Separation and purification of active ingredients in tobacco by free flow electrophoresis

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Table 1 The chemical structures of isolated compounds and purity

Fig. S1 Schematic of the overall free-flow electrophoresis unit.

Fig. S2 The pH curve was established by running 15 mmol/L NaCl solution at 300 V (left) and 400 V (right) for 1 h.

Fig. S3~24 Gas chromatogram of the NO.1~22 fraction.

Fig. S25 Mass spectrum of 2-hydroxy-2-cyclopenten-1-one.

Fig. S26 Mass spectrum of 1-(2-methyl-1,3-oxathiolan-2-yl) ethanone.

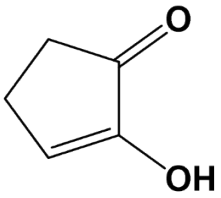
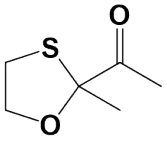
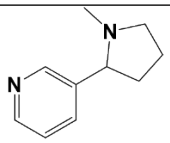
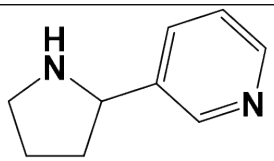
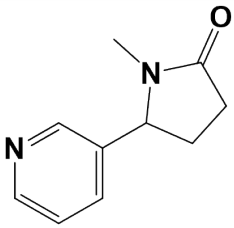
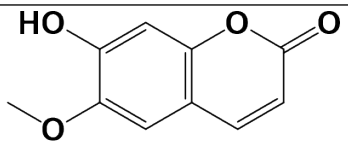
Fig. S27 Mass spectrum of nicotine.

Fig. S28 Mass spectrum of nornicotine.

Fig. S29 Mass spectrum of cotinine.

Fig. S30 Mass spectrum of scopoletin.

Table 1 The chemical structures of isolated compounds and purity

Compounds	chemical structure	purity
2-hydroxy-2-cyclopenten-1-one		4.769 % (tube 20)
1-(2-methyl-1,3-oxathiolan-2-yl) ethanone		5.44 % (tube 21)
nicotine		74.33 % (tube 19)
nornicotine		3.25 % (tube 15)
cotinine		1.20 % (tube 10)
scopoletin		2.49 % (tube 18)

The illustration of overall free-flow electrophoresis unit

The whole free-flow isoelectric focusing electrophoresis device was built with six parts: gas-liquid buffer (GCI), free-flow electrophoresis separation system, cooling device, self-balancing collection device (SBC), high-voltage power supply, and pump system. The electrophoresis buffer was transferred to GCI unit through constant flow pump. Then, the buffer was pushed into separation chamber at higher position through the capillary silica vessel. The sample with background buffer solution was separated in the separation chamber with the electric field force. The fractions flowed to the collection device and were collected through capillary silica vessel. At the same time, the cooling device was closely integrated base of separation chamber to promote heat dissipation and control temperature of the separation chamber. The pump system consists of five constant flow pumps for anode electrolyte circulation, cathode electrolyte circulation, cooling water circulation, pump-in buffer and sample loading.

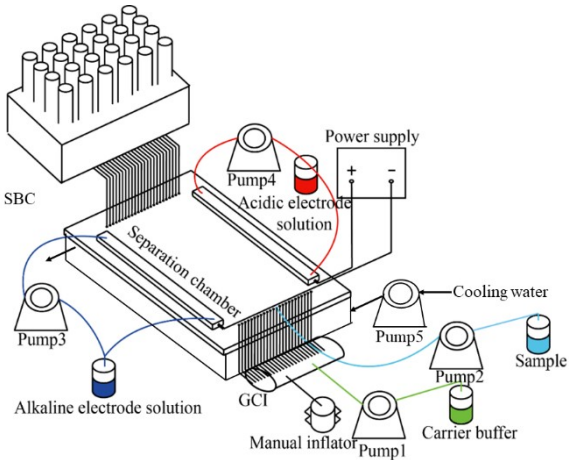


Figure S1. Schematic of the overall free-flow electrophoresis unit. Including gas-liquid buffer (GCI), separation chamber, cooling device, self-balancing collection device (SBC), power supply, and pump system consisting of buffer solution drive pump (Pump 1), sample pump (Pump 2), electrode buffer circulation pump (Pump 3,4), and cooling water circulation pump (Pump 5).

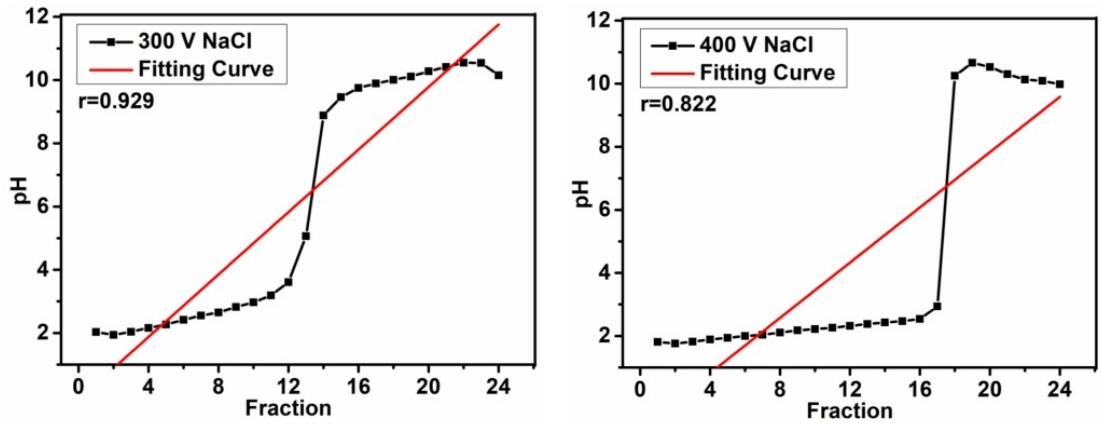


Figure S2. The pH curve was established by running 15 mmol/L NaCl solution at 300 V (left) and 400 V (right) for 1 h. The linear correlation coefficient was: 0.929 and 0.822.

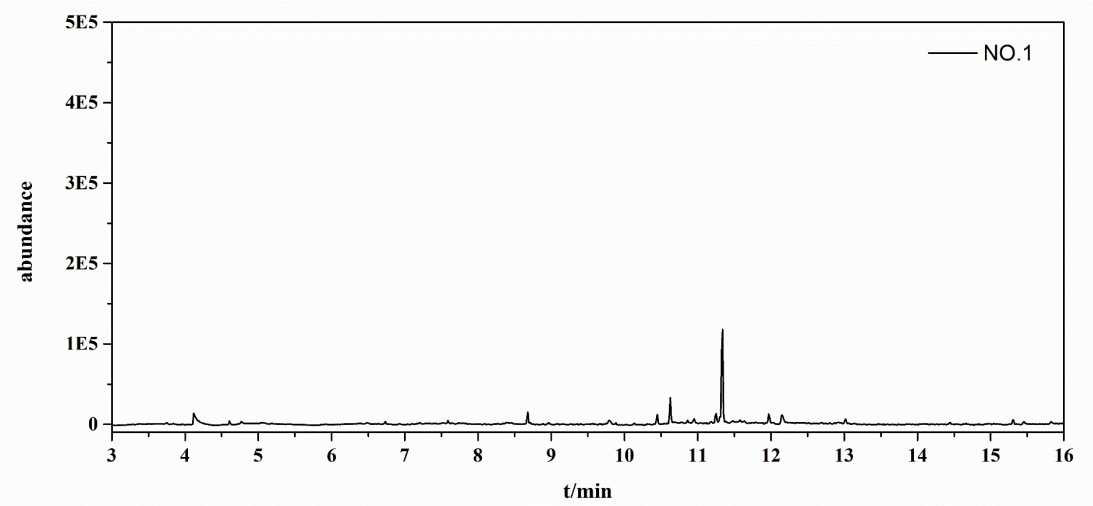


Figure S3. Gas chromatogram of the NO.1 fraction.

