Original Article

SIMULTANEOUS TRACE LEVEL DETERMINATION OF BENZENE AND 1, 2-DICHLOROETHANE BY GC-HS/GC-MS IN SEVERAL PHARMACEUTICAL DRUG SUBSTANCES

SAYEEDA SULTANA*, BALAJI NAGARAJAN²

Department of Chemistry, St. Peter's Institute of Higher Education and Research, Avadi, Chennai 600054, Tamil Nadu, India
Email: chemistry@stpetersuniversity.org

ABSTRACT

Objective: We herein report the simultaneous trace level determination of benzene and 1,2-dichloroethane in several active pharmaceutical substances by GC-HS (gas chromatograph-head space) using a DB-624 column.

Methods: This GC-HS method was developed based on an oven-programmed approach using nitrogen gas as the mobile phase. Our method is also compatible with the GC-MS (gas chromatography-mass spectrometry) technique using helium as the mobile phase instead of nitrogen. The successful separation of benzene and 1,2-dichloroethane was established by confirmation of their corresponding specific molecular masses.

Results: The retention time of benzene and 1,2-dichloroethane were found to be 34.8 min and 35.6 min, respectively. The linearity was found in the range of concentration of 0.63-4.22 ppm and 1.49-9.96 ppm for benzene and 1,2-dichloroethane. The detection limit and quantification limit for benzene were 0.2 and 0.6 ppm, while those of 1,2-dichloroethane were 0.6 ppm and 1.5 ppm. These values were calculated using our developed method with respect to the test concentration of 500 mg/ml. The recovery of benzene and 1,2-dichloroethane were found to be 89–110% and 91–105%, respectively for the various pharmaceutical drug substances. The specificity of the method was studied using 20 solvents which include benzene and 1,2-dichloroethane.

Conclusion: We expect that our method will be applicable for the simultaneous trace level determination of benzene and 1,2-dichloroethane during the control of manufacturing processes, and for use in rapid analysis for quality control in the pharmaceutical industry. Finally, this method was validated according to the International Conference on Harmonization (ICH) Validation Guidelines Q2 (R1).

Keywords: GC, GC-MS DB-624, Benzene, 1,2-dichloroethane, Class-1 solvent, Pharmaceutical substance
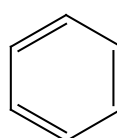
INTRODUCTION

Benzene (fig. 1) is known to cause central nervous system depression in addition to destroying bone marrow, which in turn leads to damage to the hematopoietic system. In addition, benzene has been demonstrated to be a human carcinogen (i.e., lymphatic and hematopoietic cancers), while in animal studies, Zymbal gland tumors, preputial gland tumors, skin carcinomas, mammary gland tumors, and leukemia have been reported. Although positive chromosomal aberration and DNA adducts tests have been recorded, the results of other mutagenicity tests were negative. From the data of human leukemia and benzene exposure correlations, a daily intake of 0.02 mg was found to be associated with a lifetime excess cancer risk of 10^{-5} (IRIS), and we note that the guideline value for benzene is 0.02 mg/d (2 ppm) [1–5].
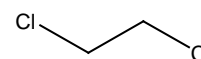
Repeated exposure to 1,2-dichloroethane (fig. 1) has been reported to induce, nausea, abdominal pain, irritation of mucous membranes, dysfunction of liver and kidney and neurological disorders. In addition, the depression of leukocytes, antibody-forming cells, and cellular immunity was also found in mice, while necrosis of the cerebellum and hyperplasia was observed in addition to inflammation of the forestomach in male rats after oral administration. Although there is no evidence of carcinogenicity in humans, forestomach cancer, hemangiosarcoma, breast cancer, uterine cancer, and respiratory tract cancer were found in rats and mice following gavage treatment. The evidence reported to date, therefore, indicates that 1,2-dichloroethane is potentially genotoxic, and excess cancer risk of 10^{-5} was reported at exposures of 0.05 mg/d for 50 kg human based on hemangiosarcoma using a linearized multistage model without body surface correction. The guideline value for 1,2-dichloroethane is 0.05 mg/d (5 ppm) [1–5].

To date, the detection and quantification of benzene and 1,2-dichloroethane have been using only gas chromatography-mass spectrometry (GC-MS) techniques [6-28]. However, previous reports in this area focus on the analysis of the benzene and do not address the detection or quantification of 1,2-dichlorethane.

