DEVELOPMENT AND VALIDATION OF DETERMINATION OF RESIDUAL BENZENE IN PANTOPRAZOLE SODIUM DRUG SUBSTANCE USING HEAD SPACE GAS CHROMATOGRAPHIC METHOD [HS-GC]

Dr. Mohammed Shabana Sultana

Associate Professor, Chebrolu Engineering College, Chebrolu, Guntur District, Andhra Pradesh, India.

Corresponding author E-mail: shabana_chem2007@yahoo.co.in

ABSTRACT

Benzene is a highly toxic solvent that should be limited to no more than 2 ppm in drug substances, as regulated by the International Council for Harmonization guideline Q3C. We herein report quantification of benzene in the Pantoprazole sodium drug substance using head space gas chromatography (HS-GC). Range of the method was from 0.67 to 3.02 ppm according to the results of linearity, accuracy and precision, and limits of detection and quantitation of the method were 0.22 and 0.67 ppm, respectively.

Key Words: Benzene, Pantoprazole Sodium drug substance, ICH guidelines, Validation.

1.0 Introduction

Benzene is a colorless, sweet-smelling, and flammable liquid that is present in crude oil and gasoline. This compound is not only widely used as a solvent, but also as a starting material for various chemical products, including plastics, adhesives, and nylon [1]. However, benzene is known to be highly toxic, particularly with regard to its cancer-causing effects [2] and has, therefore, been classified as "carcinogenic to human (Class 1)" by the International Agency for Research on Cancer [3], which is part of the World Health Organization. In the pharmaceutical industry, the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) guideline Q3C also classified benzene as a Class 1 solvent requiring a strict concentration limit of no more than 2 ppm [10]. Given these circumstances and the current trend of green chemistry, benzene in itself should not be used and has started being excluded from pharmaceutical manufacturing processes.

Various chemicals derived from benzene, however, are still often employed in pharmaceutical manufacturing processes and are known to contain benzene impurities. In particular, the general-use solvents toluene, acetone, methanol and ethanol are known to contain benzene at small concentrations as a by-product of their production process [4-7]. It is not reasonable to consider that the drug product is contaminated by benzene due to employing these solvents because the amount of benzene in these solvents is quite small (ppm order). From a risk assessment point of

view, however, the development of a well-validated analytical method for measuring residual benzene and ensuring that its amount in drug substances and products is less than the recommended maximum value, is still required [8]. The United States Pharmacopeia chapter 467 entitled "Residual Solvents" [9] and the European Pharmacopoeia chapter 2.4.24. entitled "Identification and Control of Residual Solvents" (EP 9.6 2018) both propose head space gas chromatography as a method to measure residual solvents in drug substances regulated by ICH guidelines [8], which include benzene.

However, no method was reported for the determination of residual benzene in Pantoprazole Sodium. Hence, the present work is aimed towards the development of rapid, specific and robust method for the determination of residual benzene in Pantoprazole Sodium at trace level concentration.

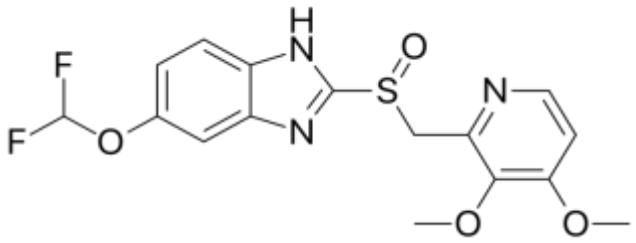

Figure 1.1: Chemical structure of Pantoprazole Sodium

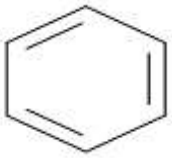

Figure 1.2: Chemical structure of Benzene

2.0 Experimental

2.1 Materials and reagents

Benzene and Dimethyl sulfoxide were obtained from Sigma-Aldrich and pure samples of Pantoprazole Sodium were obtained from synthetic division of Aurobindo pharma Limited. (R&D), Hyderabad and Telangana, India.

