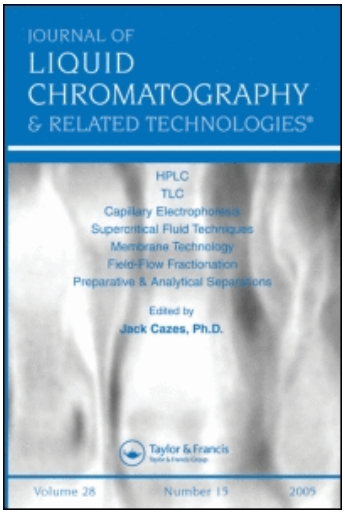

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Simultaneous Analysis of Candesartan Cilexetil and Hydrochlorothiazide in Human Plasma and Dosage Forms Using HPLC with a Photodiode Array Detector

Nevin Erk[a]
[a] Department of Analytical Chemistry, Faculty of Pharmacy, Ankara University, Ankara, Turkey

Online publication date: 28 July 2003

To cite this Article Erk, Nevin(2003) 'Simultaneous Analysis of Candesartan Cilexetil and Hydrochlorothiazide in Human Plasma and Dosage Forms Using HPLC with a Photodiode Array Detector', Journal of Liquid Chromatography & Related Technologies, 26: 15, 2581 — 2591
To link to this Article: DOI: 10.1081/JLC-120023802
URL: http://dx.doi.org/10.1081/JLC-120023802

JOURNAL OF LIQUID CHROMATOGRAPHY & RELATED TECHNOLOGIES®
Vol. 26, No. 15, pp. 2581–2591, 2003

Simultaneous Analysis of Candesartan Cilexetil and Hydrochlorothiazide in Human Plasma and Dosage Forms Using HPLC with a Photodiode Array Detector

Nevin Erk*

Department of Analytical Chemistry, Faculty of Pharmacy, Ankara University, Ankara, Turkey

ABSTRACT

A simple and fast HPLC method using a photodiode array detector was developed for the antihypertensive drugs, candesartan cilexetil, and hydrochlorothiazide in human plasma and dosage forms. The system requires a Supelcocil C_{18} (5 μm, 15 cm × 4.6 mm) column, and a mobile phase composed of 10 mM potassium dihydrogen phosphate : methanol : acetonitrile (2 : 80 : 18, v/v/v) (pH 2.5) while at a flow rate 1.0 mL min^{-1}. Candesartan cilexetil and hydrochlorothiazide were detected at 260.0 nm and were eluted in 3.5 and 6.5 min, respectively, after injection. No endogenous substances were found to interfere. The method utilises protein precipitation with acetonitrile as the only sample preparation involved prior to reversed phase-HPLC. No internal standard

*Correspondence: Nevin Erk, Department of Analytical Chemistry, Faculty of Pharmacy, Ankara University, 06100 Ankara, Turkey; E-mail: erk@pharmacy.ankara.edu.tr.

DOI: 10.1081/JLC-120023802
1082-6076 (Print); 1520-572X (Online)

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

was required. The linearity range for candesartan cilexetil and hydrochlorothiazide was 30.0–2500.0 ng mL^{-1} and 20.0–1000.0 ng mL^{-1}, respectively. The limit of detection (LOD) and limit of quantitation (LOQ) were estimated to be 2.0 and 11.0 ng mL^{-1} for candesartan cilexetil and 3.58 and 6.75 ng mL^{-1} for hydrochlorothiazide, respectively. The proposed method, which is rapid, simple, and does not require any separation step, has been successfully applied to the assay of dosage forms and human plasma containing candesartan cilexetil and hydrochlorothiazide.

Key Words: Candesartan cilexetil; Hydrochlorothiazide; Simultaneous determination; Human plasma; High performance liquid chromatography.

