

Supplementary file

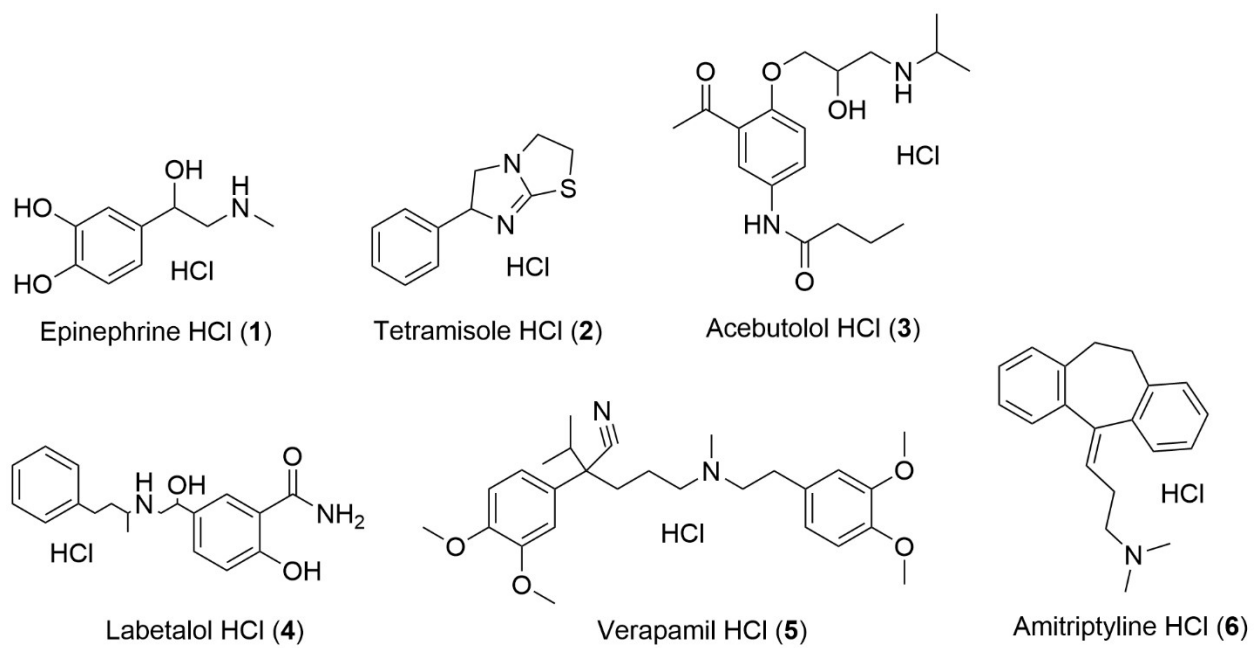
## **2-Methyl Tetrahydrofuran: A Green Organic Modifier for Eco-Friendly, Cost-Effective, and Rapid Purification in Drug Discovery**

