*Journal of Chromatography*, 417 (1987) 79–87
*Biomedical Applications*
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 3602

# SENSITIVE METHOD FOR DETERMINATION OF CAPTOPRIL IN BIOLOGICAL FLUIDS BY GAS CHROMATOGRAPHY–MASS SPECTROMETRY

TOMIHARU ITO and YASUHIKO MATSUKI*

*Hatano Research Institute, Food and Drug Safety Center, 729-5, Ochiai, Hadano, Kanagawa 257 (Japan)*

HIROYUKI KURIHARA

*Department of Chemistry, Faculty of Science, Tokai University, 1117 Kitakaname, Hiratsuka, Kanagawa 259-12 (Japan)*

and

TOSHIO NAMBARA

*Pharmaceutical Institute, Tohoku University, Aobayama, Sendai 980 (Japan)*

(First received October 27th, 1986; revised manuscript received January 15th, 1987)

## SUMMARY

A sensitive method for the determination of captopril in blood and urine by gas chromatography–mass spectrometry is described. In order to prevent oxidative degradation of captopril, its sulphhydryl group was immediately protected by treatment with N-ethylmaleimide (NEM), and the resulting NEM adduct was then converted into the bis(pentafluorobenzyl) derivative. Derivatized captopril was separated on a 2% OV-1 column, exhibiting a single peak of the correct theoretical shape. The detection limit was estimated to be 100 pg by using S-benzylcaptopril as an internal standard. The blood level and urinary excretion of unchanged captopril orally administered to dogs were determined by the proposed method. In addition, epimerization of the proline moiety and formation of the sulphoxide or sulphone through the esterification step are also described.

## INTRODUCTION

1-(3-Mercapto-2-D-methyl-1-oxopropyl)-L-proline (captopril) is known to be

0378-4347/87/$03.50 

a potent inhibitor of angiotensin-converting enzyme [1,2], and its pharmacological activity against hypertension [2,3] and its metabolic fate [4–6] in living animals have been investigated.

We have developed methods for the quantitation of captopril and its disulphide, which is a major metabolite, by gas chromatography (GC) [7] and gas chromatography–mass spectrometry (GC–MS) [8]. These procedures are applicable to the determination of captopril in biological fluids for experimental animals but are not always satisfactory for human subjects. An urgent need to clarify the pharmacokinetics of captopril in living animals in a low dose as well as in a clinical dose prompted us to develop a more sensitive and reliable method for the determination of captopril.

If it is derivatized to the hexafluoroisopropyl (HFIP) ester, captopril can be determined by GC under the established conditions, when it exhibits a single peak [8]. However, the product often provides two broad peaks with a shoulder on the gas chromatogram. This chromatographic behaviour implies that diastereoisomers might be formed from captopril through derivatization into the HFIP ester. Therefore, characterization of the shoulder peak was undertaken by means of GC–MS using the stable-isotope technique. In addition, Funke et al. [9] reported that captopril is susceptible to aerobic oxidation, resulting in formation of the sulphoxide during clean-up. These findings prompted us to clarify whether or not an oxidized product is formed from captopril-NEM during the assay procedure previously reported [8].

This paper deals with a GC–MS method for the determination of captopril in blood and urine and an investigation of the artificial formation of diastereoisomers and oxidized products.

## EXPERIMENTAL

### *Chemicals*

Captopril (SQ 14,225) was kindly donated by Sankyo (Tokyo, Japan). Pentafluorobenzyl bromide (PFBB) was purchased from Tokyo Kasei Kogyo (Tokyo, Japan), N-ethylmaleimide (NEM) from Nakarai (Kyoto, Japan) and $^2$H-labelled trifluoroethanol (TFE) ($CF_3C^2H_2O^2H$) from E. Merck (Darmstadt, F.R.G.). Other chemicals were of analytical-reagent grade.

### *Gas chromatography–mass spectrometry*

A Shimadzu Model LKB-9000B gas chromatograph–mass spectrometer connected on-line with a Shimadzu GC–mass pack 500–FDGT computer system was used. A coiled glass column (1 m × 3 mm I.D.) was packed with 2% OV-1 on Gas-Chrom Q (80–100 mesh). The flow-rate of carrier gas (helium) was 30 ml/min. The temperature of the column was 230 °C, and the injection port and ion source were kept at 270 °C. The accelerating voltage, ionization voltage and trap current were 3.5 kV, 70 eV and 60 $\mu$A, respectively.

