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Analysis of ACE inhibitors in pharmaceutical dosage forms by derivative UV spectroscopy and liquid chromatography (HPLC)

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Abstract

Derivative UV spectroscopy and high performance liquid chromatography (HPLC) were applied to the determination of angiotensin-converting enzyme (ACE) inhibitors in their pharmaceutical dosage forms. For spectrophotometric determinations, the more appropriate derivative order was selected for each drug: ramipril (third-order), benazepril (second-order), enalapril maleate (second-order), lisinopril (first- and second-order) and quinapril (first-order). Reverse phase HPLC procedures (ODS column) were developed able to provide a single, symmetric peak for each drug; mixtures A–B, where A is 20 mM sodium heptansulphonate (pH 2.5) and B is acetonitrile–THF (95:5 v/v), proved to be suitable mobile phases to obtain selective separations of the cited ACE inhibitors. At ambient temperature, a low pH value (2.5) was found to be critical to avoid peak splitting and band broadening. © 1997 Elsevier Science B.V.

Keywords: Derivative spectroscopy; High performance liquid chromatography; ACE inhibitors; Drug analysis; Pharmaceutical dosage forms

1. Introduction

The angiotensin-converting enzyme (ACE) inhibitors are established therapies for the treatment of hypertension and heart failure [1,2]. Further possible roles, such as prevention of heart failure after myocardial infarction, have already been identified. Structurally, some of them are dipeptides such as enalapril (alanil-proline) and lisinopril (lisin-proline), others bear a proline analogous moiety (quinapril and ramipril), while benazepril was derived by molecular modeling studies (Fig. 1).

Their use increased rapidly in the last few years, with a corresponding increase in analytical investigations [3–10]. In fact, the structural features of this class of drugs calls for specific analytical studies aimed to improve their detectability. In particular, the compounds exhibit a weak benzene chromophore and are characterized by low molar absorptivity values [3,5,11]; as a consequence, poor sensitivity can be achieved by conventional UV spectrophotometric methods. Moreover, reversed-phase high performance liquid chromatog-

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raphy (RP-HPLC) of these drugs, containing a proline or proline related residue, may show peak splitting owing to slow *cis-trans* isomerization, caused by hindered rotation around the *N*-substituted peptide bond [6,7].

Therefore, with a view to providing practical methods suitable for reliable quality control of ACE inhibitors, this study was concerned with the following objectives:

(a) application of derivative mode to improve the selectivity and the sensitivity of UV spectrophotometric determinations;

(b) development of a HPLC method able to avoid peak splitting and band broadening;

(c) application of both the spectrophotometric and chromatographic approaches to the analysis of commercial dosage forms (tablets) of ACE inhibitors.

2. Experimental

2.1. Materials

Enalapril maleate (Merck, Italy), ramipril (Ciba-Geigy, Italy), benazapril hydrochloride (Smith-Kline Beecham, Italy), lisinopril bihydrate (Sigma Tau, Italy) and quinapril (Malesci, Italy) were kindly supplied by their manufactures. Sodium heptansulfonate was obtained from Aldrich (Italy) and all the other chemicals were from Carlo Erba reagents (Italy). 20 mM sodium heptansulphonate solutions (pH 2.5 and 6.5) and 0.1 M phosphate buffers (pH 2.8 and 4.5) were prepared according to standard methods, using phosphoric acid or sodium hydroxide to adjust the desired pH values.

2.2. Apparatus

Spectrophotometric analyses were performed on a Jasco Uvidec 610 double beam spectrophotometer using 1 cm quartz cells with a slit width of 2 nm and a scan speed of 100 nm min^{-1}. The $\Delta\lambda$ values and the absorbance scale were selected in accordance with the nature and concentration of the analyte.

The HPLC system comprised a Waters pump and a Jasco Uvidec 100 V detector connected to a HP 3396 series integrator. Manual injections were carried out using a Rheodine model 7125 injector with a 20 μl sample loop; detector: UV 215 nm.

