Talanta 82 (2010) 1528–1537

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Determination of pK_a values of some antihypertensive drugs by liquid chromatography and simultaneous assay of lercanidipine and enalapril in their binary mixtures

Mehmet Gumustas[a,b], Senem Şanlı[a], Nurullah Şanlı[a], Sibel A. Ozkan[b,*]

[a] Department of Chemistry, Faculty of Science and Arts, Hitit University, Çorum, Turkey
[b] Department of Analytical Chemistry, Faculty of Pharmacy, Ankara University, Tandogan, Ankara, Turkey

ARTICLE INFO

Article history:
Received 14 May 2010
Received in revised form 8 July 2010
Accepted 18 July 2010
Available online 23 July 2010

Keywords:
Enalapril
Lercanidipine
Ramipril
pK_a
Liquid chromatography
Determination

ABSTRACT

In this study, pK_a values were determined using the dependence of the retention factor on the pH of the mobile phase for three ionizable substances, namely, enalapril, lercanidipine and ramipril (IS). The effect of the mobile phase composition on the ionization constant was studied by measuring the pK_a at different methanol–water mixtures, ranging between 50 and 65% (v/v), using LC–DAD method. Two simple, accurate, precise and fully validated analytical methods for the simultaneous determination of enalapril and lercanidipine in combined dosage forms have been developed. Separation was performed on an X-Terra RP-18 column (250 mm × 4.60 mm ID × 5 μm) at 40 °C with the mobile phase of methanol–water 55:45 (v/v) adjusted to pH 2.7 with 15 mM orthophosphoric acid. Isocratic elution was performed in less than 12 min with a flow rate of 1.2 mL min^{-1}. Good sensitivity for the analytes was observed with DAD detection. The LC method allowed quantitation over the 0.50–20.00 μg mL^{-1} range for enalapril and lercanidipine. The second method depends on first derivative of the ratio-spectra by measurements of the amplitudes at 219.7 nm for enalapril and 233.0 nm for lercanidipine. Calibration graphs were established for 1–20 μg mL^{-1} for enalapril and 1–16 μg mL^{-1} lercanidipine, using first derivative of the ratio spectrophotometric method. Both methods have been extensively validated. These methods allow a number of cost and time saving benefits. The described methods can be readily utilized for analysis of pharmaceutical formulations. The methods have been applied, without any interference from excipients, for the simultaneous determination of these compounds in tablets. There was no significant difference between the performance of the proposed methods regarding the mean values and standard deviations.

1. Introduction

The angiotensin-converting enzyme inhibitory drugs (ACE inhibitors) are widely used for the treatment of many cardiovascular conditions including mild to moderate hypertension and heart failure, either alone or in conjunction with other drugs [1]. Enalapril (ENA), [(2*S*)-1-[(2*S*)-2-[[(1*S*)-1-(ethoxycarbonyl)-3-phenylpropyl] amino] propanoyl]pyrrolidine-2-carboxylic acid (*Z*)-butenedioate] an ACE inhibitor, is a pro-drug. It is converted to its active metabolite, di-acid enalaprilat [2,3] and used as its maleate salt. In ENA one carboxylic group is esterified, while the second may be engaged in zwitterionic structure with the protonated basic nitrogen, depending on pH (Fig. 1). In this aspect, accurate knowledge of acidic and basic pK_a is required to assess the molecular species/pH profile.

Lercanidipine hydrochloride (LER) [2-[(3,3-diphenylpropyl) methylamine]-1,1-dimethylethyl methyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5 pyridine carboxylic ester hydrochloride] is a dihydropyridine calcium channel blocker (Ca antagonist) with actions similar to those of nifedipine. LER is member of new third generation Ca antagonist used in treatment of hypertension because of its selectivity and specificity on the smooth vascular cells [4,5]. It is administered orally as tablet dosage form. The chemical structure of LER is characterized by the presence of a side chain containing a 3,3-diphenylpropylmethylamine-2-methyl-2-propyl group that was introduced to improve the lipophilic properties and the activity duration of the drug. From a physico-chemical point of view, LER is slightly soluble in water, but it is more soluble in some widely used solvents as well as ethanol and methanol (MeOH), or mixture water–organic solvents.

Ramipril (RAM) (2-[*N*-[(*S*)-l-ethoxycarbonyl-3-phenylpropyl-lalanyl]-(1*S*,3*S*,5*S*)-2-azabicyclo [3-3-0]-octane-3-carboxylic acid, see Fig. 1) is also an orally active inhibitor of ACE, which is a pro-

* Corresponding author. Tel.: +90 312 223 82 43; fax: +90 312 223 82 43.
E-mail address: ozkan@pharmacy.ankara.edu.tr (S.A. Ozkan).

