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# Development and Validation of the Head Space Gas Chromatography Method for the Simultaneous determination and Quantification of Organic Volatile Impurities in Citalopram Hydro Bromide API and its Pharmaceutical Dosage Forms

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**Abstract:** This article describes a simple and rapid gas chromatographic method for identification and quantification of organic volatile impurities present in Citalopram hydro bromide and its pharmaceutical dosage forms. The organic solvents such as Iso-propyl alcohol (IPA), tetra hydro furan (THF), benzene, toluene and dimethyl formamide (DMF) are frequently used in manufacturing of Citalopram hydro bromide. Even after such manufacturing process, some solvents still remain in small quantities. Method for the quantification of residual solvents present in Citalopram hydro bromide was done by head space gas chromatography with flame ionization detector and utilizes the Shimadzu GC- 2010 with FID (ZB-624, 30 m × 0.53 mm, 3 µ) capillary column, nitrogen as carrier gas with a flow rate of 3.0 mL/min. The critical experimental parameters such as oven temperature, zero air, make up flow, injection temperature; split ratio, head space conditions and the selection of diluent were studied and optimized. The retention time of various residual solvents taken individually and in spiked standard solutions were determined. The retention times are5.86min for IPA,8.51min for THF,9.22 min for benzene,11.26 for toluene and 12.41 min for DMF respectively. The proposed method was statistically validated as per standard ICH guidelines. The % RSD for six injections should be NMT 10%. The percentage recovery ranges from 85-115%. The correlation coefficient ($R^2$) is NLT 0.99. The LOD and LOQ was found to be specific. Precision, method precision and intermediate precision was found to be within the acceptance limit.From the obtained validation results the proposed method has been successfully applied for the quantification of organic volatile impurities present in citalopram hydro bromide API and its pharmaceutical dosage forms.

**Key words:** Citalopram hyrobromide, Organic volatile impurities, Method development and Validation.

## I. INTRODUCTION

Citalopram HBr (figure 1) is an orally administered selective serotonin reuptake inhibitor. It is used to treat depression.The IUPAC name is 1-(3-(dimethylamino)propyl) -1-(4-fluorophenyl)-1,3-dihydroisobenzofuran -5-carbonitrile hydro bromide. And the chemical formula and molecular weight are $C_{20}H_{22}BrFN_2O$, 405.3039g/mol.

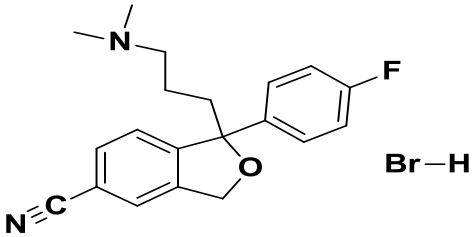


**Figure 1:** Chemical structure of Citalopram hydro bromide

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The level of these organic volatile impurities has to be determined and controlled.There has not been any literature available to simultaneously determine its residual IPA, THF, Benzene, Toluene and DMF in this Citalopram hydro bromide. These structures are followed in Figure 2.

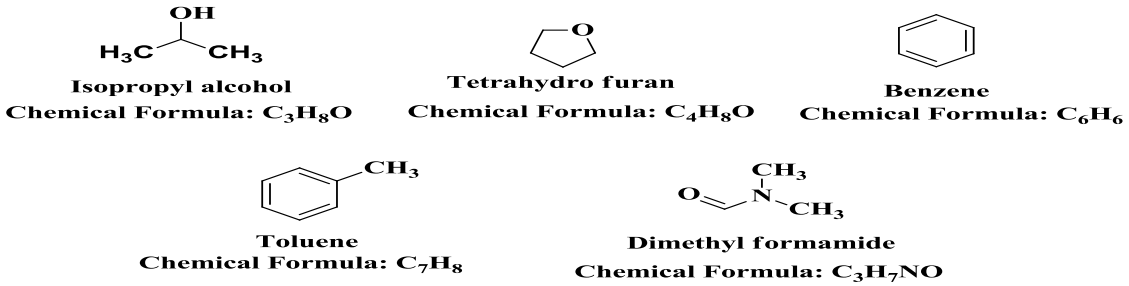


**Figure 2:** Chemical structure of Organic volatile impurities

## II. EXPERMENTAL

### *Chemicals and reagents*

Citalopram hydro bromide was a taken from local well-known laboraties. GC grade IPA, THF, Benzene, Toluene, DMF and di methyl sulfoxide (DMSO) were obtained from Merck -Mumbai.

