Journal of Chromatography, 206 (1981) 311–317
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 13,408

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYSES OF THE ANTIHYPERTENSIVE DRUG CAPTOPRIL*

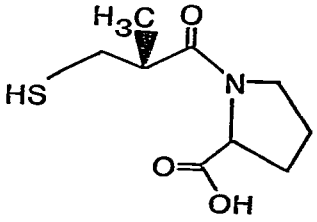
SOLOMON PERLMAN* and JOEL KIRSCHBAUM
Squibb Institute for Medical Research, New Brunswick, NJ 08903 (U.S.A.)
(First received September 15th, 1980; revised manuscript received October 9th, 1980)

SUMMARY

High-performance liquid chromatographic systems have been developed to separate and quantitate captopril. The presence of interfering excipients in various formulations necessitated the development of three assays, each with distinctly different columns and mobile phases. All work well for bulk material, providing short analysis time, high precision, and rapid sample preparation, demonstrating that there is not necessarily one, best, high-performance liquid chromatographic system. The advantages and shortcomings of the ion-exchange, amino and octadecylsilane systems are evaluated and one of them is selected as optimum for bulk and tablet analysis.

INTRODUCTION

Captopril is an orally-active antihypertensive agent[1,2] specifically designed[3,4] to inhibit competitively angiotensin-converting enzyme to prevent it from producing the potent vasoconstrictor angiotensin II. Recent studies have shown captopril to be also potentially effective in treating congestive heart failure[5]. A gas–liquid chromatographic method for quantitating derivatized captopril in body fluids has been described[6]. A method was needed to determine quantitatively captopril as bulk material and in various formulations. The assay had to be rapid, precise and stability-indicating. High-performance liquid chromatography (HPLC) was selected as meeting these requirements. On the basis of selectivity, a system utilizing a heavily-loaded octadecylsilane column was designated as optimum.



* Presented, in part, at the *Federation of Analytical Chemistry and Spectroscopy Societies Meeting, Philadelphia, PA, September 1979.*

EXPERIMENTAL

HPLC apparatus

The pump (Altex 110A), precision loop injector (Rheodyne 7010 with a 20-μl loop), variable-wavelength UV–visible detector (Hitachi 100-30 with an Altex flow cell) and recorder (Linear) were individually purchased and assembled. Filters (2 μm) were inserted before the loop injector and before the column to trap possible particulate material.

Reagents

Water is double-distilled and stored in glass. Acetonitrile and methanol are HPLC grade (Fisher Scientific, Pittsburgh, PA, U.S.A.). Phosphoric acid (85% reagent grade) and citric acid monohydrate were obtained from Mallinckrodt (St. Louis, MO, U.S.A.). Ammonium citrate dibasic and disodium ethylenediaminetetraacetate (EDTA) dihydrate were ACS grade (Fisher).

Chromatographic conditions

Anion exchange. A Partisil SAX strong anion exchange column (Whatman, Clifton, NJ, U.S.A.) was used. The mobile phase was prepared by dissolving 525 mg of citric acid monohydrate and 37.7 mg of ammonium citrate in 1 l of methanol. The apparent pH was adjusted to 3.30 with 0.1 *N* HCl. The flow-rate was 0.6 ml/min.

Amino. A μBondapak-NH_2 column (Waters Assoc., Milford, MA, U.S.A.) was used with a mobile phase of 0.01 *M* Na_2EDTA in 0.05% acetic acid–acetonitrile (95:5). The flow-rate was 1.0 ml/min at ambient temperature.

Reversed-phase octadecylsilane (5% coverage). A Partisil ODS column (Whatman) was used .The mobile phase consisted of methanol–water–85% phosphoric acid (25:75:0.1). The flow-rate was 1.0 ml/min.

Reversed-phase octadecylsilane (15% coverage). A Partisil ODS-2 column (Whatman) was used with a mobile phase of methanol–water–85% phosphoric acid (50:50:0.05). The flow-rate was 1.0 ml/min.

