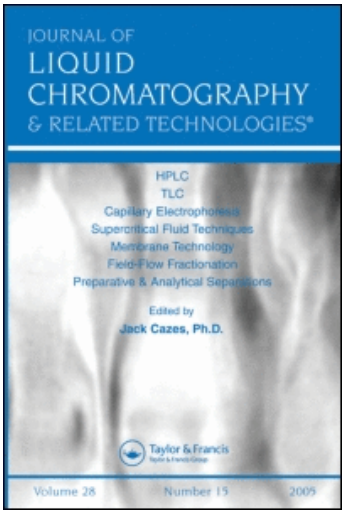


Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

The Determination of Optical Isomers of 2-[3-(2-Chloro-Penoxyphenyl)]-Propionic Acid in Rat Plasma by High Performance Liquid Chromatography

Tomozoh Tamegai[a]; Tomohide Tanaka[a]; Takeru Kaneko[a]; Satoshi Ozaki[a]; Masahiko Ohmae[a]; Kiyoshi Kawabe[a]
[a] Reseach Laboratories, Eisai Co Ltd., Tokyo, Japan

To cite this Article Tamegai, Tomozoh , Tanaka, Tomohide , Kaneko, Takeru , Ozaki, Satoshi , Ohmae, Masahiko and Kawabe, Kiyoshi(1979) 'The Determination of Optical Isomers of 2-[3-(2-Chloro-Penoxyphenyl)]-Propionic Acid in Rat Plasma by High Performance Liquid Chromatography', Journal of Liquid Chromatography & Related Technologies, 2: 4, 551 — 564
To link to this Article: DOI: 10.1080/01483917908060084
URL: http://dx.doi.org/10.1080/01483917908060084

JOURNAL OF LIQUID CHROMATOGRAPHY, 2(4), 551-564 (1979)

THE DETERMINATION OF OPTICAL ISOMERS OF 2-[3-(2-CHLORO-PENOXYPHENYL)]-PROPIONIC ACID IN RAT PLASMA BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Tomozoh Tamegai, Tomohide Tanaka, Takeru Kaneko,
Satoshi Ozaki, Masahiko Ohmae and Kiyoshi Kawabe
Reseach Laboratories, Eisai Co Ltd.,
4-6-10, Koishikawa, Bunkyo-Ku, Tokyo, Japan

ABSTRACT

The optical isomers of dl-2-[3-(2-chlorophenoxy-phenyl)]-propionic acid in rat plasma is converted to the diastereomeric derivatives with (+)-2-aminobutane and then determined by high performance liquid chromatography using a Nucleosil NH_2 (10 μm) column and cyclohexane-ethyl acetate (5:1) as a mobile phase.

The time course of the optical isomers of this drug in rat plasma after oral administration was measured.

The proposed method is specific and reproducible for the determination of the optical isomers of this drug.

INTRODUCTION

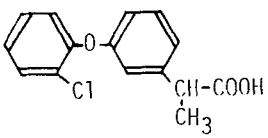
Recently, it has become clear that arylacetic acids are capable of undergoing stereospecific inversion of configuration in vivo. Kripalani et al. (1) demonstrated the stereo-inversion of l-enantiomer to d-enantiomer in dog blood after intravenous or oral administration of α-methyl-fluoren-2-acetic acid.

Also Kaiser et al. (2) demonstrated the similar inversion of the R-(-)-enantiomer to the S-(+)-enantiomer in human blood following oral administration of 2-(4-isobutylphenyl)-propionic acid (ibuprofen). These inversions have been considered to occur before metabolisms of the drugs take place (1-3). The S-(+)-enantiomer of ibuprofen was highly active as an inhibitor of prostagrandin synthesis in vitro, while both enantiomers were equivalent in the tests of analgesic and anti-inflammatory activity in vivo; that suggests the occurence of almost complete inversion (4).

In order to make sure of the pharmacological activity of an enantiomer of this kind, it is important to understand the basis for the stereospecific inversion, therefore, to develop the method for the separation and the determination of the optical isomers. However, it is difficult to separate and to determine optical isomers in biological samples by usual separation methods, because the enantiomers have the same chemical and physical properties. Diastereomeric derivatives of enantiomers with a chiral reagent have different physical properties, that enable separation on a common gas liquid chromatographic column; but many such derivatives are of low volatility and may be thermally unstable on gas chromatographic analysis.

