Analytical method development of nifedipine and its degradants binary mixture using high performance liquid chromatography through a quality by design approach

S Choiri[1,2], A Ainurofiq[2], R Ratri[3], M U Zulmi[3]

[1] Department of Pharmacy, Sebelas Maret University, Ir. Sutami 36 A, Surakarta, Indonesia 57126

[2] Faculty of Pharmacy, Gadjah Mada University, Sekip Utara, Yogyakarta, Indonesia, 55281

[3] Department of Pharmaceutical Science and Technology, Setiabudi University, Surakarta, Indonesia, 57127

Email: syaiful.apt@student.uns.ac.id

Abstract. Nifedipin (NIF) is a photo-labile drug that easily degrades when it exposures a sunlight. This research aimed to develop of an analytical method using a high-performance liquid chromatography and implemented a quality by design approach to obtain effective, efficient, and validated analytical methods of NIF and its degradants. A 2^2 full factorial design approach with a curvature as a center point was applied to optimize of the analytical condition of NIF and its degradants. Mobile phase composition (MPC) and flow rate (FR) as factors determined on the system suitability parameters. The selected condition was validated by cross-validation using a leave one out technique. Alteration of MPC affected on time retention significantly. Furthermore, an increase of FR reduced the tailing factor. In addition, the interaction of both factors affected on an increase of the theoretical plates and resolution of NIF and its degradants. The selected analytical condition of NIF and its degradants has been validated at range 1 – 16 μg/mL that had good linearity, precision, accuration and efficient due to an analysis time within 10 min.

1. Introduction

Nifedipine (NIF) as a dihydropyridine derivate is a potent and clinically active as calcium channel blockers which has a low photostability under UV light or sunlight [1]. The degradant products of NIF depend on the light exposure which become dehydronifedipine or nitroso analogs and these are clinically meaningless [2–4]. Several methods were carried out to improve the NIF stability e.g. solid dispersion [5], encapsulation using cyclodextrin [6], polymeric nano encapsulation or formulated in dosage form [7]. However, the degradation process could not be avoided in these systems. In addition, because of a high similarity of structure between NIF and its degradants, a selective analytical method has been purposed and recomended to analyze the NIF and its degradants [8,9].

High performance liquid chromatography (HPLC) is a first choice and selected method to analyze degradant products or related substances in the bulk pharmaceutical or dosage form due to cheap, selectivity, and sensitivity [9,10]. Several works have been reported in the use of liquid chromatography to analyze NIF degradant products e.g. ultra-performance liquid chromatography [8] or HPLC

[2,7,11,12]. However, in these methods did not implement the design experiment (DoE) to determine the most effective and efficient conditions of HPLC analysis.

In order to improve the efficiency and cost e.g. time to analysis in the HPLC method, the DoE was implemented in this study using a full factorial design [13]. Curvature in the design was added to improve the predictive power of model [14]. The DoE was used to determine the optimized region at a range of factor depending on the quality target product profiles [15,16]. The use of DoE was not only to determine the optimized condition but also to elucidate the effect and interaction of each factor [9,17].

The aim of this research was to develop and optimize the HPLC condition using 2^2 full factorial design with a curvature to improve the predictive power of model. Therefore, an effective, efficient, and validated analytical method was achieved to analyze the NIF and its degradant in the force degradation study.

2. Experimentals

2.1. Materials

Nifedipine (NIF) (Lot No. 400196067) was obtained from Dexa Medica (Palembang, Indonesia) Acetonitrile (ACN), methanol (MeOH), chloroform and water was obtained from Merck (Darmstadt, Germany) as a chromatography grade.

2.2. Preparation and characterization of degradation of nifedipine

Pure NIF was exposed to the sunlight during 2 min and 2 days. The degradation was characterized by a thin layer chromatography (TLC). The TLC condition was performed by a silica plate 60 GF$_{254\ nm}$ and chloroform:MeOH (9:1). An identification was performed visually or assisted by a UV lamp at a wavelength of 254 nm. A Shimadzu IR-21 Prestige Fourier transform infrared (FTIR) spectrophotometer (Kyoto, Japan) was used to confirm the degradation process of NIF. A potassium bromide method using a 1% of sample was used to characterize the NIF degradation. The sample was weighed and pressed using a hydraulic presser at 6 kN for 5 min. Samples were scanned using a 32 times iteration and resolution of 2 cm^{-1} from a range of wavenumber of 400-4000 cm^{-1}.