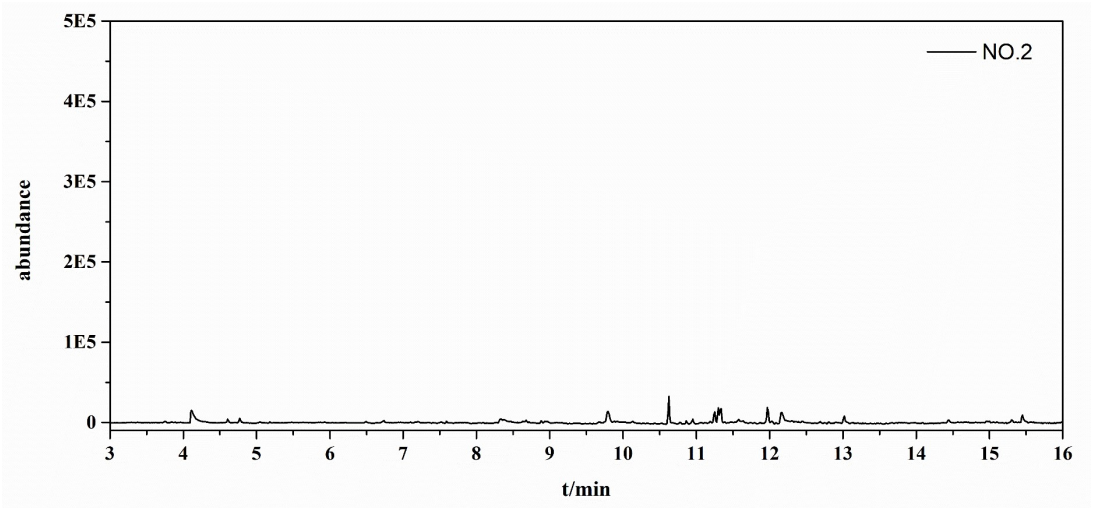


Figure S4. Gas chromatogram of the NO.2 fraction.

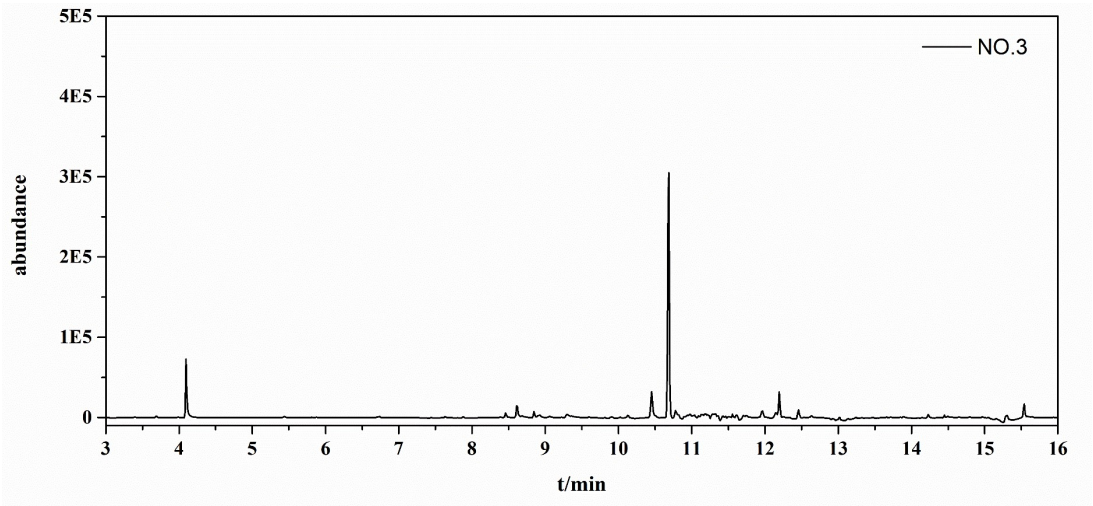


Figure S5. Gas chromatogram of the NO.3 fraction.

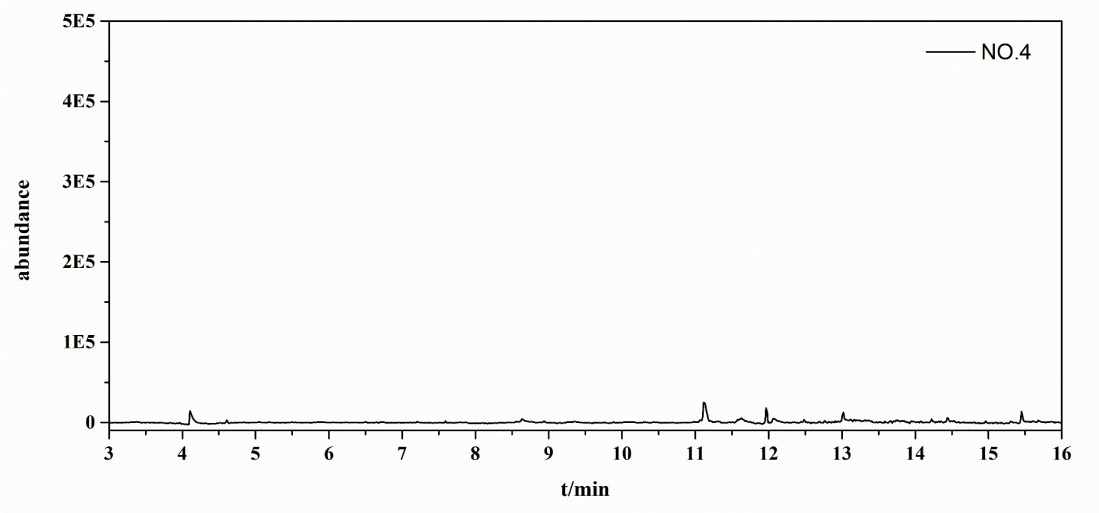


Figure S6. Gas chromatogram of the NO.4 fraction.

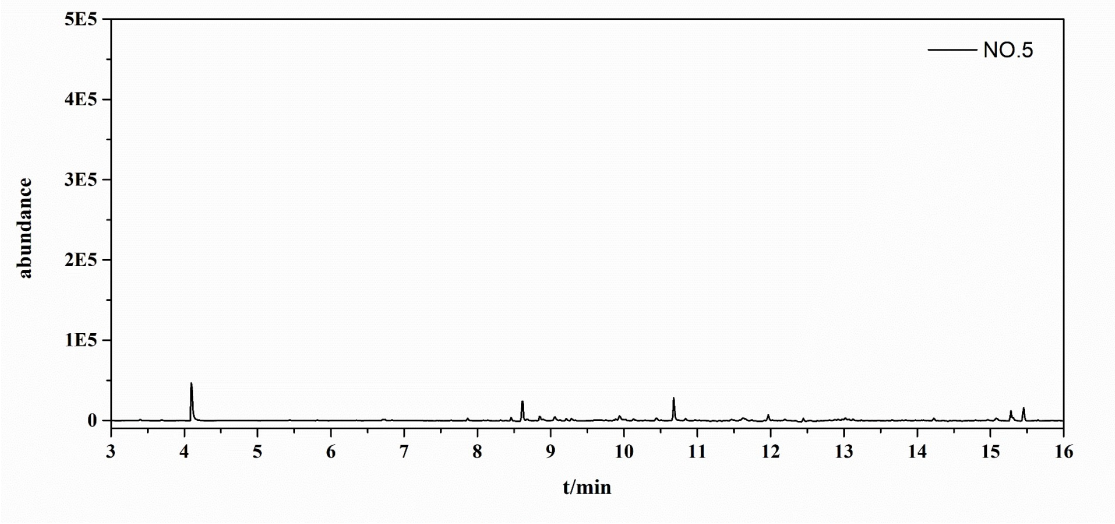


Figure S7 Gas chromatogram of the NO.5 fraction.

