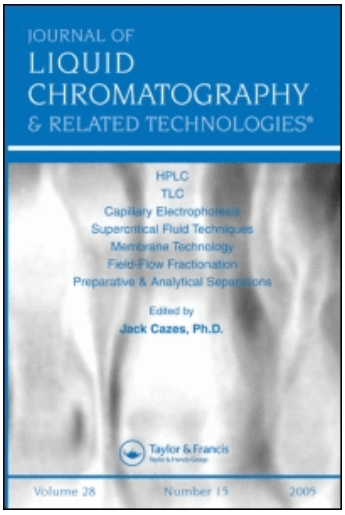

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

SEPARATION OF MULTI-MILLIGRAM QUANTITIES OF GOSSYPOL ENANTIOMERS ON POLYSACCHARIDE-BASED STATIONARY PHASES

Quezia B. Cass[a]; Regina. V. Oliveira[a]
[a] Departamento de Química, Universidade Federal de Sao Carlos, Sao Carlos, SP, Brasil

Online publication date: 23 April 2002

To cite this Article Cass, Quezia B. and Oliveira, Regina. V.(2002) 'SEPARATION OF MULTI-MILLIGRAM QUANTITIES OF GOSSYPOL ENANTIOMERS ON POLYSACCHARIDE-BASED STATIONARY PHASES', Journal of Liquid Chromatography & Related Technologies, 25: 5, 819 — 829
To link to this Article: DOI: 10.1081/JLC-120003038
URL: http://dx.doi.org/10.1081/JLC-120003038

J. LIQ. CHROM. & REL. TECHNOL., 25(5), 819–829 (2002)

SEPARATION OF MULTI-MILLIGRAM QUANTITIES OF GOSSYPOL ENANTIOMERS ON POLYSACCHARIDE-BASED STATIONARY PHASES

Quezia B. Cass* and Regina V. Oliveira

Departamento de Química, Universidade Federal de Sao Carlos, Cx. Postal 676, Sao Carlos, 13560-905, SP, Brasil

ABSTRACT

A column (200 × 7 mm ID) of cellulose tris(3,5-dimethylphenylcarbamate) coated onto naked silica (Hypersil, particle size, 5 μm; pore size, 120 Å) was used under reversed-phase condition with recycle to enantioresolve multi-milligrams quantities of gossypol enantiomers. The use of amylose derivatives and the investigation on the influence of the acidity of the supports used for the polysaccharide phases in the enantioresolution of gossypol is also discussed.

INTRODUCTION

Gossypol (Figure 1), known for its ability to inhibit maturation in human sperm (1–4) has, however, in the recent years, received attention for its potential as a potent antineoplastic agent.(5–9)

*Corresponding author. E-mail: quezia@dq.ufscar.br

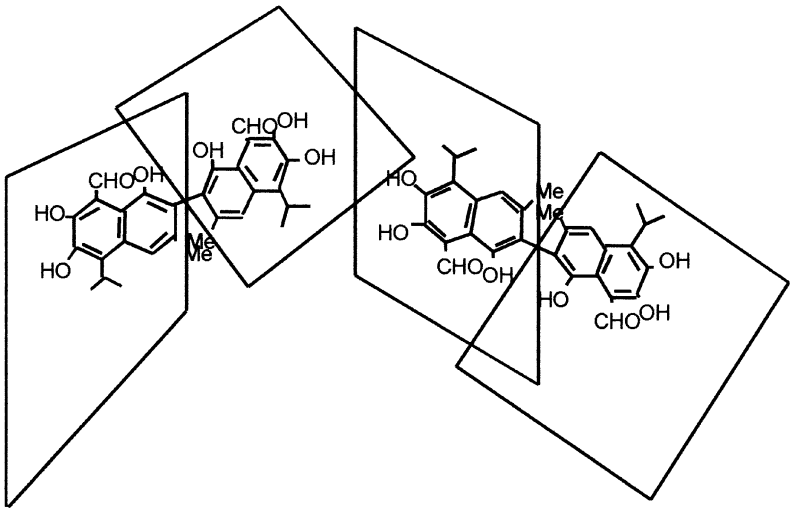

Figure 1. Enantiomers of gossypol.

Gossypol exhibits a number of interesting types of biological activity (10–15) and in all the cases the (−)-enantiomer has been shown to be substantially more active than the (+)-enantiomer.

