Application of LC-MS in Qualitative and Quantitative Analysis of Drugs

LC-MS has been widely used in many fields, such as food analysis, environmental analysis and pharmaceutical research, because it has many advantages such as high separation ability of chromatog-raphy, high selectivity and high sensitivity of mass spectrometry, and can provide relative molecular weight and structural information.

LC-MS combines the HPLC and MS with an interface which delivers samples in different material states. There are currently five commonly used ionization methods: Inductively Coupled Plasma Ionization(ICP), Electron Impact Ionization(EI), Chemical Ionization(CI), Matrix-Assisted Laser Desorption Ionization(MALD) and Atmospheric Pressure Ionization(API). API technology is most wildly used due to its features which analyzes samples with strong polarity, high melting point, low volatility and thermal instability. API technology includes three sources: electrospray ionization (ESI) or atmospheric pressure MALD(APMALDI), atmospheric pressure chemical ionization (APCI) and atmospheric pressure photoionization (APPI). The ionization of the samples in these three modes can be accomplished in an atmospheric ionization chamber. The ionization efficiency is high and the sensitivity and stability of the analysis are greatly enhanced. Currently, most LC-MS technologies use ESI and APCI.

In drug analysis, LC-MS is widely used in impurity analysis, content determination and drug metabolism studies.

Impurity Analysis

LC-MS technology can be used to fully analyze the type and source of impurities. Not only can it identify known impurities in drugs, but it can also be used to infer the structure of unknown impurities, which greatly contributes to the work of impurities. Schneider group used HPLC-UV and HPLC-ESI-MS to analyze the impurities in the generic drugs and the original drugs. It was found that there are 14 impurities in the generic drugs (not

found in the original drugs), most of which belong to amino acid analogues and side chain homologues, and these impurities would not occur in the whole process of the synthesis of the raw drugs.

In the process of active pharmaceutical ingredient production, different impurities may occur due to changes in the production process. Because these impurities are structurally similar to the active pharmaceutical ingredient or intermediate, the chemical structure of impurities can be identified directly by comparing the fragmentation characteristics of active pharmaceutical ingredient or intermediate, thereby improving the quality of active pharmaceutical ingredient. Sample pretreatment for LC-MS is simple, without derivatization, and is more effective for the separation of components that have a small content, are difficult to separate, and are easily lost during the separation process. Thus, LC-MS is widely used for impurity detection as USP prescript.

