67711 _screenshot_1.mp4

2.5. Now, turn on the power switches for each module of the liquid chromatography-evaporative light-scattering detector system [1]. Launch the LabSolutions software and open the Realtime Analysis window [2].

2.5.1. Talent switching on power for HPLC and ELSD modules.

2.5.2. SCREEN: Opening LabSolutions software and navigating to the Realtime Analysis window.00:00-00:30

2.6. Click on File and select New to create a new method file [1]. Modify the liquid chromatography stop time to 15 minutes and click Apply to all Acquisition Time [2].

2.6.1. SCREEN: Selecting File > New to create a new method file. 00:30-00:37

2.6.2. SCREEN: Modifying the liquid chromatography stop time to 15 minutes and clicking Apply to all Acquisition Time. 00:38-00:48

2.7. Then, click on Pump and modify the pump parameters [1]. Select the analysis mode as Binary High-Pressure Gradient (B.GE) (B-G-E) and set the flow rate to 1 milliliters per minute [2].

2.7.1. SCREEN: Opening Pump settings and selecting Binary High-Pressure Gradient (B.GE). 00:49-00:58

2.7.2. SCREEN: Adjusting the flow rate to 1.0 milliliters per minute. 00:59-01:04

2.8. Set the initial Pump B concentration to 80% [1]. Modify the mobile phase gradient at 3 minutes to 80%, at 4 minutes to 85%, from 5.5 to 12 minutes at 100% and at 12.1 minutes, let the Pump B return to 80% [2].

2.8.1. SCREEN: Setting the initial pump B to 80%.01:04-01:12

2.8.2. SCREEN: Adjusting mobile phase gradient: at 3 min- 80%, at 4 min- 85% from 5.5 min to 12 min- 100%, at 12.1 min-80%.01:13-01:40

2.9. Now, click on Column Oven and set the oven temperature to 55 degrees Celsius [1]. Then, click on ELSD to modify the drift tube temperature to 40 degrees Celsius [2] and save the method [3].

2.9.1. SCREEN: Setting the column oven temperature to 55 degrees Celsius. 01:41-01:47

2.9.2. SCREEN: Adjusting the drift tube temperature of the ELSD to 40 degrees Celsius. 01:48-01:53

2.9.3. SCREEN: Saving the method parameters. 01:54-02:05

2.10. Click on Download and Startup to start and equilibrate the instrument [1].

2.10.1. SCREEN: Selecting Download and Startup to initiate the equilibration process. 02:06-02:31

67711 _screenshot_2.mp4

4. HPLC Data Acquisition and Analysis

4.2. Click on Realtime Batch in the assistant toolbar of the Realtime Analysis window [1]. Next, click New in the File menu to create a new batch table [2].

4.2.1. SCREEN: Selecting Realtime Batch in the assistant toolbar. 00:00-00:07
4.2.2. SCREEN: Clicking New in the File menu to create a new batch table. 00:08-00:12

4.3. In the batch table, input the Vial# (vial number), Tray Name, Data File, and the Injection Volume of the standard and sample solutions [1]. Select the saved method file [2] and click Save Batch File in the File menu [3].
4.3.1. SCREEN: Entering Vial number, Tray Name, Data File, and Injection Volume in the batch table. 00:12-00:30
4.3.2. SCREEN: Selecting the previously saved method file. 00:30-00:33
4.3.3. SCREEN: Clicking Save Batch File in the File menu. 00:34-00:39

4.4. Wait until the liquid chromatograph pressure and the baseline of the chromatogram are stable [1]. Then, click on Start Realtime Batch in the assistant toolbar to start data acquisition [2].
4.4.1. SCREEN: Monitoring liquid chromatograph pressure and chromatogram baseline. 00:40-00:43
4.4.2. SCREEN: Clicking Start Realtime Batch in the assistant toolbar. 00:44-01:17

67711 _screenshot_3.mp4
4.5. For data analysis, open the LabSolutions software's Browser Window [1]. Drag the standard solution data into the Quantitative Results View to establish a calibration curve [2]. Modify the data processing parameters by clicking on Edit in the Method View [3].
4.5.1. SCREEN: Opening the Browser Window in LabSolutions. 00:00-00:08
4.5.2. SCREEN: Dragging standard solution data into the Quantitative Results View. 00:09-00:13
4.5.3. SCREEN: Clicking Edit in the Method View. 00:14-00:17

4.6. In the Integration Parameters window, change the slope value to 10000 [1]. Then, in the Identification Parameters, change the identification method to Band and set the Default Bandwidth to 0.1 minutes [2].
4.6.1. SCREEN: Change the integration algorithm to i-PeakFinder, and set the baseline type to Base to Base. 00:18-00:27
4.6.2. SCREEN: Selecting Band as the identification method and setting the Default Bandwidth to 0.1 minutes. 00:28-00:34

4.7. To modify the Quantitative Parameters, change the Quantitative Method to External Standard [1]. Set the # of Calibration Level (number of calibration level) to 7 [2]. Then, select the calibration curve type as Exponentially [3] and modify the Compound settings [4].
4.7.1. SCREEN: Selecting External Standard as the quantitative method. 00:35-00:40
4.7.2. SCREEN: Setting # of Calibration Level to 7. 00:41-00:43
4.7.3. SCREEN: Choosing Exponentially as the calibration curve type. 00:44-00:48
4.7.4. SCREEN: Opening the Compound settings for modification. 00:49-00:51

4.8. Input the names and standard solution concentrations of the four lipid nanoparticle

components [1]. Double-click on the peak apex to update the retention times [2]. Click View to complete the modification of the data processing parameters [3].

4.8.1. SCREEN: Entering names and concentrations of lipid nanoparticle components. 00:52-01:29

4.8.2. SCREEN: Double-clicking the peak apex to update retention times. 01:30-01:53

4.8.3. SCREEN: Clicking View to complete modifications. 01:54-01:57

4.9. Now, modify the Sample Type in the Quantitative Results View to Standard (Calc. Point) (calculate point) [1]. Set the levels of the standard solution from 1 to 7 according to the concentrations [2]. Once the calibration curves are established, click File in the menu bar and save the method file [3].

4.9.1. SCREEN: Changing the Sample Type to Standard (Calc. Point). 01:58-02:10

4.9.2. SCREEN: Setting the standard solution levels from 1 to 7. 02:11-02:22

4.9.3. SCREEN: Clicking File > Save to save the method file. 02:23-02:32

4.10. Drag the sample data into the Quantitative Results View of the browser window [1]. Observe the displayed concentration results of the four lipid nanoparticle components in the samples [2].

4.10.1. SCREEN: Dragging sample data into the Quantitative Results View. 02:33-02:39

4.10.2. SCREEN: Displaying concentration results of lipid nanoparticle components. 02:40-03:01