The chromatographic separations were performed on a 5 μm Hypersil ODS column (250 mm × 4.5 mm i.d.), using as mobile phase a mixture A–B, where A is 20 mM sodium heptasulfonate (pH 2.5) and B is acetonitrile–THF (95:5 v/v), at a flow rate of 1 ml min^{-1}. The A–B composition was adjusted as follows: 52:48 v/v (ramipril, quinapril, benazepril) and 63:37 v/v (Lisinopril and enalapril).

2.3. Calibration graphs

Standard solutions of each drug (concentrations shown in Table 1) were prepared in methanol (quinapril), methanol–water (1:1 v/v) (ramipril and benazepril)), 0.1 M phosphate buffer (pH 4.5)–methanol (80:20 v/v) (enalapril maleate) and 0.1 M phosphate buffer (pH 2.8)–methanol (75:25 v/v) (lisinopril).

2.4. Spectrophotometric method

The UV spectra of the drug standard solutions were recorded against the solvent blank using the derivative mode and the selected amplitudes ^1D, ^2D and ^3D (first-, second- and third-order derivative spectra, respectively) were plotted against the corresponding concentration to obtain the calibration graphs (Table 1).

2.5. HPLC method

Standard solutions of each drug (Table 1), containing a fixed concentration of the internal standard, were prepared in acetonitrile–20 mM sodium heptansulfonate (pH 2.5, 1:1 v/v).

A 20 μl volume of each solution was injected in triplicate and the peak height ratios (analyte to internal standard) were plotted against the drug concentration.