INTRODUCTION

Candesartan cilexetil (±-1-cyclohexyloxycarbonyloxy)ethyl-2-ethoxyl {[2′-(1H-tetrazol-5-yl)biphenyl-4-yl]-methyl}-1H-benzimidazole-7-carboxylate is a potent, long-acting angiotensin II receptor antagonist, with high selectivity for the AT1 subtype.[1] Its chemical structure is shown in Scheme 1. Hydrochlorothiazide, or 6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulphonamide-1,1-dioxide is a diuretic of the class of benzothiadiazines. Its chemical structure is shown in Scheme 1. More recently, a very new combination dosage form of candesartan cilexetil and hydrochlorothiazide is indicated in the treatment and management of edema and hypertension.

Only a high liquid chromatographic method (HPLC) with fluorimetric detection has been reported for the determination of candesartan cilexetil[2] in the open literature.

A variety of methods have also been reported for the determination of hydrochlorothiazide in pharmaceutical formulations and biological fluids including voltammetry,[3] capillary zone electrophoresis,[4–6] spectrophotometry,[7–16] and HPLC.[17–23]

The candesartan cilexetil and hydrochlorothiazide mixture is not yet official in any national pharmacopoeia. To our knowledge, no HPLC method has been described for the simultaneous determination of both drugs in human plasma and dosage forms. Therefore, it was desirable to develop a simple, accurate, and fast procedure that could be applied in quality control laboratories for the determination of both drugs in the presence of each other, and this method could also be applicable for drug monitoring and simultaneous determination of pharmacokinetic profiles for this drug combination. In this paper, a HPLC method is reported and the optimum experimental parameters is described. The proposed method was applied to the simultaneous

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

determination of both analytes in synthetic mixtures, dosage forms, and human plasma, with satisfactory results in both cases.

EXPERIMENTAL

Chemicals and Reagents

Candesartan cilexetil and hydrochlorothiazide were kindly supplied by AstraZeneca Pharm. Ind. and were used without prior purification. Analytical grade phosphoric acid and HPLC grade methanol, and acetonitrile were purchased from Merck Chem. Ind. Water was bidistilled and all the other chemicals were of analytical reagent grade and were used as received. Drug-free human plasma was obtained from the hospital blood bank.

Instrumentation and Chromatographic Conditions

The HPLC system consisted of a HP 1100 series mode quaternary pump with a HP 1100 series manual injector 20 μL fixed loop, equipped with a photodiode array and multiple wavelength UV/VIS detectors. The photodiode array detector set at 260.0 nm was used and peak areas were integrated automatically by computer using Agilent Chem-Station software programme.

The chromatographic analysis was performed at ambient temperature onto a 5 μm Supelcocil C_{18} column, 15 cm × 4.6 mm I.D., and a mobile phase composed of 10 mM potassium dihydrogen phosphate : methanol : acetonitrile (2 : 80 : 18, v/v/v). The flow rate was maintained at 1.0 mL min^{-1}.

Standards

Stock solutions (1.0 mg mL^{-1}) of candesartan cilexetil and hydrochlorothiazide were prepared daily by dissolving 10 mg of equivalent free and pure drug of each substance in methanol. Stored at +5°C in the dark, these solutions were shown to be stable during the period of study. The stock solutions were diluted with mobile phase to give working solutions at concentrations in the range 30.0–2500.0 ng mL^{-1} for cardesartan cilexetil and 20.0–1000.0 ng mL^{-1} for hydrochlorothiazide, respectively.

Preparation of Plasma Sample

The plasma samples were stored in the freezer at −17°C and allowed to thaw at room temperature before processing. The plasma samples were

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

centrifuged at 4000× *g* for 10 min. An aliquot (1.0 mL) was pipetted into a 10 mL polypropylene tube and acetonitrile (2.0 mL). The mixture was vortex mixed briefly, and after standing for 5 min at room temperature, the mixture was centrifuged at 4000× *g* for 20 min. Then, the supernatant was injected into the HPLC system.

