Supporting information

Lipidomic profiling of biological tissues using off-line two-dimensional high-performance liquid chromatography - mass spectrometry

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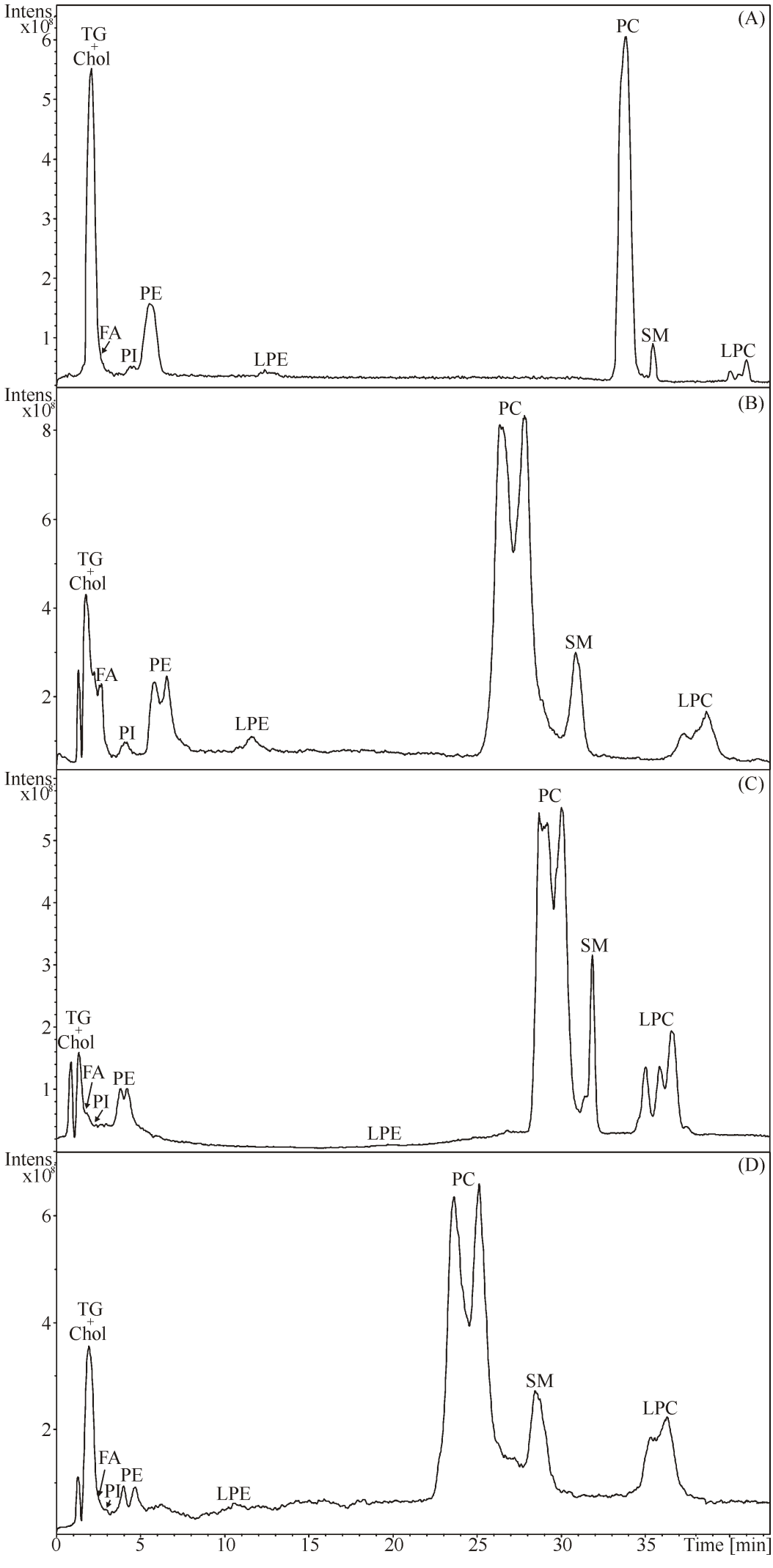

Figure S1. Comparison of HILIC separation of total lipid extract from egg yolk using various NP-HPLC and HILIC columns: (A) porous shell particles column Kinetex HILIC (150×2.1 mm, 2.6 µm, Phenomenex), (B) Spherisorb Si (150×4.6 mm, 10 µm, Waters), (C) porous shell particles column Ascentis Si (150×2.1 mm, 2.7 µm, Sigma-Aldrich), and (D) Atlantis Si (150×2.1 mm, 3 µm, Waters). HPLC conditions: flow rate 0.3 (A, C, D) and 1 (B) mL/min, separation temperature 40 ^{o}C, gradient 0 min - 96 % A + 4 % B, 60 min - 86 % A +14 % B, where A is the mixture of hexane / 2-propanol (3:4, v/v) and B is 5 mM aqueous ammonium acetate.

