Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Pharmaceutical and Biomedical Analysis 37 (2005) 621–626
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Short communication

High-performance liquid chromatographic determination of sumatriptan after in vitro transdermal diffusion studies

A. Femenía-Font[a], V. Merino[b], V. Rodilla[a], A. López-Castellano[a,*]

[a] *Departamento de Fisiología, Farmacología y Toxicología, Facultad de Ciencias Experimentales y de la Salud, Universidad Cardenal Herrera-CEU, 46113 Moncada, Spain*
[b] *Departament de Farmàcia i Tecnologia Farmacèutica, Facultat de Farmàcia, Universitat de València, 46100 Burjassot, Spain*

Received 6 May 2004; received in revised form 10 November 2004; accepted 11 November 2004
Available online 19 January 2005

Abstract

A simple, accurate, precise and rapid HPLC method with UV detection has been validated in order to determine the in vitro transdermal absorption of sumatriptan succinate. The HPLC method is a modification of that described by Nozal et al. [M.J. Nozal, J.L. Bernal, L. Toribio, M.T. Martín, F.J. Diez, J. Pharm. Biomed. Anal. 30 (2002) 285–291]. Separation was carried out on a 250 mm Kromasil C18 column at room temperature. The detector response, at 282.7 nm, was found to be linear in a concentration range between 0.145 and 145 μM. The limit of detection (LOD) was 0.019 μM and the limit of quantification (LOQ) was 0.145 μM.

Keywords: Sumatriptan succinate; Reverse-phase chromatography; HPLC; UV detection; Transdermal diffusion; Percutaneous absorption

1. Introduction

Sumatriptan succinate is a drug used in adults for the treatment of acute migraine attacks with or without aura. This compound activates a vascular 5-hydroxytryptamine receptor subtype, present on the human basilar artery and in the vasculature of human *dura mater* and thus, mediates vasoconstriction. This action in humans correlates with the relief of migraine headache. In addition to vasoconstriction, experimental data from animal studies show that sumatriptan also activates 5-HT1 receptors on peripheral terminals of the trigeminal nerve innervating cranial blood vessels. Both actions contribute to the antimigrainous effect of sumatriptan in humans.

The absolute bioavailability of sumatriptan is approximately 15, 14 and 96% after intranasal, oral and subcutaneous administration, respectively. The lower bioavailabilities are primarily due to pre-systemic metabolism and partly due to incomplete absorption. Due to the low bioavailability after oral and intranasal administration of the drug and the inconveniences related to the parenteral administration, we aim to develop a new pharmaceutical form to avoid these problems. Thus, transdermal administration for systemic distribution of drugs is considered to be a very good alternative to administration by more conventional routes such as injection or oral administration. In order to analyze if sumatriptan is a good candidate to be included in a transdermal delivery system, percutaneous diffusion experiments of this compound need to be performed.

Previous analytical methods have been described for the determination of sumatriptan succinate. They include HPLC with UV/Visible detection [1–7], MS [8–13], electrochemical detection [14,15] and capillary electrophoresis [16,17]. Several of the methods and infrastructure are not available to small laboratories. Other methods rely on complex sample preparation. Some of the methods we have tried had far too

* Corresponding author. Tel.: +34 961369000x1139; fax: +34 961395272.
E-mail address: alopez@uch.ceu.es (A. López-Castellano).

0731-7085/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2004.11.029

many interferences from endogenous compounds present in the skin, and thus were not suitable for the purpose of our research.

In this paper, we report the validation of a rapid and sensitive HPLC method with ultraviolet detection for the quantitative determination of sumatriptan succinate after in vitro transdermal permeation studies.