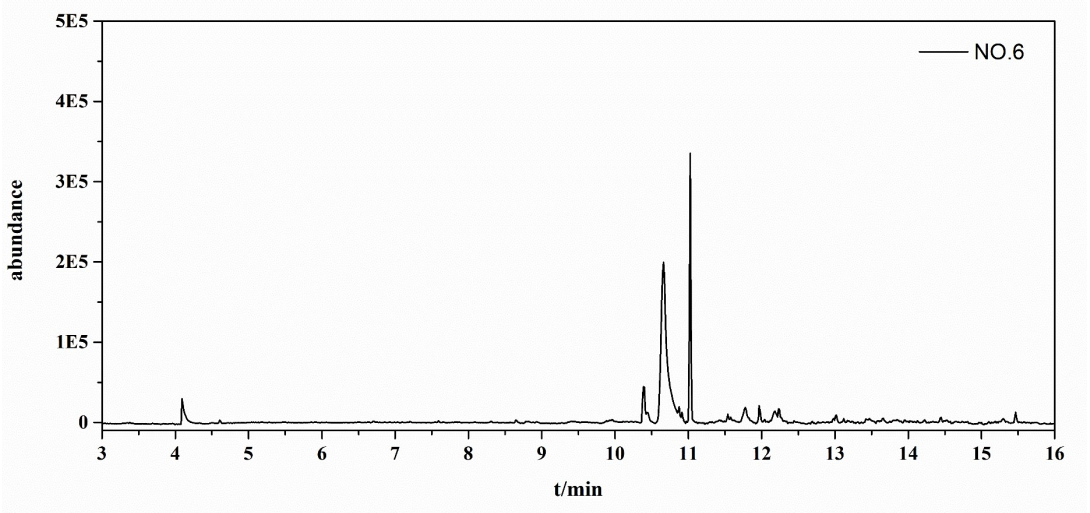


Figure S8. Gas chromatogram of the NO.6 fraction.

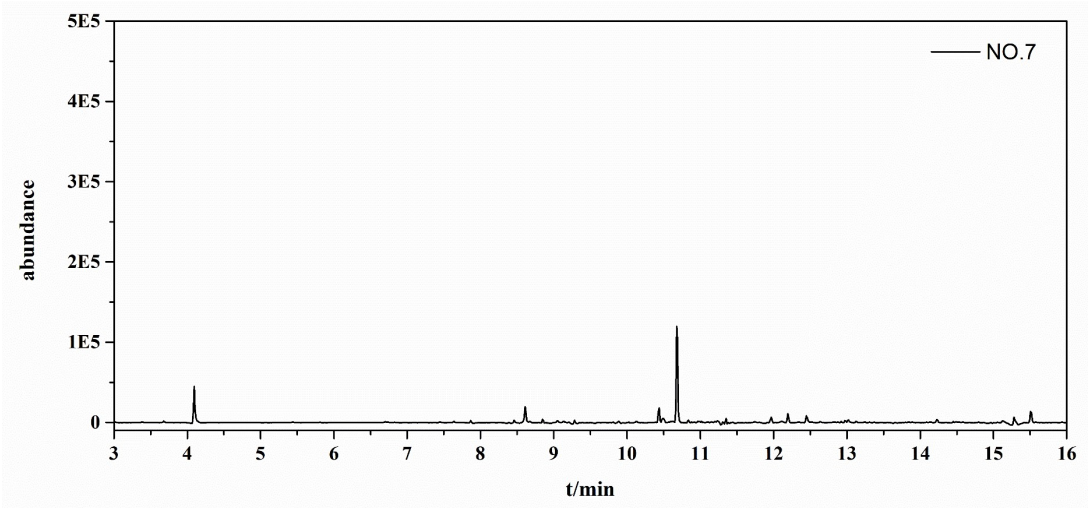


Figure S9. Gas chromatogram of the NO.7 fraction.

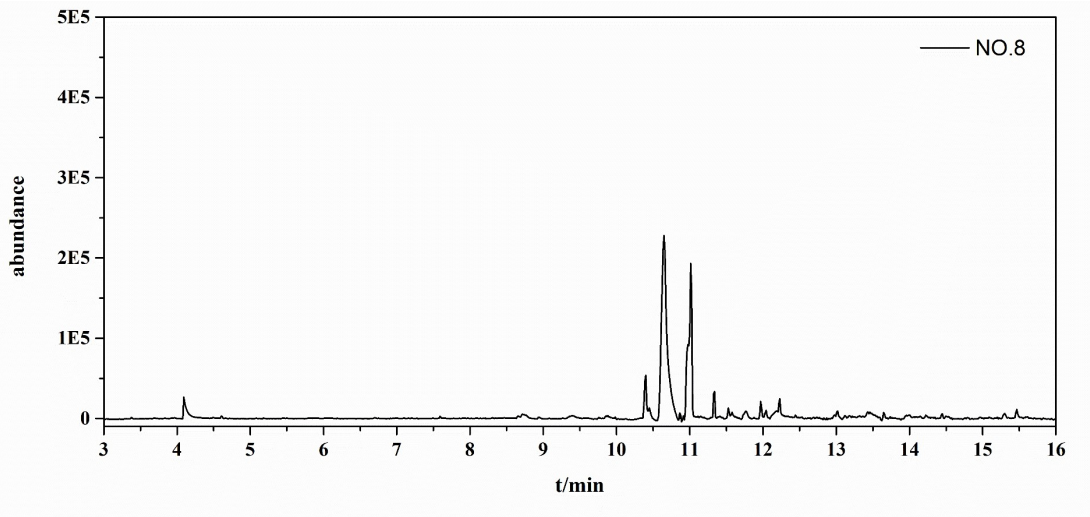


Figure S10. Gas chromatogram of the NO.8 fraction.

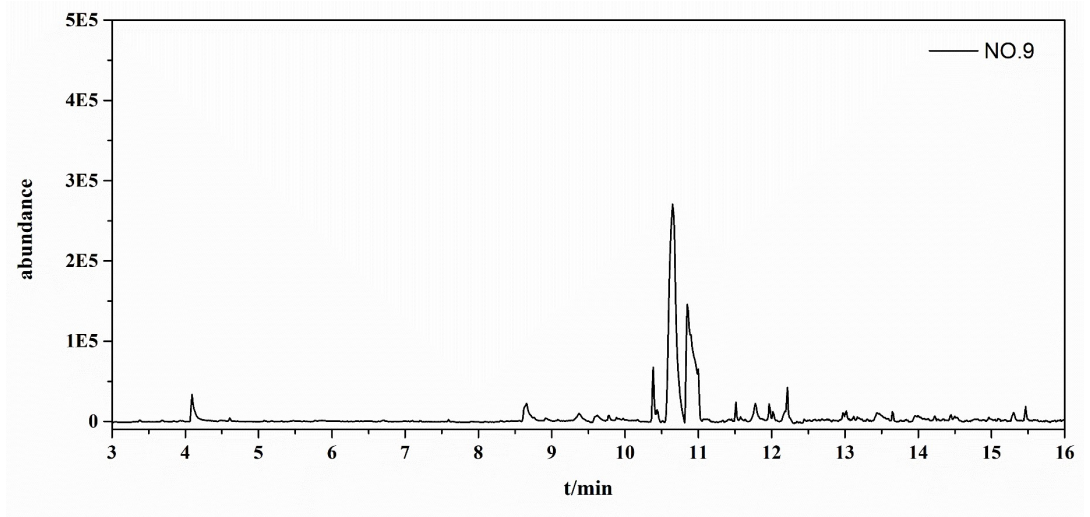


Figure S11. Gas chromatogram of the NO.9 fraction.

