



## Liquid Chromatography/ Mass Spectrometry

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## Identification of Impurities Using Accurate Mass, Sensitivity and Wide Dynamic Range of the AxION 2 TOF MS

### Introduction

Time of flight (TOF) mass spectrometry provides exact mass information and high sensitivity over a wide mass range enabling identification of unknown compounds such as impurities. Using the exact mass capabilities and proprietary TrapPulse™ technology of the PerkinElmer AxION® 2 TOF MS, we are able to describe a workflow that allows us to find impurities and identify them in common over the counter drugs. Traditional scanning instruments, such as quadrupole

mass spectrometers, suffer a large loss in sensitivity when scanning over a wide mass range. This loss in sensitivity does not occur with the AxION 2 TOF mass spectrometer due to its fast sampling rates which enables us to detect impurities that would otherwise be missed. The innovative TrapPulse mode of the AxION 2 TOF MS further enhances its sensitivity, in this instance 8X, by collecting dense packets of ions before pulsing them into the flight tube. This allows us to easily detect low level unknowns that may not be found in traditional TOF designs.

In addition to the sensitivity over a wide dynamic range, the AxION 2 TOF MS provides accurate mass enabled by real time lock mass, accurate isotope measurement, and mass resolution, all of which increases confidence for fast and accurate identification of impurities. The enhanced mass accuracy and the ability to determine precise isotope ratios provided by the AxION 2 TOF MS significantly

reduces the potential number of candidates that need to be utilized for formula identification. Typical workflows for formula identification of unknowns include the use of elemental formula calculators based upon the accurate mass and isotope pattern. The PerkinElmer AxION EC ID software further reduces the number of possibilities by matching against a database of existent formulas in addition to the accurate mass and isotope ratio matching. The formulas are then ranked in order of greatest fit to the user selected criteria for mass accuracy range and isotope ratios. This capability greatly increases the ability to choose the correct formula without the need to consider elements to include, cutting out formulas that would not occur, or performing additional experiments.

Here we present a simplification to the screening and identification of impurities using unregulated melatonin tablets as an example. Since melatonin is found in certain foods, it can be sold as a dietary over-the-counter supplement in the U.S. and may contain unknown impurities.

## Experimental

### LC Conditions:

**LC System:** Flexar™ FX-10 HPLC

**LC Column:** PerkinElmer Brownlee™ Supra column C18 (1.9  $\mu\text{m}$ , 2.1 x 50 mm)

**Column Temp:** 25  $^{\circ}\text{C}$

**Flow Rate:** 0.4 mL/min

**Mobile Phase A:** Water with 0.1% formic acid

**Mobile Phase B:** Acetonitrile with 0.1% formic acid

**Injection volume:** 2  $\mu\text{L}$

**Gradient:** 20% B to 40% B over 5 min, then to 70% B over 2 min.

### MS Conditions:

**Mass Spectrometer:** PerkinElmer AxION 2 TOF ran in ESI positive mode

**Ionization Source:** PerkinElmer Ultraspray™ 2 (Dual ESI source)

**Capillary Exit Voltage:** +100V

**Pulse Mode:** m/z 100-700

**TrapPulse Mode:** m/z 100-700

## Sample Preparation

An over the counter melatonin tablet containing 1 mg melatonin was crushed by a mortar and pestle and dissolved in 10 mL water. The mixture was vortexed and then centrifuged at 6000 rpm for 10 min. The supernatant was collected and injected on column.

## Results and Discussion

### Samples run for impurity analysis using the Flexar FX-10 HPLC coupled to the AxION 2 TOF MS

The PerkinElmer Flexar FX-10 HPLC coupled to the AxION 2 TOF MS is fully integrated and controlled by the AxION data system. The integrated software allows for easy instrument startup including Autotune™ for the AxION 2 TOF MS, method definition for all instruments on one page, and sample analysis via a single interface. Complete instrument communication through a single software package ensures minimal downtime.