The analysis of the enantiomeric ratios of gossypol in different Malvaceae species (16–20) has shown that (+)-enantiomer is found in enantiomeric excess in cottonseeds except for *G. barbadense*, which shows a modest excess of (−)-enantiomer, resulting in a need for preparative methods for the resolution of the enantiomeric mixture.

Recently, we described suitable conditions for the direct resolution of gossypol enantiomers using a chiral carbohydrate carbamate phase under reversed-phase conditions.(21) The method described here is an application of this recent method for the preparative resolution of gossypol enantiomers. The influence on the resolution by the support used for the coating of the polysaccharide phase is also discussed.

EXPERIMENTAL

Chemicals

The silicas used had the following properties: APS-Hypersil and Hypersil (Shandon, UK; particle size, 5 μm; pore size, 120 Å) and Nucleosil

(Macherey-Nagel, Germany; particle size, 7 μm; pore size, 500 Å). The Nucleosil silica was aminopropylated according to the reported procedure.(22)

Isocyanates were purchase from Aldrich, USA. Solvents were either HPLC grade from Merck (Darmstadt, Germany) or Chromar HPLC grade from Mallinckrodt Baker (St. Louis, MO, USA) or were purified as usual.(23) Cellulose used was Avicel from Merck. HPLC dead times (t_o) were estimated by using 1,3,5-tri-*tert*-butylbenzene for a normal mode of elution and acetonitrile for reversed mode.

(±)-Gossypol acetic acid was isolated from cotton seeds and its separated (−) and (+) enantiomers obtained, as described in the literature.(24) The elution order was determined by injection of each enantiomer.

Equipment

The analytical HPLC system consisted of a Shimadzu LC-10AD pump (Kyoto, Japan), a Rheodyne 7125 injector fitted with a 20 μL loop, SPD-6AV UV detector operated at 254 nm with a CBM 10A interface. A Shimadzu LC-10 AD pump, an auto injector model Sil 10A, and an SPD-10A detector with a CBM 10A interface, were also used.

The preparative HPLC system consisted of a Shimadzu LC-6AD pump, a Rheodyne 7125 injector fitted with a 1 mL loop, a recycle valve, and an SPD-6AV detector operated at 254 nm with a CBM 10A interface. Data acquisition was performed using a CLASS-VP software.

A Shandon HPLC packing pump was employed for column packing.

Columns

The columns (150 × 4.6 mm ID) were prepared as described elsewhere (25–27), and consisted of cellulose tris(3,5-dimethylphenylcarbamate) coated onto APS-Nucleosil, APS-Hypersil or Hypersil silica (20% w/w), amylose tris[(S)-1-phenylethylcarbamate] coated onto APS-Hypersil (15% w/w), and amylose tris(3,5-dimethylphenylcarbamate) coated onto APS-Hypersil (20% w/w).

The semipreparative HPLC column (200 × 7 mm ID) was prepared and packed in the same way as that of the analytical columns, using Hypersil silica (5.20 g) and cellulose tris(3,5-dimethylphenylcarbamate) (1.30 g).

The columns were evaluated in the normal mode of elution, as described elsewhere (25), before it was passed into the reversed phase mode. This was achieved by elution of 2-propanol and then acetonitrile using approximately

200 mL of each, followed by acetonitrile: $0.01\,mol \cdot L^{-1}$ phosphate buffer (pH = 3.0) (60 : 40 v/v) which was used as the mobile phase.

RESULTS AND DISCUSSION

The excellent separation obtained for the enantiomers of gossypol using a cellulose tris(3,5-dimethylphenylcarbamate) phase on the reverse mode of elution (21) prompted us to investigate conditions for scaling-up for direct separation of multimilligram quantities of gossypol enantiomers.

Amylose carbamates have been shown to have different chiral discrimination when compared with the corresponding cellulose derivatives (25), and they showed excellent resolving ability for a series of axial chiral amides (28). Since the chirality of gossypol is due to steric hindrance to rotation about the internaphthyl bond, this work was initiated by investigating the capability of these carbamates to enantioresolve gossypol.

