International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

Controlled Strategies and Estimation of Trace Toxic Solvents in Pharmaceuticals

Dharmpal Singh[1], Dr. Prachi Diwakar[2] and Dr. Priyanka Mathur[3]

[1]Department of Chemistry, Bhagwant University, Ajmer-30554, (Rajasthan) India
[2,3]Professor, Department of Chemistry, Bhagwant University, Ajmer-30554, (Rajasthan) India

Abstract
Residual solvents in pharmaceuticals refer to volatile organic chemicals that are used during the manufacture of drug substances and finished drug products. Solvents play an essential role in producing active pharmaceutical ingredients (APIs) and drug products, as their proper selection can improve reaction efficiency and influence important properties such as crystal structure, purity, and solubility. Because solvents and their related impurities are integral to the manufacturing process, they may not be fully eliminated during processing and can remain in the final product. As a result, controlling residual solvents is a critical aspect of pharmaceutical development and manufacturing. Tests for residual solvents are generally not specified in individual monographs because the types of solvents used may differ between manufacturers. These solvents can pose risks not only to the environment but also to patient safety if present above acceptable levels. Therefore, acceptable limits for residual solvents are established in the ICH guideline Q3C (R9). This guideline classifies residual solvents into three categories based on their potential risk to human health, taking into account toxicity data and environmental concerns. Class 1 solvents—including benzene (2 ppm), carbon tetrachloride (4 ppm), 1,2-dichloroethane (5 ppm), 1,1-dichloroethane (8 ppm), and 1,1-trichloroethane (1500 ppm)—should be avoided in pharmaceutical manufacturing because they are recognized human carcinogens, highly toxic, and environmentally hazardous, with some also contributing to ozone depletion. Nevertheless, trace amounts of these solvents may still be present as impurities in other commonly used solvents. For example, benzene may be found in ethanol, methanol, acetone, and isopropyl alcohol, while 1,1-dichloroethane and 1,1-trichloroethane may occur in dichloromethane. Therefore, it is essential to carefully monitor and strictly control the levels of these harmful solvents in the solvents used during pharmaceutical manufacturing.

Keywords: Gas chromatography (GC), Genotoxic impurity and carcinogenic impurity.

1. Introduction

This work was expected to create and confirm an analysis technique to measure the carcinogenic contaminant benzene in solvents employed in the production of pharmaceutical drug substances and drug products. A combined GC and flame ionization detector (FID) was used to perform the analysis [1]. The FID was chosen as it is the most frequently used detector with GC systems, and is readily accessible in most analytical laboratories.

Benzene is a carcinogenic contaminant which can be introduced as a trace contaminant solvent in toluene, isopropyl alcohol, methanol, ethanol, and acetone that are widely used as solvents in humans [2]. It is a very poisonous compound and chronic or repeated exposure may cause severe health consequences, such

as anemia, leukemia among other illnesses. These major safety concerns have led to benzene being listed under the Class 1 solvents category by the ICH with a strict limit set at 2 ppm or less [3].

Residual solvents are usually not a test specified in individual monographs due to the fact that the nature of solvents to be used can vary among manufacturers. Most of these solvents are not only dangerous to the environment, but are also toxic to patients when they occur in quantities beyond sensitivity levels [4]. Thus, the ICH guideline Q3C (R9) sets appropriate limits of residual solvents. The guideline offers a mechanism of organizing the residual solvents according to their possible risk to the human health using the toxicity data and taking into consideration their effects to the environment [5].

Method development approaches for the quantification of trace-level solvents in pharmaceuticals have been well-discussed in several publications and studied by several researchers. However, the solvents are used during the manufacturing of drugs. Solvents also have impurities that might be carcinogenic [6]. In solvents, it is highly important to control and measure these trace-level impurities. A delicate approach is therefore needed to manage and measure trace amounts of solvent impurities in solvents on otherwise these impurities will be transferred to the end drug substance and drug products which will be unproductive and potentially harmful to patients [7].

