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Short communication

Stability of ramipril in the solvents of different pH

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Abstract

The stability of ramipril in the buffer solution with different pH and the influence of acid, alkaline and oxidative medium on ramipril stability were studied. The ramipril degradation products were determined by high-performance liquid chromatography (HPLC) method. Acetonitrile:sodium perchlorate was used as the mobile phase, at a flow rate of 1.0 ml/min (linear gradient elution). A Nucleosil 100-S 5 μm C18, 250 mm × 4.6 mm i.d. was utilized as stationary phase. Detection was affected spectrophotometrically at 210 nm. The drug substance was dissolved in the ammonium phosphate buffer (pH 3, 5 and 8) and these solutions were stored at 90 °C for 1 h. The other series of test solutions were prepared from stock solution (drug substance dissolved in solvent A of the mobile phase) by dilution in acid (0.1 M HCl), alkaline (0.1 M NaOH) and oxidative (hydrogen peroxide solution) medium. More then 0.2% of impurity D (ramipril–diketopiperazine) was detected in the buffer of pH 3 and pH 5. In the buffer of pH 8 there was detected more then 1% of impurity E (ramipril–diacid). No peaks for degradation products appeared in the chromatograms above limit of quantification. The alkaline medium has the greatest effect on degradation of ramipril into impurity E (more than 50%).

Keywords: Ramipril; High-performance liquid chromatography; Stability

1. Introduction

Ramipril is potent and specific angiotensin-converting enzyme (ACE) inhibitor that lower peripheral vascular resistance without affecting heart rate. It is used in treatment of hypertension and congestive heart failure. The role of this kind of drugs is to inhibit the last step of the biosynthesis of angiotensin II, a potent vasoconstrictor, and therefore, it causes a general vasodilatation and lowers blood pressure [1–3]. Ramipril, an angiotensin-converting enzyme inhibitor, is a prodrug which is rapidly hydrolysed after absorption to the active metabolite ramiprilate. The *cis*- and *trans*-isomers of ramiprilate were investigated by nuclear magnetic resonance studies [4,5]. A radioimmunoassay (RIA) has been developed for the measurement ramipril and its active metabolite (diacid: hydrolysis product of ramipril) in serum or plasma. The RIA is specific for the active metabolite (diacid) [6]. A GLC method for the simultaneous determination of both compounds in urine are described [7]. Two sensitive, spectrophotometric and atomic absorption spectrometric procedures are developed for the determination of ramipril. Both methods are based on the formation of a ternary complex [8]. The potential presence of diastereomers as related substances significantly complicates the required chromatography due to the diastereomers' structural similarity to ramipril. High-performance liquid chromatographic methods for determining the optical purities of ramipril and its synthetic intermediates have been developed [9,10]. Separation of ramipril from its potential related substances is required for accurate and precise quantitation of degradation products potentially present in formulated pharmaceuticals. Several methods for the determination of ramipril or selected precursors have been described in the literature. The related

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substances include these precursors as well as eight additional potential related substances were presented in literature [11].

The aims of the present study were to examine the effects of pH of sample solvent on ramipril stability, to test whether the acid, alkaline and oxidative medium has the influence on ramipril degradation.

2. Experimental

2.1. Samples, solvents and reagents

Ramipril and impurity A (methylester of ramipril) are commercially available. Ammonium phosphate, sodium perchorate, triethylamine, acetonitrile (HPLC grade) were obtained from Aldrich (Germany). Ultra-pure water generated by the Milli-Q system was used, and all the other chemicals.

2.2. High-performance liquid chromatography conditions

HPLC separation was performed with a Waters liquid chromatographic system (Milford Massachusetts, USA) equipped with a separations module Alliance 2695 with thermostat and autosampler with a final injection volume of 10 μl. The photodiode array detector 2996 was set at wavelength 210 nm. The date were stored and processed by an HPLC software (Millennium 4.0, Waters). A Nucleosil 100-S 5 μm (250 mm × 4.6 mm i.d.) column was used. The column temperature was maintained by means of a Waters thermostat (40 °C).

