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Simultaneous determination of quinapril and its active metabolite quinaprilat in human plasma using high-performance liquid chromatography with ultraviolet detection

Ch. Abbara, G. Aymard*, S. Hinh, B. Diquet

Laboratoire de Pharmacocinétique, Service de Pharmacologie, Centre Hospitalier et Universitaire de la Pitié-Salpétrière Assistance-Publique Hôpitaux de Paris, 47–83 Boulevard de l'Hôpital, 75634 Paris Cedex 13, France

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Abstract

A high-performance liquid chromatography (HPLC) procedure for the simultaneous determination of quinapril and its active metabolite quinaprilat in human plasma samples is described. A one-step solid-phase extraction (SPE) with C_{18} cartridges was coupled with a reversed-phase HPLC system. The system requires two mobile phases composed of tetrabutyl ammonium hydrogensulfate (10 m*M* adjusted to pH 7)–acetonitrile (62:38, v/v) for quinapril, and (25:75, v/v) for quinaprilat elution through a C_{18} Symmetry column and detection at a wavelength of 215 nm. Calibration curves were linear over the ranges 20 to 1000 ng/ml for quinaprilat and 10 to 500 for quinapril. The limits of quantification were 20 and 10 ng/ml for quinaprilat and quinapril, respectively. Extraction recoveries were higher than 90% for quinapril and 80% for quinaprilat. This method has been successfully applied to a bioequivalence study of quinapril in healthy subjects. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Quinapril; Quinaprilat

1. Introduction

Quinapril HCl, 2-(2-{[1-(ethoxycarbonyl)-3-pheylpropyl]amino}-1-oxopropyl)-1, 2, 3, 4-tetra-hydro-3-isoquinoline carboxylic acid monohydrochloride, is a potent orally active non-sulfhydryl non-peptidic angiotensin converting enzyme (ACE) inhibitor. It is used for the treatment of hypertension and congestive heart failure [1]. Quinapril is a prodrug which is hydrolysed to the pharmacologically active metabolite, quinaprilat [2].

Quinapril alone (without quinaprilat) has been assayed by ion-pair high-performance liquid chromatography (HPLC) with UV detection in the pharmaceutical form [3].

Quantification of quinapril and quinaprilat in human plasma has been accomplished by various techniques including HPLC with fluorescence [4] or radiochemical detection [5] and by gas chromatography (GC) with negative-ion chemical ionization mass spectrometry (MS) [2] or electron-capture detection (ECD) [6].

*Corresponding author. Fax: +33-1-4216-1688.

E-mail address: pharmaco.cinetique@psl.ap-hop-paris.fr (G. Aymard).

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All of these methods are complex as they require a derivatization step and time-consuming extraction procedures [2,4–6].

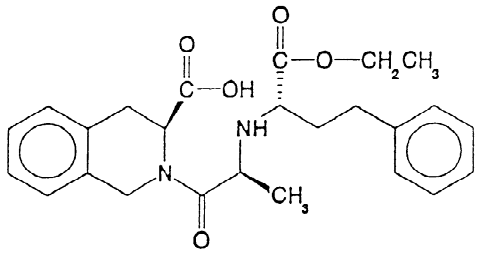
For a GC–MS method [2], the limit of quantification was 0.05 ng/ml but this method is reported for the trace analysis of quinapril and quinaprilat [2].The limit of quantification obtained from the other methods [4–6] varied from 1 to 20 ng/ml whatever the molecule.

We present a new HPLC method using UV detection after a single extraction step without derivatization procedure for use with standard equipment. Moreover it was used for a bioequivalence study in healthy subjects.

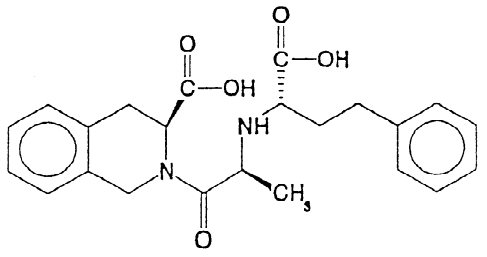
2. Experimental

2.1. Chemicals

Quinapril hydrochloride, and its active metabolite quinaprilat (Fig. 1) were a kind gift of Parke Davis.



