UTILIZING A COMPACT BENCHTOP TIME OF FLIGHT MASS SPECTROMETER FOR RAPID ACCURATE MASS INFORMATION IN A WALK-UP ENVIRONMENT.

Authors : Ashley Sage[1] , Christopher Henry [1] , Scott Campbell[2]
Affiliations : Waters Corporation, Stamford Avenue, Altrincham Road, Wilmslow, Cheshire, SK9 4AX, UK[1], SpectralWorks Ltd. Runcorn, Cheshire, WA7 4EB, UK[2]

CONNECTING SCIENCE To Solve Problems That Matter

Waters™

SpectralWorks

INTRODUCTION

Many organic chemists employ mass spectrometry (MS) as a convenient verification tool for their product, and for any impurities present in a synthetic reaction.

Single quadrupole mass spectrometers provide only nominal mass data which while useful, does not eliminate the potential of mis-assignment of the target compound, degradants, impurities, or side reaction products. Increased confidence through compound characterization is possible through the accurate mass measurements provided by Time of Flight (ToF) high resolution mass spectrometry (HRMS) which can require high levels of expertise to operate.

Within this body of work, we will demonstrate a routine high resolution mass spectrometer combined with a browser based 'walk up' software for simple access to accurate mass measurements for synthetic chemists.

METHODS

SAMPLE PREPARATION

To demonstrate this workflow a standard of ramipril, an angiotensin-converting enzyme (ACE) inhibitor was spiked at 0.1% (w/w) with four related impurities; ramipril isopropyl ester, ramipril diketopiperazine, ramipril methyl ester and hexahydroramipril labelled A-D respectively, and analyzed using the ACQUITY™ RDa™.

Method details and instrument parameters are detailed in *Table 1*.

The chemical structures of ramipril and its potential related impurities are shown in *Figure 3*.

Sample submission and processing was controlled by RemoteAnalyzer® software (SpectralWorks Ltd.), while data acquisition was carried out by UNIFI™ software with the acquired raw data remaining intact and independent of the RemoteAnalyzer processing.

METHOD CONDITIONS

LC System:	ACQUITY UPLC I-Class PLUS
Vials:	TruView™ Max Recovery Vials, PN186005668CV
Column(s):	ACQUITY UPLC™ BEH™ C_{18} 50 x 2.1mm,1.7µm
Column Temp.:	80 °C
Sample Temp.:	10 °C
Injection Volume:	1 µL
Flow Rate:	0.5 mL/min
Mobile Phase A:	2mM ammonium formate + 0.1% formic acid
Mobile Phase B:	Acetonitrile + 0.1% formic acid
Gradient	10%-50% over 1.9 minutes (4 minute runtime)

MS System:	ACQUITY RDa Detector
Ionization Mode:	ESI Positive
Acquisition Range:	50-2000 *m/z*
Capillary Voltage:	1.5 kV (default)
Cone Voltage:	30 V

Table 1. LC-MS conditions.

METHODS CONTINUED

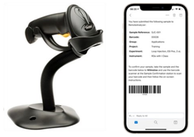
On submission of the sample, an email was sent to the submitter to confirm the analysis request was successfully received. On completion of the sample analysis, an email was received confirming successful status of the analysis as detailed in workflow steps 1-6 *Figure 2*.

Figure1. Workflow steps for RemoteAnalyzer

1. Submit

Step 1. The spiked sample was submitted to RemoteAnalyzer via a web based browser (and if necessary priority status can be assigned).

2. Scan

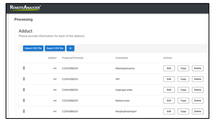


Step 2. When submitting a sample, an email is sent containing the barcode. This can then be scanned at the instrument.

3. Confirm

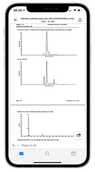
Step 3. The sample was placed into the autosampler and confirmed via instructions at the tablet.

4. Process



Step 4. The data were acquired on the ACQUITY RDa Detector and processed based on the proposed formulae entered into RemoteAnalyzer. Here the formulae for ramipril and impurities A-D were listed.

5. Email



Step 5. Once completed, the results were automatically emailed (pdf format). Here the TIC (Total Ion Chromatogram) for the analysis along with XIC (Extracted Ion Chromatogram) for the API (Active Pharmaceutical Ingredient) are displayed. A report for each impurity detected was also generated.

6. Review

Step 6. Results were also viewed within the summary tab in RemoteAnalyzer and highlights here the mass confirmation of ramipril diketopiperazine.

Figure 2. Steps 1-6 detailing the workflow steps involved when submitting and processing samples on RemoteAnalyzer.

