Research Article

Analytical Method Development to Determine Residual Solvents in Glipizide by Gas Chromatography (GC/FID) with Head Space

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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. Analysis of organic volatile impurities is of key importance for Active Pharmaceutical Ingredients (API). Most of the available methods use liquid chromatography which could be expensive and time consuming. Hence, an analytical method for the quantification of residual solvents in Glipizide was established using a headspace gas chromatography (HSGC) coupled with a flame ionization detector (FID). Methanol, acetone and ethylene dichloride as residual solvents were determined in Glipizide. Analysis was performed by headspace GC/FID method on Agilent GC 7820A system. Nitrogen was used as a carrier gas with constant flow rate of 4.2 mL/min and the separation of residual solvents was achieved on DB-624 column. The thermostat temperature was 100 °C for 30 minute for each vial and after the equilibration the vials were pressurized and injected on GC column. The %RSD for six injections obtained in acceptance criteria. The percentage recovery ranges obtained from 92.49 and 106.69%.The correlation coefficient R2 obtained greater than 0.99. The method parameters were validated included specificity, limit of detection and quantification, accuracy, linearity, precision, and robustness. A new, simple, specific, accurate and precise method was validated according to the International Conference on Harmonization (ICH) guidelines.

Keywords: GC/FID, glipizide, ICH, impurity profiling validation, residual Solvents

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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]. Residual solvents can also have an impact on the overall quality and stability of final product and an API too [2,3]. Regulatory guidance document have included acceptable limits for these residual solvents; in particular in guideline Q3C issued by the ICH for registration of pharmaceuticals for human use (ICH) [4].In recent years there have been quite a few methods developed for identifying impurities by liquid chromatography. However, the Gas Chromatography too is capable of establishing both the identity and concentration of eluting components in the carrier gas stream and responds to range of compounds with a common physical or chemical characteristic. We have developed a simple and sensitive gas chromatographic method for the determination of residual solvents in Glipizide [5, 6].

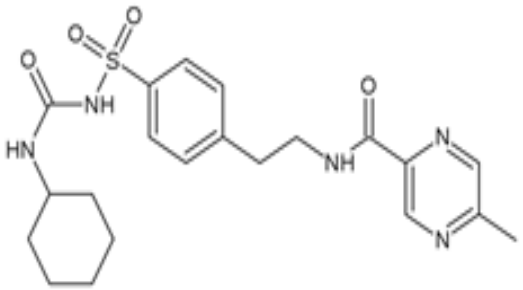

Figure 1: *N*-(4-[*N*-(cyclohexylcarbamoyl) sulfamoyl]phenethyl)-5-methylpyrazine-2-carboxamide

Glipizide is a sulfonyl urea having anti-diabetic properties, closely related to sulfa drugs. Glipizide is sold in doses of 1.25 mg, 2.5 mg and 5 mg and is used in the treatment of type II diabetes. It is also sold in combination with metformin [7,8]. Glipizide provokes a brisk release of insulin from pancreas. They act on the so called 'sulfonylurea receptors' (SUR1)on the pancreatic ß cell membrane—cause depolarization by reducing conductance of ATP sensitive K+ channels. This enhances Cainflux→ degranulation. The rate of insulin secretion at any glucose concentration is increased. In type 2DM the kinetics of insulin release in response to glucose or meals is delayed and subdued. The sulfonyl ureas primarily augment the 2nd phase insulin secretion with little effect on the 1st phase. That they do not cause hypoglycemia in pancreatectomised animals and in type 1 diabetics (presence of at least 30% functional ß cells is essential for their action) confirms their indirect action through pancreas. Hepatic degradation of insulin is slowed. (**Table 1**) indicates the solvents used in the preparation of Glipizide. The specification limit is mentioned in the column next to it.

Table 1: Residual Solvents of Glipizide

Solvents	Class of solvents (As per ICH)	Specification limit
Methanol	Class-2	3000 ppm
Acetone	Class-2	5000 ppm
Dichloroethane (EDC)	Class-1	5 ppm

MATERIALS AND METHODS

The analysis was performed on Agilent GC 7820A FID detector and Chem station software. The injection temperature was 190oC and detector temperature was 290 oC. 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 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. Nitrogen was used as a carrier gas at a constant flow rate of 4.2 mL/min [9-19].

Factor affecting and optimization:

(a)Selection of solvent (Diluent): Four diluents had been tried- Water, DMSO, DMF and NMP. Unknown peak was observed in sample so DMF was avoided as a diluent. DMSO and NMP were avoided due to recovery of EDC was not under limit of ICH guidelines. Hence, finally water is selected as a diluent which was compatible with sample.

