HPLC Evaluation of Diclofenac in the Various Forms of Therapeutic Preparations

L. HANYŠOVÁ, M. MOKRÝ, P. KASTNER, and J. KLIMEŠ

Department of Pharmaceutical Chemistry and Drug Control, Faculty of Pharmacy in Hradec Králové, Charles University in Prague, CZ-500 05 Hradec Králové, Czech Republic
e-mail: hanysova@faf.cuni.cz

Received 5 December 2003

High-performance liquid chromatography was selected for analytical evaluation of sodium diclofenac in the tablets and gel-type therapeutic preparations and also for evaluation of sodium diclofenac and lidocaine hydrochloride in the ampoule-type composite preparation. After isolation of diclofenac from these preparations the samples were analyzed on column with reversed phase, using the mobile phase methanol and phosphate buffer (pH = 3.2) and detection was at $\lambda = 282$ nm. In the composite therapeutic preparation diclofenac and lidocaine were analyzed on column with reversed phase using the mobile phase acetonitrile and phosphate buffer (pH = 3.72) and detection was at $\lambda = 220$ nm. The precision was determined by the relative standard deviation and the recovery was determined by comparing the peak area of six different concentrations with the peak area obtained after adding substances to a diluted preparation.

Diclofenac is a nonsteroidal anti-inflammatory drug used for a variety of painful and inflammatory conditions. The main adverse effects are gastrointestinal disturbances. There may be pain and, occasionally, tissue damage at the site of injection in case that diclofenac is given intramuscularly [1, 2]. Lidocaine is a local anaesthetic of the amide type and has a fast onset and an intermediate duration of action. It is employed in a wide range of anaesthetic applications. Lidocaine is also used in the treatment of ventricular arrhythmias and has been used for the control of status epilepticus as comparable to other treatments. Like with other local anaesthetics the main system adverse effects of lidocaine are on the CNS and cardiovascular system [1, 2].

Most of papers about diclofenac deal with its pharmacology. Special articles are concerned in particular with its pharmacokinetical and pharmacodynamical attributes. These papers evaluate biological availability of diclofenac after administration of individual pharmaceuticals of different drug forms (tablets, topical formulations, effervescent tablets, suppository) [3—16]. The series of the papers concerning monitoring of diclofenac and its metabolites was published in the special literature on HPLC in biological materials (most frequently in plasma, less frequently in serum, urine, ocular and synovial fluids) [17—34]. Some of publications relate stability studies of diclofenac in the pharmaceutical preparations [35—37]. *Heusler* was concerned with quantitative analyses of lidocaine and other local anaesthetics or antiarrhythmic drugs and their metabolites in plasma and blood serum at the UV detection [38]. Another papers dealt with analyses of lidocaine, the ability to separate lidocaine from its degradation products or common pharmaceuticals under a variety of conditions by RP-HPLC [39—43]. *Flanagan* was employed in separating lidocaine from other local anaesthetics by normal phase HPLC [44].

EXPERIMENTAL

Chemicals used (all anal. grade): Diclofenac sodium salt was purchased from Sigma (St. Louis, MO), lidocaine from Astra, spol. s r.o. (Prague, CR), tribusone and kebusone were purchased from Taurus a.s. (Veverská Bítýška, CR), hexane-1-sulfonic acid from Sigma-Aldrich (Steinheim, Germany), indometacine from Galenic laboratory (Ostrava, CR), sodium dihydrogenphosphate, phosphoric acid, salicylic acid, sulfanilic acid, ibuprofen, sulfanilamide, phenacetine, acetonitrile, ether, ethyl acetate were purchased from Lachema (Brno, CR), sulfisoxasole from Léčiva a.s. (Prague, CR), methanol HPLC, water was redistilled.