Preparation of the Spiked Plasma Samples

A spiked plasma sample with candesartan cilexetil and hydrochlorothiazide corresponding to the highest concentration level of the calibration points, was prepared by adding 50 μL of a working solution containing candesartan cilexetil and hydrochlorothiazide (20.0 and 12.5 $\mu g\,mL^{-1}$, respectively), to drug-free human plasma. This spiked plasma was diluted with drug-free plasma for the calibration points and quality control sample preparations. Calibration samples were prepared for each chromatographic session by diluting in a 15 mL silicone tube, known volumes (20–1000 μL) of spiked plasma with drug-free human plasma, to obtain a 1 mL final volume for each calibration samples. A calibration curve was obtained by plotting the peak area ratio of the drug against the drug concentration.

Quality control samples were prepared at the same time and stored with the subjects plasma samples at −17°C until assayed. For each drug, three levels of quality control concentrations were prepared: low level at 30.0 and 20.0 $ng\,mL^{-1}$, medium level at 1500.0 and 500.0 $ng\,mL^{-1}$, and high level at 2500.0 and 1000.0 $ng\,mL^{-1}$, for candesartan cilexetil and hydrochlorothiazide, respectively. Two quality control samples of each level were used for the in-study validation. These quality control samples were also used in the pre-study validation for the drugs.

Assay Procedure for Dosage Forms

Individual tablets were pulverized using a mortar and pestle, and completely transferred to a 100 mL conical flask. The volume was adjusted with methanol and the flask was mechanically shaken for 10 min. Five milliliter of the solution was centrifuged at 4000 rpm in a centrifuge tube for 5 min. The samples were filtered through a 0.45-μm membrane filter, then further diluted to suit the calibration graphs. Triplicate 20 μL injections were made for each solution. The amount of candesartan cilexetil and hydrochlorothiazide per tablet was calculated from the related linear regression equations.

Recovery Studies

To keep an additional check on the accuracy of the developed assay method and to study the interference of formulation additives, analytical

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

recovery experiments were performed by adding known amounts of pure drug to the preanalysed samples of commercial dosage forms. The percent analytical recovery values calculated by comparing concentration obtained from the spiked samples with actual added concentrations are also listed.

RESULTS AND DISCUSSION

The LC method proposed provides a simple procedure for the simultaneous determination of candesartan cilexetil and hydrochlorothiazide in human plasma and dosage forms.

The mobile phase, 10 mM potassium dihydrogen phosphate : methanol : acetonitrile in various proportions, was investigated after several trials. Mobile phase 10 mM potassium dihydrogen phosphate : methanol : acetonitrile (2 : 80 : 18, v/v/v) and flow rate selection was based on peak parameters (height, asymmetry, tailing), baseline drift, run time, ease of preparation of mobile phase. Internal standard was not used, as there was no extraction in the simultaneous determination of candesartan cilexetil and hydrochlorothiazide in human plasma and in pharmaceutical dosage forms. This system is quite robust. Other ODS columns have been tested, with minimal effect on the resolution of the analytes. A Supelco C_{18} column is recommended because of its demonstrated ruggedness and reproducibility in this assay. A typical chromatogram, candesartan cilexetil and hydrochlorothiazide of pure standard mixture using RP-Supelcocil C_{18} (15 cm × 4.6 mm I.D.) column at flow rate 1.0 mL min^{-1}, is shown in Fig. 1a. The optimum wavelength for detection was 260.0 nm, at which much better detector response for each drugs was obtained. In the Fig. 1a, for the estimation of candesartan cilexetil and hydrochlorothiazide a sharp and symmetrical peak was obtained with good baseline and tailing, thus facilitating the accurate measurement of the peak area. The retention times for the investigated drugs were found to be 3.5 min (candesartan cilexetil) and 6.5 min (hydrochlorothiazide). Slight variations in retention times were observed using mobile phases prepared on different days. The column-to-column reproducibility was evaluated, injecting the samples on two columns from different manufacturers and containing the same brand of packing material. The elution order and the resolution of compounds were not affected, and only slight variations in retention times were observed. No internal standard was required.