Quinapril



Quinaprilat

Fig. 1. Chemical structures of quinapril and quinaprilat.

Stock solutions (1 mg/ml) of each compound were prepared by dissolving 10 mg of equivalent free and pure base of each substance in 10 ml of methanol. Stored at −20°C in the dark, these solutions were shown to be stable during the period of study (about 4 months). The working solution was a mixture of quinapril and quinaprilat; the stock solutions were diluted with methanol to give working solutions at concentrations of 100 and 50 μg/ml for quinaprilat and quinapril, respectively. Because of the photosensitivity of both molecules, bright light has to be avoided during the preparation of solutions.

Acetonitrile and methanol (Carlo Erba, Milan, Italy) were HPLC grade. Tetrabutyl ammonium hydrogensulfate (Sigma, USA), diethylamine (Merck, Hohenbrunn, Germany), KH_2PO_4 (Merck, Darmstadt, Germany), H_3PO_4 (Merck, Darmstadt, Germany) were analytical-reagent grade. Distilled water was purchased from Fresenius France Pharma (Louvier, France). Drug-free human plasma was obtained from the hospital blood bank.

2.2. Instrumentation and chromatographic conditions

The HPLC system consisted of two 114 pumps (Beckman, Berkeley, CA, USA) set at flow-rates of 1.5 ml/min, a wisp 717 plus autosampler injector (Waters, Milford, MA, USA) set at 60 μl for sample injection volume, one switch valve (LEA switch I&T, Labmetrics Technology, Roissy, France), one Waters 2487 UV detector set at 215 nm and a System Gold 2 integrator (Beckman). Chromatography was achieved at 50°C using a column heater (temperature control system, Waters) on a 5 μm C_{18} Symmetry column of 150×4.6 mm I.D. (Waters).

Molecular spectra for quinapril and quinaprilat and peak purity were checked under the chromatographic conditions using a HPLC system consisting of a P1000XR binary pump coupled to an AS3000 autosampler injector and a UV–Vis diode array detector (Spectra Focus) coupled to PC1000 software (Thermo Finnigan, CA, USA).

Two mobile phases were prepared using acetonitrile and a solution of tetrabutyl ammonium hydrogensulfate. This solution was composed of 10 m*M* tetrabutyl ammonium adjusted to pH 7 with diethylamine and filtered through a 0.22-μm filter.

The proportions of acetonitrile–tetrabutyl ammo-

nium hydrogensulfate solution were 25:75 and 38:62, v/v, for the first and the second mobile phases, respectively. The two mobile phases were degassed ultrasonically for 10 min.

The switch was connected to the AI 406-module interface piloted by the Beckman System Gold 2 software. During the injection of the sample, the switch was in position 2 (Fig. 2): the eluent from the first mobile phase was directed to the column, and the second mobile phase to waste. The switch was activated to position 1 at time 8.5 to 23 min: the first mobile phase was then directed to the waste, and the second to the column. At time 23 min the switch was set back to position 2: the column was reequilibrated for 4 min with the first mobile phase before the next injection.

At the end of each chromatographic session the system was washed with acetonitrile–water (50:50, v/v).

2.3. Validation conditions

This method was validated according to USP XXII [7] and Shah et al.'s [8] recommendations.

2.4. Preparation of the spiked plasma samples

A spiked plasma sample with quinapril and quinaprilat corresponding to the highest concentration level of the calibration points was prepared by adding 50 μl of a working solution containing quinaprilat and quinapril (50 and 25 μg/ml, respectively), to drug-free human plasma. This spiked plasma was diluted with drug-free human plasma for the calibration point and quality control (QC) sample preparations.

Calibration samples were prepared extemporaneously for each chromatographic session by diluting in a 15-ml silicone tube, known volumes (20 to 1000 μl) of spiked plasma with drug-free human plasma to obtain a 1 ml final volume for each calibration sample. Calibration curves, based on peak height, were constructed for each assay. The linearity was assessed by unweighted least-squares regression analysis.

QC samples were prepared at the same time and stored with the subjects plasma samples at −20°C until assayed. For each molecule, three levels of QC concentrations were prepared: low level at 25 and 50 ng/ml, medium level at 250 and 500 ng/ml, and high level at 500 and 1000 ng/ml for quinapril and quinaprilat, respectively. Two QC samples of each level were used for the in-study validation. These QC samples were also used in the pre-study validation for the two molecules.

