DEVELOPMENT AND VALIDATION OF A HEADSPACE GAS CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF METHYL BROMIDE CONTENT IN ITRACONAZOLE API

MANNEM DURGA BABU\textsuperscript{1}, MEDIKONDU KISHORE\textsuperscript{2}, K.SURENDRA BABU\textsuperscript{3}

\textsuperscript{1}Research Scholar, \textsuperscript{2}Professor, \textsuperscript{3}Principal (Post Graduation)
Department of Post graduate Chemistry, SVRM College (Autonomous)
Nagaram, Andhra Pradesh, India

Corresponding Author: drmedikondukishore@gmail.com

Key words: Methyl Bromide, Head Space Gas Chromatography, Method development, Method validation.

ABSTRACT. To provide quality control over the manufacture of any API, it is essential to develop highly selective analytical methods. Gas chromatography with headspace (HSGC) is widely used for the determination of residual impurities and solvents in API’s. In the current article we are reporting the development and validation of a rapid and specific Head space gas chromatographic (HSGC) method for the determination of methyl bromide in Itraconazole API. The developed method was validated in terms of specificity, linearity, precision, accuracy, limit of detection (LOD) and limit of quantitation (LOQ). The developed method was utilized for the investigation of methyl bromide content in bulk drug.

1. INTRODUCTION

Itraconazole is a \(\text{C}_{35}\text{H}_{38}\text{Cl}_{2}\text{N}_{8}\text{O}_{4}\) which include UV spectroscopy, Reverse Phase High Performance Liquid Chromatography, Ultra Pressure Liquid Chromatography, LCMS, GC-MS methods. The present studies illustrate development and validation of simple, economical, selective, accurate, precise GC-HS method for the determination of Methyl Bromide in Itraconazole API as per ICH guidelines. In the present work a successful attempt had been made to develop a method for the determination of Methyl Bromide in Itraconazole API and validate it. The method would help in estimation of the Methyl Bromide in single run which reduces the time of analysis and does not require separate method for the drug. Thus the paper reports an economical, simple and accurate GC-HS method for the above said API’S.
Alkyl halides are categorized as genotoxic impurities (GTI) based on structure-activity relationship. During the manufacturing process of Itraconazole, formation of methyl bromide is possible due to residual methanol available in the manufacturing process and may also be formed due to thermal interaction in presence of methanol. This paper discusses analytical methodology for a specific class of GTI, the alkyl halides. GTI’s are unusually toxic material which could potentially impact genetic material by mutation. These changes to the genetic material, caused by exposure to very low levels of a genotoxin, can lead to cancer. While ICH published adequate controls for general process-related impurities (Q3A, Q3B), Pharmaceutical Genotoxic impurities (GTI) gained global prominence when regulatory bodies like European Medicines Agency (EMEA) and United States Food and Drug Administration (USFDA) [4,5] some few years back published stringent qualification thresholds lower than the default values set by ICH guidelines. These dictums confirmed that the existing ICH thresholds may not be acceptable for such DNA-reactive genotoxins or carcinogenic impurities. Genesis of these impurities could be from variety of sources, namely but not limited to starting materials, reagents, intermediates, solvents or unwanted side reactions of the active pharmaceutical ingredient (API) synthetic process that Gas chromatography, Electron capture detector, Validation. get carried over into the final product. In addition, the API itself can decompose to form genotoxic impurities or they can form in the drug product by reaction between excipients or containers and the API. In some cases, trace level of solvents could react with intermediates and form potential genotoxic impurities. Determination of methyl bromide at trace levels requires highly sensitive analytical methodology.

2. MATERIALS
Itraconazole and Methyl Bromide was obtained from Ranbaxy Research Laboratories, Gorgon, India. Milli Q water was obtained from in house Milli Q water plant. N, N-Dimethyl Acetamide was purchased from Fluka Chemical Co., Inc. (Milwaukee, WI, USA).

**Instrumentation:**
A Gas chromatograph (Shimadzu, GC 2010) equipped with a flame ionization detector, a Headspace sampler (Teledyne tekmar H) was used to load the sample. An analytical balance (XS 205 from Mettler Toledo) and autopippette (100 – 1000μL from Eppendorf) were used.

**Chromatographic conditions:**
A volume of 1ml standard and sample solution was injected into the GC injection port. The temperature of the injection port was maintained at 225°C at a split ratio of 1:10, with nitrogen as a carrier gas. The pressure was maintained at 2.1 psi with flow of 3 mL min⁻¹. The temperature of the detector was set at 250 °C. Temperature gradient was maintained at 40 °C for 5 min and then increased at a rate of 20 °C min⁻¹ up to 200 °C to a final temperature of 200 °C and maintained for 7 min.