Pharmaceutical preparations: Olfen gel, Olfen tbl., Olfen supp., Olfen amp. (Medimport). HPLC analysis proceeds at these chromatographic conditions: The sample was applied using 20 mm^3 loops to analytical columns Separon SGX C18 (150 mm × 3 mm i.d., 7 μm) from Tessek (Prague, CR) and LiChrosorb 5RP C8 (250 mm × 4.6 mm i.d.) from Tessek (Prague, CR). The mobile phase was methanol and phosphate buffer of pH 3.2 ($\varphi_r = 70:30$) and a flow rate was 0.9 cm^3 min^{-1}. Detection was performed at $\lambda = 280$ nm. The mobile phase for evaluation of diclofenac and lidocaine

was acetonitrile and phosphate buffer of pH 3.72 (φ_r = 70:30) with an addition of hexane-1-sulfonic acid (1.88 g dm^{-3}) at a flow rate 1.2 cm^3 min^{-1}. Detection was performed at λ = 220 nm.

The HPLC system consisted of a pump LCP 4100, Ecom (Prague, CR), an UV VIS detector LCD 2084, Ecom (Prague, CR), and a data station with CSW software, version 1.7., integrator from DataApex spol. s r.o. (Prague, CR).

Preparing the Samples

Gel: 55.0 mg of 1 % gel was dissolved in 5 cm^3 of the mobile phase, this sample was shaken for 30 min and then it was kept in an ultrasonic bath for 15 min. The internal standard was added and the volume was filled up to 25 cm^3 by the mobile phase. After this process the sample was centrifuged for 10 min at 3600 min^{-1}. 20 mm^3 from this sample were applied to column.

Tablets: 5 tablets were crushed into fine powder and the volume was filled up to 100 cm^3 by mobile phase. The volumetric flask was kept in an ultrasonic bath for 30 min and then it was shaken for 20 min. 0.5 cm^3 of this stock solution was transferred into the volumetric flask with the internal standard and the volume was filled up to 25 cm^3 by mobile phase. 20 mm^3 from this sample were applied to column.

Suppositories: Semifluid material from this pharmaceutical form was shaken with mobile phase for 30 min and then the internal standard was added. The volume was filled up to 25 cm^3 by mobile phase. 20 mm^3 from this sample were applied to column.

Ampoules: 2 cm^3 were transferred into the volumetric flask and the volume was filled up to 50 cm^3. 0.5 cm^3 of this stock solution was transferred into the volumetric flask with the internal standard and the volume was filled up to 25 cm^3 by mobile phase. 20 mm^3 from this sample were applied to column.

Table 1. Ten Tested Substances with their Retention Characteristics

Substance	t_R/min	k/min
Salicylic acid	1.13	0.177
Sulfisoxasole	2.41	1.510
Phenacetin	1.33	0.385
Sulfanilamide	2.01	1.094
Sulfanilic acid	2.35	1.448
Ibuprofen	7.57	6.885
Indomethacine	7.96	7.291
Kebusone	7.65	6.968
Tribusone	5.99	5.239
8-Hydroxyquinoline	19.80	19.625
Diclofenac	8.98	8.354

RESULTS AND DISCUSSION

The mixture of acetonitrile or methanol with a water component is usually used as a mobile phase in the RP-HPLC system. In this paper, we preferably applied as a mobile phase the mixture of methanol and buffer. The buffer was a mixture of equal parts by volume of solution of phosphoric acid (1 g dm^{-3}) and solution of sodium dihydrogenphosphate (1.6 g dm^{-3}) [45]. As the analyzed substance had an acid character, the mobile phase was adjusted by 10 % phosphoric acid to a pH value 3.2 and 3.72 (lidocaine amp.). The solutions from the individual drug forms were prepared so as the analyzed substance could be transferred into

