IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Review Article
May 2023 Vol.:27, Issue:2

Recent Approaches of "Impurity Profiling in Pharmaceutical Analysis of Dabigatran Etexilate Mesylate": A Review

IJPPR
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An official Publication of Human Journals
ISSN 2349-7203
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Submitted: 27 April 2023
Accepted: 02 May 2023
Published: 30 May 2023

HUMAN JOURNALS
www.ijppr.humanjournals.com

Keywords: Impurity profiling, Impurities, Identification, Analytical, Elucidation.

ABSTRACT

Impurity profiling may be described as maximum possible types of identified or unidentified impurities present in any APIs and the formulated products. These impurities can be API related impurities, process related impurities or stability related impurities. Impurity profiling is the process of acquiring and evaluating data that establishes biological safety of an individual impurity; thus, revealing its need and scope in pharmaceutical research. It is developed either during formulation, or upon aging of both API's and formulated API's in medicines. The presence of these unwanted chemicals, even in small amount, may influence the efficacy and safety of the pharmaceutical products. And it is the main scenario, why it is nowadays, mandatory by regulatory authorities. The impurities are not necessarily always present in lower limit, sometimes it may be excess due to various reasons and cause severe hazards. Highly sophisticated instrumentation, such as mass spectra meters attached to a Gas Chromatography or HPLC, are inevitable tools in the identification of minor components (drugs, impurities, degradation products, metabolites) in various matrices. There is no clear definition for impurity in the pharmaceutical world. Impurity profiling includes identification, structure elucidation and quantitative determination of impurities and degradation products in raw material, and pharmaceutical formulations. Terms such as residual solvents, byproduct, transformation products, degradation products, interaction products and related products are frequently used to define impurities. The advent of hyphenated techniques has revolutionized impurity profiling, by not only separation but structural identification of impurities as well. The present review covers various aspects related to the analytical method development for impurity profiling of an active pharmaceutical.

INTRODUCTION:

Dabigatran is an anticoagulant that works by blocking a certain substance (a clotting protein called thrombin) in your blood. Quality is not optional of any pharmaceutical product. The source of pharmaceutical products varies greatly, from plants/marine sources (natural resources), synthetic methods or recombinant DNA methods or a combination of any of these. The bulk drug industry forms base of all pharmaceutical industries as it is the source of active pharmaceutical ingredients (APIs) of specific quality. A. Common Terms of Impurities which are used by various regulatory bodies and ICH to describe the impurities: 1. Intermediate 2. Penultimate intermediate 3. By-products 4. Transformation products 5. Interaction products 6. Related products 7. Degradation products.

1. Intermediate: The compounds produced during synthesis of the desired material or as a part of the route of synthesis.

2. Penultimate Intermediate: It is the last compound in the synthesis chain prior to the production of the final desired compound.

3. By-products: The compound produced in the reaction other than the required intermediates. They can occur through a variety of side reactions, such as overreaction, incomplete reaction, demonization and rearrangement, unwanted reactions between starting materials or intermediates with chemical reagents or catalysts.

4. Transformation Products: They are related to theorized and nontheorized products that can occur in a reaction. They are similar to by-products except that more is known about these reaction products.

5. Interaction Products: These products formed either intentionally or unintentionally interaction between various chemicals involved.

6. Related Products: These are chemically similar to drug substance and may even possess biological activity.

7. Degradation Products: They are formed by the decomposition of active ingredient or other material of interest by the effect of external factors like heat, light and moisture.

B. Classification of Impurity: According to USP impurities are classified into three sections:

1. Impurities in Official Articles

2. Ordinary Impurities

3. Organic Volatile Impurities

The ICH Terminology According to ICH guidelines, impurities in drug substance produced by chemical synthesis can be broadly classified into following three categories: 1. Organic Impurities (Process and drug-related) 2. Inorganic Impurities (Reagent, ligands, catalysts) 3. Residual Solvents (Volatile solvents).

Raw Materials employed in the Manufacturing of the Pharmaceutical Substance: Pharmaceutical substances are either isolated from natural sources or synthesized from chemical starting materials. The natural sources include mineral sources, plants, animals and microbes.

