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A stability-indicating LC method for the simultaneous determination of ramipril and hydrochlorothiazide in dosage forms

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Abstract

A simple, rapid and sensitive HPLC method has been developed for the simultaneous determination of ramipril and hydrochlorothiazide in their dosage forms. Acetonitrile: sodium perchlorate solution (0.1 M) adjusted to pH 2.5 ± 0.2 with phosphoric acid (46:54 v/v), was used as the mobile phase, at a flow rate of 1.5 ml/min. A supelcosil™ LC-8 column (5 μm), 15 cm × 4.6 mm i.d. was utilized as stationary phase. Detection was affected spectrophotometrically at 210 nm. Clobazam was used as an internal standard. The method was also applied for the determination of ramipril in the presence of its degradation products. Linearity ranges for ramipril and hydrochlorothiazide were 4.5–45 and 0.6–14 μg/ml, respectively. Minimum detection limits (S/N = 2) obtained were 180 and 23 ng/ml for ramipril and hydrochlorothiazide, respectively. The proposed method was further applied to the analysis of tablets containing the two drugs, the percentage recoveries ± S.D. ($n = 5$) were $100.45\% \pm 0.63$ and $99.55\% \pm 0.78$ for ramipril and hydrochlorothiazide, respectively. © 2001 Published by Elsevier Science B.V.

Keywords: Ramipril; HPLC; Hydrochlorothiazide; Stability; Dosage forms

1. Introduction

Ramipril, 2-[N-[(S)-1-(ethoxycarbonyl)-3-phenylpropyl)]-L-alanyl]-(1S,3S,5S)-2-azabicyclo[3-3-0] octane carboxylic acid, is an angiotensin-converting enzyme (ACE) inhibitor. It acts on the renin–angiotensin aldosterone system. It inhibits the conversion of the inactive angiotensin I to the highly potent vasoconstrictor, angiotensin II, and also reduce the degradation of bradykinin [1]. Hydrochlorothiazide, 6-chloro-3,4-dihydro-2H-1,2,4-benzothia-zine-7-sulphonamide-1,1-dioxide is a thiazide diuretic. It increases sodium and chloride excretion by distal convolated tubule [1].

Literature survey reveals few analytical methods for the determination of ramipril in pharmaceutical preparations and biological fluids, viz. radioimmunoassay [2], spectrophotometry [3], potentiometry [4,5] GC, [6,7] and HPLC [8,9]. As for

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hydrochlorothiazide, several methods have been reported for its determination, either alone or in combination with other drugs. These methods include spectrophotometry [10–14], polarography [15,16], flow-injection analysis [17], and HPLC [18–21].

Ramipril is frequently co-formulated with hydrochlorothiazide in a medicinally recommended ratio of 1:5. Analysis of such mixture is challenging, because ramipril (the minor component) is poorly absorbing light in the UV region (The value of A1%/1 cm at 257 nm is ~ 8), while hydrochlorothiazide (the major component) is strongly absorbing light in the UV region (value of A1%/1 cm at 272 nm is ~ 644). Reviewing the literature revealed that, neither HPLC nor any other method was reported for the simultaneous determination of these two drugs in their dosage forms. The aim of this work is to develop a simple, rapid, sensitive and reliable HPLC assay procedure for the quality control of ramipril and hydrochlorothiazide in pharmaceutical preparations.

2. Experimental

2.1. Materials and reagents

Ramipril pure drug sample, Batch No. HA05326 and Clobazam Batch No. H030 pure drug sample were kindly provided by Hoechst, Frankfurt/M, Germany. Hydrochlorothiazide pure drug sample was kindly supplied by Ciba-Geigy, through Riyadh Central Lab. Tritace Comp(B) (Batch No. 005) containing 5 mg of ramipril and 25 mg of hydrochlorothiazide per tablet were obtained from commercial sources.

Acetonitrile (Hipersolv)® HPLC grade, (BDH, Pool, UK).

Phosphoric acid, 85%, AnalaR, (E. Merck, Darmstadt, Germany).

Sodium perchlorate (Riedel-de Haen, Germany) 0.1 M aqueous solution.

2.2. Apparatus

Waters liquid chromatograph 600 E, equipped with Waters-U6K Millipore injector, Waters-486 tunable absorbance detector and Waters-746 data module; was used. The column used was stainless steel, 15 cm × 4.6 mm i.d. packed with 5 μm Supelcosil™ LC-8 bonded material (Supelco).