Procedure

Bulk material was dissolved in methanol. Accurately weighed portions of ground tablets were extracted with 25 ml of methanol. After ultrasonication and centrifugation at 1100 *g* for 15 min, the supernatant was injected. Parenteral formulations were diluted with mobile phase. The concentration range for samples is usually 0.1–1 mg/ml. All samples were quantitated against an appropriate standard using either peak heights or peak areas. Since captopril exhibits a maximum at *ca.* 205 nm, wavelengths of 220 nm or lower were chosen for detection.

RESULTS AND DISCUSSION

In developing on HPLC system for captopril, several factors had to be considered. Sulfhydryl compounds are inherently unstable. Heavy metal ions are known to catalyze the oxidation of thiols to disulfides[7], and, of course, the loop injector, column and end-fittings are all metallic. It would thus be desirable to have a chelating agent present in the mobile phase to sequester metallic cations and minimize this

catalytic effect. Care must also be exercised in the sample preparation. Of course, the system must be able to separate captopril from its disulfide to detect this possible degradation product. In addition, molecular studies predicted hindered rotation about the "peptide" C–N bond, due to its partial double bond character and to the steric hindrance between the carboxyl and methyl groups. This effect was apparently observed on several HPLC systems in which captopril appeared as a doublet. (For example, when aqueous EDTA is used as a mobile phase with the amino column.) When the column temperature was raised to 45°C, the peaks were seen to be closer together; at 55°C, they collapsed to one peak.

The peptide-like appearance of captopril led us to try first anion-exchange chromatography with citrate buffers. The final, optimized system (Fig. 1a) turned out to be non-aqueous, required exact control of the apparent pH (±0.05 unit) and more than 300 ml of mobile phase for equilibration of the column. The latter effects are typical for ion-exchange chromatography[8]. This system was used satisfactorily to analyze captopril in bulk and tablets. The presence of 0.5% water in the mobile phase did not alter the separation, allowing for analysis of moist samples. Further assay development became necessary when an excipient, methyl paraben, in a prototype parenteral formulation interfered.

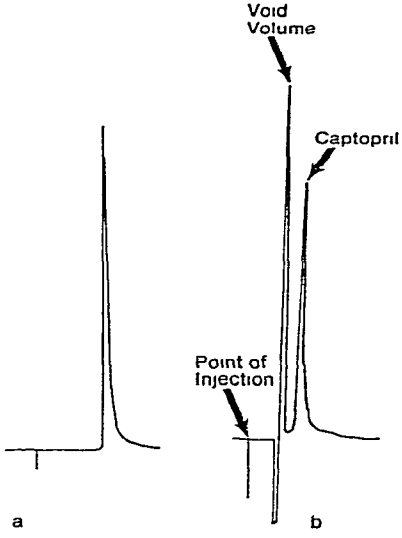


Fig. 1. HPLC of captopril. a, Anion-exchange system; b, amino system.

The amino system was chosen for theoretical considerations. To ensure the selectivity of the separation, an attempt was made to develop a system in which captopril would be separated from its immediate, S-acetyl, precursor. (The subject of selectivity will be discussed later in this paper.) It was thought that the amino column would separate them by differential hydrogen bonding between the amino hydrogens of the column and the carbonyl oxygens of the compounds. EDTA was chosen as the buffer because of its mild chelating ability. A typical chromatogram is shown in Fig. 1b. However, another excipient in a parenteral formulation (identified as sodium citrate) interfered.

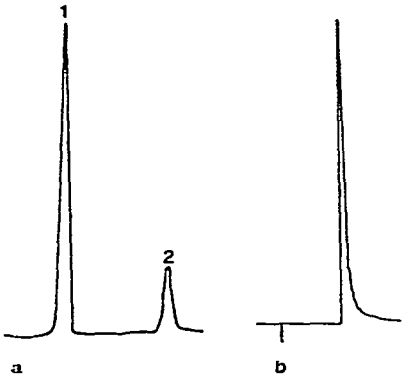


Fig. 2. Reversed-phase HPLC of captopril. a, Captopril parenteral solution; ODS column (5% load); peaks: 1 = captopril; 2 = methyl paraben. b, Captopril bulk; ODS column (15% load).

