DEVELOPMENT AND VALIDATION OF ANALYTICAL METHODS AND UNIFORMITY CONTENT OF FLUNARIZINE HYDROCHLORIDE IN TABLET PREPARATIONS

Fitra Yelli¹, Henny Lucida² and Harrizul Rivai²*

¹The Indonesian Food and Drug Authority (Indonesian FDA), Padang, Indonesia.
²Faculty of Pharmacy, Andalas University, Campus Limau Manih, Padang 25163, Indonesia.

ABSTRACT
Flunarizine dihydrochloride is an antivertigo, anti-migraine, and adjunctive therapy in epilepsy and available commercially in Indonesia in the tablet dosage form. The monograph that exists in standard literature is in the form of the capsule dosage form. This study aims to develop an analytical method of flunarizine dihydrochloride in tablets including an assay and content uniformity. Development of the assay and content uniformity has been done by High-Performance Liquid Chromatography (HPLC) with a slight modification of the method from Pharmacopoeia of The People's Republic of China (2010) for capsules. The development of methods for the assay and content uniformity of flunarizine dihydrochloride in tablets was successfully done by HPLC using C18 5 µm (4.6x150) mm column, mixture methanol, and phosphate buffer - triethylamine pH 3.5 at a ratio of (75:25) as the mobile phase at a flow rate of 1 mL/min using UV detector at 253 nm. Validation of the assay of flunarizine dihydrochloride tablets indicates that the method meets the requirements for accepting a valid, accurate, and specific method so that it can be proposed as a routine testing procedure for monitoring the flunarizine dihydrochloride tablets sold in the Indonesian market.

KEYWORDS: HPLC, flunarizine dihydrochloride, uniformity test, tablet.

INTRODUCTION
Flunarizine HCl (1- [bis (4-fluorophenyl) methyl] -4 - [(2E) -3-phenylprop-2-en-1-yl] piperazine dihydrochloride) is a difluorinated piperazine derivative.[¹] Its structure is shown
in Figure 1, formula C_{26}H_{26}F_{2}N_{2}, molar mass 404.495 g/mol, and melting point 251.5 °C (dihydrochlorides). Flunarizine HCl is a selective calcium entry blocker. Flunarizine is used in migraine prophylaxis, occlusive peripheral vascular disease, dizziness/vertigo of central or peripheral origin, and as adjunctive therapy in the management of epilepsy.

![Structure of flunarizine](image)

**Figure 1: Structure of flunarizine.**

In Indonesia, there are 19 brands of flunarizine dihydrochloride which are distributed and all are in the tablet dosage form. However, there is no official method for assay and content uniformity tests found in standard literature, including Indonesian Pharmacopoeia 2014, United States Pharmacopeia 42 2019, British Pharmacopeia 2019, Indian Pharmacopoeia 2010, and Japanese Pharmacopoeia 2016, neither is an official monograph.

The monograph of flunarizine dihydrochloride is found in Pharmacopoeia of The People's Republic of China, 2011, but in the capsule dosage form. The assay is conducted by HPLC with the mobile phase of the mixture of methanol and phosphate-triethylamine buffer at pH 3.5.

High-performance liquid chromatography (HPLC) is a sensitive method used extensively in the testing laboratory of the Indonesian Food and Drug Authority (Indonesian FDA). In the case of no available official method in Pharmacopoeia, the Indonesian FDA will develop its method which is usually used within the internal scope of the agency. Development of the analytical method and the content uniformity method of flunarizine dihydrochloride in the tablet dosage form in this study was carried out by adopting the monograph of flunarizine dihydrochloride capsule dosage form with the HPLC procedure in the Pharmacopoeia of The People's Republic of China, 2011.

