Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Supporting Information

Glycosylated diazeniumdiolate-based oleanolic acid derivatives: Synthesis, *in vitro* and *in vivo* biological evaluation as anti-human hepatocellular carcinoma agents

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Table of concents:

General Procedure for the Preparation of O^2-Glycosylated Diazeniumdiolates (21a-j). A sodium diazeniumdiolate (**20a-d**, 5.0 mmol) was dissolved in DMF (5 mL) and the solution was cooled to -20°C, during which time 2,3,4,6-tetra-*O*-acetyl-α-D-glycosyl bromide (2.26 g, 5.5 mmol) and a small amount of potassium carbonate in DMF (5 mL) was added drop wise. The reaction mixture was stirred for 12 h at -20 °C, and the mixture was poured into water (100 mL) prior to extraction with ethyl acetate (3×100 mL). The organic extracts were combined and washed sequentially with 1N HCl (3×100 mL) and saturated NaCl solution, and the organic fraction was dried over sodium sulfate. After removal of the solvent, the crude glycosylation products were purified by column chromatography (ethyl acetate/petroleum ether) to give the title compounds.

For the preparation of compound **21i** and **21j**, the corresponding intermediate crude O^2-glycosylated 1-(4-Boc-piperazine-1-yl) diazen-1-ium-1,2-diolates (**21g** and **21h**) were dissolved in a mixture of anhydrous CH_2Cl_2(5 mL) and trifluoroacetic acid (3 mL). The reaction mixture was stirred for 3-4 h at room temperature until starting materials were totally consumed, as indicated by TLC. The reaction was quenched by dropwise addition of triethylamine at 0 °C and the pH of the solution was adjusted to an alkaline pH. CH_2Cl_2 (20 mL) was added and the organic layer was washed with saturated NaCl solution, and the organic fraction was dried over sodium sulfate. After concentration, the crude products were purified by column chromatography (MeOH/CH_2Cl_2 1:10 v/v) to give**21i** and **21j**, respectively.

***O*^{2}-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl) 1-(4-hydroxylpiperidin-1-yl)diazen-1-ium-1,2-diolate (21a).** The title compound was obtained in 36% yield as a pale yellow oil. IR (KBr, cm^{-1}): 3528, 2950, 1757, 1371, 1259, 1226, 1077, 1039; ^{1}H NMR ($CDCl_3$, 500 MHz): δ2.00 (s, 6H, 2×$COCH_3$), 2.06 (s, 3H, $COCH_3$), 2.09 (s, 3H, $COCH_3$), 2.57-2.81 (m, 4H, 2×NCH_2), 3.78-3.89 (m, 1H, CH), 3.78 (ddd, *J*=2.3, 4.8, 7.2 Hz, 1H, H_5), 4.19 (dd, *J*=2.3, 12.4 Hz, 1H, H_6), 4.29 (dd, *J*=4.8, 12.4 Hz, 1H, $H_{6'}$), 5.10-5.16 (m, 1H, H_3), 5.18 (d, *J*=8.0 Hz, 1H, H_1), 5.24-5.31 (m, 1H, H_4), 5.34-5.39 (m, 1H, H_2). ESI-MS: 509 $[M+NH_4]^+$. HRMS: calculated for $C_{19}H_{19}N_3O_{12}Na[M+Na]^+$: 514.1649, found: 514.1657, PPM error 1.6.

***O*^{2}-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl) 1-(4-hydroxylpiperidin-1-yl)diazen-1-ium-1,2-diolate (21b).** The title compound was obtained in 34% yield as a pale yellow solid; mp 135-138 °C. IR (KBr, cm^{-1}): 3485, 2935, 1751, 1667, 1504, 1370, 1221, 1080; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 2.02 (s, 3H, $COCH_3$), 2.04 (s, 6H, 2×$COCH_3$), 2.08 (s, 3H, $COCH_3$), 2.67-2.81 (m, 4H, 2×CH_2), 3.56-3.67 (m, 4H, 2×NCH_2), 3.67-3.70 (m, 1H, CH), 3.82 (ddd, *J* = 2.4, 4.7, 7.1 Hz, 1H, H_5), 4.17 (dd, *J* = 2.4, 12.4 Hz, 1H, H_6), 4.27 (dd, *J* = 4.7, 12.4 Hz, $H_{6'}$), 5.13-5.17 (m, 1H, H_3), 5.21 (d, *J* = 8.2 Hz, 1H, H_1), 5.25-5.29 (m, 1H, H_4), 5.32-5.35 (m, 1H, H_2).ESI-MS: 509 $[M+NH_4]^+$. HRMS: calculated for $C_{19}H_{19}N_3O_{12}Na[M+Na]^+$: 514.1649, found: 514.1658, PPM error 1.8.

