Orthogonal Detection Techniques to Provide More Complete Characterization of an Oral Drug

Aparna Chavali, Thomas E. Wheat, Patricia McConville
34 Maple Street, Milford, MA 01757

INTRODUCTION

In any analytical method, compounds need to be separated and identified. Using a single detection technique in liquid chromatography may result in some components going undetected. HPLC with its choice of detection techniques can separate the analytes but provides little information about what a compound might be. The photodiode array can provide some information about peak purity or presence of co-eluting peaks but can only provide identification by comparing to standards in the same mobile phase. Adding a mass detector to a HPLC-PDA system can provide information for peak identification, for recognizing co-eluting chromatographic peaks and for confirming peak purity. A mass detector, however, cannot provide complete sample characterization by itself. It may not prove useful in distinguishing isomers or compounds that either poorly ionize or do not ionize under the selected conditions.

In this example, orthogonal detection techniques, in combination with liquid chromatography, are employed to help fully characterize Angiotensin II receptor blockers (ARBs). ARBs are a class of drugs used in the treatment of hypertension, diabetic neuropathy and congestive heart failure. The combination of the chromatographic configurations and software tools to combine data from these orthogonal detectors provides information to enable a more thorough characterization of the oral drug.

METHODS

LC System: ACQUITY UPLC® H-Class with PDA and ACQUITY UPLC® QDa Detector
Column: Acquity UPLC® HSS T3 1.8µm 2.1 x 100mm
Temperature: 50°C (Column)
Mobile Phase: A: 125mM Formic Acid in Water
B: 125mM Ammonium Hydroxide in Water
C: 100% Methanol
D: 100% Water
Separation Mode: Isocratic with 60% Solvent C, pH 2.99 prepared with Auto•Blend Plus™
Flow Rate: 0.55 mL/min
Injection Volume: 0.7 µL
Detection
PDA: 200-400nm, derived at 254 nm
QDa: ESI+ Full Scan Mass Range 100—700 m/z
SIR (m/z values specified in Table 1)

Sample: Angiotensin II receptor blockers mix (ARBs)
Sample Prep.: (Separate stock solutions of each of the seven components were prepared at 1.0 mg/mL in methanol. Working sample was prepared by mixing 0.1 mg/mL of Irbesartan Standard, 0.1 mg/mL of Telmisartan Standard, 0.5 mg/mL of Valsartan Standard, 0.1 mg/mL of Irbesartan Related compound A, 0.013 mg/mL of Telmisartan Related Compound A, 0.05 mg/mL of Telmisartan Related Compound B and 0.05 mg/mL of Telmisartan Impurity E. Water was used as the diluent).

RESULTS

Figure 1 shows the separation of a seven component mix of ARBs on the ACQUITY UPLC H-Class System monitored with both a photodiode array detector and mass detector.

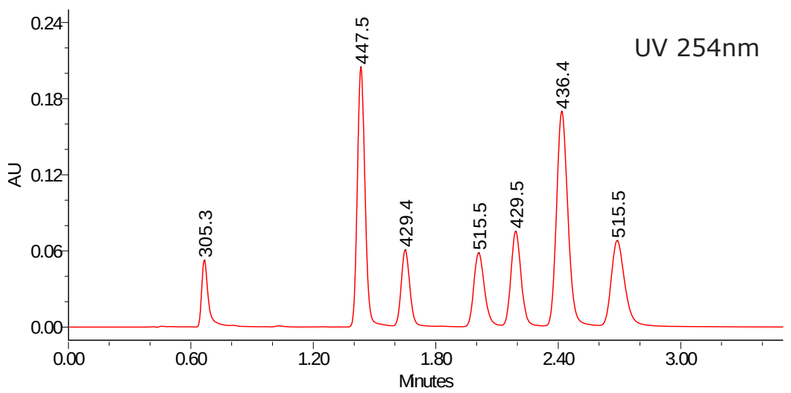

Figure 1. Separation of Angiotensin II receptor blockers with the Acquity UPLC H-Class system with ACQUITY UPLC PDA and ACQUITY UPLC QDa detectors. The separation is monitored in the UV at 254nm, and each peak is labelled automatically with the mass of the compound.

Identical UV spectra, different mass:

The UV spectra of the Irbesartan related compound A and the Valsartan standard are identical as seen in Figure 2a. One approach for peak identification that has been used traditionally is to inject individual standards in the same mobile phase. With the addition of a mass detector, the compounds can be identified based on their mass in the same analysis, an approach particularly useful for example during methods development, Figure 2.

