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Short communication

Determination of quinapril and quinaprilat by high-performance liquid chromatography with radiochemical detection, coupled to liquid scintillation counting spectrometry

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Abstract

Quinapril and quinaprilat concentrations were determined in perfusate, urine, and perfusate ultrafiltrate using a specific and sensitive reversed-phase high-performance liquid chromatographic procedure with radiochemical detection, coupled to liquid scintillation counting spectrometry. Quinapril and quinaprilat were measured in perfusate and urine after pretreatment with acetonitrile and subsequent centrifugation. Perfusate ultrafiltrate was used as collected. Two quinapril diketopiperazine metabolites, PD 109488 and PD 113413, were separated chromatographically from quinapril, quinaprilat, and from each other. Assay performance for quinapril and quinaprilat was assessed by examining precision and accuracy of the assay over four days. Using a 100-μl sample volume, the limit of quantitation for both ^{3}H-quinapril and ^{3}H-quinaprilat (sp. act. ≈ 2.0 μCi/μg) was 1 ng/ml.

1. Introduction

Quinapril (Accupril, Parke-Davis), the esterified prodrug of the active metabolite quinaprilat, is a nonsulfhydryl angiotensin converting enzyme (ACE) inhibitor used in hypertension and congestive heart failure therapy. Numerous assays for angiotensin converting enzyme inhibitors are available in the literature, including several methods for the determination of quinaprilat, or quinaprilat and quinapril, in various matrices. As described below, these assays vary in complexity, volume requirements and sample throughput, analytical range, method of detection, and specificity.

A radioimmunoassay method [1], specific for quinaprilat, was developed using sheep antiserum. The method does not require sample extraction, is highly specific (cross-reactivity of quinapril is 0.42%), and was validated in human plasma over the calibration range of 9.6–383 ng/ml using a 50-μl sample size. Although the assay has a high throughput and uses a small sample size, it does not quantitate quinapril, and the cross-reactivity of known quinapril metabolites has not been determined. General methods for quantitating ACE inhibitors have been developed by modifying commercial diagnostic kits (e.g. ACE Microvial Radioassay System; Ventrex

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Laboratories, Portland, ME, USA; Angiotensin Converting Enzyme Kit; Sigma, St. Louis, MO, USA) used for the determination of angiotensin converting enzyme levels in human plasma [2–4]. These radioassays measure only the inhibitory effect of the compounds in the matrix being studied and therefore are not specific. These methods are satisfactory if total inhibitory activity alone is needed or if it is known that the inhibitor studied will not form metabolites or degradation products under the conditions of the experiment.

A gas chromatographic method was developed [5] for quinapril and quinaprilat in human plasma and urine. The method uses electron-capture detection and allows simultaneous quantitation of quinapril and quinaprilat in human plasma (10–1000 ng/ml) and urine (50–2000 ng/ml) using a 1-ml sample size. This labor intensive assay involves both liquid–solid and liquid–liquid extractions as well as two derivatization steps prior to gas chromatographic analysis. It is unknown if quinapril metabolites other than quinaprilat are carried through the extractions or the derivatization steps and whether they are separated chromatographically from quinapril, quinaprilat, or the internal standard. A sensitive HPLC method with precolumn derivatization and fluorescence detection was developed [6] for quinapril and quinaprilat in human plasma and urine. Calibration curves were linear over the range of 20–1000 ng/ml for plasma and 100–2000 ng/ml for urine using a 1-ml sample size. Similar to the gas chromatographic method described above, this method is labor intensive and it is unknown if quinapril metabolites other than quinaprilat are carried through the extractions or derivatization steps and whether these metabolites are separated chromatographically from quinapril, quinaprilat, or the internal standard.

