SUMMARY

OF

IMPURITY PROFILING AND EXPLORATION OF THE DEGRADATION PATHWAYS FOR SOME RECENT DRUGS AND THEIR PHARMACEUTICAL FORMULATIONS

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SUMMARY

INTRODUCTION

Impurity profiling of drug substances and drug products in the Pharmaceutical industry has become a serious topic in recent years, which can make or break the approval of any pharmaceutical product by the respective regulatory agencies around the world. This approval process of NDA's (New Drug Applications) and ANDA's (Abbreviated New Drug Applications) will allow the Brand and Generic pharmaceutical companies to market the drug products in the corresponding countries in which the approval is being sought for.

The major deficiencies in the DMF's (Drug master files) and ANDA's applications submitted to agencies like USFDA and EMEA, after the review of the application are mainly revolving around the identification, reduction in levels, characterization, justification of levels, and qualification of impurities. This also includes the queries on the data related to the identification of the degradation pathways, illustration of the different degradation pathways, stability studies, validation of stability indicating analytical methods, separation of the identified and unidentified impurities from the drug substance and between the impurities itself, the stress studies under different harsh conditions and the residual solvents and the justifications of their levels.

In addition, there is lot of focus on the physical attributes of the drug substance and drug products related to the morphology of the drugs, as in polymorphic forms like crystalline, semi-crystalline or amorphous forms and in the size and shape of the crystals etc. The mixture of multiple polymorphic forms within the single crystallization procedure was observed in several instances and in some other situations the drug converts from one polymorphic form to the pseudo-polymorphic form or another crystal form when stored at different storage conditions or when the drug comes in contact with different excipients and

the packaging components. Therefore, the importance of understanding the distribution pattern and also incorporation of such type of data generated by sophisticated analytical techniques like XRPD (X-Ray Powder Diffraction chromatography), DSC (Differential Scanning calorimeter), Microscopic analysis and Raman Spectroscopy into the drug applications to the governing regulatory bodies is very critical in getting the timely approvals. Another most important physical property of drug which is severely scrutinized and reviewed and demanded in the drug applications is the particle size data (Analyzed by Malvern analyzer or other equivalent techniques) in cases of solid oral dosage forms, suspensions, inhalation products, transdermal and other topical dosage forms. The extent of distribution of various sizes of the particles in Active Pharmaceutical Ingredients in combination with the polymorphic forms has a huge bearing on the bio-equivalence of the generic drugs when formulated into the above mentioned dosage forms when compared against the brand formulations of the same active ingredient. Also, during the development of the new drugs all the above discussed physical properties play a role in understanding the bio-availability of the proposed NCE (New chemical entity) molecules which are in the pre-clinical, phase I and phase-II studies.

Hence, stringent specifications are being set for various types of impurities and the drug companies need to invest lot of funds and resources very early in the development process to comprehensively understand the impurity profiles, potential degradation pathways, possibility of by-products, interaction studies of the drug substances with various excipients and process aids etc. and also to isolate or synthesize and characterize all the possible impurities and use the impurity standards to develop the analytical methods which subsequently need to be validated as stability indicating methods. In the process of developing the drug products, one has to understand the potential toxicological profiles of each of the identified impurities and

its class either being genotoxic, mutagenic or in cases of organic volatile impurities (OVI) / residual solvents whether they are in the class I, class II or class III.

Having a thorough understanding of the impurity profiles of any drug substance and drug product helps immensely in understanding the safety and efficacy of the drug substances and also helps the pharmaceutical companies to get the approvals without any delay. Getting the approval of ANDA's in right time is very critical in this hyper competitive world of generic landscape in order for the drug companies to compete with their peers. Also, in cases of P-IV applications, the six month market exclusivity in USA will be a big factor to determine the success of the drug products commercially, hence providing the data with full details of the impurity profiling will go a long way in securing the drug approval in any country. On the other side if the impurity profiling and the degradation pathways are not understood well and if the application lacks sufficient data on these topics it will lead to a lengthy review process by way of deficiencies, eventually leading to the competitor company winning the race thereby leading to the huge financial impact on the drug companies.

