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Research Article

Analytical method development of pitavastatin-loaded SNEDDS formulation: Multivariate analysis regarding ultraviolet spectrophotometry analysis

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ABSTRACT

Herein, this work aimed to develop and assess feasibility of multivariate model of partial least square analysis for specific quantification of pitavastatin (PVT)-loaded self-nano emulsion (SNE) formulation using UV-Vis spectrophotometry.

PVT loaded into self-nano emulsion formulation comprising of Capryol-90, Tween 80, and Transcutol P under different loading levels i.e. 10-90 mg/mL. All samples scanned using UV-Vis spectrophotometer from 300-200 nm. PVT and SNE were prepared separately for estimation of interference. Multivariate model was constructed using partial least square (PLS) regression analysis as well as principal component analysis for qualitative pattern recognition. Cross-validation using a leave one out technique and goodness of fit parameters were applied for model evaluation.

The results revealed that maximum sensitivity of PVT was obtained at 244 nm. SNE formulation had different interference value and decreased exponentially as increasing the PVT loading in the SNEDDS formulation. The highest drug loading had an interference value of 7.11%. Therefore, the SNEDDS formulation interfered the PVT quantification and mainly depended on the drug loading. Finally, multivariate analysis, PLS could be applied to eliminate the placebo/formulation interference for PVT quantification independently towards drug loading level.

Keywords: SNEDDS; Pitavastatin; Multivariate analysis; PCA; PLSR;

1. Introduction

Recent years, self-nano emulsifying drug delivery system gained a great consideration owing to enhancing bioavailability of poorly solubility and permeability drugs significantly. Self-nano emulsion (SNE) formulation including in lipid base formulation comprising of oil, surfactant, and co-surfactant as an isotropic mixture formed nanodroplet when it dilutes with medium under gentle agitation (Badawy et al., 2017; Rehman et al., 2017). Both lipophilic and hydrophilic drugs can be loaded into SNE formulation (Efiana et al., 2017). Amount of the drug can be loaded depends on physicochemical characteristics and affinity between drug and SNE component in order to achieve miscible mixture (Bandyopadhyay et al., 2014; Chavan et al., 2015).

The capacity of carrier of the delivery system is depicted the drug loading. Higher drug loading promotes greater efficiency and enhances the flexibility to regulate the amount of dosage form during administration (Chavan et al., 2015).

Somehow, determination of drug content involves appropriate analytical methods in which it should eliminate the placebo or formulation blank interference in the analytical results (Choiri et al., 2018; Fukuda et al., 2018). Moreover, selection of appropriate analytical methods which had high specificity and cost-friendly i.e. efficiency is faced a challenge. High-efficiency analytical method with low time consumption i.e. fast analytical process has a limitation in eliminating the placebo interference i.e. Udv-vis spectrophotometry (Liudmil Antonov and Stefan Stoyanov, 1993; Redasani et al., 2018; Takano et al., 2017). It requires more specific analytical method which can separate the placebo interference and analytical target response i.e. high-performance liquid chromatography (HPLC) (El-Enany et al., 2007; Sahu et al., 2017). However, this analytical method requires a lot of time for each analytical process and consuming a special solvent i.e. not cost-friendly and promotes waste product i.e. environment contamination (Redasani et al., 2018; Takano et al., 2017). Therefore, Uv-vis spectrophotometer has preferable features i.e. faster, cheaper, and more efficient than HPLC, although HPLC has a better specificity parameter to eliminate the placebo interference (Cağlar and Oztunç, 2007; El-Enany et al., 2007; Grobelny et al., 2009). Hence, development of analytical method in order to enhance specificity of analytical method to the analytical target should be considered. In addition, SNE component, oil as a core and surfactant as droplet stabilizer usually use saturated or unsaturated fatty acid which has a response in high energy of UV range i.e. 250-200 nm. For instance, In this study pitavastatin (PVT) as a drug model in this work has specific transition electronic at energy level of 250-240 nm (Kukrety et al., 2015). Herein, the SNEDDS component interferes the analytical response of PVT using ultraviolet spectrophotometric assay. In addition, the previous developed analytical method did not figure out to demonstrate the placebo interference in SNEDDS formulation (Krishna and Sankar, 2007; Kukrety et al., 2015; Vadia et al., 2008). Moreover, in low drug loading, presence of high amount of SNEDDS component has a greater interference level than that of high drug loading. Hence, this study purposed to develop an analytical method using ultraviolet spectrophotometer using PVT-loaded SNEDDS formulation regarding to multivariate model. Multivariate model can consider the placebo interference or other components regarding to the SNEDDS components (Rohman et al., 2011; Rohman and Man, 2012).

2. Materials and Methods

2.1. Material

SNE formulation comprising of Capryol-90 (Gattefose; Saint-Priest, France) as oil, Tween-80 (Sigma Aldrich; St. Louis, MO) as surfactant, and Transcutol P (Gattefose; Saint Priest, France). Capitavastatin (PVT) was purchased from Thanen Chemical Co. Ltd (Xinbei District, P.R. China).

