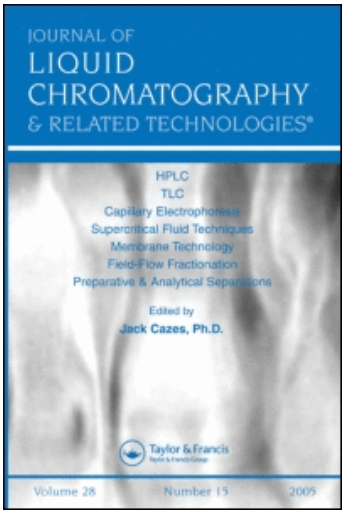

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Isocratic HPLC Separation of Several Racemic Drugs with Two Stereogenic Centers on a Pirkle Urea-Type Chiral Stationary Phase

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ISOCRATIC HPLC SEPARATION OF SEVERAL RACEMIC DRUGS WITH TWO STEREOGENIC CENTERS ON A PIRKLE UREA-TYPE CHIRAL STATIONARY PHASE

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ABSTRACT

A simple and rapid isocratic enantiomeric separation of several racemic drugs with two stereogenic centers, namely formoterol, labetalol, nadolol, indenolol, and U-54494A, was achieved under normal phase mode using a urea-type chiral stationary phase (CSP) column of (S)-indoline-2-carboxylic acid and (R)-1-(α-naphthyl) ethylamine, known as Chirex 3022 column. Labetalol and formoterol were resolved successfully into its four enantiomers. Furthermore, racemate mixture of U-54494A has been resolved into its individual enantiomers. However, nadolol and indenolol was partially separated.

INTRODUCTION

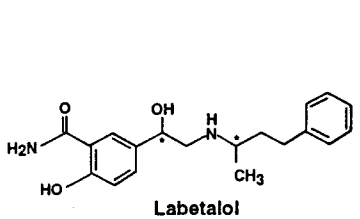
The pharmacodynamic activity of racemic drugs is exerted by specific enantiomer.[1] Furthermore, enantiomers of chiral drugs may have quite different pharmacological properties, which need not occur within the same concentration range.[2] However, when racemate are used as pharmacological tools, it is probably, not so much due to ignorance, but rather to the lack of optically pure enantiomers.[3] The trends are to develop effective new therapeutic agents in optically pure form in order to fully exploit nature and to evaluate the enantiomeric purity and toxicity/activity of any new drug introduction.[1,3,4,5] Such driving forces necessitate that fast, sensitive, and reproducible chromatographic methods must be developed for the direct separation and quantitation of enantiomers.

The separation of enantiomers of racemic compounds with one chiral center has become possible by chiral HPLC; however, the challenge is more obvious, especially when separating enantiomers of compounds with two or more stereogenic centers, especially under isocratic conditions. All the investigated drugs, namely, formoterol, labetalol, nadolol, indenolol, and U-54494 A, have two asymmetric carbon atoms in the molecule making four stereoisomers possible. The chemical structures of these drugs are shown in Figure 1. The enantiomers of β-blockers have been analyzed via chiral high performance liquid chromatography (HPLC).[6,7,8,9,10]

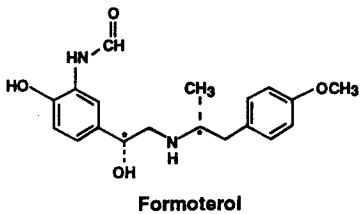
Formoterol, chemically known as 3-formylamino-4-hydroxy-α[*N*- [1-methyl-2- (*p*-methoxyphenyl) ethyl] aminomethyl] benzyl alcohol. It is a long-acting β1- and β2-selective adrenoceptor agonist, which has demonstrated significant bronchodilatory effects. It is used also for the relaxation of the tracheal smooth muscle, depression of the subtetanic contractions of the soleus muscle, and it increases the force of contraction in the electrically driven papillary muscle.[11,12,13]

Labetalol, 2-hydroxy-5- [1-hydroxy-2- [(1-methyl-3-phenylpropyl) amino] ethyl] benzamide, is an antihypertensive drug which blocks α- and β-adrenoceptors.[14,15] One of the labetalol isomers [R,R'-labetalol (dilevalol)] was later shown to cause liver toxicity.[5]

