VALIDASI METODE SPEKTROFOTOMETRI UV-VIS UNTUK STUDI KELARUTAN PENTAGAMAVUNON-0 DI DALAM PEMBAWA SELF-NANOEMULSIFYING DRUG DELIVERY SYSTEM

VALIDATION OF UV-VIS SPECTROPHOTOMETRIC METHOD FOR SOLUBILITY STUDY OF PENTAGAMAVUNON-0 IN SELF-NANOEMULSIFING DRUG DELIVERY SYSTEM VEHICLES

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ABSTRAK

Pentagamavunon-0 memiliki aktivitas anti-inflamasi yang tinggi namun kelarutan dan absorpsinya kurang, yang dapat diperbaiki dengan formulasi SNEDDS. Salah satu parameter penting optimasi SNEDDS adalah kelarutan PGV-0 dalam berbagai minyak, surfaktan, co-surfaktan dan pada produk akhir. Penelitian ini bertujuan untuk melakukan validasi metode spektrofotometri UV-Vis yang sederhana dan murah untuk perhitungan PGV-0 dalam formulasi SNEDDS. Pengujian dilakukan dengan menggunakan spektrofotometer UV-Vis, metanol sebagai pelarut dan deteksi sinar tampak dilakukan pada panjang gelombang 419 nm. Linieritas, batas deteksi (LOD), batas kuantitas (LOQ), presisi, dan keakuratan metode tersebut ditentukan. Studi kelarutan dilakukan dengan metode *shake flask* termodifikasi, menggunakan labrasol sebagai model pembawa. PGV-0 yang tidak larut dipisahkan dan dibersihkan dari filtrat, dihitung dengan spektrofotometri UV-Vis yang telah divalidasi, dan kelarutan PGV-0 ditentukan. Pengukuran validasi dilakukan pada kisaran 1-8 ppm. Hasil penelitian menunjukkan adanya linieritas yang baik dengan r=0,99949, LOD=0,29 ppm, dan LOQ=0,95 ppm. Keakuratan yang dinyatakan sebagai perolehan kembali=98,32-100,74%. Presisi metode ini baik (RSD=1,29-1,93%). Perolehan kembali hasil kelarutan PGV-0 yang diperoleh dari metode spektrofotometri UV-Vis ini dibandingkan dengan kelarutan sampel yang sama yang diperoleh dari metode standar HPLC untuk perhitungan PGV-0 adalah 100,02% yang mengindikasikan keakuratan yang baik.

Kata kunci: validasi, spektrofotometri UV-Vis, PGV-0, studi kelarutan.

ABSTRACT

Pentagamavunon-0 has a potent anti-inflammatory activity but has poor solubility and absorption properties, which can be improved by SNEDDS formulation. One of the most

critical parameters of the optimization of SNEDDS is the solubility of PGV-0 in various oils, surfactants, co-surfactant, and in the final product. This study is aimed to validate a simple and inexpensive UV-Vis spectrophotometric method for PGV-0 quantification in SNEDDS formulation. The assay was performed using UV-Vis spectrophotometer, methanol as the solvent, and Vis detection at wavelength of 419 nm. The linearity, limit of detection (LOD), limit of quantity (LOQ), precision, and accuracy of the method was determined. The solubility study was performed by modified shake flask method, using labrasol as a vehicle model. The insoluble PGV-0 was separated and cleaned from the filtrate, quantified by validated UV-Vis spectrophotometry, and the solubility of PGV-0 was determined. Validation measurement was carried out in the range of 1-8 ppm. The results showed a good linearity with r=0,99949, LOD=0.29 ppm, and LOQ=0.95 ppm. The accuracy expressed as recoveries were found to be 98.32-100.74%. The precision was good (RSD=1.29–1.93%). The accuracy expressed as recoveries were found to be 98.32 - 100.74%. The precision was good (RSD=1.29–1.93%). The recovery of solubility of PGV-0 using this UV-Vis spectrophotometric method was 100.02% compared to the solubility of the same sample obtained by the standard HPLC method for quantification of PGV-0, indicated a good accuracy.

