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Simultaneous determination of tolperisone and lidocaine by high performance liquid chromatography

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Abstract

A reversed phase high performance liquid chromatographic (RP-HPLC) method for the simultaneous determination of tolperisone (TP) and lidocaine (LD) has been developed. The drugs were separated on a column (4.60×250 mm^2) Spherisorb ODS (5 μm) using 5.5% triethylamine in 70/30 v/v acetonitrile/water as mobile phase 0.7 ml min^{-1}and UV detection at 254 nm. The detection limits for Tolperisone hydrochloride (TP·HCl) and lidocaine hydrochloride (LD·HCl) were 0.20 ng/20 μl and 100 ng/20 μl and the quantitation limits were 0.50 ng/20 μl and 250 ng/20 μl, respectively. Linear calibration curves over the ranges of 1–10, 10–100 and 150–500 μg ml^{-1} for TP·HCl and 10–500 μg ml^{-1} for LD·HCl were established. Different calibration slopes were found for TP probably owing to changes in refractive index due to increase in TP concentration. The average recoveries of the added TP in the samples (TP·HCl tablets and injection liquid). A solutions spiked with standard TP·HCl were 99.9 and 99.7% with the RSD ($n = 11$) of 0.66 and 0.67%, respectively. The average recovery of the added LD in the sample (injection) spiked with standard LD·HCl was 98.9% with the RSD ($n = 11$) of 0.59%. The proposed method has been applied to the determination of TP·HCl and LD·HCl in commercial products available in Thailand. Comparative determination of TP by UV spectrophotometry and LD by colorimetry were also carried out. The results obtained by both methods were in good agreement of those obtained by the proposed method verified by using *t*-test. The proposed RP-HPLC method is simple, accurate, reproducible and suitable for routine analysis.

Keywords: High performance liquid chromatography; Tolperisone hydrochlorides; Lidocaine hydrochlorides

1. Introduction

Tolperisone hydrochloride (TP·HCl) is used as a muscle relaxant. Very few assay methods of TP·HCl were reported, such as potentiometry, [1] spectrophotometry [2] high performance thin layer chromatography (HPTLC) [3] and high performance liquid chromatography [4]. There has

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been an increasing demand for a suitable method for the assay of TP·HCl. Because of pharmacokinetic causes, such as variable drug absorption from the gastrointestinal tract and drug–drug interactions leading to altered drug elimination, effective drug concentration might not always be reached in clinical situations [5]. HPLC has become a powerful tool for the analysis of a wide range of pharmaceutical products including muscle relaxants. However, the reported HPLC methods showed excellent separation of these drugs from their metabolites and other common drugs in several sample matrices such as blood, plasma and urine but none of the methods has been applied to the quantitation of TP.

Lidocaine (LD) is widely used as a local anesthetic. It has also achieved prominence as an antiarrhythmic agent and is now in common use particularly as emergency treatment for ventricular arrhythmias that are encountered after cardiac surgery or acute myocardial infection. A notable side effect of LD maybe caused by its metabolites rather than LD itself [6].

