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Journal of Pharmaceutical and Biomedical Analysis 40 (2006) 105–112
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
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HPLC study on stability of pyridoxal isonicotinoyl hydrazone

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Received 4 January 2005; received in revised form 13 June 2005; accepted 14 June 2005
Available online 2 August 2005

Abstract

Biocompatible iron chelators are currently under extensive investigation as promising drug candidates. Pyridoxal isonicotinoyl hydrazone (PIH) is a lead compound of the aroylhydrazone group of novel iron chelating agents. In this study, the precise and accurate HPLC analytical methods were used for the stability evaluation of water-soluble PIH salt (PIH·2HCl) in aqueous media of different pH (2.0, 3.9, 7.0, 9.0 and 12.0) as well as in two selected pharmaceutical co-solvents at both laboratory and elevated (40 °C) temperatures. The susceptibility of PIH·2HCl to oxidative decomposition was studied in the solutions of hydrogen peroxide (3 and 30%). Furthermore, the solid substance of PIH·2HCl was exposed to UV, dry and wet heat. Our experiments revealed that PIH was considerably sensitive to hydrolytic decomposition in aqueous media, resulting in the splitting of the hydrazone bond. The elevated temperature significantly accelerated the hydrolytic reaction. The lowest rate of hydrolysis of PIH was observed in the phosphate buffer of pH 7.0 and in the pharmaceutical co-solvents (30% PEG-300 and 10% Cremophor EL). No special degradation products were detected in the samples exposed to either hydrogen peroxide or co-solvents. The solid substance of PIH·2HCl was stable when exposed to UV, dry or wet heat for 33 h.

Keywords: Pyridoxal isonicotinoyl hydrazone; PIH; Stability; HPLC; Iron chelator

1. Introduction

Biocompatible iron chelators are within the biological systems capable to selectively form stable complexes with free or loosely bound iron without significant interference with other important biometals. Iron chelation therapy is now routinely used in iron-overloaded patients to prevent severe organ damage, which would ultimately lead to death. Additionally, growing body of evidence suggests that iron, even in non-overloaded subjects, plays an important role in a number of human pathologies, and thus the concept of iron chelation represents a unique approach for the novel drug development [1].

Pyridoxal isonicotinoyl hydrazone (PIH, Fig. 1) is a selective, biocompatible iron chelating agent developed by Ponka et al. [2].

Today, it is a lead compound of the large group of aroylhydrazone iron chelators. PIH is a tridentate ligand which can bind free or loosely bound Fe^{3+} via its phenolate oxygen, imine nitrogen and carbonyl oxygen atoms in a 1:2 stoichiometry. This compound can be readily synthesized by Schiff-base condensation from commercially available substances (pyridoxal and isonazid) [3]. PIH is a crystalline substance, which can exist in the form of different hydrates and salts, colored from light yellow to orange. Unfortunately the free base of PIH is poorly soluble in aqueous media of neutral pH. Since this fact represents considerable limitation for its use as a pharmaceutical, the synthesis of water-soluble salt (PIH·2HCl) has been developed and patented [4].

PIH has been shown as an effective iron chelator both in vitro and in vivo [3]. In an addition to promotion of body iron excretion, some other promising pharmacodynamic effects (antioxidative, antiproliferative and retinoprotective) have been reported in the preclinical studies [3,5,6]. The toxicity and tolerability of single and repeated administration of

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doi:10.1016/j.jpba.2005.06.021

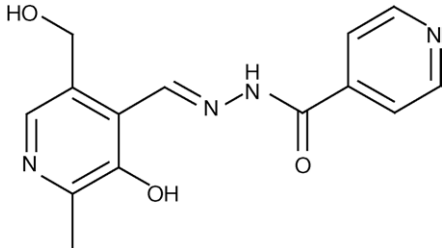

Fig. 1. The chemical structure of PIH.

PIH have been assessed in rats and rabbits [7,8]. Following the encouraging results from animal studies, phase I, clinical trial was performed. This study confirmed low toxicity and good tolerability of this chelator, although its efficacy in iron-overloaded patients was lower than in the animal studies [9]. These results might have been caused by an inappropriate pharmaceutical formulation leading to limited bioavailability [3]. This can be overcome using the water-soluble PIH salt (PIH·2HCl), which is now available.

The stability assessment of any promising drug candidate plays a vital role in the process of the novel drug development. Many environmental conditions, such as heat, light, moisture as well as the inherent chemical susceptibility of a substance to hydrolysis or oxidation can play an important role in pharmaceutical stability. These studies also provide essential information supporting pharmaceutical formulation development. Furthermore, they help to define storage and handling conditions. The exposition of the drug substance to extreme external conditions helps to revealing and identifying the likely degradation products [10]. Although some pilot data on the stability of PIH have already been reported [11,12], no systematic stress study on the drug has been performed. Since the water-soluble salt (PIH·2HCl) possessed favorable pharmaceutical properties, we focused on the stability of this salt.

