(7) A. Roos and P. H. Hinderling, *J. Pharm. Sci.*, **70**, 252 (1981).
(8) H. Ott and J. Meier, *Scand. J. Rheum., Suppl.*, **21**, 12 (1978).
(9) E. E. Ohnhaus, *Int. J. Clin. Pharmacol.*, **18**, 136 (1980).
(10) K. Siersbäck-Nielsen, J. Mølholm Hansen, J. Kampmann, and M. Kristensen, *Lancet*, **i**, 1133 (1971).
(11) M. Berman and M. F. Weiss, "User's Manual for SAAM," Public Service, Publication No. 1703, National Institutes for Health, Bethesda, Md. (1967).
(12) C. M. Metzler, "A User's Manual for NONLIN," The Upjohn Co., Technical Report 7292/69/7292/005, Kalamazoo, Mich. (1969).
(13) J. G. Wagner, *J. Pharmacokinet., Biopharm.*, **3**, 457 (1975).
(14) H. G. Boxenbaum, S. Riegelman, and R. M. Elashoff, *J. Pharmacokinet. Biopharm.*, **2**, 123 (1974).
(15) L. Z. Benet and R. L. Galeazzi, *J. Pharm. Sci.*, **68**, 1071 (1979).
(16) M. Gibaldi and D. Perrier, "Pharmacokinetics," Dekker, New York, N.Y., 1975, pp. 72–73.
(17) A. C. Guyton, "Textbook of Medical Physiology," 3rd ed., Saunders, Philadelphia, Pa., 1968, p. 325.
(18) M. Gibaldi and D. Perrier, "Pharmacokinetics," Dekkler, New York, N.Y., 1975, p. 145.
(19) K. Diem and C. Lentner (Eds), "Documenta Geigy, Scientific Tables," 7th ed. Geigy Pharmaceuticals, Division of Ciba-Geigy Corp., Ardsley, N.Y., 1975, pp. 617, 579.
(20) A. C. Guyton,"Textbook of Medical Physiology," 3rd ed., Saunders, Philadelphia, Pa., 1968, p. 419.
(21) A. S. Nies, D. G. Shand, and G. R. Wilkinson, *Clin. Pharmacokinet.*, **1**, 135 (1976).
(22) G. R. Wilkinson and D. G. Shand, *Clin. Pharmacol. Ther.*, **18**, 377 (1977).
(23) E. R. Garrett, J. Brés, K. Schnelle, and L. L. Rolf, *J. Pharmacokinet. Biopharm.*, **2**, 43 (1974).
(24) J. J. Ashley and G. Levy, *J. Pharmacokinet. Biopharm.*, **1**, 99 (1973).
(25) D. Perrier, J. J. Ashley, and G. Levy, *J. Pharmacokinet. Biopharm.*, **1**, 231 (1973).
(26) P. Veng Pedersen, *J. Pharmacokinet. Biopharm.*, **5**, 513 (1977).
(27) H. Wijnand and C. J. Timmer, *J. Pharmacokinet. Biopharm.*, **7**, 681 (1979).
(28) M. Gibaldi and D. Perrier, "Pharmacokinetics," Dekker, New York, N.Y. 1975, pp. 93–94.

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Quantitative Determination of Captopril in Blood and Captopril and Its Disulfide Metabolites in Plasma by Gas Chromatography

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Abstract □ A sensitive, quantitative gas chromatographic–electron capture (GC–EC) method for the determination of captopril in blood and captopril and its disulfide metabolites (collectively) in plasma was developed. After addition of an internal standard and *N*-ethylmaleimide to the biological samples, excess *N*-ethylmaleimide and naturally occurring interfering substances were removed by extraction with benzene followed by acidification and extraction with hexane. The *N*-ethylmaleimide adducts of captopril and of the internal standard were then extracted with benzene and converted to their hexafluoroisopropyl esters. For the assay of captopril and its disulfide metabolites, tributylphosphine was used to reduce the disulfide metabolites to captopril prior to derivatization. The hexafluoroisopropyl esters of the *N*-ethylmaleimide adducts of captopril and of the internal standard, the 4-ethoxyproline analogue of captopril, were separated by GC on a column packed with 3% OV-101 on Chromosorb W-HP. The lower limits of sensitivity were 20 ng/mL for captopril in blood and 50 ng/mL for captopril and its disulfide metabolites in plasma. Linearity, precision, and accuracy were excellent. The method was validated by comparison of results obtained for total captopril in dog plasma by the GC–EC assay with results obtained by a published GC–MS method. The assay was applied to dog and human samples to explore its general utility.

