

Supporting material

Tetraoxanes as inhibitors of Apicomplexan parasites *Plasmodium falciparum* and *Toxoplasma gondii* and anti-cancer molecules

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Synthesis

S3 - S6

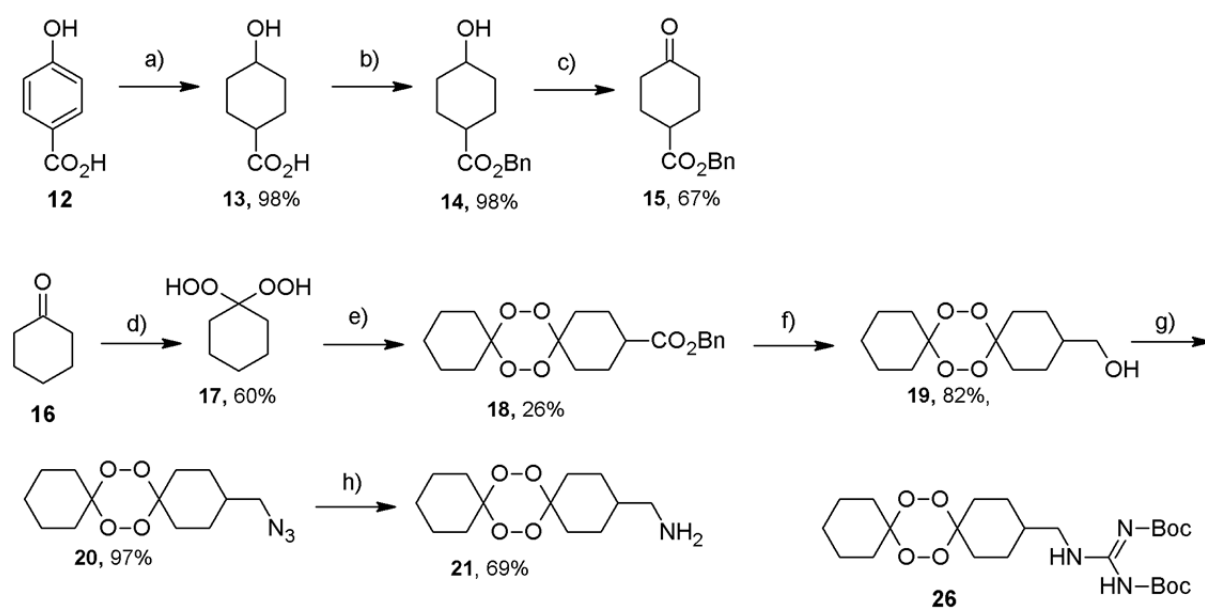
Literature

S6

HPLC purity chromatograms

S7 – S20

Scheme S1



a) 5% Rh - Al₂O₃, MeOH, 50 psi, s.t.; b) K₂CO₃, DMF, PhCH₂Cl, Δ;
 c) PCC, CH₂Cl₂, r.t.; d) Re₂O₇ (5mol%), 50% H₂O₂, CH₃CN;
 e) **15**, CH₂Cl₂, H₂SO₄ / CH₃CN; f) LiAlH₄, Et₂O; g) i) MsCl, Py; ii) NaN₃, DMF;
 h) LiAlH₄, Et₂O;

IC50 (D6) = 90.56 nM
IC50 (W2) = 54.70 nM
IC50 (TM91C235) = 116.0 nM
IC50 (TM90C2B) = 52.07 nM

Scheme S1: Reaction pathway for synthesis of derivative **21**.

Table 1S. Calculated pKa and logP values for derivatives 21, 22 and 23.^a

Compound	21	22	23
pKa	10.16	12.08	12.14
logP	1.70	3.12	1.63

^a For pKa calculations Epik, version 2.9, Schrödinger, LLC, New York, NY, 2014. Were used. For log P calculations QikProp, version 4.1, Schrödinger, LLC, New York, NY, 2014 were used.

Synthesis

4-hydroxycyclohexanecarboxylic acid (**13**)¹

Mixture of 4-hydroxybenzoic acid (15.0 g, 108.6 mmol) and 5% Rh-Al₂O₃ (1g) in MeOH (100 mL) was shaken in Parr-shaker in hydrogen atmosphere (50 psi) at r.t. After 24 hours hydrogen was exchanged with Ar, mixture was filtered through celite and solvent was removed under reduce pressure. Product was obtained as mixture of *cis/trans* isomers. Yield 15.39 g (98 %), m.p. = 120 - 123 °C. (lit. t.t. = 126 - 128 °C). IR (ATR): 3437s, 2934s, 2857m, 2601w, 1702s, 1443w, 1368w, 1312m, 1242w, 1203w, 1058m, 1026w, 949w, 913w, 736w, 587w cm⁻¹. ¹H-NMR (200 MHz, CDCl₃, δ): 4.52 (bs, OH), 3.98 – 3.84 (m, H_e-COH), 3.72 – 3.54 (m, H_a-COH), 2.54 – 2.16 (m), 2.14 – 1.86 (m), 1.84 – 1.60 (m), 1.58 -1.16 (m).

Benzyl 4-hydroxycyclohexanecarboxylate (**14**)²

Obtained as *cis/trans* mixture with 2:1 ratio of axial:equatorial hydroxyl group (1H NMR). Mixture of **13** (10.0 g, 69.4 mmol) and anhydrous K₂CO₃ (19.1 g, 138.2 mmol) in DMF (18 mL) was warmed to 55 °C and benzyl chloride (10.48 mL, 90.8 mmol) was added in drops and stirring was continued at same temperature. After 12 hours reaction was cooled to room temperature, water (25 mL) was added and mixture was extracted with CH₂Cl₂ (4 × 30 mL). Combined organic layers were washed once with sat. NaHCO₃ (15 mL), once with brine (15 mL) and dried over anh. Na₂SO₄. Crude product (white powder, 49.28 g) was used without further purification in next reaction step. Analytical sample was obtained after column chromatography purification (flash, SP Biotage, SiO₂-column, Flash 12+M, Hexane/EtOAc =

6:4). IR (ATR): 3405m, 3033w, 2938s, 2863w, 1732s, 1496w, 1454w, 1385m, 1311w, 1236m, 1169s, 1136w, 1070m, 1033m, 967m, 907w, 749m, 699m cm^{-1} . $^1\text{H-NMR}$ (200 MHz, CDCl_3 , δ): 7.40-7.30 (m, 5H-Ar), 5.12 (s, Ar- CH_2), 3.95 – 3.85 (m, H_e -COH), 2.52-2.36 (m, H_a - CO_2Bz), 2.12-1.86 (m, 3H), 1.80-1.52 (m, 5H). $^{13}\text{C-NMR}$ (50 MHz, CDCl_3 , δ): 175.10, 136.14, 128.51, 128.11, 127.98, 66.77, 66.04, 41.26, 31.94, 23.58.

