How to Realize LC-MS Quantitation with Chromeleon 7.2 CDS

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Key Words

Chromeleon 7.2 CDS, eWorkflows, AppsLab Library, One-Click Solution, Vanquish UHPLC System, Q Exactive HF Mass Spectrometer
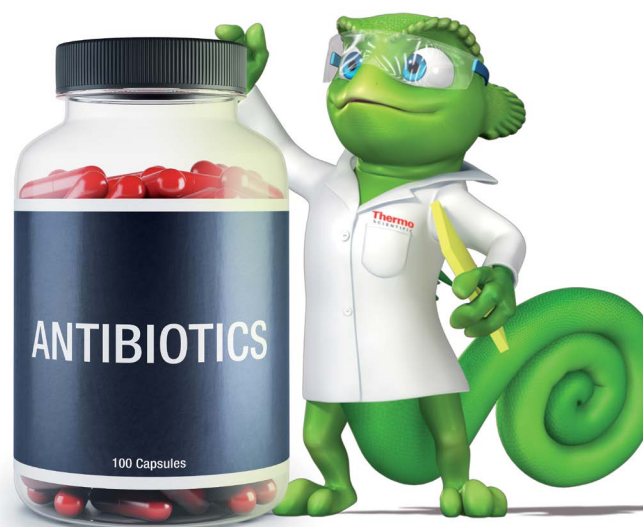
Goal

Introduce Chromeleon 7.2 CDS for LC-UV and LC-MS screening and quantitation of antibiotics.

Introduction

Many academic and industrial analytical environments, such as pharmaceutical and routine laboratories, working with liquid chromatography (LC) take benefits from both UV and mass spectrometry (MS) techniques as detection methods. More and more, these laboratories are demanding a single chromatographic software package that supports both UV and MS instrumentation.

Chromatography plays an essential analytical role in pharmaceutical companies, from drug discovery through development into manufacturing and quality assurance/quality control (QA/QC). Drug discovery and development are based on screening, identification, and isolation of both impurities and active ingredients, and often several steps of the analysis rely on LC coupled to high-resolution, accurate-mass (HRAM) MS. These analytical methods are required to be robust and reliable so they can be eventually validated and transferred to QA/QC, where the drug supply quality is monitored with LC-UV. One of the main challenges and the most time-consuming task of method transfer to QA/QC is the transfer of an MS-based method to a UV-based one. Normally, this also requires the method to be transferred to a different software package. In this regard, a single software package for both UV and MS instrumentation will facilitate the transfer.

Other examples are routine applications for analytical laboratories, which demand robust and reliable HPLC methods. For a long time, UV detection has been widely preferred owing to the simplicity of use, the minimal maintenance required, and the reliability of the results. In recent years, more and more of these laboratories are adopting MS detection due to the improved data reliability provided by the MS instruments and their increased accessibility. So again, in this case a single point of control for UV and MS systems is highly preferred.

Thermo Scientific™ Dionex™ Chromeleon™ 7.2 Chromatography Data System (CDS) software, the gold standard in chromatography data system software, responds to this rapidly growing customer demand for intuitive software to run MS analyses in routine and quantitative applications by combining both chromatography and MS workflows including instrument control, data processing, and reporting. There are several unique additional benefits:

- Tools to ensure compliance and security, such as validation, audit trails, and electronic signatures
- Ability to control, process, and report LC-MS data with remote access
- Capability to work in an enterprise (client/server) environment
- Workflow management (eWorkflows™) that guides the operator through a minimal number of choices needed to run the LC-MS method

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Here, we show how Chromeleon 7.2 CDS supports a quantitative method for targeted analysis of antibiotics with LC coupled to both UV and MS detection. The application is realized with state-of-the-art LC-MS instrumentations. The Thermo Scientific™ Vanquish™ UHPLC system is coupled to the Thermo Scientific™ Q Exactive™ HF Hybrid Quadrupole-Orbitrap™ mass spectrometer. The Vanquish UHPLC system consists of a binary parallel pump capable of operating at pressures up to 1500 bar (22,000 psi), with a LightPipe™ Diode Array Detector (DAD). The Q Exactive HF MS system combines a segmented quadrupole with a high-resolution, accurate-mass, ultra-high-field Orbitrap mass analyzer. The instrument method, data processing method, and report template are thereafter used to generate an LC-MS eWorkflow that is freely available to download in the Thermo Scientific AppsLab Library of Analytical Applications, which can be found by visiting www.thermoscientific.com/appslab.

