Original Article

HS-GC-FID Method Development and Validation for Quantification of Residual Solvents in Favipiravir

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Abstract

Residual solvents testing is an important part of quality control in pharmaceuticals and also essential part of reference material certification. In Pharmaceutical industry, in order to manufacture Favipiravir active pharmaceutical ingredient (API) the organic solvents like methanol, dichloromethane, acetonitrile and toluene is often used. According to ICH i.e., International Conference on Harmonization guidelines, the development, validation in order to identify the residual solvents in Favipiravir has been done using Head space gas chromatography having flame ionization detector. In this investigation, Agilent GC 7697A-FID detector and open labs EZ chrome software with auto injector was used for analysis. DB-624 column, (30m×0.53mm×1.4 µm coating thickness) was opted for separation where carrier gas was nitrogen and were performed at different temperature gradient programmed. At range of 50-150% of standard concentrations for solvents were noted for linearity to estimate the amount by the proposed methods resulting to be linear. Method accuracy has been estimated at three different level by recovery studies percentage conducted at the absence of interference from the diluent and API. Method was proved to be precise as recommended by the repeatability and also showing %RSD less than 10 for solvents mentioned in the method. All statistical data shown the validity of the method. This proposed method is useful in the routine analysis of estimating residual solvents in Favipiravir API. All solvents' baseline separation along with API was within 22.8 minutes. In the method ICH parameters were used to validate the developed method. The parameters for method validation are namely specificity, limit of detection, limit of quantification, Accuracy, precision, linearity, ruggedness, robustness. The developed method was simple, sensitive, rugged, reliable, reproducible and effectively applied for quantifying specified limit levels of residual solvents in Favipiravir API and marketed formulation.

KEYWORDS: Residual solvents, Gas chromatography, Head space, Favipiravir.

INTRODUCTION

In the pharmaceutical industry, organic solvents are often opted for the manufacture of active pharmaceutical ingredients, excipients, and finished pharmaceutical products. These impurities in the final product are unwanted due to several reasons such as their potential to harm the consumers, their potential impact on drug crystals' quality, and the potential unpleasantness of their odour or taste to end users. Manufacturing methods and techniques (often at higher temperature and/or lowered pressure) are used to get rid of them [1-5]. Certain solvents, while in negligible amounts, persist even after such procedures. Common names for these trace amounts of organic solvents are organic volatile impurities (OVIs) and residual solvents (RS). Among the analytical pharmaceutical jobs, identifying residual solvents in drug ingredients, excipients, or medicinal formulations is more challenging and time consuming. In addition, because polar residual solvents are notoriously difficult to extract from water or polar solvents[2], their detection in pharmaceutical formulations remains a formidable analytical issue. Quality monitoring of the various chemicals are used in API synthesis is essential for maintaining GMP (good manufacturing practice) standards. Therefore, before a GMP synthesis can begin, it is necessary to monitor and evaluate the purity of any organic residual solvents. A liquid or a solid sample can be placed in closed vessel of Headspace gas chromatography (HS-GC) till an equilibrium is reached between sample and the gas of the volatile

components and the volume of gas should be above, also known as the "headspace." Headspace is collected and injected onto a gas chromatography (GC) column for examination. For active compounds and formulations that are water-soluble, regulatory agencies and pharmacopoeias recommend using headspace gas chromatography for residual solvent testing. Specification limits for residual solvents range from a few parts per million (ppm) to hundreds of parts per million (ppm), depending on the toxicity of the solvents. In order to quantify any remaining solvents, HS-GC analysis has developed into a reliable method. Evaporation of liquid (or solid) analytes before injection into a GC system reduced contamination and wear on the GC column. Automating the equilibrium and injection technique has cut down analysis time and increased injection repeatability. [6-9]