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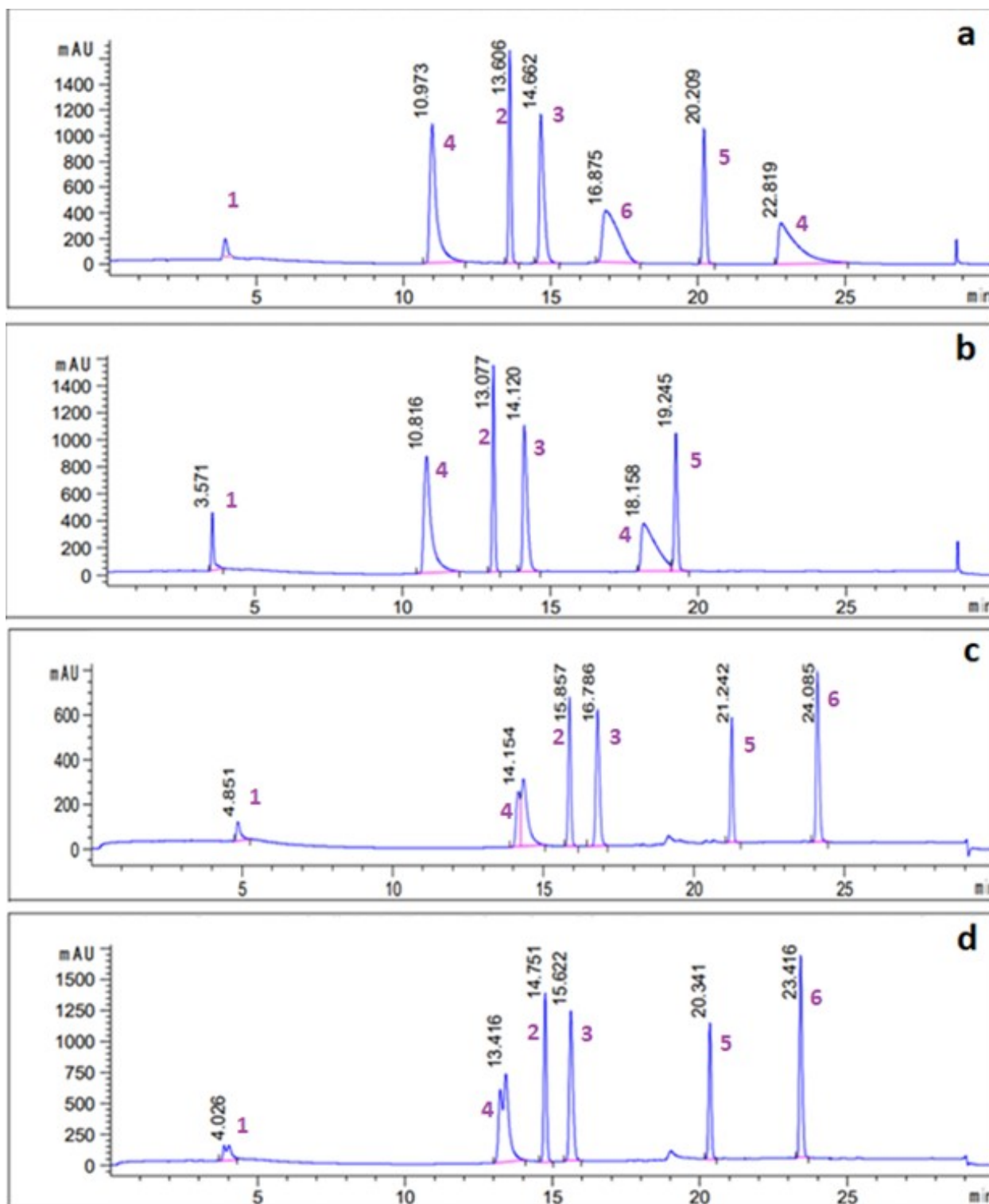
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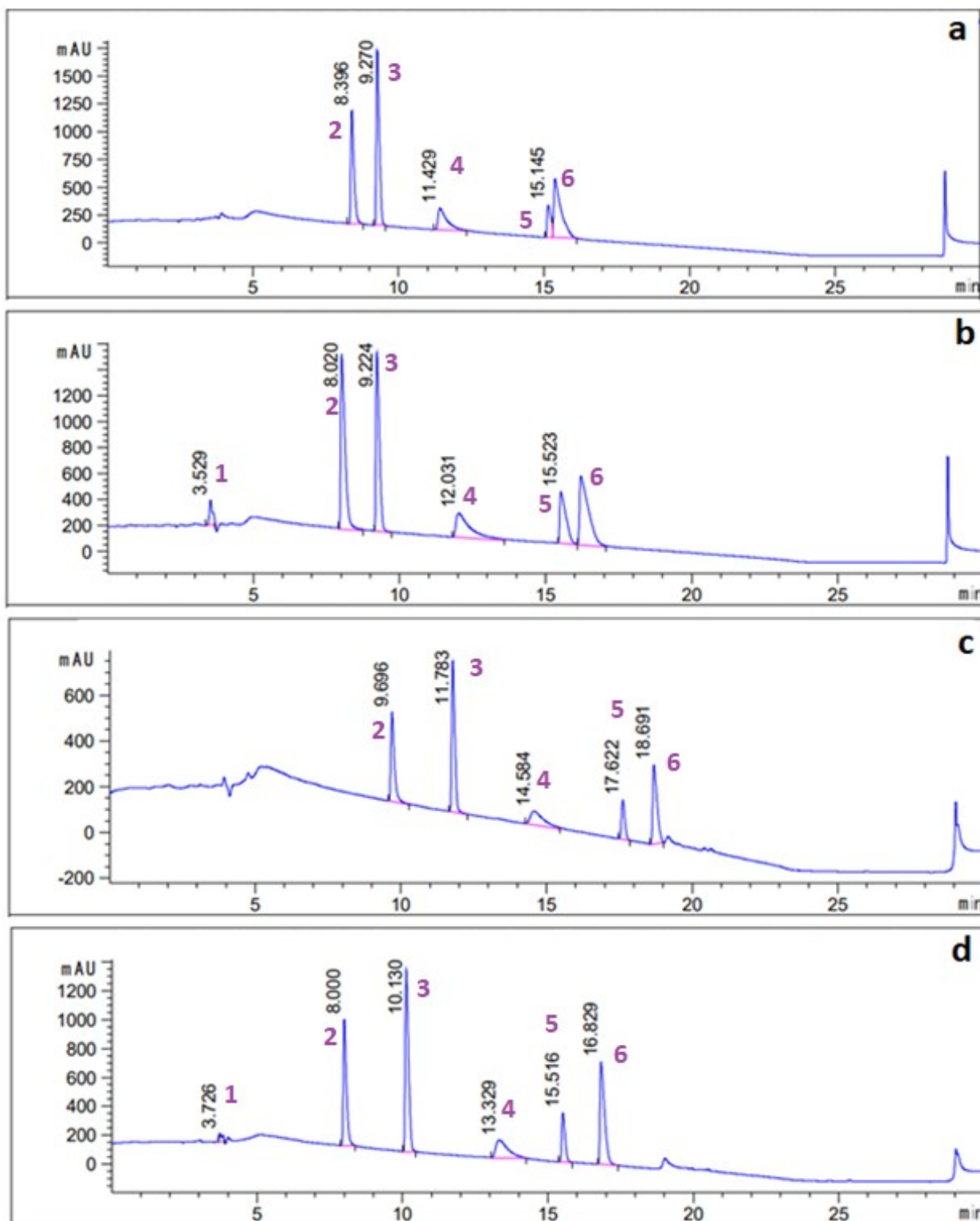
## Figures



