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Review Article

Qualitative Analysis of Plant-Derived Samples by Liquid Chromatography-Electrospray Ionization-Quadrupole-Time of Flight-Mass Spectrometry

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Abstract

Purpose: *Currently, mass spectrometry has become an effective method for the qualitative analysis of plant-derived samples. Precursor and product ions can be obtained by tandem mass spectrometry, supplying rich information for determining the structural formulas of compounds. In this work, we review the optimization of qualitative methods using liquid chromatography-electrospray ionization-quadrupole-time of flight (LC-ESI-Q-TOF), coupled with ultraviolet and infrared spectroscopy, nuclear magnetic resonance, and mass spectrometry. This paper provides a systemic reference for researchers engaged in the qualitative analysis of plant-derived samples using LC-ESI-Q-TOF.*

Keywords: *Qualitative analysis, Liquid chromatography-electrospray ionization-quadrupole-time of flight (LC-ESI-Q-TOF), Mass spectrometry, Optimization*

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INTRODUCTION

Qualitative analysis is an important part of analytical chemistry, and is commonly achieved using chromogenic reactions [1], chromatography [2-4], optical analyses [5], mass spectrometry (MS) [4,6], etc. Chromogenic reactions (CRs) are chemical reactions that can be used to determine possible functional groups in unknown compounds via distinct color changes. Qualitative analysis via chromatography is performed by comparing the retention times of samples with reference substances. Optical analyses, including ultraviolet (UV), infrared (IR), and fluorescence spectroscopy, are analytical methods based on electromagnetic radiation or interactions of matter. Because of the complexity of sample matrices, it is easy to achieve false-positive results using CRs. Furthermore, CRs can only be used to potentially classify test compounds, instead of determining their chemical structures directly. In addition, when using chromatography alone for qualitative analysis, false-positive results could be obtained in different compounds with similar chromatographic behaviors. Optical analysis methods can be used to identify functional groups and elements in test compounds; however, results obtained by optical analyses cannot be used to unequivocally determine chemical structure directly [7-12].

High-resolution mass spectroscopy (MS), such as quadrupole-time of flight-tandem MS (Q-TOF-MS/MS), can be used to address these above-mentioned problems through the collection of

abundant information about molecular and fragment ions, obtained in MS and MS/MS modes. The molecular formulas of compounds can be predicted with high precision using such data. Additionally, qualitative analysis can help researchers understand the chemical composition of samples and degradative processes [13-15]. The common process of qualitative analysis using liquid chromatography (LC)-Q-TOF-MS/MS is shown in Figure 1. Our review aims to provide a systemic reference for the qualitative analysis of plant-derived samples using LC-electrospray ionization (ESI)-Q-TOF-MS/MS or other types of MS.

SELECTION OF ION SOURCES

Due to the diversity and complexity of plant-derived samples, the development of ion sources has evolved to meet the needs of qualitative analysis. Currently, the main ion sources of MS are as follows: electron impact ionization, chemical ionization, field desorption, fast atom bombardment, liquid secondary ion, matrix-assisted laser desorption ionization, ESI, atmospheric pressure chemical ionization, and atmospheric pressure photo ionization. Moreover, multimode ionization [16] has been developed to meet the increasing demands of analyses. The applications of different ion sources are different, as shown in Table 1. Among them, ESI, a soft ionization technique, is commonly used for the qualitative analysis of natural plant-derived samples, especially in combination with reverse-phase liquid chromatography [35-38].