Several methods for the determination of lidocaine hydrochloride (LD·HCl) have been reported, such as spectrophotometry [6–8], atomic absorption spectrophotometry [9], differential pulse cathode stripping voltammetry [10], gas chromatography [11] and HPLC [12,13]. HPLC method with various detectors have been reported for the separation and determination of LD in different sample matrices [14–19]. For examples, Chen et al. [14] described an HPLC method with UV detection at 218 nm for the determination of LD and bupivacaine in human plasma. Both drugs were served as internal standard for each other. Calibration curve was linear over the range 0.05–8.0 μg ml^{-1}. The sensitivities were 0.01 μg ml^{-1} for LD and 0.06 μg ml^{-1} for bupivacaine. Achilli et al. [15] developed a reversed-phase HPLC with electrochemical detection for determining fifteen common drugs including LD. The detection limit ($S/N = 3$) was 91 pg with the RSD of 2.4%. This method was tentatively applied to the determination of drugs in extracts of human hair. A comparative assay of LD in pharmaceutical formulations by reversed phase high performance liquid chromatography (RP-HPLC) and TLC–UV densitometry [16] has been reported. The linear response was achieved up to 10 μg ml^{-1} (HPLC) and 8 mg ml^{-1} (TLC). The recoveries were in the ranges 99.6–100.2% and 99.2–100.7% for HPLC and TLC, respectively. Fabrice Mangani et al. [17] devised a selective and highly reproducible, multi-column HPLC method for the simultaneous analysis of cardiovascular drugs (including LD) in serum. The RSD values of the peak areas for spiked serum were in the range 2–5% for LD with the detection limit of 300 ng ml^{-1}. Dal Bo et al. [18] proposed a very sensitive HPLC–tandem mass spectrometric (LC–MS–MS) method for the quantitation of LD in human plasma. Linearity was ranging from 0.2 to 30 ng ml^{-1} with a limit of quantitation 0.2 ng ml^{-1}. Mohamed Abdel-Rehim et al. [19] developed a sensitive and accurate HPLC–tandem electrospray mass spectrometric procedure for determining LD and its metabolites in human plasma and urine. By using this method, the limit of quantitation (LOQ) was improved by at least ten times to those described in the literature. The LOQ was in the range 1.6–5 nmol l^{-1}. In general, dosage forms of these drugs can contain either TP·HCl or LD·HCl and both depending on the therapeutical purposes. Quantitation of these two drugs can be done separately for dosage forms. No published paper has mentioned about the simultaneous determination of TP·HCl and LD·HCl.

The purpose of this study was to develop a rapid, accurate, sensitive, simultaneous and comparatively simple method for the quantitation of TP.HCl and LD·HCl in pharmaceutical formulations.

2. Experimental

2.1. Apparatus and reagents

HPLC analyses were carried out with a Thermo Separation Product (Fremont, CA) modular chromatograph consisted of a solvent-delivery system Model P1000, with a Constametric Model 4100 pumping system, an autosampler Model AS3000 with a 20 μl loop, and a variable-wavelength UV detector Model 3200. The Spherisorb ODS ana-

lytical column (Phenomenex, 4.60 mm id × 250 mm, 5 μm) was used. An ultrasonicator was obtained from Metason (Struers, Denmark).

2.2. Reagents

Tolperisone and lidocaine hydrochlorides were of analytical reagent grade and were purchased from Sigma (St. Louis, Mo), Acetonitrile, chloroform and methanol were of HPLC grade and were obtained from Merck (Darmstadt, Germany), Triethylamine was analytical grade and was purchased from Merck (Darmstadt, Germany). The de-ionized distilled water was used throughout the experiment.

3. Procedure

3.1. Sample preparation

3.1.1. Tablets

Twenty tablets of muscle relaxant formulations (TP·HCl tablets) were accurately weighed individually, powdered and mixed thoroughly. 0.21 g portion of the drug powder was accurately weighed, dissolved in a small volume of water, transferred into a 100 ml volumetric flask to obtain final concentration of 25 mg TP·HCl/100 ml and diluted to the volume with the mobile phase, sonicated for 5–10 min and filtered. Then a solution containing 25 μg ml^{-1} of TP·HCl was prepared from this solution by appropriate dilution with the mobile phase.

3.1.2. Injectables

Two commercially available injection samples were purchased from the drug stores in Thailand with the sample codes A and B. (i) Injection A contained 100 mg TP·HCl and 2.5 mg LD·HCl per ml. Injection liquids from 10 vials were completely transferred into a 10 ml volumetric flask and mixed thoroughly. 100 μl of this liquid was pipetted, transferred into a 10 ml volumetric flask and diluted to the volume with the mobile phase. (ii) Injection B contained 20 mg LD.HCl per ml. Injection liquids from 10 vials were completely transferred into a 10 ml volumetric flask and mixed thoroughly. 100 μl of this injection liquid was pipetted, transferred into a 5 ml volumetric flask and diluted to the volume with the mobile phase.