### *High-performance liquid chromatography (HPLC)*

The apparatus used was a Shimadzu Model LC-3A equipped with a SPD-2A variable-wavelength detector setting at 240 nm. The column was Nucleosil $C_{18}$

(5 μm; 25 cm×4 mm I.D.) (Gaskuro Kogyo, Tokyo, Japan), and the mobile phase was 0.1 *M* acetic acid–acetonitrile (3:1) at a flow-rate of 0.6 ml/min.

*Preparation of captopril-NEM, its sulphoxide and sulphone*

*Deuterated TFE ester of captopril-NEM.* To a sample containing captopril-NEM (100 μg) were added [$^2H_3$]TFE (0.3 ml) and trifluoroacetic anhydride (50 μl), and the whole was allowed to stand at 50°C for 1 h. After removal of the excess reagents with the aid of a nitrogen stream, the residue was dissolved in ethyl acetate (0.5 ml), and an aliquot of this solution was subjected to GC–MS. Derivatization of captopril-NEM into the TFE ester was carried out similarly.

*Hexafluoroisopropyl (HFIP) esters of captopril-NEM sulphoxide and sulphone.* To a sample containing captopril-NEM sulphoxide (100 μg) were added hexafluoroisopropanol (0.3 ml) and trifluoroacetic anhydride (50 μl), and the whole was allowed to stand at 50°C for 1 h. After removal of the excess reagent with the aid of a nitrogen stream, the residue was dissolved in ethyl acetate (50 μl) and an aliquot of this solution was subjected to GC–MS. Captopril-NEM sulphone was similarly derivatized into the HFIP ester.

*Syntheses of authentic samples*

*S-Benzylcaptopril.* To a stirred solution of captopril (201 mg) in ethanol (4 ml) were added dropwise a solution of sodium hydride (102 mg) in ethanol (4 ml) and then benzyl chloride (0.5 ml), and the whole was heated at 60°C for 1 h. After evaporation of the solvent, the residue was diluted with water (15 ml) and washed with hexane (2×15 ml). The aqueous layer was adjusted to pH 2 with 0.5 *M* hydrochloric acid and extracted with ethyl acetate (3×15 ml). The organic layer was concentrated to give S-benzylcaptopril (297 mg) as a colourless oil. The product was homogeneous as judged by thin-layer chromatography (TLC); mass spectrum (PFB derivative): *m/z* 487 [$M^+$], 396 [$M-91$]$^+$, 294 [$M-193$]$^+$; TLC [ethyl acetate–hexane–ethanol–acetic acid (3:3:0.5:0.2)]: $R_F$ 0.33.

*Captopril-NEM sulphoxide.* To a stirred solution of captopril-NEM adduct in dichloromethane (7 ml) was added a solution of *m*-chloroperbenzoic acid (60 mg) in dichloromethane (7 ml), and the whole was stirred at −30°C for 30 min. After evaporation of the solvent, the residue was dissolved in methanol and subjected to column chromatography on silica gel. The column was washed with ethyl acetate (300 ml), and the desired sulphoxide was eluted with methanol (50 ml). After evaporation of the solvent, the residue was redissolved in methanol (1 ml) and subjected to preparative HPLC. The desired fraction was collected and evaporated to give captopril-NEM sulphoxide as a colourless oil. Mass spectrum (HFIP derivative): *m/z* 490 [$M-18$]$^+$, 264 [$M-244$]$^+$; IR: 1040 $cm^{-1}$ (S=O).

*Captopril-NEM sulphone.* To a stirred solution of captopril-NEM adduct (75 mg) in methanol (2 ml) was added dropwise 30% hydrogen peroxide (0.2 ml), and the whole was stirred at room temperature overnight. After evaporation of

the solvent, the residue was dissolved in methanol (2 ml) and subjected to preparative HPLC. The desired fraction was collected and evaporated to give captopril-NEM sulphone as a colourless oil. Mass spectrum (HFIP derivative): *m/z* 524 $[M^+]$, 264 $[M-260]^+$.

*Calibration for captopril-NEM*

To a sample containing captopril-NEM (10–500 ng) and a known amount of S-benzylcaptopril were added 2% PFBB–acetone (0.5 ml) and 0.1 *M* potassium hydroxide–methanol (0.1 ml), and the whole was heated at 50°C for 1 h. After removal of the excess reagents with the aid of a nitrogen stream, the residue was dissolved in ethyl acetate (0.1 ml) and a 1-$\mu$l aliquot of this solution was subjected to GC–MS.

