Recent Advances in Characterization of Impurities - Use of Hyphenated LC-MS Technique

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Abstract: Profiling of impurities in pharmaceutical products is an important part of the pharmaceutical manufacturing process and it is a regulatory expectation. Impurities may influence the safety and efficacy of the pharmaceutical products. Estimation of the impurity of pharmaceuticals provides excellent means for drug authorities to control the manufacturing process. To meet the challenges and to build high degree of purity in drug substances and drug products, it is required to carry out all the investigations for standards of drugs and impurities to get significant results. Different methods are available for impurity profiling; the most common analytical methods are based upon spectroscopic and chromatography separation techniques. One of the powerful tools of impurity profile is liquid chromatography (LC) coupled with mass spectroscopy (MS), and it is employed for the identification of impurities, natural products, drug metabolites, and proteins. LC-MS offers selectivity and specificity in both the chromatographic separation and detection steps, and is found as necessary steps to measure compounds at extremely low concentrations. LC-MS is steadily applied to scrutinize impurity during pharmaceutical product development and manufacturing process to support the safety evaluation of batches used in clinical studies. In this review, strategies for impurity profiling of pharmaceuticals with the applications of LC-MS, LC-MS/MS, LC-ESI/MS and LC-TOF/MS methods will be critically reviewed and discussed.

Keywords: Impurities, Impurity profiling, Liquid chromatography–mass spectrometry, Hyphenated LC–MS, LC-MS/MS, Identification, Characterization.

INTRODUCTION

Impurities in pharmaceuticals are the unwanted chemicals that remain with the active pharmaceutical ingredients (APIs), or develop during formulation development or upon aging of both APIs and formulated APIs to medicines. The definition of impurity profile is given in the guidelines of ICH (International Conferences on Harmonization). According to the ICH guidelines impurity is defined as any component of the active pharmaceutical ingredient (API) that is not the chemical entity defined as the API. ICH documented different impurities that may be present in pharmaceutical substances and classified as organic (process and drug related), inorganic and residual solvents. The ICH guidelines provide three general procedures on organic and inorganic impurities: ICH Q3A(R) deals with impurities in drug substances; Q3B(R) covers mainly degradation products and the third guideline Q3C(R) gives limits for residual solvents as impurity. According to ICH guideline, it is mandatory to know the structural details of impurities that exceed 0.1% in the bulk drugs. Impurities are usually process related compounds; they are probably, structurally similar to the synthesized target drugs [1-3].

Impurity profiling describes the route of investigating impurities associated to an active pharmaceutical compound. Impurity profile has become essential as per various regulatory requirements. Impurities are generally mediocre to API because they may not have the same intensity of pharmacological activity [4, 5]. Impurity profiling of pharmaceuticals depends upon several factors like source and quality of the starting material, reagents, solvents used during the synthesis, reaction conditions, purification steps, and drug formulations. ICH guidelines Q3A(R) and Q3B(R) describes threshold levels above which impurities are required to be reported, identified and qualified by sufficiently specific methods mandatorily is 0.1%. Organic impurities are most likely to arise during the synthesis, purification and storage of the drug substance which involve chemical reactions during synthesis of the drug. Impurity associated with raw material could contribute to new impurity of drug substances. Organic impurity can be limited to those impurities that might be due to chemical reaction involved in their synthesis and conditions involved [6]. Pharmacopoeial or other appropriate procedures are normally used for detection and identification of inorganic impurity and limit should be based on pharmacopoeial standards. Quantification of residues of the solvents used in the manufacturing process for the new drug substance is important by using analytical procedures with an appropriate level of sensitivity with particular attention to toxic solvents used in the manufacturing process [7]. Most of the methods used for profiling of impurity are based upon variety of chromatographic and a spectroscopic technique is alone or in combination with other techniques. Liquid chromatography method is extensively applied in the field of impurity profiling. The main object of the impurity profiling is to separate impurities from the main component, identification and quantification of them by using wide range of detectors and stationary phases along with its sensitive sepa-