***Key words**: validation, UV-Vis spectrophotometry, PGV-0, solubility study.*

Introduction

Pentagamavunon-0 (PGV-0) is an analog of curcumin that has anti-inflammatory activity higher significantly compared to curcumin. It is a semipolar compound that practically insoluble in water (Sardjiman, 2000; Soediman, 2003). The solubility and permeability properties of such compound can be improved by self nano-emulsifying drug delivery system (SNEDDS) formulation.

SNEDDS is an isotropic mixture of oil, surfactant, dan co-surfactant that form nanoemulsion spontaneously when introduced to aqueous medium under a gentle agitation (Patel *et al.,* 2012). One of the keys of the successful SNEDDS formulation is the selection of the vehicle (oil, surfactant, and co-surfactant) individually and collectively as a blank SNEDDS that has optimum drug dissolving ability and self-emulsification. Therefore, the prediction of PGV-0 solubility must be performed carefully with an appropriate method that has been validated to provide reliable results.

The earlier studies have reported the solubility test of PGV-0 by the shake flask method (Soediman, 2003). The amount of soluble PGV-0 in the sample was determined directly from the filtrate or indirectly from the precipitate and measured by UV-Vis spectrophotometer or HPLC. The PGV-0 quantification in a biological fluid is measured by HPLC, while the PGV-0 quantification in some of the organic solvents and in the in vitro pharmaceutical testing is measured by HPLC or UV-Vis spectrophotometer (Soediman, 2003; Irianto, 2013). The HPLC method is widely used due to its selectivity, sensitivity, and reproducibility. The UV-Vis spectrophotometry is more simple, low cost, rapid, and quite reliable for the determination of pharmaceuticals.

Due to its specifity, the matrix of the sample in the UV-Vis spectrophotometry should be as simple as possible. The vehicles may interfere with the absorption of PGV-0, so, the PGV-0 is quantified indirectly from the PGV-0 precipitate. This method is also simple because it only takes one standard curve for the various vehicles tested.

The aim of this work is to perform a solubility study of PGV-0 in various vehicles with a validated UV-Vis spectrophotometry which can be more simple and economical than HPLC

method. In this study, labrasol was used as a vehicle model.

Materials and Methods

PGV-0 was obtained from Curcumin Research Center, Gadjah Mada University. Pharmaceutical grade of labrasol was kindly provided by Gattefosse (France) via PT Mensa Group (Jakarta). Methanol L.C grade was purchased from Merck. Aquademineralisata was purchased from Bratachem (Purwokerto) and was used to prepare the samples for the UV-Vis spectrophotometry. Microtubes, micro magnetic bars, micropipettes, a vortex mixer (Genie), a magnetic stirrer (Ika RH Basic I), an ultracentrifuge (Ika RH Basic I), an oven (Memmert) were used for sample preparation and solubility study. The UV-Vis spectrophotometric method was performed using 1.0 cm quartz cell on a spectrophotometer (Shimadzu) at 420 nm. The Lab Solution software was used for absorbance measurements.

Preparation of the PGV-0 Standard Solution

Ten mg of the PGV-0 standard was accurately weighed, transferred to a 25 ml volumetric flask and dissolved properly in methanol with a sonicator. An appropriate volume of an aliquot from standard stock solution was diluted in the same volumetric flask of 5 mL capacity with methanol to give a series of working standard solution.

Preparation of the Sample Solution

The sample solution was prepared by dissolving 10 mg of PGV-0 that used for SNEDDS fomulation to a 25 mL volumetric flask. The sample stock solution then diluted with methanol in the same way as preparing the standard solution.

