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Title: Elucidation of the Material Basis of Yiqi Qingjie Formula Against IgA Nephropathy Using UHPLC-Q-Orbitrap HRMS Integrated with Network Pharmacology

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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**

Author's Note: All SCREEN shots were filmed by the videographer.

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. **Yes**

Author's Note: The testimonials were not filmed during the shoot and can be omitted.

Current Protocol Length

Number of Steps: 13

Number of Shots: 43

Interviews

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. **Lan Lin:** Our study identifies bioactive compounds in traditional Yiqi Qingjie Formula for IgA nephropathy using HRMS, serum pharmacochimistry, and network pharmacology.
 - 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. **Lan Lin:** Existing methods rely on predicted compounds rather than absorbed ones; this protocol focuses on experimentally verified blood-absorbed constituents.
 - 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. **Lan Lin:** This protocol identifies orally absorbed herbal compounds in blood and predicts their therapeutic targets and potential biological pathways.
 - 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. **Lan Lin:** Accurate compound identification using this protocol requires high-quality MS data, proper serum sampling times, and careful sample preparation.
 - 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. **Lan Lin:** Future studies can validate predicted compound–target interactions and explore molecular mechanisms of Yiqi Qingjie Formula in IgA nephropathy.
 - 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.

Testimonial Questions (OPTIONAL):

Videographer: Please capture all testimonial shots in a wide-angle format with sufficient headspace, as the final videos will be rendered in a 1:1 aspect ratio. Testimonial statements will be presented live by the authors, sharing their spontaneous perspectives.

- Testimonial statements will **not appear in the video** but may be featured in the journal's promotional materials.
- **Provide the full name and position** (e.g., Director of [Institute Name], Senior Researcher [University Name], etc.) of the author delivering the testimonial.
- Please **answer the testimonial question live during the shoot**, speaking naturally and in your own words in **complete sentences**.

~~What problem in your field of research does your JoVE article address?~~

~~1.6 — **Lan Lin, PhD Candidate, Guang'anmen Hospital, China Academy of Chinese Medical Sciences:** (authors will present their testimonial statements live)~~

~~1.6.1 — INTERVIEW: Named talent says the statement above in an interview style shot, looking slightly off camera.~~

~~What value do you expect this video article to have for other researchers working in your field?~~

~~-~~

~~1.7 — **Lan Lin, PhD Candidate, Guang'anmen Hospital, China Academy of Chinese Medical Sciences:** (authors will present their testimonial statements live)~~

~~1.7.1 — INTERVIEW: Named talent says the statement above in an interview style shot, looking slightly off camera.~~

Author's Note: The testimonials were not filmed during the shoot and can be omitted.

Ethics Title Card

This research has been approved by the Institutional Animal Care and Use Committee (IACUC) at Animal Experimental Center of Guang'anmen Hospital

Protocol

***Videographer:** Please always record an audio slate while filming each shot mentioned in the shotlist.*

2. Preparation of Yiqi Qingjie Extract Samples

Demonstrator: Lan Lin

2.1. To begin, obtain the prepared Yiqi Qingjie concentrated herbal extract and methanolic reference standard solutions [1]. Pipette 100 microliters of the Yiqi Qingjie aqueous extract into a 1.5 milliliter centrifuge tube [2]. Add 300 microliters methanol to the tube [3] and vortex for 1 minute [4].

2.1.1. WIDE: Talent arranging the prepared Yiqi Qingjie concentrated herbal extract and methanolic reference standard solutions on the work bench.

2.1.2. Talent pipetting Yiqi Qingjie aqueous extract into a 1.5 milliliter centrifuge tube.

2.1.3. Talent adding 300 μ L methanol into the centrifuge tube.

2.1.4. Talent vortexing the tube.

2.2. Centrifuge the mixture at 1800 *g*, 4 degrees Celsius for 10 minutes [1]. Confirm that the resulting supernatant is clear and free of suspended particles [2]. Add 100 microliters of the supernatant to 100 microliters ultrapure water [3]. Vortex gently to mix [4] and transfer the solution into an autosampler vial [5].

