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Review Article

APPLICATION OF LIQUID CHROMATOGRAPHY COUPLED WITH MASS SPECTROMETRY IN THE IMPURITY PROFILING OF DRUG SUBSTANCES AND PRODUCTS

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ABSTRACT

As the drug safety and efficacy is hampered in the presence of an impurity, the international regulatory agencies laid down stringent limits for the control of impurities in the active pharmaceutical ingredient and pharmaceutical formulations. The conventional approaches lack the characterization of impurities in trace levels, due to sensitivity issues, hyphenated techniques are preferred. Among the modern hyphenated techniques, liquid chromatography-mass spectrometry (LC-MS) has high sensitivity and can analyze large number of organic compounds in a short period of time. In the present study, the impurity profiling of various drug substances and products using LC-MS about past 6 years were retrospect for its importance, instrumentations, and applications.

Keywords: Impurities, International regulatory agencies, Hyphenated techniques, Liquid chromatography-mass spectrometry.

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INTRODUCTION

ICH Guidelines defines the impurity as "Any component of the medicinal product which is not the chemical entity defined as the active substance or excipients in the product" [1]**.** The impurity profile is the characterization of the identified and unidentified impurities that are present in the drug product [2].

The critical part of drug discovery and development is to establish a qualitative and quantitative impurity profile of pharmaceuticals. The quality of the pharmaceutical product is affected by impurities. Drug regulatory authorities expect to furnish in detail all impurities that might merge during the production and storage, which provides the basis for a comprehensive risk assessment.

The impurity profile can provide complete information about the manufacturing process, degradation process and it is diagnostic for the overall quality of the final product. The drug safety and quality can be ensured by knowing the identity of the impurities, thereby helps to analyze the abnormality in the production.

The impurities in pharmaceutical products are not only restricted to the process residual impurities, manufacturing impurities, and degradation products resulting from storage but also includes genotoxic impurities. Genotoxic impurities induce the genetic metamorphosis, chromosomal ruptures or chromosomal disruptions and it may cause cancer in humans.

If impurity profiling is not addressed properly, then the regulatory bodies may compel to recall the finished drug products or reject the approval of such products, or the drug may be kept on hold. In past few years the US-FDA ordered for the recall of few finished pharmaceutical products such as adagen (Pegademase bovine) injection, azelastine hydrochloride ophthalmic solution, brimonidine tartrate ophthalmic solution, budeprion XL (Bupropion HCl ER Tablets), prednisolone sodium phosphate oral solution, and topiramate 25 and 200 mg tablets due to the presence of impurities and degradation products [3].

The present review article endures the works of impurity profiling of various drug substances and product by employing the liquid chromatography-mass spectrometry (LC-MS) technique. The article mainly highlights the regulatory requirements for the control of impurities, columns, interface, ionization techniques, and mass analyzer used in the past 6 years of works on impurity profiling. Fig. 1 represents the number of research works that are undergone in the field of impurity profiling using LC-MS in past 6 years.

NEED FOR THE IMPURITY PROFILING OF PHARMACEUTICAL PRODUCTS

Benzopyridooxathiazepine derivatives are potent, antimitotic class of cytotoxic compounds. The *in vitro* cytotoxicity studies showed promising results. However, the *in vivo* activity was poor due to drug decomposition and the advent of possible adverse effect showed due to the formation of degradation products. The study showed that 10 main degradation products out of which seven structures were elucidated. The structural comparison showed that the stability problem arises due to oxathiazepine ring of BZN which could have some consequences on storage conditions [4].

MLN9708 is a small molecule proteasome which is currently under investigation developed by Millennium: The Takeda Oncology Company, on the evaluation of impurities, found that 2, 5-dichlorobenzoyl chloride (DCBC) was predicted to be mutagenic. The carryover of this impurity into drug substance was low due to multiple acid; base workup steps. However, FDA suggests to provide the verification data [5].

TYPES AND SOURCES OF IMPURITIES

As per the ICH guidelines, the impurity in pharmaceutical entities and products can be categorized into five subheadings:

- Organic impurities
- Inorganic impurities
- Residual solvents
- Elemental impurities
- Genotoxic impurities.

