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RAPID AND SENSITIVE BIOANALYTICAL STABILITY-INDICATING METHOD FOR QUANTIFICATION OF TALINOLOL, A SELECTIVE β1 ADRENOCEPTOR ANTAGONIST IN LIPID BASED FORMULATIONS USING ULTRAFAST UHPLC SYSTEMS

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Abstract: The current study evaluates the ultra high performance liquid chromatography (UHPLC) method for the quantification of talinolol in lipid-based formulations. A simple, rapid, reliable and precise reversed phase UHPLC method has been developed and validated according to the regulatory guidelines, which was composed of isocratic mobile phase; acetonitrile and phosphate buffer saline (pH 4.5) with a flow rate of 0.4 mL/min, and column HSS C_{18} (2.1 × 50 mm, 1.8 µm). The detection was carried out at 245 nm. The developed UHPLC method was found to be rapid (1.8 min run time), selective with high resolution of talinolol peak (0.88 min) from different lipid matrices and highly sensitive (limit of detection and lower limit of quantification were 0.14 ppm and 0.5 ppm, respectively). The linearity, accuracy and precision were determined as acceptable over the concentration range of 0.5–100 ppm for talinolol. The results showed that the proposed UHPLC method can be used for the estimation of talinolol in lipid-based formulation by indicating its purity and stability with no interference of excipients or related substances of active pharmaceutical ingredient.

Keywords: talinolol, UHPLC systems, lipid based formulation, method validation

Talinolol is chemically known as [1-(4-cyclohexylureidophenoxy)-2-hydroxy-3-tert-butylaminopropane], categorized as a selective β1 adrenoceptor antagonist (Fig. 1), which is structurally related to practolol (1). It is widely used in the treatment of various cardiovascular diseases such as arterial hypertension, acute and chronic tachycardiac heart arrhythmia, and hyperkinetic heart syndrome (2). Talinolol was introduced into clinical practice in 1975 under the trade-mark "Cordanum" in Germany. Talinolol is only available as 50/100 mg immediate release tablets and 5 mL *i.v.* solution (contains 10 mg of talinolol). The recommended daily doses of talinolol range from 25 to 300 mg in healthy volunteers. According to the pharmacokinetics, it is a poorly water soluble drug with solubility of 0.14 mg/mL (BCS II compound, weak base with pKa 9.4, melting point 160–162°C) and it has low, variable and incomplete absorption which leads to poor bioavailability of around 40–55% (3). Thus, it is a suitable candidate for lipid based formulation,

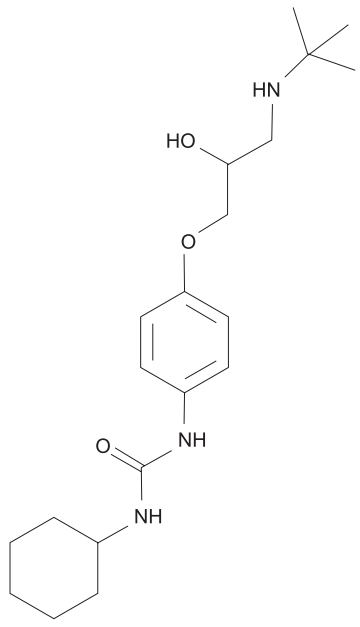

Figure 1. Chemical structure of talinolol (M.w.: 363.5, pKa: 9.4)

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which could improve the aqueous solubility and absorption rate.

In the literature, various analytical methods have been developed using reversed phase (RP) high performance liquid chromatography (HPLC) (4, 5), capillary electrophoresis (6), liquid chromatography (7), for determination of talinolol. Mostly all of these methods were used to quantify talinolol and its metabolites in plasma, urine and feces. Till date, not a single method has been developed in lipid based formulations except biological fluids and caused lengthy and tedious process, lack of required sensitivity or required special reagents and detectors. Thus, an improved analytical method for talinolol should be developed demanding more sensitivity, fast and also required to meet the stability indicating parameters. Within the experimental method development, it was also needed to carry out stability studies under forced acidic, alkaline, thermal, and oxidative degradation processes. The aim of this work was to develop a fast, reliable analysis method for the determination of talinolol using UHPLC.

