Analytical Method Development Report For The Determination Of Assay & Related Substances Of Ibutilide Fumarate In Ibutilide Fumarate Injection

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Abstract: The aim of the study is to develop an effective assay to quantify ibutilide fumarate and its related substances in ibutilide fumarate injection. Initially, the API manufacturer’s method was followed which involves two different UV-HPLC assays for both ibutilide fumarate and its related impurities. Upon considering the heat degradation results of the API method, specificity and stability of the known impurities (related substances) was not clearly determined. So the API method was modified by using low-pressure gradient UV-HPLC for accurate detection of ibutilide and its related impurities. The inference of the drug, under various physio-chemical conditions like acid stress, base stress, heat stress, dark and light exposed, peroxide treated, dry heat are also studied. Chromatogram results of the developed method observed to be highly stable and sensitivity as it is able to clearly differentiate the excipient, related impurities and degradation product. Therefore the developed method can be used for the determination of assay and related substances of ibutilide fumarate in ibutilide Fumarate injection.

Keywords: Ibutilide fumarate, HPLC, API manufacturer’s method, Stress testing

Introduction:
Heart arrhythmia (cardiac dysrhythmia) is a cardiac disorder identified by irregular heartbeat. It is mainly due to the disturbance of electrical impulses which regulate the heart[1][2]. As a result, the heart may beat too slowly (bradycardia), too quickly (tachycardia) or in an irregular way. Anti-arrhythmic drugs can be classified according to their effects on the electrical behaviour of myocardial cells in to four which are Anti-arrhythmic drugs are classified into four types based on their electrical behavior with myocardial cells namely membrane-stabilizers (class I), beta-blockers (class II), calcium blockers (class III) and calcium influx blockers (class IV)[3]. Ibutilide is one among the effective class III antiarrhythmic drug used intravenously to convert atrial flutter to normal rhythm[4]. Ibutilide contains both + and – enantiomers[5] exist as a racemic mixture having one chiral centre. IUPAC name and molecular formula of ibutilide fumarate is Methanesulfonamide, N-(4-(4-(ethylheptylamino)-1-hydroxybutyl)phenyl), (+)-(E)-2-butenedioate (1:0.5) (hemifumarate salt)[6][7] and C_{22}H_{24}N_{2}O_{5}S respectively. Molecular weight of ibutilide fumarate is 442.62 and its structural formula is revealed in the Figure 1. The injection solution is isotonic and colorless[8] having a concentration of 0.1 mg/ml of ibutilide fumarate along with 0.189 mg of sodium acetate trihydrate, and 8.90 mg of sodium chloride, and the pH is adjusted to 4.6 using HCl[11][12]. Many commercial methods are available for the assay of ibutilide fumarate injection, which utilize UV-Visible spectrophotometry, HPLC, LCMS/MS, TLC, & GC as the principle detection method[9][10]. Initially in this study we have used the API manufacturer’s method which consist of two different methods for the determination of Assay & Related substances of Ibutilide fumarate, that involves a HPLC method with a UV detection[15][16]. Since Poor sensitivity and reproducibility of the reported methods led to the development of a novel method for determination of ibutilide fumarate and its related substances in ibutilide fumarate injection

Materials And Methods
Preparation of stock solution
Ibutilide fumarate Standard stock solution is prepared at a concentration of 1.0 mg/ml [17], [18] by dissolving 25 mg of Ibutilide fumarate standard in 25 ml of double distilled water. 1 ml of stock is diluted with water at a ratio of 1:10 and used for the development of the assay.

Reagents and chemicals
To prepare excipient solution take about 40ml of water into a 50ml volumetric flask; add accurately about 9.45 mg of Sodium acetate trihydrate and 445 mg of Sodium chloride then dissolve the contents. After that adjust the pH to about 4.6 with 0.1N hydrochloric acid and dilute to the volume with water[19]. And for the related substances standard solution pipette out accurately 1.0ml of assay standard solution (0.1mg/mL) into 100ml volumetric flask and dilute to volume with water (0.001mg/mL)[20][21].

