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Determination of Residual Solvents in Dapagliflozin Amorphous by Gas Chromatography with Static Head Space Method

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ABSTRACT

The purpose of this research work was to develop a novel, sensitive and accurate analytical method for quantification of 12 residual solvents in Dapagliflozin Amorphous by using a static headspace gas chromatography (HSGC) coupled with flame ionization detector (FID). Methanol, Ethanol, Diethyl ether, Methyl acetate, Dichloromethane, Ethyl acetate, Tetrahydrofuran, Cyclohexane, Isopropyl acetate, Methyl isobutyl ketone, Toluene, Chlorobenzene as residual solvents were determined in Dapagliflozin Amorphous. The separations of 12 residual solvents were achieved on DB-624 (60 meters ×0.53 mm I.D, 3.0µm) column. Nitrogen was used as a carrier gas with constant pressure 7.0 psi. As a sample diluent in a headspace sampling, N-methyl-2-pyrrolidone was selected owing to its high capacity for dissolving Dapagliflozin Amorphous sample. Excellent correlation coeffient between peak responses and concentrations were > 0.9936. The recoveries of all 12 solvents spiked in Dapagliflozin Amorphous were in the range from 97.1% to 103.0%. Limit of quantitation for all 12 solvents were sufficiently lower than limits specified by ICH. In the proposed method, USP resolutions between all the 12 solvents were more than 1.6. The method has validated as per International Conference on Harmonization (ICH) guidelines. A precise, accurate, linear and robust Headspace Gas Chromatography method was developed for the quantification of 12 residual solvents in Dapagliflozin Amorphous.

Keywords: Dapagliflozin Amorphous**,** Residual solvents, Method development, Static headspace Gas Chromatography, Method validation, ICH guidelines.

INTRODUCTION

Residual solvents in pharmaceuticals are termed as organic volatile impurities. These chemicals are used or produced in the manufacture of drug substances. Residual solvents hazardous to health. They can modify the properties of drug substance and impact on stability of drug substance. As per the US-FDA and USP<467> general chapter specifies for the control of the residual solvents. Regulatory guidance document have included acceptable limits for these residual solvents Q3C issued by the ICH [1].Hence it is necessary to control the solvents by the procedure of the manufacturing of the drug substance.

Dapagliflozin Amorphous is chemically (2S,3R,4R,5S,6R)-2-[4-chloro-3-(4-ethoxybenzyl) phenyl]-6-(hydroxymethyl)tetrahydro-2H-pyran-3,4,5-triol.with molecular formula $C_{21}H_{25}ClO_6$. Dapagliflozin is an inhibitor of sodium-glucose co-transporter 2 (SGLT-2) in development for the treatment of Type 2 diabetes. Dapagliflozin was approved by US food and drug administration (FDA). Diabetes mellitus type 2 is a long term metabolic disorder that is characterized by high blood sugar, insulin resistance, and relative lack of insulin[2] Common symptoms include increased thirst, frequent urination, and unexplained weight loss. Symptoms may also include increased hunger, feeling tired, and sores that do not heal.[3] Often symptoms come on slowly.[2] Long-term complications from high blood sugar include heart disease, strokes, diabetic retinopathy which can result

in blindness, kidney failure, and poor blood flow in the limbs which may lead to amputations.[4] The sudden onset of hyperosmolar hyperglycemic state may occur; however, ketoacidosis is uncommon.[5][6].it occurs with increasing prevalence in the elderly and those with other comorbidities. Blood glucose control presents a challenge that is magnified by these co-existing problems. To achieve glycemic targets, many patients need more than one antidiabetic drug, and additional medications are often required as glucose control deteriorates [7].

From the literature review there were few analytical methods have been reported for Dapagliflozin such as spectrophotometry, HPLC and LC-MS/MS methods [8-14].There was no reported method for the determination of Residual solvents in Dapagliflozin Amorphous by Gas chromatographic method. The major objective of the present work is to develop a simple and robust GC method for determination of 12 Residual solvents in Dapagliflozin. Hence, a reproducible Gas Chromatography with static headspace method was developed for the quantitative determination of 12 Residual solvents in Dapagliflozin Amorphous.

This method was successfully validated according to the International Conference Harmonization (ICH) guidelines [15].

