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Original article

VALIDATION OF LIQUID CHROMATOGRAPHIC METHOD FOR SIMULTANEOUS DETERMINATION OF QUINAPRIL AND HYDROCHLOROTHIAZIDE IN PHARMACEUTICAL DOSAGE FORMS

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Abstract

The aim of this study was to develop a reversed-phase high performance liquid chromatographic (RP-HPLC) method for the simultaneous determination of quinapril (QNP) and hydrochlorothiazide (HCZ) in pharmaceutical dosage forms. In this method quinapril, hydrochlorothiazide and perindopril (internal standard) were separated using a reversed phase column (Hichrom C18; 250×4.6 mm i.d.; 10 µm) with acetonitrile: potassium dihydrogen phosphate (at pH 2.5; 0.067 M) (40:60 v/v) as a mobile phase. UV visible dedector set at 211 nm and mobile phase was pumped at 1.0 mL/min flow rate. The chromatographic separation was performed at 25 °C. In these conditions the retention times for quinapril, hydrochlorothiazide and perindoprile were 4.391, 3.237 and 3.931 min, respectively. Linearity was obtained in the concentration range of 2-30 µg/mL for quinapril and 1.25-18.75 µg/mL for hydrochlorothiazide. The proposed method has been fully validated and allows a number of cost- and time-saving benefits. It was successfully applied to the determination of QNP and HCZ in synthetic mixtures and in pharmaceutical dosage forms. The proposed method is simple, rapid and suitable for quality control (QC) applications.

Key words: *Quinapril, Hydrochlorothiazide, RP-HPLC, Simultaneous determination, Validation.*

Kinapril ve Hidroklorotiazidin Farmasötik Dozaj Formlarından Eş Zamanlı Tayini İçin Sıvı Kromatografisi Metot Validasyonu

Bu çalışmanın amacı kinapril ve hidroklorotiazidin farmasötik dozaj formlarından eş zamanlı tayini için ters faz yüksek performans sıvı kromatografisi yöntemi geliştirmektir. Bu yöntemde kinapril, hidroklorotiazid ve perindopril (iç standart) bir ters faz kolonu (Hichrom C18; 250×4.6 mm; 10 µm) ile hareketli faz olarak asetonitril: potasyum dihidrojen fosfat (pH 2.5; 0.067 M) (40:60, h/h) kullanılarak ayrılmıştır. UV görünür bölge dedektörü 211 nm'ye ayarlanmış ve mobil faz 1.0 mL /dak akış hızıyla pompalanmıştır. Kromatografik ayrım 25°C' de gerçekleştirilmiştir. Bu şartlarda kinapril, hidroklorotiazid ve perindopril için alıkonma zamanları sırasıyla 4.391, 3.237 and 3.931 dakikadır. Doğrusallık kinapril için 2-30 µg/mL ve hidroklorotiazid için 1.25-18.75 µg/mL konsantrasyon aralığında elde edilmiştir. Önerilen yöntem tamamen valide edilmiş ve maliyet ve zaman tasarrufu sağladığı bulunmuştur. Yöntem sentetik karışımlardaki ve farmasötik dozaj formlarındaki kinapril ve hidroklorotiazidin tayininde başarıyla uygulanmıştır. Önerilen yöntem basit, hızlı ve kalite kontrol uygulamaları için uygundur.

Anahtar kelimeler: *Kinapril, Hidroklorotiazid, RP-HPLC, Eş zamanlı tayin, Validasyon.*

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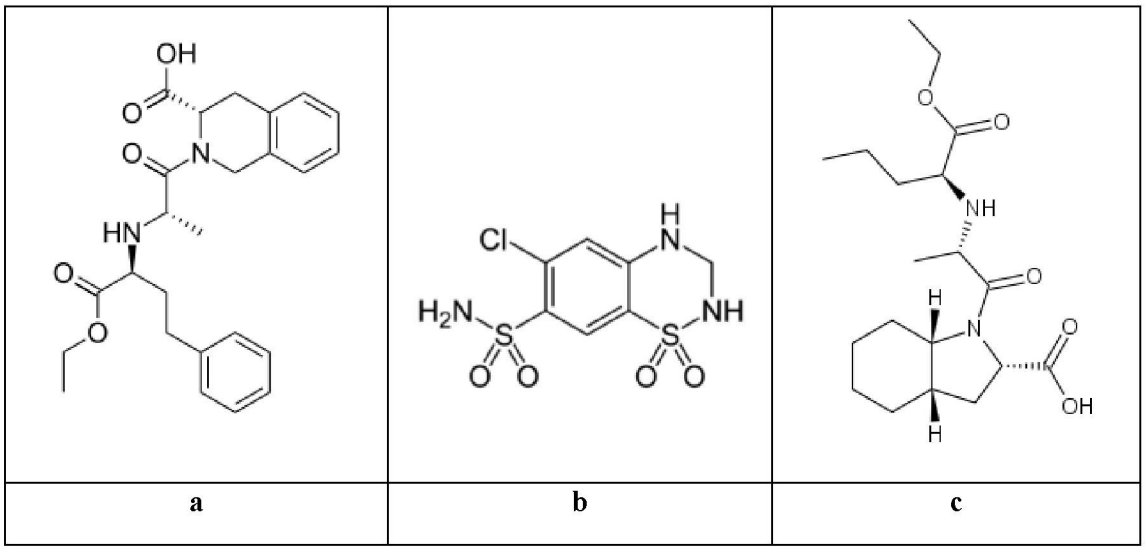
INTRODUCTION

