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A validated stability-indicating LC method for simultaneous determination of quinapril and hydrochlorothiazide in pharmaceutical samples

Short Communication

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Abstract: A stability-indicating liquid chromatographic method was developed and validated for simultaneous determination of quinapril and hydrochlorothiazide in drug substances and dosage forms. Chromatographic separation of quinapril, hydrochlorothiazide and its degradation products was achieved on a RP-18 column, using acetonitrile and phosphate buffer (pH 4.6) as mobile phase in a gradient mode and detection at 216 nm. Stress testing was performed under hydrolytic, oxidative, thermal and photolytic conditions. The degradation products were well resolved from main peaks, proving the stability-indicating power of the method. The assay was linear for quinapril and hydrochlorothiazide concentrations of 40-200 μg mL^{-1} and 25-125 μg mL^{-1}, respectively. The developed method was selective, accurate and precise for quinapril and hydrochlorothiazide determination. This method was used to quantify both drugs in combined commercial tablets. The results showed that the proposed method was found to be suitable for quantitative determination and the stability study of quinapril and hydrochlorothiazide in pharmaceutical samples.

Keywords: *Hydrochlorotiazide • Quinapril • Simultaneous determination • Stability-indicating • Stress testing*

1. Introduction

Quinapril is an angiotensin-converting enzyme inhibitor used alone or in combination with other classes of antihypertensive agents (*e.g.* thiazide diurectics) in the management of hypertension. Quinapril is a prodrug and has little pharmacological activity until hydrolyzed in the liver to quinaprilat [1]. Hydrochlorothiazide (HCTZ) is a thiazide diuretic and antihypertensive agent [2]. The association of quinapril/hydrochlorothiazide is used as combination therapy in the treatment of patients whose blood pressure is not adequately controlled with any of the substances alone [1,2].

Quinapril (Fig. 1) and hydrochlorothiazide (Fig. 2) have chemical structures susceptible to degradation, especially by hydrolytic reactions. It has been reported that quinapril is degraded mainly two degradation products; quinaprilat by hydrolysis and quinapril diketopiperazine (DKP) by cyclization intramolecular [3-6]. For hydrochlorothiazide it has been reported that is degraded to 4-amino-6-chloro-1,3-benzendisulfonamine (DSA) and formaldehyde by hydrolysis [7-11].

The stability of a pharmaceutical preparation is in relation with its potency, therefore, with its therapeutic properties, in this way is very important to keep a stable pharmaceutical product and to have the appropriate analytical methods to assess its stability; these methods must be stability-indicating [12]. The main target while developing this method is to have a single method for separation between quinapril/hydrochlorothiazide and their degradation products.

Stability-indicating methods have been reported for assays of various drugs in drug products containing only one active drug substance, only few stability-indicating methods are reported for the assay of combination drug products. A stability-indicating method for the simultaneous determination of quinapril/hydrochlorothiazide has not been documented to date; therefore arise the need to develop this method.

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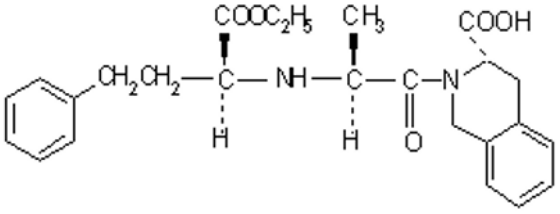

Figure 1. Chemical structure of quinapril.

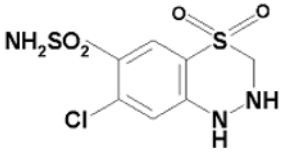

Figure 2. Chemical structure of hydrochlorothiazide.

For quinapril there are reported stability-indicating LC methods for its individual determination in an extemporaneous pediatric formulation [4] and as a drug substance [5]. For hydrochlorothiazide it has been reported stability-indicating LC methods for its simultaneous determination with losartan [7,8], enalapril [11] losartan and atenolol [13], ramipril [14], spironolactone [15], irbesartan [16] and telmisartan with amlodipine [17]. For the simultaneous determination of quinapril/hydrochlorothiazide has been reported a few methods (not stability-indicating): there is an ion pair LC method with diode array detection for determination in tablets [18], other techniques include HPTLC [19] spectrophotometry [20] and capillary electrophoresis [21], and a LC method with mass spectrometric detection for determination in plasma [22].

