CHAPTER 10

Conclusion

The presence of impurities in active pharmaceutical ingredient (API) can have a significant impact on the quality, safety and efficacy of drug products. Therefore, it is important to have a stability indicating validated method for the quantitative determination of potential impurities in the drug substance. ICH guidelines indicate that unknown impurities at or above 0.05% in the drug substance require identification depending on the maximum daily dosage. Characterization of unknown impurity is also essential to ascertain that an impurity does not have genotoxic concern; hence characterization of unknown impurities is critical to establish the quality, safety and efficacy of drug substances. As a common practice, efforts should be made to identify and characterize all unknown impurities in the drug substance due to the ever increasing demand from regulatory agencies to manufacture high purity drug substances. Impurity profiling of drugs is one of the most important issues in the modern pharmaceutical analysis for developing process technology to manufacture high purity drug substance.

A key component of the overall quality of a pharmaceutical is control of impurities, as their presence; even in small amounts may affect drug safety and efficacy. In wake of the stringent regulatory requirement, and safety concerned impurity profile of seven drug substances, which comprises of anti-Alzheimer’s, anti-epileptic, renal and genitourinary agent, iron chelating agent and anti-depressant, were carried out.

Six impurities were detected at trace level in rivastigmine tartrate drug substance by a newly developed high performance liquid chromatography method. These impurities were characterized rapidly and three impurities were found to be unknown. The unknown impurities were enriched and identified with a combination of semi-preparative HPLC and LC/MS/MS techniques. Proposed structures were further confirmed by characterization using NMR, FT-IR, and EA techniques of impurity standards. Based on the spectroscopic, spectrometric and elemental analysis data unknown impurities were
characterized as 3-[(Dimethylamino)ethyl]phenyl N-ethyl-N-methyl carbamate N-oxide, ethyl-methyl-carbamic acid 4-(1-dimethylamino-ethyl)-phenyl ester and ethyl-methyl-carbamic acid 2-(1-dimethylamino-ethyl)-phenyl ester. Major oxidative degradant impurity was identified as rivastgmine N-oxide.

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\text{Rivastgmine tartrate and new impurities}
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The LC-UV method was validated as per ICH guidelines. Newly developed HPLC method was found to be simple, sensitive, selective, cost effective and stability indicating. Detection limit for impurities was found to be as low as 0.01% indicating high sensitivity of the validated method and can be conveniently used for routine and stability studies. Regression analysis showed correlation coefficient value greater than 0.999 for rivastgmine tartrate and its impurities. Accuracy of the method was established based on the recovery obtained between 93.41 to 113.33% for all impurities. Formation of newly characterized impurities (Imp-A, Imp-B, Imp-C) can be eliminated in the drug substance by use of inert atmosphere and control of isomeric impurities in 3-hydroxy acetophenone.

Galantamine hydrobromide was subjected to oxidative stress degradation using hydrogen peroxide and analysed as per the chromatographic conditions described in European pharmacopoeia. The drug showed considerable degradation at ambient temperature resulting in the formation of two impurities at relative retention time (RRT) 0.63 and at 2.52. The minor degradant at RRT 0.63 was identified as galantamine N-oxide.

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Galantamine hydrobromide and new impurity

The principal degradant formed at RRT 2.52 was found to be unknown and has not been reported previously. The unknown impurity was identified by LC–MS/MS followed by isolation using semi-preparative HPLC. The isolated impurity was characterized using one dimensional, two dimensional nuclear magnetic resonance spectroscopy (1D and 2D NMR) and elemental analysis (EA). The principal degradant was found to be formed due to the generation of bromine and subsequent attack on the aromatic ring via in-situ reaction between hydrogen bromide and hydrogen peroxide. The unknown impurity was characterized as (4aS,6R,8aS)-5,6,9,10,11,12-hexahydro-1-bromo-3-methoxy-11-methyl-4aH-[1]benzofuro[3a,3,2-ef] [2] benazepin-6-ol.

A novel, sensitive, selective and stability indicating LC-MS method was developed for the determination of potential impurities of eslicarbazepine acetate. High performance liquid chromatographic investigation of eslicarbazepine acetate laboratory sample revealed the presence of several impurities. Three impurities were characterized rapidly and four impurities were found to be unknown.