### *Instrumentation*

Chromatography was performed on Shimadzu chromatographic system equipped with Shimadzu GC- 2010 system with FID. Samples were injected through a Teledyne tekmar HT3TM Head space. Data acquisition and integration was performed using GC-solution software.

### *Chromatographic conditions*

Column:ZB-624 (30 m, 0.53 mm ID, 3 µm); Carrier gas:Nitrogen; Flow rate: 3.0 mL/min; Injector temperature: 225ºC; Split ratio: 1:20; Oven program: initial 40ºC hold for 5 min, increase @ 20 ºC/min up to 200 ºC, hold for 12 min; Detector temperature: 250ºC; Air gas flow: 400 mL/min; hydrogen gas flow: 40 mL/min; Total run time is 25 min.

### *Headspace sampler condition*

Vial temperature: 90ºC; Needle temperature: 100ºC; Transfer line temperature: 110ºC; Vial Conditioning time: 30 min; Vial pressurize time: 3.0 min; Inject time: 1.0 min; GC cycle time: 45Min.

### Preparations

### *Standard Solutions*

According to given Specification limits for organic volatile impurities as per USP General Chapter <467>"Residual Solvents" IPA being class 3 solvent was prepared at about 5000ppm; and THF, toluene and DMF being class 2 solvents were prepared at about 720ppm, 890 ppm, 890 ppm and Benzene being class 1 solvent (Genotoxic impurity) was prepared at about 2 ppm in DMSO with respect to test solution.

***Blank preparation*:** Take 2.0 mL of Dimethyl sulfoxide in a headspace vial and seal with aluminum septum and crimp the cap.

### *Sample preparation*

Accurately weigh and transfer about 500 mg of Citalopram hydro bromide API in to a

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headspace vial, add 2 mL of diluent and seal with aluminum septum and crimp the cap.

***Preparation of Benzene standard stock solution***

Accurately weigh and transfer about100 mg of benzene in to a 10 mL volumetric flask containing about 5 mL of diluent and make up the volume with diluent. Further take 0.25 mL of above Solution into a 50 mL volumetric flask. Diluted to volume with DMSO and mixed well.

***Preparation of standard solution***

Accurately weigh and transfer about1563 mg of IPA, 225 mg of THF, 278 mg of Toluene and 275 mg of DMF in to a 25 mL volumetric flask containing about 15 mL of diluent and make up the volume with diluent. Further take 1 mL of above Solution and 0.5 ml Benzene standard stock solution into a 50 ml volumetric flask. Diluted to volume with DMSO and mixed well.

**Preparation of standard vial:** Take 2.0 mL of standard stock solution in a headspace vial and seal seal with aluminum septum and crimp the cap.

***Tablet preparation***

Twenty tablets were weighed and powdered. An amount of powder equivalent to 500 mg Citalopram hydro bromide was accurately weighed and transferred to a 2 mL head space vial, add 2 mL of diluent and seal with aluminum septum and crimp the cap.The mixture was sonicated for 5 to 10 min.

The Organic volatile impurity content (ppm) calculated by the following formula:

$$\text{Calculation (ppm):} \frac{\text{Impurity area in test solution}}{\text{Impurity area in standard solution}} \times \frac{\text{Standard concentration (mg)}}{\text{Sample concentration (mg)}} \times 10^6$$

## III.RESULTS AND DISCUSSION

***Method development***

This method development was implemented following Quality-by-Design principles including diluent selection, column selection. During the HSGC method development, in order to select the most appropriate system parameters to obtain the best separation, sensitivity and time efficiency, solvent mixtures were injected under a variety of conditions, e.g. at different GC Columns (DB-5, VF-1, ZB-624), HS temperatures, vial-room temperature (70-90ºC), needle temperature (80-110ºC), transfer line temperature (90-130ºC), detector temperatures (200-300ºC), injector temperatures(100-230ºC), GC gradients (40-230ºC, at the rate of 10–40ºC /min), carrier gas flow rates (2.0-4.0ml/min), different diluents (NMP, DMSO and DMF) etc. The final HS-GC conditions used for method validation were obtained based on optimized HS and GC parameters. Each of the solvents was injected once separately to determine method specificity and signal response sensitivity.

**Method validation**

The method validation was done by evaluating specificity, repeatability, method precision, limit of detection (LOD) and limit of quantitation (LOQ), linearity, accuracy, ruggedness, and solution stability of residual solvents as indicated in the ICH harmonized tripartite guideline[1-5].