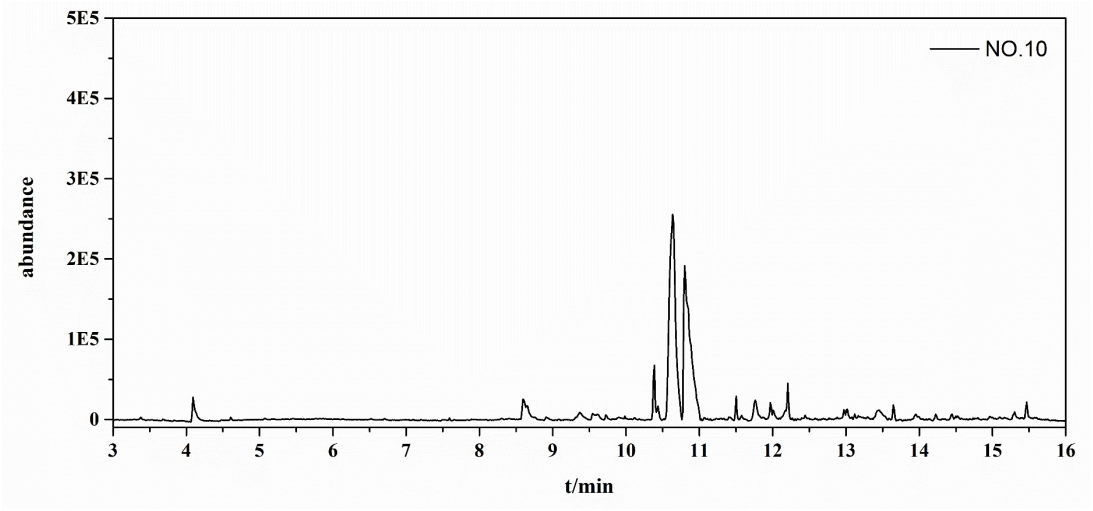


Figure S12. Gas chromatogram of the NO.10 fraction.

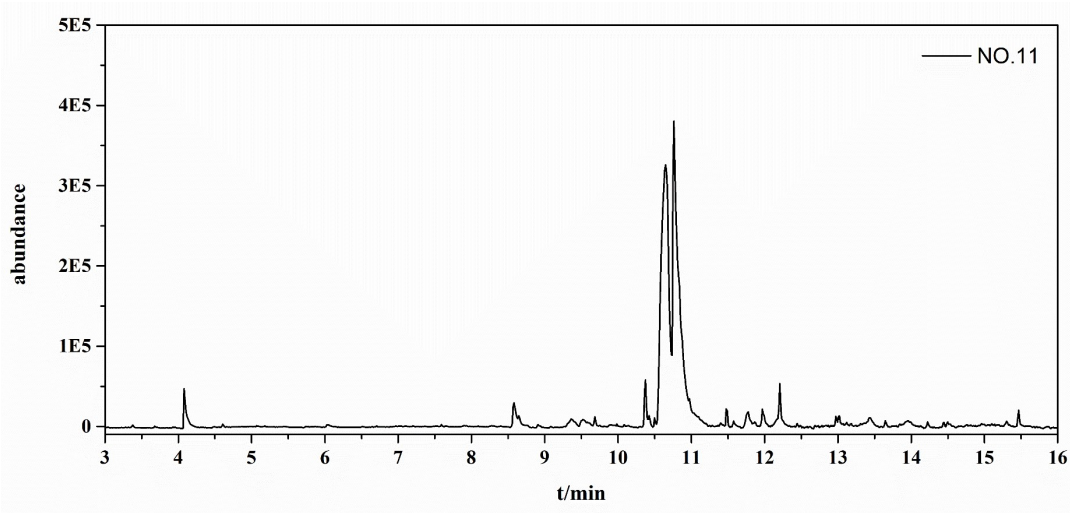


Figure S13. Gas chromatogram of the NO.11 fraction.

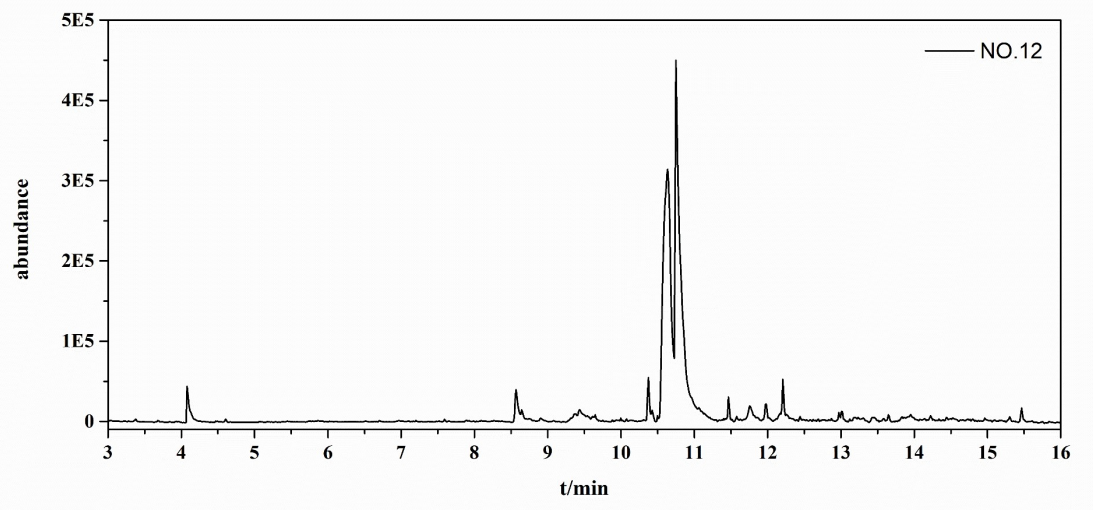


Figure S14. Gas chromatogram of the NO.12 fraction.

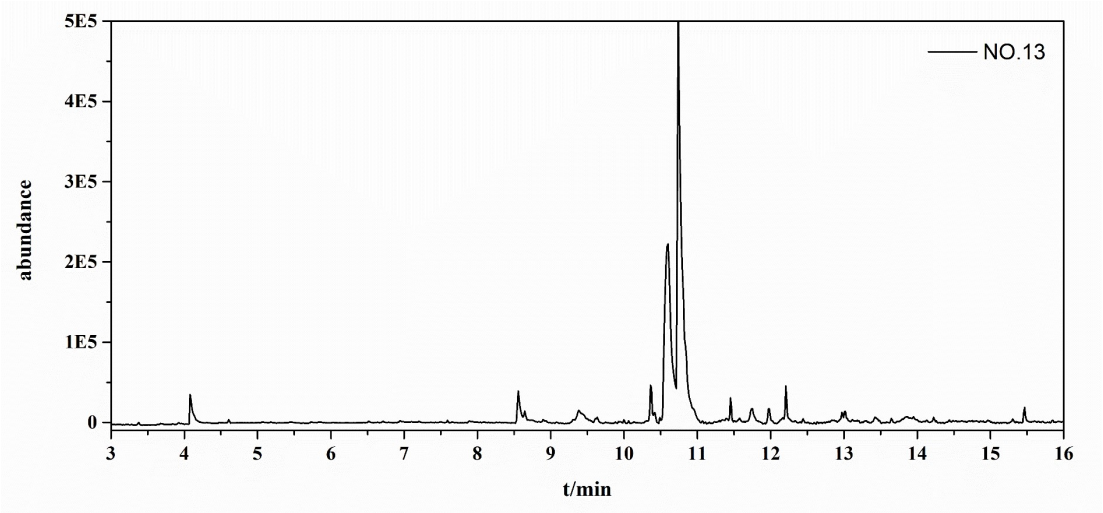


Figure S15. Gas chromatogram of the NO.13 fraction.

