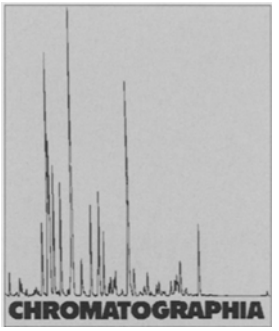
A Liquid Chromatographic Ion-pairing Method for Simultaneous Determination of Benazepril Hydrochloride, Fosinopril Sodium, Ramipril and Hydrochlorothiazide in Pharmaceutical Formulations



2001, *53*, Suppl., S-271–S-275

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Key Words

Column liquid chromatography
Ion-pair chromatography
Benazepril, fosinopril and ramipril
Hydrochlorothiazide
Pharmaceutical formulations

Summary

A rapid and accurate reversed-phase ion-pairing liquid chromatographic method with UV detection was developed for the simultaneous assay of the angiotensin-converting enzyme inhibitors benazepril hydrochloride, fosinopril sodium and ramipril, and the diuretic hydrochorothiazide.

The separation was achieved on a LC-8 (125 × 4.0 mm I. D.; 5 µm particle size) column.

The mobile phase consisted of 20 mM sodium heptanesulphonate (pH = 2.5) and methanol (32:68 *v/v*).

Validation of the method showed it to be precise, accurate and linear over the concentration range of analysis with a limit of detection of 1 ng for hydrochorothiazide, 2 ng for ramipril and benazepril and 8 ng for fosinopril.

The method developed was applied to the analysis of three different binary commercial formulations.

Introduction

Benazepril hydrochloride (I), fosinopril sodium (II) and ramipril (III) (Figure 1) are all orally active angiotensin-converting enzyme (ACE) inhibitors used in the treatment of hypertension and heart failure.

As antihypertensive agents they are administered alone, or in binary combination with the diuretic hydrochorothiazide (HCT, IV) (Figure 1) in order to increase the antihypertensive effects.

An HPLC method to quantitate benazepril and ramipril, together with other ACE inhibitors in pharmaceutical dosage forms has previously been described [1]. Two different gas-chromatography-mass spectrometry determinations for benazepril have also been developed [2, 3]. In addition a second-order derivative spectrophotometry method for the simultaneous determination of benazepril and hydrochlorothiazide in tablets has been reported [4]. However, to our knowledge no HPLC procedures for their simultaneous determination in either pharmaceutical formulations or in biological fluids have been described.

An HPLC method has been developed to evaluate the purity of fosinopril [5]; radioenzymatic and capillary zone electrophoresis procedures have been applied to its determination [6, 7]. The assay of fosinopril in pharmaceutical formulations containing HCT by multiwavelength spectrophotometry has also been reported [8]. No liquid chromatography methods are present in the literature to simultaneously quantitate fosinopril and HCT.

A literature survey based on ramipril reveals several methods for its determination in drugs or biological fluids. These included RP-HPLC [9, 1], derivative UV spectroscopy [1], atomic absorption spectrometry [10], and radioimmunoassay [11]. No analytical methods for the simultaneous assay of ramipril and HCT have been reported.

Official monographs of benazepril and fosinopril sodium are not present in any pharmacopeia.

A reversed phase HPLC assay for the quantitative determination of I, II, III and IV has been developed in order to obtain a simple and reliable single method for the quality control of different antihypertensive agents in their binary formulations.

The method was validated and applied to the analysis of some binary commercial dosage forms.

Presented at: 23rd International Symposium on Chromatography, London, UK, October 1–5, 2000

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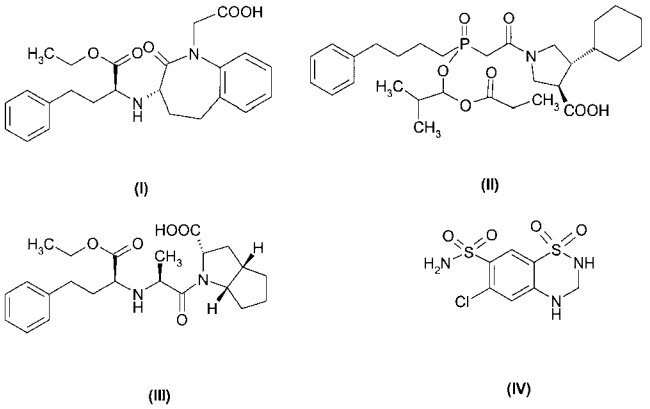

Figure 1. Chemical structures of benazepril (I), fosinopril (II), ramipril (III) and hydrochlorothiazide (IV).

