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Original Article

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF RESIDUAL SOLVENTS IN GLICLAZIDE USING GAS CHROMATOGRAPHY

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ABSTRACT

Objective: The present work discusses developing a novel method for estimation of residual solvents in Gliclazide using Gas chromatography and determining its consistency, reliability and reproducibility by performing its validation.

Methods: A Gas chromatograph equipped with aflame ionization detector, column DB-624 (60 m x 0.32 mm x 1.8 µm) with Nitrogen as carrier gas was used and the column temperature was 40 °C (hold for 15 min) and increased to 240 °C at 20 °C per min. The solutions were prepared using-Methyl-2-Pyrrolidone (NMP) diluent as per the procedure given in protocol and appropriately injected as per the sequence. The validation parameters checked were System suitability, Specificity, Linearity and Range, Accuracy, Precision, Limit of detection, Limit of quantitation, Ruggedness and Robustness.

Results: The data for each validation parameter tested is compiled and documented. It was found that the results obtained for each parameter compiled with their given acceptance criteria. Hence, the developed method was considered reproducible, reliable and consistent.

Conclusion: The method of analysis complies with all the parameters tested and it was found to be reliable, consistent and reproducible.

Keywords: Gas chromatography, Method validation, Residual solvents, System suitability, Specificity, Linearity, Range, Accuracy, Limit of Detection, Limit of Quantitation, Precision, Ruggedness, Robustness

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INTRODUCTION

Analytical method development is done to produce new methods for estimating chemical compounds like active pharmaceutical ingredients, phytoconstituents, the chemical composition of formulations and plant products, biological samples, volatile components of crude aerial parts of plants etc. using analytical instruments. Analytical instruments have gained much importance over chemical methods for estimating any compound because they give accurate results in a short period, do not require much work and consume fewer quantities of chemicals during analysis [1]. The need of designing a novel method is to make the analysis easy, accurate, cost-effective and time-efficient. The same sample was analyzed using one instrument for example, HPLC may be analyzed by another instrument, also eliciting better results and is more efficient than the one already in use. In this way, efforts are made to develop new procedures for estimating the compound or mixtures by different analytical methods and cross-validating the methods to identify the better one, which is the process called 'Analytical Method Development' and to check the new method or procedure for its accuracy and efficiency is called 'Validation' [2].

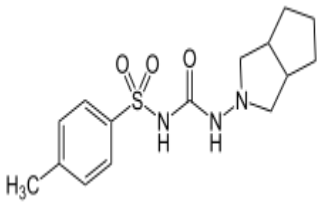

Fig. 1: Structure of gliclazide

Gas Chromatography (GC) is a widely used technique for analysing a variety of samples like biological samples, separation of volatile components in plants, impurity profiling, determination of the chemical composition of formulations etc. The technique is only suitable for estimating volatile chemicals. New procedures can be developed for the analysis of the same samples using GC. For better analysis, GC is mostly coupled with a Mass Spectrometer through which one can achieve separation as well as qualitative and quantitative estimation of individual compounds. The coupling of GC with a mass spectrometer is usually done to develop a novel method for the determination of samples [3].

Gliclazide, chemically known as 1-(Hexahydrocyclopenta[c]pyrrol-2(1H)-yl)-3-[(4-methyl phenyl) sulfonyl] urea, has the molecular formula: $C_{15}H_{21}N_3O_3S$ and molecular weight: 323.4 belonging to the chemical class of Sulfonylurea and a therapeutic class of Anti-Diabetics.