*Assay of captopril in blood and urine*

A blood sample (1 ml) was mixed with 0.5% NEM in 0.2 *M* phosphate buffer (2 ml) containing internal standard (I.S.) (100 ng) and allowed to stand at room temperature for 10 min. The resulting solution was deproteinized by treatment with ethanol (20 ml), and the precipitate was removed by centrifugation at 1600 *g* for 15 min. After evaporation of the supernatant, the residue was dissolved in 2% sodium hydrogen carbonate (3 ml) and washed with ethyl acetate (2×3 ml). The aqueous layer was acidified with 0.5 *M* hydrochloric acid (0.3 ml), saturated with sodium chloride (5 g) and extracted with ethyl acetate (2×3 ml). The organic layer was evaporated to dryness in vacuo and the residue obtained was subjected to GC–MS.

A urine specimen (0.1 ml) was mixed with I.S. (100 ng) and treated with 0.5% NEM in 0.2 *M* phosphate buffer (1 ml). The resulting solution was diluted with 2% sodium hydrogen carbonate (2 ml) and washed with ethyl acetate (2×3 ml). After removal of the solvent with the aid of a nitrogen stream, the aqueous layer was adjusted to pH 2 with 0.5 *M* hydrochloric acid and extracted with ethyl acetate (2×3 ml). The organic layer was evaporated to dryness. The subsequent procedure was carried out in the manner described above.

## RESULTS AND DISCUSSION

It has previously been demonstrated that immediate protection of the sulphydryl group with NEM is required to prevent oxidative degradation of captopril in biological fluids. Therefore our initial efforts were directed towards establishing suitable conditions for derivatization of captopril-NEM into the perfluorobenzyl (PFB) ester.

When treated with PFBB in 2% potassium hydroxide–methanol solution, captopril-NEM was converted into the bis-PFB derivative (Fig. 1). Esterification proceeded readily at 50°C and was complete in 60 min. The derivative was unequivocally characterized by means of MS (Fig. 2). The molecular ion peak, $[M]^+$, and fragment ion peak, $[M-177]^+$, formed by loss of the PFB group, appeared at *m/z* 577 and 396, respectively. The base peak at *m/z* 181 was assignable to the PFB moiety. No characteristic fragment ion peak indicating the cap-

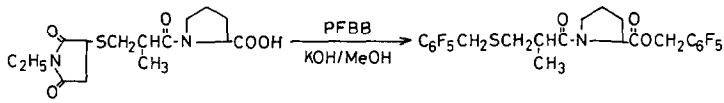


Fig. 1. Formation of captopril bis-PFB derivative.

topril-NEM structure appeared in the mass spectrum. These data indicate that captopril-NEM undergoes S-pentaflorobenzylation as well as esterification to provide captopril bis-PFB derivative.

This promising derivatization procedure was applied to the determination of captopril employing the I.S. method. Thus, S-benzylcaptopril was prepared by treatment with benzyl chloride in the presence of sodium hydride. As shown in Fig. 3, this compound exhibited a molecular ion peak, $[M]^+$, at *m/z* 487 and a fragment ion peak at *m/z* 396 on the mass spectrum, indicating the occurrence of debenzylation. Among several common stationary phases tested, OV-1 was found most suitable for the separation of captopril and I.S. A typical chromatogram obtained by selected-ion monitoring is illustrated in Fig. 4. The two compounds each gave a single peak of the correct theoretical shape.

A calibration graph was constructed by plotting the ratio of the peak area of captopril to that of I.S. against the amount of the former. Satisfactory linearity was observed in the range 0.1–5 ng of captopril; the detection limit was 100 pg.

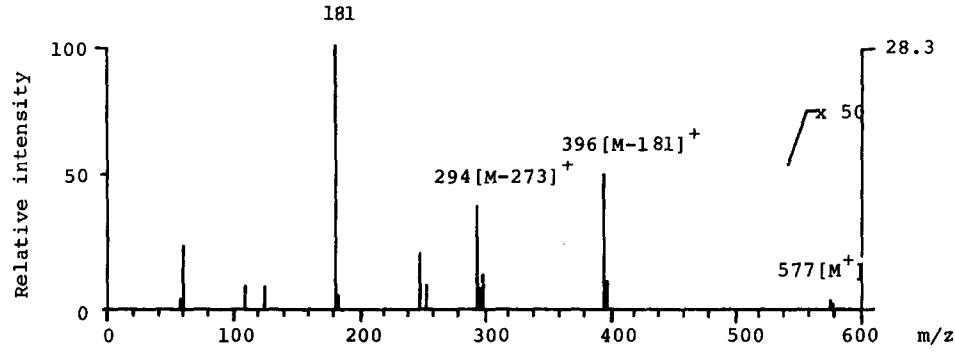


Fig. 2. Mass spectrum of captopril bis-PFB derivative.