**HEAD SPACE CONDITIONS:**

| Parameter               | Value   |
|-------------------------|---------|
| Vial temperature        | 90°C    |
| Incubation time         | 30 min  |
| Transfer line temperature| 110°C   |
| Needle temperature      | 100°C   |
| Pressurization time     | 2 min   |
| Loop fills time         | 0.5 min |
| Injection volume        | 1 ml    |
| Injection time          | 1 min   |

**GC CONDITIONS:**

| Parameter       | Value                                      |
|-----------------|--------------------------------------------|
| Column          | ZB-624, 30m length, 0.53 mm internal diameter, and 3.0 um film thickness. |
| Carrier gas     | Nitrogen                                   |
| Flow rate       | 3.0ml/min (linear velocity 22.7cm/sec)    |
| Injector temperature | 225° c                                    |
Oven program: Initial 40°C, Held for 5 minutes Increase at 20°C per Minutes to 200°C Held at 200°C for 7 minute

**Preparation of Standard Solution: Diluent:** N, N-Dimethyl acetamide was selected as the standard and sample diluent because of its ability to dissolve a wide variety of substance. It has a high boiling point that does not interfere with more volatile solvents, analyzed by GC.

**Preparation of Standard Stock Solution:** Weighed accurately 50 mg of Methyl Bromide in 50 ml of volumetric flask dissolve and make up with diluent. Transfer 1 ml of above solution into a 50 ml volumetric flask make up with diluent.

**Preparation of Standard solution:** Transfer 4.7ml of above Standard stock solution into a 50 ml volumetric flask and make up to the mark with the same diluents to get a standard solution. Then the standard vials were prepared with 2 ml of the Standard solution and seal the vial with aluminum closure. Heat the sealed vial at 90°C for 30min.

**Preparation of Sample solution:** Weighed accurately 1.0 gm of the Itraconazole API into Head Space vial, and add 2 ml of DMA solvent and seal the vial with aluminum closure. Heat the sealed vial at 90°C for 30min.

**Method Optimization**

An understanding of the nature the various Genotoxic Impurities present in API is the foremost prerequisite for successful method development in HSGC. In addition, successful method development should result in a fast, simple and time efficient method that is capable of being utilized in a manufacturing setting. Following were the stepwise strategies for the method development in our case.

**Column selection:** The primary goal of column selection was to resolve a Genotoxic Impurity which is formed during the synthesis and manufacturing of Itraconazole API. Several columns were initially investigated to finalize a single method for the separation and quantitation of solvent. Wall-coated capillary columns of various brands with a variety of phases and dimensions have been investigated, e.g., column A is VF-1 ms (30 m length, 0.32 mm i.d. with a stationary phase of 100% dimethyl polysiloxane film of 1.0 µ) and Column B is ZB-624 (30 m length, 0.53 mm i.d. with a stationary phase of 6% cynopropyl phenyl and 94% dimethyl polysiloxane film of 3.0 µ). In the above two columns, the response was found to be comparatively lower and peak shapes were found to be satisfactory in Column B. Therefore, ZB-624 with dimensions of 30 m × 0.53 mm, 3.0 µ proved to be the best column that could fulfill all the needs of the method, i.e., higher sensitivity, shorter runtime.

**Thermal program and thermal gradient:** A linear thermal gradient was chosen to provide elution of the Impurity’ peak during the isothermal segment of the chromatographic run for better quantification. An initial hold of 5 min at 40°C and a linear thermal gradient to 200°C at 20°C/min was found to give the best peak shape and retention.

**Headspace method optimization:** The headspace method was optimized in such a way that maximum amount of the Impurity present in the sample get evaporated for the detection. For this the standard and sample vials were heated at 70–110°C for 20–30 min with constant Shaking. A combination of sample vial heating at 90°C with 30 min shaking was found to be suitable for getting a good response.

**Method validation:** The method validation was done by evaluating Specificity, Repeatability, linearity and range, Accuracy, Limit of Detection (LOD) and Limit of Quantitation (LOQ), LOQ-Repeatability, LOQ-Accuracy, Ruggedness and Robustness.

