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QUANTIFICATION OF CARVEDILOL IN VARIOUS LIPIDS BY RP- HPLC/UV METHOD: APPLICATION IN DEVELOPMENT OF LIPID BASED DRUG DELIVERY SYSTEMS

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ABSTRACT

Lipid based systems have improved solubility and bioavailability of drugs than the conventional systems. In order to determine the lipid solubility of Carvedilol in various oils like labrafil M2125, capryol PGMC and oleic acid, a simple and novel RP- HPLC method was used. The method was developed using acetonitrile and Methanol in the ratio of 90/10 % v/v with a flow rate of 1 ml/ min. The drug was eluted at retention time around 3.96 min with run time of 7 minutes. The detection is performed at wavelength 242 nm. The method was validated finally according to ICH guidelines. Linearity was observed in the range of 5 to 90 mg/mL for labrafil M2125, capryol PGMC and oleic acid The intra-day precision of Carvedilol in labrafil M2125, capryol PGMC and oleic acid ranged from 0.58–0.80, 0.9–1.25 and 0.9–1.82 and the inter-day precision ranged from 0.50–1.62, 0.93–1.63 and 1.06–2.22. The percentage recoveries of carvedilol in labrafil M2125, capryol PGMC and oleic acid ranged from 99.6 to 101.25%.

KEY WORDS

Oil solubility, lipid based systems, Carvedilol, RP-HPLC.

INTRODUCTION

Carvedilol is a non selective adrenergic receptor blocker used to treat various cardiac problems. The chemical formula of carvedilol is (±)-1-9H-(carbazol-4-yloxy)-3-[[2-(2-methoxyphenoxy) ethyl]amino]-2-propanol (Figure 1). Currently, Carvedilol is used to treat hypertension, myocardial ischemia and congestive heart failure. Carvedilol has greater antioxidant activity and improves mitochondrial function. Carvedilol has been proved to reduce the risk of ischemia but is not observed with other beta blockers[1].

Several analytical methods have been reported for the determination of Carvedilol in pure drug, pharmaceutical dosage forms and in biological samples using spectrophotometer liquid chromatography, electro kinetic chromatography high performance thin layer chromatography. High-performance liquid chromatography (HPLC) with fluorescence detector, mass spectrometer or electrochemical detection has been reported for the quantification of carvedilol and its enantiomers. Estimation of cavedilol by capillary electrophoresis has also been reported[2, 3].

Currently, lipid based drug delivery systems are popular because of their advantages over conventional systems like improved solubility and bioavailabiliy of drugs. Analysis of active

pharmaceutical ingredients in bulk and conventional pharmaceutical formulations were reported in many publications but methods of quantification of drugs in oil bases were limited. In the way of development of carvedilol lipid based formulations, estimation of oil solubility of carvedilol in various types oils and lipids is necessary to select the oil or lipid to select as an excipient to develop a lipid based system[4, 5]. Methods available to estimate carvedilol in bulk and formulations were not suitable for oil solubility studies. Till now there no validated mehod published on oil solubility studies of carvedilol with RP-HPLC.

Instrumentation and chromatographic conditions

Quantitative HPLC was performed on Waters HPLC system equipped with waters 515 pump and Waters 2489 dual wavelength UV detector. Empower2 software is used for data acquisition. A Stainless steel Thermo Scientific column with dimensions 4.6 x 250mm, packed with Octadecylsilane bonded to porous silica (C18) having particle size 5 micron. Chromatographic operation was performed isocratically at room temperature. The mobile phase consisted of a mixture of acetonitrile and methanol in a ratio of 90:10 (v/v), at a flow rate of 1.0 mL/min. The eluent was monitored with a UV detector set at 242 nm.

Preparation of stock solution

A primary stock solution of carvedilol (1000 µg/ mL) was prepared by weighing and dissolving in mobile phase and oils such as labrafil M2125, capryol PGMC and oleic acid. The working solutions for calibration curve and quality control samples were prepared from stock solutions by appropriate dilution with the corresponding oils to achieve the concentrations of 5, 15, 30, 45, 60, 75 and 90 µg/mL.