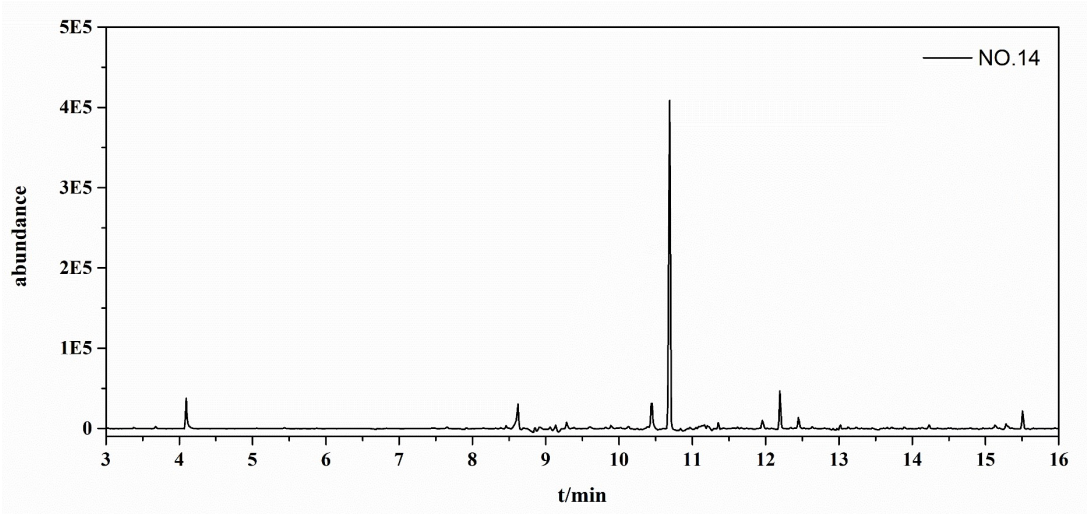


Figure S16. Gas chromatogram of the NO.14 fraction.

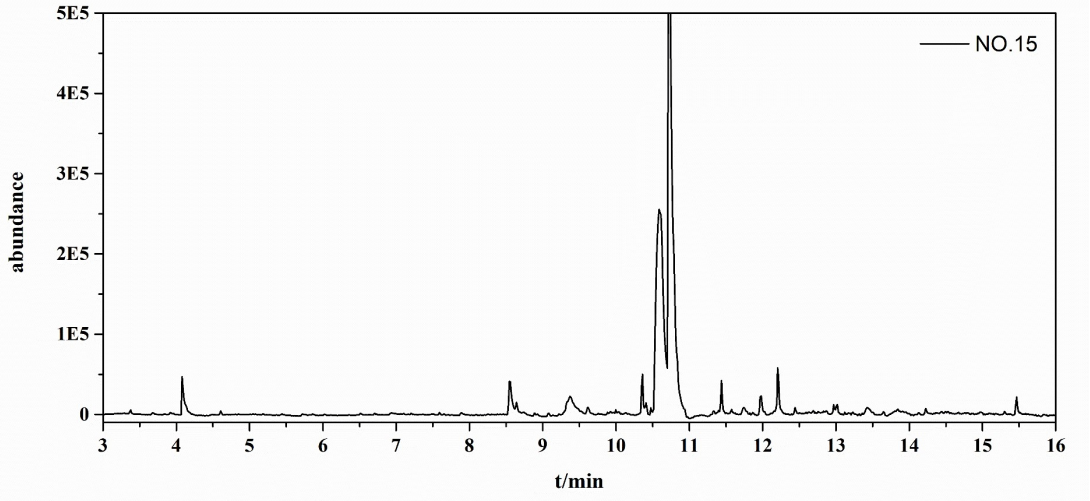


Figure S17. Gas chromatogram of the NO.15 fraction.

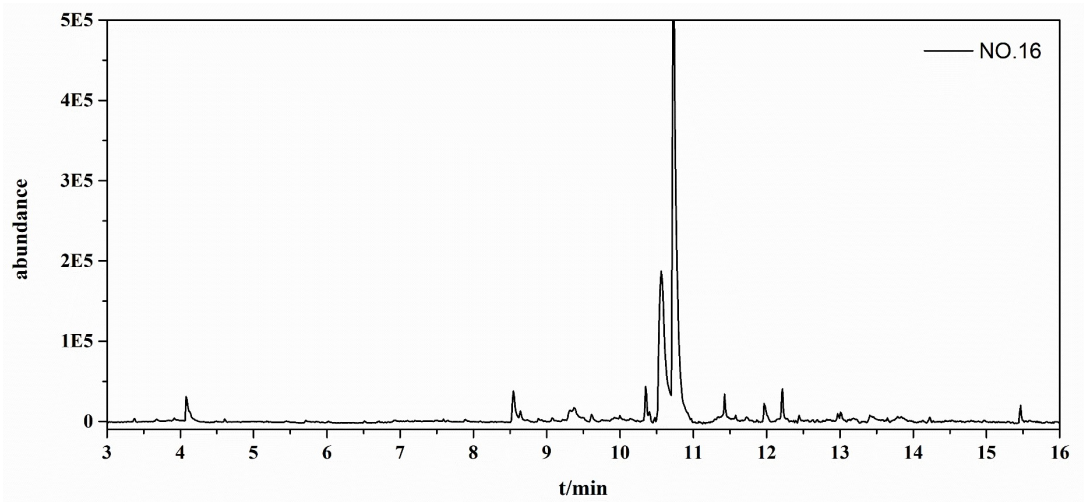


Figure S18. Gas chromatogram of the NO.16 fraction.

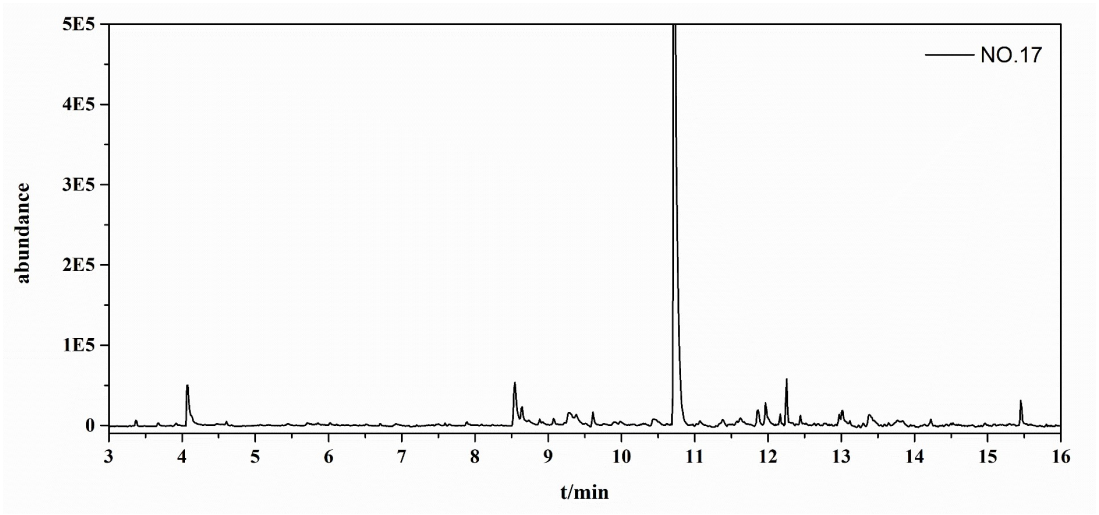


Figure S19. Gas chromatogram of the NO.17 fraction.