3.1.3. HPLC determination of tolperisone and lidocaine hydrochlorides

The chromatographic separation of TP·HCl was carried out in the isocratic mode using a mixture of 5.5% triethylamine in acetonitrile and water (70:30, v/v) as mobile phase. The column was equilibrated with the mobile phase flowing at 0.7 ml min^{-1} for about 1 h prior to sample injection. The column temperature was ambient. A liquid (20 μl) of standard (10–100 μg ml^{-1}) or sample solutions was injected automatically into the column. Subsequently, the liquid chromatographic behaviours of both drugs were monitored with a UV detector at 254 nm. Calibration curve of each drug was constructed by plotting peak areas versus various concentrations of TP or LD.

4. Results and discussion

Tolperisone and lidocaine hydrochlorides were separated and determined simultaneously by isocratic liquid chromatography on a reverse-phase C_{18} column, using mobile phase of 5.5% triethylamine in acetonitrile:water (70:30, v/v), with UV detection at 254 nm.

The aim of this work was to develop a high performance liquid chromatographic procedure for the simultaneous determination of TP and LD in pharmaceutical preparations using an UV detector. Therefore, it was of prime important to investigate a suitable wavelength for the detection of both drugs in order to achieve the highest sensitivity.

4.1. Selection of the chromatographic conditions

The detection wavelength of TP.HCl and LD.HCl after separation was investigated by scanning the UV spectra of a standard solution containing 50 μg ml^{-1} of TP·HCl and 100 μg ml^{-1} of LD·HCl in methanol, acetonitrile or in the mobile phase. The absorption maxima of

TP·HCl and LD·HCl in methanol were at 257 and 243 nm, respectively indicating that methanol and its mixture might not provide simultaneous separation and quantitation of both drugs. It was evident that both drugs exhibited maximum absorption at the same wavelength (240 nm) when acetonitrile was used as solvent, indicating that a mixture of acetonitrile and water might be used as a mobile phase for the simultaneous determination of both drugs. Appropriate mobile phase for this purpose was therefore sought.

For the separation of TP.HCl and LD.HCl by reverse phase HPLC, the following experimental conditions, namely, choice of mobile phase, phase flow rate, amounts of modifier, wavelength and injection volume have been investigated.

A mixture of standard TP.HCl and LD.HCl hydrochlorides dissolved in de-ionized distilled water was injected into a C_{18} column at flow rate of 0.5 ml min^{-1}. Subsequently, the mobile phase flow rate was varied between 0.5–0.9 ml min^{-1}. The optimum mobile phase flow rate was found to be 0.7 ml min^{-1} which was used for further optimisation of other parameters. Several mobile phases were tested using an isocratic system in all cases. Three systems of mobile phases were investigated. The first system was methanol and water at the ratio of 50:50 and 70:30 v/v, then measured the detection response at 254 nm. This system could be used to separate both drugs, but the chromatograms showed rather poor resolution. The second system was consisting of acetonitrile and water at the ratio of 50:50 and 70:30 v/v with detection at 254 nm. This solvent system gave the higher resolution of TP.HCl and LD.HCl hydrochlorides than those obtained by using the first solvent system. The mobile phase with the composition of acetonitrile:water 70:30 v/v gave higher resolution of both drugs than that with acetonitrile:water 50:50 v/v. Thus, the polarity of the second solvent system was improved by addition of triethylamine as modifier resulting in the third solvent system. The third solvent system consisted of various ratios of 5% triethylamine in the acetonitrile and water (40:60–80:20 v/v). The addition of triethylamine gave rise to bathochromic shift of the absorption maxima of the drugs. The third solvent system might be the suitable mobile phase for simultaneous determination of both drugs. The 70:30 (v/v) acetonitrile:water with 5% triethylamine gave, a good separation of the peaks of both drugs with low background signals (Fig. 1). It was seen that LD was eluted at 5.64 min and TP at 9.38 min providing complete peak separation after its optimum compositions were studied with reasonable peak areas. Although the mobile phase of acetonitrile and water of 80:20 v/v with 5% diethylamine gave the faster retention times of 4.89 and 7.25 min for LD and TP, respectively, the peak areas for both drugs were far more smaller than those obtained by the other mobile phases used. Regarding to the mobile phases of 40:60 and 50:50 v/v of 5% diethylamine in acetonitrile and water, it was seen that using both mobile phases, it took too much time for complete separation for both drugs although they provided the greater peak areas. Therefore, as a compromise the mobile phase of 70:30 of 5% driethylamine in acetonitrile and water was chosen as appropritate mobile phase for subsequent investigations.