The linearity range (upper part of the calibration curves) was tested by analysis of individual spiked plasma samples containing 2000 and 1000 ng/ml of quinaprilat and quinapril, respectively.

2.5. Sample extraction procedure

C_{18} extraction columns (Supelco–Sigma–Aldrich, St. Louis, MO, USA) were placed on a vacuum elution manifold and washed with 3 ml of methanol, 3 ml of water and 1 ml of KH_2PO_4–H_3PO_4 buffer

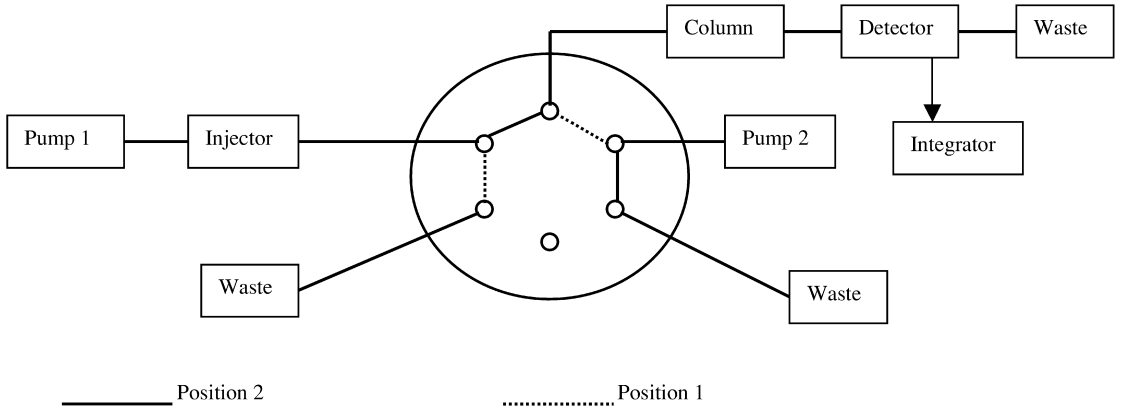


Fig. 2. Chromatographic system.

(pH 3.7). Care was taken that the columns did not dry before plasma loading. Then a mixture of 1 ml plasma sample and 1 ml of buffer was loaded onto the column. The cartridge was washed with 3 ml of water and dried for 10 min. Elution was carried out with 2×0.5 ml of methanol in a 5-ml silicone tube. The methanolic solution was evaporated under a stream of nitrogen in water bath at 40°C. The residue was reconstituted with 200 μl of the first mobile phase and vortex-mixed before injection.

2.6. Subject samples

Twenty healthy subjects (10 male and 10 female) were enrolled in a PK monocenter open-labeled non-placebo controlled cross-over two-treatment two-period study. The study aim was to assess the bioequivalence of two formulations of quinapril after a single oral dose of 40 mg given as a single 40 mg tablet (new formulation) or two 20 mg tablets (marketed formulation) with a 1 week wash out period.

The subjects were administered the drug at 08:00 h. Blood samples were collected in lithium heparinized tubes at t_0, $t_{0.5}$, $t_{0.75}$, t_1, $t_{1.25}$, $t_{1.5}$, $t_{1.75}$, t_2, $t_{2.25}$, $t_{2.5}$, t_3, t_4, t_6, t_8, t_{10}, t_{12}, t_{16} and t_{24} hours following each administration. The samples were centrifuged and plasma was separated and stored at −20°C until analysis.

All the samples of each subject (two periods) were assayed in the same chromatographic session, with six QC and nine calibration samples (51 samples/run).

3. Results and discussion

3.1. Selectivity and specificity

Fig. 3 shows at t_0 and $t_{0.75\ h}$, chromatograms of a subject receiving 40 mg of quinapril and a chromatogram of spiked plasma containing 25 ng/ml of quinapril and 50 ng/ml of quinaprilat.

The peaks of quinapril and quinaprilat were well separated. Under the described conditions, the retention times were 7 and 14 min for quinaprilat and quinapril, respectively. Blank plasma t_0 shows no interfering peak at the retention time of the studied molecules.

