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Gas Chromatographic Method for Identification and Quantification of Commonly Used Residual Solvents in Pharmaceuticals Products

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Abstract

Background: Impurities are not expected in the final pharmaceutical products. All impurities should be regulated in both drug substances and drug products in accordance with pharmacopeias and ICH guidelines. Three different types of impurities are generally available in the pharmaceutical's product specification: organic impurities, inorganic impurities, and residual solvents. Residual solvents are organic volatile chemicals used or generated during the manufacturing of drug substances or drug products. **Purpose:** The aim of this study is to develop a cost-effective gas chromatographic method for the identification and quantification of some commonly used solvents—methanol, acetone, isopropyl alcohol (IPA), methylene chloride, ethyl acetate, tetrahydrofuran (THF), benzene, toluene, and pyridine—in pharmaceutical product manufacturing. This method will be able to identify and quantify the multiple solvents within a single gas chromatographic procedure. **Method:** A gas chromatography (GC) equipped with a headspace sampler and a flame ionization detector, and a column DB 624, 30-meter-long × 0.32-millimeter internal diameter, 1,8 μm-thick, Brand-Agilent was used to develop this method. The initial GC oven temperature was 40°C and held for 5 minutes. It was then increase to 80˚C at a rate of 2˚C per minute, followed by a further increase to 225˚C at a rate of 30˚C per minute, with a final hold at 225˚C for 10 minutes. Nitrogen was used as a carrier gas at a flow rate of 1.20 mL per minute. Dimethyl sulfoxide (DMSO) was selected as sample solvent. **Results:** The developed method is precise and specific. The percent RSD for the areas of six replicate injections of this gas chromatographic method was within 10.0 and the recovery result found within 80.0% to 120.0%.

Keywords

Method Development, Gas Chromatography, Compendial Method, GDP, Specificity, Recovery

1. Introduction

Residual solvents are volatile organic compounds employed in the synthesis of complex drug products, including nanomedicines, as well as in the manufacturing of active pharmaceuticals ingredients (APIs), excipients and finished dosage forms [1] [2]. Choosing the right solvent for synthesizing a drug substance or excipient can improve the yield and influence characteristics like crystal form, purity, and solubility. Thus, the solvent can be a crucial component in the synthesis process and might not be entirely eliminated during manufacturing. Since residual solvents offer no therapeutic benefit, they should be removed as much as possible to meet safety-based limits, ingredient and product specifications, good manufacturing practices, and other quality-based requirements [2].

The primary method for analyzing residual solvents is gas chromatography, utilizing various sample introduction techniques, such as static or dynamic headspace analysis, solid phase microextraction, or direct injection of the analyte into the GC [3] [4].

There are several studies available for the detection of residual solvents in pharmaceuticals products by GC [3]-[5]. Most studies cover four to five solvents for identification by a single method. This method covers most solvents commonly used in the pharmaceutical manufacturing. It is possible to identify and quantify nine solvents in a single method in the shortest possible time.

The sample is adsorbed onto the stationary phase of the column and then separated by the carrier gas flowing through the column, based on polarity. The carrier gas will be an inert gas, such as helium or nitrogen with more than 99.99% purity. Liquid samples are vaporized prior to being injected into the carrier stream. Substances that have greater interaction with the stationary phase remain in the column longer and are thus separated from those with less interaction. Therefore, compounds eluted from the column at different times, based on their polarity, are detected by detectors, resulting in an enhanced signal. Different compounds have varying retention times (RT) based on their polarity. The response of GC detectors is proportional to the concentration of the analyte in the sample introduced. Various types of detectors used include flame ionization detectors (FID), thermal conductivity detectors (TCD), electron capture detectors (ECD), nitrogen-phosphorus detectors (NPD), and mass detectors (in both single and triple quadruple modes) [5].

The aim of this study is to develop a cost-effective gas chromatographic method for identification and quantification of some commonly used solvents—methanol, acetone, IPA, methylene chloride, ethyl acetate, THF, benzene, toluene and

pyridine—in pharmaceutical product manufacturing. This method will facilitate the identification and quantification of multiple solvents in a single gas chromatographic procedure. This simple method will help to rapid release of drug substances and products and is easy to maintain good documentation practices (GDP) contemporaneously. All analytical methods should be validated as per pharmacopeia or ICH guidelines before use [6].

