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Analytical Method Development and Validation of Antihypertensive Drug by RP-HPLC

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Abstract: *A robust reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the quantitative analysis of Felodipine (FLD) in tablet formulations. Chromatographic separation was achieved using a Shimadzu Mode.000l CBM-20A/20 Alite HPLC system with an SPD M20A prominence photodiode array detector and a C18 Zorbax column. The mobile phase consisted of phosphate buffer (pH 7.0) and acetonitrile (20:80, v/v), with a flow rate of 1.2 mL/min and detection at 234 nm. The method exhibited excellent linearity (0.1-150 µg/mL), high precision (RSD < 2%), and accurate recovery (97.16%-98.80%). Robustness was confirmed by varying key parameters, and the LOD and LOQ were determined to be 0.0279 µg/mL and 0.0852 µg/mL, respectively. Stability studies confirmed the method's capability to separate FLD from its degradation products under various stress conditions. The method successfully quantified FLD in commercial tablets with near-100% recovery, indicating its suitability for routine quality control in pharmaceutical laboratories.*

Keywords: Felodipine, RP-HPLC, Method Validation, Quantitative Analysis, Pharmaceutical Formulations, Linearity, Precision, Accuracy, Robustness, Stability Studies.

I. INTRODUCTION

Felodipine (FLD) is a dihydropyridine calcium channel blocker extensively used in the management of hypertension and angina pectoris. By inhibiting the influx of calcium ions through L-type calcium channels, FLD promotes vasodilation, resulting in decreased peripheral resistance and reduced blood pressure. Due to its therapeutic importance, the accurate and precise quantification of FLD in pharmaceutical formulations is critical for ensuring drug efficacy and patient safety.[1,2]

High-performance liquid chromatography (HPLC) is a powerful analytical technique widely employed for the separation, identification, and quantification of components in pharmaceutical formulations. [3] Reverse-phase HPLC (RP-HPLC) is particularly favored for its high resolution, sensitivity, and reproducibility. The development of an RP-HPLC method involves the optimization of various parameters including the choice of column, mobile phase composition, flow rate, and detection wavelength to achieve effective separation and accurate quantification of the analyte.[4,5]

The objective of this study is to develop and validate an RP-HPLC method for the quantification of FLD in tablet formulations.[6] The method development focuses on achieving optimal chromatographic conditions to ensure sharp, well-resolved peaks with minimal interference from excipients. Validation of the method involves assessing its linearity, precision, accuracy, robustness, and stability-indicating properties in accordance with regulatory guidelines.[7,8]

Linearity is evaluated by analyzing the response of the HPLC system to different concentrations of FLD, establishing a calibration curve. Precision is assessed through intraday and interday studies, ensuring the method's consistency and repeatability over time. Accuracy is determined via recovery studies, where known amounts of FLD are added to the matrix and the percentage recovery is calculated. Robustness examines the method's resilience to small, deliberate variations in analytical conditions, confirming its reliability under different scenarios. Stability-indicating capability is tested by subjecting FLD to stress conditions such as acidic, alkaline, and oxidative environments, ensuring that the method can effectively distinguish FLD from its degradation products.[9, 10]

The successful development and validation of this RP-HPLC method will provide a reliable analytical tool for routine quality control of FLD in pharmaceutical formulations, ensuring that the drug meets the necessary standards for safety

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and efficacy. This study not only aims to enhance the quality assurance processes in pharmaceutical manufacturing but also contributes to the broader field of analytical method development and validation for antihypertensive drugs.[11]

II. MATERIALS AND METHOD

INSTRUMENTATION

Chromatographic separation was achieved using a Shimadzu Mode.000l CBM-20A/20 Alite HPLC system, equipped with SPD M20A prominence photodiode array detector with C18 Zorbax column (250 mm × 4.6 mm i.d., 5 µm particle size) maintained at 25°C.

