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Review article

Interpretation of Full Scan Atmospheric Pressure Ionization Mass Spectra (MS) and Collision Induced Dissociation Fragmentation Spectra (MS/MS) of Small Organic Molecules – A Mini Review

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ABSTRACT
The potential of mass spectrometry (MS) is often not fully utilized due to the absence of systematic workflow of mass spectral interpretation, partially caused by the lack of simplified literature for interpretation of soft ionization methods and collision induced fragmentation spectral data interpretation. Therefore, this review summarizes the rational work flows for the interpretation of mass spectral data generated using atmospheric pressure ionization (API) and collision induced dissociation (CID) fragmentation. Designed workflow will definitely assist the readers to interpret MS and MS/MS spectral data, especially for the structural elucidation of small drug molecules and their impurities.

Key words: Mass spectrometry (MS), Tandem mass spectrometry (MS/MS), Atmospheric pressure ionization (API), Collision induced dissociation (CID), Basic rules, Rational workflow

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INTRODUCTION

Mass spectrometry (MS) is a technique that can analyze a variety of chemical and biological compounds. An enormous growth seen in the field of MS hyphenation techniques in last few decades and is still ongoing. Nowadays, high performance liquid chromatography (HPLC) and other liquid or gas -phase separation techniques coupled to mass spectrometry are routinely used in analytical laboratories as a research and quality tool. Modern mass analyzers have powerful features for the structural elucidation; for example high resolving power, high mass accuracy, tandem mass spectrometry. A numerous reviews, research articles and books on mass spectrometry technology are available to explicate its principles, instrumentation and interpretation of MS and MS/MS spectra including basic rules, workflow, challenges and perspective.[1-8] However, interpretation of full scan (MS) and tandem mass spectrum (MS/MS) is still remain complicated due to a high number of possible functional groups, structural subunits and their combinations resulting in numerous competitive fragmentation pathways.[9,10] Therefore, we made an attempt to provide a step by step rational and simplified workflow for the interpretation of mass spectra acquired by atmospheric pressure ionization (API) techniques; electrospray ionization (ESI), also for other soft ionization technique such as atmospheric pressure chemical ionization (APCI) and tandem mass spectrometry (MS/MS) spectra generated using collision induced fragmentation. The workflow for interpretation of MS and MS/MS spectra are derived from the rules given in reference text books, literature and the rational approaches proposed by experienced researchers.[1,11-14]

INTERPRETATION OF MS AND MS/MS SPECTRA

The hyphenation of tandem mass spectrometers with modern chromatography instruments, for the identification small molecules and their impurities is became an essential tool for research laboratories. The MS/MS spectra provide distinctive fragment patterns which in turn help in providing the structural information. In mass spectrometric analysis, the most essential task is to understand the mass spectra i.e. interpretation of the spectral data. Basics for mass spectrometric interpretation; nitrogen rule, accurate mass, types of ions, adducts, isotopic abundance, monoisotopic molecular weight, rearrangements and even odd electron rule need to be reminisce, to efficiently apply the workflows (Figure 1 & Figure 2) for the interpretation of acquired MS and MS/MS spectra of small organic molecules. To interpret the MS spectral data such as protonated and deprotonated ions formed during atmospheric ionization of analytes in ion source as well as mass difference of most common ion types calculated and presented in Table 1 for the quick reference of researchers. Table 1 may be quite informative, which may further help in the identification the molecular ion *m/z* value. Subsequently to classify fragments, reference table imbedded in Figure 2, will certainly helpful for assigning the right structural fragments to *m/z* values of fragments.

Especially nitrogen rule is very helpful to assign a correct elemental composition for given mass number in case of nitrogenous molecules i.e. "a compound with an odd molecular weight will have an odd number of nitrogen and compounds with an even molecular weight will have either no nitrogen or an even number of nitrogen atoms". Nitrogen rule in combination with Odd-Electron (OE) and Even-Electron (EE) rule for fragmentation interpretation is given in Table 2, which is extremely helpful to assign the correct elemental composition to fragment ions *m/z* values.

