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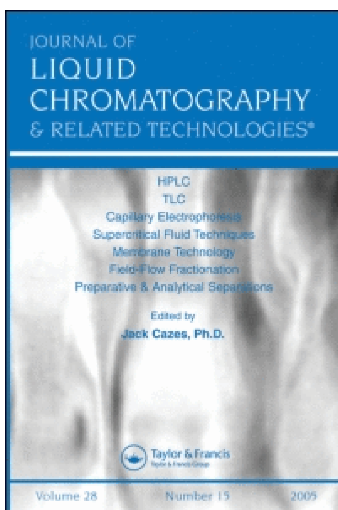

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HPLC Determination of Flunarizine Dihydrochloride in Tablets and Its Validation

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ABSTRACT

A simple, rapid, and validated HPLC method has been developed for determination of flunarizine dihydrochloride in the tablet preparations. A LiChrospher 100 RP-18 column was used with a mobile phase consisting of methanol–ion pair solution, 8 + 2, v/v. Quantitative evaluation was performed at 254 nm. The HPLC method is selective, precise, and accurate and can be used for routine analysis of the tablet preparations in pharmaceutical industry quality control laboratories.

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INTRODUCTION

Flunarizine dihydrochloride, trans-1-cinnamyl-4-(4,4′-difluorobenzenhydryl) piperazine hydrochloride, is a difluorinated derivative of cinnarizine, used for migraine prophylaxis, vertigo, and vestibular disorder, and for peripheral and cerebral vascular disorders.[1] The drugs also have antihistamine, sedative, and calcium channel blocking activity.

The Pharmacopoeia of the People's Republic of China describes titration and spectrophotometer methods for the determination of flunarizine dihydrocholoride.[2] No official method for the assay of flunarizine hydrochloride appear in USP 25-NF19,[3] BP 2000,[4] and Indonesian Pharmacopoeia 1995.[5] Wahbi et al.[6] reported the determination of flunarizine dihydrochloride and its degradation using liquid chromatography. Woestenborghs et al.[7] and Flor[8] reported the determination of flunarizine in biological fluids by using gas chromatography. Fuh and Hsieh[9] reported the determination of flunarizine in rat brain using liquid chromatography electrospray mass spectrometry.

The aim of this work was to develop a simple, validated, and rapid HPLC method for routine analysis of flunarizine dihydrochloride in tablet preparations using a photodiode array (PDA) detector.

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EXPERIMENTAL

Materials and Reagents

Flunarizine dihydrochloride (Welding GMBH & Co, Hamburg, Germany; Batch 06/01; Manufacturing date: 05/2001; Expiry date: 05/2006; Assay: 100.84%) was pharmaceutical grade. The substance was used as received for preparing laboratory-made pharmaceutical preparations. Their UV, IR spectra, and melting points were identical to the authentic flunarizine dihydrochloride (Sigma St. Louis, USA, Lot. 123 F 0307) that was used as a standard substance.

Methanol (JT Baker, USA), 1-hexanesulfonic acid sodium salt, di-*n*-butylamine (BDH, UK) were analytical grade reagents. The solvent and reagents were used without further purification. Excipients for laboratory-made tablet preparations (saccharum lactis, corn starch, calcium diphosphate, talcum, magnesium stearate, Vivapur® PVP, Tween 80, alcohol) were pharmaceutical grade substances.

Laboratory-made tablet preparations were prepared containing five different concentrations of flunarizine dihydrochloride (9.6, 10.8, 12.0, 13.2, and 14.4 mg tablet^{-1}); these were used for accuracy determination.

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Commercial tablet preparations containing flunarizine (10.0 mg tablet^{-1}; equivalent to 11.80 mg flunarizine dihydrochloride tablet^{-1}) were purchased in June 2002 from a local pharmacy in Surabaya (tablet-1, tablet-2). All the commercial pharmaceutical preparations were produced in Indonesia.

Stock standard solutions were prepared daily by dissolving accurately weighed flunarizine dihydrochloride (20.0 mg) in methanol (10.0 mL). Various standard solutions were prepared from the stock solution by dilution with methanol.

For linearity studies, flunarizine dihydrochloride (20.0, 30.0, and 40.0 mg) dissolved in methanol (10.0 mL) than diluted into various solutions contained 100, 150, 200, 250, 300, 350, 400, and 450 μg mL^{-1} of it, and each of these solutions (20 μL) was injected to HPLC.

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Sample Extraction

Twenty tablets were weighed and their mean weight was determined. The tablets were finely powdered, and an equivalent weight of a fifth tablet (equivalent to 2.4 mg flunarizine dihydrochloride) was transferred into a 10.0 mL volumetric flask containing about 9 mL of methanol, ultrasonicated for 15 min, and diluted to 10.0 mL with methanol. The solution was filtered through 0.45 μm Millipore filters before injection to the HPLC apparatus (20 μL).

