ELSEVIER

www.elsevier.nl/locate/farmac

Il Farmaco 55 (2000) 376–382

IL FARMACO

High-performance liquid chromatographic analysis of verapamil and its application to determination in tablet dosage forms and to drug dissolution studies

Yalçın Özkan [a,*], Niyazi Yılmaz [b], Sibel A. Özkan [b], İnci Biryol [b]

[a] *Department of Pharmaceutical Technology, Gülhane Military Medical Academy, 06018 Etlik, Ankara, Turkey*
[b] *Department of Analytical Chemistry, Faculty of Pharmacy, Ankara University, 06100 Ankara, Turkey*

Received 14 January 2000; accepted 7 April 2000

Abstract

A high-performance liquid chromatographic procedure with two detectors is presented for the determination of verapamil in pharmaceutical dosage forms. The procedure is based on the use of reversed-phase high-performance liquid chromatography with UV and fluorimetric detectors. Each analysis required no longer than 6 min for both detection procedures. Quantification was achieved by measurement of the ratio of the peak area of the drug to the internal standard (fluoxetine) and the detection limit was 10 ng/ml for the UV detector and 750 pg/ml for the fluorimetric detector. There was no significant difference between inter- and intra-day studies for verapamil determined for two different concentrations (0.05 and 1.00 μg/ml). This process could be used to determine verapamil concentrations in the range 0.025–50 and 0.0008–20 μg/ml for UV and fluorimetric detection, respectively. These methods were applied, without any interference from the excipients, for the determination of the drug in tablets and in drug dissolution studies. It is suggested that the proposed HPLC procedures could be used for routine quality control and dosage form assay of verapamil hydrochloride.

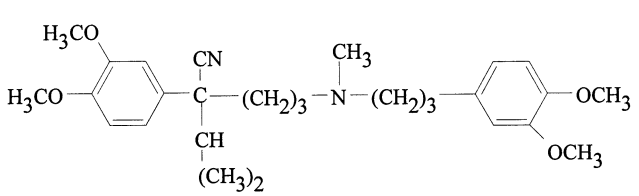
Keywords: Verapamil hydrochloride; High-performance liquid chromatography; UV detector; Fluorimetric detector; Tablet analysis; Dissolution profile

1. Introduction

Verapamil [(±)-5-[*N*-(3,4-dimethoxyphenethyl)-*N*-methylamino]-2-(3,4-dimethoxyphenyl)-2-*iso*-propylvaleronitrile] is a calcium-channel blocker and is classified as a class IV anti-arrhythmic agent [1]. It is used in the control of supra ventricular tachyarrhythmias, and in the management of classical and variant angina pectoris. It is also used in the treatment of hypertension [2]. The disposition of verapamil is described as being subject to extensive metabolism with a relatively high systemic clearence, and as such, due to high presystemic elimination, has a relatively low oral systemic bioavailability.

The molecule contains an asymmetric carbon (Scheme 1) and is clinically administered as a racemic mixture of the (+)-*R*- and (−)-*S*-enantiomers.

It has been determined by spectrophotometry [3], gas chromatography [4] and capillary electrophoresis [5]. Several high-performance liquid chromatographic (HPLC) methods have been developed for the determination of verapamil and its metabolite. They involve UV detection [6–9] or more frequently fluorescence detection [10–13] owing to the native fluorescence properties of this compound.



Scheme 1. Structure of verapamil.

* Corresponding author. Fax: +90-312-3520950.
E-mail address: yozkan@obs.gata.edu.tr (Y. Özkan).

0014-827X/00/$ - see front matter
PII: S0014-827X(00)00054-9

These chromatographic methods were developed for the determination of verapamil and its primary metabolites in biological fluids. However, these methods were not applied to pharmaceutical combinations of verapamil except in a recent work of El Ghany et al. dealing with the high-performance thin-layer chromatographic analysis [6].