Keyphrases □ Captopril—quantitative determination in blood and plasma, disulfide metabolites, gas chromatography □ Metabolites, disulfide—captopril, plasma, quantitative determination by gas chromatography □ Gas chromatography—captopril, quantitative determination in blood and plasma, disulfide metabolites

Captopril, 1-[(2*S*)-3-mercapto-2-methylpropionyl]-L-proline (I), the first orally active inhibitor of angiotensin-converting enzyme (peptidyl dipeptide hydrolase, kininase II, E.C. 3.4.15.1) to be used clinically, is a potent antihypertensive agent used to treat both renovascular and essential hypertension (1, 2). It is of particular value in treating hypertension resistant to conventional drugs (3), and shows great promise for use in congestive heart failure (2, 4).

The predominant route of excretion of captopril is *via* the kidneys (5–7). Since kidney function is often impaired in patients with hypertension, a sensitive assay for the drug in blood or plasma using readily accessible analytical equipment would be of value in optimizing dosage. Assays for both unchanged captopril and its disulfide metabolites would be useful, since interconversions occur among these compounds in rats, dogs, and humans (8–11). Metabolites of captopril include the symmetrical disulfide (IIA), captopril–L-cysteine mixed disulfide, captopril–glutathione mixed disulfide, and mixed disulfides of captopril with plasma proteins (IIB). Current methods for measurement of the drug in biological fluids are either not sensitive enough for monitoring therapeutic levels (12–14) or involve analytical equipment not available to the average clinical laboratory (15–17). Therefore, a sensitive method has been developed for quantifying captopril in blood and captopril

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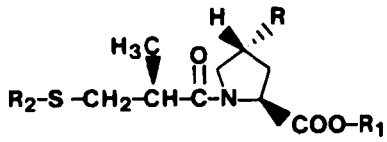
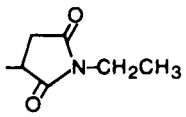
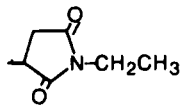
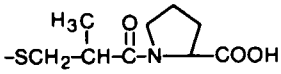
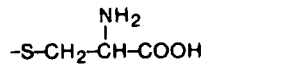
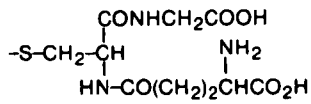
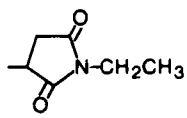


#	NAME	R	R_1	R_2
I	Captopril	H	H	H
IA	N-ethylmaleimide adduct of captopril	H	H	
IB	Ester of N-ethylmaleimide adduct of captopril	H	$-CH(CF_3)_2$	
IIA	Captopril disulfide	H	H	
IIB	Mixed disulfides of captopril	H	H	
		H	H	
		H	H	-Plasma proteins
III	SQ 25,761	$-OC_2H_5$	H	H
IIIA	Ester of N-ethylmaleimide adduct of SQ 25,761	$-OC_2H_5$	$-CH(CF_3)_2$	

and its disulfide metabolites in plasma by gas chromatography with electron capture detection (GC–EC).

EXPERIMENTAL

Materials—*N*-Ethylmaleimide[1], hexafluoro-2-propanol[2], trifluoroacetic anhydride[2], sodium chloride[3], hydrochloric acid[3], and tributylphosphine[4] were obtained from commercial sources and used without further purification. Benzene, hexane, and methanol were suitable for HPLC analysis and used as supplied[5]. Captopril and the 4-ethoxyproline analogue of captopril (SQ 25,761, the internal standard) were of pharmaceutical quality and were used as received[6].

