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Simplified Approaches to Impurity Identification using Accurate Mass UPLC-MS

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Abstract

This work demonstrates the use of Xevo G2 Tof and novel MS^E technology, and its ability to collect exact mass precursor and fragment ion information from every detectable component in a sample, in the analysis and identification of sildenafil impurities in counterfeit tablet samples.

Introduction

The profiling, identification, and quantification of impurities plays a critical role at all stages of the drug development and manufacturing process. The levels of impurities of pharmaceutical products are strictly controlled and regulated in order to ensure the safety of the administered drug product. The structure of the impurities must be identified and their pharmacology understood in order for the overall safety of the drug to be characterized.[1] In addition, pharmaceutical manufacturers monitor potential counterfeit drugs through the use of impurity profiling.

During the development process, many different routes of synthesis may be evaluated, each producing its own distinct impurity profile. It is critical that these routes of synthesis are thoroughly evaluated and that the impurities produced are identified. This activity is often performed using a combination of NMR and LC-MS/MS. Although modern nominal mass LC-MS instrumentation, such as a tandem quadrupole or ion trap mass spectrometer, can quickly produce high-sensitivity, fast scanning data, the interpretation of its data can be very time consuming and of limited utility due to low mass spectral resolving power. These techniques often require further confirmatory experiments to identify the structure of the impurities.

Modern high-sensitivity exact mass instruments with time-of-flight (Tof) technology capable of fast acquisition rates offer the opportunity to dramatically simplify the process of impurity data capture and analysis.

The use of simultaneous low and high collision energy data collection (MS^E) allows the interpretation of precursor and fragment ion data from a single analytical run. As this data is collected with a high degree of mass accuracy, elemental compositions can be obtained for both intact molecular ions and structurally significant fragments.

Sildenafil citrate is one of the approved synthetic phosphodiesterase Type-5 (PDE-5) inhibitors used in the treatment of erectile dysfunction (ED) and is widely counterfeited. In this application note, we present the use of the Xevo G2 Tof for the applicability of novel MS^E technology in the analysis and identification of sildenafil impurities present in counterfeit tablet samples.

Experimental

Sildenafil tablets were dissolved in a mixture of methanol/water (1:1), filtered, and transferred to an autosampler vial for analysis. The chromatography was performed on a 2.1 x 100 mm, 1.7-µm ACQUITY UPLC BEH C_{18} Column. The column was maintained at 45 °C. Reversed phase gradient elution was performed at 600 µL/min, using 10 mM NH_4OAC in water as the aqueous mobile phase, and acetonitrile as the organic modifier.

Positive ion electrospray, with a cone voltage of 30 V and a capillary voltage of 3k V were used. Data was collected over the mass range of 50 to 1000 *m/z*. The data was acquired in MS^E mode with the low energy data acquired using a collision energy of 4 eV. Elevated collision energy data was acquired using a collision energy ramp of 25 to 45 eV.

MassLynx 4.1 Software was used to acquire the data.

Results and Discussion

The total ion chromatogram (TIC) obtained from the analysis of a counterfeit tablet sample of sildenafil is displayed in Figure 1A. Here we can see that the parent drug elutes with a retention time of 8.49 min and there are at least eleven other peaks that elute between 2.75 and 9.79 min. Any of these peaks could represent drug-related impurities or excipient components from the tablet.

