ELSEVIER

Journal of Chromatography B, 746 (2000) 133–139

JOURNAL OF CHROMATOGRAPHY B

www.elsevier.com/locate/chromb

Solid-phase extraction and liquid chromatographic quantitation of quinfamide in biological samples

J.M. Morales[a], C.H. Jung[a,*], A. Alarcón[a], A. Barreda[b]

[a]*Departmento de Farmacia, Laboratorio 112, Conjunto E de la Facultad de Química, UNAM, Ciudad Universitaria, Mexico, 04510 D.F. Mexico*

[b]*Laboratorios Searle de México, S.A. de C.V., Mexico, D.F. Mexico*

Received 20 August 1999; received in revised form 19 May 2000; accepted 23 May 2000

Abstract

This paper describes a high-performance liquid chromatographic method for the assay of quinfamide and its main metabolite, 1-(dichloroacetyl)-1,2,3,4,-tetrahydro-6-quinolinol, in plasma, urine and feces. It requires 1 ml of biological fluid, an extraction using Sep-Pack cartridges and acetonitrile for drug elution. Analysis was performed on a CN column (5 μm) using water–acetonitrile–methanol (40:50:10) as a mobile phase at 269 nm. Results showed that the assay was linear in the range between 0.08 and 2.0 μg/ml. The limit of quantitation was 0.08 μg/ml. Maximum assay coefficient of variation was 14%. Recovery obtained in plasma, urine and feces ranged from 82% to 98%.

Keywords: Quinfamide

1. Introduction

Quinfamide is a dichloroacetyl quinolinol derivative that is widely used in the treatment of intestinal amoebasis. Its behavior in the human body makes it different from the other drugs used in the same way.

Nowadays there are drugs with intraluminal action (local) and those with systemic action, however, the systemic drugs usually have more frequent side effects [1]. If we consider that recent and/or chronic infections with *Entamoeba histolytica* are usually circumscribed to the large bowel, the existence of a drug like quinfamide with intraluminal action should be of a big therapeutic interest [2]. It is well known that quinfamide immobilizes the trofozoites of the *Entamoeba histolytica*, leading to its auto destruction, which is related to its great therapeutic efficacy; however, no information was found regarding the fraction of the dose absorbed in humans.

There is only one single work reporting the measurement of quinfamide levels in biological samples using labeled quinfamide [3]. Due to the interest of knowing the levels of quinfamide in plasma, urine and feces, the purpose of this report was to develop an HPLC method that could determine and quantify quinfamide and its main metabolite, 1-(dichloroacetyl)-1,2,3,4,-tetrahydro-6-quinolinol, in these biological samples. The method was validated according to procedures and acceptance criteria based on USP XXIII guidelines [4] and

*Corresponding author.

0378-4347/00/$ – see front matter
PII: S0378-4347(00)00307-8

recommendations of Shah et al. [5], Bresolle at al [6]. and Braggio et al. [7].

2. Experimental

2.1. Materials and instrumentation

Quinfamide and its metabolite, 1-(dichloroacetyl)-1,2,3,4,-tetrahydro-6-quinolinol, were obtained from Searle de Mexico (Mexico City). Methanol, tetrahydrofurane, hexane, dichloromethane and acetonitrile were obtained from J.T. Baker Chemical Co., Sep-Pack cartridges were obtained from Waters and Supelco, the analytical columns were obtained from Waters, Zorbax and Merck, Inc. A Schimadzu UV–VIS Spectrophotometer model 1601 was employed.

The analytical chromatographic system consisted of a pump (Beckman 126 Solvent Delivery Module), an automatic injector (Beckman 507 Autosampler) with a 100 μl sample loop, a variable wavelength UV detector (Beckman 166) and a System Gold Software Ver. 5.0 (Beckman Chromatography Systems).

2.2. Analytical method development

The chromatographic conditions and the extraction technique applied to the biological samples were developed considering the chemical structure of the quinfamide and its main metabolite, 1-(dichloroacetyl)-1,2,3,4,-tetrahydro-6-quinolinol. The software employed to establish all the analytical conditions was Statgraphics©.

In order to establish the analytical conditions for the assay of quinfamide and its main metabolite, 1-(dichloroacetyl)-1,2,3,4-tetrahydro-6-quinolinol, the Design of Experiments (DOE) was employed, analyzing the following chromatographic factors:

(a) Stationary phase's polarity: in order to evaluate the response of the compounds we used the following analytical columns, C_{18}, CN and Phenyl reverse-phase columns and a CN normal-phase column.