Recently, high performance liquid chromatography (HPLC) has been widely used for the separation of diastereomers (5-10). However, until now, an HPLC method for the determination of optical

isomers in plasma has not been described in the literature. The present paper describes a selective and rapid HPLC method for the determination of 2-[3-(2-chlorophenoxyphenyl)]-propionic acid (CPA), which has been recently developed as a non-steroidal, anti-inflammatory and analgesic drug.



[CPA]

EXPERIMENTAL

Reagents and Materials

dl-CPA was synthesized by the method of Tanaka et al.(11) Oxalyl chloride was purchased from E.Merck (Darmstadt,G.F.R.) and (+)-2-aminobutane was obtained from Norse Laboratories Inc. (Calf.,U.S.A.). The other reagents and solvents were of analytical grade.

Resolution of d- and l-enantiomer of CPA

To a solution of 27.6 g of dl-CPA in 360 ml of ethyl acetate, 12.1 g of R-(+)-phenylethylamine was added at room temperature and the mixture was heated to a clear solution, which was allowed to reflux for 10 min.. After cooling, the resultant drystals were filtered and recrystallized from ethyl acetate. After five recrystallizations, the crystals were dissolved in 173 ml of water and 60 ml ethyl ether, the solution was acidified with 6N-HCl and the aqueous layer was extracted

twice with 30 ml of ethyl ether. The extracts were washed with water, dried with $MgSO_4$ and evaporated to dryness. The residue was recrystallized five times from ethyl acetate to afford pure d-CPA. (m.p. 81.0 - 82.0 °C, $[\alpha]_D^{20}$ + 43.61.). The same procedure was repeated using S-(-)-phenylethylamine as the resolving agent to afford pure l-CPA. m.p. 81.5 - 82.5 °C, $[\alpha]_D^{20}$ - 43.07.

Circular dichroism (CD) spectra of the d- and l-enantiomers of CPA are shown in Fig. 1.

Extraction of CPA and Preparation of the (+)-2-Aminobutane Derivatives

Male Sprange-Dawley rats, 250-300 g were used for 20 mg/kg oral administration of CPA. Plasma was separated from whole heparinized blood by centrifugation and stored at - 20 °C.

Two ml of plasma was pipetted into a centrifuge tube and 2.0 ml of 15 % trichloroacetic acid in water and 5 ml of n-hexane were added. The mixture was shaken for 20 min., centrifuged at 3000 r.p.m. for 5 min., and the organic layer was separated. The aqueous layer was extracted with 5 ml of n-hexane by the same procedure and the organic layer was separated. The organic layers were combined in a round bottomed flask and organic solvents were removed to dryness under reduced pressure. The residue was dissolved in 5 ml of benzene and 0.5 ml of oxalyl chloride was added. The mixture was refluxed at 90 °C for 1 hour. The solvent and excess oxalyl chloride were removed on a water bath. The residue was dissolved in 5 ml of ethyl ether,

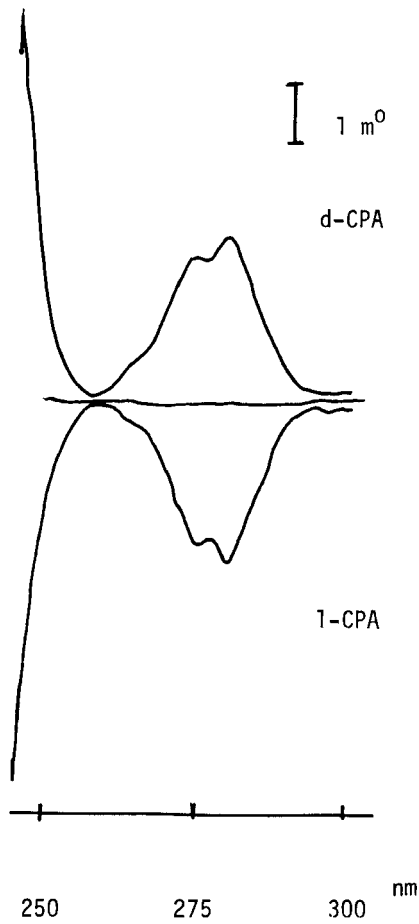


FIGURE 1

Circular dichroism spectra of the d- and l-enantiomer of CPA

Instrument : JASCO J-20 A, Solvent : Methanol
Concentration : 10.00 mg/10 ml, Cell width : 2 mm

1.0 ml of 1 % ($^{v}/_{v}$) (+)-2-aminobutane in ethyl ether was added and the mixture was allowed to stand at room temperature for 1 hour. The mixture was transfered into a separating funnel with

5 ml of ethyl ether, and washed successively with each 5 ml of 10 % HCl, sat. $NaHCO_3$ and water. The ethyl ether layer was transfered into a round bottomed flask and evaporated to dryness. The residue was dissolved in exact 0.5 ml of n-hexane and this solution was used as the sample solution.