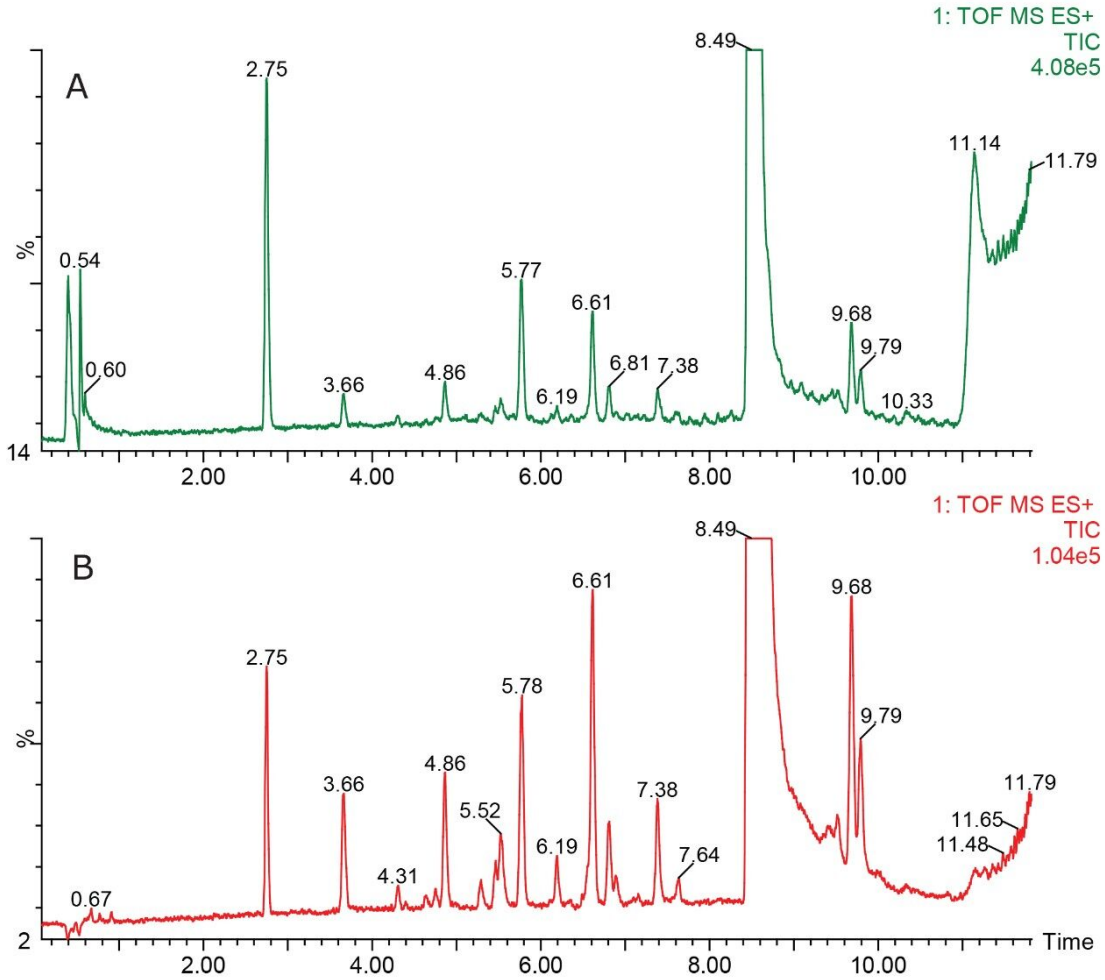

Figure 1. A) Total ion chromatogram obtained from the analysis of a counterfeit tablet of sildenafil. B) LC-MS data after filtering with the MDF.

The mass spectra displayed in Figure 2 show the elevated and low collision energy of the sildenafil peak. The low collision energy spectrum includes a peak with a measured mass of 475.2127, allowing an elemental composition of $C_{22}H_{31}N_6O_4S$ with a mass error of -0.1 mDa to be assigned. The high collision energy data gave rise to two main fragment ions, *m/z* = 100.0997 and 283.1191, which had mass errors of -0.3 mDa and -0.4 mDa, respectively.