The main purpose of an oral solid pharmaceutical dosage form is to make available a certain and defined amount of active substance to human body through the gastrointestinal system [14]. The pharmaceutical industry and the regulatory agencies focus on the evaluation of the release kinetics from dosage forms, and this study is generally performed on official or nonofficial dissolution devices [15]. The in vitro dissolution profiles obtained from dissolution rate studies have also been used in an attempt to characterise the in vivo behaviour of drugs with little success [16–18].

Up to date the dissolution rate studies of verapamil in tablet dosage forms has not been reported. The aim of the present work is to develop a simple and direct HPLC method with UV and fluorimetric detectors available for the quantitation of verapamil in dosage forms for quality control purposes. The developed HPLC method and the comparative spectrophotometric method were applied to the in vitro dissolution rate studies of the drug from the tablet dosage forms.

The main purpose of this investigation is to develop reversed phase high-performance liquid chromatographic methods simpler, cheaper and faster than USP XXIII [9] and the other HPLC methods found in literature. These methods were used to determine either formulation drug content and verapamil release from the solid dosage forms.

2. Experimental

2.1. Chemicals and reagents

Racemic Verapamil hydrochloride was kindly supplied by Knoll-Deutsche Drugs Inc. (Istanbul, Turkey). HPLC grade acetonitrile was purchased from Merck. All other chemicals (analytical grade) were obtained from Sigma or Merck. Bidistilled water was used.

The potassium dihydrogen phosphate buffer solution, prepared with bidistilled water, was filtered through WTP 0.5 μm filters (Whatman, Maidstone, UK). The internal standard fluoxetine was received from Lilly Drug Inc. (Istanbul, Turkey). For dissolution studies, working solutions of 0.1 M HCl (pH 1.2), which is similar to the physiological conditions in gastric fluids, were used.

2.2. Equipment

The chromatographic apparatus (Waters, Milford, MA, USA) consisted of a model 510 solvent delivery system, and model 481 spectrophotometric and 470 scanning fluorimetric detectors. The chromatograms were analysed with a chromatographic workstation (Baseline 810). A model 717 plus autosampler and 50 μl injection volume were used. Spectrophotometric measurements were carried out using a Shimadzu 2100 double beam UV–Vis spectrophotometer. Dissolution rate studies were carried out at a wavelength of 276 nm. The dissolution rate of verapamil from tablets was performed on Caleva 7ST dissolution apparatus (GB Caleva Inc., UK).

2.3. Chromatographic conditions

The separation was performed on a reversed-phase Supelcosil LC-18 (250×4.6 mm, 5μm particle size) column. The mobile phase consisted of a mixture of 0.05 M potassium dihydrogen phosphate:acetonitrile:*o*-phosphoric acid (69.5:30:0.5) (pH 3.60). The mobile phase was prepared daily, filtered, sonicated before use, and delivered at a flow rate of 1.5 ml/min. The UV detector was set at a wavelength of 276 nm; for fluorescence detection an excitation wavelength of 280 nm and an emission wavelength of 313 nm were selected.

2.4. Preparation of the standard solutions

2.4.1. Internal standard solution

The internal standard was prepared by dissolving 10 mg of fluoxetine in 10 ml of methanol in a 10 ml volumetric flask. Although modern injectors have generally overcome the need for an internal standard we preferred to use an internal standard to eliminate any possible interference due to the excipients of the dosage forms.

2.4.2. Standard solutions and calibration curves

A stock solution of verapamil was prepared by dissolving 10 mg of verapamil in 10 ml of methanol in a 10 ml volumetric flask. For fluorimetric detection, standard solutions for HPLC were prepared with mobile phase by varying the concentration of verapamil in the range 0.0008–20 μg/ml and maintaining a constant concentration of fluoxetine (internal standard) at 20 μg/ml. The concentration of verapamil was varied in the range 0.025–50 μg/ml and the concentration of fluoxetine (internal standard) was maintained at a constant level of 10 μg/ml for UV detection.

For the spectrophotometric method, standard solutions were prepared in 0.1 N HCl (dissolution medium) within the concentration range 4.9–39.3 μg/ml.