For our isolated perfused rat kidney (rat IPK) studies [7,8] we required a method of analysis that would simultaneously measure quinapril and its metabolites at low levels of detection using small sample volumes. None of the previously described assays were suitable for this purpose. The matrices studied included perfusate consisting of 6% bovine serum albumin [9], urine, and perfusate ultrafiltrate. The assay developed was specific, accurate, precise, and allowed for high sample throughput. It employed reversed-phase high-performance liquid chromatography with radiochemical detection (HPLC-RD), coupled to liquid scintillation counting (LSC) spectrometry. The method was sensitive enough to measure clinically relevant concentrations of quinapril and quinaprilat with minimal sample volume (100 μl used for analysis), and allowed for the detection of two diketopiperazine metabolites of quinapril. It is anticipated that the technique of combining HPLC-RD with LSC, as described in this paper, will also prove useful for radiolabeled mass balance, pharmacokinetic, and drug disposition studies in animals and humans.

2. Experimental

2.1. Reagents and materials

All chemicals were of analytical-reagent grade, unless noted otherwise. Quinapril and quinapril metabolites were obtained from Parke-Davis Pharmaceutical Research (Division of Warner-Lambert Company, Ann Arbor, MI, USA). Tritiated quinapril and quinaprilat were obtained from Amersham Buchler (Braunschweig, Germany). The purity of all quinapril related compounds was verified by HPLC and all compounds were used as received.

Quinapril

Quinapril hydrochloride (Lot W, >99.9% purity) is chemically described as [3*S*-[2[*R**(*R**)],3*R**]]-2-[2-[[1-(ethoxycarbonyl)-3-phenylpropyl]amino]-1-oxopropyl]-1,2,3,4-tetrahydro-3-isoquinolinecarboxylic acid monohydrochloride. The radiochemical purity of [^{3}H]quinapril (sp. act. 20.1 μCi/μg) was >98%. The specific activity was diluted 10-fold ($\approx$2 μCi/μg) for subsequent rat IPK studies [7,8].

Quinaprilat

Quinaprilat (Lot O, >99.9% purity), the deesterified product of quinapril, is chemically

described as [3*S*-[2[*R**(*R**)],3*R**]]-2-[2-[(1-carboxy-3-phenylpropyl)amino]-1-oxopropyl]-1,2,3,4-tetrahydro-3-isoquinolinecarboxylic acid monohydrate. The radiochemical purity of [^{3}H]quinaprilat (sp. act. 22.2 μCi/μg) was >98%. The specific activity was diluted 10-fold (≈2 μCi/μg) for subsequent rat IPK studies [7,8].

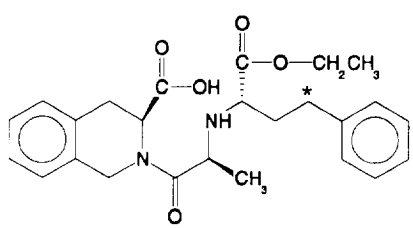
Diketopiperazine metabolites

PD 109488 (ring-closed quinapril, Lot R, 99.9% purity) and PD 113413 (de-esterified ring-closed quinapril, Lot R, 99.0% purity) are two quinapril diketopiperazine metabolites that have been identified and observed in both in vitro and in vivo systems [10,11]. While these metabolites have been found in dog, monkey and human studies, they have not been found in in vivo or in vitro rat studies. Only unlabeled PD 109488 and PD 113413 were available for study.

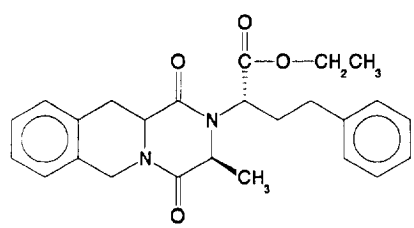
The chemical structures of [^{3}H]quinapril, [^{3}H]quinaprilat, and the two diketopiperazine metabolites of quinapril are shown in Fig. 1. Asterisks denote the location of the two tritium atoms present in each species.