2.2.1. Talent placing the tube into a centrifuge.

2.2.2. Shot of a clear supernatant without visible particles.

2.2.3. Talent adding 100 μ L supernatant to 100 μ L ultrapure water.

2.2.4. Talent gently vortexing the mixture.

2.2.5. Talent transferring the diluted solution into an autosampler vial.

3. Preparation of Drug-Containing Serum Samples

3.1. Obtain frozen, hemolysis-free serum samples collected from terminally anesthetized Sprague–Dawley rats after controlled dosing with Yiqi Qingjie extract [1]. Pipette 100 microliters of serum into a 1.5 milliliter centrifuge tube [2]. Add 300 microliters methanol [3] and vortex for 10 minutes [4]. Centrifuge the mixture at 1800 *g*, 4 degrees Celsius for 10 minutes [5].

3.1.1. Talent bringing the frozen serum samples to the clean workbench.

- 3.1.2. Talent pipetting 100 μ L serum into a 1.5 mL centrifuge tube.
- 3.1.3. Talent adding 300 μ L methanol into the tube.
- 3.1.4. Shot of the talent vortexing the tube.
- 3.1.5. Talent placing the tube into a centrifuge.

3.2. Collect 270 microliters of the supernatant and transfer it to a clean tube [1]. Use a vacuum centrifugal concentrator set at 120 g, 37 degrees Celsius for 4 hours [2] to dry the supernatant completely [3].

- 3.2.1. Talent transferring supernatant into a clean tube.
- 3.2.2. Talent placing the tube into a vacuum centrifugal concentrator.
Videographer: Please show the settings on the concentrator once it is set to 120 g, 37 °C for 4 h
- 3.2.3. CU: Shot of the solid residue after drying.

3.3. Add 90 microliters of 50 percent methanol aqueous solution to the residue [1]. Vortex for 1 minute to dissolve the residue completely [2] and centrifuge at 1800 g, 4 degrees Celsius for 10 minutes [3]. Collect 80 microliters of the supernatant and transfer it into an autosampler vial [4].

- 3.3.1. Talent adding 90 μ L of 50% methanol aqueous solution to the residue.
- 3.3.2. Talent vortexing the tube.
- 3.3.3. Talent placing the tube into a centrifuge.
- 3.3.4. Talent collecting 80 μ L of the supernatant and transferring it into an autosampler vial.

4. UHPLC-Orbitrap MS Setup and Acquisition Workflow

4.1. Switch on and initiate the ultra-high-performance liquid chromatography or UHPLC (U-H-P-L-C) system equipped with a chromatographic column [1]. Keep the binary mobile phase ready, with Phase A as water containing 0.1 percent formic acid and Phase B as acetonitrile [2]. Apply a gradient elution program at a flow rate of 0.3 milliliters per minute [3]. Set the column temperature to 40 degrees Celsius and the injection volume to 6.0 microliters [4].

- 4.1.1. WIDE: Shot of the talent initiating the UHPLC system.
- 4.1.2. Shot of the bottles with the detailed labels clearly visible.
- 4.1.3. SCREEN: The gradient and flow rate is being set
- 4.1.4. SCREEN: The temperature is being set to 40 °C, and injection volume is being set to 6.0 μ L.

Author's Note: All SCREEN shots were filmed by the videographer.

- 4.2. Next, switch on and initiate a quadrupole orbitrap mass spectrometer equipped with a heated electrospray ionization spray probe [1]. Set the resolution to 17500, auto gain control target as 1×10^5 , maximum isolation time 50 milliseconds and select up to 10 most intense parent ions with dynamic exclusion, isolation window of mass to charge 2, collision energy of 10 volts, 30 volts, and 60 volts, and intensity threshold 1×10^5 [2]. Set the ionization voltage to 3.7 kilovolts in positive mode or 3.5 kilovolts in negative mode, capillary temperature to 320 degrees Celsius, sheath gas pressure to 30 pounds per square inch, auxiliary gas pressure to 10 pounds per square inch, desolvation temperature to 300 degrees Celsius, and use nitrogen as sheath, auxiliary, and collision gas at 1.5 millitorr [3]. **Note:** The VO is added for the moved shot and the numbers are adjusted accordingly

4.2.1. WIDE: Shot of the talent switching on and initiating the mass spectrometer.

4.4.1. SCREEN: Display tandem mass spectrometry parameter configuration. **Note:** This shot and corresponding VO were moved here as per author's instructions to match the actual filming sequence.