2.2 Instruments/Equipments

Head space GC analysis was conducted using a Agilent GC-HSS 7890B series equipped with 7697A Headspace Sampler. DB-624 column (0.53 mm × 30 m, 3.0 µm; J&W Scientific Inc.) was used for analysis. A Mettler Toledo AT261 Semi-Micro Balance was also used for sample preparation.

2.3 Preparation of solutions

Preparation of diluent

Dimethyl sulphoxide used as a diluent

Preparation of standard solution

Transferred 46µL of benzene standard into a 100 mL volumetric flask containing about 50 mL of diluent and make up to volume with diluent.

Further transferred 150µL of the above solution into a 100 mL volumetric flask containing about 50 mL of diluent and make up to volume with diluent.

Preparation of sample solution

Accurately weighed 300mg of the test sample into a 20 mL head space vial added 1.0 mL of diluent and seal the vial immediately.

Preparation of sample spiked solution

Accurately weighed 300mg of the test sample into a 20 mL head space vial added 1.0 mL of benzene standard solution and seal the vial immediately.

3.0 Method Development

The objective of the general method is to determine benzene at low level with selectivity in Pantoprazole sodium drug substance, HS-GC method has intrinsic superior selectivity because this analytical technique only analyses compounds that are evaporated into the sample solution head space. Therefore, a HS-GC method was further explored for the determination of residual benzene in Pantoprazole sodium drug substance. The capillary GC column DB-624 column has reported as suitable for the analysis of a wide range of common ICH residual solvents including benzene in pharmaceutical products and thus was selected for methods development. The sample diluent, temperature program, split ratio, head space oven temperature, and other head space and GC parameters were investigated and optimised using benzene standard solution or benzene standard spiked to sample solutions.

One of the key objectives of the method development was to achieve adequate sensitivity for low level benzene analysis. The benzene method sensitivity was further optimized by the evaluating the effect of split ratio on the noise level and S/N value of a 2 ppm benzene standard solution. Several GC injection split ratios including 10:1, 5:1 and 1:1 were studied. The optimal noise level and adequate signal was observed using the 1:1 spilt ratio injection parameter.

3.1 Chromatographic and Headspace parameters conditions

For head space GC, vial equilibration temperature 90°C, Loop temperature 95°C, transfer-line temperature 100°C, equilibration time 10 min, pressurization time 0.2 min and injection time of sample 1.0 min. GC cycle time 30 minutes. The flame ionization detector (FID) detector was used split ratio 1:1, injection port temperature 180°C, detector temperature 250°C, temperature program was set to 50°C for hold time 5 minutes, then raised to 150°C with rate 10°C per

minute hold time zero minutes and then maintained at 250°C for with rate 25°C per minute hold time five minutes and carrier gas was used nitrogen.

3.2 Method validation

3.2.1 Specificity

The method specificity was validated for potential interference from blank, standard, sample and spiked sample solution. There are no detectable peaks in the chromatograms of blank, standard, sample and spiked sample. The benzene peak in the chromatogram of 2 ppm benzene spiked sample solution is sufficiently resolved from all other peaks before and after benzene peak. The retention time of benzene in the chromatogram of 2 ppm benzene spiked sample solution matches well with that from 2 ppm benzene standard solution.

Table. 1.1 Specificity results

S.No	Name	Retention Time (min)
1	Blank	ND
2	Sample solution	ND
3	Standard solution	8.701
4	Spiked sample	8.703