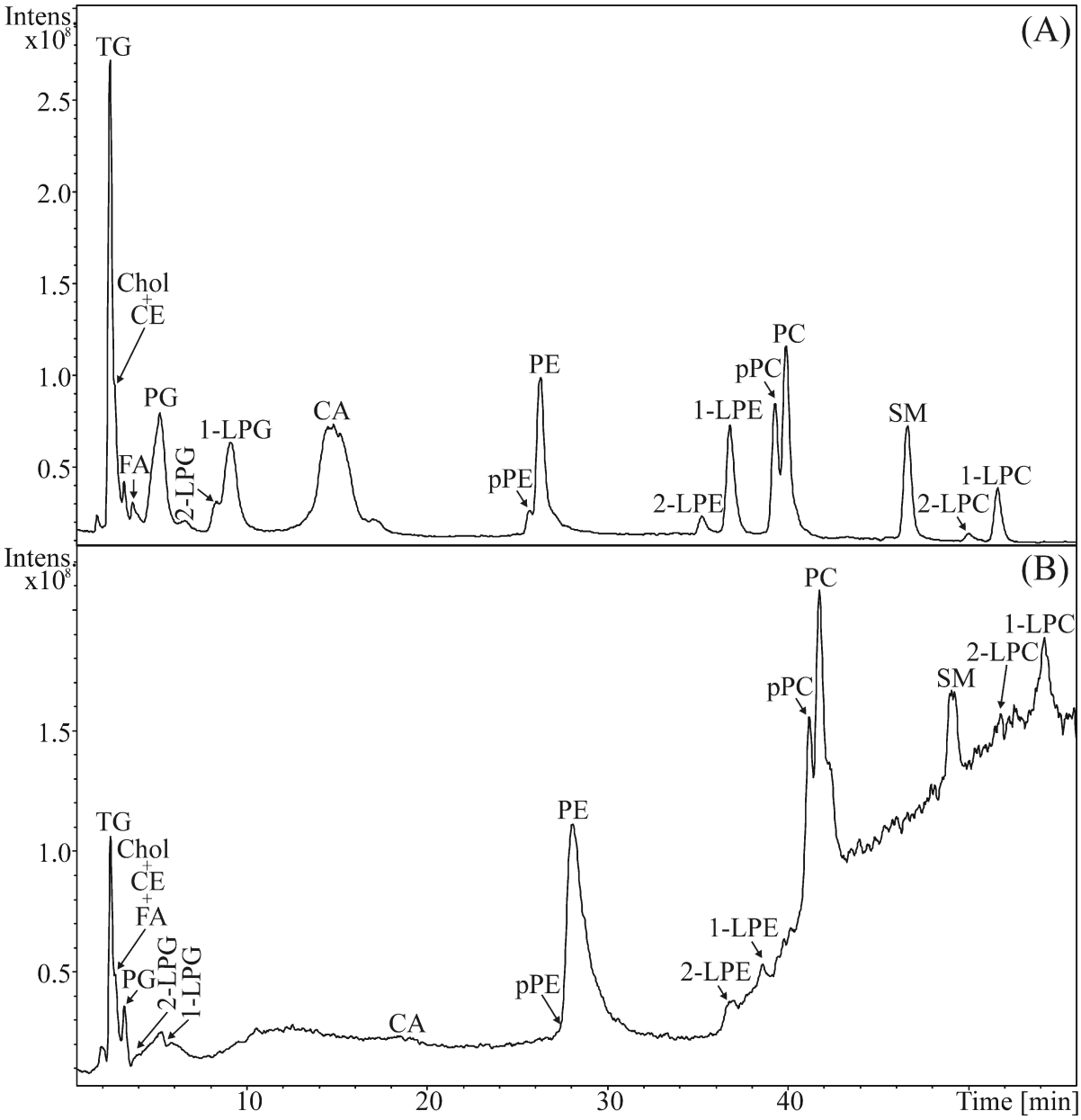

Figure S2. Influence of ammonium acetate on HILIC separation of lipid standards represented by species containing oleic acid (Δ9*cis*-C18:1). (A) 5 mM, and (B) 0 mM of ammonium acetate in water. HPLC conditions: Spherisorb Si column (250×4.6 mm, 5 μm), flow rate 1 mL/min, separation temperature 40˚C, gradient 0 min - 94% A + 6% B, 60 min - 77% A + 23% B, where A is acetonitrile and B is aqueous ammonium acetate.