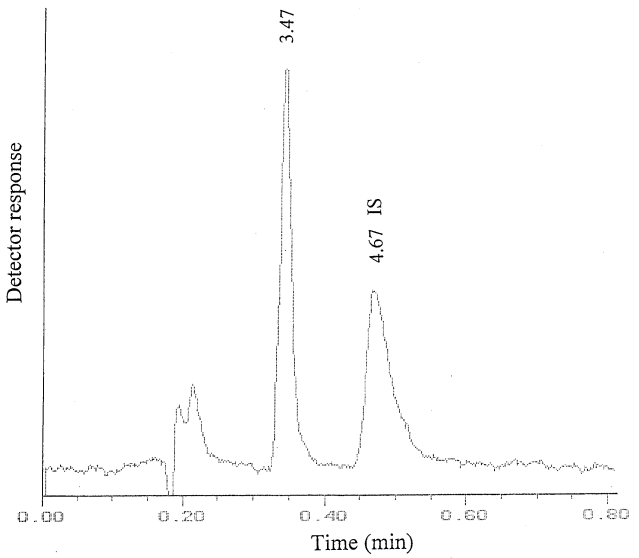


Fig. 1. Typical chromatogram of verapamil hydrochloride (1 μg/ml) and internal standard fluoxetine (10 μg/ml) obtained with UV detection.

The calibration curve for HPLC analysis was constructed by plotting the ratio of the peak area of the drug to that of internal standard against the drug concentration. Using the results of dissolution rate studies the calibration curve for spectrophotometric analysis was obtained by plotting the drug concentration against the peak-through amplitude at 276 nm in the UV spectrum.

2.5. Analysis of tablets

Ten tablets were weighed, crushed and combined. An amount of powder equivalent to about 10 mg verapamil was accurately weighed, transferred in to a 10 ml volumetric flask, diluted with methanol, sonicated for 10 min and then completed to volume with the same solution. After filtration, appropriate solutions were prepared by taking suitable aliquots of clear filtrate and adding of the appropriate internal standard, diluting them with mobile phase in order to obtain a final solution. Final solutions were used at both detectors.

2.6. Recovery studies

In order to establish the reliability and suitability of the proposed method, recovery experiments were carried out. The known amounts of the pure drug and internal standard at a constant level were added to the verapamil formulation and the mixtures were analysed by the proposed method. After three repeated experiments the recoveries were calculated.

2.7. In vitro dissolution studies

Drug release tests were carried out according to the USP 23 dissolution procedure for single-entity products with use of a USP by means of a paddle apparatus in 900 ml of 0.1 M HCl (pH 1.2, gastric medium) at a stirring rate of 75 rpm. The temperature of the cell was maintained at 37 ± 0.5°C by use of a thermostatic bath. At each sample time interval, an exact volume of sample was withdrawn from each flask and immediately replaced with an identical volume of fresh medium. A correction factor was included in the calculations to account for the drug lost in the samples. At predetermined time intervals (2, 4, 6, 10, 15, 20, 30, 45, 60, 75, 90 min), the concentration of verapamil in the dissolution medium was determined by HPLC using both detectors. Furthermore, to obtain comparative dissolution rate results, a UV spectrophotometric method at 276 nm was also applied. This spectrophotometric method was very similar to that described in the USP 23 for single-entity products. Dissolution test data were obtained on the average of six tablets.

3. Results and discussion

3.1. Development and validation of the HPLC methods

Using the described chromatographic conditions, verapamil and the internal standard, fluoxetine were well separated and their retention times were 3.47 and 4.67 min, respectively. Fig. 1 shows a typical chromatogram obtained for verapamil tablets with UV detector. For both compounds, sharp and symmetrical peaks were obtained with good baseline resolution and minimal

Table 1
Characteristics of verapamil calibration plots

Method	Linearity range (μg/ml)	Equation	Correlation coefficient	Standard error of slope	Standard error of intercept
HPLC with fluorimetric detection	0.0008–20 ($n = 12$)	$y = 22.03x + 0.075$	0.999	5.27×10^{-3}	0.034
HPLC with spectrophotometric detection	0.025–50 ($n = 10$)	$y = 1.51x + 0.039$	0.999	2.22×10^{-3}	0.041
Spectrophotometric method	4.9–39.3 ($n = 6$)	$y = 1.602x - 0.036$	0.998	6.28×10^{-4}	0.015

