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Project Page Link: <https://review.jove.com/account/file-uploader?src=21253973>

Title: Automated HPLC Separation Using LC-mate: An Integrated Repetitive Autosampler and Fraction Collector for Microscale Purification

Authors and Affiliations:

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Author Questionnaire

1. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

2. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes**

As these files are necessary for finalizing your script, please upload all screen-captured video files to your project page as soon as possible:

<https://review.jove.com/account/file-uploader?src=21253973>

3. Filming location: Will the filming need to take place in multiple locations? **No**

4. Testimonials (optional): Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **No**

Current Protocol Length

Number of Steps: 22

Number of Shots: 42

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

Authors: Please note that **the questions will not appear on screen. Please answer in *stand-alone full sentence with sufficient context.***

INTRODUCTION:

~~REQUIRED: Summarize your research focus and the main questions it aims to answer.~~

1.1 **Weimin Zhao:** Our research focuses on designing an instrument that plugs right into HPLC setups to automate all those repetitive manual steps.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the research gap or limitation in existing methods and how this protocol addresses them.~~

1.2. **Weimin Zhao:** Manual sample injection and fraction collection are very tedious. LC-mate offers a cross-platform, automated solution for HPLC sample purification.

1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

1.3. **Chengyu Shi:** This system facilitates automated repetitive purification and precise fraction collection for efficient microscale HPLC workflows.

1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the most important consideration or challenge when performing this protocol.~~

1.4. **Weimin Zhao:** Correct signal wiring, proper mode selection and parameter setting are critical for successful application of the LC-mate instrument.

1.4.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

- 1.5. **Weimin Zhao**: We're planning to boost channel capacity and integrate new detectors for better cross-platform compatibility.

- 1.5.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

Videographer: Obtain headshots for all authors available at the filming location.

Protocol

2. Integrated HPLC–LC-mate System Setup, Operation, and Automated Fraction Collection

Demonstrator: Chengyu Shi

- 2.1. To begin, verify the solvent plumbing connections between the HPLC system, the LC-mate, and the chromatographic column to ensure proper flow paths [1]. Check the connection of the signal transmission line between the ultraviolet detector and the LC-mate [2].
 - 2.1.1. WIDE: Talent visually inspecting and tracing the solvent tubing connections between the high-performance liquid chromatography system, the LC-mate, and the chromatographic column.
 - 2.1.2. Talent examining the signal transmission cable connected between the ultraviolet detector and the LC-mate.
- 2.2. Connect the black wire to the red terminal [1] and the white wire to the black terminal to match the polarity of the analog signal output [2]. Observe the LC-mate display to confirm that the analog signal waveform appears [3].
 - 2.2.1. Talent connecting the black wire to the red terminal.
 - 2.2.2. Talent connecting the white wire to the black terminal on the LC-mate.
 - 2.2.3. SCREEN: 2.2.3úĹEnglish)(updated).MOV: 00:11-00:19
- 2.3. Reverse the wire connections by attaching the black wire to the black terminal and the white wire to the red terminal if no waveform is visible [1].
 - 2.3.1. Talent switching the wire connections on the LC-mate terminals.
- 2.4. Now, check the trigger connection between the ultraviolet detector and the LC-mate [1]. Plug the single end of the data cable into the output port of the ultraviolet detector [2].
 - 2.4.1. Talent inspecting the trigger connection ports on the ultraviolet detector and LC-mate.
 - 2.4.2. Talent inserting the data cable into the ultraviolet detector output port.
- 2.5. Connect the black and white wires from the other end of the cable to the blue and black trigger terminals on the LC-mate, respectively [1]. Tighten the terminals to ensure a firm trigger connection [2].
 - 2.5.1. Talent connecting the black and white wires to the corresponding trigger terminals on the LC-mate.
 - 2.5.2. Talent tightening the trigger terminals on the LC-mate.