Organic solvents are routinely applied during the synthesis of drug substances, excipients, or 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 odour or taste, which can be unpleasant for patients [4]. To remove them, various manufacturing processes or techniques are in use. Even after such processes, some solvents still remain, albeit in small quantities. These small quantities of organic solvents are commonly known as volatile organic impurities or residual solvents. Different manufacturers produce the same pharmaceutical products using different organic solvents. Therefore, the analysis of residual solvents becomes a challenging analytical task in pharmaceutical analysis and control [5]. Residual Solvents are volatile organic chemicals used or produced in the manufacture of Active Pharmaceutical Ingredients (APIs). Residual solvent specification limits, set in accordance with the toxicity of solvents, vary from a few ppm to thousands of ppm. These are determined for their limits in APIs as per ICH–Q3C Quality Guidelines for Impurities. They are classified as per the guidelines [6]:

1. Class 1 Solvents: to be avoided
2. Class 2 Solvents: limited use

3. Class 3 Solvents: low toxic potential
4. Class 4 Solvents: no toxic data found

Methanol, Ethanol, Acetonitrile, Dichloromethane and Toluene are used as solvents in Gliclazide manufacturing steps and not removed consistently. These solvents belong to different classes of residual solvents and may be regarded as less toxic and of lower risk to human health [7].

The present work focuses on developing a novel method for estimation of residual solvents in Gliclazide using Gas chromatography and determining its consistency, reliability and reproducibility by performing its validation.

MATERIALS AND METHODS

Chemicals and reagents

Gliclazide API (purity 99.9%) was purchased from Micro labs Ltd. (API), India. Standard solvents Methanol, Ethanol, Acetonitrile, Dichloromethane and Toluene were obtained from Merck, India. N-Methyl-2-Pyrrolidone has been used as a diluent and was obtained from RCI Labscan, Thailand.

Instrumentation and chromatographic conditions

A gas chromatograph (Agilent GC system) with Headspace sampler 8697 series was used to load the sample. An analytical balance (ME204 from Mettler Toledo) and Micropipette (100–1000 µL from Eppendorf) were used. For gas chromatographic analysis, a DB-624 fused silica capillary column from Agilent (G43 phase: 6% cyanopropyl phenyl, 94% polydimethylsiloxane) (60 m length, ID 0.32 mm, and 1.8 µm film thickness) was used. The temperature of the injection port was maintained at 170 °C at a split ratio of 1:10, with nitrogen as a carrier gas (flow rate of 3.0 ml/min). The temperature of the detector was set at 250 °C. The oven temperature was programmed from 40 °C (15 min) and then increased at a rate of 20 °C/min up to 240 °C; maintained for 5 min. A volume of 1 ml solutions was injected into the GC injection port. The chromatographic system was considered suitable if the relative standard deviation of the peak area of the standard solution for six injections was not more than 15.0%.

Standard solutions and sample preparation

A common standard stock solution in N-Methyl-2-Pyrrolidone containing all the known residual solvents of Gliclazide API was prepared in such a way that it had a final concentration of 6000 ppm Methanol, 10000 ppm Ethanol, 820 ppm Acetonitrile, 1200 ppm Dichloromethane and 1780 ppm Toluene. Then, a common standard solution was prepared by diluting 1 ml of standard stock solution with 50 ml of N-Methyl-2-Pyrrolidone. Spiked sample solutions for specificity, accuracy and precision were prepared using 200 mg of Gliclazide API in 5 ml of standard stock solution. The sample solution was prepared by diluting 200 mg of Gliclazide API with 5 ml of N-Methyl-2-Pyrrolidone. Final concentrations of all dilutions were achieved using Micropipette. All vials were immediately equipped with a septum and metallic cap and crimped properly.

Method development

Estimation of residual solvents in API is important in order to limit their presence in the final product as per the quality guidelines of ICH. The present work involves the use of a gas chromatograph (Agilent technologies) for the estimation of volatile residual solvents coupled with a highly sensitive flame ionization detector. The method was developed by considering the type of column, carrier gas, oven temperature programming, and injection temperature [8].