***Specificity***

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Specificity of the method was shown by injecting the blank, Sample preparation and Standard solution and showing the resolution between all peaks are in both sample solution and Standard
Solution and there was no interference from the blank at the retention times of analyte peaks those were obtained from standard solution and resolution of more than 2.0 was obtained between two closely eluting peaks which meets the acceptance criteria. The corresponding data and chromatograms were shown in table 1and figure 3.

**Table 1:** Specificity data for organic volatile impurities

| Name | RT | Area | USP Resolution | USP Plate Count | USP Tailing |
|---|---|---|---|---|---|
| IPA | 5.86 | 636472 | ---- | 25833 | 1.09 |
| THF | 8.51 | 1122251 | 20.08 | 80003 | 1.08 |
| Benzene | 9.22 | 1673500 | 5.96 | 99117 | 1.04 |
| Toluene | 11.26 | 1046148 | 18.97 | 206758 | 1.04 |
| DMF | 12.41 | 78279 | 11.81 | 266503 | 1.36 |

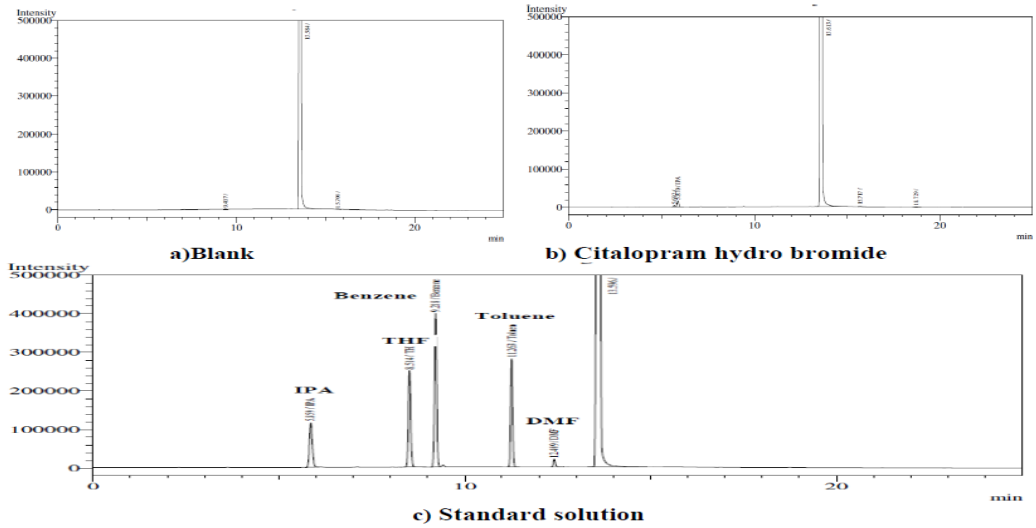


**Fig.3:** Chromatograms for Specificity

**System precision**

System precision was determined by injecting six replicate injections of standard solution respectively and analyzed as per ICH guidelines. The system precision of this method is expressed in the term of % RSD of the data. The RSD was found out to be less than 15 %. All values and chromatogrm are shown in table 2 and figure 4.

**Table 2:** System precision data for OVI'S

| OVI'S | IPA | THF | Benzene | Toluene | DMF |
|---|---|---|---|---|---|
| **Mean±STDV** | 1448594±53508 | 868478±5958 | 3857±60 | 878123±7131 | 49809±1782 |
| **%RSD** | 3.69 | 0.69 | 1.57 | 0.81 | 3.58 |

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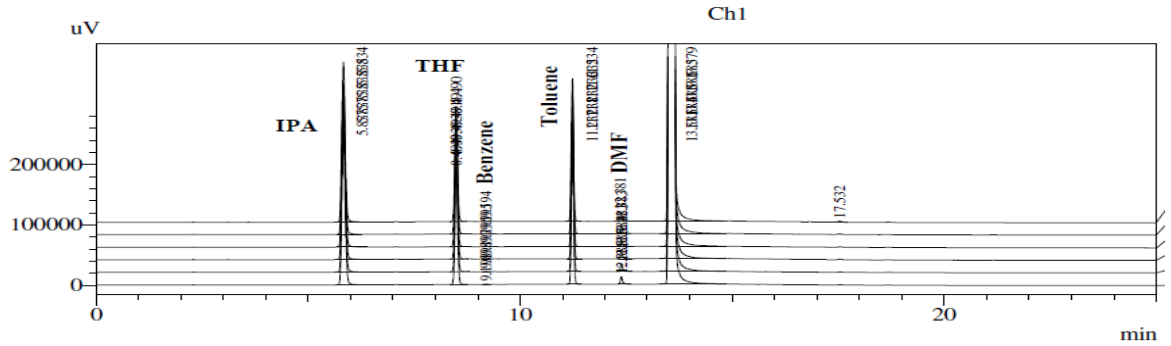