The enhanced sensitivity of the AxION 2 TOF MS provides greater insight into analysis of impurities using the TrapPulse mode. Initial analysis in traditional pulse mode showed very low amounts of impurities in the melatonin tablet (Figure 1a). However, when the analysis is performed in TrapPulse mode an increase in sensitivity of almost 8X shows impurities that were not previously able to be detected (Figure 1b). With its wide dynamic range, the AxION 2 TOF MS identifies multiple impurities in a single analysis. This ability to measure across the entire mass range with a single run enables far simpler and more rapid method development than required by scanning instruments.

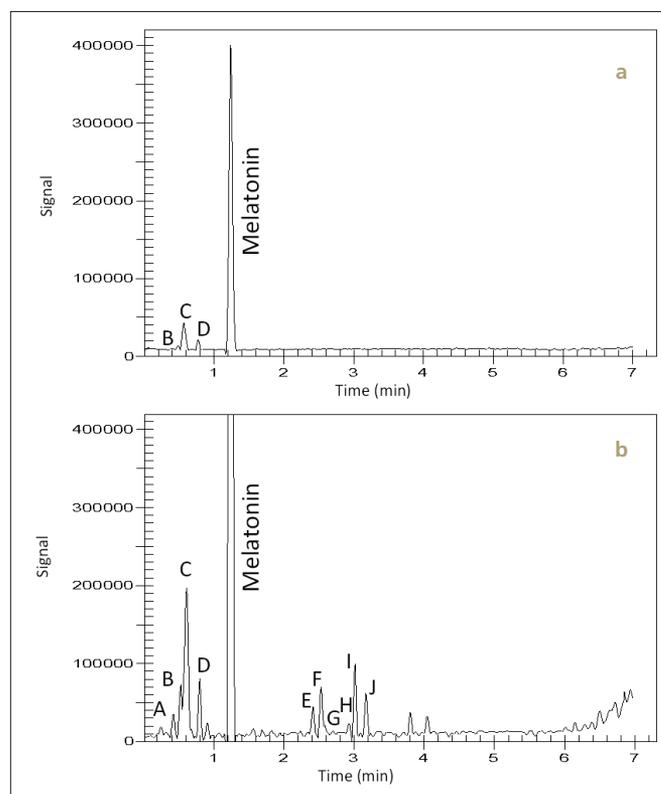


Figure 1. (a) Base peak intensity chromatogram (BIC) m/z 240-500 in pulse mode and (b) BIC m/z 240-500 in TrapPulse mode providing 8X increase in sensitivity. Impurities observed in the melatonin tablet are labeled A-J. Data obtained using positive ESI TOF MS.