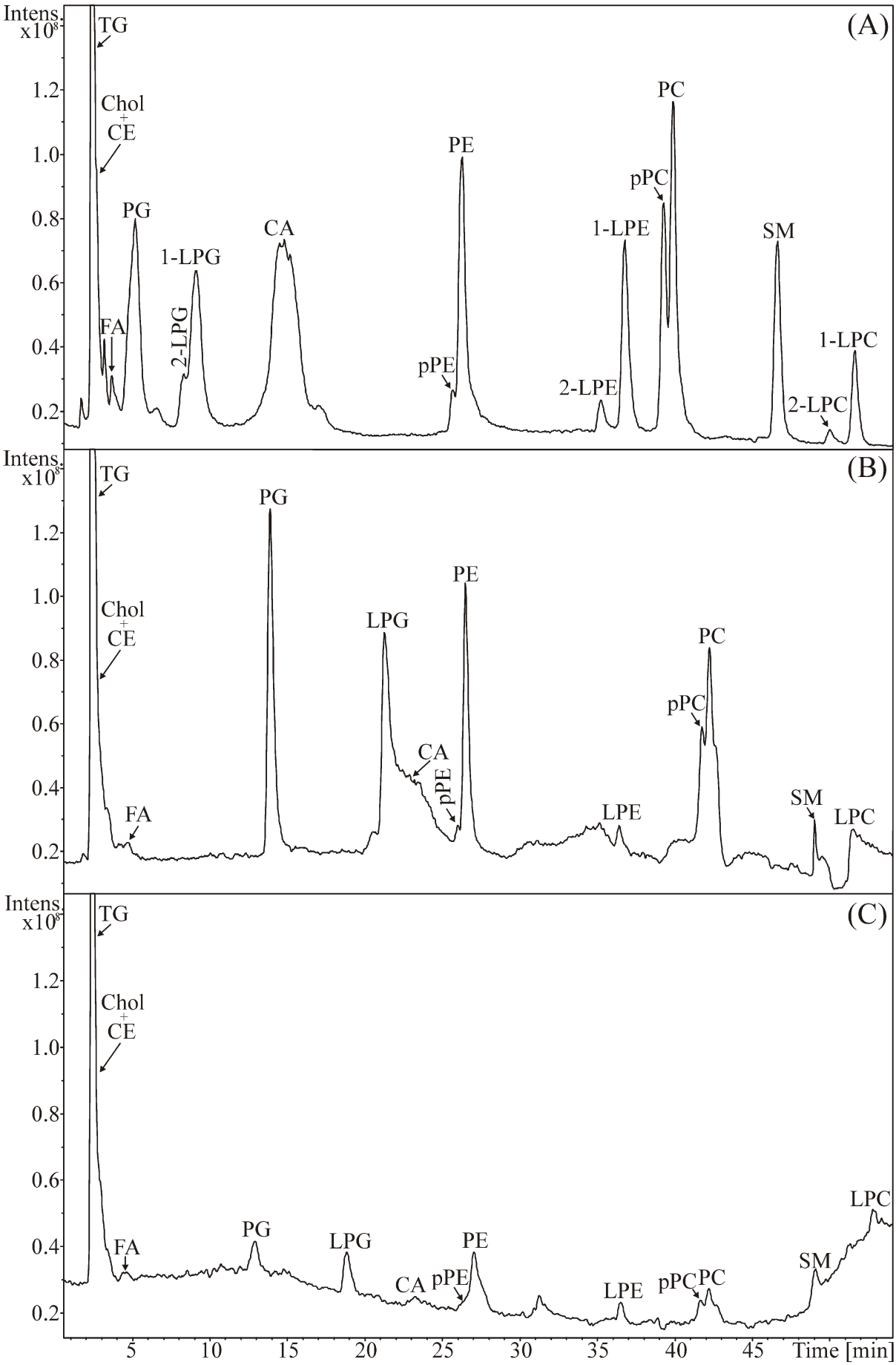

Figure S3. Influence of pH value of mobile phase on HILIC separation of lipid standards represented by species containing oleic acid (Δ9*cis*-C18:1). (A) pH = 7, (B) pH = 4.5, and (C) pH = 3.5. HPLC conditions: Spherisorb Si column (250×4.6 mm, 5 μm), flow rate 1 mL/min, separation temperature 40˚C, gradient 0 min - 94% A + 6% B, 60 min - 77% A + 23% B, where A is acetonitrile and B is 5 mM aqueous ammonium acetate.