Other researchers have documented and researched diverse method development strategies to quantify trace levels of solvents in pharmaceutical analysis. The solvents are widely used in the production of drug substances and drug products, and the solvents themselves can contain impurities, some of which are carcinogenic [8]. Thus, it is of utmost importance to control and correctly quantify those trace-level impurities in solvents. Such impurities are critical and therefore need a highly sensitive analytical procedure in order to monitor and measure them, since failure to do so might see them transferred to the final drug substance or drug product [9]. Such impurities do not have a therapeutic value and can be very dangerous to the safety of patients.

An expedited, verified procedure is needed to locate and measure trace-solvent degrees of impurity in solvents by solvent manufacturers and the pharmaceutical sector. GC has now reached a sophisticated method and is employed to determine trace amounts of impurities in solvents in the solvent/pharmaceutical process. The detectors in a modern GC are very sensitive, which means that they can measure the impurities of the solvent at the ppm level. The flame ionization detector is among the most sensitive detectors, and it can be used to determine the solvent impurities.

2. Methodology

Benzene was analyzed and determined with the help of a GC system (Agilent 7820A). Quantification of benzene was done using a DB-1HT capillary column (30 m x 0.25mm x O.10 um). The carrier gas used in the analysis was Helium, and its flow rate was kept constant at 1.5 mL/min. Temperature programming and flame ionization detection at optimal settings were used to achieve the desired perfection of benzene identification with high peak separation, sensitivity, and methodological reproducibility accurately determined in complex samples in the matrix of samples.

The GC used an oven temperature program that was controlled to the end to be able to provide maximum separation and detection of target analytes. The initial conditions were established at a temperature of 40 °C with an isothermal hold time of 2 min to allow successful concentration of volatile compounds at the column head. The temperature in the oven was then raised at 8 °C/min up to 80 °C, and a 1-minute hold period was enforced to maximize resolution of the mid-range volatility constituents. The temperature was then raised at the same rate of 8 °C/min until a temperature of 120 °C was reached, and this was maintained

at 120 °C, allowing 2 minutes also to pass before the next injection in order to elute all higher-boiling compounds and to clean the column. Introducing the samples was done through an automated headspace sampler with a split ratio of 20:1 in split mode to avoid the overloading of the column and to get sharp peaks. The temperature of the injector port was kept at 230 °C during the analytical run to ensure that the samples to be introduced were immediately vaporized and that the effects of thermal degradation or discrimination did not affect the final quantitative results, and therefore, the results were reproducible and accurate.

Chemicals

Reference samples of Benzene were obtained with the potency of 99.98% (Benzene, Batch Number-R167A24, Purity-99.98%, Vendor -Rankem), solvents were obtained with the potency of 99.99% (Methanol, Batch Number-K025A24, Purity-99.99%, Vendor -Rankem), 99.96% (2-Propanol, Batch Number-T054824, The Merck India customer had sourced dichloromethane (DCM) as a diluent, which was received as a gift sample provided by Fare Lab (Gurugram, India).

Benzene Solution

A benzene stock solution was prepared in dichloromethane (DCM) diluent at a concentration of 10 ppm (w/v) by precise gravimetric dissolution of an analytical grade benzene reference standard. Subsequently, working standard solutions at the limit concentration of 2 ppm were prepared by serial dilution of the stock solution in DCM with good mixing and homogeneity of each dilution step. To assess the precision of the method at the limit of quantitation, 6 replicate injections of a 2 ppm benzene working solution were carried out under the same chromatographic conditions using the validated GC-FID method. The highest areas for these replicate injections were recorded and statistically analyzed to determine the percent relative standard deviation (%RSD). This precision assessment at the limit level is intended for validating the suitability of the method for quantitative determination of benzene residues in order to comply with regulatory acceptance criteria, wherein the values of %RSD are not more than 10% to establish that the method is adequate for the purpose of repeatability in limit testing applications as may be required in pharmaceutical and environmental analysis applications.

Sample solution (diluent)

Mix the 20 mL of each solvent (Methanol, Ethanol, Acetone, 2-Propanol, and Toluene) with the 100 mL volumetric flask and mix well.