The unknown impurities were identified by liquid chromatography coupled with electrospray ionization, ion trap mass spectrometry (LC/ESI-IT/MS/MS). Structural confirmation of these impurities was unambiguously carried out by synthesis followed by characterization using nuclear magnetic resonance spectroscopy (NMR), infrared spectroscopy (FT-IR), mass spectrometry (MS) and elemental analysis (EA). Based on the spectroscopic, spectrometric and elemental analysis data unknown impurities were characterized as 5-Acetylid-5,11-dihydro-10H-dibenzo[b,f]azepin-10-one, N-Acetyl-5H-dibenzo[b,f]azepine-5-carboxamide, 5-Acetyl-10,11-dihydro-5H-
dibenzo[b,f]azepin-10-yl acetate and 5-Acetyl-5H-dibenzo[b,f]azepin-10-yl acetate. Major impurity formed during acidic and alkaline stress conditions was identified as licarbazepine. The newly developed LC-UV method was validated as per ICH guidelines. HPLC method was found to be simple, sensitive, selective, cost effective and stability indicating. Detection limit for impurities was found to be as low as 0.005% and was found to have excellent resolution for fifteen impurities indicating high sensitivity and selectivity of the method.

Monohydroxy carbazepine, eslicarbazepine, eslicarbazepine acetate and its new impurities

LC-MS studies were performed to get molecular weight, establish its mass fragmentation profile and identify an unknown impurity in carbamazepine active pharmaceutical ingredient.

Carbamazepine and new impurity

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Unknown impurity was isolated using semi-preparative HPLC. Studies were carried out using NMR, FT-IR and CHNS analyzer to further confirm the proposed structure and the molecular formula of unknown impurity could be deduced as C_{30}H_{30}N_{2}O_{8}S and the corresponding structure was characterized as tetra-benzof[b,f,h][a]zipino[4',5',4,5][thieno][2,3-d]azepine-3,9-dicarboxamide.

The present study describes the identification, characterization of two process impurities and major stress degradants in darifenacin hydrobromide using high performance liquid chromatography (HPLC) analysis. Forced degradation studies confirmed that the drug substance was stable under acidic, alkaline, aqueous hydrolysis, thermal and photolytic conditions and susceptible only to oxidative degradation. Impurities were identified using liquid chromatography coupled with ion trap mass spectrometer (LC-MS/MS). Proposed structures were unambiguously confirmed by synthesis followed by characterization using nuclear magnetic resonance spectroscopy (NMR), infrared spectroscopy (IR) and elemental analysis (EA). Based on the spectroscopic, spectrometric and elemental analysis data, the unknown impurities were characterized as 2-(1-[(2,3-Dihydro-benzofuran-5-yl)-2-oxo-ethyl]-pyrrolidin-3-yl]-2,2-diphenyl-acetamide (Imp-A), 2-(1-[(2-Benzofuran-5-yl-ethyl)-pyrrolidin-3-yl]-2,2-diphenyl-acetamide (Imp-B), 2-(1-[(2,3-Dihydro-benzofuran-5-yl)-ethyl]-1-oxy-pyrrolidin-3-yl]-2,2-diphenyl-acetamide (Imp-C).
and 2-{1-[2-[(7-Bromo-2,3-dihydro-benzofuran-5-y1)-ethyl]-pyrrolidin-3-y1]}-2,2-

diphenyl-acetamide (Imp-D).

The plausible mechanisms for the formation and control of these impurities have also been proposed. The method was validated as per regulatory guidelines to demonstrate specificity, precision, linearity, accuracy and the stability indicating nature. Regression analysis showed correlation coefficient value greater than 0.99 for darifenacin hydrobromide and its impurities. Accuracy of the method was established based on the recovery obtained between 86.6 to 106.7% for all impurities.

An unknown impurity was detected in deferasirox drug substance by a newly developed high performance liquid chromatography (HPLC) method. The unknown impurity was identified by liquid chromatography-tandem mass spectrometry using electrospray ionization source and Q-trap mass analyzer (LC–ESI–QT/MS/MS).

![Deferasirox and new impurity](image)

Based on LC–MS/MS data and knowledge of the synthetic scheme of deferasirox, this impurity was proposed as the regio-isomer of deferasirox. Structural confirmation of this impurity was unambiguously carried out by synthesis followed by characterization using nuclear magnetic resonance (NMR), infrared spectroscopy (IR), mass spectrometry, elemental analysis (EA) and the impurity was confirmed as 2-[3,5-bis(2-hydroxy-phenyl)-[1,2,4]-triazol-1-yl]-benzoic acid (Imp-1). The newly developed method was validated according to ICH guidelines. The resolution between Imp-1 and deferasirox was found to be more than 6.0 and the detection limit of impurities was in the range of 0.0005% to 0.01%, indicating high selectivity and sensitivity of the newly developed method.