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ration attributed to its diverse applications [8]. Often impurity profiling is considered to be a set of analytical actions aiming at the identification, detection, structure elucidation and quantitative determination of impurities in bulk drugs and pharmaceutical formulations. The conventional approaches like IR, MS and NMR are successful techniques only when there is less impurity in pharmaceuticals. The conventional approaches have certain limitations, such as it is low in throughput and the synthesized compounds may be different from the sample under examination. Mass spectroscopy (MS) along with the chromatographic separation is a first technique applied for impurity identification at minute level. MS widely recognized as yielding substantive information spectroscopic technique provides data for structural elucidation of organic molecules [9]. Hyphenated LC-MS is an instrument that is more powerful than the sum of its individual parts, LC and MS. The principle of LC-MS involves separation of the components on an LC column followed by determination of the mass-to-charge ratio in the mass spectrometer [10]. LC–MS gives information on molecular weight and structural characterization that helps in identification of an impurity. Further, recent advancements are made in tandem LC-MS such as triple quadrapole LC-MS (LC-MS/MS) available as a routine technique, atmospheric pressure chemical ionization (APCI) and electrospray ionization (ESI) modes [11]. For the quantitative analysis of many analytes, LC–MS/MS is a fast, selective and sensitive tool [12]. Impurity profiling of an active pharmaceutical ingredient is a prerequisite and it is of vital importance for medical safety reasons [13]. This review will focus on detection of different impurities found in the pharmaceuticals and their characterization by using LC condensed with MS and possible trial to elucidate structures of impurities in addition to discussion of advances in detection of impurities.

LC-MS: METHODOLOGICAL ASPECTS

The combination of LC and MS is a subject that has attracted much interest in the past many years [14, 15]. This includes high performance liquid chromatography (HPLC)-MS [16], capillary electrophoresis (CE)-MS [17] and capillary electrochromatography (CEC)-MS [18]. In impurity profiling of the pharmaceuticals, role of the liquid chromatographic technique is to provide separation of the impurity and allow their identification or quantitative determination. From qualitative perspective, the main limitation of LC in isolation is its inability to provide an unequivocal identification of the impurity [19]. LC–MS system consists of an autosampler, LC system, ionization source (which interfaces LC to MS) and MS [20]. The effect of the mobile-phase composition and flow rate is of little concern to interface LC with MS. Typical reversed phase HPLC systems connected to MS use combination of water and methanol as the mobile phase. From LC-MS perspective, the pump must deliver the mobile phase at a constant flow rate. LC–MS interfaces is designed in a way that mobile phase is not pumped directly into the source of the mass spectrometer, so as to minimize contamination and increase the time over which the interface operates at most favorable performance [21-23]. Identification is based on the comparison of the retained characteristics, simply the retention time. The role of MS lies in the fact that the mass spectra of many compounds are specific to allow their identification with a high degree of confidence. There for the capability of LC is advantageous which separates impurity with high degree of purity using the mass spectrometer meant for specific identification. Many compounds with identical retention characteristics have quite different mass spectra and can therefore be differentiated [24]. The combination of LC with MS therefore allows more definitive identification and quantitative determination of impurity. The direct coupling of the two techniques is advantageous in many respects, including the speed of analysis with ease [25]. LC-methods with ultra violet (UV) detection are also useful in the quality control of impurities because of their robustness and high sensitivity. Therefore, the impurity profiling also based upon the UV chromatogram. The main objective of LC–MS coupling is to get qualitative molecular weight information for impurity peaks detected at the same wavelength as in case of impurity test procedure. UV absorbance depends on the structure of compounds; where as the total ion-current reflects the ionization of molecules. For impurity profiling, mass spectra taken at the position of impurity peaks in the UV chromatogram, display the mass chromatogram of the found molecular weight (m/z ratio) and is compared with the UV trace. The method focuses primarily on a qualitative assignment of molecular weights to UV percentage area peaks. If the UV amount is larger than the required level then safety aspects must be considered [26]. The impurity profiling could be supported auxiliary by obtaining molecular weight, the fragmentation pattern and finally, structural identification [27]. Large number of factors are known to affect LC–MS experiment and a few of them are composition of the mobile phase employed for seperation, its pH, flow rate [24], nature and concentration of mobile-phase additives e.g. buffer or ion-pair reagent; the make-up of the solution in which the sample is injected [28], the ionization technique, spray voltage for electrospray, nebulizer temperature for APCI, nebulizing gas pressure, mass spectrometer source temperature, cone voltage in the mass spectrometer source, and the nature and pressure of gas in the collision cell if MS–MS is employed [29]. For quantification, the measurement of response was based upon the selectivity and sensitivity of the analysis. The MS/MS experiments give complete structural information through fragmentation spectra, which is a characteristic molecular fingerprint. Several instruments with ion trap technology (LC-MS^n) and multiple reaction monitoring (MRM) mode permit sequential MS/MS experiments (MS^n, where n is number of MS/MS experiments). In MS/MS (MS^2) experiment, an ion selected from the MS spectrum is fragmented to provide a fragment ion spectrum. Ions can be selected from that spectrum for further fragmentation (MS^3) and so on [30]. These spectra have extensive applications in identification and structure elucidation. In addition to that, hybrid quadrupole/time-of-flight (TOF) geometry is available these days which brings together the selectivity of MS/MS and the power of exact mass. One of the advancements in the LC-MS is the introduction of LC-MS^n/TOF instrument, having both the advantages of MS^n and exact mass [31]. Now days all these techniques are proving strong tools for the identification and structural elucidation of impurity in pharmaceuticals.