2.3. Chromatographic conditions

Chromatographic analysis was carried out at ambient temperature. The compounds were separated isocratically with a mobile phase consisting of acetonitrile: sodium perchlorate solution (0.1 M) (46:54 v/v) with the pH adjusted to 2.5 ± 0.2 using phosphoric acid. The flow rate was 1.5 ml/min. The chart speed was 0.5 cm/min and the effluent was monitored spectrophotometrically at wavelength of 210 nm and attenuation of 32. The mobile phase was filtered by passing through a 0.22 μm membrane filter (Millipore, Bradford, MA). The mobile phase was degassed by pumping pure helium gas into the solvent reservoir at a rate of 20 ml/min.

2.4. Standard solutions

- Ramipril stock solution containing 0.10 mg/ml in acetonitrile.
- Hydrochlorothiazide stock solution 0.10 mg/ml in acetonitrile was diluted with the mobile phase to give a concentration of 20.0 μg/ml.
- Clobazam internal standard stock solution 0.30 mg/ml was diluted with the mobile phase to give a concentration of 30 μg/ml.

2.5. Calibration curves

A total of 1–10 ml of the standard stock solution was transferred into 25 ml volumetric flasks; to each flask was added, 3 ml of internal standard solution and the volume was completed to the mark with mobile phase. Triplicate 10 μl injections were made for each solution and the peak area ratio of each drug to the internal standard was plotted against the corresponding concentration to obtain the calibration graph. Alternatively, the corresponding regression equation for each drug was derived.

The ruggedness and precision were checked at different days; within day ($n = 6$) and between days ($n = 15$) for three different concentrations at low, medium and high level of the standard curve. The relative standard deviations were calculated to check the ruggedness and precision of the method.

2.6. Application of the proposed method to tablets containing the two drugs

Weigh and finely powder ten tablets. Transfer an accurately weighed amount of the powder equivalent to 2.5 mg of ramipril and 12.5 mg of hydrochlorothiazide into a 50-ml beaker. Extract with 3×30 ml of acetonitrile and filter into a 100 ml volumetric flask. Wash the beaker with a few millilitres of acetonitrile and pass the washings through the same filter into the volumetric flask. Complete to the mark with the same solvent. Transfer aliquots containing suitable amounts of the drugs within the working range (Table 1) into 25 ml volumetric flasks. Add 3 ml of internal standard solution and dilute up to the mark with the mobile phase. Inject 10 μl of the final solution and calculate the peak ratio for each drug to the internal standard and calculate the concentration of each drug either from the linear regression equation or from the calibration graph.

2.7. Application of the proposed method to the degraded ramipril

A quantity of ramipril (80 mg) was transferred into a 100 ml volumetric flask and dissolved in the least volume of acetonitrile, then 50 ml of 2 M NaOH or 2 M HCl were added for alkaline or acid hydrolysis, respectively. The solutions were heated in a boiling water bath for 1 h, then cooled and diluted to the mark with the mobile phase. After neutrilization of the solutions, transfer aliquots containing suitable amounts of the degraded ramipril and a constant amount of ramipril within the working range into 25-ml volumetric flask. Add 3 ml of internal standard solution and dilute to the mark with the mobile phase. Proceed as described in Section 2.5

2.8. Recovery studies

To study the accuracy of the proposed method, and to check the interference from excipients used in the formulations, recovery experiments were carried out by the standard addition method.

A total of 5 ml of ramipril standard solution (0.025 mg/ml), 5 ml of hydrochlorothiazide standard solution (0.015 mg/ml) and 3 ml of internal standard solution (0.03 mg/ml) were transferred into a 25 ml volumetric flask (flask No. 1) to give a solution containing 5 μg/ml ramipril and 3 μg/ml hydrochlorothiazide and 3.6 μg/ml of the internal standard. The volume was completed to the mark with mobile phase.

For ramipril content: a stock sample solution (A) containing ramipril (0.025 mg/ml) and hydrochlorothiazide (0.125 mg/ml) was prepared. 5 ml of (A) were transferred into a 25 ml volumetric flask; 5 ml standard ramipril (0.025 mg/ml) were also added, followed by 3 ml of internal standard solution (0.03 mg/ml). The volume was completed to the mark with mobile phase (flask No. 2).

For hydrochlorothiazide content: 3 ml of solution A were diluted into a 25 ml volumetric flask with the mobile phase to get solution B (0.015 mg/ml of hydrochlorothiazide). 5 ml of solution B were transferred into a 25-ml volumetric flask; 5 ml of standard hydrochlorothiazide solution (0.015 mg/ml) were added, followed by 3 ml of the internal standard solution and the volume was completed to the mark with the mobile phase (Flask No. 3).

