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Stereoisomeric purity determination of captopril by capillary gas chromatography

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ABSTRACT

The GC method developed for the stereoisomeric purity determination of captopril is based on the combined information derived from the analyses of the captopril sample on two GC systems, one with a chiral and the other with an achiral column. The limit of detection has been determined to be 0.02% (w/w) for (*R,S*) or (*S,R*) and 0.03% for (*R,R*), with corresponding minimum quantifiable levels of 0.08% and 0.09%.

INTRODUCTION

Captopril, 1-[3-mercapto-2-(*S*)-methyl-1-oxopropyl]-(*S*)-proline (Fig. 1) is an inhibitor of the angiotensin-converting enzyme and is widely used for the treatment of hypertension and congestive heart failure [1]. Since the compound contains two asymmetric centers, one associated with the proline and the other associated with the 3-mercapto-2-methylpropionic acid side chain, there are three other possible stereoisomers: 1-[3-mercapto-2-(*S*)-methyl-1-oxopropyl]-(*R*)-proline, 1-[3-mercapto-2-(*R*)-methyl-1-oxopropyl]-(*S*)-proline and 1-[3-mercapto-2-(*R*)-methyl-1-oxopropyl]-(*R*)-proline. The production of captopril (*S,S*) involves the coupling of L-proline with the acid chloride of resolved 3-acetylmercapto-2-(*S*)-methylpropionic acid. Since both the acid chloride and proline may contain traces of the respective enantiomers, it is possible to form small amounts of each of the three stereoisomers, (*R,R*), (*S,R*) and (*R,S*), which could theoretically be present in the final bulk captopril product. Assuming the unlikely situation where both the proline and the acid chloride meet the

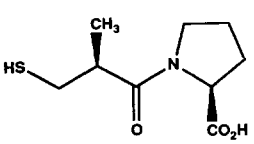

Fig. 1. Chemical structure of captopril.

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low end limit of the specifications, 99.5% and 96.0%, respectively, the theoretical amounts of the stereoisomers that can be formed have been computed: 95.5% captopril (*S,S*), 3.98% (*R,S*), 0.48% (*S,R*) and 0.02 (*R,R*). On the other hand, bulk captopril, which is obtained after extensive purification during routine processing, may not contain any of the stereoisomers. Thus, to ascertain the stereoisomeric purity of captopril (*S,S*), it was required to develop a method which is capable of quantitating any trace amounts of (*R,R*), (*R,S*) and (*S,R*) in captopril.

Gas chromatography (GC) has been used to resolve enantiomers on chiral columns [2,3] and diastereomers on achiral columns [4,5]. The GC method developed for the stereoisomeric purity determination of captopril is based on the combined information derived from the analyses of the captopril sample on two GC systems, one with a chiral and the other with an achiral column. The limit of detection (LOD) has been determined to be 0.02% (w/w) for (*R,S*) or (*S,R*) and 0.03% for (*R,R*), with corresponding minimum quantifiable levels (MQL) of 0.08% and 0.09%.

EXPERIMENTAL

Reagents and chemicals

Captopril and the other three stereoisomers were characterized products obtained from Bristol–Myers Squibb Pharmaceutical Research Institute (Chemical Process Technology Department, New Brunswick, NJ, USA). Methanol–hydrogen chloride solution (2 *M*) was prepared by adding dropwise 2.8 ml of acetyl chloride (Applied Science) to 17.2 ml of cold anhydrous methanol (Applied Science) in an ice bath. After removing from the ice bath, the solution is allowed to remain at room temperature for at least 20 minutes prior to use. The reagent is stable for at least two weeks when refrigerated. The other reagents used were pentafluoropropionic anhydride (Pierce), ethyl acetate (Burdick and Jackson, pesticide-residue grade) and *n*-butyl acetate (Burdick and Jackson, pesticide-residue grade).