Columns of amylose tris(3,5-dimethylphenylcarbamate) and tris[(S)-1-phenylethylcarbamate] adsorbed on APS-Hypersil, were prepared as usual (25,27) and evaluated on the normal mode of elution, showing good peak shape and enantioresolution for chiral standards, such as, Troger's base and *trans*-stilbene oxide. Then, they were converted to the reverse mode of elution; the separation of (±)-gossypol was investigated using acetonitrile: $0.01\,mol \cdot L^{-1}$ phosphate buffer (pH = 3.0) (60 : 40 v/v) as the mobile phase.

The amylose tris(3,5-dimethylphenylcarbamate) phase showed good enantioselectivity ($\alpha = 1.39$) but poor resolution (Rs = 0.84), while no enantioresolution was obtained for the gossypol enantiomers with amylose tris[(S)-1-phenylethylcarbamate] phase. Various changes in mobile phase composition were examined on each chiral stationary phase without success.

It is known that peak shape and resolution of polar solutes on the polysaccharides columns can be improved by the use of low acidity supports (27–29), thus, the influence of the acidity of the support used for cellulose tris(3,5-dimethylphenylcarbamate) on the enantioresolution of gossypol was also investigated.

For that, cellulose tris(3,5-dimethylphenylcarbamate) was synthesized (25,30) and three columns prepared: one with Nucleosil (Column 1), after conversion to the aminopropyl derivative, one with APS-Hypersil (Column 2), and one with Hypersil (Column 3) as the support.

The use of meso-(500 Å) and microporous (120 Å) silica as support for the polysaccharides phases with good analytical (21,25) and preparative (30) performance has already been demonstrated and, since the cellulose carbamate used was from the same batch, the difference in performance observed within

Table 1. Separation of Gossypol Enantiomers on Cellulose tris(3,5-Dimethylphenylcarbamate) Phase Coated onto Different Supports

	APS-Nucleosil Column 1	APS-Hypersil Column 2	Hypersil Column 3
k_1	8.73	8.98	5.87
α	1.40	1.32	1.37
Rs	2.34	1.72	1.47

Mobile Phase: ACN:KH_2PO_4 $0.01\,mol \cdot L^{-1}$ (pH = 3.0) (60:40 v/v); flow rate: $1\,mL \cdot min^{-1}$.

these three columns (Table 1) was credited to the difference in acidity of the three supports.

Comparing the results obtained in these three columns (Table 1) a large decrease in resolution can be observed in going from Column 1 to 3. The values of enantioselectivity (α) were also affected but not as much as the resolution. This

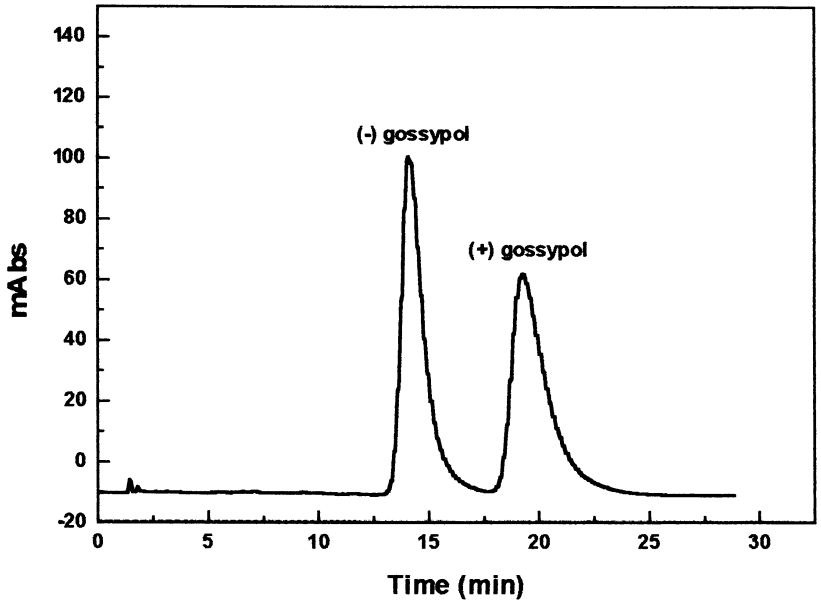

Figure 2. Enantioresolution of gossypol on cellulose tris(3,5-dimethylphenylcarbamate) coated onto APS-Nucleosil (500 Å, 7 μm) (20% w/w). Mobile Phase: ACN : KH_2PO_4 $0.01\,mol \cdot L^{-1}$ (pH = 3.0) (60 : 40 v/v) at $1.0\,mL \cdot min^{-1}$, detection at 254 nm.