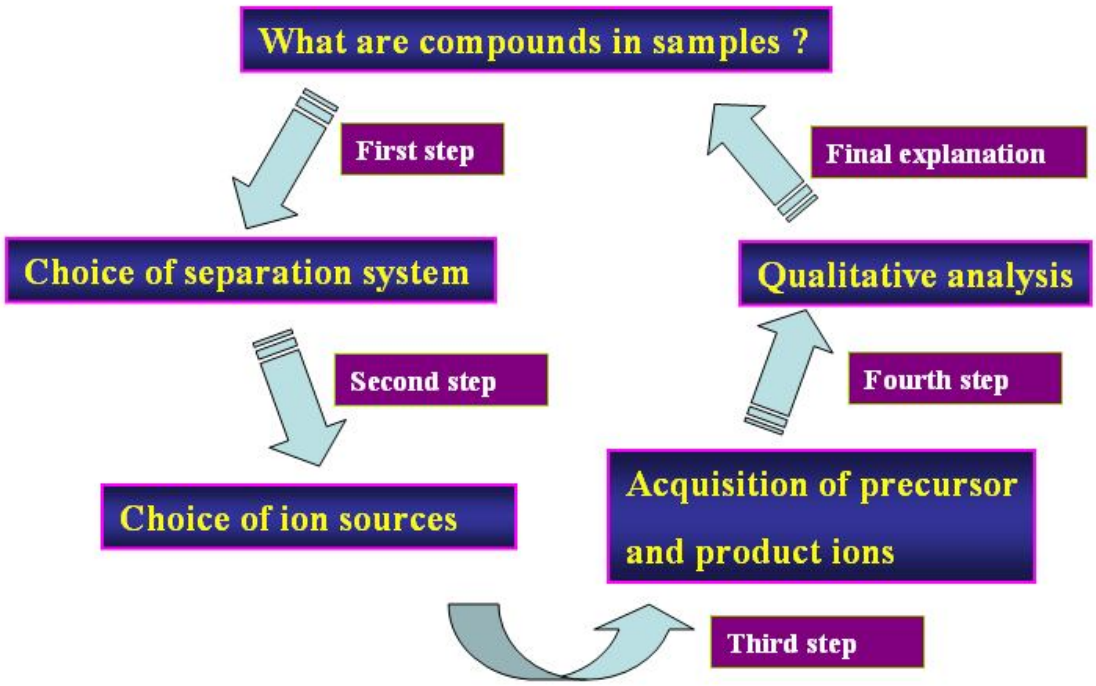

Figure 1: Qualitative analysis by LC- Q-TOF-MS/MS

Table 1: Applications of different ion sources

Ion source	Type and/or properties of suitable compounds	Reference
EI	Micro molecules (1-1000 u), low polarity, volatile	[17, 18]
CI	Micro molecules (60-1200 u), moderate polarity, volatile	[19, 20]
FD	Difficult gasification, poor stability	[21, 22]
FAB	Carbohydrates, metallo-organic compounds, proteins, nonvolatiles	[23, 24]
LSI	Carbohydrates, metallo-organic compounds, proteins, nonvolatiles	[25, 26]
MALDI	Proteins, polypeptides, nucleic acids	[27, 28]
ESI	Thermal instability, polar molecules, nonvolatile, proteins, polypeptides	[29, 30]
APCI	Micro molecules, low polarity, thermal stability	[31, 32]
APPI	non-ionizable with conventional ion sources	[33, 34]

Note: *Electron impact ionization (EI), chemical ionization (CI), field desorption (FD), fast atom bombardment (FAB), liquid secondary ion (LSI), matrix-assisted laser desorption ionization (MALDI), electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), atmospheric pressure photo ionization (APPI)*

IMPROVEMENTS IN QUALITATIVE PERFORMANCE

Only under optimal conditions, including the conditions of the chromatographic system and ion source, can LC-ESI-Q-TOF-MS/MS achieve the best performance for qualitatively analyzing plant-derived samples. In brief, good resolution and peak shape are the goals of chromatographic system optimization, and ionization and a good relative response rate are the aims of the ion source and MS system optimization, respectively.

Optimization of chromatographic conditions

Chromatographic conditions depend on the chromatographic column, column temperature, flow rate and mobile phase. Generally, the selection of the column is limited, as it cannot be changed in a timely and effective manner. Currently, reverse-phase chromatographic columns (such as a C18 column) are used widely in chromatographic analyses [39,40]. Selection of the mobile phase is crucial for the optimization of chromatographic conditions, including flow rate, composition of the mobile phase, and pH. A mobile phase with a low flow rate is commonly used to obtain good atomization and ionization efficiency of the ESI ion source [41]. Methanol, acetonitrile, and water are commonly used components of the mobile phase in reverse-phase chromatography; adjusting the ratios of these components to one another can influence peak resolution and shape [42,43]. To achieve good sample separation (such as for alkaloid samples), appropriate concentrations of volatile acids (formic acid, acetic acid), volatile alkalis (ammonium hydroxide), and volatile buffer salts (ammonium formate and ammonium acetate) are needed as components of the mobile phase [44]. In addition, vertical atomization can be used to maintain nonvolatile buffer salts in solution [45].

Triethylamine (TEA) in the mobile phase will pollute the ion source, as the molecular ion of TEA in positive ion mode is observed for a long time and can lead to incorrect results. In addition, because trifluoroacetic acid (TFA) is a very strong ion-pair reagent, it can combine charged ions, strongly suppressing their signal (especially positive ions). Moreover, as the molecular ion of TFA in negative ion mode is observed for a long time, erroneous results can be obtained.

