Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Pharmaceutical and Biomedical Analysis 41 (2006) 158–164
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Comparison of UV spectrophotometric method and high performance liquid chromatography for the analysis of flunarizine and its application for the dissolution test

Kesarin Busaranon [a], Worapot Suntornsuk [b], Leena Suntornsuk [c,*]

[a] *Faculty of Pharmacy, Rangsit University, Pratumtani, Thailand*
[b] *Department of Microbiology, King Mongkut's University of Technology Thonburi, Bangkok, Thailand*
[c] *Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Mahidol University, 447 Sri-Ayudhaya Road, Rajathevee, Bangkok 10400, Thailand*

Received 15 May 2005; received in revised form 5 November 2005; accepted 7 November 2005
Available online 13 December 2005

Abstract

This study aimed to develop a simple UV spectrophotometric method for the analysis and the dissolution test of flunarizine in capsules. The UV absorbance was both measured directly and by the first derivative measurements at 254 and 268 nm, respectively. The developed methods were validated for their linearity, accuracy, precision, limit of detection (LOD) and limit of quantitation (LOQ) in comparison with the reported HPLC method. The UV spectrophotometric method illustrated excellent linearity ($r^2 > 0.9999$) in the concentration range of 6–24 μg/mL. Precision (%R.S.D. < 1.50) and recoveries were good (%$R > 99.62$). The LOD of direct UV and first derivative measurements were 0.09 and 0.84 μg/mL, respectively, and the LOQ were 0.26 and 2.55 μg/mL, respectively. Results from the assay of flunarizine in capsules by the UV spectrophotometric methods, both direct and first derivative measurements were not significantly different from those of the HPLC method ($P > 0.05$). Additionally, the method was successfully used for the dissolution test of flunarizine capsule and was found to be reliable, simple, fast, and inexpensive.

Keywords: Flunarizine; UV spectrophotometric method; High performance liquid chromatography; Dissolution test

1. Introduction

Flunarizine, (*E*)-1-[Bis(4-fluorophenyl)methyl]-4-(3-phenyl -2-propenyl) piperazine, is a difluorinated derivative of cinnarizine (Fig. 1) [1,2]. This compound is a selective calcium channel blocker and has undergone the most extensive evaluation, and it may reduce the frequency of either classical or common migraine attacks by as much as 90%. It has antihistaminic and CNS depressant effects, but it is mainly used as an inhibitor of central and peripheral vasoconstriction. It may, also, be effective in preventing more complicated syndromes, such as childhood hemiplegic migraine [3–7].

Flunarizine is not yet official in any pharmacopoeia. Several chromatographic procedures for determination of flunarizine dihydrochloride have been described such as gas chromatography (GC) [8–11], high performance liquid chromatography (HPLC) [12–17] and high performance liquid chromatography interfaced with electrospray mass spectrometry (HPLC–ES–MS) [18,19]. These methods have been mainly used for the analysis of flunarizine and its metabolites in biological fluids. In recent years, capillary zone electrophoresis (CZE) has been applied to quantify of flunarizine in serum [20]. Determination of flunarizine in pharmaceutical dosage forms usually requires complex formation prior spectrophotometric measurement [21,22]. Voltammetric method using an activated glassy carbon electrode has, also, been developed for the analysis of flunarizine in pharmaceutical dosage form [23]. These reported methods require special instruments, which are not commonly available in routine laboratories. In addition, some procedures can be laborious and time consuming.

The aim of this study was to develop a simple UV spectrophotometric procedure for routine analysis and for the dissolution test of flunarizine in dosage forms. The analytical method for flunarizine analysis is not officially available in any pharmacopoeia and previous methods were mainly focused on the analysis of the drug in biological fluids. Thus, it is important to develop

* Corresponding author. Tel.: +66 2 644 8695; fax: +66 2 644 8695.
E-mail address: pylll@mahidol.ac.th (L. Suntornsuk).

