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PREPARATION AND EVALUATION OF GLIBENCLAMIDE-LOADED BIODEGRADABLE NANOPARTICLES

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Abstract:

The present study was designed to develop a newer method for nano emulsions with glibenclamide as a anti diabetic drug. The method was carried out using (Gemini5µ C18 110A 100×4.60mm 5micron) with mobile phase comprised of methanol: 0.2M phosphate buffer PH 7.0 in the ratio (70:30). The flow rate at 1.0 ml/min and effluent was detected at 228nm. The retention time of glibenclamide was observed at 3.2 minutes. The method was validated for specificity, accuracy, precision, linearity, and limit of detection, limit of quantification, robustness and solubility. LOD and LOQ were 200 and 800 ng/ml respectively. The calibration curve was linear in the concentration range of 1- 2 µg/ml with coefficient correlation of 0.999. The percentage recovery for the glibenclamide was 99.8% and % RSD was less than 1 %. There are scanty reports with relation to determination of gliblencamide in nanoemulsion formulation. The proposed method was used for quantitative determination of glibenclamide in nano emulsion and is authenticated using various parameters.

Key words: ***Glibenclamide, Anti diabetic drug, methanol, phosphate buffer, HPLC, Validation.***

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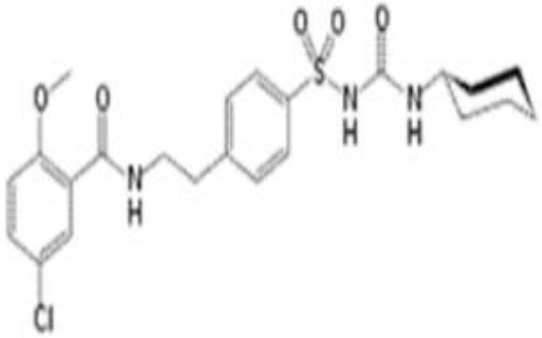
INTRODUCTION:

Glibenclamide an antidiabetic drug belonging to sulphonyl urea is a third generation drug acts by stimulating insulin release (Figure 1). It is used for the treatment of noninsulin-dependent diabetes mellitus (NIDDM). Glibenclamide is classified under class II according to biopharmaceutical classification system. The solubility of drug depends on pH. Glibenclamide exhibits very poor solubility at 37°C (7, solubility of drug is slightly increases to 0.02 mg/ml. This poor solubility may lead to poor dissolution and unpredictable bioavailability [1, 2, 5] . However, only a few reports are available showing the improved bioavailability. Determination in the lipid based formulations by using UV spectrophotometer is difficult. Hence the present study was aimed to develop a sensitive, simple and rapid analysis for lipid based formulations of glibenclamide by using RP-HPLC.

Figure 1: Chemical Structure of Glibenclamide



MATERIALS AND METHODS:

Chemicals:

Glibenclamide (API) was a gift sample from Bright labs, Hyderabad. The HPLC grade Methanol and potassium di hydrogen phosphate was purchased from Qualigens, HPLC grade water was from Merck. All other chemicals used in the study are of analytical grade. Glibenclamide nanoemulsion , contains 10 mg of Glibenclamide in 1gm of nanoemulsion base composed of 2.5% cotton seed oil, 21.5%water, and 76% Tween 80 , propylene glycol as surfactant/co surfactant mixture(S/Co S) in ratio of 1:1. Chromatographic separation was performed on the (Shimadzu LC 10A model), Japan.

Chromatographic Conditions: Columns: C18 Gemini, 100X 4.6 mm ID Mobile phase: A: Methanol (HPLC Grade) B: 0.2M phosphate buffer PH 7.0 Binary: 70:30 Flow rate: 1ml /min Detector: PDA, 228nm. Column temp.: Room temperature (25°C) Injection Volume: 20 µL sample loop.

PREPARATION OF MOBILE PHASE:

0.2M phosphate Buffer (pH 7):

Accurately measured 27grams of potassium di hydrogen phosphate dissolved in an 800ml of HPLC-grade water, and makeup to the volume with HPLC grade water in a 1000ml volumetric flask and thoroughly mixed. Measured the pH and adjusted to 7.0 ± 0.2 with 0.1N NaOH.

MOBILE PHASE:

The mobile phase was filtered through a 0.45µm membrane filter and degassed by sonication under vacuum for 5min. The contents were transferred to solvent reservoir of the LC20AD pump and purged the solvent line with 30ml of fresh mobile phase. The method development conditions for optimizing the mobile phase were depicted in Table 1.