The literature is replete with several review articles and published papers on the importance of the impurity profiling and degradation pathways for the drug products. Also there is lot of data in the form of publications on the old drugs with respect to the impurity profiling.

However, there are so many recent drug products which are approved by the state governing bodies of various countries in the form of NDA's in the last couple of decades. There is very limited literature and publications on these drugs with respect to impurity profiles and degradation pathways as it is limited to only the innovator companies work on the NCE during the developing phase and is in the confidential data submitted to the regulatory agencies. Still the drug products might not have been studied to the fullest extent in order to understand the complete spectrum of the degradation possibilities which leaves lot of scope for research. Another factor which becomes important in order to study the impurity profiles

and degradation pathways for the recent drugs is that when the drug substance becomes off-patent and is available for the rest of the world to make the generic products of the same drug, each company can synthesize the drug substance in several different ways, which might lead to new impurity profiles.

Hence, there is a strong need for conducting research in this area to systematically study the impurity profiles of the recently approved drugs as there are new inventions in the sophisticated analytical equipment's which are enabling to improve the level of understanding of the impurity profiles constantly which might not be at the innovator company's disposal during the development phase. The present research is focused on impurity profiling and exploration of degradation pathways in some recent drugs and their pharmaceutical formulations.

The impurity profiling is available in the literature for several old drugs which are approved a decade or two before to a certain extent but not to the fullest extent. The extent of work on the impurity profiling of the recently approved drugs is very limited and references are hard to find. The innovator or the brand company who submits the NDA (New drug application) shall provide partial impurity profiling information on the drugs submitted. However, when it is manufactured by other generic companies they tend to use different synthetic routes, which will lead to different impurity profiles. Therefore, it is very much in the best interest of the humans who consume these drugs and of the companies who plan to develop the recently approved drugs to know upfront the type of the impurity they should expect and what are the toxicological profiles of those impurities, if they are at or above the threshold levels as guided by the world regulatory bodies like ICH (International Conference on Harmonization) and WHO (World Health Organization).

The present thesis is aimed at understanding the impurity profiles and the most likely degradation pathways possible for the recently approved drugs. The focus is fully on all the

possible impurities like organic, inorganic and enantiomeric impurities along with the residual solvent impurities which could generate as part of the degradation pathways. The polymorphic and the particle size impurities are not considered in the present thesis.

Also, the present work focuses upon the structure elucidation and characterization of the impurities in all the selected drugs and development of the stability indicating Analytical methods using sophisticated instruments like LC-MS/MS (Liquid chromatography/Mass spectrometer), NMR (Nuclear Magnetic Resonance), HPLC (High performance Liquid chromatography), preparatory and flash HPLC, Column Chromatography etc.

The present work has been divided into three main areas of focus for some recent drugs. Total of eight different newly developed and recent molecules that are approved by the USFDA and other regulatory bodies across world like MHRA, ANVISA etc. were selected for the thesis work.

The drugs chosen for the thesis work are as follows:

- Felbamate
- Cevimeline HCl
- Lenalidomide
- Clofarabine
- Saxagliptin HCl
- Ambrisentan
- Bepotastine Besilate
- Conivaptan HCl

The present thesis covers the drug profiles, the synthetic schemes which are used for the synthesis of the drug substances, the HPLC stability indicating analytical methods developed exclusively to separate all the identified and unidentified impurities, structure elucidation and

characterization experiments along with the degradation studies for each of the drugs and the proposed pathways.

In order to study the degradation pathways for any of the drug substances or drug products the drug has to be subjected to mild to extreme conditions of heat, acidity, basicity, oxidation etc. So all the drugs studied were stressed to the following conditions and evaluated the type of impurities which will form in each and every condition.