Within the scope of the current analytical method talinolol was assayed and validated using an advanced UHPLC system, which reduced the time of analysis and the use of solvent. Apart from reducing time and solvent, the current instrument method carries many advantages such as the device, which allows the system to withstand high back pressure without any harmful effect to the analytical column or the whole device. Therefore, the column used in the UHPLC systems can last longer than any other HPLC columns. The proposed method was also successfully applied to the analysis of lipid-based formulations containing talinolol with no interference from dosage form excipients. The method was validated with respect to the standard FDA guidelines for bioanalytical method.

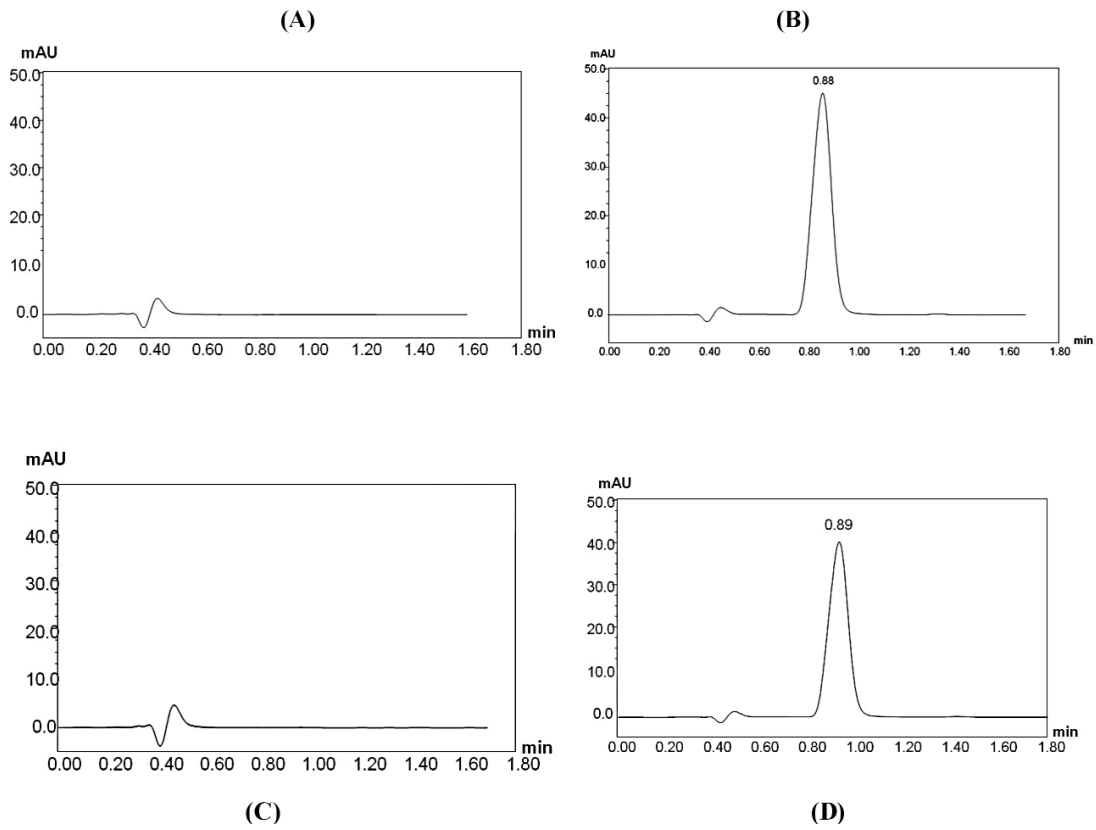

Figure 2. UHPLC chromatograms of blank sample (**A**), standard solution of talinolol at concentration 50 ppm (**B**), drug-free lipid formulation sample (Tween 80, QC_{zero}) (**C**), and drug containing lipid formulation (Tween80, QC) sample (**D**)