Benzyl-4-oxocyclohexanecarboxylate (15)^{2,3}

Mixture of alcohol **14** (25.0 g, 106.7 mmol) and PCC (34.44 g, 160.0 mmol) in CH_2Cl_2 (150 L) was stirred at r.t 2 hours. Suspension was transferred on SiO_2 column and product was collected after eluting with CH_2Cl_2 (600 mL). Solvent was removed under reduce pressure and product was obtained after column chromatography purification (flash, SP Biotage, SiO_2 -column, 40+M, eluent hexane / EtOAc gradient 85/15 \rightarrow 7/3) as pale green-yellow oil. Yield 9.57g (67%) IR (ATR): 3033w, 2954m, 1710s, 1453m, 1384m, 1303m, 1210s, 1158s, 1028w, 1004m, 965w, 746s, 698s, 495w, 421w cm^{-1} . $^1\text{H-NMR}$ (200 MHz, CDCl_3 , δ): 7.36 (s, Ar), 5.16 (s, Ar- CH_2), 2.90-2.70 (m, H_a - CO_2Bz), 2.56-1.92 (m, 8H). $^{13}\text{C-NMR}$ (50 MHz, CDCl_3 , δ): 210.02, 173.94, 135.72, 128.62, 128.36, 128.13, 66.49, 40.62, 39.62, 28.42.

Cyclohexane-1,1-diyl dihydroperoxide (17)

Into mixture of cyclohexanone (980.0 mg, 10.0 mmol) and Re_2O_7 (242.2 mg, 0.5 mmol, 5 mol %) in CH_3CN (25mL), 50% solution of H_2O_2 (1.12 mL, 40.0 mmol) was added and stirring was continued at r.t. 1 hour. Reaction was transferred on the SiO_2 column and was eluted with EtOAc. Fractions with crude product were combined, washed once with brine and dried over anh. Na_2SO_4 at 0 °C. Solvent was removed under reduce pressure and product was isolated after column chromatography (Lobar, SiO_2 -column C, eluent hexane / EtOAc = 7/3). Yield 890.2 mg (60%), colourless oil. IR (film): 3419s, 2946s, 2863s, 1712m, 1634w, 1454s, 1391s, 1278m, 1161m, 1098m, 1064s, 947m, 927m, 849m cm^{-1} . IR(CCl_4): 3424s, 2948s, 2865s, 1746m, 1722m, 1452s, 1393s, 1349m, 1162s, 951s, 922m cm^{-1} . $^1\text{H-NMR}$ (200 MHz, CDCl_3 , δ): 9.60 (bs, 2 \times HOO-C(1)), 2.0 – 1.8 (m, 4 H), 1.6 – 1.4 (m, 6 H). $^{13}\text{C-NMR}$ (50 MHz, CDCl_3 , δ): 110.94, 29.41, 25.18, 22.31.

7,8,15,16-tetraoxadispiro[5.2.5.2]hexadec-3-yl methanol (19)⁴

Flame dried two-neck round bottom flask was charged, under Ar atmosphere, with LiAlH_4 (280.0 mg, 7.3 mmol) and dry THF-u (20 mL), and solution of ester **18** (2.4 g, 4.55 mmol) in dry THF (20 mL) was added dropwise under intensive stirring, at r.t. After 2 hours reaction

was quenched with EtOAc, water was added and emulsion was transferred into separatory funnel. Water layer was acidified (pH = 2) with dilute HCl (1:1, v/v), layers were separated and water layer was extracted with EtOAc (3 × 20 mL). Combined organic layer were dried over anh. Na₂SO₄, solvent was removed under reduce pressure and product was isolated after column chromatography purification (dry-flash, SiO₂-column, eluent heptane / EtOAc = 8 / 2). Yield 1.4 g (82%). Colourless foam, softens at 116 -118 °C. IR (KBr): 3320m, 3009w, 2940s, 2861s, 1443m, 1360w, 1339w, 1310w, 1273w, 1250w, 1159w, 1094w, 1068m, 1045m, 984w, 941w, 918m, 897w, 881w, 850w cm⁻¹. ¹H NMR (500 MHz, CDCl₃, δ): 3.5 (d, *J* = 6.2 Hz, CH₂-OH), 3.12 (bs, 1H), 2.45-2.15 (m, 2H), 1.85-1.70 (m, 3H), 1.70-1.35 (m, 12H), 1.35-1.20 (m, 2H). ¹³C NMR (125 MHz, CDCl₃, δ): 108.29, 108.16, 67.41, 39.44, 31.80, 30.90, 29.52, 28.53, 25.35, 24.95, 24.45, 22.17, 21.88. (+)ESI-HRMS (*m/z*): Calculated for [M + NH₄]⁺ 276.18055, found 276.18041. Combustion analysis (C₁₃H₂₂O₅): Calculated C 60.45, H 8.58, found C 60.47, H 8.18.

