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Validation of UV-VIS Spectrophotometric Method For The Penetration Test of Pickering Emulsion Diclofenac Diethylamine

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Abstract

Pickering emulsions are emulsions stabilized by solid particles that adsorb at the interface of both phases, acting as emulsifiers without requiring amphiphilic surface properties (hydrophilic and hydrophobic segments) to stabilize the emulsion (Hutami et al., 2024). This approach allows the formulation to avoid the use of potentially toxic surfactants (Wu et al., 2020). The objective of this study was to validate an analytical method for the penetration test using a Franz diffusion cell for a diclofenac diethylamine Pickering emulsion formulation, ensuring that the UV-Vis spectrophotometric analysis method is reliable by fulfilling all validation requirements. Based on the validated parameters, the assay procedure for the penetration test (Franz diffusion cell) of the diclofenac diethylamine Pickering emulsion using UV-Vis spectrophotometry met the established criteria. The linearity with a correlation coefficient (r) of 0.9997, indicating excellent linearity. Precision testing showed a %RSD of 0.608%, indicating good repeatability. Accuracy testing at 80%, 100%, and 120% concentration levels yielded recovery results ranging from 99.822% to 101.157%. The Limit of Detection and Limit of Quantification, calculated from the absorbance at the lowest concentrations, were found to be 6.46×10^{-4} ppm and 1.96×10^{-3} ppm, respectively. Therefore, the method is considered valid, having met all analytical method validation parameters.

Keywords: *Accuracy, Diclofenac Diethylamine, Precision, Recovery, Validation Method*

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Introduction

Pickering emulsions are solid-stabilized emulsions that were first discovered by Ramsden (Ramsden, 1904) and Pickering (Pickering, 1907). They can be used to avoid the use of toxic surfactants (Wu et al., 2020). The added solid particles provide long-term stability because they accumulate at the liquid interface in the emulsion, forming a layer around the dispersed droplets and preventing droplet coalescence (Prasanthi et al., 2020). Therefore, Pickering emulsions are surfactant-free emulsions in which solid particles replace classical nonometric/micrometric particle sizes by adsorbing at the oil–water interface (Vishwakarma & Singh Panwar, 2022). Topical formulations for pain relief, particularly those containing diclofenac, are commonly prepared in emulsion forms. The salt forms of diclofenac used in topical (transdermal) preparations are diclofenac sodium and diclofenac diethylamine. These two salts have different physicochemical properties, resulting in different skin permeation capabilities. Diclofenac diethylamine (DDE) belongs to BCS Class II, characterized by low solubility and high permeability. It has a partition coefficient of 0.853, indicating relatively low lipophilicity (Windhu Wardhana et al., 2014; Hutami et al., 2024). Therefore, DDE is formulated as a Pickering emulsion to enhance its skin penetration, allowing the active compound to reach deeper layers of the skin and target treatment areas more effectively, as the drug is not retained solely on the skin surface (Nurmalia et al., 2020).

To support the therapeutic effect of the Pickering emulsion formulation, the DDE concentration needs to be determined during the penetration test. Hence, method validation is necessary to ensure a high-quality formulation that guarantees efficacy and safety. Validation is a technique used to demonstrate that every material, process, procedure, system activity, and equipment used in a procedure consistently yields expected results (Oktriana et al., 2022). The aim of the method validation in this study is to determine whether the method used for quantifying DDE in Pickering emulsion formulations is valid for penetration testing, based on specific parameters. The parameters used to evaluate the method's validity include linearity, precision, accuracy, selectivity, LOD (Limit of Detection), and LOQ (Limit of Quantification).

Material and Methods

Materials used in this study included diclofenac diethylamine (Aarti Drugs, India), sodium diclofenac (Merck, Germany), bentonite (cosmetic grade), kaolin (Aeon Procare, India), Avicel RC-591 (FMC International, Ireland), sodium aceticum (Merck, Germany),

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acidum aceticum (Merck, Germany), Natrosol 250 HX (Zhejiang, China), soybean oil (cosmetic grade) and distilled water (PT Zenith Pharmaceutical, Indonesia).