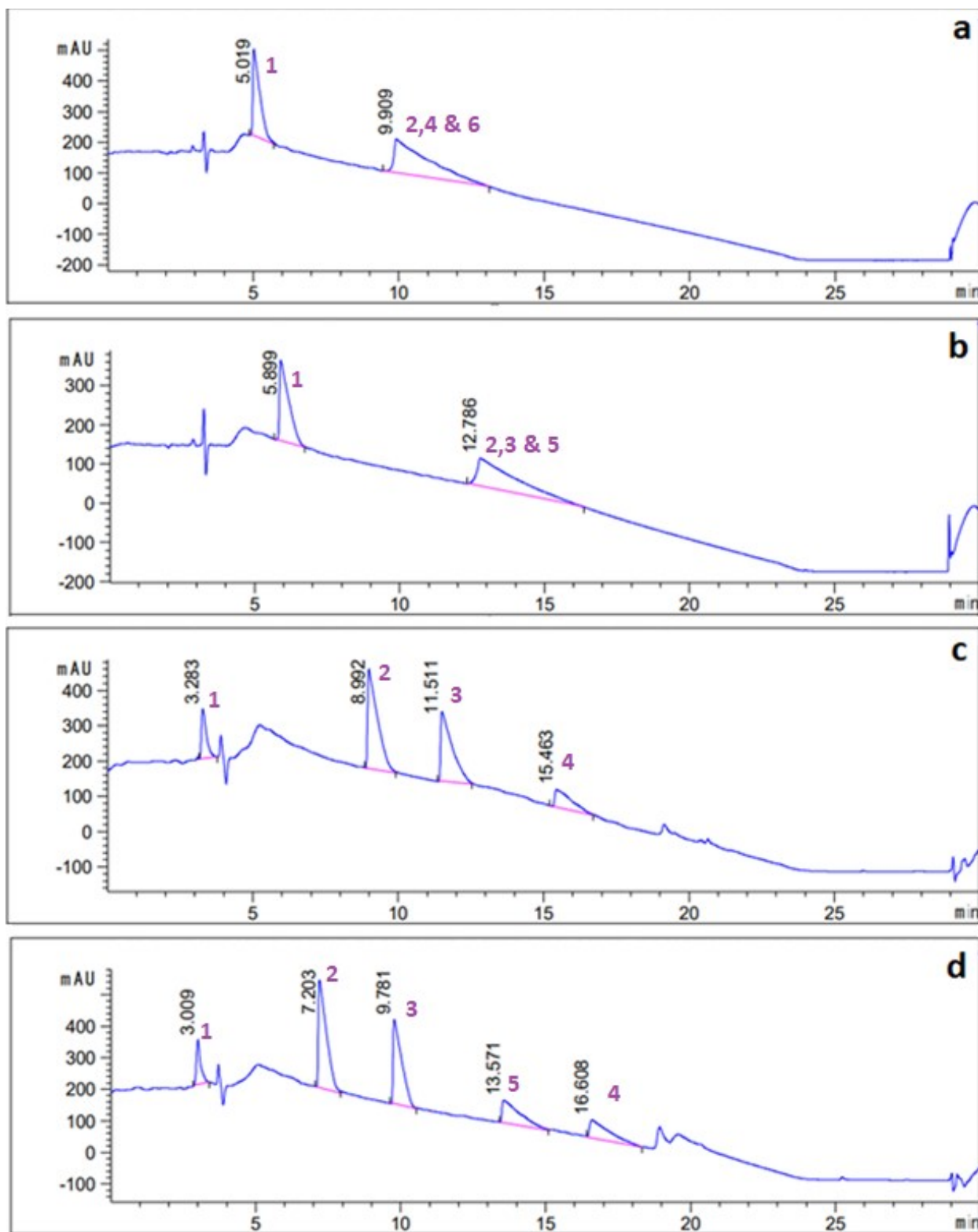
**Fig. S1.** Chemical structure of standards



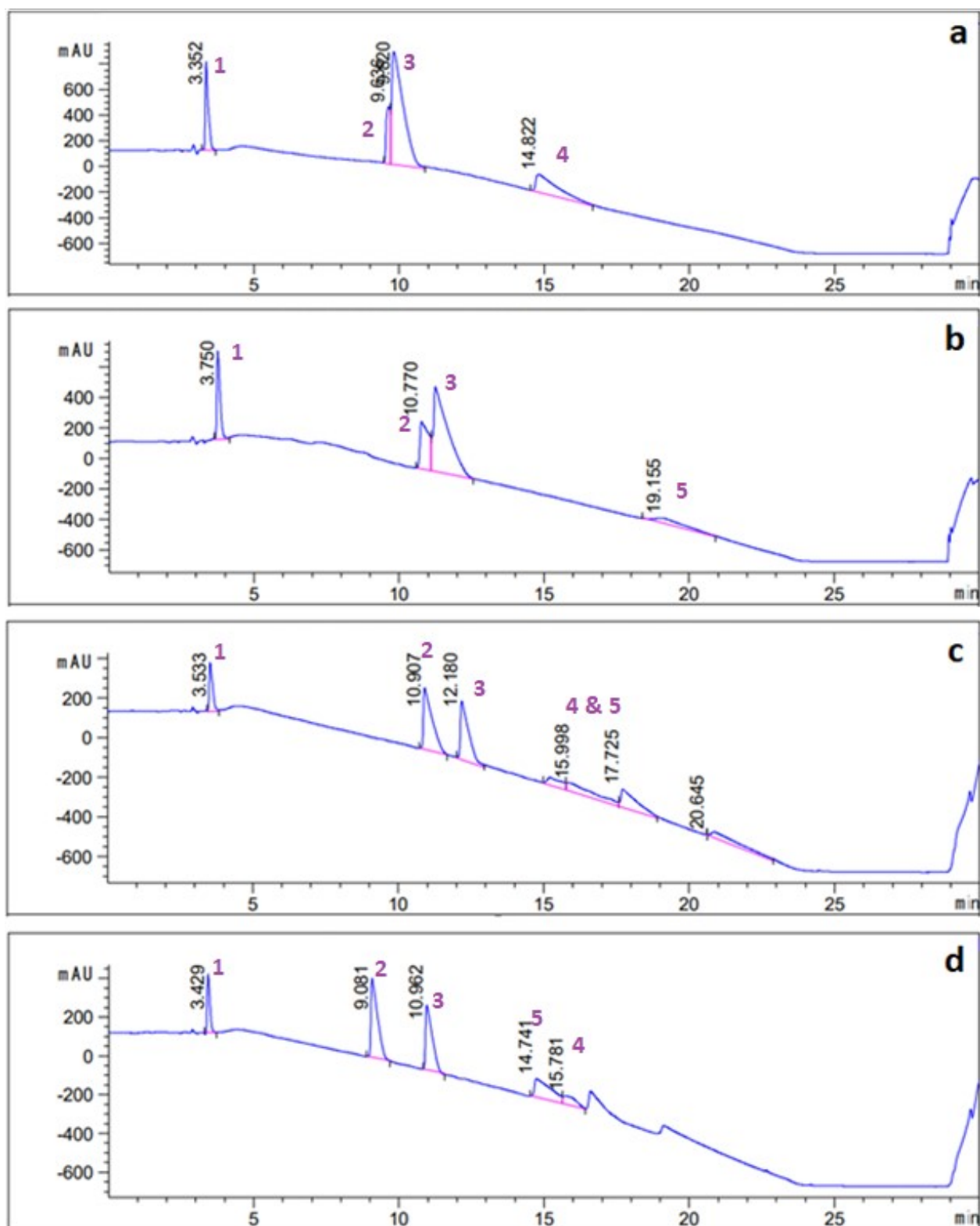
**Fig. S2.** HPLC chromatogram on YMC EXRS (250 × 4.6) mm 5μm column, using 10 mM ammonium bicarbonate in water (pH 9.5); a: acetonitrile, b: 5% MTHF in CAN, c: ACN:MeOH (1:1), d: 5% MTHF in ACN:MeOH (1:1); 1: + Epinephrine (EPI), 2: Tetramisole (TET), 3: Acebutolol (ACE), 4: Labetalol (LAB), 5: +Verapamil (VER), 6: Amitriptyline (AMI); Flow rate: 1 mL/min; Gradient elution (T/%B): 0/10, 20/100, 25/100.