- 2.6. Next, ensure the proper startup of the HPLC system [1] and LC-mate [2].
 - 2.6.1. Talent powering on and confirming the operational status of the high performance liquid chromatography system.
 - 2.6.2. Talent powering on the LC-mate device.
- 2.7. Click on the **LC-mate** icon on the interface to automatically execute the system self-test program and initialize key hardware modules [1]. Wait for the brief prompt tone and the disappearance of the Self-Testing message to confirm that initialization is complete [2].
 - 2.7.1. SCREEN: 2.7.1+2.7.2úEnglish)(updated).MOV: 00:00-00:03
 - 2.7.2. SCREEN: 2.7.1+2.7.2úEnglish)(updated).MOV 00:04-00:06
- 2.8. Configure the HPLC separation conditions and begin elution to equilibrate the column [1].
 - 2.8.1. Talent setting separation parameters on the high performance liquid chromatography system and starting elution.
- ~~2.9. Click on the **Test** icon on the left side of the LC-mate screen to open the test interface [1].~~
 - ~~2.9.1. SCREEN: Show the LC-mate main interface and select the **Test** icon. NOTE: Not provided by the authors~~
- 2.10. In the test interface, input the **Injection Volume**, **Elution Time**, and **Repetition Times** [1], then press the **Start** icon to initiate the autosampler, fraction collector, and HPLC operation [2].
 - 2.10.1. SCREEN: 2.10.1úEnglish)(updated).MOV: 00:00-end
 - 2.10.2. SCREEN: 2.10.2úEnglish)(updated).MOV: 00:01-00:04
- 2.11. Identify feasible separation conditions, including resolution, running time, retention time, injection volume, and ultraviolet wavelength [1].
 - 2.11.1. Talent observing chromatographic output and evaluating separation performance on the display.
- 2.12. Then select the **Threshold** function module on the left side of the interface to open the parameter setting screen [1]. Enter the **Injection Volume**, **Repetition Times**, **Threshold**, and **Elution Time**, and press **Start** to begin automated operation [2].
 - 2.12.1. SCREEN: 2.12.1úEnglish)(updated).PNG
 - 2.12.2. SCREEN: 2.12.2úEnglish)(updated).MOV: 00:00-00:16.
- 2.13. Monitor the status indicators. Observe **Injection in Progress** to confirm sample introduction [1]. Check the **Separation in Progress** indicator to verify that the chromatographic separation is going on [2]. Note **Syringe Emptying** indicator to identify

when the syringe is purging residual liquid [3].

2.13.1. SCREEN: 2.13.1+2.13.2úĖEnglish)(updated).MOV: 00:00-end *Video editor: Highlight tab “Injection in Progress”*

2.13.2. SCREEN: 2.13.1+2.13.2úĖEnglish)(updated).MOV: 00:00-end *Video editor: Highlight tab “seperation in”*

2.13.3. SCREEN: 2.13.3úĖEnglish)(updated).MOV: 00:00-end

2.14. To perform fraction collection based on time, select the **Timing Collection** module from the left side of the interface to open the parameter menu [1]. Input **Injection Volume**, **Repetition Times**, **Elution Time**, and the required Collection Times [2]. Press the **Start** button to initiate the repeated automated sequence [3].

2.14.1. SCREEN: 2.14.1úĖEnglish)(updated).PNG

2.14.2. SCREEN: 2.14.2úĖEnglish)(updated).MOV: 00:18-00:36

2.14.3. SCREEN: 2.14.3úĖEnglish)(updated): 00:01-00:05-00:13-00:20

2.15. Next, select the **Collect All** function module on the left side of the interface [1]. Set a collection threshold value to collect all chromatographic peaks separately [2].

2.15.1. SCREEN: 2.15.1úĖEnglish)(updated).PNG

2.15.2. SCREEN: 2.15.2úĖEnglish)(updated).MOV: 00:05-end

2.16. For pipeline cleansing, prepare a small bottle of clean solvent [1]. Insert the injection pipeline of the autosampler into the solvent [2].

2.16.1. Shot of a labeled bottled of cleaning solvent.

2.16.2. Talent placing the autosampler injection pipeline into a bottle containing clean solvent.

2.17. Select the **Cleansing** module from the left side of the interface to open the parameter menu [1]. Then set the **Injection Volume** between 0 and 1000 and the **Repetition Times** for injector pipeline cleansing [2].