Quinapril (QNP), (3S)-2-{N-((S)-1-ethoxycarbonyl-3-phenylpropyl)-L-alanyl}-1,2,3,4-tetrahydro-isoquinoline-3-carboxylic acid (Figure 1a), is a prodrug and an ACE inhibitor. Hepatic esterases transforms quinapril into active metabolite quinaprilat. QNP arrives peak concentration in 1 hour (1, 2). QNP inhibits angiotensin converting enzyme, an enzyme which catalyses the formation of angiotensin II from its precursor, angiotensin I. Angiotensin II is a powerful vasoconstrictor and increases blood pressure through a variety of mechanisms. Due to reduced angiotensin production, plasma concentrations of aldosterone are also reduced, resulting in increased excretion of sodium in the urine and increased concentration of potassium in the blood (3).

Hydrochlorothiazide (HCZ), 6-chloro-3,4-dihydro-2H-1, 2, 4-benzothiadiazine-7-sulphonamide-1,1-dioxide (Figure 1b), is a thiazide diuretic. Thiazides are moderately potent diuretics and show their diuretic effect by reducing the reabsorption of electrolytes from the renal tubules (4). HCZ is fairly rapidly absorbed from the gastrointestinal tract. It is reported to have a bioavailability of about 65 to 70%. It has been estimated to have a plasma half-life of between about 5 and 15 hours and appears to be preferentially bound to red blood cells. It is excreted mainly unchanged in the urine.

Hydrochlorothiazide and the other thiazide diuretics are used in the treatment of hypertension either alone or with other antihypertensives such as ACE inhibitors (like QNP) and beta blockers. They are also used to treat oedema associated with heart failure and with renal and hepatic disorders.



a	b	c

Figure 1. Chemical structures of quinapril (a), hydrochlorothiazide (b) and perindopril (c)

There have been a lot of issue for the determination of QNP and HCZ individually, in combination together or with other compounds. Also there have been some studies QNP and its metabolite quinaprilat but there have been no reports concerning the simultaneous determination of QNP and HCZ using HPLC-UV/visible technique from pharmaceutical dosage forms. Simultaneous determination studies for QNP and HCZ were liquid chromatographic-tandem mass spectrophotometric (5), derivative spectrophotometric (6), ratio spectra derivative spectrophotometric and chemometric (7), densitometric (8) and high performance thin layer chromatographic (HPTLC) (9) methods.

HPLC methods are useful in the determination of drugs in pharmaceutical dosage forms and biological fluids. Owing to the widespread use of HPLC in routine analysis, it is important that good HPLC methods are developed and thoroughly validated (10-13).

In the present study, simple, economical, accurate, reproducible and fully validated analytical method with good detection ranges for simultaneous estimation of QNP and HCZ in pure form, synthetic mixture and in its solid dosage forms was developed. The proposed method was aimed at developing an easy and rapid assay method for QNP and HCZ without any time-consuming sample preparation steps for routine analysis, to be adopted in quality control laboratories and, at the same time, ensure satisfactory recovery during drug estimation from pharmaceutical forms. In the proposed RP-HPLC method, there is no need to extract QNP and HCZ from the excipients matrix of pharmaceutical dosage forms, thereby decreasing the error in quantitation.

EXPERIMENTAL

Chemicals and reagents

All chemicals and solvents were of analytical reagent grade. QNP, HCZ and its pharmaceutical dosage form were kindly provided by Pfizer (Istanbul, Turkey). Perindopril used as the internal standard (IS) (Figure 1c) was kindly supplied by Msn Lab (Istanbul, Turkey).