For the mobile phase two solvents were prepared. Solvent A consisted of acetonitrile–0.02 M sodium perchlorate solution with 0.5 ml triethylamine adjusted to pH 3.6 ± 0.2 with phosphoric acid (20:80, v/v). Solvent B consisted of acetonitrile–45 mM sodium perchlorate solution with addition of 0.5 ml triethylamine adjusted to pH 2.6 ± 0.2 with phosphoric acid (70:30, v/v). Mixture of solvent A–solvent B was used as the mobile phase at a flow rate of 1.0 ml/min with gradient elution. The linear gradient used is given in Table 1.

Table 1
Linear gradient elution of the mobile phase used in HPLC method

Gradient time (min)	A mobile phase (%)	B mobile phase (%)	Profile
0–6	90	10	Isocratic
6–7	90 → 75	10 → 25	Linear gradient
7–20	75 → 65	25 → 35	Linear gradient
20–30	65 → 25	35 → 75	Linear gradient
30–40	25	75	Isocratic
40–45	25 → 90	75 → 10	Linear gradient
45–55	90	10	Isocratic

2.3. Sample preparation

Working standard solution was prepared in solvent A of the mobile phase from stock standard solution (1 mg/ml).

Resolution mixture: 5.01 mg of impurity A with 5 ml of stock solution, the volume was filled up to 10.0 ml by mobile phase B. The resolution between impurity A and ramipril was 3.09.

Several sample solutions of ramipril substance of different pH were analyzed (the final concentration of ramipril was 1 mg/ml).

(a) Ramipril substance was dissolved in solvent A of the mobile phase (control sample).
(b) Ramipril substance was dissolved in solvent A of the mobile phase (90 °C, 60 min).
(c) Ramipril substance was dissolved in 0.03 M ammonium phosphate buffer adjusted to pH 3.0 with phosphoric acid.
(d) Ramipril substance was dissolved in 0.03 M ammonium phosphate buffer adjusted to pH 5.0 with phosphoric acid.
(e) Ramipril substance was dissolved in 0.03 M ammonium phosphate buffer adjusted to pH 8.0 with phosphoric acid.

All of the sample solutions were placed in an ultrasonic bath for 15 min and then stirred with magnetic stirrer for about 30 min. The sample solutions (b–e) were heated at 90 °C for 60 min. They were centrifuged for 10 min at 5000 rpm before injection.

The influence of acid, alkaline and oxidative medium was tested on the sample solutions prepared form ramipril substance stock solution (0.5 mg/ml of ramipril substance in solvent A of the mobile phase). The final concentration of ramipril was 1 mg/ml.

(A) Five milliliter of stock solution was transferred to the 10 ml volumetric flask and made up to mark with ultra-pure water (control sample).
(B) Five milliliter of stock solution was transferred to the 10 ml volumetric flask and made up to mark with 0.1 M HCl solution.
(C) Five milliliter of stock solution was transferred to the 10 ml volumetric flask and made up to mark with 0.1 M NaOH.
(D) Five milliliter of stock solution was transferred to the 10 ml volumetric flask. A 1 ml volume of 30% H_2O_2 solution was added and the mixture was made up to mark with ultra-pure water.
(E) Five milliliter of mobile phase A with 5 ml 0.2 M HCl (blank HCl sample).
(F) Five milliliter of mobile phase A with 5 ml 0.2 M NaOH (blank NaOH sample).
(G) Five milliliter of mobile phase A 1 ml volume of 30% H_2O_2 solution was added and the mixture was made up to mark with ultra-pure water (blank H_2O_2 sample).

All of the sample solutions were kept for 1 h at laboratory temperature before injection.