Sample preparation

Into an autosampler vial containing approximately 5 mg of captopril, 1.0 ml of methanol–hydrogen chloride solution was added. After capping the vial and mixing to aid in dissolution, the solution was heated at 60°C for 30 min. After the samples had cooled, the reagents were removed by evaporation under nitrogen. To the dry residue, 250 μl of ethyl acetate and 250 μl of pentafluoropropionic anhydride were added and the resulting solution was heated at 60°C for 30 min. After cooling, the reagents were removed by evaporation under nitrogen and the dry residue was reconstituted in 0.6 ml of *n*-butyl acetate. A 1.0-μl portion of this solution was then injected into each of the two GC systems described below.

Gas chromatography

A Hewlett-Packard 5890 capillary gas chromatograph, equipped with a split/splitless injection port, a flame ionization detector and 7673A autosampler injector, was used. Injection was in the split mode, with a split flow of 50 ml/min. The split port liner was an unpacked cup splitter (Restek No. 20710). The helium carrier gas head pressure was maintained at 83 kPa (12 p.s.i.g.) and the flow-rate of the helium make-up gas for the flame ionization detector was 30 ml/min. The GC sensitivity was set at

a range of 2^2 and attenuation of 2^2. The analysis was carried out by injecting 1.0 μl of the sample solution into each of the two chromatographic systems, one chiral and the other achiral.

For the achiral column analysis, the column used was Rt_x-1 (100% dimethylpolysiloxane, Restek Corp.), 30 m × 0.32 mm I.D. and 1.0 μm stationary phase film thickness. The oven temperature was maintained at 170°C for 12 min. The injector and detector temperatures were maintained at 250°C and 300°C, respectively.

For the chiral column analysis, the column used was XE-60–*S*-valine–*S*-phenylethylamide (Chrompack), 25 m × 0.25 mm I.D. and 0.12 μm stationary phase film thickness. The oven temperature was maintained at 160°C for 15 min. The injector and detector temperatures were maintained at 250°C and 275°C, respectively.

Quantitation

Chiral column. The chiral column system resolves two of the three stereoisomers, (*S*,*R*) and (*R*,*R*), from the main peak, which is due to captopril (*S*,*S*). Thus, this system is used to determine the amounts of (*S*,*R*) and (*R*,*R*) in captopril according to the following equations:

$$\% \text{ of } (S,R) = \frac{A_{SR}}{A_{SR} + A_{RR} + A_{SS+RS}} \cdot 100$$

$$\% \text{ of } (R,R) = \frac{A_{RR}}{A_{SR} + A_{RR} + A_{SS+RS}} \cdot 100$$

where A_{SR} = area of the (*S*,*R*) peak; A_{RR} = area of the (*R*,*R*) peak; A_{SS+RS} = area of the unresolved peak for captopril and (*R*,*S*).

The (*R*,*S*), which coelutes with captopril (*S*,*S*), is determined by the combination of the results derived from the chiral and achiral systems (see below).

Achiral column. On the achiral column, the four stereoisomers elute as two peaks, composed of the co-elution of (*R*,*S*) and (*S*,*R*), and the co-elution of (*S*,*S*) and (*R*,*R*). Thus,

$$\% \text{ of } (S,R) + (R,S) = \frac{A_{SR+RS}}{A_{SR+RS} + A_{SS+RR}} \cdot 100$$

where A_{SR+RS} = the area of the peak representing the co-elution of (*S*,*R*) and (*R*,*S*); A_{SS+RR} = the area of the peak representing the co-elution of the captopril (*S*,*S*) and (*R*,*R*).