HPLC grade acetonitrile, methanol (Merck, Darmstadt, Germany), and Milli-Q water were used for preparing mobile phase solutions. All other chemicals were commercial analytical reagent grade quality (Merck or JT Baker).

Apparatus and conditions

The HPLC analysis was performed on a Shimadzu HPLC system and this system consists of a pump (Shimadzu 2550), a UV visible dedector (Shimadzu 2487) operating at 211 nm and an automatic sample injection system with 20 µL injection volume (Hewlett-Packard, Avandale, PA). The chromatographic separation was performed at 25 °C using Hickrom C18 (4.6× 250 mm, 10 µm) analytical column. The mobile phase was a mixture of acetonitrile:0.067 M KH_2PO_4 buffer and adjusted to pH 2.5 (40:60, v/v). The flow rate was 1.0 mL/min. Perindopril was used as an IS. A 20 µL amount of each solution was injected and chromatograms were recorded.

Preparation of standard solutions and calibration

Stock solutions of QNP (0.2 mg/mL), HCZ (0.125 mg/mL) and perindopril (0.2 mg/mL) were prepared in mobile phase. The concentration ranges of QNP and HCZ were 2.00-30.00 and 1.25-18.75 µg/mL, respectively and the concentration of perindopril was at a constant level of 20 µg/mL. All dilutions from standard solutions were made with mobile phase. The calibration curves were obtained by plotting the ratio of the peak area of QNP or HCZ to that of perindopril against the drugs concentrations.

Procedure for tablets

Ten tablets of Accuzide® (each tablet contains 20 mg QNP and 12.5 mg HCZ) were accurately weighed and powdered in a mortar. Portion equivalent to a tablet was weighed and transfered to 100 mL volumetric flask and completed to 100 mL with mobile phase. It was sonicated for 10 min. This solution was filtered from 0.2 µ RC filter. 1 mL of clear filtrate and appropriate perindopril solution put into a volumetric flask and diluted to 10 mL with mobile phase. The amounts of QNP and HCZ were calculated from the corresponding regression equations.

Recovery studies from tablets and laboratory-made mixtures

To study the accuracy of the proposed methods and to check the interference from excipients used in the dosage form, recovery experiments were carried out by the standard addition method. For this reason known amounts of pure QNP and HCZ and a fixed amount of perindopril were added to tablet dosage forms. The mixture was analyzed to understand whether the method influenced the excipients in the tablet form.

In order to investigate the influence of the compounds to each other, laboratory-made mixtures were prepared. The recoveries from these synthetic mixtures were calculated for each compound.

Validation procedures of the method

The proposed method was validated according to the ICH guidelines (14). The validation parameters were linearity, range, selectivity, accuracy, precision, limit of detection (LOD) and limit of quantification (LOQ). Intra-day and inter-day precision parameters were calculated at 30 μg/mL and 18.75 μg/mL of QNP and HCZ respectively, six times on the same day and on three separate days to obtain the relative standard deviations (RSD %). Accuracy was determined with recovery studies as it was mentioned above. Percent recoveries for both drugs was calculated by comparing the area before and after the addition of the standard drugs.

The LOD and LOQ of the procedure (shown in Table 1) were calculated according to the 3 s/m and 10 s/m criteria, respectively, where 's' is the standard deviation (SD) of the response of the sample and 'm' is the slope of the corresponding calibration graph.

System suitability for the proposed RP-HPLC method was evaluated. A system suitability test ensures that the method can generate results of acceptable accuracy and precision. The requirements for system suitability are usually designed after method development and validation have been completed. The criteria selected will be based on the performance of the method (shown in Table 2).

RESULTS AND DISCUSSION

RP-HPLC method

Drug analysis is undertaken during various phases of pharmaceutical development, such as formulation and stability studies, quality control and pharmacological testing in humans and animals. All of these investigations require reliable, accuracy, precision and validated analytical methods in order to determine drugs in pharmaceutical dosage forms and biological fluids. In HPLC methods, precision and accuracy can often be enhanced by the use of an appropriate IS, which also serves to correct for fluctations in the detector response (10-13). Ideally, an IS should display similar physicochemical properties compared to the analytes. However, we did not obtain good resolution and peak shape with similar compounds. The structure of perindopril (Figure 1c) is not similar to QNP and HCZ; it was chosen as the IS because it had a shorter retention time with better peak shape and resolution than other potential ISs.