***O*^{2}-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl) 1-(4-hydroxylethylpiperazine-1-yl)diazen-1-ium-1,2-diolate (21c).**The title compound was obtained in 41% yield as a brown oil; IR (KBr, cm^{-1}):

3480, 2932, 2820, 1758, 1504, 1461, 1370, 1231, 1039; ^{1}H NMR ($CDCl_3$, 300 MHz): δ2.02 (s, 3H, $COCH_3$), 2.04 (s, 6H, 2×$COCH_3$), 2.09 (s, 3H, $COCH_3$), 2.66-2.69 (m, 2H, NCH_2), 2.75-2.83 (m, 4H, 2×NCH_2), 3.56-3.65 (m, 4H, 2×NCH_2), 3.69 (t, J = 4.5 Hz, 2H, OCH_2), 3.81-3.84 (m, 1H, H_5), 4.15-4.19 (m, 1H, H_6), 4.27 (dd, J = 4.8, 12.5 Hz, 1H, $H_{6'}$), 5.12-5.37 (m, 4H, H_3, H_1, H_4 and H_2).ESI-MS: 555 $[M+Cl]^-$. HRMS: calculated for $C_{20}H_{32}N_4O_{12}Na[M+Na]^+$: 543.1914, found: 543.1920, PPM error 1.1.

***O*^{2}-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl) 1-(4-hydroxylethylpiperazine-1-yl)diazen-1-ium-1,2-diolate (21d).**The title compound was obtained in 38% yield as a brown oil; IR (KBr, cm^{-1}): 3451, 2932, 2360, 1752, 1517, 1371, 1228; ^{1}H NMR ($CDCl_3$, 300 MHz): δ2.01 (s, 3H, $COCH_3$), 2.05 (s, 6H, 2×$COCH_3$), 2.08 (s, 3H, $COCH_3$), 2.65 (t, J = 4.5 Hz, 2H, NCH_2), 2.78-2.83 (m, 4H, 2×NCH_2), 3.53-2.59 (m, 4H, 2×NCH_2), 3.69 (t, J = 4.5 Hz, 2H, OCH_2), 3.80-3.87 (m, 1H, H_5), 4.17-4.20 (m, 1H, H_6), 4.26 (dd, J = 4.9, 12.5 Hz, 1H, $H_{6'}$), 5.11-5.39 (m, 4H, H_3, H_1, H_4 and H_2).ESI-MS: 521 $[M+H]^+$. HRMS: calculated for $C_{20}H_{33}N_4O_{12}[M+H]^+$: 521.2095, found: 521.2098, PPM error 0.6.

***O*^{2}-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl) 1-(4-hydroxylethyl-*N*-methylamino)diazen-1-ium-1,2-diolate (21e).**The title compound was obtained in 41% yield as a brown oil. IR (KBr, cm^{-1}): 3546, 3416, 1755, 1646, 1501, 1371, 1232, 1040: ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.99 (s, 3H, $COCH_3$), 2.04 (s, 6H, 2×$COCH_3$), 2.13 (s, 3H, $COCH_3$), 3.09 (s, 3H, NCH_3), 3.48-3.55 (m, 2H, NCH_2), 3.74 (t, J = 4.9 Hz, 2H, OCH_2), 4.01-4.05 (m, 1H, H_6), 4.17-4.21 (m, 2H, $H_{6'}$ and H_5), 5.12 (dd, J = 3.3, 10.7 Hz, 1H, H_3), 5.20 (d, J = 8.0 Hz, 1H, H_1), 5.41-5.47 (m, 1H, H_4), 5.54-5.61 (m, 1H, H_2).ESI-MS: 483 $[M+NH_4]^+$. HRMS: calculated for $C_{17}H_{27}N_3O_{12}Na[M+Na]^+$: 488.1492, found 488.1496, PPM error 0.8.