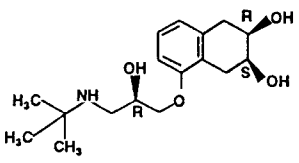
Nadolol, 2, 3 cis-1, 2, 3, 4-tetrahydro-5 [2-hydroxy-3- (tert-butylamino) propoxy]-2, 3-naphthalenediol, (SQ 11725) is a non-selective β-adrenergic blocker used for the treatment of hypertensive.[15] Nadolol has three chiral cen ters, but the 2-3 hydroxy groups are fixed in the cis configuration therefore, it



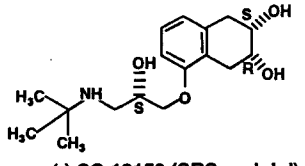
Labetalol



Formoterol

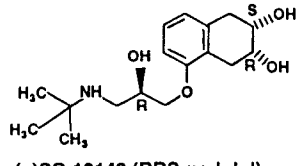


(+) SQ 12148 (RSR-nadolol)

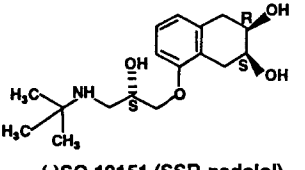


(-) SQ 12150 (SRS-nadolol)

Racemate A (SQ 12181)

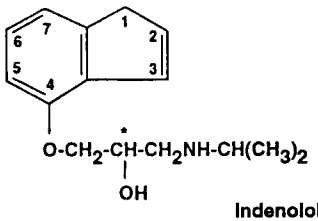


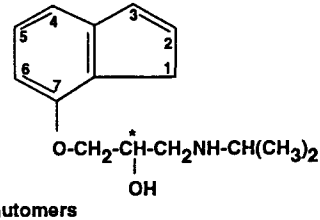
(+)SQ 12149 (RRS-nadolol)



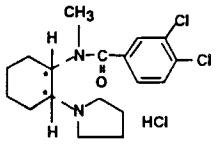
(-)SQ 12151 (SSR-nadolol)

Racemate B(SQ 12182)
Nadolol

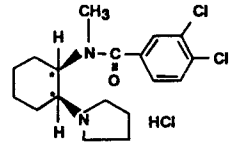




Indenolol tautomers



cis-(-)U-76531 A



cis-(+) U-76532 A

U-54494A

Figure 1. The chemical structures of labetalol, formoterol, nadolol, indenolol, and U-54494A enantiomers. Asterisk indicates the position of the chiral carbon.

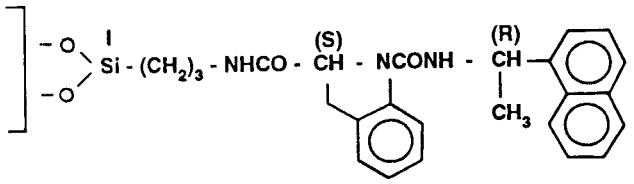


Figure 2. The structure of the CSP-Chirex 3022 made of (S)-indoline-2-carboxylic acid linked to (R)-1-(α-naphthyl) ethylamine urea.

possesses two chiral centers which allows for the presence of two racemates known as racemate A (SQ 12181) and racemate B (SQ 12182) and a total of four enantiomers. Nadolol racemate A (SQ 12181) consists of enantiomers RSR- nadolol (SQ 12148) and SRS-nadolol (SQ 12150) in 1:1 ratio, while racemate B (SQ 12182) consists of enantiomers RRS-nadolol (SQ 12149) and SSR-nadolol (SQ 12151) in 1:1 ratio.

U-54494A, (3,4-dichloro-N-methyl-N-[2-(1-pyrrolidinyl) cyclohexyl, benzamide], is a potential anticonvulsant agent, which is the racemate of U-76531A cis- (-)-, and U-76532A cis- (+)-.

