ABSTRACT
A precise and accurate chromatographic method has been developed by employing a mixed mode chromatographic column for simultaneous determination of Benzyl Alcohol (Organic analyte) and Iodide (Inorganic analyte) with appropriate sensitivity. The uniqueness of the method is that it enables measurement of both the analytes at two different wavelengths even with a single wavelength absorbance detector, in a single chromatographic run (as well as in a single chromatogram) without any conspicuous disturbance in the baseline.

KEYWORDS: Benzyl Alcohol, Iodide, Amiodarone Hydrochloride 150 mg/3mL Injection.

INTRODUCTION
Amiodarone injection is an antiarrhythmic agent indicated for initiation of treatment and prophylaxis of frequently recurring ventricular fibrillation (VF) and hemodynamically unstable ventricular tachycardia (VT) in patients refractory to other therapy.

Each mL of injection contains 50 mg of Amiodarone Hydrochloride, 20.2 mg of Benzyl alcohol, 100 mg of Polysorbate 80, and water for injection.

Benzyl alcohol is an aromatic alcohol with the formula C₇H₈O. It is used as an excipient for its preservative properties. The administration of medicines containing Benzyl alcohol to neonates or premature neonates has been associated with fatal “gasing syndrome”. As
Benzyl alcohol may cross the placenta, Amiodarone Hydrochloride 150 mg/3 mL injection needs to be used with caution in pregnancy.

Amiodarone is a potential source of large amounts of inorganic iodine and can cause either hypothyroidism or hyperthyroidism. Because of the slow elimination of Amiodarone and its metabolites, high plasma iodide levels, alter thyroid function, and abnormal thyroid-function tests may persist for several weeks or even months following Amiodarone withdrawal.

Because of these concerns associated with the Amiodarone Hydrochloride injection, monitoring of Quality perspectives of Amiodarone Hydrochloride injection mandates to monitor levels of Benzyl Alcohol and Iodide. Since, USP has indicated NMT 250 ppm limit for Iodide in Amiodarone Hydrochloride injection, method was designed to same limit for Iodide.

**Chemistry**

Amiodarone Hydrochloride has a molecular weight of 681.77 and contains 37.3% Iodine by weight. Amiodarone is a potential source of large amounts of inorganic iodine.

![Structure of Amiodarone Hydrochloride (API of Drug product)](image)

Benzaldehyde is a toxic oxidation product of the preservative Benzyl alcohol, hence presence of Benzaldehyde is associated with the presence of Benzyl Alcohol. As a result of this, it is imperative to monitor position of this compound in the chromatography having presence of Benzyl Alcohol w.r.t. specificity.

The method so developed enables elution of Benzyl Alcohol, Benzaldehyde & Iodide with proper resolution. Since, limitations of Ion pairing chromatography include long column equilibration times and the considerably high quantity of solvent and time needed to elute ion pairing agent from the column, the differential chemistry of the analytes necessitated use of
mixed mode chromatographic column for elution on a single chromatographic column in a single run. The differences in absorbance maxima of the analytes of interests (Benzyl Alcohol & Iodide) necessitated to employ two different wavelengths for quantitation.

![Figure 02: Elution of three analytes at 230 nm](image)

Data acquisition at two wavelengths was programmed sequentially based on the elution period of analyte peaks. Since, Benzyl Alcohol eluted within initial six minutes, data collection was done at 258nm to measure the response due to Benzyl Alcohol. Subsequently, data collection was done at 230nm to measure the response due to Iodide after normalizing the baseline to zero. Due to the normalization of baseline to zero, the baseline remained at zero response before elution of Iodide peak enabling smooth quantitation of the Iodide peak.

![Figure 03: Measurement at two different wavelengths using a single wavelength UV Detector](image)
MATERIALS AND METHOD

Chemicals and Reagents
All chemicals and reagents used in this study were of A.R. or equivalent Grade; Water utilised was from Milli Q water purification system and complied to USP purified water requirements.