**Fig.4:** Overlay chromatogram for System precision

***Method precision***

The method precision of the proposed method is expressed in the term of % RSD of the data. Method precision has been demonstrated by separately analyzing of sample six preparations as per the method. RSD was found to be less than 10 %. All values and chromatogrm are shown in table 3 and figure 5.

**Table 3:** Method precision data for OVI'S

| OVI'S | IPA | THF | Benzene | Toluene | DMF |
|---|---|---|---|---|---|
| **Mean±STDV** | 1433662±49979 | 913494±10277 | 4059±40 | 904817±8806 | 49199±2188 |
| **%RSD** | 3.49 | 1.13 | 0.98 | 0.97 | 4.45 |

***Linearity (Low level) for LOD and LOQ***

Linearity of the method was determined over the concentration range of 5-25% for four organic volatile impurities. Two replicates were performed at each level. Correlation coefficient ($R^2$), STEYX, SLOPE, LOD and LOQ were calculated from these linearity data and shown in table 4.

**Table 4:** Linearity (Low level) data for LOD and LOQ

| Con (%) | Avg. area of IPA | Avg. area of THF | Avg. area of Benzene | Avg. area of Toluene | Avg. area of DMF |
|---|---|---|---|---|---|
| 5 | 68906 | 43327 | 214 | 44487 | 2446 |
| 10 | 149453 | 86563 | 432 | 88146 | 4827 |
| 15 | 214186 | 129463 | 643 | 131292 | 6990 |
| 20 | 281638 | 175181 | 891 | 177276 | 9292 |
| 25 | 376838 | 218548 | 1068 | 221578 | 12660 |
| **r2** | **0.998** | **1.000** | **0.999** | **1.000** | **0.996** |
| **STEYX** | **9374** | **831** | **17** | **863** | **403** |
| **SLOPE** | **14961** | **8781** | **43** | **8866** | **498** |
| **LOD(%)** | **2.07** | **0.31** | **1.31** | **0.32** | **2.67** |
| **LOQ(%)** | **6.27** | **0.95** | **3.96** | **0.97** | **8.10** |

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***Linearity***

The linearity of the method was determined by making injections of each organic volatile impurity over the range 25-150% and LOQ level. Two replicates were performed at each level. The calibration curves were obtained with the average of peak area ratios of two replicates. The correlation coefficient (r2) values for all organic volatile impurities were found to be higher than 0.99 and the calibration curves were linear within the range. All values and linearity graph are shown in table 5 and figure 6.

**Table 5:** Linearity data for organic volatile impurities

| IPA | | THF | | Benzene | | Toluene | | DMF | |
|---|---|---|---|---|---|---|---|---|---|
| Con (ppm) | Avg Area | Con (ppm) | Avg Area | Con (ppm) | Avg Area | Con (ppm) | Avg Area | Con (ppm) | Avg Area |
| 314 | 54801 | 6.84 | 8690 | 0.1 | 166 | 8.63 | 10162 | 71.3 | 4363 |
| 1250 | 376838 | 180 | 218548 | 0.5 | 1068 | 223 | 221578 | 220 | 12660 |
| 2500 | 768455 | 360 | 438150 | 1 | 1980 | 445 | 442722 | 440 | 24921 |
| 3500 | 1061847 | 540 | 648450 | 1.75 | 3471 | 668 | 645203 | 660 | 35489 |
| 5000 | 1553949 | 720 | 862483 | 2 | 3751 | 890 | 861088 | 880 | 46640 |
| 7500 | 2492191 | 1080 | 1336492 | 3 | 5917 | 1335 | 1392368 | 1320 | 71572 |
| **r2** | **0.999** | **1.000** | | **0.999** | | **0.998** | | **1.000** | |

