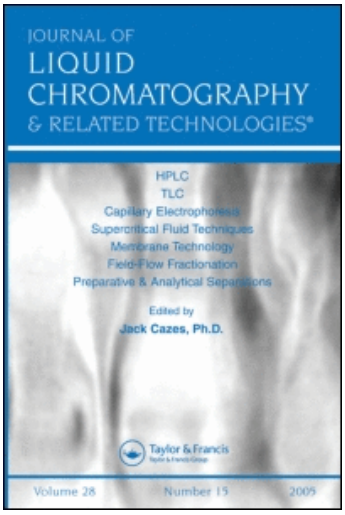

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DEVELOPMENT AND VALIDATION OF A REVERSED-PHASE HPLC METHOD FOR THE DETERMINATION OF PINDOLOL AND CLOPAMIDE IN TABLETS

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DEVELOPMENT AND VALIDATION OF A REVERSED-PHASE HPLC METHOD FOR THE DETERMINATION OF PINDOLOL AND CLOPAMIDE IN TABLETS

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ABSTRACT

A high-performance liquid chromatographic method was developed for the simultaneous determination of pindolol and clopamide in pharmaceutical dosage forms. The use of a β-cyclodextrin bonded-phase column results in satisfactory separation of both of the compounds. The mobile phase consisted of a mixture of 1.0% w/v triethylamine acetate buffer (pH = 5.5) and methanol (90:10, v/v), pumped at a flow rate 0.8 mL/min. The UV detector was operated at 245 nm.

Calibration graphs are linear (r better than 0.99997, n = 5), in concentration range 1.0–3.0 μg/mL for pindolol and 0.5–1.5 mg/mL for clopamide. The intra- and interday R.S.D. values were less than 2.97% (n = 5), while the relative percentage error E_r was less than 2.0% (n = 5). Detection limits were

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0.12 and 0.16 mg/mL for pindolol and clopamide, respectively. The method was applied in the quality control of commercial tablets and content uniformity test and proved to be suitable for rapid and reliable quality control.

INTRODUCTION

Pindolol ((±)-4-(2-hydroxy-3-isopropyl-amino-propoxy)-indole), is a non-selective β-adrenergic antagonist with intrinsic sympathomimetic activity (1) and a 5-$HT_{1A/1B}$ receptor antagonist. (2) The racemate mixture of this compound is used for the clinical treatment of angina pectoris and hypertension. (3,4) Its combination with clopamide (4-chloro-N-(2,6-dimethyl-piperidino)-3-sulphamoylbenzamide), a diuretic that reduces the reabsorption of electrolytes from renal tubules, (5) increases the antihypertensive effects.

Among the methods that have been reported in literature for the determination of pindolol are potentiometric titration, (6) and UV spectrophotometry, (7) thin-layer chromatography, (8) gas-chromatography mass spectroscopy (9) and liquid-chromatography mass spectroscopy. (10,11) Various high-performance liquid chromatographic methods have also been described for the determination of pindolol in pharmaceutical formulations (12,13) in the form of its organic salts (14) and in biological fluids (15,16) including enantioselective bioanalytical determinations. (17–20) The quantitation of clopamide has been carried out using various analytical techniques such as high-performance liquid chromatography, (21–23) derivative spectrophotometry, (24) and gas chromatography-mass spectroscopy. (25)

A few methods for the simultaneous determination of pindolol and clopamide have been published. These include derivative spectrophotometry (26,27) and high-performance liquid chromatography. (28) In the latter method, chromatographic separation was carried out under isocratic conditions on a reversed-phase C-18 column, thus, pindolol was early-eluted.

As the combination of these two compounds in antihypertensive therapy has become popular, we thought that it would be of particular interest to develop and validate a simple, selective, and reliable HPLC method for their simultaneous determination. In the present study the applicability of a β-cyclodextrin bonded-phase column to the HPLC analysis of pindolol and clopamide was evaluated, in view of the need of selective methods for their determination in pharmaceuticals. Cyclodextrin columns effect numerous chemical separations by selectively including a wide variety of organic and inorganic guest molecules in the cyclodextrin cavity. (29,30) The proposed method is applicable, as well, for routine analysis and complies well with the validation requirements in the pharmaceutical industry.