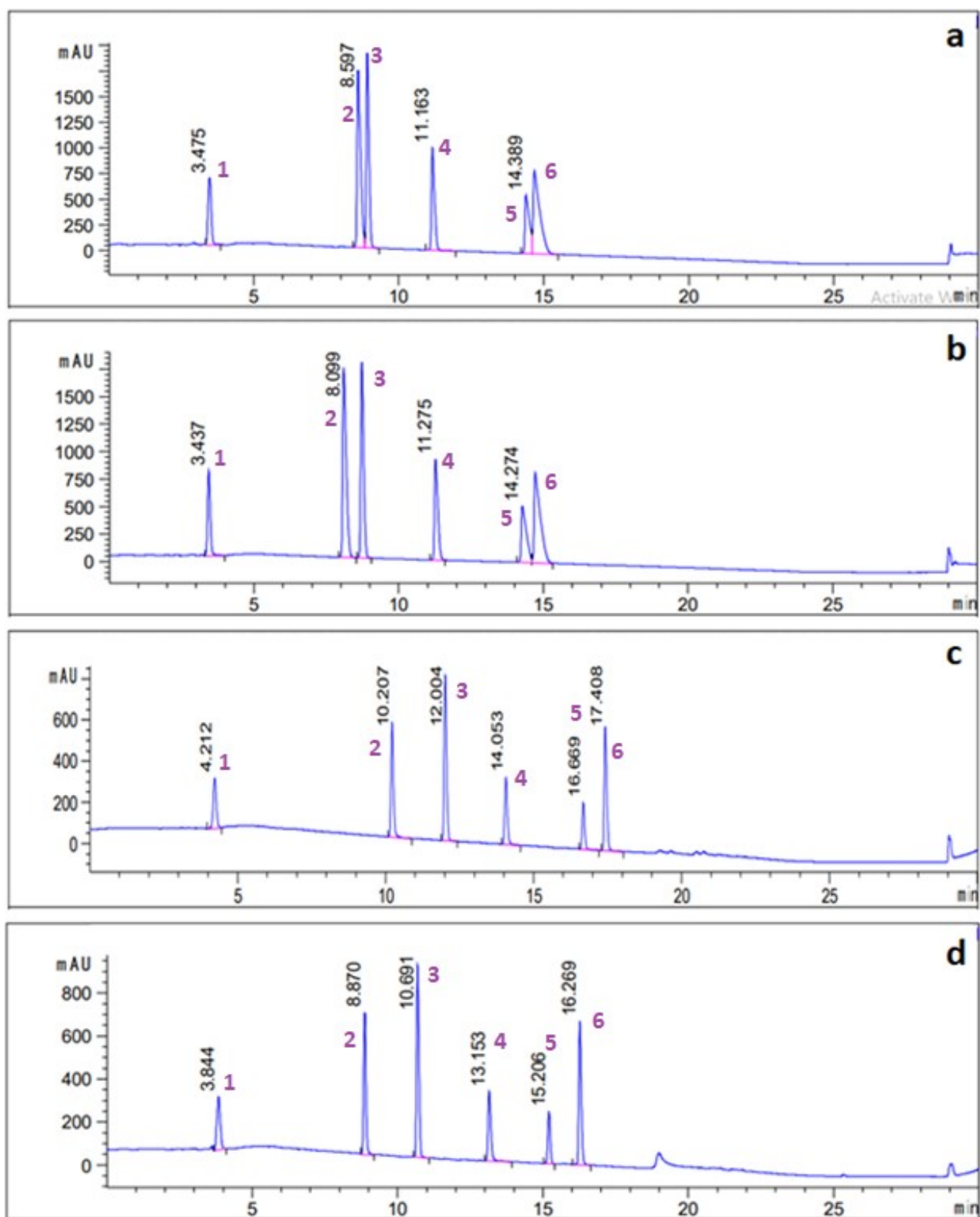
**Fig. S3.** HPLC chromatogram on YMC EXRS (250 × 4.6) mm 5 $\mu$ m column, using 10 mM ammonium acetate in water (pH 4.5); a: acetonitrile, b: 5% MTHF in ACN, c: ACN:MeOH (1:1), d: 5% MTHF in ACN:MeOH (1:1), ; 1: + Epinephrine (EPI), 2: Tetramisole (TET), 3: Acebutolol (ACE), 4: Labetalol (LAB), 5: +Verapamil (VER), 6: Amitriptyline (AMI); Flow rate: 1 mL/min; Gradient elution (T/%B): 0/10, 20/100, 25/100.