2.2. Preparation of spiked reference standards

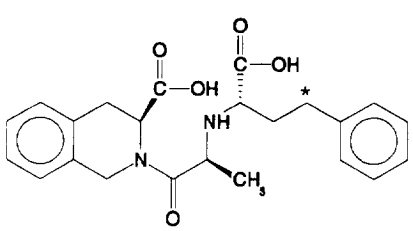
Quinapril and quinaprilat reference standards were prepared daily from separate weighings by evaporating a known amount of an aqueous standard and reconstituting in either perfusate, urine, or perfusate ultrafiltrate. Perfusate ultrafiltrate was produced by passing blank perfusate through disposable micropartition devices (Centrifree; Amicon Division, W.R. Grace and Co., Danvers, MA, USA) at 37°C.



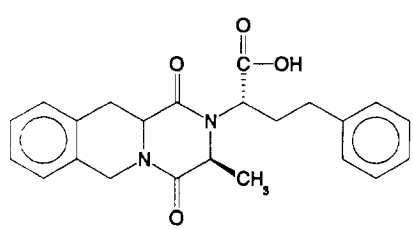
Quinapril



PD 109488



Quinaprilat



PD 113413

Fig. 1. Structures of [^{3}H]quinapril, [^{3}H]quinaprilat, PD 109488, and PD 113413. Asterisks denote the location of the two tritium atoms present in each radiolabeled species.

2.3. Sample preparation

A 100-μl aliquot of perfusate or urine was pretreated with 200 μl of acetonitrile in 1.5-ml polypropylene microcentrifuge tubes (Safe-Lock; Eppendorf/Brinkmann Instruments, Westbury, NY, USA), vortex-mixed for 5 s, sonicated for 5 min, and centrifuged at 11150 *g* for 5 min at ambient temperature. The supernatant was transferred to 300-μl polypropylene HPLC injection vials (Sun Brokers, Wilmington, NC, USA) and capped. A 300-μl aliquot of perfusate ultrafiltrate was transferred directly to 300-μl polypropylene HPLC injection vials and capped.

2.4. Apparatus and conditions

Chromatography

The reversed-phase HPLC system consisted of a solvent delivery system (Spectroflow 400; ABI Kratos Analytical Division, Ramsey, NJ, USA), an automated sampling device (ISS-100; Perkin-Elmer Norwalk, CT, USA), a programmable wavelength UV absorbance detector (Spectroflow 783; ABI Kratos Analytical Division), a flow-through radiochromatography detector system (FLO-ONE/Beta A-295; Radiomatic Instruments and Chemical Co., Meriden, CT, USA), and a reporting integrator (Model 3396A; Hewlett-Packard, Avondale, PA, USA). The absorbance detector was used only during method development to determine retention times for non-labeled drug (PD 109488 and PD 113413). The absorbance wavelength was fixed at 215 nm (maximum UV absorbance for quinapril). The tritium channel on the radiochromatography detector was set to 0–20 keV providing approximately 51.9% efficiency. The radiochromatography detector employed a 0.500-ml time-resolved liquid scintillation counting (TR-LSC) flow cell. TR-LSC technology can distinguish between β-emission spectra and energy spectra from background events. This feature allowed for higher counting accuracy, especially with low level samples.

Quinapril, quinaprilat, PD 109488, and PD 113413 were separated at ambient temperature using a C_8 column (Econosil; Alltech Associates, Deerfield, IL, USA) with 5-μm particle size, dimensions of 250 × 4.6 mm I.D., and a matching 10-mm guard column. The column contained irregularly-shaped silica, endcapped to convert any unreacted silanol functions, with a 10% carbon load. The column packing material had a 60-Å pore diameter which gave a surface area of approximately 450 m^2/g. The mobile phase was 0.01% triethylamine (pH adjusted to 2.00 with phosphoric acid)–acetonitrile (45:55, v/v), and was delivered at a flow-rate of 1 ml/min. The injection solvent wash consisted of water–acetonitrile (45:55, v/v). The mobile phase and the injection solvent wash were degassed with helium prior to use. Post-column effluent was mixed with scintillation cocktail (Flo-Scint II; Packard Instrument Co., Meriden, CT, USA) in a 1:2 ratio. A 100-μl aliquot of processed perfusate, urine, or perfusate ultrafiltrate were introduced to the system.