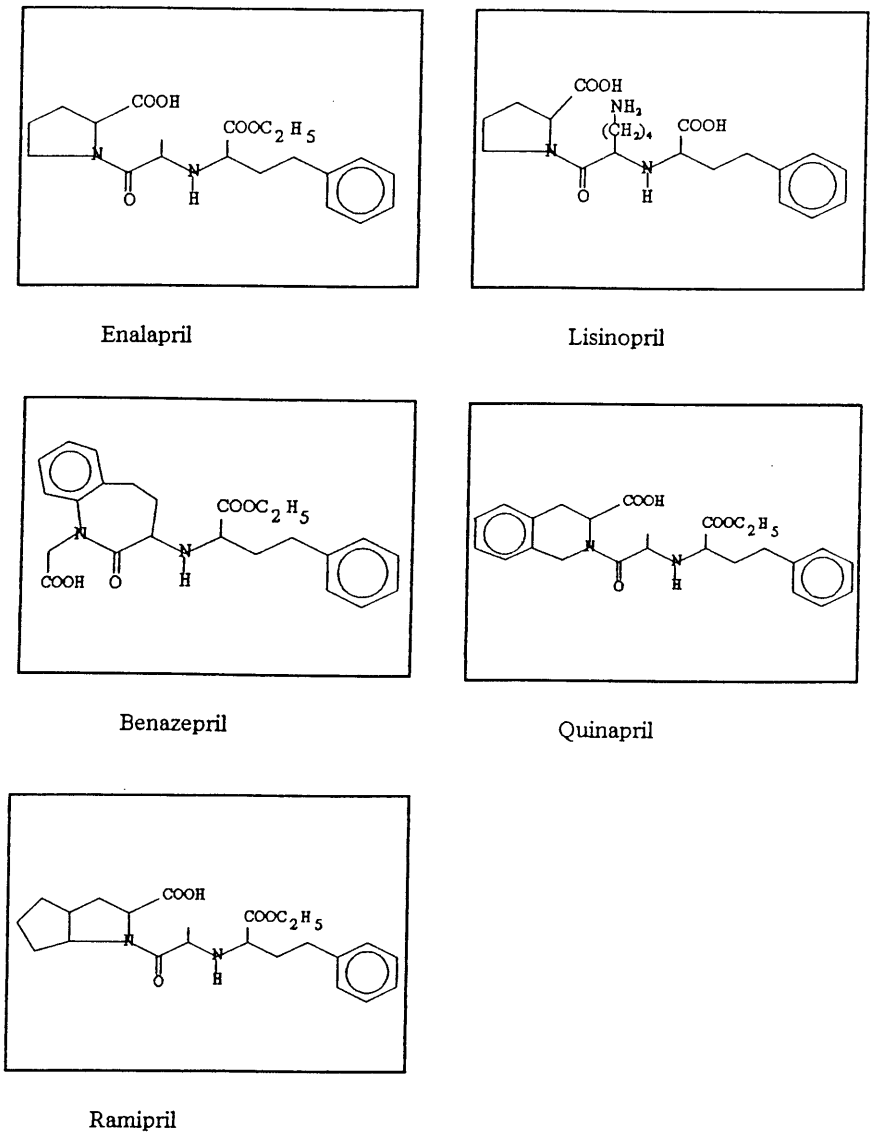

Fig. 1. Structural formulas of ACE inhibitors.

2.6. *Analysis of pharmaceutical formulations*

2.6.1. Sample preparation

A powdered sample of each drug formulation (tablets and capsules) was extracted (15 min, ambient temperature, magnetic stirring) with the same solvent system used for the calibration graphs (spectrophotometric and HPLC methods) to give a final concentration corresponding approximately to the mean value of the calibration range.

2.6.2. Assay procedure

The sample solutions were subjected to the described spectrophotometric and HPLC analysis, and the drug contents in each sample was calculated by comparison with an appropriate standard solution of the drug.

Table 1
Data of the calibration graphs for the derivative spectrophotometric and HPLC analysis of ACE inhibitors

Drug	Method	$\Delta\lambda$	Slope	Intercept	Correlation coefficient	Concentration range (mg ml^{-1})
Spectrophotometry						
Ramipril	$^2D_{222.4-218.0}$	(4)	4.82	$3.95 \cdot 10^{-5}$	0.9990	0.023–0.060
Benazepril	$^2D_{258.8}$	(4)	1.774	0.0010	0.9992	0.010–0.030
	$^2D_{258.8-241.6}$	(4)	4.826	0.0020	0.9990	0.010–0.030
	$^2D_{259.2}$	(6)	3.730	0.0020	0.9994	0.010–0.030
	$^2D_{259.2-241.0}$	(6)	10.222	0.0046	0.9990	0.010–0.030
Enalapril maleate	$^2D_{228.8}$	(4)	1.967	−0.0016	0.9992	0.021–0.071
Lisinopril	$^1D_{268.0}$	(3)	0.146	−0.0016	0.9990	0.604–2.402
	$^1D_{267.2}$	(4)	0.114	−0.0021	0.9992	0.601–2.402
	$^2D_{270.4}$	(3)	0.080	$2.11 \cdot 10^{-4}$	0.9990	0.601–2.402
	$^2D_{271.6}$	(4)	0.102	$9.83 \cdot 10^{-4}$	0.9995	0.601–2.402
Quinapril	$^1D_{272.8}$	(3)	1.785	0.0065	0.9990	0.060–0.200
HPLC	Internal standard[a]					
Ramipril	Enalapril		13.52	−0.011	0.9990	0.05–0.254
Benazepril	Enalapril		30.07	0.0324	0.9990	0.013–0.038
Enalapril	Lisinopril		13.785	0.0048	0.9993	0.040–0.100
Lisinopril	Enalapril		20.10	0.0052	0.9995	0.024–0.056
Quinapril	Enalapril		16.46	0.0112	0.9990	0.020–0.060

$n = 6$.
[a] Concentration of the internal standard solution: 40 μg ml^{-1} for all the analyses except the lisinopril determination (100 μg ml^{-1} of enalapril).

3. Results and discussion

3.1. Derivative spectroscopy

Derivative UV spectroscopy is a well established technique able to enhance the resolution of overlapping absorption bands and to discriminate sharp bands over large bands [12]. This ability has been conveniently applied to the analysis of benzenoid drugs whose UV spectra exhibit a partial fine vibrational structure. ACE-inhibitors also are characterized by UV spectra with a benzenoid profile, with maxima and shoulders (250–280 nm) which can be converted in sharp and intense peaks by the derivation process.

