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Analytical Methods and Stability Studies for Gastroretentive Superporous Hydrogels of Glipizide

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Abstract

The UV, RP-HPLC and Bio analytical method were used to estimate Glipizide. The mobile phase consisted mixture of 10mM concentration of phosphate buffer (pH adjusted to 2.1) and acetonitrile (34:66) with C18 column (250mm×4.6mm×5µ). The drug retention time was found to be 3.41 min. Representative chromatogram of glipizide was monitored at 276 nm. A linear relationship was observed in the concentration range of 1-8 mcg/ml (r^2=0.999; n= 6). The method was specific as there was no interference at the retention time of the analyte by the blank solution and the LOQ *i.e.* the limit of quantification was with acceptable precision. The accuracy at low (1mcg/ml), moderate (4 mcg/ml) and higher (8 mcg/ml) concentrations of Glipizide ranged from 98.24-101.12% and 98.88-101.51% for intra and inter day respectively.

Key words: UV, RP-HPLC and Bio analytical method

Analytical method development and validation play important roles in the discovery, development and manufacture of pharmaceuticals. Pharmaceutical products formulated with more than one drug, typically referred to as combination products, are intended to meet previously unmet patients need by combining the therapeutic effects of two or more drugs in one product. This work will discuss the development and validation of analytical method (Spectrophotometric & High performance liquid chromatography (HPLC)) for drug products containing more than one active ingredient. The official test methods that result from these processes can be used by quality control laboratories to ensure the identity, purity, potency, and performance of drug products.

Basic criteria for new method development of drug analysis:

- The drug or drug combination may not be official in any pharmacopoeias,
- A proper analytical procedure for the drug may not be available in the literature due to patent regulations,
- Analytical methods may not be available for the drug in the form of a formulation due to the interference caused by the formulation excipients,
- Analytical methods for the quantitation of the drug in biological fluids may not be available,
- Analytical methods for a drug in combination with other drugs may not be available,
- The existing analytical procedures may require expensive reagents and solvents. It may also involve cumbersome extraction and separation procedures and these may not be reliable.

SYSTEM SUITABILITY [1]:

System suitability is the checking of a system to ensure system performance before or during the analysis of unknowns. Before performing any validation experiment, you should establish that the HPLC and procedure are capable of providing data of acceptable quality. These tests are used to verify that resolution and repeatability of the system are adequate for the analysis to be performed. It is based on the concept that equipment, electronics, analytical operations and sample constitute an integral system that can be evaluated as a whole.

The purpose of the system suitability test is to ensure that the complete testing system (including instrument, reagents, columns, analysts) is suitable for the intended application.

The parameters that are affected by the changes in chromatographic conditions are,

- Resolution (R_s),
- Capacity factor (k'),
- Selectivity (α),
- Column efficiency (N) and
- Peak asymmetry factor (As).

i) Resolution (R_s)

The resolution, R_s, of two neighboring peaks is defined by the ratio of the distance between the two peak maxima. It is the difference between the retention times of two solutes divided by their average peak width. For baseline separation, the ideal value of R_s is 1.5. It is calculated by using the formula,

$$R_s = \frac{Rt_2 - Rt_1}{0.5(W_1 + W_2)}$$

Where, Rt_1 and Rt_2 are the retention times of components 1 and 2 and
W_1 and W_2 are peak widths of components 1 and 2.

ii) Capacity factor (k')

Capacity factor, k', is defined as the ratio of the number of molecules of solute in the stationary phase to the number of molecules of the same in the mobile phase. Capacity factor is a measure of how well the sample molecule is retained by a column or TLC plate during an isocratic separation. The ideal value of k' ranges from 2-10. Capacity factor can be determined by using the formula,

$$k' = \frac{V_1 - V_0}{V_0} \times S$$

Where, V_1 = retention volume at the apex of the peak (solute) and
V_0 = void volume of the system.