Chromatography

The HPLC systems used in this work was comprised of a Hitachi L-6200 intelligent pump equipped with a Hitachi LC organizer and dynamic mixer mode 655A, a Hitachi L-4500 photo diode array (PDA) detector, and a Rheodyne 7125 injector (20 μL). The analysis was carried out on a LiChrospher 100 RP-18 (5 μm; Cat. 1.50943, 12 $\times$ 0.4 cm; E. Merck, Germany).

As mobile phase, a mixture of methanol–ion pair solution $8 + 2$, v/v was used. The ion pair solution contained an aqueous solution of 1-hexanesulfonic acid sodium salt 5 mM (BDH Laboratory Supplier, UK) and di-*n*-butylamine 20 mM (BDH Laboratory Supplier, UK). The mobile phase was prepared daily, filtered through 0.45 μm Millipore filters, and ultrasonicated for 30 min.

For qualitative analysis, the eluent was monitored using a PDA detector in the range of 210–400 nm. Quantitation was performed at its λ_{max} (254 nm) via peak areas with linear regression, using at least four-point calibration. All data evaluation (identification, purity check, and quantitation) was performed using a Hitachi model D-6500 chromatography data station software, DAD system manager.

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Validation

The method was validated for linearity, homogeneity, detection limit (DL), accuracy, and range by the method of Funk et al.[10] The selectivity of the method was proven by identification and purity checks of the analyte peaks. A five-point accuracy study (80–120% of the expected value) was performed on the laboratory-made tablet preparations. For commercial preparations, accuracy studies were performed using a single-point standard addition method (30% of label claim). The precision was evaluated by analyzing six different extract aliquots from laboratory-made tablets containing 9.6, 12.0, and 14.4 mg tablet^{-1} flunarizine dihydrochloride.

RESULTS AND DISCUSSION

Figure 1 showed the HPLC chromatogram of the standard of flunarizine dihydrochloride (R_t ca. at 2.70 min), its UV spectrum, and the contour plot of the HPLC chromatogram. The λ_{max} of flunarizine dihydrochloride (254 nm) was than selected for quantitative evaluation. Extract of the excipients of the laboratory-made tablet showed no peak (Fig. 2). All HPLC chromatograms of the extracts of laboratory-made and commercial tablets showed a single peak of flunarizine dihydrochloride. All the UV-spectra of the analyte peaks showed good correlation to the standard peak ($r > 0.99$). Purity checks of the analyte peaks showed that all the peaks were pure ($r > 0.99$). This showed that the proposed HPLC method is sufficiently selective. By using a PDA detector the identity and purity of the analyte peaks could be evaluated, so the presence of degradation product of the analyte or contaminated peak can be detected easily. The presence of a contaminated analyte peak could lead to a wrong result in the analysis. This cannot be done by using HPLC apparatus equipped with a conventional UV-detector only.

Using this HPLC system, linearity of flunarizine dihydrochloride was achieved in the range of 100–450 μg mL^{-1} (linear regression line equation: $Y = 144576 + 37153X$), $n = 8$, relative process standard deviation V_{XO}(10) $= 1.25\%$, $r = 0.9996$, injection volume 20 μL. The calculated value of *Prüfgröße* X_p (for $p = 0.05$)(10) was satisfactory (17.9 μg mL^{-1}). An ANOVA regression-test for linearity testing of the regression line showed significant calculated F-value (8877.98 for $p < 0.0001$). The plots of the residuals against the quantities of the analyte confirmed the linearity of the basic calibration graph (data not shown). The residuals were distributed at random around the regression line, neither trend nor uni-directional tendency was found. This basic linear calibration curve showed variance homogeneity over the whole range. The calculated parameter *Prüfwert* (PW)[10] was 1.12.