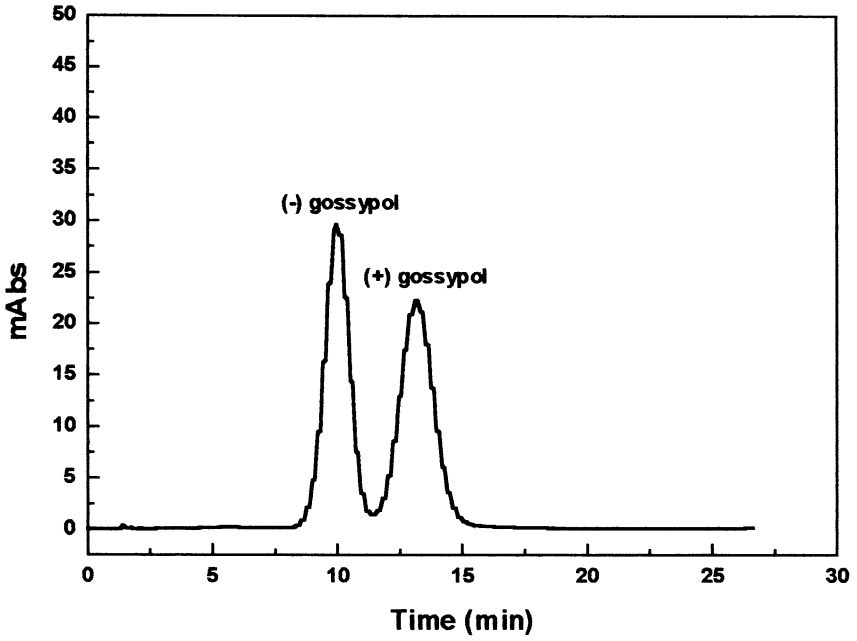

Figure 3. Enantioresolution of gossypol on cellulose tris(3,5-dimethylphenylcarbamate) coated onto Hypersil (120 Å, 5 μm) (20% w/w). Mobile Phase: ACN : KH_2PO_4 0.01 mol · L^{-1} (pH = 3.0) (60 : 40 v/v) at 1.0 mL · min^{-1}, detection at 254 nm.

is probably due to nonspecific interactions with any exposed silanol groups of the more acidic supports.(28,31)

Although the resolution obtained with the use of naked silica as the support was the smallest, the quality of the separation was unexpected due to the high polarity of gossypol. Figures 2 and 3 shows the chromatograms obtained on Columns 1 and 3.

In directing our experiments towards producing multimilligram quantities of gossypol enantiomers, the use of naked silica as the support was considered. The reason for this was that in planning a chiral preparative separation, economical reasons need also to be considered.

Not all chiral separations designed for analytical purposes are equally suited for preparative work and, although the enantioselectivity obtained with the chiral analytical column was in the range recommended for preparative separation, a resolution of 1.47 meant a difficult one for scaling-up.(30,32)

Since preparative chiral columns become more expensive as their size increases, the use of a semi-preparative column (200 × 7 mm ID) combined with