Table 1: Method development conditions

Optimization Condition	Mobile phase - A	Mobile Phase B	pH of Mobile phase	Ratio of A/B	Glibenclamide RT*(min)	Tailing Factor
1	0.2M Phosphate buffer	Methanol	7.0	70/30	14	3.4
2	0.2M Phosphate buffer	Methanol	7.0	60/40	10	2.8
3	0.2M Phosphate buffer	Methanol	7.0	50/50	6	1.8
4	0.2M Phosphate buffer	Methanol	7.0	30/70	3.2	1.05

*RT: Retention Time

METHOD DEVELOPMENT:

Methanol and 0.2M phosphate buffer pH=7 in different proportions were tried and finally Methanol: 0.2M phosphate buffer pH=7 (70:30) was selected as an appropriate mobile phase which gave good resolution and acceptable system suitability parameters [7, 9, 10, 11] . The chromatogram of working standard of Glibenclamide solution was shown in Figure 2 and the chromatogram of Glibenclamide in nano emulsion formulation is depicted in Figure 3.

Figure-2 **Typical chromatogram of Glibenclamide Standard**

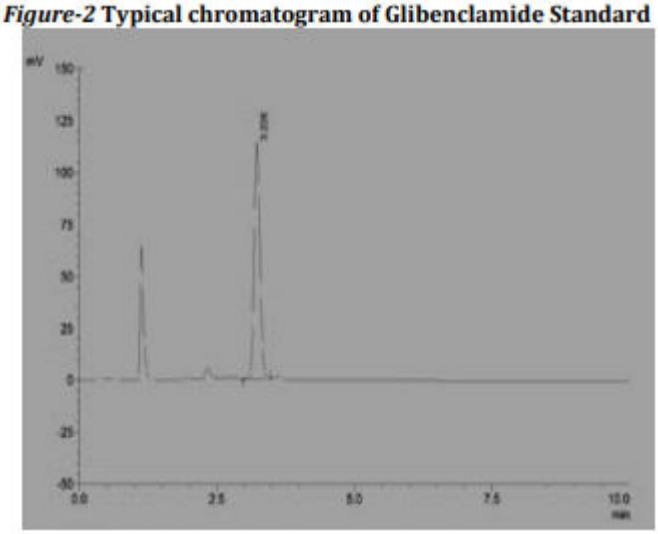
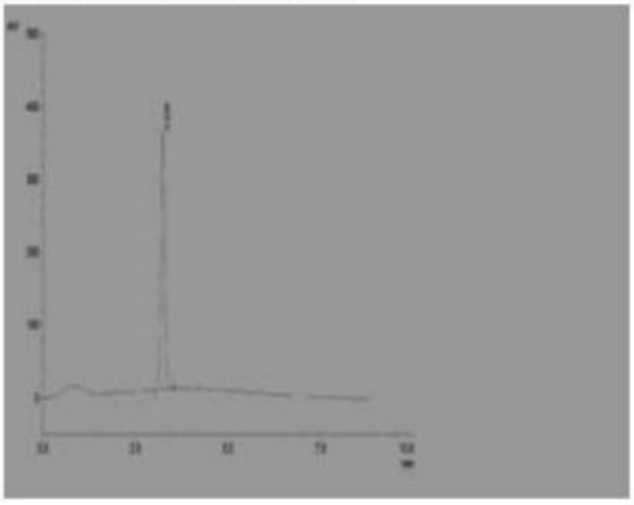


Figure 3: **Typical chromatogram of Glibenclamide in Tween+peg 400(1:1) formulation.**



PROCEDURE:

PREPARATION OF STANDARD SOLUTION:

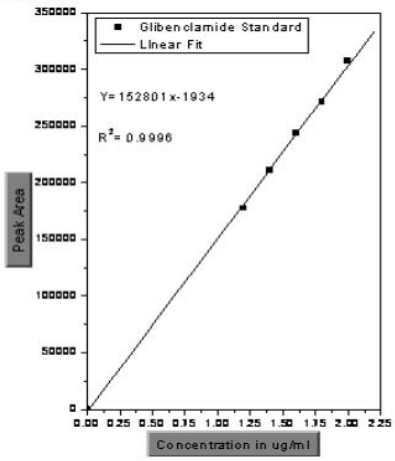
A standard stock solution was prepared by accurately weighing about 10 mg of Glibenclamide standard and transferred into 10 ml volumetric flask; added about 7 ml of mobile phase and sonicated for 5 minutes to dissolve and made up to volume with mobile phase to obtain a final concentration of 1 mg/ml.

CALIBRATION CURVE:

The required test samples were prepared freshly using the stock solution in the range of 1-2µg/ml. Triplicate 20µL injections were made for each concentration and were analyzed under the conditions prescribed chromatographic conditions. A calibration curve was obtained by plotting the response (peak area) versus concentration of drug and represented in Figure 4. Regression equation was calculated. The method was found linear over a concentration

range 1 to 2µg/ml.