Indenolol, 1-(inden-4 (or 7)-yloxy)-3- isopropylamino-2-propanol, is a non-selective β-adrenoceptor antagonist[15] that consists of two positional isomers. The racemic drug exists as tautomic mixture of 7- and 4-indenyloxy isomers in the ratio of 2:1.

In this paper an isocratic mode has been employed to analyze drugs with two stereogenic centers, using a Pirkle urea-type chiral stationary phase column of (S)-indoline-2-carboxylic acid and (R)-1-(α-naphthyl) ethylamine (Figure 2), known as Chirex 3022, column which can be used in normal phase mode.

EXPERIMENTAL

Apparatus

The chromatographic system, obtained from Waters (Milford, MA, USA), consisted of a Lambda Max 481 Variable Wavelength detector, 510 pump, a U6K injector and a 745 B integrator. The Chirex 3022 (S)-indoline-2-

carboxylic acid (R)-1-(α-naphthyl) ethylamine column (250 X4 mm ID, serial # 62923) was a generous gift from Phenomenex (Torrance, CA). Shodex OR-1 optical rotation detector (JM Sciences, NY, USA).

Chemicals

Formoterol fumarate dihydrate, a racemate mixture, Batch # 15P, was supplied by Astra Draco AB (Lund, Sweden). Labetalol hydrochloride, racemate mixture, MR 7206, racemate GR 81728A RS/SR batch # ALYT 316/12, and racemate GR 81729A RR/SS, batch # ALYT 316/15/1, were obtained from Glaxo Group Research (Ware, U.K) Ltd. Nadolol, racemate mixture (SQ 11725) batch # 02-806-34000, the two racemates, (SQ12181) lot # 2, (SQ12182) lot # 2, and the four enantiomers (SQ12148, 12149, 12150, 12151), all lot # 2, are kindly supplied by Bristol-Myers, Squibb Pharmaceutical Research Institute (Princeton, NJ, USA).

The racemate mixture U-54494A hydrochloride (Lot (A1) 0297-RND-065), its individual enantiomers U-76531A hydrochloride cis-(-), (batch # 21098KCB69A), and U-76532A hydrochloride cis-(+), (batch # 21098KCB50B-1), was a kind gift from Dr. Jeffrey S. Mehring, (Upjohn company, MI, USA). Indenolol hydrochloride, as a racemate mixture, (lot # PUP-A504) was a generous gift from Yamanouchi Pharmaceutical Corporation (Tokyo, Japan). HPLC grade n-hexane was purchased from Fisher Scientific (Springfield, NJ, USA), 1,2-dichloroethane (Rathburn Chemical Ltd. Walkerburn, Scotland, UK), trifluoroacetic acid (Sigma Chemical Co., MO, USA), and ethanol 99.7%, from (Merck, Darmstadt, Germany)

Chromatographic Parameters

Capacity factors (k') were calculated using the equation $k' = t_R - t_0/t_0$ where t_R is the elution time at peak maximum and t_0 is the elution time of unretained solute. The separation factor, (α), was calculated using the equation $\alpha = k'_2 / k'_1$ where k'_2 and k'_1 are the capacity factors for the second and first eluted peaks, respectively.

The resolution factor Rs was calculated using the equation $Rs = 2[(t_{R2} - t_{R1})/(W_{b1} + W_{b2})]$. Where t_{R2} and t_{R1} are the elution time of second and first peaks, respectively, W_{b1} and W_{b2} are the peak width at base of the first and second peaks, respectively.