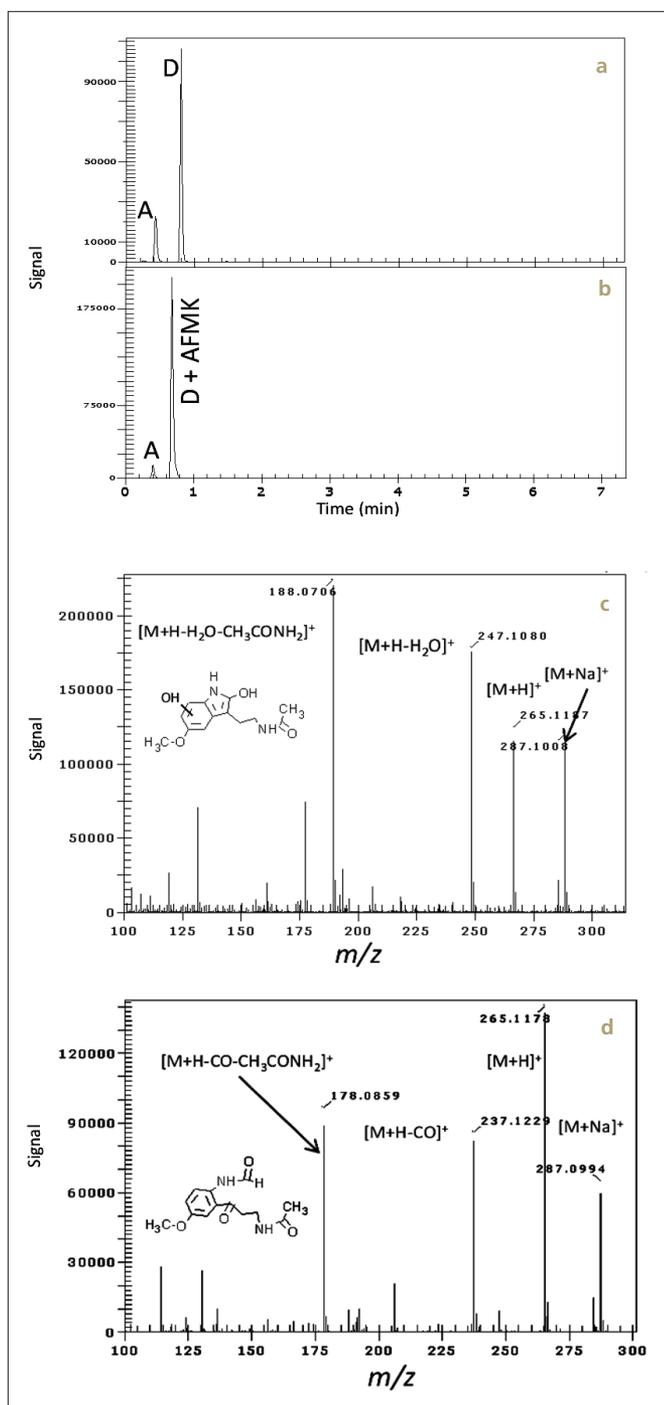


Figure 2. (a) BIC ( $m/z$   $265.132 \pm 0.050$ ) isomers A and D in melatonin tablet. (b) BIC ( $m/z$   $265.132 \pm 0.050$ ) showing co-elution of AFMK with peak D when spiked into melatonin tablet extract. (c) Mass spectrum of peak A impurity. The accurate mass of  $[M+H]^+$  and the fragments of peak A are within 2.0 ppm of theoretical value. (d) Mass spectrum of peak D. The accurate mass of  $[M+H]^+$ , and the fragments are within 2.5 ppm of theoretical value.

## Identification of unknown impurities

The accurate mass and precise isotope ratios provided by the AxION 2 TOF MS make it easy to identify unknown impurities with AxION EC ID software. Impurity peaks A and D have the same exact mass but different retention times and mass spectra indicating they are probably isomers (Figures 2a, 2b and 2d). The accurate fragment mass spectra provided by the clean and reproducible capillary exit CID provides further confirmation that these 2 peaks have the same formula but different structures. Further confirmation of the formula was obtained by AxION EC ID (Figures 3a and 3b) and an automatic PubChem search launched from AxION EC ID (Figure 3c). Using AxION EC ID with accurate mass data and isotope ratios from the AxION 2 TOF MS, only 1 possible formula comes back as a match enabling quick identification of the unknown peak. N1-acetyl-N2-formyl-5-methoxykynurenin (AFMK) was one of the hits in PubChem and is a melatonin metabolite in *in vivo* studies. This was later confirmed by spiking an AFMK standard into the sample and matching exact retention time and mass spectra of peak D (Figure 2b and 2d).

Previous studies by Williamson, *et al.* using a tandem quadrupole mass spectrometer<sup>1</sup> speculated the structure of peak D to be a substituted indoline. The elemental composition ( $C_{14}H_{20}N_2O_3$ ) of the indoline structure proposed by Williamson, *et al.*, would have an accurate mass of 265.15467. The higher accuracy provided by the AxION 2 TOF MS indicates that the structure suggested by Williamson is ~140 ppm higher than its actual accurate mass (obtained for peak D by the AxION 2 TOF), and thus suggesting the published structure to be incorrect.