Validation of the developed method

The method validation was done by evaluating system suitability, specificity, linearity and range, the limit of detection (LOD) and limit of quantitation (LOQ), accuracy, precision, ruggedness and robustness as indicated in the ICH guideline (Q3C (ICH Q3C 2006; ICH Q2 1995). System suitability of method was performed by injecting six replicates of standard solution. The system suitability was confirmed by peak resolution of closest solvents and % RSD. Specificity of the analytical method was performed by injecting system blank (empty vial), blank (only diluent), standard solution, individual solvent stock solution and spiked solution under the same chromatographic condition. For the instrumental method, LOD was determined as the lowest amount to detect and LOQ was the lowest amount to quantify by the detector. The LOD and LOQ were calculated by statistical methods (determined based on the standard deviation and slope of linearity response curves for all solvents). Detector response linearity and accuracy were assessed by investigating solutions of all solvents prepared over the range of LOQ concentration–120% of the specification limit in the diluent. System precision was determined as per system suitability criteria, while method precision was determined by analyzing six replicate injections containing Gliclazide API spiked with a standard solution. Ruggedness was determined by performing injections of six freshly prepared spiked solutions on different days and by different analysts. The robustness of the method was assessed by deliberately altering the experimental conditions such as carrier gas flow rate (±0.3 ml/min), column temperature program (±2 °C), and vial equilibration temperature (±5 °C) by keeping all the other chromatographic conditions constant as described above.

RESULTS AND DISCUSSION

System suitability

The criterion for system suitability was that the resolution between the closest solvent pair should not be less than 1.0 and it was found to be above the minimum limit. Also, %RSD for peak areas of residual solvents in six replicates of standard solution injections should not be more than 15%. Results indicate an acceptable level of precision for the analytical system. The results for the system suitability parameter are given in tables 1 and 2.

Table 1: Resolution between two closest solvents from standard solution

Name of the closest solvent pair	Resolution	Acceptance criteria
Ethanol and Acetonitrile	6.14	Not less than 1.0

Table 2: % RSD for the area of solvents from six injections of standard solutions

Name of the solvent	% RSD for area	Acceptance criteria
Methanol	2.07	Not more than 15%
Ethanol	1.78	
Acetonitrile	2.10	
Dichloromethane	1.37	
Toluene	1.59	

Specificity

The Gliclazide API sample was spiked with all residual solvents in one solution and examined for interference, if any, of the residual solvent peaks with each other. The retention time of solvents in spiked solutions was compared with the retention time of individual standard solvent solutions. The specificity results are shown in table 3. The diluent and API do not show interference at the retention time of any residual solvents. The resolution between solvent peaks was above the minimum passing limit. Hence, the method was found to be specific.

Table 3: Comparison of retention time of solvents in individual and spiked solution

Name of the solvent	Retention time from individual solution	Retention time from spiked solution
Methanol	3.252	3.248
Ethanol	4.508	4.506
Acetonitrile	5.589	5.582
Dichloromethane	7.530	7.523
Toluene	18.163	18.162

Linearity and range

The linearity of the method was determined by making five linearity levels of residual solvents spiked solutions over the range LOQ–120% of the specification limit. Two replicates were performed for LOQ to 100% level and six replicates for 120% level. The calibration curves were obtained with the average peak area of solvents. The linearity data for each solvent is shown in table 4. Thus, it can be concluded that the method is linear over the entire concentration range (LOQ–120%) for all residual solvents.

Table 4: Linearity data at different levels (LOQ, 50%, 80%, 100%, 120%)

Residual solvent	Linearity		
	Slope	Intercept	Correlation coefficient (r^2)
Methanol	732.34	23371	0.9998
Ethanol	751.97	-27670	0.9999
Acetonitrile	782.58	7438	0.9991
Dichloromethane	476.09	3827	0.9997
Toluene	1533.2	-4767.8	0.9999