The chromatographic column was glass, 1.8 m × 2 mm i.d., packed with 3% methyl silicone on 100–120 mesh chromatographic (high-performance) diatomaceous earth[7]. Nitrogen and argon–methane (95:5) of the highest available[8] purity were used.

Equipment—GC was performed using an instrument[9] equipped with a nickel-63 electron-capture detector. All extractions were carried out by shaking the samples on a reciprocating shaker[10]. An analytical evaporator[11] was used for removing solvents from extracts with a nitrogen stream. The reduction of the biological samples with tributylphosphine and subsequent esterification with hexafluoro-2-propanol were performed by incubating in a heating block[12].

Extraction, Derivatization, and Chromatography of Unchanged Captopril in Blood—For spiking blood or plasma samples, separate stock solutions of captopril and the internal standard (III) were prepared in distilled water containing excess *N*-ethylmaleimide (1:5 molar ratio). Control dog or human blood (1 mL) containing 5 mg of *N*-ethylmaleimide was spiked with aqueous solutions of captopril containing *N*-ethylmaleimide to give standard solutions containing 20–200 ng/mL of captopril. The internal standard, 400 ng in 50 μL of aqueous solution, was added to each sample. All samples were extracted in acid-washed (16 × 125-mm) glass culture tubes. Twelve milliliters of benzene was added to each sample, and the samples were shaken on a reciprocating shaker for 10 min. After centrifuging the sample for 10 min at 3000×*g*, the benzene (upper) layer was removed by aspiration and discarded. One milliliter of 1 M HCl and 2 g of NaCl were added to each tube, and the contents of each tube was mixed on a vortex mixer for ~30 s. Ten milliliters of hexane was added to each sample, and the contents of each tube was again shaken for 10 min and then centrifuged for 10 min. The hexane (upper) layer was removed by aspiration and discarded. Each sample was then extracted with 12 mL of benzene by shaking for 20 min, followed by centrifugation for 10 min.

The organic layer was transferred to a clean tube and evaporated to dryness under a stream of nitrogen. The residue in each tube was derivatized with 100 μL of hexafluoro-2-propanol and 10 μL of trifluoroacetic anhydride at 50°C for 1 h. Excess reagents were removed by evaporation under a stream of nitrogen. Each residue, which contained derivatized captopril and internal standard, was reconstituted in 1 mL of benzene;

[1] Eastman Kodak Co., Rochester, N.Y.
[2] Pierce Chemical Co., Rockford, Ill.
[3] Mallinckrodt Inc., Paris, Ky.
[4] Aldrich Chemical Co., Milwaukee, Wis.
[5] Burdick and Jackson Laboratories, Muskegon, Mich.
[6] E. R. Squibb & Sons, Inc., Princeton, N.J.
[7] 3% OV-101 on Chromosorb W-HP (100–120 mesh); Alltech Assoc., Deerfield, Ill.
[8] Linde Corp., New York, N.Y.
[9] Model 5880A; Hewlett-Packard, Avondale, Pa.
[10] Eberbach Corp., Ann Arbor, Mich.
[11] N-Evap Model 112; Organomation Assoc., Northborough, Mass.
[12] Thermolyne Corp., Dubuque, Iowa.