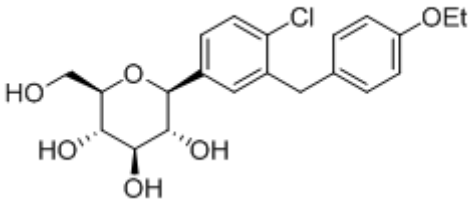

Fig 1: Structure of Dapagliflozin Amorphous

MATERIALS AND METHODS

Chemicals and reagents

Dapagliflozin Amorphous was prepared and provided by Dr.Reddys laboratories limited, IPDO, Hyderabad, India. Methanol, Ethanol, Diethyl ether, Methyl acetate, Dichloromethane, Ethyl acetate, Tetrahydrofuran, Cyclohexane, Isopropyl acetate, Methyl isobutyl ketone, Toluene, Chlorobenzene and N-methyl-2-pyrrolidone were purchased from Merck, Germany.

Instruments and software

A calibrated electronic single pan balance Mettler toledo. All analysis performed on
GC1: Agilent 6890 module equipped with FID detector and Headspace G1888.
GC2: Agilent 7890 module equipped with FID detector and Headspace G1888.
GC1 and GC2 were monitored with Empower-3 software (Waters Corporation, Milford, MA, USA). Microsoft Excel 2007 was used for analysis of validation results.

Method development and Optimization of Chromatographic conditions

The main goal of method development was to achieve separation of Methanol, Ethanol, Diethyl ether, Methyl acetate, Dichloromethane, Ethyl acetate, Tetrahydrofuran, Cyclohexane, Isopropyl acetate, Methyl isobutyl ketone, Toluene, Chlorobenzene as residual solvents were determined in Dapagliflozin Amorphous. An understanding of the nature of the various residual solvents present in API is the foremost prerequisite for successful method development in HSGC. Following were the stepwise strategies for the method development in our case.

Column selection

The primary goal of column selection was to resolve total 12 residual solvents from each other, which were used during the synthesis of Dapagliflozin Amorphous. As part of method development screened various columns, namely AT-1, AT-5, and DB-WAX were employed but no adequate separation was found with above columns. After careful screening of columns, it was observed that DB-624 column provides better resolution between all 12 solvents and it showed good system suitability parameters.

Selection of Ramping rate

Selecting appropriate ramping to get good separation between the solvents also essential. Three ramping rate were tried at 3°C/min, 8°C/min and 20°C/min. At 3°C/min the retention time was very high and runtime is long, poor separation was observed at 20°C/min. Finally, 8°C/min was optimized.

Flow rate

As the flow rate increase, the viscosity of carrier gas decreased and velocity increased. Check the Flow rate from 4 psi to 12 psi. Flow rate 7.0 psi was selected as finalized flow rate.

Selection of diluent

Diluent selection study was conducted for the headspace analysis. As residual solvents are always in the very low level it was preferred to use the headspace analysis compare to liquid injection mode of analysis. Four diluents had been tried N-methyl-2-pyrrolidone, Dimethyl formamide, Dimethyl sulphoxide and Dimethyl imidazolidine. Chloro benzene and Dimethyl formamide are close to each other. Dimethyl sulphoxide and Dimethyl imidazolidine give the interference at the solvents peaks. N-methyl-2-pyrrolidone was finalized as diluent because of no interference at the solvents peaks and sample was highly soluble in N-methyl-2-pyrrolidone.

Head space method optimization

The headspace method was optimized in such a way that maximum amount of solvents present in the sample get evaporated for the detection. For this the standard and sample vials were heated at 70 to 110°C for 5-20 min with constant shaking. A combination of sample vial heating at 90°C with 10 min shaking was found to be suitable for getting a good response.

Table 1: Optimized chromatographic conditions

Column	DB-624 60 meters ×0.53 mm I.D, 3.0µm
Flow rate	7.0 psi (Constant pressure)
Carrier gas	Nitrogen
Inlet Temperature	140°C
Injection mode	Split (1:5)
Detector	Flame Ionization Detector
Detector Temperature	250°C
Hydrogen flow	40 mL/min
Air flow	400 mL/min
Make up flow	25 mL/min
Injection volume	1 µl
Run time	45 minutes

Table 2: Column oven temperature programme

Rate (°C/min)	Temperature (°C)	Hold Time (min)
	40	10
8	190	10
50	240	5

Table 3: Optimized Headspace conditions

Oven temperature	90°C
Loop temperature	95°C
Transfer line temperature	100°C
GC cycle time	55 min
Vial equilibration time	10 min
Vial pressurization time	0.5 min
Loop fill time	0.5 min
Loop equilibrium time	0.5 min
Injection time	1.0 min
Vial shake	High