This is well illustrated by quinapril (Fig. 2) and lisinopril (Fig. 3); benazepril (Fig. 4) and enalapril (Fig. 5) show weak shoulders at high wavelength values (>250 nm), whose resolution can be improved by derivation. In general, the characteristic profiles of the derivative spectra may constitute a specific fingerprint useful for the drug identification; in particular, the ratios between the amplitudes at selected wavelength can be regarded suitable parameters useful to confirm the drug identity and purity. As an example, the maxima ratios in the second derivative spectrum of quinapril (Fig. 2) are reported in Table 2.

Using the derivative mode, resolution and sensitivity were found to be depending on the $\Delta\lambda$ value; in particular, high resolution was achieved using minor $\Delta\lambda$ values, while the sensitivity was found to increase with higher $\Delta\lambda$ values. A representative example is reported in Fig. 4 for benazepril. For quantitative applications the order of the derivative and the $\Delta\lambda$ values were chosen according to the drug spectral properties and the formulation composition (Table 1). For the analysis of ramipril dosage forms (1.25–2.5 mg per capsule), the intense peak to peak amplitude in

the third derivative spectrum $^3D_{222.4,218}$ was preferred to the weak benzenoid bands (250–280 nm) to achieve the required sensitivity. The high derivative order allowed non-specific matrix interferences to be suppressed and, using $\Delta\lambda = 4$ nm, adequate resolution was obtained which was useful to confirm the drug identity.

The second-order UV spectrum of enalapril maleate also exhibits weak benzenoid bands (Fig. 5). Second-order derivative methods with measurement at 262 nm [9] and using $^2D_{198,205}$ [8] have been reported.

On account of the draw-backs (poor sensitivity and measurement at low wavelength) of these procedures, the amplitude $^2D_{228.8}$ to the zero-line

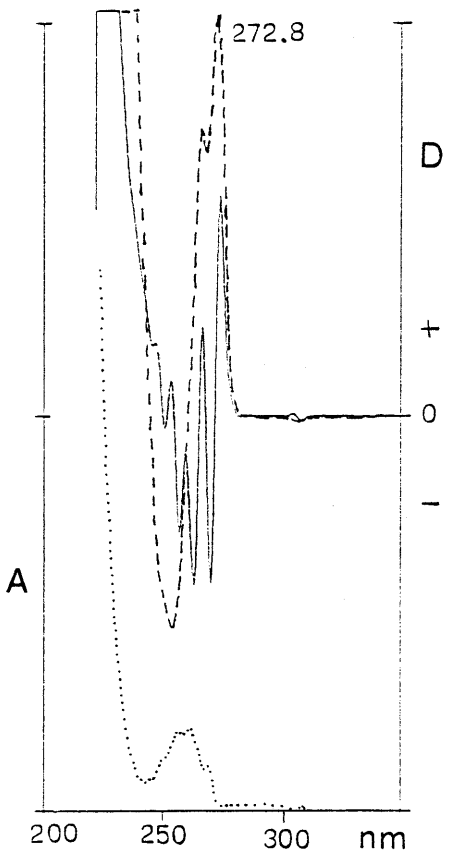

Fig. 2. Zero-order (···), first-order (- - -) and second-order (——) derivative UV spectra of quinapril hydrocloride ($\Delta\lambda = 3$ nm) in methanol.