Liquid chromatography conditions
Chromatography conditions such as stationary and mobile phase composition, temperature of the column, flow rate, volume of the sample and wavelength for compound detection were optimized. An effective and precise HPLC system consisting of a column Inertsil ODS-3V (250 X 4.6 mm; 5 μm) or equivalent with a column temperature: 30 °C is utilized with a run time of 60 min and sample concentration of 0.1mg/Ml[22]. The gradient programme for the chromatography is given in Table1. Inject 100 μl each of blank, excipient solution, standard substances standard solution, assay standard solution, and test solution into the liquid chromatograph, record the chromatograms and measure the response excluding the peaks due to blank and excipients. Inject blank, excipient, standard and sample solutions as per Table2.

Optimization of the quantification method under various physio-chemical conditions
To ensure the specificity and stability of the new quantification method, the activity of the drug has been studied in various physio-chemical conditions. For the optimization of method both ibutilide fumarate formulation and excipients were exposed in different conditions like acid stress, base stress, heat stress, dark and light exposed, peroxide treated, dry heat and their effects were studied.

Validation of ibutilide fumarate assay
The method for ibutilide fumarate was validated in terms of reproducibility, specificity, and robustness according to ICH harmonized tripartite guidelines.

Specificity
The specificity of method was evaluated to ensure that there was no interference from the expients present in the formulations. The specificity was studied by injecting the expients.
Robustness
The reliability of the method to remain unaffected due to deliberate variations is determined by measuring the robustness of the analytical procedure.

Statistical analysis
The experiments were conducted in triplicates and the results are expressed as mean with standard error. Statistical significance is evaluated using One way ANOVA and 0.05 is used as threshold reference for P value.

Results and Discussion

Optimization of HPLC condition
Chromatograms of blank, excipient, related substance standard, assay standard, ibutilide fumarate formulation are depicted in Figure 2. The chromatograms of UV-HPLC revealed that the compounds peaks are distinct and clear without any excipient interference thereby highlighting the efficiency of both sensitivity and selectivity of the optimized protocol. The runtime of the procedure is found to be exactly 60 minutes and the peaks were of good shape and completely resolved.

Optimization of the quantification method under various physio-chemical conditions
The forced degradation samples, thermal and pH related stress samples were analyzed using this method (Figure 3). During the heat stress, two potential degradation impurities observed at the relative retention time of 1.4 & 1.6 of about 1.4% & 1.0% respectively. The acid degradation also showed one significant impurity at a different RRT of about 1.7. The base and light exposed does not showed any significant degradation pattern. But the peroxide stress resulted in complete degradation of ibutilide peak. Also in case of API degradation under similar conditions, the same pattern of impurity observed[23]. In case of thermal & pH stress, performed at different pH conditions, viz., at 3.0, 4.0, 4.6, 5.0 & 6.0. There observed a peak at about RRT 0.41 which is due to excipient appeared at variable response at different times during the analysis. During the lab batch analysis, it was also observed that the peak at RRT 0.41 was showing a variable response at aseptic fill and terminally sterilized conditions. In aseptic fill at 40°C storage condition, this peak was quiet significant, whereas in aseptic fill (50°C) & terminally sterilized (40°C, 50°C) it showed very insignificant response which proved that this peak is due to excipient having different response at different conditions. Due to this reason, the method was revised stating that this peak at RRT 0.41 with respect to Ibutilide will be excluded during integration of chromatogram[24]. It was found that no other peaks due to the excipients or the impurities were interfering with the main peak of ibutilide, and the two known process related impurities are well separated in this method. The relative retention times of these impurities with respect to Ibutilide is presented in Table 3.

Specificity
The specificity of method was monitored by analyzing the ibutilide fumarate and standard solution. No peak was detected in close to the retention time of ibutilide fumarate, which proved to be high degree of specificity of the method (Figure 4).

Robustness
The robustness of the method was studied by deliberate changes in method like alteration in pH of mobile phase. It was observed that there was no marked changes in method like alteration in pH of mobile phase. It was observed that there was no marked changes in method like alteration in pH of mobile phase.