2.17.1. SCREEN: 2.17.1úĖEnglish)(updated).PNG

2.17.2. SCREEN: 2.17.2úĖEnglish)(updated).mov: 00:00-end

2.18. Click on **Cleansing** below **Cleansing Completed** to start injector pipeline cleansing [1]. Monitor the **Cycle** count to track completed wash cycles [2].

2.18.1. SCREEN: 2.18.1+2.18.2úĖEnglish)(updated).MOV: 00:20-00:26

2.18.2. SCREEN: 2.18.1+2.18.2úĖEnglish)(updated).MOV: 01:17-01:22 *Video editor: highlight “3” present in the left side*

2.19. Now, set the **Cleansing Time** for the collection pipeline cleansing [1]. Click on **Cleansing** below **Current Pipeline** to start collection pipeline cleansing [2].

2.19.1. SCREEN: 2.19.1+2.19.2úĳEnglish)(updated).MOV: 00:00-00:02

2.19.2. SCREEN: 2.19.1+2.19.2úĳEnglish)(updated).MOV: 00:02-end

2.20. Monitor the **Current Pipeline** display to confirm sequential cleaning from pipeline 0 to pipeline 10 [1].

2.20.1. SCREEN: 2.20.1úĳEnglish)(updated).MOV: 2.20.1.mp4: 00:02-00:20

2.21. Enable automatic saving to allow the system to save chromatograms after each elution cycle [1]. Alternatively, click on the **Save** button after elution to manually save data [2].

2.21.1. SCREEN: 2.21.1úĳEnglish)(updated).MOV: 00:00-end

2.21.2. SCREEN: 2.21.2úĳEnglish)(updated).pdf

2.22. After entering the metadata in the dialog box, choose the storage location from local storage, an SD card, or an external universal serial bus drive [1].

2.22.1. SCREEN: 2.21.3.mp4

Results

3. Results

- 3.1. Semi-preparative HPLC separation with LC-mate achieved continuous and stable sample injection and fraction collection during purification of 2''-O-galloyl-hyperoside [1]. Minor impurities were effectively removed to obtain purified 2''-O-galloyl-hyperoside using the LC-mate system [2].
 - 3.1.1. LAB MEDIA: Figure 3. *Video editor: Show the chromatogram panel*
 - 3.1.2. LAB MEDIA: Figure 3. *Video editor: Zoom in on the main peak labelled as 2''-O-galloyl-hyperoside*
- 3.2. Semi-preparative HPLC separation with LC-mate achieved continuous and stable sample injection and fraction collection during purification of phloridzin and trilobatin [1]. The LC-mate system achieved continuous and stable sample injection and fraction collection [2].
 - 3.2.1. LAB MEDIA: Figure 4. *Video editor: Show the chromatogram panel*
 - 3.2.2. LAB MEDIA: Figure 4. *Video editor: Zoom in on the main peaks labeled phloridzin and trilobatin.*
- 3.3. The relative standard deviation of the full ring injection peak area was 0.813 percent, indicating excellent stability and reproducibility of LC-mate injections [1].
 - 3.3.1. LAB MEDIA: Table 2. *Video editor: Highlight the row showing peak area values and the cell showing the relative standard deviation value of 0.813 percent.*

1. Chromatographic
Pronunciation link: <https://www.merriam-webster.com/dictionary/chromatographic>
IPA: /ˌkroʊ.mə.təˈgræf.ɪk/
Phonetic Spelling: kroh·muh·tuh·graf·ik
2. Ultraviolet
Pronunciation link: <https://www.merriam-webster.com/dictionary/ultraviolet>
IPA: /ˌʌl.trəˈvaɪ.ə.lət/
Phonetic Spelling: uhl·truh·vai·uh·luht
3. Transmission
Pronunciation link: <https://www.merriam-webster.com/dictionary/transmission>