The antiviral medication favipiravir (FVPR) is effective against influenza A, B, and C viruses. 1 In the International Union of Pure and Applied Chemistry (IUPAC), FVPR is known as 6-fluoro-3-hydroxy-2- pyrazine carboxamide (chemical formula: C5H4FN3O2) (Figure 1). This compound is a derivative of pyrazine carboxamide. To melt, it takes somewhere between 187 and 193 degrees Celsius. Aside from very dilute solutions, it is noted to be soluble in organic solvents such as ethanol, DMSO, and DMF while is insoluble in the water. The viral RNA polymerase is inhibited when the prodrug favipiravir ribofuranosyl-5'-triphosphate (FVPR) is ribosylated and phosphorylated inside cells to become the active favipiravir ribofuranosyl -5'-triphosphate (favipiravir RTP). [10-13] Antiviral activity of the FVPR was found against alpha-, filo-, bunya-, arena-, flavi-, and noroviruses, along with influenza, where it plays a crucial role in combating. COVID-19.

From the literature, many analytical techniques are accessible in Favipiravir determination like RP HPLC and UV,LC-MS/MS, NMR/LC-MS, visible spectroscopy, UPLC-MS/MS, voltammetry but method was not there for residual solvents in Favipiravir quantification, HS-GC technique for residual solvents' analysis in Favipiravir APIis the present research work dealt on, to develop a new method and validation to identify and quantify residual solvents in Favipiravir API and also in Dosage form. Standard solvents and the ICH standard residual solvents limit were used to make comparisons to the residual solvents. [14-25]

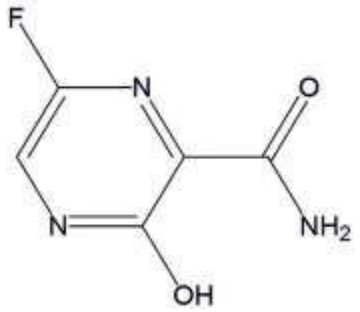

Figure 1: Structure of Favipiravir

MATERIALS AND METHODS

Reagents and chemicals Used

Dimethyl sulfoxide, Dichloromethane, Methanol, acetonitrile, and toluene were purchased from the following chemical vendors (Sigma- Aldrich, Mumbai India), Chandra Labs Hyderabad provided the Favipiravir API.

Apparatus and Instrumentation

A flame ionization detector containing gas chromatograph (Agilent Infinity 7697A), In order to load the sample, a headspace sampler of Agilent technologies 7694 E model is used, sonicator and autopipette i.e., of 100-1000 µl from Eppendorf containing analytical balance were opted.

Chromatographic conditions

Gas chromatographic analysis was carried out with the carrier gas with 30 min run time, Nitrogen, DB-624 with dimensions 30 m x 0.53 mm I.D x 1.4µm film thickness capillary column, Stationary phase- poly ethylene glycol

purchased from supelco was opted. The GC method for separation of residual solvents used a flow rate of 1.5 mL/min. Injection port temperature 180°C, and detector temperature were maintained at 220°C respectively. Split Ratio: 10:1.

Oven Programme

Oven temperature was maintained at 40°C for 4 minutes, then increased to 90°C at a rate of 15°C per minute and maintain at that Temperature for 2minutes, then increased to 200°C at a rate of 20°C per minute and maintain at that Temperature for 8 minutes, run time is 22.8 min.

Head Space Conditions

Vial Temp: 100°C, Loop Temp: 105°C, Transfer line Temp: 120°C, GC cycle Time: 42 min, Vial Equilibration Time: 30 min, Pressurization Time is 0.5 min, Loop Fill Time: 0.2 min, Loop equilibration Time: 0.05 min, Shake: 1 (Low), Injection Time: 1.0 min, Withdrawal Time: 0.5 min, Thermostat Time: 20 min.

Solution preparations

Preparation of Blank

To a headspace vial holding 200 mg of sodium chloride, transfer 5.0 ml of diluent Dimethyl Sulfoxide (DMSO) and immediately seal the Head space vial. Interfering peaks in the blank at the retention time of the analytes were not detected.

Standard stock solution-1

Weighed accurately about 60 milligrams of dichloromethane, 300 milligrams of methanol, 41 milligrams of acetonitrile,and 89 milligrams of toluene and taken into the 200 ml volumetric flask containing 50 ml of DMSO, shake well and with the diluent was made to the mark.