(b) Selection of column: Three columns had been checked for the development namely BP-01, BP-5 and DB-624. BP-01 and BP-5 were avoided due to poor peak separation, DB-624 was finalized and it showed good system suitability parameters.

(c) Selection of Ramping Rate: Increasing the ramping rate caused the retention time (RT) to decrease. Three Ramping rate were tried at 5°C/min, 10 °C/min, 15 °C/min. At 5 °C/min the RT was very high, so it was not selected. Poor separation was observed at 15 °C/min then finally 10 °C/min was optimized.

(d) Flow Rate: As the flow rate increase, the viscosity of carrier gas decrease and velocity increase. Five flow rates were applied 3 ml/min, 3.5 ml/min, 4 ml/min, 4.2ml/min, 4.5 ml/min and 5 ml/min. 4.2 ml/min was selected as a finalized flowrate.

(e) Linear Velocity: Linear velocity is the mobile phase velocity through the column which can be calculated from the length of the column divided by the retention time. Increased the linear velocity which caused decrease in the retention time. Velocity was tried at, 20 cm/sec to 35 cm/sec and finally 32 cm/sec is optimized.

f) Optimization of head space condition:

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

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

Finalized chromatographic condition:

Table 2: Instrument conditions

GC Run Time	25 min
Column Oven Temperature:	40°C-5min-@10°C/min-170°C-7 min
Injection Temperature	190^{0}
Detector Temperature	290^{0} C
Inlet Pressure	21.1 kpa (about 4.2 mL/min)
Linear velocity	32 cm/sec
Injection Volume (Head space)	1 mL
Split Ratio	1:4
Carrier Gas	Nitrogen
Detector	Flame Ionization Detector

Table 3: Head space conditions

Equilibration Temperature	100^{0} C
Equilibration Time	50 min.
Transfer line Temperature	115^{0} C
Vial Volume	20 mL
Syringe Rinsing	Thrice
Injection Volume	1 mL by Head space
Syringe Filling Speed	25 mL/min
Injection Speed	15 mL/min
GC Cycle Time	35 min

Standard solution: The required concentration for solvents was obtained by mixing appropriate aliquots of stock in dissolving solvent with respected to sample concentration. For Glipizide, the working concentration of solvents in the solution is as 3000 g/L for Methanol, 5000g/L for acetone and 5 g/L for EDC prepared in water diluent.

Diluent: Water

Preparation of Blank solution: For the Blank solution, pipette out 5 ml respective diluents into a HS vial and the vial were closed with PTFE silicon septa closure and secured the closure with an aluminum cap.

Preparation of standard solution: Standard Stock Solution-I: Accurately weigh and transfer 0.020 gm 1, 2-Dichloroethane (EDC) in to 200 ml Volumetric flask containing 140 ml Water and make up the volume to the mark with diluent.

Standard Solution-II: Accurately weigh and transfer 0.300 gm of methanol, 0.500 mg of acetone in to a 100 ml volumetric flask containing approximately 50 to 60 ml of Water and make up volume to the mark with diluent.

Working standard solution: Then further dilute 40 ml of Standard Solution-II to 200 ml volumetric flask containing approximately 50 to 60 ml of Water. Add 1 ml of Standard stock solution-I in this solution and make up the volume to the mark with diluents.

Test solution: Weight accurately about 1000mg of the substance under examination, dissolve and dilute to 5ml with diluents into a HS vial and seal the vial immediately with PTFE septa.

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, limit of detection and quantitation, linearity, accuracy, repeatability, and precision of residual solvents as was.

Specificity: Specificity denotes the resolving power of the system. The resolution of the analyte peak was well separated from the nearest peak and was not less than1.5. The specificity of the analytical method was determined by injecting blank solution and the individual and Mix solution of residual solvents under the same experimental conditions and parameters like resolution, theoretical plates, tailing factor were determined.

Detection Limit (LOD) and Quantification Limit (LOQ): By quantitative dilutions of the stock solution of solvents a series of solutions were prepared. The mean peak area was calculated by injecting each solution in triplicate. A graph of mean peak area against concentration in g/L was plotted and the equation of regression line and the residual standard deviation was determined. LOD and LOQ determined by statistical formula.

LOD= 3.3 SD/Slope

LOQ= 10 SD/Slope

Where, SD is standard deviation

RESULT AND DISCUSSION

The result for the residual solvents in the sample (in g/L) using the following formula. Where, A is the peak area of response of solvent in test preparation, B is the peak area response of solvent interference from blank preparation, C is the peak area response of solvent in standard preparation and D is the peak area response of solvent interference from blank preparation,

Ws is weight of component in standard in gm, Wt is weight of sample taken in gram, E is dilution factor.