Method Validation

The proposed method was validated in accordance with International Conference on Harmonization (ICH) guideline for its specificity, linearity, accuracy, precision, LOD, and LOQ (ICH Q2A, 2005; ICH Q2B, 2005). After validation process, the spectrophotometric method was applied to solubility test of PGV-0 in labrasol and compared the result with the solubility value from standardized HPLC method.

1. Selectivity

The selectivity was determined by observing the absorption spectrum of each PGV-0 and labrasol solution in methanol. The method is said to be selective if it is able to assess unequivocally the analyte in the presence of other components.

2. Linearity

The calibration curve was obtained from fifteen concentrations of the standard solution (1.0 ppm– 8.0 ppm). The absorbance of the solution was measured by UV-Vis spectrophotometer at the maximum absorbance wavelength. The standard curve was constructed by plotting the concentrations versus absorbances. The correlation coefficient (r) of the standard curve which indicated the linearity was calculated by the least square regression method and was defined by the linear regression analysis.

3. Accuracy

The accuracy was evaluated from three replication of determinations at three level of concentrations of the linear interval of procedure, they are 2.0, 5.0, and 7.0 ppm. The accuracy was calculated by dividing the average concentration obtained experimentally with the corresponding theoretical concentration.

4. Precision

The precision assessment was carried out based on repeatability and intermediate precision. The repeatability (within-day replication) was calculated from six replication of assays at the same concentration of 2 ppm during the same day. While the intermediate (inter-day) precision was assessed by comparing the six experimental concentrations on three different day.

5. LOD and LOQ Determination

The equation 1, 2, and 3 below were used to calculate LOD and LOQ.

$$Sy/x = \sqrt{\frac{\sum (Y - \hat{Y})^2}{N-2}} \text{ ; with } \hat{y} = a + bx \quad (1)$$

$$LOD = \frac{3 \times Sy/x}{Sl} \text{ or } LOD = \frac{3 \times Sb}{b} \quad (2)$$

$$LOQ = \frac{10 \times Sy/x}{Sl} \text{ or } LOQ = \frac{10 \times Sb}{b} \quad (3)$$

k = 3 for LOD and 10 for LOQ

Sl = slope (b at line equation y = a+bx)

The analytical response standard deviation of the blank (Sb) = Sy/x

Solubilty Study

An excessive amount of PGV-0 that accurately weighed was added to an accurately measured volume of labrasol, vortexed for 5 minutes and stirred for 24 hours. After allowed for 1 day at room temperature, the mixture was separated by ultracentrifugation at speed of 6000 rpm for 10 minutes. The supernatant was separated by decantation. The precipitate was washed with the aqua demineralisata multiple time by decantation until the aqua demineralisata became clear, colorless and showed no significant UV-Vis absorption, then dried in the oven 50^{o}C. The precipitate was diluted with methanol to an exact volume, and the absorption was measured by UV-Vis spectrophotometric method. The concentration of PGV-0 dissolved in vehicle was calculated using the equation 4. The solubility of PGV-0 which was obtained by UV-Vis spectrophotometric method was compared to the solubility value of the same sample analysed with HPLC method which is the standard method for PGV-0 quantification with T test.

$$\text{PGV}-0\ \text{solubility} = \frac{\text{initial mass of PGV-0 - mass of precipitat e}}{\text{volume of vehicle)}} \quad (4)$$

Results and Discussions

Selectivity

The spectrum of PGV-0 in methanol shows a maximum wavelength of 419 nm (Figure 1), while in the interval of 200-500 nm, labrasol shows high absorption (Figure 2). So, the significant amounts of labrasol in the PGV-0 solution in methanol can interfere the PGV-0 measurement by UV-Vis spectrophotometry. The separation together with measurement of PGV-O can be performed with HPLC, but for the measurement of many samples as in SNEDDS formulations, it is less economical compared to UV-Vis spectrophotometry.

The proposed solubility test procedure involved measuring the residue of PGV-0 from the solubility test which was separated and cleaned from the vehicle. No interfering peak from the vehicle as seen in the figure 1, so the UV-Vis spectrophotometric method was selective.