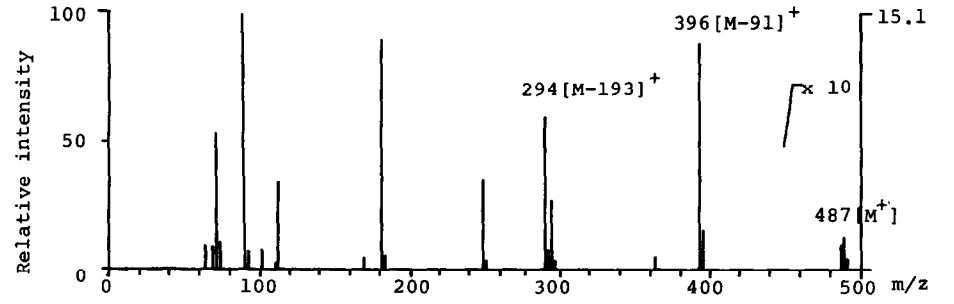


Fig. 3. Mass spectrum of S-benzylcaptopril PFB ester.

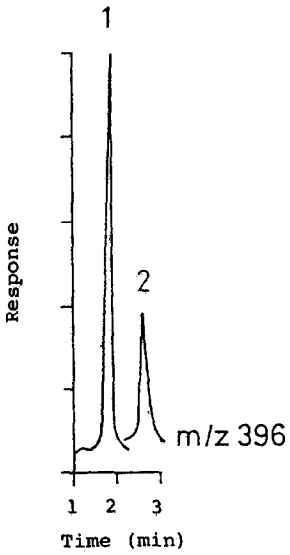


Fig. 4. Chromatogram of captopril (1) (1 ng) and internal standard (2) (0.25 ng) obtained by selected-ion monitoring.

The method was applied to the quantitation of captopril in blood and urine in dogs administered a single dose of captopril. Clean-up of captopril in blood and urine specimens was readily attained by back-extraction with ethyl acetate under basic and acidic conditions.

In order to examine the validity of the proposed method, known amounts of captopril-NEM were added to biological fluids, and their recovery rates were determined. The results obtained with blood and urine specimens are listed in Table I.

The blood and urine levels of captopril were determined after oral administration of captopril (2 mg/kg) to two dogs. The blood level of the drug is illustrated in Fig. 5. The maximum plasma level of captopril was ca. 1 μg/ml, 1 h after administration. The data for urinary excretion in dogs are collected in Table II. The cumulative amounts of unchanged captopril excreted in 24 h were 0.99 and 2.53% of the dose, respectively.

The utility of HFIP esterification for the simultaneous determination of captopril and its disulphide by GC–MS has previously been demonstrated [8]. How-

TABLE I

RECOVERIES OF CAPTOPRIL-NEM ADDED TO BLOOD AND URINE

| Sample | Amount added (ng) | Amount found (mean ± S.D., $n=3$) (ng) | Recovery (%) |
|---|---|---|---|
| Blood | 100 | 98.8 ± 4.00 | 98.8 |
| Urine | 100 | 89.1 ± 2.32 | 89.1 |

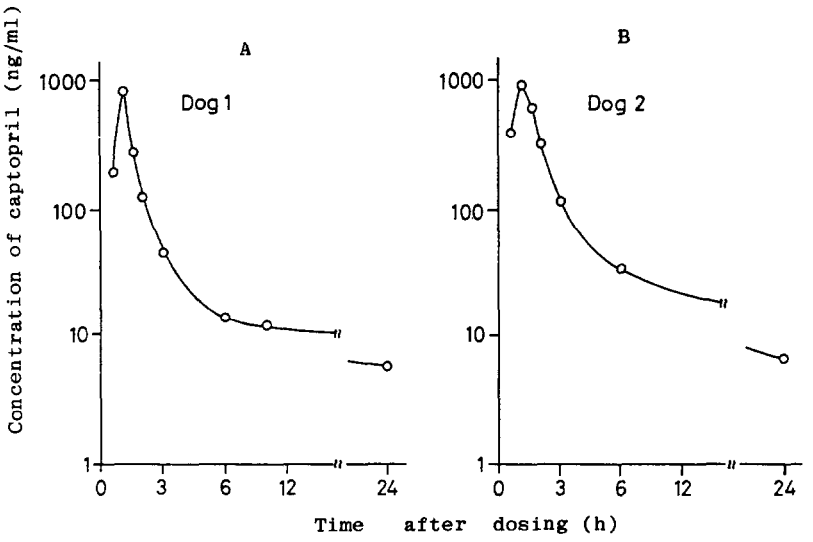


Fig. 5. Blood-level profiles of unchanged captopril in dog 1 (A) and dog 2 (B) after oral administration in a dose of 2 mg/kg.

