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Determination of Diclofenac Diethylamine Levels in Emulgel Preparations Using NIR Spectroscopy Combined with Chemometrics

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Abstract

Diclofenac is an NSAID-class drug with activity as an analgesic and anti-inflammatory recommended for treating various acute and chronic pain conditions. One of the topical preparations of diclofenac that is often used is emulgel. In this study, diclofenac diethylamine levels in emulgel preparations were determined using NIR (Near-Infrared) spectroscopy and chemometric methods. Simulation samples were prepared and divided into 24 training sets and 9 test set samples. NIR spectra of training set samples were correlated with the concentration of diclofenac diethylamine using partial least squares (PLS), principal component regression (PCR), and support vector regression (SVR). The best model was validated using leave one out cross validation (LOOCV) and external validation using test set samples. The comparison method used in this study was the validated TLC Densitometry method. The best calibration model was PLS, with an R^2 value of 0.990 and RMSE of 0.171. The results of R^2 and RMSE of LOOCV were 0.989 up to 0.990 and 0.167 up to 0.178, respectively. The result of R^2 and RMSEP external validation were 0.991 and 0.146, respectively. The precision and accuracy method showed RSD of 3.37% and a % recovery of 99.78%. The results of determining the sample levels obtained from NIR and TLC Densitometry methods tested with the Two-Paired Sample T Test and showed that the two methods have no significant differences with a significance value of more than 0.05.

Keywords: NIR spectroscopy, Chemometrics, Diclofenac diethylamine, Emulgel

Introduction

Osteoarthritis (OA) is a disease that often occurs in the elderly. Osteoarthritis is characterized by cartilage degeneration, where the damage can cause pain and loss of ability to move [1]. World Health Organization (WHO) in 2018 stated that the number of people suffering from osteoarthritis was 343 million worldwide [2]. The initial treatment for mild osteoarthritis is paracetamol, this is because paracetamol is safe, effective, and cheap. However, the U.S. Food and Drug Administration does not recommend taking more than 4,000 mg of paracetamol per day to avoid liver toxicity. When paracetamol cannot reduce symptoms, in cases of moderate to severe osteoarthritis, NSAID treatment is recommended [3]. Diclofenac is an NSAID (Non-steroidal Anti-Inflammatory Drugs) class drug that has activity as an anti-inflammatory, analgesic, and antipyretic. This drug is commonly used to treat acute and chronic pain, rheumatoid, and osteoarthritis. One of the topical preparations of diclofenac that is often used is emulgel. Emulgels are

emulsions, either oil in water or water in oil, which are mixed into gel preparations with gelling agents [4]. The use of the topical route can avoid first pass metabolism, direct administration to the target site, the administration may be more acceptable to patients to improve compliance, effective for patients who have difficulty swallowing [5].

Several research methods have been conducted to determine the levels of diclofenac diethylamine. These methods were HPLC (High-Performance Liquid Chromatography) [6], TLC (Thin Layer Chromatography) [7], UV-Vis spectroscopy [8,9], and spectrofluorometry [10]. In this study, the determination of diclofenac diethylamine levels in emulgel preparations was evaluated using NIR spectroscopy and chemometric methods. This method was chosen because there has been no analysis of diclofenac diethylamine in emulgel preparations using NIR spectroscopy. NIR spectroscopy is an effective analytical technique because it does not require solvents, does not cause contamination, does not require chemicals, and has the high analytical capability [11]. However, the NIR spectra were complicated and overlapping, so a multivariate analysis was needed. Multivariate analysis is a mathematical and statistical method that can separate data from analytical information, such as NIR spectrum information called chemometrics [12]. Chemometric techniques were used to correlate the spectrum profile and the information contained in the sample [13]. Quantitative multivariate analysis techniques were used partial least squares (PLS), principal component regression (PCR), and support vector regression (SVR) [14].

Materials and Methods

Chemical and Reagents

Diclofenac diethylamine used in this study was Pharmaceutical Grade (Aarti Drugs Ltd, India). All ingredients of emulgel preparation were pharmaceutical grade, i.e., carbopol (CV Kimia Jaya Labora), liquid paraffin, PEG 400, nipagin, nipasol (Sigma-Aldrich), propylene glycol, TEA (CV Nurra Gemilang Malang).

