Abstract
A sensitive UPLC-MS method was developed for the determination of Flecainide acetate in the presence of four its related impurities (Impurities: A, B, D, and E). The forced degradation study of Flecainide acetate was carried out under acidic, alkali, neutral, and oxidative conditions. The degradation was observed under acidic, neutral, and oxidative conditions, and four degradation products (impurities) were observed. Successful chromatographic separation of Flecainide acetate and its degradation products were achieved on a Waters Acquity BEH C18 column (100 mm x 2.1 mm x 1.7 μ) using a mobile phase of solvent A (10 mM Ammonium formate) and solvent B (acetonitrile) in gradient elution. The gradient program employed to achieve the separation was (Tmin/%Solvent B): 0/15, 1/15, 3/90, 5/90, 7/15, 9/15. The flow rate was maintained at 0.3 mL/min. The impurities were characterized and the fragmentation pathways for the impurities were proposed.

Keywords: Flecainide acetate; Forced degradation; UPLC; ICH, LC-MS method.

Introduction
Flecainide acetate, N-(2-piperidinyl-methyl)-2,5-bis(2,2,2-trifluoroethoxy) benzamide monoacetate, is an anti-arrhythmic agent that is used to treat ventricular arrhythmias by blocking sodium causing a decreased intra-cardiac conduction velocity (1) and is brand under Tambocor. Forced degradation is a process where the natural degradation rate of a drug or drug product is accelerated by the application of an additional stress (2). Stress testing is designed to estimate degradation pathways and intrinsic stability of the drug molecule. The chemical structures of Flecainide acetate and its studied impurities are presented in Fig. 1.

Some regular chromatographic methods have been available in the literature for the determination of flecainide acetate in its bulk powder, in pharmaceutical formulations or in the presence of its enantiomer, metabolites or other antiarrhythmic drugs. In the last decade, four liquid chromatographic methods (3-6), one capillary zone electrophoretic method (7), one Thin Layer Chromatographic (TLC) method (8) have been reported. Only one spectro-fluorimetric method (9) and one electrochemical method (10) were reported. Also, the stability of flecainide acetate in an extemporaneously compounded oral suspension was studied by High Performance Liquid Chromatographic (HPLC) methods (11-12) and only one stability-indicating TLC-densitometry and HPLC methods (13) were reported. No stability indicating method for the analysis of Flecainide acetate and its impurities under stress degradation conditions using LC-MS has been reported. Therefore, the aim of the present study is designed to develop a LC-MS compatible procedure for the determination of Flecainide acetate in the presence of its related substances. The present contribution of the work was to

Determination of Flecainide acetate and its degradation impurities by UPLC-MS
evaluate the opportunities offered by LC-MS for determining the impurities of the cited drug.

**Materials and Methods**

**Chemicals and Reagents:** Flecainide Acetate (purity ≥ 99%) bulk drugs were obtained as gift sample from a renowned manufacturer. 10 mM ammonium formate (Analytical-reagent grade) was purchased from Merck Pvt. Ltd., whereas acetonitrile (HPLC-grade) were purchased from Sigma Aldrich. All other reagents used like hydrochloric acid, hydrogen peroxide, and sodium hydroxide was of analytical grade (Merck Pvt. Ltd.). HPLC grade water (Milli Q water purification system) was used throughout the analysis.

**Instrumentation and Chromatographic conditions:** The separation was achieved on a UPLC separation module, Waters Acquity BEH C18 column (100 mm x 2.1 mm x 1.7 μ) using a mobile phase of solvent A (10 mM Ammonium formate) and solvent B (acetonitrile) in gradient elution. The gradient program employed to achieve the separation was (Tmin/ %Solvent B): 0/15, 1/15, 3/90, 5/90, 7/15, 9/15. The flow rate was maintained at 0.3 mL/min. The column temperature was maintained at 25 °C and the autosampler at 10 °C. The injection volume was 2 μL. This method was transferred to LC-MS analysis by LC system to Agilent Q-TOF 6540 series, Agilent Technologies. The capillary voltage applied was 4000 V. The temperature of the gas was set at 325 °C, using nitrogen as nebulizing gas and drying gas. Drying gas flow at 10 L/min, nebulizer pressure 40 psi and fragmentor 130 V. Mass spectra were acquired over an m/z range of 50-1000 and CID gas was high pure nitrogen (99.99 %). Mass Hunter Software was used for monitoring output signal, controlling acquisition and processing of the mass data.

**Sample preparation for LC-MS analysis:** Stress samples were collected and made up to volume with mobile phase whereas solid samples were directly dissolved and diluted with mobile phase. Sample concentration of 1 mg/mL was used to conduct degradation studies. All the samples were filtered through 0.22 μm membrane filter and injected into LC-MS system.