1. Method of Manufacture: The process or method of manufacture may introduce new impurities into the final product arising due to contamination by reagents, catalysts and solvents employed at various stages of the manufacturing process.

2. Regents used to eliminate other impurities:

3. Solvents: Various types of water used are:

- Tap water
- Distilled water
- Purified water
- De-mineralized water:
- Softened water
- RO Water

4. Intermediate

5. Reaction vessels

6. Atmospheric contamination during the Manufacturing Process

7. Chemical Instability of product

8. Contamination by microbes

9. Formulation Related impurity

Impurity profiling significance:

In assuring the high standard quality of drug products reached into the market, it's important in screening impurities existing in the medicinal product throughout manufacturing. As compared to the online spectrum obtained during the earlier impurity profiling study that is required for registration of the drug master file, the standard impurity spectrum is of better purity and superiority. Synthesized impurity is used for toxicological studies and also use as the standard for determining impurity. The impurity existing in the pharmaceutical product may vary the dissolution and solubility of the drug constituent and may affect the systemic circulation thus, it not only affects patient safety but also alter the biopharmaceutical behaviour of the drug substance. Thus, impurity profiling is essential for assuring the quality, efficacy and safety of pharmaceutical products.

Latest Techniques for detecting Impurity

- **Liquid chromatography-nuclear magnetic resonance spectrometry (LC-NMR)**

LC-NMR is a viable commercial technology, since about the middle of 1990. LC-NMR has sensitivity issues; here sensitivity is the capability of the NMR spectrometer to acquire sufficient data to allow for the unambiguous structure elucidation of the trace intensity in a compound mixture analyzed by HPLC. Previously many limitations of LC-NMR had obstructed its widespread application; however, this limitation has been recently overcome. Since the cryoprobes are highly sensitive as compared to the regular probes. **High-performance liquid chromatography-Mass spectrometry (HPLC-MS):** The combination of LC-MS has several advantages over the individual instrumentation LC and MS spectrometry. Owing to the high selectivity, sensitivity, dynamic range and ruggedness of LC-MS, it has been extensively employed in the pharmaceutical research area. It has excellent sensitivity for the small amount of impurity and degrading products. The innovation in the current era, increase the use of the LC-MS/MS, which is also known as tandem LC-MS. It has wide applicability in the quantitative analysis and structure elucidation of the unknown impurity.

- LC-MS/NMR

The limitation of on-flow LC-NMR is the sensitivity issue, at the magnetic field strength of 500 MHz and 1ml/min flow rate the residence time of the analyte is reduce and the detection

limit is 10 ppm. Thus, to increase the analyte residence time the flow rate should be reduced but this may increase the analysis time and also affect the resolution. Hence one of the recent advancements to increase the NMR sensitivity is LCMS/ NMR.

Directly coupled LC-MS/NMR is used universally in pharmaceutical laboratories. Coupling of the HPLC with that of MS and NMR is known as LC-MS/NMR,

Impurity Profile of Dabigatran Etexilate Mesilate:

HPLC METHOD

RUN TIME: 50 minutes

Auto sampler temperature 5ºC

Buffer: Dissolve 0.63 g of Ammonium formate in 1000mL of Milli Q-Water and mix well. Adjust its pH to 8.2 with Ammonia and filtered through 0.22 µm nylon membrane and degas it.

Mobile phase- A: Buffer

Mobile phase- B: Acetonitrile:Water (80:20) v/v

Diluent: Acetonitrile: Water (80:20) v/v

Needle wash: Diluent

Elution: Gradient

GRADIENT PROGRAMME

Time (in minutes)	Mobile Phase- A (%)	Mobile Phase B (%)
0.01	62	38
3	62	38
25	40	60
28	40	60
35	15	85
40	15	85
41	62	38
50	62	38

Note:

Use freshly prepared solution only.

Sample solution should be stabilized up to 12 hours at about 5degree Celcius and Mobile phase should be kept for 36 hours at Room temperature.

Impurities stock solution preparation Method:

Weigh each about 5mg of Methyl carbomate, Ethyl carbomate, Amide compound and Despyridyl ethyl ester into 10 ml volumetric flask, add about 7-8 ml of diluent and sonicate to dissolve. Make upto the mark with diluent and mix well.