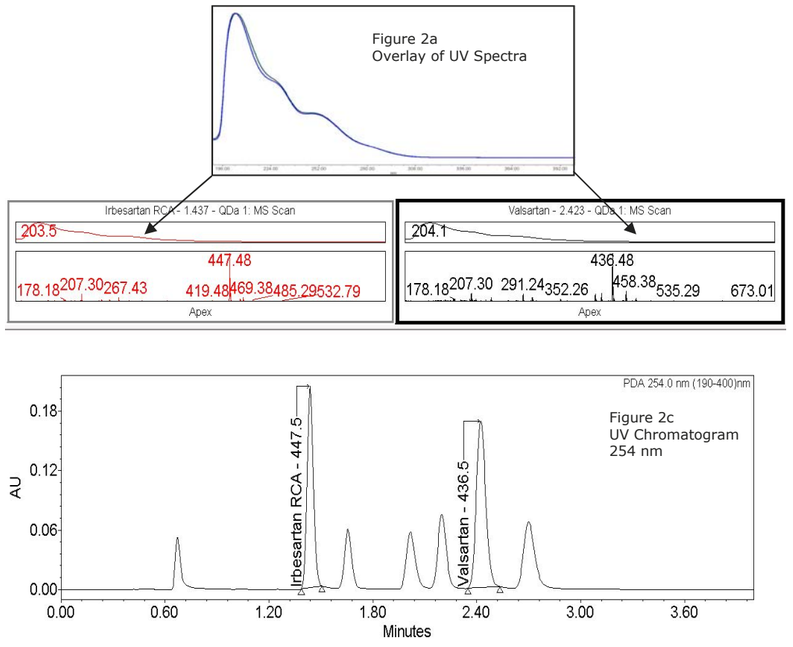

Figure 2. Mass analysis view for Irbesartan related compound A and Valsartan standard.
Figure 2a. Overlay of UV spectra for Irbesartan related compound A and Valsartan standard.
Figure 2b. Mass spectra for Irbesartan related compound A (red trace) and Valsartan standard (black trace).
Figure 2c. UV chromatogram monitored at 254nm.

Peak Number	Compound Name	Retention Time	m/z
1	Telmisartan RCA	0.672	305.3
2	Irbesartan RCA	1.437	447.5
3	Telmisartan Imp E	1.656	429.5
4	Telmisartan RCB	2.017	515.5
5	Irbesartan Standard	2.198	429.5
6	Valsartan Standard	2.423	436.5
7	Telmisartan Standard	2.699	515.5

Table 1. Retention time and m/z values for the seven components in the Angiotensin II receptor blockers mix.

Same mass, different UV spectra:

Telmisartan related compound E and Irbesartan standard are isobaric compounds. They both have a molecular mass of 428.5. The UV spectra for the two compounds can be used for distinguishing these two isobaric compounds. The mass analysis view in Empower 3 displays the UV and mass spectral information along with the chromatographic separation in a single view for analysis, Figure 3.

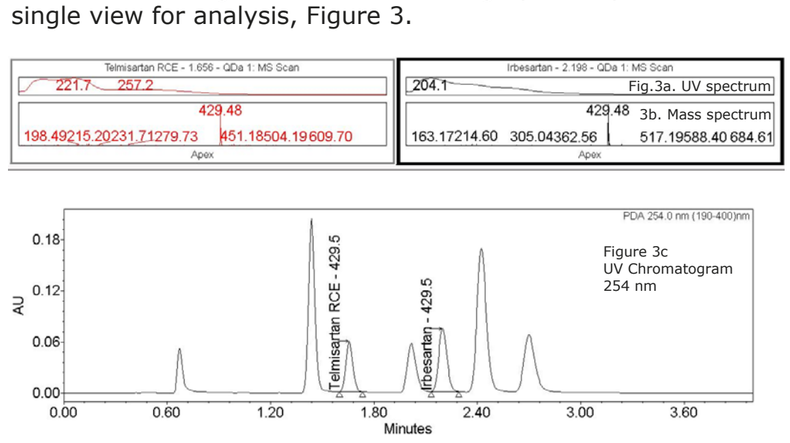

Figure 3. Mass Analysis View for Telmisartan related compound E and Irbesartan standard.
Figure 3a. UV spectra for Telmisartan related compound E (red trace) and Irbesartan standard (black trace).
Figure 3b. Mass spectra for Telmisartan related compound E (red) and Irbesartan standard (black trace).
Figure 3c. UV chromatogram monitored at 254nm.

CONCLUSION

- **Orthogonal detectors are used to confirm the identity of components in a single analysis.**
- **The ACQUITY UPLC PDA detector provides UV spectral information to distinguish isobaric compounds.**
- **The ACQUITY UPLC QDa detector provides mass spectra to distinguish peaks with identical UV spectra.**
- **Mass analysis view in Empower™ is a single user interface combining analysis of data from the orthogonal detectors.**

TO DOWNLOAD A COPY OF THIS POSTER, VISIT WWW.WATERS.COM/POSTERS