Workflow

The systematic workflow is essential for identification of unknowns in new drugs substances, to investigate the suspicious, discover the unknowns and then add to the routine analysis. Identification of molecular ion peak for trace level of impurities is very essential to assign molecular weight of analyte and its elemental formula. Flow chart with various rational steps for the identification of molecular ion peak "M" in MS spectra was established and presented in Figure 1. The rational steps are summarized in a few words; select at least 2-3 most intense *m/z* values from MS spectra. Then, refer Table 1 to calculate the mass difference

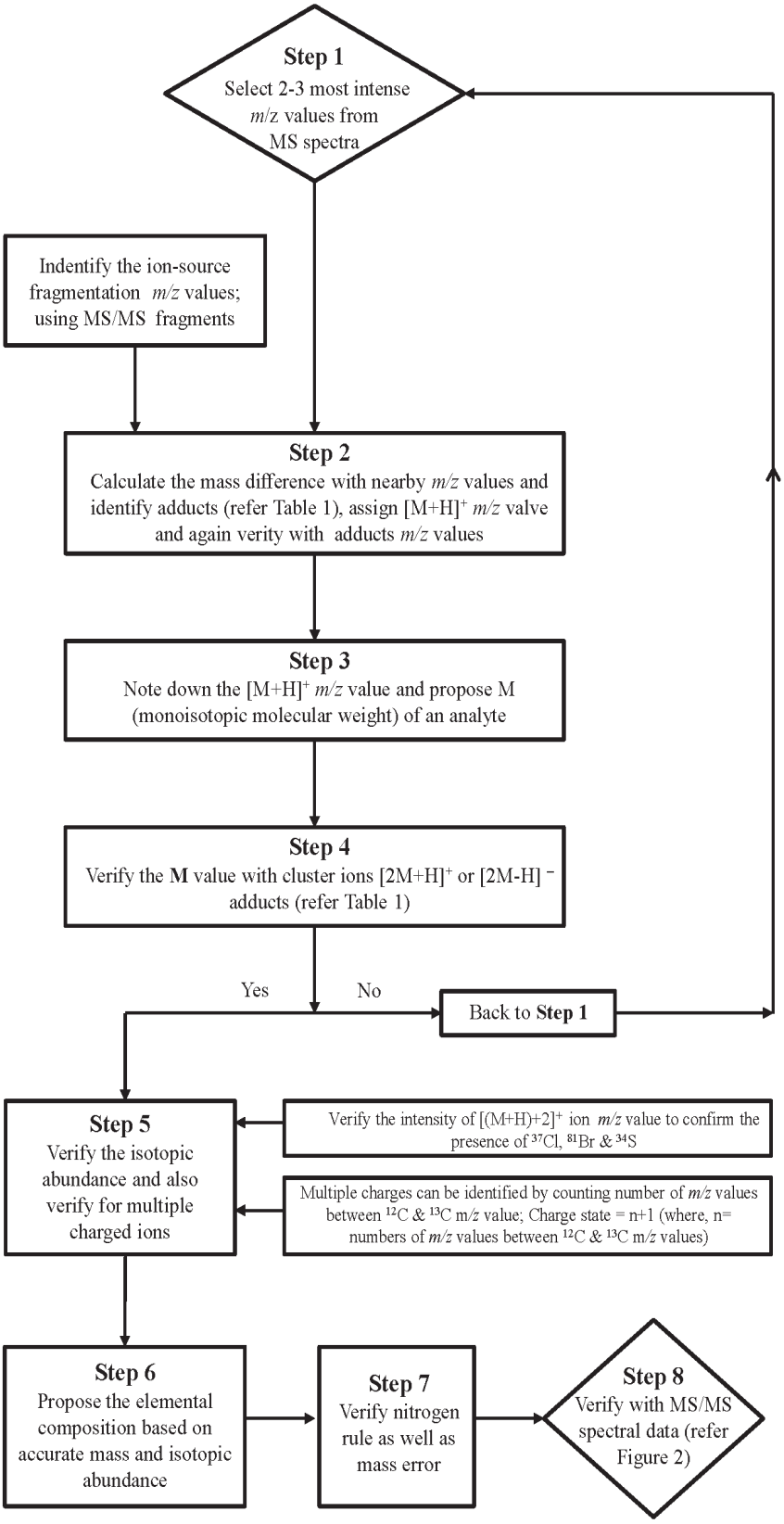

Figure 1: Rational workflow chart for the interpretation of MS spectra

Table 1: Common ions types in positive and negative ion mode

ESI Mode	Ion Type	Ion Mass*	Charge	Classification/Explanation
Positive ESI	$[M+H]^+$	M+1.01	+1	Protonated
	$[M+Na]^+$	M+22.99	+1	Adduct (Sodium)
	$[M+K]^+$	M+38.96	+1	Adduct (Potassium)
	$[M+NH4]^+$	M+18.03	+1	Adduct (Ammonium)
	$[M+H2O+H]^+$	M+19.02	+1	Adduct (Water)
	$[M+CH3OH+H]^+$	M+33.03	+1	Adduct (Methanol)
	$[M+CH3OH+Na]^+$	M+55.02	+1	Adduct (Methanol+Sodium)
	$[M+CH3OH+K]^+$	M+70.99	+1	Adduct (Methanol+ Potassium)
	$[M+CH3CN+H]^+$	M+42.03	+1	Adduct (Acetonitrile + Proton)
	$[M+CH3CN+Na]^+$	M+64.02	+1	Adduct (Acetonitrile + Sodium)
	$[M+CH3CN+K]^+$	M+79.99	+1	Adduct (Acetonitrile + Potassium)
	$[2M+H]^+$	2M+1.01	+1	Multimer
	$[2M+K]^+$	2M+38.96	+1	Multimer and adduct
	$[2M+Na]^+$	2M+22.99	+1	Multimer and adduct
	$[2M+ NH4]^+$	2M+18.03	+1	Multimer and adduct
	$[M+2H]^{++}$	M/2+1.01	+2	Multiple charged ions
	$[M+2Na]^{++}$	M/2+22.97	+2	Multiple charged ions
	$[M+2K]^{++}$	M/2+38.96	+2	Multiple charged ions
Negative ESI	$[M-H]^-$	M-1.01	-1	Deprotonated