***O*^{2}-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl) 1-(4-hydroxylethyl-N-methylamino) diazen-1-ium-1,2-diolate (21f).**The title compound was obtained in 32% yield as brown oil. IR (KBr, cm^{-1}): 3510, 2944, 1751, 1501, 1371, 1225, 1170, 1090. ^{1}H NMR ($CDCl_3$, 300 MHz): δ2.00 (s, 3H, $COCH_3$), 2.06 (s, 6H, 2×$COCH_3$), 2.16 (s, 3H, $COCH_3$), 3.13 (s, 3H, NCH_3), 3.48-3.51 (m, 2H, NCH_2), 3.79 (t, J = 4.8 Hz, 2H, OCH_2), 4.02-4.06 (m, 1H, H_6), 4.16-4.19 (m, 2H, $H_{6'}$ and H_5), 5.10 (dd, J = 3.3, 10.5 Hz, 1H, H_3), 5.18 (d, J = 8.1 Hz, 1H, H_1), 5.42-5.43 (m, 1H, H_4), 5.51-5.57 (m, 1H, H_2).ESI-MS: 483 $[M+NH_4]^+$. HRMS: calculated for $C_{17}H_{27}N_3O_{12}Na[M+Na]^+$: 488.1492, found: 488.1497, PPM error 1.0.

***O*^{2}-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl) 1-(piperazine-1-yl)diazen-1-ium-1,2-diolate (21i).** The title compound was obtained in 21% yield as a pale brown oil; IR (KBr, cm^{-1}): 3493, 2914, 2861, 1755, 1629, 1504, 1370, 1232; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.76 (s, 3H, $COCH_3$), 2.00 (s, 3H, $COCH_3$), 2.08 (s, 3H, $COCH_3$), 2.19 (s, 3H, $COCH_3$), 3.12 (t, J = 4.8 Hz, 4H, 2×NCH_2), 3.52 (t, J = 4.5 Hz, 4H, 2×NCH_2), 4.00-4.09 (m, 1H, H_6), 4.18-4.20 (m, 2H, $H_{6'}$ and H_5), 5.05 (dd, J = 3.0, 10.2 Hz, 1H, H_3), 5.14 (d, J = 8.3 Hz, 1H, H_1), 5.41-5.48 (m, 1H, H_4), 5.22-5.59 (m, 1H, H_2).ESI-MS: 477 $[M+H]^+$. HRMS: calculated for $C_{18}H_{29}N_4O_{11}[M+H]^+$: 477.1833, found: 477.1829, PPM error -0.8.

***O*^{2}-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl) 1-(piperazin-1-yl)diazen-1-ium-1,2-diolate (21j).** The title compound was obtained in 27% yield as a brown oil; IR (KBr, cm^{-1}): 3487, 2920, 2844, 1751, 1505, 1371, 1229, 1082; ^{1}H NMR ($CDCl_3$, 300 MHz): δ1.79 (s, 3H, $COCH_3$), 2.03 (s, 3H, $COCH_3$), 2.05 (s, 3H, $COCH_3$), 2.16 (s, 3H, $COCH_3$), 3.05 (t, *J* = 4.8 Hz, 4H, 2×NCH_2), 3.47 (t, *J* = 4.5 Hz, 4H, 2×NCH_2), 4.01-4.05 (m, 1H, H_6), 4.16-4.19 (m, 2H, $H_{6'}$ and H_5), 5.09 (dd, *J* = 3.0, 10.2 Hz, 1H, H_3), 5.18 (d, *J* = 8.4 Hz, 1H, H_1), 5.42-5.43 (m, 1H, H_4), 5.48-5.55 (m, 1H, H_2).ESI-MS: 477 $[M+H]^+$. HRMS: calculated for $C_{18}H_{29}N_4O_{11}[M+H]^+$: 477.1833, found: 477.1831, PPM error -0.4.

