



## Mass spectrometry

# Using walk-up software for confirmation of target compound synthesis and purity analysis by liquid chromatography coupled with single quadrupole mass detection

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## Keywords

Chromeleon XPS, Chromatography Data System, CDS, walk-up, open access, ISQ EC, ISQ EM, single quadrupole mass detector, LC-MS, Vanquish Flex, organic synthesis

## Goal

Provide a customer application of the Thermo Scientific™ Chromeleon™ XPS Open Access software and discuss strengths and usability of a walk-up solution for synthetic chemists.

## Introduction

Recent years have seen major advances in chromatography instruments and mass detector hardware as well as the associated separation techniques. These developments opened the door to new types of analyses and made them available to novice LC-MS users. In particular, single quadrupole mass spectrometry simplifies mass detection and enables selective analyte detection. For novice LC-MS users, it is crucial that instrument software provides intuitive instrument control and data analysis. Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) streamlines user-instrument interactions and supports walk-up instrument use, empowering non-analytical chemists to apply mass detection to their own research. In this customer technical note, we present Chromeleon XPS Open Access software for walk-up LC-MS instrument use. Organic and medicinal chemists who perform synthesis are able to run LC-MS analyses without having to fully master a chromatography data system. They select the appropriate analysis workflow, place the samples at the recommended positions in the autosampler, and receive an automatically generated report.

The benefits of Chromeleon XPS Open Access walk-up solution include:

- Simple sample submission with minimal user input
- Predefined instrument methods
- Automated data processing and report generation
- Direct user sample submission

## Experimental

The presented technique is mass confirmation of a synthesized target compound with subsequent compound purity determination. The chromatographic separation was performed using a Thermo Scientific™ Vanquish™ Flex Binary UHPLC system coupled with a Thermo Scientific™ ISQ™ EC single quadrupole mass detector. All the analyses were performed using liquid chromatography-mass spectrometry (LC-MS) grade solvents (Table 1). Instrument control and data analysis was performed using Chromeleon 7.2.10 CDS together with Chromeleon XPS, which provides walk-up functionality.

### Vanquish Flex Binary UHPLC system modules

- Vanquish System Base Horizon/Flex (P/N VF-S01-A)
- Vanquish Binary Pump F (P/N VF-P10-A-01) with 35 µL mixer set (P/N 6044.3870)
- Vanquish Split Sampler FT (P/N VF-A10-A-02)
- Vanquish Column Compartment H (P/N VH-C10-A-03)
- Vanquish Diode Array Detector FG (P/N VF-D11-A-01) (2.5 µL SST flow cell P/N 6083.0530)

Table 1. Solvents and additives

Reagent	Grade	Supplier	Part number
Acetonitrile	Optima™ LC-MS	Fisher Chemical	A955-212
Formic acid	Optima™ LC-MS	Fisher Chemical	A117-50
Methanol	Optima™ LC-MS	Fisher Chemical	A456-212
Water	Optima™ LC-MS	Fisher Chemical	W6500

The samples were products synthesized by organic and medicinal chemists in the laboratory at the University of Texas at San Antonio. The crude samples were filtered and diluted in methanol to obtain an approximate concentration of 1 mg/mL for analysis. All liquid chromatography and mass detection methods were created using Chromeleon CDS and stored as an eWorkflow™ procedure. The method settings are shown in Table 2. There were several different methods available for users to select for their analyses depending on the sample type. Unless otherwise stated, all analyses used the gradient shown in Table 2.