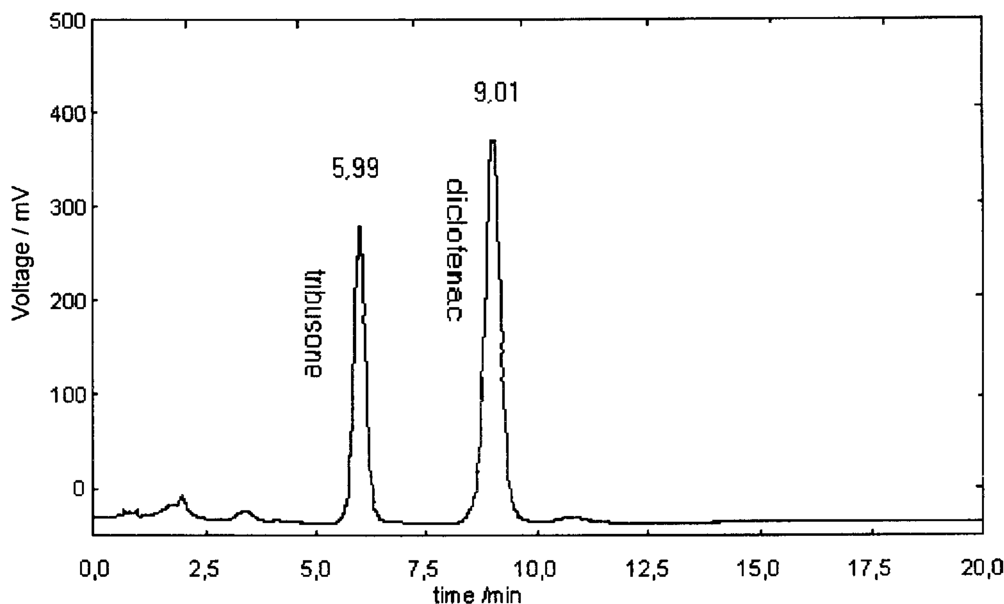

Fig. 1. Chromatogram of diclofenac and internal standard in suppository.

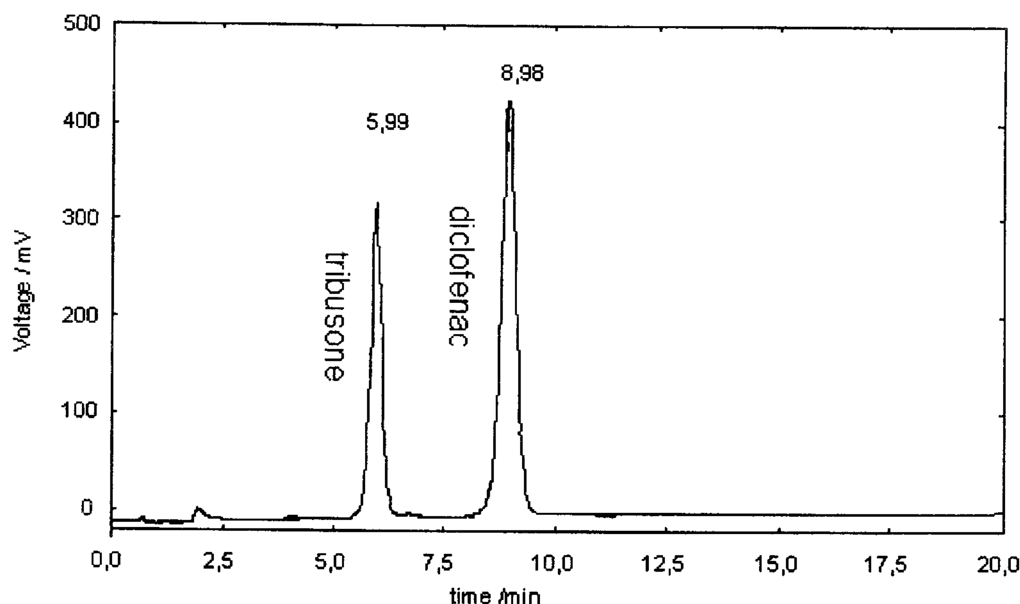

Fig. 2. Chromatogram of diclofenac and internal standard in the gel.