In this paper, a chromatographic method was developed for the simultaneous determination of QNP and HCZ on Hichrom C 18 column. To develop a reliable method, a lot of conditions were optimized including stationary phase, organic compound of mobile phase and ratio, internal standard, mobile phase flow rate. To determine the stationary phase, Hichrom C 18 (250 × 4.6 mm, 10 μ) and Thermo MOS-2 (250 ×4.6 mm, 5 μ) columns were tested. Hichrom C 18 (250 × 4.6 mm, 10 μ) column was chosen because of the better peak symmetry ratio. For organic compound of mobile phase, methanol and acetonitrile was checked and acetonitrile was selected. Than different ratios of acetonitrile were studied (20, 30, 40, 50 %; v/v) and 40 % (v/v) acetonitrile was approved. This ratio of acetonitrile provided shorter analysis time. Finally mobile phase ratio was found to be acetonitrile:KH_2PO_4 (0.067 M; at pH 2.5) (40:60; v/v). Around the flow rates of 0.9; 1.0; 1.1 mL/min, 1.0 mL/min was selected as an optimum flow rate and perindopril was selected as IS. The optimum wavelength for detection was 211 nm, at which the best detector responses for both drugs and the IS were obtained. As shown in Figure 2a, the retention times were 4.391 min for QNP, 3.237 min for HCZ and 3.931 min for perindopril (IS).

Table 1. Characteristics of the linear regression analysis of QNP and HCZ in mobile phase

	QNP	HCZ
Retention time (min)	4.391	3.237
Linearity range (µg/mL)	2.00 – 30.00	1.25 – 18.75
Slope	0.0744	0.1135
Intercept	0.0080	0.2054
Correlation coefficient	0.9995	0.9993
SE of slope	0.0005	0.0001
SE of intercept	0.0113	0.0044
Limit of detection (µg/mL)	0.0195	0.0030
Limit of quantification (µg/mL)	0.0639	0.0098
Within-day precision (RSD %)	0.5060	1.6110
Between-day precision (RSD %)	0.6520	1.3040
Equipment precision (RSD %)	0.0750	0.0670

According to U.S. Pharmacopeia (USP) 24, method <621>, system suitability tests are an integral part of an LC method. System suitability tests are used to verify that the resolution and reproducibility of the chromatographic system are adequate for the analysis. System suitability tests were carried out on freshly prepared standard stock solutions of QNP and HCZ. The results from system suitability tests are presented in Table 2 for each drug. Typically, at least two of these criteria are required to demonstrate system suitability for the proposed method.

Table 2. System suitability parameters of the proposed RP-LC method

Parameters	Observed value			Recommended value
	HCZ	IS	QNP	
Retention time (min)	3.237	3.931	4.391	
Resolution (Rs)	-	2.900	2.190	> 2
Theoretical plates (N)	2138	6181	6374	> 2000
Selectivity factor (α)	-	1.888	1.311	> 1
Asymmetry factor (A)	1.120	1.080	1.100	0.95 – 1.20

The calibration curves for quinapril or hydrochlorothiazide were obtained by plotting the peak area ratio of QNP or HCZ to perindopril versus concentrations of quinapril or hydrochlorothiazide. QNP and HCZ were linear over the range of 2.00-30.00 µg/mL and 1.25-18.75 µg/mL, respectively (r = 0.999). Table 1 includes the calibration data and related validation parameters. The LOD and LOQ values, as well as intraday and interday precision data, are also given in Table 1. Interday variability was assessed on three different days over a period of 2 weeks (Table 1). The variation in retention times among five replicate injections of QNP and HCZ standard solutions was very low, i.e. RSD 0.86 and 0.99%, respectively. The results obtained from system suitability tests are in aggrement with the USP requirements.

In order to demonstrate the validity and applicability of the proposed RP-HPLC method, recovery tests were carried out by analyzing synthetic mixtures of QNP and HCZ in reproduced different composition ratios (Table 3).

Table 3. Determination of QNP and HCZ in laboratory-made mixtures

Added (µg/mL)		Found (µg/mL)		Recovery (%)	
QNP	**HCZ**	**QNP**	**HCZ**	**QNP**	**HCZ**
20.00	1.25	-	1.23	-	98.40
20.00	5.00	-	5.05	-	101.00
20.00	6.25	-	6.18	-	98.88
20.00	12.50	-	12.45	-	99.60
20.00	18.75	-	18.65	-	99.46
Mean recovery (%)					99.47
RSD (%)					0.88
Bias (%)					0.53
2.00	12.50	2.05	-	102.50	-
8.00	12.50	8.10	-	101.25	-
16.00	12.50	16.10	-	100.63	-
20.00	12.50	20.29	-	101.46	-
30.00	12.50	30.10	-	100.33	-
Mean recovery (%)				100.23	
RSD (%)				1.21	
Bias (%)				-0.23	