4.2.2. SCREEN: The parameters are being set in this sequence: Ionization voltage: 3.7 kV (positive mode) / 3.5 kV (negative mode), capillary temperature: 320 °C, sheath gas pressure: 30 psi, auxiliary gas pressure: 10 psi, desolvation temperature: 300 °C, sheath gas and the auxiliary gas: nitrogen, collision gas: nitrogen, 1.5 mTorr.

Author's Note: All SCREEN shots were filmed by the videographer.

- 4.3. Set full scan parameters with resolution 70000, auto gain control target 1 multiplied by 10 to the power of 6, maximum isolation time 50 milliseconds, and mass to charge scan range 100 to 1500 [1]. Acquire data in **Full scan/dd-MS² (full scan data dependent tandem mass spectrometry)** mode [2].

4.3.1. SCREEN: Display full scan parameter settings in this sequence: resolution 70000, auto gain control target 1×10^6 , maximum isolation time 50 ms, and m/z scan range 100 – 1500.

4.3.2. SCREEN: Show the selection of data acquisition mode.

Author's Note: All SCREEN shots were filmed by the videographer.

- 4.4. ~~Now, set the resolution to 17500, auto gain control target as 1 multiplied by 10 to the power of 5, maximum isolation time 50 milliseconds and select up to 10 most intense parent ions with dynamic exclusion, isolation window of mass to charge 2, collision energy of 10 volts, 30 volts, and 60 volts, and intensity threshold 1 multiplied by 10 to the power of 5 [1]. Collect tandem mass spectrometry data [2].~~

- 4.4.1. ~~SCREEN: Display tandem mass spectrometry parameter configuration.~~ **Note:**
The authors requested to move this shot between 4.2.1 and 4.2.2
- 4.4.2. ~~SCREEN: Tandem mass spectrometry data is being collected.~~
Author's Note: Shot 4.4.2 was not filmed during the shoot and can be omitted.

5. Mass Spectrometry-Based Compound Identification

- 5.1. Use specialized data processing software to process the acquired mass spectrometry data [1]. Navigate to the **Import Data** module and upload raw data files [2]. Execute the peak picking module and perform peak deconvolution to group adduct ions and generate compound features [3]. Search reference compound databases, target prediction databases, and relevant literature for candidate identification [4].
- 5.1.1. SCREEN: The specialized data processing software is being opened.
- 5.1.2. SCREEN: **Import data** module is being clicked, and raw data files are being uploaded.
- 5.1.3. SCREEN: Peak picking module is being executed and peak deconvolution is being performed to group adduct ions and generate compound features.
- 5.1.4. SCREEN: The query is executed to search reference compound databases, target prediction databases, and relevant literature for candidate identification.
Author's Note: All SCREEN shots were filmed by the videographer.
- 5.2. Compare experimental data with reference standards to confirm matches [1]. Accept compound identification only when mass accuracy is within plus or minus 5 parts per million, spectral similarity is at least 0.85, isotopic matching is at least 90 percent, and retention time deviation is less than 0.2 minutes [2]. Annotate the identified compounds as MSI Level 1 [3].
- 5.2.1. SCREEN: Experimental and reference spectra are being compared.
- 5.2.2. SCREEN: Results are being checked against the validation criteria. **Note:** Shot 5.2.2 was combined with 5.2.1 and the validation criteria checking is included within the comparison process shown in shot 5.2.1.
- 5.2.3. SCREEN: The identified compounds meeting all the necessary criteria are being annotated as MSI Level 1.
Author's Note: All SCREEN shots were filmed by the videographer.
- 5.3. Use accurate mass measurements and computational spectral prediction to perform putative identification for compounds without reference standards [1]. Accept identifications when precursor ion mass error is within plus or minus 5 parts per million, fragment ion mass error within plus or minus 10 parts per million, spectral similarity is at least 0.60, isotopic matching is at least 90 percent, and retention time deviation is less than 1.0 minute [2]. Annotate the putatively identified compounds as MSI Level 2

[3].

- 5.3.1. SCREEN: Accurate mass measurements and computational spectral prediction are being used for putative identification of compounds without reference standards.
- 5.3.2. SCREEN: Results are being checked against the validation criteria.
- 5.3.3. SCREEN: The identified compounds meeting all the necessary criteria are being annotated as MSI Level 2.

Author's Note: All SCREEN shots were filmed by the videographer.

5.4. The identified compounds are further investigated using network pharmacology [1] and molecular docking to support downstream interpretation and results [2].