None of the above analytical procedures describes a method for the simultaneous determination of quinapril/ hydrochlorothiazide in the presence of their degradation products.

The objective of this study was to develop and validate a stability-indicating LC method for the simultaneous determination of quinapril and HCTZ in pharmaceutical preparations.

2. Experimental procedure

2.1. Chemicals and reagents

Standards of quinapril Hydrochloride, quinaprilat, DKP, DSA and cloxacillin sodium (> 99.0% purity) were obtained from USP (Rockville, MD, USA). Standard of HCTZ (> 99.0% purity) was obtained from Sigma (St. Louis, MO, USA). Acetonitrile and methanol LC grade, KH_2PO_4, sodium hydroxide, hydrochloric acid and hydrogen peroxide p.a. grade were from Merck (Darmstadt, Germany). Quinapril and HCTZ drug substances were obtained from Diprolab (Santiago, Chile). Quinapril / HCTZ tablets, containing 20 mg of quinapril and 12.5 mg of HCTZ were purchased from a Chilean pharmacy. The excipients in the dosage form were the following: lactose, magnesium carbonate, polyvidone, crospovidone, magnesium stearate, hydroxypropylcellulose and macrogol.

2.2. Instrumentation

Chromatography was performed by using a Perkin Elmer Series 200 liquid chromatograph (Norwalk, CT, USA) equipped with a manual injector, a 7125 Rheodyne injection valve (Cotati, CA, USA), and a 20-µL loop. An Applied Biosystems Model 785A programmable absorbance detector (Foster, CA, USA), and a Perkin Elmer Nelson Model 1022 data processor (Norwalk, CT, USA).

2.3. Chromatographic conditions

The separation was carried out using a Purospher® RP-18 column (125×4 mm, 5 µm; Merck, Darmstadt, Germany). The mobile phase consisted of acetonitrile (A) and phosphate buffer (pH 4.6; 0.01M) (B) in a gradient mode; T_{min}/A %; T_0/16; T_{10}/16; T_{13}/65; T_{21}/16. The flow rate was set to 1 mL min^{-1} with UV detector wavelength fixed at 216 nm. Cloxacillin sodium (100 µg mL^{-1}) was used as the internal standard. All analyses were performed at room temperature (23±2ºC).

2.4. Preparation of standard solutions

A stock solution of quinapril Hydrochloride and a stock solution of HCTZ were independently prepared at 2.0 mg mL^{-1} and 2.5 mg mL^{-1} respectively in methanol. A stock solution of cloxacillin sodium was prepared at 2.5 mg mL^{-1} in water. Standard solutions were prepared from the stock solutions after adequate dilution with water. A stock solution of quinaprilat, DKP and DSA were prepared in methanol at 1 mg mL^{-1} .

2.5. Preparation of sample solution

20 tablets of quinapril 20 mg and HCTZ 12.5 mg were weighed and powered. A portion equivalent to 3.0 mg of quinapril was accurately weighed and transferred to a 25 mL volumetric flask, then 5 mL of phosphate buffer (pH 4.6; 0.01M) and a portion of 1 mL of internal standard (2.5 mg mL^{-1}) were added. The solution was vortexed for 15 s and sonicated for 15 min. Then 5 mL of acetonitrile was added, and the solution was again vortexed for 15 s and sonicated for 15 min, then the volume was made up with phosphate buffer (pH 4.6; 0.01M) (final concentration of 120.0 µg mL^{-1} and 75.0 µg mL^{-1} for quinapril and HCTZ respectively). Finally the mixture was filtered.

2.6. Stability-indicating capability of the LC assay

It was established by chromatographic analysis of all stressed samples (explained in section *stress testing*) and standard solutions of the major degradation products of quinapril (quinaprilat and DKP) and HCTZ (DSA). The composition, pH, flow rate and the mobile phase gradient were changed to optimize separation between quinapril, HCTZ, internal standard and the degradation products.

2.7. Method validation

The method was validated according to the ICH guidelines [23]. The parameters validated were linearity, precision, accuracy, selectivity, limits of detection and quantification, and solution stability.

2.7.1. Linearity

The calibration graphs were obtained by plotting the ratio for peak heights of quinapril / internal standard and HCTZ / internal standard against the concentration of the drugs. The concentration range for quinapril and HCTZ were 40-200 µg mL^{-1} and 25-125 µg mL^{-1} respectively (5 different concentrations were used and each solution was injected 3 times).