3-(Azidomethyl)-7,8,15,16-tetraoxadispiro[5.2.5.2]hexadecane (20)⁴

Into solution of **19** (1.38 g, 5.34 mmol) in dry Py (11 mL) methanesulfonyl chloride (495 μL, 6.4 mmol) was added at r.t. under intensive stirring. After 2 hours, reaction was quenched with water / EtOAc mixture, transferred into separatory funnel. Water layer was acidified (pH = 5) with dilute HCl (1:1, v/v), layers were separated and water layer was extracted with EtOAc (4 × 25 mL). Combined organic layer were dried over anh. Na₂SO₄, filtered of and solvent was removed under reduce pressure. Obtained crude product was used in next reaction step without further purification. Mixture of mesylate and NaN₃ (3.47 g, 53.4 mmol) in DMF (20 mL) was stirred at 50 °C over 12 hours, cooled at r.t. and poured in to EtOAc / water mixture. Layers were separated and water layer was extracted with EtOAc (4 × 25 mL). Combined organic layers were washed with brine (2 × 25 mL), dried over anh. Na₂SO₄, filtered of and solvent was removed under reduce pressure. Product was isolated after column chromatography purification (dry-flash, SiO₂-column, eluent heptane / EtOAc = 9 / 1). Yield 1.45 g (97%). Colourless foam softens at 86-87 °C. Spectra are in accordance to literature data. IR (KBr): 2993w, 2946m, 2868w, 2096s, 1714w, 1445m, 1358w, 1338w, 1292m, 1258m, 1213w, 1183w, 1183w, 1155w, 1137w, 1091w, 1067w, 1047m, 1016w, 952w, 915m, 883w, 850w, 817w cm⁻¹. ¹H NMR (200 MHz, CDCl₃, δ): 3.18 (d, *J* = 6.2 Hz CH₂, 2 H), 3.14 (bs, 1 H), 2.27 (bs, 2 H), 1.80 – 1.26 (m, 16 H). ¹³C NMR (50 MHz, CDCl₃, δ): 108.39, 107.73, 56.74, 37.04, 31.65, 30.81, 29.48, 28.46, 25.29, 22.05. HPLC purity: method A: RT 3.140, area 96.998 %; method B: RT 1.371, area 96.81 %.

1-(7,8,15,16-Tetraoxadispiro[5.2.5.2]hexadec-3-yl)methanamine (21)⁴

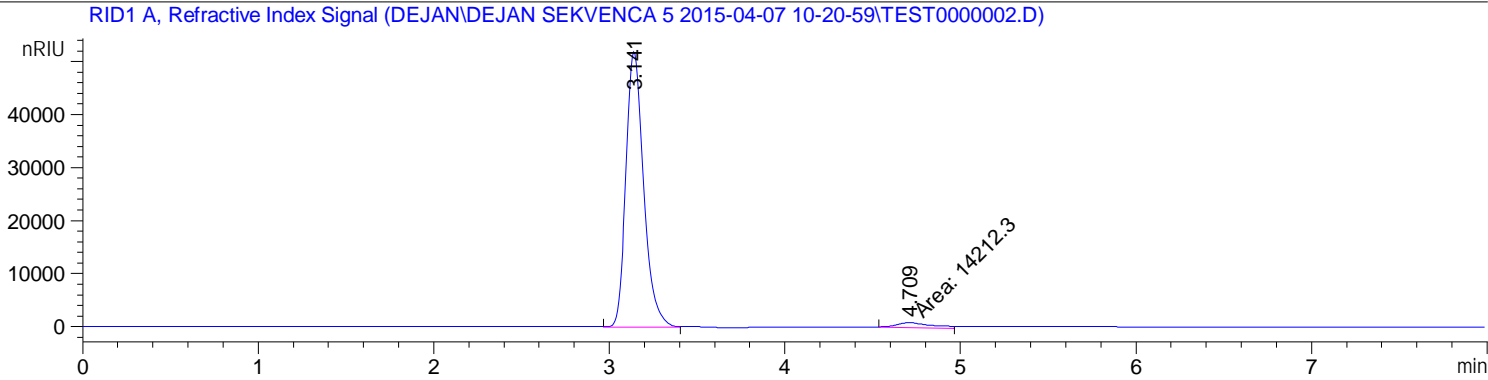
Flame dried two-neck round bottom flask was charged, under Ar atmosphere, with LiAlH₄ (217.8 mg, 5.74 mmol) and dry diethyl ether (15 mL), and solution of azide **20** (1.18 g, 4.16 mmol) in dry diethyl ether (15 mL) was added dropwise under intensive stirring, at r.t. After 1 hour reaction was quenched with water and 10 % NaOH solution, K₂Na-tartrate (4.8 g, 17.22 mmol, 3 eq. calculated to LiAlH₄) was added and mixture was stirred at r.t. until organic layer become clear (12 – 15 hours. Layers were separated, water layer was extracted with diethyl ether (3 × 25 mL) and combined organic layer were washed with brine (2 × 15 mL) and dried over anh. Na₂SO₄. Mixture was filtered of, solvent was removed under reduce pressure and product was isolated after column chromatography purification (dry-flash, SiO₂-column, eluent EtOAc / MeOH / NH₃aq = 8 / 1 / 1). Yield 0.4g (69 %) as pale yellow amorphous powder, mp. 75-77 °C. Spectra were in accordance to literature data. IR (KBr): 3378m, 3340m, 3010w, 2941s, 2862s, 1720w, 1443m, 1362w, 1341w, 1275w, 1253w, 1160w, 1096w, 1069m, 1049m, 984w, 942w, 919m, 896w, 851w, 824w cm⁻¹. ¹H NMR (200 MHz, CDCl₃, δ): 3.11 (bs, 1H) 2.58 (d, *J* = 6.0 Hz, CH₂-NH₂), 2.26 (bs, 2H), 1.90-1.11 (m, 18 H). ¹³C NMR (50 MHz, CDCl₃, δ): 108.26, 47.58, 40.20, 31.68, 31.0, 29.52, 28.73, 25.78, 25.31, 21.96. HPLC purity: method A: RT 3.139, area 97.24 %; method B: RT 1.369, area 96.93 %.

References

1. D. S. Noyce, H. I. Weingarten, *J. Am. Chem. Soc.*, **79** (1957) 3098
2. S. D. Kuduk, R. K. Chang, R. M. DiPardo, C. N. Di Marco, K. L. Murphy, R. W. Ransom, D. R. Reiss, C. Tang, T. Prueksaritanont, D. J. Pettibone, M. G. Bock, *Bioorg. Med. Chem. Lett.*, **18** (2008) 5107
3. A. Bahadoor, A. C. Castro, L. K. Chan, F. G. Keaney, M. Nevalainen, V. Nevalainen, S. Peluso, D. A. Snyder, T. T. Tibbitts, WO 2011/140190 A1
4. Igor Opsenica, Dejan Opsenica, Kirsten S. Smith, Wilbur K. Milhous, Bogdan A. Šolaja, *J. Med. Chem.*, **51** (2008) 2261.