2. Materials and Methods

2.1. Materials

The source of chemicals used in this development study from the following suppliers: Methanol (Merck, Germany), Acetone (Merck, Germany), IPA (Merck, Germany), Methylene Chloride (RCI Labscan Ltd., Thailand), Ethyl Acetate (RCI Labscan Ltd., Thailand), THF (Sigma-Aldrich, Germany), Benzene (Daejung Chemicals, Korea), Toluene (Scharlau, Spain), Pyridine (Daejung Chemicals, Korea), and DMSO (Scharlau, Spain). The Fluorometholone API was obtained from NewChem, Italy, and was used to prove the specificity and recovery of the method.

2.2. Method

2.2.1. Instrumentation

A capillary gas chromatography instrument with a flame ionization detector and a headspace sampler was utilized. Model & manufacturer: Shimadzu GC-2010, Japan. Analytical balance: SARTORIOUS CPA224S. Micropipette: 100 to 1000 µL, Eppendorf.

2.2.2. Chromatographic Conditions

Blank solution, standard solution, and sample solutions were injected into chromatographic system and record the chromatogram. The GC conditions and headspace conditions are detailed in Table 1 and Table 2, respectively.

Table 1. Gas Chromatography conditions.

Column	DB 624, 30 meters in length with a 0.32-millimeter internal diameter and a 1.8 µm film thickness, manufactured by Agilent
Oven program	Start at 40˚C and hold for 5 minutes. Increase the temperature to 80˚C at a rate of 2˚C per minute and hold for 0 minutes. Then, raise the temperature to 225˚C at a rate of 30˚C per minute and hold for 10 minutes.
Injector temperature	220˚C
Detector temperature	250˚C
Carrier gas	Nitrogen (N2)
Flow rate	1.20 mL per minute

Continued

Gases for flame ignition	Hydrogen (H2): 40 mL per minute Air flow: 400 mL per minute Makeup flow: 30 mL per minute
Makeup gas	Nitrogen
Injection mode	Split
Split ratio	10:1
GC cycle time	50 minutes
Run time	39.83 minutes

Table 2. Headspace conditions.

Equilibration temperature	85.0˚C
Sample line temperature	140.0˚C
Transfer line temperature	140.0˚C
Vial equilibration time	15 minutes
Vial pressuring time	0.3 minutes
Pressure equilibrating time	0.1 minutes
Load time	0.03 minutes
Load equilibration time	0.18 minutes
Injection time	2 minutes
Needle flush time	0 minute
Shaking level	2
Multi injection count	1
Pressurizing gas pressure	50.0 kPa

2.2.3. Standard and Sample Preparation

Blank: 2 mL of DMSO in a headspace vial. Seal the vial immediately.

Standard Stock Solution-A: Transfer about 20 mg of benzene into a 100 mL volumetric flask containing about 20 mL DMSO and volume up to mark with the same solvent. Transfer 1 mL of this solution to a 100 mL volumetric flask and volume with the same solvent.

Standard Stock Solution-B: Transfer about 300 mg of methanol, 500 mg of acetone, 500 mg of IPA, 60 mg of methylene chloride, 500 mg of ethyl acetate, 72 mg of THF, 89 mg of toluene, and 20 mg of pyridine into a 100 mL volumetric flask containing about 20 mL DMSO and volume up to mark with the same solvent.

Standard Solution: Take 10 mL of above standard stock solution-A and 10 mL

of above standard stock solution-B in 100 mL volumetric flask containing about 20 mL of DMSO and volume up to the mark with the same solvent.

Final concentration: 300 ppm methanol, 500 ppm acetone, 500 ppm IPA, 60 ppm methylene chloride, 500 ppm ethyl acetate, 72 ppm THF, 0.2 ppm benzene, 89 ppm toluene, 20 ppm pyridine.

Sample Solution: About 200 mg of the sample transfer in to a headspace vial and add 2 mL of DMSO, and seal the vial immediately.

3. Results and Discussion

All the chemicals used in this study are reagent grade. GC analysis is very sensitive to detection, so GC grade chemicals and standards should be used for analysis. Some unknown peaks were observed in the chromatograms. However, no other peaks were detected at the retention times of methanol, acetone, IPA, methylene chloride, ethyl acetate, THF, benzene, toluene, and pyridine in the blank solution. Therefore, no interference was found from the blank with the targeted peaks indicating that the method is specific for the respective solvents. From the precision study, it was observed that this method gives reproducible results. The %RSD found from the six replicate injections is less than 10.0. The recovery results of sample solutions were satisfactory and the recovery was between 80.0% and 120.0%.