2. Materials and methods

2.1. Materials

The sumatriptan succinate [3-[2-(dimethylamino) ethyl]-*N*-Methyl indole-5 methane-sulphonamide succinate (1:1)] (Eur. Ph. monograph 1573) certified standard (98.6%, w/w) was generously gifted by GlaxoWellcome (Aranda de Duero, Spain). Acetonitrile was obtained from Carlo Herba Reagenti s.r.l. Montedison Group, Rodano (MI, Italy). Ammonium phosphate monobasic (98–102%, w/w) and ortophosphoric acid (85–88%, v/v) were purchased from Panreac Montplet & Esteban S.A. (Barcelona, Spain). Hepes (*N*-[2-Hydroxyethyl]piperazine-*N*′-[2-ethanesulfonic acid]) was obtained from Sigma-Aldrich CO. (St. Louis, MO, USA). NaCl was purchased from Mallinckrodt Baker B.V. (Deventer, Holland). All reagents were of analytical or HPLC grade. Ultrapure water was obtained with a Barnstead NANOpure system (Barnstead International, Boston, MA, USA).

2.2. Preparation of standard solutions

A 14.5 mM sumatriptan succinate stock solution was prepared in isotonic buffer [NaCl (150 mM)-Hepes (20 mM), pH 7.4].

Seven standard solutions (145, 72.5, 14.5, 7.25, 1.45, 0.725 and 0.145 μM) were prepared by further dilution from the stock solution in isotonic buffer. These standard fresh solutions were used for the calibration curves.

2.3. Instrumentation and chromatographic conditions

The apparatus used for HPLC analysis was a Waters system equipped with a quaternary pump and a diode-array detector. Computerized data acquisition and treatment were performed with the Millennium Chromatography Software. Chromatographic conditions applied (flow rate and mobile phase) are described in a method previously validated for sumatriptan succinate [1]. Separation was carried out at ambient temperature on a 250 mm × 4.0 mm, reverse-phase column packed with 5 μm C18 silica particles (Kromasil C18). Absorbance was measured at 282.7 nm. Aliquots of 50 μl were injected.

2.4. Validation

The validation of the analytical method was carried out with six different concentrations of sumatriptan succinate (0.145, 0.725, 1.45, 14.5, 72.5 and 145 μM). Five aliquots from each solution were assayed to determine within-day reproducibility. Aliquots from fresh solutions were analyzed during different days to determine between-day reproducibility.

Calibration curves were obtained by the least square linear regression analysis of the peak area obtained as a function of the concentration of sumatriptan succinate. An average curve was built using all the data from all the calibration curves obtained.

The specificity of the method was investigated by analyzing 10 blank samples. Their composition was the same isotonic buffer used to prepare the standard solutions for calibration [NaCl (150 mM)-Hepes (20 mM), pH 7.4].

The linearity of the curves was tested by statistical comparison among the slopes, the intercepts of calibration curves with zero and the correlation coefficients with 1. Accuracy of the method was defined as the relative error of known concentration solutions. To be acceptable, measures should be within ±10% for all concentrations [18–20]. Precision of the method was tested as within-day and between-day reproducibilities of the assay. Precision of the method was expressed as the residual standard deviation (R.S.D.) of replicate measurements. To be acceptable, the R.S.D. should be lower than 10% at all concentrations analyzed [18–20].

The limit of detection (LOD) was determined as the sample concentration, resulting in a peak three-times higher than background. LOD was determined by the analysis of background in 10 blank samples [18–20].

The limit of quantification (LOQ) was defined as the lowest concentration, which can be determined with an accuracy and precision below 20% [18–20].

2.5. Stability

Five groups of 0.145, 0.725, 1.45, 14.5, 72.5 and 145 μM sumatriptan succinate saline-buffered solutions were stored in different conditions to determine the stability of the compound in aqueous solution.

The first group was prepared daily by dilution of a 14.5 mM sumatriptan succinate solution to obtain solutions at the appropriate concentrations. Solutions, protected from light, were injected at once and quantified.

The rest of the groups were prepared identically but the dilutions were stored under different conditions.

The second group was stored at 4 °C in darkness and was assayed to determine the residual concentration 3, 4, 8, 9 and 15 days after preparation.

