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Note d'application

Synthetic Reaction Monitoring Using the Waters ACQUITY UPLC H-Class PLUS Binary System Coupled to Photodiode Array and ACQUITY QDa Mass Detectors

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Abstract

Reaction monitoring is a critical step in the synthesis of new drug candidates. Multiple analyses can be required to assess the progress of a chemical reaction under a variety of different conditions, e.g., different solvents or catalysts. A rapid turnaround in sample analysis for reaction monitoring allows for rapid decision making and increased laboratory efficiency. Within this body of work we have demonstrated that the ACQUITY UPLC H-Class PLUS Binary System coupled to an ACQUITY PDA and ACQUITY QDa Mass Detector is capable of providing separation of atenolol, its intermediate 4-hydroxyphenylacetamide (4-HPA), and the reaction side product 4-hydroxyphenylacetic acid. Achieving these critical separations in a total runtime of 1.2 min while providing information rich mass spectral and UV data allows for easy and confident reaction monitoring, accessible to medicinal chemists with a diverse range of analytical experience.

Benefits

- Rapid sub-1-minute gradient for high sample throughput and rapid results
- PDA detection for richer sample information
- Mass detection for mass confirmation and rapid decision making

Introduction

During SAR (Structure Activity Relationship) studies in the discovery chemistry workflow of pharmaceutical drug design, medicinal chemists optimize the properties of target compounds by introducing different functional groups to known bio-active molecules. The purpose of these experiments is to obtain the most suitable drug candidate with optimal biological activities.

Once a chemical hit is found and verified, optimization of the compound's desired properties takes place. This step involves an iterative process of synthesis and reactivity measurement of the new compounds to further develop drug candidates into the lead phase.[1]

Because these reactions may take a long time, chemists need to know as soon as possible if their syntheses are proceeding as desired. This means utilizing measurement capabilities that require minimal sample preparation and provide a fast response given low detection limits. Another advantageous property of the chosen analytical technique might be the ability to measure multiple parameters (e.g., impurity formation and

optimum final product yield) simultaneously.[2]

The purpose of this application note is to demonstrate the utility of the ACQUITY UPLC H-Class PLUS Binary System's ability to perform short, ballistic gradients for rapid, high throughput chromatographic analysis. Coupled to the orthogonal detection techniques of photodiode array and mass detection, the ACQUITY UPLC H-Class PLUS Binary System provides the synthetic chemist with insight into progress of their reaction, potential side products, and any other issues that might occur in a simple and accessible solution for reaction monitoring workflows.

Experimental

Chromatographic separation was carried out on the ACQUITY UPLC H-Class PLUS Binary System. Samples were eluted in a 0.7-minute gradient (total runtime 1.2 minutes) with data collected using a PDA Detector and the ACQUITY QDa Mass Detector.

Sample Description

Preparations of atenolol (final product), 4-hydroxyphenylacetamide (4-HPA) (reaction intermediate), and 4-hydroxyphenylacetic acid (4-HPAA) (reaction side product) were prepared to simulate the progress of atenolol synthesis. All standards were sourced from Sigma Aldrich chemicals (Poole, Dorset, UK).

Method Conditions

LC Conditions

LC system:	ACQUITY UPLC H-Class PLUS Binary System
Detection:	ACQUITY Photodiode Array (PDA)
Vials:	Waters Total Recovery Vials
Column(s):	ACQUITY BEH C_{18} 30 mm x 2.1 mm, 1.7 µm
Column temp.:	45 °C

LC Conditions

Sample temp.:	10 °C
Injection volume:	0.5 µL
Flow rate:	0.8 mL/min
Mobile phase A:	0.1% v/v formic acid in water
Mobile phase B:	0.1% v/v formic acid in acetonitrile
Gradient:	5 to 95% B/0.7 min

MS Conditions

MS system:	ACQUITY QDa Mass Detector
Ionization mode:	ESI+
Acquisition range:	100–800 Da
Capillary voltage:	1.5 kV
Collision energy:	n/a
Cone voltage:	20 V

Data Management

MS software:	MassLynx v4.1

Results and Discussion

During the target compound optimization stage of the discovery workflow, rapid screening is essential for medicinal chemists to accelerate decision making and quickly identify compounds with optimal affinity and selectivity. In all cases chemists need to be able to rapidly assess if the correct product has been synthesized and if a reaction has reached its endpoint in order to quench the reaction for maximum yield.

This stage can be time consuming and labor intensive, therefore it is preferable to have a rapid and reliable means of monitoring experimental progress. In order to demonstrate this workflow, the synthesis of atenolol (Figure 1), was used as a reaction model.