Table 1. Applications of LC-MS for Analysis of Various Impurities Reported in APIs

API	Chemical Name of Impurities	Reference
Pantoprazole sodium 5-(difluoromethoxy)-2-[[(3,4-dimethoxy-2-pyridinyl)methyl]sulfinyl]-1*H*-benzimidazole	Impurity I:5-(difluoromethoxy)-2-[[(3,4-dimethoxy-2-pyridinyl)methyl]thio]-1*H*-benzimidazole Impurity II: 5-(difluoromethoxy)-2-[[(3,4-dimethoxy-2-pyridinyl)methyl]sulfonyl]-1*H*benzimidazole Impurity III:5-(difluoromethoxy)-2-[[(3,4-dimethoxy-1-oxide-2-pyridinyl)methyl]sulfonyl]-1*H*-benzimidazole Impurity IV:5-(difluoromethoxy)-2-[[(3,4-dimethoxy-2-pyridinyl)methyl]thio]-1-((3,4-dimethoxy-2-pyridinyl)methyl)-1*H*-benzimidazole Impurity V:5-(difluoromethoxy)-2-[[(3,4-dimethoxy-2-pyridinyl)methyl]sulfinyl]-1-((3,4-dimethoxy-2-pyridinyl)methyl)-1*H*-benzimidazole Impurity VI:5-(difluoromethoxy)-2-[[(3,4-dimethoxy-1-oxide-2-pyridinyl)methyl]sulfinyl]-1*H*-benzimidazole	[43]
Cefdinir 7-[2-(2-aminothiazol-4-yl)-2-hydroxyiminoacetamido]-3-vinyl-3-cephem-4-carboxylic acid	Impurity I: (6R, 7R)-7-[(z)-2-(2-aminothiazol-4-yl)-2-hydroxyiminoacetamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid-5-oxide Impurity II: (6R, 7R)-7-[(z)-2-(2-aminothiazol-4-yl)-2-hydroxyiminoacetamido]- 8-oxo-3-vinyl-5-thia-1-azabi-cyclo [4.2.0] oct-3-ene-2-carboxylic acid. Impurity III: (6R, 7R)-7-[(z)-2-(2-aminothiazol-4-yl)-2-hydroxyiminoacetamido]-8-oxo-3-methyl-5-thia-1-azabicyclo-[4.2.0]oct-2-ene-2-carboxylic acid	[42]
Ceftizoxime sodium (6*R*,7*R*)-7-[(*Z*)-2-(2-aminothiazol- 4-yl)-2-meth oxyiminoacetamido]-3-cephem-4-carboxylate	Impurity I: (6*R*,7*R*)-7-amino-3-cephem-4-carboxylic acid Impurity II: (6*R*, 7*R*)-7-[(*Z*)-2-(2-amino-4-thiazolyl)-2-(methoxyimino)acetamido]-3-cephem-1-oxo-4-carboxylic acid Impurity III: (4*RS*,6*R*,7*R*)-7-[(*Z*)-2-(2-amino-4-thiazolyl)-2-(methoxyimino) acetamido]-3,4-dihydro-3-cephem-4-carboxylic acid Impurity IV: (6*R*,7*R*)-7-[(*E*)-2-(2-amino-4-thiazolyl)-2- (methoxyimino)acetamido]-3-cephem-4-carboxylic acid Impurity V: (6*R*,7*R*)-7-[(*Z*)-2-(2-amino-4-thiazolyl)-2-(methoxyimino)acetamido]-3-cephem-*N*-(3-cephem-4-carboxy-7-yl)-4-carboxamide (impurity V); Impurity VI: (6*R*,7*R*)-7-[(*Z*)-2-[[(*Z*)-2-(2-amino-4-thiazolyl)-2-(methoxyimino)acetylamino]	[41]
Rizatriptan benzoate *N*,*N*-dimethyl-5-(1H-1,2,4-triazol-1-ylmethyl)-1H-indole-3-ethanamine monobenzoate	Impurity I: 4-(5-((1H-1,2,4-triazol-1-yl)methyl)-3-(2-(dimethylamino)ethyl)-1H-indol-1-yl)-4-(5-((1H-1,2,4-triazol-1-yl)methyl)-3-(2-(dimethylamino)ethyl)-1H-indol-2-yl)-*N*,*N*-dimethylbutan-1-amine Impurity II: [4,4-bis-(5-((1H-1,2,4-triazol-1-yl)methyl)-3-(2-(dimethylamino)-ethyl)-1H-indol-2-yl)-*N*,*N*-dimethylbutan-1-amine [rizatriptan-2,2-dimer	[40]
Piperacilin	6[6-[[2-[[(4-Ethyl-2,3-dioxo-1-iperazine1-yl)carbonyl]amino]2-phenylacetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0] heptanecarbonyl]amino]3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0] heptane-2-carboxylic acid	[39]
Piperaquine (1,3-bis-[4-(7-chloroquinolyl-4)-piperazinyl-1]propane)	1-[(5-chloroquinolin-4)-piperazinyl]-3-[(7-chloroquinolin-4)-iperazinyl]propane	[36]