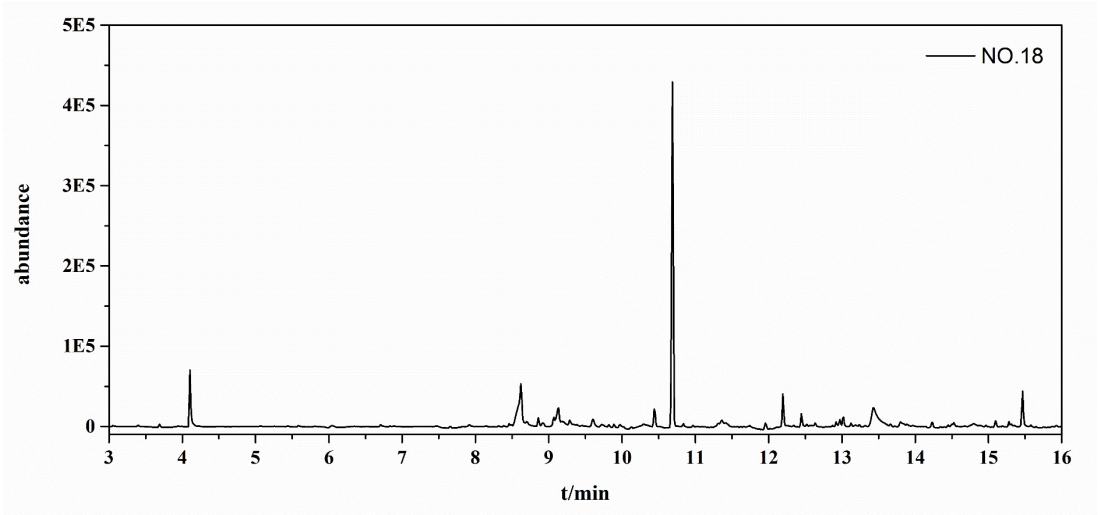


Figure S20. Gas chromatogram of the NO.18 fraction.

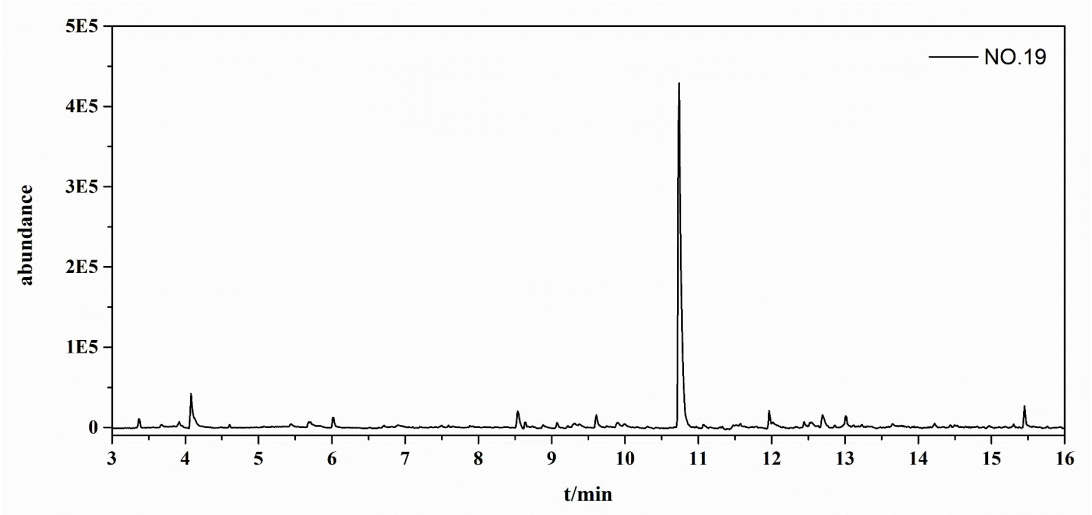


Figure S21. Gas chromatogram of the NO.19 fraction.

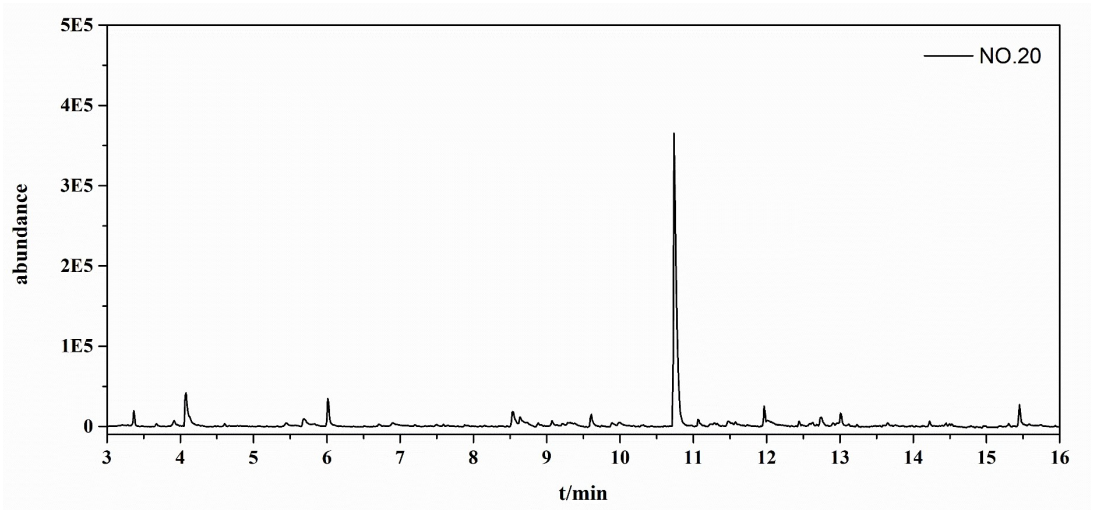


Figure S22. Gas chromatogram of the NO.20 fraction.

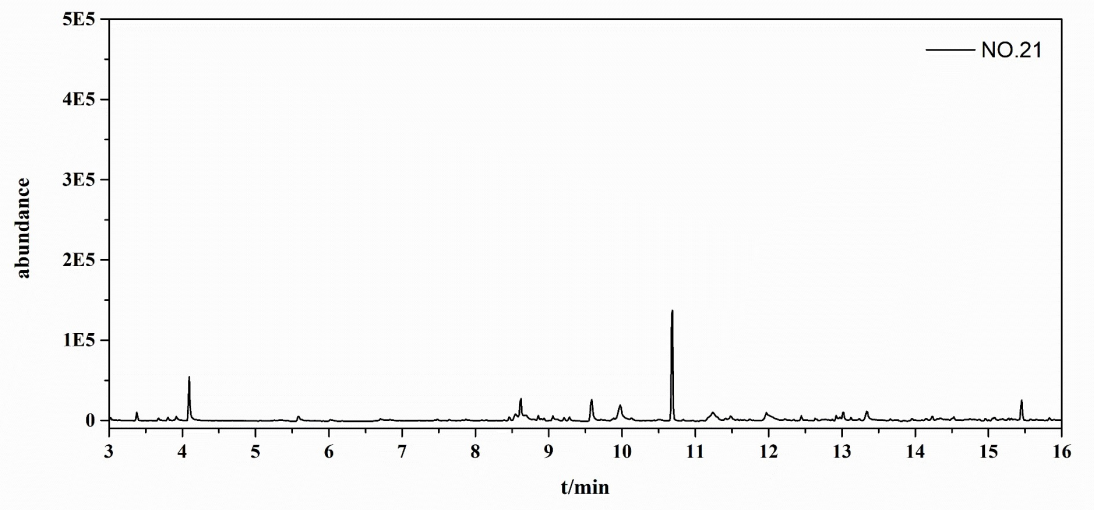


Figure S23. Gas chromatogram of the NO.21 fraction.