Using the % of (*S*,*R*) calculated from the chiral column data (see above), the % of (*R*,*S*) can be determined:

$$\% \text{ of } (R,S) = [\% \text{ of } (S,R) + (R,S)] - [\% \text{ of } (S,R)]$$

RESULTS AND DISCUSSION

The method developed for the determination of the three stereoisomers, (*R*,*R*), (*S*,*R*) and (*R*,*S*), in captopril, (*S*,*S*), is based on a two-step achiral derivatization, methylation of the carboxylic acid followed by the acylation of the sulfhydryl. The resulting product is chromatographed on two separate chromatographic systems, one using an achiral column and the other a chiral column. On the achiral column, the four stereoisomers are resolved into two peaks (Fig. 2), each peak representing a diastereomer and its enantiomer. Thus, (*R*,*S*) coelutes with (*S*,*R*) and (*S*,*S*) coelutes with (*R*,*R*). The resolution between the two peaks is 1.8. The chiral column, on the other hand, resolves the components into three peaks (Fig. 3) with the coelution of (*S*,*S*) and (*R*,*S*). The resolution between the main peak and the (*S*,*R*) peak is 1.8, whereas the resolution between the (*S*,*R*) and (*R*,*R*) peaks is 0.53, which was found to be adequate. On both systems, the minor peaks elute before the main peak, which makes integration of the minor peaks more reliable. The reagent blank chromatogram on each system (Figs. 4 and 5) showed that there were no interfering peaks in the regions of interest. By the combination of the data from the runs obtained on the two chromatographic systems, it is appropriate to determine the levels of the three stereoisomers in captopril.

The accuracy of the method was established by analyzing captopril samples spiked with varying amounts of each of the three stereoisomers. As shown in Table I, added stereoisomer was quantitatively recovered. The table also shows that precision of replicate injections is excellent. Excellent precision was also obtained for replicate preparations of captopril spiked with the stereoisomers (Table II). The data in Table II were also used to estimate the LOD and MQL of the method, by utilizing the formulae LOD = 3 × standard deviation of replicate sample preparations and

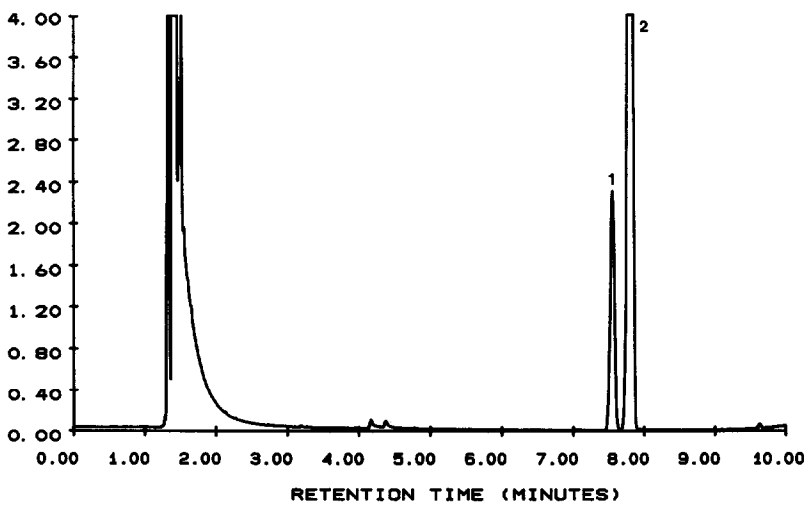

Fig. 2. Achiral column chromatogram of a solution prepared from 5 mg of captopril, (*S*,*S*), spiked with 0.5 mg of each of the three stereoisomers, (*R*,*R*), (*R*,*S*) and (*S*,*R*). Peaks: 1 = (*R*,*S*) + (*S*,*R*) (7.55 min); 2 = captopril + (*R*,*R*) (7.82 min). The resolution between the two peaks is 1.8.