Indeed, the simultaneous trace level determination of benzene and 1,2-dichloroethane in pharmaceutical drug substances has yet to be reported, and so we selected gas chromatography-head space (GC-HS) technique for the purpose of our study in combination with a DB-624 capillary column for the determination of these two compounds in pharmaceutical substances.

Fig. 1: Chemical structures of benzene and 1,2-dichloroethane

MATERIALS AND METHODS

Materials

All samples (i.e., Cabergoline, Celecoxib, Dronedarone hydrochloride, Etravirine, Fesoterodine fumarate, Gabapentin, Irinotecan hydrochloride, Levetricetam and Levothyroxine sodium with the purity of 95%) were received from Jinan Jiage Biological Technology Co, Ltd., China. Benzene, 1,2-dichloroethane, dimethyl sulfoxide, n-butyl acetate, methanol, ethanol, acetone, isopropyl alcohol, acetonitrile, dichloromethane, n-hexane, ethyl acetate, tetrahydrofuran, toluene, n-heptane, 2-butanone, cyclohexane, methyl tertiary butyl ether, methyl isobutyl ketone and diisopropyl ether solvents were purchased from Fisher Scientific with the purity of 99.5% (Mumbai, India). The DB-624 GC capillary column was obtained from LCGC (Hyderabad, India). USP grade water was employed throughout and was prepared using a Metrohm Elga

water purifier (Metrohm, Switzerland). The nitrogen gas cylinder was procured from Indo Gas agencies (Tamil Nadu, India). Development and validation studies were carried out using an Agilent 7890A GC equipped with a G1888 headspace sampler (Agilent Technologies, Singapore). Finally, an Agilent 5973C GC-MS (Agilent technologies, Singapore) was utilized for molecular mass identification of the benzene and 1,2-dichloroethane peaks.

Solution preparation

Preparation of internal standard solution

The internal standard (IS) solution was prepared by dissolving n-butyl acetate (50 mg) in dimethyl sulfoxide (50 ml).

Preparation of diluent

The diluent was prepared by mixing dimethyl sulfoxide (700 ml) with water (300 ml), followed by the addition of IS solution (2.5 ml). The resulting solution was allowed to cool to 25 °C.

Preparation of standard stock solution

The standard stock solution was prepared by diluting benzene (50 mg) and 1,2-dichloroethane (125 mg) in the diluent (50 ml). 5 ml portion of this solution was then diluted further to 100 ml using the diluent.

Preparation of standard solution

The standard solution was then prepared by diluting a portion of the standard stock solution (2 ml) to a final volume of 100 ml using the diluent. The concentrations of benzene and 1,2-dichloroethane in the standard solution were 2.0 ppm and 5.0 ppm respectively, with respect to the analyte concentration.

Preparation of sample solution

The sample solution was prepared by dilution of sample (1000 mg) in the diluent (2 ml) in a headspace vial. For the preparation of the spiked sample solutions, the sample (1000 mg) was weighed in a headspace vial, and the standard solution was added for (2 ml).

Method

GC chromatographic conditions

A DB-624 (60 m × 0.32 mm × 1.8 μm) column was employed for GC analysis. The oven temperature program began with an initial temperature about 35 °C (hold time = 45 min), Followed by heating to 240 °C at a rate of 30 °C/min, and holding at this final temperature for 25 min. The total run time was 76.83 min, a split injection mode with a split ratio of 5: 1 was used, and the column flow rate was 1.5 ml/min. The injection port and detector temperatures were both set at 240 °C.

HS Chromatographic conditions

In terms of the HS conditions, the oven temperature was 110 °C, loop temperature was 175 °C, and the transfer line temperature was 175 °C. A cycle time of 85 min was employed, along with vial equilibration time of 60 min, a vial pressurization time of 1 min, a loop filling time of 0.5 min, a loop equilibration time of 0.5 min, and an injection time of 1 min. The vial pressure was maintained at 15 psi.