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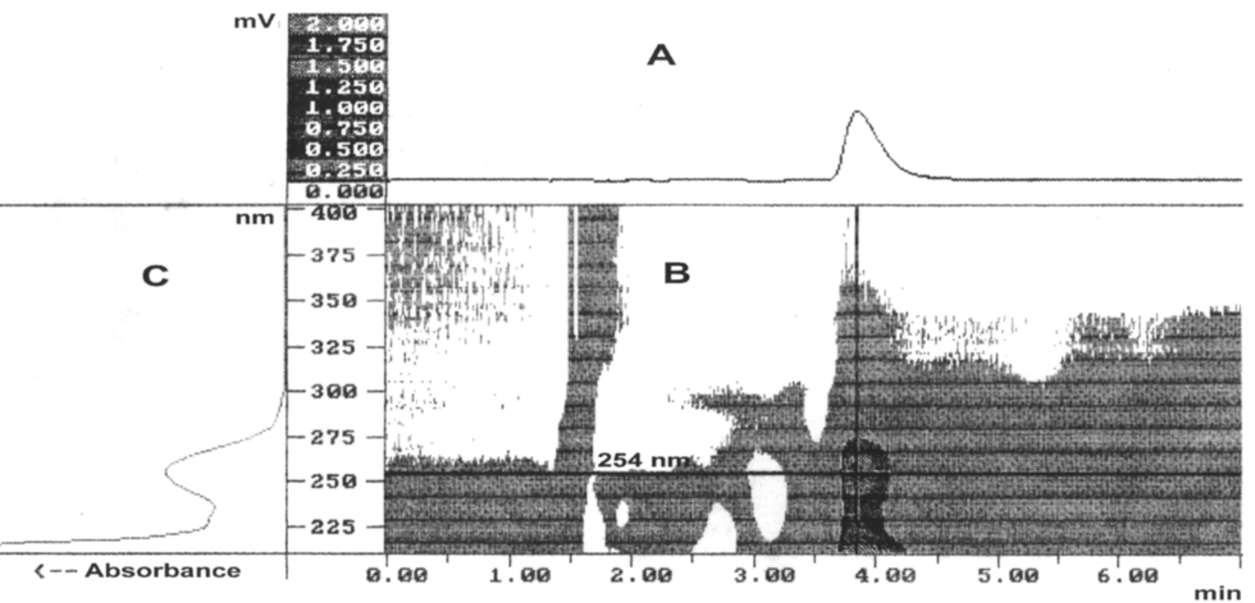

Figure 1. A typical HPLC chromatogram of flunarizine dihydrochloride (Sigma) using LiChrospher 100 RP-18 (stationary phase) and a mixture of methanol–ion pair solution 8 + 2, v/v as mobile phase, with flow rate of 0.7 mL min^{-1}. (A) HPLC chromatogram at 254 nm, (B) Contour plot of the HPLC chromatogram from 210 to 400 nm, (C) UV spectrum of flunarizine dihydrochloride peak.

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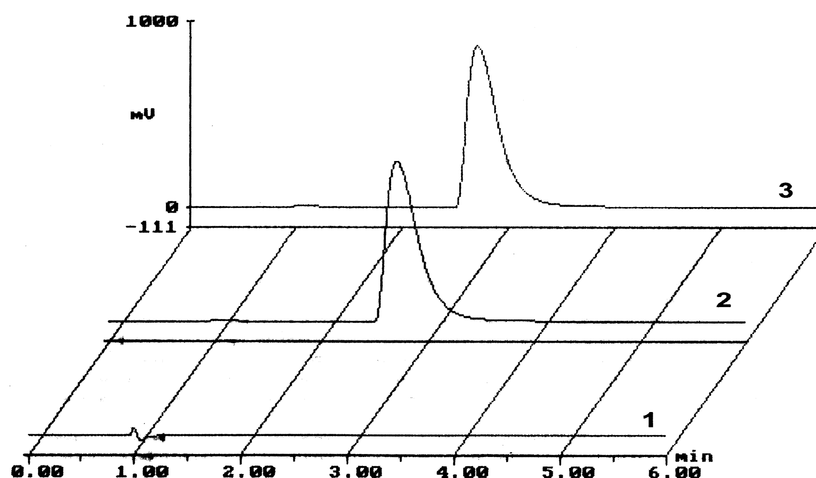

Figure 2. HPLC chromatograms at 254 nm. (1) Extract of excipient of laboratory-made tablets; (2) Extract of laboratory-made tablets; (3) Solution of flunarizine dihydrochloride (Sigma).

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The PW-values were less then the F_{table}-value (6.99; for $f_1 = 7$, $f_2 = 7$; $p = 0.01$).

Detection limit was determined by making a linear regression of relatively low concentration of flunarizine dihydrochloride (15–100 μg mL^{-1}, injection volume 20 μL) according to the method of Funk et al.[10] The calculated equation of the regression line was $Y = -3022 + 38343X$ ($n = 7$; $V_{XO} = 2.35\%$; $r = 0.9991$, $F_{\text{calculated}}$-value $= 3014.09$ for $p < 0.0001$). The calculated value of *Prüfgröße* X_p (for $p = 0.05$)(9)was 7.3 μg mL^{-1}. In this case, the value of DL $= X_p$.[10] According to Carr and Wahlich,[11] the value of the quantitation limit (QL) could be estimated as three times of the DL-value (21.9 μg mL^{-1} for injection volume 20 μL).