Solubility study

Solubility studies were conducted in the oils for Carvedilol for the development of lipid based formulation. Solubility of the drug in various oils is key determinant for the efficacy of formulation which is important for high drug loading capacity and size of the formulation. The solubility study of Carvedilol was done in three oils, labrafil M2125, capryol PGMC and oleic acid. In brief, solubility study was conducted by adding an excess amount of Carvedilol in 10 mL glass vials containing 5mL of oil separately. The vials were stoppered and placed on rotary shaker for 72 hours at 25 ^{o}C. after equilibration, the mixtures were taken into 2 mL eppendorf tubes and centrifuged ar 5000 RPM for 30 minutes. After centrifugation, supernatant was collected from three different depths. Collected samples (100µL) were solubilised in 10 mL of mobile phase and 20 µL solution was injected into HPLC. Using standard graph of Carvedilol in various oils, the solubility was estimated.

System suitability test

System suitability test was performed by injecting blank solution once and standard solution of 100% test concentration six times in to stabilized HPLC system. The system suitability was established by evaluating the system suitability parameters from the last peak obtained. System suitability parameters include retention factor (k'), repeatability, resolution (R), tailing factor (T) and theoretical plates (N)[6,7].

METHOD VALIDATION

Specificity and selectivity

The specificity of the method was determined by analyzing different oils tested for the solubility of Carvedilol. Specificity was done to confirm the non interference of oils with the peak of Carvedilol.

Calibration curve:

A calibration curve was constructed using calibration samples covering the range of 5 to 90µg/mL in various oils. For calibration curve, three replicates of each concentration were injected. The calibration curve was plotted by using the average peak area of the analyte against the concentration

of the analyte. The concentration of the analyte was calculated using the calibration curve and evaluated by linear regression equation.

Precision and accuracy

The intra-day precision was determined by analyzing three independent replicate QC samples (30, 45 and 60 µg/mL) of Carvedilol in corresponding oils on the same day under the same experimental conditions. The reproducibility (day-to-day variation, i.e., inter-day precision) of the method was determined by analyzing three sets of QC samples (30, 45 and 60 µg/mL) on two different days. Precision was determined as the coefficient of variance (CV), and expressed as percentage RSD[8, 9].

Accuracy is the closeness of agreement between the values that are accepted, either the conventionally true value or an accepted reference value, and values that are found. Accuracy was determined by analyzing three replicates at three QC levels (30, 45 and 60 µg/mL) on the same day and on three different days using optimized chromatographic conditions.

RESULTS AND DISCUSSION

Method development and Optimization

RP-HPLC method was developed utilized for the determination of Carvedilol in various oils. Method was optimized using different buffers like di-potassium hydrogen phosphate, acetonitrile, methanol with different compositions were tried. Different columns were investigated, including C18, C8.). The method was optimized finally using combination of acetonitrile and Methanol in the ratio of 90/10 % v/v with a flow rate of 1 ml/ min. The drug was eluted at retention time around 3.96 min with symmetric peak shape. The run time was set for 7 minutes. The detection is performed at wavelength 242 nm.