2.2. Preparation and incorporation of PVT into SNE formulation

SNEDDS formulation, comprising of 23.4% Capryol-90, 35.6% Tween-80, and 40.0 % Transcutol P was weighed according to the weight ratio and mixed together. PVT was weighed accurately and placed in the volumetric flask. Thereafter, SNEDDS formulation was added accurately in order to achieve drug loading of 10; 20; 30; 50; 70; and 90 mg/mL. The mixture was sonicated using ultrasonic bath (Branson U-2510; Danbury, CT) at 40°C until a clear solution was achieved. The mixture was placed in ambient temperature (26±1°C; RH 60±10%) overnight in order to ensure no precipitation of drug.

2.3. Analytical preparation and ultraviolet analysis

For pre-analytical treatment, SNE formulation, PVT-loaded SNE formulation, and PVT were simulated as aforementioned at concentration of drug loading of 10-90 mg/mL. Prior analytical methods, a 150 µL of PVT-loaded SNE and SNE formulation was withdrawn for each drug loading level and placed into 10 mL volumetric flask. Furthermore, it was diluted 100 times and methanol was used for all preparation methods including initial preparation and dilution. Sample was scanned using Shimadzu UV-2100 double beam spectrophotometer (Tokyo, Japan) from 300 to 200 nm with resolution of 1 nm and this analysis was performed triplicates. Methanol was used as a blank solution. For PVT solution, it was prepared under similar concentration of prepared solution of PVT-loaded SNE formulation. Placebo interference towards PVT response was calculated using following equation.

$$\text{Interference } (\%) = \frac{A_{SNE}}{A_{PVT}} x\ 100 \quad (1)$$

Where, A_{SNE} and A_{PVT} are UV-absorbance of SNE formulation and PVT, respectively under similar drug loading level and concentration of prepared solution.

2.4. Calibration Model

Conventional calibration model was constructed according to the linear regression model between concentration and absorbance. Meanwhile, multivariate analyses, principal component analysis (PCA) and partial least square (PLS) regression were applied for pattern recognition (qualitative) and quantification of PVT, respectively. Response, absorbance in range of 280-220 nm was used for quantification and pattern recognition. PLS model was assessed according to the goodness of fit parameter i.e. coefficient of determination (R^2), adjusted R^2, and root mean square error (RMSE). The model was validated using cross-validation with leave one out technique for calculation of predicted R^2 and RMSECV (Rohman et al., 2011). External sample was prepared for calculation of accuracy and precision using conventional and multivariate calibration models. Accuracy and precision are depicted by recovery and relative standard deviation (RSD) of recovery and they are calculated using following equation.

$$\text{Recovery} = \frac{Actual\ drug\ loading}{Theoretical\ drug\ loading}\ x\ 100\% \qquad (2)$$

$$\text{RSD} = \frac{Standard\ deviation\ of\ recovery}{Mean\ of\ recovery}\ x\ 100\% \qquad (3)$$

3. Results and Discussion

Ultraviolet spectrophotometer becomes an alternative choice for fast, cheap and efficient (Cağlar and Oztunç, 2007; Redasani et al., 2018). Therefore, in this work, we developed spectrophotometric method to analyze PVT-loaded SNE formulation. In addition, we compared the conventional calibration model in order to quantify the PVT in SNE formulation. UV-spectrophotometric band of PVT is presented in Figure 1. Maximum sensitivity of PVT was observed at 244 nm with absorptivity about 1000 $mLg^{-1}cm^{-1}$. This wavelength was set as a quantification wavelength for PVT assay. In order to assess the interference and characteristic UV band of SNE formulation, it is presented in Figure 1b. The highest sensitivity of SNE formulation was observed at 231 nm with absorptivity about 100 $mLg^{-1}cm^{-1}$. However, at the highest sensitivity of PVT wavelength (244 nm), the SNE formulation had response. Therefore, it promoted interference in quantification of PVT presence of SNE formulation owing to additive effect (Dastidar and Sa, 2009). For instance, low interference level was observed at wavelength above 250 nm, meanwhile the UV band of PVT had adequate enough response for quantification. Although, linearity can be ensured if the quantification was performed above 250 nm. In order to quantify the interference of SNE formulation to PVT absorbance under different drug load levels, the correlation of drug load level and percentage of interference is presented in Figure 2. The interference was calculated according to the absorbance of PVT towards SNE formulation at 244 nm. It depicted that interference level reduced as increase as drug loading in the PVT exponentially. The highest interference level about 44% at 10 mg/mL drug loading and the lowest interference level about 5% at 90 mg/mL drug load. The interference level was modeled using bi-exponential function with the best fitting model i.e. R^2 (0.9999) and adjusted R^2 (0.9996) close to 1.000. It revealed that the model was adequate enough for prediction of interference level as increase as the drug loading (Choiri et al., 2018). Low interference level i.e. less than 2% was achieved at drug loading of above 120 mg/mL. However, this system reached super-saturable of PVT in SNE system. The super-saturable system is unstable thermodynamically of soluble PVT in lipid base formulation. Changes of temperatures i.e. reduction temperature promoted precipitation of PVT in the system. Thereafter, it promoted crystal seeding and higher precipitation level under storage condition. Hence, low interference level could not be achieved at non super-saturable condition (Bandyopadhyay et al., 2014; Chavan et al., 2015). Thus, development of analytical model particularly ultraviolet spectrophotometric should be considered.