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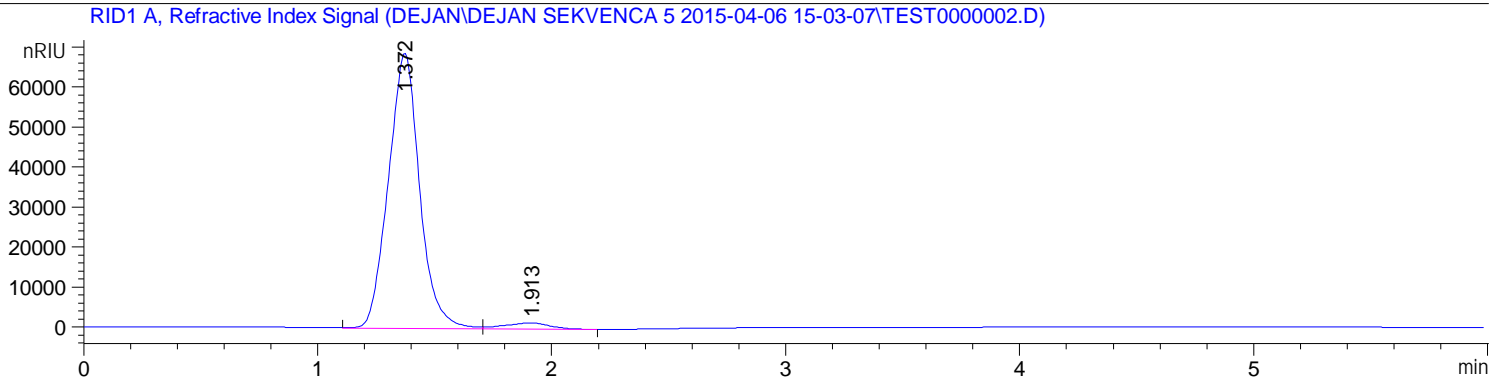
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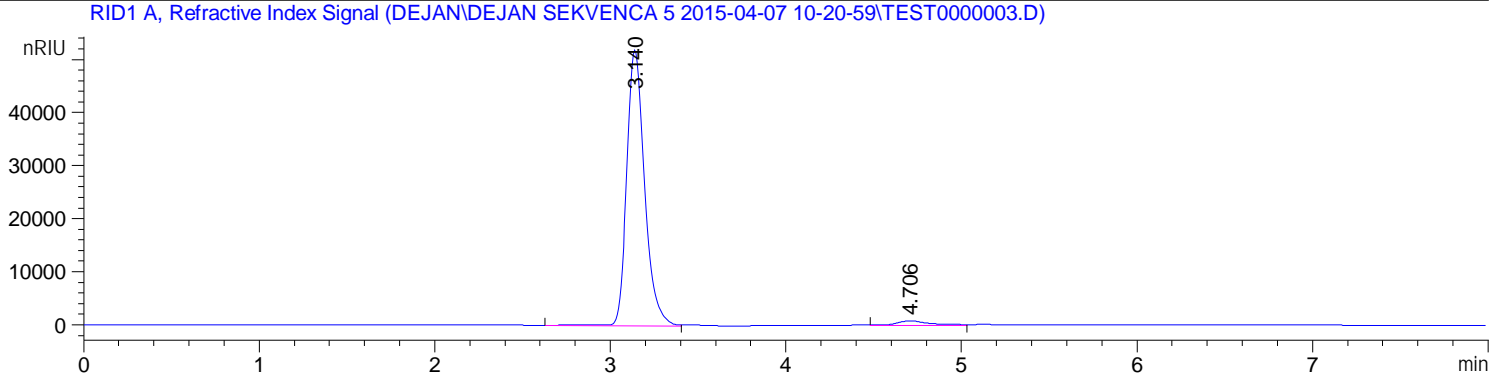
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Compound 20; Method A

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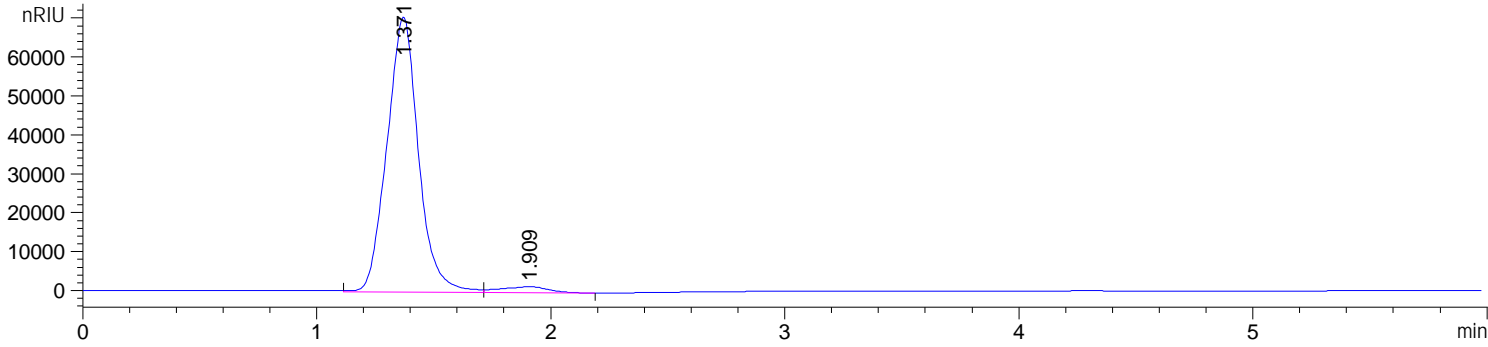
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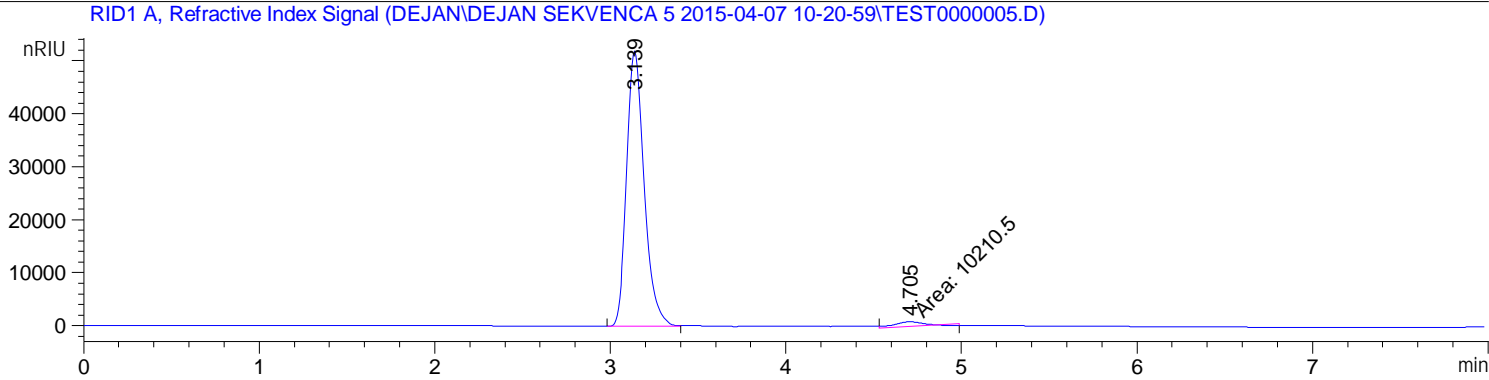
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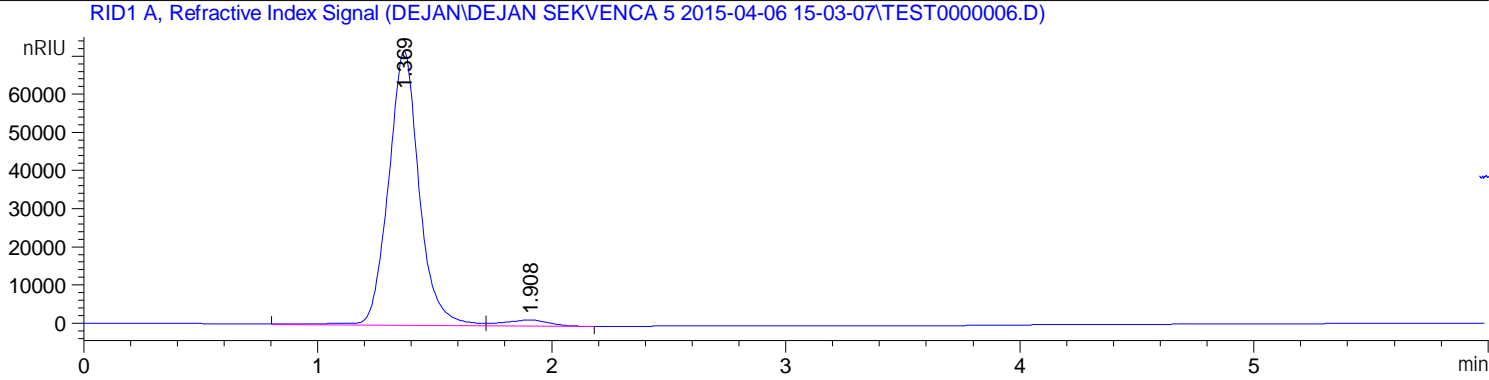
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Use Multiplier & Dilution Factor with ISTDs