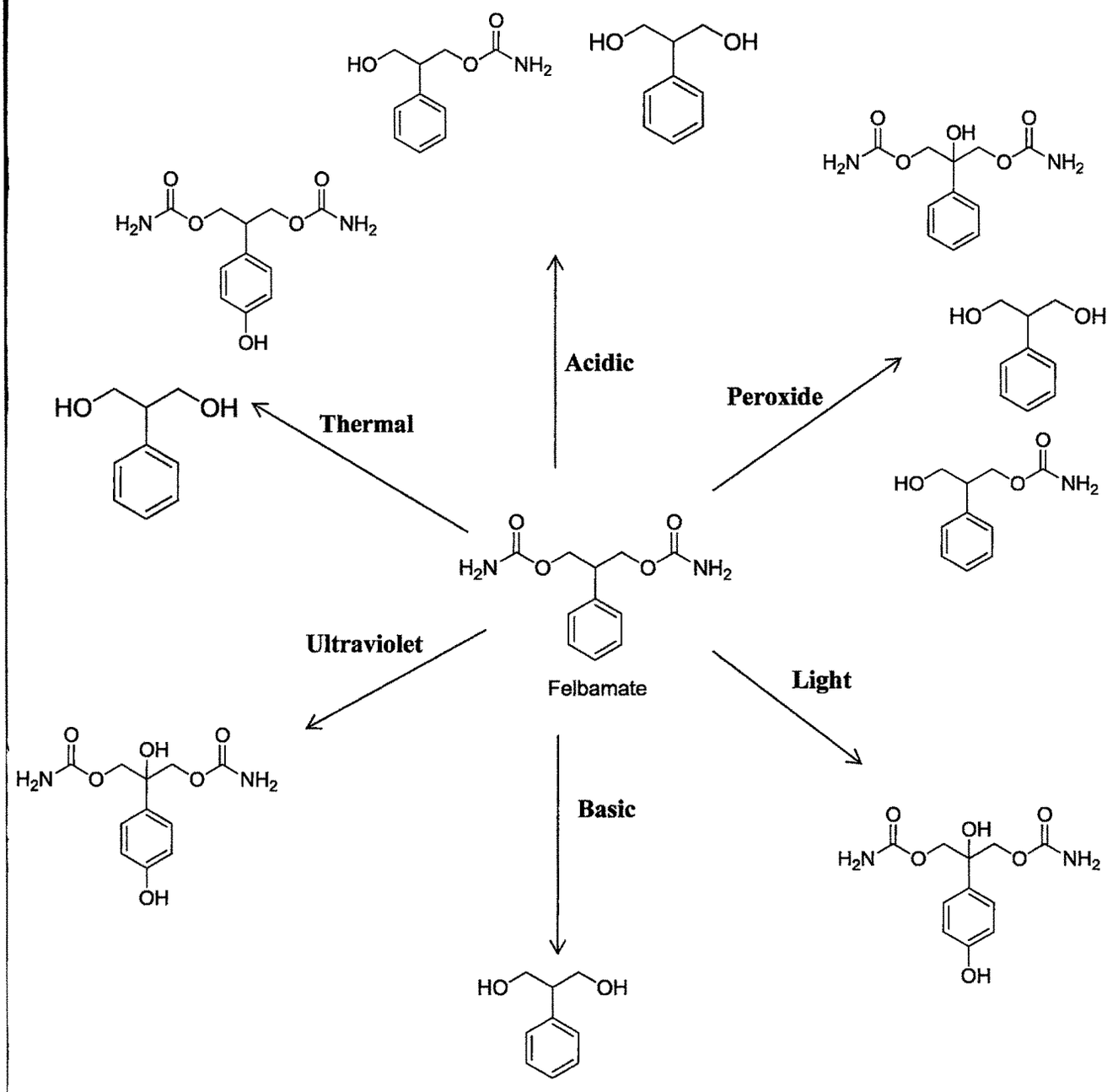
STRESS STUDIES (INDUCED DEGRADATION)