The third group was stored at −20 °C in darkness and was also analyzed to determine the existing concentration 8 and 15 days after preparation.

The fourth group was stored at ambient temperature exposed to light and was also assayed to determine the residual concentration 3 and 9 days after preparation.

The fifth group was stored at ambient temperature in darkness and was also assayed to determine the residual concentration 3 and 9 days after preparation.

2.6. Application of the method to in vitro transdermal permeation of sumatriptan succinate

The validated HPLC method was used to measure sumatriptan succinate in samples obtained from in vitro transdermal permeation experiments. These experiments were performed using standard diffusion cells (Franz type) and skin from pig ear as the membrane.

Pigs' ears were collected immediately after animal death from a local slaughterhouse (Carnes Estellés, Paterna, Spain). The skin from the outer face was excised from the ear using a surgical blade. Afterwards it was sectioned using an electric dermatome to a thickness of 600 μm. The thin sections of skin obtained with the dermatome were packed separately and stored until use at −20 °C.

For the diffusion assays the skin was placed on the standard diffusion cells with the stratum corneum facing the donor compartment to give an effective surface area available for diffusion of 0.9 cm^2. The experiments were carried out at room temperature.

Three millilitres of a sumatriptan succinate solution (14.5 mM) prepared in an isotonic buffer NaCl (150 mM)-Hepes (20 mM) pH 7.4 were placed in the donor compartment. The receptor compartment was filled with 8 ml of the same isotonic buffer (pH 7.4) and stirred by a rotating Teflon-coated magnet placed inside the cell.

One-ml samples from the receptor chamber were taken hourly during 8 h for detection and quantification of sumatriptan. The sample volume withdrawn was replaced with the same buffer.

At the end of the in vitro transdermal diffusion experiments the amount of sumatriptan retained in the skin was extracted by shaking the skin during 12 h with 3 ml of the isotonic buffer (pH 7.4) and determined by the HPLC method.

The transdermal flux (J) of sumatriptan was estimated from the slope of the linear region (steady-state portion) of the plot of the accumulated amount of sumatriptan (nmol/cm^2) against time (hours).

3. Results and discussion

In this paper, a specific and sensitive HPLC method to determine sumatriptan succinate is described. This method enables us to determine sumatriptan succinate in samples obtained from in vitro transdermal permeation experiments. The methods used for this purpose need to be highly specific, as these kinds of samples usually contain endogenous compounds released from the skin. Furthermore, the method also needs to have enough sensitivity, due to the frequently low concentration of the drug in the samples collected in these experiments.

HPLC methods with UV/Visible detection for the determination of sumatriptan have been previously described [1–7]. We have adapted the method developed by Nozal et al. [1] to obtain one which will enable us to analyze sumatriptan samples directly from transdermal experiments without sample preparation or drug extraction. The method developed by Nozal and co-workers was devised to quantify sumatriptan succinate residues in swabs collected from manufacturing equipment surfaces in order to control a cleaning procedure. To obtain the best resolution of the sumatriptan peak we have used a 250 mm (Kromasil C18). Since sumatriptan succinate spectra showed two maximum peaks of absorbance at 227.4 and 282.7 nm, we have selected 282.7 nm for the detection because there were too many interferences from skin at the retention time of sumatriptan at 227.4 nm. The wavelength selected had enough sensitivity and specificity to analyze sumatriptan in the range of concentrations of the samples collected in transdermal diffusion experiments. Under these conditions, we obtained a good separation between sumatriptan and other endogenous compounds from skin present in our samples.

Representative chromatograms from standards and samples obtained from transdermal diffusion studies are shown in Figs. 1 and 2, respectively.

The method was validated using the following criteria: specificity, linearity, precision, accuracy, LOD, LOQ, stability and applicability to in vitro transdermal diffusion studies.

Ten blank samples were analyzed to investigate the specificity of the method. There was no interference found at the retention time of sumatriptan succinate.

