Research Article

Development and Validation of Gas Chromatographic Method for Residual Solvents Determination in Difenoxin Hydrochloride

Ojas Joshi*[1], Parag Gide[2], Nitin Nimkar[1]

1. Padmaja Aerobiologicals (P) Ltd. Turbhe, Navi Mumbai 400705, India.
2. Hiranandani College of Pharmacy Ulhasnagar 421003, India.

ABSTRACT

Residual solvents in pharmaceuticals are termed as organic Volatile Impurities. These are the chemicals that are used in the manufacture of drug substance or excipients or use in the preparation of final formulation. Analysis of organic volatile impurities is of key importance for Active Pharmaceutical Ingredients (API). Most of the available methods use liquid chromatography which could be expensive and time consuming. The determination of Organic volatile impurities by Headspace GC method was developed and validated for Difenoxin hydrochloride bulk drug and for its seven residual solvents namely Dimethyl Formamide (DMF), Dichloromethane (DCM), Acetone, Toluene, Ethanol Methanol and Benzene. Analysis was performed on Agilent GC 7820A FID detector and Chemstation software with auto injector. Carrier gas Helium was used with constant flow rate of 2.5mL/min as carrier gas and the separation of residual solvents were achieved on DB-5 column. The thermostat temperature was 125 °C for 20 minute for each vial and after the equilibration the vials were pressurized and injected on GC column. FID detector was used for detection. We used ICH parameters to validate the developed method. The parameter for which the method was validated included specificity, limit of detection and quantification, linearity, precision, accuracy and robustness. The method was successfully used to quantify the levels of specified limit for residual solvents in Difenoxin hydrochloride bulk drug.

Keywords: Difenoxin hydrochloride, drug substance, headspace GC, impurity profiling, residual solvent quantification

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***Address for correspondence:**
Ojas Joshi,
Padmaja Aerobiologicals (P) Ltd. Turbhe, Navi Mumbai 400705, India.
E-mail: ojas.joshi@padmajalab.com

INTRODUCTION

Difenoxin developed in 1970 is an anti diarrheal agent and is closely related to Pethidine (meperidine) which is an analgesic drug and more distantly related to Alphaprodine and Piritramide. It is an active metabolite of the anti-diarrheal drug Diphenhydramine hydrochloride. Difenoxin has mechanism of action working primarily on various opioid receptors in the intestines [1]. Now, the Residual Solvent should either be absent in the product or should be present only below acceptable levels [2-3]. Literature [4-7] points out development of various Headspace GC methods for the identification of organic volatile impurities in pharmaceuticals drugs. However, we could not find a single method to determine the organic volatile compounds using Gas Chromatography coupled with Flame ionization detector (FID). The study reports a development and validation of Headspace GC Method for separation and estimation of organic volatile impurities in Difenoxin hydrochloride bulk drug. The QC of residual solvent [8] analysis was also discussed which gave us an idea that there should be a separate method for estimation residual solvent with specified limit for drug. Some of the methods [9-18] for identification of residual solvent in drugs are also discussed.

The Residual solvents used in the synthesis of Difenoxin hydrochloride are DMF, DCM, Acetone, Toluene, Ethanol Methanol.

Now, the actual manufacture process does not use benzene. The content of Benzene is controlled as there is use of solvents like

Toluene, Acetone and Iso-propanol. Contamination of benzene is possible if it is not controlled at the release of solvent like Isopropanol, Toluene and Acetone. Benzene is present in these solvents as impurity.