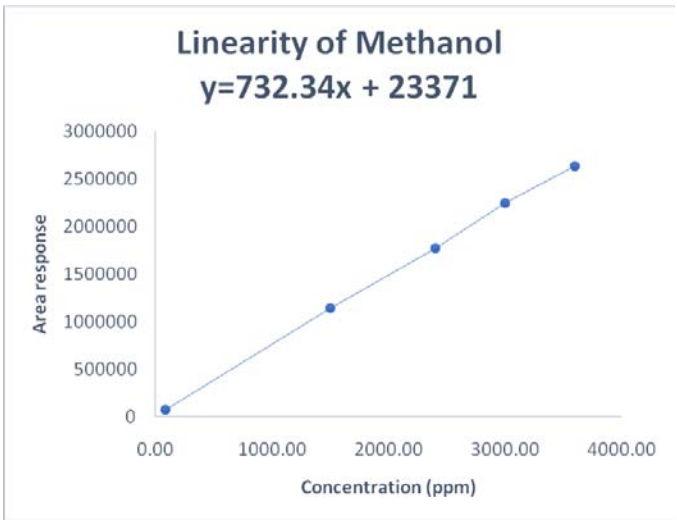

Fig. 2: Linearity curve of methanol

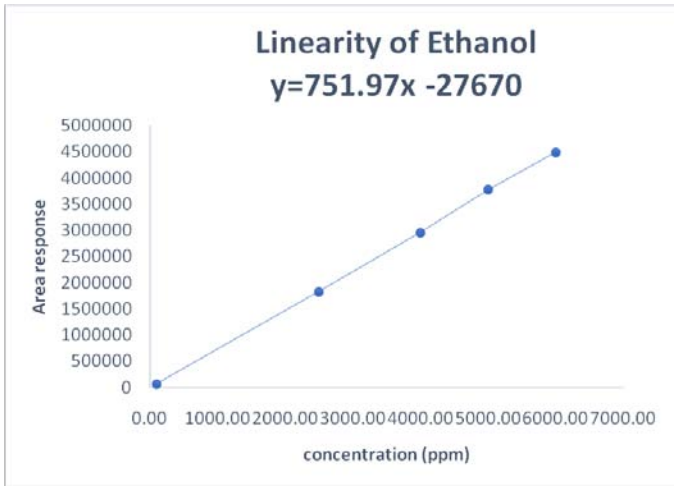

Fig. 3: Linearity curve of ethanol

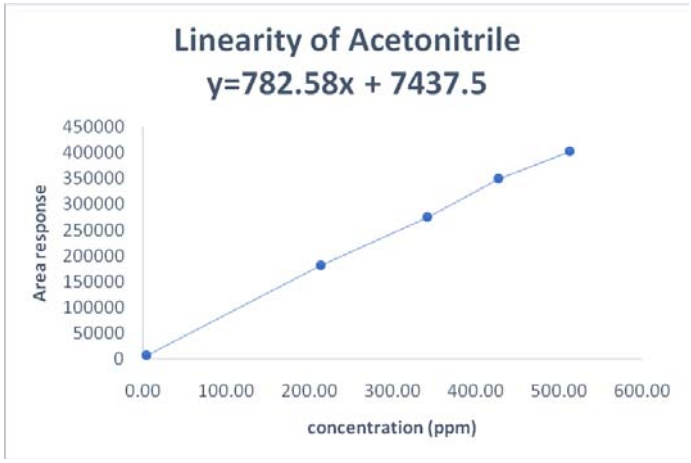

Fig. 4: Linearity curve of acetonitrile

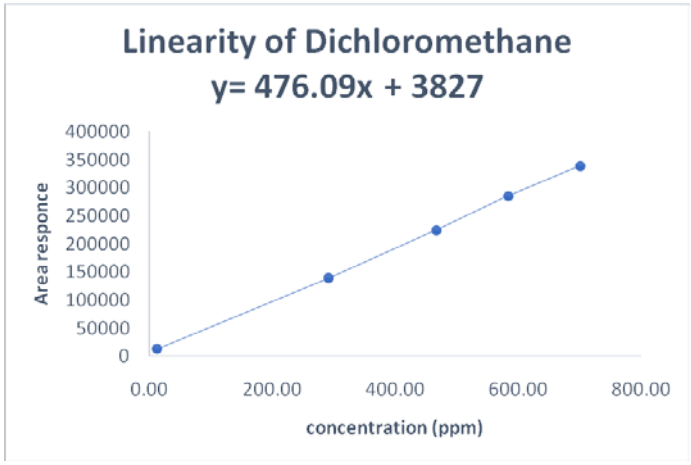

Fig. 5: Linearity curve of dichloromethane

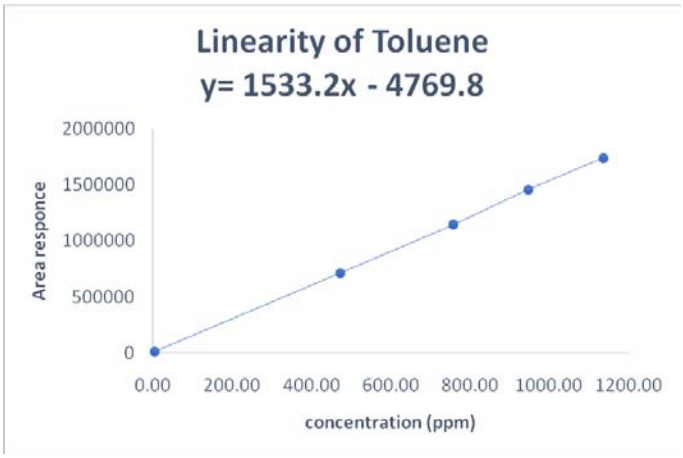

Fig. 6: Linearity curve of toluene

Table 5: LOD and LOQ concentrations of residual solvent

Name of the solvent	LOD concentration (ppm)	LOQ concentration (ppm)
Methanol	27.11	84.33
Ethanol	32.56	95.18
Acetonitrile	1.65	4.95
Dichloromethane	4.59	13.11
Toluene	2.33	5.59

Limit of detection and limit of quantitation

The LODs and LOQs of residual solvents in Gliclazide API were determined based on the standard deviation and the slope of the response curve. The values of LOD and LOQ are much less than the lower limit of the concentration range and cannot affect the accuracy of the test. The LOQ values are well below the ICH specification limit of the residual solvents. LOD and LOQ values for all solvents are given in below table 5.

Accuracy

Accuracy, the closeness of measured values and its actual value, was determined by injecting a known amount of residual solvent at accuracy levels at LOQ, 50%, 100% and 120% of standard stock solution. The % recovery of residual solvents is listed in table 6. These values are well within the prescribed limits; hence the method is accurate for the determination of residual solvents in Gliclazide API.

Table 6: Accuracy data for different residual solvents

Accuracy levels	Methanol	Ethanol	Acetonitrile	Dichloromethane	Toluene
LOQ	108.40	100.61	107.65	102.72	101.24