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Signal 1: RID1 A, Refractive Index Signal

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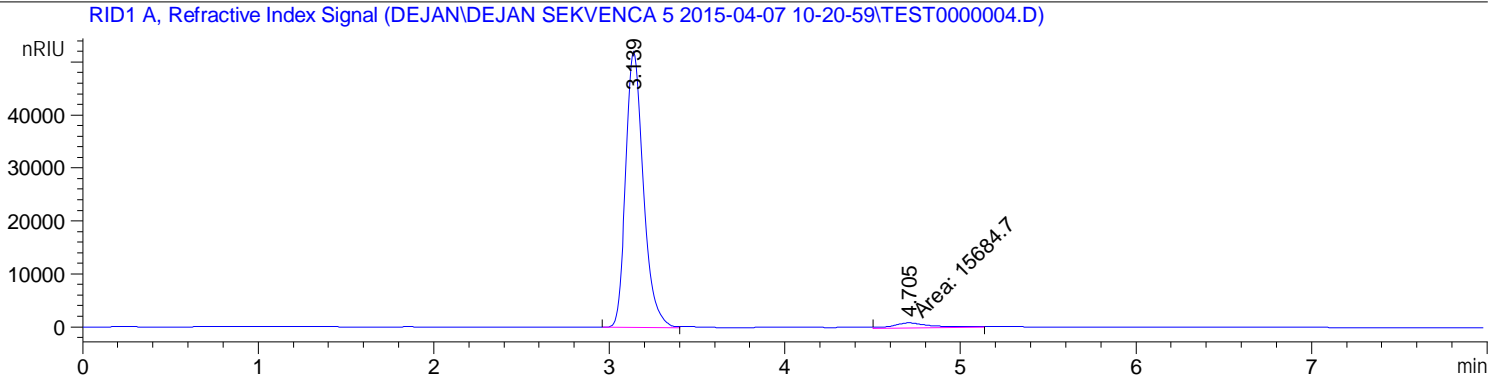
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Signal 1: RID1 A, Refractive Index Signal

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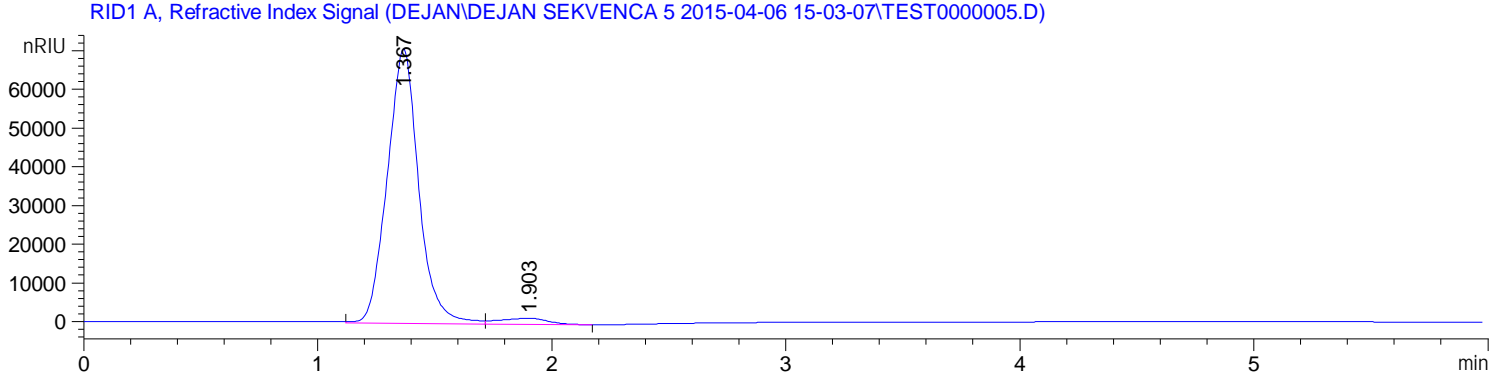
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=====
Fraction Information
=====

```

No Fractions found.