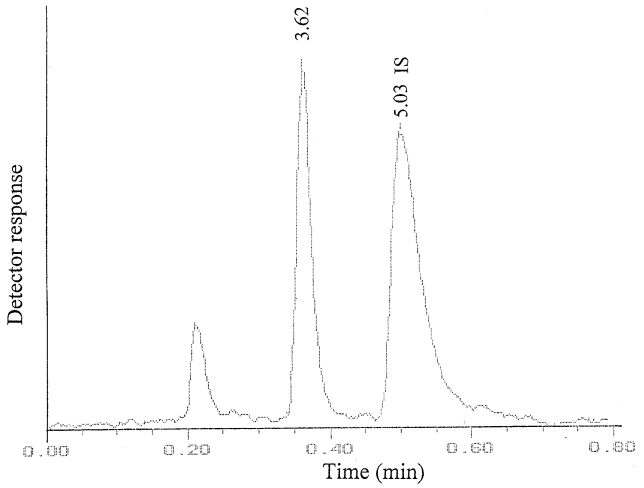


Fig. 2. Typical chromatogram of verapamil hydrochloride (0.050 μg/ml) and internal standard fluoxetine (20 μg/ml) obtained with fluorimetric detection.

tailing, thus facilitating the accurate measurement of the peak area. No interfering peaks were found in the chromatogram due to tablet excipients.

Table 1 represents a calibration plot for the peak area ratio of varying amounts of verapamil (0.025–50 μg/ml) to a constant amount of fluoxetine (10 μg/ml). Calibration characteristics are given in Table 1.

A typical HPLC chromatogram of the analysed tablet formulation with fluorimetric detector is shown in Fig. 2; the retention times were 3.62 min (verapamil) and 5.03 min (fluoxetine, internal standard), respectively. The ratio of peak area of verapamil to the internal standard versus concentration of drug plot was linear over the concentration range 0.0008–20 μg/ml. The linear regression analysis results for this detector are given in Table 1.

For the HPLC method, the lower detection limit of verapamil, defined as three times the level of the baseline noise, was 750 pg/ml for the fluorimetric detector and 100 ng/ml for the UV detector.

The results obtained for intra- and inter-day variability studies of verapamil samples are reported in Table 2. The within-day precision for the studied concentrations (0.05 and 1.0 μg/ml) showed RSD of 1.11 and 1.39%, and 0.82 and 0.34% for the UV and fluorimetric detectors, respectively. These results show the accuracy and reproducibility of the assay. Thus, it was concluded that there was no significant difference for the assay which was tested within day and between days.

3.2. Analysis of verapamil tablet dosage forms

On the basis of above results, the proposed method using both detectors was applied to the direct determination of verapamil in different tablet formulations. The results obtained from the analysis of tablet dosage forms and their recovery studies are summarised in Table 3. These results were obtained by commercially analysing available samples. The results of the analysis of verapamil tablets indicate that the proposed assay can be used for quantitation of verapamil in commercial samples.

In order to check the accuracy and precision of the developed method, we also carried out a recovery study. The results of the recovery tests, presented in Table 3, which average 98.94 and 98.18% for the UV and fluorimetric detectors, respectively, confirmed the accuracy of the proposed HPLC methods. It can be concluded from Table 3 that the proposed method is sufficiently accurate and precise to be applied to pharmaceutical dosage forms within a short analysis time (< 6 min). The method is very simple and rapid and it does not involve use of any complex instrument or complicated sample preparation. The high percentage recovery indicates that the method is not affected by the interference due to the excipients used of in the formulations. Therefore, the method can be useful in routine quality control analysis of verapamil.