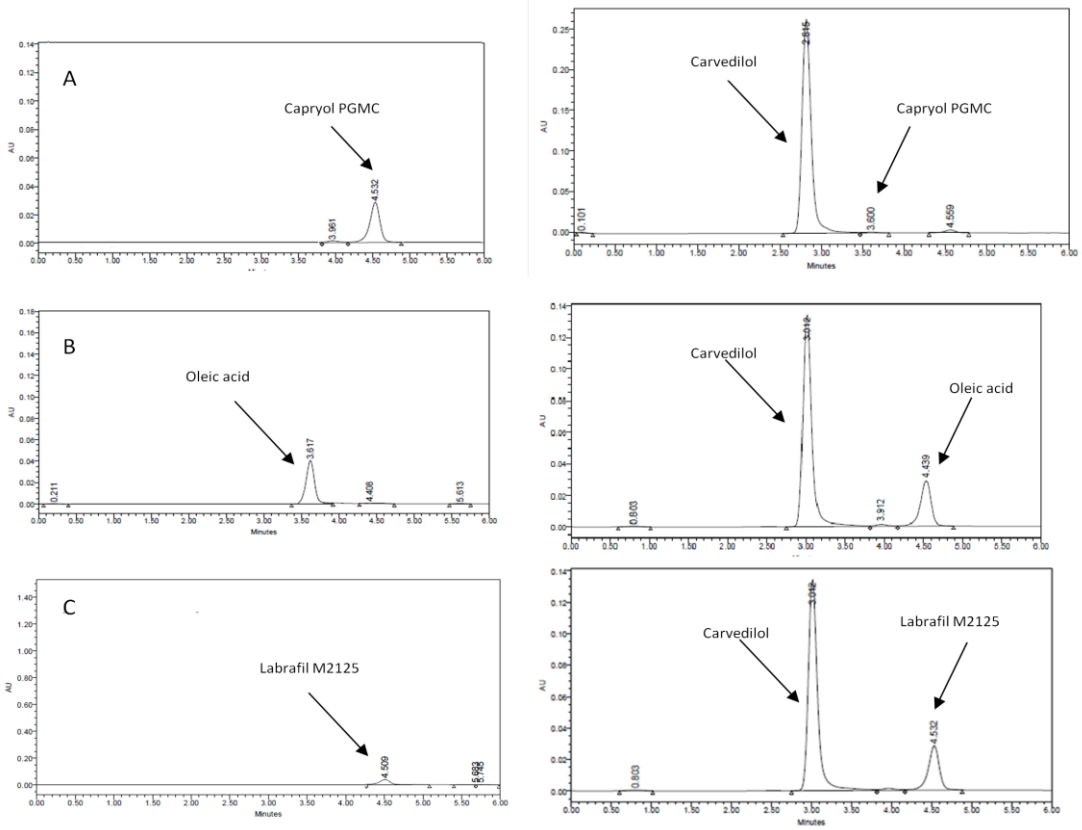

Fig I: Typical chromatograms of oils and spiked oil matrix with Carvedilol: (A) Capryol PGMC (B) Oleic acid (C) Labrafil M2125.

System suitability

For performing system suitability studies, 100% test concentration under degradation conditions was selected. System suitability test was performed by injecting blank solution once and standard solution of 100% test concentration six times in to stabilized HPLC system. The system suitability was established by evaluating the system suitability parameters from the last peak obtained. System suitability parameters include retention factor (k'), repeatability, resolution (R), tailing factor (T) and theoretical plates (N). It was performed by using the concentration of 90µg/ml. The system suitability data was given in the table.

Specificity and selectivity

The chromatograms shown in Figure 1 express that the developed method is specific and the response due to different oils do not interfere with the response of Carvedilol present in oil. Figures 1 show the chromatograms for blank oil matrix extracted with the mobile phase and the oils spiked with the analyte in labrafil M2125, capryol PGMC and oleic acid. Drug and oil excipients were all well resolved and well separated from each other. This shows that the method is specific and selective.

Linearity

The calibration curves were linear and correlation coefficients (r^2) were 0.999, 1 and 0.998 over the concentration range of 5 to 90 mg/mL in labrafil M2125, capryol PGMC and oleic acid (Figure II). Each concentration was injected in triplicate. The back calculated concentrations (mean±SD) from the representative calibration standards by HPLC determination for Carvedilol and the for each of the three oils are given in Table I.

TableI: Linearity data for carveilol in labrafil M2125, capryol PGMC and oleic acid.