The stability of this chelator was determined in the aqueous media of different pH (2.0, 3.9, 7.0, 9.0 and 12.0) and in selected pharmaceutical co-solvents (30% PEG-300 and 10% Cremophor EL) at both the laboratory (25 °C) and elevated (40 °C) temperature. The susceptibility of PIH·2HCl to oxidative decomposition was studied using the exposure to hydrogen peroxide (3 and 30%). In addition, other extreme external conditions (UV, dry and wet heat) were employed in order to describe degradation behavior of the said compound.

2. Experimental part

2.1. Material and instrumentation

2.1.1. Chemicals

PIH·2HCl was synthesized according to Lewis et al. [4]. The structure and purity of this compound was confirmed by FTIR (Nicolet Impact 400 spectrophotometer, Thermo, Madison, USA), ^{1}H and ^{13}C NMR (Varian Mercury-Vx BB 300 instrument, Palo Alto, USA, operating at 300 MHz for ^{1}H, 75 MHz for ^{13}C) and HPLC method [13]. The melting point was measured using the Kofler apparatus (Boëtius, Nagema, Germany) and it is uncorrected. The melting point (255–260 °C, degradation) and the spectra were in accordance with those previously reported [4]. Isoniazid, pyridoxal, isonicotinic acid and 1-heptanesulfonic acid sodium salt were purchased from Sigma–Aldrich (Munich, Germany). Methanol, EDTA, phosphate buffers, hydrogen peroxide, phosphoric acid and sodium hydroxide were obtained from Lachema a.s. (Brno, Czech Republic). PEG-300 and Cremophor EL were purchased from Kulich (Hradec Králové, Czech Republic). The water was purified using reverse osmosis.

2.1.2. Chromatographic systems and conditions

2.1.2.1. Isocratic method (method I). The chromatographic system (Thermo Separation Products Inc., Riviera Beach, USA), which consisted of an isocratic pump (Costametric 3500), an automatic injector (AS 1000) and a UV–vis detector (UV 3000 HR), was used for the stability evaluation. The HPLC data were processed with SpectraSystem Software-PC 1000 operated under the control of a OS/2 Warp operation system. The chromatographic conditions used in this study have been developed and discussed in our previous study [13]. The chromatographic analyses were achieved on 5 μm particle size C_{18} Nucleosil (Watrex, Prague, Czech Republic) column (250 mm × 4 mm) kept at 25 °C. The mobile phase was composed of mixture of component A (methanol) and component B (0.01 M NaH_2PO_4, with 5 mM 1-heptanesulfonic acid sodium salt, adjusted to pH 3.0) in a ratio 49:51 (v/v). EDTA was added into the aqueous part of mobile phase (component B) in the concentration 2 mM. The flow rate was 0.9 ml/min and the detector was set up to the dual mode (297 and 254 nm). While the first channel was set at the absorption maximum of PIH (297 nm), the second channel enabled the more sensitive detection of the degradation products. The injection volume was 20 μl.

2.1.2.2. Gradient method (method II). In order to reveal the possible co-elution of isoniazid and isonicotinic acid, the method was change from isocratic to gradient one. The gradient analyses were performed using chromatographic system series HP 1100 (Agilent Technologies, Palo Alto, USA) which consisted of a HP 1100 series binary pump, a vacuum degasser, a thermostated column compartment, a variable wavelength detector and an autosampler. The chromatographic data were processed using HP Chemstation (Agilent technologies). The following gradient was used: 0–6 min 80–50% (component B); 6–18 min 50–0% (component B); 18–25 min 0–80% (component B). All other HPLC conditions were the same as described above (method I).

2.1.3. Stability study instrumentation

The hydrolytic stability study was carried out using water bath U 7 (LMW, Medingen, Germany) equipped with thermostat controller. Photostability study was performed under

the UV lamp (Camag, Muttenz, Switzerland). The exposition to wet and dry heat was proceeded in oven (HS 61-A, Chirana, Prague, Czech Republic) set at 80 °C.

2.2. Stability study

2.2.1. Calibration curve, precision and accuracy—methods I and II

The stock solution of PIH (500 μg/ml) was prepared by dissolving an appropriate amount of PIH·2HCl in pure water. The calibration curve was made using standard solutions of six different concentrations of PIH (0.25, 0.5, 1, 2, 5 and 10 μg/ml). The standard solutions were prepared by diluting the stock solution with the mixture of methanol–phosphate buffer [0.1 M $(NH_4)_2HPO_4$, pH 7.0] in a ratio 1:1. The peak area ratios (detected at 297 nm) were plotted against the corresponding concentrations. The accuracy was evaluated as a percentage of recovery of the analyses of the spiked samples at three concentration levels. The precision was expressed as a R.S.D. of the analyses of these spiked samples.