No specific study was performed to evaluate the interference with the other drugs that are usually co-administered with quinapril since the method was developed for a pharmacokinetic study in healthy subjects.

To improve the specificity and the resolution, an ion-pair chromatography was performed. At the pH of the mobile phases (7), ion-pairs were formed using tetrabutyl ammonium hydrogensulfate (counter ion). When using a mobile phase containing KH_2PO_4–H_3PO_4 buffer (10 m*M*, pH 3)–acetonitrile (75:25, v/v), the retention times of both molecules were shorter as compared to those obtained after adding the counter ion (4.5 vs. 7.6 min for quinaprilat and 8 vs. 14.3 min for quinapril). Unfortunately interfering peaks were observed in the blank and broadening of both peaks was noticed.

To shorten the run time, the two pumps and the switch were replaced by a binary gradient pump. The initial conditions were 25% acetonitrile and a percentage of 50% acetonitrile was achieved in 15 min. Under these conditions, in spite of the shorter run time (20 min) and sharper peaks of quinapril and quinaprilat, the noise signal was increased and interfering peaks were noticed in the blank, hence no improvement of the sensitivity (signal-to-noise ratio) was achieved. The high noise could probably be related to the degassing system (vacuum membrane degasser) used at 215 nm.

3.2. Stability

The methods previously published suggest a storage at +4°C for the stock solutions. However, after injecting the solutions prepared from the stock solutions stored at this temperature during 2 months, the area of quinaprilat peak was smaller and an unknown peak appeared in the chromatogram at 11 min. This peak corresponds to a quinaprilat degradation product (Fig. 4) since the quinapril peak area was unchanged. In addition, the molecular spectra of quinapril, quinaprilat and this degradation product, obtained with diode array detector, were strictly similar. This degradation product does not appear in the chromatogram obtained from the stock solution freshly prepared. Therefore, the stock solutions for