Reagents used were analytical grade, i.e., methanol pro analysis (Merck), toluene, ethyl acetate, glacial acetic acid, filter paper (Whatman), distilled water, and TLC plates (Merck). Four commercial samples of diclofenac diethylamine emulgel were purchased from a pharmacy store in East Java, Indonesia, in August of 2021.

Instrumentation

The tools used in this study were a Densitometer scanner (Camag), winCATS software, NIR spectroscopy (Brimrose Luminar 3070), The Unscrambler X 10.4 software (Camo), analytical balance (Sartorius), ultrasonicator (Elmasonic), capillary micro pipette (Socorex), mortar and stamper, and glassware.

Sample Simulation Preparation

The preparation of emulgel simulation samples was based on Bhanu et al. with modification. Diclofenac diethylamine emulgel simulation samples were made in oil-in-water type with the addition of diclofenac diethylamine. Simulated emulgel samples were prepared by distinguishing between the oil phase and the liquid phase. In the aqueous phase, carbopol and distilled water were crushed in a mortar, then TEA was added. Nipagin and nipasol were dissolved in propylene glycol. In the oil phase, liquid paraffin was dissolved, and PEG 400 was heated in a cup at 75°C. The oil phase was added gradually to the water phase with continuous stirring until a fine emulsion was formed, and then spiking diclofenac diethylamine to the emulgel gradually until

varying concentrations were obtained. The simulation samples were divided into a training set and a test set sample. The training set sample consists of 24 samples with a concentration variation range of diclofenac diethylamine of 0% - 5.75%, while the test set sample consists of 9 samples with a concentration of 0.6% - 5.4%.

Determination of NIR Spectra

The samples were analyzed with a NIR instrument, Luminar 3070. Before the samples were measured, the instrument was heated for 30 min. The sample was placed on the sample holder plate. Each sample was replicated 5 times, and each replication was subjected to 5 shots. The spectra wavelength range was 850 nm - 2000 nm.

Preparation of Diclofenac Diethylamine Standard Solution

The standard solution of diclofenac diethylamine in methanol was made at a concentration of 100, 200, 400, 600, 800, 1000 and 1200 µg/mL.

Preparation of Sample

The emulgel sample was weighed 300 mg in a beaker glass and extracted with methanol, then ultrasonicated for 15 min. The extracted sample was put into a 10 mL volumetric flask and rinsed the beaker glass with the solvent, then added methanol up to 10 mL. The extracted sample was filtered using filter paper and put into a vial.

Method Validation

The determination of the levels of the training set, test set, and commercial samples was carried out after this comparison method was validated through the stages of eluent optimization, wavelength optimization, linearity, specificity, detection limit and quantitation limit, precision and accuracy [15].

Chemometrics Calibration Model and Validation

The chemometrics calibration model for quantitative analysis in this study was formed with PLS, PCR, and SVR multivariate analysis techniques. The selected calibration model was validated using LOOCV and external validation. LOOCV evaluated the model using the training set data by removing a set of data then the remaining data was used to form a new model. The process was repeated until all data was used as a validation set. External validation used an independent sample (test set) to evaluate the model by comparing the predicted value of the test set sample generated from the model with the reference value [16]. The accuracy and precision of the method were evaluated using three levels of concentration of the sample and three replication [17].

The valid calibration model was applied to the determination of diclofenac diethylamine in the commercial sample and then compared with the levels obtained from the comparison method (TLC densitometry). The comparison methods were tested with the Two Paired Samples T-test to determine whether there was a significant difference.

Results and Discussion

In this study, the diclofenac diethylamine standard solution concentration range of 0% - 5.75% was chosen as the training set because this range already covered the concentration range of diclofenac diethylamine on commercial emulgel. The simulated training set and commercial emulgel sample (Fig. 1) have similar spectral patterns. The spectra of the training set simulation

sample and the commercial emulgel sample have different transmittance values.

