An Elsevier Indexed Journal ISSN-2230-7346

Journal of Global Trends in Pharmaceutical Sciences

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF GLIPIZIDE TO DETERMINE RESIDUAL SOLVENTS BY HEAD SPACE-GAS CHROMATOGRAPHY

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ARTICLE INFO

Key Words

Gas Chromatography, Glipizide, Method development ,Validation, Residual Solvents

Access this article online
Website:
https://www.jgtps.com/
Quick Response Code:

ABSTRACT

Residual solvents in Pharmaceuticals are termed as organic volatile impurities. These are the chemicals that are used in the manufacture of drug substance or excipients or use in the preparation of final formulation. Most of the available methods use liquid chromatography which could be expensive and time consuming. Hence, an analytical methodology was developed for the quantification of residual solvents in Glipizide using a headspace gas chromatography (HSGC) with the help of flame ionization detector (FID). Methanol, acetone and dimethyl formamide as residual solvents were determined in Glipizide. Analysis was performed by headspace GC/FID method on Auto system- HS40. Nitrogen was used as a carrier gas and the separation of residual solvents was achieved by DB-Wax 0.25mm, 0.3mcm column. The thermostat temperature was 115 ℃ for 40 minutes for each vial. %RSD for nine injections obtained are in acceptance criteria. The correlation coefficient R2 obtained greater than 0.99. The method parameters were validated includes specificity, limit of detection and quantification, accuracy, linearity, precision, and robustness. According to the International Conference on Harmonization (ICH) guidelines, a new simple, specific, accurate and precise method was developed and validated.

INTRODUCTION

Residual solvents, or organic volatile impurities, are given hindmost importance in pharmaceutical products. This has been a major concern of pharmaceutical manufacturers for many years.[1] Organic solvents are routinely applied during synthesis of drug substances, excipients, or during drug product formulation. They are not desirable in the final product, mainly because of their toxicity, influence on the quality of crystals of the drug substance, and their odor or taste, which can be unpleasant for patients. These small quantities of organic solvents are commonly known as organic volatile impurities or Residual solvents. The determination of residual solvents in drug substances, excipients or drug products is known to be one of the most difficult and demanding analytical tasks in the pharmaceutical industry.[2] The role of analytical method is very crucial in the success of in-vivo studies of any formulation. The sensitivity of the analytical method should be very high especially for in-vivo studies because the amount of the drug present in the plasma is usually very less and there are many other substances present in plasma, which may interfere with the analysis.[3] The Glipizide derivative was determined by electron capture gas chromatography upto about 20ng/ml in a

plasma sample. Currently the most commonly prescribed medications for Type 2 diabetes are metformin and the second generation sulfonylureas which include glipizide, gliclazide, glibenclamide and glimperide.[4] The protein binding has been reported to be 92–98% for glipizide.[5] Glipizide can increase the secretion of insulin by stimulating islet β-cells.[6]

MATERIALS AND METHODS

The analysis was performed on Flame Ionisation Detector and Chem. station software. The injection temperature was maintained at 190ºC and detector temperature was 290ºC. Column was DB-624m (30m long, 0.53mm internal Diameter coated with 3.0um film of 6% Cyanopropylphenyl 94% Dimethyl polysiloxane). Split ratio of injection is 1:4, oven temperature was maintained at 40°C for 5 min and then raised at rate of 10°C/min to 170°C, maintained for 7 min. Total run time was 25 min and nitrogen was used as carrier gas at a constant flow rate of 4.2 ml/min.

Optimization of head space condition

Due to problem in recovery and precision of DMF increased the equilibrium time and temperature for complete evaporation of DMF solvent and after this change, better precision and recovery results observed.

Reagents: Methanol, Acetone, Dimethyl formamide (DMF) and water were used as analytical grade reagents. Glipizide bulk drug sample was obtained from Anuh Pharma ltd, Mumbai.

METHOD DEVELOPMENT

Procedure

Determine the specificity by injecting a reference solution and determining possible interferences, tailing factor and column efficiency. Determine the precision in terms of RSD of 9 injections of the 1000 ppm of acetone and 500 ppm of methanol level. Determine the accuracy by adding a known amount of acetone and of methanol on a sample of glipizide. Performed the determination at three levels (ab. 500-750 and 1000 ppm for acetone ab. 250-350-500 ppm for methanol). Determine the linearity of the method by injecting different samples at 8 levels from 10% to 150%; 3 samples for each level except the level 100% and the level 80% where 9 and 6 samples are injected respectively.