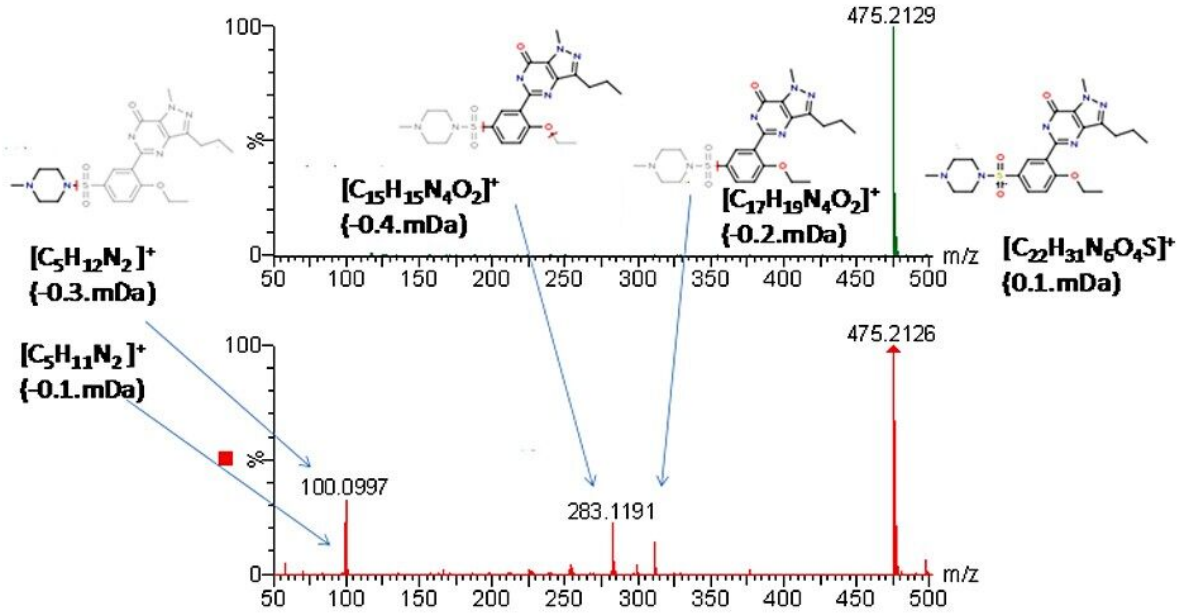

Figure 2. Mass spectra showing the elevated and low collision energy of the sildenafil peak.

The elemental composition and fragment ion structures generated using MassFragment are shown in Figure 2. MassFragment is software that automatically identifies product ion fragments using a series of chemically intelligent algorithms.[2]

The accurate mass capability of the Xevo G2 Tof mass spectrometer in conjunction with the software capabilities in MassLynx can be used to simplify data analysis. The fractional part of the exact mass of the parent drug *m/z* can be used to visualize only the drug related material by applying a mass deficiency filter (MDF) to the data. In this case, the exact mass of the sidenafil ion is 475.2127. A 20 mDa range was applied to the mass measurement after the decimal point to limit display.

The data displayed in Figure 1B shows UPLC-MS data after filtering with the MDF. Here we can see that there are at least 17 drug-related impurities. The use of MDF has greatly improved the signal-to-noise of the related components, most notably at RTs 5.52 min, 6.81 min and 7.64 min. This is due to the reduction in background noise, which allowed improved peak detection for the low concentration drug-related components.

The common fragment ions may also be used as an approach to highlight drug-related peaks. The data shown in Figure 3 shows the use of narrow window extracted ion chromatograms for the fragment ions *m/z* 283.1195 and 100.1001 using a 5 mDa window. From this data, we can see six possible drug-related impurities in each trace.

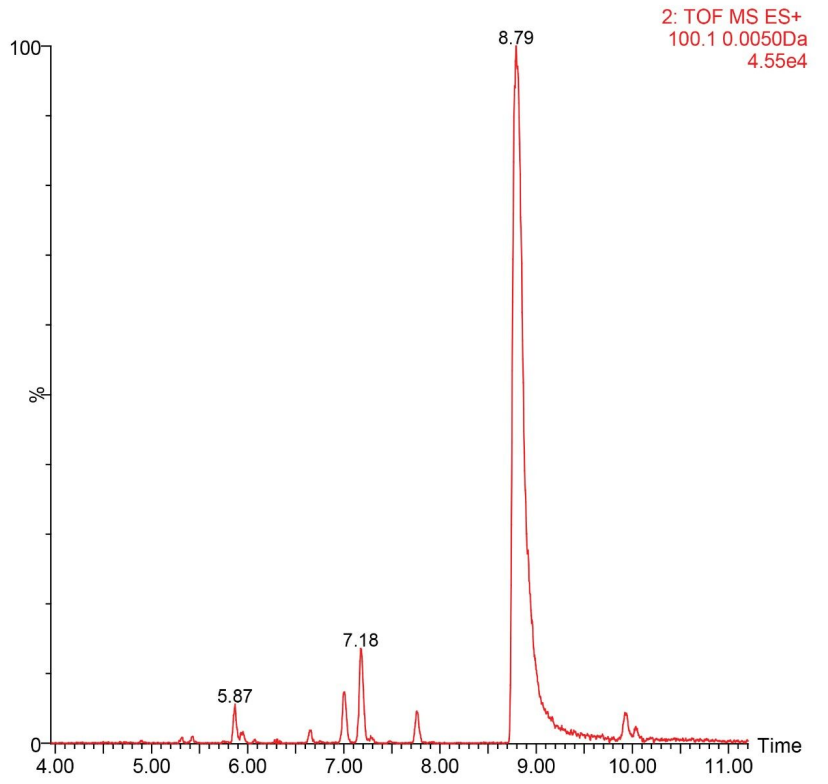

Figure 3. The use of narrow window extracted ion chromatograms for the fragment ions m/z = 283.1195 and m/z = 100.1001 using a 5 mDa window.

All of this data were available from one simple UPLC-MS experiment taking just 13 minutes.