Table 2. LC-MS conditions for the analysis

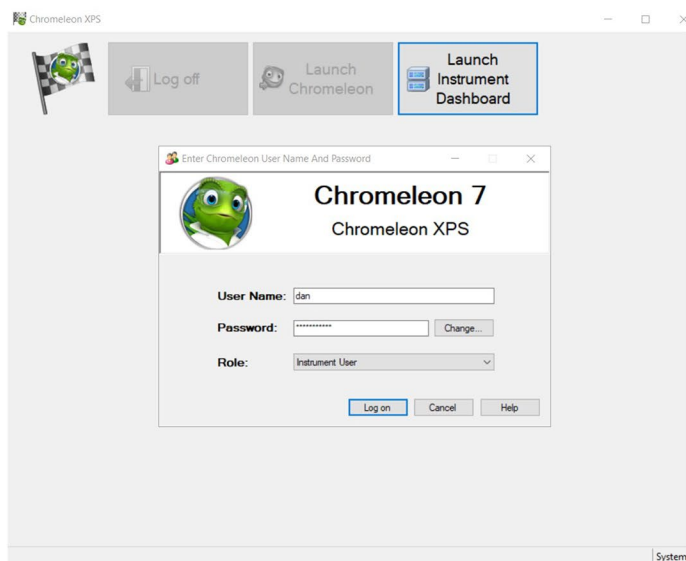
Parameter	Value										
Mobile phases	A: 0.1% formic acid in water B: 0.1% formic acid in acetonitrile										
Column	Thermo Scientific™ Accucore™ C18, 50 × 4.6 mm, 2.6 µm, P/N 17126-054630										
Gradient	<table border="1"><thead><tr><th>Time (min)</th><th>%B</th></tr></thead><tbody><tr><td>0.00</td><td>5.0</td></tr><tr><td>9.50</td><td>95.0</td></tr><tr><td>9.99</td><td>95.0</td></tr><tr><td>10.00</td><td>5.0</td></tr></tbody></table>	Time (min)	%B	0.00	5.0	9.50	95.0	9.99	95.0	10.00	5.0
Time (min)	%B										
0.00	5.0										
9.50	95.0										
9.99	95.0										
10.00	5.0										
Flow rate	1.2 mL/min										
Column temp.	25 °C										
Injection volume	1.00 µL										
UV detection	Wavelength channels 214, 254, 280, and 365 nm										
<b>Source settings</b>											
Vaporizer temp.	400 °C										
Ion transfer tube temp.	300 °C										
Sheath gas pressure	30.0 psig										
Aux gas pressure	2.0 psig										
Sweep gas pressure	1.0 psig										
<b>Full Scan (Basic Mode)</b>											
Mode	ESI										
Mass range	70–1250 <i>m/z</i>										
Polarity	Positive and Negative two separate full scans										
Source CID voltage	15 V										
Dwell time	0.12 s (Assumed peak width and scans per peak are 6.0 s and 20, respectively)										

## Results and discussion

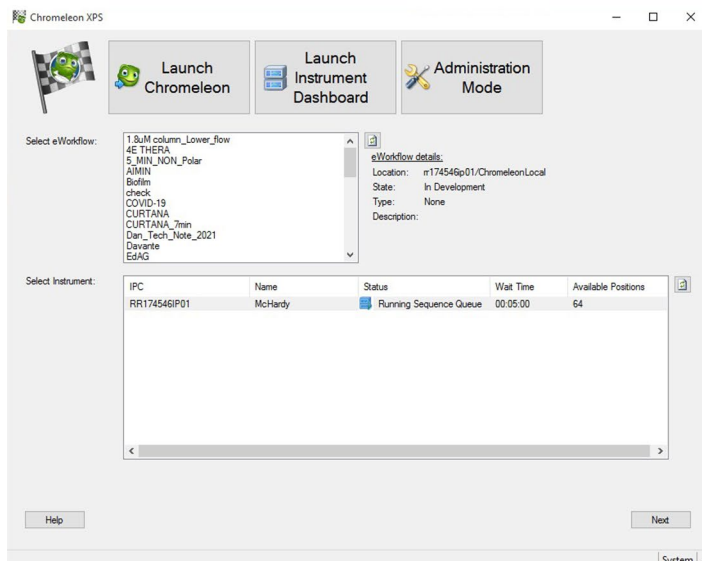
Synthetic organic and medicinal chemists are often interested in confirming whether they have obtained the correct product after every step of their synthesis. Occasionally, this requires time-consuming flash chromatography separation prior to confirmation. Traditionally this is done by performing thin layer chromatography (TLC) on the crude mixtures. After developing

the plate using an appropriate solvent system it is imaged under UV light (if a chromophore is present). For non-chromophoric compounds, various staining reagents such as anisaldehyde, ninhydrin, etc. are used. LC-MS analysis circumvents the need for manual TLC separations, but typically requires more technical knowledge of instrument hardware and software, leading to increased training requirements. One option is to submit samples to an analytical group or core-facility for analysis. However, this increases the cost per sample and can result in delays for