High Performance Liquid Chromatography

The chromatographic system was composed of an Altex 110 A pump (Berkeley, CA, U.S.A.), a Rheodyne 7120 loop injector (Calf., U.S.A.) and a JASCO UVIDEC 100-II multi-wavelength detector (JAPAN).

The column was 25 cm x 4.6 mm I.D. stainless-steel tubing slurry packed with Nucleosil NH_2 (Macherey-Nagel, Düren, G.F.R.) having an average particle diameter of 10 μm and the mobile phase consisted of cyclohexane and ethyl acetate in the ratio of 5 : 1. The flow rate was maintained at 1.5 ml/min. and the procedure was carried out at ambient temperature. Twenty μl of the sample solution were injected onto the chromatographic column and the effluent was monitored at 280 nm.

RESULT AND DISCUSSION

Earlier investigation (12,13) showed that the (+)- and (-)-isomers of aliphatic carboxylic acids could be separated by HPLC of diastereomeric amides obtained by the reaction with R(+)-α-methyl-p-nitrobenzylamine or α-(1-naphthyl)-ethylamine.

Our initial efforts were also directed toward the HPLC resolution of diastereomeric amides of CPA with α-arylethylamine. A highly sensitive limit of detection was obtained in R(+)-α-

(1-naphthly)-ethylamide derivative. However R(+)-α-(1-naphthly)-ethylamine reacted not only with CPA but also with various fatty acids in plasma and it was impossible to separate these amides from each other. When (+)-2-aminobutane was used as a chiral reagent, the optical isomers of CPA could be detected at 280 nm without interference by various fatty acids in plasma, because the amides of fatty acids have no absorption at 280 nm. A series of samples containing known amounts of CPA were prepared to determined the optimum reaction conditions for amide formation. The results indicated that reaction of CPA with oxalyl chloride was completed within 30 min. at 90 ^{o}C and amide formation from the acid chloride and excess (+)-2-aminobutane was finished within 30 min. at room temperature.

Earlier investigators have resolved diastereomers on conventional normal phase partition column systems. Therefore, an adsorption chromatographic system, silica or NH_2-bonded silica column, were chosen for the separation of CPA amides. The best result was obtained by HPLC using Nucleosil NH_2 column and cyclohexane-ethyl acetate system as a mobile phase.

Fig. 2 shows the chromatograms of rat plasma after oral administration of the racemic mixture of CPA and control rat plasma. Fig. 3 shows the chromatograms of authentic d-enantiomer, l-enantiomer and racemic mixture of CPA. The retention volumes of two peaks I and II in Fig. 2 coincided with those of authentic d- and l-enantiomer derivatives.

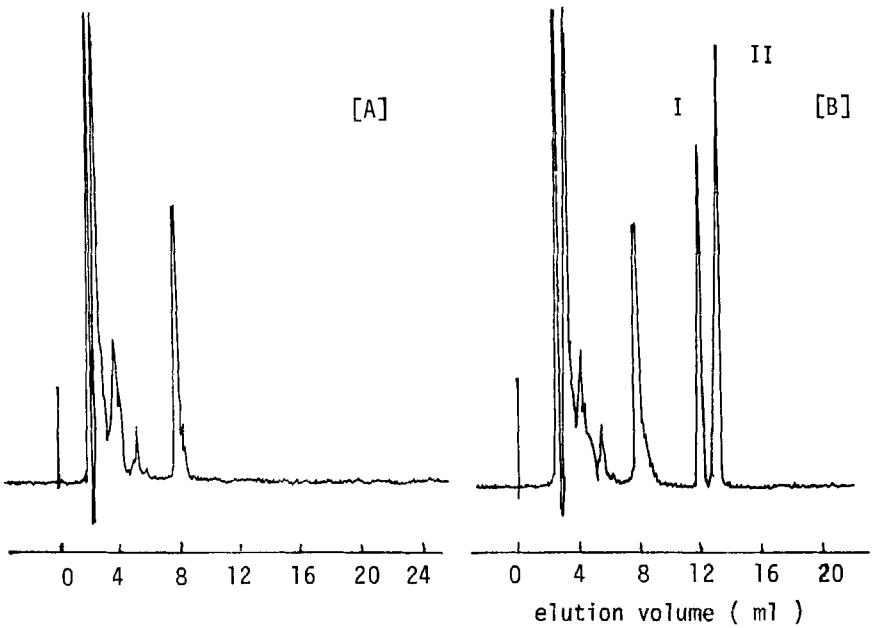


FIGURE 2

The chromatograms of rat plasma after oral administration of dl-CPA and control rat plasma