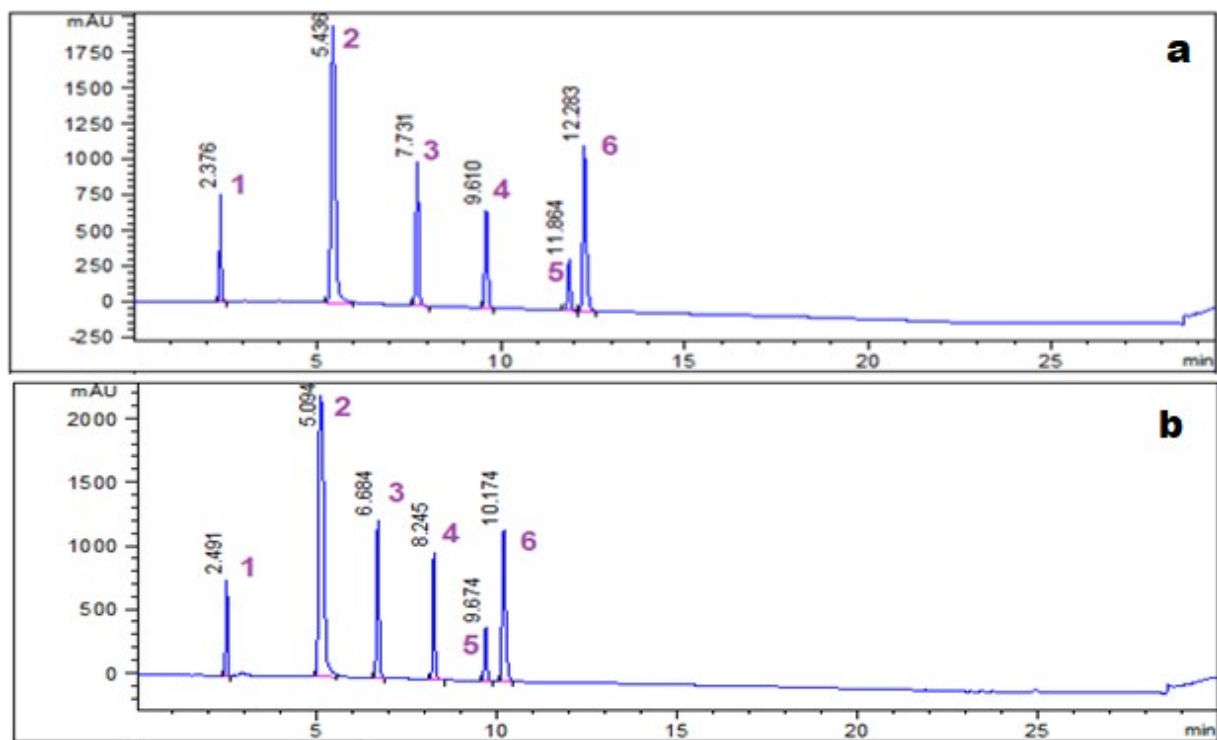
**Fig. S4.** HPLC chromatogram on YMC EXRS (250 × 4.6) mm 5µm column, using 0.1% acetic acid in water (pH 3.4); a: acetonitrile, b: 5% MTHF in CAN, c: ACN:MeOH (1:1), d: 5% MTHF in ACN:MeOH (1:1); 1: + Epinephrine (EPI), 2: Tetramisole (TET), 3: Acebutolol (ACE), 4: Labetalol (LAB), 5: +Verapamil (VER), 6: Amitriptyline (AMI); Flow rate: 1 mL/min; Gradient elution (T/%B): 0/10, 20/100, 25/100.



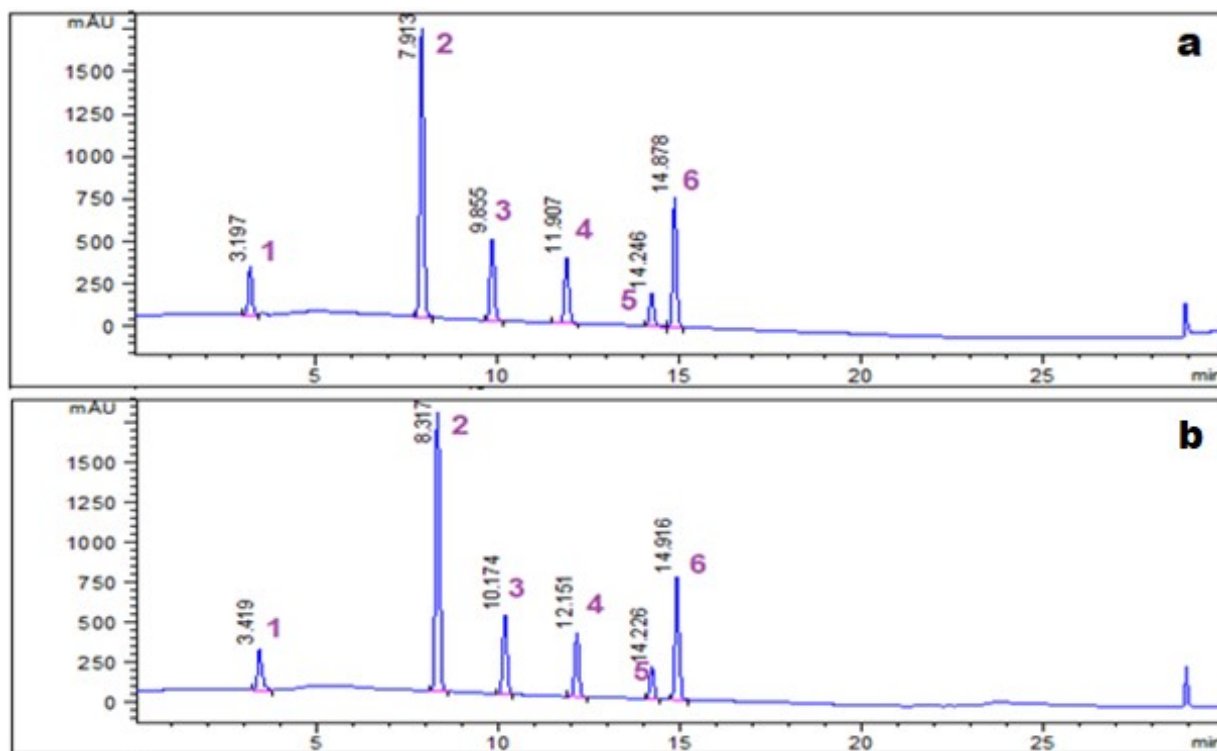
**Fig. S5.** HPLC chromatogram on YMC EXRS (250 × 4.6) mm 5μm column, using 0.1% Formic acid in water (pH 2.9); a: acetonitrile, b: 5% MTHF in CAN, c: ACN:MeOH (1:1), d: 5% MTHF in ACN:MeOH (1:1), ); 1: + Epinephrine (EPI), 2: Tetramisole (TET), 3: Acebutolol (ACE), 4: Labetalol (LAB), 5: +Verapamil (VER), 6: Amitriptyline (AMI); Flow rate: 1 mL/min; Gradient elution (T/%B): 0/10, 20/100, 25/100.