2.2.2. Stability of PIH in aqueous solutions of different pH and in the pharmaceutical co-solvents

The hydrolytic stability of PIH (at the concentration of 20 μg/ml) was studied in the following media: phosphate buffers of pH 2.0, 7.0 and 9.0; 0.3% KOH (pH 12.0); pure water and solutions of pharmaceutical co-solvents, viz. 10% Cremophor EL; 30% PEG-300. The following buffers were used in these experiments: 0.1 M NaH_2PO_4, adjusted using 10% phosphoric acid to pH 2.0; 0.1 M $(NH_4)_2HPO_4$, adjusted using 10% phosphoric acid to pH 7.0; 0.1 M $(NH_4)_2HPO_4$, adjusted using 1 M NaOH to pH 9.0. The solutions of co-solvents were prepared by dilution of appropriate amount of Cremophor EL and PEG-300 with the neutral phosphate buffer (pH 7.0). The tested solutions were prepared as follows: 0.4 ml of the standard solution were added into the 10 ml volumetric tubes and filled up with a buffer, 0.3% KOH, pure water, 10% Cremophor EL or 30% PEG-300. The tested solutions were maintained either at laboratory temperature (25 °C) or in water bath at 40 °C. At 0, 15, 60, 120, 180 and 240 min, 1 ml of each tested solution was mixed with 4 ml of the mixture of methanol–phosphate buffer (pH 7.0) in a ratio 1:1 and injected onto the column in triplicate. Each experiment was performed in duplicate and the results were expressed as means. Control samples consisting of 1 ml of the appropriate degradation medium and 4 ml of the mixture of methanol–phosphate buffer [0.1 M $(NH_4)_2HPO_4$, pH 7.0] in a ratio of 1:1 were injected onto the column before every single analysis. All samples were analyzed employing isocratic method (method I). In order to determine the relative rate of hydrolytic decomposition of the chelator, the logarithm of remaining concentration (log *C*) was plotted versus time. The linearity of the dependence was investigated using the linear regression. The half-life values ($t_{1/2}$) of the hydrolyses were calculated from the slope of the kinetic curves. The samples from the end of each study were also analyzed employing the gradient HPLC method (method II).

2.2.3. Oxidative stability

In order to reveal both the susceptibility of the chelator to oxidative decomposition and the possible oxidative degradation products, 0.4 ml of the standard solution of PIH were added into the volumetric tubes and filled up with either 3% (v/v) or 30% (v/v) hydrogen peroxide. The hydrogen peroxide (3%) was prepared by dilution of the concentrated hydrogen peroxide with the phosphate buffer (pH 7.0). The tested solutions were maintained at laboratory temperature (25 °C) or in a water bath at 40 °C. After 4 h, 1 ml of each tested solution was mixed with the mixture of methanol–phosphate buffer (0.1 M $(NH_4)_2HPO_4$, pH 7.0) in a ratio 1:1 and injected onto the column. The samples were analyzed using methods I and II.

2.2.4. Photostability and thermal stability (dry and wet heat)

The solid substance of PIH·2HCl was outlaid uniformly under the UV lamp (set at 254 nm) in the laboratory (25 °C, 60% RH) or maintained as a thin film in the oven set at 80 °C. In order to simulate the wet heat conditions, the substance was moisturized with pure water (10% of the substance weight). At the time period of 3, 18, 26 and 33 h 10 mg of substance were dissolved in water, diluted with the mixture of methanol–phosphate buffer [0.1 M $(NH_4)_2$ HPO_4, pH 7.0] in a ratio 1:1 and injected onto the column. The samples were analyzed employing method I.

3. Results and discussion

3.1. Calibration curve, precision and accuracy—methods I and II

The correlation coefficients $r = 0.9996$ and 0.9989 indicated the linearity of the isocratic and the gradient method,

Table 1
Intra- and inter-day accuracy and precision of the method isocratic method (method I)

Concentration added (μg/ml)	Intra-day ($n = 5$)		Inter-day ($n = 3$)	
	Concentration found (μg/ml) ± S.D.; R.S.D.	Recovery (%)	Concentration found (μg/ml) ± S.D.; R.S.D.	Recovery (%)
8.00	8.01 ± 0.10; 1.25	100.17	8.09 ± 0.11; 1.38	101.10
1.17	1.18 ± 0.01; 0.99	100.45	1.16 ± 0.01; 1.23	99.93
0.50	0.51 ± 0.17; 2.83	101.11	0.50 ± 0.01; 2.20	100.32

Table 2
Intra- and inter-day accuracy and precision of the gradient method (method II)

Concentration added (μg/ml)	Intra-day ($n = 5$)		Inter-day ($n = 3$)	
	Concentration found (μg/ml) ± S.D.; R.S.D.	Recovery (%)	Concentration found (μg/ml) ± S.D.; R.S.D.	Recovery (%)
10.02	10.60 ± 0.18; 1.66	103.97	10.35 ± 0.27; 2.60	103.19
1.28	1.27 ± 0.01; 1.11	99.90	1.26 ± 0.01; 1.30	99.72
0.61	0.61 ± 0.01; 1.01	99.07	0.60 ± 0.01; 1.96	98.99