Total radioactivity determination

Total radioactivity was determined as follows. A 100-μl aliquot of perfusate, perfusate ultrafiltrate, or urine was combined with 15 ml of scintillation cocktail (Ready Safe; Beckman Instruments, Fullerton, CA, USA) in 20-ml glass scintillation vials. Samples were mixed thoroughly, allowed to settle in the dark for 24 h, and each counted for 20 min or until a 1%-2σ error was reached by liquid scintillation spectrometry (LS 7500; Beckman Instruments, Arlington Heights, IL, USA). Values of counts per min and quench were converted to disintegrations per minute (dpm) using an established ^{3}H-label quench curve.

2.5. Calculations

Data from liquid scintillation counting (dpm) were multiplied by fractional chromatographic areas (drug ^{3}H-peak area/total ^{3}H-peak area) from HPLC-RD assays. Total drug amounts were then calculated using the corresponding specific activities. This calculation does not require the construction of typical HPLC calibration curves.

2.6. Pilot studies

Several pilot studies were performed to examine drug recovery and stability. Recoveries of quinapril and quinaprilat from perfusion medium were determined at 1, 20, 200, and 2000 ng/ml. Recoveries of quinapril and quinaprilat from urine were determined at 20, 400, and 8000 ng/ml. Assays were performed in quadruplicate on each of four days for each matrix at each concentration. Quinapril and quinaprilat concentrations were compared with reference water standards. Recoveries were determined as [(concentration of processed sample)/(concentration of reference sample)] · 100%. The stabilities of quinapril (10 and 1000 ng/ml) and quinaprilat (10 and 1000 ng/ml) were determined in processed perfusate samples (i.e. injection solvent). Samples were analyzed in quadruplicate after storage at room temperature for up to 96 h. The stabilities of quinapril (10 and 1000 ng/ml) and quinaprilat (10 and 1000 ng/ml) in perfusion medium were determined in quadruplicate following each of three freeze–thaw cycles.

2.7. Precision and accuracy

Assay performance for quinapril and quinaprilat was assessed by examining precision (coefficient of variation, C.V.%) and accuracy (%bias) of the assay over four days. Reference standards were analyzed in quadruplicate on four separate days for a total of sixteen determinations at each of several concentrations. Quinapril and quinaprilat concentrations were examined at 1, 20, 200, and 2000 ng/ml for perfusate, at 20, 400, and 8000 ng/ml for urine, and at 10, 100, and 1000 ng/ml for perfusate ultrafiltrate. Observed versus theoretical concentrations were compared for a given sample.

3. Results and discussion

3.1. Chromatography and total radioactivity

Representative chromatograms of injections containing quinapril, quinaprilat, PD 109488, and PD 113413 are shown in Fig. 2. Total chromatographic run time was 15 min per injection with retention times of approximately 3.4, 4.3, 5.2, and 9.3 min for quinaprilat, quinapril, PD 113413, and PD 109488, respectively. Elution order was determined by comparing these retention times with those obtained by injecting individual reference compound solutions under identical chromatographic conditions. Peak shapes were symmetric and there were no interfering components in any of the chromatograms. For HPLC-RD analyses, the signal-to-noise ratio at 1 ng/ml was ≥ 5. For LSC analyses, the response was $\geq 40\times$ background.

3.2. Pilot studies

A summary of quinapril and quinaprilat recoveries from perfusate and urine are given in Table 1. In all cases, the recovery of quinapril and quinaprilat was essentially complete ($\geq 94.2\%$) and highly reproducible. Furthermore, both compounds were stable in processed perfusate and after several freeze–thaw cycles. Quinapril and quinaprilat were stable in injection solvent for at least 96 h; perfusate concentrations were unchanged following three freeze–thaw cycles.

3.3. Precision and accuracy

The precision and accuracy of the assay method for quinapril and quinaprilat is demonstrated in Table 2. Regardless of the matrix or compound, the method was quite reproducible with C.V. values ranging from 0.69 to 8.55%. The accuracy was also excellent with values for %bias ranging from −2.37 to 3.72%. Using a 100-μl sample volume, the assay was effective down to 1 ng/ml for [^{3}H]quinapril and [^{3}H]quinaprilat (sp. act. ≈ 2.0 μCi/μg).