A simple, rapid, and validated HPLC method has been developed for the 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. A 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.[8]

Determination of flunarizine hydrochloride in human blood serum has carried out by HPLC-MS/MS. The separation was carried out on a Shimadzu ODS column (150 mm × 4.6 mm, 5 μm) at 35 °C. The mobile phase was methanol -10 mmol/L ammonium acetate (pH 3.5 adjusted by acetic acid) (75:25), running at a flow rate of 1.0 mL/min. After separation, the mobile phase was split and 0.2 mL/min was into MS. HPLC-MS/MS was performed in the MRM mode using target ions at m/z 203.4 → m/z 183.1 for flunarizine and m/z 285.4 → m/z 193.2 for diazepam as an internal standard.[9]

In this study, we tried to develop several mobile phases for determining the levels of flunarizine hydrochloride in the RP-HPLC column. The purpose of this study was to find a valid analysis method for flunarizine hydrochloride in tablet preparations. This analysis method is expected to be used to determine the uniformity of flunarizine hydrochloride content in tablet preparations.

MATERIALS AND METHODS

Materials

Flunarizine dihydrochloride reference standard was accepted from the Indonesian FDA (Indonesia) (Indonesian Pharmacopoeia reference standard). The commercial products of flunarizine dihydrochloride 10 mg tablets were purchased, consisted of 1 generic product (G) and 2 branded name products (F and S). Methanol for liquid chromatography (Merck, Germany), acetonitrile for liquid chromatography (Merck, Germany), distilled water (Brataco, Indonesia), potassium dihydrogen phosphate for analysis (Merck, Germany), triethylamine for synthesis (Merck, Germany), ortho-phosphoric acid 85% for analysis (Merck, Germany), hydrochloric acid fuming 37% for analysis (Merck, Germany), sterile water for injection (Ika Pharmindo, Indonesia) filtered with regenerated cellulose 0.45 μm membrane filter (Sartorius, Germany), PVDF 0.45 μm syringe filter (Whatman, USA), and PTFE 0.45 μm syringe filter (Whatman, USA). The following instruments were used: MSA6.6S-000-DM Micro Balance & MSA225S-100-DU Analytical Balance (Sartorius, Germany), Seven Easy pH meter (Mettler Toledo, USA), Ultrasonic Bath CPXH-8800 Branson (Branson, USA), e 2695 Alliance series High-Performance Liquid
Chromatography system with 2489 UV/Vis Detector, 2998 PDA Detector and equipped with C18 5 \( \mu m \) (4.6 x 150) mm XBridge column (Waters, USA).

Development of the HPLC assay and content uniformity methods

**Optimization of chromatographic conditions**

Optimization was carried out using two chromatographic conditions. First, mixture methanol and phosphate buffer - triethylamine pH 3.5 (solution of 1.36 g KH$_2$PO$_4$ in 1 L distilled water, added 4 mL of triethylamine, adjusted to pH 3.5 with phosphoric acid) at composition ratio A (75: 25) was used as the mobile phase with a UV detector at 253 nm.\(^7\) Second, a mixture phosphate buffer pH 4.5 (solution of 0.272 g KH$_2$PO$_4$ in 1 L distilled water, adjusted to pH 4.5 with phosphoric acid) and acetonitrile at composition ratio B1 (60: 40); B2 (55: 45) dan B3 (50: 50) as the mobile phase with a UV detector at 252 nm and 30 °C column temperature.\(^10\) Twenty \( \mu L \) of filtered standard solution equivalent to 100 ppm of flunarizine in the mobile phase was injected into the HPLC under each of the above conditions using a C18 5 \( \mu m \) (4.6 x 150) mm column with a flow rate of 1 mL/min.

**Standard solution preparation**

As a standard stock solution, flunarizine dihydrochloride is accurately weighed into a volumetric flask and dissolved with methanol to volume. The stock solution was diluted with 0.1 N HCl to produces a standard solution with 5 levels of concentration equivalent to 6; 8; 10; 12 and 14 ppm, and then all of these solutions were filtered with a 0.45 \( \mu m \) PTFE syringe filter. Before injecting each regression standard, the system suitability test is carried out by injecting one standard solution 6 times to ensure that the HPLC system is running stable.