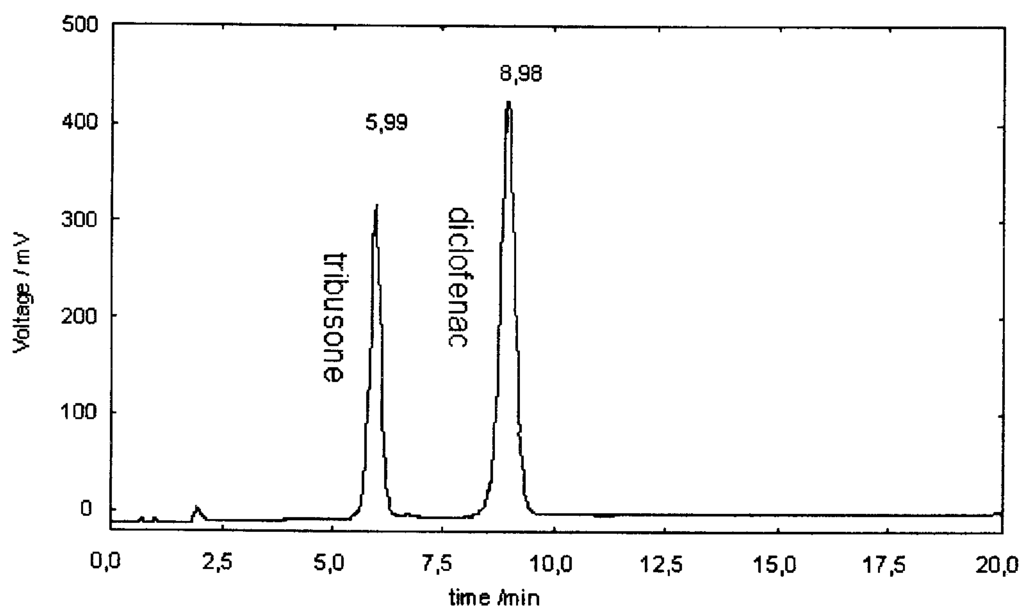

Fig. 3. Chromatogram of diclofenac and internal standard in the tablets.

the solution and separated from insoluble elements of drug preparation.

The internal standard should have retention time different in contrast to diclofenac. There have been tested 10 substances the retention characteristics of which are ordered in Table 1. Tribusone, the retention time of which was 5.99 min (tbl., supp., gel) and 6.12 min (amp.) as shown in Figs. 1—4, suited as an internal standard at the conditions of analysis.

A flow rate has been tested from 0.4 cm^3 min^{-1} to 1.2 cm^3 min^{-1} for the determination of diclofenac from the tbl., supp., and from the gel. The best effect was achieved at the flow rate of 0.9 cm^3 min^{-1} from the viewpoint of the optimal separation. UV detector worked at a wavelength in absorption maximum of diclofenac at 280 nm. The proportion of components in the mobile phase was converted to φ_r (methanol—buffer) = 70:30 and pH was modified to the values from 2.5 to 4.5. The best shape of peaks was achieved at pH 3.2. At these conditions the time of analysis was 10 min. At the determination of diclofenac and lidocaine from the ampoule, there were tested the mobile phases of methanol and buffer (φ_r = 70:30), pH between 2.8 and 4.2 and acetonitrile and buffer (φ_r = 70:30), pH between 3.2 and 4.0. The best effect of the analysis was acquired at the flow rate 1.2 cm