3. Results and Discussion

Feasibility studies have been conducted using GC to allow a better resolution and response, and the reduction of interference in Benzene estimation. Different columns having different chemistries were tried, such as DB-5MS (30 m in length, internal diameter of 0.32 mm, and 0.25-micron thickness of film), ZB-624 (30 m in length, internal diameter of 0.32 mm, and 1.8-micron thickness of film), and DB-1HT (30 m in length, internal diameter of 0.25 mm, and 0.10-micron thickness of film). GC settings, including oven temperature schedule and gas flow rate, were evaluated and optimized as well. The repercussion of maintaining the pressure in constant mode and keeping the flow in continuous mode was also evaluated. Trials based on a DB-1HT (length of 30 m, internal diameter of 0.25 mm, and thickness of the film 0.10 micrometer) column with optimum oven conditions and Gas flow rate provided more appropriate results compared to other investigated trials.

The DB-5MS column, although offering good separation, showed the benzene having wider peak shapes and a longer retention time, which may affect the sensitivity. The ZB-624 column had proven to have

improved volatility separation with an increase in baseline noise and interference of matrix components. In contrast, the DB-1HT column's non-polar dimethylpolysiloxane stationary phase with ultra-thin film thickness resulted in much better efficiency, peak symmetry, and resolution due to potential interfering compounds.

Optimization of the oven temperature program showed that an oven temperature of initially 40 °C for 2 min, followed by a ramp of 8 °C per min to 80 °C with a 1 min hold time, and then ramped to 120 °C at 8 °C per min with a hold time of 2 min, gave optimal separation. Constant flow mode at 1.5 mL/min helium carrier gas showed better reproducibility than the constant pressure mode, with the least variability of the retention times. The DB-1HT configuration eventually provided superior signal-to-noise ratios, shorter analysis time, and increased method robustness to be used for routine benzene quantification.

Validation

Specificity

The method-specific nature was characterized with the help of other solvents (Methanol, Ethanol, Acetone, 2-Propanol, and Toluene). The quantity of Benzene spiked in the sample solution mixture was ca. 2 ppm. From Figures 1 and 2, there are no observable interferences at the retention time of benzene in the chromatograms of the spiked solvent mixture. The retention times were found to be 1.68 min, 1.79 min, 1.87 min, 2.08 min, 3.06 min, and 4.02 min for Acetone, Methanol, Ethanol, 2-Propanol, Toluene, and Benzene, respectively in Table 1.

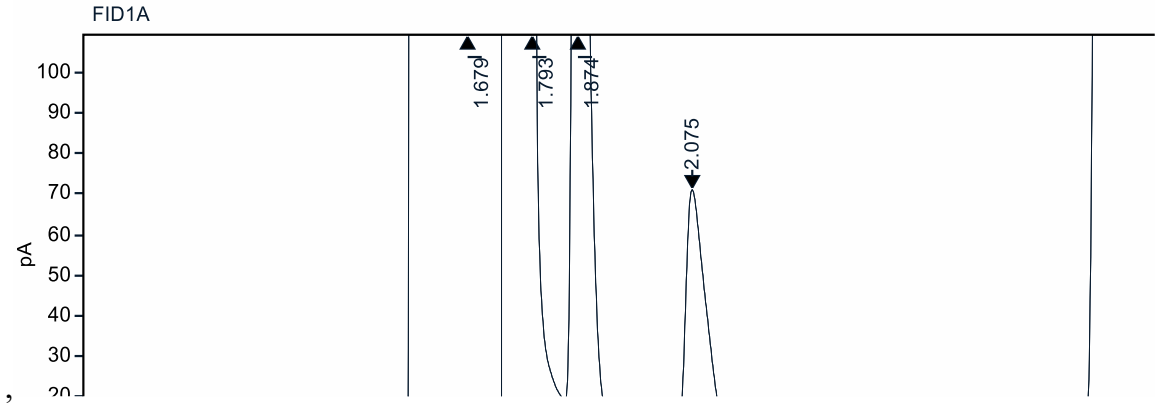

Figure 1. Specificity evaluation: Chromatogram of organic solvents showing no interference at benzene retention time