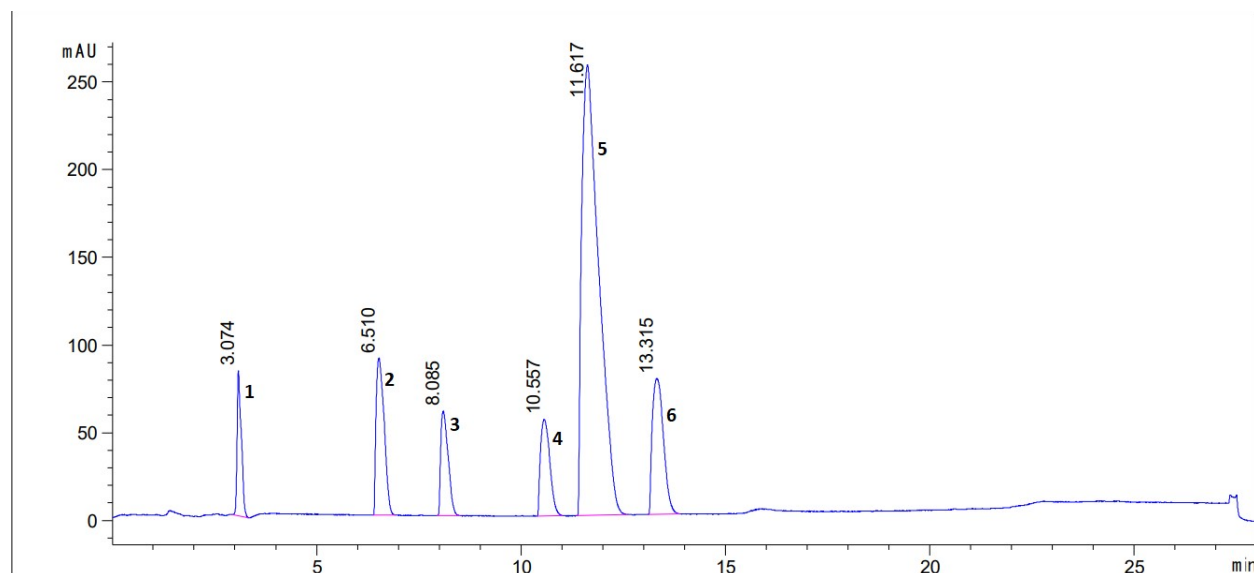
**Fig. S6.** HPLC chromatogram on YMC EXRS (250 × 4.6) mm 5μm column, using 0.1% TFA in water (pH 2.2); a: acetonitrile, b: 5% MTHF in CAN, c: ACN:MeOH (1:1), d: 5% MTHF in ACN:MeOH (1:1), ; 1: + Epinephrine (EPI), 2: Tetramisole (TET), 3: Acebutolol (ACE), 4: Labetalol (LAB), 5: +Verapamil (VER), 6: Amitriptyline (AMI); Flow rate: 1mL/min; Gradient elution (T/%B): 0/10, 20/100, 25/100.



**Fig. S7.** HPLC chromatogram on Kinetex EVO C18 (250 × 4.6) mm 5μm column, using 0.1% TFA in water (pH 2.2); a: ACN:MeOH (1:1), b: 5% MTHF in ACN:MeOH (1:1), ); 1: + Epinephrine (EPI), 2: Tetramisole (TET), 3: Acebutolol (ACE), 4: Labetalol (LAB), 5: +Verapamil (VER), 6: Amitriptyline (AMI); Flow rate: 1 mL/min; Gradient elution (T/%B): 0/10, 20/100, 25/100.