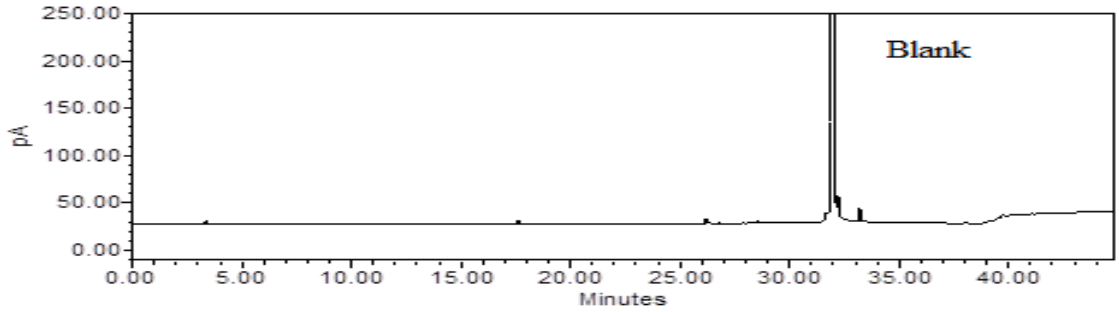

Figure 2: Typical Blank Chromatogram of Dapagliflozin Amorphous

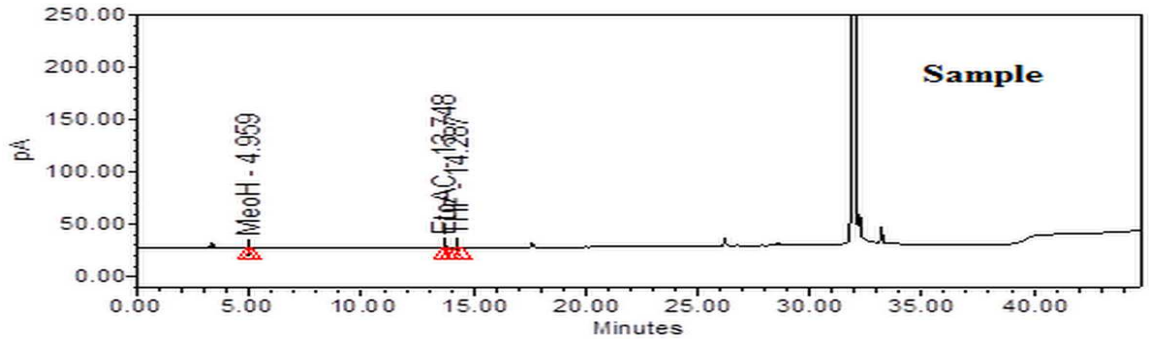

Figure 3: Typical Chromatogram of Dapagliflozin Amorphous sample

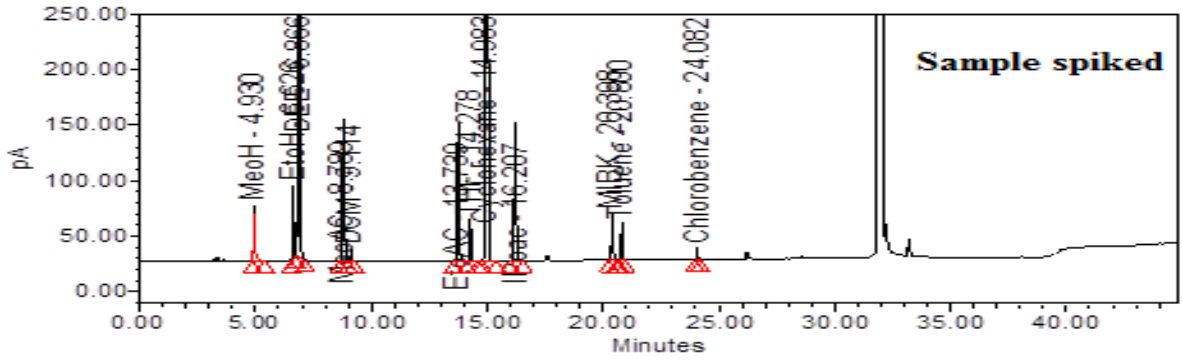

Figure 4: Typical Chromatogram of Spiked Dapagliflozin Amorphous sample

Sample solution preparation

A quantity (200 mg) of Dapagliflozin Amorphous sample was accurately weighed in an Agilent 20 mL HS sample vial and N-methyl-2-pyrrolidone (10 mL) was added. The vial was immediately capped and sealed with a Teflon-lined septum and aluminum crimp cap. The capped vial was placed in the oven of the HS sampler.