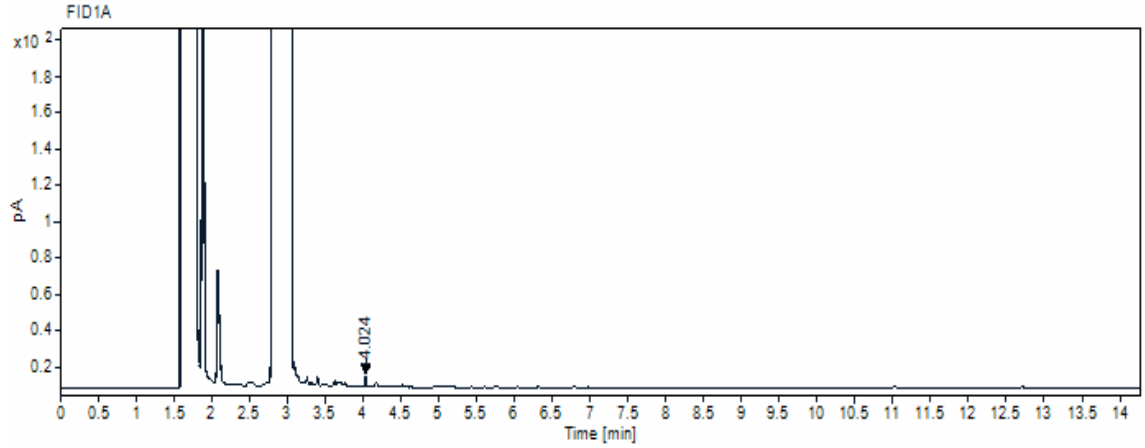

Figure 2. Chromatogram of benzene standard (2 ppm) in solvent mixture (RT: 4.02 min)

S.No.	RT [min]	Area	Area%	Name
1	1.68	321.86	46.412	Acetone
2	1.79	84.13	13.544	Methanol
3	1.87	51.91	0.145	Ethanol
4	2.08	38.49	0.036	Iso-propyl alcohol
5	3.06	524.61	39.862	Toluene
	Sum	1021.00	100.00	

Table 1: Retention time of solvents

Limits of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ for benzene were set according to the signal-to-noise (S/N) ratio evaluations of benzene at known concentrations. The LOD was found to be 0.1 ppm, corresponding to an S/N ratio of around 3:1, whereas the LOQ was confirmed at 0.3 ppm with an S/N ratio of 10:1, so quantification can be done with good to reliable quantification in Table 2. Precision at the LOQ level was determined by six replicate injections of the 0.3 ppm benzene standard solution, and the relative standard deviation (%RSD) of the peak area of responses was calculated. The obtained value of %RSD was less than 15% showing acceptable repeatability and confirming the appropriateness of the established LOQ for quantitative accurate analysis. These validation parameters collectively ensure sensitivity, reliability, and compliance with the regulations for trace-level determination of benzene in pharmaceutical or environmental samples using this method and can be used for strong quality control applications.

Table 2: Demonstrating LOD and LOQ Values of Benzene

Injection No.	LOD Level – Benzene (Area Counts)	LOQ Level – Benzene (Area Counts)
1	2.54	0.36
2	2.57	0.29
3	2.53	0.32
4	2.56	0.33
5	2.60	0.29
6	2.53	0.35
Mean	**2.5550**	**0.3233**
CV (%)	**1.1**	**9.1**

LOD – Limit of Detection; LOQ – Limit of Quantification; CV – Coefficient of Variation.

Linearity

The linearity of the analytical method for quantification of benzene was rigorously evaluated in order to ascertain the proportionality of the detector response to the analyte concentration over the working range specified. Five calibration standards were prepared at concentrations ranging from the established limit of quantitation (LOQ, 0.3 ppm) to 200% of the specification limit (2.0 ppm), resulting in a concentration range of 0.2 to 5.0 ppm. Each standard solution was injected three times under the same chromatographic conditions, and the corresponding peak areas were recorded for statistical analysis. Calibration curves were drawn between mean peak area responses and various benzene concentrations, as can be seen in Figure 3. Linear regression analysis was conducted in order to find the correlation coefficient (r^2), slope, and y-intercept values, which are summarized in Table 3. The square of the correlation coefficient (r^2) for

benzene was higher than 0.994, which showed very good linearity, and the detector response was found to be directly proportional to the analyte concentration over the range studied. Additionally, the slope and intercept values represented negligible systematic error and sensitivity appropriate for trace-level quantification. These results are collectively validated to prove that the developed GC method has acceptable linearity characteristics to meet the regulatory acceptance criteria for analytical method validation and to provide reliable quantitative determination of benzene residues in the pharmaceutical or environmental sample matrices within the specified concentration range.