The results obtained from both detectors were compared with the reversed-phase HPLC method, which is recommended by USP 23 [9] (Table 3). It is evident that the proposed method is as sensitive as the official method. According to the Student t-test, the calculated t values did not exceed the theoretical value for a significance level of 0.05. These results indicate that

Table 2
Intraday and interday precision of verapamil standards for both detectors

	Theoretical concentration	Intraday measured concentration [a]		Interday measured concentration [b]	
		Mean	RSD (%)	Mean	RSD (%)
UV detector	50 ng/ml	49.5 ng/ml	1.11	50.1 ng/ml	3.14
	1 μg/ml	0.992 μg/ml	1.39	0.995 μg/ml	1.45
Fluorimetric detector	50 ng/ml	50.46 ng/ml	0.82	50.18 ng/ml	0.95
	1 μg/ml	1.004 μg/ml	0.34	0.99 μg/ml	0.63

[a] Mean value of five different verapamil standards for each concentration.
[b] Interday reproducibility was determined from five different runs over a period of 3 weeks.

Table 3
Results of the determination and the recovery analysis of verapamil in tablet dosage forms

	UV detector		Fluorimetric detector		Official method [9]	
	Tablet	Film-coated tablet	Tablet	Film-coated tablet	Tablet	Film-coated tablet
Label claim (mg)	80.0	80.0	80.0	80.0	80.0	80.0
Mean of amount found (mg) [a]	79.43	80.58	79.52	79.78	79.33	79.75
RSD (%)	1.36	1.21	0.47	0.37	1.51	1.46
Student t-test result	$t_{calculated}$: 0.152	$t_{calculated}$: 1.32	$t_{calculated}$: 0.327	$t_{calculated}$: 0.068	Theoretical t value: 2.228 Not significant	Theoretical t value: 2.228 Not significant
Added (mg)	10	10	10	10		
Recovered (mg) [b]	9.73	9.84	9.84	9.93		
Recovery (%)	98.64	99.24	99.2	99.16		
RSD (%)	0.39	0.53	0.85	0.32		

[a] Each value is the mean of six experiments.
[b] Each value is the mean of five experiments.

there is no significant difference between the proposed and official methods with respect to precision and accuracy. However, the proposed HPLC methods are simple, inexpensive and rapid in comparison with the official method. No treatment of the sample is required before the proposed HPLC analyses. Excipients present in the tablet do not interfere with the analyses.

3.3. In vitro dissolution studies

The proposed HPLC method with fluorimetric and UV detectors and the comparative spectrophotometric method was applied to the quantitation of verapamil in dissolution rate studies. The results of linear regression analysis from standard verapamil in 0.1 M HCl (pH 1.2, artificial gastric medium) obtained with a proposed comparative spectrophotometric method are given in Table 1. Two different tablet formulations which were produced by different companies were tested according to the paddle dissolution method. The release profiles were drawn as a percentage of the drug dissolved from the tablets versus time (Fig. 3(a,b)).

In order to determine the dissolution kinetics of the tablets dissolution data were fitted according to the different models, namely zero order, first order, $Q-\sqrt{t}$ and the Weibull distribution function (RRSBW) [19,20]. All the kinetic and related rate constants and statistical parameters of the various models are summarised in Table 4. The most proper release kinetic was found to be RRSBW, since the highest determination coefficient and the lowest sum of weighed squared deviations (Table 4) were obtained for this model.

Differences between tablets and film-coated tablets were obtained as far as the $T_{63.2\%}$ and β values are concerned (Table 4). The 63.2% release of active material was attained to, after 1 h, from tablets whilst from film-coated tablets the same result was achieved after a few minutes. It may be that this result is due to the use of different excipients, and to different tableting techniques. The results obtained also show that, if no strict