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EXPERIMENTAL

Apparatus

Chromatography was performed on a Waters Model 501 pump, and a Rheodyne Model 7125 injector with a 20-μL loop. Detection was performed with a Waters Model 486 UV-Vis detector with a 8-μL flow cell. Integration of the chromatograms was made with a Hewlett-Packard Model HP-3394A integrator. A pH meter Metrohm, Model 654 Herisau was used for all pH measurements.

Materials

Solvents were of HPLC grade and were purchased from Lab-Scan Science Ltd., Ireland. Triethylamine acetate (pro analysi), and glacial acetic acid (analytical reagent grade) were purchased from Aldrich Ltd. Water was deionised and further purified by means of a Milli-Q Plus Water Purification System, Millipore Ltd. Pindolol and clopamide of pharmaceutical purity grade were kindly provided by Novartis Pharma, while sulfamerazine of pharmaceutical purity grade was kindly provided by Minerva Hellas. All substances were used without any further purification. Viskaldix tablets are products of Novartis Pharma; each tablet was labelled to contain 10.0 mg of pindolol and 5.0 mg of clopamide.

Methods

Chromatographic separations were performed on a β-cyclodextrin column (250×4.6 mm i.d.), which was obtained from Advanced Separation Technologies Inc., (Whinappy, NJ, USA) under the commercial name Cyclobond-I. When not in use the column was stored in 100% methanol.

The mobile phase, 1.0% w/v triethylamine acetate buffer (pH = 5.5) and methanol (90:10, v/v), was filtered through a 0.45 μm Millipore filter and degassed, under vacuum, prior to use. The mobile phase was pumped at a flow rate 0.8 mL/min. All chromatographic experiments were carried out at room temperature. The column eluate was monitored at 245 nm, a suitable wavelength obtained from the PND and CLP UV-spectra.

Stock Standard Solutions

Stock standard solutions of pindolol (PND), 1.0 mg/mL, clopamide (CLP), 1.0 mg/mL, and sulfamerazine, (SLF), 0.5 mg/mL, were prepared by dissolving

appropriate amounts of the compounds in methanol. These solutions were stored in the dark under refrigeration at 4°C and were found to be stable for several weeks. A series of mixed standard solutions were prepared by the appropriate dilution of the above mentioned stock standard solution in mobile phase to reach concentration ranges of 1.00–3.00 μg/mL and 0.50–1.00 μg/mL for PND and CLP, respectively. In each sample 1.20 μg/mL of the internal standard SLF was added. Standard solutions were found to be stable during the analysis time.

Assay Sample Preparation

Twenty tablets were weighed and finely pulverised. An appropriate portion of this powder, equivalent to 10.0 mg of PND and 5.0 mg of CLP was placed in a 50-mL volumetric flask with 40 mL of methanol. The solution was sonicated for 5 min and diluted to volume with methanol. A portion of this solution was centrifuged at 4000 rev/min (2890 g) for 15 min. A 5-mL aliquot was transferred to a 50-mL volumetric flask and diluted to volume with mobile phase. Consequently, a 500-mL aliquot of this solution was further diluted to 10 mL mobile phase containing 1.20 μg/mL of the internal standard, SLF; 20 μL sample was injected into the HPLC system. Peak area ratios of each compound to that of the internal standard were then measured for the determinations. The same procedure was followed for the content uniformity test, using one tablet per sample.

Calibration Procedure

Two calibration curves were constructed by assaying the above mentioned mixed standard solutions of PND and CLP in mobile phase. The concentration range covered was 1.00–3.00 μg/mL for PND and 0.50–1.50 μg/mL for CLP. Triplicate 20-mL injections were made of each solution and the peak area ratio of each drug to that of the internal standard was plotted against the corresponding concentration to obtain the calibration graph.