Materials
Benzyl Alcohol, Potassium Iodide, Potassium monobasic Phosphate, Sodium Pyrophosphate Decahydrate, Acetonitrile, Purified water, Amiodarone Hydrochloride 150 mg/3mL Injection.

Instrument

Table No. 01: Chromatographic Conditions

<table>
<thead>
<tr>
<th>Mobile Phase</th>
<th>Eluent A</th>
<th>Eluent B</th>
<th>Eluent C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column:</td>
<td>Acclaim Mixed-Mode WAX-1, 5µm</td>
<td>Solution of 0.2M KH₂PO₄ [containing 0.5g/L Sodium Pyrophosphate Decahydrate] at pH 6</td>
<td>Acetonitrile: Purified Water (90:10)</td>
</tr>
<tr>
<td>Dimensions:</td>
<td>4.6mm x 150mm</td>
<td>Purified water</td>
<td>Purified water</td>
</tr>
<tr>
<td>Flow rate</td>
<td>1.2mL/min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Injection volume</td>
<td>30 µL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Detector wavelengths</td>
<td>258 nm &amp; 230nm</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table No.02: Program for Standard runs

<table>
<thead>
<tr>
<th>Time (Minutes)</th>
<th>Wavelength (nm)</th>
<th>% A</th>
<th>% B</th>
<th>% C</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>258</td>
<td>11</td>
<td>20</td>
<td>69</td>
</tr>
<tr>
<td>7.00</td>
<td>230</td>
<td>11</td>
<td>20</td>
<td>69</td>
</tr>
<tr>
<td>8.30</td>
<td>Autozero</td>
<td>11</td>
<td>20</td>
<td>69</td>
</tr>
<tr>
<td>15.00</td>
<td>230</td>
<td>11</td>
<td>20</td>
<td>69</td>
</tr>
<tr>
<td>15.10</td>
<td>258</td>
<td>11</td>
<td>20</td>
<td>69</td>
</tr>
</tbody>
</table>

Table No. 03: Program for sample runs

<table>
<thead>
<tr>
<th>Time (Minutes)</th>
<th>Wavelength (nm)</th>
<th>% A</th>
<th>%B</th>
<th>% C</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>258</td>
<td>11</td>
<td>20</td>
<td>69</td>
</tr>
<tr>
<td>7.00</td>
<td>258</td>
<td>11</td>
<td>20</td>
<td>69</td>
</tr>
<tr>
<td>7.00</td>
<td>230</td>
<td>11</td>
<td>20</td>
<td>69</td>
</tr>
<tr>
<td>8.30</td>
<td>Autozero</td>
<td>11</td>
<td>20</td>
<td>69</td>
</tr>
<tr>
<td>12.00</td>
<td>230</td>
<td>11</td>
<td>20</td>
<td>69</td>
</tr>
</tbody>
</table>
Preparation of Diluent
A mixture of Acetonitrile and Purified water in the ratio of 50:50 (v/v).

Preparation of Iodide Standard stock solution: 6.3 ppm
Dissolve about 82 mg of Potassium Iodide in 100mL volumetric flask in about 30 mL diluent and dilute up to the mark with diluent. Further diluent 1 mL of this solution to 100mL with diluent.

Preparation of Benzyl Alcohol Standard stock solution: 10100 ppm
Weigh about 1010 mg of Benzyl alcohol in to 100mL volumetric flask and dilute up to the mark with diluent. Mix well.

Preparation of Standard Solution: 0.63ppm/1010ppm(Iodide/Benzyl Alcohol)
Transfer 2mL each of Iodide and Benzyl Alcohol standard stock solution to 20mL volumetric flask and dilute to volume with diluent.

Preparation of Test Solution
Dilute 1mL of sample solution to 20mL with diluent.

Procedure
Inject Diluent followed by six replicates of standard solution followed by Test solutions.