- IPA: /trænz'mɪf.ən/
Phonetic Spelling: tranz·mish·uhn
4. Analog
Pronunciation link: <https://www.merriam-webster.com/dictionary/analog>
IPA: /'æn.ə,lɔ:g/
Phonetic Spelling: an·uh·lawg
5. Waveform
Pronunciation link: <https://www.merriam-webster.com/dictionary/waveform>
IPA: /'weɪv,fɔ:rm/
Phonetic Spelling: wave·form
6. Polarity
Pronunciation link: <https://www.merriam-webster.com/dictionary/polarity>
IPA: /pəʊ'ler.ə.ti/
Phonetic Spelling: poh·lair·uh·tee
7. Elution
Pronunciation link: <https://www.merriam-webster.com/dictionary/elution>
IPA: /i'lu:ʃən/
Phonetic Spelling: ih·loo·shuhn
8. Equilibrate
Pronunciation link: <https://www.merriam-webster.com/dictionary/equilibrate>
IPA: /i:kwə'lib.ri.ert/
Phonetic Spelling: ee·kwuh·lib·ree·ayt
9. Autosampler
Pronunciation link: <https://www.merriam-webster.com/dictionary/autosampler>
IPA: /,ɔ:.toʊ'sæm.plə/
Phonetic Spelling: aw·toh·sam·plur
10. Chromatogram
Pronunciation link: <https://www.merriam-webster.com/dictionary/chromatogram>
IPA: /kroʊ'mæt.ə,græm/
Phonetic Spelling: kroh·mat·uh·gram
11. Resolution
Pronunciation link: <https://www.merriam-webster.com/dictionary/resolution>
IPA: /,rez.ə'lu:ʃən/
Phonetic Spelling: rez·uh·loo·shuhn
12. Retention
Pronunciation link: <https://www.merriam-webster.com/dictionary/retention>
IPA: /rɪ'ten.ʃən/
Phonetic Spelling: rih·ten·shuhn
13. Threshold
Pronunciation link: <https://www.merriam-webster.com/dictionary/threshold>
IPA: /'θref.hould/
Phonetic Spelling: thresh·hohld
14. Syringe
Pronunciation link: <https://www.merriam-webster.com/dictionary/syringe>

- IPA: /səˈrɪndʒ/
Phonetic Spelling: suh·rinj
15. Purging
Pronunciation link: <https://www.merriam-webster.com/dictionary/purging>
IPA: /ˈpɜːr.dʒɪŋ/
Phonetic Spelling: pur·jing
16. Pipeline
Pronunciation link: <https://www.merriam-webster.com/dictionary/pipeline>
IPA: /ˈpaɪpˌlaɪn/
Phonetic Spelling: pipe·line
17. Cleansing
Pronunciation link: <https://www.merriam-webster.com/dictionary/cleansing>
IPA: /ˈklen.zɪŋ/
Phonetic Spelling: klen·zing
18. Metadata
Pronunciation link: <https://www.merriam-webster.com/dictionary/metadata>
IPA: /ˈmeɪ.ə.deɪ.tə/
Phonetic Spelling: meh·tuh·day·tuh
19. Semi-preparative
Pronunciation link: <https://www.merriam-webster.com/dictionary/semi-preparative>
IPA: /ˌsem.i.prɪˈper.ə.tɪv/
Phonetic Spelling: sem·ee·prih·pair·uh·tiv
20. Hyperoside
Pronunciation link: No confirmed link found
IPA: /haɪˈpɪr.oʊ.saɪd/
Phonetic Spelling: hy·peer·oh·syde
21. Phloridzin
Pronunciation link: No confirmed link found
IPA: /ˈflɔːr.ɪd.zɪn/
Phonetic Spelling: flor·id·zin
22. Trilobatin
Pronunciation link: No confirmed link found
IPA: /traɪˈlɒʊ.bə.tɪn/
Phonetic Spelling: try·loh·buh·tin
23. Reproducibility
Pronunciation link: <https://www.merriam-webster.com/dictionary/reproducibility>
IPA: /ˌrɪ.prəˌduː.səˈbɪl.ə.ti/
Phonetic Spelling: ree·pruh·doo·suh·bil·uh·tee
24. Deviation
Pronunciation link: <https://www.merriam-webster.com/dictionary/deviation>
IPA: /ˌdiː.viˈeɪ.ʃən/
Phonetic Spelling: dee·vee·ay·shuhn
25. 2''-O-galloyl-hyperoside
Pronunciation link: No confirmed link found

IPA: /tu: ʊʊ 'gæl.ɔɪl haɪ'pɪr.ʊʊ.said/

Phonetic Spelling: too·oh·gal·oyl·hy·peer·oh·syde