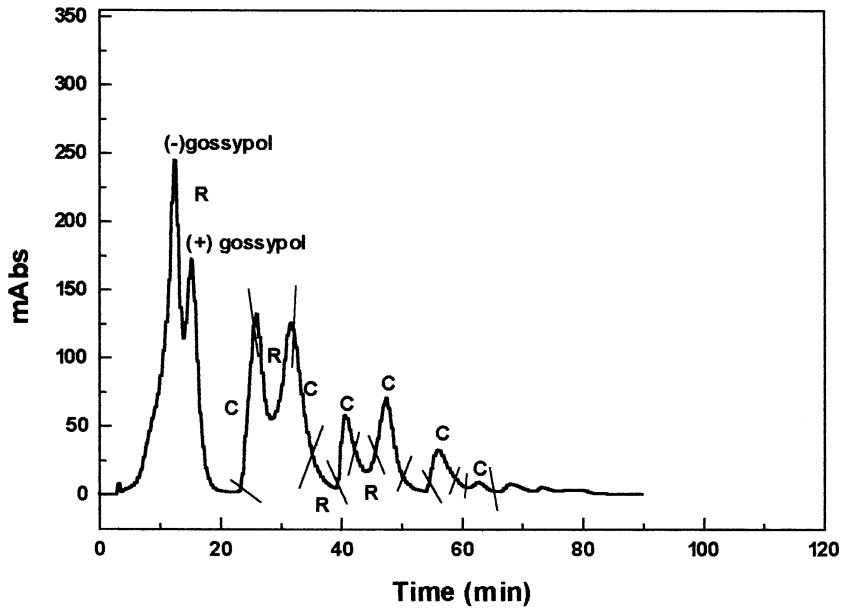

Figure 4. The recycle chromatogram of (±)-gossypol on cellulose tris(3,5-dimethylphenylcarbamate) coated onto Hypersil (120 Å, 5 μm) (200 × 7 mm ID). Mobile Phase: ACN : KH_2PO_4 0.01 mol · L^{-1} (pH = 3.0) (65 : 35 v/v) at 2.0 mL · min^{-1}, detection at 254 nm; R = recycle; C = collect.

recycling of the eluate seemed the best alternative to enhance the separating effect.

When performing a recycle preparative purification, attention must be given to the broadening effect of the eluate band during the recycling process. The flow rate should be well adjusted for effecting the separation without peak overlap during recycling. To obtain recycling in a minimum possible time, the use of peak-shave is recommended as an efficient method.(32)

The appropriate number of cycles is determined on the basis of the chromatograms. In this work, racemic gossypol was injected as an 8 mg · mL^{-1} sample into a semipreparative HPLC column, and the separation was performed at a flow rate of 2 mL · min^{-1} using acetonitrile: 0.01 mol · L^{-1} phosphate buffer (pH = 3.0) (65 : 35 v/v) as mobile phase with cycles of 20 min. The separation was monitored at $\lambda = 254$ nm. The recycle chromatogram of the separation is shown on Figure 4. No shaving was made on the first cycle. Complete separation was obtained with three further cycles. Figure 4 shows also how the shaving was carried out.

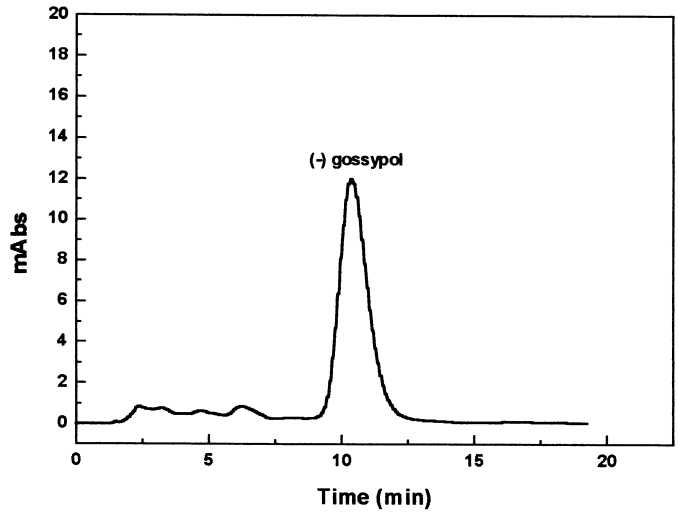

Figure 5. Analytical chromatogram of the isolated (−)-gossypol on cellulose tris(3,5-dimethylphenylcarbamate) coated onto APS-Nucleosil (500 Å, 7 μm) (20% w/w). Mobile Phase: ACN : KH_2PO_4 0.01 mol · L^{-1} (pH = 3.0) (60 : 40 v/v) at 1.0 mL · min^{-1}, detection at 254 nm.

