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METHOD DEVELOPMENT AND STABILITY-INDICATING METHOD FOR TALINOLOL EMULSION BY RP-HPLC

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ABSTRACT:
The objective of this investigation was to synthesize Talinolol emulsions at the nanoscale to enhance their release, permeability, and solubility, hence augmenting their oral bioavailability. The development and validation of analytical procedures for the measurement of talinolol in bulk and dose forms were conducted in accordance with the ICH standards. RP-HPLC methods were developed and their specificity, accuracy, precision, and robustness were validated in order to evaluate Talinolol.

Key words: Talinolol,Method development, Forced degradation studies, Talinolol emulsions

INTRODUCTION:

Stability testing and forced degradation studies play a very crucial role during drug development to elucidate the intrinsic stability of a drug substance. The stability testing of the drug substance should be carried out under different stress conditions to validate the stability indicating supremacy of the analytical methods used for the analysis of stability samples. The prime objective of studying the stability of a drug is to determine the shelf-life of the drug. The various conditions specified for the forced degradation studies should include extremes of pH, oxidative and photolytic degradation, and the effect of temperature [1–2].

Furthermore, a stability-indicating assay method provides assurance on the detection changes in identity, as well as the purity potency of the product.

In the literature, various analytical methods have been developed for determination of Talinolol in plasma, urine, and in feaces. Oertel et al. used liquid chromatography–tandem mass spectrometry (LC–MS–MS) methods for the determination of talinolol in plasma. Talinolol was assayed in serum using a high-performance liquid chromatography (HPLC)–fluorimetric detector method; Number of methods has been reported in the literature for the determination of Talinolol. As we novel emulsion formulation we prepared so, it was decided to carry out the stability study of talinolol emulsion towards acidic, alkaline, neutral, thermal,oxidative, and photolytic degradation processes specified as per the ICH guidelines for stress testing [3-5].

Material and Methods:
The physiochemical properties of drug samples containing Talinolol were assessed through the implementation of various analytical techniques: particle size analysis, differential scanning calorimetry infrared spectroscopy, and melting point analysis.
Talinalol, was attenuated to a concentration of 50 pg/ml each, and the A.max was calculated by scanning them with a UV-visible spectrophotometer in the 200–400 nm range.
The Talinalol was placed in standard aluminum containers, which were subjected to a dry nitrogen flow rate of 25 milliliters per minute and a scanning rate of 10°C/min. Drugs were thermogrammed using DSC at temperatures ranging from 50°C to 350°C.

Preparation of standard plot by UV method:
The pharmaceuticals were standard-plotted in triplicate using phosphate buffer at pH 6.8, distilled water, hydro-alcoholic solution, methanol, 0.1 N HC1, and Kreb's Ringer phosphate buffer. The concentration range of the standards was 2–20 pg/ml.

Preparation of standard plot in water/hydro-alcoholic solution:
In order to create the standard graphs for Talinolol, 10 mg of each drug was dissolved in a modest amount of alcohol. Subsequently, distilled water was added to the mixture to achieve a final volume of 100 ml for both the methanol:water and water components

Preparation of standard plot in methanol:
In order to generate standard plots for talinolol, 10 mg of each drug was dissolved in 100 cc of methanol.

Development of validated RP-HPLC method for Talinolol:
It was demonstrated that the RP-HPLC method is a dependable technique for estimating talinolol. The solubility of Talinolol in various solvents utilized during the formulation development of SEDDS/nanoemulsions was evaluated utilizing a well-established methodology.

Chromatographic conditions:
The following are the HPLC conditions used in the analytical method:

HPLC instrument: HPLC apparatus comprised of a model series LC-10 ADVP pump, an SPD-10 AVP UV-visible detector, a SCL-10 AVP system controller, and a DGU-12 A degasser, all connected to a Rheodyne 7725i injector. The information was acquired using the Spinchrom software.

Column: Column size: 250 mm x 4.6 mm 5pm; Inertsil ODS-3 C-18.