Standard solution preparation

A stock standard solution was prepared as follows. 76 µL of Methanol, 127µL of Ethanol, 140µL of Diethyl ether, 108µLof Methyl acetate, 9µL of Dichloromethane, 111 µL of Ethyl acetate, 16 µL of Tetrahydrofuran, 100 µL of Cyclohexane, 115 µL Isopropyl acetate, 56 µL Methyl isobutyl ketone, 21 µL of Toluene and 6.5 µL of Chlorobenzene into a 10 mL of volumetric flask containing about 5mL of N-methyl-2-pyrrolidone, mix and dilute to volume with the N-methyl-2-pyrrolidone. Transfer 1.0 mL of above stock solution into 100 mL volumetric flask containing small quantity of N-methyl-2-pyrrolidone, mix and dilute to volume with the N-methyl-2-pyrrolidone. Transfer 10 mL of above standard solution into a 20mL Headspace vial. The vial was immediately capped and sealed as mentioned above. System suitability results shown in Table 4.

Table 4: Result of System suitability

	Methanol	Ethanol	Diethyl ether	Methyl acetate	Dichloro methane	Ethyl acetate	Tetrahydro furan	Cyclo hexane	Isopropyl acetate	Methyl isobutyl ketone	Toluene	Chloro benzene
%RSD	1.9	2.4	0.7	1.7	2.0	2.0	1.9	1.1	2.0	2.5	2.4	2.7
Resolution	---	14.5	1.8	12.3	2.0	29.5	3.5	4.2	7.3	30.2	3.3	28.8

Method validation

The method has been validated as per ICH guidelines Q2 (R1). The method was validated for the following parameters: System suitability, Limit of quantitation (LOQ) and Limit of detection (LOD), Precision, Linearity, Accuracy, Robustness, Ruggedness and Solution stability.

Precision

The repeatability of the method was verified by injecting six individual preparations. Dapagliflozin was spiked with 12 solvents with specification limit and the % RSD was calculated for 12 solvents. The Intermediate precision (Ruggedness) of the method was also determined by repeating the same experiment on different days by different

analysts using different equipment. The % RSD for the 12 solvents was found to be less than 3 % in all the studies. The results confirmed the high precision of the method was shown in Table 5.

Table 5: Results of precision

	Methanol	Ethanol	Diethyl ether	Methyl acetate	Dichloro methane	Ethyl acetate	Tetrahydro furan	Cyclo hexane	Isopropyl acetate	Methyl isobutyl ketone	Toluene	Chloro benzene
%RSD at LOQ	2.9	2.0	1.0	1.2	1.0	0.8	1.7	1.4	0.9	2.3	2.7	2.2
%RSD at 100%	1.3	2.3	0.7	1.1	1.7	1.3	1.7	0.9	1.6	3.0	2.1	2.7
%RSD at 150%	1.6	2.8	0.2	1.1	1.9	1.5	1.6	0.5	1.8	2.6	2.3	1.9
%RSD at Ruggedness	0.9	1.9	0.8	1.4	2.1	1.9	1.1	0.9	2.9	1.9	1.9	2.8

Accuracy

Recovery experiments were conducted to determine the accuracy of the method for the quantification of the 12 solvents in Dapagliflozin. The study was conducted by spiking to the test sample (20 mg mL^{-1}) with known amount of 12 solvents at LOQ, 50, 100 and 150% in triplicate. Individual and average recoveries of three preparations and at four concentrations for 12 solvents were within 100±5% was shown in Table 6.