The over-all precision and accuracy of the chromatographic assay was evaluated by analyzing three series of mixed standard solutions of PND and CLP, at concentrations of 1.00, 2.00, and 3.00 μg/mL for PND and 0.50, 1.00, and 1.50 μg/mL for CLP. In each sample 1.20 μg/mL of the internal standard SLF was added. The precision of each method was based on the calculation of the relative standard deviation (% R.S.D.). An indication of the accuracy was based on the relative percentage error of the samples (E_r %).

In order to assess the specificity of the proposed HPLC method, the effect of the excipients used in the formulation of tablets was evaluated using the

standard addition method. (31) Thus, five equal amounts of tablets equivalent to 10.00 mg of PND and 5.00 mg of CLP, were spiked with different amounts of reference standards of PND and CLP. The samples were analyzed as mentioned in the assay procedure, while in each sample, 1.20 μg/mL of the internal standard, SLF, was added. Peak area ratios of each drug to that of the internal standard were measured for the determination of both compounds.

RESULTS AND DISCUSSION

Chromatographic Characteristics

Chromatographic separations using CD-bonded phases, are mainly the result of variations in the stability of inclusion complexes of the analytes with the cyclodextrin molecules. (32) Thus, a thorough investigation was conducted in order to choose the optimum conditions for the chromatographic separation of the analytes on a Cyclobond-I column.

The effect of composition and pH of the mobile phase on the retention time of PND, CLP, and SLF (internal standard), were investigated. Results of the effect of methanol concentration in the mobile phase are presented in Figure 1. An

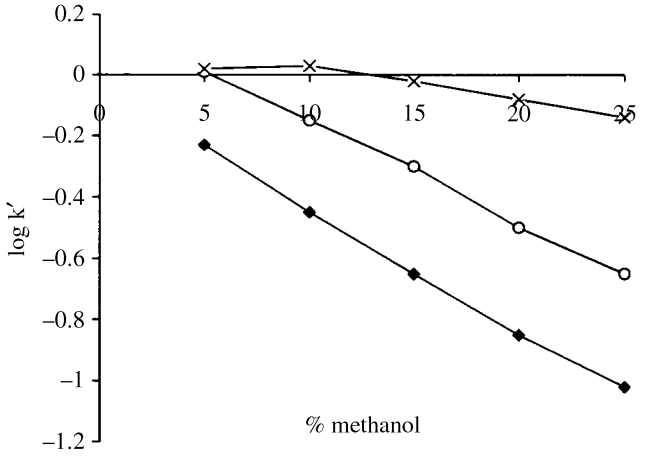

Figure 1. Effect of methanol concentration on the log k′ of pindolol, ◆, clopamide, ○, and sulfamerazine, ×. Column, Cyclobond I (250 × 4.60 mm i.d.); eluent, 1.0% w/v triethylamine acetate buffer (pH = 5.5) and methanol; flow rate, 0.8 mL/min; detection wavelength 245 nm.

increase in the percentage of methanol decreases the interaction between the analysed components and the β-cyclodextrin cavity, and results in lower degrees of retention. The optimum methanol concentration was found to be (10% v/v). The effect of pH on the retention time of the analytes was also investigated by changing the pH values of the aqueous component of the mobile phase from 4.5 to 7.0 using triethylamine acetate buffer (1.0% w/v). For all experimental pH values, the drugs are eluted in order of PND, CLP, and SLF. It is known that the non-polar and less ionic molecules form more stable inclusion complexes than the polar and ionic molecules. (33) Consequently, non-polar and uncharged compounds are, in principle, more strongly retained by cyclodextrin than the polar and ionic molecules.

Pindolol is a basic substance (pKa 8.8) and owing to protonization the retention is lower for lower pH values. On the contrary, at a pH value of 4.5 clopamide (weak acid, pKa 7.0) and sulfamerazine (weak acid, pKa 8.0) are non-ionized, thereby, forming more stable inclusion complexes. Consequently, an increase in the pH from 4.5 to 7.0 decreases the stability of CLP-β-CD and SLF-β-CD complexes, and thus, decreases the retention times (Figure 2). Therefore, at the highest pH value a loss of resolution between CLP and PND is observed. A pH value of 5.0 was chosen for the optimum separation of the compounds.