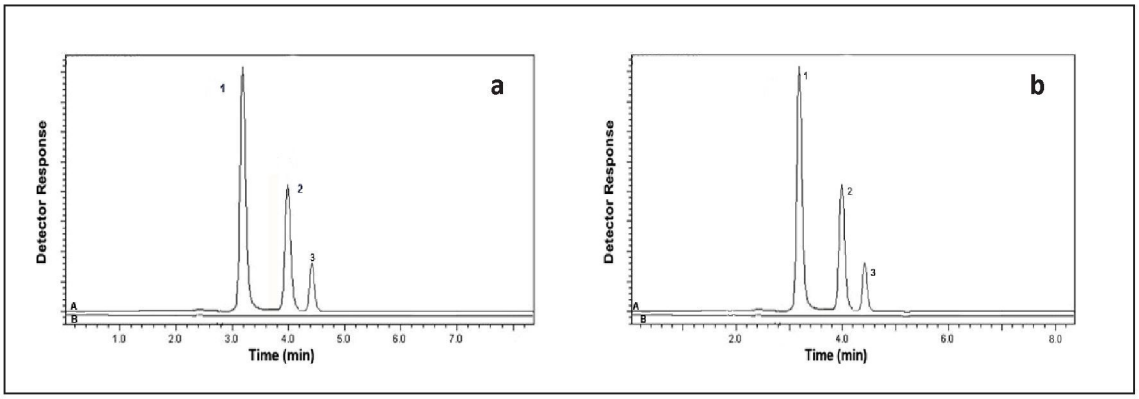


Figure 2. Chromatograms obtained from the mobile phase (B), standard solutions (a, A) and pharmaceutical dosage forms (b, A) containing 12.5 µg/mL HCZ (1), 20 mµ/mL IS (2) and 20 µg/mL QNP (3).

Analysis of tablets

Developed method could be used for the simultaneous determination of QNP and HCZ in the presence of each other and without prior separation of the excipients. Each tablet contained 20 mg QNP, 12.5 mg HCZ and the inactive ingredients: candelilla wax, crospouidone, hydroxypropyl cellulose, hypermellose, iron oxide red, iron oxide yellow, lactose, magnesium carbonate, magnesium stearate, polyethylene glycol, povidone and titanium oxide (15).

Figure 2b shows a typical chromatogram obtained for analysis of QNP and HCZ in tablets. As shown in Figure 2b, the substances formed well-shaped, symmetrical single peaks that were well-separated from the mobile phase front. Also, no interfering peaks were obtained in the chromatogram due to tablet excipient (Table 4).

Results obtained from the proposed method for the analysis of both drugs in tablets indicate that the proposed technique can be used for simultaneous quantitation and routine quality control analysis of this binary mixture in the pharmaceutical.

Recovery studies were also carried out to determine accuracy and precision of the proposed method. The recovery procedure was carried out by spiking already analyzed samples of tablets with known concentrations of standard solutions of QNP and HCZ. The results of the recovery analysis are tabulated in Table 4. It is concluded that the proposed method is sufficiently accurate and precise enough to be applied to the tablet forms. High recovery data show that the developed method is accurate and free from interference by excipients used in the formulations.

Table 4. Resuls of the assay and the recovery analysis of QNP and HCZ in tablet

	QNP	HCZ
Labeled claim (mg)	20.00	12.50
Amount found (mg)	20.02	12.46
RSD (%)	0.79	1.20
Bias (%)	-0.10	0.32
Added (mg)	20.00	12.50
Found (mg)	20.02	12.46
Recovery (%)	100.10	99.70
RSD % of recovery	0.59	0.89
Bias (%)	-0.10	0.30

Precision and accuracy studies were performed at 30 µg/mL for QNP and at 18.75 µg/mL for HCZ. Within-day and between-day precision results can be seen in Table 1.

CONCLUSIONS

The RP-HPLC method enable the simultaneous determination of QNP and HCZ with good accuracy and precision, either in laboratory-made samples or in the pharmaceuticals. The proposed HPLC method gives a good resolution between QNP, HCZ and the IS with in 5 min. Developed method is suitable for quality control laboratories, where economy and time considerations are essential. High recovery shows that the method is free from the interferences of the commonly used excipients and additives in the formulations of drugs. In addition, the run times are suitable for processing numerous samples on a daily basis.

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