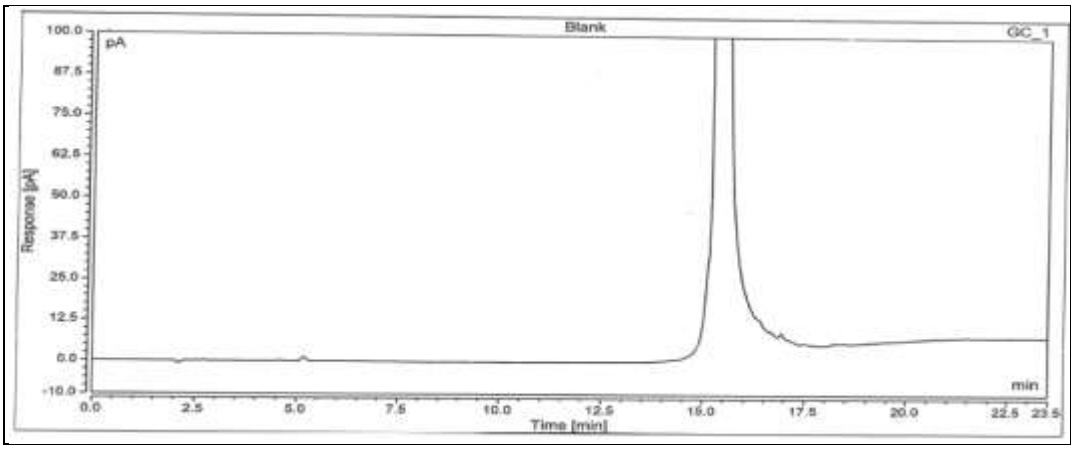

Figure 1.3: Chromatogram of blank

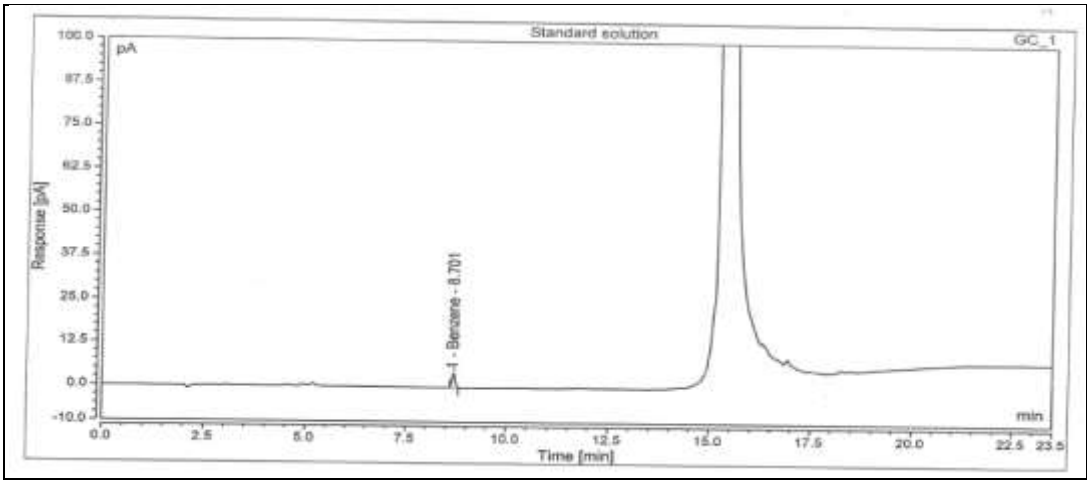

Figure 1.4: Chromatogram of standard

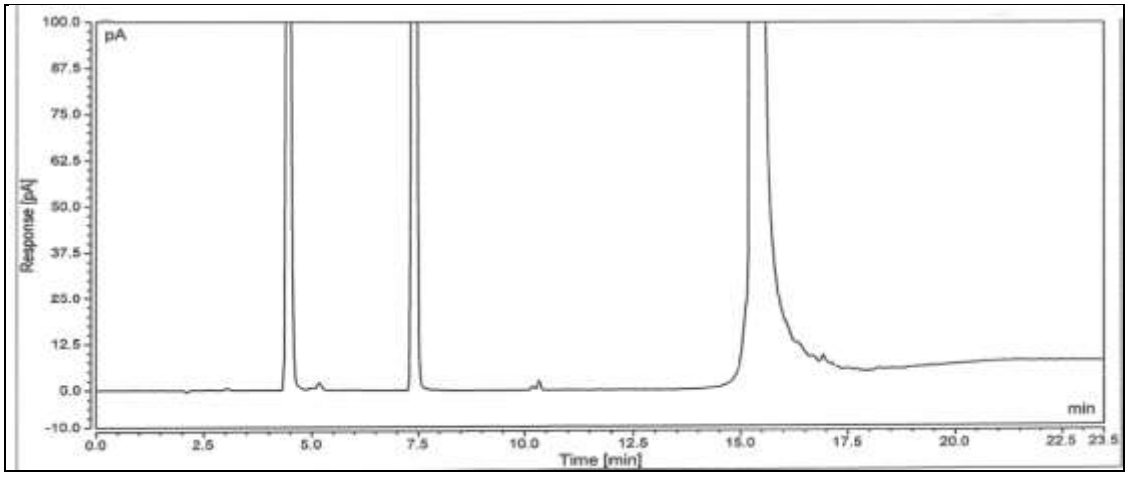

Figure 1.5: Chromatogram of sample

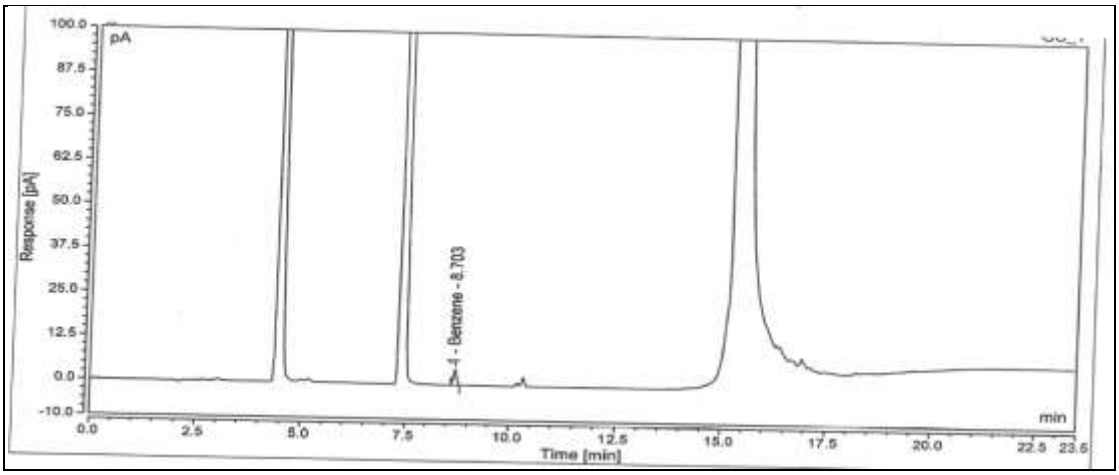

Figure 1.6: Chromatogram of spiked sample

3.2.2 System precision

System precision was demonstrated by preparing standard solution as per method and chromatographed the same into GC system in six replicated injections of standard solution. The peak areas of analyte were recorded for these standard injections. The system precision was evaluated by computing the % Relative standard deviation for the peak area of these standard injections. The observations are tabulated below.

Table. 1.2 System Precision data

Injection No.	Benzene
1	22.2509
2	22.4686
3	22.3934
4	33.4515
5	22.3858
6	22.3450
Mean area	22.3825
SD	0.08
%RSD	0.4

3.2.3 Detection limit (LOD), Quantitation limit (LOQ)

A solution containing 0.67 ppm of benzene standard was injected six times. The RSD of areas, deviations of each six replicates from the linear regression curve and average deviation for each standard were calculated. A solution containing 0.22 ppm of benzene standard was injected three times. The worst found signal to noise ratio for each peak was greater than 3 in each injection. All the peaks were detected in all the three injections.

Therefore, the quantitation limit (QL) and the detection limit (DL) was thus set at 0.67 ppm and 0.22 ppm, respectively. These S/N ratios are much greater than ICH recommended S/N value for DL (S/N~3) and QL (S/N~10).