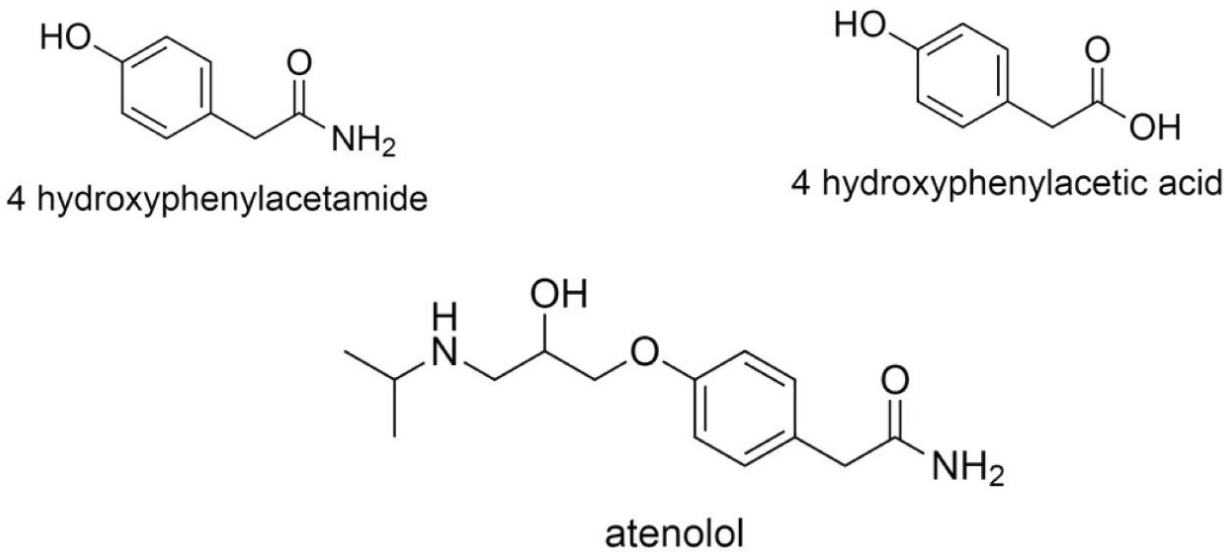

Figure 1. Structures of 4-HPA, atenolol, and 4-HPAA.

The increase in the formation of atenolol was monitored as well as the decrease in the intermediate 4 - hydroxyphenylacetamide (Figure 2). The reaction by-product 4-hydroxyphenylacetic acid was also observed.