Standard stock solution-2

Taken 5 ml from standard stock solution-I into the 25 mL volumetric flask having 20 ml DMSO and with the diluent was made to the mark. Transferred 5 ml of the standard stock solution -2 into the head space vial and add 0.2 grams of sodium chloride, and immediately the vial was sealed using a rubber septa and metallic ring closure, further heated the vial at 90°C.

Test Sample Preparation

Weigh accurately about 1000 mg of test sample (Favipiravir API) and 0.2 gm sodium chloride transferred into headspace vial and about 5 ml diluent was taken accurately to dissolve, crimp the vial and sealed immediately, vortex it for 5min. In a batch sample of Favipiravir, solvents were not detected.

RESULTS AND DISCUSSION

Method Development

Method Development was conducted through a series of trials with various diluents by changing different chromatographic conditions, by considering the physicochemical properties of analyte that was optimized in final trial by using DMSO as diluent. As stated in limits of ICH, all the parameters were optimized by injecting blank, sample and standard solution into the instruments. The Retention time of blank (DMSO) was 22.142 minutes as shown in figure 2, Retention times of standard were observed as 7.733 for Dichloromethane, 9.719 for methanol, 10.209 for Acetonitrile, 11.719 for Toluene, standard chromatograms were shown in figure 3, sample chromatogram were shown in figure 4.