50%	99.01	100.20	99.82	103.25	101.68
100%	98.49	100.05	98.41	102.10	101.11
120%	101.88	103.66	102.64	104.02	103.64

Precision

Two types of precision were performed, namely system and method precision. System precision was assessed by system suitability data of six injections of standard solution and it was found that the %RSD for peak area response of residual solvents was within the acceptable limit. Method precision was determined by six injections of the spiked solution containing Gliclazide API and residual solvents. The results concluded that %RSD for the peak area of solvents was within the acceptable limit. The results for system and method precision are summarized in tables 7 and 8. Hence, the method gave repeatable results was and considered to be precise.

Ruggedness

Intermediate precision/or reproducibility was determined by injecting the same sequence of freshly prepared solutions as in method precision but on different days and by different analysts. The % RSD is shown below in table 9 and was below the maximum acceptable limit of 15%.

Table 7: System precision: % RSD of area response from six injections of standard solutions

Injection No.	Area of methanol	Area of ethanol	Area of acetonitrile	Area of dichloromethane	Area of toluene
1	2065348	3444918	346865	292759	1377580
2	2152566	3584744	366073	299215	1413757
3	2071625	3454602	352233	292531	1371344
4	2071220	3448250	350526	293640	1380111
5	2081135	3495383	354033	293374	1394966
6	2036020	3413519	345270	290691	1365321
Mean	2079652	3473569	352500	293702	1383847
% RSD	1.87	1.74	2.10	0.99	1.28
Acceptance	% RSD Not more than 15%				

Table 8: Method precision: solvent content (spiked at specification level into samples) results