Mobile phase: Acetonitrile and potassium dihydrogen orthophosphate buffer (pH 4.4) were combined at a ratio of 27:73 to create the mobile phase.

Flow rate: 1.0 ml/min.

Temperature: 40°C.

Injection volume: 20 pi.

Detector: UV detector, max 242 nm.

Diluent: Mobile Phase.

In order to prepare the Talinolol standard stock solution, 50 ml of methanol was added to a 100 ml volumetric flask containing 100 mg of precisely weighed Talinolol powder. The flask was then sonicated for 30 seconds. One minute after the final volume of triple-distilled water was added, the mixture was vortexed.

Validation:

Linearity and range

The calibration curve of Talinolol was produced in a concentration range of 2–100 pg/ml to guarantee linearity. By plotting the peak area against the appropriate concentration and applying a linear regression analysis to the data, the calibration graph was generated.

Accuracy

Utilizing the amount of talinolol added in standard solutions, the accuracy of the procedure was determined. Talinolol was detected at three concentrations that caused spikes. Following this, the percentage recovery of the additional substance was calculated using the linearity plot.

Precision

In order to ascertain the consistency of the results both on and off days, repeatability research was undertaken. On the same day, four distinct Talinalose concentrations were injected in order to conduct intra-day precision testing.

Limit of detection and limit of quantification

The results revealed that the limits of quantitation and detection were 10 a/S and 3.3 a/S, respectively, where S represented the slope of the calibration curve (n = 6) and the y-intercept standard deviation of the regression equation. In order to ascertain the LOD and LOQ values for each drug concentration, the sample was hexaplicate-injected.

Robustness

To investigate the effect of these variables on peak resolution, the flow rate and column temperature were varied from 35°C to 45°C and 0.8 to 1.2 ml/min, respectively, for the duration of this experiment.

Forced degradation studies

Acidic conditions

In order to conduct acidic hydrolysis, various concentrations of hydrochloric acid were added to a 1 mg/ml Talinolol solution. A 100 ml volumetric flask was utilized to contain 100 mg of Talinolol, which had been 0.0IN HC1 dissolved in 5 ml of methanol. The cylinder was subsequently maintained at 25°C for two hours and 40°C for eight. Additionally, the pharmacological solutions were prepared in the same manner utilizing 0.1N HC1, refluxed for durations of 2, 8, and 12 hours, and stored at 40°C overnight.

Alkaline conditions

To facilitate our examination of degradation in an alkaline environment, a solution containing Talinolol at a concentration of 1 mg/ml was prepared. Following the dissolution of 100 mg of Talinolol in 5 ml of methanol, 0.0IN sodium hydroxide was added to bring the volume to 100 ml. The mixture was subsequently subjected to two hours at 25°C and eight hours at 40°C.

Neutral conditions

To enable the process of neutral hydrolysis, 5 ml of methanol was added to a 100 ml volumetric vial containing 100 mg of Talinolol. After adjusting the volume with 1 mg/ml of triple-distilled water, the mixture was refluxed for twelve and twenty-four hours after being heated to 25°C for two hours and 40°C for eight hours.

Thermal degradation studies

In order to determine the sensitivity of Talinolol to heat stress, both the bulk drug and the drug solution were dried-heated to 70°C in a hot air oven for fifteen days. Following a predetermined collection schedule, samples were diluted tenfold prior to analysis via the efficient RP-HPLC method.

Oxidative conditions

Analyses on oxidative deterioration were carried out utilizing solutions containing hydrogen peroxide in variable concentrations at ambient temperature. A solution of Talinolol was formulated with 3% H_2O_2 and left to react for durations of 6, 10, and 24 hours. A day later, an analogous solution containing 10% H_2O_2 was prepared and analyzed. Also investigated were medication solutions containing 30% H_2O_2 and stored at ambient temperature for duration of 48 hours.

Photo degradation studies

To examine the process of photodegradation, solutions and powdered medications were exposed to light within a photo-stability chamber. A delicate layer of the powder was spread onto a Petri dish. There was a total of 6000 lux of fluorescent illumination and 0.7 W/m of ultraviolet light at the placement site. Following a period of 15 days, the extracted samples were diluted tenfold before undergoing a proficient RP-HPLC analysis. Samples in both powder and solution forms were maintained adjacent to one another for the same duration in a dark, covered chamber.