	$[M+HCOO]^-$	M+44.997	-1	Adduct (Formate)
	$[M+CH3COO]^-$	M+59.01	-1	Adduct (Acetate)
	$[M+CF3COO]^-$	M+112.99	-1	Adduct (Trifluoroacetate)
	$[M-H-H2O]^-$	M-19.02	-1	Adduct & loss
	$[M+Cl]^-$	M+34.97	-1	Adduct
	$[M+Br]^-$	M+78.92	-1	Adduct
	$[2M-H]^-$	2M-1.01	-1	Multimer
	$[M-2H]^-$	M/2-1.01	-2	Multiple charged ions
		*Mono Isotopic		

with nearby *m/z* values and identify adducts, neutral loss and correlate adducts for the identification of molecular ion *m/z* value. Note down *m/z* value of $[M+H]^+$ and propose monoisotopic molecular weight (M) of analyte. Refer Table 1 to verify the molecular ion (*m/z* value) with cluster ions $[2M+H]^+$ or $[2M-H]^-$ and adducts. Also, verify the isotopic abundance i.e. the presence elements such as chlorine, bromine and / or sulfur, etc and also verify for multiple charged ions. Then, propose elemental composition, verify with nitrogen rule and accurate mass. Probable structure can be proposed based on the verified elemental composition. Further to verify with MS/MS information.

For the interpretation of MS/MS spectra, a flowchart containing various rational steps is presented in Figure 2. The rational steps are summarized as: first prepare the fragmentation table as shown in Figure 2 and then assign the common neutral losses ($-H_2O$, $-NH_3$, -COO, CO, HCN and other neutral losses), EE and OE fragments. Then propose the element composition and verify with the rules EE/OE rule, nitrogen rule and mass accuracy. In case of unknown impurity or metabolite, identify the modification by comparing with parent compound; verify the modification by using fragmentation pattern and possibility of formation. Propose a fragmentation pathway, based on charge migration, bond cleavage and rearrangements check for rearrangements and correlate with fragment table.

