SIMPLIFYING ACCURATE MASS CONFIRMATION IN A WALK-UP ENVIRONMENT

Jayne Kirk[1] (jayne_kirk@waters,com), Caitlyn Da Costa[1], Christopher Henry[1], Russell Mortishire-Smith[1], David Deans[1], Scott Campbell[2]
[1]Waters, Wilmslow, UK, [2]SpectralWorks LTD, Cheshire, UK

INTRODUCTION

In academia and industry, chemists and lab managers require accurate mass confirmation capabilities, whether they work, for example, in a medicinal chemistry team in the pharmaceutical industry performing reaction monitoring or synthesis confirmation, or in academia within the core analytical lab providing synthesis support.

The combination of the ACQUITY RDa Detector, a system suited to scientists with a diverse range of expertise, with the easy and intuitive RemoteAnalyzer software, provides a solution for small molecule accurate mass confirmation.

Here we demonstrate, through a simulated reaction monitoring experiment, the formation of the beta-blocker atenolol from its intermediate, 4-hydroxyphenylacetamide (4-HPA).

Data were acquired using the ACQUITY RDa Detector with sample submission and reporting through SpectralWorks' RemoteAnalyzer software.

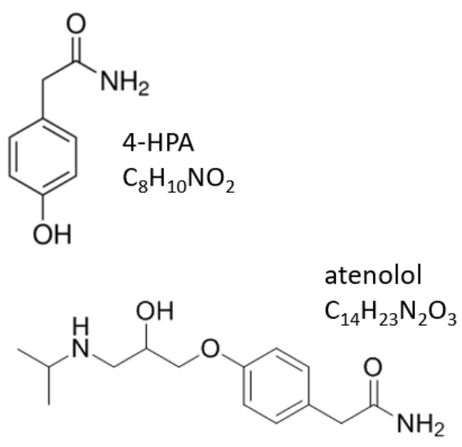


Figure 1. The intermediate 4-HPA and atenolol.

METHODS

Mixtures of atenolol and its intermediate, 4-hydroxyphenylacetamide (4-HPA) were prepared at a range of relative concentrations, to simulate 4 time points in the reaction, and the conversion of the intermediate to the final product (Figure 1).

Samples were then acquired on the ACQUITY RDa Detector (Waters Corporation) with RemoteAnalyzer software (SpectralWorks Limited).

A generic (pre defined) chromatographic gradient and MS method were selected with the RemoteAnalyzer software, with details highlighted in Tables 1 and 2.

Table 1: LC method

LC System:	ACQUITY UPLC I-Class PLUS
Detection:	ACQUITY TUV
Vials:	TruView Max Recovery Vials, PN186005668CV
Column(s):	ACQUITY BEH C18 2.1 x 100mm, 1.7µm
Column Temp.:	45°C
Sample Temp.:	8
Injection Volume:	1µl
Flow Rate:	0.4mL/min
Mobile Phase A:	Water + 0.1% Formic Acid
Mobile Phase B:	Acetonitrile + 0.1% Formic Acid
Gradient:	5% B to 100% B, (3 minutes)

MS System:	ACQUITY RDa Detector
Ionization Mode:	ESI +
Acquisition Range:	100-2000 Da
Capillary Voltage:	1.5kV (default)
Cone Voltage:	30V
Fragmentation Cone Voltage Ramp	60 – 150V
Scan Rate	10Hz
Desolvation Gas Temp	550°C