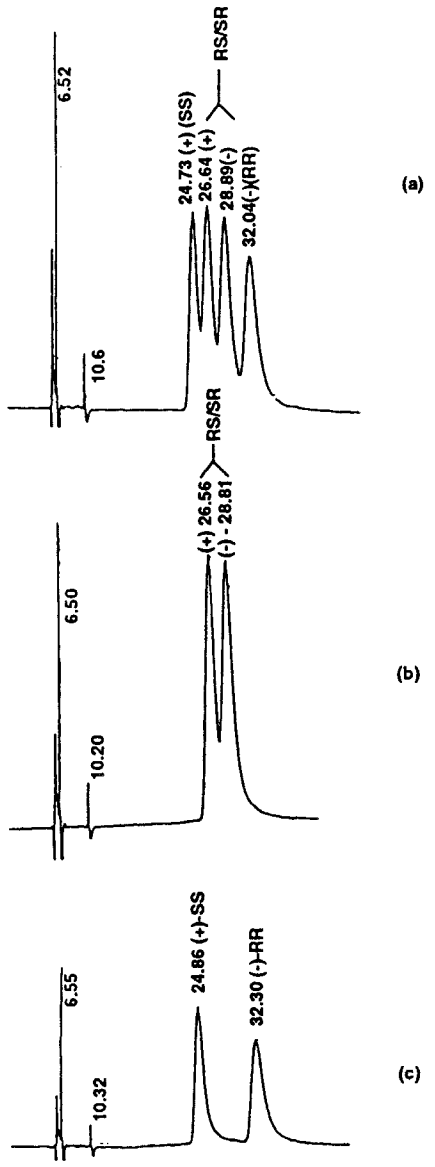


Figure 3. Chromatograms of labetolol; (a) racemate mixture, (b) racemate GR 81728A, and (c) racemate GR 81729A. Mobile phase: Hexane:1,2-dichloroethane:[ethanol + TFA (20:1) premixed]:methanol (58:35:7:0.7); flow rate: 0.4 mL/min; chart speed: 0.25 cm/min; wavelength: 308 nm.

Table 1

Chromatographic Parameters Capacity (k'), Separation (α) and Resolution (R_S) Factors for Labetalol Enantiomers

Racemate	k'1	k'2	α	R_S
GR 81729A	3.19 (+) SS	4.44 (-) RR	1.4	2.86
GR 81728A	3.50 (+)	3.88 (-)	1.11	0.78

RESULTS AND DISCUSSION

For simplicity we tried to separate all the enantiomers of these drugs using isocratic technique. Published reports have described an HPLC analysis of β-blocking agents with more than one chiral center such as nadolol,[7,16] indenolol,[6] and labetalol.[16] Optical rotation detector was used to identify the optical rotation of the enantiomers of these drugs when applicable, using the same chromatographic conditions. The optical rotation was verified by injecting individual enantiomers or racemate mixture were available.

Labetalol

The four enantiomers were separated using isocratic elution under the chromatographic conditions shown in Figure 3a. Each pair of enantiomers was determined by injecting individual racemate. Figure 3b shows the chromatogram of racemate GR 81729A (RR/SS) while Figure 3c shows the chromatogram of racemate GR 81728A (RS/SR).

With the aid of an optical rotation detector, it was confirmed that the first eluting enantiomer in Figure 3a and 3b is the dextrorotatory enantiomer, while the second eluting enantiomer in Figure 3b or the fourth in Figure 3a is the levorotatory enantiomer. Also the first and second enantiomers in Figure 3c or the second and the third in Figure 3a are the enantiomers with (+) and (-) sign, respectively.

Since the enantiomer with the RR configuration, (dilevalol), of racemate GR 81729 A had (-) sign [14], therefore, the other enantiomer with (+) sign will have the (SS) configuration. The chromatographic parameters calculated for the labetalol enantiomers are summarized in Table 1.

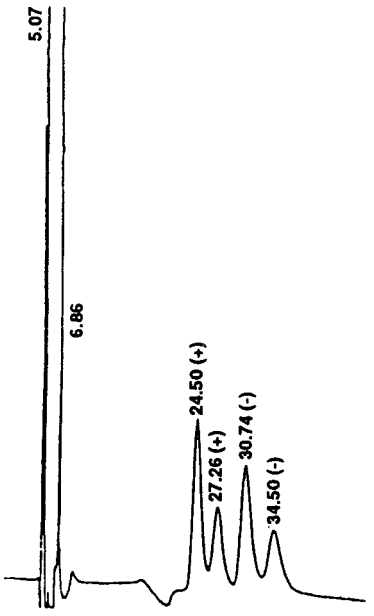


Figure 4. Chromatogram of formoterol racemate mixture. Mobile phase: Hexane:1,2-dichloroethane:[ethanol + TFA (20:1) premixed], 55:35:10; flow rate: 0.5 mL/min; chart speed: 0.25 cm/min; wavelength: 278 nm.