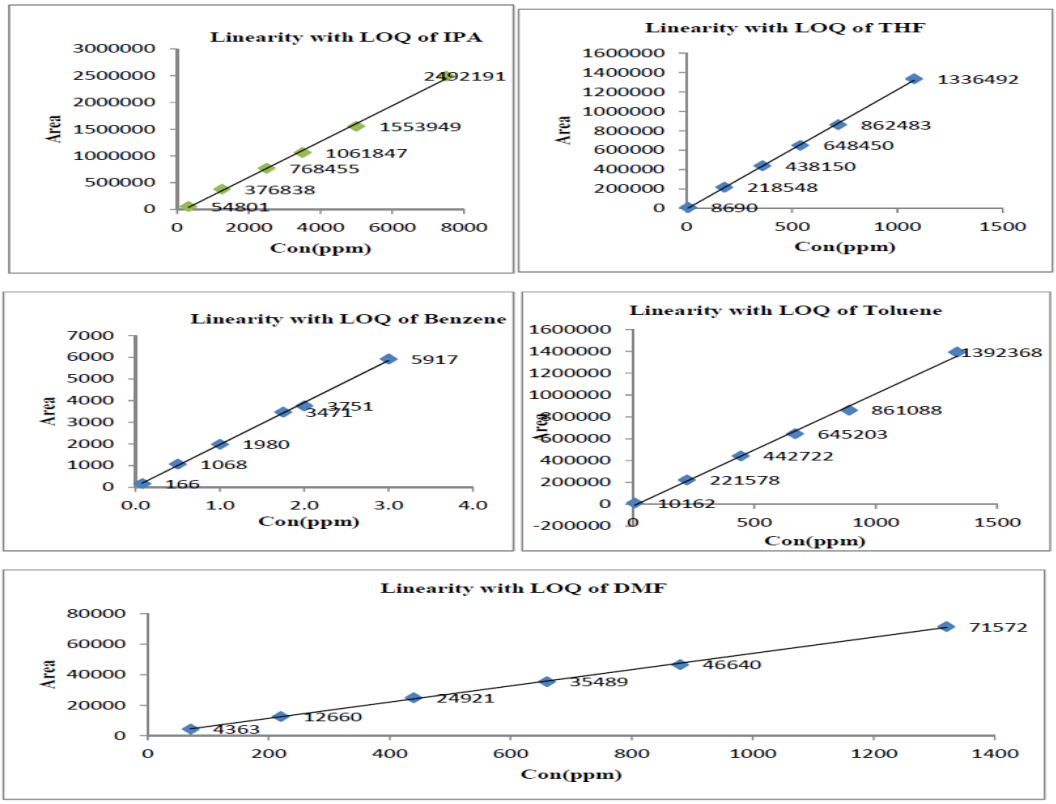


**Fig.6:** Linearity graph for OVI'S

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

The LOD and LOQ for the proposed method were determined using calibration standards and calculated using 3.3 σ /s and 10 σ/s formulae respectively, where s is the slope of the calibration curve and σ is the standard deviation of y- intercept of the regression equation. Results are shown in table 6.

**Table 6:** LOD and LOQdata of OVI'S

| Name of OVI'S | LOQ(µg/ml) | LOD(µg/ml) | Area of LOQ | Area of LOD |
|---|---|---|---|---|
| IPA | 0.314 | 0.104 | 54801 | 18598 |
| THF | 0.007 | 0.0023 | 8690 | 2711 |
| Benzene | 0.0001 | 0.00003 | 166 | 57 |
| Toluene | 0.009 | 0.003 | 10162 | 2897 |
| DMF | 0.071 | 0.024 | 4363 | 1112 |

***System precision at LOQ***

The system precision of this method is expressed in the term of % RSD of the data. System precision at LOQ concentration has been demonstrated by six replicate injections of standard solutions. The RSD was found out to be less than 10 %. Results are summarized in table 7.

**Table 7:** System precision data OVI'S at LOQ

| OVI'S | IPA | THF | Benzene | Toluene | DMF |
|---|---|---|---|---|---|
| **Mean±STDV** | 55600±1164 | 8869±453 | 163±5 | 10277±291 | 3720±166 |
| **%RSD** | 2.09 | 5.11 | 3.24 | 2.83 | 4.47 |

***Accuracy***

Accuracy of the methods was assured by applying the standard addition technique. The % recovery was calculated. The mean % recovery of each solvent at 50%, 100%,150% and LOQ levels should not be less than 85.0 and not more than 115.0. Results obtained were within the limits indicating the method as accurate and are shown in table 8.