Table 6: Results of Accuracy

	Methanol	Ethanol	Diethyl ether	Methyl acetate	Dichloro methane	Ethyl acetate	Tetrahydro furan	Cyclo hexane	Isopropyl acetate	Methyl isobutyl ketone	Toluene	Chloro benzene
%Recovery at LOQ	97.9	101.4	101.0	97.1	102.0	103.0	102.4	101.7	102.6	102.3	102.0	101.6
%Recovery at 50%	98.0	99.2	98.7	98.0	97.6	97.3	98.6	98.8	98.4	98.0	98.3	99.4
%Recovery at 100%	98.5	100.5	100.1	100.0	100.8	100.3	99.1	100.4	100.7	101.2	101.6	101.2
%Recovery at 150%	99.9	100.4	97.5	98.1	99.9	98.5	98.6	99.2	99.9	99.3	99.8	99.1

Limit of detection and limit of quantification

The limit of detection (LOD) and limit of quantification (LOQ) for 12 solvent in Dapagliflozin Amorphous were estimated at signal-to-noise ratios of 3:1 and 10:1, respectively, by injecting a series of diluted solutions with known concentrations. A precision study was also conducted at the LOQ level by injecting six individual preparations of 12 solvent in Dapagliflozin Amorphous and calculating the % relative standard deviation (RSD) of the 12 solvents areas. The accuracy at LOQ level was evaluated in triplicate for the Dapagliflozin Amorphous by spiking the 12 solvents at the estimated LOQ level to the test solution. The limit of detection (LOD) and limit of quantification (LOQ) for 12 solvent were listed in Table 7.

Table 7: Result of LOD and LOQ

	Limit of Detection (LOD) and Limit of Quantification(LOQ) with respect to test concentration in PPM											
	Methanol	**Ethanol**	**Diethyl ether**	**Methyl acetate**	**Dichloro methane**	**Ethyl acetate**	**Tetrahydro furan**	**Cyclo hexane**	**Isopropyl acetate**	**Methyl isobutyl ketone**	**Toluene**	**Chloro benzene**
LOD in PPM	14	22	2	13	34	9	5	2	9	14	8	25
LOD in PPM	50	77	5	31	143	32	18	6	32	47	27	87

Linearity

Linearity solutions for the 12 solvents were prepared by diluting individual solvent stock solutions to the required concentrations. The solutions were prepared at different concentration levels from the limit of quantification (LOQ) to 150%. The result of linearity table was shown in Table 8.

Table 8: Linearity

Linearity	Methanol	Ethanol	Diethyl ether	Methyl acetate	Dichloro methane	Ethyl acetate	Tetra hydro furan	Cyclo hexane	Isopropyl acetate	Methyl isobutyl ketone	Toluene	Chloro benzene
Slope	3.26	2.74	42.47	7.35	1.39	6.41	11.50	41.70	6.26	3.62	5.75	1.64
y-intercept	4.51	-4.11	94.85	10.33	0.68	6.25	1.93	50.91	4.53	-0.17	0.31	-0.03
Y-intercept at 100% conc.	2.3%	-1.5%	2.2%	1.4%	4.0%	1.0%	1.2%	1.5%	0.7%	-0.1%	0.3%	-0.3%
Correlation coefficient	0.9960	0.9959	0.9970	0.9968	0.9949	0.9968	0.9967	0.9972	0.9968	0.9956	0.9958	0.9936

Table 9: Robustness- Variability from column oven initial Temperature

Solvent name	% RSD			Resolution		
	35°C	40°C	45°C	35°C	40°C	45°C
Methanol	0.9	1.9	1.4	NA		
Ethanol	1	2.4	1.9	17.1	14.5	12.8
Diethyl ether	0.5	0.7	0.5	1.6	1.8	1.9
Methyl acetate	0.5	1.7	0.9	13.2	12.3	11.4
Dichloromethane	0.8	2	1.7	1.9	2	2.1
Ethyl acetate	0.6	2	1.2	29.3	29.5	28.3
Tetrahydrofuran	0.6	1.9	1.1	3.3	3.5	3.6
Cyclohexane	0.3	1.1	0.6	4.1	4.2	4.2
Isopropyl acetate	0.6	2	1.3	7.6	7.3	6.9
Methyl isobutyl ketone	1	2.5	2.5	30.1	30.2	30.1
Toluene	1	2.4	2.3	3.3	3.3	3.3
Chlorobenzene	2	3.1	3.1	28.6	28.8	29