Organic impurities emerge during the course of production and/or while storing new drug substance. Organic impurities are further classified as:

- Starting materials
- By-products
- Intermediates
- Degradation products
- Reagents, ligands, and catalyst.

Inorganic impurities arise during the manufacturing stage, and they can be classified as:

- Reagents, ligands, and catalyst
- Heavy metals or other residual metals
- Inorganic salts
- Other materials (Ex: Charcoal and filter aids) [1].

Residual solvents are the remnants of the solvents used in the production of pharmaceutical entities, excipients or in the process of formulating the drug product. Residual solvents can be classified based on their toxicity levels as:

- Class I solvents: The use of solvents present in this class must be avoided. Ex: Benzene and carbon tetrachloride.
- Class II solvents: The use of solvents present in this class must be controlled. Ex: Acetonitrile, chloroform, and Hexane.
- Class III solvents: The solvents present in this class have low lethal effects. Ex: Acetic acid, acetone, and formic acid.
- Class IV solvents: The solvents present in this class lack in required toxicological data. Ex: Isopropyl ether, petroleum ether, and trifluoroacetic acid [6].

ICH Q3B(R2) guidelines for impurities in new drug product includes the impurities that arise due to deterioration of pharmaceutical entities or the products arise due to the reaction between drug substance and excipients and/or immediate packaging materials [7].

Elemental impurities in pharmaceuticals emerge from various sources, i.e., maybe the unconsumed catalyst that was added purposefully in the process of production or may be formed due to the reaction between the manufacturing equipment or packaging systems or exist in the drug product.

Elemental impurities may be classified as:

- Class I elements: The elements of this class have minimal or no use in the manufacture of medicinal products. However, if present they have lethal effects on human beings. Ex: As, Cd, Pb, and Hg.
- Class II elements: The elements of this class have route dependent toxicity.
- Class 2A: The elements of this class have a relatively high risk of existence in the medicinal product. Ex: Co, V, and Ni.
- Class 2B: The elements of this class have a low possibility of a presence in the medicinal product. Ex: Ag, Rh, Au, Ru, and Pd.
- Class III elements: The elements of this class have comparatively low lethality by enteral route of administration, but may be toxic in case of inhalation and parenteral route.
- Other elements: The elements of this class have low elementary toxicity and/or difference in provincial regulations [8].

Genotoxic impurities are DNA reactive substances can injure DNA, when present at low levels leading to the transformation of DNA, and therefore, likely cause cancer [9]. They can be classified into two types,

- Mutagenic carcinogens: These are usually found by bacterial reverse mutation (mutagenicity) assay. Ex: DCBC is a mutagenic impurity present in MLN9708 proteasomes [5].
- Non-mutagenic genotoxicants: These have threshold mechanism, and at low levels, they do not pose a carcinogenic risk in humans. Ex: 2-bromo-2-chloro-1,1-difluoroethylene is a genotoxic degradation product of Halothane [10].

REGULATORY REQUIREMENTS

The main objective of the regulatory bodies is to establish the safety, efficacy, and quality of drug products. Presence of impurities in the pharmaceutical entities and product may lead to undesirable pharmacological and toxicological effects, which in turn effect the safety and efficacy of the drug.

Therefore, various regulatory agencies have enforced reporting, identification, qualification, and quantification impurity thresholds for assessing the safety of pharmaceutical substances and their associated dosage forms. Table 1 provides various guidelines lay down by the various regulatory agencies.

QUALIFICATION OF IMPURITIES

Qualification is the process of obtaining and assessing data that provides the biological safety of a particular impurity or given impurity profile at the level(s) described. Table 2 provides the threshold levels for reporting, identification, and qualification of impurities [1].

CONVENTIONAL APPROACHES FOR THE ISOLATION AND CHARACTERIZATION OF IMPURITIES

Before two and half decades, there were no precise methods to isolate and characterize the impurities. The characterization of impurities was based on the quantification of active ingredient content by nonspecific titrimetric [23] and photometric methods (such as UV spectroscopy [24], IR, Raman spectroscopy [25], and nuclear magnetic resonance [NMR] [26]), which were supported by the physical constants and some limit tests for known impurities. Even the pharmacopeias were suggesting various nonspecific characterization methods for determining the content of active ingredients. Due to the emergence of chromatography in mid-19th century, various chromatographic methods such as capillary electrophoresis (CE) [27], Chiral separations [28], gas chromatography (GC) [29], high-pressure liquid chromatography (HPLC) [30], supercritical fluid chromatography [31], and Thin-layer chromatography (TLC) [32] were employed for the separation of various impurities in drug substance and products. Followed by the isolation/enrichment/synthesis by solid phase extraction, liquid–liquid extraction, accelerated solvent extraction, supercritical fluid extraction, column chromatography, flash chromatography, TLC, etc., and later spectrophotometric characterization of impurities using UV spectroscopy, IR, Raman spectroscopy, and NMR.