(A-B)/(C-D) x WS/WT x E x 10^6

Specificity:

There was no interference of dissolving solvent at the retention time of methanol, acetone and EDC and all peaks were well resolved from each other. Hence the method was found specific. Specificity parameters showed in (**Table 4**).

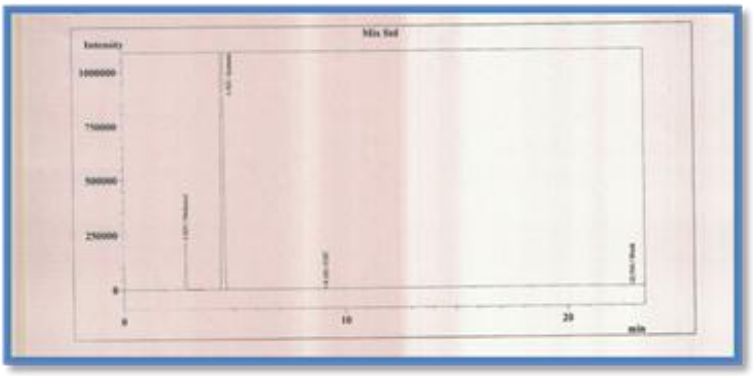

Figure 2: Graph of standard solution (Methanol, Acetone, and EDC)

Table 4: Specificity Parameters

Solvents	RT	Resolution	Theoretical Plates	Tailing Factor
Methanol	2.829	--	8057.28	1.146
Acetone	4.502	11.456	11682.63	1.028
EDC	9.103	30.401	70897.24	1.014
Acceptance Criteria		NLT 1.5	NLT 5000	NMT 1.5

Precision: Six replicate injections of standard solution for system precision were analyzed as per the proposed method and the chromatograms obtained. The standard deviation and percentage relative standard deviation (% RSD) was calculated. For the precision of method and system the % RSD for six solvents complies with acceptance criteria of less than 2%, hence the method and system is said to be precise.

Table 5: Precision Parameters

No. of Standard	Methanol		Acetone		EDC	
	RT	Area	RT	RT	RT	Area
Standard 1	2.828	1047728	4.503	14358607	9.103	69326
Standard 2	2.829	1028512	4.502	14419726	9.103	70411
Standard 3	2.825	1021782	4.499	14481633	9.100	71325
Standard 4	2.827	1055241	4.501	14465511	9.101	69801
Standard 5	2.823	1036095	4.495	14487743	9.096	70572
Standard 6	2.828	1087560	4.498	14830634	9.097	71870
Mean	2.827	1046152.90	4.500	14507308.97	9.100	70550.83
% RSD	0.080	2.26	0.065	1.14	0.033	1.33

Linearity: A linear relationship evaluated across the range of concentration of analyte solvents (1% to 150% Concentration) and calculate the correlation coefficient, y-intercept and slope of the regression line. The acceptance criteria of correlation coefficient should be more then 0.99. Linearity of solvents showed in (**Table 6**) and (**Figure 3 and 5**).

Table 6: Linearity of residual solvents of Glipizide

Methanol		Acetone		EDC	
Level (ug/L)	**RSD**	**Level (ug/L)**	**RSD**	**Level (ug/L)**	**RSD**
30	9.22	50	8.33	--	--
90	7.10	150	6.79	0.15	5.87
150	5.12	250	4.23	0.25	2.16
300	3.65	500	0.65	0.50	0.59
1500	3.91%	2500	1.21%	2.50	0.25%
2250	2.62%	3750	2.96%	3.75	3.21%
3000	0.83%	5000	0.24%	5.0	2.87%
3750	1.66%	6250	1.90%	6.25	2.39%
4500	1.26%	7500	1.45%	7.5	0.94%