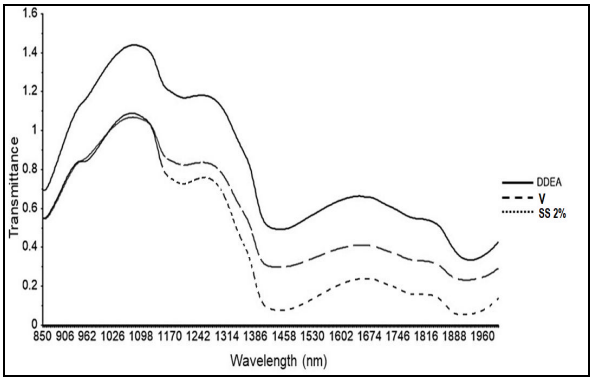

Figure 1. **NIR spectra of diclofenac diethylamine (DDEA), real sample (V), and simulation sample 2% (SS 2%)**

The TLC Densitometry results of eluent optimization were toluene: ethyl acetate: glacial acetic acid (v/v/v) = 8:2:0.3 with Rf value of 0.48 which is included in the range of optimum Rf 0.2-0.8; Rs value of 2.214 which has met the resolution requirements of greater than 1.5; the largest N value was 237.037; and the smallest H value was 0.379. The optimum wavelength was 284 nm because it had the highest reflectance value. The method used as a comparison method has been validated with the results of the parameter assessment of each validation stage listed in Table 1.

Table 1. **TLC densitometry method validation results.**

Validation Parameters	Results
Linearity	
Linierity Range (n=5)	404 – 3232 ng
Correlation coefficient (r)	0.998
Coefficient of Variation (*Vx0*)	3.867%
LOD	98.179 ng
LOQ	294.54 ng
Specificity	
Purity and identity test	R >0.99
Precision (RSD, n=9)	1.227%
Accuracy (%*recovery* ± RSD (%)	
Simulation 0.6%	100.33 ± 0.831
Simulation 1.2%	100.583 ± 0.911
Simulation 1.8%	97.44 ± 1.938

This method fulfilled the linearity requirement, i.e., correlation coefficient (r) ≥ 0.99 and the coefficient of function variation (Vxo) < 5%. The purity test was determined based on the r(s,m) value and the r(m,e) value which produces a value of more than 0.99. The identity test was determined based on the r(s,s) value and the r(s,a) value where the r(s,s) value showed the spectral correlation between the two standard tracks. In contrast, r(s,a) showed the correlation between the standard track and the analyte track in the sample. The analyte in the sample was identical to the standard if the correlation value was more than 0.99 [18]. It can be concluded that the analytes in the standard and sample are pure and identical. The assessment of the precision and accuracy fulfilled the acceptance requirement of the RSD value for the precision test of AOAC [19].

The results of the calibration model in Table 2 showed that the three calibration models formed met the criteria for a good calibration model where the R^2 value was more than 0.91. In this study, the PLS calibration model was the best model because it has the highest R^2 value of 0.990 and the smallest RMSE value of 0.171.

Table 2. **Training set sample calibration model results.**

No.	Model		RMSE	R^2
1.	PLS	Calibration	0.171	0.990
		Validation	0.176	0.989
2.	PCR	Calibration	0.492	0.918
		Validation	0.495	0.917
3.	SVR	Calibration	0.394	0.948
		Validation	0.399	0.947

The LOOCV results are shown in Table 3. LOOCV has R^2 >0.91, and the result of the RMSE value was small. The PLS model was valid in LOOCV.

Table 3. **PLS calibration model LOOCV validation results.**

Data removed		RMSE	R^2
No data removed	Calibration	0.167	0.989
	Validation	0.174	0.989
Training set 1.5%	Calibration	0.173	0.990
	Validation	0.178	0.989
Training set 4.25%	Calibration	0.169	0.990
	Validation	0.177	0.989
Training set 5.75%	Calibration	0.168	0.989
	Validation	0.176	0.989

The results of external validation shown in Fig. 2, which have an R^2 value >0.91 and an RMSE value was small, so the PLS calibration model has good reliability to be implemented on commercial samples [20]. The precision and accuracy of the method result showed an RSD of 3.37% and a % recovery of 99.78%. The results of the determination of diclofenac diethylamine levels in commercial samples of emulgel by TLC Densitometry and NIR spectroscopy methods can be seen in Table 4.