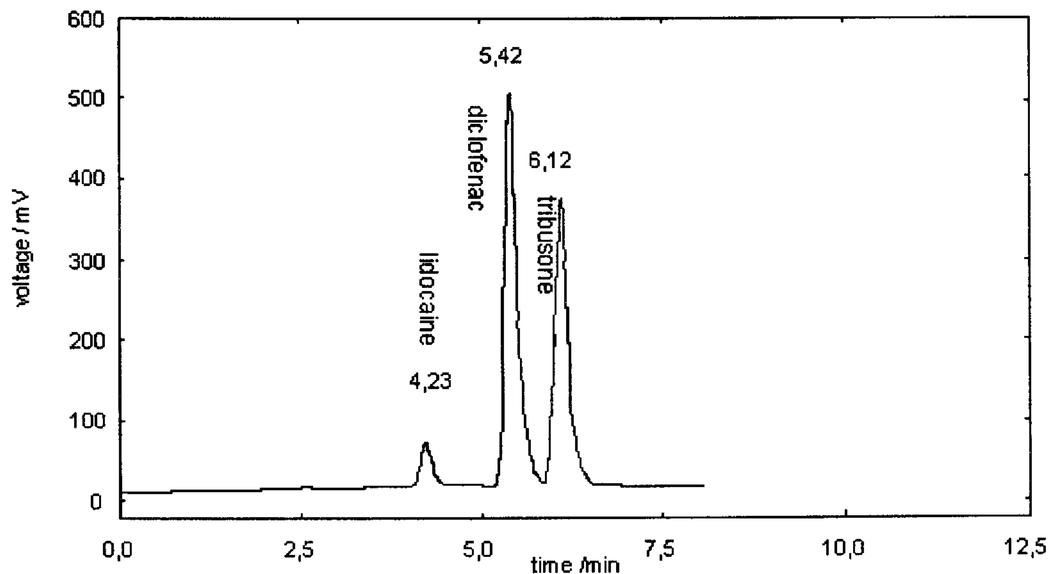

Fig. 4. Chromatogram of diclofenac, lidocaine, and internal standard in the ampoule.

Table 2. Determination of Diclofenac in the Gel from Three Measurements with Total Preparation of Samples of One Concentration Level (22.0 μg cm^{-3})

Sample	Experimental conc. $C/(\mu g\ cm^{-3})$	Recovery $R_i/\%$
1	22.4502	102.05
2	22.5872	102.66
3	22.2797	101.27
Average value	22.4391	101.99
Standard deviation	0.1541	0.4313

Table 3. Determination of Diclofenac in the Suppository from Three Measurements with Total Preparation of Samples of One Concentration Level (20.0 μg cm^{-3})

Sample	Experimental conc. $C/(\mu g\ cm^{-3})$	Recovery $R_i/\%$
1	18.6874	93.44
2	18.4965	92.48
3	18.6959	93.49
Average value	18.6266	93.13
Standard deviation	0.1128	0.5662

min^{-3}. UV detector worked at a wavelength in absorption maximum of lidocaine at λ = 280 nm. At these conditions the time of analysis was 7 min.

The quantification of diclofenac and lidocaine was based on the calibration curves constructed as the dependence of the ratios of the areas (y) of peaks of analyzed substances to the area of the peak of the internal standard on concentration numerical value (x).

Table 4. Determination of Diclofenac in the Tablets from Three Measurements with Total Preparation of Samples of One Concentration Level (22.0 μg cm^{-3})

Sample	Experimental conc. $C/(\mu g\ cm^{-3})$	Recovery $R_i/\%$
1	26.4625	105.85
2	26.4905	105.96
3	26.4924	105.97
Average value	26.4818	105.92
Standard deviation	0.0167	0.0666

The calibration curve reflects the linearity in the concentration range of 8—56 mg cm^{-3} with a correlation coefficient r = 0.9996 (gel), of 5—50 mg cm^{-3} with a correlation coefficient r = 0.9995 (tbl. and supp.), of 15—72 mg cm^{-3} with a correlation coefficient r = 0.9995 (amp. at the determination of diclofenac) and in the concentration range of 4—19.5 mg cm^{-3} with a correlation coefficient r = 0.9999 (amp. at the determination of lidocaine). There was used the method of linear regression in this paper and the equations of calibration curves were: $y = 0.06277x - 0.050$ (gel), $y = 0.0707x - 0.059$ (tbl. and supp.), $y = 0.04401x - 0.009$ (amp. at the determination of diclofenac), $y = 0.02217x - 0.0105$ (amp. at the determination of lidocaine). The concentration of diclofenac was calculated from an average value of the ratios of the areas from the three measurements of the theoretical concentration level in the gel as shown in Table 2, in the tablets as shown in Table 3, in the suppositories as shown in Table 4. In the composite therapeutic preparation partial validation was made as shown in Tables 5 and 6. There was made the recovery by the method of the

Table 5. Six Independent Analyses of Homogenized Samples, which were Carried out by Complete Procedure, Including Sample Preparation

Sample	Diclofenac $R_i/\%$ $R_p = 100.22$	Lidocaine $R_i/\%$ $R_p = 99.125$	Diclofenac $S_R/\%$	Lidocaine $S_R/\%$
1	99.52	98.35		
2	100.17	98.58		
3	100.40	100.14	0.1825	0.4384
4	100.08	98.87		
5	100.82	99.55		
6	100.31	99.26		