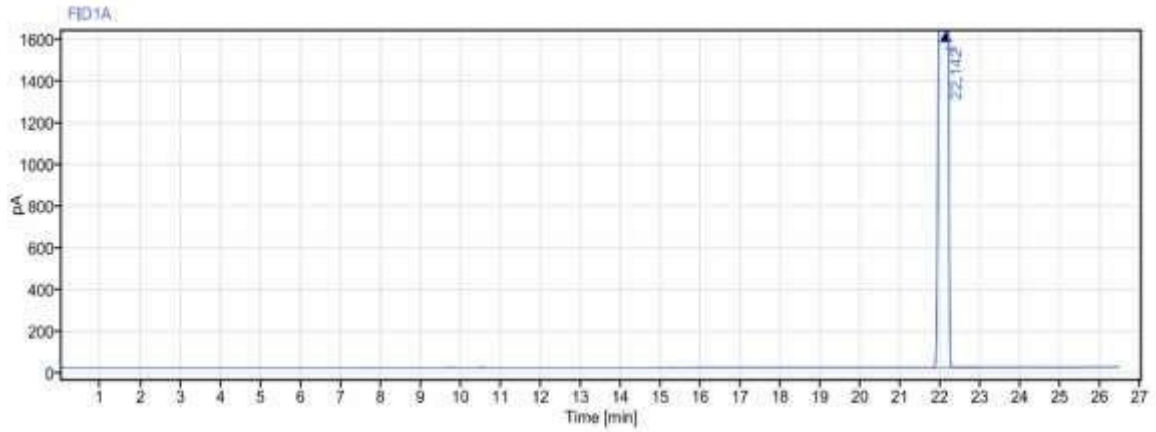

Figure 2: Chromatogram of blank

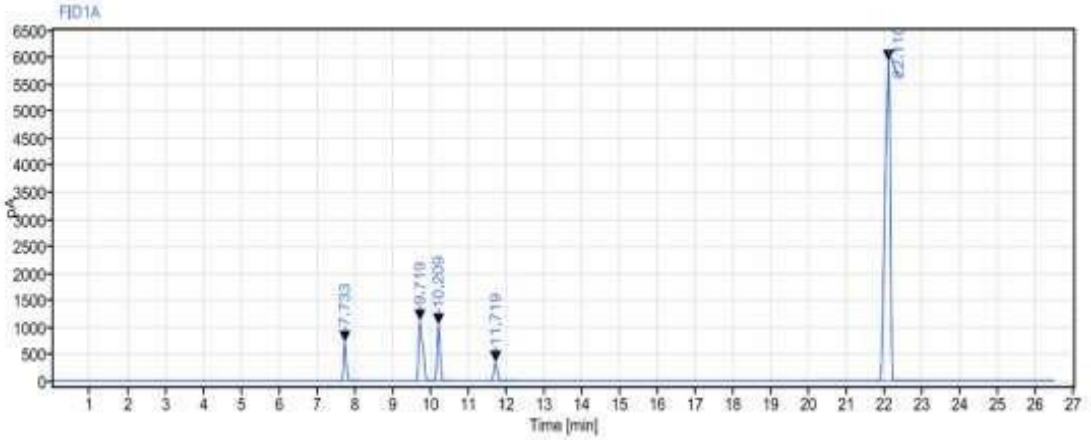

Figure 3: Chromatogram of standard mixture

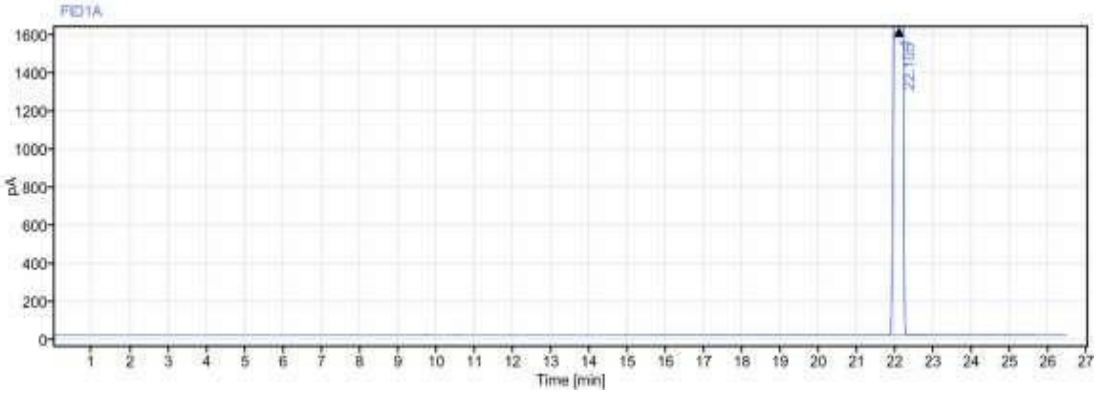

Figure 4: chromatogram of sample

Analytical Method validation Parameters

The analytical validation method of residual solvents was performed by analyzing linearity and range, specificity, Limit of Detection (LOD), Limit of Quantification (LOQ), LOQ Accuracy, LOQ Precision, accuracy, method precision, system suitability, Intermediate Precision (ruggedness), The Robustness and Batch Analysis of the residual solvents were done as mentioned in ICH guidelines. [26-36]

Specificity

By injecting blank, individual solvents, mixed standard solution of Dichloromethane, Methanol, Acetonitrile, Toluene and sample were examined for interference through chromatograph. The Retention times in Spiked injection was 7.74 min, 9.725 min, 10.215 min, 11.725 min shown in standard chromatogram of specificity of Fig.5. It is noted from the above data that solvent peaks should not interfere with each other, and no interference of each solvent in blank should be seen. Then, the method is said to be specific.