Preparation No.	Methanol (ppm)	Ethanol (ppm)	Acetonitrile (ppm)	Dichloromethane (ppm)	Toluene (ppm)
1	3063	5069	438	687	950
2	3086	5092	440	698	952
3	2941	4862	423	672	925
4	2924	4844	421	667	916
5	3110	5124	447	698	959
6	2920	4821	419	664	914
Mean	3007.33	4969	431.33	681	936
% RSD	2.93	2.82	2.73	2.26	2.13
Acceptance criteria	% RSD Not more than 15%				

Table 9: Ruggedness data for residual solvents

Ruggedness	Methanol	Ethanol	Acetonitrile	Dichloromethane	Toluene
Mean (ppm)	2981.66	4930.24	467.42	665.25	908
SD	67.38	111.42	16.64	14.90	30.69
%RSD	2.26	2.26	3.56	2.24	3.38
Acceptance criteria	%RSD Not more than 15%				

Robustness

Robustness of the method was checked by system suitability criteria with slight variation in chromatographic conditions like carrier gas flow rate (+0.3 ml/min), column oven temperature (+2 °C) and vial equilibration temperature (+5 °C) from actual values. The % RSD for all solvents in altered conditions was within an acceptable limit. The results are summarized in tables 10 and 11.

Table 10: Resolution between closest solvent pair under robustness conditions

Conditions	Resolution between closest solvent pair (ethanol and acetonitrile)
Actual column flow (3.0 ml/min)	6.14
Column flow increase (3.3 ml/min)	5.93
Column flow decrease (2.7 ml/min)	6.36
Actual oven temperature (40 °C)	6.14
Oven temperature increase (42 °C)	5.96
Oven temperature decrease (38 °C)	6.35
Actual vial equilibration temperature (70 °C)	6.14
Vial temperature increase (75 °C)	6.14
Vial temperature decrease (65 °C)	6.11
Acceptance criteria	Not less than 1.0

Table 11: %RSD of the solvent area under robustness conditions

Conditions	%RSD for a standard solution under different robustness conditions				
	Methanol	Ethanol	Acetonitrile	Dichloromethane	Toluene
Actual column flow (3.0 ml/min)	2.07	1.78	2.10	1.37	1.59
Column flow increase (3.3 ml/min)	2.38	2.57	2.58	2.13	2.26
Column flow decrease (2.7 ml/min)	4.45	4.56	5.59	4.36	4.59
Actual oven temperature (40 °C)	2.07	1.78	2.10	1.37	1.59
Oven temperature increase (42 °C)	2.31	2.43	2.28	1.50	2.30
Oven temperature decrease (38 °C)	5.13	2.65	2.61	2.79	2.60
Actual vial equilibration temperature (70 °C)	2.07	1.78	2.10	1.37	1.59
Vial temperature increase (75 °C)	3.02	2.89	3.04	2.88	2.76
Vial temperature decrease (65 °C)	1.95	2.10	1.75	1.96	1.73
Acceptance criteria	%RSD Not more than 15%				

CONCLUSION

Analytical method validation is a process of validating a newly developed method by estimating standard parameters and providing documented evidence that the method of analysis is reliable, consistent and reproducible. In the present work, a simple, rapid and highly selective gas chromatography method was developed and validated for the quantification of residual solvents present in quinabut API through an understanding of the synthetic process and nature of solvents and nature of stationary phases of columns. The parameters checked were System suitability, Specificity, Linearity and Range, Accuracy, Precision, Limit of detection, Limit of quantitation, Ruggedness and Robustness. The developed method complies with all the parameters tested and it was found to be reliable, consistent and reproducible as per ICH guidelines. The result of this validation shows that residual solvents (Methanol, Ethanol, Acetonitrile, Dichloromethane and Toluene can be analyzed in Gliclazide API according to the method described in this article with reliability for further analytical studies.

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The data used to support the findings of this study are available from the corresponding author upon request.

AUTHORS CONTRIBUTIONS

Judy jays has supervised the overall project work. Burhanuddin Madriwala carried out the method development and validation process and documented the result. The manuscript was prepared by Burhanuddin Madriwala and revised by Judy jays.

CONFLICTS OF INTERESTS

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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