ever, on an OV-1 column these derivatives were often resolved into two or three peaks, together with a shoulder peak (Fig. 6). This led to the speculation that diastereoisomers might be formed from captopril and its disulphide during derivatization into the HFIP esters. Accordingly, the stable isotope technique was used to investigate the formation of diastereoisomers during esterification of captopril-NEM. When treated with [$^2H_3$]trifluoroethanol in the presence of trifluoroacetic anhydride, captopril-NEM was readily esterified. The product was resolved into two peaks with a shoulder on a 2% OV-1 column as previously reported for the HFIP ester [8]. In their mass spectra, both peaks exhibited the

TABLE II

URINARY EXCRETION OF CAPTOPRIL AFTER ORAL ADMINISTRATION OF CAPTOPRIL (2 mg/kg)

Figures in parentheses represent the percentage of dose.

| Time (h) | Excreted amount of captopril (μg) | |
|---|---|---|
| | Dog 1 | Dog 2 |
| 0-1 | 113.9 | 58.1 |
| 1-2 | 37.6 | 389.4 |
| 2-3 | 8.5 | 56.0 |
| 3-6 | 4.4 | 37.4 |
| 6-8 | 1.5 | 109.4 |
| 8-24 | 6.5 | 3.2 |
| 0-24 | 172.4 (0.99) | 653.5 (2.53) |

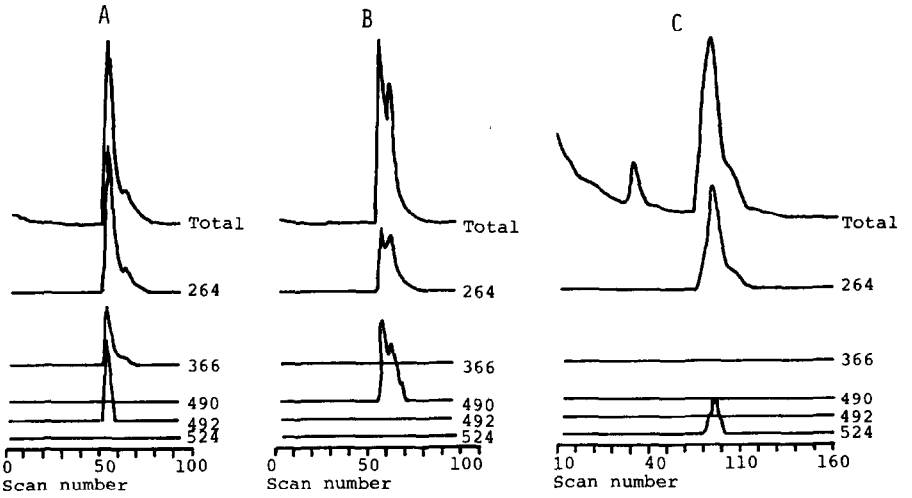


Fig. 6. Reconstructed ion profiles of captopril-NEM HFIP ester (A), its sulphoxide (B) and sulphone (C).

molecular ion, $[M]^+$, at *m/z* 427, the prominent ion, $[M-126]^+$ at *m/z* 301 formed by the loss of the NEM group, and $[M-228]^+$ at *m/z* 199 assignable to the trideuterated proline TFE ester moiety, three mass units higher than that from the non-labelled captopril-NEM TFE ester (Fig. 7). The data indicate that a hydrogen atom at the chiral centre in the proline moiety is exchanged with one