***O*^{2}-(β-D-Galactopyranosyl) 1-(4-hydroxylpiperadin-1-yl)diazen-1-ium-1,2-diolate (24).** Compound **21b** (1 g, 2 mmol) was dissolved in a 1:1 mixture of anhydrous CH_2Cl_2 and MeOH at ice-bath temperature and the pH of the solution was adjusted to 9.0 with 0.1NMeONa/MeOH.The deacetylation reaction was was monitored by TLC. Upon completion of the reaction, pH was adjusted to 7.0 with acidic ion exchange resin 001×7(732). After filtration, the filtrate was evaporated in vacuo and the resulting residue was purified by column chromatography (MeOH/$CH_2Cl_2$1:10 v/v) to furnish the title compound in 35% yield as a colorless viscous liquid; IR (KBr, cm^{-1}): 3416, 2926, 1632, 1499, 1378, 1213, 1079; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.48-1.59 (m, 2H, CH_2), 1.89-1.90 (m, 2H, CH_2), 3.45-3.69 (m, 5H, 2×NCH_2, CH), 3.62-3.67 (m, 1H, H_3), 4.53 (d, *J* = 4.5 Hz, 1H, H_5), 4.64-4.68 (m, 1H, H_4), 4.79 (d, *J* = 3.9 Hz, 1H, H_2), 4.85 (d, *J* = 8.1 Hz, 1H, H_6), 4.89 (d, *J* = 5.7 Hz, 1H, $H_{6'}$), 5.24 (d, J=5.4 Hz, 1H, H_1).ESI-MS: 346 $[M+Na]^+$. HRMS: calculated for $C_{11}H_{20}N_3O_8[M- H]^-$: 322.1250, found: 322.1254, PPM error 1.2.

HPLC assessment of compound purity.

Column: Inertex C18 (150mm×4.6mm×5μm);

Mobile phase: Methanol: Water = 80:20;

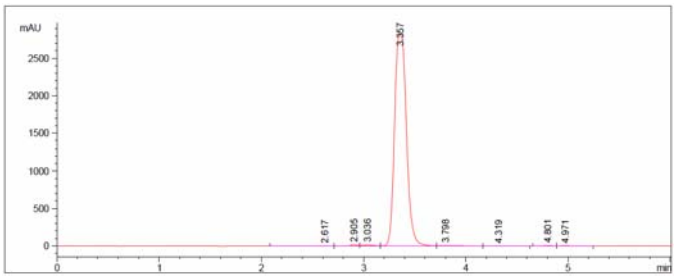
Wavelength: 254 nm;

Rate: 1 mL/min;

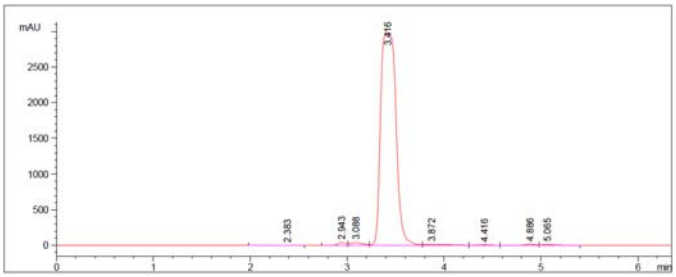
Temperature: 25 °C;

Pressure: 115-118 bar.

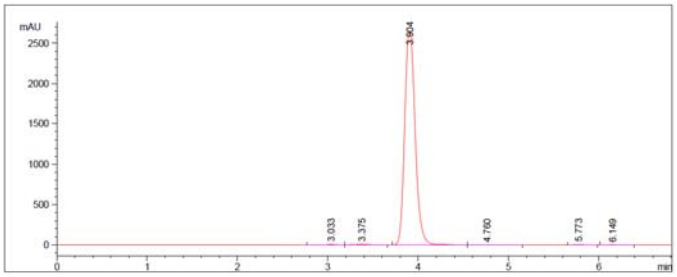
21a, 97.9%



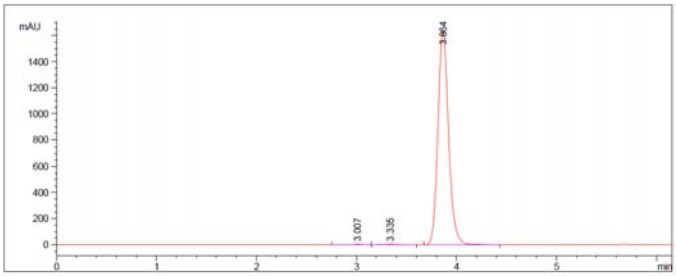
21b, 96.2%



21c, 98.4%



21d, 99.2%



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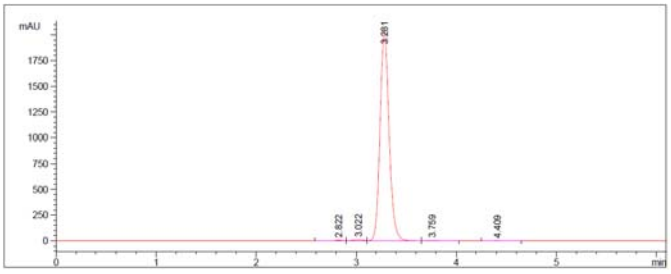