(b) Mobile phase's polarity: in order to determine the influence of this factor in the response we selected the following solvents: water, methanol, acetonitrile and acetonitrile–tetrahydrofurane (90–10 v/v).

(c) Flow rate: 0.5 to 1.0 ml/min were evaluated.

The responses measured were: retention time (*tr*), resolution (*Rs*) and absorbance of the compounds.

The optimization of the method was performed using Simplex. The mobile phase's polarity was evaluated by using water, methanol and acetonitrile. A flow-rate of 0.7 ml/min was used in order to obtain a resolution value grater than 1.6 and a retention time lower than 15 min.

For the extraction technique, we analyzed the following factors:

(a) Stationary phase's polarity by using the following cartridges: SiOH, Phenyl RP, CN NP, C_8 RP and C_{18} RP.

(b) Extracting solvent's polarity: this factor was evaluated using acetonitrile, methanol, water, chloromethane and hexane to elute the compounds. The response measured was the percent of recovery.

2.3. Data analysis

The ratio of the peak height of quinfamide to that of the internal standard was used as the assay parameter. Peak height ratios were plotted against the theoretical concentrations.

3. Results and discussion

Considering that there are no reports related to the assay of quinfamide in biological samples by HPLC, we used DOE in order to develop and improve the analytical method [8,9]. We selected the Sep-Pack cartridges to extract the compounds considering that some studies performed in our laboratory with antihelmintic agents showed that when the Sep-Pack cartridges were used as the extraction technique, good recoveries were obtained [10–12].

Fig. 1 shows the results found when the DOE was used. In this figure we can see that the best analytical response (Resolution, *Rs*) was obtained when a polar stationary phase and a mobile phase of medium polarity was used, this zone is indicated with a dotted line at the top of the figure. From these results a reverse-phase Zorbax SB-CN 5 μm, 250×4.6 mm (I.D.) column was selected. Then the sample was scanned at different wavelengths. We found that the

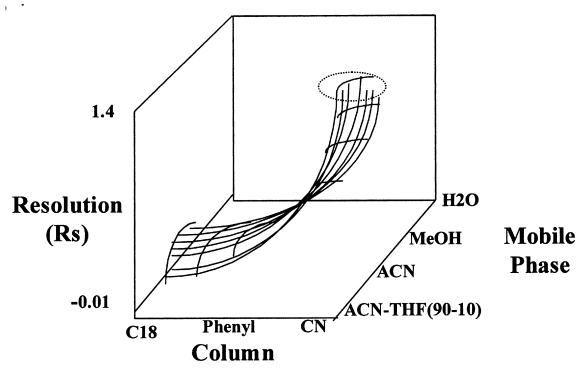

Fig. 1. Response surface plot analysis for the chromatographic conditions selection. The dotted line indicates the zone of interest.

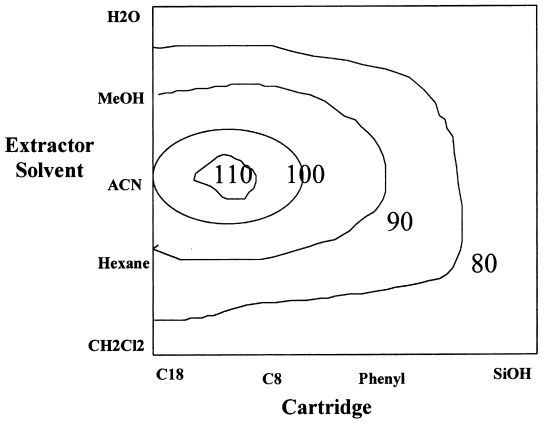

Fig. 2. Contour plot analysis for the cartridge and extractor solvent selection. The straight line indicates the recovery (%) for quinfamide and its main metabolite, 1-(dichloroacetyl)-1,2,3,4,-tetrahydro-6-quinolinol.

best analytical wavelength was 269 nm. After this, the mobile phase was optimized. Water, methanol and acetonitrile were selected from the results of the previous DOE. A Simplex design was employed in order to obtain a resolution value greater than 1.6. The linear model for the relationship between mobile phase and resolution (*Rs*) was:

$$Rs = 3.51\ H_2O + 1.16\ \text{Methanol} + 0.77\ \text{Acetonitrile}$$

With these results the final composition of the mobile phase was: water–methanol–acetonitrile (40–50–10 v/v) at a flow-rate of 0.7 ml/min.