Optimization of the ion source

Compounds in plant-derived samples can be analyzed after ionization, and the parameter settings of the ion source have important effects on atomization. The parameters of the ESI ion source include atomization gas pressure, drying gas flow, drying gas temperature, capillary voltage, and capillary outlet voltage. In general, these parameter settings are related to the composition of the mobile phase, its flow rate, and the nature of the plant-derived sample. Atomization gas pressure plays an important role in atomization. Drying gas flow and temperature play important roles in removing the solvent. Atomization gas pressure is positively related to flow rate: A high flow rate requires a high atomization gas pressure. In addition, if the mobile phase contains a high proportion of water, a higher drying gas flow is needed to remove the solvent, otherwise, the spectrum would present as a sharp peak. Drying gas temperature is negatively related to the vapor pressure of the mobile phase: A low vapor pressure of the mobile phase requires a high temperature. Capillary voltage is related to the ion modes. In general, the capillary voltage in positive ion mode is set to 3500 or 4000 V, and the capillary voltage in negative ion mode is set to 3000 or 3500 V. Importantly, when high current or a blue light (corona) is observed in the spray chamber, the capillary voltage must be turned down. The optimization of capillary outlet voltage can improve the transmission efficiency of precursor ions.

Functions of ion optics

Ionized compounds of plant-derived samples can be analyzed using ion optics (capillary, skimmer, octopole, and lens), which have an important influence on the transmission of ions. The high-vacuum environment of the mass spectrometer can be maintained by a capillary, and the vacuum can enable a mean free path for the ions: the bigger the mean free path, the higher the resolution. In addition, Q-TOF is high resolution and requires a high vacuum. Skimmers and lenses can remove excess neutral gas and solvent molecules. Octopoles can focus dispersive ions toward the quadrupole. Neutral substances can be separated using a turbo molecular pump in the octopole area, which can average the energy distribution of the ions before they are imported into the quadrupole.

Optimization of the mass analyzer

The monitoring mode (positive or negative ion mode) should first be confirmed. Generally, the positive ion mode is suitable for alkaline samples; when plant-derived samples contain secondary or tertiary ammonium compounds, the positive ion mode should be used preferentially [46]. However, the negative ion mode is suitable

for acid samples; when the plant-derived samples contain highly electronegative groups (such as chlorine, bromine, and multiple hydroxide radicals), negative ion mode should be preferentially used [47]. Other MS parameters include the mass range, acquisition rate/time and transients/spectrum, precursor ions, and collision energy; the optimization of these parameters can improve sensitivity and reduce noise.

The general steps of qualitative analysis are as follows: A high-resolution mass number of precursor ions should be obtained by the primary mass spectrum in Q-TOF mode; the target precursor ion, selected by the quadrupole mass analyzer, is then imported into the collision cell at a certain collision energy; fragment ions are then monitored by the TOF mass analyzer and MS/MS is performed; subsequently, a high-resolution mass number of precursor and product ions are obtained. Molecular and structural formulas of compounds can then be preliminarily determined using databases. Importantly, improving the relative response of the ion signals in qualitative analysis is as important as it is for quantitative analysis.

CONCLUSION

Based on its good selectivity, sensitivity, and robustness [48,49], qualitative analysis of compounds in plant-derived samples analyzed via LC-Q-TOF-MS/MS is extensively used. However, despite continuous progress, there are still many areas that must be improved in the future. Although LC-Q-TOF-MS/MS is excellent for providing sensitive and selective results for the identification of known compounds, reference compounds are also needed to confirm the preliminary results. A global and comprehensive mass database for the identification of target compounds is currently lacking. Therefore, it is urgent to devote more work to develop a solid mass database, similar to the NIST database for gas chromatography-MS [50]. It is also well known that TOF-MS can be used to identify an accurate molecular weight of target compounds in samples, and it appears that LC-Q-TOF-MS/MS could also be used to identify unknown compounds in samples [51,52]. However, it is currently difficult to identify structures of new target compounds using LC-Q-TOF-MS/MS alone, due to its complicated analysis methods and software. Consequently, improved analysis methods for compound identification that are easy to execute are urgently needed for qualitative analysis. Furthermore, using MS alone, it is difficult to identify isomerides due to their similar chemical properties and structures. For this reason, to obtain reliable and convincing results, qualitative analyses of target compounds in samples also require comprehensive data from UV and IR spectroscopy as well as from NMR methods. Recently, the combination of UV spectroscopy and LC-MS has been used to separate and identify compounds in samples. The combination of UV and IR spectroscopy, NMR, and TOF-MS has also been used for these purposes [53-56]; however, the combination of IR spectroscopy and NMR with LC-MS requires more works to achieve commercialization. Qualitative analysis will achieve historic developments if the four qualitative techniques mentioned above can be performed simultaneously.

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