Table 1 demonstrates the high accuracy as revealed by the percentage of mean recovery data (100.6–100.9%). To prove whether systemic errors did not occur, linear regression of the recovery curve of X_f (concentration of the analyte measured by the proposed method) against X_c (nominal concentration of the analyte) of the laboratory-made tablets was constructed.[10] The confidence range data ($p = 0.05$) of the intercept [$V_{B(a_f)}$] and slope [$V_{B(b_f)}$] from the recovery curves did not reveal the occurrence of constant-and proportional-systematic errors.

All the RSD values of the repeatability and intermediate precision evaluations were less than 2% (see Table 2). These values were also less

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Table 1. Results from determination of the accuracy of analysis of the laboratory-made (LM) and commercial preparations.

Sample	Amount found[a] (Mean ± SD)[e]	Amount added[a]	Percentage of recovery (Mean ± SD)	Recovery curve[b]	$V_{B(af)}$[c]	$V_{B(bf)}$[c]
LM-tablet	—	—	100.80 ± 0.68[d]	$X_f = 6.00 + 0.98X_c$	6.00 ± 10.87	0.98 ± 0.05
Tablet-1	100.56 ± 0.66	40	100.66 ± 0.58[e]	—	—	—
Tablet-2	97.79 ± 0.97	40	100.97 ± 0.54[e]	—	—	—

[a] Percentage of label claim.
[b] X_f and X_c are, respectively, the measured and nominal concentration of the analyte (μg mL^{-1}; injection volume 20 μL).
[c] For $p = 0.05$.
[d] $n = 5$.
[e] $n = 3$.

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Table 2. Results from evaluation of precision of laboratory made-tablets.

	RSD-Value ($n=6$)[b]		
Measurement[a]	Laboratory-made tablet A[c]	Laboratory-made tablet B[d]	Laboratory-made tablet C[e]
1	0.46	0.25	0.42
2	1.19	0.87	0.58
3	0.42	0.81	0.32

[a]Each measurement was performed by a different analyst on different days.
[b]Evaluated by one analyst (repeatability).
[c]Content of flunarizine dihydrochloride was 9.6 mg $tablet^{-1}$.
[d]12.0 mg $tablet^{-1}$.
[e]14.4 mg $tablet^{-1}$.

than the required values that were described by Ermer[11] (1.44%; for specification range of 95–105%; basic lower limit 97.5%; $n=6$). The three measurements were performed within one laboratory by different analysts on the different days. These results demonstrated that the accuracy and precision of the proposed method were satisfactory in the range of 80 to 120% of the expected value.

Therefore, the proposed method is suitable for the routine analysis of products of similar composition in pharmaceutical industry quality control laboratories.

REFERENCES

1. Parfitt, K. *Martindale The Complete Drug Reference*, 32nd Ed.; Pharmaceutical Press: London, 1999; 411 pp.
2. *Pharmacopoeia of The People's Republic of China*, English Ed.; Chemical Industry Press: Beijing, China, 1997; Vol. II, 256–257.
3. *The United States Pharmacopoeia 2–National Formulary 20*, Asian Ed.; United States Pharmacopoeial Convention: Rockville, MD, 2001.
4. *British Pharmacopoeia 2000*; The Stationary Office: London, 2000.
5. *Farmakope Indonesia Edisi IV (Indonesian Pharmacopoeia IV)*; Departemen Kesehatan Republik Indonesia: Jakarta, Indonesia, 1995.
6. Wahbi, A.A.; Walify, A.F.; Hassan, E.M.; Salfman, F.G.; Gendi, A. Liquid chromatography determination of flunarizine dihydrochloride in the presence of its degradation product. J. Pharm. Biomed. Anal. **1995**, *13*, 777–784.

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7. Woestenborghs, R.; Michiefsen, L.; Lorreyne, W.; Heykants, J. Sensitive gas chromatography method for the determination of cinnarizine and flunarizine in biological samples. J. Chromatogr. **1982**, *232*, 85–91.
8. Flor, S.C. Determination of the calcium antagonist flunarizine in biological fluids by gas liquid chromatography. J. Chromatogr. **1983**, *272*, 315–323.
9. Fuh, M.R.; Hsieh, C.J. Determination of flunarizine in rat brain by liquid chromatography–electrospray mass spectrometer. J. Chromatogr. B. **1999**, *736*, 167–173.
10. Funk, W.; Damman, V.; Donnervert, G. *Qualitätssicherung in der Analytischen Chemie*; VCH: Weinheim, New York, Basel, Cambridge, 1992; 12–36, 161–180.
11. Carr, G.P.; Wahlich, J.C. A practical approach to method validation in pharmaceutical analysis. J. Pharm. Biomed. Anal. **1990**, *8*, 613–618.
12. Ermer, J. Validation in pharmaceutical analysis. Part I: An integrated approach. J. Pharm. Biomed. Anal. **2001**, *24*, 755–767.

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