Table 2: MS method

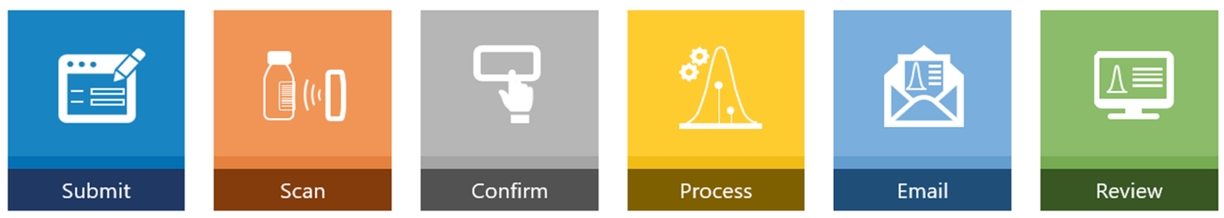
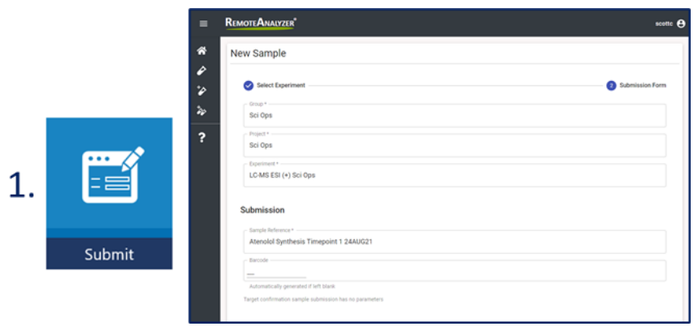


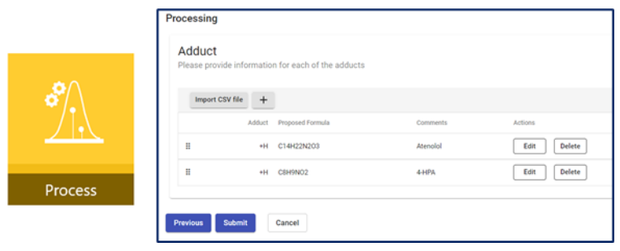
Figure 2. Workflow for submitting samples to RemoteAnalyzer for mass confirmation.



Step 1. The time points were submitted to RemoteAnalyzer via a web-based browser . Shown here is the submission of time point 1 which was then added to the queue (and if necessary priority status can be assigned).

Step 2. When submitting a sample, an email is sent containing the barcode. This was then scanned at the instrument.

Step 3. The sample was placed into the autosampler and confimed via instructions at the tablet.



Step 4. The data were acquired on the ACQUITY RDa Detector and processed based on the proposed formula entered into RemoteAnalyzer. Here the formula for atenolol was entered.

RESULTS AND DISCUSSION

The samples were submitted through the RemoteAnalyzer software via six key steps highlighted above.

The samples were submitted to RemoteAnalyzer using a web browser. Using the barcode scanner situated at the instrument samples were scanned and placed into position and the results were automatically emailed in PDF format.

Results showed a reduction of the concentration of the intermediate 4-HPA consistent with the increase in the final product atenolol over the various timepoints analyzed. The results were viewed in the fit-for purpose report where high mass accuracy confirmations of the compounds of interest were displayed.