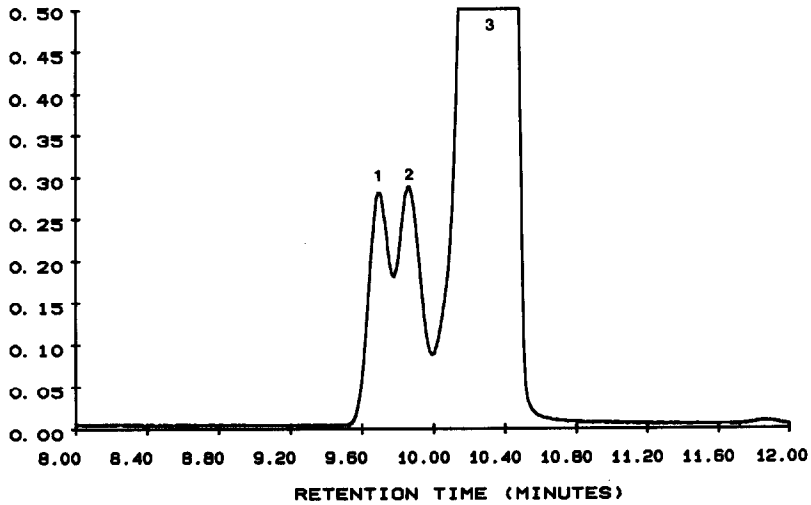

Fig. 3. Chiral column chromatogram of a solution prepared from 5 mg of captopril, (*S,S*), spiked with 0.5 mg of each of the three stereoisomers, (*R,R*), (*R,S*) and (*S,R*). Peaks: 1 = (*S,R*) (9.71 min); 2 = (*R,R*) (9.87 min); 3 = captopril + (*R,S*). The resolution between peaks 1 and 2 is 0.53; the resolution between peaks 1 and 3 is 1.8.

MQL = 10 × standard deviation of replicate sample preparations. Thus, the LOD is estimated to be 0.02% for (*R,S*) or (*S,R*) and 0.03% for (*R,R*), with corresponding MQL values of 0.08 and 0.09% (w/w).

Analyses of a large number of different batches of captopril showed that little or no stereoisomers are present in captopril. The largest level found was for the (*R,S*)

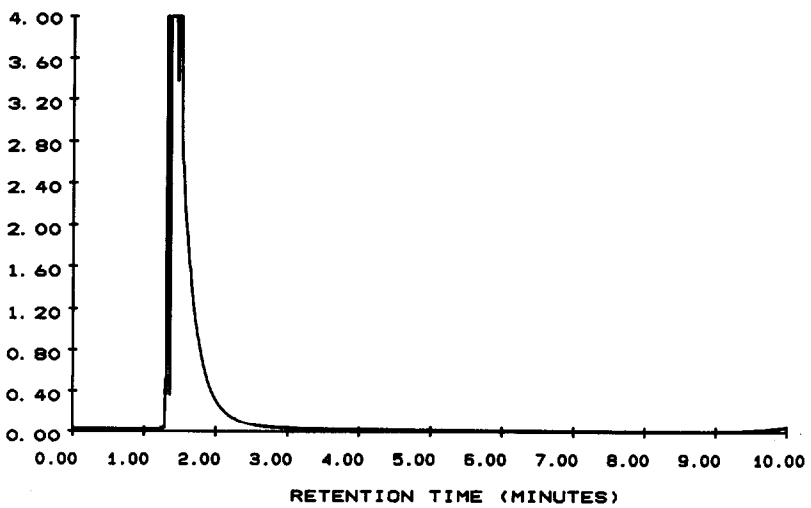

Fig. 4. Chromatogram of a reagent blank corresponding to Fig. 2.