Table 10: Robustness- Variability from carrier gas flow rate

Solvent name	%RSD			Resolution		
	6.3 psi	7.0 psi	7.7 psi	6.3 psi	7.0 psi	7.7 psi
Methanol	1.4	1.9	1.1	NA		
Ethanol	1.9	2.4	1.4	14.7	14.5	14.4
Diethyl ether	0.5	0.7	0.6	1.8	1.8	1.7
Methyl acetate	1	1.7	0.6	12.7	12.3	11.9
Dichloromethane	1.6	2	0.9	2.1	2	1.9
Ethyl acetate	1.2	2	0.8	28.3	29.5	30.5
Tetrahydrofuran	1.1	1.9	0.6	3.7	3.5	3.3
Cyclohexane	0.7	1.1	0.5	4.3	4.2	4.1
Isopropyl acetate	1.4	2	0.9	7	7.3	7.5
Methyl isobutyl ketone	2.4	2.5	2	30.3	30.2	29.9
Toluene	2.4	2.4	1.9	3.5	3.3	3.1
Chlorobenzene	2.8	3.1	2.1	28.2	28.8	29.1

Table 11: Robustness- Variability vial equilibration time

Solvent name	%RSD			Resolution		
	5 min	10 min	15 min	5 min	10 min	15 min
Methanol	1.9	1.2	1.7			
Ethanol	2.4	1.1	1.6	14.5	14.6	15
Diethyl ether	0.7	0.4	0.3	1.8	1.7	1.8
Methyl acetate	1.7	1	0.8	12.3	12.2	12.3
Dichloromethane	2	1.5	1.2	2	2.1	1.9
Ethyl acetate	2	2.1	1	29.5	29.4	29.4
Tetrahydrofuran	1.9	1.2	1	3.5	3.3	3.4
Cyclohexane	1.1	1.1	0.4	4.2	4.2	4.3
Isopropyl acetate	2	0.9	1.1	7.3	7	7.1
Methyl isobutyl ketone	2.5	1.1	1.8	30.2	30	30.1
Toluene	2.4	2	1.7	3.3	3.2	3.3
Chlorobenzene	3.1	3.4	2.6	28.8	28.5	28.6

Table 12: Robustness- Variability from vial oven temperature

Solvent name	%RSD			Resolution		
	85°C	90°C	95°C	85°C	90°C	95°C
Methanol	2.2	1.9	0.8	NA		
Ethanol	3.4	2.4	1.4	14.5	14.5	14.7
Diethyl ether	0.6	0.7	0.2	1.8	1.8	1.8
Methyl acetate	1.3	1.7	0.6	12.3	12.3	12.2
Dichloromethane	2.2	2	1	2	2	2
Ethyl acetate	1.9	2	0.8	29.6	29.5	29.6
Tetrahydrofuran	1.7	1.9	0.8	3.5	3.5	3.5
Cyclohexane	0.9	1.1	0.4	4.2	4.2	4.2
Isopropyl acetate	2.2	2	0.9	7.3	7.3	7.3
Methyl isobutyl ketone	4.1	2.5	1.7	30.2	30.2	30.2
Toluene	3.8	2.4	1.6	3.3	3.3	3.3
Chlorobenzene	3.7	3.1	2	28.7	28.8	28.8

Robustness

Robustness was studied by altering chromatographic conditions like flow rate, column oven temperature, Headspace oven temperature and Headspace vial equilibration time. The method was more robust within the normal operating range, i.e., flow rate, 7.0 ± 0.07 psi and column oven temperature, 40±5 °C, Headspace oven temperature 90±5 °C and Headspace vial equilibration time 10±5 min. demonstrating the robustness of the method shown in Table 9 to Table 11.

Solution stability

The results from solution stability experiments confirmed that sample solution and Dapagliflozin Amorphous spiked test solutions were stable up to 24 hours at Room temperature.

RESULTS AND DISCUSSION

Based on the results, the successful separation of the 12 solvents from each other. All the validated parameters were found to be within limits. System suitability for 6 injections % RSD was found to be NMT 3.8%. Precision at LOQ, 100% and 150% were found to be NMT 2.9%, Accuracy at LOQ, 50% 100% and 150% were found to be 97.1% to

103.0%. Linearity was performed from LOQ to 150% and graph obtained was linear showing correlation coefficient > 0.9936.

CONCLUSION

A simple, sensitive and cost effective Static headspace gas chromatographic method was developed and validated for the quantitative determination of the 12 Residual solvents in Dapagliflozin Amorphous. All the 12 residual solvents were well separated from each other, indicating that the developed GC method was specific. The method validation data showed satisfactory results for all tested method parameters. This simple GC method is precise, accurate, linear and rugged. Hence, it is proved that developed method can be used for routine testing in quality control laboratories for estimation of 12 residual solvents in Dapagliflozin Amorphous. The method is user-friendly and robust to operate.

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