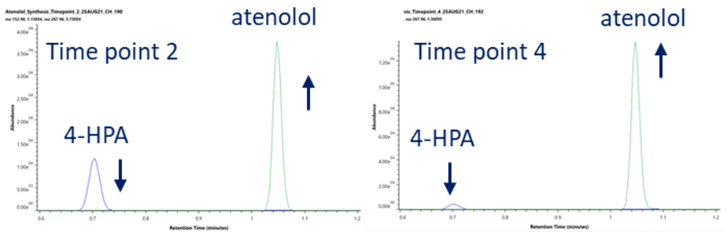
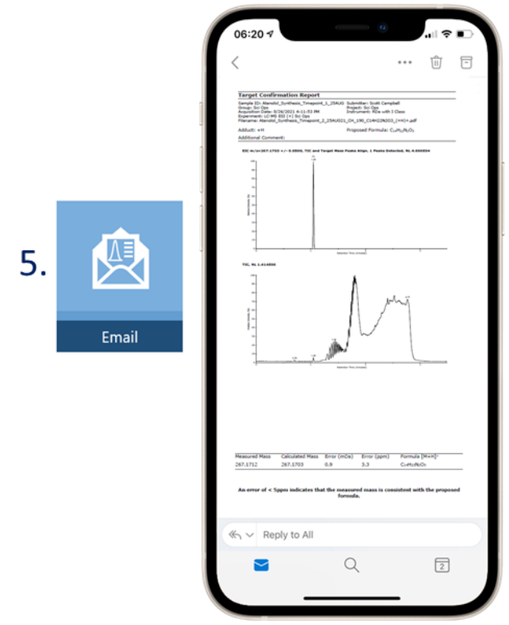
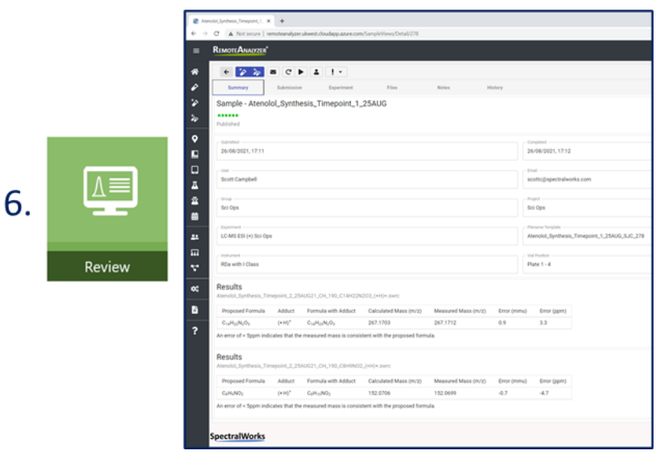


Figure 3. Extracted ion chromatograms of 4-HPA and atenolol at time points 2 and 4.



Step 5. Once completed the results were automatically emailed (PDF), which can then be used to make informed decisions in terms of next steps. Here the mass measurement of atenolol was confirmed in time point 1.



Step 6. Results were also viewed within the summary tab in RemoteAnalyzer and highlighted here the mass confirmation of atenolol and 4-HPA in time point 2.

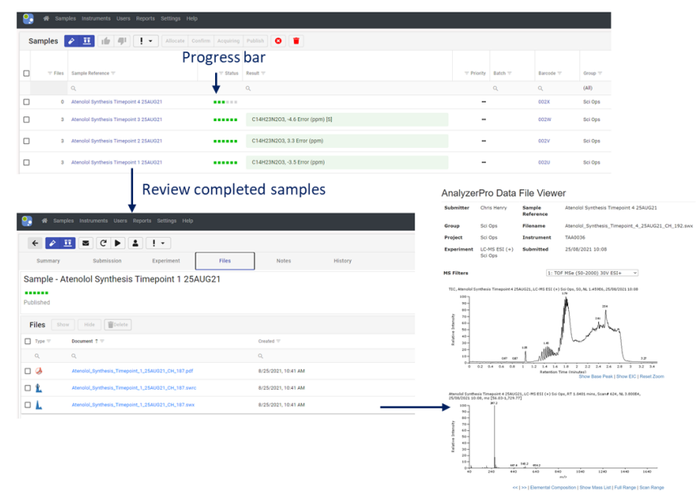


Figure 4. After samples have been acquired it is possible, if required, to interact with the data using the data file viewer from your web browser. Alternatively, data files can be downloaded and viewed or reprocessed using AnalyzerPro XD. Data shown here highlights the four time course injections, the results and data review of time point 4.

CONCLUSION

- **The RemoteAnalyzer software reported the mass measurement errors (< 5 ppm) for 4-HPA and atenolol over the time course highlighting its applicability for reaction monitoring experiments.**
- **Combining the ACQUITY RDa Detector with the RemoteAnalyzer software enables access to accurate mass measurements for reaction monitoring studies.**
- **The straightforward operation with easy-to-use hardware and web-based software provides scientists with accurate mass measurements, enabling critical decisions to be made under time constraints, and with confidence.**

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