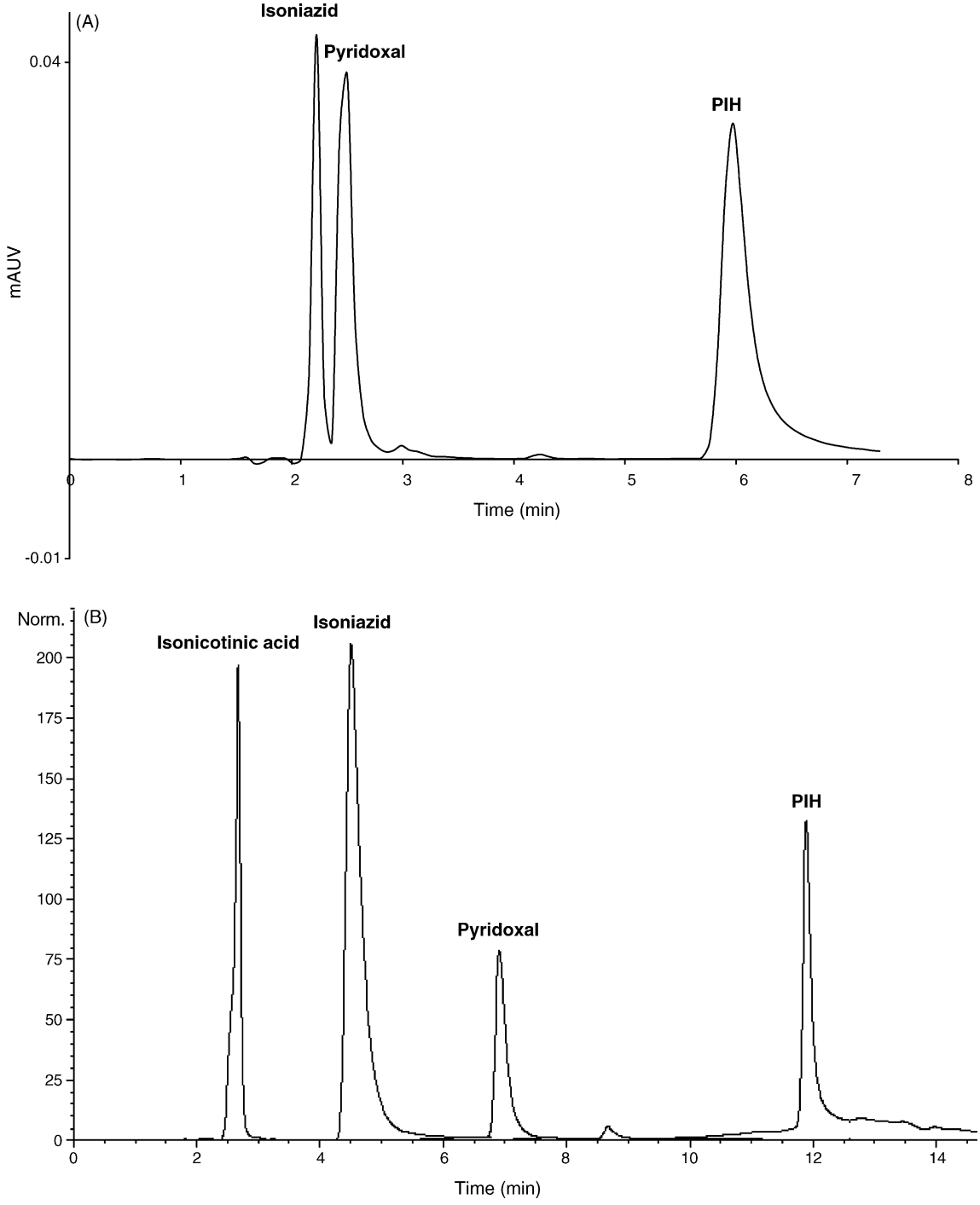

Fig. 2. Chromatograms of separations of PIH from pyridoxal and isoniazid using (A) the isocratic mode and (B) gradient mode of the analyses. The chromatograms were detected at 254 nm.

respectively. The results of the methods' accuracy and precision are shown in the Tables 1 and 2. Fig. 2 shows the chromatograms of the separation of PIH and its degradation products (pyridoxal and isoniazid) employing the isocratic and gradient chromatographic methods.

3.2. The stability of PIH in aqueous solutions of different pH and in the selected pharmaceutical co-solvents

Since hydrazone bond is known to be sensitive to both acid and alkaline hydrolysis, wide range of pH was chosen on purpose to cover acid, neutral as well as alkaline conditions and to allow the comparison among these conditions. The susceptibility of PIH to hydrolytic decomposition was determined as a decrease of the concentration of chelator during the time course of the experiment. Relatively fast degradation of PIH was observed in the acid medium (phosphate buffer, pH 2.0). At the laboratory temperature, 44% of the initial amount of PIH decomposed to isoniazid and pyridoxal within 4 h. The acid hydrolysis was markedly accelerated with the elevated temperature, where 89% of the initial amount of the chelator decomposed during the same time period. The decrease of the concentration of PIH was accompanied with the increase of the peak areas of both degradation products (pyridoxal and isoniazid). In order to determine the rate of the hydrolytic reactions (at both laboratory temperature and 40 °C) the log *C* were plotted versus time. The linear behavior of these plots indicated the pseudo-first order reaction rates (correlation coefficients $r = 0.985$ and 0.989 for laboratory and elevated temperature, respectively; Fig. 3).