The specificity of the HPLC method is illustrated in Figure 3, where complete separation of the compounds was observed. PND was eluted at 4.66 min, CLP appeared at 5.84 min, while the internal standard, SLF, was eluted at 6.80 min.

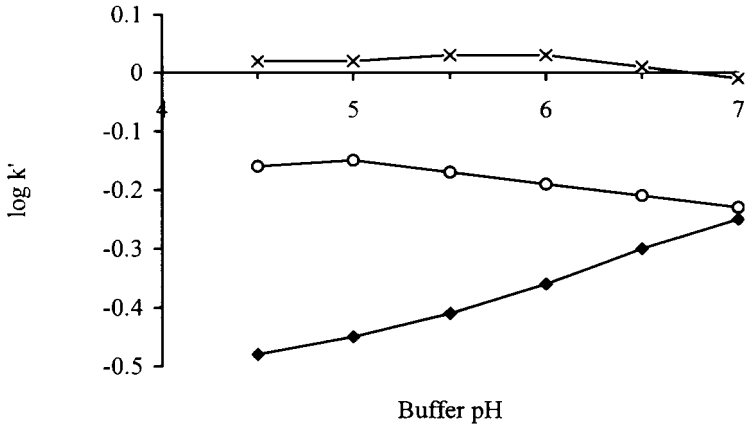

Figure 2. Effect of mobile phase pH on the log k′ of pindolol, ◆, and sulfamerazine, ×. Column, Cyclobond I (250 × 4.60 mm i.d.); eluent, 1.0% w/v triethylamine acetate buffer and methanol (90:10, v/v); flow rate, 0.8 mL/min; detection wavelength 245 nm.

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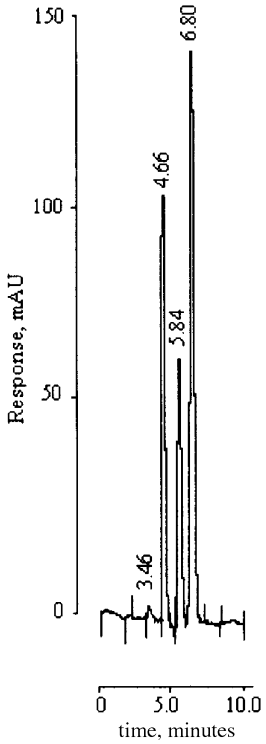

Figure 3. Representative chromatogram of a mixture of pindolol clopamide and sulfamerazine at retention times 4.66, 5.84, and 6.80 min, respectively. The chromatogram was obtained from the analysis of tablets and the chromatographic conditions were: reversed-phase HPLC on a Cyclobond-I column (250 × 4.60 mm i.d.); mobile phase, 1.0% w/v triethylamine acetate buffer (pH = 5.5) and methanol (90:10, v/v); flow rate, 0.8 mL/min; detection wavelength 245 nm.

Linearity and Reproducibility

Calibration graphs were constructed at six concentration levels in the range 1.0 to 3.00 μg/mL for PND and 0.50 to 1.50 μg/mL for CLP, and three independent determinations were performed at each concentration (n = 3). Linear relationships were obtained between the peak area ratio of each compound and the corresponding concentration, as shown by the equations presented in Table 1. The correlation coefficient (r) and the standard error of the estimate (S.E.) of the calibration lines are also given, along with the S.D. of the slopes and intercepts.

Table 1. Analytical Data of the Calibration Graphs for the Determination of Pindolol and Clopamide by High-Performance Liquid Chromatography

Linearity Range (μg/mL)		Calibration Equation[a]	r[b]
PND	CLP		
1.00–3.00	0.50–1.00	$S_{PND} = 0.720(0.003) \times C_{PND} + 0.010(\pm 0.03)$	0.99997
1.00–3.00	0.50–1.00	$S_{CLP} = 0.830(\pm 0.002) \times C_{CLP} + 0.020(\pm 0.04)$	0.99998

[a]Ratio of the peak area amplitude of each compound to that of the internal standard, S, versus concentration of each compound, C, in μg/mL; six standards.
[b]Correlation coefficient.