**Table 8:** Accuracy data for OVI'S

| OVI'S | Recovery at 50% | Recovery at 100% | Recovery at 150% | Recovery at LOQ |
|---|---|---|---|---|
| IPA | 107.89 | 107.67 | 99.83 | 107.35 |
| THF | 101.58 | 101.32 | 107.61 | 102.51 |
| Benzene | 105.92 | 102.88 | 105.58 | 104.09 |
| Toluene | 107.93 | 106.97 | 106.13 | 106.01 |
| DMF | 107.91 | 107.46 | 104.06 | 99.41 |

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***Robustness***

This study was performed by making small and deliberate variations in the method parameters. The variation in the column flow(±0.2mL/min) and vial condition temperature(±5°C) was done and the results were obtained within the acceptance criteria indicating the method is robust within the specified range. % RSD values were less than 10% as shown in table 9.

**Table 9:** Robustness data for four OVI'S

| Flow and Vial temp. change | %RSD(n=6) | | | | |
|---|---|---|---|---|---|
| | IPA | THF | Benzene | Toluene | DMF |
| Flow 2.8 ml/min | 4.49 | 1.28 | 1.21 | 0.58 | 2.80 |
| Flow 3.2 ml/min | 4.91 | 1.18 | 1.43 | 2.25 | 1.83 |
| Vial temperature 75°C | 2.10 | 1.26 | 2.73 | 2.51 | 4.53 |
| Vial temperature 85°C | 4.87 | 2.24 | 2.67 | 2.15 | 4.76 |

***Ruggedness***

Ruggedness of the method was evaluated by performing the sample analysis in six replicates using different analyst on different days and the results were obtained within the acceptance criteria indicating the method is rugged within the specified range. The %RSD values of less than 10.0%. The results are presented in table 10.

**Table 10:** Ruggedness datafor four OVI'S

| Days and Analysts | %RSD(n=6) | | | | |
|---|---|---|---|---|---|
| | IPA | THF | Benzene | Toluene | DMF |
| Day-1(Analyst-1) | 2.13 | 1.02 | 1.53 | 1.23 | 4.13 |
| Day-1(Analyst-2) | 3.63 | 2.37 | 1.69 | 2.25 | 4.00 |
| Day-2(Analyst-1) | 4.90 | 2.24 | 1.13 | 2.46 | 3.39 |
| Day-2(Analyst-2) | 4.34 | 3.25 | 3.28 | 3.46 | 4.09 |
| Cummulative Days and Analysts | %RSD(n=12) | | | | |
| | IPA | THF | Benzene | Toluene | DMF |
| Day-1(Analyst-1&2) | 2.89 | 1.76 | 1.68 | 1.73 | 3.89 |
| Day-2(Analyst-1&2) | 4.47 | 2.8 | 2.37 | 2.95 | 3.66 |
| Analyst-1(Day1&2) | 4.31 | 1.67 | 1.65 | 2.06 | 3.97 |
| Analyst-2(Day1&2) | 4.59 | 2.75 | 2.49 | 2.79 | 4.04 |

***Application of the proposed method (Analysis of Citalopram hydro bromide tablet):***

The proposed method was evaluated by the assay of commercially available Citalopram hydro bromide tablet for quantification of organic volatile impurities present in it. The results obtained for organic volatile impurities were compared with the corresponding specification limits of standard guidelines and reported in table 11. This revealed that concentration of organic

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volatile impurities present Citalopram hydro bromide tabletin ppm levels which were less than the specified limits.

**Table 11:** Assay results of commercially available Citalopram hydro bromide tablet.

| Name of The drug | Label claim(mg) | IPA (ppm) | THF (ppm) | Benzene (ppm) | Toluene (ppm) | DMF (ppm) |
|---|---|---|---|---|---|---|
| **Citalopram hydro bromide** | 20 | 240 | Not detected | Not detected | 75 | Not detected |

## IV. CUNCLUSION

A single, rapid and highly selective HS-GC method was developed and validated for the quantification of organic volatile impurities present in Citalopram hydro bromideAPI through an understanding of the synthetic process, nature of solvents and nature of stationary phases of columns. The developed gas chromatographic method has to evaluate reliable and economical result for the determination of IPA, THF, Benzene, Toluene and DMF as organic volatile impurities present in Citalopram hydro bromide. The results of various validation parameters confirmed that the method is specific, System Suitability, Limit of Detection, Limit of Quantification, Accurate (% of recovery studies) as per ICH guidelines. The method was found to be applicable for the routine analysis of the Citalopram hydro bromide API and its pharmaceutical dosage forms in pharmaceutical industry.

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