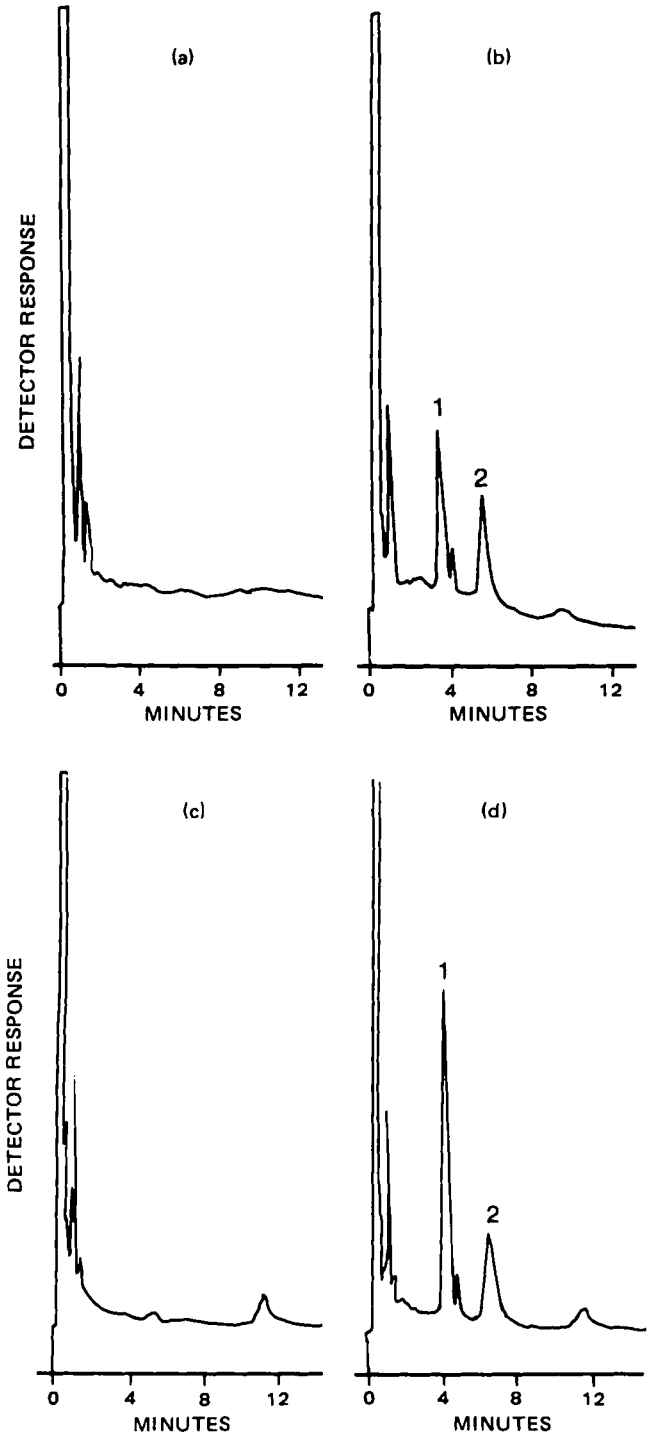


Figure 1—*Typical chromatograms of control human blood (a), control human blood containing derivatized captopril and internal standard (b), control dog plasma (c), and control dog plasma containing derivatized captopril and internal standard (d). Key: (1) captopril; (2) internal standard.*

an aliquot (≤5 μL) of each benzene solution was injected into the GC column. The column had been previously conditioned for 24 h at 250°C with the carrier gas (argon–methane, 95:5) at a flow rate of 10 mL/min. The detector and injector temperatures were 250°C and 225°C, respectively. The column operating temperature was 220°C, and the carrier gas flow rate was 40 mL/min.

Standard curves were constructed from spiked blood samples containing 0–200 ng/mL of captopril and a constant amount (400 ng/mL) of the internal standard. The peak height ratios of the unknown to the internal standard were plotted against concentrations. A new calibration curve was constructed daily. Comparison of peak height ratios from the unknown samples to those from the calibration curve permitted quantitation of the assayed samples.