- **HYDROLYTIC DEGRADATION WITH HYDROCHLORIC ACID**
- **HYDROLYTIC DEGRADATION WITH ALKALI (BASE)**
- **PREPARATION OF THERMAL DEGRADATION SAMPLE**
- **OXIDATIVE DEGRADATION WITH HYDROGEN PEROXIDE**
- **PREPARATION OF SUNLIGHT DEGRADATION SAMPLE**
- **PREPARATION OF PHOTOLYTIC DEGRADATION SAMPLE (UV)**

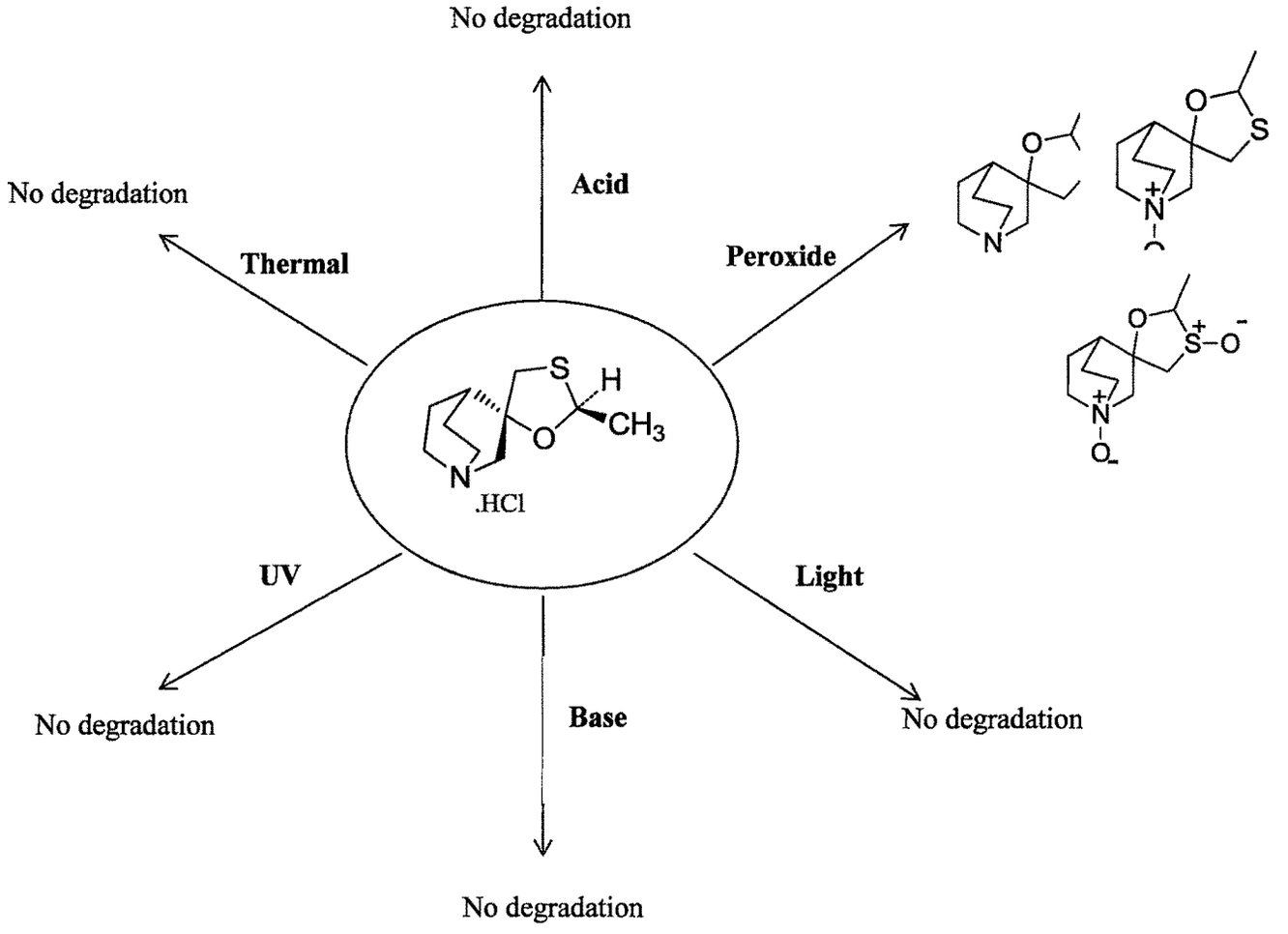
The impurities formed were either isolated through the column purification or enriched by the extreme conditions to convert the drug to the impurity completely and then isolated the pure form of the Impurity from the reaction masses. Wherever the characterization of the impurity was possible based on the data generated on some of the analytical equipment like FT-IR, LC/MS/MS and NMR, the structure was elucidated and accordingly the impurity was synthesized in the pure form and then fully characterized for the physico-chemical properties of these impurities.