System suitability parameters & Acceptance criteria
- %RSD of area response for the Benzyl Alcohol peak from the replicate system suitability standards shall be NMT 5%.
- %RSD of area response for the Iodide peak from the replicate system suitability standards shall be NMT 15%.
Calculations

**Calculation for Iodide content: (ppm)**

\[
\text{Aspl} \times \text{Wstd} \times 1 \times 2 \times \text{Dspl} \times 126.9 \times \text{P} \times (100 - \text{LOD})
\]

\[
= \frac{\text{Aspl} \times \text{Wstd} \times 1 \times 2 \times \text{Dspl} \times 126.9 \times \text{P} \times (100 - \text{LOD})}{\text{Astd} \times 100 \times 100 \times 20 \times \text{Wspl} \times 166 \times 100 \times 100}
\]

Where:

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspl</td>
<td>Area of Iodide in sample preparation</td>
</tr>
<tr>
<td>Astd</td>
<td>Average area of Iodide in Standard preparation</td>
</tr>
<tr>
<td>Wstd</td>
<td>Weight of Potassium Iodide in standard (mg)</td>
</tr>
<tr>
<td>Dspl</td>
<td>Dilution volume (mL) for sample preparation</td>
</tr>
<tr>
<td>Wspl</td>
<td>Weight of sample in mg (1mL = 50 mg Amiodarone HCL)</td>
</tr>
<tr>
<td>126.9</td>
<td>Atomic weight of Iodide</td>
</tr>
<tr>
<td>166</td>
<td>Molecular weight of Potassium Iodide</td>
</tr>
<tr>
<td>P</td>
<td>Purity of Potassium Iodide on dried basis</td>
</tr>
<tr>
<td>LOD</td>
<td>Loss on drying of Potassium Iodide</td>
</tr>
</tbody>
</table>

**Calculation for Benzyl Alcohol content: (%)**

\[
\text{Aspl} \times \text{Wstd} \times 2 \times \text{Dspl} \times \text{P} \times 100
\]

\[
= \frac{\text{Aspl} \times \text{Wstd} \times 2 \times \text{Dspl} \times \text{P} \times 100}{\text{Astd} \times 100 \times 20 \times 1 \times \text{LC}}
\]

Where:

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspl</td>
<td>Area of Benzyl Alcohol in Test preparation</td>
</tr>
<tr>
<td>Astd</td>
<td>Average area of Benzyl Alcohol in Standard preparation</td>
</tr>
<tr>
<td>Wstd</td>
<td>Weight of Benzyl Alcohol in standard (mg)</td>
</tr>
<tr>
<td>Dspl</td>
<td>Dilution volume (mL) for sample preparation</td>
</tr>
<tr>
<td>P</td>
<td>Purity of Benzyl Alcohol standard</td>
</tr>
<tr>
<td>LC</td>
<td>Label claim of Benzyl Alcohol (20.2mg/mL)</td>
</tr>
</tbody>
</table>
Figure 04: [Full scale] Typical Chromatogram for Standard Solution

Figure 05: [Magnified] Typical Chromatogram for Standard Solution
RESULTS

Analytical method validation

Specificity
There was no interference at the retention time of Benzyl alcohol from diluent and Placebo. There was no interference at the retention time of Iodide from diluent. Benzaldehyde an impurity of Benzyl alcohol eluted with more than 5 resolution factor from Benzyl Alcohol & Iodide. There was no coelution visible for Benzyl Alcohol as well as Iodide peaks in test solution. The method is specific for estimation of Benzyl Alcohol and Iodide in Amiodarone Hydrochloride 150 mg/3mL Injection.

Limit of Detection and Limit of Quantitation
Since out of Benzyl Alcohol and Iodide, only Iodide is an impurity, the Limit of Detection and Limit of Quantitation were established for Iodide based on the S/N ratio approach.