Conditions , Column:Nucleosil NH_2, 10 μm, 4.6 mm I.D. x 250 mm
Mobile phase:cyclohexane · ethyl acetate (5:1)
Detector:UV_{280}, Flow rate:1.5 ml/min.
Materials , [A] Control rat plasma, [B] Rat plasma after oral administration of dl-CPA

There was a complete baseline separation between the two diastereomers, which was a prerequisite to accurate measurment of peak height. The optical isomers eluted from the column have symmetrical peaks with retention volumes of 11.5 ml and 13.5 ml; and the resolution factor was 1.88. Chromatography of control rat plasma showed no interfering material absorbing at 280 nm.

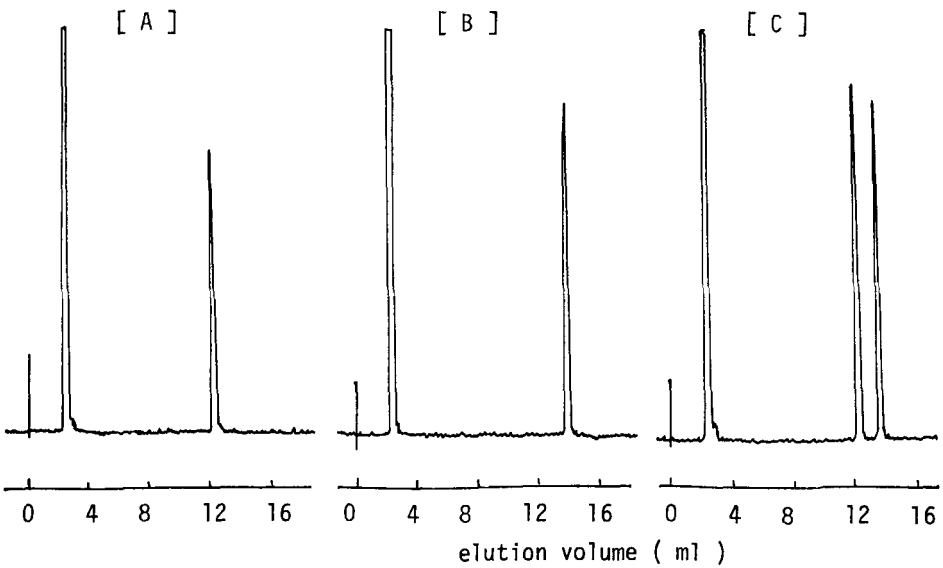


FIGURE 3

The chromatograms of authentic d-,l- and dl-CPA

Conditions , Column:Nucleosil NH_2 10 μm, 4.6 mm I.D. x 250 mm
Mobile phase:cyclohexane · ethyl acetate (5:1)
Detector:UV_{280}, Flow rate:1.5 ml/min.
Materials , [A] d-CPA, [B] l-CPA, [C] dl-CPA

The developed method is sensitive enough to measure the optical isomers in rat plasma after oral administration of CPA, although the minimum detectable amount of each enantiomer was 5 μg/ml of plasma, which was not so sensitive as compared with 0.2 μg/ml of R(+)-α-(1-naphthyl)-ethylamide.

Fig. 4 shows the standard curves of the d- and l-enantiomer added to control rat plasma, based on the peak height measurement. These curves were linear over the range of plasma concentrations from 5.0 to 91.3 μg/ml and had an intercept close to the origin.