EXPERIMENTAL

Materials

All chemicals used in the studies were obtained from commercial suppliers. Talinolol (purity > 99.2%) was obtained as a gift sample from Alfa Aesar, a Johnson Metthey Company (Ward Hill, MA). Acetonitrile, potassium dihydrogen orthophosphate and hydrochloric acid were obtained from BDH Chemicals Ltd., Poole, U.K. Sodium hydroxide and hydrogen peroxide were used as analytical grade reagents. Imwitor 308 (medium chain monoglycerides) and Tween 80 (non ionic surfactant) were used to prepare self emulsifying lipid formulations, supplied by Sasol Germany GmbH, Werk Witten, Germany. The high purity milli-Q water was obtained through a Milli-Q Integral Water Purification System (Millipore, Bedford, MA). All other reagents were of analytical grade and used without further purification.

METHODS

UHPLC chromatographic conditions

Chromatographic separation was developed and optimized with respect to the stationary/ mobile phase compositions, flow-rate, sample volume, column temperature and detection wavelength. The study employed a highly sensitive UHPLC system that consisted of a Dionex ® UHPLC binary solvent manager equipped with a Dionex® automatic sample manager and a photodiode array (PDA) eλ detector obtained from Thermo Scientific, Bedford, MA, USA. The mobile phase was an isocratic mix of HPLC-grade acetonitrile (ACN) and potassium dihydrogen orthophosphate in a ratio of 30 : 70 (v/v) and maintained at pH 4.5. The flow rate was 0.4 mL/min delivered through an Acquity® UPLC HSS C_{18} column (2.1 × 50 mm, 1.8 µm) kept at 30°C. The total run time was 1.8 min. Freshly prepared mobile phase was filtered through an online 0.20 µm filter and degassed continuously by an online degasser within the UHPLC system. The detector wavelength was set at 245 nm and the injection volume was 1.0 µL.

Preparation of buffer solution

Phosphate buffer solution was prepared by dissolving 13.61 g of potassium dihydrogen orthophosphate in 900 mL of milliQ water. The pH of the solution was adjusted to 4.5 with 0.1 M hydrochloric acid and the buffer was further diluted up to 1000 mL with water. The buffer solution was found stable without having any changes with pH and visual clarity for more than a week.

Preparation of stock solution, calibration standards and QC samples

Standard stock solution was prepared by dissolving accurately weighted 50 mg of talinolol powder in 50 mL of milliQ water, resulting in a solution containing 1000 ppm. For the purpose of calibration, the standard solutions of talinolol at seven points were prepared by appropriate serial dilutions in ACN to cover the concentration range of 0.5–100 ppm. These standards solutions were stored at 4°C temperature before use. Calibration curves were obtained by plotting peak area against standard drug concentration and regression equations were computed thereby. Four quality control (QC) samples with the selected concentration levels (2.3, 6.5, 35, 70 ppm) were prepared from stock solution to cover the desired range. QC samples were prepared by spiking the self emulsifying lipid-formulation with known amount of talinolol, and then diluting the mixture with appropriate volume of ACN.

Method validation

The above mentioned developed method in the present study has been validated for linearity, sensitivity, precision, accuracy, selectivity, recovery and stability according to the standard guidelines of bioanalytical method validation (8–10) by US Food and Drug Administration (FDA). The acceptance criteria of precision and accuracy were evaluated as the relative standard deviation of the mean expressed as a percent (coefficient of variation, CV% determined precision of the method).

Linearity and range

Appropriate volume of talinolol stock solution (1000 ppm) was utilized in the preparation of seven non-zero standard drug concentrations covering the calibration range of 0.5–100 ppm. Four different QC samples were prepared by spiking known concentrations of talinolol within the same detection range (0.5– 100 ppm).

Each standard solution (0.5, 1, 5, 10, 20, 50, and 100 ppm) has been injected as six replicates daily on three consecutive days for validation. Calibration solutions were injected in ascending order in each validation run and the other samples were distributed randomly through the run.

Linear regression equation and correlation coefficient (R^2) were employed to statistically calculate the linearity of the results (11).

Specificity

Specificity of the method was required to assess the matrix effect by comparing peak areas

between the drug and different lipid-based formulations.