5 ml of sample solution (A) were transferred into a 25-ml volumetric flask; followed by 3 ml of internal standard solution and volume was completed to the mark with mobile phase (flask No. 4). 5 ml of sample solution (B) were transferred into a 25-ml volumetric flask; followed by 3 ml of internal standard solution and the volume was completed to the mark with mobile phase (flask No. 5). Triplicate injections were made for each solution (Flasks 1–5), and added recoveries were calculated as follows:

Table 1
Performance characteristics of the proposed method

Drug	Linear range (μg/ml)	Slope	Intercept (a)	Correlation coefficient	Minimum detectability (ng/ml)	Capacity factor	Tailing factor
Hydrochloro-thiazide	0.6–14	468	5.9×10^{-3}	0.9997	23	0.48	1.17
Ramipril	4.5–45	282	−0.05	0.9999	180	4.14	1.19

Table 2
Determination of hydrochlorothiazide and ramipril in their pharmaceutical preparation by the proposed method

Preparation	Hydrochlorothiazide			Ramipril		
	Amount taken (μg/ml)	Amount found (μg/ml)	%Recovery	Amount taken (μg/ml)	Amount found (μg/ml)	%Recovery
Tritace (Comp®) hydrochlorothiazide 25 mg + Ramipril 5 mg[a,b]	3.20	3.21	100.39	6.40	6.43	100.50
	4.80	4.73	98.64	9.60	9.55	99.45
	6.40	6.40	99.95	19.20	19.39	101.02
	9.60	9.57	99.67	32.00	32.07	100.27
	12.80	12.65	98.81	24.00	24.23	100.98
Means ± S.D.			99.55 ± 0.78			100.45 ± 0.66
Added recovery mean ± S.D.			99.41 ± 1.01			100.41 ± 1.62

[a] Product of Hoechst AG, Frankfurt, Germany (Batch no. 005).
[b] Each result is the average of three separate determinations.

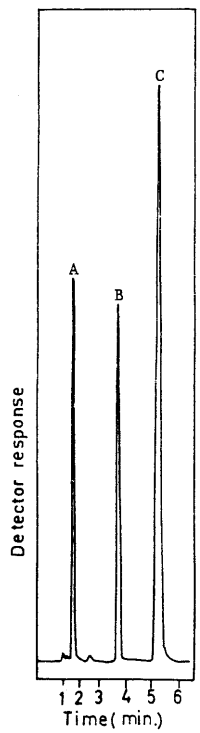

Fig. 1. Typical high-performance liquid chromatogram of mixture of ramipril and hydrochlorothiazide. Conditions: Flow rate: 1.5 ml/min; UV detection at 210 nm, 25°C, sensitivity: 32, chart speed: 0.5 cm/min. (A) hydrochlorothiazide (1.48 min); (B) clobazam internal standard (3.4 min); and (C) ramipril (5.12 min).

Table 3
Within day reproducibility and precision of hydrochlorothiazide and ramipril mixture as evaluated by peak ratio

Run	Hydrochlorothiazide[a]		Ramipril[a]	
	Peak ratio	Recovery%	Peak ratio	Recovery%
1	2.741	99.65	2.780	100.58
2	2.764	100.48	2.784	100.51
3	2.761	100.38	2.761	99.68
4	2.748	99.91	2.765	99.82
5	2.753	100.00	2.735	98.74
6	2.736	99.47	2.774	100.14
Mean	2.750	99.98	2.768	99.91
±S.D.	0.01	0.40	0.02	0.67
% R.S.D.	0.36	0.40	0.72	0.68

[a] Hydrochlorothiazide (12 μg/ml) and Ramipril (20 μg/ml).

$$P(\text{ad}) - P(\text{sp})/P(\text{std}) \times 100$$

where: $P(\text{ad})$ = peak ratio for added solution; $P(\text{sp})$ = peak ratio for sample solution; and $P(\text{std})$ = peak ratio for standard solution.

3. Results and discussion

In order to affect the simultaneous elution of the two components under isocratic conditions, different chromatographic conditions (organic modifier, flow rate, ionic strength, pH) have been investigated. Mobile phases containing methanol alone or acetonitrile alone were found to elute the two compounds unresolved. A satisfactory separation was obtained using mobile phase consisting of a mixture of acetonitrile and sodium perchlorate 0.1 M (46:54 v/v) with the pH adjusted to 2.5 ± 0.2 with phosphoric acid. The mobile phase composition was optimized. Under the described conditions, the analyte peaks were well defined, resolved and free from tailing, the tailing factors were < 1.2 for all peaks. The elution order were hydrochlorothiazide ($t_R = 1.48$ min), clobazam ($t_r = 3.4$ min) and ramipril ($t_R = 5.12$) at a flow rate of 1.5 ml/min (Fig. 1).