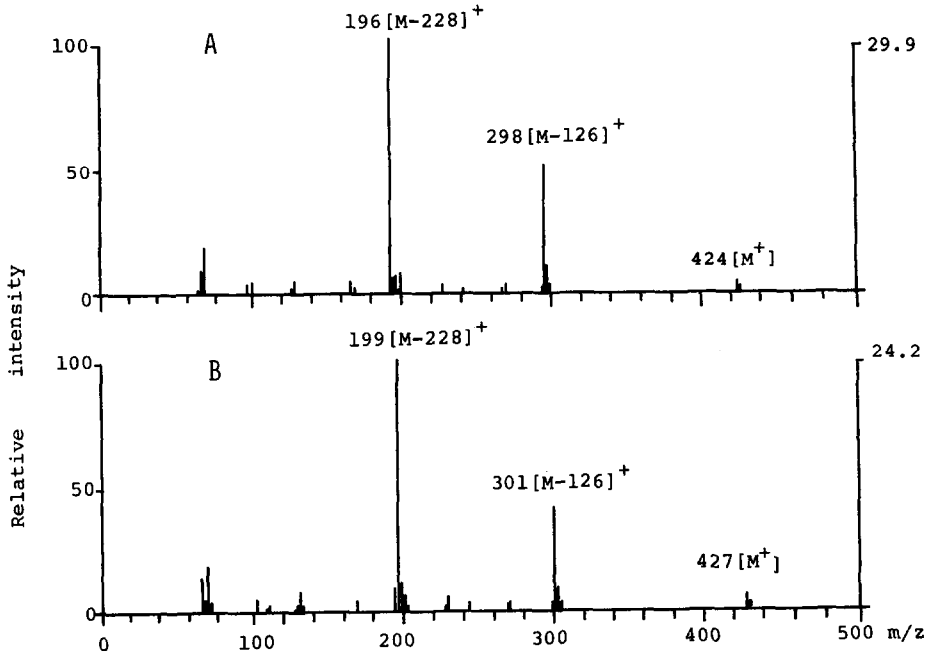


Fig. 7. Mass spectra of captopril-NEM TFE (A) and $[^2H_3]$TFE (B) ester.

deuterium atom from [$^2H_3$]TFE. These results support the assumption that two peaks of captopril-NEM HFIP derivative on the gas chromatogram would be assignable to the diastereoisomers formed during esterification. On the other hand, in the case of pentafluorobenzylation of captopril-NEM, with $NaO^2H$ as a catalyst in [$^2H_4$]methanol, no characteristic fragment ions produced by hydrogen–deuterium exchange appeared on the mass spectrum.

Next, we examined whether or not the oxidized product is formed from captopril-NEM through esterification. Funke et al. [9] reported that captopril-NEM underwent oxidation yielding the sulphoxide in the purification step. Therefore, the authentic sulphoxide and sulphone were synthesized from captopril-NEM. When chromatographed on a 2% OV-1 column, the sulphoxide exhibited a retention time close to that of captopril-NEM. Captopril-NEM in plasma gave no characteristic ions derived from the sulphoxide or sulphone in the reconstructed ion profile (Fig. 6). Thus, it was concluded that neither the sulphoxide nor the sulfone of captopril-NEM was artificially formed through esterification as well as pentafluorobenzylation in this study.

The proposed method has proved to be satisfactory with respect to simplicity, sensitivity and reliability for the determination of low levels of captopril in biological fluids. It should be noted that pentafluorobenzylation of captopril-NEM provides solely the bis-PFB derivative without the artificial formation of diastereoisomers and oxidized products.

REFERENCES

1 R.R. Vollmer, J.A. Boccagno, D.N. Harris and V.S. Murthy, Eur. J. Pharmacol., 51 (1978) 39.
2 B.H. Migdalof, M.J. Antonaccio, D.N. McKinstry, S.M. Singhvi, S.J. Lan, P. Egli and K.J. Kriparani, Drug Metab. Rev., 15 (1984) 841.
3 N. Ohara, H. Ono and K. Hashimoto, Jpn. J. Pharmacol., 29 Suppl. (1979) 102.
4 T. Ikeda, T. Komai, K. Kawai and H. Shindo, Chem. Pharm. Bull., 29 (1981) 1416.
5 T. Komai, T. Ikeda, K. Kawai, E. Kameyama and H. Shindo, J. Pharmacobio-Dyn., 4 (1981) 677.
6 K.K. Wong and J. Dreyfuss, Pharmacologist, 20 (1978) 213.
7 Y. Matsuki, K. Fukuhara, T. Ito, H. Ono, N. Ohara, T. Yui and T. Nambara, J. Chromatogr., 188 (1980) 177.
8 Y. Matsuki, T. Ito, K. Fukuhara, T. Nakamura, M. Kimura, T. Nambara and H. Ono, J. Chromatogr., 239 (1982) 585.
9 P.T. Funke, E. Ivashkiv, M.F. Malley and A.I. Cohen, Anal. Chem., 52 (1980) 1086.