Formoterol

The racemate mixture was separated into its four enantiomers, (Figure 4). The absolute configuration of the individual enantiomers was not determined. So, the chromatographic peaks were identified according to their optical rotation sign (results not shown). Referring to Figure 4, the first two eluting enantiomers carry the (+)-dextrorotatory sign while the latter two are both (-)-levorotatory. From Figure 4 we obtained the ratio of the total percent area of the first (+) and third (-) eluting enantiomers over that of the second (+) and forth (-) eluting enantiomers which was 1.8:1 and this guided us to know the composition of the racemic formoterol. Moreover, according to this result we calculated the chromatographic parameters as shown in Table 2. We noticed that formoterol degraded after several hours of preparation for analysis when the mobile phase was used as an injection solvent (data not shown). Therefore, methanol was used as an injection solvent.

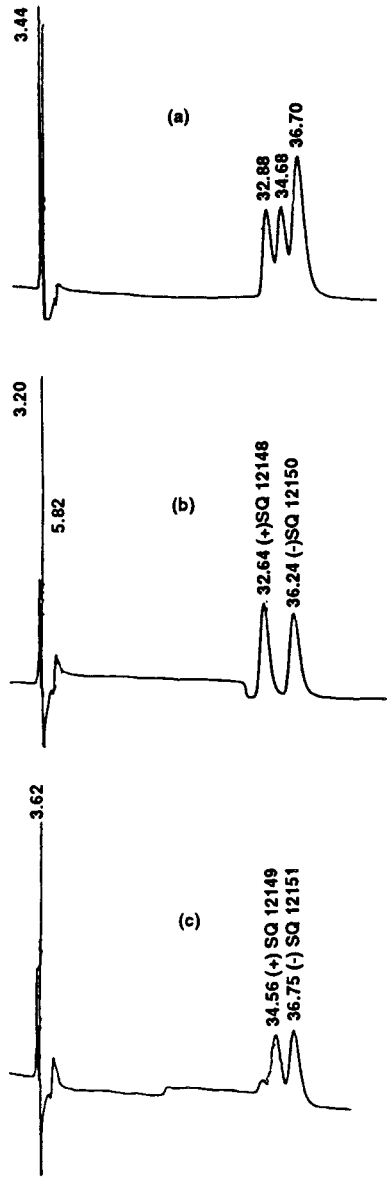


Figure 5. Chromatogram of nadolol, (a) racemate mixture SQ 11725, (b) racemate A SQ12181, and (c) racemate B SQ 12182. Mobile phase: Hexane:1,2-dichloroethane:[ethanol + TFA (20:1) premixed], (75:17:8); flow rate: 0.6 mL/min; chart speed: 0.25 cm/min; wavelength: 269 nm.

Table 2

Chromatographic Parameters Capacity (k'), Separation (α) and Resolution (R_S) Factors for Formoterol Enantiomers

Racemate	k'	k'2	α	R_S
A	4.18 (+)	5.50 (-)	1.32	2.6
B	4.76 (+)	6.29 (-)	1.32	2.5

Table 3

Chromatographic Parameters Capacity (k'), Separation (α) and Resolution (R_S) Factors for Nadolol Enantiomers

Racemate	k'	k'2	α	R_S
SQ12, 181 Racemate A	8.56 (+) SQ 12148	9.67 (-) SQ 12150	1.13	1.6
SQ12, 182 Racemate B	9.08 (+) SQ 12149	9.67 (-) SQ 2151	1.06	0.8

Nadolol

Several methods for the assay of nadolol in bulk material and pharmaceutical formulation based on HPLC[9,10,17] have been reported. Belas et al.[10] reported a reverse-phase separation of the four nadolol enantiomers after derivatization with the chiral reagent R(-)-1-(naphthyl)ethylisocyanate [R(-)-NEI]. In this method Chirex 3022 column in the normal phase mode was used for the separation of nadolol, which shows three separate peaks (Figure 5a). These peaks were identified by the injection of individual enantiomers onto the HPLC system under the same chromatographic conditions.