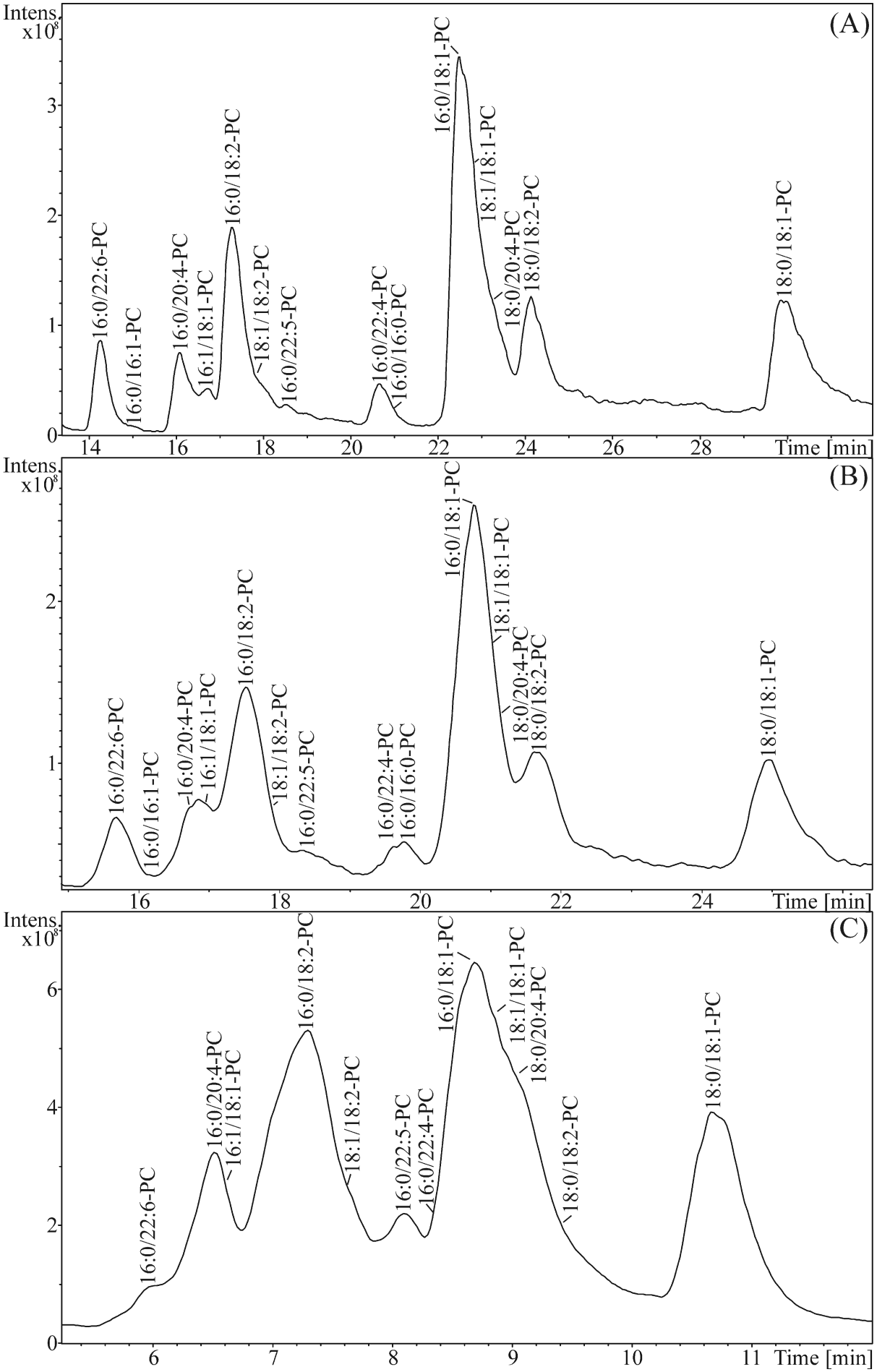

Figure S4. Comparison of RP-HPLC separation of PC fraction from egg yolk using various C_{18} columns: (A) porous shell particles column Kinetex C_{18} (150×2.1 mm, 2.6 µm, Phenomenex), (B) Luna C_{18} (250×4.6 mm, 5 µm, Phenomenex), and (C) Hypersil Gold C_{18} (150×3 mm, 5 µm, Thermo Fischer Scientific). HPLC conditions: flow rate 0.3 (A), 1 (B) and 0.6 (C) mL/min, separation temperature 40˚C, gradient 0 min - 80 % A + 20 % B, 30 min - 95 % A + 5 % B, where A is the mixture of acetonitrile / 2-propanol (3:1, v/v) and B is 5 mM aqueous ammonium acetate.