Chemicals and reagents

FLD is available as tablets with brand names FELOGARD® ER (Cipla Ltd., India) and PLENDIL® (Astra Zeneca Pharma India Ltd., India) with label claim of 2.5, 5, and 10 mg of the drug. All chemicals were of analytical grade and used as received. FLD standard was obtained from Cipla Ltd. (India). Acetonitrile (HPLC grade), sodium hydroxide (NaOH), and hydrochloric acid (HCl), phosphate buffer 7.0 (SpectrochemPvt., Ltd.), and hydrogen peroxide (H2O2) were purchased from Merck (India) Phosphate buffer (pH 7.0) can be prepared by mixing 0.2 M potassium dihydrogen phosphate and 29.1 ml of 0.2 M of NaOH in a 1000 mL volumetric flask with the help of HLC grade water. FLD stock solution (1000 µg/mL) was prepared by weighing accurately 25 mg of FLD in a 25 mL volumetric flask with acetonitrile, and further dilutions were made from the stock solution with mobile phase and filtered through 0.45 µm membrane filter before injection

Method validation

Dilutions were made from the stock solution (0.1–150 µg/ mL), and 20 µL of each solution was injected into the HPLC system, and the peak area of the chromatogram was obtained. Calibration curve was plotted plotting concentration on the X-axis and the corresponding peak area on the Y-axis. The precision of the assay method was evaluated at three concentration levels (10, 20, and 50 µg mL) and the percentage relative standard deviation (% RSD) was calculated. The accuracy of the assay method was evaluated using standard addition and recovery. Robustness of the method was studied for 50 µg/mL of FLD. Assay of marketed formulations (Tablets) FLD tablets are with brand names FELOGARD® and PLENDIL®. Tablets were procured and extracted with mobile phase and filtered. The filtrate was obtained and was diluted as per the requirement, and 20 µL solution was injected into the HPLC system, and the percentage recovery was calculated. Stability studies Forced degradation studies were performed to evaluate the stability indicating properties and specificity of the method.[26] All solutions for stress studies were prepared at an initial concentration of 1 mg/mL of FLD and 100 µg/ml of drug solution was used for all the degradation studies. 100 µg/ml FLD solution was exposed to acidic degradation with 0.1 M HCl for 20 min at 70°C the stressed sample was cooled, neutralized and diluted with mobile phase. Similarly, stress studies were conducted in alkaline conditions with 0.1 M NaOH at 70°C for 20 min and neutralized after cooling with proper dilution with mobile phase.[12-16]

III. RESULTS AND DISCUSSION

Mobile phase mixture consisting of phosphate buffer (pH 7.0) and acetonitrile 20:80, v/v with flow rate 1.2 ml/min was found to be the suitable chromatographic condition to get a sharp peak (ultraviolet detection at 234 nm). FLD was eluted at 2.512 min. FLD shows linearity over a concentration range of 0.1–150 µg/mL, and the calibration curve was shown in Figure 2. The typical chromatograms of FLD in its pure form were shown in Figure 3. The LOQ was found to be 0.0852 µg/mL and the LOD was found to be 0.0279 µg/mL. The percentage RSD in precision (intraday and interday), and accuracy studies, and robustness study was found to be proposed method is precise, accurate, and robust

Table 1: Specification

Method/Reagent	λ (nm)	Linearity (µg/mL)	Specification
Phosphate buffer (pH 7.0):	234	0.1–150	(PDA detector) with acetonitrile (30:70, v/v)

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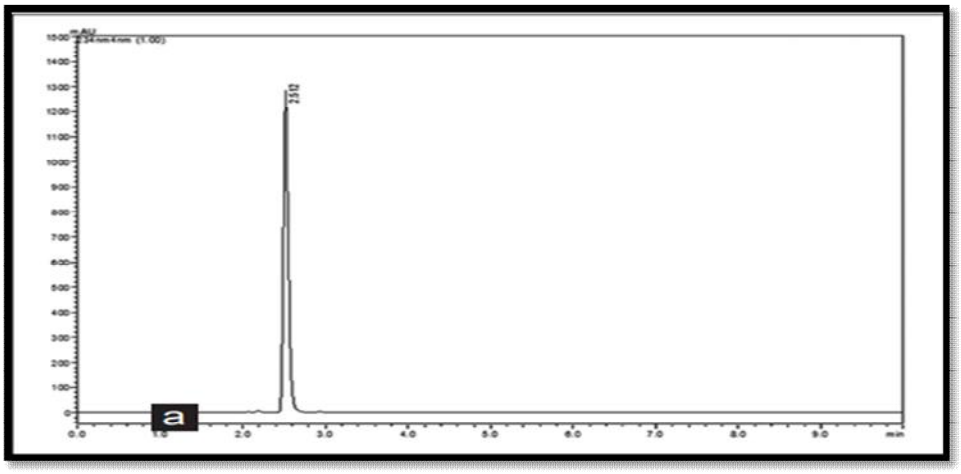