The degradation behavior was also observed in 20 μg/ml solution of PIH·2HCl in pure water. The pH of this solution was 3.9. Interestingly, the hydrolysis at the laboratory temperature did not follow the pseudo-first order rate. The concentration of PIH had reached 82% of initial concentration at 120th min of experiment and did not decrease further. On the contrary, the hydrolysis of PIH in the same solution at elevated temperature (40 °C) followed the pseudo-first order degradation ($r = 0.990$) (Fig. 3).

In neutral solution (phosphate buffer, pH 7.0) relatively slow hydrolysis of PIH was observed. At the laboratory temperature 10% of PIH decomposed during the time course of this study (240 min). The elevated temperature accelerated the hydrolytic reaction (26% of initial amount of the substance decomposed). Both the hydrolysis at the laboratory

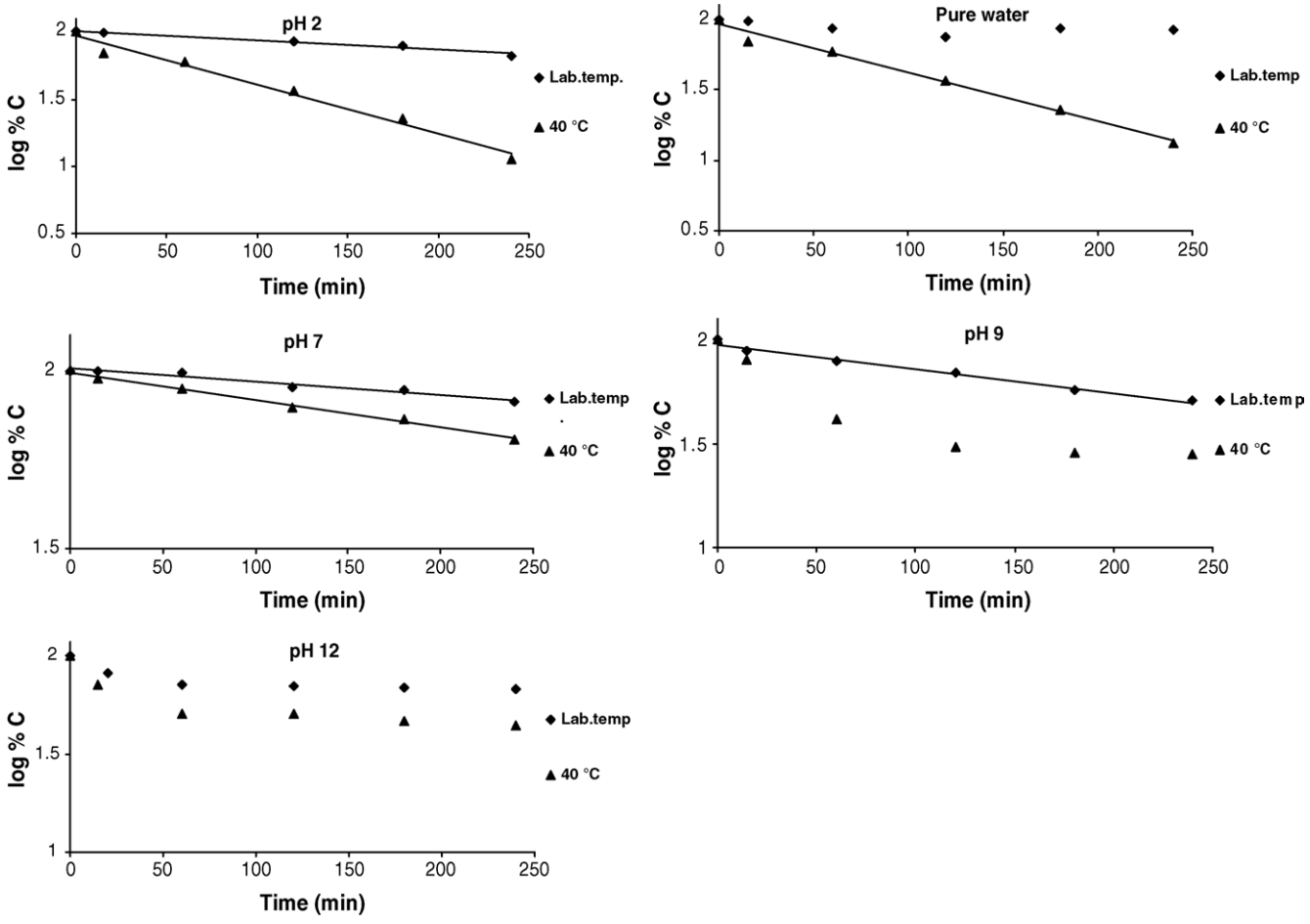

Fig. 3. The kinetic plots (log *C* vs. time) of the hydrolyses of PIH in aqueous solutions of different pH at laboratory (25 °C) and elevated (40 °C) temperatures.

and the elevated temperature followed the pseudo-first order rates (the correlation coefficients $r = 0.974$ and 0.990, respectively; Fig. 3).

