Analysis of Benzodiazepines Using Portable LC-MS Instrumentation

Authors: Sam Foster, Milton Lee

Axcend, Lehi, Utah

Abstract

Mass spectrometry (MS) is a powerful detection method that provides high sensitivity and structural information of an analyte. Liquid chromatography (LC) is commonly used as a separation technique prior to MS detection to reduce matrix interferents and resolve compounds of similar mass. Although powerful, this technique often requires considerable space as well as the need for external pumping and gas supplies, making it impractical for field analysis. Due to the growing number of drug-related deaths, the need for fast and accurate identification of drugs at the point of care is critical. In this application note, the Axcend Focus LC® was coupled to the MassTech MTE30 MS to form a portable LC-MS system. A sample containing four benzodiazepines was used to test the system, achieving baseline resolution in 15 min, with the identity of each analyte confirmed by its mass spectrum. This demonstrates the viability of compact and portable LC-MS instrumentation for use in field settings.

Introduction

LC-MS is a powerful analytical technique providing sensitive detection and structural identification capabilities. Conventional LC-MS instrumentation requires large benchtop footprints to account for the LC stack as well as the MS and its external vacuum pumps and gas supplies. This limits the portability of the technique, often restricting it to dedicated laboratory spaces. The Axcend Focus LC offers a compact and portable LC solution for capillary scale chromatographic analysis; however, the system's portability is hindered by the limited availability of portable MS instrumentation. To allow

for fully portable LC-MS analysis, the MassTech MTE 30 compact MS was selected for coupling to the Axcend Focus LC, as it features internal vacuum pumping systems and gas supplies with small dimensions of 8 x 12 x 13 inches. The MTE30 uses a 3D ion trap with a mass range of 30-2000 Da and a mass resolution of 0.5 Da. In combination, the Axcend Focus LC and MassTech MTE30 offer the ability to perform portable and sensitive LC-MS experimentation.

Portable instrumentation allows for point-of-care (POC) analysis in clinical settings. For example, illicit drugs have become an area of interest in POC analysis, as rapid detection and identification of these compounds can offer guidance for treatment without the lead time required when sending samples to a lab, improving patient outcomes. Benzodiazepines are a class of central nervous system depressant drugs often used to treat anxiety disorders, insomnia, and muscle spasms. Although they have clinical benefits, rates of death by overdose of benzodiazepines have increased ~10x from 1999 to 2021. Due to the number of different benzodiazepines and their similar structures/reactivities, identification of the specific analyte through more conventional immunoassays proves challenging. In this application note, we demonstrate the LC-MS analysis of four benzodiazepines using the Focus LC coupled to an MTE30 MS.

Materials and Methods

LC-MS-grade water and acetonitrile were used for the preparation of all mobile phases and samples. A sample mixture of 10 ppm alprazolam, diazepam, flunitrazepam, and flurazepam was prepared in water. The LC and MS operating conditions are listed in Tables 1 and 2, respectively.

Table 1. Operating Conditions for the Axcend Focus LC with Heated Column Cartridge

Column	Fortis Evosphere C18/AR, 100 x 0.3 mm i.d., 5 µm
Mobile Phase	(A) Water with 0.1% Formic Acid, (B) 100% ACN
Temperature	Ambient
Flow Rate	2 µL/min
Elution Mode	Gradient 5 to 95% B over 10 min, then 5 min hold at 95% B
Run Time	15 min
Injection Method	250 nL external injection loop toggled for 0.02 min for 40 nL injection

Table 2. Operating Conditions for the MassTech MTE30 MS

ESI Mode	Positive, 2.5 kV
Capillary Temperature	210 °C
Capillary DC Offset	10 V
Hexapole 1 DC Offset	5 V
Conductance Limit DC Offset	3 V
Hexapole 2 DC Offset	-3 V
Hexapole RF Amplitude	50 V_{p-p}
Detector High Voltage Multiplier	-0.58 kV
Conversion Dynode High Voltage	-3.80 kV
Mass Range	200-500 m/z

Results and Discussion

The LC-MS analysis of benzodiazepines was performed using the Axcend Focus LC coupled to the MassTech MTE 30. The total ion chromatogram (TIC) is shown in Figure 1, with the elution times of flurazepam, alprazolam, flunitrazepam, and diazepam being 10.7 min, 11.4 min, 11.8 min, and 12.4 min, respectively. The extracted-ion-chromatogram for each compound is shown in Figure 2 between the time range of 9 to 14 min to provide a more focused view of each analyte, with its peak mass listed to the right. All compounds were baseline resolved and were identified by their mass spectra.