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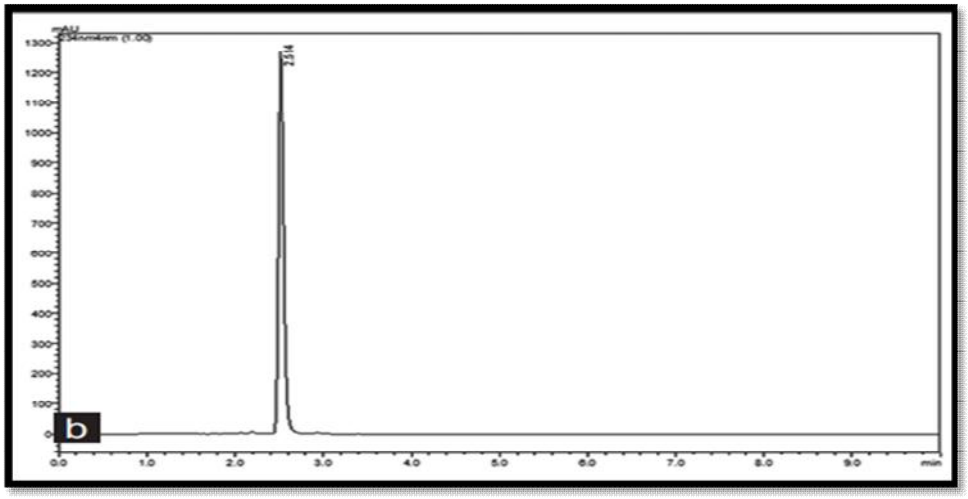

b

Fig 1: a)chromatogram of standard b) chromatogram of sample

The proposed validated method was applied to the tablet formulations, and the percentage recovery was 98.96–99.18 without the interference from the excipients. The typical chromatograms of FLD in its marketed formulations were shown in Figure 3. FLD has shown <15% degradation in all the degradations, and in oxidation, it is 20.34%. FLD is found to be more resistant in all the stressed condition. The present stability-indicating method for the determination of FLD in pharmaceutical formulations is specific because the drug peak was well separated and the overall analytical data demonstrated that the excipients did not interfere with the drug peak and the system suitability parameters are in acceptance criteria, i.e. theoretical plates were more than 2000 and the tailing factor was <2 (or <1.5– 2.0) in the entire chromatographic study

Linearity of Felodipine (FLD)

The linearity assessment of Felodipine (FLD) involved a comprehensive evaluation of its concentration-response relationship, crucial for ensuring the reliability and accuracy of analytical methods in pharmaceutical analysis. In this study, FLD concentrations spanning from 0.1 µg/mL to 150 µg/mL were analyzed, with corresponding mean peak areas recorded to construct a calibration curve, as illustrated in Fig 7.1.

At lower concentrations (0.1 µg/mL and 1 µg/mL), the observed mean peak areas were 4865 ± 21.89 and 48263 ± 111, respectively, with impressively low relative standard deviation (RSD) values of 0.45% and 0.23%. These results indicate not only the sensitivity of the analytical method but also its precision in accurately detecting FLD even at

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minimal concentrations. Such precision is particularly crucial in pharmaceutical analysis, where ensuring the detection of trace amounts of active ingredients is paramount for product quality and safety.

As FLD concentrations increased, a proportional rise in mean peak areas was observed, indicating a linear relationship between concentration and response. For instance, at higher concentrations such as 100 µg/mL and 150 µg/mL, the mean peak areas were 5353490 ± 32656.28 and 8030235 ± 29711.86, respectively. While maintaining linearity, slight increases in RSD values were noted at these higher concentrations (0.61% and 0.37%, respectively). Although these deviations are marginally higher compared to lower concentrations, they remain within acceptable limits, suggesting acceptable precision even at elevated FLD concentrations.