At the laboratory temperature, hydrolysis of PIH in alkaline phosphate buffer (pH 9.0) followed the pseudo-first order reaction rate ($r = 0.999$). On the contrary, a non-linear profile of the dependence log C versus time was obtained in the experiment carried out at the elevated temperature. The initial rapid decrease of the concentration of PIH (obtained during the first 60 min of the experiment) was followed by the more gradual fall by the end of the study. Under the conditions of alkaline hydrolysis, 49 and 72% of the initial amount of PIH decomposed at the laboratory and elevated temperature, respectively.

The stability of PIH was also studied in the strong alkaline solution (pH 12.0). In this medium, hydrolysis of the chelator did not follow the pseudo-first rate either at laboratory temperature or at 40 °C. The initial fast degradation rate (seen in first 60 min of hydrolysis) was followed by slower one by the end of experiment. 33 and 60% of the initial amount of PIH decomposed in KOH solution (pH 12) at laboratory and elevated temperature, respectively (Fig. 3).

Besides the hydrazone bond, PIH contains also another hydrolytically sensitive bond—hydrazide one. Considering the hydrolytic splitting of this bond, the presence of isonicotinic acid on chromatograms would be expectable. Since the isocratic mode of the analysis was unable to separate isonicotinic acid from isoniazid, the chromatographic mode was changed to gradient one. The most stressed samples were analyzed employing the gradient method developed for this purpose. Only minor amount of isonicotinic acid (less then 10% of the peak area of isoniazid) was detected. Thus, based on this observation, it was confirmed that pyridoxal and isoniazid should be considered as the main hydrolytic degradation products of PIH, whereas the isonicotinic acid is only the minor one. The results of this study indicate that PIH·2HCl is sensitive to hydrolytic decomposition in aqueous solutions. The reaction data are summarized in Table 3.

Since considerable rate of hydrolysis could been observed in all tested media already at the laboratory temperature, the degradation of PIH in aqueous solution should be taken into account in further investigations employing this chelator. At the laboratory temperature, hydrolytic reaction proceeds faster in the alkaline solution than in the appropriate acidic one. This observation is in agreement with the outcomes of the study performed by Richardson et al. [11], although the hydrolytic conditions employed are not completely identical. Interestingly, the hydrolysis rate in the solution of pH 12 was determined to be lower than the decomposition in the phosphate buffer of pH 9 (at both laboratory and elevated temperature). However, the hydrolysis in pH 12 did not follow the pseudo-first order kinetic either at laboratory temperature or at 40 °C. This observation could be likely explained by the possible backward reaction (condensation), which can take place in the alkaline solution. The significant condensation of pyridoxal and isoniazid in the solution of pH 12 (resulting into the formation of hydrazone) was also reported [11].

At laboratory temperature, the alkaline hydrolysis (pH 9) of PIH was faster than the acid ones (pH 2 and 3.9). Nevertheless, the elevated temperature affected the acid hydrolysis more significantly. At 40 °C, the PIH concentrations in acid conditions were at the end of the experiment even lower than those obtained in alkaline ones. The less pronounced effect of temperature on alkaline hydrolysis might be associated with the acceleration of the backward reactions in these conditions.

Since acid hydrolysis of PIH (pH 2) followed pseudo-first order rate (at both temperatures), the backward reactions played a minimum role. By contrast, the hydrolysis in pure water (pH 3.9) followed the pseudo-first order rate only when performed at elevated temperature, which leads us to an assumption that at the laboratory temperature the backward reaction might take place as well.

The slowest, however still important, degradation was observed in the neutral phosphate buffer. The calculated half-life of neutral hydrolysis is different from that obtained in HEPES buffer (spectrophotometric study) [11]. Nevertheless the degradation rate at elevated temperature is comparable with degradation of PIH in phosphate buffer saline, pH 7.5 at 37 °C [12]. Fig. 4 shows the representative chromatogram of analyses of stressed samples.

Table 3
The summary of kinetic characteristics of hydrolyses of PIH in aqueous media and two co-solvents

Medium	Laboratory temperature			Elevated temperature (40 °C)		
	Portion of decomposed PIH at the end of experiment (%)	k (min^{-1})	$t_{1/2}$ (h)	Portion of decomposed PIH at the end of experiment (%)	k (min^{-1})	$t_{1/2}$ (h)
Phosphate buffer pH 2	44	0.001642	7.0	89	0.008890	1.30
Pure water pH 3.9	16	–[a]	–[a]	87	0.008613	1.34
Phosphate buffer pH 7	10	0.000387	29.8	26	0.001214	9.50
Phosphate buffer pH 9	49	0.00269	4.3	72	–[a]	–[a]
0.3% KOH	33	–[a]	–[a]	60	–[a]	–[a]
30% PEG-300	9	0.000307	37.6	18	0.000907	12.7
10% Cremophor EL	10	0.000389	29.8	15	0.000636	18.2

The rates of hydrolyses are expressed as both the percentage of PIH decomposed in the time of the experiments and the half-lives.
[a] Due to the non-linearity of the plot log C vs. time, the k and $t_{1/2}$ were not calculated.