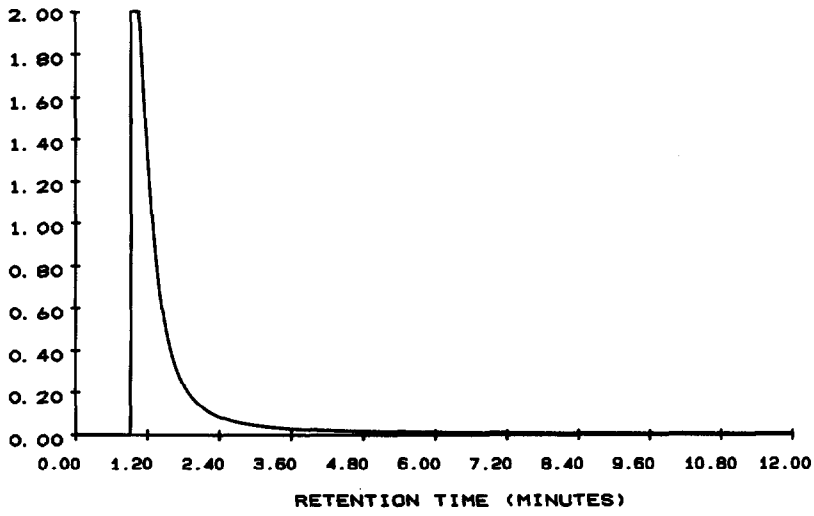

Fig. 5. Chromatogram of a reagent blank corresponding to Fig. 3.

TABLE I

RECOVERY OF THE STEREOISOMERS ADDED TO CAPTOPRIL

The four values shown for each level of a stereoisomer represent replicate injections of the same solution.

Added (%, w/w)	(*R*,*S*) Stereoisomer recovered (%, w/w)	(*S*,*R*) Stereoisomer recovered (%, w/w)	(*R*,*R*) Stereoisomer recovered (%, w/w)
4.4	4.9, 4.9, 4.9, 4.9	4.3, 4.4, 4.4, 4.4	4.9, 4.9, 4.9, 4.9
0.97	1.1, 1.1, 1.1, 1.0	0.99, 0.97, 0.95, 0.98	1.2, 1.2, 1.2, 1.2
0.49	0.54, 0.53, 0.52, 0.54	0.49, 0.52, 0.52, 0.52	0.49, 0.57, 0.54, 0.48
0.20	0.22, 0.23, 0.25, 0.22	0.22, 0.24, 0.22, 0.24	0.22, 0.21, 0.22, 0.16
0.10	0.10, 0.10, 0.12, 0.10	0.13, 0.12, 0.13, 0.12	0.10, 0.087, 0.082, 0.10

TABLE II

REPRODUCIBILITY OF REPLICATE PREPARATIONS OF CAPTOPRIL SPIKED WITH 0.1% (w/w) OF THE STEREOISOMERS

S.D. = Standard deviation; R.S.D. = Relative standard deviation.

Replicate No.	(*R*,*S*) Stereoisomer recovered (%, w/w)	(*S*,*R*) Stereoisomer recovered (%, w/w)	(*R*,*R*) Stereoisomer recovered (%, w/w)
1	0.11	0.10	0.084
2	0.11	0.12	0.081
3	0.11	0.11	0.094
4	0.11	0.12	0.076
5	0.12	0.11	0.10
6	0.12	0.12	0.083
7	0.11	0.11	0.095
8	0.13	0.11	0.081
Mean	0.12	0.11	0.086
S.D.	0.0076	0.0071	0.0084
R.S.D.(%)	6.6	6.3	9.7

stereoisomer as expected, but the level was lower than the MQL even for this stereoisomer.

Before the adoption of the method described here, several different investigations were undertaken. The resolution obtained on the chiral column after just methylation, without subsequent acylation, was not satisfactory. The use of another amino acid-based chiral column, Chiralsil-VAl III, did not give the resolution obtained on the XE-60-*S*-valine-*S*-phenylethylamide column.

The method described above could be cleanly accomplished by mutidimensional GC. The sample would first be injected into the achiral column which separates the four compounds into two components, (*R*,*S*) and (*S*,*R*) coeluting first, and (*S*,*S*) and (*R*,R) coeluting next. The two peaks would then be directed separately to the chiral column, with each producing two peaks on the chiral column. Thus, the first peak from the achiral column will produce two peaks on the chiral column, that of (*S*,*R*) eluting first and (*R*,*S*) eluting next, well separated from each other.

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