An assay for captopril in parenteral solutions was finally developed using an octadecylsilane column with 5% (w/w) hydrocarbon load. The interfering excipient (methyl paraben in the chromatogram shown) is separated from captopril. The chromatogram is shown in Fig. 2a.

When the Whatman ODS-2 column with its 15% (w/w) hydrocarbon load was substituted for the more lightly loaded ODS column, and the methanol content of the mobile phase increased to 50%, it was found that methyl paraben was again eluting very close to captopril. But for bulk and tablets, the peak shape was improved. Fig. 2b shows a typical chromatogram. Even though the reversed-phase systems do not have a sequestering agent in the mobile phase, no oxidation *in situ* of captopril to disulfide has been observed.

Thus, only one of the HPLC systems described will separate captopril in parenteral solutions containing methyl paraben and citric acid, but all are useful for analyzing bulk material and captopril in tablets. In all systems, the precision is excellent with a relative standard deviation (R.S.D.) less than 0.8% for at least six repetitive injections (Table I). The plot of response at 214 nm *vs.* concentration is linear from 50 to 1000 μg/ml and passes through the origin. The anion-exchange and ODS systems have been successfully utilized to analyze tablets. Some comparative data, with recoveries from spiked placebos, are listed in Table II. The anion-exchange system is the least desirable because of the long equilibration time and strict control of the apparent pH of the mobile phase. With factors such as cost, convenience, ease

TABLE I

PRECISION OF THE SYSTEMS

HPLC system	*Height of repetitive injections (cm)*	$\bar{x}$	*R.S.D.*
Anion exchange	10.60, 10.59, 10.70, 10.73, 10.68, 10.75	10.68	0.6
Amino	9.72, 9.69, 9.77, 9.79, 9.76, 9.72	9.74	0.4
5% ODS	13.13, 13.08, 13.05, 13.02, 13.03, 13.08	13.07	0.3
15% ODS	11.20, 11.08, 11.09, 11.09, 11.10, 11.10	11.11	0.4

TABLE II

COMPARATIVE DATA FOR THE ANION-EXCHANGE AND ODS SYSTEMS

Ten individual captopril tablets were analyzed. The same solution was injected into both systems. Theoretical potency, 25 mg/tablet; average tablet weight, 100 mg.

	Potency		*Difference*
	Anion exchange	*ODS*	
	25.0	24.2	+0.8
	25.4	24.7	+0.7
	25.7	25.7	—
	25.3	25.3	—
	24.3	24.8	−0.5
	24.5	25.0	−0.5
	24.2	24.6	−0.4
	24.0	24.9	−0.9
	24.5	24.9	−0.4
	23.8	24.4	−0.6
Total difference			−1.8
Mean potency	24.7	24.9	
R.S.D.	2.6	1.7	
Recovery from spiked placebos (%)	99.5	100.5	

of preparation, availability and ruggedness being more or less equal for the other systems, it was decided to focus on selectivity. A desirable feature of any HPLC system is its ability to separate the compound in question from its synthetic intermediates, precursors and solvents used in its synthesis. If the prime candidates for possible impurities have been separated from the main peak, the results are more reliable. Also, the HPLC system can be applied to problems related to the purity of these related compounds. Table III shows the relative retention times of precursors

TABLE III

RELATIVE RETENTION TIMES OF CAPTOPRIL INTERMEDIATES

*Compound**	*HPLC system*			
	Anion exchange	*Amino*	*C_{18} (5%)*	*C_{18} (15%)***
Captopril (IV)	1.00	1.00	1.00	1.00
S-Acetyl captopril (III)	0.99	0.98	1.90	1.17
Disulfide of captopril	1.27	2.51	>4	1.64
Thioacetic acid (I)	0.94	>4	0.34	0.90
Thioisobutyric acid	0.90	0.71	0.65	1.06
Thioisobutyric acid disulfide	0.95	>4	0.35	2.03****
				2.23
S-Acetyl thioisobutyric acid (II)	0.87	>4	1.44	1.25

* Numbers in parentheses refer to Fig. 3.