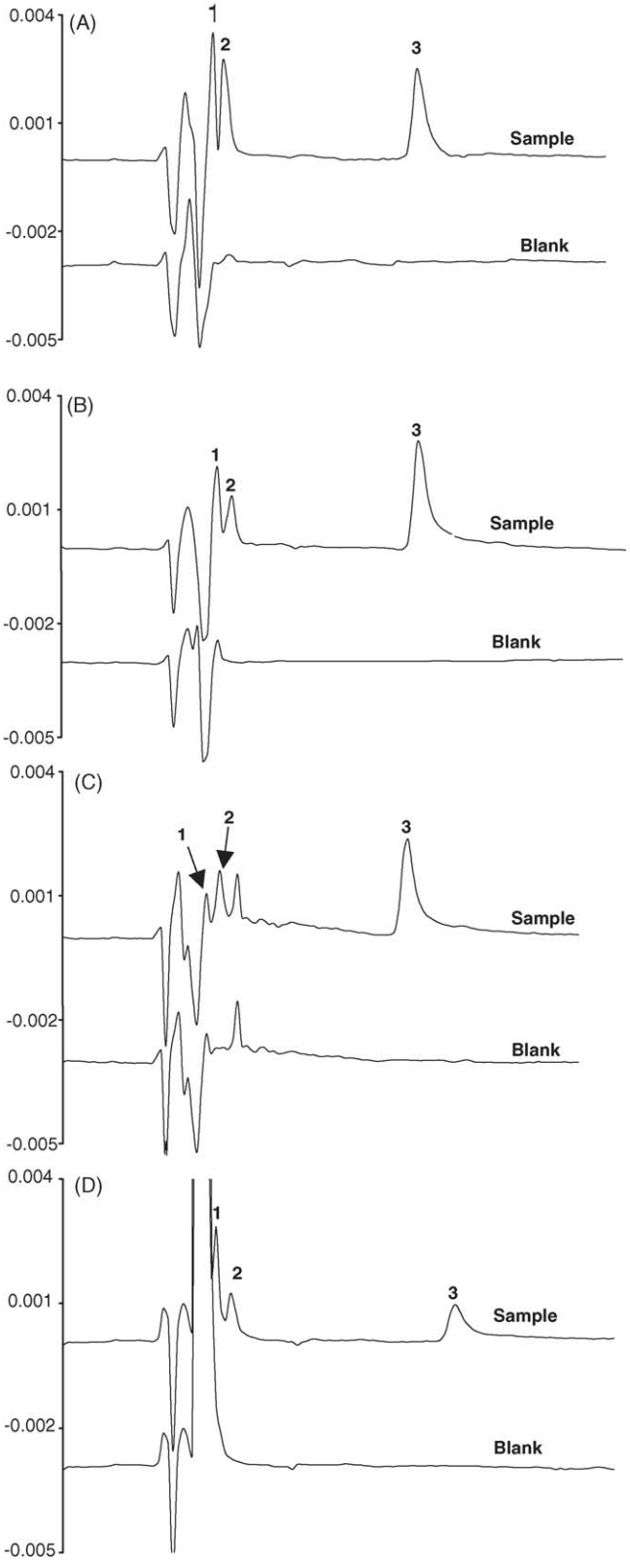

Fig. 4. The representative chromatograms of the samples stressed in the different media (40 °C) for 4 h. The samples were analyzed employing method I. (A) Phosphate buffer, (B) 10% Cremophor EL, (C) 30% PEG and (D) 30% H_2O_2. (1) Isoniazid; (2) pyridoxal; (3) PIH.

3.2.1. The stability of PIH in the pharmaceutical co-solvents

The stability of PIH was also defined in the selected pharmaceutical co-solvents (10% Cremophor EL and 30% PEG-300). Ten percent aqueous solution of Cremophor EL has been already employed as a vehicle for PIH in the preclinical studies [5,7,8]. PEG-300 was chosen as another suitable pharmaceutical co-solvent. The experiment revealed the similar pattern degradation of PIH in both co-solvents. 9 and 18% of the initial amount of the substance decomposed in 30% PEG-300 along the course of this experiment performed at the laboratory and elevated temperature, respectively. The correlation coefficients obtained from the plots log *C* versus time ($r = 0.995$ and 0.977) indicated the pseudo-first order reaction rate at both temperatures. In the case of 10% Cremophor EL, 10% of the initial amount of the substance was decomposed at the laboratory temperature and 15% at the elevated temperature. The correlation coefficients for the laboratory and higher temperature were calculated to be 0.968 and 0.987, respectively. Furthermore, the rates of degradation of PIH in both co-solvents were comparable with the degradation rate of hydrolysis in neutral phosphate buffer. Fig. 5 shows corresponding kinetic plots.