Other antihypertensive drugs (valsartan, lisinopril, losartan, and hydroflumethiazide) are extracted by this procedure. Nevertheless, under the chromatographic conditions described, these compounds elute at retention times different from those of candesartan cilexetil and hydrochlorothiazide, and therefore, do not interfere with the analysis. Potentially co-administered anti-

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

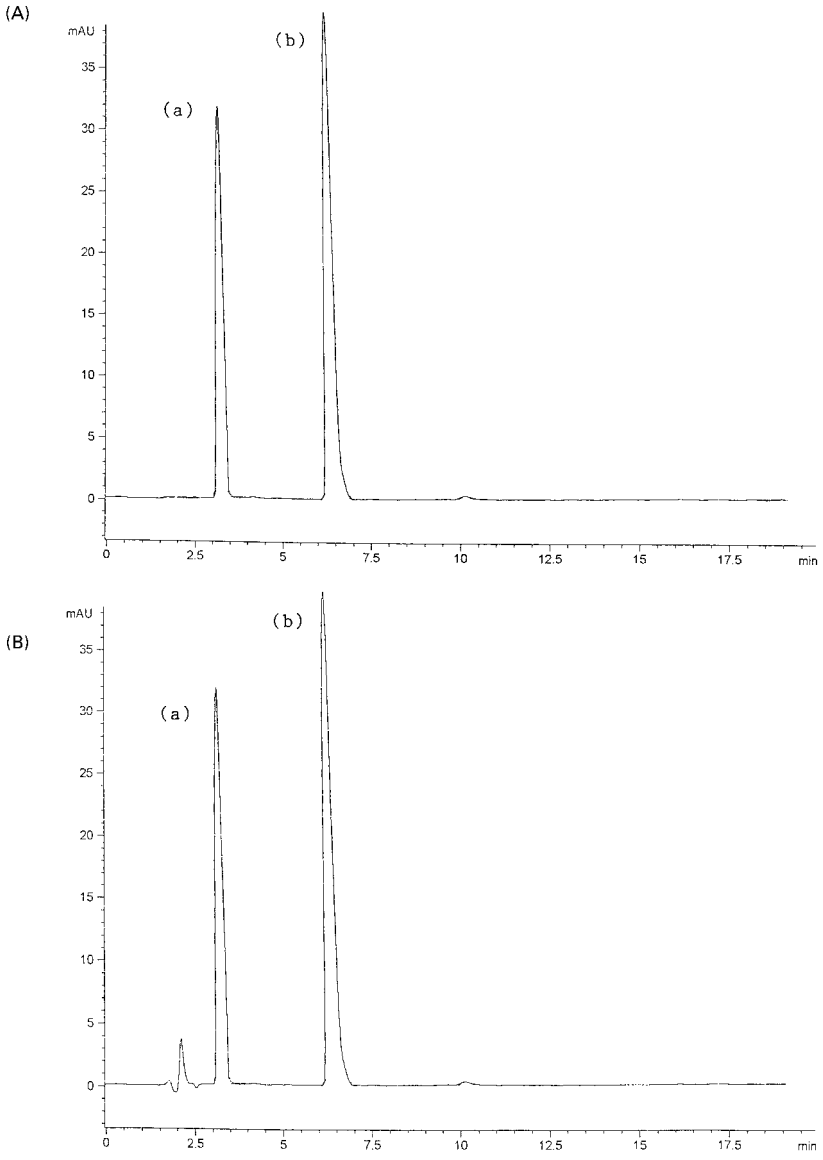

Figure 1. (A) HPLC chromatogram of a 20 µL injection containing (a) 1000.0 ng mL^{-1} of Candesartan cilexetil; (b) 625.0 ng mL^{-1} of hydrochlorothiazide. (B) HPLC chromatogram of human plasma spiked with (a) 1000.0 ng mL^{-1} of Candesartan cilexetil; (b) 625.0 ng mL^{-1} of hydrochlorothiazide.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

hypertensive drugs tested were detected at different retention times (benazepril hydrochloride 4.5 min, lisinopril 7.3 min, cilazapril 2.5 min, and hydroflumethiazide 11.0 min), or were not detected under the described analytical condition (valsartan, amiloride hydrochloride, and losartan hydrochloride).