The two enantiomers SQ 12150 and SQ 12151 were overlapping in the third peak of nadolol racemate mixture. However, the method can separate the enantiomers of both racemates A and B as shown in Figures 5b and 5c. The chromatographic parameters are presented in Table 3.

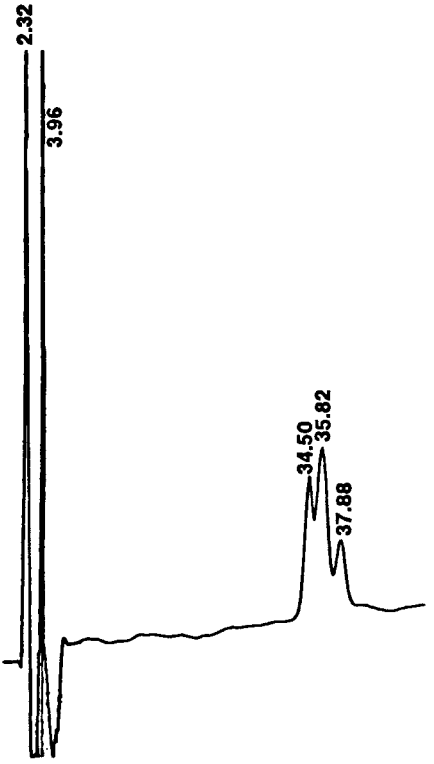


Figure 6. Chromatogram of indenolol racemate mixture. Mobile phase: Hexane:[ethanol + TFA premixed], (92:8); flow rate: 0.7 mL/min; chart speed: 0.25 cm/min; wavelength: 250 nm.

Indenolol

Indenolol has been separated to its four enantiomers using gradient elution on a cellulose stationary phase.[6] However, in the method described isocratic mode was used to separate indenolol racemate mixture to its four enantiomers; however, only three peaks were separated (Figure 6), which indicates that there is an overlapping in the second peak. Since the composition of racemic indenolol is a tautameric mixture of the 7- and 4-indenyloxy isomers in the ratio of 2:1 (as stated by the manufacturer), the enantiomers can be grouped under the tautomer that they belong to, by calculating the area under each peak via integration. The ratio of the first peak over the third peak, also the ratio of

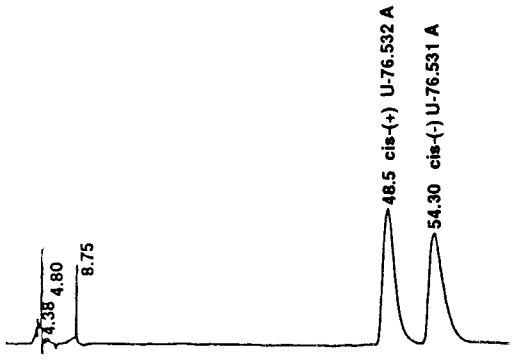


Figure 7. Chromatogram of U-54494A; Mobile pahse: Hexane:1,2-dichloro-ethane:[ethanol + TFA premixed] (80:5:5); flow rate: 0.7 mL/min; chart speed: 0.25 cm/min; wavelength: 278 nm.

Table 4

Chromatographic Parameters Capacity (k'), Separation (α) and Resolution (R_S) Factors for Indenolol Enantiomers

Racemate	k'	k'2	α	R_S
7-Indenyloxy tautomer	13.94	14.51	1.04	0.53
4-Indenyloxy tautomer	14.51	15.40	1.06	0.82

Table 5

Chromatographic Parameters Capacity (k'), Separation (α) and Resolution (R_S) Factors forU-54494A by Hydrochloride Racemates

Racemate Mixture	k'	k'2	α	R_S
U-54494A	11.44 (+) U-76532A	12.92 (-) U-76531A	1.13	1.81

the second peak over the first peak and the ratio of the second peak over the third peak confirm the manufacturer's composition of racemic indenolol. These results conclude that the first peak and one of the overlapping enantiomers in the second peak are the corresponding enantiomers for the 7-indenyloxy tautomer. Furthermore, the third peak and one of the overlapping enantiomers in the second peak are the corresponding enantiomers for the 4-indenyloxy tautomer. The chromatographic parameters calculated for the indenolol enantiomers are summarized in Table 4. We noticed a severe worsening of the peak shapes resulting in lower resolution factor when methanol was used as an injection solvent (data not shown), therefore we used the mobile phase as an injection solvent.