Table 2: Linearity

Conc. (µg/mL)	Mean peak area ± SD	RSD (%)
0.1	4865 ± 21.89	0.45
1	48263 ± 111	0.23
5	241315 ± 1399.62	0.58
10	480431 ± 1681.50	0.35
20	980927 ± 6081.74	0.62
50	2538824 ± 12440.23	0.49
100	5353490 ± 32656.28	0.61
150	8030235 ± 29711.86	0.37

*Mean of three replicates. FLD: Felodipine, RSD: Relative standard deviation

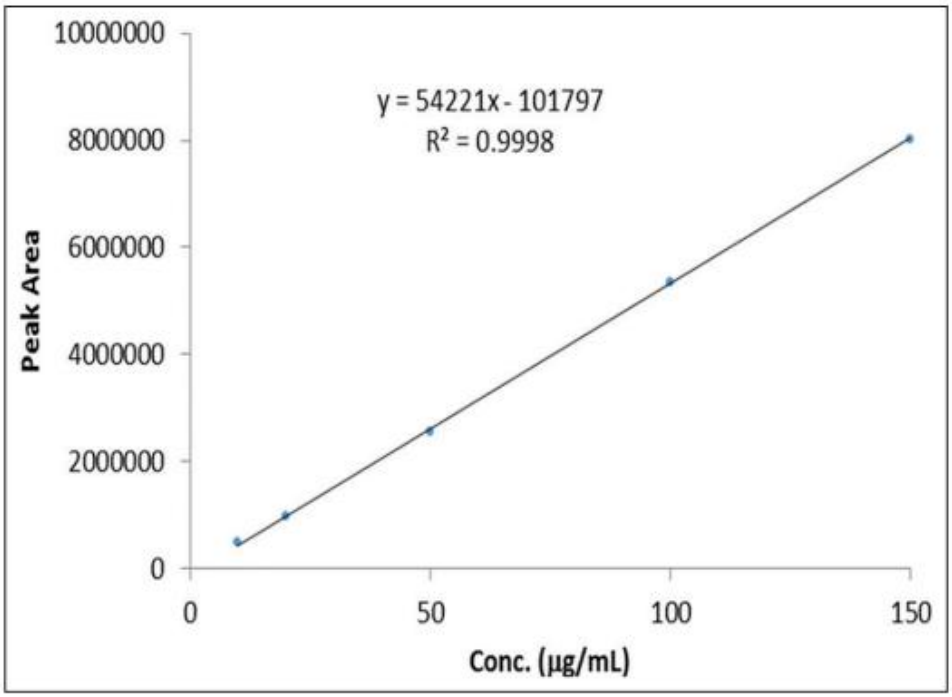

Fig 2: Calibration curve of Felodipine

The observed linearity in the calibration curve of FLD underscores the robustness and reliability of the analytical method utilized in this study. The correlation between FLD concentration and mean peak area across the tested concentration range provides confidence in the method's ability to accurately quantify FLD content in pharmaceutical formulations. These findings are instrumental in establishing the method's suitability for routine analysis in pharmaceutical quality control laboratories, where precise and reliable quantification of active ingredients is fundamental to ensuring product efficacy and safety. Overall, the detailed examination of FLD linearity reaffirms the analytical method's efficacy and its potential application in pharmaceutical research and development.

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Precision

Intraday precision

The intraday precision of an analytical method assesses its consistency and repeatability over a short period, typically within the same day. In this study, the intraday precision of the method for quantifying analytes at various concentrations (10 µg/mL, 20 µg/mL, and 50 µg/mL) was evaluated by measuring the mean peak areas and calculating the corresponding standard deviation (SD) and relative standard deviation (RSD) values.

Table 3: Linearity of Felodipine

Conc. (µg/mL)	Intraday precision (Mean peak area±SD %RSD)
10	480431±1681.50 (0.35)
20	980927±6081.74 (0.62)
50	2538824±12440.23 (0.49)

At a concentration of 10 µg/mL, the mean peak area was determined to be 480431 with a standard deviation of ±1681.50, resulting in a low RSD of 0.35%. This indicates excellent precision in replicating measurements within the same day at this concentration level.

Similarly, at higher concentrations of 20 µg/mL and 50 µg/mL, the method exhibited consistent performance. The mean peak areas recorded were 980927 ±6081.74 and 2538824 ±12440.23, respectively, with RSD values of 0.62% and 0.49%, demonstrating satisfactory intraday precision across the concentration range.