Several drugs were tested for selection of a suitable internal standard, clobazam was found to be a suitable internal standard for this study. Regression analysis of the calibration curve indicated a linear relation between peak-ratio (Y) and concentration (X) (Table 1).

The proposed method was applied to the determination of hydrochlorothiazide and ramipril in their pharmaceutical preparation (Tritace Comp®), the results in Table 2 indicate satisfactory accuracy and precision of the method. Tablets excipients, such as talc, starch, lactose, gum, magnesium stearate and avisil did not interfere with the assay. The % recovery ± S.D. ($n = 5$) of the added hydrochlorothiazide and ramipril was 99.41 ± 1.01 and 100.41 ± 1.62, respectively, the results are shown in Table 2. The precision and ruggedness of the proposed method were assessed by the follow up of within-day, between days data for low, medium and high concentrations. R.S.D.s in all three cases were $< 2\%$, indicating good reproducibility and precision of the

Table 4
Ruggedness and precision of the hydrochlorothiazide and ramipril mixture as evaluated by peak ratio between days using three different concentrations[a]

Days	Hydrochlorothiazide			Ramipril		
	Conc. taken (μg/ml)			Conc. taken (μg/ml)		
	3.2	8.0	12.8	8.0	16.0	22.4
Initial	0.948	2.001	3.054	1.041	2.057	2.874
After 7 days	0.945	2.014	3.032	1.074	2.095	2.966
After 12 days	0.942	1.997	3.042	1.063	2.107	2.870
Mean	0.945	2.008	3.043	1.054	2.086	2.903
±S.D.	0.003	0.009	0.011	0.017	0.026	0.054
%R.S.D.	0.32	0.045	0.36	1.61	1.25	1.86

[a] Each result is the average of five separate determinations.

method, the results are shown in Tables 3 and 4. Furthermore, the proposed method was extended to be used as stability-indicating assay for the determination of intact ramipril in presence of its degradation products. It has been previously reported that, diketopiperazine is the major degradation product of ramipril [22]. A typical chromatogram of hydrochlorothiazide, ramipril and its alkaline degradation products is shown in Fig. 2.

The presence of the degradation products of ramipril did not interfere with its determination. The drug was completely degraded in alkaline medium at once and could not be detected by proposed method. On the other hand, in acidic medium, the drug was not completely degraded, therefore, a small amount of ramipril could be detected. The results of determination of ramipril in the presence of its degradation products are shown in Table 5.

4. Conclusion

A simple, accurate and precise method was developed for the analysis of the binary mixture of hydrochlorothiazide and ramipril in their pharmaceutical preparations. The sensitivity, reproduciblity, simplicity, and short analysis time of the method makes it valuable in the routine analysis of this mixture.

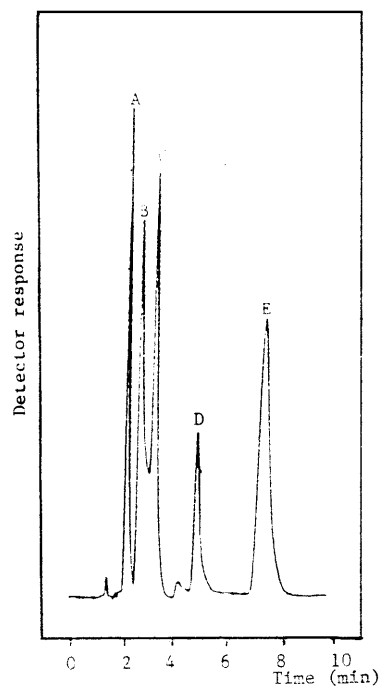

Fig. 2. Typical high-performance liquid chromatogram of ramipril and its alkaline degradation products. Conditions: The same as in Fig. 1 except flow rate: 1 ml/min. (A) hydrochlorothiazide (2.15 min); (B–C) degradation products of ramipril (2.64 and 3.20 min, respectively); (D) clobazam internal standard (4.84 min); and (E) ramipril (7.36 min).

Table 5
Determination of ramipril in presence of its alkaline degradation products by the proposed method[a]

Added conc. (μg/ml)		Found ramipril (μg/ml)	% Recovery
Ramipril	Deg.[b] ramipril		
24.00	16.00	24.01	100.03
24.00	24.00	24.06	100.27
24.00	32.00	24.13	100.56
24.00	40.00	23.39	98.75
Mean			99.90
±S.D.			0.80

[a] Each result is the average of three separate determinations.
[b] Deg., alkaline degradation product.

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