When the DOE was used to determine the extraction technique we found that 1.0 ml of the sample must be delivered into the Sep-Pack C_{18} cartridge, washed with 12 ml of water, and then eluted with 1.0 ml of acetonitrile. The cartridge activation was made as directed by the manufacturer. The results are shown in Fig. 2. In this case the straight line indicates the recovery (%) of the compounds and it can be seen that there were various combinations to obtain the response measured. We selected a non-polar cartridge (C_{18}) and an extracting solvent of medium polarity, acetonitrile, to elute the compounds.

Using the chromatographic conditions and the extraction technique described above, we obtained a resolution value greater than 2.0, a retention time equal to 13 min and a recovery (%) equal to 82–100% in the range of concentrations assayed.

3.1. Chromatographic conditions

From the results of the DOE the final chromatographic conditions were: a mobile phase water–methanol–acetonitrile (40–50–10 v/v) delivered at 0.7 ml min^{-1}. It was filtered through a 0.22 μm Millipore membrane, degassed and passed through a reverse-phase Zorbax SB-CN 250×4.6 mm (I.D.) column. The eluent was monitored by UV detection at 269 nm, the sensitivity of the detector was 0.01 AUFS. The chromatographic analysis was performed at room temperature.

3.2. Sample preparation

From the results obtained by using the DOE, the final extraction technique was: 1 ml of the biological sample (for the feces 10 mg of lyophilized feces were dissolved in 10 ml of acetonitrile) was spiked with the compounds in the range of 0.08 to 2.0 μg/ml and passed through a Sep-Pack C_{18} cartridge. After the spiked sample had been passed through the cartridge it was washed with 12 ml of HPLC water, the compounds were then eluted with 1.0 ml of acetonitrile. The eluting solvent was mixed by vortex and injected directly (20 μl) into the HPLC system.

The Sep-Pack cartridge was activated by the elution of 2 ml of methanol, 1 ml of the methanolic

standard (diazepam, 5 μg/ml) and 2 ml of HPLC water. A small volume of water was left in the cartridge to prevent drying before loading the sample.

Typical chromatograms of the extracted plasma, urine and feces are shown in Fig. 3. The retention times for the quinfamide's metabolite, the internal standard and quinfamide were 6.5, 8.5 and 9.7 min respectively. No interfering peaks occurred at these times. Any endogenous contaminants remaining in the extracts were eluted before the quinfamide's metabolite, so that the sample could be injected after 13 min.

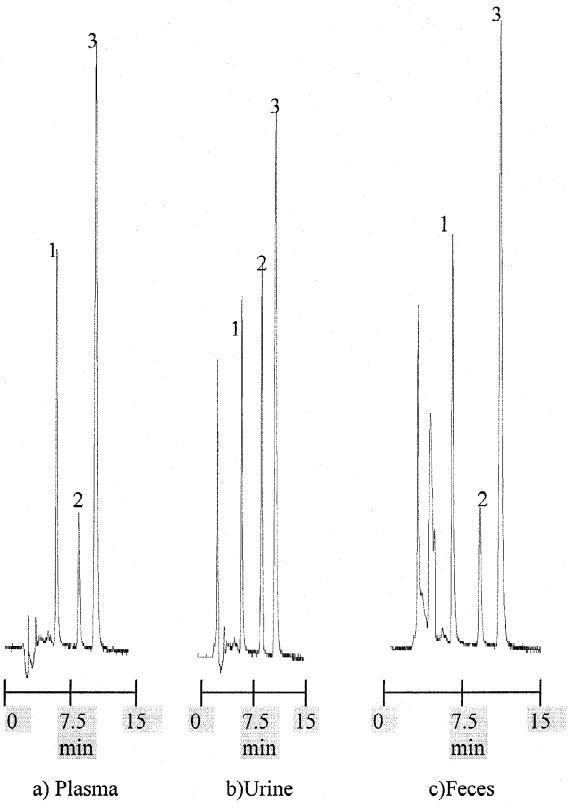

Fig. 3. Typical chromatograms obtained from a) plasma, b) urine and c) feces; spiked with 2.0 μg/ml of quinfamide, 2.0 μg/ml of metabolite, 1-(dichloroacetyl)-1,2,3,4,-tetrahydro-6-quinolinol and 5.0 μg/ml of Diazepam. The peaks are (1) metabolite, 1-(dichloroacetyl)-1,2,3,4,-tetrahydro-6-quinolinol, $tr = 6.38$ min, (2) Diazepam, internal standard, $tr = 9.08$ min, (3) quinfamide, $tr = 11.13$ min.