The specificity of the method was evaluated through the whole assay period using drug free lipid formulation samples (QC_{zero}) and talinolol analyte. The retention times of the drug free lipid components were compared with that of talinolol analyte. In addition, towards the establishment of the method, specificity was also studied by determination of the intact drug in terms of resolution (R) between the drug peak and the nearest degradation product if present (12).

Accuracy and precision

The intra-day accuracy and precision were assessed by analyzing six replicates of each seven talinolol standards within the same day. Similarly, the inter-day accuracy and precision were also determined during the three consecutive days using six replicates analysis of the LLOQ, low, medium and high QC samples. The overall precision of the method was expressed as relative standard deviation (RSD) and accuracy of the method was expressed as % drug recovered.

Limit of detection (LOD) and lower limit of quantification (LLOQ)

The LOD and LLOQ levels were determined by serial dilutions of talinolol stock solutions in order to obtain signal to noise (S/N) ratio of at least ≈ 3 : 1 for LOD and ≈ 10 : 1 for LLOQ (13).

RESULTS AND DISCUSSION

UHPLC peak separation

Separation and detection of talinolol peak without any minimum interference was ideal by the developed UHPLC assay. The chromatographic results of UHPLC technique in the current analysis

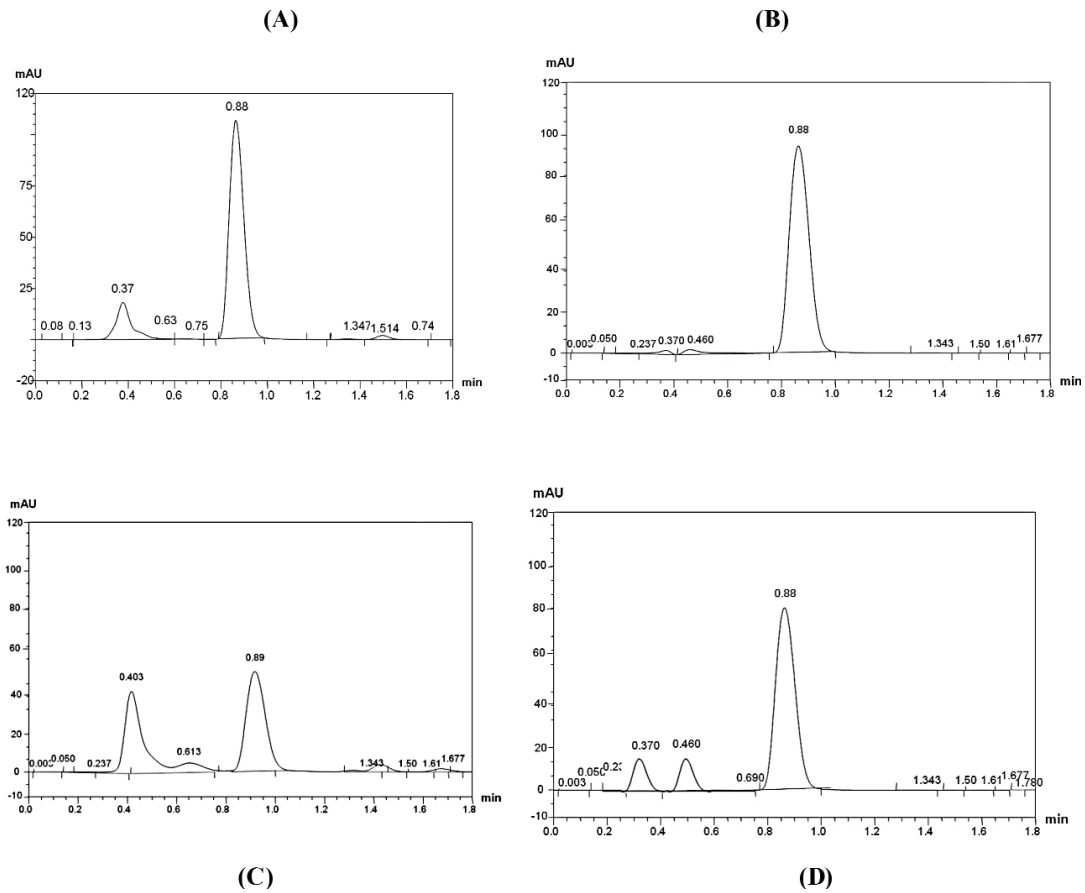

Figure 3. Typical UHPLC chromatograms of: (**A**) acid hydrolysis degraded talinolol, (**B**) base hydrolysis degraded talinolol, (**C**) oxidative degraded talinolol and (**D**) thermally degraded talinolol