Experimental

Chemicals

Benazepril hydrochloride and hydrochlorothiazide (Crinos, Italy), ramipril (Aventis Pharma, Italy) and fosinopril sodium (Menarini, Italy) were kindly supplied by their manufacturers. Sodium heptansulphonate was obtained by Acros Organics (New Jersey, USA). HPLC-grade methanol was from Carlo Erba (Milan, Italy). Deionized, double-distilled water was used for the mobile phase preparation. 20 mM sodium heptanesulphonate solution at pH = 2.5 was prepared according to standard methods using phosphoric acid to adjust the pH to the desired value.

Commercial tablet formulations containing hydrochlorothiazide in binary combination with fosinopril sodium, ramipril and benazepril hydrochloride were obtained from the national market.

Chromatographic System

The chromatographic apparatus consisted of a W600E pump equipped with a W717plus autosampler and a W996 Photodiode Array Detector all from Waters (Milford, MA, USA).

A Waters Millennium32 Chromatographic Manager software was used for data collection and calculation.

Isocratic separation was achieved using a Lichrospher 100 RP-8 column, particle size 5 µm, 125 × 4.0 mm I. D. (Merck, Darmstadt, Germany), thermostated at 38 °C.

The mobile phase was 20mM sodium heptanesulphonate (pH = 2.5) – methanol (32:68 *v/v*).

The flow rate was set at 1.0 mL min^{-1}. The monitoring wavelength was 220 nm and the injection volume was 20 µL.

Preparation of Standard and Sample Solutions

Standard stock solutions of fosinopril sodium, ramipril, benazepril hydrochloride and HCT at a concentration of about 1 mg mL^{-1} were prepared in mobile phase and stored for two weeks at most. The stability of the standard stock solutions was checked over this period by daily preparing and injecting a diluted solution of each analyte.

The working standard solutions at the concentrations in the calibration range (Table I) were prepared daily by appropriate dilution of the stock solutions with the mobile phase.

Ten tablets of each formulation were weighed and finely pulverised to prepare sample solutions. An appropriate portion of these powders (equivalent to 12.5 mg of hydrochlorothiazide and 10 mg of benazepril, 25 mg of hydrochlorothiazide and 5 mg of ramipril, 12.5 mg of hydrochlorothiazide and 20 mg of fosinopril sodium) was transferred to a 50 mL volumetric flask with 40 mL of methanol.

The solution was sonicated for 30 min, diluted to volume with methanol and then filtered through 0.45 µm nylon syringe filters.

Sample solutions were further diluted with the mobile phase to give working concentrations in the calibration range and injected immediately after preparation.

Results and Discussion

Several reversed-phase eluents, like acetic or phosphate buffers in mixture with acetonitrile or methanol, as described in former reports [12, 13], were tested with the objective of obtaining an acceptable and rapid separation of I, II, III and IV.

The separation was tried using either the columns previously described in the literature or alternative stationary phases.

The main problems encountered using these elution systems were the lack of resolution between benazepril and ramipril, and excessive retention time (more than 30 minutes) for fosinopril due to its very different chromatographic behaviour compared to the other three analytes.

In order to solve these problems, the effects of mobile phase pH changing on the separation were explored. Higher pH values caused peak broadening and tailing. Use lower pH, as previously reported [14], gave sharper peaks and shorter retention time but with no adequate separation between ramipril and benazepril.

Use of an ion pairing method was attempted due to the difficulty of obtaining separation between ramipril and benazepril with usual reversed-phase eluents. As previously reported [1], sodium heptanesulphonate at an acidic pH, could be an useful ion pairing reagent to improve peak shape, retention and resolution of ana-

Table I. Sensitivity and linearity data.

	LOD (µg mL^{-1})*	LOQ (µg mL^{-1})*	Linearity range (µg mL^{-1})*	Slope	Intercept	Number of data points	Standard error
HCT	0.05	0.5	0.5 – 50	$1.24\ 10^8$	$9.8\ 10^3$	5	$1.4\ 10^4$
Benazepril	0.1	1.0	1 – 100	$2.08\ 10^7$	$5.2\ 10^3$	6	$7.9\ 10^3$
Ramipril	0.1	1.0	1 – 50	$1.77\ 10^7$	$-8.6\ 10^2$	6	$1.4\ 10^3$
Fosinopril	0.4	5.0	5 – 200	$1.20\ 10^7$	$1.5\ 10^2$	6	$7.3\ 10^3$

* Injection volume: 20 µL.

Table II. Inter and intra-day repeatability.