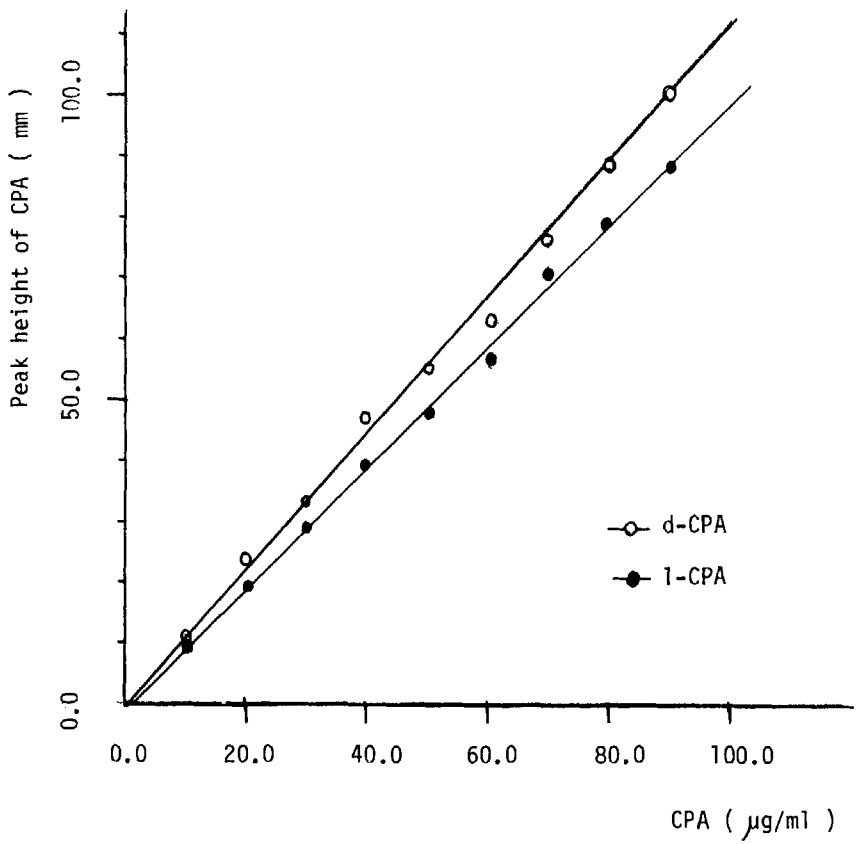


FIGURE 4

The standard curves of d- and l-CPA added to rat plasma

The slopes coincided with those obtained from additional standard curves of authentic d- and l-enantiomer, which shows that the extractability of CPA from rat plasma is effective. However, since the analysis was carried out without the use of an internal standard, it was necessary that at least one standard sample was put on assay.

As shown in Table 1, percent recovery and standard deviation of d- and l-CPA in this method were 97.9 %, 2.69 % and 98.3 %, 1.69 % respectivity for 51.5 μg/ml of rat plasma.

In order to explore whether the l-isomer was converted to the d-isomer, the time course of the CPA plasma levels were investigated. Fig. 5 shows the plasma levels of the d- and l-enantiomer in rat after oral administration (20 mg/kg) of the d- and l-isomer of CPA respectively There was a facile epimerzation of the l-isomer to d-isomer, but d-isomer was not converted to the l-isomer.

TABLE 1

Percent recovery of d- and l-CPA added to control rat plasma

Sample	Recovery %	
d-CPA	102.6 97.4 94.6 96.5 98.4 99.8 95.8	$\bar{X}$ = 97.9 % σ_{n-1} = 2.69 %
l-CPA	101.1 98.9 96.3 97.4 96.5 98.7 99.2	$\bar{X}$ = 98.3 % σ_{n-1} = 1.69 %