Figure 4 : **Standard Graph of Glibenclamide in nano emulsion formulation.**



PREPARATION OF GLIBENCLAMIDE NANOEMULSION:

A nanoemulsion consisting of 2.5% cotton seed oil, 21.5%water, and 76% Tween 80: propylene glycol as surfactant/co surfactant mixture(S/CoS) in ratio of 1:1 was prepared. The composition of the nanoemulsion was chosen according to the pseudo ternary phase diagram. Cotton seed oil and S/CoS mixture were mixed in the chosen concentrations, and then water was added portion wise with continuous stirring.[12-14]100 mg of glibenclamide was incorporated in the 10ml of prepared emulsion, and the mixture was sonicated by using probe sonicator (Ultrasonic, USA) for about 10 min for size reduction.

PROCEDURE FOR ANALYSIS OF NANOEMULSION:

Equivalent proportions of nanoemulsion (1ml) and glibenclamide (10mg) was accurately weighed and transferred to a 10ml volumetric flask containing 5ml of mobile phase. The content of the flask was allowed to stand for 15 minutes with intermittent sonication to ensure complete solubility of the drug and made up to the required volume with mobile phase. The above solution was filtered through 0.45µm membrane filter and the filtrate wad diluted (10x). The appropriate dilutions were made with mobile phase to obtain concentration in the calibration range. By using the optimized chromatographic conditions, a steady baseline, working standard solution was injected and further chromatogram was recorded. The retention time of glibenclamide was found to be 3.2 min. The proposed method was found to be specific with no interference from common nanoemulsion base vehicles such as s/cos mixture, cotton seed oil etc. The response factors of the standard solutions and sample solutions were calculated. The assay was calculated from the regression equation. The assay procedure was repeated for 6 times and the percentage of drug in the formulation was calculated. The results of analysis showed that the amount of drug was in good agreement with the label claim of formulation (table 2).

Table 2: **Analysis of Glibenclamide Nanoemulsion formulation**

Formulation	Analyte	Label claim(mg)	%Label claim estimated
Nano emulsion	Glibenclamide	10	99.8

(*mean of six samples)

METHOD VALIDATION

LINEARITY

The method was linear in the range of 1 to 2µg ml for glibenclamide standard and results of linear regression data was tabulated in Table 3.

Table 3: **Linear Regression data for calibration curves**

Parameters	Glibenclamide
Linearity range (ng/ml)	200ng/ml – 600ng/ml
Correlation coefficient	0.999
Regression equation	Y=15209X-1214
slope	15209
intercept	-1214

PRECISION

The precision of the method was demonstrated by inter day and intraday variation studies. In the intraday studies, solutions of standard and sample were repeated thrice in a day and percent relative standard deviation (%RSD) for response factor was calculated. The intraday %RSD of glibenclamide was found to be 0.1581%. In the inter day variation studies, injections of standard and sample solutions were made on three consecutive days and %RSD was calculated. The inter day %RSD for glibenclamide was found to 0.5499. From the data obtained the developed RP-HPLC method was found to be precise.

ACCURACY

The accuracy of the method was determined by recovery experiments. Known concentration of working standard was added to the fixed concentration of the pre-analyzed micro emulsion gel solution. Percent recovery was calculated by comparing the area before and after the addition of working standard. The recovery was performed in the same way. The recovery studies were performed in triplicate. The standard addition method was performed at 50%, 100%, 150% level and the percentage recovery was calculated. Percent recovery was within the range of 99.2% to 100.8% for glibenclamide which indicates that the method was accurate.

LIMIT OF DETECTION AND LIMIT OF QUANTIFICATION

The Limit of detection and quantification were calculated using standard deviation of the response and slope of calibration curve. The LOD for glibenclamide was found to be 200ng/ml. The LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified. The LOQ for glibenclamide was found to be 800ng/ml.

ROBUSTNESS

Robustness of the method was checked by making slight changes in chromatographic conditions like mobile phase ratio, pH of buffer, flow rate. It was observed that there were no marked changes in chromatograms, which demonstrated that the developed RP-HPLC method is robust and is represented in Table 5.

Table 5: **Robustness**

Condition	% RSD
pH of the buffer (± 0.2)	0.1582
Organic phase ratio (± 2%)	0.1573

SOLUBILITY STUDIES:

Solubility of glibenclamide in various components oils, surfactant, co surfactants and their ratios are determined by taking 10ml of each of selected vehicle was added to each cap vial containing an excess amount of Glibenclamide. After sealing the mixture was shaken well until suspension was formed. Formed suspensions were then shaken by using orbital shaking incubator at 25°c for 48hrs after shaking the solution was filtered by whattman filter paper and filtrates were centrifuged at 5000rpm for 1hr. The concentration of Glibenclamide in each vehicle and their ratios were quantified by RP-HPLC and were graphically represented in Figure 5. UV detector is used by keeping λmax at 228nm.