The values of k' of individual band increase or decrease with changes in solvent strength. In reverse phase HPLC, solvent strength increases with the increase in the volume of organic phase in the water / organic mobile phase. Typically an increase in percentage of the organic phase by 10 % by volume will decrease k' of the bands by a factor of 2-3.

iii) Selectivity (α)

The selectivity (or separation factor), α, is a measure of relative retention of two components in a mixture. The ideal value of selectivity is 2. It can be calculated by using the formula,

$$\alpha = \frac{V_2 - V_0}{V_1 - V_0}$$

Where, V_0 is the void volume of the column and V_2 and V_1 are the retention volumes of the second and the first peak respectively.

iv) Column efficiency (N)

Efficiency, N, of a column is measured by the number of theoretical plates per meter. It is a measure of band spreading of a peak. Smaller the band spread, higher is the number of theoretical plates, indicating good column and system performance. Columns with N ranging from 2000 - 100,000 plates/meter are ideal for a good system. Efficiency is calculated by using the formula,

$$N = 16\frac{Rt^2}{W^2},$$

Where, Rt is the retention time and W is the peak width.

v) Peak asymmetry factor (As)

Peak asymmetry factor, can be used as a criterion of column performance. The peak half width, b, of a peak at 10 % of the peak height, divided by the corresponding front half width, a, gives the asymmetry factor.

$$As = \frac{b}{a}$$

For a well packed column, an asymmetry factor of 0.9 to 1.1 should be achievable.

VALIDATION [2]:

Validation is derived from Latin which means "strong ness".

Validation is documented evidence that provides a high degree of assurance that a specific process will consistently produce a product that meets its predetermined specifications and quality attributes.

VALIDATION AS DEFINED BY DIFFERENT AGENCIES

1. USFDA: According to this " Validation is the process of establishing documented evidence which provides a high degree of assurance that a specific process will consistently produce a product meeting its predetermined specifications and quality attributes.

2. WHO: Defines Validation as an action of providing any procedure, process, equipment, material, activity or system actually leads to the expected results.

3. EUROPEAN COMMITTEE: Defines Validation as an action of providing in accordance with the principles of GMP that any procedure, process, material, activity or system actually lead to expected results.

Benefits of Validation:

a. Produces quality products
b. Helps in process improvement technology transfer, related product validation, failure investigation, and increased employee awareness.
c. Cost reduction by increasing efficacy, few reject, longer equipment life, production of cost effective products
d. Helps in optimization of process or method.
e. Regulatory affairs- produces approved products and increased ability to export.

ANALYTICAL METHOD VALIDATION [1]:

"Validation of an analytical method is the process by which it is established by laboratory studies, that the performance characteristics of the method meet the requirements for the intended analytical application.

Types of Analytical Procedures to be validated [3]:

The discussion of the validation of analytical procedures is directed to the four most common types of analytical procedures:

- Identification tests;
- Quantitative tests for impurities' content;
- Limit tests for the control of impurities;
- Quantitative tests of the active moiety in samples of drug substance or drug product or other selected component(s) in the drug product.

Typical analytical parameters used in assay validation according to ICH guidelines:

1. Accuracy
2. Precision
3. Specificity
4. Limit of detection
5. Limit of quantitation
6. Linearity
7. Range
8. Robustness

Category-I: Analytical methods for quantitation of major components of bulk drug substances or active ingredients (including preservatives) in finished pharmaceutical products.

Category-II: Analytical methods for determination of impurities in bulk drug substances or degradation compounds in finished pharmaceutical products. These methods includes quantitative assays and limit tests.

Category-III: Analytical methods for determination of performance characteristics (e.g. dissolution, drug release).

The type of method and its intended use dictates which parameters are required to be investigated. They are illustrated in the following Table

Data elements required for assay validation

Analytical Performance Parameters	Assay category-I	Assay category –II		Assay category –III
		Quantitative	Limit Test	
Accuracy	Yes	Yes	*	*
Precision	Yes	Yes	No	Yes

Specificity	Yes	Yes	Yes	*
LOD	No	No	Yes	*
LOQ	No	Yes	No	*
Linearity and range	Yes	Yes	No	*
Ruggedness	Yes	Yes	Yes	*

UV Method: (Method described in USP was followed**)**

Stock solution: 100mg of Glipizide was dissolved in methanol and the volume was made upto 100ml with pH 1.2 to obtain 1mg/ml solution.

Scanning: From the stock solution, a 15 µg/ml solution of glipizide was prepared in SGF and scanned between 200-400nm. The spectrum is reproduced in Figure 1. The absorption maxima of 276nm was selected and used for further studies.