In order to further evaluate the linearity of the proposed method, five calibration equations were constructed over a period of four weeks. The average regression equation for PND:

$$S_{PND} = 0.730(\pm 0.014) \times C_{PND} + 0.030(\pm 0.08)$$

and for CLP:

$$S_{CLP} = 0.832(\pm 0.009) \times C_{CLP} + 0.080(\pm 0.54)$$

where S is ratio of the peak area amplitude of each compound to that of the internal standard, and C is the appropriate concentration expressed in mg/mL. The slopes of the calibration equations of PND and CLP had R.S.D. values of 1.94 and 1.08, respectively, while the correlation coefficient invariably exceeded 0.9998.

Intra-day data for the precision and accuracy of the method given in Table 2, indicate R.S.D.% = 1.32–2.0 and $E_r = -1.0$–1.0 for PND and R.S.D.% = 1.01–1.99 and $E_r = -1.0$–2.0 for CLP. Moreover, the inter-day R.S.D.% values (Table 2) for the determination of PND and CLP ranged from 2.02 to 2.97 and 1.99 to 2.04, respectively.

The limit of detection attained as defined by IUPAC,(34) $LOD_{(k=3)} = k \times S_a/b$ (where b is the slope of the calibration graph and S_a is the standard deviation of the blank signal) was found to be 0.12 and 0.16 μg/mL for PND and CLP, respectively. The limits of quantitation LOQ were also attained according to the IUPAC definition, $LOD_{(k=10)} = k \times S_a/b$, and were found to be 0.42 and 0.48 μg/mL for PND and CLP, respectively.

The statistical evaluation of the HPLC method revealed its good linearity and reproducibility, and led us to the conclusion that it could have been used for the reliable determination of CLP and PND in tablets.

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Table 2. Precision and Accuracy of Within- and Between-Run Analysis for the Determination of Pindolol and Clopamide by High-Performance Liquid Chromatography

Nominal Concentration (μg/mL)		Assayed Concentration of Pindolol (μg/mL)			Assayed Concentration of Clopamide (μg/mL)		
Pindolol	Clopamide	Mean ± s.d.	RSD%[a]	E_r(%)[b]	Mean ± s.d.	RSD%[a]	E_r(%)[b]
Intra-day (n = 5)							
1.0	0.5	0.99 ± 0.02	2.02	−1.0	0.51 ± 0.01	1.96	2.0
2.0	1.0	1.99 ± 0.03	1.50	−0.5	0.99 ± 0.01	1.01	−1.0
3.0	1.5	3.03 ± 0.04	1.32	1.0	1.51 ± 0.03	1.99	0.7
Inter-day (n = 5)							
1.0	0.5	1.01 ± 0.03	2.97	1.0	0.49 ± 0.01	2.04	−2.0
2.0	1.0	1.98 ± 0.04	2.02	−1.0	1.02 ± 0.02	2.04	2.0
3.0	1.5	3.05 ± 0.07	2.30	1.7	1.52 ± 0.05	1.99	1.3

[a]Percentage relative standard deviation.
[b]Relative percentage error.

Assay of Pharmaceutical Formulations

The proposed method was evaluated in the assay of commercially available tablets containing a mixture of PND and CLP in the proportion 2.0:1.0. Ten replicate determinations were carried out on an accurately weighed amount of the pulverised tablets equivalent to 10.00 mg of PND and 5.00 mg of CLP. The results obtained gave a mean of 9.89 ± 0.22 with a R.S.D. of 2.22 for PND, and a mean of 4.99 ± 0.03 with a R.S.D. of 0.60 for CLP.

The method proved to be suitable for the content uniformity test, where a great number of assays on individual tablets are required. Commercially available tablets, containing mixtures of PND and CLP in proportion 2.0:1.0, were analysed using the proposed methodology and the results are given in Table 3. Recoveries achieved were in accordance with the actual content of PND and CLP in tablets.