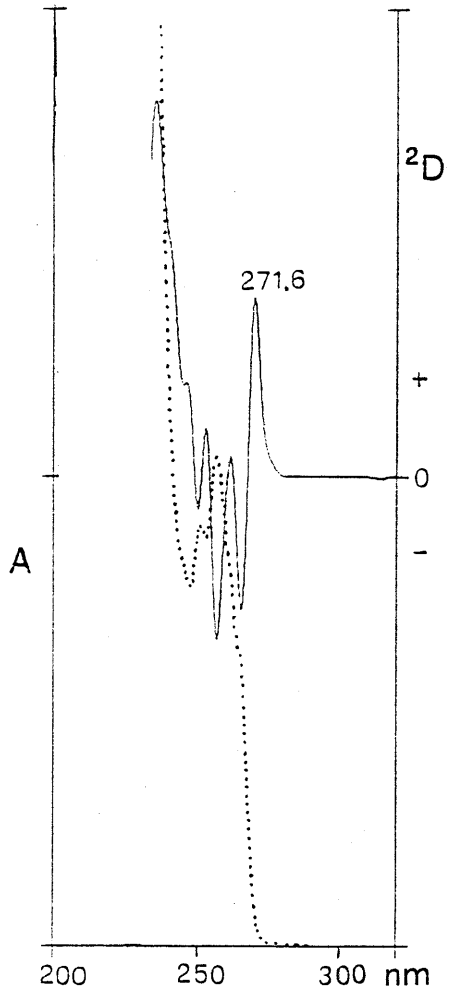

Fig. 3. Zero-order (···) and second-order (——) derivative UV spectra of lisinopril ($\Delta\lambda = 4$ nm) in 0.1 M phosphate buffer (pH 2.8)–methanol 75:25 (v/v).

was chosen in the present work (Table 1). It was verified that maleic acid significantly contributes to the amplitude of this positive peak; thus, using $^2D_{228.8}$ enalapril maleate (1:1 molar ratio) is determined. When the molar ratio was altered by adding maleic acid to enalapril maleate, the $^2D_{228}$ amplitude was affected, but this modified composition was clearly shown by marked alterations in the second-order spectrum profile over the 200–220 nm range. Thus, using derivative mode, useful

qualitative (identification and enalapril to maleate molar ratio) and quantitative (drug content) information on enalapril maleate preparation can be achieved.

For all the other ACE-inhibitors examined, the amplitudes of the benzenoid bands were found to be suitable for quantitative applications. Linear relationships between the selected amplitudes and the drug concentration were obtained for each drug (Table 1). Commercial dosage forms were then analyzed and the results obtained were in agreement with the claimed drug content with good inter-day precision (Table 3).

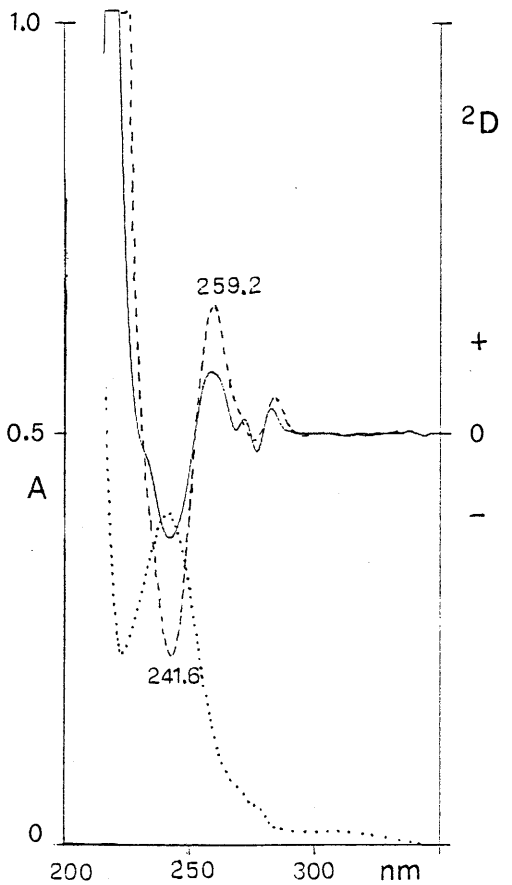

Fig. 4. Zero-order (···) and second-order ($\Delta\lambda = 4$, ——; $\Delta\lambda = 6$,- - -) derivative UV spectra of benazepril hydrochloride in methanol–water (1:1 v/v).