Various percentages of triethylamine presented in acetonitrile ranging from 4.0 to 6.0% were also studied. Maximum peak areas of TP and LD were observed, when the mobile phase was consisting of 5.5% of triethylamine in acetonitrile/water (70:30 v/v) as shown in Fig. 1.

The effect of the flow rates of the mobile phase on the separation of TP and LD was evaluated, working in isocratic mode, to obtain an appropriate mixture of 5.5% triethylamine in acetonitrile and water (70:30 v/v), Subsequently, 20 μl of standard drug mixture/solution was injected into the column at varying flow rate from 0.5 to 0.9 ml min^{-1}. It was found that the elution times and the peak areas of both drugs under study decrease with increasing the mobile phase flow rates. However, as a compromise a flow rate of 0.7 ml min^{-1} was chosen as suitable in order to achieve the highest resolution although the elution times of both drugs were rather slower (0.6–1.1 min for LD and 1–1.8 min for TP) and the peak areas were slightly smaller than those with the higher flow rates (0.8 and 0.9 ml min^{-1}).

4.2. Validation of the method

4.2.1. Detection limit and quantitation limit

The detection limit of the method was investigated by injecting standard solutions of TP.HCl and LD.HCl into the HPLC column. The limit detection is defined as that concentration of the analyte producing a peak height and/or peak area signal which is at least twice that of the baseline noise ($S/N = 2$) measured from peak to peak [20]. The detection limits for LD and TP were 100 ng/20 μl and 0.20 ng/20 μl, respectively. The quantitation limit is defined as analyte that concentration of the analyte producing the signal which is at least five times of the baseline noise ($S/N = 5$) [21] which were found to be 250 ng/20 μl and 0.50 ng/20 μl for LD.HCl and TP·HCl, respectively.

4.2.2. Linearity of calibration curves

The linearity of responses to TP.HCl and LD.HCl were determined. Linear calibration curves of TP·HCl over the concentration ranges of 1–10, 10–100 and 150–500 μg ml^{-1} were established. Over these three concentration ranges, linear regression analysis of the TP peak area (y) versus TP concentration (x) ($n = 5$) yielded the following equations:

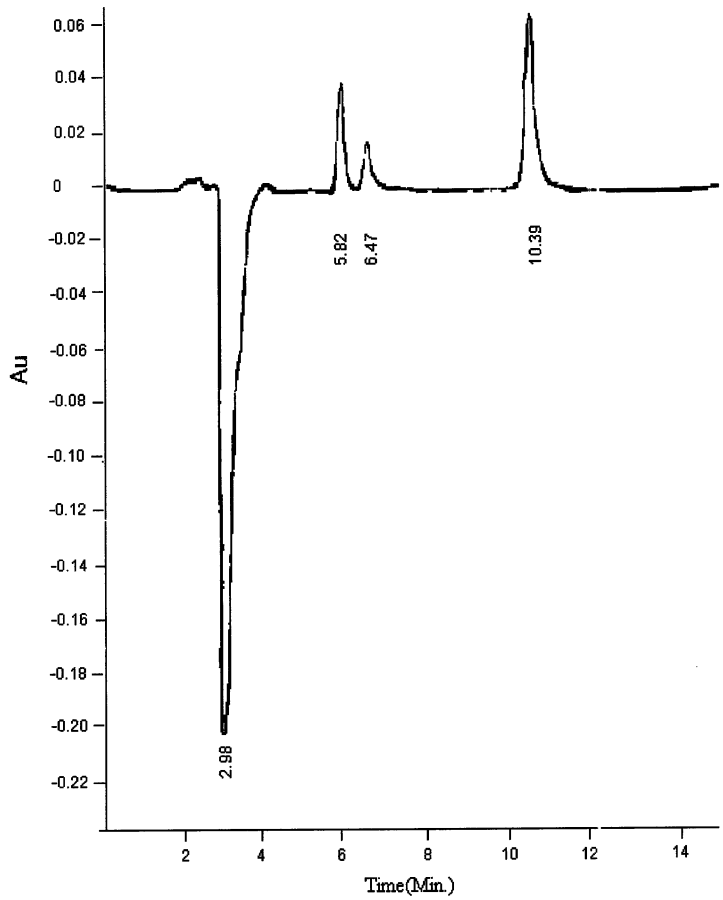

Fig. 1. Chromatogram of a standard mixture of tolperisone hydrochloride (20 μg ml^{-1}) and lidocaine hydrochloride (50 μg ml^{-1}). A 20 μl volume was injected onto Spherisorb ODS column. Mobile phase consisted of acetonitrile and water (70:30, v/v) in the presence of 5.5% triethylamine at a flow rate of 0.7 ml min^{-1}. UV detection at 254 nm.

$y = 380.77x + 1067.66$ ($r = 0.9994$)

$y = 226.81x + 2782.31$ ($r = 0.9998$) and

$y = 142.95x + 48992.40$ ($r = 0.9999$).

It was seen that different calibration slopes were found for TP which might be due to limitation of Beer's law for TP probably owing to changes in refractive index with increase in TP concentrations leading to a slight depression of absorbance. For LD·HCl, peak areas of concentrations ranging from 10 to 500 μg ml^{-1} yielded a linear calibration curve with the linear equation viz: $y = 15.48x + 221.19$ ($r = 0.990$).

4.2.3. Reproducibility and accuracy

The reproducibility and accuracy of the proposed method were verified by analysing the aliquotes of tablet extract and injection sample solutions spiked with various concentrations of standard TP.HCl or LD.HCl using the proposed procedure. The average recoveries of the spiked TP in the tablet extracts and the injection solution were found to be 99.9 and 99.7% with the relative standard deviations of 0.66 and 0.67%, respectively. With respect to the average recovery of spiked LD in the aliquate of injection liquid sample A and B spiked with various concentrations of standard LD·HCl, it was evident that the average recovery of the spiked LD were 99.3 and 99.0% with the coefficient of variations of 1.09 and 0.59%, respectively. These indicated that the recommended method was highly accurate and reproducible. In addition, the coefficient of variation ($n = 11$) for the assay of TP and LD in the samples solutions (without addition of standard) were found to be 0.63 and 0.55%, respectively.

4.2.4. Stability of drugs

Stock solutions of TP and LD were prepared in the mobile phase at concentrations of 50 μg ml^{-1} TP and 100 μg ml^{-1} LD, stored at room temperature (25–28 °C) and at 4 °C followed by assaying every hour over a period of 24 h. The peak-height of the drugs at different time intervals were compared with those of the initial ones. It was showed that no changes in both TP and LD concentration of stock solutions containing both drugs was observed over a period of a few weeks when stored at 4 °C. When the standard TP·HCl and LD·HCl stock solutions were kept at room temperature, the solutions were stable over a period of at least a week.