Table 6. Six Different Concentrations were Applied to Column for Recovery of Method

Sample		Theoretical conc. C_o / $\mu g\ cm^{-3}$	Experimental conc. C / $\mu g\ cm^{-3}$	Analytical recovery $R_i/\%$
1	Lidocaine	7.2	7.144	99.22
	Diclofenac	27	26.75	99.07
2	Lidocaine	8.0	7.996	99.96
	Diclofenac	30	30.22	100.73
3	Lidocaine	8.8	8.920	101.36
	Diclofenac	33	33.05	100.16
4	Lidocaine	9.5	9.42	99.14
	Diclofenac	36	36.07	100.18
5	Lidocaine	10.4	10.29	98.94
	Diclofenac	39	38.72	99.28
6	Lidocaine	11.2	11.10	99.12
	Diclofenac	42	42.11	100.25

standard addition described in this paper.

LOQ of diclofenac was 0.1578 mg dm^{-3} and LOQ of lidocaine was 0.2476 mg dm^{-3}.

CONCLUSION

The method has been worked out for HPLC analysis of diclofenac, so it enables its evaluation in the final preparation of the type of gel, tbl., supp. and no excipient has been interfered with analysis. The HPLC method for the assay of diclofenac and lidocaine in composition pharmaceutical preparation has been developed and partially validated. Even though this method was rapid, no excipients have been interfered with analysis. The results confirmed that the method is selective and accurate.

Acknowledgements. The study was implemented with the support of the Research Project MSM 111600001.

REFERENCES

1. Abdel-Rehim, M., Bielenstein, M., Askemark, Y., Tyrefors, N., and Arvidsson, T., *J. Chromatogr., B: Biomed. Appl. 741*, 175 (2000).
2. Raynolds, J., *Martindale 31*, 1332, 36, (1996).
3. Ravlsankar, S., Vasudevan, M., Nanjan, M. J., and Bijukurian, S. B., *Indian Drugs 34*, 663 (1997).
4. Palmero, M., Bellot, J. L., Alcoriza, N., Garcia-Canabes, C., and Orts, A., *Ophthalmic Res. 31*, 309 (1999).
5. Terhaag, B., Hoffmann, A., Barkworth, M., and Vens-Cappell, B., *Int. J. Clin. Pharmacol. Ther. 38*, 546 (2000).
6. Dimitrova, B., Doytchinova, I., and Zlatkova, M., *J. Pharm. Biomed. Anal. 23*, 955 (2000).
7. Silva, L. C., Simoes, I. G., Lerner, F. E., Belem, G. R., De-Moraes, M. E., and De-Nucci, G., *Arzneim.-Forsch. 49*, 920 (1999).
8. Marzo, A., Dal-Bo, L., Verga, F., Ceppi-Monti, N., Abbondati, G., Tettamanti, R. A., Uhr, M. R., and Ismaili, S., *Arzneim.-Forsch. 50*, 43 (2000).
9. Bender, T., Schafer, M., and Bariska, J., *Clin. Rheumatol. 19*, 89 (2000).
10. Shinde, V. M., Desai, B. S., and Tendolkar, N. M., *Indian J. Pharm. Sci. 57*, 35 (1995).
11. Zhang, X., Duan, J., and Ye, G., *Clin. Pharm. J. 34*, 159 (1996).
12. Zmeili, S., Hasan, M., Najib, N., Sallam, E., Deleq, S., and Shubair, M., *Int. J. Clin. Pharmacol. Ther. 34*, 564 (1996).
13. Sengupta, S., Velpandian, T., Kabir, S. R., and Gupta, S. K., *Eur. J. Clin. Pharmacol. 54*, 541 (1998).
14. Šatínský, D., Solich, P., Chocholou, P., and Karlíček, R., *Anal. Chim. Acta 499*, 205 (2003).
15. Schaffazick, S. R., Pohlmann, A. R., Dalla-Costa, T., and Guterres, S. S., *Eur. J. Pharm. Biopharm. 2003*, 56.