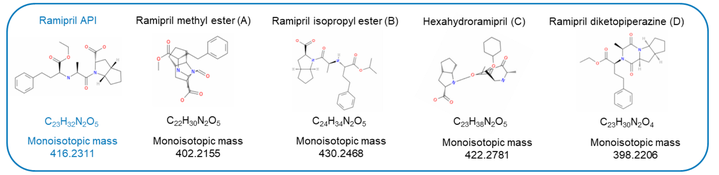


Figure 3. Ramipril API and four related impurities

RESULTS

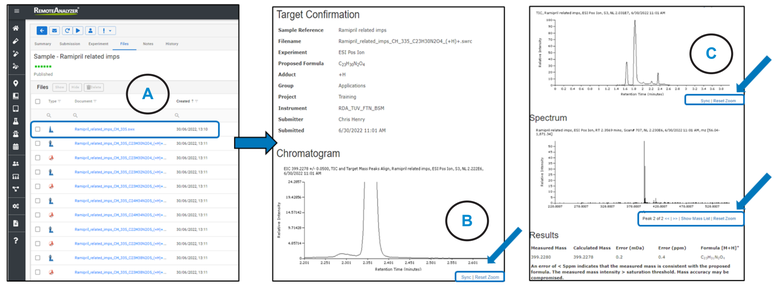


Figure 4. Results page with interactive chromatograms/spectra .

The emailed result contained a pdf. document for every proposed formula. In addition, a hyperlink was present in the email which when clicked takes the analyst to a sample review page within the RemoteAnalyzer software *Figure 4A*.This contained copies of the pdf's along with links to .swrc. files (RemoteAnalyzer propriety file format) for each component detected. When .swrc files are selected, an interactive results page opens with 'zoom enabled' TIC *Figure 4B*, along with EIC's and extracted spectrum for each analyte *Figure 4C*.

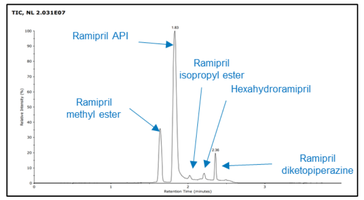


Figure 5. TIC from RemoteAnalyzer software .

Compound	Proposed Formula	Calculated Mass [M+H]+	Measured Mass [M+H]+	ppm error
Ramipril	$C_{23}H_{32}N_2O_5$	417.2384	417.2379	-1.2
Ramipril methyl ester (A)	$C_{22}H_{30}N_2O_5$	403.2228	403.2220	-1.9
Ramipril isopropyl ester (B)	$C_{24}H_{34}N_2O_5$	431.2541	431.2540	-0.1
Hexahydroramipril (C)	$C_{23}H_{38}N_2O_5$	423.2854	423.2862	2.0
Ramipril diketopiperazine (D)	$C_{23}H_{30}N_2O_4$	399.2278	399.2280	0.4

Table 2. Summarised results for ramipril and related impurities

Figure 5. shows the TIC from the RemoteAnalyzer software report demonstrating the successful separation of all five components within the four minute runtime. The results for the measured mass and ppm error for the five compounds have been summarized in *Table 2*. All mass errors were calculated as less than or equal to 2 ppm.

NOTE: 'ACQUITY', 'RDa', 'UPLC', 'BEH', 'UNIFI' and 'TruView' are all registered trademarks of Waters Corporation. 'RemoteAnalyzer' is a registered trademark of SpectralWorks Ltd.

DISCUSSION

The ACQUITY RDa Detector was set up automatically, including detector, auto-tune and mass calibration. Following this routine set up, MS full scan accurate mass data were acquired at a capillary voltage of 1.5 kV and a cone voltage of 30 V.

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.

The chromatography showed slight co-elution of the isopropyl ester impurity and the API however due to the increased specificity of full scan time of flight detection, this allows for the accurate quantitative integration and assignment of closely eluting peaks[2].

All compounds of interest exhibited excellent mass accuracy of ≤ 2 ppm.

Sample submission and processing was controlled by the RemoteAnalyzer software, while data acquisition was carried out by the UNIFI application with the acquired raw data remaining intact and independent of the RemoteAnalyzer processing.

CONCLUSION

- **Combining the ACQUITY RDa Detector with the RemoteAnalyzer software provides routine access to accurate mass measurements for synthetic chemists.**
- **Ramipril and the four spiked related substances were all detected with excellent mass accuracies of ≤2ppm using a four minute UPLC run time.**
- **More data points collected across a peak compared to single quadrupole detection the RDa detector enables the use of rapid UPLC gradients without compromising qualitative mass measurements for efficient, reliable sample turnaround time.**
- **With sample submission and processing controlled by the browser based RemoteAnalyzer software, chemists can retrieve and review data remotely from anywhere without needing to return to the instrument controlling PC.**
- **The straightforward operation with easy-to-use hardware and web-based software provided scientists access to accurate mass measurements, enabling critical decisions to be made under time constraints with confidence.**

References

1. A Review of Waters Hybrid Particle Technology. Part 2: Ethylene Bridged [BEH Technology™] Hybrids and Their Use in Liquid Chromatography. 720001159EN
2. Alelyunas YW, Wrona MD, Cook K, McDonald S, Rainville P: Effect of MS Scan Speed on UPLC Peak Separation and Metabolite Identification: Time-of-Flight HRMS v Orbitrap. March 2013 720004762EN

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

©2023 Waters Corporation