Table I—Precision of the GC–EC Assay of Captopril and Captopril plus its Disulfide Metabolites

Actual Conc., ng/mL	Observed Conc., ng/mL	Mean ± *SD*	*CV*, %
	Captopril in Human Blood		
20	16	22 ± 4.5	20.5
	20		
	26		
	23		
	27		
100	112	101 ● 8.1	8.1
	94		
	96		
	102		
0200	196	204 ± 6.8	3.3
	201		
	207		
	214		
	203		
	Captopril and its Disulfide Metabolites in Dog Plasma		
50	55	49 ± 4.4	9.8
	45		
	49		
	52		
	45		
100	102	102 ± 3.7	3.7
	99		
	101		
	108		
	106		
500	464	491 ± 25.2	5.2
	465		
	502		
	521		
	504		
1000	1018	1023 ± 40.3	3.9
	1016		
	1027		
	927		

Extraction, Derivatization, and Chromatography for Quantitation of Captopril and its Disulfide Metabolites in Plasma—One-milliliter samples of plasma containing 800 ng of the internal standard (III) were transferred to acid-washed glass culture tubes (16 × 125 mm); 0.1 mL of a 2% solution of tributylphosphine in methanol was added to each sample. All tubes were mixed on a vortex mixer for ~30 s and then were heated for 30 min at 50°C. The samples were washed with 10 mL of hexane to remove excess tributylphosphine. The plasma layer was then mixed with 0.2 mL of a freshly prepared 2.5% aqueous solution of *N*-ethylmaleimide and allowed to stand for 15 min at room temperature. Further extraction, derivatization, and GC analyses then were performed as outlined for captopril in blood. Standard curves were constructed from spiked plasma samples containing 0–1000 ng/mL of captopril and 800 ng of the internal standard. Calibration curves, constructed on each day that analyses were performed, were used to quantitate the unknown samples.

Gas Chromatography–Mass Spectrometry—The derivatives of captopril and the internal standard were subjected to GC–MS analyses to confirm their structures. Mass spectra were obtained on a hyperbolic quadrupole mass spectrometer[13] in the electron-ionization mode.

RESULTS AND DISCUSSION

Derivatization of Captopril—Because of the reactivity of the thiol moiety of captopril, it was necessary to prevent oxidative degradation of captopril by adding *N*-ethylmaleimide to the samples of blood. *N*-Ethylmaleimide immediately reacts with captopril to form a stable thioalkyl derivative (IA) and prevents formation of the symmetrical (IIA) or mixed disulfides (IIB). The assay procedure for captopril and its disulfide metabolites is based on the quantitative reduction of its disulfide metabolites with tributylphosphine, as reported by Kawahara *et al.* (13).

[13] Hewlett-Packard, Palo Alto, Calif.