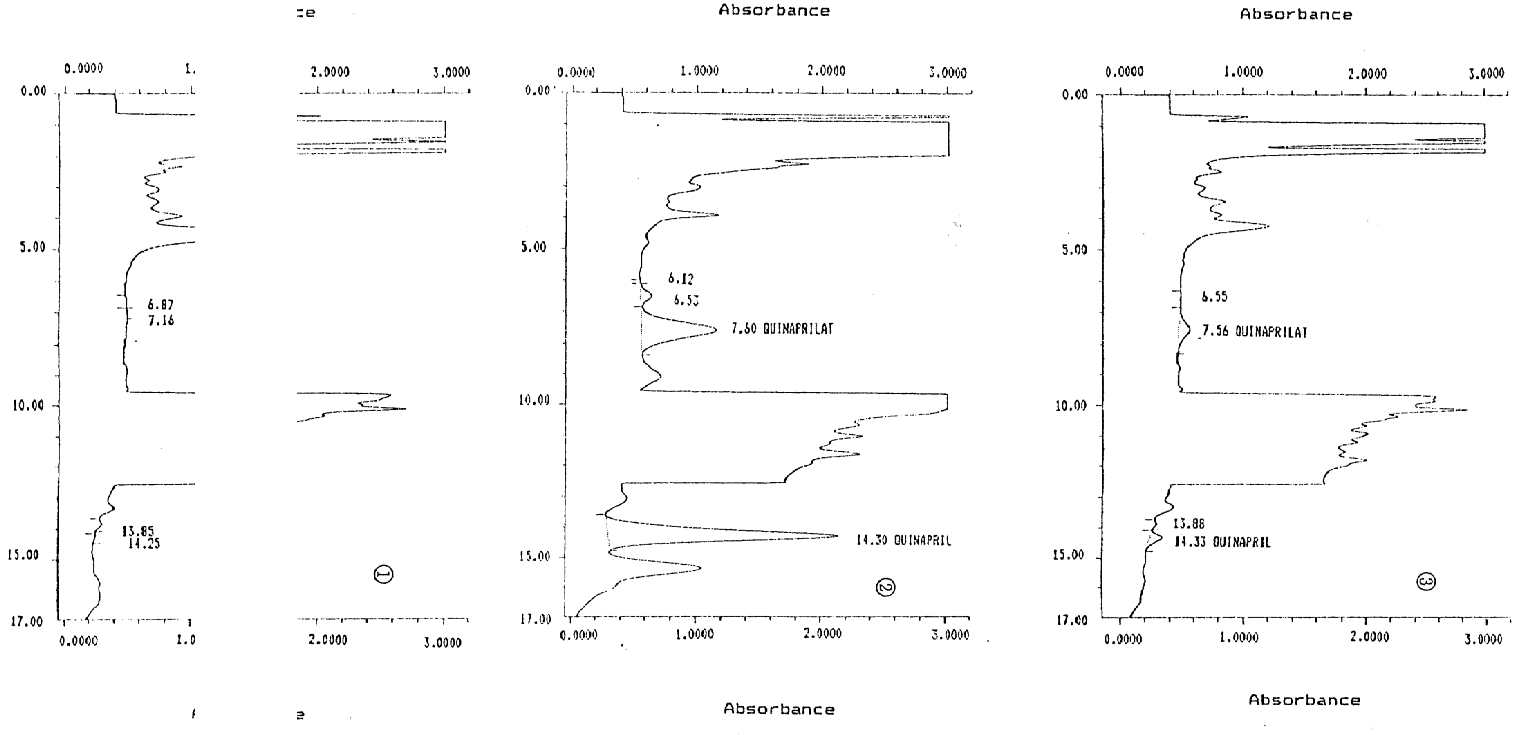


Fig. 3. Chromatograms obtained under described conditions from (1) subject sample at t_0; (2) subject sample at $t_{0.75\ h}$ containing 303 ng/ml of quinapril and 522 ng/ml of quinaprilat; (3) spiked plasma containing 25 ng/ml of quinapril and 50 ng/ml of quinaprilat (calculated concentrations are 26 and 48 ng/ml, respectively).