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Two impurities were detected during the high performance liquid chromatographic (HPLC) analysis of citalopram hydrobromide sample by United States Pharmacopoeia (USP) method-1. One of the impurities of the order of 0.25% was found to be not reported previously. Unknown impurity was identified by a liquid chromatography-mass spectrometry (LC-MS) compatible reverse phase isocratic method using electrospray ionization source and ion trap mass analyzer. Impurity was isolated by semi-preparative HPLC followed by characterization using nuclear magnetic resonance spectroscopy (NMR), infrared spectroscopy (FT-IR) and elemental analysis (EA).

![Citalopram hydrobromide and new impurity](image)

**Citalopram hydrobromide and new impurity**

Structure of this impurity was unambiguously confirmed by synthesis and the impurity was characterized as 1-[(1-3-Dimethylamino-propyl)-1-(4-fluoro-phenyl]-1,3-dihydro-isobenzofuran-5-yi etherone. A logical mechanism for the formation of this impurity is also proposed.

In order to serve the public with best possible pharmaceutical products, various regulatory agencies across the globe are putting stringent regulatory guidelines for the identification, qualification, determination and control of impurities as low as possible to consistently manufacture high quality drug products. This work provides detailed study from synthetic sequence of pharmaceutical substance, stability indicating method development for the quantitative determination of impurities, preparation of impurities either by semi-preparative isolation or by synthesis. Characterization of impurities by combination of chromatographic, spectroscopic, spectrometric and elemental analysis. Method validation was carried out as per ICH guidelines. Forced degradation study conducted to understand the degradation behavior of drug substance and to recommend suitable packaging and storage conditions.
LIST OF PUBLICATIONS

1.0 Semi-preparative isolation and characterization of a principal oxidative degradation impurity in galantamine hydrobromide by LC-ESI-MS<sup>n</sup> and 2D-NMR
   *Acta Chromatographica, communicated*
   Saji Thomas, Saroj Kumar Paul, Ashutosh Agarwal, Chandra S Mathela

2.0 Characterization of an unknown impurity in citalopram hydrobromide active pharmaceutical ingredient by semi-preparative isolation and LC-ESI/MS<sup>n</sup> and NMR
   *Journal of Chromatography and related technologies, Accepted manuscript*
   Saji Thomas, Ashutosh Agarwal, Raghavendra Desai Rao, Chandra S Mathela

3.0 Identification and structural elucidation of two process impurities and stress degradants in dafilnacin hydrobromide active pharmaceutical ingredient by LC-ESI/MS<sup>n</sup>
   *Analyst, 2012, 137, 3571-3582*
   Saji Thomas, Saroj Kumar Paul, Sanjeev Shendilya, Ashutosh Agarwal, Nitesh Saxena, Arun Kumar Awashi, Hari Babu Matta, Dharam Vir, Chandra S Mathela

4.0 Identification, characterization and quantification of a new impurity in deferasirox active pharmaceutical ingredient by LC-ESI-QT/MS/MS
   *Journal of Pharmaceutical and Biomedical Analysis, Volume 63, 7 April 2012, Pages 112-119*
   Saji Thomas, Subhash Chandra Joshi, Dharam Vir, Ashutosh Agarwal, Raghavendra Desai Rao, I. Sridhar, Clj O M. Xavier, Chandra S. Mathela

5.0 Highly efficient, selective, sensitive and stability indicating RP-HPLC–UV method for the quantitative determination of potential impurities and
characterization of four novel impurities in eslicarbazepine acetate active pharmaceutical ingredient by LC/ESI-IT/MS/MS
*Journal of Pharmaceutical and Biomedical Analysis, Volume 61, 5 March 2012, Pages 165-175*
Saji Thomas, Amber Bharti, Fawan Kumar Maddhesia, Sanjeev Shandilya, Ashutosh Agarwal, Dharam Vir, Sujay Biswas, Vikas Bhansal, Ashish Kumar Gupta, Praveen Kumar Tewari, Chandra S. Mathela

6.0 Identification, characterization and quantification of new impurities by LC-ESI/MS/MS and LC-UV methods in rivastigmine tartrate active pharmaceutical ingredient
*Journal of Pharmaceutical and Biomedical Analysis, Volume 57, January 2012, Pages 39-51*
Saji Thomas, Sanjeev Shandilya, Amber Bharati, Saroj Kumar Paul, Ashutosh Agarwal, Chandra S. Mathela

7.0 Identification and structural elucidation of an unknown impurity in carbamazepine active pharmaceutical ingredient by liquid chromatography-tandem mass spectrometry and semi-preparative chromatographic isolation
*Journal of Pharmaceutical and Biomedical Analysis, Volume 56, Issue 2, 10 September 2011, Pages 423-428*
Saji Thomas Chandra S. Mathela, Ashutosh Agarwal, Saroj Kumar Paul