VALIDATION

The validation was done as indicated in the International Conference on harmonization (ICH) guidelines Q2B "validation of analytical procedures and the following parameters were taken into consideration specificity, linearity, accuracy, limit of detection and quantitation, robustness, and precision of residual solvents .

RESULTS AND DISCUSSION

Accuracy

The accuracy was determined using three different levels of spliked solvents, employing the standard addition quantitation as per the method. The initial amount was 505.6 ppm of acetone and 252.8 ppm of methanol (= 50% of the stated limits), the final amount was 1011.2 - 1213.44 and 1516.8 ppm of acetone and 505.6 - 606.72 and 758.4 ppm of methanol.

Precision

The precision of an analytical procedure defines the degree of agreement between a series of results obtained from multiple sampling of the same homogeneous sample. The determination of the precision was performed by 9 injections of a sample containing an added amount of solvents corresponding to a residual solvent content in glipizide equal to 1011.2 ppm of acetone and of 505.6 ppm of methanol (level 100).

Linearity and range:

The linearity of an analytical procedure lies within the test results which are directly proportional to the concentration of the analyte in the sample. Linearity is determined using linear regression analysis to deduce the relation between instrumental response and the known concentration of analyte present in samples in a given interval. For a good rule atleast five analyte concentrations should be analyzed. The levels should be equally spaced throughout the given interval and spanning the intended operating concentration of the assay method.

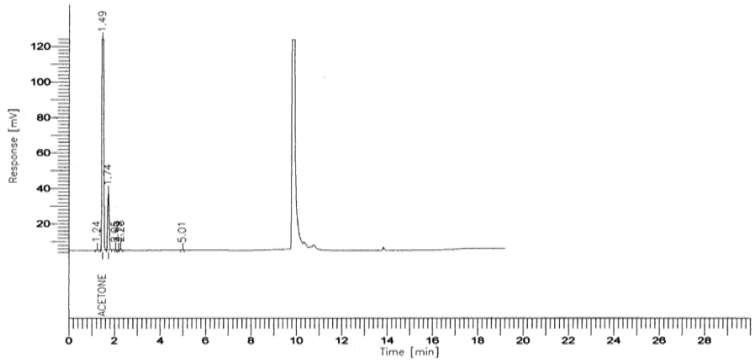

Fig 1: Glipizide solvent residue

Table 1: Glipizide peak values

Peak	Time(min)	Area	Component name
2	1.486	1077986	acetone
3	1.737	140058	methanol

Table 2(a): Accuracy values of acetone in glipizide

ACETONE	PPM added	PPM found	%
Level 1	505.6	499.50	98.79
Level 2	707.84	719.52	101.65
Level 3	1011.2	1029.45	101.80

Accuracy mean : **100.75 +/- 1.70 %**

Table 2(b): Accuracy values of methanol in glipizide

METHANOL	PPM added	PPM found	%
Level 1	252.8	262.53	103.85
Level 2	352.92	368.45	104.10
Level 3	505.6	538.46	106.50

Accuracy mean : **104.82 +/- 1.46%**

Table3: Precision values of acetone and methanol in glipizide

N= 9	ACETONE: 1.65%	METHANOL : 2.06%

Table 4: Linearity and range values of acetone and methanol in glipizide

Level 150	Acetone	1516.8	Methanol	758.4	n=3
Level 120	Acetone	1213.44	Methanol	606.72	n=3
Level 100	Acetone	1011.2	Methanol	505.6	n=9
Level 80	Acetone	808.96	Methanol	404.48	n=6
Level 50	Acetone	505.6	Methanol	252.8	n=3
Level 40	Acetone	404.48	Methanol	202.24	n=3
Level 20	Acetone	202.24	Methanol	101.12	n=3
Level 10	Acetone	101.12	Methanol	56.56	n=3