Table 1: Residual solvents in Difenoxin hydrochloride

Sr.No.	Residual solvent	Limit	Limit as per ICH	Class
1.	Methanol	Max 100 ppm	3000 ppm	3
2.	Ethanol	Max 100 ppm	5000 ppm	3
3.	Acetone	Max 200 ppm	5000 ppm	3
4.	DCM	Max 200 ppm	600 ppm	2
5.	Benzene	Max 2 ppm	2 ppm	1
6.	Toluene	Max 100 ppm	890 ppm	2
7.	DMF	Max 400 ppm	880 ppm	2

MATERIALS AND METHODS

Reagents

Difenoxin hydrochloride bulk drug sample was obtained from Vasudha Pharma Chem Ltd.with certificate of analysis. DCM, DMF, Methanol, Ethanol, Acetone, Benzene and Toluene used were Analytical grade reagents.

Standard Solution Preparation

Mixture of requisite concentration for solvents was obtained by mixing appropriate aliquots of stock for the above seven solvents in dissolving solvent (DS). The DS was prepared by mixing Benzyl alcohol: Diethylene glycol in the ratio 1:3.

The working concentration of solvents in the solution is as follows:

1. 100µglmL each of methanol, ethanol and toluene.
2. 200µglmL of dichloromethane and acetone.
3. 400µglmL of dimethyl formamide.
4. 2µglmL of benzene.

1 mL of this solution was transferred into each of the 6 HS vials. 1mL of DS was added to each vial. These vials were closed and secured the closure with an aluminium cap.

Test Preparation

About 0.5g of the test sample was taken into each of the two HS vials. Add 2mL of DS to make a fine suspension. The prepared vials were closed with PTFE silicon septa closure and the closures were secured with an aluminum cap.

Blank Preparation

Bland was prepared by adding 2mL of DS into two HS vials. The vials were closed with PTFE silicon septa closure and the closures were secured with an aluminum cap.

Headspace GC Instrumentation

Agilent GC 7820A FID detector and Chem station software. The peaks of residual solvent were resolved on Megabore column with 2.65 micron film thickness, 0.53mm id and 30 mt lengths with stationary phase 5% phenyl polysiloxane (DB-5 J&W make suitable). The chromatographic conditions are listed below for the head- space analysis.

Operating conditions

Table 2: Operating conditions

Carrier gas flow	2.5mL/min.
Carrier gas	Helium
Detector	FID
Column temperature	50°C for 12 mins.
Programme rate-1	10°C/min
Final temperature-1	90°C
Final time-1	0 min.
Programme rate-2	40°C/min.
Final temperature-2	210°C
Injector temperature	200°C
Detector temp.	250°C
Vial temp.	125°C for 20 mins.
Head space needle temperature	135°C
Transfer temperature	135°C
Gas Chromatography cycle time	25 mins.
Pressurizing time	1 min.
Injection time	0.05 min.
Withdrawal time	0.4 min.
Attenuation	-4

Validation

Specificity

The Dissolving Solvent was used to prepare the mix and individual solution at the working concentration level for each Toluene, Benzene, Methanol, Ethanol, and Acetone, DCM and DMF. The blank and

individual solution were chromatographed using the headspace conditions after transferring them in HSS vials

Table 3 : Retention time of residual solvents in Difenoxine hydrochloride

Methanol	2.0 mins
Ethanol	2.4 mins
Acetone	2.7 mins.
Dichloromethane	3.3 mins.
Benzene	7.0 mins.
Toluene	14.1 mins.
Dimethyl formamide	14.5 mins.

Quantification Limit (QL) and Detection Limit (DL)

A number of solutions were prepared to obtain solutions in the range of 1% to 20%. This was achieved by quantitative dilutions of the stock solution of DCM, methanol, benzene, ethanol, acetone, toluene and DMF. Mean peak area of individual solutions was calculated after injection on individual solution. The RSD and the equation of regression line were determined by plotting a graph of mean peak are vs concentration in ppm. The following formula was used to do the calculations