4.2.5. Specificity and selectivity

A representative RP-HPLC chromatogram of the tablet sample extract solution spiked containing both drugs with certain amounts of standard TP·HCl and LD·HCl is very similar to that obtained by the artificial drug sample prepared by mixing 20 μg ml^{-1} TP·HCl and 50 μg ml^{-1} standard LD·HCl (Fig. 1). It is demonstrated that no matrix components were found to interfere with the simultaneous elution of both TP and LD.

4.2.6. Application

In order to test the applicability of the proposed procedure, an artificial sample of TP.HCl and LD.HCl prepared in the mobile phase (100 mg ml^{-1} TP·HCl and 2.5 mg ml^{-1} LD·HCl) and analyzed by the proposed RP-HPLC method. The proposed method has been also applied to the analysis of one tablet formulation (containing TP·HCl 50 mg/tablet) and two injection formulations (injection A containing 100 mg ml^{-1} TP·HCl and 2.5 mg ml^{-1} LD·HCl; and injection B containing only 20 mg ml^{-1} LD·HCl) which were commercially available in Thailand under the identical experimental conditions.

It was revealed that the TP·HCl contents found in the artificial sample, TP tablets and the injection liquid A were 99.7, 98.9 and 99.0%, with the standard errors of 0.3, 1.1 and 1.0%, respectively. With respect to the LD·HCl contents in the artificial sample, injection liquid A, and injection liquid B; it was found that the LD·HCl found in samples were 99.2, 99.2 and 97.3% with the standard errors of 0.8, 0.8 and 2.7%, respectively.

In order to evaluate the proposed RP-HPLC method, comparative determinations of both drugs using standard methods of pharmacopoeias [1,2] and the published paper [9] were carried out. TP determination was performed by UV spectrophotometry based on the measurement of the maximum absorption at 257 nm of the drug in

methanolic medium [2] and the potentiometric titration with 0.1 M perchloric acid [1]. The TP·HCl contents were found to be 96.2% for the artificial sample, 97.0% for TP tablets, and 97.4% for injection liquid A, using UV spectrophotometry. When these three samples were assayed by potentiometric titration; it was found that the TP contents were 97.4, 97.2 and 97.5%, respectively.

In order to validate the proposed RP-HPLC method for the determination of LD·HCl, comparative determination of LD·HCl in the artificial sample, injection liquid A, and injection liquid B by colorimetry [9] and the standard HPLC method [22] was performed. The colorimetric method [9], involved the reaction between LD·HCl with bromocresol green in an acetate buffer (pH 4.5) solution followed by extraction with chloroform. After complete separation, the chloroform extract was taken, 0.25 g of sodium sulphate was added, and appropriate volume of the supernatant liquid was pipitted then appropriate volume of 30% (v/v) triethanolamine in 95: ethanol was added, mixed well and the absorbance of the sample solution was measured at 625 nm. The LD·HCl contents found in the drug samples were 95.2, 95.9 and 96.4% for the artificial sample, injection liquid A and B, respectively. In the standard HPLC method, separation and assay of LD·HCl was carried out on a C_{18} Column with a mobile phase consisting of four volume of the mixture of 50 ml glacial acetic acid and 930 ml water, and adjusted the pH to 3.40 with 1 N NaOH and mixed with one volume of acetonitrile with the flow rate of 1.5 ml min^{-1}. Operate at temperature between 20 and 25 °C using UV detector at 254 nm. The results were found to be 99.4, 97.5 and 98.9%, respectively. The results obtained by the proposed RP-HPLC compared with those obtained by using the standard methods using the student *t*-test. It was evident that the *t*-values for TP·HCl determination by comparison the results obtained by proposed method with those obtained by UV spectrophotometry and potentiometric titration were 1.88 and 1.93, respectively.

Similarly, for LD·HCl determination the *t*-values by comparison the results obtained by the recommended method with those obtained by colorimetry and the standard HPLC method were 1.77 and 0.04, respectively. It was seen that the experimental *t*-values for both drug assays were smaller than the theoretical valve (2.78) with a confidence interval of 95% indicating that the differences between the proposed RP-HPLC and the standards methods for both TP·HCl and LD·HCl were insignificant.