0731-7085/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2005.11.008

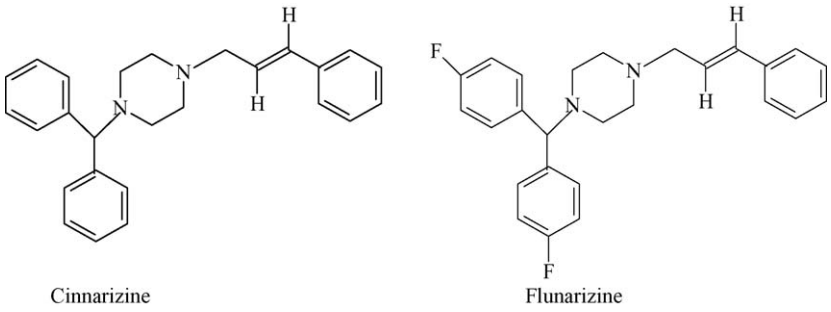

Fig. 1. Structures of cinnarizine and flunarizine.

a method, which is applicable for routine quality control of the drug. The developed method was validated for its linearity, accuracy, precision, limit of detection (LOD) and limit of quantification (LOQ). The validities of the spectrophotometric method were statistically compared with the reported HPLC method [13]. Finally, the method was applied for the quantitation and dissolution test of flunarizine dihydrochloride in capsules since they are two common tests in most monographs, which are important for quality control of pharmaceuticals in solid dosage forms. To the best of our knowledge, this was the first paper, which described the UV spectrophotometric method for the analysis of flunarizine in pharmaceutical dosage forms and its application for the dissolution test.

2. Experimental

2.1. Apparatus and conditions

The UV absorbance was measured by direct and first derivative measurements at 254 and 268 nm, respectively, by Hitachi U-2000 spectrophotometer (Hitachi High Technology, Japan), with a fixed slit width of 2 nm, in a 1-cm quartz cuvette. The spectrophotometric method was measured against methanol as a blank.

Reversed-phased HPLC was performed on a Knauer Welchrome System (Germany) equipped with a K-1001 pump and connected to a K-2006 multiple wavelength UV detector. Separations were carried out using a HIQ sil C18 (4.6 mm i.d. × 150 mm) column (Kya Tech, Japan). Mobile phase consisted of methanol–water (75:25 v/v) containing sodium chloride (0.5%, w/v) and triethanolamine (0.2%, v/v) (pH 6.6) [13]. The pH of the mobile phase was adjusted with hydrochloric acid (30%, v/v). The injection volume was 20 μL with a flow rate of 1.5 mL/min and the detector wavelength was at 254 nm. To avoid the effect of chloride generated in the HPLC instrument, the system was thoroughly washed and cleaned up the system after each injection.

2.2. Materials and reagents

Flunarizine and triethanolamine were from Fluka (Buchs SG, Switzerland), methanol and other solvents of HPLC grade were from Labscan Asia (Bangkok, Thailand). All chemicals were of analytical grade and were used without further purification.

2.3. Standard and sample solutions

Stock standard solution of flunarizine dihydrochloride was freshly prepared at a concentration of 15 μg/mL in methanol for both spectrophotometric and HPLC methods. Working standard solutions was prepared by diluting the stock standard solution to appropriate concentrations.

Four brands of flunarizine capsules were purchased from local drug stores. Twenty capsules from each brand were sampled and the content was mixed to homogeneous powder. The powder was weighed and dissolved in methanol to give a concentration of 15 μg/mL. All sample solutions were filtered through a 0.2 μm membrane and diluted to appropriate concentrations prior analysis.

2.4. Method validation

The UV spectrophotometric method was validated comparing with the reported HPLC method [13]. Linearity was studied by analyzing six standard solutions covering the range of 6–24 μg/mL ($n = 3$). Repeatability was determined at three points of the calibration curve (6, 15 and 24 μg/mL, $n = 6$). Intra-day and inter-day variability was determined by analyzing six standard solutions of the calibration curve ($n = 6$) within 1 day and on six different days ($n = 6$), respectively. Recovery (%R) of the methods was performed in four brands of flunarizine capsules using standard addition method. Standard flunarizine in a range of 50, 80, 90, 100, 110, 120, 150% of the nominal sample concentration (15 μg/mL) was added into the sample solution, which corresponded to the final concentrations of 22.5, 27, 28.5, 30, 31.5, 33 and 37.5 μg/mL, respectively. Each concentration was analyzed in triplicates. To cover the concentration range in the recovery study, additional calibration curves for the direct UV, D_1 measurements and HPLC method were established in a range of 5–50 μg/mL. For spectrophotometric method LOD and LOQ were calculated by Eqs. (1) and (2), respectively, where δ is the standard deviation of blank and s is slope of calibration [24]:

$$\mathrm{LOD} = \frac{3.3\delta}{s} \quad (1)$$

$$\mathrm{LOQ} = \frac{10\delta}{s} \quad (2)$$

For HPLC method, the concentration of flunarizine, which could be detected with a signal to noise ratio (S/N) of 3, was considered to be the LOD and the lowest concentration, which could be quantified with a S/N of 10, was defined as the LOQ.

2.5. Quantitative analysis and dissolution test of flunarizine capsules

The developed spectrophotometric method was applied for the analysis of flunarizine in four different brand capsules comparing with the HPLC method modified from Ref. [13]. The results from both techniques were statistically compared using SPSS program.

The dissolution test was performed on a dissolution apparatus (Hanson, USA) by dissolving each flunarizine capsule in a rotating vessel consisting of 900 mL of 0.1N HCl as the medium. The temperature of the medium was controlled at 37 ± 1 °C and the vessel was rotated at a speed of 50 rpm for 60 min. Ten milliliters of the medium were sampled after 5, 15, 30, 45 and 60 min, filtered through a Whatman no. 1 paper and analyzed by UV spectrophotometry. Ten milliliters of the fresh medium were replaced into each vessel after the sampling. To avoid interference from capsule shell in the dissolution test, blank solutions were prepared by dissolving the empty capsule shell of each brand into the same medium and performed the dissolution test in the same manner as the samples. Solutions from blank were sampled at specific intervals (5, 15, 30, 45 and 60 min) as in the samples and subjected for direct UV and D_1 measurement. Any absorbance obtained from the blank solutions was subtracted from the absorbance of the sample solutions. The dissolution test for each brand was performed in six replicates.

3. Results and discussion

3.1. UV spectrophotometric and HPLC conditions

Flunarizine was completely soluble in methanol, whereas the solution was turbid in the presence of water. Methanol was selected as the solvent for flunarizine because it provided the highest solubility and UV absorbance without interference from sample matrix for both direct UV and first derivative measurements. The first derivative was performed to prove whether sample matrices of the investigated capsules would interfere with the flunarizine spectrum. Results showed that both direct UV and first derivative measurements are feasible for the analysis of flunarizine without interference from sample matrices. The detection wavelength at 254 and 268 nm were selected for direct UV and first derivative measurement, respectively. The absorptivity was 396.5 and 240.0 L/g cm with the Sandell's sensitivity of 18.12 and 177.28 ng/cm^2 at 254 and 268 nm for direct UV and first derivative measurement, respectively. Typical original absorption and first derivative spectra of standard flunarizine in methanol are shown in Fig. 2.

The HPLC condition in the current study was adopted from Ref. [13] since the method can be used for the quantitation of flunarizine in the presence of its degradation products and was referred as a stability indicating method. Currently, the flow rate of 1.5 mL/min was employed instead of 2.0 mL/min due to the high back-pressure. Flunarizine was eluted within 8 min without interference from other ingredients in the formulations (Fig. 3). Although the chromatogrms showed slight fronting peak of flunarizine, this did not intefere with the peak integration. Additionally, no degradation products were observed during the analysis.