$$LOD = \frac{3.3\ \sigma}{S}$$

$$LOQ = \frac{10\ \sigma}{S}$$

Where,

σ = Residual SD

S = Slope

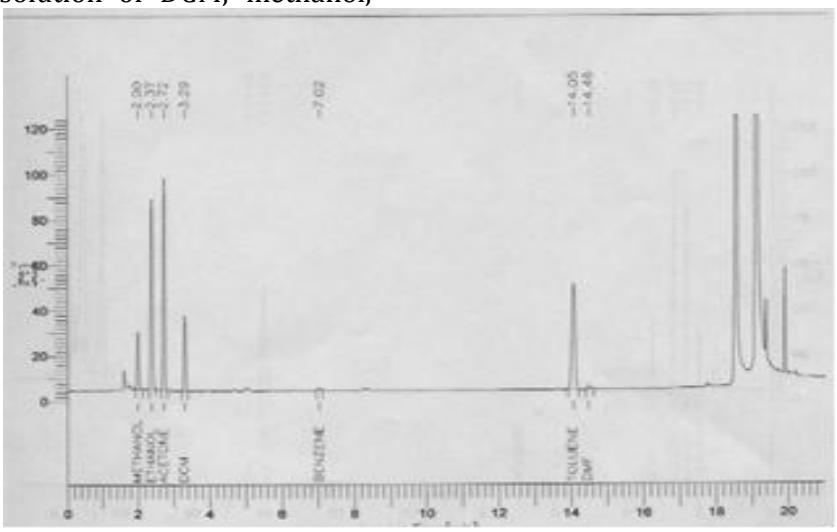

Figure 1: Typical chromatogram for mix standard of solvents in Difenoxine hydrochloride

Linearity

Linearity was calculated by preparing solutions by quantitative dilutions of the stock solution. Each of DCM, Acetone, Ethanol, Benzene, Toluene and DMF was prepared to obtain solutions which were in the range from the Quantification Limit to 160% of the working concentration. The estimation of Mean peak area was done by injecting each solution in duplicate.

The plot of mean peak area against concentration in ppm gave a Regression line. The characteristic of regression line viz- The slope, intercept and correlation coefficient were reported.

Precision

a. <u>System Precision</u>

A standard solution of DCM, DMF, Methanol, Ethanol, Acetone, Benzene and Toluene which was of working concentration was prepared as described in the Methodology section and injected in six replicates into the chromatograph. The mean, SD and relative SD was calculated from the peak areas that were recorded for each solvent.

b. <u>Repeatability</u>

Difenoxine hydrochloride sample was weighed in six different HSS vials. Each of these samples was spiked with DCM, DCF, Acetone, Benzene, Toluene, Ethanol,

Toluene and Benzene so as to obtain six solutions at the concentration level of the 50% limit of DCM, DCF, Acetone, Benzene, Toluene, Ethanol and Benzene. Similar solutions were prepared at 100% and 160% level of working concentration. The mean, SD and RSD of the results was calculated by analyzing each level against a freshly prepared standard of DCM, DCF, Acetone, Benzene, Toluene, Ethanol and Toluene.

c. Intermediate Precision

The process of repeatability mentioned above was repeated on a different date and the RSD, mean and SD of the results was calculated.

Accuracy

The Sample preparations were analyzed in a manner as described in repeatability section.