APPLICATIONS OF LC-MS IN IMPURITY PROFILING: RECENT FINDINGS

Applications of LC-MS are extensive with retention time and molecular weight emerging as essential analytical features [32-35]. Application of LC–MS for identification and determination of impurity has been well explained in many quality research papers. The main aim of the present review is to give an overview on the progress till date, by using LC-MS and LC–MS/MS for the quantitative analysis and characterization of drug impurity. N. Lindegardh *et al*. [36] applied LC-MS and 2D NMR spectroscopy to profile significant amount of impurities in piperaquine (1, 3-bis-[4-(7-chloroquinolyl-4)-piperazinyl-1] propane). These impurities are positional isomers 1-[(5-chloroquinolin-4)-piperazinyl]-3-[(7-chloroquinolin-4)-piperazinyl] propane, and they developed due to contamination of batches of 4, 7-dichloroquinoline (a precursor in the synthesis of piperaquine) with 4, 5-dichloroquinoline (Table **1**). Recently

Table 2. Applications of LC-MS/MS for Analysis Various Impurities Reported in APIs

API	Impurities	Reference
Simvastatin [1*S*-[1α3α,7β,8β(2*S**,4*S**),8αβ]]-1,2,3,7,8,8a-hexahydro-3, 7-dimethyl- 8- [2-(tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl)ethyl]-1-naphtalenyl-2,2-dimethylbutanoate	Ethyl ester, Methyl ester and Methyl ether of Simvastatin	[45]
Fluconazole 2-(2,4-difluorophenyl)-1,3-bis-(1*H*-1,2,4-triazol-1-yl)-2-propanol	1-(1-*H*-1,2,4-triazole-1-yl) propane-2,3-diol *Z*-2-(2,4-difluorophenyl)-3-(1-*H*-1,2,4-triazole-1-yl)-2-propen-1-ol	[46]
Ezetimibe 1-(4-fluorophenyl)-3(R)-[3-(4-fluorophenyl)-3(S)-hydroxypropyl]-4(S)-(4-hydroxy phenyl)-2-azetidinone	2-(4-hydroxybenzyl)-N,5-bis-(4-fluorophenyl)-5-hydroxypentanamide	[48]
Avermectins	24-demethylH2B1a, 3'-demethylH2B1a, 3"-demethyl H2B1a and 24a-hydroxy B2a	[50]