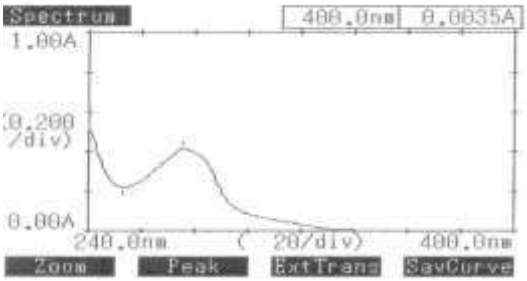

Figure 1: UV spectra of glipizide in pH 1.2

Standard plot: From the stock solution (1mg/ml), 10 ml was pipetted and transferred into a 100ml volumetric flask and diluted upto the mark with pH 1.2 buffer solution. From this, aliquots of 0.5ml, 1.0ml, 1,5ml, 2.0ml, 2.5ml, 3.0ml and 3.5ml were transferred to a series of 10ml volumetric flasks and the volumes were made upto 10ml with pH 1.2 buffer to obtain 5, 10, 15, 20, 25, 30, 35mcg/ml solutions of glipizide respectively. The absorbance of these solutions was measured at 276nm.The data for the standard curve is reported in Table 1. A calibration graph was plotted (Figure 2).

Table 1: Standard plot data of glipizide in pH 1.2.

Concentration (mcg\ml)	Absorbance Mean± S.D.*
5	0.140±0.001
10	0.265±0.003
15	0.403±0.003
20	0.521±0.006
25	0.655±0.002
30	0.771±0.003
35	0.899±0.002

*Standard deviation n=6

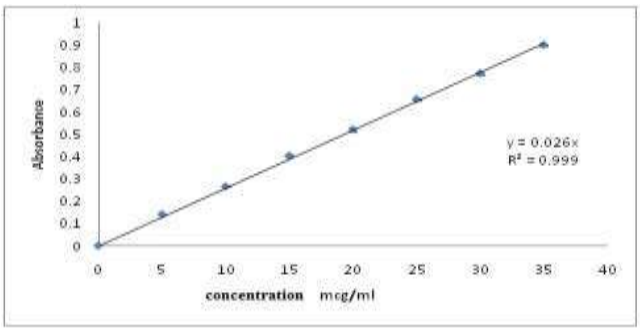

Figure 2: Calibration curve of Glipizide in pH 1.2

HPLC Method: Analytical method

The RP-HPLC method was used to estimate glipizide. The mobile phase consisted mixture of 10mM concentration of phosphate buffer (pH adjusted to 2.1) and acetonitrile (34:66) with

C18 column (250mm×4.6mm×5µ). The drug retention time was found to be 3.41 min. Representative chromatogram of glipizide monitored at 276 nm. A linear relationship was observed in the concentration range of 1-8 mcg/ml (r^2=0.999; n= 6). The method was specific as there was no interference at the retention time of the analyte by the blank solution and the LOQ *i.e.* the limit of quantification was with acceptable precision. The accuracy at low (1mcg/ml), moderate (4 mcg/ml) and higher (8 mcg/ml) concentrations of glipizide ranged from 98.24-101.12% and 98.88-101.51% for intra and inter day respectively. The data for the standard curve is reported in Table 2. A calibration graph was plotted (Figure 4).

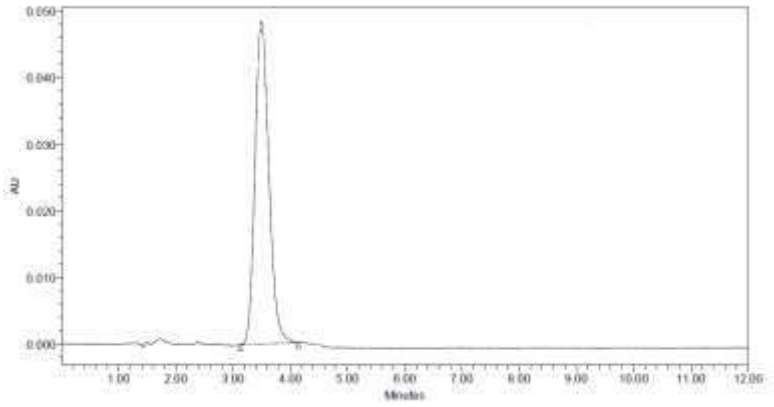

Figure 3: Typical chromatogram of glipizide by RP-HPLC

Table 2: Standard calibration data of glipizide by RP-HPLC

Concentration(mcg/ml)	Area Mean ± SD*
1	3662.54±321.20
2	7112.72±475.28
4	13997.46±523.10
6	21205.34±702.46
8	28182.49±789.22