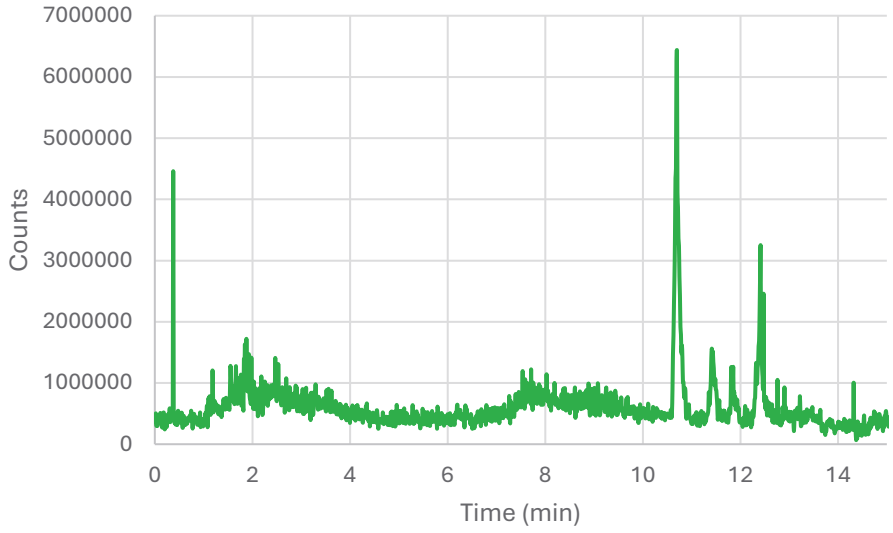

Figure 1. Total-ion-chromatogram of a separation of four benzodiazepines.

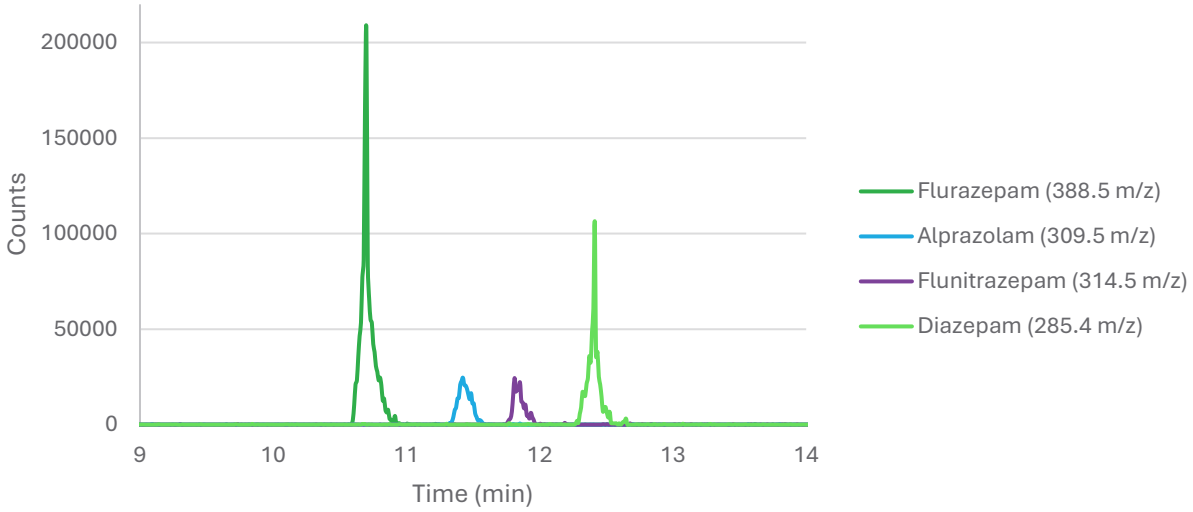

Figure 2. Extracted-ion-chromatograms of flurazepam, alprazolam, flunitrazepam, and diazepam.

Conclusions

LC-MS analysis is a critical technique for identification and quantification of analytes in complex matrices. With the growing rate of drug-related overdoses, there is a need for rapid and accurate testing at the point of care to inform treatment options and improve patient outcomes. The Axcend Focus LC and the MassTech MTE30 MS combine to form a compact and portable LC-MS instrument capable of POC analysis of drugs of abuse. Four benzodiazepines were separated in 15 min, with each analyte being identifiable from its mass spectrum. This illustrates the viability of combining both compact instruments for portable analysis.

Reference(s)

(1) Drug Overdose Deaths: Facts and Figures. National Institute on Drug Abuse. https://nida.nih.gov/research-topics/trends-statistics/overdose-death-rates.