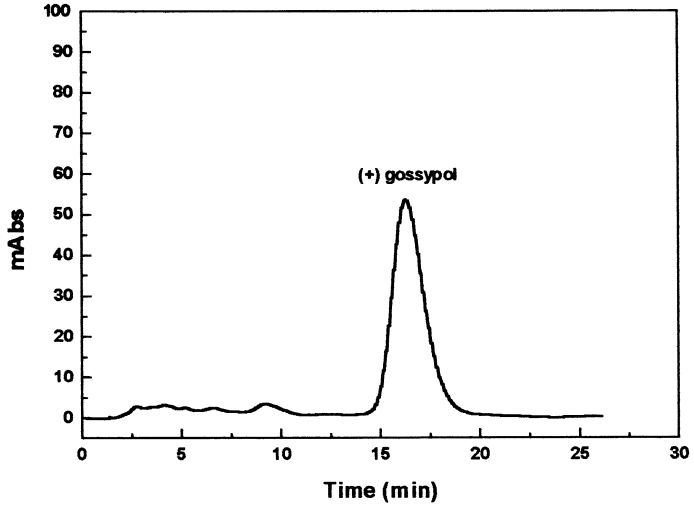

Figure 6. Analytical chromatogram of the isolated (+)-gossypol on cellulose tris(3,5-dimethylphenylcarbamate) coated onto APS-Nucleosil (500 Å, 7 μm) (20% w/w). Mobile Phase: ACN : KH_2PO_4 0.01 mol · L^{-1} (pH = 3.0) (60 : 40 v/v) at 1.0 mL · min^{-1}, detection at 254 nm.

With this method (−)-gossypol (15.7 mg) and (+)-gossypol (16.2 mg) were isolated from 40 mg of racemic gossypol in 98% of enantiomeric purity. The enantiomers were isolated, from the mobile phase by extraction of the aqueous phase with dichloromethane (3 × 150 mL) after evaporation of the acetonitrile *in vacuum*. This procedure was responsible for lowering the recovery of the enantiomers, which were isolated in 78.3% and 81.0%, respectively. Figures 5 and 6 show the chromatograms of the isolated enantiomers.

The use of recycling increases the production rate and also causes a decrease in eluent consumption; in this work these two conditions were achieved and also made possible the use of a much more economical support for the polysaccharide cellulose column.

The enantiomers of gossypol has been previously separated in semi and preparative scale only by conversion to diastereomeric Schiff's base derivatives (24,33); the method here described is the first practical procedure for the direct separation of multi-milligram quantities of gossypol enantiomers.

ACKNOWLEDGMENTS

The authors acknowledge the financial support and grants from FAPESP. The grant from CNPq is also acknowledged.

REFERENCES

1. Segal, S.J. *Gossypol: A Potential Antifertility Agent for Males*; Plenum Press: New York, 1985; 1–271.
2. Sonenberg, M.; Huang, J.T.; Ren, Y.F.; Su, T.L.; Watanabe, K.A.; Haspel, H.C.; Corin, R.E.; Hoffer, A.P. Contraception **1988**, *37* (3), 247–255.
3. Waites, G.M.H.; Wang, C.; Griffin, P.D. Int. J. Andrology **1998**, *21* (1), 8–12.
4. Coutinho, E.M.; Athayde, C.; Atta, G.; Gu, Z.P.; Chen, Z.W.; Sang, G.W.; Emuveyan, E.; Adekunle, A.O.; Mati, J.; Otubu, J.; Reidenberg, M.M.; Segal, S.J. Contraception **2000**, *61* (1), 61–67.
5. Seidman, A.D.; Rosen, P.; Ligueros, M.; Salinas, L.; Norton, L.; Sonenberg, M.; Reidenberg, M.M. J. Invest. Med. **1998**, *46* (3), 213A–213A.
6. Bushunow, P.; Reidenberg, M.M.; Wasenko, J.; Winfield, J.; Lorenzo, B.; Lemke, S.; Himpler, B.; Corona, R.; Coyle, T. J. Neuro-Oncol. **1999**, *43* (1), 79–86.