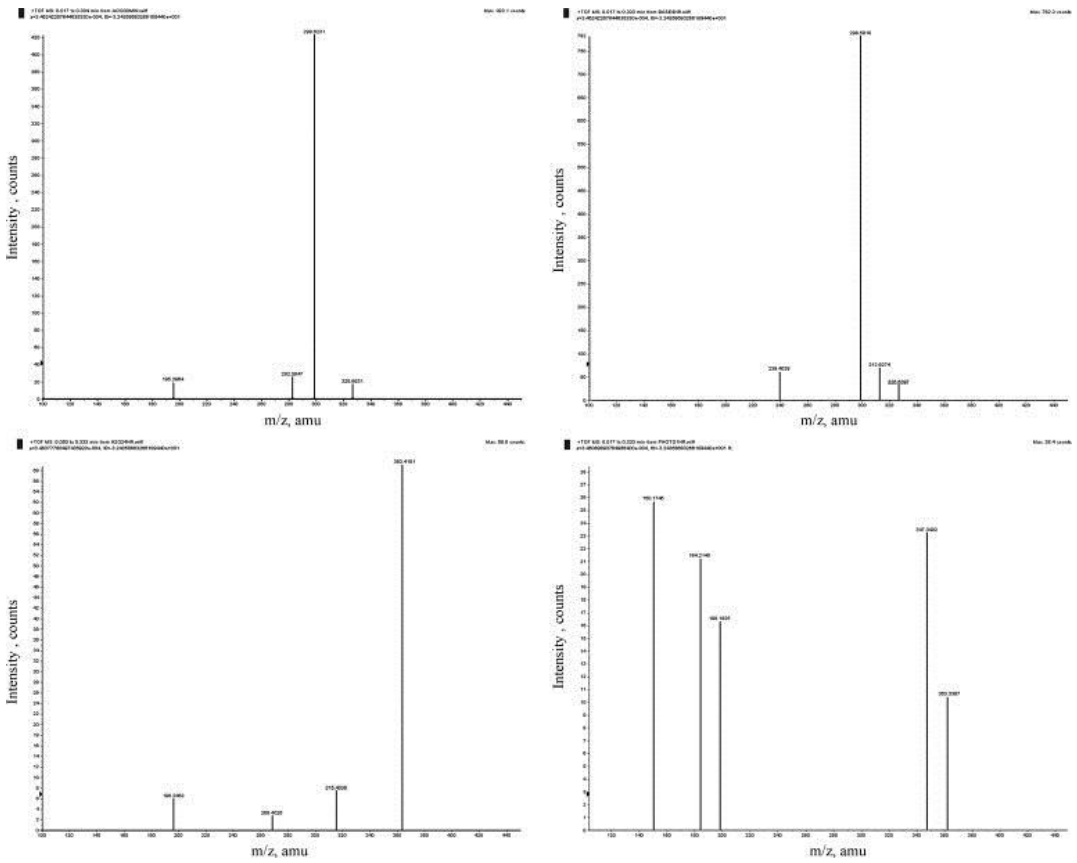

Fig 1 Representative positive ESI-Quadrapole (+Q1) mass spectra of tenatoprazole.[1]

Identification and Content Determination

Most of the samples used in drug analysis are mixed substances. The advantages of LC-MS are its high efficiency, rapidity and sensitivity. Its detection limit can be as low as pg grade which is suit for small quantities content test. Separation of the components of the product is more effective with LC, which provides an efficient and feasible solution for the research and analysis of natural products and drugs.

Drug Metabolism Analysis

For drug metabolites analysis, LC-MS technology can provide better separation, reduce the detection limit of analytes, and improve the analysis speed, so as to achieve rapid and high-throughput analysis of complex samples *in vivo*.

At present, two-stage or multi-stage tandem mass spectrometry (MS / MS or MSn) is widely used in drug metabolism analysis *in vivo*. There are 4 kinds of data acquisition methods for tandem mass spectrometer. (1) ion scanning: this method is especially suitable for analyzing the structural information of metabolites. Usually the matrix is obtained first. The molecular ion spectrum of the drug molecule, followed by the metabolite of the ion spectrum, allows us to obtain rich structural information based on biotransformation or metabolic sites. (2) Mother ion Scanning: This method can help trace the source of daughter ions, and can quickly screen a class of compounds that produce characteristic fragment ions. This scanning function is very important in drug metabolism. (3) Neutral Loss Scanning: Neutral Loss Spectrum best reflects the specific functional group of the compound. If there is a neutral loss of 18 Da functional molecule, it means the loss of a molecule of water (-H20). Neutral loss scans are widely used to determine phase II metabolic processes, such as glucuronic acid (- 176 Da) and sulfate (- 80 Da). (4) Multiple Reaction Monitoring or Selective Reaction Monitoring (MRM or SRM): This method is very suitable for selecting a specific mass number of substances from many complex systems, typically for quantitative analysis of trace components. In

addition, IDA(information-dependent) and Energy-related two-stage mass spectrometry data acquisition methods are also wildly used in metabolites study.

LC-MS/MS can deduce some or even complete molecular structures based on the specific cleavage rules in the study of drug metabolism. The main reason is that the metabolites of most drugs retain the skeleton structure of the prototype drug molecules, so the metabolites may have similar splitting rules with the parent drug, so the possible metabolites can be quickly found. For example, different biotransformations will cause different quality changes, which can be used to infer the metabolic reactions of prototype drugs *in vivo*.

LC-MS/MS method not only reduces the development time of analytical methods, sample preparation and analysis, but also provides higher sensitivity and the ability to identify metabolites. At the same time, it can simultaneously analyze the parent drug and its metabolites in a sample. With the development of entire drug industry, LC-MS will play an increasingly important role in the study of drug metabolism.

References

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