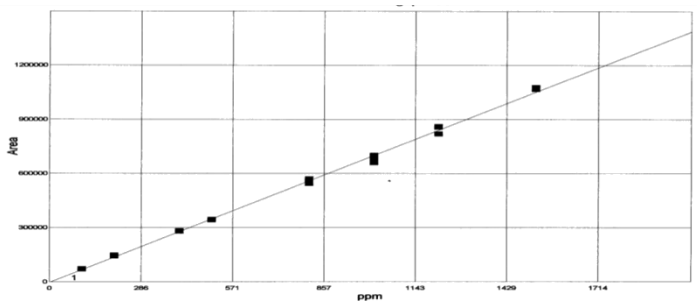

Fig 2(a): Acetone in glipizide

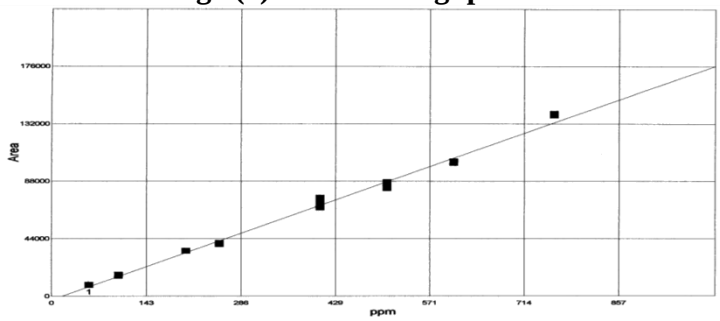

Fig 2(b): Methanol in glipizide

Table 5 (a) : LOD and LOQ values of acetone and methanol in glipizide

Level 40	ACETONE	404.48 ppm	METHANOL	202.24 PPM
level 20	ACETONE	202.24 ppm	METHANOL	101.12 PPM
level 10	ACETONE	101.12 ppm	METHANOL	56.56 PPM

Table 5 (b) : LOD and LOQ values of acetone and methanol in glipizide

LOD VALUES		LOQ VALUES	
Acetone	4ppm	Acetone	15ppm
Methanol	5ppm	Methanol	16ppm

Fig. 3: LOD and LOQ Peak values of Glipizide

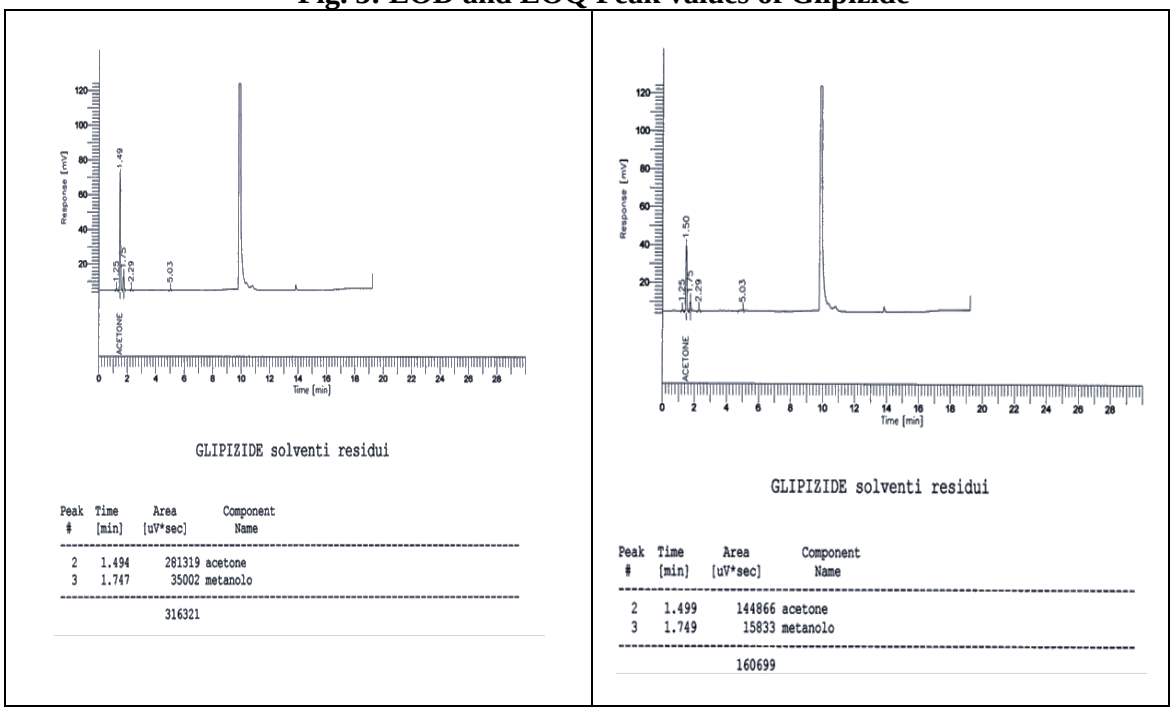

Table 6 (a) : Ruggedness values of acetone and methanol in glipizide

Level 100	Acetone	1011.2ppm	Methanol	505.6 ppm
level 80	Acetone	808.96 ppm	Methanol	404.48 ppm

The analyses were performed in two different days and by one/two different operators.