3.3. Validation

A linear relationship ($r = 0.998$) was found when the ratio of the peak-height of quinfamide or its metabolite, 1-(dichloroacetyl)-1,2,3,4-tetrahydro-6-quinolinol, to the peak height of the internal standard were plotted against various concentrations ranging from 0.08 to 2.0 μg/ml. The equation by the least-squares method was $Y = 2.2768X - 0.0675$ for quinfamide and $Y = 1.6309X + 0.0344$ for quinfamide's metabolite in plasma and $Y = 2.6063X + 0.0778$ for quinfamide and $Y = 1.30901X + 0.0131$ for quinfamide's metabolite in urine.

The intra-day precision and accuracy of the method was evaluated by analyzing on the same day three replicates of spiked samples at each of all concentrations. The results are shown in Tables 1 and 2.

Inter-day precision and accuracy were assessed by performing analysis samples at same concentrations. The procedure was repeated on different days. The results are shown in Tables 1 and 2.

The recovery of quinfamide and its metabolite, determined by comparing peak height from plasma or urine, spiked with known amounts of the drugs, ranging from 0.08 to 2.0 μg/ml and processed according to the described method versus the same concentrations of the compound prepared in methanol and injected directly onto the analytical column, showed that the recoveries of quinfamide and its metabolite from plasma and urine were similar and ranged from 82 to 100%.

The limit of quantitation (LOQ) was determined from the peak and the standard deviation of the noise level, S_N. The limit of quantitation was defined as the

Table 1
Accuracy and precision for quinfamide (Qfda) in plasma

Conc. (μg/ml)	Accuracy Qfda (%)	Accuracy metabolite (%)	C.V. Qfda (%)	C.V. metabolite (%)
2	98	97	9.9	5.6
1	82	98	4.5	8.2
0.6	89	99	5.1	5.6
0.4	87	99	6.5	1.9
0.2	92	101	5.7	3.9
0.1	96	97	4.2	9.8
0.08	96	98	7.1	2.3

Table 2
Accuracy and precision for quinfamide (Qfda) in urine

Conc. (μg/ml)	Accuracy Qfda (%)	Accuracy metabolite (%)	C.V. Qfda (%)	C.V. metabolite (%)
2	98	99	2.0	4.9
1	97	98	7.6	6.1
0.6	95	97	3.6	2.7
0.4	96	96	4.1	4.1
0.2	96	98	4.0	5.6
0.1	98	99	1.7	1.4
0.08	98	99	7.8	8.2

sample concentration resulting in a peak height of 10 times S_N. The limit of detection (LOD) was considered as the sample concentration resulting in a peak height of 3 times S_N. The LOD was 0.05 μg/ml and LOQ for quinfamide and its metabolite was 0.08 μg/ml.

The short-term stability assessed at 0 and 24 h in acetonitrilic solution at ordinary laboratory conditions (room temperature and daylight exposure) showed that quinfamide and its metabolite are stable at this condition.

The long-term stability of quinfamide in frozen human plasma and urine (−17°C) was determined by periodic analysis over a week. Samples were analyzed immediately after preparation and after storage. Prior to their analysis, samples were brought to room temperature and vortex-mixed well, showing that quinfamide is quickly hydrolyzed in human plasma and the stability in urine was 1 week.

The stability of quinfamide at different pH values was evaluated as well, from pH 2 to pH 10. The test was carried out during 4 h, showing that at pH values greater than pH 9.0 quinfamide is rapidly hydrolyzed (less than 2 h).

3.4. Hydrolysis of quinfamide in plasma

In order to determine the effect of the plasma components over the hydrolysis of quinfamide, samples of 5 ml of human plasma were spiked with three different concentrations of quinfamide: 1.0 μg/ml, 1.5 μg/ml and 2.0μg/ml (triplicates of each one were assayed); and maintained at 37°C±0.5 without agitation in open tubes in a water bath, following the methodology described by Hung et al. [13,14] and Hansen et al. [15], for similar compounds. The methodology was chosen due to the facilities of our laboratory. Samples of 1.0 ml of plasma were drawn at 0, 0.25, 0.5, 0.75, 1.0, 1.5 and 2.0 h (one tube for each sampling time), and quinfamide and its metabolite were determined by the method described above. The remained quinfamide concentrations after drug incubation in human plasma for different time intervals are plotted in Fig. 4 where it can be seen that hydrolysis rates were linearly dependent on drug concentrations. A good relationship for each set of data ($r^2 > 0.99$) was achieved. The apparent half life for quinfamide hydrolysis in human plasma was 7.4 min. It was found that between 71% and 92% of the original quinfamide in human plasma was converted into the metabolite.