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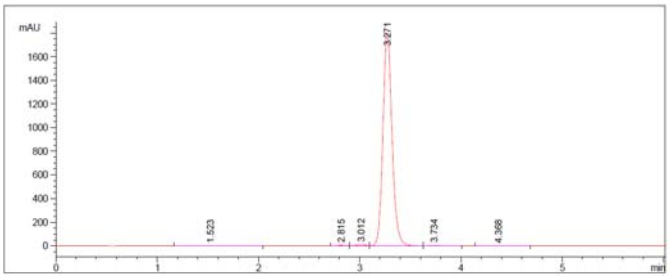
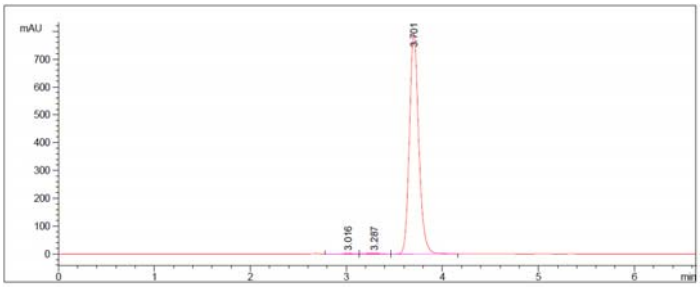
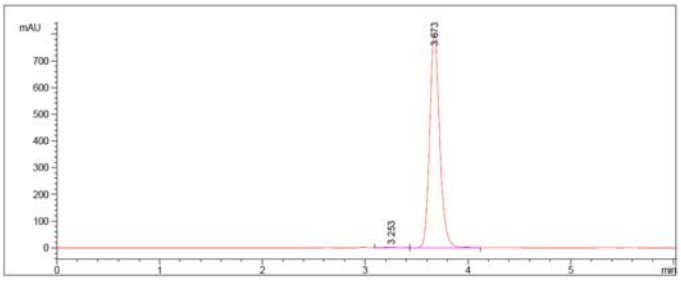


21i, 98.9%

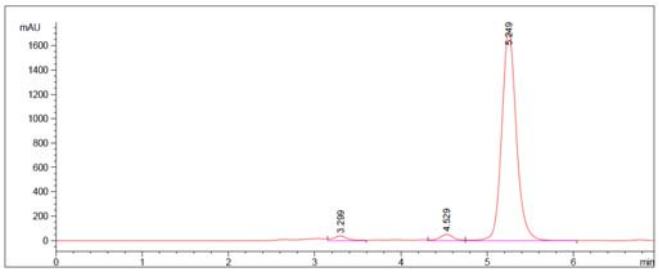


21j, 99.5%



4, 96.7%

5, 95.8%



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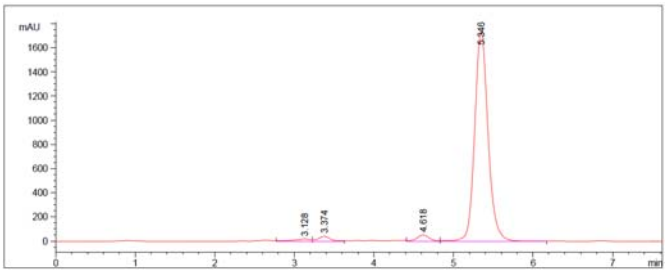

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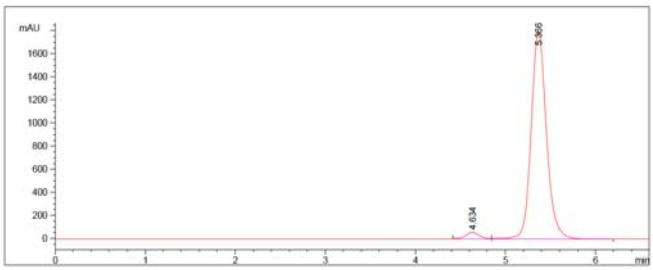