Table 6 (b) : Ruggedness values of acetone in glipizide

ACETONE	Operator A-1	Operator A-2	Operator B
Level 100 ACETONE % error	1.7%	-1.2%	-0.4%
Level 80 ACETONE % error	-0.53%	0.53%	---

Table 6 (c) : Ruggedness values of methanol in glipizide

METHANOL	Operator A-1	Operator A-2	Operator B
Level 100 METHANOL % error	-1.77%	-0.24%	2.01%
Level 80 METHANOL % error	-3.17%	3.17%	---

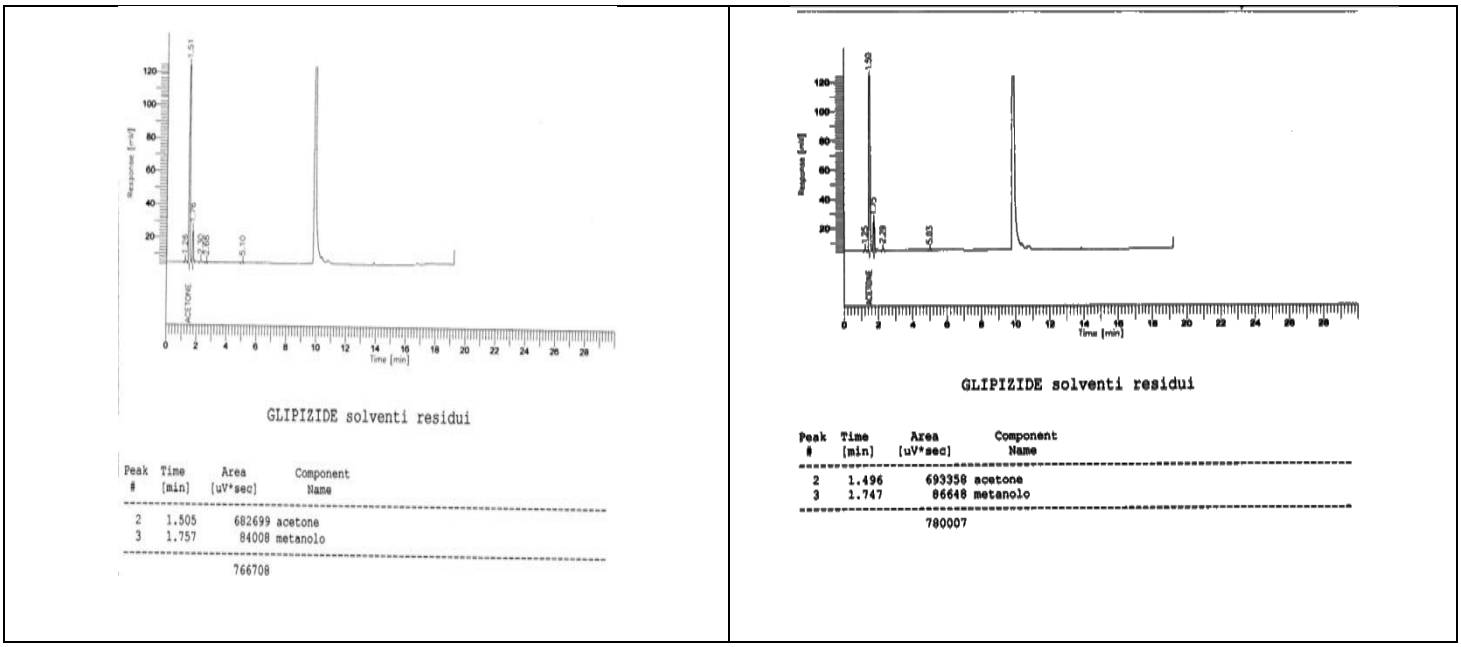

Fig. 4: Peak values of acetone and methanol in Ruggedness

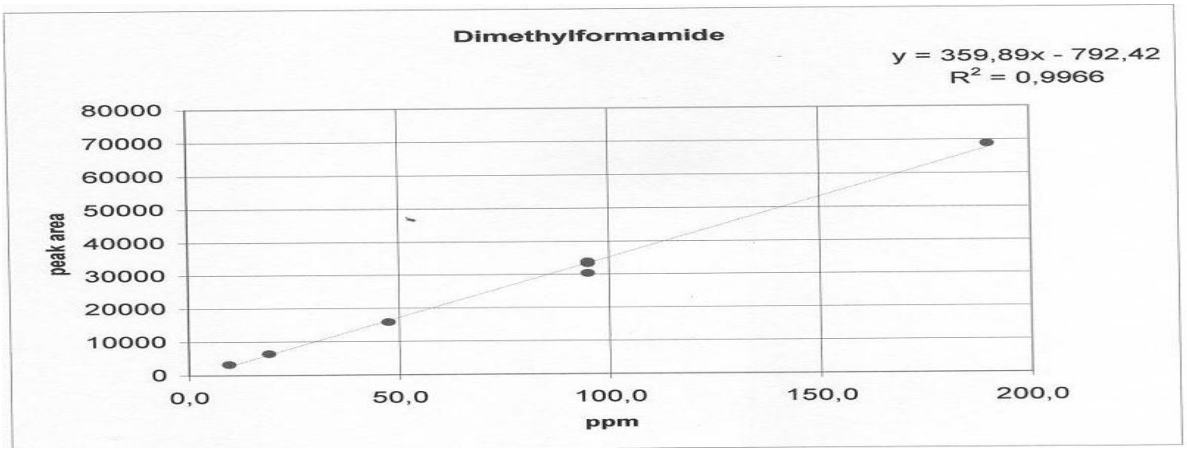

Fig. 5: Linearity and range plotted graph of dimethylformamide(DMF)

ppm	Peak area
190.0	68859
95.0	30412
95.0	33861
95.0	33346
47.5	15945
19.0	6441
9.5	3257
9.5	3257

Equation of the line: peak area=359.9 ppm – 792.4, Slope: 359.9, Intercept: -792.4, Correlation: R^2 = 0.9966 > 0.99

Table 7: Linearity and range values of dimethylformamide (DMF)

x	Y found	Y calculated value	% diff.	Y	Corresponding x value	Calculated x	% diff.
190.0	68859	67586.7	1.85	68859	190.00	193.5	-1.86
95.0	30412	33397.1	-9.82	30412	95.00	86.7	8.73
95.0	33861	33397.1	1.37	33861	95.00	96.3	-1.36
95.0	33346	33397.1	-0.15	33346	95.00	94.9	0.15
47.5	15945	16302.4	-2.24	15945	47.50	46.5	2.09
19.0	6441	6045.5	6.14	6441	19.00	20.1	-5.78
9.5	3257	2626.5	19.36	3257	9.50	11.3	-18.44
9.5	3257	2626.5	19.36	3257	9.50	11.3	-18.44

For most purposes, the linearity associated with the analytical procedure can be estimated by correlation coefficient which should be as close to 1.0000 as possible. Any method with a correlation coefficient less than 0.99 or more than 1.01 may be insufficiently precise or non-linear. The linearity determination was performed by analyzing 8 levels of added amount of solvents.

Limits of detection and quantitation: The limit of detection (LOD) of an analytical procedure is the lowest amount of analyte in the sample which can be reported to be present, with a given limit of confidence, using a specified experimental procedure. Similarly, the limit of quantitation (LOQ) of an analytical procedure is the lowest amount of analyte in the sample which can be quantitatively determined, with acceptable precision and accuracy, when using a specified experimental procedure. Both quantities are expressed in units of concentration. The determination of the LOD and LOQ was performed using 3 levels of added amount of solvents.