Equipment used in this study included an analytical balance (Ohaus), an ultrasonicator (Elmasonic, Germany), a pH meter (Eutech Instruments), a UV-Vis spectrophotometer (Hitachi), glassware (Pyrex, Japan), and other non-glass laboratory equipment.

Linearity

A standard solution of diclofenac diethylamine at a concentration of 1000 ppm was prepared by accurately dissolving 50 mg of diclofenac sodium in 50 mL of methanol, and subsequently diluting the solution to a final volume of 100 mL with methanol in a volumetric flask. The solution was sonicated using an ultrasonicator for 30 minutes until completely dissolved. Methanol was then added to reach the final volume. A calibration curve of diclofenac diethylamine was prepared by diluting the stock solution to concentrations of 8, 12, 16, 20, and 24 ppm. The absorbance of each solution was measured at its maximum wavelength, and a linear regression equation ($y = a + bx$) was established between concentration (ppm) and absorbance (Kumar et al., 2015).

Precision and Accuracy

Precision testing was conducted using a 100% concentration solution, which was measured six times. The relative standard deviation (RSD) of the measured concentrations was calculated, with the acceptance criterion of RSD ≤ 2%.

Accuracy testing was performed using the standard addition method, where diclofenac diethylamine was added to a placebo Pickering emulsion matrix. The Pickering emulsion preparations contained diclofenac diethylamine at 80%, 100%, and 120% levels. Each concentration level was evaluated in triplicate, and the percent recovery was calculated to assess method accuracy. The acceptance range for percent recovery was set at 98.0%–102.0% (Muslich et al., 2020).

For the accuracy assessment, diclofenac diethylamine was weighed at three concentration levels: 21.025 mg (80%), 26.281 mg (100%), and 31.537 mg (120%). Each sample was dissolved in phosphate buffer pH 7.4 and diluted to volume in a 50 mL volumetric flask. Subsequently, 1 mL of each solution was transferred into separate 50 mL volumetric flasks for further analysis. The placebo matrix was prepared by dissolving 123.55 mg of placebo in phosphate buffer pH 7.4 up to the mark in a 50 mL volumetric flask. Volumes of 0.8 mL (80%), 1.0 mL (100%), and 1.2 mL (120%) of the placebo solution were

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P-ISSN : 2715-3053

then added into the corresponding 50 mL volumetric flasks containing the drug solutions, followed by phosphate buffer addition up to the mark. For calculating the percent recovery, following equation was utilized : (Nugroho & Alrayan, 2024):

$$\% \text{ Recovery} = \frac{\text{The concentration obtained}}{\text{The actual concentration}} \text{ x } 100\%$$

The relative standard deviation was calculated using the following equation:

$$\text{RSD} = \frac{\text{SD}}{\text{X}} \text{ x } 100\%$$

Where :

RSD : Relative Standart Deviation

SD : Standart Deviation

X : The average concentration of the active substance

Selectivity

The selectivity test was performed to ensure that the UV–Vis spectrophotometric method is capable of specifically measuring diclofenac diethylamine without interference from other components present in the Pickering emulsion matrix. Selectivity refers to the precise and careful measurement of a specific analyte in the presence of other components that may be present in the sample matrix, such as impurities or excipients (Harmita, 2004). The test was conducted by comparing the absorbance of the diclofenac diethylamine standard and the absorbance of the diclofenac diethylamine Pickering emulsion with that of the placebo in the Pickering emulsion formulation.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The Limit of Detection (LOD) and Limit of Quantitation (LOQ) are interrelated analytical parameters, with the LOD defined as the minimum analyte concentration that can be reliably detected but not necessarily quantified, and the LOQ as the lowest concentration that can be quantitatively determined with acceptable precision and accuracy under the established experimental conditions(Andaririt et al., 2025). The LOD and LOQ were estimated using the standard deviation of the analytical response and the slope derived from the calibration curve, in accordance with established validation guidelines (Harron, 2013). For calculating the LOD & LOQ, following equation was utilized :

$$\text{LOD} = \frac{3{,}3 \text{ x Sy}}{\text{b}}$$

$$\text{LOD} = \frac{10 \text{ x Sy}}{\text{b}}$$

Where Sy is the standard deviation and b is the slope.

