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The use of microcalorimetry and HPLC for the determination of degradation kinetics and thermodynamic parameters of Perindopril Erbumine in aqueous solutions

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Abstract

Perindopril Erbumine (PER) is one of the newly used angiotensin-converting enzyme inhibitors (ACE inhibitors) and is used for the treatment of patients with hypertension and symptomatic heart failure. It has two main degradation pathways, i.e. the degradation by hydrolysis and the degradation by cyclization. An isothermal heat conduction microcalorimetry (MC) and high pressure liquid chromatography (HPLC) were used for the characterization of aqueous solutions of PER and its stability properties. The rates of heat evolved during degradation of perindopril were measured by MC as a function of temperature and pH and from these data rate constant and change in enthalpy of the reactions were determined. With the HPLC method the concentration of perindopril and its degradation products were measured as a function of time in aqueous solutions of different pH that were stored at different temperatures. We demonstrated that reactions of degradation of perindopril at observed conditions follow the first order kinetics. The Arrhenius equation for each pH was determined. At pH 6.8 only one degradation pathway is present, i.e. the degradation by hydrolysis. Degradation constants for this pathway calculated from MC data are in good agreement with those obtained from HPLC. MC as a non-specific technique was shown to be useful in studies of PER when one reaction was present in the sample and also when more chemical and physical processes were simultaneously running.

Keywords: Isothermal microcalorimetry; Chromatography; Perindopril; Solution stability; Degradation kinetic

1. Introduction

Stability studies are an integral part of the drug development program and have a very important role in the registration documentation for each individual pharmaceutical product and drug substance. According to International Conference on harmonization (ICH) a note for guidance on stability testing should be followed: stability testing of new drug substances and products (CPMP/ICH/2736/99), long term and accelerated stability studies have to be carried out to prove the stability of the marketed product and to ensure its shelf life.

Finished product formulations are often manufactured using granulation procedure where substance is dissolved in buffer at elevated temperatures and than sprayed to other tablet excipients to produce a granulate. Dissolving can increase the degradation of the substance and knowing the critical factors that can influence the stability of the active substance in solutions, such as temperature and pH can therefore be of high importance in pharmaceutical development.

Perindopril Erbumine (PER), angiotensin-converting enzyme inhibitor (ACE inhibitor) is used in the treatment of hypertension. It is a *tert*-butylamine salt of 1-{(2*S*)-2-[(1*S*)-1-carbethoxybutyl)amino]-1-oxopropyl}-(2*S*,3*aS*,7*aS*)-perhydroindole-2-carboxilic acid.

According to perindopril monographs in European Pharmacopoeia and findings described in this report its main degradation products are perindoprilate (PAT) and diketopiperazine (DKP). The main degradation paths are presented in Fig. 1.

The main aim of this study was to present kinetic and thermodynamic data for the degradation of PER as a new substance

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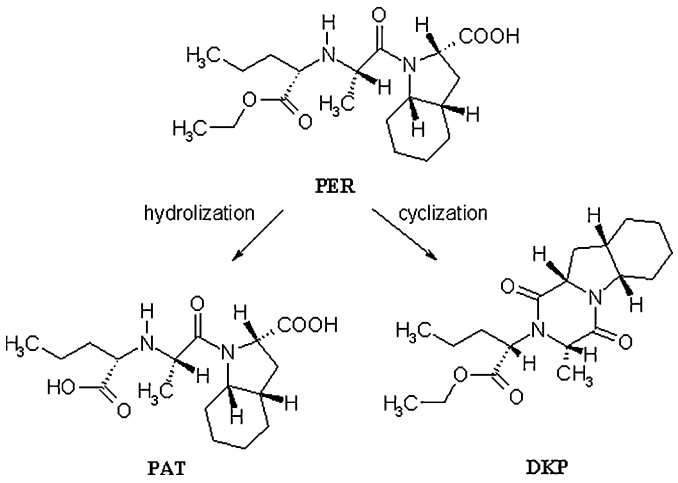

Fig. 1. Degradation of PER through the two main degradation paths.

since no data on kinetics of degradation could be found in the published literature. Our goal was to identify different degradation paths in solutions with different pH values and also to determine rate constants for individual degradation paths. We focused on solutions with pH 2.0 and 6.8 because besides obtaining degradation information in different solution we wanted to mimic in vivo conditions that a drug is exposed to when administered.

