



Detection of UV-transparent Compounds by Addition of a Mass Detector to an Existing High Performance Liquid Chromatography System with Photodiode Array Detection

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This is an Application Brief and does not contain a detailed Experimental section.

Abstract

This application brief demonstrates that mass detection, in combination with UV detection, ensures more complete sample characterization.

Benefits

The addition of mass detection to an existing LC system with PDA detection enables detection of UV-transparent compounds.

Introduction

Liquid chromatography is widely used to separate a broad range of analytes. While UV detection is used for qualitative analysis and quantitative analytical measurements, the compound of interest must have a UV chromophore. For compounds that have a weak or non-existent UV chromophore, pre-column or post-column derivatization of the sample, among many other alternative approaches, may be employed. However, pre- or post-column sample derivatization may not always be an attractive option. Some pre-column derivatization reactions can be slow and time-consuming, while post-column derivatization often requires additional tubing between the column and detector to provide reagent mixing and sufficient reaction time. This extra tubing increases extra-column band broadening, which deteriorates the quality of the separation. Adding a mass detector to an HPLC-UV system, as a complement to UV detection, can prove to be a more effective approach than derivatization in the analysis of non-chromophoric samples by HPLC, since mass detection does not rely on the compound having a UV chromophore. Mass detection, however, cannot be used for compounds that either poorly ionize or do not ionize under the selected conditions.

Therefore, using the two detectors in one system will help increase the opportunity of detecting compounds that do not share common functional groups for one mode of detection. However, the typically differing sensitivities of UV and MS detectors to the same compound may mean that the detectors are not operating in the linear dynamic ranges at a given sample concentration.

Results and Discussion

The addition of the ACQUITY QDa Detector to an existing HPLC separation monitored by UV provides an orthogonal mass detection technique to detect compounds that cannot solely be analyzed using the diode-array detector. The ACQUITY QDa Detector, with its small footprint, ease of use, and affordability, offers a convenient way to bring mass spectral information to chromatographic separations. The restrictor module in the ACQUITY Isocratic Solvent Manager (ISM) divides the flow from the column between the Waters 2998 Photodiode Array (PDA) Detector and the ACQUITY QDa Detector, aligning the dynamic range of both detectors in the same analysis (Figure 1).

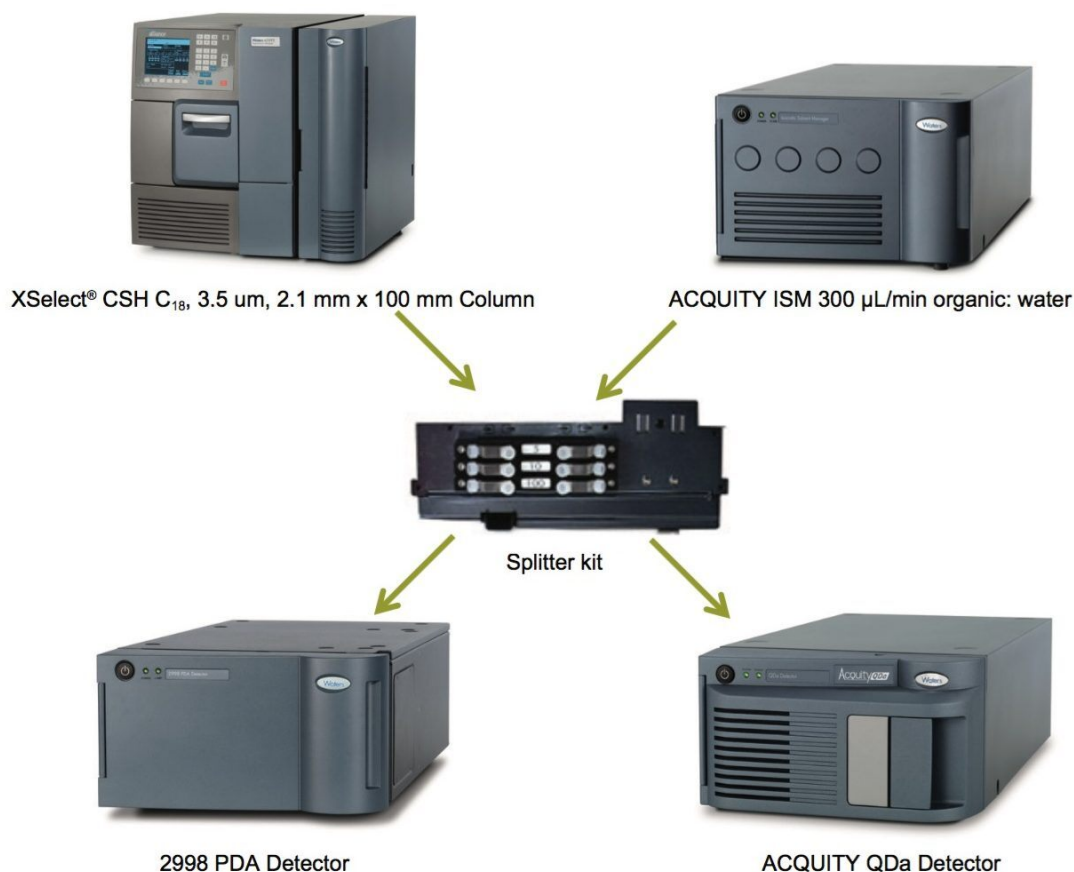


Figure 1. System configuration with Split-and-Dilute.