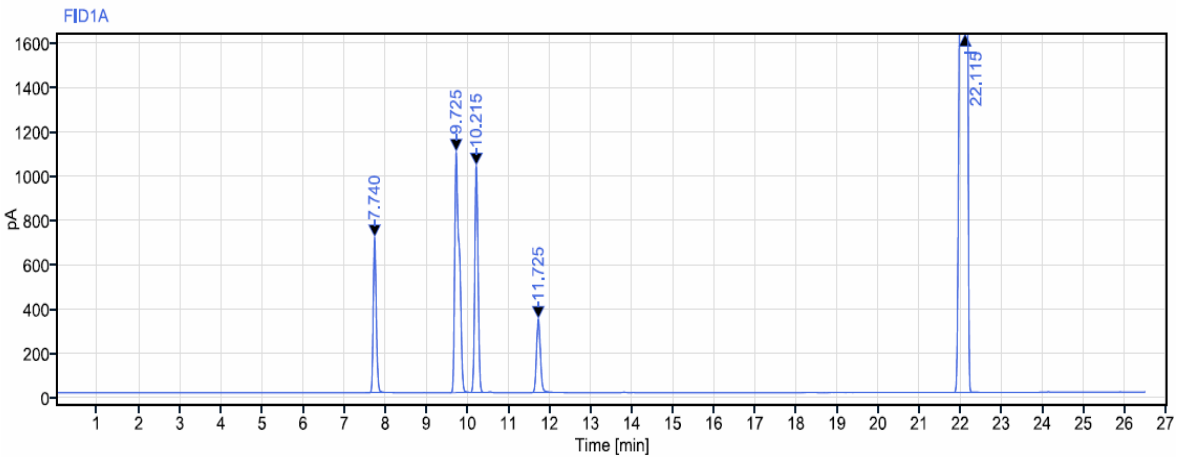

Figure 5: Chromatogram of Specificity

Linearity and Range

The determination of linearity method was done through injecting each residual solvents through specification limit range that is 50%, 80%, 100%, 120%, 150% concentration level, injected into the chromatographic system and evaluated the linearity of detector response. By plotting the corresponding concentration on the x-axis and the average peak areas of the solvents on the Y-axis. From the statistical analysis, correlation co-efficient and regression equations, linearity is known, as shown in Table 1 and Linearity graphs were shown in figure 6. A minimum of five concentration level ranges from 50%-150%. The residual solvents' correlation co-efficient values were noted to be higher than 0.998 and the calibration curves were linear within the range.

Table 1: Correlation Coefficient of Linearity Data and Range

Solvent	Correlation coefficient	Regression Equation	Concentration (%) Range
Dichloromethane	$R^2 = 0.9998$	y = 23.33x - 1118.4	50-150%
Methanol	$R^2 = 0.9994$	y = 39.105x - 3415.7	50-150%
ACN	$R^2 = 0.9996$	y = 177.33x - 1772.2	50-150%
Toluene	$R^2 = 0.9993$	y = 31.125x - 666.58	50-150%