Calibration models of PVT and PVT-loaded SNE formulation are presented in Figure 3a and Figure 3b, respectively. Both models had good linearity. However, intercept value of PVT calibration model had insignificant manner ($p>0.1$), yet PVT-loaded SNE formulation had very significant intercept ($p<0.001$). The significant intercept was affected by placebo effect which placebo has a great impact on PVT response. Therefore, it resulted in different drug load according to concentration of prepared solution of PVT-loaded SNE formulation. In other words, interference level of low concentration of prepared solution had a greater effect than that of high concentration of prepared solution of PVT-loaded SNE formulation. In addition, for quantification of placebo interference throughout all concentration in calibration model, the interference level was calculated according to the percentage of different slope of PVT and PVT-loaded SNE formulation to slope of PVT model. Thus, it had value of 7.11%.

For instance, a multivariate calibration of PVT-loaded SNE formulation should be constructed for addressing afore discussed issue. UV-band of PVT-loaded SNEDDS formulation is presented in Figure 4a. It showed that different pattern was observed at different drug loading levels. Low drug loading (10 and 20 mg/mL) has a similar pattern. Meanwhile, drug loading of 30-90 mg/mL had a similar pattern. Therefore, the presence of SNE formulation altered the UV pattern of the PVT. It proved that presence SNE

formulation interfered the PVT response. For pattern recognition, PCA analysis using score plot is presented in Figure 4b. Multivariate analysis for factor reduction was applied for pattern recognition using PCA and it showed that 2-principal component was applied. The contribution of principal component 1 (99.97%) was higher than that of principal component 2 (0.03%). Score plot (Figure 4b) showed that it divided by two quadrants owing to negligible effect of principal component 2. Drug loading of 10-50 mg/mL had a similar pattern, while drug loading of 60-90 mg/mL located in quadrant 2. According to principal component 1, score of each drug load has lied on linear pattern which principal component standardized value increased as increasing as the drug loading. Principal component 2 promoted random standardized score particularly drug loading of 60 and 80 mg/mL.

Calibration model using multivariate analysis, partial least square regression (PLSR), is presented in Figure 5a. The model had the best fitting model with the goodness of fit parameter of R^2 0.9999 and adjusted R^2 of 0.9999 as well as RMSR value of 0.25. These parameters indicated that the model had adequate enough and had a good relationship between actual and predicted drug loading using PLS (Kurniawati et al., 2014). Cross-validation model using leave one out technique was carried out for model evaluation and validation. The results showed that the cross-validated model had predicted R2 of 0.9999 and RMSEP of 0.094%. This result proved that the model was highly adequate for prediction the drug loading. Residual of observed drug load and normal distribution plot are depicted in Figure 5b and 5C, respectively. Low residual value indicated that the predicted and actual drug loading closed to each other and all drug loading was normally distributed (Figure 5C; $p>0.05$). According to the coefficient of PLSR (Figure 5d), the greater coefficient value, the higher contribution was. Around maximum sensitivity of PVT, the coefficient had low contribution value. The highest contribution value was achieved in range of a 255-275 nm. In addition, response in 220-240 nm had high enough effect on contribution. Therefore, it was affected by the interference of placebo which it had a negligible effect on maximum sensitivity of PVT.

In order to evaluate multivariate model along with conventional calibration model, accuracy and precision from each model are presented in Table 1. For instance, multivariate model has a better results in both accuracy and precision compared to the conventional calibration model. It was affected by high recovery (around 110-140%) in low drug loading level (10-30 mg/mL) and drug loading of 40-90 mg/mL had recovery of 96-104%. It indicated that SNE formulation was considerable for quantification of PVT in low drug loading level.

4. Discussion

Presence of SNE formulation for PVT quantification appeared in placebo interference regarding the drug loading level. The higher drug loading, the lower placebo interference level was. Herein, the analytical method for quantification of PVT in PVT-loaded SNE formulation has been successfully developed and validated. Multivariate analysis, PLSR, provided better accuracy and precision compared to the conventional calibration model. It calculated and considered SNE formulation effect for quantification PVT in PVT-loaded SNE formulation. Therefore, it was independent with PVT loading level. Finally, multivariate analysis could be applied to eliminate the placebo/formulation interference for drug quantification independently.

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