2.7.2. Precision

The intra-day precision was determined by carrying out three independent assays in concentrations of 96.0, 120.0 and 144.0 µg mL^{-1} for quinapril and 60.0, 75.0 and 90.0 µg mL^{-1} for HCTZ on the same day. The inter-day precision was studied by comparing the assays on three different days.

2.7.3. Accuracy

To evaluate the accuracy of the method, recovery test were performed by adding known amounts of standard of quinapril and HCTZ in the level 80, 100 and 120% of the quinapril and HCTZ levels in the tablets (three replicates of each level) to common tablet excipients (lactose, starch and magnesium stearate). The accuracy of the assay was determined by comparing the found amount with the added amount.

2.7.4. Selectivity

The selectivity of the method was evaluated through the stress studies in order to demonstrate the separation between quinapril, HCTZ, and their degradation products. The selectivity was also evaluated by observing any interference from excipients used in the tablets; therefore a sample of the commercial product was analyzed.

2.7.5. Limits of detection and quantification

LOD and LOQ were calculated by using the equations: LOD = $3.3 \times \sigma / S$; LOQ = $10 \times \sigma / S$, where σ is the standard deviation of y-intercepts of regression lines and S is the slope of the calibration curve.

The obtained LOQ were validated by triplicate analysis of samples prepared at 1.01 µg mL^{-1} and 2.09 µg mL^{-1} for quinapril and HCTZ, respectively.

2.7.6. Robustness

To evaluate the robustness of the method, the chromatographic conditions were intentionally altered and the resolution between all peaks was evaluated. The flow rate was changed by 0.2 units, from 0.8 to 1.2 mL min^{-1} and pH of phosphate buffer of mobile phase was changed from 4.4 to 4.8.

2.7.7. Solution stability

Solution stability of standard of HCTZ was evaluated previously by us [11]; therefore only solution stability of quinapril is described. The stability of the standard solution of quinapril was evaluated at room temperature (23±2ºC), 8±1ºC and -20±0.5ºC, for 3, 6 and 20 days, respectively. The stability was determined by comparing the concentration at each time with the initial concentration.

2.8. Stress testing

Stress testing was carried out according to the ICH stability testing guidance [24]. Forced degradation studies of HCTZ were developed previously by us [11]; therefore only stress testing of quinapril is described. Quinapril was stressed under various conditions until to facilitate approximate 5-20% degradation [25]. For each condition, a blank solution was prepared and was subjected to stress in the same manner as the drug, also a control solution was prepared, which was stored without the stress condition.

2.8.1. Hydrolysis acidic, neutral and alkaline

A solution of quinapril 10 mg mL^{-1} was prepared in methanol, then an aliquot of 1 mL was transferred to a 10 mL volumetric flask and diluted with 0.1 N HCl, water and 0.1 N NaOH to volume (1 mg mL^{-1}). Samples of 3 mL were kept on a hot plate at 70ºC for 6 h for acid and neutral hydrolysis, and for 5 min for basic hydrolysis. After which they have been cooled to room temperature, then they were transferred quantitatively to a 25 mL volumetric flask, neutralized, a 1 mL of internal standard (2.5 mg mL^{-1}) was added, and they were diluted to volume (120 µg mL^{-1}).

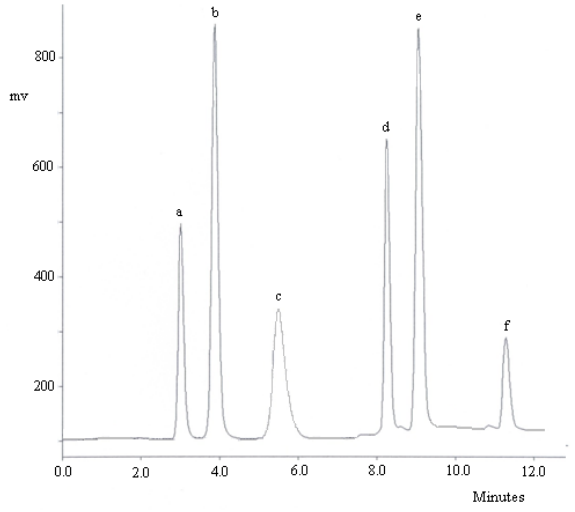

Figure 3. Representative chromatogram of quinapril, HCTZ and their degradation products: (a), DSA (b), HCTZ (c), quinaprilat (d), cloxacillin sodium (e), quinapril Hydrochloride (f), DKP. 1 mV: 0.001 AU.