Table 3, Linearity results of Benzene

Level (%)	**Concentration (ppm)**	Response
QL level	0.2	1.32
15% level	0.3	2.57
50% level	1.0	4.72
100% level	2.5	11.47
200% level	5.0	18.79
Slope		**3.6103**
Intercept		**1.2755**
Correlation coefficient		**0.99485**

The resulting regression line was calculated in accordance with the equation: y = m * x + c

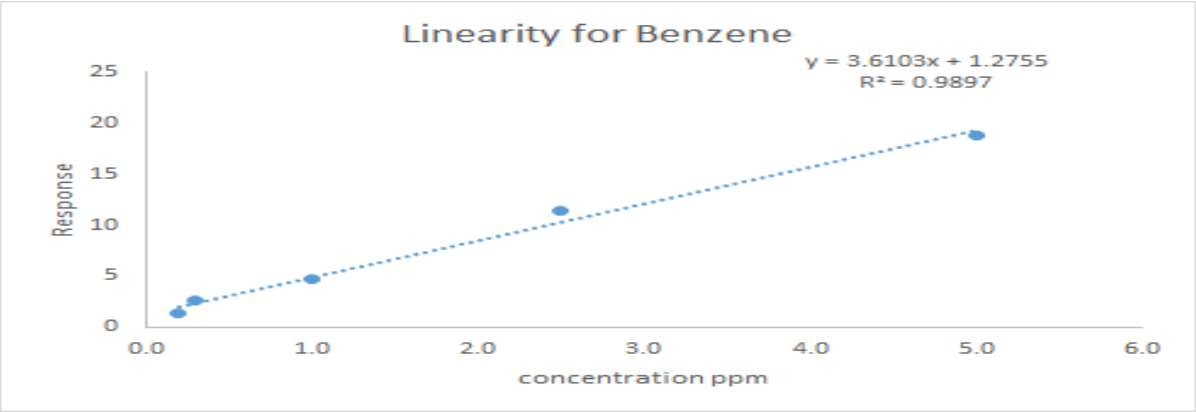

Figure 3. Linearity of Benzene

Accuracy

The accuracy of the method was determined by the study of a mixture of solvents spiked with Benzene in triplicate at LOQ (0.3 ppm) and in six repetitions at 100% of the limit value specified (2 ppm). Recovery studies for Benzene were at the limit of quantitation (LOQ, 0.3 ppm) and 100% specification level (2.0 ppm) with results summarized in Tables 4 to 8. The percent mean recovery values for Benzene at both concentration levels were consistently within the accepted value range of 80-120% of acceptance. These results show that the developed GC method has acceptable accuracy and reliability in quantitative determination of Benzene Residues and is a precise measurement in the analytical range, and can be used in routine quality control applications for pharmaceutical or environmental testing purposes.

Table 4, Accuracy at QL Level of Benzene

Solvent	Amount Present (ppm)	Response found before spiked sample			Response found after spiked sample		
		Inj-1	Inj-2	Inj-3	Inj-1	Inj-2	Inj-3
Benzene	0	2.54	2.57	2.53	2.56	2.60	2.53

Table 5, Accuracy at QL Level of Benzene

Recovery (%)				
Solvents	Inj-1	Inj-2	Inj-3	Mean recovery
Benzene	101.2	101.2	100.0	100.8

Acceptance criteria

The recovery should be between 70% to 130% at the QL level.

Conclusion

The recovery was found to be between 100.0% to 101.2% of Benzene.