Experimental

Sample Preparation

Prepare stock solutions of the seven antibiotics shown in Table 1 by dissolving 1 mg in 1 mL of water/acetonitrile (1:1, v/v). Perform serial dilutions with water/acetonitrile (90:10, v/v) to produce premixed working solutions in the range 1–100 ng/mL (1–100 ppb).

Table 1. Antibiotics with relative MS and UV properties.

Compound	Molecular Formula	[M+H]+ m/z	UV Absorption at 260 nm
Sulfamethizole	$C_9H_{10}N_4O_2S_2$	271.032344	yes
Sulfamethazine	$C_{12}H_{14}N_4O_2S$	279.091573	yes
Sulfachloropyridazine	$C_{10}H_9ClN_4O_2S$	285.021301	yes
Sulfadimethoxine	$C_{12}H_{14}N_4O_4S$	311.081403	yes
Penicillin G	$C_{16}H_{18}N_2O_4S$	335.106555	no
Cefamandole	$C_{18}H_{18}N_6O_5S_2$	463.085837	yes
Erythromycin	$C_{37}H_{67}NO_{13}$	734.469069	no

Instrumentation

- Vanquish UHPLC system including:
  - System Base Vanquish (P/N VH-S01-A)
  - Binary Pump H (P/N VH-P10-A)
  - Split Sampler HT (P/N VH-A10-A)
  - Column Compartment H (P/N VH-C10-A)
  - Diode Array Detector HL (P/N VH-D10-A)
  - LightPipe flow cell, standard (P/N 6083.0100)
- Q Exactive HF hybrid quadrupole-Orbitrap Mass Spectrometer
- Vanquish MS connection kit (P/N 6720.0405)

LC Conditions

Column	Thermo Scientific™ Hypersil GOLD™ 1.9 µm, 50 x 2.1 mm (P/N 25002-052130)
Mobile Phases	A: Water with formic acid 0.1% (v/v, %)
	B: Acetonitrile with formic acid 0.1% (v/v, %)
Gradient	0–1.50 min: 15–60% B
	1.50–1.75 min: 60–90% B
	1.75–1.76 min: 90–15% B
	1.76–4.00 min: 15% B
Flow Rate	0.400 mL/min
Temperature	25 °C
Inj. Volume	5 µL
Detection	260 nm
	50 Hz
	0.10 s response time
	4 nm slit width
	4 nm bandwidth
Flow Cell	LightPipe, 10 mm

MS Conditions

Ionization Conditions	HESI
Polarity	Positive
Mass Range	m/z 100–1000
Resolution	30,000
Automatic Gain Control (AGC) Target	3×10^6
Maximum Ion Injection Time (IT)	200 ms
Spray Voltage	3.5 kV
Transfer Temperature	320 °C
S-Lens RF-Level	50 V
Heater Temperature	350 °C
Sheath Gas	40 arb units
Aux Gas	10 arb units

Data Acquisition and Processing

Chromeleon 7.2 CDS SR2

Results and Discussion

Instrument Method and Data Acquisition

A mixture containing seven antibiotics at five calibration levels (1–100 ppb) was used to create a calibration curve. The compounds were separated in less than 2 min on the Vanquish UHPLC system using a Hypersil GOLD, 1.9 µm, 50 x 2.1 mm column and detected with DAD at 260 nm and MS in full scan mode. The DAD and the MS detectors were connected on-line.