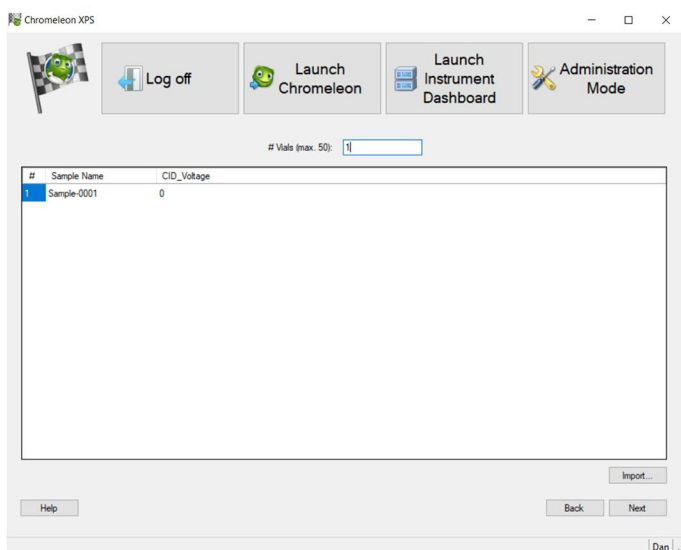
sample characterization. With the availability of Chromeleon XPS, synthetic chemists can obtain reliable and accurate LC-MS data without relying on assistance from technicians. When using the Chromeleon XPS Open Access walk-up solution, each user has a separate login with predefined privileges, e.g., which instruments or which methods they can use. Users can submit samples with minimal input by following the steps (Figure 1) and will receive an automatically generated report afterwards.<sup>1</sup>



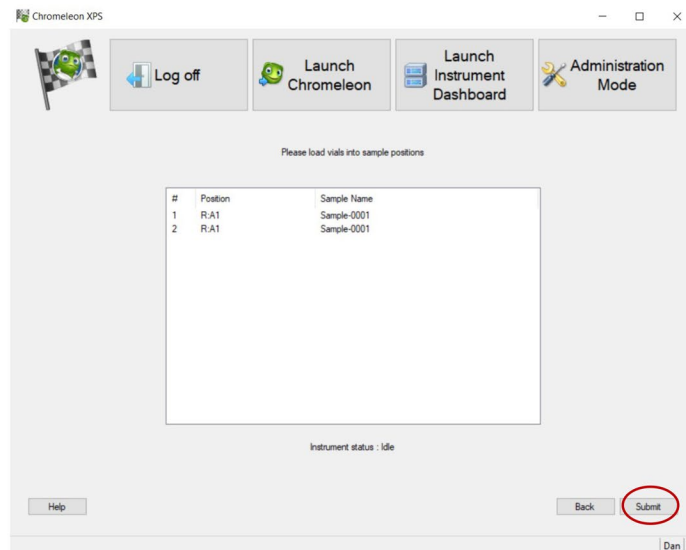
Step 1: User login



Step 2: Select eWorkflow



Step 3: Enter number of samples and names



Step 4: Submit sample(s) for analysis

**Figure 1. Steps in the sample submission using Chromeleon XPS Open Access.** First, a user needs to log in using Chromeleon login credentials. Then, select the eWorkflow procedure and the instrument. After that, enter the number of vials and sample name(s). Finally, submit the sample(s) for analysis. The results will be automatically generated, and the report can be sent by email.

Chromeleon CDS allows user profiles with different levels of privileges, which helps meet compliance standards in regulated environments. Those who have Chromeleon software login profiles can log into Chromeleon XPS using the same credentials. Once users are logged in using their credentials, they will be able to see all the personally available eWorkflow procedures. When an eWorkflow procedure is selected, users can see a list of instruments for which that particular eWorkflow template is assigned. Then, the user can select the instrument with the shortest wait time for the analysis. Samples are inserted, the number of samples to be analyzed is entered, sample name and any eWorkflow template specific parameters are also recorded. After all user inputs have been completed, samples are submitted for analysis. Data processing and reporting can be automated with the results sent via email as soon as analysis is complete. This saves time and allows the user to focus on other tasks.