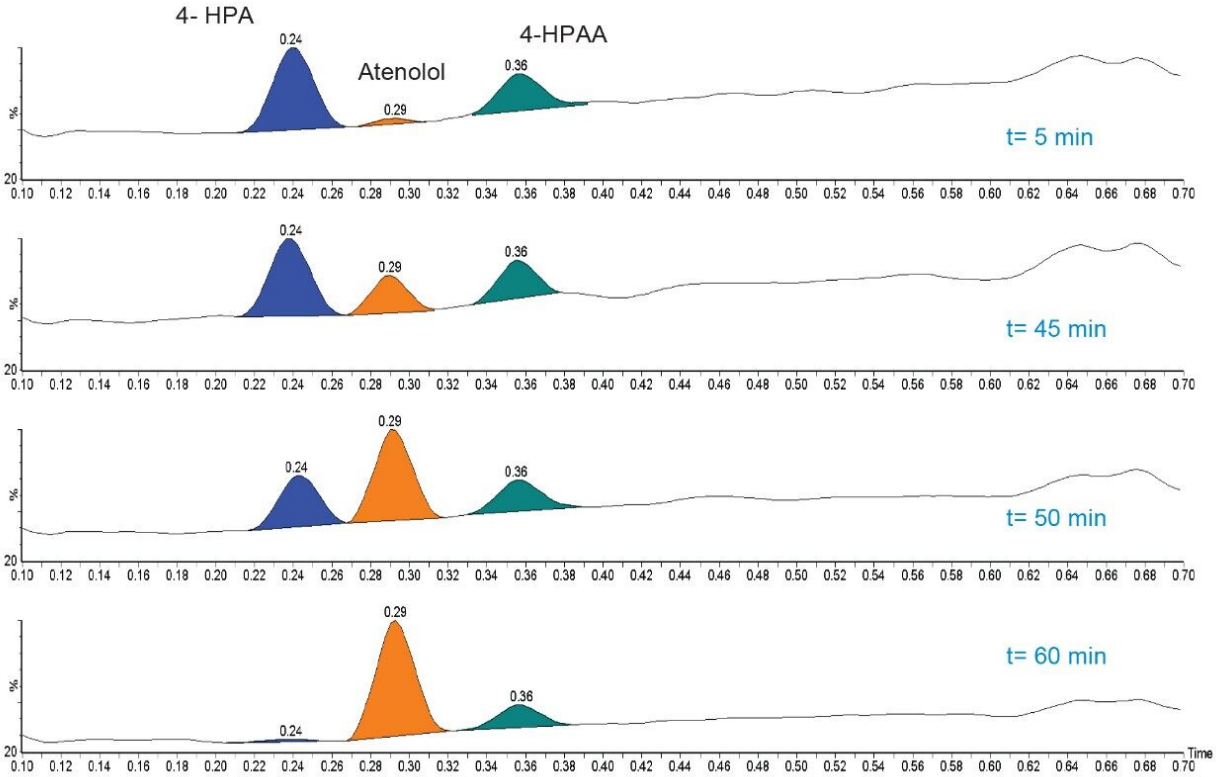

Figure 2. TIC chromatograms of the reaction mixture showing increasing levels of atenolol (orange) and decreasing levels of 4-HPA (blue) over time.

The combination of PDA and ACQUITY QDa detectors provides a simple orthogonal detection system to monitor compounds with different physio-chemical attributes. The 4-HPAA side product gives a strong response in UV with atenolol and 4-HPA providing relatively weak UV responses. With the ACQUITY QDa detection (ESI+ mode) the responses of atenolol/4-HPA are significantly better (Figure 3).

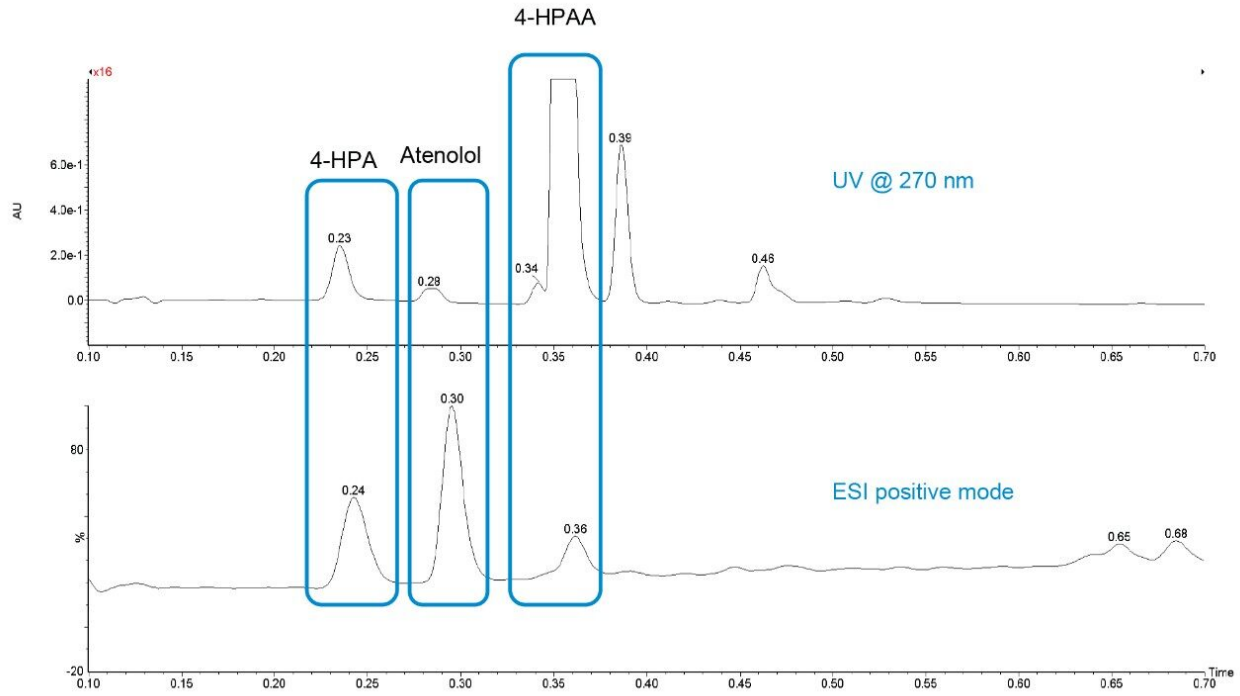

Figure 3. Comparison of PDA and ACQUITY QDa responses for all three compounds.

The integrated ACQUITY QDa Mass Detector gives easy access to mass confirmation, which is a significant benefit giving chemists increased confidence of reaction success. Analysis of the mass spectrum of the data generated shows *m/z* values corresponding to the expected compounds (Figure 4).