3.5. Application of the method

Following the optimization of the chromatographic conditions and validation of the assay, the method was used in the analysis of samples from two healthy volunteers, who received 300 mg of quinfamide in a single oral dose. Blood samples were taken into heparinized tubes from 0 to 24 h, plasma was separated by centrifugation and immediately assayed for quinfamide and its metabolite. Urine samples were collected in tubes containing 0.25 ml of toluene from 0 to 48 h and immediately assayed for quinfamide and its metabolite and feces samples were collected from 0 to 48 h. Results showed, as expected, that quinfamide was not detected at any sampling time, only its metabolite, 1-(dichloroacetyl)-1,2,3,4,-tetrahydro-6-quinolinol, was detected from 15 min to 6 h post-dosing but could not be quantified. This does not occur with animal models, where the authors found high levels of unaltered quinfamide in plasma (2.3 μg/ml) at 7 h post-dosing [3]. In urine quinfamide could not be detected, only the metabolite, 1-(dichloroacetyl)-1,2,3,4,-tetrahydro-6-quinolinol, could be quantified from 0.5 to 24 h post-dosing. Fig. 5 shows the obtained results. It can be seen that less than 2% was excreted in this sample. Our results indicate a low absorption of quinfamide through the gastrointestinal tract. In feces 72.02% of the given dose was excreted

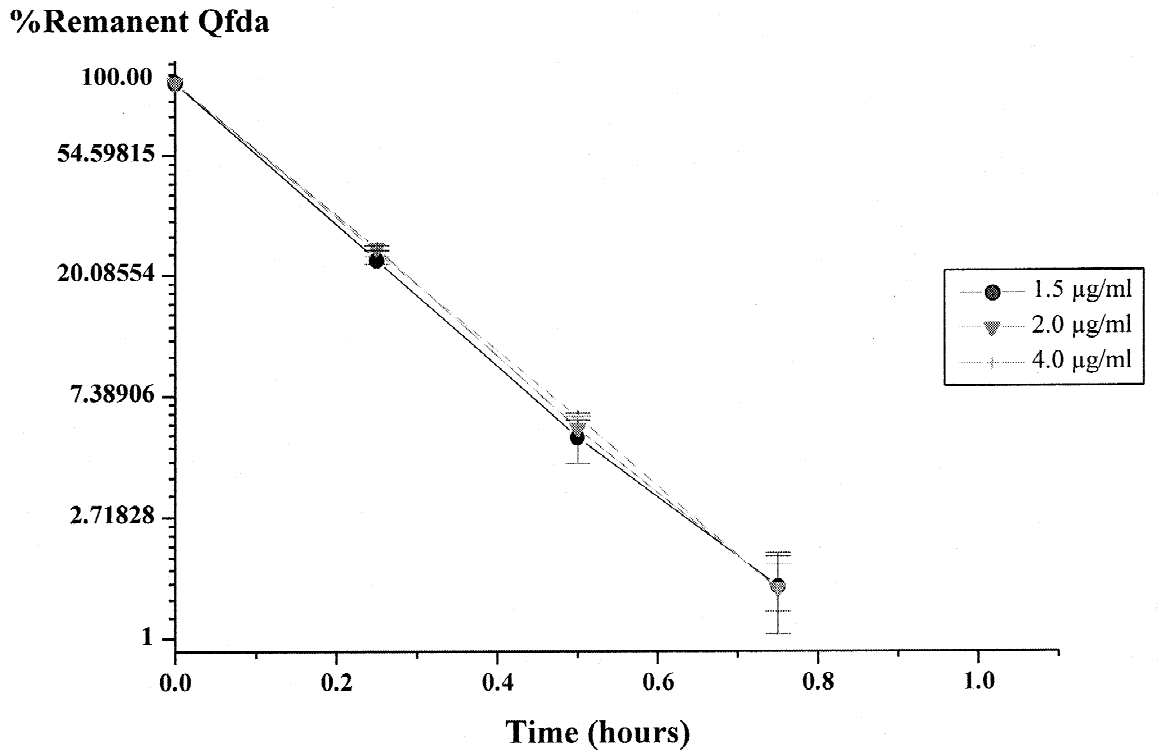

Fig. 4. Kinetics of quinfamide hydrolysis in human plasma. Percentage of amount non-hydrolyzed in plasma: $Y = -5.6263X + 4.5906$; $r^2 = 0.9936$.