- 5.4.1. SCREEN: The identified compound is being investigated using network pharmacology. *Videographer: Please capture a representative shot of the talent investigating the compound using network pharmacology on as a backup.*
- 5.4.2. SCREEN: Molecular docking studies being performed using the identified compound. *Videographer: Please capture a representative shot of the talent performing molecular docking studies as a backup.*

Author's Note: All SCREEN shots were filmed by the videographer.

Results

6. Results

- 6.1. The Base Peak Ion chromatogram of the Yiqi Qingjie aqueous extract was obtained in positive ion mode [1] and in negative ion mode [2].
 - 6.1.1. LAB MEDIA: Figure 1. *Video editor: Highlight panel A*
 - 6.1.2. LAB MEDIA: Figure 1. *Video editor: Highlight panel B*
- 6.2. In the PPI (*P-P-I*) network, node size and color varied with parameter values [1]. TP53 (*T-P-53*), SRC (*S-R-C*), AKT1 (*A-K-T-1*), ESR1 (*E-S-R-1*), and STAT3 (*stat-3*) ranked among the top five core targets [2].
 - 6.2.1. LAB MEDIA: Figure 5. *Video editor: Show the network and emphasize that some circles are larger and darker while others are smaller and lighter*
 - 6.2.2. LAB MEDIA: Figure 5. *Video editor: Highlight the node labeled TP53, SRC, AKT1, ESR1, STAT3*
- 6.3. The molecular docking binding energy heatmap presented the binding energies between core targets and key blood-absorbed compounds [1] with ligands represented along the vertical axis [2] and receptor proteins along the horizontal axis [3].
 - 6.3.1. LAB MEDIA: Figure 9. *Video editor: Show the full heatmap including the color scale and all rows and columns.*
 - 6.3.2. LAB MEDIA: Figure 9. *Video editor: Sequentially highlight the rows labeled Deacetylasperulosidic Acid, Dioscin, and Geniposidic Acid, quickly highlighting each row one at a time.*
 - 6.3.3. LAB MEDIA: Figure 9. *Video editor: Sequentially highlight the column labeled CASP3, IGF1R, JUN, STAT3, PRKCB, and PIK3CA, quickly highlighting each column one at a time.*

Pronunciation Guide:

1. Methanolic
 Pronunciation link: <https://www.merriam-webster.com/dictionary/methanolic>
 IPA: /ˌmɛθəˈnɑːlɪk/
 Phonetic Spelling: meh·tuh·naa·lik
2. Centrifuge
 Pronunciation link: <https://www.merriam-webster.com/dictionary/centrifuge>
 IPA: /ˈsɛntrəˌfjuːdʒ/
 Phonetic Spelling: sen·truh·fyooj

3. Supernatant
Pronunciation link: <https://www.merriam-webster.com/dictionary/supernatant>
IPA: /ˌsuːpərˈneɪtənt/
Phonetic Spelling: soo·per·nay·tuhnt
4. Ultrapure
Pronunciation link: <https://www.merriam-webster.com/dictionary/ultrapure>
IPA: /ˌʌltrəˈpjʊr/
Phonetic Spelling: uhl·truh·pyur
5. Autosampler
Pronunciation link: <https://www.merriam-webster.com/dictionary/autosampler>
IPA: /ˌɔːtoʊˈsæmplər/
Phonetic Spelling: aw·toh·sam·pler
6. Hemolysis
Pronunciation link: <https://www.merriam-webster.com/dictionary/hemolysis>
IPA: /hɪˈmɑːləsɪs/
Phonetic Spelling: hih·maa·luh·suhs
7. Anesthetized
Pronunciation link: <https://www.merriam-webster.com/dictionary/anesthetized>
IPA: /əˈnɛsθəˈtaɪzd/
Phonetic Spelling: uh·nes·thuh·tyzd
8. Centrifugal
Pronunciation link: <https://www.merriam-webster.com/dictionary/centrifugal>
IPA: /sɛnˈtrɪfjʊɡəl/
Phonetic Spelling: sen·trif·yuh·guhl
9. Concentrator
Pronunciation link: <https://www.merriam-webster.com/dictionary/concentrator>
IPA: /ˈkɒːnsənˌtreɪtər/
Phonetic Spelling: kaan·suhn·tray·ter
10. Residue
Pronunciation link: <https://www.merriam-webster.com/dictionary/residue>
IPA: /ˈrɛzəˌduː/
Phonetic Spelling: rez·uh·doo
11. Chromatographic
Pronunciation link: <https://www.merriam-webster.com/dictionary/chromatographic>
IPA: /ˌkroʊmətəˈgræfɪk/
Phonetic Spelling: kroh·muh·tuh·graf·ik
12. Acetonitrile
Pronunciation link: <https://www.merriam-webster.com/dictionary/acetonitrile>
IPA: /ˌæsətoʊˈnɛtriːl/
Phonetic Spelling: a·suh·toh·ny·treel
13. Gradient
Pronunciation link: <https://www.merriam-webster.com/dictionary/gradient>