The method exhibited linearity between the response (y) and the corresponding concentration of sumatriptan succinate (x) over the 0.145–145 μM range of concentrations assayed. An average calibration curve was built (y = 14108.878 (±16.557) x + 303.008 (±1019.616)). The results of the least square linear regression analysis showed a correlation coefficient ≥0.999. The slope of the calibration curve was statistically different from zero, and the intercept was not statistically different from zero.

For each concentration of calibration standard the relative error values were computed (Table 1). Accuracy was within acceptable limits: the values obtained for all concentrations were below 10%, except for the lowest one (0.145 μM − 11.840%).

The results of the between-day and within-day precision determined are shown in Table 2 and were below 10% in all cases.

The limit of detection and the limit of quantification of sumatriptan were 0.019 and 0.145 μM, respectively. The LOQs reported by other authors were smaller due to the detector employed. For example the LOQs for the methods with MS-MS detection were 0.0006 μM [10], 0.006 μM [11], 0.001 μM [12] and 0.002 μM [13], respectively. The LOQ with electrochemical detection was 0.003 μM in plasma and 0.0006 μM in urine [14]. Dunne and co-workers also using an electrochemical detector estimated

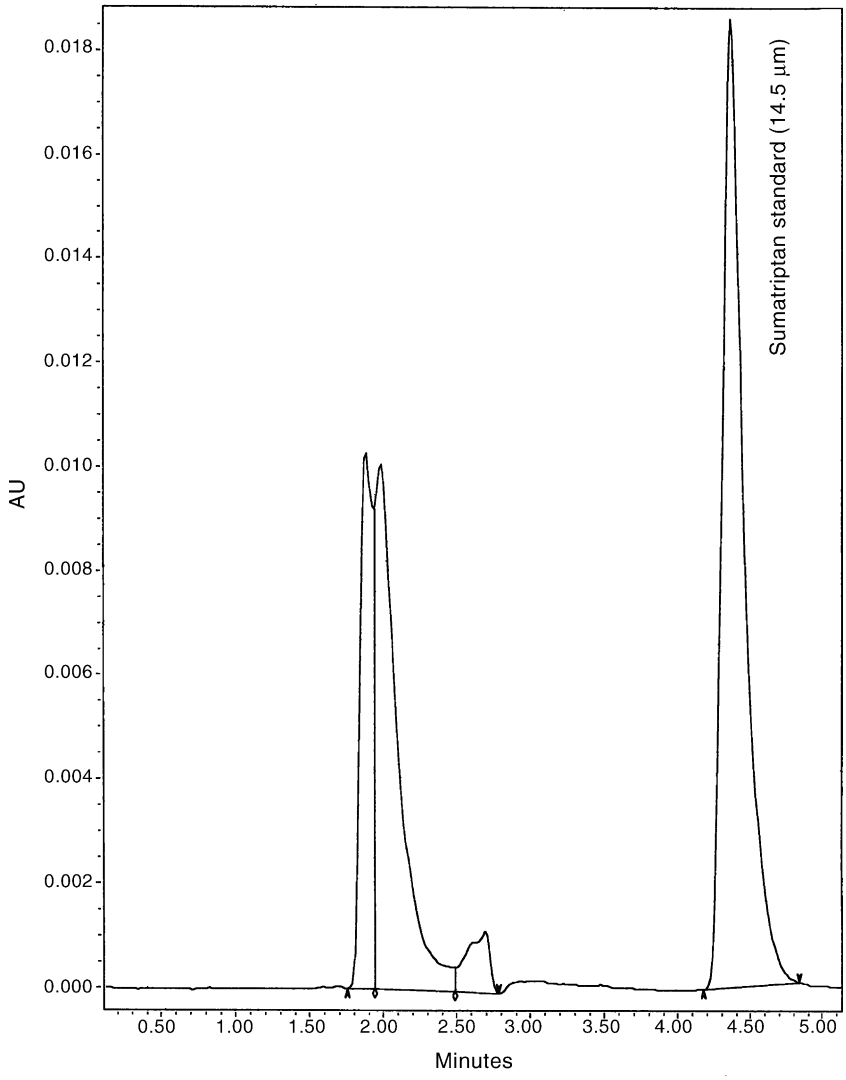

Fig. 1. Chromatogram of 14.5 μM sumatriptan succinate standard solution.

the LOD as 0.003 μM also with electrochemical detector [15].