CONCLUSION

MS technology has been used an exploratory analytical technique from several decades in the research arena of chemistry. In fact, HPLC coupled with MS has become a powerful analytical tool for the identification of drugs, especially small drug molecules and their impurities. In this technical review, interpretation workflows were designed for interpretation of MS spectral data (Figure 1 and Figure 2) surely beneficial for readers. Notably, adduct ions information presented in Table 1 is very useful for identification of ions generated by ESI and APCI ion source. The information and the complex approach existed in the literature is gathered and presented in Figure 1 and Figure 2 in a simple way, which is easy to apply for the interpretation. The established workflow is efficient and can

Table 2: Reference table for applying for even-odd electron fragments

Mass	Odd Electron Ions	Even Electron Ions
Even	No Nitrogen / Even Nitrogen	Odd Nitrogen
Odd	Odd Nitrogen	No Nitrogen / Even Nitrogen

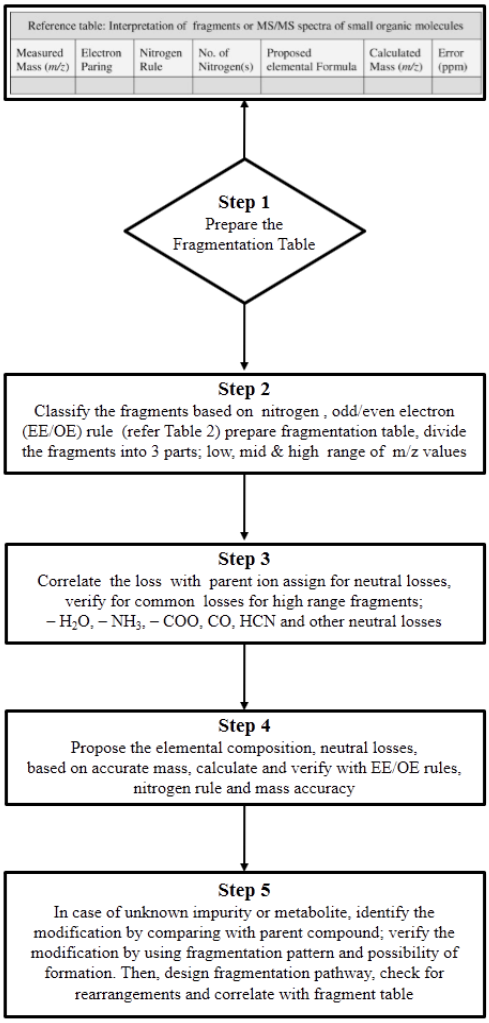

Figure 2: Rational workflow chart for the interpretation of MS / MS spectra

be applied for structure verification studies and for structure elucidation studies of unknown small drug molecules and their impurities.

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CONFLICTS OF INTEREST

Declared none

ABBREVIATIONS USED

LC: Liquid chromatography; **HPLC**: High performance liquid chromatography; **MS**: Mass spectrometry; **MS/MS:** Tandem mass spectrum; ***m/z:*** mass-to-charge ratio; **API:** Atmospheric pressure ionization; **APCI:** Atmospheric pressure chemical ionization; **ESI:** Electrospray ionization; **CID:** Collision induced dissociation ; **OE**: Odd Electron; **EE**: Even Electron

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SUMMARY

- In this article, an efficient workflow was given for the interpretation of MS (molecular ion peak) and MS/MS collision induced dissociation (CID) spectra of small drug molecules
- The information in this review can help the reader to interpret MS and MS/MS spectral data generated by ESI or APCI ion source

AUTHOR PROFILE

Shandilya D. K: A competent professional with over 18 years of experience in *Analytical Research & Development of Pharmaceutical Research*. He is expert in analytical research in various phases of drug development cycle (drug substance synthesis; DMF and Formulation development; ANDA), method development and validation. He is familiar with GC, HPLC, LC-MS/MS (Triple Quard & Q-TOF), XRD, GC-MS, NMR, DSC, TGA, ICP-MS, Ion-chromatograph and other analytical instruments. He has obtained his M.Sc. in Analytical Chemistry from Gurukula Kangari University, Haridwar in 1998 and currently pursuing Ph.D. from Bhagwant University, Ajmer, Rajasthan, India.