STRUCTURAL CHARACTERIZATION USING MODERN HYPHENATED TECHNIQUES

As the process of impurity profiling takes more time for the identification, separation, characterization and quantification using conventional methods, researchers prefer to use one equipment for all the purpose. This can be achieved by employing the modern hyphenated techniques that possess both the separation and characterization of impurities in single equipment. The commonly used modern hyphenated techniques for the isolation and characterization are as follows GC-MS [33], LC-MS [34], LC-MS-MS [35], LC-inductively coupled plasma (ICP)-MS [36], CE-ICP-MS [37], etc.

HISTORY OF LC-MS

The mass spectrometer is considered as a better detector for LC applications as LC requires sensitivity, specificity, and versatility to provide both qualitative and quantitative analysis of a wide variety of compounds. Mass spectrometer was discovered by Francis Aston in early 1920s based on Thomson's discovery of the electron. Victor Tal'roze was pioneer in attempting to couple LC with MS in 1960s by directly spraying liquid into the ionization chamber of low-pressure electron impact mass spectrometer. In the same year, Malcom Dole demonstrated electron spray ionization. In early 1970s Tal'roze provided three strategies to address the interface issue, they are atmospheric pressure chemical ionization, moving wire system, which led to the moving belt interface and direct liquid introduction [38] Table 3.

ADVANTAGES OF USING LC-MS OVER OTHER ANALYTICAL TECHNIQUES

Most of the analytical techniques lack sensitivity and are not able to deduce the structure of the unknown impurities. Hence, LC-MS is considered as an essential and versatile tool for the structural elucidation of impurities. It is handy and efficient since it provides rapid and effective separation. The structures from the unknown impurities can be obtained by characterization on the basis of mass separations from the drug in the form of ions; molecular formulae can be determined from specific mass measurements [39]. As the instrument has high sensitivity, it can detect impurities in trace levels up to fematogram (1fg=10^{-15} g) level in bulk samples [40]. The structural elucidation with LC-MS can help in the determination of route cause for arise of impurities and thereby helps in controlling the impurity levels in the drug substance or product.

INSTRUMENTATION OF LC-MS

LC-MS instrumentation can be classified into two parts, i.e., HPLC system and mass spectrometry [41]. The LC-MS instrumentation is briefly given in Fig. 2.

HPLC system

The HPLC system works as follows, the mobile phase is pumped from a reservoir and the sample is injected through injector which enters the column along with mobile phase. The separation occurs in the column, and the separated constituents are detected by the detectors. The most commonly used pumps are piston pumps. The pistons are commonly constructed from beryl glass, called sapphire pistons. The introduction of sample successfully into the columns is achieved using loop injectors and valve injectors [41].

In most of the studies, 0.1% aqueous formic acid with the organic solvents with or without formic acid was used, as they have various advantages when compared to acetic acid and trifluoroacetate. Formic acid has less molecular weight than acetic acid and trifluoroacetate; hence, they cause less interference in the mass spectrum. For the analysis of basic compounds, acidic mobile phases are needed. The use of low pH mobile phase keeps residual silanol in an undissociated state. Formic acid has lower contamination levels than acetic acid [42].

Column is considered as the heart of HPLC system. The most commonly used silica column is C18 column or octadodecyl silica column. It is a non-polar organic phase chemically bonded to silica. In few cases, C8 columns are preferred. Table 4 gives the various commercially available C18 and C8 columns which were used in the impurity profiling works carried out from 2012 to 2017.

The detectors in LC-MS system is mass spectrometers. However, other HPLC detectors are either placed in series or a part of mobile phase can be diverted to mass spectrometer by employing splitters. Secondary detectors used may be refractive index detector, conductivity detector, ultraviolet detector or fluorimeters.