The limits reported using UV detection were also lower than ours (LOD = 0.009 μM and LOQ = 0.026 μM) [1]. It is important to note that in the work of Nozal and co-workers sumatriptan succinate was measured at 228 nm and the absorbance at this wavelength was higher than 282.7 nm. As stated earlier we carried out our detection and quantification at 282.7 nm because at 228 nm there were interferences from endogenous compounds from skin. Hence, we have compromised between a lower sensitivity but a better resolution and a higher specificity.

Table 1
Accuracy (n = 5) of the HPLC method for determining sumatriptan succinate concentrations in saline-buffered samples, pH 7.4

Real concentration (μM)	Concentration found (mean ± S.D.) (μM)	Accuracy (%) (n = 5)
0.145	0.128 ± 0.003	11.840
0.725	0.733 ± 0.035	1.121
1.450	1.481 ± 0.021	2.129
7.250	7.383 ± 0.303	1.832
14.500	14.816 ± 0.304	2.176
72.500	76.336 ± 1.847	5.291
145.000	145.381 ± 4.503	0.262

Stability was investigated using five different groups stored under different conditions. The results obtained are shown in Table 3. The group stored exposed to the light and at ambient temperature showed the biggest degradation of the sumatriptan succinate in saline-buffered solution (pH 7.4). On the other hand, the compound stored at −20 °C (darkness) suffered no detectable degradation since it kept the same concentration during the 15 days of the study. The groups stored at ambient temperature and at 4 °C, both in darkness, showed an increasing degradation of the compound during the time of the study. Taking into account these results, and to avoid a significant degradation, sumatriptan succinate must be analyzed immediately. If not, it should be stored at −20 °C in