0039-9140/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2010.07.037

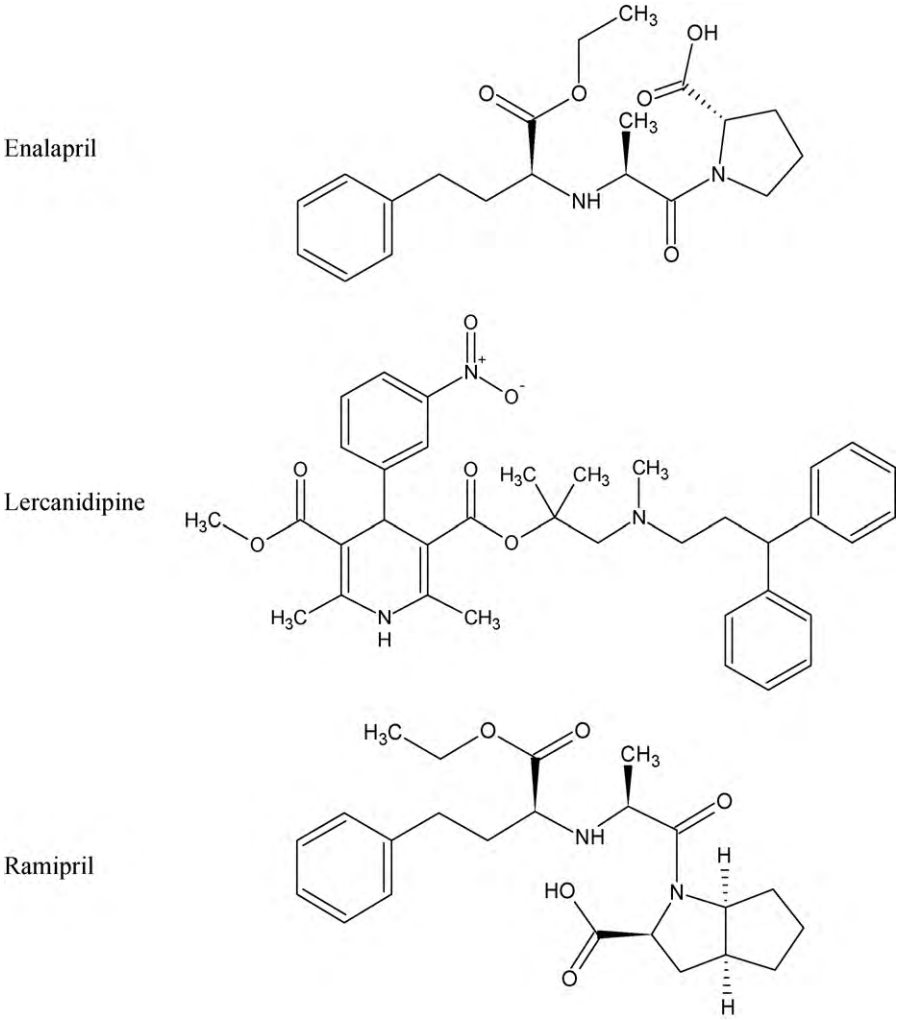

Fig. 1. Structures of compounds studied.

drug used in the treatment of all forms of hypertension, heart failure and following myocardial infarction to improve survival in patients with clinical evidence of heart failure [1–4]. In this study, RAM is used as an internal standard during separation studies because of its shorter elution time and similar structure.

The ionization constant is an important physico-chemical parameter of a drug and the knowledge of this parameter is of fundamental importance in a wide range of applications and research areas. The chromatographic retention and electrophoretic behavior of ionizable compounds strongly depend on the pK_a of the compound and the mobile phase pH. A satisfactory knowledge of the acid–base behavior of substances in hydro-organic media such as methanol–water is therefore essential to predict the influence of pH on selectivity and on retention in LC and also to optimize analytical procedures for the separation of ionizable compounds by different techniques [6,7]. Although methanol–water mobile phases have been used in RP-LC separation procedures, the pK_a values of ENA, LER and RAM have not yet been determined in methanol–water binary mixtures.

A methodological approach of choice is pK_a estimation by isocratic reversed-phase LC [8,9]. In fact, as most of the organic compounds tend to be poorly soluble in water, the classical potentiometric techniques for studying acid–base equilibrium are not practical. Another advantage of the LC method is that it only requires a small amount of sample. The determination of pK_a values by RP-LC is based on the relationship between the retention factors and the pH values of the mobile phase [10,11]. This procedure is limited by working pH range of the LC column, the optimum conditions being when the pK_a corresponds to the equilibrium between a neutral species and a charged species (this is, $H_2A^+ \leftrightarrow HA$, $HA \leftrightarrow A^-$, or $B \leftrightarrow HB^+$).

Several analytical methods have been reported in the literature for the analysis of ENA and LER, individually, in pharmaceutical dosage forms. The techniques include spectrophotometry [12], atomic absorption spectroscopy [13] and the number of high performance liquid chromatographic (LC) methods have also been reported for these drugs using ultraviolet (UV) as well as mass (MS) detectors [14–18]. Some of the reported methods require solid-phase extraction or expensive equipments, which are not economically feasible for routine use in pharmacokinetic and pharmaceutical studies, where numerous samples should be analyzed.

Owing to the widespread use of LC in routine analysis, it is important that specific LC methods are developed and that these are thoroughly validated [19–22]. LC–UV detection offers important advantages, such as rapid set-up of instrumentation, versatility and low cost, and has proved to be a valuable method in the quality control of drug compounds.

The ratio-spectra derivative spectrophotometric method permits the determination of a component in their binary mixtures at the wavelengths corresponding to a maximum or minimum and also the use of the peak-to-peak measurements between consecutive maximum and minimum. Moreover, the presence of a number