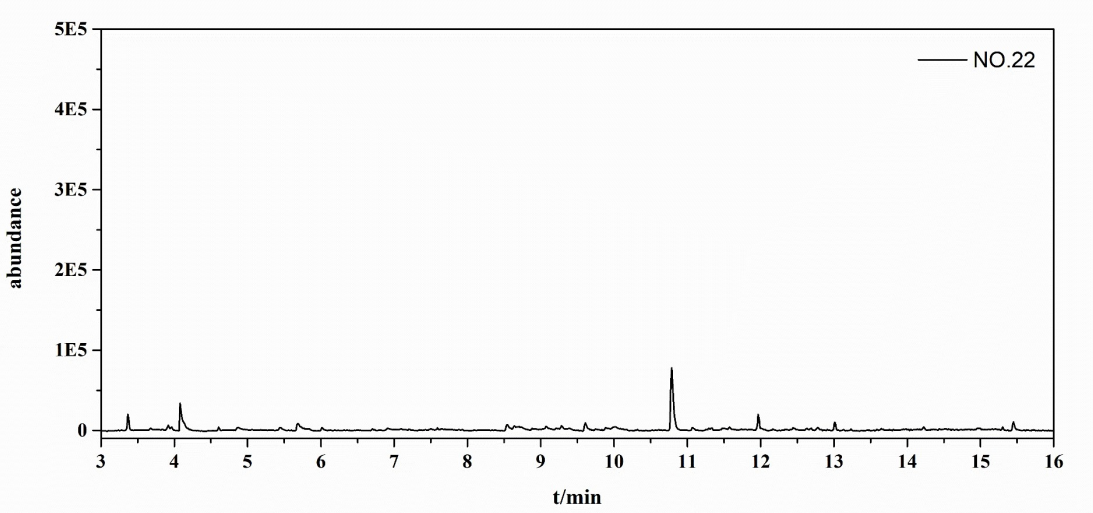


Figure S24. Gas chromatogram of the NO.22 fraction.

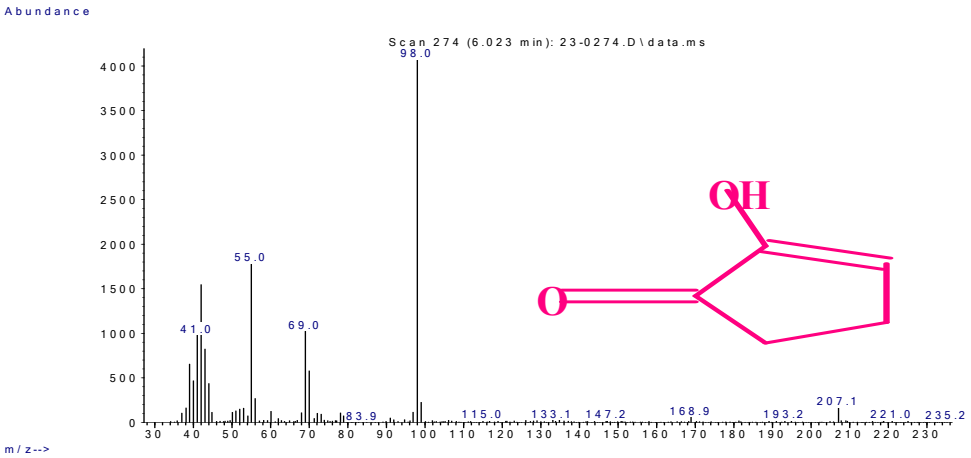


Figure S25. Mass spectrum of 2-hydroxy-2-cyclopenten-1-one.

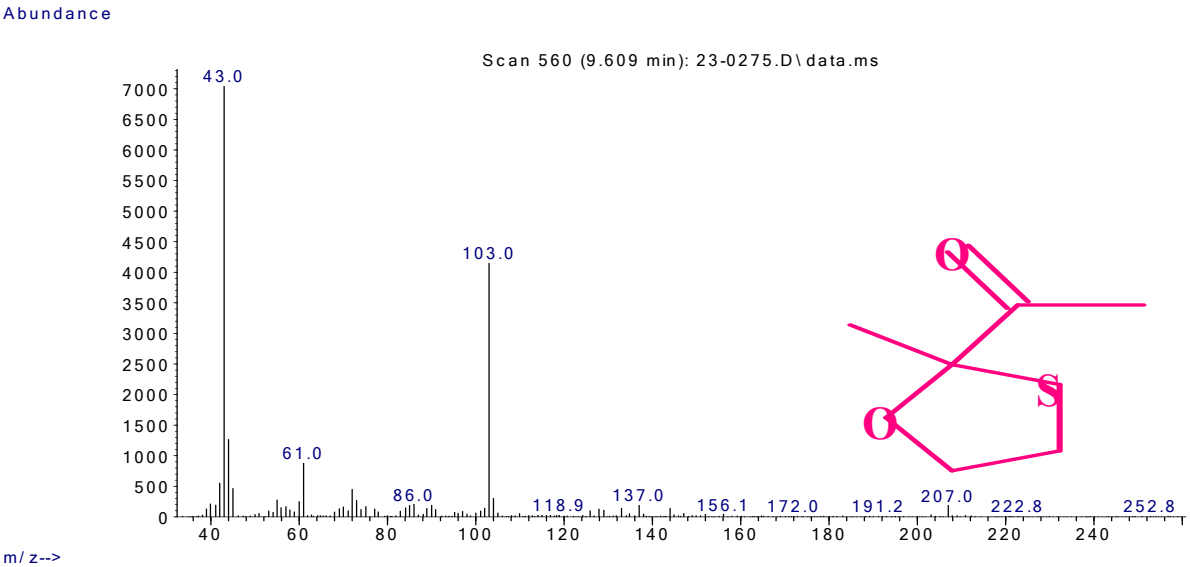


Figure S26. Mass spectrum of 1-(2-methyl-1,3-oxathiolan-2-yl) ethanone.

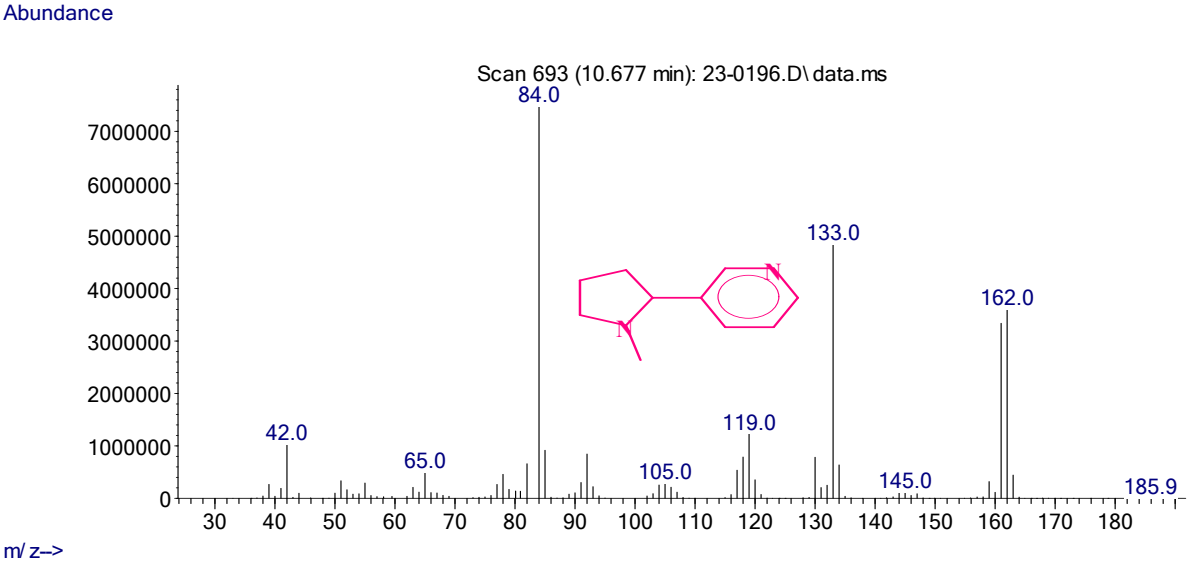


Figure S27. Mass spectrum of nicotine.