Alireza S. Kord *et al.* [37] found trace level genotoxic impurities from pazoapanib hydrochloride a VEGFR tyrosine kinase inhibitor in phase III clinical development for the treatment of renal cell carcinoma by LC-MS method. Five trace analysis LC/MS methods were developed and validated for testing all the genotoxic impurities in the pazopanib drug substances intended for clinical use, to ensure patient safety.

Predrag Novak *et al.* [38] worked upon the detection of unknown impurities of 5-aminosasycilic acid by LC-NMR and LC-MS. The impurity is a structural analogue of the main drug. The characterization was made by the LC-MS technique and that of impurity was done by NMR. Magesh Vijayan *et al.* [39] worked upon the impurity profile of the piperacilin that is genotoxic. They used LC-MS techniques for identification and characterization of piperacilin impurities. Hemant Sharma *et al.* [40] identified, isolated and characterized process-related impurities in rizatriptan benzoate by LC–MS. Ramesh Dandala *et al.* [41] studied the impurity profiling of ceftizoxime sodium by HPLC method and detected eight impurities which were process impurities (Table **1**). The paper describes identification, isolation and characterization of impurities present in ceftizoxime by LC-MS. Ramesh Dandala *et al*. [42] detected three unknown impurities in cefdinir bulk drug at levels below 0.2% by high performance liquid chromatography (HPLC). They isolated these impurities from crude sample of Cefdinir by LC–MS analysis. J. Moses Babu *et al.* [43] detected six impurities in Pantoprazole sodium bulk drug substance by applying LC-MS method (Table **1**).

Derivatization LC-MS Approach

Jianguo A. *et al.* [44] developed derivatization LC-MS methodology for determining a group of commonly encountered alkyl esters of sulfonates at low level. Authors used trimethylamine as the derivatizing reagent for ethyl/propyl/isopropyl esters and triethylamine for methyl esters. The resulting quaternary ammonium derivatization products are highly polar (ionic) and can be retained by a hydrophilic interaction liquid chromatography (HILIC) column. Sulfonic acids (Methanesulfonic, benzenesulfonic, *p*-toluenesulfonic and sulfuric acids) are commonly used for salt formation of APIs. Methanol, ethanol, propanol and isopropanol are frequently used as solvents for crystallization or purification of drug substances. Interactions between sulfonic acids (or sulfonyl chlorides) and alcohols could lead to the formation of their corresponding alkyl ester.

HPLC Tandem Mass Spectrometry (LC-MS/MS)

Tandem mass spectrometry (MS/MS) is a term which covers a number of techniques in which one stage of mass spectrometry is used to isolate desired ion. The second stage is used to probe the relationship of this ion with others from which it may have generated on decomposition. Liquid chromatography in combination with multi-stage mass spectrometry (HPLC/MS^n) is useful for characterizing impurity structure due to its capability to afford both molecular mass and structural information.

Marko Vuletic *et al.* [45] detected unknown impurities in simvastatin using LC with UV (DAD) detection. Structures of impurities (Table **2**) were elucidated through direct hyphenation LC to high-resolution MS with electrospray ionisation interface. The structures proposed for all impurities revealed modifications of simvastatin molecule on the lactone ring. Vaijanath G. Dongre *et al.* [46] detected three impurities (Table **2**) in the LC-MS analysis of fuconazole bulk drug substance. These impurities were structurally characterized by LC-MS/MS using electrospray ionization source and an ion trap mass analyzer. The structures were finally confirmed by the authors by synthesizing the impurities. K. Vyas *et al.* [47] carried out impurity profile study of troglitazone, a clinically useful hypoglycemic drug for NIDDM (non-insulin-dependent diabetes mellitus) patients and four process-related impurities have been detected by LC-MS/MS. The Authors synthesized, characterized and co-injected with the sample containing impurities and found the retention time match of the impurities. Bhanu Raman *et al.* [48] detected a major process-related impurity associated with the synthesis of ezetimibe by LC–MS. They proposed