1) **Specificity:** The Itraconazole API sample was spiked with methyl bromide and sample was chromatographed to examine interference, if any, of the residual solvent peaks with each other. The retention time for standard methyl bromide 2.99 min, respectively.
Figure 1: Typical chromatograms of (a) Blank (DMA) (b) Methyl Bromide (c) Itraconazole, (d) Methyl Bromide and Itraconazole Spiked
2) **Repeatability:** The Methyl Bromide was prepared at 1.88 ppm absolute with respect to Sample concentration and injected in six replicates. The RSD (n=6) values obtained for the area of Methyl bromide is 3420. The %RSD for methyl bromide peak area response of Standard six injections should not more than 15%.

![Typical chromatogram showing %RSD for methyl bromide.](image)

Table 1: Repeatability data for Methyl Bromide

| S.No. | Methyl Bromide Area |
|-------|---------------------|
| 1     | 3447                |
| 2     | 3896                |
| 3     | 2808                |
| 4     | 3771                |
| 5     | 3668                |
| 6     | 2927                |

Average area: 3420

Standard Deviation: 454

% of RSD: 13.27

3) **Linearity & Range:** The linearity of the method was determined by making injections of Standard Methyl Bromide solvent over the range 50-150% LOQ. Three replicates were performed at each level. The calibration curves were obtained with the average of peak area ratios of three replicates. The correlation coefficient (r2) value for methyl bromide was found to be higher than 0.997 and the calibration curves were linear within the range. These results revealed an excellent linearity. The linearity values for the Methyl bromide as shown in figure.

![Figure 2: Typical chromatogram showing %RSD for methyl bromide.](image)

Table 2: Linearity data for Methyl Bromide

| S.No. | Concentration level | Run-I Area | Run-II Area | Run-III Area | Average Area |
|-------|---------------------|------------|-------------|--------------|--------------|
| 1     | 50%                 | 1504       | 1537        | 1467         | 1503         |
| 2     | 80%                 | 2423       | 2556        | 2418         | 2466         |
| 3     | 100%                | 2886       | 3083        | 3120         | 3030         |
| 4     | 120%                | 3841       | 3718        | 3831         | 3797         |
| 5     | 150%                | 4956       | 4676        | 4459         | 4697         |
Figure 3: Linearity Graph. Table 3: Linearity Graph data for Methyl Bromide (Right)

4) Accuracy (%recovery):
A known amount of sample (about 1.0 gr) was taken separately in three different vials and spiked with 2.0 ml of methyl bromide at three different levels (50, 100 & 150 % of Quantization Limit) in triplicate. From accuracy data, the % recovery of methyl bromide was found within the limits (100 ±15%). Results indicates that the method has an acceptable level of accuracy. The results are presented in below table.

Table 4: Accuracy data for Methyl Bromide

| S.No | 50% Area | 100% Area | 150% Area |
|------|----------|-----------|-----------|
| 1    | 1504     | 2886      | 4956      |
| 2    | 1537     | 3083      | 4676      |
| 3    | 1467     | 3120      | 4459      |
| Average area | 1503     | 3030      | 4697      |
| %Recovery | 87.89    | 88.60     | 91.56     |
| Standard Avg. Area : 3420 |

5) Limit of Detection (LOD) and Quantitation (LOQ):
The LOD and LOQ were calculated by instrumental and statistical methods. For the instrumental method, LOD is determined as the lowest amount to detect, and LOQ is the lowest amount to quantify, by the detector. The LOD and LOQ of Methyl bromide in Itraconazole API was determined based on Linearity. The area of Methyl Bromide at LOD Concentration (0.20 ppm) is 706 and Standard average area of Methyl Bromide at LOQ Concentration (0.61 ppm) is 767. The linearity also passed at LOQ Concentration. The data and Chromatograms of LOD and LOQ as shown in figure.

Table 5: Linearity Graph data for Methyl Bromide at LOQ Concentration

| S.No. | Conc, ppm | Area |
|-------|-----------|------|
| 1     | 1.875     | 1503 |
| 2     | 3.000     | 2466 |
| 3     | 3.750     | 3030 |
| 4     | 4.500     | 3797 |
| 5     | 5.625     | 4697 |
| Correlation Coefficient | 0.999 |
| Slope | 857      |
| STEYX | 52       |
| LOD   | 0.20 ppm  |
| LOQ   | 0.61 ppm  |
6) **Repeatability at LOQ Concentration**: The methyl bromide was prepared at LOQ level (0.61 ppm) absolute and injected in six replicates. The RSD (n=6) values obtained for the area of Methyl bromide is 767. The %RSD for methyl bromide peak area response of Standard six injections should not more than 15%.
Table 6: Repeatability data for Methyl Bromide at LOQ Concentration

| S.No. | Methyl Bromide Area |
|-------|---------------------|
| 1     | 827                 |
| 2     | 705                 |
| 3     | 741                 |
| 4     | 833                 |
| 5     | 756                 |
| 6     | 738                 |

Average Area 767
Standard Deviation 52
% of RSD 6.76

Figure 6: Typical %RSD chromatograms at LOQ.