Memantine is an orally active noncompetitive NMDA receptor antagonist used to treat Parkinson's Disease and movement disorders. While memantine and its impurities lack good UV chromophores, the ACQUITY QDa Detector achieves sensitive detection. Waters Empower CDS enables acquisition and analysis of data

acquired from the PDA and mass detectors in a single user interface (Figure 2).

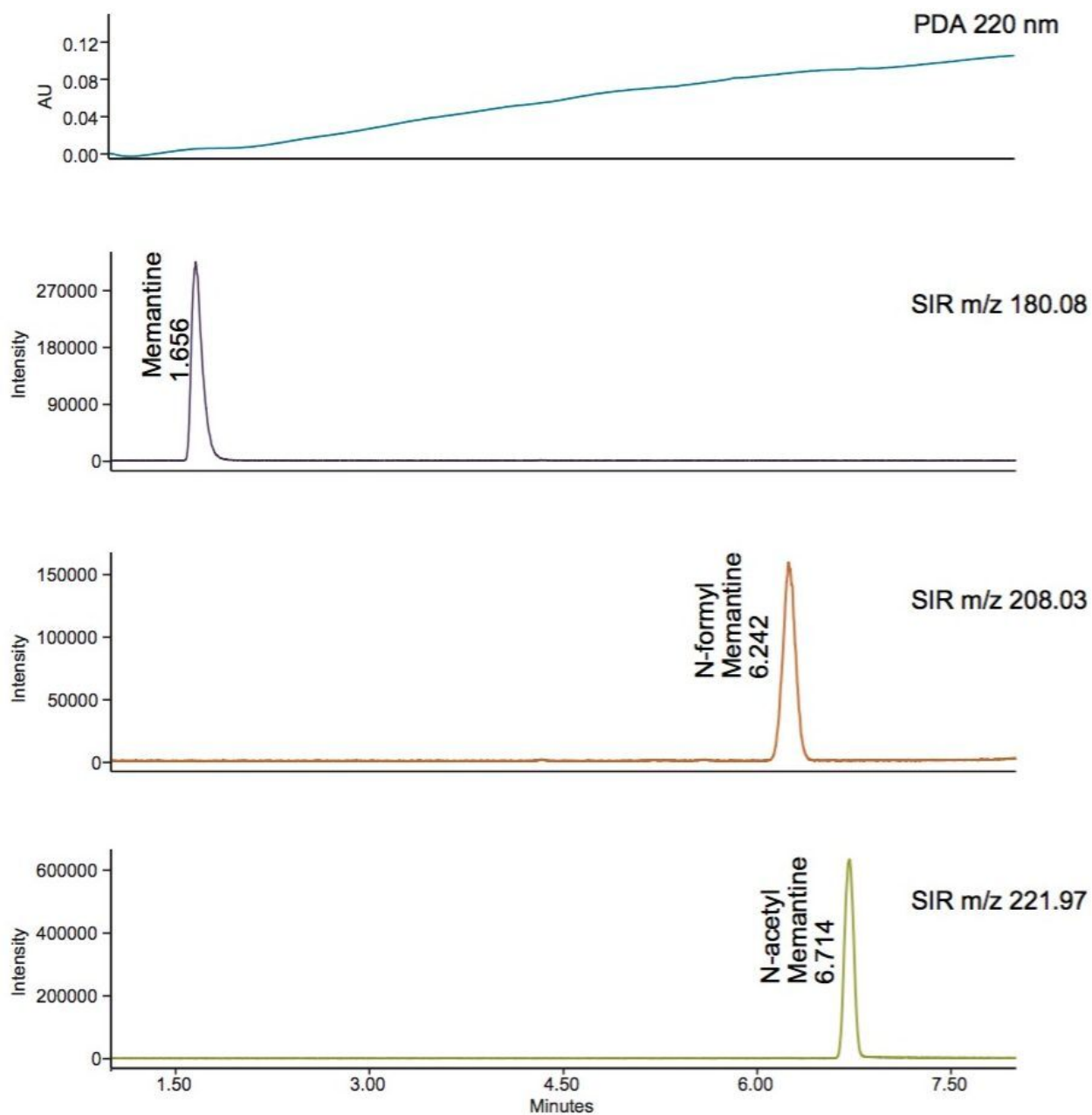


Figure 2. Memantine and impurities that lack UV chromophores show no UV trace at 220 nm and are detected with the addition of the ACQUITY QDa Detector.

Retention time and area reproducibility values for five injections prepared at 0.2 $\mu\text{g}/\text{mL}$ concentration for memantine API and its impurities are shown in Figure 3. Retention time and area %RSD values are within 0.25 and 3.00, respectively.

%RSD (n = 5)	Retention time	Area
Memantine API	0.20	2.47
N-formyl memantine	0.12	2.50
N-acetyl memantine	0.10	2.86

Figure 3. Retention time and area %RSD for memantine and its impurities (using the ACQUITY QDa Detector). Area %RSD < 3.0 for memantine API and impurities.

Conclusion

Mass detection allows the detection of compounds that have weak or no chromophores, such as memantine and its impurities. The combination of the ACQUITY QDa Detector and a UV detector can detect components that do not share functional groups for a single mode of detection. In this example, the retention time and area %RSD for memantine and impurities were within 0.25 and 3.00, respectively, when measured with the ACQUITY QDa Detector. The addition of the ACQUITY QDa Detector to the Alliance HPLC System with the 2998 PDA Detector and Empower CDS (software capable of combining data from orthogonal detectors) increases confidence in the analysis of non-chromophoric samples.

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