**Assay**

As a test solution, homogeneous powders from 20 tablet samples were each weighed accurately equivalent to 10 mg of flunarizine to a 100 mL volumetric flask, added 10 mL of methanol, sonicated for 15 minutes, diluted with 0.1 N HCl to volume, mixed well and filtered. Each filtrate was diluted with 0.1 N HCl to obtain a 10 ppm flunarizine solution and filtered with a 0.45 \( \mu m \) PTFE syringe filter. Test solutions and standard solutions were injected 20 \( \mu L \) into the HPLC system with chromatographic conditions based on an optimized method and selected as a method for assay of flunarizine HCl in the tablet dosage form.
Content uniformity
This procedure was carried out on 10 tablets from each sample. Each tablet was put into a 100 mL volumetric flask, added 10 mL of methanol, and approximately 20 mL of 0.1 N HCl, sonicated for 30 minutes, diluted with 0.1 N HCl to volume, mixed well and filtered. Each filtrate was diluted with 0.1 N HCl to obtain a 10 ppm flunarizine solution and filtered with a 0.45 μm PTFE syringe filter. Each 20 μL of this solution was injected into the HPLC under the same conditions as in the assay.

Validation of the assay
The precision was determined through an assay procedure of 10 replications using one of the sample tablets by calculation the RSD value. Using the same HPLC conditions as in the assay, accuracy was tested by determining the recovery of flunarizine dihydrochloride reference standard which added to the analyte in tablet samples (using the standard addition method). The total amount of flunarizine analyte in the test solution varying from 80%; 100% to 120% (each has 3 replications), each solution consisted of 70% analytes from the tablet sample powder and 30% from the standard solution of flunarizine dihydrochloride added. In specificity testing, solvents (blank), standard solution (10 ppm), test solution (precision test solution) and solution of a mixture of sample and standard (100% accuracy test solution) were injected into the HPLC successively using the same method as in the assay, but with a PDA detector at a wavelength of 210 - 400 nm. The limit of detection and the limit of quantitation were also calculated.

RESULT AND DISCUSSION
Data on retention time, tailing factors and theoretical plates for flunarizine dihydrochloride standard with various mobile phase compositions can be seen in Table 1. The standard solution of flunarizine dihydrochloride (equivalent to 10,023 mg/100 mL) which injected using method A,[7] produces a chromatogram peak at a retention time of 3.676 with the tailing factor value is 0.884 and the number of theoretical plates is 4204. Optimization then was carried out to avoid the use of triethylamine in the mobile phase of method A, because of its more toxic and corrosive nature. Another alternative is to use the mobile phase in the B1 method, which is a combination of phosphate pH 4.5 and acetonitrile (60: 40).[10] But this combination produces peaks with longer retention times (26.947 minutes) and less ideal tailing factors (T = 2.762). Furthermore, several other combinations were tried at a pH of 4.5 and acetonitrile, but they still did not produce peaks with better performance. Another
combination of the methods B2 (55:45) and B3 (50:50) can accelerate the retention time but produce a peak fronting with the tailing factor values of 0.777 and 0.627, respectively. Besides the chromatogram profile is worse. Profiles of flunarizine dihydrochloride chromatograms in methods A and B can be seen in Figure 2.

Table 1: Data of retention times, tailing factors and theoretical plates for flunarizine dihydrochloride standard solution with various mobile phase compositions.

<table>
<thead>
<tr>
<th>Mobile phase compositions</th>
<th>Ratio</th>
<th>Method ID</th>
<th>Retention times (minute)</th>
<th>Tailing factors (T)</th>
<th>Theoretical plates (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphate buffer-triethylamine pH 3.5 : methanol</td>
<td>(25 : 75)</td>
<td>A</td>
<td>3.676</td>
<td>0.884</td>
<td>4203.778</td>
</tr>
<tr>
<td>Phosphate buffer pH 4.5 : acetonitrile</td>
<td>(60 : 40)</td>
<td>B1</td>
<td>26.947</td>
<td>2.762</td>
<td>3918.335</td>
</tr>
<tr>
<td></td>
<td>(55 : 45)</td>
<td>B2</td>
<td>14.311</td>
<td>0.777</td>
<td>3104.949</td>
</tr>
<tr>
<td></td>
<td>(50 : 50)</td>
<td>B3</td>
<td>9.563</td>
<td>0.627</td>
<td>883.488</td>
</tr>
</tbody>
</table>

Figure 2: (a) Chromatogram of flunarizine dihydrochloride by method A, and (b) method B (the composition of phosphate buffer pH 4.5: acetonitrile) at comparison B1 (60:40); B2 (55:45) and B3 (50:50)

