

Waters Integrity System Applications

Authentication of Dietary Supplements Using ThermoBeam™ EI Mass Spectra and Automated Library Search Capabilities

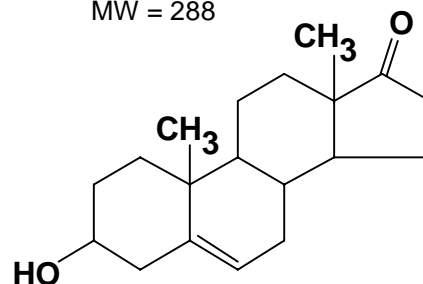
Highlights: Confidence in the correct identification of commercially available dietary supplements for authentication purposes is obtained using the Waters benchtop Integrity LC/MS System featuring ThermoBeam Electron Ionization (EI) capabilities.

The ability to obtain positive compound identification is critical in the modern analytical laboratory. Chromatographers, especially, need an easy to use, reliable and accurate tool to identify unknown peaks in a separation. LC/MS is the technique of choice for the chromatographer who needs either positive compound identification of unknown compounds or compound confirmation of known compounds. Positive compound identification of unknown compounds using LC/MS refers to the ability of MS data to unequivocally determine the identity of a peak based on classical, irrefutable information. Compound confirmation of known compounds by LC/MS refers to the ability of MS data to corroborate the suspected identity of a known compound based on limited, but useful, information. The former can be accomplished by generating an EI "fingerprint" of a compound which can either be compared to standard material, searched against a library database or scientifically interpreted. The latter is accomplished by generating molecular weight information which becomes a single parameter known about a suspected compound that can be used to verify the possible identity of such material.

Although there are various LC/MS interfaces and ionization techniques available in both benchtop and research grade mass spectrometers, there is only one combination of interface and ionization technique that offers the chromatographer positive compound identification as opposed to compound confirmation. The Waters ThermoBeam interface creates a desolvated analyte "particle beam" from the HPLC effluent so it may then be ionized by Electron Ionization (EI). This process produces a reproducible, positive ion EI mass spectrum "fingerprint" of the analyte of interest. Other softer LC/MS ionization techniques such as ESI and APci do not result in such specific mass fingerprints even when Collision Induced Dissociation (CID) is used. CID results in limited, method subjective, fragmentation which is only safely used to generate confirming ions rather than a complete, reproducible mass spectrum which can be automatically searched against a commercial library for identification purposes.

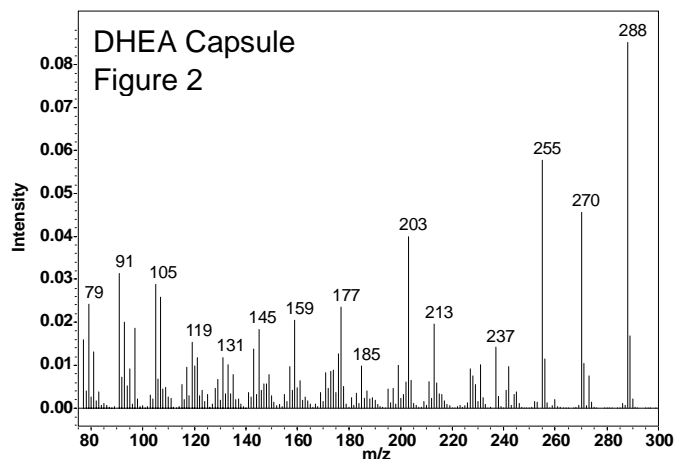
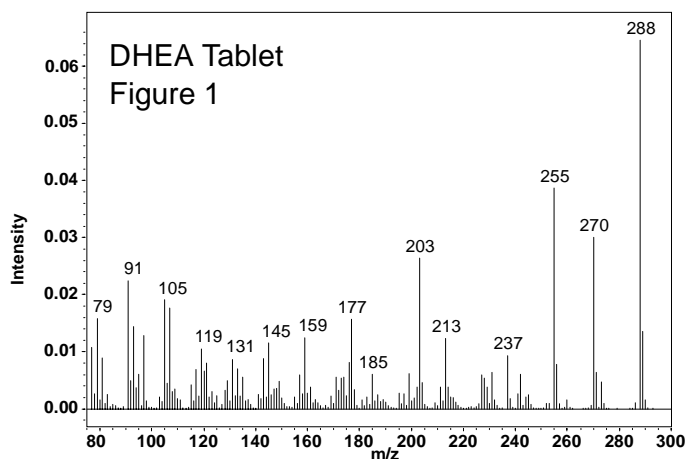
This study focuses on the ability of the Waters Integrity LC/MS System to obtain a positive ion EI mass spectrum of a commercially available dietary supplement, Dehydroepiandrosterone (DHEA). DHEA is normally produced in the adrenal glands and is most abundant in young adults and declines in most people as they age. In human studies, the effects of DHEA have been linked to mood and memory improvement and protection against heart disease and influenza. In order to authenticate this compound obtained from two distributors, an EI spectrum was generated using the ThermoBeam interface on the Integrity System. A common public objection to such dietary supplements is that they are not subject to the same FDA scrutiny as are pharmaceuticals. To counter suspicion, some products are labeled, "verified by HPLC". However, positive compound identification by ThermoBeam EI LC/MS yields greater confidence in verifying compound authenticity.