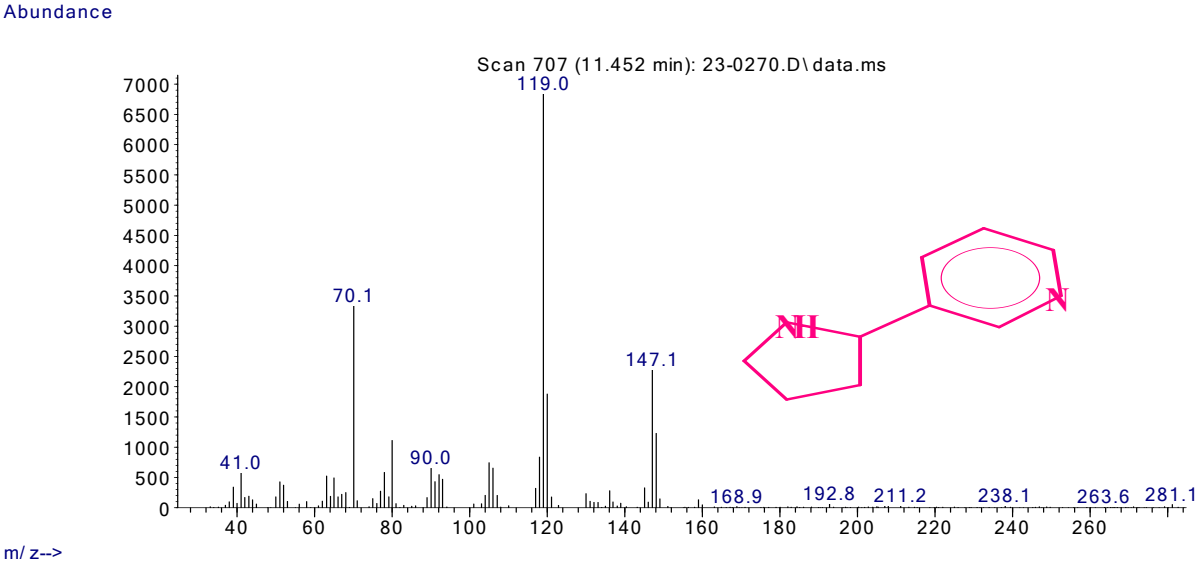


Figure S28. Mass spectrum of nornicotine.

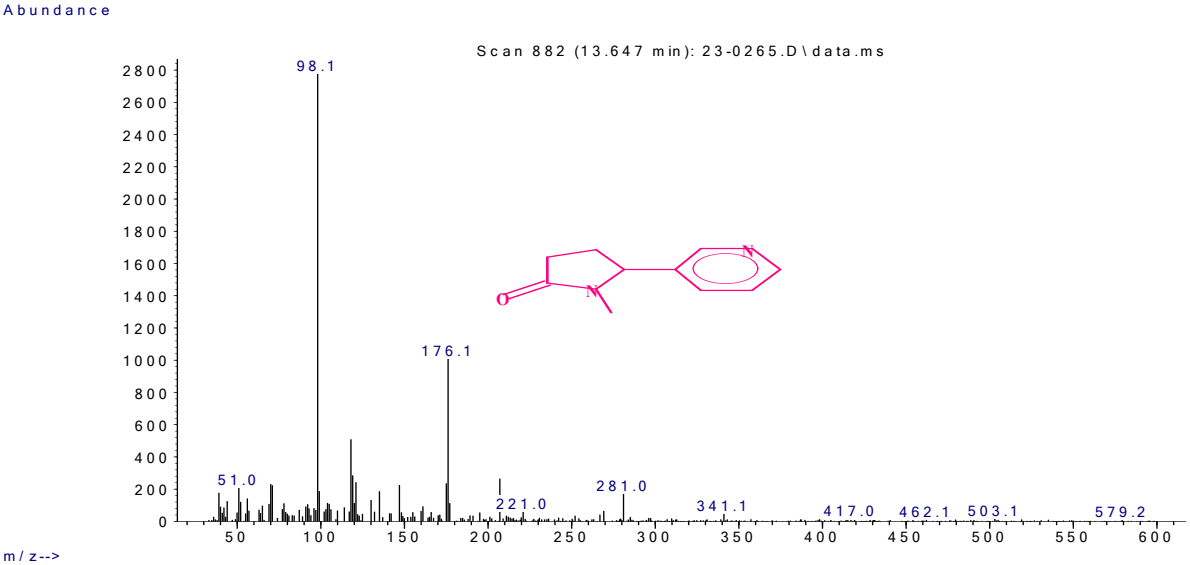


Figure S29. Mass spectrum of cotinine.

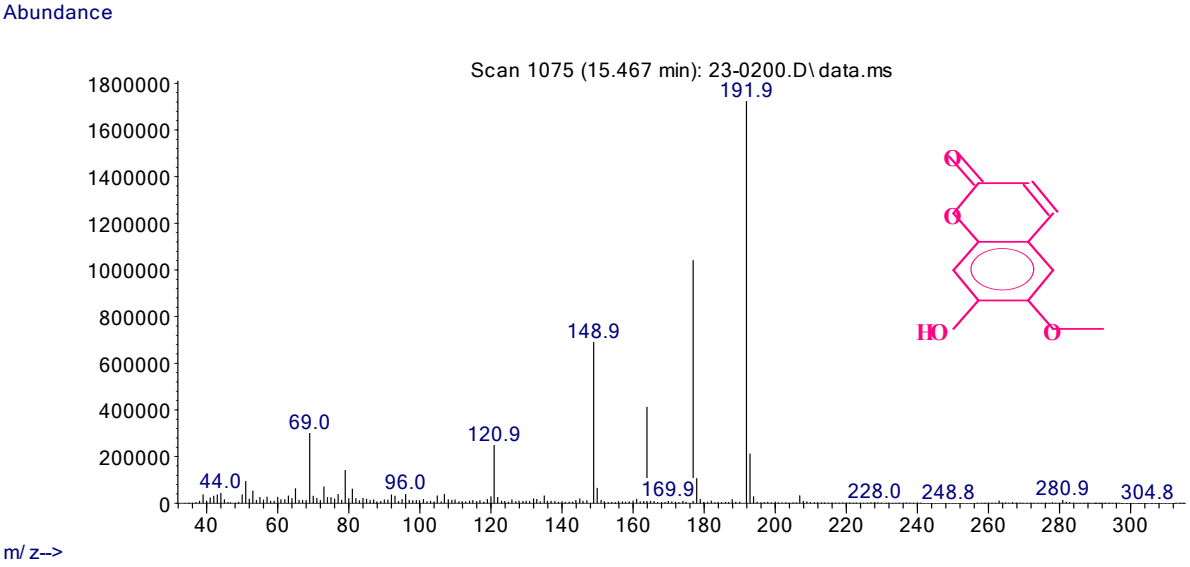


Figure S30. Mass spectrum of the scopoletin.