RESULTS AND DISCUSSION:

Values observed in the IR spectrum of Talinolol:1447.9 -C-C stretches,1509.6 -C-O-C stretch,1570.8- NH stretch**,**1626.9 -C=0 amide carbonyl**,**2933.5 -C-H stretch (methyl),3103.6-CH stretch (aromatic)**,**3311.4 -NH stretch.

Particle size Distribution

The average particle diameters of Talinolol were calculated to be 19.36 um based on the plots presented.

Analytical method development for Talinolol:

Preparation of standard plots of Talinolol by UV method:

(a) Preparation of standard plot of Talinolol in water: methanol (95:5 %v/v)

The standard plot of talinolol in water:methanol was determined to be linear across the concentration range of 2–20 pg/ml, as indicated by the E_1cm^1% value of 450.

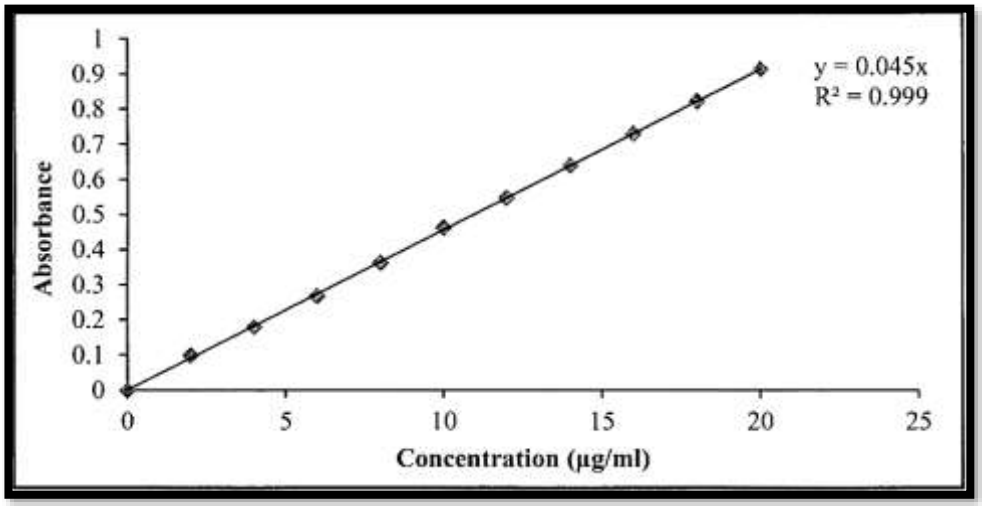

Figure.1: Standard solution for Talinolol in water: methanol.

Preparation of standard plot of Talinolol in phosphate buffer (pH 6.8):
The standard plot of Talinolol in phosphate buffer (pH 6.8) was found to be linear in the concentration range of 2–20 pg/ml with an E_1cmvalue of 420.

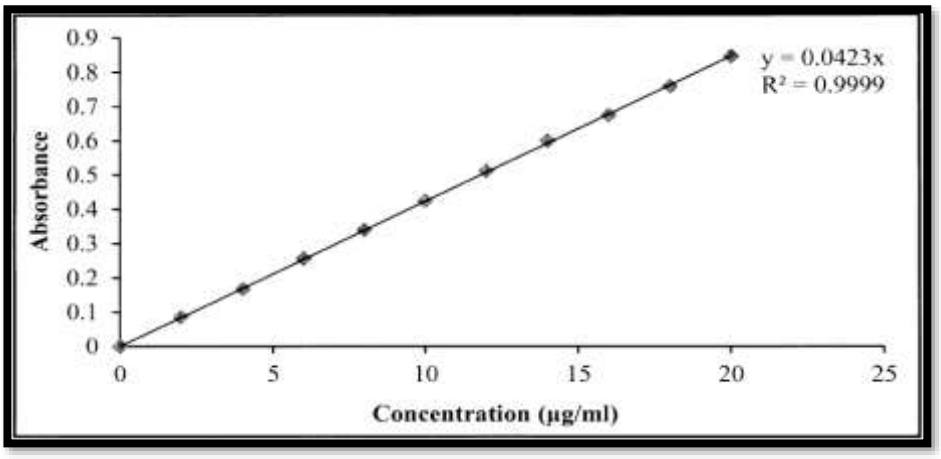

Figure 2: Standard plot of talinazolol in phosphate buffer.