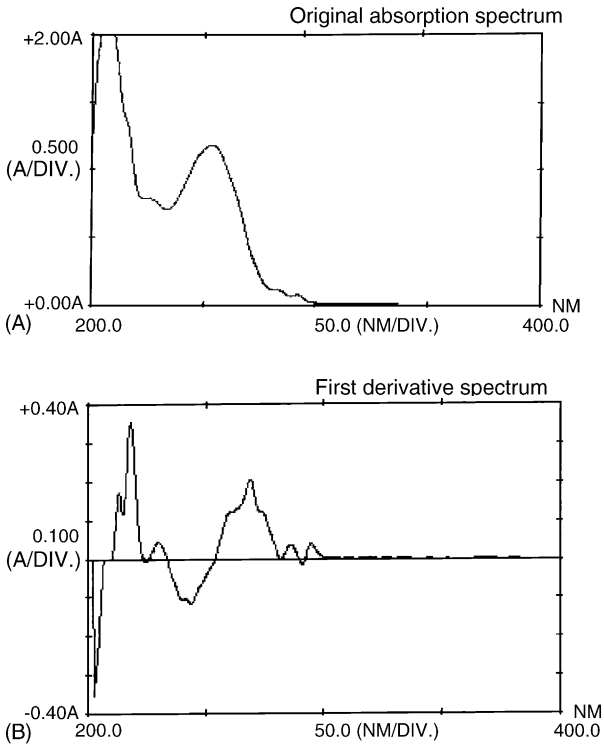

Fig. 2. Typical spectra of flunarizine in methanol (15 μg/mL) from: (A) original absorption spectrum and (B) first derivative spectrum.

3.2. Method validation

Analytical characteristics of the proposed spectrophotometric methods, direct UV and first derivative measurement, were

Table 1
Linearity and precision of spectrophotometric method and HPLC[a]

	UV spectrophotometric method		HPLC
	Direct UV measurement	D_1 measurement	
Slope	0.0537 (0.75)	0.0055 (2.09)	54662 (1.45)
Intercept	0.0016	−0.0001	3572
Correlation coefficient (r^2)	0.9999	1.0000	0.9999
Repeatability ($n = 6$)	0.02	0.01	1.97[b], 0.40[c]
Intra-day precision ($n = 6$)	0.25	1.50	1.97[b], 0.95[c]
Inter-day ($n = 6$)	0.30	1.33	0.62[b], 1.78[c]
LOD (μg/mL)	0.09	0.84	1.80
LOQ (μg/mL)	0.26 (2.58)	2.55 (0.29)	6.00 (1.89)

[a] Numbers in parentheses represent %R.S.D.
[b] Calculated from retention times.
[c] Calculated from peak areas.