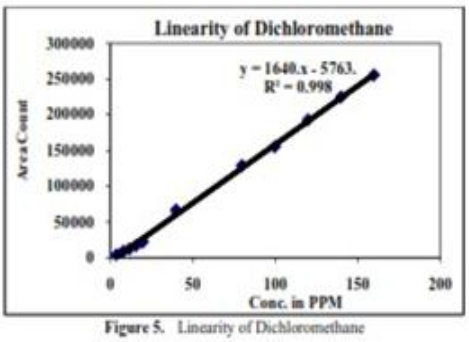

Figure 5. Linearity of Dichloromethane

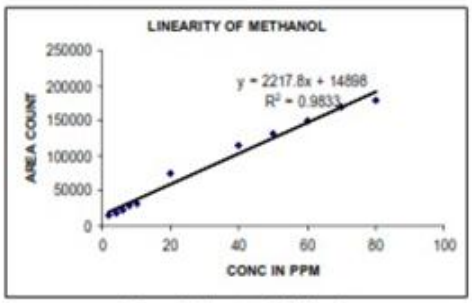

Figure 2. Linearity of Methanol

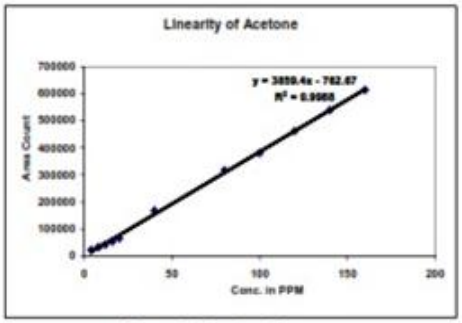

Figure 6. Linearity of Acetone

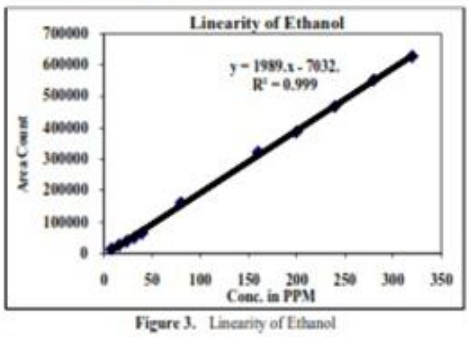

Figure 3. Linearity of Ethanol

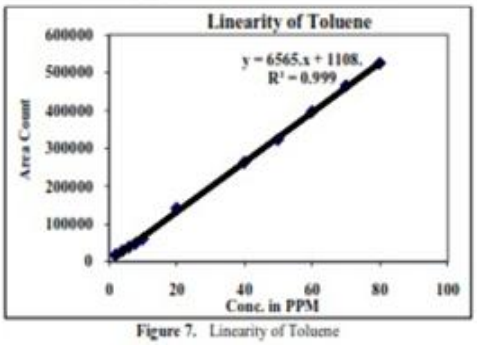

Figure 7. Linearity of Toluene

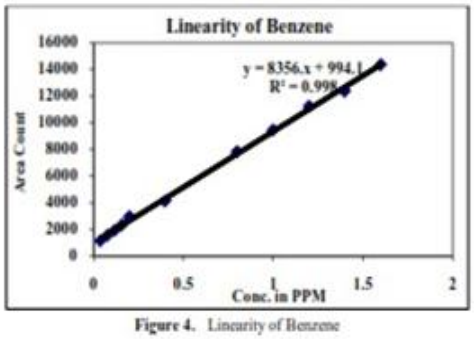

Figure 4. Linearity of Benzene

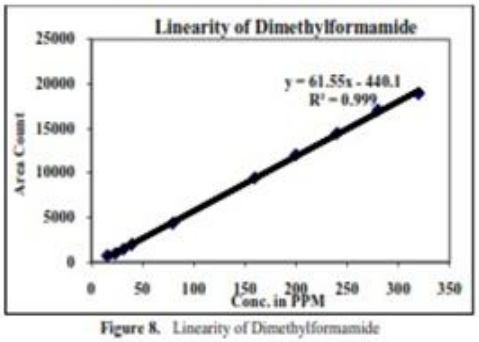

Figure 8. Linearity of Dimethylformamide

Range

Range was defined after linearity, accuracy and precision had been established.

Robustness

The analysis was carried out using the method outlined in the methodology section and by spiking the sample with DCM, DCF, Acetone, Benzene, Toluene, Ethanol and Benzene at the working concentration level. The following alteration in the chromatographic conditions was carried out. Change in flow rate of carrier gas (2.5mLlmin± 0.3mLlmin)

The difference between the results obtained in accordance with the normal method and analysis by altered method was calculated and the system suitability test criteria were evaluated for each condition. The difference in the results of DCM, DCF, Acetone, Benzene, Toluene, Ethanol and Benzene obtained by the normal method and those obtained by carrying out deliberate changes in the method were within ±10%. The system suit- ability criteria were not affected by the deliberate changes made in the method.