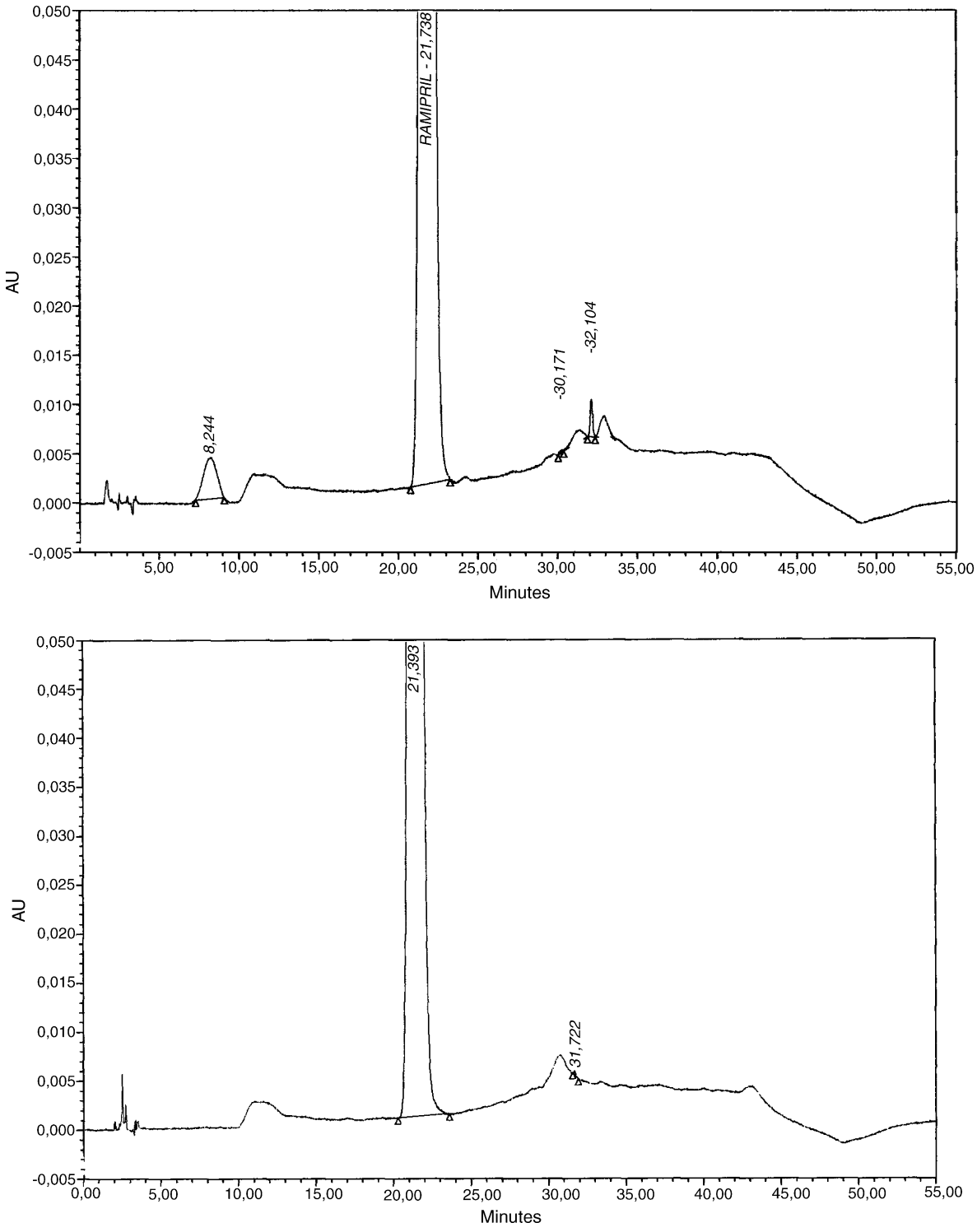

Fig. 1. HPLC chromatogram of ramipril substance dissolved in phosphate buffer adjusted to pH 5. (a) The peak with retention time 8.2 min is impurity E, the peak with retention time 21.7 min is ramipril, the peak with retention time 30.17 min is impurity C and the peak with retention time 32.1 min is impurity D (sample preparation see Section 1.3, results are presented in Table 1). HPLC chromatogram of ramipril substance dissolved in 0.1 M HCl. (b) The peak with retention time 21.4 min is ramipril and the peak with retention time 31.7 min is impurity D (sample preparation see Section 1.3, results are presented in Table 3). HPLC chromatogram of ramipril substance dissolved in 0.1 M NaOH. (c) The peak with retention time 6.6 min is impurity E and the peak with retention time 21.3 min is ramipril (sample preparation see Section 1.3, results are presented in Table 3).