Table 1. Precision determined during method validation.

	Concentration (μg mL^{-1})	Relative standard deviation (%)	
		intra-day [a]	inter-day [b]
Quinapril	96.00	2.43	3.14
	120.00	1.03	1.18
	144.00	2.71	1.08
HCTZ	60.00	0.75	1.20
	75.00	1.58	0.87
	90.00	0.14	1.77

a) Analyzed on the same day (n=3).
b) Analyzed on three different days (n=9).

2.8.2. Oxidation

A solution of quinapril 10 mg mL^{-1} was prepared in water, then an aliquot of 1 mL was transferred to a 10 mL volumetric flask, and diluted with 6% H_2O_2. This solution was kept at room temperature (25±2ºC) for 7 days in the dark. Then a solution of quinapril 120 μg mL^{-1} was prepared.

2.8.3. Thermal degradation

Solid drug of quinapril was exposed to dry heat at 70ºC in an oven for 10 days. Then a solution of quinapril 120 μg mL^{-1} was prepared.

2.8.4. Photostability

Photodegradation studies were carried out according to option 2 of the ICH Q1B guidelines [26]. Samples of quinapril 1 mg mL^{-1} in water and solid drug in 1 mm layer in a petri-plate, were exposed to light for an overall ilumination of 1.2 million lux hours and a integrated near ultraviolet energy of 200 watt hours m^{-2}.

Then a solution of quinapril 120 μg mL^{-1} was prepared. Dark controls were run simultaneously.

3. Results and discussion

3.1. Method development and optimization

The main purpose while developing this method was to get the separation between quinapril, HCTZ and their degradation products. Initially, the compounds were analyzed under isocratic mobile phase, but DKP eluted with a very long retention time, therefore it was necessary to use a gradient mobile phase.

The gradient program was optimized to provide sufficient selectivity in a short separation time. Optimal chromatographic separation, with good peak shapes, was achieved using acetonitrile as solution A and phosphate buffer (pH 4.6; 0.01M) as solution B in a gradient mode. System suitability parameters were found to be within the suitable range: *Rs* > 2.2 between all peaks (range Rs ≥ 2); *T* (peak tailing factor) 1.2 for quinapril and HCTZ (range 1 ≤ T < 1.5); and *N* 11960 for quinapril and 3160 for HCTZ (range N > 2000). As shown in Fig. 3, the method was able to resolve all the compounds; quinapril, HCTZ, quinaprilat, DSA, DKP and internal standard. The method thus proved to be stability-indicating.

3.2. Method validation

3.2.1. Linearity

The equations of the calibration curves were $y = 0.006x + 0.070$; r^2= 0.991 and $y = 0.010\ x + 0.109$; r^2= 0.994 for quinapril and HCTZ, respectively. According to statistical analyses by ANOVA, both calibration curves were linear (p< 0.005).

3.2.2. Precision

The results of precision study are shown in Table 1. The obtained values show a suitable precision for the analytical method.

3.2.3. Accuracy

The results of accuracy study are shown in Table 2. The obtained values confirm the accuracy of the proposed method.

3.2.4. Selectivity

Results from the stress testing studies indicated that the method is selective towards quinapril, HCTZ, degradation products and internal standard as shown in Fig. 3. The chromatograms of the commercial product

Table 2. Recovery determined during method validation.

	Sample Level (%)	Added Amount (mg)	Found Amount (mg) [a]	Recovery (%) [b]
Quinapril	80	2.40	2.39 ± 0.08	99.52
	100	3.00	3.00 ± 0.27	99.97
	120	3.60	3.60 ± 0.10	100.14
HCTZ	80	1.50	1.54 ± 0.01	102.65
	100	1.88	1.88 ± 0.03	100.37
	120	2.25	2.31 ± 0.00	102.78

a) Mean ± SD (n= 3).
b) (Found concentration/ Added concentration) x 100.

showed that there is no interference or overlap of the excipients with the quinapril, HCTZ, degradation products or internal standard peaks. These results proved the selectivity of the proposed method.