** Method of choice. An ODS-2 column (Whatman) is used.

*** The presence of this doublet has been confirmed. At present, its origins have not been further investigated.

and intermediates in the synthesis of captopril (Fig. 3) on all systems. Also included in the table is the disulfide, a metabolite[9]. On the basis of these data, the system utilizing the more heavily loaded ODS-2 column has been designated as optimum. Some representative tablet data with this preferred system are listed in Table IV. The other systems were used twice as alternatives when formulations containing interfering excipients were analyzed.

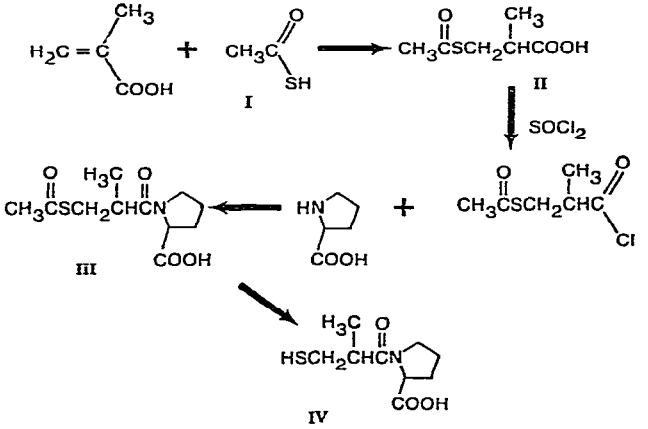


Fig. 3. Synthesis of captopril.

TABLE IV

INDIVIDUAL CAPTOPRIL TABLET ASSAYS USING THE PREFERRED HPLC SYSTEM

Theoretical potency (mg/tablet)	*Mean tablet weight (mg)*	*Potency obtained (mg/tablet)*
100	400	96.8, 97.6, 98.9, 99.5, 95.4, 103.6, 97.5, 100.1, 98.2, 100.1 $\bar{x} = 98.8$ R.S.D. = 2.2
50	200	47.6, 49.1, 48.1, 46.9, 47.8, 46.5, 47.4, 48.7, 50.2, 47.6 $\bar{x} = 48.0$ R.S.D. = 2.3
25	100	24.2, 24.7, 25.7, 25.3, 24.8, 25.0, 24.6, 24.9, 24.9, 25.4 $\bar{x} = 25.0$ R.S.D. = 1.7

ACKNOWLEDGEMENTS

We are grateful to Dr. Glenn Brewer, Dr. Klaus Florey and Mr. R. Poet for their critical reading of this manuscript, and to Dr. Bhupendra Patel and Dr. Steve Taylor for stimulating discussions.

REFERENCES

1 H. Gavras, H. R. Brunner, G. A. Turini, G. R. Kershaw, C. P. Tifft, S. Cuttlerod, R. A. Vukovich and D. N. McKinstry, *New England J. Med.*, 298 (1978) 991.
2 J. Koch-Weser, *New England J. Med.*, 299 (1978) 363.
3 M. A. Ondetti, B. Rubin and D. W. Cushman, *Science*, 196 (1977) 441.
4 D. W. Cushman, H. S. Cheung, E. F. Sabo and M. A. Ondetti, *Biochemistry*, 16 (1977) 5484.
5 R. Davis, H. Ribner, E. Keung, E. Sonnenblick and T. Le-Jemtel, *New England J. Med.*, 301 (1979) 117.
6 Y. Matsuki, K. Fukuhara, T. Ito, H. Ono, N. Ohara, T. Yui and T. Nambara, *J. Chromatogr.*, 188 (1980) 177.
7 S. Oae (Editor), *Organic Chemistry of Sulphur*, Plenum, New York, 1977, p. 316.
8 J. J. Kirkland (Editor), *Modern Practice of Liquid Chromatography*, Wiley-Interscience, New York, 1971, p. 311.
9 K. K. Wong, S. J. Lan and B. H. Migdalof, *Pharmacologist*, 21 (1979) 173.