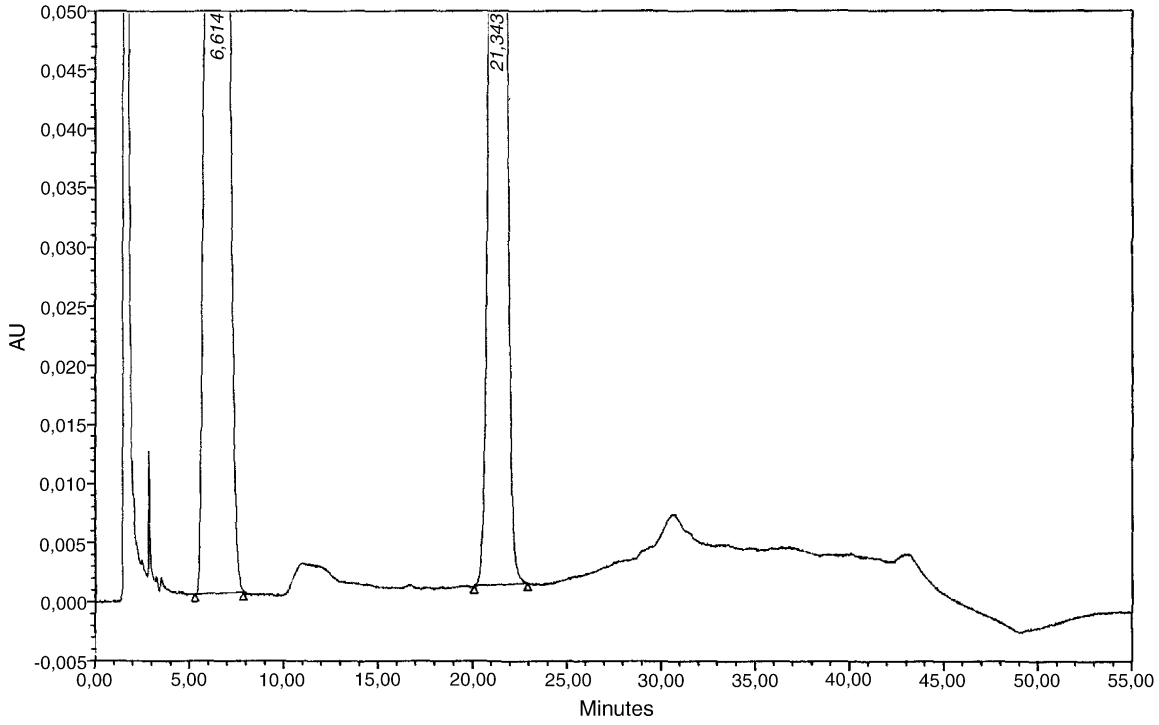

Fig. 1. (*Continued*).

3. Results and discussion

Optimal separation of related substances from each other and from ramipril was achieved with an acetonitril–sodium perchlorate buffer mobile phase gradient. The mobile phase gradient employed is described in Table 1. A column temperature of 40 °C was employed for the separation. The resolution between the impurities C and D has been better at this column temperature than at higher column temperature. The resolution between impurity A (methylester ramiprilu) and ramipril was 3.09. This impurity is the nearest related substance of ramipril on chromatogram. The analysis run on the column with reverse phase as known in literature, but with different parameters. The single peak of ramipril is obtained on the chromatogram, when the separation run on C18 column. Unlike the use of cation-exchange column, where the elution of ramipril as a bimodal, broad peak may be due to a difference in acidity for the *cis*- and *trans*-rotamers [4].

There were described the studies about the effect of sample sonication time on leaching of ramipril from pharmaceutical preparations in literature [11]. It was clear, that the proposed method with total sonication time of 30 min was sufficient from the theory described in this literature.

All stressed samples were compared to a control sample. The values of pH were measured again after 60 min in drying chamber and these values are shown in Table 2. The impurity D increases by heat as known in literature [11]. In this paper, the impurity D has increased in the sample prepared from tablets after 60 min, in the control sample and in acid medium.

