effects of ampelopsin on human lung cancer GLC-82 implanted in nude mice[J]. J Chin Med Mater(中药材), 2004, 27(11): 842-845.

[9] NI F, GONG Y, LI L, et al. Flavonoid ampelopsin inhibits the growth and metastasis of prostate cancer *in vitro* and in mice[J]. PLoS One, 2012, 7(6): e38802. Doi: 10.1371/journal.pone.0038802.

[10] KOU X J, SHEN K Y, AN Y H, et al. Ampelopsin inhibits H_2O_2-induced apoptosis by ERK and Akt signaling pathways and up-regulation of heme oxygenase-1[J]. Phytother Res, 2012, 26(7): 988-994.

[11] RUAN L P, YU B Y, FU G M, et al. Improving the solubility of ampelopsin by solid dispersions and inclusion complexes[J]. J Pharm Biomed Anal, 2005, 38(3): 457-464.

[12] HUANG R J, DENG Y R. Development of a fluorescence analysis method for determining ampelopsin in rabbit plasma[J]. Chin J Mod Appl Pharm(中国现代应用药学), 2011, 28(1): 70-74.

[13] HE Z F, LIU D Y, ZENG S, et al. Study on preparation of ampelopsin liposomes[J]. China J Chin Mater Med(中国中药杂志), 2008, 33(1): 27-30.

[14] HUANG R J, YAN X L, CHEN H B. Preparation and *in vitro* evaluation of ampelopsin-loaded nanomicelles[J]. China J Chin Mater Med(中国中药杂志), 2016, 41(6): 1054-1058.

[15] KEMP D C, FAN P W, STEVENS J C. Characterization of raloxifene glucuronidation *in vitro*: Contribution of intestinal metabolism to presystemic clearance[J]. Drug Metab Dispos: Biol Fate Chem, 2002, 30(6): 694-700.

[16] JEONG E J, LIN H, HU M. Disposition mechanisms of raloxifene in the human intestinal Caco-2 model[J]. J Pharmacol Exp Ther, 2004, 310(1): 376-385.

[17] VILLALOBOS-HERNÁNDEZ J R, VILLAFUERTE-ROBLES L. Effect of carrier excipient and processing on stability of indorenate hydrochloride/excipient mixtures[J]. Pharm Dev

Technol, 2001, 6(4): 551-561.
[18] CORNAIRE G, WOODLEY J, HERMANN P, et al. Impact of excipients on the absorption of P-glycoprotein substrates *in vitro* and *in vivo*[J]. Int J Pharm, 2004, 278(1): 119-131.
[19] REN S, PARK M J, KIM A, et al. *In vitro* metabolic stability of moisture-sensitive rabeprazole in human liver microsomes and its modulation by pharmaceutical excipients[J]. Arch Pharmacal Res, 2008, 31(3): 406-413.
[20] Guidance for industry: bioanalytical method validation[S]. US Food and Drug Administration, Available: http: //www. fda. gov/ucm/groups/fdagov-public/@fdagov-drugs-gen/documents /document/ucm368107. pdf, 2013 September.
[21] NEWTON D J, WANG R W, LU A Y. Cytochrome P450 inhibitors. Evaluation of specificities in the *in vitro* metabolism of therapeutic agents by human liver microsomes[J]. Drug Metab Dispos: Biol Fate Chem, 1995, 23(1): 154-158.
[22] LAKSHMI V M, ZENSER T V, DAVIS B B. Rat liver cytochrome P450 metabolism of *N*-acetylbenzidine and *N*, *N*'-diacetylbenzidine[J]. Drug Metab Dispos, 1997, 25(4): 481-488.
[23] TRANSON C, LEEMANN T, DAYER P. *In vitro* comparative inhibition profiles of major human drug metabolizing cytochrome P450 isozymes(CYP2C9, CYP2D6 and CYP3A4) by HMG-CoA reductase inhibitors[J]. Eur J Clin Pharmacol, 1996, 50(3): 209-215.
[24] ZHANG X, WANG R B, ZHOU W, et al. Antitumor activity of DMAKO-05, a novel shikonin derivative, and its metabolism in rat liver microsome[J]. AAPS PharmSciTech, 2015, 16(2): 259-266.
[25] BOURRIÉ M, MEUNIER V, BERGER Y, et al. Cytochrome P450 isoform inhibitors as a tool for the investigation of metabolic reactions catalyzed by human liver microsomes[J]. J Pharmacol Exp Ther, 1996, 277(1): 321-332.
[26] KAKKAR T, BOXENBAUM H, MAYERSOHN M. Estimation of *K*i in a competitive enzyme-inhibition model: Comparisons among three methods of data analysis[J]. Drug Metab Dispos: Biol Fate Chem, 1999, 27(6): 756-762.
[27] UBEAUD G, HAGENBACH J, VANDENSCHRIECK S, et al. *In vitro* inhibition of simvastatin metabolism in rat and human liver by naringenin[J]. Life Sci, 1999, 65(13): 1403-1412.
[28] KRIPPENDORFF B F, LIENAU P, REICHEL A, et al. Optimizing classification of drug-drug interaction potential for CYP450 isoenzyme inhibition assays in early drug discovery[J]. J Biomol Screen, 2007, 12(1): 92-99.
[29] XIA Z L, YING J Y, SHENG R, et al. *In vitro* metabolism of BYZX in human liver microsomes and the structural elucidation of metabolite by liquid chromatography-mass spectrometry method[J]. J Chromatogr B Analyt Technol Biomed Life Sci, 2007, 857(2): 266-274.
[30] BRAVO GONZÁLEZ R C, HUWYLER J, BOESS F, et al. *In vitro* investigation on the impact of the surface-active excipients Cremophor EL, Tween 80 and Solutol HS 15 on the metabolism of midazolam[J]. Biopharm Drug Dispos, 2004, 25(1): 37-49.
[31] REN S, PARK M J, SAH H, et al. Effect of pharmaceutical excipients on aqueous stability of rabeprazole sodium[J]. Int J Pharm, 2008, 350(1/2): 197-204.

收稿日期：2019-10-12
(本文责编：蔡珊珊)