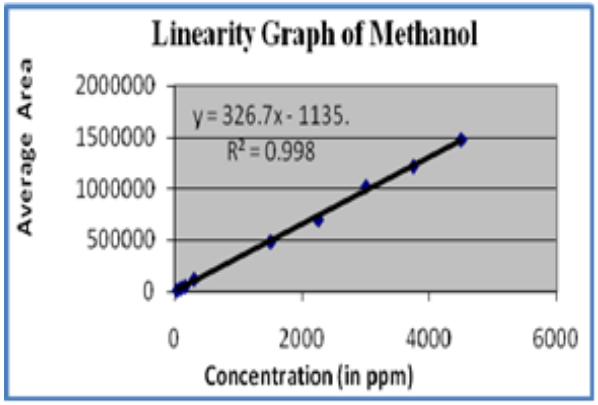

Figure 3: Linearity graph of Methanol

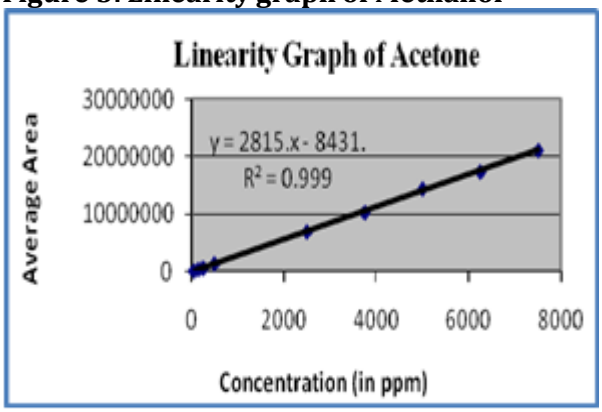

Figure 4: Linearity graph of Acetone

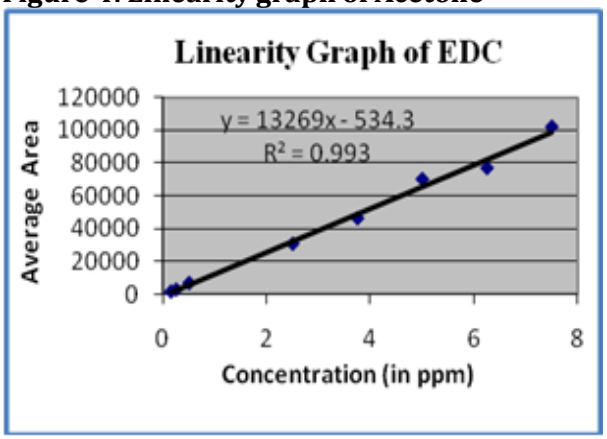

Figure 5: Linearity of EDC LOD and LOQ (limit of detection and limit of quantification)

The LOD and LOQ were calculated by instrumental method. LOD is determined as the lowest amount to detect and LOQ is the lowest amount to quantify by the detector. The value for the limit of detection and limit of quantification showed in (**Table 5**).

Accuracy Recovery (By Standard Addition Method):

Accuracy of the method was ascertained by standard addition method at 3 levels. Standard solution quantity equivalent to 50%, 100% and 150 % were added in Sample. The amount recovered by the method was compared to the amount added. Percent deviation was calculated at each levels and a grand average across all the levels was also calculated. The acceptances criteria of recovery at each level are 90.0 - 110.0%. [20] % Recovery = (Area of solvent in spiked sample-Area of solvent in Sample)*100/Area of solvent in standard % Recovery calculated showed in (**Table 8**).

Robustness:

There was no significant difference in the results for Methanol, Acetone, EDC obtained by the normal method and those obtained by carrying out deliberate changes in the method. Hence the method was found robust with respect to change in the flow rate for the carrier gas and incubation temperature in head space. It should show the reliability of an analysis with respect to deliberate variations in method parameters.

Ruggedness:

The ruggedness was established by determining residual solvents using the same chromatographic system and the same column by two analysts on a different day. The assay result indicated that the method was capable with high precision. Additionally, good separations were

achieved, which indicates that the method is selective for all components under the test.

Table 7: Limit of detection and Limit of quantification

Solvents	Linearity % RSD Range	2	Slope	LOD	LOQ (g/L)
Methanol	0.83 - 9.23%	0.998	326.7	8.43	25.54
Acetone	0.24 - 8.33%	0.999	2815	9.56	28.97
EDC	0.25 - 5.87%	0.993	13269	0.05	0.15

Table 8: Accuracy Recovery

Solvents	Range (%, g/L)	Recovery 1	Recovery 2	Recovery 3	% RSD
	1500 g/L (50 %)	103.6	101.53	100.78	2.75
Methanol	3000 g/L (100%)	100.19	99.36	101.12	1.47
	4500 g/L (150%)	98.11	98.74	99.87	0.643
	2500 g/L (50 %)	96.69	93.47	96.98	1.82
Acetone	5000 g/L (100%)	99.47	92.49	98.18	0.54
	7500 g/L (150%)	96.18	93.20	97.41	0.62
	2.5 g/L (50 %)	103.3	97.35	96.58	3.84
EDC	5 g/L (100%)	104.34	99.03	98.34	3.04
	7.5 g/L (150%)	97.12	103.24	106.69	5.37

CONCLUSION

The method developed for the analysis of residual solvents in Glipizide, is rapid, sensitive, accurate and rugged. The method is quite faster with a run time of 25 minutes and achieves to address the residual solvents at the prescribed range of limits. The method exhibits a good range of quantization.

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