Table: 1.3 LOQ results of Benzene

S.No	Area of Benzene
1	7.1753
2	6.3559
3	7.1843
4	7.097
5	6.7405
6	6.8683
Avg.	**6.9036**
Std.Dev	**0.32**
%RSD	**4.7**

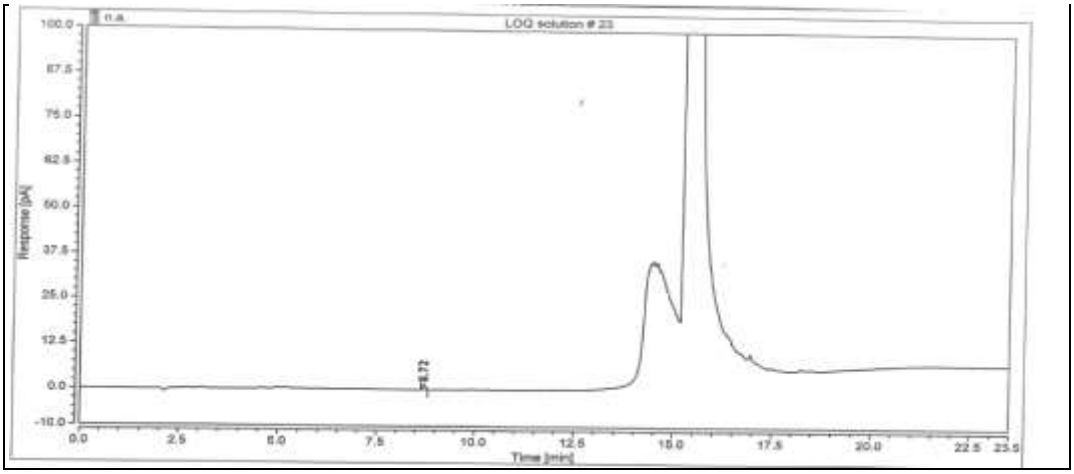

Figure 1.7: Chromatogram of LOQ

3.2.4 Method precision

Precision (repeatability) was evaluated from the recovery data. Recovery data was determined by injecting six sample solutions spiked benzene 2 ppm at specification level. The samples were prepared as per the analytical method.

Table. 1.4 Method precision

S.No.	Benzene (ppm)
1	2.11
2	2.11
3	2.15
4	2.10
5	2.12
6	2.12
Average	2.12
SD	0.02
%RSD	0.9

3.2.5 Linearity and Range

The linearity of benzene was evaluated from 0.67ppm to 3.02 ppm (six levels with duplicate preparations at each level. The peak areas were plotted against the corresponding concentrations and the linear regression was performed. The range 0.67-3.02 ppm was established by meeting the acceptable criteria of linearity.

Table: 1.5 Linearity studies for Benzene by proposed method

%Level	Concentration (ppm)	Area of Benzene
LOQ	0.67	6.8600
50	1.01	10.4796
75	1.51	15.7719
100	2.01	20.8045
125	2.52	26.5136
150	3.02	32.1023
Correlation co-efficient		0.9997
Slope		10.6915
Intercept		-0.3824
%Y-intercept		-1.84

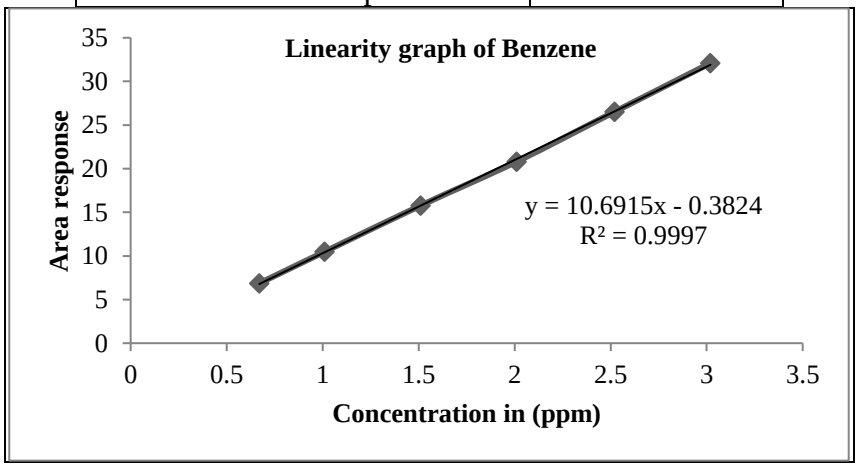

Figure.1.8 Calibration curve for Benzene

3.2.6 Accuracy

Accuracy was determined by analyzing the triplicate preparation of benzene standard at LOQ to 150% levels in the presence of Pantoprazole drug substance, as per the analytical method. The

accuracy as % recovery was calculated from the experimental concentrations of benzene standards by the theoretical concentrations. The recovery of ranged from 104.9% to 109.5% were obtained for the three concentrations levels.