3.1. Specificity

Each solvent—methanol, acetone, IPA, methylene chloride, ethyl acetate, THF, benzene, toluene, and pyridine—was spiked individually to confirm the interference between solvents. The retention time for methanol, acetone, IPA, methylene chloride, ethyl acetate, THF, benzene, pyridine, and toluene were found to be 4.18, 6.59, 6.97, 7.73, 12.00, 12.66, 14.85, 23.63, and 23.97 min, respectively. **Figure 1** shows the chromatogram for the spiked sample.

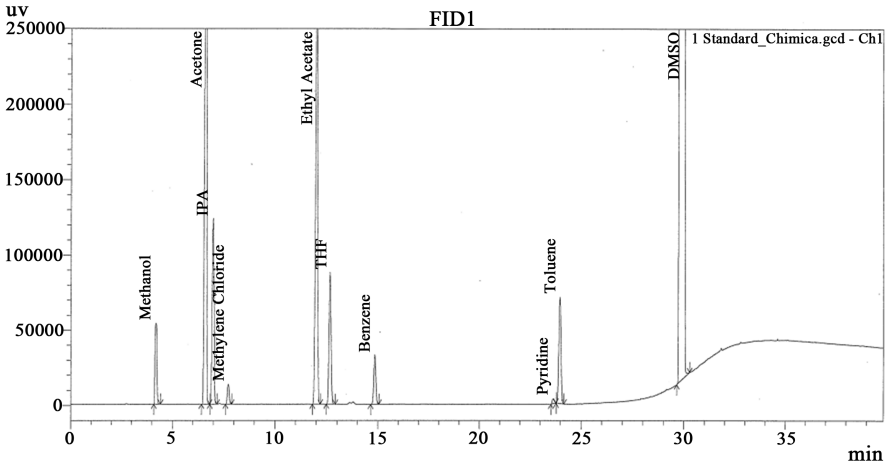

Figure 1. Spiked sample chromatogram.

All the samples were prepared individually, and injected to the chromatographic

system to confirm the identification of retention time. Chromatograms of identification solution is presented from Figure 2-11.

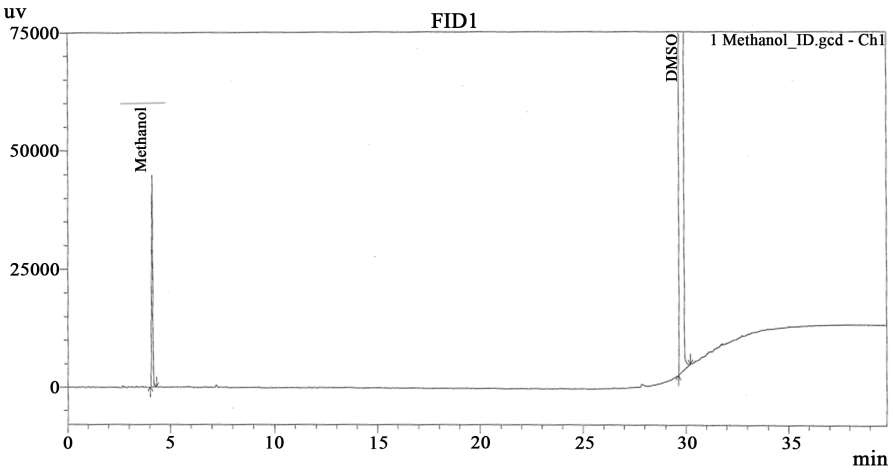

Figure 2. Chromatogram of methanol.

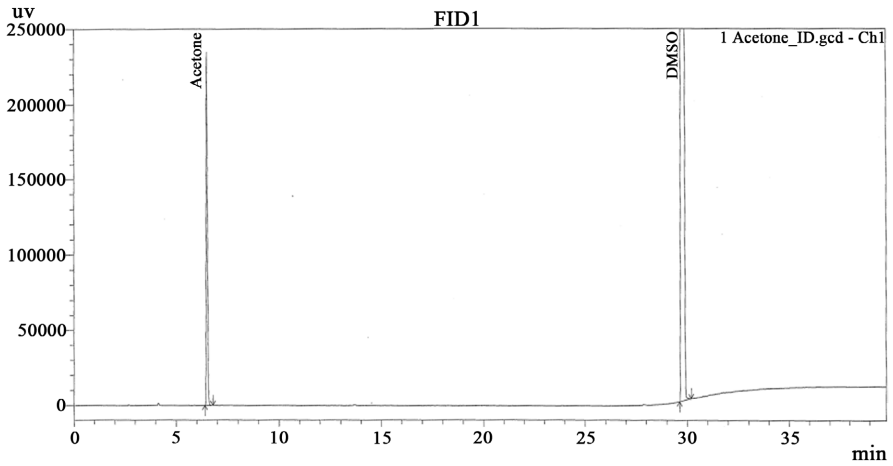

Figure 3. Chromatogram of acetone.