Concentration	Measured concentration (µg/ ml)		
	Labrafil M2125	Capryol PGMC	Oleic acid
5	5.08	5.12	5.18
15	15.12	15.35	15.58
30	30.11	30.26	30.64
45	45.09	45.18	45.51
60	59. 98	60.28	60.36
75	75.14	74.84	74.54
90	89.84	90.36	90.64
Regression Equation	y = 14747x + 89099	y = 14311x + 10241	y = 14470x + 11194
r^2	0.998	0.999	1

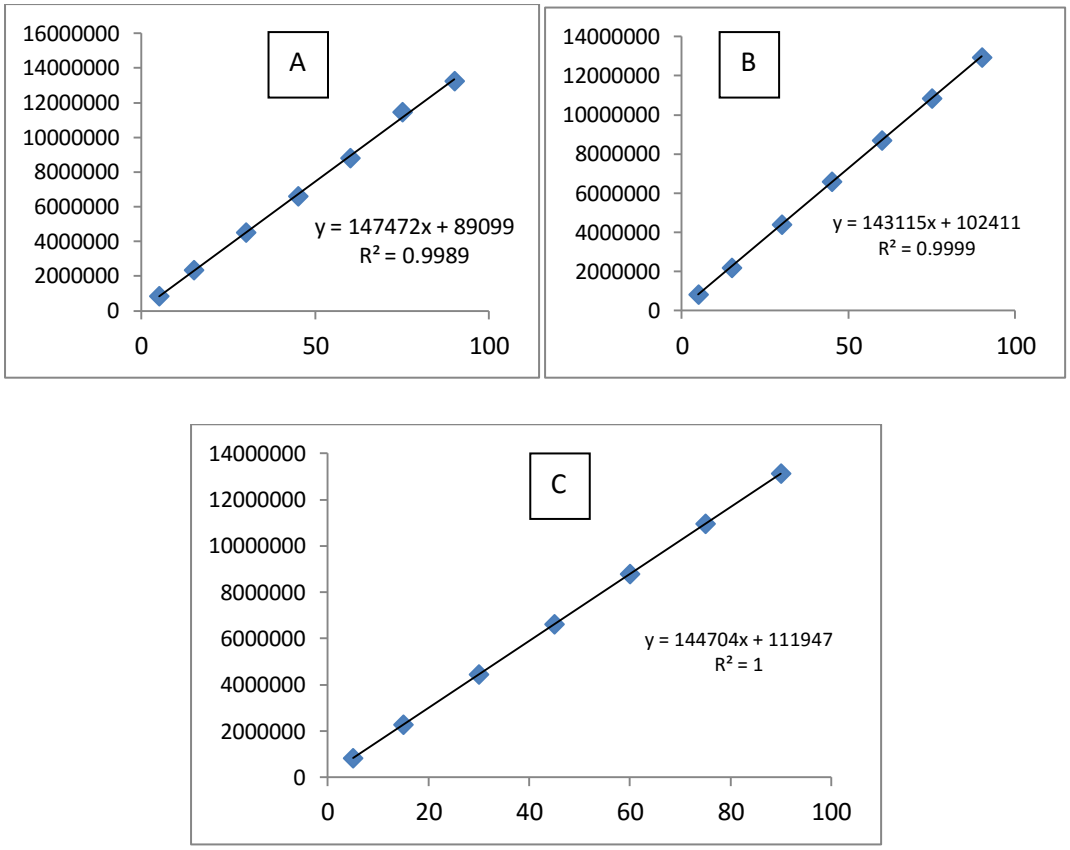

Fig II: Linearity plots of Carvedilol in (A) Labrafil M2125 (B) Capryol PGMC (C) oleic acid.

Precision and accuracy

Precision was evaluated by determining the RSD of the repeatability and intermediate precision for six measurements. The intra-day precision of Carvedilol in labrafil M2125, capryol PGMC and oleic acid ranged from 0.58–0.80, 0.9–1.25 and 0.9–1.82 and the inter-day precision ranged from 0.50–1.62, 0.93–1.63 and 1.06–2.22. Results are shown in Table II).