Chromeleon 7.2 CDS allows controlling and editing the instrument method of both detectors in a single user-friendly interface (Figure 1). The properties and current settings of each module are displayed in ePanels. A dedicated MS ePanel gives access to the MS tune information and shows the TIC trace and the MS spectrum during the acquisition (Figure 2). The injection sequence displays a selected plot (i.e. UV, TIC, Pressure, or Temperature) in MiniPlots™ (Figure 3).
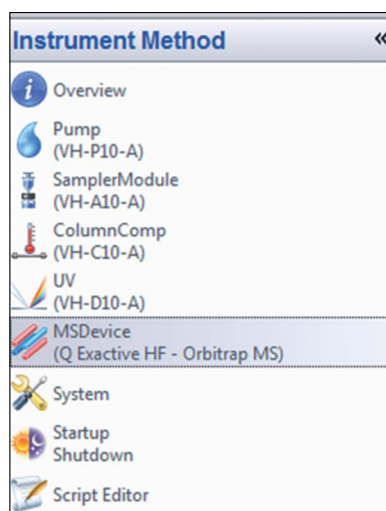

Figure 1. HPLC modules and MS are displayed in a single instrument method editor.
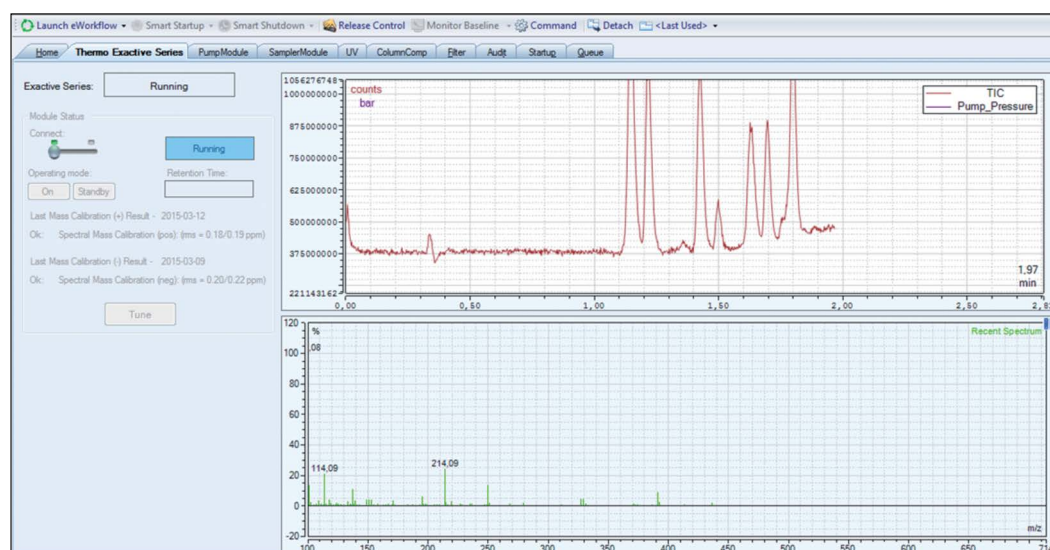

Figure 2. MS ePanel with TIC trace and the MS spectrum.