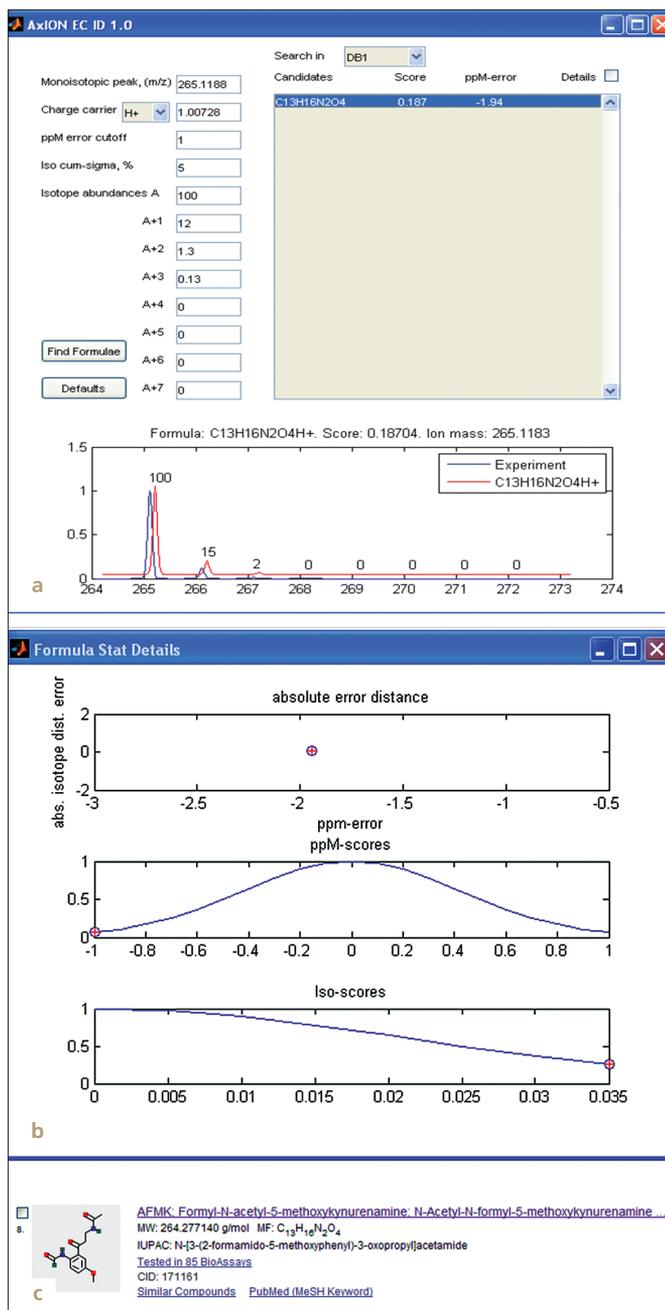


Figure 3. (a) AxION EC ID with user defined mass accuracy and isotope pattern confidence. (b) Additional fit statistics of the experimental data versus the chosen formula match. (c) Automatic PubChem match of the chosen formula from AxION EC ID.

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## Conclusion

Improved and simplified analysis of impurities is achieved using the AxION platform consisting of the PerkinElmer Flexar FX-10 HPLC coupled to the AxION 2 TOF MS and innovative post processing software. The analysis is fast, easy, and accurate. The platform's integrated software which combines all instrument functionalities in a single interface ensures reliable startup and robust sample analysis. Further, the platform enables rapid and easy interpretation of results for confident identification of unknown impurities.

## References

1. B.L. Williamson, A.J. Tomlinson, P.K. Mishra, G.J. Gleich, S. Naylor, Chem. Res. Toxicol. 11 (1998) 234.