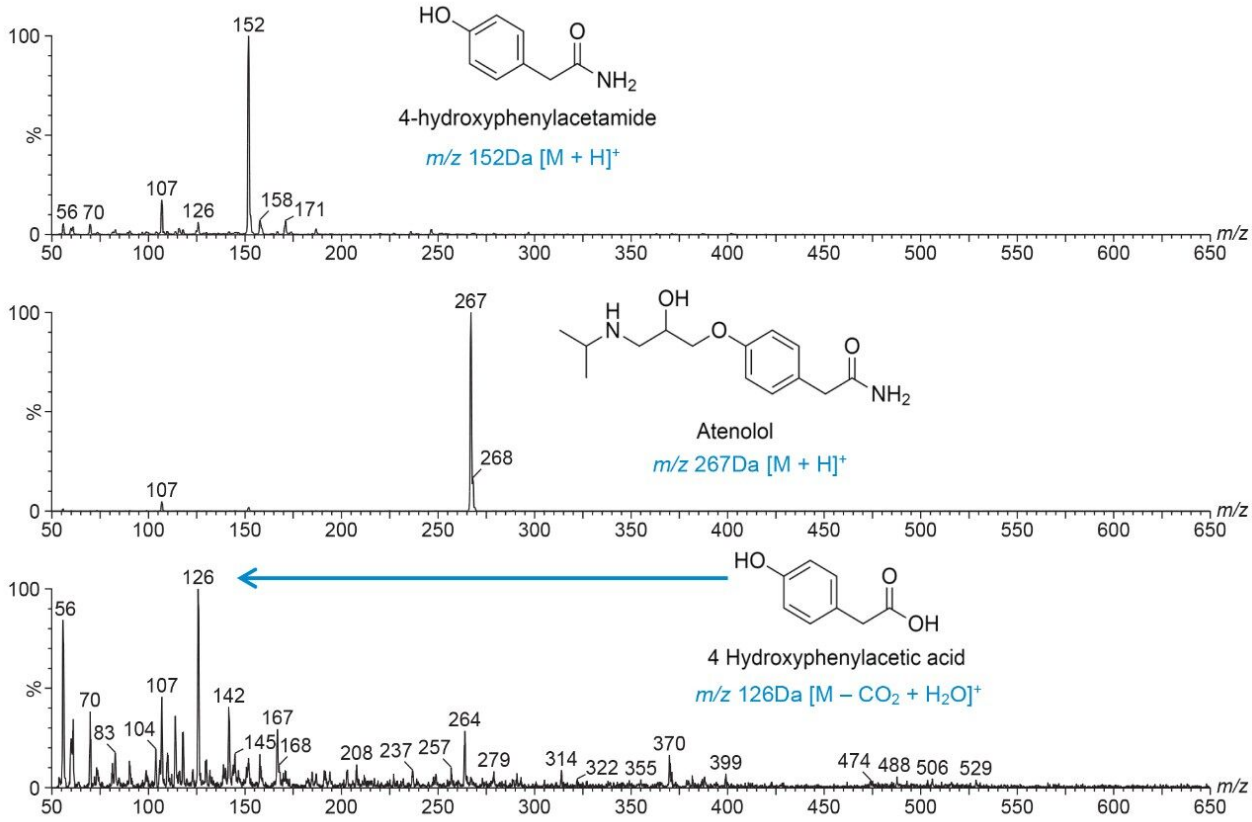

Figure 4. Mass spectra of the three compounds using ACQUITY QDa Mass Detector.

Conclusion

By using the ACQUITY UPLC H-Class PLUS Binary System with PDA/ACQUITY QDa detection it was possible for this workflow to achieve UPLC cycle time of 1.2 minutes separating desired reaction product, reaction intermediate and side product compounds.

Mass detection enabled easy mass confirmation of all compounds with the PDA giving additional UV spectral information within the same injection.

The use of this platform gives non-expert mass spectrometry users access to vital mass spectral information during the reaction monitoring progress, thus accelerating the drug discovery process.

References

1. Twohig, M.; Shave, D.; LeFebre, P.; Plumb, R. Synthetic Reaction Monitoring Using UPLC-MS. Waters Application Note, 2007, 720002258EN. <https://www.waters.com/nextgen/us/en/library/application-notes/2007/synthetic-reaction-monitoring-using-uplc-ms.html>
2. Analytical and Purification methods in Combinatorial Chemistry, *Wiley-Interscience* (5): 87–123.
3. Dahlin, J.; Walters, M. The Essential Roles of Chemistry in High Throughput Screening Triage. *Future Med Chem*., 2014, July; 6(11): 1265–1290.

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