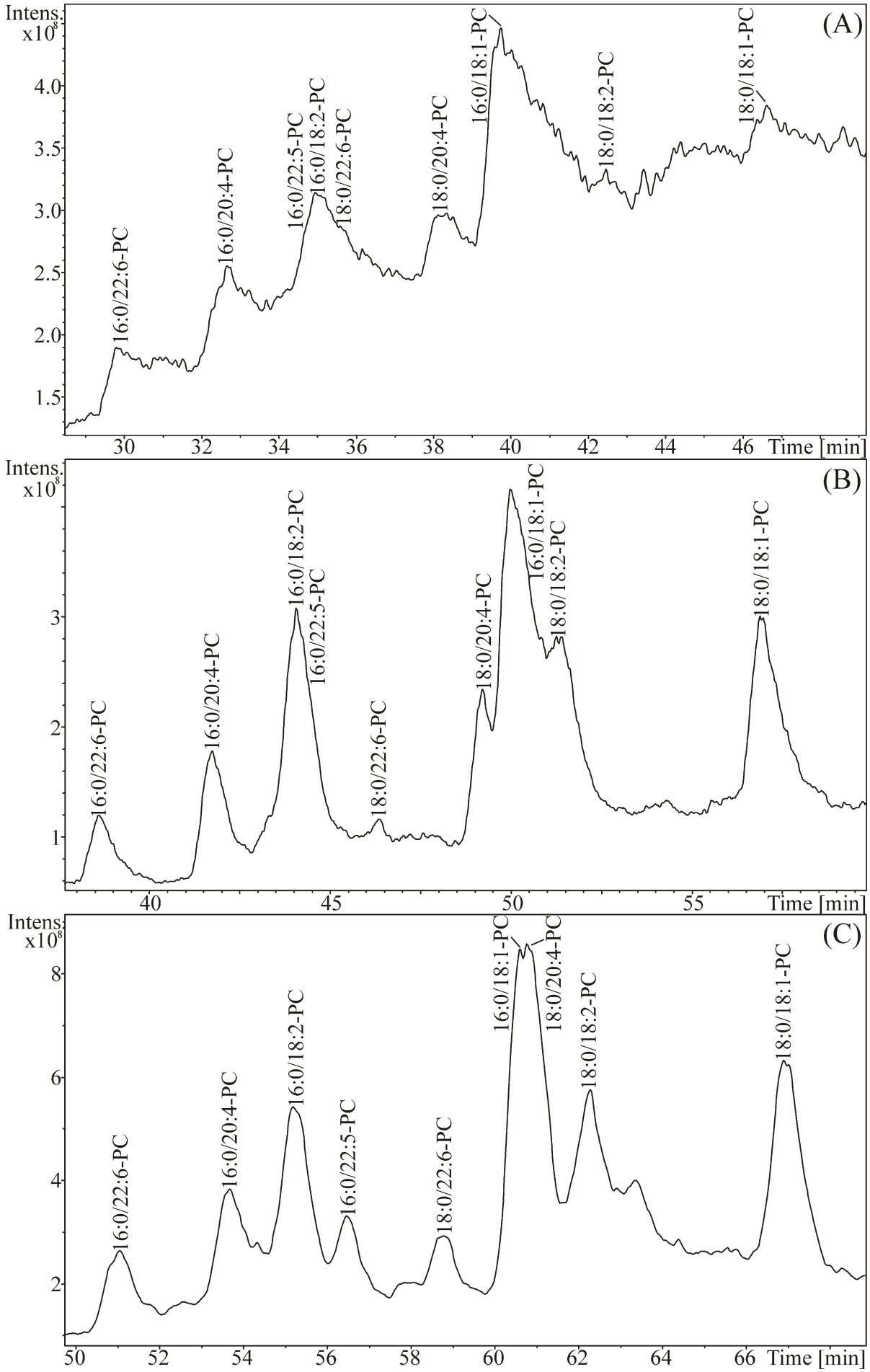

Figure S5. Influence of water concentration in RP-HPLC analysis of PC fraction from egg yolk. (A) 5%, (B) 10%, and (C) 15% of water. HPLC conditions: Luna C_{18} column (250×4.6 mm, 5 μm, Phenomenex), flow rate 1 mL/min, separation temperature 40˚C, gradient (A) 0 min - 85% A + 10% B + 5% C, 60 min - 25% A + 70% B + 5% C, (B) 0 min - 80% A + 10% B + 10% C, 60 min - 20% A + 70% B + 10% C, and (C) 0 min - 75% A + 10% B + 15% C, 60 min - 15% A + 70% B + 15% C, where A is acetonitrile, B is 2-propanol and C is 5 mM aqueous ammonium acetate.