Validation of HPLC method for Talinolol estimation

1. Linearity and range

The linearity curve indicated that the drug exhibited perfect linearity of reaction throughout the concentration range of 2 to 100 pg/ml. The percentage R.S.D. indicated a significant correlation of 0.998 and a slope of 33.51±1.13. Figure 5.9 presents the chromatograms of Talinolol at various concentrations.

Table 1: parameters of the regression equation for the talinolol measurement produced in the HPLC technique.

Parameters	**Values**
Calibration Range(pg/ml)	2-100
Detection limit (pg/ml)	0.125
Quantitation limit (pg/ml)	0.378
Regression equation(Y)[a]	
Slope(b)	33.51
Standard deviation of the slope(S_b)	0.31
Relative standard deviation of the slope (%)	1.13
Intercept (a)	0
Correlation coefficient (r)	0.998

2. Accuracy

Table 2 displays the data acquired during the recovery investigations. The results indicated that the range of drug recovery from a mix of deteriorated samples was 102.36% to 100.5%. The gathered data indicates that the technique shows no discernible interference and a high degree of accuracy in identifying Talinolol and its degraded samples.

Table 2: conclusions drawn from three recovery studies that using the HPLC method.

Spiked Concentration.(µg/ml)	**Calculated spiked con.(µg/ml)±S.D;%R.S.D**	**Recovery%**
2	1.02±1.70;2.38	500.1
50	41.45±3.85;0.61	602.31
100	601.02±1.70;2.38	501.41

3. Precision

It was demonstrated that the percent R.S.D. for experiments with repeatable accuracy ranged from 0.19% to 1.68%. For investigations with moderate precision the percent R.S.D. values ranged from 0.18% to 1.8%. The study's findings demonstrated how remarkably accurate the method is in predicting Talinolol, with percent R.S.D. values of less than 2%.

Table 3: Findings from the precision study on the HPLC technique

Actual Concentrations (µg/ml)	Measured Concentrations.(µg/ml)±S.D;%R.S.D	
	Repeatability(n=6)	Intermediate precision(n=6)
2	8.00±1.20;1.62	0.94±1.24;1.81
10	00.15±2.10;0.62	8.95±0.61;0.19
50	90.44±3.03;0.10	39.96±17.84;1.14
100	101.73±23.17;0.67	600.65±9.09;0.21

5. Limit of quantitation and limit of detection

The results of the measurements showed that the limits of detection and quantification were, respectively, 0.378±0.003 pg/ml and 0.125±0.002 pg/ml.

6. Robustness

The little variation in resolution between Talinolol and its breakdown products under purposefully altered chromatographic conditions demonstrated the robustness of the approach.

7. System suitability test parameters

For 1.39 minutes, the unretained apex was maintained within the permitted range of $0.8 < T < 1.5$. When talinolol breaks down, TL1, TL2, and TL3 are produced.