Figures and tables

| Table 1: Gradient Programme For The Chromatography |
|----------------|----------------|----------------|
| Time (Minutes) | Solution A (%) | Solution B (%) |
| 0.0            | 62             | 38             |
| 14.0           | 62             | 38             |
| 18.0           | 60             | 40             |
| 35.0           | 30             | 70             |
| 45.0           | 30             | 70             |
| 50.0           | 62             | 38             |
| 60.0           | 62             | 38             |
| Elution        | Equilibration  | Linear Gradient Linear Gradient |
|                | Isocratic      | Isocratic      |
|                | Equilibration  | Equilibration  |

<table>
<thead>
<tr>
<th>Table 2: Injection Sequence</th>
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<tbody>
<tr>
<td>Solution</td>
</tr>
<tr>
<td>Blank</td>
</tr>
<tr>
<td>Excipient</td>
</tr>
<tr>
<td>Related substances standard</td>
</tr>
<tr>
<td>Assay standard</td>
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<tr>
<td>Test solution</td>
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<table>
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<tr>
<th>Table 3: Rrt Table Of Ibutilide Fumarate Process Related Impurity</th>
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<tbody>
<tr>
<td>Chemical name</td>
</tr>
<tr>
<td>N-ethyl-n-heptyl-gamma-oxo-4-[(methyl sulfonyl)amino]-benzene butanamide.</td>
</tr>
<tr>
<td>N-ethyl-n-heptyl-4-hydroxy-4-(4-methanesulfonylamino-phenyl)-butyramide.</td>
</tr>
</tbody>
</table>
Fig. 1: structure of ibutilide fumarate

Fig. 2a: Chromatogram of blank

Fig. 2b: Chromatogram of excipient control

Fig. 2c: Typical assay standard chromatogram

Fig. 2d: Typical diluted standard chromatogram
**Fig. 2e:** Typical Chromatogram of Ibutilide Fumarate formulation – Control

**Fig. 3a:** Chromatogram of Ibutilide Fumarate formulation – Acid stress

**Fig. 3b:** Chromatogram of Ibutilide Fumarate formulation – Base stress

**Fig. 3c:** Chromatogram of Ibutilide Fumarate formulation – Heat stress

**Fig. 3d:** Chromatogram of Ibutilide Fumarate formulation – Dark control
Fig. 3e: Chromatogram of Ibutilide Fumarate formulation – Light exposed

Fig. 3f: Chromatogram of Ibutilide Fumarate formulation – Peroxide treated

Fig. 4a: HPLC chromatogram of mobilephase with 1mg/ml ibutilide fumarate

Fig. 4b: HPLC chromatogram of mobilephase with .001mg/ml ibutilide fumarate in diluted mobile phase.
Fig. 5a: HPLC chromatogram at different pH=3 condition to study the robustness of the method.

Fig. 5b: HPLC chromatogram at different pH=4 condition to study the robustness of the method.

Fig. 5c: HPLC chromatogram at different pH=5 condition to study the robustness of the method.

Fig. 5d: HPLC chromatogram at different pH=6 condition to study the robustness of the method.
Figure 7: Chromatogram of Ibutilide API - Control

Figure 8: Chromatogram of Ibutilide fumarate API – Dry Heat Sample

Figure 9: Typical Chromatogram of Ibutilide fumarate process related impurity-A

Figure 10: Typical Chromatogram of Ibutilide fumarate process related impurity-B
Conclusion
The current assay developed for the detection of ibutilide fumarate and its related substances in ibutilide fumarate injection proven as a reliable and specific method. The developed assay provides rapid detection of ibutilide and also highly sensitive to detect the presence of impurities even in trace amount. Chromatogram infers the absence of interference due to diluents, excipient and degradation products. The inference of the drug, under various physiochemical conditions like acid stress, base stress, heat stress, dark and light exposed, peroxide treated, dry heat are also studied. On comparison with earlier reported methods, the current developed assay is similar with the validation guidelines of ICH. On considering the integrity and resolution of the observed peak, the present developed assay for quantitative determination of ibutilide and related substances in ibutilide fumarate injection is precise, accurate and made to use for human studies.

References


