Waters Integrity System Applications

The Use of the Waters Integrity System for the Identification of Minor Impurities in Trimethoprim

Highlights: Detection and identification of Trimethoprim impurities using combined PDA/MS detection and automated library search capabilities.

The identification of impurities in compounds is of paramount importance in many industries. What follows demonstrates the use of the Integrity system for the detection and identification of such impurities in Trimethoprim, an antibacterial agent which is used in drugs for urinary tract infections such as Bactrim.

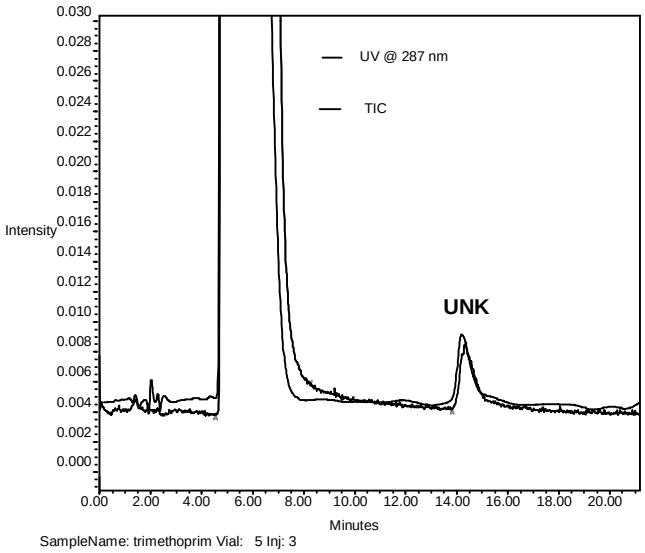
Trimethoprim Experimental Conditions:

Waters Integrity System
- ThermaBeam mass detector
- 996 Photodiode Array detector
- 2690 Separations Module
- Millennium 2010 Chromatography Manager

LC Conditions
- Mobile Phase: 80% Water 20% Acetonitrile @ 0.25ml/min
- Column: Waters Symmetry C-8 150 X 2.1 mm ambient temp.
- PDA : 220-400 nm @ 1.2 nm resolution
- TMD: 65-350 u

Positive compound identification is achieved through the ability of Integrity to generate Electron Ionization (EI) spectra on chromatographic peaks. The dual detector capability of Integrity also enhances compound ID. The system ensures low dispersion allowing a greater degree of well characterized peaks along with the ability to overlay the PDA and MS chromatograms for identification purposes. The PDA is very useful in this example since it was used to determine that the impurity is related to Trimethoprim. There is also a need to confirm compound ID by more than one analytical technique, so LC/MS in general has been experiencing increasing interest in the pharmaceutical industry.

Trimethoprim with Impurity

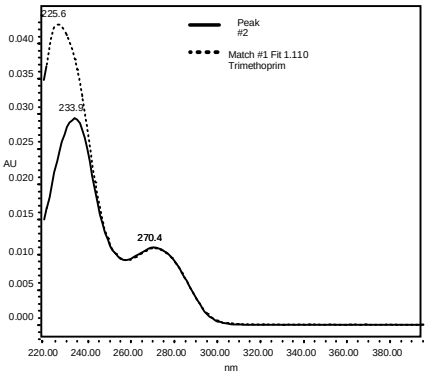


SampleName: trimethoprim Vial: 5 Inj: 3

Overlay of Both UV and MS Chromatograms for Trimethoprim.

The chromatography indicates that the UNK peak is less polar than the main peak.

UV Spectral Overlays

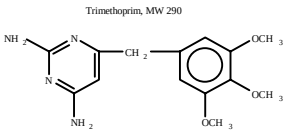


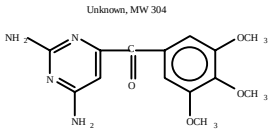
This represents the overlay of the UV Spectrum of the Unknown with the UV Spectrum of Trimethoprim.

This is the overlay of the UV spectrum from the impurity and the library spectrum of Trimethoprim. While the match is not a good match, it is clear that the spectra are related and, therefore, the impurity is probably a related compound.

Possible Impurity

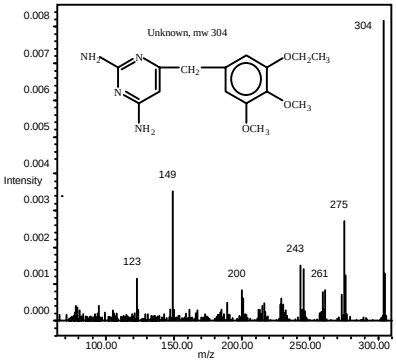
A possible impurity of Trimethoprim is illustrated here which would give a molecular ion at 304. It was thought that the impurity was an oxidation product, and the formation of the carbonyl would be consistent with a molecular ion at m/z 304, however this would be more polar and would elute before Trimethoprim in reverse phase. Also the oxygen would go to either the da-amino ring or the trimethoxy ring upon fragmentation. We don't see that in the mass spectrum.





Possible oxidation product of Trimethoprim
More polar than Trimethoprim

Mass Spectrum of Trimethoprim Impurity



- The impurity is less polar than Trimethoprim
- Spectrum shows logical loss of m/z 29 - (CH_2CH_3)
- Ethoxy-dimethoxy benzaldehyde is a known impurity in the synthesis of Trimethoprim

This represents the most likely structure for the Trimethoprim impurity. This would be less polar than Trimethoprim and would elute later. The next major fragment below 304 is m/z 275, a loss of 29, an ethyl group. The replacement of one of the three methoxy groups with an ethoxy group yields the only structure that allows a loss of 29 from the molecule. However, the location of the ethoxy group is uncertain. Ethoxy-dimethoxy benzaldehyde is a known impurity in one of the intermediates in the synthesis of Trimethoprim.

Positive compound identification of minor impurities is easily achieved using the Integrity LC/MS System. In this example, the chromatographic information for Trimethoprim indicates that the impurity must be less polar than Trimethoprim; the PDA information shows similarity in structure; the MS spectrum of the impurity confirms its structure. 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.