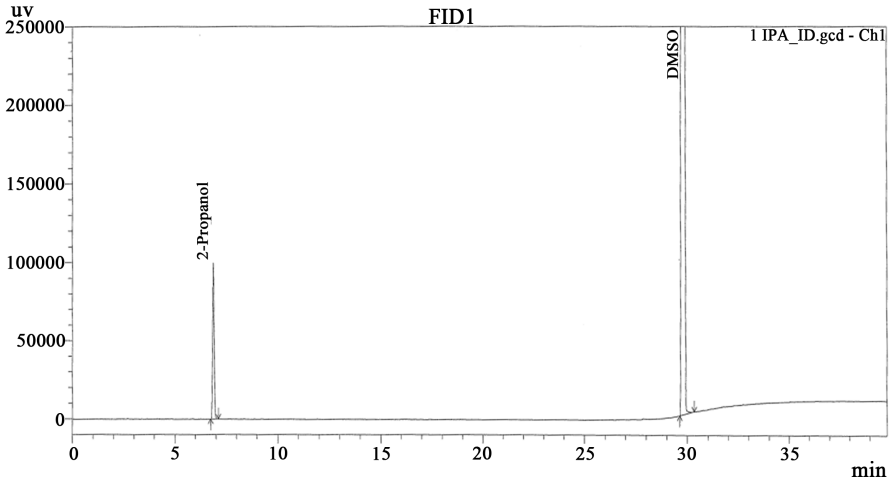

Figure 4. Chromatogram of IPA or 2-propanol.

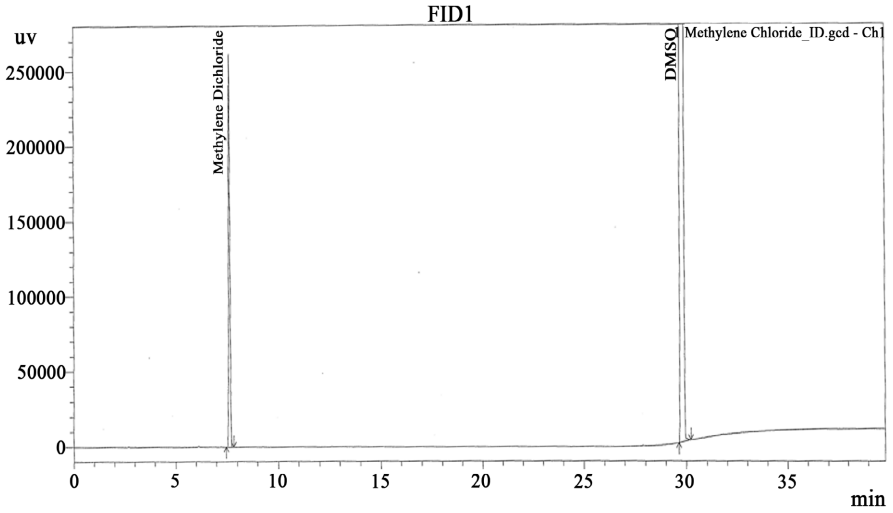

Figure 5. Chromatogram of methylene chloride.