2.3. Design of Experimental of HPLC analysis

A 2^2 full factorial design using an addition of center point as a curvature was used to obtain the design space of HPLC analysis. Flow rate (FR) and mobile phase composition (MPC) i.e. ACN to MeOH ratio were used as factors using different levels of factors which are presented in Table 1. A 4 runs was constructed based on a two factors and levels, respectively and a one run was added in the center point as the curvature. The design space was determined by several parameters i.e. time retention (tR), tailing factor (tf), theoretical plates (N), and resolution (Rs).

Table 1. Factors and levels of HPLC analysis using 2^2 full factorial design with a curvature

Factor	Level		
	-1 (low level)	0 (curvature)	+1 (high level)
Flow rate (mL/min)	0.8	1.05	1.3
ACN to MeOH ratio	0.25	2.125	4

2.4. HPLC condition

A Shimadzu HPLC (Kyoto, Japan) using a LiChrosphere 100 RP-18 (250x4.6; 5μm) column (Merck; Darmstadt, Germany) and ACN to MeOH under several ratios at determined FR depending on DoE (Table 1) with isocratic system was used to assay of NIF from a mixture with degraded NIF. NIF and degraded NIF were mixed and dilute with a mobile phase. The sample was filtered using a 0.22 μm membrane filter and a volume of 20 μL of sample was injected into the HPLC instrument at a column temperature of 40ºC.

2.5. Parameter analysis and determination of design space

Each parameter was analyzed by a multiple linear regression analysis (MLRA) model. Several model criteria were used to determine the best fitting model including significant model, not significant curvature, coefficient of determination (R^2) more than 0.7, and difference between adjusted R^2 and predicted R^2 (Adj. R^2-Pred R^2) of 0.2. The best models were used to construct the contour plot. The design space was determined by a desirability function of the superimposed contour plot at several requirements i.e. tR less than 10 min, tf less than 2.5, and Rs higher than 2.

2.6. Validation of optimized condition

Several validation parameters were performed in the optimized condition i.e. linearity, limit of detection (LoD), limit of quantification (LoQ). Cross validation using a leave one out technique was carried out to validate especially the accuration and precision.

Linearity was prepared by several point calibrations. The range concentration from 1 to 16 µg/mL. Each concentration was filtered using a 0.22 µm membrane filter and 20 µL was injected into the HPLC instrument. A calibration model was constructed based on calculated and observed data.

LoD and LoQ were calculated based on a noise to signal ratio and slope of regression coefficient (b). The noise was determined by standard deviation of noise (s) in the baseline of HPLC chromatogram. LoD and LoQ were calculated based on following equations.

$$LoD = 3.3 \times s/b \qquad (1)$$
$$LoQ = 10 \times s/b \qquad (2)$$

3. Results and Discussion

A good performance of analytical method i.e. selective, effective and efficient was needed to a routine analysis especially for an easily degradable drug [8]. NIF is a photodegradable drug that produce more than two degradation products depending on its mechanism and light exposure [1,2,4]. The NIF degradant product was confirmed by TLC and FITR spectra (data not shown). The TLC results showed that a similar pattern in chromatograms during 2 min and 2 days exposures was observed. It proved that a short time exposure of sunlight promoted the degradation of NIF. Meanwhile, the amount of degradation product increased during exposure. The FTIR spectra confirmed that a new peak was observed due to a C=N bonding vibration at a wavenumber of 1726.29 cm^{-1}. It showed that NIF degradants had a similar structure and distinct in a one functional group. Therefore, a good performance of analytical method to analyze the NIF and degradants must be developed [8]. FR and MPC were the most factors affecting the suitability test system on the HPLC analysis [10,13,18].

In order to develop validated, cheap, effective and efficient analytical methods, a 2^2 full factorial design with a curvature was applied. This system enhanced the validity and predictive power of the model due to a presence of a curvature. The curvature implied the value of intercept of MRLA depending on the observed data [14]. A not significant term of curvature was required to select the suitable model.

All models had significant models ($p<0.05$) and not significant of curvatures ($p>0.05$). The significant model indicated that the model could be used to describe the effect of independent variables on responses. Therefore, it was a first requirement in the model selection [16]. The curvature was used to verify the intercept of each model, a not significant term of curvature showed a not significant difference between the observed value in the middle level of each factor and intercept value. tR was selected as a response due to an efficient reason of analytical process in the HPLC. The shorter analysis time, the more efficient of analytical process using HPLC [19,20]. Hence, the effect and interaction of ACN to MeOH ratio and FR on tR can be shown in Table 2 and Fig. 1a. An increase the ACN to MeOH ratio and FR had significant effect ($p<0.05$) on reducing the tR. Although, an interaction of both factors increase the tR. An increase the ACN fraction comparted to the MeOH fraction reduced the tR due to ability of ACN to increase the elution power [21]. As same as the FR, increasing the FR reduced the time analysis due to reducing the interaction between compound and stationary phase [10,19]. Depending on contour plot of tR (Fig. 1a) showed that the highest tR was obtained at a low level of

ACN to MeOH ratio and FR. On the other hand, the lowest tR was obtained at a high level of both factors.