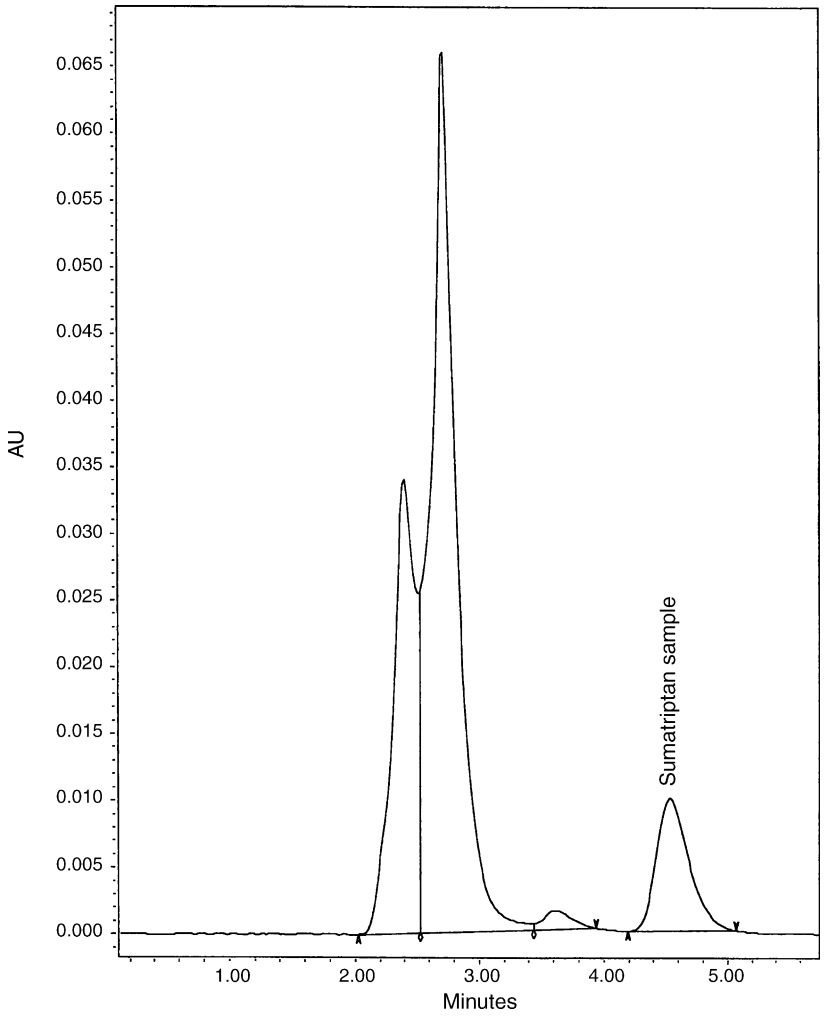

Fig. 2. Chromatogram of a sumatriptan succinate sample, obtained from in vitro transdermal permeation experiments.

Table 2
Between- and within-day variabilities of the HPLC method for determining sumatriptan succinate concentrations in saline-buffered samples, pH 7.4

Real concentration (μM)	Between-day variability ($n = 10$)		Within-day variability ($n = 5$)	
	Concentration found (μM)	R.S.D. (%)	Concentration found (μM)	R.S.D. (%)
0.145	0.123	4.373	0.128	2.490
0.725	0.720	6.362	0.733	4.811
1.450	1.413	0.929	1.481	1.408
7.250	7.309	3.962	7.383	4.098
14.500	14.350	2.895	14.816	2.051
72.500	72.806	5.783	76.336	2.420
145.000	144.872	2.378	145.381	3.097

Table 3
Stability results obtained for sumatriptan succinate in standard solutions

	Recovery (%) ± S.D. (%) ($n = 5$)				
	Day 3	Day 4	Day 8	Day 9	Day 15
Darkness (ambient temperature)	94.01 ± 1.47			75.96 ± 3.14	
Light (ambient temperature)	93.32 ± 2.82			68.13 ± 1.78	
Darkness (4 °C)	95.63 ± 4.22	90.42 ± 4.84	87.07 ± 4.00	83.32 ± 6.84	79.18 ± 8.81
Darkness (−20 °C)			101.28 ± 1.55		103.53 ± 2.60

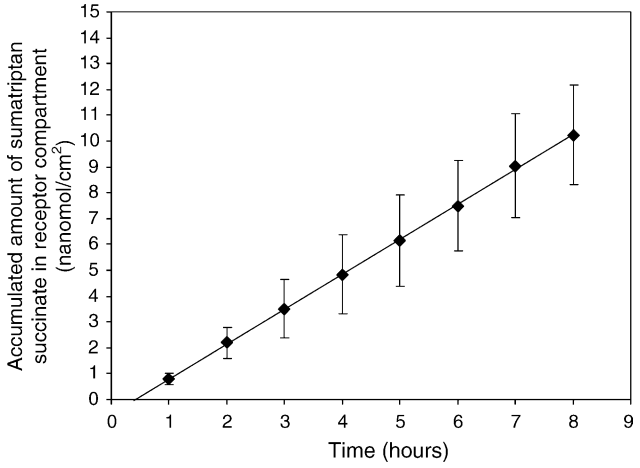

Fig. 3. Accumulated amount of sumatriptan succinate found in the receptor chamber versus time. Error bars represent standard deviation.

darkness, as in these conditions there is no degradation of the compound for at least 15 days.