Table 6: Accuracy at 100% Level of Benzene

Solvents	Amount Present (ppm)	Response found before spiked sample					
		Inj-1	Inj-2	Inj-3	Inj-4	Inj-5	Inj-6
Benzene	0	11.53	11.58	11.60	11.52	11.57	11.45

Table 7: Accuracy at 100% Level of Benzene

Solvents	Amount Present (ppm)	Response found after spiked sample					
		Inj-1	Inj-2	Inj-3	Inj-4	Inj-5	Inj-6
Benzene	0	11.49	11.66	11.62	11.47	11.45	11.36

Table 8, Accuracy at 100% Level of Benzene

Recovery (%)								
Solvents	Inj-1	Inj-2	Inj-3	Inj-4	Inj-5	Inj-6	Mean recovery	CV
Benzene	99.7	100.7	100.2	99.7	99.0	99.2	99.8	0.6

Acceptance criteria

The recovery should be between 80% to 120% at the QL level.

Conclusion

The recovery was found to be between 99.0% to 100.7% of Benzene.

5. Conclusion

The developed and validated GC method showed excellent analytical performance in the simultaneous determination of genotoxic impurities of solvent matrices. Comprehensive validation studies have shown that the method is specific, precise, sensitive, linear, and accurate within the concentration ranges. The performance of the robust analytical protocol showed reliable detection and quantification capabilities, satisfactory recovery rates, and little interference from matrix components. Consequently, this validated GC method becomes a reliable, efficient, and reproducible tool for routine quality control applications for effective monitoring and control of the genotoxic impurities in pharmaceutical-grade solvents. Its implementation supports compliance with the regulatory guidelines and ensures the consistent evaluation of solvent quality along the manufacturing processes, finally contributing to improved product safety and quality assurance of pharmaceutical development and production workflows.

Acknowledgement

The authors wish to thank the Fare lab for providing the infrastructure required to conduct this research.

References

1. Parys, W., Dołowy, M., & Pyka-Pająk, A. (2022). Significance of Chromatographic Techniques in Pharmaceutical Analysis. *Processes*, *10*(1), 172. https://doi.org/10.3390/pr10010172
2. Gautam, A., & Cressey, P. J. (2025). Benzene in cosmetic products: Non cancer and cancer risk assessment. *Toxicology reports*, *15*, 102135. https://doi.org/10.1016/j.toxrep.2025.102135
3. Smith M. T. (2010). Advances in understanding benzene health effects and susceptibility. *Annual review of public health*, *31*, 133–following148. https://doi.org/10.1146/annurev.publhealth.012809.103646
4. B'Hymer, C. (2003). Residual solvent testing: A review of gas-chromatographic and alternative techniques. *Pharmaceutical Research, 20*(3), 337–344. https://doi.org/10.1023/A:1022693516409
5. Guerassimoff, L., & Nicolas, J. (2026). Towards safer medicines: Management of residual solvents and green alternatives. *Journal of Controlled Release, 391*, 114643. https://doi.org/10.1016/j.jconrel.2026.114643
6. Manchuri, K. M., Shaik, M. A., Gopireddy, V. S. R., Naziya Sultana, & Gogineni, S. (2024). Analytical Methodologies to Detect N-Nitrosamine Impurities in Active Pharmaceutical Ingredients, Drug Products and Other Matrices. *Chemical research in toxicology*, *37*(9), 1456–1483. https://doi.org/10.1021/acs.chemrestox.4c00234
7. Ayon N. J. (2023). High-Throughput Screening of Natural Product and Synthetic Molecule Libraries for Antibacterial Drug Discovery. *Metabolites*, *13*(5), 625. https://doi.org/10.3390/metabo13050625
8. Alsante, K. M., Huynh-Ba, K. C., Baertschi, S. W., Reed, R. A., Landis, M. S., Furness, S., Olsen, B., Mowery, M., Russo, K., Iser, R., Stephenson, G. A., & Jansen, P. (2014). Recent trends in product development and regulatory issues on impurities in active pharmaceutical ingredient (API) and drug products. Part 2: Safety considerations of impurities in pharmaceutical products and surveying the impurity landscape. *AAPS PharmSciTech*, *15*(1), 237–251. https://doi.org/10.1208/s12249-013-0061-z
9. Pilely, K., Johansen, M. R., Lund, R. R., Kofoed, T., Jørgensen, T. K., Skriver, L., & Mørtz, E. (2022). Monitoring process-related impurities in biologics-host cell protein analysis. *Analytical and bioanalytical chemistry*, *414*(2), 747–758. https://doi.org/10.1007/s00216-021-03648-2