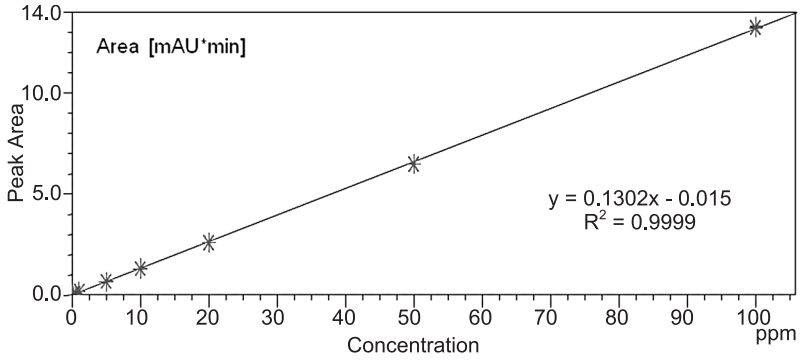

Figure 4. UHPLC calibration curve of talinolol in ACN

Table 1. UHPLC data of intra-day back-calculated talinolol concentrations of the calibration standards in ACN.

Nominal concentration (ppm)	Back calculated concentrations (ppm)									
	1st	2nd	3rd	4th	5th	6th	Mean	S.D.	Precision %	Accuracy %
0.5	0.55	0.53	0.56	0.56	0.57	0.52	0.55	0.022	3.378	109.59
1	1.05	1.02	1.06	1.06	1.08	1.04	1.05	0.022	2.095	105.16
5	5.08	5.06	5.12	5.10	5.10	5.09	5.09	0.020	0.399	101.84
10	9.96	9.92	9.91	9.90	9.94	9.92	9.93	0.019	0.194	99.26
20	19.57	19.71	19.65	19.63	19.53	19.66	19.62	0.065	0.331	98.12
50	49.23	49.33	48.98	49.16	49.00	49.24	49.16	0.138	0.281	98.31
100	100.46	100.41	100.55	100.54	100.57	100.45	100.50	0.067	0.066	100.50

show that talinolol can be determined well enough in the self emulsifying lipid formulations within the highest sensitivity and selectivity of this analytical procedure and in a very short time. In the previous studies, Sinha and Damanjeet (5) developed talinolol method using conventional HPLC and showed the talinolol peak at 12.5 min. Our current analysis has a great advantage over the previous studies, which reduced the analysis time and solvent consumption significantly. Figure 2 shows the representative chromatograms of blank sample (2A), standard solution of talinolol (2B), drug-free lipid formulation (QC_{zero}) sample (2C), and drug-containing lipid formulation QC sample (2D). The talinolol analyte was well separated from the solvent peak (used as mobile phase as well as sample dilutions) at retention time of ~0.88 min, while there was no degradation product detected in the sample formulation (Fig. 2 A–D). The total chromatographic run time was ~ 1.8 min and the talinolol peak was of good shape and completely resolved.

Forced degradation study

The forced degradation study was conducted by treating model drug talinolol with 1 M HCl, 1 M NaOH, 3% H_2O_2 under 60°C temperature for 12 h in water bath. In addition, the thermal degradation experiment was carried out under 120°C temperature in an oven. All the talinolol sample solutions used in forced degradation studies were prepared with final concentrations of 100 µg/mL (100 ppm). The results showed by the degradation study that the amounts of talinolol in the samples were lowered compared to the original concentrations. This degradation study was particularly important for the analysis of lipid based formulation, which was not studied previously (5).