#	TIC	Name	Type	Level	Position	Volume [µl]	Instrument Method	Processing Method
1		blank	Blank		R:A1	5.00	Method_Antibiotics	Processing_Antibiotics
2		Antibiotics 1 ppb	Calibration Standard	01	R:A2	5.00	Method_Antibiotics	Processing_Antibiotics
3		Antibiotics 1 ppb	Calibration Standard	01	R:A2	5.00	Method_Antibiotics	Processing_Antibiotics
4		Antibiotics 1 ppb	Calibration Standard	01	R:A2	5.00	Method_Antibiotics	Processing_Antibiotics
5		Antibiotics 5 ppb	Calibration Standard	02	R:A3	5.00	Method_Antibiotics	Processing_Antibiotics
6		Antibiotics 5 ppb	Calibration Standard	02	R:A3	5.00	Method_Antibiotics	Processing_Antibiotics
7		Antibiotics 5 ppb	Calibration Standard	02	R:A3	5.00	Method_Antibiotics	Processing_Antibiotics
8		Antibiotics 10 ppb	Calibration Standard	03	R:A4	5.00	Method_Antibiotics	Processing_Antibiotics
9		Antibiotics 10 ppb	Calibration Standard	03	R:A4	5.00	Method_Antibiotics	Processing_Antibiotics
10		Antibiotics 10 ppb	Calibration Standard	03	R:A4	5.00	Method_Antibiotics	Processing_Antibiotics
11		Antibiotics 50 ppb	Calibration Standard	04	R:A5	5.00	Method_Antibiotics	Processing_Antibiotics
12		Antibiotics 50 ppb	Calibration Standard	04	R:A5	5.00	Method_Antibiotics	Processing_Antibiotics
13		Antibiotics 50 ppb	Calibration Standard	04	R:A5	5.00	Method_Antibiotics	Processing_Antibiotics
14		Antibiotics 100 ppb	Calibration Standard	05	R:A6	5.00	Method_Antibiotics	Processing_Antibiotics
15		Antibiotics 100 ppb	Calibration Standard	05	R:A6	5.00	Method_Antibiotics	Processing_Antibiotics
16		Antibiotics 100 ppb	Calibration Standard	05	R:A6	5.00	Method_Antibiotics	Processing_Antibiotics
17		blank	Blank		R:A1	5.00	Method_Antibiotics	Processing_Antibiotics

Figure 3. Injection sequence with TIC MiniPlots.

Data Processing

The data were quickly processed using an inclusion list with all the information about compound names, accurate masses, polarity, and extraction windows. Chromeleon CDS contains already preconfigured templates ideal for MS quantitation reports. A MS quantitation channel that displays all the extracted ion chromatograms of the MS quantitation peaks and a component list were generated from the inclusion list (.csv format) (Figure 4).

For each component at each calibration level the extracted ion chromatogram, along with the mass spectrum and calibration curve were displayed (Figure 5).

The simultaneous detection with DAD and MS detectors provides complementary information for impurity profiling. The MS component channel with the extracted ion chromatograms of all seven antibiotic standards was overlaid to the UV trace. The two traces could be perfectly aligned by taking into account the delay time between the two detectors. This simple comparison revealed the presence of a UV active impurity, as shown in Figure 6.
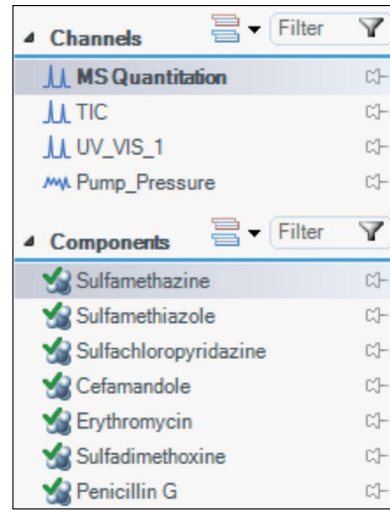

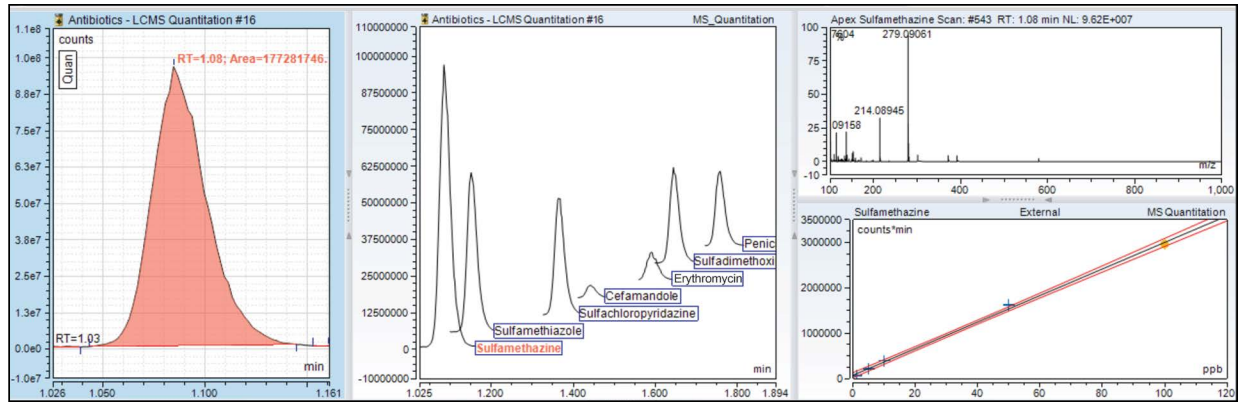Figure 4. MS quantitation channel and component list.