Typical Degradation pathways were seen as follows for some of the drugs. The thesis covers all the drugs studied.

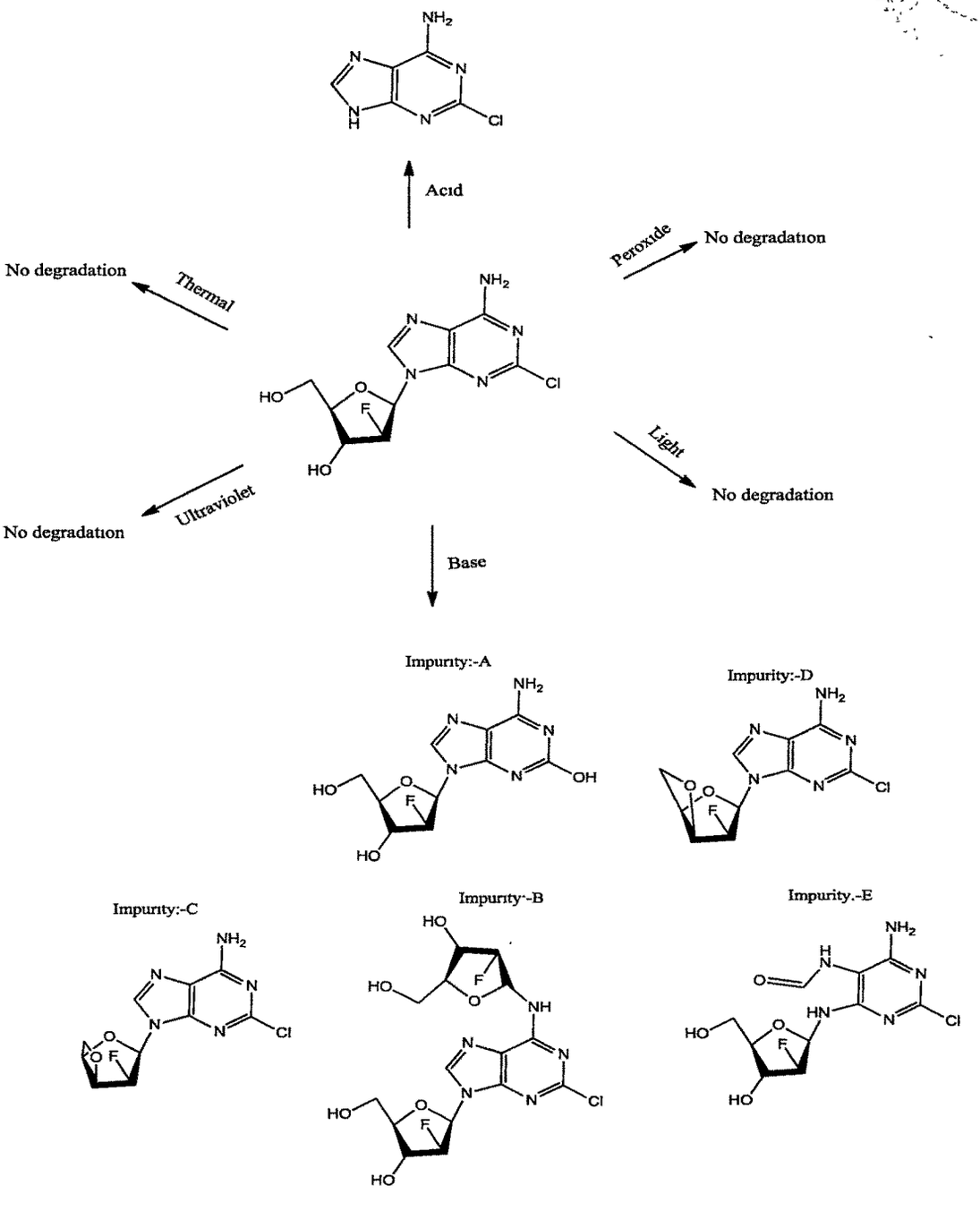
DEDRADATION PATHWAYS FOR FELBAMATE:



Cevimeline


No degradation
Acid
No degradation
Thermal
Peroxide
UV
Light
No degradation
Base
No degradation
No degradation
.HCl

Clofarabine Degradation Pathways



The HPLC methods were developed for all these drugs which separates the degradation impurities, synthetic process related impurities and all others from the drug peaks. One of the example of the HPLC method developed for Bepotsatine besilate is as follows:

ASSAY (% W/W ON DRIED BASIS) AND RELATED SUBSTANCE (% W/W ON AS IS BASIS BY HPLC)

1.1 Instrumentation

- A High Performance Liquid Chromatograph equipped with Gradient elution capability, Ultraviolet Spectrophotometer as detector and an auto sampler. (Shimadzu LC 2010 system or Waters 2690 or equivalent was used)

- **Data handling system**

  LC Solution Software, Empower 2 or equivalent chromatographic software

- **Column**

  A stainless steel column of length 150 mm, internal diameter 4.6 mm and filled with Octadecyl Silane chemically bonded to porous silica particles of 5µ diameter or Inertsil ODS-3V (150 x 4.6mm x 5µ) or equivalent.

1.2 Reagents

1. Distilled Water
2. Monobasic potassium phosphate

   (Potassium dihydrogen orthophosphate, KH_2PO_4)
3. Triethyl amine
4. Orthophosphoric acid
5. Tetrahydrofuran

1.3 Preparation of buffer [Mobile phase-A]

1.3 g of Potassium dihydrogen orthophosphate (KH_2PO_4) was weighed and dissolved in 1000 mL distilled water and mixed well. 10 ml of Triethyl amine was added and

mixed well. The pH was adjusted to 2.4 ± 0.1 with Ortho-phosphoric acid and filtered through 0.45µ or finer porosity membrane filter.

1.4 Preparation of Mobile phase B

ACN: MeOH: THF: 800:200:1

1.5 Elution Program (Gradient Composition)

Initial program: Mobile Phase A: Mobile phase B: 90: 10

Time in minutes	**Mobile phase A (% v/v)**	**Mobile phase B (% v/v)**
0.01	90	10
5.00	90	10
40.00	50	50
45.00	50	50
50.00	90	10
60.00	90	10

1.6 Chromatographic parameters

Flow rate : 1.0 mL/minute

Detection : UV at 210 nm

Injection Volume : 20 µL

Column Oven Temperature : 40°C

Run time : Not less than 60 minutes

1.7 Preparation of diluent

Water: Acetonitrile: 90:10

1.8 Preparation of Bepotastine Besilate Standard solution for assay and RS

50 mg of Bepotastine Besilate standard was weighed accurately and transferred into a 50 mL volumetric flask. 20 mL of diluent was added and sonicated to dissolve the solid with occasional shaking. It is diluted to volume with diluent and mixed well. This is stock standard solution-S

5.0 mL of above stock solution-S was accurately transferred in to 50 mL volumetric flask and diluted to volume with diluent and mixed. This is diluted standard solution-S_1