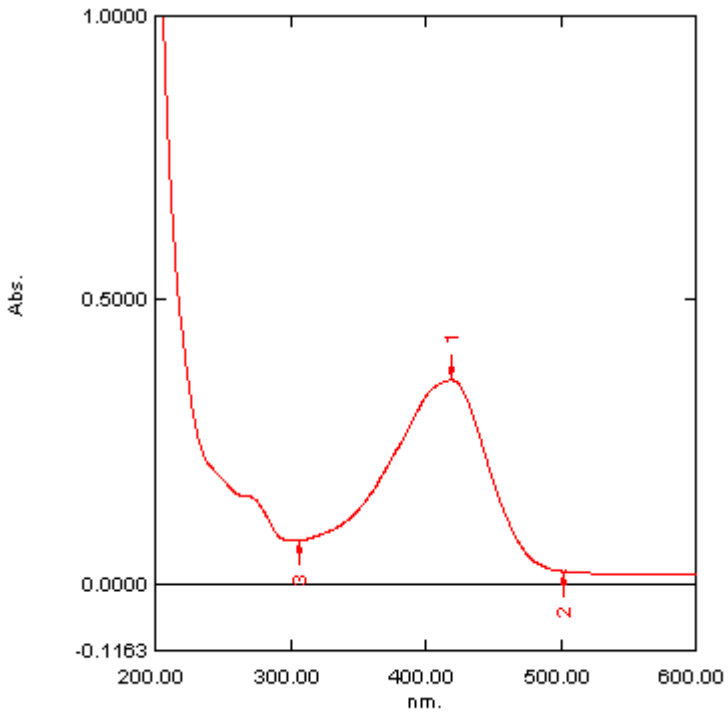

Figure 1. Spectrum of PGV-0 in methanol.

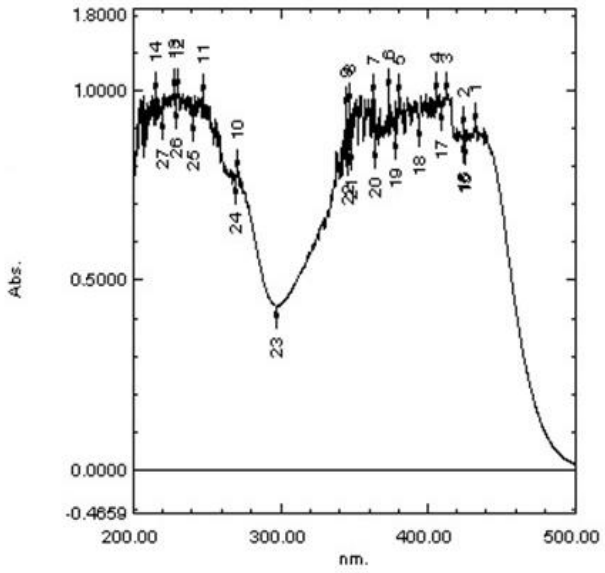

Figure 2. Spectrum of labrasol in methanol.

Linearity

The calibration curve of PGV-0 was prepared by plotting the absorbances of samples at wavelength of 419 nm wavelength vs. their concentrations (Figure 2). The correlation coefficient (r) of determination for active ingredients should be ≥ 0.999 (Kazakevich and LoBrutto, 2007). The curve shows a good linearity within 1.0–8.0 ppm with the value of r of 0.9995 and the regression equation was y = 0.17736x + 0.00508. The absorbance of the test results proved to be directly proportional to the concentration, therefore by entering the absorbance to the equation, the solubility of PGV-0 can be determined.