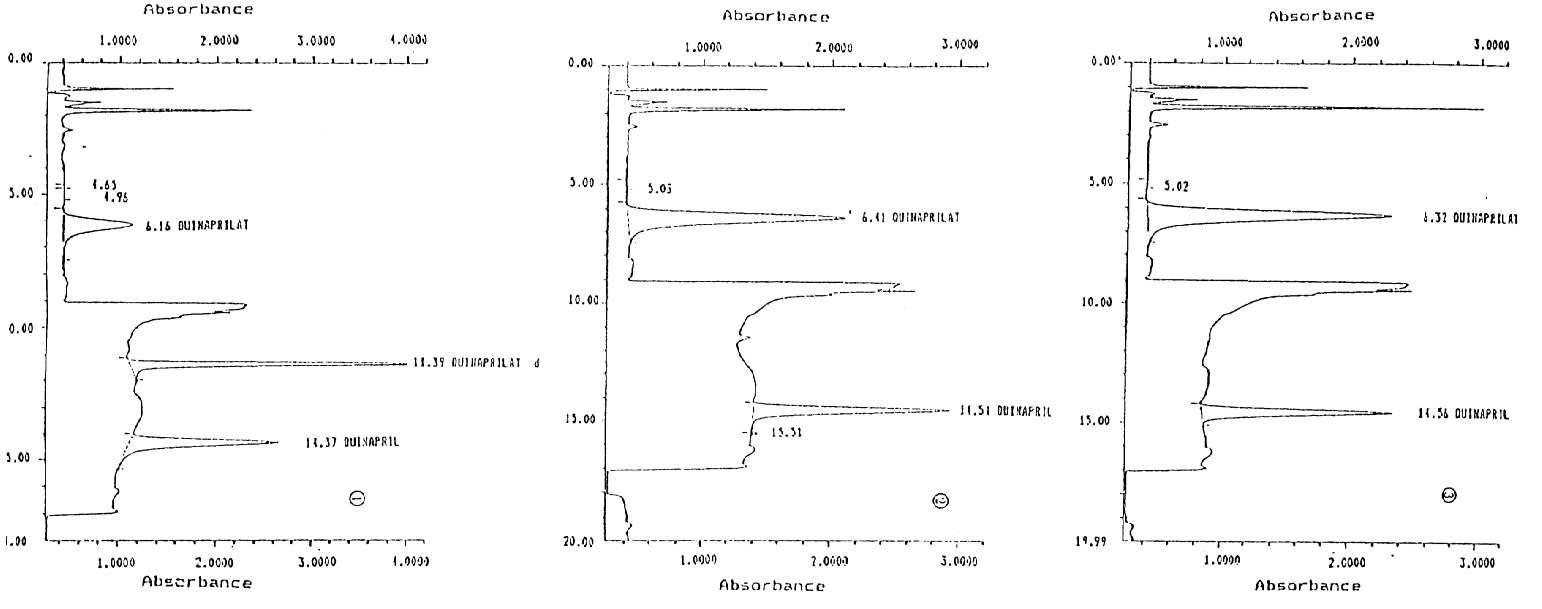


Fig. 4. Chromatograms obtained from the direct injection containing quinapril and quinaprilat at 2500 and 5000 ng/ml, respectively, in the first mobile phase of: (1) stock solution stored at +4°C for 2 months; (2) stock solution stored at −20°C for 2 months; (3) stock solution freshly prepared.

both molecules were stored at −20°C, and they were shown to be stable for months.

QC samples at the three levels (for the two molecules) were stable at −20°C when avoiding the light during the whole study period (4 months).

Autosampler stability was also checked, and no degradation was observed in the 51 samples injected at each chromatographic session, so both molecules were stable in the first mobile phase for 30 h at least at room temperature.

3.3. Limit of quantification (LOQ)

The limits of quantification obtained under the described conditions were 20 ng/ml for quinaprilat and 10 ng/ml for quinapril. The previously published methods obtain LOQs varying from 1 to 20 ng/ml for both molecules using a 1-ml plasma sample volume: 5 ng/ml by ECD [6]; 20 ng/ml by fluorescence detection [4]; or 1 ng/ml by radiochemical detection [5].

3.4. Linearity and reproducibility

Calibration curves were linear in the range of 20 to 1000 ng/ml for quinaprilat and from 10 to 500 ng/ml for quinapril. Correlation coefficients were higher than 0.99 in these ranges of concentration during pre-study and in-study validation. When the spiked plasma tested for the maximum linearity range for quinaprilat and quinapril was included in the calibration curves (20 to 2000 ng/ml for quinaprilat and 10 to 1000 ng/ml for quinapril), correlation coefficients were higher than 0.99 ($n=4$). The mean calculated concentrations (RSD) of this spiked plasma were 2056 ng/ml (9.3%) for quinaprilat and 1007 ng/ml (10.6%) for quinapril.

Within-day and between-day reproducibility data are reported in Table 1. Precision (RSD) and accuracy (mean deviation, %) were lower than 11.6 and 5.8%, respectively, for the two molecules.

3.5. Recoveries

The recoveries were calculated by comparing peak height of extracts to those obtained after direct injection of the same amounts of quinaprilat and quinapril. The recoveries [mean (RSD); $n=21$] were 95% (5.5%) and 86% (8.8%) for quinapril and quinaprilat, respectively. In this method the solid-phase extraction was carried out at pH 3.7. Other pH levels were tested but the recoveries were less than 70%, and interfering peaks were observed in the blank.

No internal standard was used in this method as one extraction step is not suspected to induce a large variability of the extraction recoveries; furthermore the use of an autosampler favors the low variability.

3.6. Subject samples

For pharmacokinetic purposes, over 700 plasma

Table 1
Within-day and between-day precision [mean (RSD, %)] and accuracy (mean deviation, %) for the analysis of quinapril and quinaprilat in human plasma samples ($n=7$)

Theory concentration (ng/ml)		Mean calculated concentration (RSD, %) in ng/ml and mean deviation (%)			
Quinapril	Quinaprilat	Between-day reproducibility		Within-day reproducibility	
		Quinapril	Quinaprilat	Quinapril	Quinaprilat
25	50	26 (7.6) +4.4	52 (10.3) +3.8	26 (6.6) +2.9	49 (11.6) −2.3
250	500	249 (4.2) −0.5	499 (3.5) −0.2	263 (4.2) +5.4	506 (9.5) +1.1
500	1000	503 (1.1) +0.6	996 (2.4) −0.4	521 (5.6) +4.3	1058 (5.3) +5.8