1.9 Preparation of Benzene sulfonic acid Standard solution

50 mg of Bepotastine Besilate standard was accurately weighed and transferred into a 50 mL volumetric flask. 20 mL of diluent was added and sonicated to dissolve the solid with occasional shaking. It is diluted to volume with diluent and mixed well. This is stock standard solution-S_2

5.0 mL of above stock solution-S was transferred accurately in to 50 mL volumetric flask and diluted to volume with diluent and mixed. This is diluted standard solution-S_3

1.10 Preparation of Sample solution for assay

50 mg of Bepotastine Besilate sample was weighed accurately and transferred in duplicate into individual 50 mL volumetric flask. 20 mL of diluent was added into each volumetric flask and sonicated to dissolve the solid with occasional shaking. It is diluted to volume with acetonitrile and mixed well. This is stock sample solution-A and B

5.0 mL of each stock sample solution-A and B was accurately transferred into individual 50 mL volumetric flask and diluent was added to volume and mixed well. This is diluted sample solution-A_1 and B_1

1.11 Preparation of Sample solution for related substances (RS)

50 mg of Bepotastine Besilate sample was weighed accurately and transferred into 50 mL volumetric flask. 20 mL of diluent was added to the volumetric flask and sonicated to dissolve the solid with occasional shaking. It is diluted to volume with acetonitrile and mixed well. This is sample solution-C for related substances.

1.12 Procedure

The diluent, standard preparation and sample preparation were separately injected into the chromatograph and the chromatograms were recorded. The peak responses were measured only for the major peak in the chromatogram of standard and sample for assay. The peak responses were determined of all eluting peaks in the chromatogram of the sample solution for related substances. Diluent chromatogram was examined for any extraneous peaks, and disregarded the corresponding peaks observed in the chromatogram of the sample solution for related substances.

The injection sequence as mentioned below was followed.

Sr. no.	**Sample**	**No. of injections**
1	Diluent	1
2	Diluted Standard S_1 (system suitability)	6
3	Benzene sulfonic acid	1
4	Sample preparation for Assay – 1	1
5	Sample preparation for Assay – 2	1
6	Sample preparation (RS)	1

The table below for retention and relative retention time of known impurities was followed

Name of compound	Retention time (Minutes)	Relative retention time (RRT)
Besilate	6.2	0.28
Bepotastine	22.2	1.0
Bepotastine Besilate related compound – A	29.6	1.33

Typical results and conclusions for the studies performed, characterized and methods developed for the recent drugs in order to research the Degradation pathways is captured in conclusions section like below for all the drugs studied.

CONCLUSION

Based on the studies performed to study extensively the various degradation pathways we have observed that different impurities can be formed due to the stress effects on the Clofarabine drug substance. Not all stress conditions are generating the same impurities, however the impurities formation mechanism is pretty similar to other nucleoside compounds which are approved in the market.

When the exercise was initiated on drug product also, we have observed the similar trend in the impurity profiles of the Clofarabine since the drug product is the solution of Clofarabine in sterile vial with buffers, therefore exactly similar impurity profiles will be observed.

The impurity isolations and characterizations of the same, led to develop a better HPLC method which is a stability indicating method in order to monitor the related substances present in the drug substances and drug products. This method can be used at Quality Control laboratories which are required to test and release the Clofarabine drug substance and drug products for human use.

The knowledge of the possible degradation pathways achieved in this project prompted us to understand the impurities behavior in the Humans when administered either orally or systemic, and to get the toxicology information of the same. After thorough literature search, we have found that the impurities found via forced degradation studies were partially similar to the metabolites found in human patients and animal studies performed on Clofarabine as a part of clinical studies. These metabolites are already studied for its safety since Clofarabine dosed to human patients has seen extensive metabolism to give rise to the metabolites which are similar to our degradation impurities. Other process and degradation impurities should be controlled by Pharmaceutical companies in order to get the drug approvals for marketing.