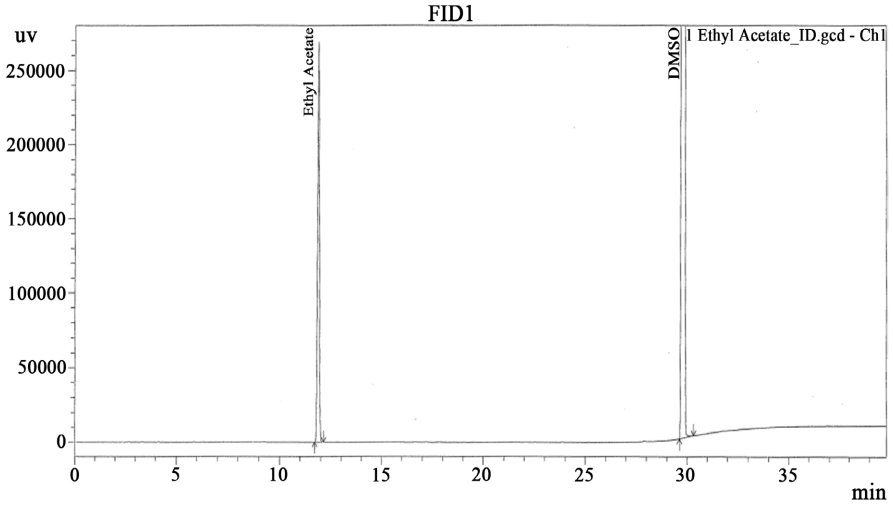

Figure 6. Chromatogram of ethyl acetate.

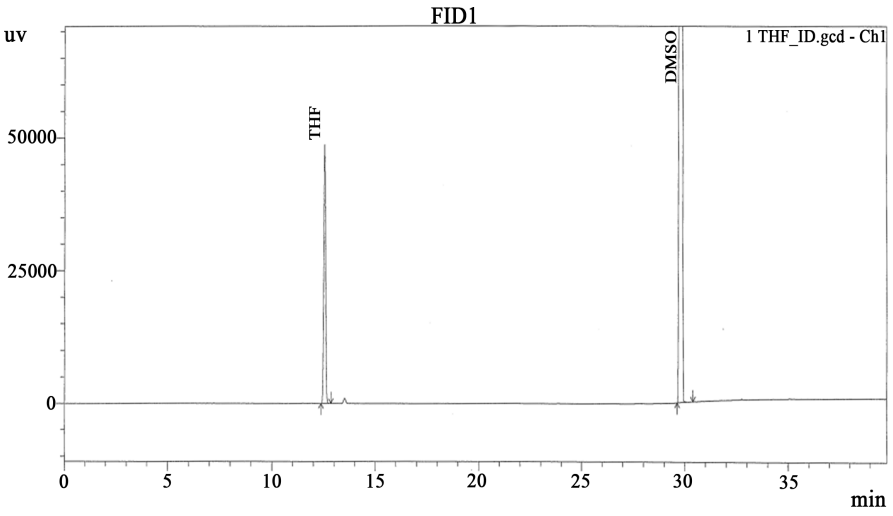

Figure 7. Chromatogram of THF.

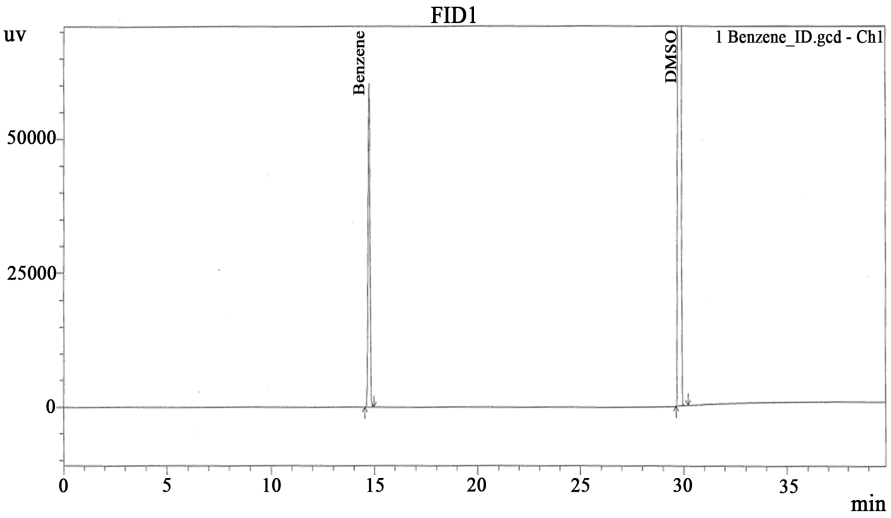

Figure 8. Chromatogram of benzene.