Deshexyl compound stock solution preparation: Weigh about 5 mg of Deshexyl Compound into 10 ml volumetric flask, add 7-8 ml of Acetonitrile: water (1:1) solution and sonicate to dissolve and make up the mark with same solution.

Note: Deshexyl compound is not prepared in specified diluent, hence prepared separately.

System suitability solution: Weigh about 15 mg of Dabigatran Etexilate Mesilate standard into a 10 ml volumetric flask, add about 7-8 ml of diluent sonicate to dissolve. Add 45 microlitre of Impurities stock solution and 45 microlitre of Deshexyl compound stock solution. Make upto volume to mark and mix well.

Impurities blend solution preparation: Weigh about 15 mg of impurities blend sample into a 10 ml volumetric flask, add about 7-8 ml of diluent, mix well to dissolve. Make upto the volume with diluent and mix well.

Note: The above solution is only to identify the individual components and not for quantification.

Acceptance criteria for SST Solution:

Resolution between Amide compound and Dabigatran Etexilate peak should not be less than 2.0.

Theoretical plates for Dabigatran Etexilate peak should ne not less than 3000.

Tailing factor for Dabigatran Etexilate peak should not be more than 2.0.

Reference Solution preparation: Weigh about 15 mg of Dabigatran Etexilate mesilate standard into a 10 ml volumetric flask, add about 7-8 ml of diluent, mix well to dissolve. Make upto the mark with diluent and mix well. Transfer 100 µL of above solution into a 100 ml volumetric flask, containing about 60 ml of diluent. Make upto the mark with diluent and mix well.

Acceptance criteria for Reference Solution.

Detector sensitivity for Dabigatran Etexilate from reference solution of first injection, the S/N ratio should not be less than 30.

% RSD of reference solution should not be more than 5.0%.

Test Solution preparation: Weigh about 15 mg of test sample and transfer into 10 ml volumetric flask. Add about 7-8 ml of diluent and mix well to dissolve. Make upto the mark with diluent and mix well.

PROCEDURE: After equilibrating the column inject diluent as blank, System suitability test solution to test the system suitability criteria. Once the system suitability test passes then inject diluent as blank, reference solution six injections and followed by test solution preparation and system suitability solution into liquid chromatographic system and record the chromatograms. Disregard the peaks due to blank.

Record the results.

Impurities Calculation: Identify known impurities based on retention time of peaks present in the chromatogram of the test solution corresponding peaks present in the system suitability solution/impurities blend solution. Calculate the amount of impurities area percentage taken by using the following formula:

$$\% \text{ Impurity: } \frac{\text{SAMA}}{\text{STD A}} \times \frac{\text{SAM V}}{\text{SAM W}} \times C \times 100$$

Total Impurities + Highest individual unspecified impurity + other total impurities

Note: Report the average results from sample solution preparations.

Where, SAM A: Peak area of individual related compound in sample preparation.

STD A: Average peak area for Dabigatran Etexilate from duplicate injections of reference solution preparations.

SAM V: Volume in mL of sample preparation.

SAM W: Sample weight in mg in sample preparation.

C: Concentration of Dabigatran Etexilate Mesylate in reference solution preparation in mg/ml

100: Conversion factor for percent

Acceptance criteria:

The % of each impurity and total impurities in individual sample solution preparation should be within the specification limit.

The practical impurity Profile data of Dabigatran Etexilate Mesylate is listed below after chromatographic condition of HPLC performance as stated above.

First Chromatogram is of BLANK.

Chromatogram of Test

Chromatogram of SSS with all impurities identified

CALCULATION OF IMPURITIES AS PER METHOD

CONCLUSION

According to the regulatory guideline, the analytical monitoring of impurity in a new drug substance is a mandatory requirement for market authorization. It is good as far as health and safety of people is concerned. Each and every related impurities of Dabigatran Etexilate Meylate was reported practically performing HPLC METHOD. And all the data are compiled showing the raw material is approved for its use.

Impurity profiling has gained importance in modern pharmaceutical analysis due to the fact that unidentified, potentially toxic impurities are hazardous to health and in order to increase the safety of drug therapy, impurities should be identified and determined by selective methods as per guidelines or monograph or specification.

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