Other general chromatographic conditions

Nitrogen and helium were used as the mobile phase for GC-HS and GC-MS analysis respectively. All other parameters were as described for GC-HS above. Each headspace vial was closed tightly with PTFE septum and sealed by the crimping of an aluminium cap. The vials were introduced into the chromatograph using a headspace auto sampler. For the specificity study, desired solvent (10–20 mg) was added to a headspace vial with the diluent. Each solvent vial was closed tightly with PTFE septum and sealed by the crimping of an aluminium cap. The chromatograms of the blank and standard solutions are shown in fig. 2.
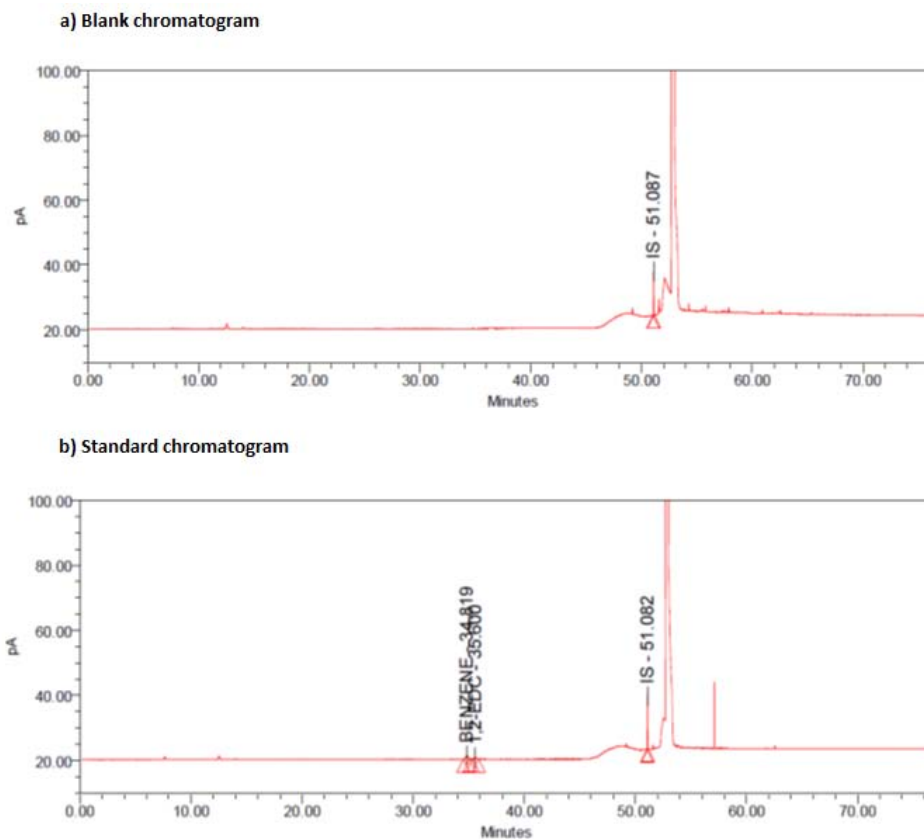

Fig. 2: Chromatograms of (a) the blank and (b) the standard solutions

RESULTS AND DISCUSSION

Development

In recent years, several trials have been performed involving the investigation of different capillary stationary phases for the detection of benzene [6-28]. However, we selected the DB-624 capillary column as the stationary phase for our study due to its unique properties. The selection of pharmaceutical drug substances was based on the availability and cost. In addition, the solvents employed for the specificity study were selected based on the reported synthetic process of their respective product patents.

The resolution of<0.8 was obtained between benzene and 1,2-dichloroethane using 20 and 30 m capillary GC columns. As the boiling points of benzene and 1,2-dichloroethane are similar. (i.e., 80.1 °C and 83.5 °C, respectively), increasing the column length was expected to improve the resolution. Thus, a column measuring 60 m in length with an internal diameter of 0.32 mm and a particle size of 1.8 μm was employed.

Validation

The system suitability and precision, limit of detection (LOD), limit of quantitation (LOQ), linearity and range, recovery, specificity, robustness, and solution stability of this method for the analysis of benzene and 1,2-dichloroethane were determined as per the ICH validation guidelines Q2, (R1) [29]. Further details regarding each of the above points can be found in the following subsections.

System suitability and precision

As indicated in table 1, a resolution of 1.9 was established between the benzene and 1,2-dichloroethane upon analysis of the standard solution under the optimized conditions. To determine the precision of this analytical system (i.e., an expression of the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions) [29], the standard solution was injected into the chromatograph six times and the percentage relative standard deviation (%RSD) was calculated. The obtained %RSD of<3% indicates that this system was precise (%RSD limit≤15%) [29], and as such, is suitable for analysis of the benzene and 1,2-dichloroethane contents. Further details regarding determination of the system precision are outlined in table 1 below.

Method precision

To determine the precision of this method, six spiked sample solutions were initially prepared. Using the above-described method, the % RSD for the benzene and 1, 2-dichloroethane content were<0.5% for the method precision (limit =<5%) [29], and these values were within 1.0% for the intermediate precision when performed by different analysts, on different columns and instruments on different days. These observations, in combination with the detailed results outlined in table 2, indicate that our developed technique was suitably precise for the system of interest.