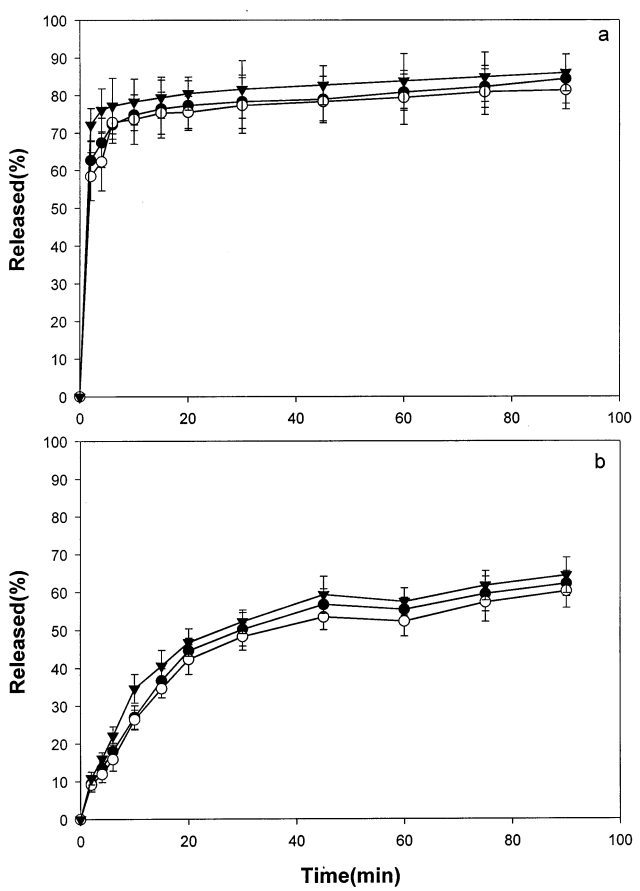


Fig. 3. Dissolution profiles obtained for: (a) film-coated tablets, and (b) tablet formulations using the HPLC method with UV detection (---○---); with fluorimetric detection (—▼—); and using the spectrophotometric method (—●—).

Table 4
Kinetic assessment of dissolution data [a]

Method	Sample	First order			Zero order			$Q-\sqrt{t}$			RRSBW			
		k_r	r^2	SWSD	k_r°	r^2	SWSD	K	r^2	SWSD	$T_{63.2\%}$	β	r^2	SWSD
HPLC with UV detection	Tablet	0.54	0.849	0.36	26.11	0.779	0.44	3335.1	0.909	0.074	78.08	0.63	0.957	0.046
	Film-coated tablet	0.42	0.683	0.42	8.74	0.574	0.418	9216.2	0.721	0.68	2.58	0.156	0.890	0.019
HPLC with fluorimetric detection	Tablet	0.58	0.834	0.56	25.85	0.75	0.64	4080.5	0.891	0.14	62.92	0.58	0.954	0.051
	Film-coated tablet	0.38	0.895	5.3	5.86	0.82	5.3	10443	0.93	2.6	0.15	0.1	0.98	0.0013
Spectrophotometry	Tablet	0.57	0.844	0.417	26.81	0.773	0.50	3663.9	0.906	0.093	70.01	0.62	0.962	0.050
	Film-coated tablet	0.45	0.816	0.43	8.48	0.709	0.44	9615.4	0.838	0.12	2.48	0.094	0.959	0.009

[a] k_r, release rate constant of first order kinetic; k_r°, release rate constant of zero order kinetic; K, release rate constant of $Q-\sqrt{t}$ kinetic; r^2, determination coefficient; SWSD, sum of weighed squared deviations; β, shape factor; $T_{63.2\%}$, value stands for the time for 63.2% dissolution.

requirements on dissolution control of final product are issued, drugs with a large difference in dissolution characteristics can reach the market. The need of dissolution control in drug production is indispensable to ensure drug quality. It is necessary to consider the behavioral differences of tablet dosage forms in an in vitro method when judging the applicability of a dissolution test. The physical–chemical properties of drug substances have also great importance.

4. Conclusions

The proposed HPLC methods are simple, rapid, accurate, precise, sensitive and easy to apply for the determination of verapamil in pharmaceuticals, as can be seen from validation data. Hence it can be easily and conveniently adopted for routine quality control analysis.

It was shown that these HPLC methods can be successfully applied to the determination of dissolution rate studies of verapamil in tablet dosage forms.

