7. Summary and Conclusion

In present study degradation behavior of ASP and ATR was carried out at length alone as well as in combination. Main aim of this study was to check the chemical incompatibility of between ASP and ATR. Afterwards degradation behavior of TAP and FBX was studied by subjecting TAP and FBX to various degradation conditions as recommended by ICH guidelines. Subsequently major degradation products of TAP and FBX were characterized with the help of LC-MS/MS. From the LC-MS/MS data fragmentation pattern for parent drug as well as major degradation products was also postulated. In addition plausible mechanism of formation of degradation products was also proposed and probable structures of degradation products was also laid down.

A fixed-dose combination of ATR and ASP is widely used for the treatment of myocardial infarction. A comprehensive study of the stress degradation behavior of ATR and ASP was carried out in accordance with ICH guidelines, alone as well as in combination of 1:1 and 1:7.5 ratios, respectively. The degradation products of ASP as well as atorvastatin were successfully separated by a developed simple, selective, and precise stability-indicating reversed-phase HPLC method. Chromatographic separation was achieved on the Phenomenex Luna analytical column, 150 mm x 4.6 mm, 5µm. The mobile phase consisted of 0.1% glacial acetic acid in water and acetonitrile in the ratio of 50:50 v/v at a flow rate of 1.0 ml/min. UV detection was performed at 246 nm. The extent of degradation was significantly influenced when both of the drugs were present in combination. Stress degradation behavior of atorvastatin was highly influenced by aspirin under acid hydrolysis, thermal degradation, and oxidative stress conditions. Similarly, the stress degradation behavior of aspirin was affected by atorvastatin especially under neutral hydrolysis, thermal degradation, and oxidative stress conditions. Additionally, the combination ratio of aspirin and atorvastatin also influenced the percentage degradation of each other. A mixture of aspirin and atorvastatin was also analyzed after a one-month stability study at 40 °C and 75% RH. All the results indicate chemical incompatibility of both aspirin and atorvastatin if present in combination.

A novel, simple, isocratic as well as LC-MS compatible RP-HPLC stability indicating assay method was developed for TAP in bulk. Forced degradation of TAP was carried out in accordance with ICH guidelines. The chromatographic separation was achieved on Inertsil ODS C18 column, (250×4.6 mm i.d., with 5µm particle size) by using mobile phase, ammonium acetate buffer, (12.5 mM, pH 3.60 ± 0.02) and acetonitrile (75:25,v/v). The flow
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was 1 mL/min and detection was carried out at 285nm. TAP found stable under all forced degradation conditions, except oxidative stress. TAP showed two major degradation products in oxidative stress condition. Developed method was able to separate TAP along with both the degradation product generated after stress degradation. Developed method was successfully validated by assessing various validation, parameters as recommended by ICH. Furthermore both oxidative degradation products were characterized with the help of LC-MS/MS technique using same mobile phase. Furthermore its fragmentation pathway and plausible mechanism for generation of degradation product was also proposed. From the study probable structure for oxidative degradation products of TAP was also proposed. Oxidative degradation product DP-I is, 3-(1-(dimethylamino)-3-hydroxy-2-methylpentan-3-yl) phenol n-oxide and degradation product DP-II is, 3-(1-(dimethylamino)-2-methylpentan-3-yl) phenol n-oxide (i.e. N-oxide of Tapentadol). No previous report was available in the literature regarding the characterization of degradation product of TAP.

A new, simple, isocratic as well as LC-MS compatible RP-HPLC stability indicating assay method was developed for FBX in bulk as well as in its pharmaceutical dosage form. Degradation behavior of FBX in bulk as well as its marketed formulation was investigated under various stress degradation conditions as recommended by ICH guidelines. FBX was found instable under alkaline hydrolytic conditions, in all other stress conditions it was found stable. The chromatographic separation and quantitation was achieved on, Phenomenex Luna C18 column, having dimensions, 250 × 4.6 mm i.d., with 5 µm particle size. Mobile phase consisted of acetonitrile and water (0.1 % GAA and 0.1 % ammonia in it) in ratio of 43:57, v/v. The flow was 1 mL/min and detection was carried out at 315nm. Developed method was able to separate FBX and its major alkaline degradation product. Later method was successfully validated by evaluating various validation, parameters as recommended by ICH guidelines. Alkaline degradation product was characterized by LC-MS/MS analysis with the help of same mobile phase. Furthermore fragmentation pathway of alkaline degradation product was laid down. Subsequently, possible mechanism for formation of generation of degradation product was also postulated. Therefore the alkaline degradation product of FBX is 2-(3-carbamoyl-4-isobutoxyphenyl)-4-methyl-1, 3-thiazole-5-carboxylic acid (amide of FBX).

All the developed methods of selected drugs as discussed above can be expediently used in the quality control laboratory for routine analysis as well as for the evaluation of stability study samples.