Isothermal MC is a non-specific thermo-analytical method that is used by pharmaceutical industry, especially to determine the stability, compatibility and amorphicity properties. Isothermal MC can also be used to determine thermodynamic and kinetic parameters of the long term reactions (Gaisford and Buckton, 2001; Beezer et al., 1999; Buckton, 1995; Wilson et al., 1995a,b; Buckton et al., 1991). Several authors have shown the applicability of this method for studying various aspects of stability (Simončič et al., 2007; Roškar and Kmetec, 2005; Chadha et al., 2003; Schmitt et al., 2001; Zaman et al., 2001a,b; Jakobsen et al., 1997; Wilson et al., 1995a,b; Buckton, 1995; Pikal and Dellerman, 1989). The present study was undertaken to explore the potential of isothermal MC together with the HPLC for the determination of the stability of PER at different pH values under conditions that can arise during production of drug product. The kinetic and thermodynamic parameters of the PER degradation were determined.

2. Materials and methods

2.1. Materials

PER was Ph. Eur. grade, Batch No. 06PR040300, produced by Krka, d.d., Novo mesto.

Studied samples were prepared as aqueous solutions in pH 2.0 and pH 6.8 phosphate buffers. Concentration of samples studied by the HPLC technique was 1 mg of PER/ml (2.26 mmol/l) and for MC studies it was 100 mg of PER/ml (226 mmol/l). Higher concentrations were used in MC studies with the aim to get better responses.

Phosphate buffers with pH 2.0 or 6.8 were prepared by dissolving 136 mg of potassium dihydrogenphosphate in 800 ml of water, adjusting the pH to 2.0 or 6.8 with phosphoric acid and diluting with water to 1000 ml. Buffer capacity was not high enough for the sample with a higher concentration (100 mg of PER/ml; 226 mmol/l) at pH 2.0 and therefore, phosphate buffer with pH 2.0 was prepared at a 1000 times higher concentration.

For HPLC assays the following reagents were used: acetonitrile (Ph. Eur., quality for liquid chromatography) and buffer solution with pH 2.0 (prepared by weighting 0.92 g of sodium heptanesulphonate (p.a.) into 1000 ml volumetric flask with the addition of 1 ml of triethylamine (p.a.), diluting to volume with water and adjusting the pH with perchloric acid (p.a.)).

2.2. Methods

A Micro DSC III (Setaram) calorimeter, operating in the isothermal mode at various temperatures was used together with Hastelloy closed batch vessels. Temperature was maintained with a precision $\pm 1 \times 10^{-4}$ °C. A calorimeter measures the heat conduction out of the sample cell to a heat sink so that the output presents exothermic processes as positive heat flows and endothermic processes as negative heat flows.

The calorimeter was calibrated using the Joule effect method (as described in Micro DSC III User Manual by Setaram) in the range from 20 to 80 °C before experiment set.

For the experiments 850 μl of sample was placed into the sample vessel and the same volume of the buffer was placed into the reference vessel. The thermograms of degradation were monitored at four temperatures (40, 50, 70 and 80 °C) for at least 45 h.

We also monitored the thermograms with the buffer in the sample cell and in the reference cell. The subtraction of this buffer curve from the PER degradation curves was our final thermogram. This was necessary in order to eliminate any instrumental differences arising from the fact that both calorimeter cells are not completely the same. All of the observed effects are thus a consequence solely of the degradation of PER. Studies in the microcalorimeter were performed in three repetitions and the results obtained from separate measurements did not differ significantly. The reported values are average ones.

HPLC area percent method was used for the determination of the contents of PER, PAT and DKP. HPLC instrument (Hewlett Packart 1100 Series) with a variable UV detector and column thermostat was used. Analyses were performed under the following conditions: Hypersil ODS, 5 μm particles, 250 mm × 4 mm i.d. column at temperature 70 °C and mobile phase buffer (pH 2.0) and acetonitrile. The flow was gradient. UV detection was performed at 215 nm.

Studied samples were prepared as solutions of PER in buffers with pH 2.0 or 6.8. Samples were stored at elevated temperatures (40, 50 and 80 °C) for a prescribed time period (1, 2, 4, 8 and 24 h) and afterwards the HPLC was used to determine the content of PER, PAT and DKP present in the sample.