Figure 5. Extracted ion chromatogram of sulfamethazine at 100 ppb concentration, with relative mass spectrum and calibration curve. The central plot displays the quantitation channel showing the extracted ion chromatograms of all the components at 100 ppb concentration.
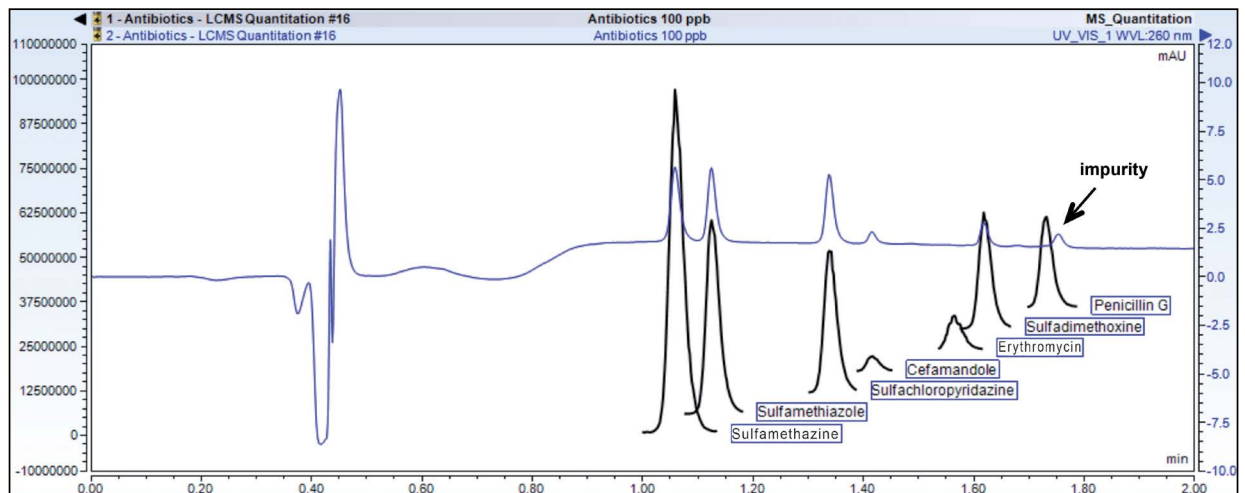

Figure 6. Overlay of MS Quantitation channel and UV trace at 260 nm of the sample mixture with antibiotics at 100 ppb concentration.

eWorkflows and AppsLab Library

An eWorkflow is an electronic procedure that contains all the information required to run a sequence. A specific eWorkflow for the quantitation of the antibiotics was generated in few steps, adding the instrument method, data processing method, report template, and view settings files. All the documents relevant to the analysis can be attached to the eWorkflow. In this case, the antibiotic inclusion list was attached (Figure 7). Finally the injection list was pre-defined including sample names, sample types (e.g. calibration standard), calibration level, number of replicates and injection order.

After creation, the eWorkflow can be used by any operator worldwide; they simply select their LC-MS instrument and the starting vial position in the autosampler before beginning the analysis. Chromeleon CDS then runs the analysis, processes the data, and produces the final results.