7) Accuracy at LOQ Concentration: A known amount of sample (about 1.0 gr) was taken separately in three different vials and spiked with 2.0 ml of methyl bromide at LOQ Level (0.61 ppm) in triplicate. From accuracy data, the % recovery of Methyl Bromide was found within the limits (100 ± 20%). Results indicate that the method has an acceptable LOQ level of accuracy. The results are presented in below table.

Table 7: Accuracy data for Methyl Bromide at LOQ Concentration

| S.No | LOQ Level Area |
|------|----------------|
| 1    | 858            |
| 2    | 779            |
| 3    | 872            |

Average area 836
Standard Average Area 767
% Recovery 91.74%

8) Ruggedness: Ruggedness of the method was evaluated by performing the sample analysis in six replicates using different analyst on different days and the results are summarized as shown in bellow table. The RSD values of less than 15.0% for Methyl Bromide content indicate that the method adopted is rugged.
Table 8: Ruggedness data for Methyl Bromide

| SST Parameter | Day-1 | Day-2 | Analyst-1 | Analyst-2 | Day-1&2 | Day-1&2 |
|---------------|-------|-------|-----------|-----------|---------|---------|
| %RSD          | 6.42  | 7.18  | 6.56      | 6.84      | 6.68    | 6.48    |

9) Robustness: This study was performed by making small but deliberate variations in the method parameters. The effect of variations in flow rate of carrier gas and Vail temperature was studied. Under all the variations, system suitability requirement is found to be within the acceptance criteria and hence the proposed method is robust.

The relative standard deviation of area counts for Methyl Bromide peak obtained from six replicate injections of standard solution should be not more than 15.0%. The data of Robustness is following table.

Table 9: Methyl Bromide Robustness (Flow variation)

| System Suitability Parameter | 2.59mL/min (Flow Minus) | 3mL/min (Control) | 3.5mL/min (Flow Plus) |
|-----------------------------|--------------------------|-------------------|----------------------|
| % RSD                       | 6.5                      | 5.11              | 7.21                 |

Table 10: Methyl Bromide Robustness (Vail Temperature variation)

| System Suitability Parameter | 35°C (Temperature Minus) | 40°C (Control) | 45°C (Temperature Plus) |
|-----------------------------|--------------------------|----------------|-------------------------|
| % RSD                       | 7.37                     | 7.07           | 6.72                    |

10) Batch Analysis: Weighed accurately 1.0 gm of three different Batches of Itraconazole API into three Head Space vial, and dissolve 2 ml of DMA and seal the vial with aluminum closure. Heat the sealed vial at 90°C for 30 min. Finally, the Methyl Bromide was not detected in three batches. The acceptance criterion of Methyl Bromide in Itraconazole API is NMT 3.75 ppm. The Chromatograms of three batches is as follows.

3. DISCUSSION

Avoidance of potential genotoxic impurities through drug substance synthesis process may be impractical in many instances, present at trace levels, organohalides due to their reactive nature, are invariably unstable posing a major challenge to the analytical chemist in terms of recovery, reproducibility and sensitivity. The simple GC method described in this research paper for Methyl Bromide in finished pharmaceutical product obviates the need to derivatize the sample to enhance detectability. The method can be easily transferred from R&D lab to manufacturing quality control site since it does not involve complex sample preparation or very sophisticated analytical setup. This robust method demonstrated excellent linearity, specificity, precision, sensitivity and accuracy which can be routinely used for the quantification of Methyl Bromide in Itraconazole API. This method can be extended to other different pharmaceutical products.

4. CONCLUSION

A single, rapid and highly selective HSGC method was developed and validated for the quantification of Methyl Bromide present in Itraconazole API through an understanding of LOD, LOQ, nature of stationary phases of columns. The residue methyl bromide was determined in ppm levels also. The method was shown to be specific for Itraconazole API and was applied successfully.
to monitor and control impurity level. The method was found to be applicable for the routine analysis of the Itraconazole API in pharmaceutical industry.

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