RUGGEDNESS: The ruggedness of an analytical method is to obtain the degree of reproducibility of test results by performing analysis of the same sample under a variety of conditions such as different analysts, different days etc. Ruggedness was performed by using two levels of added solvent.

Dimethylformamide (DMF)

Precision: The determination of the precision was performed by repetitive 3 injections of the Standard vial level B, corresponding to an addition of 95 ppm of DMF. The peak of DMF appears with a retention time of 10.96 min. The found peak areas were:30412, 33861, 33346. The mean value, standard deviation and % relative standard deviation was found to be 32539.7, 1860.5 and 5.7% respectively. The obtained precision = 5.7% complies with the prescription of the P. Eur. which reports that the relative standard deviation of the peak area should be less than 15.0%.

Linearity and range: The linearity was checked on five levels ranging from 9.5 to 190 ppm of added DMF. The range corresponds to about 1%-20% of the stated ICH limit for DMF=880 ppm. Eight determinations were used to calculate the equation of the line.

SUMMARY

Different validation parameters were studied after the method development. Nitrogen is used as carrier gas**.** By using the method the retention time was found to be acetone-1.499 minutes, methanol-1.759 minutes and DMF-10.96 minutes. Tailing factor of acetone- 0.972, methanol-1.0, DMF- 0.99.

CONCLUSION

Linearity of the solution was demonstrated for 5 injections**.** Accuracy was demonstrated as reported. Recovery and % of RSD are within the recommended limits. Injection reproducibility was demonstrated and the % of relative standard deviation for retention time and area were within the limits. Specificity was also demonstrated and there was no interference. Hence the method can be adopted as a stability indicating method.

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