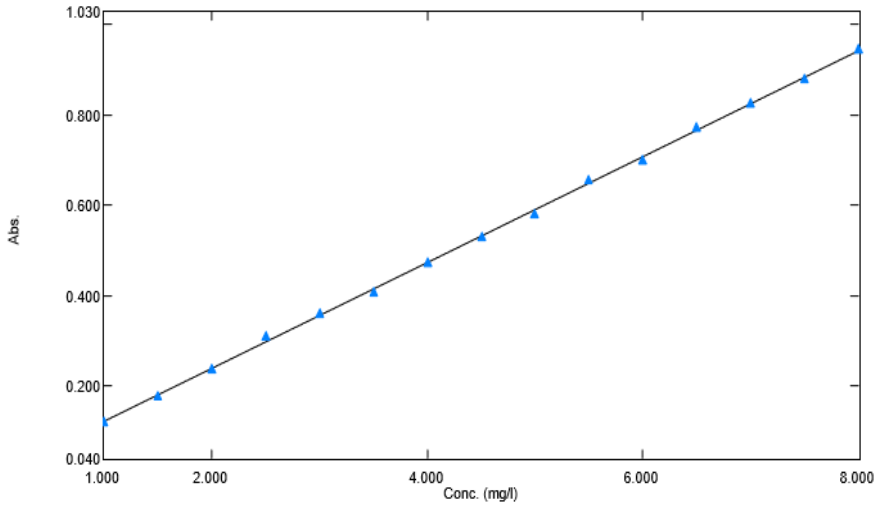

Figure 3. The calibration curve of PGV-0 in methanol.

Accuracy

Accuracy is the closeness of the test result with the corresponding true value. The parameter of accuracy was % recovery. The precision and accuracy were assessed from six replication of three concentration levels (2, 5, and 7 ppm) of the PGV-0 solution. The result is presented in Table 1.

Table 1. Precision and accuracy of the PGV-0 in methanol (n=3)

Concentration (ppm)	Accuracy (% recovery)		Standard Deviation		Precision (% RSD)	
	Within-day	Inter-day	Within-day	Inter-day	Within-day	Inter-day
2.00	98.81	99.81	0.03	0.04	1.75	1.88
5.00	98.66	98.32	0.06	0.09	1.29	1.93
7.00	98.71	100.74	0.10	0.12	1.44	1.68

The recovery of the method was in the range of 98.32-100.74% (within day) and 98.32-100.74% (interday). It is meet the acceptance limit of accuracy, i.e. 98–102% (Synder *et al.*, 2010) and the method is accurate over the desired range.

Precision

Precision is the parameter that shows the closeness of the test results of homogeneous samples with minimum three replication. The precision was expressed as relative standard deviation (RSD). The acceptance limit of precision is RSD ≤ 2 (Synder *et al.,* 2010). The result of precision determination is shown in Table 1. The repeatability in all three levels were 1.29 – 1.75%, indicated a good precision. The inter-day precisions (1.68 – 1.93%) also fulfill the precision requirement.

LOD and LOQ

The sensitivity of measurement is described by LOD and LOQ. The LOD in this study, i.e. the lowest of PGV-0 concentration could be detected by the method but not be required for analysis was 0.29 ppm. While the LOQ, i.e. the minimum concentration of PGV-0 in the sample that could be measured accurately was 0.95 ppm. The sensitivity of the proposed method is sufficient for solubility measurement in SNEDDS ingredients.

Solubility Study

Table 2 shows that the $t_{count} > t_{table}$, so the solubility of PGV-0 in labrasol obtained by the UV-Vis spectrophotometric method is not significantly different from that of HPLC. The accuracy of the proposed method was 100.02%, indicated the solubility study by indirect method measurement was accurate.

Table 2. Comparison of solubility study of PGV-0 in labrasol

Method used	Solubility (mg/mL)	RSD (%)	t_{count}	t_{table}
HPLC	14.46	100.02	0.606	0.05
UV-Vis spectrophotometry	14.48 ± 0.05			

Conclusion

In the recent study, the proposed method for the estimation of solubility of PGV-0 in various SNEDDS ingredients as a vehicle was found to be economical, simple, and sensitive with good accuracy and precision. The method is also selective without interference of vehicle.

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