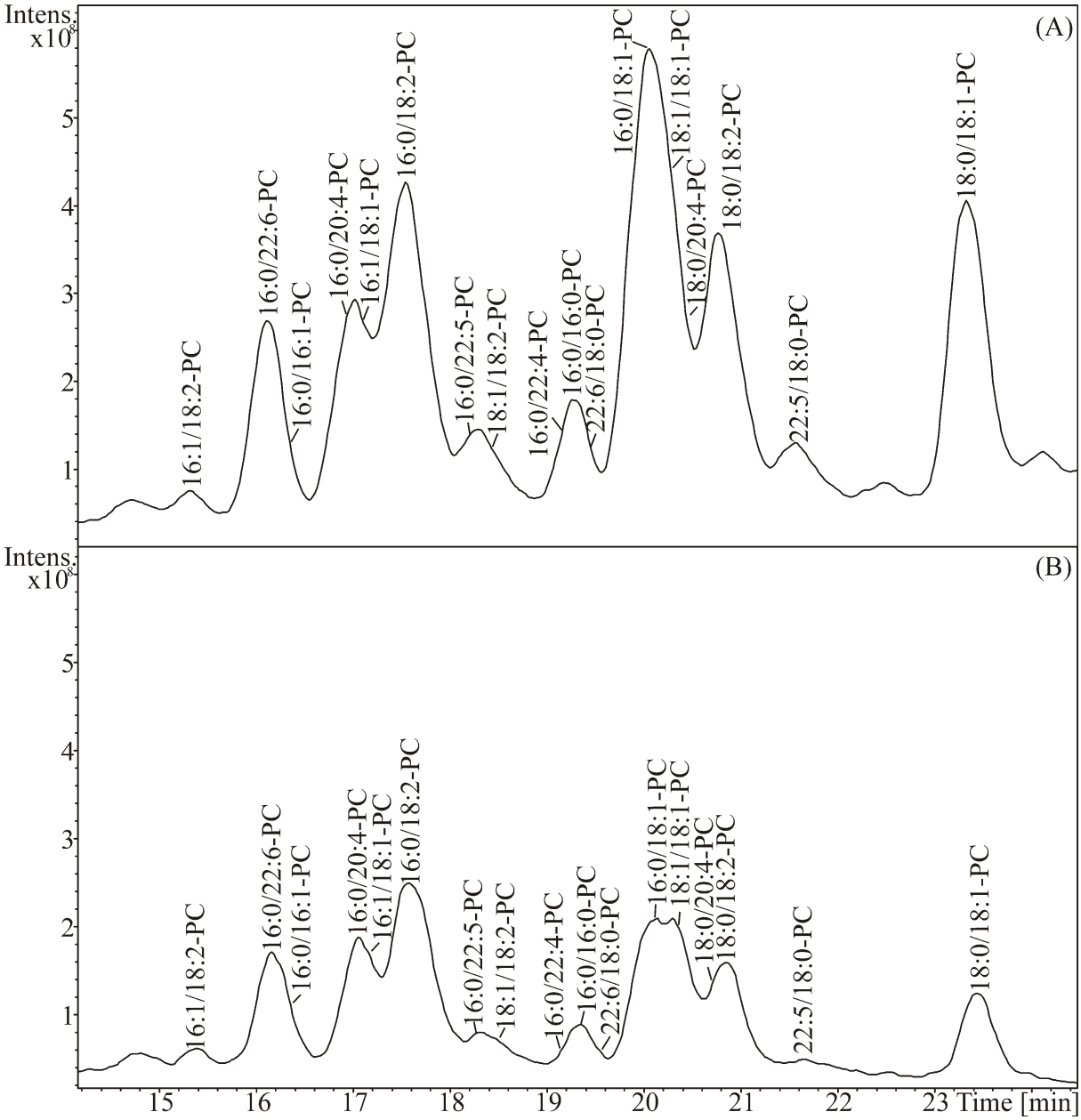

Figure S6. Influence of ammonium acetate on RP-HPLC separation of PC fraction from egg yolk. (A) 5 mM, and (B) 0 mM of aqueous ammonium acetate. HPLC conditions: Luna C_{18} column (250×4.6 mm, 5 µm, Phenomenex), flow rate 1 mL/min, separation temperature 40˚C, gradient 0 min - 80 % A + 20 % B, 30 min - 95 % A + 5 % B, where A is the mixture of acetonitrile / 2-propanol (3:1, v/v) and B is aqueous ammonium acetate.