```

=====
Area Percent Report
=====

```

```

Sorted By      :      Signal
Multiplier     :      1.0000
Dilution       :      1.0000
Use Multiplier & Dilution Factor with ISTDs

```

Signal 1: RID1 A, Refractive Index Signal

Peak #	RetTime [min]	Type	Width [min]	Area [nRIU*s]	Height [nRIU]	Area %
1	1.367	VV	0.1369	6.61517e5	7.09264e4	96.7679
2	1.903	VB	0.1932	2.20950e4	1581.47388	3.2321

```
Totals :                6.83612e5  7.25079e4
```

```

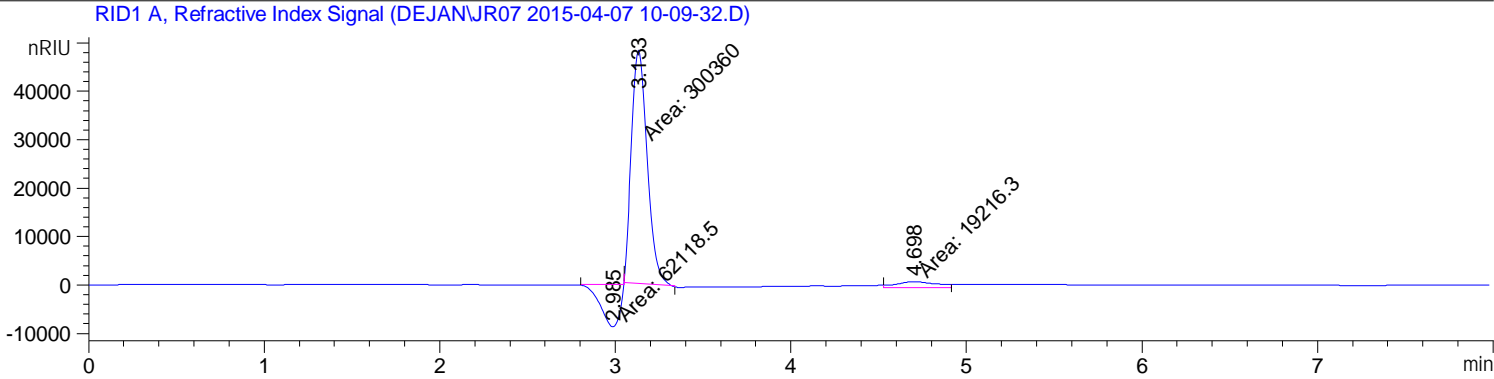
=====
*** End of Report ***

```

```

=====
Acq. Operator   : SYSTEM
Acq. Instrument : HPLC-Solaja           Location : Vial 2
Injection Date  : 4/7/2015 10:10:41 AM
                                           Inj Volume : 0.600 µl
Acq. Method     : C:\CHEM32\1\METHODS\IZOKRATSKI 1.M
Last changed    : 4/7/2015 10:09:23 AM by SYSTEM
                  (modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\DEF_LC.M
Sample Info     : Poroshell 70%MeOH/30%H2O
    
```

Additional Info :



Fraction Information

No Fractions found.

Area Percent Report

```

Sorted By      : Signal
Multiplier     : 1.0000
Dilution       : 1.0000
Use Multiplier & Dilution Factor with ISTDs
    
```

Signal 1: RID1 A, Refractive Index Signal

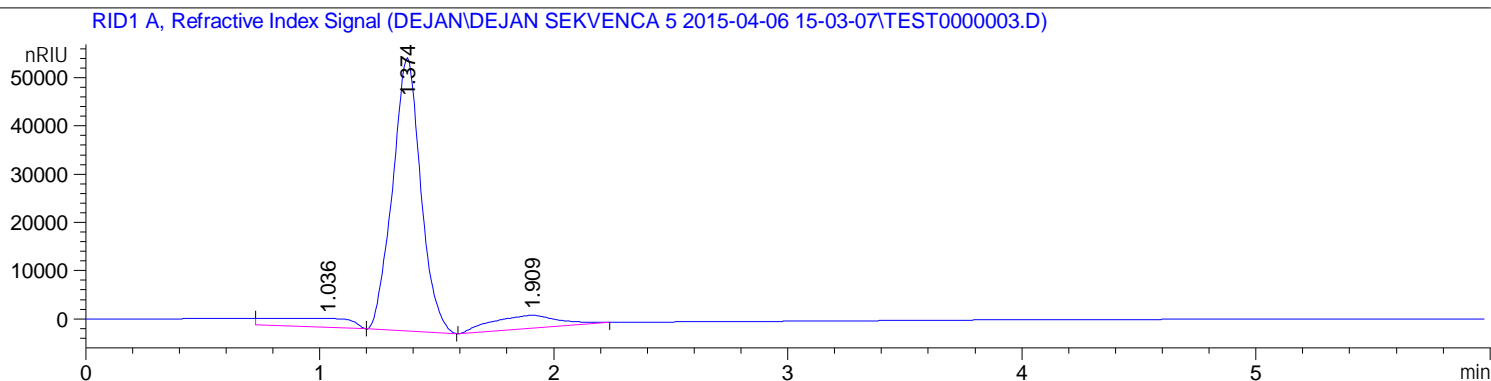
Peak #	RetTime [min]	Type	Width [min]	Area [nRIU*s]	Height [nRIU]	Area %
1	2.985	MM N	0.1179	6.21185e4	8780.76953	16.2744
2	3.133	MM	0.1040	3.00360e5	4.81253e4	78.6912
3	4.698	MM	0.2645	1.92163e4	1210.69263	5.0345

Totals : 3.81695e5 5.81167e4

*** End of Report ***

```

=====
Acq. Operator   : SYSTEM                      Seq. Line :    3
Acq. Instrument : HPLC-Solaja                 Location  : Vial 2
Injection Date  : 4/6/2015 3:19:24 PM        Inj       :    1
                                                Inj Volume: 1.000 µl
Method          : C:\CHEM32\1\DATA\DEJAN\DEJAN SEKVENCA 5 2015-04-06 15-03-07\IZOKRATSKI 1.M
                (Sequence Method)
Last changed    : 4/6/2015 3:03:07 PM by SYSTEM
Additional Info :
=====
    
```



=====
 Fraction Information
 =====

No Fractions found.
 =====

=====
 Area Percent Report
 =====

```

Sorted By      :      Signal
Multiplier     :      1.0000
Dilution       :      1.0000
Use Multiplier & Dilution Factor with ISTDs
    