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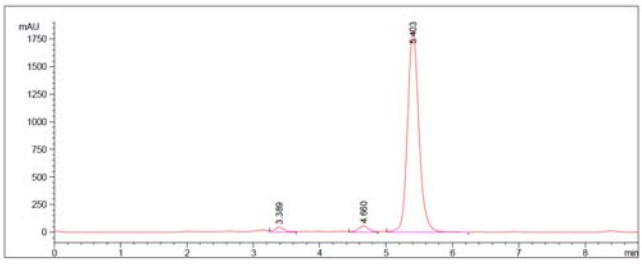

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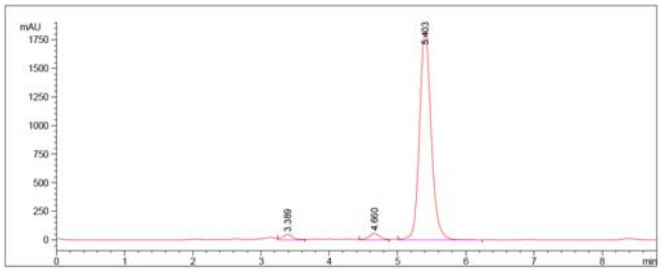

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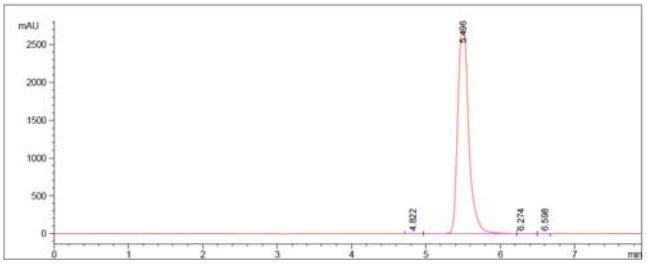

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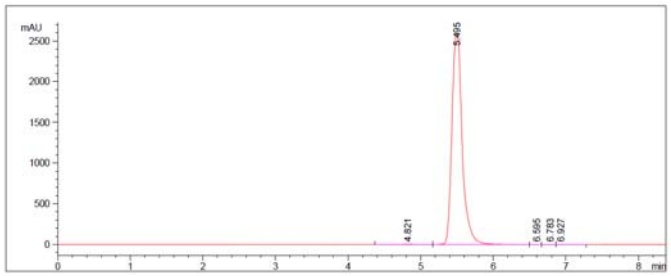

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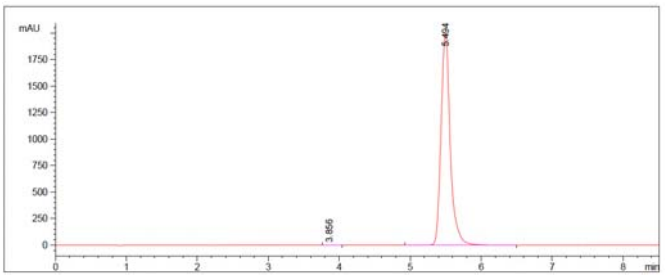

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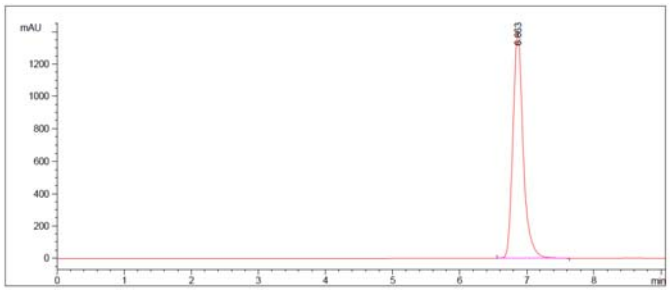