Acid degradation

Ten milligrams of talinolol powder was transferred into a 100 mL round bottom flask and then10 mL of 1 M hydrochloric acid solution was added. The contents of the flasks were mixed well and kept for overnight at 60°C temperature. After cooling the solution at room temperature, 10 mL of 1 M sodium hydroxide solution was added in 100 mL volumetric flask for neutralization and then diluted to 100 mL with ACN. The chromatogram of the acid degradation is shown in Figure 3A.

Basic degradation

Ten milligrams of talinolol powder was transferred into a 100 mL round bottom flask and then10 mL of 1 M sodium hydroxide solution was added. The contents of the flasks were mixed well and kept for overnight at 60°C. After cooling the solution to room temperature, 10 mL of 1 M hydrochloric acid solution was added in 100 mL volumetric flask for neutralization and then diluted to 100 mL with ACN. The result of the basic degradation is shown in Figure 3B.

Oxidation

Ten milligrams of talinolol powder was transferred into a 100 mL round bottom flask with the addition of 10 mL of 3% hydrogen peroxide solution. The contents of the flasks were mixed well and kept for overnight at 60°C. After cooling the solution to room temperature, it was diluted to 100 mL with ACN. The chromatogram of the oxidation data is shown in Figure 3C.

Thermal degradation

Ten milligrams of talinolol powder was transferred into a 100 mL round bottom flask and kept in oven at 120°C for 24 h. After cooling the powder yo room temperature, the ACN was added up to 100 mL as diluent. The chromatogram of the thermal degradation study is shown in Figure 3D.

Table 2. UHPLC data of inter-day accuracy and precision of talinolol QC samples.

Day of analysis	QC1	QC2	QC3	QC4
	2.30 ppm	6.50 ppm	35 ppm	70 ppm
1st	2.23	6.61	34.93	69.68
	2.09	6.67	35.08	70.48
	2.33	6.47	35.05	69.32
	2.36	6.55	34.94	69.98
	2.33	6.60	35.08	69.43
	2.31	6.66	34.93	69.76
2nd	2.38	6.99	35.15	70.04
	2.29	6.70	35.38	70.28
	2.35	6.49	35.06	70.08
	2.26	6.55	35.33	69.89
	2.35	6.71	35.15	69.48
	2.31	6.60	35.19	70.19
3rd	2.39	6.44	34.37	68.42
	2.35	6.39	34.74	69.06
	2.33	6.44	34.93	69.05
	2.41	6.33	34.33	68.73
	2.45	6.38	34.16	68.17
	2.38	6.65	34.36	68.88
Mean	2.327	6.567	34.897	69.497
SD	0.060	0.117	0.376	0.681
Precision %	2.591	1.778	1.077	0.980
Accuracy %	101.159	101.026	99.705	99.281

Table 3. Statistical data of the regression equation for the determination of talinolol obtained from the proposed method.

Parameters	UHPLC Method
Concentration range	0.5–100 ppm
Intercept	0.015
Slope	0.1302
Correlation coefficient (r)	0.9999
Limit of detection (LOD)	0.14 ppm
Limit of quantification (LOQ)	0.5 ppm

Table 4. Systems suitability parameters of talinolol.

System suitability parameter	Talinolol
Retention time	0.88 min
% RSD	0.067%
Peak tailing	1.05
Theoretical plate number	2829

Bioanalytical method validation

The precision and accuracy of the method show an excellent workability of the method. The precision was 20% or better for LLOQ (% RSD) and 15% or better for the remaining concentrations and the acceptable accuracies were 100 ± 20% or better for LLOQ and 100 ± 15% or better for the remaining concentrations.

Linearity and range

A seven point standard calibration curve was constructed to see the linearity within the concentration range. The peak area response of talinolol was linear over the concentration range between 0.5 and 100 ppm (Fig. 4). The result of linear regression gives the following mean equation:

$$y = 0.1302x - 0.015$$

where y and x denote: the peak area and the concentration of the analyte, respectively. This result shows an excellent linearity (approaching a straight line function) over the interval studied. The correlation coefficient (r) was higher than 0.9995 with an average value of 0.9999 (Table 3) (11).