E-ISSN : 2715-0518
P-ISSN : 2715-3053

Results and Discussion

Determination of Maximum Wavelength (λmax)

The maximum wavelength was determined by dissolving diclofenac diethylamine in phosphate buffer at pH 7.4, resulting in a λmax of 275.2 nm with an average absorbance value of 0.646. Theoretically, the maximum wavelength of diclofenac sodium is approximately 276 nm (Kemenkes, 2020). Therefore, the analysis at a wavelength of 275.2 nm can be applied for the determination of penetrated DDE content using the Franz diffusion cell method.

Linearity

Linearity is the ability of the analytical method to elicit test results that are directly proportional to the concentration of analyte within a specified range, as demonstrated by appropriate statistical and mathematical methods. The linear relationship between diclofenac diethylamine concentration and absorbance was established by preparing five concentrations and measuring their absorbance using UV-Vis spectrophotometry at the maximum wavelength of 275.2 nm. The DDE standard series ranged from 10 to 20 ppm. The regression equation of the diclofenac diethylamine calibration curve followed the model y = ax + b and is presented in Figure 1, showing a strong correlation with an r-value of 0.9997. According to the Kemenkes, (2020), a correlation coefficient (r) of ≥ 0.999 is required for an analytical method to be considered valid. A correlation value close to 1 indicates a strong linear relationship between the measured absorbance and the analyte concentration (Nurmalia et al., 2020).

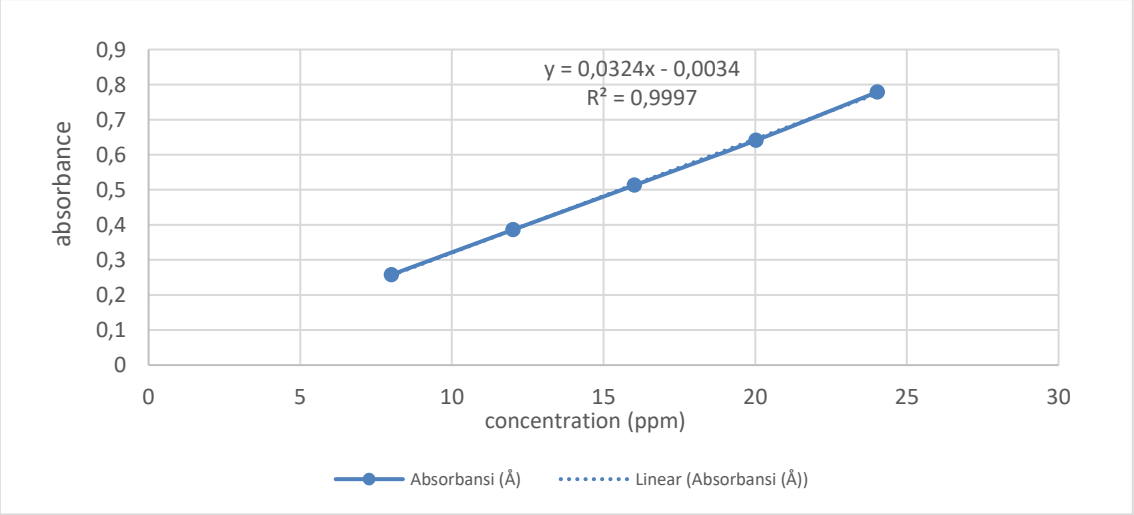

Figure 1. Calibration Curve of Diclofenac Diethylamine Standard

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P-ISSN : 2715-3053

Precision

Precision is a measure that indicates the degree of agreement between individual test results and the mean when the procedure is repeated on samples taken from a homogeneous mixture (Harmita, 2004). Precision is determined based on the relative standard deviation (RSD) to assess possible errors in the analytical process. An analytical method is considered to have good precision if it produces an RSD value of less than 2%. In this study, an RSD value of 0.608% was obtained, indicating that the method meets the established criteria. The results of the precision test calculations are presented in Table 1.