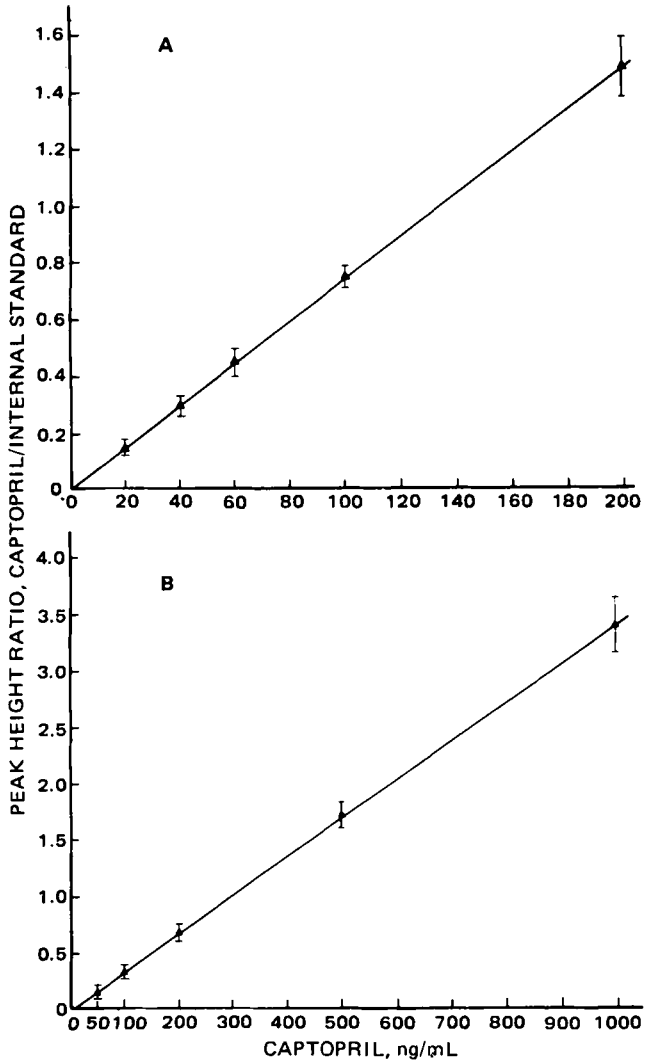


Figure 2—*Calibration curves for unchanged captopril in spiked human blood (A) and captopril and its disulfide metabolites measured as derivatized captopril in spiked dog plasma (B). Each point is the mean of six (A) or five (B) sets; the bars represent the ranges for each mean.*

The liberated captopril was treated with *N*-ethylmaleimide to form the stable derivative (IA). Compound IA formed from either unchanged captopril or captopril arising from reduced disulfides was further derivatized to give the hexafluoroisopropyl ester. The structures of the esters of the thioalkyl derivatives of both captopril (IB) and the internal standard (IIIA) were confirmed by GC–MS. The molecular ion peak (*m/z* 492), base peak (*m/z* 264), and other fragment ion peaks (*m/z* 366 and 228) obtained in the spectrum of derivatized captopril were in good agreement with those reported by Matsuke *et al.* (12). The characteristic fragmentation pattern of the derivatized internal standard (IIIA) agreed well with the known structure (*m/z* M^+ 536, 410, and 264).

The retention times of the derivatized captopril and internal standard were 3.4 and 5.8 min, respectively. No interfering peaks were observed in the extracts of the control human or dog blood. Typical chromatograms of an extract of human blood are shown in Fig. 1a and b. After treatment of plasma samples with tributylphosphine, no interfering peaks were observed in extracts of control human or dog plasma samples. Typical chromatograms of an extract of dog plasma are shown in Fig. 1c and d.

Linearity and Reproducibility—Known amounts of captopril and the internal standard were added to control dog or human blood to give standards suitable for captopril analysis. A plot of chromatographic peak height ratios *versus* concentrations was linear for captopril from 20 to 200 ng/mL (Fig. 2A); the linearity and reproducibility of the assay for captopril by the GC–EC method in human blood was demonstrated by six consecutive calibration curves. Linearity and reproducibility of the GC–EC method for captopril and its disulfide metabolites (50–1000 ng/mL) in dog plasma was demonstrated by five consecutive calibration curves (Fig. 2B). The correlation coefficient and the *y*-intercept for the straight lines were 0.999 and 0.004, respectively, for the captopril assay, and 0.999 and −0.001, respectively, for the assay of captopril and its disulfide metabolites. The average coefficient of variation (*CV*) for all the peak height ratios for both procedures was ±8%.

Table II—Analysis for Captopril and Captopril plus its Disulfide Metabolites in Spiked Human Blood and Plasma

Actual Conc., ng/mL	Observed Conc., ng/mL	Percent Deviation[a]
	Captopril in Human Blood	
0	0	—
50	52	+4.0
60	63	+5.0
70	71	+1.4
90	93	+3.3
100	111	+11.0
150	150	0
200	184	−8.0
500	512	+2.4
500	525	+5.0
	Captopril and its Disulfide Metabolites in Human Plasma	
0	0	—
67	68	+1.5
82	90	+9.7
96	98	+2.1
170	182	+7.1
202	167	−17.3
256	274	+7.0
287	286	−0.3
470	458	−2.5
690	682	−1.1
832	839	+0.8
878	967	+10.1

[a] Percent deviation = [(Observed Conc. − Actual Conc.)/Actual Conc.] × 100.

Precision and Accuracy—The precision of the method for captopril was determined by analysis of replicate human blood samples spiked at three concentrations (20, 100, and 200 ng/mL). Captopril and its disulfide metabolites were also assayed in replicate samples of dog plasma spiked at four concentrations (50, 100, 500, and 1000 ng/mL). For unchanged captopril assays, the *CV* was 20% at 20 ng/mL and 3% at 200 ng/mL; the *CV* for the assays of captopril and its disulfide metabolites was <10% (Table I).