Accuracy and precision

The intra-day and inter-day accuracies were calculated as the % of drug recovered after analyzing six replicates of the QC samples at four nominal concentration levels. The intra-day (Table 1) accuracies were found in between 98 and 109.59% and the inter-day (Table 2) accuracies were in between 99.70 and 101.154%. The results from the drug recovery studies suggest that the accuracy of the assay method was within the acceptable limits according to the FDA guidelines (11).

Precision

The developed method was found to be precise as the intra-day standard deviation (SD) values (Table 1) of six replicate analyses were within the range of 0.02–1.38 ppm. Within the analytical concentration range of 0.5–100 ppm, % CV values were less than 3.38%. In addition, the inter-day (Table 2) accuracies of six replicates during the three consecutive days were between 0.06 and 0.68 ppm, whereas the % CV values were less than 2.59%. These low values of both SD and % CV during the intra-day and inter-day analysis thus met the accepted requirements of precision for the current method (14).

Specificity

The specificity of the developed UHPLC method was investigated in order to measure the analyte response in the presence of its degradation products. Specificity was established by determining the purity of the talinolol peak using a PDA detector. In addition, the resolution factor of the drug peak was determined with respect to the near-

est resolving peak. The developed method was found to be specific for talinolol without having any possible interference from the degradation product(s). The result from a sample of lipid solubility studies (talinolol loaded in lipid formulation) in Figure 2D shows that there was no degradation products present in the sample containing talinolol. It seems that talinolol compound can be recovered completely from the lipid formulation (Fig. 2D). Therefore, the R value in this assay can be calculated based on the availability of the talinolol peak only. In addition, there were no significant interfering peaks present in randomly selected drug free lipid formulation (QC_{zero}) samples at talinolol retention time (Fig. 2C), that suggests that talinolol compound can be analyzed predominantly from lipid based formulations.

Limit of detection (LOD) and lower limit of quantification (LLOQ)

The LLOQ in the assay was 0.5 ppm, which was estimated to be the lowest concentration in the standard curve that can be measured with acceptable accuracy and precision for the talinolol analyte with S/N ratio of 79.7. To the best of our knowledge, this concentration was relatively low as compared to other available bioanalytical methods that developed using the similar instruments. On the other hand, LOD was 0.14 ppm with S/N ratio of 27.8. Both LLOQ and LOD were experimentally verified by six replicate injections of talinolol standard concentrations (Table 3).

Suitability of the systems

Systems suitability parameters were taken into consideration to confirm the highest precision of the systems. The variation (% RSD) in the peak area from six replicates injections was around 0.067%, which proves that the system is precise. The results of other chromatographic parameters such as peak tailing and theoretical plate numbers (showing column efficiency) are shown in Table 4. The overall analysis results show the acceptable performance of the system as the % RSD and the tailing peak are not more than 2.0 % and 1.5, respectively, along with the theoretical plates, which are not less than 2000.

Application

Within the scope of the current research, the developed UHPLC method has been successfully used for the quantification of talinolol compound in the studies of equilibrium solubility, and dissolution profiles of self emulsifying lipid-based formulations (SEDDS/SMEDDS) (15).

CONCLUSION

The developed UHPLC analytical method provides a reliable, reproducible and specific assay for talinolol in pure form and pharmaceutical formulations. The described method is sensitive enough to detect as low as 0.14 ppm and exclusively offer a rapid determination of talinolol (peak at 0.88 min within 1.8 min run time). No significant interferences were caused by the formulation excipients, diluents and/or degradation products.

The validation of the method allows quantification of talinolol in the range between 0.5 to 100 ppm. Compared to previously reported methods, the present assay method assessed extensive validation parameters according to FDA guidelines. The method has shown acceptable precision, accuracy and adequate sensitivity and demands to be in use for further studies.

The established method satisfies the system suitability criteria, peak integrity, and resolution of the drug peak. The overall results clearly indicate that the current method is attractive due to the good selectivity for quantitative determination of talinolol in lipid-based formulation and also suitable for stability measurements.

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