Table 1: International guidelines outlining the regulatory requirements for the control of impurities in drug substances and drug products

Regulatory agency	Guidelines	Issue date	References
ICH (USA, Europe and Japan)	Q3A (R2) Impurities in new drug substances	25th October 2006	[1]
	Q3B (R2) Impurities in new drug products	2nd June 2006	[7]
	Q3C (R6) Impurities guideline for residual solvents	20th October 2016	[6]
	Q3D Guideline for elemental impurities	16th December 2014	[8]
	M7(R1) Assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk	31st March 2017	[9]
EMA (Europe)	EMA/CHMP/CVMP/QWP/450653/2006 Assessment of the quality of medicinal products containing existing/known active substances	9th February 2009	[11]
	CPMP/QWP/1529/04 Control of impurities of pharmaceutical substances	22nd April 2004	[12]
	CPMP/SWP5199/02 and EMEA/CHMP/ QWP/251344/2006 Guidelines on the limit of genotoxic impurities	28th June 2006	[13]
	EMEA/CHMP/SWP/4446/2000 Guideline on the specification limits for residues of metal catalyst or metal reagent	21st February 2008	[14]
	EMA/CHMP/CVMP/QWP/199250/2009 Guideline on setting specification for related impurities in antibiotics	30th June 2013	[15]
US-FDA	NDAs: Impurities in new drug substances	February 2000	[16]
	ANDAs: Impurities in new drug substances	June 2009	[17]
	ANDAs: Impurities in new drug products	November 2010	[18]
	Elemental impurities in drug products (draft)	June2016	[19]
	Genotoxic and carcinogenic impurities in drug substances and products: Recommended approach (draft)	December 2008	[20]
Health Canada	Impurities in existing drug substances and products	6th September 2005	[21]
TGA (Australia)	Guidance 18: Impurities in drug substances and drug products	9th August 2013	[22]

Table 2: Reporting, identification and qualification thresholds for the impurities in pharmaceuticals

Maximum Daily dose	Reporting threshold	Identification threshold	Qualification threshold
≤2 g/d	0.05%	0.10% or 1.0 mg/d intake (whichever is maximum)	0.15% or 1.0 mg/day intake (whichever is maximum)
>2 g/d	0.03%	0.05%	0.05%

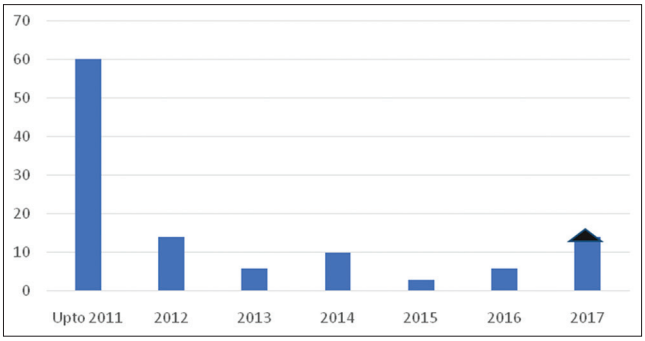

Fig. 1: Articles published on the liquid chromatography-mass spectrometry in the impurity profiling of pharmaceuticals