Table II: Accuracy and Precision - intraday(A) and interday(B)

Spiked concentration	A: Intraday precision (n=3)		
	Mean ± SD (µg/ml)	Accuracy %	% CV
Carvedilol in Labrafil M2125			
30	29.9 ± 0.102	99.6 - 100.98	0.98
45	44.94± 0.111	99.86 - 100.95	0.75
60	59.89 ± 0.098	99.81 - 101.08	1.05
Carvedilol in Capryol PGMC			
30	29.86 ± 0.12	99.53 - 101.1	0.56
45	44.97± 0.181	99.93 - 100.95	1.02
60	59.79 ± 0.108	99.65 - 101. 12	0.46
Carvedilol in Oleic acid			
30	29.91 ± 0.182	99.7 - 100.02	1.02
45	44.98± 0.11	99.95 - 101.1	1.32
60	59.85 ± 0.18	99.75 - 101.25	1.08

Spiked concentration	B: Interday precision (n=6)		
	Mean ± SD (µg/ml)	*Accuracy %	% CV
Carvedilol in Labrafil M2125			
30	30.09 ± 0.012	100.65 - 100.98	1.05
45	45.09± 0.1	100.36 - 100.95	1.85
60	59.85 ± 0.2	99.019 - 101.02	1.55
Carvedilol in Capryol PGMC			
30	30.06 ± 0.12	100.12 - 101.32	1.25
45	44.88± 0.181	99.68 - 101.25	1.58
60	59.89 ± 0.108	99.85 - 101. 19	1.26
Carvedilol in Oleic acid			
30	30.86 ± 0.165	100.08 - 101.22	1.52
45	45.09± 0.186	100.08 - 101.36	1.21
60	59.82 ± 0.152	99.89 - 101.65	1.12

***Accuracy= (measured concentration/ Spiked concentration) X 100**

Solubility studies:

Solubility of carvedilol in various oils was determined and shown in fig III. solubility was checked for each oil and combination with oleic acid. Carvedilol showed maximum solubility in mixture of oleic acid and labrafil M2125 in 1:1 w/w ratio is 296.54±12.54mg/ml.

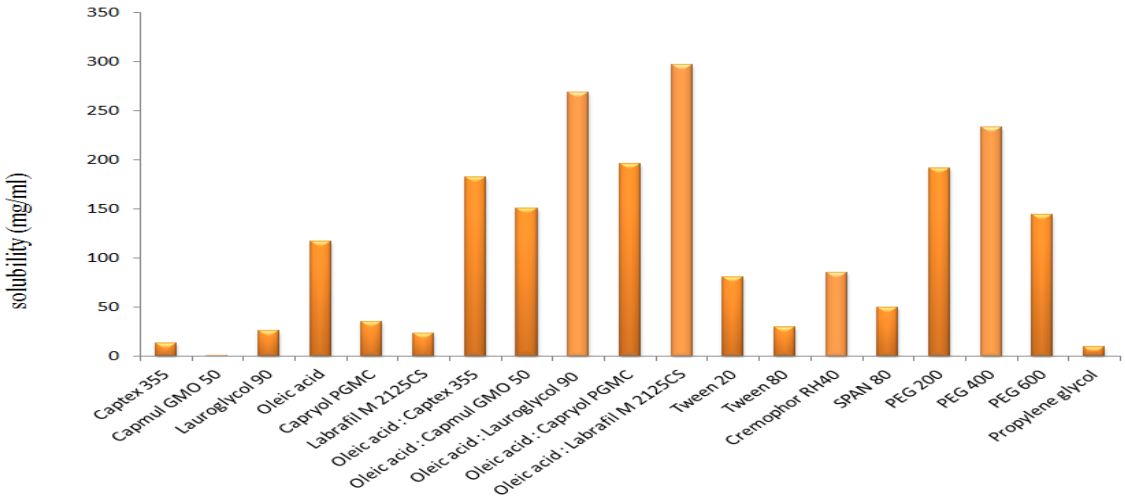

Fig III: solubility of Carvedilol in various oils

CONCLUSION

A simple, specific, precise, accurate HPLC method was developed and validated to quantify Carvedilol in different oils. Extraction procedure from different oils was developed to determine Carvedilol. From the results, it can be concluded that the present method will be useful for the determination of Carvedilol in oils like labrafil M2125, capryol PGMC and oleic acid.

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