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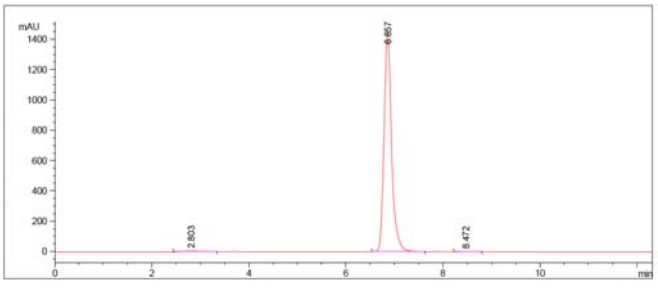

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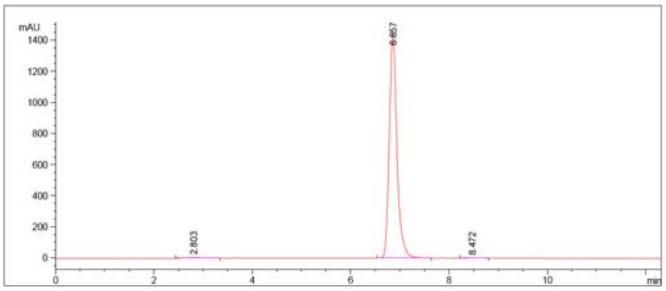

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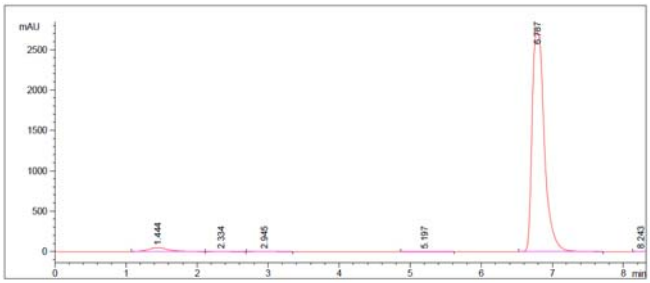

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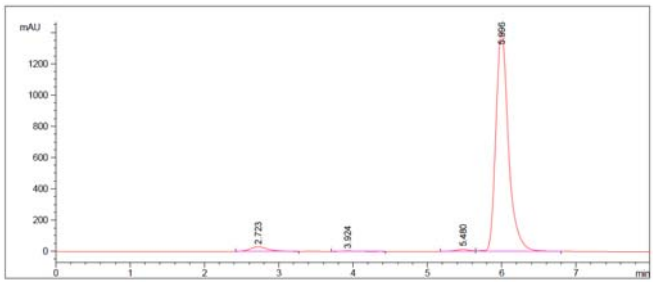

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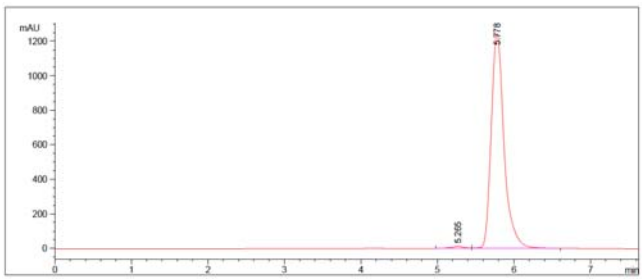

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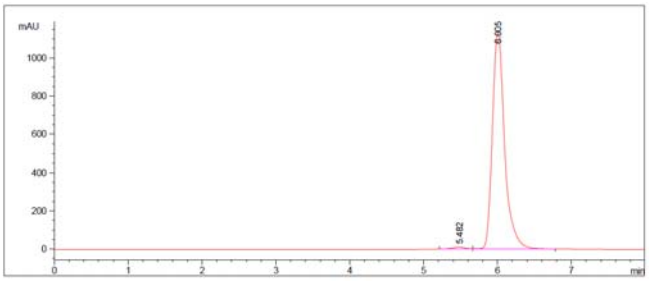

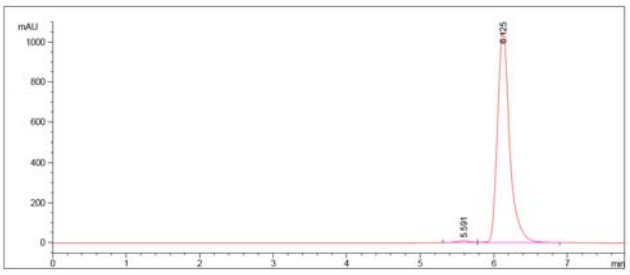

24, 100%