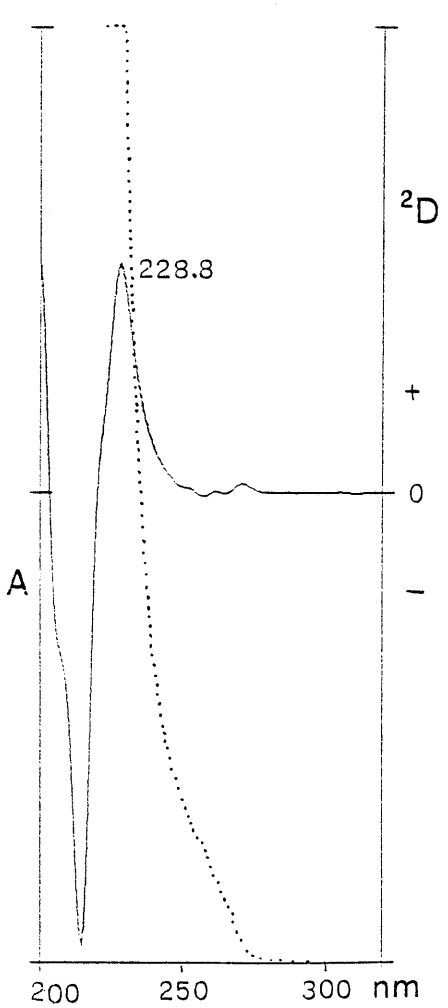

Fig. 5. Zero-order (···) and second-order (——) derivative UV spectra of enalapril maleate ($\Delta\lambda = 4$) in 0.1 M phosphate buffer (pH 4.5)–methanol (80:20 v/v).

The method accuracy was verified by analysing samples fortified with 30% of the claimed drug content; quantitative recoveries (98.8–99.7%) were obtained in each case with adequate inter-day precision (R.S.D. not more than 1.6%; $n = 3$).

3.2. Chromatography

Peak splitting and broadening in reversed phase liquid-chromatography (RP-HPLC) of ACE-inhibitors were observed and this phenomenon prompted investigation of the influence of the

Table 2
Maxima ratios in second-order derivative UV spectrum of quinapril

Selected amplitude	$\Delta\lambda$	Ratio	R.S.D. %
$^2D_{254}/^2D_{258}$	3	0.499	2.00
$^2D_{268}/^2D_{263}$	3	0.528	2.27
$^2D_{268}/^2D_{271}$	3	0.580	2.41
$^2D_{274}/^2D_{271}$	3	1.226	0.80

operating conditions (pH, temperature, organic modifier, ion pairing agent) on the analyte chromatographic behavior [6,7]. Generally, high temperature and/or low pH values were found to improve the peak shape and the resolution.

In this work, a characteristic peak splitting [6], with two evident separated peaks, was observed for enalapril, ramipril and lisinopril when the separations were carried out at ambient temperature and pH 6.5. At higher temperature (70°C), as proposed by USP [13], a single peak was obtained. In order to provide more practical experimental conditions ambient temperature was chosen in combination with low pH values.

Table 3
Assay results for the derivative spectrophotometric and HPLC analyses of ACE inhibitors in commercial dosage forms

Drug	Method	$\Delta\lambda$	% Found	R.S.D. %
Ramipril (1.25 mg)	$^3D_{222.4-218.0}$	4	95.55	0.59
Ramipril (2.5 mg)	$^3D_{222.4-218.0}$	4	96.30	1.16
Benazepril	$^2D_{258.8}$	4	99.76	2.00
	$^2D_{258.8-241.6}$	4	98.34	2.40
	$^2D_{259.2}$	6	99.49	1.70
	$^2D_{259.2-241.6}$	6	99.99	1.47
	HPLC		99.83	1.42
Enalapril maleate	$^2D_{228.8}$	4	100.14	0.61
Lisinopril	$^1D_{268.0}$	3	100.74	0.72
	$^1D_{267.2}$	4	99.97	0.64
	$^2D_{270.4}$	3	101.90	0.91
	$^2D_{271.6}$	4	101.43	1.27
	HPLC		101.35	1.32
Quinapril	$^1D_{272.8}$	3	101.14	2.20
	HPLC		99.83	1.42

The data are expressed as a percentage of the claimed content and are the average of five determinations.