Table 3. Determination of Clopamide and Pindolol in Commercial Formulations by High-Performance Liquid Chromatography

Sample	Pindolol; Found mg/Tablet		Clopamide; Found mg/Tablet	
	Mean ± s.d. (n = 10)	Recovery (%)[a]	Mean ± s.d. (n = 10)	Recovery (%)[a]
Viskaldix	9.94 ± 0.03	99.4	4.95 ± 0.05	99.0

[a]Mean and standard deviation for ten determinations; percentage recovery from the label claim amount.

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Recovery studies were also performed, by analyzing spiking sample powders with appropriate amounts of the reference standard of both compounds. Two calibration curves were then constructed by plotting the amount of the drug found (mg) versus the amount of the drug added (mg) for each one of the two compounds. The following linear regression equations were obtained through regression analysis of data:

$$C^{f}_{PND} = 0.990(\pm 0.017) \times C^{a}_{PND}\ 9.93(\pm 0.17),\ r = 0.9996$$

$$C^{f}_{CLP} = 1.018(\pm 0.011) \times C^{a}_{CLP} + 4.98(\pm 0.09),\ r = 0.9998$$

where C^{f}_{PND} and C^{f}_{CLP} are the amounts (mg) found for PND and CLP, respectively, while C^{a}_{PND} and C^{a}_{CLP} are the amounts (mg) added for PND and CLP, respectively; r is the correlation coefficient of the calibration equation. The y-axis intercept of the above mentioned linear regression equations indicate the amount (mg) of the drug found in the powdered tablets, while the percentage recoveries were calculated as: % recovery = slope × 100. The results presented in Table 4 indicate that there is no interference from the excipients used in the formulation of the tablets.

The proposed method was evaluated by comparison with a zero-crossing, first-order derivative spectrophotometric method developed in our laboratory. (27) Commercially available tablets containing mixtures of 10.00 mg of PND and 5.00 mg of CLP were analysed by derivative spectrophotometry and the proposed HPLC method. The results obtained by both methods are demonstrated in Table 5.

Table 4. Recoveries of Pindolol and Clopamide in Spiked Commercial Samples

Drug	Amount Added (mg)	Amount Found (mg)	m[a]	Recovery[b]
Pindolol	5.0	14.85	0.990	99.0
	8.0	17.93		
	10.0	19.75		
	12.0	21.96		
	14.0	23.70		
Clopamide	2.0	7.09	1.018	101.8
	5.0	7.09		
	7.0	12.10		
	9.0	14.06		
	12.0	17.29		

[a]m is the slope of the linear regression analysis of the amount found versus the amount added.
[b]Recovery (%) = m × 100.

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Table 5. Determination of Pindolol and Clopamide in Commercial Formulation by High-Performance Liquid Chromatography and First-Order Derivative Spectrophotometry

	Pindolol; Found mg/Tablet		Clopamide; Found mg/Tablet	
	$^1D_{262.4}$	HPLC	$^1D_{272.6}$	HPLC
Mean ± sd (n = 10)	9.94 ± 0.17	9.95 ± 0.03	5.05 ± 0.04	4.97 ± 0.05
R.S.D (%)	1.71	0.30	0.79	1.01
E_r (%)	−0.6	−0.5	1.0	−0.6

In conclusion, the proposed high-performance liquid chromatographic method was evaluated for linearity, reliability, and specificity, and proven to be convenient and effective for the analysis of clopamide and pindolol in commercial formulations. Moreover, the proposed method offers a short analytical run time of 8.00 min and achieved good resolution between PND, CLP, and the internal standard, SLF. The method was successfully applied to the determination of clopamide and pindolol mixture in tablets.