IPA: /'greɪdiənt/

Phonetic Spelling: gray·dee·uhnt

14. Quadrupole

Pronunciation link: <https://www.merriam-webster.com/dictionary/quadrupole>

IPA: /'kwɑːdrəˌpɔːl/

Phonetic Spelling: kwah·druh·pohl

15. Spectrometer

Pronunciation link: <https://www.merriam-webster.com/dictionary/spectrometer>

IPA: /spɛk'trɑːmɛtər/

Phonetic Spelling: spek·trom·uh·ter

16. Electrospray

Pronunciation link: <https://www.merriam-webster.com/dictionary/electrospray>

IPA: /ɪ'lektroʊˌspreɪ/

Phonetic Spelling: ih·lek·troh·spray

17. Ionization

Pronunciation link: <https://www.merriam-webster.com/dictionary/ionization>

IPA: /ˌaɪənə'zeɪʃən/

Phonetic Spelling: eye·uh·nuh·zay·shuhn

18. Capillary

Pronunciation link: <https://www.merriam-webster.com/dictionary/capillary>

IPA: /'kæpəˌlɛri/

Phonetic Spelling: kap·uh·lair·ee

19. Desolvation

Pronunciation link: <https://www.merriam-webster.com/dictionary/desolvation>

IPA: /diˌsɔːl'veɪʃən/

Phonetic Spelling: dee·saal·vay·shuhn

20. Resolution

Pronunciation link: <https://www.merriam-webster.com/dictionary/resolution>

IPA: /ˌrɛzə'luːʃən/

Phonetic Spelling: rez·uh·loo·shuhn

21. Isolation

Pronunciation link: <https://www.merriam-webster.com/dictionary/isolation>

IPA: /ˌaɪsə'leɪʃən/

Phonetic Spelling: eye·suh·lay·shuhn

22. Milliseconds

Pronunciation link: <https://www.merriam-webster.com/dictionary/millisecond>

IPA: /'mɪləˌsekənd/

Phonetic Spelling: mil·uh·sek·uhndz

23. Spectrometry

Pronunciation link: <https://www.merriam-webster.com/dictionary/spectrometry>

IPA: /spɛk'trɑːmɛtri/

Phonetic Spelling: spek·trom·uh·tree

24. Deconvolution
Pronunciation link: <https://www.merriam-webster.com/dictionary/deconvolution>
IPA: /ˌdiːkɔːnvəˈluːʃən/
Phonetic Spelling: dee·kon·vuh·loo·shuhn
25. Adduct
Pronunciation link: <https://www.merriam-webster.com/dictionary/adduct>
IPA: /ˈædʌkt/
Phonetic Spelling: ad·uhkt
26. Isotopic
Pronunciation link: <https://www.merriam-webster.com/dictionary/isotopic>
IPA: /ˌaɪsəˈtɔːpɪk/
Phonetic Spelling: eye·suh·taa·pik
27. Retention
Pronunciation link: <https://www.merriam-webster.com/dictionary/retention>
IPA: /rɪˈtɛnʃən/
Phonetic Spelling: rih·ten·shuhn
28. Deviation
Pronunciation link: <https://www.merriam-webster.com/dictionary/deviation>
IPA: /ˌdiːviˈeɪʃən/
Phonetic Spelling: dee·vee·ay·shuhn
29. Pharmacology
Pronunciation link: <https://www.merriam-webster.com/dictionary/pharmacology>
IPA: /ˌfɑːrməˈkɔːlədʒi/
Phonetic Spelling: far·muh·kaa·luh·jee
30. Docking
Pronunciation link: <https://www.merriam-webster.com/dictionary/docking>
IPA: /ˈdɔːkɪŋ/
Phonetic Spelling: daak·ing