LC-MS analyses usually follow the same general workflow: sample injection, analyte separation, analyte detection, and result reporting. The main differences lie in the specific instrument and software conditions for each step, creating complexity in finding the most appropriate workflow for a given analyte. eWorkflow procedures simplify instrument operation by capturing all the unique aspects of the chromatography and mass spectrometry workflow and guiding the operator to enter minimal inputs, such as instrument conditions.<sup>2,3</sup> As a result, Chromeleon XPS utilizing eWorkflow procedures allows inexperienced users to submit and run samples quickly and successfully. In fact, instead of creating methods, users simply select an eWorkflow procedure for a given instrument and the method is predefined by the lab supervisor. Therefore, it is the lab supervisor's responsibility to create and maintain the required methods. The lab supervisor

can create analyses in the form of eWorkflow templates and assign instruments to that eWorkflow procedure. The procedures include instrument method files, data processing files, and report templates. For labs with multiple instruments, updates to eWorkflow procedures are automatically applied to all relevant instruments.

In this example, analysis of a Buchwald-Hartwig amination, the chemist is synthesizing a bis-aniline (Figure 2). The starting material is a dibromide with a molecular weight of 325 that ionizes in negative mode (RT = 1.39 min,  $m/z$  324, with a two-bromine isotopic pattern). Upon analysis, the desired product is observed with a molecular weight of 324 that ionizes in positive mode (RT = 0.757 min,  $m/z$  325, without bromine). A non ionizable by-product, which is a homo-coupled dimer, can be detected by UV only (RT = 1.12 min).

A report generated for an analysis performed for the mass confirmation and sample purity calculation is shown in Figure 3. In this method, the product of the amination reaction is confirmed by mass detection, and the purity calculation is based on the UV detection. The same analysis could alternatively be performed without UV data where the purity calculation can be done using the mass detector data (data not shown here). The report provides the percentage of the target compound and the mass confirmation of the target compound as well as the impurity masses from the mass spectrum in the first pane of the report. This report contains much more sample information than obtained by running a TLC. LC-MS analysis provides analyte quantitation and improved selectivity. Therefore, Chromeleon XPS software enables chemists to obtain detailed results faster, increasing lab productivity.

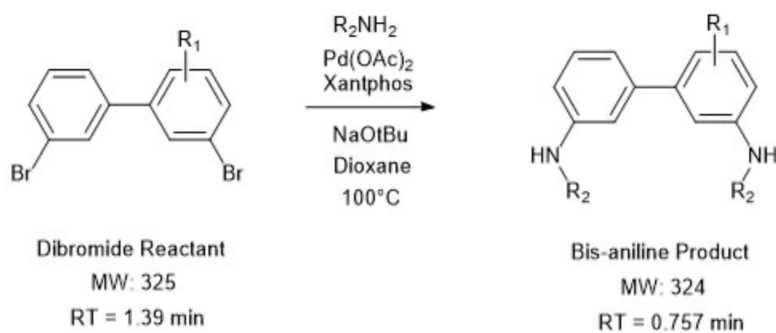


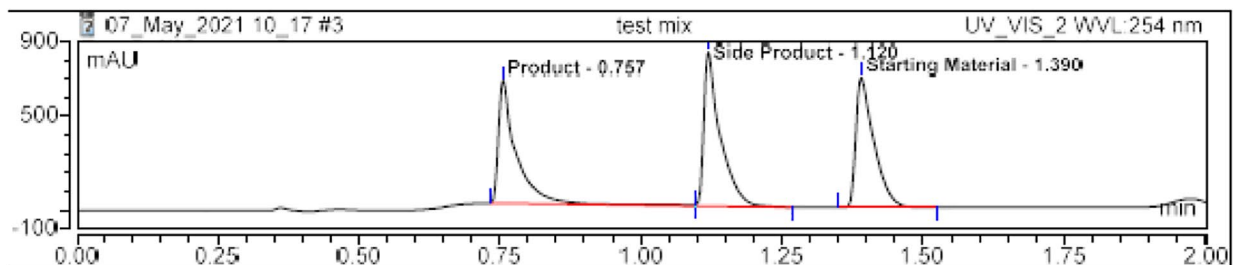
Figure 2. Buchwald-Hartwig amination reaction