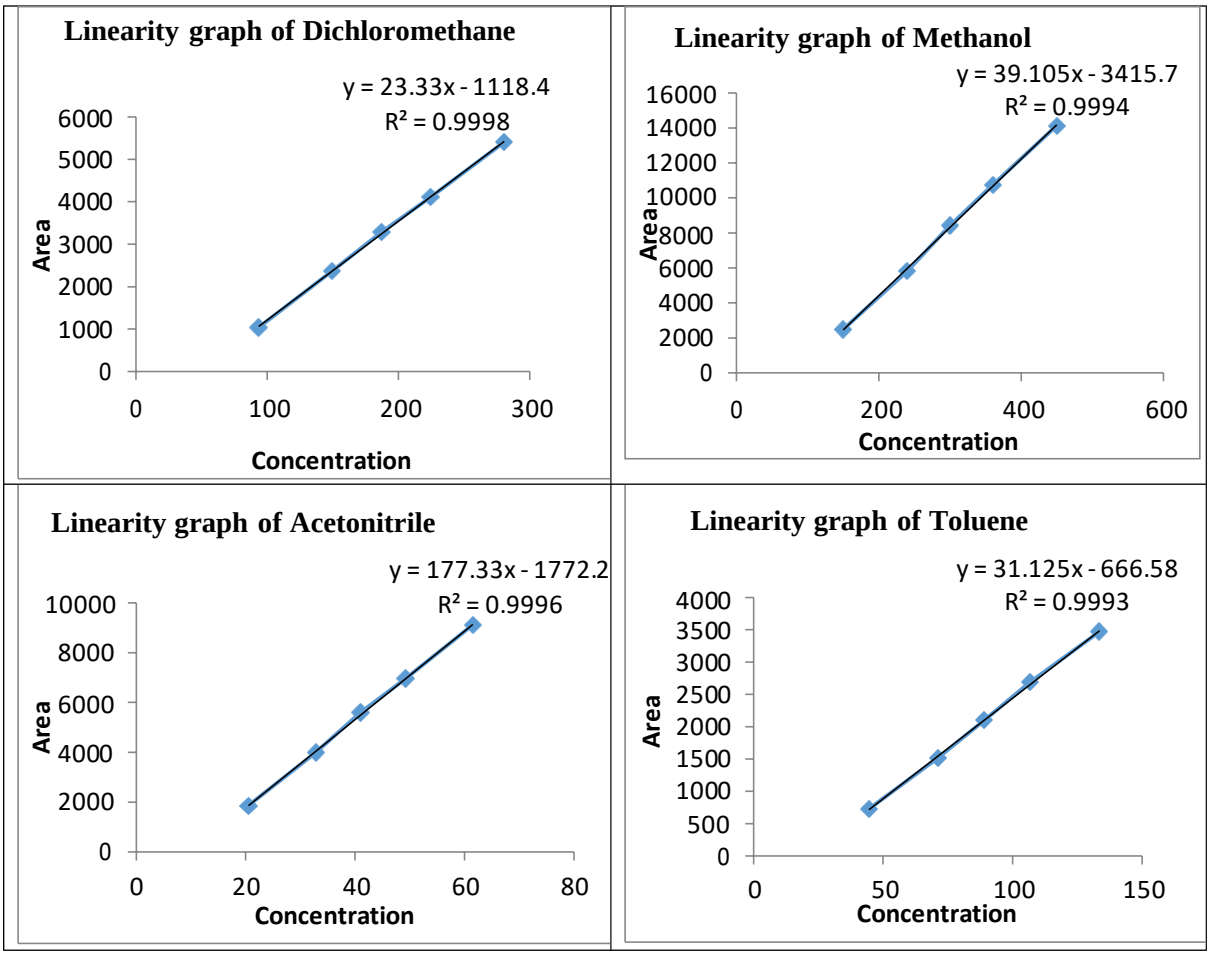

Figure 6: Linearity Graphs of Solvents

Limit of Detection (LOD) and Quantitation (LOQ)

Instrumental and statistical approaches were used to determine the LOD and LOQ. The limits of detection (LOD) and quantification (LOQ) are identified by the detector for the instrumental approach. When calculating the LODs for left over solvents in Favipiravir API, a signal-to-noise ratio of 3:1 was used. With a signal-to-noise ratio of 10:1, the LOQs of left-over solvents were calculated. Depending on signal to noise ratio, the ratio of all solvents for LOQ and LOD met the acceptance criteria and LOQ and LOD concentrations are established. Thereof, the LOQ and LOD obtained has met the requirements. The values are as displayed in table 2.

Table 2: The values for the LOD and LOQ

Name of the Parameter	Dichloromethane S/N Ratio	Methanol S/N Ratio	Acetonitrile S/N Ratio	Toluene S/N Ratio
Limit of Detection	4.02	10.23	3.30	2.22
Limit of Quantification	12.19	31.00	10.00	6.7

LOQ Accuracy

The accuracy has been determined at limit of quantification level. Prepared six sample solutions having Dichloromethane,Methanol, Acetonitrile, Toluene at about limit of quantification level on test sample and injected into system. Calculated the % recovery of Dichloromethane, Methanol, Acetonitrile, Toluene from six preparations at LOQ level solution. The % RSD of solvents i.e., Dichloromethane, Methanol, Acetonitrile, Toluene at LOQ level should not be more than 10%, and recovery values was in between 70-130%. LOQ Accuracy was met the requirements.The values are depicted in Table 3.

LOQ Precision

Determined the Precision at limit of quantification level, Injected LOQ solution six times having Dichloromethane, Methanol, Acetonitrile, Toluene at limit of quantification level into system. Calculated the % RSD for area of Dichloromethane, Methanol, Acetonitrile, Toluene from six injections were below the acceptance criteria. The values are depicted in Table 3.