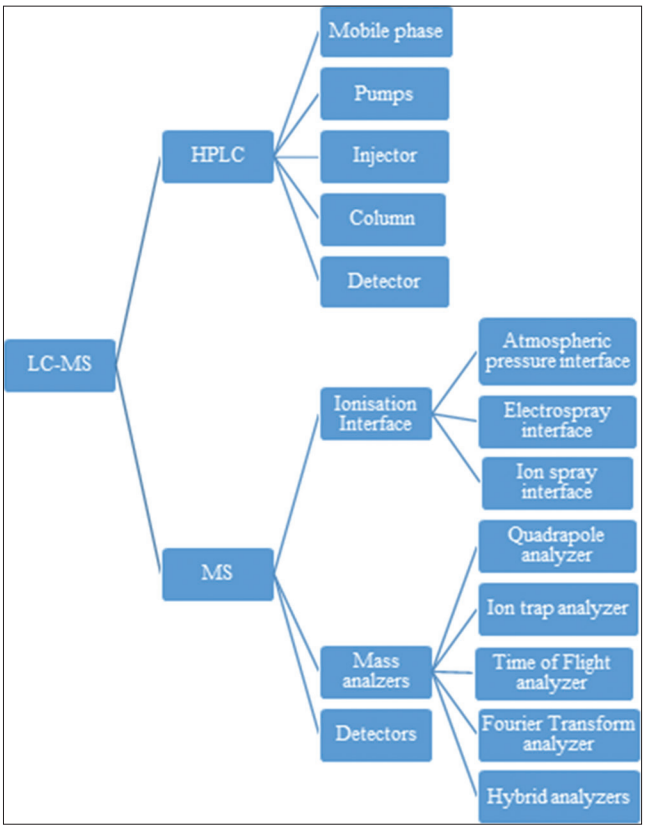

Fig. 2: Instrumentation of liquid chromatography-mass spectrometry

Mass spectrometry

The role of interface in the LC-MS is that it must specifically retain the compound of interest by removing the volatile solvents and additives. As most of the target compounds are uncharged in the HPLC eluent, the interface helps in the ionization of the molecules. Atmospheric pressure interfaces for LC-MS system are of two types, electron spray and ion spray or atmospheric pressure chemical ionization. The commercially built mass spectrometers contain both interfaces which allow the switching of modes based on the area of interest. Positive ionization mode gives rise to molecular ions and solvent adducts, whereas, negative ionization mode gives rise to deprotonated ions. Electron spray interfaces can be mainly used for compounds with high polarity and ionization properties. Ion spray interfaces are recommended when the flow rate from the HPLC system is up to 2 mL/min, and the effluent polarity is very low [41].

The heart of mass spectrometer is mass analyzer. Quadrupole is less expensive mass analyzer whereas ion trap analyzers are 10 - 100 times more sensitive than quadruple and they require less maintenance. Time of flight mass analyzers can analyze very large biological molecules. Fourier transform analyzers are non-destructive LC-MS detectors as they produce mass spectra using ion cyclotron resonance. But in most of the impurity profiling studies, hybrid mass analyzers were employed. The determination of trace levels of impurities cannot be achieved using single analyzer. Few of the hybrid mass analyzers employed in the studies were as follows Triple Quadrupole, Orbitrap, Q-Trap, Q-IT, Q-TOF, IT-TOF, etc. The commonly used detectors of ions are electron multiplier tube, faraday cup, scintillation counter, etc. [41].

Table 3: List of discoveries in the field of LC-MS

Year	Discoveries
1907	Discovery of electron by Thomson
1913	First spectrum that demonstrates two different isotopes of Neon by Thomson
1919	Development of First mass spectrometer by Francis Astom
1922	Use of First electron ionization method by Smyth
Late 1960s	First attempt to couple LC with MS by Victor Tol'roze
Late 1960s	Demonstration of Electron spray at atmospheric pressure
Early 1970s	APCI, the moving wire system and direct liquid introduction were studied by Victor Tol'roze
1979	Incorporation of direct liquid injection probe approach into first available LC-MS interfaces by Hewlett-packard
1980s	Commercialization of a moving belt LC-MS interface by Finnigan
1984	Development of thermospray LC-MS by Marvin Vestal
1989	Commercialization of triple quadrupole LC-MS/MS by AB Sciex

LC-MS: Liquid chromatography-mass spectrometry

UTILIZATION OF LC-MS IN IMPURITY PROFILING

Role of LC-MS in process related impurity profiling

Process-related impurities are potential impurities that emerge during the API manufacturing process which includes the starting material, intermediate material, and by-products, which may be organic or inorganic in nature.

Impurity analysis of bitespiramycin by Ming *et al.* [40], provided information on 9 major components, 5 minor components, 12 new related substances, and 12 unknown related substances which were partially characterized. Few of the proposed related substances characterized were 4-propionyl SPM I, 4-(iso)butyryl SPM I, 4-(iso) hexadienyl SPM II, etc.

The process of structural elucidation can be simplified by employing various characterization tools. Andras *et al.* [47] were able to reveal the presence of a novel by-product, 4-phenyl-naphtopyran indapoxetine by utilizing the results obtained from NMR along with the results obtained from LC-MS for the structural elucidation. In the same way, Musty *et al.* [90] were able to characterize two process-related impurities using NMR results along with LC-MS.