MW = 288

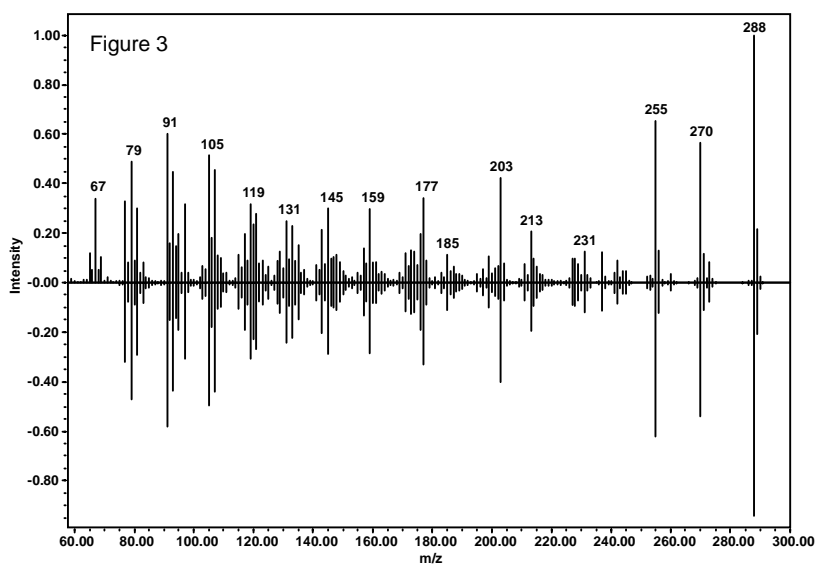


Dehydroepiandrosterone (DHEA)

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Two samples of DHEA from two distribution sources were analyzed. A capsule sample was emptied into ether (non-quantitative), shaken, filtered and flow injected into the Integrity System. A tablet sample was ground up, dissolved in ether, shaken filtered and flow injected. Figure 1 shows the EI mass spectrum of the tablet sample while Figure 2 is the EI mass spectrum of the capsule sample. Within Millennium software, automated search of a user generated library and/or of the Wiley library is possible. The Wiley library contains 262,000 spectra, 190,000 compounds and 160,000 structures. Figure 3 represents the Wiley library search results from an automated Millennium library search. The top spectrum is the Wiley mass spectrum of DHEA while the bottom spectrum is the tablet sample spectrum. The "fit" for this comparison is 97 which is considered to be an excellent match. A "fit" value for each library search result indicates the accuracy of the library match. If a target analyte is not in the Wiley library, identification of that material is still possible by either a spectrum match with a standard or by spectral interpretation. In this example, both DHEA samples are authenticated by a good match against the standard Wiley spectrum. Molecular weight alone would not be enough to identify this material especially in a multi-component biological extract. There are 969 entries in Wiley alone with a molecular weight of 288.



In addition to the ability to obtain classical EI mass spectra, the Waters Integrity LC/MS System affords the chromatographer an easy to use, easy to maintain complete LC/MS system in a compact design which also features a Photodiode Array detector for peak homogeneity information. The Waters "No Tools" approach to system maintenance allows the user to easily perform routine system maintenance without tools. Millennium software automates the entire data collection and results management process for control of all system operations from a single keyboard.