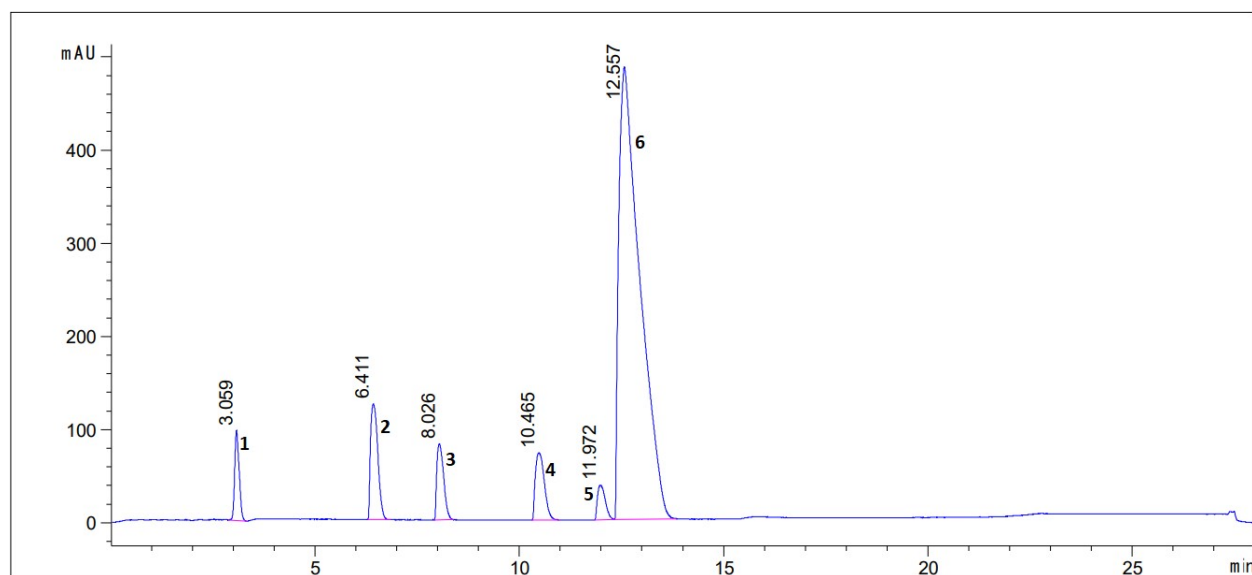


**Fig. S8.** HPLC chromatogram on Sunfire C18 (250 × 4.6) mm 5 $\mu$ m column, using 0.1% TFA in water (pH 2.2); a: ACN:MeOH (1:1), b: 5% MTHF in ACN:MeOH (1:1), ; 1: + Epinephrine (EPI), 2: Tetramisole (TET), 3: Acebutolol (ACE), 4: Labetalol (LAB), 5: +Verapamil (VER), 6: Amitriptyline (AMI); Flow rate: 1 mL/min; Gradient elution (T/%B): 0/10, 20/100, 25/100.



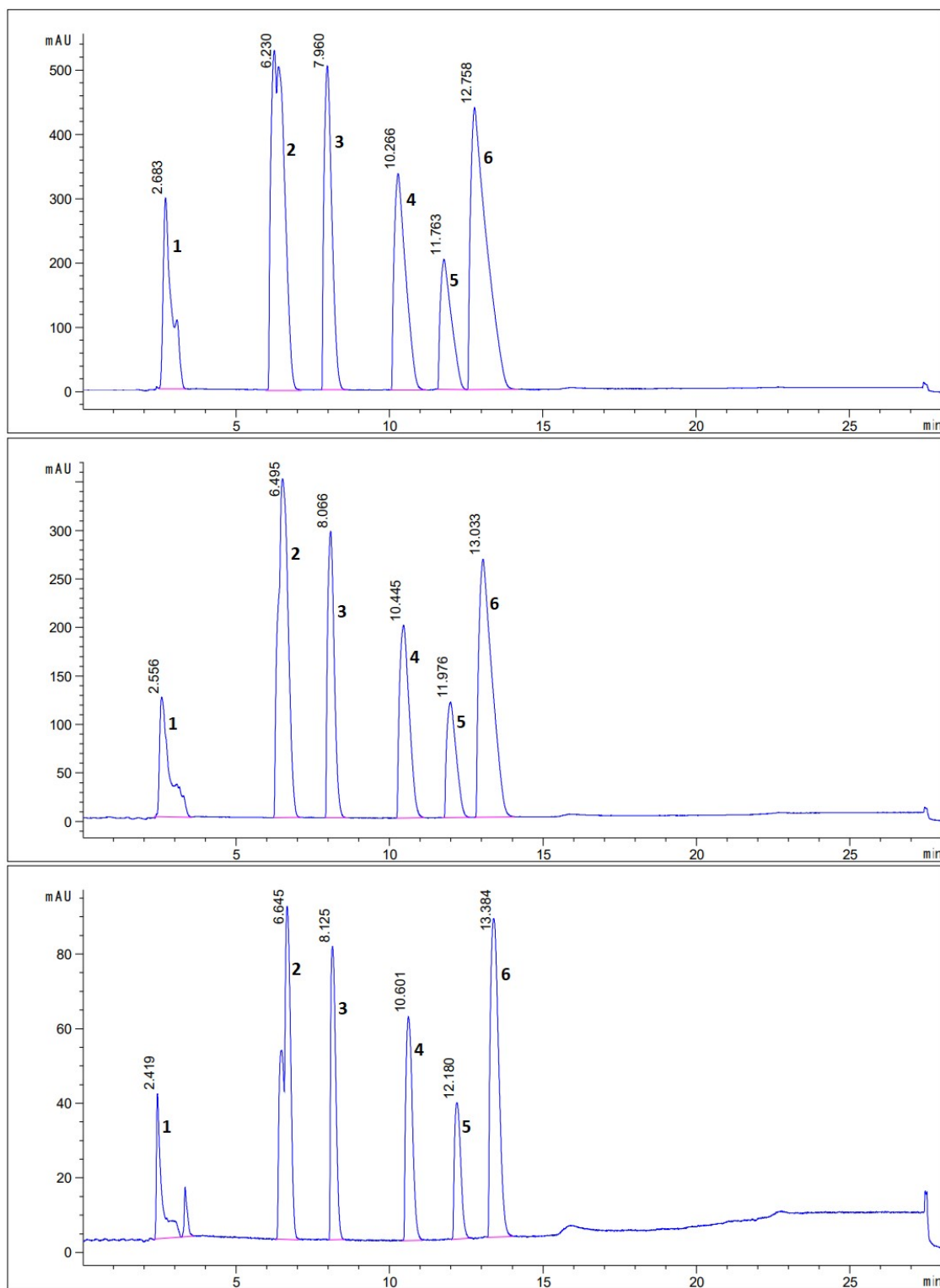
**Fig. S9.** Recovery study at 10% level, Sample A.

Chromatogram depicting the resolution of a mixture consisting of six analytes, with the concentration of compound 5 being tenfold greater than that of the other compounds. 1: + Epinephrine (EPI), 2: Tetramisole (TET), 3: Acebutolol (ACE), 4: Labetalol (LAB), 5: +Verapamil (VER), 6: Amitriptyline (AMI); Chromatographic condition at preparative HPLC scale: Column: YMC EXRS (250 × 20) mm 5 $\mu$ m. Mobile phase A: 0.1% TFA in water; Mobile phase B: 10% MTHF in ACN:MeOH 9(1:1), Gradient (T/%B): 0/10, 14/73, 14.01/100, 16/100. Flow rate: 20 mL/min. It demonstrates excellent separation of compound 4, which elutes at the front and is present at approximately one-tenth the concentration of compound 5.