Few drugs of microbial origin such as antibiotics gain impurities during the process of fermentation. Chopra *et al.* [86] analyzed the impurities in tylosin. The study revealed the presence of 4 major impurities and 23 unknown impurities arised during the process of fermentation. The unknown impurities were easily characterized and quantified using LC-MS technique.

Role of LC-MS in impurity profiling of product-related impurities

Product-related impurities emerge during the manufacture and/or storage due to the molecular variation of the intended product structure and degradation products. In most of the cases, product-

Table 4: List of columns used in the impurity separation

Manufacturer	Column name	Column dimensions	References
Agilent	Zorbax C18 Column	50 mm×3 mm ID, 3.5 µm dp	[43]
	Poroshell 120SB C18 Column	150 mm×4.6 mm ID, 2.7 µm dp	[44]
	Zorbax Eclipse Plus C18 Column	100 mm×4.6 mm ID, 3.5 µm dp	[47]
	Varian Polaris C18 A Column	150 mm×2 mm ID, 5 µm dp	[48]
	Poroshell 120EC C-18 Column	100 mm×4.6 mm ID, 2.7 µm dp	[5]
	Zorbax SB C18 column	150 mm×4.6 mm ID, 5 µm dp	[49]
	Eclipse Plus C18 RRHD Column	100 mm×2.1 mm ID, 1.8 µm dp	[50]
	Poroshell HPH C18 Column	50 mm×2.1 mm ID, 2.7 µm dp	[51]
	Zorbax SB-CN Column	200 mm×4.6 mm ID, 5 µm dp	[52]
	Zorbax RX C8 Column	250 mm×4.6 mm ID, 5 µm dp	[53]
Akzo Nobel	Kromosil 100-5 C18 Column	150 mm×4.6 mm ID, 5 µm dp	[54]
	Kromosil C18 Column	250 mm×4.6 mm ID, 5 µm dp	[55]
Discovery	C8 Column	25 cm×4.6 mm ID, 5 µm dp	[56]
GL Sciences	Inertsil ODS- 3V Column	250 mm×4.6 mm ID, 5 µm dp	[57]
Grace	Alltima C18 Column	250 mm×3 mm ID, 5 µm dp	[58]
Macherey-Nagel	Nucleosil C18 Column	150 mm×4.6 mm ID, 5 µm dp	[59]
Merck	LiChrospher RP18 column	250 mm×2 mm ID, 5 µm dp	[60]
Nacalai Tesque	Cosmosil C18 Column	250 mm×4.6 mm ID, 5 µm dp	[61]
Phenomenex	Gemini C18 Column	50 mm×2 mm ID, 3 µm dp	[45]
	Gemini C18 Column	150 mm×3 mm ID, 3 µm dp	[62,63]
	Gemini NX C18 Column	250 mm×4.6 mm ID, 5 µm dp	[64-68]
	Luna C18 Column	250 mm×4.6 mm ID, 10 µm dp	[69]
	Luna C18 Column	150 mm×3 mm ID, 5 µm dp	[70]
	Gemini C18 Column	150 mm×4.6 mm ID, 3 µm dp	[71]
Shiseido	CAPCELL PAK C18 Column	150 mm×2 mm ID, 5 µm dp	[44,72]
Shimadzu	Shim pack GISS C18 Column	50 mm×2.1 mm ID, 1.9 µm dp	[73]
	Shim pack XR-ODS Column	75 mm×3 mm ID, 2.2 µm dp	[74]
Supelco	Ascentis Express HILIC Column	150 mm×2.1 mm ID, 2.7 µm dp	[75]
Thermo Fischer Scientific	Hypersil BDS C18 Column	250 mm×4.6 mm ID, 5 µm dp	[76,77]
	Thermo Hypersil Gold Column	100 mm×2.1 mm ID, 1.9 µm dp	[78]
Welch	Xtimate C18 Column	250 mm×4.6 mm ID, 5 µm dp	[79]
	Xtimate SEC-120 Column	30 cm×7.8 mm ID, 5 µm dp	[80]
Waters	Acquity HSS T3 C18 Column	100 mm×2.1 mm ID, 1.7 µm dp	[4]
	Acquity UPLC BEH C18 Column	150 mm×2.1 mm ID, 1.7 µm dp	[46]
	Acquity UPLC BEH C18 Column	100 mm×2.1 mm ID, 1.7 µm dp	[39,81]
	Xterra RP18 Column	250 mm×4.6 mm ID, 5 µm dp	[82,83,84]
	Sunfire C18 Column	250 mm×4.6 mm ID, 5 µm dp	[85]
	Xterra C18 Column	250 mm×2.1 mm ID, 5 µm dp	[86]
	Spherisorb C8 Column	250 mm×4.6 mm ID, 5 µm dp	[87]
	BEH Glycon Column	100 mm×2.1 mm ID, 1.7 µm dp	[88]
YMC	C18 Pro Column	150 mm×4 mm ID, 3 µm dp	[89]