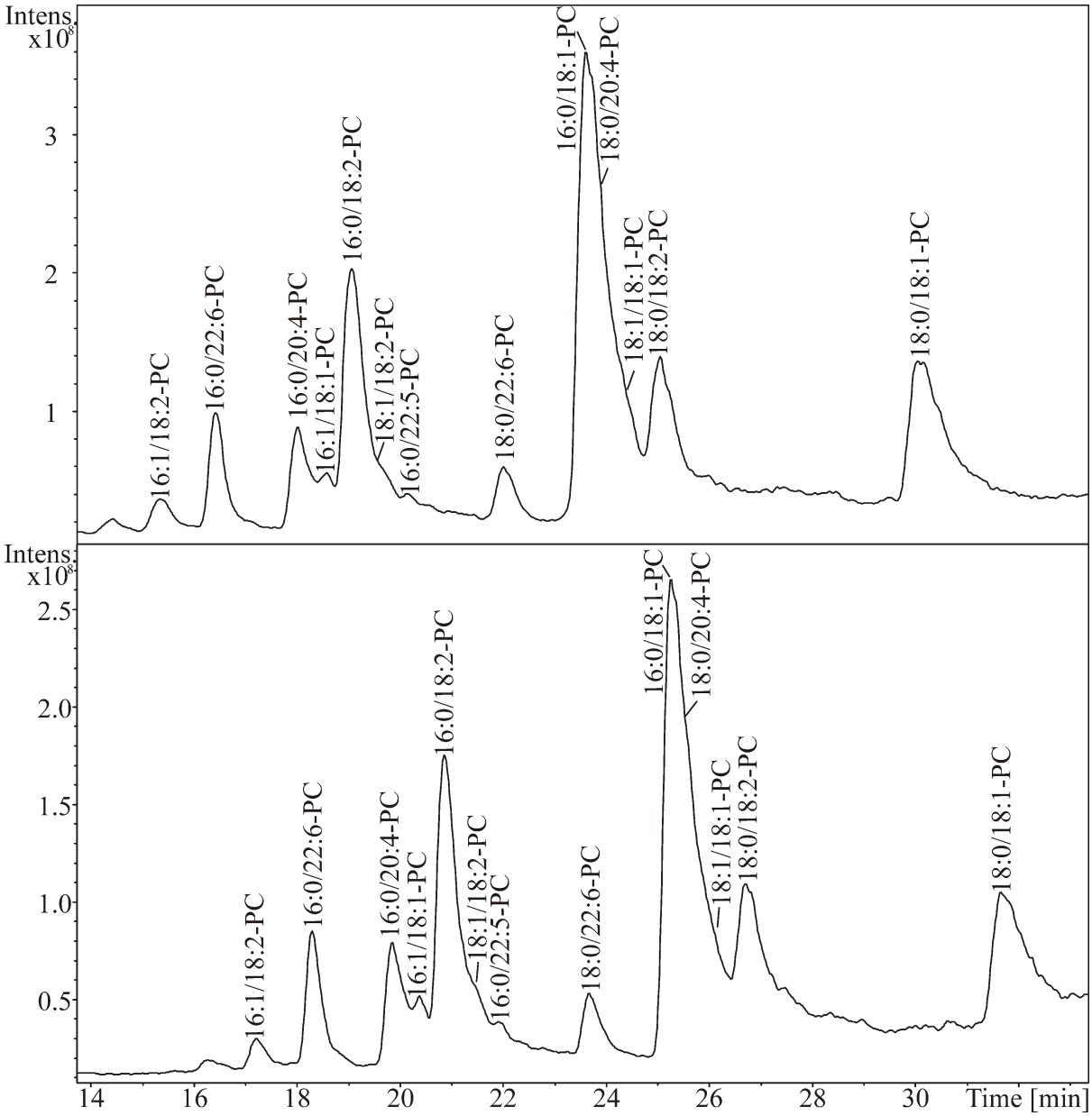

Figure S7. Influence of pH value of mobile phase on RP-HPLC separation of PC fraction from egg yolk. (A) pH = 7, and (B) pH = 3. HPLC conditions: Luna C_{18} column (250×4.6 mm, 5 μm, Phenomenex), flow rate 1 mL/min, separation temperature 40˚C, gradient 0 min - 80 % A + 20 % B, 40 min - 100 % A, where A is the mixture of acetonitrile / 2-propanol (1:3, v/v) and B is 5 mM aqueous ammonium acetate.