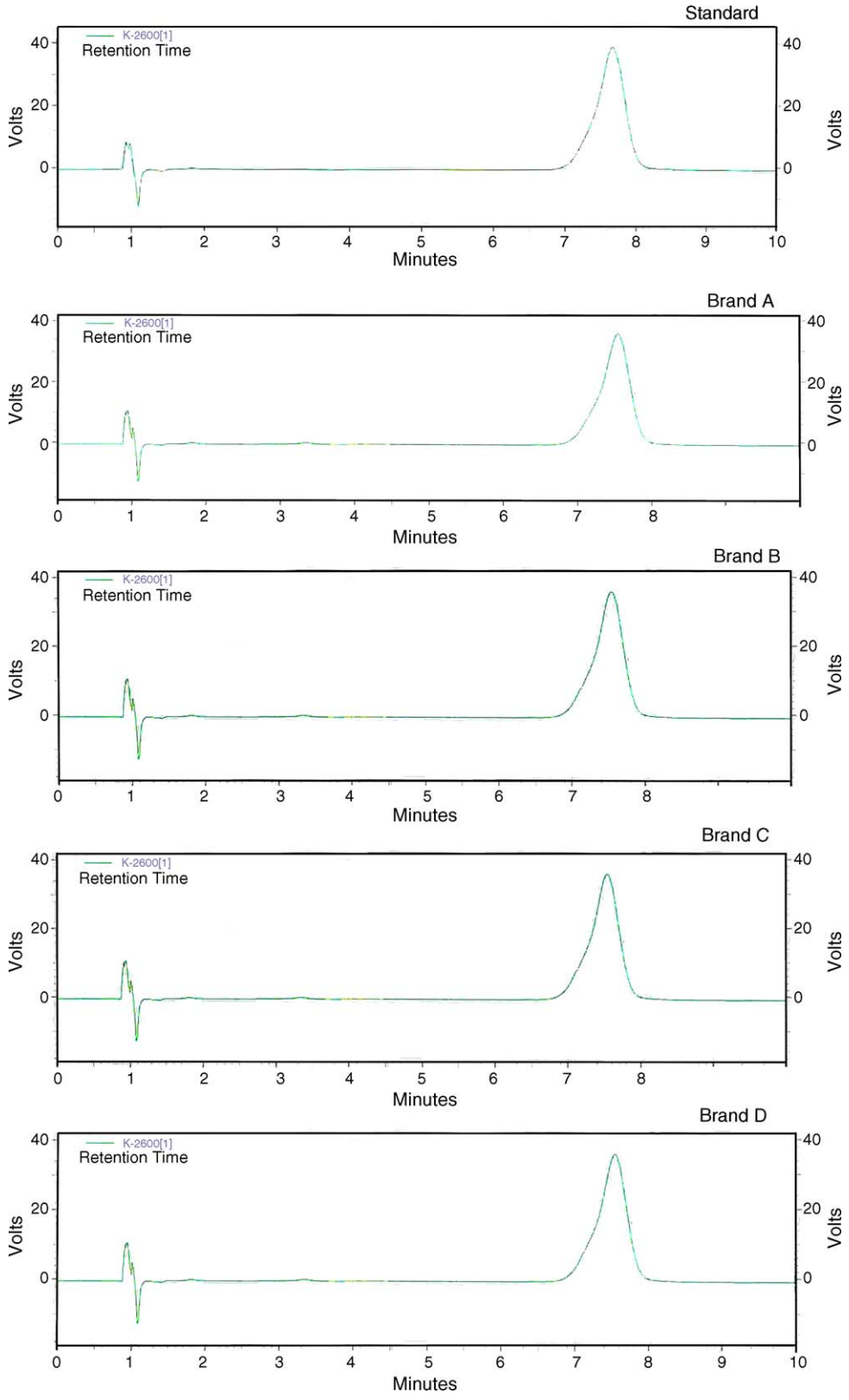

Fig. 3. Chromatograms of flunarizine standard and flunarizine in different brands of capsules (15 μg/mL). Chromatographic conditions as described in Section 2.

evaluated comparing with the HPLC method, which included linearity, precision, recovery, LOD and LOQ (Table 1). All methods showed good linearity ($r^2 > 0.9999$) in the concentrations of 6–24 μg/mL. Repeatability, intra-day and inter-day variability were evaluated for the precision of the methods. In most cases, the %R.S.D.s of the UV spectrophotometric methods was within 1.50% and of the HPLC method they were less than 1.97 and 1.78% for the retention time and peak area,

Table 2
%Recovery of spectrophotometric method and HPLC ($n = 3$)[a]

	UV Spectrophotometric method		HPLC
	Direct UV measurement	D_1 measurement	
Brand A	101.01–101.67 (0.13–0.20)	100.32–100.66 (0.14–0.31)	99.84–100.54 (0.10–0.40)
Brand B	100.17–100.66 (0.04–0.41)	100.29–100.61 (0.01–0.36)	100.78–101.32 (0.04–1.06)
Brand C	99.90–100.83 (0.10–0.540)	100.01–100.56 (0.17–0.31)	99.80–100.55 (0.06–0.35)
Brand D	100.06–100.69 (0.04–0.17)	100.12–100.48 (0.09–0.33)	99.65–101.21 (0.06–0.57)

[a] Numbers in parentheses represent %R.S.D.

Table 3
Percent label amounts of flunarizine in capsules ($n = 4$)[a]

	UV spectrophotometric method		HPLC
	Direct UV measurement	D_1 measurement	
Brand A	101.09 (0.17)	100.31 (0.45)	99.78 (0.67)
Brand B	101.90 (0.21)	101.54 (0.23)	102.25 (0.59)
Brand C	101.20 (0.12)	100.68 (0.23)	100.50 (0.43)
Brand D	101.78 (0.13)	101.27 (0.29)	101.07 (0.59)

[a] Label amount of flunarizine is 5 mg/capsule and numbers in parentheses represent %R.S.D.