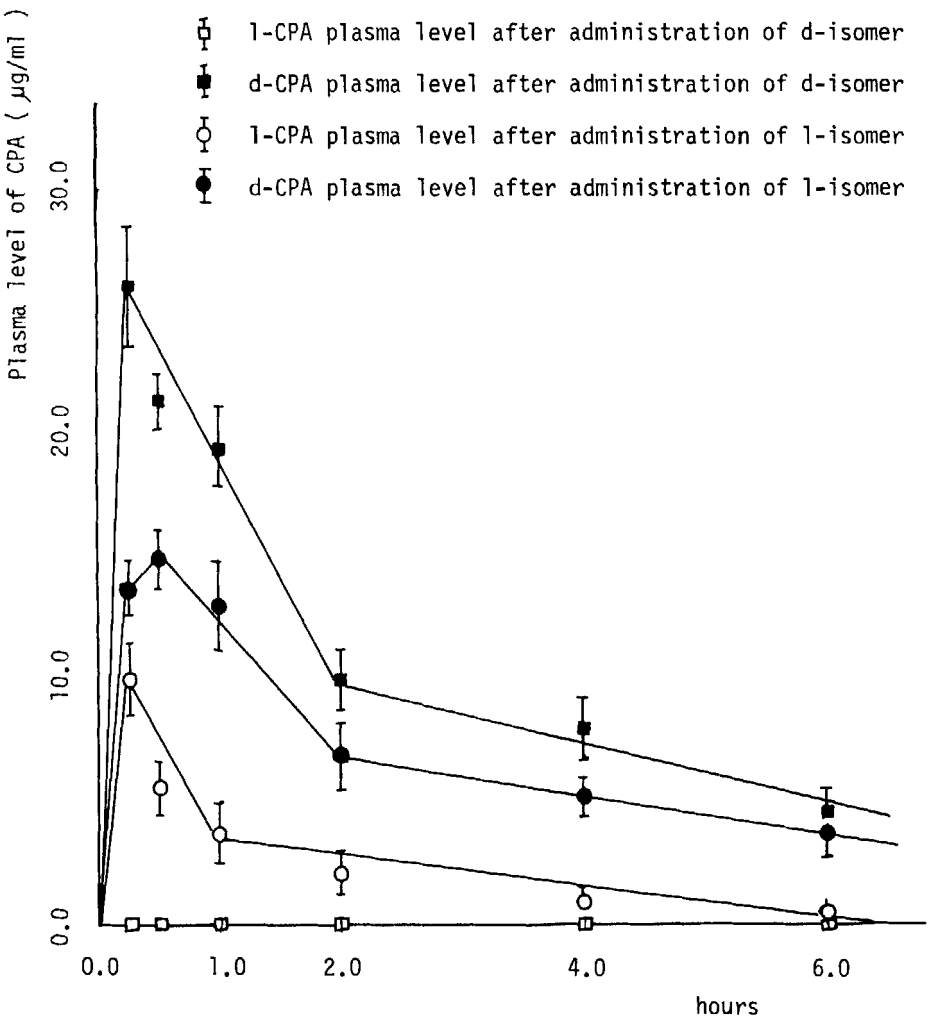


FIGURE 5

The time course of CPA plasma levels after oral administration of d- and l-enantiomers

This result corresponded to the data of Kripalani (1) for α-methylfluoren-2-acetic acid in rat, monkey and man, and of Kaiser (2) for ibuprofen in humans.

Futher studies should be done in order to elucidate the mechanism and reaction sequence of this isomerization and enzymatic pathway involved.

CONCLUSION

We have developed an HPLC method for the determination of the optical isomers of dl-2-[3-(2-chlorophenoxyphenyl)]-propionic acid in plasma. The sensitivity and specificity of this method were compatible with stereo-invertion studies in rat.

REFERENCES

1 K.J.Kripalani, A Zein el-abdin, A.V.Dean and E.C.Schreiber Xenobiotica, 6, (1976) 159

2 D.G.Kaiser, G.J.Vangiessen, R.J.Reischer and W.J.Wechter, J.Pharm.Sci., 65, (1976) 269

3 W.J.Wechter, D.G.Loughhead, R.J.Reisuher, G.J.Vangiessen and D.G.Kai Biochem. Biophys. Res. Commun., 61, (1974) 833

4 S.S.Adams, P.Bresloef and C.G.Mason, J.Pharm.Pharmacol., 28, (1976)

5 M.Koreeda, G.Weiss and K.Nakanishi, J.Amer.Chem.Soc., 95 (1973) 239

6 G.Helmcher and W.Strubent, Chromatographia, 7, (1974) 713

7 K.Imai and S.Murano, Tetrahedron Lett., (1976) 1211

8 C.G.Scott, M.J.Petrin and T.McCorkle, J.Chromatogr., 125 (1976) 157

9 G.Helmchen, H.Volter and W.Schuhle, Tetrahedron Lett., (1977) 1417

10 J.Goto, M.Hasegawa, S.Nakamura, K.Shimada and T.Nambara, J.Chromatogr., 152, (1978) 413

11 S.Tanaka, N.Hashimoto, M.Watanabe, S.Munakata, Y.Kato and T.Tazima, The 98 th Anual Meeting of the Pharmaceutical Society of Japan, Okayama, Apr., (1978) P 263

12 D.Valentine Jr., K.Kchan, C.G.Scott, K.K.Johnson, K.Toth and G.Sancy, J.Org.Chem., 41, (1976) 62

13 B.J.Bergot, R.J.Anderson, D.A.Schooley and C.A.Henrick, J.Chromatogr., 155, (1978) 97