Formulation	HCT/Benazepril						HCT/Ramipril						HCT/Fosinopril					
	HCT			Benazepril			HCT			Ramipril			HCT			Fosinopril		
Concentration ($mg\,mL^{-1}$)	0.020	0.025	0.030	0.016	0.020	0.024	0.020	0.025	0.030	0.004	0.005	0.006	0.020	0.025	0.030	0.032	0.040	0.048
Intra-day precision (RSD%)[a]	1.2	0.59	0.44	1.0	0.26	0.30	0.67	1.5	0.62	0.53	0.95	0.40	0.32	0.21	0.78	0.90	1.4	1.1
Inter-day precision (RSD%)[a]	0.67	0.73	0.49	1.2	1.4	0.45	0.93	1.3	1.1	1.1	1.3	2.2	0.57	1.2	1.3	1.0	1.4	1.2

[a] n = 3

lytes containing acidic and basic functions. In our work using a mixture of 20 mM sodium heptanesulphonate at pH 2.5 and methanol (32:68 *v/v*) as the mobile phase on an RP-8 column, resolution factors between benazepril and ramipril greater than 1.6 were obtained; retention time of fosinopril was about 20 minutes. Furthermore, maintaining the column temperature at 38 °C reduced the analysis time to 16 min without unduly affecting resolution between benazepril and ramipril. A representative chromatogram of a mixture of the four analytes is shown in Figure 2.

Much shorter time of analysis could be obtained using a gradient elution; however, owing to the slow equilibration of the ion-pair reagent on the column, changes in the organic-solvent concentration of the mobile phase create an inherently unstable system: consequently isocratic elution was preferred.

Detection wavelength was set at 220 nm in order to have a good sensitivity for the three ACE inhibitors and especially for fosinopril and ramipril characterized by low molar absorptivity values at higher wavelengths.

Sensitivity and Linearity

Limits of detection were determined by visual inspection of the chromatograms, and were 1 ng for HCT, 2 ng for benazepril and ramipril and 8 ng for fosinopril.

Linearity was satisfactory for all the analytes in a large range of concentrations, giving correlation coefficients greater than 0.999.

Limits of quantitation were 10 ng for HCT, 20 ng for benazepril and ramipril and 100 ng for fosinopril.

LOD and LOQ for each compound, along with linearity data, are reported in Table I.

Table III. Accuracy data.

Formulation	Amount added (mg tablet ^{1})	Amount found (mg/tablet ^{1})	Slope	Intercept	Correlation coefficient
HCT	0	12.30	1.027	12.22	0.996
	2.5	14.68			
	5	17.31			
	7.5	19.98			
Benazepril	0	10.28	1.031	10.29	0.996
	2	12.42			
	4	14.32			
	6	16.52			
HCT	0	24.61	1.008	24.59	0.997
	5	29.71			
	10	34.44			
	15	39.84			
Ramipril	0	5.10	1.022	5.11	0.998
	1	6.12			
	2	7.19			
	3	8.15			
HCT	0	12.22	1.027	12.17	0.998
	2.5	14.70			
	5	17.24			
	7.5	19.93			
Fosinopril	0	19.42	1.042	19.36	0.999
	4	23.41			
	8	27.73			
	12	31.82			

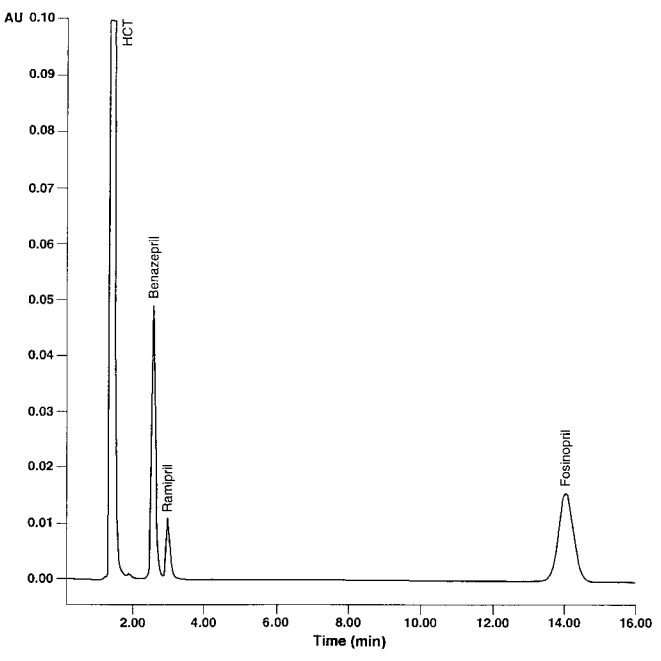

Figure 2. Representative HPLC separation of a standard mixture of HCT (0.025 mg/ml), Benazepril hydrochloride ($0.020\,mg\,mL^{-1}$), Ramipril ($0.005\,mg\,mL^{-1}$) and Fosinopril sodium ($0.04\,mg\,mL^{-1}$). Column: Lichrospher 100 RP-8 125 × 4.0 mm I. D., 5 µm; mobile phase, 20mM sodiumheptansulphonate (pH 2.5) – Methanol (32:68 *v/v*) at a flow rate of $1.0\,ml\,min^{-1}$. UV detection at 220 nm. Injection volume 20 µL.