Solution Stability

Sample and Standard solution was prepared as described in the methodology. These solutions were analyzed against freshly prepared standard after keeping the sample solution at room temperature for 24 hours. The initial results were then compared with the results at 24 hours. The difference between the two observed values for standard preparation was within ± 5%.

RESULTS AND DISCUSSION

Specificity

No interference of any solvent at the retention time of DCM, DCF, Acetone, Benzene, Toluene, Ethanol and Benzene peaks. The peaks were well re- solved from each other and hence the method was found specific.

Linearity

Correlation Coefficient was more than 0.99 for each solvent and therefore the method is linear in the range of LOQ to 160% of the working concentration.

1. Methanol: The Method is found linear in the range of 2ppm to 160ppm.
2. Ethanol: The Method is found linear in the range of 8ppm to 640ppm.
3. Acetone: The Method is found linear in the range of 4ppm to 320ppm.
4. DCM: The Method is found linear in the range of 4ppm to 320ppm.
5. Benzene: The Method is found linear in the range of 0.08ppm to 3.2ppm.
6. Toluene: The Method is found linear in the range of 2ppm to 160ppm.
7. Dimethyl formamide: The Method is found linear in the range of 16ppm to 640ppm.

Table 4: Experimental Calculated Detection Limit (DL) and Quantification Limit (QL) of various residual solvents

	Methanol	Ethanol	Acetone	DCM	Benzene	Toluene	DMF
Limit of Detection	0.67ppm	0.98ppm	1.06ppm	0.90ppm	0.02ppm	0.18ppm	5.23ppm
Limit of Quantitation (LOQ)	2.04ppm	2.96ppm	3.20ppm	2.27ppm	0.07ppm	0.53ppm	15.84ppm
Established LOQ	2ppm	8ppm	4ppm	4ppm	0.08ppm	2ppm	16ppm

Precision

a. System Precision
   The RSD of the results for Precision experiment was less than 15.0% for all solvents.
b. Repeatability
   The RSD of the results of test for repeatability experiment was less than 15.0% for all solvents.
c. Intermediate precision Experiment
   The relative SD of the results of system precision for intermediate precision experiment was less than 15.0% for all solvents. The relative SD of the results for test of intermediate precision experiment was less than 15.0% for all solvents.

Accuracy

Each Solvents individual recovery at 50%, 100% and 160% level were within 70.0% to 130.0%. The mean preparation for initial

analysis and the analysis after keeping for 24 hour at room temperature. There was no significant change in the results for test preparation after 24 hour. Hence the solution prepared for standard and test were stable for 24 hour.

Range

The Method Complies with parameters of Precision, Linearity and Accuracy in the defined range of limit of detection to 160% of working concentration of each solvent.

1. Methanol: The range of the method was 2 to 160ppm.
2. Ethanol: The range of the method was 8 to 640ppm.
3. Acetone: The range of the method was 4 to 320ppm.
4. DCM: The range of the method was 4 to 320ppm.
5. Benzene: The range of the method was 0.08 to 3.2ppm.
6. Toluene: The range of the method was 2 to 160ppm.
7. DMF: The range of the method was 16 to 640ppm.

Robustness

There was no significant difference in the results for DCM, DCF, Acetone, Benzene, Toluene, Ethanol and Benzene obtained by the normal method and those obtained by carrying out deliberate changes in the method. Hence the method was found robust with respect to change in the flow rate for the carrier gas.

Solution Stability

The difference in the two observed values for standard recovery was within 80.0% to 120.0% for all the solvent other than Acetone. The mean recovery was within 75.0% to 125.0% for Acetone.

CONCLUSION

The suggested method can be successfully used to estimate the residual solvent present in the Difenoxin hydrochloride bulk drug.

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