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Review

Critical practical aspects in the application of liquid chromatography–mass spectrometric studies for the characterization of impurities and degradation products

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ABSTRACT

Liquid chromatography–mass spectrometry (LC–MS) is considered today as a mainstay tool for the structure characterization of minor components like impurities (IMPs) and degradation products (DPs) in drug substances and products. A multi-step systematic strategy for the purpose involves high resolution mass and multi-stage mass studies on both the drug and IMPs/DPs, followed by comparison of their fragmentation profiles. Its successful application requires consideration of many practical aspects at each step. The same are critically discussed in this review.

Abbreviations: $[M+H]^+$, molecular ion; 2-MeTHF, 2-methyltetrahydrofuran; 2NFP-APB, [3-(2-nitro-4-trifluoromethylphenyl) aminophenyl] dihydroxyborane; 4-APEBA, 4-(2-((4-bromophenethyl) dimethylammonio) ethoxy) benzenaminium dibromide; AAPH, 2,2′-azobis(2-amidinopropane) dihydrochloride; ACN, acetonitrile; AIBN, azobisisobutyronitrile; AMDSA, 2-acrylamino-2-methyl-propanesulfonic acid; APCI, atmospheric pressure chemical ionization; API, atmospheric pressure ionization; APPI, atmospheric pressure photoionization; APZ, 4-(4-methyl-1-piperazyl)-3-nitrobenzoyl azide; BMC, 4-bromomethyl-7-methoxycoumarin; CHEFOG, chemical formula generator; CID, collision-induced dissociation; Da, Dalton; DAABD-AE, 4-[2-(N,N-dimethylamino) ethylaminosulfonyl]-7-(2-aminoethylamino)-2,1,3-benzoxadiazole; DAABD-MHz, 4-[2-(N,N-dimethylamino) ethylaminosulfonyl]-7-N-methylhydrazino-2,1,3-benzoxadiazole; DAPB, dansyl-3-aminophenylboronic acid; DBA, di-n-butyl amine; DCU, dicyclohexyl urea; DFT, density functional theory; DIPEA, diisopropylethylamine; DMAE, 2-(N,N-dimethyl-amino) ethyl acrylate; DMAP, dimethyl amino pyridine; DMEQTAD, 4-[2-(6,7-dimethoxy-4-methyl-3-oxo-3,4-dihydroquinoxalyl)ethyl]-1,2,4-triazoline-3,5-dione; DMF, dimethylformamide; DMIS, 1,2-dimethylimidazole-4-sulfonyl; DMSO, dimethyl sulfoxide; DNPH, 2,4-dinitrophenylhydrazine; Dns-Cl, dansyl chloride; Dns-Hz, dansyl hydrazine; DPs, degradation products; ESI, electrospray ionization; ESI-MS, electrospray ionization-mass spectrometry; ESI-MS/TOF, electrospray ionization mass spectrometry/time of flight; FT-ICR, fourier transform-ion cyclotron resonance; GirP, 1-(carboxymethyl) pyridium chloride hydrazide; GirT, 1-(carboxymethyl) trimethylammonium chloride hydrazide; H/D, hydrogen/deuterium; HILIC, hydrophilic interaction liquid chromatography; HMP, 2-hydrazino-1-methyl-pyridine; HP, 2-hydrazinopyridine; HPLC, high performance liquid chromatography; HRMS, high resolution mass spectrometry; IHD, index of hydrogen deficiency; IMPs, impurities; IR, infrared; IUPAC, International Union of Pure and Applied Chemistry; LC, liquid chromatography; LC-ESI-MS, liquid chromatography-electrospray ionization-mass spectrometry; LC–MS, liquid chromatography–mass spectrometry; LOQ, limit of quantification; MBO-TAD, 4-[4-(6-methoxy-2-benzoxazolyl) phenyl]-1,2,4-triazoline-3,5-dione; MDMAES, mono(dimethylaminoethyl) succinyl imidazolide; mM, millimolar; mmu, milli mass unit; MNBDH, N-methyl-4-hydrazino-7-nitrobenzofurazan; MS, mass spectrometry; MS-TOF, mass spectrometry-time of flight; MS^n, multi stage mass spectrometry; NA, isonicotinoyl azide; NBA, 2-nitrobenzaldehyde; NBD-Cl, 7-chloro-4-nitro-2,1,3-benzoxadiazole; NBD-F, 7-fluoro-4-nitro-2,1,3-benzoxadiazole; NBDPZ, 4-nitro-7-piperazino-2,1,3-benzoxadiazole; NFPH, 2-nitro-4-trifluoromethylphenylhydrazine; NH_4OH, ammonium hydroxide; NIT, naphthylisothiocyanate; NMR, nuclear magnetic resonance; NPTAD, 4-(4-nitrophenyl)-1,2,4-triazoline-3,5-dione; NSAIDs, non-steroidal anti-inflammatory drugs; PABA, para-amino benzoic acid; PAHs, polycyclic aromatic hydrocarbons; PBS, 4-(1H-pyrazol-1-yl) benzenesulfonyl; PFB-Br, pentafluorobenzyl bromide; PFPA, pentafluoropropionic acid anhydride; PGF2α, prostaglandin F2α; ppm, parts per million; PPZ, 1-(2,4-dinitro-5-fluorophenyl)-4-methylpiperazine; PS, pyridine-3-sulfonyl; Q-TOF, quadrupole-time of flight; RDB, ring plus double bonds; RP-HPLC, reversed phase-high performance liquid chromatography; SRM, selected reaction monitoring; TFA, trifluoroacetic acid; TFE, 2,2,2-trifluoroethanol; THAS, 4-(trimethylammonium) anilyl-N-hydroxysuccidimidyl carbamate iodide; THF, tetrahydrofuran; TMAE, trimethyl amino-ethylalcohol; TMPP, tris(2,4,6-trimethoxyphenyl) phosphine; TMPP-AcPFP, S-pentafluorophenyl tris(2,4,6-trimethoxyphenyl) phosphonium acetate bromide; TMPP-PrG, (4-hydrazino-4-oxobutyl) tris(2,4,6-trimethoxyphenyl) phosphonium bromide; TOF, time of flight; TSH, p-toluenesulfonylhydrazide; UV, ultra violet; XRD, X-ray Diffraction.