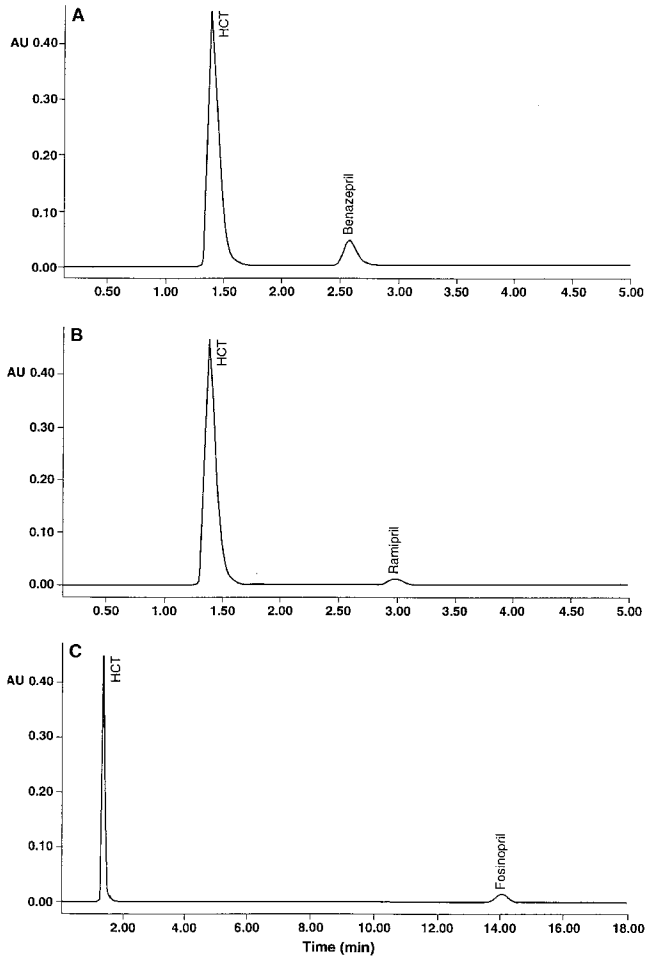

Figure 3. Typical chromatograms of sample solutions of the drugs: (**a**) HCT/ Benazepril 12.5/10 formulation; (**b**) HCT/Ramipril 25/5 formulation; (**c**) HCT/Fosinopril 12.5/20 formulation. Chromatographic conditions are the same as Figure 2.

Table IV. Assay of commercial binary dosage forms.

Formulation (label claim in mg)	HCT			ACE inhibitors		
	Found[a] (mg)	%	RSD (%)	Found[a] (mg)	%	RSD (%)
HCT/Benazepril (12.5/10)	12.2	97.6	0.6	10.1	101.0	1.0
HCT/Ramipril (25/5)	25.1	100.4	1.1	4.93	98.6	1.1
HCT/Fosinopril (12.5/20)	12.7	101.6	1.2	19.8	99.0	1.1

[a] Mean of five independent determinations.

Precision and Accuracy

Method precision, expressed as inter- and intra-day precision, was determined for each of the three formulations by replicate analyses of a pooled sample of 10 tablets. Each sample was carried through the entire sample preparation scheme and assayed. The study was conducted at three different concentration levels for each compound. The relative standard deviations ranged from 0.21 to 1.5% for intra-day repeatability and from 0.49 to 2.2% for inter-day repeatability. The results are shown in Table II.

Accuracy was verified using the standard addition method with known amounts of standard being added to sample solutions. A calibration curve for each compound was then constructed by plotting the amount of the drug found versus the amount added. The results are reported in Table III.

Ruggedness

During the method development it was observed that mobile phases prepared in different days by different analysts caused slight variations in retention times. On no occasion, however, the resolution factor between benazepril and ramipril was significantly affected.

The column-to-column reproducibility was evaluated injecting a reference solution containing all the analytes on three columns from different manufacturers which contained the same type of packing material. The elution order and the resolution factors of the compounds were not affected and only slight variations in retention times were observed.

Analysis of Pharmaceuticals Formulations

The validated HPLC method was then applied to the analysis of commercial formulations containing hydrochlorothiazide, in combination with either benazepril, ramipril or fosinopril. Sample chromatograms for each formulation are shown in Figure 3.

The amount of the active ingredients ranged from 97.6% to 101.6% of the label amount. Results are shown in Table IV.

Conclusions

The proposed ion-pairing HPLC procedure was found to be suitable to obtain complete separation of the ACE inhibitors benazepril, ramipril and fosinopril and the diuretic hydrochlorothiazide.

It provides a simple and reliable method for routine analysis of commercial preparations containing the above-named antihypertensive agents alone or in combination with hydrochlorothiazide.

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Received: Oct 8, 2000
revised manuscript
reseived: Feb 5, 2001
Accepted: Mar 5, 2001