respectively. Interestingly, both the LOD and LOQ of the proposed spectrophotometric methods were lower than those of the HPLC method (Table 1). Among the methods, direct UV measurement provided the lowest LOD and LOQ for the analysis of flunarizine. Recoveries of the methods were performed in four brands of flunarizine capsules using standard addition method covering the range of 50–150% of the nominal sample concentration (15 μg/mL), which corresponded to the final concentrations of 22.5–37.5 μg/mL. Thus, additional calibration curves in a range of 5–50 μg/mL was established for the direct UV, D_1 measurements and HPLC method. Results showed the correlation coefficients of more than 0.9998 for all three methods, although, for the spectrophotometric methods, the absorbance were higher than 1.0 at concentrations of 25 μg/mL and higher. Table 2 shows that the % recoveries were within 99.90–101.67 (%R.S.D. = 0.54), 100.01–100.66 (%R.S.D. = 0.36) and 99.65–101.32 (%R.S.D. = 1.06) for direct UV, first derivative and HPLC method, respectively. The best recovery with the smallest %R.S.D. was by the UV spectrophotometric method using first derivative measurement. The selectivity of the spectrophotometric method was performed by using a placebo. Results showed no interference from the placebo.

3.3. Applications

3.3.1. Determination of flunarizine in capsules

The developed spectrophotometric methods, direct UV and first derivative measurement, were applied for the analysis of flunarizine capsules comparing with the modified HPLC method [13]. The change in this work was the flow rate, which was 1.5 mL/min instead of 2.0 mL/min. The NaCl content in the mobile phase of 0.5% (w/v) did not cause problems with our HPLC system after a thorough wash and clean up of the system following each analysis. This HPLC condition was chosen since it can be used for the determination of flunarizine in the presence of its degradation products and was referred as a stability indicating method. Our results show no degradation products were observed during the analysis. Results from the assay of flunarizine by direct UV, first derivative measurement and the modified HPLC are shown in Table 3. Typical spectra and chromatograms of flunarizine in samples are shown in Figs. 2 and 3, respectively. The assay data obtained from the three methods showed non-significant difference at the confidence level of 95% ($P > 0.05$). Thus, all methods are applicable for the determination of flunarizine in commercial capsules. However, the spectrophotometric method was more economical, in terms of cost and time, than the HPLC method.

3.3.2. Dissolution test of flunarizine capsules

The release of the drug substance from the drug product, the dissolution of the drug under physiological conditions and the permeability across the gastrointestinal tract are rate determining steps that affect the drug absorption from a solid dosage form after oral administration. In vitro dissolution test can be used to predict the release and the dissolution of the drug, hence, the in vivo performance of the drug. The dissolution test is, now, routinely employed for lot-to-lot quality control of pharmaceuticals in solid dosage forms. Since flunarizine is not officially

Table 4
Percent of drug dissolved in flunarizine capsules ($n = 6$)[a]

Time (min)	Brand A		Brand B		Brand C		Brand D	
	Direct UV	D_1	Direct UV	D_1	Direct UV	D_1	Direct UV	D_1
5	51.65 (0.61)	53.62 (2.49)	28.83 (1.10)	23.62 (3.58)	25.94 (1.46)	24.98 (2.67)	28.49 (0.94)	26.89 (1.57)
15	71.79 (0.32)	73.94 (1.91)	66.51 (0.57)	66.16 (1.50)	54.96 (0.79)	57.71 (0.73)	58.83 (0.71)	55.66 (2.53)
30	92.28 (0.41)	93.44 (3.59)	78.06 (0.76)	80.62 (1.12)	81.65 (0.39)	84.71 (1.33)	81.86 (1.01)	82.80 (2.06)
45	95.03 (0.24)	95.34 (2.15)	96.08 (0.68)	97.94 (0.46)	94.53 (0.23)	96.03 (1.97)	96.01 (0.85)	97.39 (0.34)
60	94.39 (0.89)	94.94 (4.13)	96.37 (0.66)	98.34 (0.74)	98.69 (0.50)	98.21 (0.97)	98.48 (2.00)	100.25 (1.39)

[a] Numbers in parentheses represent %R.S.D.