To determine the relative accuracy of the methods, coded, spiked human blood and plasma samples were analyzed for captopril and captopril and its disulfide metabolites. The plasma samples spiked with various amounts of captopril (I), captopril disulfide (IIA), and captopril–L-cysteine mixed disulfide (IIB) were analyzed for captopril and its disulfide metabolites. Good agreement was observed between the actual concentrations and the experimentally determined concentrations for

Table III—Comparison of GC–MS and GC–EC Assays for Captopril and its Disulfide Metabolites in Dog Plasma After an Oral 2.5-mg/kg Dose of Captopril

Dog	Time After Dosing, h	Plasma Concentration, ng/mL GC–MS Method	Plasma Concentration, ng/mL GC–EC Method	Percent Deviation[a]
HO-56	0	0	0	—
	0.5	1551	1512	−2.5
	1	2955	3222	+9.0
	1.5	2122	1996	−6.9
	2	1633	1444	−11.6
	2.5	1038	1036	−0.2
	3.5	744	676	−9.1
	4.5	518	506	−2.3
	6	339	364	+7.4
HO-60	0	128	104	−18.8
	0.5	2946	2678	−9.1
	1	2465	2420	−1.8
	1.5	2057	2050	−0.3
	2	1532	1436	−6.3
	2.5	1005	978	−2.7
	3.5	734	738	+0.5
	4.5	487	482	−0.1
	6	578	322	−44.3

[a] Percent Deviation = [(GC–EC Value − GC–MS Value)/GC–MS Value] × 100.

both assays (Table II). The accuracy of the methods was further validated by comparing results from the assay of captopril and its disulfide metabolites by the GC–EC method with results from the GC–MS assay (15) in plasma from dogs that had been given oral doses of captopril (2.5 mg/kg). The generally excellent agreement obtained for the two methods is shown in Table III.

The GC–EC assay described appears suitable for determining blood concentrations of unchanged captopril and plasma concentrations of captopril and its disulfide metabolites in samples from dogs and humans. In the range of blood concentrations (captopril) from 20 to 200 ng/mL and plasma concentrations (captopril and its disulfide metabolites) from 50 to 1000 ng/mL, the linearity, reproducibility, precision, and accuracy of the assays were generally excellent. The lower limits for quantitation of captopril and its disulfide metabolites by the GC–EC method are comparable to the existing GC–MS (15) and liquid chromatography–electrochemical detector methods (16, 17). The thin-layer radiochromatographic method (18) is more sensitive when high specific activity [^{14}C]captopril is used. The GC–EC method is 4- to 10-fold more sensitive than the other quantitative methods (12, 14) previously reported.

REFERENCES

(1) M. A. Ondetti, B. Rubin, and D. W. Cushman, *Science*, **196**, 441 (1977).

(2) A. B. Atkinson and J. I. Robertson, *Lancet*, **ii**, 836 (1979).

(3) N. J. White and H. Yahya, *Lancet*, **ii**, 108 (1980).

(4) V. J. Dzau, W. S. Colucci, G. H. William, G. Curfman, L. Meggs, and N. K. Holkenberg, *N. Engl. J. Med.*, **302**, 1373 (1980).

(5) R. C. Heel, R. N. Brogdon, T. M. Speight, and G. S. Avery, *Drugs*, **20**, 409 (1980).

(6) K. J. Kripalani, D. N. McKinstry, S. M. Singhvi, D. A. Willard, R. A. Vukovich, and B. H. Migdalof, *Clin. Pharmacol. Ther.*, **27**, 636 (1980).

(7) S. M. Singhvi, D. N. McKinstry, J. M. Shaw, D. A. Willard, and B. H. Migdalof, *J. Clin. Pharmacol.*, **22**, 135 (1982).