Table: 1.6 Recovery studies for Benzene by proposed method

Accuracy Levels	% Recovery	Mean Recovery
Accuracy at LOQ-1	107.3	109.5
Accuracy at LOQ-2	109.6	
Accuracy at LOQ-3	111.6	
Accuracy at 50%-1	104.6	104.9
Accuracy at 50%-2	107.2	
Accuracy at 50%-3	102.9	
Accuracy at 100%-1	104.8	105.7
Accuracy at 100%-2	105.1	
Accuracy at 100%-3	107.2	
Accuracy at 150%-1	105.2	105.8
Accuracy at 150%-2	105.7	
Accuracy at 150%-3	106.5	

3.2.7 Solution stability

The stability of standard, sample and spiked sample solutions were prepared in duplicate and stored at ambient laboratory conditions (25±2°C) respectively. Therefore, the standard solution, sample solution and spiked sample solution were stable for 24 hrs at room temperature.

Table.1.7 Solution stability of standard at room temperature

Time Interval	similarity factor
Initial	-
12 hrs	0.99
24 hrs	0.94

Table. 1.8 Solution stability of sample at room temperature

Time Interval	Benzene (ppm)	% difference in (ppm)
Initial	Not detected	NA
12 hrs	Not detected	NA
24 hrs	Not detected	NA

Table. 1.9 Solution stability of spiked sample at room temperature

Time Interval	Benzene (ppm)	% difference in (ppm)
Initial	2.11	NA
12 hrs	2.08	0.03
24 hrs	2.04	0.07

4.0 Results & Discussion

A simple, economic, accurate and precise GC method was successfully developed. In this method it was carried out by using DB-624 column (0.53 mm x 30 m, 3.0 µm**.** The results obtained were accurate and reproducible. The method developed was statistically validated in terms of Selectivity, accuracy, linearity, precision, and stability of solution.

For selectivity, the chromatograms were recorded for blank, standard, sample and spiked sample solutions of benzene and pantoprazole drug substance. Selectivity studies reveal that the peak is well separated from each other. Therefore the method is selective for the determination of benzene in pantoprazole drug substance. There is no interference of blank at retention time of benzene standard peak. The elution order and the retention time of benzene in standard and spiked sample solution obtained from individual standard preparation and spiked sample solution preparations are comparable.

The limit of quantitation and limit of detection of benzene 0.67 µg/mL and 0.22 µg/ respectively. The linearity results for benzene in the specified concentration range are found satisfactory, with a correlation coefficient found to be 0.9997.

The accuracy studies were shown as % recovery for benzene at LOQ-150% level. The limit of % recovered shown is in the range of 80 and 120% and the results obtained were found to be within the limits. Hence the method was found to be accurate.

For precision studies (system precision and method precision) were performed. %RSD was determined from the peak areas of benzene for system precision and ppm is calculated for method precision. The acceptance limit should be not more than 10, and the results were found to be within the acceptance limits.

A study to established the solution stability of the standard, sample and spiked sample on bench top was conducted initial and 24 hours. The standard, sample and spiked sample solutions were stable for 24 hours on bench top.

5.0 Conclusion

The Head space Gas chromatographic presented in this report, successfully achieved the main objective of method development, which was to obtain a method that can be used as general method to determine residual benzene in various pharmaceutical drug substances. Therefore, this new method can be used as a general method to determine residual benzene because it has a good potential to work either as-is or with minor modifications for other liquid pharmaceutical drug substances.

Conflict of interests

The authors claim that there is no conflict of interest.

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