3.4. Application of method

The proposed HPLC-RD-LSC assay has been used for the determination of quinapril and quinaprilat in perfusate, urine, and perfusate

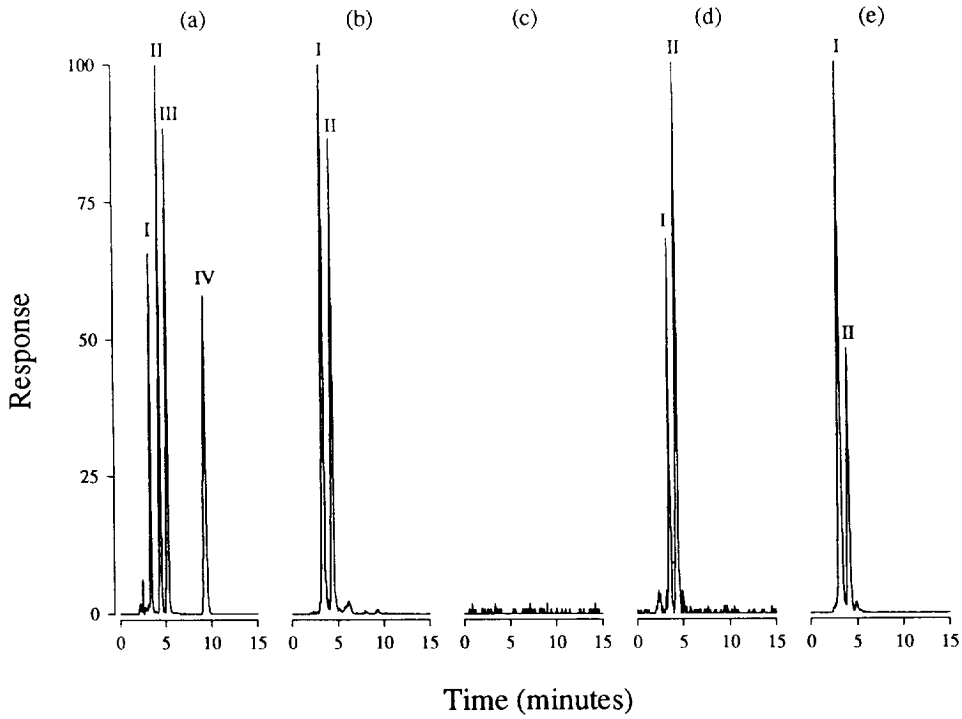


Fig. 2. Representative HPLC chromatograms from (a) an aqueous solution containing all four compounds (UV detection), (b) the same injection as (a) but with ^{3}H-label detection, (c) a blank perfusate sample, (d) a perfusate sample collected 10 min into a quinapril rat IPK study, and (e) a plasma sample collected 15 min into a quinapril dog study. Peaks: I = quinaprilat, II = quinapril, III = PD 113413, and IV = PD 109488. Response for (b)–(e) is ^{3}H-label detection.

ultrafiltrate, as derived from rat IPK studies [7,8]. Representative chromatograms of perfusate from a rat IPK experiment (200 μCi bolus dose of quinapril) is shown in Figs. 2c,d. In addition, this assay has been successfully applied to in vivo studies in dogs. A representative chromatogram of plasma from a dog experiment (500 μCi i.v. bolus dose of quinapril) is shown in

Table 1
Recovery of quinapril and quinaprilat from perfusate and urine reference standards[a]

Perfusate			Urine		
Theoretical concentration (ng/ml)	Recovery (%)		Theoretical concentration (ng/ml)	Recovery (%)	
	Quinapril	Quinaprilat		Quinapril	Quinaprilat
20	101 (3.85)	97.5 (9.06)	20	100 (4.34)	99.8 (10.9)
200	102 (1.82)	95.5 (4.53)	400	99.7 (2.46)	101 (3.27)
2000	102 (1.36)	94.2 (2.24)	8000	99.7 (1.27)	101 (1.46)

[a] Data are reported as the mean (%C.V.) of samples analyzed in quadruplicate over four separate days (n = 16).