Stability of candesartan cilexetil and hydrochlorothiazide in solution, and in biological matrix, was determined at different temperatures. No significant changes in the concentration of candesartan cilexetil and hydrochlorothiazide at 5°C, 25°C, and 40°C were observed during 3 days. The test performed in triplicate for one low and one high concentration of each compound, ensures that no degradation occurred during steps before loading the plasma into column. Candesartan cilexetil and hydrochlorothiazide showed to be stable, also, in the eluate from the column. No degradation were noticed over a 8 hr interval.

The linearity of the method was determined by injection of candesartan cilexetil and hydrochlorothiazide standard solutions, at five concentration levels in the range of 30.0–2500.0 ng mL^{-1} for candesartan cilexetil and 20.0–1000.0 ng mL^{-1} for hydrochlorothiazide, respectively. Each concentration was tested in triplicate. Least-square regression calibration curves were constructed by plotting peak areas of candesartan cilexetil and hydrochlorothiazide as a function of the drug concentration in the standard working solution. The calibration curves could be represented by the following regression equations.

$$y\,(\text{Candesartan cilexetil}) = 0.039x + 0.071\,(r = 0.9991,\ n = 5)$$
$$y\,(\text{Hydrochlorothiazide}) = 0.063x + 0.018\,(r = 0.9996,\ n = 5)$$

where x is the concentration of candesartan cilexetil or hydrochlorothiazide in ng mL^{-1} and y is the peak area ratio.

The equations of the regression linear with the coefficients of the determination and the linear response ranges are presented. In all cases, the intercepts were estimated as negligible by using the student test ($p = 0.05$).

Limits of detection and quantification (LOD and LOQ) were estimated in accordance with the baseline noise. The baseline noise was evaluated by recording the detector response over a period of as much as 10 times the peak width. Limit of detection was obtained as the sample concentration that causes a peak three times as high as the baseline noise level, and the LOQ was calculated as being ten times as high as the baseline noise level.[24] Using the parameters mentioned above, LOD and LOQ were estimated to be 2.0 and 11.0 ng mL^{-1} for candesartan cilexetil and 3.58 and 6.75 ng mL^{-1} for hydrochlorothiazide, respectively. Repeatability is given as inter- and intra-day precision and accuracy were evaluated by analyzing three different concentration of candesartan cilexetil and hydrochlorothiazide. Accuracy of the method

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Table 1. Intra- and inter-day precision and accuracy of candesartan cilexetil and hydrochlorothiazide ($n = 5$).

Compound	Theoretical concentration ($ng\,mL^{-1}$)	Intra-day concentration mean	Measured[a] ($ng\,mL^{-1}$) RSD (%)	Inter-day[b] concentration mean	Measured[a] ($ng\,mL^{-1}$) RSD (%)
Candesartan cilexetil	30.0	28.9	0.56	29.7	1.42
	1500.0	1500.8	0.79	1498.4	1.59
	2500.0	2497.4	0.72	2503.8	1.42
Hydrochlorothiazide	20.0	19.5	0.98	19.2	0.68
	500.0	501.4	1.25	498.7	1.25
	1000.0	1000.2	1.36	998.5	1.86

[a]Mean values represent five different sample standards for each concentration.
[b]Inter-day reproducibilty was determined from five different runs on three different days.

was checked for 3 days, at three concentration levels at 30.0, 1500.0, and 2500.0 ng mL^{-1} for candesartan cilexetil and 20.0, 500.0, and 1000.0 ng mL^{-1} for hydrochlorothiazide, each in triplicate. Solutions for the standard curves were prepared fresh every each day. The results are given in Table 1. The precision of the assays is demonstrated by RSD of lower than 1.86%.