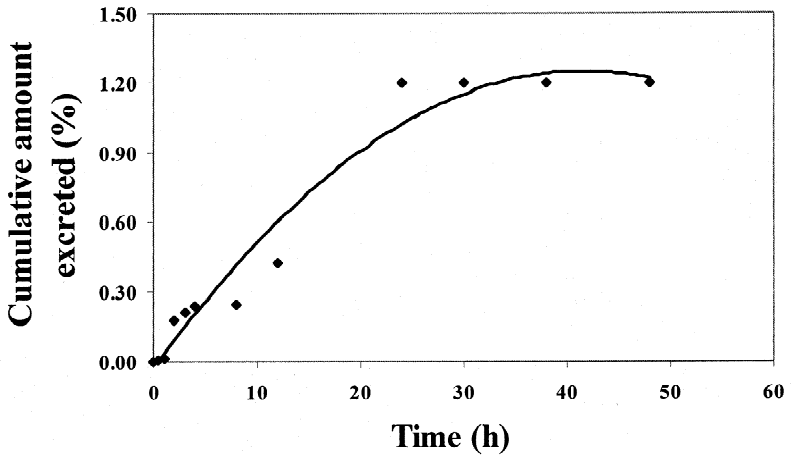

Fig. 5. Cumulative amount excreted of quinfamide's metabolite, 1-(dichloroacetyl)-1,2,3,4-tetrahydro-6-quinolinol in urine.

as unchanged drug and 16.53% was eliminated as the metabolite.

4. Conclusions

A sensitive HPLC assay was developed for the evaluation of quinfamide and its metabolite using 1 ml of biological fluid and UV detection at 269 nm. Quinfamide, its metabolite (1-(dichloroacetyl)-1,2,3,4-tetrahydro-6-quinolinol) and the internal standard, diazepam, yield a high response in the chromatographic system. This HPLC method is simple, sensitive and fast. It also can be used as a reliable assay in the study of the biopharmaceutics of the drug.

Acknowledgements

The authors are indebted to Searle de Mexico S.A. de C.V. and to M. en C. Juan Manuel Rodríguez for the support to this work.

References

[1] P. Fernandes, L. Brunet de Sá, C.C. de Oliveira, A Folha Med. 92 (1986) 8.
[2] C.P. Robinson, Drugs of Today 20 (1984) 479–482.
[3] J.F. Baker, P.E. O'Melia, D.P. Benziger, J. Edison, Arth. Int. Pharmacodyn. 258 (1982) 29–38.
[4] US Pharmacopoeia XXIII, The United States Pharmacopeial Convention, Rockville, MD, 1994, p. 1929
[5] V.P. Shah, K.K. Midha, S. Dighe, I.J. McGilvery, J.P. Skelly, A. Yacobi, T. Layloff, C.T. Viswanathan, C.E. Cook, R.D. McDowall, K.A. Pittman, S. Spector, J. Pharm. Sci. 81 (1992) 309.
[6] F. Bresolle, M. Bromet-Petit, M. Audran, J. Chromatogr. B 686 (1996) 3.
[7] S. Braggio, R.J. Barnaby, P. Grossi, M. Cugola, J. Pharm. Biomed. Anal. 14 (1996) 375.
[8] J.A. Lewis, L.R. Snyder, J.W. Dolan, J. Chromatogr. A 721 (1996) 15–29.
[9] K.L. Lee, P.R. McAllister, Drug Develop. and Ind. Pharm. 22 (1996) 891–908.
[10] E.D.F. Gonzalez, O.C. Morano, R.M. Sánchez, M.J. Sotelo, C.H. Jung, J. Chromatogr. 613 (1993) 174–178.
[11] M. Hurtado, M.T. Medina, M.J. Sotelo, C.H. Jung, J. Chromatogr. (Biomed. Appl.) 494 (1989) 403–407.
[12] L.M.R. Del Rivero, C.H. Jung, R. Castillo, A. Hernández-Campos, J. Chromatogr. B 712 (1998) 237–241.
[13] H.P. Hung, J.W. Ayres, J. Pharm. Sci. 77 (1988) 104–109.
[14] H.P. Hung, J.W. Ayres, J. Pharm. Sci. 77 (1988) 285–298.
[15] K.T. Hang, P. Faarup, H. Bundgaard, J. Pharm. Sci. 8 (1991) 793–798.