Optimization of chromatographic conditions was chosen based on observations of the retention times, tailing factors, and theoretical plates. However, the use of the method A mobile phase produces peaks with better performance, faster retention times, tailing factors, and theoretical plates that meet the ideal peak requirements. Therefore, mixture methanol and phosphate buffer - triethylamine pH 3.5 (solution of 1.36 g KH₂PO₄ in 1 L distilled water, added 4 mL of triethylamine, adjusted to pH 3.5 with phosphoric acid) at composition ratio A (75:25) in method A was still selected as the mobile phase in this study.
The results of the assay and content uniformity of flunarizine in the tablet samples (Table 2) showed that method A (which is the adoption of the flunarizine dihydrochloride analysis method in capsules) can also be used for the analysis of flunarizine dihydrochloride in tablet dosage forms, where all tablet samples used have percent levels ranging from 90% - 110%. However, there are changes in the solvent used in the preparation procedure, which is methanol, replaces the function of ethanol (as in the procedure for assay of flunarizine dihydrochloride capsules in Pharmacopoeia of The People’s Republic of China monograph) because of flunarizine dihydrochloride is slightly soluble in water, ethanol (0.1 – 1 %), and HCl concentration. The use of methanol in the early stages of the preparation aims to increase the solubility because the highest solubility of flunarizine HCl is in methanol.\textsuperscript{[11]} The use of methanol also aims to minimize the variations of the solvent to be more similar to the mobile phase conditions in method A to help reduce the amount of noise on the chromatogram.

**Table 2: The result of assay and content uniformity of flunarizine HCl tablets.**

<table>
<thead>
<tr>
<th>Tablet samples name</th>
<th>Assay (%)</th>
<th>Content uniformity AV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G</td>
<td>100.34 ± 0.099</td>
<td>6.294</td>
</tr>
<tr>
<td>F</td>
<td>101.59 ± 0.248</td>
<td>6.605</td>
</tr>
<tr>
<td>S</td>
<td>97.31 ± 0.411</td>
<td>3.823</td>
</tr>
</tbody>
</table>

In the content uniformity test, it is necessary to add 0.1 N HCl together with methanol at the beginning of the preparation. This aims to help the process of disintegration of tablets with the help of aqueous solution molecules which cannot be achieved if only pure methanol is used as the initial solvent. The content uniformity test results are represented as AV values in which all tablet samples used to meet the requirements (Table 2) by Indonesian Pharmacopoeia 2014 (AV ≤ 15 %).\textsuperscript{[12]}

The development of a method for the assay of flunarizine dihydrochloride in the tablet dosage form has been validated (as shown in Table 3). It provides linearity (regression equation is \( y = 58599x – 25922 \) with \( R^2 \) value 0.9999) (Figure 3), precision (100.94 ± 0.573 % with RSD 0.568 %) and accuracy test results (98.26 % to 101.93 %) that meet the acceptance requirements.

**Table 3: The summary of the assay validation of flunarizine dihydrochloride in tablet dosage form.**
Assay validation parameters | Result | Acceptance criteria
--- | --- | ---
Linearity | $R^2 : 0.9999$ | $R^2$ value $\geq 0.999$
Precision | RSD : 0.568\% | RSD $\leq 2.0\%$
Accuracy | 98.26\% to 101.93\% | 98.0\% to 102.0\%
Specificity | The spectrum profile of the tablet sample solution is the same as the standard solution of flunarizine dihydrochloride | The spectrum profile of the sample solution is the same as the standard solution

<table>
<thead>
<tr>
<th>Injected solution</th>
<th>Retention time</th>
<th>Purity Angle</th>
<th>Purity Threshold</th>
<th>Retention time of sample solution = standard solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solvent</td>
<td>3.336</td>
<td>4.355</td>
<td>4.308</td>
<td></td>
</tr>
<tr>
<td>Standard</td>
<td>3.354</td>
<td>0.060</td>
<td>0.228</td>
<td>Purity angle &lt; purity threshold</td>
</tr>
<tr>
<td>Sample</td>
<td>3.358</td>
<td>0.066</td>
<td>0.239</td>
<td></td>
</tr>
<tr>
<td>Standard spiked sample</td>
<td>3.353</td>
<td>0.086</td>
<td>0.232</td>
<td></td>
</tr>
</tbody>
</table>

Percentage of the area of the solvent compared to the area of the standard solution : 0.503\%

Range | 80.45\% to 121.01\% | -
Limit of detection | 0.2251 ppm | -
Limit of quantitation | 0.7502 ppm | -

Figure 3: Calibration curves for regression standard solution of linearity test for assay of flunarizine dihydrochloride in tablet dosage form.