7. Shelley, M.D.; Hartley, L.; Groundwater, P.W.; Fish, R.G. Anti-Cancer Drugs **2000**, *11* (3), 209–216.
8. Wang, X.H.; Wang, J.; Wong, S.C.H.; Chow, L.S.N.; Nicholls, J.M.; Wong, Y.C.; Liu, Y.; Kwong, D.L.W.; Sham, J.S.T.; Tsao, S.W. Life Sci. **2000**, *67* (22), 2663–2671.
9. Shelley, M.D.; Hartley, L.; Fish, R.G.; Groundwater, P.; Morgan, J.J.G.; Mort, D.; Mason, M.; Evans, A. Cancer Letters **1999**, *135* (2), 171–180.
10. Lin, T.; Schinazi, R.; Griffith, B.P.; August, E.M..; Eriksson, B.F.H.; Zheng, D.; Huang, L.; Prusoff, W.H. Antimicrob. Agents Chemother. **1989**, *33* (12), 2149–2151.
11. Gomez, M.S.; Piper, R.C.; Hunsaker, L.A.; Royer, R.E.; Deck, L.M.; Makler, M.T.; Vander Jagt, D.L. Mol. Biochem. Parasitol. **1997**, *90* (1), 235–246.
12. Razakantoanina, V.; Phung, N.K.P.; Jaureguiberry, G. Parasitol. Res. **2000**, *86* (8), 665–668.
13. Kulp, S.K.; Rikihisa, Y.; Lin, Y.C.; Moh, P.P.; Li, P.K.; Gu, Y. Parasitol. Res. **1993**, *79* (4), 279–285.
14. Deck, L.M.; Royer, R.E.; Chamblee, B.B.; Hernandez, V.M.; Malone, R.R.; Torres, J.E.; Hunsaker, L.A.; Piper, R.C.; Makler, M.T.; Vander Jagt, D.L. J. Med. Chem. **1998**, *41* (20), 3879–3887.
15. Rajarajeswari, V.; Subbarao, I.V. Indian J. Agric. Sci. **1997**, *67* (7), 293–295.
16. Zhou, R.H.; Lin, X.D. Contraception **1988**, *37* (3), 239–245.
17. Cass, Q.B.; Tiritan, M.E.; Matlin, S.A.; Freire, E.C. Phytochemistry **1991**, *30* (8), 2655–2657.
18. Jaroszewski, J.W.; Strom-Hansen, T.; Hansen, S.H.; Thastrup, O.; Kofod, H. Planta Med. **1992**, *58*, 454–458.
19. Cass, Q.B.; Tiritan, M.E.; Bassi, A.L.; Degani, A.L.; Freire, E.C. 18th Annual Meeting of Brazilian Chemical Society, 1995; PN 107.
20. Schmidt, J.H.; Wells, R. J. Agric. Food Chem. **1990**, *38*, 505–508.
21. Cass, Q.B.; Bassi, A.L. Chirality **1999**, *11* (1), 46–49.
22. Jones, D.; Burns, I.W.; Sellings, S.G.; Cox, J.A. J. Chromatogr. **1977**, *144*, 169–180.
23. Perin, D.; Armarego, D.; Perin, D.R. *Purification of Laboratory Chemicals*, 2nd Ed.; Pergamon Press: Oxford, 1980.
24. Matlin, S.A.; Belenguer, A.; Tyson, R.G.; Brookes, A.N. J. High Resol. Chromatogr. & Chromatogr. Comm. **1987**, *10*, 86–91.
25. Matlin, S.A.; Tiritan, M.E.; Crawford, A.J.; Cass, Q.B.; Boyd, D.R. Chirality **1994**, *6*, 135–140.
26. Cass, Q.B.; Bassi, A.L.; Calafatti, S.A.; Matlin, S.A.; Tiritan, M.E.; Campos, L.M.M. Chirality **1996**, *8*, 143–146.

27. Matlin, S.A.; Tiritan, M.E.; Cass, Q.B.; Boyd, D.R.; Chirality **1996**, *8*, 147–153.
28. Cass, Q.B.; Degani, A.L.G.; Tiritan, M.E.; Matlin, S.A.; Curran, D.P.; Balog, A. Chirality **1997**, *9*, 109–112.
29. Grieb, S.J.; Matlin, S.A.; Beleguer, A.M.; Ritchie, H.J. Chirality **1994**, *6*, 129–134.
30. Tiritan, M.E.; Cass, Q.B.; Alamo, A.D.; Matlin, S.A.; Grieb, S.J. Chirality **1998**, *10*, 573–577.
31. Cass, Q.B.; Tiritan, M.E.; Calafatti, S.A.; Matlin, S.A. J. Liq. Chrom. & Rel. Technol. **1999**, *22* (20), 3091–3099.
32. Charton, F.; Bailly, M.; Guiochon, G. J. Chrom. A **1994**, *687*, 13–31.
33. Matlin, S.A.; Zhou, R. J. High Res. Chrom. & Chrom. Comm. **1984**, *7*, 629–631.

Received October 1, 2001
Accepted October 25, 2001
Manuscript 5674