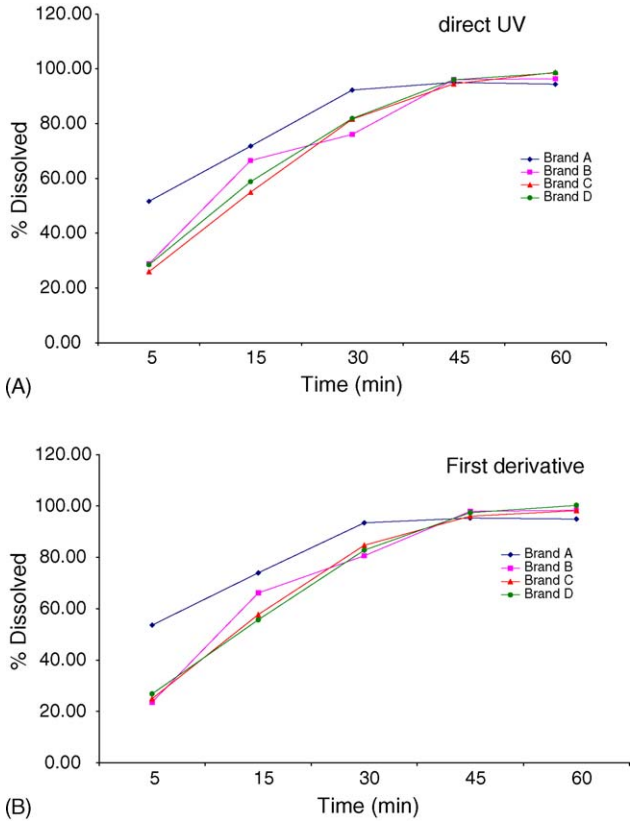

Fig. 4. Dissolution profile of flunarizine in capsules from: (A) direct UV and (B) first derivative measurements.

available in pharmacopoeia, we have developed the dissolution testing condition for this drug. The condition was to dissolve a flunarizine capsule in 900 mL of 0.1N HCl using the apparatus 2 (paddle) rotating at 50 rpm for 60 min. The acidic medium was chosen in order to simulate the physiological condition of the stomach where the drug is highly absorbed. Apparatus 2 was selected instead of the apparatus 1 (basket) since the latter caused the clogging of the dissolved drug in the sieves of the basket. The amount of drug dissolved was sampled and analyzed by the proposed UV spectrophotometric method. The dissolution test data of flunarizine capsules are shown in Table 4 and the dissolution profiles were illustrated in Fig. 4. In most cases, flunarizine was slowly dissolved in 0.1N HCl in the first 5 min with the percents of the dissolved drug of 24–29%. Except for brand A, the amount of the drug dissolved was up to 50% after 5 min. This difference might due to the excipient in the formula, which could be varied among brands. However, flunarizine was readily dissolved after 15 min and the percents of the dissolved drug at 60 min were within 94–100% for all brands (Table 4). The percents of the dissolved drugs calculated by the direct UV and D_1 measurements were not significantly different ($P > 0.05$). In this study, it was very important to perform the dissolution test of capsule shells as blanks in the same manner as the samples. Particularly at the dissolution time of 45 and 60 min, absorbencies from capsule shell solutions were pronounce, varying from 0.013–0.089, and should be subtracted from those of the samples.

4. Conclusion

A reliable, simple and fast UV spectrophotometric method was developed and validated for the analysis of flunarizine in capsules. Results from the UV spectrophotometric method show no significant difference from those obtained from the HPLC method ($P > 0.05$). In addition, the dissolution procedure for flunarizine capsules was, also, established in 900 mL of 0.1N HCl using rotating paddle apparatus. The proposed spectrophotometric method will not replace the presently known methods available for the analysis of flunarizine. However, it can serve as an alternative where advanced instruments (e.g. HPLC) are not available for routine analysis. Lack of high technology instrument is usually a problem in developing countries. Both the proposed assay and dissolution test methods of flunarizine will be valuable to facilitate the lot-to-lot quality control process for local pharmaceutical manufacturers prior marketing of the drug.