In spite of the failing attempts to optimize the separation of these overlapping peaks under isocratic condition, this method can separate the pair of enantiomers of both nadolol and indenolol individual racemates.

U-54494A

U-54494A has two chiral centers with four possible enantiomers, however, only the cis-configuration of the drug was available. Accordingly, U-54494A racemate contains two enantiomers U-76531A and U-76532A, which are present in 1:1 ratio, and were separated as shown in Figure 7. The chromatographic parameters for the resolution of these two enantiomers are shown in Table 5.

This simple, direct and rapid separation method of these drugs using Pirkle urea-type CSP column, provides a possibility to the separation of bulk quantity of these drugs. Also this method can be used for the analysis of these drugs in bulk and pharmaceutical dosage forms.

REFERENCES

1. J. Trofast, K. Osterberg, B. Kallstrom, B. Waldeck, Chirality, **3**, 443-450 (1991).

2. B. Waldeck, Chirality, **5**, 350-355 (1993).

3. B. Testa, W. F. Trager, Chirality, **2**, 129-133 (1990).

4. S. Ahuja "The Importance of Chiral Separations in Pharmaceuticals," chapter 10, in **The Impact of Stereochemistry in Drug Development and Use,** edited by H. Y. Aboul-Enein, I. W. Wainer, John Wiley and Sons, New York, 1997, pp. 287-315.

5. D. R. Abernethy, N. S. Andrawis, "Stereoisomeric Drugs in the Therapeutics, Clinical Perspectives" in **Drug Stereochemistry; Analytical Meth ods and Pharmacology**, Irving W. Wainer, ed., Marcel Dekker, Inc., New York, 1993, pp. 385-397.

6. H. Y. Aboul-Enein, V. Serignese, J. Liq. Chromatogr. & Rel. Technol., **19**, 933-938 (1996).

7. H. Y. Aboul-Enein, L. I. Abou-Basha, J. Liq. Chromatogr., **19**, 383-392 (1996).

8. H. Y. Aboul-Enein, M. R. Islam, J. Chromatogr., **511**, 109-114 (1990).

9. N. R. Srinivas, W. C. Shyu, V. R. Shah, D. A. Campbell, R. H. Barbhaiya, Biomed. Chromatogr., **9**, 226-228 (1995).

10. F. J. Belas, M. A. Phillips, N. R. Srinivas, R. H. Barbhaiya, I. A. Blair, Biomed. Chromatogr., **9**, 140-145 (1995).

11. D. Faulds, L. M. Hollingshead, K. L. Goa, Drugs, **42**, 115-137 (1991).

12. C. G. Lofdahl, P. J. Barnes, Acta Pharmacol. Toxicol. Suppl., **58**, 91-111 (1986).

13. **Annual Drug Data Report Vol. 3**, J. R. Prous, ed., J. R. Prous, S.A., Inter national Publishers, Barcelona, Spain, 1981, pp. 1-272.

14. **Annual Drug Data Report Vol. 5**, J. R. Prous, ed., J. R. Prous, S.A., Inter national Publishers, Barcelona, Spain, 1983, pp. 1-210.

15. **Annual Drug Data Report Vol. 2**, J. R. Prous, ed., J. R. Prous, S.A., Inter national Publishers, Barcelona, Spain, 1981, pp. 1-252.

16. T. Cleveland, J. Liq. Chromatogr., **18**, 649-671 (1995).

17. M. Hoshino, K. Yajima, Y. Suzuki, A. Okahira, J. Chromatogr. B, 661, 281-289 (1994).

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