The potential use of this method has been demonstrated by the study of in vitro transdermal diffusion of sumatriptan succinate. The accumulated amounts of sumatriptan in receptor compartments (nmol/cm^2) were plotted against time (hours) (see Fig. 3). The transdermal flux, estimated from the slope of the linear region (steady-state portion) of the plot, was found to be 1.322 ± 0.775 nmol/(cm^2 h) (mean $\pm$ S.D.; $n = 10$). The amount of sumatriptan retained in skin was 0.113 ± 0.043 μmol/cm^2 (mean $\pm$ S.D.; $n = 10$). Although the sumatriptan permeation flux values and its accumulated amount in the receptor compartment are low, the amount of the compound in all biological samples can be calculated properly.

4. Conclusions

A simple chromatographic method has been validated for the rapid and precise determination of sumatriptan succinate. The results of the validation of the HPLC method are considered to be satisfactory since the specificity, limit of detection and quantification, the accuracy and precision of the method allow quantifying the amount of sumatriptan succinate contained in the samples obtained from the in vitro transdermal permeation experiments.

Acknowledgements

The authors wish to thank *Agència Valenciana de Ciència i Tecnologia (Generalitat Valenciana) (CTIDIA/2002/61)*, the *Fondo de Investigaciones Sanitarias (Ministerio de Sanidad y Consumo) (PI030616)* and the *Universidad Cardenal Herrera-CEU (PRUCH-03/39)* for their financial support. They also thank Glaxo Wellcome, S.A. for their generous gift of sumatriptan succinate.

References

[1] M.J. Nozal, J.L. Bernal, L. Toribio, M.T. Martín, F.J. Diez, J. Pharm. Biomed. Anal. 30 (2002) 285–291.
[2] S. Shigh, R. Jain, Indian Drugs 34 (1997) 527–531.
[3] A.B. Avadhanulu, J.S. Srinivas, Y. Anjaneyulu, Indian Drugs 33 (1996) 334–337.
[4] D.N. Tipre, P.R. Vavia, Indian Drugs 36 (1999) 501–505.
[5] A.B. Avadhanulu, K.S. Srinivas, Y. Anjaneyulu, Indian Drugs 33 (1996) 36–40.
[6] F. Franklin, J. Odontiadis, E.M. Clement, J. Chromatogr. B 2 (1996) 416–420.
[7] V.A. Shirsat, S.Y. Gabhe, S.G. Deshpande, Indian Drugs 35 (1998) 404–407.
[8] B.D. Dulery, M.A. Petty, J. Schoun, M. David, N.D. Huebert, J. Pharm. Biomed. Anal. 15 (1997) 1009–1020.
[9] K. Vishwanathan, M.G. Bartlett, J.T. Stewart, Rapid Commun. Mass Spectrom. 14 (2000) 168–172.
[10] K.N. Cheng, M.J. Redrup, P.N. Williams, J. Pharm. Biomed. Anal. 17 (1998) 399–408.
[11] J. Oxford, M.S. Lant, J. Chromatogr. B 88 (1989) 137–146.
[12] D.A. McLoughlin, T.V. Olah, J.D. Ellis, J.D. Gilbert, R.A. Halpin, J. Chromatogr. A 726 (1996) 115–124.
[13] D.W. Boulton, G.F. Duncan, N.N. Vachharajani, Biomed. Chromatogr. 17 (2003) 48–52.
[14] P.D. Andrew, H.L. Birch, D.A. Philipot, J. Pharm. Sci. 82 (1993) 73–76.
[15] M. Dunne, P. Andrew, J. Pharm. Biomed. Anal. 14 (1996) 721–726.
[16] K.D. Altria, S.D. Filbey, Anal. Proc. 30 (1993) 363–365.
[17] K.D. Altria, S.D. Filbey, J. Liq. Chromatogr. 16 (1993) 2281–2292.
[18] A. Doliwa, S. Santoyo, M.A. Campanero, P. Ygartua, J. Pharm. Biomed. Anal. 26 (2001) 531–537.
[19] D.R. Jenke, J. Liq. Chromatogr. Relat. Technol. 19 (1996) 719–736.
[20] D.R. Jenke, J. Liq. Chromatogr. Relat. Technol. 19 (1996) 737–757.