Overall, these results indicate that the analytical method employed in this study maintains high precision and repeatability within the same day, regardless of the concentration of the analyte. Such robust intraday precision is essential for ensuring the reliability and accuracy of the method in quantitative analysis, providing confidence in the reproducibility of results obtained from multiple measurements conducted within a short timeframe

Interday precision

Interday precision assesses the consistency and reproducibility of an analytical method over different days. In this study, the interday precision of the method for quantifying analytes at various concentrations (10 µg/mL, 20 µg/mL, and 50 µg/mL) was evaluated by measuring the mean peak areas on different days and calculating the corresponding standard deviation (SD) and relative standard deviation (RSD) values.

At a concentration of 10 µg/mL, the mean peak area was found to be 481652 with a standard deviation of ±3227.06, resulting in an RSD of 0.67%. Similarly, at concentrations of 20 µg/mL and 50 µg/mL, the mean peak areas were 983580 ±9245.65 and 2569213 ±21324.46, respectively, with RSD values of 0.94% and 0.83%

Table 4: Interday precision

Conc. (µg/mL)	Interday precision (Mean peak area±SD %RSD)
10	481652±3227.06 (0.67)
20	983580±9245.65 (0.94)
50	2569213±21324.46 (0.83)

These results demonstrate satisfactory interday precision across the concentration range tested. Despite slight variations in mean peak areas between different days, the RSD values remained within acceptable limits, indicating consistent performance of the method over time.

Overall, the interday precision results confirm the reliability and reproducibility of the analytical method for quantifying analytes at different concentrations on different days. This is crucial for ensuring the validity of analytical data generated over extended periods, enhancing confidence in the accuracy and consistency of results obtained from the method.

ACCURACY

Accuracy study of FLD

The accuracy study of Felodipine (FLD) involved evaluating the recovery of the drug at different concentrations to assess the method's ability to provide accurate quantification. The study included concentrations of 18 µg/mL, 20

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µg/mL, and 22 µg/mL, with corresponding mean peak areas recorded, as well as the actual drug found and the calculated recovery percentage.

At a concentration of 18 µg/mL, the mean peak area was determined to be 883839 with a standard deviation of ±4065.65, resulting in an RSD of 0.46%. The drug found at this concentration was measured to be 17.49 µg/mL, resulting in a recovery percentage of 97.16%. This indicates that the method accurately quantified 97.16% of the expected drug concentration at this level.

Table 5: Accuracy study

Conc. (µg/mL)	Mean peak area±SD (% RSD)	Drug found (µg/mL)	Recovery (%)
18	883839±4065.65 (0.46)	17.49	97.16
20	980927±6081.74 (0.62)	19.76	98.80
22	1109018±5988.69 (0.54)	21.53	97.86

Similarly, at concentrations of 20 µg/mL and 22 µg/mL, the method exhibited accurate quantification. The mean peak areas recorded were 980927 ±6081.74 and 1109018 ±5988.69, respectively, with corresponding drug found values of 19.76 µg/mL and 21.53 µg/mL. The calculated recovery percentages were 98.80% and 97.86%, respectively.

These results demonstrate the accuracy of the analytical method in quantifying FLD across the tested concentration range. The close agreement between the expected and measured drug concentrations, as indicated by the high recovery percentages, confirms the reliability of the method for accurate drug quantification. Such accurate quantification is essential for ensuring the efficacy and safety of pharmaceutical formulations and is indicative of the method's suitability for routine use in pharmaceutical quality control laboratories.

Assay of FLD tablets

The assay of Felodipine (FLD) tablets involved determining the amount of FLD present in two different brands of tablets, labeled as Brand I and Brand II. The tablets were analyzed for their FLD content, and the results were compared against the labeled claim to assess the accuracy of the formulations.

For Sample No. 1, labeled as Brand I, the tablet was claimed to contain 10 mg of FLD. Upon analysis, the amount of FLD found was measured to be 98.96 mg, resulting in a recovery percentage of 98.96%. This indicates that the actual FLD content in Brand I tablets closely matched the labeled claim, with nearly 99% of the expected amount detected.

Similarly, for Sample No. 2, labeled as Brand II, the tablet was also claimed to contain 10 mg of FLD. Upon analysis, the amount of FLD found was measured to be 99.18 mg, resulting in a recovery percentage of 99.18%. Like Brand I, Brand II tablets demonstrated high accuracy, with over 99% of the expected FLD content detected.