related impurities emerge from the reaction of drug component with excipients such reactions were observed in gabapentin capsules where GBP-Lactose conjugate impurity was formed. This impurity was characterized by Buchi *et al.* [87] by employing LC-MS technique.

The storage of formulation in extreme conditions may give rise to various product-related impurities, which was observed in the parenteral infusion of an amino acid supplement containing L-alanyl-L-glutamine by Simone *et al.* [63]. Authors were able to identify and quantify 21 impurities in the infusion after stress studies by employing liquid chromatography tandem mass spectrometry.

Role of LC-MS in the profiling of degradation of drug substance

Degradation is the inability of a particular drug substance in a specific container to remain within particular chemical, microbiological, therapeutical, physical, and toxicological specifications.

Development of degradation pathway can provide the information on possible impurities that may arise in the drug substance. In this regard, Thomas *et al.* [83] subjected eslicarbazepine drug to forced degradation studies and developed degradation pathway by separating the impurities by LC-UV followed by characterization using LC-MS/MS, NMR, and IR spectroscopy. Trandolapril degradation pathway was developed by Dendeni *et al.* [39], included the mechanism of degradation under chemical stress in both neutral and acidic conditions using UPLC-ESI-tandem mass spectrometry. In the same way, Mayuri *et al.* [91] developed the degradation pathway of Fenoxazoline by employing HPLC-UV for separation and LC-MS/MS for the characterization of degradation products.

Role of LC-MS in the profiling of degradation of drug product

Development of degradation pathway is important not only in drug substances but also in drug products. Nagdeep *et al.* [57] employed LC/ESI-MS/MS method for developing degradation pathway of dabigatran etexilate in both bulk drug and capsule formulation. Authors were able to identify 15 impurities among which two impurities were unknown. Degradation pathway was developed for the unknown impurities based on the information obtained from LC-MS, FTIR, Proton, and ^{13}C NMR spectroscopies.

Degradation of the drug product may also occur during the process of administration. Tiaziana *et al.* [75] identified degradation products for sodium risedronate granules for oral solution and effervescent tablets by carrying out oxidative degradation study. Authors employed LC-ESI-MS for determining the main degradation product.

Role of LC-MS in the profiling of genotoxic impurities

Genotoxic impurities cause the deleterious change in the genetic material regardless of the mechanism by which the change takes place.

Katerina *et al.* [59] and Szekely *et al.* [45] extensively worked on the analysis of potential genotoxic impurities in meropenem and glucocorticoids, respectively. Use of LC-MS/MS in the study helped in identifying genotoxic impurities in trace levels, reducing the cost per

analysis, ultra-low limit of quantitation and obtaining high throughput results.

CONCLUSION

The above discussion, explains the importance of impurity profiling and various guidelines were enforced by various regulatory bodies to maintain the safety and quality of pharmaceutical products. The study also reveals that LC-MS is a better option for the structural elucidation of both known and unknown impurities. Most of the research work on impurity profiling includes the use of C-18 column and mobile phases containing 0.1–1% of formic acid. Electron spray ionization under atmospheric pressure was preferred by the researchers for the impurity profiling mostly in positive ionization mode. Hybrid analyzers were preferred especially Q-Trap and Q-TOF. The present study may help the researchers in the selection of column, mobile phase, ionization technique, and analyzers for the LC-MS instrument and understand the ease with which the impurity profiling may be carried out using LC-MS.

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