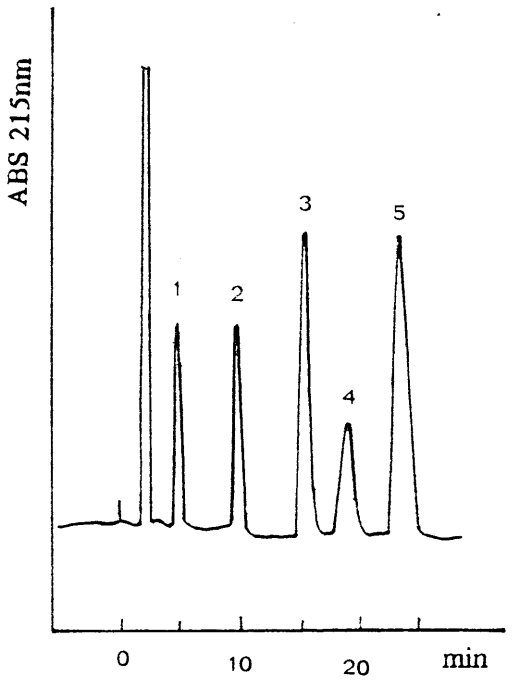

Fig. 6. Representative HPLC separation of a standard mixture of ACE inhibitors: lisinopril (1), enalapril (2), benazepril (3), ramipril (4) and quinapril (5). Column: 5 μm Hypersil ODS (250 mm × 4.5 mm i.d.). Mobile Phase: 20 mM sodium heptansulfonate (pH 2.5)–acetonitrile (5% THF) (63:37 v/v) at a flow rate of 1 ml min^{-1}. UV detection at 215 nm.

At pH 2.5, triethylamine (TEA), used as a mobile phase amine modifier, was found to be responsible for peak splitting, whereas sodium heptansulfonate proved to be a useful ion pairing agent able to improve the analyte retention and resolution, providing a single symmetric peak for each analyte. The tailing factor ($T = W0.05/2f$), a measure of peak symmetry [13], for the analyte peaks was not more than 1.5.

Thus, mixtures A–B, where A is 20 mM heptansulfonate (pH 2.5) and B is acetonitrile–THF (95:5 v/v), proved to be suitable for the separation of the examined ACE-inhibitors; the A–B ratio was adjusted according to the required analysis (Section 2). A representative separation of ACE-inhibitors is illustrated in Fig. 6.

When sodium heptansulfonate was used at pH 6.5, peak splitting was observed; the critical, favorable effect of a decrease in pH to obtain single

peaks can be explained by a higher isomerization rate [6].

The chromatographic conditions of Fig. 6 allows a complete separation of the examined ACE-inhibitors; these conditions are not indispensable for analysing commercial single-component dosage forms, however they are useful for drug identification and the appropriate choice of the internal standard.

For quantitative applications, linear relationships between the peak height ratios (analyte to internal standard) and the analyte concentration (Table 1) were obtained.

The HPLC method was applied to the analysis of commercial formulations of selected ACE-inhibitors and the results (Table 3) were found in close agreement with the claimed content and the spectrophotometric data. The results obtained by the spectrophotometric and HPLC methods were compared by applying the F-test and t-test at the 95% confidence level; no significant differences between the proposed methods were found.

The accuracy of the methods was verified by analyzing commercial samples fortified at the level of 130% of the claimed content; quantitative recoveries (98.7–99.8%) were obtained with an R.D.S. ($n = 3$) of not more than 1.4%.

4. Conclusion

Derivative UV spectroscopy proved to be a useful and simple technique suitable for rapid quality control of ACE inhibitors dosage forms; its high resolution allows unambiguous identification and selective determination of each drug to be achieved. On the other hand, RP-HPLC procedures, under controlled experimental conditions (temperature and/or pH), were found to be suitable to obtain complete separations of ACE inhibitors. At ambient temperature, a pH decrease was confirmed to be essential to improve the peak shape, avoiding peak splitting and band broadening.

Acknowledgements

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