Table 1: Determination of the system suitability of % RSD along with resolution and precision for benzene and 1,2-dichloroethane

%RSD of peak area ratios			
Inj. #	Benzene	1,2-Dichloroethane	Criteria
1	0.5394	0.2351	≤15%
2	0.5414	0.2335	
3	0.5464	0.2393	
4	0.5481	0.2437	
5	0.5401	0.2472	
6	0.5471	0.2413	
Mean	0.5437	0.2400	
SD	0.00	0.01	
%RSD	0.71	2.16	
Inj. #	Resolution		Criteria
1	1.9		≥1.2

*Abbreviations: Each value is represented as the mean+SD of 6 measurements (n = 6), SD: standard deviation, RSD: relative standard deviation, # Acceptance criteria<15%.

Table 2: Method and intermediate precision results for benzene and 1,2-dichloroethane

Preparation No.	Benzene content (ppm)	1,2-Dichloroethane content (ppm)
1	2.145	5.151
2	2.147	5.152
3	2.149	5.150
4	2.150	5.153
5	2.150	5.149
6	2.151	5.148
Mean	2.148	5.150
SD	0.00	0.00
%RSD	0.10	0.03
1	2.105	5.056
2	2.107	5.025
3	2.140	5.015
4	2.102	5.006
5	2.109	4.998
6	2.114	5.015
Mean	2.112	5.019
SD	0.01	0.02
%RSD	0.65	0.40

*Abbreviations: Each value is represented as the mean+SD of 6 measurements (n = 6), SD: standard deviation, RSD: relative standard deviation, # Acceptance criteria<5%.

Limit of detection and limit of quantification

The method detection limit (MDL) and method quantification limit (MQL) (i.e., the LOD and the LOQ) were determined based on the signal to noise (S/N) ratio method as outlined in the ICH guideline Q2 (R1). Upon injecting the solution sequence of predetermined known concentrations (0.1–2.5 ppm for benzene and 0.5–5.5 ppm for 1,2-dichloroethane), the S/N ratio for the LOD was determined to be 3:1, while that of the LOQ was determined to be 10:1. Thus, the MDL for benzene and 1,2-dichloroethane were 0.2 and 0.5 ppm, respectively; the MQL for benzene and 1,2-dichloroethane were 0.6 and 1.5 ppm, respectively. These results indicate that our method was sufficiently sensitive for simultaneous trace level determination of the benzene and 1,2-dichloroethane contents in the of pharmaceutical drug substances examined herein.

Linearity and range

The linearity of an analytical procedure reflects its ability to produce results that are directly proportional to the concentration of an analyte in the sample [29]. In this case, linearity tests were performed from the LOQ to 200% of this limit for an analyte concentration. The results of this test and the corresponding correlation coefficients are shown in table 3, while the linearity plots are provided in fig. 3 and 4. As shown, the correlation coefficient was close to 1, indicating that the developed method was indeed linear. Furthermore, the statistical linear regression results indicate that the validated method was linear for pharmaceutical drug substances examined herein, and that this linearity was satisfactory over the defined concentration range (i.e., 0.6–4.2 ppm for benzene and 1.5–9.9 ppm for 1,2-dichloroethane).
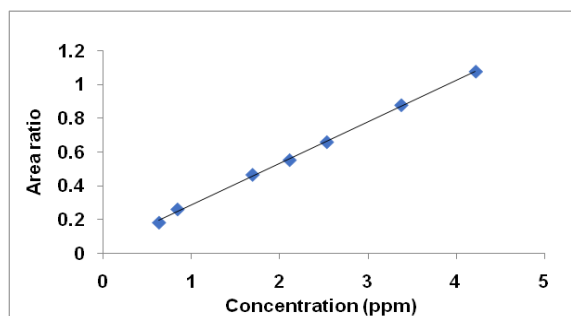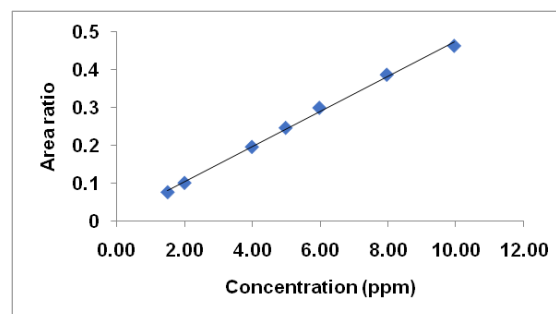