```

Signal 1: RID1 A, Refractive Index Signal

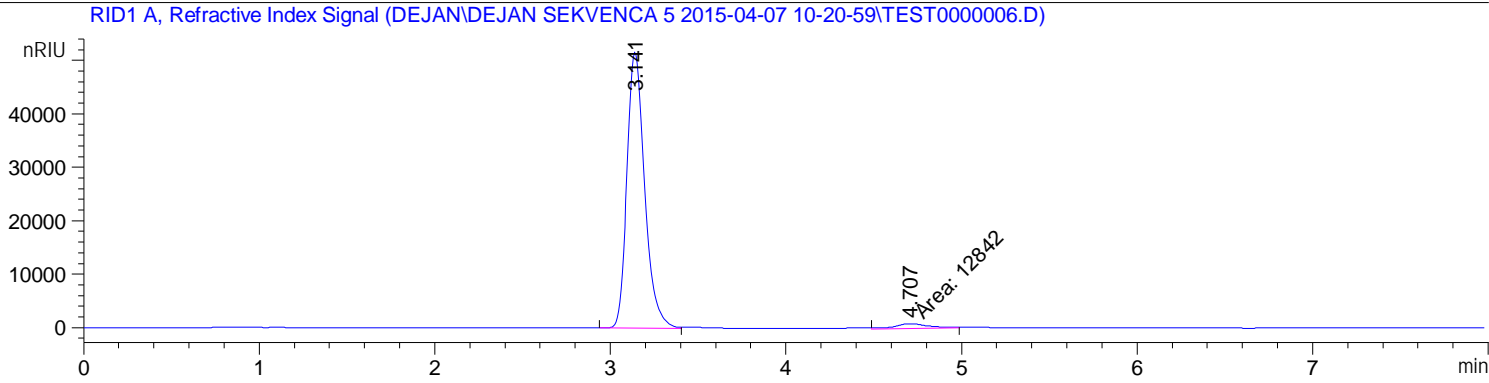
Peak #	RetTime [min]	Type	Width [min]	Area [nRIU*s]	Height [nRIU]	Area %
1	1.036	VB	0.3214	4.30415e4	1848.04871	7.5191
2	1.374	BB	0.1257	4.74381e5	5.66800e4	82.8717
3	1.909	BB	0.2733	5.50058e4	2663.60571	9.6092

Totals : 5.72429e5 6.11917e4

=====
 *** End of Report ***


```

=====
Acq. Operator   : SYSTEM                      Seq. Line :    6
Acq. Instrument : HPLC-Solaja                 Location  : Vial 8
Injection Date  : 4/7/2015 11:09:53 AM      Inj       :    1
                                                Inj Volume: 0.600 µl
Method          : C:\CHEM32\1\DATA\DEJAN\DEJAN SEKVENCA 5 2015-04-07 10-20-59\IZOKRATSKI 1.M
                  (Sequence Method)
Last changed    : 4/7/2015 10:20:59 AM by SYSTEM
Additional Info :
    
```



Fraction Information

No Fractions found.

Area Percent Report

```

Sorted By      :      Signal
Multiplier     :      1.0000
Dilution       :      1.0000
Use Multiplier & Dilution Factor with ISTDs
    
```

Signal 1: RID1 A, Refractive Index Signal

Peak #	RetTime [min]	Type	Width [min]	Area [nRIU*s]	Height [nRIU]	Area %
1	3.141	VV	0.1076	3.60992e5	5.15494e4	96.5648
2	4.707	MM	0.2276	1.28420e4	940.18732	3.4352

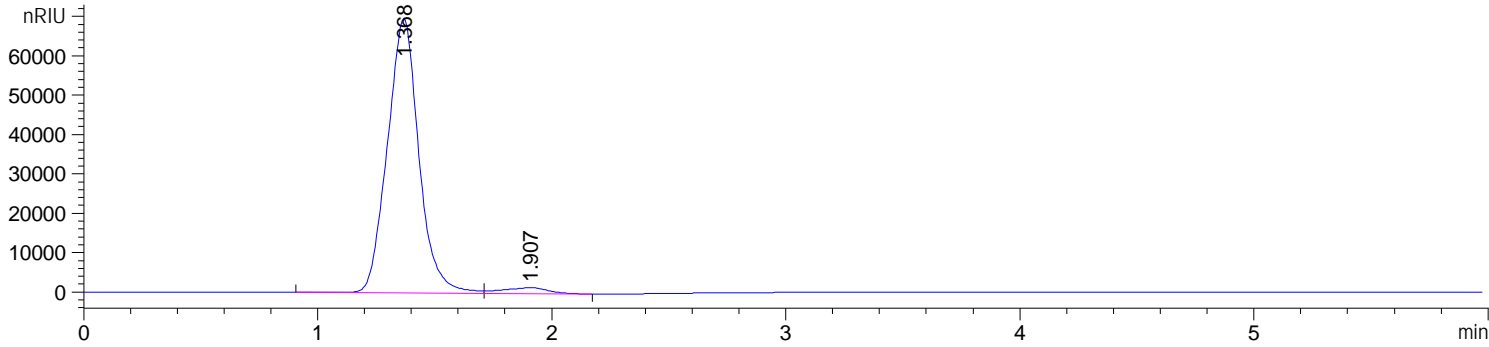
Totals : 3.73834e5 5.24896e4

*** End of Report ***

```

=====
Acq. Operator   : SYSTEM                      Seq. Line :    7
Acq. Instrument : HPLC-Solaja                 Location  : Vial 8
Injection Date  : 4/6/2015 3:49:32 PM        Inj       :    1
                                                Inj Volume: 1.000 µl
Method          : C:\CHEM32\1\DATA\DEJAN\DEJAN SEKVENCA 5 2015-04-06 15-03-07\IZOKRATSKI 1.M
                (Sequence Method)
Last changed    : 4/6/2015 3:03:07 PM by SYSTEM
Additional Info :
    
```

RID1 A, Refractive Index Signal (DEJAN\DEJAN SEKVENCA 5 2015-04-06 15-03-07\TEST0000007.D)



Fraction Information

No Fractions found.

Area Percent Report

```

Sorted By      : Signal
Multiplier     : 1.0000
Dilution       : 1.0000
Use Multiplier & Dilution Factor with ISTDs
    