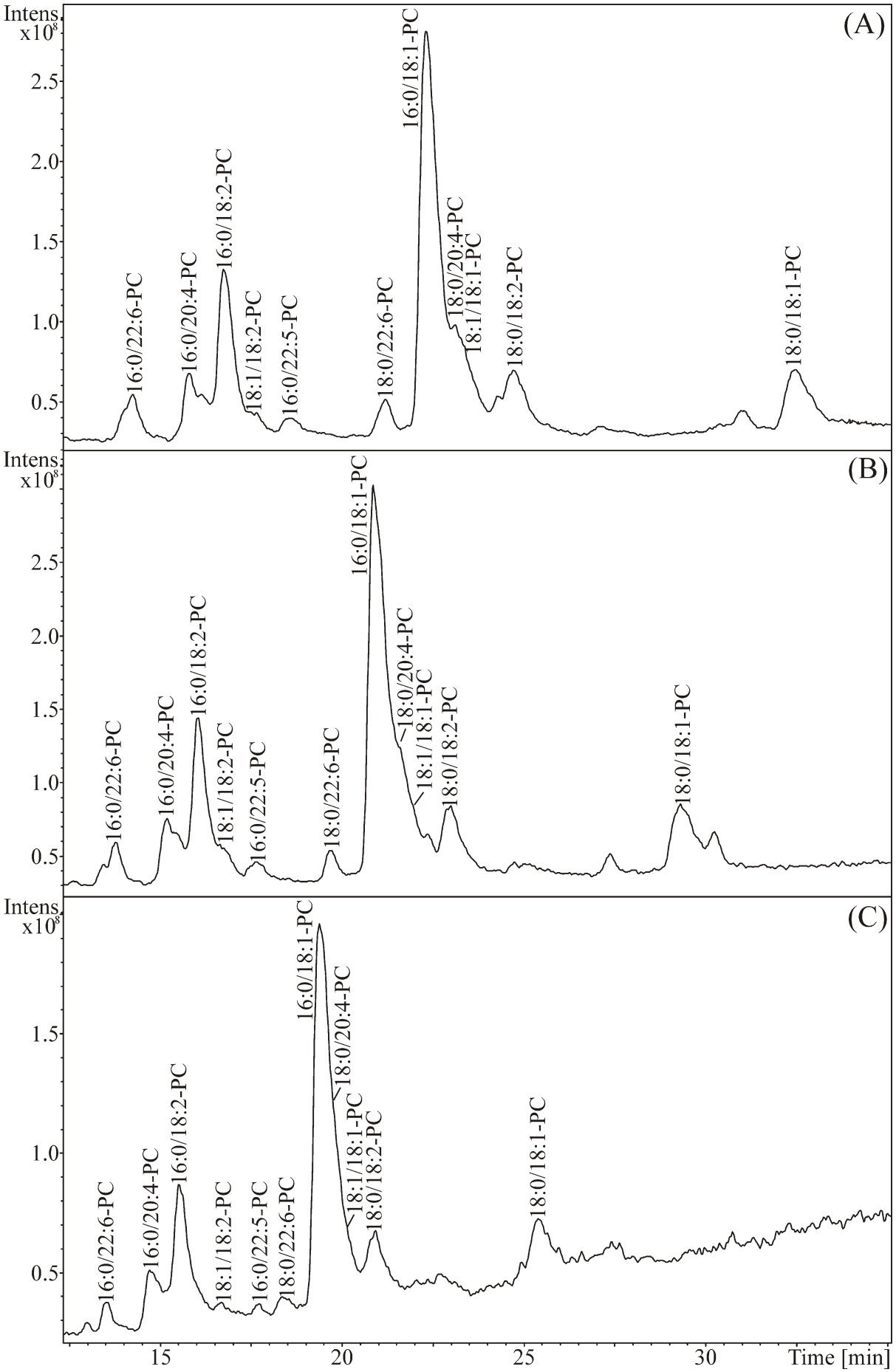

Figure S8. Influence of gradient steepness on RP-HPLC separation of PC fraction from egg yolk. Gradient from 0 min - 80 % A + 20 % B to 96 % A + 4 % B in (A) 160 min, (B) 100 min, and (C) 50 min, where A is the mixture of acetonitrile / 2-propanol (1:1, v/v) and B is 5 mM aqueous ammonium acetate. HPLC conditions: porous shell particles column Kinetex C_{18} (150×2.1 mm, 2.6 μm, Phenomenex), flow rate 0.3 mL/min, separation temperature 40˚C.