**Fig. S10.** Recovery study at 10% level, Sample B.

Chromatogram depicting the resolution of a mixture consisting of six analytes, with the concentration of compound 6 being tenfold greater than that of the other compounds. 1: + Epinephrine (EPI), 2: Tetramisole (TET), 3: Acebutolol (ACE), 4: Labetalol (LAB), 5: +Verapamil (VER), 6: Amitriptyline (AMI); Chromatographic condition at preparative HPLC scale: Column: YMC EXRS (250 × 20) mm 5 $\mu$ m. Mobile phase A: 0.1% TFA in water; Mobile phase B: 10% MTHF in ACN:MeOH 9(1:1), Gradient (T/%B): 0/10, 14/73, 14.01/100, 16/100. Flow rate: 20 mL/min. It shows effective separation of compound 5, which elutes at the front and is about one-tenth the concentration of compound 6.



**Fig. S11.** HPLC chromatograms of a sample mixture comprising compounds 1-4, with compounds 5 and 6 sourced equally from their respective tablet formulations. The sample was analyzed at three different concentrations: 100%, 50%, and 10%. 1: + Epinephrine (EPI), 2: Tetramisole (TET), 3: Acebutolol (ACE), 4: Labetalol (LAB), 5: +Verapamil (VER), 6: Amitriptyline (AMI); Chromatographic condition: Column at preparative HPLC scale: YMC EXRS (250 × 20) mm 5 $\mu$ m. Mobile phase A: 0.1% TFA in water; Mobile phase B: 10% MTHF in ACN:MeOH 9(1:1), Gradient (T/%B): 0/10, 14/73, 14.01/100, 16/100. Flow rate: 20 mL/min.

**Table S1. Peak resolutions between closely eluting compounds (5 & 6) at different sample loading and mobile phase combinations.**

| Sample Loading ( $\mu\text{L}$ ) | ACN:MeOH (1:1)<br>(without MTHF) | 5% MTHF in<br>ACN:MeOH (1:1) | 10% MTHF in<br>ACN:MeOH (1:1) |
|----------------------------------|----------------------------------|------------------------------|-------------------------------|
| 50 $\mu\text{L}$                 | 1.067                            | 1.925                        | 1.911                         |
| 100 $\mu\text{L}$                | 0.709                            | 1.516                        | 1.47                          |
| 150 $\mu\text{L}$                | 0.583                            | 1.325                        | 1.284                         |
| 200 $\mu\text{L}$                | 0.547                            | 1.209                        | 1.178                         |
| 250 $\mu\text{L}$                | 0.512                            | 1.125                        | 1.108                         |

Column: YMC EXRS (250  $\times$  20) mm 5 $\mu\text{m}$ . Mobile phase A: 0.1% TFA in water; Mobile phase B: ACN:MeOH, 5% MTHF in ACN:MeOH, and 10% MTHF in ACN:MeOH. All the ACN:MeOH combinations used were in 1:1 ratio.

Compound **5**: +Verapamil (VER), and Compound **6**: Amitriptyline (AMI).

**Table S2. Recovery study for peaks eluting at tailing and fronting at 10% level**

| Compounds        | Sample mixture A |                   |                   | Sample mixture B |                   |                   |
|------------------|------------------|-------------------|-------------------|------------------|-------------------|-------------------|
|                  | Quantity used    | % Sample recovery | % Fraction purity | Quantity used    | % Sample recovery | % Fraction purity |
| EPI ( <b>1</b> ) | 0.25 mg          | 90.4              | 99.9              | 0.25 mg          | 93.4              | 99.9              |
| TET ( <b>2</b> ) | 0.25 mg          | 97.5              | 99.9              | 0.25 mg          | 97.8              | 99.9              |
| ACE ( <b>3</b> ) | 0.25 mg          | 93.1              | 99.9              | 0.25 mg          | 93.0              | 99.9              |
| LAB ( <b>4</b> ) | 0.25 mg          | 92.9              | 99.9              | 0.25 mg          | 93.6              | 99.9              |
| VER ( <b>5</b> ) | <b>2.5 mg</b>    | 98.3              | 99.9              | 0.25 mg          | 94.8              | 99.9              |
| AMI ( <b>6</b> ) | 0.25 mg          | 99.1              | 99.9              | <b>2.5 mg</b>    | 95.0              | 99.9              |

**Table S3. Application of optimized method in drug products (tablet formulations)**

| Compounds | 100% Level<br>(2.5 mg of each compound) |                   | 50% Level<br>(1.25 mg of each compound) |                   | 10% Level<br>(0.25 mg of each compound) |                   |
|-----------|-----------------------------------------|-------------------|-----------------------------------------|-------------------|-----------------------------------------|-------------------|
|           | % Sample recovery                       | % Fraction purity | % Sample recovery                       | % Fraction purity | % Sample recovery                       | % Fraction purity |
| EPI (1)   | 97.3                                    | 99.9              | 98.8                                    | 99.9              | -                                       | 99.9              |
| TET (2)   | 97.8                                    | 99.9              | 98.9                                    | 99.9              | 97.6                                    | 99.9              |
| ACE (3)   | 95.6                                    | 99.9              | 98.8                                    | 99.9              | 90.8                                    | 99.9              |
| LAB (4)   | 96.8                                    | 99.9              | 98.9                                    | 99.9              | 89.6                                    | 99.9              |
| VER (5)*  | 96.8                                    | 99.9              | 99.1                                    | 99.9              | 91.9                                    | 99.9              |
| AMI (6)*  | 95.6                                    | 99.9              | 99.4                                    | 99.9              | 83.3                                    | 99.9              |

An equal amount of compound was obtained from the corresponding tablet formulation