Table 1. The results of the precision test

No	Weight (g)	Sample dilution	Drug content (mg)	Sample concentration (mg/ml)	Sample absorbance	Concentration (%)
1	2,281	1250	11,609	0,02118	0,567	100,662
2	2,287	1250	11,609	0,02124	0,563	99,690
3	2,289	1250	11,609	0,02126	0,562	99,426
4	2,291	1250	11,609	0,02128	0,565	99,869
5	2,281	1250	11,609	0,02118	0,562	99,774
6	2,278	1250	11,609	0,02116	0,568	100,972
					SD	0,609
					RSD	0,608

Accuracy

Accuracy evaluation aims to assess the closeness of the measured values to the true concentration of the analyte. It is expressed as the percentage recovery of the analyte added to the sample matrix. Accuracy is considered more important than precision, as accuracy ensures the correctness of the analytical results, while precision ensures the repeatability or consistency of the measurements. In this study, accuracy was determined by precisely weighing diclofenac diethylamine, mixing it with a placebo, and preparing emulsions containing diclofenac diethylamine at three concentration levels: 80%, 100%, and 120% of the target concentration. An analytical method is considered accurate when the percentage recovery falls within the acceptable range of 98.0% to 102.0%. The accuracy results in this study showed recovery values ranging from 99.822% to 101.157% (table 2), indicating compliance with the established acceptance criteria. Based on the accuracy test results at three concentration levels (80%, 100%, and 120% of the target concentration), the percentage recovery was found to be within the range of 98.0–102.0%. These values comply with the accuracy criteria specified in the ICH Q2(R1) guidelines, indicating that the

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analytical method employed is accurate for the quantification of diclofenac diethylamine in the emulsion formulation (ICH Q2(R2), 2023).

Table 2. The results of the accuracy test

Concentration (%)	Replication	Sample Absorbance	The concentration obtained (%)	The actual concentration (%)	Recovery (%)
80	1	0,453	17,159	17,000	100,938
	2	0,45	17,046	17,000	100,269
	3	0,451	17,084	17,000	100,492
				Rata – rata	100,566
100	1	0,566	21,440	21,250	100,891
	2	0,564	21,364	21,250	100,535
	3	0,56	21,212	21,250	99,822
				Rata – rata	100,416
120	1	0,682	25,834	25,501	101,306
	2	0,681	25,796	25,501	101,157
	3	0,681	25,796	25,501	101,157
				Rata – rata	101,207

Selectivity Testing

Based on the selectivity test, the placebo showed an absorbance value of 0.000, indicating that the excipients used did not interfere with the analysis of the active ingredient (diclofenac diethylamine). The sample of pickering emulsion diclofenac diethylamine showed an absorbance of 0.589, while the diclofenac diethylamine standard showed an absorbance of 0.561 at a wavelength of 275.2 nm.

LOD and LOQ

The Limit of Detection (LOD) is defined as the lowest concentration of analyte in a sample that can be detected, though not necessarily quantified with exact precision. The Limit of Quantification (LOQ) represents the lowest concentration that can be quantitatively determined with acceptable levels of accuracy and precision. Both LOD and LOQ were statistically estimated using the standard deviation of the response and the slope (b) of the calibration curve. The results obtained in this study were LOD is $6{,}46 \times 10^{-4}$ ppm and LOQ is $1{,}96 \times 10^{-3}$ ppm. A low LOD indicates that the analytical method is capable of detecting very small analyte concentrations with high accuracy, whereas a high LOD suggests that the method can only detect higher analyte concentrations with lower accuracy. Similarly, a low LOQ signifies that the analytical method can quantitatively determine very small analyte concentrations with high accuracy and reproducibility, while a high LOQ indicates that the method can only measure higher analyte concentrations with lower accuracy and reproducibility (Harmita, 2004).

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Conclusion

Based on the validated parameters, the procedure for determining the concentration of diclofenac diethylamine in the Pickering emulsion formulation using the Franz diffusion cell for penetration testing with a UV-Vis spectrophotometer has met the required analytical criteria. The results showed a correlation coefficient (r) of 0.9997, relative standard deviation (RSD) of 0.608%, percent recovery ranging from 99.822% to 101.157%, LOD of 6.46×10^{-4} ppm, and LOQ of 1.96×10^{-3} ppm. Therefore, the method is considered valid, as it fulfills the necessary validation parameters for an analytical method.

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The authors declare that there is no conflict of interest associated with this publication.

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