Table 04: S/N ratio and concentration at LOD & LOQ Levels

<table>
<thead>
<tr>
<th></th>
<th>S/N ratio</th>
<th>Concn;ppb (soln strength)</th>
<th>Concn;ppm (w.r.t. Test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOD</td>
<td>8.7</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>LOQ</td>
<td>12.0</td>
<td>15</td>
<td>6</td>
</tr>
</tbody>
</table>
Linearity and Range
Method exhibited linearity for Benzyl Alcohol from 50% to 200% of target level concentration and for Iodide from 6 ppm to 506 ppm w.r.t. test concentration i.e. from LOQ (2.4%) to about 200% of limit level concentration.

Figure 07: Overlay of Linearity responses

Figure 08: Linearity of Benzyl Alcohol

\[ y = 47.725x - 1997.357 \]
\[ R^2 = 1.000 \]
**Linearity of Iodide**

\[ y = 2748.467x - 49.817 \]
\[ R^2 = 0.999 \]

![Linearity of Iodide](image)

**Figure 09: Linearity of Iodide**

**Precision**
Precision of the method was established by evaluating six individual Test solutions prepared from a single batch of Amiodarone Hydrochloride 150 mg/3mL Injection by spiking Iodide at Limit level. Method was observed to be precise with % RSD of Benzyl Alcohol assay results as 0.71% and %RSD of Iodide content results as 0.79%.

**Ruggedness**
Ruggedness of the method was established by evaluating six individual Test solutions prepared from a single batch of Amiodarone Hydrochloride 150 mg/3mL Injection by different analyst while the analytical exercise was carried out on a different day. Method observed to be rugged with % RSD of Benzyl Alcohol assay results as 1.45% and %RSD of Iodide content results as 1.88%.

**Accuracy**
The accuracy of method was established for Benzyl alcohol by spiking Placebo at 50 %, 100 %, 150% and 200% levels with respect to target level concentration. For evaluation of accuracy of the method for Iodide, prepared the accuracy solutions by spiking test solutions at LOQ, 50%, 100%, 150% & 200% of limit level concentration. Recoveries of Benzyl alcohol were observed to be ranging from 99 % to 105%. Recoveries of Iodide were observed to be ranging from 99 % to 106%.
Robustness

Robustness of the method was established by carrying out the analytical exercises by deliberately changing chromatographic parameters like flow rate, Ionic strength of the eluent, Organic modifier composition of eluent & eluent buffer pH at two different variations. The chromatography of the method was found to be sensitive to changes in eluent buffer pH. The increase in eluent buffer pH from pH 6.0 (standard condition) to pH 6.2, jeopardizes the resolution between Iodide and Benzaldehyde, a toxic oxidation product impurity of Benzyl Alcohol. However, method was found to be robust when eluent buffer pH was varied from pH 6.0 to pH 6.1. Method was concluded robust at all other studied conditions.

Solution stability

Standard solution and Test solution spiked with Iodide at Limit level concentration were studied initially and on third day of storage at control room temperature condition on the bench top. The solutions were observed to be stable.

DISCUSSION

Research work has culminated into a specific, accurate, Precise, Linear, Rugged and robust method having good sensitivity. The uniqueness of the method is that it enables elution and quantitation of both Inorganic (Iodide) & Organic (Benzyl Alcohol) analytes of interest simultaneously at concentrations mandated by regulations. It enables measurement of both the analytes at two different wavelengths even with a single wavelength absorbance detector, in a single run as well as in a single chromatogram without any conspicuous disturbance in the baseline.

Utilisation of this method will not only reduce the burden of additional resources required for two different methods necessitated by differential chemistry of analytes of interest, but will also reduce the turnaround time drastically. The method so developed can be executed both on Ion chromatographic system having UV detector in configuration as well as on conventional High performance liquid chromatographs.

CONCLUSION

Even though the two analytes - Benzyl Alcohol and Iodide have differential chemistry (analytes are organic and inorganic in nature respectively), method so developed and successfully validated for simultaneous determination of these analytes in Amiodarone
Hydrochloride 150 mg/3mL Injection provides a technically sound and economic alternative to multiple methods available for compliance to mandated regulations.

REFERENCES

1. *Handbook of Ion Chromatography*, Joachim Weiss  Dionex GmbH.