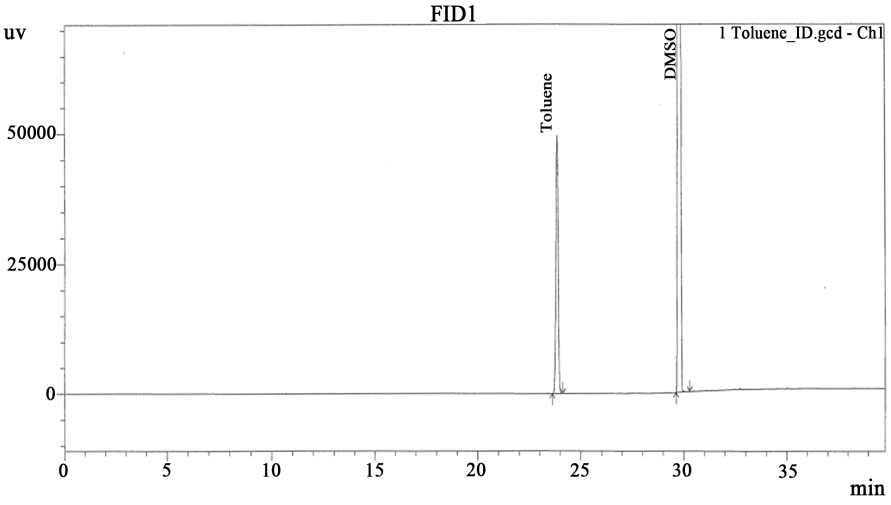

Figure 9. Chromatogram of toluene.

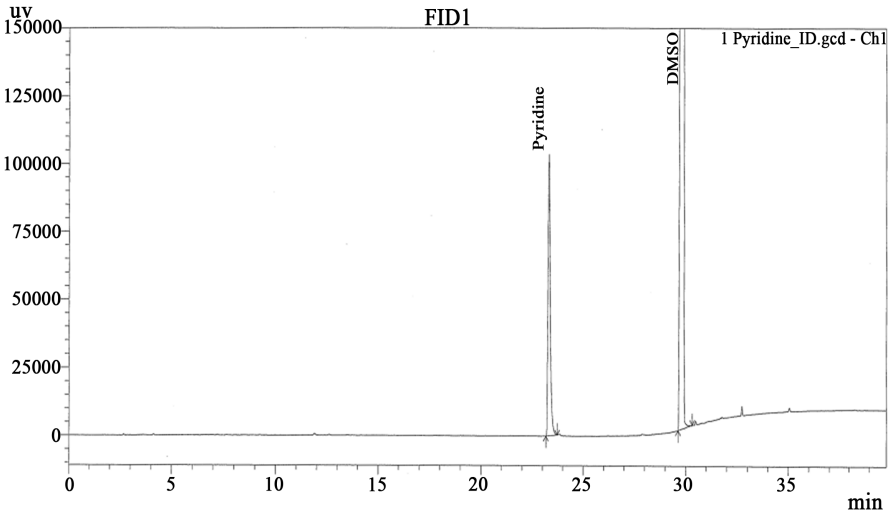

Figure 10. Chromatogram of pyridine.

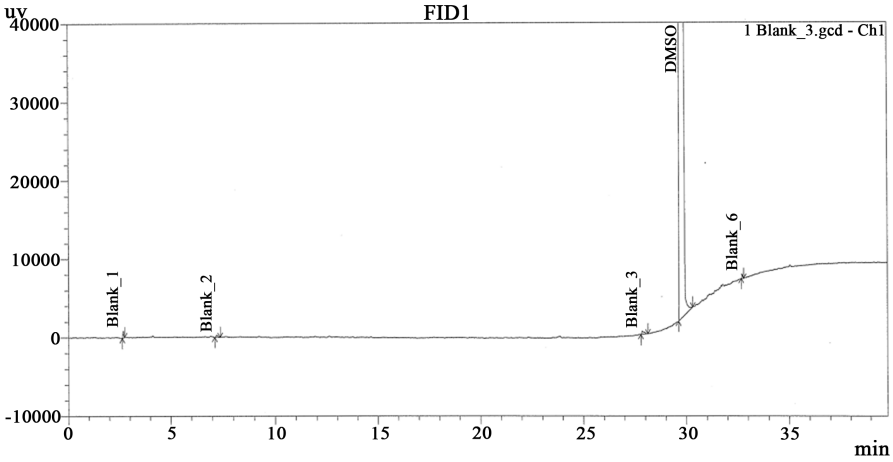

Figure 11. Chromatogram of DMSO (Blank).