The load effect on the substance was observed in chromatogram as a fraction of the peak area. The identifications of the related substances of ramipril were possible from relative retention times, known from European Pharmacopoeia. There have found 0.27% of the impurity D in the sample (1 mg/ml) prepared from five tablets Tritace 5 without heating. In the case of dissolving of ramipril substance in mobile phase it was found only 0.03% impurity D, but 0.18% impurity D after heating for 60 min. The increasing of impurities after loading of samples in different pH is shown in Table 2. The results of stress tests in alkaline and acid medium are observed in Table 3. By oxidation process, the impurity C and D increased as shown in Table 3. Ramipril is stable to oxidative decomposition. In comparison the stress samples with blank samples, it was possible to determine peak areas and volume of impurities as % from all peak area. It is clear, that the analysis of blank samples were necessary as shown in the chromatograms in Fig. 1, especially in the case of determination of increase in impurities C and D.

Table 2
The stability of ramipril in the buffer solution with different pH–percentages of relative substances of ramipril as obtained in sample chromatogram under area percentage column

	pH 8 (7.7)		pH 5 (4.8)		pH 3 (3.1)	
	t_R	Area (%)	t_R	Area (%)	t_R	Area (%)
Impurity E	7.5	5.96	8.2	1.20	8.3	0.17
Ramipirl	21.7	93.66	21.7	98.60	21.7	99.04
Unknown impurity	24.2	0.27	–	–	–	–
Impurity D	32.1	0.12	32.1	0.21	32.0	0.79

9.7

Table 3
Influence of acid, alkaline and oxidative medium on ramipril stability–percentages of relative substances of ramipril as obtained in sample chromatogram under area percentage column

	3% H_2O_2		0.1 M NaOH		0.1 M HCl	
	t_R	Area (%)	t_R	Area (%)	t_R	Area (%)
Impurity E	–	–	6.6	52.16	–	–
Ramipirl	21.4	99.94	21.4	47.84	21.3	99.98
Impurity C	29.9	0.03	–	–	–	–
Impurity D	31.6	0.02	–	–	31.7	0.02

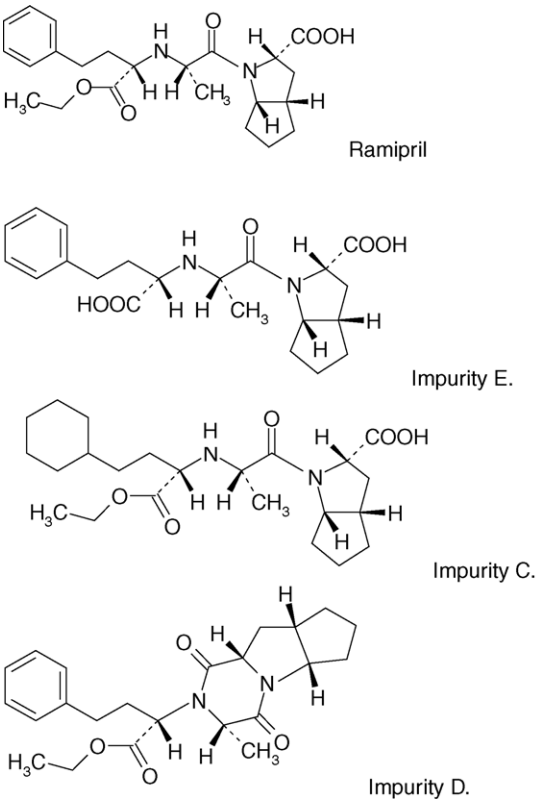

Fig. 2. Structures of the ramipril and of the related substances of ramipril.

The structure formulas of all identified impurities are shown in Fig. 2.

4. Conclusion

This paper has presented, how the substance of ramipril is stable, particularly in alkaline medium from results of stability tests. There increased the impurity E by hydrolysis of ester bond in 0.1 M NaOH and also in the buffer pH 8. This reaction also run in the buffer pH 5. This drug is also unstable in acid medium, but there increase impurity D. This degradation is also observed in the control sample (ramipril substance in mobile phase A). Stress by heat produced ramipril–diketopiperazin. In the case of stress by oxidation, the impurities C and D were observed.

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