Data was processed using MetaboLynx, an Application Manager within MassLynx Software. MetaboLynx MS^E fragmentation analysis was used to interrogate both low and high collision energy data simultaneously. This enables the visual alignment of precursor with collision induced dissociation fragment ions for sildenafil and its impurities. The automated structure elucidation tool, MassFragment, was employed to rationalize and identify fragment ion structures.

The elucidation of the impurity structures is facilitated by the elemental composition, which was obtained for both the fragment ions and parent compound molecular mass after data processing with MetaboLynx. The data in Figure 4 shows the elemental composition of the sildenafil-related impurities found in the sample.

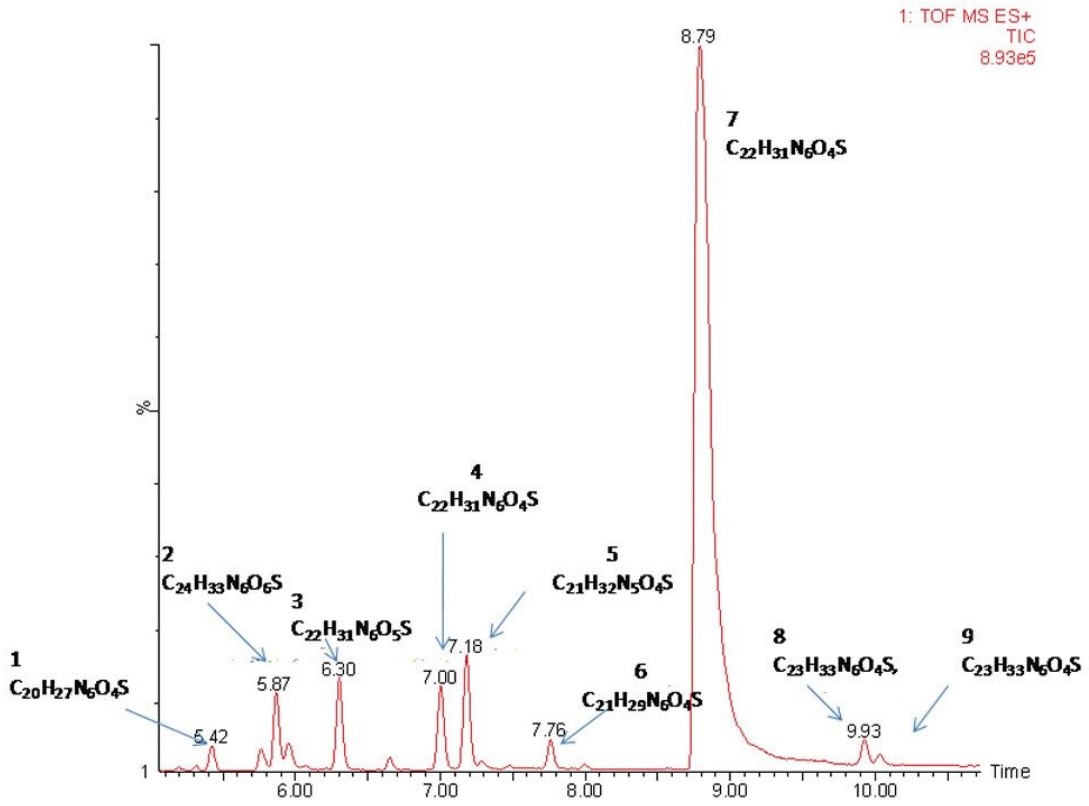

Figure 4. The elemental composition of the sildenafil related impurities found in the sample.

Conclusion

The Xevo G2 Tof operating in MS^E mode provides an excellent approach for the analysis of impurities in pharmaceutical products. The major benefits include:

- Precursor mass and fragment ion data is obtained in one simple acquisition, decreasing the need to re-inject samples for targeted ion MS/MS experiments.
- All the data is collected in accurate mass mode, allowing the elemental composition of parent and fragment ions to be derived.
- The Mass Defect Filter simplifies the task of detecting drug-related impurities by increasing the selectivity of data extraction and improving the visibility of detection for related components.
- The use of MetaboLynx and MassFragment allows rapid turnaround from data analysis to data interpretation and reporting.

References

1. Ahuja S and Alsante K. *Handbook of Isolation and Characterisation of Impurities in Pharmaceutical Compounds* . 2003; Elsevier.

2. Jones M.D *et al*. *Identification and Characterization of an Isolated Impurity Fraction: Analysis of an Unknown Degradant Found in Quetiapine Fumarate*. Waters Application Note. 2009; 720003079en.

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