3.2. Recovery Study

Methanol, acetone, IPA, methylene chloride, ethyl acetate, THF, benzene, toluene, and pyridine were spiked with sample to check the acceptable level of recovery. Table 3 shows the recovery data of different residual solvents. The % recovery of these solvents ranged from 80% to 120%, and the % RSD of areas of all solvents was below 10.0. These results demonstrate that the method achieves an acceptable level of recovery.

Table 3. Recovery data of different residual solvents.

100% spiking of different solvents	Spiked Conc. (ppm)	Recovered Conc. (ppm)	% Recovery
Methanol	3166.2	3228.2	102
Acetone	4992.6	4827.9	97
IPA	5065.5	5037.1	99
Methylene Chloride	623.4	596.9	96
Ethyl Acetate	5069	4834.2	95
THF	744.8	692.9	93
Benzene	1.957	1.841	94
Pyridine	872.5	933.7	107
Toluene	195	182.4	94

3.3. Precision Study

As a part of this study, system precision was performed. For the system precision, standard solution was injected for six times and observe the chromatogram. Table 4 represents the system precision results. The % RSD of areas of each solvent was found below 10.0. Also, Table 5 shows the % RSD of RT which proves the suitability of the method.

Table 4. System precision data (%RSD of area).

System Precision									
	Methanol	Acetone	IPA	Methylene Chloride	Ethyl Acetate	THF	Benzene	Toluene	Pyridine
% RSD of area	1.2	0.9	1.3	0.7	0.9	1.0	1.1	1.0	5.8

Table 5. %RSD of RT from precision data.

System Precision									
	Methanol	Acetone	IPA	Methylene Chloride	Ethyl Acetate	THF	Benzene	Toluene	Pyridine
Average RT	4.11	6.50	6.87	7.63	11.88	12.54	14.73	23.83	23.51
% RSD of RT	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

3.4. System Suitability

Resolution between the critical pairs was taken as the system suitability criterion, i.e., resolution between acetone and IPA, resolution between pyridine and toluene. The system suitability criteria were that the resolution between both pairs should not be less than 1.5 and the results shows that it was found to be well above the minimum criteria. The results are presented in **Table 6**.

Table 6. System suitability data of different parameters.

Study parameter	Resolution between acetone and IPA	Resolution between pyridine and toluene
Specificity	2.3	1.7
Recovery	3.0	1.7
Precision	3.0	1.7

4. Conclusion

To develop a simple, cost-effective GC method for the identification and quantification of nine residual solvents, this study was conducted. According to pharmacopeia and ICH guidelines, residual solvents are divided into 3 categories: class 1, class 2, and class 3. Class 1 solvents should be avoided, class 2 solvents should be limited, and class 3 solvents are less toxic and pose lower risk to human health. If only class 3 solvents are used in manufacturing process, a loss on drying (LOD) test with a 0.5% limit would be acceptable. If multiple category solvents are used in manufacturing process, they should be identified and quantified within the specified limits as per guidelines. This method was developed considering these three categories of solvents. Pharmaceutical manufacturing companies, quality control scientists, and researchers will benefit from this method. Additionally, it can serve as study material for students learning about method development through headspace gas chromatography. This method can be employed to quantify residual solvents—methanol, acetone, IPA, methylene chloride, ethyl acetate, THF,

benzene, toluene, and pyridine—in drug substances and drug products. The developed method is precise, specific, and accurate, and should be validated according to ICH guidelines before being used to release the commercial products.

List of Abbreviations

RT:	Retention Time
IPA:	Isopropyl alcohol
THF:	Tetrahydrofuran
DMSO:	Dimethyl sulfoxide
GDP:	Good Documentation Practices
API:	Active pharmaceuticals ingredient
GC:	Gas Chromatography
FID:	Flame Ionization Detectors
TCD:	Thermal Conductivity Detectors
ECD:	Electron Capture Detectors
NPD:	Nitrogen Phosphorus Detectors
ICH:	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
LOD:	Loss on drying
µm:	Micrometer
mL:	Milliliter
RSD:	Relative Standard Deviation
ppm:	Parts Per Million
Conc.:	Concentration

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Authors' Contribution

This research was designed and performed by Sreekanta Nath Dalal. The co-author reviewed the content, data presentation, and overall layout of the study.

Statement of Ethical Approval

The current research does not include any studies involving animal or human subjects conducted by any of the authors.

Conflicts of Interest

The authors declare no conflict of interest regarding the publication of this paper.

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