## Peak Analysis

### Injection Details

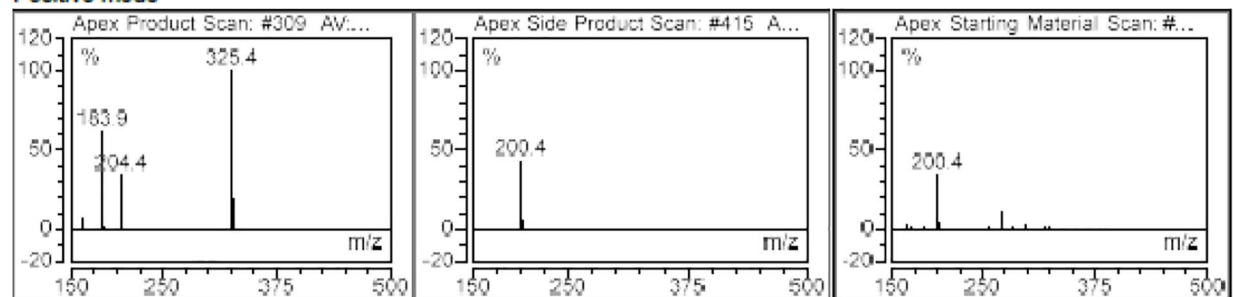
Injection Name:	test mix	Run Time (min):	2.00
Vial Number:	G:A1	Injection Volume:	1.00
Injection Type:	Unknown		
Instrument Method:	Thermo_Accucore_4.6x50		
Processing Method:	Quantitative Dan	Dilution Factor:	1.0000
Injection Date/Time:	07/May/21 10:23	Sample Weight:	1.0000

### Chromatogram

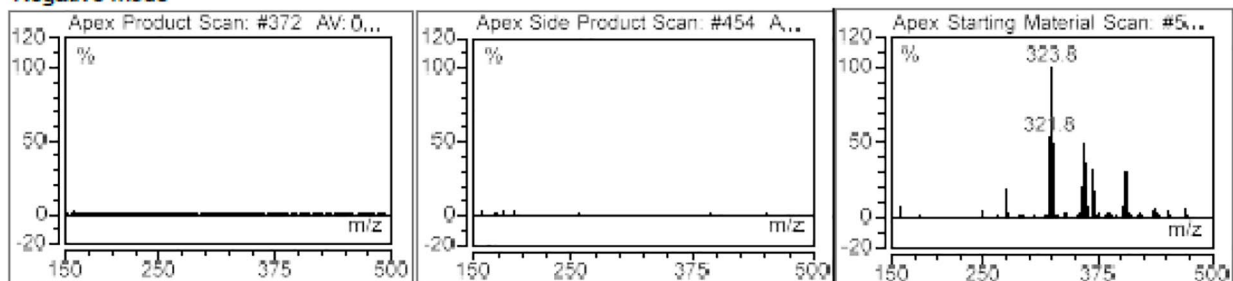


### Mass Spectra

#### Positive mode



#### Negative mode



### Table

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %
1	Product	0.757	24.345	652.351	31.40	30.18
2	Side Product	1.120	27.601	817.326	35.60	37.82
3	Starting Mat	1.390	25.595	691.676	33.01	32.00
Total:			77.541	2161.354	100.00	100.00

Figure 3. Peak analysis report generated in Chromeleon XPS. The first pane shows the chromatogram from the UV detector and the second pane shows mass spectra for the detected analytes in both positive mode and negative mode. The table shows the peak area calculated from the UV detector data and the relative peak area.

## Conclusion

Chromeleon XPS Open Access is a powerful tool that delivers a walk-up solution for using LC-MS instrumentation. The simplicity of use of the software makes mass detection and purity determination readily available to scientists without prior LC-MS experience. Sample analysis is straightforward and requires only a few mouse clicks, with the results sent to users via email once data acquisition is complete. This whole process leads to streamlined operations, reducing training requirements and simplifying system administration.

## Acknowledgement

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## References

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3. Chromeleon eWorkflows, AppsLab library. <https://appslab.thermofisher.com/Content/GettingStarted/AppsLab%20Library%20-%20eWorkflow%20-%20English.pdf>

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