Table 3. Applications of RPHPLC-MS for Analysis of Various Impurities Reported in APIs

API	Impurities	Reference
Diacerein 4,5-diacetoxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxylic acid,	Impurity I: 5-acetoxy-4-hydroxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxylic acid Impurity II: 4-acetoxy- 5-hydroxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxylic acid	[52]
Clindamycin palmitate hydrochloride	Impurity I: Clindamycin palmitate sulphoxides α -/ β -isomers Impurity II: Clindamycin laurate Impurity III: Lincomycin palmitate Impurity IV: Clindamycin myristate Impurity V: Epiclindamycin palmitate Impurity VI: Clindamycin palmitate 3-isomer Impurity VII: Clindamycin pentadecanoate Impurity VIII: Clindamycin B-palmitate Impurity IX: Clindamycin heptadecanoate Impurity X: Clindamycin stearate	[53]
Piperaquine phosphate 1,3-bis-[4-(7-chloroquinolyl-4)-piperazinyl-1]-propane phosphate	1-(1-5-chloro-4-quinolyl-4-piperazinyl)-3- (1-7-chloro-4-quinolyl-4-piperazinyl) propane	[54]

the structure of impurity to be 2-(4-hydroxybenzyl)-N, 5-bis-(4- fluorophenyl)-5-hydroxypentanamide based on LC-MS/MS studies and accurate mass data. The proposed structure was confirmed with the help of the NMR and IR analyses of a synthetically obtained sample. Robyn A. Rourick *et al.* [49] developed the LC/MS and LC/MS/MS methodologies for elucidation of structures of impurities and degradants. Cefadroxil was used as a model compound for the evaluation of a predictive strategy for the production and elucidation of impurities and degradants induced by acid, base, and heat. Andreas Abend *et al.* [50] identified and characterized four process impurities in bulk of Avermectin, using a combination of LC-MS and LC-MS/MS. The result indicates that these impurities are a product of biosynthesis of avermectin. Fenghe Qiu *et al.* [51] proposed structure of an unknown by-product formed during the synthesis of nevirapine analogue. The impurities were identified by using a combination of low resolution, high resolution and H/D exchange LC/MS and LC/MS/MS.

Reverse-Phase High Performance Liquid Chromatography –Mass Spectroscopy (RPHPLC-MS)

Chaudhari Ashok *et al.* [52] found two impurities in the crude sample of diacerein. Diacerein is chemically known as 4, 5-diacetoxy-9, 10-dioxo- 9, 10-dihydroanthracene-2-carboxylic acid. These impurities (Table **3**) were isolated from crude sample of diacerein by reverse-phase preparative liquid chromatography. The molecular weights of the impurities were determined by LC–MS. Ramesh Dandala *et al.* [53] detected total 12 impurities by isocratic reverse-phase high performance liquid chromatography (HPLC). The molecular weights of impurities were determined by LC–MS analysis. The authors describe identification of impurities present in clindamycin palmitate hydrochloride, detection of masses by LC–MS, isolation by preparative HPLC and characterization of impurities using spectral data. Vaijanath G. Dongre *et al.* [54] detected four impurities in piperaquine phosphate bulk drug substance by a newly developed gradient reverse phase HPLC method. These impurities were identified by LC-MS/MS and the structures of impurities (Table **3**) were confirmed by spectroscopic studies (NMR and IR) using synthesized authentic compounds. Andrej Kocijan *et al.* [55] detected an unknown impurity in analysis of pravastatin sodium sample by RPHPLC-MS; the structure of an impurity was finally determined by the proposed fragmentation mechanisms for statins.