REFERENCES

1. Hansson, L.; Lindholm, L.H.; Ekbom, T. Lancet (North America Edition) **1999**, *354*, 1751–1756.
2. Lesch, K.P.; Poten, B.; Sohne, K.; Schulte, H.M. Eur. J. Clin. Pharmacol. **1990**, *39*, 17–19.
3. Goodman & Gilman's, *The Pharmacological Basis of Therapeutics*; McGraw Hill: New York, **1992**; 234–235.
4. Murai-Kushiya, M.; Okada, S.; Kitamura, T.; Hasegawa, R. J. Pharm. Pharmacol. **1993**, *45*, 225–228.
5. Gotzen, R.; Faupel, R.P.; Hammerschmidt, D. Med. Welt **1981**, *32*, 1450–1455.
6. *European Pharmacopeia*, 2nd Ed.; Council of Europe, Maissonneuve S.A.: France, 1989; 634.
7. *British Pharmacopeia*; Health Minister: London, 1993; 1061.
8. Spahn, H.; Prinoth, M.; Mutschler, E.J. J. Chromatogr, **1986**, *342*, 458–464.
9. Delbeke, F.T.; Debackere, M.; Desmet, N.; Maertens, F. J. Chromatogr. **1988**, *426*, 194–201.
10. Abdel-Hamid, M.E. Il Farmaco **2000**, *55* (2), 136–145.
11. Needham, S.R.; Brown, P.R.; Duff, K.; Bell, D. J. Chromatogr. A **2000**, *869*, 159–170.

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12. *The United States Pharmacopeia, XXIII Revision, Pharmacopeial Conversion*; Rockville, MD, 1995; 1230–1231.
13. Erram, S.; Tippnis, H.P. Indian Drugs **1992**, *29* (14), 651–654.
14. Pietiläinen, H.; Saesmaa, T. J. Liq. Chrom. & Rel. Technol. **1996**, *19* (4), 583–591.
15. Smith, H.T. J. Chromatogr. **1987**, *415*, 93–103.
16. Wanwimolruk, S. J. Liq. Chromatogr. **1991**, *14* (9), 1699–1706.
17. Hsyu, H.P.; Giacomini, K.M. J. Pharm. Sci. **1986**, *75* (6), 601–601.
18. Zhang, H.L.; Stewart, J.T.; Ujhelyi, M. J. Chromatogr. B **1995**, *668* (2), 309–313.
19. Beal, J.L.; Tett, S.E. J. Chromatogr. B **1998**, *715*, 409–415.
20. Yan, H.; Lewander, T. Eur. Neuropharmacol. **1999**, *10*, 59–62.
21. Wanwimolruk, S. J. Liq. Chromatogr. **1991**, *14* (9), 1707–1714.
22. Begona-Barroso, M.; Jimenez, R.M.; Alonso, R.M. J. Liq. Chrom. & Rel. Technol. **1997**, *20* (4), 637–650.
23. Guchelaar, H.J.; Chandi, L.; Schouten, O.; van den Brand, W.A. Fresenious J. Anal. Chem. **1999**, *363* (7), 700–705.
24. Bedair, M.M.; Korany, M.A.; Ebdel-Hay, M.A.; Gazy, Analyst **1990**, *115*, 449–453.
25. Stuber, W.; Blume, H.; Sorgel, F.; Stenzhorn, G. J. Pharm. Sci. **1989**, *78* (8), 679–682.
26. Panderi, I.; Parissi-Poulou, M. Int. J. Pharm. **1993**, *99*, 327–331.
27. Panderi, I.; Parissi-Poulou, M. J. Pharm. Biomed. Anal. **1994**, *12* (2), 151–156.
28. El-Walily, A.F.M.; El-Yazbi, F.A.; Betal, S.F.; Razak, O.A. Anal. Lett. **1995**, *28* (5), 893–907.
29. Ward, T.J.; Armstrong, D.W. *Cyclodextrin Stationary Phase in Chromatographic Chiral Separations*; Chromatographic Science Series; Marcel Dekker: New York, 1984; Vol 40, 131–163.
30. Piperaki, S.; Parissi-Poulou, M.; Koupparis, M.. J. Liq. Chromatogr **1993**, *16* (16), 3487–3508.
31. Miller, J.N. Analyst **1991**, *116*, 3–14.
32. Piperaki, S.; Perakis, A.; Parissi-Poulou, M. J. Chromatogr. **1994**, *660*, 339–350.
33. Abidi, S.L. J. Chromatogr. **2001**, *362*, 33–39.
34. Long, G.L.; Winefordner, J.D. Anal. Chem. **1983**, *55*, 712A–721A.

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