References

[1] J.E.F. Reynolds (Ed.), Martindale, The Extra Pharmacopoeia, 29th ed., Pharmaceutical Press, London, 1989, pp. 89–91.
[2] J.G. Hardman, L.E. Limbird (Eds.), Goodman & Gilman's The Pharmacological Basis of Therapeutics, 9th ed., McGraw-Hill, New York, 1996, p. 855.
[3] Y. Long, J. Feng, S. Tong, Spectrophotometry of verapamil tablets with charge transfer reaction, Zhongguo Yiyao Gongye Zazhi 24 (1993) 267–270.
[4] O.H. Drummer, S. Horomidis, S. Kourtis, M.L. Syrjanen, P. Tippett, Capillary gas chromatographic drug screen for use in forensic toxicology, J. Anal. Toxicol. 18 (1994) 134–138.
[5] H. Soini, M.L. Riekkala, M.V. Novotny, Chiral separations of basic drugs and quantitation of bupivacaine enantiomers in serum by capillary electrophoresis with modified cyclodextrin buffers, J. Chromatogr. 608 (1992) 265–274.
[6] M.F.A. El Ghany, A.A. Moustafa, B.E. Elzeany, J.T. Stewart, High-performance thin-layer chromatographic analysis of verapamil hydrochloride in drug substance and dosage forms, J. Planar. Chromatogr. Mod. TLC 9 (1990) 388–390.
[7] A.M. Rustum, Measurement of verapamil in human plasma by reversed-phase high-performance liquid chromatography using a short octyl column, J. Chromatogr. 528 (1990) 480–486.
[8] M.A. Garcia, J.J. Aramayana, M.A. Bregante, L.J. Fraile, C. Solans, Simultaneous determination of verapamil and norverapamil in biological samples by high-performance liquid chromatography using ultraviolet detection, J. Chromatogr. B 693 (1997) 377–382.
[9] US Pharmacopoeia, 23rd rev., 18th ed., Easton, Rand McNally, Taunton, MA, 1995, p. 1625.
[10] A. Sioufi, F. Marfil, J. Godbillon, Automated determination of an angiotensin II receptor antagonist CGP 48 933, in plasma by high-performance liquid chromatography, J. Liq. Chromatogr. 17 (1994) 2179–2186.
[11] A. Ceccato, P. Chiapi, P. Hubert, B. Toussaint, J. Crommen, Automated determination of verapamil and norverapamil in human plasma with on-line coupling of dialysis to high-performance liquid chromatography and fluorometric detection, J. Chromatogr. A 750 (1996) 351–360.
[12] C. Köppel, A. Wagemann, Plasma level monitoring of DL-verapamil and three of its metabolites by reversed-phase high-performance liquid chromatography, J. Chromatogr. 570 (1991) 229–234.
[13] I. Jane, A. McKinnon, R.J. Flanagan, High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II: Application of UV, fluorescence and electrochemical oxidation detection, J. Chromatogr. 323 (1985) 191–225.
[14] A.B. Morrison, J.A. Campbell, Tablet disintegration and physiological availability of drugs, J. Parm. Sci. 54 (1) (1965) 1–8.
[15] R.N. Jashnani, P.R. Byron, R.N. Dalby, Validation of an improved wood's rotating disk dissolution apparatus, J. Pharm. Sci. 82 (1993) 670–671.
[16] C. Anchisi, B.M. Baroli, A.M. Fadda, M. Manconi, C. Sinico, D. Valenti, Dissolution properties of Flurazepam capsules, Farmaco 53 (1998) 662–666.
[17] J.B. Satiropoulus, T. Deutsch, F.M. Plakogiannis, Comparative bioavailability of three commercial acetaminophen tablets, J. Pharm. Sci. 70 (1981) 422–425.
[18] J.S. Rowe, J.E. Carless, Comparison at the in vitro dissolution behavior of various indomethacin formulations with their in vivo bioavailability, J. Parm. Pharmacol. 33 (1981) 561–565.
[19] F. Langenbucher, Parametric representation of dissolution rate curves by the RRSBW distribution, Pharm. Ind. 38 (1976) 472–477.
[20] T. Higuchi, Mechanism of sustained action medication, J. Pharm. Sci. 52 (1963) 1145–1149.