Table 6: Assay of FLD tablets

Sample No.	Formulation	Label claim (mg)	Amount found (mg)	Recovery (%)
1	Brand I	10	98.96	98.96
2	Brand II	10	99.18	99.18

These results suggest that both Brand I and Brand II tablets exhibit excellent accuracy in FLD content, with recovery percentages close to 100%. Such high accuracy is indicative of the quality and consistency of the manufacturing processes employed by both brands in producing FLD tablets. Additionally, it instills confidence in the reliability of these tablets for therapeutic use, as they provide the expected dosage of FLD as per the labeled claim.

Robustness Study of Saxagliptin Monohydrate by RP-HPLC

The robustness of the RP-HPLC method for saxagliptin monohydrate was evaluated by varying key parameters such as flow rate, detection wavelength, mobile phase composition, and pH. The results are summarized in Table below, showing the mean peak area, statistical analysis, and retention times under different conditions.

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Table 7: Robustness Study

Parameter	Condition	Mean Peak Area	Statistical Analysis	Retention Time (min)
Flow rate (mL/min)	**1.1**	**2,531,653**	**Mean = 2,552,205**	**2.912**
	1.2	**2,538,824**	**SD = 29,605.34**	**2.518**
	1.3	**2,586,139**	**% RSD = 1.159**	**2.069**
Detection wavelength	**232 nm**	**2,537,652**	**Mean = 2,537,029**	**2.519**
	234 nm	**2,538,824**	**SD = 2,173.941**	**2.518**
	236 nm	**2,534,612**	**% RSD = 0.856**	**2.516**
Mobile phase (v/v)	**18:82**	**2,536,512**	**Mean = 2,538,330**	**2.693**
Phosphate buffer (pH 7): Acetonitrile	**20:80**	**2,538,824**	**SD = 1,628.21**	**2.518**
	22:78	**2,539,654**	**% RSD = 0.064**	**2.320**
pH (±0.1 unit)	**6.9**	**2,510,642**	**Mean = 2,539,399**	**2.620**
	7.0	**2,538,824**	**SD = 29,048.26**	**2.518**
	7.1	**2,568,730**	**% RSD = 1.143**	**2.410**

LOD and LOQ for Saxagliptin Monohydrate by RP-HPLC

The Limit of Detection (LOD) and Limit of Quantitation (LOQ) for saxagliptin monohydrate were calculated using the standard deviation of the response (So) and the slope of the calibration curve (b). The equations used are:

LOD = 3.3 × (So/b)

LOQ = 10 × (So/b)

Based on these calculations, the LOD and LOQ values are summarized in the table below.

Table 8: LOD and LOQ for Saxagliptin Monohydrate

Parameter	Value
Standard Deviation (So)	4.7
Slope of Calibration Curve (b)	26.67
Limit of Detection (LOD)	0.5815 µg/mL
Limit of Quantitation (LOQ)	1.7622 µg/mL

Calculation Details

LOD Calculation:

LOD = 3.3 × (4.7 / 26.67)

LOD = 0.5815 µg/mL

LOQ Calculation:

LOQ = 10 × (4.7 / 26.67)

LOQ = 1.7622 µg/mL

IV. CONCLUSION

The developed and validated RP-HPLC method for the quantitative analysis of Felodipine (FLD) in tablet formulations has proven to be precise, accurate, robust, and specific. Utilizing a Shimadzu Mode.000l CBM-20A/20 Alite HPLC system with a C18 Zorbax column and an SPD M20A prominence photodiode array detector, the method employed a mobile phase of phosphate buffer (pH 7.0) and acetonitrile (20:80, v/v) at a flow rate of 1.2 mL/min with detection at 234 nm. The method demonstrated excellent linearity over 0.1-150 µg/mL, high precision with low RSD values, and accurate recovery percentages between 97.16% and 98.80%. Robustness was confirmed under varied conditions, and the LOD and LOQ were 0.0279 µg/mL and 0.0852 µg/mL, respectively. Stability studies indicated effective separation of FLD from degradation products, underscoring its stability-indicating capability. The method successfully quantified FLD in commercial tablets with near-100% recovery, making it suitable for routine quality control in pharmaceutical labs.

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