In addition, the effect and interaction of ACN to MeOH ratio and FR on tf are presented in Table 2 and Fig 1b. Only the ACN to MeOH ratio had a significant effect on an increase the tf ($p < 0.05$). Increasing the tf was caused by an interaction between NIF and stationary phase. The higher interaction, the higher tf value. Fig. 1b showed that only the change of MPC affected the tf value, meanwhile the change of FR had no significant effect ($p > 0.05$). There was no significant interaction between two factors ($p > 0.05$). On the other hand, only FR significant affected on N ($p < 0.05$) and had no interaction between both factors. The effect and interaction of ACN to MeOH ratio and FR on the N parameter can be deeply elucidated using N's contour plot (Fig. 1c). A significant color graduation could be observed at a change of FR. Although, an alteration of MPC had no significant effect ($p > 0.05$). There was no interaction of two factors, an interaction was observed by a non-parallel or intersect patterns. Rs was selected as a factor due to important parameter to distinguish between main and degradant peaks of NIF. A high resolution was required to determine the design space of degradant analysis [10]. The effect and interaction of both factors are presented in Table 2 and Fig. 1d. MPC and FR had a significant effect on reducing the Rs ($p < 0.05$), although there was no significant interaction of both factors ($p > 0.05$). An increase the ACN fraction in mobile phase increased the elution power but the resolution was decreased. As similar as the flow rate, an increase the flow rate reduced the tR and reduced the resolution. Depending on the contour plot of resolution (Fig. 1d), it proofed that the MPC had a higher effect than flow rate on Rs and there was no specific interaction between each factor.
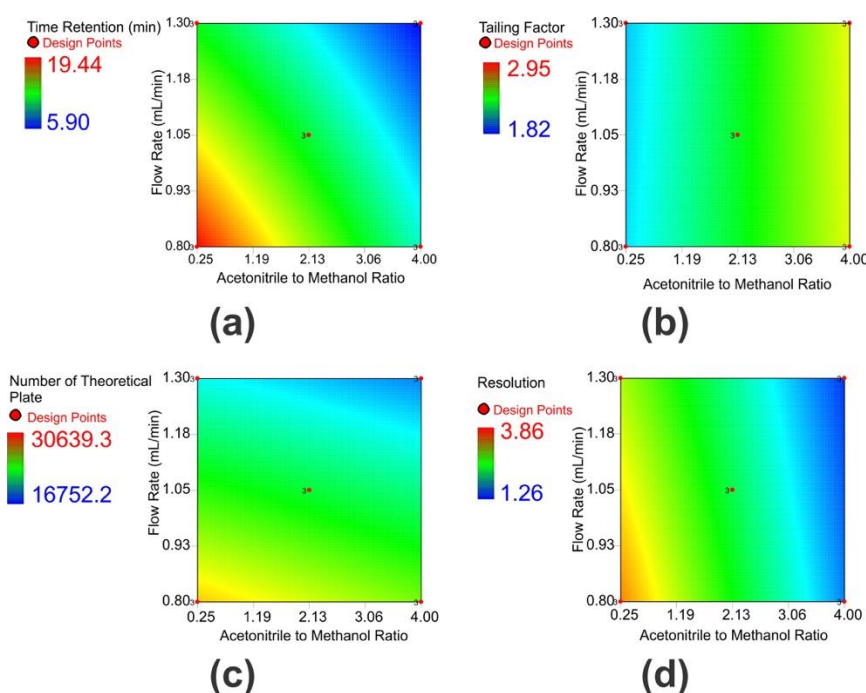

Figure 1. Contour plot of retention time (a), tailing factor (b), number of theoretical plates (c), and resolution (d)

Selection adequate model depended on how the model fit well. Goodness of fit parameters was selected as criteria to determine an appropriate model which could be used to design the optimum region [15]. R^2 measured the effect of independent variables on responses, R^2 more than 0.7 was a criterion in model selection to minimize the error and lack of effect. All models had R^2 more than 0.7. The Adj. R^2-Pred R^2 measured how adequate of model to predict the response, the model can be used to predict the response without a misleading if the Adj. R^2-Pred R^2 was less than 0.2 [15]. The Adj. R^2-Pred R^2 of tf and N responses had higher than 0.2, thus it indicated there was a potential miss-leading in the model to

predict the response. Therefore, a verification step was required to obtain an adequate model. PRESS measured the residual between predicted and observed data, the PRESS could be compared between each factor using a relative value with a mean. Adequate precision measured the signal to noise ratio. The adequate precision more than 4 indicated that the model could be used to estimate or predict the response adequately [14,15]. Depending on the all fitting parameters, all models had a potential ability to predict and estimate the response, thus the optimum region could be determined by these models.