Table 2
Precision and accuracy of quinapril and quinaprilat from perfusate, urine, and perfusate ultrafiltrate reference standards[a]

Matrix	Theoretical concentration (ng/ml)	Observed concentration (ng/ml)	
		Quinapril	Quinaprilat
Perfusate	1	1.01 (7.28)	1.01 (7.21)
		%bias: 1.16	%bias: 0.80
	20	20.3 (7.88)	20.7 (8.55)
		%bias: 1.61	%bias: 3.72
	200	198 (3.75)	196 (4.28)
		%bias: −1.19%	bias: −2.37
	2000	1990 (3.16)	1970 (2.52)
		%bias: −0.42%	bias: −1.35
Urine	20	20.5 (6.45)	20.0 (5.88)
		%bias: 2.53%	bias: 0.18
	400	395 (1.67)	395 (1.37)
		%bias: −1.29%	bias: −1.22
	8000	7900 (0.91)	8080 (0.79)
		%bias: −1.24	%bias: 1.03
Perfusate Ultrafiltrate	10	10.1 (5.40)	9.90 (5.60)
		%bias: 1.11	%bias: −0.99
	100	98.6 (2.69)	100 (2.01)
		%bias: −1.41	%bias: 0.44
	1000	1000 (1.27)	1010 (0.69)
		%bias: 0.30	%bias: 0.55

[a] Data are reported as the mean (%C.V.) of samples analyzed in quadruplicate over four separate days ($n = 16$).

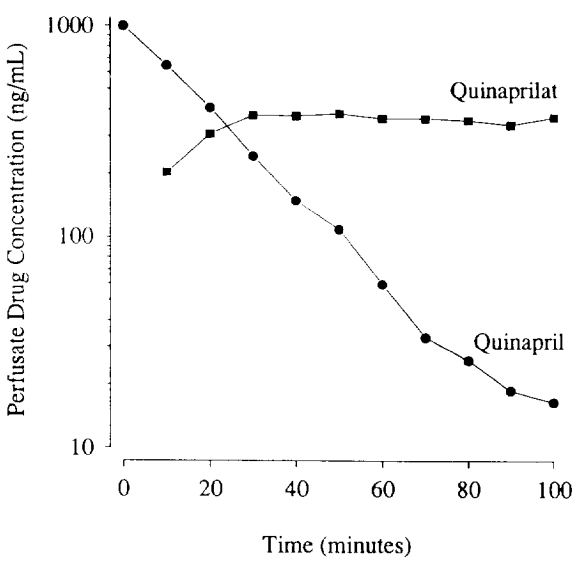


Fig. 3. Perfusate quinapril and quinaprilat concentration–time profiles after quinapril administration in a representative rat IPK study. Concentration data are plotted on a log scale.

Fig. 2e. In both rat IPK (Fig. 3) and in vivo dog studies, quinapril was rapidly and extensively converted to quinaprilat.

4. Conclusions

An HPLC-RD method coupled to LSC has been developed for the specific and sensitive assay of quinapril, quinaprilat, and the two diketopiperazine metabolites of quinapril. Using a 100-μl sample volume, this method can measure quinapril and quinaprilat concentrations (sp. act. ≈ 2.0 μCi/μg) down to 1 ng/ml. Although it was unnecessary for our purposes, increased sensitivity could be achieved by using the undiluted drug (i.e. at higher specific activity) or by using larger sample/injection volumes. The proposed assay method has been successfully used for isolated perfused rat kidney experiments as well as for in vivo pharmacokinetic studies in

dogs. Further, the proposed assay can be easily modified for quantitation of other radiolabeled ACE inhibitors. The method, as described, should have widespread use for the study of radiolabeled drug in absorption, distribution, metabolism, and excretion experiments in animals and humans.

Acknowledgements

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