Fig. 3: The linearity plot for benzene

Fig. 4: The linearity plot for 1,2-dichloroethane

Table 3: Linearity data for benzene and 1,2-dichloroethane

Benzene			
Sample No.	**% Level**	**Concentration (ppm)**	**Area ratio**
1	LOQ	0.63	0.1808
2	40	0.84	0.2595
3	80	1.69	0.4645
4	100	2.11	0.5517
5	120	2.53	0.6581
6	160	3.38	0.8772
7	200	4.22	1.0761
Slope		0.246	
Y-intercept		0.0386	
Correlation co-efficient squared (r^2)		0.9991	
1,2-Dichloroethane			
Sample No.	**% Level**	**Concentration (ppm)**	**Area ratio**
1	LOQ	1.49	0.0756
2	40	1.99	0.1002
3	80	3.98	0.1954
4	100	4.98	0.2458
5	120	5.97	0.2988
6	160	7.97	0.3863
7	200	9.96	0.4626
Slope		0.0464	
Y-intercept		0.011	
Correlation co-efficient squared (r^2)		0.9975	

*Abbreviations: Each value is an average of 7 measurements (n = 7), LOQ: limit of quantitation, ppm: parts per million, # Acceptance criteria (r^2)>0.98.

Accuracy

The accuracy of an analytical procedure indicates the closeness of understanding between the quality which is acknowledged either as a true conventional value or an accepted reference value and the value found [29]. For quantitative approaches, a minimum of nine determinations across a specified range should be obtained [29]. In our case, the accuracy (%) for detecting the benzene and 1, 2-dichloroethane in LOQ and in 50%, 100% and 150% levels for the various pharmaceutical drug substances were 89–110% and 91–105%, respectively. These results indicate that our developed method was accurate for the present analytical system, as the mean accuracy value was within the standard 80–120% limit.

Furthermore, the accuracy of this method at the LOQ and at 50, 100, and 150% levels of benzene and 1, 2-dichloroethane is outlined in tables 4 and 5.

Specificity

Specificity is the ability to assess the analyte unequivocally in the presence of other components that may be present in the mixture. These may typically include solvents, impurities, degradants, and matrix components, among others [29]. Thus, the specificity of our method was determined by examination of the interference. No interference was observed either from the blank or from all solvents. Furthermore, the solvent spiking results (i.e., with sample spiking) indicate that benzene

and 1, 2-dichloroethane were not co-eluted with the other solvents. These results confirm the specificity/homogeneity of our developed method for the detection of benzene and 1, 2-dichloroethane. This was also confirmed by GC-MS, and the mass spectra are given in fig. 5 and 6.

Table 4: Accuracy of this method for the detection of benzene in various pharmaceutical drug substances (% recovery)

Pharmaceutical drug substance	LOQ	50%	100%	150%
Cabergoline	89%	92%	91%	101%
Celecoxib	92%	95%	89%	93%
Dronedarone hydrochloride	93%	95%	100%	90%
Etravirine	96%	97%	105%	99%
Etoricoxib	97%	96%	98%	98%
Fesoterodine fumarate	98%	93%	96%	95%
Gabapentin	101%	92%	93%	99%
Irinotecan hydrochloride	98%	91%	89%	102%
Levetricetam	95%	94%	102%	101%
Levothroxine sodium	93%	89%	101%	110%