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Contents

1. Introduction

The definition of quality of pharmaceuticals has changed in recent times. From previous focus on purity, a greater emphasis is being paid today on impurities (IMPs), degradation products (DPs), etc. The characterization of such trace to minor analytes is not only important during drug and product development, but also from the perspective of regulatory approvals. Fortunately, the advent of a variety of sophisticated hyphenated techniques has made this task much simpler [1], against the time-consuming exercise involving isolation and enrichment of targeted compounds to milligram quantities, followed by acquisition of the spectral (mass, UV, NMR and IR) and elemental (CHN) data. In particular, liquid chromatography–mass spectrometry (LC–MS) is a technique of choice among all other hyphenated techniques due to its sensitivity and ease of use. Also, it carries potential in itself to provide unequivocal characterization of the structure of most trace to minor components, except enantiomers and epimers. A variety of LC–MS instruments are available today, which vary in (i) atmospheric pressure ionization (API) sources, like electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI) and/or atmospheric pressure photo ionization (APPI); and (ii) analyzers, like quadrupole, ion trap, time of flight (TOF), Orbitrap, etc. Usually ESI and APCI interfaces along with ion trap, TOF and Orbitrap analyzers are more useful for providing qualitative information on component structures.

The unequivocal structure elucidation of unknown analytes by LC–MS techniques is possible through the strategy put forth by us earlier [1,2]. It entails the generation of molecular ion and fragment spectra of both the drug and its related substances; determination of their accurate and exact masses, elemental formula, ring plus double bonds and number of nitrogens; determination of the number of labile hydrogens through hydrogen/deuterium (H/D) exchange mass studies; MS^n study on molecular ions and fragments, and establishment of fragmentation pathways, followed by their comparison. Introductory details on each of these steps were provided by us in our previous publication [1]. The strategy has been duly validated in our laboratories and even by other researchers by application to the characterization of process impurities [3], drug degradation products [4–6], drug–drug interaction products [7,8], drug-excipient interaction products [9], and metabolites of a variety of drugs [10,11].