Table 3: % RSD values of LOQ Precision and LOQ Accuracy

Solvents	%RSD LOQ Accuracy	%RSD LOQ Precision	Acceptance Criteria	%Recovery	Acceptance Criteria
Dichloromethane	4.1	1.4	NMT* 10%	96.9	70-130%
Methanol	2.08	1.7	NMT* 10%	92.6	70-130%
Acetonitrile	2.36	1.8	NMT* 10%	102.3	70-130%
Toluene	1.42	1.3	NMT* 10%	103.3	70-130%

* Not more than

Accuracy (recovery)

The method accuracy has been determined using recovery studies. Recovery analyses confirmed the reliability of the procedure. The Residual solvents were added to the API (pre-analyzed sample) at the concentrations of 50%, 100%, and 150%. For three consecutive times the recovery studies were performed and the percentage recovery and mean percentage recovery was taken to calculate the drug. The table 4 displays the results of three separate recovery levels, in which the proportion of recovered drugs and their mean recovery rate were determined. Recoveries of 50%, 100%, and 150% were performed by mixing known amounts of standard drug solution with the already-analyzed sample solution to verify the method's Accuracy. In accordance with the ICH standard, the percent recovery of residual solvents was determined to be within the allowed range (70% - 130%), and the percent RSD for area was below 10 percent for all the solvents. The outcomes show that the strategy achieves a satisfactory degree of accuracy.

Table 4: Recovery results for Solvents

S. No	Name of the Parameter	Dichloromethane	Methanol	Toluene	Acetonitrile
01	50% Recovery	100.1	101.3	101.7	101.8
02	100% Recovery	100.6	100.7	100.6	100.3
03	150% Recovery	101.3	101.8	101.4	101.9
Average		103.4	100.7	101.3	101.2
%RSD		2.3	0.60	0.55	0.57

Method Precision

In order to gauge the method Precision, one blank injection and same sample at 100% concentration level six replicate injections of standard solution were performed. Each solvent's percentage relative standard deviation (RSD) was determined to be less than 10 percent, as depicted in Table 5. Hence the method was deemed suitable and precise.

Table 5: % Relative standard deviation results for Method Precision

Solvent Name	Dichloromethane		Methanol		Acetonitrile		Toluene	
S. No	Rt	Area	Rt	Area	Rt	Area	Rt	Area
1	7.74	3591.98	9.726	8394.03	10.216	5683.83	11.726	2220.16
2	7.739	3646.42	9.726	8486.67	10.217	5766.41	11.727	2242.52
3	7.739	3550.7	9.724	8262.46	10.215	5615.66	11.726	2194.41
4	7.741	3573.93	9.727	8264.4	10.217	5639.54	11.728	2220.97

5	7.746	3578.11	9.732	8319.43	10.224	5659.01	11.735	2206.27
6	7.75	3528.37	9.737	8160.87	10.228	5570.79	11.741	2191.7
Average	7.742	3578.25	9.7286	8314.64	10.2195	5655.87	11.7305	2212.67
St dev	0.005	40.253	0.005	113.853	0.005	66.532	0.006	19.132
%RSD	0.1	1.1	0.1	1.4	0.1	1.2	0.1	0.9

System suitability

The system suitability has been prepared, injected six replicates of standard solution into the chromatographic system and evaluated system suitability parameters and system precision. Parameters between the peaks of System suitability like Retention time, peak area, resolution has been evaluated. Calculated the percentage relative standard deviation of standard peak area response of all solvents from six replicate injections, so this parameter proved that instrument functioning correctly and system is suitable for validating the method, The percentage of the relative standard deviation has to be below 10%, values were depicted in Table 6.