*Abbreviations: Each value is an average of 10 measurements (drugs) (n = 10), LOQ: limit of quantitation, # Acceptance criteria 80-120%.
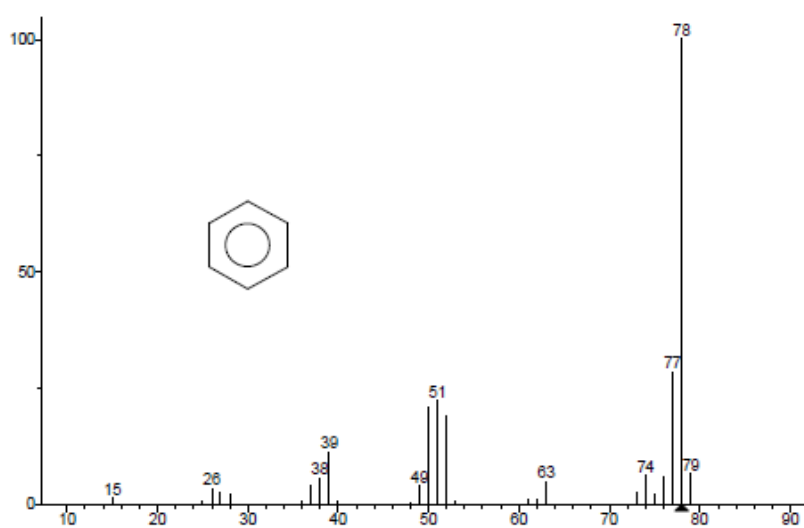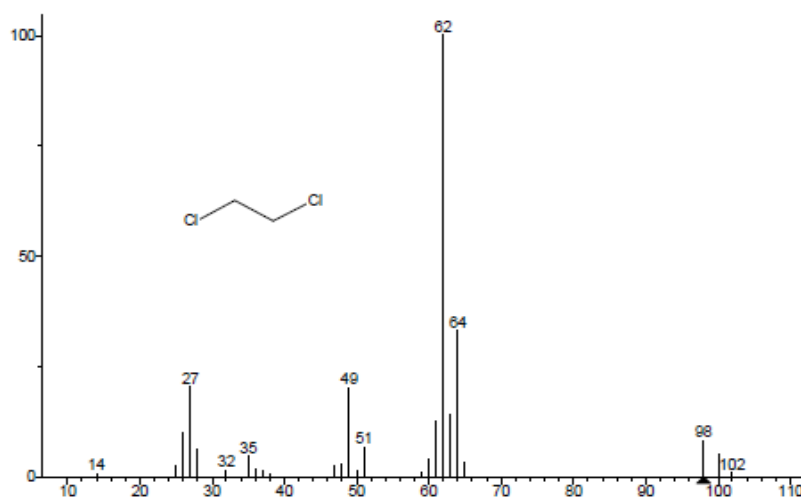

Fig. 5: The mass spectrum of benzene

Fig. 6: The mass spectrum of 1,2-dichloroethane

Robustness

We then examined the effect of chromatographic conditions on the resolution between the benzene and 1,2-dichloroethane. As the original nitrogen gas flow rate was 1.5 ml/min, we varied the flow rate from 1.4 to 1.6 ml/min to investigate its effect on the resolution.

In addition, the column oven temperature was set at 30, 35, or 40 °C to examine the effect of temperature.

Finally, the headspace oven temperature was varied between 100 and120 °C. Interestingly, the resolution was>1.0 under all conditions studied, thus demonstrating the robustness of our method.

Table 5: Accuracy of this method for the detection of 1,2-dichloroethane in various pharmaceutical drug substances (% recovery)

Pharmaceutical drug substance	LOQ	50%	100%	150%
Cabergoline	91%	96%	97%	101%
Celecoxib	102%	100%	101%	98%
Dronedarone hydrochloride	98%	98%	102%	105%
Etravirine	88%	95%	93%	98%
Etoricoxib	91%	97%	100%	102%
Fesoterodine fumarate	95%	102%	100%	101%
Gabapentin	101%	102%	103%	100%
Irinotecan hydrochloride	98%	97%	101%	99%
Levetricetam	92%	101%	103%	98%
Levothroxine sodium	97%	99%	98%	100%

*Abbreviations: Each value is an average of 10 measurements (drugs) (n = 10), LOQ: limit of quantitation, # Acceptance criteria 80-120%.

Solution stability

Finally, the solution stability was determined by examination of a freshly prepared standard solution in a sealed vial at 25 °C over 24 h. The % Difference between the peak areas of benzene and 1,2-dichloroethane at 0 h and 24 h<15% for standard solution [29]. The obtained results thereby confirm that the standard solution was stable under these conditions.

CONCLUSION

We herein reported the versatile gas chromatographic method for the simultaneous quantitative determination and separation of benzene and 1,2-dichloroethane in pharmaceutical drug substances. More specifically, a DB-624 column was employed in our precise and accurate method, yielding acceptable and repeatable recoveries in addition to low limits of detection and quantification. This authenticated method is expected to be applicable in the regular analysis of benzene and 1,2-dichloroethane in quality control laboratories of pharmaceutical drug substances. However, further studies are required to decrease the run time of our method, as this was not possible through simply increasing the column flow rate.

AUTHORS CONTRIBUTIONS

All the author have contributed equally

CONFLICTS OF INTERESTS

The authors declare no conflict of interest

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