**Forced degradation study:** Forced degradation studies were performed as per ICH guidelines. All stress decomposition studies were performed with control solution i.e. prepared and treated similarly to the respective stress conditions without active component. Acidic degradation was performed by refluxing of sample at 1 mg/mL of 1 N HCl at 70 °C for 22 h. Alkaline degradation was performed by refluxing of sample with 1 mg/mL of 0.1 N NaOH at 70 °C for 28 h. The neutral degradation was performed by refluxing of sample with 1 mg/mL of H2O for 48 h. Oxidative degradation was performed with 1 mg/mL of 10 % H2O2 at room temperature for 48 h. All the forced degraded samples were filtered and then injected into LC-MS system.

**Results and Discussion**

**Optimization of the method:** Flecainide Acetate is relatively non polar compound and was found to retain on traditional C18 bonded phases. To improve the selectivity and retention and further the peak shape bonded phases of stationary phase was tried. The initial trials were carried out with aqueous buffer solutions of pH 3.0, 4.0, 5.0, 5.5 and 6.0 with organic modifier methanol or acetonitrile. The method was optimized in keeping view of adequate separation of the impurities from the main peak.

**Different columns with variable chemistries like Acquity CSH C18, Acquity HSS C18, Acquity HSS Cyano were tried with different combinations of mobile phase containing different proportions of buffer system and organic modifiers. The critical aspect of optimizing the method was based on the resolution obtained among the peaks. After a thorough screening of various buffers, organic modifiers and other chromatographic parameters the final separation was achieved on a Waters Acquity BEH C18 column (100 mm x 2.1 mm x 1.7 μ) using a mobile phase of solvent A (10 mM Ammonium formate)
and solvent B (Acetonitrile) in gradient elution. The final chromatogram of Flecainide acetate standard was shown in Fig. 2.

**Forced degradation studies**: In the present study, Flecainide acetate was subjected to different stress conditions, including acid, alkali and oxidative degradation as stated by International Conference on Harmonization guidelines (ICH, 2003). The drug shows the absence of degradation products under basic conditions (Figure 3). Flecainide acetate was degraded up to 6.09% and 25.95% during neutral and peroxide degradation, respectively followed by formation of one major degradation product (Impurity-A) and the respective chromatograms are shown in Figures 4 & 5. Another degradation product (Impurity-D) was found under acidic condition and Flecainide acetate was degraded up to 4.55% (Figure 6). The separation of all the impurities (Impurity-A, B, D & E) using the final optimized chromatographic conditions is shown in Figure 7, indicates the selectivity of the method.

The identification of impurity products was also very effective for knowing the pathways of impurity of drug substances or drug products. Therefore, the impurity products were subjected to LC-MS study to elucidate structural details. Results were tabulated in Table 1 and mass spectrums were shown in Figures 8 & 9.

Mass spectrum shows pure drug at m/z 415.14, Impurity–A, with m/z 397.13 was obtained in neutral and peroxide degradation, Impurity–D

<table>
<thead>
<tr>
<th>Impurity products</th>
<th>Experimental mass</th>
<th>Best possible molecular formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Impurity - A</td>
<td>397.13</td>
<td>C_{17}H_{20}F_{6}N_{2}O_{2}</td>
</tr>
<tr>
<td>Impurity - B</td>
<td>115.12</td>
<td>C_{6}H_{15}N_{2}</td>
</tr>
<tr>
<td>Impurity - D</td>
<td>319.04</td>
<td>C_{11}H_{8}F_{6}O_{4}</td>
</tr>
<tr>
<td>Impurity - E</td>
<td>409.09</td>
<td>C_{17}H_{14}F_{6}N_{2}O_{3}</td>
</tr>
</tbody>
</table>

**Fig. 1**: Structures of Flecainide acetate and its impurities A, B, D & E

Determination of Flecainide acetate and its degradation impurities by UPLC-MS
Fig. 2: Chromatogram of Flecainide Acetate standard

Fig. 3: Chromatogram of Flecainide acetate under base degradation

Fig. 4: Chromatogram of Flecainide Acetate under neutral degradation

Fig. 5: Chromatogram of Flecainide Acetate under peroxide degradation

Fig. 6: Chromatogram of Flecainide Acetate under acidic degradation

Fig. 7: Representative chromatogram showing the selectivity of the method

Fig. 8: MS/MS spectra of a) Flecainide acetate; b) Impurity A; c) Impurity B

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with m/z 319.04 in acidic degradation and Impurity–B & E with m/z 115.12, 409.09 along with A & D in mix and the fragmentation pathways of impurity products were shown in Figures 10-13.

**Conclusion**

The degradation behaviour of Flecainide acetate was studied under various stress conditions. The degradation study indicated that the selected drug was stable to alkali treatment while susceptible to neutral, peroxide and acidic stress. LC-MS study results reveal the formation of four impurity products in the chromatogram and the fragmentation pathways of the so used impurities were also identified. The developed method can be used to construct a profile for Flecainide acetate.
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Conflict of interest: The authors declare that there is no conflict of interests regarding the publication of this paper.

References

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