Table 2. Coefficient regression, statistical and fitting parameters on responses

	Time retention	Tailing Factor	Number of Theoretical Plates	Resolution
Coefficient Regression				
Intercept	11.96	2.35	23053	2.38
A (ACN to MeOH ratio)	-3.90	0.29	-1124.21*	-0.87
B (Flow rate)	-2.75	-0.006*	-3523.84	-0.18
AB	0.72	0.012*	181.67*	0.083*
Fitting parameters				
R^2	0.9937	0.6858	0.7803	0.9491
Adjusted R^2	0.9918	0.5915	0.7144	0.9339
Predicted R^2	0.9858	0.2930**	0.5057**	0.8856
PRESS	3.99	1.02	1.04×10^8	1.16
Adequate precision	54.72	4.85	7.48	16.108
p-value (CI=95%)				
Model	<0.0001	0.0071	0.0013	<0.0001
Curvature	0.1702*	0.6285*	0.6493*	0.1977*

* = no significant different (p>0.05)

** = difference between adjusted and predicted R^2 more than 0.2
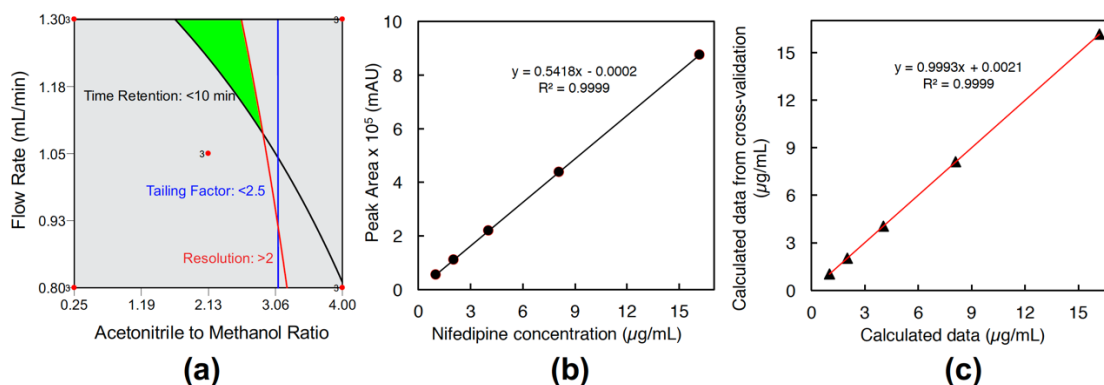

Figure 2. Superimposed of contour plot (a), calibration model (b), and cross-validation calibration model (c)

The optimum region as a controlled space was determined by several criteria. Furthermore, it is presented in the superimposed of each contour plot (Fig. 2a). In order to determine the controlled space, each parameter had a priority to determine the design space. Priority of tR and Rs responses was higher twice than that of the tf and N responses. ACN to MeOH ratio of 2.06 and flow rate of 1.30 mL/min was the optimized condition. This condition was used to construct the calibration model (Fig. 2b). The calibration equation had high R^2 0.9999 and adjusted R^2 of 0.9999. The linearity of concentration was obtained at a range from 1 to 16 µg/mL. Furthermore, the calibration model of cross validation (Fig. 2c) had a high predicted R^2 of 0.9999. There was an error of cross validation of 0.07% and had precision of 100.01% (RSD=0.27%). Based on the noise to signal ratio of baseline, LoD and LoQ were 8.9 and 27.0 ng/mL, respectively. The optimized condition conformed the requirement of validation parameter.

4. Conclusion

A 2^2 full factorial design with a curvature was successfully implemented to optimize the HPLC condition of NIF and its degradant analysis. FR and ACN fraction in MPC had a positive effect on an increase the efficiency of analytical process. However, an increase of both factors reduced the separation ability and an appropriate selection of region in the design space as the controlled strategy had the better results. The optimized condition was validated using cross-validation with a leave one out technique and had good linearity, sensitivity, precision and accuration.

Acknowledgement

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