```

Signal 1: RID1 A, Refractive Index Signal

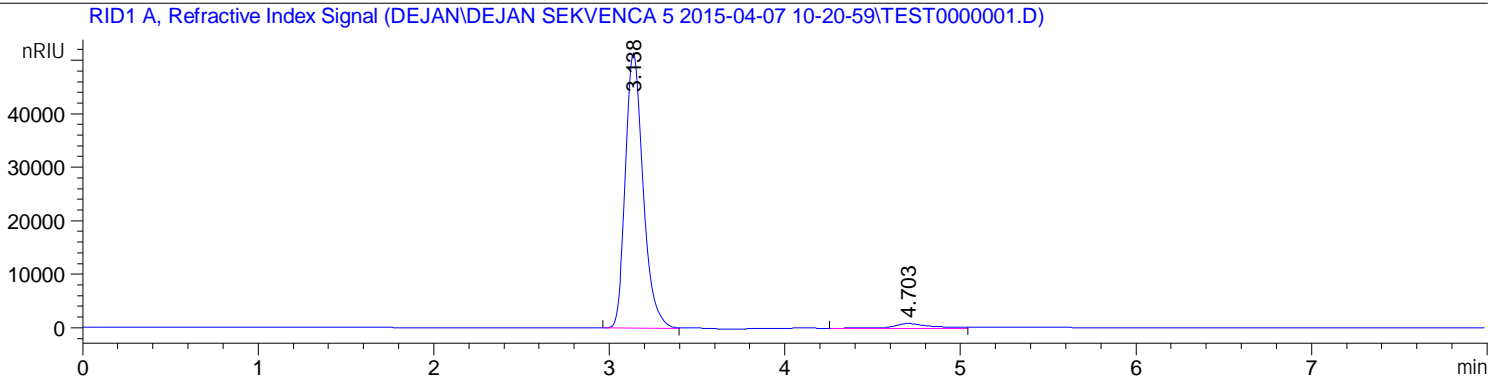
Peak #	RetTime [min]	Type	Width [min]	Area [nRIU*s]	Height [nRIU]	Area %
1	1.368	BV	0.1368	6.50110e5	6.97456e4	96.9697
2	1.907	VB	0.1872	2.03159e4	1509.72192	3.0303

Totals : 6.70426e5 7.12553e4

*** End of Report ***

```

=====
Acq. Operator   : SYSTEM                      Seq. Line :    1
Acq. Instrument : HPLC-Solaja                 Location  : Vial 6
Injection Date  : 4/7/2015 10:22:13 AM      Inj       :    1
                                                Inj Volume: 0.600 µl
Method          : C:\CHEM32\1\DATA\DEJAN\DEJAN SEKVENCA 5 2015-04-07 10-20-59\IZOKRATSKI 1.M
                  (Sequence Method)
Last changed    : 4/7/2015 10:20:59 AM by SYSTEM
Additional Info :
=====
    
```



Fraction Information

No Fractions found.

Area Percent Report

```

Sorted By      : Signal
Multiplier     : 1.0000
Dilution       : 1.0000
Use Multiplier & Dilution Factor with ISTDs
    
```

Signal 1: RID1 A, Refractive Index Signal

Peak #	RetTime [min]	Type	Width [min]	Area [nRIU*s]	Height [nRIU]	Area %
1	3.138	BV	0.1077	3.60537e5	5.14312e4	96.3422
2	4.703	BV	0.2172	1.36885e4	895.57855	3.6578

Totals : 3.74225e5 5.23268e4

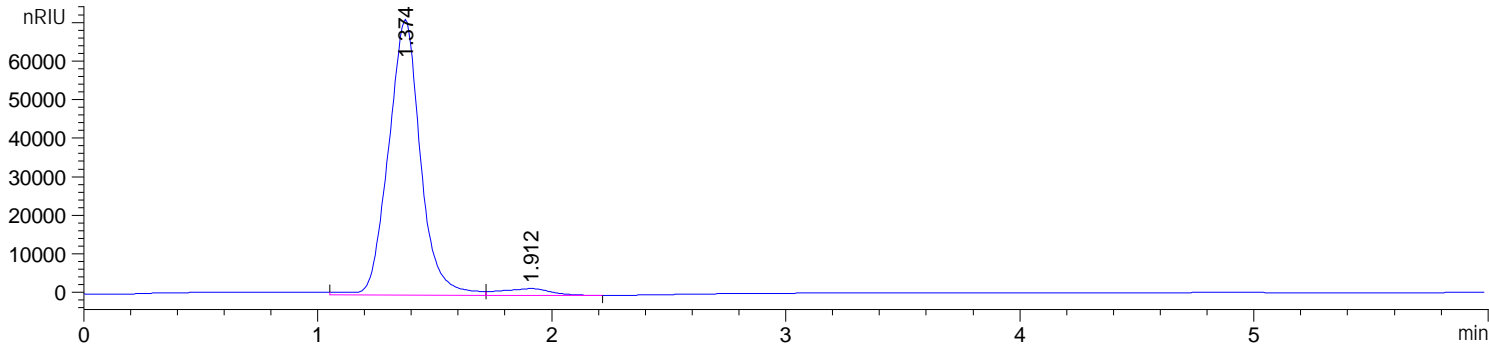
*** End of Report ***

```

=====
Acq. Operator   : SYSTEM                      Seq. Line :    1
Acq. Instrument : HPLC-Solaja                 Location  : Vial 6
Injection Date  : 4/6/2015 3:04:22 PM        Inj       :    1
                                                Inj Volume: 1.000 µl
Method          : C:\CHEM32\1\DATA\DEJAN\DEJAN SEKVENCA 5 2015-04-06 15-03-07\IZOKRATSKI 1.M
                  (Sequence Method)
Last changed    : 4/6/2015 3:03:07 PM by SYSTEM
Additional Info :
=====

```

RID1 A, Refractive Index Signal (DEJAN\DEJAN SEKVENCA 5 2015-04-06 15-03-07\TEST0000001.D)



```

=====
Fraction Information
=====

```

No Fractions found.

```

=====
Area Percent Report
=====

```

```

Sorted By      :      Signal
Multiplier     :      1.0000
Dilution       :      1.0000
Use Multiplier & Dilution Factor with ISTDs

```

Signal 1: RID1 A, Refractive Index Signal

Peak #	RetTime [min]	Type	Width [min]	Area [nRIU*s]	Height [nRIU]	Area %
1	1.374	VV	0.1390	6.78876e5	7.14253e4	96.4019
2	1.912	VB	0.2010	2.53385e4	1731.30737	3.5981

```
Totals :                7.04215e5  7.31566e4
```

```

=====
*** End of Report ***
=====

```