Table 6: System suitability for Solvents

Solvent Name	Dichloromethane		Methanol		Acetonitrile		Toluene	
S. No	Rt	Area	Rt	Area	Rt	Area	Rt	Area
1	7.74	3584.76	9.725	8394.3	10.215	5683.83	11.725	2211.69
2	7.739	3639.06	9.724	8486.66	10.216	5766.42	11.726	2226.51
3	7.739	3543.94	9.723	8262.49	10.215	5615.66	11.725	2187.85
4	7.74	3567.39	9.727	8264.38	10.217	5639.54	11.729	2210.9
5	7.745	3569.93	9.732	8319.35	10.224	5659.15	11.736	2194.92
6	7.749	3523.93	9.737	8160.85	10.228	5570.79	11.741	2187.27
Average	7.742	3571.502	9.728	8314.672	10.21917	5655.898	11.73033	2203.19
St dev	0.004	39.451	0.005	113.891	0.005	66.537	0.007	15.701
%RSD	0.1	1.1	0.1	1.4	0.1	1.2	0.1	0.7

Intermediate Precision (Ruggedness)

Intermediate precision is the degree of repeatability of the test results attained through same sample analysis, and the method's ruggedness has been studied by evaluating the variation from analyst to analyst through assay done by 2 different analysts, on two varied days and two varied instruments by injecting the six individual sample preparations. Calculate relative standard deviation of the standard peak area response of all solvents from six replicate injections should be not more than 10%. The values were displayed in table 7.

Table 7: The ruggedness of Analyst 1 day one and Analyst 2 day two and Instrument 1, 2.

Solvents	Ruggedness % RSD System suitability	Ruggedness % RSD ppm at 100% spiked solution	Acceptance Criteria
Dichloromethane	1.1	0.60	NMT* 10%
Methanol	1.4	1.06	NMT 10%
Acetonitrile	1.2	0.75	NMT 10%
Toluene	0.9	1.47	NMT 10%

*Not more than

Robustness

The method's robustness was demonstrated by making minor changes to optimized parameters, i.e., effect of variation in column initial temperature and flow to demonstrate the robustness, prepare a standard solution and spiked sample solution as per the test procedure. Evaluate system suitability and content of solvents using nominal condition of column oven temperature was 100°C, robustness condition column oven temperature changed to 95°C and 105°C and optimal column flow was 1.5 mL/min is changed to 1 mL/min and 2 mL/min. Cumulative %

RSD for content of Dichloromethane, Methanol, Acetonitrile, and Toluene met the acceptance criteria, that is below 10%. Results are met the requirements in robustness parameters hence method is robust. The values are as depicted in Table 8.

Table 8: Robustness of Flow 1ml/min, 2 ml/min and Vial Temp 95 ^{0}c and 105 ^{0}c.

Solvents	% R.S.D. Flow 1 ml/ min and Vial Temp 95^{0}c	% R.S.D. Flow 2 ml/ min and Vial Temp 105^{0}c	Acceptance Criteria
Dichloromethane	1.1	1.1	NMT* 10%
Methanol	1.4	1.4	NMT 10%
Acetonitrile	1.2	1.2	NMT 10%
Toluene	0.8	0.7	NMT 10%

* Not more than

Batch analysis

Drug sample in pure form was injected to perform batch analysis and marketed formulation into the head space instrument, in batch analysis of Favipiravir for two batches, Residual solvents were not detected.

The chromatogram is shown in figure 4, and results for Favipiravir pure and marketed formulation are shown in table 9.

Table 9: Results from Residual Solvent Testing in Drug Substances in different batches and marketed drug

S. No	Solvents	Pure sample batch 1	Pure sample batch 2	Result
1	Dichloromethane	Not detected	Not detected	Accepted
2	Methanol	Not detected	Not detected	Accepted
3	Acetonitrile	Not detected	Not detected	Accepted
4	Toluene	Not detected	Not detected	Accepted

CONCLUSION

In view of the nature of solvents and nature of stationary phase of column, synthetic process, simplicity, fastness and good selectivity HS-GC technique has been devised and method validation for identifying residual solvents contained in Favipiravir API. For quantifying residual solvents in Favipiravir API HS-GC is opted and marketed formulations and method could be opted for routine sample analysis in industries and laboratories. According to ICH criteria, the established approach is specific, accurate, exact, and robust.

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