16. Hasan, N. Y., Abdel-Elkawy, M., Elzeany, B. E., and Wagieh, N. E., *Il Farmaco 58*, 91 (2003).
17. Zecca, L. and Ferrario, P., *J. Chromatogr. 495*, 303 (1989).
18. Chawla, J. L., Sodhi, R. A., and Sane, R. T., *Indian Drugs 33*, 171 (1996).
19. Sawchuk, R. J., Maloney, J. A., Cartier, L. L., Rackley, R. J., Chan, K. K. H., and Lau, H. S. L., *Pharm. Res. 12*, 756 (1995).
20. Hanses, A., Spahn-Langguth, H., and Mutschler, E., *Arch. Pharm. 328*, 257 (1995).
21. Mohamed, F. A., Jun, H. W., Elfaham, T. H., Sayed, H. A., and Hafez, E., *J. Liq. Chromatogr. 17*, 1065 (1994).
22. Riegel, M. and Ellis, P. P., *J. Chromatogr., B: Biomed. Appl. 654*, 140 (1994).
23. Webster, L., Pacey, M., Winchester, T., Johnson, P., and Jezequel, S., *Appl. Microbiol. Biotechnol. 49*, 371 (1998).
24. Gupta, S. K., Velpandian, T., Mathur, P., and Sengupta, S., *Pharmacology 56*, 137 (1998).
25. Kuhlmann, O. and Krauss, G. J., *J. Pharm. Biomed. Anal. 16*, 553 (1997).
26. Tabaka, K., Yamaoka, K., Fukuyama, T., and Nakagawa, T., *Drug Metab. Dispos. 24*, 216 (1996).
27. Tabaka, K., Yamaoka, K., Fukuyama, T., Nakagawa, T., and Yasui, H., *J. Pharm. Pharmacol. 48*, 866 (1996).
28. Cwiertnia, B., Hladon, T., and Stobiecki, M., *J. Pharm. Pharmacol. 51*, 1213 (1999).
29. Kuhlmann, O., Struck, H. G., and Krauss, G. J., *J. Pharm. Biomed. Anal. 17*, 1351 (1998).
30. Hye-Suk-Lee, Chang-Kyun-Jeong, Sung-Jin-Choi, Sang-Beom-Kim, Mi-Hyun-Lee, Geon-Il-Ko, and Dong-Hwan-Sohn, *J. Pharm. Biomed. Anal. 23*, 775 (2000).
31. Guo, T., Sui, Y., Dang, D. S., and Shi, G. B., *Chin. Pharm. J. 235*, 467 (2000).
32. Mayer, B. X., Namiranian, K., Dehghanyar, P., Stroh, R., Mascher, H., and Miller, M., *J. Pharm. Biomed. Anal. 33*, 745 (2003).
33. Hilton, M. J. and Thomas, K. V., *J. Chromatogr. 1015*, 129 (2003).
34. Roškar, R. and Kmetec, V., *J. Chromatogr., B: Biomed. Appl. 788*, 57 (2003).
35. Csoka, G., Marton, S., Zelko, R., and Racz, I., *Pharm. Sci. 10*, 415 (2000).
36. Ye, H. and Zhang, M., *Chin. Pharm. J. 35*, 192 (2000).
37. Gaudiano, M. C., Valvo, L., Bertocchi, P., and Manna, L., *J. Pharm. Biomed. Anal. 32*, 151 (2003).
38. Heusler, H., *J. Chromatogr. 340*, 273 (1985).
39. Horvath, C. and Melander, W., *Am. Lab.* (Boston) *10*, 17 (1977).
40. Waraszkiewicz, S. M., *J. Pharm. Sci. 70*, 1215 (1981).
41. Crommen, J., *J. Chromatogr. 186*, 705 (1979).
42. Wolff, M., *Pharmazie 38*, 891 (1983).
43. Archari, R. G. and Jacob, J. T., *J. Liq. Chromatogr. 3*, 81 (1980).
44. Flanagan, R. J., *J. Chromatogr. 247*, 15 (1982).
45. *Pharmacopoea Bohemica MCMXCVII*, p. 1567. Grada Publishing, Prague, 1997.