Therefore, it can be concluded, that the tested pharmaceutical co-solvents had no distinct effect on the stability of the chelator. No additional peak, which might indicate any different degradation product, could be detected. Fig. 4 shows the typical chromatograms of the analyses. Thus, the hydrolysis was the most important decomposition mechanism for both co-solvent solutions. Interestingly, a thiosemicarbazone iron chelator Triapine was reported to be stable in the infusion formulation (25 °C, pH 3–4) consisting of the co-solvents (PEG, ethanol) and saline more than 8 h. Based on our experiments, the aroylhydrazone chelator PIH seems to be less stable than Triapine [14].

3.3. Oxidative stability

In 3% H_2O_2, only 10 and 23% of the initial amount of the chelator decomposed at laboratory and elevated temperature, respectively. The concentrated (30%) H_2O_2 was used to accelerate the potential oxidative process. While 13% of the initial amount of PIH decomposed in the sample exposed to the 30% H_2O_2 for 4 h at laboratory temperature (25 °C), 60% decomposed at the same solution maintained at 40 °C. The degradation of PIH in 3% H_2O_2 (both temperatures) as well as in 30% H_2O_2 (laboratory temperature) resembles the results obtained in the experiments employing neutral phosphate buffer. Interestingly, elevated temperature substantially accelerated the degradation of the chelator especially in 30% H_2O_2. However, no special peak of a putative oxidative degradation product was detected in either chromatogram. The results presented above suggest that PIH is more sensitive to hydrolysis than to oxidation. The representative chromatogram of analysis of sample exposed to hydrogen peroxide is shown in Fig. 4.

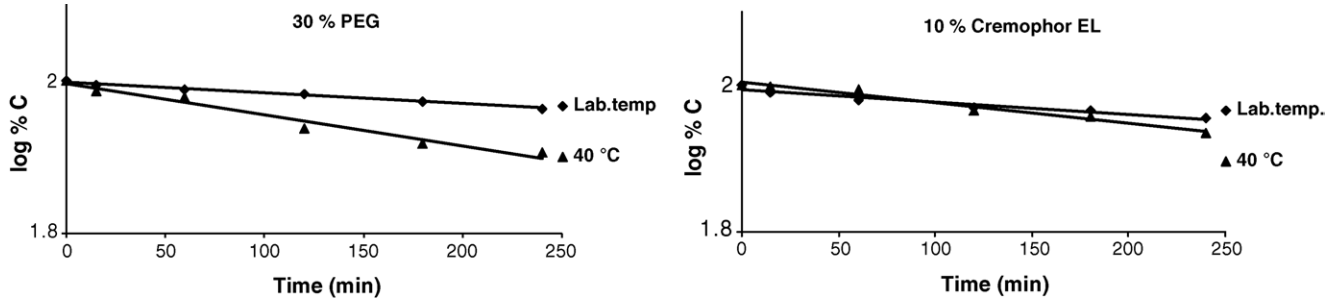

Fig. 5. The kinetic plots of hydrolyses of PIH in selected pharmaceutical co-solvents at laboratory (25 °C) and elevated (40 °C) temperature.

3.4. Photolytic and thermal stability

The solid substance of PIH·2HCl was stable when exposed to UV and heat (wet and dry) for 33 h. However, the change of the color of the substance (from yellow to slightly orange) was observed at 3 h of exposition to wet heat. The change could be most likely attributed to the formation of a hydrate [4].

4. Conclusion

In this study, for the first time, HPLC analytical technique was employed for the stability evaluation of water-soluble salt of PIH. The hydrolysis of this iron chelator was studied in the aqueous solutions of different pH (from 2.0 to 12.0) and in the selected pharmaceutical co-solvents (30% PEG-300 and 10% Cremophor EL) at both the laboratory (25 °C) and elevated temperature (40 °C). Furthermore, the susceptibility of PIH·2HCl to decomposition under the oxidative, photolytic, dry and wet heat conditions was studied. PIH was shown to be sensitive to the hydrolysis in the aqueous media and the observed degradation should be considered in further studies. Pyridoxal and isoniazid were identified as main degradation products, whereas isonicotinic acid is only a minor one. The degradation behavior of the chelator in hydrogen peroxide resembled that those seen in the neutral phosphate buffer (with exception of 30% H_2O_2 at 40 °C). No special oxidative product was detected. The solid substance of PIH·2HCl was stable under the influence of other external conditions (UV, heat). Information presented herein can be used for the definition of optimal handling and storage conditions and use of the pharmaceutical excipients.

Acknowledgement

This study was supported by the Research Project MSMT 0021620822 from The Ministry of Education, Young and Sport.

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