Table 4. **Results of diclofenac diethylamine level determination in commercial samples.**

Sample	Diclofenac diethylamine content w/w (%)	
	NIR Spectroscopy ± SD	TLC Densitometry ± SD
A	1.129 ± 0.028	1.140 ± 0.027
M	1.150 ± 0.027	1.153 ± 0.031
F	1.152 ± 0.027	1.149 ± 0.016
V	1.148 ± 0.024	1.151 ± 0.031

The results of the determination of diclofenac diethylamine levels showed that the normality test value >0.05, meaning that the data in both methods are normally distributed. The two paired samples t-test has a significant value (2-tailed)>0.05, so it can be concluded that there is no significant difference between the NIR spectroscopy and TLC Densitometry methods [21].

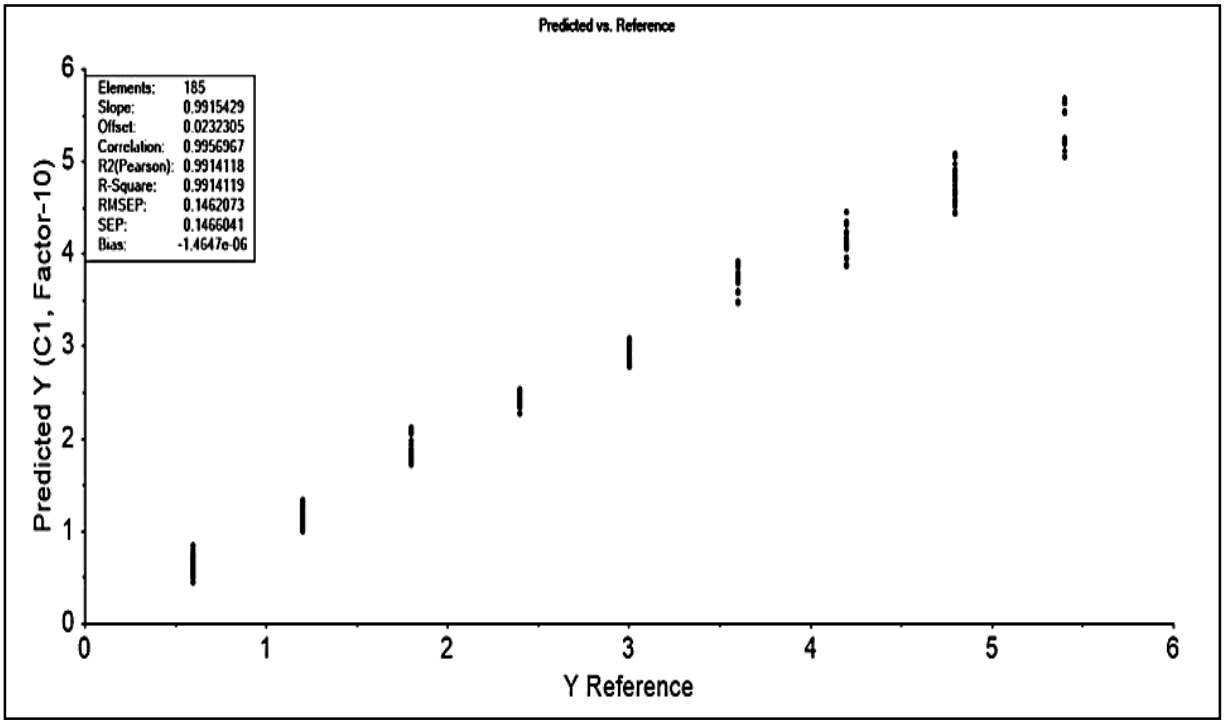

Figure 2. **Results of external validation method using test set samples**

Conclusion

From this research, it can be concluded that the diclofenac diethylamine levels in emulgel can be determined by NIR spectroscopy combined with chemometric methods using the best calibration model, namely PLS with an R^2 value of 0.990 and RMSE of 0.171. There is no significant difference in the determination of diclofenac diethylamine levels using TLC Densitometry and NIR-Chemometric evidenced by the results of two paired samples T-test with a significance value (2-tailed) > 0.05.

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Conflict of Interest

The authors declare no conflict of interest for this article.

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