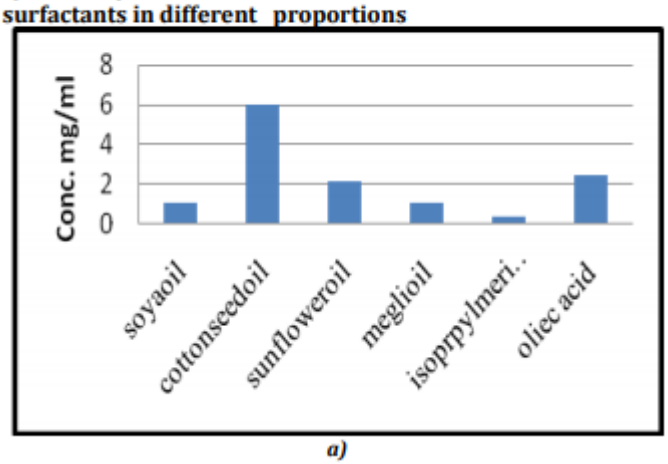
RESULTS AND DISCUSSION:

The developed method is specific and reproducible for the quantitative determination of glibenclamide in nano emulsion formulation with good resolution in short run time (3.2 min.) and high sensitivity. The method developed was found to be linear in the concentration range of 1 to 2 µg/ml. The method was specific since vehicles in the formulation did not interfere in the estimation of Glibenclamide. Accuracy of the method was indicated by recovery values from 99.2% to 100.8% for Glibenclamide. Precision is reflected by %RSD values less than 2. The LOQ for Glibenclamide was found to be 800ng/ml. These low values suggest sensitivity of the developed method the concentration of Glibenclamide in each vehicle were quantified. Validation parameters were summarized in Table 4.

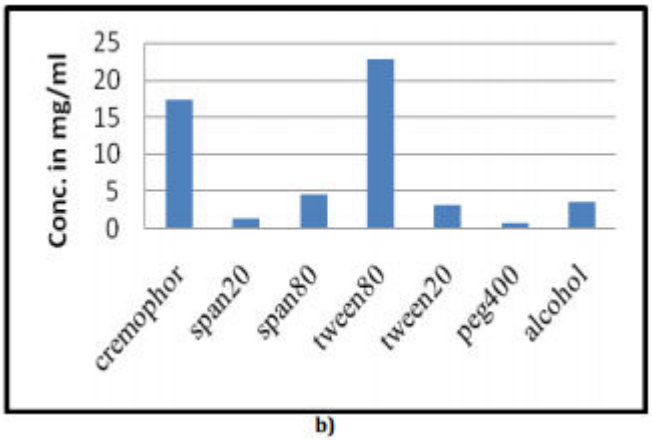
Table 4: **Summary of validation parameters**

Parameters	Glibenclamide
LOD (ng/ml)	200
LOQ (ng/ml)	800
Mean % recovery	99.8%
Precision (% RSD)	
Inter day (n=3)	0.5499
Intra day (n=3)	0.1581
Robustness	0.1582
Retention time	3.2
Theoretical plates	3889.917
Tailing factor	1.051

Figure 5**: a) Solubility of Glibenclamide in different oils. b) Solubility of Glibenclamide Surfactants and Co-surfactants. c)Solubility of Glibenclamide in Surfactants and Co-surfactants in different proportions**



a)



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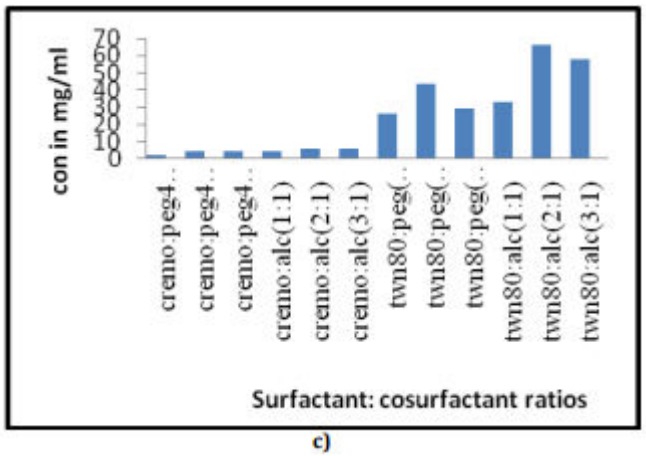

c)

CONCLUSION:

The developed rp-hplc method was simple, sensitive, precise and accurate and hence can be used in routine analysis of glibenclamide in bulk as well as in pharmaceutical preparations.

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