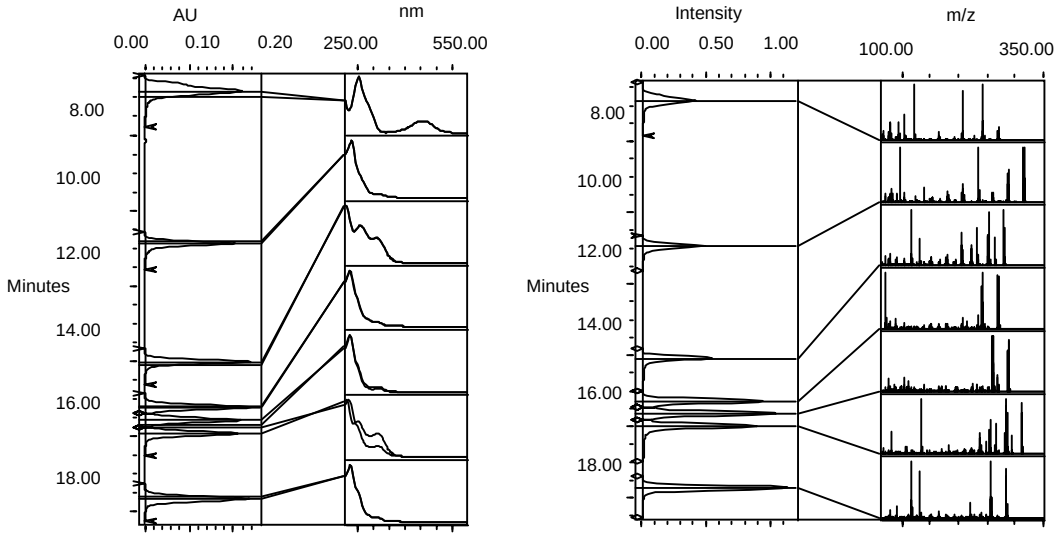
Waters Integrity System Applications

Reverse Search Capabilities Using the Waters Integrity System for the Detection and Identification of Benzodiazepines

Highlights: Detection and identification of coeluting Medazepam and Bromazepam (tranquilizers) using combined PDA/MS detection and automated library search capabilities.

Impurity identification is becoming increasingly significant in regulated environments, and detection and identification of impurities at the ~0.1% level is critical. Since compound identification based solely on retention time is not absolute, the dual detection capabilities of the Integrity System is extremely useful for the absolute identification of impurities in pharmaceutical and industrial materials. Low dispersion chromatography is also becoming more and more desirable in all industries. In this example, a flow injection analysis of Medazepam and Bromazepam deliberately creates a perfect coelution of the two compounds in order to study the ability of the Integrity System to identify coeluting sample components. The benefit of being able to identify each component of the coelution utilizing reverse library search capabilities in Millennium software on the Integrity LC/MS System will be demonstrated.

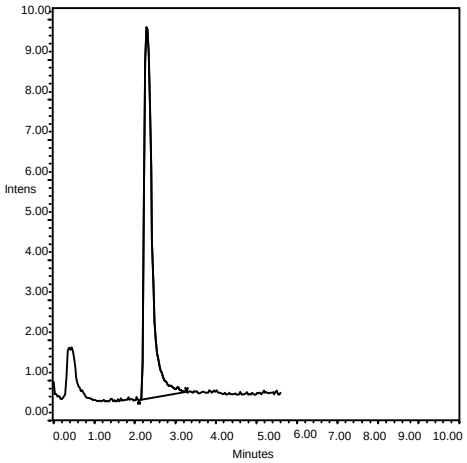
Benzodiazepine Separation
UV and MS Spectrum Index Plots



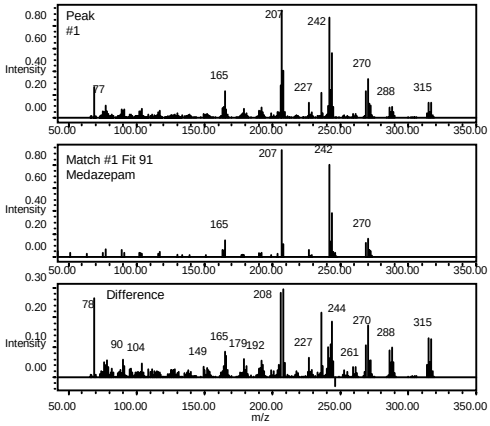
The power of the combined detection capabilities of the Integrity system allows for simultaneous acquisition of PDA and MS data. Consequently, PDA and MS spectra can be displayed above each chromatographic peak as in the Spectrum Index Plots illustrated here. The UV Spectrum Index Plot also has the ability to display the point of maximum impurity for each chromatographic peak. Similar retention times for the PDA and MS chromatograms shows minimal loss of chromatographic resolution from the PDA detector through the ThermaBeam interface

The following illustrates the reverse search capabilities of Palisade's PBM algorithm. The chromatogram to the right shows a perfect coelution of two benzodiazepines by flow injection of the compounds with no column in line. We then searched a "mixed' mass spectrum of the flow injected peak.

TIC of Flow injection of a mixture of Medazepam and Bromazepam

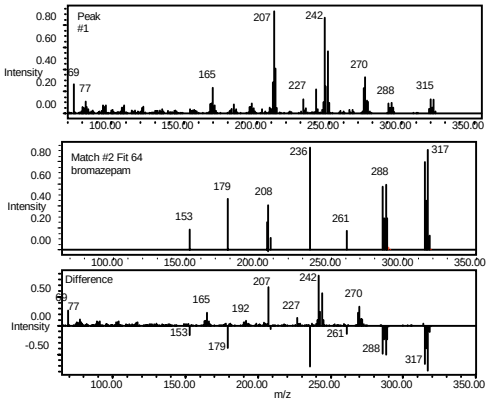


Triple Plot of Coeluted Peak Match # 1



The search results show the first hit to be an excellent 91 fit but with high contamination of about 68% indicating nearly seventy percent of the peaks in the spectrum are not from the primary compound Medazepam. In reverse search, the Medazepam spectrum is then subtracted from the mixed peak spectrum and the resulting spectrum is then searched against the library giving match #2.

Triple Plot of Coeluted Peak Match # 2



This is match # 2; identification of Bromazepam which is the second compound in the mix. The "Difference" spectrum at the bottom of the Triple Plot is the spectrum of Medazepam.

Positive compound identification of minor impurities is easily achieved using the Integrity LC/MS System. Simultaneous acquisition of PDA and MS data on a single injection and seamless processing and reporting of both channels of data as well as automated library search capabilities offer confidence in peak identification.