(8) B. H. Migdalof, K. K. Wong, S. J. Lan, K. J. Kripalani, and S. M. Singhvi, *Fed. Proc. Fed. Am. Soc. Exp. Biol.*, **39**, 2589 (1980).

(9) K. K. Wong, S. J. Lan, and B. H. Migdalof, *Pharmacologist*, **21**, 173 (1979).

(10) K. J. Kripalani, A. V. Dean, and B. H. Migdalof, *Abstracts, APhA Acad. Pharm. Sci.*, **12**, 39 (1979).

(11) K. K. Wong and J. Dreyfuss, *Pharmacologist*, **20**, 213 (1978).

(12) Y. Matsuke, K. Fukuhara, T. Ito, H. Ono, N. Ohara, T. Yue, and T. Nambara, *J. Chromatogr.*, **188**, 177 (1980).

(13) Y. Kawahara, M. Hisaoka, Y. Yamazaki, A. Inaga, and T. Morioka, *Chem. Pharm. Bull.*, **29**, 150 (1981).

(14) B. Jarrott, A. Anderson, R. Hooper, and W. J. Louis, *J. Pharm. Sci.*, **70**, 665 (1981).

(15) P. T. Funke, E. Ivashkiv, M. F. Malley, and A. I. Cohen, *Anal. Chem.*, **52**, 1086 (1980).

(16) K. Shimada, M. Tanaka, T. Nambara, Y. Inai, K. Abe, and K. Yeshinagae, *J. Chromatogr.*, **227**, 445 (1982).

(17) D. Perrett and P. L. Drury, *J. Liq. Chromatogr.*, **5**, 97 (1982).

(18) B. H. Migdalof, S. M. Singhvi, and K. J. Kripalani, *J. Liq. Chromatogr.*, **3**, 857 (1980).

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Cascade Impactor Method for the Droplet Size Characterization of a Metered-Dose Nasal Spray

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Abstract □ A relatively rapid and simple method was developed to characterize the droplet size of a metered-dose nasal spray. The study primarily concerned the measurement of the relative proportion of small to large droplets. A small droplet could potentially reach bronchi or alveoli, depending on its size, and was therefore undesirable for the topical corticosteroid therapy of rhinal disease. The nasal spray was a solution of flunisolide, a topically active anti-inflammatory corticosteroid, administered by a manually operated, metered-dose pump spray system. The method utilized a cascade impactor fitted with a glass chamber; the cascade impactor collected and sized droplets into six fractions 0.5–16 μm in diameter, while the glass chamber collected droplets >16 μm in diameter as another fraction. Results showed that the majority of the spray droplets deposited in the glass chamber. Less than 0.5% by weight of the spray dose was delivered in droplets <8 μm aerodynamic diameter. These results are in good agreement with the droplet size distribution obtained by laser holography. The cascade impactor method showed that the number of undesirable small droplets produced by the flunisolide nasal spray unit was negligible. The method can be used with other aerosols where there is a similar concern for the inhalation of small particles.

Keyphrases □ Cascade impactor method—droplet-size characterization, metered-dose nasal spray □ Nasal spray—metered-dose, cascade impactor method for the droplet-size characterization □ Droplet-size characterization—cascade impactor method, metered-dose nasal spray

The particle or droplet size of an aerosol spray dosage form is important for both efficacy and toxicity. Inhalation aerosols intended for local activity in the lung must have the majority (by weight) of the particles in the size range of 2–6 μm in diameter to reach the terminal bronchi and alveoli (1). Particles <0.5 μm may fail to remain in this area (2), while particles >10 μm in diameter are mostly deposited in the upper respiratory tract (3).

In contrast, a nasal spray such as the metered-dose flunisolide nasal solution requires a majority of droplets having diameters >10 μm to localize delivery in the nose and to avoid possible undesired effects resulting from the material reaching the lung. It is therefore important, from an efficacy standpoint, to ensure that the nasal spray unit primarily produces droplets >10 μm in diameter. However, droplets <10 μm in diameter inevitably exist in small quantity in a spray. The ability of these small droplets to advance in the trachea–bronchi system depends on their

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