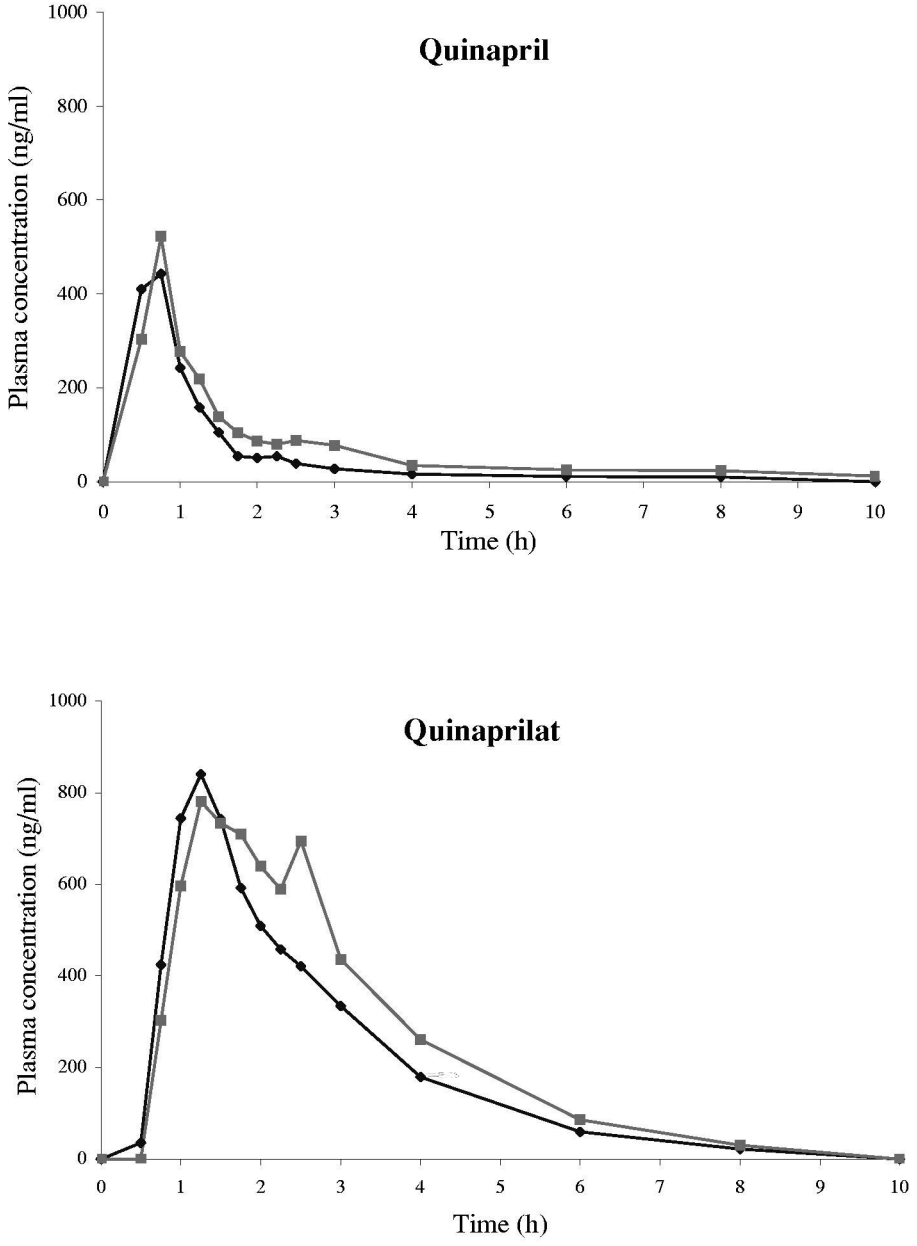


Fig. 5. Pharmacokinetic profiles of quinapril and quinaprilat from the same subject after oral administration of the new (©) and the marketed formulations (j).

samples from 20 subjects have been assayed with this method. Fig. 5 shows the pharmacokinetic profiles of quinapril and quinaprilat from one subject after oral administration of the two formulations.

4. Conclusion

The method described in this paper allows a quantification of quinapril and quinaprilat with ap-