5. Conclusion

A RP-HPLC method for the simultaneous assay of TP and LD had been developed and validated. The results showed that the method is very selective no significant interfering peak were detected; accurate with the percentage recoveries of 99.7–99.9 and 99.0% for TP and LD, respectively and very reproducible with the RSD of 0.66–0.67% and 0.59–1.09% for TP and LD, respectively. The method is sensitive as little as 0.20 ng/20 μl and 100 ng/20 μl of TP·HCl and LD·HCl, respectively can be detected with the quantitation limit of 0.50 ng/20 μl and 250 ng/20 μl, respectively. The proposed RP-HPLC method has been applied to the quantitation of both drugs in pharmaceutical formulations. Results obtained by the recommended method are compared favorably with those obtained by the standard methods evaluated by using the student *t*-test. The method is simply, accurate, reproducible, rapid and suitable for routine analysis.

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References

[1] The Pharmacopoeia of Japan, 11th ed., 2 (1986) 1027.
[2] The Pharmacopoeia of Japan, 12th ed. (1991) 554.

[3] S. Liawruangrath, B. Liawruangrath, J. Pharm. Biomed. Anal. 20 (1999) 401.
[4] E. Mikami, S. Yamada, N. Noda, Y. Fuji, N. Kawamura, J. Hayakawa, Iyakuhin Kenkyu 23 (1992) 185.
[5] N. Bahata, M.C. Nahata, Aus. Pharmacother 26 (1992) 46–55.
[6] A.G. Gilman, L.S. Goodman, T.W. Rall, F. Murad, Goodman and Gilman's The Pharmacological Basis of Therapeutics, 7th ed, Macmillan, New York, 1980, p. 310, 767.
[7] L.J. Zivanovic, S. Agatonovic, M. Vasiljevic, L. Markovic, I. Nemcova, Pharmazie 49 (1994) 927.
[8] G.A. Saleh, H.F. Askal, Anal. Lett. 28 (1995) 2663.
[9] S.A. Ismaiel, D.A. Yassa, L.N. Gad-El-Rub, Pharmazie 30 (1975) 408.
[10] M.A. El-Ries, F.M. Abou Attia, F.M. Abdel-Gawad, S.M. Abu El-Wafa, J. Pharm. Biomed. Anal. 12 (1994) 1209.
[11] C. Liu, Y. Zhang, K. Jian, Fenxi Ceshi Xuebao 12 (1993) 14.
[12] M. Terrada, C. Wakasugi, Hochudoku 11 (1993) 142.
[13] J. Liu, H. Wang, Y. Shen, X. Zhang, Zhongguo Yaoxue Zazhi 29 (1994) 48.
[14] X.C. Chen, K. Chen, W. Hu, W. Yun, Sepu 11 (1993) 371.
[15] G. Achilli, G.P. Cellerins, G.V. Melzi d'Eril, F. Tagliaro, J. Chromatogr. A. 729 (1996) 273.
[16] L. Zivanovic, S. Agatonovic-Kustrin, M. Vasiljevic, I. Nemcova, J. Pharm. Biomed Anal. 14 (1996) 1229.
[17] F. Mangani, G. Luck, C. Fraudeau, E. Verette, J. Chromatogr. A. 762 (1997) 325.
[18] L. Dal Bo, P. Mazzucchelli, A. Marzo, J. Chromatogr. 854 (1999) 3.
[19] M. Abdel-Rehim, M. Bielenstein, Y. Askemark, N. Tyrefors, T. Arvidsson, J. Chromatogr. B. 741 (2000) 175.
[20] J.M Miller, Chromatography, Concepts and Contrasts, Wiley, New York, 1988, p. 96.
[21] J. Saatre Yorano, H.-J. Guchelaar, J. Chromatogr. B 720 (1998) 89.
[22] United State Pharmacopoeia, USP 24, NF 19, 2000, p. 971.