3.2.5. Limits of detection and quantification

LOD was 0.35 µg mL^{-1} and 0.61 µg mL^{-1} for quinapril and HCTZ respectively, and the respective LOQ were 1.06 µg mL^{-1} and 1.85 µg mL^{-1}. These values are adequate for determination in pharmaceutical samples.

For validation of LOQ, the concentration of quinapril and HCTZ was found to be 1.09 ± 0.10 µg mL^{-1} and 2.03 ± 0.12 µg mL^{-1} respectively.

3.2.6. Robustness

After modifications of flow rate and pH, the resolution between all picks was greater than 2, this demonstrate the robustness of the method.

3.2.7. Solution stability

Recovery of quinapril at 23ºC for 3 days, 8ºC for 6 days and -20ºC for 20 days, were 101.2%, 100.3% and 101.3% respectively. These results confirm that all the solutions were stable, as there the concentration remained almost unchanged and there was no formation of degradation products.

3.3. Stress testing

Quinapril was found to degrade *via* hydrolysis. After hydrolysis in water, 0.1 N hydrochloric acid and 0.1 N sodium hydroxide the percentage of degradation was 26.3%, 37.0% and 30.8% respectively. It was observed the formation of two degradations products identified as quinaprilat and DKP by comparing the chromatograms

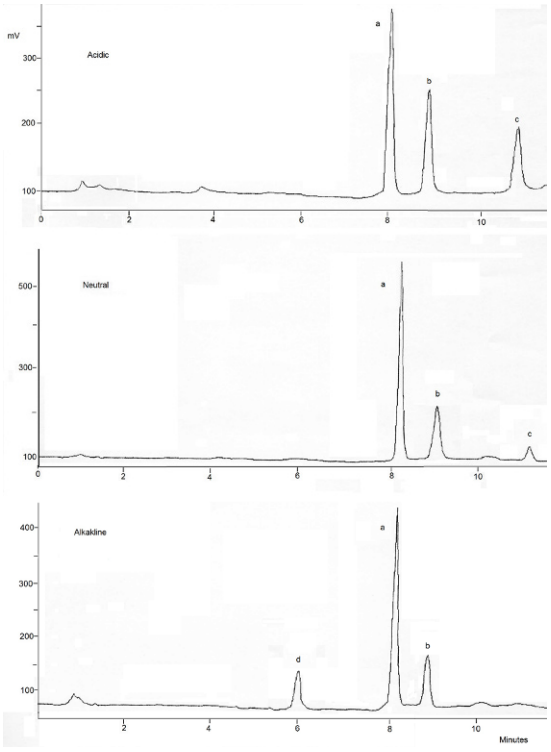

Figure 4. Representative chromatograms of quinapril after acidic, neutral and alkaline hydrolysis: (a), cloxacillin sodium (b), quinapril Hydrochloride (c), DKP (d), quinaprilat. 1 mV: 0.001 AU.

of stressed samples with chromatograms of standard solution of these compounds. Under alkaline stress, quinapril degraded principally to quinaprilat, and under neutral and acidic condition, quinapril degraded to DKP (Fig. 4). Quinapril also degrades under thermal stress; the percentage of degradation was 11.7%, with formation of DKP. Quinapril was found to be stable under photolytic and oxidative conditions.

According to a previous investigation developed by us [11] HCTZ degrades to one degradation products identified as DSA.

3.4. Determination of quinapril and hydrochlorothiazide in combined commercial tablets

This method was used for the quantification of commercial product of quiapril / HCTZ tablets containing 20 of quinapril and 12.5 mg of HCTZ. The percentage of quinapril and HCTZ was found to be 97.2 ± 2.3% and 96.4±2.0% (mean ± SD, n = 3) respectively, these values are within the specified limits of 90 to 110% according to USP.

4. Conclusion

A stability-indicating LC method for analysis of binary mixtures of quinapril and HCTZ in the presence of its degradation products has been developed and validated. The method has the required linearity, precision, accuracy, selectivity, robustness, LOD and LOQ necessary for the quantitative determination of quinapril and HCTZ in drug substance and tablets. Thus the developed method can be used for routine analysis and to check the stability of samples of quinapril and HCTZ.

The results obtained from the stress testing show that quinapril is unstable under hydrolysis and thermal stress. Therefore, care should be taken in the manufacturing process and during storage of this product in order to avoid degradation, because if the drug is degraded it could result in diminution of the therapeutic activity and safety.

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