This ready-to-use LC-MS eWorkflow is available for downloading from AppsLab Library, a Thermo Scientific repository comprising over 1000 applications with detailed method information, chromatograms, and related compound information.
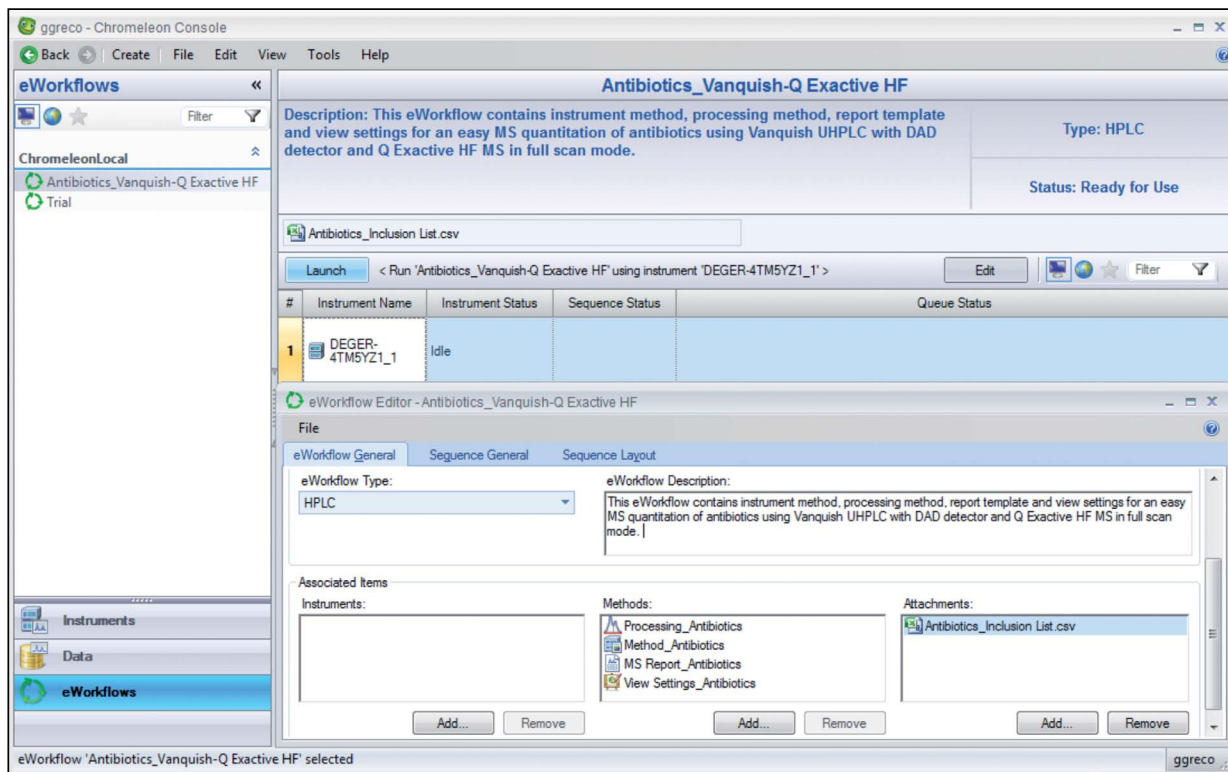

Figure 7. Antibiotics LC-MS eWorkflow with instrument method, data processing method, report, view settings, and compound inclusion list.

Conclusion

Chromeleon 7.2 CDS contains the necessary MS-specific data views, data processing, and reporting capabilities to streamline chromatography and MS quantitation workflows in a single application.

All the information used to acquire and process the data for the quantitation of antibiotics was collected in an eWorkflow. The eWorkflow, created for LC-UV-MS analysis of antibiotics, is available from the AppsLab Library. Any operator worldwide can download it free of charge and the method will be ready to run on compatible LC-MS instrumentation.

Useful Links

AppsLab Library

The eWorkflow and the Chromeleon Backup (cmbx) file can be downloaded at AppsLab Library:

https://appslab.thermoscientific.com/

www.thermofisher.com/chromatography

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