

## Analysis of Benzodiazepines in Whole Blood Using Selected Ion Monitoring Electron **Ionization Mass Spectrometry**

Highlights: The analysis of very low levels of benzodiazepines in whole blood samples is easily achieved using the techniques of Selected Ion Monitoring (SIM) and Electron Ionization (EI) mass spectrometry. Simultaneous Photodiode Array (PDA) detection as well as the ability to monitor characteristic ions by SIM using the Waters Integrity <sup>™</sup> system provides sensitive and selective chromatographic peak detection.

The detection and identification of low levels of materials in complex matrices is of paramount importance in today's analytical laboratory. This study describes the utility of the Waters Integrity system, a benchtop LC/MS which combines a Photodiode Array detector (PDA) with the specificity of the ThermaBeam<sup>™</sup> Mass Detector (TMD) featuring Electron Ionization (EI). The PDA is useful for determining peak homogeneity, generating UV spectra for searching against user-built libraries and for quantification. El provides mass spectra that are characteristic compound "fingerprints" which can be interpreted and/or searched against commercial or user-built reference libraries for positive identification. Once an EI spectrum of a compound has been acquired, one, two or several representative ions from that spectrum can be selected for subsequent SIM analysis. By monitoring one or a few characteristic ions from a compound instead of the entire mass spectrum, the specificity and the signal-to-noise of the acquisition can be increased. The specificity of the analysis is improved due to the acquisition of signal from only chemically significant ions to the exclusion of all else. Signal-to-noise is subsequently increased as a result. Two benzodiazepines, Estazolam and Triazolam, were spiked into, and extracted from, whole blood samples. Estazolam was used as an internal standard. UV spectra and selected masses were generated for each compound in a single injection. Peak homogeneity was determined for each chromatographic peak using the unique Spectral Contrast <sup>™</sup> algorithm in Millennium<sup>™</sup> software.

## Waters Integrity System:

ThermaBeam Mass Detector 996 Photodiode Array Detector 616 Pump and 717+ Autosampler Millennium 2010 Chromatography Mgr.

## LC Conditions:

Mobile Phase: 60% 50mM Ammonium Acetate 40% Acetonitrile @ 0.25ml/min Column: Waters Symmetry C-18 150 X 2.1 mm 28 deg.C PDA: 220-350nm @ 2.4 nm resolution TMD: 70-350 Da

**Sample Preparation** Samples were whole blood spiked with one of two benzodiazepines.

Samples were extracted by liquid-liquid extraction (LLE) into n-chlorobutane and evaporated. n-chlorobutane contained Estazolam as an internal standard

Samples were reconstituted in mobile phase and analyzed by LC/MS

Estazolam and Triazolam are common sedatives. Detection and identification of low levels of these compounds in complex matrices such as biological materials is often sought. In addition to the generation of an EI compound "fingerprint", the Integrity system is capable of utilizing SIM to increase the sensitivity of analysis.



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MW = 294

MW = 342

## Triazolam PDA Results 0.02 ug/mL spiked in blood



Estazolam PDA Results



Figures 1 and 3 illustrate the results from the Waters 996 PDA detector. The UV spectrum of a compound (Triazolam and Estazolam, respectively) is searched against a user-built library containing a standard spectrum of the same compound. Peak homogeneity results are shown at the right of each figure. Figure 2 illustrates the EI mass spectrum of Triazolam, and Figure 4 similarly shows the EI mass spectrum of Estazolam. The specificity and selectivity of SIM are illustrated in Figure 5. The signal for the ions at m/z 342 and 259 are acquired respectively for Triazolam and Estazolam. These are chemically significant ions for both of these compounds. The signals for these ions are collected to the exclusion of all else. This enhances the signal-to-noise and the specificity of analysis for these materials.



**Estazolam El Mass Spectrum** 





In addition to the positive compound identification capability of the Waters Integrity system that can be achieved through search and/or interpretation of full scan spectra, SIM can be used to enhance detection sensitivity and specificity. Simultaneous acquisition of PDA and MS data on a single injection, and seamless processing and reporting of both channels of information also affords greater confidence in compound identification.

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