In order to access the validity and applicability of the developed method, recovery studies were performed by analysing synthetic mixtures of each drug in different ratios. The percentage recoveries of candesartan cilexetil and hydrochlorothiazide in synthetic mixtures ranged from 96.9% to 101.0% with RSD values less than 1.14% and 97.9% to 100.7% with RSD values less than 1.31%, respectively. The selectivity of the proposed method for the estimation of the drugs in presence of various tablet excipients such as starch, lactose, talc, and magnesium stearate were investigated. A placebo comprising starch 10%, lactose 40%, talc 2%, and magnesium stearate 1% were prepared. A 1 : 1 blend of drug and placebo was prepared. The recoveries (%) obtained in the determination of both compounds are very acceptable. The developed method was applied to candesartan cilexetil and hydrochlorothiazide in three batches of commercial formulations, respectively. The results presented in Table 2 are in good agreement with the labeled content. All data represent the average of 10 determinations.

The specificity of the HPLC method is illustrated in Fig. 1b where complete separation of each of candesartan cilexetil and hydrochlorothiazide from biological endogenous components in the human plasma was noticed, and no interfering peaks at the retention times of candesartan cilexetil and hydrochlorothiazide peaks was observed in the blank human plasma.

The optimized HPLC procedure was also successfully used for the simultaneous determination of candesartan cilexetil and hydrochlorothiazide

Table 2. Assay results of tablets containing 20.0 mg candesartan cilexetil and 12.5 mg hydrochlorothiazide.

	Mean[b] ± RSD[c]	
Drug[a]	Candesartan cilexetil	Hydrochlorothiazide
Batch no 1	20.3 ± 1.02	12.3 ± 0.45
Batch no 2	20.1 ± 1.30	12.5 ± 0.63
Batch no 3	20.3 ± 1.12	11.8 ± 0.42

[a]Atacand® Plus tablets were labeled to contain 20.0 mg candesartan cilexetil and 12.5 mg hydrochlorothiazide per tablets.
[b]Each value is the mean of 10 experiments.
[c]Relative standard deviation.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Table 3. Results obtained for candesartan cilexetil and hydrochlorothiazide analysis from human plasma.

Compound	Taken concentration ($ng\,mL^{-1}$)	Found concentration ($ng\,mL^{-1}$)	Error (%)	RSD (%)	Average recovery (%)
Candesartan cilexetil	30.0	29.1	+0.33	0.75	97.0
	1500.0	1497.8	−0.39	0.63	99.8
	2500.0	2507.2	+1.00	0.84	100.3
Hydrochloro-thiazide	20.0	19.8	+0.98	1.00	99.0
	500.0	501.5	−0.77	0.36	100.3
	1000.0	997.1	+1.36	0.54	99.7

Note: Mean values represent five different sample standards for each concentration.

in protein-free spiked human plasma. Human plasma samples were spiked with different concentrations with candesartan cilexetil and hydrochlorothiazide. The amounts of candesartan cilexetil and hydrochlorothiazide in human plasma were calculated from related linear regression equations. The determination results and recoveries of known amounts of candesartan cilexetil and hydrochlorothiazide added to human plasmas are presented in Table 3. The percentage recoveries of candesartan cilexetil and hydrochlorothiazide in human plasma ranged from 97.0% to 100.3% with RSD values less than 0.84%, and 99.0% to 100.3% with RSD values less than 1.00%, respectively. Good recoveries of candesartan cilexetil and hydrochlorothiazide were achieved from human plasma samples with the proposed method.