Specificity test evaluations were carried out on the value of the solvent area and UV absorption spectrum characterization using EmpowerTM 3. This method is specific if the purity angle value is smaller than the value of each purity threshold and the value of the solvent area does not exceed 1\% compared to the standard area. Specificity test results of the
assay indicate the same identity and shape of the UV spectrum between the standard solution and the sample solution and standard spiked sample solution, as shown in Figure 4, which provides maximum wavelengths at 252.3 nm each.

![Figure 4: UV spectrum of a) solvent, b) standard solution, c) sample solution and d) standard spiked sample solution in assay specificity test of flunarizine dihydrochloride](image)

The 2-dimensional UV spectrum profile of the solvent looks similar to the standard solution of flunarizine dihydrochloride, but its 3-dimensional UV spectrum profile explains the difference, as shown in Figure 5. PDA analysis results on the standard solution, sample solution, and standard spiked sample solution showed that flunarizine has a purity angle of 0.060; 0.066 and 0.086, respectively, where these values are smaller than the value of the purity threshold, 0.228; 0.239 and 0.232 respectively and this shows the peak purity of flunarizine dihydrochloride at the retention time. The percentage value of the solvent area compared to the standard solution is 0.503 %, which means that it meets the requirements of ≤ 1 %, indicating the solvent does not affect the results of the determination of flunarizine dihydrochloride levels. Overall based on the results of validation, the assay of flunarizine dihydrochloride in tablets can be declared accurate and valid as long as it is used according to the range (80.45 % to 121.01 %) and limits of detection (0.2251 ppm) and quantitation (0.7502 ppm) that have been calculated.
Figure 5: 3-dimensional chromatogram a) solvents, b) standard solutions, c) sample solutions and d) standard spiked sample solutions in assay specificity test of flunarizine dihydrochloride.
Several alternatives in the selection of non-toxic mobile phases have been carried out to develop a method for assay flunarizine dihydrochloride in the tablet dosage form. However, after being optimized, the mobile phase adopted from Pharmacopoeia of The People's Republic of China,\(^7\) in the assay procedure of flunarizine dihydrochloride monograph in capsule dosage form, remains the best choice as the mobile phase for assay of flunarizine dihydrochloride in the tablet dosage form. Modification of ethanol solvents which are replaced with methanol in the preparation of the assay procedure aims to make the conditions of the solvent in the test solution more similar to the mobile phase used. While the use of HCl with a concentration of 0.1 N aims to make the solvent conditions in the test solution as same as the dissolution media so that the chromatographic conditions can be used simultaneously with the dissolution test. Content uniformity was carried out by modifying the replacement of ethanol solvents with methanol and adding 0.1 N HCl at the beginning of preparation to help the process of tablet breakage.

CONCLUSIONS
In summary, a new HPLC method has been successfully developed for assay and content uniformity of flunarizine dihydrochloride tablet dosage form by HPLC using C18 5 μm (4.6 x 150) mm column, mixture methanol and phosphate buffer - triethylamine pH 3.5 (solution of 1.36 g KH\(_2\)PO\(_4\) in 1 L distilled water, added 4 mL of triethylamine, adjusted to pH 3.5 with phosphoric acid) at composition ratio A (75: 25) as the mobile phase at a flow rate of 1 mL/min using UV detector 253 nm. The results of the validation of the assay of flunarizine HCl in tablets dosage form indicate that the method meets the acceptance requirements as a valid, accurate, and specific method so that it can be proposed as a routine testing method for monitoring flunarizine dihydrochloride tablets sold in the Indonesian market.

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CONFLICT OF INTEREST
The authors declare that there are no conflicts of interest.

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