5.5 Forced degradation studies

Table 4: For Talinolol, several stress thresholds were used

Stress	Strength	Conditions Exposed	%Amount remaining)±S.D
Acidic	0.01N HCl	25^0 C,2h	400±0.01
		40^0 C,8h	500±0.01
	0.1N HCl	40^0 C,24h	75.15±4.49
		2h reflux	75.15±2.79
		8h reflux	50.15±2.71
Alkaline	0.01NNaOH	25^0 C,2h	500±0.51
		40^0 C,8h	800±0.61
	0.1N NaOH	40^0 C,24h	900±0.91
		2h reflux	800±0.99
		8h reflux	851.50±1.04
Neutral	Water	25^0 C,2h	600±1.01
		40^0 C,8h	100±1.51
		12h reflux	52.11±2.67
		24h reflux	25.37±1.01
Thermal	0.01N HCl	70^0 C,15 day	56.23±1.36
	Drug Powder	70^0 C,15 day	500.23±1.61
Oxidative	30%H_2O_2	RT 6h	600.23±1.00
		RT 10h	500.23±0.65
		RT 24h	400.23±0.61
	10%H_2O_2	RT 24h	200.23±0.31
	30%H_2O_2	RT 48h	91.97±10.29
Light(Wrapped Samples)	0.1NHCl	Cool white	200.23±0.60
	Water:Methanol	Fluorescent & UV light 15 Day	500.23±0.61
	0.1N NaOH	-	200.23±0.21
	Drug Powder	-	500.23±0.51

Light(Unwrapped Samples)	0.1NHCl	Cool white	500.23±0.62
	Water:Methanol	Fluorescent & UV light 15 Day	400.23±0.61
	0.1N NaOH	-	600.23±0.71
	Drug Powder	-	300.23±0.31

Alkaline conditions

It was shown that the drug exhibited greater resistance to alkaline hydrolysis in comparison to acidic hydrolysis. As a result, RT 2.5 and 3.29 minutes were when the largest amount of degradation products was discovered. Unlike the results of acidic hydrolysis, a second peak was seen at 3.29 minutes, indicating the formation of a third chromophoric molecule.

Neutral conditions

The Talinolol was subjected to neutral hydrolysis after 12 and 24 hours of reflux, resulting in effective breakdown rates of 278.99% and 84.63%, respectively. HPLC examination of a 24-hour deteriorated sample revealed that the medication suffered two major degradation products, each with a relative retention time of 4.17 minutes and 2.53 minutes.

Thermal degradation studies

Temperature stress did not weaken or distort Talinolol. On the other hand, after 15 days at 70°C, 34% of the drug's concentration broke down. Three additional peaks that showed the fall in the drug peak area appeared at RT values of 2.5, 3.3, and 4.17, according to HPLC analysis.

CONCLUSION:

The well-established RP-HPLC technique is simple, sensitive, repeatable, selective, and stability-indicating for the medication as well as any breakdown products generated, based on the results of stress testing tests carried out in accordance with ICH recommendations for Talinolol emulsion. Talinolol emulsion method may be used for the routine testing of pharmaceutical formulations.

REFERENCES:

1. He Juan, Bernd Terhaag , Yang Li-Ying , Zhang Bi-Kui , Su Fen-Li , Zhu Yun-Gui , Song Juan , Tang Jing , Liu Xiao-Lei , Peng Wen-Xing Determination of talinolol in human plasma by high performance liquid chromatography–electrospray ionization mass spectrometry: Application to pharmacokinetic study Journal of Chromatography B, 2007, 853: 1–2, 15,275-2802.
2. Shriram M Pathak , Prashant B Musmade, Krishnamurthy M Bhat, Nayanabhirama Udupa Validated HPLC method for quantitative determination of talinolol in rat plasma and application to a preclinical pharmacokinetic study .Bioanalysis. 2010,2(1):95-104.
3. V.R. Sinha and Damanjeet Ghai RP-HPLC Stability-Indicating Assay Method for Talinolol and Characterization of its Degradation Products Journal of Chromatographic Science, 2011 Vol. 49, 786-795.
4. Mohsin K , Al-Qahtani S , Alanazi FK Rapid and sensitive bioanalytical stability-indicating method for quantification of talinolol, a selective β1 adrenoceptor antagonist in lipid based formulations using ultrafast UHPLC systems. Acta Poloniae Pharmaceutica, 2014, 71(5):737-745
5. H. Mahgoub, A. Hanafy Development and Validation of a Gradient Elution High-Performance Liquid Chromatographic Method for Determination of Talinolol in Rat Plasma: Application to a Preclinical Food–Drug Interaction Study Acta Chromatographica.2015, 27:1, 67–79.