CONCLUSION

The newly HPLC method is a suitable technique for the simultaneous determination of candesartan cilexetil and hydrochlorothiazide in multi component formulations and in human plasma without interference of each other. The HPLC method gives a good resolution between candesartan cilexetil and hydrochlorothiazide within a short analysis time (<6.5 min). The developed method is a simple and accurate procedure requiring inexpensive reagents that could be used for rapid, and reliable clinical and pharmacokinetic studies of candesartan cilexetil and hydrochlorothiazide.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

ACKNOWLEDGMENT

The author gratefully acknowledges AstraZeneca Pharm. Ind. for providing the reference substance and its pharmaceutical formulations.

REFERENCES

1. Melian, E.B.; Jarvis, B. Drugs **2002**, *62*, 787.
2. Gonzalez, L.; Lopez, J.A.; Alonso, R.M.; Jimenez, R.M. J. Chromatogr. A. **2002**, *949*, 49.
3. Ghandour, M.A.; Kasım, E.A.; El-Haty, M.T. Anal. Lett. **2002**, *35*, 239.
4. Prieto, J.A.; Alonso, R.M.; Jimenez, R.M. Electrophoresis **2002**, *23*, 102.
5. Al-Majed, A.A.; Belal, F.; Al-Warthan, A.A. Spectrosc. Lett. **2001**, *34*, 211.
6. Prieto, J.A.; Akesolo, U.; Jimenez, R.M. J. Chromatogr. A. **2001**, *916*, 279.
7. Kargosha, K.; Sarrafi, A.H.M. J. Pharmaceut. Biomed. Anal. **2001**, *26*, 273.
8. Erk, N. Anal. Lett. **2002**, *35*, 283.
9. El-Gindy, A.; Ashour, A.; Abdel-Fattah, L. J. Pharmaceut. Biomed. Anal. **2001**, *25*, 299.
10. Erk, N. J. Pharmaceut. Biomed. Anal. **2001**, *24*, 603.
11. Luis, M.L.; Fraga, J.M.G.; Jimenez, A.I.; Arias, J.J. Talanta **2001**, *53*, 761.
12. Sağlık, S.; Sağırlı, O.; Atmaca, S.; Ersoy, L. Anal. Chim. Acta **2001**, *427*, 253.
13. Lapa, R.A.S.; Lima, J.L.F.C.; Santos, J.L.M. Anal. Chim. Acta **2001**, *407*, 225.
14. Dinç, E. Anal. Lett. **2002**, *35*, 1021.
15. Dinç, E.; Üstündağ, Ö. J. Pharmaceut. Biomed. Anal. **2002**, *29*, 371.
16. Dinç, E.; Baleanu, D. J. Pharmaceut. Biomed. Anal. **2002**, *30*, 715.
17. Manna, L.; Valvo, L.; Alimonti, S. Chromatographia **2001**, *53*, S271.
18. Atay, O.; Tamer, U.; Ařıkan, D. Anal. Lett. **2001**, *34*, 1153.
19. Carlucci, G.; Di Carlo, V.; Mazzeo, P. Anal. Lett. **2000**, *33*, 2491.
20. Carlucci, G.; Palumbo, G.; Mazzeo, P. J. Pharmaceut. Biomed. Anal. **2000**, *23*, 185.
21. Argekar, A.P.; Sawant, J.G. Anal. Lett. **2000**, *33*, 869.
22. Belal, F.; Al-Zaagi, I.A.; Gadkariem, E.A. J. Pharmaceut. Biomed. Anal. **2001**, *24*, 335.
23. Hassib, S.T.; El-Sherif, Z.A.; El-Bagary, R. Anal. Lett. **2000**, *33*, 3225.
24. Altria, K.D.; Chanter, Y.L. J. Chromatogr. **1993**, *652*, 459.

Received February 10, 2003
Accepted April 9, 2003
Manuscript 6085

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016