Acknowledgements

This work was financially supported by the Graduate School, Mahidol University. The authors also thank the Faculty of Pharmacy, Mahidol University and Rangsit University for providing all facilities and Prof. Wolfgang Buchberger, Institute of Analytical Chemistry, Johannes Kepler University, Linz, Austria for his valuable discussion.

References

[1] M. Windholz (Ed.), The Merck Index—An Encyclopedia of Chemicals and Drugs, 11th ed., Merck & Co., Inc., Whitehouse Station, New Jersey, 2001, p. 4172.
[2] M.R. Kilbourn, Int. J. Rad. Appl. Instr. [A] 42 (1991) 109–111.
[3] J.E.F. Renolds (Ed.), Martindale's the Extra Pharmacopoeia, 30th ed., The Pharmaceutical Press, London, 1993, pp. 939–940.
[4] P.R. Saxena, M.O. Den Boer, J. Neurol. 238 (1991) s28–s33.
[5] G. Micieli, G. Sances, C. Pacchetti, M. Trucco, M. Magri, D. Piazza, Int. J. Clin. Pharmacol. Res. 4 (1984) 239–245.
[6] M. Thomas, M. Behari, G.K. Ahuja, Headache 31 (1991) 613–615.
[7] R. Schmidt, W. Oestreich, J. Cardiovasc. Pharmacol. 18 (1991) s27–s30.
[8] R. Woestenborghs, L. Michielsen, W. Lorreyne, J. Heykauts, J. Chromatogr. 232 (1982) 85–91.
[9] S.C. Flor, J. Chromatogr. 272 (1983) 315–323.
[10] I.M. Kapetarovic, C.D. Torchin, W.D. Yonekawa, H.J. Kupferberg, J. Chromatogr. 383 (1986) 223–228.
[11] A. Yamaji, K. Kataoka, M. Oishi, N. Kanamori, T. Tagawa, T. Mimaki, J. Chromatogr. 421 (1987) 372–376.
[12] M. Ahnoff, B.A. Persson, J. Chormatogr. 531 (1990) 181–213.
[13] A.A.M. Wahbi, A.F.M. El-Walily, E.M. Hassan, F.G. Saliman, A. El-Gendi, J. Pharm. Biomed. Anal. 13 (1995) 777–784.
[14] L.F. Tang, J.H. Wu, L.C. Li, H.Y. Chen, B.Y. Tan, Z.W. Li, Fenxi. Ceshi. Xuebao. 19 (2000) 75–79.
[15] H.X. He, Y.D. Zhou, G.Q. Yao, Z.B. Zhu, Yaowu. Fenxi. Zazhi. 20 (2000) 157–160.
[16] M.R. Fuh, Y.F. Chan, W.W.H. Pan, I.P. Huang, J. Liq. Chromatogr. Rel. Tech. 22 (1999) 2525–2533.
[17] W.F. Kartinasari, H. Chufianty, G. Indrayanto, J. Liq. Chromatogr. Rel. Tech. 26 (2003) 1059–1067.
[18] M.R. Fuh, C.J. Hsieh, Y.L. Tai, Talanta 49 (1999) 1069–1075.

[19] M.R. Fuh, C.J. Hsieh, J. Chromatogr. B: Biomed. Sci. Appl. 736 (1999) 167–173.
[20] R.F. Fan, X.Y. Shi, J.L. Gu, Y.L. Deng, R.N. Fu, Sepu 18 (2000) 357–360.
[21] A.F.M. El Walily, A. El Gindy, A.A.M. Wahbi, J. Pharm. Biomed. Anal. 13 (1995) 53–58.
[22] K. Kelani, L.I. Bebawy, L. Abdel-Fattah, J. Pharm. Biomed. Anal. 18 (1999) 985–992.
[23] B. Uslu, N. Yilmaz, N. Erk, S.A. Ozkan, Z. Senturk, I. Biryol, J. Pharm. Biomed. Anal. 21 (1999) 215–220.
[24] H.D. Ravansiddappa, B. Manju, J. Pharm. Biomed. Anal. 25 (2001) 631–637.