LC Coupled to Time of-Flight Mass Spectrometry (TOF/MS)

LC coupled to TOF/MS for impurity profiling has been developed in the last few years [56]. The resolving power enables MS accuracy for small molecules and charge-state identification of multiply- charged ions. T.J. Novak [57] and colleagues identified impurities in raltegravir belonging to a new class of compounds. It was selected as inhibitors of HIV integrase, by LC-MS incorporating a TOF/MS analyzer. Further MS/MS was used for the determination of atomic composition. The study describes the fragmentation pattern of raltegravir as a model compound and ion spectra of an impurity were compared to both the model fragmentation pattern and the atomic composition to deduce a structure. Robert S. plumb *et al.* [58] showed that LC-MC technique is the one of the fast profiling technique for recognizing the impurities in simvastatin. The ultra performance LC with TOF/MS applied with PCA (principal components analysis) to evaluate impurities of simvastatin.

High Performance Liquid-Electrospray Ionization Tandem Mass Spectrometric (HPLC-ESI-MS)

Yuanjiang P. *et al.* [59] developed high-performance liquid-electrospray ionization tandem mass spectrometric (HPLC-ESI-MS^n) method for identification of clindamycin

Table 4. Applications of LC-ESI-MS for Analysis of Various Impurities Reported in APIs

API	Impurities	Reference
Eprosartan (4-[2-butyl-5-(2-carboxy-3-thiophen-2-yl-propenyl)-imidazol-1-ylmethyl]-benzoic acid)	4,4'-(5,5'-(1E,1'E)-3,3'-(4,4'-methylenebis(thiophene-4,2-diyl))bis(2-carboxyprop-1-ene-3,1-diyl)bis(2-butyl-1H-imidazole-5,1-diyl))bis(methylene) dibenzoic acid	[60]
Puerarin (8- β -d-glucopyranosyl-7 and 4-dihydroxyisoflavone)	Neopuerarin A, Neopuerarin B and Isoflavone-C-glycosides	[61]
Citalopram	1-(1,1-bis (4-fluorophenyl)-1,3-dihydroisobenzofuran-5-yl)-4-(dimethylamino) butan-1-one hydrobromide	[63]

and its related minor impurities in bulk drug. According to the fragmentation mechanism of MS and HPLC-UV-ESI-MS^n data, six impurities of clindamycin have been identified. Positive ion mode extracted ion current (EIC) method has been used to separate and identify compounds. Cuirong Sun *et al.* [60] developed HPLC-ESI/MS for eprosartan, a new antihypertensive agent as an angiotensin II receptor antagonist. The fragmentation behavior of eprosartan and the impurity in negative mode was investigated. Haijiang Zhanga *et al.* [61] established qualitative and quantitative analysis method for profiling and quantification of isoflavone-C-glycosides impurities in puerarin injection. A total of nine impurities were detected and eight of them were identified as isoflavone-C-glycosides. Oliver M. Denk *et al.* [62] studied LC-ESI/MS analysis of samples of pholcodine and shown that two of the previously unidentified compounds had mass spectra with molecular ions which differed from pholcodine. From this observation and other experimental data the authors concluded that impurities were hydroxy derivatives of pholcodin. Bhanu Ramana *et al.* [63] developed a new LC–ESI/MS method for the identification of two impurities (Table **4**) in citalopram bulk drug. The structure of the impurity was proposed on the basis of MS^n data obtained by using ion trap mass analyzer and accurate mass obtained using Q-TOF mass analyzer.

CONCLUSION

Control of impurities is considered to be an essential aspect to ensure the quality, potency and purity of pharmaceuticals. An addition of extra dimensions to chromatographic separations by hyphenated techniques offers exclusive possibilities of ensuring the quality and safety of pharmaceuticals. This review demonstrates that LC-MS method has drawn great attention to the analysis of pharmaceuticals for identification of the impurities. The method has allowed researchers to study the structures of impurities. A large number of works related to identification of impurities by application of LC-MS is presented in this review. The examples used in this review clearly demonstrated that LC-MS is a very powerful technique for impurity profiling in pharmaceuticals. We believe that this method will hasten up the process of structure elucidation of impurities.

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