*Standard deviation n=6

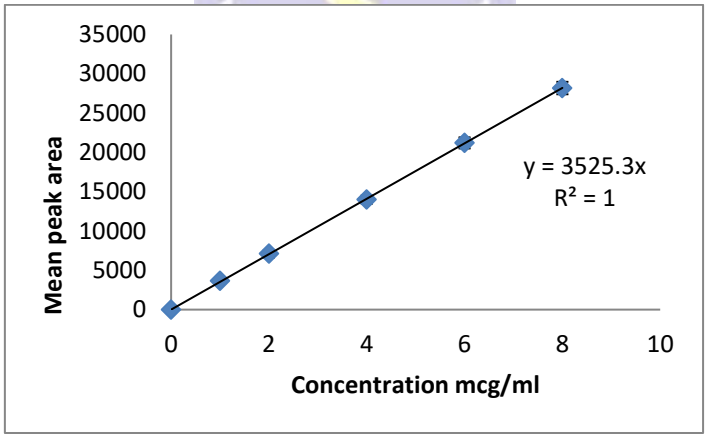

Figure 4: Standard calibration curve of glipizide by RP-HPLC

Bio analytical method

The HPLC bio analytical method used for glipizide analysis was rapid and simple. Protein precipitation method was used (Acetonitrile) for extraction, the clear supernatant liquid was separated, filtered (0.45µ filter) and injected into the HPLC system. Drug retention time was 3.45 min. A linear relationship was observed in the concentration range of 1-8 mcg/ml (r^2 = 0.998; n = 6). The results show the linearity between the peak area and the concentration of the analyte. The developed method was specific as there was no interference by the matrix components at the retention time of the analyte and the LOQ *i.e.* the limit of quantification was with acceptable precision. The accuracy at low (1mcg/ml), moderate (4mcg/ml) and higher (8 mcg/ml) concentrations of glipizide ranged from 98.04-101.31% and 95.11-103.42% for intra and inter day respectively. The data for the standard curve is reported in table 3. A calibration graph was plotted (Figure 5).

Table 3: Standard calibration data of glipizide by RP-HPLC

Concentration (mcg/ml)	Mean Peak Area Mean ± SD*
1	3562.23±200.12
2	7012.42±323.41
4	13897.88±868.10
6	21605.92±901.21
8	29982.65±769.45

* Standard deviation: n=6

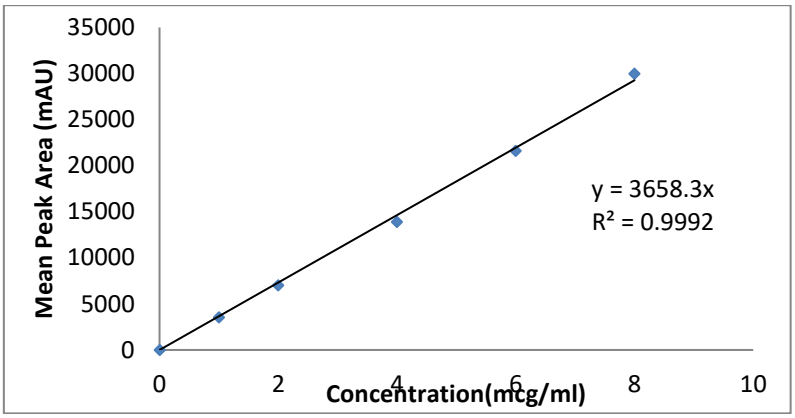

Fig 5: Calibration curve for the estimation of glipizide in plasma samples by HPLC method

STABILITY STUDIES:

The objective of stability studies is to predict the shelf life of a product by accelerating the rate of decomposition, preferably by increasing the temperature and RH. The optimized nanoparticle formulation was subjected to stability studies according to ICH guidelines by storing at 25ºC/60% RH, 30ºC/65% RH and 40ºC/75% RH for 6 months. These samples were analyzed and checked for changes in physical appearance and drug content at regular intervals. The observations of accelerated conditions. There are no significant changes in the parameter even when it was subjected to stress testing for a period of six months.

Conclusion:

All the methods UV, RP HPLC and The HPLC bio analytical method used for glipizide analysis were rapid and simple and validated as per ICH guidelines.

Protein precipitation method was used (Acetonitrile) for extraction, the clear supernatant liquid was separated, filtered (0.45µ filter) and injected into the HPLC system. Drug retention time was 3.45 min. A linear relationship was observed in the concentration range of 1-8 mcg/ml (r^2 = 0.998; n = 6). The results show the linearity between the peak area and the concentration of the analyte. The developed method was specific as there was no interference by the matrix components at the retention time of the analyte and the LOQ *i.e.* the limit of quantification was with acceptable precision. The accuracy at low (1mcg/ml), moderate (4mcg/ml) and higher (8 mcg/ml) concentrations of glipizide ranged from 98.04-101.31% and 95.11-103.42% for intra and inter day respectively.

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